Indian Journal of Chemistry
Vol. 56B, July 2017, pp. 732-739

Synthesis, characterization and anti-inflammatory evaluation of novel substituted tetrazolodiazepine derivatives

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Received 4 July 2016; accepted (revised) 2 June 2017

In this paper, has been described the synthesis of some novel tetrazolodiazepine derivatives starting from piperidin-4-ones, with a view to test the novel compounds for various biological activities. The structures of the synthesized compounds have been established by IR, ^{1}H and ^{13}C NMR, mass and elemental analysis. Representative compounds have been characterized by COSY, HETCOR and DEPT 135 spectra. The representative compounds of the tetrazolodiazepine have been tested for anti-inflammatory activity by carrageenan induced paw edema method. From the results of the *in vivo* study, it is observed that the compounds taken for the study possess fairly good activity. Among the synthesized compounds, 6,8-bis-(3-chlorophenyl)-5-methyl-6,7,8,9-tetrahydro-5*H*-tetrazolo[1,5-*d*][1,4]diazepine **3c** shows potent anti-inflammatory activity.

Keywords: Synthesis, characterization, two dimensional NMR studies, tetrazolodiazepine, anti-inflammatory activity

Various diazepines have been reported as fungicidal and herbicidal[1]. Substituted 1,4-diazepine and their derivatives possess anti-HIV activity[2] which is lesser than that of zidovudine (3′-azidothymidin = AZT). They also show platelet activating factor (PAF) antagonistic and serotonergic S_3 antagonistic activities[3,4]. Benzodiazepine receptor systems have been related to the development and evolution of various neurological and psychiatric disorders, including epilepsy[5], Huntington's disease[6] and Alzheimer's disease[7]. It has also been reported that benzodiazepam binds to benzodiazepine receptors in the brain and potentially useful radiopharmaceutical for PET (positron emission tomography) studies of cerebral benzodiazepine receptor. 1,5-Benzodiazepines have been tested against breast cancer and have shown moderate activity. Other than their biological importance, benzodiazepine derivatives are also commercially used as dyes for acrylic fibres[8]. Moreover, 1,5-benzodiazepine derivatives are valuable synthons that can be used in the preparation of other fused ring compounds, such as triazolo-, oxadiazolo-, oxazino-, or furano- benzodiazepines[9]. Research in this area is still very active and is directed towards the synthesis of compounds with enhanced pharmacological activity.

The tetrazole ring system has attracted much attention in medicinal chemistry[10]. Tetrazoles are reported to exhibit antihypertensive, anti-allergic and antibiotic activity[11,12]. For example, they are currently used as activators and anticonvulsants, as well as in cancer and AIDS treatment. Many patents are available concerning their varied biological activities, such as muscle relaxation, anti-inflammatory, antiarthritic, analgesic, ulcer therapeutic and coccidiostatic properties. Tetrazoles are also used as plant growth regulators, herbicides and fungicides in agriculture[13], as stabilizers in photography and photoimaging[14,15]. Another important application of tetrazoles is in the preparation of imidoylazides[16]. Due to high enthalphy of formation, tetrazole decomposition results in the liberation of two nitrogen molecules and significant amount of energy. Therefore, several tetrazole derivatives have been explored as explosives, propellent components for missiles and as gas generators for air bags in the automobile industry. Furthermore, the tetrazole ring has strong electron withdrawing properties and the tetrazole halides are employed in synthesis as derivatising agents for the chemical modification of alcohols[17].

Inflammation is the body effort to inactivate or destroy invading organisms, remove irritants and set

the stage for tissue repair. It is a normal protective response to tissue injury. Inflammation may be caused by microbiological agents, noxious chemicals or trauma. It can also be triggered by innocuous agents, such as pollen or by an autoimmune response, as in some asthmas or rheumatoid arthritis. In such cases, the defence reactions themselves may cause progressive tissue injury. Anti-inflammatory or immune depressive drugs may be required to modulate the inflammation process. The inflammation subsides when the healing is complete. Inflammation is triggered by the release of chemical mediates from the injured tissues and migrating cells. These specific chemical mediators may vary depending upon the type of inflammatory process. The chemical mediators include amines such as histamine and 5-hydroxytryptamine. Lipids such as prostaglandins, small peptides such as bradykinin and larger peptides such as interleukin-1 are some of the chemical mediators. Discovery of such a variety of chemical mediators has clarified the apparent paradox that different drugs are effective in treating one form of inflammation but not others. Thus, a drug may interfere with the action of a particular mediator important in one type of inflammation, but ineffective in the inflammatory processes not involving the target mediator.

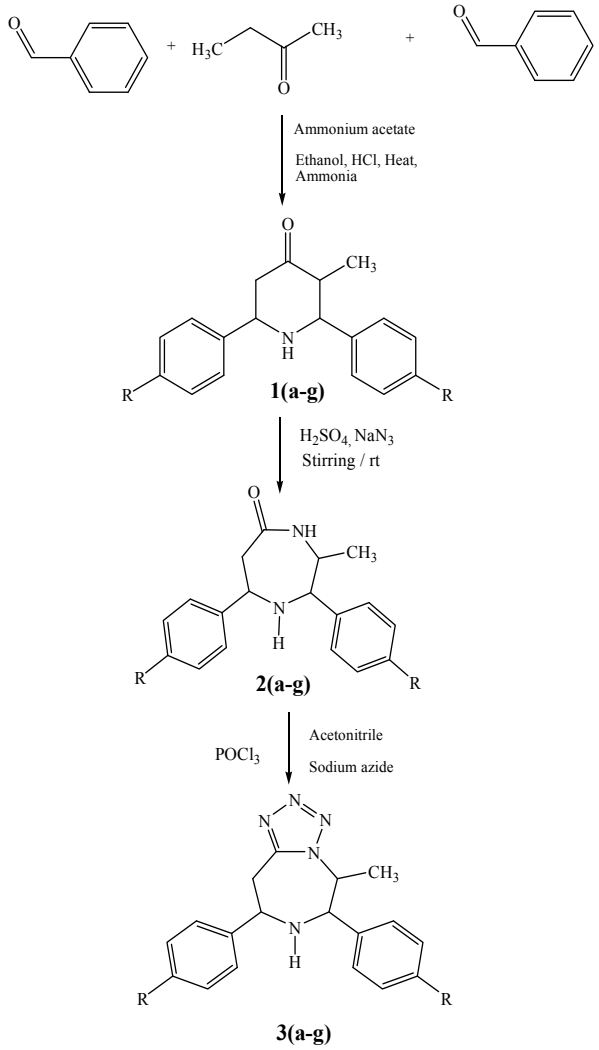
Results and Discussion

Characterization

The FTIR spectrum of the compound, *viz*., 5-methyl-6,8-diphenyl-6,7,8,9-tetrahydro-5*H*-tetrazolo[1,5-*d*][1,4]diazepine (**3a**, Scheme I) shows an absorption band at 1643 cm^{-1} for the –C=N functional group. The absorption band at 1450 cm^{-1} is due to the presence of –N=N functional group in the compound. The presence of –N−N=N functional group is evidenced by the appearance of an absorption band at 1237 cm^{-1}. The presence of the tetrazole ring in the compound is confirmed by the appearance of absorption bands at 1023 cm^{-1} and 1095 cm^{-1}. The –N−H stretching frequency of the compound is evidenced by the appearance of an absorption band at 3425 cm^{-1}.

The proton NMR spectrum of the compound **3a** shows all expected signals. The presence of methyl protons is evidenced by the appearance of a doublet at δ 1.5 (J = 7 Hz). A double doublet appearing in the range δ 3.35-3.40 can be assigned to the methylene proton H6b at C6. The H6a proton appears as a double doublet centered on δ 3.65 (J = 2 Hz). A doublet appearing at δ 3.83 (J = 8.5 Hz) correspond to the methine proton at C2. A doublet of doublet appearing at δ 4.1 (J = 2 Hz) corresponds to the methine proton H7 at C7. The methine proton at C3 appears as a multiplet centered on δ 4.81. The multiplet observed in the range δ 7.30-7.44 is due to the presence of the aromatic protons of the phenyl rings. The 1H (N-H) proton of the tetrazolodiazepine compound is undetectable in the ^{1}H NMR spectrum.

In the ^{13}C NMR spectrum of the compound, *viz*., 5-methyl-6,8-diphenyl-6,7,8,9-tetrahydro-5*H*-tetrazolo[1,5-*d*][1,4]diazepine **3a**, the characteristic tetrazole carbon resonates at δ 153.73. The methyl carbon shows signal at δ 17.69. The peak at δ 32.03 is attributed to



3a = R = -H, 3b = R = - NO_2, 3c = R = -Cl,
3d = R = $(-OCH_3)_2$, 3e = R = -Br, 3f = R = -OH
and 3g = R = *Trans* Cinnalmaldehyde

Scheme I

the C6 carbon of the diazepine ring. The signals at δ 59.11, 63.06 and 65.23 are due to the C2, C7 and C3 carbon atoms of the diazepine ring respectively. The carbon signals recorded at δ 141.53 and 143.80 is attributed to the carbon atoms of the phenyl ring attached to the diazepine ring. The aromatic carbon atoms of the phenyl rings are observed around δ 126.49-129.36. The carbonyl absorption peak which appeared in the diazepine compound, *viz*., 3-methyl-2,7-diphenyl-[1,4]diazepan-5-one (**2a**, Scheme I) does not appear in the ^{13}C NMR of this tetrazolodiazepine compound, evidencing the formation of tetrazole ring.

The DEPT 135 spectrum is an excellent tool to distinguish among the methyl, methylene, methine and quaternary carbons in the ^{13}C NMR spectrum. The DEPT 135 spectra augments the evidence for the assigned structure to the compound, *viz*., 5-methyl-6,8-diphenyl-6,7,8,9-tetrahydro-5*H*-tetrazolo[1,5-*d*][1,4]diazepine **3a**. The earlier assignment of the methyl carbon at δ 18.21 is further confirmed by the appearance of a positive peak (odd number of protons) at δ 17.33 in the DEPT 135 spectrum. The negative peak appearing at δ 34.13 is due to the presence of CH_2 group (even number of protons). The positive peaks appearing at δ 61.54, 64.01 and 69.00 are assigned to the methine carbons at C2, C7 and C3 respectively (odd number of protons). The aromatic carbon atoms of the phenyl rings show carbon peaks at δ 126.43, 127.45, 128.34, 128.75, 128.97 and 129.17.

The COSY spectrum of the compound, *viz*., 5-methyl-6,8-diphenyl-6,7,8,9-tetrahydro-5H-tetrazolo[1,5-*d*][1,4]diazepine **3a** shows distinct spots on a diagonal, extending from the upper right corner of the spectrum down to the lower left corner. In the spectrum, we can extend a horizontal line from the spot at δ 1.5 (which is labelled **A** and corresponds to methyl protons). This horizontal line eventually encounters an off-diagonal spot **B** that corresponds to the methine proton peak at δ 4.8. A vertical line drawn from this off-diagonal spot intersects the spot on the diagonal that corresponds to the methine proton (**K**). The presence of this off-diagonal spot **B**, which correlates the methyl proton spot and the methine proton spot, confirms that the methyl protons are coupled to the methine protons, as we would have expected.

We can extend a horizontal line from the spot at δ 3.3 (which is labelled **C** and corresponds to the methylene proton (H6b)). This horizontal line encounters off-diagonal spots at **D** and **E** that correspond to methylene (H6a) and methine proton (H7). If a vertical line is drawn from these off-diagonal spots, they intersect with the spots **F** and ***J*** on the diagonal that corresponds to H6a and H7 protons. This confirms that the H6b proton is coupled to H6a proton and H7 proton, as per our expectation. The H6b proton undergoes self coupling which is evidenced by the spot F on the diagonal and correlates with H6b proton which is evidenced by the off-diagonal spot at **G**. A horizontal line is drawn from the spot at δ 3.8 (which is labelled **H** and corresponds to the methine proton (H2)). This horizontal line eventually encounters an off-diagonal spot at **I** that corresponds to the methine proton peak at δ 4.8 (H3). This indicates that the H2 methine proton correlates with the H3 methine proton.

Further confirmation of the assigned structure of the compound **3a** is evidenced by recording the Heterocosy spectrum. In the Heterocosy spectrum, the methyl carbon which appears at δ 17.33 in the carbon spectrum (vertical axis) shows a doublet for methyl protons at δ 1.5 in the ^{1}H NMR spectrum (horizontal axis). A spot is obtained if a vertical line from the proton spectrum and a horizontal line from the methyl peak of the carbon spectrum is drawn. The two lines intersect exactly at this point. The spot indicates that the protons at δ 1.5 and carbons at δ 17.33 represent the same position of the molecule. The carbon peak at δ 69.00 and a proton doublet at δ 3.82 correspond to the C2 methine group. The carbon peak at δ 64.01 and multiplet at δ 4.8 correspond to the C3 methine group. The carbon peak at δ 1.5 and a proton doublet at δ 4.1 correspond to the C7 methine group. The carbon peak at δ 34 and a proton doublet and multiplet at δ 3.65 and δ 3.35 correspond to the H6a and H6b protons respectively. The cluster of carbon peaks around δ 126.42-129.16 and proton multiplet centered on δ 7.30-7.44 correspond to the aromatic protons of the phenyl rings of the compound.

In compound **3b**, the tetrazole ring frequency appears at 1022 cm^{-1} and 1097 cm^{-1}. The absorption bands at 1023 cm^{-1}, 1128 cm^{-1}; 1025 cm^{-1}, 1091 cm^{-1}; 1011 cm^{-1}, 1071 cm^{-1}, 1010 cm^{-1}, 1095 cm^{-1} and 1031 cm^{-1}, 1120 cm^{-1} confirm the presence of the tetrazole ring in the compounds **3c-g** (Scheme I) respectively. A similar observation was reported by Rauf and Parveen for the tetrazole ring synthesized by them. Dayanithi *et al*. have also reported bands at about 1100 cm^{-1} and 1000 cm^{-1}, which were characteristic for the CN_4 (tetrazole ring) in the

following compound (**189**): The presence of an absorption bands at 821 cm^{-1} (**3e**) and 794 cm^{-1} (**3c**) show the presence of C-Br and C-Cl groups respectively. The presence of nitro group in the compound **3b** is confirmed by the appearance of an absorption band at 1350 cm^{-1}. The –N−H absorption band appears around 3400 cm^{-1} in all the tetrazolodiazepine compounds synthesized in the present investigation. Further evidence for the formation of target compounds was obtained from the ^{1}H NMR spectra, which proved to be the diagnostic tool for the positional elucidation of protons.

The compound, *viz*., 5-methyl-6,8-bis-(3-nitrophenyl)-6,7,8,9-tetrahydro-5*H*-tetrazolo[1,5-*d*][1,4]diazepine **3b**, a doublet appearing at δ 1.5 (J = 1.5 Hz) is due to the methyl protons. The H6a and H6b protons of the compound exhibit double doublet peaks centered on δ 3.7 (J = 2.0 Hz) and δ 3.45 (J = 10 Hz) respectively. The H2 proton shows a doublet at δ 4.1, J = 2.0 Hz whereas the H3 proton appears as a multiplet at δ 4.95. A double doublet appearing at δ 4.3 (J = 2 Hz) is due to the H7 proton of the compound. The aromatic protons of the compound exhibit a multiplet centered on δ 7.5.

The compound, *viz*., 6,8-bis-(3-chlorophenyl)-5-methyl-6,7,8,9-tetrahydro-5*H*-tetrazolo[1,5-*d*][1,4] diazepine **3c** also shows all the expected signals. The methyl protons of the compound exhibit a doublet at δ 1.5 (J = 7.5 Hz). As stated earlier, the H6a and H6b protons of the compound show double doublets centered on δ 3.61 (J = 2 Hz) and δ 3.31 (J = 10 Hz) respectively. The H2 proton appears as a doublet at δ 3.8 (J = 8.5 Hz), whereas the H3 proton appears as a multiplet centered at δ 4.75. The H7 proton exhibits a double doublet at δ 4.04 (J = 2 Hz). The cluster of signals centered on δ 7.3 is due to the presence of aromatic protons.

The methyl protons of the compound, *viz*., 6,8-bis-(3,4-dimethoxyphenyl)-5-methyl-6,7,8,9-tetrahydro-5*H*-tetrazolo[1,5-*d*][1,4]diazepine **3d** exhibit a doublet at δ 1.5 (J = 6.5 Hz) in its ^{1}H NMR spectrum. The H6a and H6b protons appear as double doublets centered on δ 3.62 (J = 2 Hz) and δ 3.33 (J = 10 Hz) respectively. The H2 proton of the compound exhibits a doublet at δ 3.76 (J = 8.5 Hz). The H3 proton appears as a multiplet centered on δ 4.76 and the H7 proton appears as a double doublet centered on δ 4.02 (J = 2 Hz). The aromatic protons of the compound show a multiplet around δ 6.82-6.97. The methoxy protons of the compound appear as intense peaks at δ 3.89.

In compound **3e**, the appearance of a doublet at δ 1.5 (J = 7 Hz) indicates the presence of methyl protons. A double doublet centered on δ 3.82 (J = 8.5 Hz) indicates the presence of H6a proton. The H6b proton appears as a double doublet at δ 3.65 (J = 1.5 Hz). The H2 proton appears as double doublets at δ 4.07 (J = 2 Hz). The multiplets centered on δ 4.31 and δ 4.7 indicate the H3 and H7 protons respectively. The aromatic protons of the compound appear as a multiplet centered on δ 7.5.

In compound **3f**, methyl protons appear as a doublet at δ 1.5 (J = 7 Hz), as expected. The double doublets appearing at δ 3.62 (J = 2 Hz) and δ 3.31 (J = 10 Hz) may be assigned to the H6a and H6b protons. The H2 proton exhibits a doublet at δ 3.8 (J = 8.5 Hz). The H7 proton of the compound appears as a double doublet centered on δ 4.05 (J = 2 Hz). The H3 proton shows a multiplet centered on δ 4.75. The phenolic protons may be merged with the multiplet at δ 4.7. The protons *ortho* to the –OH group appear as a double doublet centered on δ 7.52 and the protons *meta* to the –OH group appear as a multiplet centered on δ 7.30.

In the ^{13}C NMR spectrum of the compound, *viz*., 6,8-bis-(3-chlorophenyl)-5-methyl-6,7,8,9-tetrahydro-5*H*-tetrazolo[1,5-*d*][1,4]diazepine **3c**, the carbon signal at δ 16.58 ppm is attributed to the methyl carbon atom. The CH_2 carbon atom of the diazepine ring is observed at δ 32.01. The C2 and C3 carbon atoms of the diazepine ring show absorptions at δ 59.48 and 63.45 respectively, whereas the C7 carbon atom of the diazepine ring resonate at δ 62.26. The *ipso* carbon atoms of the phenyl rings show the downfield shift in ^{13}C NMR due to the presence of an electronegative chlorine atom, which is evidenced by the appearance of carbon signals at δ 134.11 and 134.44. The carbon atoms of the phenyl ring attached to the diazepine ring are observed at δ 142.95 and 141.08. The other carbon atoms of the phenyl ring resonate in the region δ 125.94-130.69. The carbon peak at δ 153.90 is attributed to the tetrazole carbon atom flanked by two nitrogen atoms. A similar interpretation can be given to the ^{13}C NMR spectrum of the compound **3d**. The compound **3d** shows expected carbon signals. The appearance of a carbon signal at δ 17.42 is due to the methyl carbon atom. The C6 carbon of the diazepine ring shows a carbon signal at δ 37.12. The C2, C7 and C3 carbon atoms of the diazepine ring were observed at δ 61.29, 64.06 and 68.62 respectively. The carbon atoms of the phenyl rings linked to the −OCH_3 groups show

signals around δ 148.90-149.44. The -OCH_3 carbon shows carbon signals at δ 55.98 and 56.08. The carbon atoms of the phenyl ring *ortho* to the –OCH_3 group show carbon signals at δ 118.46 and 119.84, whereas the carbon atoms *meta* to the –OCH_3 group show carbon signals at δ 109.58-111.36. The signals at δ 133.24 and 135.74 are attributed to the carbon atoms attached to the diazepine ring. The carbon atom of the tetrazole ring resonates at δ 154.28.

Anti-inflammatory Activity

The anti-inflammatory activity of the test compounds by Carrageenan Induced Paw Edema Method is represented in table (Table I). Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergents or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of this chronic illness. Although it is a defence mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases. Edema which develops after carrageenan inflammation is a biphasic event. The initial phase is attributed to the release of histamine and serotonin. The edema maintained between the first and the second phase is due to kinin like substances. The second phase is said to be promoted by prostaglandin like substances. It has been reported that the second phase of edema is sensitive to drugs like hydrocortisone, phenylbutazone and indomethacin. Currently used anti-inflammatory drugs are associated with some severe side effects. Therefore, the development of potent anti-inflammatory drugs with fewer side effects is necessary.

From the results of the study, it is evident that the compound **3c** shows more percentage inhibition (71.42%). The percentage inhibition shown by the bromo-substituted tetrazolodiazepine **3e** is found to be 57.14. The –OH substituted compound **3f** shows 55% inhibition to paw edema. The least inhibition is observed in the case of unsubstituted compound **3a**. It is observed that introducing −Cl group has enhanced the anti-inflammatory activity of the compound **3c** remarkably.

Table I — Anti-inflammatory activity of tetrazolodiazepine derivatives against carrageenan induced paw edema in albino rats

Treatment Group	Paw Thickness at 0 h (mm)	Paw Thickness at 3 h (mm)	% Inhibition
Control	0.567 ± 0.08	1.967 ± 0.29	–
Indomethacin	0.65 ± 0.15	0.93 ± 0.13	80
3a	0.67 ± 0.12	2.03 ± 0.12	2.85
3b	0.65 ± 0.10	1.33 ± 0.52	51.43
3c	0.70 ± 0.09	1.1 ± 0.17	71.42
3d	1.13 ± 0.52	1.9 ± 0.18	45
3e	0.65 ± 0.10	1.25 ± 0.24	57.14
3f	0.77 ± 0.10	1.4 ± 0.19	55

Experimental Section

Infrared spectra were recorded on a Perkin-Elmer FTIR 1600 spectrometer using KBr disks. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Bruker spectrometer. COSY and HETCOR (500 MHz), DEPT 135 (125 MHz) were recorded on Bruker spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard and coupling constants (*J*) are given in Hz. Signals were characterized as s (singlet), d (doublet), t (triplet), and m (multiplet). Melting points were measured by a digital melting point apparatus and are uncorrected. Progress of the reactions were followed by TLC using solvent systems of different polarities. The mass spectra were recorded on JEOL GCmate mass spectrometer. Synthesis of 3-methyl-2,6-diphenylpiperidin-4-one (**1a-g**) was carried out according to the reported procedure[18].

General procedure for the synthesis of 3-methyl-2,7-diphenyl-[1,4]diazepan-5-one, 2a-g

The powdered 3-methyl-2,6-diphenylpiperidin-4-one (**1a**) (2.65 g, 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 to RT. Sodium azide (0.64 g, 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 *p*H of the mixture was adjusted to ≈8.0 using 2N NaOH solution. The white solid separated was isolated by filtration. The crude product was purified by recrystallization from ethanol. The same experimental procedure was followed for the preparation of remaining target compounds **2b-g**.

General procedure for the synthesis of 5-methyl-6,8-diphenyl-6,7,8,9-tetrahydro-5*H*-tetrazolo [1,5-*d*][1,4]diazepine, 3a-g

A mixture of 3-methyl-2,7-diphenyl-1,4-diazepan-5-one (**2a**) (2.80 g, 0.01 mol), sodium azide (0.64 g,

0.01 mol) and phosphorus oxychloride (1.64 mL, 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 by sodium bicarbonate. The precipitated crude product was purified by recrystallization from alcohol to get the target compound. The same experimental procedure was followed for the preparation of remaining target compounds **3b-g**.

Yield 86%. m.p.162-64°C. FTIR (KBr): 3425 (-NH), 2922 (Ar-CH), 1643 (-C=N), 1450 (-N=N), 1237 (N-N=N), 1095, 1023 cm^{-1} (Tetrazole ring); ^{1}H NMR (500 MHz, 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]. Anal. Calcd: C, 68.29; H, 6.98; N, 20.78. Found: C, 68.12; H, 6.94; N, 20.78%.

5-Methyl-6,8-bis-(3-nitrophenyl)-6,7,8,9-tetrahydro-5*H*-tetrazolo[1,5-*d*][1,4]diazepine, 3b: Yield 75%. m.p.78-80°C. FTIR (KBr): 3415 (NH), 2923 (Ar-CH), 1649 (-C=N), 1383 (-N=N), 1097, 1022 cm^{-1} (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): δ 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]. Anal. Calcd: 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-5*H*-tetrazolo[1,5-*d*][1,4]diazepine, 3c: Yield 74%. m.p.110-12°C. FTIR (KBr): 3430 (-NH), 2923 (Ar-CH), 1598 (-C=N), 1468 (-N=N), 1128, 1023 cm^{-1} (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): δ 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+2]. Anal. Calcd: 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-5*H*-tetrazolo[1,5-*d*][1,4]diazepine, 3d: Yield 72%. m.p.78-80°C. FTIR (KBr): 3437 (NH), 2921 (Ar-CH), 1615 (-C=N), 1420 (-N=N), 1091, 1025 cm^{-1} (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): δ 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]. Anal. Calcd: 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-5*H*-tetrazolo[1,5-*d*][1,4]diazepine, 3e: Yield 79%. m.p.88-90°C. FTIR (KBr): 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): δ 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* 465 [M+2]. Anal. Calcd: 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-5*H*-tetrazolo[1,5-*d*][1,4]diazepine-6,8-diyl)diphenol, 3f: Yield 69%. m.p.243-45°C. FTIR (KBr): 3449 (NH), 2931 (Ar-CH), 1642 (-C=N), 1392 (-N=N), 1095, 1010 cm^{-1} (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]. Anal. Calcd: C, 63.29; H, 6.47; N, 19.25. Found: C, 63.13; H, 6.51; N, 19.22%.

5-Methyl-6,8-distyryl-6,7,8,9-tetrahydro-5*H*-tetrazolo[1,5-*d*][1,4]diazepine, 3g: Yield 70%. m.p.239-41°C. FTIR (KBr): 3411 (-NH) 2921 (Ar-CH), 1638 (-C=N), 1383 (-N=N), 1120, 1031 cm^{-1} (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]. Anal. Calcd: C, 63.29; H, 6.47; N, 19.25. Found: C, 63.13; H, 6.51; N, 19.22%.

Acute Toxicity Study – OECD 423 (Ref 19)

Three healthy young adult rats of either sex were fasted prior to dosing over-night. 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 the 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 central nervous systems, somatomotor activity and behaviour pattern. Attention was given to the observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma.

Anti-inflammatory Activity

Carrageenan Induced Hind Paw Edema Method (Ref 20)

Albino rats of either sex weighing 150-250 g were divided into eight groups of six animals each. Group one served as control which received only saline. Group two animals received the standard drug indomethacin (10 mg/kg po). The remaining groups of animals were administered with test drugs (50 mg/kg po). All the drugs were administered orally. After 1 h of the administration of the drugs, 0.1 mL of 1% w/v carrageenan solution in normal saline was injected into the sub-plantar region of the left hind paw of the rat. The paw volume of the rats were measured in the digital plethysmometer (Ugo-basile, Italy) at the end of 0 min and 180 min. The percentage increase in paw edema of the treated group was compared with that of the control and the inhibitory effect of the drugs were studied. The relative potency of the drugs under investigations was calculated based upon the percentage inhibition of inflammation.

Conclusion

In the present research work, seven novel tetrazolodiazepine derivatives were synthesized. The compounds were synthesized from piperidones *via* diazepine derivatives. A representative sample of the diazepine was characterized by IR, ^{1}H and ^{13}C NMR, COSY spectrum, HETCOR spectrum and DEPT 135 spectrum. The assigned structure to the tetrazolodiazepine compound was confirmed by recording COSY, HETCOR and DEPT 135 spectra for a representative sample. The FTIR spectra of all the tetrazolodiazepine compounds showed absorption bands around 1600-1650 cm^{-1} (−C=N), 1400-1500 cm^{-1} (−N=N), 1100-1200 cm^{-1} (−N−N=N) and 900-1100 cm^{-1} (Tetrazole ring). The ^{1}H and ^{13}C NMR spectra also gave convincing evidence for the structure assigned to the compounds synthesized in the present investigation. Final proofs for the structures were obtained by recording their mass spectra, which exhibited molecular ion peaks corresponding to their molecular weight.

The representative compounds were tested in animal models for the preliminary pharmacological activities. Acute toxicity study was also carried out. The LD_{50} values of the compounds were fixed as 300 mg/kg.

The anti-inflammatory activity of the representative compounds was tested against Carageenan Induced Paw Edema Method. Indomethacin was used as the standard drug. The results showed that the synthesized compounds possess fairly good anti-inflammatory activity. However, the activity is less than that of the standard drug.

Acknowledgements

The authors are most grateful to the management of SRM University, Ramapuram Campus, Chennai for their continuous encouragement and financial support to carry out this research work.

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