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Synthesis, characterisation, stereochemistry and antimicrobial activity of N_5-piperazino- and N_5-morpholinoacetyl-2,2,4-trimethyl-1, 5-benzodiazepines

S PONNUSWAMY[a,*], A AKILA[a], D DEEPA RAJAKUMARI[a], V SHREEVIDHYAA SURESSH[b] and G USHA[c]

[a]P G & Research Department of Chemistry, Government Arts College (Autonomous), Coimbatore 641 018, Tamil Nadu, India
[b]Department of Physics, Anna Adarsh College for Women, Chennai 600 040, Tamil Nadu, India
[c]P G & Research Department of Physics, Queen Mary's College, Chennai 600 004, Tamil Nadu, India
e-mail: kspons2001@gmail.com

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Abstract. Three 1,5-benzodiazepines *viz*., N_5-chloroacetyl-, N_5-piperazinoacetyl- and N_5-morpholinoacetyl-2,2,4-trimethyl-1H-1,5-benzodiazepines have been synthesized. The structural characterisation and the conformational preferences of the compounds have been carried out using IR, 1D and 2D NMR spectral data. The NMR spectral data show that the *N*-acetyltetrahydro-1,5-benzodiazepines prefer to exist in boat conformation with *exo* orientation of >C=O at N_5 position in the solution state. The X-ray crystal structure of N_5-morpholinoacetyl-2,2,4-trimethyl-1H-1,5-benzodiazepine also supports boat conformation in the solid state. The antimicrobial activity for *N*-acetyltetrahydro-1,5-benzodiazepines have been carried out. *N*-morpholinoacetyl-2,2,4-trimethyl-1H-1,5-benzodiazepine demonstrated better antibacterial and antifungal activities.

Keywords. *N*-acetyl-1,5-benzodiazepines; NMR spectra; boat conformation; X-ray crystal structure; antimicrobial activity.

1. Introduction

Benzodiazepines are bicyclic heterocyclic compounds having benzene nucleus fused to a seven membered ring containing two nitrogen atoms which form an important class of biologically and medicinally active compounds. Some benzodiazepine derivatives are used as anti-inflammatory,[1] anti-convulsant, anti-anxiety, anti-fungal, anti-bacterial, anti-feedant, analgesic, sedative, anti-depressive and hypnotic agents.[2–5] Certain derivatives *viz.,* lofendazam, clobazam and triflubazam are used for the treatment of anxiety and neuroses including psychomatic disturbances. A few 2,4-diaryl-7,8-dimethyl-2,3-dihydro-1*H*-1,5-benzodiazepines have been tested against breast cancer and have shown moderate activity.[6]

The introduction of the acyl groups at N_1 and N_5 of tetrahydro benzodiazepines[7] results in perihydrogen interaction between the acyl groups and the ortho hydrogen of the benzene ring which could lead to interesting conformational changes.[8–10] The amide units with piperazinoacetyl moiety are anticipated to possess very useful and significant biological activity.[11] Hence, the present work involves the incorporation of amide and piperazine units together into the nitrogen site of benzodiazepine and study their conformational preferences as well as biological activity. So far, to the best of our knowledge, no benzodiazepine has been reported with piperazine/morpholino moiety.

2. Experimental

2.1 *Materials, methods and instruments*

All the reported melting points were taken in open capillaries and are uncorrected. The IR spectra were recorded using a BRUKER FT-IR alpha model spectrometer using KBr pellets. The ^{1}H and ^{13}C NMR spectra were recorded in a $CDCl_3$ solution using TMS as the internal standard in Bruker DRX 500 and 125 MHz Bruker AMX 400 and 100 MHz NMR spectrometers and the chemical shifts were referenced to TMS. A 0.05 M solution of the sample prepared in $CDCl_3$ was used for obtaining the 2D NMR spectra. The tubes used for recording the NMR spectra were of 5-mm diameter. Electron impact mass spectra were recorded using a

*For correspondence

JEOL GS mate spectrometer. Unless otherwise stated, all the reagents and solvents were of high grade and purchased from Aldrich and Merck. All the solvents were distilled prior to use. The parent tetrahydro-1,5-benzodiazepines, **1-2** were prepared by following the literature procedure.[7]

2.2 *Synthesis of N-chloroacetyl-2,2,4-trimethyl-1H-1,5-benzodiazepine **3***

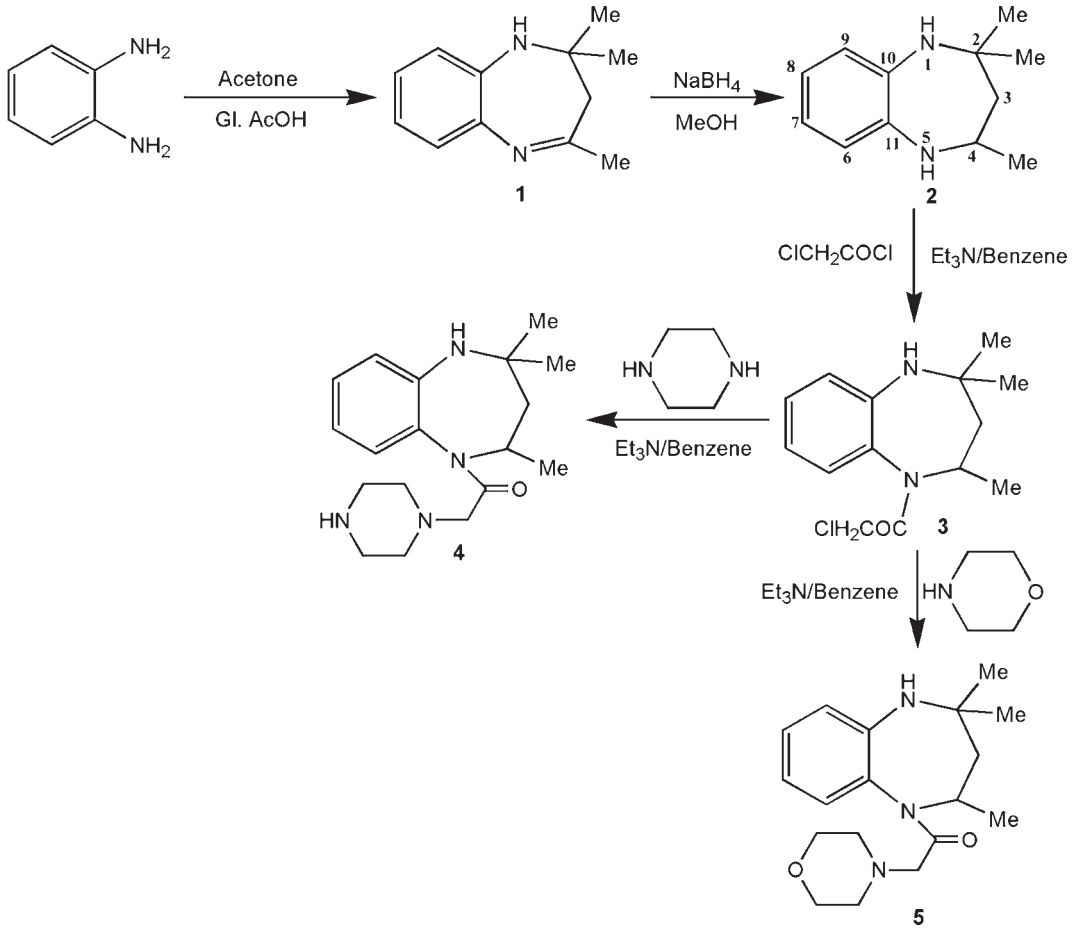
To an ice-cold solution of tetrahydrobenzodiazepine **2** (1.9 g, 10 mmol) in anhydrous benzene (50 mL), triethylamine (4 mL, 30 mmol) and chloroacetyl chloride (2.4 mL, 30 mmol), were added (scheme 1). The reaction mixture was stirred at RT for 2 h. The precipitated ammonium salt was filtered off and the filtrate was washed with water (4x20 mL). The benzene solution was dried over anhydrous Na_2SO_4 and concentrated after passing through a short column of silica. The resulting solid was purified by recrystallization from benzene to yield colorless crystals of **3**. The analytical data of the compound **3** are furnished in table 1.

Table 1. Analytical data for the compounds **3-5**.

Compounds	Molecular formula (Mol.Wt.)	Melting point (°C)	Yield (%)
3	$C_{14}H_{19}N_2OCl$ (266)	142-144	72
4	$C_{18}H_{28}N_4O$ (316)	260-262	55
5	$C_{18}H_{27}N_3O_2$ (317)	82-84	50

2.3 *Synthesis of N-piperazinoacetyl-2,2,4-trimethyl-1H-1,5-benzodiazepine **4***

To a solution of *N*-chloroacetylbenzodiazepine **3** (1.33 g, 5 mmol) in anhydrous benzene (50 mL), triethylamine (2.8 mL, 20 mmol) and piperazine (1.72 g, 20 mmol) were added (scheme 1). The reaction mixture was stirred at 60°C for 6 h. The resulting solution was washed with water (4×20 mL), dried over anhydrous Na_2SO_4, and passed through a short column of silica and concentrated. The residue thus obtained was purified by recrystallization from benzene and pet-ether (60-80°C) in the ratio of 95:5. The analytical data of the compound **4** are furnished in table 1.



Scheme 1. Synthesis of *N*-acetylterahydro-1,5-benzodiazepines **3-5**.

2.4 *Synthesis of N-morpholinoacetyl-2,2,4-trimethyl-1H-1,5-benzodiazepine* **5**

To a solution of *N*-chloroacetylbenzodiazepine **3** (1.33 g, 5 mmol) in anhydrous benzene (50 mL), triethylamine (2.8 mL, 20 mmol) and morpholine (1.8 mL, 20 mmol) were added (scheme 1). The reaction mixture was stirred at 60°C for 8 h. The benzene solution was washed with water (4x20 mL), dried over anhydrous Na_2SO_4, and passed through a short column of silica and concentrated. Purification of resulting mass by recrystallization from benzene afforded colorless crystals of **5**. The analytical data of the compound **5** are furnished in table 1.

2.5 *X-ray data collection*

A colorless diffraction quality crystal 0.20 × 0.18 × 0.18 mm of **5** was obtained as blocks by slow evaporation technique using benzene as solvent. The intensity data were measured with graphite monochromated MoKα radiation using scans on Bruker SMART APEX area detector diffractometer,[12a] up to $\theta_{max=}$28.58°. The data covered over a hemisphere of reciprocal space by a combination of three sets of exposures; each set had a different φ angle (0, 88, 180°) for the crystal and each exposure of 10 seconds covered 0.3° in ω.

The detector was at a distance of 4 cm from the crystal and the swing angle of the detector was -35°. Cell refinement and data reduction were carried out. Decay was monitored by repeating thirty initial frames and analyzing the duplicate reflections. For the crystals of the *N*-morpholinoacetyl-2,2,4-trimethyl-1*H*-1,5-benzodiazepine **5**, the decay was found to be negligible. Lorentz and polarization corrections were applied but none for absorption or extinction. Absorption correction was neglected because μ t=0.092 mm^{-1} only.

The compound crystallizes in monoclinic system. The Laue group assignment, systematic absences and intensity statistics were consistent with space group C2/c. A total of 16955 reflections were collected out of which only 4536 reflections had I>2σ (I) and these reflections were considered as observed and included in the refinement.

2.5a *Structure determination and refinement*: The structure of the compound **5** was solved by direct methods using the program SHELXS-97[12b,c] with E's ≥ 1.2. An E-map showed clearly all the non-hydrogen atoms of the molecule. The trial structure of the molecule was refined with full-matrix least-squares on F^2 procedures for 212 parameters along with isotropic thermal parameters and later anisotropic thermal parameters for the non-hydrogen atoms and isotropic parameters for the hydrogen atoms using the SHELXL-97[12b,c] programs. The position of the hydrogen atoms were fixed by geometry and were treated as riding on the parent C atoms, with aromatic C-H distances of 0.93- 0.98 Å, N-H distance of 0.86 Å, and with U_{iso} (H) = 1.5 U_{eq} (C) for methyl H and 1.2 U_{eq} (C) for other H. The refinement was terminated when mean shift/σ for non-hydrogen atoms was ≤ 0.001. The final R-value for 4536 observed reflections converged to 0.056; wR_2 = 0.162; S =1.02; $\Delta\rho$ = 0.42 to -0.66 eÅ^{-3} and (Δ/σ_{max}) ≤ 0.001.

3. Results and Discussion

In the IR spectrum of the parent benzodiazepine **2**, the NH stretching bands were observed around 3332 and 3350 cm^{-1} due to N_1-H and N_5-H, respectively. However, one of the N-H stretching bands around 3330 cm^{-1} was absent in the IR spectrum of the compound **3** indicating the formation of monosubstituted product. Furthermore, the greater deshielding of proton at C_4 of **3** (4.84 ppm) compared to the parent compound **2** (3.22 ppm) confirms the substitution at N_5. In the IR spectra of the compounds **3-5**, the presence of NH peak at 3356, 3335 and 3355 cm^{-1} and appearance of a new carbonyl stretching frequency at 1646, 1654 and 1638 cm^{-1}, respectively, confirmed the formation of the compounds **3-5**. In the mass spectra, the presence of molecular ion peaks at m/z 266, 316 and 317 for the compounds **3-5**, respectively, and their fragmentation pattern confirms the proposed substitution at N_5.

The preferred conformation of the N_5-chloroacetyl-, N_5-piperazinoacetyl- and N_5-morpholinoacetyl-2,2,4-trimethyl-1H-1,5-benzodiazepines **3-5**, respectively, has been derived from the ^{1}H and ^{13}C NMR spectral data in comparison with those of the tetrahydro-2,2,4-trimethyl-1H-1,5-benzodiazepine **2**. Furthermore, 2D NMR spectra (^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC) were also used for the unambiguous assignment of the NMR signals of compounds **3-5**. The ^{1}H and ^{13}C NMR spectral data of the compounds **3-5** are presented in tables 2 and 3. The vicinal coupling constants $^3J_{3e,4a}$ and $^3J_{3a,4a}$ of the compounds **3-5** extracted from their ^{1}H NMR spectra were used to estimate the dihedral angles between the vicinal protons at C_3 and C_4 using DAERM[13] (Dihedral Angle Estimation by Ratio Method) and are presented in table 4.

Table 2. ^{1}H Chemical shift values (δ ppm) of *N*-acetylterahydro-1,5-benzodiazepines (**3-5**) and parent amine **2**.

Compound	H_4	H_{3a}	H_{3e}	CH_3 at C_2	'CH_3 at C_2	CH_3 at C_4	Pip	Mor	NH	CH_2 of $NCOCH_2$	Aromatic protons
3	4.84	1.48	1.55	1.27	1.20	1.13	–	–	3.11	3.81,3.62	7.27-6.72
4	4.82	1.43	1.49	1.22	1.17	1.07	2.42	–	3.09	2.89,2.51	7.27-6.67
5	4.84	1.44	1.50	1.24	1.19	1.10	–	2.40,3.67	3.14	2.96,2.56	7.29-6.70
2	3.22	1.56-1.67		1.33	1.08	1.24	–	–	–	–	6.62-6.90

Table 3. ^{13}C Chemical shift values (δ ppm) of *N*-acetylterahydro-1,5-benzodiazepines (**3-5**) and parent amine **2**.

Compound	C_2	C_3	C_4	CH_2 of $NCOCH_2$	CO of $NCOCH_2$	Pip	Mor	CH_3 at C_2	'CH_3 at C_2	CH_3 at C_4	Aromatic Carbons	*Ipso* carbons
3	53.2	43.3	46.3	42.6	165.8	–	–	33.5	29.2	18.8	130.1-120.0	144.5,125.4
4	53.0	43.3	45.1	60.1	169.0	53.1	–	33.5	29.1	18.9	130.4-119.8	144.5,126.2
5	53.1	43.3	45.2	60.4	168.7	–	53.6,66.8	33.5	29.2	18.9	130.3-119.7	144.5,126.0
2	51.7	51.2	47.8	–	–	–	–	32.8	25.9	23.9	119.7-121.6	140.2,137.6

Table 4. The vicinal coupling constant data (in Hz) and the corresponding dihedral angles (in degrees) estimated using DAERM of the compounds **3-5** and parent amine **2**.

Compound	$J_{3e,4a}$	$J_{3a,4a}$	$\phi_{3e,4a}$	$\phi_{3a,4a}$
3	5.0	12.0	48	168
4	5.5	12.0	46	166
5	5.5	12.0	46	166
2	2.0	11.2	62	182

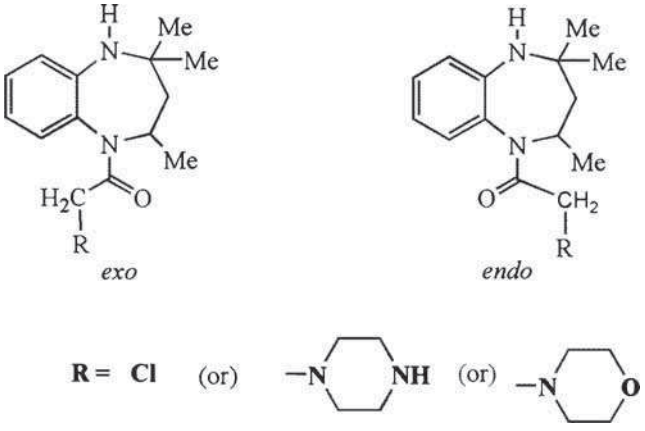


Figure 1. Relative orientation of aryl groups (*exo, endo*).

3.1 *Orientation of >C=O group at N_5 in **3-5***

The ^{1}H and ^{13}C NMR spectra of *N*-acetyl-2,2,4-trimethyl-1*H*-1,5-benzodiazepines **3-5** showed isochronous nature of the proton and carbon signals at RT indicating that either rotation about *N*−CO bond may be fast or the *N*-CO group might be locked in one of the possible orientations *viz., exo* or *endo*. Between the two possible planar orientations of the *N*−CO group, the one in which oxygen is directed towards the benzene ring is designated as *endo* and the other in which oxygen is away from the benzene ring as *exo* (figure 1).

The shielding/deshielding of C_4 carbon signal in the ^{13}C NMR spectra of the compounds **3-5** compared to that of the parent **2** was used to decide the orientation of acetyl groups at N_5 end. It was observed that the C_4 carbon signals of compounds **3-5** were shielded significantly compared to those of the parent benzodiazepines **2** (Table 3). The *syn* orientation of C=O with reference to the α-carbon would result in an eclipsing interaction between N_5-C_4/N_5-C_{11} and CO bonds and the α-carbon is expected to be shielded.[14] It was observed that one of the *ipso* carbon signals, C_{10} and C_{11}, of **3-5** was deshielded by 4.3 ppm, while the C_4 carbon signals were shielded by 1.6, 2.7, 2.6 ppm, respectively, compared to those of parent tetrahydrobenzodiazepines **2**. Hence, the $COCH_2R$ group at N_5 position adopts an *exo* orientation (*syn* to C_4). The coupling constants were calculated from the signals of C_3 protons which are comparable with those obtained by irradiating the C_4-methyl doublets (table 4).

3.2 *Assignment of ^{1}H and ^{13}C NMR spectra*

In the ^{1}H NMR spectra of the compounds **3-5**, the signals at 3.11, 3.09 and 3.14 ppm, respectively, were assigned for NH protons based on D_2O exchange studies. The α-protons of **3-5** at C_4 appeared as multiplet at 4.84, 4.82 and 4.84 ppm, respectively. The *N*-$COCH_2$ protons of **3-5** appeared at 3.81 and 3.62, 2.89 and 2.51 and 2.96 and 2.56 ppm, respectively, due to their diastereotopic nature. The piperazine protons appeared at 2.42 ppm, whereas morpholine protons appeared at

2.40 and 3.67 ppm. The complete assignment of all the proton signals is presented in table 2.

In the ^{13}C NMR spectra of the compounds **3-5**, the peaks at 46.3, 45.1 and 45.2 were assigned for C_4 carbons, respectively. The CO peak of *N*-$COCH_2$ carbons appeared at 165.8, 169.0 and 168.7 ppm for the compounds **3-5**, respectively. The piperazine carbons appeared at 53.1 ppm whereas morpholine carbons appeared at 53.6 and 66.8 ppm. The assignment of all the carbon signals is presented in table 3.

3.3 *Preferred conformation of the ring*

The parent benzodiazepine **2** prefers to exist in a chair conformation[7] (figure 2) . The *N*-acetyl derivatives of the compounds **3-5** may adopt any one of the following conformations (figure 3).

I Chair conformation **CA**
II Chair conformation **CE**
III Boat conformation **BA**
IV Boat conformation **BE**
V Twist form T**B**.

The boat forms **BA** and **BE** are obtained by flipping the C_2-C_3-C_4 part of the ring or aromatic part of the ring (*i.e.*, N_4-C_{10}-C_{11}-N_5) from the parent chair, respectively. In the boat form **BA**, flipping of C_2-C_3-C_4 part of the ring would move C_2 and C_4 equatorial methyl groups into axial position resulting in 1,3-diaxial interaction between C_2 and C_4 axial methyl groups. In the chair **CA** and boat **BA** forms, both the coupling constants $^3J_{3a,4e}$ and $^3J_{3e,4e}$ are expected to be around 2-5 Hz. However, the observed coupling constants were found to be 12.0 and 5.0, 12.0 and 5.5 and, 12.0 and 5.5Hz for **3-5**, respectively (table 4). Furthermore, analysis using Dreiding models indicated that the chair **CA** and boat **BA** conformations require an approximate *cis* ($\phi_{3a,4e}$) and *trans* ($\phi_{3e,4e}$) angle of 60°.[15] But the *cis* and *trans* angles calculated using DAERM from the coupling constant values of **3-5** are 168° and 48°, 166° and 46° and, 166° and 46°, respectively (table 4). Hence,

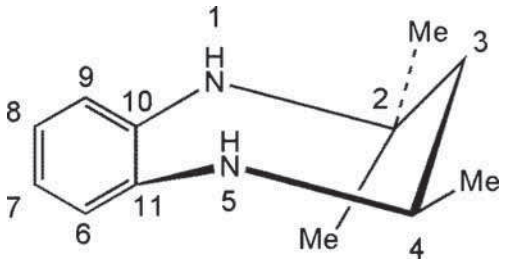


Figure 2. Preferred conformation of the compound **2**.

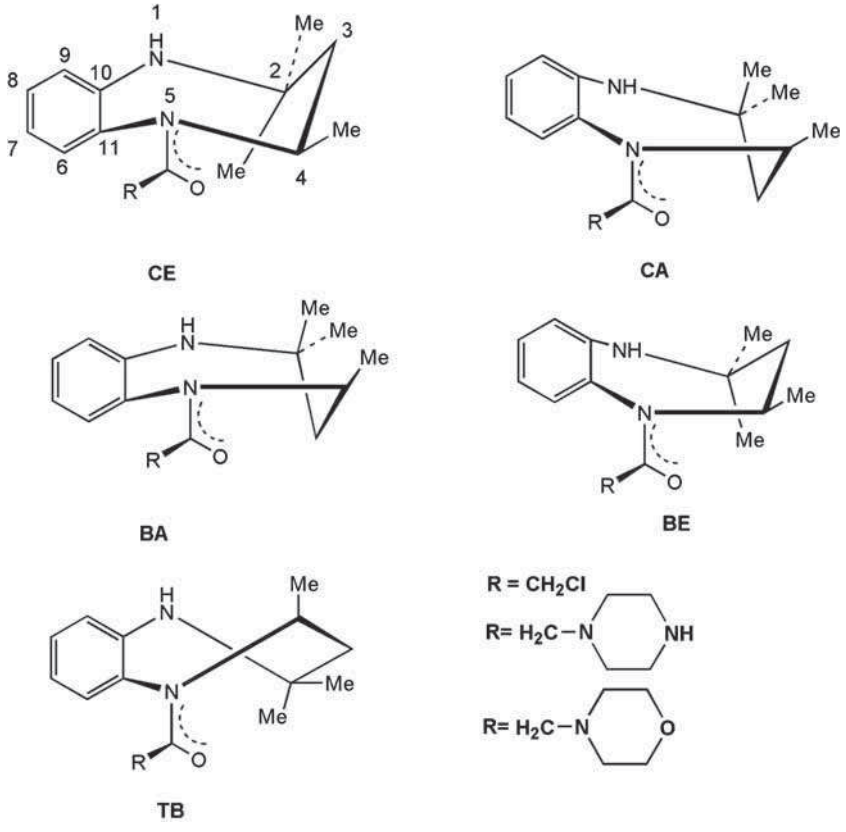


Figure 3. Possible conformations of 1,5-benzodiazepines **3-5**.

the observed coupling constants and estimated dihedral angles eliminated the possibility of chair **CA** and boat **BA** conformations.

In the twist boat conformations **TB**, the dihedral angles between the protons at C_3 and C_4 are expected to be around 60°. Hence, the observed dihedral angle 166° eliminates the possibility of the twist boat conformation **TB** also.

The extracted coupling constants and the estimated dihedral angles may be accounted using both the conformations **CE** and **BE**. Furthermore, it is not possible to use the vicinal coupling constants to decide the possibility between the conformations **CE** and **BE**, since the C_2-C_3-C_4 part of the chair **CE** and boat **BE** conformations are almost similar. However, the choice between the conformations **CE** and **BE** could be decided by considering the difference in chemical shift between the methyl groups at C_2. Analysis of Dreiding models indicated that in the chair conformation **CE** one of the methyl groups at C_2 would fall into the periphery of the aromatic ring. But in the boat form **BE** both the methyl groups are away from the aromatic ring. Hence, the chemical shift difference between methyl groups at C_2 may be expected to be smaller in boat conformation **BE** compared to that of parent chair conformation **CE**. In the case of parent tetrahydrobenzodiazepine **2**, the chemical shift difference between methyl groups at C_2 were found to be 0.25 ppm for protons and 6.9 ppm for carbons. But the corresponding values were smaller in the compounds **3-5** (**3**:0.06 and 4.4, **4**:0.05 and 4.4, **5**:0.04 and 4.4 ppm). Hence, it was concluded that the compounds **3-5** prefer to adopt boat conformation **BE** with *exo* orientation of $COCH_2R$ groups at N_5 positions (figure 4).

The dihedral angles $\phi_{3a,4a}$ and $\phi_{3e,4a}$ of **3-5** were found to be decreased to 166° and 46° compared from those of the parent tetrahydrobenzodiazepine **2** (182° and 62°) by 16° which may be due to the *exo* orientation of acetyl ($COCH_2R$) groups resulting in $A^{1,3}$ strain,[16] between carbonyl group and equatorial methyl group at C_4. In order to avoid the $A^{1,3}$ strain, the methyl group may deviate from the equatorial orientation. Analysis using Dreiding models indicates that the deviation would result in decrease of both ϕ_{trans} and ϕ_{cis} angles. The deviation of methyl group from equatorial position would also move the H_4 axial proton at 1.6 ppm towards the amide plane. Hence, the deshielding effect of H_4 axial proton may be explained on the basis of Paulsen and Todt's models for the anisotropic effect of amides.[17]

On the basis of the above observations, it was concluded that the N_5-chloroacetyl-, N_5-piperazinoacetyl-, N_5-morpholinoacetyl-2,2,4-trimethyl-1H-1,5-benzodiazepines **3-5**, prefer to adopt a boat conformation **BE** with *exo* orientation of acetyl group at N_5 positions.

3.4 *Molecular structure determination from crystal data*

The ORTEP plot of the molecule **5** is shown in figure 5. The benzodiazepine ring in compound **5** adopts a

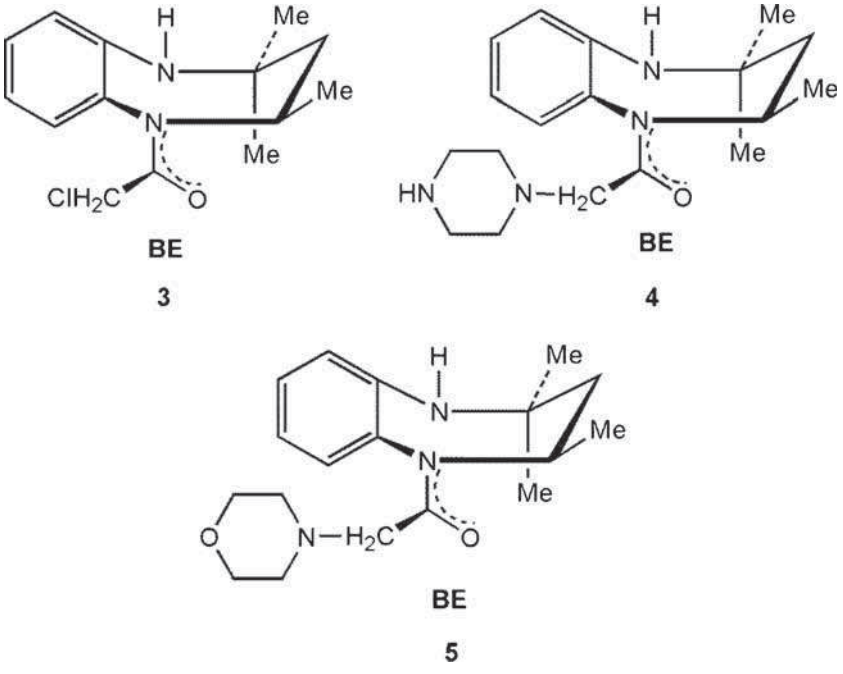


Figure 4. The preferred conformations of 1,5-benzodiazepines **3-5**.

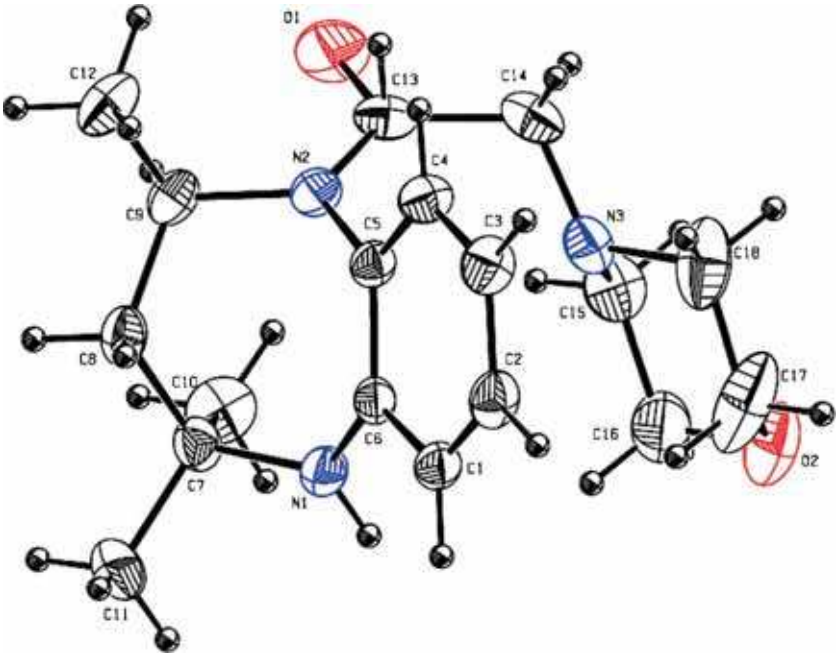


Figure 5. ORTEP diagram for the compound **5**.

distorted boat conformation and the carbonyl group is coplanar with the C5-N2-C9 plane (figure 6). C-C Bond distances for aromatic ring are in the range of 1.372(2) -1.404(2) Å and for benzodiazepine ring they are in the range of 1.513 (3) -1.531(3) Å, and are in good agreement with the literature values.[18] The C-N distances are ranging from 1.381(2) Å to 1.478(2) Å and these values agree well with the values of related reported structures.[19,20] The distances C13-C14 = 1.515(3) Å, C13-N2 = 1.352(2) Å and C13- O1 =

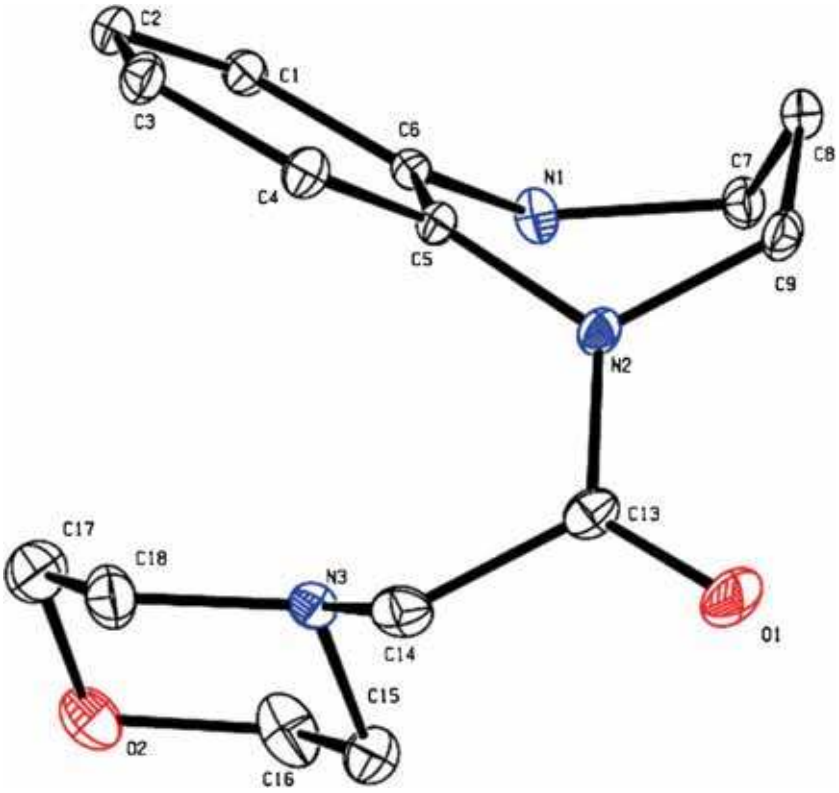


Figure 6. Solid state conformation for the compound **5**.

1.223(3) Å are comparable with the values of the related reported structures.[20] The C-C, C-N and C-O bond distances in morpholine ring are comparable with the literature values.[18] The crystal data and other relevant parameters are given in tables 5–7.

The bond angles in all the three ring systems are in the normal range and are comparable with values of the related reported structures.[19,20] The bond angles C5-N2-C13 = 123.6(1)°, C9-N2-C13 = 119.2(1)°, C14-C13-N2 = 118.0(2)°, C14-C13-O1) = 120.4(2)° and N2-C1-O1 = 121.6(2)° are somewhat different from the literature values and are comparable with the values of related reported structures.[20] Torsion angles C6-C1-C2-C3 = 1.7(3)°, C2-C1-C6-N1 = 177.4(2)°, C3-C4-C5-N2 =-176.2(2)° are comparable with values of related reported structures.[19] The sum of the angles around N_2 and N_3 are [360° and 337.7(2)°] which indicates sp^2 and sp^3 hybridization of the respective atoms. The morpholine ring adopts a chair conformation as evidenced by the puckering parameters Q = 0.556(3) Å, θ = 180.0(3)°, Φ = 64.0(9)°.[21]

3.4a *The crystal packing features*: The molecular structure of the compound **5** is stabilized by intramolecular O-H. . .N, C-H. . .O and N-H. . .O type hydrogen bonds. The crystal packing is stabilized by the N-H. . .O type hydrogen bond (figure 7). The molecule at (x,y,z) is linked to its symmetry related molecule at (1/2-x, -1/2+y,1/2-z), through an N-H. . .O type hydrogen bond forming a chain along the crystallographic 'c' axis. These chains are not linked by any direction-specific bonds.

3.5 *Antibacterial activity*

The compounds **2-5** were screened for their *in vitro* growth inhibitory action against different strains of pathogenic bacteria *viz.*, *Proteus mirabilis*, *Staphylococcus*, *Escherichia coli*, *Enterococcus faecalis* and *Klebsiella* using Muller-Hint agar medium by disc diffusion technique. Sterile Muller-Hinton agar plates were prepared and the agar surface was inoculated with the bacteria. Compounds **2-5** were dissolved in 1 mL

Table 5. Crystal data and structure refinement details for the compound **5**.

Compound 5	
CCDC	1038659
Empirical formula	C_{36} H_{44} N_6 O_4
Formula weight	624.77
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system, space group	C2/c, Monoclinic
Unit cell dimensions	a=16.0616(8) Å
	b=10.0135(6) Å
	c=22.6857(14)Å
	β =101.655(3)°
Volume	3573.4(4)Å 3
Z	4
Calculated density	1.161 Mg/m3
Absorption coefficient	0.077 mm-1
F(000)	1336
Crystal size	0.20 × 0.18 × 0.18 mm
Theta range for data collection	1.83 to 28.58°
Limiting indices	−21≤h≤21
	−8≤k≤13
	−23≤l≤30
Reflections collected / unique	16955 / 4536 [R(int)=0.023]
Completeness to theta = 28.58°	99 %
Refinement method	Full-matrix least-squares on F2
Data / restraints / parameters	4536 / 0 / 212
Goodness-of-fit on F^2	1.019
Final R indices [I>2sigma(I)]	R1=0.056
	wR2=0.162
R indices (all data)	R1=0.082
	wR2=0.184
Largest diff. peak and hole	0.427 and−0.66 e. Å−3

Table 6. Selected bond lengths (Å) and bond angles (degrees) for compound **5**.

Atoms	Bond Length	Atoms	Bond Angle
C1-C2	1.373(2)	C2-C1-C6	121.7(2)
C1-C6	1.404(2)	C1-C2-C3	120.6(2)
C2-C3	1.371(3)	C2-C3-C4	118.7(2)
C3-C4	1.382(2)	C3-C4-C5	121.5(2)
C4-C5	1.384(2)	C4-C5-C6	120.2(2)
C5-C6	1.394(2)	C4-C5-N2	120.2(1)
C5-N2	1.435(2)	C6-C5-N2	119.6(1)
C6-N1	1.381(2)	C1-C6-C5	117.2(1)
C7-C8	1.520(3)	C1-C6-N1	119.6(1)
C7-C10	1.531(3)	C5-C6-N1	123.1(1)
C7-C11	1.531(3)	C8-C7-C10	111.7(2)
C7-N1	1.478(2)	C8-C7-C11	108.8(2)
C8-C9	1.519(3)	C8-C7-N1	111.4(1)
C9-C12	1.513(3)	C10-C7-C11	109.0(2)
C9-N2	1.471(2)	C10-C7-N1	109.3(1)
C13-C14	1.515(3)	C11-C7- N1	106.5(2)
C13-N2	1.352(2)	C7-C8-C9	116.9(2)
C13-O1	1.223(3)	C8-C9-C12	111.7(2)
C14-N3	1.440(3)	C8-C9-N2	110.8(2)
C15-C16	1.495(4)	C12-C9-N2	110.9(2)
C15-N3	1.441(3)	C14 -C13-N2	118.0(2)
C16-O2	1.407(4)	C14-C13-O1	120.4(2)
C17-C18	1.480(5)	N2-C13-O1	121.6(2)
C17-O2	1.399(3)	C13-C14-N3	113.4(2)
C18-N3	1.441(3)	C16-C15-N3	110.7(2)
		C15-C16-O2	111.7(2)
		C18-C17-O2	111.9(2)
		C17-C18-N3	110.1(2)
		C6-N1-C7	124.6(1)
		C5-N2-C9	117.2(1)
		C5-N2-C13	123.6(1)
		C9-N2-C13	119.2(1)
		C14-N3-C15	113.6(2)
		C14-N3-C18	115.0(2)
		C15-N3-C18	109.2(2)
		C16-O2-C17	109.9(2)

Table 7. Geometry of the Hydrogen bonds (Å, °) for the compound **5**.

D-H...A	D-H	H...A	D...A	<(DHA)
N1-H1A...O2*	0.86	2.50	3.182(2)	137

*[6545] = 1/2-x, -1/2 +y, 1/2-z

of DMSO in various concentrations in separate tubes. Commercially available sterile discs were soaked in the preparation for half an hour. It was then placed in empty petri plates for air-drying. Using sterile forceps, the discs were placed on the surface of the agar plates and gently pressed on to the agar surface. The culture plates were inverted and incubated for 24-48 h at 37°C. After incubation, zone of clearance was observed and measured its diameter using microscope. Zone of inhibition of extracts was compared with standard Chloramphenicol for antibacterial activity. The results showed that the synthesized compounds possessed a broad spectrum of activity against the tested microorganisms and showed relatively better activity against all the five bacterial strains.

Among the benzodiazepines **2-5**, the compound containing morpholinoacetyl moiety **5** showed greater activity against *Enterococcus faecalis,* and better activity against *Klebsiella*, *Proteus mirabilis*, *Staphylococcus*, *Escherichia coli*. The compound **2** showed good activity against all the bacterial strains. The chloroacetyl compound **3** exhibited greater activity against *Klebsiella* and moderate antibacterial activity against all the bacterial strains whereas the compound containing piperazino acetyl moiety **4** showed significant activity against *Klebsiella* and moderate activity against all the remaining strains. The reports clearly indicate that the compounds **2-5** showed significant antibacterial activity against all the organisms at all concentrations but they showed better activity at 250 μ g/mL.

3.6 *Antifungal activity*

The compounds **2-5** were screened for their antifungal activity against strains *viz.*, *Aspergillus niger, Aspergillus flavus, Aspergillus oryzae, Aspergillus fumigates, Tricoderma viride.* For antifungal assays, inhibition of mycelia growth on agar media was measured. Compounds **2**-**5** were dissolved in 1 mL of sterile Dimethyl sulfoxide (DMSO) serving as a stock solution. Then, it was transferred it to 4 mL Sabouraud dextrose agar (SDA) growth media in separate tubes and autoclaved at 121°C for 15 min. These tubes were allowed to cool to 50°C and non-solidified SDA of each tube was loaded with various concentration of drug solution. Tubes were then allowed to solidify at room temperature. Then each glass tube was inoculated with 4 mm diameter piece of inoculums removed from the 7 days old culture of fungus. All these tubes were incubated at 28 ± 1°C for 10 days. A relative humidity was maintained at 40–50% in the incubation room. Growth in the media was determined by measuring linear growth (mm) of the compounds **2**-**5**, and compared with chloramphenicol which was used as a standard reference.

Among the benzodiazepine compounds **2**-**5**, the morpholinoacetyl compound **5** showed greater activity against *Tricoderma viride,* better activity against *Aspergillus flavus*, and moderate activity against *Aspergillus*

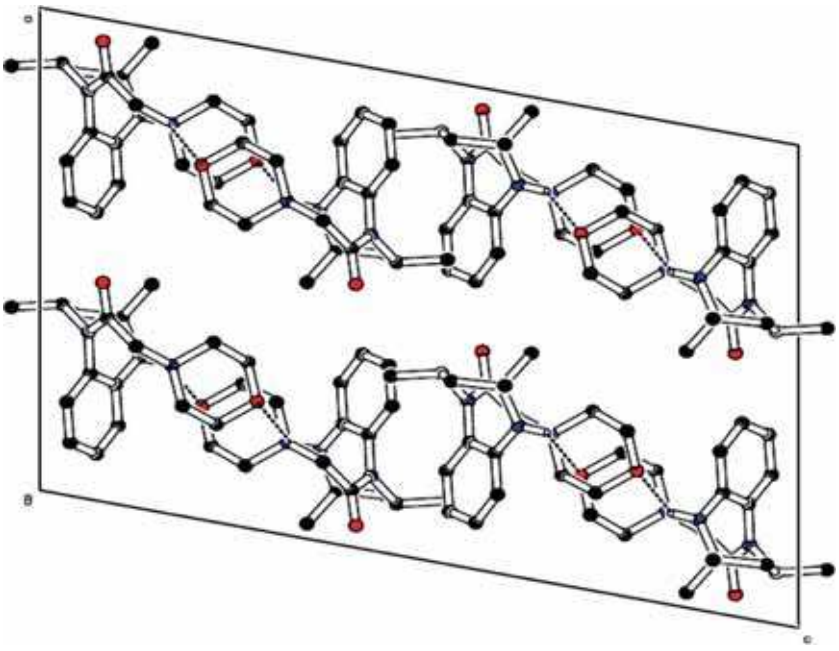


Figure 7. The packing of the molecules, viewed along the 'b' axis for the compound **5**.

niger, Aspergillus oryzae and *Aspergillus fumigates*. The compound **2** showed greater activity against all the fungal strains. The chloroacetyl compound **3** exhibited significant activities against *Aspergillus niger, Aspergillus flavus* and *Tricoderma viride*, moderate activity against *Aspergillus oryzae* and *Aspergillus fumigates* whereas the compound containing piperazino acetyl moiety **4** showed moderate activity against all the strains. The reports clearly dictate that the compounds **2**-**5** showed significant antifungal activity against all the organisms at all concentrations but it showed better activity at 250 μ g/mL.

4. Conclusions

The N_5-chloroacetyl-, N_5-piperazinoacetyl-and N_5-morpholinoacetyl-2,2,4-trimethyl-1H-1,5-benzodiazepines **3-5**, have been synthesized. The stereochemistry of **3-5** was studied with the help of ^{1}H, ^{13}C, DEPT and 2D NMR (^{1}H-^{1}H COSY and HSQC) spectral data. In addition, mass spectra were also recorded. On the basis of NMR spectra, it is inferred that the compounds **3-5** prefer to adopt a boat conformation **BE** with *exo* orientation of acetyl group at N_5 positions. The X-ray crystal structure for the compound N_5-morpholinoacetyl-2,2,4-trimethyl-1H$-$1,5-benzodiazepine also supports the boat conformation. The antibacterial and antifungal studies showed that the compound containing morpholinoacetyl group exhibited greater activity against all the tested microorganisms when compared to the standard reference drug.

Supplementary Information

Copies of IR spectra, ^{1}H NMR, ^{13}C NMR, DEPT spectra, COSY NMR spectra, HSQC NMR spectra and Mass spectra of the compounds **3** (figures S1-S7), **4** (figures S8-S14), **5** (figures S15-S21), graphical representation of antibacterial activities (figure S22) and antifungal activities (figure S23) have been included. Tables containing IR spectral assignments (table S1), antibacterial activities (table S2) and antifungal activities (table S3) have been included. Furthermore, CIF (word file) and check CIF (pdf file) have been included for X-ray crystallography. Supplementary Information is available at www.ias.ac.in/chemsci.

Crystallographic data has been deposited at the Cambridge Crystallographic Data Centre as supplemental publication no. CCDC 1038659. Copies of data can be obtained free of charge on application to CCDC, 12, Union Road, Cambridge, CB21EZ, UK. (Fax: +44 01223 336033 or email: deposit@ccdc.cam.ac.uk.)

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