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Synthesis, Characterization, Stereochemistry and Antimicrobial Evaluation of *N*-Acyltetrahydrobenzodiazepin-2-ones

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A few N_5-substituted (**1**) and N_1,N_5-disubstituted tetrahydro-1,5-benzodiazepin-2-ones (**2-8**) *viz.* N_5-chloroacetyl-, N_5-formyl-, N_5-dichloroacetyl-, N_1,N_5-diethoxycarbonyl-, N_1,N_5-bischloroacetyl-, N_5-piperazinoacetyl- and N_5-morpholinoacetyltetrahydro-1,5-benzodiazepin-2-ones have been synthesized. The characterization and conformational analysis of these compounds **2-8** have been carried out using IR and ^{1}H, ^{13}C, DEPT & 2D (COSY & HSQC) NMR spectral techniques. The coupling constants for compounds **3-6** were determined by irradiating the C_4-methyl doublet. The appearance of *major* and *minor* conformers has been found in the case of benzodiazepin-2-ones (**3** and **6**) and the spectral data confirm the equilibrium due to ring inversion over the *N*-C=O rotation. The spectral data and the extracted coupling constant values revealed that the substituted tetrahydro-1,5-benzodiazepin-2-ones (**2-8**) prefer to adopt boat conformation. The antimicrobial activity of compounds **1-8** has also been evaluated.

Keywords: *N*-Acyltetrahydrobenzodiazepin-2-ones, Boat conformation, Biological activity.

INTRODUCTION

Benzodiazepines are bicyclic heterocyclic mixes having benzene nucleus combined to a seven membered ring containing two nitrogen atom which form a significant class of biologically and medicinally active compounds [1-3]. Benzodiazepines are an important class of pharmacologically active compounds [4,5], which are finding applications as anti-inflammatory, anticonvulsant, antianxiety, antifungal, antibacterial, analgesic, antifeedant, seditative, antidepressive and hypnotic agents [6-10]. Certain derivatives like lofendazam, clobazam and triflubazam are used for the treatment of anxiety and neuroses including psychomatic disturbances and 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 [11]. Some benzodiazepine derivatives are used as valuable synthons for the preparation of other fused compounds such as triazolo [12], oxadiazolo [13], oxazino [14] and furobenzodiazepines [15]. Moreover, the derivatives of some benzodiazepines are used as anticancer [16], antithrombotic [17,18], protein kinase inhibitory and anti-inflammatory agents [19,20].

The introduction of acyl groups at N_1 and N_5 of tetrahydrobenzodiazepines results in perihydrogen interaction between the acyl groups and the *ortho*-hydrogen of benzene ring, which could lead to interesting conformational changes [21-31]. The conformations of diazepines play a key role in deciding their biological activity [32-36]. Hence, it is of interest to introduce the conformation directing moieties, such as acyl groups at the nitrogen site of benzodiazepines and to study the stereochemical consequences on the seven membered rings of benzodiazepines. In continuation of the work on *N*-acyltetrahydro-1,5-benzodiazepines [21-23,37] and other related systems [38-43], we report, herein, the synthesis and stereochemistry of N_5-chloroacetyl-, N_5-formyl-, N_5-dichloroacetyl-, N_1,N_5-diethoxycarbonyl- and N_1,N_5-bischloroacetyl-, N_5-piperazinoacetyl- and N_5-morpholinoacetyltetrahydro-1,5-benzodiazepin-2-ones (**2-8**), using NMR spectra.

EXPERIMENTAL

Unless otherwise stated, all the reagents and solvents used were of high grade and purchased from Aldrich and Merck.

All the solvents were distilled prior to use. All the reported melting points were taken in open capillaries and are uncorrected. IR spectra were recorded in Shimadzu FT-IR 8400s and Bruker α-model spectrometers using KBr pellets. The ^1H & ^{13}C NMR spectra were recorded in $CDCl_3$ solution using TMS as the internal standard in Bruker AMX 400 & 100 MHz NMR spectrometer, respectively, with the chemical shifts referenced to TMS. 0.05 M solutions of the sample prepared in $CDCl_3$ were used for recording 2D NMR spectra. Electron impact mass spectra were recorded in JEOL GS mate spectrometer and microanalyses were performed on Carlo Erba 1108 CHN analyzer.

The parent benzodiazepin-2-one (**1**) was prepared according to the literature procedure [44,45].

General method for the synthesis of compounds 2-6: To a solution of tetrahydro-4-methyl-1,5-benzodiazepin-2-one (**1**) (0.88 g, 5 mmol) in anhydrous benzene (50 mL) were added triethylamine (2.8 mL, 20 mmol) and the acylating agents [**2**: chloroacetylchloride (0.8 mL, 10 mmol), **3**: acetic-formic anhydride (15 mL), **4**: dichloroacetylchloride (0.95 mL, 10 mmol), **5**: ethylchloroformate (1.91 mL, 20 mmol), **6**: chloroacetylchloride (1.6 mL, 20 mmol)] for the synthesis of compounds **2-6**, respectively. The reaction mixture was allowed to reflux on a water bath for 6 h. The course of the reaction was monitored by TLC (silica, $CHCl_3$ as eluent). The resulting solution was washed with water (4 × 25 mL). The organic layer was separated, dried using anhydrous Na_2SO_4, passed through a short column of silica (eluent: $CHCl_3$), evaporated and crystallized from ethanol (for compounds **2**, **4**, **5** and **6**), petroleum ether (60-80 ºC) and benzene (for compound **3**). All the synthesized compounds were obtained in good yield.

5-(2-Chloroacetyl)-4-methyl-1,3,4,5-tetrahydrobenzo-[*b*][1,4]diazepin-2-one (2): Yield: 73%; m.p.: 161-163 ºC; ^1H NMR: δ ppm: 7.24-7.49 (m, 4H, aromatic protons), 9.15 (brs, 1H, NH), 5.32 (m, 1H, H_{4a}), 3.77 (d, 1H, H_A of $COCH_2Cl$) 3.66 (d, 1H, H_B of $COCH_2Cl$), 2.45 (dd, 1H, H_{3e}), 2.36 (t, 1H, H_{3a}), 1.34 (d, 3H, CH_3); ^{13}C NMR: 18.9 (CH_3 at C_4), 40.2 (C_3), 55.2 (C_4), 41.9 (CH_2 of $NCOCH_2Cl$), 123.3-136.8 (aromatic carbon), 165.7 (CO of $NCOCH_2Cl$), 173.0 (C_2); Anal. calcd. (found) % for $C_{12}H_{13}N_2O_2Cl$: C 57.04 (56.82); H 5.19 (5.12); N 11.09 (11.22)

2-Methyl-4-oxo-2,3,4,5-tetrahydro-benz[*b*][1,4]diazepine-1-carbaldehyde (3): Yield: 75.4 %; m.p.: 244-245 ºC; ^1H NMR: δ ppm: 8.40 (s, 1H, CHO_{Minor}), 8.12 (s, 1H, CHO_{Major}), 7.14-7.48 (m, 4H, aromatic protons), 8.33 (brs, 1H, NH), 5.16 (m, 1H, $H_{4a\ Major}$) & 4.65 (m, 1H, $H_{4a\ Minor}$), 2.54 (m, 2H, H_3), 1.26 (d, 3H, $CH_{3\ Major}$), 1.33 (d, 3H, $CH_{3\ Minor}$); ^{13}C NMR: 19.4 (CH_3 at C_4), 39.8 (C_3), 53.2 (C_4), 122.6-129.4 (aromatic carbon), 136.0 & 130.7 (aromatic *ipso* carbons), 162.0 (CHO), 172.2 (C_2); Anal. calcd. (found) % for $C_{11}H_{12}N_2O_2$: C 64.69 (64.38); H 5.92 (5.85); N 13.72 (13.87).

5-(2,2-Dichloroacetyl)-4-methyl-1,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-2-one (4): Yield: 76.6 %; m.p.: 151-153 ºC; ^1H NMR: δ ppm: 7.25-7.54 (m, 4H, aromatic protons), 8.83 (brs, 1H, NH), 5.30 (m, 1H, H_{4a}), 5.64 (s, 1H, H of $COCHCl_2$), 2.47 (dd, 1H, H_{3e}), 2.35 (t, 1H, H_{3a}), 1.26 (d, 3H, CH_3); ^{13}C NMR: 18.7 (CH_3 at C_4), 40.1 (C_3), 56.1 (C_4), 63.9 (CH of $NCOCHCl_2$), 123.9-130.9 (aromatic carbon), 137.0 & 129.2 (aromatic *ipso* carbons), 172.8 (CO of $NCOCHCl_2$), 163.3 (C_2); Anal. calcd. (found) % for $C_{12}H_{12}N_2O_2Cl_2$: C 50.19 (49.95); H 4.21 (4.29); N 9.76 (9.54).

2-Methyl-4-oxo-3,4-dihydro-2*H*-benzo[*b*]diazepine-1,5-dicarboxylic acid diethyl ester (5): Yield: 59.3 %; m.p.: 117-119 ºC; ^1H NMR: δ ppm: 7.21-7.39 (m, 4H, aromatic protons), 4.93 (m, 1H, H_{4a}), 2.48 (dd, 1H, H_{3e}), 2.16 (t, 1H, H_{3a}), 1.09-1.32 (m, 9H, CH_3 & $COOC_2H_5$); ^{13}C NMR: 13.9 &14.4 (C_2H_5 of N_1 & N_5), 18.7 (CH_3 at C_4), 42.6 (C_3), 54.5 (C_4), 61.7 & 63.5 (CH_2 of $NCOOCH_2CH_3$), 152.1 & 154.3 (CO of $NCOOCH_2CH_3$), 127.2-130.8 (aromatic carbon), 136.4 & 132.2 (aromatic *ipso* carbons), 169.7 (C_2); Anal. calcd. (found) % for $C_{16}H_{20}N_2O_5$: C 59.99 (59.73); H 6.29 (6.34); N 8.74 (8.66).

1,5-Bis-(2-chloroacetyl)-4-methyl-1,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-2-one (6): Yield: 75.9 %; m.p.: 125-127 ºC; ^1H NMR: δ ppm: 7.13-7.54 (m, 4H, aromatic protons), 5.16 (m, 1H, $H_{4a\ Major}$) & 5.27 (m, 1H, $H_{4a\ Minor}$), 3.79 (d, 1H, H_A of $COCH_2Cl$ at N_1), 4.81 (d, 1H, H_A of $COCH_2Cl$ at N_5), 3.70 (d, 1H, H_B of $COCH_2Cl$ at N_1), 4.76 (d, 1H, H_B of $COCH_2Cl$ at N_5), 2.50 (m, 1H, $H_{3e\ Major}$), 2.41 (m, 1H, $H_{3e\ Minor}$), 2.19 (m, 1H, $H_{3a\ Major}$), 2.29 (m, 1H, $H_{3a\ Minor}$), 1.12 (d, 3H, $CH_{3\ Major}$), 1.19 (d, 3H, $CH_{3\ Minor}$); ^{13}C NMR: 18.3 (CH_3 at C_4), 41.5 (CH_2 of $NCOCH_2Cl$ at N_1), 47.1 (CH_2 of $NCOCH_2Cl$ at N_5), 42.3 (C_3), 53.0 (C_4), 129.4-130.0 (aromatic carbon), 128.2-135.9 (aromatic *ipso* carbons), 169.1 (CO of $NCOCH_2Cl$ at N_1), 169.5 (CO of $NCOCH_2Cl$ at N_5), 171.0 (C_2); Anal. calcd. (found) % for $C_{14}H_{14}N_2O_3Cl_2$: C 51.08 (50.87), H 4.29 (4.18), N 8.51 (8.62).

Synthesis of N_5-piperazinoacetyltetrahydro-4-methyl-1,5-benzodiazepin-2-one (7): A mixture of N_5-chloroacetyltetrahydro-4-methyl-1,5-benzodiazepin-2-one (**2**) (2.53 g, 10 mmol), piperazine (0.86 g, 10 mmol) and triethylamine (2.8 ml, 20 mmol) in anhydrous benzene (30 ml) was stirred at room temperature for 7 h. The precipitated ammonium salt was washed with water (4 × 10 mL). The resulting benzene solution was dried over anhydrous Na_2SO_4, passed through a short column of silica and concentrated. The pasty mass was purified by crystallization from benzene and petroleum ether (60-80 ºC) in the ratio of 95:5. Yield: 63 %; m.p.: 280-282 ºC; ^1H NMR: δ ppm: 9.42 (s, 1H, NH), 7.08-7.41 (m, 4H, aromatic protons), 5.34 (m, 1H, H_{4a}), 3.11 (d, 1H, H_A of $COCH_2$), 2.1 (m, 8H, piperazine protons), 2.80 (d, 1H, H_B of $COCH_2$), 2.43 (m, 2H, H_3), 1.18 (d, 3H, CH_3); ^{13}C NMR: 19.2 (CH_3 at C_4), 52.1 (piperazine carbons), 60.3 (CH_2 of $NCOCH_2$), 40.8 (C_3), 55.3 (C_4), 122.6-130.2 (aromatic carbon), 131.3 & 137.1 (aromatic *ipso* carbons), 168.9 (CO of $NCOCH_2$), 174.2 (C_2); Anal. calcd. (found) % for $C_{16}H_{22}N_4O_2$: C 63.58 (63.65), H 7.28 (7.33), N 18.54 (18.46).

Synthesis of N_5-morpholinoacetyltetrahydro-4-methyl-1,5-benzodiazepin-2-one (8): A mixture of N_5-chloroacetyltetrahydro-4-methyl-1,5-benzodiazepin-2-one (**2**) (1.25 g, 5 mmol), morpholine (1.8 mL, 20 mmol) and triethylamine (2.8 mL, 20 mmol) in anhydrous benzene (30 mL) was stirred at room temperature for 7 h. The precipitated ammonium salt was washed with water (4 × 10 mL). The resulting benzene solution was dried over anhydrous Na_2SO_4, passed through a short column of silica and concentrated. The pasty mass was purified by crystallization from benzene and petroleum ether (60-80 ºC) in the ratio of 95:5. Yield: 40.5 %; m.p.: 226-228 ºC; ^1H NMR: δ ppm: 8.51 (s, 1H, NH), 7.14-7.41 (m, 4H, aromatic

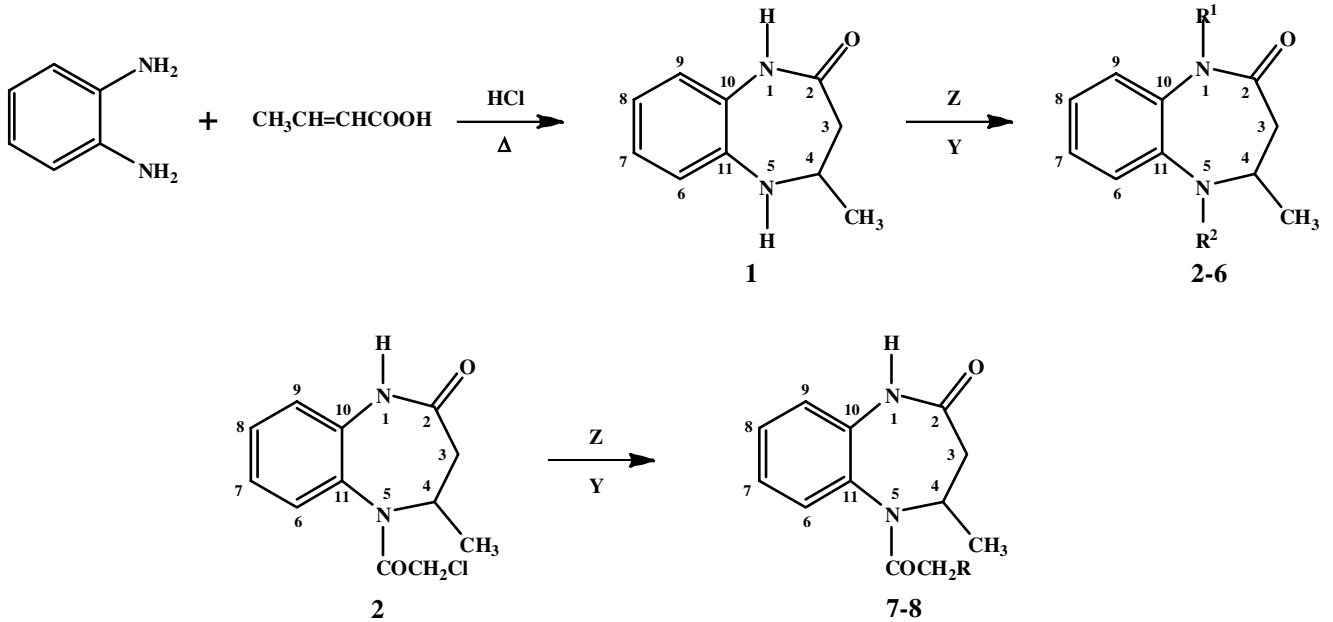
protons), 5.31 (m, 3H, H_3 & H_{4a}), 2.85 (d, 1H, H_A of $COCH_2$), 2.29 & 3.58 (m, 8H, morpholine protons), 2.66 (d, 1H, H_B of $COCH_2$), 2.06 (d, 3H, CH_3); ^{13}C NMR: 19.2 (CH_3 at C_4), 60.3 & 55.3 (morpholine carbons), 60.0 (CH_2 of $NCOCH_2$), 40.3 (C_3), 53.1 (C_4), 122.6-130.2 (aromatic carbon), 131.2 & 137.1 (aromatic *ipso* carbons), 168.9 (CO of $NCOCH_2$), 174.2 (C_2); Anal. calcd. (found) % for $C_{16}H_{21}N_3O_3$: C 63.36 (63.21); H 6.93 (6.98); N 13.86 (13.75).

RESULTS AND DISCUSSION

Tetrahydrobenzodiazepin-2-ones (**2-6**) were synthesized by the action of chloroacetyl chloride, acetic-formic anhydride, dichloroacetyl chloride, ethylchloroformate and chloroacetyl chloride, respectively, on tetrahydrobenzodiazepin-2-one (**1**) in dry benzene and using triethylamine as catalyst (**Scheme-I**), while compounds **7** and **8** were synthesized by the action of piperazine and morpholine, respectively, on N_5-chloroacetyltetrahydro-1,5-benzodiazepin-2-ones (**2**) in dry benzene and using triethylamine again as catalyst (**Scheme-I**). Only monoacyl derivatives **3** & **4** were obtained by the reaction of acetic-formic anhydride and dichloroacetyl chloride on compound **1**. However, a controlled reaction of chloroacetyl chloride with compound **1** gave monoacyl derivative **2** and the addition of excess reagent yielded diacyl product **6**. However, there was a difficulty in isolating monoethoxycarbonyl derivative and only diethoxycarbonyl compound **5** was isolated.

In IR spectra of compounds **2-4**, a stretching band for amine NH was absent (3296 cm^{-1}) and for benzodiazepin-2-ones **5** & **6**, the stretching bands for both amine NH and amide NH (3254 cm^{-1}) were absent, which confirms the formation of products **2-6**. The presence of amide NH peak at 3230 & 3382 cm^{-1} for compounds **7** & **8**, respectively, confirms the formation of compounds **7** & **8**. The ring C=O stretching values of compounds **2-4** were increased to 1694, 1686 & 1695 cm^{-1}, respectively, when compared to the parent **1** (1664 cm^{-1}) due to the -I effect of acyl groups attached at N_5. The C=O stretching vibrations of *N*-acyl moiety in compounds **2-4** were observed at 1658, 1642 & 1643 cm^{-1}, respectively. In the case of N_1,N_5-disubstituted compounds **5** & **6**, there is a remarkable increase in the ring C=O stretching vibrations due to the substitution at N_1 site (**5**: 1705 & **6**: 1754 cm^{-1}) and the C=O stretching vibrations at N_1 & N_5 positions were observed below 1680 cm^{-1} (**5**: 1667 & **6**: 1678, 1628 cm^{-1}). The C=O stretching vibrations of ring and *N*-acyl moiety of compounds **7** & **8** were observed around 1677 & 1655 cm^{-1} as broad peaks.

In the ^1H NMR spectra of compounds **2-4**, an absence of amine NH signals (δ 3.50 ppm) confirms the monoacylation. The absence of both amine NH (δ 3.50 ppm) and amide NH (δ 8.2 ppm) signals in compounds **5** & **6** revealed the acylation of both sites. In mass spectra of compounds **2-4**, the molecular ion peaks were observed at *m/z* 252, 204 and 287 and their fragmentation patterns correspond to chloroacetyl, formyl and dichloroacetyl derivatives, respectively. The preferred confor-



Z	Y	R^1	R^2	R	Compound
$ClCOCH_2Cl$	Et_3N/benzene	H	$COCH_2Cl$	–	**2**
$HCOOCOCH_3$	Benzene	H	CHO	–	**3**
$ClCOCHCl_2$	Et_3N/benzene	H	$COCHCl_2$	–	**4**
$ClCOOCH_2CH_3$	Et_3N/benzene	COOEt	COOEt	–	**5**
$ClCOCH_2Cl$	Et_3N/benzene	$ClCOCH_2Cl$	$ClCOCH_2Cl$	–	**6**
Piperazine	Et_3N/benzene	–	–	$C_4H_9N_2$	**7**
Morpholine	Et_3N/benzene	–	–	C_4H_8NO	**8**

Scheme-I: Synthesis of N_5-acyl and N_1,N_5-diacyltetrahydro-1,5-benzodiazepin-2-ones (**2**-**8**)

mation of benzodiazepin-2-ones (**2-6**, **7** & **8**) were derived from the ^{1}H & ^{13}C NMR spectral data in comparison with those of the parent amines **1** and **2**. The DEPT and 2D (^{1}H-^{1}H COSY & HSQC) NMR spectra were also used for the assignments. The coupling constants $J_{3a,4a}$ and $J_{3e,4a}$ were determined by irradiating the C4-methyl doublet and the corresponding dihedral angles were estimated using dihedral angle estimation by ratio method (DAERM) [46] and are presented in Table-1. The parent tetrahydrobenzodiazepin-2-one (**1**) has been reported to exist in a boat conformation on the basis of the coupling constant values 7.5 ($J_{3a,4a}$) and 4.2 Hz ($J_{3e,4a}$) observed between H_{3a}/H_{3e} and H_4 protons and also by X-ray crystallography [45].

TABLE 1
VICINAL COUPLING CONSTANT DATA (Hz) AND THE CORRESPONDING DIHEDRAL ANGLES (°) ESTIMATED USING DAERM OF BENZODIAZEPIN-2-ONES (**2-8**) AND PARENT AMINE (**1** and **2**)

	Compounds	$^3J_{3e,4a}$	$^3J_{3a,4a}$	$\phi_{3e,4a}$	$\phi_{3a,4a}$
2		5.2	12.8	48	168
3	Major & minor	5.5	12.0	46	166
4		5.0	12.5	48	168
5		6.0	12.0	44	164
6	Major & minor	5.0	13.0	49	169
7		6.0	12.8	45	165
8		5.6	12.0	45	165
1		4.2	7.5	41	161

***N*$_5$-Chloroacetyl and *N*$_5$-dichloroacetyltetrahydro-1,5-benzodiazepin-2-ones (2 and 4):** The ^{1}H and ^{13}C NMR spectra of *N*$_5$-acyltetrahydrobenzodiazepin-2-ones (**2** and **4**) show only isochronous nature of proton and carbon signals at room temperature. This observation might be explained due to either a fast rotation about *N*-C=O bond (or) the *N*-C=O moiety due to the arrest in one of the possible orientations *viz. exo* or *endo*. Between these two possible planar orientations of *N*-C=O 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 is *exo* (Fig. 1).

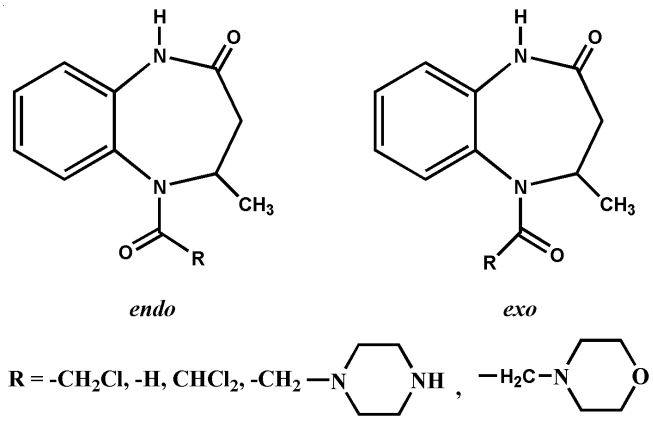


Fig. 1. Relative (*endo/exo*) orientations of acyl groups in benzodiazepin-2-ones **2-4** and **7-8** at *N*$_5$

The shielding of α-carbon signals in ^{13}C NMR spectra of compounds in **2** and **4** compared to that of parent compound **1** is used to fix the orientation of acyl group at *N*$_5$. If C=O group is oriented towards the α-carbon (*syn* orientation), it would result in an eclipsing interaction between N5-C4/N5-C11 and C=O bonds and the α-carbon is expected to be shielded [47]. Even if there was a fast *N*-C=O rotation, α-carbons would encounter shielding effect [47]. However, C_4-carbon signal for compounds **2** and **4** does not show a significant shielding/deshielding effect compared to that of parent diazepine **1**. Hence, X-ray crystal structures of compounds **2** and **4** were used to predict the orientation of acyl groups at *N*$_5$. The X-ray crystal structures [48,49] showed that acyl groups in compounds **2** and **4** at *N*$_5$ prefer to adopt an *exo* orientation (Fig. 1, *exo*).

Ring conformations of compounds 2 and 4: The *N*$_5$-acyl derivatives **2** and **4** may prefer to adopt the chair conformations **CE** & **CA** or any of the boat conformations **BE** & **BA** (Fig. 2). In chair **CA** and boat **BA** forms, the coupling constants $J_{3a,4a}$ and $J_{3e,4a}$ were expected to be around 2-5 Hz. But one of the observed coupling constant of compounds **2** and **4** was larger (Table-1). In addition, analysis using Dreiding models indicates that chair **CA** and boat conformations **BA** require an approximate *cis* ($\phi_{3a,4a}$) and *trans* ($\phi_{3e,4a}$) angle of 60º. However, the *cis* and *trans* angles calculated using DAERM [46] obtained from the coupling constant values were 48º and 168º for compounds **2** and **4**, respectively. Hence, on the basis of the observed coupling constants and calculated dihedral angles, the possibility of chair **CA** and boat **BA** conformations was ruled out.

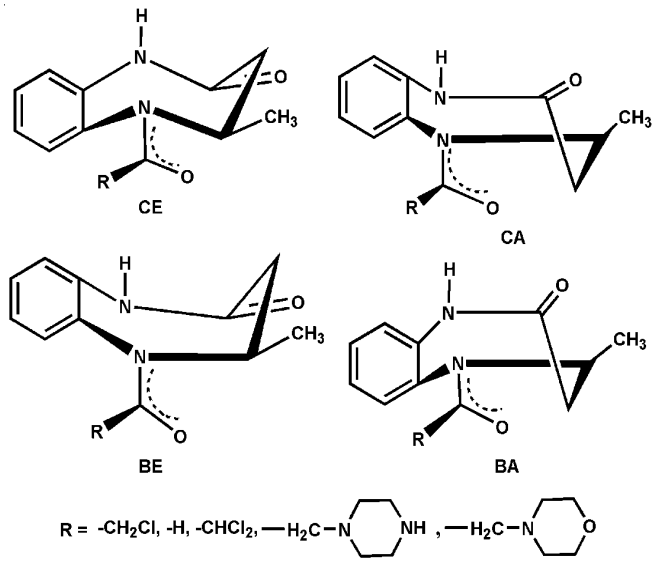


Fig. 2. Possible conformations of *N*$_5$-acyltetrahydro-1,5-benzodiazepin-2-ones **2-4** and **7-8**

The coupling constants cannot be used to differentiate between the conformations **CE** and **BE** since the C2-C3-C4 parts of them were similar. Thus, a choice between the conformations **CE** and **BE** can be decided by using Dreiding models, which indicate that the dihedral angle between the planes C10-N1-C2 and N1-C2-C3 would be around 60º for chair conformation **CE** and around 0º for boat conformation **BE**. The X-ray crystal structures [48,49] of compounds **2** and **4** were used to decide the conformation and the preferred conformation was found to be **BE** for both compounds. Hence, it is concluded that compounds **2** and **4** prefer to adopt a boat conformation **BE** with *exo* orientation of -$COCH_2Cl$, -$COCHCl_2$ groups at *N*$_5$ position, respectively (Fig. 3).

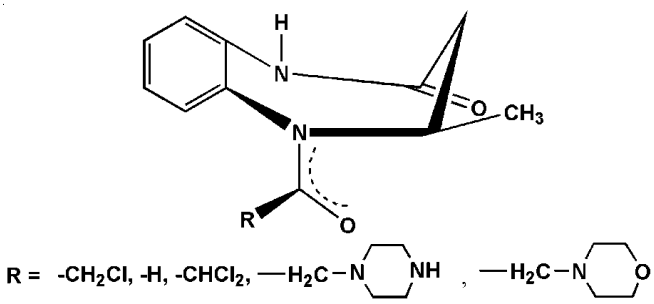


Fig. 3. Preferred conformation of N_5-acyltetrahydro-1,5-benzodiazepin-2-ones **2**-**4** and **7**-**8**

***N*$_5$-Formyltetrahydro-1,5-benzodiazepin-2-one (3)**: The ^{1}H NMR spectrum of N_5-formyltetrahydro-1,5-benzodiazepin-2-one (**3**) shows two sets of proton signals for CH_3 & H_a at C_4 and CHO due to *major* and *minor* conformers in equilibrium. However, the intensity of *minor* conformer is as low as 8 %. Hence in ^{13}C NMR spectrum, the chemical shifts were observed only for *major* conformer. The comparable coupling constant and dihedral angle values of the *major* conformer of compound **3** (Table-1) with those of compounds **2** and **4** indicate that the *major* conformer of compound **3** also prefers to adopt **BE** conformation with *exo* orientation of C=O group. The $^3J_{3e,4a}$ & $^3J_{3a,4a}$ values for the *minor* & *major* conformers were found to be the same (Table-1). Hence, the C2-C3-C4 parts of them should be similar. However, a perusal of ^{1}H NMR data indicates that C_4-H_a of *major* is more deshielded (5.16 ppm) compared to that of minor (4.65 ppm). In the case of N_1,N_5-diformyl-2,2,4-trimethyl-1*H*-tetrahydro-1,5-benzodiazepine, the *major* conformer was found to be boat in which C_4-H_a was more deshielded compared to that of the *minor* conformer which prefers to exist in a chair conformation [22]. Hence, based on the above discussion, similar conformational equilibrium due to ring inversion over the *N*-C=O rotation was expected for compound **3** also and the *minor* conformer may prefer to adopt the chair conformation **CE**. Hence, the conformational equilibrium between boat **BE** and chair **CE** conformation with *exo* orientation of *N*-C=O is preferred by compound **3** (Fig. 4). The X-ray crystal structure of compound **3** [50] also confirmed that acyl group at N_5 prefers *exo* orientation.

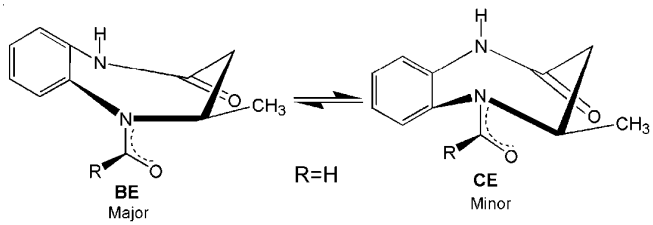


Fig. 4. Major and minor isomers of N_5-formyltetrahydro-1,5-benzodiazepin-2-one **3**

***N*$_1$,*N*$_5$-Diethoxycarbonyltetrahydro-1,5-benzodiazepin-2-one (5):** The NMR spectra of N_1,N_5-diethoxycarbonyltetrahydro-1,5-benzodiazepin-2-one (**5**) also showed a isochronous nature of proton and carbon signals at room temperature. The CH_2 protons of ethoxycarbonyl groups at N_1 & N_5 showed an unsymmetrical quartet at δ 4.27 & 4.10 ppm, respectively. Furthermore, except at N_1 site, most of the NMR spectral data of compound **5** were comparable to that of compounds **2** and **4**. The possible orientations of C=O groups at N_1 and N_5 are shown in Fig. 5. One of the *ipso* carbons was deshielded by 4.2 ppm and the other is shielded by 1.9 ppm when compared to the parent **1**. Hence, similar to compounds **2** and **4**, the ethoxycarbonyl group at N_5 prefers *exo* orientation (*syn* to C4, C11 is deshielded), while ethoxycarbonyl group at N_1 position prefers to adopt an *endo* orientation (*syn* to C10, C10 is shielded). Thus, it is concluded that N_1,N_5-diethoxycarbonyltetrahydro-1,5-benzodiazepin-2-one (**5**) also prefers to adopt a boat conformation **BE** with *endo* and *exo* orientation of the ethoxycarbonyl groups at N_1 and N_5 positions, respectively (Fig. 6).

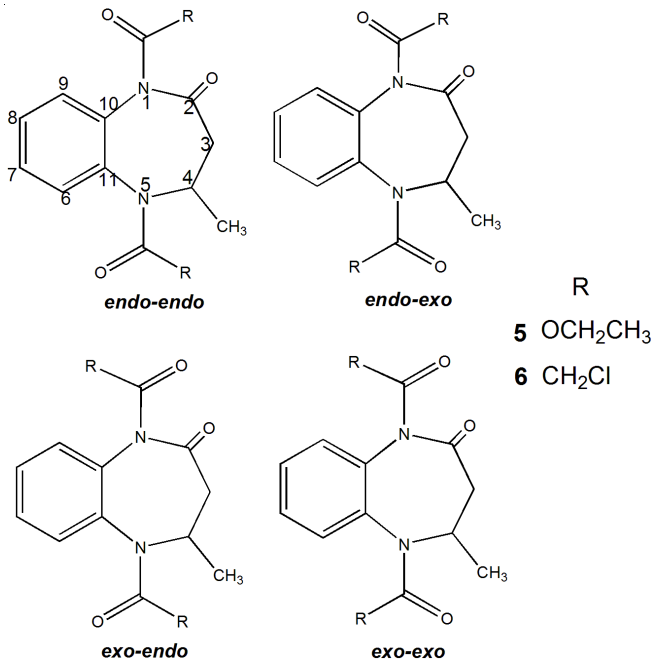


Fig. 5. Relative (*exo*/*endo*) orientations of diacyl groups in benzodiazepin-2-ones **5** & **6** at N_1 and N_5

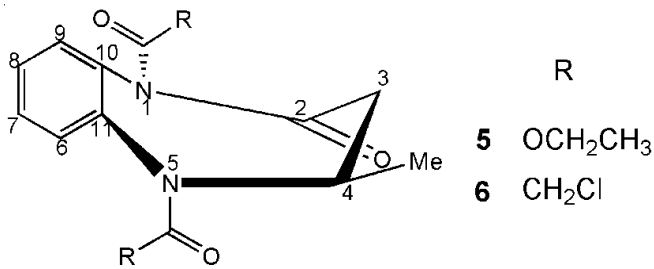


Fig. 6. Preferred conformation of N_1,N_5-diacyltetrahydro-1,5-benzodiazepin-2-ones **5** & **6**

***N*$_1$,*N*$_5$-Bischloroacetyltetrahydro-1,5-benzodiazepin-2-one (6):** The NMR spectral data of N_1,N_5-bischloroacetyltetrahydro-1,5-benzodiazepin-2-one (**6**) were also similar to that of compound (**5**) for the *major* conformer. Hence, the *major* conformer of compound (**6**) prefers a boat conformation with *endo* and *exo* orientation of C=O groups at N_1 & N_5 positions. In this case also, an intensity of *minor* conformer is 20 %. Furthermore, the coupling constants and estimated dihedral angles were similar to compound **3**. Hence, similar to compound **3**, compound **6** also expected to exist in a conformational equilibrium between the boat **BE** (major) & chair **CE** (minor) with *endo* & *exo* orientation of C=O of N_1 & N_5, respectively (Fig. 7).

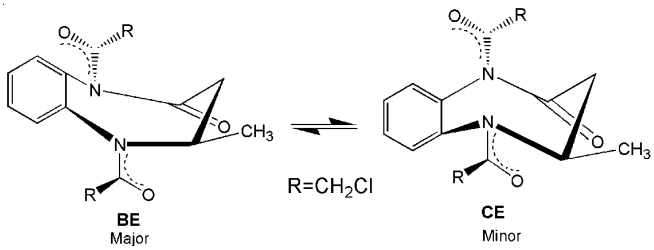


Fig. 7. Major and minor isomers of N_1,N_5-bischloroacetyltetrahydro-1,5-benzodiazepin-2-one **6**

***N*$_5$-Piperazinoacetyl- and *N*$_5$-morpholinoacetyltetrahydro-1,5-benzodiazepin-2-one (7 & 8):** The C_4-carbon signal for compound **7** was shielded by 1.7 ppm which confirms the *exo* orientation of C=O. However, compound **8** did not show significant shielding/deshielding effect compared to that of parent diazepine **2**. Therefore, the X-ray crystal structure of compound **2** was used to predict the orientation of acyl groups at N_5 and it shows that chloroacetyl group at N_5 prefers to adopt an *exo* orientation. Hence, it was decided that *N*-CO groups at N_5 position of compounds **7** and **8** also adopt *exo* orientation.

The *N*-acyl derivatives of benzodiazepinones **7** and **8** may prefer any one of the possible conformations (Fig. 2). As discussed earlier, N_5-chloroacetyltetrahydro-1,5-benzodiazepin-2-one (**2**) prefers to adopt boat conformation **BE** with *exo* orientation of the -$COCH_2Cl$ group at N_5-position (Fig. 3). In the chair **CA** and boat **BA** conformations, the coupling constants $J_{3a,4a}$ and $J_{3e,4a}$ are expected to be around 2-5 Hz. However, one of the observed coupling constants was larger for compounds **7** and **8** (**7**: 6.0 & 12.8 Hz and **8:** 5.6 & 12.0 Hz). In addition, analysis using Dreiding models indicates that chair **CA** and boat **BA** forms require an approximate *cis* and *trans* dihedral angle of 60º. But observed dihedral angles (45º and 165º calculated using DAERM) eliminate the possibility of **CA** and **BA** forms.

In **CE** and **BE** conformations, the C_2-C_3-C_4 part is almost similar, and the coupling constant cannot be used to decide the possibility of conformations between **CE** and **BE**. Thus, a choice between the conformations **CE** and **BE** can be decided using Dreiding models, which indicate that expected dihedral angles would be around 60º for **CE** and around 0º for boat conformation **BE**. The preferred conformation of parent compound **2** , observed coupling constants and dihedral angles were used to decide the conformation of compounds **7** & **8** and the preferred conformation was assigned to be **BE** for compounds **7** & **8** (Fig. 3).

Thus, it is concluded that *N*-piperazinoacetyl- and *N*-morpholinoacetyltetrahydro-4-methylbenzodiazepin-2-ones **7** and **8** also preferred to adopt boat conformation **BE** with *exo* orientation of the acyl groups at N_5 position.

Antibacterial activity: The synthesized compounds **1-8** were screened for their *in vitro* growth inhibitory action against different strains of bacteria *viz*., *Staphylococcus aureus, Staphylococcus albus, Escherichia coli, Salmonella paratyphi* and *Klebsiella pneumonia* using Muller-Hint agar medium by disc diffusion technique [51]. Sterile Muller-Hinton agar plates were prepared and the agar surface was inoculated with the bacteria. Compounds **1-8** were dissolved in 1mL of DMSO in various concentrations in separate tubes. Commercially available sterile discs were soaked in the preparation for 0.5 h. It was then placed in empty petri plates for air-drying. Using sterile forceps, the discs were placed on the surface of agar plates and gently pressed onto the agar surface. The culture plates were inverted and incubated for 24-48 h at 37 ºC. After incubation, zone of clearance was observed and its diameter measured using microscope. Zone of inhibition of compounds was compared with standard ciprofloxacin for antibacterial activity. The results showed that the synthesized compounds possess a broad spectrum of activity against the tested microorganisms (Table-2).

Among benzodiazepinones, *N*-chloroacetyl benzodiazepinone (**2**) showed a better antibacterial activity against *E. coli* and *S. aureus*. The introduction of piperazine moiety at N_5 position retains the activity and morpholine moiety at N_5 position of compound **2** increases the activity against *S. aureus* and decreases the activity of other organisms.

All the compounds, in general, dictate superior antibacterial activity against *S. aureus* and in particular the compounds **4** & **8** are more active than the standard. All of them show better activity against *E. coli*, moderate activity against *K. pneumonia* and less activity against *S. albus* & *S. paratyphi*.

Antifungal activity: Compounds **1-8** were also screened for their antifungal strains *viz*. *Aspergillus niger, Aspergillus fumigates, Candida albicans*, *Monascus ruber* and *Aspergillus parasites.* Assays measuring inhibition of mycelia growth on agar media were used. Compounds **1-8** were dissolved in 1 mL of sterile DMSO serving as a stock solution. Then, it was transferred to 4 mL Sabouraud dextrose agar (SDA) growth media in separate tubes and autoclaved at 121 ºC for 15 min. These tubes were allowed to cool to 50 ºC and non-solidified SDA of each tube was loaded with various concentrations of drug solution. Then, the tube was inoculated with 4 mm diameter piece of inoculums removed from 7 days old culture of fungus [52,53]. All these tubes were incubated at 28 ± 1 ºC for 10 days. A relative humidity was maintained at 40-50 % in the incubation room. Growth in the media was determined [54]

TABLE-2
ANTIBACTERIAL ACTIVITY OF THE COMPOUNDS **1-8**

Organisms	Ciprofloxacin (10 µg/L)	Zone of inhibition (mm) Sample (100 µg/L)							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Staphylococcus aureus	8	8	8	8	9	7	8	8	9
Staphylococcus albus	25	8	9	8	7	8	9	7	7
Escherichia coli	11	8	10	9	8	7	9	6	6
Salmonella paratyphi	26	6	10	6	8	7	9	8	8
Klebsiella pneumonia	20	8	8	9	9	10	9	8	8

TABLE-3
ANTIFUNGAL ACTIVITY OF THE COMPOUNDS **1-8**

Organisms	Zone of inhibition (mm)								
	Clotrimazole (10 µg/L)	Sample (100 µg/L)							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Aspergillus niger	12	8	9	8	8	8	20	8	7
Aspergillus fumigates	11	8	9	10	8	10	8	9	13
Candida albicans	15	8	7	7	7	8	13	8	9
Monascus ruber	13	11	10	12	9	10	9	15	13
Aspergillus parasites	15	9	11	9	10	7	9	9	8

by measuring linear growth (mm) of the compounds **1-8**, and compared with clotrimazole which was used as a standard reference (Table-3).

The parent benzodiazepinone **1** exhibits superior antifungal activity against *Monascus ruber*, better activity against *Aspergillus fumigates*, good activity against *Aspergillus niger* and *Aspergillus parasites* and moderate activity against *Candida albicans*. The chloroacetyl benzodiazepinone **2** shows superior activity against *Aspergillus fumigates*, better activity against *Monascus ruber*, *Aspergillus niger* and *Aspergillus parasites* and moderate activity against *Candida albicans.*

The diformyl benzodiazepinone **3** shows superior activity against *Monascus ruber* and *Aspergillus parasites*, better activity against *Aspergillus niger*, good activity against *Aspergillus parasites* and moderate activity against *Candida albicans*. The compound containing dichloroacetyl group at N_5 position **4** shows better activity against *Aspergillus fumigates*, *Monascus ruber*, *Aspergillus niger* and *Aspergillus parasites* and moderate activity against *Candida albicans*. The diethoxy carbonyl benzodiazepinone **5** exhibits superior antifungal activity against *Aspergillus fumigates* and better activity against *Monascus ruber* and *Aspergillus niger*, good activity against *Candida albicans and* moderate activity against *Aspergillus parasites*. The introduction of two chloroacetyl groups at N_1 & N_5 positions of benzodiazepinone **1**, results in the formation of compound **6** and it shows marked improvement in activity against all the organisms. The bischloroacetyl benzodiazepinone **6** has superior activity against *Aspergillus niger* and *Candida albicans*, better activity against *Monascus ruber* and *Aspergillus fumigates* and good activity against *Aspergillus parasites*.

Compound **7**, obtained by the substitution of piperazine moiety in compound **2** exhibits superior antifungal activity against *Monascus ruber* and *Aspergillus fumigates,* better activity against *Aspergillus niger*, good activity against *Candida albicans* and *Aspergillus parasites.* The replacement of morpholine in the place of piperazine of compound **7** results in the formation of compound **8** and it shows superior activity against *Monascus ruber* and *Aspergillus fumigates* and good activity against *Aspergillus niger*, *Candida albicans and Aspergillus parasites*.

All the compounds have superior activity against *Aspergillus fumigates* and *Monascus ruber*. Compounds **6**, **7** & **8** were more active than standard and among these the bischloroacetyl benzodiazepinone **6** showed a superior antifungal activity against *Aspergillus niger.* Introduction of piperazine and morpholine moieties at N_5 position of compound **2** increased the activity against *Monascus ruber* and *Candida albicans* and decreases the activity against *Aspergillus niger* & *Aspergillus parasites*. However, when compared to the activity of compound **2** against *Aspergillus fumigates*, compound **7** possessed a comparable activity and compound **8** was more active.

Conclusion

On the basis of the above observations, it is concluded that N_5-chloroacetyl, N_5-formyl and N_5-dichloroacetyl-, N_5-piperazinoacetyl- and N_5-morpholinoacetyltetrahydro-1,5-benzodiazepin-2-ones **2-4** and **7-8**, respectively, prefer a boat conformation (**BE**) with *exo* orientation (*syn* to C_4) of acyl groups at N_5 position and N_1,N_5-diethoxycarbonyl and N_1,N_5-bischloroacetyltetrahydro-1,5-benzodiazepin-2-ones **5** & **6**, prefer a boat conformation (**BE**) with *endo* orientation (*syn* to C_{10}) of acyl groups at N_1 position and *exo* orientation (*syn* to C_4) of acyl groups at N_5 position. The synthesized compounds **1-8** display a superior and better antimicrobial activity against selected the antibacterial and fungal strains when compared to the standard.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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