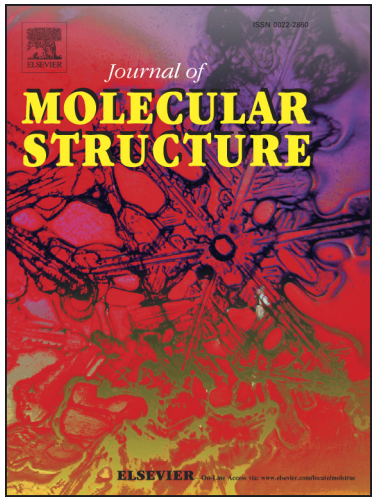


Synthesis, characterisation, stereochemistry and biological activity of *N*-formylpiperidin-4-ones

P. Sakthivel, S. Ponnuswamy

PII: S0022-2860(14)00567-5
DOI: http://dx.doi.org/10.1016/j.molstruc.2014.05.053
Reference: MOLSTR 20652

To appear in: *Journal of Molecular Structure*

Received Date: 10 December 2013
Revised Date: 21 May 2014
Accepted Date: 23 May 2014

Please cite this article as: P. Sakthivel, S. Ponnuswamy, Synthesis, characterisation, stereochemistry and biological activity of *N*-formylpiperidin-4-ones, *Journal of Molecular Structure* (2014), doi: http://dx.doi.org/10.1016/j.molstruc.2014.05.053

Synthesis, Characterisation, Stereochemistry and Biological Activity of *N*-formylpiperidin-4-ones

P. Sakthivel and S. Ponnuswamy*

P. G. & Research Department of Chemistry, Government Arts College (Autonomous), Coimbatore 641 018, Tamil Nadu, India

* Corresponding author. E-mail address: kspons2001@gmail.com

Mobile number: +919244645744

Abstract

A new series of *N*-formyl-2,6-bis(4-methoxyphenyl)piperidin-4-ones **5**-**8** has been synthesised and characterised using IR, mass and ^{1}H, ^{13}C, DEPT and 2D (COSY and HSQC) NMR spectral techniques. The NMR spectral data indicated that the *N*-formylpiperidin-4-ones **5**-**8** prefer to exist in a conformational equilibrium between a *syn* rotamer with a twist boat conformation (**TB1)** and an *anti* rotamer with a twist boat conformation (**TB2)** in solution. The stereodynamics of these systems have been studied by recording the dynamic ^{1}H NMR spectra of compound **5**, and the energy barrier for the N-CO rotation was determined to be 64.3 kJ/mol. All of the synthesised compounds (**5**-**8**) were screened for their biological activity.

Keywords :*N*-formylpiperidin-4-one; NMR spectra; twist boat conformation; *syn* and *anti* rotamers; energy barrier; biological activity

1. Introduction

The conformational equilibrium between the *syn* and *anti* rotamers based on the restricted rotation at the N-C bond in several N-acyl derivatives of azacycles is known to be fast at RT, and their conformations can be drastically different due to the influence of N-acyl functions [1-22]. Resonance stabilisation in the *N*-nitroso and *N*-acetyl derivatives of *cis*-2,6-dimethylpiperidine has been observed [23,24]. Competition between $A^{1,3}$-strain [25] and resonance stabilisation in these cases leads to dynamic equilibria due to restricted rotation around the N-N bond of the *N*-nitroso group or N-C bond of the *N*-acetyl group. The ring has been shown to prefer flipped chair conformation with diaxial methyl groups (Figure 1) [23,24]. However, when the *cis*-2,6-substituents are aromatic groups, the rings prefer, twist chair, twist boat or flattened boat conformations [1-22] even though the flipped chair conformation has also been observed in a few cases in the solid state [2,22,26-28]. Because the presence of *cis*-2,6-diaryl groups in *N*-acetyl and *N*-nitrosopiperidines exerts unpredictable conformational changes [1-22] in the piperidine ring, there are significant differences between the steric influences of an α-aryl group and an α-alkyl group on the conformational preferences of 2,6-disubstituted piperidines containing heteroconjugate groups (i.e., groups capable of delocalising the lone pair of electrons on nitrogen, such as NO, CHO and COMe).

A comparison of the rotational barriers of various 2,6-dimethylpiperidines containing hetero conjugate groups indicates that the rotational barrier for the *N*-CHO derivatives [23,24] was lower than that of *N*-nitroso derivatives but larger than that of any other derivatives (e.g., $COCH_3$, COPh and CONHPh) [1-19,29]. The *N*-formyl

derivatives of 2,6-diphenylpiperidines were reported to favour flattened boat conformations [8].

To study the relative influences of allylic strain, torsional strain, resonance energy due to the delocalisation of the lonepair electrons on nitrogen to the carbonyl π-cloud and 1,3-diaxial strains over the preferred conformations of the piperidine ring, four new *N*-formyl-*cis*-2,6-bis(4-methoxyphenyl)piperidin-4-ones **5**-**8** were synthesised and their stereochemistry was studied using ^{1}H, ^{13}C, DEPT and 2D (COSY and HSQC) NMR spectral data and dynamic ^{1}H NMR spectra. In addition, the antibacterial and antifungal activities have also been tested.

2. Experimental

2.1 *Materials, methods and instruments*

All of the reported melting points were taken in open capillaries and are uncorrected. The IR spectra were recorded using a SHIMADZU FT-IR 88400s spectrometer using KBr pellets. The ^{1}H NMR and ^{13}C NMR spectra were recorded in a $CDCl_3$ solution at 243 K with a Bruker AV 300 & 75 MHz, Bruker DRX 500 & 125 MHz and Bruker (Avance III) 500 & 125 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 5-mm diameter. Electron impact mass spectra were recorded using a JEOL GS mate spectrometer, and microanalyses were performed on a Carlo Erba 1108 CHN analyser. Unless otherwise stated, all of the reagents and solvents were of high grade and purchased from Sigma-Aldrich chemicals, Bangalore, India and Merck chemicals, Worli,

Mumbai. All of the solvents were distilled prior to use. The parent piperidin-4-ones were prepared by following the literature procedure [30-33].

2.2 *General procedure for the synthesis of compounds* **5-8**

An ice-cold solution of acetic-formic anhydride was prepared from acetic anhydride (10ml) and 85% formic acid (5ml), and this solution was slowly added to a cold solution of piperidin-4-ones [**1**: (1.625g, 5mmol), **2, 3** and **4**: (1.69g, 5mmol)] in benzene (30ml) to synthesise compounds **5**-**8**, respectively. The reaction mixture was allowed to stir at room temperature for 5 hrs. The organic layer was separated, dried over anhydrous Na_2SO_4 and concentrated. The resulting product was purified by crystallisation from benzene-petroleum ether (333-335K) in a 1:1 ratio. The analytical data of compounds **5**-**8** are reported in Table 1.

3. Results and discussion

In the IR spectra of the compounds **5**-**8**, the amide >C=O stretching bands were observed at approximately 1662-79 cm^{-1}. In addition, the ring carbonyl stretching band was observed at approximately 1704-1716 cm^{-1}. The NH stretching band at approximately 3300 cm^{-1} that was observed for parent compounds **1**-**4** was absent in *N*-formylpiperidin-4-ones **5**-**8** (Table 2). In the mass spectra, the presence of molecular ion peaks at m\z 353, 367, 367 and 367 for compounds **5**-**8**, respectively, and their fragmentation pattern confirmed the structures.

The RT NMR spectra showed the doubling of signals with broadening. Therefore, all of the NMR spectra were recorded at 243 K for N-formylpiperidin-4-ones **5**, **7** and **8**, which showed well resolved signals for each of the protons and carbons

corresponding to the *syn* and *anti* rotamers. For compound **6**, a well-resolved doubling of the NMR signals was observed at 283 K. These observations indicated the existence of conformational equilibria in compounds **5**-**8**.

The ^{1}H NMR signals of *N*-formylpiperidin-4-ones **5**-**8** were assigned based on their chemical shifts, multiplicities and intensities as well as in comparison with those of parent piperidin-4-ones **1**-**4**, respectively. Additional COSY spectra were also used to perform the assignments. The ^{13}C NMR spectral data of compounds **5**-**8** were assigned by comparison with its parent amines **1**-**4**, respectively, and within themselves. In addition, DEPT and HSQC spectra were used for unambiguous assignments.

3.1 *Orientation of -N-C=O group*

Each of the protons and carbons in *N*-formylpiperidin-4-ones **5**-**8** exhibited anisochronous nature in their ^{1}H and ^{13}C NMR spectra at RT. Doubling of the proton and carbon signals in their NMR spectra indicated that the delocalisation of the lone pair of electrons on the nitrogen of compounds **5**-**8** into the carbonyl π- cloud is sufficient to create a substantial double bond character along the -N-C=O bond. This non-equivalence arises due to the *syn* and *anti* orientations of the formyl group. Therefore, the presence of the anisochronous nature of the proton and carbon signals in compounds **5**-**8** indicated the presence of rotational equilibrium between the *syn* and *anti* rotamers in these systems, and the -N-C=O moiety adopts a coplanar orientation with the C_2-N-C_6 plane of the piperidine ring.

To confirm the presence of restricted rotation around the N-CO bond, variable temperature ^{1}H NMR spectra were recorded for formamide **5**, and the energy barrier for

N-CO rotation ($\Delta G^{\#}$) was calculated using the modified Eyring equation [34-36]. $\Delta G^{\#} = 1.914 \times 10^{-2} \times T_c[9.97 + \log T_c/\delta v]$ kJ mol^{-1} where $\Delta G^{\#}$ is the free energy of activation, T_c is the coalescence temperature (K) and δv is the chemical shift difference in Hz at T_c. The change in the shapes of the signals of the benzylic protons was followed (Chart 1) to calculate the energy barrier for N-CO rotation. The chemical shift difference was plotted as a function of the temperature, and the chemical shift difference (δv) at the coalescence temperature (T_c) was determined by extrapolating the plot. The T_c and δv were determined to be 325 K and 114 Hz, respectively. The energy barrier for the N-CO rotation in compound **5** was calculated to be 64.3 kJ mol^{-1}. A comparison of the rotational barriers of *N*-formylpiperidin-4-one **5** with that of *N*-nitroso analogue **5** ($\Delta G^{\#}$ = 76.3 kJ mol^{-1}) [37] indicated that the formyl derivatives exhibit lower rotational barriers than the nitroso derivatives. This result may be due to the greater polarisation of the carbonyl group than the nitroso group resulting in an increase in the repulsion between the charge cloud on oxygen and the π-electrons of the aromatic rings.

3.2 *Assignment of the ^{1}H and ^{13}C NMR signals to the syn and anti rotamers*

For *N*-formylpiperidin-4-ones **5**-**8**, when N-C=O is *syn* to the C_2 carbon, it is designated as a *syn* rotamer, and when N-C=O is *anti* to the C_2 carbon, it is designated as an *anti* rotamer (Figure 2). For N-formyl derivatives **5**-**7** (unsymmetrical $H_2 \neq H_6$), the H_2 and H_6 protons are expected to exhibit doubling of the signals, and in compound **8** (symmetrical $H_2 = H_6$), only two signals are expected for the H_2 and H_6 protons of the *syn* and *anti* rotamers.

To understand the preferred conformation of the individual rotamers (i.e., *syn* and *anti*) the identification of the NMR signals corresponding to the *syn* and *anti* rotamers was necessary (Figures 3, 4 and 5). The assignment of the *syn* and *anti* signals could be better achieved using HSQC spectra. This method is based on the observation that the *syn* carbons to the –X=Y functions in compounds containing N-X=Y functions, such as *N*–NO, *N*-COR and,*N*-CHO are always more shielded than the *anti* carbons [38-40]. For example, for compound **5**, between the two benzylic ^{13}C NMR signals (i.e., 58.1 and 62.8), the signal that was more shielded (58.1) was assigned to the C_2 carbon of the *syn* rotamer (C_2 *syn* to C=O), and the signal at 62.8 was assigned to the C_2 carbon of the *anti* rotamer (C_2 *anti* to C=O). Because these two carbon signals at 58.1 and 62.8 correlated with the benzylic doublets at 5.26 and 4.66 in the HSQC spectrum, respectively, the former signal (5.26) was assigned to the α - proton *syn* to the formyl group and the latter signal (4.66) to the *anti* α - proton. From the benzylic carbons and protons signals, the C_3 and C_5 NMR signals were assigned using COSY and HSQC spectra. In addition, the population of the rotamers was also used for the assignment. Similar assignments were extended to *N*-formylpiperidin-4-ones **6**-**8**.

As a result of the attachment of the *N*-acyl groups at the nitrogen of the heterocyclic systems, the benzylic proton that is *syn* to the N-C=O function would be more deshielded compared to the proton that is *anti* based on the Paulsen and Todt's model for anisotropy of amides [41]. According to this model, the syn α-protons are within the deshielding cone of the amide (in-plane region) and are deshielded. Therefore, the signals corresponding to the H_2 proton of the *syn* rotamer and the H_6 proton of the *anti* rotamer are more deshielded than that of the *anti* and *syn* isomers,

respectively. This fact also supports the assignment of the benzylic proton signals using the HSQC spectra. The complete assignment of the ^{1}H and ^{13}C NMR signals of **5-8** corresponding to the *syn* and *anti* rotamers is presented in Tables 3 and 4.

3.3 *Preferred conformations of N-formylpiperidin-4-ones **5-8***

The coupling constant data of *N*-formylpiperidin-4-ones **5-8** extracted from their ^{1}H NMR spectra are listed in Table 5 along with the dihedral angles estimated using DAERM [42] (Dihedral Angle Estimation By Ratio Method). The possible conformations of N-formylpiperidin-4-ones **5**-**8** are shown in Figure 6. Compounds **5**-**8** may prefer to adopt any one of the conformations where the destabilising interactions, such as $A^{1,3}$ strain, 1,3-diaxial interactions and torsional strain, are minimised while retaining the coplanarity of the N-C=O moiety, which results in the maximum resonance energy for the molecule.

The conformational preference for N-formyl derivatives **5**-**8** was based on the following factors (i) the relative strain factors involved in each of the possible conformations (Figure 6); (ii) the observed vicinal coupling constant and estimated dihedral angle values; (iii) the orientation of protons derived from the anisotropic influence of the formyl group based on Paulsen and Todt's model for the anisotropic effects of the amides (Table 6) and (iv) shielding of the C2 and C6 carbons (Table 7). Parent piperidin-4-ones **1**-**4** have been previously reported to exist in a chair conformation with equatorial orientation of the alkyl and aryl groups [33,43-45].

3.4 *r-2, c-6-Bis(4-methoxyphenyl)-t-3-methyl-N-formylpiperidin-4-one* (**5**)

In *N*-formylpiperidin-4-one **5**, four broad signals were observed for benzylic protons H_2 and H_6 at RT. When the temperature was lowered to 243 K, four resolved signals were obtained for benzylic protons H_2 and H_6 at 5.26 and 4.66 and 5.22 and 6.02 corresponding to the *syn* and *anti* rotamers (Figure 3). The signals at 5.26 and 4.66 appeared as doublets (J=8.5 Hz and J=10.5 Hz, respectively) and were assigned to the H_2 proton of the *syn* rotamer (H_2 is *syn* to C=O) and the H_2 proton of the *anti* rotamer (H_2 is *anti* to C=O), respectively. The signal at 5.22 that appeared as a triplet (J=5.5 Hz) was assigned to the H_6 proton of the *syn* rotamer (H_6 is *anti* to N-C=O), and the signal at 6.02 was not resolved well even at 243 K. This signal was assigned to the H_6 proton of the *anti* rotamer (H_6 is *syn* to N-C=O). Because the signal for the H_6 proton of the *anti* rotamer at 6.02 was broad, the *trans* and *cis* vicinal coupling constants were calculated from the corresponding coupling partners at C_5 ($^3J_{5HA6H}$= 6.0 Hz and $^3J_{5HB6H}$= 2.0 Hz).

If the molecule is in equilibrium between the chair conformations **CE** (*syn* and *anti* rotamers), the *trans* and *cis* coupling constants are expected to be approximately 10-12 Hz and 2-4 Hz, respectively. In addition, the observed deshielding of the benzylic protons (Δδ=+1.09-2.23) from those of the parent cannot be convincingly explained based on the Paulsen and Todt's model [41]. Therefore, the possibility of equilibrium between chair conformations **CE** has been ruled out. In the flipped chair conformation **CA** (*syn* and *anti* rotamers), the $A^{1,3}$-strain is completely relieved because the aryl groups are in the axial position. However, the 1,3-diaxial interaction between the two

anisyl groups at the C_2 and C_6 positions would destabilise the conformations. Benzylic protons H_2 and H_6 have equatorial orientations, and the *trans* and *cis* coupling constants are expected to be in the range of 2 to 4 Hz. Although the observed deshielding can be reasonably explained based on the Paulsen and Todt's model [41], the observed vicinal coupling constants (J_{2H3H}=10.5 Hz) for the H_2 proton of the *anti* rotamer ruled out the possibility of an equilibrium between the flipped chair conformations (**CA**). In boat conformations **B5** (*syn* and *anti* rotamers), although the $A^{1,3}$-strain is relieved, the 1,3-diaxial interaction between the anisyl groups would destabilise the conformations. The vicinal coupling constants between the H_2 and H_3 protons (=H_5 and H_6 protons) are expected to be approximately 8 Hz and 4 Hz. However, the observed vicinal coupling constant (J_{2H3HA}=10.5 Hz) for the H_2 proton of the *anti* rotamer ruled out the possibility of an equilibrium between the boat conformations **B5** (*syn* and *anti* rotamers). $A^{1,3}$-strain and bond eclipsing interactions destabilise boat conformations **B6** (*syn* and *anti* rotamers) and similar to **B5**, the observed vicinal coupling constant ruled out the possibility of an equilibrium between boat conformations **B6** (*syn* and *anti* rotamers).

In boat conformations **B1** (*syn* and *anti* rotamers), the H_2 and H_6 benzylic protons occupy the equatorial and axial orientations, respectively, and are not chemical shift equivalent. If the molecule exhibits equilibrium between boat conformations **B1** (*syn* and *anti* rotamers), the *trans* and *cis* coupling constants are expected to be approximately 10-12 Hz and 2-4 Hz, respectively. However, the observed vicinal coupling constants ruled out the possibility of equilibrium between boat conformations **B1**. In boat conformations **B2** (*syn* and *anti* rotamers), the benzylic protons at C_2 and C_6 occupy the axial and equatorial orientations, respectively. If an equilibrium exists

between the *syn* rotamer with **B1** conformation and *anti* rotamer with **B2** conformation, the signals for the H_2 proton in the *syn* rotamer of **B1** and the H_2 proton in the *anti* rotamer of **B2** would appear as doublets with vicinal coupling constants at approximately 2-4 Hz and 10-12 Hz, respectively, and the signals for the H_6 proton in the *syn* rotamer of **B1** and the H_6 proton in the *anti* rotamer of **B2** would appear as a doublet of doublets with vicinal coupling constants at approximately 10-12 and 2-4 Hz and 2-4 and 2-4 Hz, respectively. The signals corresponding to the H_2 proton of the *syn* rotamer and the H_6 proton of the *anti* rotamer should be more deshielded. From the vicinal coupling constants and dihedral angles *r*-2, *c*-6-bis(4-methoxyphenyl)-*t*-3-methyl-*N*-formylpiperidin-4-one (**5**) preferred to adopt an equilibrium between the *syn* rotamer with **B1** conformation and the *anti* rotamer with **B2** conformation with a twist along the C6-N1-C2-C3 portion of the *syn* rotamer and C2-N1-C6-C5 portion of the *anti* rotamer (Figure 7). Therefore, equilibrium would be established between twist boat conformations **TB1** and **TB2**. The X-ray crystal structure of **5** also corresponds to the *anti* rotamer of boat conformation **B2** with a twist along C2-N1-C6-C5 [46]. In addition, the more populated *anti* rotamer (70%) crystallised in the solid state.

3.5 *r-2, c-6-Bis(4-methoxyphenyl)-t-3-ethyl-N-formylpiperidin-4-one* (**6**)

In the ^{1}H NMR spectrum of **6**, the H_3, H_{5a} and H_{5e} proton signals of both the *syn* and *anti* rotamers were merged. The signals at 5.90 and 4.89 correspond to the *syn* and *anti* rotamer of H_2. The signal at 4.89 appeared as a doublet (J=6.0 Hz). The signals at 5.10 ($J_{6a,5a}$=7.5 Hz and $J_{6a,5e}$=7.5 Hz) and 5.90 were assigned to H_6 of the *syn* and *anti* rotamer, respectively. The signal at 5.90 (H_6 of the *anti* rotamer) was merged

with H_2 of the *syn* rotamer. The observed deshielding of the benzylic protons and shielding of the benzylic carbons are similar to that in compound **5**. Therefore, compound **6** also prefers to exist in equilibrium between the *syn* rotamer with boat conformation **B1** and *anti* rotamer with boat conformation **B2** with a twist along the C6-N1-C2-C3 portion of the *syn* rotamer and the C2-N1-C6-C5 portion of the anti rotamer (Figure 7). However, there is more twisting in compound **6** based on the following observations: (i) the deshielding of H_2 *syn* α-protons (2.23) is more than H_6 (1.89); (ii) shielding of C_2 *syn* α-carbon (13.1) is more than that of C_6 (11.1) compared to those of compound **5** and (iii) significant changes in the coupling constant and dihedral angle values. *N*-formylpiperidin-4-one **6** exhibited a well-resolved doubling of the signals even at 283K. Therefore, the energy barrier ($\Delta G^{\#}$) of the N-C=O rotation in this compound is expected to be higher than that in the remaining compounds.

3.6 *r-2, c-6-Bis(4-methoxyphenyl)-c-3-t-3-dimethyl-N-formylpiperidin-4-one* (**7**)

For *N*-formylpiperidin-4-one **7**, four broad signals were observed for benzylic protons H_2 and H_6 at RT. When the temperature was decreased to 243 K, four well-resolved signals were observed for benzylic protons H_2 and H_6 at 5.79, 4.78, 5.21 and 5.70 corresponding to the *syn* and *anti* rotamers. The signals at 5.79 and 4.78, which appeared as singlets, were assigned to H_2 in the *syn* rotamer and H_2 in the *anti* rotamer, respectively. The signals at 5.21 and 5.70, which appeared as triplets (*J*=8.5 and 6.5 Hz and *J*=7.0 & 7.0 Hz, respectively), were assigned to H_6 of the *syn* rotamer and H_6 of the *anti* rotamer, respectively. Based on the discussion for *N*-formylpiperidin-4-one **5**, equilibrium between the *syn* rotamer with boat conformation **B1** and the *anti* rotamer

with boat conformation **B2** with a twist along the C6-N1-C2-C3 portion of the *syn* rotamer and the C2-N1-C6-C5 portion of the *anti* rotamer (Figure 7) as considered for compound **7**. In addition, the signals corresponding to the H_2 proton of the *syn* rotamer and the H_6 proton of the *anti* rotamer are more deshielded. The X-ray crystal structure of **7** also corresponds to the *syn* rotamer with boat conformation **B1** with a twist along the C6-N1-C2-C3 portion [47]. Similar to **5**, the molecule crystallised in a more populated *syn* rotamer (61%) in the solid state.

3.7 *r-2, c-6-Bis(4-methoxyphenyl)-t-3-t-5-dimethyl-N-formylpiperidin-4-one* (**8**)

For compound **8**, broad signals were observed at RT, and when the temperature was decreased to 243 K, two doublets were observed for benzylic protons H_2 and H_6 at 5.41 and 4.66 corresponding to the *syn* and *anti* rotamers. The signal at 5.41 was assigned to the H_2 proton of the *syn* rotamer (=H_6 proton of *anti* rotamer), and the signal at 4.66 was assigned to the H_2 proton of the *anti* rotamer (=H_6 proton of *syn* rotamer). The coupling constant for the H_2 proton of the *syn* rotamer (=H_6 proton of *anti* rotamer) was calculated to be 5.0 Hz. The coupling constant for the H_2 proton of the *anti* rotamer (=H_6 proton of *syn* rotamer) was calculated to be 8.0 Hz. The coupling constant value for the *anti* rotamer was larger than the syn rotamer. In boat conformations **B1** and **B2**, benzylic protons H_2 and H_6 are not chemical shift equivalent. If an equilibrium exists between boat conformations **B1** or **B2** of the *syn* and *anti* rotamers, four signals would be expected for benzylic protons H_2 and H_6 in both cases. The observations of only two signals for the benzylic protons eliminated the possibility of the above equilibrium. Therefore, the observation of only two signals for the benzylic protons, the vicinal

coupling constants and the observed deshielding of the protons as well as the shielding of carbons can only be explained if an equilibrium exists between the *syn* rotamer with boat conformation **B1** and the *anti* rotamer with boat conformation **B2** with a twist along the C6-N1-C2-C3 portion of the *syn* rotamer and the C2-N1-C6-C5 portion of the *anti* rotamer (Figure 7). The X-ray crystal structure of **8** corresponds to the *anti* rotamer with boat conformation **B1** with a twist along the C6-N1-C2-C3 portion [48].

3.8 *^{13}C NMR Spectra*

The *syn* α-carbons (8.7 – 13.1) are more shielded than the *anti* α-carbons (1.1 – 6.0). The higher shielding of the *syn* α-carbons may be due to the γ-eclipsing interaction between the -N-C=O bond of the formyl group and the N_1-C_2/N_1-C_6 bonds. In addition, this observation also supports the coplanar orientation of N-C=O with the C2-N-C6 plane.

4. Antimicrobial Screening

N-formylpiperidin-4-ones **5**-**8** were subjected to preliminary screening for their antibacterial and antifungal activities using a disc diffusion technique. Sterile Muller-Hinton agar plates were prepared, and the agar surface was inoculated with the following bacteria: *Escherichia Coli*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Staphylococcus aureus.* The antifungal activity of the test compounds was determined against the following fungi: *Aspergillus flavus, Fusarium oxysporum, Mucor indicus, Penicillium chrysogenum* and *Trichoderma viride*. Compounds **5**-**8** were

dissolved in 1ml of DMSO in various concentrations in separate tubes. Commercially available sterile discs were soaked in the preparation for half an hour. Then, the discs were 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 hrs at 37°C. After incubation, a zone of clearance was observed, and its diameter was measured using a microscope. The zone of inhibition of the extracts was compared with standard ciprofloxacin for antibacterial activity and amphotericin-B for antifungal activity. The results are reported in the Tables 8 and 9, and the results indicated that the synthesised compounds possessed a broad spectrum of activity against the tested microorganisms and exhibited relatively better activity against all five bacteria. In comparison to the reference disks (ciprofloxacin 5 µg/disk), compounds **5, 7** and **8** exhibited greater activity against *E. coli*. Compound **6** exhibited better activity against *E. coli* and *Staphylococcus aureus*. As shown in Table 8, all of the tested compounds, which were applied in 12-fold concentrations (60 µg) compared to the ciprofloxacin (5 µg) reference, exhibited less activity against the bacteria. Compounds **5** and **7** exhibited better activity against *Trichoderma viride* compared to other fungal strains but less activity than the amphotericin-B reference.

5. Conclusion

Four new *N*-formylpiperidin-4-ones **5-8** have been synthesised and characterised using IR, mass, ^{1}H, ^{13}C, DEPT and 2D (^{1}H, ^{1}H-COSY and ^{1}H-^{13}C-HSQC) NMR spectra. *N*-Formylpiperidin-4-ones **5-8** existed in conformational equilibrium between a *syn*

rotamer with twist boat conformation **TB1** and an *anti* rotamer with twist boat conformation **TB2** in solution. By performing variable temperature NMR spectral studies, the barrier for the N-C rotation in *N*-formylpiperidin-4-one **5** was determined to be 64.3 $kJmol^{-1}$. The antibacterial and antifungal activities of formylpiperidin-4-ones **5**-**8** were assessed. The antibacterial activity of the test compounds was determined against *Escherichia Coli* sp, *Bacillus cereus* sp, *Klebsiella pneumonia* sp, *Proteus vulgaris* sp, *Staphylococcus* sp and *Pseudomonas* sp. Antifungal activity of the test compounds was determined against *Aspergillus* sp*, Fusarium* sp*, Mucor* sp*, Trichoderma* sp *and Penicillium* sp*.* The results clearly indicated that all four compounds exhibited significant antibacterial and antifungal activity.

Acknowledgements

One of the authors (SP) wishes to thank UGC for financial assistance in the form of a Major Research Project [No. F. 39-724/2010 (SR)]. We wish to thank MKU Madurai, (SAIF) IIT Chennai and (SIF) IISC Bangalore for the NMR spectra.

REFERENCES

[1] T. Ravindran, R. Jeyaraman, R.W. Murray, M. Singh, J. *Org. Chem.* 56 (1991) 4833-4840.

[2] T. Ravindran, Synthesis, *Stereodynamics and reactivity of N-nitrosopiperidines and N-nitrosoazabicyclo[3,3,1]nonanes*, Ph.D. Thesis, Bharathidasan University, India,1993.

[3] J.C. Thenmozhiyal, *Synthesis and Stereodynamics of Piperidines and 3-Azabicyclo(3.3.1)nonan-9-ones Containing N-X-Y Functions, Ph.D. Thesis, Bharathidasan University, India,* 1995.

[4] R. Krishnakumar, M. Krishnapillay, *Indian J. Chem.* 35B (1996) 418-425.

[5] K. Pandiarajan, A. Manimekalai, N. Kalaiselvi, *Magn. Reson. Chem.* 35 (1997) 372-378.

[6] R. Jeyaraman, S. Ponnuswamy, *Indian J. Chem*. 36B (1997) 730-737.

[7] R. Jeyaraman, S. Ponnuswamy, *J. Org. Chem*. 62 (1997) 7984-7990.

[8] R. Jeyaraman, J.C. Thenmozhiyal, R. Murugadoss, M. Venkatraj, *Indian J. Chem*. 38B (1999) 325-336.

[9] R. Jeyaraman, J.C. Thenmozhiyal, R. Murugadoss, M. Muthukumar, *J. Indian Chem. Soc.* 76 (1999) 527-536.

[10] D. Kumaran, M.N. Ponnuswamy, G. Shanmugam, J.C. Thenmozhiyal, R. Jeyaramam, K. Panneerselvam, M. Soriano-Garcia, J. Chem. Crystallogr. 29 (1999) 769-775.

[11] N. Bhavani, D. Natarajan, A. Manimekalai, *Indian J. Chem*. 39B (2000) 16-20.

[12] M. Krishnapillay, R. Krishnakumar, A. Nagarajan, G. Jeyaraman, *Indian. J. Chem.* 39B (2000) 419-425.

[13] R. Jeyaraman, J.C. Thenmozhiyal, R. Murugadoss, M. Venkatraj, P. Laavanya, K. Panchanatheswaran, M. Bhadbhade, *Indian J. Chem*. 39B (2000) 497-503.

[14] R. Jeyaraman, R. Murugadoss, *Indian J. Chem*. 39B (2000) 826-835.

[15] S. Ponnuswamy, M. Venkatraj, R. Jeyaraman, M. Sureshkumar, D. Kumaran, M.N. Ponnuswamy, *Indian J. Chem*. 41B (2002) 614-627.

[16] M. Venkatraj, S. Ponnuswamy, R. Jeyaraman, Indian *J. Chem.* 45B (2006) 1531-1540.

[17] S. Ponnuswamy, R. Murugadoss, R. Jeyaraman, A. Thiruvalluvar, V. Parthasarathy, *Indian J. Chem*. 45B (2006) 2059-2070.

[18] J. Jayabharathi, A. Manimekalai, T. Consalta Vani, M. Padmavathy, *Eur. J. Med. Chem*. 42 (2007) 593-605.

[19] J.C. Thenmozhiyal, M. Venkatraj, S. Ponnuswamy, R. Jeyaraman, *Indian J. Chem*. 46B (2007) 1526-1536.

[20] G. Aridoss, S. Balasubramanian, P. Parthiban, S. Kabilan, *Spectrochim Acta*. 68 (2007) 1153-1163.

[21] G. Aridoss, S. Balasubramanian, P. Parthiban, R. Ramachandran, S. Kabilan, *Med. Chem. Res*. 16 (2007) 188-204.

[22] M. Gdaniec, M.J. Mileswka, T. Polonski, *J. Org. Chem*. 60 (1995) 7411-7418.

[23] Y.L. Chow, C.J. Colon, J.N.S. Tam, *Can. J. Chem*. 46 (1968) 2821-2825.

[24] R.R. Fraser, T.B. Grindley, *Tetrahedron Lett*. 15 (1974) 4169-4172.

[25] F. Johnson, S.K. Malhotra, *J. Am. Chem. Soc*. 87 (1965) 5492-5493.

[26] Prathebha, K.; Revathi, B. K.; Usha, G.; Ponnuswamy, S.; Basheer, S. A. Acta Cryst. 2013, E69, o1424.

[27] A. Thangamani, J. Jayabharathi, A. Manimekalai, *J. Struct. Chem*. 50 (2009) 628-639.

[28] A. Manimekalai, K. Selvaraju, T. Maruthavanan, *Indian J. Chem*. 46B (2007) 160-169.

[29] L. Lunazzi, D. Macciantelli, G. Cerioni, *J. Org. Chem*. 47 (1982) 4579-4581.

[30] C.R. Noller, V. Baliah, *J. Am. Chem. Soc.* 70 (1948) 3853-3855.

[31] V. Baliah, V. Gopalakrishnan, *J. Indian Chem. Soc*. 31 (1954) 250-252.

[32] V. Baliah, A. Ekambaram, T.S. Govindarajan, *Curr. Sci*. 23 (1954) 264.

[33] V. Mohanraj, *M.Phil., Dissertation*, Bharathiar University, India, 2008.

[34] M. Oki, *Applications of Dynamic NMR Spectroscopy to OrganicChemistry* (VCH, Florida), 1985, Chapter I.

[35] J. Sandstrom, *Dynamic NMR Spectroscopy* (Academic Press, London), 1982**.**

[36] H. Shanon-Atidi, K.H. Bar-Eli, *J. Phy. Chem.* 74 (1970) 961-963.

[37] P. Sakthivel, S. Ponnuswamy, (unpublished results).

[38] F.W. Werhli, T. Wirthlin, "Interpretation of Carbon-13 NMR Spectra", Heydon & Son, London.1976.

[39] J.P. Gouesnard, G.J. Martin, *Org. Magn. Reson.* 12 (1979) 263-270.

[40] F.A.L. Anet, A.J.R. Bourn, *J. Am. Chem. Soc.* 87 (1965), 5250-5251.

[41] H. Paulsen, K. Todt, *Angew Chem. Int. Ed. Engl.* 5 (1966) 899-900.

[42] K.N. Slessor, A.S. Tracey, *Can. J. Chem*. 49 (1971) 2874-2884.

[43] K. Pandiarajan, R.T. Sabapathy Mohan, R. Krishnakumar, *Indian J. Chem*. 26B (1987) 624-627.

[44] T. Kavitha, S. Ponnuswamy, V. Mohanraj, S.S. Ilango, M.N. Ponnuswamy, *Acta Cryst.* E63 (2007) o3985.

[45] S. Ponnuswamy, V. Mohanraj, P. Gayathri, A. Thiruvalluvar, R.J. Butcher, *Acta Cryst*. E64 (2008) o2328.

[46] P. Gayathri, P. Sakthivel, S. Ponnuswamy, A. Thiruvalluvar, R.J. Butcher, *Acta Cryst*. E65 (2009) o2813.

[47] T. Kavitha, S. Ponnuswamy, P. Sakthivel, K. Karthik, M.N. Ponnuswamy, *Acta Cryst*. E65 (2009) o856.

[48] T. Kavitha, P. Sakthivel, S. Ponnuswamy, S.S. Ilango, M.N. Ponnuswamy, *Acta Cryst*. E65 (2009) o2818.

Scheme 1

Synthesis of *N*-formylpiperidin-4-ones **5**-**8**.

Figure captions

Figure 1

Allylic strain or $A^{1,3}$ strain.

Figure 2

Designation of *syn* and *anti* rotamers in **5**-**8**.

Figure 3

^{1}H NMR spectrum of **5** showing doubling of the signals (*syn* and *anti* rotamers).

Figure 4

^{13}C NMR spectrum of **7** showing doubling of signals (*syn* and *anti* rotamers).

Figure 5

HSQC NMR spectrum of **8**.

Figure 6

Possible conformations of the *N*-formylpiperidin-4-ones **5-8**.

Figure 7

Conformational equilibrium between the *syn* and *anti* rotamers of *N*-formylpiperidin-4-ones **5**-**8**.

Chart 1

Dynamic ^{1}H NMR spectra of **5**.

Table captions

Table 1:

Analytical data for compounds **5-8**.

Table 2:

IR spectral data for compounds **5**-**8**.

Table 3:

Proton chemical shift values (δppm) for compounds **5**-**8**.

Table 4:

^{13}C Chemical shift values (δppm) of the compounds **5**-**8**.

Table 5:

Vicinal and geminal coupling constants and dihedral angles of *N*-formylpiperidin-4-ones **5-8** compared to parent piperidin- 4-ones **1**-**4**.

Table 6:

Formyl induced ^{1}H NMR chemical shifts of α-protons in *N*-formylpiperidin-4-ones **5**-**8**.

Table 7:

Formyl induced ^{13}C NMR chemical shifts of α-carbons in *N*-formylpiperidin-4-ones **5**-**8**.

Table 8:

Result of the antibacterial activity of *N*-formylpiperidin-4-ones **5-8**.

Table 9:

Result of the antifungal activity of *N*-formylpiperidin-4-ones **5-8**.

Table 1

Compounds	Molecular formula (Mol.Wt.)	Melting point (°C)	Yield (%)	Elemental analysis Found (Calcd) % C	H	N
5	$C_{21}H_{23}NO_4$ (353)	106-108	84.9	71.09 (71.37)	6.61 (6.56)	3.82 (3.96)
6	$C_{22}H_{25}NO_4$ (367)	85-87	64.3	71.64 (71.91)	6.80 (6.86)	3.92 (3.81)
7	$C_{22}H_{25}NO_4$ (367)	98-100	65.3	72.12 (71.91)	6.79 (6.86)	3.75 (3.81)
8	$C_{22}H_{25}NO_4$ (367)	85-87	79.0	71.72 (71.91)	6.93 (6.86)	3.98 (3.81)

ACCEPTED MANUSCRIPT

Table 2

Compound	IR (cm^{-1})
5	2966, 2933, 2881, 1716(C=O stretching), 1670(amide C=O stretching), 1654, 1514, 1365, 1033, 840, 582, 682, 563.
6	2954, 2933, 2835, 1704(C=O stretching), 1662(amide C=O stretching),1616, 1514, 1419, 1249, 1184, 1035, 827, 671, 514.
7	2993, 2954, 2831, 1716(C=O stretching), 1666(amide C=O stretching),1610, 1514, 1251, 1024, 838, 651, 547, 491.
8	2975, 2937, 2835, 1716(C=O stretching), 1679(amide C=O stretching),1654, 1514, 1247, 1178, 1033, 835, 686, 541.

ACCEPTED MANUSCRIPT

Table 3

Compounds		H_2	H_3	H_{5a}	H_{5e}	H_6	$2\times OCH_3$	CH_3 at C_3	Aromatic	CHO	CH_2 of ethyl at C_3	Population (%)/NH
5	*syn* rotamer	5.26(d)	3.15(m)	2.80-2.83,**a**	3.23(dd)	5.22(t)	3.78(s),3.82(s), 3.84(s)	1.08(d)	6.74-7.29(m)	8.48(s)		30
	anti rotamer	4.66(d)	2.80-2.83,**a**	2.99(dd)	3.33(dd)	6.02(unsym.dd)		0.81(d)		8.30(s)		70
6	*syn* rotamer	5.90,**c**	2.73(dd),2.87-3.06(m),**b**			5.10(t)	3.76(s),3.75(s), 3.74(s)	1.04(t)	6.67-7.37(m)	8.49(s),8.42(s)	1.50-1.79(m)	48.5
	anti rotamer	4.89(d)				5.90,**c**		0.91(t)				51.5
7	*syn* rotamer	5.79(s)	-	3.14(dd)	3.02(dd)	5.21(t)	3.79(s),3.74(s)	1.41(s),1.24(s)	6.59-7.11(m)	8.31(s)		61
	anti rotamer	4.78(s)	-	3.07(d),3.09(d)		5.70(t)	3.78(s),3.71(s)	1.36(s),1.22(s)		8.60(s)		39
8	*syn* rotamer	5.41(d)	3.23(m)	3.05(m)	-	4.66(d)	3.84(s),3.83(s)	1.17(d,CH_3 & C_5 of *anti)*	6.85-7.10(m)	8.39(s)		50
	anti rotamer	4.66(d)	3.05(m)	3.23(m)	-	5.41(d)		0.96(d,CH_3 & C_5 of *syn)*				50
1		3.57(d)	2.7(m)		2.58(dd)	4.03(dd)	3.80(s),3.81(s)	0.82(d)	6.74-7.38(m)			2.03(s)
2		3.67(d)	2.57(m)	2.71(t)	2.57(m),**d**	4.01(dd)	3.80(s),3.83(s)	0.78(t)	6.87-7.63(m)		1.17(m),1.58(m)	1.99(s)
3		3.76(s)		2.90(unsym t)	2.45(dd)	3.99(dd)	3.81(s)	0.94(s),1.19(s)	6.85-7.43(m)			1.90(s)
4		3.55(d)	2.74(m)		-	3.55(d),**e**	3.80(s)	0.82(d)	6.86-7.55(m)			2.0(b,s)

a-H5a *of syn* rotamer merged with H3 of *anti* rotamer, **b**-H3 of *syn* & *anti* rotamers merged with H5a & H5e of syn & *anti* rotamers, **c**- H6 of *anti* rotamer merged with H2 of *syn* rotamer, **d**-H5e merged with H3, **e**-H6 merged with H2

ACCEPTED MANUSCRIPT

Table 4

Compounds		C_2	C_3	C_4	C_5	C_6	CH_3 at C_3	OCH_3	Aromatic Carbons	Aromatic (ipso)Carbons	CHO	CH_2 of CH_2CH_3
5	*syn* rotamer	58.1	45.6	209.5	43.0	55.5	14.0	55.5	113.6,114.1,114.4, 127.6,128.3,128.9	131.3,132.5, 158.7,159.3	164.0	
	anti rotamer	62.8	47.1		41.5	48.8	11.9				165.1	
6	*syn* rotamer	53.1	52.3 & 52.7	209.4	43.4	56.4	11.8	55.2	113.6,113.9,128.0, 128.5	130.7,132.1, 159.0,	163.8 & 164.1	22.1 & 23.8
	anti rotamer	60.2			41.4	50.1	10.8					
7	*syn* rotamer	60.1	46.9	211.8	43.7	56.6	26.9, 26.7 & 21.5	55.2	130.1,129.8,128.6, 127.2,127.0,113.8, 113.3, 113.2	158.5,158.3,158.06, 157.9, 132.4, 131.8, 130.3	164.0	
	anti rotamer	67.7	47.6	211.9	41.4	52.2					163.1	
8	*syn* rotamer	56.4	44.5	211.9	45.7	62.1	15.2	55.5 & 55.4	128.7, 128.6, 114.3, 113.8	159.2, 158.6, 132.3, 131.8	165.1	
	anti rotamer	62.1	45.7		44.5	56.4	13.4					
1		68.3	52.3	210.5	51.5	61.4	10.6	55.7	129.1, 128.1, 114.4, 114.2	159.6, 159.5, 135, 134.6		
2		66.2	58.7	209.5	51.7	61.2	12.3	55.3	113.8-159.3			17.9
3		68.8	50.0	213.2	47.3	61.0	19.9 & 20.4	55.2 & 55.1	129.7, 127.6, 113.9, 113.0	159.0, 135.4, 131.4		
4		67.9	51.8	210.8	51.8	67.9	10.3	54.9	128.4, 113.5	158.9, 134.1		

Table 5

	Compounds	$^{3}J_{2a,3a}$	$^{3}J_{6a,5e}$	$^{3}J_{6a,5a}$	$^{2}J_{5a,5e}$	$\Phi_{6a,5e}$*cis*	$\Phi_{6a,5a}$*trans*
5	*syn rotamer*	8.5	5.0	5.5	18.5	30	150
	anti rotamer	10.5	2.5	5.5	18.0	45	165
6	*syn rotamer*	-	7.5	7.5	-	27	147
	anti rotamer	6.0	-	-	-		
7	*syn rotamer*	-	6.5	8.5	17.5	34	154
	anti rotamer	-	7.0	7.0	-	27	147
8	*syn rotamer*	5.0	-	8.0	-		
	anti rotamer	8.0	-	5.0	-		
1		10.3	2.3	11.5	13.1	60	180
2		10.5	3.0	12.0	-	57	177
3		-	3.0	12.2	13.2	57	177
4		10.5	-	10.5	-		

ACCEPTED MANUSCRIPT

Table 6

Formyl induced ^{1}H NMR chemical shifts of α-protons in *N*-formylpiperidin-4-ones **5**-**8**

Compounds		H_{2a}	H_{6a}
5	*syn* rotamer	+1.69	+1.19
	anti rotamer	+1.09	+1.99
6	*syn* rotamer	+2.23	+1.09
	anti rotamer	+1.22	+1.89
7	*syn* rotamer	+2.03	+1.22
	anti rotamer	+1.02	+1.71
8	*syn* rotamer	+1.86	+1.11
	anti rotamer	+1.11	+1.86

+ = deshielding

Table 7

Formyl induced ^{13}C NMR chemical shifts of α-carbons in *N*-formylpiperidin-4-ones **5**-**8**

Compounds		C_2	C_6
5	*syn* rotamer	-10.2	-5.9
	anti rotamer	-5.5	-12.6
6	*syn* rotamer	-13.1	-4.8
	anti rotamer	-6.0	-11.1
7	*syn* rotamer	-8.7	-4.4
	anti rotamer	-1.1	-8.8
8	*syn* rotamer	-11.5	-5.8
	anti rotamer	-5.8	-11.5

- = shielding.

Table 8

Compounds	Disk potency 60µg Diameter of inhibition zone (mm) *Escherichia coli*	*Bacillus cereus*	*Klebsiella pneumoniae*	*Proteus vulgaris*	*Staphylococcus aureus*
5	20	9	9	15	15
6	20	8	10	10	20
7	25	20	10	8	15
8	27	10	9	10	12
Ciprofloxacin (5µg/disk)	35	28	15	21	21

ACCEPTED MANUSCRIPT

Table 9

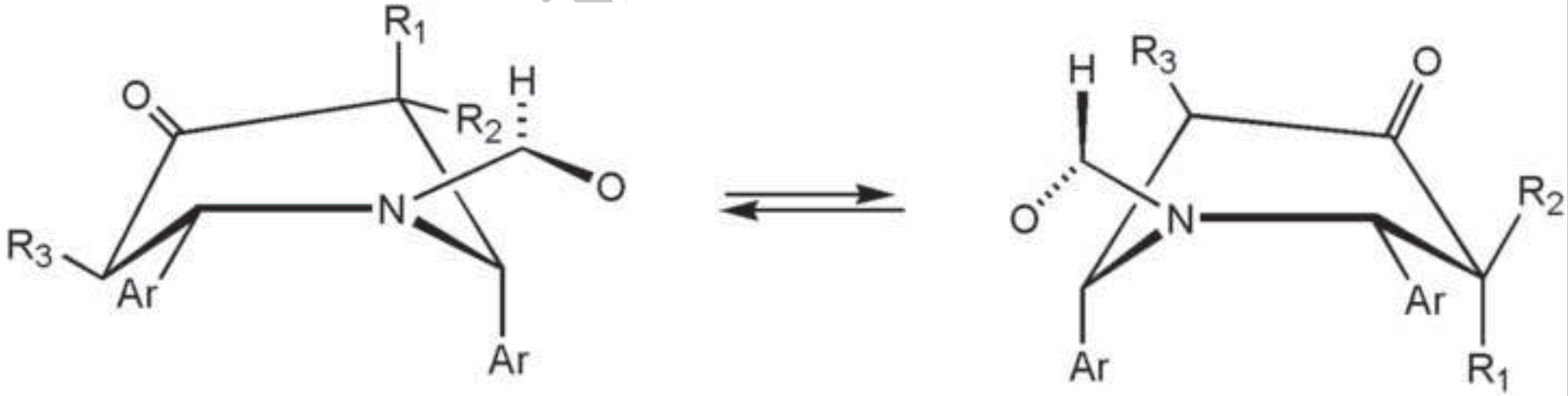
Disk potency 60µg Diameter of inhibition zone (mm)					
Compounds	*Aspergillus flavus*	*Fusarium oxysporum*	*Mucor indicus*	*Penicillium chrysogenum*	*Trichoderma viride*
5	8	9	9	9	11
6	12	7	10	10	7
7	10	11	10	8	13
8	9	9	9	11	8
Amphotericin-B (5µg/disk)	17	21	16	19	23

ACCEPTED MANUSCRIPT

Highlights

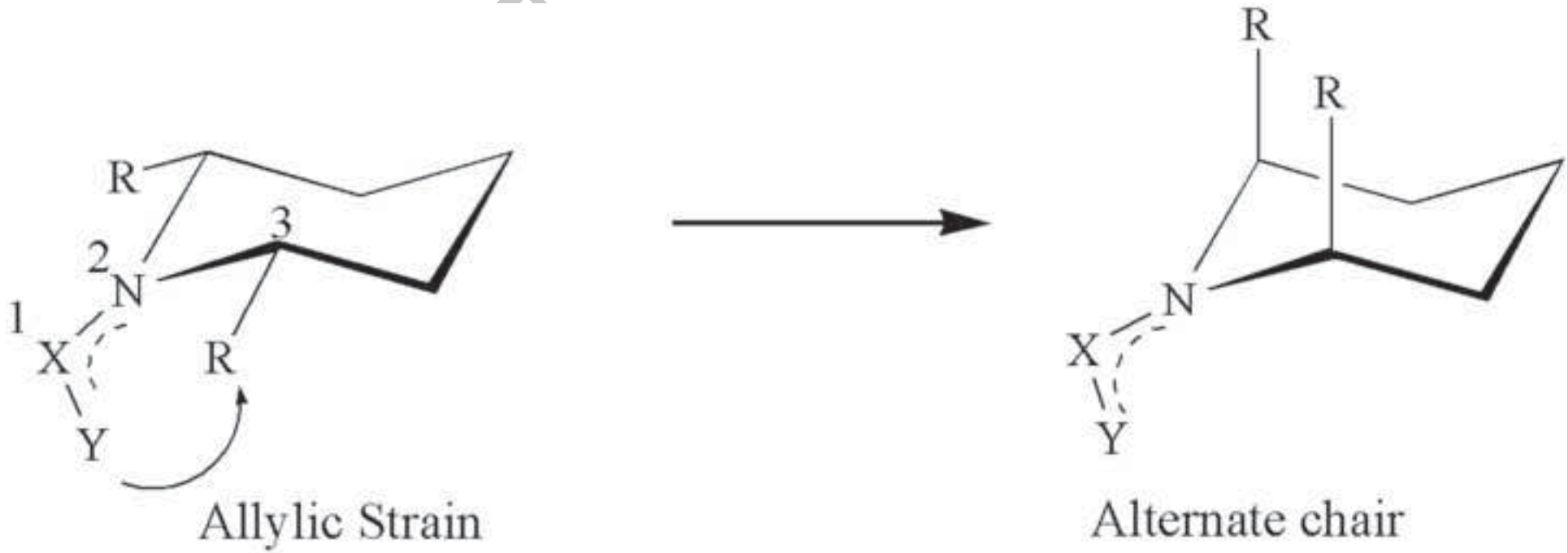
- Four new compounds have been synthesized and are characterized using IR, mass, advanced NMR techniques.
- *N*-formylpiperidin-4-ones showed doubling of all the signals for each of the protons and carbons corresponding to *syn* and *anti* rotamers at RT.
- The energy barrier for *N*-CO rotation of one of the compounds was determined using dynamic ^{1}H NMR spectral study ($\Delta G^{\#}$ = 64.3 kJ/mol).
- The *N*-formylpiperidin-4-ones have been found to prefer equilibrium between the *syn* rotamer of **TB1** conformation and *anti* rotamer of **TB2** conformation.
- All the compounds were screened for their antibacterial & antifungal activities.

Graphical Abstract

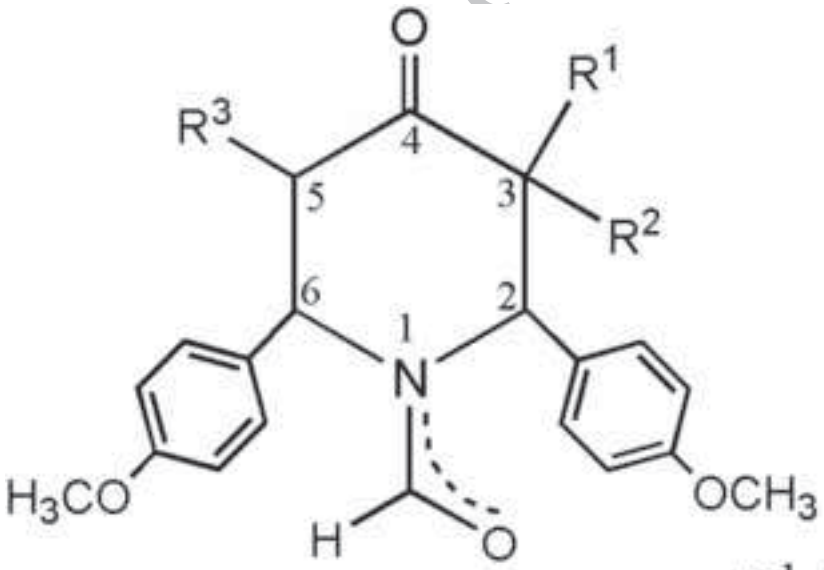


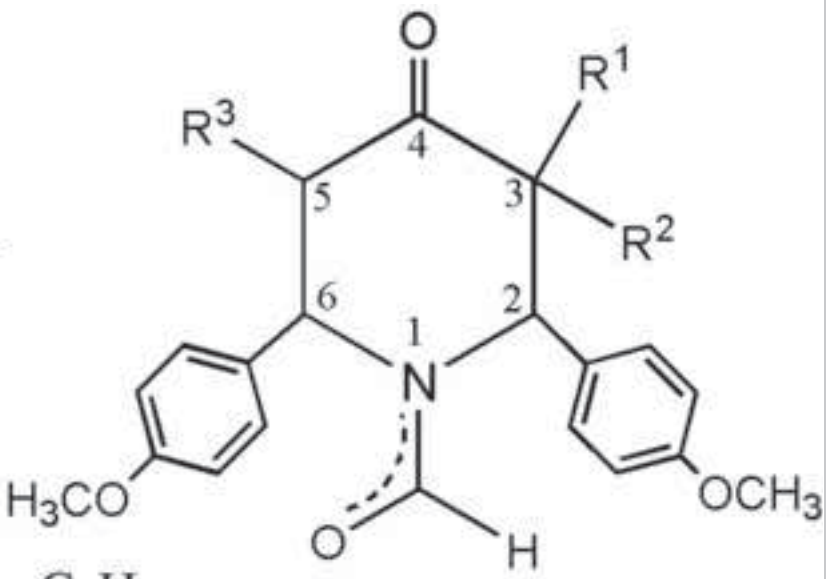
TB1 *syn rotamer* **TB2** *anti rotamer*

Ar=$C_6H_4OCH_3$; R^1, R^2, R^3=CH_3, C_2H_5, H



X=Y: NO, $COCH_3$, R: CH_3





R^1, R^2, R^3=CH_3, C_2H_5

syn rotamer

N-C=O *syn* to C_2

H_2 is *syn* to -C=O

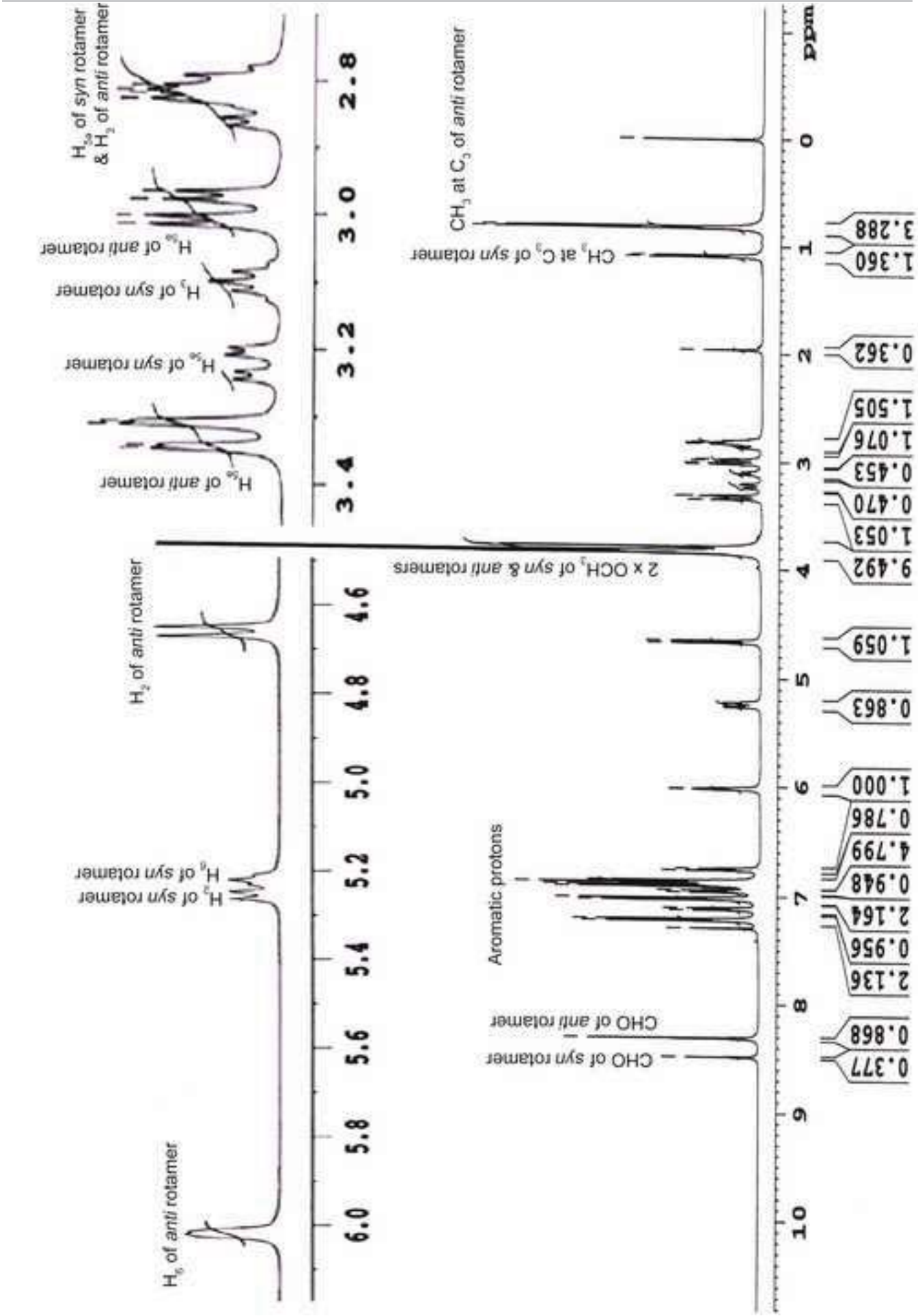
H_6 is *anti* to -C=O

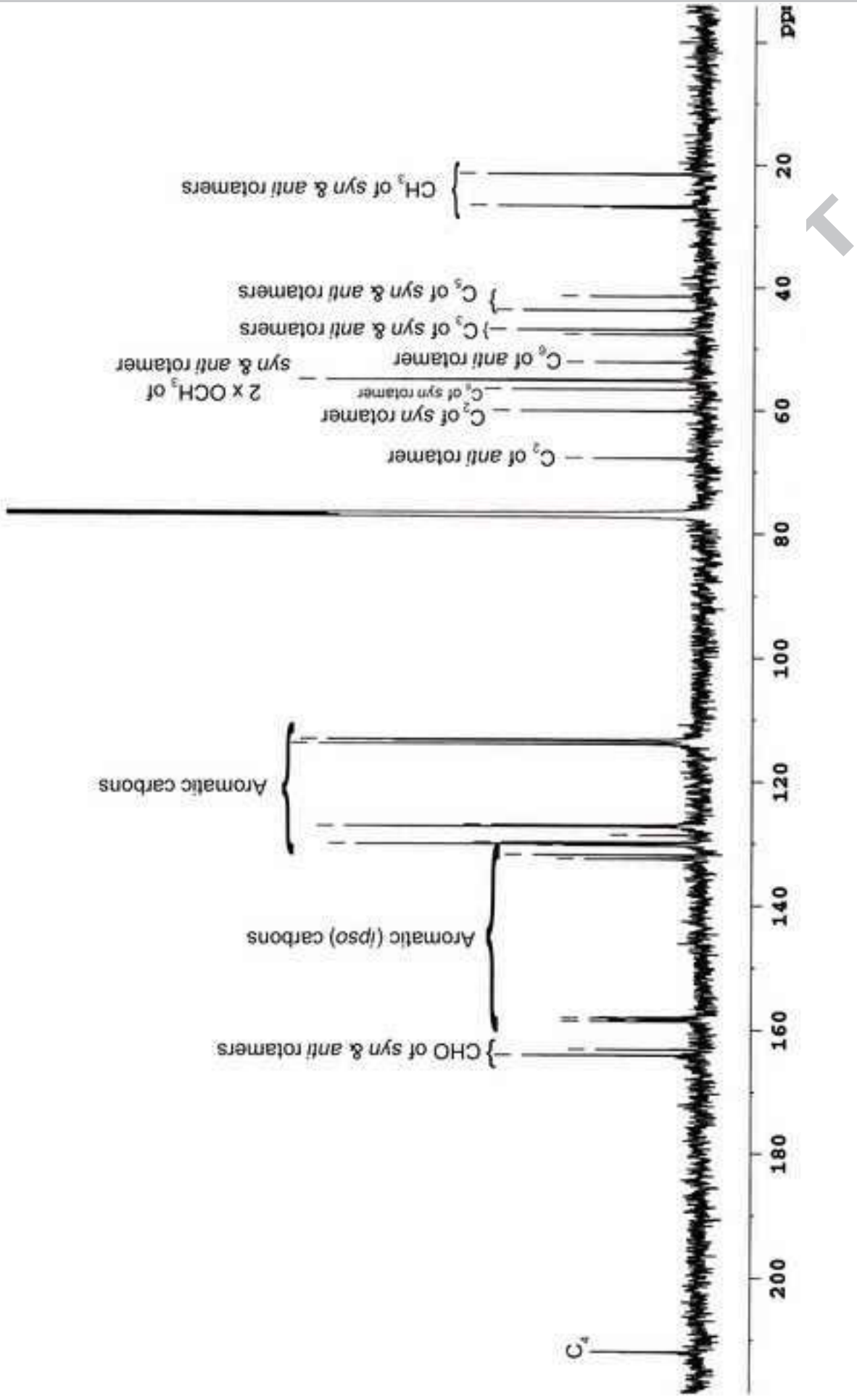
anti rotamer

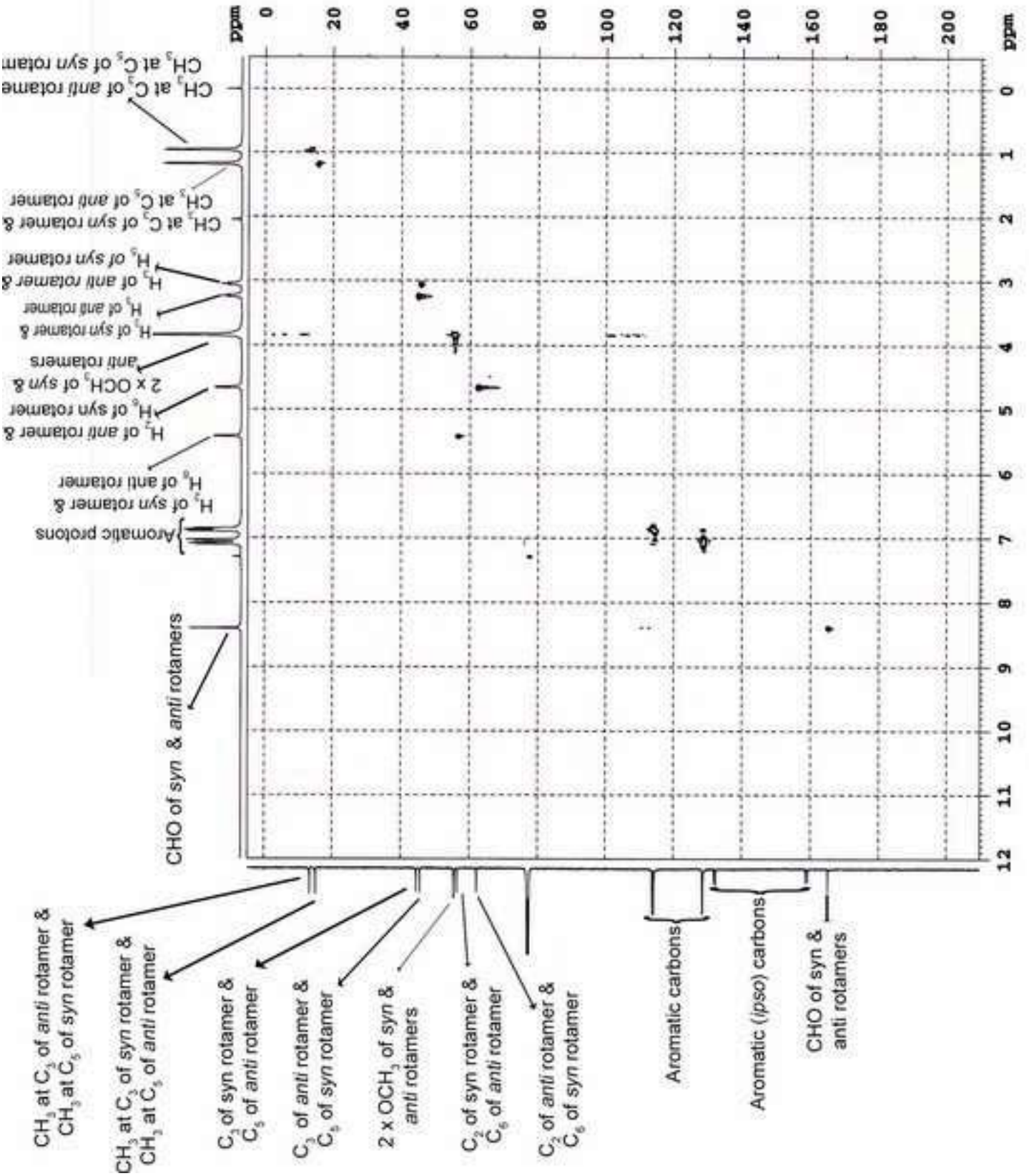
N-C=O *anti* to C_2

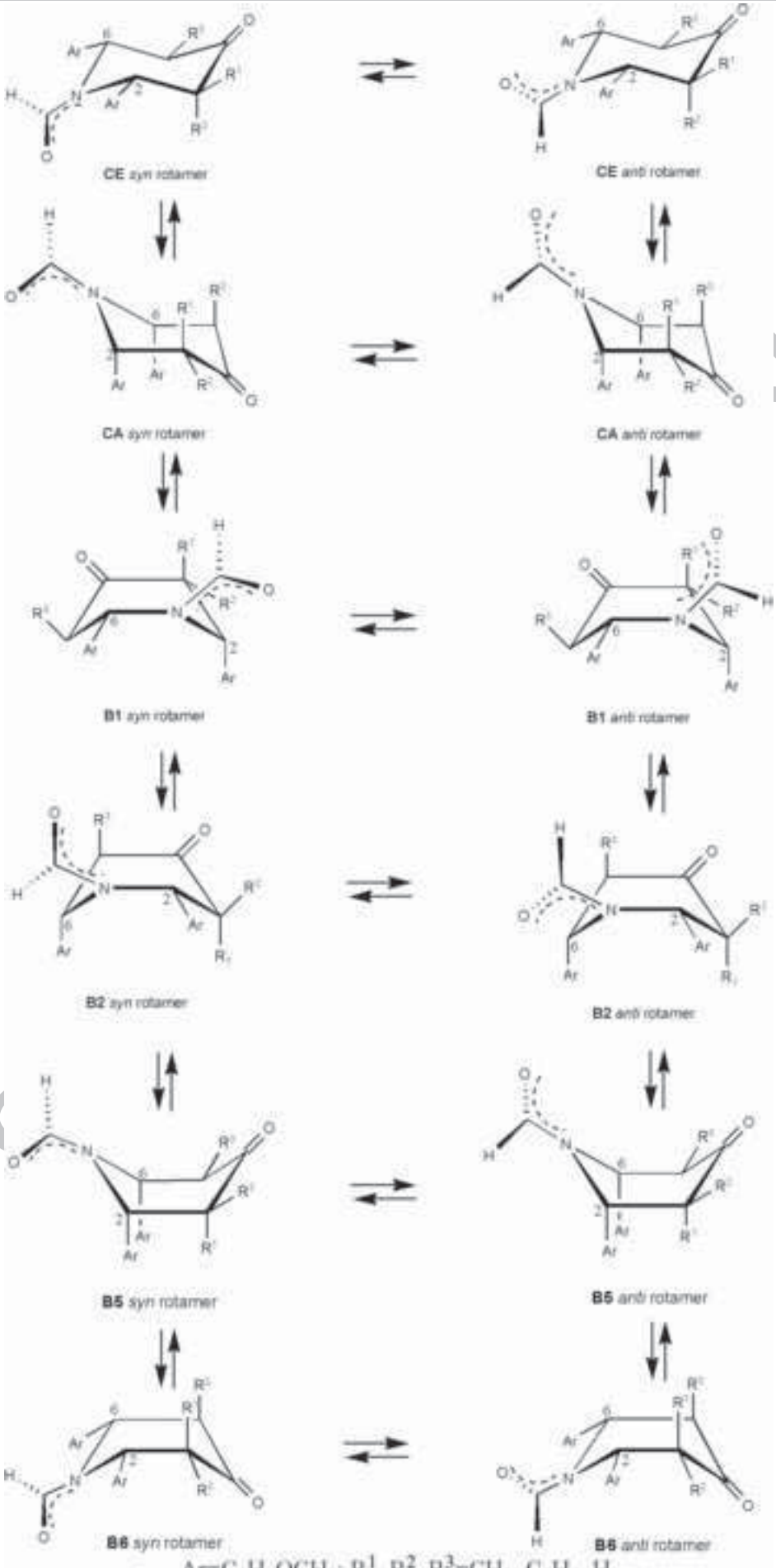
H_2 is *anti* to -C=O

H_6 is *syn* to -C=O









CE *syn* rotamer

CE *anti* rotamer

CA *syn* rotamer

CA *anti* rotamer

B1 *syn* rotamer

B1 *anti* rotamer

B2 *syn* rotamer

B2 *anti* rotamer

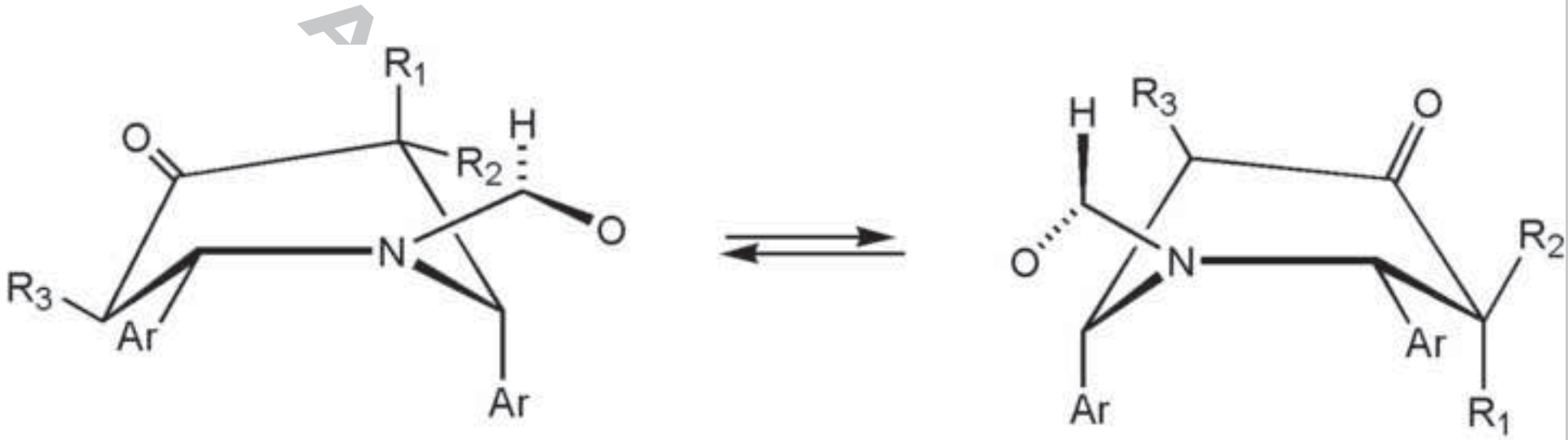
B5 *syn* rotamer

B5 *anti* rotamer

B6 *syn* rotamer

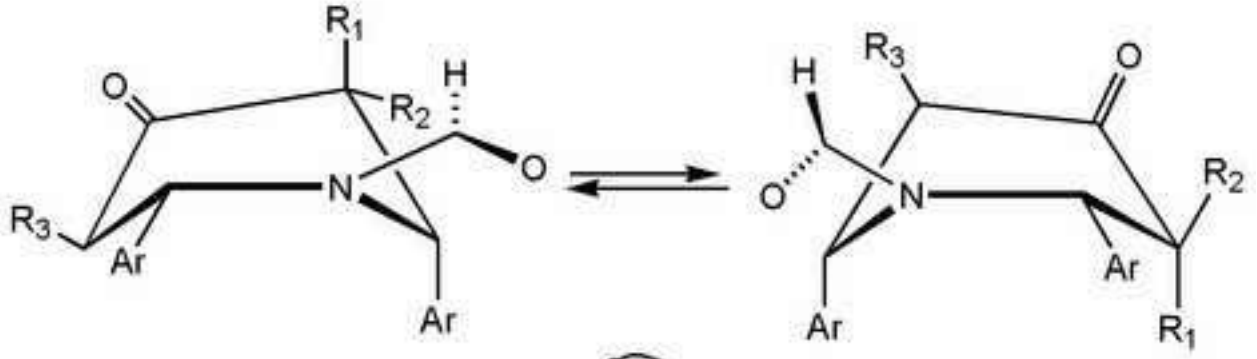
B6 *anti* rotamer

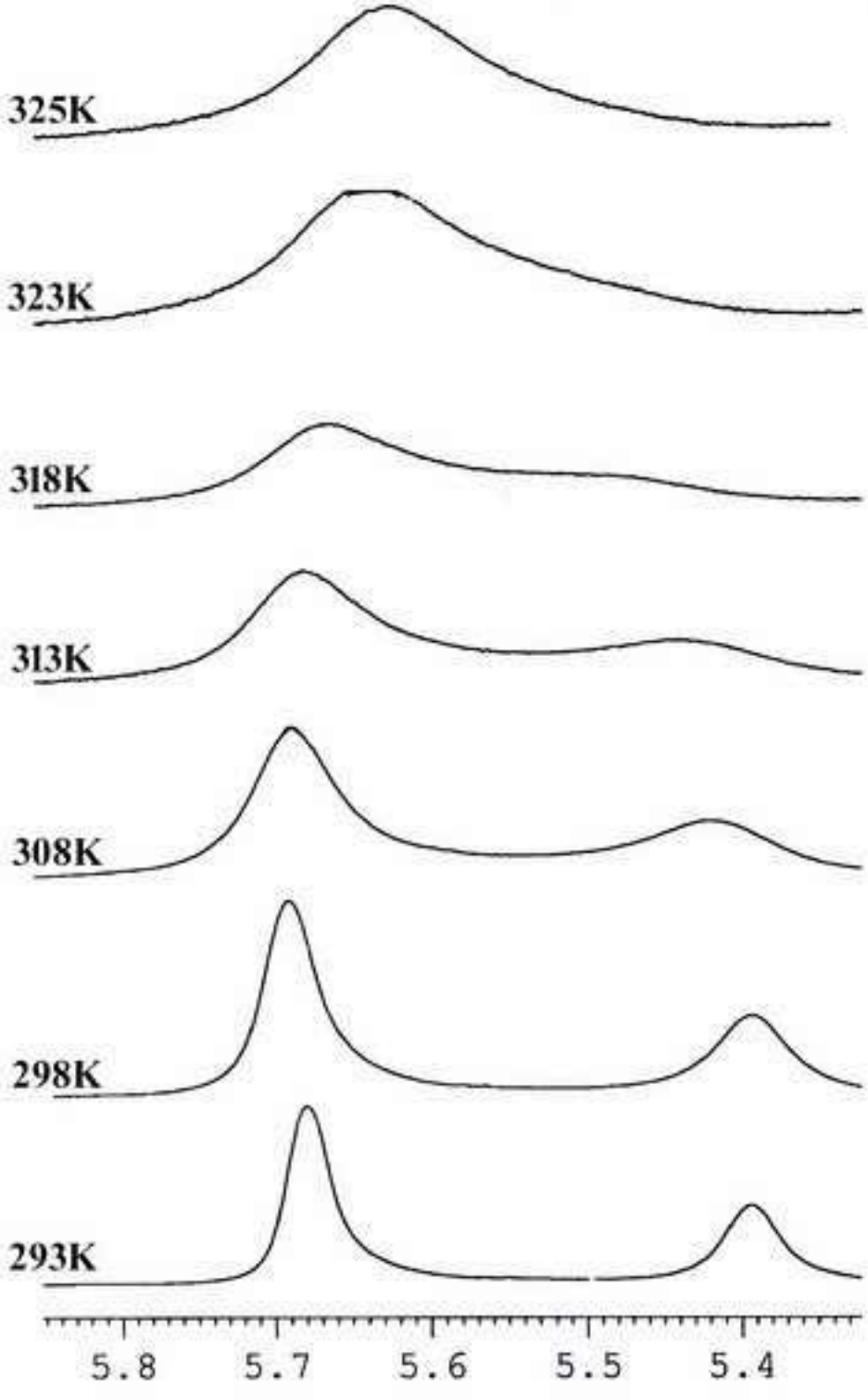
Ar=$C_6H_4OCH_3$; R^1, R^2, R^3=CH_3, C_2H_5, H

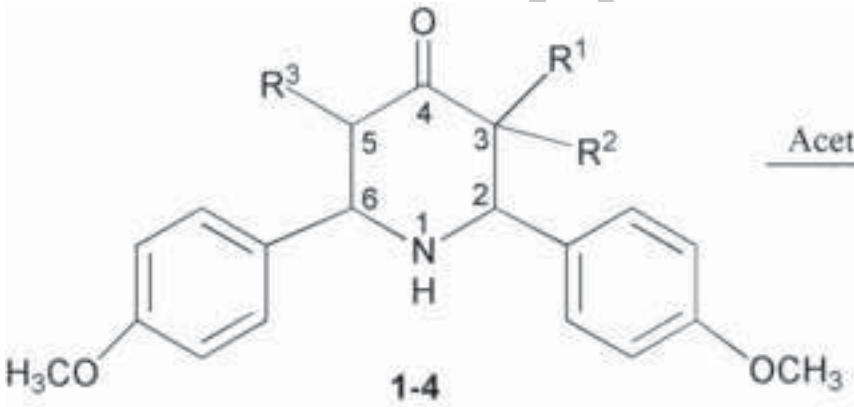


TB1 *syn rotamer* $Ar=C_6H_4OCH_3$ **TB2** *anti rotamer*

	R^1	R^2	R^3
5	CH_3	H	H
6	C_2H_5	H	H
7	CH_3	CH_3	H
8	CH_3	H	CH_3

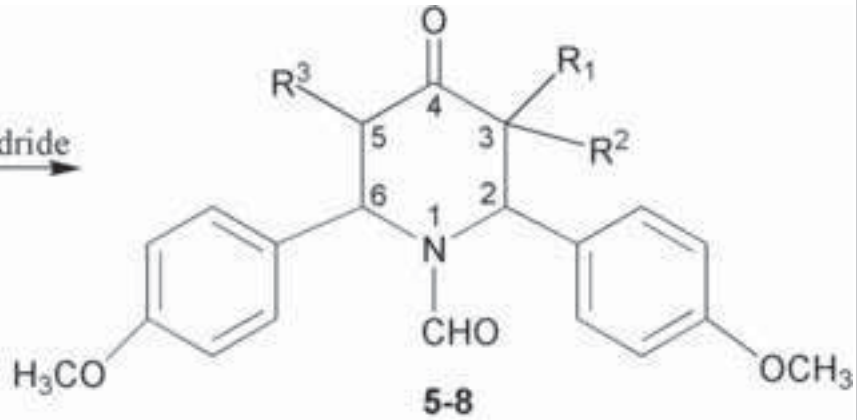






1-4

Acetic-formic anhydride



5-8

	R^1	R^2	R^3	
1	CH_3	H	H	**5**
2	C_2H_5	H	H	**6**
3	CH_3	CH_3	H	**7**
4	CH_3	H	CH_3	**8**