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Research Article

# A STUDY ON STRUCTURAL ASPECTS AND MICROBIAL ACTIVITY OF (E)-4-PYRIDINECARBOXALDEHYDE-3-HYDROXY-5-(HYDROXYMETHYL)-2-METHYL-OXIME HYDROCHLORIDE: AN EXPERIMENTAL AND COMPUTATIONAL APPROACH

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**ABSTRACT**

An experimental and computational studies including antibacterial activity have been carried out with an objective to find the structural and biological aspects of (E)-4-Pyridinecarboxaldehyde-3-hydroxy-5-(hydroxymethyl)-2-methyl-oxime(PCHHMMO)hydrochloride.The crystal structure of (E)-4-Pyridinecarboxaldehyde-3-hydroxy-5-(hydroxymethyl)-2-methyl-oxime hydrochloride was determined from three-dimensional X-ray data with Z=4, with space group P-1 and cell dimensions: a=6.6067(15) Å, b=9.726(2) Å and c=14.805(3) Å. The data obtained confirmed that the title compound crystallizes in the non planar form, assembled as 3-D tetramer, involving inter and intramolecular hydrogen bonding. The equilibrium studies were carried out to ascertain metal binding sites(*p*Ka1=5.0, *p*Ka2=8.2) in molecule. The HyperChem 7.5 tools were employed to generate computed structural and spectral data as well as molecular properties. The energies and orientation of HOMO and LUMO are computed to understand the affinity of compound to bind with metal ions for the formation of corresponding complexes.

**Keywords:** (E)-4-Pyridinecarboxaldehyde-3-hydroxy-5-(hydroxymethyl)-2-methyl-oxime (PCHHMMO) hydrochloride, X-ray diffraction, Hydrogen bonding, HyperChem 7.5.

## INTRODUCTION

3-Hydroxy- 5-(hydroxymethyl)-2-methyl-4-pyridinecarboxaldehyde 4-pyridinylcarbonyl hydrazone (Pyridoxal Isonicotinoyl Hydrazone PIH) [1] and 3-Hydroxy-5-(hydroxymethyl)-methyl 4-pyridinecarboxaldehyde benzoyl hydrazone (Pyridoxal Benzoyl Hydrazone PBH) [2], have been identified as promising candidates for replacing desferrioxamine (DFO), the only therapeutically safe drug in clinical use for the treatment of iron overload in Thalasemic patients [3]. Some of the analogues of PIH with different substituents were synthesized [4] in order to replace the drug DFO which are more efficient than DFO in mobilizing non-heme iron from the precursors of red cell [5,6,7]. PIH also had immense activities in DNA synthesis [8], cell proliferation differentiation [9], cancer chemotherapy [10], malaria chemotherapy [11] and immunology [12]. As the title compound 4-Pyridinecarboxaldehyde-3-hydroxy-5-(hydroxymethyl)methyl-oxime is one of the analogues of PIH class, keeping in view of its biological importance, in the present investigation it is planned to evaluate its structural properties by experimental results and computed data generated by employing HyperChem 7.5 tools.

## MATERIALS AND METHODS

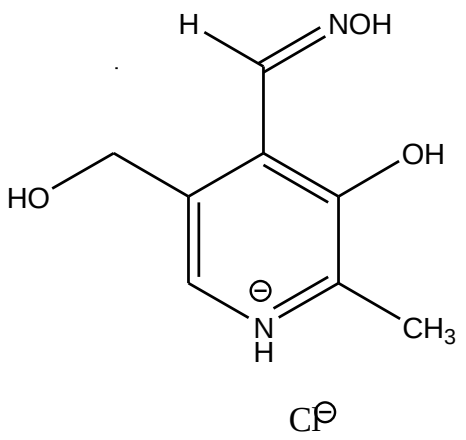


**Fig. 1: 4-Pyridinecarboxaldehyde-3-hydroxy-5-(hydroxymethyl)-2-methyl- oxime hydrochloride(PCHHMMO)**

All the chemicals used are AR Grade. Pyridoxal Hydrochloride is procured from Sigma Aldrich and Hydroxylamine from MERCK.

The 4-Pyridinecarboxaldehyde-3-hydroxy-5-(hydroxymethyl)-2-methyl-oxime hydrochloride (PCHHMMO) (**Fig.1**) was synthesized using known procedure [4]. Colourless crystalline product was obtained in 70% yield and purity was checked by TLC (m.p 226-228°C). It is recrystallized in ethanol and was allowed to evaporate slowly for crystal growth. The single crystal X-ray diffraction studies were carried out with the above developed crystals. High resolution single crystal X-ray diffraction data were collected at 293K on BRUKER SMART APEX diffractometer, equipped with CCD area detector system, graphite monochromatic and a MoKα, fine focus sealed tube[λ(Mo-Kα,)]=0.71073Å. A single crystal was mounted in a Lindmann capillary and 2400 frames were recorded with scanning angle ω of 0.3°, each for 5 sec exposure with 0.5 mm collimated X-ray. The crystal to detector distance was kept 60 mm. Collected data was reduced by, SAINT-PLUS V6.45[13]. An empirical absorption correction was applied to the collected reflections with SADABS[14] . The structures were solved with direct methods using SHELXS-97 and the refinement was done against $F^2$ using SHELXL-97[15]. All non-hydrogen atoms were refined anisotropically and hydrogens were introduced on calculated positions and included in the refinement riding on their respective parent atoms.

The pH measurements were made using a digital ELICO electronic model LI 120 pH meter in conjunction with a combined glass and calomel electrode. The pH meter was calibrated at different pH values 4.0, 7.0 and 9.2 using the appropriate standard (BDH) buffers with necessary temperature corrections.

Because protons and metal ions compete for the donor group of the ligands, complex formation is investigated most accurately and conveniently by pH-metry. Irving-Rossotti pH [16,17,18] titration technique was employed for the determination of dissociation constants. The elemental analyses (C, H, N) of compound under study was carried out on Perkin-Elmer 240C elemental analyzer. The LC-MS data was collected on Shimadzu LCMS-2010A. The chromatogram was obtained by injecting 5µL of the sample dissolved in methanol into $C_{18}$ column, using methanol : water mobile phase mixture 90:10, with a flow rate 0.2 mL/min and UV (254 nm) detector. The mass spectrum is obtained by using Atmospheric Pressure Chemical Ionization in Negative Mode. Conductance was measured on SYSTRONICS model No.304 digital

conductometer using conductivity cell. IR spectrum (KBr) was recorded on a Perkin–Elmer 435 spectrophotometer. $^{1}$H-NMR and $^{13}$C-NMR spectra were recorded on Bruker WH (270 MHz) spectrometer. UV spectrum was scanned on UV-3600 Schimadzu spectrophotometer.

The molecular modeling program HyperChem 7.5 is employed for computational studies.

**RESULTS AND DISCUSSION**

LC chromatogram of the compound showed a single peak at 0.712 min (**Fig. 2**). The mass spectrum displayed [M-1] peak at m/z 217 (base peak), at m/z 181 due to loss of HCl and other peaks observed at m/z 163, 147 due to fragmentation. The C H N analyses are also carried out to ascertain the composition; Found% (calculated%): C:44.28 (44.13); H: 4.68 (4.59); N:12.75 (12.87) for $C_8H_{11}N_2O_3Cl$.

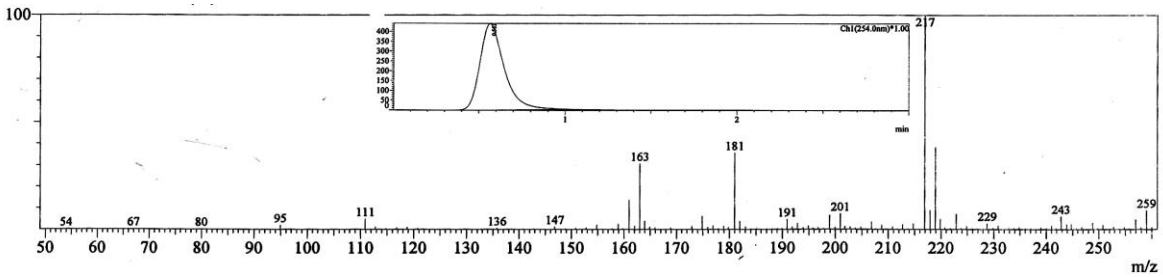


**Fig. 2: Liquid Chromatogram and mass spectrum of PCHHMMO**

The conductivity measurements with $1x10^{-6}$ M solution in ethanol medium at room temperature showed molar conductance of $2x10^{-4}$ Mhos indicating the existence of compound in ionic form.

IR spectrum of compound displayed peaks at 3500-3350 $cm^{-1}$ (phenolic OH, NOH, $CH_2OH$, $\nu_{O-H}$), 3202 $cm^{-1}$ ($\nu_{N-H}$), 3180 $cm^{-1}$ ($\nu_{C-H(Arom)}$), 2950 $cm^{-1}$ ($CH_3$, $\nu_{C-H}$), 1620 $cm^{-1}$ ($\nu_{C=N}$), 1525 $cm^{-1}$ ( $\nu_{C=C}$), 1277 $cm^{-1}$ ($CH_3$ bending) and 1089 $cm^{-1}$ ($\nu_{C-O}$). The appearance of shoulder and broad nature of the band above 3000 $cm^{-1}$ indicate that extensive inter and intra molecular hydrogen bonding is present in the compound.

The $^{1}$H-NMR spectrum in DMSO-d6 showed peaks (δ) at 8.4(s, Ar-CH), 8.6(s, CH=N), 13.2(s, NOH) ppm in down field region. The peak at 2.7 ppm corresponds to $CH_3$ protons, the peak at 4.4 ppm is a admixture of $CH_2$ and aliphatic OH group which readily exchanged with $D_2O$ resulting in decrease in intensity of peak. The signal pertaining to phenyl OH group admixed in the region 8.4-8.6 ppm which disappeared on deuteration. The $D_2O$ exchangeable peak recorded in the region 2.5-2.7 ppm admixed with peak of methyl protons, corresponds to ring $NH^{+}$ proton. The $^{13}$C-NMR recorded signals (DMSO-d6, δ) at 163, 156, 149, 132, 124, 120, 63 and 18.9 ppm correspond to azomethine, aromatic and aliphatic carbons respectively.

The electronic spectrum of title compound in dil. acetic acid (1:1) showed absorption maxima at wavelengths λ of 275 nm and 326 nm. These peaks may be assigned to π→ π* (C=C) and n→ π* (HC=N) transitions respectively.

**Crystal studies**

The overall view of molecule with atom labeling is shown in the (**Fig.3)**. The single crystal X-ray diffraction study indicates the presence of E conformer in the crystal. The structure is supported by above mentioned IR, $^{1}$H-NMR and $^{13}$C-NMR experimental spectral data.

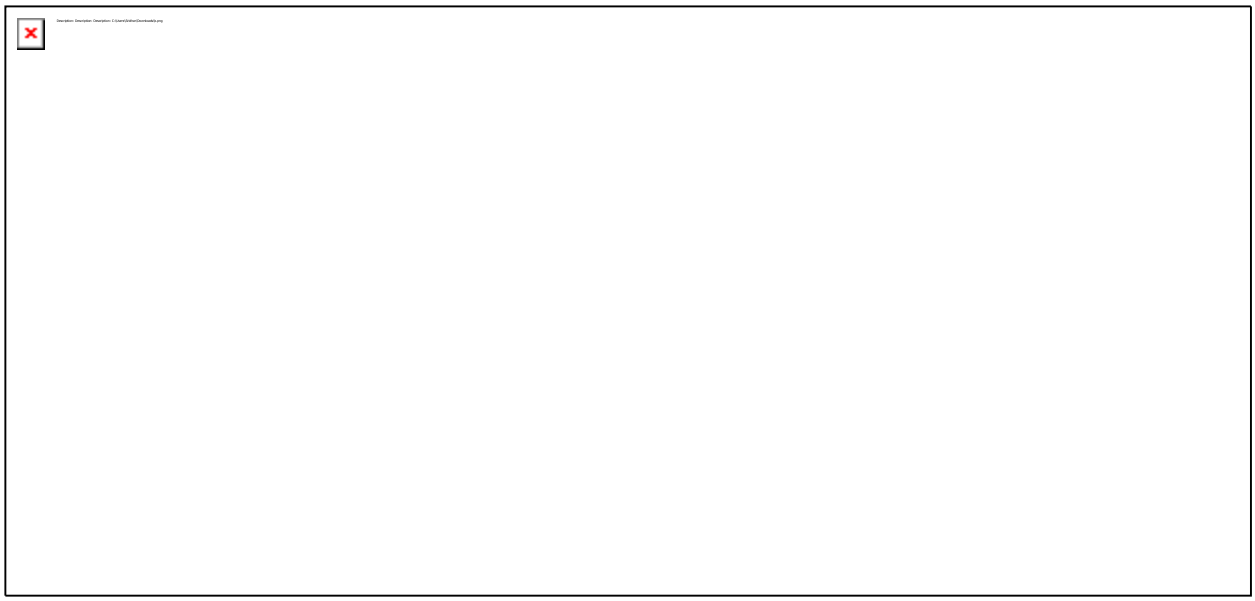

**Fig.3: ORTEP diagram of PCHHMMO with atom labeling scheme and thermal ellipsoid at 50% probability**

The title molecule contain two nitrogen atoms, three oxygen atoms, eight carbon atoms, eleven hydrogen atoms and one chloride ion outside the molecule having probability for H-bonding interactions. The unit cell parameters, experimental details and refinement parameters are listed in Table-1. The crystal system is triclinic and P-I space group with 4 molecules of the title compound per unit cell.Analysis of bond lengths and bond angles reveal that the two benzene rings are non planar with small dihedral angle due to slight tilting. The 'H' atoms were placed geometrically. These results are comparable with crystal parameters reported earlier for its analogue compounds [19].

**Table 1: Single Crystal data and structure refinement**

| | | |
|---|---|---|
| Empirical formula | $C_8H_{11}ClN_2O_3$ | |
| Formula weight | 218.64 | |
| Temperature | 298(2) K | |
| Wavelength | 0.71073 Å | |
| Crystal system | Triclinic | |
| Space group | P-1 | |
| Unit cell dimensions | a = 6.6067(15) Å | α= 87.180(4)°. |
| | b = 9.726(2) Å | β= 88.119(4)°. |
| | c = 14.805(3) Å | γ = 87.856(4)°. |
| Volume | 949.1(4) $Å^3$ | |
| Z | 4 | |
| Density (calculated) | 1.530 $Mg/m^3$ | |
| Absorption coefficient | 0.385 $mm^{-1}$ | |
| F(000) | 456 | |
| Crystal size | 0.42 x 0.32 x 0.20 $mm^3$ | |
| Theta range for data collection | 1.38 to 25.00°. | |
| Index ranges | -7<=h<=7, -11<=k<=11, -16<=l<=17 | |
| Reflections collected | 6551 | |
| Independent reflections | 3211 [R(int) = 0.0337] | |
| Completeness to theta = 25.00° | 96.0 % | |
| Absorption correction | Semi-empirical from equivalents | |
| Max. and min. transmission | 0.9270 and 0.8551 | |
| Refinement method | Full-matrix least-squares on $F^2$ | |
| Data / restraints / parameters | 3211 / 0 / 261 | |
| Goodness-of-fit on $F^2$ | 1.045 | |
| Final R indices [I>2sigma(I)] | R1 = 0.0635, wR2 = 0.1744 | |
| R indices (all data) | R1 = 0.0751, wR2 = 0.1842 | |
| Largest diff. peak and hole | 0.760 and -0.364 $e.Å^{-3}$ | |

A total of four molecules are present in unit cell (**Fig.3**). Out of four molecules, two are in one plane and other two are in another plane. Orientation of molecules in different planes allows intermolecular hydrogen bonding apart from intramolecular hydrogen bonding.

**The Hydrogen bonding interactions**

The molecule has good number of H-bonding donors and H-bonding acceptors. It is evident from diffraction studies in the present investigation (**Fig.5** and **Table- 2**) that there are a total of eight intermolecular interactions and three intramolecular interactions. The aromatic hydrogen showed only intramolecular hydrogen bonding while the hydrogen atoms on ring nitrogen and methyl group participated only in intermolecular hydrogen bonding interactions. The methylene and azomethine hydrogens are not involved in hydrogen bonding interactions. While the hydroxyl protons of molecule participated in both intra and inter molecular hydrogen bonding interactions.

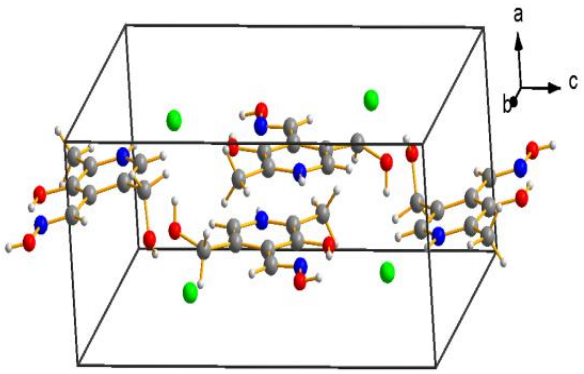


**Fig. 4: Alignment of molecules in the unit cell**

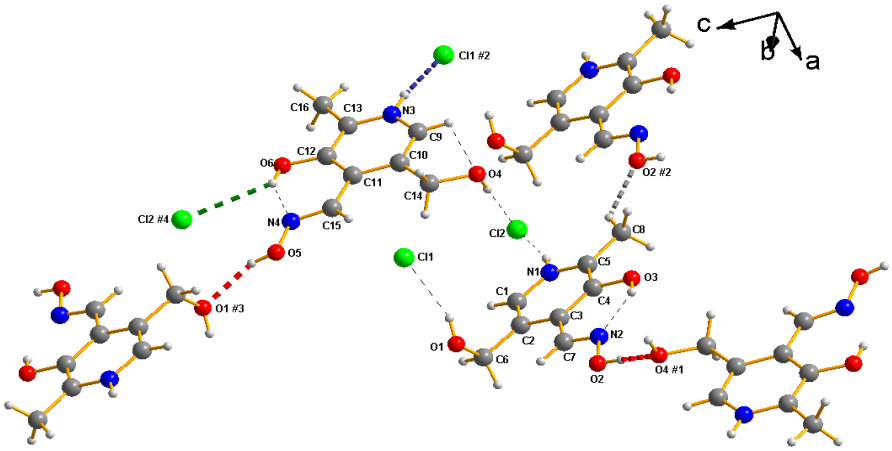


**Fig. 5: Hydrogen bonding interactions among pyridoxal oxime cation and chloride anion.**

**Table 2: Hydrogen bonding parameters (Å, deg) of compound**

| D–H···A | *d*(D–H) | *d*(H···A) | D(D···A) | ∠DHA |
|---|---|---|---|---|
| **Intermolecular H-bonds** | | | | |
| O2–H2···O4 #1 | 0.82 | 1.82 | 2.608(3) | 160 |
| C8–H8A···O2 #2 | 0.96 | 2.41 | 3.161(4) | 135 |
| O5–H5···O1 #3 | 0.82 | 1.96 | 2.722(4) | 155 |
| N1–H1A···Cl2 | 0.86 | 2.19 | 3.026(3) | 164 |
| O1–H1B···Cl1 | 0.82 | 2.31 | 3.092(3) | 159 |
| N3–H3···Cl1 #2 | 0.86 | 2.22 | 3.068(3) | 170 |
| O4–H4···Cl2 | 0.82 | 2.22 | 3.042(3) | 179 |
| O6–H6···Cl2 #4 | 0.82 | 2.82 | 3.371(2) | 126 |
| Intramolecular H-bonds | | | | |
| O3–H3A···N2 | 0.82 | 1.93 | 2.634(4) | 143 |
| O6–H6···N4 | 0.82 | 1.90 | 2.601(4) | 143 |
| C9–H9···O4 | 0.93 | 2.40 | 2.742(2) | 102 |

Symmetry transformations used to generate equivalent atoms: #1: 1-x,2-y,-z; #2: x,-1+y,z; #3: 1-x,2-y,1-z; #4: 1-x,1-y,1-z.

**Equilibrium studies**

As the title compound is one of the analogue to PIH class which is a therapeutically safe drug in clinical use for the treatment of iron overload, to understand its chelation properties an attempt is made to study its potential donor sites that bind with metal ions.

To understand metal chelation, it is prerequisite to determine dissociation constants of dissociable protons and protonation constants at probable hydrogen binding sites. In the present study, Irving-Rossotti pH titration technique was employed for the determination of dissociation constants as the candidate compound has dissociable protons.

The pH-Metric titrations were carried out in aqueous medium at 303ºK and 0.1M ($KNO_3$) ionic strength (**Fig.6**). The dissociation constant values were calculated using Irving-Rossotti titration technique. From the titration data obtained, dissociation constants have been calculated from the linear plots of Log (2- $\bar{n}_A$)/ ($\bar{n}_A$-1), Log(1- $\bar{n}_A$)/ $\bar{n}_A$, Vs pH (**Fig.7 and 8**). The results indicated the presence of two dissociable protons corresponding to ring $NH^+$ proton ($pKa_1$=5.0) and phenolic OH group of PCHHMMO ($pKa_2$=8.2).

The titration curves clearly indicated the release of dissociable protons more easily in presence of metal ion indicating formation of corresponding complexes in solution.

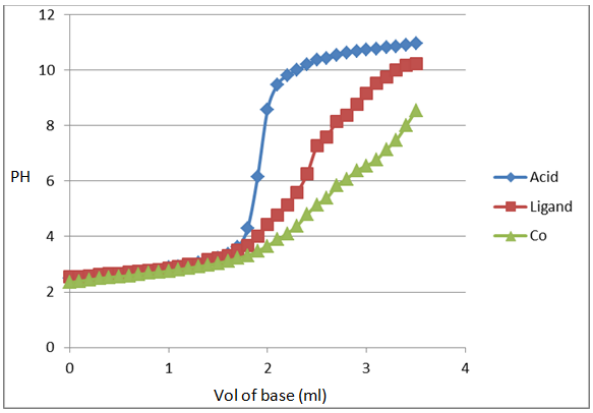


**Fig. 6: pH titration curves of PCHHMMO system in aqueous medium at 303K and 0.1 M ionic strength**

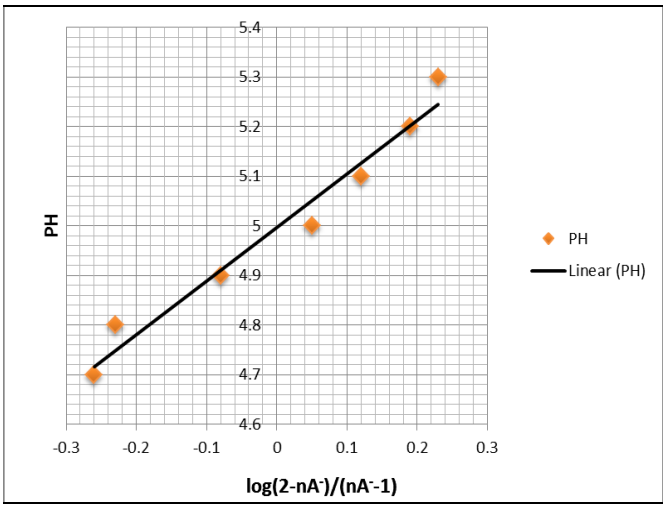


**Fig. 7: Plots of Log (2- $\bar{n}_A$)/ ($\bar{n}_A$-1) Vs pH of PCHHMMO in aqueous medium**

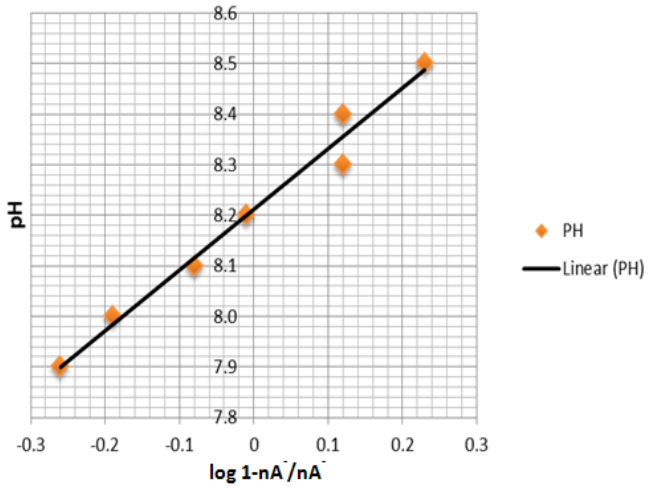


**Fig. 8: Plots of Log (1- $\bar{n}_A$)/ ( $\bar{n}_A$) Vs pH of PCHHMMO in aqueous medium**.

These results infer that $pKa_1$ (5.0) value corresponds to dissociation of proton from $NH^+$ group and $pKa_2$ (8.2) from phenolic OH group.

**Biological Activity**

The antibacterial activity of the title compound 4-Pyridinecarboxaldehyde-3-hydroxy-5- (hydroxymethyl)-2-methyl-oxime was studied by applying the disc diffusion method which is one of the most precise and reliable methods for determining the degree of sensitivity of microbes to antibiotics. The actively growing cultures were mixed in soft agar (1% Nutrient agar were used for bacteria respectively) and plated, to permit fast and good growth yields for many bacterial species. Extract was loaded onto 6mm sterile filter paper discs separately. The discs were then placed on the pre-seeded agar medium and incubated for 24 hrs at 37°C and observed for zone of growth inhibition. The compounds were tested for antibacterial activity against *Staphylococcus aureus, Bacillus cereus* (Gram positive) and *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Gram negative) bacteria. The comparison of biological activity with different strains of bacteria revealed that the compound under investigation is highly active against both gram positive and negative bacteria. The results are tabulated in table-3. The studies revealed that PCHHMMO showed high activity against gram-negative bacteria and gram-positive bacteria.

**Table 3: Antibacterial activity of PCHHMMO**

| Name of the bacteria | Activity |
|---|---|
| **Gram positive** | |
| *Staphylo coccus aureus* | ++ |
| *Bacillus cereus* | ++ |
| **Gram negative** | |
| *Pseudomonas aeruginosa* | + |
| *E.Coli* | ++ |
| *Klebsiella pnemoniae* | ++ |
| + <10mm (slightly active), ++ >10mm (moderately active), +++ > 35mm (highly active) | |

**Computational studies**

In the present investigation the HyperChem 7.5 software was used for quantum mechanical calculations to generate spectral data. After building molecule by HyperChem tools[20-25], the geometry optimization was carried out using Ab Initio method. The IR spectral data is generated with semi empirical single point PM3 method approximation for the title compound. QSAR properties allows calculation and estimation of a variety of molecular descriptors commonly used in quantitative structure activity relationship (QSAR) studies[26,27].

Quantum chemical calculations have been widely used to study donor and acceptor properties of molecules. The values of energy of the highest occupied molecular orbitals ($E_{HOMO}$), the lowest unoccupied molecular orbitals ($E_{LUMO}$) and the energy gap between these ($E_{LUMO-HOMO}$) were computed.

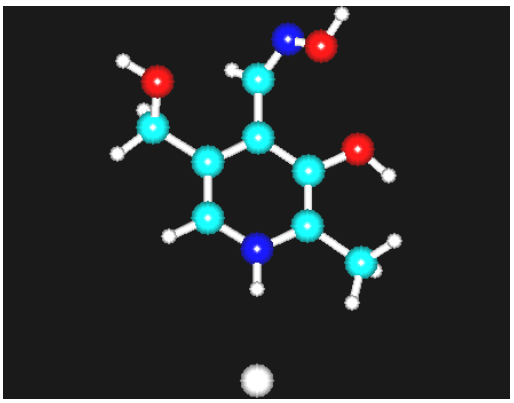

**Fig. 9: The geometry optimized structure of PCHHMMO in the HCl form**

**Vibrational Analysis**

HyperChem calculates all of the fundamental modes, both IR and Raman, as well as the IR intensities. The experimental IR spectral data and corresponding computed data (**Table.4**) generated through semi empirical single point AM1 method for optimized molecule (**Fig.9**), are in good agreement.

**Table 4: IR Spectral data of PCHHMMO**

| Compound | $Cm^{-1}$ | | | | | |
|---|---|---|---|---|---|---|
| PCHHMMO | $\upsilon_{CH2OH}$, $\upsilon_{OH}$, $\upsilon_{NOH}$ | $\upsilon_{NH}$ | $\upsilon_{CH\ aro}$ | $\upsilon_{CH3}$ | $\upsilon_{C=N}$ | $\upsilon_{C=C(ring)}$ |
| Exp | 3500-3350 | 3202 | 3180 | 2950 | 1620 | 1525 |
| AM1 | 3519-3390 | 3209 | 3301 | 2937 | 1680 | 1628 |

**NMR Spectral Simulation**

$^{1}$H-NMR and $^{13}$C-NMR data were also computed and compared with the experimentally obtained corresponding spectral data (**Table-5, 6**). From the analysis of data it is clear that there is deviation in chemical shift values with respect to protons attached to electronegative groups. Such deviations are attributable to hydrogen bonding interactions.

**Table 5: $^{1}$H-NMR δ(ppm) Spectral Data of PCHHMMO**

| PCHHMMO | δ (HC=N) | δ ring (OH) | δ(NOH) | δ CH (aromatic) | δOH | δCH | δ $CH_3$ | δ NH |
|---|---|---|---|---|---|---|---|---|
| Exp | 8.6 | 8.4 | 13.2 | 8.4 | 4.4 (admixed) | | 2.7 - 2.5 (admixed) | |
| AM1 | 12.49 | 8.11 | 9.22 | 9.32 | 6.83 | 3.19 | 3.09 | 2.51 |

**Table 6: $^{13}$C-NMR Spectral data**

| PCHHMMO | δ ppm | | | |
|---|---|---|---|---|
| | Aromatic carbons | HC=N | $CH_2$ | $CH_3$ |
| Exp | 120, 124, 132, 156 &163 | 149 | 63 | 18.9 |
| AM1 | 139, 142, 152, 159 &166 | 249 | 51 | 19.5 |

**Molecular Orbitals**

The molecule has dissociable protons which are replaceable with metal ion for the formation of a metal complex. Hence energies of molecular orbitals are computed with single point calculation for the geometrically optimized charged deprotonated structure. The orientation of highest occupied molecular orbitals (HOMO) and lowest unoccupied molecular orbitals (LUMO) are generated (**Fig. 10, 11**) to understand the metal binding sites. The energy difference between the HOMO (-5.959eV) and LUMO (0.456eV) implies to HOMO-LUMO gap (5.503eV). The optimum HOMO–LUMO gap implies the formation of ionic species through proton dissociation with ease.

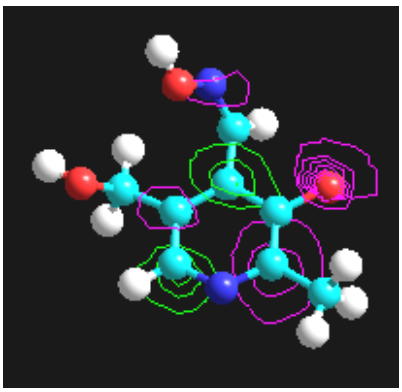

**Fig. 10: Highest Occupied Molecular Orbitals (HOMO) in Ionized Form**

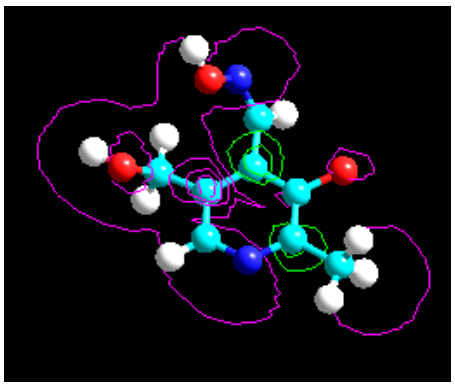

**Fig. 11: Lowest Unoccupied Molecular Orbitals (LUMO) in Ionized Form**

The HOMO orientations confirm that, the non bonding orbital oriented along oxygen of phenyl ring is more suitable for bonding. Subsequent chelate formation occurs with the involvement of oxime nitrogen which has lone pair of electrons in an orbital with slight bonding character.

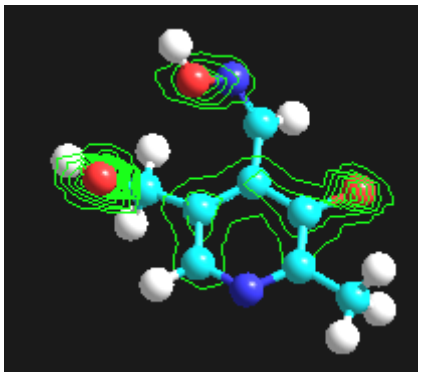

**Fig. 12: Total Charge Density Distribution in Ionized Form**

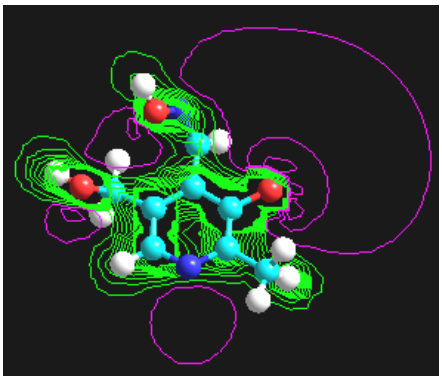

**Fig. 13: Electrostatic potential Orientations in Ionized Form**

The charge density sites are localized on oxygen atoms of molecule and electrostatic potentials are more on phenolic oxygen and ring nitrogen **(Fig.12)**. Though electrostatic potential is observed on ring nitrogen **(Fig.13)**, the metal ion binding is preferred at phenolic oxygen because of presence of one more nearest coordination site at oxime nitrogen. As the present study is to understand chelation sites in title compound, the experimental pH-metric studies and computed diagrams shown above are useful to draw theoretical predictions.

**Quantitative structure-activity relationship (QSAR)**

Quantitative structure-activity relationship (QSAR) is a computational process that relates the chemical structure of compounds with biological activity. QSAR properties like surface area, volume, hydration energy, logP, refractivity, polarisability, mass, total energy etc. and other molecular properties were computed (Table-7). Log P is critical parameter as it gives information about how molecules cross the cell membrane and is important in receptor interactions in biological systems. The low Log P value in PCHHMMO indicates its hydrophilic nature.

**Table 7: QSAR and Molecular properties of PCHHMMO**

| **QSAR properties** | |
|---|---|
| Surface area(Aprox) | 388.01 Å $^2$ |
| Surface area(Grid) | 427.59 Å $^2$ |
| Volume | 658.49 Å $^3$ |
| Hydration energy | -16.54 kcal/mol |
| Log P | 1.56 |
| Refractivity | 55.08 Å $^3$ |
| Polarisability | 20.84 Å $^3$ |
| Mass | 218.64 amu |
| **Molecular properties** | |
| Total energy | -683354.75 kcal/mol |
| Dipole moment | 3.21 D |

**CONCLUSIONS**

The theoretical and experimental study on the title compound is informative in understanding various physicochemical aspects of compounds. The computed IR data is nearly in good agreement with experimental data. While the NMR data computed is in accordance with experimental data except with some disparity which is ascribable to hydrogen bonding interactions and solvent effects. The Log P value indicates greater hydrophilicity of molecule indicating more polar character and less infiltrating capacity through cell membrane. The HOMO and LUMO frontier orbitals computed for the ionized form of title compound indicate phenoxide oxygen and oxime nitrogen, as its potential binding sites with metal ions.

**Supplementary material**

Crystallographic data (including structure factor) for the reported structures have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 866656.

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