Synthesis and crystal structure of *N*'-hydroxy-4-methylbenzimidamide for biological activity

Sreedhara R [a], Vinaya K. [b*]

[a] Government First Grade College NH-206, B.H. Road Kadur.

[b] Government First Grade College,NH-206, B.H. Road Kadur.

ABSTRACT

Synthesis of the intermediate compounds N'-hydroxy-4-methylbenzimidamide and N'- hydroxy-4-methoxybenzimidamide by reacting 4-methylbenzonitrile and 4- methoxybenzonitrile respectively with hydroxylamine hydrochloride in the presence of sodium carbonate at 60-70^{o}C for 4 hrs the presence of oxime proton peak in ^{1}H-NMR and IR spectra confirmed the formation of compounds.

A series of 1-benzhydryl-sulfonyl-piperazine derivatives 12(a-g) were designed by a nucleophilic substitution reaction of 1-benzhydryl-piperazine with various sulfonyl chlorides and characterized by H nuclear magnetic resonance (NMR), Fouriertransformation infrared (FTIR) and elemental analysis. Our research is focused on identifying synthetically occurring chemotherapeutic substances capable of inhibiting, retarding or reversing the process of multistage carcinogenesis. The title compounds were evaluated for their efficacy in inhibiting MDA-MB-231 breast cancer cell proliferation. Compound 1-benzhydryl-4-(4-tert-butyl-benzenesulfonyl)-piperazine showed significant inhibitory activity.

Key words: NMR, FTIR, IR Spectra, Breast cancer.

1. Introduction

Heterocyclic compounds are organic compounds that contain a ring structure composed of at least one atom other than carbon. These non-carbon atoms, known as heteroatoms, can be nitrogen, oxygen, sulfur, or other elements. Heterocyclic compounds are incredibly diverse and play a significant role in various biological processes and pharmaceutical applications. Heterocyclic compounds can be either aromatic or non-aromatic. Common heterocyclic rings include pyridine (with nitrogen), furan (with oxygen), thiophene (with sulfur), and pyrrole (with nitrogen). The presence of heteroatoms in the ring influences the chemical reactivity and properties of these compounds. The heteroatoms can participate in hydrogen bonding, coordinate with metals, and engage in various chemical reactions, making heterocycles versatile building blocks in synthesis. Many natural products and biomolecules, such as nucleic acids, vitamins, and hormones, contain heterocyclic structures. Heterocycles are integral to the structure of DNA and RNA, with purines (adenine, guanine) and pyrimidines (cytosine, thymine, uracil) being crucial heterocyclic bases.

Heterocyclic compounds form the backbone of many pharmaceuticals, including antibiotics, antiviral drugs, anti-inflammatory agents, and anticancer medications [1, 2]. Examples include penicillin (a β-lactam antibiotic), quinine (an antimalarial compound), and many synthetic drugs like benzodiazepines (used as anxiolytics and sedatives) [3]. Heterocyclic compounds often act as enzyme inhibitors, blocking the activity of specific enzymes involved in disease processes. For instance, sulfa drugs, which contain a heterocyclic

sulfonamide group, inhibit bacterial enzyme dihydropteroate synthase. Many heterocyclic compounds interact with biological receptors to modulate physiological functions. An example is the heterocyclic compound morphine, which binds to opioid receptors to provide pain relief.

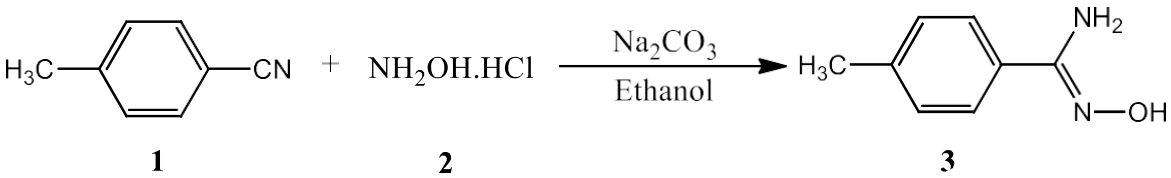
Certain heterocyclic compounds are used as diagnostic agents and imaging tools. Fluorescent heterocycles are employed in bioimaging to visualize cellular structures and track biological processes. Heterocyclic compounds are used in agriculture as pesticides, herbicides, and fungicides to protect crops from pests and diseases. Examples include pyrimidine-based herbicides like chlorotoluron and imidazole-based fungicides like prochloraz.

Researchers continuously develop new synthetic methods to create heterocyclic compounds with desired biological activities. Structural modifications of existing heterocycles can lead to compounds with improved efficacy, reduced side effects, and better pharmacokinetic properties [4]. High-throughput screening techniques are used to rapidly evaluate the biological activity of large libraries of heterocyclic compounds. This accelerates the discovery of new drug candidates and bioactive molecules.

2. Synthesis

For the synthesis of the key intermediate compound **(3)**, the reaction sequences outlined in**Scheme 1** were followed. *N*'-hydroxy-4-methylbenzimidamide **(3)** was synthesized by reacting 4-methylbenzonitrile **(1)** (1.0 eq) with hydroxylamine hydrochloride (2.5 eq) in presence of sodium carbonate (1.6 eq) at 60-70^{o}C for 4 hr. The presence of oxime proton peak in ^{1}H-NMR and IR spectra confirmed the formation of compound **3**.

Scheme 1



1 **2** **3**

3. Procedure for the synthesis of *N*'-hydroxy-4-methylbenzimidamide(3)

A solution of 4-methylbenzonitrile **(1)** (1 g, 5.644 mmol) was taken in methanol; hydroxylamine hydrochloride (0.980 g, 14.111 mmol) and sodium carbonate (0.957 g, 9.031 mmol) was added slowly under stirring condition. The reaction mixture was heated at 60-70^{o}C for 4 hr. Progress of the reaction was monitored by TLC. Upon completion, the solvent was removed under reduced pressure and residue was taken in water and extracted with ethyl acetate. The organic layer was washed with brine solution. Finally water wash was given to organic layer and dried with anhydrous sodium sulphate, the solvent was evaporated to get crude product which was purified by column chromatography over silica gel (60-120 mesh) using hexane: ethyl acetate (8:2) as eluent. Awhite amorphous solid compound with 88% yield was obtained. The product obtained was dissolved in ethyl acetate. White crystals were developed after 4 days due to the slow evaporation of the solvent.

M. P: 135 ^{0}C. ^{1}H NMR (DMSO-*d6*, 400 MHz) δ: 9.67 (bs, 1H, N-OH), 7.79 (d, 2H, Ar-H), 7.05 (d, 2H, Ar-H), 5.86 (bs, 2H, N-H), 2.29 (s, 3H, -CH3). IR (KBr, cm^{-1}): 1669, 1042, 1258. Anal.calcd. for C8H10 N2O2 (in %): C-63.98, H-6.71, N-18.65. Found C-63.94, H-6.73, N-18.68.

4. Crystallographic Analysis

Table-1: Crystal data and structure refinement Table

Empirical formula	$C_8H_{10}N_2O_1$
Formula weight (g mol^{-1})	600.72
Temperature (K)	296(2)
Wavelength (*Å*)	1.54178
Crystal system	Triclinic
Space group	$P\bar{1}$
Unit cell dimension	
a(*Å*)	6.4166(9)
b(*Å*)	14.948(2)
c(*Å*)	17.443(2)
α	104.500(6)
β	95.870(6)
γ	94.346(7)
Cell volume ($Å^3$)	1602.3(4)
Z	2
Calculated density (g cm^{-3})	1.245
Absorption coefficient (μ) (mm^{-1})	0.687
F(000)	640
Theta range for data collection (degree)	2.637 – 64.538
Index ranges	-7 < h < 7 -17 < k < 17 -20 < l < 20
Reflections collected	5195
Independent reflections	2266
Absorption correction	
Refinement method	Full-matrix least-square on F^2
Data/restraints/parameters	5195 / 0 / 402
Goodness of fit on F square	1.036
Final R indices [I> 2 sigma(I)]	0.0754
R indices (all data)	0.1650
Delta row max/delta row min ($e.Å^{-3}$)	
Extinction coefficient	0.0029(10)
Largest diff. peak and hole	0.396 and -0.341

Table-2: Fractional Atomic coordinates and equivalent thermal parameters of non-hydrogen atoms

C1A	0.79251	0.00170	0.92875	0.07675
C2A	0.66040	0.00445	0.85274	0.05260
C3A	0.72175	-0.03504	0.77890	0.05042
C4A	0.60444	-0.03177	0.70889	0.04471
C5A	0.41803	0.01060	0.70987	0.03616
C6A	0.35430	0.04862	0.78394	0.04876
C7A	0.47340	0.04600	0.85404	0.05633
C8A	0.29671	0.01619	0.63442	0.03924
N9A	0.39442	0.00441	0.57276	0.05504
N10A	0.09283	0.03272	0.63298	0.05873
O11A	0.25859	0.01266	0.50526	0.06752
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C1B	0.26445	0.25743	0.43491	0.08280
C2B	0.14165	0.25025	0.35486	0.05457
C3B	0.22843	0.28632	0.29761	0.04933
C4B	0.11573	0.28129	0.22433	0.04373
C5B	-0.09005	0.23695	0.20512	0.03570
C6B	-0.17867	0.20014	0.26139	0.04800
C7B	-0.06528	0.20773	0.33508	0.05349
C8B	-0.21197	0.23111	0.12617	0.03753
N9B	-0.10757	0.25105	0.07225	0.05108
N10B	-0.42394	0.20672	0.11439	0.05555
O11B	-0.24654	0.24481	0.00141	0.06426
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C1C	1.26341	0.75814	0.43462	0.08222
C2C	1.14025	0.74985	0.35418	0.05027
C3C	1.22942	0.78644	0.29753	0.05052
C4C	1.11795	0.78046	0.22407	0.04343
C5C	0.91089	0.73732	0.20448	0.03795
C6C	0.82368	0.70079	0.26149	0.04712
C7C	0.93702	0.70741	0.33528	0.05741
C8C	0.79147	0.73179	0.12610	0.03906
N9C	0.89227	0.75098	0.07179	0.05248
N10C	0.58195	0.70792	0.11458	0.05803
O11C	0.75299	0.74584	0.00102	0.06389
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C1D	0.20780	0.49821	0.07194	0.08073
C2D	0.33920	0.49579	0.14750	0.05187
C3D	0.27852	0.53488	0.22146	0.04938
C4D	0.39666	0.53200	0.29138	0.04299
C5D	0.64648	0.45152	0.21612	0.03590
C6D	0.52713	0.45464	0.14651	0.04621
C7D	0.58188	0.48919	0.28995	0.05574
C8D	0.70269	0.48361	0.36520	0.03657

N9D	0.60617	0.49552	0.42721	0.05453
N10D	0.90735	0.46734	0.36683	0.05914
O11D	0.74119	0.48713	0.49468	0.06948

Table-3: Selected Bond Lengths in *Å*

Atom(I)	Atom(J)	Observed	Calculated

O11A	- N9A	1.4263	1.4316
N9A	- C8A	1.2835	1.2965
N10A	- C8A	1.3498	1.3630
C1A	- C2A	1.5101	1.5156
C2A	- C3A	1.3891	1.4024
C2A	- C7A	1.3876	1.4012
C3A	- C4A	1.3766	1.3816
C4A	- C5A	1.3927	1.4063
C5A	- C6A	1.3910	1.4043
C5A	- C8A	1.4818	1.4872
C6A	- C7A	1.3816	1.3866

Table-4: Selected Bond angles (in degrees)

O11A - N9A - C8A	110.47	C1A - C2A - C3A	120.75
C1A - C2A - C7A	121.76	C3A - C2A - C7A	117.49
C2A - C3A - C4A	121.16	C3A - C4A - C5A	121.59
C4A - C5A - C6A	117.14	C4A - C5A - C8A	120.99
C6A - C5A - C8A	121.87	C5A - C6A - C7A	121.18
C2A - C7A - C6A	121.41	N9A - C8A - N10A	123.13
N9A - C8A - C5A	116.86	N10A - C8A - C5A	120.02
N9A - O11A- H11A	109	C8A - N10A - H10E	120
C8A - N10A- H10F	120	H10E - N10A - H10F	120
C2A - C1A - H1A1	109	C2A - C1A - H1A2	109
C2A - C1A - H1A3	109	H1A1- C1A - H1A2	109
H1A1 - C1A - H1A3	109	H1A2- C1A - H1A3	109
C2A - C3A - H3A	119	C4A - C3A - H3A	119
C3A - C4A - H4A	119	C5A - C4A - H4A	119
C5A - C6A - H6A	119	C7A - C6A - H6A	119
C2A - C7A - H7A	119	C6A - C7A - H7A	119

Table-5: Selected Torsion angles (in degrees)

C1A	C2A	C3A	C4A	-179.13	C7A	C2A	C3A	C4A	1.05
C1A	C2A	C7A	C6A	179.50	C3A	C2A	C7A	C6A	-0.68
C2A	C3A	C4A	C5A	-0.04	C3A	C4A	C5A	C6A	-1.33
C3A	C4A	C5A	C8A	178.22	C4A	C5A	C6A	C7A	1.70
C8A	C5A	C6A	C7A	-177.85	C4A	C5A	C8A	N9A	-19.05
C4A	C5A	C8A	N10A	160.94	C6A	C5A	C8A	N9A	160.48
C6A	C5A	C8A	N10A	-19.53	C5A	C6A	C7A	C2A	-0.72
N10A	C8A	N9A	O11A	0.55	C5A	C8A	N9A	O11A	-179.47

C8A	N9A	O11A	H11A	180	H1A1	C1A	C2A	C3A	56
H1A1	C1A	C2A	C7A	-124	H1A2	C1A	C2A	C3A	176
H1A2	C1A	C2A	C7A	-4	H1A3	C1A	C2A	C3A	-64
H1A3	C1A	C2A	C7A	116	C1A	C2A	C3A	H3A	1
C7A	C2A	C3A	H3A	-179	C1A	C2A	C7A	H7A	0
C3A	C2A	C7A	H7A	179	C2A	C3A	C4A	H4A	180
H3A	C3A	C4A	C5A	180	H3A	C3A	C4A	H4A	0
H4A	C4A	C5A	C6A	179	H4A	C4A	C5A	C8A	-2
C4A	C5A	C6A	H6A	-178	C8A	C5A	C6A	H6A	2
C5A	C6A	C7A	H7A	179	H6A	C6A	C7A	C2A	179
H6A	C6A	C7A	H7A	-1	N9A	C8A	N10A	H10E	0
N9A	C8A	N10A	H10F	-180	C5A	C8A	N10A	H10E	-180
C5A	C8A	N10A	H10F	0					

Table-6: Hydrogen Bond Geometry

No.	Type	Donor --- H....Acceptor	D - H	H...A	D...A	D - H...A
1	Intra	N10C --H10A ….O11C	0.86	2.20	2.5281	102
2		N10C --H10A O11B	0.86	2.27	3.0424	149'
3	Intra	N10B --H10A ….O11B	0.86	2.21	2.5330	102
4		N10B --H10A O11C	0.86	2.26	3.0254	149'
5	Intra	N10D --H10C ….O11D	0.86	2.20	2.5279	102
6		N10D --H10C ..O11D	0.86	2.25	3.0345	151'
7	Intra	N10A --H10E ….O11A	0.86	2.20	2.5283	102
8		N10A --H10E O11A	0.86	2.25	3.0316	151'
9		O11A --H11A N9A	0.82	2.00	2.7163	146
10		O11B --H11B ….N9C	0.82	2.01	2.7132	143
11		O11C --H11C ….N9B	0.82	2.04	2.7293	142
12		O11D --H11D N9D	0.82	2.01	2.7287	146

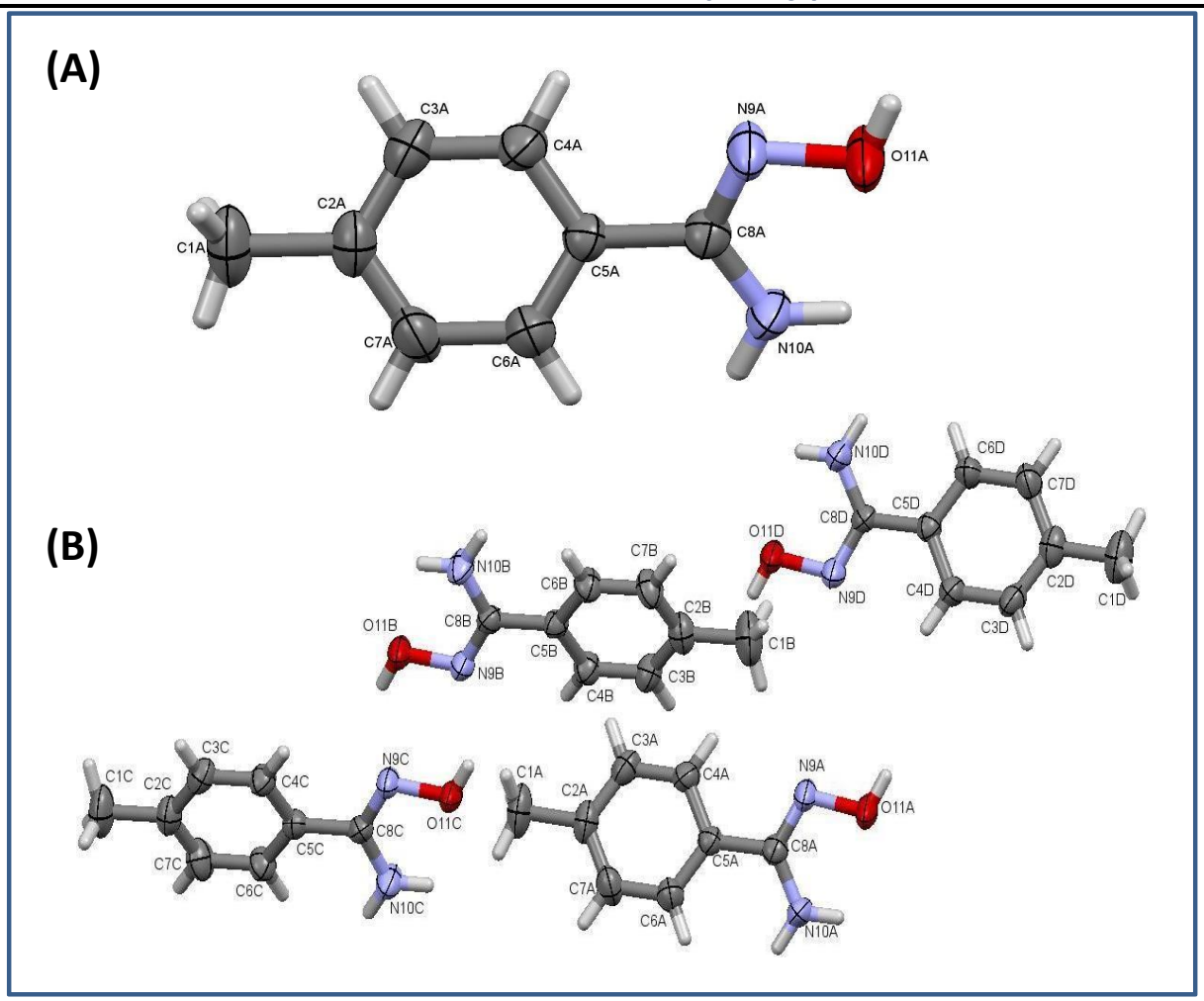

Figure-1 **(A-B) The ORTEP of the molecule with thermal ellipsoids of atoms drawnwith a probability of 50 %**

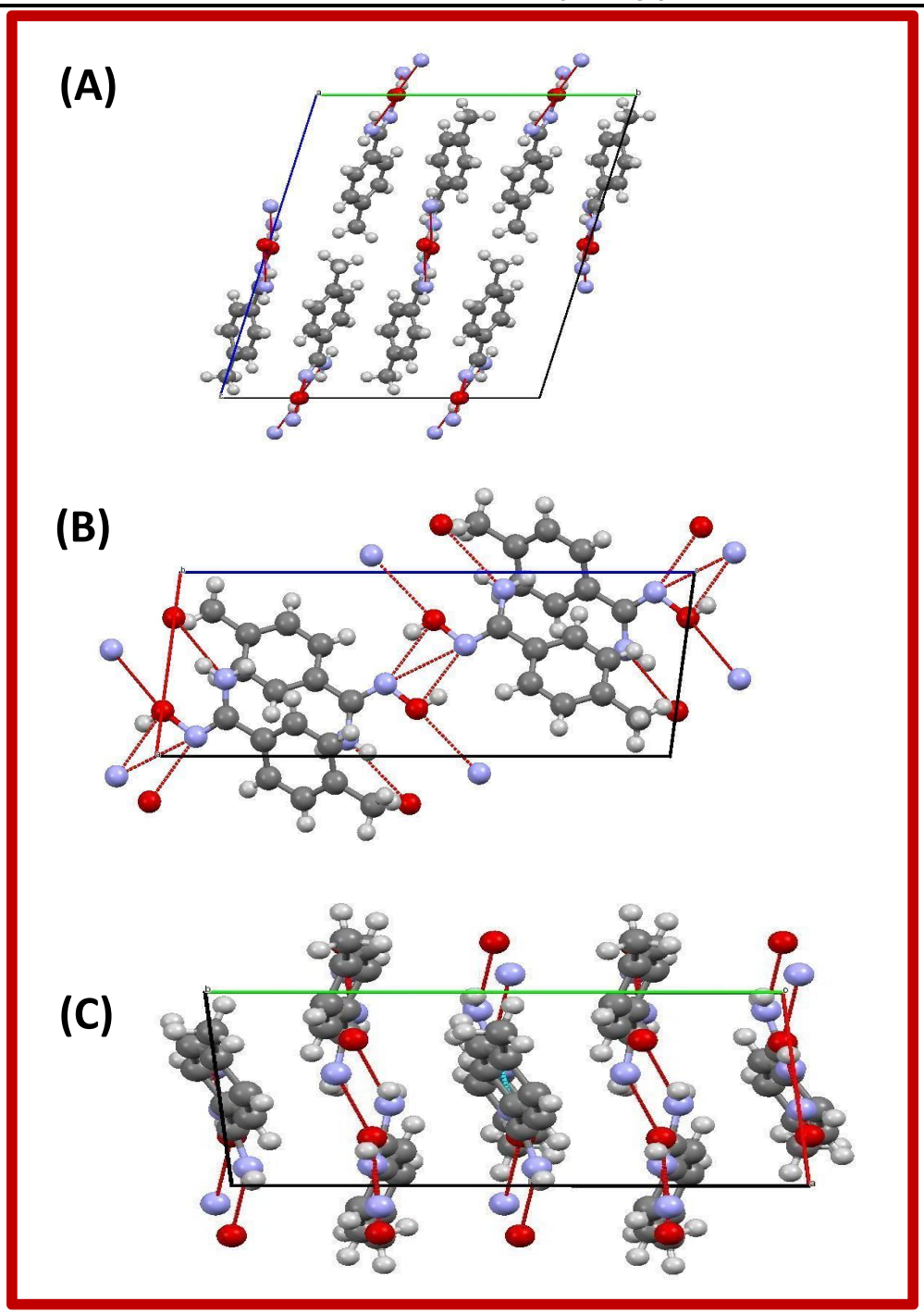

Figure-2 (A, B & C): The packing diagram of the molecule as viewed along a-axis, b-axis andc-axis respectively. The dashed red lines represent the hydrogen bonds.

A disc shaped crystal of dimension 0.2x0.2x0.2 mm^3 was chosen for x-ray diffraction study. X-ray intensity measurements were done using Bruker single crystal x-ray diffractometer. The crystal to detector distance was maintained at 46 mm. Space group of the crystal was detected and the lattice parameters were estimated using initially collected 24 frames of data. The complete data was collected with 0.5 degree rotation between the limits specified in Table-1 and three second of exposure per frame.

The cell refinement and data reduction is carried out using APEX2 (Bruker, 2013). The data reduction was also done with SAINT program. All frames could be indexed with the triclinic primitive lattice. The structure was solved by direct methods using SHELXS. All the non hydrogen atoms were revealed in the first Fourier map itself. Full-matrix least squares refinement method was employed using SHELXL. The

refinement was carried out using 2266 unique reflections out of 5195 reflections collected, which converged to residual value 7.54% and goodness of fit 1.036.

ORTEP diagram of the title molecule is plotted using MERCURY is as shown in **Figure 1.** Bond lengths and bond angles agree very well with the usual standard values. And we observed that the bond distance of C3-C5 (1.4818) is slightly more than the standard C-C bond, due to the presence of oxime group which may result in delocalization of electrons. This behavior has been observed in similar structures. The torsional angle of -19.05 and -19.53 between N(9)C(8)C(5)C(4) and N(10)C(8)C(5)C(6) respectively shows negative syn- periplanar confirmation. The torsional angle of -0.55 degree between O(11)N(9)C(8)N(10)also exhibits negative syn-periplanar confirmation. The torsional angle of C(5)C(8)N(9)O(11) is -179.47 has negative anti-periplanar confirmation. Above torsion angles suggest that the plane of oxime group is parallel with the plane of benzene ring.

The crystal structure possesses both intermolecular and intra-molecular hydrogen bonds. The observed hydrogen bonds are tabulated in **Table-6**. The packing diagram of the molecule as observed down the *a*-axis, *b*-axis and *c*-axis are shown in **Figure-3, 4** and **5** respectively. From the packing diagram, one can observe that molecules linked through hydrogen bonds form a pair wise chain like structure.

The crystal data and refinement statistics are summarized in **Table-1**. Bond lengths and bond angles of the molecule are listed in **Table-3 and 4 respectively.** The torsional angles are given in the **Table-5**. The hydrogen bond geometry is given in **Table-6**. The final atomic coordinates and equivalent thermal parameters of the non-hydrogen atoms are listed in **Table-2**. The bond lengths and bond angles are in good agreement with the standard values.

5. Results and discussion

The presence of N–H proton N-OH proton at 5.86 □ and 9.64 □ value in *N*'-hydroxy-4-methoxybenzimidamide and the absence of this proton peak in proton NMR spectra confirms the formation of product **(3)**. The oxime group is almost parallel to the benzene ring with an angle about ±(0.55 to 0.53) degree. The bond connecting the benzene ring and oxime group is elongated due to delocalization of electrons caused by oxime group [5]. Bond lengths and bond angles are agree with the similar related structures

6. Conclusion

Heterocyclic compounds are a cornerstone of medicinal chemistry and play an indispensable role in modern drug discovery and development. Their unique structural characteristics and diverse biological activities make them invaluable in the design of therapeutics, diagnostics, and agricultural chemicals. On-going research into heterocyclic chemistry continues to expand our understanding and utilization of these compounds in various biological applications.

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