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CRYSTAL STRUCTURE OF ISATIN ANCHORED AZOMETHINE LIGAND: DFT AND MOLECULAR DOCKING STUDIES ON 5HFZ PROTEIN

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ABSTRACT
A novel azomethine compound was formulated *via* the condensation of Isatin and 3-Isopropylaniline. The synthesised azomethine compound was characterised by spectral analysis, mass spectrometry and SCXRD. DFT calculations showed that the energy gap between the highest occupied and lowest empty orbitals is 2.8471 eV, which means the compound is moderately reactive and stable. The calculated electronegativity (4.3029 eV) and chemical potential (–4.3029 eV) indicate a strong tendency to attract electrons and are thermodynamically stable. Docking analysis revealed that the compound IPSB has a potential binding tendency with the protein 5FHZ.
Keywords: Azomethine, Isatin, SCXRD, DFT, Docking.
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INTRODUCTION

Azomethine compounds are synthesised using a primary amine with aryl or aliphatic aldehydes or ketones. Azomethine compounds, commonly known as Schiff bases, were first synthesised in 1864 by Hugo Schiff. They have wide applications in pharmaceutical, agricultural and industrial fields. In 1841, Erdmann and Laurent isolated Isatin from indigo. Since then many research has concentrated and produced more compounds from isatin due to their high potential biological properties.[1-5] Rajaram *et al.* (2010) reported azomethine compound from *p*-phenylenediamine, and Isatin showed analgesic activity.[6] Savitha *et al.* (2023) reported 20 isatin-derived azomethine compounds that were explored the antioxidant properties, which were confirmed by *in vitro* and *insilico* studies.[7] Meshram *et al*. (2025) examined two sets of azomethine compounds that were reported to have antitumor activities.[8] The researchers have reported that various isatin-based Schiff bases have been described in many pharmaceutical applications and drug discovery. The very long history and frequently reported high potential properties of isatin make interested to create a simple system of compounds from isatin. In this work, we formulated a novel azomethine compound from Isatin and 3-Isopropylaniline and examined the structural formation through spectral analysis and biological activity by *insilico* methods.

EXPERIMENTAL

Material and Methods

Isatin, 3-Isopropylaniline, and other solvents were purchased from Hyma Synthetic Chemicals. FT-IR recorded on Shimadzu using a KBr pellet (4000-400 cm^{-1}). NMR recorded in $CDCl_3$ on Bruker, Avance III 400MHz, UV recorded on Specord 210 plus spectrometer, HRMS recorded in Waters USA, XEVO-G2-XS-QTOF. SCXRD recorded on Bruker D8 QUEST FIXED CHI diffractometer, DFT performed by Gaussian 09 software.

General Procedure

1.1371 g (7.7 mmol) of Isatin and 1.0450 g (7.7 mmol) of 3-Isopropylaniline were mixed with ethanol and refluxed for 4 hours. TLC monitoring confirmed the formation of the new compound. The red-orange clear solution was filtered and cooled. Upon cooling, the azomethine compound precipitated, filtered out, washed with hot ethanol, and recrystallised with ethanol. Crystals for SCXRD studies are obtained in ethanol solvent within a week. The scheme of the azomethine compound was represented in Fig.-1. The

newly synthesised compound was designated as IPSB. The compound is soluble in ethanol, methanol, $CHCl_3$, acetonitrile, DMF and DMSO. Yield: 84%, m.p. 185 °C.

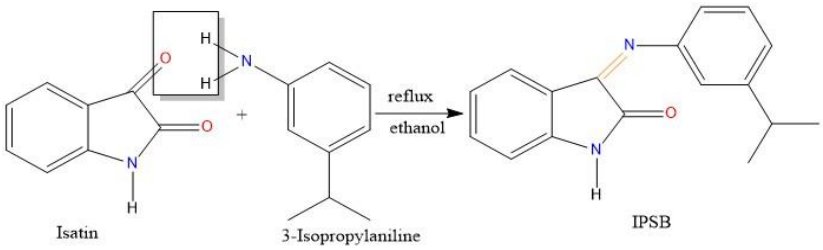

Fig.-1: Scheme of Formulation of IPSB

RESULTS AND DISCUSSION

The synthesised IPSB was characterised and confirmed by the spectral analysis. From FT-IR (cm^{-1}), the azomethine is confirmed by the 1661 (-C=N-), 2868 – 2961 (isopropyl -C-C-, -C-H), 1746 (-C=O) and 3459 (-N-H). UV-Visible results showed absorption bands at 232, 428 and 454 nm due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ corresponding to the presence of aromatic π bonds and hetero atoms like O and N. From ^1H NMR, the chemical shift values 9.841 ppm (s, 1H, -NH), 6.632to 6.688 ppm (q, 2H, aromatic H), 5.794 to 6.814 ppm (d, 1H, aromatic H), 6.853 to 6,879 ppm (d, 1H, aromatic H), 6.898 ppm (s, 1H, phenyl H), 7.03 to 7.05 ppm (d, 1H, phenyl H), 7.189 to 7.302 (m, 2H, aromatic H), 2.831 to 2.900 ppm (m, 1H, isopropyl), 1.176 and 1.193 (d, 6H, methyl H) justifies the total number of protons.

From ^{13}C NMR,23.91 ppm corresponds to aliphatic -$CH(CH_3)_2$, 34.15 ppm is assigned for aliphatic -$CH(CH_3)_2$,111.93 to 134.28 ppm for aromatic C, 150.05 and 150.42 ppm show amide carbon, 165 ppm for imine carbon. Mass spectrometry, the m/z $[M+H]^+$265.13 had a good agreement with the calculated mass 264.13. The structure was examined and confirmed by SCXRD analysis.

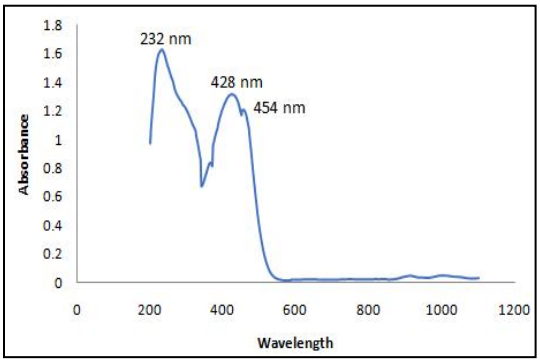

Fig.-2: UV-Vis for IPSB

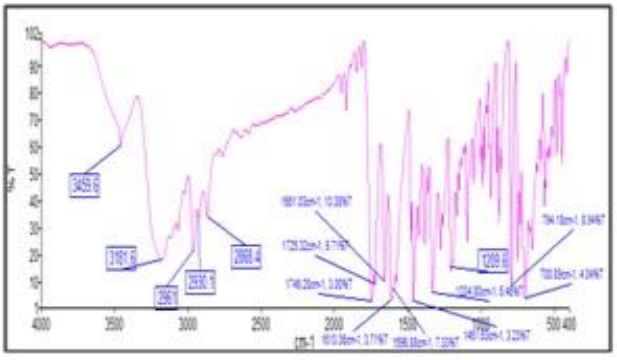

Fig.-3: FT-IR for IPSB

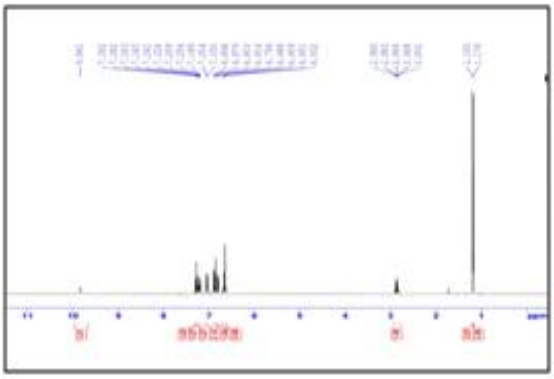

Fig.-4: ^1H NMR for IPS B

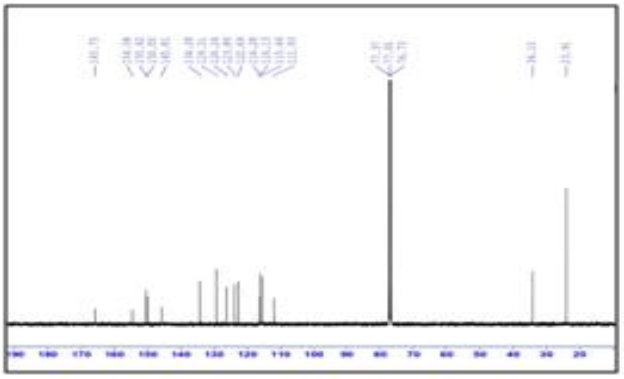

Fig.-5: ^{13}C NMR for IPSB

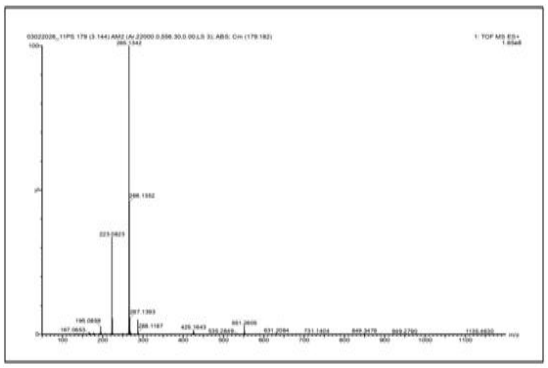

Fig.-6: Mass Spectrum for IPSB

SCXRD

IPSB is a red, block-shaped crystal. At 303(2) K and 0.71073 Å, radiation was maintained and analyzed in a Bruker D8 QUEST diffractometer. Using SAINT V8.41, SHELXT 2018/2 full-matrix least-squares methods revealed 32342 reflections, of which 3451 were independent reflections. Intrinsic Phasing methods were used to solve and refine the crystal structure. CCDC number 2532398. Crystal data and structural fine-tune of the IPSB at 303 K revealed that the size of the crystal is 0.157 × 0.247 × 0.432 mm^3, Monoclinic crystal system, $P2_1/c$ (14) space group (number), unit cell parameters intercepts and angles a = 13.2608 Å, b = 9.7316 Å, c = 10.8398 Å, α = β = 90° and γ = 97.659, the number of molecule in one unit cell (Z) is 4, density 1.266 gcm^{-3}, $C_{17}H_{16}N_2O$ molecular formula, 264.32 molecular mass, the volume 1386.4 $Å^3$, absorption coefficient μ= 0.08 mm^{-1}, Bragg's diffracting angle 2θ range 5.21 to 56.63 (0.75 Å), collected reflection 32342 and extinction coefficient 0.020.

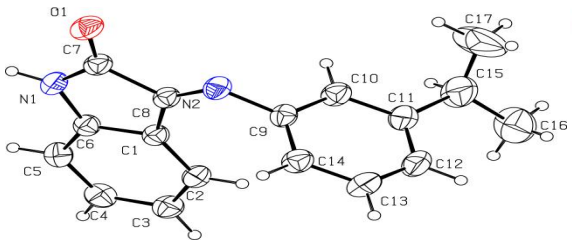

Fig.-7: Atom Numbering Scheme of IPSB

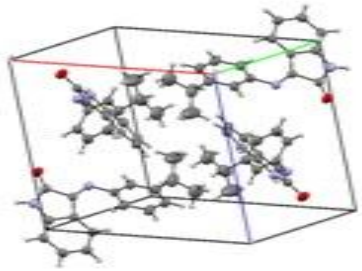

Fig.-8: Crystal Packing of IPSB

Table-1: Selected Bond Length and Bond Angle of IPSB from SCXRD

Atom–Atom	Length [Å]		Atom–Atom–Atom	Angle [°]	
N_2–C_8	1.273(3)		C_7–N_1–C_6	111.20(17)	
N_2–C_9	1.426(3)		C_8–N_2–C_9	118.99(18)	
C_{15}- C_{17}	1.401(6)		N_2–C_8–C_1	135.23(19)	
C_{15}-C_{16}	1.444(5)				

Frontier Molecular Orbitals

The FMO provides significant insight into the reactivity and stability of the investigated molecule.[9] The calculated HOMO and LUMO energies were found to be –5.7264 eV and –2.8793 eV, yielding an energy difference (ΔE) of 2.8471 eV. A relatively moderate HOMO–LUMO gap suggests enhanced intramolecular charge transfer and potential biological activity. It denotes moderate chemical reactivity and good kinetic stability of the compound. The ionisation potential (5.7264 eV) and electron affinity (2.8793 eV) were derived using Koopmans' theorem, confirming the molecule's balanced electron-donating and electron-accepting abilities. The calculated electronegativity (4.3029 eV) and chemical potential (–4.3029 eV) indicate a strong tendency to attract electrons and thermodynamic stability. Furthermore, the chemical hardness in eV (η) 1.4236 and softness (σ) 0.3512 eV values suggest that the molecule possesses optimal stability with sufficient reactivity for intermolecular interactions. The electrophilicity index (6.5029 eV) reveals significant electrophilic character, implying its ability to participate effectively in charge transfer processes and molecular binding interactions. The detailed

quantum chemical parameters are displayed in Table-2 and Fig.-9 indicated the HOMO–LUMO energy diagram of IPSB.

Table-2: The Calculated Frontier Molecular Orbital of IPSB

Parameters	Values	Parameters	Values
HOMO (eV)	-5.7264	Energy gap(eV)	2.8471
LUMO (eV)	-2.8793	EN	4.3029
IP	5.7264	Chemical potential	-4.3029
EA	2.8793	Chemical hardness	1.4236
Chemical softness	0.3512	Electrophilicity index	6.5029

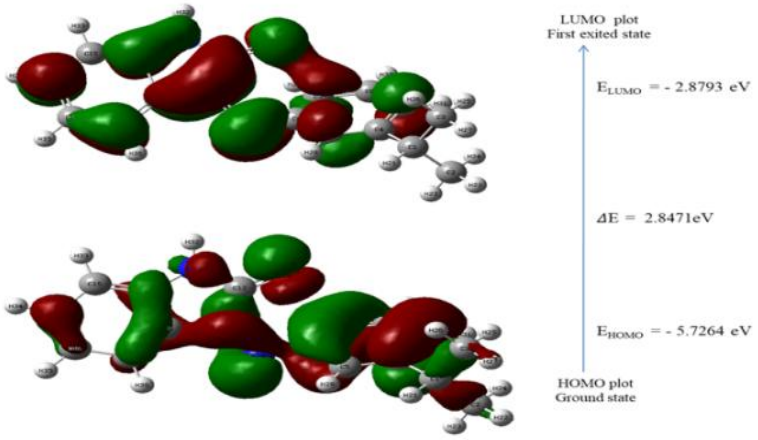

Fig.-9: Frontier Molecular Orbitals Structure of IPSB

Surface MEP

The MEP surface was mapped to identify the reactive sites and charge distribution of the optimised molecule.[10] The MEP map ranges from –6.140 × 10^{-2} to +6.140 × 10^{-2} a.u., where the red region represents electron-rich areas, while the blue region corresponds to electron-deficient regions. The pronounced negative potential observed around the electronegative heteroatoms, particularly oxygen and nitrogen, indicates a favourable area for electrophilic attack, whereas the positive potential regions confined over hydrogen atoms hint at possible nucleophilic attack. The green regions represent neutral potential zones, indicating balanced charge distribution over the aromatic framework. Such charge separation supports intramolecular charge transfer and enhances intermolecular interaction capability, which is crucial for molecular recognition and biological binding mechanisms. The MEP surface analysis (Fig.-10) thus confirms the presence of distinct electrophilic and nucleophilic reactive centres within the molecule.

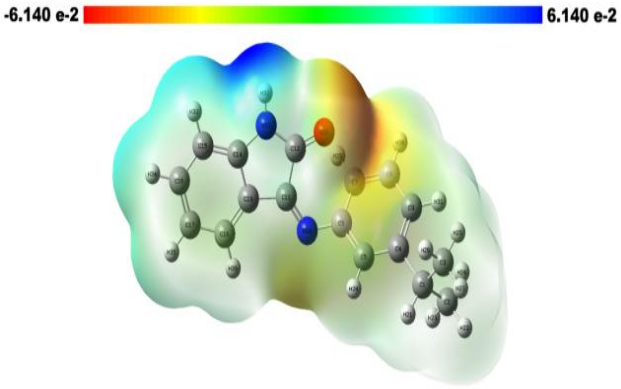

Fig.-10: MEP of IPSB

Mulliken Atomic Charges

The Mulliken atomic charge distribution analysis was performed to understand the electronic charge localisation within the optimised molecule.[11] The calculated atomic charges reveal significant charge separation across the molecular framework, indicating possible reactive centres. Notably, carbon atom C19 exhibits a high positive charge (1.2219), suggesting strong electron deficiency and its potential role as an electrophilic centre. In contrast, several carbon atoms such as C14 (–0.7955), C15 (–0.7866), and C6 (–0.6915) display substantial negative charge densities, indicating electron-rich regions favourable for electrophilic attack. The oxygen atom (O20) carries a negative charge (–0.2299), confirming its electron-withdrawing nature and participation in charge delocalisation. Nitrogen atoms show differentiated behaviour, with N10 (0.2053) exhibiting positive character and N13 (–0.1671) showing slight negative character, reflecting intramolecular charge transfer effects. Hydrogen atoms generally possess positive charges, consistent with their electropositive nature. The observed charge distribution supports the presence of distinct nucleophilic and electrophilic reactive sites, thereby influencing molecular stability, intermolecular interactions, and biological activity. The Fig.-11 represents the detailed Mulliken atomic charge values and the graphical representation of charge distribution.

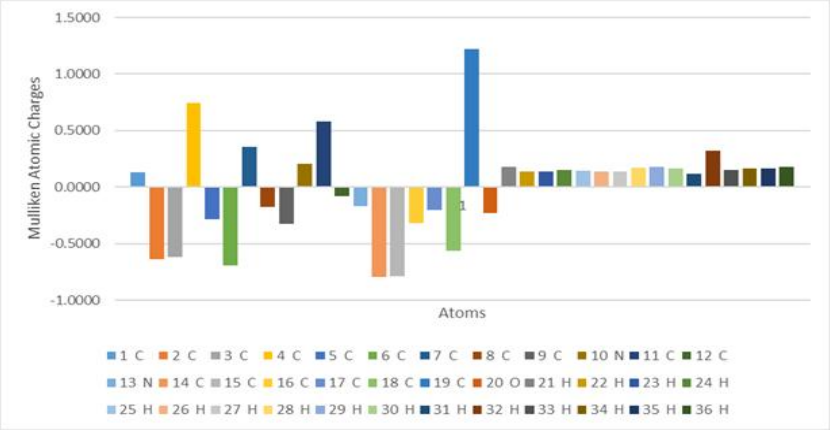

Fig.-11: The Histogram of Mulliken Atomic Charges

Molecular Docking Analysis

The molecular docking assay of IPSB with the protein target 5FHZ revealed a stable binding orientation characterised by a significant hydrogen bonding interaction with the active site residue ASN469, as summarized in illustrated in Fig.-12. The crystal structure of human ALDH1A3 (PDB ID: 5FHZ) provides the structural basis for understanding ligand accommodation within the catalytic pocket. The ligand forms a strong H---O bond with ASN469 at 2.0 Å bond length, which falls within the optimal range for effective hydrogen bond stabilisation and indicates proper geometrical alignment inside the binding cavity. Hydrogen bonds in the range of 1.8–2.5 Å are known to contribute significantly to protein–ligand stability by providing favourable enthalpy interactions. The calculated binding energy of −7.17 kcal·mol^{-1} reflects a thermodynamically favourable interaction consistent with micromolar affinity predictions in molecular docking studies. The intermolecular energy value of −7.77 kcal·mol^{-1} suggests that electrostatic interactions, physical force, and hydrogen bonding stabilise the docked complex. The estimated inhibition constant (Ki) of 5.53 µM further supports moderate binding affinity toward the 5FHZ target, as docking-derived Ki values are commonly correlated with predicted free binding energies. In addition to hydrogen bonding, complementary hydrophobic interactions within the surrounding active site residues likely contribute to the overall stabilisation of the complex, as reported in structure-based drug design studies. Although the reference RMSD value is reported as 0.9769 Å, unusually high RMSD values in docking outputs may arise due to reference pose misalignment and should therefore be interpreted cautiously. Overall, the results presented in Fig.-12 confirm that ASN469 plays a significant role in ligand recognition and stabilisation within the 5FHZ protein target, suggesting its importance for further inhibitor optimisation and experimental validation.

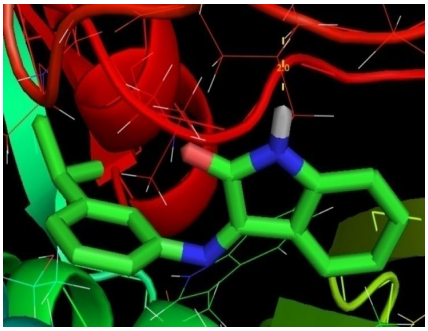

Fig.-12: Hydrogen Bonding and Molecular Docking with Protein Target (5FHZ)

CONCLUSION

A successfully synthesized azomethine IPSB compound was structurally elucidated by FT-IR, UV, NMR, SCXRD and good agreement with mass spectrometric analysis. Computational studies revealed the potential electrostatic surface in the molecule. The docking pose clearly demonstrates the spatial orientation of the ligand within the active pocket and highlights ASN469 as the key interacting residue responsible for hydrogen bond formation. It plays a significant role in ligand recognition and stabilisation within the 5FHZ protein target, suggesting its importance for further inhibitor optimisation and experimental validation. This work creates a path for further inhibitor optimisation and experimental confirmation, aiding effective drug design.

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CONFLICT OF INTERESTS

The authors declared that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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