Design and Synthesis of some novel aromatic compounds for biological evaluation using schiff base synthesis method

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***Abstract*—A series of novel isatin-based hydrazone derivatives were synthesized through condensation reactions involving isatin-3-carbohydrazide and substituted aromatic aldehydes. Three derivatives, namely (E)-N′-benzylidene-2-oxoindoline-3-carbohydrazide (D1), (E)-N′-(4-chlorobenzylidene)-2-oxoindoline-3-carbohydrazide (D2), and (E)-N′-(4-methoxybenzylidene)-2-oxoindoline-3-carbohydrazide (D3), were successfully prepared using a simple reflux method in ethanol with glacial acetic acid as catalyst. The synthesized compounds were obtained in good yields ranging from 76.44% to 77.26% and exhibited high melting points, indicating excellent thermal stability and purity. Structural characterization was carried out using Thin Layer Chromatography (TLC), Fourier Transform Infrared (FTIR) spectroscopy, Proton Nuclear Magnetic Resonance (^1H NMR) spectroscopy, and Mass Spectrometry. FTIR spectra confirmed the formation of the azomethine (C=N) linkage and the presence of characteristic functional groups, while ^1H NMR spectra verified the aromatic framework and substituent-specific proton environments. Mass spectral analysis revealed molecular ion peaks corresponding to the calculated molecular weights of the synthesized compounds, confirming their molecular structures. TLC analysis demonstrated the formation of single, well-defined spots, indicating successful synthesis and purification. The influence of electron-withdrawing (chloro) and electron-donating (methoxy) substituents on physicochemical properties and spectral behavior was also investigated. The results demonstrated that structural modifications significantly affected thermal stability and electronic characteristics. Owing to their stable hydrazone framework and conjugated aromatic system, these derivatives represent promising candidates for further studies involving fluorescence properties, biological evaluation, and medicinal chemistry applications.**

***Index Terms*—Isatin, Schiff Base, Hydrazone Derivatives, Azomethine Linkage**

I. INTRODUCTION

Schiff bases represent an important class of organic compounds characterized by the presence of an azomethine (–C=N–) functional group formed through the condensation of primary amines with aldehydes or ketones. Since their first report by Hugo Schiff in 1864, these compounds have attracted considerable attention owing to their structural diversity, facile synthesis, and broad spectrum of biological and physicochemical properties. The incorporation of aromatic and heterocyclic moieties into Schiff base frameworks enhances molecular conjugation, resulting in improved stability, electronic delocalization, and the ability to interact effectively with biological and chemical systems.

Among various heterocyclic scaffolds employed in medicinal chemistry, isatin (1H-indole-2,3-dione) occupies a unique position because of its diverse pharmacological activities and favorable electronic characteristics. Isatin-derived Schiff bases and hydrazone derivatives have been extensively investigated for their antimicrobial, antioxidant, anticancer, antiviral, anti-inflammatory, and enzyme inhibitory activities. The biological activity of these compounds is largely attributed to the presence of the azomethine linkage, which facilitates interactions with biological macromolecules such as proteins, enzymes, nucleic acids, and cellular receptors. Furthermore, the aromatic framework contributes to enhanced lipophilicity and membrane permeability, thereby improving biological efficacy.

In addition to their medicinal importance, Schiff base derivatives have emerged as attractive fluorescent materials due to their extended π-conjugated systems and tunable electronic properties. The presence of electron-donating and electron-withdrawing substituents significantly influences intramolecular charge transfer processes, resulting in distinctive absorption and emission characteristics. Such compounds have found applications in fluorescence sensing, molecular recognition, bioimaging, metal ion detection, and optoelectronic devices. The fluorescence behavior of Schiff bases can be further enhanced through structural rigidification, increased conjugation length, and incorporation of heteroatoms capable of participating in charge-transfer interactions. Recent advances in fluorescence chemistry have demonstrated that Schiff base fluorophores exhibit high sensitivity toward environmental changes such as solvent polarity, pH, and metal ion concentration. Their ease of synthesis and structural modification make them ideal candidates for the development of fluorescent probes and chemosensors. In particular, isatin-based Schiff bases possess favorable photophysical characteristics owing to the presence of both electron-rich and electron-deficient centers within the molecular framework. These features facilitate efficient π→π* and n→π* electronic transitions, which are essential for fluorescence emission.

Another significant aspect of Schiff base chemistry is their ability to act as multidentate ligands toward transition metal ions. Coordination of Schiff bases with metal ions frequently leads to remarkable changes in fluorescence intensity and emission wavelength, enabling their application as fluorescence-based sensing platforms. Moreover, metal complexation often enhances biological activity through the chelation effect, thereby providing multifunctional molecules possessing both therapeutic and analytical utility.

Despite extensive investigations on Schiff base derivatives, the development of novel aromatic compounds with combined fluorescence and biological properties remains an active area of research. The increasing prevalence of antimicrobial resistance and the demand for multifunctional molecular systems necessitate the exploration of new Schiff base architectures with improved physicochemical and biological profiles. Rational structural modification of aromatic and heterocyclic frameworks offers opportunities to optimize both fluorescence performance and biological activity.

II. MATERIALS AND METHODS

2.1. Materials

All chemicals and reagents used in this study were of analytical grade and used without further purification. Isatin (1H-indole-2,3-dione), benzohydrazide, p-chlorobenzaldehyde, cobalt salts, and nickel salts were procured from Loba Chemie (Mumbai, India). Ethanol, methanol, dimethyl sulfoxide (DMSO), and glacial acetic acid were purchased from Finar Chemicals (Ahmedabad, India). Deionized water was used throughout the study. All reagents were stored under appropriate laboratory conditions to maintain their purity and stability.

2.2. Instrumentation

Fourier-transform infrared (FTIR) spectra were recorded on a Shimadzu FT-IR 8400 spectrophotometer using the KBr pellet technique in the range of 4000–400 cm^{-1}. Proton nuclear magnetic resonance (^1H NMR) spectra were recorded on a Bruker Avance II 400 MHz spectrometer using DMSO-d_6 as solvent and tetramethylsilane (TMS) as the internal standard. Mass spectral analyses were performed using electron ionization (EI) mode at 70 eV. UV–Visible absorption measurements were carried out using a UV–Visible spectrophotometer. Fluorescence emission spectra were recorded at ambient temperature using a fluorescence spectrophotometer equipped with xenon lamp excitation.

2.3. Synthesis of Schiff Base Ligand

Isatin (1.47 g, 10 mmol) was dissolved in hot ethanol (25 mL) in a 100 mL round-bottom flask. In a separate vessel, 2-aminophenol (1.09 g, 10 mmol) was dissolved in ethanol (15 mL). The ethanolic solution of 2-aminophenol was added dropwise to the isatin solution under continuous stirring. Glacial acetic acid (2–3 drops) was added as a catalyst, and the reaction mixture was refluxed at 78 °C for 2–3 h.

The progress of the reaction was monitored by thin-

layer chromatography (TLC). Upon completion, the reaction mixture was cooled to room temperature to obtain a colored precipitate. The product was filtered, washed with cold ethanol, recrystallized from DMSO, dried in a desiccator over silica gel, and stored for further characterization.

2.4. Synthesis of Isatin-3-Benzoylhydrazone (Derivative 1)

Isatin (0.147 g, 1 mmol) was dissolved in absolute ethanol (20 mL), followed by the addition of benzohydrazide (0.136 g, 1 mmol). Glacial acetic acid (2–3 drops) was added as a catalyst, and the reaction mixture was refluxed for 4 h. After cooling to room temperature, the resulting precipitate was filtered, washed with cold ethanol, and recrystallized from ethanol to afford pure Isatin-3-benzoylhydrazone.

III. RESULTS AND DISCUSSION

3.1. Physicochemical Characterization of Synthesized Derivatives

Three Schiff base hydrazone derivatives were successfully synthesized through condensation reactions between isatin-3-carbohydrazide and substituted aromatic aldehydes. The synthesized compounds were obtained as crystalline solids with satisfactory yields ranging from **76.44% to 77.26%**. The percentage yields obtained indicate that the adopted synthetic methodology was efficient and reproducible for the preparation of isatin-based hydrazone derivatives.

The physicochemical properties of the synthesized compounds, including molecular formula, molecular weight, percentage yield, and melting point, are presented in Table 3.1.

Physicochemical Properties of Synthesized Derivatives

Compound	IUPAC Name	Molecular Formula	Molecular Weight (g/mol)	Theoretical Yield (g)	Practical Yield (g)	Yield (%)	Melting Point (°C)
D1	(E)-N′-benzylidene-2-oxoindoline-3-carbohydrazide	C15H11N3O2	265.27	3.65	2.82	77.26	238–241
D2	(E)-N′-(4-chlorobenzylidene)-2-oxoindoline-3-carbohydrazide	C15H10ClN3O2	299.71	3.99	3.05	76.44	246–249
D3	(E)-N′-(4-methoxybenzylidene)-2-oxoindoline-3-carbohydrazide	C16H13N3O3	295.29	3.95	3.03	76.71	232–235

The synthesized derivatives exhibited good yields, confirming successful condensation between the hydrazide and aldehyde moieties. Among the synthesized compounds, D1 showed the highest percentage yield (77.26%**),** whereas D2 exhibited a slightly lower yield (76.44%). The methoxy-substituted derivative D3 produced a yield of 76.71%**.**

The minor variation in percentage yields can be attributed to the electronic effects of substituents present on the aromatic aldehyde ring. The electron-withdrawing chloro substituent in D2 reduces electron density around the carbonyl carbon and may slightly affect the reaction kinetics, resulting in a marginally lower yield. Similarly, the electron-donating methoxy

group in D3 influences the condensation process through resonance effects. However, the differences among the yields were minimal, indicating that all derivatives were synthesized efficiently under identical reaction conditions.

3.2. Melting Point Analysis

Melting point determination was performed to assess the purity and thermal stability of the synthesized compounds. All derivatives exhibited sharp melting point ranges, indicating high purity and successful recrystallization.

Among the synthesized compounds, D2 showed the highest melting point (246–249°C), followed by D1 **(**238–241°C) and D3 **(**232–235°C**).** The higher melting point observed for D2 may be attributed to the presence of the chlorine atom, which increases molecular mass and intermolecular attractive forces within the crystal lattice. The electron-withdrawing nature of chlorine also enhances molecular packing efficiency, contributing to increased thermal stability.

In contrast, the methoxy-substituted derivative D3 exhibited the lowest melting point. The methoxy group acts as an electron-donating substituent and introduces steric effects that may reduce crystal packing efficiency, resulting in comparatively lower thermal stability.

3.3. Effect of Structural Modification

Structural modification of the benzylidene moiety significantly influenced the physicochemical properties of the synthesized derivatives. Introduction of electron-withdrawing and electron-donating substituents altered molecular weight, crystal packing, and thermal behavior.

The unsubstituted derivative D1 served as the parent compound and exhibited excellent yield and moderate thermal stability. Incorporation of a chlorine atom in D2 increased molecular weight from 265.27 g/mol to 299.71 g/mol and enhanced thermal stability as reflected by the higher melting point. Conversely, introduction of a methoxy group in D3 increased molecular weight to
295.29 g/mol but resulted in a slightly lower melting point due to steric and electronic factors.

3.4. Structure–Property Relationship

The observed physicochemical characteristics suggest that substitution on the aromatic ring plays an important role in determining the overall molecular properties of the hydrazone derivatives. The presence of conjugated aromatic systems and azomethine linkages contributes to molecular rigidity and stabilization through resonance. Furthermore, intermolecular hydrogen bonding involving the hydrazide and carbonyl functionalities may contribute to the high melting points observed for all derivatives.

The synthesized compounds exhibited melting points above 230°C, indicating considerable thermal stability and strong intermolecular interactions. Such properties are advantageous for pharmaceutical and analytical applications because they often correlate with improved storage stability and structural integrity.

3.5. Mass Spectral Analysis

Mass spectrometry was employed as a confirmatory analytical technique to determine the molecular weights of the synthesized hydrazone derivatives and to verify their proposed molecular structures. The mass spectra of compounds D1, D2, and D3 exhibited molecular ion peaks corresponding to their respective molecular masses, providing strong evidence for successful synthesis and structural integrity of the target compounds.

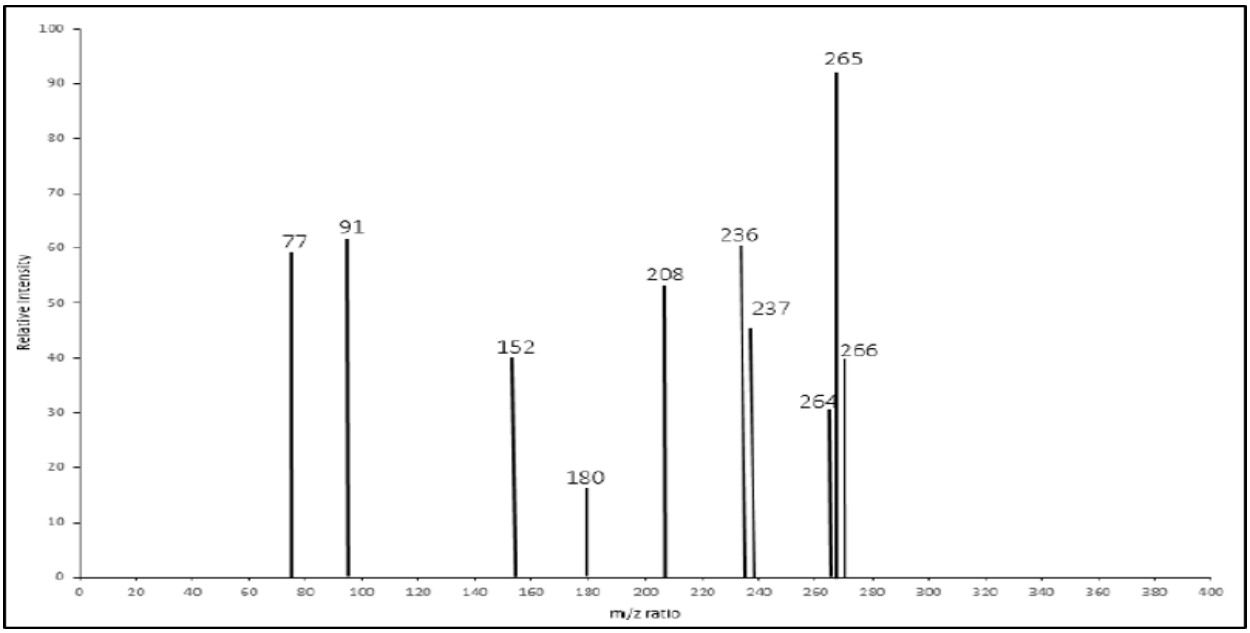

Fig : Mass Spectra of N′-benzylidene-2-oxoindoline-3-carbohydrazide

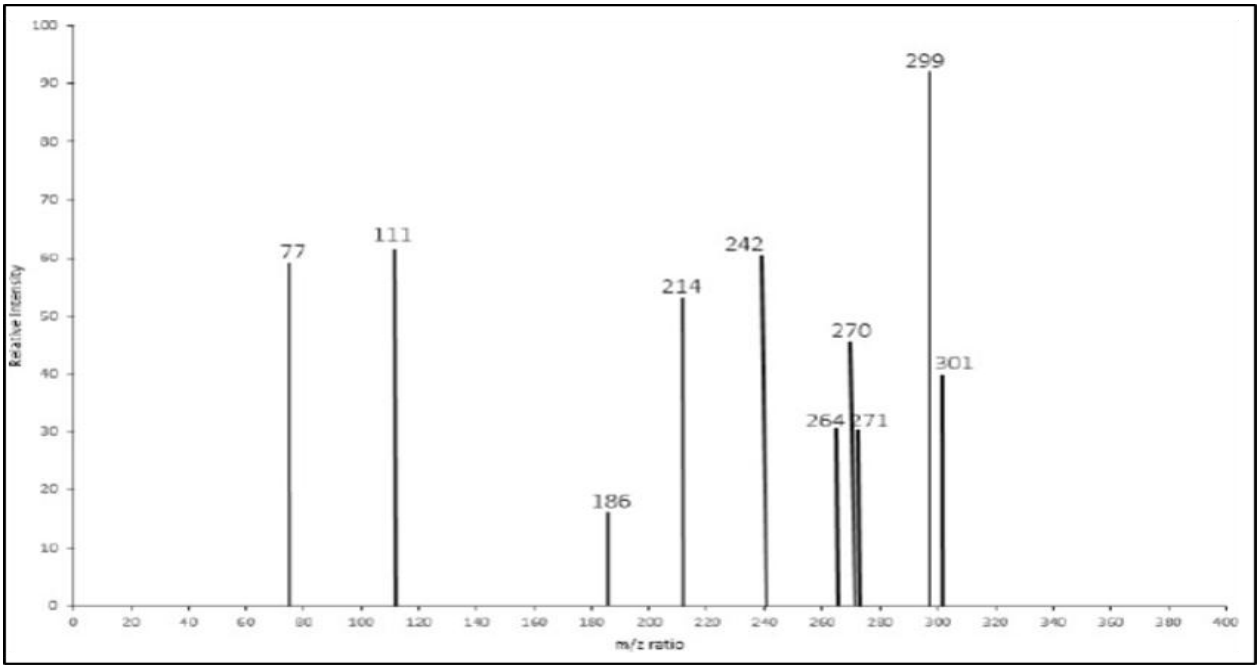

Fig : Mass Spectra of N′-(4-chlorobenzylidene)-2-oxoindoline-3-carbohydrazide

The molecular ion peak **(**M^+**)** observed at m/z 265 for D1 [(E)-N′-benzylidene-2-oxoindoline-3-carbohydrazide] corresponded closely to its calculated molecular weight of 265.27 g/mol. Similarly, D2 [(E)-N′-(4-chlorobenzylidene)-2-oxoindoline-3-carbohydrazide] exhibited a molecular ion peak at m/z 299, which agreed with its theoretical molecular weight of 299.71 g/mol**.** For D3 [(E)-N′-(4-methoxybenzylidene)-2-oxoindoline-3-carbohydrazide**]**, the molecular ion peak appeared at m/z 295, corresponding to the calculated molecular weight of 295.29 g/mol. The close agreement between observed and theoretical molecular masses confirmed the successful formation of the desired compounds and demonstrated the reliability of the synthetic procedure.

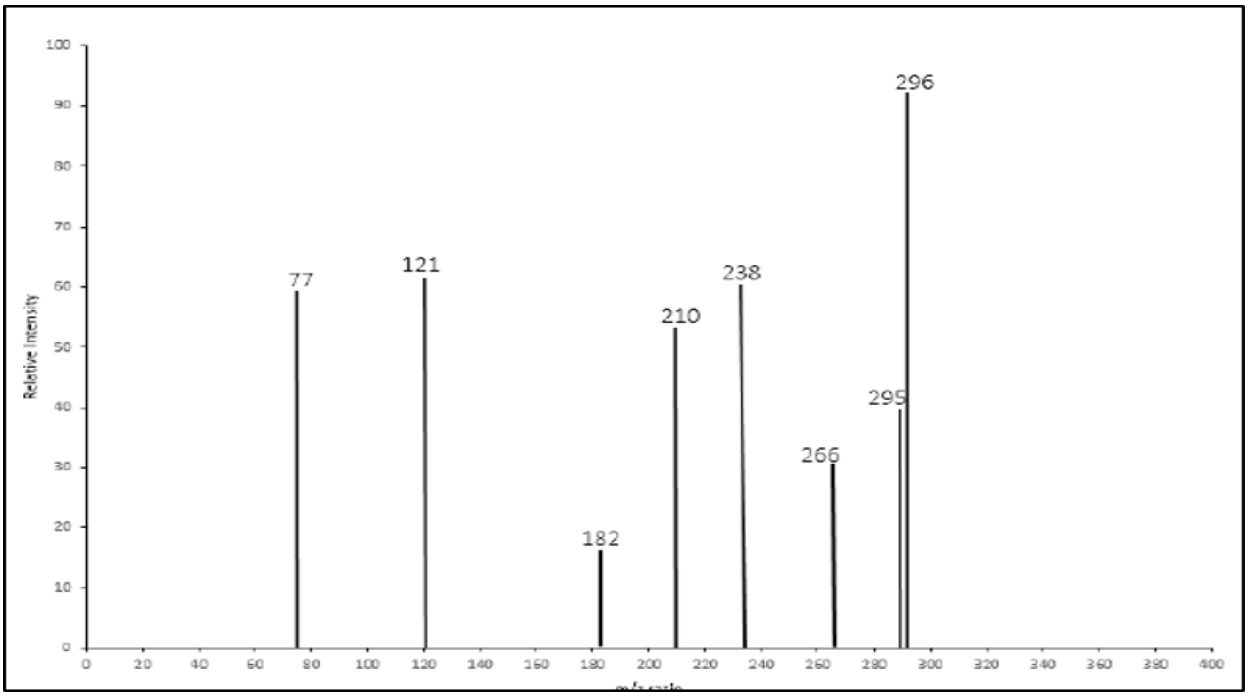

Fig: Mass Spectra of N′-(4-methoxybenzylidene)-2-oxoindoline-3-carbohydrazide

A particularly significant feature was observed in the mass spectrum of D2, which contained a characteristic isotopic peak at m/z 301 in addition to the molecular ion peak at m/z 299. This isotopic pattern is a diagnostic feature of chlorine-containing compounds and arises from the natural abundance of the two stable chlorine isotopes, ^{35}Cl (75.8%) and ^{37}Cl (24.2%)**.** The appearance of molecular ion peaks separated by two mass units with an approximate intensity ratio of 3:1 is considered strong evidence for the presence of a chlorine atom within the molecula structure. Therefore, the isotopic peak at m/z 301 conclusively confirmed successful incorporation of the chloro substituent in derivative D2.

The fragmentation behavior of the synthesized compounds further supported their proposed structures. The molecular ion peaks underwent characteristic fragmentation pathways involving cleavage of relatively weak bonds within the hydrazone framework. One of the common fragmentation routes involved the loss of carbon monoxide (CO, 28 amu) from the isatin carbonyl group, producing stable fragment ions of lower mass. The elimination of carbon monoxide is a frequently observed fragmentation process in carbonyl-containing heterocyclic compounds and reflects the presence of the lactam carbonyl functionality within the isatin nucleus.

Another important fragmentation pathway involved the loss of formyl (CHO) or related carbonyl-containing fragments, resulting in the formation of resonance-stabilized aromatic ions. Such fragmentations are characteristic of hydrazone derivatives containing conjugated carbonyl systems and provide additional confirmation of the molecular framework.

Cleavage of the hydrazide linkage (–CONH–N=CH–) also generated prominent fragment ions in the spectra. The hydrazone bond is susceptible to fragmentation under electron ionization conditions, leading to the formation of stable aromatic and heterocyclic fragments. These fragments are often highly stabilized by resonance within the conjugated aromatic system and therefore appear as significant peaks in the mass spectrum.

In addition to these fragmentation pathways, several aromatic fragment ions were observed for all derivatives. These fragments originated from cleavage of the benzylidene ring and isatin moiety and produced characteristic ions that are commonly associated with

aromatic hydrazone compounds. For D2, aromatic fragments containing chlorine retained the characteristic isotopic pattern, providing further confirmation of the chloro-substituted structure. Similarly, D3 generated fragment ions associated with the methoxy-substituted aromatic ring, supporting the presence of the methoxy functionality.

The observed fragmentation patterns were entirely consistent with the proposed molecular structures and complemented the FTIR and ^{1}H NMR findings. The combination of molecular ion peaks, chlorine isotopic distribution in D2, and characteristic fragmentation pathways provided definitive evidence for the successful synthesis of D1, D2, and D3. Therefore, mass spectral analysis served as a powerful tool for confirming molecular identity, structural integrity, and substituent incorporation in the synthesized hydrazone derivatives.

IV. CONCLUSION

Three novel isatin-based hydrazone derivatives were successfully synthesized and characterized using mass spectrometry. The compounds were obtained in good yields and exhibited high thermal stability and purity. Spectral analyses confirmed the successful formation of hydrazone linkages and verified the presence of the intended substituents. The electron-withdrawing chloro group and electron-donating methoxy group significantly influenced the spectral behavior of the derivatives. These compounds provide valuable scaffolds for future investigations involving fluorescence studies, biological evaluation, and medicinal chemistry applications.

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