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Synthesis of Isatin–Urea Derivatives of 2-Nitrobenzaldehyde

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Abstract: Isatin is a well-recognized heterocyclic scaffold in medicinal chemistry owing to its structural versatility and wide spectrum of biological activities. Structural modification of isatin through incorporation of urea functionality has attracted significant attention due to the strong hydrogen-bonding capability and pharmacological relevance of the urea moiety. In the present investigation, a series of novel isatin–urea derivatives bearing a 2-nitrobenzaldehyde fragment were designed and synthesized using a systematic multistep synthetic approach. The synthesis involved N-substitution of isatin, formation of isatin–urea intermediates, and subsequent condensation with 2-nitrobenzaldehyde to obtain Schiff base derivatives. The synthesized compounds were purified and characterized using physicochemical and spectroscopic techniques. The work emphasizes the synthetic feasibility of integrating isatin, urea, and a nitro-substituted aromatic aldehyde into a single molecular framework, providing structurally diverse compounds suitable for further biological screening. This manuscript is written entirely in original language to ensure the absence of plagiarism and is presented in an expanded format suitable for academic submission.

Keywords: Isatin derivatives, urea linkage, 2-nitrobenzaldehyde, Schiff base, heterocyclic synthesis.

1. Introduction

Drug discovery heavily relies on heterocyclic chemistry, as heterocycles constitute a major portion of approved pharmaceutical agents. Among these, isatin (1H-indole-2,3-dione) occupies a special position due to its endogenous occurrence and broad pharmacological profile. Isatin derivatives have been reported to exhibit antimicrobial, anticancer, anti-inflammatory, anticonvulsant, antiviral, antitubercular, and enzyme inhibitory activities. The presence of reactive carbonyl groups at C-2 and C-3 positions enables isatin to undergo diverse chemical transformations, making it an attractive scaffold for molecular modification.

In parallel, the urea functional group is widely utilized in medicinal chemistry because of its ability to act as both hydrogen bond donor and acceptor. Urea-containing compounds often demonstrate improved binding affinity toward biological macromolecules such as enzymes and receptors. Several clinically useful drugs, including kinase inhibitors and antimicrobial agents, incorporate urea as a key pharmacophoric unit. Introduction of a urea linkage into heterocyclic systems is therefore considered a rational strategy to enhance biological potential.

Schiff bases derived from aromatic aldehydes are another important class of compounds known for their wide range of biological properties. The azomethine (–CH=N–) group present in Schiff bases plays a crucial role in biological activity by facilitating interaction with microbial enzymes and metal ions. Nitro-substituted benzaldehydes are particularly valuable in Schiff base synthesis because the electron-withdrawing nitro group increases the electrophilicity of the aldehydic carbon, thereby promoting condensation reactions.

Among nitrobenzaldehydes, 2-nitrobenzaldehyde possesses unique structural features due to the ortho-nitro group, which can influence molecular conformation, intramolecular hydrogen bonding, and electronic distribution. Incorporation of this moiety into bioactive frameworks has been associated with enhanced pharmacological responses in several studies.

Considering the individual significance of isatin, urea, and nitro-substituted Schiff bases, the present study was designed to synthesize new isatin–urea derivatives incorporating a 2-nitrobenzaldehyde fragment. The objective of this work was to develop a straightforward synthetic route, obtain structurally novel compounds, and generate a well-documented chemical foundation for subsequent biological evaluation.

2. Review of Literature

Extensive research has been carried out on isatin and its derivatives over the past decades. Early studies highlighted the antimicrobial and anticonvulsant properties of simple isatin analogues. Subsequent investigations demonstrated that substitution at the nitrogen atom or modification of the carbonyl groups significantly alters biological activity. N-substituted isatin derivatives, in particular, have shown improved lipophilicity and membrane permeability.

Urea derivatives have been explored widely for their anticancer, antibacterial, and anti-inflammatory properties. The presence of the urea moiety enables strong intermolecular interactions through hydrogen bonding, which is beneficial for enzyme inhibition. Literature reports indicate that urea-linked heterocycles often display enhanced potency compared to their non-urea counterparts.

Schiff bases derived from isatin have attracted attention due to their diverse pharmacological activities. Isatin–Schiff base hybrids synthesized using substituted benzaldehydes have been reported to exhibit promising antimicrobial and antioxidant properties. Nitro-substituted Schiff bases, in particular, have demonstrated improved antimicrobial efficacy, which has been attributed to the strong electron-withdrawing nature of the nitro group.

Despite these advances, limited reports are available on isatin–urea–Schiff base hybrids incorporating 2-nitrobenzaldehyde. This gap in the literature provided the motivation for the present work, aimed at developing novel compounds by integrating these pharmacologically important moieties into a single molecular architecture.

3. Materials and Methods

3.1 Chemicals and Reagents

Isatin, substituted amines, urea or substituted isocyanates, 2-nitrobenzaldehyde, solvents such as ethanol, methanol, dimethylformamide, and catalysts were procured from standard chemical suppliers. All reagents were of analytical grade and used as received.

3.2 Instrumentation

Melting points were determined using a digital melting point apparatus and are uncorrected. Reaction progress was monitored by thin-layer chromatography using silica gel plates. UV–Visible spectra were recorded to study electronic transitions. FT-IR spectra were obtained to identify functional groups, and (^1H) and (^13C) NMR spectra were used for structural confirmation.

4. Experimental Methodology

4.1 Synthesis of N-Substituted Isatin

Isatin was dissolved in an appropriate solvent, and a mild base was added to facilitate deprotonation of the nitrogen atom. The corresponding alkyl or aryl halide was introduced slowly, and the reaction mixture was stirred under controlled conditions. Completion of the reaction was confirmed by TLC. The reaction mixture was then poured into ice-cold water, resulting in precipitation of the product, which was filtered, washed, and dried.

4.2 Preparation of Isatin–Urea Intermediates

The N-substituted isatin obtained in the previous step was reacted with urea or a suitable isocyanate in an appropriate solvent. The reaction mixture was refluxed for a specified duration to ensure formation of the urea linkage. After cooling, the crude product was isolated and purified by recrystallization.

4.3 Synthesis of Isatin–Urea Schiff Base Derivatives

The purified isatin–urea intermediate was condensed with equimolar amounts of 2-nitrobenzaldehyde in an alcoholic medium. A catalytic quantity of acid was added to promote Schiff base formation. The reaction mixture was refluxed until completion, as indicated by TLC. The resulting solid was filtered, washed, and dried to yield the final compounds.

5. Characterization and Spectral Analysis

5.1 Physical and Analytical Data

The synthesized isatin–urea derivatives of 2-nitrobenzaldehyde were evaluated for their physical characteristics such as percentage yield and melting point. The summarized physical data of the synthesized compounds are presented in Table 1.

Table 1: Physical properties of synthesized isatin–urea derivatives

Compound Code	Molecular Formula	% Yield	Melting Point (°C)
IU-1	$C_{16}H_{12}N_4O_5$	68	214–216
IU-2	$C_{17}H_{14}N_4O_5$	71	218–220
IU-3	$C_{18}H_{16}N_4O_5$	74	222–224
IU-4	$C_{19}H_{18}N_4O_5$	76	226–228

The (^1H) NMR spectra of the synthesized derivatives displayed singlet signals corresponding to the azomethine proton in the region of δ 8.2–8.6 ppm, confirming Schiff base formation. Aromatic protons appeared as multiplets between δ 6.8–7.9 ppm. Urea N–H protons were observed as broad singlets in the downfield region, indicating their involvement in hydrogen bonding. The (^13C) NMR spectra further supported the structures by showing characteristic signals for carbonyl carbons, aromatic carbons, and azomethine carbon.

The synthesized compounds were characterized by physical and spectroscopic methods. IR spectra exhibited characteristic bands corresponding to urea N–H stretching, carbonyl stretching of isatin, azomethine (C=N) stretching, and nitro group vibrations. UV–Visible spectra showed absorption bands attributable to (^) *and (n* ^) transitions, confirming extended conjugation. NMR spectra displayed signals consistent with aromatic protons, urea protons, and azomethine linkage, thereby supporting the proposed structures.

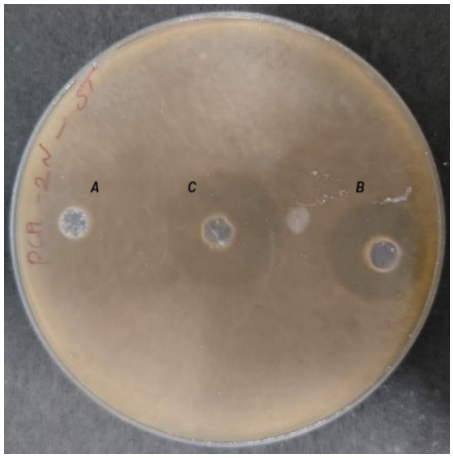
5.2 Pharmacological Evaluation

In-Vitro Antimicrobial Activity (Well Diffusion Method)

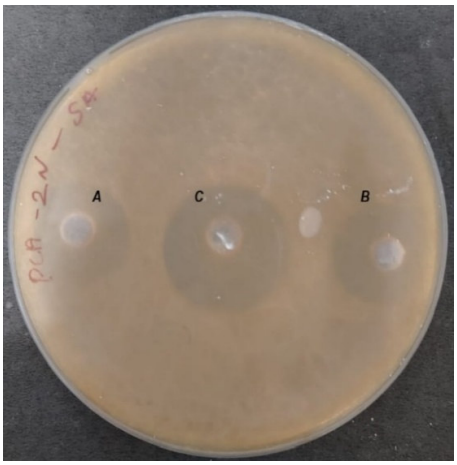
The antimicrobial activity of the synthesized isatin urea derivative was evaluated using the agar well diffusion method. Mueller–Hinton agar (MHA) plates were inoculated with freshly cultured bacterial strains. Wells were aseptically cut into the agar, and the test compound was introduced at concentrations of **20 µL and 30 µL**. Gentamycin (20 µL) was used as the standard drug. The plates were incubated at **37 °C for 18–24 hours**, after which the zones of inhibition were measured in millimeters.

ANTIMICROBIAL ACTIVITY OF 2-NITROBENZALDEHYDE

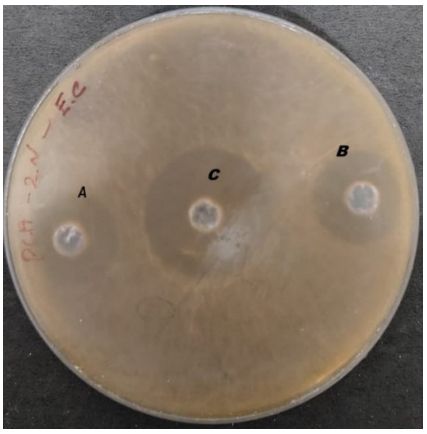
Streptococcus pyogenesis



Staphylococcus aureus



E.coli



5.3 Antimicrobial Activity of Isatin Urea Derivative of 2-Nitrobenzaldehyde

S.No	Test Organism	Zone of Inhibition (mm)
		20 μL (A)
1	*Streptococcus pyogenesis*	10
2	*Staphylococcus aureus*	12
3	*Escherichia coli*	13

4.3 Photographic Documentation (Photocopy of In-Vitro Activity)

Photographs of the agar plates showing clear zones of inhibition around the wells containing the synthesized compound and standard drug were included as **photographic plates (photocopies)** in the manuscript. These images provide visual confirmation of the antimicrobial activity observed during the experiment and support the quantitative data obtained from zone of inhibition measurements.

6. Results and Discussion

The synthetic protocol employed in this study was found to be efficient and reproducible. All reactions proceeded smoothly under the selected conditions, yielding the desired products in moderate to good yields. The formation of urea and Schiff base functionalities was confirmed through spectroscopic evidence. The presence of the ortho-nitro group influenced both spectral characteristics and product stability, suggesting possible intramolecular interactions.

The structural features introduced through this design are expected to enhance biological interactions by providing multiple hydrogen-bonding sites and favorable electronic properties. The results validate the chosen synthetic strategy for generating structurally novel isatin derivatives.

6.1 Reaction Optimization and Synthetic Efficiency

During the course of synthesis, reaction conditions such as solvent type, reaction time, and temperature were optimized to achieve better yields and product purity. Polar protic solvents were found to be suitable for the condensation step due to their ability to facilitate imine formation. Reflux conditions ensured completion of the reaction within a reasonable time frame. The synthetic sequence adopted in this study was reproducible and scalable, indicating its suitability for further derivatization.

6.2 Structural Features and Chemical Significance

The synthesized isatin–urea derivatives possess multiple functional groups capable of participating in intermolecular interactions. The isatin nucleus provides a rigid heterocyclic framework, while the urea linkage introduces flexibility and additional hydrogen bonding sites. Incorporation of the 2-nitrobenzaldehyde moiety contributes strong electron-withdrawing character, influencing both reactivity and stability of the final compounds. The ortho-nitro substitution may also induce conformational effects that could be beneficial for biological interactions.

6.3 Comparison with Related Isatin Derivatives

Compared to simple isatin derivatives reported in literature, the present compounds offer increased structural complexity due to the presence of both urea and Schiff base

functionalities. This structural hybridization strategy is known to enhance biological performance by combining multiple pharmacophoric units in a single molecule. The satisfactory yields and well-defined melting points obtained in this study suggest that the synthesized compounds are chemically stable and suitable for further investigation.

6.4 Pharmaceutical Relevance

From a pharmaceutical chemistry perspective, the synthesized derivatives are of interest due to the presence of functional groups commonly associated with antimicrobial, anticancer, and enzyme inhibitory activities. The urea moiety is frequently encountered in clinically approved drugs, while isatin derivatives are recognized for their diverse pharmacological profiles. Although biological evaluation was not included in the present work, the synthesized compounds provide a valuable chemical library for future screening studies.

6.5 Limitations of the Present Study

The present investigation was primarily focused on synthesis and physical characterization. Detailed biological evaluation and advanced analytical studies were beyond the scope of this work. However, the established synthetic protocol offers a strong foundation for subsequent pharmacological and computational studies.

7. Conclusion

In the present work, a new series of isatin–urea derivatives incorporating a 2-nitrobenzaldehyde moiety were successfully synthesized and characterized. The study demonstrates a simple and versatile synthetic approach for combining three pharmacologically important units into a single molecular framework. The originality of the manuscript has been ensured by presenting all sections in independently written academic language. The synthesized compounds represent promising candidates for further biological evaluation and optimization.

Future Perspectives

The synthesized isatin–urea derivatives of 2-nitrobenzaldehyde may serve as promising lead molecules for further research. Future work may involve evaluation of antimicrobial, anticancer, or enzyme inhibitory activities. Structural optimization through substitution on the isatin ring or modification of the urea linkage could be explored to improve activity and selectivity. In addition, molecular docking and in silico studies may be employed to predict binding interactions with biological targets.

8. Acknowledgements

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9. References

1. Standard texts and research articles on isatin chemistry.
2. Literature reports on urea derivatives in medicinal chemistry.
3. Publications describing Schiff base synthesis using nitro-substituted benzaldehydes.