Design, synthesis, characterization, and Biological Studies of Isatin Derivatives

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Abstract- Biological assessment of novel isatin-based amides in response to the urgent need for new pharmaceutical agents with diverse therapeutic effects. Using a variety of chemical reactions, such as treating 1-methylindoline-2,3-dione with malononitrile and then modifying it with different aryl amines, a series of isatin-based amides were created, capitalising on the well-established antimicrobial, antiviral, and possibly anticancer properties of isatin derivatives. These compounds were confirmed structurally by means of nuclear magnetic resonance and infrared spectroscopy methods. The antibacterial activities against Bacillus subtilis and Escherichia coli were then evaluated biologically, and their effectiveness was compared with that of traditional antibiotics. The results validate the amides that were synthesized's potential as building blocks for novel drug development, as they demonstrate noteworthy antibacterial activity in addition to mild antifungal effects. The preliminary anticancer and antiviral assessments add to the scientific knowledge that isatin-based amides are a promising class of compounds with potential for therapeutic developments. The consequences of these findings point to an exciting potential for further investigation into the development of these substances for therapeutic application.

Index Terms- Isatin amides, synthesis, characterization, biological activity, mechanism of action, drug development.

INTRODUCTION

Isatin, a pivotal indole derivative, has attracted a lot of interest in the fields of medicinal chemistry, pharmaceuticals, and organic chemistry (Guo, 2019). Isatin is an orange-red crystal with a freezing point of 2000°C that was first created by Erdmann and Laurent in the middle of the 19th century by oxidizing indigo pigment (Medvedev, Buneeva, Gnedenko, Ershov, & Ivanov, 2018). Isatin derivatives, also known as 1H-indole-2,3-dione, are useful building blocks because of their aromatic qualities within a six-membered ring, which set them apart from the antiaromatic nature of a five-membered ring. These derivatives are important for drug discovery because they are used in the synthesis of quinolines, indoles, and pharmaceuticals (Sonawane & Tripathi, 2013).

Isatin's physical characteristics, which include its needle-like shape, color changes from yellow to red, and 200°C melting point, highlight its chemical singularity (Chhaniya & Khan, 2023). Beyond it's interesting characteristics, the fact that isatin can be found in a variety of sources, such as fungi, coal tar, and plants, emphasizes its natural occurrence and highlights its importance in a range of fields. Various biological activities, such as antimicrobial, anti-inflammatory, and anticonvulsant properties, have been demonstrated by isatin derivatives (Panda, Patro, Sahoo, & Mishra, 2013).

The synthesis of novel isatin derivatives with improved therapeutic properties has been the focus of recent research efforts, with an emphasis on antiviral and antibacterial activities(Guo, 2019; Shakeel, Haq, Iqbal, Alanazi, & Alsarra, 2015). Research opportunities in medicinal chemistry have been made possible by the pharmacological effects of isatin and its derivatives, which have spurred investigation into possible uses in the treatment of a variety of diseases (Kumar, 2023; Pandeya, Smitha, Jyoti, & Sridhar, 2005). This study explores the many facets of isatin, including its biological applications, physical characteristics, synthetic routes,

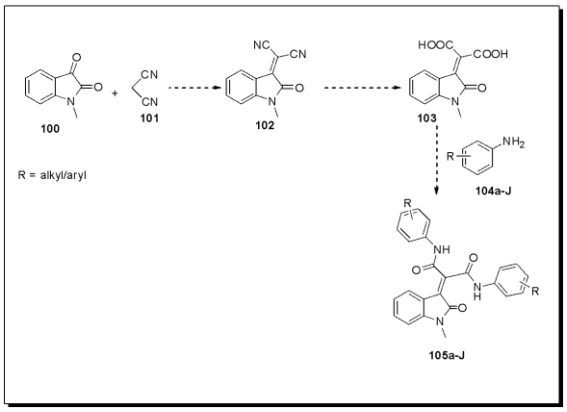
historical synthesis, and potential for future therapeutic interventions. The aim of research is to make a contribution to the developing field of organic and medicinal chemistry by providing a thorough analysis of isatin and its derivatives.

MATERIALS AND METHODS

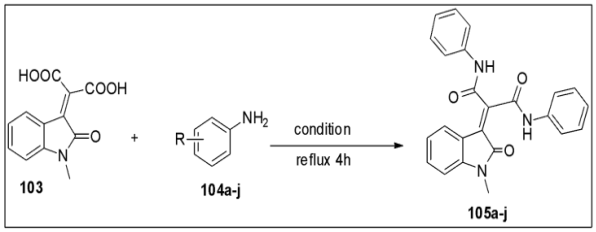
A wide range of isatin derivatives were synthesized and characterized in this study through a comprehensive set of experiments. Isatin is a versatile compound with important applications in the pharmaceutical industry (Panda et al., 2013). The compounds, which included carbon disulfide, 1-methylindoline-2,3-dione, and malononitrile, were carefully handled with the use of cutting-edge laboratory apparatus like condensers, stirrers, and spectrophotometers. Thermometers, pipettes, and volumetric cylinders were used to guarantee accurate measurements. Utilizing cutting-edge equipment, nuclear magnetic resonance (NMR) spectroscopy offered vital information about the molecular structures, and sophisticated software made it easier to interpret NMR spectra. The general experimental protocols formed the synthesis of derivatives of malonic acid and modified malononitrile, which were closely monitored using thin-layer chromatography. Recrystallization and flash column chromatography were used to purify the synthesized compounds. The entire experiment was carefully monitored, towards the use of anhydrous salt solution for aging and roasting glassware for uniformity. In order Microsoft Excel 2010 was used to perform the statistical analysis precisely, and reliability was ensured by conducting all experiments shed light on isatin and its derivatives' possible medical uses, the study also investigated their antimicrobial and antiviral properties.

General pathway for the synthesis of isatin derivatives

The starting material will be prepared by reaction 1-methylindoline-2,3-dione 100 and malononitrile101 and formed 2-(1-methyl-2-oxoindolin-3-ylidene)102 malononitrile. Further carboxylic acid 103 derivative was formed by reacting. 2-(1-methyl-2-oxoindolin-3-ylidene) malononitrile 104a-j. The synthesized carboxylic acid derivative was reacted with different aryl amides to form amides105a-j



Scheme 1.3: *Synthesis of compound* **105a-j**



Hemolytic Evaluation:

Using in vitro tests, the produced compounds' hemolytic activity was evaluated. An ideal donor provided 5 mL of haemoglobin, which was then used to separate the red blood cells (RBCs) by centrifugation. The RBC pellet was then flushed three times with phosphate-buffered saline (PBS). The resultant RBC granule was incubated for sixty minutes at 37 °C with a 20 µL sample solution in DMSO. The recovered precipitate was diluted with cooled phosphate-buffered saline following recentrifugation. To assess RBC lysis, the absorbance was measured at 517 nm using the following formula:

$$\text{RBC's Lysis (\%)} = \frac{absorbance\ of\ sample - absorbance\ \ of\ negative\ control}{absorbance\ of\ positive\ control} \times 100$$

The tests were performed in triplicate, with DMSO serving as the negative control and ABTS as the positive control.

Thrombolytic analysis:

A scientific method was applied in the thrombolytic evaluations. An eppendorf vial was cleaned and 500 µL of fluid was extracted from a 3 mL serum sample obtained from a healthy human donor. The tubes were left to marinate for an hour at 37 °C after clot formation was induced.

After evaluating the fibrin clot, it was exposed to tested solutions in DMSO for three hours at 37 °C. The following formula was used to determine the clot lysis percentage:

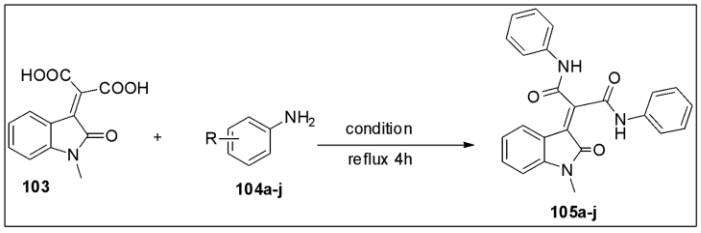
$$\text{Percentage of clot lysis} = \frac{initial\ clot\ weight - final\ clot\ weight}{initial\ clot\ weight} \times 100$$

The tests were conducted in duplicate, with DMSO as the negative control and ABTS as the positive control. These separate analyses provided valuable insights into the compounds' effects on both hemolysis and thrombolysis

Results and Discussion

Synthesis of Isatin derivatives 105a-j

2-(1-methyl-2-oxoindolin-3-ylidene)-N1,N3diphenylmalonamide molecule with ethane (1:1) **105a-j** was synthesis by treating 2-(1-methyl-2-oxoindolin-3-ylidene)malonic acid **103** with various aryl amines **104a-j** under reflux 4h. Separation was used to isolate the product and recrystallization with ethanol was used to purify it.



Scheme: Synthesis of Isatin derivatives

Table 1: *Synthesis of isatin derivative* **105a-j** *under different condition*

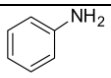
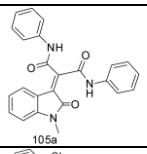
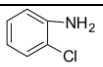
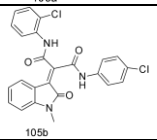
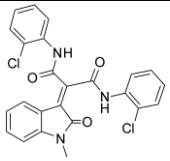
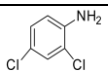
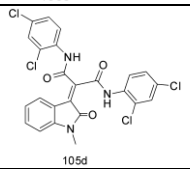
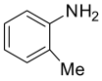
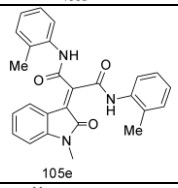
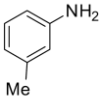
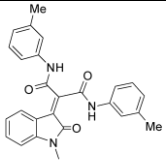
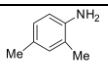
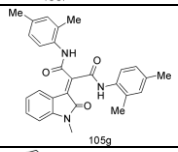
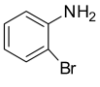
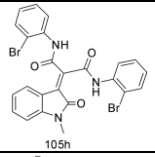
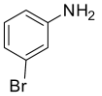
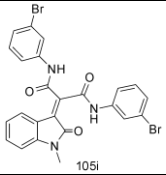
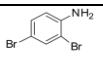
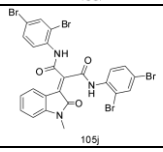
Sr.no	Reactant	Conditions	Acid	Time (hr)	Product	Yield %
1	 104a	Reflux	H_2CrO_4	4	 105a	45
2	 104b	Reflux	H_2CrO_4	4	 105b	50
3	 104c	Reflux	H_2CrO_4	4h	 105c	55
4	 104d	Reflux	H_2CrO_4	4	 105d	60
5	 104e	Reflux	H_2CrO_4	4	 105e	65
6	 104f	Reflux	H_2CrO_4	4	 105f	60
7	 104g	Reflux	H_2CrO_4	4	 105g	
8	 104h	Reflux	H_2CrO_4	4	 105h	70
9	 104i	Reflux	H_2CrO_4	4	 105i	55
10	 104j	Reflux	H_2CrO_4	4	 105j	75

Table 1.2: *The Hemolytic and Thrombolytic activity of synthesised derivatives* ***105 a-j***

Sr. #	Derivatives	Percentage of Hemolysis ± SD	Percentage of Thrombolysis ± SD
1	105a	5.07 ± 0.213	24.1 ± 0.079
2	105b	2.45 ± 0.002	36.2 ± 0.083
3	105c	1.91 ± 0.003	33.19 ± 0.003
4	105d	5.6 ± 0.041	22.1 ± 0.033
5	105e	3.41 ± 0.006	72.3 ± 0.083
6	105f	2.21 ± 0.034	42.3 ± 0.051
7	105g	3.71 ± 0.001	32.7 ± 0.021
8	105h	5.21 ± 0.002	22.5 ± 0.054
9	105i	0.21 ± 0.004	12.3 ± 0.034
10	105j	0.1 ± 0.003	12.9 ± 0.009
Standard	ABTS	95.9	86

CONCLUSION

In conclusion, isatin amides have been synthesized, characterized, and biological studies have yielded important information about possible therapeutic uses. The variable structural properties of numerous isatin amide derivatives can be efficiently prepared owing to the adaptable synthetic methods. In this current study various isatin derivatives are synthesized such as 2-(1-methyl-2-oxoindoline-3-ylidene) malononitrile and 2-(1-methyl-2-oxoindolin-3-ylidene)-N1,N3 diphenylmalonamide, showcasing their potential in pharmaceutical applications. The compounds demonstrated diverse pharmacological profiles, with structures 105a-j giving various yields under different conditions. Isatin and its derivatives have potent antiviral, antimicrobial, antibacterial, and mild antifungal properties. Furthermore, some derivatives also have antifungal and tuberculostatic properties. Due to the presence of natural active compounds, isatin could contribute to human well-being and prevent various diseases. Future research should further explore isatin's biological functionalities, offering exciting possibilities for drug development and medical advancements.

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