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DESIGN SYNTHESIS AND BIOLOGICAL EVALUATION OF ISATIN DERIVATIVES

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ABSTRACT

Isatin and its derivatives exhibit a wide range of biological activities, including anticancer, antiviral, antibacterial, antifungal, anti-inflammatory, and antihistaminic effects. In this study, 16 N-substituted isatin derivatives have shown significant potential against bacterial infections. Molecular docking and modeling studies reveal that these derivatives interact effectively with targets like SARS-CoV-2 main protease (PDB IDs: 6VXX, 7V7Q, 7DF4, 7V8B). These interactions occur via hydrogen bonding and hydrophobic interactions, leading to promising activity against main protease.

The structural modifications, including substitutions at C2, C3, and N positions, enhance the biological efficacy of isatin derivatives. This study synthesized and evaluated a library of isatin derivatives for their activity against Gram-Positive and Gram-negative microbial strains such as *Staphylococcus aureus, Escherichia coli*. The results suggest that these derivatives are promising candidates for further development as therapeutic agents are reflected in their minimal inhibitory concentration value.

Keywords: Isatin, Molecular docking, Synthesis, antibacterial

1. INTRODUCTION

Due to versatile pharmacological activity, Isatin is mainly used in pharmaceutical industry as intermediate, and in wide range of pharmaceutical drug designing **[1]**. Naturally this Pharmcophore occurred in many alkaloids **[2]**. Bacterial infectious diseases pose a significant health risk worldwide, leading to increased mortality and creating challenges for hospitals by placing a heavy burden on healthcare systems **[1]**. Diseases are

caused by various pathogens, including Gram-positive bacteria like *Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus pneumoniae,* and *enterococci* such as *Enterococcus faecalis* and *Enterococcus faecium.* Gram-negative pathogens include members of the *Enterobacteriaceae* family like *Escherichia coli* and *Klebsiella spp.,* as well as *Chlamydophila pneumoniae, Pseudomonas aeruginosa, Legionella pneumophila, Mycoplasma pneumoniae,* and *Acinetobacter spp*. **[3]**. Isatin efficiently show activity against these bacteria with noticeable structures, bromo, methoxy, phenoxy, carbonitrile, fluoro have wide range of biological and pharmacological activity **[3, 4]**. We designed some compounds by using molecular docking approach against main protease enzyme from target SARS-CoV-2 and docking score obtained between -4 to -6. Protease is helping to form different protein in microbes on ribosomes of gram-positive and gram-negative microbes **[2-5]**.

In the conclusion from molecular docking study, physicochemical properties of designed isatin, derivatives found good antimicrobial agent **[2-7]**.

2. MATERIALS AND METHODS

All chemicals were procured from *Omkar* Traders and *Sahyadri* Chemicals, all solvents and reagents are of laboratory grade, The melting point of the synthesized compounds was determined using the Veego VMP-D digital melting point apparatus and was uncorrected. The purity of all final compounds was checked by thin-layer chromatography (TLC). TLC plates of 4 cm × 1 cm were prepared using "Silica Gel G 60, F 254" from Merck. The Rf values of all compounds were observed. The TLC plates were visualized using iodine vapor in a chamber or under UV-visible light **[8-10]**.

Fourier-transform infrared spectroscopy (FT-IR) spectra were recorded using KBr on the "JASCO FTIR-4100" and are reported in cm^{-1}. Proton Nuclear Magnetic Resonance Spectroscopy (^1H NMR) was recorded using DMSO as a solvent on the "BRUKER AVANCE II 400 MHz NMR Spectrometer" (NIPER, Mohali, Punjab), with trimethylsilane (TMS) as an internal standard. Chemical shifts are expressed in parts per million (ppm) **[11]**.

Experimental

General Procedure for synthesis of Isatin

Firstly, the target compounds were synthesized according to the mentioned procedure. Sandmeyer Isatin synthesis is a condensation reaction between arylamine and chloral hydrate in the presence of hydroxylamine and aqueous sodium sulfate.

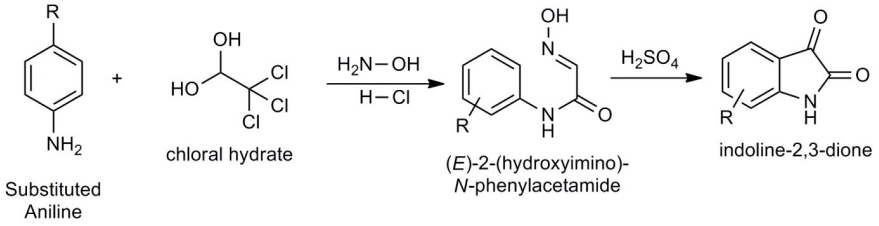
Stolle Isatin synthesis is a cyclization reaction between aniline and oxalyl chloride to obtain chlorooxalylanilide in the presence of acid. The designed derivatives were prepared from isatin using the Suzuki-coupling reaction **[11-14]**.

The progress of the reaction was monitored using TLC with different concentrations of ethyl acetate and n-hexane (3:1, 1:3, 2:2, etc.). The Rf values were recorded as mentioned in the table below. The melting point was determined using a melting point apparatus. All compounds were solid and crystalline, ranging in color from yellow to dark orange powder. One compound was found to be sticky in nature, with a lower melting point and less purity compared to the others **[1, 15]**.

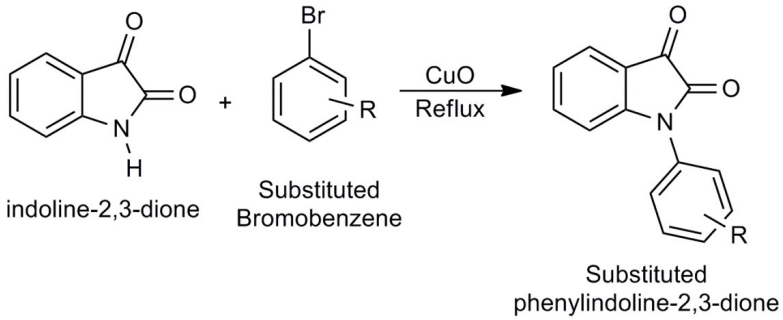
Synthesis of Isatin derivatives:

Synthetic scheme for derivatives (D1-D15)

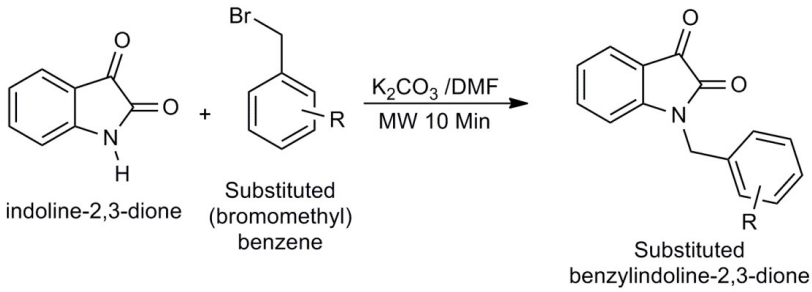
Step-I



Scheme-I



Scheme-II



Where R is D1-CH_3, D2-OCH_3, D3-OCH_3,OCH_3, D4-$CHCHCOOCH_3$, OCH_3, D5-Br, OCH_3, D6-Br, OCH_3, D7-Br,Br, OCH_3, D8-F,OCH_3, D9-Phenyl ring, OCH_3, D10-Br-Ph, OCH_3, D11-CN,OCH_3, D12-OH,OCH_3, D13-F, Cl, OCH_3, D14-OCH_3, OCH_3,OCH_3, 4-bromo-2-fluorophenyl

1-(3-methylbenzyl)indoline-2,3-dione

Melting Point (MP)(ºC)- 198-203, Yield: 70%, IR (KBr)cm^{-1}: 1372 (C-N, str), 1853-1729 (C=O, str), 1621-1529 (C=C, str-Ar) ^1H NMR (500 MHz, DMSO) δ 7.72 – 7.42 (m, 8H), 5.49 (s, 2H), 2.30 (s, 3H)

1-(3-methoxybenzyl)indoline-2,3-dione

Melting Point (MP)(ºC)- 204-208, Yield: 72%, IR (KBr)cm^{-1}: 1379 (C-N, str), 1707-1642 (C=O,str), 1048 (-O-,str), 1213-1592 (C=C, str-Ar) 1H NMR (500 MHz, Chloroform-*d*) δ 6.69-7.93 (m, 8H), 5.48-5.49 (s, 2H), 3.7 (s, 1H)

2,9-dimethoxyindolo[2,1-b]quinazolin-12(6H)-one

Melting Point (MP)(ºC)- 175-180, Yield:68%, IR (KBr)cm^{-1}: 1037-1153 (C-N, str), 1774 (C=O, str), 1347-1598 (C=C, str-Ar) ^1H NMR (500 MHz, Chloroform-*d*) δ 6.64 – 7.75 (m, 6H), 3.79 – 3.83 (d, 6H), 3.4 (s, 2H)

(E)-methyl 3-(1-(4-methoxyphenyl)-2,3-dioxoindolin-5-yl)acrylate

Melting Point (MP)(ºC)- 230-234, Yield:62%, IR (KBr)cm^{-1}: 1100 (C-N str), 1704-1603 (C=O str), 1509-1603 (-O- str-Ar) ^1H NMR (500 MHz, Chloroform-*d*) δ 6.98 – 8.16 (m, 7H), 6.39 – 6.42 (d, 2H), 3.76-3.80 (d, 6H).

5-bromo-1-(4-methoxybenzyl)indoline-2,3-dione

Melting Point (MP)(ºC)- 250-253, Yield:67%, IR (KBr)cm^{-1}: 1055 (C-N str), 1675 (C=O str), 1210 (-O- str), 1675-1258 (C=C str-Ar), 759 (Br) ^1H NMR (500 MHz, Chloroform-*d*) δ 6.82 – 8.06 (m, 7H), 5.48 (s, 2H), 3.80 (s, 3H)

6-bromo-1-(4-methoxybenzyl)indoline-2,3-dione

Melting Point (MP)(ºC)- 249-253, Yield:64%, IR (KBr)cm^{-1}:1028 (C-N str), 1661 (C=O str), 1131 (-O- str), 1589-1308 (C=C, str-Ar), 750 (Br). ^1H NMR (500 MHz, Chloroform-*d*) δ 6.82-8.01 (m, 7H), 5.47 (s, 2H), 3.79 (s, 3H).

5,6-dibromo-1-(4-methoxybenzyl)indoline-2,3-dione

Melting Point (MP)(ºC)- 253-257, Yield:70%, IR (KBr)cm^{-1}: 1059 (C-N str), 1760-1650 (C=O str), 1325 (-O- str), 1650-1428 (C=C str-Ar), 895 (Br). ^1H NMR (500 MHz, Chloroform-*d*) δ 8.09 (s, 1H), 7.80 (s, 1H), 7.20 – 6.82 (dd, 2H), 5.47 (s, 2H), 3.74 (s, 3H).

5-fluoro-1-(4-methoxyphenyl)indoline-2,3-dione

Melting Point (MP)(ºC)- 278-280, Yield:68%, IR (KBr)cm^{-1}:1061 (C-N str), 1753 (C=O str), 1276 (-O- str), 1421 (C=C str-Ar), 875 (F). ^1H

NMR (500 MHz, Chloroform-*d*) δ 6.99-7.01 (dd, 2H), 7.35-7.92 (m, 3H), 3.80 (s, 3H)

5-(4-bromophenyl)-1-(4-methoxyphenyl)indoline-2,3-dione

Melting Point (MP)(ºC)- 266-270, Yield: 65%, IR (KBr)cm^{-1}:1040 (C-N str), 1753-1706 (C=O str), 1217 (-O- str), 1612-1477 (C=C str-Ar). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 6.99 – 8.34 (m, 11H), 3.8 (s, 3H).

1-(4-methoxyphenyl)-2,3-dioxoindoline-5-carbonitrile

Melting Point (MP)(ºC)- 245-252, Yield:62%, IR (KBr)cm^{-1}: 1050 (C-N str), 1853-1729 (C=O str), 1621-1529 (C=C str-Ar). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 6.99 – 8.23 (m, 7H), 3.8 (s, 3H).

5-hydroxy-1-(4-methoxyphenyl)indoline-2,3-dione

Melting Point (MP)(ºC)- 238-245, Yield:63%, IR (KBr)cm^{-1}: 1037 (C-N str), 1610-1681 (C=O str), 1610-1445 (C=C str-Ar), 1265 (-O- str). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 6.99-8.23 (m, 7H), .8 (s, 3H).

5-chloro-6-fluoro-1-(4-methoxyphenyl)indoline-2,3-dione

Melting Point (MP)(ºC)- 245-252, Yield: 68%, IR (KBr)cm^{-1}: 1038 (C-N str), 1708-1643 (C=O str),1254 (-O- str), 1341-1605 (C=C str) 3594 (-OH). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 6.99-8.08 (m, 6H), 3.8 (s, 3H).

5,6-dimethoxy-1-(4-methoxyphenyl)indoline-2,3-dione

Melting Point (MP)(ºC)- 228-234, Yield:70%, IR (KBr)cm^{-1}: 1038 (C-N str), 1608-1677 (C+O str) 1152-1264 (-O- str), 1608-1264 (C=C str-Ar). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.67-6.99 (m, 9H), 3.80-3.90 (t, 9H).

1-(4-bromo-2-fluorophenyl)indoline-2,3-dione

Melting Point (MP)(ºC)- 241-249, Yield: 70%, IR (KBr)cm^{-1}:1161 (C-N str) 1806-1677 (C=O str) 1612-1268 (C=C str), 975 (F), 862 (Br). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.47 – 7.98 (m, 7H).

Molecular Docking Study

Ten Isatin derivative are docked and mentioned in research paper. This study concludes that molecular hybrids of isatin and its derivatives exhibit high binding affinity towards EGFR inhibition. The strong binding affinity and interaction at the inhibition site suggest their potential as synthetic agents for EGFR inhibition. Further experimental studies are required to evaluate the efficacy of these agents in EGFR inhibition and lung cancer treatment **[1, 11, 16]**.

***In vitro* antimicrobial activity**

The antimicrobial assay was conducted by comparing the inhibition of microbial growth using measured concentrations of the test antimicrobial agents against a standard antibiotic with known activity. Three bacterial strains were selected as test organisms: *Escherichia coli* (MTCC 1573), *Staphylococcus aureus* (MTCC 1430), and *Pseudomonas aeruginosa*, to evaluate the antibacterial spectrum of the test compounds **[14, 17, 18]**. Growth media were prepared according to the instructions provided in the package insert. The test organisms, *Escherichia coli* (MTCC 1573), *Staphylococcus aureus* (MTCC 1430), and *Pseudomonas aeruginosa*, were inoculated in nutrient broth. A specific volume of this bacterial suspension was mixed with nutrient agar, cooled to 40°C, and poured into Petri dishes to achieve a uniform thickness **[1, 11-17]**.

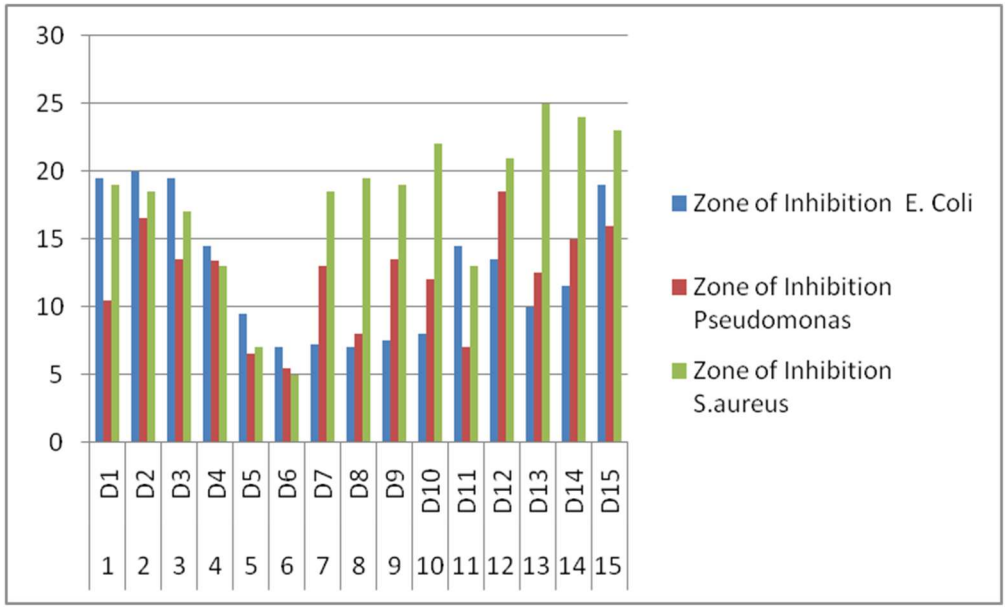
The surface of the agar plates was punctured using a sterile cork borer to create wells. These wells were then filled with equal volumes of antimicrobial agent solutions (D1–D15). After a pre-incubation diffusion period, the plates were incubated face-up for a specific duration at the optimal temperature for each bacterial strain. The diameters of the zones of inhibition were measured to assess the antimicrobial activity of the test compounds **[11-13]**.

Table 1: Antibacterial Activity of Isatin Derivatives

Sr. No.	Compounds	Zone of Inhibition		
		E. coli	*Pseudomonas*	*S.aureus*
1	D1	19.5	10.5	19
2	D2	20	16.5	18.5
3	D3	19.5	13.5	17
4	D4	14.5	13.4	13
5	D5	9.5	6.5	7
6	D6	7	5.5	5
7	D7	7.2	13	18.5
8	D8	7	8	19.5
9	D9	7.5	13.5	19
10	D10	8	12	22
11	D11	14.5	7	13
12	D12	13.5	18.5	21
13	D13	10	12.5	25
14	D14	11.5	15	24
15	D15	19	16	23

To perform an ANOVA on this data, data is organized in Treatment groups: The samples (D1, D2, D3... D15) are the different treatment groups that are being tested and Measurements: The zone of inhibition in millimeters for each of the three test organisms, *E.coli, Pseudomonas,* and *S.aureus*, are the variables. Null Hypothesis (H0): There is no significant difference in the mean zone of inhibition between the samples

for a specific microorganism. Alternative Hypothesis (H1): There is a significant difference in the mean zone of inhibition between at least two of the samples for a specific microorganism. In above result *F* crit < *F* so, we reject null hypothesis and accept alternative.



Graph 1: Zone of Inhibition

3. RESULT AND DISCUSSION

The synthesized isatin derivatives demonstrated significant antimicrobial potential, exhibiting activity against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacterial. Molecular docking studies revealed effective interactions with targets like the SARS-CoV-2 main protease, primarily through hydrogen bonding and hydrophobic interactions, suggesting a mechanism for their biological activity. Structural modifications at the C2, C3, and N positions were found to enhance the efficacy of the derivatives, highlighting the importance of chemical modifications. The antimicrobial activity varied across the different derivatives, as shown by the zones of inhibition, with some, like D15, D14, and D13, displaying high activity against *S. aureus*, and D2 showing good results against *E. coli* and *P. aeruginosa*. Statistical analysis through ANOVA confirmed significant differences in the mean zone of inhibition among the tested derivatives. Furthermore, molecular docking scores ranging from -4 to -6 against the SARS-CoV-2 main protease, suggest a good binding affinity for the target

enzyme, supporting their potential as antimicrobial agents.

4. CONCLUSION

The synthesized isatin derivatives exhibit significant antimicrobial activity against both Gram-positive and Gram-negative bacteria. Molecular docking studies showed effective interactions with the SARS-CoV-2 main protease. Structural modifications enhance efficacy. Derivatives D15, D14, and D13 were highly active against *S. aureus*, while D2 was effective against *E. coli* and *P. aeruginosa.* These findings suggest that these isatin derivatives are promising candidates for further development as therapeutic agents, and molecular hybrids of isatin could potentially inhibit EGFR

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Conflict of Interest: Authors do not have Conflict of Interest

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