ejpmr, 2025, 12(10), 264-275

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN (O): 2394-3211

ISSN (P): 3051-2573

SYNTHESIS AND EVALUATION OF NOVEL ISATIN DERIVATIVES FOR ANTIMICROBIAL ACTIVITY

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DOI: https://doi.org/10.5281/zenodo.17275381

Article Received on 14/08/2025 **Article Revised on 04/09/2025** **Article Accepted on 25/09/2025**

ABSTRACT

This study details the synthesis of 1-benzyl indoline-2,3-dione and its derivatives through a series of chemical reactions, followed by their characterization and evaluation of biological activities. The method starts with the reaction of indoline-2,3-dione with benzyl chloride, leading to 1-benzyl indoline-2,3-dione, which undergoes further transformations to form ethyl 2-(1-benzyl-2-oxoindolin-3-ylidene)acetate and acetohydrazide derivatives. These compounds were characterized by their physical properties, melting points, and solubility profiles, with further analysis through Thin Layer Chromatography (TLC), IR, and NMR spectroscopy. The biological activity of the synthesized compounds was evaluated for antibacterial and antifungal properties, revealing significant inhibition against various pathogens. These results indicate the potential of these compounds as bioactive agents, with compound B showing promising antimicrobial effects.

KEYWORDS: 1-benzyl indoline-2,3-dione, acetohydrazide derivatives, antimicrobial activity, antibacterial, antifungal, NMR, IR spectroscopy.

1. INTRTODUCTION

"Chemistry is the science of molecules and their transformations. It is the science not so much of the one hundred elements but of the infinite variety of molecules that may be built from them". Science can be viewed as a containing human effort to systematize knowledge for describing and understanding nature. That present in nature and changes in them in daily life. curd formation from milk , formation of vinegar from sugarcane juice on keeping for prolonged time and rusting of iron are some of the examples of changes which we come across many times. For the sake of convenience, science is sub-divided into various disciplines: chemistry, physics, biology, geology, etc. The branch of science that studies the preparation, properties, structure and reactions of material substances is called chemistry.

1.1 Branches Of Chemistry

- **Organic chemistry:** It is concerned with the study of most carbon based compounds.
- **Inorganic chemistry:** It deals with considered to organic, which may contain any of over 100 elements (including carbon).
- **Physical chemistry:** It deals with the application of physical laws to chemical change and chemical systems.
- **Biochemistry:** It concerned with the chemistry of life processes and living organisms.
- **Analytical chemistry:** It concerned mainly with the various techniques and laboratory methods to determine the composition of matter.

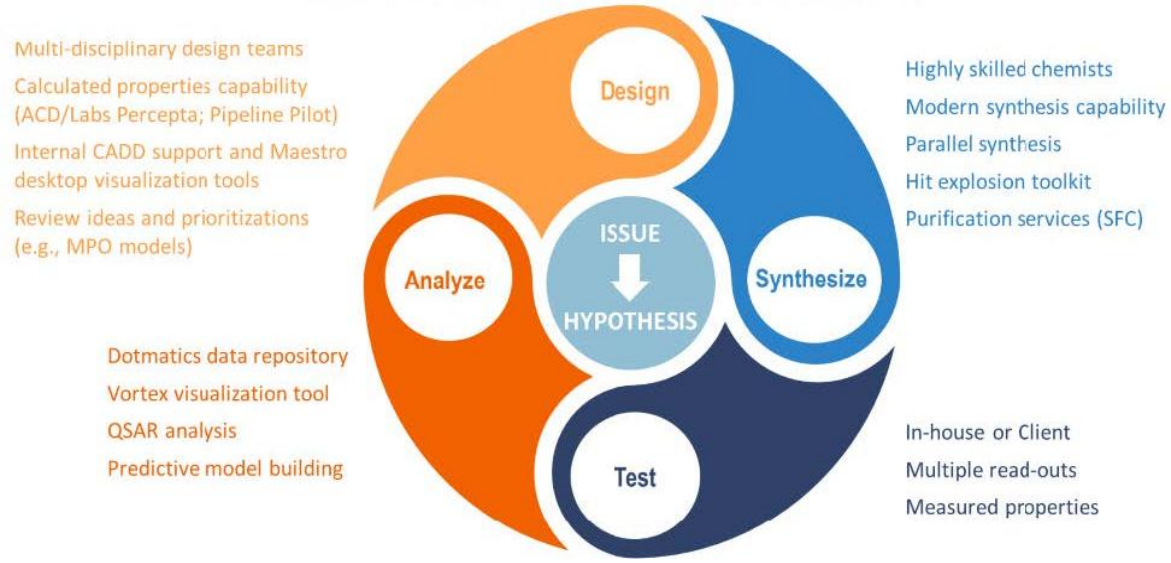


Fig. 1: Medicinal Chemistry.

1.2 Medicinal Chemistry

Medicinal Chemistry is the science that deals with the discovery or design of new therapeutic chemicals and their development into useful medicines. It may involve synthesis of new compounds, investigations of their relationships between the structure of natural or synthetic compounds and their biological activities, elucidations of their interactions with receptors of various kinds, including enzymes and DNA, the determination of their absorption, transport, and distribution properties, and studies of the metabolic transformations of these chemicals into other chemicals. Medicinal chemistry, in its crudest sense, has been practiced for several thousand years. Man has searched for cures of illnesses by chewing herbs, berries roots, and barks. Some of these early clinical trials were quite successful, however, not until the last 100 year has knowledge of the active constituents of these natural sources been known The earliest written records of the Chinese, Indian, South American, and Mediterranean cultures described the therapeutic effects of various plant concoctions. If the approach to drug discovery continued as in ancient times, few diseases would be treatable today. Natural products make up a small percentage of drugs on the current market. Typically, when a natural product is found to be active, it is chemically modified in order to improve its properties. As a result of advances made in synthesis and separation methods and biochemical techniques since the late 1940s, a more rational approach to drug.

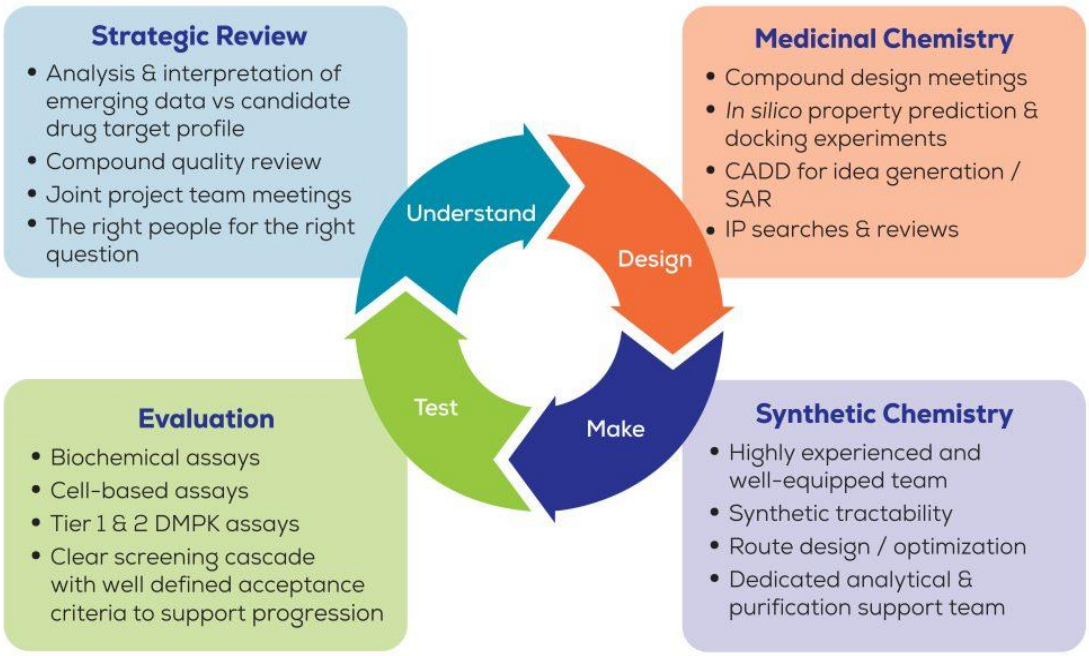


Fig. 2: Drug Design.

1.3 Isatin

Isatin (1H-indole-2, 3- dione) are synthetically versatile substrates, where they can be used for the synthesis of a large variety of heterocyclic compounds, such as indole and quinoline and as raw material for drug synthesis. Isatin have also been found in mammalian tissue and their function as a modulator of biochemical processes has been the subject of several discussions. The advances in the use of isatin for organic synthesis during the last twenty-five years. (Isatin (1*H*-indole-2, 3- dione) was first discovered by Erdmann and Laurent in 1841, independently as a product from oxidation of indigo by nitric and chromic acids. Isatin (1*H*-indole-2, 3- dione) is a versatile molecule and possesses wide range of biological activities.

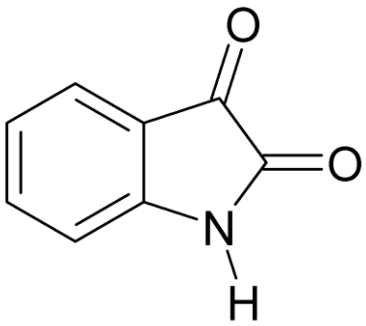


Fig. 3: Structure of Isatin.

Isatin, a heterocyclic compound, is known for its distinct chemical structure and several notable physicochemical properties. Here's an overview of its key properties:

a) Chemical Structure

- **IUPAC Name:** 1H-Indole-2,3-dione
- **Molecular Formula:** $C_8H_7NO_2$
- **Molar Mass:** 161.15 g/mol
- **Structure:** It is an indole derivative with a ketone group attached to the 2-position of the indole ring, making it an aromatic heterocycle.

b) Physical Properties

- **Appearance:** Yellow crystalline solid.
- **Melting Point:** 172–174 °C.
- **Boiling Point:** Around 400 °C (decomposes before boiling).
- **Density:** 1.33 g/cm³.
- **Solubility:** Isatin is moderately soluble in water (about 2.5 g/L), more soluble in polar solvents like ethanol, methanol, and acetone, but is generally insoluble in non-polar solvents like benzene or chloroform.
- **Odor:** Odorless or faintly aromatic.

2 Chemical Properties

- **Acidity (pKa):** The pKa values for isatin suggest that it behaves as a weak acid. This is due to the acidic nature of the NH group and the carbonyl group in the structure.

c) Spectroscopic Properties

- **UV-Vis Absorption:** Isatin shows characteristic UV absorption maxima in the range of 280–300 nm, associated with π-π* transitions in the aromatic ring.
- **Infrared (IR):** absorption bands typically appear around: 3200–3400 cm^{-1} (N-H stretch),
- 1700 cm^{-1} (C=O stretch, carbonyl group).
- **Nuclear Magnetic Resonance (NMR): ^{1}H NMR:** Peaks associated with the aromatic protons (around 7–8 ppm), along with a broad singlet for the NH group. **13C NMR:** Peaks around 120–140 ppm for the carbons in the aromatic ring and around 180 ppm for the carbonyl group.
- **Mass Spectrometry:** The molecular ion peak (M+) appears at **161 m/z**, consistent with the molecular weight of isatin.

d) Thermal Properties

- **Stability:** Isatin is relatively stable under normal conditions but can decompose when heated to high temperatures (>400 °C).
- **Decomposition:** Upon heating, isatin may decompose, releasing toxic fumes like nitrogen oxides and carbon monoxide.

e) Biological Properties

- **Pharmacology:** Isatin exhibits a variety of biological activities including antimicrobial, antiviral, anticancer, and anti-inflammatory effects. This has spurred its use in pharmaceutical research, particularly in the development of drugs targeting various conditions.
- **Toxicity:** Generally, isatin is not considered highly toxic, but as with many organic compounds, prolonged exposure or high doses could have adverse effects.

f) Synthesis

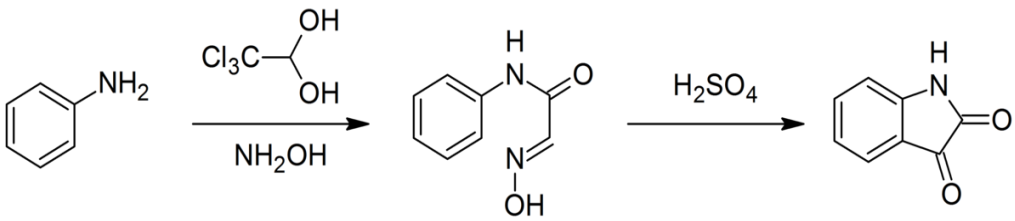


Fig. 4: Synthesis of Isatin.

2. SCHEME OF WORK

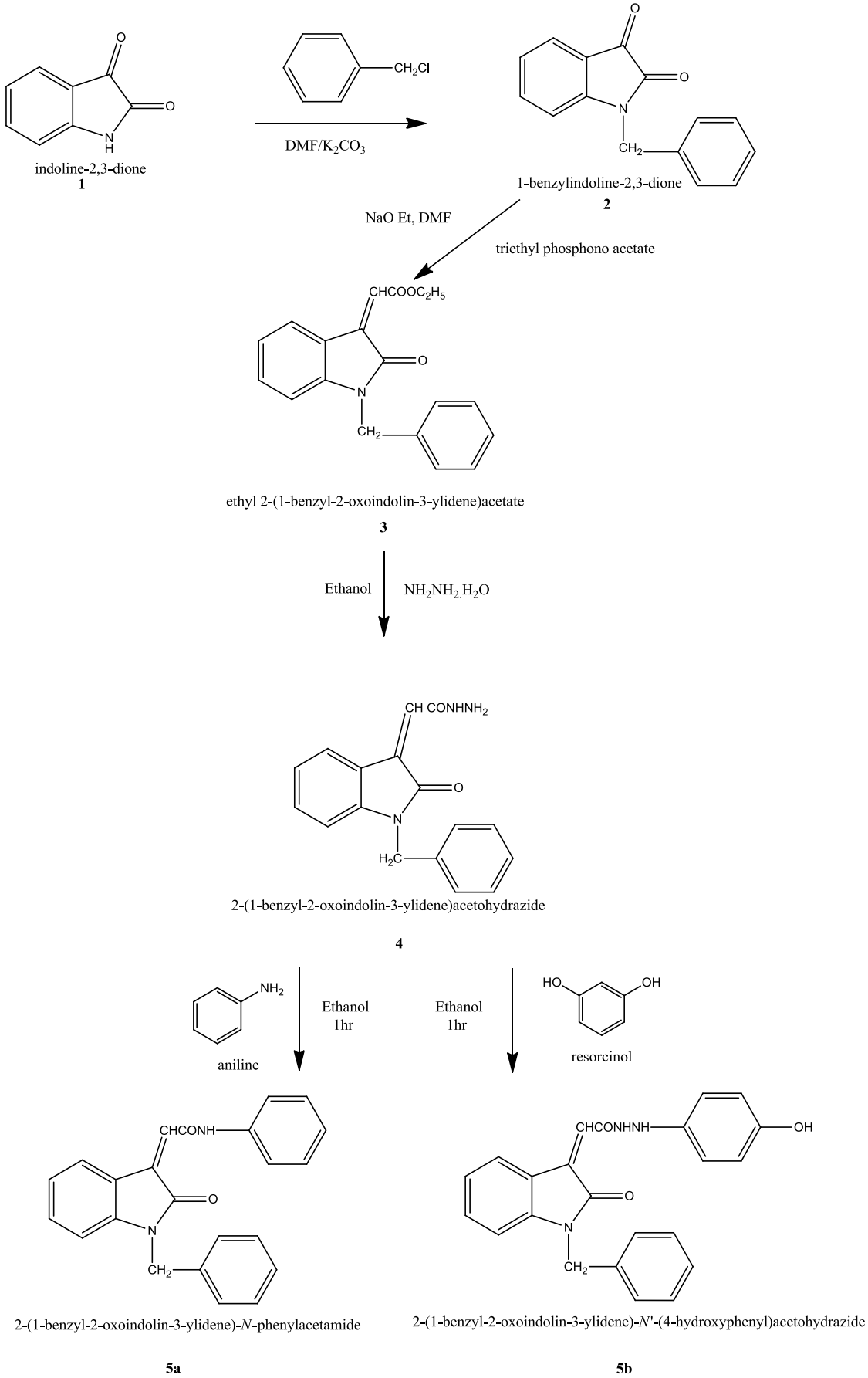
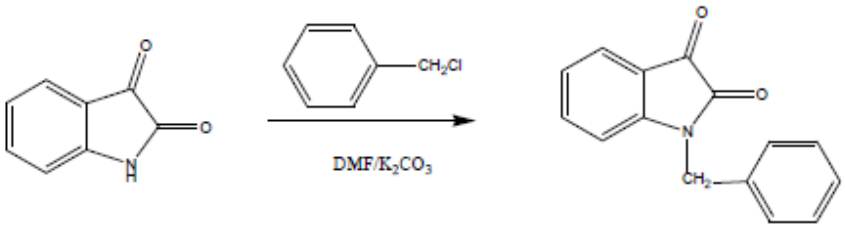


Fig. 5: Scheme of Work.

1. Method of preparation of 1-benzyl indoline-2, 3-dione from indoline- 2,3-dione (2)

In the round bottom flask take indole–2, 3-dione (isatin) 0.8 gm. (0.00337M) and equimolar quantity of benzyl chloride i.e. 6.5 ml (0.00337), mix with 20 ml of Di-methyl formamide (DMF), and this mixture add 2 gm of potassium carbonate (K2CO3). After gentle mixing of this reaction mixture, reflux for 2 hour, cool, and pour to 100 ml of ice cold water. The resultant orange red precipitate collected wash with water and dried and recrystallised from acetonitrile.

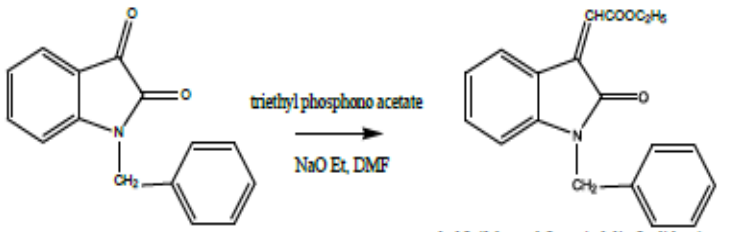


indoline-2,3-dione
1

1-benzylindoline-2,3-dione
2

2. Synthesis of ethyl 2-(1-benzyl-2-oxoindolin-3-ylidene)acetate (3)

Equimolar quantities of 1-benzyl indoline-2, 3-dione (20 mmol), triethylphosphono acetate (20 mmol), Sodium Ethoxide (20 mmol) and in DMF (20 ml) was kept overnight at room temperature. The reaction mixture was refluxed on a steam bath for about 3 hr. It was cooled filtered recrystallized with ethanol.

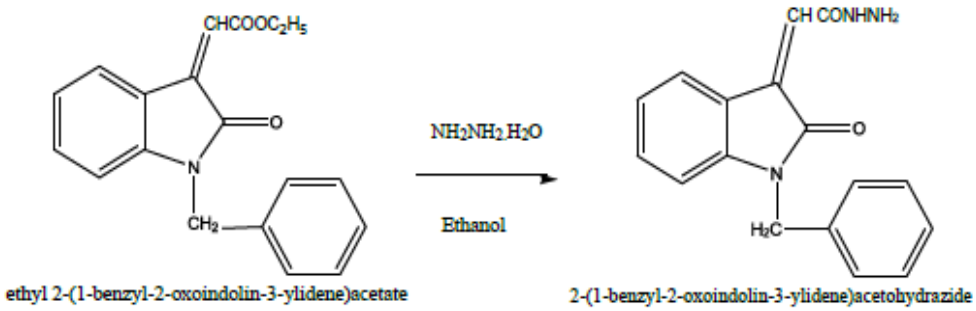


1-benzylindoline-2,3-dione
2

ethyl 2-(1-benzyl-2-oxoindolin-3-ylidene)acetate
3

3. Synthesis of 2-(1-benzyl-2-oxoindolin-3-ylidene) acetohydrazide (4)

A mixture of compound **3** (20 mmol) and hydrazine hydrate (100%; 20 mmol) in dry ethanol (40 ml) was refluxed on a steam bath for 6 hrs the excess solvent was removed under reduced pressure and the product crystallized from ethanol to obtain compound 2-(1-benzyl-2-oxoindolin-3- ylidine) acetohydrazide **4**.

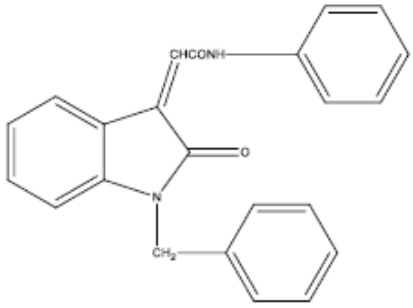


ethyl 2-(1-benzyl-2-oxoindolin-3-ylidene)acetate
3

2-(1-benzyl-2-oxoindolin-3-ylidene)acetohydrazide
4

4. Synthesis of 2-(1-benzyl-2-oxoindolin-3-ylidene) acetohydrazide derivatives (A) & (B)

A mixture of compound **4** (30 mmol) and Aniline (a), Resocinol (b) (5 mmol) in dry ethanol (40 ml) was refluxed on a steam bath for 1 hrs the excess solvent was removed under reduced pressure and the product crystallized from ethanol.

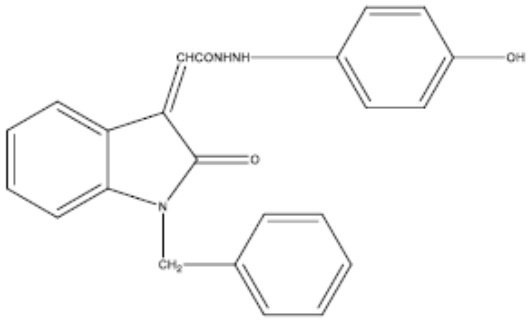


2-(1-benzyl-2-oxoindolin-3-ylidene)-*N*-phenylacetamide

Chemical Formula: $C_{23}H_{18}N_2O_2$

Molecular Weight: 354.40

COMPOUND A



2-(1-benzyl-2-oxoindolin-3-ylidene)-*N'*-(4-hydroxyphenyl)acetohydrazide

Chemical Formula: $C_{23}H_{19}N_3O_3$

Molecular Weight: 385.42

COMPOUND B

Fig. 6: Compound A & B.

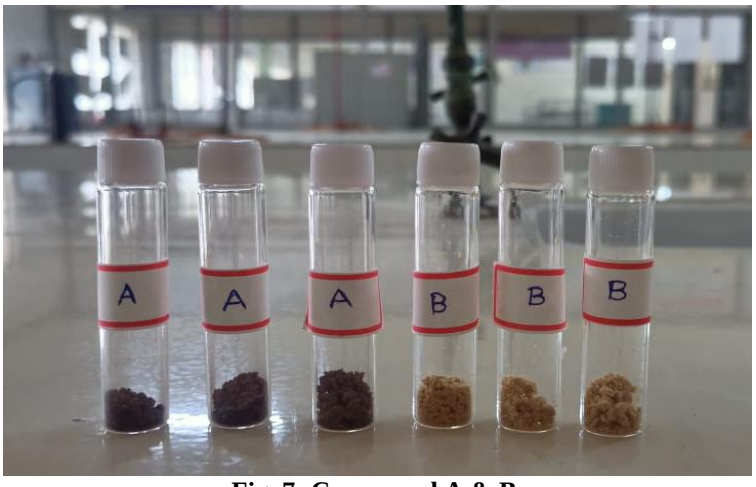


Fig. 7: Compound A & B.

3. MATERIALS AND METHODS

3.1 Melting Points & Appearance

Melting points were determined using melting point apparatus and are uncorrected. The melting point physical characterization of all synthesized compounds

3.2 Solubility

At the room temperature, solubility of all synthesized compound was determined different solvent.

3.3 Thin Layer Chromatography

The purity of all synthesized compounds was monitored on TLC.

- Adsorbent used : Pre coated silica gel-G plate
- Mobile phase : Chloroform: Methanol (3:7)
- Detecting technique : Iodine chamber

3.4 Spectral Analysis

The objectives of the spectral analysis is to confirm the chemical structures of the synthesized compounds and the various functional groups in final compounds. The IR spectra of the starting compounds and intermediates where taken to confirm the changes at the reactive functional groups and then final compounds were confirmed by IR, NMR analysis.

3.5 Biological Screening

3.5.1 Evaluation Of Anti Fungal Activity

Preparation Of Agar Medium: Prepare MHA from the dehydrated medium according to the manufacturers instructions. media should be prepared using distilled water or deionized water. Heat with frequent agitation and boil to dissolve the medium completely. Sterilized by autoclaving at 121:c for 15minutes. Check the PH of each preparation after it is sterilized, which should be between 7.2 and 7.4 at room temperature. This is done by macerating a small amount of medium in a little distilled water or by allowing a little amount of medium to gel around a PH meter electrode. Cool the agar medium to 40 to 50:c. Pour the agar into sterile glass or plastic petri dish on a flat surface to a uniform depth of 4mm. Allow to solidified. Prior to use, dry plates at 30-37:c in an incubator, with lids partly agar, for not more than 30minutes or until excess surface moisture have evaporated. media must be moist but free of water droplets on the surface. presence of water droplets may result to swarming bacterial growth, which could give in accurate results. They are also easily contaminated.

Inoculum Preparation: From a fungal culture (not more than 48 hours, old except for slow growing organism) take 4 or 5 colonies with a wire loop. Transfer colonies to 5ml of trypticase soy broth or 0.9% saline. Incubate the broth at 30:c at an optimum growth temperature until it achieves or exceeds the turbidity of 0.5 macfarland standard (prepared by adding 0.5ml of 0.048m Bacl2 to 99.5ml of 0.36 NH2SO4; commercially available). Compare the turbidity of the test bacterial suspension with that of 0.5 macfarland (vigorously shaken before use) against a white background with contrasting black line under adequate light. Arrow points to tube with correct turbidity. Reduce turbidity by adding sterile saline or broth.

Inoculation of Plates

- Dip a sterile cotton swab into the standardized fungal suspension.
- Remove excess inoculum by lightly pressing the swab against the tube wall at a level above that of the liquid.
- Innoculate the agar by streaking with the swab containing the inoculum.
- Rotate the plate by 60:c and repeat the rubbing procedure. Repeat two times. This will ensure an even distribution of the inoculum.
- Allow the surface of the medium to dry for 3-5 minutes but not longer than 15 minutes to allow for absorption of excess moisture.

3.5.2 Evaluation Of Anti Bacterial Activity

The bacterial strains were sub cultured to get fresh cultures of bacteria for this purpose, a single colony from bacterial strain was inoculated on nutrient broth. The broth was incubated for 24 h at 37 °C. 14 gm of nutrient agar media was dissolved in 1 L of distilled water at PH 7 and autoclaved for 20 min at 121 °C. The media were allowed to cool down to 45 °C and poured to petri plates for preparing 75 ml of solid media. using sterile cork borer 7 wells per plate were made in the solidified media. Agar diffusion method was used for antibacterial activity. Bacterial culture was inoculated on the surface of solid media. The crude synthesized compound A and B afractions were dissolved in dimethylsulfoxide (DMSO) at the same concentration of 2 mg/ml to prepare stock solutions. from the stock solution, 1000 µl was poured into respective wells. Ciprofloxacin was used as a positive control and DMSO was used as a negative control. The zone of inhibition of crude extract and fractions were measured in mm after 24 h of incubation at 37 °C and compared with the zone of inhibition of standard drug Ciprofloxacin.

4. RESULTS AND DISCUSSION

4.1 Physical Characterization

Table 1: Physical Characterization.

S.NO.	CODE	MOLECULAR FORMULA	MOLECULAR WEIGHT	COLOR	NATURE	PERCENTAGE YIELD
1	1	$C_8H_5NO_2$	147.13	Reddish brown	Powder	-
2	2	$C_{15}H_{11}NO_2$	237.25	Reddish brown	Crystal	91.55%
3	3	$C_{19}H_{17}NO_3$	307.34	Orange	Crystal	85.31%
4	4	$C_{17}H_{15}N_3O_3$	293.32	Orange	Crystal	87.35%
5	A	$C_{23}H_{18}N_2O_2$	354.40	Brown	Crystal	88.91%
6	B	$C_{23}H_{19}N_3O_3$	385.42	Light Orange	Crystal	93.45%

4.2 Physical Properties

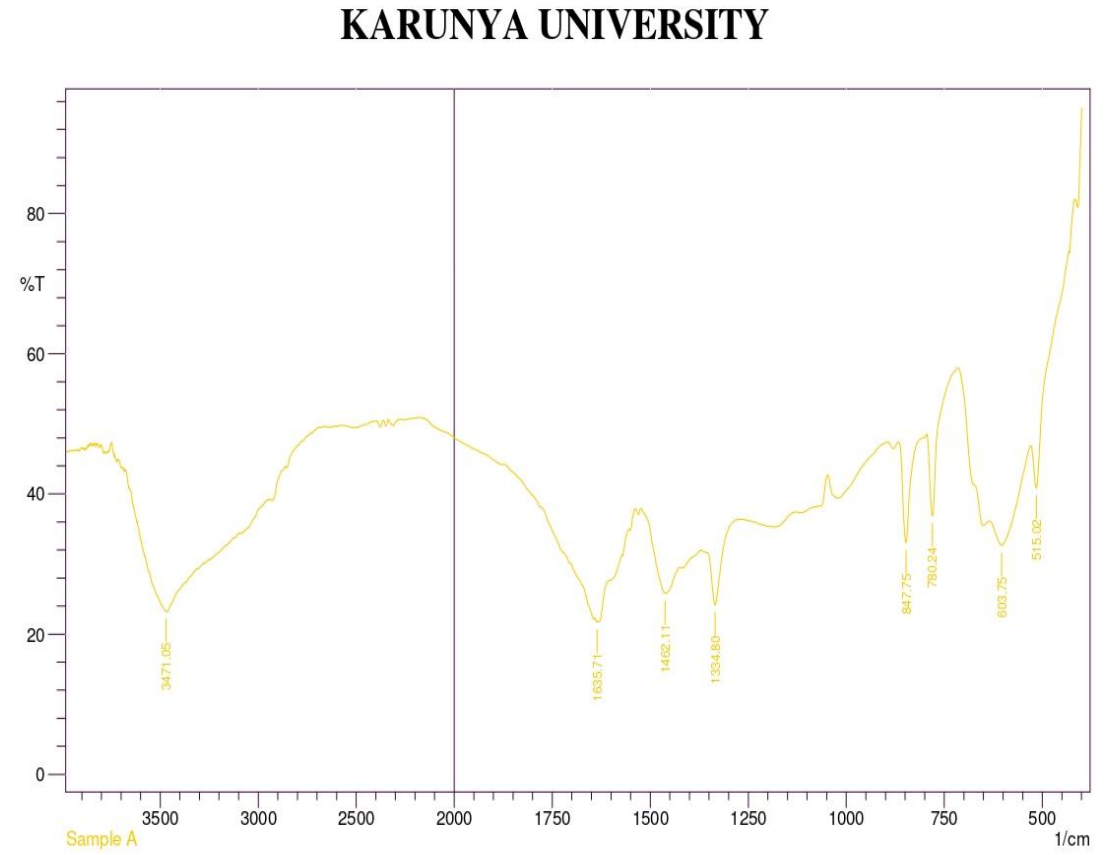
Table 2: Physical Properties.

CODE	MELTING POINT	RF VALUE	METHANOL	ETHANOL	CHLOROFORM	DMF	DMSO
1	145-149	0.61	+	+	+	+	+
2	127-130	0.75	+	+	+	+	+
3	145-148	0.71	+~	+~	+~	+~	+~
4	136-140	0.69	+~	+~	+~	+~	+~
A	131-135	0.65	+~	+	+~	+	+
B	136-140	0.68	+~	+	+~	+	+

Where
+ → soluble,
- → insoluble,
~ +→ slightly soluble

4.3 Spectral Conformation

4.3.1 IR Spectrum Analysis



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	515.02	40.841	10.179	529.48	432.07	23.27	1.076
2	603.75	32.675	6.628	634.61	529.48	44.969	4.454
3	780.24	36.855	13.373	794.71	714.66	23.896	1.853
4	847.75	33.072	14.615	867.04	794.71	26.198	3.106
5	1334.8	24.195	8.588	1354.09	1275.97	39.464	2.77
6	1462.11	25.81	6.885	1523.83	1424.49	51.674	4.428
7	893.08	21.746	0.168	1641.49	1633.78	5.084	0.015
8	3471.05	23.286	0.04	3492.27	3470.09	13.887	0.029

Fig. 8: IR Spectrum of Compound A.

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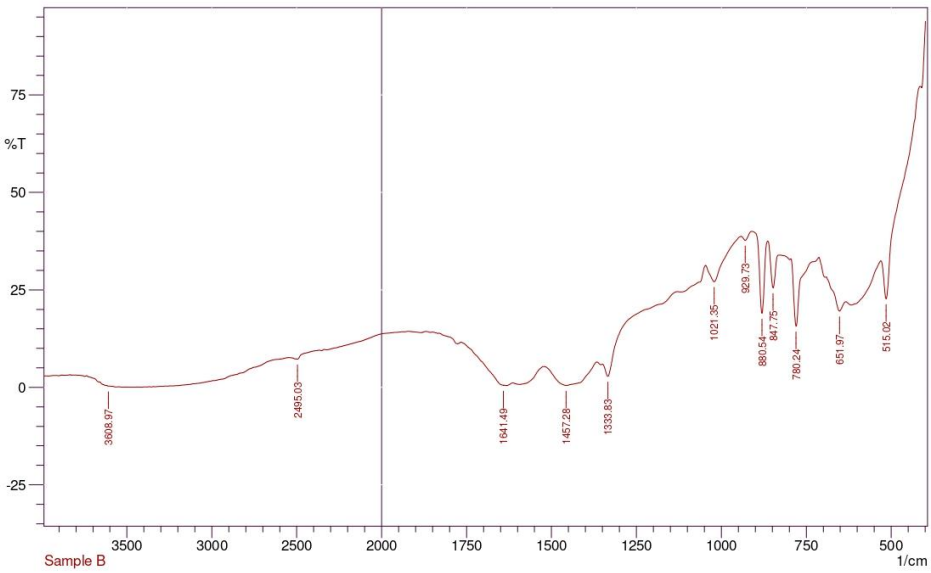

No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	515.02	22.724	15.699	530.45	414.71	38.221	3.437
2	651.97	19.564	4.406	691.51	633.64	36.91	2.004
3	780.24	15.665	17.126	795.67	731.05	37.161	5.698
4	847.75	25.515	10.347	863.18	829.43	17.158	2.044
5	880.54	18.983	19.447	909.48	863.18	23.197	4.124
6	929.73	37.723	1.503	942.27	909.48	13.515	0.24
7	1021.35	27.097	5.98	1046.43	942.27	50.791	3.057
8	1333.83	2.842	4.52	1350.23	1130.33	178.921	-22.676
9	1457.28	0.5	5.33	1520.94	1366.62	267.102	77.454
10	1641.49	0.512	0.161	1765.91	1640.53	166.726	-31.71
11	2495.03	7.229	0.233	2503.71	2367.72	144.09	-2.522
12	3608.97	0.298	0.055	3624.4	3606.08	44.064	0.17

Fig. 9: IR Spectrum of Compound B.

4.3.2 H1NMR Spectrum Analysis

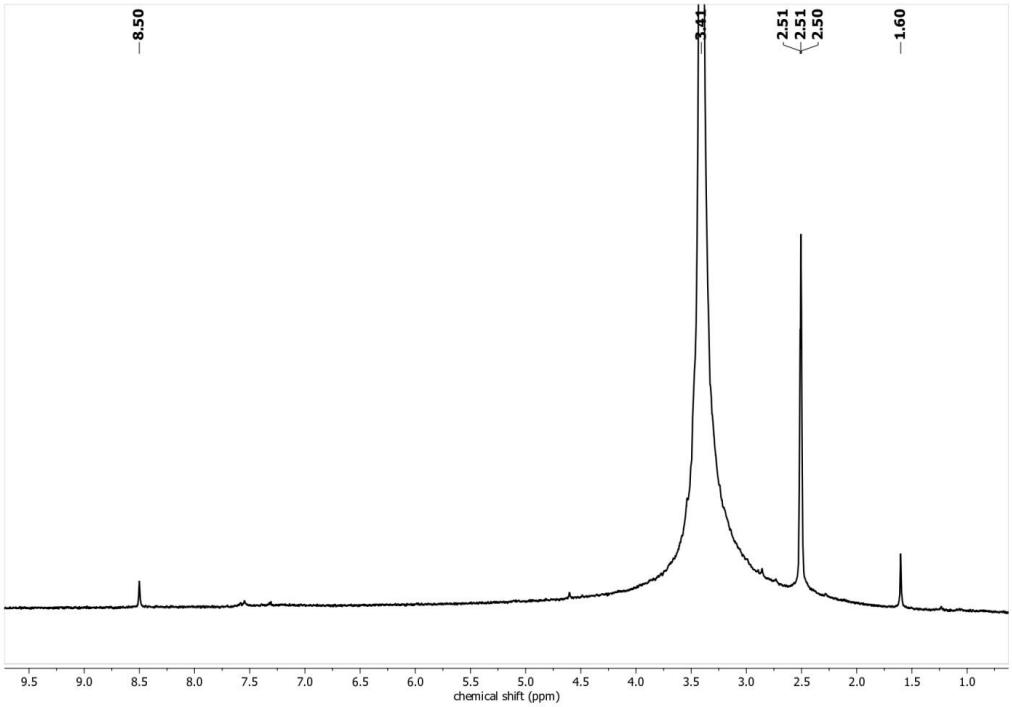

Fig. 10: NMR Spectrum of Compound A.

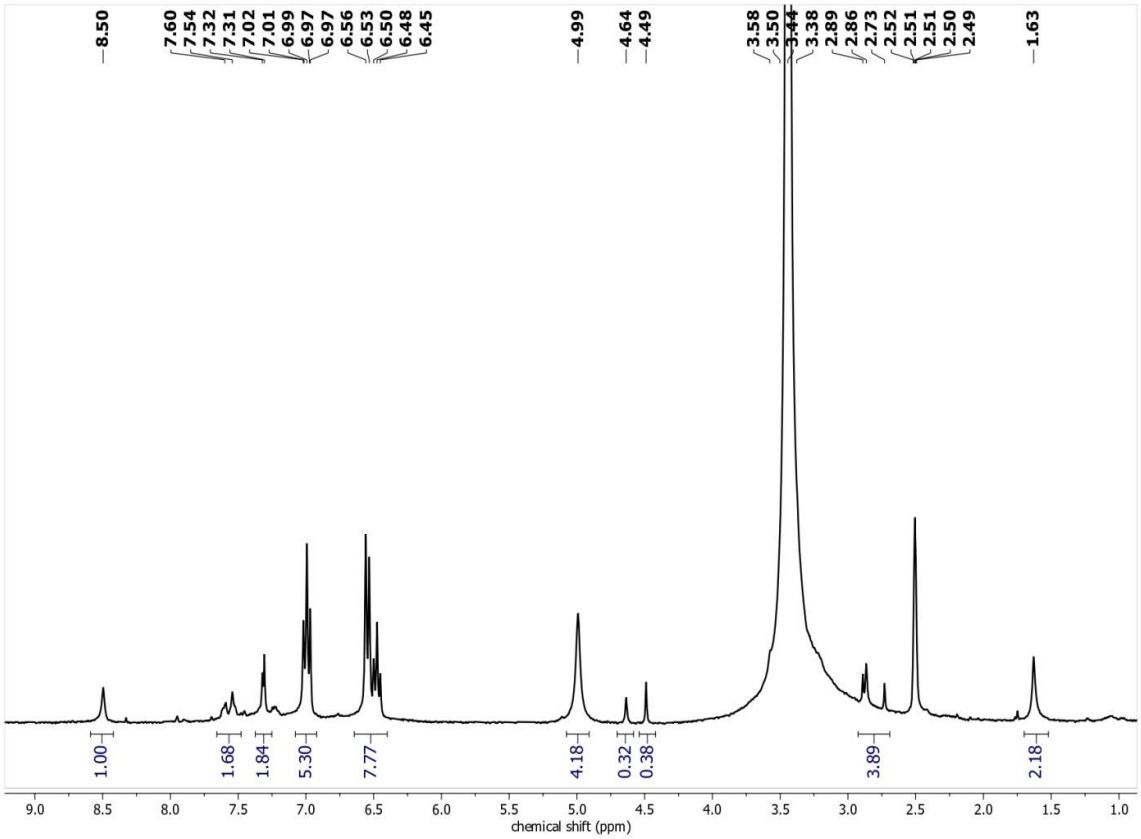

Fig. 11: NMR Spectrum of Compound B.

4.4 Biological Screening

4.4.1 Anti – Bacterial Activity

Table 3: Anti – Bacterial Activity.

S.NO	Microorganisms	Control	A	B	Ciprofloxacin
		Zone of inhibition in mm			
1.	***Bacillus cereus***	-	10	12	35
2.	***Escherichia coli***		08	15	32

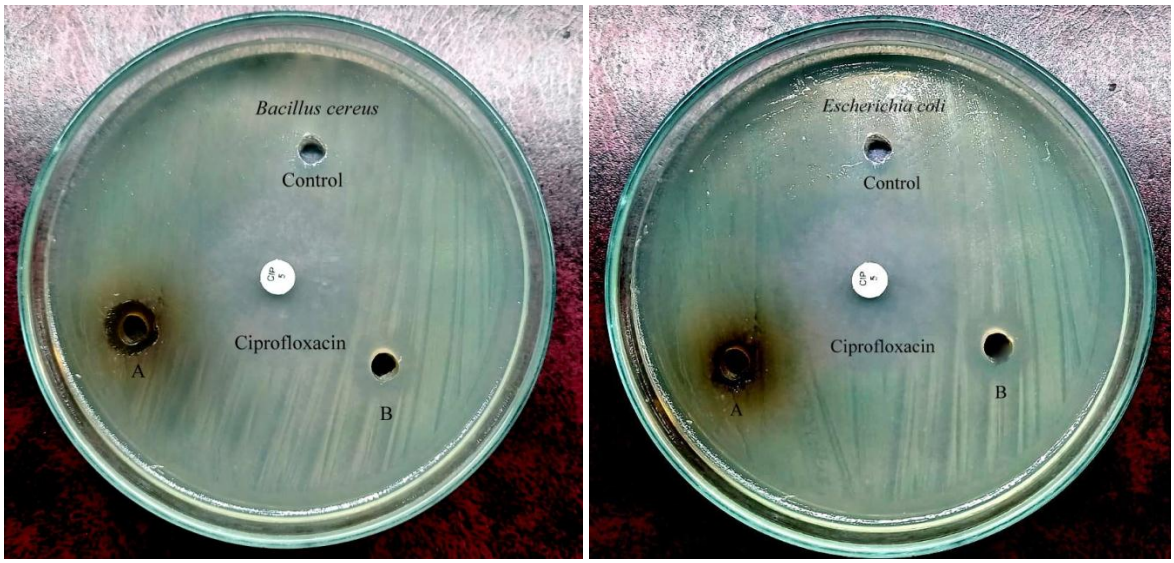

Fig. 12: Anti – Bacterial Activity.

4.4.2 Anti – Fungal Activity

Table 4: Anti – Fungal Activity.

S.NO	Microorganisms	Control	A	B	Amphotericin -B
		Zone of inhibition in mm			
1.	***Trichpphyton rubrum***	**-**	**21**	**42**	**16**
2.	***Fusarium oxysporum***		**07**	**55**	**14**

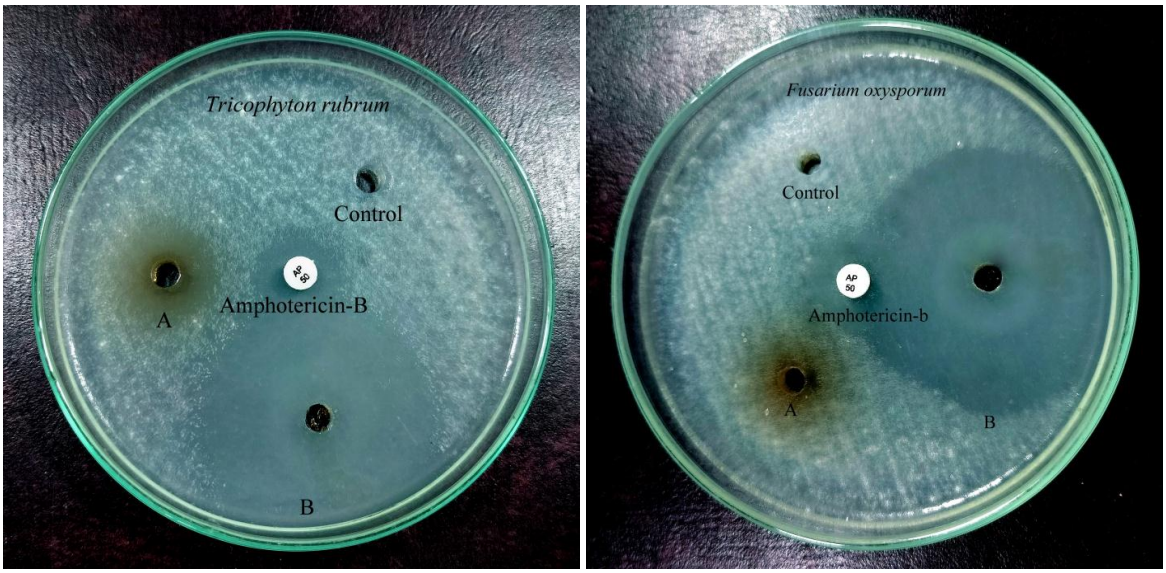

Fig. 13: Anti – Fungal Activity.

DISCUSSION

The synthesis of 1-benzyl indoline-2,3-dione and its derivatives involved several key steps, each carefully monitored for purity and yield. The initial reaction between indoline-2,3-dione and benzyl chloride, facilitated by potassium carbonate in DMF, resulted in a high yield of 1-benzyl indoline-2,3-dione (91.55%). This intermediate was then reacted with triethylphosphono acetate, sodium ethoxide, and DMF to yield ethyl 2-(1-benzyl-2-oxoindolin-3-ylidene)acetate with an 85.31% yield. Further reaction with hydrazine hydrate produced acetohydrazide derivatives, which were characterized by their color, solubility, and spectral data.

The compounds were subjected to various physical characterization methods, including melting point determination and TLC analysis, to assess their purity and physical properties. The IR and NMR spectra confirmed the chemical structures of the final products, providing additional evidence for their successful synthesis. The biological screening demonstrated that compound B exhibited significant antibacterial and antifungal activity, particularly against Escherichia coli and Trichophyton rubrum, suggesting its potential for therapeutic applications. Compounds A and B, in particular, displayed moderate inhibition zones, comparable to the standard drugs ciprofloxacin and amphotericin-B.

5. CONCLUSION

The synthesized 1-benzyl indoline-2,3-dione derivatives, including ethyl 2-(1-benzyl-2-oxoindolin-3-ylidene)acetate and its acetohydrazide derivatives, were successfully prepared and characterized. The results of biological screening revealed that these compounds possess significant antibacterial and antifungal activities, with compound B showing the most promising results in inhibiting microbial growth. This study highlights the potential of indoline-2,3-dione derivatives as lead compounds for the development of novel antimicrobial agents, warranting further investigation into their mechanisms of action and therapeutic potential.

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