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Novel Bis-Schiff Base Derivatives Of Isatin And Acenaphthoquinone: Synthesis, Spectral Characterization And Antibacterial Investigations

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Abstract
Bacterial infections, including drug-resistant strains, are a significant health threat. This study explores the antibacterial potential of acenaphthoquinone and isatin derivatives. Isatin and acenaphthoquinone were reacted with diamino alkane derivatives through a nucleophilic elimination reaction using PTSA as a catalyst in ethanol. The aim was to investigate the enhanced antibacterial activity, with the goal of developing new antibiotics to combat bacterial infections in humans. In the present study, two novel Schiff base derivatives, 5a and 5b, were synthesized and evaluated for their in-vitro antibacterial potential against Escherichia coli and Bacillus subtilis. Compound 5b exhibited superior antibacterial activity compared to 5a, particularly against E. coli, where it showed a maximum zone of inhibition of 5.33 ± 0.58 mm at 100 µg/mL, as compared to 2.33 ± 0.58 mm for 5a. A similar trend was observed against B. subtilis, where 5a demonstrated slightly better activity at higher concentrations. The synthesized compounds were characterized using FT-IR, and NMR. Our findings suggest that these synthesized compounds have potential antibacterial activity and warrant further investigation against other bacterial strains in future studies.
Keywords: *Acenaphthoquinone; Isatin; Imines, Oxindole; Synthesis; Antibacterial*

INTRODUCTION

Bacterial infections, caused by both Gram-positive (e.g., *Staphylococcus aureus* (S. aureus), *Staphylococcus epidermidis* (S. epidermidis), *Streptococcus pneumoniae* (S. pneumoniae), *Bacillus subtilis* (*B. subtilis*) and Gram-negative pathogens (e.g., *Escherichia coli* (*E. coli*), account for the majority of infections in human [1,2]. Furthermore, bacteria have developed resistance to nearly all current antibiotics due to the prolonged, widespread, and often improper use of these drugs, worsening the global situation. Each year, approximately 0.7 million deaths are attributed to drug-resistant pathogens, and this number could rise to 10 million by 2050,if current trends persist[3]. Consequently, there is an urgent need to develop new antibiotics effective against both drug-sensitive and drug-resistant bacteria. Acenaphthylene-1, 2-dione or acenaphthoquinone is a water insoluble yellow solid which is used as a precursor in the synthesis of agrochemicals and dyes. If the chemical undergo oxidation, reduction, protonation, elimination or other reactions, various derivatives of acenaphthoquinone are produced. These derivatives possess various pharmacological activities like antibacterial and antifungal [4]. On comparing the activities of acenaphthoquinone with other compounds that have oxygen and nitrogen-containing heterocyclic compounds, it was inferred as both compounds have different biological applications therefore each compound is used for unique activities [5]. Acenaphthoquinone derivatives are most probably studied for their antitumor, antiviral and antimicrobial activities whereas the nitrogen containing heterocyclic compound like Isatin is analysed for their ability on interacting with biological receptors. The exploration of medicinally relevant heterocyclic scaffolds is a key focus in drug discovery. The isatin (1H-indole-2,3-dione, Fig. 1) moiety is widely found in nature, and its derivatives exhibit diverse pharmacological properties, including anti-cancer [6,7], anti-tubercular [8,9], anti-HIV [10,11], anti-malarial [12,13], and anti-bacterial [14–16] activities. As building blocks isatin derivatives can be modified at nearly all positions, with the N-1, C-3, and C-5 being the most common sites for chemical alterations [17]. Some isatin-based compounds, such as semaxanib and indirubin, have already been used in clinical settings or

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are under clinical trials for treating various diseases [18,19]. The broad spectrum of biological activities, coupled with extensive structural modifications and successful clinical applications, has inspired further research into isatins, leading to the creation of a wide array of structurally diverse derivatives. In recent years, various isatin derivatives have been evaluated for their antibacterial properties, with some demonstrating promising in vitro and in vivo efficacy.

If the studies on the synthesized compounds were examined carefully according to the composition of the compounds, there will be development in synthesizing the drugs and therapeutic compounds. Acenaphthylene-1,2-dione, also known as acenaphthoquinone, is a yellow, water-insoluble solid commonly used as a precursor in the synthesis of agrochemicals and dyes. Through oxidation, reduction, protonation, elimination or other chemical reactions, various derivatives of acenaphthoquinone can be produced. These derivatives exhibit a range of pharmacological activities, including antibacterial and antifungal properties. Some of the derivatives synthesized from acenaphthoquinone possess more pharmacological activities [20] like spasmolytic activity, diuretic activity, anti-coagulant activity, anti-anaphylactic activity [20–22], anti-cancer activity [23], cytotoxic activity [24,25] , anti-HIV activity [26], anti-inflammatory activity [27], anti-malarial activity [28], anti-bacterial activity [29], anti-tubercular [30] anti-hyperglycemic activity [31], anti-dyslipidemic activity [32], anti-neurodegenerative activity for diseases like Alzheimer's disease, Parkinson's disease and Huntington's disease [33].

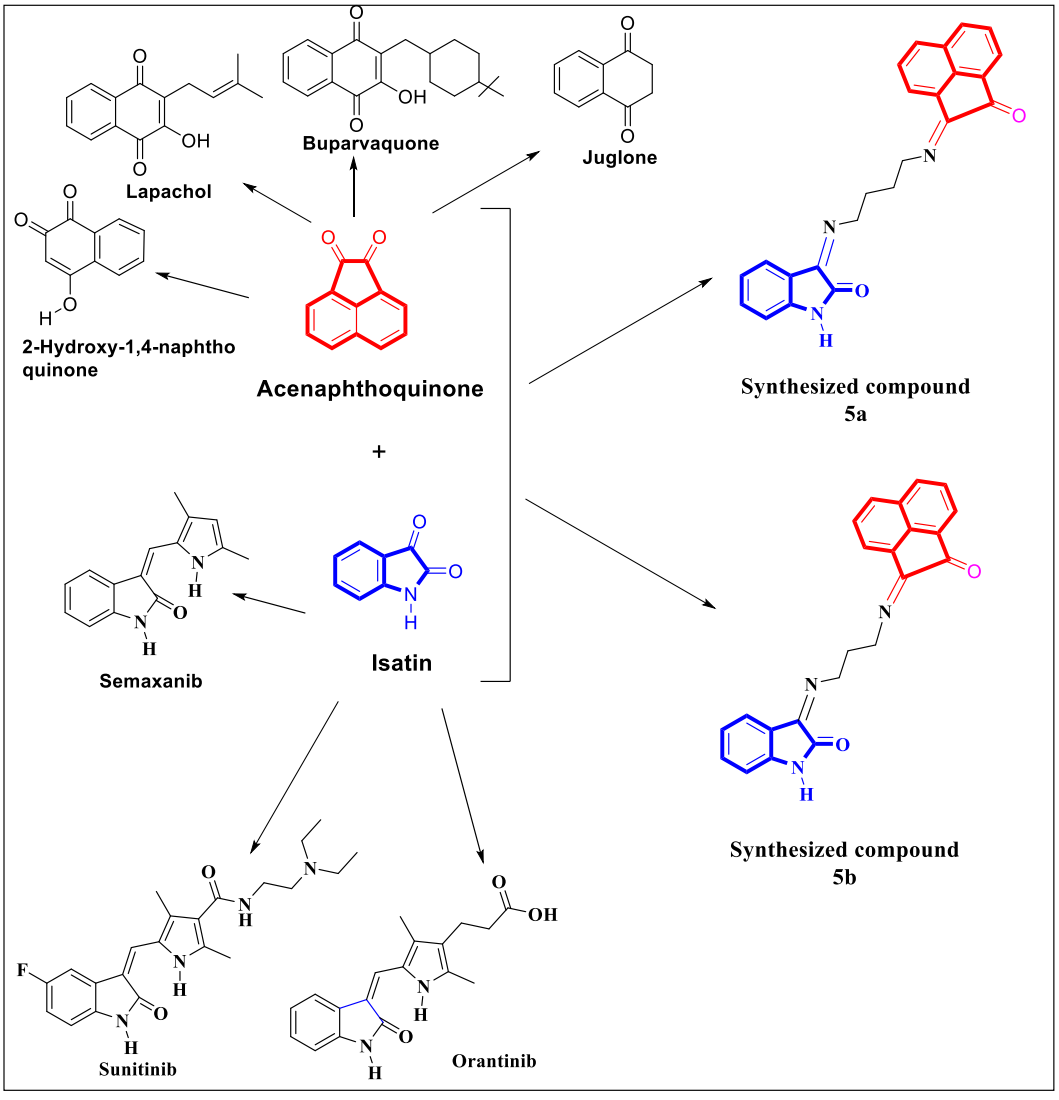


Fig.1. Structural representation of Acenaphthoquinone and Isatin-derived drugs and newly synthesized drug like molecules 5a and 5b

Herein designs an isatin and acenaphthoquinone hybrid (bis-oxindole-imine derivatives) with the potent antibacterial activities (**Figure 1).** In synthesis, highly active reactants named dioxindole, diaminoalkane, and acenaphthoquinone were selected for the synthesis of oxindole-containing bis-Imine

derivatives using nucleophilic elimination reactions. Synthesized derivatives were characterized using, FT-IR, ^{1}H and ^{13}C NMR spectroscopy.

MATERIALS AND METHODS

Materials and Instrumentation

All reagents and chemicals were procured from Sigma-Aldrich and used without further purification. Melting points were determined using a digital melting point apparatus (Gentek-934-35) and are reported uncorrected. Infrared (IR) spectra were recorded using an FT-IR spectrometer (Alpha-II 210966) over the range of 3500–500 cm^{-1} at the IIRC-CIF facility, Integral University, Lucknow-226026, India. Proton (^{1}H) and carbon-13 (^{13}C) NMR spectra were recorded on a JEOL JNM-FCZ400S spectrometer operating at 400 MHz and 100 MHz, respectively, using DMSO-d_6 as the solvent. NMR analyses were conducted at Babasaheb Bhimrao Ambedkar University, Lucknow-226025, India.

Experimental

Synthesis of 3a-b

Ethane-1,2-diol was used as a protector for the one carbonyl group of acenaphthene-1,2-dione in the form of 2H-spiro[acenaphthylene-1, 2-[1,3]dioxolan]-2-one (**4a)** that was owing to reaction proceed in straight direction for the formation of imine. In a 100 ml round bottom flask dioxindole (**1**) was reacted with diaminoalkane (n = 4 and 3) (**2**) derivatives in ethanol by stirring 20-30 minutes in equimolar amounts at room temperature to synthesized *mono*-Schiff base derivatives (**3a-b**) as intermediates, further separated using standard work-up procedures and then crystallized in ethanol to obtain pure compounds. The purity of the synthesized *mono*-Schiff bases was verified by thin layer chromatography (TLC) using a solvent system of diethyl ether and ethyl acetate (ratio 8:2).

(Z)-3-((4-aminobutyl)imino)indolin-2-one (3a):FT-IR (KBr, 500–3500 cm^{-1}): ν = 3306 (N–H), 3086 (Ar–H), 2921, 2883 (C–H, aliphatic), 1754, 1721 (C=O), 1640 (C=N), 1611, 1463 (C=C, aromatic), 1359–1154 (C–N, C–O), 999–725 (C–H bending), 666, 594 cm^{-1}.^{1}HNMR (400 MHz, DMSO-d_6, δ ppm): 10.76 (s, 1H, CH=N), 7.76, 7.74 (each d, J ≈ 8.0 Hz, 2H, Ar–H ortho-coupled), 7.41–7.37 (dd, J ≈ 8.0, 1.8 Hz, Ar–H ortho/meta), 7.03–6.88 (m, Ar–H), 4.03–3.34 (m, 4H, CH_2–N/O), 2.49–0.83 (m, aliphatic CH_2, CH_3).^{13}CNMR (100 MHz, DMSO-d_6, δ ppm): 159.68, 154.54, 153.55, 145.83, 144.30, 133.32, 132.79, 127.27, 127.14, 122.16, 122.11, 121.61, 121.53, 116.69, 111.03, 110.50 (aromatic and imine carbons); 63.66, 53.43, 50.96, 50.89, 39.71, 39.50, 39.29 (aliphatic CH_2); 28.77, 28.72, 28.53, 28.49 (methyl/methylene groups).

(Z)-3-((4-(((E) -2-oxoacenaphthylen-1(2H)-ylidene)amino)butyl)imino)indolin-2-one(5a): FT-IR (KBr, 500–3500 cm^{-1}): ν = 3189 (N–H), 2928, 2853 (aliphatic C–H), 2360 (C–N^+ stretching), 1747, 1720 (C=O), 1656, 1643 (C=N), 1604, 1487–1420 (C=C aromatic), 1374–1211 (C–N/C–O stretching), 937–743 (Ar C–H out-of-plane bending) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.66 (s, 1H, CH=N), 8.43, 8.42, 8.07, 8.05 (each d, J ≈ 8 Hz, 4H, Ar–H ortho-coupled), 7.90–7.70 (dd, Ar–H, ortho/meta), 7.39, 7.01, 6.90, 6.88 (m, 4H, Ar–H), 3.93 (t, 2H, CH_2–O/N), 3.34 (t, 2H, CH_2–N), 2.49–0.81 (m, aliphatic CH_2 and CH_3).^{13}CNMR (100 MHz, DMSO-d_6, δ ppm): 187.60 (C=O), 144.32 (C=N), 132.31, 128.83, 128.48, 122.15, 121.49, 121.27, 111.05, 110.47 (aromatic C), 53.56, 51.15 (CH_2–N/O), 39.71, 39.50, 39.29 (aliphatic CH_2), 30.78, 30.57, 26.89 (alkyl CH_2/CH_3).

(Z)-3-((3-aminopropyl)imino)indolin-2-one(3b): FT-IR (KBr, cm^{-1}): 3232 (N–H), 3089–2931 (C–H,aromatic and aliphatic), 1748, 1715 (C=O), 1649 (C=N, imine), 1611, 1462, 1429 (C=C aromatic), 1352–1153 (C–N and C–O), 936–726 (C–H bending), 656, 590.^{1}H NMR (400 MHz, DMSO-d_6,δ, ppm): 10.77 (s, 1H, CH=N), 7.85, 7.83 (each d, J≈8.0 Hz, 2H, Ar–H ortho-coupled), 7.39, 7.02 (dd, J≈8.0, 1.5 Hz, Ar–H ortho/meta), 6.89–6.87 (m, Ar–H), 4.13 (m, 2H, CH_2–N/O), 3.34 (m, 2H, CH_2–N),2.49–1.04 (m, aliphatic CH_2 and CH_3).^{13}CNMR (100 MHz, DMSO-d_6, δ ppm): 138.41, 126.97, 124.122.80, 122.37, 122.23, 121.56, 121.51, 112.23 (aromatic and imine carbons); 56.06 (CH_2–N/O), 39.92,39.71, 39.50, 39.29, 39.08 (aliphatic CH_2), 18.56 (CH_3).

(Z)-3-((3-(((Z)-2-oxoacenaphthylen-1(2H)-ylidene)amino)propyl)imino)indolin-2-one (5b): FT-IR (KBr, 500–3500 cm^{-1}): ν = 3231 (N–H stretching), 3088–2930 (aromatic and aliphatic C–H stretching), 1758 and 1715 (C=O stretching), 1649 (C=N stretching), 1609–1152 (aromatic skeletal and C–N/C–O stretching), and bands below 900 (aromatic C–H out-of-plane bending).^{1}HNMR (400 MHz, DMSO-d_6, δ ppm): 10.75 (s, 1H, –CH=N), 7.85 and 7.83 (d, J ≈ 8 Hz, 2H, Ar–H), 7.39 and 7.02 (dd, 2H, Ar–H), 6.89–6.87 (m, 2H, Ar–H), 4.32–3.36 (m, 4H, CH_2–N), 2.49–1.02 (m, aliphatic CH_2 and CH_3).^{13}CNMR

(100 MHz, DMSO-d_6, δ ppm): 163.6 (C=O), 155.0 (C=N), 145.0–110.5 (aromatic carbons), 51.5 (CH_2–N), 40.1–32.0 (aliphatic CH_2 and CH_3).
The supplementary information includes figures of the FT-IR, ^{1}H NMR, and ^{13}C NMR spectra for the synthesized compounds.

Antibacterial activity

The antibacterial activity of samples **5a** and **5b** was determined using the agar well diffusion technique against gram-positive (*Bacillus subtilis*) and gram-negative (*Escherichia coli*) bacterial strains. Bacterial cultures were swabbed evenly onto nutrient agar medium (NA) plates, and three different volumes of samples were placed in 5 mm diameter wells. Bacterial cell suspensions were adjusted to an optical density of 0.1 at 600 nm, or approximately 10^8 colony-forming units per milliliter (CFU/mL), using an Eppendorf spectrometer (AG, Germany). The inhibitory effects of synthesized compounds (**5a** & **5b**) at various doses (50, 75, and 100 µg/mL) were compared to control. Gentamycin (1 µg/mL) was used as a positive control and DMSO (dimethyl sulfoxide) as a negative control.

RESULTS AND DISCUSSION

Chemistry of mono-Schiff base derivatives (3a-b)

Compounds **3a** and **3b** were synthesized via a condensation reaction of isatin (1) with respective linear diamines in ethanol at room temperature, yielding (Z)-3-((4-aminobutyl)imino)indolin-2-one (**3a**) and (Z)-3-((3-aminopropyl)imino)indolin-2-one (**3b**) in good yields. The reaction was rapid and proceeded efficiently under mild conditions, giving yellow solid products within 30 minutes. Compound **3a,** chemical structure $C_{11}H_{13}N_3O$ and molecular weight: 203.25 was isolated in 88% yield as a yellow solid powder, m.p. 248 °C, while compound **3b** chemical structure $C_{10}H_{11}N_3O$ and molecular weight: 189.22 was obtained in 83% yield, m.p, 242 °C also as yellow solid powder.
The structure of **3a** was confirmed by FT-IR, which showed a broad absorption at 3306 cm^{-1}, indicative of N–H stretching. Bands at 1754 and 1721 cm^{-1} correspond to C=O stretching vibrations, while a strong peak at 1640 cm^{-1} confirms the presence of the C=N (imine) group. Additional peaks in the 1450–1200 cm^{-1} range represent aromatic C=C and C–N stretching, and bands below 800 cm^{-1} correspond to out-of-plane C–H bending in substituted aromatic rings. The 1H NMR spectrum of compound **3a** (DMSO-d_6) displayed a sharp singlet at δ 10.76 ppm, attributed to the imine proton (–CH=N). Aromatic protons appeared in the δ 6.90–7.77 ppm region as a series of doublets and doublet of doublets, indicative of ortho- and meta-coupled systems. Specifically, doublets at δ 7.76 and 7.74 ppm (J ≈ 8 Hz) are consistent with ortho-coupled protons, while doublet of doublets at δ 7.41–7.37 ppm likely arise from protons coupled both ortho and meta to adjacent protons (J ≈ 8.0 and 1.8 Hz). Additional signals in the δ 7.03–6.88 ppm range reflect meta and para-substituted aromatic environments. Methylene protons adjacent to heteroatoms appeared as multiplets at δ 4.03–3.34 ppm, while aliphatic side-chain signals were observed between δ 2.49 and 0.83 ppm. The coupling patterns further validate the substitution pattern of aromatic rings in the Schiff base framework. The ^{1}*C NMR spectrum of 3a (DMSO-d_6) showed Downfield resonances between δ 153.5–159.6 ppm were attributed to C=N (imine) and conjugated aromatic carbons. Peaks at δ 145.8 to 122.1 ppm correspond to quaternary and tertiary aromatic carbons, while δ 121.6–116.7 ppm reflect substituted aromatic systems. Signals at δ 111.0 and 110.4 ppm indicate additional aromatic or heterocyclic carbons. A prominent signal at δ 63.6 ppm suggests a methylene group adjacent to nitrogen or oxygen. Peaks between δ 53.4–39.2 ppm are assigned to aliphatic methylene carbons, and those at δ 28.4–28.7 ppm are due to alkyl side chains. These data support the successful construction of the target molecule.

For compound **3b**, FT-IR analysis showed a broad absorption at 3232 cm^{-1}, corresponding to N–H stretching, along with peaks at 3089–2931 cm^{-1} for aromatic and aliphatic C–H stretches. Strong carbonyl absorptions were observed at 1748 and 1715 cm^{-1}, while the C=N (imine) stretching appeared prominently at 1649 cm^{-1}. Peaks in the 1611–1350 cm^{-1} region were assigned to C=C aromatic, C–N, and C–O stretching vibrations. Bands below 900 cm^{-1} indicate out-of-plane C–H bending typical of substituted aromatic rings.^{1}H NMR spectrum of compound **3b** (DMSO-d_6) exhibited a singlet at δ 10.77 ppm, corresponding to the imine proton (–CH=N). Aromatic protons appeared as doublets at δ 7.85 and 7.83 ppm (J ≈ 8 Hz, ortho-coupled) and as doublet of doublets around δ 7.39 and 7.02 ppm, consistent with ortho/meta-coupled protons in a substituted benzene ring. Peaks at δ 6.89–6.87 ppm suggest meta-substituted aromatic protons. Multiplets at δ 4.13 and 3.34 ppm were assigned to methylene groups adjacent to heteroatoms. Additional aliphatic proton signals at δ 2.49–1.04 ppm represent side chain methylene and methyl groups, supporting the aliphatic substitution pattern of the Schiff base framework. ^{13}C NMR spectrum of compound **3b** confirmed its structural integrity through distinct resonances. A downfield signal at δ 138.4 ppm corresponds to a quaternary aromatic carbon, while signals in the δ 126.9–121.5 ppm range represent aromatic CH and C=N carbons. The peak at δ 112.2 ppm is consistent with a substituted aromatic carbon. A signal at δ 56.0 ppm suggests a methylene group adjacent to a nitrogen or oxygen atom. Multiple resonances between δ 39.0–39.9 ppm indicate several aliphatic methylene carbons, and a peak at δ 18.5 ppm corresponds to a terminal methyl group.

The spectral and analytical data thus support the formation of (Z)-configured *mono*-Schiff bases through selective condensation of isatin, offering potential for further derivatization or biological evaluation.

Chemistry of bis-Schiff base derivatives (5a-b)

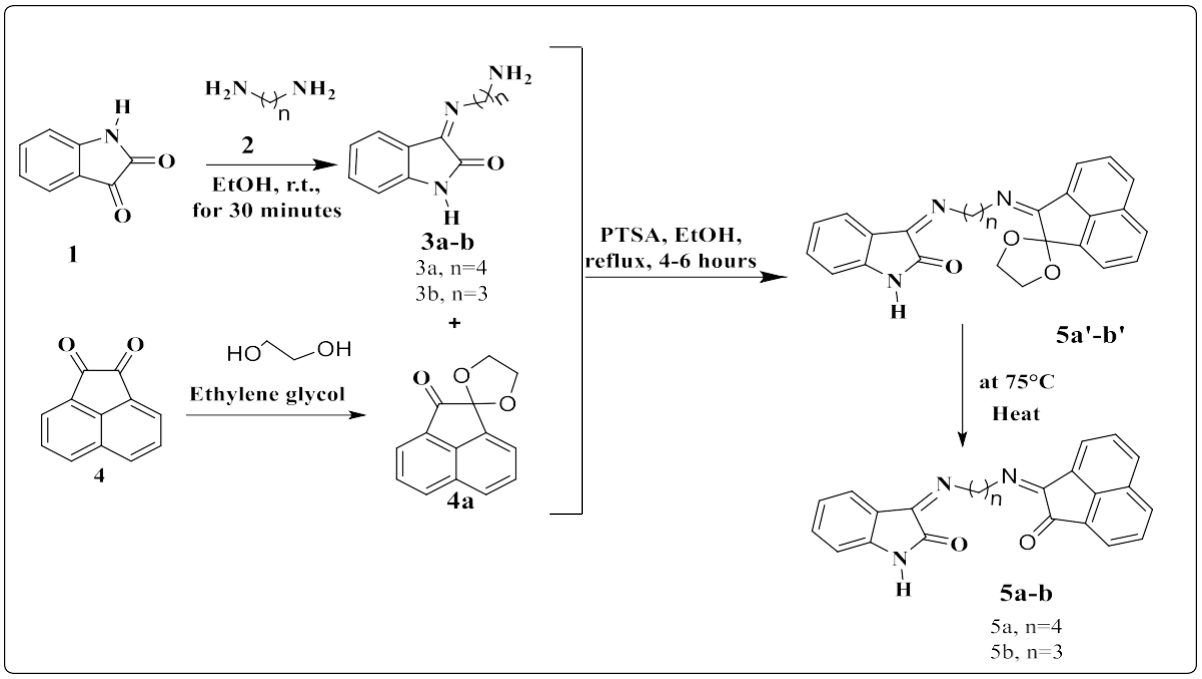
The key intermediates 5a′–b′, obtained via condensation of compounds 3a–b with the monoacetal derivative 4a of acenaphthoquinone, were subjected to thermal deprotection at 75 °C, resulting insitu hydrolysis of the acetal group. This step effectively regenerated the masked carbonyl group and allowed intramolecular nucleophilic attack by the pendant amine, yielding the *bis*-Schiff base derivatives **5a–b** with good yields and purity.

Compound **5a**, showing chemical structure $C_{24}H_{19}N_3O_2$, molecular weight: 381.44 corresponding to the longer alkyl chain (n = 4), was isolated as a dark yellow-brown solid powder, with a melting point of 210 °C and 78% yield. The FT-IR spectrum of compound 5a displayed a broad absorption at 3189 cm^{-1}, indicative of N–H stretching, and bands at 2928 and 2853 cm^{-1} corresponding to aliphatic C–H stretching. Strong carbonyl stretches appeared at 1747 and 1720 cm^{-1}, while the imine (C=N) and aromatic C=C stretches were observed at 1656–1604 cm^{-1}. Peaks in the 1487–1211 cm^{-1} range were attributed to aromatic skeletal vibrations, C–N, and C–O stretching. Absorptions below 900 cm^{-1}, including bands at 830, 799, and 743 cm^{-1}, suggest characteristic out-of-plane C–H bending in substituted aromatic rings. The ^{1}H NMR spectrum of 5a (400 MHz, DMSO-d_6) showed a singlet at δ 10.66 ppm attributed to the imine proton (–CH=N). Multiple aromatic protons appeared as closely spaced doublets and doublet of doublets in the δ 8.43–6.88 ppm range, consistent with ortho- and meta-coupled protons in a substituted aromatic system. Notably, doublets at δ 8.43 and 8.07 ppm (J ≈ 8 Hz) confirmed ortho-coupled protons, while dd patterns around δ 7.90–7.01 ppm indicated meta and para-substituted aromatic environments. Signals at δ 3.93 and 3.34 ppm correspond to methylene groups adjacent to nitrogen or oxygen. The aliphatic region (δ 2.49–0.81 ppm) revealed multiplets for methylene and methyl groups from alkyl substituents, supporting the proposed hybrid Schiff base structure. ^{13}C NMR spectrum of compound 5a exhibited a strong downfield signal at δ 187.6 ppm, characteristic of a carbonyl carbon (C=O). The signal at δ 144.3 ppm corresponds to the imine (C=N) carbon, while peaks in the δ 132.3–121.2 ppm range represent aromatic carbons, including both protonated and quaternary centres. Signals at δ 111.0 and 110.4 ppm indicate substituted aromatic carbons. The resonance at δ 53.5 and 51.1 ppm is attributed to methylene carbons adjacent to nitrogen or oxygen, and the peaks at δ 39.7–26.8 ppm are due to aliphatic methylene and methyl groups present in the alkyl chain.

Compound **5b** (n = 3), showing chemical structure $C_{23}H_{17}N_3O_2$, molecular weight: 367.41 also a yellow-brown solid, melted at 198 °C and was obtained in 66% yield. The FT-IR spectrum of compound 5b exhibited a broad band at 3231 cm^{-1}, corresponding to N–H stretching, and peaks at 3088–2930 cm^{-1} due to aromatic and aliphatic C–H stretches. Strong absorptions at 1758 and 1715 cm^{-1} were attributed to C=O stretching, while a distinct band at 1649 cm^{-1} confirmed the presence of the C=N (imine) group.

Aromatic skeletal vibrations and C–N/C–O stretches appeared in the 1609–1152 cm^{-1} region. Out-of-plane C–H bending in the aromatic ring was observed below 900 cm^{-1}, supporting the aromatic substitution pattern. ^{1}H NMR spectrum of compound 5b (DMSO-d_6) displayed a singlet at δ 10.75 ppm, corresponding to the imine proton (–CH=N). Aromatic protons appeared as well-resolved doublets at δ 7.85 and 7.83 ppm (J ≈ 8 Hz), indicative of ortho-coupled protons, while signals at δ 7.39 and 7.02 ppm exhibited doublet of doublet patterns, suggestive of protons involved in both ortho and meta coupling. Additional aromatic resonances between δ 6.89–6.87 ppm confirmed a substituted phenyl system. Multiplets in the δ 4.32–3.36 ppm region was attributed to methylene groups adjacent to the nitrogen. Aliphatic proton signals appearing between δ 2.49 and 1.02 ppm correspond to methylene and methyl protons, consistent with the presence of an aliphatic side chain. ^{13}C NMR spectrum of compound **5b** (100 MHz, DMSO-d_6) confirmed the proposed structure. A prominent signal at δ 163.6 ppm was assigned to the carbonyl carbon (C=O), while the signal at δ 155.0 ppm corresponds to the imine carbon (C=N). Multiple resonances in the δ 145.0–110.5 ppm range were attributed to aromatic carbons of the substituted phenyl rings. The signal at δ 51.5 ppm indicated a methylene carbon adjacent to nitrogen, and the cluster of peaks between δ 40.1–32.0 ppm represents aliphatic methylene and methyl groups, confirming the presence of an alkyl chain.

These analytical data confirmed the successful construction of the desired *bis*-Schiff bases **5a-b** through an efficient, regioselective strategy enabled by temporary carbonyl protection and sequential activation.



Scheme 1. Proposed reaction for the synthesis of di-oxindole bis-Schiff base derivatives

Antibacterial activities

The antibacterial activities of the synthesized Schiff bases (**5a** and **5b**) against *Escherichia coli* and *Bacillus subtilis* strains at concentrations of 50, 75 and 100 µg/mL were evaluated. Gentamycin (50 µg/mL) was used as a standard antibiotic for comparison, and dimethyl sulfoxide (DMSO) was used as a control (Figure 2 & 3).

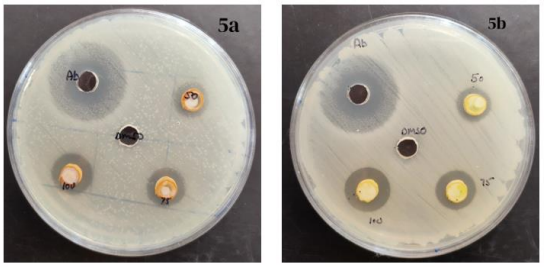


Fig. 2. Antibacterial activity of synthesized compounds (5a and 5b) against *Bacillus subtilis*

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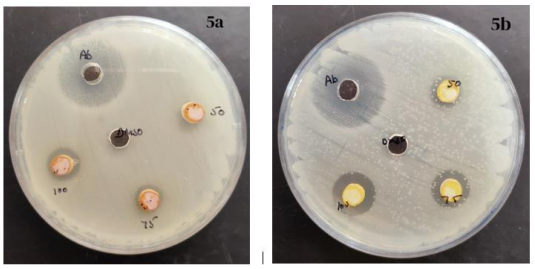

Fig.3.Antibacterial activity of synthesized compounds (5a and 5b) against *Escherichia coli*

In-vitro antibacterial activity was assessed against *Bacillus subtilis* and *Escherichia coli* using the agar well diffusion method. The antibacterial activity of the synthesized compouds **5a** and **5b** demonstrated the antibacterial activity with a zone of inhibition at three different concentrations (50, 75, and 100 µg/mL) against gram positive *Bacillus subtilis* and gram-negative *Escherichia coli* bacterial strain. The results indicate a dose-dependent increase in inhibition zones for all bacterial strains, with higher concentrations exhibiting greater antibacterial effects. However, the inhibition zones of the compound remained significantly lower compared to the standard antibiotic Gentamycin, which demonstrated consistent and lower antibacterial activity. Among the *E. coli* strains, **5b** exhibited the highest sensitivity to the compounds, whereas **5a** showed minimal inhibition at lower concentrations. Similarly, for *B. subtilis*, **5a** and **5b** both showed relatively better susceptibility. In contrast, the control substance (DMSO) showed no inhibition zones, and positive control (Gentamycin) showed the zone of inhibition confirming the antibacterial efficacy of the above compounds.

Table 1: Antibacterial activity of compounds 5a and 5b against gram positive and gram-negative bacteria. Values are means ±standard deviation

S.No.	**Bacterial strain**	**Concentration of compound (mm)**			**Gentamycin (Standard) (mm)**	**Control (DMSO)**
		50 µg/mL	75 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL
E. Coli						
1	**5a**	0.0±0.0	1.33±0.58	2.33±0.58	11.67±0.58	0.0±0.0
2	**5b**	2.67±0.58	3.33±0.58	5.33±0.58	12.67±0.58	0.0±0.0
B. Subtilis						
3	**5a**	2.67±0.58	3.67±0.58	5.33±0.58	11.67±0.58	0.0±0.0
4	**5b**	1.33±0.58	3.67±0.58	4.67±0.58	11.67±0.58	0.0±0.0

The observed concentration-dependent antibacterial effect highlights the potential of these Schiff bases as candidates for antibacterial drug development. Future studies could focus on elucidating the exact mechanism of action and optimizing structural modifications to enhance their activity.

CONCLUSION

The synthesis of two novel bis-oxindole di-imine derivatives, 5a and 5b, was successfully achieved using para toluene sulfonic acid (PTSA) as the sole Bronsted acid catalyst and ethanol as the solvent. While the precursor compounds 3a and 3b were synthesized efficiently under mild, catalyst-free conditions at room temperature, yielding 88% and 83% respectively, the formation of the advanced bis-oxindole frameworks 5a and 5b at elevated temperatures (reflux) and acid catalysis to achieve satisfactory yields of 78% and 66%. These findings underscore the significance of optimizing reaction parameters such as solvent, temperature, and catalyst concentration in multi-step syntheses. The synthesized compounds were thoroughly characterized to confirm their molecular structures. Biological evaluation revealed that both 5a and 5b exhibit promising antibacterial activity against *Escherichia coli* and *Bacillus subtilis*, with compound 5b showing comparatively greater efficacy, particularly against *Escherichia coli*. Although the

observed activity was moderate compared to the standard antibiotic gentamycin, the concentration-dependent response indicates the potential of these bis-oxindole derivatives as lead scaffolds for the development of new antimicrobial agents. Thus, the strategic design and successful synthesis of *bis*-oxindole di-imines **5a–b**, exhibiting promising biological activity, provide a solid foundation for further structural optimization and pharmacological investigations aimed at the treatment of bacterial infections.

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