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Research Article

## Imidazole Phenanthroline Derivatives: A Promising Application in Modern Medicine

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### Abstract

**Purpose:** The primary objective of this study is to create a new class of imidazole phenanthroline compounds that target the 1,10-phenanthroline core for its antifungal and antibacterial properties.

**Methods:** Commercially available 1,10-phenanthroline (phen) was nitrated with potassium bromide in the presence of sulfuric and nitric acids to get 1,10-phenanthroline-5,6-dione (phendione), an intermediate molecule, which served as the starting compound for the synthesis of 1H-imidazo [4,5-f] [1,10] phenanthroline compounds. This intermediate product was dissolved in glacial acetic acid and then reacted with different benzaldehydes while ammonium acetate acted as a catalyst. Thin Layer Chromatography (TLC) was used to track the reaction's progress and the purity of the product. Their biological efficacy against in vitro bacterial and fungal growth was also investigated. The antimicrobial potential of the investigated compounds when compared to the standards Clotrimazole and Streptomycin, respectively, revealed impressive antifungal and antibacterial properties.

**Results:** A series of compounds were synthesized successfully and characterized by various analytical techniques such as NMR, IR and ESI-mass spectroscopy. The compounds possess remarkable antibacterial and antifungal potential.

**Conclusion:** A series of imidazole phenanthrolines were synthesized and found to have antimicrobial activities.

**Keywords:** phenanthrene, imidazole phenanthroline, 1,10-phenanthroline, potassium bromide, 1,10-phenanthroline-5,6-dione, 1H-imidazo [4,5-f] [1,10] phenanthroline

## INTRODUCTION

The growing concern over antimicrobial resistance (AMR) has escalated the demand for novel antimicrobial drugs. As the incidence of infections caused by antibiotic-resistant microorganisms rises, existing therapies often become ineffective. This critical situation underscores the urgency for innovative solutions to combat resistant strains and protect public health.

1,10-Phenanthroline (commonly known as phen) is a heterocyclic organic substance with distinct structure and versatile chemical properties. Its molecular formula is  $C_{12}H_8N_2$  and has a molar mass of 180.21 g/mol, with melting and boiling points of approximately 118.56 °C and 409.2 °C, respectively <sup>1</sup>. The compound is primarily encountered as a white, crystalline substance that dissolves in a range of organic liquids and polar solvents like acetone and ethanol <sup>2</sup>.

1,10-Phenanthroline has gained attention for its significant biological activities. Research has indicated its effectiveness against various Gram-positive bacteria and selected fungi, making it a candidate for antimicrobial applications <sup>3,4</sup>. Additionally, metal

complexes of 1,10-phenanthroline have been studied for their anticancer properties, particularly in relation to binding and cleaving DNA, which leads to cytotoxic effects <sup>5</sup>. The compound has also shown antioxidant properties by scavenging free radicals <sup>6</sup>, further expanding its therapeutic potential. In analytical contexts, 1,10-phenanthroline is employed for the detection of ferric and ferrous ions, forming coloured complexes that can be quantitatively measured using spectrophotometry <sup>7</sup>.

Phenanthroline derivatives are not only synthetically produced but also occur naturally in various organisms. Many psychotropic medications, such as opiate analgesics and cough suppressants like codeine and dextromethorphan, include these compounds. Also, phenanthrene, an essential component of phenanthroline, has been identified in the mineral ravatite, illustrating its natural occurrence in organic matter. The presence of phenanthrenoids in flowering plants further highlights the significance of these compounds, particularly within the *Orchidaceae* family, as they possess various biological activities <sup>8</sup>.

Phendione, a derivative of 1,10-phenanthroline, is characterized by its formula  $C_{12}H_6O_2N_2$  and a yellow solid appearance. With its unique structure featuring two carbonyl groups at the 5 and 6 positions, phendione exhibits both chelating and redox-active properties due to its di-iminic functionalities and o-quinoid moiety<sup>9,10</sup>. Phendione is notable for its broad-spectrum antimicrobial properties, exhibiting significant antifungal efficacy against various *Candida* species. It also displays antibacterial activity against Gram-positive bacteria<sup>11,12</sup>. Most compounds derived from the phenanthroline frameworks have been employed in medicinal contexts to form metal-complexes with enhanced therapeutic effects<sup>13</sup>.

Among phenanthroline derivatives, imidazole phenanthroline derivatives have garnered attention because of their varied biological activity and structural adaptability. The integration of imidazole rings into the phenanthroline core leads to compounds exhibiting a range of anti-inflammatory, antibacterial, and anticancer pharmacological actions. Numerous analytical techniques, including X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, infrared (IR) spectroscopy, and mass spectrometry (MS) facilitate detailed analyses of physical and chemical properties, aiding in the development of effective therapeutic agents<sup>14,15</sup>.

Evaluating the antibacterial properties of imidazole phenanthroline derivatives is a critical step in their development as antimicrobial agents. Techniques such as Minimum Inhibitory Concentration (MIC) and Zone of Inhibition (ZOI) assays are employed to determine the effectiveness of these compounds against various microbiological strains, including Gram-positive and Gram-negative bacteria, as well as fungi. These assays provide essential insights into the compounds' potential

utility in clinical settings and their capacity to address the challenges posed by resistant microorganisms<sup>16</sup>.

## EXPERIMENTAL

### Reagents and Instrumentation

The solvents and chemicals utilized in this study were sourced from commercial vendors without additional purification. 1,10-Phenanthroline, potassium bromide, sulfuric acid, nitric acid, ammonium acetate, glacial acetic acid, ethanol, chloroform, 4-chloro-2-methoxy benzaldehyde, 5-chloro-2-hydroxy-3-iodo benzaldehyde, 3-iodo-4-methoxybenzaldehyde and 2-bromo-4-chlorobenzaldehyde were all purchased from Sigma Aldrich. Bacterial and fungal pathogens were procured from CRMAS, Trivandrum, Kerala: Centre for Research on Molecular and Applied Sciences.

The Bruker Avance III 500MHz/54mm was used to record the compounds' NMR spectra in DMSO with TMS serving as the internal reference. The Shimadzu IR Prestige 21 Spectrometer was used to record infrared spectra in KBr medium between  $4000\text{ cm}^{-1}$  and  $400\text{ cm}^{-1}$ . The Waters Q-ToF Micro Mass Spectrometer provided the ESI-Mass spectra. The Carlo Erba Elemental Analyser EA1108 was used to measure the proportion of C, H, N, and S. Melting point equipment was used to measure the melting points of produced compounds. To guarantee reaction completeness and product purity, TLC was employed.

### Synthesis

The procedure for synthesis with both steps A and B, showing the starting compound 1,10-phenanthroline, the ultimate desired products and the intermediate molecule 1,10-phenanthroline-5,6-dione are depicted in Figure 1 below.

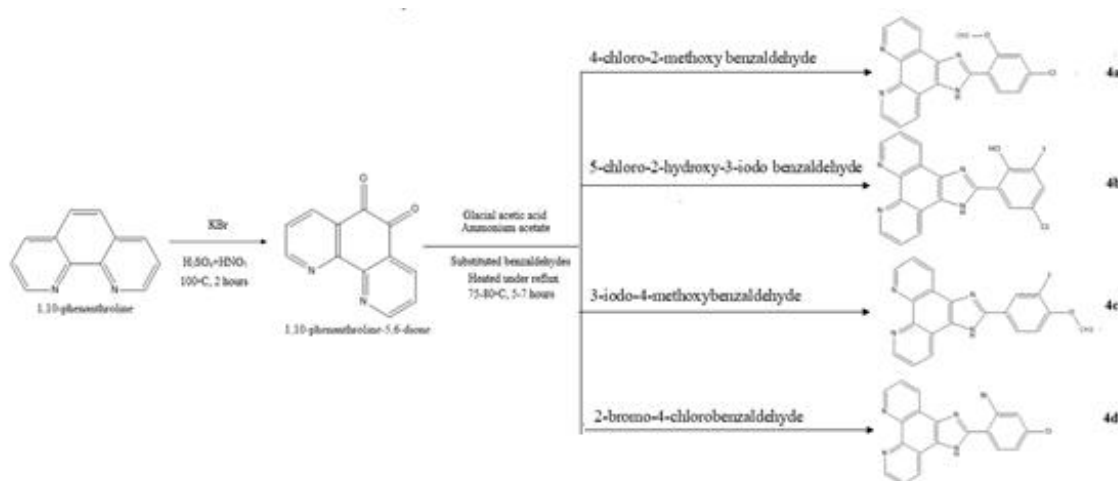


Figure 1: An overview of 1H-Imidazo[4,5-f][1,10]phenanthrolines synthesis scheme

#### Step A: Production of 1,10-phenanthroline-5,6-dione

1,10-phenanthroline was nitrated using potassium bromide in the initial step. This was done using a combination of oxidizing agents such as sulphuric acid and nitric acid to give 1,10-phenanthroline-5,6-dione,

which is a dione structure (double ketone) at the 5<sup>th</sup> and 6<sup>th</sup> positions of the phenanthroline ring.

1,10-phenanthroline [50 mmol, 10.0 g] as well as potassium bromide [76 mmol, 9 g] was thoroughly mixed using a mortar and pestle to minimize its particle size and to ensure effective blending for successive

reactions. This solid mixture was subsequently moved to a round-bottom flask that contained a 100 mL cooled sulfuric acid and nitric acid (50 mL) combination maintained at 0-5 °C. It was then refluxed for 2 hours at 100 °C. This reaction produces bromine fumes and hence requires caution. The liquid was poured over ice when it had reached room temperature, which precipitated the organic product and produced an aqueous phase that contained excess of sulfuric and nitric acids and any water-soluble by-products. Careful neutralization with NaOH solution to a pH 4-5 at a temperature lower than 10°C was necessary. At elevated pH levels, the blend looked dark green. However, the inclusion of acid to keep a pH of 4-5 brought back the yellow hue of the suspension. Maintaining a constant pH value was important to ensure that the compound retained its structural form. The yellow colour of the suspension was an indicator of the presence of the desired compound.

The suspension containing the desired organic product (1,10-Phenanthroline-5,6-dione) and the aqueous phase were subjected to extraction with chloroform, a non-polar solvent. Chloroform being less polar than water, was effective in dissolving organic compounds while leaving behind polar substances (like acids and water-soluble impurities) in the phase of water. To improve separation efficiency, this extraction process was carried out twice. The organic phase which contained 1,10-Phenanthroline-5,6-dione, was cleaned with water to remove any remaining residual acids or water-soluble impurities that may have been carried over into the organic phase during extraction. A drying agent anhydrous magnesium sulfate ( $\text{MgSO}_4$ ) was added in the organic phase after washing to adsorb any traces of water, which was then filtered off. A rotary evaporator was then used to concentrate the dry organic solution at a lower pressure to extract the solid from the chloroform. The evaporation process resulted in a yellow solid product, which relates to the desired 1,10-Phenanthroline-5,6-dione. This crude solid was melted in warm methanol and recrystallized for further purification to get a pure product 1,10-Phenanthroline-5,6-dione <sup>17</sup>.

#### **Step B: Synthesis of 1H-Imidazo[4,5-f][1,10]phenanthrolines from benzaldehydes**

20 mg of 1,10-phenanthroline-5,6-dione, which had been acquired in the preceding stage, were combined with 5 mL of warm glacial acetic acid to guarantee complete dissolution. This was then reacted with various substituted benzaldehydes. This mixture was stirred for 10 minutes initially for proper mixing of the reactants. Ammonium acetate served as a catalyst. To generate imidazo[4,5-f][1,10]phenanthrolines, it was heated under reflux for five to seven hours at 75 to 80°C <sup>17</sup>.

Using the fast and efficient technique of TLC, or thin-layer chromatography, the purity of every compound made, and the course of reactions were tracked. From a variety of solvent systems that were tested in varying ratios, the mobile phase was chosen to be a 2:1 blend of chloroform and methanol. This allows the separation of compounds differing in polarities. The non-polar nature

of chloroform facilitates the movement of non-polar compounds, while the polar methanol helps in eluting more polar substances. Iodine vapours were used to identify the spots <sup>18,19</sup>.

The blend was moved to a separating funnel that held chloroform and water in a ratio of 1:1. The separation of organic compounds into the non-polar chloroform layer is made possible by the biphasic system created by the equal proportions of chloroform and water. The funnel was shaken vigorously with its cap closed, for around 15-20 minutes. Care should be taken to release the developed pressure inside the separating funnel occasionally to avoid accidents. The separation of organic and aqueous phases into two separate layers facilitates efficient partitioning of the compounds. The aqueous layer was eliminated, and the necessary product was collected when it emerged in the chloroform layer. This procedure was carried out twice to ensure that most of the product is transferred into the chloroform layer and hence get maximum yield. Evaporating the collected chloroform layer removes the solvent, leaving behind the purified organic product. IR spectroscopy, NMR spectroscopy, and ESI-MS spectrophotometry were some of the techniques employed to describe the compounds that were produced.

#### **Antimicrobial studies**

Antimicrobial tests are essential to guarantee the safety and effectiveness of new antimicrobial agents for clinical application. The Zone of Inhibition (ZOI) and Minimum Inhibitory Concentration (MIC) tests for antimicrobials were used in this study. These tests provide important data regarding the effectiveness of any new drug.

#### **ZOI determination**

The antimicrobial activity of a material may be assessed using the Agar Well Diffusion technique. The synthesized chemicals were permitted to proliferate across the medium and engage with an agar plate newly inoculated with microorganisms. The resultant inhibitory zones' diameter formed in a circular pattern is measured in millimetres. The results were compared with established standards to classify microorganisms as susceptible, intermediate or resistant.

Bacterial cultures of *Staphylococcus aureus* and *Escherichia coli* were seeded using 20 millilitres of Muller Hinton Agar Medium on petri dishes. A sterilized well cutter was used to cut wells in the agar of each plate that were about 10 mm in diameter. The same compound was added in varying amounts to the wells: 250µg, 500µg, and 1000µg and 37°C was used to incubate the plates for a whole day. A well without any sample was the negative control. To ascertain the antibacterial action, the width was recorded for the well's surrounding inhibitory zone with a ruler. The ZOI was contrasted with that of the standard antibacterial agent Streptomycin, which served as the positive control <sup>20</sup>. Proper aseptic conditions were maintained throughout the procedure to avoid any contamination.

### MIC determination

The lowest concentration of an antibiotic (in µg/mL) that stops a particular bacterial strain from growing is referred to as the MIC, or Minimum Inhibitory Concentration. It can be shown by growing microorganisms in liquid media or on solid growth medium plates. A lower minimum inhibitory concentration (MIC) value indicates that less medication is required to stop the organism's development. In summary, antibacterial agents with lower MIC scores are more effective.

The two-fold serial dilution approach was used to find the lowest inhibitory concentration. The broth dilution test was conducted on a microtiter plate with 96 wells. The *E. coli* conidial inoculum preparations were added to each well of the plate in an amount of 100 microliters, that had been double diluted, for a total amount of 200µl per well. After dissolving the substance, 10 mg/mL as the final dosage in DMSO was obtained. After that, it was added to the wells progressively at increasing concentrations of 62.5µg, 125µg, 250µg, 500µg, and 1000µg. At room temperature, it was then incubated for the whole night. It was maintained under control on its own.

An identical process was done for *S. aureus*. Proper aseptic conditions were maintained throughout the procedure to avoid any contamination. Growth was seen visually and measured employing an ELISA plate reader, in terms of optical density (OD) at 630 nm. The growth inhibition for the test wells at each extract was calculated using the following procedure.

Inhibition percentage = (OD of control - OD of test) / (OD of control) × 100

OD of Control: optical density of the control group, without any treatment

OD of Test: optical density of the test group, where organism is treated with the compound.

## RESULTS

### Spectral data

#### 1,10-phenanthroline-5,6-dione

Yellow solid; yield (90%); mp 260.00°C; IR: 1700cm<sup>-1</sup>(C=O), 3100 cm<sup>-1</sup>(aromatic); <sup>1</sup>H-NMR (δ ppm): δ=7.26 (dd, 2H), 7.94 (dd, 2H), 8.73 (dd, 2H); <sup>13</sup>C-NMR: 121.4,130.3,136.9,151.7,154.6,173.3; MS (ESI): m/e 211.04 (M<sup>+</sup>); analytically calculated values for C<sub>12</sub>H<sub>6</sub>N<sub>2</sub>O<sub>2</sub>: %C 66.57, %H 2.88, %N 13.33, %O 15.22.

#### Compound 4a: 2-(4-chloro-2-methoxyphenyl)-1H-imidazo[4,5-f] [1,10] phenanthroline

Yellow solid; yield (83%); mp 600.72 °C; IR: 3061.03 cm<sup>-1</sup>(double bond, aromatic); <sup>1</sup>H-NMR (δ ppm): δ=3.83 (s, 3H), δ=7.11 (dd, 2H), 7.25 (s, 1H), 7.58 (t, 1H), 7.62 (dd,1H), 8.38 (dd,1H), 8.83 (dd,1H), 12.81(s,1H); <sup>13</sup>C-NMR: 56.1, 115.6, 121.6, 129.9, 132.3,149.9, 116.1,121.6, 125.2,126.4,135.2,152.9, 124.1,122.8,136.5,154.1,158.7; MS (ESI): m/e 361.09 (M<sup>+</sup>); analytically calculated values for C<sub>20</sub>H<sub>13</sub>ClN<sub>4</sub>O: %C 66.67, %H 3.69, %N 15.55, %O 4.44, %Cl 9.83.

#### Compound 4b: 4-chloro-2-(1H-imidazo[4,5-f] [1,10] phenanthroline-2-yl)-6-iodophenol

Yellow solid; yield (85%); mp 737.00 °C; IR: 3061 cm<sup>-1</sup>(double bond, aromatic); <sup>1</sup>H-NMR (δ ppm): δ=7.44 (s, 1H), δ=7.58 (t, 3H), 7.83 (s, 1H), 8.38 (d, 2H), 8.83 (d,2H), 9.82 (s,1H), 12.81 (s,1H); <sup>13</sup>C-NMR: 89.4, 121.5, 122.8, 124.1, 125.2, 126.4, 127.7, 129.0, 132.3, 135.2, 139.2, 149.9, 152.9, 154.1, 162.8; MS (ESI): m/e 471.95 (M<sup>+</sup>); analytically calculated values for C<sub>19</sub>H<sub>10</sub>ClIN<sub>4</sub>O: %C 48.28, %H 2.13, %N 11.85, %O 3.38, %Cl 7.50, % I 26.85.

#### Compound 4c: 2-(3-iodo-4-methoxyphenyl)-1H-imidazo[4,5-f][1,10]phenanthroline

Yellow solid; yield (82%); mp 628.86 °C; IR: 3061cm<sup>-1</sup>(double bond, aromatic); <sup>1</sup>H-NMR (δ ppm): δ=3.83 (s,1H), 6.82 (d,2H), 7.58 (t,3H), 7.75 (s,1H), 7.96(d,2H), 8.38 (d,2H), 8.83 (d,2H); <sup>13</sup>C-NMR: 54.9, 86.4, 116.4, 117.6, 121.5, 122.8, 124.1, 125.2, 126.4, 129.2, 132.3, 135.2, 137.8, 149.9, 152.9, 154.1, 159.4; MS (ESI): m/e 452.01 (M<sup>+</sup>), analytically calculated values for C<sub>20</sub>H<sub>13</sub>IN<sub>4</sub>O: %C 53.12, %H 2.90, %N 12.39, %O 3.54, % I 28.06.

#### Compound 4d: 2-(2-bromo-4-chlorophenyl)-1H-imidazo[4,5-f] [1,10] phenanthroline

Yellow solid; yield (80%); mp 627.02 °C; IR: 3045 cm<sup>-1</sup>, 3726 cm<sup>-1</sup> (double bond, aromatic); <sup>1</sup>H-NMR (δ ppm): δ=7.49 (s,2H), 7.58 (s,3H), 7.62 (t,2H). 7.88 (s,1H), 8.38 (d,2H), 8.83 (d,2H), 12.81 (s, 1H); <sup>13</sup>C-NMR: 121.5, 122.8, 124.1, 126.4, 128.3, 130.8, 131.1, 132.3, 135.2, 137.9, 149.9, 152.9, 154.1; MS (ESI): m/e 407.97 (M<sup>+</sup>), analytically calculated values for C<sub>19</sub>H<sub>10</sub>BrClN<sub>4</sub>: %C 55.70, %H 2.46, %N 13.68, %Cl 8.65, % Br 19.50.

### Biological screening

The area of inhibition (ZOI) results for the synthesized compounds (4a to 4d) against *E. coli* are shown in Table 1. ZOI measures the efficiency of each compound through their capacity to suppress the development of this bacteria, in millimetres (mm).



**Table 1: ZOI of *Escherichia coli* and *Staphylococcus aureus* by synthesized compounds at different concentrations**

Organism	Name of compound	Concentration ( $\mu$ g)	Zone of inhibition (mm)
<i>E. coli</i>	Streptomycin (standard)	100	32
		250	32
		500	34
		1000	35
	4a	250	35
		500	37
		1000	38
		250	31
	4b	500	33
		1000	35
		250	29
		500	33
<i>S. aureus</i>	Streptomycin (standard)	100	27
		250	31
		500	32
		1000	33
	4a	250	30
		500	33
		1000	35
		250	34
	4b	500	36
		1000	38
		250	34
		500	36
	4c	1000	38
		250	34
		500	36
		1000	38

The pictorial representations of zones of inhibition of *E. coli* by the synthesized compounds are represented by Figure 2 and that of *S. aureus* by Figure 3.

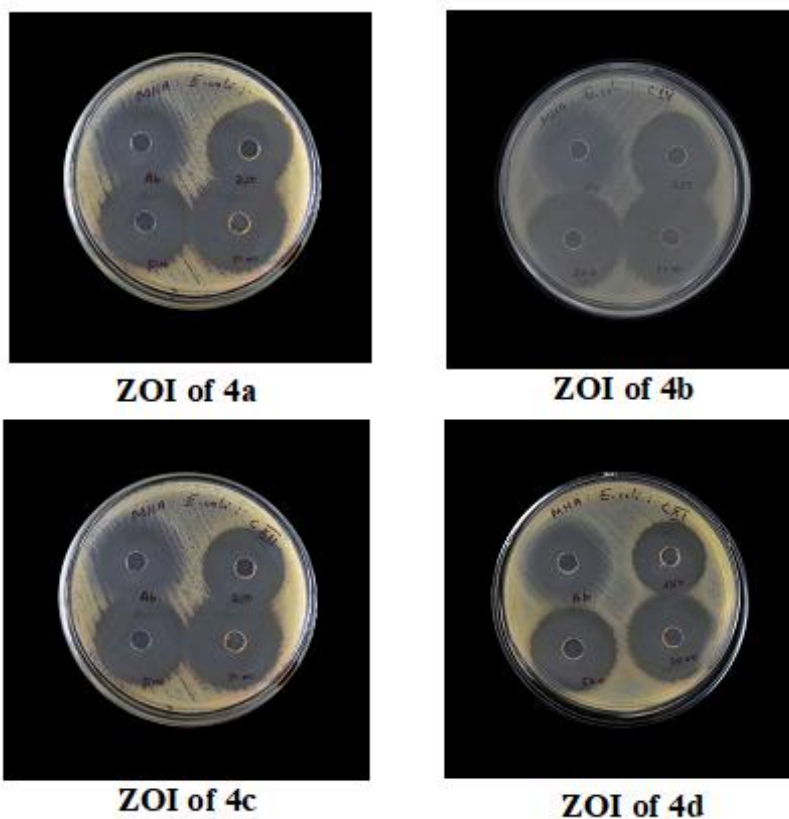


Figure 2: ZOI of *Escherichia coli* by synthesized compounds

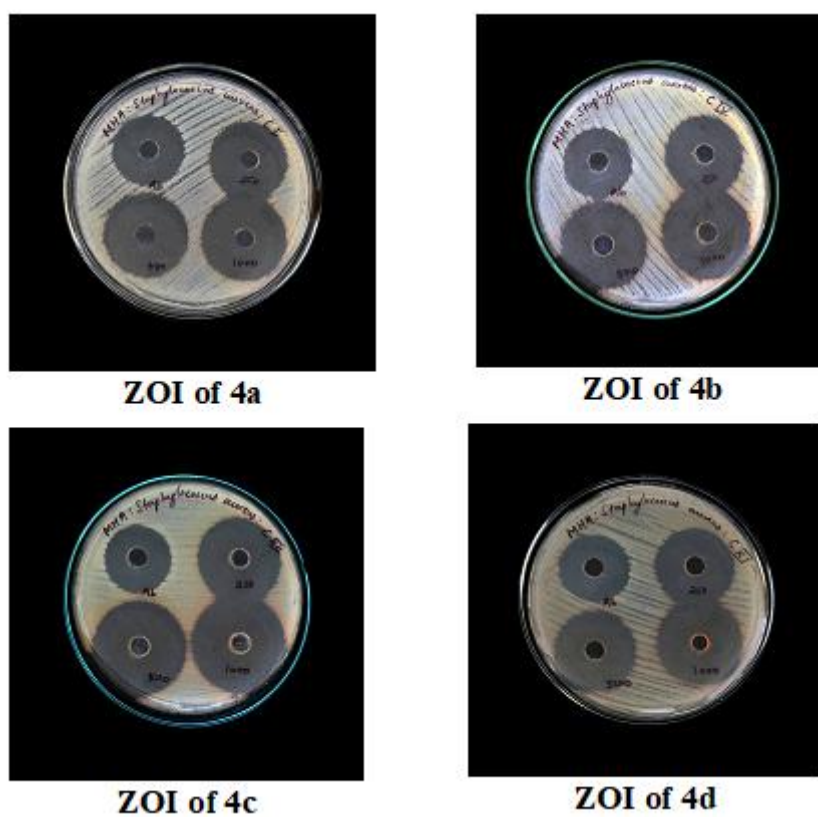


Figure 3: ZOI of *Staphylococcus aureus* by synthesized compounds

**Table 2: MIC of *Escherichia coli* by synthesized compounds at different concentrations**

Organism: <i>E. coli</i> (Gram negative)						
Compounds	Concentration (µg)	OD 1	OD 2	OD 3	Average	Inhibition percentage
4a	Control	0.5714	0.5680	0.5898	0.5764	
	62.5	0.3983	0.3477	0.3889	0.3783	34.36
	125	0.1234	0.1173	0.1181	0.1196	79.25
	250	0.0926	0.0899	0.0921	0.0915	84.11
	500	0.0585	0.0442	0.0470	0.0499	91.34
	1000	0.0125	0.0220	0.0118	0.0154	97.32
4b	Control	0.5617	0.5677	0.5508	0.5600	
	62.5	0.2865	0.2869	0.2881	0.2871	48.72
	125	0.1048	0.1065	0.1077	0.1063	81.01
	250	0.0745	0.0770	0.0621	0.0712	87.28
	500	0.0412	0.0336	0.0488	0.0412	92.64
	1000	0.0120	0.0135	0.01256	0.0126	97.73
4c	Control	0.5713	0.5679	0.5897	0.5763	
	62.5	0.3982	0.3476	0.3888	0.3782	34.37
	125	0.1233	0.1172	0.1180	0.1195	79.26
	250	0.0925	0.0898	0.0920	0.0914	84.13
	500	0.0584	0.0441	0.0469	0.0498	91.35
	1000	0.0125	0.0220	0.0118	0.0154	97.32
4d	Control	0.5008	0.5034	0.5151	0.5064	
	62.5	0.2995	0.2825	0.2849	0.2889	42.93
	125	0.1175	0.1076	0.1123	0.1124	77.79
	250	0.0778	0.082	0.0812	0.0803	84.13
	500	0.0465	0.0384	0.0332	0.0393	92.22
	1000	0.0225	0.0145	0.0123	0.0164	96.75

**Table 3: MIC of *Staphylococcus aureus* by synthesized compounds at different concentrations**

Organism: <i>S. aureus</i> (Gram positive)						
	Concentration (µg)	OD 1	OD 2	OD 3	Average	Inhibition percentage (%)
4a	Control	0.4719	0.4677	0.4672	0.4689	
	62.5	0.2922	0.2804	0.2865	0.286367	38.92
	125	0.1103	0.1031	0.1067	0.1067	77.24
	250	0.0786	0.0821	0.0803	0.080333	82.86
	500	0.0586	0.0413	0.0355	0.045133	90.37
	1000	0.0124	0.0102	0.0118	0.011467	97.55
4b	Control	0.3978	0.3708	0.4357	0.4014	
	62.5	0.2862	0.2088	0.2845	0.2598	35.03
	125	0.1100	0.1113	0.1070	0.1094	72.25
	250	0.0854	0.0889	0.0741	0.0828	78.84
	500	0.0486	0.0556	0.0561	0.0534	86.10
	1000	0.0123	0.0139	0.0158	0.0140	95.86
4c	Control	0.4273	0.5123	0.5144	0.4846	
	62.5	0.3895	0.3459	0.3369	0.3574	26.24
	125	0.1211	0.1157	0.1153	0.1173	75.78
	250	0.0982	0.0981	0.0845	0.0936	80.68
	500	0.0652	0.0521	0.0532	0.0568	88.27
	1000	0.0145	0.0122	0.0114	0.0127	97.37
4d	Control	0.4718	0.4676	0.4671	0.4688	
	62.5	0.2921	0.2803	0.2864	0.2862	38.93
	125	0.1102	0.1030	0.1066	0.1066	77.26
	250	0.0785	0.0820	0.0802	0.0802	82.88
	500	0.0585	0.0412	0.0354	0.0450	90.39
	1000	0.0123	0.0101	0.0117	0.0113	97.57

## DISCUSSION

The intermediate compound 1,10-phenanthroline-5,6-dione and new compounds 4a-4d were synthesized as per procedure. Various spectroscopic methods were used to characterize their structures. The biological actions of the compounds were also evaluated, and their results are interpreted as follows.

### Interpretation of ZOI results

When compared to the control strain, the test compound efficiently inhibits the bacteria if the zone width of the test organism is 20mm or more. If the zone diameter falls between 15mm and 19mm, indicate a moderate level of inhibition by the test compound. If the diameter is less than or equal to 14mm, no significant antibacterial activity can be concluded. Bacterial cultures of *E. coli* and *S. aureus* at concentrations of 250, 500, and 1000µg/ml were used. Streptomycin (100µg) served as the reference medication.

Regarding the data for *E. coli*, compounds 4b demonstrated the largest inhibitory zones, measuring 38mm, at the maximum dose of 1000µg. Other compounds also showed a relatively consistent inhibition zone. At the highest dosage of 1000µg, compound 4c and 4d demonstrated a 38mm zone of inhibition in the findings of *Staphylococcus aureus*. Other compounds also showed good response with most zones around 30-36mm at the tested concentrations. Comparing the compounds to the conventional medicine with zone of inhibitions, they demonstrated good efficacy greater than 20mm, suggesting a remarkable antibacterial inhibitory activity.

### Interpretation of MIC results

The MIC was computed using ED50 PLUS V1.0 software based on optical density readings. More than 95% microbial inhibition rates at maximum concentration of 1000µg were shown by all compounds.

The bacterial growth was visually inspected and the optical density (OD) at 630 nm was quantified using an ELISA plate reader. The percentage of bacterial growth inhibition in each extract was calculated for the dilution in the test wells. All compounds showed promising antibacterial properties with significant growth inhibition at various concentrations.

## CONCLUSION

Numerous microbes were discovered to be the source of infectious illnesses in humans due to the resistance developed by these bacteria to modern drugs over time in several ways. The current scenario presents a serious threat to world health with fewer effective treatment options for antimicrobial infections. Strong infection control techniques and the careful use of presently available treatments are needed to prevent the development of such microbes. To find new antimicrobial agents, imidazo-phenanthroline derivatives have gained attention among the various classes of compounds, due to their diverse biological activities and structural versatility. In conclusion, an efficient and convenient method was developed for the

synthesis of some imidazole phenanthroline derivatives with promising antibacterial applications.

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