

Synthesis and Characterization of Novel Naphthalene-Based Chelators and Their Biological Application

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Abstract

Naphthalene-based chelators have generated significant attention due to their special coordination properties and biological applications. In this study, two novel naphthalene-derived chelators, HLa ($C_{15}H_{12}N_2O_2$) and HLb ($C_{17}H_{12}NO_2Cl$), were synthesized via reflux condensation reactions and were characterized using methods like melting point determination, Fourier Transform Infrared (FTIR), and Nuclear Magnetic Resonance (^1H and ^{13}C NMR) spectroscopy. The synthesized compounds exhibited good thermal stability with HLa exhibiting melting point of 203-205 °C and that of HLb was 205-207 °C, both showing melting points above 200 °C. Both compounds were highly soluble in dimethyl sulfoxide ($(CH_3)_2SO$) but slightly soluble in most polar organic solvents. The spectroscopic investigation confirmed the successful formation of the target chelators and the presence of key functional groups. FTIR analysis of HLa revealed characteristic absorptions at 3029 cm^{-1} (aromatic C-H), 1673 cm^{-1} (C=O), and 1589 cm^{-1} confirmed imine (CH=N) groups while HLb showed bands at 3120 cm^{-1} (O-H), 3061 and 1618 cm^{-1} confirmed aromatic rings and 1618 cm^{-1} (C=N) imine linkage. The ^1HNMR spectrum for LHa displayed signals at δ 10.80 ppm (NH), 8.10-7.50 ppm (pyridine protons), and 2.35 ppm (CH_3), while its $^{13}CNMR$ spectrum showed carbonyl carbons at δ 180-182 ppm. For HLb, ^1HNMR signals at δ 9.90-9.68 ppm (OH) and 9.47 ppm (CH=N), together with $^{13}CNMR$ signals at δ 160-165 ppm (ArC-OH) and 155-160 ppm (CH=N), confirmed the proposed structures. Antimicrobial screening at 10 $\mu g/mL$ revealed that HLa showed notable inhibition against *Klebsiella pneumoniae* (15.5 ± 0.5 mm), *Escherichia coli* (11.5 ± 0.5 mm), and *Pseudomonas aeruginosa* (10.5 ± 0.5 mm). Whereas, HLb exhibited a strong inhibitory effect against *Staphylococcus aureus* (23.0 ± 1.0 mm), but moderate activity against *Staphylococcus epidermidis* (11.5 ± 0.5 mm) and *Bacillus subtilis* (10.5 ± 0.5 mm). These results underscore the potential of naphthalene-based chelators for medicinal and coordination chemistry applications.

Keywords: Chelators, FTIR, NMR spectroscopy, antimicrobial.

Introduction

Chelating agents are a fascinating group of organic compounds that can bind metal ions through multiple coordination sites, creating stable complexes (Cilibrizzi et al., 2018; Chioma et al., 2023). They play crucial roles in various fields like medicinal chemistry, catalysis, environmental cleanup, and materials science (Malinowski et al., 2020). This versatility stems

from their ability to control metal ion levels and mitigate metal-induced toxicity (Hameed et al., 2017; Ceramella et al., 2022). Recently, there has been a surge of interest in designing chelators that are structurally tailored for better selectivity, stability, and biological activity. Among these, naphthalene-based chelators have stood out as promising options due to their unique structural and electronic characteristics (Behera et al., 2026). Naphthalene, with its fused aromatic hydrocarbon structure, offers a rigid, flat framework that boosts π - π interactions, electron delocalization, and coordination capabilities when paired with the right donor groups. Adding functional groups like Schiff base linkages ($-C=N-$), hydroxyl groups, or heteroatoms (N, O, S) to the naphthalene structure can significantly enhance its chelating efficiency and its ability to form stable metal complexes (Singh et al., 2016; Malik et al., 2017). The extended conjugation of the naphthalene ring also improves its photophysical and redox properties, making these compounds appealing for both biological and material applications (Sharma et al., 2024).

The importance of naphthalene-based chelators in biology is closely tied to the crucial roles that metal ions play in our physiological systems (Liu et al., 2021). Transition metals like iron, copper, and zinc are key players in processes such as enzymatic reactions, electron transport, and cellular signaling. However, when these metal ions are out of balance, it can lead to oxidative stress and various health issues, including cancer and infections. Naphthalene-derived chelators can help regulate the availability of these metal ions and interact with biological targets, making them promising candidates for therapeutic use (Hossain et al., 2018).

Recent research has shown that naphthalene-based metal complexes possess notable biological activities, such as antimicrobial, antifungal, antioxidant, and anticancer effects (Sinicropi et al., 2022). These biological activities are due to increased lipophilicity, better membrane permeability, and stronger interactions with biological macromolecules like DNA and proteins (Uddin et al., 2019; Hassan et al., 2022; Jorge et al., 2024). Additionally, the flat aromatic structure of naphthalene allows it to intercalate with DNA, which enhances its cytotoxic and anticancer properties (Tadele & Tsega, 2019). When it comes to their biological significance, naphthalene-based chelators are making waves in both analytical chemistry and materials science (Bhosale et al., 2021). Due to their impressive coordination capabilities and adjustable electronic properties, they are perfect for applications in electrochemical sensors, catalysis, and cutting-edge functional materials (Oiyee et al., 2019; Pour et al., 2019; Xu et al., 2020). Plus, adding naphthalene groups to polymeric and nanostructured systems has been shown to boost stability, conductivity, and biological activity, which really expands their potential uses (Khan et al., 2024).

Although, numerous aromatic-based chelators and their metal complexes have demonstrated promising biological activities, relatively few studies have investigated naphthalene-derived Schiff base chelators, particularly how their structure modifications influence biological activity. This study dives into creating new naphthalene-derived chelators, exploring their structures through cutting-edge spectroscopic techniques, and assessing their biological activity. We are particularly focused on unraveling how molecular structure ties into coordination behavior and biological effectiveness. These insights are crucial for designing the next wave of chelating agents that could have exciting applications in medicinal chemistry, environmental science, and advanced materials.

Materials and Methods

Chemicals and Instruments

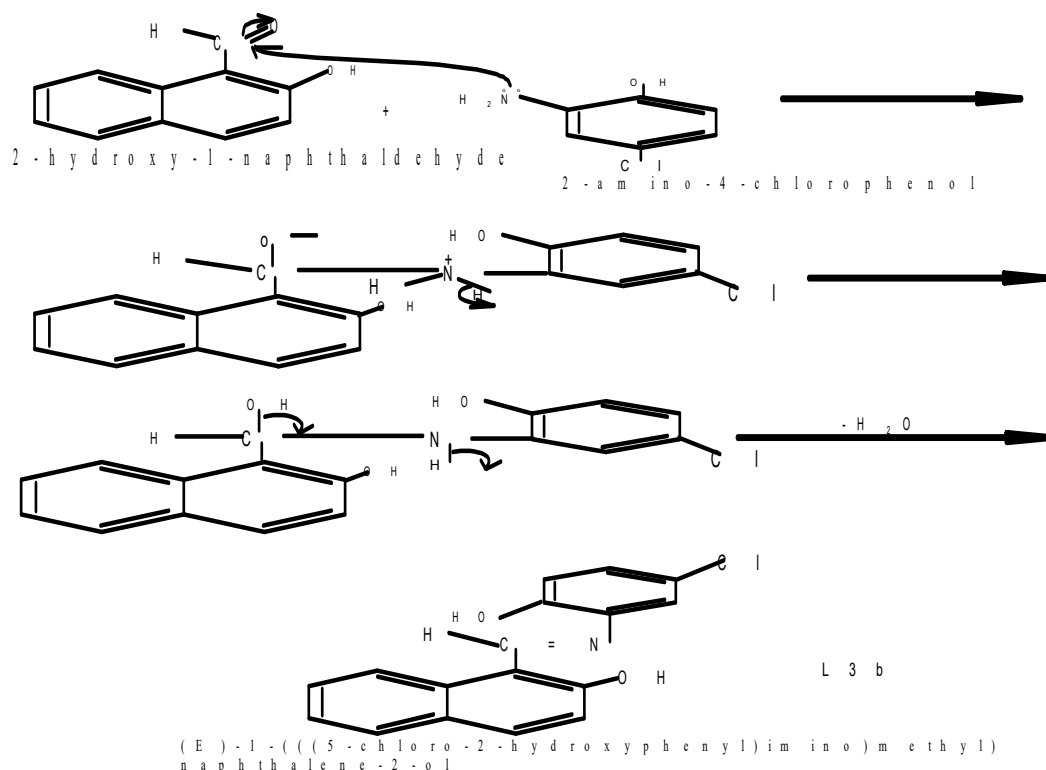
All the chemicals, reagents and solvents used in the chemical synthesis of the compounds were of analytical grade and were purchased from Sigma Aldrich. The melting point of the

synthesised organic chelators was determined using an Electrothermal Melting Point Apparatus with open-ended glass capillary tubes. The Fourier Transform Infrared (FTIR) spectra of the synthesised compounds were recorded using a Perkin Elmer FTIR Spectrum BX spectrophotometer while the ^1H and ^{13}C Nuclear Magnetic Resonance (NMR) spectra of the synthesised compounds were recorded at room temperature using a Bruker Avance III 300 MHz spectrophotometer.

Synthesis of the chelators

The organic chelators were synthesised using reflux condensation reaction method.

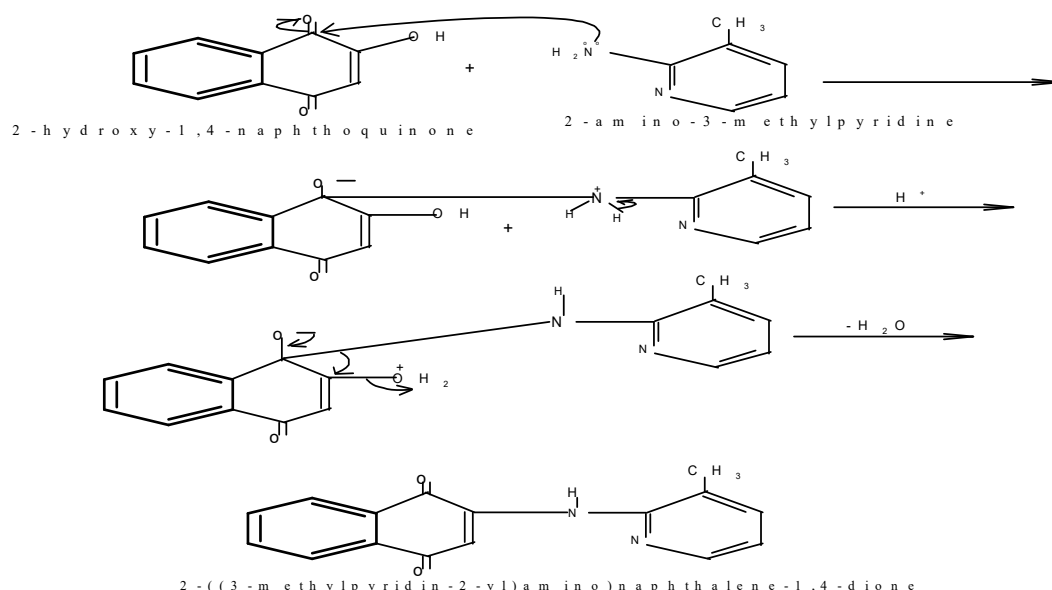
Synthesis of HL_a: A stoichiometric mixture of 1.74g (10 mmol) of 2-hydroxy-1,4-naphthoquinone and 1.08g (10 mmol) of 2-amino-3-methylpyridine were dissolved in 50 mL of ethanol within a 250 mL round-bottom flask equipped with a magnetic stir bar. The reaction mixture was stirred and refluxed at specific temperature range for 3-6 hours to facilitate condensation. During the reflux period, drops of glacial acetic acid were added to promote product formation. The mixture was then allowed to cool to room temperature in a water bath, leading to the gradual formation of a solid orange precipitate. The precipitate was separated by gravity filtration, thoroughly washed with cold ethanol to eliminate impurities, and air-dried to afford the purified product.



Scheme 1: Mechanism of reaction for the synthesis of HL_a

Synthesis of HL_b: A 250 mL round-bottom flask equipped with a magnetic stir bar was charged with 50 mL of absolute ethanol. To this, 1.72 g (10 mmol) of 2-hydroxy-1-naphthaldehyde and 1.56 g (10 mmol) of 2-amino-4-chlorophenol (97% pure) were added sequentially. The reaction mixture was stirred and heated under reflux at specific temperature for 3-6 hours. During this

period, drops of glacial acetic acid were added to promote the completion of the condensation reaction. Upon cooling to room temperature, a solid brown precipitate formed, which was collected by gravity filtration using a Buchner funnel. The crude product was washed thoroughly with cold ethanol (10 mL) to remove unreacted starting materials and side products. The solid was then air-dried at room temperature to yield the pure product as a yellow solid, which was later stored in a dry sample bottle and kept safely for further analysis.



Scheme 2: Mechanism of reaction for the synthesis of HLb

Antibacterial Screening

The synthesised chelators **HLa** and **HLb** were tested against seven Gram negative and Gram positive bacteria, namely *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Pseudomonads aeruginosa*, *Klebsiella pneumoniae*, *Bacillus subtilis*, and *Proteus mirabilis*. All the tests were performed using Agar-well diffusion method. The solvent used for the screening was Dimethyl Sulfoxide (CH₃)₂SO and for all the tested samples, concentrations were 10 µg/mL. Comparison of data obtained was achieved using Streptomycin as reference standard for the antibacterial screening following it's widespread application as positive controls in antibacterial susceptibility studies

Melting Point

The melting point of the synthesised compounds are reported in Table 1.0. Both **HLa** and **HLb** showed melting points above 200 °C indicating good thermal stability and strong intermolecular interaction within the molecular framework. The high and sharp melting points further confirm the successful formation of stable, highly crystalline Schiff base compounds (Festus et al., 2022).

Table 1.0: Physicochemical properties of the synthesised compounds

Compound	Molecular formula	Molecular weight	Colour	Melting Point (°C)
HLa	C ₁₅ H ₁₂ N ₂ O ₂	252.27	Orange	203 -305
HLb	C ₁₇ H ₁₂ NO ₂ Cl	296.73	Brown	205-207

Solubility

The solubility study of the synthesised chelators showed difference in the degree of solubility of the synthesised chelators in different solvents with different degree of polarity. The compounds showed very good solubility in Dimethyl sulfoxide, (CH₃)₂SO, while **HLa** was slightly soluble in Dimethylformamide, (CH₃)₂NCHO. Both **HLa** and **HLb** were slightly soluble in organic solvents that showed higher degree of polarity such as ethanol, CH₃CH₂OH, methanol, CH₃OH, dichloromethane, CH₂Cl₂, but completely insoluble in water, H₂O.

FTIR Characterization of the compounds

The FTIR spectrum of the synthesized chelator **HLa** confirmed the condensation between 2-hydroxy-1,4-naphthoquinone and 2-amino-3-methylpyridine, resulting in the formation of a Schiff base (imine) linkage. The parent compound, 2-hydroxy-1,4-naphthoquinone, exhibits a characteristic quinone C=O stretching vibration within the 1660–1680 cm⁻¹ region. In **HLa**, this band appeared at 1673 cm⁻¹, indicating that the quinone carbonyl group remained intact and did not participate directly in bond formation (More et al., 2019). The primary amine (–NH₂) stretching bands of 2-amino-3-methylpyridine, normally observed between 3300 and 3500 cm⁻¹, disappeared completely in the product spectrum, confirming consumption of the amino group during condensation. A new absorption band at 1589 cm⁻¹, assigned to azomethine (C=N) stretching, verified Schiff base formation (Berhanu et al., 2019; More et al., 2019). Furthermore, the aromatic C–H stretching band at 3029 cm⁻¹ confirmed that the aromatic naphthoquinone and pyridine ring systems remained structurally intact after synthesis (Berhanu et al., 2019).

The FTIR spectrum of **HLb** provides solid evidence for the successful synthesis between 2-hydroxy-1-naphthaldehyde and 2-amino-4-chlorophenol, leading to the synthesis of the chelator. In the original reactants, 2-hydroxy-1-naphthaldehyde shows a distinct aldehydic carbonyl (C=O) stretching vibration around 1660–1680 cm⁻¹, while 2-amino-4-chlorophenol features primary amine (–NH₂) stretching bands in the 3300–3500 cm⁻¹ range. The absence of these aldehydic C=O and amine N–H stretching and in the spectrum of **HLb**, along with the appearance of a strong azomethine ν(C=N) absorption at 1618 cm⁻¹, confirms the formation of the imine linkage (–CH=N–). Additionally, the broad O–H band suggests that the phenolic hydroxyl group is still present, while the aromatic C–H stretching bands at 3061 cm⁻¹ and 3025 cm⁻¹, along with the aromatic C=C vibration at 1546 cm⁻¹, confirm the existence of the conjugated naphthalene and chlorophenyl rings, supporting the proposed structure of the synthesized chelator (Uddin et al., 2020; Fabbriizzi, 2020).

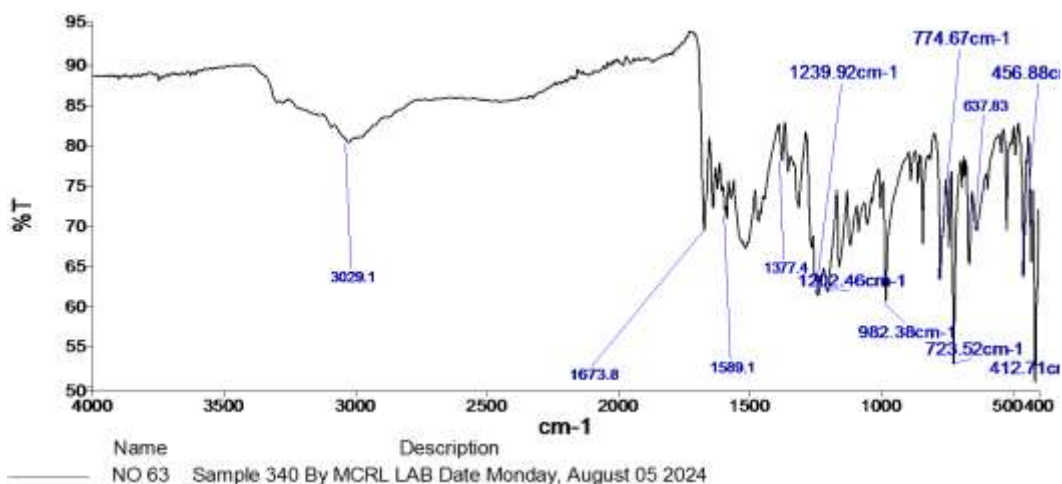


Fig. 1: FTIR Spectrum of HLa

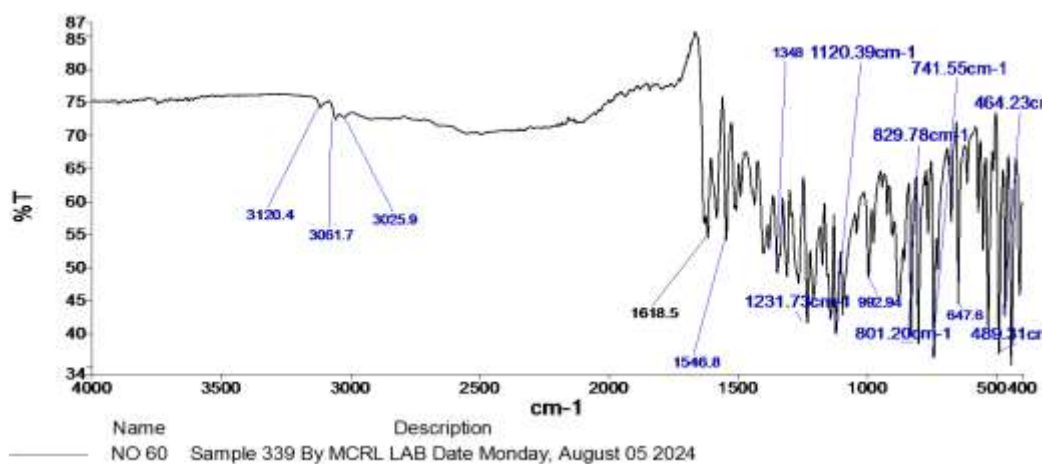


Fig. 2: FTIR Spectrum from L3b

Nuclear Magnetic Resonance (NMR) Spectrum of the compounds

The NMR spectra of **HLa** confirmed the successful condensation of 2-hydroxy-1,4-naphthoquinone with 2-amino-3-methylpyridine. In the starting amine, the primary amino ($-NH_2$) protons normally appear as a broad signal at δ 4–6 ppm. In contrast, the product exhibited a broad singlet at δ 10.80 ppm, assigned to the secondary $-NH$ proton formed after condensation. The disappearance of the characteristic amino proton resonance further confirmed complete condensation (Don-Lawson & Chioma, 2021; Mu et al., 2021). The aromatic proton resonances at δ 8.10–7.50 ppm (naphthoquinone) and δ 8.20, 7.75, and 7.35 ppm (pyridyl ring) reflected the expected deshielding effects of the extended π -conjugated system, consistent with related aminonaphthoquinone Schiff bases (Bala et al., 2021). The ^{13}C NMR spectrum showed signals at δ 180 and 172 ppm for the quinone carbonyl carbons, indicating retention of the quinone functionality. Resonances at δ 122–140 ppm and δ 125–140 ppm were assigned to naphthoquinone and pyridine carbons, respectively, while the methyl carbon signal at δ 21–25

ppm confirmed the methyl substituent, consistent with similar naphthoquinone Schiff bases (Bala et al., 2021).

The ^1H NMR spectrum of **HLb** confirmed the successful condensation of 2-hydroxy-1-naphthaldehyde with 2-amino-4-chlorophenol. A broad resonance at δ 9.90–9.68 ppm was assigned to the hydrogen-bonded phenolic –OH proton. The disappearance of the aldehyde proton, normally observed around δ 10 ppm, together with the appearance of a new singlet at δ 9.47 ppm for the azomethine (–CH=N–) proton, confirmed Schiff-base formation, consistent with related 2-hydroxy-1-naphthaldehyde-derived chelators (Mu et al., 2021). The aromatic proton resonances at δ 8.04–7.22 ppm were assigned to the naphthalene and chlorophenol rings. The ^{13}C NMR spectrum showed signals at δ 160–165 ppm for the phenolic C–OH carbons and δ 155–160 ppm for the azomethine carbon. Resonances between δ 115–145 ppm corresponded to the aromatic carbons of the naphthalene and chlorophenol rings, supporting the proposed structure of **HLb** and agreeing with published NMR data for structurally related naphthaldehyde Schiff bases (Don-Lawson & Chioma, 2021).

Table 2: ^1H NMR and ^{13}C NMR data of the synthesised chelators

^1H NMR		^{13}C NMR	
Site (HLa)	Chemical Shift, δ (ppm)	Site (HLa)	Chemical Shift, δ (ppm)
-N-H	10.80.	-CH=O	180-182
-OH	-	-ArC-NH	150-156
Ar-H (pyridine)	8.20-7.75	Ar-CH (pyridine)	125-140
Ar-H (naphthal)	8.10+7.50	Ar-C (naphthal)	122-140
CH3	2.35	CH3	21-25
(HLb)		HLb	
-OH	9.90-9.68.	ArC-OH	160-165
-CH=N-	9.47	-CH=N-	155-160
Ar-H (naphthal)	8.04-7.23	ArC (phenyl)	155-145
Ar-H (phenol)	7.05-6.99	ArC (naphthal)	120-135

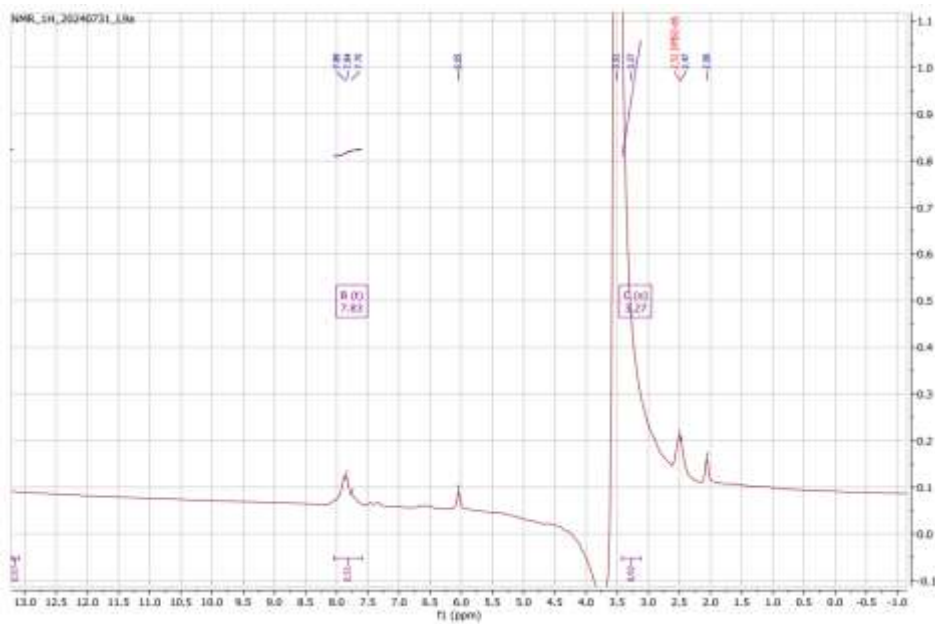


Fig 3: ^1H NMR spectrum of HLa

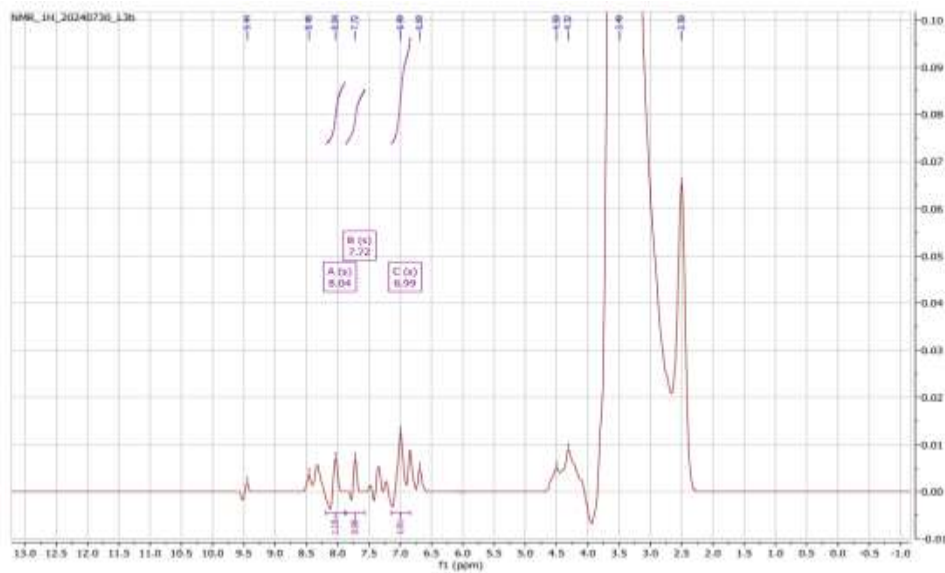


Fig 4: ^{13}C NMR spectrum of HLa

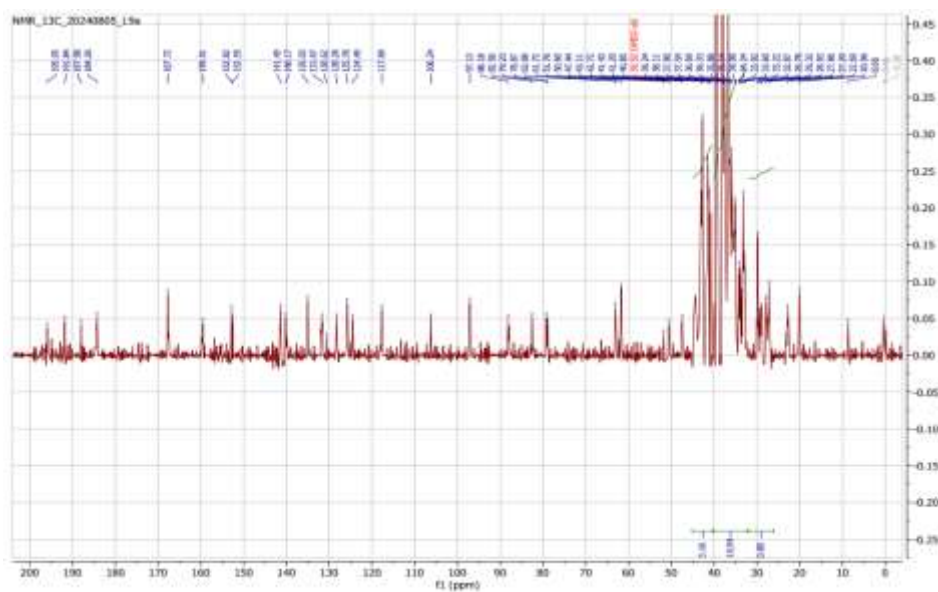


Fig 5: ^1H NMR spectrum of HLb

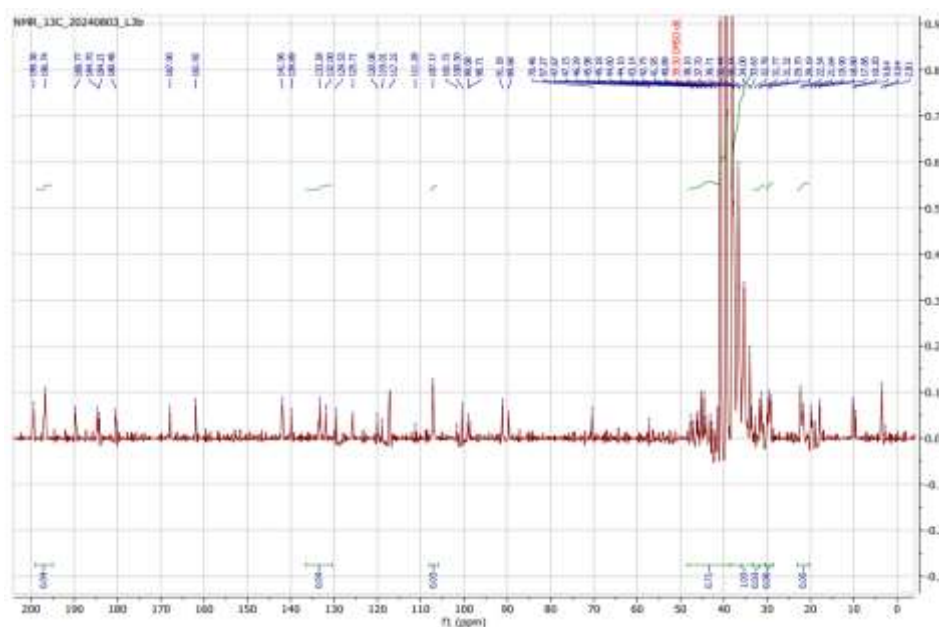


Fig 6: ^{13}C NMR spectrum of HLb

Antibacterial Screening

The anti-bacterial screening results are reported in Table 5.0. The results revealed that both chelators showed selective effectiveness against the tested bacterial strains. **HLa** had its strongest inhibition against *Klebsiella pneumoniae*, measuring 15.5 ± 0.5 mm, which was better than streptomycin's 11.5 ± 0.5 mm. It also demonstrated activity against *Escherichia coli* (11.5 ± 0.5 mm) and *Pseudomonas aeruginosa* (10.5 ± 0.5 mm), but had no inhibition against

Staphylococcus aureus and *Bacillus subtilis*. On the other hand, **HLb** proved to be very effective against *Staphylococcus aureus*, with an inhibition of 23.0 ± 1.0 mm, outperforming streptomycin's 15.0 ± 1.0 mm. It also showed moderate inhibition against *Staphylococcus epidermidis* and *Bacillus subtilis*. These results indicated that the structural differences between **HLa** and **HLb** played a role in how susceptible the bacteria are, with variations in lipophilicity and electronic properties influencing how well they penetrated membranes and interact with crucial microbial biomolecules. This selective antibacterial behavior aligns with previous studies indicating that Schiff base chelators have antimicrobial activity that depends on their structure, affecting both Gram-positive and Gram-negative bacteria (Festus et al., 2019).

Table 3: Anti-bacterial screening values of the synthesised compounds

Chelator	<i>E. coli</i>	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>B. subtilis</i>	<i>P. mirabilis</i>
HLa	11.5±0.5	0.0±0.0	5.5±0.5	10.5±0.5	15.5±0.5	0.0±0.0	7.0±1.0
HLb	0.0±0.0	23.0±1.0	11.5±0.5	0.0±0.0	5.5±0.5	10.5±0.5	5.0±1.0
Streptomycin	13±1.0	15±1.0	0.0±0.0	9.5±0.5	11.5±0.5	19.5±0.5	8.5±0.5

HLa = $C_{15}H_{12}N_2O_2$, **HLb** = $C_{17}H_{12}NO_2Cl$

Conclusion

We successfully synthesized and characterized two new naphthalene-based chelators, **HLa** and **HLb**, using FTIR and NMR spectroscopic techniques. These compounds demonstrated impressive thermal stability and showed unique structural features that align with their proposed molecular designs. When we conducted biological screening, we found that they exhibited selective antibacterial activities, with **HLb** standing out for its strong effectiveness against *Staphylococcus aureus*. These results underscore the potential of naphthalene-derived chelators as exciting candidates for the production of antibacterial medicine and in coordination chemistry.

Acknowledgements

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Conflict of Interest

The authors confirm that there is no known conflict of interest with respect to this article

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