



A Novel Cyclohexyl-Naphthyl Bis-Hydrazone Schiff Base: Synthesis, Characterization and Antibacterial Activity

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Abstract

A novel bis-hydrazone Schiff base, (2E)-1-(cyclohexyl(phenyl)methylene)-2-(naphthalen-1-ylmethylene)hydrazine (CycPh–NapHz), was synthesized through a straightforward condensation reaction between (Z)-(cyclohexyl(phenyl)methylene)hydrazine and 1-naphthaldehyde in ethanol under reflux conditions. The synthetic procedure was efficient, yielding the target compound as a yellow crystalline solid in 81% yield. The synthesized compound (CycPh–NapHz) was characterized through FT-IR and ¹H NMR spectroscopy. The antibacterial activity of the synthesized hydrazone was evaluated against the Gram-positive bacterium *Staphylococcus aureus* and the Gram-negative bacterium *Pseudomonas aeruginosa* using the disc diffusion assay. CycPh–NapHz exhibited moderate antibacterial

effects, with inhibition zones of 14 mm against *S. aureus* and 12 mm against *P. aeruginosa*, in comparison with 28 mm observed for the standard antibiotic tetracycline. The antibacterial behavior of the compound can be attributed to the presence of the azomethine functionality along with an extended aromatic framework, which may enhance interactions with bacterial membranes and intracellular targets. Overall, these findings demonstrate that the synthesized molecule represents a useful structural framework for further modification, and that additional investigations, particularly minimum inhibitory concentration studies and the synthesis of related metal complexes, may contribute to improving its antibacterial efficacy, as metal coordination has been shown to enhance biological performance in comparable systems.

Keywords: Air pollution; Health impacts; Mardan; Particulate matter; Urban–rural comparison; Vehicular emissions

Introduction

Schiff bases, also known as imines or azomethines, are an important class of organic compounds characterized by the presence of a carbon-nitrogen double bond (C=N). These compounds are typically formed through the condensation of a primary amine with an aldehyde or ketone under mild reaction conditions (F. Nicotra et al., 2014; N. N. G. Wood et al., 1997). First discovered by Hugo Schiff in 1864, Schiff bases have attracted considerable attention due to their structural diversity, straightforward synthesis, and versatile chemical properties (A. S. Alpan et al., 2007). The imine functionality not only serves as a reactive site but also contributes to the formation of conjugated systems that can influence the electronic, optical, and chemical behavior of these molecules (S. V. Gaikwad et al., 2018). Their relatively simple synthetic procedures, combined with the possibility of structural modification, make Schiff bases a valuable platform for developing novel organic compounds with potential applications in both fundamental and applied chemistry (R. R. Gupta et al., 1998). Over the years, these compounds have found utility in a wide array of fields, including coordination chemistry, catalysis, analytical chemistry, material science, and medicinal chemistry, due to their ability to interact with various chemical species and biological targets (C. D. Silva et al., 2011).

Schiff bases are particularly notable for their ability to act as ligands in coordination chemistry. The nitrogen atom of the imine group possesses a lone pair of electrons that can be donated to metal centers, forming stable complexes with both transition metals and main group metals. Such complexes often exhibit unique catalytic, magnetic, and electronic properties that are not observed in the free ligand (Abd El-Halim, H. F et al., 2017). Incorporating aromatic or heteroaromatic substituents into the Schiff base framework generally enhances the stability of these metal complexes and can introduce additional π -conjugation, which may further modulate their chemical and biological properties. Among Schiff bases, hydrazone derivatives—formed by the condensation of hydrazines with carbonyl compounds—have drawn significant attention (J. Kumar et al., 2017; S. Avram et al., 2021; S. Ejaz et al., 2019; M. A. Bhat et al., 2019). These compounds display greater rigidity and extended conjugation compared to simple imines, which can improve their coordination behavior and chemical stability. This enhanced structural framework makes hydrazones particularly attractive for applications ranging from metal ion sensing to the development of biologically active compounds (Alalwiat, Ahlam A et al., 2025; A. Naqvi et al., 2009; M. Zarei et al., 2011).

In recent years, Schiff bases and their derivatives have been extensively investigated for their biological activities. Numerous studies have reported their antimicrobial, antifungal, anticancer, antiviral, and antioxidant properties. The biological activity of Schiff bases is highly influenced by the nature of the substituents attached to the imine group, as well as the overall three-dimensional molecular structure (Pandey, V et al., 2012; Kargar, H et al., 2021; Ur Rahman, Hameedrt al., 2024; G. T. Tigineh et al., 2015). For instance, the presence of cyclic groups, such as cyclohexyl rings, introduces conformational flexibility and hydrophobic interactions, while planar aromatic systems like naphthalene provide extended conjugation and potential π - π stacking interactions with biological macromolecules. By carefully selecting substituents, researchers can modulate lipophilicity, cellular uptake, and target specificity, thereby designing Schiff bases with improved pharmacological profiles (P. Kamaria et al., 2011; Khan, Shahab et al., 2025; A. Mermer et al., 2019). This makes them highly valuable in medicinal chemistry, where structure-activity relationship (SAR) studies can guide the rational development of potent and selective bioactive compounds (E. Umofia

et al., 2016; G. T. Tigineh et al., 2015; Alalwiat, Ahlam A et al., 2025). Hydrazone derivatives, in particular, are of great interest due to their versatile chemical properties and broad biological potential. These compounds can form stable complexes with metals, which can be applied in catalysis, sensing, and drug delivery systems (P. Silku et al., 2016; Nurudeen, Saidu et al., 2015; Phaniband, M. A et al., 2009; M. S. Alam et al., 2012). The presence of conjugated π -systems and electron-rich nitrogen atoms enables hydrazones to act as fluorescent probes and molecular sensors, facilitating detection and quantification of metal ions and other analytes (Muhammad, M et al., 2025; K. Andiappan et al., 2018; Khan, Maaz et al., 2024; X. Wang et al., 2014; X. D. Jin et al., 2011). Moreover, bis-Schiff bases, which contain two imine functionalities within a single molecule, provide additional sites for coordination, enabling multidentate ligand formation. Such structural features open possibilities for multifunctional applications in both chemical and biological systems, including the design of new catalytic systems, metal-based therapeutics, and diagnostic tools (A. Jarrahpour et al., 2007; Khan, Maaz et al., 2025; K. M. Khan et al., 2014; F. Rahim et al., 2020; M. Bashiri et al., 2021).

This study focuses on the synthesis and characterization of a novel bis-hydrazone derivative, **(2E)-1-(cyclohexyl(phenyl)methylene)-2-(naphthalen-1-ylmethylene)hydrazine** (CycPh–NapHz) **compound**. This compound is derived from cyclohexyl phenyl ketone and 1-naphthaldehyde, chosen for their complementary structural and electronic properties. The cyclohexyl ring contributes a flexible aliphatic component that can influence the overall molecular conformation, solubility, and steric environment, while the naphthyl group adds a rigid, planar aromatic system that enhances π -conjugation and potential intermolecular interactions. Together, these structural elements are expected to influence not only the chemical reactivity and stability of the hydrazone derivative but also its potential biological activity. The design of this compound aims to integrate structural rigidity, electronic conjugation, and steric factors, resulting in a molecule with enhanced functional versatility and promising properties for further exploration.

Research OBJECTIVES

1. To synthesize a new bis-hydrazone Schiff base through a straightforward condensation reaction carried out under controlled reflux conditions.
2. To confirm the successful formation and structural features of the synthesized compound using FT-IR and ^1H NMR spectroscopy.
3. To investigate the antibacterial activity of the prepared compound against representative Gram-positive and Gram-negative bacterial strains.
4. To assess how key structural elements, particularly the azomethine linkage and aromatic groups, influence the observed antibacterial response.
5. To provide baseline experimental data that may support future work on structural modification, metal complex formation, and more detailed biological evaluation.

Significance of the study

The synthesis and investigation of Schiff base and hydrazone derivatives remain an active area of research because of their simple preparation methods and wide range of applications. In this work, a new bis-hydrazone Schiff base incorporating both flexible aliphatic and rigid aromatic components has been successfully developed, adding to the growing library of structurally diverse hydrazone systems. The presence of two azomethine groups, together with phenyl, naphthyl, and cyclohexyl moieties, gives the molecule a balanced combination of conjugation and hydrophobic character. These features are important not only for structural stability but also for potential biological interactions. The moderate antibacterial activity observed against both tested bacterial strains suggests that the compound has the ability to interact with microbial systems, even in its unmodified ligand form.

Although the antibacterial performance is lower than that of the standard antibiotic, the results are encouraging when compared with similar hydrazone derivatives reported in the literature. More importantly, the study highlights the compound's suitability as a starting point for further development. Structural optimization and metal coordination, which are known to enhance biological activity in Schiff base systems, may lead to improved antimicrobial properties.

Overall, this research contributes useful experimental and biological insight into bis-hydrazone Schiff bases and supports their continued exploration as adaptable scaffolds for future chemical and medicinal applications.

Literature Review

Hydrazone and bis-hydrazone Schiff bases are most often prepared through straightforward condensation reactions involving hydrazine derivatives and aldehydes or ketones. This synthetic route has remained popular because it is simple, reliable, and compatible with a wide range of functional groups. In most reported studies, the reaction is carried out in alcoholic solvents such as ethanol or methanol under reflux, which provides sufficient thermal energy to drive imine formation while maintaining good solubility of the reactants. The addition of a small amount of acid, commonly glacial acetic acid, is frequently used to activate the carbonyl group and promote dehydration of the intermediate. Reaction times typically range from several hours to overnight, depending on the steric and electronic nature of the substrates. Aromatic aldehydes and ketones, particularly those containing extended conjugated systems, are known to give stable hydrazone products that readily crystallize from the reaction medium, allowing isolation without extensive purification. When asymmetrical bis-hydrazone compounds are required, a stepwise condensation strategy is often preferred, as it allows better control over the reaction sequence and minimizes the formation of undesired side products (Jarrahpour et al., 2007).

Although a variety of alternative synthetic techniques have been developed in recent years, conventional reflux condensation remains the method of choice for many hydrazone systems, especially those incorporating bulky or rigid structural units (Naqvi, A et al., 2009). Microwave-assisted and ultrasonic methods have been reported to significantly reduce reaction times, while solvent-free and aqueous protocols have been introduced in line with green chemistry principles. Despite these advances, such methods are not always ideal for sterically demanding substrates, where controlled heating and longer reaction times are necessary to ensure complete conversion. The literature consistently shows that ethanol-based reflux conditions provide a good balance between reaction efficiency, reproducibility, and scalability, particularly for compounds combining flexible aliphatic groups with large

aromatic moieties. In systems containing cyclohexyl-substituted ketones and polycyclic aromatic aldehydes, gradual imine formation under reflux conditions helps produce well-defined, crystalline hydrazone products with high yields and structural integrity (Tigineh, G. T et al., 2015; Mermer, A et al., 2019; Kumar, J et al., 2017). As a result, this classical synthetic approach continues to be widely employed for the preparation of complex bis-hydrazone Schiff bases similar to those investigated in the present work.

Experimental

Chemicals and Instruments

In this research, work analytical grade reagents and solvent were used. The chemicals and reagents employed were ethanol, potassium carbonate and different acid halides. The product purities were examined by means of TLC cards coated with silica. TLC profiles for all reactions were established by n-Hexane and ethyl acetate used as solvent systems. TLC was used to determine the time for the completion of reaction to obtain the excellent product yield. The UV lamp was used for visualizing spots on the TLC strips.

Borosilicate and Pyrex clean and dry glassware were used in order to obtain pure product. Chemicals of high purity and synthetic grade reagents were used and all the reactions were carried according to the definite protocol. Standard procedures were followed for synthesizing the cyclohexyl phenyl ketone bis Schiff base derivatives. All of the synthetic grade reagent and solvents used obtained from Merck, Aldrich and BDH. For FT-IR and proton NMR characterization of the synthesized compound were carried out through FT-IR spectrophotometer (Schimadzu-1802 Japan) and Bruker (Advance III MHz) spectrometer.

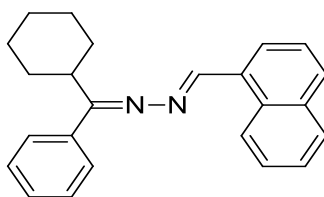
Synthesis of (2E)-1-(cyclohexyl(phenyl)methylene)-2-(naphthalen-1-ylmethylene)hydrazine (CycPh–NapHz)

For the synthesis, (Z)-(cyclohexyl(phenyl)methylene)hydrazine (starting materials) (0.1196 mmol, 0.04 g) was first dissolved in ethanol in a round-bottom flask and stirred on a hotplate to ensure complete mixing. After about 15 minutes, a few drops of glacial acetic acid were added to activate the reaction. The mixture was allowed to stir for an additional 10 minutes before introducing an equimolar amount of 1-naphthaldehyde. The reaction was then

maintained under reflux at 70 °C for approximately 10 hours. Throughout the process, the progress of the reaction was monitored using a solvent system of ethyl acetate and n-hexane. Once the reaction reached completion, the mixture was poured into cold water, which prompted the formation of a solid product. The precipitated solid was collected by filtration, washed, and dried thoroughly to obtain the final compound.

Physical data

Molecular-formula	C ₂₄ H ₂₄ N ₂
Molecular-Weight	340.46 g/mol
Solubility	DMSO, DMF, Chloroform.
Melting point	140 °C
Yield	81%
Color	Yellow



(*2E*)-1-(cyclohexyl(phenyl)methylene)-2-(naphthalen-1-ylmethylene)hydrazine

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Characterization

¹HNMR (CD₃Cl, 400Hz, δ (ppm): δ =1.59-1.21 (m, 6H, 3CH₂-Cyclic), δ =1.95-1.68 (m, 4H, 2CH₂-Cyclic), δ =3.27 (tt, *J* = 11.6 Hz, 3.2 Hz, 1H, 1CH- Cyclic), 7.71-7.51 (m, 8H, Ar-H), δ =8.15 (d, *J*=7.2 Hz 1H, Ar-H), δ =8.95 (d, *J*= 8.6 Hz, 1H, Ar-H), 9.47 (s, 1H, Ar-CH=N). FT-IR data of *2E*-1-(cyclohexyl(phenyl)methylene)-2-(naphthalen-1-ylmethylene)hydrazine (CycPh-NapHz) is given in the Table 1.

Antibacterial Activity

The antibacterial potential of the synthesized hydrazone, (2E)-1-(cyclohexyl(phenyl)methylene)-2-(naphthalen-1-ylmethylene)hydrazine (CycPh–NapHz), was investigated using the standard disc diffusion technique. The study was carried out against *Staphylococcus aureus* as a representative Gram-positive strain and *Pseudomonas aeruginosa* as a Gram-negative strain. Actively growing bacterial cultures were prepared and adjusted to a turbidity equivalent to the 0.5 McFarland standard. Mueller–Hinton agar plates were uniformly inoculated with the respective bacterial suspensions. Sterile filter paper discs were then impregnated with a solution of the test compound at a fixed concentration and carefully placed on the inoculated agar surface. Tetracycline was employed as the reference antibacterial agent, while discs treated with the solvent alone were used as negative controls. All plates were incubated at **37 °C for 24 h**. After incubation, antibacterial activity was evaluated by measuring the diameters of the inhibition zones formed around the discs, and the results were recorded in millimeters (mm).

Results and discussion

Characterization of CycPh–NapHz

The ¹H NMR and FT-IR spectra of the synthesized (2E)-1-(cyclohexyl(phenyl)methylene)-2-(naphthalen-1-ylmethylene)hydrazine (CycPh–NapHz) is given below:

¹H NMR

The ¹H NMR spectrum of Compound 2E-1-(cyclohexyl(phenyl)methylene)-2-(naphthalen-1-ylmethylene)hydrazine (**Figure 1**) shows two well-defined multiplets appearing in the region of δ 1.95–1.21. These signals, integrating for **6H** and **4H**, clearly correspond to the protons of the **cyclohexyl ring**, reflecting its characteristic proton environment. In addition, a **triplet of triplets** observed at δ 3.24 with an integral of **1H** is also attributed to the cyclohexyl framework, indicating the presence of a proton situated in a slightly deshielded position. In the aromatic portion of the spectrum, two prominent **doublets** at δ 8.97 and δ 8.15, each integrating for **2H**, along with a broad **multiplet** spanning δ 7.71–7.51 and integrating for **8H**, represent the collection of **aromatic protons** distributed across the phenyl

and naphthyl rings. A sharp and easily identifiable **singlet** at δ 9.47 (1H) is assigned to the **imine proton** ($-\text{CH}=\text{N}-$), serving as a key indicator of the formation of the hydrazone linkage in Compound 2E-1-(cyclohexyl(phenyl)methylene)-2-(naphthalen-1-ylmethylene)hydrazine.

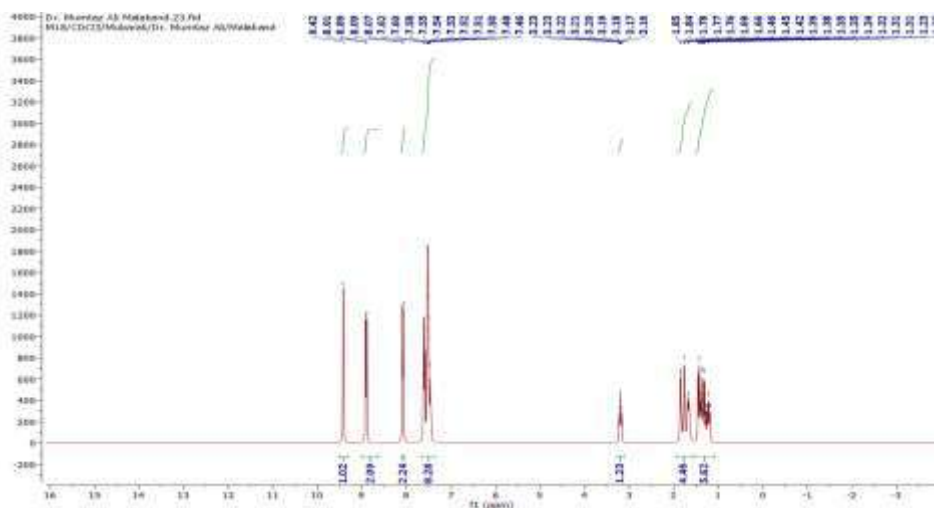


Figure 1: ^1H NMR Spectrum of CycPh–NapHz

FT-IR

FTIR spectrum of Compound (CycPh–NapHz) as shown in (Figure 2) conform the synthesis of expected compound because of two absorption frequency bands at 1608 and 1622 cm^{-1} which corresponds to two imine bonds expected in the molecules. The absorption frequency bands from 2920 to 2820 cm^{-1} and from 1420 to 1340 cm^{-1} show the aliphatic C-H stretching and bending frequency of cyclohexyl ring respectively and absorption bands from 3130 to 3040 and 950 to 643 show the aromatic C-H stretching frequency and bending frequency respectively. The infrared stretching absorption frequency bands occur above 1700 cm^{-1} for carbonyl compounds and the imine ($\text{C}=\text{N}$) stretching frequency bands occur below 1660 cm^{-1} and also the infrared stretching absorption frequency of $\text{C}=\text{N}$ depends on the nature of R_1 and R_2 ($\text{R}_1\text{-C}=\text{N-R}_2$), for saturated alkyls the stretching bands occur from 1664 to 1672 cm^{-1} but when R_1 and R_2 are unsaturated alkyl or phenyl group in conjugation with imine ($\text{C}=\text{N}$) the stretching absorption frequency shifted to lower frequency up to 1400 cm^{-1} according to the conjugation.

Table 1: FT-IR Spectral data of CycPh–NapHz

Functional group	Stretching frequency (ν)	Bending frequency (ν)
$\begin{array}{c} \text{Ar} \\ \diagdown \\ \text{C}=\text{N}- \\ \diagup \\ \text{R}_1 \end{array}$	1608
$\begin{array}{c} \text{R} \\ \diagdown \\ \text{C}=\text{N}- \\ \diagup \\ \text{H} \end{array}$	1622
$\begin{array}{c} \\ \text{---C---} \text{ Cyclohexyl} \\ \\ \text{H} \end{array}$	2920-2820	1420-1340
H–Ar	3130-3040	950-643

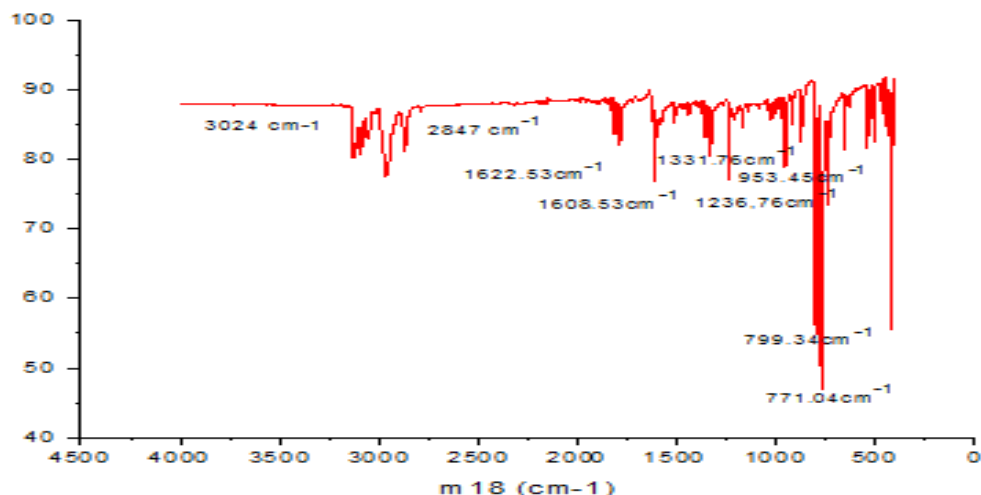


Figure 2: FT-IR Spectrum of CycPh–NapHz

Antibacterial Activity

The antibacterial behavior of *(2E)-1-(cyclohexyl(phenyl)methylene)-2-(naphthalen-1-ylmethylene)hydrazine* (CycPh–NapHz) was examined against *Staphylococcus aureus* and *Pseudomonas aeruginosa* using the disc diffusion assay. The results indicated that the

compound possesses a noticeable level of antibacterial activity against both microorganisms. Inhibition zones of **14 mm** and **12 mm** were recorded for *S. aureus* and *P. aeruginosa*, respectively. As expected, the reference drug tetracycline exhibited substantially stronger activity, producing inhibition zones of **28 mm** under the same experimental conditions. The slightly higher sensitivity of *S. aureus* compared to *P. aeruginosa* can be rationalized by differences in their cell wall structures. Gram-negative bacteria possess an additional outer membrane that often limits the uptake of hydrophobic compounds, whereas Gram-positive bacteria lack this barrier, making them more susceptible. The antibacterial activity observed for the synthesized hydrazone may be linked to the presence of the **azomethine (C=N) group**, which is commonly associated with antimicrobial effects in Schiff base derivatives. This functional group is believed to interact with bacterial enzymes and membrane components, leading to impaired cellular function. Moreover, the extended aromatic system provided by the phenyl and naphthyl moieties may increase lipophilicity, promoting better interaction with bacterial membranes. Although the activity of the compound is lower than that of the standard antibiotic, the inhibition zones obtained are comparable to those reported for related hydrazone derivatives. These findings indicate that the synthesized molecule can serve as a useful structural framework for further development. Additional studies, particularly **minimum inhibitory concentration (MIC) measurements** and the synthesis of corresponding **metal complexes** may enhance the antibacterial efficacy, as metal coordination has been shown to improve biological performance in similar systems.

Table 2: Antibacterial Activity of CycPh–NapHz

Compound	<i>S. aureus</i>	<i>P. aeruginosa</i>
CycPh–NapHz	14 mm	12 mm
Tetracycline (standard)	28 mm	28 mm



S. aureus

P. Aeruginosa

Figure 3: Antibacterial activity of (2E)-1-(cyclohexyl(phenyl)methylene)-2-(naphthalen-1-ylmethylene)hydrazine (CycPh–NapHz)

CONCLUSION

A new bis-hydrazone Schiff base, CycPh–NapHz, was successfully synthesized using a simple and efficient condensation method, yielding a pure yellow crystalline product. Spectroscopic analysis by FT-IR and ¹H NMR confirmed the formation of the expected molecular structure, including two azomethine groups and the cyclohexyl, phenyl, and naphthyl components. The compound showed moderate antibacterial activity against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa*) bacteria. The slightly higher activity against *S. aureus* is likely related to differences in the bacterial cell wall structure. The presence of the azomethine groups, combined with aromatic conjugation and hydrophobic cyclohexyl moieties, appears to contribute to the observed antibacterial effects. Although its activity is lower than tetracycline, the results are consistent with those of related hydrazone derivatives. The structural features of CycPh–NapHz provide a promising platform for further modifications. Future studies could explore minimum inhibitory concentrations, structural optimization, and metal complex formation to enhance biological activity. Overall, this work highlights the potential of bis-hydrazone Schiff bases as scaffolds for developing new antibacterial agents and multifunctional chemical compounds.

Conceptualization, Maaz Khan; methodology, Malik Muhammad Shoaib, Maaz Khan; software, Maaz Khan, Malik Muhammad Shoaib, Muhammad Ali Shah, Mubark Jan; validation, Maaz Khan, Talha Mahmood; investigation, Malik Muhammad Shoaib, Madiha Aslam and Maaz Khan; resources, Kalim Ullah; writing—original draft preparation, Maaz Khan, Akhtar Ali, Kiran Jabeen, Arif Ullah Khan; writing—review and editing; Habib Ur Rahman, Fozia Farid; Supervision, Maaz Khan; funding; Maaz Khan, Muhammad Ali Shah. Authors have read and agreed to publish the manuscript.

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