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Design, Synthesis, Characterization and Antibacterial Evalution of a Novel Methoxy-Substituted Cyclohexyl Phenyl Bis-Schiff Base against Escherichia coli and Staphylococcus Aureus

Najam Ud Din Jamali[1], Tania Tabussam[2], Khalid Hussain[3], Madiha Aslam[4*], Maaz Khan[5], Pooja Bai[6], Abdul Ghaffar Chandio[7], Muhammad Faisal[7], Fozia Farid[8], Sabba Gul[8], Muhammad Ali Shah[5], Akhtar Ali[3]

[1]Najam Ud Din Jamali, Dr. M.A. Kazi Institute of Chemistry, University of Sindh, Jamshoro.

[2]School of chemistry and chemical engineering, Beijing institute of technology, Beijing, 102488, China.

[3]Department of chemistry, University of Sindh Jamshoro.

[4]Institute of Chemistry, University of Sargodha, 40100 Sargodha, Punjab.

[5]Department of Chemistry, University of Malakand, KPK, Pakistan.

[6]Institute of chemistry shah Abdul Latif University Khairpur Mir's.

[7]Deptt.National Centre of Excellence in Analytical Chemistry university of Sindh Jamshoro.

[8]Institute of chemical sciences university of science and technology Bannu

*** Correspondence:** Madiha Aslam

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ABSTRACT: In this work, a novel Bis-Schiff base compound, (2E)-1- (cyclohexyl (phenyl) methylene)-2-(2,4-dimethoxybenzylidene) hydrazine was synthesized by the condensation of the cyclohexyl phenyl ketone in a two-step reaction. The structure of the synthesized compound was verified by ^1H NMR and FT-IR, revealing the presence of azomethine (–C=N–) bonds and methoxy groups. The antibacterial activity was tested in vitro against Gram-negative and the Gram-positive bacteria strains (E.coli and S. aureus, respectively) using the agar well diffusion technique. The synthesized compound showed moderate dose-dependent antibacterial activity. At concentrations of 50, 100, and 200 µg/mL, zones of inhibition against E. coli were 8 ± 0.5, 12 ± 0.7, and 16 ± 0.6 mm, respectively, while against S. aureus they were 10 ± 0.6, 14 ± 0.5, and 18 ± 0.7 mm, respectively. The standard drug ampicillin (100 µg/mL) exhibited higher activity with zone of 22 ± 0.5 mm, (E. coli) and 24 ± 0.6 mm (S. aureus), while DMSO was inactive. The compound appears to be more active against the Gram-positive bacteria and its activity seems to be dependent on structural features, such as the presence of two imine groups and methoxy substituents. These results indicate that the compound could be used in near futrue for the development of new antibiotics.

Keywords: Schiff base; Bis-Schiff base; Azomethine; Hydrazone; E. coli; S. aureus

1. INTRODUCTION

Schiff bases are commonly referred as the azomethine compounds, are very important class of organic molecules that contain a characteristic imine (–C=N–) functional group [1]. These compounds are usually formed through the condensation between the primary amines and the carbonyl compounds such as the aldehydes or ketones. Since their discovery by Hugo Schiff, they have been widely studied due to their simple synthesis, structural flexibility, and broad range of applications in chemistry and related fields [2, 3]. Due to their various biological activities, Schiff base derivatives, over the years, have drawn considerable attention, especially in medicinal chemistry [4]. A number of these compounds are reported to possess antimicrobial, antifungal, antiviral, anti-inflammatory and anticancer activities. Biological importance of Schiff base is frequently associated with the presence of azomethine (–C=N–) group which can form hydrogen bond or coordinate with the biological systems involving metal ion [5, 6]. The interactions can disrupt the critical biological processes in microorganisms, which renders Schiff bases as attractive candidates for drug development. Bis-Schiff bases (BSBs), which are compounds with two imine groups, have been progressively gaining popularity among the various Schiff base varieties. There are two azomethine linkages in a single molecule which increases the stability and enhances the biological activity of the molecule than the mono-Schiff bases. This is primarily because of the fact that extra functional groups enable better interactions with biological targets. Hence, bis-Schiff base derivatives have been found to be more efficient binding with enzymes or interfering in the functions of microbes [7-10]. With the emergence of the frontier of antibiotic resistance at an alarming rate, there is an urgent need for new antimicrobial agents [11]. Pathogenic bacteria like Escherichia coli and Staphylococcus aureus cause a variety of infections, and have developed resistance to frequently-used antibiotics. Treatment of infections caused by Gram-negative bacteria like E. coli is one of the main challenges due to the presence of an outer membrane which impedes the penetration of several antimicrobial agents [12-16]. Gram-positive bacteria, such as S. aureus, have a much simpler cell wall structure and are therefore vulnerable to some compounds. Hence, the design of molecules which could be effective against both types of bacteria is an area of interest [17]. Schiff base derivatives have exhibited good antibacterial activity against different bacterial strains. They exert their effect through several mechanisms such as altering cell membranes, inhibiting enzymes and interfering with metabolic pathways [18]. Furthermore, the lipophilic character of numerous Schiff bases facilitates the penetration of their bacterial cell membrane and thus their effectiveness. These can be further enhanced by structural modifications such as an electron-donating group like methoxy substituents, which would enhance their biological activity by increasing their lipophilicity and thereby improve their interactions with the microbial target [19-23].

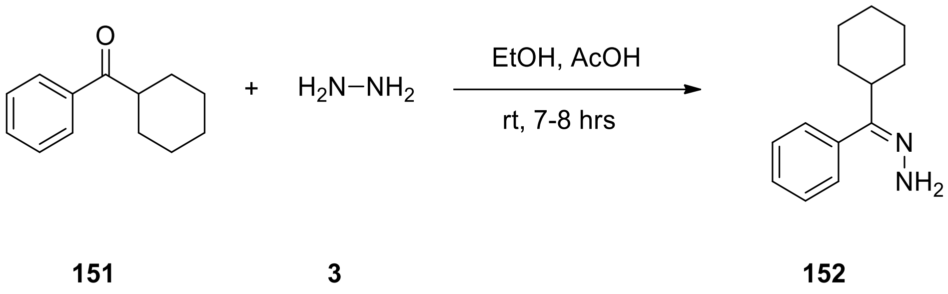
Cyclohexyl phenyl ketone is a useful starting material in the synthesis of Schiff bases due to its structural features. The cyclohexyl ring is important for the hydrophobic nature of the molecule, possibly favoring membrane permeability, and the phenyl group for conjugation and stability. The introduction of these structural features into Schiff base derivatives can affect their chemical properties as well as biological activities [24-29]. Hydrazine derivatives also are used extensively in the preparation of Schiff bases, especially hydrazones, by reaction with carbonyl compounds. The intermediates are important because of their biological relevance and because they are important building blocks in the formation of more complex molecules. Cytotoxic effects may be provided by the presence of nitrogen atoms in hydrazones, as they are able to form hydrogen bonds and interact with biological systems, especially with microorganisms [30-36]. A new bis-Schiff base derivative (2E)-1-(cyclohexyl(phenyl)methylene)-2-(2,4-dimethoxybenzylidene)hydrazine was synthesized from 2,4-dimethoxybenzaldehyde as a component of its preparation.A new bis-Schiff base derivative, (2E)-1-(cyclohexyl(phenyl)methylene)-2-(2,4-dimethoxybenzylidene)hydrazine was synthesized as a component of its preparation. The synthesis was carried out by forming a hydrazone intermediate and then condensation with 2,4-dimethoxybenzaldehyde. It was hoped that the introduction of methoxy groups on aromatic rings and dual imine groups would increase the biological activity of the compound due to the increase of conjugation and lipophilicity, and the interaction with microbial systems [37-41]. The synthesized compound was characterized by the traditional spectroscopic methods such as proton nuclear magnetic resonance (^{1}H NMR) and Fourier-transform infrared (FT-IR) spectroscopy. These methods were used to confirm the molecular structure and the presence of important functional groups, such as the azomethine linkage. The antibacterial activity of the compound was assessed against gram positive and gram negative bacteria by agar well diffusion method for its biological potential. These results were compared to a standard antibiotic, ampicillin, to evaluate its effectiveness as compared to this antibiotic. In general, the purpose of this research is to make contribution to the development of new Schiff base derivatives for having antibacterial activity. This could help the design of better compounds in the future by investigating the structure/activity relationship. The results of this work indicate that bis-Schiff base derivatives have great potential as future candidates for antimicrobial studies.

2. EXPERIMENTAL

Synthetic grade Solvents and chemicals were obtained from BDH, Alfa Aesar, Merck and Aldrich for synthesis. Dry glassware was used for all of the reactions. Thin layer chromatography was used to monitor the reaction, using various solvent systems and for detection, a UV lamp was used. Standard procedures were followed to get the various bis-Schiff base derivatives of the cyclohexyl phenyl ketone.

2.1 Synthesis of (*Z*)-(cyclohexyl*(*phenyl*)*methylene*)*hydrazine as starting material

Hydrazine hydrate was added to the solution of cyclohexyl phenyl ketone in ethanol solvent and few drops of the glacial acetic acid were added to the solution and the reaction mixture was stirred for 7-8 hours at 80 ^{o}C. The reaction was monitored by TLC using n-hexane ethyl acetate solvent system, once the reaction was completed the product was precipitated in cold water, filtered and dried.

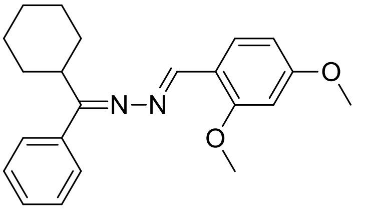


2.2. Synthesis of (*2E*)-1-*(*cyclohexyl*(*phenyl*)*methylene*)*-2-(2*,*4 dimethoxybenzylidene*)*hydrazine

A 0.1196 mmol (0.042 g) of (Z)-(cyclohexyl(phenyl)methylene)hydrazine acetohydrazide was carefully added to a clean round-bottom flask in which ethanol was used as the solvent. The reactants were thoroughly dissolved and mixed by stirring the reaction mixture on a hot plate. The solution was stirred continuously for about 15 minutes at moderate temperature to get a homogenous solution. Following this initial stirring, a few drops of acetic acid were added to the reaction mixture as a catalyst and then 2,4-dimethoxybenzaldehyde was added. The contents of the flask were thoroughly mixed and the reaction mixture was refluxed for 10 hours at 80 °C to complete the condensation reaction. Thin layer chromatography (TLC) was used to check the progress of reaction at periodic times. A solvent system made up of n-hexane and ethyl acetate was used in TLC analysis to monitor the depletion of the starting materials and the appearance of the desired product. Reaction mixture was left to cool down to the room temperature after the completion of the reaction was confirmed by TLC. This product was then precipitated by casting into cold water with stirring. The precipitated solid product was filtered off and washed to wash-out impurities and the excess of reagents and then dried under appropriate conditions to yield the purified final product.

Physical data

Molecular formula	$C_{22}H_{26}N_2O_2$
Molecular Weight:	350.45 g/mol
Color	pink
Solubility	Chloroform, DMSO, DMF
Melting point	177 ^{o}C
Yield	72%



(2*E*)-1-(cyclohexyl(phenyl)methylene)-2-(2,4-dimethoxybenzylidene)hydrazine

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2.3. Antibacterial Activity

The in vitro anti-bacterial activity of the synthesized, (2E)-1-(cyclohexyl(phenyl)methylene)-2-(2,4-dimethoxybenzylidene)hydrazine was evaluated against two selected bacterial strain viz., Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive) using the agar well diffusion method, under aseptic laboratory conditions. New overnight bacterial culture was done individually in sterile nutrient broth and cultured at 37 °C for around 18–24 hours for the growth of active bacterial cells. The turbidity of each bacterial suspension was adjusted and standardized to the 0.5 McFarland standard (CFU/mL ~ 1×108) by turbidimetric method to have the same bacterial concentration throughout the experiment. Sterile Mueller–Hinton agar plates were made and left to dry under sterile conditions. The spread plate method was used to obtain a confluent bacterial lawn by uniformly spreading the standardized bacterial inocula on the entire surface of the agar plates with a sterile cotton swab. Cylindrical wells of about 6 mm diameter were carefully made in the inoculated agar medium by using a sterile cork borer in aseptic conditions. Accurately dissolved in dimethyl sulfoxide (DMSO) for obtaining various test concentrations of the test compound, 50, 100 and 200 µg/mL. 50µL of each prepared concentration was then carefully added to the corresponding wells with a micropipette. Ampicillin (100µg/mL) was used as positive control for the antibacterial assay and DMSO was used as negative control for the assay to ensure that the solvent did not have any inhibitory effect on the bacterial growth. Those plates of inoculated agars were then placed at 37 °C for 24 hours in an aerobic state to enable the growth of the bacteria and penetration into the agar medium. After incubation the antimicrobial activity of the prepared compound was determined by measuring the zone of inhibition (in millimeters, mm) around each well. The results of the various experiments were carried out in triplicates for reproducibility and reliability and the mean inhibition zone values were calculated and recorded as the final result of the antibacterial activity.

3. RESULTS AND DISCUSSION

3.1. Characterization

3.1.1. ^{1}H NMR

^{1}HNMR (CD3Cl, 400Hz, δ(ppm): δ =1.94-1.26 (m, 10H, 5-CH2-Cyclic), δ =2.64 (tt, J =11.4 Hz, 3.4 Hz, 1H, -CH-Cyclic), δ=3.85 (s, 6H, 2CH3-O-Ar) δ =6.43 (d, J= 2.4, 3H, H-Ar), δ =6.54 (dd, J= 8.7 Hz, 2.4 Hz, 2H, Ar-H), δ =7.41-7.22 (m, 2H, Ar-H), δ =8.08 (d, J= 8.6 Hz, 1H, Ar-H), δ =9.02(s, 1H, Ar-CH=N-).

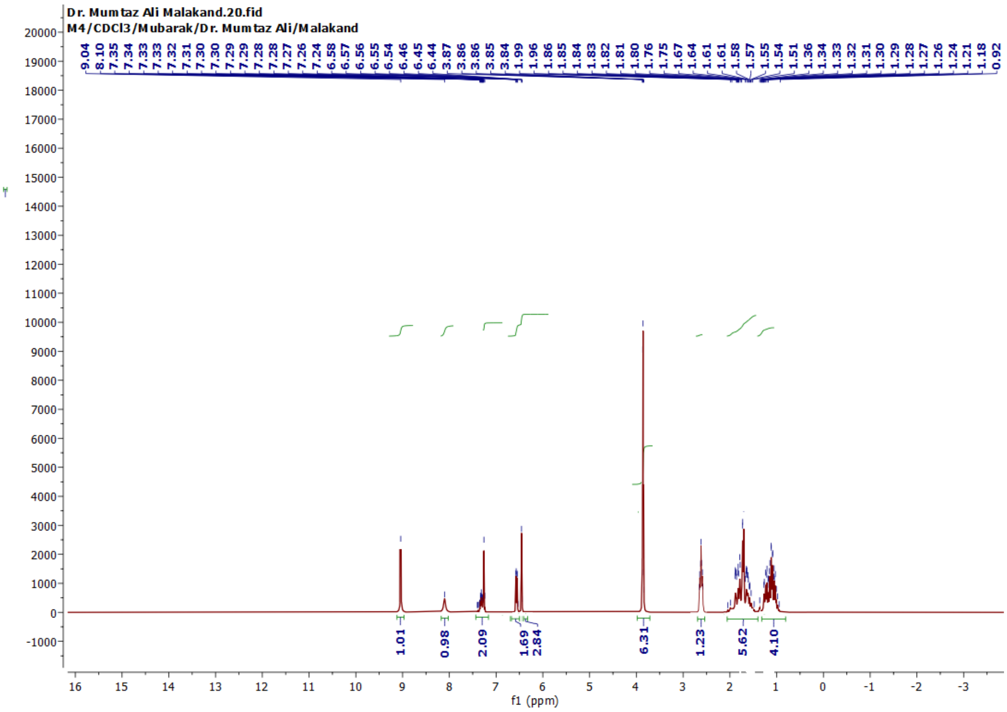


Figure 1: ^{1}HNMR Spectrum of *(2E)*-1-(cyclohexyl(phenyl)methylene)-2-(2,4-dimethoxybenzylidene)hydrazine

The ^{1}H NMR spectrum of the synthesized compound (2E)-1-(cyclohexyl(phenyl)methylene)-2-(2,4-dimethoxybenzylidene)hydrazine (Figure 1) offered strong support for the successful synthesis and characterization of the desired product. The spectrum showed typical proton signals for the cyclohexyl ring, aromatic protons and imine proton as well as methoxy groups in the molecule. The proposed molecular structure of the synthesized compound was confirmed by careful interpretation of the chemical shifts, multiplicities and integral ratio. In the aliphatic region of the spectrum, two multiplet signals were observed at δ 1.94 and 1.26 ppm with the ratio of integral of 4H and 6H, and assigned to the methylene protons of the cyclohexyl ring. The different magnetic environment and conformational flexibility of the cyclohexane part resulted in these signals being observed as overlapping multiplets. In addition, a quintet signal with an integral ratio of 1H was detected at δ 2.64 ppm, which was assigned to the methine proton of the cyclohexyl group. The multiplicity and chemical shift of this proton agreed with the coupling interactions of the adjacent methylene protons in the saturated cyclic system. The aromatic region of the spectrum further confirmed the presence of substituted phenyl rings in the structure. The singlet signal with an integral of 1H appeared at δ 8.08 ppm while a multiplet signal with an integral of 7H appeared in the range of δ 7.27 to 6.43 ppm was assigned to the aromatic protons of the two phenyl rings present in the compound. The chemical shift range observed and splitting patterns were well consistent with the protons of substituted aromatic systems, in particular those bearing an electron donating methoxy group and a conjugated imine function. This difference in chemical shift can be explained by the different electronic environment of the aromatic protons due to the effect of the substituents on the benzene rings. The azomethine (–CH=N–) or imine proton of the amide gave a single peak at δ 9.02 ppm with an integral value of 1H. Strong deshielding from the adjacent electronegative nitrogen atom and the conjugation with the aromatic rings accounted for the downfield location of this signal. The appearance of this characteristic imine proton signal was also a good indication of successful condensation reaction and the formation of the Schiff base structure in the synthesized molecule. Moreover, a sharp singlet signal observed at δ 3.85 ppm with an integral value of 6H was assigned to the two methoxy groups (–OCH3) on the aromatic ring. Both methoxy groups were chemically equivalent or close enough in the molecular environment to look like a single intense singlet with the integral of 6 protons. The chemical shift was in accord with methoxy groups directly attached to an aromatic ring system via oxygen. In conclusion, the observed ^{1}H NMR spectral data (chemical shift values, multiplicities and proton integration ratios) was found to be in excellent agreement with the proposed structure of *(*2E*)*-1-*(*cyclohexyl*(*phenyl*)*methylene*)*-2-*(*2*,*4-dimethoxybenzylidene*)*hydrazine*.* The spectrum clearly showed the presence of all the desired structural fragments and functional groups, indicating successful target compound synthesis and purity.

3.1.2. FT-IR

The FT-IR spectrum of the synthesized compound *(*2E*)*-1-*(*cyclohexyl*(*phenyl*)*methylene*)*-2-*(*2*,*4-dimethoxybenzylidene*)*hydrazine is shown in Figure 2*,* which is crucial for confirming the successful synthesis of the desired Schiff base derivative. The infrared spectrum showed a number of absorption bands which were in good agreement with the structural features and functional groups that were expected from the proposed molecular structure. The characteristic stretching and bending vibrations were analysed carefully to confirm the presence of imine linkages, aromatic rings, methoxy substituents and the cyclohexyl moiety in the synthesized compound. The stretching vibrations of the two azomethine or imine (C=N) groups in the molecule were found in the FT-IR spectrum as two separate absorption frequency bands at 1574 cm^{-1} and 1611 cm^{-1}. These characteristic bands were observed and it strongly confirmed the successful condensation reaction and formation of the Schiff base framework. The imines have characteristic stretching vibration below 1660 cm^{-1} in general and its exact position is greatly dependent on the electronic environment and the substituents attached to the carbon and nitrogen atom of the imine bond. The decrease in the C=N stretching frequency in the present compound may be explained by the conjugation of the imine units with the unsaturated systems such as aromatic phenyl rings in the molecule. The aliphatic C–H stretching vibrations of the cyclohexyl ring also showed absorption frequency bands in the FT-IR spectrum in the range 2850-2925 cm^{-1}. The presence of the cyclohexane moiety in the synthesized compound was confirmed by these bands, which are characteristic of saturated aliphatic hydrocarbons. Further, the absorption bands in the range of 1445 to 1304 cm^{-1} were attributed to the bending or deformation vibrations of the aliphatic C–H bonds of the cyclohexyl ring. The presence of these stretching and bending vibrations further confirmed the presence of the saturated cyclic aliphatic group in the structure. Characteristic aromatic C–H vibrations were also clearly observed in the FT-IR spectrum. The absorption bands in the range of 3170–3010 cm^{-1} were attributed to the C–H stretching vibrations of aromatic rings of phenyl group present in the compound. In addition, the bands which were observed between 950-695 cm^{-1} indicated the out-of-plane bending modes of the aromatic C–H bonds. Such bands have been usually seen in substituted benzene derivatives and it was confirmed that there exist aromatic ring systems in the synthesized molecule. The distribution of these bands also suggested the substitution of the aromatic rings as a result of methoxy substituents and imine functionalities. The presence of the C=O functional group is well known and carbonyl compounds are known to have strong stretching absorption bands above 1700 cm-*1*. This range was scanned in the FT-IR spectrum of synthesized compound and no absorption band was found in this region, which implies that the carbonyl functionality has not been detected and therefore it is assumed that the condensation reaction is complete, using the precursor materials. Rather, the presence of strong imine (C=N) stretching bands indicated that the condensation of the carbonyl functionalities of the starting aldehyde had occurred to good effect, resulting in azomethine linkages. The frequency of the stretching vibration of the imine bond is strongly affected by the nature of the substituents on the carbon and nitrogen atoms in a general structure R1–C=N–R2. The C=N stretching vibration usually occurs in the region of 1664-1672 cm-1 for simple saturated alkyl groups. If the imine group is linked to unsaturated alkyl groups, to aromatic phenyl rings, or other π-electron systems, then the delocalization decreases the bond order of the C=N bond to a certain degree. This causes the stretching absorption frequency to decrease in value. When resonance stabilization and extended π-conjugation effects occur in highly conjugated systems the absorption frequency can be lowered significantly. The two imine groups are conjugated with the aromatic rings and methoxy groups, which accounts for the observed decrease of the C=N stretching frequency to 1611 and 1574 cm^{-1}.

Furthermore, methoxy groups on the aromatic ring have resonance donation, adding more electrons to the rest of the molecule, and also contributing to the shift in the vibrational frequencies that are observed in the IR spectrum. The aromatic conjugation, the electron delocalization and the interaction between the substituents were in this case the cause of the typical features in the spectra of the synthesized compound. In conclusion, the FT-IR spectral analysis proved to be a very good confirmation of the successful synthesis of (2E)-1-(cyclohexyl(phenyl)methylene)-2-(2,4-dimethoxybenzylidene)hydrazine. The presence of imine group, aromatic and aliphatic C–H vibrations and the absence of carbonyl stretching frequencies were provided the support for the proposed structure and confirmed the successful synthesis of the desired Schiff base compound.

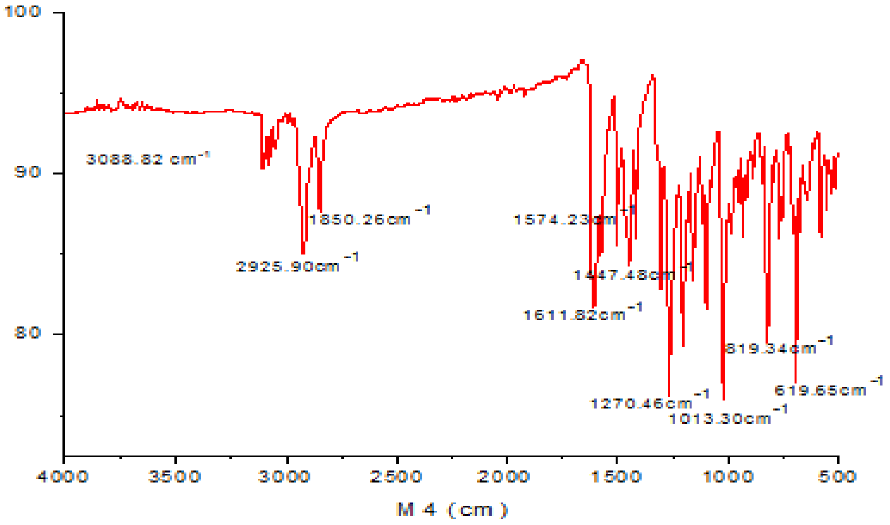
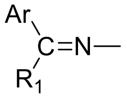
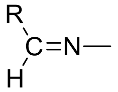
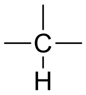


Figure 2: FT-IR Spectrum of *(2E)*-1-(cyclohexyl(phenyl)methylene)-2-(2,4-dimethoxybenzylidene)hydrazine

Table 1: FT-IR Spectral data of *(2E)*-1-(cyclohexyl(phenyl)methylene)-2-(2,4-dimethoxybenzylidene)hydrazine

Functional group	Stretching frequency (v)	Bending frequency (v)
 $Ar(R_1)C{=}N{-}$	1574	……………
 $R(H)C{=}N{-}$	1611	…………….
 $-CH-$ Cyclohexyl	2850-2925	1445-1304
H−Ar	3170-3010	950-695

3.2. Anti-Bacterial Activity

The antibacterial activity of the synthesized compound, *(2E)*-1-(cyclohexyl(phenyl)methylene)-2-(2,4-dimethoxybenzylidene)hydrazine, against two bacterial strains (Escherichia coli and Staphylococcus aureus) was investigated using the agar well diffusion method in a controlled laboratory setting. The synthesized Schiff base compound has been tested on Gram negative and gram positive bacteria in the antibacterial assay to check its antibacterial activity. The standard positive control (100 µg/mL) used was Ampicillin with well-known antibacterial activity while the negative control (0 µg/mL) was dimethyl sulfoxide (DMSO) to ensure that the solvent does not inhibit bacterial growth. The results of the experiments showed moderate antibacterial activity of the synthesized compound against both the bacterial strains tested. The activity was concentration dependent since the inhibition zone diameter was increased with the increase in concentration of the compound. Smaller inhibition zone at lower concentration and larger zone at higher concentration of compound suggests that it has a greater antibacterial activity with increased amount of dosage. The wells with the DMSO only were not inhibited, which showed that the effect on the bacteria was not the result of the DMSO that was used to dissolve the compound, but rather of the synthesized compound. The results also indicated that the compound was more effective against Staphylococcus aureus than E. coli. This difference in sensitivity to antibiotics between gram-positive and gram-negative bacteria could be due to the differences in the architecture of their cell walls and in their membrane composition. Some gram-negative bacteria such as Escherichia coli have an extra outer lipopolysaccharide membrane outside the peptidoglycan layer which acts as a good permeability barrier, preventing many antimicrobial chemicals from entering the bacterial cell. Gram-positive bacteria, on the other hand, lack this outer membrane, and have a relatively simpler cell wall structure that consists of a thick peptidoglycan layer which is more susceptible to penetration and action of antibacterial compounds. The synthesized compound is found to have antibacterial activity which could be significantly related with the biologically active functional groups present in the compound. The azomethine or imine (–C=N–) groups found in the Schiff base structure are recognized to play a significant role in antimicrobial activity in particular. These functional groups could enter in coordination or hydrogen-bonding interaction with the essential enzymes of microbial cells or with essential proteins of cells, and consequently interfere with important biochemical and metabolic processes essential to the survival and growth of bacteria. The azomethine bond-containing Schiff base compounds have been well reported in the literature for their biological activities such as anti-bacterial activity, anti-fungal activity, etc., which are related to their interference with cellular functions of microbes. Moreover, the methoxy ($-OCH_3$) groups on the aromatic ring could also be vital to improving the bioactivity of the molecule. The methoxy groups contribute to the electron donating capability of the molecule which can enhance the lipophilic character of the molecule and thus its ability to penetrate the lipid rich bacterial cell membranes. Increased permeation of the membrane could help the compound enter the bacterial cell to inhibit its growth more effectively. Additionally, the conjugation of aromatic rings with azomethine groups might enhance the electron delocalisation within the molecule and might also affect the interaction of the compound with the biological target. The synthesized compound was found to possess good antibacterial activity against both strains but its activity was observed to be less as compared to the standard antibacterial drug ampicillin. This observation indicates that the compound has promising antibacterial activity, but further structural modifications and optimizations might enhance its activity and extend its antimicrobial spectrum. Future studies could involve introduction of other electron donating or withdrawing substituents, metal complexation or modification of the aromatic framework, which may improve the biological activity of the compound. In general, the antibacterial screening results showed that (*(2E)*-1-(cyclohexyl(phenyl)methylene)-2-(2,4-dimethoxybenzylidene)hydrazine was moderate in its antibacterial activity against both Gram-positive and Gram-negative bacteria with relatively higher activity against Staphylococcus aureus. The observed activity has been explained by the complementary effect of the azomethine linkage, the aromatic system and methoxy substituents of the molecular structure and thus the compound can be considered as a promising molecule for developing new antibacterial drugs.

Table 2: Antibacterial Activity of *(2E)*-1-*(*cyclohexyl*(*phenyl*)*methylene*)*-2-*(*2,4-dimethoxybenzylidene*)*hydrazine

S.No	**Sample Control**	**Concentration (µg/mL)**	**Zone of Inhibition (mm) – *E. coli***	**Zone of Inhibition (mm) – *S. aureus***
1	Test Compound	50	8 ± 0.5	10 ± 0.6
2	Test Compound	100	12 ± 0.7	14 ± 0.5
3	Test Compound	200	16 ± 0.6	18 ± 0.7
4	Ampicillin	100	22 ± 0.5	24 ± 0.6
5	DMSO	-	No inhibition	No inhibition

Overall, the results indicate that the synthesized bis-Schiff base derivative possesses dose-dependent antibacterial activity, with relatively better efficacy against Gram-positive bacteria, making it a potential candidate for further investigation and development.

Conclusion

A new bis-Schiff base derivative with cyclohexyl, phenyl and methoxy-substituted aromatic rings has been successfully synthesized and characterised in this study. The compound was successfully synthesized and confirmed by spectroscopy, including the presence of azomethine (–C=N–) groups. Antibacterial studies revealed that the compound has moderate and dose-dependent antibacterial effects against E. coli and S. aureus. In fact, the compound exhibited inhibition zone from 8-16 mm against E. coli and 10-18 mm against S. aureus as the concentration increased from 50-200 µg/mL. The greater sensitivity of S. aureus indicates that the compound is more active against Gram-positive bacteria, probably because of variations in cell wall architecture. While the activity is less than that of the control antibiotic ampicillin, the antibacterial action demonstrates that this bis-Schiff base has biological activity. The conjugated double bonds (–C=N) and electron-donating methoxy groups are likely to increase the lipophilicity and binding interaction with microbial cells, thus enhancing its activity. In summary, the findings underline the significance of structural features in the bioactive behavior and suggest that structural modifications and optimisation of these compounds can lead to more effective antibacterial agents.

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