

Synthesis and Antibacterial Activity of Phenylhydrazone Schiff Base Derivatives and their Copper(II) Complexes

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Abstract

Hydrazone derivatives have exhibited several interesting biological properties such as antimicrobial, antiviral, anti-inflammatory, antiplatelet, antimalarial, and anticancer. In present study, hydrazone of phenylhydrazine, 4-methoxybenzaldehyde, 4-nitrobenzaldehyde and their respective benzenesulfanohydrazones (**1-3**) and Cu(II) complexes (**4-5**) were synthesized. Synthesized ligands were characterized by IR, ¹H NMR, and ¹³C NMR whereas Cu(II) complexes were characterized by elemental analysis and IR. The IR spectra of ligand and its metal complexes imply that the schiff base ligand behaves as basic bidentate ligand coordination through the azomethine nitrogen atom and nitrogen atom. *In-vitro* antibacterial test revealed Cu(II) complexes of compound **2** showed better activity (15mm zone of inhibition) against *K.pneumoniae* compared to the standard ceftriaxone (11 mm zone of inhibition) which might be due to increase in lipophilicity of the compound.

Keywords: antibacterial activity, benzenesulfanohydrazones, complexes, hydrazone

Introduction

Transition metal complexes are cationic, neutral or anionic species in which a transition metal is coordinated by ligands [1]. Metal based drugs have demonstrated wide scope of pharmacological application and may offer unique therapeutic opportunities [2]. The Schiff bases are considered as 'privileged ligands' because of their capability to stabilize different metals in various oxidation states and their metal chelates are widely studied owing to the synthetic flexibility, sensitivity and selectivity toward various metal ions [3]. Over the years, many hydrazone derivatives have exhibited several biological properties such as antimicrobial, antiviral, anti-inflammatory, antiplatelet, antimalarial, anticancer [4-6]. Hydrazones are also used as ligands to coordinate a variety of metal ions, the resulting metal complexes exhibited a broad spectrum of biological activities such as antitubercular activity against *Mycobacterium tuberculosis* [7], cytotoxic [8-9], and anticancer activity [10]. In this study, phenylhydrazone derivative and their Cu(II) complexes were synthesized and antibacterial activity of the schiff base and complexes were conducted on selected strains of microorganisms.

Materials and methods

The chemicals and reagents that were used in this investigation are phenylhydrazine, 4-methoxybenzaldehyde, 4-nitrobenzaldehyde, benzenesulphonylchloride, anhydrous Cu(CH₃COO)₂.H₂O. Solvents such as ethanol, methanol, chloroform, dimethylsulfoxide (DMSO), distilled water, glacial acetic acid, and tetramethylsilane (TMS) were used. All the chemicals and reagents utilized in this work were analytical grade and are of pure grade Aldrich.

Instrumentation

Melting or decomposition point was determined by Stuart SMP3 digital melting point apparatus. IR spectra were recorded using Perkin-Elmer FT-IR spectrophotometer in the range of 4000-450 cm⁻¹. ¹H and ¹³C NMR was recorded using Bruker Avance 400MHz spectrometer using DMSO-*d*₆ as solvent and tetramethylsilane (TMS) as an internal standard. Elemental analysis of CHNS was done by using Exter Analytical CE 440 elemental analyzer. The purity of the phenylhydrazone Schiff base derivative and its Cu(II) complexes were checked by thin layer chromatography (TLC) using silica Gel-G Pellets with layer thickness of 0.3 mm. TLC visualization was realized by UV-light.

Synthesis of Hydrazone Schiff base

Equimolar quantities (0.984mL, 0.01 moles) of phenylhydrazine and the corresponding aldehyde 4-methoxybenzaldehyde (1.21 mL, 0.01 moles)/4-nitrobenzaldehyde (1.51g, 0.01 moles) were dissolved in 40 mL ethanol. Glacial acetic acid (2 mL) were added and refluxed for about 6 hr at 60 °C. The reaction progress was monitored by TLC. The product was poured on crushed ice. A precipitate was obtained. This precipitate was filtered and washed with ethanol and dried in vacuum desiccators overnight and recrystallized by ethanol. The purity was checked by TLC.

Synthesis of Phenylhydrazone Schiff base derivatives

The phenylhydrazone Schiff bases (0.01 moles) was dissolved in 40 mL of ethanol and equimolar benzenesulphonylchloride were added. The mixture was refluxed for 6 hr. The completion of reaction was monitored by TLC using ethyl acetate:*n*-hexane (3:7) as eluent. After the completion of reaction, the mixture was poured in ice cold water and the product obtained was filtered, washed with ice cold water and dried at room temperature. The crude product was recrystallized using ethanol and placed in desiccators to obtain solid. The purity was checked by TLC (ethyl acetate: *n*-hexane (3:7)).

Synthesis of Copper (II) complexes

Cu(II) complexes of hydrazone derivative schiff base ligand were prepared by mixing 0.2M of hydrazone derivative schiff base with 40 mL ethanolic solution of Cu(CH₃COO)₂.H₂O keeping ligand-metal ratio 2:1. Glacial acetic acid (2 drop) was added. The result mixture was refluxed for 8 hr while monitoring the P^H of the solution. After completion of the reaction, the solution was cooled and precipitate was formed. The precipitate was filtered and washed by 50 mL ethanol three times to purify and remove excess of ligand. Finally, the precipitate was dried in desiccators overnight and recrystallized from ethanol. The purity was checked by TLC (ethyl acetate:*n*-hexane (3:7)).

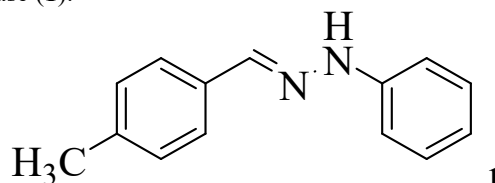
Antibacterial activity

Antibacterial activities of test samples were evaluated using disc diffusion method against the test strains .The plates were allowed to solidify for 5min and 0.1% inoculums suspension of tested organisms were swabbed uniformly and the inoculums were allowed to dry for 5min. 20 μL of synthesized compound (**1-5**) were loaded on 6 mm sterile individual paper discs (HiMedia) and thoroughly dried in air draft to remove traces of the solvent. Negative control was prepared using DMSO. 25 mg/mL solution of Cefraxone (20 μL/disc) was used as positive control. The fortified discs were placed on the surface of medium using a disc template and incubated at 37°C for 24hr. Inhibition zones formed around the discs were measured with transparent ruler (in millimeters) [11].

Result and Discussion

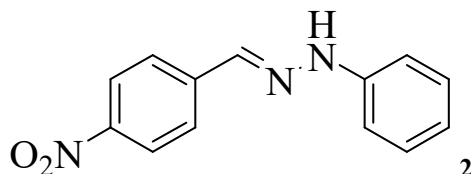
Characterization of the ligands

Compound **1** was obtained as a white crystals with melting point of 113-114°C. Its IR spectrum showed two characteristic absorptions at 3483 and 3316 cm⁻¹ assignable to ν(N-H) and ν(CH=N), respectively. Strong band of azomethine group (C=N) occurred at 1597 cm⁻¹. The ¹H NMR spectrum showed peaks at δ_H 10.15 and 7.83 attributed to N-H and N=CH moiety, respectively, with integration values corresponding to one proton for each. A singlet peak appeared at δ_H 3.80 (3H) belongs to methoxy group (OCH₃). The presence of 1,4-substituted benzene ring with AA'BB' spin system was observed at δ_H 7.60 (2H, H-2',6') and 6.95 (2H, H-3',5') whereas the presence of monosubstituted benzene ring was observed at δ_H 7.22 (2H, H-3,5), 7.07 (2H, H-2,6) and 6.74 (1H, H-4). The ¹³C NMR spectrum of showed signals at δ_C 137.02 and δ_C 159.77 attributed to azomethine carbon (C=N) and sp²oxygentaed quaternary carbon, respectively. The presence of eight aromatic peaks were observed between δ_C 112.3-159.8 of which three of them are quaternary at δ_C 146.0, 159.8, and 128.9. The former suggests sp² quaternary carbon connected to nitrogen whereas the middle one suggests sp² quaternary carbon connected to oxygen. In agreement with the ¹H NMR, the presence of methoxy group was confirmed at δ_C55.6. Thus, based on the above spectral data the following structure was proposed which is in agreement with the synthesis protocol where the first moiety (monosubstituted phenyl ring) and the second moiety (disubstituted phenyl ring) are from the hydrazine and 4-methoxybenzaldehyde counterpart, respectively confirming the formation of hydrazone Schiff base (**1**).

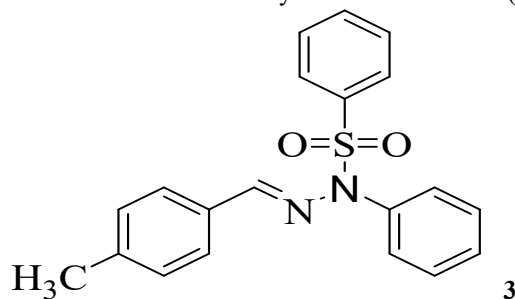


Compound **2** was obtained as a red crystals with melting point of 145-147°C. Its IR spectrum showed two characteristic absorptions at 3500 and 3305 cm⁻¹ assignable to ν(N-H) and ν(CH=N), respectively. The strong band observed at 1600 cm⁻¹ and 1330 cm⁻¹ suggests the presence of azomethine group (C=N) and nitro functional groups respectively . The ¹H NMR spectrum showed peaks at δ_H 10.85 and 7.88 attributed to N-H and N=CH group protons, respectively, with integration values corresponding to one proton for each one. The presence of 1,4-substituted benzene ring with AA'BB' spin system was observed at δ_H 8.18 (2H, H-3',5') and 7.15 (2H, H-2',6') whereas the presence of monosubstituted benzene ring was observed at δ_H7.79 (2H, H-2,6), 7.25 (2H, H-

3,5) and 6.85 (1H, H-4). The ^{13}C NMR spectrum showed signal at δ_{C} 134 attributed to azomethine carbon (C=N). The presence of eight aromatic peaks were observed between δ_{C} 113.0-146.4 of which three of them are quaternary with δ_{C} 146.4, 144.4 and 143.0. The former suggests sp^2 quaternary carbon connected to nitrogen (C-1)) whereas the middle one suggests sp^2 quaternary carbon connected to nitro group (C- NO_2). Thus, based on the above spectral data the following structure was proposed which is in agreement with the synthesis protocol where the first moiety (monosubstituted phenyl ring) and the second moiety (disubstituted phenyl ring) are from the hydrazine and 4-nitrobenzaldehyde counterpart, respectively confirming the formation of hydrazone Schiff base (2).

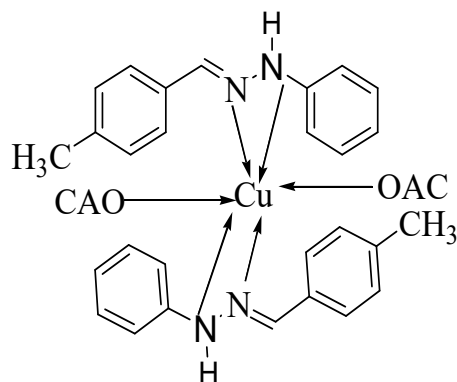


Compound 3 was obtained as gray solid with melting point of 213-215°C. Its IR spectrum showed characteristic absorptions at 3347 assignable to $\nu(\text{CH}=\text{N})$. Strong bands observed at 1614 cm^{-1} , 1510 cm^{-1} and 1256 cm^{-1} suggest the presence of azomethine group (C=N), and two (S=O) respectively. The IR bands of the hydrazone derivative were compared with those of compound 1. The two bands at 1500 cm^{-1} and 1256 cm^{-1} is due to the presence of benzenesulphonyl. The ^1H NMR spectrum showed a singlet peak at δ_{H} 7.85 attributed to N=CH group, with integration values corresponding to one proton. A singlet peak appeared at δ_{H} 3.80 (3H) belongs to methoxy group (OCH₃). The ^{13}C NMR spectrum showed signals at δ_{C} 136.82 (C-1''') and δ_{C} 159.7 attributed to azomethine carbon (C=N) and sp^2 oxygenated quaternary carbon (C-4'), respectively. The presence of 12 aromatic peaks were observed between δ_{C} 112.2-159.7 of which four of them are quaternary with δ_{C} 144.2, 159.7, 137.6 and 129.0. The first suggests sp^2 quaternary carbon connected to nitrogen (C-1), the second one suggests sp^2 quaternary carbon connected to oxygen (C-4'), third one suggests sp^2 quaternary carbon connected to sulfur (C-1'') whereas the fourth one attributed to C-1'. In agreement with the ^1H NMR, the presence of methoxy group was confirmed at δ_{C} 55.6. Thus, based on the above spectral data the structure shown below was proposed which is in agreement with the synthesis protocol where the first moiety (diarylhydrazones) and the second moiety (monosubstituted phenyl ring) are from compound 1 and benzenesulphonylchloride counterpart, respectively confirming the formation of benzenesulfanohydrazone derivative (3).



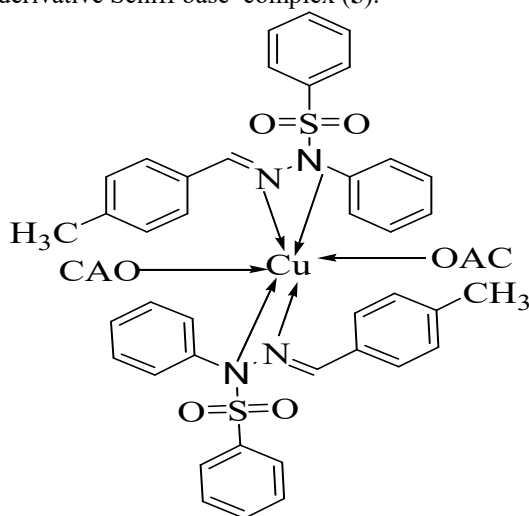
Characterization of the complexes

Compound 4, Cu(II) complex of compound 2, was obtained as a light brown solid crystals with melting point of 140-141°C. The elemental analysis result of Cu(II) complexes (table 1) is in good agreement with the theoretical values. The infrared spectra showed sharp absorption at 1616 cm^{-1} attributed to azomethine groups (C=N). The sharp absorption at 3013 cm^{-1} was assigned to H-C=N band of the azomethine group. The imine bond C=N stretching shifted to higher value in the spectra of the complexes than the ligand indicating the coordination of azomethine nitrogen to the metal ions [12]. This was further supported by the appearance of a new band in the region 606 cm^{-1} due to metal ligand M-N bonding. The presence of coordination and/or lattice water molecules in the binary complexes is indicated by a broad band within the range 3350-3550 cm^{-1} due to $\nu(\text{OH})$ vibrational modes of the water molecules [13]. Based on the elemental analysis, IR spectral data and literature indications octahedral geometry was suggested in which bidentate ligand coordination through the azomethine nitrogen atom, nitrogen atom, the fifth and sixth coordination sites being occupied OAC as shown below which is in agreement with the synthesis protocol where ligand (diarylhydrazone) and $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ undergo reaction confirming the formation of Cu(II)hydrazone complex (4).



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Compound **5**, Cu (II) complex of compound **3**, was obtained as a dark brown crystals with melting point of 190-192°C. The elemental analysis result of Cu(II) complexes (table 1) is in good agreement with the theoretical values. The infrared spectra showed strong absorption band at 1611 cm^{-1} assigned to the vibrations of azomethine groups (C=N). The imine bond C=N stretching shifted to lower value in the spectra of the complexes than the ligand indicating the coordination of azomethine nitrogen to the metal ions [12]. This was further supported by the appearance of a new band in the region 652 cm^{-1} in the complexes due to metal ligand M-N bonding. The presence of coordination and or lattice water molecules in the binary complexes is indicated by a broad band within the range 3350–3550 cm^{-1} due to $\nu(\text{OH})$ vibrational modes of the water molecules [13]. Based on the elemental analysis, IR spectral data and literature indications octahedral geometry was suggested in which bidentate ligand coordination through the azomethine nitrogen atom, nitrogen atom, the fifth and sixth coordination sites being occupied OAc as shown below which is in agreement with the synthesis protocol where the ligand (hydrazone derivative Schiff base) and $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ are undergo reaction and confirming the formation of Cu(II)hydrazone derivative Schiff base complex (**5**).



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Table 1. Elemental analysis results for copper complexes.

Complex	Molecular weight(g/mlo)	%C		%N		%H	
		Calc.	Found	Calc.	Found	Calc.	Found
4	681	52.86	52.42	12.33	12.12	3.23	3.14
5	931	56.71	52.21	6.01	5.82	4.73	4.68

Antibacterial activity results of ligands and Cu(II) complexes

The synthesized hydrazone schiff base derivatives and Cu(II) complexes were tested for their antibacterial activity *in-vitro* against *S.aureus*, *E.coli*, *K.pneumonia* and *P.aeruginos*. Ceftriaxone was used as standard antibacterial drug. The zone of inhibition values indicates that all the compounds exhibited a varied range 6-15 mm of antibacterial activity (table 2) against all the tested bacterial strains. Compound **1** showed moderate activity against *E.coli* and *P.aeruginos*. Compounds **2** and **3** showed no activity against all the tested bacterial strains. Compound **5**, Cu(II) complex of **3**, demonstrated moderate activity than the ligand **3** against *S.aureus* and no activity against *E.coli*, *P.aeruginos* and *K.pneumonia*. Compound **4**, Cu(II) complex of Compound **2**, showed moderate activity against *E.coli*(10mm zone of inhibition) and better activity against *K.pneumoniae* (15mm zone of inhibition) compared to the positive control (11mm zone of inhibition). Results of the

antibacterial activity show that Cu(II) complexes exerted excellent antibacterial activities than the ligand which might be due to increase in lipophilicity of the compound.

Table 2. *In-vitro* antibacterial activity values of the synthesized hydrazone schiff base and Cu(II) complexes.

Compound	Zone of Inhibition (mm)			
	<i>S.aureus</i>	<i>E.coli</i>	<i>K.pneumonia</i>	<i>P.aeruginosa</i>
1	6	8	6	7
2	6	6	6	6
3	6	6	6	6
4	9	10	15	6
5	8	6	6	6
+Ve. Control	24	23	11	14
-Ve. Control	6	6	6	6

Conclusion and Recommendation

In present study hydrazone derivatives of phenylhydrazine and 4-methoxybenzaldehyde and 4-nitrobenzaldehyde and their respective benzenesulfanohydrazones and Cu(II) complexes were successfully synthesized. The synthesized ligand characterized by FT-IR, ¹H NMR, ¹³C NMR and the complex were characterized by FT-IR and Elemental analyzer. The elemental analysis result of the both Cu(II) complexes are in good agreement with the calculated. Anti-bacterial activities of the synthesized compounds were examined. Of all synthesized compounds, Cu(II) complexes of compound 2 showed better activity against *K.pneumoniae* and moderate activity against *E. coli* compared to positive control Ceftriaxone. Results of the antibacterial activity show that the Cu(II) complex of ligand having electron withdrawing substituent's (nitro substituted) exerted excellent antibacterial activities which might be due to increase in lipophilicity of the compound. These observations promote further research work with structure-activity relationships studies to have a better understanding of structural and chemical parameters required to develop potent compounds. Additional research efforts are also recommended to determine the concentration and target of the compounds and application of computational analysis to have a deeper understanding of the molecular interaction.

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