Egypt. J. Chem. **Vol. 67** No. 3 pp. 379 - 386 (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Schiff base transition metal (II) complexes: spectral analyses and biological application

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Abstract

New metal complexes have been successfully synthesized and thoroughly characterized using various analytical techniques, including elemental analysis, spectroscopy (1H NMR-FT-IR, mass, UV-visible), and molar conductance. These analyses consistently indicate a 1:1 ratio of metal to ligand in the complexes.

The significance of this study lies in the investigation of the antimicrobial properties of both the ligand and its metal complexes. Specifically, the Schiff base-metal complexes of Co (II), Ni (II), Cu (II), and Cd (II) have been explored for their potential biological applications. The primary focus of this research is to assess their synthesis, characterization, and in vitro antibacterial and antifungal activities. In the context of the in vitro evaluations, the prepared metal complexes have exhibited substantially enhanced antibacterial and antifungal properties compared to the metal-free ligand. Notably, the highest level of antibacterial activity was observed against E. coli, as determined through the disc diffusion method, and this activity was found to be comparable to that of the standard antibiotic Ampicillin. Ultimately, it was conclusively demonstrated that the Co (II) complex exhibited the most pronounced biological efficacy in terms of both in vitro antibacterial and antifungal investigations.

Keywords: Schiff base ligand; metal complexes; spectroscopy; antibacterial activity; antifungal activity

1. Introduction

Schiff base metal complexes are a crucial category of pharmaceutical components and materials due to their diverse range of biological activities, including anticancer, antimicrobial, antiviral, antioxidant, anticonvulsant, anti-inflammatory, antidiabetic effects, and their ability to bind with DNA [1-5]. Moreover, the synthesis of organic compounds containing N, O, or N, S donor sites has sparked significant interest in their capacity to bond with various metal ions, continuously expanding their potential in the realm of biological activity [6-10]. It is worth noting that numerous research studies have been published,delving into the biological activities of these compounds after they undergo metalation with different transition metal ions, such as Co (II), Cu (II), and Ni (II) [10-16].Considering their biological characteristics (such as anticancer and antibacterial), Schiff bases are among the organic compounds that are thought to play a significant role in the pharmaceutics industry [1-7]. They are widely used in several sectors. Schiff bases exhibit versatile functionality as they can act as catalysts in various organic redox reactions, highly effective anticorrosion agents, organic semiconductors, filaments, deodorizers, light stabilizers, dental materials, cross-linked polymers, and fragrances [3-5]. The ability of Schiff bases to engage in complex formation, aided by the donation of their lone

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Receive Date: 06 August 2023 Revise Date: 06 September 2023 Accept Date: 11 September 2023

DOI: 10.21608/EJCHEM.2023.226655.8376

electron pairs for enhanced stability, is contingent upon the specific substitution groups present on these compounds. This capability stems from the presence of heterocyclic nitrogen, oxygen, and sulfur atoms. Thanks to their ease of preparation and wide-ranging structural diversity, Schiff base complexes involving transition metals are frequently recommended as primary stereochemical models in the field of coordination chemistry [6,7].. As biochemical, analytical, and antibacterial reagents, they are becoming increasingly important [8-12]. A major contribution to the synthesis of metal-ligand bonds is also contributed by the lone pair of electrons that are available on heteroatoms. Due to their extraordinary capacity for building complexes and extensive use as spectrometric and gravimetric reagents in analytical chemistry, Schiff base ligands with N, O, and S donor atoms have drawn a lot of attention over the years. Additionally, it has been claimed that administering these chemicals as metal complexes increases their action [12-18].

In the current study, a tridentate Schiff base ligand [L] and its Co(II), Ni(II) and Cu(II) complexes were developed and characterized utilizing analytical methods such as CHN analysis, UV-Vis spectroscopy, FT-IR spectroscopy, ^{1}H NMR, EI-mass spectral analysis, and conductivity measurements. This study's primary goal is to demonstrate that the synthesized compounds are strong competitors for diverse biomedical applications by analyzing the coordination properties of the novel Schiff base with various M(II) [18-20].

2. Materials and methods

2.1. Experimental

2.1.1. Chemicals and reagents

The (*2-hydrazineyl-2-oxoacetamide* and 1-(2-(p-tolyl)hydrazineylidene)propan-2-one were purchased from the Merck group. The metal salts $CoCl_2 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$ and $CuCl_2 \cdot 2H_2O$ (Sigma-Aldrich) were obtained from the Merck group. Absolute ethanol, diethylether and dimethyl formamide (DMF) were employed as organic solvents. All of the chemical utilized was provided in the highest purity attainable and all preparations were prepared using deionized water.

2.1.2. Solutions

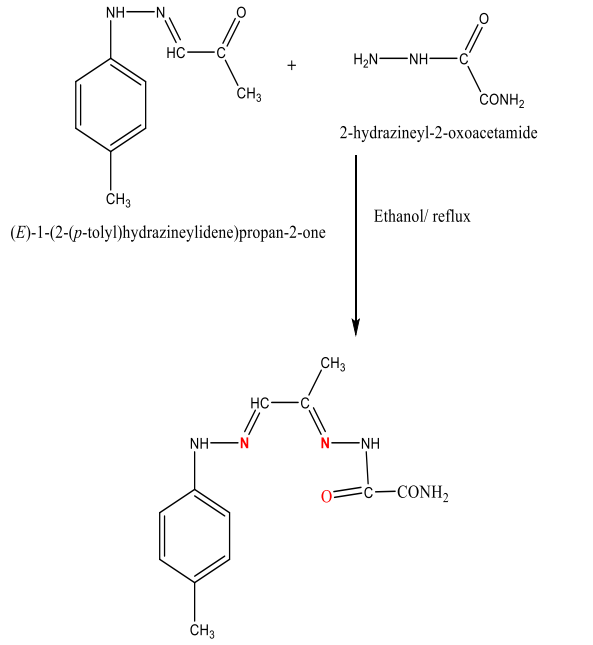
For conductivity experiments, metal complex solutions (1×10^{-3} M) have been synthesized in ethanol [19, 20]. Both the Schiff base ligand and its complexes were prepared in solutions with a concentration of 1×10^-4 M for UV-Vis spectral analysis.

2.1.3. Instrumentation

The microanalysis of carbon, hydrogen, and nitrogen was carried out using a CHNS-932 (LECO) Vario Elemental analyzer at Cairo University's Microanalytical Center in Egypt. Melting point determination was performed using a triforce XMTD-3000 instrument. Fourier transform infrared (FT-IR) spectra were acquired using a Perkin-Elmer 1650 spectrometer, employing KBr disks, within the spectral range of 4000–400 cm−1. For 1H NMR spectroscopy, measurements were taken at room temperature utilizing a 300-MHz Varian-Oxford Mercury spectrometer in dimethyl sulfoxide-d6 (DMSO-d6) solutions, with tetramethylsilane serving as an internal standard. The molar conductance of solid complex solutions in ethanol, with concentrations of 10−3 M, was determined using a Jenway 4010 conductivity meter. Mass spectra were obtained using an MS-5988 GS-MS instrument from Hewlett-Packard, employing the electron ionization method at 70 eV. A Perkin-Elmer Model Lambda 20 automated spectrophotometer was employed to record the spectral data of solutions across a wavelength range spanning from 200 to 700 nm. The antimicrobial research was conducted at Cairo University's Microanalytical Center. Procedures

2.2 Synthesis of the Schiff base ligand [L]

According to the suggested technique, a novel Schiff base ligand was synthesized. This was accomplished by condensing 2-hydrazineyl-2-oxoacetamide (5 mmol, 0.515 g), which was dissolved in hot 100% ethanol (60°C), and 1-(2-(p-tolyl)hydrazineylidene)propan-2-one (5 mmol, 0.88 g), which was dissolved in hot ethanol in a 1:1 molar ratio. The reaction mixture was then placed in reflux for 4 hours. Subsequently, pale brown solid compound was isolated, filtered out, and recrystallized using ethanol and diethyl ether to produce a pure Schiff base with a yield of 92 %. Scheme (1) illustrates both the structure and the overall formation reaction of the Schiff base ligand.



Scheme. 1: Synthesis pathway of the Schiff base ligand (L).

2.3. Synthesis of the metal complexes

The metal complexes were synthesized by combining a hot ethanolic solution (70°C) of the Schiff base ligand (1 mmol, 0.26 g) within a hot absolute ethanol (20 ml) solution of metal salts (0.23-g $CoCl_2 \cdot 6H_2O$, 0.23-g $NiCl_2 \cdot 6H_2O$ and 0.14-g $CuCl_2 \cdot 2H_2O$). After 3 hours of stirring in reflux, the resultant complexes began to precipitate from the mixtures. The precipitates were collected by filtration, cleaned by repeated washings, and dried under a vacuum over anhydrous calcium chloride. Consequently, the pure metal Complexes resulted through the recrystallization process.

2.4.Antimicrobial activity

To evaluate the in vitro antibacterial and antifungal properties of the compounds, we employed the disc diffusion technique. As positive controls for Gram-positive bacteria, Gram-negative bacteria, and fungi, gentamycin, ampicillin, and amphotericin B were used, respectively [21, 22]. The bacterial strains included Gram-positive bacteria such as *Bacillus subtilis*, *streptococcus faecalis*, and *Staphylococcus aureus*, Gram-negative bacteria including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Neisseria gonorrhoeae*, and fungal strains consisting of *Candida albicans* and *Aspergillus flavus*. The Schiff base ligand and its complexes were dissolved in DMSO to prepare the stock solutions (1 mmol). For the antibacterial activity assessment, a nutrient agar medium was meticulously prepared and subsequently cooled to a temperature of 47°C. Following this, it was inoculated with microorganisms. Once the medium had solidified, a sterile cork borer was employed to create holes with a diameter of 5 mm. The investigated compounds, namely, the Schiff base ligand and its metal complexes, were dissolved in DMSO at a concentration of 1×10−3 M and added to Petri dishes, each containing 0.1 ml of the solution. The growth plates of bacteria and fungi were then placed in an incubator for 20 hours at 37°C. Then the inhibition zones were subjected to diameter measurements in millimeters. The average of the final reading of antimicrobial activity assessments was determined by carrying out the antimicrobial activity experiments in triplicate [23].

3. Results and Discussion

The newly synthesized L Schiff base ligand (*2-oxo-2-(2-((1E,2E)-1-(2-(p tolyl)hydrazineylidene)propan-2-ylidene)hydrazineyl)acetamide (L)*underwent elemental analysis, electron ionization-mass spectrometry (EI-MS), IR, UV-Vis, and ^{1}H NMR spectrum studies. The findings of the elemental analyses (C, H, and N) are in good agreement with the formulas suggested in Table 1 and Fig. 1.

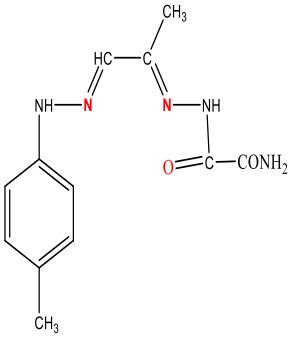


Figure 1. Depicts the structural representation of the Schiff base ligand (L).

The purity of the Schiff base ligand (L) was demonstrated by its sharp melting point. The distinctive stretching vibration bands for the L ligand can be seen in the IR data (Table 2) at 3399, 3127, 1654 and 1614 cm^{-1} for $\upsilon(NH_2)$, $\upsilon(NH)$ $\upsilon(CO)$ and $\upsilon(C=N)$ azomethine.

Table 1: provides the physical and analytical data for both the Schiff base ligand (L) and its corresponding metal complexes.

Compound (chemical formula)	Color Yield (%)	M.p. (°C)	Found (Calcd)				A_m (Ω^{-1} mol^{-1} cm^2)
			C (%)	H (%)	N (%)	M (%)	
L **($C_{12}H_{15}N_5O_2$)**	brown (92)	145	55.13 (55.17)	5.33 (5.74)	26.61 (26.82)	—	—
[Co(L)Cl_2.H_2O].H_2O	Brown (88)	195	33.32 (33.72)	4.35 (4.45)	16.24 (16.39)	13.71 (13.82)	30
[Ni(L)Cl_2.H_2O]	Brown (87)	210	34.92 (35.20)	4.01 (4.16)	16.98 (17.11)	14.22 (14.43)	19
[Cu(L)Cl]Cl.H_2O	Dark Brown (87)	255	٣٤.٥٢ (٣٤.٨٧)	3.95 (4.12)	16.16 (16.95)	15.02 (15.25)	71

Table 3 contains the ^{1}H NMR spectrum of the ligand. Two signals from the NH resonance can be seen in the ^{1}H NMR spectrum at 13.05 and 10.92 ppm for the free L proving that the two amine environments are not equivalent. A characteristic singlet proton signals at 8.35, 8.01, 2.49 and 2.07 ppm are assigned to NH_2, azomethine CH and two CH_3 groups respectively. In addition to this, the multiplet signals in the 6.90-7.52 ppm range are due to aromatic protons [37].

Table 3 ^{1}H NMR spectral data of the Schiff base ligand (L).

Compound	Chemical shift, (δ) ppm	Assignment
L	13.05	(s, H, CONH)
	10.92	(s, H, ph-NH)
	8.35	(s, 2H, NH_2)
	8.01	(s, H, azomethine CH)
	6.90-7.52	(m, 4H, aromatic)
	2.49	(s, 3H, ph-CH_3)
	2.07	(s, 3H, CH_3)

A recording of the Schiff base ligand's mass spectrum was presented. The resulting molecular ion (m/z) peak at 261 amu supported the proposed formulation, in which the ligand moiety was $C_{12}H_{15}N_5O_2$. In addition, several peaks in the mass spectrum were shown that each one corresponding to a different stage in the ligand's breakdown.

3.1 Elemental analyses and molar conductivity measurements.

the complexes of Co(II), Ni(II) and Cu(II) are all air-stable. They are largely soluble in polar organic solvents including EtOH, MeOH, DMF, and DMSO. They cannot dissolve in water, though. Additionally, elemental analysis was used to corroborate the stoichiometry and formulation of the Schiff base (L) ligand and its metal complexes which were confirmed to have a metal/ligand ratio of 1:1 in the complexes. This was done by measuring the metal contents of the complexes as well as their carbon, hydrogen and nitrogen (Table 1). The ligand and their complexes elemental studies show good agreement with the suggested structures. It was observed that the molar conductivity (Λm) values of metal complexes in DMF (10^{-3} M) at 25 2°C ranged from 30 to 71 Ω^{-1} mol^{-1} cm^2, indicating that the complexes of Ni(II), Co(II) were non electrolyte where the Cu(II) complex was ionic in nature and 1:1 electrolyte. The low molar conductance values for the Ni(II) and Co(II) complexes suggested that chloride anions were found inside the coordination sphere. Table 1 presents the findings.

3.2 FT-IR spectral data

The vibrational spectra of the produced compounds in the 4000-400 cm^{-1} range were recorded and the results were shown in Table 2. Comparing the infrared spectra of the parent ligand and the corresponding metal complexes allows one to investigate the coordination mechanism of the ligand toward the metal centers. The azomethine group in this study displayed a distinctive strong band at 1614 cm^{-1}, which is shifted in the complexes to 1595-1606 cm^{-1}, showing that coordination occurred through the nitrogen atoms of the azomethine groups [26-28]. Moreover, due to the presence of CO group, the complexes produced

a band at 1641-1678 cm^{-1}. Additionally, all complexes exhibited nonligand bands in the range of 421–503 cm^{-1} and 510-544 cm^{-1} corresponding to υ(M−N) and υ(M−O), respectively, greatly corroborating the idea that coordination took place via the nitrogen atoms in the azomethine and carbonyl oxygen [33, 36]. The data of IR revealed that the Schiff base behaved as neutral tridendate ligand.

Table 2 The significant infrared frequencies in the range of 4000–400 cm^{-1} for both the Schiff base ligand (L) and its metal complexes

Compound	υ(CO)	υ(C=N)	υ(M−O)	υ(M−N)
L	1654m	1614sh	------	------
[Co(L)Cl_2.H_2O].H_2O	1678s	16٠٤s	544s	416s
[Ni(L)Cl_2.H_2O]	1650s	1606s	546s	430s
[Cu(L)Cl]Cl.H_2O	1641s	1595w	510s	415w

3.3 Mass spectra

The EI-MS technique was used in this work to corroborate the mass of the ligand [38] L and its complexes by examining the intense molecular ion peaks in the spectra shown at *m/z* = 261 $[M]^+$ (L), 409 $[M\text{-}H_2O]^+$ (Co complex), 410 $[M\text{-}H_2O]^+$ (Ni complex) and 416 $[M+1]^+$ (Cu complex).The molecular ions results confirmed that the 1:1 metal-to-ligand stoichiometric ratio.

3.4 UV–Vis spectral study

The parent ligand and its complexes UV-Vis absorption spectra were studied in the 200–800 nm range in the room-temperature DMF solution. The ligand's spectrum exhibits strong bands at 229, 258 and 370 nm in the ultraviolet region. The π–π* and n–π* intramolecular transitions of the ligand are responsible for these bands [32, 33]. Furthermore, as a result of their coordination with metal ions in the ranges of 216-240, 244-251, and 344-383 nm, respectively, these bands were shifted in all complexes.

3.5 Thermal analysis

In the case of the Schiff base (L), the TG curve exhibited three distinct stages of mass loss within the temperature range of 30 to 600 °C. The initial two decomposition stages, occurring between 88 and 235 °C, were attributed to the loss of $C_3H_6N_2O_2$ molecules, resulting in an estimated mass loss of 38.88% (calculated as 39.08%). The third stage, observed between 235 and 500 °C, indicated the elimination of $C_9H_9N_3$ molecules, with an estimated mass loss of 61.01% (calculated as 60.92%). Overall, the cumulative weight loss amounted to 99.99% (calculated as 100%).

For the Co (II) chelate, a total of six decomposition steps were observed. The initial two decomposition stages, in the temperature range of 30–180 °C, were possibly attributed to the loss of 2H2O, resulting in an estimated mass loss of 8.45% (calculated as 8.43%). The third stage, occurring between 180 and 415 °C, indicated the release of Cl_2 gas, with an estimated mass loss of 16.75% (calculated as 16.63%). The final three steps, observed in the range of 415–1000 °C, corresponded to an estimated weight loss of 57.23% (calculated as 57.37%), signifying the removal of the organic moiety and leaving CoO as the residue.

3.6 Antimicrobial activity

To assess the antibacterial and antifungal activity of the synthesized ligand and its metal (II) complexes, we employed the disc diffusion method. The bacterial strains selected for testing included Gram-positive bacteria such as *Bacillus subtilis, streptococcus faecalis,* and *Staphylococcus aureus*. Additionally, we tested Gram-negative bacteria, including *Escherichia coli, Pseudomonas aeruginosa,* and *Neisseria gonorrhoeae.* In addition to bacteria, we also evaluated the compounds' effectiveness against fungal strains, specifically *Candida albicans* and *Aspergillus flavus* (Fig 2). The observations of antibacterial and antifungal activities are listed in Table 4. The observed outcomes demonstrate the present analysis's complexes' efficacy against both Gram-positive and Gram-negative bacterial strains [41,42]. Only ***Aspergillus flavus*** exhibited strong antifungal activity in the Co(II) complex (Table 4). According to the overall findings, the synthesized Co complex was more effective than the parent ligand and the other complexes against various bacterial and fungal species. Based on the chelation theory, the enhanced antibacterial and antifungal activity of the produced metal Schiff base complexes presented here can be adequately explained [43].

Table 4: Biological activity of L ligand and its metal complexes Ampicillin: Standard antibacterial agent; Amphotericin B: Standard antifungal agent.

Sample		Inhibition zone diameter (mm / mg sample)							
		Gram-positive bacterial species			Gram-negative bacterial species			Fungi	
		Staphylococcus faecalis	*Bacillus subtilis*	*Staphylococcus aureus*	*E.coli*	*Neisseria gonorrhoeae*	*Pseudomonas aeruginosa*	*Aspergillus flavus*	*Candida alibicans*
L		**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
$[Co(L)Cl_2.H_2O].H_2O$		**26**	**23**	**18**	**21**	**20**	**22**	**NA**	**23**
$[Ni(L)Cl_2.H_2O]$		**14**	**15**	**17**	**15**	**13**	**16**	**20**	**15**
$[Cu(L)Cl]Cl.H_2O$		**14**	**17**	**10**	**15**	**18**	**19**	**NA**	**22**
Standard	**Ampicillin**	**26**	**26**	**21**	**25**	**28**	**26**	**----**	**-----**
	Amphotericin B	**-----**	**-----**	**-----**	**-----**	**-----**	**-----**	**17**	**21**

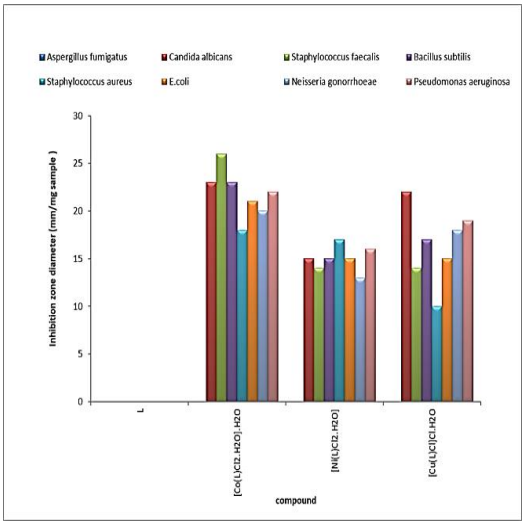


Figure 2: illustrates the biological activity of the Schiff base ligand and its corresponding metal complexes when tested against various bacterial and fungal species. This figure provides a visual representation of their antimicrobial properties and their effectiveness against different microorganisms.

4. Conclusions

The newly synthesized L ligand was prepared. With the help of two imine nitrogen and carbonyl oxgyen, the Schiff base L functioned as a neutral tridentate ligand. Following a thorough characterization of the complexes, it was observed that all chelates displayed greater biological activity than the free ligand. The antibacterial behavior of ligands is thus significantly impacted by metal chelation. In light of the chelation principle, the antibacterial activity was clearly demonstrated. Among the produced complexes, the Co (II) complex can be regarded as the most promising, powerful, and all-purpose antibacterial chemical. The synthesized compounds' antifungal efficacy demonstrated Co (II) outstanding activity showing promise, but more extensive research on both humans and animals is still needed.

5. Conflicts of interest

There are no conflicts to declare.

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