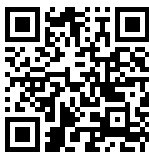


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Synthesis, Characterization and Biological Activity of Fe(II), Cu(II), and Cd(II) with Schiff Base Ligands (SBLs): Biological Screening of Divalent Metal Chelated Complex

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ABSTRACT

This study aimed to develop novel Schiff base ligands (SBLs), namely *N*¹,*N*²-Bis(4-nitrobenzylidene)benzene-1,2-diamine (1), 4,6-Dimethyl-*N*-((5-nitrofuran-2-yl)methylene)pyrimidin-2-amine (2) and *N*¹,*N*²-Bis((5-nitrofuran-2-yl)methylene)benzene-1,2-diamine (3). It also aimed to examine how they coordinate with various bidentate transition metal ions to form metal complexes. The goal was to properly characterize the newly synthesized chemicals and investigate their possible uses in chemical and pharmaceutical industries, with an emphasis on the drug industry. Thin-layer chromatography (TLC) was used to methodically monitor and confirm the synthesis process at each step. The compounds were then put through characterization procedures such as Fourier transform infrared spectroscopy (FTIR), urease inhibition assays, and assessments of antioxidant activity.

Keywords: azomethine group, chelation, metal complex, Schiff base ligands (SBLs), transition metal ion

1. INTRODUCTION

An excellent type of ligands and a common source of ligands in coordination chemistry are the chemical compounds known as Schiff bases that contain the (-C=N-) azomethine group. In organic synthesis, the development of the carbon-nitrogen double bond (-C=N-) is crucial. This is accomplished through the reactivity of carbonyl compounds with amines in

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an acidic media, resulting in the formation of Schiff bases (imines). Over the past decade, a lot of research has been conducted on Schiff bases and their bioactive complexes. These imines may react with metal ions to form a variety of coordination metal complexes, which are utilized in numerous industries [1]. They have substantial biological properties, such as anticancer [2], antitumor [3], anti-inflammatory [4], insecticidal [5], antibacterial [6], antituberculosis [7], antimicrobial [8], anticonvulsant [9], antioxidant [10], and anthelmint [11] properties. Schiff bases are also utilized in nucleophilic addition with an organometallic reagent and cycloaddition processes as flexible components [12].

Macrocyclic ligands that possess more donor atoms linked to their rings have obtained significant scholarly attention, recently. This is primarily because of their unique capacity to bind and transport metal ions, allowing for the synthesis and analysis of their mixed-valence forms. These ligands also serve as valuable models for metalloproteins. Moreover, their applications span across various scientific disciplines, making them an object of great interest in both basic and applied sciences, particularly in the field of coordination chemistry. To address these research interests, three Schiff base ligands (SBL) were synthesized and characterized through condensation reactions. These ligands exhibit enhanced biological activity when complexed with transition metals, thereby highlighting their potential applications in medicinal chemistry. Furthermore, both the metal and the ligand are present in the complex, which lessens the host's cytotoxicity and further enhances their suitability for biomedical applications [13–16]. It was followed by complexation with divalent metals, that is, Fe (II), Cu (II), and Cd (II). This study intended to achieve biologically active compounds from biologically inactive or less active compounds via chelation. After the chelation of SBLs, these compounds were screened for their biological activities with excellent results.

2. EXPERIMENTAL SETUP

2.1. Materials and Chemicals

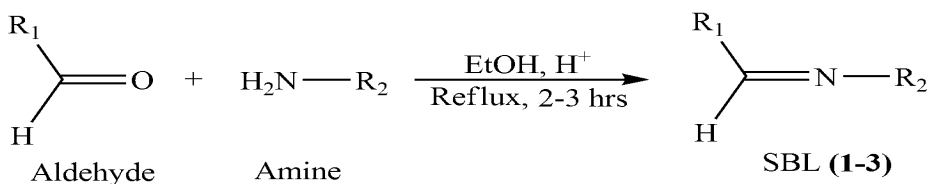
All of the chemicals and solvents were used exactly as they were purchased from Merck. Metal salts, specifically metal (II) sulphate or chloride, were used as hydrated forms [FeSO₄·7H₂O, CuSO₄·5H₂O, and CdCl₂·2H₂O].

2.2. Physical Measurements

A type AL 204 electric Mettler Toledo balance was used for weighing. On the Gallen Kamp equipment, melting points were observed. The IR spectra were recorded using the KBr pallet and a Thermo Nicolet Avatar 320 FTIR spectrophotometer. For preparative TLC, pre-coated silica gel G-25-U was utilized. Furthermore, a hot-magnetic plate, 1000W microwave, and magnetic beads were used.

2.3. Synthesis

2.3.1. Schiff Base Ligand (1-3). Aldehyde solution (0.01 mole in 50 ml of ethanol) was added to a round bottom flask while being stirred continuously and the reaction mixture was refluxed for 5 minutes. Afterwards, 3-5 drops of CH₃COOH were added into it and the mixture was refluxed further for 15 minutes. Amine solution (0.01 mole in 50 ml ethanol) was added to the reaction mixture, which was then refluxed for 2-3 hours with continuous stirring. After regular interval of time, the reaction mixture was filtered in hot state. The filtrate was then kept at room temperature for 7 to 15 days for crystallization. The resultant product followed a cold, absolute EtOH/MeOH/n-hexane wash and was then crystallized by an equal mixture of absolute MeOH and ethyl acetate. Reduced pressure was used to dry the recrystallized products over anhydrous CaCl₂ or P₂O₅. The recrystallized products were air-dried. TLC was routinely checked for confirmation when reactions were completed (Scheme 1) [13].



Scheme 1. Synthesis of SBLs (1-3)

SBL	R ₁	R ₂
1	4-Nitrobenzaldehyde	1,2-Phenylenediamine
	<i>N</i> ¹ , <i>N</i> ² -Bis(4-nitrobenzylidene)benzene-1,2-diamine (1)	
2	5-Nitro-2-furaldehyde	2-Amino-4,6-dimethylpyrimidine
	<i>4,6-Dimethyl-N</i> -(5-nitro-2-furyl)methylene)pyrimidin-2-amine (2)	
3	5-Nitro-2-furaldehyde	1,2-Phenylenediamine
	<i>N</i> ¹ , <i>N</i> ² -Bis((5-nitro-2-furyl)methylene)benzene-1,2-diamine (3)	

2.3.2. Schiff Base Metal Complexes (4-12). The methanolic solution containing the appropriate salts [$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, and $\text{CdCl}_2 \cdot 2\text{H}_2\text{O}$] was slowly added to the heated, stirring ligand (SBL) methanolic solution in a 2:1 (L: M) molar ratio. The reaction mixture was refluxed for 45 minutes. To keep the pH of reaction mixture appropriate for the synthesis of complexes and their precipitation, 1M NaOH was added dropwise in MeOH. The reaction medium's color changed once NaOH was introduced. The precipitates were filtered and vacuum-dried on anhydrous CaCl_2 after being rinsed with ice-cold methanol. The resulting product was recrystallized with ethyl acetate and methanol at a ratio of 1:1, then dried in desiccators over P_2O_5 . TLC was used to examine the resulting items' purity (Figure 1) [13].

SBL (1)

Black color, M. P.: 269 °C; yield: 89%; IR (KBr) ν_{max} cm^{-1} : 1570.7 (C=N).

SBL (2)

Amorphous solid, black color; M. P.: 230 °C; yield: 90%; IR (KBr) ν_{max} cm^{-1} : 1552.8 (C=N)

SBL (3)

Amorphous solid, black color; M. P.: 280 °C; yield: 85%; IR (KBr) ν_{max} cm^{-1} : 1560.1 (C=N)

SBL (1) - Fe(II) (4)

Amorphous solid, orange brown color; M. P. (decomp.): > 400 °C; yield: 85 %; IR (KBr) ν_{max} cm^{-1} : 1433.2 (C=N), 853.6 (Fe-N)

SBL (1) - Cu(II) (5)

Amorphous solid, dirty brown color; M. P. (decomp.): > 400 °C; yield: 80 %; IR (KBr) ν_{max} cm^{-1} : 1422.1 (C=N), 855.4 (Cu-N).

SBL (1) - Cd(II) (6)

Amorphous solid, yellow brown color; M. P. (decomp.): > 400 °C; yield: 75 %; IR (KBr) ν_{max} cm^{-1} : 1431.3 (C=N), 862.9 (Cd-N).

SBL (2) - Fe(II) (7)

Amorphous solid, orange brown color; M. P. (decomp.): > 400 °C; yield: 70 %; IR (KBr) ν_{max} cm^{-1} : 1433.2 (C=N), 862.9 (Fe-N).

SBL (2) - Cu(II) (8)

Dark brown color; M. P. (decomp.): > 400 °C; yield: 87 %; IR (KBr) ν_{\max} cm^{-1} : 1427.6 (C=N), 864.7 (Cu-N)”

SBL (2) - Cd(II) (9)

Amorphous solid, brown color; M. P. (decomp.): > 400 °C; yield: 76 %; IR (KBr) ν_{\max} cm^{-1} : 1435.0 (C=N), 862.9 (Cd-N).

SBL (3) - Fe(II) (10)

Amorphous solid, orange brown color; M. P.: > 400 °C (decomp.); yield: 69 %; IR (KBr) ν_{\max} cm^{-1} : 1422.0 (C=N), 877.8 (Fe-N)

SBL (3) - Cu(II) (11)

amorphous solid, dark brown color; M. P.: > 400°C (decomp.); yield: 75 %; IR (KBr) ν_{\max} cm^{-1} : 1433.2 (C=N), 862.9 (Cu-N).

SBL (3) - Cd (II) (12)

Amorphous solid, dirty brown color; M. P.: > 400°C. (decomp.); yield: 70 %; IR (KBr) ν_{\max} cm^{-1} : 1431.3 (C=N), 860.1 (Cd-N).

2.3.3. Biological Activities.

2.3.3.1. DPPH Radical Scavenging Activity Assay. The DPPH solution (0.3 mm) was prepared in ethanol. Before 95 μl of an ethanol-based DPPH solution was added, each sample was dissolved in 5 μl of methanol at a varied concentration (between 5 and 500 μg). Afterwards, the mixture was spread out across a 96-well plate and let to stand at 37°C for 30 minutes. Absorbance at 515 nm was then measured using a microtitre plate reader (Spectramax plus 384 Molecular Device, USA). As a standard for comparison, butylated hydroxyanisole (BHA) was used. The following formula was used to calculate the percent radical scavenging activity in contrast to the methanol-treated control:

$$\text{DPPH scavenging effect (\%)} = \frac{\text{Absorbance of control (DMSO treated)} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The IC₅₀ values of compounds were calculated by observing the effects at various levels (1-1000 μM) using the EZ-fit enzyme kinetic program (Pellera Scientific Inc. Amherst, USA [17]).

2.3.3.2. Urease Inhibition Assay . Phosphate buffer (0.01M LiCl₂, 1 mM EDTA, and K₂HPO₄.3H₂O) was used to make the urease enzyme solution. For this purpose, 0.125 units of the enzyme were added to each well containing 80µl of 0.05 M potassium phosphate buffer (pH 8.2) and 10µl of the test material (concentration range 5-500 M). The contents were then mixed. The mixture was incubated at 30°C for the next 15 minutes. To start the reaction, 40µl of urea substrate solution (50 mM) was added to each well except for well B. Each well then contained 40µl of the phenol reagent (1% phenol and 0.005% w/v sodium nitroprusside) and 70µl of the alkaline reagent (0.5% NaOH and 0.1% active NaOCl). After 50 minutes, absorbance at 630 nm was measured after the reaction mixture was spread out on well plates. By observing how increasing test drug concentrations affected the degree of inhibition, IC₅₀ values were identified [18].

3.RESULTS AND DISCUSSION

The condensation process between aldehyde and amine yielded the SBLs (1-3) (Scheme 1) which were chelated with divalent metal ions (Fe²⁺, Cu²⁺ and Cd²⁺) (Figure 1). SBL (1) was synthesized as an amorphous solid of black color with the melting point of 269°C. The Fe(II), Cu(II), and Cd(II) complexes (4-6) of SBL (1) were obtained as amorphous solids of orange brown, dirty brown, and yellow brown colors, respectively. SBL (2) was obtained as an amorphous solid of black color with the melting point of 230°C. The complexes of SBL (2) with Fe(II), Cu(II), and Cd(II) (7-9) were of orange brown, dark brown, and brown colors, respectively. SBL (3) was obtained as an amorphous solid of black color with the melting point of 280°C. The complexes of SBL (3) with Fe(II), Cu(II), and Cd(II) (10-12) were of orange brown, dark brown, and dirty brown colors, respectively. All these prepared metal complexes decomposed above 400°C. The temperatures at which these complexes decomposed show that they are rather stable substances. Even though insoluble in water and other common organic solvents, all of the complexes (4–12) remain soluble in dimethyl sulfoxide (DMSO) and dimethyl formamide (DMF).

3.1.IR Spectra

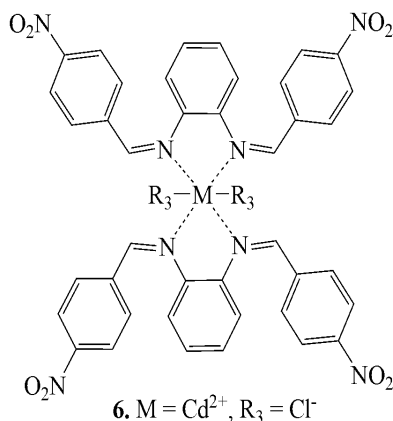
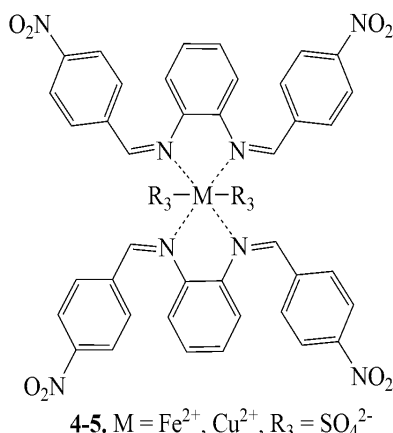
The IR spectra of the free SBLs and the spectra of ligand-metal complexes were studied in order to better understand the binding mechanism of ligands with metals. 4-nitrobenzaldehyde's distinctive

carbonyl group $\nu(\text{C}=\text{O})$ peak and 1,2-phenylenediamine's absence of an amino group $\nu(-\text{NH}_2)$ absorption frequency disappeared. It showed that the condensation reaction was completed.

At $1570.7 - 1552.8 \text{ cm}^{-1}$ [9], a prominent band of the azomethine group $\nu(\text{C}=\text{N})$ of ligands (1-3) was observed. At around $1435 - 1422.0 \text{ cm}^{-1}$, the absorption frequency shifted towards a lower frequency region, indicating the coordination of metal ion with the nitrogen atom of the azomethine group ($-\text{C}=\text{N}-$) [10]. The peak below 3000 cm^{-1} in all complexes (4–12) indicated the presence of an aromatic C–H bond. The peaks appearing at $853.6, 855.4,$ and 862.9 cm^{-1} were assigned to metal-nitrogen bond in Fe(II), Cu(II), and Cd(II) complexes (4-6) of ligands (1). Also, the peaks appearing at $862.9, 864.7,$ and 862.9 cm^{-1} were assigned to metal-nitrogen bond in Fe(II), Cu(II), and Cd(II) complexes (7-9) of ligands (2). Similarly, the peaks appearing at $877.8, 862.9,$ and 860.1 cm^{-1} were assigned to metal-nitrogen bond in Fe(II), Cu(II), and Cd(II) complexes (10-12) of ligands (3). The reason the peak for the azomethine group ($-\text{C}=\text{N}-$) emerged at such a lower value might be related to the group's attachment to metal ion (M-N) and the single bond character enforced by the resonance of the nitrogen lone pair [13].

3.2. Proposed Structures of Complexes (4-12)

SBL (Scheme 1) and metal complexes were characterized using the spectroscopic data. Analytical and spectral data were used to support the theory that nitrogen atom in the azomethine group links the Schiff base to metal ion. In this regard, the suggested structures for complexes (4–12) are shown below in Figure 1.



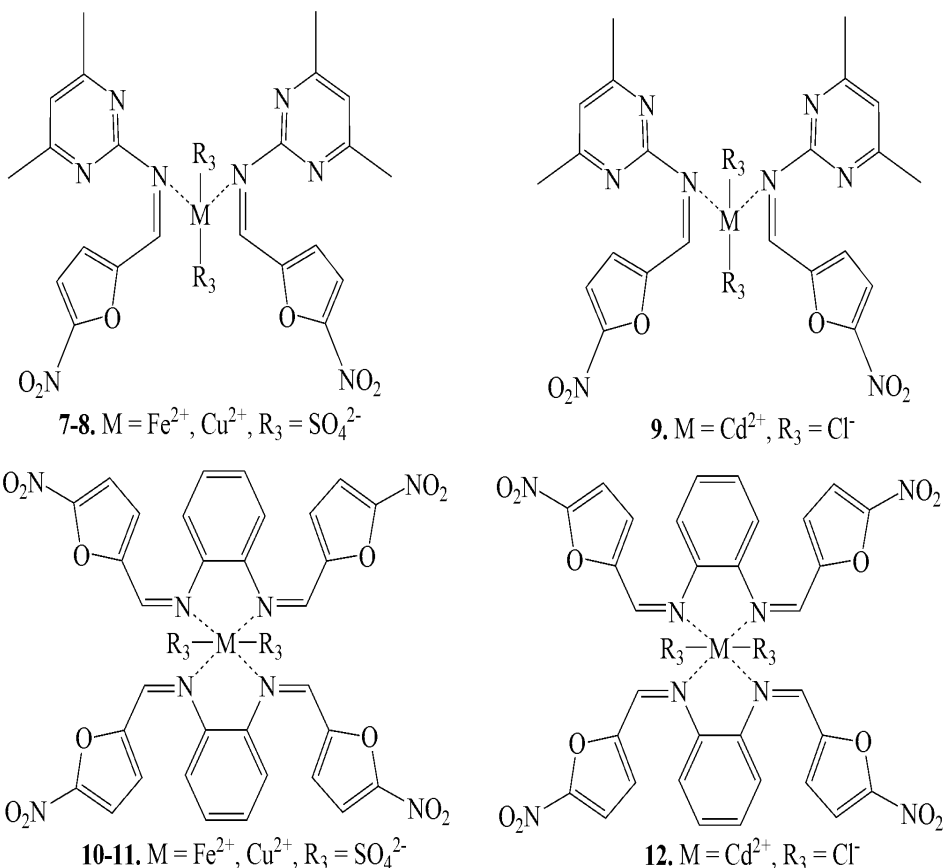


Figure 1. Proposed Structures of SBL Metal Complexes (4-12)

3.3. Biological Activities

3.3.1. Antioxidant: DPPH Radical Scavenging Activity Assay. The well diffusion method and the reference chemical BHA were used to evaluate the synthetic compounds' ability to scavenge free radicals. The substances' antioxidant activity was evaluated based on their ability to neutralize the stable radical DPPH'. UV-Vis spectrophotometer was used to quantify the decline in absorbance at a wavelength of 517 nm, which caused the colour to shift from purple to yellow when DPPH' was reduced. Complexes 4-6 and 9-10 showed little activity, whereas SBL 1-3 displayed somewhat moderate activity. As compared to standard (BHA), complexes 7 and 12 showed moderate activity. The other two compounds, namely 8 and 11, showed potent antioxidant activity (Table 1) [19].

3.3.2. Urease Inhibition Assay. The urea amidohydrolase enzyme (EC 3.3.1.5), which produces urease, has nickel as a cofactor. It is found in a wide range of species including bacteria, plants, and fungus. Urea is broken down into ammonia and carbonic acid with the help of urease. At physiological pH, carbonic acid undergoes further hydrolysis to produce an additional urea and carbonic acid molecule [20, 21]. The pathogenic bacteria *Helicobacter pylori* (*H. pylori*) can survive in an acidic environment and establish colonization due to urease overexpression, which acts as a virulence factor. The pathogenesis of gastric and peptic ulcers is influenced by this fact. Moreover, urease is linked to a number of other health problems, such as the development of kidney stones, hepatic coma, and urinary tract infections (UTIs). Consequently, targeting urease has emerged as a significant approach for treating *H. pylori* infections. Several remedies have been identified to inhibit the excessive activity of urease in *H. pylori*. The compounds 2 and 6 showed slightly moderate activity but compounds 4 and 5 showed moderate to significant activity. Compounds 8 and 11 showed non-significant activity, while all others showed strong potent urease activity (Table 1).

Table 1. Antioxidant and Urease Inhibition Activities of Compounds and Standard Reagents

Compounds	IC ₅₀ value (uM)	
	Antioxidant	Urease inhibition
1	86.8±0.38	15.8±0.14**
2	75.5±0.82	42.6±0.29
3	83.9±0.81	9.5±0.07**
4	93.2±0.28	31.4±0.90
5	93.4±0.74	33.6±0.60
6	95.7±0.26	39.2±0.32
7	72.8±0.93	11.6±0.14**
8	13.9±0.38**	Non-Significant
9	85.6±0.48	12.2±0.11**
10	85.6±0.32	13.8±0.15**
11	11.8±0.26**	Non-significant
12	69.8±0.86	16.3±0.89**
BHA*	44.2 ± 0.24	
Thiourea*		22.4 ± 0.29

Note. * Standards, ** Potent

3.4. Conclusion

This study aimed to synthesize, characterize, and assess the biological properties of metal complexes with Schiff base ligands (SBLs). The characterization process involved utilizing the Cary 630 FTIR Spectrometer, which allowed to collect data and confirm the presence of functional groups in both SBLs and their corresponding complexes. Biological screening was carried out to evaluate the activity of synthesized compounds which showed that upon completion, the activity of these compounds changed from slightly moderate to significant. Some compounds showed potent activity. This change in activity was due to the change in the structure of SBLs and their complexes.

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CONFLICT OF INTEREST

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

DATA AVAILABILITY STATEMENT

Data availability is not applicable as no new data was created.

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