



Heterocyclic Schiff Base Complexes of Bivalent Transition Metals: Microwave-Assisted Green Synthesis, Structure Elucidation and Antimicrobial Studies

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Authors' contributions

This work was carried out in collaboration between both authors. Author Shalinee conceptualized and investigated the study did formal analysis. Authors Shalinee and SK supervised the study, wrote, reviewed and edited the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

A heterocyclic Schiff base ligand [LH] namely {5-[(E)-[(1H-pyrrol-2-yl)methylidene]amino]-1,3,4-thiadiazole-2-thiol}(NNN donor) and its divalent transition metals (Co²⁺, Ni²⁺, Cu²⁺ and Zn²⁺) mononuclear complexes were synthesised efficiently in relatively very short times (from one-two hours to 6-15 minutes) with higher yields [from 59% (conventional method) to 90% (green method)] by microwave-assisted greener process is presented here. The ligand and its complexes were characterised by elemental analysis, molar conductance, and magnetic susceptibility measurements and spectroscopic (IR, electronic, and NMR) analyses. All investigated complexes

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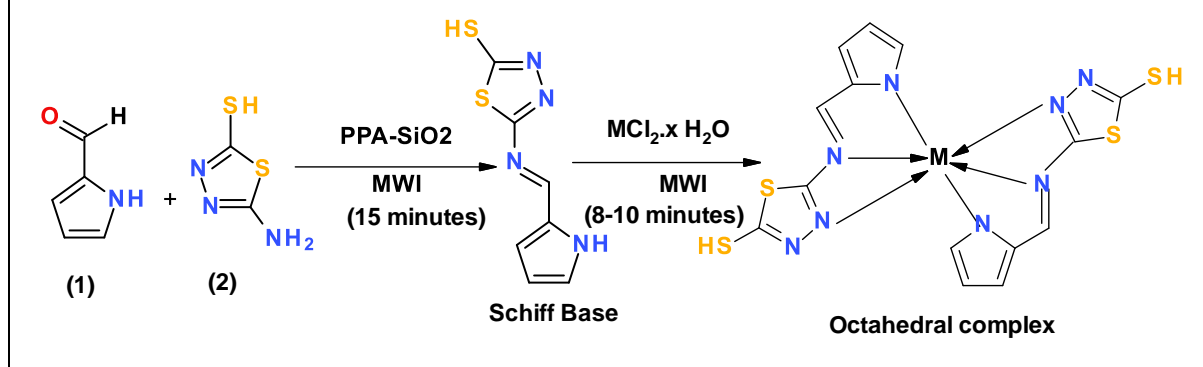
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were found to be six coordinated ML₂ [1:2 (metal: Ligand) ratio] type which possessed mononuclear octahedral geometry. The ligand and its metal complexes were screened for antimicrobial activity by the disc diffusion method, and the results indicate that the metal complexes are better antimicrobial agents compared to the Schiff base and the metal complexes have followed antimicrobial trends:

Cu(II) complex > Co(II) complex > Ni(II) complex > Zn(II) complex > Schiff base

Graphical Abstract



Keywords: Heterocyclic schiff base; transition metal complexes; microwave irradiation; antimicrobial activity; octahedral geometry.

1. INTRODUCTION

The conventional synthetic approaches have several disadvantages, such as long reaction periods, lower yields, the need for higher temperatures, the use of an additive to complete the reaction, the use of expensive or difficult-to-find starting materials, the use of toxic reagents or catalysts, and are polluting, non-facile, and inefficient. To overcome such disadvantages, synthetic researchers are now focusing on greener or sustainable processes for synthesising compounds like drugs or bioactive chemicals. A microwave-assisted synthetic approach uses less energy, accelerates the rate of reactions, takes less time than conventional heating, produces less chemical waste and ultimately reduces health hazards, meeting all the requirements of a greener or sustainable process [1-9].

Schiff base compounds (SBs) are imines or azomethine (-N=C) compounds which are formed by the catalytic condensation of primary amines and carbonyl compounds in acid or alkali. Because SBs have structural versatility and an easy synthetic approach, they have multifunctional and vital roles in industrial, pharmaceutical, biological, and chemical fields, as well as wide applications as guest-selective binders, sensitizers, catalysers, biological mimickers, developers, copolymers, molecular

engineers, and so on [10-14]. The versatility of SB ligands and the biological, analytical, synthetic, industrial, and material applications of their metal complexes with multifaceted activities make additional research in this area highly desirable. There has been a great deal of interest in the synthesis and applications of coordination compounds of Schiff base ligands because of their practical convenience, versatile operational flexibility, and very interesting structural highlights [15]. Schiff base-metal complexes have multifunctional applicability and play an important role in maintaining modern human life standards. Recently, common trends in educational, economic, environmental, and social fields towards the design, synthesis, characterisation, and multifunctionality of metal complexes of SBs have been enhanced beyond all doubt [16].

Several researchers have reported the usability of Schiff bases [17] and their transition metal complexes in versatile applications as promising research materials but there are very few reports in terms of heterocyclic Schiff bases derived from heterocyclic amine (5-amino-1,3,4-thiadiazole-2-thiol) and heterocyclic aldehyde (1H-pyrrole-2-carbaldehyde) giving rise to {5-[(E)-[(1H-pyrrol-2-yl)methylidene]amino]-1,3,4-thiadiazole-2-thiol}N donor (NNN donor sites) and their transition metal complexes under greener or sustainable synthetic process with

multifaceted properties, which is the rationale for this research article.

2. MATERIALS AND METHODS

The heterocyclic amine (5-amino-1,3,4-thiadiazole-2-thiol), heterocyclic aldehyde (1H-pyrrole-2-carbaldehyde) and other chemicals & solvents used in this research project were of Anal R grade and obtained from the local supplier. The progress of the synthetic reaction was monitored by performing TLC using Al sheets precoated with Merck 60 F₂₅₄ that was visualised using a UV lamp. A modified microwave oven model 2001 ETB (Bajaj Electricals Limited, Mumbai, India) with rotating tray and a power source 230V, microwave energy put out 800W and microwave frequency 2450 MHz was used for green synthesis of ligand as well as undertaken M(II) complexes. The Carlo Ebra-1106 elemental analyzer was used to analyse the C, H, and N content of the compound / complexes in the microanalytical laboratory. The metal contents were estimated using standard methods. Molar conductance (10⁻³ M) was measured by Elico-conductivity bridge at room temperature. The magnetic susceptibility measurements were carried out on a Gouy balance at room temperature using Hg[Co(SCN)₄] as the calibrant. Electronic spectra were recorded (in DMSO at 10⁻³ M) on a Shimadzu UV-Vis-160A spectrophotometer (λ = 200-1100 nm). Shimadzu 8400-S FT-IR spectrophotometer (λ = 4000-400 cm⁻¹) was used to record the infrared spectra using KBr pellets. The NMR Varian-Mercury 400MHz spectrometer was used for recording the ¹H NMR spectra in DMSO-d₆ using TMS as an internal standard. The chemical shift was measured in ppm on the δ scale and the coupling constants were measured in Hertz.

2.1 Preparation of Green Catalyst (PPA-SiO₂)

SiO₂ (5g 200-400 mesh) was added to the polyphosphoric acid (PPA) solution in chloroform at 320K and stirred for one hour until solid formation. To remove the unused chloroform, a rotary evaporator was used and the resulting solid was dried under vacuum at 298K [18].

2.2 Green Synthesis of Heterocyclic Schiff Base Ligand (LH)

An equimolar mixture of heterocyclic amine (1) and heterocyclic aldehyde (2) with PPA-SiO₂ was

grounded thoroughly using mortar and pestle. 5-6 mL of the triturated reaction mixture in aqueous ethanol was irradiated in the microwave oven for 15 minutes (360W) at 350K and the reaction was monitored by TLC (Scheme 1). The resulting reaction product was filtered and the yellow solid product recovered by recrystallisation with aqueous ethanol and identification was confirmed by IR, NMR spectra and the melting point [yield = 89% (conventional 65%), m p = 395K]. AQZ

2.3 Green Synthesis of Heterocyclic Schiff Base Transition Metal (II) Complexes

An aqueous-ethanolic solution of ligand (LH) and the respective transition metal (II) salt was thoroughly mixed in the stoichiometric ratio of 1:2 (metal: ligand) stoichiometric ratio and irradiated in the microwave oven adding 0.1% ethanolic KOH solution to adjust the pH within the range of 7-8 for a period of 8-10 minutes and produced a better yield (75-90 %) of the respective metal complexes (Scheme 1) compared to the conventional method (59-65%). The resultant coloured solid products were filtered and recrystallised from DMF, washed with ethyl acetate and then dried under reduced pressure over anhydrous CaCl₂ in a desiccator.

The synthetic reaction completion time and the yield of the products using a green synthetic approach and conventional method are presented in Table 1 for comparison.

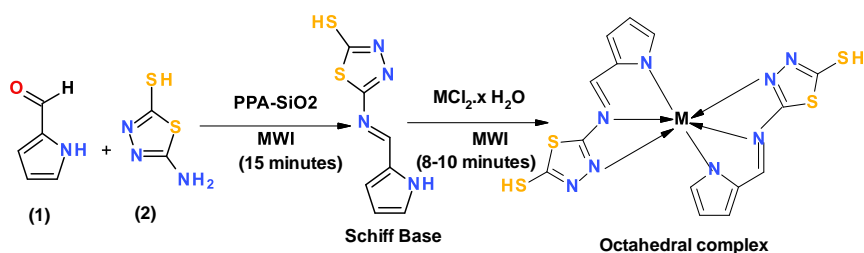
2.4 Antimicrobial Activity

"The *in vitro* antimicrobial (anti-bacterial and anti-fungal) activities of the investigated ligand and its transition metal (II) complexes were evaluated by disc-diffusion method" [19]. "The *in vitro* anti-bacterial activity of the undertaken compounds was achieved against two Gram-positive bacteria {*Staphylococcus aureus* (SA) & *Bacillus subtilis* (BS)} and two Gram-negative bacteria {*Escherichia coli* (EC) & *Salmonella typhi* (ST)} using chloramphenicol as standard reference. The anti-fungal activity of the undertaken compounds was performed against fungal strains {*Candida albicans* (CA) & *Aspergillus niger* (AN)} using griseofulvin as standard reference of the same concentration under identical conditions" [17].

Table 1. Microanalytical & physical data with the comparative results of conventional and green synthetic methods of the investigated compounds

Mol. Formula of Compound (colour)	Mol. Weight (in u)	Melting Point (in K)	Elemental analysis % calculated (Found)				Conductance (ohm ⁻¹ cm ² mol ⁻¹)	Reaction Time CM (GM)	% Yield CM (GM)
			C	H	N	M			
C ₇ H ₆ N ₄ S ₂ (Yellow)	210.3	490	39.94 (40.0)	2.85 (2.90)	26.63 (26.70)	--	--	2h (15m)	65 (89)
[Co(C ₇ H ₅ N ₄ S ₂) ₂] (Orange-red)	477.5	505	35.2 (35.0)	2.1 (2.0)	23.5 (23.4)	12.3 (12.4)	12.85	1h (10m)	59 (75)
[Ni(C ₇ H ₅ N ₄ S ₂) ₂] (Light green)	477.3	502	35.2 (35.3)	2.1 (2.0)	23.5 (23.6)	12.3 (12.4)	14.60	1h (9m)	60 (81)
[Cu(C ₇ H ₅ N ₄ S ₂) ₂] (Dark green)	482.1	533	34.8 (35.0)	2.1 (2.0)	23.2 (23.3)	13.2 (13.0)	10.45	1h (8m)	65 (90)
[Zn(C ₇ H ₅ N ₄ S ₂) ₂] (Off white)	484.0	515	34.7 (34.5)	2.1 (2.3)	23.1 (23.2)	13.5 (13.4)	7.54	1h (9m)	61 (85)

CM = Conventional method, time in hours; GM = Green method, time in minutes



Scheme 1. Microwave-assisted green synthesis of ligand and its metal (II) complexes

“The green synthesized ligand and its transition metal (II) complexes were dissolved separately in DMSO (without inhibitory activity) to appropriate concentration (100 µg/mL stock solution and 25 µg/mL after dilution). The sterile disks were drenched in investigated test compounds and were carefully placed on incubated agar surface separately. The petri-dishes were incubated for 24 hours at 643 K for bacteria and for 48 hours at 643K for fungal strain” [17]. Finally, the zone of inhibition was carefully measured separately for each compound tested. Each test was performed in triplicate in an individual experiment, and the mean is reported in Table 4.

3. RESULTS & DISCUSSION

It is observed that the microwave-assisted synthetic reactions were completed in shorter time with higher yields compared to the conventional thermal synthetic methods, which is probably due to the increased homogeneity of reaction mixture by rotation of the reaction platform tray.

The heterocyclic Schiff base (HL) was synthesised using a microwave-assisted green approach by condensation of heterocyclic aldehyde with thiadiazole amine in the presence of PPA-SiO₂ as a solid acid catalyst under clean and environmentally benign conditions. The condensation involves the nucleophilic attack of NH₂ group on the electrophilic H-C=O group followed by dehydration to form corresponding Schiff base (Scheme 2). Obviously, the reaction proceeds through the nucleophilic substitution mechanism. The catalyst used (PPA-SiO₂) was recycled by simple filtration and can be used repeatedly; therefore, it acts as a green catalyst and the present synthetic approach complies with the principle of sustainable chemistry or green chemistry.

3.1 Microanalytical & Physical Studies

“The investigated heteronuclear Schiff base and all the transition metal (II) complexes are stable and non-hygroscopic coloured solid at room

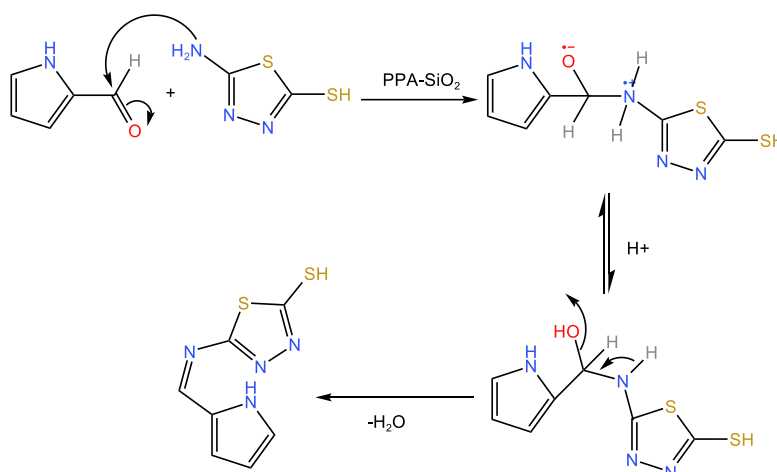
temperature. The complexes have sharp and characteristic melting points. The complexes are insoluble in common organic solvents but soluble in DMF and DMSO” [18]. The comparative results of conventional and green synthetic approaches, analytical data of the investigated compounds together with their physical properties are consistent with proposed molecular formula are presented in Table 1. “The spectroscopic and micro-analytical data of the studied complexes revealed that their composition corresponds to [ML₂] {1:2 (metal: ligand)} stoichiometry and mononuclear nature. The values of observed molar conductance of the metal (II) complexes (7.54 to 14.60 ohm⁻¹cm²mol⁻¹) were too low to account for their any dissociation in DMF at room temperature, which revealed their non-electrolytic nature” [20].

3.2 Infrared Spectral Studies

The infrared spectral data of the undertaken compounds {ligand & its metal(II) complexes} are listed in Table 2. Comparison of the IR spectra of the ligand and that of metal (II) complexes reveals the involvement of coordination sites in chelation. The IR spectral data of the metal (II) complexes contained all the major absorption bands of the ligand and some new bands indicative of the tridentate coordination of the ligand with the metal (II) ion in the complexes.

The IR spectrum of the undertaken Schiff base (HL) exhibited the azomethine (HC=N) and thiadiazole stretching at 1637 and 1618 cm⁻¹ respectively. The SH stretching appeared in the 2585-2590 cm⁻¹ region along with a band resulting from the NH stretching of pyrrole moiety at 3215 cm⁻¹.

“Comparison of the infrared spectra of the Schiff base ligand was performed as a reference and its metal (II) complexes showed that the ligand was principally tridentate coordinated with the central metal (II) ions. The absorption band appearing at 1637 cm⁻¹ due to azomethine stretching vibration is shifted to a lower frequency by 18 cm⁻¹, indicating the participation



Scheme 2. Proposed mechanism for acid-catalysed Schiff base synthesis

Table 2. Observed IR bands (cm^{-1}) of Schiff base & its metal (II) complexes

Compound	$\nu(\text{C}=\text{N})_{\text{azomethine}}$	$\nu(\text{C}=\text{N})_{\text{thiadiazole}}$	$\nu(\text{N}-\text{H})_{\text{pyrrolyl}}$	$\nu(\text{N}-\text{M})_{\text{azomethine}}$	$\nu(\text{N}-\text{M})_{\text{thiadiazole}}$	$\nu(\text{N}-\text{M})_{\text{pyrrolyl}}$
(HL)	1637	1618	3215	--	--	--
[Co(L ₂)]	1620	1605	--	525	525	540
[Ni(L ₂)]	1620	1605	--	525	520	535
[Cu(L ₂)]	1625	1610	--	520	525	540
[Zn(L ₂)]	1620	1610	--	525	530	535

of azomethine nitrogen in complexation" [21]. "The absorption band at 1618 cm^{-1} assigned to the thiadiazole ring ($\text{C}=\text{N}$) vibration is also shifted to lower frequency by 15 cm^{-1} , which is indicative of the involvement of the thiadiazole ring in complexation" [22]. Furthermore, "an absorption band at 3215 cm^{-1} attributed to NH stretching in the ligand disappeared in the respective metal (II) complexes, indicating deprotonation of the NH moiety during coordination and revealed the monoanionic nature of the ligand" [23]. Finally, conclusive evidence of coordination of the ligand with metal atoms was established by far IR spectra in which new absorption bands at $520\text{-}525$, $520\text{-}530$, and $535\text{-}540 \text{ cm}^{-1}$ assigned to M-N (azomethine), M-N (thiadiazole), and M-N (pyrrolyl) were observed in the spectra of the metal (II) complexes, which were not present in the ligand spectra.

Thus, infrared spectral studies revealed that the investigated Schiff base ligand acted as a monoanionic tridentate (designated as NNN donor) ligand.

3.3 ^1H NMR Spectral Studies

"The ^1H NMR spectrum of the investigated Schiff base displayed the SH proton as singlet at δ

12.97 ppm , the azomethine ($\text{HC}=\text{N}$) proton appeared as singlet at δ 9.05 ppm and the NH proton also appeared at δ 10.23 ppm as a broad singlet. $\text{C}_{10}\text{-H}$ and $\text{C}_{12}\text{-H}$ of the pyrrole moiety appeared as doublets at δ 6.59 ppm and δ 6.98 ppm respectively, thus showing almost no electronic effect of the thiol function. Similarly, $\text{C}_{11}\text{-H}$ appeared as a double doublet at δ 6.27 ppm , again showing almost no electronic effect of the thiol group" [24] (Fig. 1).

3.4 Electronic Spectral Studies & Magnetic Properties of Complexes

"Electronic or ultraviolet-visible (UV-vis) spectral studies along with the observed magnetic moment values of the metal (II) complexes are quite useful for the nature of the ligand field around the metal ion and thus can be used for the determination of their geometry or stereochemistry in association with other spectroscopic methods. The structure of the coordination complexes can be assigned based on the position and number of peaks of the $d\text{-}d$ transitions in their electronic spectra" [18]. The observed $d\text{-}d$ transitions and determined magnetic moment values of the investigated metal (II) complexes with their plausible geometry are presented here in Table 3.

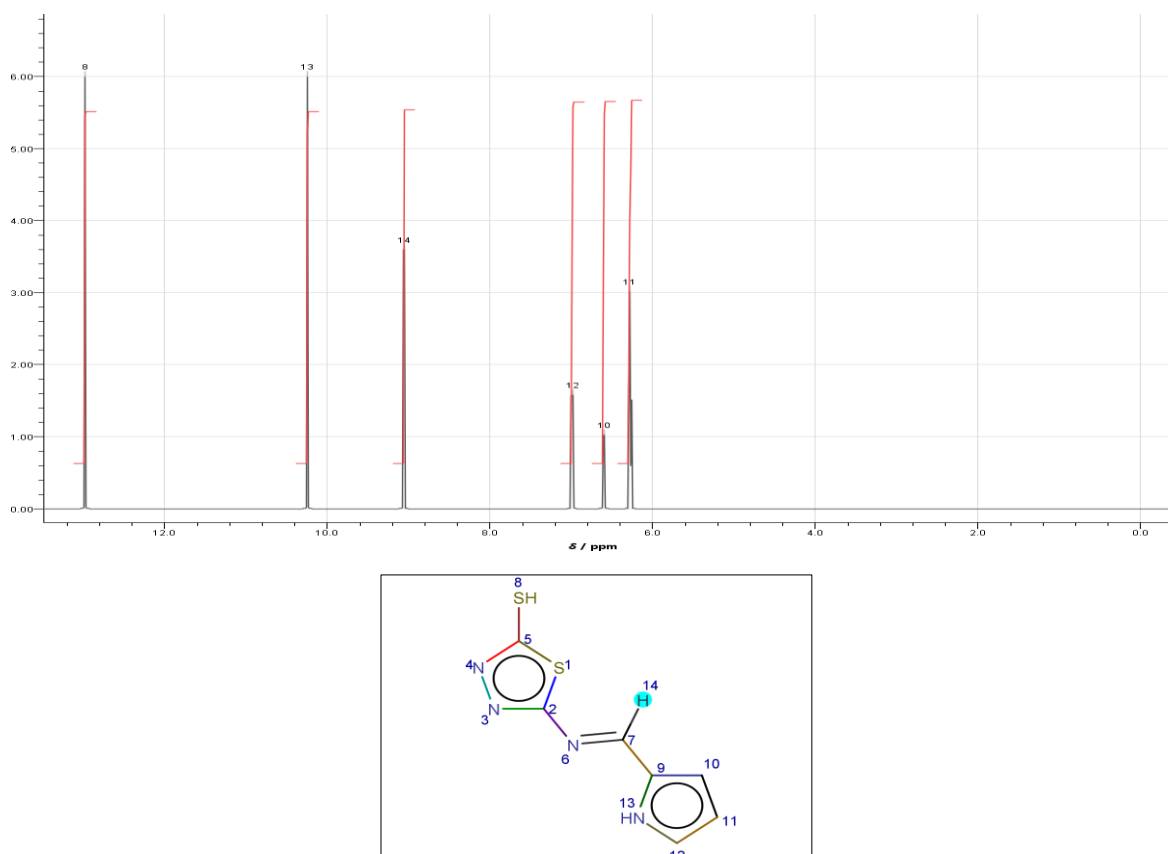
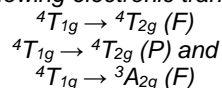


Fig. 1. ^1H NMR spectrum of the investigated Schiff base

Table 3. Observed electronic bands, magnetic moments, and geometry of M(II)-complexes

M-complexes	d-d transition bands (nm)	Charge Transfer Bands (cm^{-1})	μ_{eff} (BM)	Stereochemistry of M(II) Complexes
[Co(L ₂)]	1095, 550, 330	355-360	4.4	Octahedral
[Ni(L ₂)]	1010, 620, 350	353-355	3.4	Octahedral
[Cu(L ₂)]	326, 450	353-355	1.4	Distorted octahedral
[Zn(L ₂)]	--	350-355	0.0	Octahedral

The electronic spectrum of the investigated Co (II) complex consisted of three absorption bands corresponding to the following electronic transitions:

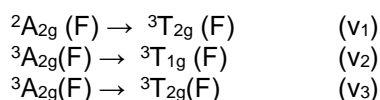


These revealed its octahedral geometry [25] around the central metal ion and is substantiated by the observed magnetic moment value (4.4 BM) corresponding to three unpaired electrons per Co^{2+} ion in an octahedral environment [26-27].

“The electronic spectrum of the investigated Cu(II) complex showed absorption bands between $10Dq$ band for octahedral geometry corresponding to the ${}^2E_g \rightarrow {}^2T_{2g}$ transition. The determined magnetic moment value of the Cu(II) complex was 1.4 BM, which is indicative of one unpaired electron per Cu^{2+} ion suggesting that

the investigated complex had structure within the range consistent with spin-free distorted octahedral geometry” [28]. “The EPR parameters from the X-band ESR spectrum of the Cu(II) complex at room temperature without hyperfine structure suggest that the 9th unpaired electron of Cu^{2+} ion is in the $d_{x^2-y^2}$ orbital (energy order: $d_{x^2-y^2} > d_{z^2}$) which is inconsistent with its distorted octahedral geometry” [27-29]. The EPR parameters of the Cu(II) complex are: $g_{\parallel} = 2.104$, $g_{\perp} = 2.012$, $g_{\text{av}} = 2.054$ G = 2.48

The three absorption bands observed correspond to the electronic transitions:



in the electronic spectrum of the Ni(II) complex showed its octahedral geometry. The determined magnetic moment value of the Ni (II) complex was 3.4 BM which corresponds to two unpaired electrons per Ni²⁺ ion for the ideal six-coordinated octahedral configuration [30].

The Zn (II) complex was found to be diamagnetic. The diamagnetic zinc complex did not show any *d-d* bands and its electronic spectrum was dominated only by the charge transfer bands at 350-355 nm. In general, six coordinated Zinc (II) complexes would have octahedral geometry.

3.5 Proposed Structure of the M(II)-Complexes

On the basis of the aforesaid experimental evidences, we tentatively propose the octahedral /distorted octahedral structure of the investigated M(II)-complexes (where M = Co, Ni, Zn /Cu) in which the heterocyclic Schiff base ligand acts as a monobasic tridentate (NNN Donor) ligand. Fig. 2 presents the common tentative octahedral geometry/structure of the complexes.

3.6 Antimicrobial Activities

The experimental results of the antimicrobial activity of the undertaken heterocyclic Schiff base ligand and its transition metal (II) complexes are given in Table 4.

“Bases on the experimental values of minimum inhibitory concentration (MIC) of the undertaken Schiff base ligand and its M (II) complexes, the moderate antimicrobial activity of the compounds is observed which is quite anticipating and up to expectation. The M(II) complexes have slightly higher antimicrobial activity than the free Schiff base ligand; although the investigated complexes displayed poor activity than the used references. Obviously, the antimicrobial activity enhancement is observed in ligand due to complexation or coordination with the metal. Such induction or enhancement in biological activity of the metal complexes can be explained by Overtones’ concept” [31] and chelation theory [32]. According to Overtone’s concept, the liposolubility controls the antimicrobial activity of the bioactive compounds. “The chelation theory states that the chelation increases the lipophilicity of the complex by reducing polarity of central metal ion and increasing the delocalization of π -electrons over the whole chelate ring. The increased lipophilicity enhances the penetration of the complexes into lipid membranes and blocking of metal binding sites on the enzymes of the microorganisms. Apart from this, solubility, conductivity, and dipole moment are influenced by the presence of metal ions may also be amongst the possible reasons causing enhancement of the antimicrobial activity of the metal complexes as compared to the uncomplexed of free Schiff base compounds” [33]. The observed trend of antimicrobial activity follows the order:

Cu(II) complex > Co(II) complex > Ni(II) complex > Zn(II) complex > Schiff base

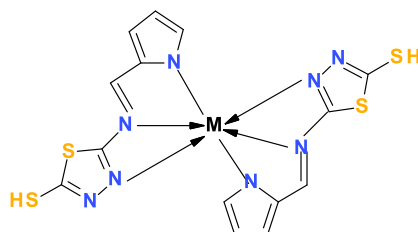


Fig. 2. Proposed octahedral structure of the investigated M(II) complexes

Table 4. *In vitro* Antimicrobial activity of Schiff base ligand and its M(II) complexes

Compounds	MIC ($\mu\text{g} / \text{mL}$)					
	Bacteria				Fungi	
	Gram-positive		Gram-negative			
	SA	BS	EC	ST	CA	AN
(HL)	13	11	13	10	18	22
[Co(L ₂)]	7	7	8	9	12	15

Compounds	MIC ($\mu\text{g} / \text{mL}$)					
	Bacteria				Fungi	
	Gram-positive		Gram-negative			
	SA	BS	EC	ST	CA	AN
[Ni(L ₂)]	9	10	10	9	15	18
[Cu(L ₂)]	6	6	9	8	11	12
[Zn(L ₂)]	11	10	12	10	17	21
Chloramphenicol	0.25	1.20	2.3	1.5	3.2	3.5
Griseofulvin	--	--	--	--	8	11

4. CONCLUSION

A highly efficient, facile, environmentally benign and economical method has been applied for the synthesis of mono-anionic tridentate heterocyclic Schiff base ligand (LH) by condensation of heterocyclic amine (5-amino-1,3,4-thiadiazole -2-thiol) and heterocyclic aldehyde (1H-pyrrole -2-carbaldehyde) under microwave irradiation in solvent-free condition in presence of PPA-SiO₂ as green catalyst. The results were compared with the conventional synthetic methods. The bivalent transition metal complexes of green synthesized ligand have been obtained in an environmentally safe microwave protocol. The infrared and ¹H NMR spectral studies and elemental analysis indicate the mono-anionic tridentate (NNN donor) nature of the Schiff base ligand. The non-electrolytic and mononuclear nature of the investigated complexes are determined by elemental analysis and molar conductance measurements. The octahedral geometry of the undertaken mononuclear M(II) complexes has been proposed on the basis of elemental analysis, molar conductance and magnetic moment measurements, IR, UV-visible and ¹H NMR spectral observations. The observed antimicrobial activity of the M(II) complexes clearly indicates that the complexes are found to be more active than the free and uncomplexed ligand towards the tested microbes owing to enhanced lipophilicity by chelation and follows the order:

Cu(II) complex > Co(II) complex > Ni(II) complex > Zn(II) complex > Schiff base

The present eco-friendly methodology offers very attractive features such as simple experimental work-up, higher yields and economic viability compared to other methodologies and it will have wide scope in synthetic chemistry.

In conclusion, this research article describes a simple, proficient, and green approach for the synthesis of heterocyclic Schiff base ligand and its mononuclear bivalent transition metal complexes bearing possible pharmacophore activity.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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CONFLICTS OF INTEREST

Authors declared no conflicts of interest or personal relationship that could have appeared to influence the research work reported in this paper.

DATA AVAILABILITY STATEMENT

The data that has been used is confidential.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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