Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
*J. Serb. Chem. Soc.* *79* (4) 421–433 (2014)
*JSCS–4596*
UDC 546.562'742'732'472:547.853+547.442+547.466:542.9+547.571+547.551
*Original scientific paper*

# Synthesis and characterization of bioactive binuclear transition metal complexes of a Schiff base ligand derived from 4-amino-1*H*-pyrimidin-2-one, diacetyl and glycine

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(Received 23 January, revised 27 November 2013)

*Abstract*: A series of novel binuclear transition metal complexes was synthesized by reaction of a Schiff base ligand 2-((1-methyl-2-((2-oxo-1,2-dihydro-pyrimidin-4-yl)imino)propylidene)amino)acetic acid) ($L^aH$) derived from 4-amino-1*H*-pyrimidin-2-one, diacetyl, glycine and the corresponding chloride salt of Cu(II), Ni(II), Co(II) and Zn(II) metals in a 1:1 (metal:ligand) mole ratio. The compounds were characterized by elemental analyses, molar conductance measurements, magnetic moment measurements and various spectral studies *viz.* IR, UV–Vis, $^1$H-NMR, $^{13}$C-NMR, EPR and ESI-MS. The molar conductance measurements revealed the non-electrolytic nature of the metal complexes. Electronic absorption spectral data, electronic paramagnetic resonance parameters and magnetic moment values revealed an octahedral geometry for the binuclear metal complexes. A cyclic voltammetric study of Ni(II) complex shows a couple of one-electron anodic responses near 0.70 and 1.10 V. The *in vitro* biological activity of the Schiff base ligand and the binuclear complexes was assessed against bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi*) and fungi (*Candida albicans* and *Candida parapsilosis*) to ascertain their antibacterial and antifungal properties.

*Keywords*: pyrimidine; diketone; amino acid; octahedral geometry; antimicrobial properties.

## INTRODUCTION

Considerable attention has been paid to pyrimidines and related *N*-heterocyclic derivatives as ligands for transition metal ions because these compounds show multifunctional coordinating ability and are present in many biological systems.[1–3] Amino heterocycles containing two or more potential donor centers play an important role in the study of the comparative reactivity of ambidentate ligand

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doi: 10.2298/JSC130123148S

systems.[4] Heterocyclic diazines, such as pyridazine and pyrimidine, are known to act as bidentate or tridentate ligand when coordinated to metal ions. Due to the multifunctional coordinating ability of pyrimidines and their derivatives, they have been used for the synthesis of mononuclear and binuclear transition metal complexes.[5–7] Transition metal complexes containing pyrimidine ligand are commonly found in biological media and play important role in processes such as catalysis of drug interaction with biomolecules. Recently, a number of transition metal complexes of pyrimidine derivatives were prepared that showed good biological activity *viz.* antibacterial and antifungal.[8–10] Complexes that contain glycine and its derivative as potent ligands were synthesized to assess their biological property.[11,12] Recently synthesized Cu(II), Ni(II), Co(II) and Zn(II) complexes show the current interest of researchers in the field of coordination chemistry of these metal ions.[13–15] Some other transition metal complexes were also synthesized for the study of their biological activities *viz.* antimicrobial, fluorescence quenching study in proteins, toxicity and DNA interaction.[16–22] In view of the importance of metal complexes, the preparation, characterization and *in vitro* antibacterial and antifungal properties of a new Schiff base ligand derived from 4-amino-1*H*-pyrimidin-2-one, diacetyl, glycine and its binuclear metal complexes are reported herein.

## EXPERIMENTAL

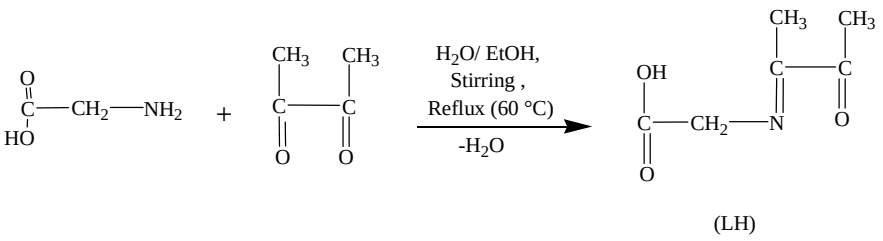
All chemicals and solvents were of analytical reagent grade and used as obtained without further purification. Methanol, ethanol, diethyl ether, DMF, DMSO and metal salts were purchased from Qualigens (Mumbai, India). Diacetyl was purchased from Sigma–Aldrich. 4-Amino-1*H*-pyrimidin-2-one and glycine were purchased from SD-Fine (Mumbai). Silica gel $F_{254}$ TLC plates (20 cm×20 cm) were purchased from Merck (India). Elemental analyses (C, H, N) were performed using a VarioEL Elementar Analysensystem. The metals and chlorides were determined volumetrically[23] and gravimetrically,[24] respectively. Melting points were recorded on an electro-thermal melting point apparatus and are uncorrected. The IR spectra were recorded as KBr discs using a Perkin-Elmer-621 spectrophotometer covering the frequency range 4000–200 $cm^{-1}$. The electronic absorption spectra in the 200–900 nm range were measured in DMF on a Systronic UV–visible spectrophotometer at room temperature. The $^{1}$H-NMR and $^{13}$C-NMR spectra were recorded at room temperature in DMSO-$d_6$ on a Bruker Avance II 400 NMR spectrometer. The chemical shifts ($\delta$) were measured down-field with reference to TMS (tetramethylsilane, 0.0 ppm). The ESI-mass spectra were obtained on an AB-Sciex Q-Star LCMS-MS spectrometer. The molar conductance measurements were determined in DMSO ($\approx 10^{-3}$ M) at room temperature using a Jenway model 4070 conductivity meter. The magnetic moment measurements were realized by the Gouy method at room temperature using $Hg[Co(SCN)_4]$ as the calibrant. The electrochemical behavior of the binuclear Ni(II) complex was studied (in acetonitrile solution) on a CHI620A electrochemical analyzer using a platinum electrode. Tetraethylammonium perchlorate (TEAP) was used as the supporting electrolyte and the potentials are referenced to a saturated calomel electrode (SCE) without junction correction. The cyclic voltammgram was recorded at a scan rate of 50 mV $s^{-1}$ with *iR* compensation. The EPR spectra of the Cu(II) and Co(II) complexes were recorded as

polycrystalline sample on a Varian E-112 spectrometer in the X-band region with a frequency of 9.1 GHz under a magnetic field strength of 3200 G using TCNE (tetracyanoethylene) as the field marker ($g$ = 2.0027).

*Synthesis of Schiff base ligand 2-((1-methyl-2-((2-oxo-1,2-dihydropyrimidin-4-yl)imino)propylidene)amino)acetic acid, $L^aH$*

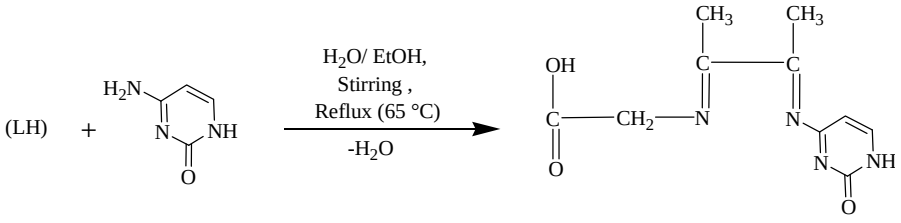
To an aqueous solution of glycine (10 mmol, 0.75 g), an ethanolic solution of diacetyl (10 mmol, 0.87 mL) was added dropwise under constant stirring. The resulting solution was stirred for 30 min and refluxed at 60 °C for 1 h. The precipitated cream-colored solid product 2-((1-methyl-2-oxopropylidene)amino)acetic acid (LH) was filtered off, washed with water, ethanol and diethyl ether and dried in vacuum desiccator over anhydrous calcium chloride. Then, an ethanolic solution of (LH) (10 mmol, 1.30 g) was stirred with an aqueous ethanolic solution of 4-amino-1*H*-pyrimidin-2-one (10 mmol, 1.40 g) for 45 min and refluxed at 65 °C for 3 h. Completion of reaction was monitored by thin layer chromatography (TLC). The reaction mixture was cooled in a refrigerator overnight. The obtained yellow solid Schiff base ligand ($L^aH$) was filtered off, washed with water, methanol, ethanol and diethyl ether and dried in a vacuum desiccator over anhydrous calcium chloride (Scheme 1).

**Step I**




(LH)

**Step II**




($L^aH$)

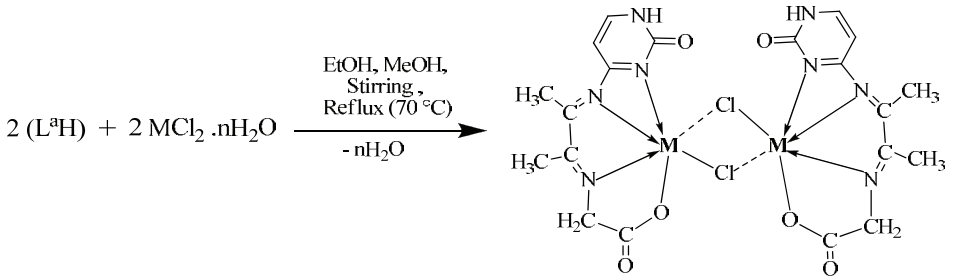
Scheme 1. Synthesis of Schiff base ligand ($L^aH$).

Yield: 74 %; color: yellow; m.p. 204 °C; Anal. Calcd. for $C_{10}H_{12}N_4O_3$ (FW: 236.22): C, 50.84; H, 5.12; N, 23.72 %. Found: C, 50.75; H, 5.06; N, 23.62 %.

*Synthesis of binuclear metal complexes **1**–**4***

To an ethanolic solution of the Schiff base ligand ($L^aH$) (2 mmol, 0.47 g), a methanolic solution of metal salt (2 mmol, $CuCl_2{\cdot}2H_2O$ (0.34 g), $NiCl_2{\cdot}6H_2O$ (0.47 g), $CoCl_2{\cdot}6H_2O$ (0.48 g) or $ZnCl_2$ (0.27 g)) was added dropwise under constant stirring. The resulting solutions were

stirred for 1.5 h and refluxed at 70 °C for ≈10–12 h. Completion of the reaction was monitored by thin layer chromatography (TLC). The reaction mixture was cooled in a refrigerator over night. The colored solid products (except for the Zn(II) complex) of the metal complexes were filtered off, washed with water, methanol, ethanol and diethyl ether, and dried in a vacuum desiccator over anhydrous calcium chloride (Scheme 2).




Scheme 2. Synthesis of the binuclear metal complexes $[M_2(L^a)_2Cl_2]$ (**1**–**4**) of the ligand ($L^aH$).

In vitro *antibacterial activity*

Antibacterial activity of synthesized ligand and binuclear complexes were screened *in vitro* against two Gram-positive (*Staphylococcus aureus* MTCC 1144 and *Bacillus subtilis* MTCC 2423) and two Gram-negative (*Escherichia coli* MTCC 739 and *Salmonella typhi* MTCC 733) bacteria using the agar well diffusion method.[25,26] Streptomycin was used as reference antibacterial drug. Bacterial strains stored in Mueller–Hinton broth (Merck) were sub-cultured for testing in the same medium and grown at 37 °C. Test compounds (ligand, metal complexes and streptomycin) were dissolved in DMSO at a concentration of 2 mg $mL^{-1}$. Stock solutions were prepared and dilutions were made according to the guidelines in the NCCLS approved standard document M7-A4 using the microdilution broth procedure.[27] Bacterial cells were suspended according to the 0.5 McFarland protocol in saline solution to produce a suspension of $10^4$–$10^6$ CFU $mL^{-1}$. Serial dilutions of the test compounds were prepared in test tubes to final concentrations of 1024, 512, 256, 128, 64, 32, 16, 8, 4 and 2 μg $mL^{-1}$. All strains were incubated at 37 °C for 24 h with different concentrations of compounds in Mueller–Hinton broth. Wells were created in medium with the help of a sterile cork borer of 8 mm diameter and the nutrient agar broth was prepared by dissolving beef extract (1.0 g), yeast extract (2.0 g), peptone (5.0 g), NaCl (5.0 g), agar (15.0 g) in one liter of distilled water.. The pH of the solutions was adjusted to 7.2 by the addition of the appropriate amount of sodium hydroxide. The resulting solution was autoclaved for 25 min at 15 psi and seeded with 100 μL of prepared inocula containing approximately $10^6$ CFU $mL^{-1}$. The Petri plates were prepared by pouring 70 mL of seeded nutrient agar. The antibacterial activity was determined by measuring the diameter of the inhibition zone (in mm). For quantitative measurement of growth inhibition, the calculation was performed according to a literature procedure.[26] Minimum inhibitary concentrations of each chemical compound were recorded as the lowest concentration of each chemical compound in the tubes with no growth (*i.e.* no turbidity) of the inoculated bacteria. Each assay was performed in duplicate and repeated three times.

In vitro *antifungal activity*

The antifungal activity of the synthesized ligand and all the binuclear complexes were tested *in vitro* against two fungi *Candida albicans* MTCC 227 and *C. parapsilosis* MTCC 2509 strains by using the poison food technique.[28] The fungal strains were grown on Sabouraud dextrose agar (SDA) at 25 °C for 7–8 days. One-week old cultures of the fungi were used as inoculums for determining the antifungal activity of test compounds. Fluconazole was used as a reference antifungal drug and the medium using DMSO as the solvent was used as a negative control. Solutions of the test compounds (ligand and metal complexes) and the reference drug were dissolved in DMSO at a concentration of 2 μg $mL^{-1}$. Molten SDA was poisoned by the addition of 100 μL of the prepared inocula and poured into sterile Petri plates. The prepared plates containing the test compounds were inoculated with fungal plugs (6 mm diameter) obtained from the activity growing margins of the fungal plates. The plates were incubated at 25 °C for one week. Each assay was performed in duplicate and repeated three times. Antifungal activity data of all compounds were expressed as percent inhibition calculated from the diameter of inhibition zone. The percent inhibition was determined using the formula:

$$\text{Inhibition} = 100(C - T)/C$$

where $C$ is the diameter of the fungal colony in the control plate and $T$ is the diameter of the microbial colony in the tested plate after the same incubation period.

## RESULTS AND DISCUSSION

The Schiff base ligand was synthesized in two steps. In first step, glycine was condensed with diacetyl in a 1:1 mole ratio to form a Schiff base 2-((1--methyl-2-oxopropylidene)amino)acetic acid (LH) and in second step, condensation of LH with 4-amino-1*H*-pyrimidin-2-one produced corresponding Schiff base ligand 2-((1-methyl-2-((2-oxo-1,2-dihydropyrimidin-4-yl)imino)propylidene)amino)acetic acid ($L^aH$). The newly synthesized Schiff base ligand ($L^aH$) was effectively employed for the isolation of model binuclear metal complexes **1**–**4**. The Schiff base ligand and binuclear metal complexes were quite stable at room temperature in the solid state. The Schiff base ligand was soluble in common organic solvents but the metal complexes were soluble in DMF and DMSO. Single crystal of the compounds, suitable for X-ray diffraction studies, could not be crystallized by various methods, such as crystallization using solvent mixtures, low temperature crystallization, but the analytical and spectral data, presented in the Supplementary material to this paper, were consistent with the proposed molecular formula and structure of the Schiff base ligand and metal complexes.

The positions of the molecular ion peaks in the mass spectra of the compounds were consistent with their empirical formulae and formula weight. The molar conductance values of the metal complexes, found in the range 3.4–8.3 $\Omega^{-1}$ $cm^2$ $mol^{-1}$ (in DMSO), were too low to account for any dissociation, hence the complexes were considered non-electrolyte in nature.[29] Based on the electronic

spectral data, EPR spectral data and magnetic moment values, octahedral geometry was assigned for all the binuclear metal complexes.

*IR spectra*

The most relevant IR absorption bands from the spectra of Schiff base ligand ($L^aH$) and binuclear metal complexes **1**–**4** are summarized in Table S-I of the Supplementary material to this paper.

In the IR spectrum of Schiff base ligand ($L^aH$), there are no bands of unreacted $–NH_2$ or ketonic groups. The absence of these bands and appearance of a new band at 1642 $cm^{-1}$, which may be assigned to azomethine group (ν(–C=N)) vibrations, indicates the condensation of amino groups of glycine and 4-amino-1*H*-pyrimidin-2-one with the carbonyl groups of diacetyl and the formation of the proposed Schiff base ligand.[30,31] The band in the IR spectrum of ligand at 1696 $cm^{-1}$ may be assigned to the (N–C=O) group of the pyrimidine ring.[32] The appearance of band at 1532 $cm^{-1}$ may arise from ν(C=N) of the pyrimidine ring. The band at 3074 $cm^{-1}$ may be due to the characteristic stretching vibration of the heterocyclic –NH group of the pyrimidine ring.[32] The Schiff base ligand ($L^aH$) showed two characteristic bands at 1748 and 1236 $cm^{-1}$, assigned to the asymmetric and symmetric vibrations of the (–COOH) group, respectively.[33] The IR spectra of metal complexes **1**–**4** show significant changes compared to the free ligand ($L^aH$).

The IR spectra of the binuclear complexes exhibit characteristic bands in the range of 3072–3078 $cm^{-1}$ due to the stretching vibration of the (–NH) groups of the pyrimidine ring. The band in the range 1688–1694 $cm^{-1}$ may be assigned to the non-coordinated (N–C=O) group of the pyrimidine ring.[10,32] In the IR spectrum of metal complexes **1**–**4**, the absence of the bands at 1748 and 1236 $cm^{-1}$ revealed that the (–COOH) group of the Schiff base ligand was deprotonated on complexation.[34] Instead of these bands, two new bands in the ranges 1604–1565 $cm^{-1}$ and 1396–1348 $cm^{-1}$ appeared that may be assigned to asymmetric and symmetric vibrations of the ($–COO^–$) group, respectively. The pragmatic $\Delta\nu$ ($\nu_{as} - \nu_s$) values for the synthesized complexes were in the range of 206–232 $cm^{-1}$, which, being larger than 200 $cm^{-1}$, indicates the unidentate fashion of coordination of carboxylato group with the central metal ion.[35] Coordination through the oxygen atom of the deprotonated carboxyl group is further supported by the appearance of a new band in the range of 508–524 $cm^{-1}$, which may be assigned to ν(M–O) vibrations.[36,37] The band observed at 1642 $cm^{-1}$ in the spectrum of the free ligand was shifted to 1580–1592 $cm^{-1}$ in the spectra of the complexes, indicating coordination of the azomethine group (–C=N).[38] The band at 1532 $cm^{-1}$ of the ν(C=N) of the pyrimidine ring in free ligand was shifted to 1490––1506 $cm^{-1}$, indicating the participation of (C=N) group of the pyrimidine ring in the coordination.[39] Coordination through nitrogen atom of azomethine group and

the (C=N) group of the pyrimidine ring is further supported by the presence of a new band in the range of 462–495 $cm^{-1}$, which is assignable to ν(M–N) vibrations.[37,40] The bands observed in the ranges 345–338 $cm^{-1}$ and 305–290 $cm^{-1}$ may be assigned to ν(M–Cl) and bridged metal chloride (ν(M–Cl–M)) vibrations, respectively.[41,42]

*$^{1}$H-NMR and $^{13}$C-NMR spectra*

The $^{1}$H-NMR and $^{13}$C-NMR spectra of Schiff base ligand ($L^aH$) and $Zn_2[(L^a)_2Cl_2]$ complex (**4**) were recorded in DMSO-$d_6$ and the resulting data are given in Table S-II of the Supplementary material to this paper.

The $^{1}$H-NMR spectrum of ligand ($L^aH$) does not display a signal corresponding to a primary amine proton, which suggests the condensation of amino group of glycine and 4-amino-1*H*-pyrimidin-2-one with the carbonyl group of diacetyl. A sharp singlet at 11.36 ppm and broad singlet at 11.10 ppm indicate the presence of characteristics (–OH) proton of carboxyl group and (–NH) proton of pyrimidine ring in synthesized ligand ($L^aH$), respectively. In the $^{1}$H-NMR spectrum of the $Zn_2[(L^a)_2Cl_2]$ complex, the signals of the ($CH_3$–C=N) protons, and the (–NH) and (–CH) protons of the pyrimidine ring were shifted compared to the corresponding protons in the spectra of the free ligand, suggesting coordination through the nitrogen atoms of the azomethine group and the (C=N) group of the pyrimidine ring. The absence of the signals of the (OH) protons indicates deprotonation of the carboxyl group present in the Schiff base.[43]

In the $^{13}$C-NMR spectrum of the Zn(II) complex, the changes in the chemical shift values compared to those of the free ligand ($L^aH$) show coordination through nitrogen atom of the azomethine group and the (C=N) group of the pyrimidine ring and the oxygen atom of the carboxyl group present in the Schiff base. On the other hand, no change in the chemical shift value of the carbon of the carbonyl group of the pyrimidine ring indicates that the oxygen atom of this group did not participate in the coordination.

Thus, the $^{1}$H-NMR and $^{13}$C-NMR spectral data support the proposed structure of ligand and its Zn(II) complex, as well as the coordination behavior of ligand.[43]

*Mass spectra*

The formation of Schiff base ligand ($L^aH$) and metal complexes **1**–**4** were studied through their ESI-MS spectra. The proposed molecular formula of compounds was confirmed by comparing their molecular formula weight with the *m*/*z* values. In the mass spectra of the compounds, peaks were attributed to the molecular ions, *m/z*: 236.12 $[M]^+$ for the Schiff base compound ($L^aH$); 742.30 $[M+1]^+$ for complex **1**; 732.62 $[M+1]^+$ for complex **2**, 734.11 $[M+2]^+$ for complex **3** and 839.32 $[M]^+$ for complex **4**. These data are in good agreement with the proposed

molecular formula of the Schiff base compound ($L^aH$) and its binuclear metal complexes **1**–**4**. In addition to the peaks due to the molecular ion, the spectra exhibited peaks assignable to various fragments arising from the cleavage of the compounds in interaction with the accelerated electrons.

*Electronic absorption spectra and magnetic moment measurements*

The electronic absorption spectra of the binuclear metal complexes **1**–**4** were measured in DMF and the data along with their magnetic moment values are summarized in Table S-III of the Supplementary material to this paper.

The electronic absorption spectrum of $Cu_2[(L^a)_2Cl_2]$ (**1**) exhibits a band at 762 nm that was assigned to the $^2E_g \rightarrow {}^2T_{2g}$ transition, suggesting octahedral geometry around the Cu(II) ion.[44] The obtained $\mu_{eff}$ value for Cu(II) complex is 1.62 $\mu_B$, indicating that magnetic exchange occurs between the two copper sites. The electronic absorption spectrum of the $Ni_2[(L^a)_2Cl_2]$ complex (**2**) shows a sharp peak at 242 nm which was assigned to an intra-ligand transition, while the broad peaks around 376 and 552 nm, assigned to $^3A_{2(g)} \rightarrow {}^3T_{1g}$ and $^3A_{2(g)} \rightarrow$ $\rightarrow {}^3T_{2g}$ transitions, respectively, showed d–d transition of Ni(II), consistent with an octahedral geometry of the complex.[44,45] The magnetic moment value of Ni(II) complex was 2.82 $\mu_B$, which indicates the presence of two unpaired electrons per Ni(II) ion, that also confirmed the octahedral geometry of the binuclear Ni(II) complex. The electronic absorption spectral data of the $Co_2[(L^a)_2Cl_2]$ complex (**3**) show absorptions at 590 and 462 nm, which may be assigned to the transitions $^4T_{1g} \rightarrow {}^4A_{2g}$ and $^4T_{1g} \rightarrow {}^4T_{1g}$, respectively. These transitions revealed the octahedral geometry of Co(II) complex.[44] Furthermore, the octahedral geometry for the Co(II) complex was also proved by its magnetic moment value at room temperature of 4.56 $\mu_B$ per Co atom. The electronic absorption spectrum of $Zn_2[(L^a)_2Cl_2]$ (**4**) shows two bands at 328 and 356 nm which may be assigned to charge transfer transitions from ligand to the Zn(II) ion (LMCT). The Zn(II) complex is diamagnetic.[44]

*EPR spectra*

The X-band EPR spectra of the Cu(II) and Co(II) complexes were recorded and the data are given in Table S-III. The X-band EPR spectrum of $Cu_2[(L^a)_2Cl_2]$ complex (**1**) was recorded at a frequency of 9.1 GHz under a magnetic field strength of 3200 G at room temperature (298 K) while the spectrum of $Co_2[(L^a)_2Cl_2]$ complex (**3**) was recorded at liquid nitrogen temperature (77 K) as a polycrystalline sample. Their $g_{||}$ and $g_{\perp}$ were determined from EPR spectra and $g_{av}$ values were calculated from the formula:

$$g_{av}^2 = \frac{\left(g_{||}^2 + 2g_{\perp}^2\right)}{3}$$

Analysis of the EPR spectrum of the Cu(II) complex gives $g_{||}$ 2.124, $g_{\perp}$ 2.056 and $g_{av}$ 2.080. The trend $g_{||} > g_{\perp} > 2.002$ observed for the complex under study indicates that the unpaired electron is localized in the $d_{x2-y2}$ orbital of the Cu(II) ion.[46] Analysis of EPR spectrum of the Co(II) complex gives $g_{||}$ 2.314, $g_{\perp}$ 2.014 and $g_{av}$ 2.112. The trend $g_{||} > g_{\perp} > 2.002$ observed for the Co(II) complex under study was due to a large angular momentum contribution. Thus, the EPR values also support octahedral geometry for the Cu(II) and Co(II) complexes.[47]

*Cyclic voltammetric study of the $[Ni_2(L^a)_2Cl_2]$ complex*

The electrochemical behavior of the $Ni_2[(L^a)_2Cl_2]$ complex (**2**) was studied by cyclic voltammetry in acetonitrile solution at a platinum electrode *versus* SCE. The complex exhibits two one electron anodic responses near 0.70 and 1.10 V. The anodic responses were assigned to the Ni(II)–Ni(II) to Ni(II)–Ni(III) and Ni(II)–Ni(III) to Ni(III)–Ni(III) transitions. This result is consistent with other reported results for binuclear Ni(II) complexes.[48]

In vitro *antibacterial activity*

The newly synthesized Schiff base ligand ($L^aH$), its binuclear metal complexes **1–4** and the standard drug streptomycin were screened *in vitro* separately to assess their antibacterial activity against two Gram-positive bacteria (*S. aureus* and *B. subtilis*) and two Gram-negative bacteria (*E. coli* and *S. typhi*). The synthesized compounds show greater toxicity towards the Gram-positive strains than towards the Gram-negative strains. The reason is the difference in the complexity of the structure of the cell walls of Gram-positive and Gram-negative bacteria. The antibacterial screening concentrations of the compounds were estimated from the minimum inhibitory concentration (*MIC*) value, which were in the range 4–64 μg/mL$^{-1}$. The *MIC* values presented in Table I clearly indicate that the $Co_2[(L^a)_2Cl_2]$ complex (**3**) was the most potent antibacterial compound with *MIC* values 16, 16, 32 and 32 μg mL$^{-1}$ and the $Cu_2[(L^a)_2Cl_2]$ complex (**1**) was the least potent antibacterial compound with *MIC* values 32, 32, 64 and 64 μg

TABLE I. Minimum inhibition concentration (*MIC* / μg mL$^{-1}$) values for the Schiff base ligand ($L^aH$), its binuclear metal complexes **1**–**4** and the standard drug; metal complexes: **1** = $Cu_2[(L^a)_2Cl_2]$, **2** = $Ni_2[(L^a)_2Cl_2]$, **3** = $Co_2[(L^a)_2Cl_2]$, **4** = $Zn_2[(L^a)_2Cl_2]$; streptomycin = standard drug

| Microorganism | Ligand ($L^aH$) | Metal complex | | | | Streptomycin |
|---|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** | |
| Gram-positive | | | | | | |
| *S. aureus* | 64 | 32 | 32 | 16 | 32 | 4 |
| *B. subtilis* | 64 | 32 | 16 | 16 | 16 | 4 |
| Gram-negative | | | | | | |
| *E. coli* | 128 | 64 | 32 | 32 | 32 | 8 |
| *S. typhi* | 128 | 64 | 64 | 32 | 64 | 8 |

$mL^{-1}$ as compared to the other studied complexes against *S. aureus*, *B. subtilis*, *E. coli* and *S. typhi*, respectively.

In vitro *antifungal activity*

The antifungal activites of synthesized Schiff base ligand ($L^aH$) and its binuclear metal complexes **1**–**4** were determined *in vitro* against two fungi *C. albicans* and *C. parapsilosis* and compared with the standard antifungal drug fluconazole at the same concentration. The antifungal activity data are summarized in Table II. Among all the synthesized compounds, the $Zn_2[(L^a)_2Cl_2]$ complex (**4**) was the most active against the studied fungi and showed a higher activity against *C. parapsilosis*. The activity was greatly enhanced at the higher concentration. The DMSO control showed a negligible activity as compared to the synthesized compounds. All the metal complexes exhibited good antifungal activity against *C. albicans* and *C. parapsilosis* as compared to the activity of the standard drug fluconazole. The antifungal activity data showed that the activity of complexes depended on the type of metal ion present in complex and thus, it was observed that the $Zn_2[(La)_2Cl_2]$ complex was the most active, the $Ni_2[(L^a)_2Cl_2]$ complex was the least active, while the $Cu_2[(L^a)_2Cl_2]$ and $Co_2[(L^a)_2Cl_2]$ complexes exhibited good activity against the studied fungi.

TABLE II. *In vitro* antifungal screening data of the Schiff base ligand ($L^aH$), its binuclear metal complexes **1**–**4** and the standard drug`

| Compound | Mycelial growth inhibition, % | |
|---|---|---|
| | *C. ablicans* | *C. parapsilosis* |
| ($L^aH$) | 35.3 | 32.6 |
| $[Cu_2(L^a)_2Cl_2]$ (**1**) | 55.4 | 57.4 |
| $[Ni_2(L^a)_2Cl_2]$ (**2**) | 45.3 | 48.6 |
| $[Co_2(L^a)_2Cl_2]$ (**3**) | 58.2 | 61.5 |
| $[Zn_2(L^a)_2Cl_2]$ (**4**) | 65.8 | 69.6 |
| Fluconazole (standard drug) | 79.2 | 85.8 |

In the present study, the low activity of some of the metal complexes may be due to their low lipophilicity because of which penetration of the complex through the lipid membrane was decreased and hence, they could neither block nor inhibit the growth of the microorganism. The variation in the antimicrobial activity of different metal complexes against different microorganisms depends on their permeability into the cell or differences in ribosomes in the microbial cell.[49] The lipid membrane surrounding the cell favors the passage of any lipid soluble material and it is known that liposolubility is an important factor controlling antimicrobial activity.[50]

## CONCLUSIONS

The newly synthesized Schiff base ligand and its binuclear metal complexes were characterized by various physicochemical techniques. The data obtained from various studies are in good agreement with proposed structure of the Schiff base ligand and its metal complexes. Octahedral geometry of the complexes was proved by their electronic absorption spectra, EPR spectra and magnetic moment values. The molar conductance values show the non-electrolyte nature of all the metal complexes. *In vitro* antibacterial and antifungal studies showed that the Schiff base ligand and its binuclear metal complexes were biologically active. The $Co_2[(L^a)_2Cl_2]$ complex showed the best activity against the studied bacteria and the $Zn_2[(L^a)_2Cl_2]$ complex showed the best activity against the studied fungi.

## SUPPLEMENTARY MATERIAL

Physical and analytical data for complexes **1**–**4** are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

*Acknowledgements.* The authors are thankful to ACBR, Delhi for providing the spectral data, IIT Roorkee for providing the elemental analyses data, and SAIF, Punjab University, Chandigarh for providing the $^1$H-NMR and $^{13}$C-NMR spectral data. The authors are also thankful to the authorities of Meerut College, Meerut for providing the necessary research facilities.

## И З В О Д

## СИНТЕЗА И КАРАКТЕРИЗАЦИЈА БИОЛОШКИ АКТИВНИХ БИНУКЛЕАРНИХ КОМПЛЕКСА ПРЕЛАЗНИХ МЕТАЛА СА ШИФОВОМ БАЗОМ КАО ЛИГАНДОМ КОЈИ ЈЕ ДОБИЈЕН У РЕАКЦИЈИ ИЗМЕЂУ 4-АМИНО-1*H*-ПИРИМИДИН-2-ОНА, ДИАЦЕТИЛА И ГЛИЦИНА

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Синтетизована је серија нових бинуклеарних комплекса прелазних метала са Шифовом базом као лигандом 2-((1-метил-2-((2-оксо-1,2-дихидропиримидин-4-ил)имино)-пропилиден)амино)сирћетне киселина, $L^aH$, који је добијен у реакцији 4-амино-1*H*-пиримидин-2-она, диацетила и глицина. Све реакције између Шифове базе и одговарајућег хлорида прелазног метала (Cu(II), Ni(II), Co(II) или Zn(II)) су извођене у 1:1 молском односу. Комплекси су окарактерисани елементалном микроанализом, мерењем моларне проводљивости и магнетног момента, као и различитим спектроскопским методама (IR, UV–Vis, $^1$H-NMR, $^{13}$C-NMR, EPR и ESI-MS). Мерење моларне проводљивости је показало да су сви синтетисани комплекси електронеутрални. На основу спектроскопских података и вредности магнетног момента закључено је да испитивани бинуклеарни комплекси прелазних метала имају октаедарску геометрију. У цикличном волтамограму Ni(II) комплекса јављају се два анодна пика на приближно 0,70 и 1,10 V. Извршена су *in vitro* испитивања антибактеријске и антифунгалне активности лиганда Шифове базе и одговарајућих бинуклеарних комплекса на различитим сојевима бактерија (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* и *Salmonella typhi*) и гљива (*Candida albicans* и *Candida parapsilosis*).

(Примљено 23. јануара, ревидирано 27 новембра 2013)

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