

## Synthesis, Characterization, Antimicrobial and Cytotoxic Evaluation of a Bidentate Schiff Base Ligand: *{5-Chloro-2-[(4-nitrobenzylidene)amino]phenyl}(phenyl)methanone* and its Transition Metal (II) Complexes

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**mmary:** A Schiff base ligand (SBL): *{5-chloro-2-[(4-nitrobenzylidene) amino] phenyl}(phenyl)methanone*, was synthesized from the reaction of 4-nitrobenzaldehyde and 2-amino-5-chlorobenzophenone followed by complexation with transition metal (II) ions (**1-5**). Their structures were elucidated on the basis of infrared, <sup>1</sup>H-NMR, FAB-MS spectral, elemental analyses and molar conductance data. The octahedral geometry for complexes (**1-4**) and square planar geometry for complex (**5**) was proposed on the basis of electronic and magnetic moment data. The non-electrolytic nature of the complexes (**1-5**) was suggested from the conductivity data. The complexes (**1-5**) showed higher *in vitro* antimicrobial activity and *in vivo* lethality to shrimp larvae than the parent Schiff base ligand.

Keywords: Schiff base; Transition metal; Antimicrobial activity; Brine shrimp bioassay.

### Introduction

Schiff bases are the imperative chelating ligands in metal co-ordination chemistry [1]. These are derived from the condensation of amines and ketones or aldehydes and form stable complexes with most of the metals in acidic or basic medium [2]. The biological activity of the Schiff base ligand increases after the complexation with transition metals and also the cytotoxic effects of both the metal and the ligand decrease on host [3]. The azomethine linkage can be developed from the reactions of Schiff base which is useful for designing the mechanism of transamination and racemization reactions in living systems [4]. The different biological activities are reported for azomethine moiety such as herbicidal [5], antimicrobial [6], anti-inflammatory, anti-tumor, anti-tuberculosis, anti-convulsant, and anti-HIV activities [7]. Keeping the above facts in mind, we report the preparation and characterization of Schiff base ligand, *{5-chloro-2-[(4-nitrobenzylidene)amino]phenyl}(phenyl)methanone*, derived from the condensation of 4-nitrobenzaldehyde with 2-amino-5-chlorobenzophenone, followed by complexation with Pb (II), Ni (II), Co (II), Cu (II) and Cd (II)

metal ions and also their biological activities such as antimicrobial and cytotoxic were investigated.

### Results and Discussion

The Schiff base ligand: *{5-chloro-2-[(4-nitrobenzylidene)amino]phenyl}(phenyl)methanone* (SBL) was obtained as a yellow transparent crystalline solid, soluble in water and common organic solvents except ether. The complexes (**1-5**) of the ligand (SBL) were obtained as crystalline solids and are fairly stable compounds as indicated by their decomposition temperatures. All the complexes (**1-5**) are soluble in dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO) but insoluble in water and other common organic solvents.

#### Infrared Spectra

The IR spectrum of free Schiff base ligand was compared with the ligand-metal complexes spectra to understand the binding mode of the ligand with metals. The disappearance of characteristic peaks of carbonyl group,  $\nu(\text{C}=\text{O})$  for 4-nitrobenzaldehyde and absence of absorption frequency of amino group  $\nu(-\text{NH}_2)$  for 2-amino-5-

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chlorobenzophenone suggested the completion of condensation reaction [8]. The strong band of azomethine group  $\nu(\text{C}=\text{N})$  of ligand was observed at  $1657\text{ cm}^{-1}$  [9]. The shifting of this absorption frequency towards lower frequency region at about  $1514\text{-}1610\text{ cm}^{-1}$  indicated that nitrogen atom of azomethine group ( $\text{C}=\text{N}$ ), in IR spectrum of complexes, is involved in coordination with the metal ion [10]. The keto group of benzophenone appeared as a characteristic band at  $1765\text{ cm}^{-1}$  [11]. The probable coordination of carbonyl oxygen with metal was supported by shifting of carbonyl band towards lower frequency region *i.e.*  $1691\text{-}1670\text{ cm}^{-1}$ , in the IR spectra of complexes. The same is further supported by the appearance of peaks at  $403\text{-}463\text{ cm}^{-1}$  and  $531\text{-}588\text{ cm}^{-1}$  due to the presence of M-O and M-N bonds respectively [12]. Out of plane bending vibration  $\nu(\text{C-H})$  of aromatic ring was observed at  $729\text{-}816\text{ cm}^{-1}$ . Similarly  $\nu(\text{C}=\text{C})$  stretching was observed at  $1403\text{-}1476\text{ cm}^{-1}$  and  $919\text{-}1097\text{ cm}^{-1}$  [13]. The symmetric stretching vibrations of  $\nu(\text{COO}^-)$  for acetate complexes (**1-2**) were observed at  $1276$  and  $1236\text{ cm}^{-1}$  respectively. However, the bands for asymmetric stretching of carboxyl group in complex (**1-2**) were observed at  $1631$  and  $1573\text{ cm}^{-1}$  which indicated the coordination of carboxylic group with metal ion in a unidentate fashion. The appearance of bands at  $3545$  and  $3439\text{ cm}^{-1}$  for complexes (**3-4**) respectively, indicated that water molecules coordinated with them [14]. The stretching of  $\nu(\text{C-Cl})$  and  $\nu(\text{NO}_2)$  were observed at  $616\text{-}681\text{ cm}^{-1}$  and  $1323\text{-}1370\text{ cm}^{-1}$  respectively. The absorption band at  $2834\text{-}3104\text{ cm}^{-1}$  was assigned to  $\nu(\text{C-H})$  stretching vibration of azomethine group [15].

#### <sup>1</sup>H-NMR Spectra

The <sup>1</sup>H-NMR spectrum of ligand (SBL) showed a singlet at  $8.75\text{ ppm}$  due to C-H of azomethine group, which confirmed the formation of azomethine linkage in SBL and the signals at  $8.11\text{-}7.48\text{ ppm}$  were due to aromatic protons

#### FAB Mass Spectra

The stoichiometric composition of free SBL and its complexes (**1-5**) were compared with the aid

of their FAB (+ve) spectra. The molecular ion peak  $[\text{M}+\text{H}]^+$  at  $m/z\ 365.3$  appeared for the free SBL which coincides with the theoretical molecular weight *i.e.*  $364.0$  and appearance of this peak confirmed that the metal-ligand are present in the ratio 1:2. These values were found to show good agreement with those of elemental analysis.

#### Electronic Spectra and Magnetic Moments

The stereochemistry of metal ion in complexes (**1-5**) was assigned by making use of electronic spectra and considering charge transfer and number of d-d transitions. The octahedral geometry for compounds (**1-4**) and square planar for complex (**5**) was suggested by the electronic spectral and magnetic moment values [16] (Table-1). It was further supported by the low values of molar conductance which in turn describes then non-electrolytic nature of complexes (**1-5**) [17].

#### Molar Conductance

The molar conductance ( $\lambda_m$ ) of  $0.001\text{ M}$  solutions of all the complexes (**1-5**) was measured in DMSO at room temperature. The non-electrolytic nature of all these complexes (**1-5**) was suggested by observed molar conductance values [18] of these complexes (**1-5**) which were in the range of  $8.3\text{-}21.1\text{ ohm}^{-1}\text{ cm}^{-1}\text{ mol}^{-1}$ .

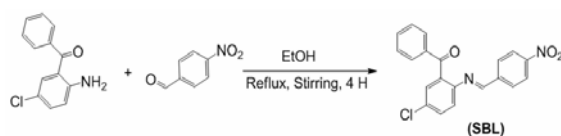
#### Single Crystal XRD

The (SBL) was characterized by spectral data and X-Ray crystallography [19] as shown in Scheme-I.

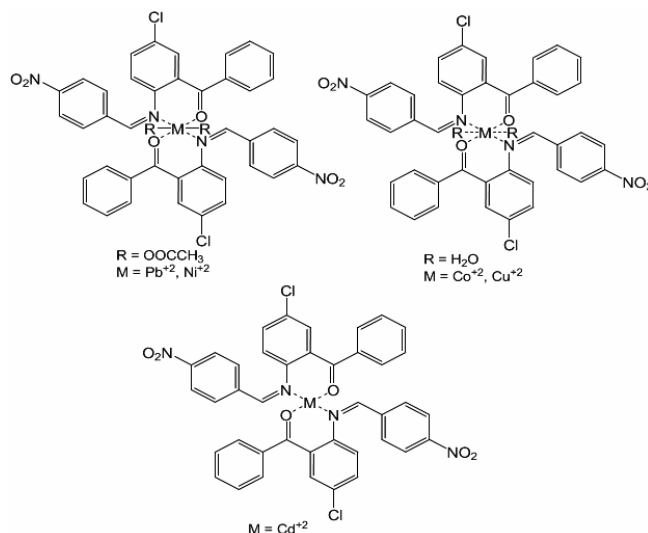
The spectral data and x-ray crystallography was employed to characterize the Schiff base ligand (Scheme-I). The linkage of Schiff base to metal ion through nitrogen atom of azomethine group and oxygen atom of carbonyl group was supported by analytical and spectral data. In this regard, the proposed structures for complexes (**1-5**) are shown in Scheme-II.

Table-1: Electronic and magnetic moment data of Schiff base ligand complexes (**1-5**).

Compound	Electronic spectral Bands ( $\text{cm}^{-1}$ )	Magnetic moment(B.M.)	Assignment	Geometry
1	9450, 14450, 190500	5.03	${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{2g}(\text{F}), {}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{A}_{2g}(\text{F}), {}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{1g}(\text{P})$	Octahedral
2	12100, 18500, 25300	3.17	${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{2g}(\text{F}), {}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{1g}(\text{F}), {}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{1g}(\text{P})$	Octahedral
3	9950, 17500, 21500	5.13	${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{2g}(\text{F}), {}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{A}_{2g}(\text{F}), {}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{1g}(\text{P})$	Octahedral
4	11400, 18400	1.87	${}^2\text{B}_{1g} \rightarrow {}^2\text{B}_{2g}, {}^2\text{B}_{1g} \rightarrow {}^2\text{E}_{1g}$	Octahedral
5	21350, 28500, 31550	4.72	${}^1\text{A}_{1g} \rightarrow {}^1\text{T}_{1g}$ , Charge transfer, Charge transfer	Square-planar



Scheme-1: Synthesis of Schiff base ligand.



Scheme-II: Proposed structures of complexes (1-5).

### Biological Activities

#### Antimicrobial Activity

The microorganisms used for these studies include different fungi *e.g.*, *Trichophyton longifusus*, *Fusarium solani* and *Aspergillus flavus* and bacteria *e.g.*, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*. The antimicrobial study of these compounds was conducted *in vitro* against the said strains of bacteria and fungi. Table-2 describes the MIC values of different compounds against growth of microorganisms. It was observed that SBL was less active than the metal chelates [20] and is in complete agreement to chelation theory and overtone's concept [21]. The overtone concept describes that only lipid-soluble materials are allowed to pass through the lipid membrane, surrounding the cell and the main controlling parameter of antimicrobial activity is the liposolubility. On the other hand, chelation concept explains that polarity of metal ion is reduced due to partial sharing of positive charge of metal ion with the co-ordinating groups and overlap of ligand orbitals with metal ion [22]. The complexes (1-5) become more and more stable due to delocalization of  $\pi$ -electrons which enhances the capability of complexes for blocking metal binding sites in the enzymes of pathogens and penetration of complexes

into lipid membrane. The synthesis of proteins in pathogens is blocked by the complexes *via* interference in respiratory process and in this way growth of pathogens is restricted [23].

#### Brine Shrimp Bioassay

*In vivo* shrimp lethality can be employed for initially monitoring newly synthesized bioactive compounds because shrimp larvae are often affected by the toxicity of bioactive compounds. This test is a very fine and economical method which forms the basis for large scale *in vivo* experiments on animals. The free Schiff base ligand and the complexes were screened *via* brine shrimp lethality and exhibited significant activity in opposition to brine shrimp nauplii. Compound (2) was found most active among compounds (1-5) with  $LD_{50}$  value of 0.043  $\mu\text{mL}$ . However,  $LD_{50}$  values for compounds (1, 3-5) were 45.3, 3.23, 46.03 and 27.57  $\mu\text{mL}$  respectively which showed that these compounds are active to a good extent.  $LD_{50}$  value for SBL was 50.13  $\mu\text{mL}$ .

### Experimental

#### Materials and Methods

All reagents and solvents were used as purchased from Merck. The metal salts were used as metal (II) acetate or chloride in hydrated form

[Pb(CH<sub>3</sub>COO)<sub>2</sub>.3H<sub>2</sub>O, Ni(CH<sub>3</sub>COO)<sub>2</sub>.4H<sub>2</sub>O, CoCl<sub>2</sub>.6H<sub>2</sub>O, CuCl<sub>2</sub>.2H<sub>2</sub>O, Cd(CH<sub>3</sub>COO)<sub>2</sub>.2H<sub>2</sub>O].

#### Physical Measurements

The electric Mettler Toledo balance, model AL 204 was used for the weighing. Melting points were recorded on Gallenkamp apparatus and are uncorrected. The IR spectra were taken on Thermo Nicolet Avatar 320 FTIR spectrophotometer by using the KBr pallet. The <sup>1</sup>H-NMR spectra were recorded on a Bruker AMX-400 spectrometer in DMSO-*d*<sub>6</sub>. The chemical shifts (δ) are given in ppm, relative to tetramethylsilane as an internal standard, and the scalar coupling constants (*J*) are reported in Hertz. The FAB mass spectra were obtained from JEOL SX102/DA-6000 mass spectrometer using glycerol as matrix and ions are given in *m/z*. Elemental analysis was carried out on Perkin Elmer 2400 Series II elemental analyzer. The molar conductances were determined on Jenway 4010 at room temperature in DMSO solution (1 × 10<sup>-3</sup> M). The magnetic moments were recorded on Guy-type magnetic balance (Hertz SG8SHJ) while the electronic spectra were carried out on Specord 200 UV-Vis spectrophotometer. Preparative TLC was performed using pre-coated silica gel G-25-UV<sub>254</sub> plates followed by detection at 254 and 366 nm.

#### Synthesis

##### Synthesis of {5-Chloro-2-(4-nitrobenzylideneamino)phenyl}(phenyl)methanone (SBL)

The solution of 4-nitrobenzaldehyde (0.01 mole in 50 mL ethanol) was added to 2-amino-5-chlorobenzophenone (0.01 mole in 50 mL ethanol) along with 3 drops of conc. H<sub>2</sub>SO<sub>4</sub>. Then mixture was stirred at 70°C for about 5 hours. The resulting solution was concentrated to one third of its volume by evaporating excess solvent on rotary evaporator and it was cooled after acetone addition. Yellow coloured crystals were obtained by keeping reaction mixture overnight at ambient temperature, recrystallized with methanol and dried over anhydrous CaCl<sub>2</sub>. Progress of the reaction was

monitored by TLC (*Scheme-1*). Yield: 78.12 %; m.p.: 169 °C; IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 3104 (C-H), 1657 (C=N), 1403, 1073, 760 (benzene ring), 1370 (NO<sub>2</sub>), 616 (Cl); <sup>1</sup>H-NMR (DMSO *d*<sub>6</sub>, 400 MHz) δ: 8.75 (1H, s, H-1'''), 8.11 (2H, d, *J* = 8.8 Hz, H-2'', -6''), 7.74 (1H, d, *J* = 8.8 Hz, H-3), 7.69 (2H, d, *J* = 8.8 Hz, H-3'', -5''), 7.68 (1H, dd, *J* = 8.8, 2.4 Hz, H-4), 7.59 (1H, dd, *J* = 8.4, 2.4 Hz, H-4'), 7.62 (1H, d, *J* = 2.4 Hz, H-6), 7.51 (2H, d, *J* = 8.8, 2.4 Hz, H-2', -6'), 7.48 (2H, dd, *J* = 8.8, 8.4 Hz, H-3', -5'); FAB-MS (+ve) *m/z*: 365.3 [M+H]<sup>+</sup> (calcd. 364.0 for C<sub>20</sub>H<sub>13</sub>ClN<sub>2</sub>O<sub>3</sub>); Elemental analysis (%): C 66.04, H 3.91, N 7.51 (clacd. C 65.85, H 3.59, N 7.68).

##### {5-Chloro-2-(4-nitrobenzylideneamino)phenyl}(phenyl)methanone (SBL)

Yield: 78.12 %; m.p.: 169 °C; IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 3104 (C-H), 1657 (C=N), 1403, 1073, 760 (benzene ring), 1370 (NO<sub>2</sub>), 616 (Cl); <sup>1</sup>H-NMR (DMSO *d*<sub>6</sub>, 400 MHz) δ: 8.75 (1H, s, H-1'''), 8.11 (2H, d, *J* = 8.8 Hz, H-2'', -6''), 7.74 (1H, d, *J* = 8.8 Hz, H-3), 7.69 (2H, d, *J* = 8.8 Hz, H-3'', -5''), 7.68 (1H, dd, *J* = 8.8, 2.4 Hz, H-4), 7.59 (1H, dd, *J* = 8.4, 2.4 Hz, H-4'), 7.62 (1H, d, *J* = 2.4 Hz, H-6), 7.51 (2H, d, *J* = 8.8, 2.4 Hz, H-2', -6'), 7.48 (2H, dd, *J* = 8.8, 8.4 Hz, H-3', -5'); FAB-MS (+ve) *m/z*: 365.3 [M+H]<sup>+</sup> (calcd. 364.0 for C<sub>20</sub>H<sub>13</sub>ClN<sub>2</sub>O<sub>3</sub>); Elemental analysis (%): C 66.04, H 3.91, N 7.51 (clacd. C 65.85, H 3.59, N 7.68).

##### General Procedure for Synthesis of Schiff Base Metal (II) Complexes (1-5)

The methanolic solution of respective salts [(Pb(CH<sub>3</sub>COO)<sub>2</sub>.3H<sub>2</sub>O, Ni(CH<sub>3</sub>COO)<sub>2</sub>.4H<sub>2</sub>O, CoCl<sub>2</sub>.6H<sub>2</sub>O, CuCl<sub>2</sub>.2H<sub>2</sub>O, Cd(CH<sub>3</sub>COO)<sub>2</sub>.2H<sub>2</sub>O] in 2:1 (L:M) molar ratio, were slowly added to hot stirring methanolic solution of ligand (SBL). The reaction mixture was further refluxed for 45 min. The 1N NaOH in MeOH was added dropwise to maintain the pH for complex formation and precipitation of complexes. The precipitates were filtered, washed with cold methanol and dried over anhydrous CaCl<sub>2</sub> under vacuum.

Table-2: Antimicrobial activities of the compounds and standard reagents.

Compound	Antibacterial activity (MIC x 10 <sup>-2</sup> M)			Antifungal activity (MIC x 10 <sup>-2</sup> M)		
	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>A. flavus</i>	<i>F. solani</i>	<i>T. longifusus</i>
SBL	5.5	4.3	5.8	5.1	3.9	4.3
1	9.3	7.6	15.9	5.2	6.2	9.1
2	10.0	8.9	11.4	6.9	5.3	6.6
3	9.6	9.8	13.1	4.1	5.9	8.2
4	8.5	6.9	12.3	7.3	4.3	5.9
5	9.3	7.3	14.5	5.6	6.2	9.0
Imipenem*	12.0	10.9	16.1	-	-	-
Amphotericin B*	-	-	-	8.0	7.3	11.7

\*Standards

*{5-Chloro-2-(4-nitrobenzylideneamino)phenyl}(phenyl)methanone-lead (II)* [Pb(SBL)<sub>2</sub>(OAc)<sub>2</sub>] (**1**)

Lemon chiffon solid; yield: 71 %; m.p.: 241 °C (decomp.); IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 2865 (C-H), 1631 (COO)<sub>as</sub>, 1555 (C=N), 1440, 1097, 753 (benzene ring), 1345 (NO<sub>2</sub>), 1276 (COO)<sub>s</sub>, 681 (Cl), 531 (Pb-N), 403 (Pb-O); Molar conductance (DMSO)  $\lambda_m$  (Ohm<sup>-1</sup>cm<sup>2</sup>mol<sup>-1</sup>): 13.9; FAB-MS (+ve) *m/z*: 1055.4 [M+H]<sup>+</sup> (calcd. 1054.1 for C<sub>44</sub>H<sub>32</sub>Cl<sub>2</sub>N<sub>4</sub>O<sub>10</sub>Pb); Elemental analysis (%): C 52.10, H 3.34, N 5.49, Pb 20.09 (clacd. C 50.10, H 3.06, N 5.31, Pb 19.64).

*{5-Chloro-2-(4-nitrobenzylideneamino)phenyl}(phenyl)methanone-nickel (II)* [Ni(SBL)<sub>2</sub>(OAc)<sub>2</sub>] (**2**)

Yellow green solid; yield: 63 %; m.p.: 187 °C (decomp.); IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 2834 (C-H), 1573 (COO)<sub>as</sub>, 1514 (C=N), 1403, 919, 816 (benzene ring), 1323 (NO<sub>2</sub>), 1236 (COO)<sub>s</sub>, 638 (Cl), 535 (Ni-N), 463 (Ni-O); Molar conductance (DMSO)  $\lambda_m$  (Ohm<sup>-1</sup>cm<sup>2</sup>mol<sup>-1</sup>): 21.1; FAB-MS (+ve) *m/z*: 905.2 [M+H]<sup>+</sup> (calcd. 904.0 for C<sub>44</sub>H<sub>32</sub>Cl<sub>2</sub>N<sub>4</sub>O<sub>10</sub>Ni); Elemental analysis (%): C 59.34, H 3.19, N 6.19, Ni 6.03 (clacd. C 58.31, H 3.56, N 6.18, Ni 6.48).

*{5-Chloro-2-(4-nitrobenzylideneamino)phenyl}(phenyl)methanone-cobalt (II)* [Co(SBL)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>] (**3**)

Dark violet solid; yield: 68 %; m.p.: 219 °C (decomp.); IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 3545 (OH), 3064 (C-H), 1610 (C=N), 1476, 1034, 783 (benzene ring), 1340 (NO<sub>2</sub>), 673 (Cl), 537 (Co-N), 410 (Co-O); Molar conductance (DMSO)  $\lambda_m$  (Ohm<sup>-1</sup>cm<sup>2</sup>mol<sup>-1</sup>): 14.3; FAB-MS (+ve) *m/z*: 824.3 [M+H]<sup>+</sup> (calcd. 823.0 for C<sub>40</sub>H<sub>30</sub>Cl<sub>2</sub>N<sub>4</sub>O<sub>8</sub>Co); Elemental analysis (%): C 58.41, H 3.71, N 6.84, Co 7.18 (clacd. C 58.27, H 3.67, N 6.80, Co 7.15).

*{5-Chloro-2-(4-nitrobenzylideneamino)phenyl}(phenyl)methanone-copper (II)* [Cu(SBL)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>] (**4**)

Olive drab solid; yield: 60 %; m.p.: 203 °C (decomp.); IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 3439 (OH), 2971 (C-H), 1601 (C=N), 1455, 1039, 751 (benzene ring), 1367 (NO<sub>2</sub>), 674 (Cl), 588 (Cu-N), 444 (Cu-O); Molar conductance (DMSO)  $\lambda_m$  (Ohm<sup>-1</sup>cm<sup>2</sup>mol<sup>-1</sup>): 11.5; FAB-MS (+ve) *m/z*: 828.3 [M+H]<sup>+</sup> (calcd. 827.0 for C<sub>40</sub>H<sub>30</sub>Cl<sub>2</sub>N<sub>4</sub>O<sub>8</sub>Cu); Elemental analysis (%): C 57.35, H 3.70, N 6.95, Cu 6.31 (clacd. C 57.94, H 3.65, N 6.76, Cu 7.66).

*{5-Chloro-2-(4-nitrobenzylideneamino)phenyl}(phenyl)methanone-cadmium (II)* [Cd(SBL)<sub>2</sub>] (**5**)

Navajo white solid; yield: 66 %; m.p.: 197 °C (decomp.); IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 2930 (C-H), 1566

(C=N), 1408, 1045, 729 (benzene ring), 1359 (NO<sub>2</sub>), 661 (Cl), 542 (Cd-N), 409 (Cd-O); Molar conductance (DMSO)  $\lambda_m$  (Ohm<sup>-1</sup>cm<sup>2</sup>mol<sup>-1</sup>): 8.3; FAB-MS (+ve) *m/z*: 843.1 [M+H]<sup>+</sup> (calcd. 842.0 for C<sub>40</sub>H<sub>26</sub>Cl<sub>2</sub>N<sub>4</sub>O<sub>6</sub>Cd); Elemental analysis (%): C 57.97, H 3.47, N 6.91, Cd 13.64 (clacd. C 57.06, H 3.11, N 6.65, Cd 13.35).

*Biological Studies**Antimicrobial Activity*

*Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* were used as bacterial strains to determine the effects of synthesized compounds during *in vitro* biological screening and imipenem was used as a standard drug during these studies by employing method of well diffusion [19-21]. The nutrient medium used during these studies was agar [24]. The fungal species *viz.*, *Aspergillus flavus*, *Fusarium solani* and *Trichophyton longifusus* were used for the screening of the compounds through the same method as used for antibacterial activity, using amphotericin B as standard drug, cultured on a medium of potato dextrose agar [25]. The MIC (minimum inhibitory concentration) values were determined by gradually diluting the stock solution (1 x 10<sup>-2</sup> M) of compounds in dimethyl sulfoxide (DMSO). The reported literature [26] showed that inoculated medium (*i.e.* agar) was used for making wells by using microorganisms. The test solution was introduced into the well using the micropipette and incubation of plate for 72 hours in case of fungi and 24 hours for bacteria at 35 °C. During this time, the growth of inoculated microorganisms was affected. The concentration of test solutions was recorded during the development of zone of inhibition.

*Brine Shrimp Lethality Bioassay*

The artificial sea water was prepared by dissolving 3.8 g sea salt per liter in double distilled water followed by filtration. 1.0 mg of shrimp eggs (*Artemia salina*) were added to the sea water in small tank. Aluminum foil was used to darken the wall of the tank. The shrimp larvae were attracted by illuminated partition through perforation in the dam. It was allowed to stand at 25 °C for 24 hours for the larvae to hatch. The different concentration of the test samples (50, 100, 150 µg/mL) were prepared in DMSO. Three replicates were prepared for each concentration with the total of 18 vials. After two days, the shrimp larvae got matured. Then 5 mL sea water and 12 shrimps were added per vial, and were placed under illumination for 24 hours. After 24 hours, the LD<sub>50</sub> values were determined from the

counting of surviving shrimps and the data was analyzed with the help of Finney computer program [27].

### Conclusion

This study presents the synthesis of transition metal complexes with bidentate Schiff base ligands which were derived from 2-amino-5-chlorobenzophenone and 4-nitrobenzaldehyde. These complexes were characterized on the basis of various spectral and analytical techniques. The non electrolytic nature of the complexes is deduced from the conductance data and the stoichiometric ratio of metal to ligand is 1:2, which is indicated from FAB (+ve) mass spectra and elemental analyses of the ligand (SBL) and its complexes (1-5). The geometry of the complexes was assigned on the grounds of magnetic moment and electronic data, square planar for complex (4) and octahedral for complexes (1-4). The IR spectra indicated that oxygen and nitrogen are coordinated in the complexes, new bands appeared for  $\nu(\text{M-N})$  and  $\nu(\text{M-O})$  at  $531\text{-}588\text{ cm}^{-1}$  and  $403\text{-}463\text{ cm}^{-1}$  respectively. The metal complexes showed higher *in vitro* antimicrobial activity than the Schiff base ligand and also have significant *in vivo* lethality to shrimp larvae.

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