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Synthesis and Structure of 4-Chloro-2-{[5-(diethylamino)-2-hydroxybenzylidene]amino}phenol and Its Metal Complexes

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Abstract—The present study is devoted to the synthesis of 4-chloro-2-{[5-(diethylamino)-2-hydroxybenzylidene]-amino}phenol and its transition metal complexes. Synthesis of the ligand has been achieved by the condensation reaction of *N,N*-diethylsalicylaldehyde with 4-chloro-2-aminophenol in acidic medium. Metals complexes of the ligand with different transition metal ions [M^{2+}= Mn(II), Fe(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Hg(II), and Pd(II)] have been accumulated in alcoholic media and characterized by spectroscopic methods. UV-Vis analysis of the complexes indicates the ligand coordination to the metal ions via both OH groups and the azomethine nitrogen atom, acting as a tridentate ligand. Anticancer tests of selected complexes demonstrate moderate in vitro activity of Cu(II) complex against HeLa cell line.

Keywords: anticancer activity, HeLa cell line, azomethine, condensation reaction, spectroscopic methods

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INTRODUCTION

Schiff base metal complexes display a wide range of biological applications such as anticancer, antibacterial, antivirus, and antifungal [1–4]. Schiff bases containing oxygen atoms are important chelating ligands due to the presence of N and O donor atoms that can coordinate via azomethine nitrogen and/or an oxygen containing group [5–8].

Objective of the current research was the synthesis of a novel tridentate Schiff base ligand and its coordination complexes with Mn(II), Fe(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Hg(II), and Pd(II), and study of their biological activity.

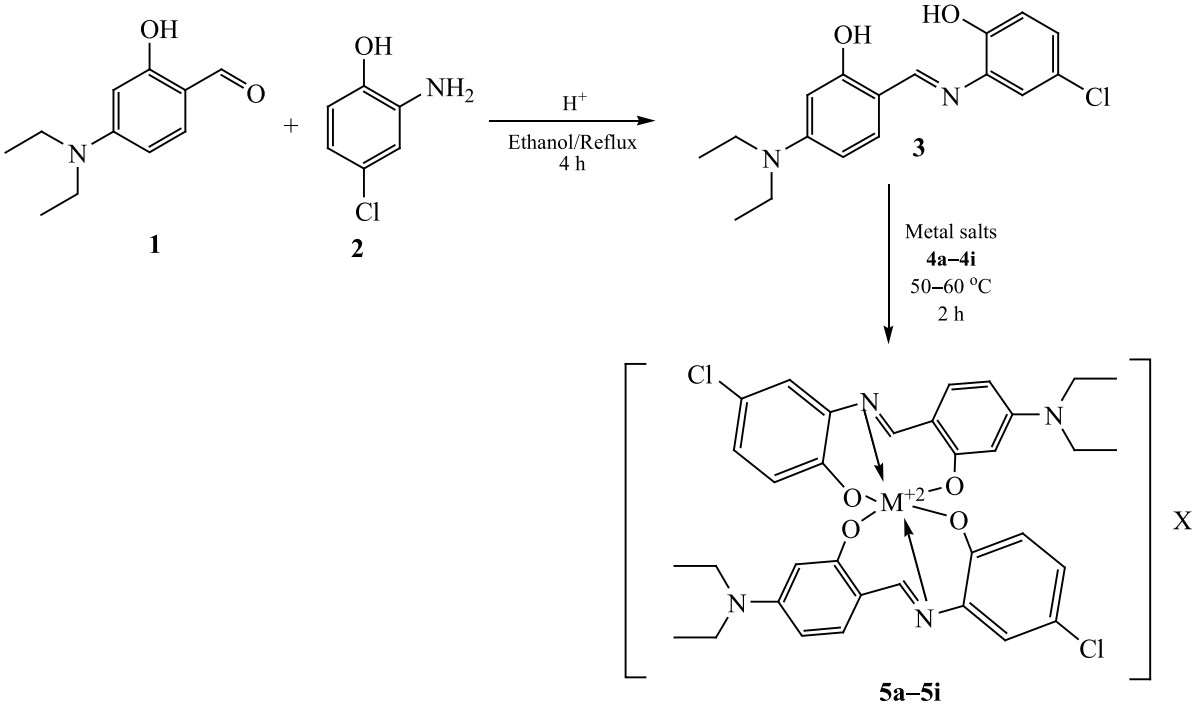
EXPERIMENTAL

All chemicals used were of analytical reagent grade (AR). 4-Chloro-2-aminophenol, 4-diethylaminosalicyl-aldehyde and hydrochloric acid were purchased from Aladdin Chemicals, China. Metal salts were purchsed from Sigma Alrich. The organic solvents were purchased from Daejung Chemicals (Korea) and were spectroscopically pure.

IR spectra were recorded on a FTX-3000 MX Bio Rad Merlin, (Excalibur Model) spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured on a Bruker-300 MHz spectrometer using TMS as an internal reference. UV-Vis spectra were recorded on a Schimadzu 1700 UV-Vis Spectrophotometer. Elemental analysis was carried out on a CHNS 932 LECO instrument. Progress of reactions was monitored by TLC using precoated silica gel aluminum sheets 2.0×5.0 cm (layer thickness 0.2 mm, HF_{254}, Merck).

Synthesis of the Schiff base 3. Solution of *N,N*-diethylsalicylaldehyde (1.93 g, 0.01 mol) in 25 mL of ethanol was mixed with 1-2 drops of H_2SO_4 (98%) and alcoholic solution of 4-chloro-2-aminophenol (1.43 g, 0.01 mol) in 25 mL of ethanol upon constant stirring and then refluxed for 4 h. The progress of reaction was monitored by TLC until consumption of both reactants was observed. On completion of reaction, the reaction mixture was cooled to room temperature resulting in

Scheme 1. Syntheses of the Schiff base ligand **3** and its metal complexes **5a**–**5i**.



$MnCl_2·4H_2O$ (**4a**), $FeSO_4·7H_2O$ (**4b**), $CoCl_2·6H_2O$ (**4c**), $NiCl_2·6H_2O$ (**4d**), $CuCl_2·2H_2O$ (**4e**), $Zn(CH_3COO)_2·2H_2O$ (**4f**), $Cd(CH_3COO)_2·2H_2O$ (**4g**), $Hg(CH_3COO)_2$ (**4h**), $K_2[PdCl_4]$ (**4i**); $M^{+2} = Mn^{+2}$, X = $2Cl^{-1}$ (**5a**), $M^{+2} = Fe^{+2}$, X = SO_4^{-2} (**5b**), $M^{+2} = Co^{+2}$, X = $2Cl^{-1}$ (**5c**), $M^{+2} = Ni^{+2}$, X = $2Cl^{-1}$ (**5d**), $M^{+2} = Cu^{+2}$, X = $2Cl^{-1}$ (**5e**), $M^{+2} = Zn^{+2}$, X = $2CH_3COO^{-1}$ (**5f**), $M^{+2} = Cd^{+2}$, X = $2CH_3COO^{-1}$ (**5g**), $M^{+2} = Hg^{+2}$, X = $2CH_3COO^{-1}$ (**5h**), $M^{+2} = Pd^{+2}$, X = $2Cl^{-1}$ (**5i**).

the formation of yellow precipitates which were filtered off, washed with diethyl ether and dried over anhydrous calcium chloride, and recrystallized from absolute ethanol to give pure product, yield 80%.

Synthesis of metal complexes 5a–5i. A hot ethanol solution of the corresponding metal salt Mn(II), Fe(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Hg(II), or Pd(II) was mixed with the hot ethanol solution of 4-chloro-2-{[5-(diethylamino)-2-hydroxybenzylidene]amino}phenol in 1 : 2 molar ratio. The reaction mixture was stirred at 70°C for 2 h, then 2–3 drops of TEA were added, and the precipitates of complexes were obtained (Scheme 1). The products were filtered off, left over for drying, washed by diethyl ether, crystallized from methanol, and dried over anhydrous $CaCl_2$.

$C_{17}H_{19}ClN_2O_2$ (3). Yield 80%, mp 194–197°C. IR spectrum, ν, cm^{-1}: 580 (C–Cl), 808 (Ph–H), 1206 (C–O), 1375 (CH_2), 1489–1570 (C=C_{Ph}), 1608 (C=N), 1739 (C=O), 2970 (C=C–H), 3342–3446 (OH). ^1H NMR spectrum, δ, ppm: 1.11 t (6H, 2Me), 3.37 q (4H, $2CH_2$), 6.00 s (1H, C_6H_5), 6.29–6.92 m (2H, C_6H_5), 7.04–7.27 m (2H, C_6H_5), 7.38 s (1H, C_6H_5), 8.68 s (1H, Ph–CH=N–Ph), 9.91 s (1H, OH), 13.86 s (1H, OH). ^{13}C NMR spectrum, δ_C, ppm: 13.04, 44.41, 97.44, 104.45, 109.33, 117.82, 118.59, 123.57, 125.91, 134.80, 136.58, 149.73, 152.36, 160.27, 165.49. Found, %: C 49.41; H 4.59; N 6.71. $C_{17}H_{19}ClN_2O_2$. Calculated, %: C 49.22; H 4.62; N 6.75. *M* 318.

$C_{34}H_{38}Cl_4MnN_4O_4$ (5a). Yield 65%, mp >350°C. IR spectrum, ν, cm^{-1}: 519 (Mn–O), 910 (C=C–H), 1229 (C–O), 1474–1573 (C=C), 1380 (CH_2), 1590 (C=N), 1738 (C=O), 2922 (CH_2), 2972 (C=C–H). Found, %: C 59.67; H 5.93; N 8.70. $C_{34}H_{38}Cl_4MnN_4O_4$. Calculated, %: C 59.14; H 5.25; N 8.11. *M* 763.

$C_{34}H_{38}Cl_2FeN_4O_8S$ (5b). Yield 63%, mp >350°C. IR spectrum, ν, cm^{-1}: 512 (Fe–O), 787 (C–Cl), 915(C=C–H), 1220 (C–O), 1366 (CH_2), 1499–1586 (C=C), 1610 (C=N), 2922 (CH_2), 2970 (C=C–H). Found, %: C 59.67; H 5.93; N 8.70; S 4.06. $C_{34}H_{38}Cl_2FeN_4O_8S$. Calculated, %: C 59.06; H 5.15; N 8.10; S 4.46. *M* 789.

$C_{34}H_{38}Cl_4CoN_4O_4$ (5c). Yield 69%, mp >350°C. IR spectrum, ν, cm^{-1}: 514 (Co–O), 931 (C=C–H), 2970 (C=C–H), 783 (C–Cl), 1217 (C–O), 1372 (CH_2), 1513–1571 (C=C), 1598 (C=N), 2922 (CH_2). Found, %:

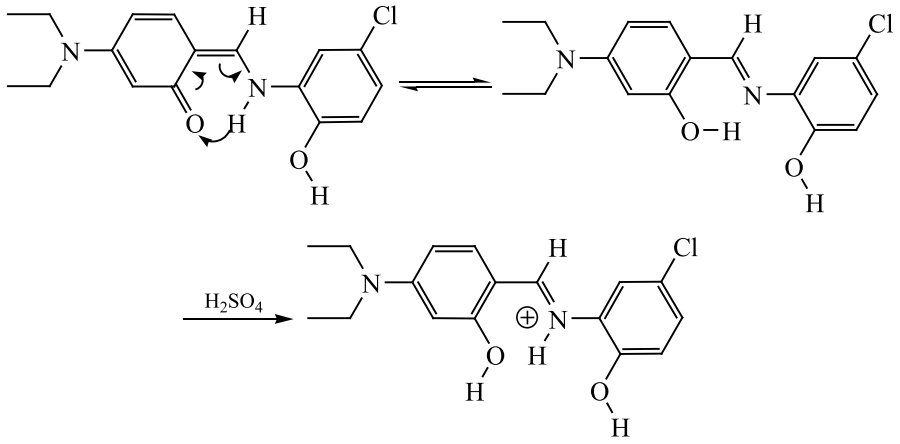


Fig. 1. Keto-amine tautomerism appearing in ligand **3**.

C 58.61; H 5.93; N 8.23. $C_{34}H_{38}Cl_4CoN_4O_4$. Calculated, %: C 58.80; H 5.22; N 8.07. *M* 767.

$C_{34}H_{38}Cl_4N_4NiO_4$ (5d). Yield 62%, mp >350°C. IR spectrum, ν, cm^{-1}: 508 (Ni–O), 788 (C–Cl), 916 (C=C–H), 1225 (C–O), 1351 (C–H), 1612 (C=N), 1474–1590 (C=C), 2970 (CH_2), 3015(C=C–H). Found, %: C 58.67; H 5.93; N 8.70. $C_{34}H_{38}Cl_4N_4NiO_4$. Calculated, %: C 58.82; H 5.23; N 8.07. *M* 767.

$C_{34}H_{38}Cl_4CuN_4O_4$ (5e). Yield 61%, mp >350°C. IR spectrum, ν, cm^{-1}: 510 (Cu–O), 920 (C=C–H), 1240 (C–O), 1382 (CH_2), 1489–1593 (C=C), 1631 (C=N), 2926 (CH_2), 3021(C=C–H). Found, %: C 58.62; H 5.87; N 8.31. $C_{34}H_{38}Cl_4CuN_4O_4$. Calculated, %: C 58.41; H 5.19; N 8.01. *M* 772.

$C_{38}H_{44}Cl_2N_4O_8Zn$ (5f). Yield 68%, mp >350°C. IR spectrum, ν, cm^{-1}: 508 (Zn–O), 922 (C=C–H), 1225 (C–O), 1382 (C–H), 1476–1581 (C=C), 1618 (C=N), 2915 (CH_2), 3027(C=C–H). Found, %: C 58.53; H 5.55; N 8.01. $C_{38}H_{44}Cl_2N_4O_8Zn$. Calculated, %: C 58.26; H 5.18; N 7.99. *M* 821.

$C_{38}H_{44}CdCl_2N_4O_8$ (5g). Yield 64%, mp >350°C. IR spectrum, ν, cm^{-1}: 490 (Cd–O), 920 (C=C–H), 1232 (C–O), 1335 (CH_2), 1497–1586 (C=C), 1628 (C=N), 2919 (CH_2), 3030 (C=C–H). Found, %: C 54.87; H 4.76; N 7.98. $C_{38}H_{44}CdCl_2N_4O_8$. Calculated, %: C 54.59; H 4.85; N 7.49. *M* 868.

$C_{38}H_{44}Cl_2HgN_4O_8$ (5h). Yield 67%, mp >350°C. IR spectrum, ν, cm^{-1}: 481 (Hg–O), 916 (C=C–H), 1227 (C–O), 1340 (CH_2), 1491–1593 (C=C), 1615 (C=N), 2922 (CH_2), 3015 (C=C–H). Found, %: C 48.93; H 4.36; N 6.70. $C_{38}H_{44}Cl_2HgN_4O_8$. Calculated, %: C 48.84; H 4.34; N 6.70. *M* 956.

$C_{34}H_{38}Cl_4N_4O_4Pd$ (5i). Yield 21%, mp >350°C. IR spectrum, ν, cm^{-1}: 474 (Pd–O), 910 (C=C–H), 1210 (C–O), 1352 (CH_2), 1489–1593 (C=C), 1622 (C=N), 2942 (CH_2), 3026 (C=C–H). Found, %: C 54.13; H 4.93; N 7.79. $C_{34}H_{38}Cl_4N_4O_4Pd$. Calculated, %: C 55.04; H 4.89; N 7.55; *M* 814.

Anticancer activity. Cytotoxic activity of compounds was evaluated in 96-well flat-bottomed micro plates by using the standard MTT colorimetric assay. HeLa Cells (Cervical Cancer) were cultured in Minimum Essential Medium Eagle, supplemented with 5% of fetal bovine serum (FBS), 100 IU/mL of penicillin and 100 µg/mL of streptomycin in 75 cm^3 flasks, and stored in a 5% CO_2 incubator at 37°C. Exponentially growing cells were harvested, counted with haemocytometer and diluted with the particular medium. Cell culture with the concentration of 6×104 cells/mL was prepared and introduced (100 µL/well) into 96-well plates. After overnight incubation, the medium was removed and 200 µL of fresh medium were added with different concentrations of compounds (1–30 µM). After 48 h, MTT (200 µL, 0.5 mg/mL) was added to each well and incubated further for 4 h. Subsequently, 100µL of DMSO were added to each well. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 570 nm, using a micro plate reader (Spectra Max plus, Molecular Devices, CA, USA). Cytotoxicity (IC50) was recorded. The results (% inhibition) were processed by using Soft- Max Pro software (Molecular Device, USA).

RESULTS AND DISCUSSION

The novel imine compound has been synthesized following the known procedure by nucleophilic addition of amine to aldehyde (Scheme 1).

Usually the inner-chelate complexes of Schiff bases with metal ions were formed via nitrogen atom of the imine group and oxygen atoms of hydroxyl groups [9].

Table 1. Physical properties of ligand **3** and its metal complexes **5a**–**5i**

Chromophore	Molecular formula	Color	λ_{max}, nm/absorbance	Solvent
3 (ligand)	$C_{17}H_{19}ClN_2O_2$	Yellow	370/0.688	DMSO
5a	$Mn(C_{17}H_{19}ClN_2O_2)_2$	Reddish brown	430/0.740	DMSO
5b	$Fe(C_{17}H_{19}ClN_2O_2)_2$	Bluish brown	420/0.079	DMSO
5c	$Co(C_{17}H_{19}ClN_2O_2)_2$	Rusty brown	435/0.707	DMSO
5d	$Ni(C_{17}H_{19}ClN_2O_2)_2$	Yellow	415/0.874	DMSO
5e	$Cu(C_{17}H_{19}ClN_2O_2)_2$	Green	425/0.673	DMSO
5f	$Zn(C_{17}H_{19}ClN_2O_2)_2$	Light brown	428/0.824	DMSO
5g	$Cd(C_{17}H_{19}ClN_2O_2)_2$	Bright Yellow	435/0.619	DMSO
5h	$Hg(C_{17}H_{19}ClN_2O_2)_2$	Dark brown	350/0.854	DMSO
5i	$Pd(C_{17}H_{19}ClN_2O_2)_2$	Bluish brown	350/1.154	DMSO

Table 2. Geometry of complexes **5a**–**5i**

Number	Chromophore	Metals	Geometry
1	**5a**	Mn^{2+}	Octahedral
2	**5b**	Fe^{2+}	Octahedral
3	**5c**	Co^{2+}	Octahedral
4	**5d**	Ni^{2+}	Tetrahedral
5	**5e**	Cu^{2+}	Octahedral
6	**5f**	Zn^{2+}	Square planer
7	**5g**	Cd^{2+}	Tetrahedral
8	**5h**	Hg^{2+}	Tetrahedral
9	**5i**	Pd^{2+}	Tetrahedral

Appearance of weak but sharp bands in IR spectrum of the ligand **3** at 3446 and 3342 cm^{-1} were attributed to stretching modes of OH phenol group. The specific profiles of those peaks were largely due to intra-molecular hydrogen bonding between OH group and azomethinic nitrogen in **3** (Fig. 1). Disappearance of the hydroxyl group bands in the range of 3442–3346 cm^{-1} in the spectrum of **5a** indicated deprotonation of the phenolic group upon coordination with manganese ion (Mn^{+2}). In comparison with C=N band of ligand **3**, the imine group stretching band of **5a** has been shifted to lower wavenumber 1586 cm^{-1} which evidenced the nitrogen atom coordination with metal atoms. Appearance of new weak bands at 519 cm^{-1} in IR spectrum of **5a** was attributed to the Mn–O bond formation upon complexation.

The UV-Vis spectra of all synthesized complexes (Table 1, Fig. 2) demonstrated bathochromic shift with the exception of **5h** and **5i**. Most of the compounds exhibited hyperchromic effect as compared to the absorption intensity of the ligand **3**. Bathochromic shifts in some metal complexes spectra corresponded to the increased electron density on the ligand moiety which required less energy to excite the electrons. This was ascribed to the presence of CH=N chromophore and substituted amine group. The π–π* and *n*–π* transitions occurred in the ligand which showed the absorptions in this region The John–Teller distortion was obvious in UV-Vis spectra of **5c** and **5e**. The metal–ligand ratio was determined to be 1 : 2 for all complexes. MLCT and LMCT was also established in this series which corresponded to the hypochromic and hyperchromic effects. Geometries of

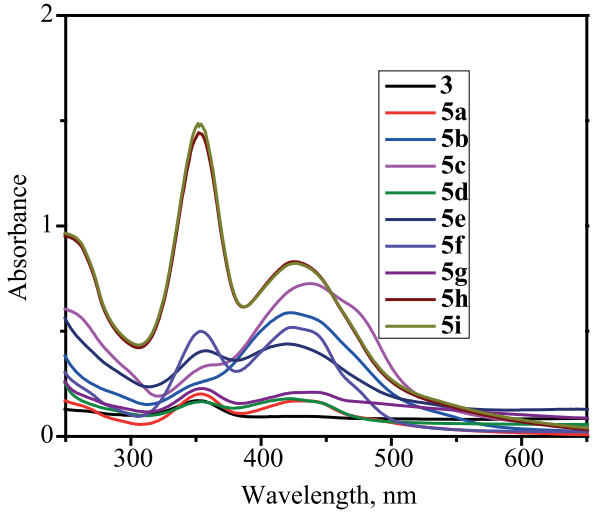


Fig. 2. UV-Vis spectra of ligand **3** and its metal complexes **5a**–**5i**.

Table 3. Cytotoxicity of ligand **3** and its metal complexes **5a**–**5i**

Compound	Concentration, mg/mL	Inhibition, %	IC_{50}±SD
3a (ligand)	30	82	2.3±0.19
5a	30	65	Not determined
5b	30	63	Not determined
5e	30	91	1.9±0.25
5f	30	09	Not determined
5i	30	40	Not determined
Streptomycin	30	94	1.5±0.4

the complexes determined by the slope ratio method are presented in Table 2.

Anticancer activity of ligand 3 and its metal complexes 5a–5i. The structural elucidation of the synthesized compounds were also achieved by NMR studies, In which some of the interesting facts about keto-imine tautomerism were also realized. The ligand **3** and selected complexes were tested for their anticancer activity against Hela Cancer line (Table 3). The ligand **3** and complex **5e** demonstrated promising results as compared to the standard drug. Such activity could be attributed to the planar structure of the compound, molecules of which could align between the nitrogenous bases of DNA pair of the opposing strands causing anticancer action.

CONCLUSIONS

The new ligand 4-chloro-2-{[5-(diethylamino)-2-hydroxybenzylidene]amino}phenol has been synthesized by traditional method, and its structure has been confirmed by spectroscopic methods like NMR, Single Crystal XRD, FT-IR, UV-Vis. Transition metal complexes of the ligand have been synthesized and their geometries have been elucidated from UV-Vis spectra involving the slope ratio method. Anticancer activity of ligand **3** and its metal complexes **5a**–**5i** was tested against Hela cancer lines, and the complex of copper **5e** has demonstrated promising activity probably due to its planar molecular structure and intercalation binding mode with DNA.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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