Indian Journal of Chemistry
Vol. 61, September 2022, pp. 965-975
DOI: 10.56042/ijc.v61i9.66350

Synthesis and characterisation of novel tetradentate Schiff base complexes: Biological evaluation exploring anticancer activity towards MCF 7 cell breast cancer lines with *in vitro* docking studies

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Received 16 November 2021; accepted (revised) 15 July 2022

A method to synthesize novel tetradentate ligand and a new series of metal complexes comprising Co (II), Fe (II), Cu (II) and Zn (II) (1-4) using 3,5-dichlorosalicylaldehyde and trans 1,2-diaminocyclohexane is described. The structural features and mode of bonding exhibited by Schiff base have been characterized by spectral methods like UV-visible, FT-IR and ESI-MS. Schiff base ligand and (1-4) complexes have displayed superior antibacterial towards both Gram positive and Gram negative bacteria. The antioxidant studies have revealed significant free radical scavenging activity towards the free radical DPPH. Theoretical molecular docking studies are performed to elucidate the interactions of synthesized compounds with biomolecules. A model illustrating specific DNA-binding of synthesized compounds and groove binding mode of DNA interaction is evolved. Cu(II) complex (3) with more pronounced biological activity (antibacterial and antioxidant) has been subjected to cytotoxicity studies against MCF-7 breast cancer cell lines through MMT assay. Cu (II) complex have exhibited stronger anti-cancer activity in comparision to ligand in that way the synergistic effects of signifying Cu Schiff base complexes illustrate the higher inhibiting efficiency of 77.2% at 320 µg/mL. The proposed study, therefore discloses potentiality of the synthesized new Schiff base derived complexes as a prodrug for cancer treatment.

Keywords: Tetradentate Schiff base, New transition metal complex, Biological evaluation, Docking with DNA, Microbial studies and cytotoxicity

With the advancement of inorganic medicinal chemistry, metal-based drugs have been put in the foreground in combating cancer. Among these bio-essential metals cobalt, iron, copper and zinc are widely studied[1,2]. Schiff base complexes derived from these metals display significant antifungal, antibacterial, anticancer properties. Homeostasis and metabolism are critical for all types of human cancers. Due to various physicochemical properties, biological and metalloenzyme activities, these complexes have gained growing attention. Many metal ions play important role in living systems. Usually, metal ions appear to lose electrons quickly from the familiar elemental or metallic state to produce charged ions that are vulnerable to biological fluid solubility. The chief factor that metals play their part in biology during this cationic form is caused by this property[3]. Most of the biological molecules such as proteins and DNA are electron rich, while metal ions are electron deficient. The attachment of those opposing charges results in a general propensity for metal ions to bind to biological molecules and interact with them[4-7]. Typically, the tetradentate Schiff bases are documented to coordinate with different metal ions with nitrogen and oxygen donor atom set, and this has grabbed the interest of many researchers[8,9]. The complexes of Schiff base ligands are studied for their dioxygen and oxidative catalysis property. In addition, significant attention has been given to transition metal complexes, which include salicylaldehyde and diamine derivatives. This is also due to their ability for oxygen insertion into an organic substrate as catalysts.

Cisplatin is one of the principal and broadly utilized metal-based anticancer medications for disease treatment. In spite of the fact that it goes about as a compelling medication against malignancy, it has experienced many side impacts, for example, going bald, abatement in bone marrow adequacy, neurotoxicity and harm to the covering of the gastrointestinal plot because of the medication obstruction wonder and restricted movement and selectivity in disease cells[10-14]. Along these lines, significant endeavors are being made to supplant this medication with reasonable choices, and various progress metal complexes of Co(II), Fe(II), Cu(II) and Zn(II) have been combined and tried for their

cytotoxicity. Its viability is restricted by its high poisonousness and frequency of medication obstruction. This has given inspiration to the quest for progress of metal-based medications with a more extensive range of action and lower fundamental poison levels.

There is an extensive interest in the plan of small molecules, which respond at explicit destinations along the DNA strand, as receptive models for protein-nucleic corrosive communications, as tests of DNA structure, as a guide to drug plan, and as tools of molecular biology[15]. The other novel progress in metal complexes is the creation of antitumor specialists and a portion of the mixtures is under clinical trial. Generally, the physicochemical features of metal complexes such as planarity, hydrophobicity, nature of co-ligands and coordination sites of ligands or geometry of the complexes play an essential role in interactions with DNA. The available research has shown that the action of metal-based cancer therapy is due to the ligand system since this organic ligand can govern the absorption, distribution, and metabolism of metals in biological systems and thus mitigate toxic side-effects, increase efficiency, improve delivery, and provide better drug resistance cell operations.

Significant research efforts are devoted for exploring salicylaldehyde derived Schiff base metal complexes[16,17]. 5-Bromosalicylaldehyde derived Schiff base metal complexes with good antimicrobial activity were reported earlier[18]. Also, 5-chloro-salicylaldehyde based Schiff base metal complexes were investigated[19]. Recently, Schiff base ligands derived from halogenated salicylaldehyde with homopiperonylamine was synthesized and its metal complexes were shown to exhibit cytotoxicity towards different tumor cell lines selectively, interact with DNA in an intercalative way by the electrostatic bonding[20]. The nickel complex of diimine Schiff base N,N-bis(salicylaldehyde)-o-phenylenediamine displayed good binding affinity for both DNA and protein[21].

The metal complexes of complexes 5-bromosalicylaldehyde and β-alanine also displayed favourable ligand-protein interaction when docked with enzymes[22]. These results demonstrated that halogenated derivative of salicylaldhyde perofrm better in comparis on to other salicylaldahyde ligands. Of late, Schiff base was prepared using 2-aminopyridine with 3,5-dichloro-salicylaldehyde by Anju *et al*., to obtain tellurium complex[23]. Besides, the ligand was derived from phenylacetic hydrazide and 3,5-dichlorosalicylaldehyde to obtain vanadium complex[24].

Our earlier studies on novel histidine derived Schiff base metal complexes also revealed that the synthesised Schiff base metal complexes are potential anticancer molecules[25]. A little attention has been paid to develop Schiff base ligand systems using dichloro derivatives of salicylaldehyde. In this regard, to shed light on these dicholorosystems, the present investigation intends to analyze synthesis of a novel tetradentate Schiff base ligand derived from the condensation reaction of 3,5-dichlorosalicylaldehyde and trans-1,2-diamino-cyclohexane. Four new series of metal complexes of Co(II), Ni(II), Cu(II) and Zn(II) were characterized by various analytical and spectral methods. Their spectral, biological activities (anti-bacterial and scavenging studies) and DNA cleaving nature are discussed in detail. Cu(II) complex exhibited in stronger anti-cancer activity in that way the synergistic effects of signifying Cu Schiff base complexes illustrating higher inhibiting efficiency of 77.2% at 320 μg/mL. Therefore, the present research reveals the capability of the synthesized new Schiff base derived complexes as a chemotherapy prodrug.

Experimental Details

Materials and methods

All the reagents and solvents were acquired from the commercial suppliers and utilized without any additional purification. The starting materials *viz*., 3,5-dichlorosalicylaldehyde and trans-1,2-diaminocy-clohexane were bought from Sigma Aldrich and every metal precursor salts used was of Merck products. The solvents methanol, ethanol, dimethylsulfoxide, dimethylformamide, agar agar were collected from Himedia.

The C, H and N analysis have been taken on Elemental Vario EL III CHNOS elemental analyzer. The UV -visible spectra have been measured using Shimadzu 2450 spectrophotometer. The infrared spectra were carried out by FT-IR Shimadzu 8400S spectrophotometer within the range of 4000 - 400 cm^{-1} by using KBr pellets. The ESI-MS spectra of the compounds have been recorded on DMSO UPLC-TQD Mass Spectrometer. Powder XRD was recorded on X-ray diffractometer with Cu Kα radiation. The docking studies were carried out using Autodock vina program and discovery studio software.

General synthetic procedure

Synthesis of ligand (L)

3,5-dichlorosalicylaldehyde (20 mmol) in methanol (20 mL) was added drop wise to trans-1,2-di

aminocyclohexane (10 mmol) in methanol (10 mL). The mixture was stirred continuously for 24 h to get a bright yellow solid at RT. Then the solid substance was filtered and washed with cold methanol, followed by washing with ether (2:1) solution. Then the resultant ligand was dried and used for further studies.

Chemical formula: $C_{20}H_{18}Cl_4N_2O_2$, elemental analysis (calculated): C, 52.20; H, 3.94; Cl, 30.82; N, 6.09; O, 6.95%; IR (KBr pellets) υ (cm^{-1}): 3447 (υ O-H), 1648 (υ C=N), 1628 (υ C=O); ^{1}H NMR ($CDCl_3$, 400MHz): (OH, J=5.0, 1.0, 1H, HPy); 8.57 (s, 1H, HC=N); 8.62 (dt, J=7.8, 1.0, 1H, HPy); 7.81 (tq, J=7.8, 1.8, 1H, HPy); 7.38–7.35 (m, 1H, HPy); 7.38–7.35 (m, 3H, HPh).

Synthesis of metal complexes

Metal complexes were synthesised by refluxing metal precursor with ligand (L). The synthesized ligand (1mmol) were dissolved in 4 mL of hot methanol and metal salts (Co, Cu, Fe and Zn acetate salts) (1 mmol) was dissolved in 5 mL of methanol and added drop by drop through the wall of the RB. The insoluble solid mass was thrown out within 1 h. The metal complexes were filtered and washed with 5 mL methanol: water (1:1) mixture.

Synthesis of Cobalt(II) (L) metal complexes

Cobalt acetate (1 mmol) and (L) (1 mmol), refluxing time 1 h, colour of the complex: brown, yield (86%), chemical Formula: $C_{20}H_{16}Cl_4CoN_2O_2$. Elemental analysis (calculated): C, 43.43; H, 3.64, N, 5.06; O, 11.57. IR (KBr pellets) υ (cm^{-1}): 3370 (υ O-H), 1618 (υ C=N).

Synthesis Copper(II) (L) metal complexes

Copper acetate (1 mmol) and (L) (1 mmol), refluxing time 1 h, colour of the complex: dark green, yield (71%), chemical formula: $C_{20}H_{16}Cl_4CuN_2O_2$, elemental analysis (calculated): C, 44.11; H, 3.88; N, 4.90; O, 11.19. IR (KBr pellets) υ (cm^{-1}):3180 (υ O-H), 1632 (υ C=N).

Synthesis of Iron(II) (L) metal complexes

Iron citrate (1 mmol) and (L) (1 mmol) were refluxed for 1 h, colour of the complex: brick red, yield (75%), chemical formula: $C_{20}H_{16}Cl_4FeN_2O_2$, elemental analysis (calculated): C, 45.95; H, 3.67; N, 5.10; O, 8.74%; IR (KBr pellets) υ (cm^{-1}): 3368 (υ O-H), 1629 (υ C=N).

Synthesis of Zinc(II) (L) metal complexes

Zinc acetate (1 mmol) and (L) (1 mmol), refluxing time 1 h, colour of the complex: yellow, yield (85%), chemical formula: $C_{20}H_{16}Cl_4ZnN_2O_2$, elemental analysis (calculated): C, 45.40; H, 3.63; N, 5.04; O, 8.64%: IR (KBr pellets) υ (cm^{-1}): 3330 (υ O-H), 1629 (υ C=N).

Molecular docking analysis

Molecular docking study is one of the tool for binding biomolecules with synthesised drugs. Molecular docking studies were carried out using AutoDock Tools (ADT) v1.5.6, Auto Dock v 4.2.5.1 and AutoDockvina 4.2. The crystal structure of the biomolecules such as double chain, 583 sequence BSA protein (PDB ID: 4F5S) and synthetic DNA dodecamerd $(CGCGAATTCGCG)_2$ (PDB ID: 1BNA) were downloaded from Protein Data Bank. The copper atom parameter values such as RDW radii 0.96 Å and depth 0.01 kcal mol^{-1} were fixed. The optimised structure of the metal complexes was converted pdb format using mercury v3.8 software. For the receptor molecule kollman charges and hydrogen atoms were added. The bonds between the atoms were permitted to rotatable one. The grid box value was created using Auto Dock as 120 × 120 × 120 for the X*Y*Z dimensions and the spacing (Å) value of 0.921 point in which almost involved the entire DNA/BSA molecule. The water molecule and the ligands present in the biomolecules were removed using Discovery studio 2017 R2. The output results were collected using Discovery studio 2017 R2.

Antioxidant activity

The antioxidant activity of (L) and metal complexes (1-4) were carried out using the free radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH). Usually $DPPH^{\bullet}$ is purple colour in the solution state and gives absorbance peak at 519 nm. The mechanism of the antioxidant activity using DPPH free radical is $DPPH^{\bullet}$ absorbs the free radical by breaking hydrogen radical from the external source like ligand or metal complexes. So, the absorbance at 519 nm is decreasing due to the lack of free radicals in the DPPH. The various concentrations of (L) and (1-4) (0, 20, 40, 60, 80, 100 μM) was added to the fixed concentration of $DPPH^{\bullet}$ (50μM). Ascorbic acid was used as standard and the values of (L) and (1-4) were comparing with the standard. The free radical scavenging activity percentage was calculated using the following Eqn (1).

$$\text{DPPH scavenging (\%)} = (A_0 - A_{sample}/A_0) \times 100 \quad \ldots (1)$$

Where A_0 is the absorbance of the control and A_{sample} is the absorbance of the sample. IC_{50} values were calculated for (L) and (1-4) metal complexes. The IC_{50} value is defined as the sufficient concentration to reach the 50% of the maximum scavenging activity.

Antimicrobial activity

Antimicrobial activities of the compounds were determined using well diffusion method. It was performed by sterilizing Mueller Hinton agar media. After solidification, wells were cut on the Mueller Hinton agar using cork borer. The test bacterial pathogens were swabbed onto the surface of Mueller Hinton agar plates. The cut pieces of thin film of samples were placed on the well. The plates were incubated at 37 °C for 24 h, and then the zone of inhibition was measured in millimetres. Each antibacterial assay was performed in triplicate and mean values were reported (Table 1).

Cytotoxic studies

In vitro growth inhibitory effect of four synthesised compounds (L) and (1-4) were treated against tumour cell lines using MTT (tetrazolium salt reduction) assay on human breast cancer cells (MCF-7). The layers of the cancer cells were detached with trypsin-ethylene diamine tetra acetic acid (EDTA) to make single cell suspensions and viable cells. The cells were counted by using hemocytometer and diluted with medium containing 5% FBS to give final density of 1×10^5 cells/mL. The environment of the cell line is maintained as 100% relative humanity by maintaining at 37°C, 5% CO_2, 95% air. One hundred micro litres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well. After 36 h the cells were treated with serial concentrations of the Cu complex. The samples were dissolved in dry dimethylsulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. There will be four dilutions were made to provide a total of five sample concentrations. The cancer cells without samples were served as control.

Table 1 — Antibacterial activity of compound 1-4

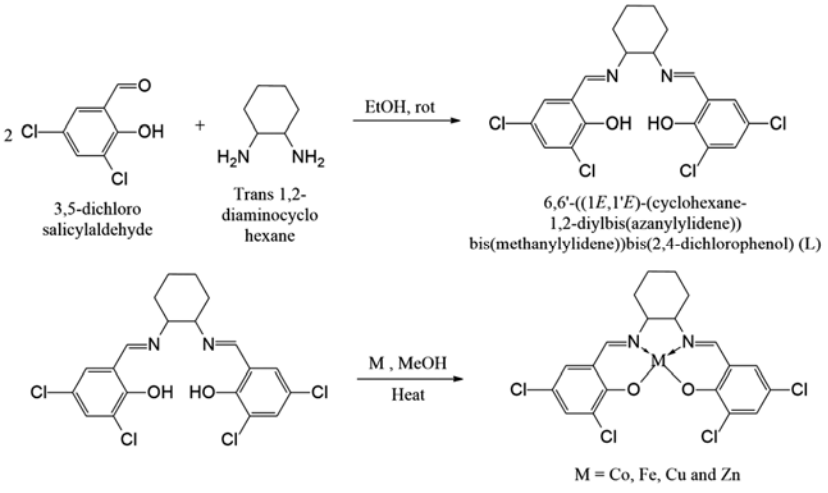
Compound	Zone of inhibition in (*mm*)			
	Control	*Escherichia coli*	Control	*Staphylococ-cus aureus*
1	21	19	15	17
2	21	18	15	12
3	21	17	15	17
4	21	17	15	17

Results and Discussion

The synthesized ligand and metal complexes are stable in RT. The tetradentate ligand 6,6'-((1E,1'E)-(cyclohexane-1,2-diylbis(azanylyli-dene))bis(methan-ylylidene))bis(2,4-dichlorophenol) (L) which chemically reacted with metal ions (Co(II), Cu(II), Ni(II) and Zn(II)) to form the corresponding complexes with regular structures. The metal complexes are soluble in DMSO solvents. The proposed reaction pathway was depicted in Scheme 1.

FT-IR and NMR spectra

FT-IR spectrum shows the preliminary confirmation of the synthesized materials. The spectrum results also give the outline of the complex formation. On the first hand ligand shows 3447 cm^{-1} range broad peak which indicate the –OH stretching peak of phenyl molecule and 1628 cm^{-1} range peak confirms the formation of –C=N-azomethine peak. The 1200-1370 cm^{-1} having three peaks which confirm the aromatic ring benzene involves the structure. For the cobalt complex (1) the azomethine peak shift into 1618 and also for complexes (2-4) the peaks shifts to 1629, 1623 and1629 cm^{-1}, respectively. All the complexes have M-N and M-O bonds in the range of 540-630 cm^{-1}. The FT-IR spectra are shown in Fig. 1.



Scheme 1 — The proposed reaction scheme of Schiff base ligand (L) and corresponding metal complexes comprising Co(II), Cu(II), Fe(II) and Zn(II)

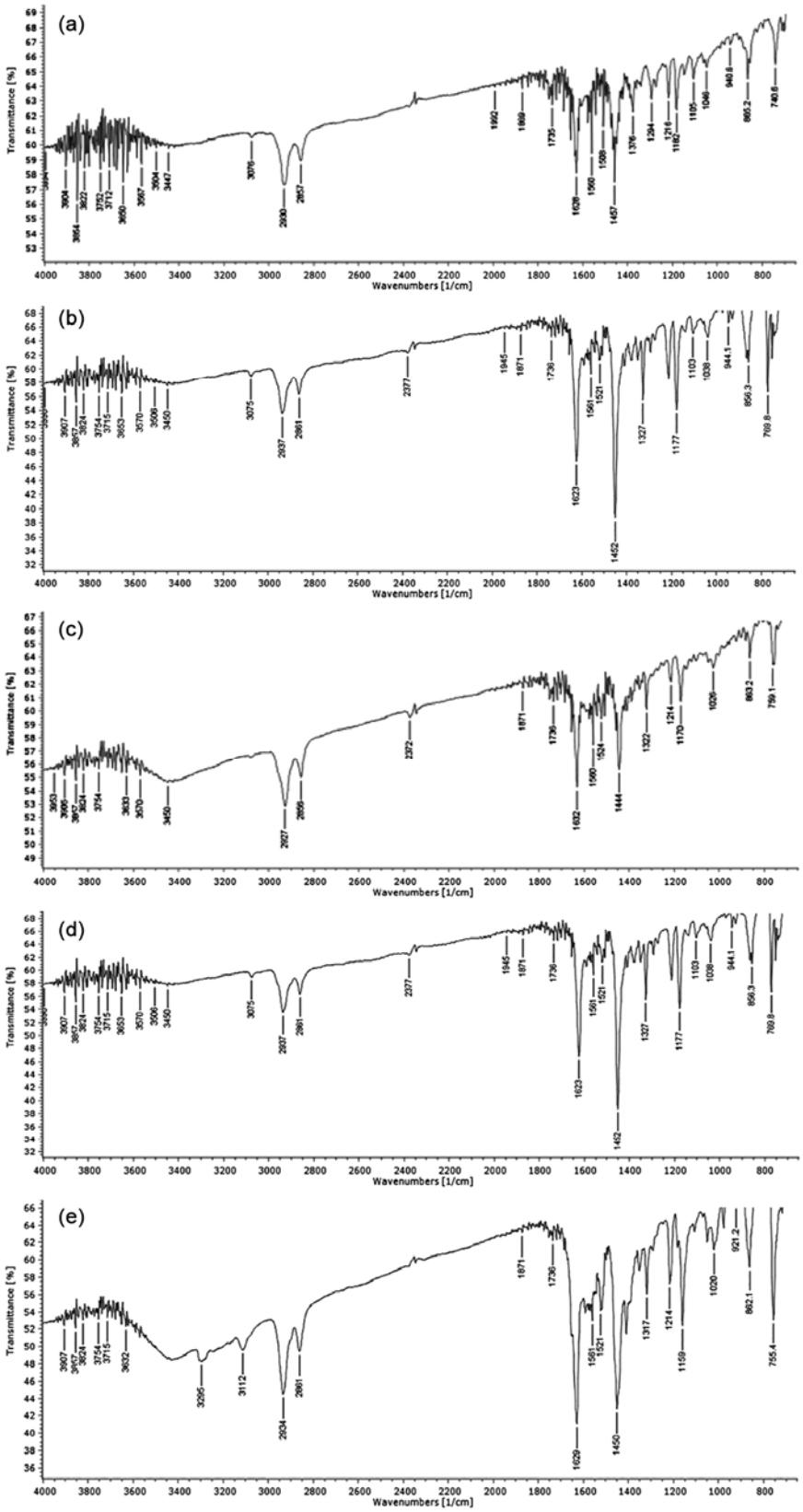


Fig. 1 — FT-IR spectra for (a) L and (b-e) Co(II), Cu(II), Fe(II) and Zn(II), respectively

The proton NMR studies show the number of proton presents in the structure. The NMR studies are taken using DMSO-D6 solvent. The single intensity peak of 5ppm range denotes the –OH of the phenyl ring. The two azomethine bond confirmed by the 8.57 & 8.62 ppm range peaks. The aromatic protons appear at the range of 7.38-7.35 ppm. The proton NMR image showed in Fig. 2.

Electronic spectra

There are three suggested band appears for (L) in the range of 260, 330 and 432 nm. The 260 nm bands represents the $\pi \rightarrow \pi^*$ transition of the inner shell electrons of (L). The ring -C=C- represents at the range of 330 nm by the same $\pi \rightarrow \pi^*$ electronic moderate transition. The major –C=N- represent the $n \rightarrow \pi^*$ transition which confirms the formation of Schiff base. For metal complexes the corresponding bands of the ligand shifts, which confirm the formation of the metal complexes. The d-d electronic transition band of the transition metal complex will appear in the range of 800-500 nm. The spectrum of the cobalt which shows the d-d band at 543 nm denotes the $2E_g \rightarrow 2T_{2g}$ and having an octahedral geometry .The major spectrum of copper complex shows the d-d band at 630 nm, which shows the same $2E_g \rightarrow 2T_{2g}$ transition and confirms octahedral structure. Fe(II) complexes having the band shift at the range of 285, 320 and 420 nm which confirms the formation of the complex. Zn(II) complex there is no d-d transition appears and having new bands at the range of 290 and 370 nm which confirms the formation of Zn(II) complex. The UV-visible spectra results were shown in Fig. 3.

ESI-mass spectral studies

The ESI-MS spectrum of ligand was represented in Fig. S1. The ESI-MS spectrum confirms the predicted structure. For ligand (L) shows a mass peak in the range of m/z 462. It implies the M+2 peak formation. The fragment peak 288 m/z represent that the 1:1 ligand peak. The Co (II) complex shows peak at

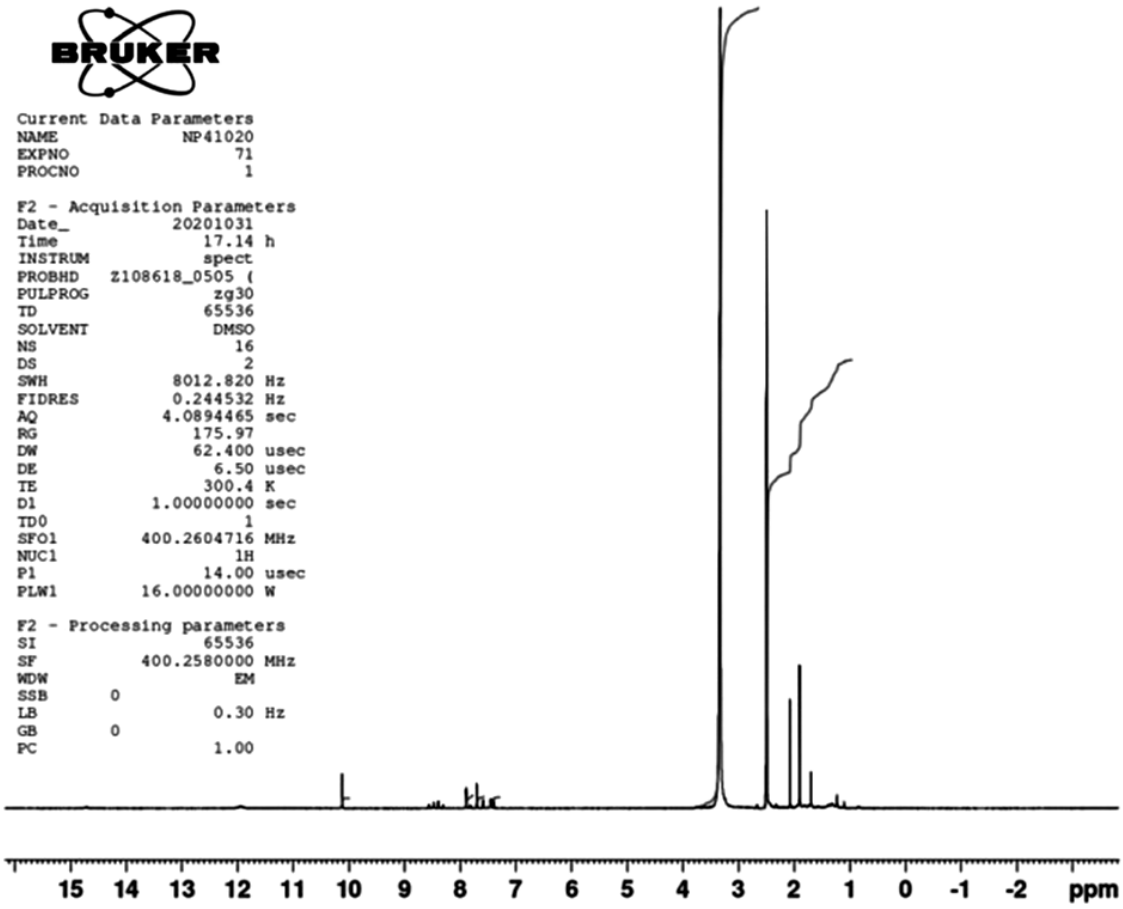


Fig. 2 — NMR spectra of ligand (L)

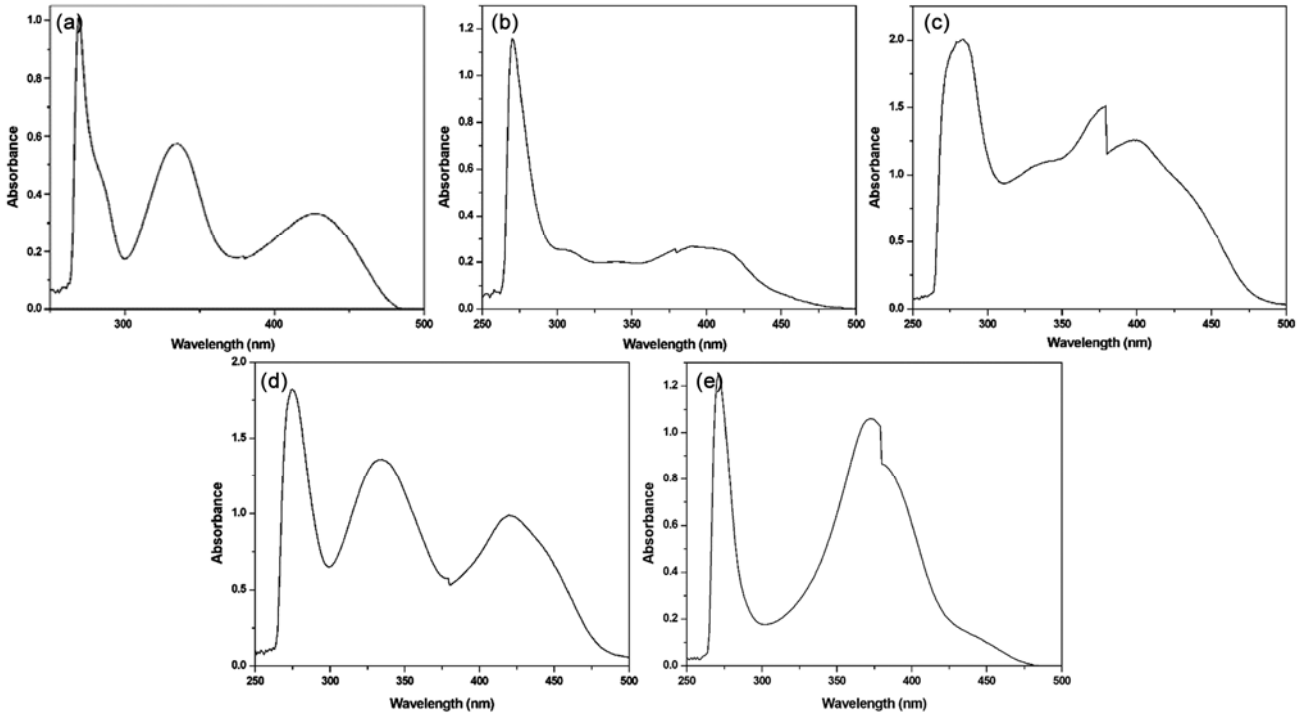


Fig. 3 — UV-visible spectra for (a) L, (b-e) Co(II), Cu(II), Fe(II) and Zn(II), respectively

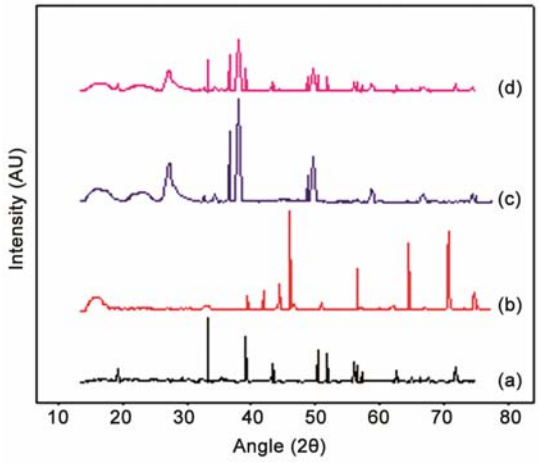


Fig. 4 — Powder XRD pattern of metal complexes (1-4)

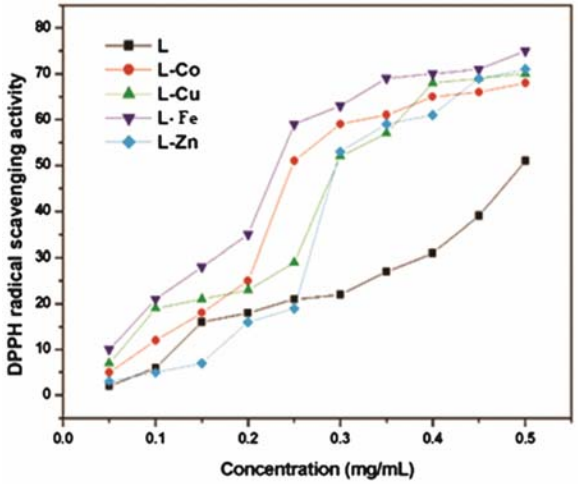


Fig. 5 — Antioxidant activity of (L) and metal complexes (1-4)

518 m/z which denotes the metal complex formation by M+1. Copper complex shows the additional water and methanol coordination 575 m/z and gives octahedral structure with M+3 molecular mass. For iron complex M+2 metal complex formations take place with one additional water coordination. Zn(II) metal complex shows the M+2 metal ion which denotes at 554 m/z.

XRD studies

The powder X-ray diffraction pattern recorded for metal complexes to detect the degree of crystalline nature of metal complexes and is represented by Fig. 4. The presence of sharp crystalline regions demonstrates the crystalline phase nonetheless broadened peaks relate to amorphous phases (1-4).

Biological evaluation DPPH radical scavenging assay

Fig. 5 represents both the antioxidant activity of ligand (L) and metal complexes (1-4). The active oxygen and free radicals exist in physical body within the sort of superoxide peroxide (H_2O_2) and hydroxyl

(OH). As normal metabolic action happening in physical body, active oxygen and free radicals are constantly formed. If they reach high levels, oxidative stress in physical body would be created, which leads to a spread of biochemical and physiological lesions and sometimes results in metabolic impairment and cell death. At the same time the various higher levels of active oxygen and free radicals could also cause lipid oxidation problems which led to a highly deteriorative process and intolerable properties of the injected foods and also loss in nutritional value. The activity of dynamic oxygen and free extremists is restricted by a decent arrangement of cancer prevention agent safeguards, including cell reinforcement mixes and proteins.

The antioxidant property of the four synthesized complexes and their corresponding ligand (ODA) was determined by using DPPH radicals. The scavenging nature of the free radical DPPH with the synthesised compounds are shown in the Fig. 6. The IC_{50} values of the (1-4) metal complexes and (L) were 0.242, 0.253, 0.223, 0.258 and 0.531 (mg/mL). Table S1 depicts the IC50 values. In the antioxidant experiment, the absorbance peak at 517 nm was decreased respect to the addition of (L) and complex (1-4). Based on the results the complex having higher antioxidant property than the (L) and marginally lesser than the standard ascorbic acid. IC_{50} values for the complex (1) concentration is less than 10 mg mL^{-1} which denotes that complex (1) has efficient antioxidant property. Complex (1) has a lower absorbance value and higher free radical scavenging activity. The order of the antioxidant ability is 3>1>2>4>L.

Docking study

Docking with BSA protein

The synthesized metal complexes (1-4) are analysed for docking with BSA protein (PDB ID: 3V03) molecule using the Autodock vina program. Two chains (A, B) in the BSA protein, are interacting with the (1-4) complexes. We can easily predict the binding amino acid residue using the Autodock vina, The B chain predominantly binds with the (1-4) complexes comparing with A. We can establish the binding sites, binding mode and binding energy in the basis of drug-BSA interactions. All the synthesised metal complexes (1-4) having the tendency to bind with the amino acid of the BSA molecule. The synthesised complexes (1-4) having binding energy -8.9, -6.2, -7.3 and -7.1 kcal mol^{-1}, respectively. In complex 1 atom C12, C13-C25-C28 binds with LEU115 amino acid residue by Pi-Sigma bond. In complex 2 Cl10 atom interact with PRO113 with conventional H-bond. In the complex (3) mainly C12, C13-C25-C28 atom binds with ARG427 amino acid residue using pi-sigma bond. Overall complex (1-4) binds with amino acid residues which are shown in (Table 2). The complex-BSA interactions and non-bonding binding and distance were shown in Fig. 6. The order of the binding with respect to binding energy is 1>3>4>2.

Table 2 — Complexes (1-4) with BSA interactions

Atoms	Interaction	Distance
Complex 1		
C1-C6	PRO117	4.12
Cl9	LEU178	3.59
C12,C13-C25-C28	LEU115	4.96
C16-C21	LEU115	5.12
Cl24	ARG144	3.93
Complex 2		
C1-C6	LEU115	5.43
C1-C6	GLU140	3.47
Cl10	PRO113	4.42
Cl23	LEU115	3.16
C16-C21	ILE141	3.74
Cl24	ARG185	3.81
Complex 3		
C12,C13-C25-C28	ILE522	2.16
C12,C13-C25-C28	ARG427	3.12
C1-C6	PRO420	5.49
Cl19	PRO420	3.12
Cl10	PRO420	4.68
C16-C21	ARG458	3.94
Complex 4		
C12,C13-C25-C28	LYS114	3.29
Cl24	ILE141	3.71
Cl24	TRY137	4.01
C16-C21	LEU115	4.16
Cl10	PRO117	3.95
Cl23	TRY160	4.26

Docking with DNA

Restraint of DNA is indispensable in the structuring of chemotherapeutic drugs, so as to capture the replication venture in the cell cycle. The edifices obstruct the cell capacities by destabilizing the DNA structure. Using the molecular docking study, we can easily understand the drug–biomolecular interaction for all the conformers of the ligand. So, it is a very useful tool for the drug discovery. We can identify the binding site, energy and mode using this software. The

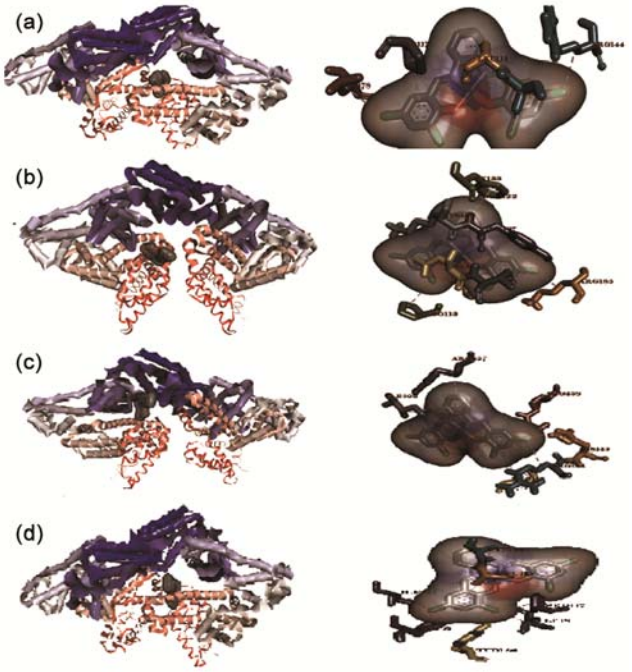


Fig. 6 — BSA binding of (L) and metal complexes (1-4)

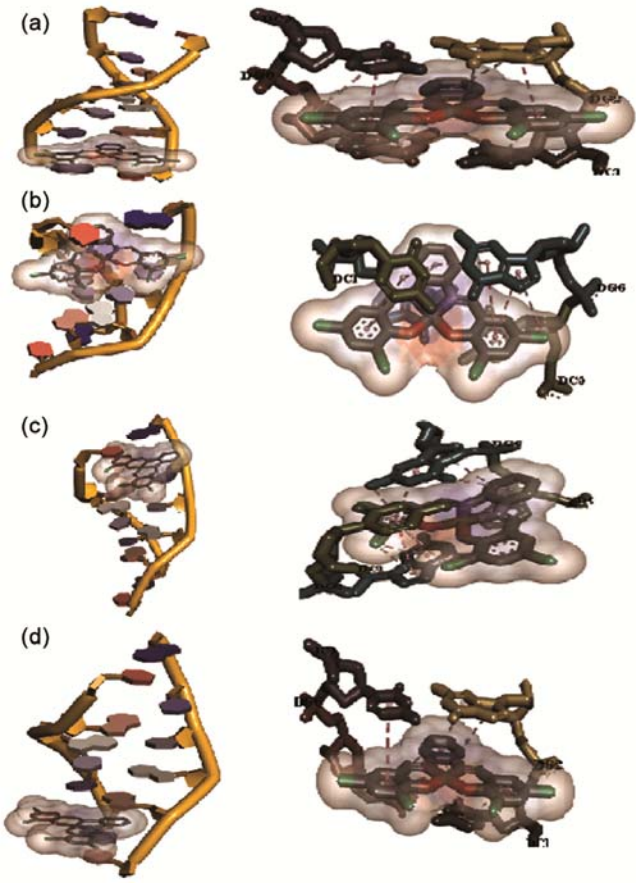


Fig. 7 — DNA binding of (L) and metal complexes (1-4)

synthesized complexes (1-4) used to dock with DNA hexamer d (CGATCG) 2 (PDB ID: 1Z3F). The complexes (1-4) shows intercalation mode of binding towards the biomolecule DNA. The complexes (1-4) which binds in-between two nucleotides are with great binding mode and binding constant value. There are nine possible conformers of the complex which can bind with DNA. The lowest binding energy value confirmation is the best mode of binding which shown in Fig. 7. The binding interaction parameters are shown in Table 3. The complexes (1-4) having binding energy values -7.9, -7.1, -7.7 and -7.3 kcal/mol, respectively. All the complexes having tendency to bind with DNA as inferred from docking studies. Observed outcomes are in correlation with earlier studies that investigate on interactive binding strategy of synthesised Schiff base with DNA/protein[21-25]. Complex (1) having the lowest negative binding value which suggests that complex (1) is capable of interacting with DNA strongly.

Antimicrobial activity

In vitro antimicrobial studies of newly synthesized complexes (1-4) were carried out using one gram positive (*Staphylococcus aureus*) and one gram negative (*Escherichia coli*) bacteria Table S2 depicts the antibacterial results of complexes (1-4). The

Table 3 — Complexes (1-4) with DNA interactions

Atoms	Interaction	Distance
Complex 1		
C12,C13-C25-C28	DG2	4.32
C12,C13-C25-C28	DG6	2.36
C1-C6	DC5	3.18
C1-C6	DG6	2.13
C16-C21	DG2	2.53
C16-C21	DC1	2.96
Complex 2		
C1-C6	DC1	4.69
C1-C6	DG2	4.26
C12,C13-C25-C28	DC1	3.51
C12,C13-C25-C28	DG6	2.83
Complex 3		
C12,C13-C25-C28	DC5	3.15
C12,C13-C25-C28	DG6	4.21
C1-C6	DC1	3.93
C1-C6	DG2	3.61
Complex 4		
C1-C6	DC5	3.21
C1-C6	DG6	3.68
C12,C13-C25-C28	DG5	4.02
C16-C21	DC1	3.96
C12,C13-C25-C28	DG2	4.33

microbial study was investigated by using Agar well diffusion method. The microbial results were compared with chloramphenicol as a standard. The growth of the microorganisms after incubation with the complexes were compared to the respective control plates using Vincent Eqn as follows.

% Inhibition = 100 (C - T) / C

Where C is the diameter of the microbial growth in the control plate and T is the diameter of the bacteria growth in the test plate. Effect of the microorganisms growth before and after incubation with the compounds were shown in Fig. S2. The copper complex shows good results towards the microbial studies. Cu(II) complex exhibits pronounced anti-oxidative and antibacterial properties along with a strong tendency to have binding interaction with DNA/BSA protein. Based on these results, anticancer studies are performed with Cu(II) as a model compound. All synthesised metal complexes may have anticancer activity but we have performed only for copper complex to demonstrate ability of synthesised complexes.

It is noteworthy that biological activities are enhanced after complex formation because that the creation of metal-ligand coordination bonding which clearly enhances the activity and the type of metal ions also determines this capacity. Therefore, bioactivity of the metal complex is higher as compared to free ligand molecule, which is well-documented in the literature. The metal complexes are more dynamic than Schiff base, which can be clarified Tweedy's chelation hypothesis[26,27].

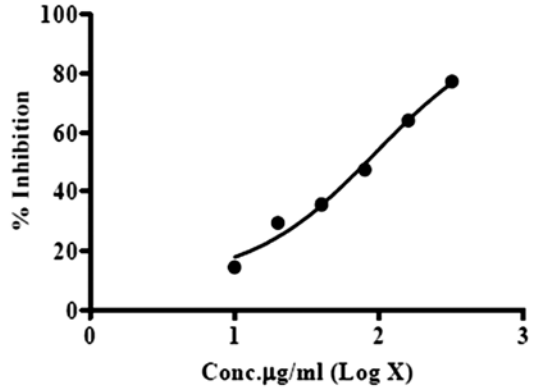


Fig. 8 — Plot for %inhibition with concentration of copper complex in MTT assay using MCF-7 cells

Table 4 — IC_{50} value determination of copper complex using various concentrations against MCF-7 cells

Sample	Conc. µg/mL	OD @ 590 nm	%Inhibition	IC50 µg/mL
Control	0	0.614	0.00	
	10	0.524	14.66	
	20	0.432	29.64	
LIC	40	0.395	35.67	94.76
	80	0.323	47.39	
	160	0.221	64.01	
	320	0.140	77.20	

The cytotoxic studies

The copper complex was treated against MCF-7 cells for determining the cytotoxicity by using MTT assay that measures mitochondrial dehydrogenase activity as an indication of the cell viability. After 48 h of incubation, the compounds can arrest the proliferation of the corresponding tumour cells. The results were examined by the cell viability curves and correlate with the IC_{50} values of the studied concentration range from 0.1-100 µM. The cytotoxic assay against MCF7 cell lines under varied concentration of synthesised LCu is displayed in Fig. S3. IC_{50} values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response. The cytotoxicity of copper complex CuL_2 is provided in Fig. S4. These results show the nature of primary ligand, ligand chelation with the metal ion, size of an atom, presence various parameter, pharmacokinetic factors and binding affinity of the metal complexes which are essential for anticancer activities of these newly synthesized complexes[28,29]. IC_{50} values for cytotoxicity tests were derived from a nonlinear regression analysis (curve fit) based on sigmoid dose response curve (variable) and computed using Graph Pad Prism. The IC_{50} value for copper complex is 94.76 (Fig. 8 and Table 4). IC_{50} of a drug can be determined by constructing a dose-response curve and examining the effect of different concentrations of antagonist on reversing agonist activity. Anticancer cell line testing with MCF 7 studies indicated that Cu(II) complex arrested the cell cycle efficiently and promoted tumor cell destruction *via* a reactive oxygen species (ROS)-mediated mitochondrial pathway. The Cu(II) complexes show great activity towards the MCF-7 cell lines.

Conclusion

The present study described synthesis and characterization of novel tetradentate ligand that belong to least explored dichlorosalicylaldehyde derivative and its metal complexes Co(II), Fe(II),

Cu(II) and Zn(II) (1-4). The antioxidant studies of the Schiff base ligand and (1-4) complexes show good results against DPPH and the order is 3>1>2>4 and (L). The theoretical studies were carried out using docking software to understand the interactions between the synthesized compounds and biomolecules. Based on the binding energy the best complex for interaction with BSA and DNA was complex (1). The cytotoxicity activities of (1-4) were carried out against MCF-7 cancer cell lines. The microbial studies were carried out for the synthesized metal complexes using gram positive and negative bacteria. The results suggest that the copper complex having a good anticancer activity and microbial activity. Cu (II) complex exhibited in stronger anti-cancer activity by synergistic effects of signifying copper Schiff base complexes exemplifying higher inhibiting efficiency of 77.2% at 320 µg/mL. Therefore, this investigation discloses potentiality of the synthesized new Schiff base derived complexes as a prodrug for cancer treatment, future aspects will be considered for better efficiency.

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

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