Indian Journal of Chemistry
Vol. 60A, May 2021, pp. 682-691

Synthesis, spectral elucidation, antibacterial, antioxidant and DNA studies of ONNO tetradentate Schiff base metal(II) complexes derived from Benzene-1,4-dicarboxaldehyde

Kumarasamy Savitha & Subramaniam Vedanayaki*

Department of Chemistry, Kandaswami Kandar's College, ParamathiVelur (TK), Tamil Nadu 638 182, India

*E-mail: ksavitha39@gmail.com (KS)/ varshuvishal@gmail.com (SV)

Received 18 December 2019; revised and accepted 10 February 2021

The new dibasic tetradentate (ONNO) Schiff base ligands ($L_1$and L_2) have been synthesized by the reaction of terephthalaldehyde with 2-amino 4-chlorophenol and X (where, X=2-amino 4-methylphenol, 2-aminophenol) in 1:1:1 molar ratio. The macrocyclic binuclear Co(II), Ni(II) and Cu((II) metal complexes are prepared in ligand to metal ratio 2:2. The elements, metal structure and binding sites of Schiff bases and its complexes are established by diverse studies like elemental, molar conductance, UV, magnetic moment, FT-IR, H^1 and C^{13} NMR, ESI-mass, ESR, thermal and powder-XRD. The spectral studies reveal that the ligands are tetradentate and its metal complexes possess a square planar geometry. All the compounds are screened for antibacterial, antioxidant and DNA cleavage and the results show high activity for metal complexes than the ligand. The DNA binding studies of Cu(II) complexes were measured by electronic absorption method. *In vitro* cytotoxicity assay of Cu(II) complexes have been tested for their tumour inhibiting potential against MCF-7 human breast cancer cell by using MTT method**.**

Keywords: Schiff bases, Square planar, Antimicrobial, Antioxidant, DNA studies, Antitumour activity

Schiff base ligands played central role as chelating ligands in the main group. Chelating Schiff bases having mono, di and polydentate donor atoms of O, N and S show biological activity and these chelating ligands are very useful for organometallic chemistry. It is bound to metal ions and Schiff base metal complexes give small changes in the geometry which depends upon its ligand having hard and soft donor atoms[1,2]. N_2O_2, NNNN and ONO donor atoms of polydentate ligands give stable complexes with transition metal ions due to their close proximity of the donor sites and it gives four, five and six member chelate rings. Symmetrical diimines (~N=HC-Ar-CH=N~ (or) ~HC=N-Ar-N=CH~) formed from the condensation of one mole of an aromatic diamine and two moles of aldehyde (or) one mole of an aromatic dialdehyde and two moles of primary amine was prepared directly. Schiff base including two different imino groups attached to the same aromatic ring form an asymmetric diimine (~N=HC-Ar-N=CH~). Recently many literatures concerning synthesis of asymmetrical diimines have been published[3]. Biological applications of Schiff base complexes are one of the most completely studied topic in coordination chemistry due to its best activities than the non-Schiff base metal complexes[4,5]. Schiff base metal complexes have an extensively diverse structure from the coordination of metal with either mono-, bi-, tri and tetra dentate ligand which is depends upon the carbonyl groups and amines. The asymmetrical tetradentate ligand complexes have been used as catalyst in some chemical process and suggested as very useful biological kinds in understanding no regular interaction of peptides[6]. Schiff base ligands are resulted in small changes in its structure can affect significantly their antiprolifer active activity and considerable cytotoxicity activities due to its varied structure[7]. Schiff base transition metal complexes has more strong properties of structural, chemical and spectral character depends upon the nature of its ligand, which are particular attention to bio-inorganic and inorganic chemistry[8,9].

Schiff bases, containing the imine group (C=N) as the central structural form, have been the subject of prevalent research in the various field of chemical industry as these are used as model analogues of certain metal enzymes. In recent years, metal-based drugs act very important role in medicinal field such as drugs for the cancer treatment, diabetes, cardiovascular diseases and anti-inflammatory[10,11].

Transition metal complexes of 2-aminophenol based compounds have vast application fields in antidepressants, antiphlogogistic, nematocide and other medicinal agents[12]. Schiff base complexes are widely studied due to synthetic flexibility, selective and sensitivity towards a diversity of organisms, potential application in fields like as oxidation catalysis, analytical and electrochemistry, dye & food industry, agrochemical and pharmaceutical fields[13-15]. Intramolecular hydrogen bonding between hydroxyl group (OH) hydrogen and imino group (CH=N) nitrogen atoms of the Schiff base and their metal complexes plays a considerable role in many fields of anti-tubercular, anti-HIV, anthelmintic, anti-amoebic, antinociceptive, antimouse hepatitis virus (MHv), inhibition of herps simplex virus type-1(HSV-1), adenovirus type-5 (AD-5), antimalarial, pesticidal, herbicidal, antipyretic, antiviral, antioxidant, antibacterial and antifungal[16-23]. Moreover, copper metal is a very important between other transition metals and it is the third most abundant metallic element in human body following the metals of iron and zinc. It plays many roles in the enzyme catalysis, necessary for the growth, maintenance and development of bones, connectivity tissues, heart, brain and many other body organs[24].

Based on the above fact, herein we have reported the synthesis of new asymmetric Schiff base ligands (4-chloro-2-((E)-(4-((E)-((2-hydroxy-5-methyl phenyl) imino)methyl)benzylidene)amino) phenol) (L_1) and 4-chloro-2-((E)-(4-((E)-((2-hydroxyphenyl)imino)methyl)benzylidene)amino-phenol (L_2) containing the azomethine (CH=N) and hydroxyl groups (OH) as potent chelating sites and its Co(II), Ni(II) and Cu(II) binuclear metal complexes evaluated by diverse physicochemical technique and their applications were analyzed for antibacterial, antioxidant, DNA studies (cleavage and binding) and antitumour activity (*in vitro* cytotoxicity).

Materials and Methods

Analytical and physical measurements

Terephthalaldehyde, 2-amino 4-chlorophenol, 2-amino 4-methylphenol, 2-aminophenol and metal salts were purchased from Sigma Aldrich. Ethanol, methanol, dimethyl solphoxide (DMSO), dimethyl formamide (DMF) and acetone were purchased from Loba and Merck chemicals, respectively. The purity of all compounds was tested by thin layer chromatography (TLC). C, H and N elements were determined on a Thermo Finningan Flash EA 1112 series elemental analyzer. Molar conductance of the compound was measured by ELICO CM 180 Conductivity Bridge. Magnetic moment values were observed using Gouy balance calibrated with $Hg[Co(SCN)_4]$ experiment. Electronic absorption spectrum was measured using Perkin Elmer Lambda - 25 spectrometer. FT-IR spectra were recorded on a Shimadzu FT-IR-8300 spectrometer using KBr pellet technique. H^1 and C^{13}-NMR spectra were obtained on a BRUKER ADVANCED III 400 MHz spectrometer using TMS used as reference. ESI-mass spectra were recorded on a Perkin- Elmer R MU-6E instrument. ESR spectra were measured on JES-FA200 ESR spectrometer with X-band frequency. Thermal analysis studies were carried out at 0-1000 ºC using SDT-Q600 V20.9 Build 20 thermal analyzer. The PXRD was recorded on Perkin Elmer TA/SDT-2960 and Philips 3701 instrument.

Synthesis of asymmetrical tetra dentate Schiff base L_1 (compound 1)

The mixture of 1 mmol of terephthalaldehyde with 1 mmol of 2-amino 4-chlorophenol and 1 mmol of 2-amino 4-methylphenol were dissolved in methanol. The solution was kept under stirring for 2 h, the formed yellow precipitate was separated by filtration, washed and purified by methanol solvent. The Schiff base solid was recrystalized from ethanol. Yellow solid, Molecular weight: 364.82, Melting point: 240 ºC, Yield: 80%, IR (KBr, cm^{-1}): 3345 (OH), 1621 (C=N), 1278 (C-O); ^{1}H-NMR (DMSO-d_6, δ, ppm): 8.75 (s, 1H, C=N), 10.1 (s, 1H, OH), 3.4(s, 3H, CH_3), 6.9-8.2 (m, Ar—H); ^{13}C-NMR (100 MHz, $CDCl_3$): 112, 113, 114, 116, 120, 124, 129, 135, 137, 147, 161; Elemental analysis (%) of $C_{21}H_{17}ClN_2O_2$: calculated (found): C, 69.14 (68.86); H, 4.70 (4.50); N, 7.68 (7.98); ESI-mass: m/z: $(M+1)^+$ 365.

Synthesis of asymmetrical tetra dentate Schiff base L_2 (compound 5)

The procedure is the same as L1 using terephthalaldehyde with 2-amino 4-chlorophenol and 2-aminophenol. The dark yellow precipitate was formed. Molecular weight: 350.08, Melting point: 250 ºC, Yield: 80%, IR (KBr, cm^{-1}): 3346 (OH), 1621 (C=N), 1369 (C-O); ^{1}H-NMR (DMSO-d_6, δ, ppm): 8.80 (s, 1H, C=N), 9.4 (s, 1H, OH), 6.8-7.3 (m, Ar—H); ^{13}C-NMR (100 MHz, $CDCl_3$): 110, 115, 116,120, 123, 124, 126, 127, 128,130, 137, 138, 148, 150, 152; Elemental analysis (%) of $C_{20}H_{15}ClN_2O_2$: calculated

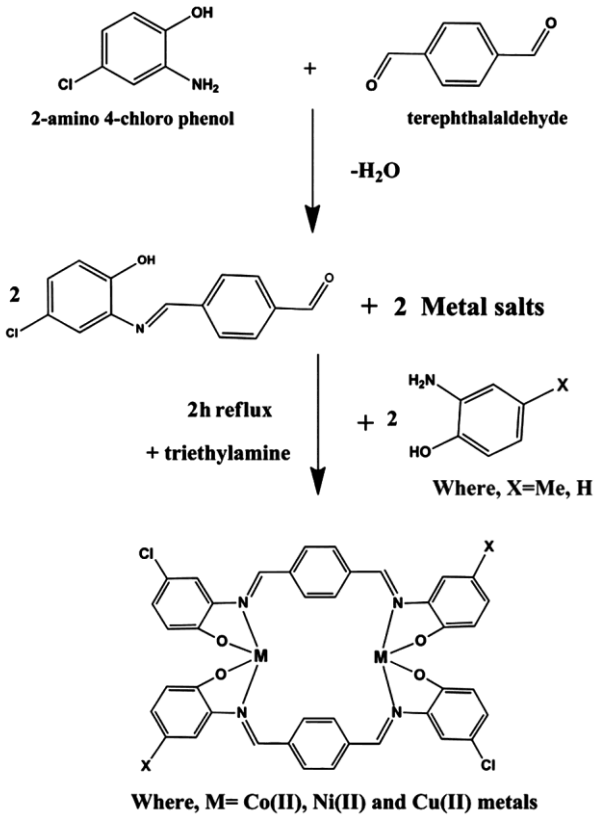
(found): C, 68.48 (67.71); H, 4.31 (4.13); N, 7.99 (8.12); ESI-mass: m/z: $(M+1)^+$ 351.

Synthesis of binuclear metal(II) complexes

The homo binuclear metal(II) complexes were synthesized by using template method[25]. The mixture of terephthalaldehyde (2 mmol) with 2-amino 4-chlorophenol (2 mmol) and X (where, X = 2-amino 4-methylphenol, 2-aminophenol) (2 mmol) were dissolved in methanol, which were added to the methanolic solution of metal salts (2 mmol) like Co, Ni and Cu. The mixture was stirred and few drops of tri ethylamine were added to the mixture. It was stirred for 1 h and refluxed for 3 h. The product was partly evaporated, cooled at room temperature. The obtained metal complexes were separated by filtration, washed with methanol and diethyl ether, stored in room temperature. The structure of metal complexes was show in Scheme 1.

Schiff base binuclear Co(II) complex (compound 2)

Brownish black solid, Molecular weight: 903.62, Melting point: >300 ºC, Yield: 78%, IR (KBr, cm^{-1}): 1606 (C=N), 1384 (C-O), 518 (M-O), 492 (M-N); Elemental analysis (%) of $C_{46}H_{42}Cl_2Co_2N_4O_4$: calculated (found): C, 61.14 (60.80); H, 4.68 (4.51); N, 6.20 (6.18); M, 13.04 (12.98); Molar conductance (Ω^{-1} cm^2 mol^{-1}) 10.6.



Scheme 1 — Synthesis of metal complexes by template method

Schiff base binuclear Ni(II) complex (compound 3)

Reddish yellow solid; Molecular weight: 903.14; Melting point: >300 ºC; Yield: 76%; IR (KBr, cm^{-1}): 1565 (C=N), 1385 (C-O), 515 (M-O), 467 (M-N); Elemental analysis (%) of $C_{46}H_{42}Cl_2N_4Ni_2O_4$: calculated (found): C, 61.17 (61.12); H, 4.69 (4.53); N, 6.20 (6.21); M, 13.00 (12.81); Molar conductance (Ω^{-1} cm^2 mol^{-1}) 11.3.

Schiff base binuclear Cu(II) complex (compound 4)

Black solid; Molecular weight: 912.85; Melting point: >300 ºC; Yield: 79%; IR (KBr, cm^{-1}): 1601 (C=N), 1397 (C-O), 513 (M-O), 462 (M-N); Elemental analysis (%) of $C_{46}H_{42}Cl_2Cu_2N_4O_4$: calculated (found): C, 60.52 (60.35); H, 4.64 (4.55); N, 6.14 (6.04); M, 13.92 (13.82); Molar conductance (Ω^{-1} cm^2 mol^{-1}) 13.5.

Schiff base binuclear Co(II) complex (compound 6)

Wine red solid; Molecular weight: 875.57; Melting point: >300 ºC; Yield: 79%; IR (KBr, cm^{-1}): 1601 (C=N), 1385 (C-O), 583 (M-O), 468 (M-N); Elemental analysis (%) of $C_{44}H_{38}Cl_2Co_2N_4O_4$: calculated (found): C, 60.36 (59.81); H, 4.37 (4.17); N, 6.40 (6.30); M, 13.46 (13.25); Molar conductance (Ω^{-1} cm^2 mol^{-1}) 10.3.

Schiff base binuclear Ni(II) complex (compound 7)

Yellowish brown solid; Molecular weight: 875.09; Melting point: >300 ºC; Yield: 75%; IR (KBr, cm^{-1}): 1583 (C=N), 1383 (C-O), 598 (M-O), 443 (M-N); Elemental analysis (%) of $C_{44}H_{38}Cl_2N_4Ni_2O_4$: calculated (found): C, 60.39 (60.15); H, 4.38 (4.19); N, 6.4 (6.30); M, 13.41 (13.19); Molar conductance (Ω^{-1} cm^2 mol^{-1}) 11.7.

Schiff base binuclear Cu(II) complex (compound 8)

Black solid; Molecular weight: 884.79; Melting point: >300 ºC, Yield- 78%; IR (KBr, cm^{-1}): 1572 (C=N), 1394 (C-O), 581 (M-O), 477 (M-N); Elemental analysis (%) of $C_{44}H_{38}Cl_2Cu_2N_4O_4$: calculated (found): C, 58.73 (58.82); H, 4.33 (4.22); N, 6.33 (6.21); M, 14.36 (13.10); Molar conductance (Ω^{-1} cm^2 mol^{-1}) 13.8.

Pharmacological Studies

Antibacterial activity

In vitro antibacterial activity was performed by the Disc-agar well diffusion experiment. The asymmetric

tetradentate Schiff base ligands and their homo binuclear complexes were screened against bacteria of gram-positive (*S. aureus*, *B. subtillis*) and gram-negative (*K. neumoniae*, *E. coli*). Tetracycline was used as standard drug for antibacterial activity and the tested compounds were dissolved in DMSO. The antibacterial activities were maintained in nutrient agar well plates at 4 ºC. The 100 µL concentration of culture supernatant was placed on agar well plate and then it was incubated for 24 h at 37 ºC. The antibacterial activity was determined by measuring the diameter of the zone indicating complete inhibition [26].

Antioxidant activity

Schiff base ligands (compound: 1, 5) and their metal (compounds: 2, 3, 4, 6, 7, 8) complexes were investigated by using its free radical scavenging activity on the stable DPPH free radicals described in the literature[27]. The scavenging activity investigates the antiradical power of an antioxidant by calculating the decrease in the absorbance wavelength of DPPH at 510 nm. The ligands and its metal complexes exhibit DPPH free radical scavenging activity at different concentrations (like as 10, 20, 30, 40 and 50 µL). The percentage of DPPH free radical scavenging ability was measured by using the following formula,

$$I\ (\%) = \ (A_c - A_s)/A_c \times 100$$

where, A_c and A_s are the absorbance of the control and tested sample, respectively. IC_{50} values were calculated for ligand and its complexes, which showed the significant ability and it is defined as concentration sufficient to generate 50% of maximum scavenging activity. Ascorbic acid was used as standard.

DNA binding studies

Electronic absorption spectroscopy

Electron absorption spectroscopy is one of the most useful technique to determine the interaction between the metal complexes with DNA from the stock solution of calf thymus (CT)[28]. DNA was prepared by 5 mM Tris-HCL/20 mM NaCl buffer (p^H=7.2) at room temperature. The stock solution was stored at 4 ºC and used up to 4 days. The buffer solution of CT- DNA gave a ratio of ~1.8-1.9 and the absorbance at 260 to 280 nm. The concentration of CT-DNA was determined using the known molar extinction coefficient value of 6600 M^{-1} cm^{-1} at absorption intensity is 260 nm. The stock solution concentrations of the complexes were prepared by dissolving the Cu(II) complexes (compounds 4, 8) in DMSO. All the experiments were maintained at proper dilution with corresponding buffer to the required concentration. The binding constant (K_b) was calculated by using the following equation,

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_a - \varepsilon_f) + 1/K_b\ (\varepsilon_a - \varepsilon_f)$$

where, [DNA] is molar concentration of DNA in base pairs, ε_a, ε_f are apparent extinction coefficient, the K_b values were obtained from the ratio between the equation of DNA/ (ε_a-ε_f) versus [DNA] in each case.

Agarose gel electrophoresis assay

The cleavage of super coiled pBR322 DNA was investigated by gel electrophoresis method[29]. The agarose gel electrophoresis studies were carried out incubation of the mixtures containing 20 µL pBR322 DNA, 50 mM of NaCl, 50 mM of metal complexes and 50 mM H_2O_2 in Tris-HCl buffer (p^H=7.4) at 37 ºC for 1 h. After incubation, the sample compounds were electrophoresed at 60 ºC for 2 h on 1% of agarose gel using TAE (Tris-Acetic acid-EDTA) buffer (p^H=8.0). After 0.5 µg/mL of ethidium bromide was used, the gel was stained. Thus all the experiments were performed at room temperature and photographed under UV light at 360 nm.

Antitumor activity

In vitro cytotoxicity was carried out on a MCF-7 cell line in which cell viability was tested using MTT (3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide) activity[30]. In vitro effect of growth inhibition of Cu(II) complexes (4, 8) were measured by spectrophotometric experiment. This experiment was used to determine the MTT conversion into "formazan" by living cells and it was maintained in a 96-well microplate having DMEM/F12 plain media supplemented with 10% heat inactivated FBS (Fetal Bovine Serum) having 5% of mixture of 100 µg/mL of streptomycin and 100 units/ml of penicillin were incubated at 37 ºC for 24 h in the presence of 5% CO_2. In this study, various concentrations of 10, 20, 40, 80, 160 and 320 µL of the stock solution was prepared in DMSO, which were added to relevant wells having 100 µL of the DMSO medium. After incubation, 100 µL of MTT stock solution (5 mg/10 mL of MTT in PBS) was added to each well and further incubated for 4 h. After

the supernatant was removed, the plates were gently shaken to form solubilise formazan crystals by adding 100 µL of DMSO. The absorbance was measured at wavelength 590 nm by microplate reader. The activity was performed in triplicate and used to calculate the mean.

Results and Discussion

The synthesized ligands and their metal complexes were investigated by diverse physicochemical methods. The resultant compounds are soluble in DMF and DMSO, partially soluble in ethanol and methanol. The molar conductance of ligands and their complexes were measured at 25 ºC in DMF solution (10^{-3} M) indicates low conductance values (10.3 to 13.8), which shows that the complexes are non-electrolytic nature[31, 32].

Electronic absorption spectral data

The UV-visible spectral data of the ligand and its complexes were measured within a 200-800 nm wavelength at room temperature in DMSO solution. In the electronic spectra of the mixed ligands (L_1 and L_2) were exhibit absorption bands at 300, 299 and 392, 388 nm, which are referred to the aromatic benzene rings ($\pi\rightarrow\pi^*$) and imine group ($n\rightarrow\pi^*$), respectively[33]. The diffuse reflectance spectrum of the macrocyclic metal(II) compounds 2, 3,4, 6, 7 and 8 show bands at 442, 439, 440, 436, 414 and 438 nm, which may be attributed to the ligand to metal co-ordinations (L→M transitions) in square planar geometry of the compounds (given in Table 1). The magnetic moment and absorption spectra is used to determine the geometry of the metal complexes[34].

FT-IR studies

IR spectrum is used to investigate of the existence of intra molecular hydrogen bonding, the nature of the co-ordination mode in the metal complexes and presence of the tautomeric forms in the solid states. The important vibrational frequency of the free ligands (L_1 and L_2) were exhibited bands at 1621 and 1621 cm^{-1}, which indicates the formation of imine groups(C=N) [35]. However, on complexation this band (C=N) is shifted to lower energy range 1606-1565 $cm^{-1,}$ suggesting the participation of the azomethine nitrogen atom in co-ordination to the metal ion. The sharp broad bands around 3345 and 3346 cm^{-1} in the ligands (L_1 and L_2) were assigned to the phenolic –OH groups. The absence of ν(OH) bands in the metal complexes, indicates the co-ordination of the ν(OH) groups after deprotonation. The ν(C=O) band at 1278 and 1369 cm^{-1} for L_1 and L_2 were lower frequency than the band at about 1700 cm^{-1} for ν(C=O) , this frequency change shows the formation of azomethine group(ligand). The appearance of new bands at 443-492 and 513-598 cm^{-1} in the vibrational spectra of the complexes were assigned to $\nu_{(M-N)}$ and $\nu_{(M-O)}$ frequencies, respectively[36]. These vibrations were clearly suggesting the co-ordination of the metal ions with the azomethine nitrogen and phenolic oxygens in the complex.

Table 1 — UV absorption data of ligands and their metal(II) complexes

S. No.	Compounds	$\pi-\pi*$	$n\rightarrow\pi*$	L→M	Geometry	μ_{eff} (BM)
1	[L1]	300	392	---	---	---
2	$[Co_2L_2]$	251	374	442	Square planar	1.82
3	$[Ni_2L_2]$	253	370	439	Square planar	---
4	$[Cu_2L_2]$	252	378	440	Square planar	1.71
5	[L2]	299	388	---	---	---
6	$[Co_2L_2]$	271	354	436	Square planar	1.80
7	$[Ni_2L_2]$	251	370	414	Square planar	---
8	$[Cu_2L_2]$	279	369	438	Square planar	1.76

NMR spectral studies

^{1}H-NMR spectra

The ^{1}H NMR spectra of the ligands of L_1 and L_2 were recorded at 25 ºC (RT) in DMSO-d^6 solvent with TMS as internal standard. The azomethine (CH=N) proton signal was obtained at δ 8.75 and δ 8.80 ppm, this signal indicates the formation of mixed Schiff base ligands (Fig. 1)[37]. The new peaks of phenolic –OH protons of the aminophenol and aromatic protons were observed at δ 10.1, δ 9.4 ppm, and δ 6.9-8.2, δ 6.8-7.3 ppm[38]

^{13}C-NMR spectra

^{13}C- NMR spectra of the synthesized ligands (L_1 and L_2) are shifted to δ 161 and δ 152 ppm for azomethine carbon atom. The peaks observed at δ 147, δ 150 ppm and δ 112-129, δ 137-110 ppm are due to phenolic –OH carbons by aminophenol and aromatic benzene ring carbon atoms for ligands L_1 and L_2 are shown in Fig. 2. The peaks appeared at δ 137, δ 138 ppm are due to substituted Cl atoms by aminophenol for L_1 and L_2. The signal appeared at δ 135 ppm was referred to the methyl group of the 2-aminophenol moiety for L_1 [39].

ESI-mass spectrum

The mass spectra of Schiff base ligands (L_1, L_2) and their copper complexes (compounds 4, 8) as

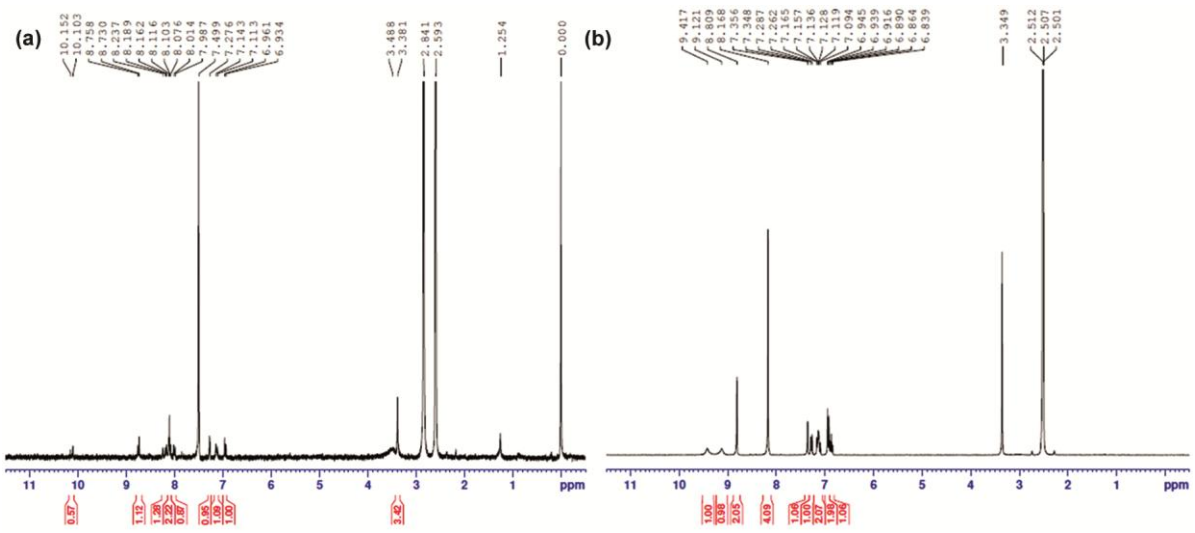


Fig. 1 — H^1-NMR spectra of (a) L_1 and (b) L_2

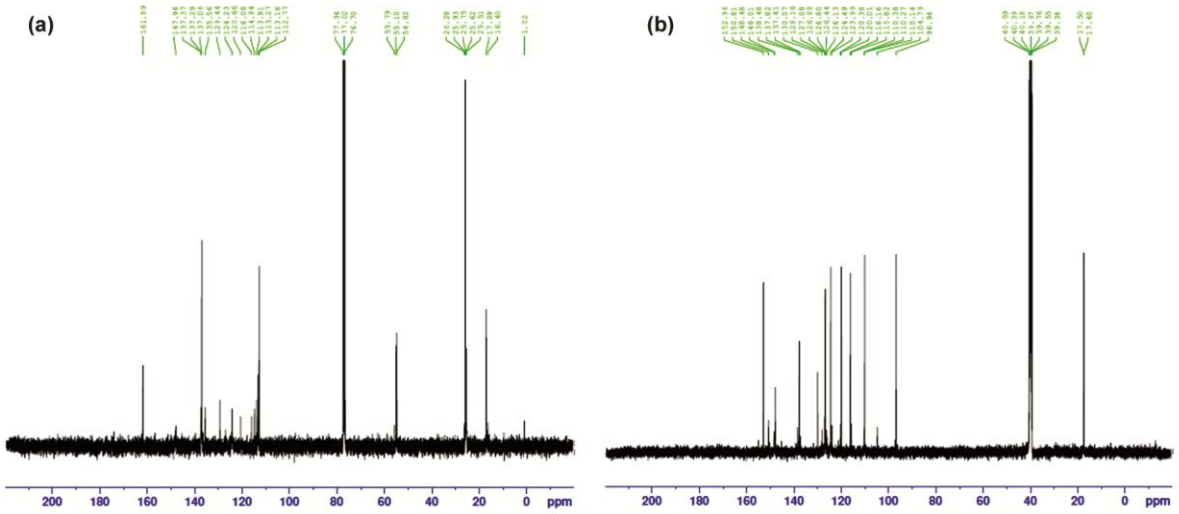


Fig. 2 — C^{13}-NMR spectra of (a) L_1 and (b) L_2

shown in Supplementary Data, Fig. S1, have molecular ion peaks at m/z 365, 351 $(M+1)^+$ and 913, 885 $(M+1)^+$, which corresponds to the proposed molecular formula of L_1 ($C_{21}H_{17}ClN_2O_2$), L_2 ($C_{20}H_{15}ClN_2O_2$) and their Cu complexes 4 ($C_{46}H_{42}Cl_2Cu_2N_4O_4$), 8 ($C_{44}H_{38}Cl_2Cu_2N_4O_4$), respectively[40]. The mass spectral data of ligands (L_1 and L_2) and their copper (II) complexes (compounds 4, 8) were confirmed by comparing their molecular formula weights with (m/z) mass values, which is in good agreement for these ligands (compound 1 and 5) and Cu (compound 4 and 8) complexes.

Electron paramagnetic resonance spectra

The electron paramagnetic resonance (epr) studies of the Cu(II) complexes (compound 4 and 8) gives information about hyperfine and superhyperfine structures and about the nature of the bonding between the copper ion and their ligand[41]. The epr spectra of compound 4(Cu_2L_2) and compound. 8(Cu_2L_2) exhibit an axial symmetry at X-band frequencies in the solid state as shown in Fig. 3. The obtained g-values are g_{II} = 2.2391, $g_{\perp}$ = 2.0554 and g_{II} = 2.2418, $g_{\perp}$= 2.0498 for compounds 4 and 8, which are related by $G=(g_{II}-2)/(g_{\perp}-2)=4.0$. If G value is more than 4 i.e., (G>4), it indicates no considerable exchanging interaction between the two copper centers in the complex, whereas if G value is less than 4 (G<4), it shows that the exchange interaction occurs in the solid state complex. From the observed G value of Cu(II) complexes are 4.31 and 4.85 for compounds

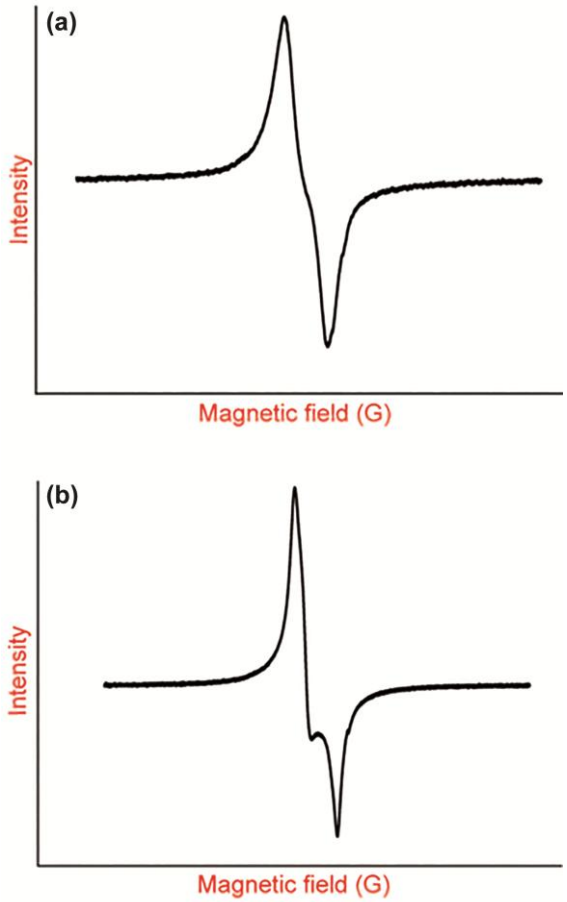


Fig. 3 — EPR spectra of Cu(II) complexes: (a) compound 4 and (b) compound 4

4 and 8. It is clear that $g_{II}>g_{\perp}>2.0023$, which indicates d_{x2-y2} in orbital ground state for square planar structure of the Cu(II) complexes 4 and 8.

Thermal studies

The thermal analysis (TG & DSC) was used to determine thermal stability of compounds in the presence of air atmosphere at the temperature range between 0 to 1000 ºC[42]**.** Thermograms of the ligands (L1 and L2) and their complexes (2, 3, 4, 6, 7 and 8) are shown in Supplementary Data, Fig. S2. The both TG and DSC analysis of compounds have three steps of decomposition process each. The first, second and third steps correspond to the elimination of small groups of substituted Cl atoms by 2-aminophenol, the removal of total ligand moiety and the formation of metal oxide residue.

Powder XRD analysis

The structure of Schiff bases and their binuclear metal complexes were investigated using P-XRD

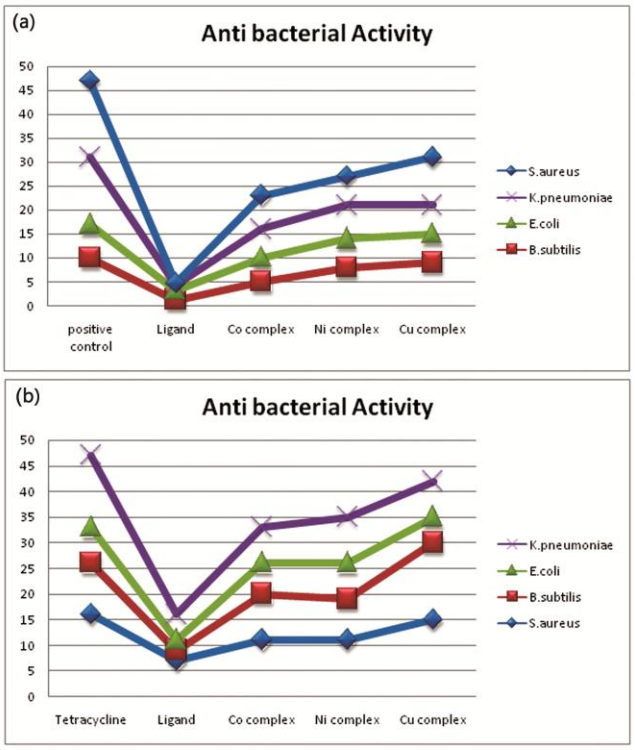


Fig. 4 — Antibacterial activity of (a) ligands (1 and 2) with (b) their metal (Co(II), Ni(II) and Cu(II)) complexes

analysis which indicates the amorphous orthorhombic crystal[43] nature of compounds within the range 10-90 ºC (2θ) and the wavelength is 1.5406 Å (Supplementary Data, Fig. S3). Thus all the compounds show crystalline nature from its observed peaks. The powder-XRD provides the d-value, relative intensity and 2θ value for each peak.

Biological applications

Antibacterial assay

The antibacterial results showed that the Schiff base ligands have very low or no inhibition activity compared to their metal complexes[44]. Thus, Cu(II) complexes (compounds 4, 8) have exhibited higher antibacterial activity than the other Co(II) (compounds 2, 6) and Ni(II) (compounds 3, 7) complexes. Antibacterial activities of L1 and L2 with their metal complexes are shown in Fig. 4.

Antioxidant assay

DPPH free radical scavenging activity

The ligands (compounds 1, 2) and their Co(II) (compounds 2,6), Ni(II) (compounds 3, 7) and Cu(II) (compounds 4, 8) complexes were screened for their DPPH free radical scavenging ability using Ascorbic acid as standared[4] . The ligands have very low activity

when compared to all the complexes as shown in Fig. 5. The scavenging activity of Cu(II) complexes are higher than that of other metal complexes. The IC_{50} values are determined for all compounds and the IC_{50} values of [Cu_2 ($C_{46}H_{42}N_4O_4Cl_2$)] and [Cu_2 ($C_{44}H_{38}N_4O_4Cl_2$)] have shown significant activity compared to other complexes and ligands. The order of scavenging activity of all complexes according to their IC_{50} values is given below.

Ascorbic acid > [Cu(II) complexes] > [Ni(II) complexes]> [Co(II) complexes]> ligands

DNA- copper complex interaction studies

The interactions of CT-DNA and metal complexes (4, 8) were determined by the electronic absorption spectroscopy technique. From the studies it is revealed that the intensity changes of the intra ligand $\pi \rightarrow \pi^*$ transition band occur at 250-280 nm[46]. The UV absorption experiments of Cu(II) complexes (compounds 4, 8) in presence of buffer solution are performed by using a fixed concentration of complex to which increments of the stock solution are added.

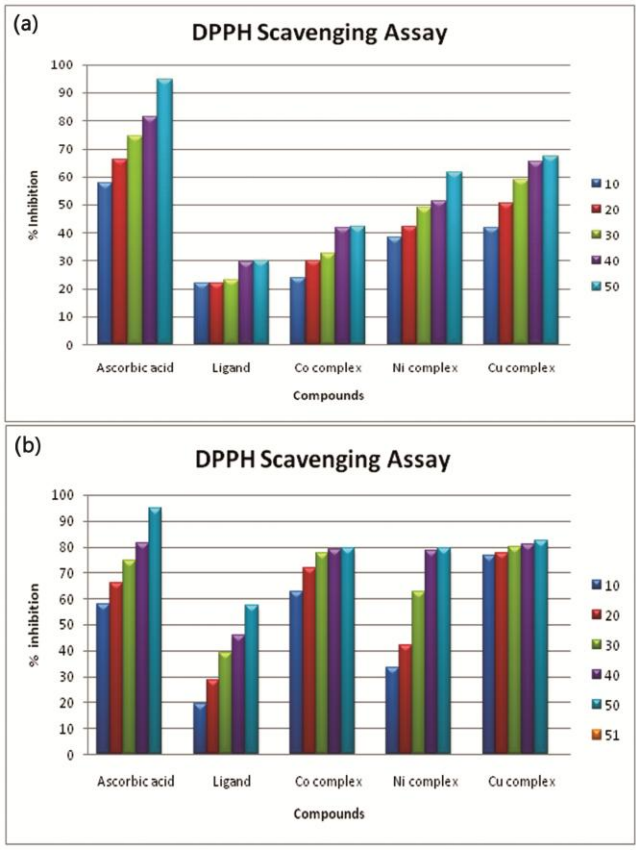


Fig. 5 — Antixoidant activity of (a) ligands (1 and 2) with (b) their metal (Co(II), Ni(II) and Cu(II)) complexes

The interaction of [Cu_2 ($C_{46}H_{42}N_4O_4Cl_2$)] and [Cu_2 ($C_{44}H_{38}N_4O_4Cl_2$)] complexes with duplex DNA led to a decrease in the intensities and a small amount of red shift in the electronic absorption spectra and the concentration of DNA increases with the absorption bands of Cu(II) complexes are affected to a considerable extent. This absorption spectra indicate clearly that the addition of CT-DNA to the Cu(II) complexes yields hypochromic and red shift (shown in compound 8) to the ratio of [DNA]/[Cu] for the compounds 4, 8 complexes and the complexes interact with CT-DNA most likely through a binding mode that involves $\pi \rightarrow \pi^*$ stacking interaction between the aromatic chromophore and the base pair of DNA as shown in Fig. 6.

H_2O_2 induced DNA damage/ production assay

Gel electrophoresis activity is a method to determine different binding modes of newly synthesized complexes to super coiled pBR322 DNA. The natural- derived plasmid pBR322 DNA has three forms of closed-circle super coiled form (form-I),

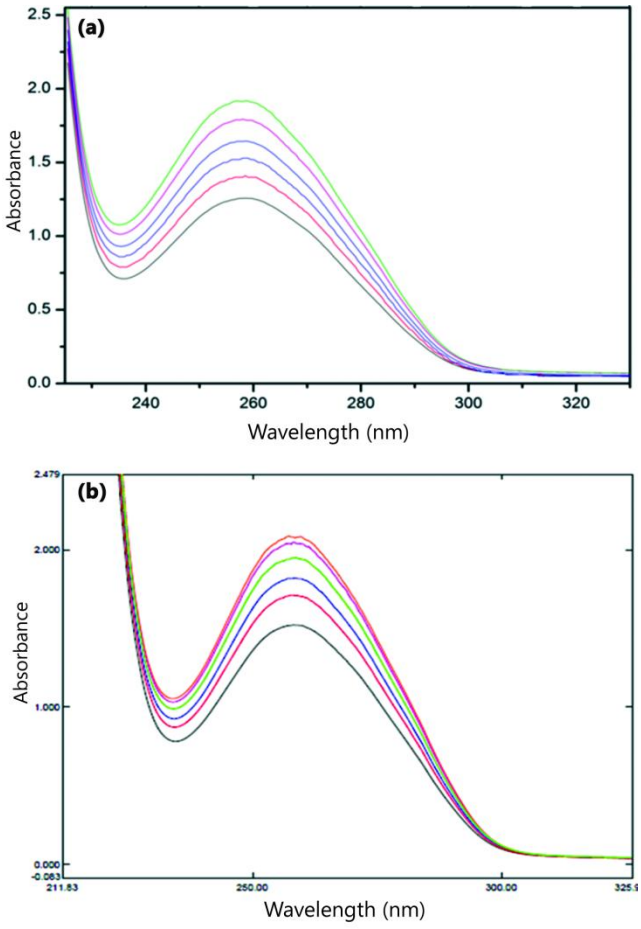


Fig. 6 — Absorption spectra of Cu complexes (compd. 4 and 8)

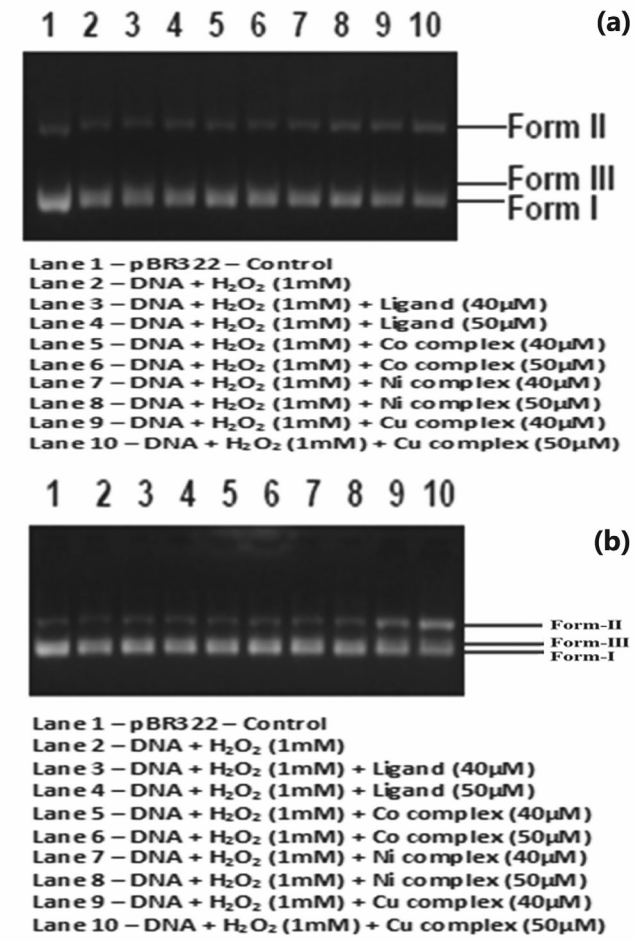


Fig. 7 — DNA cleavage studies of (a) ligands (1 and 2) and (b) their metal (Co(II), Ni(II) and Cu(II)) complexes

nicked form (form-II) and linear form (form-III)[47]. The circular plasmid DNA is conducted by electrophoresis, relatively the fasted migration measured for the super coiled form (form-I). If cleavage occurs on one strand, the super coils will relax to generate slowed moving open circular form (form-II). If both of strands are cleaved a form-III (linear form), it will be produced that moves in between super coiled form and open circular form. From the results (Fig. 7), it indicates that the gel electrophoretic separation of plasmid pBR322 DNA, interaction with metal complexes in the presence of H_2O_2. The addition of the complexes with the mixture of form-II and form-III, which form the cleavage of super coiled DNA. The reports show that the compounds 2, 3, 4, 6, 7 and 8 metal complexes induced intensively the cleavage of DNA in the presence of H_2O_2. These observations suggested that all dinuclear complexes effectively cleave the plasmid pBR322 DNA.

Table 2 — Antitumor activity of Cu(II) complexes

Compound name	Concentrations (μg/mL)	Absorbance at 590 nm	Toxicities (%)	IC_{50} (μg/mL)
Control (MCF-7)	0	0.984	0.00	
Cu(II) complex (4)	10	0.846	14.06	53.7
	20	0.732	25.57	
	40	0.578	41.21	
	80	0.403	59.04	
	160	0.318	67.73	
	320	0.152	84.56	
Cu(II) complex (8)	10	0.812	17.48	84.6
	20	0.702	28.70	
	40	0.638	35.21	
	80	0.513	47.89	
	160	0.387	60.69	
	320	0.274	72.12	

In vitro cytotoxicic assay

To determine the cytotoxicity effect, the newly synthesized homo binuclear Cu(II) complexes (compounds 4, 8) were treated with human breast cancer cell line (MCF-7) by MTT experiments method[48]. The absorbance value is lower than the control cell which indicates a reduction in the rate of cell proliferation. Contrary, a higher absorbance rate show an increase in cell proliferation cell survival is almost 50% after 24 h of incubation with Cu(II) complexes (compounds 4, 8) (Fig. 7). The anticancer results of complexes revealed that Cu(II) complexes (compounds 4, 8) exhibit significant cytotoxic effect. The IC_{50} value and percentage of inhibition of Cu(II) complexes (compounds 4, 8) are given in Table 2.

Conclusions

Macrocyclic binuclear metal complexes of compounds 2, 3, 4, 6, 7 and 8 of terephthalaldehyde with 2-amino 4-chlorophenol and X (X = 2-amino 4-methylphenol, 2-aminophenol) were synthesized, characterised by using physicochemical techniques and various spectroscopic studies. The spectral data showed that the all metal complexes are four coordinated and possess square planar structure around the metal ion. The antibacterial, antioxidant and DNA cleavage activity of the metal complexes reveal more potent than the free ligands. The antitumour and DNA binding studies of Cu(II) complexes of compounds 4, 8 have significant activity. The Cu(II) complexes gives the more biological efficiency of the above biological activites (antimicrobial, DNA cleavage, binding, antioxidant

and antitumour) when compared to study of all binuclear Co(II) and Ni(II) complexes.

Supplementary Data

Supplementary data associated with this article are available in the electronic form at http://nopr.niscair.res.in/jinfo/ijca/IJCA_60A(05)682-691_SupplData.pdf.

Reference

1 Maurya RC, Patel P & Rajput S, *Synth React Inorg Met Org Chem*, 33 (2003) 801.
2 Deligonul N, Tumer M & Serin S, *Trans Met Chem*, 31 (2006) 920.
3 Gungor O Gurkan P, *Spectrochimica Acta Part A*, 77 (2010) 304.
4 Crans D C, Woll K A, Prusinskas K, Johnson M D & Norkus E, *Inorg Chem*, 52 (2013) 12262.
5 Nagesh G Y & Mruthyunjayaswamy B H M, *J. Mol Struc*, 1085 (2014) 198.
6 Boghaei D M & Mohebi S, *Tetrahedron*, 58 (2002) 5357.
7 Vieira A P, Wegermann C A, Da Costa & Ferreira A M, *New J Chem*, 42 (2018) 13169.
8 Yilmaz I, Temel H & Alp H, *Polyhedron*, 27 (2008) 125.
9 Ziyadanogullari B, Cevizic D, Temel H & Gullari R Z, *J Hazard Mater*, 150 (2008) 285.
10 Fricker S P, *Dalton Trans*, 43 (2007) 4903.
11 Crichton R R, Dexter D T & Ward R J, *Coord Chem Rev*, 252 (2008) 1189.
12 Benjamin Chibuzo Ejelonu, *IOSR J Appl Chem*, 9 (2016) 12.
13 Das P & Linert W, *Coord Chem Rev*, 311 (2016) 1.
14 Maher A & Mohammed S R, *Int J Cur Res Rev*, 7 (2015) 6.
15 Sobola A O, Watkins G M &Van Brecht B, *S Africa J Chem*, 67 (2014) 45.
16 Mishra P, Rajak H & Mehta A, *J Gen Appl Microbia*, 51 (2005) 133.
17 Mathew B, Vakketh S S & Kumar S S, *Der Pharma Chemica*, 2 (2010) 337.
18 Omar T N, *Iraqi J Pharm Sci*, 16 (2007) 5.
19 Melnyk P, Leroux V, Sergheraert C & Grellier P, *Bioorg Med Chem Lett*, 16 (2006) 31.
20 Harpstrite S E, Collins S D, Oksman A, Goldberg D E & Sharma V, *Med Chem*, 4 (2008) 392.
21 Aydogan F, Ocal N, Turgut Z & Yolacan C, *Bull Korean Chem Soc*, 22 (2001) 476.
22 Kumar K S, Ganguly S, Veerasamy R & Clercq E D, *Eur J Med Chem*, 45 (2010) 5474.
23 Alam M S, Choi J H & Lee D U, *Bioorg Med Chem*, 20 (2012) 4103.
24 Iftikhar B, Javed K, Saif Ullah Khan M, Akhter Z, Mirza B & Mckee V, *J Mol Stru*, 1155 (2018) 337.
25 Naeimi H, Rabiei K & Salimi F, *J Coord Chem*, 62 (2009) 1199.
26 Jayaseelan P, Prasad S, Vedanayaki S & Rajavel R, *Eur J Chem*, 2 (2011) 480.
27 Kavitha P, Saritha M & Laxma Reddy K, *Spectrochim Acta Part A*, 102 (2012) 159.
28 Srinivasulu K, Reddy K H, Anuja K, Dhanalakshmi D & Ramesh G, *Asian J Chem*, 31 (2019) 1905.
29 Raman N, Dhaveethu Raja J & Sakthivel A, *J Chem Sci*, 119 (2007) 303.
30 Domotor O, de Almeida R F M, Corte-Real L, Matos C P, Marques F, Matos A, Real C, Kiss T, Enyedy E A, Garcia M H & Tomaz A I, *J Inorg Biochem*, 168 (2016) 27.
31 Khedr A M & Marwani H M, *Int J Electrochem Sci*, 7 (2012) 10074.
32 Radhika P, Muhammed Basheer U & Krishnankutty K, *Arch Appl Sci Res*, 4 (2012) 2223.
33 Singh N P, Tyagi V P & Ratnam B, *J Chem Pharm Res*, 2(1) (2010) 473.
34 Abdalrazaq E A, Al-Ramadane O M & Al-Numa K S, *Am J Appl Sci*, 7 (2010) 628.
35 Thakor Y J, Patel S G & Patel K N, *J Chem Pharm Res*, 2 (2010) 518.
36 Halli M B, Vithal Reddy P, Sumathi R B & Basavaraja A, *Der Pharma Chemica*, 4(3) (2012) 1214.
37 Shebl M, Khalil S M E, Ahmed S A & Medien H A A, *J Mol Struct*, 980 (2010) 39.
38 Pawar R K, Sakhare M A & Arbad B R, *Int J Chem Sci*, 14 (2016) 2575.
39 Senthil Kumaran J, Priya S, Jayachandramani N & Mahalakshmi S, *J Chem Pharma Res*, 5(7) (2013) 56.
40 Sakthi M & Ramu A, *J Mol Struct*, 1149 (2017) 779.
41 Vamsikrishna N, Pradeep Kumar M, Ramesh G, Ganji N, Daravath S & Shivaraj, *J Chem Sci*, 129 (2017) 609.
42 Prashanthi Y & Shiva Raj, *J Sci Res*, 2 (2010) 114.
43 Biradar V D & Mruthyunjayaswamy B H M, *World J*, (2013) 1.
44 Raman N, Sobha S & Mitu L, *J Saudi Chem Soc*, 17 (2013) 151.
45 Colak, Terzi U, Col M, Karaoglu S A, Karabocek S, Kuçukdumlu A & Ayaz F A, *Eur J Med Chem*, 45 (2010) 5169.
46 Hu K Liu C Li J Liang F, *Med Chem Commun*, 9 (2018) 1663.
47 Akila E Usharani M Rajavel R, *Int J Bio Tech Res* 3 (2013) 61.
48 Marques M P M, Girao T, Pedroso D, Lima M C, Gameiro A, Pereira E & Garcia P, *Biochem Biophy Acta*, 1589 (2002) 63.