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Synthesis, Characterization and *in vitro* Anticancer Studies of New Co(II), Ni(II), Cu(II) and Zn(II) Complexes of (*E*)-4-((Quinoline-8-ylimino)methyl)benzene-1,2,3-triol Ligand

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ABSTRACT

Herein, a new tridentate (NNO) Schiff base ligand, (*E*)-4-[(quinoline-8-ylimino)methyl]benzene-1,2,3-triol derived from the condensation of 8-aminoquinoline with 2,3,4-trihydroxy benzaldehyde is reported. The ligand was complexed with certain metal ions like Co(II) (**1**), Ni(II) (**2**), Cu(II) (**3**), Zn(II) (**4**) and were characterized by various spectroscopic and analytical techniques such as FT-IR, UV-Vis, ^{1}H NMR, ^{13}C NMR, ESI-Mass, ESR, elemental analysis and magnetic susceptibility. Spectral data revealed octahedral geometry for cobalt(II), nickel(II), copper(II) complexes and tetrahedral geometry for zinc(II) complex. All the metal(II) complexes along with the Schiff base ligand were screened for their anticancer activities. The CT-DNA binding studies revealed high binding propensity for metal complexes with K_b values 1.50×10^4 M^{-1} for **1**; 3.62×10^4 M^{-1} for **2**; 2.53×10^4 M^{-1} for **3** and 1.8×10^4 M^{-1} for **4**, respectively. Anticancer studies against A549 & MCF-7 demonstrated excellent antiproliferative activity with IC_{50} values in the range 17.62-48.82 µM. A standard drug cisplatin was employed to compare the activity of metal complexes. The complexes exhibited remarkable antitumour activity due to their high binding ability with DNA. It is interesting to observe that the complexes did not produce any cytotoxicity towards the normal cell lines.

KEYWORDS

Tridentate ligand, 8-Aminoquinoline, 2,3,4-Trihydroxy benzaldehyde, Intercalative binding, Anticancer activity, MTT assay, Octahedral, Cisplatin.

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INTRODUCTION

Cancer is world's second leading cause of mortality by accounting for nearly 9.6 million deaths in the year of 2018 (WHO; Cancer today; Factsheet). Abnormal cell growth within the human tissues is triggered by genetic or environmental factors. Mutations result in cancer and the genetically altered cells keeps on multiplying and start propagating to form tumours [1]. Cancer can affect any part of the body and the most common tumours are found in lungs and breasts cancers. The search for new chemotherapeutic agents is never ending as the tumours adopt drug resistance so quickly [2]. Apart from the drug

resistance, no approved drug in the market is free from producing side effects. All most all the drugs produce severe side effects or the tumour cells develop resistance within 3 to 6 months of their administration [3]. Hence, there is need to identify effective drug candidates that can treat cancer while producing minimal or no side effects. In light of this, efforts have been made to design, synthesize new quinoline based Co(II), Ni(II), Cu(II) and Zn(II) complexes and evaluated their antitumour activities against selected cancer cells.

Design and synthesis of coordination complexes by using Schiff base ligands acknowledged prevalent consideration in the last few years due to their applications in the field of molecular sensing, catalysis [4,5], supramolecular interactions, magnetism, excellent biological properties, *etc.* [6]. Transition metal complexes with multidentate organic ligands have been the subject of extensive research because they not only have interesting spectral and magnetic properties but also possess a various spectrum of biological activities [7,8]. In Schiff base ligands, the presence of azomethine group (-C=N) leads to various medicinal properties including antifungal, antibacterial [9-19], antitumour, antioxidant, anti-inflammatory, antispasmodics, antimalarial, antiviral and herbicidal activities [20]. Herein, the metal complexes of the Schiff bases ligand derived from 8-aminoquinoline and substituted salicylaldehyde were synthesized and examined for their biological properties [21-23]. In continuance of this type of work, we have focused on scheming a sequence of biologically active Co(II), Ni(II), Cu(II) and Zn(II) metal complexes in complexation with NNO tridentate ligand *i.e.* (*E*)-4-((quinoline-8-ylimino)methyl)-benzene-1,2,3-triol.

EXPERIMENTAL

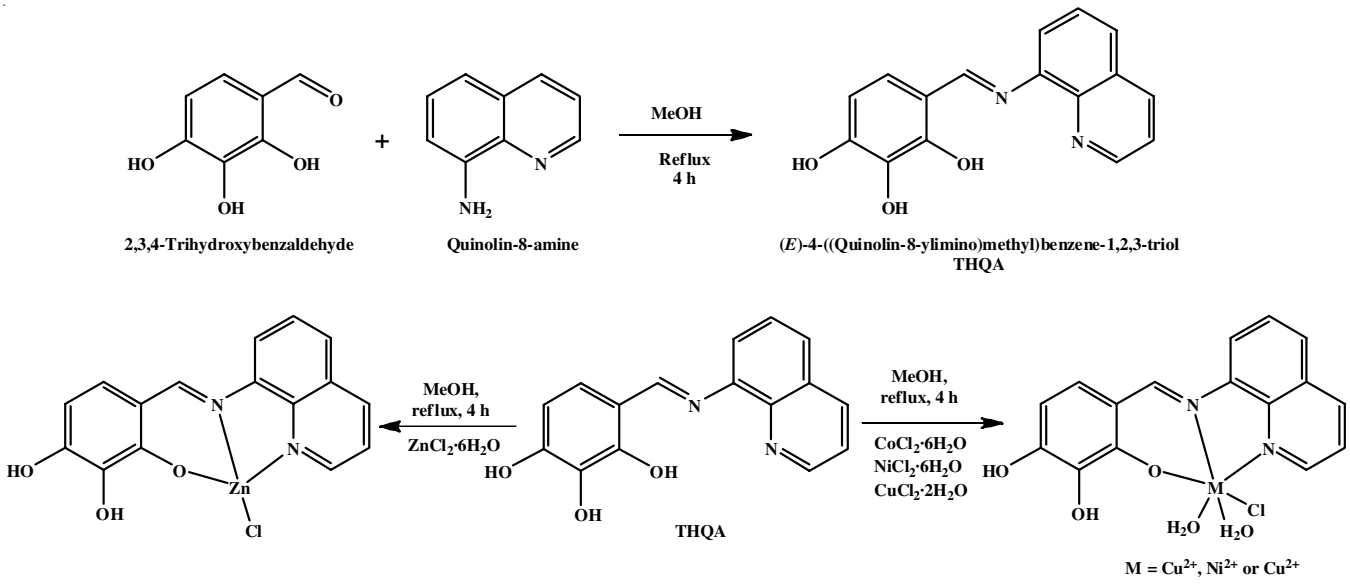
The chemicals and solvents used in this work were of LR grade. All the metal salts were purchased from Avra chemicals, Hyderabad, India. High purity amine and aldehyde were purchased from Sigma-Aldrich, Germany. The solvents were purified by standard measures before usage and double distilled water was utilized to carry out the reactions. The purity of ligand was checked by using Merck silica gel 60 F_{254} pre-coated aluminium sheet TLC plates. CT-DNA, Tris-HCl buffer and cell lines for anticancer studies were purchased from Fluka (Switzerland), Bangalore Genei (India) and NCCS (Pune, India), respectively. Culture media DMEM and RPMI-1640 for MTT assay was purchased from Sigma-Aldrich, Germany. Buffer solution made up of 5 mM Tris-HCl/50 mM NaCl was utilized to perform DNA binding experiments. ESI-Mass of synthesized ligand and complexes were obtained from Quattro LC micro mass (Manchester, UK) instrument. Electronic absorption spectra were recorded using Shimadzu 160A UV-visible spectrophotometer. Bruker-400 and 101 MH_Z spectrometer was utilized to record proton and ^{13}C NMR spectra, respectively. Infrared spectra were recorded using Perkin-Elmer FT-IR spectrometer in KBr phase in the range 4000-400 cm^{-1}. Electron spin resonance spectroscopy (ESR) of Cu(II) complex was measured using JEOL, X-band ESR spectrometer. The magnetic susceptibilities of metal complexes were studied at room temperature using Faraday balance (CAHN-7600). Carlo Erba 1108 elemental analyzer was used obtain elemental analysis of metal complexes.

CT-DNA binding by UV-Vis method: The CT-DNA binding experiments were carried out using 5 mM Tris-HCl, 50 mM NaCl buffer solution and 1 mM complex concentrations. A fresh round bottom flask containing double distilled water was added with CT-DNA, Tris-HCl, NaCl and continued to stir overnight to get a clear solution. The solution was then filtered and kept at low temperature, ideally at 0-4 ºC. The pH of resulting clear solution was found to be 7.5. To know whether the DNA was sufficiently free from protein an absorption ratio at 260 and 280 nm was taken. The ratio (A_{260}/A_{280}) was observed to be 1.9 and an indication that the DNA is sufficiently free from protein contamination [24]. The concentration of the CT-DNA stock solution was measured from its absorption intensity at 260 nm and taking extinction coefficient as 6600 M^{-1} cm^{-1} [25]. The concentration of a well-known intercalator, ethidium bromide, was calculated using absorption intensity at 480 nm and taking 5860 M^{-1} cm^{-1} as extinction coefficient [26]. The absorption titrations were carried on Shimadzu 160A UV-visible spectrophotometer with 1 cm pathlength cuvettes. In the reference cell only, buffer was taken. During the titrations the concentrations of complexes were kept constant (10 µM) and that of CT-DNA was varied from 0-10 µM. The intrinsic binding constants (K_b) for the synthesized complexes were calculated using Wolfe-Shimmer equation [27].

Ethidium bromide displacement assay: The ethidium displacement assay was carried using electronic absorption titrations on Shimadzu 160A UV-visible spectrophotometer with 1 cm pathlength cuvettes. During the titrations, the concentration of ethidium bromide and CT-DNA were kept constant at 40 µM and the concentration of complexes varied between 0-50 µM.

***In vitro* anticancer activity:** The anticancer properties of the synthesized ligand metal complexes (**1-4**) were examined using MTT colorimetric assay. Two culture media namely DMEM and RPMI-1640 were used to maintain the cells. For optimal cell growth and to prevent unwanted bacterial infection to the media both foetal bovine serum (10%) and penicillin-streptomycin (1%) were added. The tumour and normal cell lines were then seeded onto the 96-well plates with 5000 cells per well density and incubated at 37 ºC in 5% CO_2 atmosphere. Six different concentrations (2.5, 10, 20, 30, 40 and 50 µM) for each of the compound were utilized to treat A549, MCF-7 and for 24, 48 and 72 h, respectively. After the completion of incubation period MTT reagent (5 mg/mL) was added to each well and plate and the cell lines were allowed to incubate for another 3 h [28-30]. The purple-coloured precipitates formed due to the addition of MTT reagent were made to soluble using 100 µL DMSO. The absorbance values were taken at 560 nm and IC_{50} calculated using origin software. Cell viability was examined with three independent experiments (in triplicate for accuracy) and the corresponding half maximal inhibitory concentration (IC_{50}) values were measured in µM.

Synthesis of ligand: The synthesis of Schiff base ligand (*E*)-4-((quinoline-8-ylimino)methyl)benzene-1,2,3-triol (THQA) is depicted in **Scheme-I**. The hot methanolic solution of 8-aminoquinoline (2 mmol, 0.218 g, 10 mL), was stirred magnetically in an round-bottom flask to which a methanolic solution of 2,3,4-trihydroxy benzaldehyde (2 mmol, 0.308 g,



Scheme-I: Synthesis of THQA ligand and its metal complexes

15 mL) was added dropwise in 1:1 ratio. The solution was refluxed for 4-5 h with constant stirring on an oil bath at 60-70 ºC. The reaction progress was monitored by thin layer chromatography, with (4:6) ethyl acetate:hexane solution as an eluent. The orange-coloured precipitate was obtained and extracted by using petroleum ether and the hot methanol. The resulting product was kept in anhydrous $CaCl_2$ desiccators to remove unwanted moisture. Yield: 86%, Colour: orange, m.p.: 183 ºC. ^{1}H NMR (400 MHz, DMSO-d_6): δ 15.22 (d, J = 5.2 Hz, 1H), 9.58 (s, 1H), 9.07-8.90 (m, 2H), 8.45 (dd, J = 8.3, 1.6 Hz, 2H), 8.01-7.91 (m, 1H), 7.89-7.83 (m, 1H), 7.76-7.60 (m, 2H), 6.87 (d, J = 8.9 Hz, 1H), 6.28 (d, J = 8.8 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.42, 157.54, 150.17, 149.55, 140.11, 139.80, 136.31, 133.43, 128.58, 126.88, 125.05, 124.22, 122.32, 115.34, 111.67, 108.75. UV-Vis [λ_{max}, nm (ε, M^{-1} cm^{-1})] (DMSO): 268 (3.95×10^4), 272 (3.67×10^4), 385 (3.51×10^4). IR (KBr, ν_{max}, cm^{-1}): 3442, 1627, 1470. ESI-Mass: *m/z*, 281 $[M+1]^+$. CHN analysis calcd. (found) % for $C_{16}H_{12}N_2O_3$: C, 68.56 (68.24); H, 4.32 (4.09); N, 9.99 (9.85).

Synthesis of metal(II) complexes: A hot methanolic solution (15 mL) of metal(II) chloride hexahydrate (0.2 mmol, dissolved in 10 mL of methanol) of zinc(II), cobalt(II) and nickel(II) chloride dihydrate (0.611 mmol, dissolved in 10 mL of methanol) of Cu(II) was added dropwise to a stirred hot methanolic solution of Schiff base ligand (0.2 mmol, 0.05 g, 15 mL) in 1:1 molar ratio. The resulting solution was refluxed for 4-6 h at 60-70 ºC, the reaction mixture was filtered out and the precipitation of all the products was cleaned with petroleum ether, hot methanol and dried at room temperature for 24 h. A pink colour [Co(II)(THQA)(Cl)$(H_2O)_2$], pale green [Ni(II)-(THQA)(Cl)$(H_2O)_2$], green colour [Cu(II)(THQA)(Cl)$(H_2O)_2$] and white colour [Zn(II)(THQA)(Cl)] complexes were obtained. The synthetic route for the preparation of metal complexes is depicted in **Scheme-I**.

[Co(II)(THQA)(Cl)$(H_2O)_2$] (1): Yield: 78%, Colour: pink, m.p.: 210 °C. UV-Vis [λ_{max}, nm (ε, M^{-1} cm^{-1})] (DMSO): 260 (4.05×10^4), 275 (3.83×10^4), 420 (2.31×10^4). IR (KBr, ν_{max}, cm^{-1}): 3508, 1604, 1425, 549, 1415, 493. ESI-Mass: *m/z*, 410 $[M+1]^+$. CHN analysis calcd. (found) % for $C_{16}H_{15}N_2O_5ClCo$: C, 46.91 (46.45); H, 3.69 (3.53); Cl, 8.65 (8.60); Co, 14.38 (14.32); N, 6.84 (6.65); O, 19.53 (19.50).

[Ni(II)(THQA)(Cl)$(H_2O)_2$] (2): Yield: 85%, Colour: pale green, m.p.: 224 ºC. UV-Vis [λ_{max}, nm (ε, M^{-1} cm^{-1})] (DMSO): 255 (4.25×10^4), 280 (3.52×10^4), 410 (2.55×10^4). IR (KBr phase, ν_{max}, cm^{-1}): 3350, 1606, 1454, 547, 487. ESI-Mass: *m/z*, 409 $[M+1]^+$. CHN analysis calcd. (found) % for $C_{16}H_{15}N_2O_5ClNi$: C, 46.94 (46.75); H, 3.69 (3.42); Cl, 8.66 (8.65); Co, 14.33 (14.35); N, 6.84 (6.77); O, 19.54 (19.52).

[Cu(II)(THQA)(Cl)$(H_2O)_2$] (3): Yield: 80%, Colour: green, m.p.: 225 ºC. UV-Vis [λ_{max}, nm (ε, M^{-1} cm^{-1})] (DMSO): 260 (4.12×10^4), 285 (3.41×10^4), 418 (2.38×10^4). IR (KBr, ν_{max}, cm^{-1}): 3350, 1606, 1454, 547, 487. ESI-Mass: *m/z*, 414 $[M+1]^+$. CHN analysis calcd. (found) % for $C_{16}H_{15}N_2O_5ClCu$: C, 46.39 (46.31); H, 3.65 (3.58); Cl, 8.56 (8.53); Cu, 15.34 (15.32); N, 6.76 (6.62); O, 19.31 (19.30).

[Zn(II)(THQA)(Cl)] (4): Yield: 75%, Colour: white, m.p.: 202 ºC. UV-Vis [λ_{max}, nm (ε, $M^{-1}cm^{-1}$)] (DMSO): 265 (4.08×10^4), 288 (3.32×10^4), 410 (2.54×10^4). IR (KBr, ν_{max}, cm^{-1}): 3541, 1593, 1435, 574, 493. ESI-Mass: *m/z*, 379 $[M+1]^+$. CHN analysis calcd. (found) % for $C_{16}H_{11}N_2O_3ClZn$: C, 50.56 (56.45); H, 2.92 (2.76); Cl, 9.33 (9.32); Zn, 17.20 (17.23); N, 7.37 (7.29); O, 12.63 (12.60).

RESULTS AND DISCUSSION

All the synthesized metal (II) complexes were tested for their solubility and found to be soluble in dichloromethane, dimethylformamide, chloroform and dimethyl sulfoxide. The compounds were stable, non-hygroscopic and decomposed at elevated temperatures. The complexes were non-electrolytic in nature as evident from their low conductance values in methanol and methanol + water (1:10 parts) mixture. Magnetic moment value (μ_{eff}) of Cu(II) was found to be 1.80, which indicates the presence of one unpaired electron. For Ni(II) and Co(II) complexes the magnetic moment values (μ_{eff}) were found to be 2.83 and 3.87 indicating the presence of two and three unpaired electrons, respectively.

ESI-Mass spectra: The mass spectra of the synthesized Schiff base ligand (TQHA) depicted a base peak at *m/z* 281 which indicates the formation of ligand as $[M+1]^+$ molecular ion. All the metal(II) complexes produced base peaks at *m/z* 410 for Co(II), *m/z* 409 Ni(II), *m/z* 414 for Cu(II) and *m/z* 379 for Zn(II) indicating the formation of metal complexes as $[M+1]^+$ molecular ions.

FT-IR spectra: A sharp azomethine band of THQA ligand was observed at 1627 cm^{-1}. The absence of peaks at 3400 cm^{-1} and 1695-1685 cm^{-1} corresponding to free -CHO and -NH_2 groups, respectively is a clear sign of the formation of new Schiff base ligand [31]. The IR spectra of all the metal complexes showed stretching frequency for azomethine (-C=N) group in the range 1604-1593 cm^{-1}. The azomethine stretching frequency shifted towards downfield region by 10-30 cm^{-1} in all the metal complexes, indicating that the azomethine nitrogen of the THQA Schiff base ligand was coordinated with the corresponding metal ions. All the metal(II) complexes showed M-O and M-N stretching frequencies in the range 480-580 cm^{-1}. The characteristic IR stretching frequencies that include aromatic C=N, azomethine C=N, M-O and M-N are presented in Table-1. The presence of coordinated water molecules in the complexes is evident from the broadband bands in the region of 3600-3200 cm^{-1}. Two weak bands were observed in the range 840 & 758-740 cm^{-1} due to ν(-OH) rocking & wagging mode of vibrations, respectively [32].

Electronic spectra: The UV-Vis spectra of THQA Schiff base ligand and its Co(II), Ni(II), Cu(II) and Zn(II) complexes were recorded at room temperature in DMSO solution in the region 200-800 nm (Fig. 1). The UV absorption bands at 260 nm for Co(II), 255 nm for Ni(II), 260 nm for Cu(II) and 265 nm for Zn(II) complex were assigned to π–π* transitions of aromatic chromophore. The bands at 275 nm for Co(II), 280 nm for Ni(II), 285 nm for Cu(II) and 288 nm for Zn(II) complex were assigned to n–π* transitions of azomethine group. In the UV spectrum of all the metal(II) complexes, the transitions of free ligand were shifted due to the ligand coordination to metal ion. Cobalt(II) complex exhibited *d-d* band at with $^4T_1g(F) \rightarrow {}^4T_1g(P)$ transition indicated the octahedral geometry around Co(II) ion [33]. Ni(II) complex showed *d-d* band at with $^3A_1g(F) \rightarrow {}^3T_1g(P)$ transition also indicated the octahedral geometry around Ni(II) ion [34]. The absorption spectra of copper(II) complexes showed broad *d-d* bands in the range of 400-720 nm suggestive of a distorted octahedral geometry with weak equatorial coordination of solvent/anion molecules [35]. Due to the fully filled *d*-orbitals zinc(II) metal complex did not show *d-d* band. Zinc(II) complex with four coordinate generally, would have tetrahedral geometry.

Thermal studies: The water molecules associated with metal complexes are of generally two types *i.e.*, lattice water and coordinated water. In general, the loss corresponding to lattice water is observed in the temperature range 80-150 ºC, while that of coordinated water are lost in the temperature range 150-250 ºC. In the present investigation, all the metal(II) complexes showed weight loss in the temperature range 180-220 ºC. For complexes **1**, **2** and **3**, the observed weight loss was 8.89%, 8.85% and 8.76%, respectively which confirms the presence of two coordinated water molecules. All the complexes decomposed in two stages, the first stage decomposition is attributed to the loss of water molecules and while that of the second stage is ascribed to loss of THQA ligand.

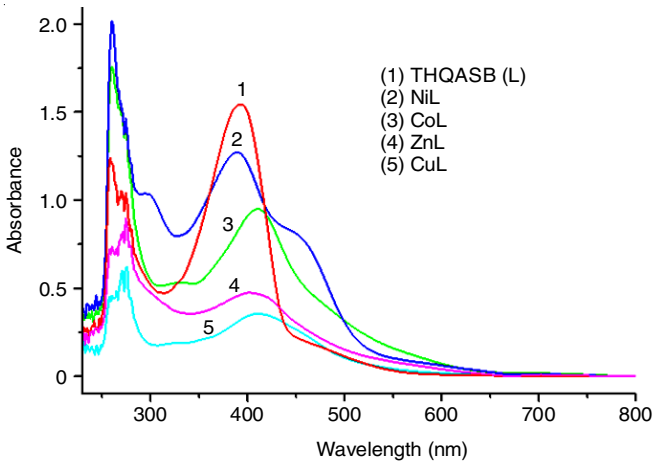


Fig. 1. UV-visible absorption spectra of THQA ligand and its Co(II), Ni(II), Cu(II) and Zn(II) complexes

ESR spectra: The geometry and environment around the metal ions is conveniently estimated by analysing ESR spectra. The structural information of [Cu(II)(THQA)$(H_2O)_2$Cl] (**3**) complex was obtained by its electron spin resonance spectra at room temperature (Fig. 2). The g values play key role in analyzing the nature of unpaired electron in Cu(II) ion. In the

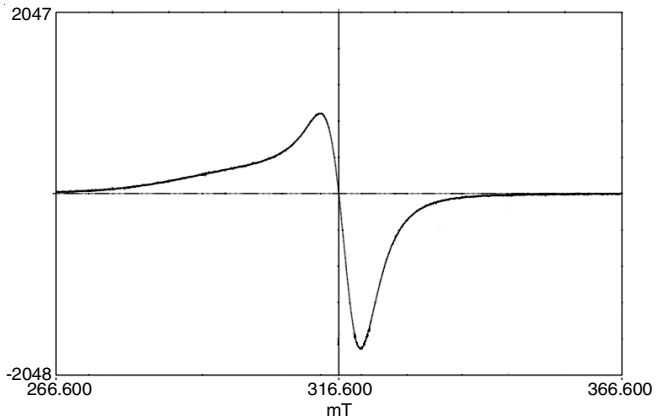


Fig. 2. Electronic spin resonance spectra of [Cu(II)(THQA)(Cl)$(H_2O)_2$] (**3**) complex

TABLE-1
KEY INFRARED BANDS (cm^{-1}) OF METAL COMPLEXES (**1-4**)

Ligand/complex	ν(O-H)	ν(C=N) azomethine	ν(C=N)	ν(M-O)	ν(M-N)
THQA	3442	1627	1470	–	–
[Co(II)(THQA)(Cl)$(H_2O)_2$]	3508	1604	1425	549	493
[Ni(II)(THQA)(Cl)$(H_2O)_2$]	3350	1606	1454	547	487
[Cu(II)(THQA)(Cl)$(H_2O)_2$]	3404	1597	1431	542	495
[Zn(II)(THQA)(Cl)]	3541	1593	1435	574	493

present analysis, the observed g values *i.e.*, $g_{\parallel}$ (2.0947), $g_{\perp}$ (2.0734) for **3** follow a general order, $g_{\parallel} > g_{\perp} > g_e$ (2.0023). This order is typical of a tetragonal distortion around Cu(II) ion and infers that the unpaired electron lies in $d_{x^2-y^2}$ ground state [36]. If the g values are less than or more than 2.3 the environment around Cu(II) would be covalent or ionic, respectively. The g value which is evidently less than 2.3 clearly suggests that the environment around Cu(II) ion is covalent. The exchange interaction parameter (G) values were also calculated using the equation, $G = (g_{\parallel} - 2.0023)/(g_{\perp} - 2.0023)$ and obtained a value of 1.29 for **3**. The G value ($G < 4$) indicates the presence of an appreciable exchange coupling between two neighbouring copper(II) ions in solid state [37].

Biological studies

DNA binding: The DNA binding experiment involves the gradually increasing addition of CT-DNA (0-10 µM) concentration to the fixed amount of concentration (10 µM) of the metal(II) complexes (**1-4**). The conformational changes of DNA are interpreted using the UV electronic absorbance data. If at all the synthesized metal complexes intercalate with the base it results in the lengthening of DNA strand and subsequently changes conformational flexibility of giant DNA molecule [38]. There are characteristic shifts which determine the intercalative mode of binding of complexes with DNA base pairs *i.e.*, bathochromic and hypochromic shifts of absorption bands in the UV spectra. The changes in the absorption bands of electronic spectra are of direct consequences from intercalation of complexes into the base pairs of DNA molecule [31]. The 200-300 nm region from the Fig. 3 also depicts the hypochromic and bathochromic changes to the electronic absorption bands for complexes **1-4** and it confirms the intercalative mode of interaction for synthesized complexes with CT-DNA. The strong π–π stacking interaction of aromatic chromophores of complexes and base pairs of DNA results in hypochromism [39,40]. Whereas, the bathochromism results when the π* orbital of the intercalated complex further couples with π-orbital of DNA base pairs, which ultimately decrease the π–π* transition energy [41]. The K_b values for the complexes found to be 1.50×10^4 M^{-1} for **1**; 3.62×10^4 M^{-1} for **2**; 2.53×10^4 M^{-1} for **3** and 1.8×10^4 M^{-1} for **4** and they indicate a higher binding affinity towards the DNA molecule. The

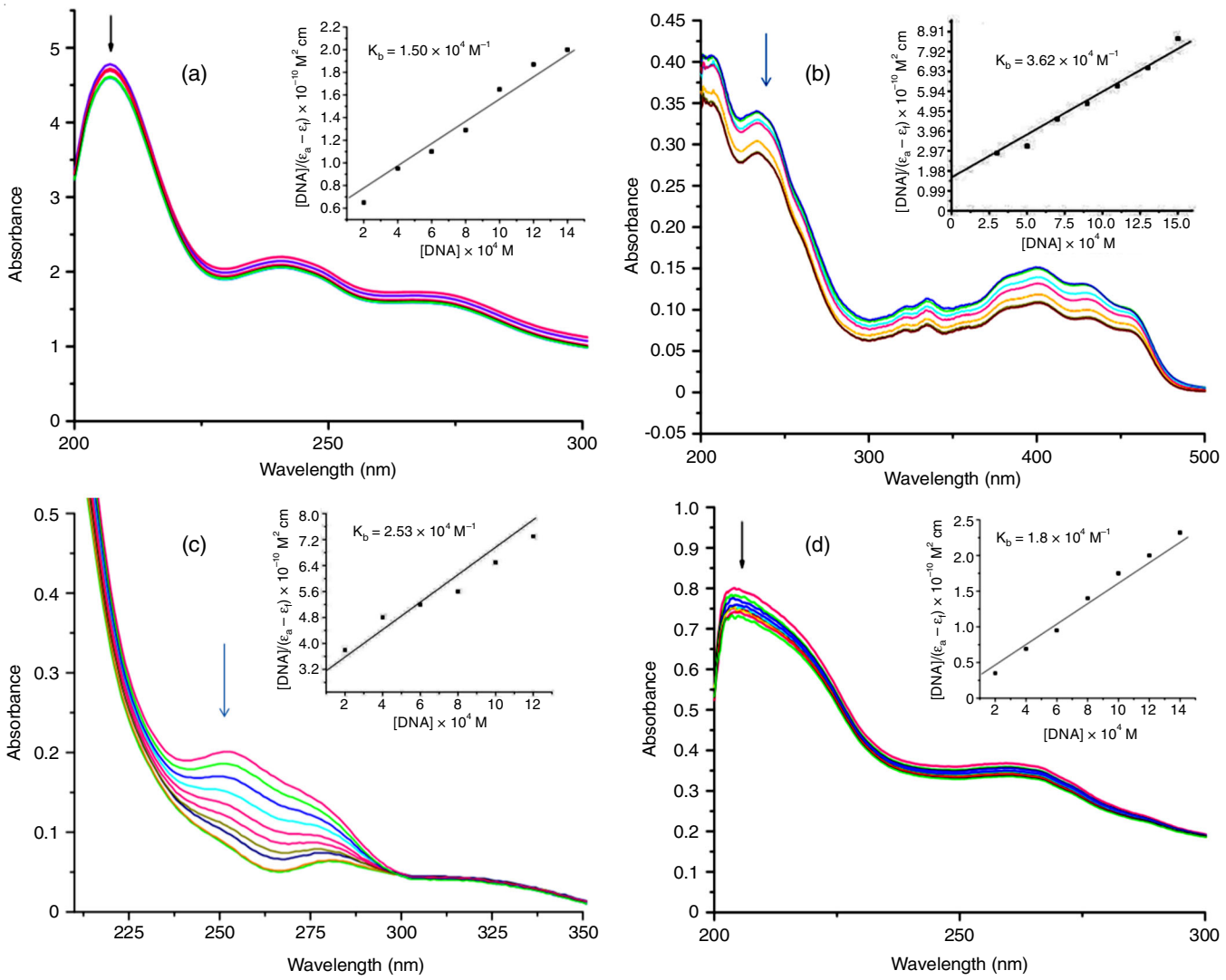


Fig. 3. Electronic absorption spectra of (a) [Co(II)(THQA)(Cl)(H_2O)$_2$] (**1**), (b) [Ni(II)(THQA)(Cl)(H_2O)$_2$] (**2**), (c) [Cu(II)(THQA)(Cl)(H_2O)$_2$] (**3**) and (d) [Zn (II)(THQA)(Cl)] (**4**) depicting the absorption intensity variations upon the increasing additions of CT-DNA from 0-10 µM, while the complex concentration is fixed at 10 µM. Insets represent intrinsic binding constant (K_b)

observed intrinsic binding values are near to classical intercalator EB (ethidium bromide) *i.e.*, $K_b = 1.4 \times 10^6$ M^{-1} calculated in 25 mM Tris-HCl/40 mM NaCl; pH = 7.9) [42].

Competitive binding: To further prove the intercalative mode of binding for complexes (**1** to **4**), competitive binding experiments were carried out using buffer solution. The aim of the competitive binding is to estimate the competitiveness of metal complexes in replacing the ethidium bromide (a well-known intercalator) from the DNA base pairs. Ethidium bromide (EB) shows its absorption intensity maxima at 480 nm. The treatment of EB with DNA will shift the absorption maxima to higher wavelength with a decrease in the absorption intensity. This clear shift is an indication for intercalative mode of binding for EB between the base pairs of DNA [43]. The solution with DNA and EB was further treated with increasing concentrations of metal complexes (**1-4**) which showed gradual increase of absorption intensity. The electronic absorption changes of EB + DNA system upon the addition of metal complexes clearly suggest that the complexes competitively replace EB from DNA. This is a confirmation for the intercalative mode of interaction for synthesized complexes. Fig. 4 represents the replacement of intercalatively bound EB by complexes **1-4**.

Anticancer studies: The synthesized ligand THQA and its metal complexes **1-4** were tested against A549, MCF-7 tumour and NIH/3T3 normal cell lines using standard MTT assay. A reference drug cisplatin was employed to compare the activity of compounds. The anticancer activity of metal(II) complexes is a consequence of their ability to interact with DNA. As evident from the DNA binding studies, the complexes found to inhibit the growth of cancer cells with IC_{50} values ranging from 17.62 + 1.52 to 48.82 ± 1.35 µM. Among all, complex **4** showed excellent activity with IC_{50} 20.84 ± 1.23 against MCF cancer cells after 24 h treatment. The cancer cells A549 & MCF-7 both were found to be sensitive toward the treatment of metal complexes. The corresponding IC_{50} values and morphology of anticancer activity are presented in Table-2 and Fig. 5, respectively. Interestingly, the metal complexes did not harm the normal cells *i.e.* NIH/3T3 by producing any cytotoxicity. Cisplatin was taken as a reference drug and the activity of metal complexes was comparable to the standard drug. The presence of azomethine functional group, free hydroxyl groups, metal ions and aromatic chromophores contribute to the observed anticancer activity. Interaction of metal complexes with DNA restricts vital nuclear processes like replication and

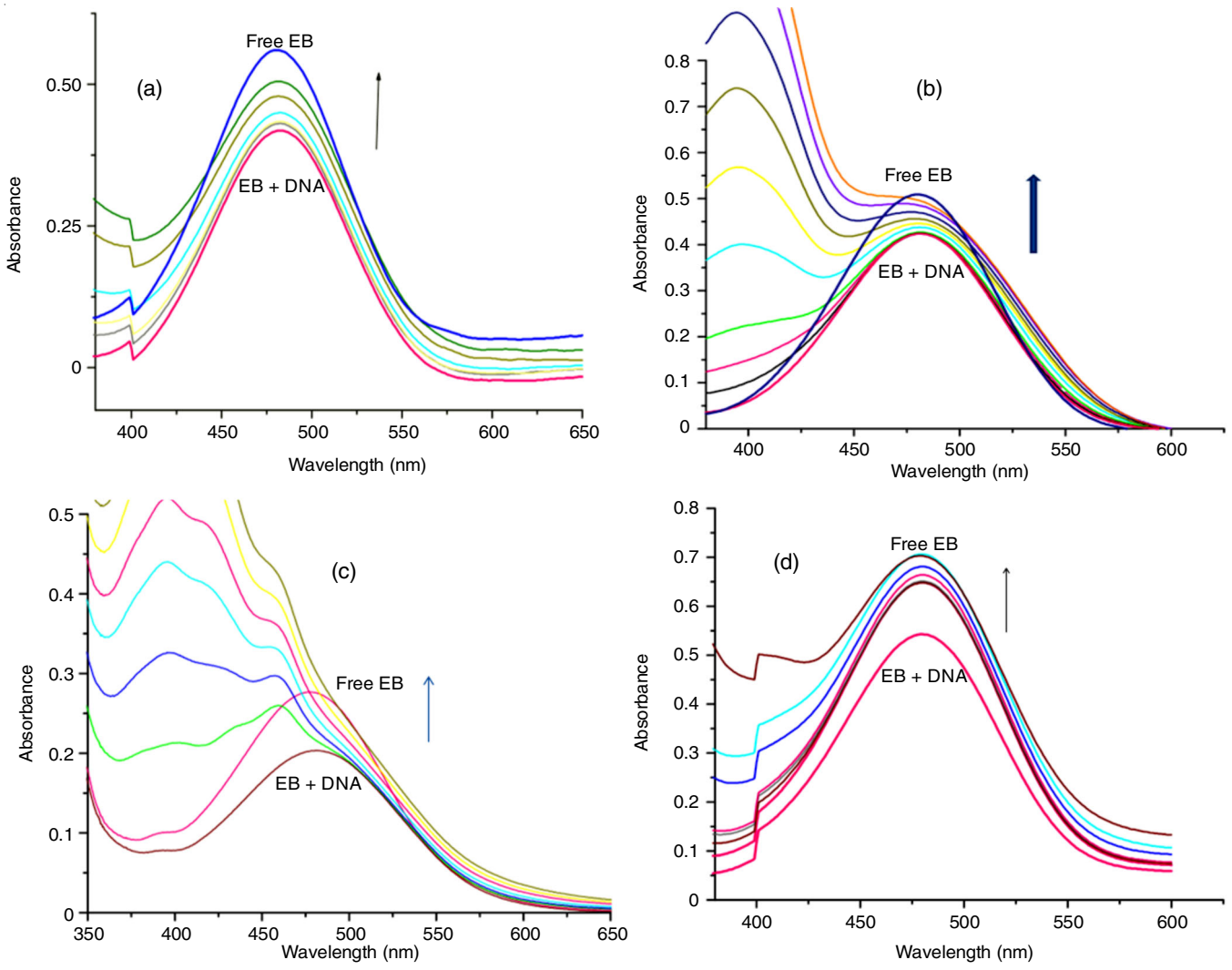


Fig. 4. Competitive binding spectra of (a) [Co(II)(THQA)(Cl)(H_2O)$_2$] (**1**), (b) [Ni(II)(THQA)(Cl)(H_2O)$_2$] (**2**), (c) [Cu(II)(THQA)(Cl)(H_2O)$_2$] (**3**) and (d) [Zn (II)(THQA)(Cl)] (**4**) depicting the replacement well known intercalator, ethidium bromide. Upward arrow represents increase in absorption intensity upon the addition of increasing amounts of complex **1** from 0-50 µ

TABLE-2
IC_{50} VALUES OF METAL COMPLEXES AGAINST A549 AND MCF-7 CANCER CELLS AFTER 24, 48 AND 72 h TREATMENT

Compound	A549			MCF-7			NIH/3T3
	24 h	48 h	72 h	24 h	48 h	72 h	
THQA	55.57 ± 0.69	49.10 ± 1.29	45.94 ± 1.18	42.78 ± 1.12	38.25 ± 0.65	34.56 ± 1.28	Not determined
1	48.82 ± 1.35	43.87 ± 1.01	38.31 ± 1.26	24.89 ± 0.73	22.67 ± 1.45	18.54 ± 1.32	Not determined
2	34.55 ± 1.34	29.43 ± 1.51	26.11 ± 1.10	31.33 ± 1.87	28.92 ± 1.72	21.38 ± 1.09	Not determined
3	28.59 ± 1.27	26.12 ± 1.21	22.85 ± 1.77	26.62 ± 1.45	22.98 ± 1.35	19.72 ± 1.59	Not determined
4	26.98 ± 1.14	23.94 ± 0.95	20.24 ± 1.62	20.84 ± 1.23	17.62 ± 1.52	15.53 ± 1.15	Not determined
Cisplatin	26.37 ± 0.7	23.96.13 ± 0.5	21.48 ± 0.4	26.37 ± 0.7	23.96.13 ± 0.5	21.48 ± 0.4	Not applicable

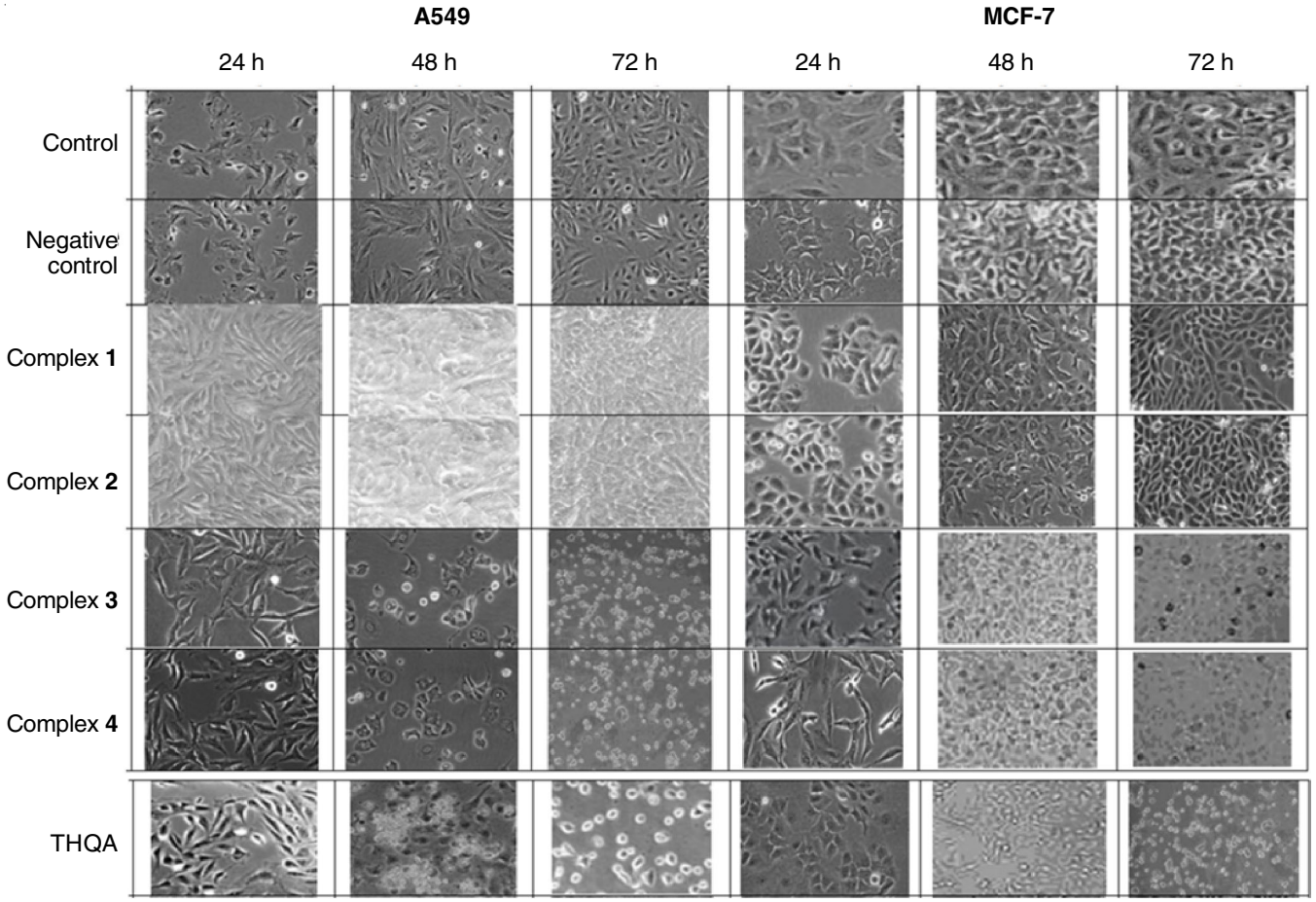


Fig. 5. Morphology of A549 (lung cancer) and MCF-7 (breast cancer) cells after the treatment of 50 μM concentration of compounds (THQA & complexes **1-4**) for 24, 48 and 72 h respectively

transcription. These processes are essential for the tumour cells to grow, multiply and spread. It is important for the molecules to contain few important functional groups that interact with cancer cell DNA and stop its multiplication. The free hydroxyl groups of metal complexes form hydrogen bonds with phosphate groups to induce the DNA damage. At the same time, planar aromatic groups interact with base pairs of DNA of cancerous cells through intercalation and hampers the cell growth.

Conclusion

The synthesis of new Schiff base ligand (*E*)-4-((quinoline-8-ylimino)methyl)benzene-1,2,3-triol (THQA) and its Co(II), Ni(II), Cu(II) and Zn(II) is reported by non-template technique and characterized them using FT-IR, UV-VIS, ^{1}H NMR,^{13}C NMR, ESI-Mass and elemental analysis. The Schiff base ligand acts as a tridentate NNO donor ligand. The complexes assumed the octahedral geometry for Co(II), Ni(II), Cu(II) and tetrahedral geometry for Zn(II) complex. The binding interaction of the metal complexes was proved to be intercalative mode, which is a prerequisite for anticancer activity of drug candidates. The complexes produced better tumour cell inhibition than the Schiff base ligand. Among all the complexes, Zn(II) and Cu(II) complexes showed better anticancer activity with IC_{50} values 26.98 μM, 20.84 μM and 28.59 μM, 26.62 μM, respectively against A549 and MCF-7 cancer cells after 24 h treatment. The IC_{50} values of both Zn(II) and Cu(II) were comparable to the standard drug, cisplatin. Moreover, all of the metal(II) complexes and TQHA ligand were non-toxic towards normal cells.

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