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Synthesis, DNA binding and anticancer properties of new Cu(II) and Zn(II) complexes of a Schiff base ligand containing a triphenylphosphonium as a lipophilic cation

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ABSTRACT

A new quinoline-Schiff base ligand (L_{TPP}) containing the lipophilic triphenylphosphonium cation and its complexes [Cu(L_{TPP})Cl]Cl and [Zn(L_{TPP})Cl]Cl were synthesized and characterized. The structures of the ligand and metal complexes were characterized by FTIR, elemental analysis, NMR (for ligand), UV–Vis absorption and fluorescence spectroscopies. DNA binding interactions of the ligand and its metal complexes were investigated by UV–vis absorption and fluorescence spectroscopies as well as viscosity measurements. The DNA binding affinity (K_b) for the ligand and complexes [Cu(L_{TPP})Cl]Cl and [Zn(L_{TPP})Cl]Cl) obtained from absorption spectra were found as 4.33, 4.66 and 6.01 ($\times 10^5$ M^{-1}), respectively. The ligand and its Cu(II) complex showed similar propensities to interact with DNA, yet the DNA binding affinity of the Zn(II) complex was relatively higher than free ligand and its Cu(II) complex. Docking studies were conducted in order to further investigate the DNA binding interactions. The ligand and its complexes were screened for their cytotoxic properties towards malignant mesothelioma (H2452) and healthy human umbilical vein endothelial (HUVEC) cells. The Cu(II) and Zn(II) complexes that were loaded into the cells (H2452) confirm their intracellular uptake by fluorescence-based cell imaging. Complex [Zn(L_{TPP})Cl]Cl emitted fluorescent light even after entering the cell suggesting that the compound can be used for cell tracking purposes. In order to find out whether the compounds we synthesized had the potency to enter human cells, we measured the florescent light emission of H2452 cells treated with the products at their IC_{50} concentration for 24 h. In vitro antioxidant properties of the ligand and its metal complexes were also studied.

1. Introduction

Metal complexes make up a significant proportion of the compounds that are biologically important. Metal complexes are widely used as contrast agents in medicine as anticancer, anti-inflammatory, antibacterial, antirheumatic, antimalarial, and contrast agents [1]. Many chemists have been intrigued by the success of cisplatin and carbo-platin as anticancer agent [2]. Although platin based compounds are effective anticancer agents, they suffer from selectivity and severe side effects. To overcome the disadvantages of these drugs, scientists have focused [3] on developing new metal-based drugs. In addition to being necessary for numerous biological processes, copper is also important for the growth and spread of malignancies [4]. Its versatile activities encompass pivotal roles in DNA synthesis and repair. Serving as an indispensable component in DNA-binding proteins featuring Zn-fingers, it plays a crucial role in shaping transcription factor functionality and facilitating the translation of genetic messages. Furthermore, it assumes a central position in overseeing cell metabolism and providing vital protection against oxidative damage [5]. The presence of zinc is essential for the survival of cells and the maintenance of tissues, in particular for cell proliferation, differentiation, apoptosis, immunity and reproduction. Moreover, zinc complexes, along with numerous other metal (II) complexes, are being explored as promising candidates for novel anti-cancer drugs, offering a metal-based alternative to traditional platinum derivatives [6]. This is mainly due to the fact that zinc(II) is not significantly toxic at higher doses compared to other metals (e.g. Fe, Cu, Hg), and then zinc

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complexes undergo rapid ligand exchange, promoting Lewis acid activation and DNA damage. Recently, compounds containing delocalized lipophilic cation such as quaternary ammonium and phosphonium ions have taken considerable attention due to their better cell membrane penetration and targeting mitochondria [7]. Because of their large hydrophobic surface area, triphenyl phosphonium (TPP) cations pass directly through phospholipid layers, lowering the activation energy of uptake. It has been also known that mitochondria-targeted antioxidants can show antimicrobial properties [8]. Besides, the Schiff base metal complexes containing triphenyl phosphonium side unit(s) as lipophilic cation exhibited more potent cytotoxic properties against human hepatoma (HepG2) and HeLa cell lines than 5-fluorouracil. The higher cytotoxic properties of these compounds have been attributed to their strong DNA binding interactions [6,9,10].

Due to the potential anticancer properties of Schiff base metal complexes having triphenyl phosphonium side unit as lipophilic cation [11–30] and in continuation of our interest in the preparation of new anticancer therapeutics, we have prepared a new quinoline-based Schiff base ligand (L_{TPP}) containing a triphenyl phosphonium moiety and its Cu(II) and Zn(II) complexes. The synthesized ligand and its Cu(II) and Zn(II) complexes were screened for their DNA binding, cytotoxic and antioxidant properties. Synthesis scheme of the ligand and its Cu(II) and Zn(II) complexes is given in Fig. 1. The compounds were characterized by FT-IR, ^{1}H NMR (for ligand), mass spectra and elemental analysis. Antiproliferative properties of the synthesised compounds were investigated against HUVEC and H2452 cell lines using the MTS method.

2. Experimental

2.1. General methods

8-Aminoquinoline (CAS: 578–66-5), Triphenylphosphine (CAS: 603–35-0), Salicylaldehyde (CAS: 90–02-8), Formaldehyde (CAS: 50–00-0), Zinc chloride (CAS): 108816.1000), Copper(II) chloride dihydrate (CAS: 10125–13-0), Hydrochloric acid (CAS: 7647–01-0) materials and organic solvents were purchased from commercial sources and used as received, unless noted otherwise. 3-Formyl-4-hydroxybenzyltriphenylphosphonium chloride (L) was synthesised according to the reported procedure (**Fig. S1**) [31]. FT-IR spectra were collected on a Perkin Elmer Spectrum 400 and FTIR spectra are given in **Figs. S2-S4**. The ^{1}H/^{13}C NMR spectra were taken on a Bruker AVANCE III 400 MHz (**Figs. S5&S6**). The UV–vis spectra were performed on Hitachi U3900H spectrophotometer. Emission spectra were taken by using Perkin Elmer Spectrum LS55.

2.2. Synthesis of the Schiff base ligand L_{TPP}

3-Formyl-4-hydroxybenzyltriphenylphosphonium chloride (L) (1 mmol) was dissolved in 20 mL of methanol and followed by addition of 8-aminoquinoline compound (1 mmol). The reaction mixture was then refluxed for 24 h. As a result of slow evaporation of the solvent, a red powder was obtained. The product was filtered and washed with diethyl ether.

L_{TPP}; Molecular Formula**:** $C_{35}H_{28}PN_2OCl$. Yield: 58 %. M.P. $>$ 250°C (decompose). M.W: 559.23 g/mol. Colour: Red. Λ_o (S cm^2 mol^{-1}): 50.3 (MeOH). FT-IR (cm^{-1}): 3350 (–OH), 2998 (-C-H), 1608 (-C = N), 1585, 1435, 1366, 1284, 1106, 995, 831, 742, 732, 687, 490. Elementel Anal. Calc. for $C_{35}H_{28}PN_2OCl$ (559.23 g/mol): C, 75.20; H, 5.05; N, 5.01. Found: C, 75.04; H, 4.88; N, 4.95 %. ^{1}H NMR (DMSO-d_6, TMS, ppm 400 MHz,) 11.08 (s, 1H, OH); 8.77 (s, 1H, CH = N); 7.93–7.89 (d, 4H, aromatic CH); 7.77–7.73 (m, 9H, aromatic CH); 7.70 (s, 2H, aromatic CH); 7.68–7.67 (d, 2H, aromatic CH); 7.65 (s, 1H, aromatic CH); 7.42 (s, 2H, aromatic CH); 7.26 (s, 2H, aromatic CH), 7.13 (s, 2H, aromatic CH); 6.97–6.92 (q, 2H, aromatic CH); 5.14–5.10 (d, 2H, CH_2). ESI-MS, *m/z*: 523.05 for $\{L_{TPP}\}^+$.

2.3. Preparation of Cu(II) and Zn(II) complexes

To the stirring solution of ligand L_{TPP} (1 mmol) in 20 mL of methanol, $CuCl_2{\cdot}2H_2O$ or $ZnCl_2$ was added. The reaction mixtures were then

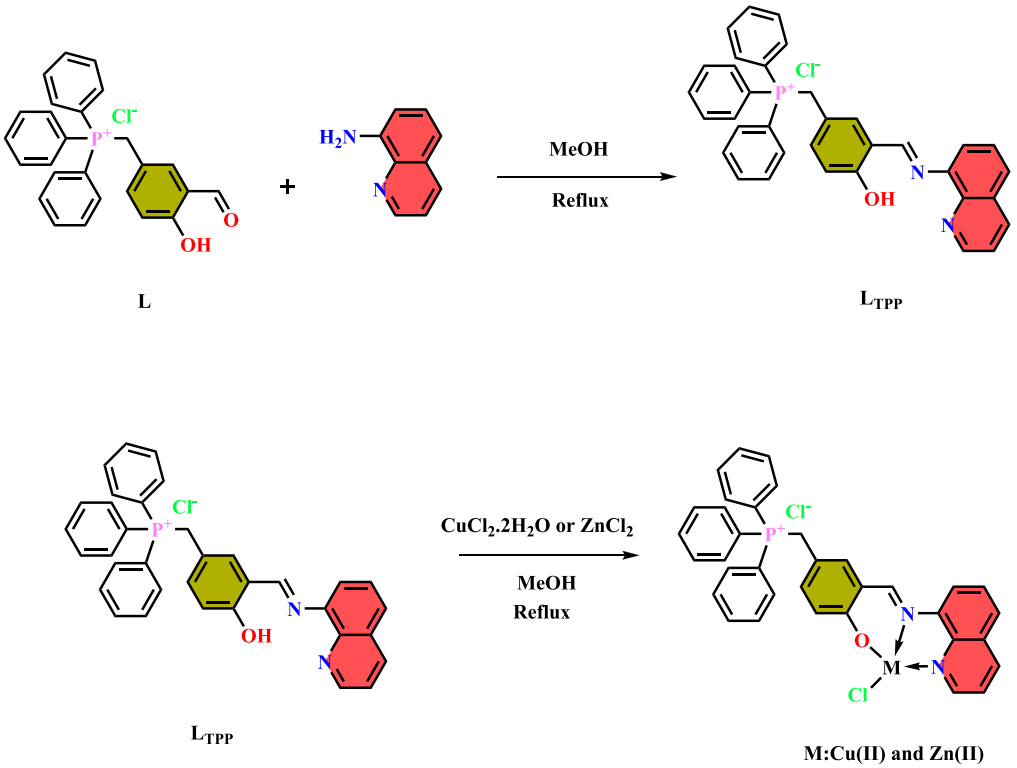

Fig. 1. Synthesis scheme of the ligand L_{TPP} and its Cu(II) and Zn(II) complexes.

refluxed for 24 h. As a result of slow evaporation of the solvent, light orange for Zn(II) complex and dark green for Cu(II) complex were obtained in powder form. The product was filtered and washed with diethyl ether.

[Cu(L_{TPP})Cl] Cl: $C_{35}H_{29}PN_2O_2Cl_2Cu$. Yield: 59 %. M.P. > 250 °C; MW: 675.05 g/mol. Colour: Dark green. Λ_o (S cm^2 mol^{-1}): 81.1 (MeOH), FT-IR (cm^{-1}): 3319, 2785, 1606, 1585, 1503, 1422, 1322, 1215, 1108, 1039, 832, 714, 480. Elementel Anal. Calc. for $C_{35}H_{29}PN_2O_2Cl_2Cu$ (675.05 g/mol): C, 62.27; H, 4.33; N, 4.15. Found: C, 62.14; H, 4.17; N, 4.11 %. ESI-MS, *m/z*: 621 for $\{[Cu(L_{TPP})Cl]\}^+$.

[Zn(L_{TPP})Cl] Cl: $C_{35}H_{29}PN_2O_2Cl_2Zn$. Yield: 62 %. M.P. > 250 °C. MW: 676.88 g/mol. Colour: Yellow. Λ_o (S cm^2 mol^{-1}): 86.7 (MeOH). FT-IR (cm^{-1}): 3320, 2923, 1614, 1585, 1435, 1322, 1217, 1106, 1039, 995, 830, 713, 688. Elementel Anal. Calc. for $C_{35}H_{29}PN_2O_2Cl_2Zn$ (676.88 g/mol): C, 62.10; H, 4.32; N, 4.14. Found: C, 62.02; H, 4.09; N, 4.05 %. ESI-MS, *m/z*: 623 for $\{[Zn(L_{TPP})Cl]\}^+$.

2.4. DNA binding studies

2.4.1. Spectral measurements

Methods of all biological activity (DNA binding assay, DNA-EB (ethidium bromide) displacement, DNA-MB (Methylene blue) displacement and DNA-RB (Rhodamine B) displacement assay and Viscosity assay) studies of compounds are given in the supplementary section.

2.4.2. Molecular docking with DNA

The molecular modelling protocol is provided in the supplementary file.

2.5. Cytotoxicity studies

Malignant mesothelioma (H2452; CRL-5946) and healthy human umbilical vein endothelial (HUVEC; PCS-100–10) cells were obtained from the American Type Cell Culture (ATCC, USA). Both cells were cultured in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10 % FBS (Fetal Bovine Serum) and 1 % penicillin–streptomycin, and incubated at 37 °C with the CO_2 pressure of 5 %. The synthesized Schiff base ligand and its Cu(II) and Zn(II) complexes were dissolved in DMSO at suitable concentrations (Table 1).

2.5.1. Determining the IC_{50} values

The IC_{50} values of the synthesized compounds on HUVEC and H2452 cells were determined using the MTS (Methylthiazolyl Tetrazolium Assay) kit (Promega, USA). The IC_{50} values were calculated with modifications to the protocol presented by Comertpay et al., [32]. The mean absorbance values of the cell-free media were subtracted from the values obtained for the wells containing cells. In order to calculate the relative viability of the treated wells, the cells incubated in media only were considered as 100 % vital. The obtained values were plotted on an XY graph, and the IC_{50} value was determined using GraphPad Prism software. Subsequently, the IC_{50} values determined for HUVEC and H2452 cells were compared using Two-Way ANOVA.

2.6. Antioxidant properties of the compounds

The MTS method was also used to determine the antioxidant properties of the synthesized compounds through the method proposed by Liu et al., [33] with some modifications. The same concentrations of the compounds used for determining the IC_{50} values (Table 1) were also used in the antioxidant assay. The compound-containing culture media were added to the wells of a 96-well plate without cells, and MTS assay was performed. The value measured for the media without synthetic compounds was considered as "1.00″ and all other values were normalized against it. The obtained values were plotted on a "Column" graph, and a student *t*-test was used for comparison.

Table 1
The concentrations (μg/mL) of the compounds applied to the cells.

Compounds	Concentration (μg/mL)
L_{TPP}	0 μg/mL, 150 μg/mL, 300 μg/mL, 450 μg/mL
[Zn(L_{TPP})Cl]Cl	0 μg/mL, 75 μg/mL, 150 μg/mL, 225 μg/mL, 300 μg/mL, 375 μg/mL
[Cu(L_{TPP})Cl] Cl	0–30 μg/mL, 60 μg/mL, 90 μg/mL, 120 μg/mL, 150 μg/mL

2.7. Statistical analysis

The statistical analyses for the experimental findings were performed using the GraphPad Prism software (CA, USA). For the determination of the IC_{50} value, the 'EC50 Shift' function within the 'Non-linear regression' option in the 'Analyze' tab was used. The comparison of IC_{50} values for the synthesized compounds was made *via* Two-Way ANOVA test, and the difference between the values was considered statistically significant only when the p-values for 'interaction' were less than 0.05. For antioxidant detection, unpaired t-tests were used to compare each concentration against each other, and for the p-values lower than 0.05, the difference was accepted as statistically significant. The symbols *, **, ***, and **** represent p-values less than 0.05, 0.01, 0.001, and 0.0001, respectively.

2.8. Cellular intake

In order to find out whether the compounds we synthesized had the potency to enter human cells, we measured the florescent light emission of H2452 cells treated with the products at their IC_{50} concentration for 24 h. The cells were washed and kept in no-product containing media during visualization with EVOS PL (photoluminescence) Cell Imaging System (ThermoFisher, USA). Since phenolic red in regular DMEM interfered with the fluorescence, DMEM without Phenol Red (Gibco; Thermo Scientific, USA) was used for experimental solutions.

3. Results and discussion

A new Schiff base ligand (L_{TPP}) containing a triphenyl phosphonium moiety and its Cu(II) and Zn(II) complexes were prepared (Fig. 1). The ligand and its Cu(II) and Zn(II) complexes were obtained in high yield and purity. Elemental analysis was performed in order to confirm the purity of the compounds. The structures of the ligand and its metal complexes were characterized by FTIR, NMR, mass spectroscopies, elemental analysis, conductivity measurements. Experimental C, H and N values are in good agreement with the theoretical values of proposed structures which confirm the purity of the compounds.

3.1. Characterization of the compounds

FT-IR spectra of 3-formyl-4-hydroxybenzyltriphenylphosphonium chloride (L), the Schiff base ligand (L_{TPP}) and its Cu(II) and Zn(II) complexes were in the region of 4000–450 cm^{-1}. In the spectrum of 3-formyl-4-hydroxybenzyltriphenylphosphonium chloride (L), the broad medium intensity band at around 3700 cm^{-1} and sharp medium intensity band at around 1700 cm^{-1} are due to the υ(O-H) and υ(CH = O) vibrations, respectively. In the spectrum of the Schiff base ligand (L_{TPP}), a sharp medium intensity band at 1611 cm^{-1} and abroad medium intensity band at around 3650 cm^{-1} can be attributed to the υ(O-H) and υ(CH = N) vibrations, respectively. The absence of the aldehyde group vibration and presence of new imine bond vibration confirmed the formation of the Schiff base ligand (L_{TPP}). In the spectra of complexes [Cu(L_{TPP})Cl]Cl and [Zn(L_{TPP})Cl]Cl, the imine bond vibrations υ(CH = N) were observed at 1606 and 1614 cm^{-1}, for complexes [Cu(L_{TPP})Cl]Cl and [Zn(L_{TPP})Cl]Cl, respectively. The slight shift of the imine bond vibrations in the spectra of the complexes compared to the free ligand

suggested the coordination of the Schiff base ligand to Cu(II) or Zn(II) ion through the imine nitrogen atom. In the spectra of the complexes, the broad band due to the phenolic group υ(O-H) was absent and this suggest the deprotonation of phenolic group and coordination bond formation between the phenolate oxygen and metal ions.

In the ^{1}H NMR spectrum of 3-formyl-4-hydroxybenzyltriphenylphosphonium chloride (L) singlet signals at 11.26 and 10.63 ppm were assigned to the phenolic and aldehydic protons, respectively. In the spectra of the ligand L_{TPP}, the singlet signals at 11.08 and 8.77 ppm are assigned to the phenolic and imine group (CH = N) protons and these signals confirmed the successful formation of the Schiff base ligand (L_{TPP}).

The ESI mass spectra for the ligand LTPP and its Cu(II) and Zn(II) complexes were performed and the data are given in the Supplementary file (**Fig. S7**). Molecular ion peaks at *m/z*: 523.05 for $\{L_{TPP}\}^+$, 621 for $\{[Cu(L_{TPP})Cl]\}^+$, 623 for $\{[Zn(L_{TPP})Cl]\}^+$ were observed in the mass spectra.

The molar conductance data of the free ligand and complexes [Cu(L_{TPP})Cl]Cl and [Zn(L_{TPP})Cl]Cl were obtained in 0.001 M solutions of MeOH are shown in the experimental part. The molar conductance were obtained as 50 S cm^2 mol^{-1} for free ligand (L_{TPP}), 81.1 S cm^2 mol^{-1} for [Cu(L_{TPP})Cl]Cl and 86.7 S cm^2 mol^{-1} for [Zn(L_{TPP})Cl]Cl. The conductivity data showed the 1:1 electronic nature of the ligand and its metal complexes. Moreover, conductive nature of the complexes suggest that one chloride ion remains uncoordinated.

3.2. UV–vis absorption and emission properties

UV–vis absorption and emission spectra of the Schiff base ligand and its Cu(II) and Zn(II) complexes were analysed in DMSO solution (10^{-5} M). UV–vis absorption and emission spectra of the ligand and its Cu(II) and Zn(II) complexes are provided in the supplementary documents (**Fig. S8**). In the spectrum of the free ligand, two absorption bands at 300–550 nm range ($\lambda_{max:}$ 343 and λ_{max}: 476 nm) were observed and these electronic absorption bands were assigned to the π-π* and n-π* electronic transitions of aromatic rings or azomethine group of the ligand. In the spectra of complex [Cu(L_{TPP})Cl]Cl and [Zn(L_{TPP})Cl]Cl complexes, the absorption bands due to the π-π* and n-π* transitions shifted to shorter wavelengths (blue shift) confirming the formation of the metal complexes. The emission spectra of the Schiff base ligand showed a weak emission band at 450–600 nm range when irradiated at 349 nm (λ_{exc}). Schiff base compounds derived from salicylaldehyde exhibits weak emission properties due to the C = N isomerisation (enol-keto tautomerism) [34]. Complex [Cu(L_{TPP})Cl]Cl showed emission an emission band at 450–600 nm when excited at 303 nm (λ_{exc}). Compared to the emission spectrum of the free ligand, the position of the emission band was almost unchanged yet the emission intensity considerably increased. The increase in the emission intensity of complex may be due to the inhibition C = N isomerisation upon coordination to the metal ion. The dramatic increase in the emission intensity was observed in the case of complex [Zn(L_{TPP})Cl]Cl. Additionally, a considerable red shift was observed in the emission band with respect to the free ligand.

3.3. DNA binding studies

3.3.1. UV–vis spectral titrations

Electronic absorption titration method is among the most widely used methods to determine the DNA binding mechanism of ligand and metal complexes. When determining the interaction between DNA and molecules, it is determined from the shifts of the spectral position to the right or to the left (bathochromic or hyperchromic) and the changes in the decrease or increase in the peak intensity (hypochromic or hyperchromic). The hypochromic and hyperchromic effects involve changes in the DNA double helix structure at the main groove. The ability of the molecule to bind to DNA by intercalation is indicated by the bathochromic effect observed in the spectrum and the significant hypochromic effect. The π* orbital of the DNA binding molecule couples with the π-orbital of the DNA base pairs which leads to a decrease the π-π* transition energy. This leads to a bathochromic effect. The empty π* orbital of DNA binding molecule is partially filled by electrons, reducing the transition probability and this causes hypochromism. When molecules are electrostatically bound, they can form an electrostatic interaction with DNA, causing the absorption intensity to become hyperchromic. This is the case where there is little or no bathochromic effect in groove binding and a hypochromic effect is seen. This can be attributed to the overlap of electronic states of the chromophore of the molecule with the nitrogenous bases in the DNA grooves [35,36]. The absorption spectra of the compounds in the presence of increasing amount of DNA were measured (Fig. 2). In the absorption spectra of the Schiff base ligand (L_{TPP}), there is a broad absorption band at 300–400 nm range (λ_{max}: 346 nm). The copper complex showed two absorption maximums at 320 and 340 nm while the Zn complex showed two absorption peaks at 310 and 385 nm. All these transitions are assigned to the π-π* transitions. Upon addition of DNA, the compounds were found to have a hypochromic effect in the absorption spectra. Simultaneously, DNA addition resulted in a small bathochromic shift in the spectra of the compounds. The hypochromic effect occurs when the DNA double helix becomes disrupted as a result of combining with compounds binding noncovalently to DNA or forming more embedded bases in the exposed DNA. The interactions of the ligand and the metal complexes with the groove walls and the phosphate groups of the DNA structures of the compounds may lead to stabilisation of these compounds. Spectroscopic changes showed that the complexes exhibited remarkable groove/surface binding to FSdsDNA (double stranded fish sperm DNA). The intrinsic binding constants (K_b) values for the Schiff base ligand (L_{TPP}), [Cu(L_{TPP})Cl]Cl and [Zn(L_{TPP})Cl]Cl complexes were found to be in the range of 4.33 to 6.01 x 10^5 M^{-1} (Table 2). The result indicates minor groove binding mode of interactions between the compounds and DNA. The values of the binding constant (K_b) of these complexes were lower than those observed for the typical intercalators, ethidium bromide, with binding constants of 1.4 x 10^6 M^{-1}. When the binding constants (K_b) of the compounds were examined, it was determined that the binding constant of complex [Zn(L_{TPP})Cl]Cl was higher than cisplatin and 5-fluorouracil (5-Fu) (2.0 x 10^4 and 6.6 x 10^4 M^{-1}).

3.3.2. Fluorescence competitive EB, MB and RB binding studies

Fluorescence spectral titration of displacement studies for the Schiff base ligand **L_{TPP}** and its complexes with ethidium bromide (EB), methylene blue (MB) and rhodamine-b (RB) were performed to further assess DNA binding affinity. It is known from the literature that EB and MB are able to bind to DNA by means of intercalation, whereas RB binds to the minor groove of DNA [37]. In general, there will be a significant increase in emission intensity with the addition of DNA to EB. However, when small molecules and metal complexes are added to EB-DNA, they are replaced by EB. This leads to a decrease in emission intensity. To determine the binding mode, the interaction of EB-DNA with the Schiff base ligand **L_{TPP}** and metal complexes was first investigated. Fig. 3**a, 4a, 5a** shows the spectra of the EB-DNA adduct in the presence and absence of compounds. Partial emission intensity reductions of the EB-DNA system were observed, these reductions are thought to bind to the EB site of the compounds where some parts of EB intercalating with DNA are cleaved, releasing EB. Alternatively, Cu(II) and Zn(II) complexes may bind to small grooves in the DNA and reduce the emission strength. The Schiff base ligand (L_{TPP}) and its Cu(II) and Zn(II) complexes caused the decrease in the emission band of EB-DNA system by 52 %, 69 % and 65 %, respectively. It is assumed that when a certain concentration is reached, some of them are bound to DNA. Others may remain bound to different regions of the DNA macromolecule. In this case, the Scatchard equation can be used to determine the bound and unbound compounds, where $\log[(I_0\text{-}I)/I] = \log[K_b] + n\log[Q]$, where K and n are the binding sites and I_0 and I are the fluorescence intensities in the absence and presence of the quencher, respectively. The binding constant is

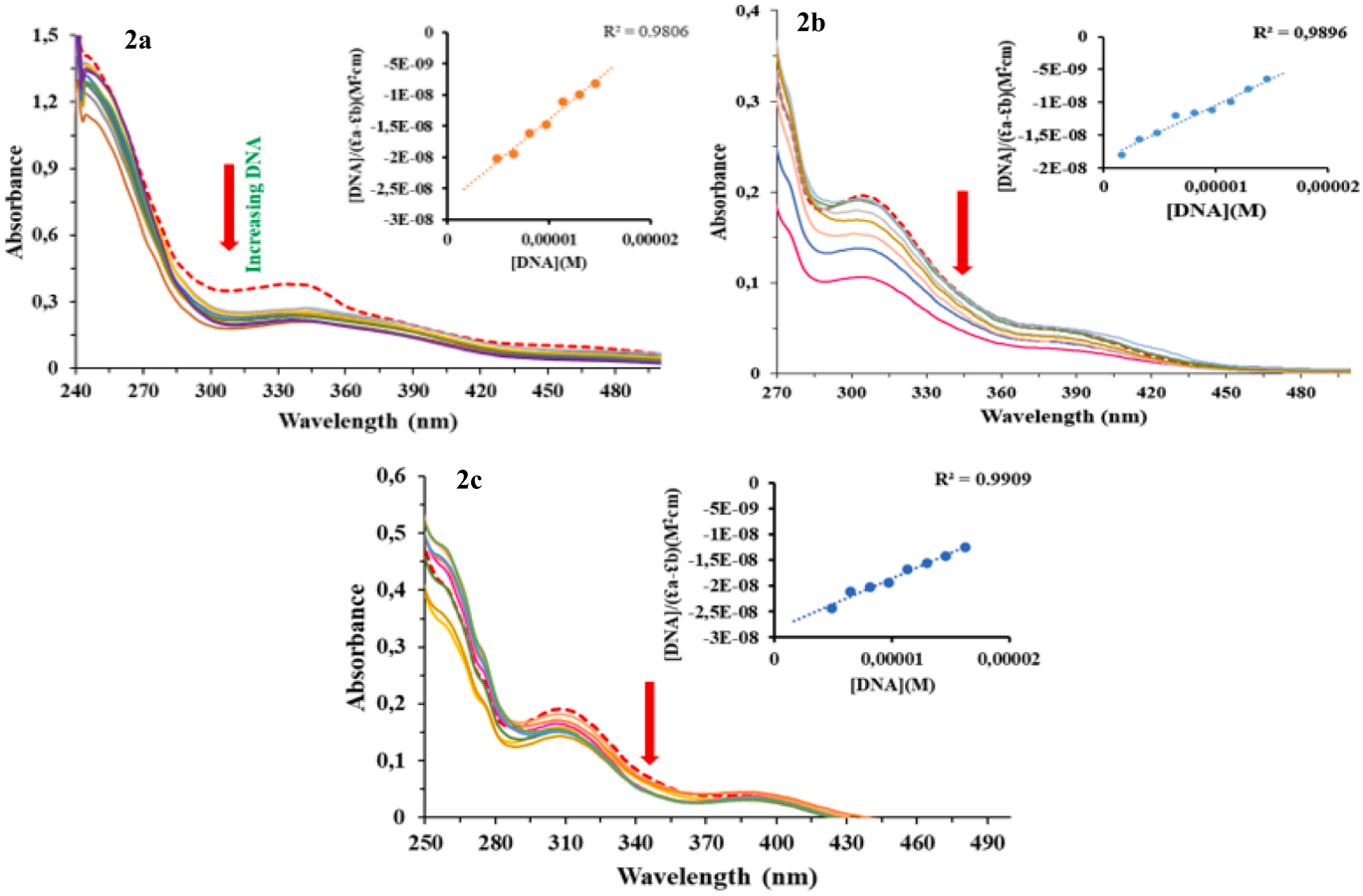

Fig. 2. A-c UV–vis spectral changes of L_{TPP} (2a), [Cu(L_{TPP})Cl]Cl (2b) and [Zn(L_{TPP})Cl]Cl (2c) in the presence of incremental addition of DNA. The arrow indicates the change in absorption with increasing DNA concentration. The [DNA] vs. [DNA]/(εf-εa) plot is shown as inset.

Table 2
DNA binding parameter for the compounds.

Compound	[a]K_b (10^5 M^{-1})		[b]K_{sv} 10^4 (M^{-1})	[c]K_f 10^{11} ($M^{-1}S^{-1}$)	[d]n	logK_b	[e]K_{app} 10^4 ($M^{-1}S^{-1}$)
L_{TPP}		EB	9.73	9.73	1.036	6.78	2,36
	4.33	MB	6.56	6.56	–	–	–
		RB	3.46	3.46	2.04	9.56	0.28
[Cu(L_{TPP})Cl]Cl		EB	4.05	0.192	0.94	4.35	3.42
	4.66	MB	1.90	1.46	–	–	–
		RB	8.80	9.59	1.87	8.64	0.48
[Zn(L_{TPP})Cl]Cl		EB	6.61	6.61	1.17	5.69	0.27
	6.01	MB	9.49	9.49	–		–
		RB	3.46	3.46	1.24	6.48	4.15

[a] Binding constants. [b]Stern-Volmer constant. [c]Quenching constants. [d]Number of binding sites. [e]Apparent binding constant.

calculated from the log[(I_0-I)/I] and log[Q] plots (Fig. 3**d, 4d, 5d**). Table 2 shows the calculated quenching constants. The quenching constants of the compounds are in the range of 4.05 – 9.73 × 10^4 M^{-1}, which is in agreement with what was observed from the electronic absorption spectral studies.

In order to provide further evidence for the above results, a study of the attachment of competitors to MB was carried out. The emission intensity of the MB itself is quite high. A significant decrease in emission intensity is observed when DNA solution is added. The addition of ligands or metal complexes causes an increase in emission intensity, possibly indicating that the synthesized compounds displaced MB from DNA-MB system. In this case, the change in emission intensity was studied by titration of the Schiff base ligand L_{TPP}, Cu(II) and Zn(II) complexes with increasing concentrations, while the ratio of MB to DNA was kept constant. An increase in emission intensity was observed with the addition of compounds (Fig. 4**b**). This is thought to be due to the free residual of MB. The increased emission intensity was found to increase by 50, 45 and 65 %, for ligand and its Cu(II) and Zn(II) complexes, respectively. Nevertheless, it is remarkable that the binding constant of the components (Table 2) is lower than that of MB (2.1 x 10^5 M^{-1}). However, the different binding sites of the compounds showed that there was no release of MB, indicating that the compounds bound to DNA *via* small grooves rather than intercalating (Fig. 3**d,4d,5d).**

A minor groove of dsDNA is known to bind Rhodamine B. As with EB, a partial increase in the intensity of the emission was observed with the addition of DNA to which the intensity of Rhodamine B was partially weak. The same procedure was used for Rhodamine B [38]. The decrease in emission intensity was studied by the addition of increasing concentrations of the Schiff base ligand L_{TPP} and its metal complexes to the mixture of RB and DNA (Fig. 5**c**). No red or blue shift was observed,

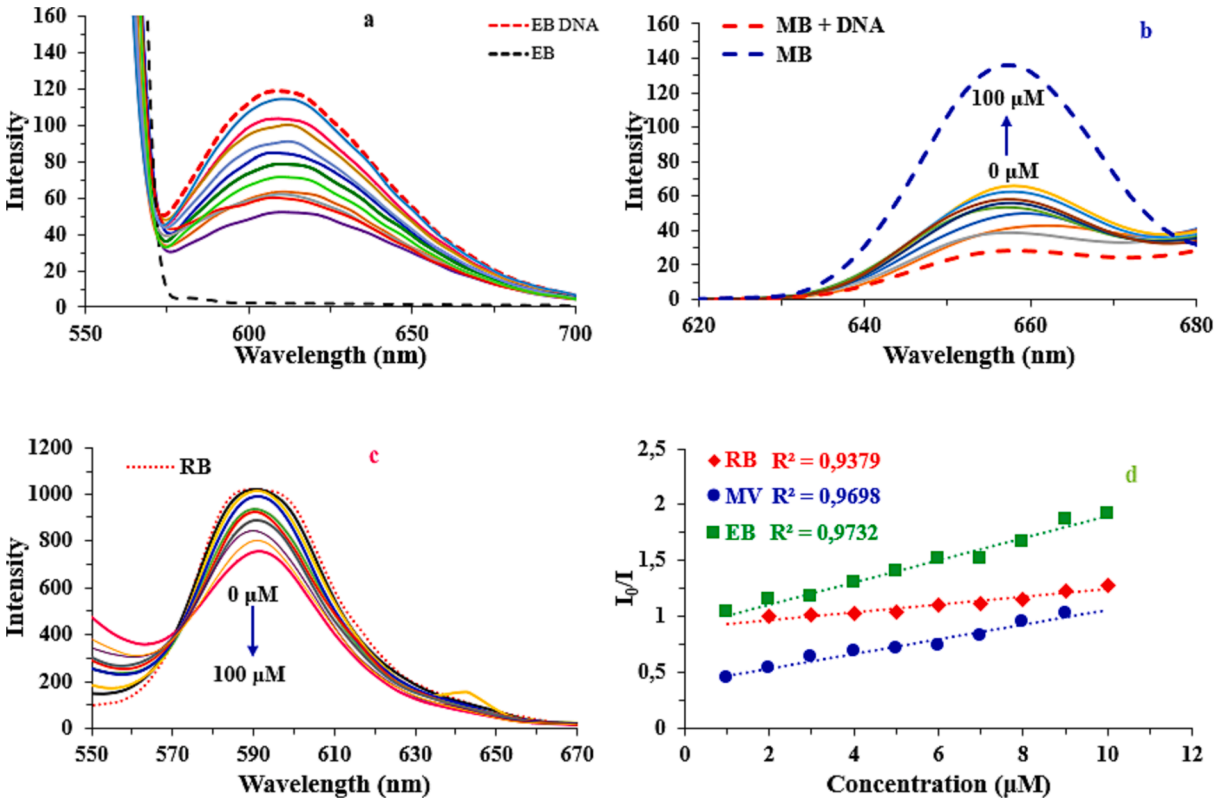

Fig. 3. A-d Effect of addition of the Schiff base ligand L_{TPP} on the emission intensity of the EB-FSdsDNA complex (a), the MB-FSdsDNA complex (b), the RB-FSdsDNA complex (c) at different concentrations in 2 µM Tris-HCl buffer (pH 7.1) and Stern-Volmer plot of fluorescence titrations of the L_{TPP} with EB-FSdsDNA (d).

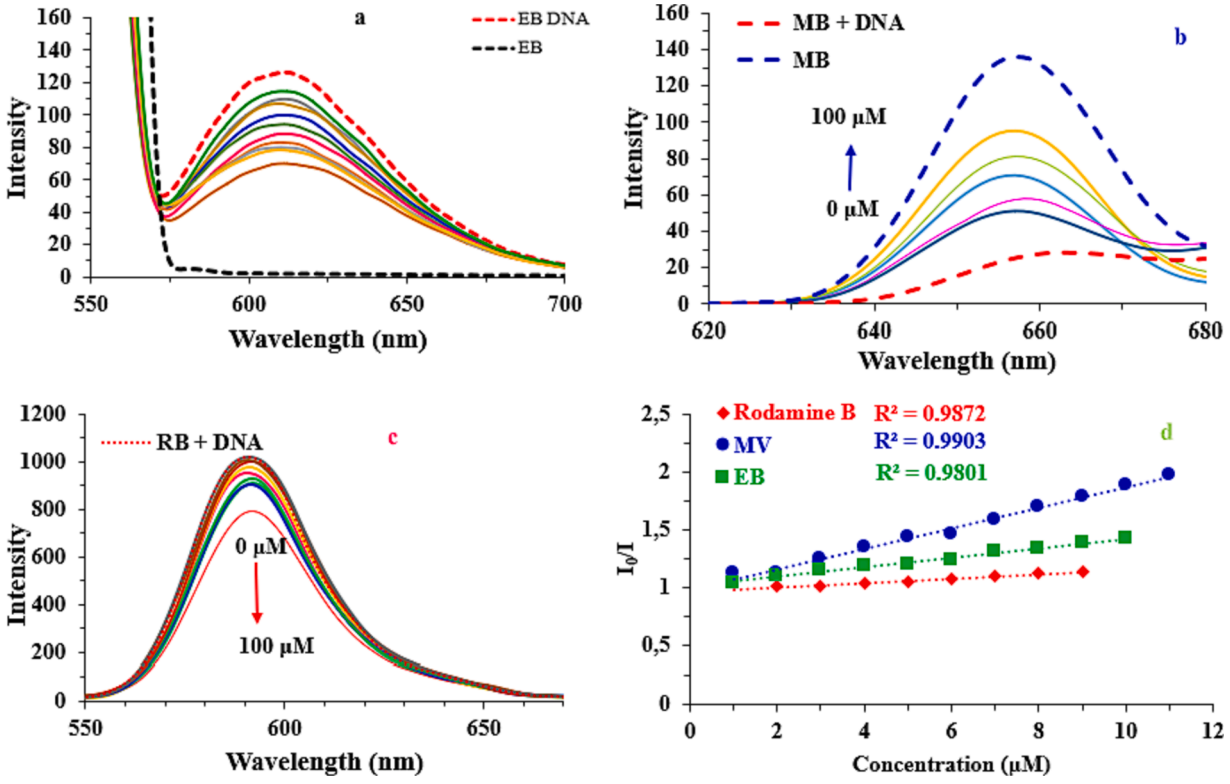

Fig. 4. A-d Effect of addition of complex [Cu(L_{TPP})Cl]Cl on the emission intensity of the EB-FSdsDNA complex (a), the MB-FSdsDNA complex (b), the RB-FSdsDNA complex (c) at different concentrations in 2 µM Tris-HCl buffer (pH 7.1) and Stern-Volmer plot of fluorescence titrations of the [Cu(L_{TPP})Cl]Cl with EB-FSdsDNA (d).

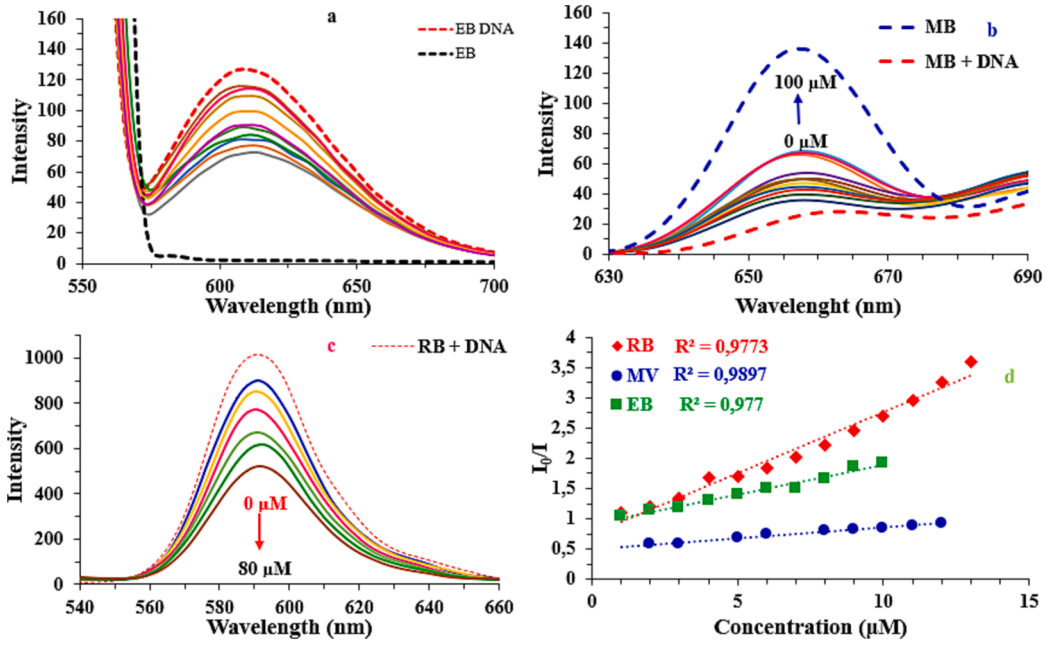

Fig. 5. A-d Effect of addition of complex [Zn(L_{TPP})Cl]Cl on the emission intensity of the EB-FSdsDNA complex (a), the MB-FSdsDNA complex (b), the RB-FSdsDNA complex (c) at different concentrations in 2 µM Tris-HCl buffer (pH 7.1 and Stern-Volmer plot of fluorescence titrations of the compound [Zn(L_{TPP})Cl]Cl with EB-FSdsDNA(d).

but a significant decrease in emission intensity was observed. The quenching of the fluorescence of a compound is associated with a variety of intermolecular interactions such as molecular rearrangements, ground state complex formation, energy transfer and excited state reactions. As mentioned above, K_{sv} values were calculated from Stern-Volmer plots (Table 2). K_{sv} values were found between 3.46–8.80x10^4 M^{-1}. An indication that the synthesised compounds bind to DNA *via* the minor groove was the fact that the substitution of the compounds with Rhodamine B (Fig. 3**c,4c,5c**) was almost 2 times higher than that of EB and MB. The fact that the binding constant is higher than that of the others, as can be seen in Table 2, is also an indication of this. The number of times the DNA was bound (n) is given in the table.

3.3.3. *Viscosity measurements*

Another method for the determination of the mode of binding of compounds and metal complexes to DNA is the use of viscosity experiments. DNA viscosity increases when molecules are introduced between adjacent base pairs, indicating that the DNA molecule and compound are bound by intercalation [39]. This indicates electrostatic or groove surface binding of the compounds if there is no significant change in DNA viscosity [40]. Ethidium bromide (EtBr), a well-known DNA intercalator, strongly increased the relative viscosity by lengthening the DNA double helix. In the present experiment, EtBr was considered as control. When EtBr was added to FSdsDNA, a dramatic increase in the viscosity of the solution was observed. Taking into account the above explanations, the viscosity measurement of the Schiff base ligand L_{TPP},

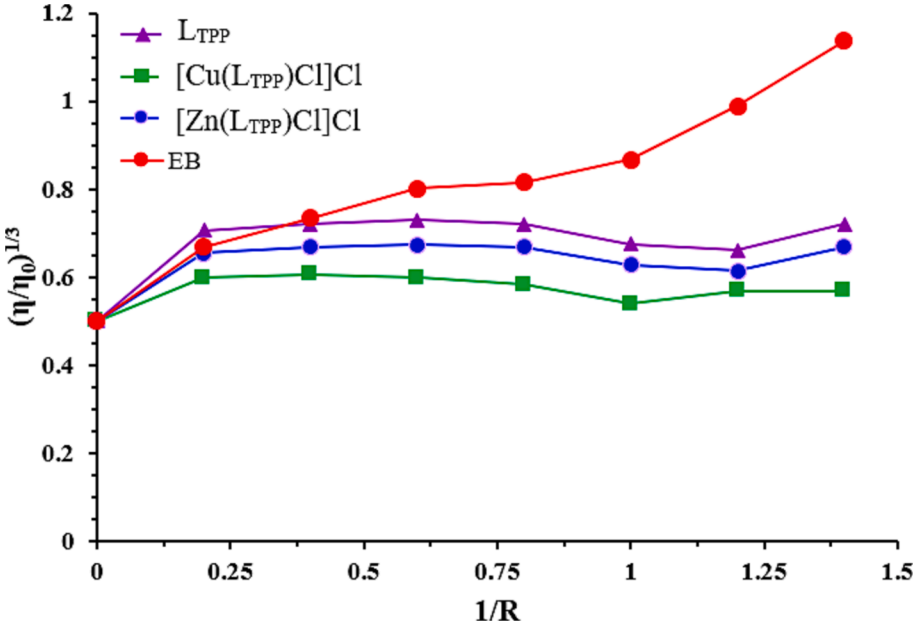

Fig. 6. Viscosity change of DNA in the presence of gradual addition of the compounds.

Cu(II) and Zn(II) complexes did not show a significant change in the viscosity of the DNA molecule as compared to the EB (Fig. 6). An interaction with groove binding rather than intercalation is indicated by the very low slopes of EB (0.38), the Schiff base ligand L_{TPP} (0.07), Cu(II) (0.064) and Zn(II) (0.0504).

3.3.4. Molecular docking

Molecular docking studies were performed using AutoDock Tools [41,42] and AutoDock Vina to determine the molecular mechanism, binding modes and all possible configurations with DNA of the triphenyl phosphonium-containing Schiff base ligand and its metal complexes with DNA dodecamers $d(CGCGAATTCGCG)_2$ (PDB: 1BNA). Compounds can interact with DNA *via* both covalent and non-covalent interactions. Non-covalent DNA interactions are the interactions of compounds with the DNA helical through intercalative, electrostatic and groove binding [43]. As seen in in the Fig. 7, the compounds preferred the groove binding mode. The most stable conformations of all compounds were preferred the minor groove binding mode with DNA. As a result of docking, L_{TPP}, $[Cu(L_{TPP})Cl]Cl$ and $[Zn(L_{TPP})Cl]Cl$ settled in the same region of the DNA. The binding affinities of the most stable conformations of the molecularly aligned compounds were calculated as −8.7 kcal/mol for L_{TPP}, −7.9 kcal/mol for $[Cu(L_{TPP})Cl]Cl$ and −9.0 kcal/mol for $[Zn(L_{TPP})Cl]Cl$. As a result of docking of L_{TPP} with DNA, nucleotide DA5 (-2.9 Å) with the nitrogen atom in the quinoline ring and nucleotide DG22 (-2.7 Å) with the hydroxyl group formed a hydrogen bond. In addition, shown that the hydroxyl group makes π-π T-shaped and hydrophobic interactions with the phenyl ring to which it is attached. The Cu(II) complex involved in Van der Waals and hydrophobic interactions with DNA. Zn(II) complex has been observed adapts well to the minor groove by making π-sigma, Van der Waals and hydrophobic interactions between functional groups and nucleotides. As a result of docking, the binding affinities of the compounds and experimental findings appear to be compatible with each other. $[Zn(L_{TPP})Cl]Cl$ both the K_b value obtained experimentally and the binding affinity obtained as a result of docking showed that it has stronger activity than L_{TPP} and $[Cu(L_{TPP})Cl]Cl$ compounds.

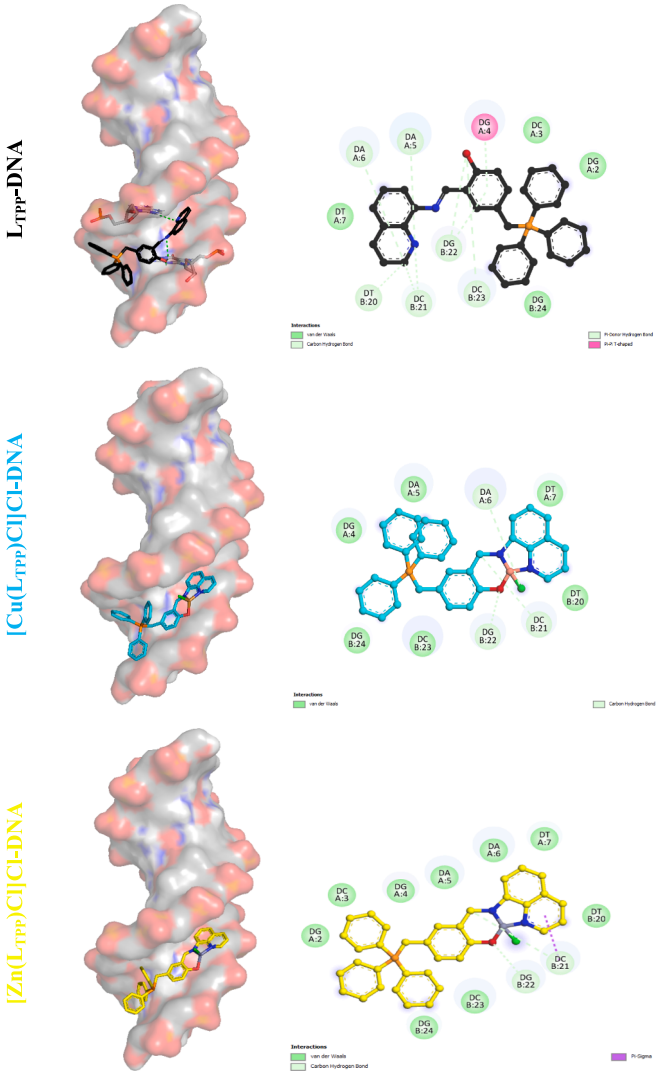

Fig. 7. 3D and 2D binding modes of the most stable conformations by docking of L_{TPP}-DNA, $[Cu(L_{TPP})Cl]Cl$-DNA and $[Zn(L_{TPP})Cl]Cl$-DNA.

3.4. Cytotoxic properties

The cytotoxic properties of the synthesized compounds on HUVEC and H2452 cell lines were determined through the conducted experiments. The graphs presenting these values are shown below.

The IC_{50} value of compound the Schiff base ligand L_{TPP} on HUVEC was found to be 452.9 μg/mL, while it was 575.35 μg/mL on the H2452 cell line (Fig. 8**a**). When the graphs generated using these values were evaluated, it was determined that the effect of compound the Schiff base ligand L_{TPP} on these two cell populations was similar ($p > 0.05$). On the other hand, the IC_{50} value of the synthetic complex $[Zn(L_{TPP})Cl]Cl$, which was produced by adding zinc metal to the Schiff base ligand L_{TPP}, on HUVEC was found to be 149.4 μg/mL, whereas it was 120.3 μg/mL for H2452 (Fig. 8**b**) with no statistically significant difference ($p > 0.05$). The IC_{50} values of the complex formed by the Schiff base ligand L_{TPP} with copper, $[Cu(L_{TPP})Cl]Cl$, however, were found to be 48.76 μg/mL and 31.89 μg/mL for HUVEC and H2452, respectively (Fig. 8**c**). When these values were analysed through Two-Way ANOVA, it was observed that $[Cu(L_{TPP})Cl]Cl$ was more effective in killing H2452 cells than HUVEC cells ($p < 0.05$).

The IC_{50} values were determined varied among the compounds and the cell type studied (Fig. 8**a-c**). However, when IC_{50} values are compared to those reported in the literature for similar compounds, it was observed that our values were relatively higher. Through our investigations into the reasons for this difference, it became apparent that there were various parameters involved. For instance, when examining the study conducted by Asadi et al., [13], we observed that they used use different cell lines such as A549 (Lung Carcinoma), Jurkat (Human T-Cell Leukemia), and Raji (Burkitt Lymphoma), they seeded the cells at different density. (7500 and 15,000 per well) and the duration of cell exposure to compounds was varied (48 h). In another study, Mardani et al., [44] used the K562 (Human Leukemia) cell line, with a cell count of 4000, and the cells were exposed to compounds for either 48 or 72 h. In addition, the synthesized compounds examined in these studies were also different. Therefore, considering these variables between their studies and ours, it is not unexpected to see some variations for the IC_{50} values obtained.

Furthermore, when the IC_{50} values of the synthesized compounds were compared among themselves, it was realized that IC_{50}'s of the Schiff base ligand L_{TPP} on both cell populations were higher than complexes $[Zn(L_{TPP})Cl]Cl$ and $[Cu(L_{TPP})Cl]Cl$. Besides, $[Zn(L_{TPP})Cl]Cl$ exhibited a higher IC_{50} value than $[Cu(L_{TPP})Cl]Cl$. In other words, the calculated IC_{50} values are ranked from low to high within each cell line as $[Cu(L_{TPP})Cl]Cl < [Zn(L_{TPP})Cl]Cl <$ ligand L_{TPP}. When the literature was carefully examined, it became evident that both Zn(II) and Cu(II) complexes bound to the same ligand usually increase the tumour selectivity of the compound by killing the cancerous cells more easily than the healthy ones [45]. However, when these two complexes were compared to each other, the Cu(II) complex turned out to have a higher antitumor activity than its Zn(II) counterpart [46,47]. Moreover, in another study conducted by Zhong *et al.,* [48], which compared Zn(II), Mn(II), Ni(II), Co(II), and Cu(II) complexes bound to the same ligand, it

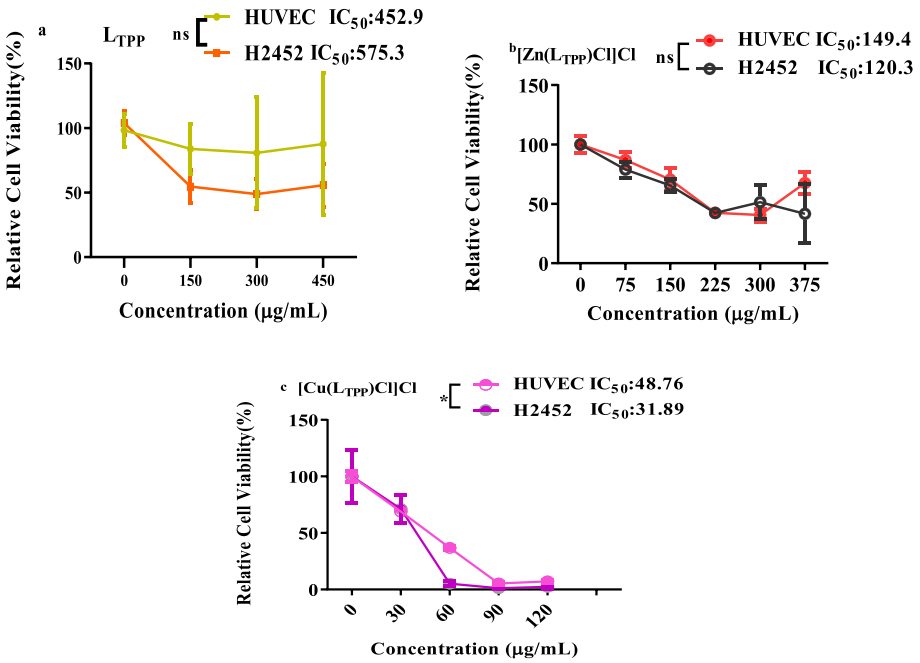

Fig. 8. A-cCytotoxicity of compounds of the Schiff base ligand L_{TPP} (a), [Zn(L_{TPP})Cl]Cl (b) and [Cu(L_{TPP})Cl]Cl (c) on both HUVEC and H2452 cells. The graphs illustrate the percentage viability of the cells exposed to increasing concentrations of each compound * indicates statistical significance at $p < 0.05$. ns: no statistically significant difference ($p > 0.05$).

was shown that the Cu(II) complex presented the highest antitumor activity compared to other metals [48]. Taken altogether, we can state that our findings are consistent with the literature in this manner.

The cells treated with [Cu(L_{TPP})Cl]Cl and [Zn(L_{TPP})Cl]Cl separately were visualized at 532 nm (green light). As discussed in section 3.2, complex [Zn(L_{TPP})Cl]Cl is highly emissive at 400–600 nm range and its emission intensity was found to be considerably higher than the free ligand and its Cu(II) complex. The strong emissive properties of Zn(II) complex has led us to investigate whether the complex enter the cancer cells. As can be seen in Fig. 9, the fluorescence emission of [Zn(L_{TPP})Cl]Cl inside the cells was clearly visible, while [Cu(L_{TPP})Cl]Cl emitted almost no light.

When images taken at a wavelength of 532 nm under green light were examined, it was observed that the compound [Zn(L_{TPP})Cl]Cl emitted fluorescent light even after entering the cell. As stated by Fernández-Moreira et al., [49], such a compound can be used for cell tracking purposes.

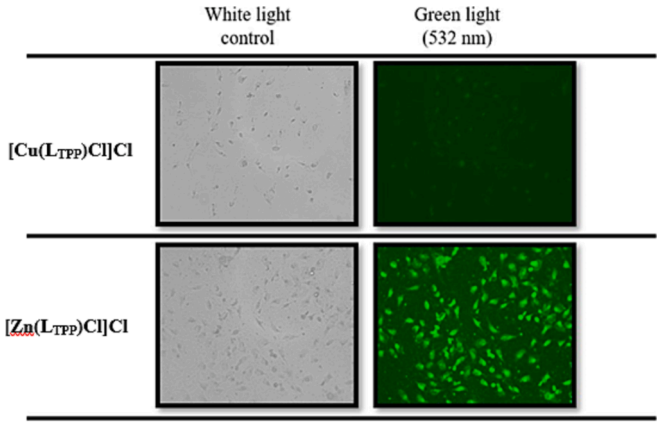

Fig. 9. Images captured under fluorescent light for [Cu(L_{TPP})Cl]Cl and [Zn(L_{TPP})Cl]Cl compounds. White light has been used as the control. Green light images for both compounds were taken at 532 nm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.5. Antioxidant properties

The MTS method was utilized to reveal the differences in antioxidant levels exhibited by the synthesized compounds at increasing concentrations. While it was found that the antioxidant levels of the Schiff base ligand L_{TPP} (Fig. 10**a**) and [Zn(L_{TPP})Cl]Cl (Fig. 10**b**) significantly increased with the increase of the concentrations compared to the control (0 µg/mL) ($p < 0.05$), the increase of antioxidant potential of [Cu(L_{TPP})Cl]Cl compared to no-compound media started at 150 µg/mL and kept rising as the concentration escalated (Fig. 10**c**)($p < 0.05$).

The antioxidant properties of the synthesized compounds using the water-soluble form of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) compound, known as MTS was determined. While the use of this agent for cell viability assessment is very common, its application for antioxidant measurement is not encountered frequently [33]. However, when the principle on which the technique is based is carefully evaluated (Methylthiazol Tetrazolium Assay, Promega, USA, G3582), it becomes apparent that such usage is not incorrect. In fact, in a comprehensive screening study published by Liu and Nair in 2010, which included plant extracts and pure compounds, it was demonstrated that MTT was an effective and economical method that could be used to measure antioxidant activity [33]. Therefore, we believe that other findings are legit and they reflect the real nature of the compounds in terms of antioxidant potentials.

Moreover, when the results of the MTS tests conducted on the compounds used in our study were analysed, it was found that the Schiff base ligand L_{TPP}, both in its pure form and in complex forms with metals, exhibited increasing antioxidant properties with increasing concentrations. In order to further investigate the changes induced by the ligand when forming complexes with metals, we compared the antioxidant value observed at the highest concentration (450 µg/mL) of the compound in its pure form to the values measured for the complexes (data not shown). Since we have not observed a statistically significant difference among these three compounds in terms of their antioxidant properties, we concluded that forming complexes with metals did not

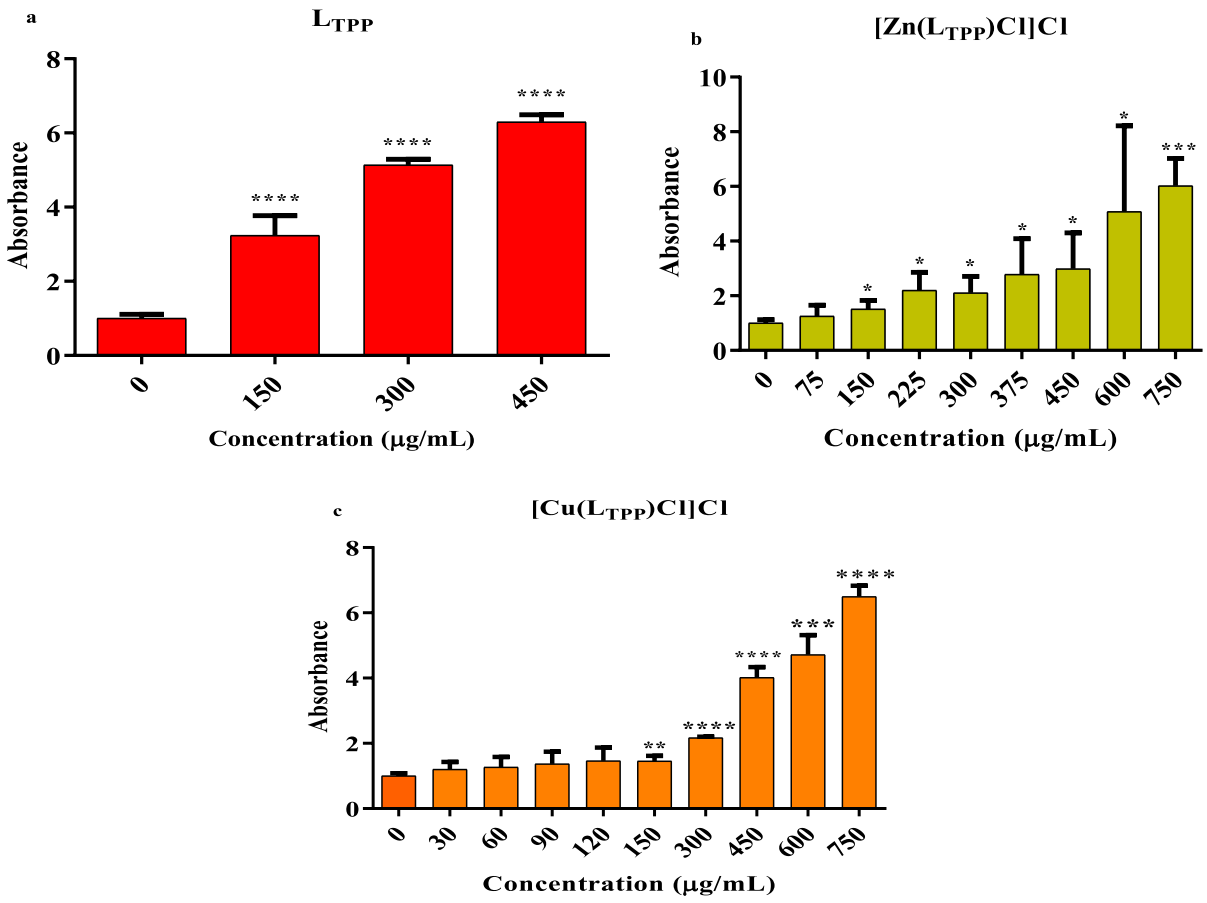

Fig. 10. Antioxidant level of the Schiff base ligand L_{TPP} (a), [Zn(L_{TPP})Cl]Cl (b) and [Cu(L_{TPP})Cl]Cl (c). In general, there is a tendency of antioxidant potential of each compound to rise with increasing concentrations (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

affect the antioxidant potential of the Schiff base ligand L_{TPP}. However, unfortunately, this observation did not align with the literature because metal complexes are thought to increase the antioxidant potential of the ligands. For instance, in a study published by Liu et al., it was stated that the binding of Zn(II) and Cu(II) metals to ligand compounds increased the antioxidant level of the compound dramatically [50] and it was explained by the conformational change and the metal's own antioxidant properties. We believe that this discrepancy may be attributed to the difference in the ligand we used in the present study.

4. Conclusions

In the course of this work, new Cu(II) and Zn(II) Schiff base complexes were synthesized and characterized. The DNA binding properties of ligand (L_{TPP}) and its complexes were examined by UV–vis absorption, fluorescence spectra and viscosity studies. From spectroscopic findings, it was found that, the ligand and its complexes exhibited groove binding interactions with DNA. Molecular docking studies also showed that the compounds interact with DNA in the minor groove binding mode. As a result of molecular docking, the binding affinities of the compounds and experimental findings are compatible with each other. The ligand and its complexes were screened for their cytotoxic properties towards malignant mesothelioma (H2452) and healthy human umbilical vein endothelial (HUVEC) cells. IC_{50} values are found to be in order as [Cu(LTPP)Cl]Cl < [Zn(LTPP)Cl]Cl < ligand LTPP.

CRediT authorship contribution statement

Ozge Gungor: Writing – original draft, Methodology, Investigation, Data curation. **Abdulmecit Gul:** Writing – original draft, Methodology, Investigation, Data curation. **Seyit Ali Gungor:** Writing – original draft, Methodology, Data curation, Investigation. **Sabahattin Comertpay:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Conceptualization. **Muhammet Kose:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jphotochem.2023.115453.

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