Molecular docking, QSAR properties and DNA/BSA binding, anti-proliferative studies of 6-methoxy benzothiozole imine base and its metal complexes

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ABSTRACT

The molecular and QSAR (Quantitative Structure–Activity Relationship) properties of title compound 2-((6-Methoxybenzo[d]thiazol-2-ylimino)methyl)-6-ethoxyphenol (HL) were evaluated employing HyperChem 7.5 tools. The interaction of the 1a–1e complexes of HL with calf thymus DNA (CT-DNA) was investigated by absorption titrations, Fluorescence quenching and viscosity measurements. The experimental data suggest that these complexes bind to CT-DNA through an intercalative mode, wherein DNA-binding affinity of 1e is found to be greater compared to other complexes. The tryptophan emission-quenching with bovine serum albumin (BSA) experiment revealed stronger binding of 1e than other complexes in the hydrophobic region of protein. The photo-cleavage of plasmid pBR322 DNA investigated in the presence of the title complexes inferred conversion of supercoiled form of DNA plasmid to circular nicked form. Free-radical scavenging activity studies of HL and its metal complexes determined by their interaction with the stable free-radical DPPH have shown promising antioxidant property. Further cytotoxicity studies with HeLa and MCF-7 cell lines indicated that the compounds can efficiently inhibit the cell proliferation in a dose dependent manner. The DAPI staining assay studies revealed the higher potency of 1e to induce apoptosis.

ABBREVIATIONS: BSA: Bovine serum albumin protein; CT-DNA: Calf thymus DNA; DMSO: Dimethyl sulfoxide; DAPI: 4',6-Diamidino-2-phenylindole dihydrochloride; ESI–MS: Electrospray ionization mass spectrometry; IC50: Half-maximal inhibitory concentration; MTBTE: 2-((6-methoxybenzo[d]thiazol-2-ylimino)methyl)-6-ethoxyphenol; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS: Phosphate-buffered saline; Tris: Tris(hydroxymethyl)aminomethane

Introduction

Metallo-anticancer drug innovation is one of the significant fields encouraging pharmaceutical research. Metal complexes which can bind and cleave DNA under physiological circumstances have the potential to be used as therapeutic agents for medicinal purposes (Ammavasi et al., 2017; Barnett, VamCamp, Trosko, & Mansour, 1969; Mathiyan, Nattamai, & Anandaram, 2016). The earlier reports revealed that the platinum containing drug cisplatin which has the affinity to bind with DNA through covalent bonding interactions is effective as anticancer drug. But later the use of platinoid drugs has often been limited due to their various side effects and drug immune systems. Such limitations of cisplatin have prompted wide-ranging investigation into other metal based anticancer agents (Ammavasi et al., 2017; Chandrasekhar et al., 2018; Jegathalaprabhan et al., 2017; Karuthamohamed, Mookkandi, Kumar, Lokesh, & Jegathalaprabhan, 2018).

The anticancer efficacy of benzothiozole imine bases (Amnerkar & Bhusari, 2010; Aziz, Elantabli, Moustafa, & Medani, 2017; Negm et al., 2011, Zha, Xia, & Hu, 1999) was initially represented to be due to their action as inhibitors of Ribonucleotide reductase, which catalyzes the rate-limiting step of DNA synthesis. DNA binding and cleavage are two important studies for gene mutation and cancer in biological study (Aveli, Pradeep Kumar, Tejaswi, Vamsikrishna, & Shivaraj, 2016; Bathini et al., 2014; Ravi et al., 2015; Sreenu et al., 2017; Sreenu, Vamsikrishna, Ganji, Venkateswarlu, & Shivaraj, 2018). Therefore, DNA binding agents may have great viewpoint for use as artificial nucleases, DNA structural probes or gene selective drugs. Earlier research studies both in vitro and in vivo revealed that benzothiozole imine base metal complexes have exhibited pronounced cytotoxicity (Bradshaw et al., 2002; Musser et al., 1984; Vicini et al., 2003; Yoshida et al., 2005).

In the investigation of metal complexes which have binding ability with target DNA strand and amino acid residues of target protein, the metal complexes of copper, nickel and cobalt were found to have a broad range of therapeutic uses (Aveli, Pradeep Kumar, Nirmala, Sreenu, & Shivaraj, 2019; Bathini et al., 2014; Carlo et al., 2014; Jayaseelan, Prasad, Vedanayaki, & Rajavel, 2016; Jayaseelan, Akila, Usha Rani, & Rajavel, 2016; Jain, Gupta, Ganeshpure, & Raison, 2005; Pradeep et al., 2015; Shabbir et al., 2017). Though many transition metal complexes having antitumor properties were

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discovered earlier, because of their various incompatible and detrimental effects, developing new potential biocompatible metal complexes has turned as a challenge of present day researchers.

The demonstrated antibacterial activity of imine bases led researchers to investigate their possible use as anticancer agents. Moreover, a wide number of different heterocyclics with imine side chain and having potential binding ability with metal ions such as Co(II), Cu(II), Ni(II) etc., showed many applications in various fields (Aveli et al., 2019; Bathini et al., 2014; Carlo et al., 2014; Jain et al., 2005; Jayaseelan, Prasad et al., 2016, Jayaseelan, Akila, 2016; Pradeep et al., 2015; Shabbir et al., 2017).

Keeping in view the various potential applications of imine bases of heterocyclics, we reported earlier (Ravi et al., 2014) from our laboratory the synthesis and characterization of 2-((6-Methoxybenzo[d]thiazol-2-ylimino)methyl)-6-ethoxy-phenol (HL) and its complexes. In continuation of this work, we report herein quantum chemical and structural activity parameters as well as in vitro biological activity studies of HL and its complexes 1a–1e. In continuation of this work, we report herein quantum chemical and structural activity parameters as well as in vitro biological activity studies of HL and its complexes 1a–1e. In continuation of this work, we report herein quantum chemical and structural activity parameters as well as in vitro biological activity studies of HL and its complexes 1a–1e. In continuation of this work, we report herein quantum chemical and structural activity parameters as well as in vitro biological activity studies of HL and its complexes 1a–1e.

**Molecular docking**

In silico analysis was performed in Dell Precision T7610 workstation (12 processors; 8 GB RAM; NVIDIA 3GB graphics; Discovery Studio 2.1) running on windows environment. The molecular docking of complexes 1a, 1b, 1c, 1d and 1e was performed using Ligandfit (Rabindra Reddy, Kumara Swamy, & Satyanarayana, 2018a). CHARMm force field was used throughout the simulation before running Ligandfit. Ligandfit is a high algorithm for docking ligands into an active binding site on the receptor. The crystal structure of human DNA topoisomerase 1 (TOP1) receptor was downloaded from RCSB database (PDB ID-1T8I) and then all the water molecules of the protein were removed by using Discovery studio. As the receptors have many active sites, the one of the suitable receptor is selected. All the chemical structures of title compounds are drawn in chemdraw software and then docked into the target binding site. The docked structures were visualized in molecular graphics software of ssviewer (Rabindra Reddy, Kumara Swamy, & Satyanarayana, 2018b) and discovery studio Visualizer (Porkodi, Arunadevi, & Raman, 2019).

**Materials and experimental methods**

All the solvents and chemicals used were of analytical reagent grade. Calf thymus DNA, BSA protein, Cisplatin, MTT and 99% Tris–HCl buffer (1.0 × 10−5 M, pH 7.1) were purchased from Sigma Chemicals. The super coiled pBR-322 DNA stored at −20 °C (Genie, Bangalore, India) was used instantaneously within few hours. All other chemicals and...
solvents were procured from local available sources. All the experiments involving the interaction of the ligand and its transition metal complexes with CT DNA were carried out in double distilled water and ethanol-DMSO solvent mixture. Solution of CT DNA in the buffer gave UV absorbance peaks ratio of about 1.8–1.9:1.0 at 260 and 280 nm, indicating that the CT DNA was sufficiently free of protein. The CT DNA concentration per nucleotide was determined spectrophotometrically by employing an extinction coefficient of 6,600 M$^{-1}$ cm$^{-1}$ at 260 nm. The ligand and its metal complexes were dissolved in a solvent mixture of ethanol and 1% DMSO (Futera, Li, Tateyama, Li, & Higuchi, 2011).

Elemental analysis (C, H and N) was carried out using an Elemental Perkin Elmer – 2400 analyzer. Melting points were determined by Veego Melting Point VMP-III apparatus. The IR spectra were obtained in KBr discs on a Shimadzu FT-IR spectrophotometer in the 4,000–400 cm$^{-1}$ region. $^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker DMSO-d$_6$ with TMS as an internal standard. Mass spectra were recorded on VG – AUTOSPEC instrument using ethanol as solvent/mobile phase. The UV-Vis spectra were recorded on Shimadzu UV-2600 spectrophotometer. Fluorescence measurements were recorded on Shimadzu RF-5301PC Spectrophotometer at room temperature. DNA cleavage studies were carried out on GeNei gel electrophoresis instrument, and then the components on gel were viewed using gel documentation system. Subsequently the viewed images were photographed using a CCD camera (BIO-RAD Gel Doc 1000™). Fluorescence microscopy of apoptosis assays was performed with an OX31 fluorescence microscope (Olympus, Japan) and confocal images viewed in Confocal Microscope (LSM 510 META) Zeiss.

**Biomolecular applications**

**DNA binding and photocleavage experiment**

The imine base, its 1a–1e metal complexes were tested for their binding abilities with CT-DNA by absorption spectroscopy (Biswas et al. 2019). Characterized stock solution of Calf thymus DNA was stored at 4 ºC and used within five days. Concentrated stock solutions of metal complexes were prepared by dissolving calculated amount of metal complexes in ethanol and diluting with corresponding buffer to the concentration required for all the experiments. The solutions were incubated for 5 minutes before recording the absorption spectra. Absorption spectral titration experiment was performed by keeping concentration of the complex constant and varying the DNA concentration. The intrinsic binding constants ($K_b$) of the metal complexes with CT-DNA are evaluated by using following Wolfe–Shimer equation (1) (Ravi et al., 2015)

\[
\frac{[\text{DNA}]/(i_a - i_t)}{[\text{DNA}]/(i_b - i_t)} = 1 + \frac{1}{K_b} - \frac{i_b - i_t}{i_a - i_t}
\]

where [DNA] represents the concentration of DNA, and $i_a$, $i_t$ and $i_b$ correspond to the apparent extinction coefficient ($i_a = A_{obs}/[M]/L$), the extinction coefficient of the free metal complex ($i_t$) and the extinction coefficient of the metal complex in the fully bounded form with DNA ($i_b$). The $K_b$ is the binding constant of specific compound with DNA and is obtained from the plot of $[\text{DNA}]/(i_a - i_t)$ vs. [DNA] in respective systems (Ammavasi et al., 2017; Chandrasekhar et al., 2018; Jegathalappritahban et al., 2017; Karuthamohamed et al., 2018; Ravi et al., 2019).

**Protein binding study**

The stock solution of BSA protein prepared by dissolving the appropriate amount of solid BSA in buffer solution (5 mM Tris–HCl/50 mM NaCl at pH 7.2) followed by stirring for 1 h, was kept at 4 ºC. The solutions ($2.0 \times 10^{-4}$ M) of title complexes were prepared by dissolving in ethanol and diluting with Tris-HCl buffer. The BSA concentration was found spectrophotometrically by recording absorbance at 280 nm and substituting reported molar extinction coefficient value ($i_{280} = 44,300$ M$^{-1}$ cm$^{-1}$) (Ray, Seth, Pal, & Basu, 2012).

UV-absorption titrations were executed by keeping the BSA concentration constant (40 μM) and rising the concentration of the complex (0.00–40 μM) in all the systems under investigation. The change in absorbance of the characteristic band of the BSA at $\lambda$ max = 280 nm was recorded after each incremental addition. The binding constant value is calculated from the linear plot of $[1/(A-A_0)]$ Vs $[1/[\text{Metal complex}]]$ where $A_0$ and $A$ are absorbance of the BSA at 280 nm in the absence and presence of metal complex (Anjomsho, Fatemi, Mahani, & Hadadzadeh, 2014).

**Fluorescence measurements**

The quenching constants of the metal complexes to calf thymus DNA were determined by fluorescence spectral method using the emission intensity of Ethidium Bromide (EB) as a probe, which fabricates highly fluorescent EB-DNA adduct with fluorescence emission property at 560 nm. The fluorescence intensity of EB-DNA can decrease gradually upon the addition of the metal complex in small increments due to competitive binding of complexes. The interactions of complexes of 1a–1e with CT-DNA were determined with an EB-bound CT-DNA solution in 5 mM Tris–HCl/NaCl buffer (pH = 7.2). Stern–Volmer equation (Sedighipoor, Kianfar, Kamil Mahmood, & Azarian, 2017) and Scatchard equation (Das, Nasani, Saha, Mobin, & Mukhopadhyay, 2015) were used to calculate $K_{sv}$ and $K_b$ values. The observed fluorescence reducing curve of EB-bound CT-DNA by complexes is in good agreement with the classical Stern–Volmer (2) and Scatchard equations (3)

\[
\frac{I_0}{I} = K_{sv}[Q] + 1
\]

\[
\log\left(\frac{I_0}{I}\right) = \log K_b + n \log [Q]
\]

($I_0$ is initial fluorescence intensity, $I$ quenched intensity)

Quenching of the tryptophan residues of BSA experiments was performed by using phosphate buffer (pH = 7.2). Quenching titrations were manually done by using a micro pipette for the addition of 1a, 1c and 1e complexes as quenchers. The emission intensity of tryptophan residues of
BSA at 344 nm was recorded after each addition of the quencher and then by using 2 and 3 equations, the number of binding sites \( n \) and quenching constant \( Q \) were calculated.

**Viscosity measurements**

Viscosity measurements were performed with viscometer at a constant temperature of 28 ± 0.1 °C in a thermostatic bath. A digital stopwatch was used to measure the flow time, and each sample was measured three times to obtain the average flow time. The result in each system was presented as plot of \( (\eta/\eta_0)^{1/3} \) versus the ratio of complex concentration to DNA concentration (\( \eta \) and \( \eta_0 \) are respectively the viscosity of DNA in presence and in absence of the complex) (Jian et al., 2014; Mahadevan & Palaniandavar, 1998; Vuradi et al., 2017, 2018).

**pBR322 photo cleavage study**

The cleavage of supercoiled pBR322 DNA was carried out in agarose gel electrophoresis instrument. The imine base and its metal complexes were treated with supercoiled DNA (1 μL) in buffer to prepare respective solutions. Subsequently these solutions were then irradiated at room temperature with UV Lamp (365 nm) for 5 min. Then, the samples were mixed with loading buffer (3 μL) containing 25% bromo phenol blue, 0.25% xylene cyanol and 30% glycerol (2 μL) and electrophoresed for 1 h at 50–100 eV on 1% agarose gel using tris-acetic acid EDTA buffer (pH 7.2). Subsequently the gel was stained using 1 mg/1 mL ethidium bromide (EB) and viewed with gel documentation system and photographed using a CCD camera (Padithem, Eshwari, Ravi, Zaheer, & Sarala Devi, 2015; Yan & Cowan, 2005).

| QSAR properties | Molecular properties |
|-----------------|----------------------|
| Surface area    | 442.91 Å²  | Total energy  | –8417.99 Kcal/mol |
| Surface area (Grid) | 548.34 Å²  | Dipole moment  | 4.293 D          |
| Volume          | 929.88 Å³  | RMS gradient  | 0.1865 Kcal/mol  |
| Hydration energy| –11.07 kcal/mol|                |                  |
| Log P           | 1.16                   |                |
| Refractivity    | 99.99 Å³               |                |
| Polarisability  | 28.63 Å³               |                |
| Mass            | 328.39 amu             |                |

**Figure 1.** Geometry optimized structures and electrostatic potentials of HL in molecular and ionized forms.
Radical scavenging activity

The percentage of free radical scavenging activity assay is completely based on decrease in absorbance value of DPPH free radical at 517 nm on addition of $1a$–$1e$. The experiment involves diluting the working solution of the metal complex as well as the ascorbic acid standard (700, 600, 500, 400, 300 and 200 $\mu$g/mL) in methanol. A series of solutions for each metal complex system as well as for standard were prepared by keeping concentration of DPPH constant and varying the concentration of a metal complex. These solutions were shaken vigorously and kept in dark for 30 min at room temperature. Then, the absorbance was measured at 517 nm in spectrophotometer (Satish & Dilipkumar, 2005). The whole experiment was carried out using spectroscopic grade methanol solvent at 25°C. The scavenging ratio was determined using the following equation.

$$\text{Scavenging activity} \% = \left( \frac{A_0 - A_i}{A_0} \right) \times 100$$

($A_i$ is the absorbance of solution of the ligand or its complexes after dilution factor correction and $A_0$ is the absorbance of blank).

In vitro cytotoxicity assay

The in vitro cytotoxic effects of the $1a$–$1e$ complexes were measured in human breast adenocarcinoma cell lines (MCF-7) and Human cervical cancer cell lines (HeLa). Cells were cultured according to the American Type Culture Collection (ATCC) instructions and then maintained at 37°C and 5% CO$_2$ in a humidified incubator. MTT assay is used for the measurement of cytotoxicity (Gao, Sun, Liu, & Duan, 2006). HeLa cells were treated with prepared metal complexes
(50–150 μM) in three different concentrations in DMSO medium for 48 h using 96-well culture plates. In parallel, the cells were treated with only 0.5% (v/v) DMSO and were used as control. At the end of incubation, MTT (20 μL of 5 mM) was added in each well and further incubated for 4 h in cell culture incubator (Ferrari et al., 1990). Then the medium was replaced with 150 μL of MTT solvent (4 mM HCl, 0.1% NP-40 in isopropanol) and further incubated for 15 min at room temperature. Finally, the absorbance readings at 595 nm were collected in an ELISA plate reader (Bio-Rad, Model 680XR) (Rey et al., 2009). The data from cancer cell lines were acquired from three independent cell passages and the IC₅₀ values were calculated from the plot of cell viability against concentration of complexes.

**Live cell confocal microscopy**

DAPI staining was performed on HeLa cancer cells pretreated with the complexes of 1a–1e was studied by confocal microscopy. The HeLa cells were cultured on cover slips (corning 22 × 22 mm), incubated for 24 h until they reached 70% confluence. These cells were then serum starved overnight, followed by incubation with 50 Mm of respective
Confocal microscopy imaging was carried out on one set without any staining and other set was stained with DAPI (nuclear stain) to identify localization of the complex and any nuclear disintegration in each system (Chennam et al., 2016).

Results and discussion

**QSAR properties and molecular orbital properties**

QSAR properties like surface area, volume, hydration energy, log P, refractivity, polarisability, mass, total energy etc. are

Table 3. Binding Constant ($K_{bd}$), Quenching Constant ($K_{eq}$), Number of Binding Sites ($n$) and R for the Interactions of Complexes with CT-DNA.

| Metal Complexes | Absorption binding | Emission quenching |
|-----------------|---------------------|--------------------|
|                 | $K_b$ | $K_{eq}$ | $K_{bd}$ | $K_{eq}$ | n   | R   |
| 1a              | $2.3 \times 10^3$ M$^{-1}$ | $1.7 \times 10^4$ M$^{-1}$ | $1.5 \times 10^4$ M$^{-1}$ | 0.99 | 0.96 |
| 1b              | $1.5 \times 10^4$ M$^{-1}$ | $1.1 \times 10^4$ M$^{-1}$ | $1.4 \times 10^4$ M$^{-1}$ | 0.99 | 0.97 |
| 1c              | $2.5 \times 10^5$ M$^{-1}$ | $1.9 \times 10^5$ M$^{-1}$ | $1.9 \times 10^5$ M$^{-1}$ | 0.99 | 0.98 |
| 1d              | $2.7 \times 10^2$ M$^{-1}$ | $2.2 \times 10^4$ M$^{-1}$ | $2.1 \times 10^4$ M$^{-1}$ | 0.99 | 0.98 |
| 1e              | $3.5 \times 10^3$ M$^{-1}$ | $3 \times 10^4$ M$^{-1}$ | $2.3 \times 10^4$ M$^{-1}$ | 0.99 | 0.97 |
computed for above optimized molecule using HyperChem tools. The data are presented in Table 1. The one of the QSAR parameters Log P is critical parameter to express the competence of the molecule to cross the cell membrane to reach receptor site in biological systems. The more positive Log P for HL indicates more hydrophilicity rather than lipophilicity. The frontier molecular orbital energies (i.e. $E_{\text{HOMO}}$ and $E_{\text{LUMO}}$) (HyperChem, 2006) are significant parameters for the prediction of the reactivity of a chemical species. The $E_{\text{HOMO}}$ is often associated with the electron donating ability of a molecule while $E_{\text{LUMO}}$ with the ability of the molecule to accept electrons. The minus Eigen value of $E_{\text{HOMO}}$ corresponds to binding energy value of electrons present in HOMO energy levels. Therefore lesser the binding energy value for $E_{\text{HOMO}}$ electrons indicates higher tendency for the donation of electrons to the appropriate acceptor molecule with low energy and empty molecular orbitals (Padmaja, Laxmi, & Sarala Devi, 2011).

The contour maps presented above portray orientation of HOMO and LUMO orbitals both in molecular and ionized forms. Figure 1. The Eigen values of $E_{\text{HOMO}}$, $E_{\text{LUMO}}$ and $E_{\text{LUMO-HOMO}}$ were found to be 8.869, −0.5647 and 8.306 eV for neutral molecule and −3.805, 2.58 and 6.386 eV for ionized form. The large difference in energy in HOMO and LUMO orbitals indicates higher stability in molecular form, while in ionized form less energy difference ($E_{\text{LUMO-HOMO}}$) predicts high labile character Figure 2 (Padmaja, Begum, Raghavaiah, & Sarala Devi, 2011). Moreover, the ionization energy of electrons in different orbitals defined as equivalent to their corresponding minus Eigen values are the measure to predict the order of ionization potentials of the electrons from different levels. The lowest binding energy of electrons 3.805 eV and 2.58 eV from highest occupied molecular orbitals in molecular and ionised forms respectively, infer relatively more electron donor property in ionized form. Thus the contour maps and the binding energies predict the probable donor sites and magnitude of binding

Figure 5. Emission spectra of EB–DNA in the absence and presence of the complexes in Tris- HCl/NaCl buffer (pH = 7.2). The arrow indicates the emission intensity changes of EB–DNA upon increasing the concentration of the complexes 1d: A, 1e: B, 1a: C, 1b: D and 1c: E. Insert is the Stern–Volmer and Scatchard plot.
Figure 6. Absorption spectra of BSA with A for 1d, B for 1e, C for 1a, D for 1b and E for 1c. The arrow shows the absorbance changes with increasing complexes concentration. Insert shows the plot of 1/(A–A₀) vs. 1/[Complex].

Table 4. Binding Constant (K_{b}), Quenching Constant (K_{sv}), Number of Binding Sites (n) and R for the Interactions of Complexes with BSA.

| Metal Complexes | Absorption binding | Emission |
|-----------------|--------------------|----------|
|                 | K_{b}              | K_{sv}   | n  | R  |
| 1a              | 6.7 × 10^4 M⁻¹     | 4.7 × 10^4 M⁻¹ | 1 × 10^4 M⁻¹ | 0.99 | 0.98 |
| 1b              | 1.4 × 10^5 M⁻¹     | –        | –  | –   | 0.97 |
| 1c              | 1.8 × 10^5 M⁻¹     | 8.2 × 10^5 M⁻¹ | 1.1 × 10^4 M⁻¹ | 0.99 | 0.99 |
| 1d              | 1.9 × 10^5 M⁻¹     | –        | –  | –   | 0.99 |
| 1e              | 2.2 × 10^5 M⁻¹     | 1.9 × 10^5 M⁻¹ | 5.8 × 10^4 M⁻¹ | 0.99 | 0.98 |
strength with metal ions to form stable metal complexes which comprise dative bonds (Winchester & Doyle, 1992).

**Molecular docking**

Minimization of the DNA Topoisomerase I was performed using Discovery studio. The CHARMM force field is applied for identification of binding sites in the DNA Topoisomerase I receptor which plays important role in replication of DNA. The molecular docking revealed the interactions of all title compounds in their best suitable pose with more negative value of dock score corresponding to lowest binding energy in all the systems studied. The interactions and docking scores of the complexes with the active site pocket residues of human DNA TOP1 were tabulated in Table 2 1a, 1b, 1c, 1d and 1e. Further, it is evidenced by the molecular docking studies that the active site pocket residues of receptor are involved in hydrogen bonding with docked title compounds in respective systems. A high minus dock indicates a stronger binding affinity of guest title molecules with host receptor sites (Vijayalakshmi, Subramanian, Nair, & Sarma 2002). The measured biological interactions were shown in Figure 3a.

**DNA binding studies**

**Electronic absorptions titrations**

The changes in the absorption spectra of the 1a–1e complexes in the presence of CT-DNA, at fixed concentration of free metal complexes are shown in Figure 4. It has been observed that the gradual incremental addition of CT-DNA for a fixed concentration of complexes in respective systems, the band positions of the complexes altered due to hypochromic shift and slight red shift. The extent of hypochromism and bathochromism are expected to be generated through an intercalative mode of binding involving a $\pi \rightarrow \pi^*$ stacking interaction between the

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Figure 7. The emission spectrum of BSA in the presence of increasing amounts of compounds 1e: A, 1a: B and 1c: C. The arrow shows the fluorescence quenching upon increasing the concentrations of the compounds. The insets show the Stern–Volmer plot and Scatchard plot of the complexes with BSA.

Figure 8. Effect of increasing concentrations of the complexes 1a–1e on the relative viscosity of CT-DNA at 28 ± 0.1 °C.
aromatic chromophores of the intercalated complexes and the base pairs of DNA duplex (Chennam et al., 2016). The extent of interaction gives a measure of the strength of the intercalative binding. The above recorded absorption spectral data were then substituted in the Wolfe–Shimer equation to obtain intrinsic binding constant $K_b$ (Martins et al., 2017; Nagula et al., 2018). The higher $K_b$ value of 1e compared to 1a, 1b, 1c and 1d is ascribable to the presence of relatively more number of aromatic moieties as the complex contains two coordinated ligand groups (ML2).

The binding constant ($K_b$) values presented in Table 3 for five metal complexes confirm their competent interaction with CT-DNA. These $K_b$ values which reflect binding affinities of complexes with DNA follow the order; 1e > 1d > 1c > 1a > 1b.

Fluorescence quenching measurements

To further confirm the binding of 1a–1e complexes with DNA through intercalation, emission titrations were performed. Since, no luminescence was observed for metal complexes in the presence of CT-DNA, the competitive binding studies using ethidium bromide (EB) were performed as it produces intense fluorescence emission due to intercalation between base pairs of DNA Duplex. The emission intensity of EB bound to DNA is used as a structural probe as fluorescence quenching occurs when other molecule competitively binds through intercalation with DNA already bound with EB. Such a significant change in the nature of fluorescence of EB bound to DNA is used to understand the relative DNA binding strength of the second molecule. The addition of 1a–1e complexes to DNA pretreated with EB, showed noticeable fluorescence quenching at 560 nm (270 nm excitation) Figure 5. Relative binding of these complexes to CT DNA is measured from the extent of reduction in emission intensity. The Stern–Volmer constant $K_{sv}$ (Sedighipoor et al., 2017 and Scatchard (Das et al., 2015). binding constant $K_b$, and binding ratio $n'$ were evaluated for all complexes, and presented in Table 3. These values indicate relatively strong binding of 1e with CT-DNA among all the systems studied.

BSA binding studies

Electronic absorption titrations

The absorption spectral data of BSA after every successive addition of specific complex indicated hypochromic and red shift due to its binding with BSA. Such a binding would result in polarity variation in the microenvironment of tyrosine and tryptophan region of BSA (Anjomsho et al., 2014). The higher $K_b$ value of 1e compared to 1a, 1b, 1c and 1d is ascribable to the presence of relatively more number of aromatic moieties as the complex contains two coordinated ligand groups (ML2).

The binding constant ($K_b$) values presented in Table 3 for five metal complexes confirm their competent interaction with CT-DNA. These $K_b$ values which reflect binding affinities of complexes with DNA follow the order; 1e > 1d > 1c > 1a > 1b.
groups. This method was used to characterize the interactions of metal complexes with the BSA. Upon successive addition of complexes (1–110 μM) to BSA solution, decrease in the fluorescence intensity of tryptophan was observed.

The emission intensity of tryptophan residues of BSA at 344 nm was recorded after each addition of the quencher and then by using equations (2) and (3) $K_v$, $K_w$, and number of binding sites ($n$) values are calculated (Das et al., 2015) and are given in Table 4 and Figure 7. Among the three $1_e > 1_d > 1_c$ complexes, the former complex has better interaction with BSA than the latter two complexes. The calculated value of $n$ is around 1.0 for all of the complexes indicating 1:1 binding ratio.

Viscosity measurements

To further investigate the binding nature of the metal complexes to CT-DNA, viscosity studies have been carried out. Viscosity measurements sensitive to length change are regarded as the least ambiguous and the most critical test of binding model in a solution. As per classical intercalation model, lengthening of DNA helix occurs as base pairs get separated to insert ligand/complex (Nagula et al., 2018) and thus results in increase of its viscosity. In contrast, partial or non-classical intercalation of ligand/complex, may decrease the length of DNA duplex, and subsequently decrease its viscosity. In present investigation, relative viscosity of the DNA increased steadily upon addition of $1_e > 1_d > 1_c > 1_a > 1_b$ complexes indicating intercalative mode of binding of complexes to DNA Figure 8.

DNA photocleavage cleavage

The interaction of plasmid pBR 322 DNA with metal complexes was carried out by gel electrophoresis. The gel electrophoresis is widely used technique for the exploration of pBR 322 DNA cleavage property upon its treatment with metal complexes. After the migration of cleaved fragments on gel under the influence of an electric field, the separated components are viewed with gel documentation system and then snapped for image using a CCD camera. The DNA cleavage ability of $1_a$–$1_e$ and HL were enumerated by evaluating the conversion of the SC form into NC form with the increase of concentration of the complexes, the DNA cleavage activity is enhanced as shown in Figures 9A,B. Thus, overall results signify the competence of title complexes to cleave plasmid DNA proficiently.

Radical scavenging activity

Antioxidant property was evaluated by using DPPH method. Test compounds were dissolved in DMSO solution, as shown in Figure 10 and measured at different concentration of compounds between 200 μg/mL to 700 μg/mL. Radical scavenging activity was increased with increasing concentration of compounds. Ascorbic acid is used as reference antioxidant to compare free radical scavenging activities of complexes (Bathini et al., 2014; Blois, 1958). The metal scavenging activity which is the measure of antioxidant property in the concentration range 200–700 μg/mL follows the order: $1_e > 1_d > 1_c > 1_a > 1_b$. The variation in scavenging behavior of compounds under
investigation is attributable to structural factors influenced by metal ion associated in respective systems.

**In vitro cytotoxicity assay**

Cytotoxic studies were evaluated by MTT assay against a two-tumor cell line panel of HeLa and MCF-7. The cells were exposed to different concentrations (50 μM, 100 μM, and 150 μM) of samples. Comparing the IC$_{50}$ values, the cytotoxicity is compatible with the DNA binding affinities of all the compounds. The percentage of cell viability gradually decreased with increasing the concentration of prepared compounds. This phenomenon indicates that the ligand and the 1a–1e complexes show different cytotoxic activity against two tumor cell lines. Cell death was obtained by treating the HeLa cancer cells and MCF-7 (Jeyalakshmi et al., 2015; Loganathan et al., 2014) at 50–150 μM of sample for 24 h of incubation and then growth inhibition of cells is observed by adopting DAPI staining. The increase in number of abnormal cells with increase in concentration of compounds reveals the concentration effect of compounds as showed in Figure 11. The observed morphological changes in 24 h such as; nuclear swelling, cytoplasmic bubbling and late apoptosis indication of dot-like chromatin condensation are shown in Figure 12. The results predict the order for apoptosis-inducing effect as 1e > 1d > 1c > 1a > 1b > HL. The IC$_{50}$ values of all the

**Table 5. The IC$_{50}$ values for complexes against HeLa and MCF-7 cell lines.**

| Compound | HeLa (μM) | MCF-7 (μM) |
|----------|-----------|------------|
| 1a       | 59 ± 2.2  | 58 ± 1.5   |
| 1b       | 63 ± 2.0  | 65 ± 2.6   |
| 1c       | 53 ± 1.8  | 52 ± 1.7   |
| 1d       | 44 ± 2.8  | 49 ± 2.1   |
| 1e       | 41 ± 2.1  | 45 ± 0.9   |
| HL       | 80 ± 2.8  | 85 ± 3.8   |
| Cisplatin| 9 ± 2.0   | 11 ± 2.2   |
compounds in Table 5 infer their efficient antitumor activity (Tabrizi, Faranak, & Hossein, 2016).

Conclusions

The molecular and QSAR properties evaluated by HyperChem tools are informative to understand explicitly structural related properties. The absorption titration and fluorescence quenching studies revealed that the metal complexes of present investigation are efficient binders of CT-DNA. The steady increase of relative viscosity of the DNA upon addition of complexes in respective systems infers intercalative mode of binding of complexes with DNA. The binding studies of title compounds showed a good interaction with BSA. DNA cleavage ability enumerated by evaluating the conversion of the SC form into NC form indicated that 1d and 1e is relatively more active among all the investigated metal complexes.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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