



## DNA binding, photoactivated DNA cleavage and cytotoxic activity of Cu(II) and Co(II) based Schiff-base azo photosensitizers



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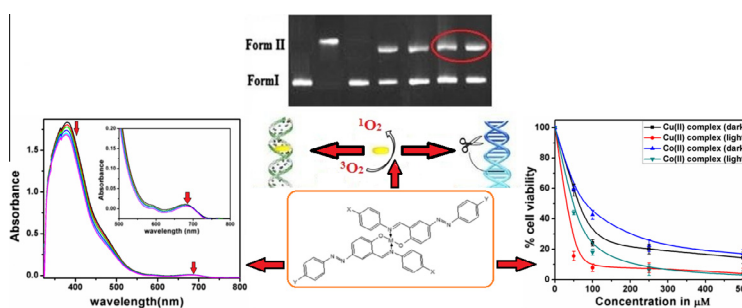
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### HIGHLIGHTS

- We have synthesized Cu(II) and Co(II) based Schiff-base azo photosensitizers.
- Both the complexes interacted with CT-DNA via intercalative mode.
- Both the complexes act as efficient photocleavers of SC pUC19 DNA in UV light.
- The  $\Phi_{\Delta}$  of  $^1\text{O}_2$  generation of complexes was found to be 0.38 and 0.36, respectively.
- Photocytotoxicity of the complexes against MCF7 and A549 cells was studied in UV-A light.

### GRAPHICAL ABSTRACT



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### ABSTRACT

A new class of Cu(II) and Co(II) complexes of azo-containing Schiff base of the type  $[\text{Cu}(\text{L}_1)_2]$  and  $[\text{Co}(\text{L}_1)_2]$ , where  $\text{L}_1 = 4\text{-}[(E)\text{-}\{2\text{-hydroxy-3-}[(E)\text{-}(4\text{-bromophenyl)diazenyl}\}\text{benzylidene}\}\text{amino}\}\text{benzoic acid}$  have been synthesized and characterized. Extension of conjugation and the presence of free carboxylic acid group of the ligand  $\text{L}_1$  increased the wavelength of the complexes from visible region to the near IR region (620–850 nm). The Cu(II) and Co(II) complexes interacted with CT-DNA via intercalative mode with the respective  $K_b$  value of  $3.2 \times 10^4 \text{ M}^{-1}$  and  $2.9 \times 10^4 \text{ M}^{-1}$  and acted as proficient photocleavers of SC pUC19 DNA in UV-A light, forming  $^1\text{O}_2$  as the reactive oxygen species with the quantum yield of 0.38 and 0.36, respectively. Furthermore, the Cu(II) and Co(II) complexes showed photocytotoxicity toward two selected tumor cell lines MCF-7 and A549 by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) method, and the Cu(II) complex exhibits higher photocytotoxicity than Co(II) complex against each of the selected cell lines, this result is identical with their DNA binding ability order.

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### Introduction

Photocleavage of nucleic acids by transition metal complexes has emerged as a field of great interest to improve the development of new biotechnological and therapeutic approaches [1–4]. Basis of photoinduced DNA cleavage is the photogeneration of reactive

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oxygen species (ROS), which abstract hydrogen atoms from the DNA sugar moiety or oxidize nucleobases, especially guanine [1,5,6]. Such compounds are useful in photodynamic therapy (PDT) applications. Following the success of PDT, a number of so called “second generation” photosensitizers have been developed. These include modified porphyrins, chlorins, bacteriochlorins, phthalocyanines, naphthalocyanines, pheophorbides and purpurins and they showed increased efficacy in PDT for many different reasons [7–13]. Despite of their efficacy, they have shown some side effects such as, long-lasting photosensitization, absorption at wavelengths below the optimal tissue penetration, dark toxicity and hepato-toxicity etc. [14,15]. Therefore, the current effort was aimed to produce a photosensitizer which as a single compound should hold a large difference between cytotoxicity in the presence and absence of irradiation. The wavelength of activation should preferably lie within a photochemotherapeutic window of 620–850 nm, which would give rise to more excited photosensitizer at deeper tissue sites and hence cause more tumor damage [16], besides they should also have high quantum yield or efficiency.

As described earlier, the activation of the photochemotherapeutic compounds depend on the property of the photosensitizer. Therefore, many strategies have been applied to improve the photophysical properties of photoactivated metal based drugs. These include the utilization of fused, planar, aromatic molecules as ligands with the introduction of electron donating or withdrawing substituent's into the parent ligands and also by the use of redox active metal ions for complex formation [17]. Larger substituent's such as phenyl groups are, despite their highly conjugated structure, not as favorable in enhancing the absorption properties, as noted for Foscan [18] and other macrocyclic compounds containing hetero atoms [19–21]. The large aromatic substituents increase the wavelengths but they also increase the hydrophobicity of the compounds, making them less soluble in aqueous solution [18]. The azo linked Schiff base ligands having carboxylic acid group in *ortho*-position involved in the complexation with first row transition metal ions showed absorption values corresponding to  $\pi$ - $\pi^*/n$ - $\pi^*$ ,  $-N=N-$ , characteristic charge transfer and d-d transitions in the region 300–398 nm, 480–520 nm and 576–684 nm, respectively [22,23].

Hence, in the present work we investigated the influence of the position of axial substituents on the parent azo linked Schiff base compound with respect to absorption wavelength, solubility and extent of photophysical properties, in order to obtain the most satisfying combinations. The availability of free carboxylic acid group in the complexes held significant impact on the absorption properties. The Cu(II) and Co(II) complexes of the ligand  $L_1$  containing free carboxylic acid group at *para*-position showed low-energy metal-to-ligand charge transfer (MLCT) absorption bands around 590 nm and d-d transitions in the NIR region of 650–715 and 690 nm, respectively. They are soluble in DMSO-H<sub>2</sub>O solvent system and are photostable. *In-vitro* DNA binding studies revealed that these complexes are avid CT DNA binding agents; thereby they can act as strong chemotherapeutics. Furthermore, DNA cleavage activity of Cu(II) and Co(II) complexes with supercoiled (SC) pUC19 DNA has been carried out. The complexes were tested for their toxicity in MCF-7 and A549 cancer cell lines and the results revealed that they are nontoxic in dark and toxic upon light activation, providing greater potency as the approved photosensitizers.

## Experimental

### Materials and physical measurements

All reagents and solvents required were of AR grade, purchased commercially. All the solvents were purified according to the reported procedures [24]. Metal salts (CoCl<sub>2</sub>·6H<sub>2</sub>O and CuCl<sub>2</sub>·2H<sub>2</sub>O),

sodium azide (NaN<sub>3</sub>), Tris-HCl and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) were purchased from Merck (India), and used without further purification. Calf thymus (CT) DNA and supercoiled (SC) pUC19 DNA was purchased from Bangalore Genie (India), *p*-amino benzoic acid, 2-hydroxybenzaldehyde, *p*-bromoaniline, Agarose (molecular biology grade) and Ethidium bromide were purchased from Hi-media. Tris-HCl buffer solution used for binding and cleavage studies was prepared using deionised double distilled water.

The melting points were determined by open capillary methods and are uncorrected. The UV-visible spectra were recorded on a Shimadzu model impact 1650 UV-visible double beam spectrometer and the FT-IR spectra on a Shimadzu model impact 8400S FT-IR spectrometer (KBr pellets, 3 cm<sup>-1</sup> resolution). Elemental analysis was performed using a Vario ELCHNOS elemental analyzer. Conductivity measurements were determined in DMF (10<sup>-3</sup> M) using an ELICO-CM82 Conductivity Bridge. Magnetic measurements were carried out by the Gouy method at room temperature (28 ± 2 °C) using Hg[Co(SCN)<sub>4</sub>] as calibrant. <sup>1</sup>H NMR spectra on a Bruker 400 MHz and mass spectra are recorded on LCMS Shimadzu, Japan 800 MHz spectrometer.

### Synthesis

#### Synthesis of azo linked Schiff base 4-[(*E*)-[2-hydroxy-3-[(*E*)-(4-bromophenyl)diazenyl]benzylidene]amino]benzoic acid ( $L_1$ )

Azo-linked Schiff base ligand ( $L_1$ ) was prepared in a two-step process. In the first step, *para*-bromoaniline (5 mmol) was mixed with hydrochloric acid (37%, 4 mmol) in distilled water (3 mL) and diazotized below 5 °C with sodium nitrite (4 mmol) in distilled water (3 mL). The diazotized *para*-bromoaniline compound was then coupled with salicylaldehyde in alkaline media below 5 °C. The pH value during the coupling was maintained between 7 and 8.5. Coupling to the salicylaldehyde occurred in basic media at the *para*-position to the hydroxyl group.

In the second step, the ligand ( $L_1$ ) was synthesized according to the known condensation method [25]. About 2 mmol of the 2-hydroxy-5-[(4-bromophenyl)diazenyl]benzaldehyde (**1a**) and 2 mmol of *para*-amino benzoic acid were dissolved in 50 mL dry ethanol with few drops of glacial acetic acid as a catalyst (Scheme 2). The mixture was refluxed and stirred magnetically for 2 h at 80 °C on a hot plate. After cooling, the solution of the azo-linked Schiff base was filtered, and the solid was washed several times with methanol. All organic impurities were then extracted by washing with small portions of diethyl ether. The ligand  $L_1$  was dried in vacuo over calcium chloride, and was recrystallized several times from ethyl alcohol. Further purification was performed by column chromatography using CHCl<sub>3</sub> as eluent. The purity of the ligand was evaluated by thin layer chromatography.

Yield: (90%). Color: red, m.p. 248 ± 2 °C, Anal. (%) Calc. for [C<sub>20</sub>H<sub>14</sub>BrN<sub>3</sub>O<sub>3</sub>]: C, 56.62; H, 3.33; N, 9.90. Found: C, 56.64; H, 3.35; N, 9.89. LC-MS: *m/z*: 424 M<sup>+</sup>, 426 [M+2H]<sup>+</sup>.

IR data on KBr pellet  $\nu$  (cm<sup>-1</sup>): 3421 (OH) phenolic, 1707 (C=O) -COOH, 1600 (-C=N), 1348 (-N=N-). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz, ppm):  $\delta$  9.50 (s, -CH=N); 10.50 (s, -OH, 1H), 10.90 (s, 1H, -COOH), 7.07–8.32 (m, Ar-11H). UV-visible in DMF [ $\lambda_{max}/nm$  ( $\epsilon/M^{-1} - cm^{-1}$ ): 549 (9363), 401 (3453), 329 (1880).

#### Synthesis of photoreactive Cu(II) and Co(II) complexes using $L_1$

Cu(II) and Co(II) complexes were synthesized by the addition of appropriate metal salts (0.5 mmol, in 15 mL absolute ethanol) to a solution of  $L_1$  (1 mmol, in 20 mL absolute ethanol). The resulting solutions were refluxed for 2 h and then the volume of the solution was reduced to one-half by evaporation. The obtained solution was left for 1 h in the freezer; compounds were filtered off, washed

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