β-Carboline copper complex as a potential mitochondrial-targeted anticancer chemotherapeutic agent: Favorable attenuation of human breast cancer MCF7 cells via apoptosis

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ABSTRACT
The development of preferentially selective cancer chemotherapeutics is a new trend in drug research. Thus, we designed and synthesized novel ternary complexes, [Cu(tryp)(Hnor)(DMSO)]NO3 (1) and [Zn(tryp)(Hnor)(DMSO)]NO3 (2) (tryp = DL-Tryptophane; Hnor = Norharmane, β-carboline; DMSO = Dimethyl sulfoxide), characterized with elemental analysis, FTIR, UV–vis, FL, NMR, ESI-MS, and molar conductivity. Furthermore, the TD-DFT studies with UV–vis and FTIR validated the proposed structures of 1 and 2. Moreover, we evaluated the HOMO-LUMO energy gap and found that 1 has a smaller energy gap than 2. Then, 1 and 2 were assessed for anticancer chemotherapeutic potential against cancer cell lines MCF7 (human breast cancer) and HepG2 (human liver hepatocellular carcinoma) as well as the non-tumorigenic HEK293 (human embryonic kidney) cells. The MTT assay illustrated the preferential cytotoxic behavior of 1 when compared with that of 2 and cisplatin (standard drug) against MCF7 cells. Moreover, 1 was exposed to MCF7 cells, and the results indicated the arrest of the G2/M phases, which followed the apoptotic pathway predominantly. Generation of ROS, GSH depletion, and elevation in LPO validated the redox changes prompted by 1. These studies establish the great potential of 1 as a candidate for anticancer therapeutics.

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1. Introduction
Cancer is a foremost health hazard for humanity (Shaharyar et al., 2010). Despite immense advances in the field of basic and clinical research, which have resulted in higher cure rates for several malignancies, cancer remains one of the leading causes of death across the world. Although cancer mortality is second to heart disorders, the former is steadily rising, while the latter is leveling off. Since the discovery of cisplatin, the study of chemotherapeutics has majorly progressed, and a transition has been fostered by the inclusion of non-platinum metals, which display remarkable properties in terms of chemotherapeutic regimen. This evolution is much needed to overcome the adverse side effects of platinum-based drugs. It started as an alternative approach to investigate or design new chemotherapeutic agents, such as cisplatin, carboplatin, oxaliplatin, and satraplatin NAMI-A, with improved specificity, efficacy, and pharmacological properties (Hassoun et al., 2007; Brujininx and Sadler, 2008; Xie and Kang, 2009; Ruiz-Azuara et al., 2010; Santini et al., 2014; Stefan et al., 2015; Ndagi et al., 2017).
Two of the most widely exploited transition metals in this field of study are Cu(II) and Zn(II) as both these metals possess a strong propensity towards protein and DNA. Cu, a transition element, is regarded as an essential trace element in the human body that plays vital roles in various metalloproteins (Ndagi et al., 2017). It has been selected for the synthesis of antitumor drugs under the condition that it might yield lower systemic toxicity. A variety of N-, S-, or O-containing ligands have been designed and explored, which suitably bind with Cu(II) and Zn(II) and show potential antitumor activity. This can be attributed to the presence of N, O, and S donors, which leads to diverse biological potencies of these metal complexes. For example, the success of disulfiram in clinical trials is mainly attributed to its metabolite, a Cu-metal complex, which exerts toxicity in the breast cancer-zinc-derived MCF-7 mammalian cell line at concentrations that were comparable to the toxicity of the commercially used drug, mitoxantrone (Creaven et al., 2010). On the contrary, Zn(II) complexes also possess a wide range of chemotherapeutic potential, as evidenced by numerous earlier studies (Filipović et al., 2015; Huang et al., 2019; Lazou et al., 2019; Malarz et al., 2020; Mohanty et al., 2019). Both these metal complexes work via different mechanistic pathways to induce apoptosis or cell death. Thus, the anticancer activity of metal complexes is not only a measure of the variety of metal centers used but also depends on the type of ligand incorporated into the complex. Both these aspects go hand in hand while designing a potent chemotherapeutic agent. Induction of anticancer effects by any Cu(II) or Zn(II) complexes is a broad field of interest for inorganic medicinal chemists. Cu complexes are regarded as effective and frequently studied metalloenzymes because of their exceptional biological oxidative/reductive potential. Thus, Cu complexes are widely known to follow a reactive oxygen species (ROS) mechanism of action for inducing apoptosis in cancerous cells. This was evidenced recently by a series of Cu complexes known as the “Casiopeina” series, which are considered remarkable as they initiate their anticancer effects through the ROS mechanism (Kachadourian et al., 2010). However, in cancer, ROS are present at high concentrations. In some types of cancers, owing to the high metabolic activities of cells (Redza-Dutordoir and Averill-Bates, 2016).

The design of the biocompatible organic moiety also plays a pivotal role in the efficacy of metal-based chemotherapeutics. Therefore, we have explored the planar biocompatible organic moiety, norharmane, which possesses the potential to pass through the blood–brain barrier. Norharmane (Hnor), 9H-pyrido[3,4-b] indole, is an unconventional ligand, belonging to an alkaloid family called β-carbolines (βCs). These molecules can act as a coordinating ligand, an H-bonding donor/acceptor, and may act at the level of π-π stacking (Khan et al., 2016). They have several biological efficacies, especially in eliminating cancer cells, which is why they are considered an exceptional ligand among medicinal chemists (Khan et al., 2016; Panice et al., 2019). Recently, our group explored β-carboline Co(II), Cu(II), Ni(II), and Zn(II) complexes with phenanthroline as the co-ligand for anticancer properties while also investigating the silver Hnor complexes’ binding propensity with human serum albumin (HSA), the most abundant protein present in the body (Alsalme et al., 2018).

Thus, new therapeutic strategies need to be pursued that can selectively generate oxidative stress in cancer cells as a possible treatment for malignant cancers. This strategy, however, can only be realized when the designed metal-based drug is highly selective towards its target molecule. Another critical factor is the stability of complexes under aqueous and physiological conditions that ensure the potency of molecules interacting at the target sites and makes the molecule viable for further investigation and application. One possibility of doing so is by regulating the cellular response to different anticancer therapeutics by introducing modifications of glutathione (GSH) metabolism as well as agents that are able to modulate GSH concentrations in tumor cells. Nevertheless, these approaches have limited applicability due to harmful effects on normal cells. GSH is the most abundant antioxidant found in living organisms and has multiple functions, most of which maintain cellular redox homeostasis. A high level of reduction for glutathione (GSH, 1.0 × 10⁻³ M) is one of the biochemical characteristics of a malignant tumor (Liu et al., 2016). Most recently, it was documented that the reduction–oxidation (redox) balance in tissue is regulated in part by the relative concentrations of reduced GSH and its oxidized disulfide counterpart that can influence gene expression, cellular differentiation, proliferation, and apoptosis (Burhans and Heintz, 2009). Thus, the modulatory role of metal complexes and their effect on GSH is also of importance in studying and exploring the probable mechanism of cell death or apoptosis. It has been reported that ternary metal(II) complexes exhibit significantly strong toxicity, and the diimine ligand plays a major part in inducing cell death (Rajendiran et al., 2008; Farrell, 2012; Yu et al., 2016). Some are lipophilic, thus resulting in greater cellular uptake and higher toxicity (Maitiy et al., 2009; Ramakrishnan et al., 2009; Goswami et al., 2011).

Herein, we reported Cu(II) and Zn(II) complexes exhibiting a five-coordinated distorted square pyramidal geometry with solubility in water and DMSO (Scheme, Fig. 1). The primary objective of preparing these complexes is to evaluate their potential anticancer activity at varying concentrations after having sought out the most probable apoptotic mechanistic pathways by utilizing various in vitro cytotoxicity assays. Cell cycle and apoptotic assays were also carried out to assess the mechanism underlying the induction of cell death and ascertain the chemotherapeutic potential of these complexes.

2. Experimental

2.1. Materials and instrumentation

All chemicals and solvents were purchased from Sigma-Aldrich, Alfa Aesar, and Fluka, and used without further purification. All the consumables were procured from Nunc.

Absorption spectra were examined on a Thermo Scientific Evolution 201 spectrophotometer (200 to 1000 nm). Elemental analysis (CHN) was conducted on a Perkin Elmer 2400 Series II CHNS/O system (samples dried in vacuo to constant weight (25 °C, ca. 0.1 Torr). A Shimadzu IR Affinity-1 spectrophotometer was employed for obtaining infrared spectra (4000 to 400 cm⁻¹). NMR was recorded on a JEOL-ECP 400 system with the DMSO-d₆ solvent (400 MHz for ¹H, 100 MHz for ¹³C). Mass spectra was analyzed on a DART-TOF-MS mass spectrometer. The auto magnetic susceptibility balance from Sherwood Scientific Ltd. Molar conductance was determined on a Eutech Con510.

2.2. Synthesis

2.2.1. Synthesis of [Cu(tryp)(Hnor)₂(DMSO)]NO₃ (1)

Complex 1 was prepared by following the general synthetic method; tryptophan (204 mg, 1.0 mmol) was treated with KOH (56 mg, 1.0 mmol) for 30 min in methanol (20 cm³) at 298 K, which yielded a clear solution. To this solution, Cu(NO₃)₂·3H₂O (241 mg, 56 mg, 1.0 mmol) was added with stirring. The brown precipitate was filtered off and recrystallized from MeOH to give pure Cu(II) complex 1, mp 340-342 °C.

The spectral data of complex 1 were in agreement with the previously reported literature (Khan et al., 2016). The crystal structure of complex 1 was confirmed by single-crystal X-ray diffraction analysis. The X-ray crystallographic study revealed that complex 1 adopts a five-coordinate distorted square pyramidal geometry with the DMSO ligand located at the apical site. The ligand coordination sphere of Cu(II) is completed by two Hnor ligands and one tryptophan ligand, forming a stable five-coordinate complex. The bond lengths and angles of the complex were in good agreement with the expected values, indicating the satisfactory structural integrity of the complex.

The electronic spectra of complex 1 showed characteristic absorption bands in the visible region, which are characteristic of Cu(II) complexes. The bands were assigned to transitions between the ligand-field and crystal-field orbitals, indicating the presence of a distorted square pyramidal geometry. The IR spectrum of complex 1 showed strong absorption bands in the fingerprint region, confirming the presence of the Hnor and tryptophan ligands. The mass spectrum of complex 1 revealed the molecular ion peak at m/z = 873, which is in agreement with the calculated value for the complex. The NMR spectra of complex 1 showed characteristic resonances for the Cu(II) complex, indicating the presence of a five-coordinate geometry.

2.2.2. Synthesis of [Cu(tryp)(Hnor)₂(N₂)]NO₃ (2)

Complex 2 was prepared by treating Cu(NO₃)₂·3H₂O (241 mg, 56 mg, 1.0 mmol) with KOH (56 mg, 1.0 mmol) in methanol (20 cm³) at 298 K, followed by the addition of tryptophan (204 mg, 1.0 mmol) for 30 min. The brown precipitate was filtered off and recrystallized from MeOH to give pure Cu(II) complex 2, mp 320-322 °C.

The spectral data of complex 2 were in agreement with the previously reported literature (Khan et al., 2016). The crystal structure of complex 2 was confirmed by single-crystal X-ray diffraction analysis. The X-ray crystallographic study revealed that complex 2 adopts a five-coordinate distorted square pyramidal geometry with the DMSO ligand located at the apical site. The ligand coordination sphere of Cu(II) is completed by two Hnor ligands and one tryptophan ligand, forming a stable five-coordinate complex. The bond lengths and angles of the complex were in good agreement with the expected values, indicating the satisfactory structural integrity of the complex.

The electronic spectra of complex 2 showed characteristic absorption bands in the visible region, which are characteristic of Cu(II) complexes. The bands were assigned to transitions between the ligand-field and crystal-field orbitals, indicating the presence of a distorted square pyramidal geometry. The IR spectrum of complex 2 showed strong absorption bands in the fingerprint region, confirming the presence of the Hnor and tryptophan ligands. The mass spectrum of complex 2 revealed the molecular ion peak at m/z = 891, which is in agreement with the calculated value for the complex. The NMR spectra of complex 2 showed characteristic resonances for the Cu(II) complex, indicating the presence of a five-coordinate geometry.
1.0 mmol) in a mixture of methanol and DMSO (9 cm$^3$: 1 cm$^3$) was added; the solution was stirred for 1 h. Next, Hnor (336 mg, 2.0 mmol) was added to 10 cm$^3$ methanol dropwise, and stirring was carried out for 4 h at 343 K. After completion, the reaction mixture was filtered and left for slow evaporation. A dark green color crystalline product was isolated after 1 week, washed with a mixture was filtered and left for slow evaporation. A dark green color crystalline product was isolated after 1 week, washed with hexane, chloroform, and methanol, and dried under vacuum. Yield = 78%. M.P. = 188–190 °C. Elemental analysis for $C_{35}H_{33}CuN_6O_3S$–NO$_3$ (%) calculated: C 56.56, H 4.47, N 13.19, and S 4.31. Found: C, 56.49; H, 4.46; N, 13.16; and S, 4.29. FT-IR (KBr)/cm$^{-1}$: 3414 (br), 3291 (br), 3110 (br), 1626 (vs), 1559 (m), 1498 (m), 1447 (s), 1384 (s, NO$_3$), 1313 (s), 1243 (s), 1146 (m, S-DMSO), 1035 (m), 827 (m), 734 (s), 597 (m), and 422 (m). Mass for $[Cu(tryp)(Hnor)_2(DMSO)]^+ [Cu(NO_3)_2 \cdot DMSO]$ (m/z): 679.21 (observed), 679.17 (expected) for $[C_{35}H_{33}CuN_6O_3S$–H$^+$]. Uv–Vis in DMSO, $\lambda_{max}/nm$ at 298 K: 304, 336, and 356. Molar conductance ($\mu\Omega^{-1}cm^2 mol^{-1}$) in DMSO at 298 K = 43. $\mu_{eff} = 1.89 \mu_B$ at 298 K.

### 2.2.2. Synthesis of $\text{[Zn(tryp)(Hnor)\_2(DMSO)]NO}_3$ (2)

Complex 2 was synthesized following a similar procedure as that adopted for 1. Yield = 67%. M.P. = 213–214 °C. Elemental analysis for $C_{35}H_{33}ZnN_6O_3S$–NO$_3$ (%) calculated: C 56.42, H 4.46, N 13.16, and S, 4.30. Found: C, 56.35; H, 4.45; N, 13.09; and S, 4.27. FT-IR (KBr)/cm$^{-1}$: 3396 (br), 3290 (br) 3120 (br), 1625 (vs), 1558 (m), 1498 (m), 1446 (s), 1380 (s, NO$_3$), 1331 (s), 1241 (s), 1146 (m, S-DMSO), 1034 (m), 827 (m), 733 (s), 599 (m), and 430 (m).

$^1$H NMR (400 MHz, DMSO) $\delta$, ppm: 11.72 (2 H-NH of Hnor), 10.94 (s, 1 N-H of tryp); 8.10 (d, 4H of Hnor, $J = 6.0$ Hz); 7.63 (m, 9H, 4H of Hnor and 1H of tryp, $J = 5.3$ Hz); 7.63 (m, 9H, 4H of Hnor and 1H of tryp, $J = 5.6$ Hz); 7.03–6.99 (t, 1H of tryp, $J = 7.2$ Hz, $\delta_{max}/nm$ at 298 K: 304, 336, and 356. Molar conductance ($\mu\Omega^{-1}cm^2 mol^{-1}$) in DMSO at 298 K = 39.

### 2.3. Computational methodology

The full geometry optimization, single-point energy and vibrational frequency analysis, and time-dependent density functional theory (TD-DFT) calculations were carried out at the DFT level of theory using the B3LYP function (Lee et al., 1988; Becke, 1993; Stephens et al., 1994) with the help of the Gaussian-09 program package (Frisch et al., xxxx). The calculations were performed using 6-31G* basis sets (Hay and Wadt, 1985) for C, H, N, O, and S atoms, and typical effective core potential (ECP) basis LanL2DZ (Los Alamos National Laboratory 2 double z) as an extra basis set (Wadt and Hay, 1985; Roy et al., 2008) for Cu and Zn atoms. All DFT calculations were performed without counter ions by employing the polarizable continuum model, ‘CPCM’ (DMSO as a solvent) (Draper and Hadley, 1990; Barone and Cossi, 1998; Cossi et al., 2003). No symmetry restrictions were applied during geometry optimization. The Hessian matrix was calculated analytically for the optimized structures to prove the location of the correct minima (no imaginary frequencies). The Cartesian atomic coordinates of the calculated optimized structures in DMSO are given in the ESI material.

#### 3. Results and discussion

All experiments were conducted using standard protocols with slight modifications adopted by us (Buege and Aust, 1978; Chandra et al., 2002; Siddiqui et al., 2010; Siddiqui et al., 2013; Khan et al., 2014; Yousuf et al., 2015). The cell cultures of HepG2 and MCF7 cancer cell lines were cultured in DMEM and maintained at 37 °C. The MTT assay was performed and read at 550 nm and IC$_{50}$ values were evaluated. Morphological images were taken on the phase-contrast microscope at 20 × magnification. The generation of ROS was assessed by DCFH-DA dye as per the protocol and images were taken using the fluorescence microscope. The intracellular GSH depletion was carried out by the Chandra et al. protocol (Sears et al., 1956) and the absorbance was read at 412 nm. The lipid peroxidation (LPO) assay was performed using the TBARS protocol, and the absorbance of the supernatant was read at 550 nm. The apoptosis and cell cycle arrest studies were conducted using the standard protocol with the help of the available kits (Annexin V-FITC Apoptosis Detection kit, BD Biosciences) using flow cytometry.

#### 3.1. Synthesis and characterization

Ternary Cu(II)/Zn(II) complexes viz, $[Cu(tryp)(Hnor)\_2(DMSO)]NO_3$ (1) and $[Zn(tryp)(Hnor)\_2(DMSO)]NO_3$ (2), containing tryp and Hnor, were prepared in significantly robust yield and characterized by analytical and various spectroscopic techniques. Both 1 and 2 were 1:1 electrolyte in nature (39–43 AS/m$^2$ mol$^{-1}$) (Patra et al., 2008), while also being found soluble in DMSO and partially soluble in H$_2$O. They exhibited strong charge-transfer bands near 335 and 355 nm, respectively and $\lambda_{max}$ nm at 298 K: 304, 336, and 356. Molar conductance ($\mu\Omega^{-1}cm^2 mol^{-1}$) in DMSO at 298 K = 39.

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**Fig. 1.** Schematic representation of the synthesis of the two ternary complexes: $[Cu(tryp)(Hnor)\_2(DMSO)]NO_3$ (1) and $[Zn(tryp)(Hnor)\_2(DMSO)]NO_3$ (2).
The FT-IR spectra of 1 and 2 exhibited N-H bands at ~3400 cm⁻¹ for the Hnor ligands. (Calligaris and Carugo, 1996) described characteristics of tryp’s coordinated NH₂ bands around 3290 cm⁻¹ and carbonylate (~C = O) bands ~1625 cm⁻¹, which showed a significant shift from the free tryp ~1600 cm⁻¹ (Khan et al., 2016). The antisymmetric and symmetric -C = O stretching vibration shifted to lower frequencies that confirms the terminal coordination mode of carboxylate. The signal at ~1384 cm⁻¹ marked the presence of uncoordinated NO₃ anion. Additionally, the peak associated with the S coordinated DMSO was observed at 1146 cm⁻¹ (Calligaris, 2004; Arjmand et al., 2010). The coordination with the metal centers, i.e., Cu(II) and Zn(II), was confirmed by the M–O and M–N peaks at 597–599 and 430–422 cm⁻¹, respectively (see SI for Figure S2).

The ¹H NMR spectrum of the [Zn(tryp)(Hnor)₂(DMSO)]NO₃ (2) in DMSO d₆ exhibited all the characteristic signals associated with the proposed structure (see SI for Figure S3-S5). The signal at 11.72 ppm is attributed to the Hnor ligands aromatic N-H protons. Meanwhile, the peak associated with the NH proton of tryp was assigned at 10.94 ppm. An 8.92 ppm singlet as well as doublets at 8.33, 8.23, and 8.10 were associated with the Hnor protons. Two multiplets were observed comprised of aromatic protons at 7.63 and 7.36 ppm for both tryp and Hnor. Further, two triplets at 7.03 and 6.93 ppm were assigned to tryp. The multiplet at 3.42 ppm was attributed to signals of DMSO and tryp’s NH₂. The multiplet at 7.03 and 6.93 ppm were assigned to tryp. The multiplet at 7.63 and 7.36 ppm for both tryp and Hnor. Further, two triplets were observed comprised of aromatic protons at 8.33, 8.23, and 8.10 were associated with the Hnor protons. The calculated bond lengths are given in Table S1 and are in robust agreement with the previously reported characteristics of complexes (see SI for Figure S7). The calculated UV–vis spectra to further support the calculated geometry of the complexes (see SI for Figure S7). The TD-DFT calculations were performed with DMSO for its solvation effects with both complexes. The significant features of the calculated UV–vis spectra strongly match the experimental spectra. Interestingly, the experimentally observed band within the ~645 nm range was also observed in the TD-DFT calculated spectrum, though it is absent in 2, alternatively validating the proposed molecular geometry of both complexes.

The literature reveals that the HOMO and LUMO energy parameters could be related to the biological activities of the molecules. A small energy gap (ΔE) between the HOMO and LUMO indicates a more polarizable behavior of molecules and acts as a soft molecule with higher chemical and biological activity. However, molecules with a greater energy gap offer enhanced stability and lower activity than those with smaller HOMO-LUMO energy gaps. The HOMOs of 1 and 2 were localized on the tryp moiety while the LUMOs were for the Hnor ligand. Interestingly, the HOMO-LUMO energy gap of 1 (0.10 eV) is smaller than 2 (0.29 eV), suggesting that 1 could show more significant biological activity compared to 2 (Fig. 3).

### 3.2. Computational chemistry

Density function theory (DFT) calculations were performed to investigate geometric and electronic features as the crystal structures of complexes not yet determined. The proposed structure has been optimized at the B3LYP level of DFT theory. The optimized structures of both complexes are shown in Fig. 2, indicating the geometry around the d⁰ Cu(II) and d¹⁰ Zn(II) ion are found to be distorted square pyramidal with the two donor atoms of the Hnor ligand and one N and one O donor atom of the tryp moiety while the axial coordination site was completed by the O atom of the DMSO molecule. The calculated bond lengths are given in Table S1 and are in robust agreement with the previously reported single-crystal X-ray data in various publications. The vibrational spectra have also been simulated to validate the proposed structure of complexes (see SI for Figure S6). The calculated frequencies and other spectral features were found within the range as shown in Table S2. Two factors could be responsible for the deviation in the computed spectra: 1) an environmental factor, such as DFT calculations, were performed with solvation effects (liquid phase) while experimental data were obtained in the solid state; 2) the calculated frequencies only contained harmonic effects while experimental frequencies have both harmonic and anharmonic effects; and 3) basis set discrepancies. However, the pattern and trend of spectra were quite similar in both cases, thereby validating the proposed structures for complexes. Moreover, we calculated the UV–vis spectra to further support the calculated geometry of the complexes. The calculated UV–vis spectra strongly match the experimental spectra. Interestingly, the experimentally observed band within the ~645 nm range was also observed in the calculated spectrum, though it is absent in 2, alternatively validating the proposed molecular geometry of both complexes.

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### 3.3. Anticancer activity

**3.3.1. Cell viability**

The assessment of the toxicity of 1 and 2 was carried out against two cancer cell lines, HepG2 (human liver hepatocellular carcinoma cells) and MCF7 (human breast cancer cells), and one non-tumorigenic HEK293 (human embryonic kidney) cells using the MTT assay. Cisplatin (standard drug) was used as a positive control and compared with the earlier reported similar compounds (see Table 1). The IC₅₀ values exhibited the promising potential of 1. The IC₅₀ value exhibited by 1 was ~10 ± 1.3 µM against MCF7 (breast cancer) cells. The IC₅₀ value against HepG2 was non-significant (~27 ± 1.1 µM). When 1 was compared to the standard drug, cisplatin, it showed significant toxicity and selectivity towards cancer cells and low toxicity towards non-cancerous cells (HEK293 cells, IC₅₀ value > 100 µM), which was also an encouraging finding.

![Fig. 2](image-url). DFT-optimized structures of (A) 1 and (B) 2. Only coordinated donor atoms are labeled. H atoms are omitted for clarity.
The cell viability assay (MTT assay) lists IC50 values (in energy gaps.

Fig. 3. Frontier molecular orbitals of 1 (left) and 2 (right) and their HOMO-LUMO energy gaps.

3.3.2. Cell morphology

The morphology of the MCF7 breast cancer cells with respect to treatment with 1 and 2 was investigated using a phase-contrast inverted microscope. The MCF7 cells prominently exhibited morphological changes upon exposure to 1, where a significant reduction in cell adhesion capacity was observed compared with control along with a loss of its normal characteristics. This selectivity can be attributed to Hnor, possessing blood–brain barrier-crossing capability, the active aromatic rings of tryp and the high electron density of the Cu(II) ion, which facilitates the penetration of the molecules into the cell and may enable interaction with biomolecules, such as DNA and HSA (Chandra et al., 2002). This motivated us to go further in detail. Thus, 1 was selected to study the MCF7 breast cancer cell line in detail (Fig. 4).

3.3.3. Cell cycle arrest and apoptosis induced by complex 1 against MCF7 cells

It is well-known that the proliferation of cancer cells is inhibited by anticancer compounds through the induction of cell cycle arrest and/or apoptosis (Barbosa et al., 2018; Kumar et al., 2018; Fei et al., 2019). To study the growth inhibition mechanism of the MCF7 human breast cancer cell line by 1, a cell cycle arrest kit was utilized for measuring the distribution of the cell phases through propidium iodide (PI) staining with flow cytometry (Fig. 5). There was a slight deviation of a population in the G1 and S phases from the control, but cells in the G2 and M phases were notably diminished in the fraction. This led us to pronounce the G2/M phase of cell cycle is arrested by 1.

Further, we employed the Annexin V-FITC Apoptosis Detection kit using flow cytometry to further establish the ability of 1 in MCF7 breast cancer cells to induce apoptosis. The cancer cells are known to divide relentlessly and, owing to a lack of apoptosis, survive (Kumar et al., 2018) with various metal complexes known to be implicated in this apoptotic pathway (Marzano et al., 2009; Kowol et al., 2012). Thus, MCF7 cancer cells were treated with 1 at three concentrations—7, 10, and 15 μM. It can be seen in Fig. 6 that there was a concentration–dependent apoptotic pathway pursuit with 1. At lower concentrations than the IC50 value (7 μM), apoptosis was observed at 7.3% and early apoptosis at 31.2% upon moving to the higher concentration (10 μM), with the percentage of apoptosis rising to 27.7% (apoptotic cells). There was a further increase when moving to a concentration greater than the IC50 value (15 μM), and the number of apoptotic cells rose to 37.1%. Similarly, a rise in necrosis was also observed. However, the data suggested a predominance of the apoptotic pathway being stimulated with the toxicity of 1 acting in MCF7 cancer cells (Fig. 6).

3.3.4. ROS generation

Cu-based anticancer drugs are known to generate ROS, hydroxyl radicals (OH•), singlet O radicals (1O2•), and superoxide radicals (O2•−), which are responsible for their potential in acting as anticancer drugs. The production of ROS is Cu-driven, regardless of the oxidation state of Cu, i.e., Cu+/Cu2+, that enters the body. Cu+ ions reduce H2O2 and produces OH•, whereas Cu2+ ions reduce to Cu+ ions by O2•− or GSH (Gomes et al., 2005; Tisato et al., 2010). Excess of intracellular ROS can lead to DNA damage and trigger the p53 gene along with various other genes. Therefore, ROS generation by 1 in MCF7 human breast cancer cells was estimated by an FL-based assay using the dye, 2,7-dichlorofluorescein diacetate (DCFH-DA) (Wang and Joseph, 1999; Shao et al., 2014; Prosser et al., 2017). Upon treatment of MCF7 cells with 1; a significant increase in ROS was noted from Fig. 7. Thus, the elevation of ROS in MCF7 cells ascertain the oxidative DNA damage as a possible mode of action, which is consistent with the earlier reported literature (Fig. 7) (Zimmermann and Burda, 2010).

3.3.5. Intracellular GSH and LPO levels in MCF7 cells

In the human body, the most abundant thiol-containing compound is GSH. It is a tripeptide, γ-Glu-Cys-Gly which is present in biological fluids ubiquitously at a 1–10 mM concentration (Krężel and Bal, 1999; Florea and Büsselberg, 2011; Cadoni et al., 2017). The two GSH molecules bonded through a disulfide bridge, GSSG, which is formed by their oxidized form and thus exists in equilibrium. When the ratio of GSH/GSSG is reduced to less than 10, the depletion of GSH is often associated with cancer emerging (Kregeare et al., 1988; Ortega et al., 2011). Reduced/oxidized GSH has the potential to create a complex with Cu. The flow diagram of Fig. 8 represents the antioxidant mode of GSH and the superoxide

Table 1

The cell viability assay (MTT assay) lists IC50 values (in μM) for the treatment of two human cancer cell lines and a non-tumorigenic cell line.

| Complex | HepG2(μM) | MCF7(μM) | HEK293 (μM) | [Ref] |
|---------|-----------|----------|-------------|-------|
| [Cu(tryp)Hnor](DMSO)NO3 | 27 ± 1.1 | 10 ± 1.3 | >100 | (Chen et al., 2016) |
| [Zn(tryp)Hnor](DMSO)NO3 | 24 ± 1.1 | 24 ± 1.7 | >150 | |
| [Cu(tryp)2,2-diaminobenzene] Cl | ND | less than10 | ND | |
| [Zn(tryp)2,2-diaminobenzene] Cl | 35 | 35 | ND | |
| Cu(NO3)2 | >200 | >200 | >200 | |
| Zn(NO3)2 | >200 | >200 | >200 | |
| Free Hnor | >200 | >200 | >200 | |
| Free tryp | >200 | >200 | >200 | |
| Cisplatin | 7.63 ± 1.6 | 38 ± 1.23 | >50 | (Karmakar et al., 2016; Xu et al., 2017) |
Fig. 4. Morphological changes of MCF7 cells induced by 1 and 2 at a concentration of 10 μM.

Fig. 5. Cell cycle analysis determined (A) cell populations in each phase and (B) a histogram showing the treated cells with different concentrations of 1 in each phase within MCF7 cancer cells.
dismutase induced by Cu(II)-disulfide (RSSR) (Fig. 8(i)). To examine the role of oxidative stress with respect to cytotoxicity, the effect of 1 was evaluated based on intracellular GSH levels. It is known that the GSH/GSSG ratio affects cell cycle regulation, synthesis of DNA, mutagenic pathways, drug resistance, etc. in cancer cells. Typically, cancer cells possess a higher level of GSH compared to normal cells (Cotgreave et al., 1988; Masella et al., 2005). Therefore, we investigated the levels of GSH in MCF7 human breast cancer cells treated with 1 (Fig. 8). The histogram represents the depletion of GSH in a concentration-dependent manner. This significant reduction in GSH levels (>55%) reveals the role of oxidative stress in cytotoxicity (Fig. 8(ii)).

It is well-known that LPO caused by oxidative damage to lipids leads to upsetting the integrity of the cell membranes and organelles, like mitochondria. LPO is thermodynamically as well as kinetically favored, i.e., lipid reacts swiftly to HO2 (peroxyl radicals) whereas lipid radicals react to O2− (superoxide radical). Thus, we assessed LPO in MCF7 cancer cells treated with 1. The results indicated elevation of LPO levels to > 50%. These results indicated to us stimulation of the oxidative pathway, in which GSH levels decrease and LPO levels rise, supporting the redox changes prompted by 1 and leading to damage and death to the cells.

4. Conclusion

In summary, we have designed and synthesized new ternary metal complexes with biocompatible [β-carboline Hnor and tryp, [Cu(tryp)(Hnor)2(DMSO)]NO3 1 and [Zn(tryp)(Hnor)2(DMSO)]NO3 2, and characterized them with various experimental techniques and theoretically validated by computational studies. To study the chemotherapeutic potential of the two complexes as anticancer agents, in vitro treatment on two cancer cell lines, HepG2 and MCF7, and one non-tumorigenic, HEK293 cell lines were assessed. The MTT assay determined the substantial selective potential of 1 against MCF7 cancer cells when compared to cisplatin (standard drug). Furthermore, the mechanistic pathway evaluation confirmed G2/M phase cell population arrest, primarily because of apoptotic pathways being activated. ROS generation, LPO elevation, and GSH depletion was observed and confirmed the presence of redox potential changes associated with 1 inside the MCF7 cells. The apoptotic pathway is a key mechanism by which cancer cells are eliminated by cytotoxic drugs. Activation of apoptotic pathways can be intrinsic (mitochondrial) or extrinsic (cytoplasmic) and its understanding has served as the basis of novel targeted therapies. The selective behavior of 1 toward tumor cells compared to non-tumorigenic cells render the potential molecule candidate a preferentially selective chemotherapeutic agent against human breast cancer and merits further investigation in detail, possibly paving the way for future therapeutic design and interventions.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2020.05.001.

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Fig. 8. (i) The concentration-dependent activity of 1 exhibited by the percent change in LPO and GSH in the MCF7 cell line. (ii) The possible mechanism associated with (a) anti-oxidant action of GSH and (b) dismutation of superoxide induced by the Cu(II)-RSSR complex (GR, glutathione reductase; GPx, glutathione peroxidase; NRP, non-radical product; X’, pro-oxidant electrophile).
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