Synthesis of $\text{N,N}^{\prime}$–di–$\beta$–D–glucopyranosyl ethylenediamine

The ligand $[\text{C}_{14}\text{H}_{28}\text{N}_{2}\text{O}_{10}]$ was synthesized by the slight modification of procedure described earlier by E. Mitts and R. M. Hixon [220]. To the methanolic solution of ethylenediamine 3.5 g (0.06 mol) was added D–glucose 21 g (0.12 mol). This mixture was refluxed gently on a water–bath until the glucose was completely dissolved. After two hours, the refluxing was discontinued, the flask cooled and left in the refrigerator overnight. The compound obtained was isolated as a white precipitate, washed with cold methanol and dried in vacuo. Yield = 78%. M.P. = 152–154 °C (dec). Anal.Calcd for $\text{C}_{14}\text{H}_{28}\text{N}_{2}\text{O}_{10}$ (L): C, 43.75; H, 7.34; N, 7.29. Found: C, 43.79; H, 7.11; N, 7.01. IR (KBr) ($\nu_{\text{max}}/\text{cm}^{-1}$): 3442 $\nu$(O–H, broad); 3336 $\nu$(N–H); 1640 $\delta$(N–H), 1467 $\delta$(OCH, CH$_2$, CCH); 1077 $\nu$(CO, CC); Uv–vis. (1 x $10^{-3}$ M, H$_2$O, nm) 233. $^1$H NMR (400MHz, DMSO, $\delta$): 4.70–4.69 (NH(en)); 3.93–3.53 (OH of D–glucose); 3.40–2.71 (CH$_2$(en), CH of D–glucose). $^{13}$C NMR (100 MHz, DMSO, $\delta$): 93.44 (C$_1$), 60.66 (C$_2$), 76.90 (C$_3$), 74.06 (C$_4$), 75.87 (C$_5$), 69.64 (C$_6$) (6C, Glc); 42.46 (2C, amine) ESI–MS: (m/z, H$_2$O): 385.3 $[\text{C}_{14}\text{H}_{28}\text{N}_{2}\text{O}_{10}]^+$. 

Synthesis of $[\text{C}_{22}\text{H}_{42}\text{N}_{6}\text{O}_{11}\text{CuSnCl}_2]$ 

A suspension of $\text{N,N}^{\prime}$–di–$\beta$–D–glucopyranosyl ethylenediamine (L) (0.384 g, 1 mmol) in 30 ml methanol was added CuCl$_2$.2H$_2$O (0.171 g, 1 mmol), the solution was refluxed with continuous stirring for ca. 2 h at 70 °C until the homogenous green colored solution appeared. To this in situ generated copper complex, dichlobis(imidazole)dimethyltin(IV) (0.352 g, 1 mmol) in 10 ml methanol was added slowly and was kept on stirring for 24 h at room temperature. The solvent was further removed by rotary evaporator to give a dark
green colored solid product, which was filtered, washed with methanol, and dried in vacuo. Yield= 53%. M.P. = 224 °C (dec). Anal.Calcd for C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}: C, 32.23; H, 5.16; N, 10.25. Found: C, 32.21; H, 5.15; N, 10.21. IR(KBr) (\nu_{\text{max}}/\text{cm}^{-1}): 3428 \nu(\text{O–H, broad}); 3298 \nu(\text{N–H}); 1613 \delta(\text{N–H, HOH}); 1573 \nu(\text{C=\text{N}}); 1419 \delta(\text{OCH, CH}_2, \text{CCH}); 1038 \nu(\text{CO, CC}); 1319 \nu(\text{C–N}); 531 \nu(\text{Sn–N}); 475 \nu(\text{Cu–N}); Molar Conductance, \Lambda_M (1\times10^{-3} \text{ M, H}_2\text{O}): 19.0 \Omega^{-1}\text{cm}^2\text{mol}^{-1} (\text{non–electrolyte}); Uv–vis. (1 \times 10^{-3} \text{ M, H}_2\text{O, nm}) 236, 259, 653. ESI–MS: (m/z, MeOH): 822.7 [C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}+3\text{H}]^+. 

**Synthesis of [C\text{_{22}H42}N\text{\textsubscript{6}}O\text{\textsubscript{11}}NiSnCl\text{\textsubscript{2}}]**

This complex was prepared by similar method as for [C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}] with NiCl_{2}.6H_{2}O (0.237 g, 1 mmol). A dark brown colored solid product formed was washed with methanol and dried in vacuo. Yield= 48%. M.P. = 215 °C (dec). Anal.Calcd for C_{22}H_{42}N_{6}O_{11}NiSnCl_{2}: C, 32.43; H, 5.19; N, 10.31. Found: C, 32.42; H, 5.13; N, 10.35. IR (KBr) (\nu_{\text{max}}/\text{cm}^{-1}): 3428 \nu(\text{O–H, broad}); 3325 \nu(\text{N–H}); 1638 \delta(\text{N–H, HOH}), 1543 \nu(\text{C=\text{N}}); 1456 \delta(\text{OCH, CH}_2, \text{CCH}); 1071 \nu(\text{CO, CC}); 1329 \nu(\text{C–N}); 575 \nu(\text{Sn–N}); 452 \nu(\text{Ni–N}); Molar Conductance, \Lambda_M (1\times10^{-3} \text{ M, H}_2\text{O}): 23.0 \Omega^{-1}\text{cm}^2\text{mol}^{-1} (\text{non–electrolyte}); Uv–vis. (1 \times 10^{-3} \text{ M, H}_2\text{O, nm}) 235, 271, 623. \textsuperscript{1}H NMR (400 MHz, D_{2}O, \delta): 7.35–7.21 (CH Imidazole); 3.11–4.72 (skeleton protons of D–glucose and (CH\textsubscript{2}(en)). \textsuperscript{13}C NMR (100 MHz, D_{2}O, \delta): 144.04, 135.70, 121.61 (ArC, Imidazole); 89.94 (C\textsubscript{1}), 60.56 (C\textsubscript{2}), 76.69 (C\textsubscript{3}), 74.15 (C\textsubscript{4}), 75.79 (C\textsubscript{5}), 69.95 (C\textsubscript{6}, Glc); 44.90 (2C, amine). \textsuperscript{119}SnNMR (149.19 MHz, D_{2}O, \delta): -301.23. ESI–MS: (m/z, MeOH): 817.0 [C\text{_{22}H54}N\text{\textsubscript{6}}O_{17}NiSnCl\text{\textsubscript{2}}+3\text{H}]^+.

**Results and discussion**

The glycoconjugate heterobimetallic entities [C\text{_{22}H42}N_{6}O_{11}CuSnCl\text{\textsubscript{2}}] and [C\text{_{22}H42}N_{6}O_{11}NiSnCl\text{\textsubscript{2}}] were synthesized by reacting dichlorobis(imidazole)
dimethyltin(IV) (A) [221] with corresponding 1:1 metal complex of N,N’–di–β–D–glucopyranosyl ethylenediamine N–glycoside ligand [C_{14}H_{28}N_{2}O_{10}], derived from ethylenediamine and β–D–glucose (scheme II). The compositions of the complexes were confirmed by elemental analysis and spectral data. On the basis of spectral studies, the coordination geometry of the central metal ion (Cu^{II}, Ni^{II}) was found to be square pyramidal while the Sn^{IV} atom was present in hexacoordinated environment. Both the complexes [C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}] and [C_{22}H_{42}N_{6}O_{11}NiSnCl_{2}] were highly hygroscopic in nature and soluble in H_{2}O and DMSO. The growth of single crystals of these complexes for X–ray studies was very difficult, however, XRPD measurements confirmed the crystalline nature of the complexes. The molar conductance (\lambda_M) value at 25 °C suggests their non–electrolyte nature (19–23 \Omega^{-1} cm^{-2} mol^{-1}).

Scheme II. Synthetic route for the heterobimetallic complexes [C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}] and [C_{22}H_{42}N_{6}O_{11}NiSnCl_{2}].
**IR spectroscopy**

The IR spectrum of ligand \([C_{14}H_{28}N_{2}O_{10}]\) displayed a characteristic band in the range 3500–3200 cm\(^{-1}\) assignable to ν(O–H) stretching vibration of free saccharides. However, complexes \([C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}]\) and \([C_{22}H_{42}N_{6}O_{11}NiSnCl_{2}]\) exhibited merged and broadened band at 3444 cm\(^{-1}\) as compared to intense band of free saccharide moieties, due to overlapping with the –OH stretching vibration band of the coordinated H\(_2\)O molecule [222]. The absorption bands observed in the range of 3325–3298 cm\(^{-1}\) was attributed to ν(N–H) stretching vibration of the coordinated secondary amino functions of the complexes [223]. Other medium intensity bands observed at 1638–1613 cm\(^{-1}\) were attributed to δ(N–H) vibration of the glycosidic ligand [224]. The strong characteristic IR bands at 1456–1419, 1071–1038, and 892–860 cm\(^{-1}\) were attributed to δ(OCH, CH\(_2\), CCH), ν(CO, CC) and δ(CCH, CH), as well as ν(CC, CO) vibrations, respectively, indicating the presence of metal–saccharide adducts. Strong coupling between the bands resulted in merging and broadening in the spectra and complicated the assignment of these to individual vibrational modes [225]. Additionally, the signals at 1573–1543 cm\(^{-1}\) were assigned to ν(C=N) vibration of the imidazole ring [226]. The coordination of metal ion was further supported by the appearance of new medium intensity bands at 475, 452 and 575–531 cm\(^{-1}\) assigned to ν(Cu–N), ν(Ni–N) and ν(Sn–N) stretching vibration, respectively.

**NMR spectroscopy**

The \(^1\)H and \(^{13}\)C spectra of ligand \([C_{14}H_{28}N_{2}O_{10}]\) was recorded in DMSO–d\(_6\) solution exhibited signal at 4.70–4.69 ppm attributed to C\(_1\) and glycosyl –NH proton, indicating the glycosylation process followed by the condensation between the two glucose rings
and amino protons of linker ethylenediamine at C₁ centre [227]. The signals at 3.53–3.93 ppm were assigned to C₂, C₃, C₄, C₆ hydroxyl protons [228]. The signals at 2.91~2.85 and 2.71~2.78 ppm were attributed to –CH₂ proton of linker ethylenediamine at C–1, together with other distinct sugar proton resonances at 3.40~3.32 ppm (C₆ proton), 3.28~3.24 ppm (C₃ and C₅ proton), 3.08~3.13 ppm (C₂ and C₄ proton) [229]. However, ¹H and ¹³C NMR spectra of the complex [C₂₂H₄₂N₆O₁₁NiSnCl₂] was recorded in D₂O and the assignments was made by relative comparison with those of free ligand. The ¹H NMR spectra of complex [C₂₂H₄₂N₆O₁₁NiSnCl₂] revealed a series of multiplets in the range of 3.11–4.72 ppm were attributed to the strongly coupled skeletal proton signals of saccharides moiety, and CH₂ protons of linker ethylenediamine. Due to these factors, the assignment of the individual resonances was could not be resolved. The aromatic protons of the imidazole ring were observed at 7.21–7.35 ppm. Additionally, the sharp signal at 0.77 ppm corresponded to methyl proton bound to tin atom.

The ¹³C NMR spectra of the [C₁₄H₂₈N₂O₁₀] and the complex [C₂₂H₄₂N₆O₁₁NiSnCl₂] corroborated well with the proposed structures. In the ¹³C NMR spectrum of [C₁₄H₂₈N₂O₁₀], the sharp signals at 42.46 ppm attributed to the two C₇–atoms of the ethylenediamine (en)–based linker, together with six distinct carbon atoms of the D–glucose resonances at 93.44 (C₁), 60.66 (C₂), 76.90 (C₃), 74.06 (C₄), 75.87 (C₅), and 69.64 (C₆). However, significant changes were observed in the spectrum of complex [C₂₂H₄₂N₆O₁₁NiSnCl₂] for the aromatic carbons of imidazole which appeared in the range 144.04–121.61 ppm, and the two methyl groups attached to tin atom displayed chemical shift signal at 9.11 and 9.15 ppm [230, 231]. The ¹¹⁹Sn NMR spectrum recorded
a sharp single peak at $\sim$301.23 ppm, which was quite in agreement with the hexacoordinated environment of diorganotin (IV) metal ion (Figure. 55) [232].
Figure 55. (a) $^1H$, (b) $^{13}C$ and (c) $^{119}Sn$ N.M.R. spectrum of heterobimetallic complex $[C_{22}H_{42}N_6O_{11}NiSnCl_2]$.  

**EPR spectrum**

The X–band EPR spectra of complex $[C_{22}H_{42}N_6O_{11}CuSnCl_2]$ (Figure 56a and b) were recorded at room temperature in solid state and at liquid nitrogen temperature (LNT) in aqueous solution using TCNE as a field marker ($g = 2.00277$).

![EPR spectrum](image)

**Figure 56.** X–band EPR spectrum of complex $[C_{22}H_{42}N_6O_{11}CuSnCl_2]$ at (a) LNT (in liquid state) and (b) RT (in solid state).

The spectrum shows an isotropic pattern and exhibits axial symmetrical line shape with $g_\parallel = 2.125$ and $g_\perp = 2.082$ and $g_{\text{iso}} = 2.09$ computed from the formula $g_{\text{iso}} = (g_\parallel + 2g_\perp)/3$, consistent with square pyramidal geometry [233]. The trend $g_\parallel > g_\perp > 2.0023$ revealed...
that the unpaired electron is present in the $d_{x^2-y^2}$ orbital [234]. For a Cu(II) complex, $g_\parallel$ is a parameter sensitive enough to indicate covalence. For a covalent complex, $g_\parallel$ is $< 2.3$ and for an ionic environment, $g_\parallel = 2.3$ or more. In the present complex $g_\parallel < 2.3$ indicates an appreciable metal–ligand covalent character [235].

**UV–Vis spectra**

The electronic spectrum of ligand $[\text{C}_{14}\text{H}_{28}\text{N}_2\text{O}_{10}]$ shows a sharp hump at 233 nm in UV region exhibiting $\pi–\pi^*$ transition. However, the UV–vis spectrum of complex $[\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]$ revealed a broad band in the lower frequency region centered at 653 nm attributed to $dxz, dyz \rightarrow dx^2-y^2$ ligand field transition suggesting a square pyramidal geometry around Cu(II) ion [236]. The electronic spectrum of complex $[\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{NiSnCl}_2]$ displayed, spin allowed transition at 623 nm assigned to $^3\text{B}_1(\text{F}) \rightarrow^3\text{E}(\text{F})$ transition suggesting a pentacoordinate geometry around Ni(II) metal ion [237]. Additionally, electronic spectra of both complexes $[\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]$ and $[\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{NiSnCl}_2]$ exhibit an intense absorption bands at 268–271 nm followed by a shoulder at 285 nm attributed to the intraligand $\pi–\pi^*$ transition and the ligand to metal charge transfer (LMCT) band.

**X–ray diffraction analysis**

X–ray diffraction was performed to obtain further evidence about structure of the metal complexes. The diffractograms obtained for the metal complexes $[\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]$ and $[\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{NiSnCl}_2]$, indicating the crystalline nature of complexes (Figure. 57a and b).
Figure 57. The XRD pattern of (a) $[C_{22}H_{42}N_6O_{11}CuSnCl_2]$ and (b) $[C_{22}H_{42}N_6O_{11}NiSnCl_2]$ showing crystalline nature of the complexes.

DNA binding studies

Absorption titration with CT DNA

Electronic absorption spectroscopy was employed to ascertain the binding modes of ligand $[C_{14}H_{28}N_2O_{10}]$, complexes $[C_{22}H_{42}N_6O_{11}CuSnCl_2]$ and $[C_{22}H_{42}N_6O_{11}NiSnCl_2]$ with DNA followed by the changes in the absorbance and shift in the wavelength. In the UV region, the intense absorption bands at 233–271 nm were observed in the spectra of the $[C_{14}H_{28}N_2O_{10}]$, $[C_{22}H_{42}N_6O_{11}CuSnCl_2]$ and $[C_{22}H_{42}N_6O_{11}NiSnCl_2]$ attributed to the intraligand π–π* transition. Binding of the complexes to DNA is expected to perturb the ligand centered transitions of complexes. Upon addition of increasing amounts of CT DNA ($0–33.3 \times 10^{-6}$ M) to $[C_{14}H_{28}N_2O_{10}]$, $[C_{22}H_{42}N_6O_{11}CuSnCl_2]$ and $[C_{22}H_{42}N_6O_{11}NiSnCl_2]$ of fixed concentration ($6.67 \times 10^{-6}$ M), resulted in hyperchromism of 26, 38 and 33%, respectively with no band shift (Figure. 58a–c). Hyperchromism was attributed to the presence of synergic non–covalent interactions: external contact (electrostatic binding), hydrogen bonding and groove surface binding (major or minor) along outside of DNA helix [238]. Since the observed hyperchromic effect revealed no band shift (bathochromic or hyposochromic shift), it can be inferred that
[C_{14}H_{28}N_{2}O_{10}], [C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}] and [C_{22}H_{42}N_{6}O_{11}NiSnCl_{2}] exhibit groove binding interactions.

In order to quantify the binding affinity of [C_{14}H_{28}N_{2}O_{10}], [C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}] and [C_{22}H_{42}N_{6}O_{11}NiSnCl_{2}] with CT DNA, the intrinsic binding constant, $K_b$, were calculated from Wolfe–Shimer Eq. (2) and were found to be $2.98 \times 10^3$, $9.41 \times 10^4$ and $6.28 \times 10^4$ M$^{-1}$, respectively, indicated that [C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}] has higher binding affinity as compared to [C_{14}H_{28}N_{2}O_{10}] and [C_{22}H_{42}N_{6}O_{11}NiSnCl_{2}] due to the dual mode of action of Cu$^{II}$ and Sn$^{IV}$ metal ion (both hard Lewis acid); Cu showing preference for N7 position of guanine while Sn$^{IV}$ prefer to binds to the vicinal anionic phosphate oxygen of DNA helix.

Figure 58. Absorption spectral traces of (a) Ligand [C_{14}H_{28}N_{2}O_{10}] (b) complex [C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}] and (c) complex [C_{22}H_{42}N_{6}O_{11}NiSnCl_{2}] in 5 %DMSO/ 5mM Tris HCl/ 50 mM NaCl buffer at pH 7.2 upon addition of CT DNA. Inset: Plots of [DNA]/ $\varepsilon_a$–$\varepsilon_f$ (m$^2$ cm) vs [DNA] for the titration of CT DNA with complexes ■, experimental data points; full lines, linear fitting of the data. [Complex] $6.67 \times 10^{-6}$ M, [DNA] 0–33.3 $\times 10^{-6}$ M.
Additionally, a heterocyclic imidazole ring in [C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}] and [C_{22}H_{42}N_{6}O_{11}NiSnCl_{2}] bound to Sn^{IV} atoms could contribute to partial intercalation. Moreover, complexes bearing –NH– and –OH– groups or the ligand–N atom may be involved in the secondary interactions like hydrogen bonding with DNA possessing several hydrogen bonding sites accessible both in major and minor grooves [240]. The ligand framework possessing carbohydrate functionality plays a crucial role in molecular recognition in many anticancer drugs, as it binds to DNA in a sequence selective manner. Therefore, carbohydrate conjugate scaffold in complex drives it to attain electrostatic interactions with negatively charged sugar phosphate backbone of DNA. Similar effect of carbohydrate residues have been studied under calicheamicin γ_{1} antibiotic, which belongs to a class of compound called enediyne antibiotics [241].

**Ethidium bromide displacement assay**

To further investigate the mode of binding of the ligand [C_{14}H_{28}N_{2}O_{10}], complexes [C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}] and [C_{22}H_{42}N_{6}O_{11}NiSnCl_{2}] with DNA, the ethidium bromide displacement assay was carried out. The extent of quenching of the fluorescence of EB bound to DNA would reflect the extent of DNA binding of complexes. EB (3,8–diamino–5–ethyl–6–phenylphenanthrium bromide), a phenanthridine fluorescence dye, a typical indicator of intercalation form soluble complexes with nucleic acids and emits intense fluorescence when intercalated into the base pairs of DNA. The addition of a second DNA–binding molecule can quench the DNA–EB adduct emission by either replacing the EB and/or by accepting the excited–state electron of the EB through a photoelectron transfer mechanism [242]. Upon addition of [C_{14}H_{28}N_{2}O_{10}], [C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}] and [C_{22}H_{42}N_{6}O_{11}NiSnCl_{2}], to CT DNA pretreated with EB ([DNA]/[EB]=1) solution caused
appreciable reduction in emission intensities (Figure. 59a–c), indicating that the replacement of the EB fluorophore by the \([C_{14}H_{28}N_{2}O_{10}], [C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}]\) and \([C_{22}H_{42}N_{6}O_{11}NiSnCl_{2}]\), which results in a decrease of the binding constant of ethidium bromide to DNA.

![Emission spectra of EB bound to DNA in the absence and presence of ligands](image)

**Figure 59.** Emission spectra of EB bound to DNA in the absence and presence of (a) Ligand \([C_{14}H_{28}N_{2}O_{10}]\) (b) complex \([C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}]\) and (c) complex \([C_{22}H_{42}N_{6}O_{11}NiSnCl_{2}]\) in 5 mM Tris–HCl/50 mM NaCl buffer. Arrows show the intensity changes upon increasing concentration of the complexes. Inset: Plots of \(I_0/I\) vs \([\text{complex}]/[\text{DNA}]\). (■) experimental data points; full lines, linear fitting of the data.

The quenching efficiency for \([C_{14}H_{28}N_{2}O_{10}], [C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}]\) and \([C_{22}H_{42}N_{6}O_{11}NiSnCl_{2}]\) is evaluated by the classical Stern–Volmer constant \(K_{sv}\), which varies with the experimental conditions. The \(K_{sv}\) values for \([C_{14}H_{28}N_{2}O_{10}], [C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}]\) and \([C_{22}H_{42}N_{6}O_{11}NiSnCl_{2}]\) was found to be 0.67, 1.92 and 0.94, respectively. The highest \(K_{sv}\) value of \([C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}]\) suggesting its stronger ability to displace EB from the EB–DNA system as compared to \([C_{14}H_{28}N_{2}O_{10}]\) and \([C_{22}H_{42}N_{6}O_{11}NiSnCl_{2}]\).
DNA cleavage activity

The DNA cleavage ability of complex \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]\) was studied by agarose gel electrophoresis using supercoiled pBR322 plasmid DNA as a substrate in a medium of 5mM Tris–HCl/50 mM NaCl buffer, at pH 7.2. The activity of complex \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]\) was assessed by the conversion of DNA from Form I (supercoiled form) to Form II (nicked circular) and then to Form III (linear form). With increase in concentration of complex \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]\), the amount of Form I gradually diminishes whereas Form II increases (Lane 2–6), suggesting the single strand DNA cleavage. At 30 µM concentration, complex \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]\) exhibited efficient nuclease activity and there was complete conversion of Form I into Form II without concurrent formation of Form III (Figure. 60a).

**Figure 60.** Agarose gel electrophoresis diagram showing cleavage of pBR322 supercoiled DNA (300 ng) by complex \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]\) at 310 K after 45 min of incubation; Lane 1, DNA control; Lane 2, 10 µM of \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]+\text{DNA}\); Lane 3: 15 µM of \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]+\text{DNA}\); Lane 4: 20 µM of \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]+\text{DNA}\); Lane 5: 25 µM of \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]+\text{DNA}\); Lane 6: 30 µM of \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]+\text{DNA}\); (b) in presence of different activating agents; Lane 1: DNA Control; Lane 2: 9 µM of \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]+\text{H}_2\text{O}_2 (0.4 M)+\text{DNA}\); Lane 3: 9 µM of \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]+\text{MPA (0.4 M)}+\text{DNA}\); Lane 4: 9 µM of \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]+\text{GSH (0.4 M)}+\text{DNA}\); Lane 5: 9 µM of \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]+\text{Asc (0.4 M)}+\text{DNA}\); Lane 6: 9 µM of \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]+\text{DMSO (0.4 M)}+\text{DNA}\); Lane 7: 9 µM of \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]+\text{tert–butyl alcohol (0.4 M)}+\text{DNA}\); Lane 8: 9 µM of \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]+\text{sodium azide (0.4 M)}+\text{DNA}\); Lane 9: 9 µM of \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]\) SOD (15 units)+DNA.

Many copper complexes have been proven to cleave DNA more efficiently in the presence of exogenous agents viz; \(\text{H}_2\text{O}_2\), ascorbate (Asc), 3–mercaptopropionic acid (MPA) and glutathione (GSH). The cleavage activity of \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]\) was
significantly enhanced by these activators which follows the order MPA≈GSH>Asc>H₂O₂ (Figure. 60b). Thus, complex [C_{22}H_{42}N_{6}O_{11}CuSnCl₂] exhibited a significant DNA cleavage activity in the presence of 3-mercaptopropionic acid which could be correlated to other previous studies on bis(o-phenanthroline) copper(II) complexes [243].

Mechanistic investigations were done using various additives to understand the nature of reactive oxygen species (ROS) such as hydroxyl radical scavengers (DMSO, EtOH), singlet oxygen scavenger (NaN₃) and a superoxide scavenger (SOD) involved in the DNA cleavage reactions (Figure. 60b). The experiment results showed that the DNA breakdown mediated by [C_{22}H_{42}N_{6}O_{11}CuSnCl₂] was clearly inhibited in the presence of DMSO and ter-butyl alcohol (Lane 5 and 6), indicating that the freely diffusible hydroxyl radical is one of the reactive species involved in the DNA strand scission. On the other hand, addition of NaN₃ and SOD did not show significant quenching of the cleavage revealing that singlet oxygen and superoxide anion were not involved in the cleavage process (Lane 8 and 9). Since, the complex [C_{22}H_{42}N_{6}O_{11}CuSnCl₂] was able to cleave DNA in the absence of any reducing agent, which implies that DNA might be cleaved by a discernible hydrolytic pathway. Hydrolytic pathways usually depend on the Lewis acidity of the central metal ion, which serves to activate the phospho–diester bonds towards nucleophilic attack via charge neutralization. Also the presence of aqua ligands in the complex provide an inbuilt nucleophile in the complex, which attack the phosphorus atom thereby leading to direct hydrolysis of the diester bonds [244]. The coordinated water molecule of complex [C_{22}H_{42}N_{6}O_{11}CuSnCl₂] facilitates the nucleophilic attack of water oxygen to phosphorus, followed by a five-coordinate
phosphate intermediate and subsequent rearrangement of the phosphate allows the DNA to be cleaved readily.

The potential interacting site of \( [C_{22}H_{42}N_6O_{11}CuSnCl_2] \) with pBR322 DNA was further explored in presence of the minor groove binding agent, DAPI and the major groove binding agent, methyl green. The supercoiled DNA was treated with DAPI or methyl green prior to the addition of \( [C_{22}H_{42}N_6O_{11}CuSnCl_2] \). The DNA cleavage patterns demonstrated that the activity of \( [C_{22}H_{42}N_6O_{11}CuSnCl_2] \) was inhibited in presence of DAPI while it remains unaffected in the presence of methyl green indicating minor groove–binding propensity of the complex \( [C_{22}H_{42}N_6O_{11}CuSnCl_2] \) (Figure. 61).

**Figure 61.** Agarose gel electrophoresis pattern for the cleavage of pBR322 supercoiled DNA (300 ng) by complex \( [C_{22}H_{42}N_6O_{11}CuSnCl_2] \) in presence of DNA minor binding agent DAPI and major binding agent methyl green at 310 K after incubation for 45 min. Lane 1: DNA control; Lane 2: 9 µM of \( [C_{22}H_{42}N_6O_{11}CuSnCl_2]+DNA+DAPI \) (8 µM); Lane 3: 9 µM of \( [C_{22}H_{42}N_6O_{11}CuSnCl_2]+DNA+methyl \) green (2.5 µL of a 0.01mg/ml solution).

**T4 DNA ligation experiment**

Direct evidence for a hydrolytic mechanism was carried out from ligation experiments of the linear DNA cleavage products generated by complex \( [C_{22}H_{42}N_6O_{11}CuSnCl_2] \) in which supercoiled pBR322 DNA was treated with T4 ligase enzyme and subjected to gel electrophoresis. It is well known that in DNA hydrolytic cleavage 3′–OH and 5′–OPO\(_3\) (5′–OH and 3′–OPO\(_3\)) fragments remain intact and that these fragments can be enzymatically ligated and end–labeled. In some cases, the hydrolytic products either did not end at the required 5′–phosphate and 3′–OH (ribose) termini or the complex
sometimes bound to the termini of cleaved DNA [245]. Complex \([\text{C}_{22}\text{H}_{42}\text{N}_{6}\text{O}_{11}\text{CuSnCl}_2]\) yielded linearized DNA which was religated by using T4 ligase enzyme (Figure. 62). In our case, DNA religation was complete and after ligation there was complete repair which was quantitatively ascertained by comparison of complex treated DNA and with the control DNA alone in supercoiled form. Hence, this result implied that the process of DNA cleavage by \([\text{C}_{22}\text{H}_{42}\text{N}_{6}\text{O}_{11}\text{CuSnCl}_2]\) occurs via a hydrolytic pathway. Complex \([\text{C}_{22}\text{H}_{42}\text{N}_{6}\text{O}_{11}\text{CuSnCl}_2]\) could therefore, be useful not only in drug design but also in elucidating the precise role of metal ions in enzyme catalysis [246].

![Figure 62. Agarose gel electrophoresis pattern for the ligation pBR322 plasmid DNA linearized by complex \([\text{C}_{22}\text{H}_{42}\text{N}_{6}\text{O}_{11}\text{CuSnCl}_2]\): Lane 1: DNA control; Lane 2: pBR322 plasmid DNA cleaved by \([\text{C}_{22}\text{H}_{42}\text{N}_{6}\text{O}_{11}\text{CuSnCl}_2]\); Lane 3: ligation of linearized pBR322 plasmid DNA by T4 DNA ligase. Topo–I inhibition assay

A plasmid DNA cleavage assay was used to investigate the effect of complex \([\text{C}_{22}\text{H}_{42}\text{N}_{6}\text{O}_{11}\text{CuSnCl}_2]\) on the inhibitory activity of human–Topo–I by agarose gel electrophoresis. This assay provides a direct means of determining whether the drug affects the unwinding of a supercoiled (SC) duplex DNA to nicked open circular (NOC) and relaxed (R) DNA. When catalytic activity of topoisomerase I was assayed, complex \([\text{C}_{22}\text{H}_{42}\text{N}_{6}\text{O}_{11}\text{CuSnCl}_2]\) inhibited this process in a concentration–dependent manner [247]. With increasing the concentration of complex \([\text{C}_{22}\text{H}_{42}\text{N}_{6}\text{O}_{11}\text{CuSnCl}_2]\) (5–20 μM), the levels of relaxed form were inhibited (Lane 3–6). At 20 μM, the DNA relaxation
effect caused by Topo–I was significantly inhibited by complex \([ \text{C}_{22} \text{H}_{42} \text{N}_{6} \text{O}_{11} \text{CuSnCl}_{2} ]\) (Figure. 63). These observations suggest that complex \([ \text{C}_{22} \text{H}_{42} \text{N}_{6} \text{O}_{11} \text{CuSnCl}_{2} ]\) inhibited topoisomerase I catalytic activity due to the relatively strong DNA binding affinity of complex \([ \text{C}_{22} \text{H}_{42} \text{N}_{6} \text{O}_{11} \text{CuSnCl}_{2} ]\), which prevents the enzyme from efficiently binding to the DNA and exercising its function. Thus, the metal–based drug entity can exert its antitumor activity via topoisomerase inhibition as it was found that cancerous cell showed significantly increased intracellular levels of topoisomerase, which is required for rapid and unchecked proliferation of cancer cells [248].

**Figure 63.** The cleavage patterns of the agarose gel electrophoresis diagram showing effect of different concentration of complex \([ \text{C}_{22} \text{H}_{42} \text{N}_{6} \text{O}_{11} \text{CuSnCl}_{2} ]\) on the activity of DNA topoisomerase I (Topo–I); Lane 1, DNA control; Lane 2: Topo–I+DNA; Lane 3: 5 \(\mu\)M of \([ \text{C}_{22} \text{H}_{42} \text{N}_{6} \text{O}_{11} \text{CuSnCl}_{2} ]\)+DNA+Topo–I; Lane 4: 10 \(\mu\)M of \([ \text{C}_{22} \text{H}_{42} \text{N}_{6} \text{O}_{11} \text{CuSnCl}_{2} ]\)+DNA+Topo–I; Lane 5: 15 \(\mu\)M of \([ \text{C}_{22} \text{H}_{42} \text{N}_{6} \text{O}_{11} \text{CuSnCl}_{2} ]\)+DNA+Topo–I; Lane 6: 20 \(\mu\)M of \([ \text{C}_{22} \text{H}_{42} \text{N}_{6} \text{O}_{11} \text{CuSnCl}_{2} ]\)+DNA + Topo–I.

**Cellular uptake**

The cellular uptake of metal–based anticancer drugs is of importance for its effectiveness against tumors and their antiproliferative efficacies. Literature revealed that *in vitro* and *in vivo* monitoring of glucose utilization in rapidly growing cancer cells has attracted much attention due to over–expression of glucose transporters (GLUTs), particularly GLUT–I, in rapidly growing HeLa cells which catabolize glucose at high rates and entering the cells through a GLUT–mediated glucose–uptake pathway [249]. In order to assess the intracellular localization of complex \([ \text{C}_{22} \text{H}_{42} \text{N}_{6} \text{O}_{11} \text{CuSnCl}_{2} ]\) inside the human cervical carcinoma (HeLa) cells, confocal microscopic experiment was
conducted. The emission for $[C_{22}H_{42}N_6O_{11}CuSnCl_2]$ was observed at 561 nm, while the DNA stain was observed at 740 nm. The untreated HeLa cells showed negligible background fluorescence at the 510 nm emission wavelength (no auto fluorescence emanates from unstained cells; cell without staining).

**Figure 64.** Confocal microscopic visualization of cellular uptake and localization of complex $[C_{22}H_{42}N_6O_{11}CuSnCl_2]$ in HeLa cells. (a) Cells incubated solely for 4 h with complex $[C_{22}H_{42}N_6O_{11}CuSnCl_2]$ (20 $\mu$M) at 37 °C showed the efficient cellular uptake in the cytoplasm and nearby nucleus region [red fluorescence]. (b) DNA was counterstained blue with DAPI [blue fluorescence]. (c) Overlaid image of complex $[C_{22}H_{42}N_6O_{11}CuSnCl_2]$ and DAPI.

However, after incubation of HeLa cells with the $[C_{22}H_{42}N_6O_{11}CuSnCl_2]$ at 37 °C under a 5% CO$_2$ atmosphere for 4 h resulted in efficient cellular uptake. The weak red fluorescence in the cytoplasm with no specific localization revealed that $[C_{22}H_{42}N_6O_{11}CuSnCl_2]$ was taken by any cytoplasmic organelle and thus suggesting very little accumulation of $[C_{22}H_{42}N_6O_{11}CuSnCl_2]$ in the cytoplasm (Figure 64a). However, intense luminescence was observed in the nuclear region indicating the nuclear localization of the $[C_{22}H_{42}N_6O_{11}CuSnCl_2]$ in HeLa cells. To confirm its nuclear localization, the cell was subjected to staining for which the cell treated with $[C_{22}H_{42}N_6O_{11}CuSnCl_2]$ was thoroughly washed, stained with a blue nuclear dye, DAPI.
(4',6-diamidino-2-phenylindole), a DNA selective fluorescent probe and after fixation [250], the images were recorded (Figure. 64b). The superimposed images (Figure. 64c), clearly indicated that the \([C_{22}H_{42}N_6O_{11}CuSnCl_2]\) was localized in DNA–containing areas.

**Molecular docking with DNA**

To study the molecular basis of interaction mode and affinity of binding, ligand \([C_{14}H_{28}N_2O_{10}]\), complexes \([C_{22}H_{42}N_6O_{11}CuSnCl_2]\) and \([C_{22}H_{42}N_6O_{11}NiSnCl_2]\) were docked into DNA duplex of sequence \(d(CGCGAATTCGCG)\)\(_2\) dodecamer (PDB ID: 1BNA). Targeting the minor groove of DNA through binding to a small molecule has long been considered as an important tool in molecular recognition of specific DNA–sequence. The resulting docked pose (Figure. 65), revealed that the \([C_{14}H_{28}N_2O_{10}]\) strongly binds to the narrow minor groove region of DNA within G–C rich region and lead to van der Waals interactions and hydrophobic contacts with DNA functional groups that define the groove. Moreover, –NH and –OH groups of the ligand \([C_{14}H_{28}N_2O_{10}]\) may be engaging in hydrogen–bonding interactions with DNA nucleobases available in the minor grooves [251]. Additionally, \([C_{22}H_{42}N_6O_{11}CuSnCl_2]\) and \([C_{22}H_{42}N_6O_{11}NiSnCl_2]\) (Fig. 5b and c) exhibited more effective \(\pi–\pi\) stacking interactions between imidazole rings and DNA base pairs. The resulting relative binding energy of docked \([C_{14}H_{28}N_2O_{10}]\), complexes \([C_{22}H_{42}N_6O_{11}CuSnCl_2]\) and \([C_{22}H_{42}N_6O_{11}NiSnCl_2]\) with DNA were found to be \(-204.6, -258.6\) and \(-248.9\) eV respectively, follows the order \([C_{22}H_{42}N_6O_{11}CuSnCl_2]>[C_{22}H_{42}N_6O_{11}NiSnCl_2]>[C_{14}H_{28}N_2O_{10}]\) which is also in accordance with our hypothesis that \([C_{22}H_{42}N_6O_{11}CuSnCl_2]\) is more prominent DNA binder than \([C_{22}H_{42}N_6O_{11}NiSnCl_2]\) and free ligand \([C_{14}H_{28}N_2O_{10}]\). Thus, we can conclude that there is a mutual complement between spectroscopic techniques and molecular
docked model, which can substantiate our experimental results and provide valuable information about the mode of interaction between complex and DNA.

Figure 65. Molecular docked model of (a) Ligand \([C_{14}H_{28}N_{2}O_{10}]\) (b) complex \([C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}]\) and (c) complex \([C_{22}H_{42}N_{6}O_{11}NiSnCl_{2}]\) with DNA [dodecamer duplex of sequence \(d(CGCGAATTCGCG)_{2}\) (PDB ID: 1BNA)]

**Molecular docking with human–DNA–Topo–I**

To further validate the observed Topo–I inhibitory assay, complex \([C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}]\) was successively docked with the human–DNA–Topo–I complex (PDB ID: 1SC7) to search the exact binding site. The X–ray crystallographic structure of the human–DNA–Topo–I complex (PDB ID: 1SC7) from Protein Data Bank was uploaded in which Topo–I is bound to the oligonucleotide sequence 5’–AAAAAGACTTsX–GAAAATTTTT–3’, where ‘s’ is 5’–bridging phosphorothiolate of the cleaved strand and ‘X’ represents any of the four bases A, G, C or T. The phosphoester bond of G12 in 1SC7 was rebuilt and SH of G11 on the scissile strand was changed to OH [252].
Figure 66. Diagram showing in (a) human–DNA–Topo–I (70 kDa) (PDB ID: 1SC7) (b) molecular docked model of complex \([C_{22}H_{42}N_{6}O_{11}CuSnCl_2]\) in the cleavage active site of human DNA topoisomerase I (PDB ID: 1SC7).

The resulting docking model (Figure. 66) revealed that the heterocyclic imidazole rings of \([C_{22}H_{42}N_{6}O_{11}CuSnCl_2]\) approaching towards the DNA cleavage site in the Topo–I–DNA complex and forming a stable complex through \(\pi–\pi\) stacking interactions between the G11 (+1) and pyrimidine ring of T10 (−1) in the minor groove on the scissile strand and C112 and A113, on the non–scissile strand, parallel to the plane of base pairs, whereas the OH groups of sugar probably interacting through hydrogen bond to Arg 364, which is considered an essential amino acid that interacts with the ligand in the DNA–Topo–I active site. On the other hand, the ethylenediamine group of \([C_{22}H_{42}N_{6}O_{11}CuSnCl_2]\) was involved in H–bond with the oxygen atom of G11 ribofuranose in the scissile strand, which could strongly block the religation process, subsequently leading to inhibitory effect on Topo–I [253, 254]. Furthermore, DNA intercalating forces was much more important than hydrogen bonding of the ligand to the surrounding amino acids residues of the protein, or to the base pairs [255]. Our molecular
docking study proved the importance of DNA intercalating ability of [C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}] as well as H–bond with the enzyme in the cleavage site. This result suggests that blocking the religation of the G11 hydroxyl group could be the main design point for novel Topo–I inhibitors.

**Antitumor activity assays**

*In vitro* cytotoxic activity of complex [C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}] has been evaluated in terms of GI_{50}, TGI and LC_{50} (Table 4) values against nine different human carcinoma cell lines of different histological origin: 786–O, A498 (kidney), Zr–75–1 (Breast), SiHa (Cervix), A549, Hop–62 (Lung), SW620, HCT15 (Colon), MIAPACA2 (Pancreatic). The *in vitro* anti–tumor screening of [C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}] was evaluated by employing microculture Sulforhodamine B test (SRB) [219]. The result showed high potential of the complex [C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}] as drug candidate, as expected from the *in vitro* DNA binding studies and topoisomerase I inhibition assay. Surprisingly, complex [C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}] exhibits promising antitumor activities (with GI_{50} <10 μg/ml) on a panel of human carcinoma cell lines, indicating that its remarkable potential to act as efficacious metal–based anticancer drug.
Table 4: Summary of the screening data of \([C_{22}H_{42}N_{6}O_{11}CuSnCl_{2}]\) for the in vitro anti–tumor activity (in μg/ml).

<table>
<thead>
<tr>
<th>Human Tissue of origin</th>
<th>Kidney</th>
<th>Breast</th>
<th>Cervix</th>
<th>Lung</th>
<th>Colon</th>
<th>Lung</th>
<th>Colon</th>
<th>Kidney</th>
<th>Pancreatic</th>
</tr>
</thead>
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<tr>
<td>Cell line</td>
<td>786–O</td>
<td>Zr–75–1</td>
<td>SiHa</td>
<td>Hop62</td>
<td>HCT15</td>
<td>A549</td>
<td>SW620</td>
<td>A498</td>
<td>MIAPACA2</td>
</tr>
<tr>
<td>(\text{GI}_{50})</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>(\text{ADR})</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
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<td>&lt;10</td>
</tr>
<tr>
<td>(\text{TGI})</td>
<td>20.6</td>
<td>&lt;10</td>
<td>15.5</td>
<td>&lt;10</td>
<td>24.7</td>
<td>11.2</td>
<td>16.8</td>
<td>10.2</td>
<td>&lt;10</td>
</tr>
<tr>
<td>(\text{ADR})</td>
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<td>&lt;10</td>
<td>14.9</td>
<td>10.1</td>
<td>37.0</td>
<td>42.1</td>
<td>17.0</td>
<td>13.9</td>
<td>20.4</td>
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<tr>
<td>(\text{LC}_{50})</td>
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<td>19.7</td>
<td>35.0</td>
<td>22.9</td>
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<td>38.3</td>
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</tr>
<tr>
<td>(\text{ADR})</td>
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<td>66.9</td>
<td>77.8</td>
<td>34.3</td>
<td>35.3</td>
<td>46.8</td>
</tr>
</tbody>
</table>

Where:
- \(\text{GI}_{50}\) = Growth inhibition of 50 % \((\text{GI}_{50})\) calculated from \(\left[(\text{Ti}–\text{Tz})/(\text{C}–\text{Tz})\right] \times 100 = 50\), drug concentration result in a 50% reduction in the net protein increase.
- \(\text{ADR}\) = Adriamycin (taken as positive control compound).
- \(\text{TGI}\) = Tumor growth inhibition
- \(\text{LC}_{50}\) = Lethal concentration of 50 % \((\text{LC}_{50})\)
Conclusion

In this work, we have designed and synthesized new metal–based cancer chemotherapeutic glycoconjugate entities containing heterobimetallic core \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]\) and \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{NiSnCl}_2]\) derived from \(\text{N,N’–di–β–D–glucopyranosyl ethylenediamine N–glycoside ligand \([\text{C}_{14}\text{H}_{28}\text{N}_2\text{O}_{10}]\)\). The general hydrophilic nature of carbohydrate molecule and their high density of hydroxyl groups (hydrogen bond donors and acceptors) could increase the cellular uptake of the compounds as well as their facile transport at the molecular level. *In vitro* DNA binding studies revealed that complex \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]\) binds more strongly to CT DNA than \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{NiSnCl}_2]\) and \([\text{C}_{14}\text{H}_{28}\text{N}_2\text{O}_{10}]\) *via* electrostatic groove binding mode, synergized by partial intercalation in G–C rich sequences. Complex \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]\) cleave pBR322 DNA double strand scission, which proceeds *via* hydrolytic pathway as further validated by T4 DNA ligase assay. Complex \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]\) is also a notable Topo–I inhibitor exhibiting remarkably high Topo–I inhibition activity at a very low concentration (20 μM). Furthermore, complex \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]\) showed potent *in vitro* cytotoxicity (GI\(_{50}\) value < 10 μg/ml) on a panel of selected human cancer cell lines; also confocal cell imaging studies using the HeLa cell line depicted localization in the cells of its nuclear localization. Thus, complex \([\text{C}_{22}\text{H}_{42}\text{N}_6\text{O}_{11}\text{CuSnCl}_2]\) is an exceptional anticancer agent which involves Topo–I inhibition mechanism and at the same time can also be utilized as a biocompatible fluorescent probe. Furthermore, molecular docking studies were performed with molecular target DNA and the active site of Topo–I in order to validate the experimental results.