Synthesis

Synthesis of \( \text{N,N'-di-\beta-D-glucopyranosyl ethylenediamine (\([\text{C}_{14}\text{H}_{28}\text{N}_{2}\text{O}_{10}\])\)} \)

The ligand \([\text{C}_{14}\text{H}_{28}\text{N}_{2}\text{O}_{10}\]) was synthesized by the slight modification of procedure described earlier in the chapter IV.

Synthesis of monometallic complexes \([\text{C}_{22}\text{H}_{52}\text{N}_{6}\text{O}_{13}\text{Cu}]\) and \([\text{C}_{22}\text{H}_{60}\text{N}_{6}\text{O}_{17}\text{Ni}]\)

The monometallic complexes \([\text{C}_{22}\text{H}_{52}\text{N}_{6}\text{O}_{13}\text{Cu}]\) and \([\text{C}_{22}\text{H}_{60}\text{N}_{6}\text{O}_{17}\text{Ni}]\) were prepared by following procedure.

To a methanolic suspension (30 ml) of ligand \([\text{C}_{14}\text{H}_{28}\text{N}_{2}\text{O}_{10}\]) (0.384 g, 1 mmol) was added CuCl\(_2\).2H\(_2\)O (0.171 g, 1 mmol) and allowed to reflux at 70 °C for 2 h until the homogenous green colored solution appeared. A solution of piperazine hexahydrate (0.388g, 2 mmol) in 10 ml methanol was added slowly to the reaction mixture, a fluorescent green colored precipitate formed was filtered and washed thoroughly with methanol and dried in vacuo.

\([\text{C}_{22}\text{H}_{52}\text{N}_{6}\text{O}_{13}\text{Cu}]\) [Yield: ~61%]. M.P. 234 °C (dec), Anal.Calcd for \(\text{C}_{22}\text{H}_{52}\text{N}_{6}\text{O}_{13}\text{Cu}: \text{C}, 39.31; \text{H}, 7.80; \text{N}, 12.50\). Found: \(\text{C}, 39.32; \text{H}, 7.81; \text{N}, 12.52\).

IR(KBr) (\(\nu_{\text{max}}/\text{cm}^{-1}\)):
- 3386 \(\nu(\text{O–H, broad})\);
- 3342 \(\nu(\text{N–H})\);
- 1649 \(\delta(\text{N–H, HOH})\);
- 1542 \(\nu(\text{C=N})\);
- 1412 \(\delta(\text{OCH, CH}_2, \text{CCH})\);
- 1024 \(\nu(\text{CO, CC})\);
- 1348 \(\nu(\text{C–N})\);
- 534 \(\nu(\text{Sn–N})\);
- 419 \(\nu(\text{Cu–N})\); Molar Conductance, \(\Lambda_M\) (1·10\(^{-3}\) M, DMSO): 16.0 \(\Omega^{-1}\text{cm}^2\text{ mol}^{-1}\) (non-electrolyte); Uv–vis. (1 x 10\(^{-3}\) M, DMSO, nm) 244, 292, 590. ESI–MS: (m/z, DMSO): 634.9 \([\text{C}_{22}\text{H}_{52}\text{N}_{6}\text{O}_{13}\text{Cu–2H}_2\text{O–2H}]^+\).

\([\text{C}_{22}\text{H}_{60}\text{N}_{6}\text{O}_{17}\text{Ni}]\) [Yield: 56%], M.P. 228 °C (dec), Anal.Calcd for \(\text{C}_{22}\text{H}_{60}\text{N}_{6}\text{O}_{17}\text{Ni}: \text{C}, 35.73; \text{H}, 8.18; \text{N}, 11.37\). Found: \(\text{C}, 35.52; \text{H}, 8.14; \text{N}, 11.32\). IR (KBr) (\(\nu_{\text{max}}/\text{cm}^{-1}\)):
- 3386 \(\nu(\text{O–H, broad})\);
- 3299 \(\nu(\text{N–H})\);
- 1627 \(\delta(\text{N–H, HOH})\), 1574 (C=N);
Synthesis of heterobimetallic complexes \([C_{22}H_{50}N_6O_{13}CuSnCl_2]\) and \([C_{22}H_{58}N_6O_{17}NiSnCl_2]\)

The heterobimetallic complexes \([C_{22}H_{50}N_6O_{13}CuSnCl_2]\) and \([C_{22}H_{58}N_6O_{17}NiSnCl_2]\) were prepared by refluxing methanolic solution of the monometallic complexes \([C_{22}H_{52}N_6O_{13}Cu]\) and \([C_{22}H_{59}N_6O_{17}Ni]\) (0.672/0.739 g, 1 mmol) and dropwise addition of SnCl\(_4\)-5H\(_2\)O (0.350g, 1 mmol). After 5–6 h the solutions were filtered and the filtrate was reduced to one–third of its volume on a vacuum evaporator, affording the heterobimetallic complexes.

\([C_{22}H_{50}N_6O_{13}CuSnCl_2]\): [Yield: 46%]. M.P. >280 °C (dec). Anal.Calcd for C\(_{22}\)H\(_{50}\)N\(_6\)O\(_{13}\)CuSnCl\(_2\): C, 30.73; H, 5.86; N, 9.77. Found: C, 30.72; H, 5.81; N, 9.71. IR (KBr) \((v_{\text{max}}/\text{cm}^{-1})\): 3420 \(v(O-H, \text{ broad})\); 3246 \(v(N-H)\); 1646 \(\delta(N-H, \text{ HOH})\); 1559 (C=N); 1436 \(\delta(OCH, CH_2, CCH)\); 1079 \(v(CO, CC)\); 1321 \(v(C-N)\); 559 \(v(Sn-N)\); 420 \(v(Cu-N)\). Molar Conductance, \(\Lambda_M\) (1·10\(^{-3}\) M, DMSO): 28.0 \(\Omega^{-1}\) cm\(^2\) mol\(^{-1}\) (non–electrolyte); Uv–vis. (1 x 10\(^{-3}\) M, DMSO, nm) 247, 296, 630. ESI–MS: (m/z, DMSO): 841.8 \([C_{22}H_{58}N_6O_{17}NiSnCl_2-H_2O]^+\)

\([C_{22}H_{58}N_6O_{17}NiSnCl_2]\) [Yield: ~37%]. M.P. > 265 °C (dec). Anal.Calcd for C\(_{22}\)H\(_{58}\)N\(_6\)O\(_{17}\)NiSnCl\(_2\): C, 28.50; H, 6.31; N, 9.07. Found: C, 28.51; H, 6.32; N, 9.05. IR
(KBr) ($v_{\text{max}}$/cm$^{-1}$): 3428 $v$(O–H, broad); 3301 $v$(N–H); 1634 $\delta$(N–H, HOH), 1559 $v$(C=N); 1440 $\delta$(OCH, CH$_2$, CCH); 1071 $v$(CO, CC); 1321 $v$(C–N); 575 $v$(Sn–N); 456 $v$(Ni–N). Molar Conductance, $\Lambda_M$ (1·10$^{-3}$ M, DMSO): 28.0 $\Omega^{-1}$cm$^2$ mol$^{-1}$ (non-electrolyte); Uv–vis. (1 x 10$^{-3}$ M, DMSO, nm) 245, 295, 674. $^1$H NMR (400 MHz, DMSO–$d_6$, $\delta$): 5.19–5.00 (N–H(en)); 3.19–4.43 (CH$_2$(en), H$_2$O, CH$_2$(ppz), skeleton protons of D–glucose). $^{13}$C NMR (100 MHz, DMSO–$d_6$, 25°, $\delta$): 89.74–60.86 (6C, Glc); 46.22–40.28 (CH$_2$ of amine and piperazine). $^{119}$SnNMR (149.19 MHz, DMSO–$d_6$, $\delta$): −570.47 ppm. $^{119}$SnNMR (149.19 MHz, D$_2$O, $\delta$): −570.47. ESI–MS: (m/z, DMSO): 801.1 [C$_{22}$H$_{58}$N$_6$O$_{17}$NiSnCl$_2$–7H$_2$O+H]$^+$

**Results and discussion**

The synthesis of heterobimetallic complexes [C$_{22}$H$_{50}$N$_6$O$_{13}$CuSnCl$_2$] and [C$_{22}$H$_{58}$N$_6$O$_{17}$NiSnCl$_2$] were achieved by reacting monometallic complexes [C$_{22}$H$_{52}$N$_6$O$_{13}$Cu] and [C$_{22}$H$_{60}$N$_6$O$_{17}$Ni] with SnCl$_4$·5H$_2$O in 1:1 stoichiometric ratio (scheme 1). Empirical formulae and proposed structure were ascertained by elemental analysis and various spectroscopic techniques (IR, UV–vis, ESI–MS and NMR spectroscopy). All the complexes were hygroscopic in nature and soluble in H$_2$O and DMSO. Molar conductance ($\Lambda_M$) values of complexes in DMSO (16–28 $\Omega^{-1}$cm$^2$ mol$^{-1}$) at 25 °C suggest their non–ionic nature. On the basis of spectral studies, the coordination geometry of the central metal ions Cu$^{II}$/Ni$^{II}$ was square pyramidal, while the Sn(IV) atoms was present in a hexacoordinated environment.
**Scheme III:** Synthetic route for the ligand, monometallic and heterobimetallic complexes

**Spectral characterization**

**IR spectroscopy**

The IR spectrum of ligand \([\text{C}_{14}\text{H}_{28}\text{N}_{2}\text{O}_{10}]\) displayed a characteristic band in the range 3500–3200 cm\(^{-1}\) assignable to \(\nu(\text{O–H})\) stretching vibration of free saccharides. However, IR spectra of the complexes exhibited a characteristic broad envelop at around 3400 cm\(^{-1}\) due to the stretching vibrations of coordinated water molecule [256]. The absorption bands observed in the range of 3342–3246 and 1649–1627 cm\(^{-1}\) were assigned to \(\nu(\text{N–H})\) stretching and \(\delta(\text{N–H and HOH})\) bending vibration of the coordinated secondary amino functions of the complexes. The strong characteristic IR bands at 1503–1412, 1079–1024 and 883–864 cm\(^{-1}\) were attributed to \(\delta(\text{OCH, CH}_{2}, \text{ CCH})\), \(\nu(\text{CO, CC})\) and \(\delta(\text{CCH, CH})\).
vibrations, respectively, indicating the presence of sugar moiety [257]. Additionally, the bands at 1321–1348 cm\(^{-1}\) was assigned to \(\nu(C-N)\) stretching vibration of the piperazine ring. The medium intensity bands around 419–439 and 575–559 cm\(^{-1}\) were attributed to \(\nu(Cu/Ni-N)\) and \(\nu(Sn-N)\), respectively revealing the complex formation.

**NMR spectroscopy**

The \(^1\)H and \(^{13}\)C NMR spectra of ligand \([\text{C}_{14}\text{H}_{28}\text{N}_{2}\text{O}_{10}]\) was recorded in DMSO–\(d_6\) solution. \(^1\)H NMR spectra of \([\text{C}_{14}\text{H}_{28}\text{N}_{2}\text{O}_{10}]\) exhibited signal at 4.70–4.69 ppm attributed to \(C_1\) and glycosylic \(-NH\) proton, indicating the condensation between the hydroxyl group of saccharides positioned at C–1 center and amino protons of ethylenediamine. The signals at 3.53–3.93 ppm were assigned to \(C_2, C_3, C_4, C_6\) hydroxyl protons. The signals at 2.91–2.85 ppm were attributed to \(-CH_2\) proton of linker ethylenediamine at C–1, together with other distinct sugar proton resonances at 3.40–3.32 ppm (\(C_6\) proton), 3.28–3.24 ppm (\(C_3\) and \(C_5\) proton), 3.08–3.13 ppm (\(C_2\) and \(C_4\) proton). However, \(-NH\) proton shifted downfield to 4.82–4.79 and 5.19–5.00 ppm in complexes \([\text{C}_{22}\text{H}_{60}\text{N}_6\text{O}_{17}\text{Ni}]\) and \([\text{C}_{22}\text{H}_{58}\text{N}_6\text{O}_{17}\text{NiSnCl}_2]\), respectively, which revealed the coordination of glycosylic nitrogen to the metal centre. The \(^1\)H NMR spectra of complexes \([\text{C}_{22}\text{H}_{60}\text{N}_6\text{O}_{17}\text{Ni}]\) and \([\text{C}_{22}\text{H}_{58}\text{N}_6\text{O}_{17}\text{NiSnCl}_2]\) revealed broad envelope at 3.48–4.50 and 3.19–4.43 ppm, respectively were attributed to the skeletal protons signal of saccharides moiety, \(-CH_2\) proton of linker ethylenediamine and piperazine ring [258]. Due to the saccharide–typical strongly coupled system, the resonances arising from different saccharide protons generally overlap in the same region resulting in the broadening of signals, and thereby individual resonances was difficult to identify [259, 260]. The \(^{13}\)C NMR spectra of the \([\text{C}_{14}\text{H}_{28}\text{N}_{2}\text{O}_{10}]\) and the complex \([\text{C}_{22}\text{H}_{60}\text{N}_6\text{O}_{17}\text{Ni}]\) and \([\text{C}_{22}\text{H}_{58}\text{N}_6\text{O}_{17}\text{NiSnCl}_2]\)
corroborated well with the proposed structures. In the $^{13}$C NMR spectrum of ligand [C$_{14}$H$_{28}$N$_2$O$_{10}$], the signal at 42.46 ppm attributed to the C$_7$–atom of the ethylenediamine (en)–based linker, together with six distinct carbon atoms of the D–glucose resonances at 93.44 (C$_1$), 60.66 (C$_2$), 76.90 (C$_3$), 74.06 (C$_4$), 75.87 (C$_5$), and 69.64 (C$_6$). However, the $^{13}$C NMR spectrum of complex [C$_{22}$H$_{60}$N$_6$O$_{17}$Ni] and [C$_{22}$H$_{58}$N$_6$O$_{17}$NiSnCl$_2$] exhibited distinct signals at 44.00–39.09 and 46.22–40.28 ppm, respectively attributed to –CH$_2$ carbons of piperazine and ethylenediamine moiety. The peaks for the carbon atoms of the D–glucose were observed in the range of 92.02–60.50 and 89.74–60.86 ppm for complex [C$_{22}$H$_{60}$N$_6$O$_{17}$Ni] and [C$_{22}$H$_{58}$N$_6$O$_{17}$NiSnCl$_2$], respectively. $^{119}$Sn NMR spectroscopy has been found to be a useful technique for structure elucidation and the nature of coordination of tin atom in complexes. The $^{119}$Sn chemical shift, $\delta$ ($^{119}$Sn) is sensitive to the chemical environments of the tin atom. $^{119}$Sn NMR spectrum of the heterobimetallic complex [C$_{22}$H$_{58}$N$_6$O$_{17}$NiSnCl$_2$] displayed a sharp single peak at $-570.47$ ppm, which was in agreement with the hexacoordinated geometry of tin metal atom (Figure. 67) [261].
Figure 67. (a) $^1H$, (b) $^{13}C$ and (c) $^{119}Sn$ N.M.R. spectrum of heterobimetallic complex $[C_{22}H_{58}N_6O_{17}NiSnCl_2]$

EPR spectrum

The X–band EPR spectra of complex $[C_{22}H_{56}N_6O_{17}CuSnCl_2]$ (Figure. 68) was recorded at liquid nitrogen temperature (LNT) under the magnetic field strength 3000 ± 1000 G using tetracyanoethylene as a field marker. The spectrum shows an anisotropic signal
with $g_\parallel = 2.32$ and $g_\perp = 2.05$ and $g_{av} = 2.14$ computed from the expression $g_{av}^2 = (g_\parallel^2 + 2g_\perp^2)/3$. The trend of $g$ values ($g_\parallel > g_\perp > 2.0023$) revealed that the unpaired electrons lies predominantly in the $\{dx^2−y^2\}^1$ ground state of the Cu(II) ion, in a square pyramidal geometry [262]. For a Cu(II) complex, $g_\parallel$ is a parameter sensitive enough to indicate covalence. For a covalent complex, $g_\parallel < 2.3$, and for ionic environment, $g_\parallel = 2.3$ or more. In the present complex $g_\parallel < 2.3$ indicates an appreciable metal–ligand covalent character. The exchange interaction parameter $G < 4$ (2.42), indicates considerable exchange interaction between the Cu(II) centers in the solid phase [263].

![Figure 68. X–band EPR spectrum of complex \([C_{22}H_{50}N_6O_{13}CuSnCl_2]\) at LNT.](image)

**UV–Vis spectra**

The electronic absorption spectra of ligand \([C_{14}H_{28}N_2O_{10}]\) and complexes \([C_{22}H_{52}N_6O_{13}Cu], [C_{22}H_{60}N_6O_{17}Ni], [C_{22}H_{50}N_6O_{13}CuSnCl_2]\) and \([C_{22}H_{58}N_6O_{17}NiSnCl_2]\) were recorded in DMSO solution at room temperature in the range of 200–1100 nm. The ligand displayed high–energy band at 233 nm in UV region corresponds to $\pi\rightarrow\pi^*$ transition. The electronic absorption spectra of complexes displayed intense absorption
bands at 244–247 nm followed by a shoulder at 292–296 nm attributed to the intraligand \( \pi-\pi^* \) transition and the ligand to metal charge transfer (LMCT) band. Besides this the electronic spectrum of complex \([C_{22}H_{52}N_6O_{13}Cu]\) displayed a low intensity broad band at 590 nm attributed to a d–d transition, typical for square pyramidal geometry around the Cu(II) metal ion [264]. Similarly, complex \([C_{22}H_{60}N_6O_{17}Ni]\) also exhibits a low energy metal centered d–d absorption band at 641 nm which was assigned to \(^3\text{B}_1(F)\rightarrow^3\text{E}(F)\) transition suggesting a pentacoordinate geometry around Ni(II) metal ion [265]. The heterobimetallic complexes \([C_{22}H_{50}N_6O_{13}CuSnCl_2]\) and \([C_{22}H_{58}N_6O_{17}NiSnCl_2]\) exhibited a broad and intense d–d band in the visible region with a maximum at 630 nm and 674 nm, respectively, consistent with the pentacoordinate geometry around Cu(II)/Ni(II) metal.

**DNA binding studies**

DNA is the primary pharmacological target of antitumor drugs and therefore, it is essential to explore the interactions of metal complexes with DNA for the development of effective chemotherapeutic agents. Particularly, metal complexes in a well–tailored ligand framework are responsible for specific shape and surface features that complement the molecular target site usually major/minor groove of the DNA double helix and metal complex–DNA interactions are therefore, of paramount importance. The mode and propensity of binding of the ligand and its metal complexes to CT DNA were examined by using absorption and emission spectra.

**Absorption titration with CT DNA**

The absorption spectra of ligand \([C_{14}H_{28}N_2O_{10}]\) and the complexes \([C_{22}H_{52}N_6O_{13}Cu]\), \([C_{22}H_{60}N_6O_{17}Ni]\), \([C_{22}H_{50}N_6O_{13}CuSnCl_2]\) and \([C_{22}H_{58}N_6O_{17}NiSnCl_2]\) exhibited intense
absorption bands around 233–249 nm, attributed to π–π* intraligand transition. Upon addition of incremental amount of CT–DNA (0–33.3 x 10⁻⁶ M) to a fixed concentration of Ligand/complex (6.67 x 10⁻⁶ M) resulting in “hyperchromism” at the intraligand absorption bands (Figure 69a–e).

**Figure 69.** Absorption spectral traces of (a) Ligand \([C_{14}H_{28}N_{2}O_{10}]\) (b) complex \([C_{22}H_{52}N_{6}O_{13}Cu]\) (c) complex \([C_{22}H_{60}N_{6}O_{17}Ni]\) (d) complex \([C_{22}H_{50}N_{6}O_{13}CuSnCl_{2}]\) (e) complex \([C_{22}H_{58}N_{6}O_{17}NiSnCl_{2}]\) in 5 %DMSO/ 5mM Tris HCl/ 50 mM NaCl buffer at pH 7.2 upon addition of CT DNA. Inset: Plots of \([\text{DNA}] / \varepsilon_{o} – \varepsilon_{f} (M^2 \text{ cm})\) vs \([\text{DNA}]\) for the titration of CT DNA with complexes ■, experimental data points; full lines, linear fitting of the data. \([\text{Complex}] 6.67 x 10^{-6} \text{M}, [\text{DNA}] 0–33.3 x 10^{-6} \text{M}.

**Table 5:** Change in spectral features of Ligand/complexes on interaction with CT–DNA in 5 mM Tris–HCl/50 mM NaCl buffer (pH 7.2).

<table>
<thead>
<tr>
<th>Ligand/complexes</th>
<th>(K_b) (M⁻¹)</th>
<th>Monitored at (nm)</th>
<th>% Hyperchromism</th>
</tr>
</thead>
<tbody>
<tr>
<td>([C_{14}H_{28}N_{2}O_{10}])</td>
<td>2.98 x 10⁴</td>
<td>233</td>
<td>44</td>
</tr>
<tr>
<td>([C_{22}H_{52}N_{6}O_{13}Cu])</td>
<td>6.20 x 10³</td>
<td>244</td>
<td>46</td>
</tr>
<tr>
<td>([C_{22}H_{60}N_{6}O_{17}Ni])</td>
<td>4.35 x 10³</td>
<td>246</td>
<td>48</td>
</tr>
<tr>
<td>([C_{22}H_{50}N_{6}O_{13}CuSnCl_{2}])</td>
<td>8.67 x 10¹</td>
<td>247</td>
<td>52</td>
</tr>
<tr>
<td>([C_{22}H_{58}N_{6}O_{17}NiSnCl_{2}])</td>
<td>4.00 x 10⁴</td>
<td>245</td>
<td>51</td>
</tr>
</tbody>
</table>

The “hyperchromic effect” was due to the dissociation of ligand aggregates or due to its external contact (electrostatic binding) with the duplex by engaging in hydrogen–bonding
interactions between coordinated –NH and –OH with functional groups positioned on the edge of DNA bases. Since, complexes are appended with the carbohydrate functionality which features novelty not only from the structural point of view but also it provides molecular recognition at the specific site; it drives the complexes towards the phosphate sugar backbone of DNA double helix [266]. Hyperchromic effect and hypochromic effects are the spectral features of DNA concerning its double helix structure. Hyperchromism results from the damage of DNA double helix structure while hypochromism results from contraction of DNA in the helix axis [267]. Furthermore, there was no change in the position of the absorption bands of the complexes in the presence of DNA, indicating the possibility of groove binding for all complexes to DNA. The intrinsic binding constants (\(K_b\)) were calculated (Table 5), which follows the order 

\[
[C_{22}H_{50}N_6O_{13}CuSnCl_2] > [C_{22}H_{58}N_6O_{17}NiSnCl_2] > [C_{22}H_{52}N_6O_{13}Cu] > [C_{22}H_{60}N_6O_{17}Ni] > [C_{14}H_{28}N_2O_{10}],
\]

consistent with our hypothesis that heterobimetallic complexes are more prominent DNA binders than the monometallic in comparison to the free ligand. The higher binding propensity of heterobimetallic \([C_{22}H_{50}N_6O_{13}CuSnCl_2]\) and \([C_{22}H_{58}N_6O_{17}NiSnCl_2]\) is due to the introduction of Sn cations in the monometallic core; tin ions have a hard Lewis acid which has preferential selectivity for phosphate sugar backbone of the DNA double helix [268–270], in addition to the presence of second metal ion Cu\(^{II}\)/Ni\(^{II}\) which prefers coordinate covalent binding to nucleobases of DNA helix.

**Ethidium bromide displacement assay**

In order to further investigate the interaction modes of the ligand/complexes with DNA, fluorimetric competitive binding experiment was carried out using ethidium bromide as a
probe. Ethidium bromide (EthBr) emits intense fluorescence in the presence of DNA due to its strong intercalation between the adjacent DNA base pairs [271]. This enhanced fluorescence can be quenched by the addition of a second DNA binding molecule, which can be used to monitor the mode of binding thereby indicating the ability of a molecule to prevent intercalation of EthBr to DNA [272]. On addition of ligand/complexes to CT DNA pretreated with EthBr ([DNA]/[EthBr] = 1), there was reduction in emission intensity (Figure. 70a–e), indicating that they competitively bound to DNA and displaces the EthBr.

Figure 70. Emission spectra of EB bound to DNA in the absence and presence of (a) Ligand [C₁₄H₂₈N₂O₁₀] (b) complex [C₂₂H₅₂N₆O₁₃Cu], (c) complex [C₂₂H₆₀N₆O₁₃Ni] (d) complex [C₂₂H₅₀N₆O₁₃CuSnCl₂] (e) complex [C₂₂H₅₈N₆O₁₇NiSnCl₂] in 5 mM Tris–HCl/50 mM NaCl buffer. Arrows show the intensity changes upon increasing concentration of the complexes.

The $K_{sv}$ values for ligand [C₁₄H₂₈N₂O₁₀] and complexes [C₂₂H₅₂N₆O₁₃Cu], [C₂₂H₆₀N₆O₁₃Ni], [C₂₂H₅₀N₆O₁₃CuSnCl₂] and [C₂₂H₅₈N₆O₁₇NiSnCl₂] were found to be
0.67, 1.82, 1.51, 2.66 and 2.31, respectively. The highest $K_{SV}$ value of complex $[C_{22}H_{50}N_6O_{13}CuSnCl_2]$ suggesting its stronger ability to displace EB from the EB–DNA system. Since EB was not completely displaced, the electrostatic mode of binding cannot be ruled out.

**Gel electrophoresis**

Since heterobimetallic complexes $[C_{22}H_{50}N_6O_{13}CuSnCl_2]$ shows maximum binding propensity with CT DNA, therefore the DNA cleavage activity of $[C_{22}H_{50}N_6O_{13}CuSnCl_2]$ was evaluated by agarose gel electrophoresis using supercoiled pBR322 plasmid DNA in a medium of 5 mM Tris–HCl/50 mM NaCl buffer solution (pH 7.2). The reaction mixture was subjected to agarose gel electrophoresis with increasing complex concentrations incubated at 310 K for 45 min.

**Figure 71.** Agarose gel electrophoresis diagram showing cleavage of pBR322 supercoiled DNA (300 ng) by complex $[C_{22}H_{50}N_6O_{13}CuSnCl_2]$ at 310 K after 45 min of incubation (a) at different concentration; Lane 1, DNA control; Lane 2, 10 μM+DNA; Lane 3: 15 μM+DNA; Lane 4: 20 μM+DNA; Lane 5: 25 μM+DNA; Lane 6: 30 μM+DNA. (b) in presence of reactive oxygen species at 310 K after incubation for 45 min. Lane 1: DNA Control; Lane 2: DNA+$[C_{22}H_{50}N_6O_{13}CuSnCl_2]$+DMSO (0.4 M); Lane 3: DNA+$[C_{22}H_{50}N_6O_{13}CuSnCl_2]$+ethyl alcohol (0.4 M); Lane 4: DNA+$[C_{22}H_{50}N_6O_{13}CuSnCl_2]$+sodium azide (0.4 M); Lane 5: 9 μM of $[C_{22}H_{50}N_6O_{13}CuSnCl_2]$+SOD (15 units)+DNA.

The activity of complex $[C_{22}H_{50}N_6O_{13}CuSnCl_2]$ was assessed by the conversion of DNA from Form I (Supercoiled Form) to Form II (Nicked Circular Form) and then to Form III (Linear Form). It was observed that both the complex $[C_{22}H_{50}N_6O_{13}CuSnCl_2]$ was found to exhibits nuclease activity at different concentration (10–30 μM), followed by
conversion of supercoiled DNA (Form I) into NC DNA (Form II) without concurrent formation of Form III, suggesting single strand DNA cleavage (Figure 71a).

In order to investigate the DNA cleavage mechanism (oxidative or hydrolytic) induced by complex \([C_{22}H_{50}N_6O_{13}CuSnCl_2]\), we have monitored the quenching of DNA cleavage in the presence of some standard radical scavengers like DMSO and ethyl alcohol as hydroxyl radical scavenger (HO\(^{•}\)), sodium azide (NaN\(_3\)) as singlet oxygen (\(^{1}\)O\(_2\)) quencher and superoxide dismutase as superoxide anion radical (O\(_2^{-}\)) scavenger (Figure 71b). The experiment results revealed that the DMSO and ethyl alcohol showed complete inhibition of nuclease activity (lane 2 and 3), indicating that the hydroxyl radicals is one of the reactive species involved in the DNA strand scission. Thus, OH free radicals participate in the oxidation of the deoxyribose moiety, followed by hydrolytic cleavage of the sugar phosphate backbone of DNA [273]. On the other hand, addition of NaN\(_3\) and SOD did not show any significant inhibition of the DNA strand scission, and even in presence of NaN\(_3\) the cleavage reaction is enhanced suggesting the non–involvement of the singlet oxygen radical and superoxide anion radical in the mechanistic pathway of DNA cleavage (lane 4 and 5).

**Figure 72.** Agarose gel electrophoresis for the ligation of pBR322 plasmid DNA nicked by complex \([C_{22}H_{50}N_6O_{13}CuSnCl_2]\) Lane 1: DNA control; Lane 2: pBR322 plasmid DNA cleaved by complex \([C_{22}H_{50}N_6O_{13}CuSnCl_2]\); Lane 3: ligation of nicked pBR322 plasmid DNA by T4 DNA ligase in presence of \([C_{22}H_{50}N_6O_{13}CuSnCl_2]\).
Since the complexes cleave DNA in the absence of any external agents, the mechanism occurred through a hydrolytic pathway [274]. Therefore, hydrolysis promoted by metal ions mainly depend on the Lewis acidity, which serves to activate the phospho-diester bonds towards nucleophilic attack via charge neutralization as well as metal–coordinated water molecules provide an inbuilt nucleophile, which attack the phosphate back bone of DNA double helix to give a five–coordinate phosphate intermediate and thereby leading to direct hydrolysis of the diester bonds [275]. To ascertain the hydrolytic nature of the DNA cleavage reaction mediated by complex \([C_{22}H_{50}N_6O_{13}CuSnCl_2]\) DNA religation experiment was performed in which supercoiled pBR322 DNA was treated with T4 ligase enzyme and subjected to gel electrophoresis [276]. The complex \([C_{22}H_{50}N_6O_{13}CuSnCl_2]\) yielded nicked DNA which was religated by using T4 DNA ligase enzyme; nicked form (Form II) was religated to a large extent in the presence of T4 ligase enzyme in comparison to control DNA alone in supercoiled form (Figure. 72).

**DNA cleavage in presence groove binding agents**

The potential interacting site of complex \([C_{22}H_{50}N_6O_{13}CuSnCl_2]\) with pBR322 DNA was determine in presence of minor groove binding agent, DAPI and the major groove binding agent, methyl green.

![Figure 73. Agarose Gel electrophoresis for the cleavage of pBR322 supercoiled DNA (300 ng) by complex \([C_{22}H_{50}N_6O_{13}CuSnCl_2]\) 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 \(\mu\)M of \([C_{22}H_{50}N_6O_{13}CuSnCl_2]\)+DNA+methyl green (2.5 \(\mu\)L of a 0.01mg/ml solution); Lane 3: 9 \(\mu\)M of \([C_{22}H_{50}N_6O_{13}CuSnCl_2]\)+DNA+DAPI (8 \(\mu\)M).](image-url)
When supercoiled pBR322 was treated with DAPI or methyl green prior to the addition of complex, the cleavage reaction mediated by [C$_{22}$H$_{50}$N$_{6}$O$_{13}$CuSnCl$_{2}$] was quenched in presence of DAPI while it enhances in the presence of MG indicating minor groove–binding preference of the complex [C$_{22}$H$_{50}$N$_{6}$O$_{13}$CuSnCl$_{2}$] (Figure. 73).

**Topoisomerase II inhibitory activity**

The enzyme–mediated supercoiled pBR322 DNA relaxation assay was used to investigate the effect of complex [C$_{22}$H$_{50}$N$_{6}$O$_{13}$CuSnCl$_{2}$] on the inhibitory activity of human–Topo–II by gel electrophoresis. When enzymatic activity was assayed supercoiled plasmid DNA converted to relaxed circular and then linear DNA. The presence of each type of DNA indicates a different behavior by the enzyme: relaxed DNA reveals that the enzyme's isomerase activity remains intact; supercoiled DNA suggests that the enzyme's action was inhibited; linear DNA reveals the formation of permanent double strand breaks during the catalytic cycle [277]. The effect of dose–dependent Topo–II inhibition assay of complex [C$_{22}$H$_{50}$N$_{6}$O$_{13}$CuSnCl$_{2}$] was observed in the relaxation assay using supercoiled pBR322 plasmid DNA in the presence of ATP (Figure. 74). It was observed that the treatment with 10 µM (Lane 3) of [C$_{22}$H$_{50}$N$_{6}$O$_{13}$CuSnCl$_{2}$] afforded partial inhibitory activity. As the concentration increases (20–40 µM), complex [C$_{22}$H$_{50}$N$_{6}$O$_{13}$CuSnCl$_{2}$] successively inhibited the DNA relaxation activity of Topo II. These observations suggest that complex [C$_{22}$H$_{50}$N$_{6}$O$_{13}$CuSnCl$_{2}$] was selective towards Topo–II inhibition via ATPase binding domain due to the presence of hydroxyl moiety which may anchor in the entrance of ATP–binding pocket resulting in increased binding affinity and reduced ATP competition, thereby leads to a higher Topo–II catalytic inhibitory activity. Thus, we conclude that [C$_{22}$H$_{50}$N$_{6}$O$_{13}$CuSnCl$_{2}$] was
catalytic inhibitor and not topoisomerase poisons because topoisomerase poisons give rise to permanent DNA cleavage by binding to the Topo–DNA complex covalently, stabilizing it and preventing the religation of the cleaved DNA by the Topo enzyme. On other hand, Topo–II catalytic inhibitors interact non–covalently with enzyme (e.g. substrate competition) to impede enzymatic activity during the catalytic cycle, thus, alter their normal functioning resulting in cell death.

![Figure 74. The cleavage patterns of the agarose gel electrophoresis diagram showing effect of different concentration of complex \([C_{22}H_{50}N_6O_{13}CuSnCl_2]\) on the activity of DNA Topo–II α (Topo–II, 5 units); Lane 1: DNA control; Lane 2: Topo–II control (Topo–II+DNA); Lane 3: 10 μM of \([C_{22}H_{50}N_6O_{13}CuSnCl_2]+DNA+Topo–II\); Lane 4: 20 μM of \([C_{22}H_{50}N_6O_{13}CuSnCl_2]+DNA+Topo–II\); Lane 5: 30 μM of \([C_{22}H_{50}N_6O_{13}CuSnCl_2]+DNA+Topo–II\); Lane 6: 40 μM of \([C_{22}H_{50}N_6O_{13}CuSnCl_2]+DNA+Topo–II\).

**Molecular docking with DNA**

Molecular docking technique is a well–documented computational tool to understand the Drug–DNA interactions for the development of rational drug design and to predict the exact binding site available at the molecular target DNA mainly in a non–covalent fashion, which can substantiate the spectroscopic results. Different structural properties lead to different binding modes; infact, one of the most important factors governing the binding mode is the molecular shape.

Herein, molecular docking studies of the ligand and complexes with DNA duplex of sequence \(d(CGCGAATTCGCG)_{12}\) dodecamer (PDB ID:1BNA) were performed in order to predict the proper binding site along with preferred orientation of the ligand.
[C_{14}H_{28}N_{2}O_{10}] molecules inside the DNA minor groove. The resulting docked pose (Figure. 75a–e), revealed that the ligand [C_{14}H_{28}N_{2}O_{10}] 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 attached to the complexes acts as strong H–bond donor or acceptor were engaged in hydrogen–bonding interactions with DNA nucleobases available in the minor grooves. Furthermore, the potential energies of complex–DNA–binding system after intercalation are much less than those of the system before intercalation, which may be attributed with electrostatic interaction and space matching. The total potential energy of complex–DNA binding system formed by complex intercalating into C5G6 region from minor groove is less than those of other systems, illustrating that the minor groove binding of the complex in C5G6 region is the most preferential binding interactions between the complex and DNA base pairs. Although a narrow minor groove is considered to enhance ligand–DNA interaction in the formed complex, it may not represent the most favorable geometry for the approximation and fit of the ligand [278].

Figure 75. Molecular docked model of (a) Ligand [C_{14}H_{28}N_{2}O_{10}] (b) complex [C_{23}H_{50}N_{6}O_{13}Cu] (c) complex [C_{22}H_{64}N_{6}O_{17}Ni] (d) complex [C_{23}H_{54}N_{6}O_{13}CuSnCl_{2}] (e) complex [C_{23}H_{64}N_{6}O_{17}NiSnCl_{2}] with DNA [dodecamer duplex of sequence d(CGCGAATTCGCG)_{2} (PDB ID: 1BNA)].
The resulting relative binding energy of docked ligand $[\text{C}_{14}\text{H}_{28}\text{N}_2\text{O}_{10}]$ and complexes $[\text{C}_{22}\text{H}_{52}\text{N}_6\text{O}_{13}\text{Cu}]$, $[\text{C}_{22}\text{H}_{60}\text{N}_6\text{O}_{17}\text{Ni}]$, $[\text{C}_{22}\text{H}_{50}\text{N}_6\text{O}_{13}\text{CuSnCl}_2]$ and $[\text{C}_{22}\text{H}_{58}\text{N}_6\text{O}_{17}\text{NiSnCl}_2]$ with DNA were found to be -204.6, -248.2, -227.9, -292.4 and -267.5 eV, respectively, correlating well with the experimental DNA binding studies and minor groove binder using DAPI assay. The larger negative value of the binding energy establishes the greater binding potential of the metal complexes with DNA.

**Molecular docking with topoisomerase**

To study the molecular basis of interaction and rationalize the observed enzymatic activity, molecular docking studies of complex $[\text{C}_{22}\text{H}_{50}\text{N}_6\text{O}_{13}\text{CuSnCl}_2]$ with the Topo–II was carried out using the genetic algorithm docking program HEX version 6.3. The complex $[\text{C}_{22}\text{H}_{50}\text{N}_6\text{O}_{13}\text{CuSnCl}_2]$ was docked separately into the DNA binding site (PDB ID: 2rgr) and N–terminal domain in ATP binding sites (PDB ID: 1zxm) of Topo–II.

![Figure 76. Molecular docked model of complex $[\text{C}_{22}\text{H}_{50}\text{N}_6\text{O}_{13}\text{CuSnCl}_2]$ into the human Topo II bound to DNA.](image)
The docked conformation into the DNA binding site of Topo–II revealed that complex [C\textsubscript{22}H\textsubscript{50}N\textsubscript{6}O\textsubscript{13}CuSnCl\textsubscript{2}] was located in the hydrophilic pocket of the enzyme and it is in close proximity to residues Asn 828, Gly 766 and Tyr 760 (Figure 76). Moreover, the hydroxyl group of sugar moiety was potentially involved in hydrogen bonding contact with the carboxylate of Asp 687.

Figure 77. (a) Molecular docked model of complex [C\textsubscript{22}H\textsubscript{50}N\textsubscript{6}O\textsubscript{13}CuSnCl\textsubscript{2}] in to the ATP binding pocket of human Topo IIα parallel to the Mg\textsuperscript{2+} ion. (b) The ATP–binding domain is represented as a molecular surface (purple), and complex [C\textsubscript{22}H\textsubscript{50}N\textsubscript{6}O\textsubscript{13}CuSnCl\textsubscript{2}] in stick representation. The green ball represents Mg\textsuperscript{2+} ion.

It also forms a water mediated hydrogen bond with Asn 769, which is considered an important amino acids that interact with the ligand in the DNA binding site of Topo II, subsequently leading to inhibitory effect on Topo II [279]. The presence of the hydroxyl group modifies the binding properties of the molecule to the DNA–binding domain of the Topo II enzyme and serves to stabilize the enzyme–DNA complex (the “cleavable complex”). The docking model of complex [C\textsubscript{22}H\textsubscript{50}N\textsubscript{6}O\textsubscript{13}CuSnCl\textsubscript{2}] with ATP–binding domain of human Topo IIα revealed that the sugar moiety of complex [C\textsubscript{22}H\textsubscript{50}N\textsubscript{6}O\textsubscript{13}CuSnCl\textsubscript{2}] was fitted into the middle of the cavity, and stabilized by strong
hydrophobic interactions with Asn91, Asn95, Lys123, Gly124 and Arg184 residues (Figure 77). Other hydrophobic residues such as Ile217, Ile118, Ile88 and Ala92, form a small subpocket at the bottom of the binding site filled with water molecules that establish a hydrogen bond network connecting the side chain of Asn120 to that of Asn91 [280]. On the opposite side, three residues, Ile125 together with Arg98 and Ser149, constitute the entrance of the cavity. Moreover, the hydroxyl group from the side chain forms hydrogen bonds with Ser149. These multiple interactions between complex [C$_{22}$H$_{50}$N$_6$O$_{13}$CuSnCl$_2$] and residues in the ATPase domain and the magnesium ion suggest that the metal complex can form a strong binding interaction with Topo II, preventing the entry of ATP. The resulting relative binding energy of docked complexes [C$_{22}$H$_{50}$N$_6$O$_{13}$CuSnCl$_2$] with DNA binding site of Topo II and ATP domain were found to be $-284.6$ and $-312.9$ eV, respectively, indicating the best results into the ATP pocket than for the DNA binding site.

**Antitumor activity assays**

The observations of the biological studies for complex [C$_{22}$H$_{50}$N$_6$O$_{13}$CuSnCl$_2$] viz. *in vitro* DNA binding studies, pBR322 plasmid DNA cleavage and Topo--II inhibition revealed remarkable potential of complex [C$_{22}$H$_{50}$N$_6$O$_{13}$CuSnCl$_2$] to act as cancer chemotherapeutic agents, therefore their *in vitro* cytotoxic activity was evaluated. The activity of complex [C$_{22}$H$_{50}$N$_6$O$_{13}$CuSnCl$_2$] was evaluated in terms of GI$_{50}$, TGI and LC$_{50}$ 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$_{50}$N$_6$O$_{13}$CuSnCl$_2$] was evaluated as a consequence of their binding affinity towards
Table 6: Summary of the screening data of complex \([\text{C}_{22}\text{H}_{50}\text{N}_6\text{O}_{13}\text{CuSnCl}_2]\) 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>
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<tr>
<td><strong>GI (_{50})</strong></td>
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<tr>
<td>([\text{C}<em>{22}\text{H}</em>{50}\text{N}<em>6\text{O}</em>{13}\text{CuSnCl}_2])</td>
<td>35.9</td>
<td>76.1</td>
<td>40.0</td>
<td>&gt;80</td>
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<td>53.4</td>
<td>&gt;80</td>
<td>25.0</td>
<td>14.7</td>
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<tr>
<td>([\text{C}<em>{22}\text{H}</em>{50}\text{N}<em>6\text{O}</em>{13}\text{CuSnCl}_2])</td>
<td>68.6</td>
<td>&gt;80</td>
<td>74.1</td>
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<td>46.8</td>
<td>&gt;80</td>
<td>&gt;80</td>
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<tr>
<td>ADR</td>
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<td>42.1</td>
<td>17.0</td>
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<td>20.4</td>
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<tr>
<td><strong>LC (_{50})</strong></td>
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<tr>
<td>([\text{C}<em>{22}\text{H}</em>{50}\text{N}<em>6\text{O}</em>{13}\text{CuSnCl}_2])</td>
<td>&gt;80</td>
<td>&gt;80</td>
<td>&gt;80</td>
<td>&gt;80</td>
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<td>&gt;80</td>
<td>&gt;80</td>
<td>62.6</td>
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<tr>
<td>ADR</td>
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<td>22.2</td>
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<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 % (GI\(_{50}\)) calculated from \([(\text{Tz}–\text{CTz})/(\text{C}–\text{CTz})] \times 100 = 50\), drug concentration result in a 50% reduction in the net protein increase.
- ADR= Adriamycin (taken as positive control compound).
- TGI = Tumor growth inhibition
- \(\text{LC}_{50}\) = Lethal concentration of 50 % (LC\(_{50}\))
DNA by applying microculture Sulforhodamine B test (SRB) [219]. The initial cytotoxic screening data (Table 6) shows that the \([\text{C}_{22}\text{H}_{50}\text{N}_6\text{O}_{13}\text{CuSnCl}_2]\) act as a potential selective anticancer agent with a significant GI\(_{50}\) values specifically towards MIAPACA2, A498 and HCT15 tumor cell lines.

**Conclusion**

In the present work, we have described the synthesis and characterization of cancer chemotherapeutic carbohydrate–conjugated heterobimetallic core \([\text{C}_{22}\text{H}_{50}\text{N}_6\text{O}_{13}\text{CuSnCl}_2]\) and \([\text{C}_{22}\text{H}_{58}\text{N}_6\text{O}_{17}\text{NiSnCl}_2]\) derived from their monometallic complexes \([\text{C}_{22}\text{H}_{52}\text{N}_6\text{O}_{13}\text{Cu}\)]\) and \([\text{C}_{22}\text{H}_{60}\text{N}_6\text{O}_{17}\text{Ni}\]). *In vitro* DNA–binding profile of ligand \([\text{C}_{14}\text{H}_{28}\text{N}_2\text{O}_{10}\)]\) and complexes \([\text{C}_{22}\text{H}_{52}\text{N}_6\text{O}_{13}\text{Cu}\]}, \([\text{C}_{22}\text{H}_{50}\text{N}_6\text{O}_{13}\text{CuSnCl}_2]\) and \([\text{C}_{22}\text{H}_{58}\text{N}_6\text{O}_{17}\text{NiSnCl}_2]\) were carried out by using various biophysical techniques which reveal strong electrostatic binding mode via phosphate backbone of DNA helix, in addition to selective binding to the minor groove of DNA. Complex \([\text{C}_{22}\text{H}_{50}\text{N}_6\text{O}_{13}\text{CuSnCl}_2]\) cleaves supercoiled plasmid pBR322 DNA via hydrolytic pathway (supported by DNA relegation assay employing T4 DNA ligase). The Topo–II inhibition activity of the complex \([\text{C}_{22}\text{H}_{50}\text{N}_6\text{O}_{13}\text{CuSnCl}_2]\) has been evaluated using pBR322 plasmid DNA cleavage assay which showed inhibitory effect at a concentration \(\sim 40 \mu\text{M}\). This work therefore, features the design of novel metal–based topoisomerase inhibitors, which are specific DNA groove binders. Furthermore, *in vitro* antitumor activity of \([\text{C}_{22}\text{H}_{50}\text{N}_6\text{O}_{13}\text{CuSnCl}_2]\) was evaluated by SRB assay, which exhibited moderate results against MIAPACA2 (human Pancreatic carcinoma), HCT15 (human Colon carcinoma), and A498 (human Renal carcinoma) cell lines.