Synthesis and characterization of new transition metal {Cu(II), Ni(II) and Co(II)} L-phenylalanine-DACH conjugate complexes: *In vitro* DNA binding, cleavage and molecular docking studies
Synthesis and Characterization

Synthesis of [C$_{15}$H$_{29}$N$_3$O$_4$CuCl$_2$]

The complex was prepared by a general synthetic method in which L-phenylalanine (0.165 g, 1 mmol) in 15 mL methanol was added to a methanolic solution (10 mL) of CuCl$_2$·2H$_2$O (0.170 g, 1 mmol) with stirring for ca. 12 h followed by the addition of the 1,2-diaminocyclohexane solution (0.121 mL, 1 mmol) and stirred for 4 h. On completion of the reaction, the reaction mixture was kept at room temperature, which yielded a light bluish product, washed with hexane and diethyl ether and dried in vacuo.

(Yield: 70%). M.p. 260 °C, Anal. Calc. for [C$_{15}$H$_{29}$N$_3$O$_4$CuCl$_2$] (%): C, 40.05; H, 6.50; N, 9.34, Found: C, 40.12; H, 6.53; N, 9.41. Selected IR data (KBr/nujol, v, cm$^{-1}$): 3389 ν(O–H)$_{\text{water}}$; 3031 ν(N–H)$_{\text{L-phe}}$; 1622 ν(C=O); 1575 ν$_{\text{as}}$(COO); 1325 ν$_{\text{s}}$(COO); 1325 ν(C–N); 841 δ(H$_2$O); 753 (Ar); 555 ν(C=O); 416 ν(Cu–N). Molar Conductance, $\Lambda_M$ (1×10$^{-3}$ M, DMSO): 150 Ω$^{-1}$cm$^2$mol$^{-1}$ (1:2 electrolyte). UV–vis absorption: (DMSO, 10$^{-4}$ M), $\lambda_{\text{max}}$/nm: 656, 264. ESI–MS (m/z$^+$, DMSO): 451.1 [C$_{15}$H$_{29}$N$_3$O$_4$CuCl$_2$+2H]$^+$. EPR (LNT, 3000G): $g_\parallel = 2.195$, $g_\perp = 2.05$, $g_{\text{av}} = 2.10$.

Synthesis of [C$_{15}$H$_{31}$N$_3$O$_5$NiCl$_2$]

The complex was synthesized by a similar procedure as described for complex [C$_{15}$H$_{29}$N$_3$O$_4$CuCl$_2$] with NiCl$_2$·6H$_2$O (0.237 g, 1 mmol). Light green coloured product was isolated.

(Yield: 65%). M.p. 280 °C, Anal. Calc. for [C$_{15}$H$_{31}$N$_3$O$_5$NiCl$_2$] (%): C, 38.91; H, 6.75; N, 9.08, Found: C, 38.83; H, 6.73; N, 8.99. Selected IR data (KBr/nujol, v, cm$^{-1}$): 3357 ν(O–H)$_{\text{water}}$; 3031 ν(N–H)$_{\text{L-phe}}$; 1635 ν(C=O); 1599 ν$_{\text{as}}$(COO); 1345 ν$_{\text{s}}$(COO); 1385 ν(C–N); 813 δ(H$_2$O); 750 (Ar); 551 ν(Ni–O); 418 ν(Ni–N). Molar Conductance, $\Lambda_M$ (1×10$^{-3}$ M, DMSO): 180 Ω$^{-1}$cm$^2$mol$^{-1}$ (1:2 electrolyte). UV–vis absorption: (DMSO, 10$^{-4}$ M), $\lambda_{\text{max}}$/nm: 632, 263. $^1$H NMR (DMSO–d$_6$, 400 MHz) δ(ppm): 17.12 (COOH); 7.90–6.99 (Ar–H); 3.27–2.49 (CH$_2$ + NH$_2$)$_{\text{L-phe}}$; 2.01–1.06 (–CH$_2$–)$_{\text{DACH}}$; $^{13}$CNMR (DMSO–d$_6$, 100 MHz) δ(ppm): 173.9 (O–C=O); 135.0–127.6 (Ar–C); 36.28–22.87 (–CH$_2$). ESI–MS (m/z, DMSO): 463.1 [C$_{15}$H$_{31}$N$_3$O$_5$NiCl$_2$]$^+$. 

Synthesis of [C$_{15}$H$_{29}$N$_3$O$_4$CoCl$_2$]

The complex was synthesized by a similar procedure as described for monometallic complex [C$_{15}$H$_{29}$N$_3$O$_4$CuCl$_2$] with CoCl$_2$·6H$_2$O (0.237 g, 1 mmol). Dark purple coloured product was isolated.
(Yield: 75%). M.p. 240 °C, Anal. Calc. for [C_{15}H_{29}N_{5}O_{4}CoCl_{2}] (%): C, 40.46; H, 6.56; N, 9.44. Found: C, 40.42; H, 6.61; N, 9.46. Selected IR data (KBr/nujol, ν, cm⁻¹): 3383 ν(O–H)water; 3019 ν(N–H)L-phen; 1637 ν(C=O); 1586 νas(COO); 1337 νs(COO); 1388 ν(C–N); 846 δ(H₂O); 751 (Ar); 365 ν(Co–O); 439 ν(Co–N). Molar Conductance, muş (1×10⁻³ M, DMSO): 200 Ω⁻¹cm²mol⁻¹ (1:2 electrolyte). Uv–vis absorption: (DMSO, 10⁻⁴ M), λmax/nm: 525, 258. ESI–MS (m/z, DMSO): 446.1 [(C_{15}H_{29}N_{5}O_{4}CoCl_{2}+ H)]⁺.

Results and discussion

New transition metal–based {Cu(II), Ni(II) and Co(II)} molecular complexes [C_{15}H_{29}N_{5}O_{4}CuCl_{2}], [C_{15}H_{31}N_{5}O_{5}NiCl_{2}] and [C_{15}H_{29}N_{5}O_{4}CoCl_{2}] derived from amino acid auxiliary ligand viz., L–phenylalanine and 1,2–diaminocyclohexane were synthesized (Scheme 1) and thoroughly characterized by using elemental analysis, IR, UV–vis, NMR, EPR spectral studies and XRPD. The complexes were stable towards air and moisture and readily soluble in DMSO and H₂O. The ESI–MS studies confirmed the formula proposed for [C_{15}H_{29}N_{5}O_{4}CuCl_{2}], [C_{15}H_{31}N_{5}O_{5}NiCl_{2}] and [C_{15}H_{29}N_{5}O_{4}CoCl_{2}] complexes. The molar conductance values of complexes in H₂O (150–200 Ω⁻¹ cm⁻³ mol⁻¹) suggested their 1:2 electrolytic nature.

\[
\text{L-Phenylalanine} + \text{1,2-DACH} \rightarrow \text{MeOH} \quad \text{Stirring, 12h}
\]

\[
\begin{align*}
\text{MeOH} & \quad \text{Stirring, 4h} \\
\text{CoCl}_2·2\text{H}_2\text{O} & \quad \text{NiCl}_2·6\text{H}_2\text{O} \\
\text{CoCl}_2·6\text{H}_2\text{O} & \quad \text{NiCl}_2·6\text{H}_2\text{O} \\
\text{CuCl}_2·2\text{H}_2\text{O} & \quad \text{NiCl}_2·6\text{H}_2\text{O} \\
\end{align*}
\]

Scheme 1. Synthesis scheme of complexes [C_{15}H_{29}N_{5}O_{4}CuCl_{2}], [C_{15}H_{31}N_{5}O_{5}NiCl_{2}] and [C_{15}H_{29}N_{5}O_{4}CoCl_{2}].
Infrared spectra
The IR spectra of complexes \([C_{15}H_{29}N_3O_4CuCl_2]\), \([C_{15}H_{31}N_3O_3NiCl_2]\) and \([C_{15}H_{29}N_3O_4CoCl_2]\) exhibited characteristic broad envelope at ca. 3031–3019 cm\(^{-1}\) corresponding to the coordinated amino group of L-phenylalanine and 1,2-diaminocyclohexane, which shifted from 3041 to 3400 cm\(^{-1}\), suggesting the coordination of -NH\(_2\) group to the metal ions [158]. The \(\Delta \nu = [\nu_{as}(CO_2) - \nu_s(CO_2)]\) value was used to determine the nature of binding of carboxylate to transition metal ion. In general, the difference in \(\Delta \nu\) between asymmetric \(\nu_{as}(CO_2)\) and symmetric \(\nu_s(CO_2)\) absorption frequencies below 200 cm\(^{-1}\) suggests the bidentate carboxylate moiety, while greater than 200 cm\(^{-1}\) implicates the unidentate carboxylation. In all cases, the \(\Delta \nu\) values were above 245 cm\(^{-1}\) suggestive of coordination of the carboxylate group in a monodentate fashion [159]. Further, the presence of v(O–H) stretching vibrations and the rocking mode of water at \(~3389–3357\) cm\(^{-1}\) and \(~846–813\) cm\(^{-1}\), respectively, suggested the presence of the coordinated H\(_2\)O in these complexes. Moreover, the formation of complexes \([C_{15}H_{29}N_3O_4CuCl_2]\), \([C_{15}H_{31}N_3O_3NiCl_2]\) and \([C_{15}H_{29}N_3O_4CoCl_2]\) were also revealed by the presence of medium intensity v(M–N) and v(M–O) bands at ca. 420 and 555 cm\(^{-1}\), respectively in far IR region.

NMR spectral studies
The \(^1\)H and \(^{13}\)C NMR spectra of complex \([C_{15}H_{31}N_3O_3NiCl_2]\) in deuterated DMSO exhibited well resolved signals with significant chemical shifts. As a typical spectroscopic feature, the acidic carboxylic proton (COOH) of \([C_{15}H_{31}N_3O_3NiCl_2]\) appeared as a broad downfield shifted singlet at 17.12 ppm suggesting the participation of carboxylic group in the coordination to Ni(II) without deprotonation. Peaks with chemical shift values at \(\delta 7.90–6.99\) ppm were ascribed to the aromatic protons of “L-phenylalanine”. While the presence of superimposed multiplet peaks in the range \(\delta 3.27–2.49\) ppm, were assigned to the protons of -NH\(_2\) and -CH\(_2\) of L-phenylalanine functionality. Further, the ring protons of the 1,2-diaminocyclohexane moiety were monitored in the range of \(\delta 2.01–1.06\) ppm (Figure 30) [160].
Figure 30. \(^1\)H NMR spectra of complex \([C_{15}H_{31}N_3O_3NiCl_2]\).

The \(^{13}\)C NMR spectrum of \([C_{15}H_{31}N_3O_3NiCl_2]\) was characterized by various resonances due to O–C=O and Ar–C carbons at \(\delta\) 173.9 and 135.0–127.6 ppm, respectively of the coordinated L-phenylalanine. Additionally, the spectra also revealed aliphatic carbons at 36.28–22.07 ppm confirming the presence of 1, 2-diaminocyclohexane moiety in the complex (Figure 31) [161].

Figure 31. \(^{13}\)C NMR spectra of complex \([C_{15}H_{31}N_3O_3NiCl_2]\).
Mass spectroscopy
The formation of metal complexes and the speciation of various ionic forms in DMSO solution were studied with ESI–MS. The spectra of the complexes displayed prominent peaks corresponding to the molecular ion fragment. The complex [C_{13}H_{29}N_3O_4CuCl_2] revealed major peak at m/z$^+$ 451.1 corresponding to the fragment [C_{13}H_{29}N_3O_4CuCl_2+2H]$^+$. While the complex [C_{13}H_{31}N_3O_4NiCl_2] exhibited peaks at m/z$^+$ 463.1 and 409.1 corresponding to the fragments [C_{13}H_{31}N_3O_5NiCl_2]$^+$ and [C_{13}H_{31}N_3O_5NiCl_2–3H_2O+H]$^+$, respectively. Similarly, complex [C_{13}H_{29}N_3O_4CoCl_2] showed fragmentation peaks at m/z$^+$ 446.1 and 338.1 ascribed to the [(C_{13}H_{29}N_3O_4CoCl_2+H)]$^+$ and [C_{13}H_{29}N_3O_4CoCl_2–2Cl_2+2H_2O]$^+$ fragments, respectively.

Electronic absorption spectra
The UV–visible spectra of complexes [C_{13}H_{29}N_3O_4CuCl_2], [C_{13}H_{31}N_3O_5NiCl_2] and [C_{13}H_{29}N_3O_4CoCl_2] at room temperature were carried out in the region 190–1100 nm in DMSO. The spectra similarity suggests that all the complexes present the same chromophore in solution. The complex [C_{13}H_{29}N_3O_4CuCl_2], revealed d–d transitions in the lower frequency region centered at 656 nm and a band in UV region at 264 nm consistent with their octahedral geometry. The UV–vis spectra of complex [C_{13}H_{31}N_3O_5NiCl_2] revealed prominent band at 632 nm attributed to d$_{xz}$, d$_{yz}$→d$_{x^2-y^2}$ ligand field transitions and a strong band in the UV region at 263, assignable to ligand to metal charge transfer (LMCT). These observations were typical of square planar geometry around Ni(II) ion. The electronic spectra of complex [C_{13}H_{29}N_3O_4CoCl_2] exhibited d–d transitions at ~525 nm and a ligand–based transitions at 258 nm in UV region consistent with octahedral environment around the Co(II) ion [162].

X–ray diffraction analysis
To obtain further evidence about the structure of the metal complexes, X–ray powder diffraction studies of the complexes were performed as it was difficult to isolate single crystals suitable for single crystal X–ray crystallography. The XRPD pattern obtained for the metal complexes [C_{13}H_{29}N_3O_4CuCl_2], [C_{13}H_{31}N_3O_5NiCl_2] and [C_{13}H_{29}N_3O_4CoCl_2] revealed well defined crystalline peaks indicating that the complexes is crystalline in phase due to inherent crystalline nature.
The X-ray diffractogram of the complex [C$_{15}$H$_{29}$N$_3$O$_4$CuCl$_2$], exhibited reflecting peaks at 2θ scattering angles of 22.96, 45.36, 48.10, 54.96, 54.87, 57.01 and 64.64 assigned to (023), (410), (402), (082), (443), (415) and (551) crystal planes respectively, characteristic of a well ordered monoclinic arrangement of Cu(II) atoms (JCPDF: No.04–0838) (Figure 32(a)). The XRPD pattern of complex [C$_{15}$H$_{21}$N$_3$O$_5$NiCl$_2$] as depicted in Figure 32(b) reveals peaks at 2θ scattering angles of 21.40, 23.19, 27.42, 37.27 and 42.86 assigned to (400) (331), (432), (402) and (544) crystal planes, respectively characteristic of a well ordered monoclinic arrangement of Ni atoms.

![Figure 32. X-ray diffraction pattern of complexes (a) [C$_{15}$H$_{29}$N$_3$O$_4$CuCl$_2$], (b) [C$_{15}$H$_{21}$N$_3$O$_5$NiCl$_2$] and (c) [C$_{15}$H$_{29}$N$_3$O$_4$CoCl$_2$].](image)

Similarly, the XRD pattern of the complex [C$_{15}$H$_{29}$N$_3$O$_4$CoCl$_2$], exhibited peaks at 2θ scattering angles of 23.83, 26.84, 38.63, 46.66, 48.84 and 52.40 assigned to (104), (007), (112), (117), (212) and (209) crystal planes, respectively which revealed that structure is same as that of bulk (JCPDF No. 27–1029) (Figure 32(c)). These parameters further indicated the hexagonal crystal structure arrangement of Co atoms. A summary of the refined XRPD parameters is given in Table 1.
Table 1. Summary of the XRPD data and the refinement parameters for complexes [C₁₅H₂₉N₃O₄CuCl₂], [C₁₅H₃₁N₃O₃NiCl₂] and [C₁₅H₂₉N₃O₄CoCl₂].

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DNA binding Studies

Electronic spectral studies

Electronic absorption spectroscopy is one of the most convenient tools to examine the binding mode of CT DNA with metal complexes. The interaction between the complexes and DNA is expected to perturb the ligand centered transitions of complex.

In the UV region, the complexes [C₁₅H₂₉N₃O₄CuCl₂], [C₁₅H₃₁N₃O₃NiCl₂] and [C₁₅H₂₉N₃O₄CoCl₂] exhibit intense absorption bands at ~264 nm attributed to π→π* intraligand transition. Upon addition of CT DNA, complexes exhibited an increase in molar absorptivity (Δε), (hyperchromism; 45–54%, {Figure 33(a–c)}) without significant shifts in band position. The observed "hyperchromism" suggested that the binding of complexes to CT DNA could be attributed to electrostatic interaction with the exterior phosphates of DNA duplex [163]. There was negligible shift in band position of complexes suggesting the involvement of the complexes in DNA groove binding through hydrophobic interaction of the aromatic functionality of L-phenylalanine.

To quantify the binding strength of the complexes with DNA, the intrinsic binding constant, $K_b$ was determined using Wolfe–Shimer equation (2). The intrinsic binding constant $K_b$ values obtained for complexes were found to be $5.30 \times 10^3$, $3.41 \times 10^4$ and
2.74 × 10^4 M⁻¹, respectively revealing higher binding propensity of complex [C_{12}H_{28}N_5O_4CuCl_2] and as compared to [C_{15}H_{31}N_5O_3NiCl_2] and [C_{15}H_{29}N_5O_4CoCl_2]. The Cu(II) complex exhibited higher DNA binding affinity than the analogous Ni(II) and Co(II) complexes, which could be expected due to the smaller size of Cu(II) complex which fits well into the DNA grooves than the bulkier Ni(II) or Co(II) cations, a typical variation in stabilities of transition metal complexes [164].

![Absorption spectral traces of complexes](image)

**Figure 33.** Absorption spectral traces of complexes (a) [C_{12}H_{28}N_5O_4CuCl_2] (b) [C_{15}H_{31}N_5O_3NiCl_2] and (c) [C_{15}H_{29}N_5O_4CoCl_2] in 5mM Tris HCl / 50 mM NaCl buffer at pH 7.2 upon addition of CT DNA. Inset: Plots of [DNA]/ [DNA] vs [DNA] for the titration of CT DNA with complexes ■ experimental data points; full lines, linear fitting of the data. [Complex] 1.00 × 10^{-4} M, [DNA] 0.00–0.23 × 10^{-4} M.

However, tethering an amino acid moiety to the metal complex introduces chirality which enhances the pharmacological behavior of the complex by adopting a specific conformation and may also confer selective binding affinity for the chiral DNA. Nevertheless, as the DNA double helix possesses many hydrogen-bonding sites positioned on the edges of the DNA bases, it is quite possible that the amine groups of 1,2-diaminocyclohexane (DACH) functionality could participate in hydrogen bonding with the DNA base pairs, which further define the complex–DNA stability.
Interaction studies of complex \([C_{13}H_{29}N_3O_4CuCl_2]\) with 5'-GMP

To obtain concrete information and to determine the coordination of the metal ion to the specific site, interaction with low molecular building blocks of large DNA molecules viz., guanosine 5'-monophosphate becomes mandatory. Cu(II) complexes, exhibits specificity towards guanine nucleotide of DNA. On addition of 5'-GMP to the complex \([C_{13}H_{29}N_3O_4CuCl_2]\), there was a sharp increase "hyperchromic" effect in the absorption bands at 264 nm (Figure 34). This observation can be corroborated with the results of absorption spectral studies and demonstrate the specific recognition of copper(II) complex, \([C_{13}H_{29}N_3O_4CuCl_2]\) for guanine.

![Graph showing absorption bands](image)

**Figure 34.** Variation of UV–vis absorption for complex \([C_{13}H_{29}N_3O_4CuCl_2]\) with increase in the concentration of 5'-GMP (0.067 × 10⁻⁴ – 0.33 × 10⁻⁴ M) in buffer (5mM Tris–HCl/50 mM NaCl, pH = 7.2) at room temperature. Inset: plot of [5'-GMP]/\(ε_{a-e1}\) vs [5'-GMP] for the titration of 5'-GMP.

**Fluorescence spectral studies**

Fluorescence spectral technique is an effective method to study metal interaction with DNA. In the absence of CT DNA, complexes \([C_{13}H_{29}N_3O_4CuCl_2]\), \([C_{13}H_{31}N_3O_5NiCl_2]\) and \([C_{13}H_{29}N_3O_4CoCl_2]\) exhibited luminescence at ~380 nm in Tris–HCl buffer at physiological pH, when excited at 260 nm. Upon addition of increasing concentration of CT DNA over a range 0.00–2.33 × 10⁻⁵ M, to the fixed amounts of complexes (1 × 10⁻⁵ M), the emission intensity of \([C_{13}H_{29}N_3O_4CuCl_2]\), \([C_{13}H_{31}N_3O_5NiCl_2]\) and \([C_{13}H_{29}N_3O_4CoCl_2]\) increased appreciably with no apparent change in the shape and position of the emission bands (Figure 35(a)-(e)). This implies that the complex strongly interacts with CT DNA, probably due to the inaccessibility of the solvent water molecules to the hydrophobic environment inside
the DNA helix, and the mobility of the complexes is restricted at the binding site, ultimately leading to a decrease in the vibrational mode of relaxation [165]. The affinity constant, \( K \) estimated for the complexes \([\text{C}_{13}\text{H}_{29}\text{N}_3\text{O}_4\text{CuCl}_2] \), \([\text{C}_{13}\text{H}_{31}\text{N}_3\text{O}_5\text{NiCl}_2] \) and \([\text{C}_{13}\text{H}_{29}\text{N}_3\text{O}_4\text{CoCl}_2] \) by the Scatchard equation were found to be \( 4.4 \times 10^4 \), \( 3.4 \times 10^4 \) and \( 2.21 \times 10^5 \text{M}^{-1} \), respectively [166].

**Figure 35.** Emission enhancement spectra of the complexes (a) \([\text{C}_{13}\text{H}_{29}\text{N}_3\text{O}_4\text{CuCl}_2] \), (b) \([\text{C}_{13}\text{H}_{31}\text{N}_3\text{O}_5\text{NiCl}_2] \) and (c) \([\text{C}_{13}\text{H}_{29}\text{N}_3\text{O}_4\text{CoCl}_2] \) in presence of CT DNA. Arrow indicates the change in the intensity upon increasing DNA concentration.

**Effect of \( K_4[\text{Fe(CN)}_6] \)**

To demonstrate the interaction strength of the complexes \([\text{C}_{13}\text{H}_{29}\text{N}_3\text{O}_4\text{CuCl}_2] \), \([\text{C}_{13}\text{H}_{31}\text{N}_3\text{O}_5\text{NiCl}_2] \) and \([\text{C}_{13}\text{H}_{29}\text{N}_3\text{O}_4\text{CoCl}_2] \) with CT DNA, steady state emission quenching experiments using \([\text{Fe(CN)}_6]^{3-} \) as quencher were also performed [167]. The quenching efficiency for complexes was evaluated by the classical Stern–Volmer equation (4). The emission intensity of complexes in the absence of DNA was lower than that in the presence of CT DNA under the same concentration of \( K_4[\text{Fe(CN)}_6] \) thereby, reflecting that complexes were protected by the DNA helix. Stern–Volmer plots with anionic quencher for complexes \([\text{C}_{13}\text{H}_{29}\text{N}_3\text{O}_4\text{CuCl}_2] \), \([\text{C}_{13}\text{H}_{31}\text{N}_3\text{O}_5\text{NiCl}_2] \) and \([\text{C}_{13}\text{H}_{29}\text{N}_3\text{O}_4\text{CoCl}_2] \) in the absence and presence of CT DNA are illustrated in **Figure 36** (a–c). The decrease in emission intensity of the complexes was due to the
repulsion of highly negative charged $[\text{Fe(CN)}_6]^{4-}$ from the DNA polyanion backbone, which hinders access of $[\text{Fe(CN)}_6]^{4-}$ to the DNA–bound complexes [168]. The $K_{sv}$ values of complexes $[\text{C}_{13}\text{H}_{29}\text{N}_3\text{O}_4\text{CuCl}_2]$, $[\text{C}_{13}\text{H}_{31}\text{N}_3\text{O}_3\text{NiCl}_2]$ and $[\text{C}_{15}\text{H}_{29}\text{N}_3\text{O}_4\text{CoCl}_2]$ in absence of DNA were found to be $4.8 \times 10^4$, $3.1 \times 10^4$ and $1.3 \times 10^4 \text{ M}^{-1}$, respectively. In the presence of DNA the quenching curve was depressed reflecting the protection of complexes by the DNA helix and the $K_{sv}$ values of complexes $[\text{C}_{13}\text{H}_{29}\text{N}_3\text{O}_4\text{CuCl}_2]$, $[\text{C}_{13}\text{H}_{31}\text{N}_3\text{O}_3\text{NiCl}_2]$ and $[\text{C}_{15}\text{H}_{29}\text{N}_3\text{O}_4\text{CoCl}_2]$ decreased to $9.7 \times 10^4$, $3.7 \times 10^4$ and $1.63 \times 10^4 \text{ M}^{-1}$, respectively. The greater decrease in $K_{sv}$ value for complex $[\text{C}_{15}\text{H}_{29}\text{N}_3\text{O}_4\text{CuCl}_2]$ indicated its higher DNA binding ability as compared to $[\text{C}_{13}\text{H}_{31}\text{N}_3\text{O}_3\text{NiCl}_2]$ and $[\text{C}_{13}\text{H}_{29}\text{N}_3\text{O}_4\text{CoCl}_2]$.

**Figure 36.** Stern–Volmer quenching plots of complexes (a) $[\text{C}_{13}\text{H}_{29}\text{N}_3\text{O}_4\text{CuCl}_2]$, (b) $[\text{C}_{13}\text{H}_{31}\text{N}_3\text{O}_3\text{NiCl}_2]$ and (c) $[\text{C}_{15}\text{H}_{29}\text{N}_3\text{O}_4\text{CoCl}_2]$ in absence (—) and presence (—) of CT DNA.

**Circular dichroic studies**

Circular dichroism (CD) is a powerful, sensitive and sophisticated tool for identifying conformational changes of DNA as upon change in conformation of DNA from B–form to A–form. The double helical right–handed B–DNA is characterized by a positive band at 275 nm and a negative band around 245 nm while A–DNA is α–helical double helix (right–handed) fairly similar to the B–DNA form, is characterized by a positive band at 260 nm and a negative band at 210 nm in CD spectrum. Simple groove binding and electrostatic interaction of the complexes with
DNA show less or no perturbations on the base stacking and helicity bands, while intercalation can stabilize the helix conformation of B-DNA, and enhances the intensities of both the CD bands [169]. The observed CD spectrum of CT DNA consists of a positive band at 275 nm due to base stacking and a negative band at 245 nm due to helicity, characteristic of DNA in right handed canonical B-form. On incubation with complexes [C_{15}H_{29}N_3O_4CuCl_2], [C_{15}H_{31}N_3O_3NiCl_2] and [C_{15}H_{29}N_3O_4CoCl_2] the signatures corresponding to B-DNA were perturbed with marginal changes both in positive and negative (ellipticity and helicity) bands retaining the basic shape (Figure 37). Thus, observed decrease in intensities in both the positive and negative bands without any shifts suggested that the complexes induce distortion of the secondary structure of B-DNA without the alteration in its conformation. The CD spectral changes clearly rules out the intercalative mode of binding and also demonstrate non-covalent interaction between the complexes and DNA, probably via electrostatic mode.

![Figure 37. CD spectra of CT DNA alone (a), in the presence of [C_{15}H_{29}N_3O_4CuCl_2], in the presence of [C_{15}H_{31}N_3O_3NiCl_2] (b) and in the presence of [C_{15}H_{29}N_3O_4CoCl_2] (d). [Complex] = 1×10^{-4} M, [DNA] 1×10^{-4} M.](image)

**Figure 37.** CD spectra of CT DNA alone (a), in the presence of [C_{15}H_{29}N_3O_4CuCl_2], in the presence of [C_{15}H_{31}N_3O_3NiCl_2] (b) and in the presence of [C_{15}H_{29}N_3O_4CoCl_2] (d). [Complex] = 1×10^{-4} M, [DNA] 1×10^{-4} M.

**Thermal denaturation study**

The binding of the complexes to CT DNA was studied by measuring changes in the melting temperature (T_m) of DNA that characterizes the transition from a double-stranded (ds) DNA to a single-stranded one. When ds-DNA is heated slowly, it gradually dissociates to single strands, which generates a hyperchromic effect on the absorption spectra (λ_{max} = 260 nm). In general, groove binding or electrostatic
interaction gives rise to only a small change in thermal denaturation temperature, while intercalation leads to a significant rise in thermal denaturation temperature of DNA due to the stabilization of the Watson–Crick base-paired duplex. Therefore, thermal denaturation experiment of DNA provides a convenient tool for detecting binding and also assessing relative binding strengths. In the present work, the melting curves of CT DNA in the absence and the presence of complexes $[\text{C}_{15}\text{H}_{29}\text{N}_{3}\text{O}_{4}\text{CuCl}_{2}]$, $[\text{C}_{15}\text{H}_{31}\text{N}_{3}\text{O}_{3}\text{NiCl}_{2}]$ and $[\text{C}_{15}\text{H}_{29}\text{N}_{3}\text{O}_{4}\text{CoCl}_{2}]$ are presented in (Figure 38). The $\Delta T_m$ values in the range of 2–3 °C for complexes $[\text{C}_{15}\text{H}_{29}\text{N}_{3}\text{O}_{4}\text{CuCl}_{2}]$, $[\text{C}_{15}\text{H}_{31}\text{N}_{3}\text{O}_{3}\text{NiCl}_{2}]$ and $[\text{C}_{15}\text{H}_{29}\text{N}_{3}\text{O}_{4}\text{CoCl}_{2}]$, indicate the stabilization of DNA double helix is due to the interaction of complexes with CT DNA. The moderate increase in the melting temperature of CT DNA primarily suggests the non-intercalative binding behavior of the complex [170].

![Figure 38. Thermal melting curves of CT DNA (pink), DNA + complex $[\text{C}_{15}\text{H}_{29}\text{N}_{3}\text{O}_{4}\text{CuCl}_{2}]$ (blue) an DNA+ complex $[\text{C}_{15}\text{H}_{31}\text{N}_{3}\text{O}_{3}\text{NiCl}_{2}]$ (green) and DNA+ complex $[\text{C}_{15}\text{H}_{29}\text{N}_{3}\text{O}_{4}\text{CoCl}_{2}]$ (Dark purple).](image)

**DNA cleavage activity**

The characteristic agarose gel pattern of plasmid DNA consists of two distinguishable conformations, one corresponding to the supercoiled DNA SC (Form I), the nicked-circular DNA NC (Form II). The nicked form of plasmid DNA is generated due to the cleavage of one of the DNA strands. Moreover, if both the strands are cleaved, a slow moving linear form LC (Form III) generates between SC (Form I) and NC (Form II) [171].

**In presence of increasing concentration**

Since, complex $[\text{C}_{15}\text{H}_{29}\text{N}_{3}\text{O}_{4}\text{CuCl}_{2}]$ exhibited greater binding propensity to CT DNA, the cleavage activity of $[\text{C}_{15}\text{H}_{29}\text{N}_{3}\text{O}_{4}\text{CuCl}_{2}]$ was studied using supercoiled plasmid
pBR322 DNA as a substrate in a medium of 5 mM Tris–HCl/50 mM NaCl buffer (pH 7.2) under physiological conditions. A concentration dependent DNA cleavage by \([C_{15}H_{29}N_3O_4CuCl_2]\) was performed at increasing complex concentrations (5–30 \(\mu\)M) exhibiting significant cleavage at 30 \(\mu\)M with the formation of Form III, suggesting efficient double strand DNA cleavage (shown in Lane 2–7, Figure 39). It is well documented that double strand breaks in duplex DNA are more significant sources of cell lethality in comparison to single strand breaks, because they appear to be less readily repaired by DNA repair mechanisms [172]. Thus, results are of importance towards designing and developing new metal–based DNA cleaving agents that are capable of exhibiting double strand break of DNA.

Figure 39. The cleavage patterns of the agarose gel electrophoresis diagram showing cleavage of pBR322 supercoiled DNA (300 ng) by complex \([C_{15}H_{29}N_3O_4CuCl_2]\) at 25 \(^\circ\)C after 45 minutes of incubation. Lane 1, DNA control; Lane 2, 5 \(\mu\)M of complex + DNA; Lane 3, 10 \(\mu\)M of complex + DNA; Lane 4, 15 \(\mu\)M of complex + DNA; Lane 5, 20 \(\mu\)M of complex; Lane 6, 25 \(\mu\)M of complex; Lane 7, 30 \(\mu\)M of complex. With the increase of concentration of complex was converted from Form I (SC) to Form II (NC) and then to Form III (LC).

In presence of activators

The nuclease efficiency of the metal complex at cellular level markedly depends on the activators to produce DNA strand scission. Thereof, DNA cleavage activity of complex \([C_{15}H_{29}N_3O_4CuCl_2]\) at a concentration of 30 \(\mu\)M was evaluated in the presence of activators such as ascorbate (Asc), 3-mercaptopropionic acid (MPA), \(H_2O_2\) and glutathione (GSH) (Figure 40, Lanes 2–5). The cleavage activity was significantly enhanced by these activators following the order: \(H_2O_2\rangle MPA \rangle Asc \rangle GSH\), suggesting that \(H_2O_2\) plays a key role to aid the copper(II) complex in DNA degradation by oxidative cleavage.
Figure 40. Agarose gel electrophoresis pattern for the cleavage pattern of pBR322 plasmid DNA (300 ng) by complex [C_{15}H_{29}N_{3}O_{4}CuCl_{2}] (0.20 mmol) in the presence of different activating agents at 37 °C after incubation for 45 min: Lane 1, DNA control; Lane 2, DNA + complex + H_{2}O_{2} (0.4 μM); Lane 3, DNA + complex + MPA (0.4 μM); Lane 4, DNA + complex + Asc (0.4 μM); Lane 5, DNA + complex + GSH (0.4 μM); Lane 6, DNA + complex + DMSO (0.4 μM); Lane 7, DNA + complex + tert-butyl alcohol (0.4 μM); Lane 8, DNA + complex + NaN_{3} (0.4 μM); Lane 9, DNA + complex + SOD (15 units).

Effect of reactive oxygen species on DNA cleavage

The involvement of ROS (reactive oxygen species) (hydroxyl, superoxide, singlet oxygen–like species, hydrogen peroxide) in the nuclease mechanism can be inferred by monitoring the quenching of the DNA cleavage in the presence of ROS scavengers. The interaction between metal complexes and dioxygen or redox reagents are believed to be a major cause of DNA damage. To probe the potential mechanism of DNA cleavage mediated by complex [C_{15}H_{29}N_{3}O_{4}CuCl_{2}], some standard radical scavengers were used prior to the addition of [C_{15}H_{29}N_{3}O_{4}CuCl_{2}] to DNA solution (Figure 40). The complex [C_{15}H_{29}N_{3}O_{4}CuCl_{2}] did not attenuate the DNA strand scission in presence of hydroxyl radical scavengers DMSO (Lane 6) and tert-butyl alcohol (Lane 7) suggestive of non–involvement of diffusible (OH) hydroxyl radicals. The DNA cleavage of the plasmid was efficiently inhibited in the presence of NaN_{3} (Lane 8), suggesting that ¹O_{2} is likely to be the reactive species responsible for the nuclease activity [173]. Similarly, in the presence of SOD (Lane 9), the cleavage was inhibited, which indicated that O^{-2} might be an inhibitor in the cleavage process and reducing the amount of O^{-2} can improve the cleavage effect. Based on the above results, the mechanistic pathway mediated by complex [C_{15}H_{29}N_{3}O_{4}CuCl_{2}] involves Cu(II) center that is initially reduced to Cu(I) species and subsequently reacts with dioxygen to form a peroxo Cu(II) derivative, which could generate ROS responsible for initiating DNA strand scission [174].

\[
\begin{align*}
\text{Cu(II) + DNA} & \rightarrow \text{Cu(II) \cdots \cdots DNA} \\
\text{Cu(II) + e^{-} } & \rightarrow \text{Cu(I)} \\
\text{Cu(I) + O}_{2} & \rightarrow \text{Cu(II) + O}_{2}^{-} \\
\text{O}_{2}^{-} + 2\text{H}^{+} & \rightarrow \text{H}_{2}\text{O}_{2} + \text{O}_{2} 
\end{align*}
\]
Cu(I) + H₂O₂ → Copper-oxo-species
Copper-oxo-species + DNA → DNA cleavage

DNA cleavage in presence of recognition elements
Minor groove binding agent DAPI, and major groove binding agent methyl green (Figure 41, Lanes 1 and 2) were used to examine the potential interacting site of complex [C₁₅H₂₉N₅O₄CuCl₂] with supercoiled plasmid DNA. The cleavage patterns, demonstrated that complex [C₁₅H₂₉N₅O₄CuCl₂] preferred minor groove binding, which was further validated by molecular docking studies.

\[ \text{Figure 41. Agarose gel electrophoresis pattern for the cleavage of pBR322 plasmid DNA (300 ng) by complex [C₁₅H₂₉N₅O₄CuCl₂] (0.20 mmol) in the presence of DNA minor groove binding agent DAPI and major binding agent MG at 310 K after incubation for 45 min. Lane 1, DNA + complex + MG; Lane 2, DNA + complex + DAPI (8 mM); Lane 3; DNA control.} \]

Topoisomerase I inhibition
A standard plasmid DNA cleavage assay of the complex [C₁₅H₂₉N₅O₄CuCl₂] was used to investigate the influence of the complex on the activity of human Topo-I by agarose gel electrophoresis. Complex [C₁₅H₂₉N₅O₄CuCl₂] inhibited Topo-I activity in a concentration-dependent manner. As shown in Figure 42, supercoiled DNA SC (Form I) was fully converted into nicked circular NC (Form II) (Lane 2) in the absence of complex.

\[ \text{Figure 42. Agarose Gel electrophoresis cleavage patterns showing cleavage showing effect of different concentrations of complex [C₁₅H₂₉N₅O₄CuCl₂] on the activity of DNA topoisomerase I (Topo I); Lane 1, DNA control; lane 2, Topo I + DNA; Lane 3, 5 μM of complex + DNA + Topo I; Lane 4: 10 μM of complex + DNA + Topo I; Lane 5: 15 μM of complex + DNA + Topo I; Lane 6: 20 μM of complex + DNA + Topo I.} \]
However, upon increasing concentrations of the complex \([\text{C}_{13}\text{H}_{29}\text{N}_{3}\text{O}_{4}\text{CuCl}_{2}]\) (5–20 \(\mu\)M), the levels of NC (Form II) were diminished (Lanes 3–6). The DNA relaxation effects caused by Topo-I were totally inhibited by complex \([\text{C}_{13}\text{H}_{29}\text{N}_{3}\text{O}_{4}\text{CuCl}_{2}]\) at 20 \(\mu\)M (IC\(_{50}\) ~20\(\mu\)M). These observations suggest that complex \([\text{C}_{13}\text{H}_{29}\text{N}_{3}\text{O}_{4}\text{CuCl}_{2}]\) inhibited Topo-I catalytic activity which may be attributed to i) its strong DNA binding affinity or ii) direct interference with the enzyme (i.e. stabilization of topoisomerase–DNA cleavable complexes) and inactivating its proper functioning, iii) ligand that occupies the topoisomerase binding site may suppress the association of topoisomerase with DNA, thus influencing the topoisomerase activity [175].

**Molecular docking with DNA**

Docking calculations were carried out on complexes \([\text{C}_{13}\text{H}_{29}\text{N}_{3}\text{O}_{4}\text{CuCl}_{2}],\) \([\text{C}_{13}\text{H}_{31}\text{N}_{3}\text{O}_{3}\text{NiCl}_{2}]\) and \([\text{C}_{15}\text{H}_{29}\text{N}_{3}\text{O}_{4}\text{CoCl}_{2}]\) with the DNA duplex of sequence \(\text{d(CGCGAAATTCGCG)}\))\(_{2}\) dodecamer (PDB ID: 1BNA) in order to understand and rationalize the mode of DNA binding of the complexes.

![Molecular docked model of complex (a) \([\text{C}_{13}\text{H}_{29}\text{N}_{3}\text{O}_{4}\text{CuCl}_{2}]),\) (b) \([\text{C}_{13}\text{H}_{31}\text{N}_{3}\text{O}_{3}\text{NiCl}_{2}]\) and (c) \([\text{C}_{15}\text{H}_{29}\text{N}_{3}\text{O}_{4}\text{CoCl}_{2}]\) with DNA dodecamer duplex of sequence \(\text{d(CGCGAAATTCGCG)}\))\(_{2}\) (PDB ID: 1BNA).

Figure 43. Molecular docked model of complex (a) \([\text{C}_{13}\text{H}_{29}\text{N}_{3}\text{O}_{4}\text{CuCl}_{2}]),\) (b) \([\text{C}_{13}\text{H}_{31}\text{N}_{3}\text{O}_{3}\text{NiCl}_{2}]\) and (c) \([\text{C}_{15}\text{H}_{29}\text{N}_{3}\text{O}_{4}\text{CoCl}_{2}]\) with DNA dodecamer duplex of sequence \(\text{d(CGCGAAATTCGCG)}\))\(_{2}\) (PDB ID: 1BNA).

The minimum energy docked pose \((\text{Figure 43 (a–c)})\), revealed that complexes \([\text{C}_{13}\text{H}_{29}\text{N}_{3}\text{O}_{4}\text{CuCl}_{2}]),\) \([\text{C}_{13}\text{H}_{31}\text{N}_{3}\text{O}_{3}\text{NiCl}_{2}]\) and \([\text{C}_{15}\text{H}_{29}\text{N}_{3}\text{O}_{4}\text{CoCl}_{2}]\) snuggly fitted into the curve contour of DNA minor groove, within A–T regions of the dodecamer, and slightly bends the DNA in such a way that a part of the aromatic ring of L–phenylalanine ligand makes more effective \(\pi–\pi\) stacking interactions between DNA
base pairs and lead to van der Waals interaction with the DNA functional groups which define the stability of groove [176]. Complexes exhibited additional stabilization through the strong intermolecular hydrogen bonding interaction with the hydrogen atom of the axial water molecules with the phosphate oxygen atom of DNA helix. The resulting relative binding energy of docked structures of complexes \([C_{13}H_{29}N_3O_4CuCl_2], [C_{15}H_{31}N_3O_5NiCl_2]\) and \([C_{15}H_{29}N_3O_4CoCl_2]\) with DNA were found to be \(-241.4, -232.8\) and \(-220.1\) KJmol\(^{-1}\), respectively indicating the more potent binding affinity between DNA and complex \([C_{15}H_{29}N_3O_4CuCl_2]\). The more negative the relative binding energy, greater the binding propensity of the complex with DNA, which correlated well with the experimental DNA binding studies and minor groove binder using DAPI assay. Thus, we can conclude that there is a mutual complement between spectroscopic and molecular docking techniques, which can substantiate our experimental results and provide valuable information about the mode of interaction between complex and DNA.

**Molecular docking with topoisomerase-I**

In order to further validate the observed Topo-I inhibitory assay, complex \([C_{15}H_{29}N_3O_4CuCl_2]\) 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 was bound to the oligonucleotide sequence 5′-AAAAAGACTTsX-GAAAAATTTTT-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. The resulting docking model (Figure 44 (a) and (b)) with minimum relative binding energy of \(-235.46\) KJ mol\(^{-1}\) indicated that the complex \([C_{15}H_{29}N_3O_4CuCl_2]\) approached towards the DNA cleavage site in the Topo-I–DNA complex and forming a stable complex through π–π 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 O atom of L-phenylalanine probably interacting through hydrogen bond to Arg 364, which is located at the binding site of the enzyme in the minor groove region of the DNA in the DNA–Topo–I conjugate [177].
Figure 44. (a) A molecular docked model of complex [C\textsubscript{13}H\textsubscript{29}N\textsubscript{3}O\textsubscript{4}CuCl\textsubscript{2}] in the cleavage site of human DNA topoisomerase I (PDB ID: ISC7). (b) View is a cut–out of sequence 5’-AAAAGACTTsX-GAAAATTTTT-3’ showing complex [C\textsubscript{13}H\textsubscript{29}N\textsubscript{3}O\textsubscript{4}CuCl\textsubscript{2}] (space filling model) docked in DNA sequence between scissile and non–scissile strand.

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. This result suggests that blocking the religation of the G11 hydroxyl group could be the main design point for novel Topo–I inhibitors. Thus, computer–aided molecular docking studies provide a structural explanation of drug binding mode in the active site of an enzyme, which leads to the rational design of new classes of anticancer drugs targeting Topo–I.
Conclusion

Amino acid–DACH conjugate complexes are novel in chemotherapeutic response (nuclear DNA inhibition) as DACH ligand scaffolds are better targeted and show improved cell penetration and possibly a different binding mode as compared to classical chemotherapeutics. In this work, synthesis and structural characterization of new transition metal {Cu(II), Ni(II) and Co(II)) complexes \( [\text{C}_{15}\text{H}_{29}\text{N}_3\text{O}_4\text{CuCl}_2] \), \( [\text{C}_{15}\text{H}_{21}\text{N}_3\text{O}_3\text{NiCl}_2] \) and \( [\text{C}_{15}\text{H}_{29}\text{N}_3\text{O}_4\text{CoCl}_2] \) of L-phenylalanine–DACH conjugate was carried out. Since DNA is the primary target of most of chemotherapeutic agents, therefore, \textit{in vitro} DNA binding studies of these complexes were carried out by employing UV–vis, fluorescence, circular dichoric, and thermal denaturation studies. The corroborative results of above experiments suggested electrostatic mode of binding with CT DNA preferably \textit{via} minor groove. The binding affinity of these complexes with CT DNA followed the order \( [\text{C}_{15}\text{H}_{29}\text{N}_3\text{O}_4\text{CuCl}_2] \) > \( [\text{C}_{15}\text{H}_{21}\text{N}_3\text{O}_3\text{NiCl}_2] \) > \( [\text{C}_{15}\text{H}_{29}\text{N}_3\text{O}_4\text{CoCl}_2] \), with complex \( [\text{C}_{15}\text{H}_{29}\text{N}_3\text{O}_4\text{CuCl}_2] \) displaying a higher binding propensity as compared to other complexes. The cleavage activity of complex \( [\text{C}_{15}\text{H}_{29}\text{N}_3\text{O}_4\text{CuCl}_2] \) with plasmid pBR322 DNA was carried out by electrophoretic mobility assay. Interestingly, complex \( [\text{C}_{15}\text{H}_{29}\text{N}_3\text{O}_4\text{CuCl}_2] \) presents an efficient cleavage of plasmid DNA converting the Form I to Form II and ultimately leading to the formation of linearized Form III. The complex \( [\text{C}_{15}\text{H}_{29}\text{N}_3\text{O}_4\text{CuCl}_2] \) exhibited remarkable ability to affect DNA scission by an oxidative mechanism involving the generation of ROS. Furthermore, Topoisomerase–mediated DNA relaxation assay result showed that complex \( [\text{C}_{15}\text{H}_{29}\text{N}_3\text{O}_4\text{CuCl}_2] \) could remarkably inhibit the activity of Topo–I at a very low concentration ~20 \text{μM}. 

62