Three New Copper (II) Complexes with CHIRAL SCHIFF BASES: Synthesis, Characterization, DNA Binding and DNA-Cleavage Studies

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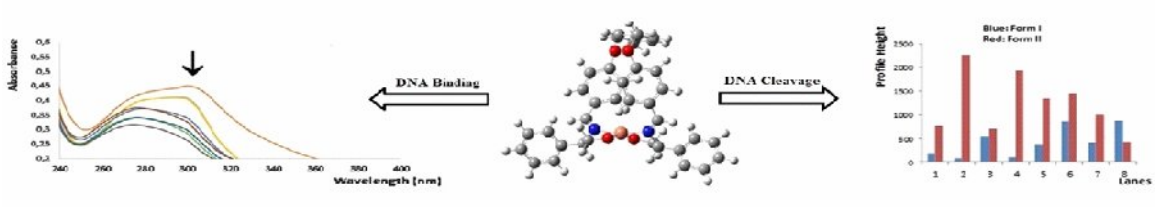
Summary: New mononuclear copper (II) complexes (1, 2 and 3) were synthesized from Schiff bases (H_2L) of chiral amino alcohols. The structures of the copper complexes were proposed by a combination of elemental analyses, FTIR, LCMS, magnetic susceptibility and molar conductance measurement methods. Spectroscopic and analytical data of the complexes suggest four-coordinated structures. Geometry optimization carried out with DFT/6-31G (d,p) were proposed to be distorted square planar geometry for the complexes. The similarity between experimental and theoretical IR spectra confirms the proposed structures. The interaction of copper (II) complexes with calf thymus (CT-DNA) was investigated using absorption titration method. The results suggest that the complex **1** and **2** can bind to DNA by intercalation. Binding constants K_b were found to be 2.46×10^5 for **1**, 5.41×10^5 for **2** and 7.00×10^4 for **3**. Moreover, agarose gel electrophoresis assay demonstrates that all complexes were found to cleavage of plasmid pentry/d-topo plasmid DNA. Complex **2** shows the best cleavage activity (5 μM).



Keywords: Schiff base complexes, DNA binding, DNA cleavage, DFT, Copper (II) complexes.

Introduction

Although metal ions have either boon or bane for living organisms [1]. They are crucial for cellular activity thanks to play role as co-factor. Metal ion deficiency or overloading of these ions can cause pathological conditions such as anemia because of iron deficiency and Wilson's disease thanks to copper overloading in the liver. Treatments of this situation are required same strategies. If someone has Wilson's syndrome S/he can treat with chelating agent to remove poisoned metal ion [2]. On the other hand, metallodrugs are not only therapeutics but also they are used for diagnostic purpose and even they have been used as theranostic agents recently [3].

The second review by G. Pappalardo and co-authors is focused on the re-purposing of copper-chelating compounds for treating neurodegenerative disorders, such as Alzheimer's (AD) and Parkinson's (PD). Together with zinc and iron, copper plays a key role in neuronal functions and its levels are finely regulated to avoid the onset of various age-related diseases. Their vocation is to trigger cellular effects by redox and/or by coordinative processes. Several complexes with different metals such as Pt, As, Au, Co, Tc, Gd, are approved by FDA and they are used as therapeutic, anticancer, antisyphillis, antiarthritic, antimicrobial and diagnostic agent, in single photon emission computed tomography respectively [4].

Copper, an endogenous element for several aerobic organisms, is less toxic to normal cells than cancer cells. For this reason, copper complexes possess anticancer activities [5-7]. In the past decades, Schiff bases copper complexes have attracted much attention in the improvement of novel therapeutic agents because they have a various pharmacological activity such as anticancer, antifungal, anti-inflammatory antioxidation, antibacterial, antimalarial, DNA nuclease activity and so forth [8-12]. Beside anticancer activity of metallodrugs containing copper as coordinated ion. They are attacted considerable attention in view of neurological diseases

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such as ALS, Parkinson's Disease [13] and epilepsy [14] in recent years.

Chiral molecules exhibit different chemical and physical properties in the chiral environment. Therefore, chiral biological active compounds for instance pharmaceuticals, agrochemicals; nutrients are sold as single enantiomers [15]. Enantioselectivity is the important criterion for metal complex-DNA interactions and drug design because the best fitting complexes for DNA helical structure exhibit the highest binding affinity [16]. On the other hand, chiral ligands increase the chirality [17] of the complexes and improve the pharmacological behavior of metal complexes. Therefore, there is a great demand for enantiomeric metallodrugs.

Although a large number of research on the chiral metal complexes have been published so far [18-28] few studies have been carried out on chiral copper(II) complexes [29-36] and their DNA binding and biological properties [37-39]. In this regard, we have synthesized and characterized three new copper(II) complexes (**1 -3**) bearing chiral Schiff bases of chiral amino alcohols. The CT-DNA binding and supercoiled pentry/d-topo plasmid DNA cleavage activities of copper complexes were also evaluated.

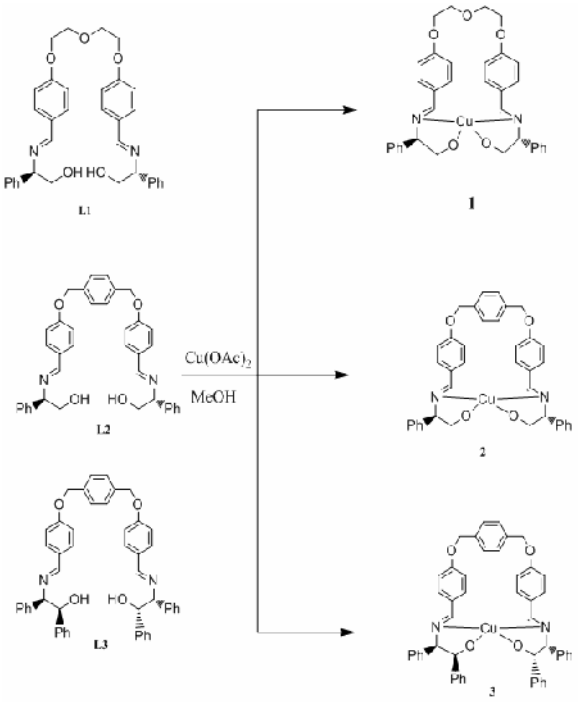
Experimental

Materials and Physical Measurements

All solvents and reagents were purchased from Sigma-Aldrich and used without further purification. IR spectra were recorded on a Nicolet-6700 ATR-FT-IR spectrophotometer in the 4000 – 400 cm^{-1} region. UV–Vis spectra were recorded on Thermo Scientific Genesys 10S spectrophotometer. Elemental analyses for C, H and N were carried out with an LECO CHNS-932 auto elemental analyzer. Melting points were measured using a Thermo Fisher Scientific Electrothermal 9100 apparatus. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker DPX-400 high performance digital FT-NMR spectrometer. Mass spectra were recorded with Thermo Scientific TSQ Quantum Access Max LC-MS spectrometers in methanol/acetonitrile mixture.

Synthesis of the compounds

Chiral Schiff bases L1 (1,4-Bis(phenoxy) ethane, (4,4'-(E,'E)-(R,)--hydroxy-2-phenylethylimino) methyl)), L2 (1,4-Bis-(4,4'-(E,'E)-(R,)--hydroxy-2-phenylethylimino) methyl) benzene), L3 (1,4-Bis-(4,4'-(E,'E)-(1S,2R)--hydroxy-1,2-diphenylethylimino) methyl) benzene) were synthesized according to our previous reported procedure [40]. The Cu(II) complexes **(1, 2** and **3)** were synthesized using the following general procedure (Scheme 1). A methanolic solution of (10 mL) of the ligand (0.064 mmol) was added dropwise to methanolic solution (10 mL) of a copper (II) acetate (0.064 mmol, 11.7 mg). 2-3 drop of the trimethylamine was added to the mixture, then, refluxed for six hours at 60 ^{o}C. The solution was allowed to cool and waited for 2 days at room temperature. The green solid was collected by vacuum filtration and washed twice with 3–5 mL aliquots of ice-cold methanol and dried under vacuum.



Scheme-1: Synthesis route of the copper (II) complexes.

Synthesis of [CuL^{1}] **(1)**

Yield: 0.031 g (88.9%), M.p.: 104-106 ^{o}C. LC-MS (m/z): 615.06 [M+H] (calc. 614.16). FTIR data (neat, v/cm^{-1}): 3034, 2896, 1680, 1599, 1570, 1505, 1424, 1395, 1309, 1251, 1145, 1047, 951, 921, 837, 802, 761. Anal. calcd. for $C_{34}H_{34}CuN_2O_5$: C, 66.49; H,5.58; N,4.56. Found: C, 65.97; H, 5.42; N, 4.75. Λ_m^{o}: 12.11 $ohm^{-1}cm^{2}mol^{-1}$. μ_{eff}:1.92 BM.

Synthesis of [CuL^{2}] **(2)**

Yield: 0.036 g (82.9%), M.p.: 122-124 ^{o}C. LC-MS (m/z): 647.02 [M+H] (calc. 646.25). FTIR data

(neat, v/cm^{-1}): 3086, 2938, 2874, 1678, 1600, 1570, 1504, 1420, 1381, 1305, 1243, 1158, 1103, 1048, 997, 886, 827, 796. Anal. calcd. for $C_{38}H_{34}CuN_2O_4$: C, 70.63; H, 5.30; N, 4.33. Found: C, 71.04; H, 5.17; N, 4.26. Λ_m^o: 11.25 ohm^{-1}cm^{2}mol^{-1}. μ_{eff}: 1.85 BM.

Synthesis of [CuL^3] **(3)**

Yield: 0.042 g (79.4%), M.p.: 123-125 ^{o}C. LC-MS (m/z): 799.02 [M+H] (calc. 798.44). FTIR data (neat, v/cm^{-1}): 3102, 3025, 2966, 2944, 1741, 1678, 1599, 1569, 1503, 1421, 1374, 1306, 1223, 1159, 1040, 999, 904, 827. Anal. calcd. for $C_{50}H_{42}CuN_2O_4$: C, 75.22; H, 5.30; N, 3.51. Found: C, 75.11; H, 5.62; N, 3.39. Λ_m^o: 14.45 ohm^{-1}cm^{2}mol^{-1}. μ_{eff}: 1.83 BM.

Computational method

All density functional theory (DFT) calculations were made using Gaussian 03 program package using the standard B3LYP functional [41]. For the geometric optimization of the copper (II) complexes (multiplicity =2, charge = 0) initial geometry was obtained by semi-empirical PM6 method. Then, obtained structures were reoptimized using B3LYP/6-31G (d,p) basis set for all atoms in the gas phase. The vibrational frequency calculations were also performed using B3LYP/6-31G(d,p) level. All the frequencies are found to be positive for the structures, indicating them to be a local minima. The choice of B3LYP/6-31G(d,p) level is based on its good performance found in earlier computational studies on copper(II) complexes.

DNA binding experiment

CT-DNA binding studies was conducted in Tris buffer (5 mM, pH 7.1). UV–Vis absorbance at 260 and 280 nm of the solution of CT-DNA in the buffer about 1.9:1 indicating that the DNA was free from protein [42]. The amount of DNA concentration was calculated by absorption spectroscopy using the molar extinction coefficient (6600 M^{-1} cm^{-1}) at 260 nm [43]. The intrinsic binding constant K_b for the interaction of our Cu (II) complexes with CT-DNA was calculated based on the absorption spectral changes after the addition of increasing DNA. The binding constant was calculated according to the following equation [44-45].

$$[DNA]/(\varepsilon a - \varepsilon f) = [DNA]/(\varepsilon b - \varepsilon f) + 1/Kb(\varepsilon b - \varepsilon f)$$

[DNA] represent the concentration of DNA in base pairs; the apparent absorption coefficient ε_a, ε_f and ε_b correspond to A_{obs}/[M], the extinction coefficient of the free and the extinction coefficient of the compound when fully bound to DNA, respectively. The plot of $[DNA]/(\varepsilon a - \varepsilon f)$ vs [DNA] gave a straight line with a slope of $1/(\varepsilon b - \varepsilon f)$ and an intercept of $1/Kb(\varepsilon b - \varepsilon f)$; K_b was determined from the ratio of the slope to intercept. Uv-vis titration experiments were performed by keeping the concentration of complexes constant and changing the nucleic acid/nucleotide concentration. The absorbance (A) was recorded after consecutive additions of CT-DNA. CT-DNA was added to both the compound solution and the reference solution to avoid the absorbance of the CT-DNA itself.

DNA cleavage experiment

The DNA cleavage activity of our complexes was examined using supercoiled pentry/d-topo plasmid DNA. Plasmid DNA was purified by using Perfect Prep Endo Free Plasmid Maxi Kit from Qiagen according to manufacturer instructions [46]. After purification, plasmid DNA amount was measured at 260 nm by nanodrop spectrophotometer. Pure DNA preparation has a ratio of $A_{260}/A_{280} > 1.8$ and $A_{260}/A_{230} = 1.80$. A stock solution (500 µM) of the complexes was prepared. Plasmid DNA was treated with increasing complex concentrations (20-150 µM) and enough buffer solution for a final volume of 50 µL (22 µL plasmid DNA + required volume of complexes to achieved desired concentration and volume are completed to 50µL). The samples were incubated in the dark for 12 h at 37 ^{o}C. The DNA cleavage experiments were analyzed by 1% agarose gel electrophoresis using 1XTris–Acetate–EDTA (TAE) running buffer. 20 µL of each reaction solution together with 10 µL of the loading dye was loaded into the wells and subjected to electrophoresis at 75 V for 90 min. The gel was stained with ethidium bromide and documented using a Gene Tools imaging system from Syngene.

Results and Discussion

Structure of the copper (II) complexes

The molar conductivities of the complexes dissolved in DMF (1.00×10^{-3} M) were measured at room temperature. The molar conductance values (Λ_m^o) were found to be 12.11 for **1**, 11.25 for **2** and 14.45 for **3**. Obtained low conductivity data indicate that the copper(II) complexes are non-electrolytes [47].

The magnetic moments of the complexes were found to be 1.92 for **1**, 1.85 for **2** and 1.83 BM for **3** at room temperature. These values are quite close to the values expected for spin-only monomeric copper(II) complexes and are characteristic of $3d^9$ electronic configuration.

ESI-MS spectra of the copper(II) complexes were depicted in Fig 1. The spectra recorded in the

positive mode of the complexes **(1-3)** were exhibited indicating the presence of $[M+H]^+$ ions for **1**,**2** and **3**. These molecular ions confirm the stoichiometry of the copper(II) complexes as [CuL] type. Briefly, analytical data of the complexes were in agreement with a 1:1 (Cu:L) molar ratio.

Synthesis and single crystal X-ray structure of several copper (II) complexes bearing Schiff base prepared from different chiral amino alcohols have been reported in the literature [48-51]. In these complexes, Schiff bases coordinate to copper(II) atom with imine nitrogen atom and undeprotonated or deprotonated alkoxy-oxygen atom. It is known that different copper salts give rise to different geometry [52]. In this study, copper(II) acetate and trimethylamine were used in the synthesis, and deprotonation occurred. ^{1}H-NMR spectrum of the complex **1,** not given here due to poor resolution, confirm the deprotonation. In addition, disappearing of υ_{OH} stretching vibration (Fig. 2), a broad band between 3500 - 3550 cm^{-1} of the Schiff bases with complex formation also confirm the deprotonation. On the basis of these findings, four- coordinated copper(II) complexes bearing four-dentate Schiff bases ligands having two imine nitrogen atoms and two deprotonated alkoxy-oxygen atoms were proposed for **(1-3).**

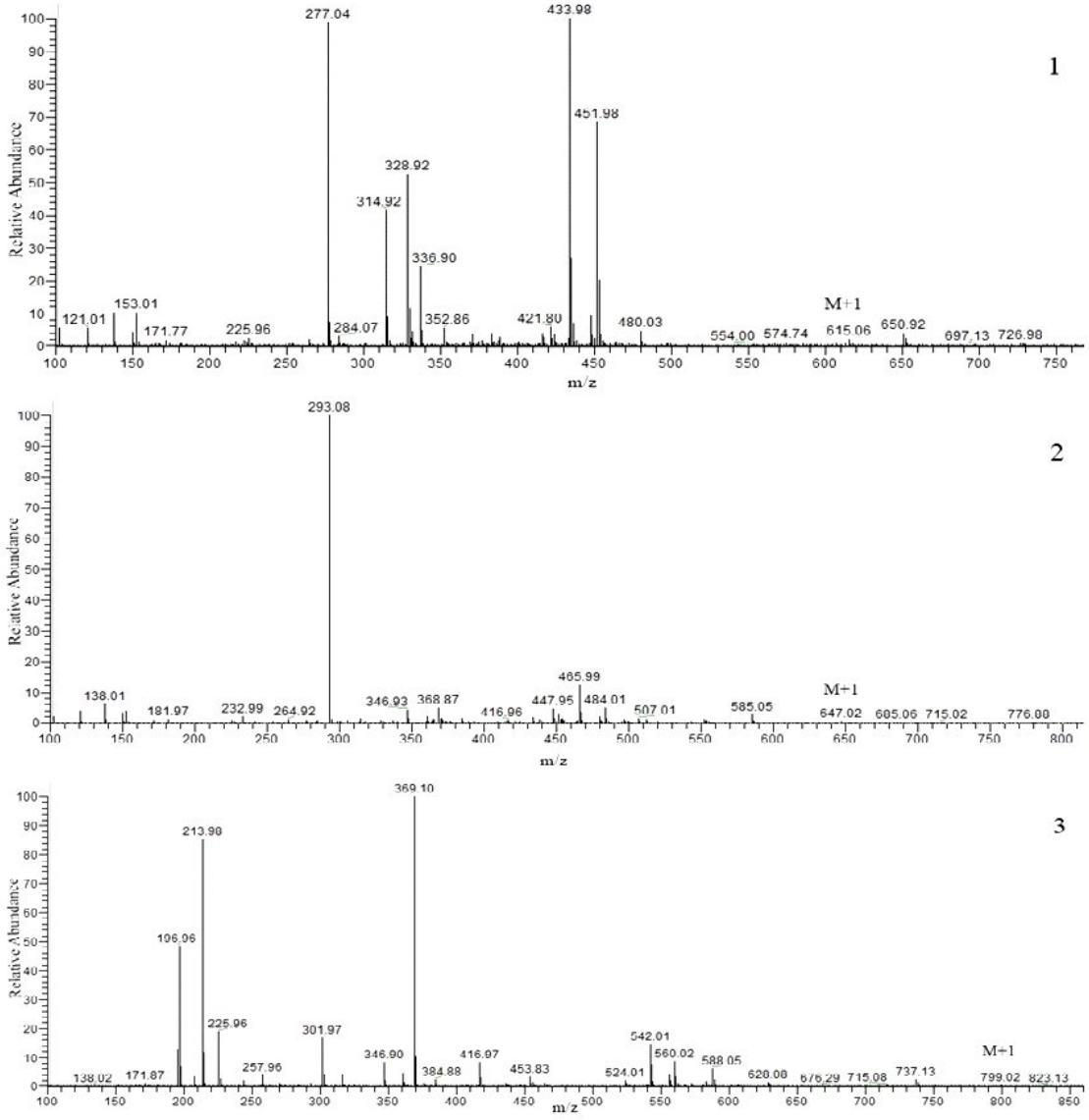


Fig. 1: LC-MS spectra of the copper (II) complexes.

DFT calculation of the copper (II) complexes

The geometry optimization of the proposed complexes **(1-3)** was performed at B3LYP/3-21G(d) level for different starting geometries, and several local minima were found. Then, two low-energy conformers were selected and optimized with B3LYP/6-31G(d,p) level. Their optimized structures are shown in Fig. 3. Calculated electronic energies, some selected bond angles, and bond length were given in Table-1. The calculated bond length of Cu-N and Cu-O were in agreement with the single crystal X-ray data obtained from similar copper(II) complexes reported in the literature [47-52]. Initial geometries of (a) and (b) were square planar and tetrahedral structures, respectively. However, after optimization, both got the distorted square planar geometry. As seen from Table 1, square planar (a) structures have lower energy than tetrahedral (b) structures for three complexes **(1-3)**. O-Cu-O and N-Cu-N bond angles of (a) are wider than the corresponding (b). If the octahedral structure was taken as starting geometry with additional two phenolic oxygen atoms of Schiff base, after optimization, it returns to the *pseudo* square planar structure. In this structure, the Cu-O distance was calculated as 6.5 Å, indicating that there is no bond between Cu and phenolic oxygen atoms.

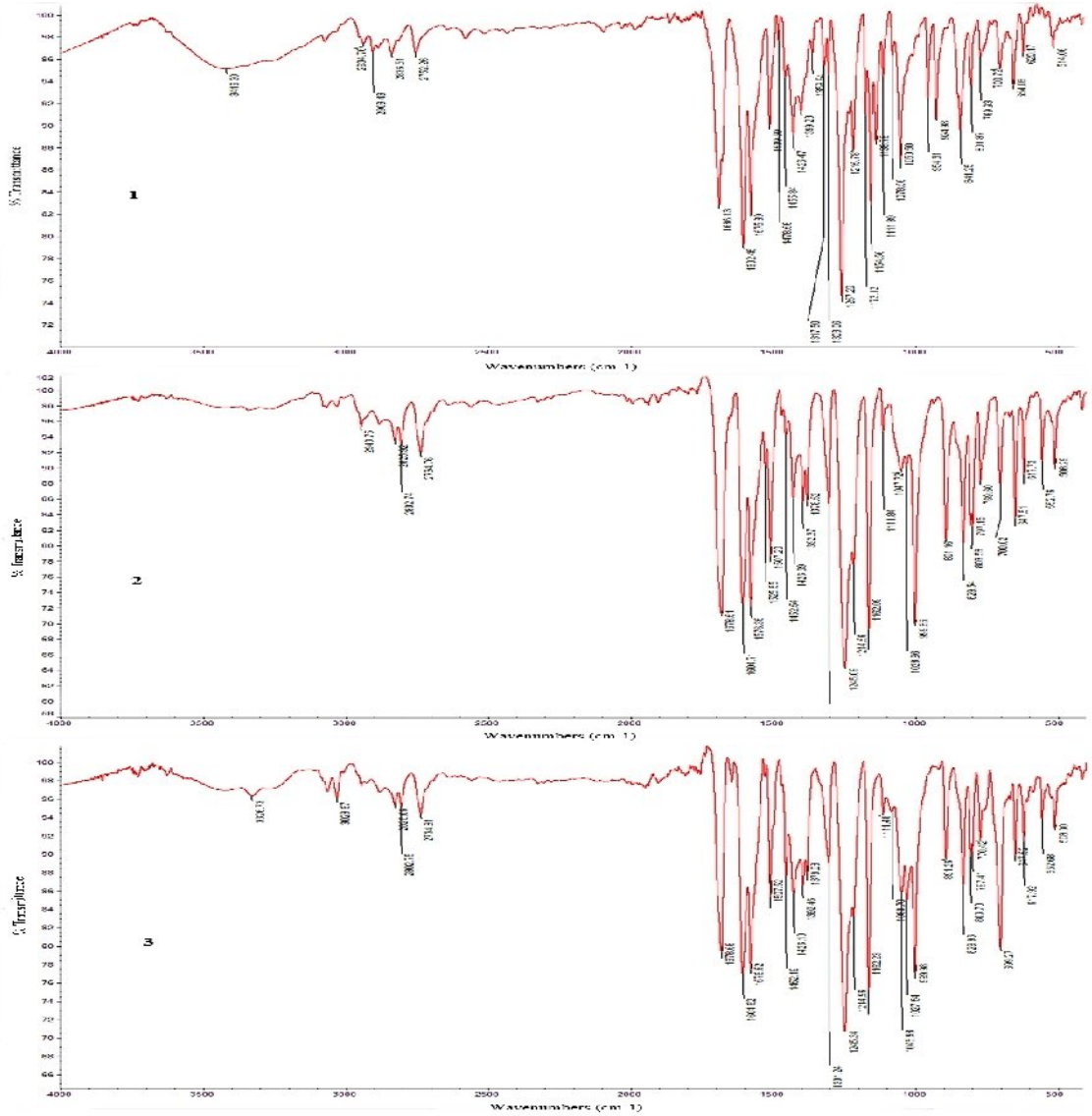


Fig. 2: FT-IR spectra of the copper (II) complexes.

Table-1: Total electronic energies (Hartrees) and selected bong angles () and bond length (Å) for two structures of the copper(II) complexes optimized via DFT/B3LYP/6-31G(d,p)

	$E_{electonic}$ (a.u)	O-Cu-O	N-Cu-N	Cu-N	Cu-O
1a	**-3442.0434**	**152.65**	**161.22**	**2.01**	**1.88**
1b	**-3442.0315**	**115.00**	**122.18**	**1.99**	**1.85**
2a	**-3519.2707**	**158.13**	**170.06**	**2.01**	**1.87**
2b	**-3519.2695**	**153.58**	**169.97**	**2.01**	**1.87**
3a	**-3981.3856**	**159.65**	**173.16**	**2.02**	**1.88**
3b	**-3981.3802**	**153.03**	**170.67**	**2.02**	**1.89**

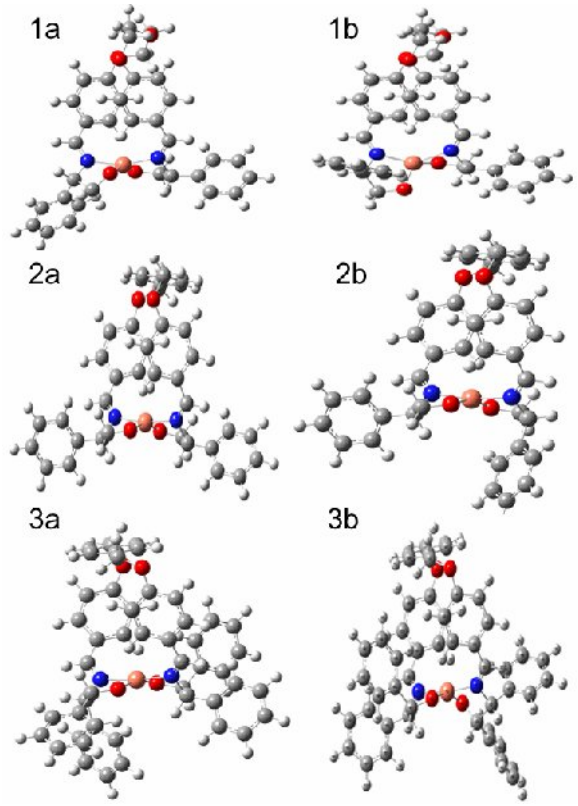


Fig. 3: Optimized structures of square planar (a) and tetrahedral (b) the copper(II) complexes calculated by B3LYP/6-31G(d,p).

Harmonic vibrational frequencies of ground state optimized geometries (a) were calculated using the DFT/B3LYP /6-31G(d,p). Calculated IR spectra (Table-2) showed good agreement with the experimental IR spectra (Fig. 2). The differences were related to the packing effects of the solid state structure, with respect to the isolated complexes at the gas phase. The second reason is that calculated values are harmonic frequencies while the experimental values contain anharmonic vibrational frequencies. However, the spectral patterns are quite similar to each other, confirming the proposed structures. It is well known that the calculated wavenumbers are higher than their experimental equivalents, for this reason, empirical scaling factors are used to obtain considerably better agreement with the experimental data. We have used the scaling factors of 0.98-0.96. Afterward, we compared and assigned the vibrational modes by matching peak intensities and using scaled peak frequencies. Experimental and calculated wave numbers of selected vibrational modes (for intensity higher than 40 km/mol) of the complexes were given in Table-2.

Table-2: Experimental and calculated wavenumbers of selected vibrational modes of the complexes.

1		**2**		**3**		**Assignments***
exp	**calc**	**exp**	**calc**	**exp**	**calc**	
-	**3202**	**-**	**3199**	**3028**	**3204**	**υ_{CH}(ar)**
-	**3199**	**-**	**3173**	**-**	**3198**	**υ_{CH}(ar)**
-	**3147**	**-**	**3138**	**-**	**3151**	**υ_{CH}(ar)**
2935	**3056**	**2943**	**3052**	**2945**	**3053**	**υ_{CH2}**
2836	**2970**	**2825**	**2900**	**2826**	**2947**	**υ_{CH2}**
2752	**2907**	**2734**	**2899**	**2734**	**2847**	**υ_{CH2}**
1686	**1716**	**1678**	**1718**	**1678**	**1714**	**$\upsilon_{C=N}$**
1602	**1661**	**1604**	**1660**	**1604**	**1665**	**υ_{ring}**
1575	**1659**	**1576**	**1659**	**1576**	**1660**	**υ_{ring}**
1509	**1657**	**1507**	**1656**	**1507**	**1658**	**υ_{ring}**
1426	**1496**	**1426**		**1426**	**1460**	**$\delta_{CH(ar)}$**
1399	**1470**	**1392**		**1392**	**1395**	**$\delta_{CH(ar)}$+ δ_{CH2}**
1359	**1365**	**1378**		**1379**	**1361**	**$\delta_{CH(ar)}$+ δ_{CH2}**
1257	**1286**	**1245**	**1286**	**1245**	**1287**	**δ_{CH2}+δ_{CH}(ar)**
1173	**1207**	**1162**	**1202**	**1162**	**1202**	**δ_{CH}(ar) bridge**
1111	**1130**	**1111**	**1132**	**1111**	**1132**	**$\upsilon_{C-O(alkoxy)}$ +δ_{CH}(ar)**
1078	**1109**	**1080**	**1098**	**1080**	**1097**	**$\upsilon_{C-O(alkoxy)}$+ δ_{CH}(ar)**
1050	**1082**	**1047**	**1062**	**1045**	**1060**	**$\upsilon_{C-O(phenolic)}$**
-	**-**	**1028**	**1044**	**1027**	**1043**	**$\delta_{CH(ar)}$**
-	**-**	**1000**	**1013**	**999**	**1012**	**$\delta_{CH(ar)}$ + δ_{CH2}**
954	**964**	**-**	**-**	**-**	**-**	**υ_{C-N}**
924	**934**	**891**	**902**	**891**	**902**	**$\delta_{CH2(aliphatic)}$+**
841	**850**	**829**	**846**	**829**	**845**	**$\delta_{CH2(aliphatic)}$**
801	**816**	**803**	**829**	**803**	**828**	**CH (ar-bridge)**
769	**788**	**797**	**819**	**797**	**819**	**CH (ar-bridge)**
700	**717**	**769**	**783**	**770**	**783**	**CH (ar-terminal)**
654	**662**	**647**	**846**	**647**	**659**	**ring(ar)**
620	**653**	**617**	**631**	**617**	**632**	**ring(ar)**
514	**554**	**552**	**574**	**552**	**574**	**υ_{as}Cu-O**
-	**-**	**508**	**522**	**508**	**522**	**skeleton**

*ν: stretching, δ: in-plane bending, γ:out-of-plane bending

DNA Binding Studies

The absorption spectra of the titration of the copper(II) complexes (20 μM) with CT-DNA from 0 to 100 μM are shown in Fig. 4. Absorption band in the range 250 -300 nm (λ_{max} is 280 nm) correspond to $\pi \rightarrow \pi^*$ transition of the aromatic rings of the copper(II) complexes. As seen in Fig.4, the band height at 280 nm gradually decreases for **1** and **2**, but increases for **3**. On titration with CT-DNA, complex **1** exhibits slight hypochromic and narrow bathochromic effects; whereas, complex **2** displays significant hypochromic and large bathochromic effects; moreover, complex **3** shows slight hyperchromic and narrow hypsochromic effects. Hypochromism is caused by parallel stacking of base pairs in a double helix and refer to the presence of intercalation or at last partial intercalation for **2** and

1. The extent of the hypochromic is usually proportional to the strength of the interaction [53]. Whereas, hyperchromism is an indication of the reduction in the amount of parallel stacking in double helix resulting from 1,2-intrastrand crosslinking. The extent of the hyperchromism is indicative of the partial or non-intercalative binding modes [54].

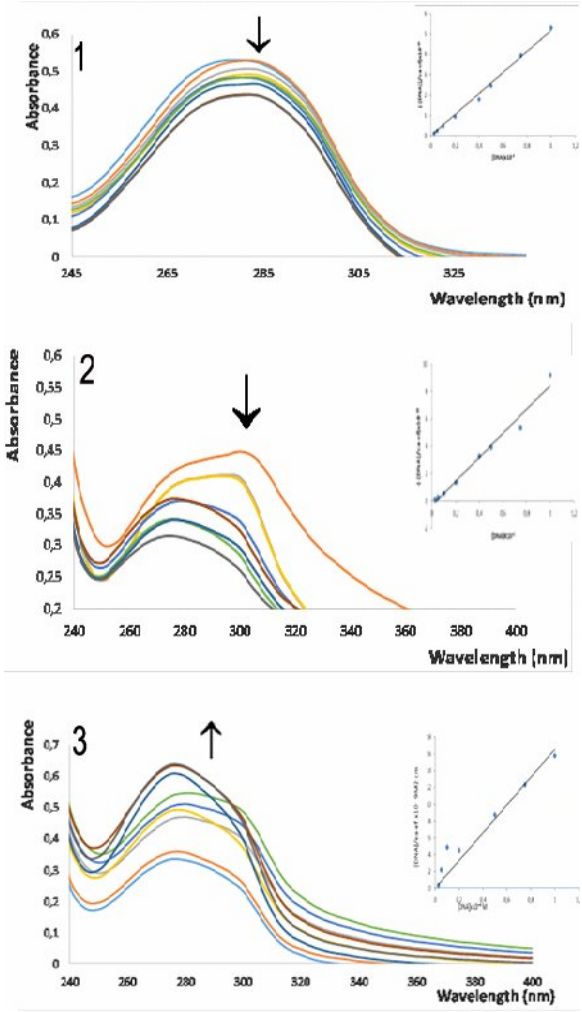


Fig. 4: Absorption spectra of the copper(II) complexes (20 µM) in buffer solution (5 mM Tris/ 50 mM NaCl, pH 7.5) upon addition of CT-DNA from 0 to 100 µM. Arrow indicates the changes in absorbance upon increasing the DNA concentration. Inset: Plots of -[DNA]/åa-åf vs. [DNA] for the titration of CT-DNA. (1 and 3 cyan represents 0 M [DNA] and 2 orange represents 0 M [DNA]. Directions of Arrows indicate increasing DNA concentration.)

Cis-platin and its analogs were reported to have hyperchromic and bathochromic shifts [55]. In order to compare the DNA-binding strengths of these complexes, the intrinsic binding constant K_b was determined from the changes in absorbance. The values of K_b were 2.46×10^5 **(1),** 5.41×10^5 **(2)** and 7.00×10^4 **(3)**, respectively (Table-3). It was observed that the K_b value increased when the aromatic moiety was replaced by the aliphatic ether group on the bridge at **2**. It was expected due to the strong stacking interaction between the aromatic chromophore and the base pairs of DNA. On the contrary, K_b value decreased when the number of phenyl substituent increased at **3**. Much increase in molecular volume caused an opposite effect. The copper(II) complexes having $[Cu_8]^{8+}$ and $[Cu_4]^{8+}$ units of the chiral alkanol amine Schiff base ligands were reported to exhibit hyperchromism and low K_b values as $3.42x10^3$ and $3.04x10^4$, respectively [49].

Table-3: Absorption Spectroscopic properties of the complexes binding to CT DNA.

Complexes	Change in absorbance	Shifting	Shifting Δλ	K_b/ M^{-1}
1	Hypochromism	Bathochromic	+5	$2.46x10^5$
2	Hypochromism	Bathochromic	+25	$5.41x10^5$
3	Hyperchromism	Hypsochromic	5	$7.00x10^4$

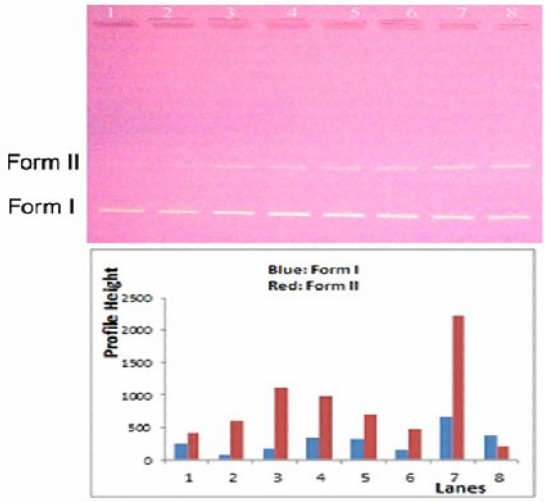


Fig. 5: Gel electrophoresis pattern showing cleavage of plasmid DNA (300 ng) treated with complex 1 in a Tris buffer (5 mM Tris/ 50 mM NaCl, pH 7,5) at 37 ^oC with an incubation time of 12 h. Lane1: Control DNA; Lane 2: DNA + 1 (5 µM), Lane 3: DNA+ 1 (10 µM), Lane 4: DNA+ 1 (25 µM), Lane 5: DNA+ 1 (50 µM), Lane 6:DNA+1 (100 µM), Lane 7: DNA+1 (150 µM), Lane 8: DNA+1 (200 µM) (b) Comparison profiles of the lanes.

Plasmid DNA cleavage

The DNA cleavage activity of the complexes for supercoiled pentry/d-topo plasmid DNA was investigated by gel electrophoresis. When supercoiled DNA is subject to electrophoresis, relative migration will be observed for the intact supercoiled form (Form I). If cutting occurs on one strand (nicking), the supercoiled form will relax and generate a slower-moving singly nicked form (Form II). If both strands are cleaved, a double nicked linear form (Form III) that migrates between Form I and Form II will be generated. Plasmid DNA electropherogram traces of the complexes **(1-3)** with increasing concentration (5-200 µM) were given in Fig 5-7. DNA cleavage activity of the complexes is not concentration dependent. While the percentage conversion to Form I to Form II is mostly at line **2** (10 µM), the maximum conversion was observed at 150 µM (line 7) for complex **1** (Fig 5). Complex **2** exhibited the best cleavage at 5 µM in Fig. 6 (line 2), and the complex **3** shows the best conversation to Form II at 10 µM (Fig. 7, line 3). These results do not correspond to their binding capabilities.

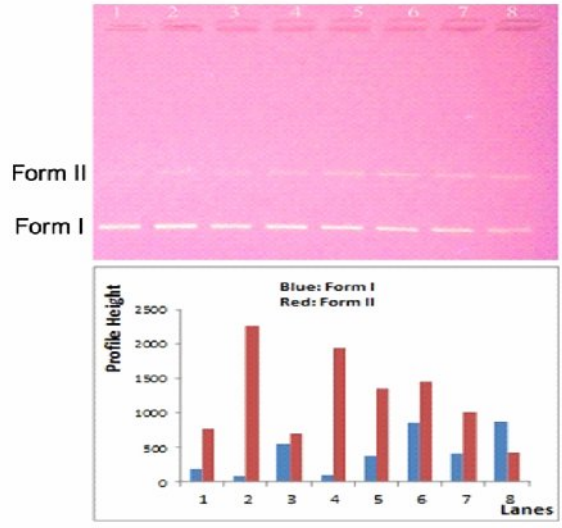


Fig. 6: Gel electrophoresis pattern showing cleavage of plasmid DNA (300 ng) treated with complex 2 in a Tris buffer (5 mM Tris/ 50 mM NaCl, pH 7,5) at 37 ^{o}C with an incubation time of 12 h. Lane1: Control DNA; Lane 2: DNA + 2 (5 µM), Lane 3: DNA+ 2 (10 µM), Lane 4: DNA+ 2 (25 µM), Lane 5: DNA+ 2 (50 µM), Lane 6:DNA+2 (100 µM), Lane 7: DNA+2 (150 µM), Lane 8: DNA+2 (200 µM) (b) Comparison profiles of the lanes.

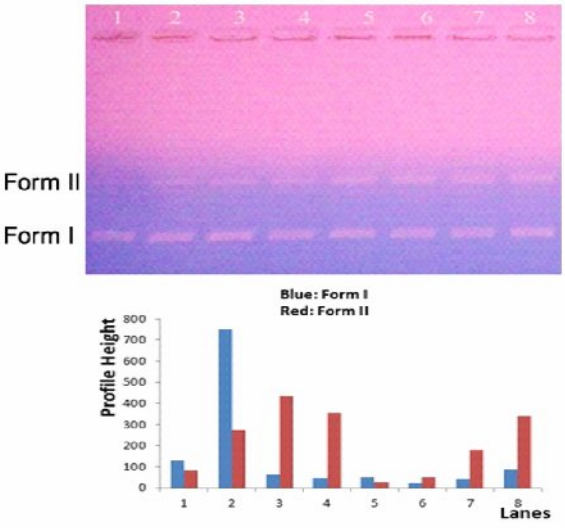


Fig. 7: Gel electrophoresis pattern showing cleavage of plasmid DNA (300 ng) treated with complex 3 in a Tris buffer (5 mM Tris/ 50 mM NaCl, pH 7,5) at 37 ^{o}C with an incubation time of 12 h. Lane1: Control DNA; Lane 2: DNA + 3 (5 µM), Lane 3: DNA+ 3 (10 µM), Lane 4: DNA+ 3 (25 µM), Lane 5: DNA+ 3 (50 µM), Lane 6:DNA+3 (100 µM), Lane 7: DNA+3 (150 µM), Lane 8: DNA+3 (200 µM) (b) Comparison profiles of the lanes.

Conclusion

Three new mononuclear copper(II) complexes possessing ONNO type Schiff bases of chiral amino alcohol were synthesized. On the basis of analytical and experimental data, the Schiff bases were coordinated to Cu(II) ion with four donor atoms, two imine nitrogens and two deprotonated alkoxy-oxygen atoms. Deprotonation is achieved by the addition of trimethylamine to the reaction mixture. Geometry optimization with B3LYP/6-31G(d,p) in the gas phase was proposed to be a distorted square planar geometry of the complexes. The similarity between experimental IR spectra and theoretical IR spectra obtained from calculated harmonic vibrational frequencies of the complexes confirm the proposed structures. The CT-DNA binding mode of these complexes has been evaluated using absorption titration method. Intercalation was proposed for complex **1** and **2,** which exhibit hypochromic and bathochromic effects. Whereas, complex **3** displays slight hyperchromic effect and hypsochromic shift. The binding constants K_b of the complexes were determined in the following order **2** > **1** > **3**. The number of the phenyl group play an

important role in the DNA binding values. K_b value increased when the aromatic moiety was replaced by the aliphatic ether group on the bridge at **2**. However, K_b value decreased when two more phenyl groups join the structure of **3**. Much increase in molecular volume caused an opposite effect. The concentration-dependent DNA cleavage experiments using plasmid pentry/d-topo plasmid DNA were also performed. Complex **2** shows the best DNA cleavage activity (5 μM). We consider that there is no correlation between the cleavage activities and DNA binding capabilities. We tested the correlation between DNA cleavage and binding properties of compouns by Pearson Analysis by Excell and we concluded that no correlation is present between these two features with 95% fonfidence ($p<0.05$).

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