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Mechanochemical synthesis of six Cu(II) complexes with selected thiols, their physicochemical characterization and interaction with DNA

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ABSTRACT

Cysteine together with histidine (His_2Cys) has been identified as one of the ligands in Type 1 copper protein active sites, and Cu-S(Cys) interaction determines their unique spectroscopic features. This work reports the synthesis of model Cu(II)-thiolates, which could mimic the Type 1 sites. The Cu(II)-complexes with *l*-cysteine, *N*-acetylcysteine, *l*-glutathione, *l*-penicillamine, mercaptosuccinic acid and *dl*-dithiothreitol were synthesized by solvent-free mechanochemical methods. The complexes were found to be of the ML_2 type as revealed by solid-state analytical techniques including FT-IR (ATR) spectroscopy, Raman spectroscopy, electronic absorption spectroscopy (diffuse reflectance), powder X-ray diffraction and desorption electrospray ionization mass spectrometry. These compounds are difficult, in some cases impossible, to isolate from solution because of rapid oxidation of thiols to disulfides and reduction of Cu(II) to Cu(I) or Cu(0). However, in the solid state these complexes were found to be highly stable and exhibited spectroscopic features similar to those in the Type 1 site. Interaction of the complexes with genomic DNA isolated from human blood was studied by electrophoresis, which showed that Cu(II)-mercaptosuccinic acid and Cu(II)-*N*-acetylcysteine caused extensive degradation, whereas Cu(II)-penicillamine amd Cu(II)-glutathione significantly degraded the DNA. Cu(II)-cysteine appears to form DNA adducts. Docking of Cu(II)-cysteine with thioredoxine reductase suggests that the complex has the potential to inhibit the activity of the enzyme.

1. Introduction

Copper exhibits 1+, 2+, 3+ and 4+ oxidation states in different environments. Copper, being an essential element, is present in human body (1.4–2.1 mg kg^{-1} body weight), where it plays vital roles including formation of red blood cells, absorption and utilization of iron, metabolism of cholesterol and glucose, and in inflammation. In the human body copper exists in both 1+ and 2+ oxidation states as a part of various proteins and a pool of small molecular complexes [1]. Copper having variable valency is capable of undergoing redox reactions in the body. The Type 1 class of copper protein active sites have been recognized as redox centers in biological systems. Cysteine together with histidine (His_2Cys) has been identified as one of the ligands in all of these sites, and Cu-S(Cys) interaction determines their unique spectroscopic features. Efforts are being continued to synthesize model Cu(II)-thiolates, which could mimic the Type 1 sites.

Positive standard reduction potentials of copper ions [2], due to $Cu^{2+}(aq) + e^- \rightarrow Cu^+(aq)$ $E^\circ = +0.153V$, $Cu^+(aq) + e^- \rightarrow Cu(s)$ $E^\circ = +0.52V$ and $Cu^{2+}(aq) + 2e^- \rightarrow Cu(s)$ $E^\circ = +0.34$ V, indicate the ease of reducibility to elemental copper. Cu^{2+} forms stable complexes with hard ligands containing O and N donor atoms, whereas Cu^+ forms stable complexes with soft ligands containing S and P as donor atoms. Thiols are among the soft ligands, and

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they play very important roles in biological systems. When Cu^{2+} interacts with thiols in solution, it is readily reduced to Cu^{+} and Cu^{0}. It is very difficult and sometimes impossible to make stable Cu^{2+} complexes with thiols due to higher oxidizability of thiols (e. g. $E^{\circ} = -440$ mV for cysteine and $E^{\circ} = -383$ mV for glutathione) [3]. Thus there has been little success in synthesis of stable Cu(II)-thiolates *in vitro*. We believe that such compounds isolated as solids, being less stable in solution, will exhibit high catalytic and biological activities.

It has been reported that cysteine metal complexes exist *in vivo* and perform a variety of functions in the biological system [4,5]. In fact, Cu(II)–cysteine (Cu(II)–Cys) complexes are considered as models for 'Type I' blue proteins, where the copper ions are bonded to two N and two S atoms [6]. Some of the copper-thiolates are of interest due to their cytotoxic and antitumor properties [7]. It has been shown that the combination of cysteine (Cys), glutathione (GSH), and Cu can inactivate HIV protease [8].

Most of the studies regarding Cu(II)-cysteine complexes discuss their formation in solution without isolation [9–11]. Dokken et al. [12] were able to isolate dinuclear and trinuclear Cu(II)-cysteine complexes from solution. Most of the papers discussing the synthesis of Cu(II)–cysteine complexes in aqueous solution have reported mixed results. In these cases, usually Cu^{2+} is reduced to Cu^{+} forming cystine (two cysteines connected by a disulfide bond) complex where the Cu(I) is bound to two amino groups and two carboxylate groups. Rigo et al. [9] reported that cuprous ions form a polymeric species containing a bridging complex with the thiol of cysteine in a 1:1.2 ratio (Cu(I):Cys) in H_2O containing 0.1 M phosphate at pH 7.0. Cupric ions were reduced to Cu(I) and formed cystine complexes at Cu(I):cystine ratios of 0.45:1. Masoud and El-Hamid have claimed the synthesis of a pale green Cu(II)-cysteine dehydrate [13], which was characterized by elemental analysis, electronic absorption and FT-IR spectra. Their findings suggest that both carboxylate and thiol groups are involved in binding copper. However, based on their data there is likelihood of this being an oxidized Cu(I)-cysteine complex or an impure Cu(II)–cystine complex similar to that reported by Gale and Walker [14]. In the present work we report the mechanochemical synthesis of some biologically important Cu(II)-thiolates, their interaction with genomic DNA (gDNA) and docking with thioredoxine reductase-like protein from thermus thermophilus HB8 (PDB ID: 2ZBW).

2. Materials and methods

2.1. Materials

The chemicals used in this study were: Copper acetate monohydrate (Riedel-de Haën), *l*-cysteine (Sigma-Aldrich, St. Louis, MO, USA), *N*-acetylcysteine (Sigma-Aldrich), glutathione reduced (Sigma-Aldrich), mercaptosuccinic acid (Sigma-Aldrich), dithiothreitol (Phyto Technology Laboratories, USA), and *D*-penicillamine (Sigma-Aldrich). These were used without further purification. The spray solvents used for desorption electrospray ionization-mass spectrometry (DESI-MS) analysis were: methanol (Sigma-Aldrich), formic acid (Sigma-Aldrich), and dimethyl sulfoxide (DMSO, Fisher Scientific, Waltham, MA). All solvents utilized were of HPLC grade. Deionized water was acquired from a Barnstead Ultrapure purification system. The following materials were used for the drug-DNA interaction study (all from Merck Chemicals, Darmstadt, Germany, unless stated otherwise): sucrose, Tris base, hydrochloric acid, sodium hydroxide, magnesium chloride, Triton X-100, ethylenediaminetetraacetic acid, sodium chloride, sodium hypochlorite solution, proteinase K solution, isopropyl alcohol, ethanol, glycerol, agarose powder, ethidium bromide, glacial acetic acid, and bromothymol (Tedia, Fairfield, OH, USA).

2.2. Synthesis

Copper acetate monohydrate (0.400 g, 2.0 mmol) and the appropriate ligand (4.0 mmole) were accurately weighed and transferred to an agate mortar. The mixture was ground with an agate pestle. On grinding a change in color and a weight loss due to evolution of acetic acid vapors were observed. The grinding was continued until the acetic acid vapors ceased to evolve; this process took about 10 min. The complex thus obtained could be impure because of the presence of the unreacted ligand or the copper salt; nonetheless characterization was carried out by using the complex obtained as such in order to avoid any conversion during purification process. For comparison purposes, reaction products purified by washing with water, methanol and ether were also analyzed.

2.3. Preparation of Cu(II)-cysteine

Cu(II)-cysteine was prepared by grinding copper acetate monohydrate (0.400 g, 2.0 mmol) and *l*-cysteine (0.4840 g, 4.0 mmol), producing a purple-hued complex. Elemental analysis for $Cu(CyS)_2$ - Calculated: C (23.72%), H (3.98%), N (9.22%), S (21.11%), Cu (20.91%). Experimental: C (23.49%), H (4.30%), N (9.32%), S (21.44%), Cu (20.56%); Melting point (decomp.): >250°C; Absorption spectrum λ_{max} (diffused reflectance, nm): 421 (N-Cu), 644, 703 (πS-Cu); IR (neat solid, cm^{-1}): 3595, 3518, 3449, 3296, 3228 (νNH), 3047, 2987 (νOH), 2353 (ν=NH_2^+), 2100 (νCH), 1626 ($\nu_{asym}COO^-$), 1493 (def CH), 1400 ($\nu_{sym}COO^-$), 1352 (νOH), 1209 (νCN), 1121, 1063 (νCO), 966 (δCH), 918 (δOH), 849 (δCN); Raman (cm^{-1}) 474 (N-Cu), 421 (S-Cu); DESI-MS: $[(CyS)_2Cu]^+$ (*m/z* 303.9), $[CySCu]^+$ (*m/z* 183.8).

2.4. Preparation of Cu(II) n-acetylcysteine

Cu(II) *N*-acetylcysteine was prepared by grinding copper acetate monohydrate (0.400 g, 2.0 mmol) and *N*-acetylcysteine (0.6527 g, 4.0 mmol), producing a sea blue-hued complex. Elemental analysis for $Cu(N\text{-}AC)_2$ - Calculated: C (30.96%), H (4.16%), N (7.22%), S (16.53%), Cu (16.38%). Experimental: C (31.00%), H (4.01%), N (7.40%), S (16.25%), Cu (16.40%); Melting point (decomp.): >200°C; Absorption spectrum λ_{max} (diffused reflectance, nm): 340 (S-Cu), 447, 401 (πS-Cu); IR (neat solid, cm^{-1}): 3339 (νNH), 2924 (νOH), 2561 (νSH), 1711 (νCO), 1601, 1549 ($\nu_{asym}COO^-$), 1423 ($\nu_{sym}COO^-$), 1215 (νCN), 1038 (νCO), 837 (δCN); Raman (cm^{-1}) 470 (N-Cu), 423 (S-Cu); DESI-MS: $[(N\text{-}AcCyS)_2Cu]^+$ (*m/z* 387.9), $[N\text{-}AcCySCu]^+$ (*m/z* 225.7)

2.5. Preparation of Cu(II)-glutathione

Cu(II)-glutathione was prepared by grinding copper acetate monohydrate (0.400 g, 2.0 mmol) and GSH (1.2290 g, 4.0 mmol), producing a blue/green-hued complex. Elemental analysis for $Cu(GSH)_2$ - Calculated: C (35.53%), H (4.77%), N (12.43%), S (9.48%), Cu (9.40%). Experimental: C (35.33%), H (4.82%), N (12.29%), S (9.48%), Cu (9.51%); Melting point (decomp.): >230°C; Absorption spectrum λ_{max} (diffused reflectance, nm): 260 (π-π^*), 306 (S-Cu) and 700 nm (πS-Cu); IR (neat solid cm^{-1}): 3339 (νNH), 3250 (νNH), 3028 (νOH), 2349 (ν=NH_2^+), 1975 (δCH), 1713 (νCO), 1607 ($\nu_{asym}COO^-$), 1539 (δNH_3^+), 1410, 1335 ($\nu_{sym}COO^-$), 1263 (νCN), 1061, 930 (δOH), 822 (ωCH), 764 (δCH), 638 (δCOO^-); Raman (cm^{-1}) 472 (N-Cu), 422 (S-Cu); DESI-MS: $[(GS)_2Cu]^+$ (*m/z* 676.2); $[GSCu]^+$ (*m/z* 369.9).

2.6. Preparation of Cu(II)-d-penicillamine

Cu(II)- *D*-penicillamine was prepared by grinding copper acetate monohydrate (0.400 g, 2.0 mmol) and *D*-penicillamine (0.5968 g,

4.0 mmol), producing a purple-hued complex. Elemental analysis for $Cu(Pen)_2$ - Calculated: C (33.37%), H (5.60%), N (7.78%), S (17.82%), Cu (17.56%). Experimental: C (33.22%), H (5.49%), N 7.70%), S (17.71%), Cu (17.72%); Melting point (decomp.): >200°C; Absorption spectrum λ_{max} (diffused reflectance, nm): 255 nm (π-π^*) and 520 nm (πS-Cu); IR (neat solid cm^{-1}): 3701 (νOH), 3069, 2959 (νCH), 2343.51 (ν=NH_2^+), 1599 ($\nu_{asym}COO^-$), 1482 (δNH_4^+), 1377 (νCO), 1246 (δOH), 1192, 1113 (νCO), 885, 773, 679 (δCH), 563 (γCOO); Raman (cm^{-1}) 473 (N-Cu), 419 (S-Cu); DESI-MS: $[(Pen)_2Cu]^+$ (*m/z* 359.9); $[PenCu]^+$ (*m/z* 211.7).

2.7. Preparation of Cu(II)-mercaptosuccinic acid

Cu(II)-mercaptosuccinic acid prepared by grinding copper acetate monohydrate (0.400 g, 2.0 mmol) and mercaptosuccinic acid (0.6006 g, 4.0 mmol, producing a grayish-green hued complex. Elemental analysis for $Cu(MSA)_2$ - Calculated: C, 26.55; H, 2.79; S, 17.72; Cu, 17.56. C (26.55%), H (2.79%), S (17.72%), Cu (17.56%). Experimental: C (26.61%), H (2.59%), S (17.76%), Cu (17.61%); Melting point (decomp.): >200°C; Absorption spectrum λ_{max} (diffused reflectance, nm): 208 nm (π-π^*), 610 nm (πS-Cu); IR (neat solid cm^{-1}): 3505, 3296, 3267 (νNH), 3048, 2978 (νOH), 2349 (ν=NH_2^+), 1593 ($\nu_{asym}COO^-$), 1431 ($\nu_{sym}COO^-$), 1267 (δOH), 1040 (νCO), 856 (δCH), 820 (ωCH), 689 (δCH); Raman (cm^{-1}): 471 (N-Cu), 420 (S-Cu); DESI-MS: $[(MSA)_2Cu]^+$ (*m/z* 361.8), $[MSACu]^+$ (*m/z* 212.8).

2.8. Preparation of Cu(II)-dithiothreitol

Cu(II)-dithiothreitol was prepared by grinding copper acetate monohydrate (0.400 g, 2.0 mmol) and dithiothreitol (0.6170 g, 4.0 mmol); producing a parrot green-hued complex. Elemental analysis for $Cu(DTT)_2$ - Calculated: C (25.97%), H (4.90%), N (9.22%), S (34.66%), Cu (17.17%). Found: C, 25.35; H, 4.72; Cu, 17.82; S, 34.86. C (25.35%), H (4.72%), S (34.86%), Cu (17.82%); Melting point (decomp.): >200°C; Absorption spectrum λ_{max} (diffused reflectance, nm): 336 (S-Cu), 702 (πS-Cu); IR (neat solid cm^{-1}): 3480, 3366, 3270 (νOH), 2980 (νCH), 2561 (νSH), 2351 (ν=NH_2^+), 1603 (νCO), 1437 (νCH), 1042 (νCO), 925.83, 689 (δCH), 550 (γCOO); Raman (cm^{-1}) 470 (N-Cu), 422 (S-Cu); DESI-MS: $[(DTT)_2Cu]^+$ (*m/z* 370.9), $[DTTCu]^+$ (*m/z* 216.8).

2.9. Characterization

The products were characterized by elemental analysis, FT-IR (ATR) spectroscopy, Raman spectroscopy, electronic absorption (diffuse reflectance) spectroscopy, pXRD, DESI-MS and TGA. The samples were subjected to these analyses in the solid-state, probing the native state of the mechanochemical syntheses performed.

Elemental analysis was performed by use of Leco CHN628 analyzer. Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectra of the samples were recorded on FTIR 7600 (Lambda Scientific, Australia). The compound was evenly distributed on the surface of diamond crystal and the spectra were recorded at 4 cm^{-1} resolution in the transmission mode from 4000 to 450 cm^{-1}.

Raman spectra were recorded on LabRAM HR Evolution (Horiba Scientific, Japan) Raman spectrometer. Samples were placed on glass slide under optical microscope and focused using white light. After focusing the laser beam was switched on and spectra were collected from 200 scans.

The electronic spectra were recorded in diffused reflectance mode by using a Perkin Elmer Lambda 12 UV-visible spectrometer equipped with the diffuse reflectance accessory. The pXRD spectra was recorded on X'Pert PRO X-ray diffractometer (PANalytical, The Netherlands) using CuK_α radiation (λ = 1.582 Å) in the 2θ range 20–80°.

Mass spectral data was collected for solid-state samples via DESI-MS, which has been shown to allow probing of mechanochemical synthetic products [15–17] without the altering effects of liquid-phase methodologies (e.g., direct infusion electrospray ionization or LC-MS). Direct DESI-MS analysis was conducted from unprepared, solid products by placing a small (<1 mg) deposit of the powdered sample onto double-sided adhesive tape (Scotch Brand, Avon, OH) that was pre-mounted on a glass microscope slide; excess sample was removed by using a gentle stream of nitrogen gas. No significant background ions originating from the tape surface and adhesive were observed in collected spectra. Mass spectra were collected using a LCQ Fleet quadrupole ion trap (Thermo Scientific, San Jose, CA, USA) mass spectrometer in both base scan and MS^n scan modes for both molecular weight and structural information. DESI-MS was performed with the Prosolia (Indianapolis, IN) Omni Spray ionization source with a manual x,y,z translational stage for sample manipulation; the prepared sample slide fits directly into this stage, allowing full control of DESI analysis point, and standard DESI-MS parameters were utilized [18,19]. Positive-ion mode spectra were collected using a spray solvent flow rate of 2.5 µL min^{-1} and spray voltage of 4.5 kV. Inlet capillary temperature was maintained at 200°C. Multiple spray solvent compositions were investigated in order to efficiently desorb and ionize the solid-phase synthetic products. Ultimately, a 1:1:1 methanol:water:DMSO with 0.1% formic acid spray solvent showed optimal solubility and ionization efficiency for these studies..

Thermal analysis was carried out in the thermogravimetric (TGA) mode using the SDT-Q600 (TA Instruments, SA) thermal analyzer. The sample (2-3 mg) was heated from ambient to 1000 °C at 10 °C min^{-1} under nitrogen flow.

2.10. Interaction of the synthesized copper(II) complexes with DNA

The gDNA was extracted from a healthy human blood according to a standard protocol [20]. Interaction of the complexes was investigated by agarose gel electrophoresis as follows.

Preparation of solutions and 0.4% Agarose Gel

A 50 × TAE buffer (500 mL, pH 8) was prepared by mixing of tris base (121.0 g), glacial acetic acid (28.5 mL) and 0.5 M EDTA solution (50 mL). The working/running buffer was prepared by diluting this buffer 100 times using distilled water.

Agarose powder (0.4 g) was transferred to a conical flask containing 0.5 × TAE buffer (100 mL). The flask was gently swirled and heated in a microwave oven for 1 min. The flask was then gently swirled and again heated for 1-2 min until the solution became clear. The solution was allowed to cool to approximately 50–60°C and ethidium bromide (8 µL of 10 mg mL^{-1}) was carefully added. The solution was poured into the casting tray containing well former template/comb, carefully, to avoid bubble formation. The casting tray was placed on a flat surface and left undisturbed for 90 min so that gel could dry. The samples were pipetted into the wells and electrophoresis was carried out. Bromothymol dye was used as a loading dye during electrophoresis in order to make the DNA molecule denser. Bromothymol (0.2 g) was mixed with 50% glycerol (6 mL) and distilled water (4 mL).

Stock solutions of the five Cu(II) thiol complexes were prepared by adding 100 mg of each complex in five different 1.5 mL Eppendorf tubes containing DMSO (1 mL) followed by vigorous shaking. These were labeled as stock solutions.

2.11. Electrophoresis

The stock solution (5 µL) of each complex was transferred into five separate fresh Eppendorf tubes followed by addition of DNA

solution (5 µL). Blank solution contained gDNA solution (5 µL) in DMSO (5 µL). Both, blank and gDNA-complex solutions were placed in a china dish and carefully kept in a laboratory oven. The solutions were allowed to incubate overnight at 37°C, before performing agarose gel electrophoresis, the following day. To begin with the electrophoresis, 0.5x TAE buffer was filled in the electrophoresis tank till the maximum fill mark. A clean and small piece of paper tape was secured on the working shelve. Each of the gDNA-complex solution (3 µL) was transferred from the Eppendorfs onto this piece of tape with a 10 µL micropipette. Bromothymol dye (3 µL) was added from above and mixed several times (via micropipette) with each drop (3 µL) of the DNA-complex solution already present on the piece of tape. The mixture of loading dye and gDNA-complex were then sequentially loaded carefully in the wells of agarose gel with the help of a micropipette. Electrophoresis was performed at 125 V for 30 min using 0.5 × TAE buffer. Elec-

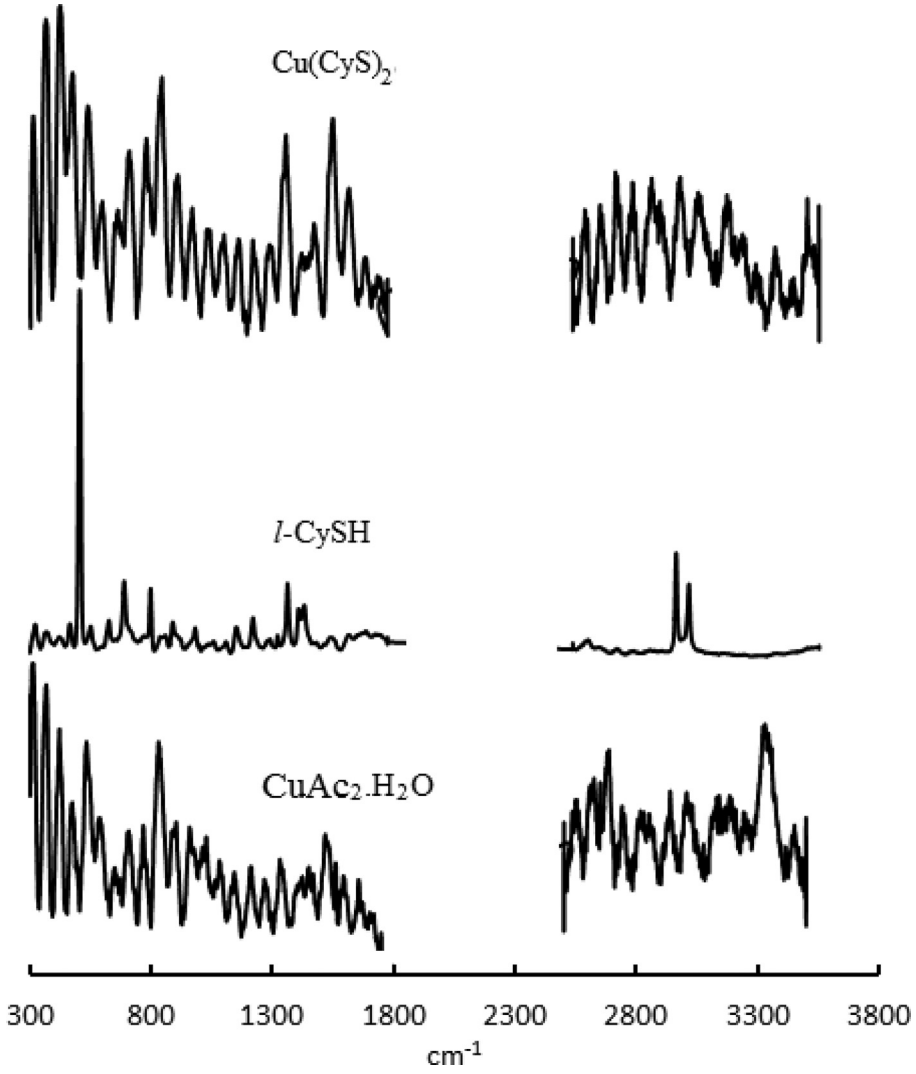

Fig. 1. Raman spectra of $CuAc_2.H_2O$, *l*-Cysteine and Cu(II)-cysteine.

Table 1
Electronic absorption spectral data of charge transfer bands of the complexes in DMF.

Complex	S-Cu/N-Cu charge transfer, nm (ε, M^{-1} cm^{-1})	πS-Cu charge transfer, nm (ε, M^{-1} cm^{-1})	Refs.
Cu(II)-cysteine	520 (3500)	703 (5800)	
Cu(II)-glutathione	490 (3000)	700 (5750)	
Cu(II)-penicillamine	550 (2920)	695 (5680)	
Cu(II)-mercaptosuccinic acid	500 (2560)	702 (4980)	
Cu(II)-*N*-acetyl-*l*-cysteine	575 (3210)	703 (5760)	
Cu(II)-dithiothreitol	545 (2830)	698 (5550)	
Plastocyanin	597 (4500)		[21]
Cu(II)-N_2S(thiolate)-S(thioether), $C_{44}H_{56}CuN_2OS$	566 (1400)	738 (5600)	[22]

trophoresis of each of the five samples was repeated three times to ensure reproducibility. After electrophoresis, the bands were visualized under UV light (254 nm).

Fluorouracil (5-FU) was selected as the standard drug for this study since it is reported to bind to DNA via intercalative mode, and like most Cu based complexes, thiolates of Cu(II) used in this work were also found to bind to gDNA through intercalation. In order to compare the extent of DNA degradation and cytotoxicity of Cu(II) thiolates, similar concentration of 5-FU was incubated overnight with DNA and electrophoresis was performed under similar conditions.

2.12. Docking of Cu(II)-cysteine with thioredoxine reductase

The structure of Cu(II)-cysteine were optimized by DFT using B3LYP basis sets in HyperChem Release 8.0.10 (Hypercube, Inc., USA) and docked with thioredoxin reductase-like protein from thermus thermophilus HB8 (PDB ID: 2ZBW). PatchDock server was used for docking study and Chimera (UCSF Chimera, USA) was used to visualize the images.

3. Results and discussion

3.1. Synthesis

The complexes were easily synthesized by the experimental procedure described above in very high yields (> 95%). The formation of the complexes was witnessed by (i) loss in weight during grinding, (ii) change in color, and (iii) evolution of acetic acid.

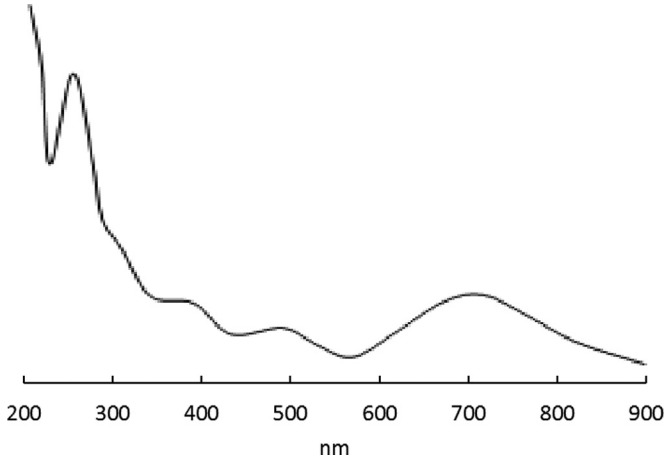

Fig. 2. Electronic absorption spectrum (diffuse reflectance) of Cu-GS.

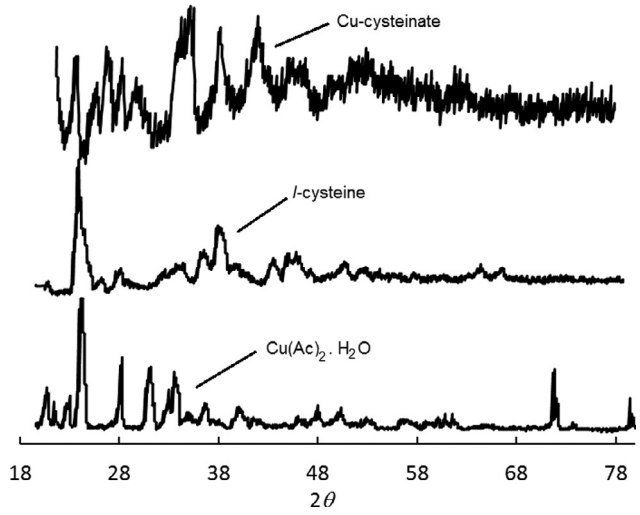

Fig. 3. Powder XRD spectra of copper acetate monohydrate, *l*-cysteine and Cu(II)-cysteine.

Table 2
DESI-MS data of the complexes with MeOH:H_2O:DMSO (1:1:1) as spray solvent (*obs.* = observed ion mass-to-charge, *theo.* = theoretical ion mass-to-charge).

Reactants	Ligand Exchange Products (m/z)	Ligand Scrambling Products (m/z)
$(CH_3COO)_2Cu$ + CySH Cu (II) Cysteine complex	$[(CyS)_2Cu]^+$ (*obs.* 303.9, *theo.* 303.96) $[CySCu]^+$ (*obs.* 183.8, *theo.* 183.95)	$[CySH]^+$ (*obs.* 122.0, *theo.* 122.03) $[CyS]^-$ (*obs.* 120.0, *theo.* 120.01) $[CySSCy]^-$ (*obs.* 239.0, *theo.* 239.02)
$(CH_3COO)_2Cu$ + GSH Cu (II) Glutathione complex	$[(GS)_2Cu]^+$ (*obs.* 676.2, *theo.* 676.09) $[GSCu]^+$ (*obs.* 369.9, *theo.* 370.01)	$[GSH]^-$ (*obs.* 306.1, *theo.* 306.08) $[GSHH]^+$ (*obs.* 307.9, *theo.* 308.09) $[GSSG]^-$ (*obs.* 612.0, *theo.* 612.15)
$(CH_3COO)_2Cu$ + Penicillamine Cu(II) Penicillamine complex	$[(Pen)_2Cu]^+$ (*obs.* 359.9, *theo.* 360.02) $[PenCu]^+$ (*obs.* 211.7, *theo.* 211.98)	$[PenH]^+$ (*obs.* 150.1, *theo.* 150.06) $[(PenSSPen]^-$ (*obs.* 296.9, *theo.* 297.09)
$(CH_3COO)_2Cu$ + Mercaptosuccinic Acid (Cu Thiomalic Acid) complex	$[(MSA)_2Cu]^+$ (*obs.* 361.8, *theo.* 361.92) $[MSACu]^+$ (*obs.* 212.8, *theo.* 212.93)	$[MSA]^-$ (*obs.* 152.1, *theo.* 151.99) $[MASSMA]^-$ (*obs.* 297.1, *theo.* 296.97)
$(CH_3COO)_2Cu$ + *N*-Acetyl-*l*-CySH Cu (II) *N*-Acetyl-*l*-CySH complex	$[(N\text{-AcCyS})_2Cu]^+$ (*obs.* 387.9, *theo.* 387.98) $[N\text{-AcCySCu}]^+$ (*obs.* 225.7, *theo.* 225.96)	$[N\text{-AcCyS}]^-$ (*obs.* 161.9, *theo.* 162.02) $[N\text{-AcCySSCyNAc}]^-$ (*obs.* 322.9, *theo.* 323.04)
$(CH_3COO)_2Cu$ + Dithiothreitol Cu(II) Dithiothreitol complex	$[(DTT)_2Cu]^+$ (*obs.* 370.9, *theo.* 370.95) $[DTTCu]^+$ (*obs.* 216.8, *theo.* 216.94)	$[DTT]^-$ (*obs.* 153.0, *theo.* 153.00) $[DTSSDT]^-$ (*obs.* 304.9, *theo.* 305.00)

Completion of reaction was ascertained by continuing grinding till the evolution of acetic acid vapors ceased. The products thus obtained were characterized without further manipulation and after purification by washing with water, methanol and ether for comparison purpose. As there was no significant difference in the values, the data reported herein are those of the unpurified complexes. The CHN analysis suggested a ML_2 (M: metal ion, L: ligand) composition of all the synthesized complexes.

3.2. Characterization

3.2.1. FT-IR and Raman spectroscopy

The FT-IR spectra of the complexes exhibited the entire absorption band present in the ligands with a disappearance of νSH around 2550 cm^{-1} and appearance of a new band around 420 cm^{-1} due to νS-Cu, indicating the bonding through the thiolate ion. The band at about 420 cm^{-1} was more prominent in the Raman spectra (Fig. 1). A shift in the νNH_2 band around 2975 cm^{-1} in the ligands to $>$ 3200 cm^{-1} was observed; this suggested coordination through the NH_2 group, whereas no significant change in νCOO^- was observed. Based on this data it could be concluded that Cu binds to the ligands through thiolate and amino groups.

3.2.2. Electronic absorption spectroscopy

A high energy band in the range 208–260 nm due to π-π^*, a lower energy band in the range 306–575 nm due to ligand to metal (S-Cu/N-Cu) charge transfer, and the lowest energy band in the range 695–703 nm due to πS-Cu charge transfer transitions were observed. In order to determine the extinction coefficients, the solution spectra were recorded in DMF and the spectral data of the complexes under investigation and the reference complexes are listed in Table 1. These charge transfer transitions compare well, in terms of energy and extinction coefficient, with similar complexes reported in literature [21,22]. The spectra also support the bonding of the ligands to Cu through thiolate and amino groups. A typical spectrum of Cu(II)-glutathione is shown in Fig. 2. The spectra exhibited features similar to Type 1 model complexes [6].

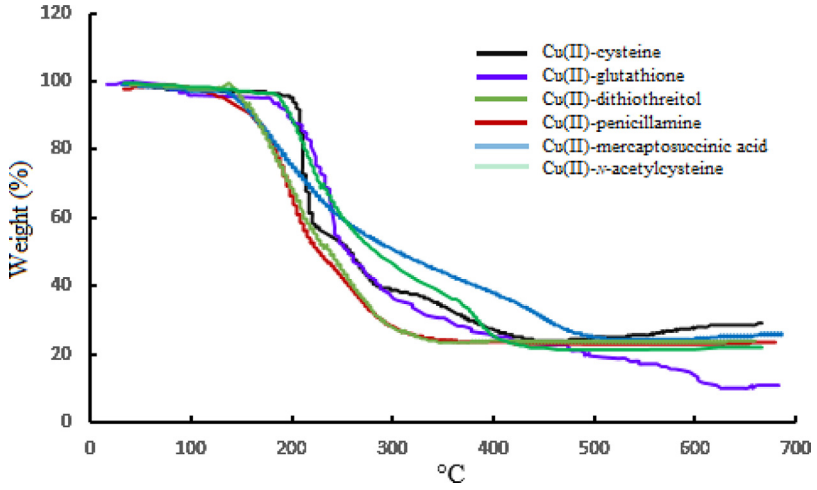

Fig. 4. TGA scans of the complexes studied in this work.

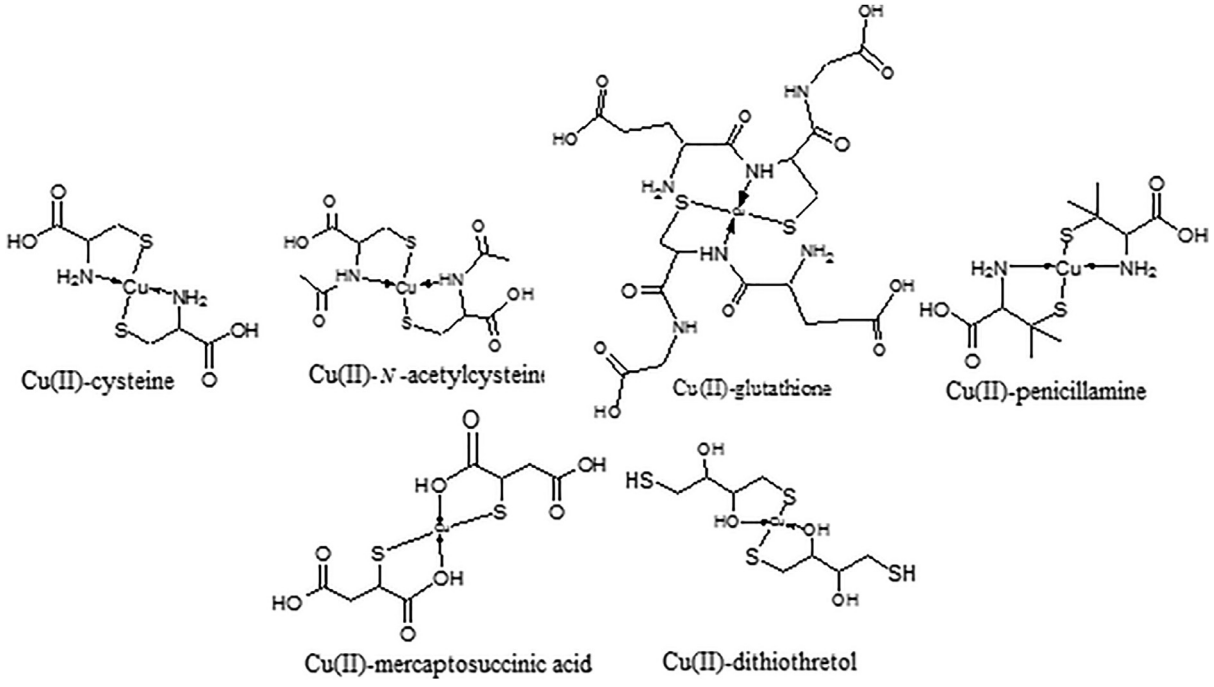

Fig. 5. Proposed structures of the complexes.

Table 3
TGA data of the complexes.

Complex	Decomposition range (°C)	Weight loss (%) (Calculated)	Remarks
Cu(II)-cysteine	200–220	38.5(39.5)	Loss of one cysteine
	220–290	14.9(15.2)	Loss of CO
	290–423	20.1(18.8), Residue 26.5	Loss of remaining part of second cysteine with CuO residue
Cu(II)-*N*-acetylcysteine	175–400	75.4(75.4), Residue 24.5	Loss of two *N*-acetylcystein ligands with CuS residue
Cu(II)-glutathione	210–600	88.3(88.2), Residue 11.7	Loss of two glutathione ligands with CuO residue
Cu(II)-penicillamine	172–300	78.0(77.9), Residue 22.0	Loss of two penicillamine ligands with CuO residue
Cu(II)-mercaptosuccinic acid	169–500	73.5(73.6), Residue 26.5	Loss of two mercaptosuccinic acid ligands with CuS residuue
Cu(II)-dithiothreitol	158–300	78.5(78.5), Residue 21.5	Loss of two dithiothreitol ligands with CuO residue

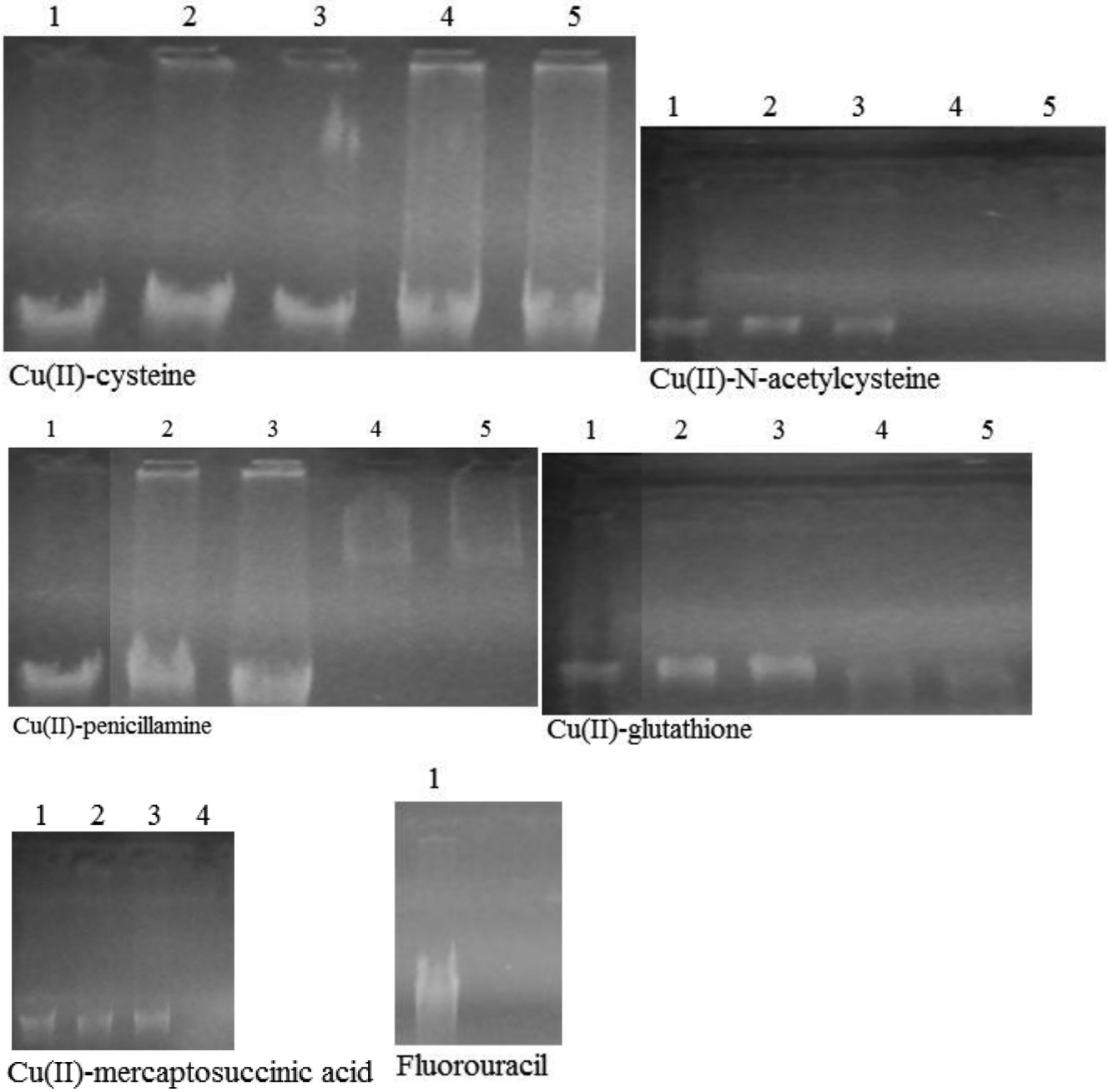

Fig. 6. Electrophoretograms showin interaction of the Cu(II) complexes with genomic DNA.

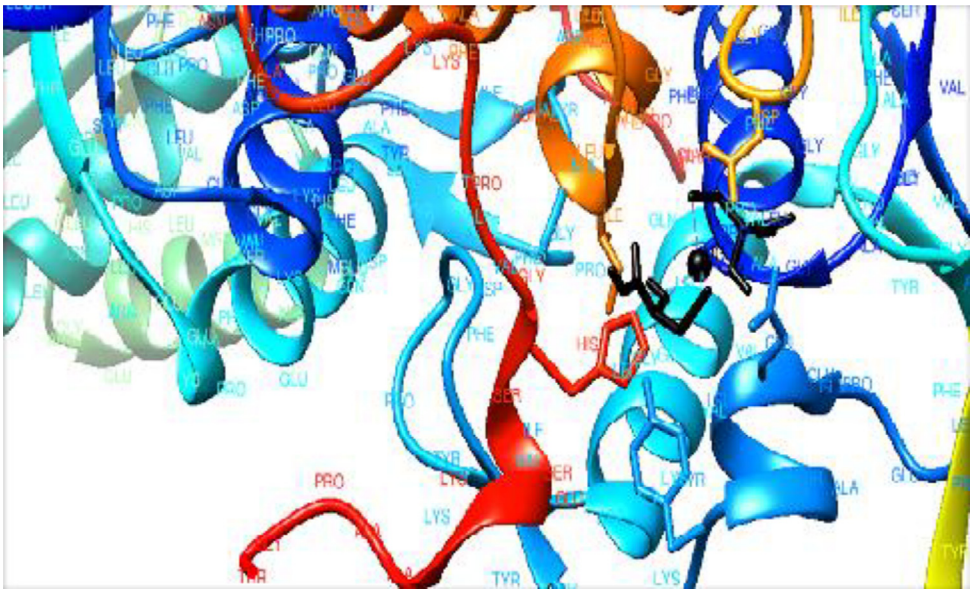

Fig. 7. Docking of Cu(II)-cysteine at the active site of thioredoxine reductase-like protein from thermus thermophilus HB8 (PDB ID: 2ZBW).

3.2.3. Powder XRD spectra

The powder XRD spectra of the complexes significantly differed with those of the ligands (Fig. 3) in peak positions and intensities, providing a strong evidence of phase change depicting the formation of the complexes.

3.2.4. DESI-MS analysis

The DESI-MS data (Table 2) confirms the ML_2 type composition of the complexes under investigation due to the presence of their molecular ion peaks. The peaks due to scrambling of ligands were also present. In addition to these, the peaks due to formation of disulfides from the ligands were also visible. The absence of peaks due to copper acetate and acetate ions further confirmed the completion of reaction. The data also confirmed the anhydrous nature of the complexes.

3.2.5. Thermal analysis

Thermal analysis data are presented in Table 3, and TGA scans for the complexes are shown in Fig. 4. A stepwise removal of the ligands was recorded in the case of Cu(II)-cysteine, whereas in all other cases a single step decomposition was observed, leaving behind CuO as the residue except in two complexes, Cu(II)- *N*-acetylcysteine and Cu(II)-mercaptosuccinic acid, where the residue came out to be CuS. These data also confirm the ML_2 composition of the complexes.

The analytical and spectroscopic evidence obtained in this work supports square-planar structures of the complexes under investigation as shown in Fig. 5.

3.3. Interaction of the copper(II) complexes with DNA

The electrophoretograms for the interaction of the synthesized Cu(II) complexes with DNA is shown in Fig. 6. The results are discussed as follows:

Cu(II)-cysteine : The denser band (lane # 2) indicates that Cu(II) acetate has formed an adduct with DNA; the band in lane # 3 is similar to that obtained for the blank (lane # 1), showing that cysteine did not interact with DNA, whereas Cu(II)-cysteine (lane # 4), like Cu(II) acetate, appeared to form an adduct. This association of the complex with the DNA may be through a ligand exchange mechanism or an extension in coordination number of Cu(II) from 4 to 5 or 6. This complex did not result in extensive degradation of DNA, like 5-fluorouracil.

Cu(II)-N-acetylcysteine and Cu(II)-mercaptosuccinic acid : These complexes completely degraded the DNA, the activity better than that of 5-fluorouracil, suggesting that Cu(II)- *N*-acetylcysteine and Cu(II)-mercaptosuccinic acid are highly active as cytotoxic agents. Thus, these complexes can be the potential candidates for cancer treatment.

Cu(II)-glutathione and Cu(II)-penicillamine: These complexes extensively, but not completely, degraded the DNA as some diffused bands with lower density were visualized. Thus, they show comparable activity to that of 5-flurouracil. Like 5-flurouracil, these complexes have the tendency to change the conformation and structure of DNA [23].

Lane # 1: DMSO + DNA, 2: Cu(II) acetate + DNA, 3: Ligand + DNA, 4 : Cu(II)-complex + DNA

3.4. Docking of Cu(II)-cysteine with thioredoxine reductase

The result of docking of the optimized structure of Cu(II)-cysteine complex with thioredoxine reductase-like protein from thermus thermophilus HB8 (PDB ID: 2ZBW) is depicted in Fig. 7. The enzyme plays an active role in cell growth and survival and is considered as a target for anti-cancer therapy. The enzyme is upregulated in various types of cancer [24,25]. The low molecular-weight thioredoxin reductase has recently been demonstrated to be a target for auranofin and ebselen [26]; its use has also been suggested for antibiotic resistant bacterial infections [27]. The complex docked in the active site of the enzyme with docking score of 3556 and binding energy of -7.22 kCal mol^{-1}. This preliminary docking study suggests that the potential of copper(II)-thiolates should be explored as anti-cancer, anti-fungal and anti-bacterial agents.

4. Conclusions

Anhydrous Cu(II)-thiolates having ML_2 composition were successfully synthesized in solid state and characterized by different analytical techniques. The thiol ligands were: *l*-cysteine, *N*-acetylcysteine, *l*-glutathione, *l*-penicillamine, mercaptosuccinic acid and *dl*-dithiothreitol. The complexes possessed square-planar geometry as revealed by spectroscopic data. All the complexes interacted with gDNA; Cu(II)- *N*-acetylcysteine and Cu(II)-

mercaptosuccinic acid completely degraded it whereas the others formed adducts.

Authors' contributions

All authors contributed equally.

Compliance with ethical standards

The study was approved by the Institutional Review Board of FC College in 2019 and was conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki 1964).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Syed G.T. Kazimi: Methodology, Data curation, Validation, Investigation. **Mohammad S. Iqbal:** Conceptualization, Visualization, Writing – original draft, Writing – review & editing, Supervision. **Christopher C. Mulligan:** Methodology, Data curation, Writing – review & editing. **Maryam Baseer:** Methodology, Data curation, Validation. **Atta U. Rehman:** Data curation, Validation. **Fatima Farooqi:** Methodology, Software, Validation. **Jonathan R. Person:** Methodology, Data curation, Validation.

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