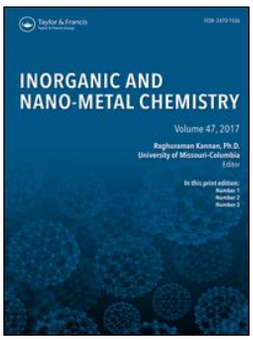

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A guanylurea ligand and its Cu(II), Ni(II) and Zn(II) complexes: antibacterial activities and DNA binding properties

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ABSTRACT

New Cu(II), Ni(II), Zn(II) complexes of a guanylurea ligand were synthesized and their structures were characterized by spectroscopic and analytical methods. The ligand was prepared by the hydrolysis of dicyandiamine in the presence of glacial acetic acid and obtained as acetate salt (HL·OAc). Molecular structure of the ligand and its Cu(II) complex were determined by single crystal X-ray diffraction studies. In the structure of the Cu(II) complex, each Cu(II) ion is four-coordinate with an approximate square planar geometry. The structures of the ligand and its Cu(II) complex were stabilized by hydrogen bond interactions. Antibacterial activity of the complexes was evaluated by single disk method. The complexes exhibit antibacterial activity. The synthesized compounds were screened for their FSds-DNA properties and Zn(II) complex showed higher binding affinity than free ligand and its Cu(II) and Ni(II) complexes.

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KEYWORDS
Dicyanamide; metal complexes; X-ray; antibacterial activity; DNA binding

Introduction

Recently, metal structures have begun to attract great attention in the world of science with the expansion of their uses and physical chemical and mechanical properties.[1] Dicyandiamide (DCDA) or cyanoguanidine has found medicinal and industrial applications.[2,3] In industry, DCDA is used for melamine production and the basic ingredient of amino plastics and resins.[4] It is also widely used in the synthesis of several organic compounds such as fertilizers, fire retardant agents, epoxy laminates, powder coatings and adhesives, leather, rubber and explosive industries.[4] DCDA and its derivatives are also of biological interest.[5–7] DCDA has been used extensively as a building block in supramolecular chemistry and crystal engineering.[5] DCDA and cyanamide, monomer of DCDA, have been reported that they interact with aldehyde dehydrogenases in the livers.[8] The DCDA derivatives have been showed to exhibit a wide range of biological activities including anti-hypertensive, anti-cancer, anti-histaminic and anti-microbial propeerties.[6,9] The DCDA is susceptible to the acid and base. In the presence of dilute acid, for example, it forms guanylurea while in the presence of base (ammonia or amines) it forms biguanide.[10] The reaction of DCDA with alcohols in the presence of Cu(II), Ni(II) and Pd(II) ions results is the formation of 1-amidino-O-alkylureas.[11–13] The DCDA and its derivatives have also been used to prepare supramolecular structures *via* hydrogen bonding interactions.[14–16] Dicyandiamide and its derivatives are also of biological interest that antibacterial and antifungal activity have been investigated as medical, crop protection agents and antiseptics for industry products, food and other goods for daily use.[17]

In the current work, we prepared an acetate salt of guanyurea (HL·OAc) ligand and its Cu(II), Ni(II) and Zn(II) complexes. The synthesized compleounds were characterized by spectroscopic and analytical methods. Solid state structures of the ligand and its Cu(II) complex were determined by single crystal X-ray diffraction studies. Antibacterial and antifungal activity of complexes was determined. Finally, DNA binding properties of complexes were examined.

Experimental

Materials

Dicyandiamide (DCDA), $Cu(ClO_4)_2.6H_2O$, $Ni(ClO_4)_2.6H_2O$ and $ZnCl_2.6H_2O$ were purchased from Aldrich Chemical Company and used as received. All other reagents used in this study were purchased from commercial sources. Glacial acetic acid was purchased from Sigma Aldrich and used without further purification.

Caution: Salts of perchlorate and their metal complexes are potentially explosive and should be handled with great care and in small quantities.

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Supplemental data for this article can be accessed at the publisher's website

Physical measurements

X-ray crystallographic data collection and cell refinement for HL·OAc and $[Cu(L)_2]\cdot(ClO_4)_2$ were carried out using a Bruker D8 QUEST diffractometer and data reduction was performed using Bruker SAINT. Diffraction data were measured at 293(2) K using Mo-$K\alpha$ radiation with a Bruker ApexII diffractometer.[18] SHELX-2014/6 was used to solve and refine the structures.[19,20] The structures were solved by direct methods and refined on F^2 using all reflections. All the non-hydrogen atoms were refined using anisotropic atomic displacement parameters. Details of the crystal data and refinement are given in Table 1.

Synthesis of guanylurea acetate (HL·OAc)

Dicyandiamide (2 mmol) was added to solution of glacial acetic acid-water mixture (20 mL-) and refluxed 24 h. When mixture was cooled, precipitates were collected and dried in air.

Yield: 1.71 g, 89%. Color: White. M.p.: 213–218 °C. Elemental analysis Calc. (Found) for $C_4H_{10}N_7O_3$ (162.15 g/mol): C, 29.63 (29.15); N, 34.55 (34.22); H, 6.22 (5.98)%.

^{1}H-NMR (d_6-DMSO, ppm): 2.00 (1H, s, NH), 2.10 (3H, *s*, -$CH_{3(acetate)}$), 6.20 (2H, *s*, NH_2), 6.60 (2H, *s*, NH_2), 7.88 (2H, *s*, NH_2). ^{13}C-NMR (d_6-DMSO, ppm): 20.8 ($CH_{3(acetate)}$), 155.0 ($C_{guanylurea}$), 158.5 ($C_{guanylurea}$), 188.5 ($C_{acetate}$). FT-IR (cm^{-1}): 618, 681, 761, 815,924, 1028, 1122, 1202, 1350, 1396, 1464, 1580, 1610, 1692, 1714, 3372, 3449.

Synthesis of the Cu(II) and Ni(II) complexes

Guanylurea acetate (HL·OAc) (1 mmol) was dissolved in MeOH (10 mL) and NaOH (1 mmol) was added. The reaction mixture was refluxed for 2 h and followed by addition of $Cu(ClO_4)_2\cdot6H_2O$ (0.5 mmol) or $Ni(ClO_4)_2\cdot6H_2O$ (0.5 mmol).The resulting solution was refluxed for 24 h. When cooled to the room temperature, precipitates were filtered, washed with MeOH and dried in air.

$[Cu(L)_2]\cdot(ClO_4)_2$: Yield: 0.228 g, 63%. Color: Purple. M.p.: 248–250 °C (decomp.). Elemental analysis Calc. (Found) for $C_4H_{12}CuN_8O_{10}Cl_2$ (466.64g/mol): C, 10.30 (10.12); N, 24.01 (23.45); H, 2.59 (2.44)%. FT-IR (cm^{-1}): 618, 674, 738, 930, 1080, 1246, 1360, 1487, 1625, 1673, 3341, 3386, 3458, 3489.

$[Ni(L)_2]\cdot(ClO_4)_2$: Yield: 0.225 g, 61%. Color: Orange. M.p. = 240–242 °C. (decomp.). Elemental analysis Calc.(Found) for $C_4H_{12}Cl_2NiN_8O_{10}$ (461.79 g/mol):) : C, 10.40 (10.21); N, 24.27 (24.05); H, 2.62 (2.38)%. FT-IR (cm^{-1}): 612, 638, 740, 767, 941, 1088, 1262, 1292, 1425, 1544, 1644, 3362, 3461.

Synthesis of the Zn(II) complex

Guanylurea acetate (HL·OAc) (1 mmol) was dissolved in MeOH (10 mL) and NaOH (1 mmol) was added. The reaction mixture was refluxed for 2 h and followed by addition of $ZnCl_2\cdot4H_2O$ (1 mmol). The resulting solution was refluxed for 24 h. When cooled to the room temperature, precipitates were filtered, washed with MeOH and dried in air.

Table 1. Crystallographic data.

Identification code	HL·OAc	$[Cu(L)_2]\cdot(ClO_4)_2$
Empirical formula	$C_4H_{10}N_4O_3$	$C_4H_{12}N_8O_{10}Cl_2Cu$
Formula weight	162.16	466.66
Temperature (K)	293(2)	293(2)
Crystal system	*Monoclinic*	*Monoclinic*
Space group	$P2_1$	$P2_1/c$
Unit cell		
a (Å)	7.74830(10)	5.5397(15)
b (Å)	9.1045(2)	11.850(4)
c (Å)	11.4601(2)	12.802(4)
α (°)	900	90
β (°)	91.4160(10)	115.64
γ (°)	90	90
Volume (Å^3)	808.20(2)	757.6(4)
Z	4	2
Calculated density (g/cm^3)	1.333	2.046
Abs. coeff. (mm^{-1})	0.113	1.867
Refl. collected	11557	16050
Ind. reflections [R_{int}]	3781 [0.0219]	1548 [0.0887]
R1, wR2 [$I > 2\sigma$ (I)]	0.0334, 0.0860	0.0461, 0.1045
R1, wR2 (all data)	0.0386, 0.0897	0.0692, 0.1152
Goodness-of-fit on F^2	1.057	1.082
Completeness to theta	99.7%	99.9%
CCDC Number	1990705	1990706

$[Zn(L)Cl_2]$: Yield: 0.215 g, 55%. Color: White. M.p.: 243–249 °C. Elemental analysis Calc. (Found) for $C_2H_6Cl_2N_4OZn$ (238.38 g/mol):) : C, 10.08 (9.65); N, 23.50 (23.32); H, 2.54 (2.32)%. FT-IR (cm^{-1}): 735, 813, 921, 1044, 1144, 1337, 1453, 1587, 1682, 1728, 3202, 3277, 3363, 3449.

Antibacterial activity of the compounds

Preparation and cultivation of strains

In this study, gram-positive (+); *Staphylococcus Aureus* Rosenbach ATCC-6538, *Bacillus Subtilis* Ehrenberg ATCC-14028, *Bacillus Cereus* ATCC 7064, gram- negative (-); *Escherichia Coli* ATCC-8739, Citrobacter sp. *Salmonella Typhimurium* strains were inoculated to Muller Hilton Broth (MHB) and incubated for 18 h at 37 ± 1 °C to provide activation. For antimicrobial activation Müller Hilton Agar (MHA) was used as the broth. Bacteria standardized with 0.5 McFarland Standard was inoculated to sterile prepared petri dishes and incubated for 1 h at 37 ± 1 °C.[21] To control disk included DMSO Amikacin (AK: 30 μg) and Gentamisin (CN: 10 μg) were used.

Preparation of disks from complexes and application

Antimicrobial activation of complexes synthesized was determined using Kirby-Bauer Disk Difussion Method.[22,23] The synthesized complexes were dissolved in 10% DMSO and impregnated with disks at a concentration of 25 μL to disks made of blank sterile whatman papers of 6 mm diameter. Prepared disks were placed on the cultivation of bacteria in the MHA. Disks were incubated at 37 ± 1° C for 18–24 ± 2 h to determine inhibition zones.[24,25] The study was performed in three replicates and the mean values were given.

Minimum inhibition concentration

For the determination of minimum inhibition concentration (MIC) values of the synthesized complexes, the minimum

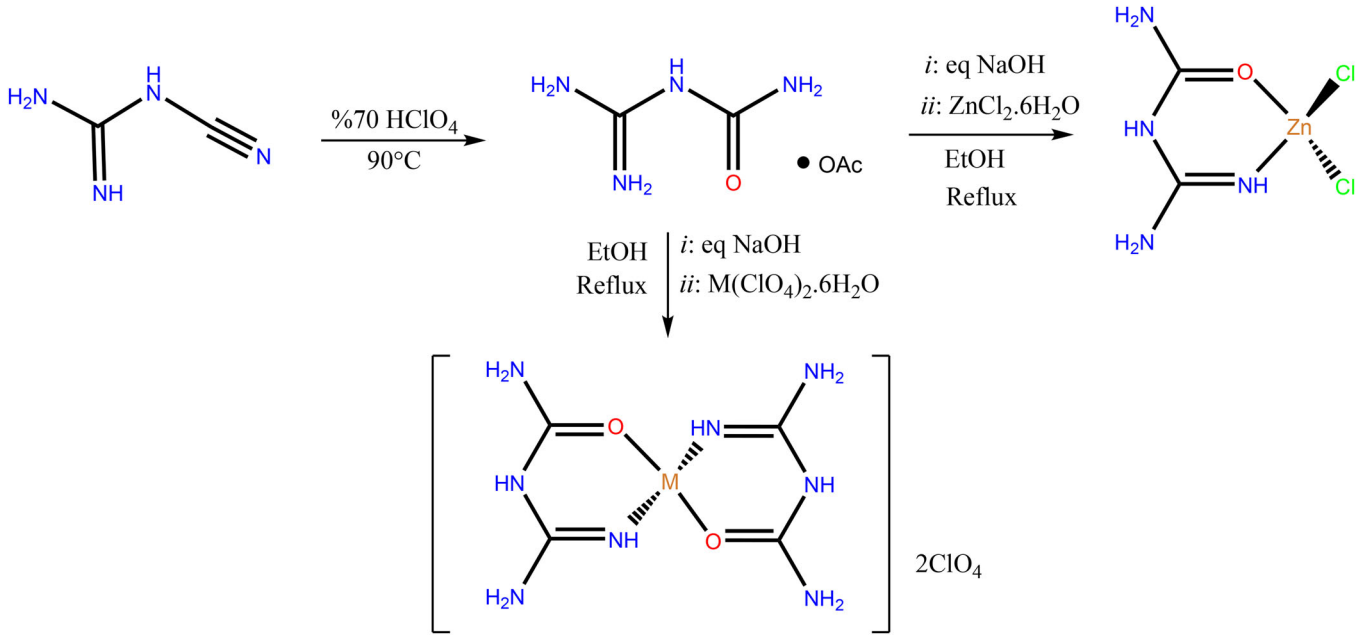

Figure 1. Synthesis of the ligand and its complexes.

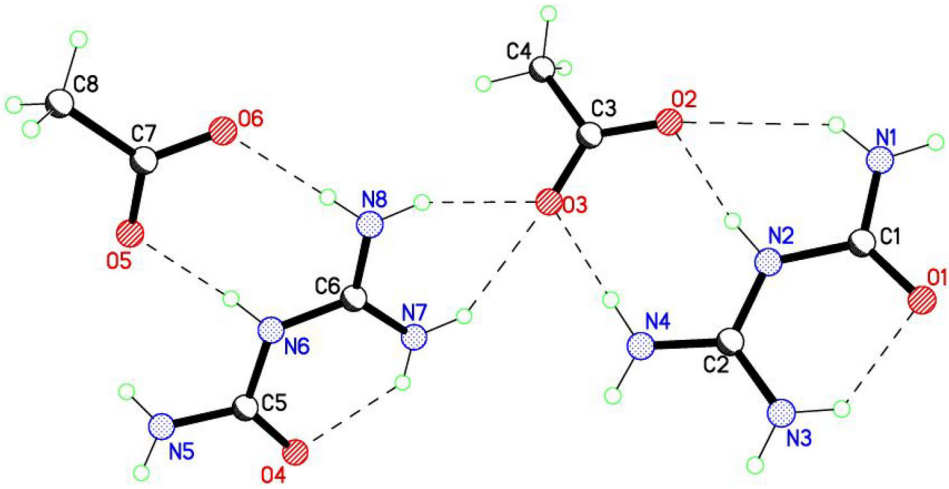

Figure 2. Molecular structure of [HL·OAc]. The NH···O hydrogen bonds are shown as dashed lines.

effect values determined for gram positive (+) and gram negative (-) bacteria strains were determined by dissolving with 10% DMSO to 12500 μg/mL, 1250 μg/mL and 125 μg/mL.

DNA binding properties of complexes

In order to determine the DNA binding capacity of the ligand and metal complexes, double-strand FSds-DNA (Aldrich) was used as received without further purification. The DNA stock solution was prepared in Tris-HCl buffer (20 mM Tris-HCl, 20 mM NaCl, pH 7.0) at room temperature and stored at 4 °C. In UV titration experiments, the spectra of FSdsDNA in the presence of HL·OAc, $[Cu(L)_2]\cdot(ClO_4)_2$, $[Ni(L)_2]\cdot(ClO_4)_2$ and $[Zn(L)Cl_2]$ have been recorded for a constant FSds-DNA concentration in diverse [Compound]/[FSdsDNA] mixing ratios (r). The intrinsic binding constant, Kb, of the with FSds-DNA has been determined through the UV spectral measurements. The stock solution of the ligand and complexes were prepared in DMSO (1×10^{-5} M).

Results and discussion

In this work, guanylurea acetate (HL·OAc) was first obtained *via* hydrolysis of dicyandiamide in glacial acetic acid in high yield and purity. The monoprotonated compound was then neutralized by an equivalent NaOH before complexation with Cu(II), Ni(II) and Zn(II) ions. The reaction of two equivalents guanylurea ligand with an equivalent $Cu(ClO_4)_2\cdot6H_2O$ or $Ni(ClO_4)_2\cdot6H_2O$ in MeOH gave metal complexes with general formulae of $[M(L)_2](ClO_4)_2$ (where metal: Cu(II) or Ni(II)) complexes with 2:1 ratio (ligand: metal). The mononuclear Zn(II) complex was prepared by the reaction of eqivalents amount of ligand and $ZnCl_2$ in MeOH (Figure 1).

FT-IR spectra of the guanylurea acetate (HL·OAc) and its Cu(II), Ni(II) and Zn(II) complexes were recorded in the

range of 4000–450 cm^{-1} and the data are given in experimental section. In the FT-IR spectra of the synthesized complexes, the stretching peaks observed at 3300–3400 cm^{-1} are due to asymmetric and symmetric ν(N-H) vibrations. The stretching vibrations of the nitrile group (C≡N) observed in the FT-IR spectrum of the starting dicyandiamide molecule completely disappeared in the spectra of the all complexes.[26] The disappearance of the band due to nitrile group confirms the addition of water molecule to the nitrile group. In the spectra of the complexes, the characteristic ν_s(C-O-C) and ν_a(C-O-C) vibrations were observed at 1028 and 1122 cm^{-1}, respectively. In the FT-IR spectrum of the guanylurea acetate (HL·OAc), the sharp strong peaks at 1692 and 1714 cm^{-1} are due to the ν(C=O) and $\nu(COO)_{acetate}$ stretchings. The characteristic ν(C=N) vibration band of the guanylurea was observed in 1610 cm^{-1}. In the spectra of the complexes, $\nu(COO)_{acetate}$ stretchings were completely absent and the carbonyl group stretching ν(C=O) was observed at lower wavenumbers suggesting the coordination of this group to the metal center. The imine group stretchings ν(C=N) in the spectra of the complexes

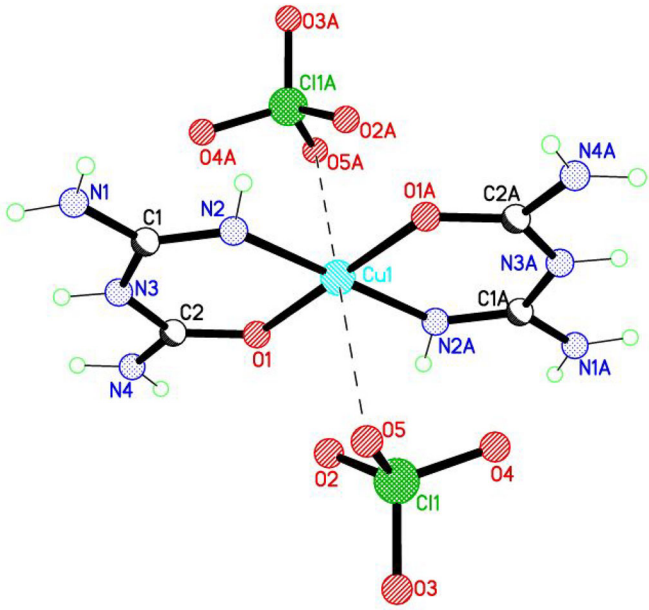

Figure 4. Molecular structure of $[Cu(L)_2]\cdot(ClO_4)_2$. The NH····O hydrogen bonds are shown as dashed lines.

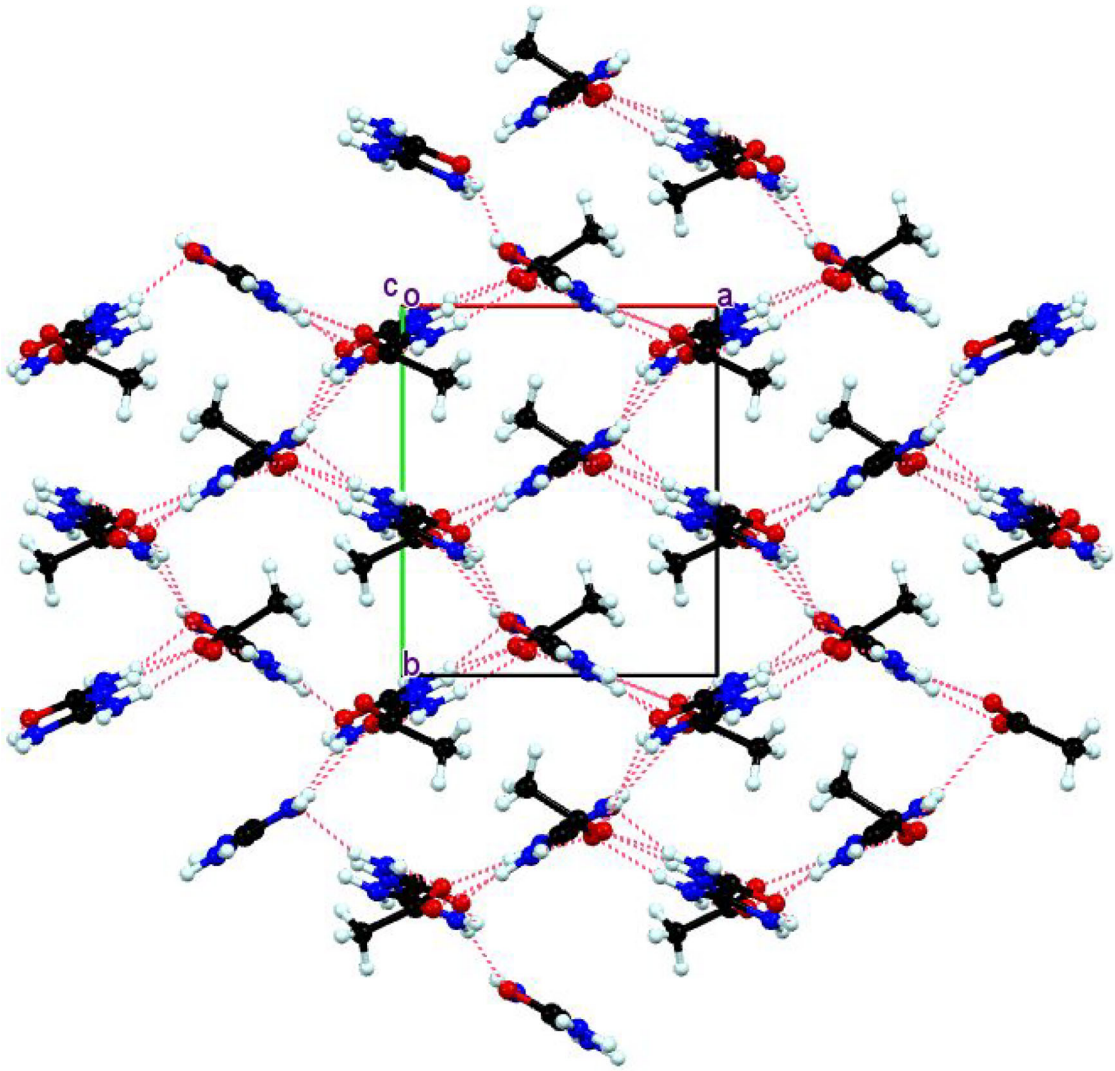

Figure 3. The NH···O hydrogen bond network in [HL·OAc]. Hydrogen bonds are shown as dashed lines.

shifted to higher wavenumbers confirming the coordination of this group to the metal centers. Moreover, the sharp perchlorate $\nu(ClO_4)$ stretchings were seen at around 1080 and 610 cm^{-1} in the spectra of the Cu(II) and Ni(II) complexes indicating the presence of the perchlorate ions as counter ions.

Crystal structure of guanylurea acetate (HL·OAc)

The single crystals of guanylurea acetate were obtained from slow evaporation of ethanol solution of the compound. The compound crystalizes in monoclinic unit cell $P2_1$ space-group. The assymetric unit contains two independent monoprotonatedguanylurea cations and two acetate anions balancing the positive charge. The perspective view of the assymetric unit is shown in Figure 2. The X-ray crystallographic data showed that the two independent cations in the assymetric unit are almost same differing only in the intermolecular hydrogen bond interactions. In the structure of the compound, the O(1)-C(1) bond distance of 1.227(2) Å is slightly longer than characteristic C=O double distance. The C1-N1 bond is shorter (1.323(3) Å) than that of C-N single bond suggesting the π-electron delocalization in ureic fragment of the cation. The C-N distances in the guanide part of the cation confirms the the π-electron delocalization in the whole mono-protonated cation and these values are in well angrement with the chloride or perchlorate salts of the same cation.[27] Both cations showed intra molecular

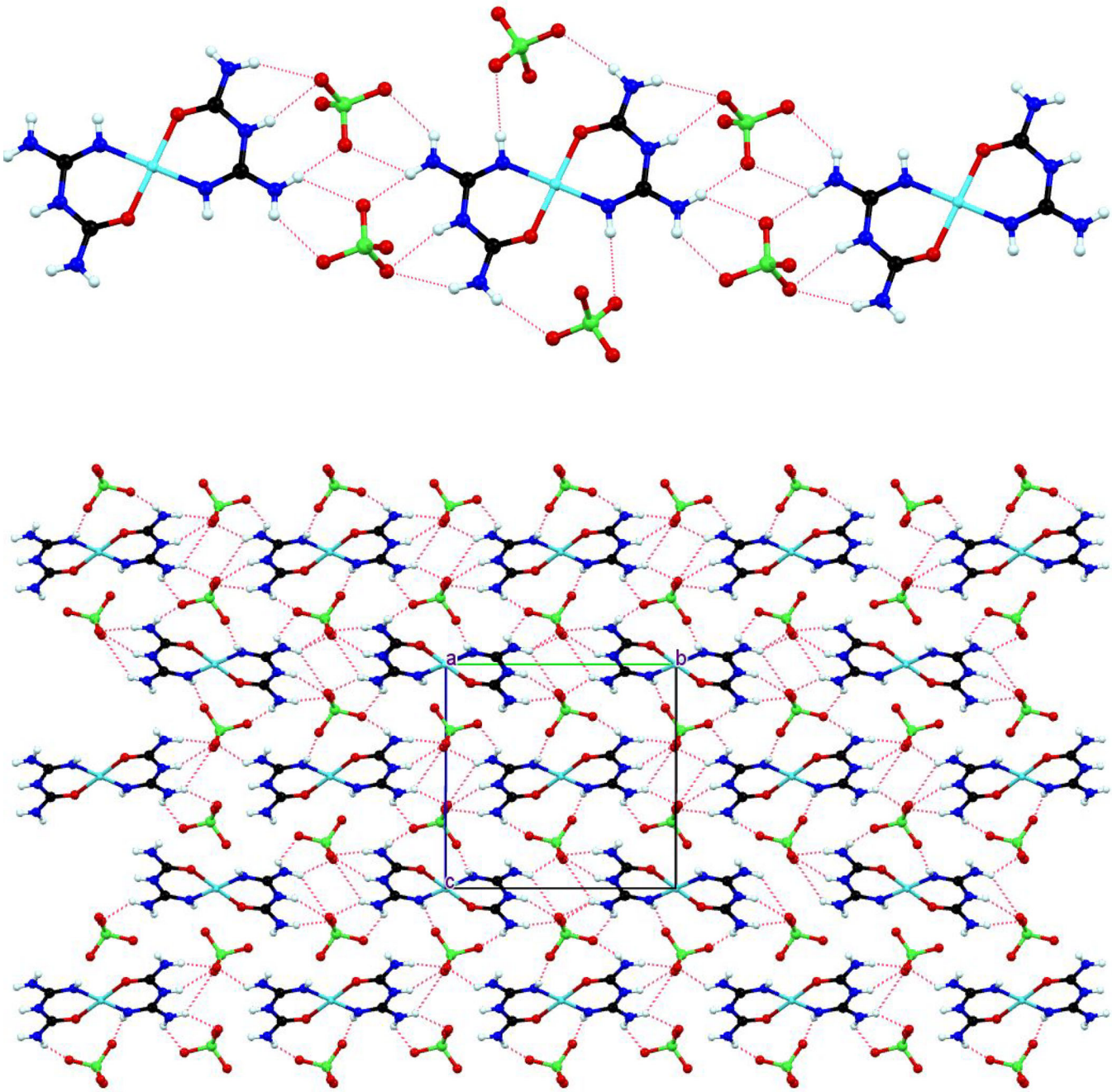

Figure 5. The 2D hydrogen bond networks in and $[Cu(L)_2]\cdot(ClO_4)_2$. Hydrogen bonds are shown as dashed lines.

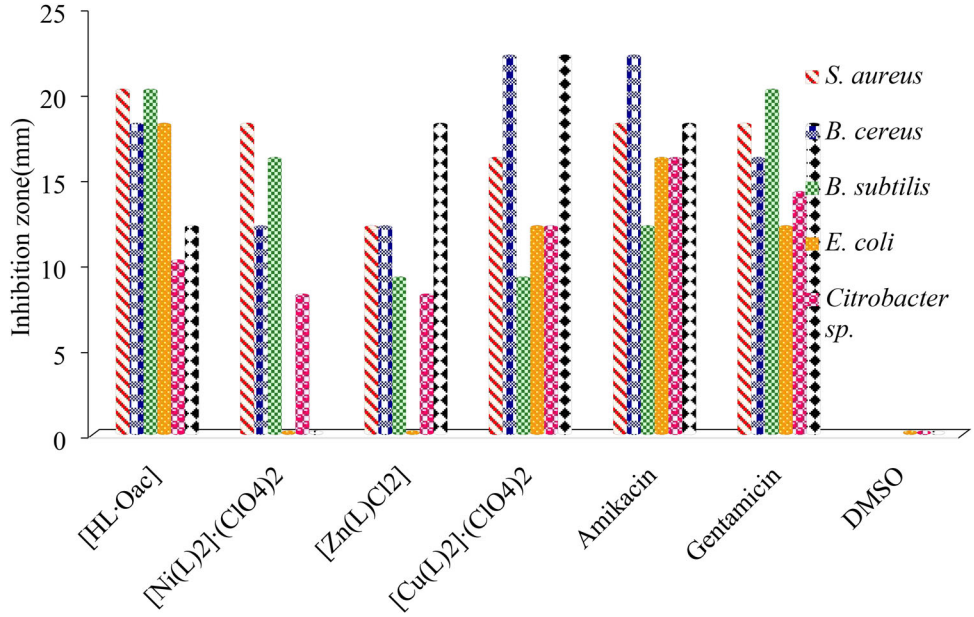

Figure 6. Antibacterial activity of the complexes showing inhibition zones in (mm).

NH·····O hydrogen bond formin a S(6) graph motif and uretic oxygen atoms also involve in intermolecular hydrogen bonds with the -NH_2 groups of the adjacent molecules. The uretic O1 atom makes two hydrogen bonds while the other uretic oxygen atom O4 in the second cation makes three hydrogen bonds. The acetate anions further link cations via hydrogen bonding interactins. The oxygen atoms of the acetate anions make hydrogen bonds with the bridging -NH- and terminal -NH_2 groups reslting in $R^2_2(8)$ and $R_2(6)$ hydrogen bond graph sets. The NH·····O hydrogen bond contacts form 3 D supramolecular network as show in Figure 3.

Crystal structure of $[Cu(L)_2]\cdot(ClO_4)_2$

The single crystals of the Cu(II) complex was obtained from slow evaporation of the reaction solution. The structure of the complex was solved in *monoclinic* unit cell $P2_1$ space group. In the structure, the Cu(II) ion locates on an inversion center and the asymmetric unit contains half of the complex cation, a perchlorate counter ion resulting in a general formulae of $[Cu(L)_2](ClO_4)_2$. The structure of the complex is shown in Figure 4. In the structure, two guanylurea ligands are coordinated to the Cu(II) center *vi* carbonyl (C=O) oxygen and imine nitrogen (C=N) donor atoms. The two ligands around the Cu(II) center are *trans-located* and the geometry around the metal ion is square planar. One of the oxygen atom of the perchlorate anion is weakly interacts with the Cu(II) center with Cu1-O5 distance of 2.869(5) Å. In the structure, the C2-O1 and C1-N2 bond distances are 1.260(5) and 1.274(5) Å, respecetively, within the range of C=O and C=N double bond distances. The complex cations $[Cu(L)_2]^{2+}$ are linked by NH·····$OClO_3$ hydrogen bonds forming a 3 D hydrogen bond network (Figure 5). In the complex cations, all of the N-H groups involve in hydrogen bond interactions with perchlorate ions and each perchlorate ion make eight hydrogen bonds. Hydrogen bond parameters are given in the supplementary documents.

Antibacterial activity of the compounds

The biological activities of the ligand and metal complexes used in the study are shown in Figure 6. In the study, it was determined that the guanylurea acetate (HL·OAc) and complexes showed 8–22 mm diameter inhibition zones against gram positive and gram-negative microorganisms. Guanylurea acetate (HL·OAc) showed considerable activity toward microorganisms tested when compared to the standart antibiotics. The acvitiy of the guanylurea acetate (HL·OAc) was higher than $[Ni(L)_2]\cdot(ClO_4)_2$ and $[Zn(L)Cl_2]$. Both Ni(II) and Zn(II) complexes are not effective to that of *E. Coli*. The Cu(II) complex $[Cu(L)_2]\cdot(ClO_4)_2$ showed the highest inhibiton zones with 28 mm against the *Salmonella typhimurium* strain. It has been determined that it has more effect than the standart antibiotic disks (amikacin and gentamicin). In the same way, for the Cu(II) complex, the 20 mm inhibition zone against the gram-positive *Bacillus Cereus* was found to be more effective than the antibiotics Amikacin (18 mm) and Gentamicin (18 mm).

The MICs of complexes on microrganism strains are given in Table 2. Metal complexes showed different antimicrobial activity on microorganism strains. Gram-positive and gram-negative strains generally had a minimum inhibition concentration of 12500 μg/mL. At this concentration, complexes $[Ni(L)_2]\cdot(ClO_4)_2$ and $[Zn(L)Cl_2]$ are not effective toward *E. Coli*. The Ni(II) complex is also not effective against to *S. Typhimurium* at 12500 μg/mL. The Cu(II) complex is effective against Citrobacter *sp*. strain as low concentration as 1250 μg/mL.

DNA binding properties

UV absorption spectra analysis

UV-vis spectrophotometric method is one of the most common and simple methods used to determine the interaction of nucleic acids with small molecules. The easiest way to determine the interaction is to monitor the changes in the

Table 2. Minimum inhibition concentrations (MIC) of the compounds to strains.

MIC (μg/mL)

	Gram negative			Gram positive		
Compound	*E. coli*	*Citrobacter sp.*	*S. typhimurium*	*S. aureus*	*B. cereus*	*B. subtilis*
HL·OAc	12,500	12,500	12,500	12,500	12,500	12,500
$[Ni(L)_2]\cdot(ClO_4)_2$	—	12,500	—	12,500	12,500	12,500
$[Zn(L)Cl_2]$	—	12,500	12,500	12,500	12,500	12,500
$[Cu(L)_2]\cdot(ClO_4)_2$	12,500	1250	12,500	12,500	12,500	12,500

Table 3. K_b and K_{sv} values of complexes.

Compound	K_b	K_{sv}
HL·OAc	5.3×10^4	2.02×10^3
$[Cu(L)_2]\cdot(ClO_4)_2$	4.7×10^4	1.73×10^4
$[Ni(L)_2]\cdot(ClO_4)_2$	6.2×10^4	2.75×10^4
$[Zn(L)Cl_2]$	2.3×10^5	1.23×10^5

drug molecule bands before and after the interaction. The interaction with the DNA of the drug shows different behaviors depending on the binding mode. These attachment modes are grouped in three groups. These are, respectively, DNA double-helix structure to connect to large and small grooves, electrostatic interactions and DNA is intercalation between base pairs.

Interactions of novel ligand and metal complexes with DNA can indeed provide valuable finds out the development of effective drugs. Therefore, UV-visible absorption titration and fluorescence spectroscopy were performed to explore the interaction of the synthesized compounds with DNA.

A single absorption band was observed in the ligand, whereas two absorption bands were observed in the complexes. The UV-visible spectra of the ligand, Cu(II), Ni(II) and Zn(II) complexes in different concentrations of FSds-DNA are presented. The absorption band of the ligand exhibited residuals hypochromism and minor bathochromism as a result of the increasing FSds-DNA concentration. The results of the UV − visible absorption titration specified the interaction of the ligand with DNA.[28] As this band is absent in the spectra during the addition of the FSds-DNA to the [HL·OAc] in Tris − HCl buffer (Figure 7). The K_b value of the ligand obtained from the absorption data is 5.3×10^4 M^{-1}.

A single absorption band was observed in the ligand, whereas two absorption bands were observed in the complexes. The complexes $[Cu(L)_2]\cdot(ClO_4)_2$ and $[Ni(L)_2]\cdot(ClO_4)_2$ and $[Zn(L)Cl_2]$ exhibit absorption bands in the UV region at 252, 263 and 270 nm, respectively. By the addition of increasing amounts of FSds-DNA to the complex solutions, a sharp hyperchromic effect in the absorption bands with a moderate red shift of 5, 3 and 10 nm, respectively is observed. Hyperchromic effect reflects the corresponding changes of DNA in its conformation and structure after the complex–DNA interaction has occurred. The binding constants of the complexes were calculated as 4.7×10^4, 6.2×10^4 and 2.3×10^5, respectively. Binding constants of ligands and complexes are given in Table 3.

It was observed that the binding constant (K_b) of $[Zn(L)Cl_2]$ was higher than that of the ligand and its Cu(II) and Ni(II) complexes. The binding constant of Zn(II) complex to the FSds-DNA is higher than to that of the ethidium bromide (an intercalating agent) ($K_b = 1.23 \pm 0.07 \times 10^5$).[29–31] The higher binding affinity of the Zn(II) complex can be assigned to the coordinated chloride group which can hydrolyze in water solution and intercalates between base pairs.

Fluorescence emission spectroscopy

The fluorescence technique is the most sensitive and an effective method to study the interaction between DNA and binder molecules. The mode of binding of drugs to DNA can be specified by fluorescence spectroscopy and the several analytical tools based on fluorescence emission can also ensure useful information. The orientation of fluorophoric ligands and their affinity to the DNA couple of bases can be studied by fluorescence anisotropy or fluorescence resonance energy transfer. Fluorescence quenching experiments give additional information relevant the localization of the drugs and their mode of interaction with DNA. To find out the mode of DNA-binding interaction, ethidium bromide (EB) supersedence experiments were performed. The fluorescene of EB increases in the presence of FSds-DNA, due to its strong intercalation between the adjacent FSds-DNA base pairs. It was previously reported that the improved fluorescence can be quenched by the addition of a competitor molecule. Thus, if the competitor molecule intercalates into DNA, it causes to a decline in the fluorescence intensity of the EB-DNA because it will displace EB from DNA. The extent of fluorescence quenching of EB bound to FSds-DNA can be used to determine the extent of binding between the competitor molecule and FSds-DNA.[32–33]

The fluorescence emission spectrum of FSds-DNA–EB system in the absence and presence of ligand analog is shown in Figure 8. The *Ksv* value of the [HL·OAc] was calculated as 2.02×10^3. When FSds-DNA–EB system was excited at 526 nm, a maximum emission was observed at 629 nm. The fluorescence intensity of DNA–EB system gradually reduces upon incremential addition of guanylurea acetate (HL·OAc) without major variance in the wavelength of maximum emission.

The fluorescence intensity of DNA–EB system gets decreased upon increasing concentration of the Cu(II), Ni(II) and Zn(II) complexes without major changes in the wavelength of maximum emission, which might be due to the three possible reasons.[29] The quenching of EB bound to FSds-DNA by both complexes is in good agreement with the linear Stern–Volmer equation, which provides further evidence that the complexes bind to DNA. The Ksv values for Cu(II), Ni(II) and Zn(II) complexes are 1.73×10^4 M^{-1} and 2.75×10^4 M^{-1} and 1.23×10^5 M^{-1}, respectively. The data suggest that the interaction of Zn(II) complex with CT-DNA is stronger than that of HL·OAc, Cu(II) and Ni(II) complexes.

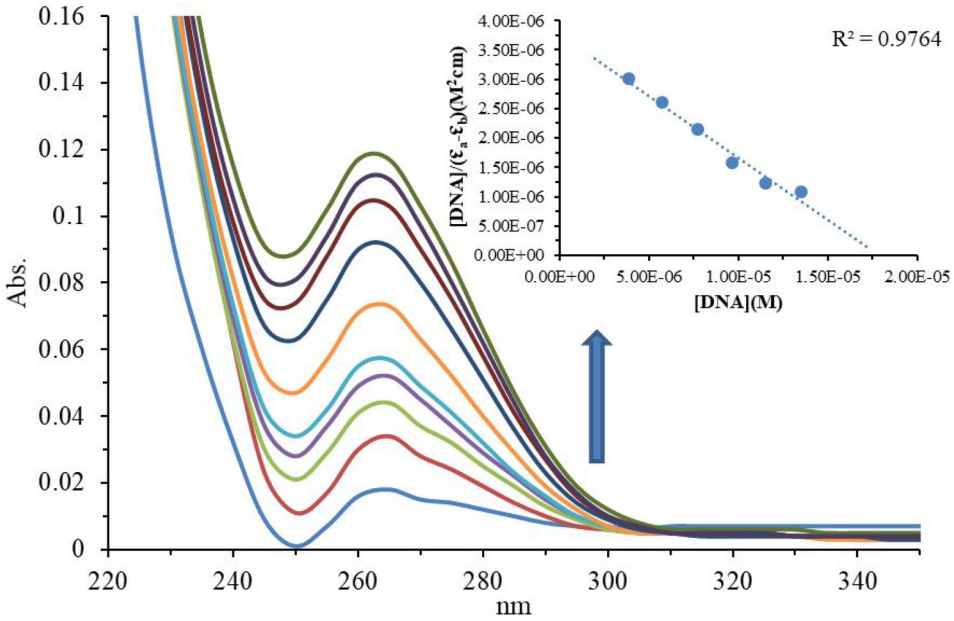

Figure 7. UV-Vis. spectra of the [HL·OAc] upon addition of FSds-DNA.

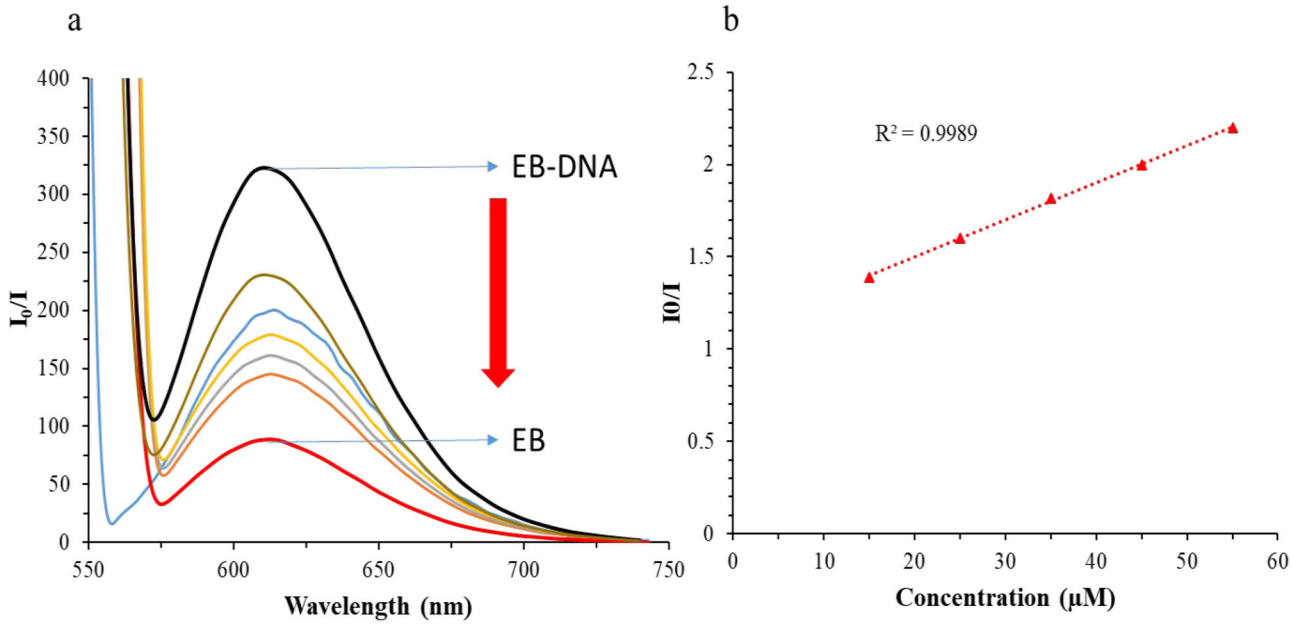

Figure 8. Effect of addition of [HL·OAc] (a) the emission intensity of the FSdsDNA-bound ethidium bromide (75 μM) at different concentrations (0–65 μM) in 2 mM Tris-HCl buffer (pH 7.1), (b) Stern-Volmer plot of fluorescence titrations of the complexes with FSdsDNA.

Conclusion

In this work, Cu(II), Ni(II) and Zn(II) complexes of guanylurea acetate (HL·OAc) were prepared and characterized. Solid state structures of the guanylurea acetate and Cu(II) complex were studied by single crystal X-ray diffraction method. Antibacterial activity of the complounds was studied using single disk method. The the guanylurea acetate ligand and its Cu(II), Ni(II) and Zn(II) complexes showed considerable antimicrobial activity toward some gram (+) and gram (-) bacteria when compared to the standart antibiotics. Finally, DNA binding properties of the compounds were examined and the Zn(II) complex showed higher FSds-DNA binding affinity than the ethidium bromide.

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