Synthesis, characterization and antitumour activity of Copper (II) complexes of some phosphonates

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Abstract- Copper (II) complexes of three phosphonate ligands were prepared and fully characterized by elemental analysis, spectral and thermal studies. The IR spectral data showed that the phosphonate compounds behave as bidentate ligands coordinating to the copper ion through the P=O and NH groups. The electronic spectroscopic data support a distorted octahedral geometry around copper ion. The activity of the phosphonate ligands and their copper (II) complexes as small molecules were investigated towards DNA cleavage. The phosphonate ligands showed no activity, while the copper complexes showed DNA cleavage. The copper (II) complex of Diphenyl (3-hydroxyphenylamino)(2-hydroxyphenyl)methylphosphonate showed the highest activity towards DNA cleavage.

Index Terms- phosphonate; spectroscopic studies; DNA cleavage; antitumor.

I. Introduction

Cancer and tumoral malignancies remain among the most widespread and difficult to treat diseases. Despite tremendous efforts to improve therapy, the spectrum of available effective drugs is comparably limited and there is a considerable need for the development of new drugs and treatment alternatives. The first step in metastasis is tumor cell invasion, involving penetration of the basement membranes by tumor cells that can locally initiate a proteolytic cascade. Metastasis of a primary tumor to vital organs is the dominant cause of cancer related deaths.[1] Tumor cell invasion is a complex process involving cell adhesion, motility (migration) and the degradation of tissue and extracellular matrix (ECM) barriers by different proteases secreted by tumor cells. Invasive malignant cells are able to degrade the extracellular matrix and basement membranes, presumably through the secretion of proteolytic enzymes, including matrix metalloproteinases (MMPs), urokinase-type plasminogen activator (uPA).

Metal complexes constitute an important class of compounds endowed with biological interest. This type of compounds is widely used in medicine as a contrast agent in image magnetic resonance (MRI), in radiopharmaceuticals, in the treatment of arthritis, ulcers and in cancer chemotherapy [2–6]. In recent years, many researches [7,8] have focused on interaction of small molecules with DNA. DNA is generally the primary intracellular target of anticancer drugs, so the interaction between small molecules and DNA can cause DNA damage in cancer cells, blocking the division of cancer cells, and resulting in cell death [9,10]. Among the metal ions regarded as coordination centers of potential anticancer agents, platinum and ruthenium ions are the most widely investigated up to now [11,12]. However, there is a growing interest in the synthesis of cheaper first-row transition metal complexes as efficient DNA binders with potential cytotoxic activity [13,14].

Phosphonates represent an important class of organophosphorus compounds. Their use in a variety of applications is well documented and their importance in a range of fields is increasing. Phosphonates have a lot of industrial applications as effective chelating agents, corrosion inhibitors in cooling systems [15-17] and water softeners[18]. They also used as stabilizers in oxidation processes associated with the pulp, paper, and textile industries [19]. In addition, The phosphonate functionality has been incorporated into a range of clinically useful drugs. Acyclic nucleoside phosphonates have shown potential as therapeutics for pathogenic species [20], and HIV protease inhibitors [21]. phosphonate containing protease inhibitors also have shown great potential for the treatment of Hepatitis C virus [22]. some have shown potential as cancer therapies [23] to inhibit growth of malignant cell lines [24,25] and also as anti-parasitic agents. [26,27].

In this study, small phosphonate molecules were prepared and characterized. The copper (II) complexes of the phosphonate ligands were also prepared and fully characterized. The ability of the phosphonates and their copper (II) complexes to cleave the DNA were investigated.

II. Experimental Section

2.1. Materials.

Perchloric acid, benzaldehyde, acetaldehyde, aniline, 2-nitroaniline and 3-hydroxyaniline were purchased from Sigma-Aldrich Chemical Co.

2.2. Synthesis of phosphonate ligands

2.2.1. Diphenyl (2-nitrophenylamino) (phenyl)methylphosphonate (DNPP)

$HClO_4$ (0.201 g, 2 mmol) was added to a solution of the benzaldehyde (1.06g, 0.01 mol) and aniline (0.93 g, 0.01 mol) in acetonitrile. The mixture was stirred for 15 min and then triphenyl phosphite (3.1 g, 0.01 mol) was added. After completion of the reaction (6 h), the reaction mixture was quenched with aq. saturated $NaHCO_3$ followed by brine solution

and then extracted with CH_2Cl_2, dried over Na_2SO_4, and concentrated under vacuum. The crude mixture was purified by washing with mixture of ether and pet. ether to afford the product.

2.2.2. Diphenyl (2-nitrophenylamino)(2-hydroxyphenyl) methylphosphonate (DNHP) and Diphenyl (3-hydroxyphenylamino)(2-hydroxyphenyl) methylphosphonate (DHHP)

$HClO_4$ (2 mmol) was added to a solution of the salcylaldehyde (0.01 mol) and 2-nitroaniline or 3-hydroxyaniline (0.01 mol) in acetonitrile. The mixture was stirred for 15 min and then triphenyl phosphite (3.1 g, 0.01 mol) was added. After completion of the reaction (6 h), the reaction mixture was quenched with aq. saturated $NaHCO_3$ followed by brine solution and then extracted with CH_2Cl_2, dried over Na_2SO_4, and concentrated under vacuum. The crude mixture was purified by washing with mixture of ether and pet. ether to afford the product.

2.3. Synthesis of metal complexes

Copper (II) chloride (0.01 mole) dissolved in about 50 ml absolute ethanol was added to the ethanolic solution of the selected ligand (0.01 mole). Small amount of solid sodium acetate was added to the solution with heating and contentious stirring. The precipitate was obtained, filtered off and washed many times with ethanol and then dried in an oven at 80 ^oC.

2.4. Physical methods

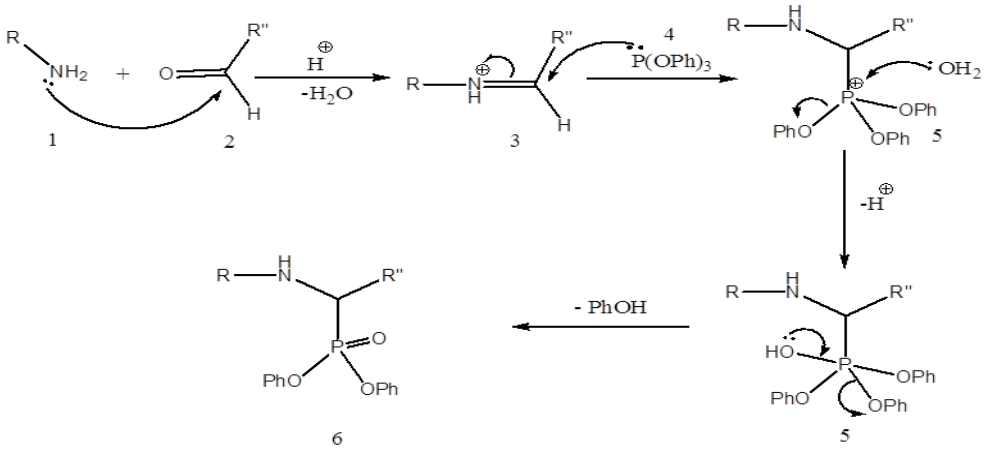
Carbon, hydrogen and nitrogen contents were determined at the microanalytical unit, Cairo University, Egypt. IR spectra of the ligand and its solid complex were measured in KBr on a Mattson 5000 FTIR spectrometer. The electronic spectra were performed using Varian Cary 4 Bio UV/VIS spectrophotometer. ^1H- NMR spectrum of the ligand was recorded on Joel-90Q Fourier Transform (200 MHz) spectrometers in [D_6] DMSO. The mass spectra of the phosphonate ligands were recorded on a Shimadzu GC-S-QP 1000 EX spectrometer using a direct inlet system. Thermal analysis measurement (TGA) was recorded on a Shimadzu thermo-gravimetric analyzer model TGA-50 H, using 20 mg sample. The flow rate of nitrogen gas and heating rate were 20 cm^3 min^{-1} and 10°C min^{-1}, respectively. The magnetic susceptibility measurements for the copper (II) complexes were determined by the Gouy balance using $Hg[Co(NCS)_4]$ as a calibrant at room temperature.

2.5. Nuclease-like activity assay (DNA cleavage)

Genomic DNA extracted from mammalian blood by salting out method was used to examine the DNA cleavage activity of examined ligands and their copper (II) complexes. DNA purity and concentration were examined spectrophotometrically at 260/280 nm. The cleavage reactions were carried out in a total volume of 15 µl containing 5 µl genomic DNA, 5 µl of ligand or complex in the range of (1.0 nM to 1mM), and 5 µl TE buffer (25.0 mM Tris–HCl containing of 50.0 mM NaCl pH 7.2). Genomic DNA alone or genomic DNA in the buffer was used as a control. The reactions were carried out at 37 ˚C at different time intervals (0, 0.5, 1, 2, 6, 12 hours). A solution of loading dye (0.05% bromophenol blue, 5% glycerol, and 2 mM EDTA) was added to the reactions mixtures prior to running the gel. Dose dependent and time dependent experiments were carried out for each ligand and its complex used in this study. The prepared compounds were run on 1.0% agarose slab gel at a constant voltage of 100 V for 30 min in TBE (Tris– Borate-EDTA) buffer. Gels were stained with ethidium bromide and visualized under UV trans-illuminator (Syngene gel documentation
system with digital camera).

III. Results and Discussion

The phosphonate ligands were prepared by stirring the mixture of the aldehyde, amine and triphenyl phosphite as one-pot reaction as shown in scheme 1.



	L2	L3	L4
R	*O*-NO_2-Ph	*O*-NO_2-Ph	*m*-NO_2-Ph
R"	Phenyl	*O*-OH-Ph	*O*-OH-Ph

Scheme 1. The mechanism of the phosphonate preparation.

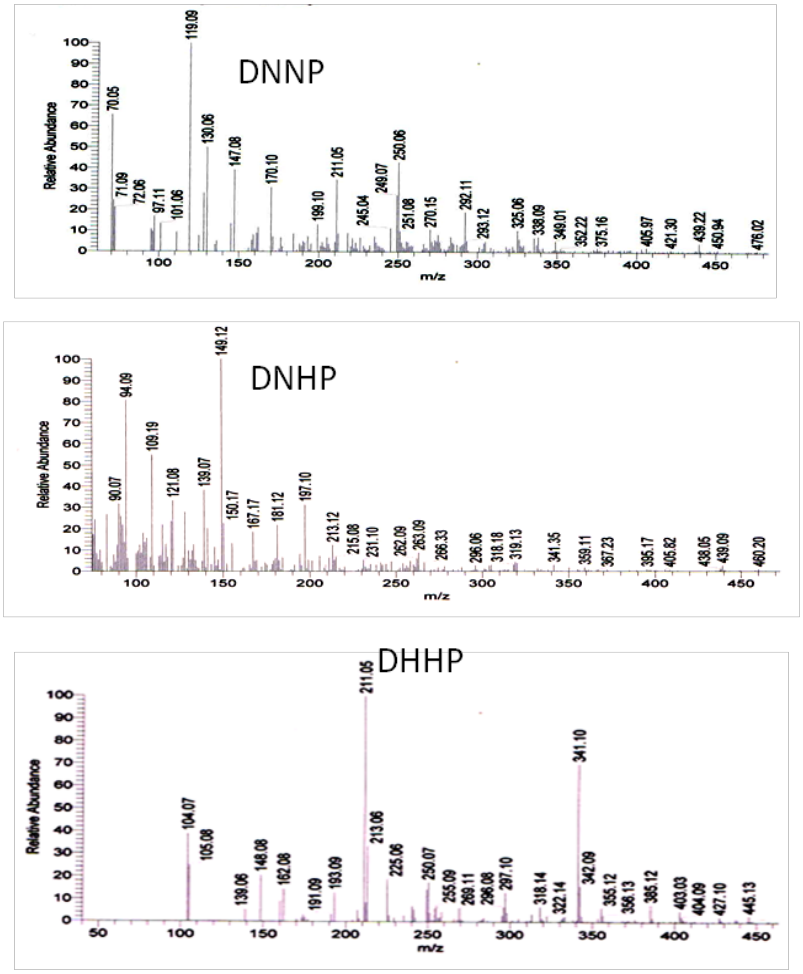


Figure 1. Mass spectroscopy of the phosphonate ligands

3.1. IR spectra

IR spectral data of the organic ligands show bands at 3430 cm^{-1} in case of DNHP and DHHP attributed to νOH. The bands appear at 3008 – 3336 cm^{-1} are corresponding to νNH. The bands corresponding to νP=O appear at 1214-1220 cm^{-1}[28]. The spectral data show bands at 1605-1680 cm^{-1} and 1413-1420 cm^{-1} 1440 cm^{-1}attributed to NH and OH bending, respectively. ^{1}H-NMR spectrum of DNNP shows δ = 4.66 (d, 1H, CH), 6.64-7.78 (m, 19H, Ar-H) and 9.33 (brs, 1H, NH). The bands at δ = 4.61 (d, 1H, CH), 5.61 (brs, 1H, OH), 6.62-7.78 (m, 18H, Ar-H) and 7.98 (brs, 1H, NH) are shown for DNHP. AT the same time the ^{1}H-NMR spectrum of DHHP shows δ = 4.62 (d, 1H, CH), 5.62 (brs, 2H, 2xOH), 6.60-7.68 (m, 18H, Ar-H) and 7.99 (brs, 1H, NH). The molecular weights for the prepared ligands were determined by mass spectroscopy (Figure1). The spectra show molecular ion peaks 460, 476 and 447 for DNNP, DNHP and DHHP, respectively. The IR and ^{1}H-NMR and mass spectral data suggest the structure of the phosphonate ligands as shown in Figure 2.

IR spectra of the copper (II) complexes were recorded to confirm their structures. The vibrational mode assignments of the metal complexes were supported by comparison with the vibrational frequencies of the free ligands (Figure 3). By comparing the IR spectral data of the prepared ligands with their copper (II) complexes, it is found that the phosphonate ligands are coordinating with the copper ion by P=O oxygen and NH nitrogen atoms. This suggestion is supposed by shifting in the position of νP=O band from1214-1220 cm^{-1} in the ligands to 1243-1267cm^{-1} in the copper (II) complexes. At the same time, the position of νNH band at the ligands which appear at 3008-3336 in the phosphonate ligands are shifted to 3009- 3460 cm^{-1}. The binding with NH is supported by shifting in the position of NH bending from to 1605-1670 to 1617-1628 cm^{-1} in the copper (II) complexes.

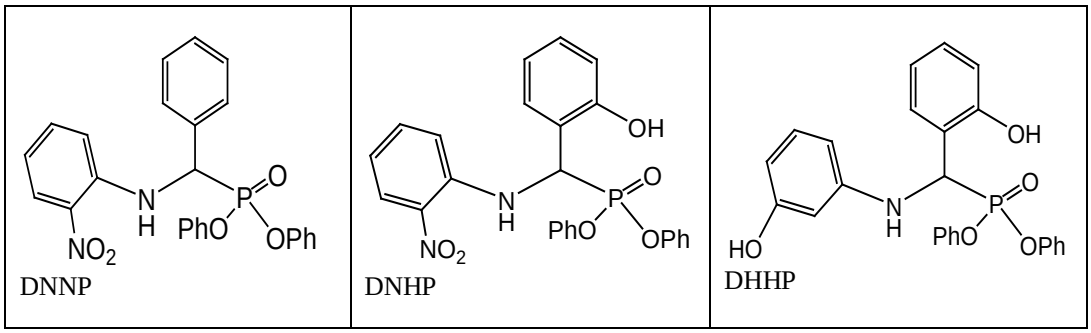


Figure 2. Structure of the phosphonate ligands

3.2. Electronic spectra

The electronic spectra of the copper complexes Cu^{II}DNNP, Cu^{II}DNHP and Cu^{II}DHHP (Figures 4) recorded at room temperature, in ethanol solution, show a broad band at ~695 nm. The copper(II) complexes with d^9 configuration are expected to experience Jahn-Teller distortion which leads to further splitting of the ^2E_g and $^2T_{2g}$ levels. Moreover, they give rise to the $^2B_{1g}\rightarrow{}^2A_{1g}$ (ν_1), $^2B_{2g}$ (ν_2) and ^2E_g (ν_3) transitions which are expected to be close in energy and generally appears as a broad band. Therefore the broad band centered at 695 nm is assigned to the envelope of $^2B_{1g}\rightarrow{}^2A_{1g}$, $^2B_{2g}$ and ^2E_g transitions [29] which support (with the magnetic moment =1.8-1.9 BM) a distortion octahedral geometry around copper (II) in the complex.

3.3. Thermal analysis

The TGA analysis is commonly used to measure and confirm solvent inside and/or outside the coordination sphere and gives information about the stability of the compound. The thermogram of the three prepared complexes shows 3-4 stages of mass loss from 25-1000 ^oC. The Cu^{II}DNNP complex shows three inflection stages. The first stage at the temperature range of 43-125 ^oC with weight loss of (Calcd. 5.71%, found5.78%) corresponding to two water molecules. The second stage in the temperature range of 230-330 ^oC with weight loss of (Calcd.21.88%, found 22.19%) corresponding to $PhNH_2NO_2$. The third stage in the temperature range of 746-984 ^oC with weight loss of (Calcd. 42.18 %, found 42.17%) corresponding to two molecules of PhOH and one molecule of Ph.

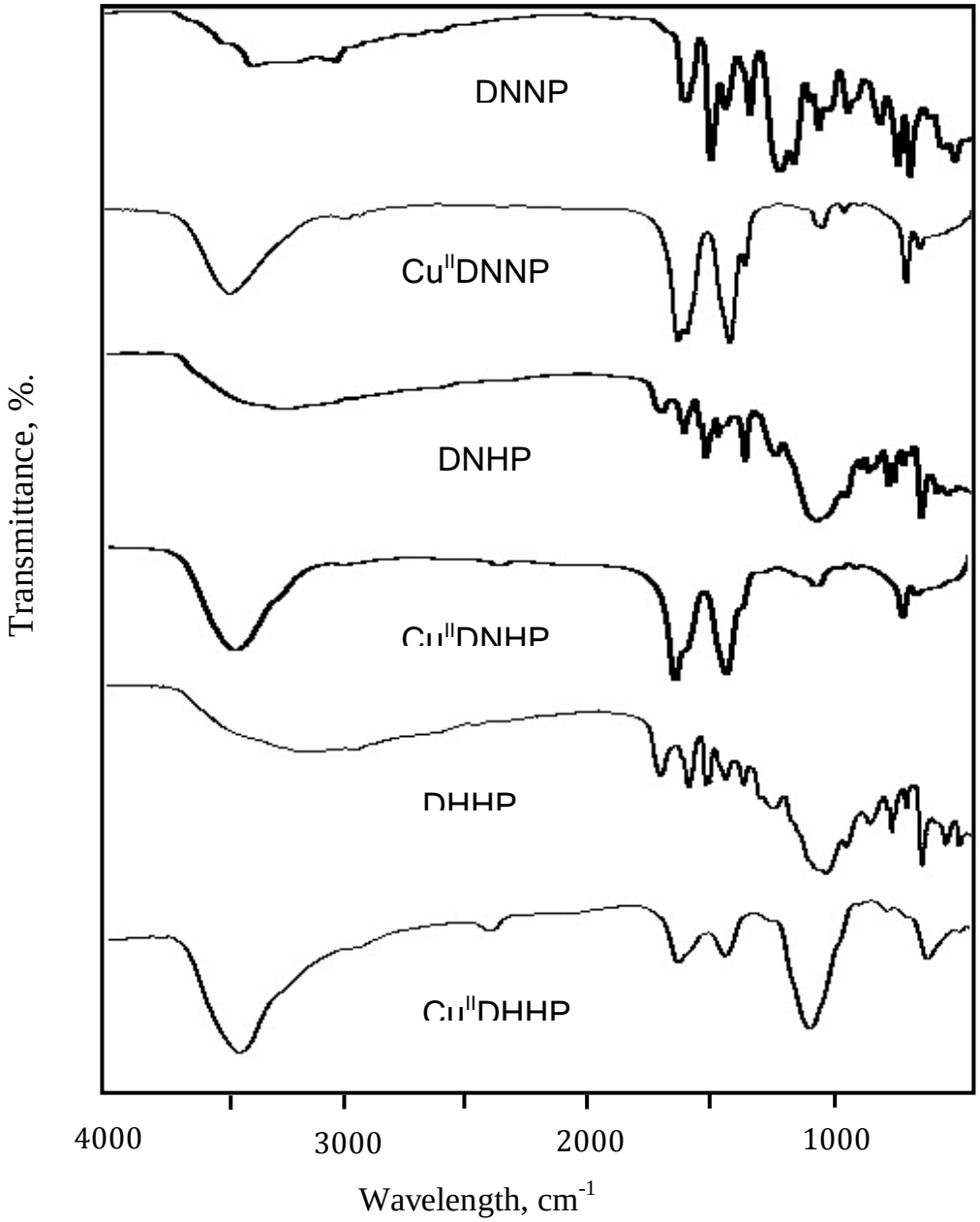


Figure 3. IR spectra of the ligands and their copper (II) complexes

The TGA of Cu^{II}DNHP complex also shows three degradation stages. The first stage in the temperature range of 40-129 ^{o}C is assigned to removal of two water molecules (Calcd. 5.57%, found 5.70%). The second stage in the temperature range of 130-325 ^{o}C is assigned to removal of $PhNO_2NH_2$.HCl with weight loss of (Calcd. 26.91%, found 27.318%). The third stage in the temperature range of 715-1000 ^{o}C with weight loss of (Calcd. 24.13%, found 24.10%) is corresponding to removal of two molecules of PhOH. On the other hand the TGA of Cu^{II}DHHP shows four degradation stages from 25- 1000 ^{o}C. The first stage in the temperature range of 43-125 ^{o}C corresponds to removal of two water molecules with weight loss (Calcd. 5.82%, found 5.79%). The second degradation stage in the temperature range of 150-270 ^{o}C is assigned to removal of NO_2 with weight loss (Calcd. 7.44%, found 7.35%). The third stage with weight loss (Calcd. 15.05 %, found 14.59%) in the range of 352-511 ^{o}C corresponds to removal of $PhNH_2$. The fourth stage in the temperature range of 680-914 ^{o}C with weight loss (Calcd. 25.26 %, found 25.52%) corresponds to removal of two Ph molecules. The total weight loss of the Cu(II)complexes Cu^{II}DNNP, Cu^{II}DNHP and Cu^{II}DHHP are 46.40, 42.90 and 30.24 % till 1000 ^{o}C. The weight remaining in case of Cu(II) complexes shows that Cu^{II}DHHP is the most stable of the prepared complexes, then Cu^{II}DNHP and finally Cu^{II}DNNP. The elemental analysis (Table 1) with the spectroscopic tools data suggest the structure of the copper (II) complexes; $CuLCl_2.2H_2O$ where L is DNNP, DNHP or DHHP as shown in Figure 5.

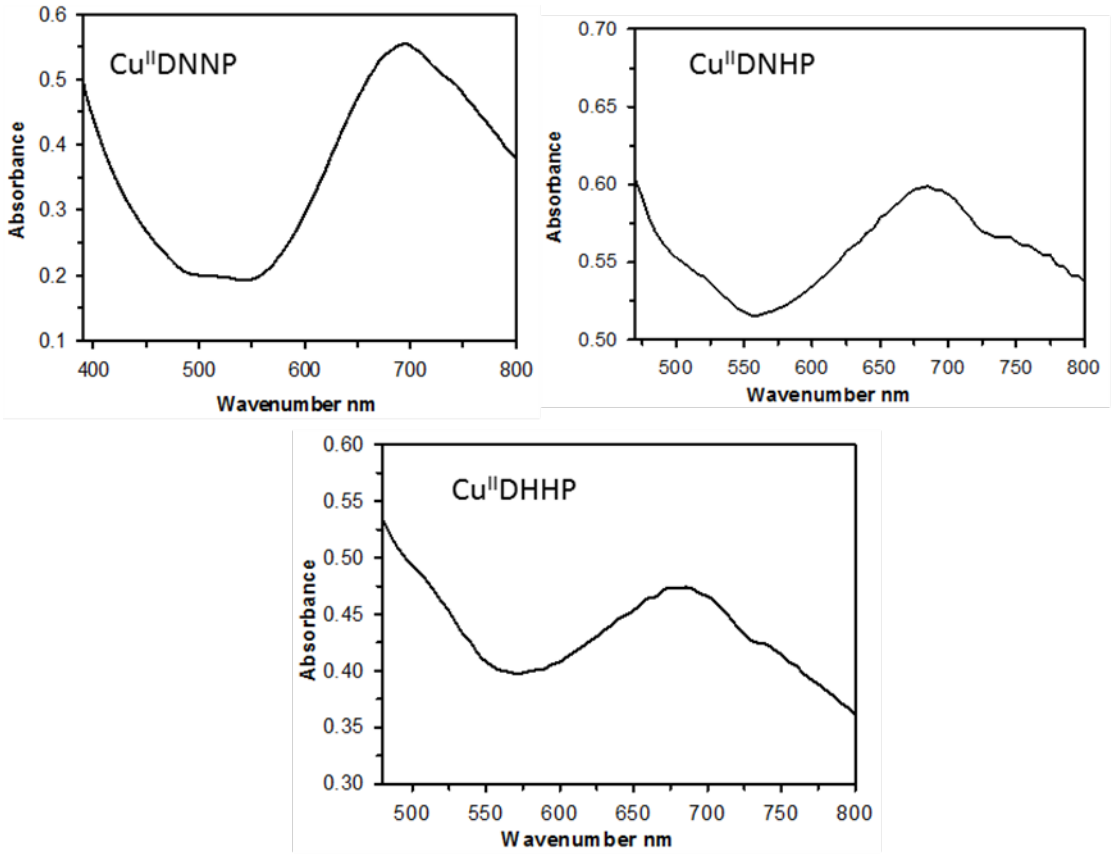


Figure 4. Electronic spectra of the copper (II) complexes

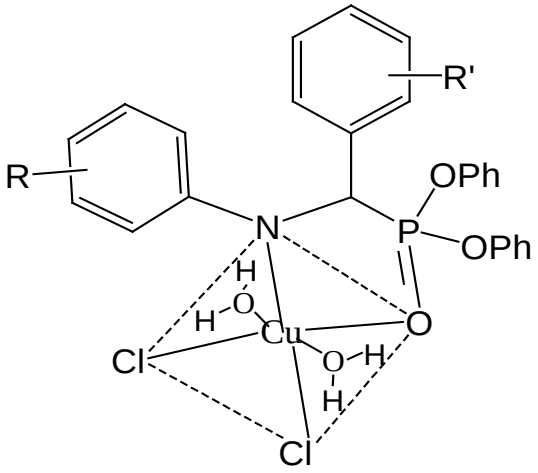
Table 1. Elemental analysis for all complexes.

Compound	M.wt.	C% calc. (Found)	H% calc. (Found)	N% calc. (Found)	Cu% calc. (Found)
Cu^{II}NNP	630.62	47.57 (47.29)	3.96 (4.16)	4.44 (4.49)	10.07 (10.01)
Cu^{II}NHP	646.61	46.39 (46.37)	3.86 (3.87)	4.33 (4.38)	9.82 (9.88)
Cu^{II}HHP	617.62	48.57 (59.42)	4.21 (4.79)	2.27 (2.47)	10.28 (10.31)

3.4. Gel electrophoresis

Particular attention has been devoted to transition metal complexes endowed with planar aromatic side groups, which can bind with DNA by both metal ion coordination and intercalation of the aromatic moiety [30].

The effect of the organic ligands and their copper (II) complexes on DNA cleavage was studied by DNA migration in agarose gel. The genome DNA was incubated for 24 h at 37 ^{o}C in the presence of the ligand or its copper complex with increasing concentrations. The samples were run in 1.0 % agarose gel in TBE (tris– Borate- EDTA) buffer of pH 7.4 at 2 V/cm for 30 min.



complex	Cu^{II}DNNP	Cu^{II}DNNHP	Cu^{II}DHHP
R	NO_2	NO_2	OH
R'	H	OH	OH

Figure 5. Structure of copper (II) complexes

The gel was stained with EtBr and photographs were taken in a syngene gel documentation system. As seen in Figure 6, the DNA migration as well as the intensity of the super coiled DNA did not change in the presence of phosphonate ligands. Thus, suggest no cleavage activities were achieved for ligands towards DNA. On the other hand the migration and intensity of the DNA bands were affected in the presence of Cu^{II}DNHP and Cu^{II}DHHP complexes (Figure 7). The disappearance of the supercoiled DNA in the presence of copper complexes together with the decreasing in the brightness of the DNA bands suggests the cleavage of the genome DNA.

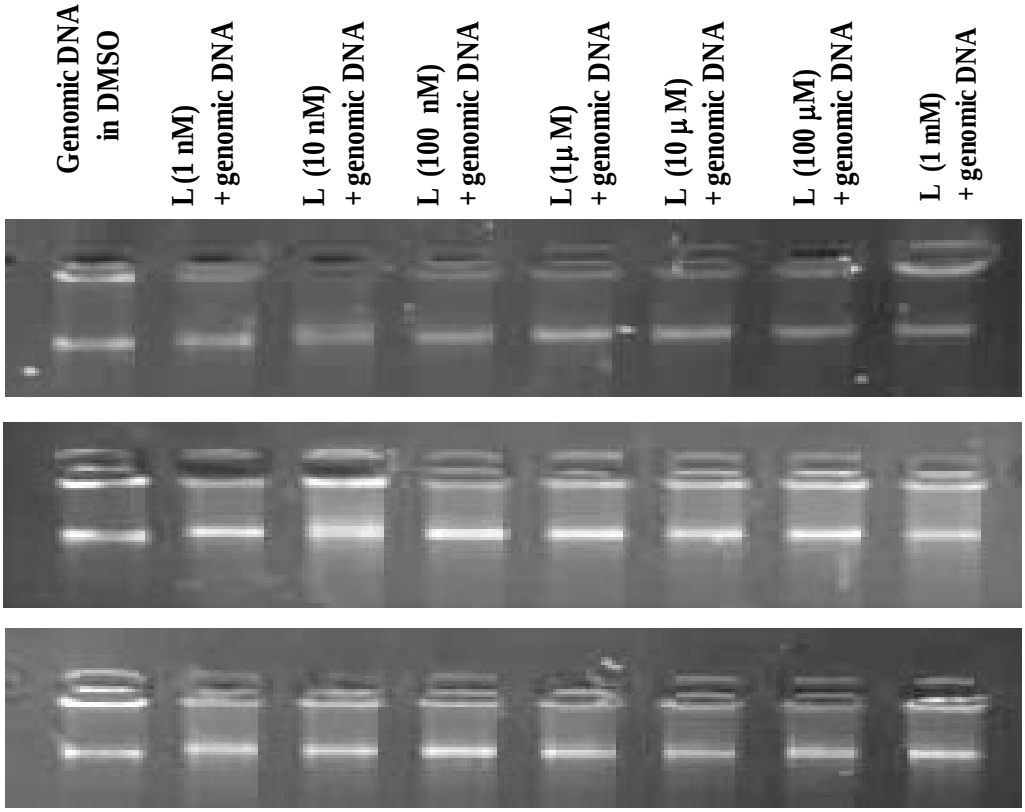


Figure 6. The DNA binding activity of the ligands where L = DNNP, DNHP and DNHHP from up to below. Various concentrations of the ligands were incubated with genomic DNA (100 ng) for 24 hours at 37 ºC.

At the same time, the copper complex Cu^{II}DNNP didn't show any activity towards the DNA binding. It was found that the more concentration of the copper (II) complex the more strongly cleavage effect on the genomic DNA. In order to study the effect of time on the DNA binding, the copper (II) complexes were incubated with the genomic DNA for different time courses. It was found that the complex Cu^{II}DHHP has the greatest ability to cleave the genomic DNA (Figure 8).

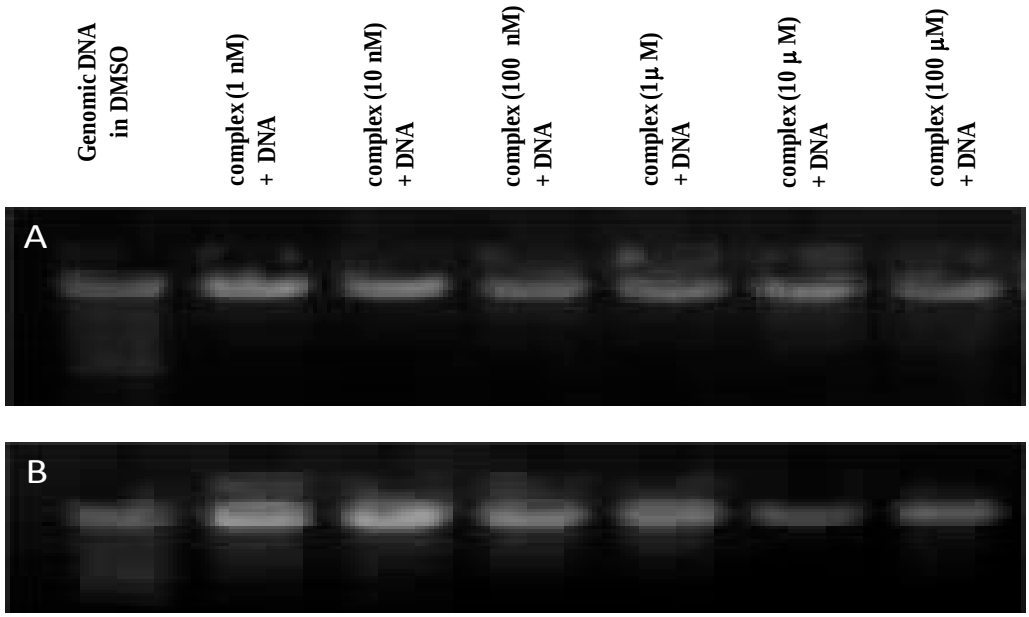


Figure 7. The DNA binding activity of Cu^{II}DNHP (A) and Cu^{II}DNHHP (B) complexes. Various concentrations of the complexes were incubated with genomic DNA (100 ng) for 24 hours at 37 ºC. DNA.

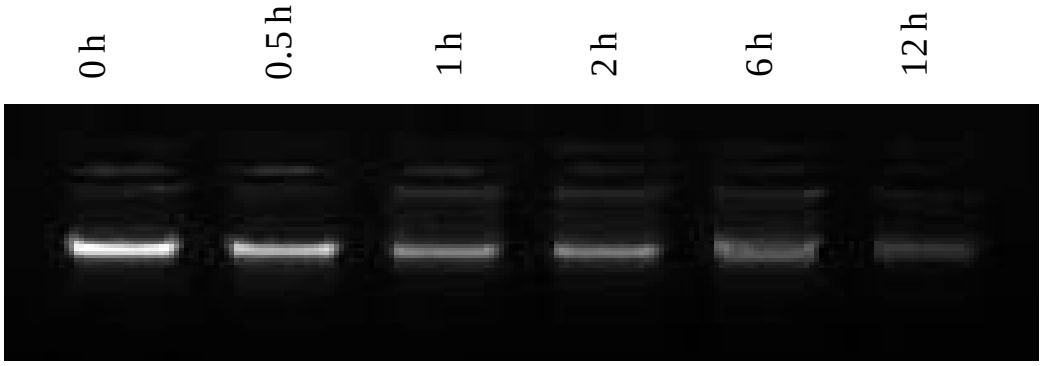


Figure 8. Time dependant effect of Cu^{II}DNHHP complex on DNA binding activity. The complex (100 mM) was incubated with genomic DNA for different time intervals at 37 ºC.

IV. Conclusion

Three phosphonate molecules were prepared and characterized by different physicochemical tools. The copper (II) complexes of these phosphonates were also synthesized and characterized. the mode of binding was studied and the thermal analysis of these complexes showed that the Cu^{II}DHHP is the most stable complex of the all complexes under investigation. The anticancer activity of these complexes was investigated using DNA cleavage study and showed that Cu^{II}DHHP reveal the highest ability to cleavage the genomic DNA.

Acknowledgments

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