

Research Article



DOI: <http://dx.doi.org/10.22192/ijcrpcs.2019.06.11.001>

# Synthesis, Characterization and DNA binding of Copper (II) complexes with mixed ligands of 1, 10 - Phenanthroline / 2, 2'- Bipyridyl, L-Valine: studies on Anti-microbial and Anti-cancer activities

P. C. Saravanan, D. Ezhilarasan and M. N. Arumugham\*

Department of Chemistry, Thiruvalluvar University, Vellore – 632 115, Tamilnadu, India.

E-mail: [aru\\_mugham@yahoo.com](mailto:aru_mugham@yahoo.com)

## Abstract

Ternary copper(II) complexes [Cu(phen)(L-val)TSC] 1 & [Cu(Bpy)(L-val)TSC] 2 (phen = 1,10-phenanthroline Bpy = bipyridyl, L-Valine and TSC = Thiosemicarbazide), have been synthesized and characterized by CHN analysis, molar conductance, electronic absorption, IR and EPR spectral studies. They have been tested for their in vitro DNA binding activities by the spectroscopic methods such as UV-Visible, Cyclic volumetric and viscosity measurement. Further, complexes 1 and 2 displayed significant cytotoxicity when examined *in-vitro* on a panel of cancerous cell line - human liver cancer cell line - HepG-2 cells (IC50= 62.5 µg/ml). Further complexes 1 & 2 were tested for their antimicrobial activities and it was found to have good antimicrobial activities.

**Keywords:** Copper (II) Complexes, L- Valine and TSC- Thiosemicarbazide, DNA Binding & Cytotoxicity.

## 1.1 Introduction

Copper complexes of 1, 10-phenanthroline and its derivatives were exhibited numerous biological activities, for example antitumor<sup>1</sup>, antiCandida<sup>2</sup>, antimycobacterial<sup>3</sup>, and antimicrobial<sup>4</sup> activity. By using electron paramagnetic resonance method the orientation of Cu (II) complexes of phenanthroline and amino acids in DNA, resulting that the complexes bind to DNA with different binding modes, such as non intercalative binding in the minor groove of DNA and/or intercalative binding<sup>5</sup>. Recently, the interaction of Cu (II) complexes with nucleic acid has attracted attention due to the study of mutation of genes and therapeutic approaches<sup>6-10</sup>. Regarding phen homoleptic Cu-complexes, [Cu(phen)<sub>2</sub>]<sup>2+</sup> is one of the most studied Cu-complex from a biochemical point of view. There is a large amount of evidence supporting its DNA non-covalent binding to the minor groove of

the double stranded DNA, cleaving DNA at its binding region<sup>11, 12</sup>.

In addition, a number of heteroleptic phen-containing Cu(II) complexes show DNA binding and nuclease activity, thus preventing cellular growth<sup>13,14</sup>. It has been reported that the tumor cells can be destroyed by stopping the replication of DNA<sup>15</sup>. Metal-based anticancer drug discovery remains as one of the advanced areas of pharmaceutical research. Metal complexes which can efficiently bind and cleave DNA under physiological conditions are considered as potential candidates for utility as therapeutic agents in medicinal applications and for genomic research<sup>16-21</sup>. The role of ternary copper(II) complexes in biological systems is well known<sup>22</sup>. Among the transition metal based DNA cleaving agents, particularly copper phenanthroline complexes, is primarily sugar directed. They are responsible for direct strand scission by

hydrogen atom abstraction from the deoxyribose moiety. Structure-activity relationships of compounds showing photo chemotherapeutic activity are important to enhance the biological activity of the drug molecules by modifying various structural or functional parameters, viz., steric and electronic controls, lipo and hydrophilicity, and the stability of the molecules derived from the parent drug by suitable modification of the substituent(s)<sup>23</sup>. Already our group synthesized some of copper complexes and discussed anticancer and DFT properties<sup>24-26</sup>. In this chapter, we report the synthesis and characterization of new copper(II) complexes containing heterocyclic bases, amino acid and thiosemicarbazide as ligands, DNA binding, antimicrobial and cytotoxic studies also carried out for these complexes.

## 1.2 Experimental

### 1.2.1 Synthesis of [Cu(phen)(L-valine)TSc]NO<sub>3</sub> (1)

The complex [Cu(phen)(L-val)(H<sub>2</sub>O)](NO<sub>3</sub>) was synthesized according to a published method<sup>27</sup>. To the aqueous solution of [Cu(phen)(L-val)(H<sub>2</sub>O)](NO<sub>3</sub>) (1 mmol) was added thiosemicarbazide (1 mmol) the colour of the solution change from blue to light yellow. The resulting solution was stirred for 6 hrs and then solution of complex was filtered. The filtrate was kept

for slow evaporation, after two weeks, yellow colored complex **1** was separated out. Yield: 65%; Anal. (%) Calc. for C<sub>18</sub>H<sub>23</sub>CuN<sub>7</sub>O<sub>5</sub>S: C, 42.14; H, 4.52; N, 19.11. Found: C, 41.59; H, 4.41; N, 18.51. IR (KBr pellet): 3517, 3291, 3137, 2936, 2413, 1623, 1349, 1076, 874, 721 cm<sup>-1</sup>. UV-Vis ( , nm): 268, & 602 nm.

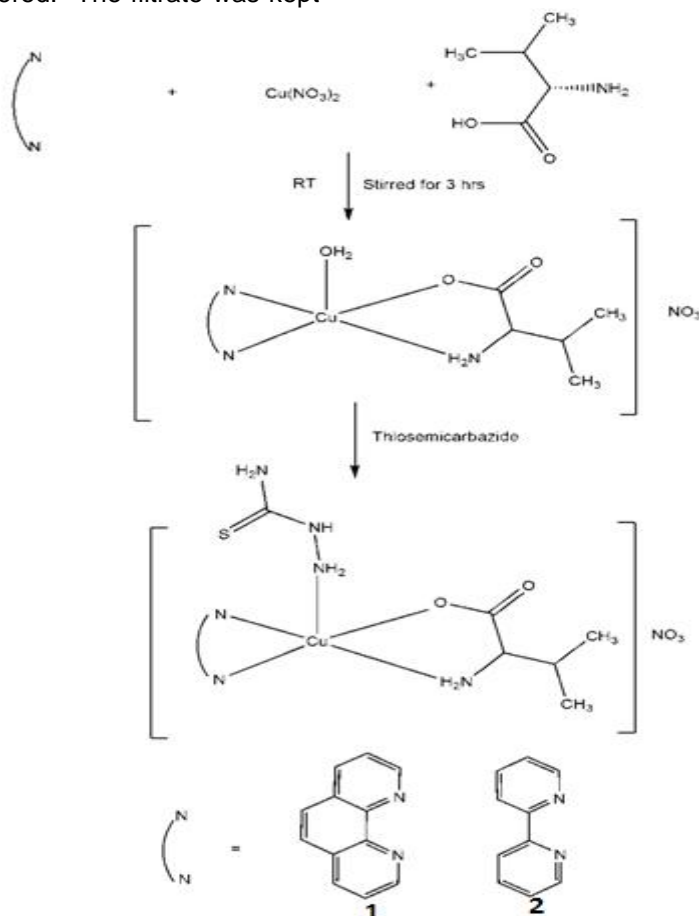
### 1.2.2 Synthesis of [Cu(bpy)(L-val)TSc]NO<sub>3</sub> (2)

Preparation of complex **2** is same as described above. Yield: 62%; Anal. (%) Calc. for C<sub>16</sub>H<sub>23</sub>CuN<sub>7</sub>O<sub>5</sub>S: C, 39.30; H, 4.74; N, 20.05. Found: C, 37.60; H, 4.65; N, 19.61. IR (KBr pellet): 3448, 3305, 2923, 2412, 1649, 1356, 1153, 1033, 771 cm<sup>-1</sup>. UV-Vis ( , nm): 300 & 608 nm.

## 1.3 Results and Discussion

### 1.3.1 General Aspects

These complexes are synthesized by ligand substitution method; the synthetic strategy of the complexes is outlined in Scheme 1.1 given below. The synthesized complexes are more stable and they are soluble in water and in other organic solvents. The elemental analysis data of the copper(II) complexes agree with the theoretical values.



Scheme 1.1: Synthesis of complexes **1** and **2**.

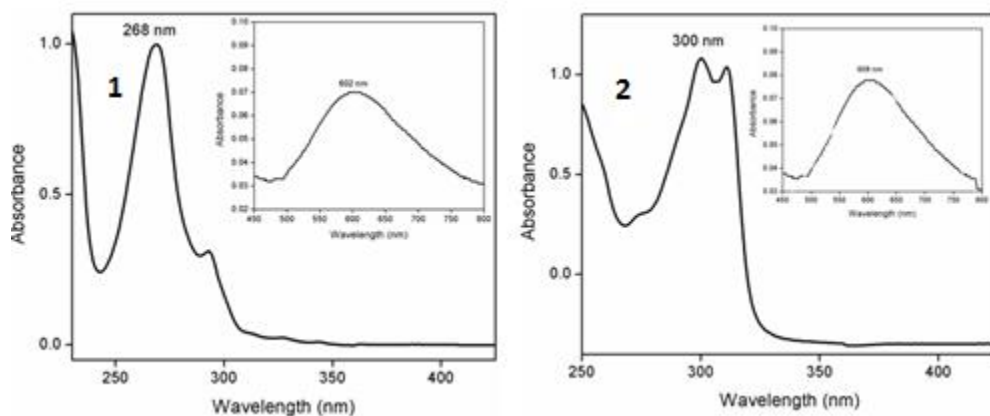


Figure 1.1: UV-Visible spectra of complexes 1 and 2.

In the UV region, the complex presented in Figure 1.1, bands around 286 and 300 nm which can be attributed to  $\pi \rightarrow \pi^*$  transition of the coordinated phenanthroline ligand, and the complexes 1 and 2 exhibits d-d band at UV-Visible spectra. The complexes are in good agreement with the previously reported square pyramidal geometry of the complexes.

In the IR region, for complexes the band around  $3517\text{ cm}^{-1}$  for complex (1) and  $3448\text{ cm}^{-1}$  for complex (2) can be assigned to (N-H) stretching frequency of amino acid. The coordination of nitrogen atoms of a heterocyclic base with copper metal ion can be examined by (C-H) for phenanthroline  $853\text{ cm}^{-1}$  is shifted to  $874\text{ cm}^{-1}$ , which confirms the metal coordinate with and the band around  $1349\text{ cm}^{-1}$  (1)

and  $1356\text{ cm}^{-1}$  (2) has been assigned to (N-O) of nitrate ion (Figure 1.2).

The solid state EPR spectra of the copper (II) complexes were recorded in X-band frequencies (Figure 1.3). At room temperature, complexes 1 and 2 exhibit well defined single isotropic lines. Such isotropic lines are usually the results of intermolecular spin exchange, which broaden the lines. This intermolecular type of spin exchange is caused by the strong spin coupling which occurs during a coupling of two paramagnetic species. EPR spectra of mononuclear complexes copper(II) species with  $S=1/2$ , those with two signals ( $g(x,y) > g(z)$ ,  $B(x,y) < B(z)$ ) representing the elongated axial symmetry of the spin tensor.

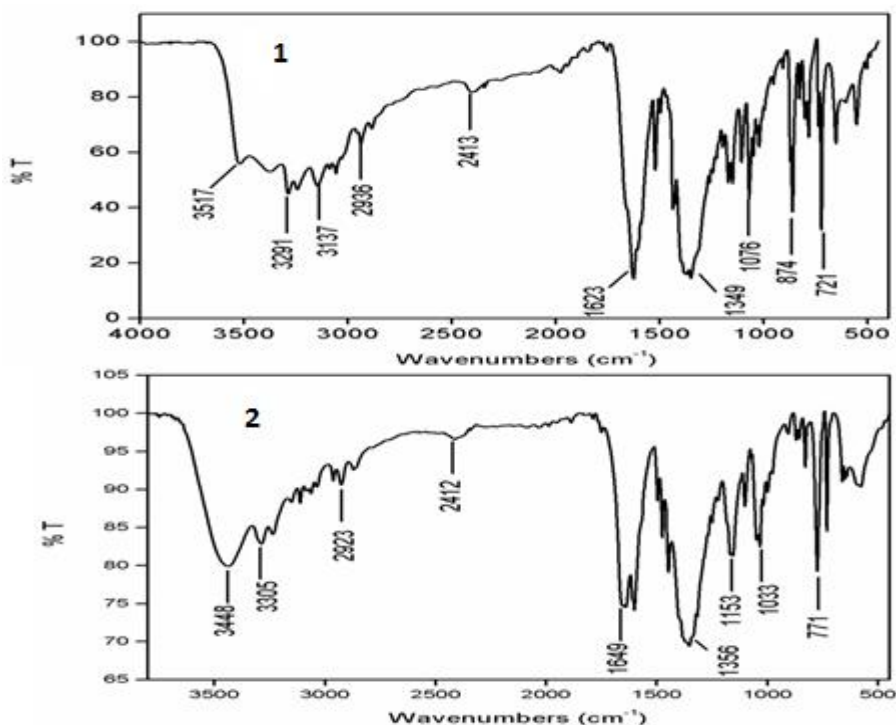


Figure 1.2: Infrared spectra of complexes 1 and 2.

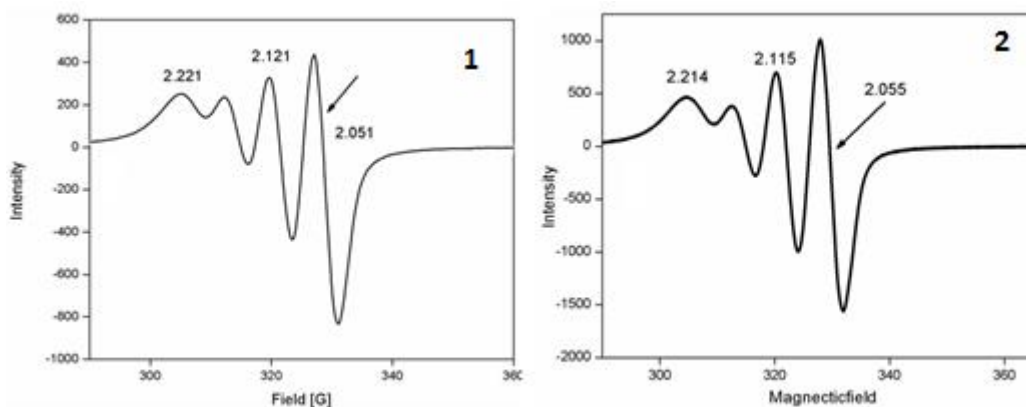


Figure 1.3: EPR spectra of complexes 1 and 2.

## 1.4 DNA binding studies

### 1.4.1 Electronic spectral studies

Electronic absorption spectroscopy was an effective method to examine the binding mode of DNA with metal complexes. In general, hypochromism and red shift are associated with the binding of the complex to the helix by an intercalative mode, involving strong stacking interaction of the aromatic chromophore of the complex between the DNA base pairs. Figure 1.4 shows the UV absorption spectral study of copper (II) complex in the absence and presence of DNA. The absorption intensity of the complexes 1 and 2 increased (hyperchromism and blue shift) evidently

after the addition of DNA, which indicated the interactions between DNA and the complex through intercalative mode.

On comparing the Kb values ( $8.5 \times 10^4 \text{ M}^{-1}$ ) (1) and  $7.3 \times 10^4 \text{ M}^{-1}$ ) (2) of complexes, complex 1 have higher value than complex 2. So the binding propensity of the phen complex 1 is high due to the presence of an extended planar aromatic ring in phen. Earlier studies on bis-phen copper complex have shown that this complex binds to DNA either by partial intercalation or binding of one phenanthroline ligand to the minor groove while the other phen making favourable contacts within the groove<sup>28,29</sup>.

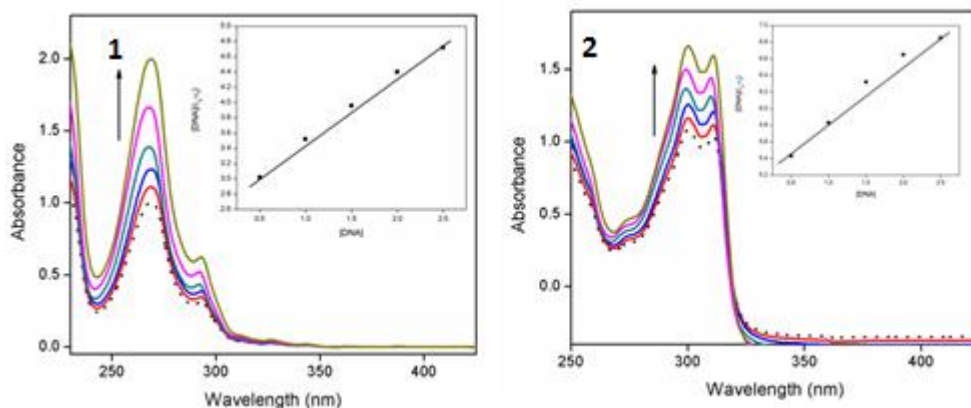


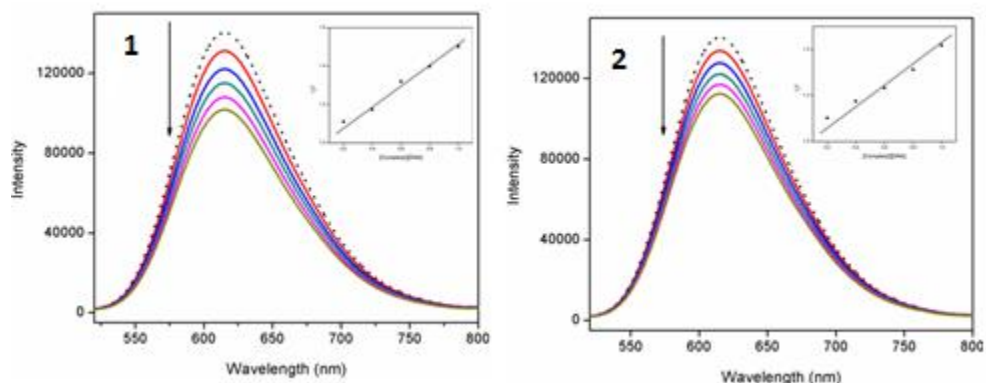
Figure 1.4: Absorption spectral traces on addition of CT DNA to complexes 1 and 2 (shown by arrow). Inset plot of  $[DNA]/[a-f]$  vs  $[DNA]$  for absorption titration of CT DNA with complexes.

### 1.4.2 Fluorescent spectral studies

As the copper (II) complexes are non-emissive, competitive binding studies with EtBr were carried out to gain support for the mode of binding of the complexes with DNA. The study involves addition of the complexes to DNA pretreated with EtBr ( $[DNA]/[EtBr] = 1$ ) and then measurement of intensity of

emission. The observed enhancement in the emission intensity of EtBr bound to DNA is due to intercalation of the fluorophore in between the base pairs of DNA and stabilization of its excited state (Figure 1.5). Addition of all the complexes to CT-DNA incubated with EtBr decreases the DNA induced enhancement in emission to the same extent. This suggests that the complexes displace DNA-bound EtBr and bind to DNA

the intercalation sites with almost the same affinity, which is consistent with the above spectral results suggesting partial intercalation of the phenanthroline ring<sup>30</sup>.



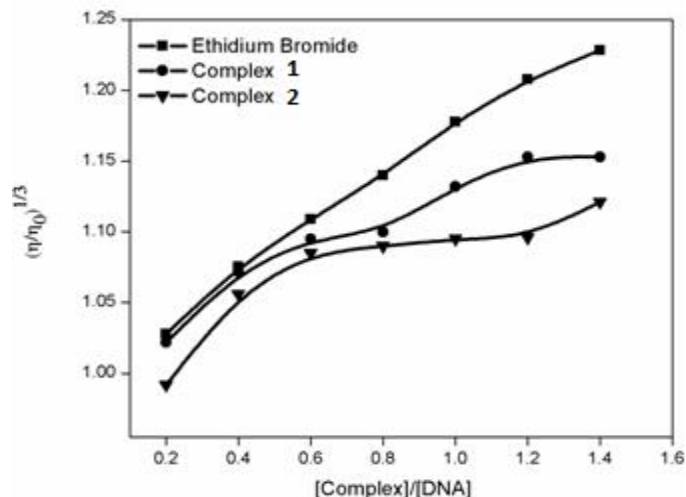
**Figure 1.5:** Emission spectra of EB bound to DNA in the absence (dotted line) and the presence (dashed line) of complexes **1** and **2**. An arrow ( ) shows the intensity changes upon increasing the concentration of the complex. Inset: Stern–Volmer quenching curves.

### 1.4.3 Viscosity measurements

To further explore the binding mode of the copper(II) complex with DNA, viscosity measurements were carried out. Since the relative specific viscosity ( $\eta/\eta_0$ ) ( $\eta$  and  $\eta_0$  are the specific viscosities of DNA in the presence and absence of the complex, respectively) of DNA reflects the increase in contour length associated with separation of DNA base pairs caused by intercalation, a classical intercalator such as ethidium bromide could cause a significant increase in viscosity of DNA solutions. In contrast, a partial and/or non-classical intercalation of the ligand could bend or kink DNA, resulting in a decrease in its effective length with a concomitant increase in its viscosity, while the electrostatic and groove binding cause little or no effect on the relative viscosity of DNA solutions. Therefore viscosity measurements, which are

sensitive to the changes in the contour length of DNA, are useful to probe for DNA intercalation by complexes.

The plots of relative specific viscosities versus  $1/R = ([Complex]/[DNA])$  are shown in Figure 1.6. The relative specific viscosity increases with increasing concentration of the complex. However, the increase in the viscosity was much less compared to that of classical intercalators like ethidium bromide in the same DNA concentration range. This observation supports the above spectral studies which suggest that the complex **1** intercalates with the DNA base pairs and complex **2** involve through groove binding. Intercalation results in lengthening of the DNA helix due to base pairs being separated to accommodate the binding ligand, leading to an increase in viscosity of the solution<sup>31, 32</sup>.

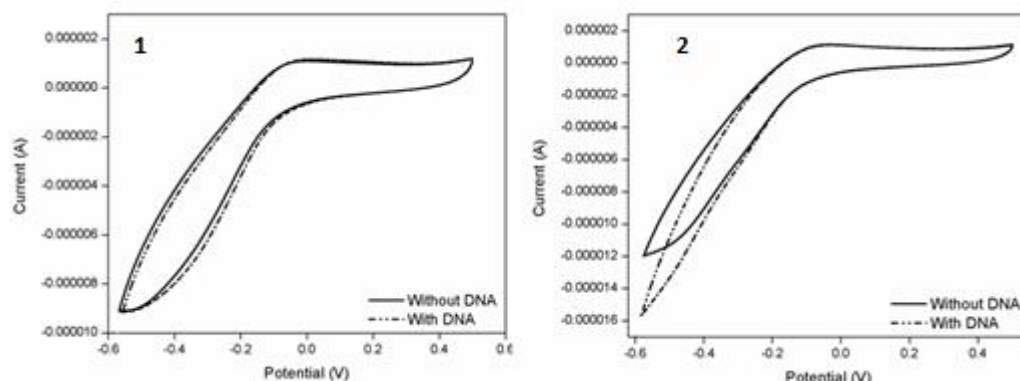


**Figure 1.6:** Viscosity of complexes **1** and **2** with CT-DNA.

#### 1.4.4 Cyclic voltammetry studies

The cyclic voltammetric (CV) response for complexes **1** and **2** in Tris-HCl buffer (pH 7.28) in the presence and absence of CT DNA is shown in Figure 1.7. In the forward scan, a single cathodic and anodic peak were observed, which corresponds to the reduction and oxidation of complexes, which indicates that the process is reversible. When CT-DNA is added to a solution of complexes, marked decrease in the peak

current and potential values was observed. The cyclic voltammetric behavior was not affected by the addition of very large excess of DNA, indicating that the decrease of peak current of complexes after the addition of DNA due to the binding of the complex to the DNA<sup>33</sup>. When the concentration of DNA increased, the changes in peak current and potential become slow. This reveals that the complexes were interacting with Calf thymus-DNA.



**Figure 1.7:** Cyclic voltammogram of complexes (**1** and **2**) in the absence and presence of CT DNA.

#### 1.4.5 Antibacterial and antifungal activity

The copper (II) complexes were tested in vitro for its antibacterial and antifungal activity against certain pathogenic bacterial and fungal species using disc diffusion method. The complexes were found to exhibit considerable activity against bacteria and the fungus. Our group recently, reported that amino acid containing complexes has good antimicrobial activity<sup>34</sup>. Copper complexes show remarkable activity against the bacteria, the copper(II) complexes with L-

phenylalanine has exhibited considerable activity against some human pathogens (Figure 1.8). In our biological experiments, using copper(II) complexes, we have observed antibacterial activity antifungal activity. The complex **1** and **2** exhibit higher antibacterial activity (Table 1.1) against *staphylococcus aureus*. On comparing complexes **1** and **2**, complex **1** has shown high antifungal activity against *Aspergillus fumigatus*. It may be concluded that our complexes **1** and **2** inhibits the growth of bacteria and fungi to a greater extent.

**Table 1.1:** Antibacterial and antifungal activity of complexes **1** and **2**.

S. No.	Micro organisms	Complex 1	Complex 2	Copper Nitrate	Ciproflaxacin/Amphotericin-B
		Zone of Inhibition (mm)			
<b>Bacteria</b>					
1	<i>Escherichia coli</i>	24	23	11	26
2	<i>Enterococcus faecalis</i>	19	22	16	24
3	<i>Staphylococcus aureus</i>	23	21	19	32
<b>Fungi</b>					
4	<i>Aspergillus fumigatus</i>	19	21	12	18
5	<i>Mucor sps</i>	17	18	11	20

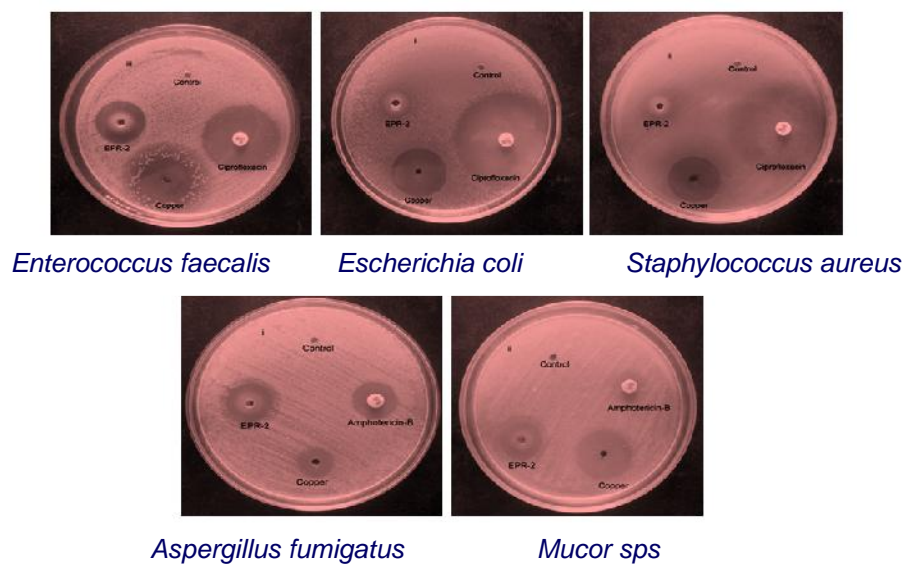


Figure 1.8: Antibacterial and antifungal activity of complex 1.

#### 1.4.6 MTT assay

The cytotoxicity of the complexes 1 and 2 to be used as anticancer agents were studied using MTT assay (Table 1.2). The ability of the complexes on HepG2 cells was tested with or without various concentrations (7.8–1000 µg/ml). Cells incubated with different concentration of Doxorubicin served as positive control. After incubation period, MTT assay was carried out to calculate the cell death percentage. For

each concentration, of the complexes cells were incubated in triplicate<sup>35</sup>. The (Figure 1.9) clearly illustrates that there is a clearly damage in the live cells number in the cells incubated with complex in a concentration dependent manner. Viability of cells incubated without any compound was considered as 100% and the percentage of live cells incubated with compound are given as relative to the control. The IC<sub>50</sub> value of the complexes 1 and 2 are 62.5 µg/ml.

Table 1.2: MTT Assay of complexes 1 and 2.

Concentration (µg/ml)	Dilutions	Absorbance (O.D)		Cell viability (%)	
		Complex 1	Complex 2	Complex 1	Complex 2
1000	Neat	0.198	0.192	20.75	21.34
500	1:1	0.260	0.250	27.25	27.52
250	1:2	0.329	0.322	34.48	33.58
125	1:4	0.399	0.385	41.82	45.27
62.5	1:8	<b>0.459</b>	<b>0.455</b>	<b>48.11</b>	<b>51.46</b>
31.2	1:16	0.527	0.521	55.24	60.48
15.6	1:32	0.598	0.590	62.68	64.18
7.8	1:64	0.660	0.661	69.18	70.04
Cell control	-	0.954	0.859	100	100

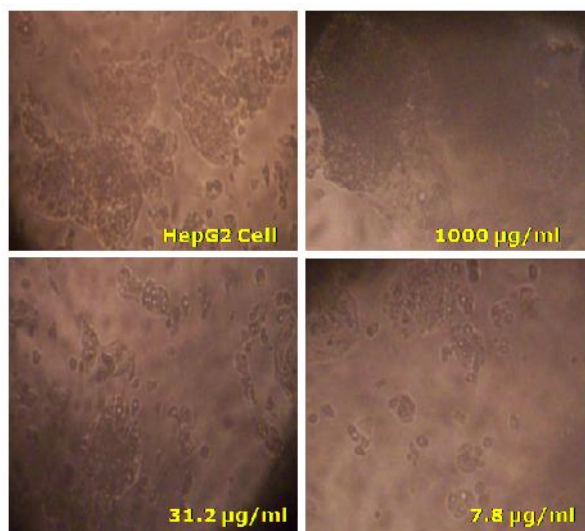


Figure 1.9: HepG2 Cell morphology of complex 1

## 1.5 Conclusion


The synthesis, characterization, DNA binding and biological activities of complexes was reported.  $\lambda$ -value suggested that copper(II) complexes are five coordination geometry. The DNA binding results revealed that the complex **1** can bind through intercalation mode and complex **2** interact through partial intercalation. As the mixed ligand complexes containing amino acid and heterocyclic bases show a unique DNA binding property, good antibacterial and antifungal agents. Complexes **1** and **2** have significant role against HepG2 cells<sup>35-37</sup>.

## 1.6 References

1. C. Marzano, M. Pellei, F. Tisato and C. Santini, *Anti-Cancer Agents Med. Chem.*, **2009**, 9, 185.
2. Ezhilarasan Dharmalingam, Arumugham, M. N., *Journal of Chemical, Biological and Physical Sciences*, 2017, 7(4), **896-905**.
3. (a) J. K. Barton and *J. Biomol. Struct. Dyn.*, **1983**, 1, 621. (b) S. Neidle and Z. Abraham, *CRC Crit. Rev. Biochem.*, **1984**, 17, 73. (c) J. K. Barton, *Commun. Inorg. Chem.*, **1986**, 19, 180. (d) J. K. Barton, *Science*, **1986**, 223, 727.
4. Dhakshanamoorthy, S.; Krishnan, M. Murali; Arumugham, M. N. *Indian Journal of Advances in Chemical Science*, 2018, 6(1), **53-58**.
5. (a) S. J. Lippard, *Acc. Chem. Res.*, **1978**, 11, 211. (b) J. J. Roberts and A. J. Thomson, *Prog. Nucleic Acid Res. Mol. Biol.*, **1979**, 22, 71. (c) S. M. Hecht, *Acc. Chem. Res.* **1986**, 19, 383. (d) J. Reedijk, *Pure Appl. Chem.*, **1987**, 59, 181.
6. F. Bregant, S. Pacor, S. Ghosh, S. K. Chattopadhyay and G. Sava, *Anti Cancer Res.*, **1993**, 13, 1007.
7. A. H. Li, L. X. Dai, and V. K. Aggarwal, *Chem. Rev.*, **1997**, 97, 2341,
8. Baskaran, S.; Murali Krishnan, M.; Arumugham, M. N., *Inorganic and Nano-Metal Chemistry*, 2017, 47(2), **269-277**.
9. E. A. Ambundo, M. V. Deydier, A. J. Grall, N. Aguera-Vega, L. T. Dresel, T. H. Cooper, M. J. Heeg, L. A. Ochrymowycz and D. B. Rorabacher, *Inorg. Chem.*, **1999**, 38, 4233.
10. C. H. Ng, K. C. Kong, S. T. Von, P. Balra, P. Jensen, E. Thirthagir, H. Hamada and M. Chikira, *Dalton Trans.*, **2008**, 4, 447.
11. M. Chikira, *J. Inorg. Biochem.*, **2008**, 102, 1016.
12. M. Chikira, Y. Tomizawa, T. Fukita, D. Sugisaki, N. Sugawara, T. Yamazaki, A. Sasano, H. Shindo, M. Palaniandavar and W. E. Antholine, *J. Inorg. Biochem.*, **2002**, 89, 163.
13. T. Hirohama, Y. Kuranuki, E. Ebina, T. Sugizaki, H. Arie, M. Chikira, P. T. Selvi and M. Palaniandavar, *J. Inorg. Biochem.*, **2005**, 99, 1205.
14. K. Hussain Reddy and P. Sambasiva Reddy, *Transition Met. Chem.*, **2000**, 25, 505.
15. I. Bertini, H. B. Gray, S. J. Lippard and J. S. Valentine, *Bioinorganic Chemistry. University Science Books, Mill Valley.*, **1995**.
16. J.K. Barton, J.M. Goldberg, C.V. Kumar and N.J. Turro, *J. Am. Chem. Soc.*, **1986**, 108, 2081.
17. T. Boulikas and M. Vougiouka, *Oncol. Rep.*, **2003**, 10, 1663.
18. E. Wong and C.M. Giandomenico. *Chem. Rev.*, **1999**, 99, 2451.
19. Saravanan, P. C.; Krishnan, M. Murali; Arumugham, M. N., *Indian Journal of Advances in Chemical Science*, 2017, 5(4), **324-329**.
20. Ezhilarasan D, Krishnan M. Murali, Arumugham M. N., *International Journal of Current Research in Chemistry and Pharmaceutical Sciences*, 2017, 4(8), **44-54**.



21. M. Sabat, in, A. Sigel and H. Sigel, *Marcel Dekker, New York, Basel*, **1996**, 32.
22. G. Dehghan, J. E. N. Dolatabadi, A. Jouyban, K. A. Zeynali, S. M. Ahmadi and S. Kashanian, *DNA Cell Biol.*, **2010**, 30, 195.
23. S. Selvaraj, S. Krishnaswamy, V. Devashya, S. Sethuraman and U.M. Krishnan, *RSC Adv.* **2012**, 2, 2797.
24. S. Dhakshanamoorthy, M. Murali Krishnan and M. N. Arumugham, *Asian Journal of Research in Chemistry*, **2017**, 10, 312.
25. D. Ezhilarasan, M. Murali Krishnan and M. N. Arumugham, *Journal of Chemistry and Chemical Sciences*, **2017**, 7, 477.
26. S. Dhakshanamoorthy, M. Murali Krishnan and M. N. Arumugham, *International Journal of Chemical and Physical Sciences*, **2017**, 6, 39.
27. J. Li, J. Dong, H. Cui, T. Xu and L. Li, *Transition Metal Chemistry*, **2012**, 37, 175.
28. J. M. Veal, and R. L. Rill, *Biochemistry*, **1991**, 30, 132.
29. Ezhilarasan, D.; Arumugham, M. N. *International Journal of Pharmacy and Pharmaceutical Research*, 2019, 14(2), **167-180**.
30. Saravanan, P. C.; Krishnan, M. Murali; Arumugham, M. N, *International Journal of Pharmaceutical Sciences and Research*, 2019, 10(1), **148-156**.
31. Baskaran, S.; Murali Krishnan, M.; Arumugham, M. N.; Kumar, R., *Journal of Coordination Chemistry*, 2019, 72(5-7), **941-961**.
32. M. C. Prabakara and H. S .B. Naik, *Biometals.*, **2008**, 21, 675.
33. P. Santhakumar and M. N. Arumugham, *International Journal of Recent Scientific Research*, **2012**, 3, 459.
34. H. Gopinathan, N. Komathi and M. N. Arumugham, *Inorganica Chimica Acta*, **2014**, 416, 93.
35. J. B. LePecq and C. Paoletti, *J. Mol. Biol.*, **1967**, 27, 87.
36. Haq, P. Lincoln, D. Suh, B. Norden, B. Z. Chowdhry and J. B. Chaires, *J. Am. Chem. Soc.*, **1995**, 117, 4788.
37. B. D. Wang, Z. Y. Yang, P. Crewdson and D. Q. Wang, *J. Inorg. Biochem.*, **2007**, 107, 1492.

Access this Article in Online	
	Website: <a href="http://www.ijcrcps.com">www.ijcrcps.com</a>
	Subject: <b>Chemistry</b>
Quick Response Code	
DOI: <a href="https://doi.org/10.22192/ijcrcps.2019.06.11.001">10.22192/ijcrcps.2019.06.11.001</a>	

How to cite this article:

P. C. Saravanan, D. Ezhilarasan and M. N. Arumugham. (2019). Synthesis, Characterization and DNA binding of Copper (II) complexes with mixed ligands of 1, 10 - Phenanthroline / 2, 2'- Bipyridyl, L-Valine: studies on Anti-microbial and Anti-cancer activities. *Int. J. Curr. Res. Chem. Pharm. Sci.* 6(11): 1-9.  
DOI: <http://dx.doi.org/10.22192/ijcrcps.2019.06.11.001>