IN-VITRO ANTICANCER ACTIVITY AND DNA CLEVAGE OF BIOLOGICALLY ACTIVE VO(II) COMPLEX WITH ETHYL-4-AMINOBENZOATE AND OXALATE ION

B. Sathiyamoorthy, K.Rajasekar*, S. Balasubramaniyan, R. Selvarani and C. Veravel
PG and Research Department of Chemistry, Ariyalur-621713, Tamil Nadu, India
(Affiliated to Bharathidasan University, Tiruchirappalli- 620 024)
*E-mail: yokkesh11@gmail.com

ABSTRACT
The mononuclear complex of VO(II) with ethyl-4-aminobenzoate (4-EAB) and oxalate ion (OX) have been synthesized and characterized based on physico-chemical, spectral and bio-potential activities. The conductance nature of the complex was confirmed by molar conductance of $10^{-3}$ M solution. The mononuclear nature and formula of the complex were confirmed by its metal estimation and elemental analysis studies. Probable geometry and ligating ability were also confirmed by its IR, electronic spectra and magnetic moment. The metal coordinated atom ability of the complex was confirmed by its Far-IR spectral study. The bio-potential activities of the complex were carried out Agar well diffusion method and compared with those for the ligand as well as standards (Chloramphenicol). The in-vitro antibacterial activity of VO(II) complex was screened for gram-positive S.aureus. The DNA cleavage of super coiled pBR322 DNA by metal complex also carried out and find out the cleavage nature of ligand and complex. The in-vitro anticancer activity of the ligand and VO(II) complex was carried out by using a human breast cancer cell line (Michigan Cancer Foundation-7 (MCF-7). Cytotoxicity assay, inhibition Concentration (IC$_{50}$) and absorbance at 570 nm were evaluated by the MTT reduction assay and UV- Spectrophotometer.

Keywords: Ethyl-4-aminobenzoate, Oxalate Ion, VO(II) Complex, S.aureus, Anticancer, DNA Cleavage

INTRODUCTION
Ethyl-4-aminobenzoate is an ethyl ester of para-aminobenzoic acid. It is synthesized from Fisher esterification using ethanol and para-aminobenzoic acid. Ethyl-4-aminobenzoic acid have one hydrogen bond donor and three hydrogen bond acceptor sites. The formal charge is zero so it is used as a neutral ligand in coordination chemistry. Local anesthetics are the compounds which are available naturally and synthetically. Ethyl-4-aminobenzoate was one of the active compound of many drugs because they have lot of applications in skin creams, dry powder for skin ulcer, throat lozenges and teething formulation for young children’s. Reducing pain in mucous membranes they are sore throat, cold sores, mouth ulcer, toothache, sore gums and denture irritations. Many pharmaceutical compounds consist of Ethyl-4-aminobenzoate in the form of tablets, syrup, and solution for the treatment of oral, tongue and gastric ulcer. The ointment form is used for the treatment of hemorrhoid disease. Microwave assisted synthesis reactions are low cost, eco-friendly, high yield method. Many metal complexes with organic ligands where of great attention due to their biological and pharmaceutical activities.

EXPERIMENTAL
All the chemicals such as Vanadylsulphate, solvents and reagents were of AnalaR grade (99% pure) used as such without further purification. Ethyl-4-aminobenzoate was purchased from Alfa Aesar Company.
DNA Cleavage
The DNA cleavage of super coiled pBR322 DNA promoted by metal complex was proceeded by the addition of the reaction mixture (20μl). The reaction mixture 20μl containing pBR322 DNA, 50 mM Tris–HCl, pH 7.4, 50 mM NaCl, 10 mM H₂O₂ added in a different volume, followed by adding Millipore water for the final volume. Then the mixed solutions were incubated at 37º C for 1 hr. They checked by Agarose gel electrophoresis method.

Synthesis of Metal Complexes
The mononuclear vanadium complex was prepared by mixing ethyl-4-aminobenzoate 2.61 g (15.81 mmol) in ethanol, oxalate ion 0.529 g (3.95 mmol) in water to Vanadylsulphate 1g (3.95 mmol) in methanol. The mixture was irradiated on a microwave oven (CATA-R, model). The precipitated pale green color complex was filtered, washed, dried in desiccators and kept in an air-tight glass container. The complex is stable under ordinary conditions.

Elemental analysis was carried out using elemental Vario make EL-III model instrument at 950-1200°C temperature. The metal ions were estimated after decomposing a known weight of complex in acids by colorimetric method using 8-quinolinol. Molar conductance of 10⁻³ M complex solution was carried out using the Systronic Conductivity Bridge. The Cyclic voltammogram of the complex was recorded in the DMSO solution at room temperature on Versa Stat (Princeton Applied Research-Make) electrochemical analyzer. The magnetic moment of VO(II) complex was measured using a Lake Shore 7410 Vibrating Sample Magnetometer (VSM) at room temperature. The solid-state diffused reflectance spectral (DRS) methods of UV-Visible spectra of the complex were recorded by using Varian make, CARY-5000 model, UV-VIS-NIR Spectrophotometer. Using Shimadzu, FT-IR, 8400 S Model IR spectrometer, IR spectra of ligands and its metal complex were recorded. The Far IR spectra of the complex were recorded in a Bruker, Germany makes 3000 Hyperion Microscope with Vertex 80 FTIR system model instruments.

Antimicrobial Assay
Antibiogram of ligand and VO(II) complex was done by Agar well diffusion method. The Petri plates were prepared by pouring 30 ml of NA/PDA medium for bacteria. The test organism was inoculated on a solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mins. The surfaces of media were inoculated with bacteria from a broth culture. A sterile cotton swab is dipped into a standardized bacterial test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing *Staphylococcus aureus* bacterium were spread on Nutrient agar plates. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the samples (50μl, 100μl and 150μl) were laid down on the surface of the inoculated agar plate. The plates were incubated with 24hrs and each sample was tested in triplicate.

Cytotoxicity Assay
The Cytotoxicity of ligand and its metal complex was evaluated by the reduction of MTT [3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium]. The single well suspensions were made by the detachment monolayer cells using tripsin-EDTA. The cell viability was measured using hemocytometer. The medium was used to dilute the cell suspension which was containing 5% of FBS. 96 well plates were used in the cell suspension. The plating density is 10,000 cells/well with one hundred microlitres of cell suspension and it was incubated at 37º C they have 5% CO₂, 95% air and 100% relative humidity. Aliquots of 100 μl of different concentrations of metal complex and ligands (12.5, 25, 50, 100, 200 and 400μg/ml) dissolved in DMSO (1%) were added to the appropriate wells already containing 100 μl of the medium, and incubated for 48hrs at 37ºC, 5% CO₂, 95% air and 100% relative humidity. After 48hrs of incubation, to each well 20μl of 0.5% (MTT) phosphate- buffer saline solution were added and incubated 4 hrs at 37ºC. Then, 100μl of 0.1% DMSO was added to each well to dissolve the MTT metabolic product. Then the plate was shaken at 150 rpm for 5 mins. The cell viability was measured by using 570nm absorbance and the concentrations required for absorbance are inhibition Concentration (IC₅₀) was determined graphically using UV- Spectrophotometer. The medium without samples served as control and triplicate were maintained for all concentrations. The effect of the samples on the antiproliferation of MCF-7 was expressed as the % Cytotoxicity using the following formulas:
% Cytotoxicity = 100 - \[\frac{\text{Abs (sample)}}{\text{Abs (control)}}\] \times 100.

% Cell Viability = \[\frac{\text{Abs (sample)}}{\text{Abs (control)}}\] \times 100.

**RESULTS AND DISCUSSION**

The elemental analysis and metal estimation of the complex shows the ligand and metal stoichiometry based on the data obtained from elemental analysis is in good agreement with the metal estimation. The molar conductance of 10^{-3} M solution of complex solution was measured and the lower value indicating their non-electrolyte (1:0 type) complex in acetonitrile solution.  

**Analytical Data for Ethyl-p-aminobenzoate**

IR (\(\nu_{\text{max}}\)): 3423 cm\(^{-1}\) (\(\nu \text{NH}_2\) sym), 3342 cm\(^{-1}\) (\(\nu \text{NH}_2\) asy), aromatic C-C at 3047 cm\(^{-1}\), aromatic C-H at 3223 cm\(^{-1}\), 1695 cm\(^{-1}\) (\(\nu \text{C}=\text{O}\) carboxylate ester); UV-Vis (nm): 261 (LMCT), 326 (MLCT); NMR for C\(_9\)H\(_{11}\)NO\(_2\): \(^1\text{H}\) (500 MHz, DMSO-d\(_6\)): \(\delta\) 1.27-1.26 (t, J = 7.54, 2H), \(\delta\) 4.22-4.20 (q, J = 7.04, 3H), \(\delta\) 5.94 (NH\(_2\), broad singlet), \(\delta\) 6.60-6.57 (d, J = 8.50, 2H-ortho), \(\delta\) 7.66-7.65 (d, J = 7.00, 2H-meta); \(^{13}\text{C}\) (125 MHz, DMSO-d\(_6\)): \(\delta\) 166.36(C5), 153.89(C4), 131.50(C2), 116.60(C1), 113.11(C3), 59.92(C6), 14.78 (C7).

**Analytical Data for [VO(4-EAB)\(_4\)(OX)]**

IR (\(\nu_{\text{max}}\)): 3428 cm\(^{-1}\) (\(\nu \text{NH}_2\) sym), 3350 cm\(^{-1}\) (\(\nu \text{NH}_2\) asy), 2984 cm\(^{-1}\) (\(\nu \text{C}-\text{C}\) aromatic), 1670 cm\(^{-1}\) (\(\nu \text{C}=\text{O} \text{ carboxylate ester}\), 1444 cm\(^{-1}\) (\(\nu \text{O}=\text{C}-\text{O}\) asy), 1370 cm\(^{-1}\) (\(\nu \text{O}=\text{C}-\text{O}\) sym), 1023 cm\(^{-1}\) (\(\nu \text{C}-\text{C}\) stretching), 772 cm\(^{-1}\) (\(\nu \text{O}-\text{C}-\text{O}\) plane deformation); UV-Vis (nm): 259, 331, 626, three transition, octahedral \(\mu_{\text{eff}}\): 3.75 BM; Analytical cal. for [VO(PAB)\(_4\)(OX)]: Cal: C 53.92, H 5.20, N 6.62, O 22.70, M 6.02.30; Found: C 53.85, H 5.13, N 6.69, O 22.60, M 6.50.

**Measurement of the Zone of Inhibition**

The antibacterial potential test of 4-EAB and VO(II) complex was measured using Agar well diffusion method by selecting S. aureus bacterial strain. The zone of inhibition was measured in millimeters from the values of the diameter of the zone, the activity of the complex compared with those for the ligand EAB. The MIC values of the tested microorganisms confirming the complex and ligand shows moderate activity at higher concentrations due to the metal-binding site and neutral nature of the complex. (Fig.-1) The lipophilicity is another factor for the antibacterial activity.

**In-vitro Anticancer Activity of Ligand and Metal Complex**

Michigan Cancer Foundation-7 (MCF-7) is a human breast cancer cell line and is useful for in-vitro breast cancer studies because the cell line has retained several ideal characteristics particular to the mammary
epithelium. These include the ability of MCF-7 cells to process estrogen via estrogen receptors. The cell growth inhibition of the ligand and VO(II) complex was tested against Michigan Cancer Foundation-7 (MCF-7) cell line at different concentrations (25, 50, 100 and 200µg/ml). The results indicating that the ligand and VO(II) complex should increase in the cell growth inhibition but the ligand found 16.71 % at 50µg/ml and highest growth inhibition up to 84.13% observed at 200 µg/ml concentration whereas the VO(II) complex found to be lowest as much as 12.21% at 50µg/ml shows highest growth inhibition up to 80.73% observed at 200 µg/ml concentration. The IC\textsubscript{50} value of ligand is 102.71µg/ml but in the complex, it is 111.94µg/ml. Fig.-2 and 3 show both ligand and complex posses potential anticancer activity. The most identifiable morphological features of apoptosis (indicated as block arrow). Fig.-4 were observed by inverted light microscopy in the VO(II) complex treated cells. The VO(II) complex cells appeared like cells undergoing apoptosis with prominent features such as detaching from the culture plate, cytoplasmic condensation, cell shrinkage and condensation and aggregation of the nuclear chromatin, and loss of contact with neighboring cells. However, the untreated cells appeared normal (Control) and were confluent.\textsuperscript{13-14}

The interaction of super coiled PUC18 DNA on free ligand ethyl-4-aminobenzoate and [VO(4-EAB)\textsubscript{4}(OX)\textsubscript{2}] complex at 10% DMSO–50 mM Tris–HCl buffer with the pH of 7.2 was carried out by Agarose Gel Electrophoresis method. The DNA (base pairs) was incubated with different concentrations of 40µM and 50µM of 4-EAB and VO(II) complex for about 1 hour and it was subjected to Gel Electrophoresis. The Gel Electrophoresis separation of PUC18 DNA in the absence of oxidizing agent (lane 1), with the presence of oxidizing agent H\textsubscript{2}O\textsubscript{2} (lane-2 in Fig.-5) and oxidizing agent with 4-EAB and VO(II) complex (50 µM) (lane-3). From the photograph, it was mentioned that there was slight cleavage at 50µM shown in form-III (lane-3, 4 for VO(II) complex and 7,8 for 4-EAB).\textsuperscript{15}
CONCLUSION

From the results of elemental analysis, metal estimation and molar conductance, the VO(II) complex are mononuclear, neutral (non-electrolyte nature) were confirmed. Octahedral geometry, complex formation and metal ligating ability, and magnetic property of the complex confirmed by its UV-Visible, magnetic moment, IR and Far-IR spectral studies. The compound shows moderate bio-potential activity but it is a good anticancer drug against human breast cancer cell line Michigan Cancer Foundation-7 (MCF-7). The interaction of supercoiled PUC18 DNA on free ligand ethyl-4-aminobenzoate and [VO(4-EAB)_{4}(OX)] complex is moderate.

ACKNOWLEDGMENT

The authors are thankful to the Principal and Head Department of Chemistry, Govt. Arts College, Ariyalur for allowing using the facilities available in the department and my extent the thanks to the Head, SAIF, IIT and the Director, STIC, Cochin for providing the spectral data collection. The authors also thank the director, Harman Research Institute for providing biological activities of the complex.

REFERENCES

1. Serhiji Plotycya, Oksana Stront Sitska, Solomiya Pysarevska, Mykola Blazheyevskiy and Liliya Dubenska, *International Journal of Electrochemistry*, 2018, 1(2018), *DOI*: 10.1155/2018/1378231.
2. Magdalena Paczkowska, Gabriela Wiergowska, Andrzej Miklaszewski, Anna Krause, Magdalena Mroczkowka, Przemyslow Zalewski and Judyta Cielecka-Pitootek, *Molecules*, 23(1737), 1(2018), *DOI*: 33901/Molecules 23071737.
3. Elena Pahontu, Diana-Carolina Ilies, Sergiu Shova, Codruta Paraschivescu, Mihaela Badea, Aurelian Gulea and Tudor Rusu, *Molecules*, 20, 5771(2015), DOI:10.3390/Molecules 200457771.

4. B. K. Sinha and Vasantha Pattabhi, *Journal of Chemical Science*, 98(3), 229(1987), DOI:10.1007/BF02900724.

5. Mirela-Fernanda Zaltariov, Maria Cazacu, Mihaela Avadanei, Sergiu Shova, Mihaela Balan, Nicoleta Vornicu, Angelica Vlad, Anatolie Dobrov, Cristian-Dragos Varganici, *Polyhedron*, 100(4), 121(2015), DOI:10.1016/j.poly.2015.07.030.

6. Sulekh Chandra, Rajiv Kumar, Rajeev Singh, Akash Kr. Jain, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 65(3-4), 852(2006), DOI:10.1016/j.saa.2006.01.005.

7. S. Baskaran, M. Murali Krishnan, M.N. Arumugham and R. Kumar, *Journal of Coordination Chemistry*, 72(5-7), (2019), DOI:10.1080/00958972.2019.1584295.

8. Xinlong Wang, Chao Qin, Enbo Wang, Yang guang Li, Na Hao, Chang wen Hu, Lin Xu, *Inorganic Chemistry*, 43(6), 1850(2004), DOI:10.1016/j.ice.2003.10.030.

9. R.V. Singh, Pratibha Chaudhary, Shikha Chauhan, Monika Swami, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 72(2), 260(2008), DOI:10.1016/j.saa.2008.09.017.

10. Prasoon Kumar Kaushik, V.K Varsihney, Pawan Kumar, Pallavi Bhatia and S. V. Shukla, *Synthetic Communications*, 46(24), 2052(2016), DOI:10.1080/00397911.2016.1245749.

11. Tarek A. Fahad, Kawther A. F. Al Radi and Enas A. Bady, *World Journal of Pharmaceutical Research*, 8(1), 265(2019), DOI:10.20959/wjpr20191-13920.

12. Jigna Parekh, Pranav Inamdhar, Rathish Nair, Shipra Baluja and Sumitra Chanda, *Journal of Serbian Chemical Society*, 70(10), 1155(2005)

13. Mokhles M. Abd-Elzaher, Anmar A. Labib, Hanan A. Mousa, Samia A. Moustafa, Mamdouh M. Ali and Ahmed A. El-Rashedy, *Bene-Suef University Journal of Basic and Applied Sciences*, 5(1), 85(2016), DOI:10.1016/j.bjbas.2016.01.001.

14. Mariana A. A. Aleixo, Tais M. Garcia, Diego B. Carvalho, Luiz H. Viana, Marcos S. Amaral, Najla M. Kassab, Marilin C. Cunha, Indiara C. Pereira, Palimécio G. Guerrero Jr., Renata T. Perdomo, Maria F. C. Matosb and Adriano C. M. Baroni, *Journal of Brazilian Chemical Society*, 29(1), 109(2018), DOI:10.21577/0103-5053.20170119.

15. Mouayed A. Hussein, Teoh S. Guan, Rosenani A. Haque, Mohamed B. Khadeer Ahamed and Amin M.S. Abdul Majid, *Journal of Coordination Chemistry*, 67(4), 714(2014), DOI:10.1080/00958972.2014.893430

[RJC-5668/2019]