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Synthesis, Characterization and Comparative study of Anti -Cancer Activity of Tetra Pyridyl Porphyrin and Zn (II) Tetra Pyridyl Porphyrin.

Uthayanila S[a] , and Neeraja P[b]*.

[a]Department of Chemistry, AVS Engineering College, Salem, Tamil Nadu, India – 636003.
[b]Department of Chemistry, Adhiyamaan College of Engineering, Hosur, Tamil Nadu, India 635109.

ABSTRACT

The tetra(4-pyridyl)porphyrin entity in its free-base (TPyP) as well as its metallated (ZnTPyP) creates the base for major biological processes and more and more wide application of porphyrins in an engineering, biology and practical medicine.The compound 5,10,15,20 tetra pyridyl porphyrin (TpyP) and the complex 5, 10, 15, 20-tetrapyridyl Zinc porphyrin (ZnTPyP) were synthesized by modified Alder method. These compounds are characterized by UV-Visible Spectroscopy,FT-IR Spectroscopy, ^{1}H-NMR spectroscopy, Fluorescence Spectroscopy and Cyclic Voltametry. In-Vitro anticancer activity of the compounds have been evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay method. Among the investigations,one focus is about the comparative study of spectroscopy and anticancer activity of tetrapyridyl porphyrin and its Zn complex of different concentration and different time dependent.

Keywords: MTT, anticancer activity, Cyclic Voltametry, UV-Visible, ^{1}H-NMR.

Corresponding author

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INTRODUCTION

Porphyrins are one of the vital chemical units essential for several life processes on the earth. Many biological molecules function with prosthetic groups essentially made of these units. A large variety of synthetic porphyrins and their metalloderivatives were made over the years to study the porphyrin based natural system [1-7].The medium in cancerous tissues is often more acidic than in normal tissues [8]. A hydrophilic molecule accumulates in cells mainly in lysosomes [9] where medium is acidic. There is evidence that porphyrin molecules can accumulate in tumor organs selectively [10].

Pyridyl porphyrins are intriguing ligands that yield, when coordinated to metal porphyrin complexes or to other metal complexes, unique supramolecular assemblies with multi electron redox and photochemical activities [11-14]. Studies shown that significant differences exist in the photo physical and redox properties of tetrapyridyl porphyrins as compared to those of tetra phenyl porphyrins. In general tetra pyridyl porphyrins are considerably more acidic than the tetra phenyl porphyrin based systems [15].

Quimby and Longo et al [16] reports shows that the effects caused by zinc insertion vary according to the porphyrin structure. Zinc porphyrins show stronger binding to both synthetic and biological membrane than their respective free base analogues. Zinc porphyrins have better efficiency compared with metal free porphyrins[17].This is because the addition of Zinc increases the hydrophilic character and decreases the hydrophobic character.

EXPERIMENTAL

Materials

All the chemicals and reagents used were analar grade obtained from Merck, India and were used as received without further purification. RPMI Medium, penicillin and streptomycin antibiotic solutions and fetal boivine serum (FBS) were purchased from ATCC (USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO) were purchased from Invitrogen (Carlsbad, CA, USA) and Sigma-Aldrich (St. Louis, MO, USA).

Synthesis

Synthesis of 5,10,15,20 tetra pyridyl porphyrin (TPyP)

Synthesis of TPyP was done according to literature data [18]: A mixture of 9.41 ml (100 mmol) of 4-pyridinecarboxaldehyde and 13 ml (100 mmol) propionic anhydride was dissolved in 300 ml propionic acid and 6.93 ml (100 mmol) pyrrole dissolved in 100 ml of propionic acid, was added drop wise to the mixture heated to reflux, for 1/2 h. The whole reaction mixture was refluxed for over 2h. After cooling to room temperature, the solvent was evaporated to dryness, and the oily residue was repeatedly washed with hot water, neutralized by aqueous ammonia (25%), and washed again with hot water. The purple solids obtained by this procedure were filtered and dried. The dry solid material was treated with three portions of 80 ml of dichloromethane, each followed by filtration. To the combined organic phases, 15 g of basic alumina (Brockman activity grade II) were added, and the solvent was evaporated to dryness.
Separation of 5,10,15,20-tetra(4-pyridyl) porphyrin was achieved by column chromatography, using as eluent a mixture of chloroform, dichloromethane and ethanol.

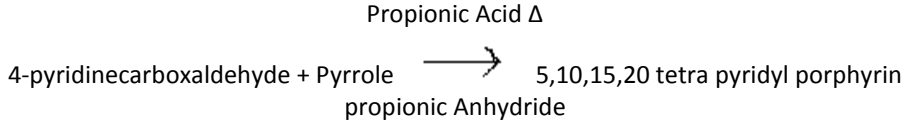

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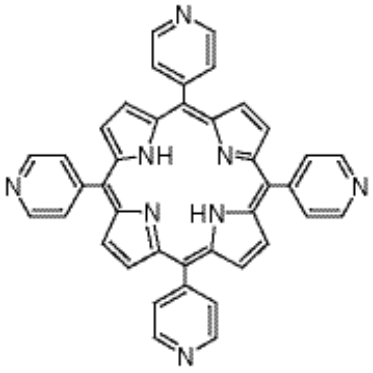
Fig.1a



5,10,15,20 tetra pyridyl porphyrin

Synthesis of 5,10,15,20-tetra(4-pyridyl) Zinc porphyrin (ZnTPyP)

Synthesis of ZnTPyP was done according to literature data [19] by using 0.108 g (0.175 mmol) TPyP and 0.173 g (0.87 mmol) Zn acetate, heated to reflux for 2 h in a mixture of 20 ml glacial acetic acid and 20 ml DMF. After cooling, the crystals are washed with water to remove the excess of Zn acetate and after drying it were purified by column chromatography using Al_2O_3 as stationary phase and as eluent a mixture consisting of 95% $CHCl_3$ and 5% methanol.

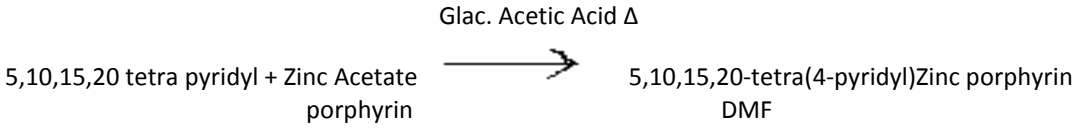
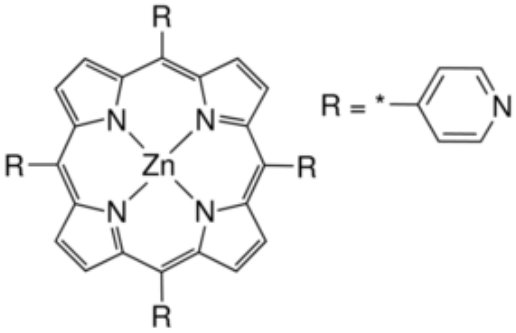


Fig.1b



Characterization techniques

UV-Visible Spectroscopy

UV-Visible Spectra were recorded at room temperature using Schimadzu 1501 spectrophotometer with 2 nm resolution.

NMR Spectroscopy

^{1}H NMR were run on a Bruker 400 MHz spectrometer using DMSO-D6 as solvent and TMS (Tetra Methyl Silane) as the internal standard.

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Cyclic Voltametry

Cyclic voltammograms were recorded using a one-compartment, three electrode cell, CH-Instruments, equipped with a platinum wire auxiliary electrode. The working electrode was a 2.0-mm diameter platinum disk from CH Instruments.

Anticancer Activity Studies

Cell culture

The U937 (human histiocytic lymphoma cell line) was procured from American Type Culture Collection (ATCC, USA). The cells were cultured in RPMI medium with 10% Fetal bovine serum (FBS), 100 U/mL penicillin and 100 µg/mL streptomycin in T25cm^2, T75 cm^2, and 96-well culture plates at 37°C in a humidified atmosphere containing 5% CO_2. All experiments were performed using cells from passage 15 or less.

Cell viability assay

The MTT assay was carried out as previously described by Mossman (1983). Briefly, U937 cells were plated at a density of 1.5 x 10^4 cells per well in 200µL of fresh culture medium. After overnight growth, cells were treated at different concentrations (25–125 µg/mL) of complexes 1-3 for 24 and 48 h. After incubation, 20 µL of MTT solution (5 mg/mL in phosphate-buffered saline (PBS) were added to each well. The plates were wrapped with aluminum foil and incubated for overnight at 37 °C. The plates were centrifuged and supernatant was carefully removed. Purple color formazan crystals were dissolved by the addition of 100µL of DMSO to each well. The absorbance was monitored at 570 (measurement) and 630 nm (reference) using a 96-well plate reader (Bio-Rad, CA, USA). Data were collected for three replicates each and used to calculate the mean. The percentage inhibition was calculated, from this data, using the formula:

$$\frac{\text{Mean } \textit{abasorbance} \text{ of untreated cells (control)-Mean } \textit{abasorbance} \text{ of treated cells}}{\text{Mean } \textit{absorbance} \text{ of untreated cells (control)}} \times 100$$

3. RESULTS AND DISCUSSION

UV-Visible Spectroscopy

The absorption spectrum of tetra pyridyl porphyin (Fig. 2a) comprises π-π* absorption bands characteristics of free base type porphyrin. An intense B band (Soret) with a peak around 360 nm and also with a slight shoulder at 294 nm due to electronic transitions. The Q bands are located in the visible spectral region around 404,520,600nm.

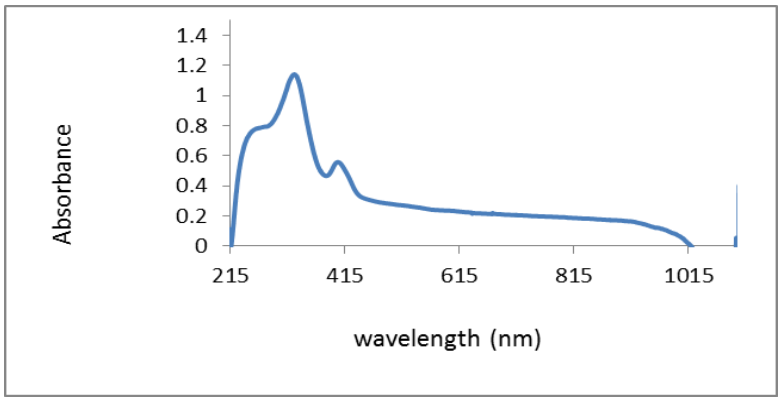

Fig. 2a UV-Visible spectrum of TPyP

The Zn metallated tetra pyridyl porphyin exhibits absorption (Fig. 2b) Soret band at 412nm and shows shoulder peak at 315nm.The shift in absorbance value were observed due to metallation. The Q bands are located in the visible spectral region around 548,620,720nm.

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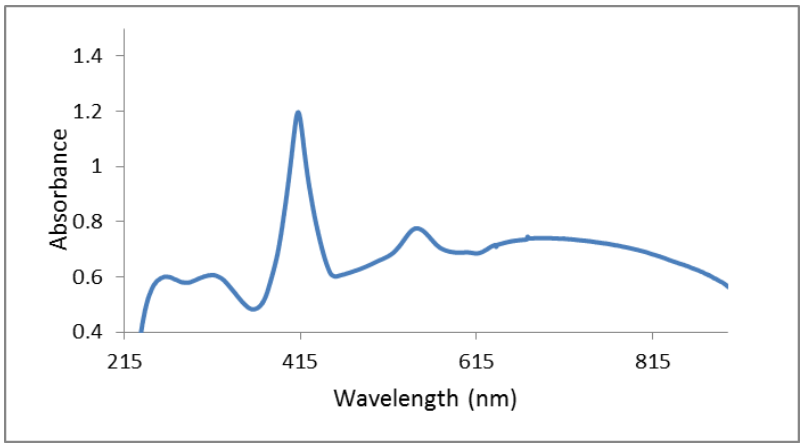

Fig. 2b UV-Visible spectrum of ZnTPyP

Tetra pyridyl porphyrin and its complexes are usually more stable to pH change than the corresponding Tetra phenyl porphyrin analogue, because the pyridyl substituents act as a sponge for surface protons. The equilibrium involved in this processes are complex and two different steps are involved in this case, the exterior protons are those attached to the pyridyl nitrogens while the interior ones are bonded to the pyrrole nitrogen.

NMR studies

The authenticities of the complexes were ascertained through ^{1}H NMR spectroscopy. ^{1}H NMR spectroscopy of porphyrins reveal the aromatic nature of porphyrin molecule. The Proton NMR spectrum [20, 21] of the compound TPyP and complexes ZnTPyP already have been reported. TPyP contains four chemically nonequivalent sets of protons gives four doublets peaks and ZnTPyP contains three different chemical environments gives three peaks.

Cyclic voltammetry studies

The oxidation and reduction potentials can be determined by cyclic voltammetry experiments. In the cathodic direction, the initial scan from 0 to - 2.0 V causes the reduction of the metals while the reverse scans +2.o to 0V causes the oxidation of metals producing a peak in a downward direction. The height of the peak is related to both concentration and reversibility of the reaction. Tetra pyridyl porphyrin (Fig.3a) results cycled in the anodic direction and there is a weak irreversible oxidation process slightly +ve with 1.0 V which is due to porphyrin oxidation. In the cathodic direction reversible redox couple with -1.1 V associated with sequential one electron reduction of the porphyrin ring to form the radical anion. [22]

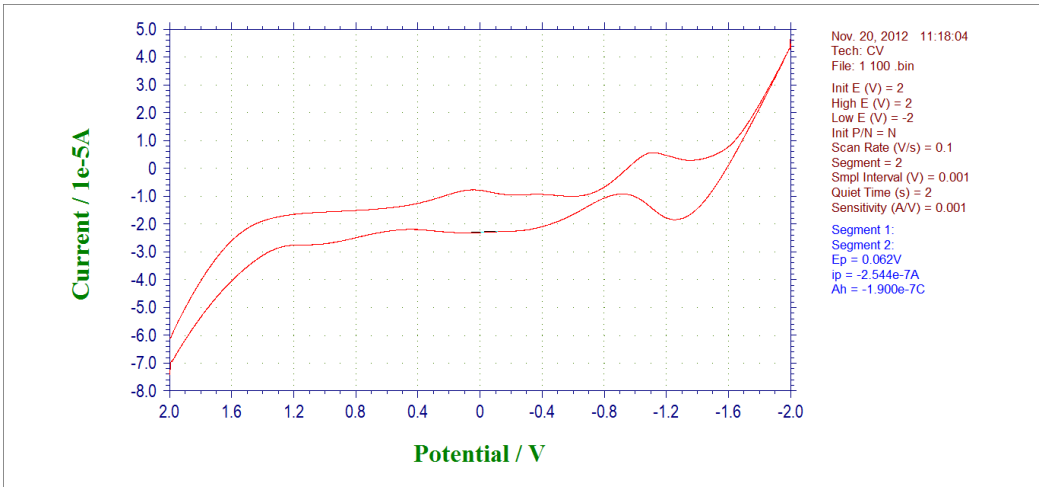

Fig.3a Cyclic voltagram of TPyP

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Zinc-Tetra pyridyl porphyrin (Fig.3b) complex is cycled in the anodic direction there are two irreversible redox couple with +0.3 V and +0.1 V associated with oxidation of Zn(II) to Zn(III) and also porphyrin oxidation .In the cathodic direction reversible redox couple with -0.9 V associated with reduction.

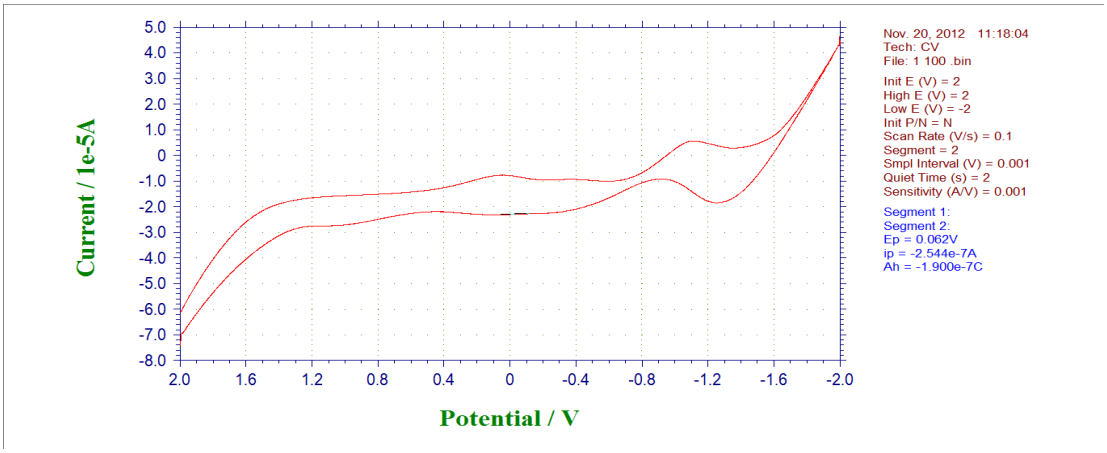

Fig. 3b Cyclic voltagram ZnTPyP

Anti-cancer activity studies

Our aim was to compare anticancer activity of the compound TPyP(Fig.4a) and the complex ZnTPyP(Fig.4b) by appropriate design of the structure of the complex to fulfill the requirements towards pharmacological profiles such as membrane permeability, target delivery, and modulation of cancer cell death. The cytotoxic effects of as prepared complexes were examined on cultured U937 human histiocytic lymphoma cell line by exposing cells for 24 and 48 h to a medium that contained the respective complexes at 25, 50, 75, 100, 125 µg/mL for 24 and 48 h. Overall TPyP and ZnTPyP induced cytotoxicity was concentration-dependent but not time-dependent. The percentage death increases by using the compound ZnTPyP compared to TPyp.(Table.1)

Table.1 Anti-cancer activity of TPyP and ZnTPyP

S. No.	Dose in µg/mL	% Death at 24 hrs for TPyP	% Death at 24 hrs for ZnTPyP	% Death at 48 hrs for TPyP	% Death at 48 hrs for ZnTPyP
1	0	0	0	0	0
2	25	2.81	8.72	6.82	12.93
3	50	3.69	18.58	8.96	30.06
4	75	8.93	25.49	11.93	34.46
5	100	11.81	42.12	19.96	43.11
6	125	27.47	45.99	35.75	51.52

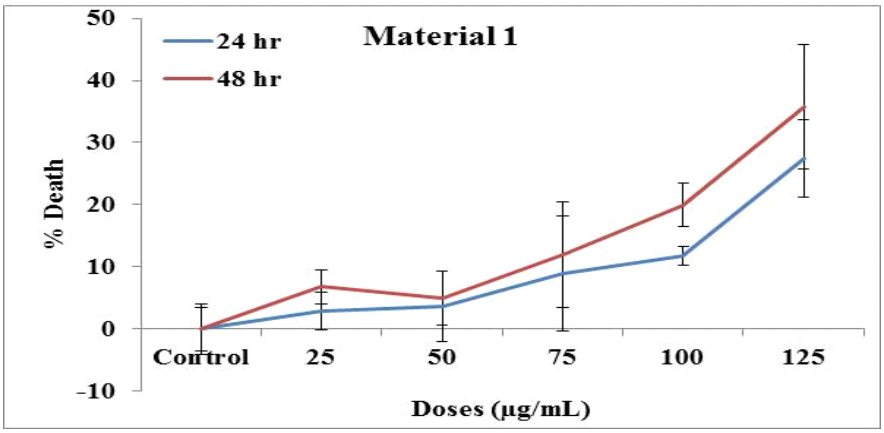

Fig. 4a Anti-cancer activities of TPyP at 24 and 48 hr on U937 lymphoma cancer cell line (material-1)

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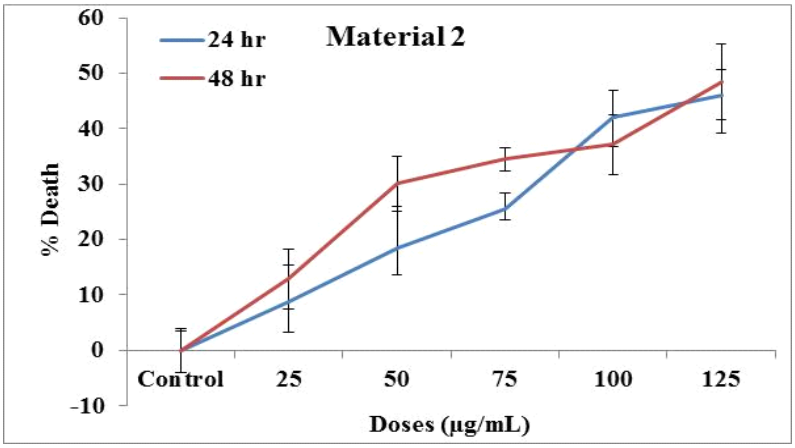

Fig.4b Anti-cancer activities of material ZnTPyP at 24 and 48 hr on U937 lymphoma cancer cell line (material-2)

CONCLUSIONS

The inhibitory activity of TPyP and Zn TPyP shows concentration-dependent and no time dependent was observed. Also this investigation may be extended to study about the detailed mechanism of inhibitory activity.

REFERENCES

[1] Nicolas PE, Oliver Z, Paul J, Bruno Therrin. Australian J Chem 2010;63:1529- 1537
[2] Antonio EH et al. Molecules 2011;16:5807-5821.
[3] Bruno Therrien. Top Curr Chem 2012;319:35-36
[4] Irigoyen JR, M.Balaco L, Lopez ST. Int J Electrochem Sci 2012;7:11246-11256.
[5] Longo J, Lozzi S, simioni A, Morais P, Tedesco A and Azevedo R. J Photochem Photobiol B 2009;94:143.
[6] Gianferra, et al. DaltonTrans 2009;48(1):742-10756.
[7] Irena Kostava. Drug Discovery2006;1:1-22.
[8] Keene JP, Kessel D, Land EJ, Redmod RW, Trustcott TG. Photochem Photobiol 1986;117:43.
[9] Wessels JM, Strauss W, Seidlitz HK, Ruck A, Schneckenburger H. J Photochem Photobiol B Biol 1992;275:12.
[10] Lotther C, Knuechel R, Bernhardt G, Brunner H. Cancer Lett 2004;215:167.
[11] Siboni G, Amit- Patito I, weizman E, Waintraubportat M, weitman H, Ehrenberg B, Malik Z. Cancer Lett 2003;196:57.
[12] Stylli SS, Hoews M, Mac Gegor L, Rajendra P, Kaye AH. J Clin Neurosci 2004;11:584.
[13] Mei W-J,Wei X-Y,Liu J,Lu W-G. Transition Metal Chem 2007;32:685.
[14] Kalyanasundaram K. Inorg Chem 1984;23:2453-2459.
[15] Hiroki Kon, Kiyoshi Tsuge, Taira Imamura, Yoichi Sasaki, Shoji Ishizaka, Noboru Kitamura. Inorg Chem 2006;45:6875-6883.
[16] DJ Qumiby and FR Longo. J Am Chem Soc 1975;97(18):5111.
[17] Christiane Pavani, et al. Photochem Photobiol Sci 2009;8:233-240.
[18] Aviezer D, et al. Cancer Res 2000:2973:60.
[19] Fleischer EB. Inorg Chem 1962;493:3.
[20] Saeed Zakavi, Aida Ghanbelanie Mojarrad, and Tahere Mokary Yazdely. Macroheterocycles 2012;5(1) :67-71.
[21] Henrique E. Toma and Koiti Araki. Coord Chem Rev 2000;196: 307 - 329
[22] Sullivan BS,Salmon DJ and Meyer JJ. Inorg Chem1978;3334:17.