Synthesis, characterization and anti-tumour activity of VOF_3 and Cu (II) porphyrine complexes

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Abstract

Metal complexes of the ligand, 10, 15, 20-Tetra-p-tolyl-porphyrine abbreviated as H_2TPP were prepared by reaction of salts of VOF_3 and Cu (OAC) $_2$ with H_2TPP in dry DMF. Characterization of the ligand and its complexes was made by microanalyses, FT-IR, ^1H NMR and UV–Visible spectroscopy. These new complexes showed excellent anti-tumor activity against two kind of cancer cells that are K562 (human chronic myeloid leukemia) cells and Jurkat (human T lymphocyte carcinoma) cells.

Keywords: 10, 15, 20-Tetra-p-tolyl-porphyrine, H_2TPP, VOF_3, Cu (II), complex, anti-tumor activity against, K562, Jurkat.

Introduction

The porphyrins are a class of naturally occurring macro cyclic compounds, which play a very important role in the metabolism of living organisms. These have been extensively studied due to their biological importance as well as analytical applications [1]. Particular attention has been given to porphyrins as the highly sensitive chromogenic reagents for spectrophotometric determination of several metal ions based on the use of the so-called Soret band at 400 - 500 nm [2-7]. The photodynamic therapy of cancer (PDT) is used a combination of photosensitize drug and light giving rise to reactive oxygen species in the tumor environment, leading to tumor death .In recent years, several papers have been published on the mechanism and kinetics of metal ions incorporation into the porphyrin nucleus [8 -11]. Metalloporphyrins have been extensively studied for many years because of their biological and catalytic functions [12–14]. Several spectroscopic techniques such as EPR [15], NMR [16], and Mossbauer spectroscopy [17] have been used to directly probe the metal centers in metalloporphyrin systems. Metal-porphyrins are used as homogeneous catalysts in a variety of oxidation reactions [18, 19]. Kinetic study of porphyrin metalation is indispensable in order to understand in vivo metal. Incorporation processes leading to the formation of natural metaloporphyrins. Generally, porphyrins are synthesized in a metal-free form and metal ions are subsequently inserted in the processes catalyzed by enzymes [20]. Synthetic metaloporphyrins are suitable sensitizers for photodynamic therapy of cancer [21]. From these points of view, it is interesting to study different types of transition metal

complexes of these biologically active ligands. In this paper, the synthesis, characterization and anti-tumor properties of a number of the first row transition metal complexes with one of the above ligands have been studied.

Results and discussion

Preparation for Ligand, H_2TPP and V(V) and Cu(II) complexes
The reaction of V(V) and Cu(II) salts with the ligand, H_2TPP, results in the formation of [ML] for M= V(V) and Cu(II).The complex probable structure is shown in Figure1.

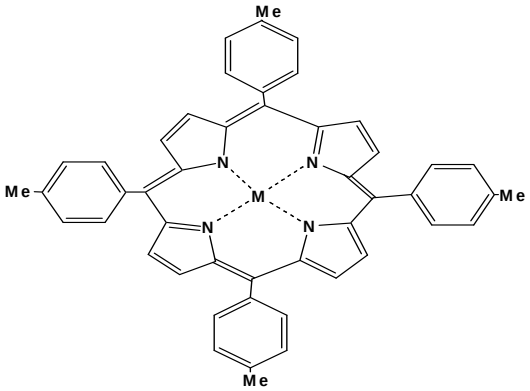

Figure. 1. Metallated 10, 15, 20-Tetra-p-tolyl-porphyrine

The Vanadium (V) and copper (II) complexes were characterized by several techniques using FT-IR, electronic spectra and molar conductance measurements. The molar conductance measurements reveal the presence of 1:1 electrolytic nature complexes. All complexes are quite stable and could be stored without any appreciable change. They are insoluble in common organic solvents, such as ethanol, methanol, acetone and DMF; however, they are soluble in chloroform. Their structures were characterized by elemental analysis, ^1H NMR and IR. The spectral data of the complexes have good relationship with the literature data. [22, 23]

Cytotoxicity studies
H_2TPP ligand and $[V(C_{44}H_{36}N_4)O]F_3$ and $[Cu(\ C_{44}H_{36}N_4\ )](OAC)_2$ compounds have been tested against two human cancer cell lines: K562 and Jurkat. The IC_{50} cytotoxicity values of the complexes were compared to those found for the starting organic bases as well as for some of the anti-cancer agents used nowadays that are cisplatin and oxaplatin compounds [24]. The general method used for testing on anti-tumor properties of these compounds is the standard testing method that has been previously described in greater detail in some papers [25, 26] and abbreviated in following:

After preincubation lasting 12h at 37°C in a 5% CO_2 atmosphere and 100% humidity, the tested compounds in the concentration rang 0.1-28μM for H_2TPP, 0.1-20μM for $[V(C_{44}H_{36}N_4)O]F_3$ and 0.1-25μM for $[Cu(\ C_{44}H_{36}N_4\ )](OAC)_2$ were added. The incubation lasted 72 h and at the end of this period IC_{90} and IC_{50} of the dead cells and live cells was measured by Trypan blue. IC_{90} and IC_{50} values that are the compounds concentrations lethal

for 90% and 50% of the tumour cells were determined both in control and in compounds concentrations lethal for both in compounds-treated cultures. The complexes were first dissolved in DMSO and then filtrated. The corresponding 50% and 90% inhibitory dose (IC_{50} and IC_{90}) values are shown in Table1.

Table1. 72 hour IC_{50} and IC_{90} values (μM) obtained for three compounds

Compound	IC_{50} for Cell line		IC_{90} for Cell line	
	K562	Jurkat	K562	K562
H_2TPP	>10	>10	-	-
$[V(C_{44}H_{36}N_4)O]F_3$	> 8.5	>8	>70	>65
$[Cu(C_{44}H_{36}N_4)](OAC)_2$	>9	>9	>40	>30

Experimental Section

Materials and measurements

Vanadium (V) oxy tri fluorides, copper (II) acetate monohydrate, were either Merck chemicals and were used without further purification. Organic solvents were reagent grade. Electronic spectra were recorded by Cam spec UV–Visible spectrophotometer model Wpa bio Wave S2 100. The IR spectra were recorded using FT-IR Bruker Tensor 27 spectrometer. ^{1}H-NMR were recorded on a Bruker AVANCE DRX 500 spectrometer at 500 MHz respectively. All the chemical shifts are quoted in ppm using the high-frequency positive convention; ^{1}H NMR spectra was referenced to external $SiMe_4$. The percent composition of elements was obtained from the Micro analytical Laboratories, Department of Chemistry, OIRC, Tehran.

Cell culture

The human chronic myeloid leukemia: K562 cell line and the human T lymphocyte carcinoma: Jurkat cell line, used for treatment with the drugs, was provided. K562 and Jurkat cells were grown at 37 $^{\circ}$C in an atmosphere containing 5% CO_2, with RPMI-1640 MEDIUM HEPES Modification with L-glutamine and 25mM HEPES (SIGMA-ALDRICH CHEMIE GmbH) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco), 2.7% sodium bicarbonate and 500 mg/L ampicillin.

Synthesis of 10, 15, 20-Tetra-p-tolyl-porphyrine

Synthesis methods for the preparation of 10, 15, 20-Tetra-p-tolyl-porphyrine [22] are summarized in follow.

A 25-mL round-bottom flask fitted with a water condenser is heated in a sand bath or heating mantle. Propionic acid, 12 mL, is added to the flask and brought to reflux (≈141°C).To the refluxing propionic acid 0.06 mL of benzaldehyde is added using a 0.1-mL graduated pipette. Pyrrole, 0.04 mL, from a freshly opened bottle or distilled at low pressure (30-40 mmHg) within a few days of use, is also added using a pipette. The colour darkens to orange-yellow and becomes dark brown-black as the reaction proceeds. The reaction mixture is refluxed for a total of 30 min. After cooling to room temperature, the mixture is added to a flask

containing 10 mL of methanol. This is chilled in an ice bath with stirring. Crystallization is induced by scratching the sides of the flask with a glass rod. The deep-purple crystals are filtered using a Buchner funnel. The crystals are washed with three 0.5-mL portions of methanol and three 0.5-mL portions of out ling-hot distilled water. The crystals are air-dried on the filtration funnel and stored in a vacuum desiccators over a drying agent until the next laboratory period. The resulting H_2TPP is pure enough for the subsequent metalation process. ^{1}HNMR (δ ppm $CDCl_3$, 300MHz): 7.12 and 7.36 [4d, 16H, arom]; 6.78 [2d, 8H, Pyrrole]; 5.6 [s, 2H, NH]; 2.56 [s, 12H, CH_3]. IR absorptions (cm^{-1} KBr): 1635.42, 1434.3, 1449.04, 985, 602-966. Anal. Calc. for $C_{44}H_{38}N_4$: theory: C, 84.88; H, 6.1; N, 9; found: C, 85.21; H, 6.42; N, 9.35. UV- vis (MeCN): λ 272 nm (ε 19), λ_{max} 313 nm (ε 272.4), λ 515 nm (ε 10.6), λ 548 nm (ε 4.4), λ nm591(ε 2.8), λ 646nm(ε 2.4).

General conditions for synthesis of complex

A solution of metal salt (VOF_3 (0.02 g, 0.16mmol) and Cu $(OAC)_2$ (0.032g, 0.16 mmol) dissolved in acetonitrile added gradually to a stirred N,N-dimethylformamide (DMF) solution of the ligand (H_2TPP(0.1g, 0.16mmol)), in the molar ratio 1:1 (metal: ligand). The reaction mixture was further refluxed for 4-5h to ensure of the completing and precipitation of the formed complexes. The precipitated solid complexes were filtered, washed several times with 50% (v/v) ethanol–water to remove any traces of the unreacted starting materials. Finally, the complexes were washed with diethyl ether and dried in vacuum desiccators over anhydrous $CaCl_2$.

Analysis of Vanadium (V) 10, 15, 20-Tetra-p-tolyl-porphyrine

^{1}HNMR (δ ppm $CDCl_3$, 300MHz): 8.11-8.88 [4d, 16H, arom]; 7.29-7.91[2d, 8H, Pyrrole]; 1.55 and 2.64 [s, 12H, CH_3]. IR absorptions (cm^{-1} KBr): 1627.59, 1410.43, 1150, 992, 985, 601-799.8. UV- vis (MeCN):λ 278nm (ε 40), λ 357 nm (ε 60), λ_{max} 440 nm (ε 400), λ 522 nm (ε 20), λ 554nm (ε 20), λ 600nm (ε 50), λ 655nm (ε 120) Figure 2.

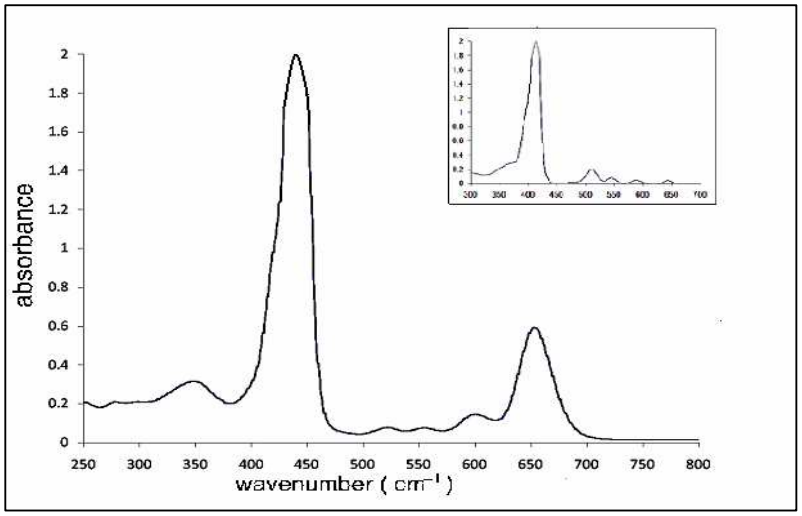

Figure. 2. UV–visible spectra of $[V(C_{44}H_{36}N_4)O]F_3$ in acetonitril

Analysis of Copper (II) 10, 15, 20-Tetra-p-tolyl-porphyrine
IR absorptions (cm^{-1} KBr): 1632.9, 1588, 1343.77, 601-793,470. UV- vis (MeCN): λ 351nm (ε 26.4), λ 417 nm (ε 251.2), λ_{max} 440 nm (ε 260), λ 522 nm (ε 380), λ 554nm (ε 15), λ 600nm (ε 12), λ 654nm (ε 40) Figure 3.

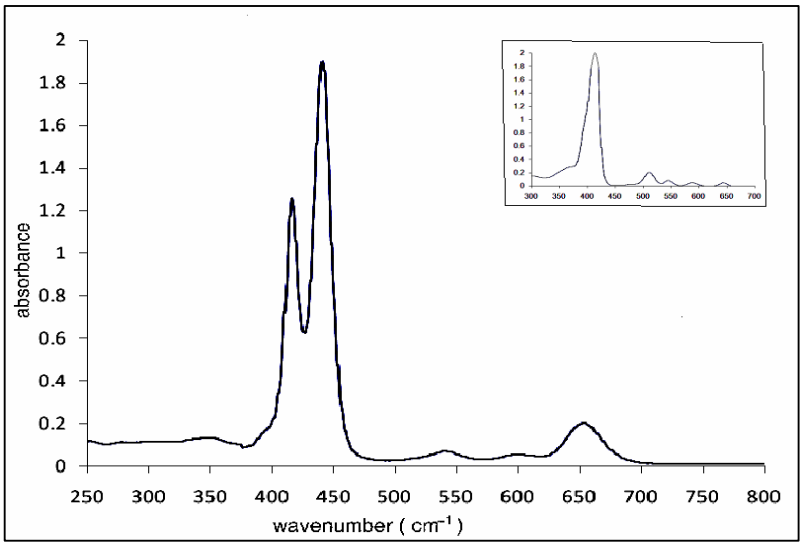

Figure. 3. UV–visible spectra of [Cu ($C_{44}H_{36}N_4$)]$(OAC)_2$ in acetonitrile

In Vitro Activities
H_2TPP ligand and [$V(C_{44}H_{36}N_4)O]F_3$ and [$Cu(C_{44}H_{36}N_4)](OAC)_2$ compounds were assayed for cytotoxicity in vitro against K562 (human chronic myeloid leukemia) cells and Jurkat (human T lymphocyte carcinoma) cells.
The two cell lines were provided by the Pasteur Institute Laboratory of natural and Biomimetic in Iran. The procedure for cytotoxicity studies was similar to that reported earlier [27]. Briefly, in order to calculate the concentration of each drug that produces a 50% inhibition of cell growth (IC_{50}), 190 mL of cell suspension (5×10^4 cell/mL) were exposed to various concentrations of ligand and complexes dissolved in sterile DMSO. The final concentration of DMSO in the growth medium was 2% (v/v) or lower, concentrations without effect on cell replication [25, 26]. After incubation periods 72 h for all cell lines, the cell concentrations were determined both in control and in drug-treated cultures. All experiments were carried out in six times and series.

Conclusion

It is clear from the above discussion that H_2TPP, [$V(C_{44}H_{36}N_4)O]F_3$ and [$Cu(C_{44}H_{36}N_4)](OAC)_2$ compounds offer a new outlook for chemotherapy.
The results of antitumor activity show that the metal complexes exhibit anti-tumour properties and it is important to note that they show enhanced inhibitory activity compared to the parent ligand. The mechanism by which these complexes act as antitumor agents is apoptosis. It has also been proposed that concentration plays a vital role in increasing the

degree of inhabitation. [28, 29]

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