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Anti-tumor activity of N^4[(E)-1-(2-hydroxyphenyl)methylidene]iso -nicotinohydrazide and Its Ti (IV) and Cu (II) complexes on K562 and Jurkat

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Abstract

N^4[(E)-1-(2-hydroxyphenyl)methylidene] isonicotinohydrazide abbreviated as NHPM were synthesized and characterized. Ti(IV) and Cu(II) metal complexes of this ligand prepared by reaction of fluoride salt of Ti(IV) and acetate salt of Cu(II) with NHPM in dry acetonitrle. Characterization of the ligand and its complexes was made by microanalyses, FT-IR, ^{1}H NMR, ^{13}C NMR and UV–Visible spectroscopy. These new complexes showed excellent antitumor activity against two kind of cancer cells that are K562 (human chronic myeloid leukemia) cells and Jurkat (human T lymphocyte carcinoma) cells.

Keywords: N^4[(E)-1-(2-hydroxyphenyl)methylidene] isonicotinohydrazide; Ti(IV) and Cu(II) complexes; Synthesis, Anti-tumor, K562, Jurkat.

Introduction

Presently, there is a growing interest in the coordination chemistry of structurally modified bio-ligands. Transition metal complexes with potential biological activity are the focus of extensive investigations [1]. Metal complexes of biologically important ligands are sometimes more effective than the free ligands [2]. Metal based drugs have been used in medicine for many centuries, but very often only in an empirical fashion. Nowadays there is enormous scope for the design of novel therapeutic compounds, for example, the well known cis-platin is a transition metal based drug which forms highly reactive, charged, platinum complexes that bind to nucleophilic groups such as GC-rich sites in DNA, inducing DNA

cross-links that result in apoptosis and cell growth inhibition [3]. Due to the severe adverse effects of cis-platin, researchmoved to a second-generation of platinum compounds like carboplatin, nedaplatin, satraplatin and other closely related platinum antitumor agents, some of which are still used for the treatment of certain types of tumors [4-7]. It has long been known that metal ions involve in biological processes of life and have been subject of interest. The modes of action of these metal ions are often complex but are believed to involve bonding to the hetero-atoms of the heterocyclic residues of biological molecules, i.e., proteins, enzymes, nucleic acids, etc. [8]. Schiff bases and their metal complexes played an important role in the development of coordination chemistry, resulting in an enormous number of publications, ranging from pure synthetic work to physicochemical [9] and biochemically relevant studies of metal complexes [10–16] and found wide range of applications.

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, characterizition and anti-tumor properties of a number of the first row transition metal complexes have been studied.

Materials and Methods

2.1.1. Reagents and materials

Titanium (IV) tetra fluorides, copper chloride and 4-Pyridinecarboxylic acid hydrazide were Merck chemicals and were used without further purification. Organic solvents were reagent grade. Electronic spectra were recorded by Camspec UV–Visible spectrophotometer model Wpa bio Wave S2 100. The IR spectra were recorded using FT-IR Bruker Tensor 27 spectrometer. ^{1}H NMR and ^{13}C-NMR were recorded on a Bruker AVANCE DRX 500 spectrometer at 500 and 125MHz respectively. All the chemical shifts are quoted in ppm using the high-frequency positive convention; ^{1}H and ^{13}C- NMR spectra were referenced to external $SiMe_4$. The percent composition of elements was obtained from the Microanalytical Laboratories, Department of Chemistry, OIRC, Tehran.

2.1.2. 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.

2.2. Synthesis of the ligand

To a magnetically stirred mixture of 4-Pyridinecarboxylic acid hydrazide (1.37 g, 10mmol) in hot methanol (20 mL) was added to the saclicylaldehyde (1.22 g,1mmol) via a syringe and heated for 45 min at 60°C. After cooling to room temperature, the resulting yellow precipitate was filtered and washed with hexane (20 mL) (Figure 1) [17].

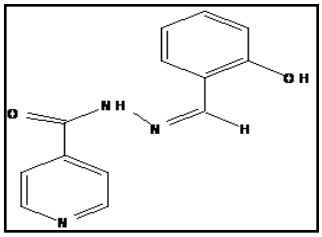


Figure1. Structure of the ligand, NHPM

The solid residue was dried and crystallized from CH_3OH (1:2) to yield as yellow crystals (1.5 g, 75%). Mp 244–246.6°C. IR (KBr) (υ_{max}, cm^{-1}): 3181 (O-H) 3003 (N-H), 1682 (C=O), 1612 (C=N), 1567 and 1489(C=C), 1289(C-N), 1157(N-N). ^{1}H NMR ($CDCl_3$, Me_4Si): δ_H 7.1 and 7.28(5H, 2q, $^3J_{HH}$=7.37, arom), 8.16(1H, s, NCH), 8.06 and 9.33(4H, 2d, $^3J_{HH}$ =5.28, pyridine), 11.04(1H, d, OH), 12.99(1H, s, NH). ^{13}C NMR ($CDCl_3$, Me_4Si): δ_C 117.14, 119.65, 128.24, 130.62 and 155.74 (arom), 126.05, 138.92 and 148.79(pyridine), 151.2(C_{11}), 165.28(OCN). Anal. calcd for $C_{13}H_{11}N_3O_2$ (241.26): C, 64.66; H, 4.55; N, 17.40%. Found: C, 67.05; H, 5.01; N, 17.83%.

2.3. Synthesis of the metal complexes; General Method

A solution of metal salt dissolved in acetonitrile added gradually to a stirred acetonitrile solution of the ligand (NHPM), in the molar ratio 1:1 (metal: ligand). The reaction mixture was further stirred for 2–3h 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 $CaCl_2$.

2.4. Analysis of $[Ti(C_{13}H_{10}N_3O_2)]F_4$ (NHPMTF)

^{1}HNMR (δ ppm $CDCl_3$, 300MHz): 8.69-10.36 [5H, 2q, arom], 6.59-7.64[4H, 2d, pyridine); 7.92(1H, s, NCH). IR absorptions (cm^{-1} KBr): 1640 ν(C=N), 1605 ν(C=O), 1504 ν(C=C), 1357 ν(C-N), 1155 ν(N-N), 702-975 ν(C-H), 676 ν(Ti-N), 593 ν(Ti-F). Anal. Calc. for $[Ti(C_{13}H_{10}N_3O_2)]F_4$: theory: C, 42.82; H, 2.74; N, 11.53; found: C, 43.15; H, 2.95; N, 11.89. UV- vis (MeCN): λ_{max} 284 nm (ε 40), λ 298nm (ε 36), λ 327 nm (ε 38) and λ454nm(ε 8).

2.5. Analysis of $[Cu(C_{13}H_{10}N_3O_2)](OAC)_2$ (NHPMCA)

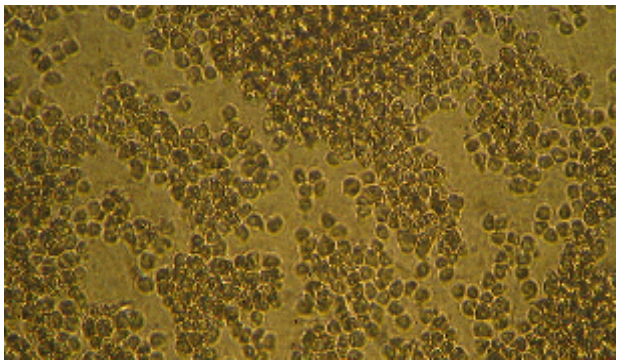
IR absorptions (cm^{-1} KBr): 1606 ν(C=N), 1569 ν(C=O), 1465 ν(C=C), 1198 ν(C-N), 1129 ν(N-N), 693-957 ν(C-H), 507 ν(Cu-N), 465ν(Cu-O). Anal. Calc. for $[Cu(C_{13}H_{10}N_3O_2)](OAC)_2$: theory: C, 36.97; H, 2.37; N, 10.01 ; found: C, 37.24; H, 2.64; N, 10.36. UV-vis (MeCN): λ 287 nm (ε 32), λ 325nm (ε 28), λ 421nm (ε 27) and λ_{max} 455nm(ε 27).

2.6. In Vitro Activities

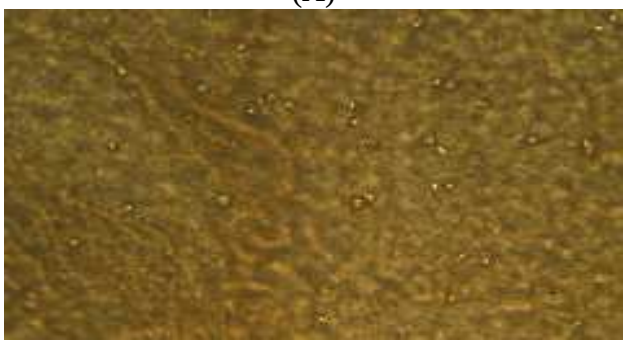
NHPM, $[Ti(C_{13}H_{10}N_3O_2)]F_4$ and $[Cu(C_{13}H_{10}N_3O_2)](OAC)_2$ three complexes were assayed for cytotoxicity in vitro against K562 (human chronic myeloid leukemia) cells and Jurkat (human T lymphocyte carcinoma) cells.

The two cell lines were provided by the Pastour Instutiute Laboratory of natural and Biomimetic in Iran. The procedure for cytotoxicity studies was similar to that reported earlier [18]. 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 [19, 20]. 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. (Figure 2)



(A)



(B)

Fig.2. (A) Tumor cell after 72h without NHPMTF compound
(B) Tumor cell after 72h with NHPMTF compound

Results and Discussion

3.1. Preparation for Ligand, NHPM, and Ti (IV) and Cu (II) complexes

The reaction of Ti(IV) and Cu(II) salts with the ligand, NHPM, results in the formation of [ML] for M= Ti(IV) and Cu(II). All complexes are quite stable and could be stored without any appreciable change. The NHPM ligand have 244-246.6°C melting point, but the $[Ti(C_{13}H_{10}N_3O_2)]F_4$ and $[Cu(C_{13}H_{10}N_3O_2)](OAC)_2$ complexes do not have sharp melting point but decompose above 243°C and 390°C respectively. They are insoluble in common organic solvents, such as ethanol, methanol, chloroform or acetone. However, they are soluble in DMSO and DMF. Their structures were characterized by elemental analysis, ^1H NMR and IR. Their elemental analyses are in accord with their proposed formula. The spectral data of the complexes have good relationship with the literature data.

3.2. Cytotoxicity studies

NHPM ligand and $[Ti(C_{13}H_{10}N_3O_2)]F_4$ and $[Cu(C_{13}H_{10}N_3O_2)](OAC)_2$ complexes 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 anticancer agents used nowadays, that are cis platin and oxaplatin compounds [21].

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

[19, 20] 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- 610μM for NHPM, 0.1-400μM for $[Ti(C_{13}H_{10}N_3O_2)]F_4$ and 0.1-190μM for $[Cu(C_{13}H_{10}N_3O_2)](OAC)_2$ 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 blu. IC_{90} and IC_{50} values, that is 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 doses (IC_{50} and IC_{90}) values are shown in Table 1.

Table 1. 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	Jurkat
NHPM	>100	>89	-	-
NHPMTF	>70	>60	>98	>95
NHPMCA	> 75	>75	>100	>98

Conclusion

It is clear from the above discussion that Ti (IV) and Cu(II) complexes 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. [22-24]

Acknowledgments

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