Synthesis, characterization and anti-tumor activity of Ti (IV) and Mn (II) complexes of N^4[(E,2E)-3-Phenyl-2-propenylidene] isonicotinohydrazide

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Abstract

N^4[(E,2E)-3-Phenyl-2-propenylidene] isonicotinohydrazide abbreviated as NPPI were synthesized and characterized. Ti (IV) and Mn(II) metal complexes of this ligand prepared by reaction of fluoride salt of Ti(IV) and chloride salt of Mn(II) with NPPI in dry acetonitrile. 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,2E)-3-Phenyl-2-propenylidene] isonicotinohydrazide; Ti(IV) and Mn(II) complexes; Synthesis, Anti-tumor, K562 and Jurkat cells.

Introduction

Though transition metals occupy many key positions in biological processes [1], metal-based drugs are traditionally undervalued by the pharmaceutical industry, which is dominated by organic chemistry [2]. Nevertheless, a number of coordination compounds have been applied in the therapy of various diseases [3] (e.g., historically salvarsan against syphilis [4], gold complexes against arthritis [5], bismuth compounds as antiulcer drugs [6], or platinum compounds against cancer [7–9].

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 cisplatin 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 [10]. Schiff base ligands are considered “privileged ligands” because they are easily prepared by the condensation between aldehydes and imines. Stereogenic centers or other elements of chirality (planes, axes) can be introduced in the synthetic design. Schiff base ligands are able to coordinate many different metals [11–13], and to stabilize them in various oxidation states. The Schiff base complexes have

been used in catalytic reactions [14] and as models for biological systems [15, 16]. During the past two decades, considerable attention has been paid to the chemistry of the metal complexes of Schiff bases containing nitrogen and other donors [17–19]. This may be attributed to their stability, biological activity [20] and potential applications in many fields such as oxidation catalysis [21], electrochemistry [22]. The complexes make these compounds effective and stereo specific catalysts for oxidation, reduction and hydrolysis and they show biological activity, and other transformations of organic and inorganic chemistry. It is well known that some drugs have higher activity when administered as metal complexes than as free ligands [23]. 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 have been studied.

Results and Discussion

Preparation for Ligand, NPPI, Ti (IV) and Mn(II) complexes

The reaction of Ti(IV) and Mn(II) salts with the ligand, NPPI, results in the formation of [ML] for M=Ti(IV) and Mn(II). All complexes are quite stable and could be stored without any appreciable change. The NPPI ligand and the [Ti($C_{15}H_{12}N_3O$)]F_4 complex have 190-193°C and 182-184°C melting point respectively , but the [Mn($C_{15}H_{12}N_3O$)]Cl_2 complex do not have sharp melting point but decompose above 200°C. These complexes 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, ^{1}H 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.

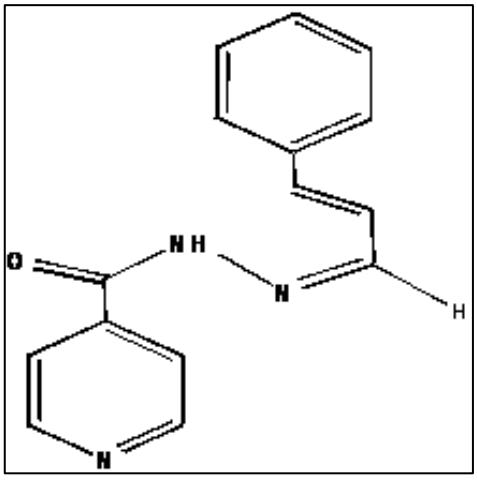

Fig.1. Structure of the ligand, NPPI

Cytotoxicity studies

The NPPI ligand and [Ti($C_{15}H_{12}N_3O$)]F_4 and [Mn($C_{15}H_{12}N_3O$)]Cl_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 [24].

The general method used for testing on anti tumor properties of compounds viz after pre incubation lasting 12h at 37°C in a 5% CO_2 atmosphere and 100% humidity, the tested compounds in the concentration rang 0.1- 450μM for NPPI, 0.1-400μM for [Ti($C_{15}H_{12}N_3O$)]F_4 and 0.1-600μM for [Mn($C_{15}H_{12}N_3O$)]Cl_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 is the compounds concentrations lethal for 90% and 50% of the tumor 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. IC_{50} and IC_{90} values (μM) of the compounds after 72hrs

Compound	**IC_{50} for Cell line**		**IC_{90} for Cell line**	
	K562	***Jurkat***	***K562***	***Jurkat***
NPPI	**>90**	**>80**	**-**	**-**
NPPITF	**>70**	**>65**	**>186**	**>178**
NPPIMC	**> 75**	**>75**	**>190**	**>185**

Materials and Methods

4-Pyridinecarboxylic acidhydrazide, Titanium (IV) tetra fluoride and manganese chloride, 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.

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 the ligand

To a magnetically stirred mixture of 4-Pyridinecarboxylic acidhydrazide (1.37 g, 10mmol) in hot methanol (20 ml) was added to the cinnamaldehyde (1.32g ,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 n-hexane (20 ml) (Fig. 1). The analytical and physical data of the ligand are given in follow: Yellow crystals (1.84 g, 92%). Mp 200–203.3°C. IR (KBr) (υ_{max}, cm^{-1}): 3233 (N-H), 1676(C=O), 1640 (C=N), 1451(C=C), 1308(C-N), 1174(N-N). ^{1}H NMR ($CDCl_3$, Me_4Si): δ_H 6.06(1H, t, CHC), 6.94(1H, d, NCH), 7.25 and 7.44(5H, 2q, $^3J_{HH}$=2.12, 5.28, arom), 7.51(1H, q, $^3J_{HH}$ =15.97, CHCH), 8.50 and 9.21(4H, 2d, $^3J_{HH}$ =7.83, pyridine) . ^{13}C NMR ($CDCl_3$, Me_4Si): δ_C 124.76(C_{13}), 127, 128.92, 129.14 and 135.81(arom), 130.42(C_{12}), 144.62(C_{11}), 122.83, 143.55 and 151.06(pyridine), 165.05(OCN).Anal. calcd for $C_{15}H_{13}N_3O$ (251.30): C, 62.70; H, 5.17; N, 16.71%. Found: C, 63.12; H, 5.64; N, 17.07%.

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 (NPPI), 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 $CaCl_2$.

Analysis of $[Ti(C_{15}H_{12}N_3O)]F_4$ (NPPITF): Yellow crystals. Yield 86%. ^{1}HNMR (δ ppm $CDCl_3$, 300MHz): 7.09-7.65 [5H, 2q, arom]; 7.81- 8.79[4H, 2d, pyridine); 7.92[1H, s, NCH]. IR absorptions (cm^{-1} KBr): 1675 ν(C=N), 1643 ν(C=O), 1407 ν(C=C), 1308 ν(C-N), 1174 ν(N-N), 678-988 ν(C-H), 754 ν(Ti-N), 648 ν(Ti-O) and 593 ν(Ti-F). UV- vis (MeCN): λ_{max} 380 nm (ε 130), λ 402nm (ε 90).

Analysis of $[Mn(C_{15}H_{12}N_3O)]Cl_2$ (NPPIMC): Dark Yellow crystals. Yield 95%. IR absorptions (cm^{-1} KBr): 1669 ν(C=N), 1632 ν(C=O), 1543 ν(C=C), 1310 ν(C-N), 1139 ν(N-N), 683-992 ν(C-H), 508.6 ν(Mn-N), 455 ν(Mn-O). UV- vis (MeCN): λ_{max} 320 nm (ε 200).

In Vitro Activities

NPPI, [Ti($C_{15}H_{12}N_3O$)]F_4 and [Mn($C_{15}H_{12}N_3O$)]Cl_2 three 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 [25]. 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 complexes dissolved in sterile DMSO. The final concentration of DMSO in the growth medium was 2%v/vor lower, concentrations without effect on cell replication [26, 27].

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 done in six times.

Conclusion

It is clear from the above discussion that Ti (IV) and Mn (II) complexes offer a new outlook for chemotherapy. The results of antitumor activity show that the metal complexes exhibit antitumor 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].

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References

[1] A. Sigel, H. Sigel (Eds.), *Metal Ions in Biological Systems*, **1974–2004**, vol. 1–42.

[2] Z. Guo, P.J. Sadler, Angew. *Chem. Int., Ed. Engl*. **1999**, 38, 1512–1531.

[3] K.H. Thompson, C. *Orvig, Dalton Trans*., **2006**, 761–764.

[4] N.C. Lloyd, H.W. Morgan, B.K. Nicholson, R.S. Ronimus, Angew. *Chem., Int. Ed*,

2005, 44, 941–944.

[5] L. Messori, G. Marcon, in: A. Sigel, H. Sigel (Eds.), *Metal Ions in Biological Systems*, **2004,** vol.41, pp. 279–304.

[6] H. Sun, L. Zhang, K.-Y. Szeto, in: A. Sigel, H. Sigel (Eds.), *Metal Ions in Biological Systems*, **2004**, vol. 41, pp. 333–378.

[7] M.D. Hall, R.C. Dolman, T.W. Hambley, in: A. Sigel, H. Sigel(Eds.), *Metal Ions in Biological Systems*, **200**4**,** vol. 42 , pp. 297–322.

[8] M.A. Jakupec, M. Galanski, B.K. Keppler, *Rev. Physiol. Biochem. Pharmacol*, **2003,** 146, 1–53.

[9] K.R. Barnes, S.J. Lippard, in: A. Sigel, H. Sigel (Eds.), *Metal Ions in Biological Systems*, **200**4, vol. 42, pp. 143–177.

[10] G. Ciarimboli, T. Ludwig, D. Lang, H. Pavenstadt, H. Koepsell, H.-J. Piechota, J. Haier, U. Jaehde, J. Zisowsky, E. Schlatter, *Am. J. Pathol*, **2005**, 167, 1477-1484.

[11] S.M.E. Khalil, *Chem. Papers*, **2000,** 54, 12.

[12] A.H. Osman, *Transition Met. Chem*, **2006,** 31, 35.

[13] S.A. Sallam, *Transition Met. Chem*, **2006**, 31, 46.

[14] D.E. Hamilton, R.S. Drago, A. Zombeck, *J. Am. Chem. Soc*. ,**1987**, 109, 374.

[15] D. Chen, A.E. *Martel*, *Inorg. Chem*, **1987,** 26**,** 1026.

[16] J. Costamagna, J. Vargas, R. Latorre, A. Alvarado, G. Mena, *Coord. Chem. Rev*, **1992,** 119, 67.

[17] S.S. Djebbar, B.O. Benali, J.P. Deloume, *Polyhedron*, **1997,** 16, 2175.

[18] P. Bhattacharyya, J. Parr, A.T. Ross, *J. Chem. Soc. Dalton* , **1998**, 3149.

[19] L. He, S.H. Gou, Q.F. Shi, *J. Chem. Crystallogr*, **1999**, 29, 207.

[20] C.M. Liu, R.G. Xiong, X.Z. You, Y.J. Liu, K.K. *Cheung, Polyhedron*, **1996**, 15, 4565.

[21] S.S. Djebbar, B.O. Benali, J.P. Deloume, *Transition Met. Chem*, **1998**, 23, 443.

[22] Y.J. Hamada, *IEEE Trans. Electron Devices*, **1997,** 44, 1208.

[23] R. Ramesh, M. Sivagamasundari, *Synth. React. Inorg. Met.-Org. Chem*, **2003**, 33, 899.

[24] Y. S. Kim et al. *Journal of Inorganic Biochemistry*, **2004,** 98, 98–104.

[25] G. Zhao, H. Lin, S. Zhu, H. Sun, Y. Chen, *J. Inorg. Biochem*, **1998**, 70**,** 219-226.

[26] J. Ishida et al. *Bioorganic & Medicinal Chemistry Letters*, **1999**, 9, 3319-3329.

[27] J, K, Son et al. *Journal of Medicinal Chemistry***, 2007**, 1- 8.

[28] F. Shabani et al. *Bioinorganic Chemistry and Applications*, **2008,** 10.1155, 501021.