Regular Article

INORGANIC CHEMISTRY RESEARCH
Published by the
Iranian Chemical Society
www.inorgchemres.org
info@inorgchemres.org

Inorg. Chem. Res., *Vol. 4, No. 1, 123-131, July 2020*
DOI: 10.22036/icr.2020.234661.1070

Vanadium Complexes with Maltol and Deferiprone Ligands: Synthesis, Characterization and *In vitro* Antiproliferative Activity toward Different Cancer Cells

M. Nazari[a], S. Salehi[a,b], S.M. Mashmoul Moghadam[a], M. Babaei[c] and A.Sh. Saljooghi[a]

[a]*Department of Chemistry, Ferdowsi University of Mashhad, Mashhad 91735-654, Iran*
[b]*Department of Chemistry, Faculty of Science, University of Mazandaran, Babolsar, Iran*
[c]*Pharmaceutical Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran*
(Received 10 June 2020, Accepted 2 August 2020)

In a systematic effort to identify a potent antiproliferative agent, four complexes of vanadium containing maltol and deferiprone ligands were synthesized and evaluated for their cytotoxic activity against five human and animal cancer cell lines, including human breast cancer cells (MCF-7), human cervix epithelial carcinoma (HeLa), human colon cancer cell line (HT-29), human leukemia cell line (K-562), and mouse neuroblastoma cell line (Neuro-2a) using cisplatin as a comparative standard by the MTT assay. The results revealed that the vanadium complexes induce apoptosis in cancer cell lines. The flow cytometry results confirmed that complex 4 exhibits a high population of apoptotic cells (65.8%) and 2-fold higher than cisplatin (32.1%) at the same concentration and induces apoptosis of K-562 cancer cells.

Keywords: Antiproliferative activity, Vanadium complexes, Maltol, Deferiprone

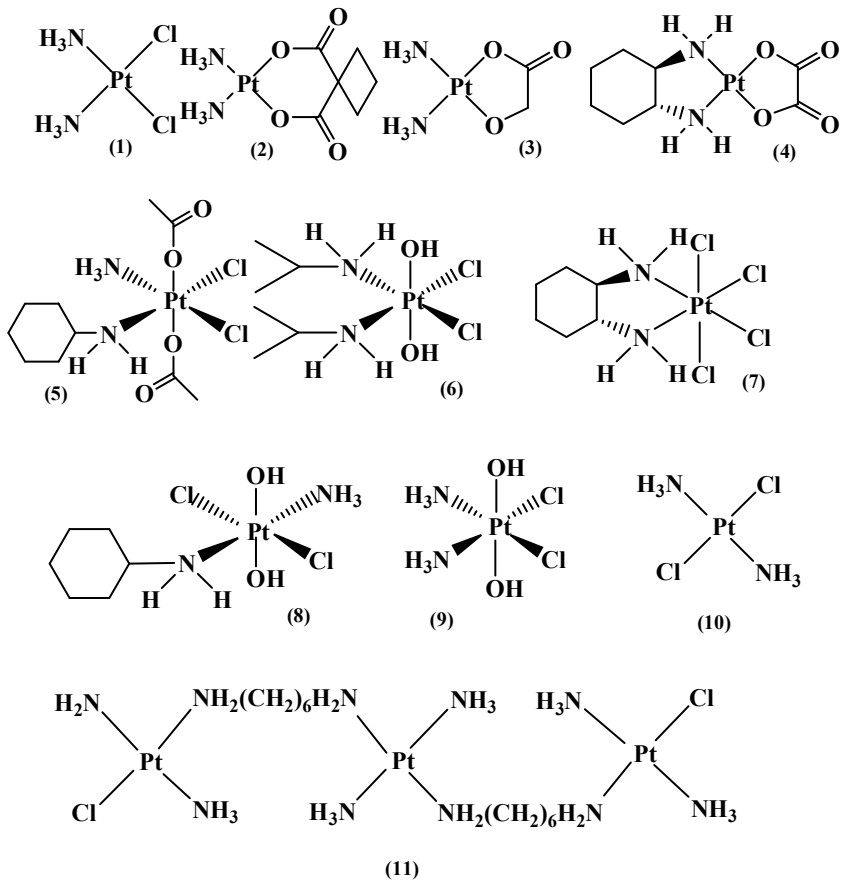
INTRODUCTION

Cancer is a mass of cells with malignant neoplasm, which divides uncontrollably, invades and spreads to distant organs, and develops secondary disease. Cancer is one of four major non-communicable diseases (*i.e.*, heart diseases, cancers, lung diseases and diabetes) that are the leading causes of deaths worldwide. A 2013 survey by the World Health Organization (WHO) estimated that non-communicable diseases (NCDs) kill more than 36 million people each year. Cancer is the second in the list and killed approximately 21% of these 36 million people, so that 8.2 million people worldwide died from cancer in 2012. Even though the incidence of cancers is comparatively higher than other diseases, improvements in cancer therapy have significantly reduced the number of related deaths.

Cancer is a fatal and tolerable disease. Unlike other infectious diseases in which cells are infected by the agents creating altered cellular biochemistry that can lead to disease, in the case of cancer, the altered cellular genetics itself causes the disease. In some cases, infectious agents such as ENKO viruses (*e.g.*, human papilloma, hepatitis B, and hepatitis C virus) and bacteria (*e.g.*, Helicobacter pylori) can also alter cellular genes and cause cancer [1]. Normally, cellular functions are operated by a network of cooperative interactions between various cellular pathways, such as cell cycle regulation, apoptotic process, cellular receptor-ligand interactions, regulators of signal transduction networks, and active DNA-repair mechanisms [2]. Cancers are caused by mutant tumor suppressor proteins, activated angiogenesis, inactive cell cycle checkpoints, loss of anti-pathotic property, active growth signaling, and DNA repair mechanisms.

Chemotherapy is today increasing cure rates in many forms of human cancer. Because of our day-to-day progress in understanding the effect of drugs on cells, both normal and cancerous, there has been a continued improvement in this mode of cancer treatment. The ease and success of

*Corresponding author. E-mail: saljooghi@um.ac.ir

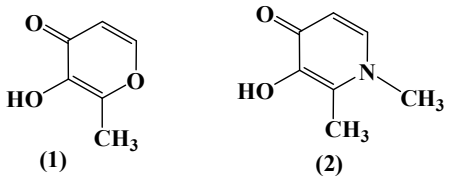


Scheme 1. Platinum(II) chemotherapeutic compounds; cisplatin (1) and cisplatin analogues: carboplatin (2), nedaplatin (3) and oxaliplatin (4). Platinum(IV) complexes; satraplatin (5), iproplatin (6) and tetraplatin (7). *Trans*-coordinated platinum complexes; *trans*-ammine (cyclohexylaminedichlorodihydroxo) platinum(IV) (JM335) (8); oxoplatin (9), transplatin (10) and the multinuclear platinum coordination complex; BBR3464 (11)

finding better drugs for any disease depends on how best we can rationalize the design of the drugs [3].

Application of metal complexes for chemotherapy goes back to the discovery of cisplatin (Scheme 1) by Rosenberg and his colleagues in 1965 [4]. The discovery of metal-based chemotherapeutic agents had a different kinetics and mechanism of action than other conventional organic drugs [5]. However, side effects, toxicity, and drug resistance are the most important problems in using these drugs [6]. Bioinorganic and pharmaceutical chemists are exploring different strategies for overcoming problems, including targeted transfer of clinical drugs [7-8], and are developing strategies to design of new platinum (Scheme 1) and non-platinum metal complexes that have different structural and reactivity attributes [9].

Vanadium complexes have also emerged as effective antiproliferative compounds *in vivo* [10]. The first report on cytostatic effects of vanadate appeared in 1965 [11], first reports on the potential of an organo-vanadium compound, namely vanadocene Cp_2VCl_2, in the treatment of, for example, Ehrlich as cites tumor, in 1986 [12]. Vanadium complexes demonstrate low toxicity in humans and effective antiproliferative activity [10]. Anti-tumor activity may be due to inhibition of cellular protein tyrosine phosphatises (PTPases) and/or activation of tyrosine phosphorylases, which activate signal transduction pathways, leading either to apoptosis and/or activation of tumor suppressor genes. Vanadium compounds have been



Scheme 2. Chemical structure of maltol (1) and deferiprone (Hdmpp) (2)

reported to be able to interdict the cell cycle or toxic effects through DNA cleavage and membrane cleavage [13]. In addition, vanadium compounds may also act as anti-metastatic agents due to their reactive properties [14]. However, the mechanism of *in vivo* activity of vanadium complexes is not well understood and requires further investigation [15-16].

In recent reports [17-18], vanadium complexes are seen as additional options for further extension because they have shown promising activity against the cisplatin-resistant cell lines [19]. To use vanadium compounds as potential anticancer agents, their limited aqueous solubility problem must be resolved. One way to overcome this limitation is to replace chloride donor groups with chelated oxygen donors. Two suitable ligands for use as an oxygen-based chelate would be 3-hydroxy-2-methyl-4-pyrone (maltol) [20] and also deferiprone (3-hydroxy-1,2-dimethyl-4-pyridinone), also known as L1, CP20, or Ferriprox (Scheme 2). Deferiprone is a low-molecular-weight bidentate orally active iron chelator, attached to 3-hydroxy-4-pyridinone group compounds [21]. Deferiprone is effective in excreting iron in iron-storage diseases and aluminium in patients on hemodialysis [22]. Pharmacokinetic studies of orally administered deferiprone have shown that in most patients, it is rapidly absorbed from the stomach and appears in blood within minutes [23-24]. In recent studies after 3 years of therapy, deferiprone has been shown effective in reducing myocardial iron and improving ventricular function in thalassemia patients [25]. Maltol, 3-hydroxy-2-methyl-4-pyron, belongs to the compounds of the hydroxy pyron group, which has long been known for its increased bioavailability and significant toxicity effects [26]. Maltol has been suggested as an oral agent that can be used to remove the excess of iron ions in thalassemia or hemochromatosis [27].

EXPREMENTAL

Chemicals

All solvents purchased from Merck. Vanadyl sulfate trihydrate, $VOSO_4.3H_2O$ and 40% aqueous methylamine were purchased from Sigma-Aldrich. Sodium dithionite was purchased from Fisher. (Acetylacetonato) oxovanadium(IV) $[VO(acac)_2]$, maltol and deferiprone were purchased from Merck without further purification. RPMI-1640 medium, Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from GIBCO (Gaithersburg, USA). Penicillin and streptomycin were purchased from Biochrom AG (Berlin, Germany). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) and cisplatin were purchased from Sigma-Aldrich.

Synthesis of Oxovanadium Complexes

Synthesis of bis(maltolato)methoxyoxovanadium(V), $[VO(OCH_3)(mal)_2]$ (1). The synthesis of complex (1) has been reported previously [28] and their purities were confirmed by mass and infrared spectroscopy; in that study, we used a modified method.

Maltol (0.126 g, 1 mmol) was added to MeOH-H_2O (1:1) mixture (10 mL) and stirred. The resulting mixture was treated with 0.50 mmol (0.13 g) of (acetylacetonato) oxovanadium(IV) $[VO(acac)_2]$ in MeOH-H_2O (1:1) mixture (10 mL). When the solution of $VO(acac)_2$ (green) was added to the solution of maltol (colorless), the color of the solution turned into dark brown. After refluxing for 2 h, the solvent was evaporated, and the crude product was purified by gel chromatography with Sephadex LH 20 with MeOH as an eluent to give the oxovanadium complex as dark brown powders. Yield 68%. m. p.: 305 °C. FT-IR (cm^{-1}): 3075, 1619 (C=O), 1581, 1477, 1269, 1206, 1057, 967, 852 (V=O). MS (+ESI) m/z = 347 $[M + H]^+$.

Synthesis of bis(3-Hydroxy-1,2-dimethyl-4-pyridinonato)oxovanadium(IV), $[VO(dpp)_2]$ (2). 3-Hydroxy-1,2-dimethyl-4-pyridinone (Hdpp) (0.013 g, 0.10 mmol) was added to MeOH-H_2O (1:1) mixture (10 mL) and stirred. The resulting mixture was treated with 0.05 mmol (0.013 g) of (acetylacetonato) oxovanadium(IV) $[VO(acac)_2]$ in MeOH-H_2O (1:1) mixture (10 mL). When the solution of $VO(acac)_2$ (green) was added to the solution of the Hdpp (colorless), the solution color changed into dark purple. After refluxing for 2 h, the solvent was evaporated, and the crude product was purified by gel chromatography with Sephadex LH 20 with MeOH as an eluent to yield oxovanadium complex as a dark purple powder.

Yield 77%. m. p.: 355 °C. IR (KBr, cm^{-1}): 3434, 1614 (C=O), 1556, 1505, 1349, 1278, 951, 827 (V=O). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.4 (C=O), 153.1 (C-OH), 140.8 (C-CH_3), 138.5 (CH-N), 125.3 (CH-CO), 79.6 (N-CH_3), 12.5 (C-CH_3). MS (+ESI) *m/z* = 342 $[M + H]^+$.

Synthesis of bis(maltolato)oxovanadium(IV), $[VO(mal)_2]$ (3) and tris(3-hydroxy-1,2-dimethyl-4-pyridinonato)vanadium(III) dodecahydrate, $[V(dpp)_3].12H_2O$ (4) have been reported previously [28, 29]. For Complex 3: yield 81%. m. p.: 310 °C. IR (KBr, cm^{-1}): 1610, 1550, 1485 (C-O and C-C); 995 (V-O). EIMS *m/z* = 317 (M+, $[C_{12}H_{10}O_7V]^+$) and for complex 4: yield 86%. m. p.: 317 °C. IR (KBr, cm^{-1}): 1606, 1550, 1501, 1461 (pyridinone ring vibrations), 706 (V-O). Mass spectrum (LSIMS): *m/z* 327 ($[V(dpp)_2]^+$, 100%). The structures of complexes 1 to 4, are displayed in Scheme 3.

BIOLOGICAL STUDIES

Cell Culture Methods

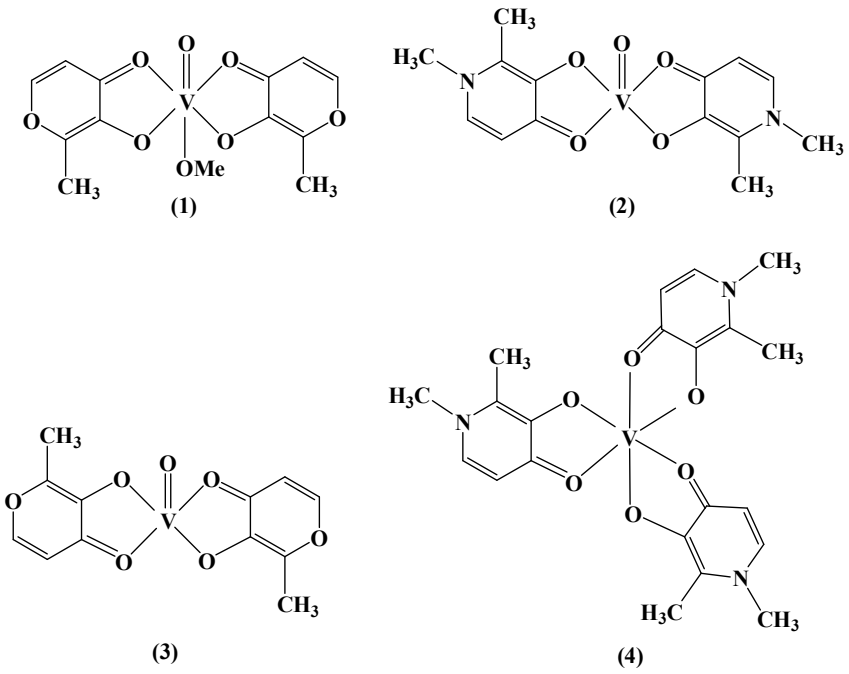
The HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 0.1 mM nonessential amino acids, 2 mML-glutamine, 1.0 mM sodium pyruvate, and 5% fetal bovine serum, at 37 °C in an atmosphere of 5% CO_2. The cells were plated in 96-well sterile plates at a density of 1×10^4 cells/well in 100 µL of medium and incubated for 1 h. Also, the MCF-7 and HT-29 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, 100 units/mL of penicillin, and 100 µg mL^{-1} of streptomycin. The K-562 cells were cultured in RPMI-1640 containing 10% fetal bovine serum, 100 units mL^{-1} of penicillin, and 100 µg mL^{-1} of streptomycin.

MTT Assay in Human Cancer Cell Lines

Complexes 1-4 were screened for antiproliferative activity against human breast cancer cells (MCF-7) human cervix epithelial carcinoma (HeLa), human colon cancer cell line (HT-29), human leukemia cell line (K-562), and mouse neuroblastoma cell line (Neuro-2a). cisplatin was used as the comparative standard. The cell viability was evaluated using a colorimetric method based on the tetrazolium salt MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]), which is reduced by living cells to yield purple formazan crystals. The cells were seeded in 96-well plates at a density of 2-5 × 10^4 cells of MCF-7, HeLa, HT-29, K-562 and Neuro-2a per well in 200 µL of culture medium and left to incubate overnight for optimal adherence. After careful removal of the medium, 200 µL of a dilution series of the compounds in fresh medium was added and incubated at 37 °C/5% CO_2 for 24 or 72 h. complexes 1-4 were first solubilized in DMSO, diluted in medium, and then added to the cells in final concentrations between 16 nM and 160 µM. The percentage of DMSO in the cell culture medium did not exceed 1%. Cisplatin was first solubilized in saline and then added at the same concentrations used for the other compounds. At the end of the incubation period, the compounds were removed and the cells were incubated with 200 µL of MTT solution (500 µg mL^{-1}). After 3-4 h at 37 °C/5% CO_2, the medium was removed and the purple formazan crystals were dissolved in 200 µL of DMSO by shaking. The cell viability was evaluated by measurement of the absorbance at 570 nm using a STAT FAX-2100 micro plate reader (Awareness Technology, Palm City, FL, USA). The cell viability was calculated dividing the absorbance of each well by that of the control wells (cells treated with medium containing 1% DMSO). Each experiment was repeated at least three times and each point was determined in at least four replicates.

Apoptosis Assay for Complex 4 by Flow Cytometry

To study the cellular death pathway (necrosis or apoptosis) in the presence of vanadium compounds, the flow cytometry assay was performed on complex 4 and



Scheme 3. Structures of the metal complexes prepared in this study

cisplatin was used as the reference [30]. The compounds were incubated for 24 h at a concentration close to the IC_{50} and the results are shown in Figs. 1 and 2. Four areas in the diagrams stand for the necrotic cells (Q1, low Annexin V-FITC, and high PI signal, left square on the top), late apoptosis or necrosis cells (Q2, high Annexin V-FITC and high PI signal, right square on the top), live cells (Q3, low Annexin V-FITC, and low PI signal, left square at the bottom), apoptosis cells (Q4, high Annexin V-FITC and low PI signal, right square at the bottom), respectively. As indicated in Figs. 1 and 2, complex 4 exhibits high population of the apoptotic cell (65.8%) and 2-fold higher than cisplatin (32.1%) at the same concentration. The results demonstrated that the newly synthesized complexes can induce apoptosis of K-562 cancer cells, however the proapoptotic property needs further investigation to better understand the precise mechanism of action of the complexes. In Fig. 3, complex 4 induces apoptosis in the NALM-6 (A, A') cells were incubated with 10 mM of 1-3 for 24 h, fixed, permeabilized, and visualized for DNA degradation in the TUNEL assay using dUTP-labeling.

STATISTICAL ANALYSIS

All experiments were carried out in triplicate. The data were reported as mean ± SD and statistical significance was analyzed by Student's t-test. The P values ≤ 0.05 were deemed statistically significant.

RESULTS AND DISCUSSION

In this study, the potential of four complexes of vanadium containing maltol and deferiprone ligands as effective antiproliferative compounds *in vitro* has been investigated. The ability of maltolato complexes to inhibit tumor cell growth *in vitro* and *in vivo* provides a strong rationale for continued research into the development of new compounds with this ligand improved therapeutic application. In recent years, the ability of iron chelators such as deferiprone to treat cancer has been highlighted. As previously mentioned, vanadium complexes demonstrate low toxicity in humans and effective antiproliferative activity [10]. Consequently, it seems the association of vanadium with mentioned ligands, can be effective in the

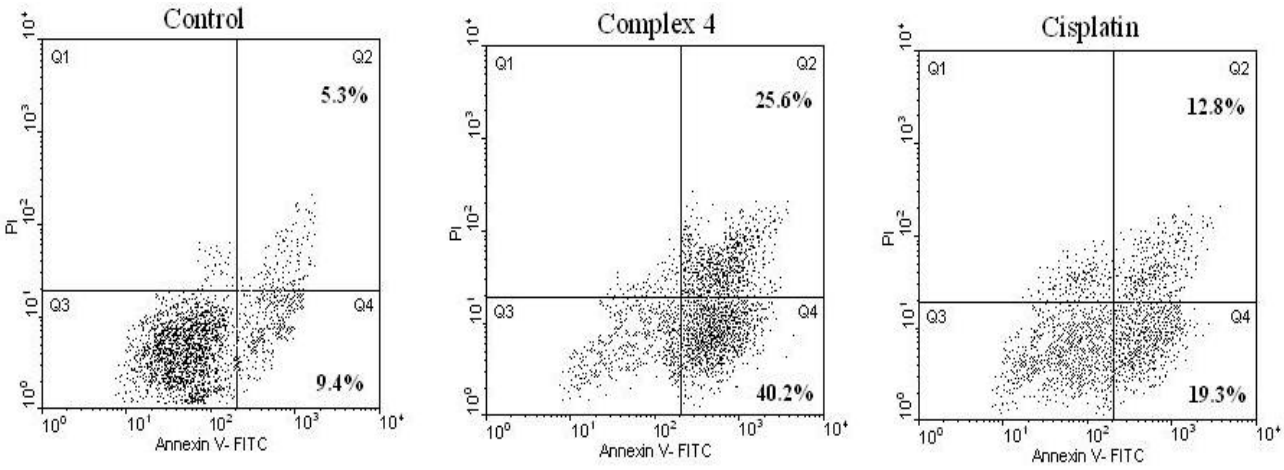


Fig. 1. Flow cytometric results after the exposure of NALM-6 cell lines to the active complex 4 and cisplatin. Four areas in the diagrams represent four different cell states: necrotic cells (Q1), late apoptotic or necrotic cells (Q2), living cells (Q3) and apoptotic cells (Q4).

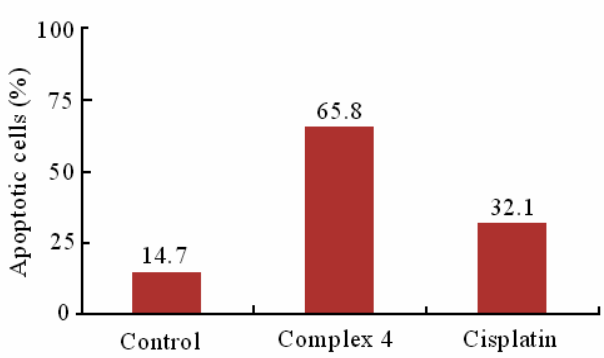


Fig. 2. Percentage of apoptotic NALM-6 cell in total cells following with complex 4 compared with cisplatin.

cancer therapy. In order to gain this aim, the in vitro cytotoxicities of 1-4 against human breast cancer cells (MCF-7) human cervix epithelial carcinoma (HeLa), human colon cancer cell line (HT-29), human leukemia cell line (K-562), and mouse neuroblastoma cell line (Neuro-2a) were determined by the MTT-based assays (Table 1).

For complexes 1-4 and cisplatin as a comparative standard, we have measured their IC_{50} values in all cell lines (Fig. 4). Such measurements were done after 72 h of incubation and using concentrations of the several compounds in the range 16 nM and 160 μM. The values determined for these complexes spanned between 4.35 and 105 μM, while those found for the comparative standard ranged between 0.45 an up to 200 μM (Table 1). These values confirmed the presence of weak to moderately cytotoxic compounds, being complex 4 the one that showed the strongest cytotoxicity in both cell lines. If set aside some cases in Table 1, the cytotoxicity in this assay followed the general trend: 4 > 2 > 1 > 3. The greater cytotoxicity of compound 1 compared to the other complexes reflects the higher stability of this complex. The complexes generally exhibited higher cytotoxic activities against human breast cancer cells (MCF-7) compared to other cell lines (Table 1). However, the highest cytotoxic activity was observed in

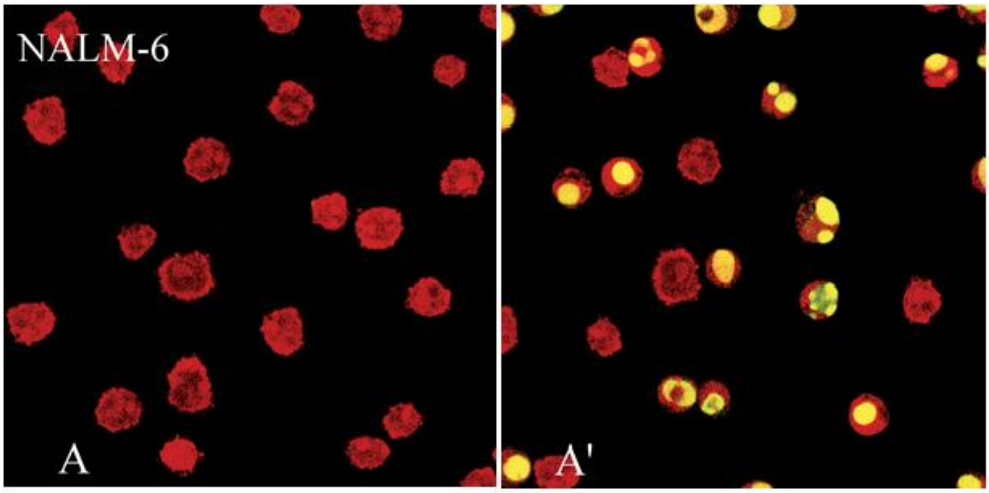


Fig. 3. $[V(dpp)_3].12H_2O$ (4), induces apoptosis in the NALM-6 (A, A') cells were incubated with 10 mM of 1-3 for 24 h, fixed, permeabilized, and visualized for DNA degradation in the TUNEL assay using dUTP-labeling. Red fluorescence, nuclei stained with propidium iodide. Green or yellow (*i.e.*, superimposed red and green) fluorescence, apoptotic nuclei containing fragmented DNA. When compared with controls, treated with 0.3% DMSO, several of the cells incubated with complex 4, (A') exhibited apoptotic nuclei.

Table 1. Cytotoxic Activities of Complexes 1-4 Tested against MCF-7, HeLa, HT-29, NALM-6, and Neuro-2a Cancer Cell Lines after 72 h Continuous Exposure

$IC_{50} \pm SD$ (μM)[a]						
Compound	MCF-7	HeLa	HT-29	NALM-6	Neuro-2a	L929
1	8.46 ± 2.65	16.8 ± 3.42	85.9 ± 6.95	83.7 ± 6.74	55.9 ± 5.23	143 ± 13.1
2	5.95 ± 1.87	17.1 ± 2.98	82.4 ± 7.10	65.4 ± 6.35	43.4 ± 5.74	127 ± 12.6
3	11.9 ± 3.34	37.5 ± 4.66	67.0 ± 6.72	105 ± 11.7	65.3 ± 6.89	199 ± 17.8
4	2.35 ± 1.23	5.22 ± 1.27	45.7± 5.20	4.50 ± 1.25	27.5 ± 3.00	>200
Cisplatin	6.50 ± 2.11	0.45 ± 0.12	15.4 ± 4.93	24.0 ± 2.93	>200	>200

[a]The concentration of the complex required to inhibit cell growth by 50%. The experiments were done in triplicate. Data were expressed as the mean of the triplicate. $IC_{50} > 100$ μM is considered to be inactive.

complex 4, which was approximately six and three times more potent than cisplatin against the two examined cancer cell lines (MCF-7 and K-562). The general pattern observed in the cytotoxicity values for the complexes across the two cell lines showed higher activities for 4 and 2. Also, these values in Table 1 confirmed that the alteration of an O atom in 1 and 3 with N (compounds 2 and 4) shows lower toxicity among the five types of cancer cell lines tested.

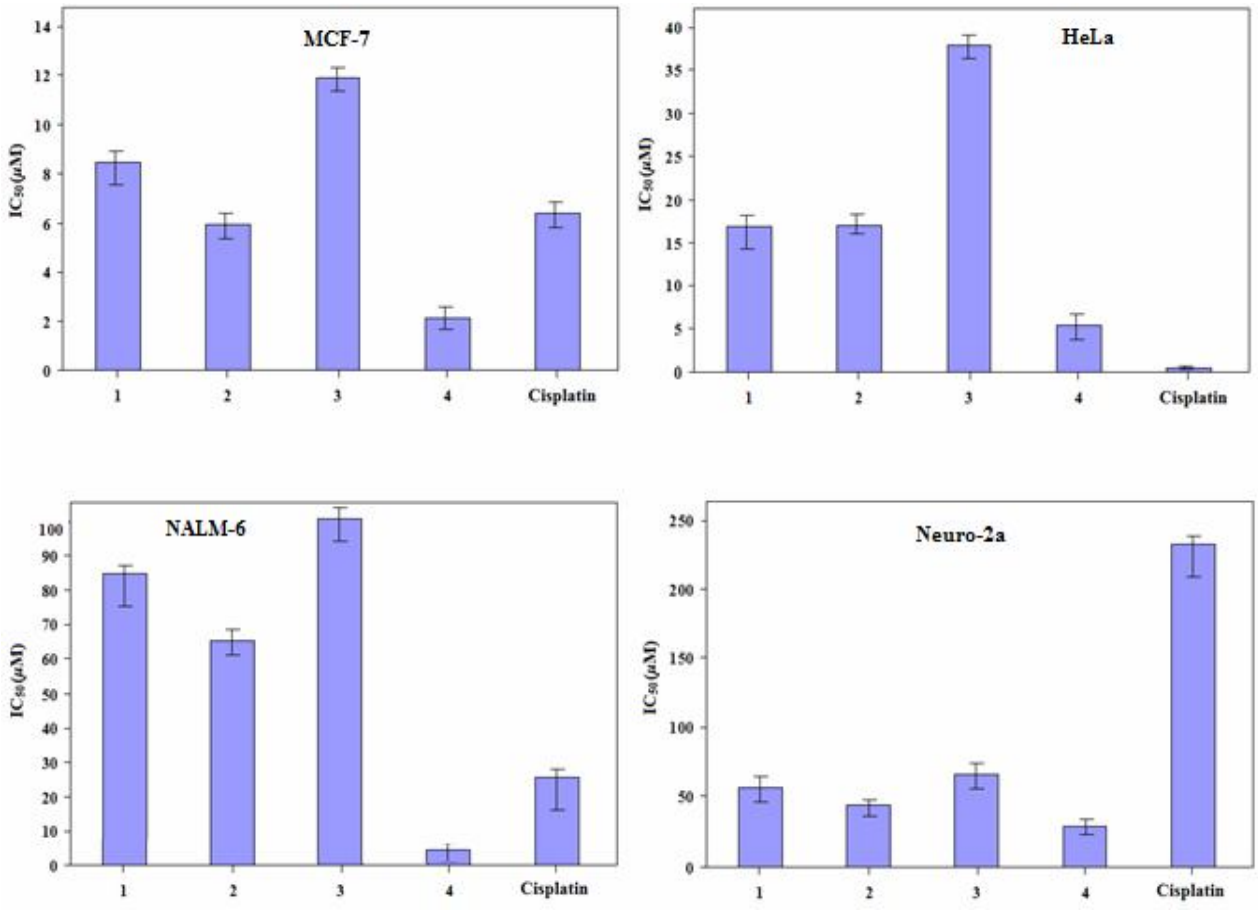


Fig. 4. IC_{50} values for complexes 1-4 and cisplatin.

Since the stability of the complexes is greatly increased by type and number of chelating ligands, it seems that the highest cytotoxic activity was observed in complex 4, which can be attributed to higher stability of this complex in biological systems.

CONCLUSIONS

In this work, the synthesis, spectroscopic characterization and antiproliferative activity of four complexes containing maltol and deferiprone ligands, were reported. Because of the structural similarity of two used ligands and their potency in chemotherapy, the complexes were used in this investigation. The results obtained can be summarized as follows:

1. Besides the anti-diabetic effects for which it is now so well known, vanadium also exhibits a number of other therapeutic effects including antiproliferative activities, that our results confirmed the excellent cytotoxic activities of these vanadium complexes against human breast cancer cells (MCF-7) compared to the other cell lines, especially.

2. Maltol and deferiprone show an interesting coordinating ability to metal ion, behave as bidentate ligands, and give high stability to formed complexes because of their chelate effect. Our results confirmed that the highest cytotoxic activity of these complexes belongs to compounds with higher stability.

3. The pharmacological results suggest that the obtained complexes are potent antiproliferative agents. Besides, the cytotoxicity of the complexes with deferiprone ligands is

higher than that of the maltol ligand, so that higher activities observed for 4 and 2.

REFERENCES

[1] X. Chen, S. Wong (Eds.), Cancer Theranostics. Elsevier Inc., 2014.

[2] F. Bunz, Principles of Cancer Genetics. Springer Sciences.1st ed., 2008.

[3] A.C.F. Caires, Med. Chem. 7 (2007) 484.

[4] B. Rosenberg, L.V. Camp, T. Krigas, Nature 205 (1965) 698.

[5] B. Babu, S. Kumar, B. Bhabatosh, N. Ganesh, R.C. Akhil, Inorg. Chim. Acta 400 (2013) 142.

[6] L. Kelland, Nat. Rev. Cancer 7 (2007) 573.

[7] X. Wang, Z. Guo, Chem. Soc. Rev. 42 (2013) 202.

[8] J.S. Butler, P.J. Sadler, Curr. Opin. Chem. Biol. 17 (2013) 175.

[9] N.P. Barry, P.J. Sadler, Chem. Comm. 49 (2013) 5106.

[10] K.B. Garbutcheon-Singh, M.P. Grant, B.W. Harper, A.M. Krause-Heuer, M. Manohar, N. Orkey, J.R. Aldrich-Wright, Curr. Top. Med. Chem. 11 (2011) 521.

[11] Y. Dong, R.K. Narla, E. Sudbeck, F.M. Uckun, J. Inorg. Biochem. 78 (2000) 321.

[12] H. Joanne, Murray, M.H. Margaret, J. Med. Chem. 37 (1994) 13.

[13] A. Evangelou, Oncol Hematol. 42 (2002) 249.

[14] A. Papaioannou, M. Manos, S. Karkabounas, R. Liasko, V. Kalfakakou, I. Correia, A. Evangelou, J.C. Pessoa, T.J. Kabanos, Inorg. Biochem. 98 (2004) 959.

[15] H. Sakurai, A. Katoh, T. Kiss, T. Jakusch, M. Hattori, 2 (2010) 670.

[16] S. Maria, B. Anna, M. Antonio, M. Silvana, C.T. Maria, P. Claudio, J. Cell Physiol. 228 (2013) 2202.

[17] R.J. Holmes, M.J. McKeage, V. Murray, W.A. Denny, W.D. McFadyen, J. Inorg. Biochem. 85 (2001) 209.

[18] B. Anupam, W. Abhijeet, A.P. Mehool, C. Malay, Cancer Lett. 294 (2010) 1.

[19] M.D. Temple, W.D. McFadyen, R.J. Holmes, W.A. Denny, V. Murray, Biochem. 39 (2000) 5593.

[20] M. Carland, K.J. Tan, J.M. White, J. Stephenson, V. Murray, W.A. Denny, W.D.J. McFadyen, Inorg. Biochem. 99 (2005) 1738.

[21] D.M. Katja, O. Chris, Chem. Rev. 114 (2014) 4540.

[22] G.J.A. Kontoghiorghes, Rev. Analyst 120 (1995) 845.

[23] G.J. Kontoghiorghes, A.N. Bartlett, A.V. Hoffbrand, Br. J. Haematol. 76 (1990) 295.

[24] G.J. Kontoghiorghes, J.G. Goddard, A.N. Bartlett, Clin. Pharmacol. Ther. 48 (1990) 255.

[25] L.J. Anderson, B. Wonke, E. Prescott, Lancet. 360 (2002) 516.

[26] K.H. Thompson, C.A. Barta, C. Orvig, Chem. Soc. Rev. 35 (2006) 545.

[27] S.I. Ahmed, J. Burgess, J. Fawcett, S.A. Parsons, D.R. Russell, S.H. Laurie, Polyhedron 19 (2000) 129.

[28] P. Caravan, L. Gelmini, N. Glover, F.G. Herring, H. Li, J.H. McNeill, S.J. Rettig, I.A. Setyawati, E.D. Shuter, Y. Sun, A.S. Tracey, V.G. Yuen , C. Orvig, J. Am. Chem. Soc. 117 (1995) 12759.

[29] M. Melchior, S.J. Rettig, B.D. Liboiron, K.H. Thompson, V.G. Yuen, J.H. McNeill, C. Orvig, Inorg. Chem. 40 (2001) 4686.

[30] A. Krishan, J. Cell Biol. 66 (1975) 188.