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Two novel compounds of vanadium and molybdenum with carnitine exhibiting potential pharmacological use

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ABSTRACT

The reaction of sodium orthovanadate with carnitine hydrochloride molecule results in the precipitation of decavanadate compound of carnitine whereas the reaction of metallic molybdenum with hydrogen peroxide and carnitine results in the peroxo-molybdenum complex of carnitine. The decavanadate compound as well as the molybdenum complex of carnitine have been characterized by means of elemental analysis, IR, electronic spectra, ^{1}H NMR, 2D-COSY-NMR (=correlation spectroscopy) and thermo-gravimetric analysis (TGA). In addition decavanadate compound of carnitine was fully characterized by X-ray crystallography. The analytical data were in good agreement with the empirical formulae of both, decavanadate compound and molybdenum complex. The two compounds were also evaluated for cell toxicity and their anticancer activity by the MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)-based assay method, using primary cells and tumor cell lines of both human and murine origins and the results show that compound **1** shows an increased biological activity in comparison with compound **2**. Moreover using confocal microscopy and antibodies against cleaved caspase 3 we further analyzed the cell toxicity and we conclude that the apoptotic pathway is triggered efficiently with tumor specificity by compound 1 and not by compound 2.

1. Introduction

Carnitine is a trimethylated amino acid with the chemical name L-beta hydroxyl-γ-N, N trimethylamino butyric acid and is synthesized in the liver from the amino acids lysine and methionine [1]. Carnitine has methylated tertiary nitrogen that carries a fixed positive charge. This tertiary nitrogen is typically balanced by an equally negative charge on the carboxylate group of the molecule. It plays a crucial biological role as it transports long chain fatty acids into mitochondria, providing in this way cells with energy [2]. It is worth mentioning that carnitine due to its relevance to long chain free fatty acids, and the aforementioned energy production is ideal as a dietary supplement for weight loss. It acts also as an antioxidant preventing in this way some of the damage that free radicals cause in the cells and protecting in such a way from all stress related diseases such as lipid peroxidation, heart failure, aging, and HIV. Carnitine's antidiabetic activity is noticeable [1,3].

Furthermore, carnitine has been found to inhibit carcinogenesis. More particularly, the role of L-carnitine in carcinogenesis is evident by data that support its inhibitory role on cancer development as well as its supportive role in prevention or minimization of side effects from cisplatin induced injury of kidney and intestine. In addition, it was found that L-carnitine treatment, prevented GP7 (4-4″-(2″,2″,6″,6″-tetramethyl-l″ piperidinyloxy)amino)-4′-demethyl epipodophyllotoxin) induced caspase 3-activation in HL-60 and Jurkat leukemia cell lines, with subsequent inhibition of GP-7 induced apoptotic internucleosomal DNA fragmentation only in HL-60 cells [4]. Another study reveals that carnitine treatment of the same two cell lines prevented IDA (idarubicin) induced caspases 3 and 7 activation, without affecting IDA induced internucleosomal DNA fragmentation. It is worth mentioning that L-carnitine promotes differentiation of a certain number of atypical cells for the myeloid lineage cells. This observation may account for the inhibitory effect of L-carnitine over GP-7 and IDA induced distinct apoptotic hallmarks. But most interesting is the anticancer drug bis(guanylhydrazone)(mitoguazone) which depresses the carnitine-dependent oxidation of long chain fatty acids in cultured mouse leukemia cells whereas exposure of the leukemia cells to the drug in the presence of carnitine abolished the inhibition of fatty acid oxidation and prevented the drug-induced mitochondrial damage [4].

Additionally, Karaliota and her co-workers synthesized two dinuclear copper (II) complexes of L-carnitine $[Cu_2(L\text{-carnitine})_4(H_2O)_2](ClO_4)_4\cdot H_2O\cdot CH_2Cl_2$ (**1**) and $[Cu_2(L\text{-carnitine})_2Cl_2(H_2O)_2]Cl_2$ (**2**) and tested the cytotoxic effect on two leukemia cells, HL-60 and K562.

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Table 1
Crystallographic data for compound **1·MeOH**.

Formula	$C_{15}H_{68}N_2Na_4O_{51}V_{10}$
Fw	1694.07
Space group	$P-1$
a (Å)	10.4234(2)
b (Å)	11.8039(2)
c (Å)	12.6276(2)
α (°)	88.750(1)
β (°)	89.468(1)
γ (°)	64.457(1)
V (Å^3)	1401.47(4)
Z	1
T (°C)	−113
Radiation	Cu Kα
ρ_{calcd} (g cm^{-3})	2.007
μ (mm^{-1})	14.756
Reflections with $I > 2\sigma(I)$	3929
R_1[a]	0.0487
wR_2[a]	0.1308

[a] $w = 1/[\sigma^2(F_o^2) + (\alpha P)^2 + bP]$ and $P = [\max(F_o^2,0) + 2F_c^2]/3$, $R_1 = \Sigma(|F_o| - |F_c|)/\Sigma(|F_o|)$ and $wR_2 = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$.

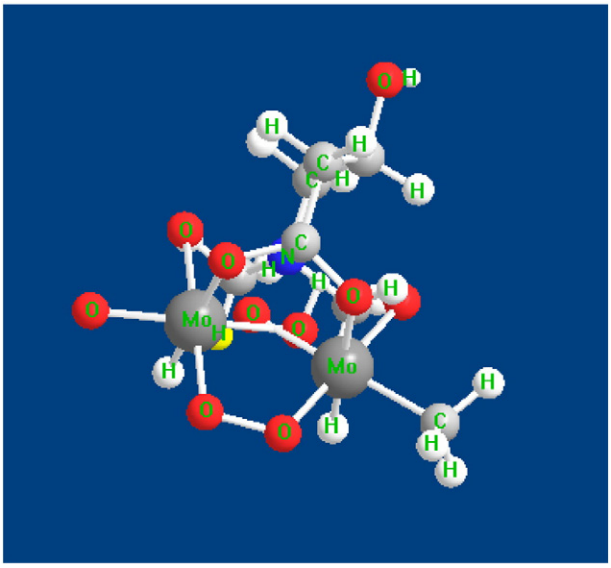

Fig. 1. Proposed structure of the peroxo-molybdenum complex of carnitine.

The results showed that the aforementioned complex (**2**) could specifically initiate cell death in these cell lines.

Vanadium is an element that is found in many organisms and possesses a wide range of biochemical processes. Vanadium compounds exert antitumor activity by modifying various xenobiotic enzymes as well as by inhibiting carcinogen-derived active metabolites [5]. Additionally, vanadium compounds exert anticancer activity by targeting a variety of molecules such as the Nuclear Factor Kb, Akt, activator protein-1, inducible nitric oxide synthase, etc. [6]. Vanadium compounds have been proven to be efficient against leukemia, breast adenocarcinoma, testicular, renal, and gastrointestinal cancers and hepatomas. Polyoxovanadates are an arising field with applications in biology and medicine [7]. The magnitude of polyoxometalates arise from their reactive oxo (O2−) groups which are efficient electron donor to the metal ions. More specifically, the metal oxoclusters have demonstrated antiviral, anticancer, antibacterial and antitumor activities [8]. Among various vanadate compounds, decavanadate has been referred to be of biochemical importance and is considered the major protein-bound species [9]. In addition decavanadate interacts with several proteins. Adenylate kinase was the first enzyme inhibited by decavanadate whereas hexokinase, phosphofructokinase and inositol were what followed. Its interaction with calcium ATPase, myosin and actin suggests that it can affect several processes such as muscle contraction, actin polymerization and calcium homeostasis [10]. Salifoglou and his co-workers refer to binary decavanadate–betaine composites and their cytotoxic profile against the MCF-7 and A549 cell lines whereas Yuan Tuan Li et al. evaluate their anti-proliferation activity of two ammonium decavanadate compounds against the A549 and P388 tumor cell lines [5,6].

The same behavior is referred for polyoxomolybdate clusters. Particularly, Yamasa and his co-authors refers a polyoxo-molybdenum cluster that induces cell death in human pancreatic cancer cell AsPC-1 and possesses antitumor activity against human Co-4 colon, MX-1 breast and lung cancers [11,12]. Furthermore, Karaliota and her co-workers in a recent research evaluate a polymeric oxo-molybdenum complex of 2,5 dihydroxybenzoic acid against two leukemia cell lines, HL-60 and K542 and the results show that this complex could potentiate and sensitize both human leukemia lines to a more pronounced decrease of cell viability [13].

Peroxo complexes have received great attention because of their importance in a variety of biological, pharmaceutical and industrial processes whereas they take part in the basic biochemical processes in cell systems [14]. Additionally, these complexes play an important role as active centers in biological processes involving dioxygen species. According to literature peroxo-molybdenum complexes exert antitumor activity [15–17]. Peroxo-molybdenum complexes of aroylhydrazones used in the treatment of tumors, tuberculosis, leprosy and mental disorders. Their biological activity was attributed to the complex forming abilities of the ligands with metal ions present in the cell and involvement of molybdenum in oxotransferase enzymes [18].

In the present paper, as a continuation of our previous work, we report the synthesis of a decavanadate compound with carnitine as compensatory, as well as a peroxo-molybdenum complex of carnitine. For the characterization of these compounds the following spectroscopic and analytical techniques were employed: elemental analysis, IR, UV–visible (UV–vis) spectroscopy, thermogravimetric analysis (TGA), ^{1}H NMR and X-ray crystallography. We also evaluate their cytotoxicity and anticancer role using normal primary fibroblasts and various tumor cell lines of human and murine origins.

2. Experimental section

2.1. Materials

Carnitine HCl, Na_3VO_4, and solvents used in syntheses were of analytical grade. Carnitine HCl and Na_3VO_4 obtained from Sigma were used without further purification. Methanol obtained from Merck was used as received. Deuterated solvents for use in NMR (d6-DMSO) and (D_2O) were purchased from Merck.

Table 2
Characteristic IR bands in (cm^{-1}) of complexes **1** and **2** and L-carnitine HCl.

	V(V–O–V)	V(V–O)	V(Mo═O)	V(O–O)	$V(CO_2)_{asym}$	$V(CO_2)_{sym}$
L-Carnitine HCl	–	–	–	–	1725	−1359
Compound **1**	958	750,833	–	–	–	–
Complex **2**	–	–	962	916	1578	1432