FINAL

Send Orders for Reprints to reprints@benthamscience.ae

Current Medicinal Chemistry, 2016, *23*, 1-37

REVIEW ARTICLE

Vanadium, Ruthenium and Copper Compounds: A New Class of Non-platinum Metallodrugs with Anticancer Activity

Ignacio Esteban León*, Juan Fernando Cadavid-Vargas, Ana Laura Di Virgilio and Susana Beatriz Etcheverry*

Centro de Quimica Inorganica (CEQUINOR, CONICET), Facultad de Ciencias Exactas, Universidad Nacional de la Plata Facultad, 47 Y 115, La Plata 1900, Argentina

ARTICLE HISTORY

Received: March 01, 2016
Revised: June 17, 2016
Accepted: July 25, 2016

DOI: 10.2174/0929867323666160824162546

Abstract: Cancer is a group of diseases involving abnormal cell growth. The cells grow uncontrollably with the potential to invade and spread to other parts of the body.

This disease is one of the principal death causes in the world, thus becoming a significant topic of scientific research. On the other hand, transition metals play a fundamental role in different living systems. In particular, Metallodrugs represent new and powerful tools for diverse therapeutic applications. To date, various metallodrugs display interesting biological activities for chemotherapy. In this field, cisplatin was the first inorganic compound with high relevance in cancer treatment. This compound was a leader agent in clinical use. Toxicity and resistance problems trigger the development of other platinum drugs with better clinical perspective and also raise the scientific interest for the putative antitumor properties of V, Ru and Cu compounds. Several scientific articles show that complexes of these metals are the new metal-based drugs used in the treatment of several cancers, such us, lung, colon, breast, bladder, *etc.*

In this review we recapitulate current information and new advances on antitumor *in vitro* effects of several organic and inorganic compounds derived from copper, ruthenium and vanadium. These metal derived compounds targeting DNA or cell proteins involved in cell signaling pathways related to cancer. The mechanisms of cell death of these metallodrugs have also been comprehensibly reviewed.

The knowledge of these mechanisms of death and the relationship between chemical structure and biological activity may be useful for the design of new metal-based drugs with promising pharmacologic applications as anticancer agents.

Keywords: Anticancer agents, vanadium, ruthenium, copper, cell in culture, mechanism of action.

1. INTRODUCTION

Metal ions regulate a vast array of essential cellular processes with high specificity and selectivity. The ability of some metal ions to prevent and cure cancer in humans has since long been known, with early documented cases dating back to the 16th century [1]. In this sense, medicinal chemistry has arisen as a field covering the design and synthesis of novel drugs, based on knowledge related to their function at the molecular level. This new prospect enabled the development of metal coordination compounds (a) acting as useful metallodrugs, which interact with a specific molecular target (pharmacodynamics), and (b) being capable of reaching that target (pharmacokinetics). Medicinal Inorganic Chemistry has arisen as a field covering the design and synthesis of novel drugs, based on the knowledge related to the function of metals at cellular and molecular levels. This new perspective enabled the

*Address correspondence to these authors at the Centro de Quimica Inorganica (CEQUINOR, CONICET), Facultad de Ciencias Exactas, Universidad Nacional de la Plata Facultad, 47 Y 115, La Plata 1900, Argentina; E-mails: etcheverry@biol.unlp.edu.ar; Ileon@biol.unlp.edu.ar

0929-8673/16 $58.00+.00

development of metal coordination compounds (a) acting as useful metallodrugs, which interact with a specific molecular target (pharmacodynamics), and (b) increasing through coordination the possibility to reach the cellular and molecular targets (pharmacokinetics).

The extensive range of coordination numbers, physicochemical properties, redox states and geometries, of the metal ions and ligands enable different reactivities that are useful tools for research in this field. Considering these facts, Inorganic Biochemistry presents good opportunities for the design of appropriate therapeutic agents [2,3]. Furthermore, this area has taken advantage of carbohydrate derived ligands to increase the solubility and molecular targeting of metallodrug candidates. As a result of such activity, a significant number of anticancer metal based drugs have emerged in the last years [4-6]. Outstanding such metallodrugs are platinum complexes. It is worthy to mention the success of cisplatin in medicine for the treatment of several kinds of tumors. Its use positioned the metallodrugs in an interesting place for cancer treatment [3, 7]. Cis-$[Pt(NH_3)_2Cl_2]$ (cisplatin) has been used in a significant number of cancer with high incidence in society but it is usually combined with radiation or other medication. Different metallodrugs that have been used for a cancer treatment have a similar mechanism of action of cisplatin and the design and synthesis of these are based on structural similarities with cisplatin.

On the other hand, serious side effects and resistance limit the therapy with cisplatin [8]. New discoveries in drug design try to reduce toxicity and also open the range of tumors to be treated with some compounds [9,10]. In fact, the disadvantage of cis-Pt has been partially ameliorated by the use of new platinum drugs such as carboplatin and oxaliplatin [11]. Cancer has been and remains to be considered yet as one of the fatal diseases worldwide. Cancer is the general name of any malign neoplasia and involves more than 100 diseases which share an uncontrolled growth of cells that invade organs and tissues placed even at a distance from the original tumor (metastasis) [12]. Cancer cure requires deep knowledge of the processes initiating and developing the chemical and biological phenotypes of the disease. The main features of cancer comprise several biological skills that involve the capacity of evading growth suppressors, resisting cell death, inducing angiogenesis, invasion and metastasis, and evading immune system [13]. The study of these concepts is expected to increase and stimulate the design and synthesis of new metal based drugs with anticancer properties and development of alternative therapies.

Among the ten most active metal derived drugs it is worthy to discuss vanadium compounds [14]. In the case of vanadium, notable is the presence of vanadocene, a promising anticancer agent belonging to the family of metallocenes [15]. However, other forms of vanadium, extending from simple inorganic salts to more defined coordination compounds involving organic and inorganic ligands have also been proposed as anticancer agents [16,17]. Future work will help to decide on their applicability in the fight against cancer, based on the strict chemical criteria governing solubility, bioavailability and molecular specificity at the subcellular level. Other interesting metal based drugs with potential anticancer properties are ruthenium derivatives [18,19]. Ruthenium is particularly attractive as the ligand exchange kinetics in its complexes can be similar to those of platinum complexes [20]. Several scientific reports show the interaction of ruthenium compounds with different molecular targets such us DNA, mitochondria, among others[21,22]. Another metal interesting pharmacological properties and different targets for cancer treatment is copper [23]. Copper is an endogenous metal with important functions. It is know that these kinds of components may be less lethal for cells with normal phenotypes than for tumor cells. Nevertheless, copper can also be toxic due to its affinity for binding sites and redox effects. The transformed metabolic processes of tumors provoke differential responses to copper compounds comparing to normal cells [6]. In this present survey, the state of knowledge referred to copper, ruthenium and vanadium derivatives with potential anticancer effects has been reviewed.

2. ANTITUMOR ACTIVITY OF VANADIUM COMPOUNDS

In the last years, the antitumor activity of several vanadium derivatives has been remarked, but the mechanisms of action are not well understood. The principal molecular targets for the anticancer effects of vanadium compounds are the disruption of cellular metabolism through the generation of ROS, the depletion of GSH, the alterations of cellular organelles, the spindle key proteins such as cyclins, cytoskeleton proteins, some signal transduction pathways and caspases, which in turn play a role in cell cycle arrest and programmed cell death.

2.1. Vanadocenes

Within metallocenes field, one of the most important metal based drug are titanocenes and derivatives. The anticancer activity of titanocene dichloride was widespread in the early of 1980. Then, this compound

was studied in phase I clinical trials in 1993 [24] and while phase I clinical trials were not as satisfactory, several phase II clinical trials with patients with breast and a renal tumor have been carried out detecting a very low activity [25]. Nevertheless, in the last ten years, different works of many groups showed that these kinds of metals exhibited anticancer activity toward several types of tumor such us prostate [26], cervix, melanoma, colon and lung, and these results renewed the interest in this field [27-29].

The biological results showed that titanocene Y (bis-[(p-methoxybenzyl)cyclopentadienyl] titanium(IV) dichloride) and C (Bis-(N,N-dimethylamino-2(N-methylpyrrolyl) methylcyclopentadienyl) titanium (IV) dichloride) are the most promising metallodrugs as antitumor agents and in particular show promise for use in combination therapies. The main mechanism of action of these compounds involved to induce apoptosis in a dose-dependent manner, induce DNA damage and a differential damage response and reduced the clonogenic capacity of the cells without affecting the cell cycle progress in prostate tumor cells. Besides, Titanocene Y activated cell signaling affected c-Jun N-terminal kinase and it increased the phosphorylation levels of adenosine monophosphate (AMP)-activated protein kinase α1 (AMPKα1) in small lung cancer cells. On the other hand, Titanocene Y triggered apoptosis in leukemia cells, regardless of the expression of anti-apoptotic Bcl-2 and pro-apoptotic smac. Moreover, the titanium compounds are also effective against leukemia cells that are multidrug resistant due to overexpression of P-gp [30].

Other interesting group of compounds with antitumor properties within metallocenes field are vanadocene derivatives [31]. Vanadocene, is a metallocene, belonging to a group of organometallic derivatives, with a metal ion sandwiched between two cyclopentadienyl rings. The first vanadocene that showed relevant pre-clinic results was the bis(cyclopentadienyl) dichloro-V(IV), vanadocene dichloride, $[VCp_2Cl_2]$ (1), [32]. This compound exhibits a higher *in vitro* activity against tumor cell lines upon direct comparison with $[TiCp_2Cl_2]$ (2) (see Fig. **1**). Besides, *in vivo* studies revealed that vanadocene compounds exhibit significant antitumor properties with vanadocene dichloride being one of the most promising among metallocenes [33]. Recently, speciation studies have shown that $[VCp_2Cl_2]$ transforms at physiological pH to $[VCp_2(OH)_2]$ and carbonate, oxalate, lactate, phosphate are able to displace the two OH- ions to yield the adducts with these ligands $[VCp_2(ox)]$, $[VCp_2(CO_3)]$, $[VCp_2(lactH^{(-1)})]$, and $[VCp_2(HPO_4)]$ [34]. Several vanadocene derivatives, especially the methyl-and methoxy-substituted vanadocene dichlorides showed cytotoxic effects against T-lymphocytic leukemia cells with MOLT-4 evidencing more activity than their corresponding titanocene analogs. Activation of apoptosis by vanadocene compounds is very similar to the mechanism used by cisplatin, as it triggers DNA injury and involves p53 activation [35]. p53 is a tumor suppressor factor that normally functions in apoptotic mechanisms, cell cycle regulation, and maintenance of genomic stability.

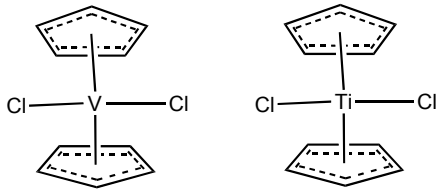


Fig. (1). Schematic structures of vanadocene dichloride (1) and, titanocene dichloride (2).

On the other hand, vanadocenes are efficient agents against human testicular cell lines [36]. Vanadocene compounds containing phenanthroline (phen) ligands are promising chemotherapeutic agents, mostly due to their high anticancer activity, solubility and stability properties [37]. In recent years, scientific reports have shown the design and synthesis of new benzyl-substituted vanadocenes, including 3, 4 and 5 which were modeled after bis-[5-(p-methoxybenzyl) cyclopentadienyl] titanium(IV) dichloride (Titanocene Y) and its derivative compound (6) (Fig. **2**). The three vanadocene caused slightly better activity than cis-platin in several cancer cell lines [38]. Besides, other novel compounds such as benzyl- substituted vana-docenes with p-methoxybenzyl)cyclo-pentadienyl], bis-[(3,4-dimethoxybenzyl)cyclopenta-dienyl] and bis-[(3,4,5-trimethoxybenzyl)cyclopenta-dienyl] showed high toxicity on pig kidney epithelial cell line [39].

2.2. Vanadium IV and V Complexes

Another interesting class of vanadium compounds with potential application in cancer treatment is formed by some oxidovanadium(IV) complexes. One of the most important and promising example in this area is bis(4,7-dimethyl-1,10-phenanthroline) sulfatooxovanadium (IV) Metvan (Fig. **3**). This complex induces cell damage through apoptosis activation in several human cancer cell lines such as multiple myeloma cells, leukemia cells, and solid tumors derived from breast, ovarian, prostate and testis [4,40,41]. One of the main therapeutic advantages of Metvan is the high effectiveness toward ovarian and testicular cancer cell resistant

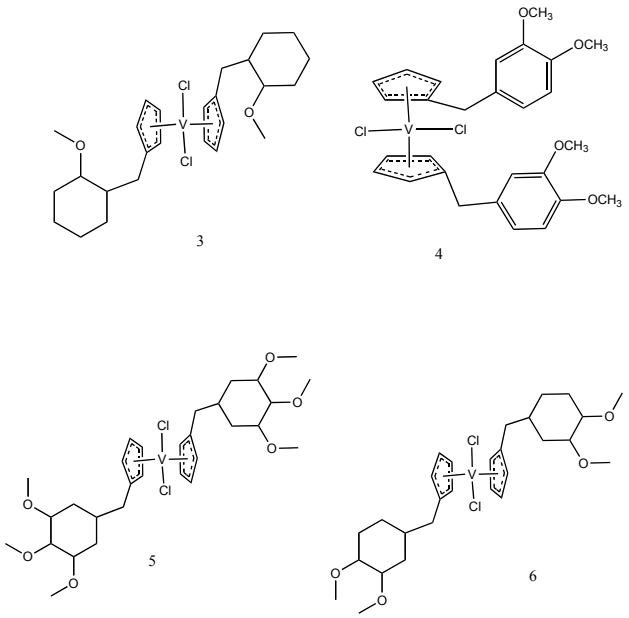


Fig. (2). Schematic structures of bis(η5-(2-methoxybenzyl) cyclopentadienyl)-vanadium(IV) dichloride (3), bis(η5-(3,4-dimethoxybenzyl)cyclopentadienyl)-vanadium(IV) dichloride (4),bis(η5-(3,4,5-trimethoxybenzyl)cyclopentadienyl)-vanadium (IV) dichloride (5), and bis(η5-(3,4-dimethoxy-benzyl) cyclopentadienyl)-titanium(IV) dichloride (6).

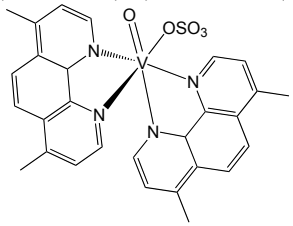


Fig. (3). Schematic structure of bis(4,7-dimethyl- 1,10-phenanthroline) sulfatooxovanadium(IV) "Metvan".

to cisplatin. On the other hand, Metvan shows promising anticancer actions on glioblastoma and breast cancer in invivo models. The wide range of Metvan antitumor activity with auspicious pharmacodynamics features and its low toxicity highlight that this compound has potential to be the first vanadium complex as an alternative to the classical platinum treatment [42]. Flavonoids are a group of natural polyphenols predominantly synthesized by higher plants with numerous pharmacological properties. The main therapeutic actions of flavonoids include antiviral, anticancer, antibacterial and antioxidative activities [43]. On the other hand, metal complexation has an important role in the improvement of the pharmacological activities of metal ions. The anticancer properties of a series of flavonoids (quercetin, hesperidin, morin, silibinin, chrysin) and their complexes with oxidovanadium(IV) were investigated against normal (MC3T3E1) and tumor (UMR106) osteoblasts-like cells [44-46]. Table **1** highlights the effects of flavonoids and VO-flavonoids complexes against normal (MC3T3E1) and tumor (UMR106) osteoblasts-like cells. The comparison was performed at 100 μM concentration. As it can be seen, hesperidin, quercetin and silibinin only altered slightly normal osteoblasts whilst chrysin produced a cytotoxic effect on proliferation of these cell lines and this effect could be probably attributed to structural differences due to the lack of substituents on B ring of the cynnamoyl system. In view of these previous results, VOsil and VOchrys (Fig. **4**) have been thoroughly investigated in the human osteosarcoma cells (MG-63). This cell line is an excellent model to study the effects of potential anticancer drugs for osteosarcoma treatment. In human osteosarcoma cells, oxidovanadium(IV), chrysin and VOchrys impaired cell viability in a concentration-dependent manner. Moreover, VOchrys was the strongest antitumor drug in human osteosarcoma cells having a lower IC_{50} value (16 μM) than vanadyl cation and chrysin (>100 μM) [47]. In addition, in the same cell line, VOsil, decreased the cell viability in a dose dependent manner with a greater potency than silibinin and vanadyl cation. The complex also displayed a concentration effect both in cyto- and genotoxicity processes. Investigation of the redox status of the cells was determined through the ROS level and GSH/GSSG ratio. These parameters were the most representative for the events involved in the deleterious effects of the complex on human osteosarcoma cells. Moreover, the compound induced cell cycle arrest (G_2/M phase) and the activation of caspase 3 that conveyed the cells to apoptosis [48]. From the comparison of the anticancer properties of VOchrys, VOsil and cisplatin in MG-63 cells, it could be concluded that VOchrys was the strongest antiproliferative effects (see Table **2**). Table **2** gives IC50 values of VOchrys, VOsil and cisplatin in MG-63 cell line. ROS generation, alterations of the GSH/ GSSG ratio, changes in the mitochondria membrane potential (MMP), induction of caspase-3 and DNA damage are the main mechanisms involved in the antitumor activity of VOchrys and VOsil.

All these mechanisms finally trigger the human osteosarcoma cells to apoptosis. Besides, VOchrys and VOsil showed promissory anticancer effects against human colon adenocarcinoma cells. These vanadium compounds caused cytotoxicity and genotoxicity in a concentration dependent manner. The main mechanisms of action of VOchrys involved a decrease in GSH/GSSG ratio and cell cycle deregulation whilst VOsil employed several mechanisms that include a decrease in GSH, programmed cell death activation,

Table 1. Effects of flavonoids and VO-flavonoids complexes against normal (MC3T3E1) and tumor (UMR106) osteoblasts-like cells.

Flavonoids	MC3T3E1 (%) basal (100 μM)	UMR106 (%) basal (100 μM)	Vanadyl and VO-flavonoid complexes	MC3T3E1 (%) basal (100 μM)	UMR106 (%) basal (100 μM)
			$(VO)^{2+}$	71	115
Hesperidin	90	85	VOhesp	20	20
Quercetin	95	78	VOquer	60	82
Silibinin	94	60	VOsil	46	33
Chrysin	65	40	VOchrys	50	42
Morin	70	65	VOmor	30	65

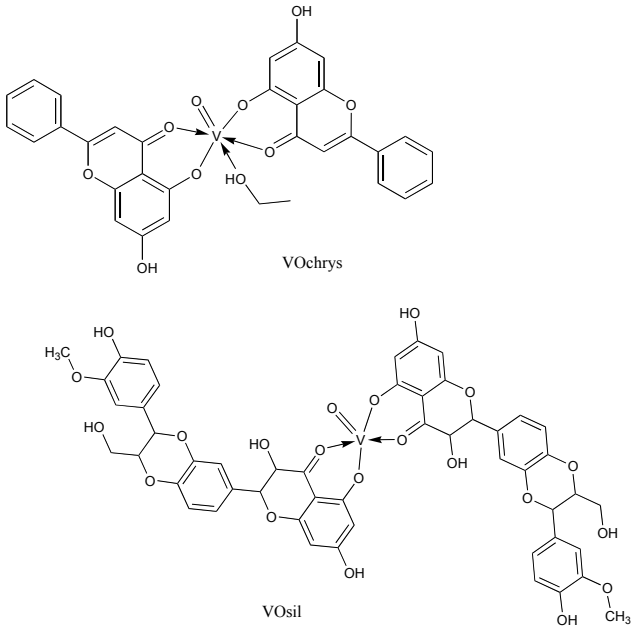


Fig. (4). Schematic structures of oxidovanadium(IV) complexes with chrysin (VOchrys) and silibinin(VOsil).

Table 2. IC_{50} values of VOchrys, VOsil and cisplatin on MG-63 cell line.

Compounds	IC_{50}
VOchrys	16 μM
VOsil	74 μM
Cisplatin	43 μM

attenuation of NF-kB pathway among others [49]. Another interesting group of vanadium based drugs complexes of oxidovanadium(IV) with ligands that hold multiple donor atoms is able to coordinate with metal centers.

Chelating ligands are relevant in living systems since they can facilitate the uptake and transport of metallodrugs inside the cells. Oxodiacetate (oda), $O(CH_2COO^-)_2$, is a very versatile ligand that coordinates metal ions by forming chelate rings [50]. $V^{IV}O$-complexes of oda, $V^{IV}O$(oda), as well as $V^{IV}O$(oda)(bipy) and $V^{IV}O$(oda)(phen) (Fig. **5**), showed significant effects in osteoblast like cells. The three complexes were tested in two cell lines derived from osteoblasts (MC3T3-E1 from mouse calvaria and UMR106 from a rat osteosarcoma cells). $V^{IV}O$ (oda) inhibited cell viability in both cell lines, but the inhibitory effect was stronger in the normal than in the tumor osteoblasts [51]. The principal mechanisms of action involved in the anticancer activity of $V^{IV}O$(oda) are ROS generation, depletion of GSH, disruption of the mitochondria membrane potential (MMP) and activated ERK cascade phosphorylation [51]. Besides, $V^{IV}O$(oda)(phen) impaired cell viability in both cell lines (MC3T3-E1 and UMR106), but the cell damage was stronger in the normal than in the tumor cells [52]. On the contrary, $V^{IV}O$(oda)(bipy) showed promissory antitumor activity since the cytotoxicity was stronger in the tumor than in the normal cells [53]. Recent reports have shown that the three complexes caused a concentration dependent inhibition of cell viability in human osteosarcoma cells in culture [54]. In this order, $V^{IV}O$(oda)(phen) produced strong cytotoxicity affecting several organelles such us lysosomes and mitochondria from 2.5 μM) while $V^{IV}O$(oda) and $V^{IV}O$(oda)(bipy) only caused these effects at higher concentrations (100 μM). Moreover, complex with phen and bipy derivatives triggered apoptosis as a relevant mechanism of cell death. Besides, the nuclease activity of the three compounds revealed that DNA cleavage caused by $V^{IV}O$(oda) and $V^{IV}O$(oda)(bipy) was similar, whilst $V^{IV}O$(oda)(phen) showed a stronger effect. In this way, a very interesting relationship between the bioactivity of the complexes and their struc-

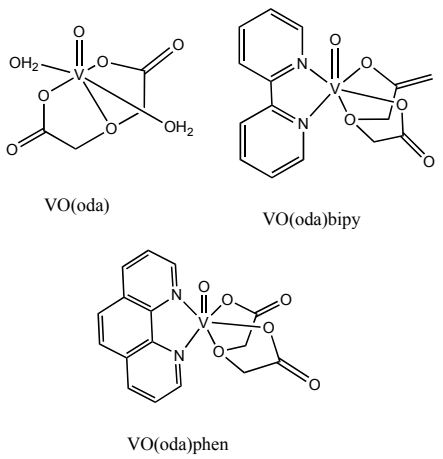


Fig. (5). Schematic structures of oxidovanadium(IV) complexes with oxodiacetate VO(oda), oxodiacetate and 2,2 bipiridine VO(oda)bipy, oxodiacetate and 1.10 phenantroline VO(oda)phen.

tures was achieved. $V^{IV}O$(oda)(phen) showed the most potent anticancer action in human osteosarcoma cells followed by $V^{IV}O$(oda)(bipy) and subsequently by $V^{IV}O$(oda)) in agreement to the number of intercalating heterocyclic moieties [54]. Others organically chelated vanadium compounds also showed interesting anticancer activity against lymphoma derived cells. Quinoline and pyridinone molecules are attractive ligands because of their pharmacological activity. The antitumor actions of quinoline complexes on U937 cells showed slight relation with the length and shape of the alkyl chain in the relationship structure-activity analysis [55]. One of these compounds showed stronger antitumor effects than cisplatin. Moreover, this complex activated apoptosis but through a pathway independent of caspase activation [55]. Besides, hydroxiquinoline derived vanadium complexes displayed specifically anticancer activity against cisplatin sensitive/resistant ovarian cells (A2780/A2780cisR). The bioactivity of these vanadium compounds toward the A2780 cell line was dependent on incubation time and concentrations showing IC_{50} values in the range of 3-14 μM. In this order, these compounds were significantly more active than cisplatin (22 μM), in the A2780 cells and in the cisplatin-resistant cells A2780cisR (4-8 μM *vs*. 75.4) [56]. A huge family of vanadyl compounds also includes bound organic ligands based on the salen ligand. A pyridoxal-based vanadium(IV) complex showed selective cytotoxicity for tumor cell lines in contrast with healthy cells. The complex impaired cell viability was stronger in melanoma and lung carcinoma cells than in normal epidermal keratinocytes, lung cells and peripheral blood mononuclear cells [57]. Oxidovanadium (IV) complexes with a Schiff base and thiosemicarbazones as mixed- ligands showed interesting antitumor effects on several colon cancer cell lines (HTC-116, Caco-2, and HT-29) and also with non-cancerous colonic myofibroblasts (CCD18-Co). In general, these compounds exhibited antitumor activity on cancer cells, but did not affect normal cells. Nevertheless, compounds 8-10 (Fig. **6**) showed less inhibitory effects on CCD-18Co cells, suggesting a possible cytotoxic selectivity against colon cancer cells and may have a potential in chemotherapy (see Table **3**) [58]. Table III shows Antitumor activity of compounds derivatives 8-10 and cisplatin, on different colon adenocarcinoma cell lines after 24, 48, and 72 h of treatment. Data are expressed as IC_{50} (μM). IC_{50} values are shown as mean ± S.D. obtained from three independent experiments. Others organic vanadium complexes with pharmacological activities are acetylacetonate derivatives.

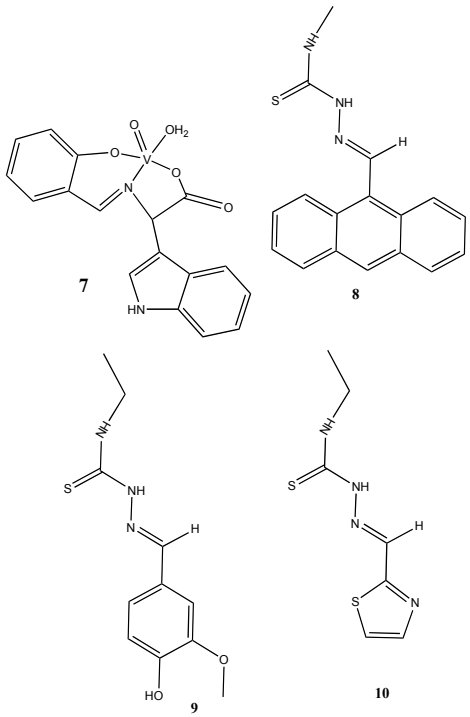


Fig. (6). Schematic representations of oxidovanadium(IV) complex with with a Schiff base and thiosemicarbazones (7) and different thiosemicarbazones derivatives of ligands (8, 9, 10).

Oxidovanadium(IV) complexes with ferrocenyl-terpyridine and acetylacetonate ligands exhibited selective antitumor effects in cervix and breast cancer cell lines (HeLa and MCF-7) with low cytotoxicity in normal fibroblast 3T3 cells [59]. Moreover, Vanadyl bisacetylacetonate (VO(acac)2) arrested cell cycle at G1 phase in a concentration- and time-dependent manner in hepatocarcinoma cells. The phosphorylation of ERK and Akt pathways was greatly activated and these are two major mechanisms of action related to the anti

Table 3. Antitumor activity of compounds derivatives 8–10 and cisplatin, on different colon adenocarcinoma cell lines after 24, 48, and 72 hours treatment. Data are expressed as IC_{50} (μM). IC_{50} values are shown as mean ± S.D. from three independent experiments.

Compounds	HT-29			Caco-2		
	24 h	48h	72h	24 h	48h	72h
8	277.1±10.2	239.6±7.9	100.3±5.1	349.1±19.5	277.1±16.3	147.1±10.8
9	244.2±8.8	169.6±15.1	87.9±0.5	367.0±21.5	281.6±14.4	152.5±12.7
10	128.6±10.7	82.4±9.6	47.8±5.5	242.1±25.1	166.2±15.3	85.4±14.0
Cis-Pt	84.7± 1.9	80.6± 1.6	69.1±3.2	32.0± 1.6	22.8± 1.5	17.9±1.8
	HCT-116			CCD18Co		
	24 h	48h	72h	24 h	48h	72h
8	203.4±7.7	181.3±9.2	115.0±6.5	495.6±23.5	339.2±16.7	208.0±11.9
9	227.4±6.8	192.7±11.6	110.3±10.1	490.6±27.6	329.5±15.4	203.6±12.5
10	161.3±4.6	134.9±3.9	89.5±14.5	382.7±21.9	246.2±11.4	152.2±12.0
Cis-Pt	53.8±2.1	49.7±1.3	41.0±2.7	83.0±1.1	72.0±1.5	64.1±1.6

cancer activity of the complex [60]. On the other hand, several V^V-compounds were reported to exhibit antitumor properties in different experimental models [4,14,16]. The development of V(V)-peroxide species in binary as well as ternary combinations emerged as a promising approach for novel cancer therapy. To this end, different V(V)- peroxide complexes have been designed and synthesized as potential antitumor compounds. The most common form of a bis(peroxido) vanadium complex is $M[VO(O_2)2L]n{\cdot}xH_2O$ and that of a mono(peroxido)vanadium complex is $M[VO(O_2)L]n{\cdot}xH_2O$. In peroxidovanadate complexes, the peroxido ligands are bound to the vanadium center almost exclusively in a side-on fashion in which the geometry around vanadium is octahedral or pentagonal bipyramidal, with varying numbers of water molecules or hydroxido ions. *In vitro* assays showed that peroxidovanadium complexes displayed inhibitory effects of protein phosphotyrosines suggesting that the effects of protein phosphotyrosines are one of the key mechanisms of action of peroxidovanadium complexes as antitumor agents [61]. V(V)-oxido and V(V)-oxidoperoxide compounds with carboxylic acids $K_3[VO(O_2)(C_2O_4)_2]{\cdot}1/2H_2O$ and $K_3[VO_2(C_2O_4)_2]{\cdot}3H_2O$ containing oxalate ligands showed interesting anticancer activity against human osteosarcoma cells. In this way, the monoperoxide complex showed a very strong antitumor action, whereas the dioxide compound was inactive [62]. Moreover, bis(peroxido)vanadium(V) complexes like (Pr4N)[$VO(O_2)$(ox)(phen)] (Vphen)[ox = oxalate (2-) and Pr4N+= tetra(n- propyl)ammonium], promote DNA cleavage in human lymphocytes in a concentration- dependent manner [63]. Further screening of probable anticancer activity on a murine leukemia cell line (L1210 cells) showed that they exhibited significant antitumor activity in a concentration and exposure time-dependent manner [63]. Several peroxide species were investigated in order to identify correlations of organic ancillary ligands with their related structures and the relationship to the physiological and antitumor effects. The nature and denticity of employed ligands were variable adopting a pentagonal bipyramidal geometry around the vanadium ion in these compounds. This geometry is typical for a monoperoxido V(V) species with an organic ancillary ligand. In addition, half-maximal inhibitory concentration (IC_{50}) values in different cancer cell lines could be related to the transition energies (LMCT) of the peroxide group, especially among complexes with analogous ligands. Djordjevic proposed that intramolecular electron transfer between the peroxide group and the metal center would affect the anticancer activity of the peroxidevanadium species and these result were in agreement [29]. It is suggested that ROS generated through intramolecular electron transfer in V(V)- peroxide species, may be one of the mechanisms of action responsible for the observed cytotoxicity. V(V)-peroxide species bearing variable ligands show different antitumor actions and these effects may be ascribed to the distinct electronic contribution of the bound peroxide moiety. Therefore, cytotoxicity and physiological actions of V(V)-peroxide species, correlating structure and activity, can ride

strongly on the nature and electronic properties of the auxiliary ligand, thus guiding the design and synthesis of new peroxidovanadium compounds that may be used as anticancer agents [64,65]. On the other hand, anthraquinone derivatives compounds exhibited specifically higher inhibition on human colorectal adenocarcinoma (HCT-8 cells) than the clinical anticancer drug Fu-5. Along the same lines, the anticancer activity of three different vanadium complexes bearing pyridinone Schiff and pyrimidinone-based ligands (Fig. **7**) was studied in two different cell lines. The study showed that the V(V)-species resulting from the V(V)-MHCPE are more toxic for tumor cells (HeLa) than for normal cells (3T3-L1 fibroblasts), thereby indicating potential anticancer activity [66,67]. Other relevant Schiff base with pharmacological properties is N,N' bis(salicylidene)ethylenediamine, more commonly referred to as salen. VO-salen (Fig. **8**) inhibited K562 leukemia cell proliferation in the range of 6-32 μM and the complex enhanced taxol anticancer activity. These conclusions support that the combined use of VO-salen and taxol might establish an effective novel approach against leukemia [68]. Semicarbazones are versatile ligands with therapeutic effects. Different semicarbazones and vanadium complexes were tested in three different human tumor cell lines derived from breast, colon and kidney cancer for then activity as potential anticancer drugs, showing selective antitumor activity on kidney derived cells. Results showed that changes in the semicarbazone moiety structure could have a relevant effect on the antitumor activity of the vanadium complexes [69]. Moreover, a complex of vanadium(V) with salicylaldehyde semicarbazone (V(V)-Salsem) (Fig. **9**) exhibited anticancer effects on tumor osteoblasts through the production of ROS, the ERK pathway activation and the apoptosis induction [70].

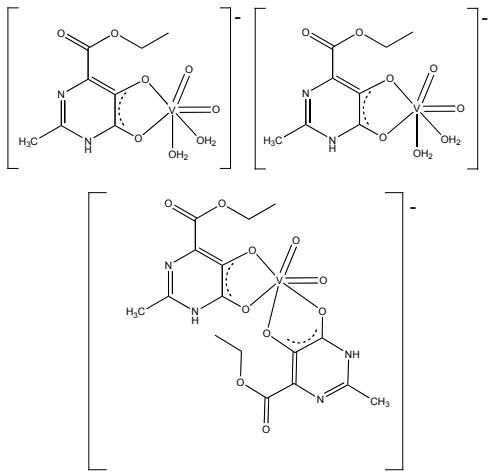


Fig. (7). Schematic illustration of plausible forms of complex V(V)MHCPE.

2.3. Polyoxovanadate compounds

Polyoxometalates (POMs) are clusters with transition metal oxygen anion that contain combinations of metal cations (usually the d^0 species V(V), Mo(VI), and W(VI)) associated by oxide anions**.** The structural, electronic and physicochemical properties (shape, stability under physiological conditions, polarity, redox potential, etc), make them attractive for different uses in diverse fields. POMs have pharmacological properties as antiviral and anticancer agents [71,72]. Polyoxovanadates are a prominent subclass of POMs that are relatively less investigated in comparison to the Mo and W compounds. These compounds show different topologies and structures such as $[V_4O_{12}]^{4-}$, $[V_5O_{14}]^{3-}$ and $[V_{10}O_{28}]^{6-}$ [73]. Other important features of polyoxovanadates are their diverse structural chemistry, combining tetrahedral (VO4), square pyramidal (VO5) and octahedral (VO6) units, a variability of oxidation states and the potential encapsulation of small molecules or ions.

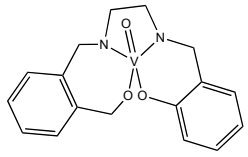


Fig. (8). Schematic representation of VO-salen.

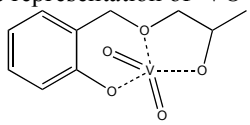


Fig. (9). Schematic structure of vanadium(V) complex with salicylaldehyde semicarbazone (V(V)-Salsem).

Polyoxovanadates form diverse anionic species in solution that can coexist simultaneously in equilibrium, like mononuclear $[VO_4]^{3-}$, dinuclear $[V_2O_7]^{4-}$, tetranuclear$[V_4O_{12}]^{4-}$ and decanuclear $[V_{10}O_{28}]^{6-}$. The pH of solutions is a relevant property to study the speciation of oxovanadates. In this sense, a decrease in pH increased the generation of the protonated forms. The pharmacological effects of polyoxovanadates include antiviral, antibacterial and antitumor activities. Scientific reports showed the antitumor activity of octacosaoxidodecavana-date(V)$Na_4Co(H_2O)_6V_{10}O_{28}{\cdot}18\ H_2O$ (CoV10) against liver and ovary cancer cell lines [74]. Moreover, octacosaoxidodecavanadate(V) diammonium derivatives, $(H2tmen)_3V_{10}O_{28}{\cdot}6H_2O$ and (H2en) $3V_{10}O_{28}.2H_2O$, decreased the cell viability showing a high anticancer activity in lung adenocarcinoma and leukemia cells [75]. The structures of these compounds are very similar (skeletal structure of octacosaoxidodecavanadate(V) anion and cation), however, the main

difference is the four methyl (CH3) substituents of cation in the former species. Therefore, the compound (H2tmen)3$V_{10}O_{28}$·6H_2O has higher anticancer activity since its amphiphilic structure facilitates its permeation through the monolayer cell membrane. In the last year, several mixed-valence V(IV)/V(V) octacosaoxidodecavanadates were interestedcompounds due to their structural, spectroscopic, nuclease activity and DNA-protecting actions. One of the more relevant compounds is (Me4N)6[$V_{15}O_{36}$Cl] that have important chemical interaction properties with alkylating agents [76]. Different DNA interaction studies showed the chemoprotective action of this compound toward alkylation agents such as diethylsulphate (DES) and dimethylsulphate(DMS) using pUC19 plasmid DNA. The main mechanism of action elucidated for this chemoprotective activity exposed the progressive generation of V(IV), V(V) and mixed-valence aggregates, suggesting mechanistically preferential transfer of the alkyl group to the vanadate instead of DNA [76]. One of the most important biomedical polyoxovanadates are the decavanadate derivatives $[V_{10}O_{28}]^{6-}$ that play a key role in many biological processes [77]. These compounds may act as effective ion pump inhibitors and have the potential therapeutic applications in the treatment of different diseases such us cancer, diabetes, ischemic heart disease, ulcers, *etc.* [78]. Recently scientific reports showed the antidiabetic actions of metforminium decavanadate (H2Metf)3[$V_{10}O_{28}$]·8H_2O in Wistar rats as biological model. This compound has therapeutic properties as a hypoglycemic, lipid-lowering and metabolic regulator [79]. Considering other promising biological roles of these kinds of compounds, several groups reported novel synthesis of different polyoxovanadates based on decavanate structure [80,81].

3. ANTITUMOR ACTIVITY OF RUTHENIUM COMPOUNDS

Historically, cisplatin discovery opened the perspective to use drugs with a metal core for cancer treatment, despite its good effects over tumor cells, it also showed a narrow therapeutic window, with severe and undesirable side effects on patients [1]. This safety issue with the cisplatin raised the number of investigations related to platinum and non-platinum metallodrugs in order to find substances capable of killing cancer cell but with better toxicological profile. Within the transition metal group, the platinum like elements have played an important role in the research and development of new active pharmaceutical ingredients (API). Among them, ruthenium (Ru) showed attractive chemical properties like its range of oxidation states (8, 6, 4, 3, 2, 0 and -2), atomic radius and the variable kind of ligands that can form complexes or bonds with Ru. Especially for the medicinal chemistry, the oxidation states 3 and 2 have a particular importance due to their stability under physiological pH and the equilibrium between those species make them suitable for potential pharmaceutical uses [82]. The ruthenium compounds with biological interest can be classified into the following main categories: coordination and organometallic ruthenium derivatives.

3.1. Coordination Compounds

As models of coordination compounds, the well-known and vastly explored NAMI-A, KP1019, KP418 and NKP-1339 were found. The main feature in these complexes is related to their six-coordinated sphere of coordination, achieving an octahedral geometry, where ligands containing soft nitrogen and sulphur atoms (*e.g.* imidazole, indazole and DMSO) are found in the axial positions and are the responsible of the steric and electronic properties of the complex [83,84]. Four axillary positions, occupied with ligands can be easily replaced or exchanged (*e.g.* chloride), depending on the environmental conditions (see Fig. **10**). The interaction of these complexes with blood components *in vitro* and *in vivo*, proved strong relation between the ability of the complexes to bound to serum albumin and transferrin with their pharmacokinetic profile and the final therapeutic effect. Such proteins have a main role in transportation and storage of ruthenium complexes [85]. In the case of trans-tetrachloridobis(1H-indazole) ruthenate(III)], Jarosz *et al.* showed that the complex can bind to transferrin in apo and holo forms, out of the Fe binding site and is still recognizable by the transferrin receptor in the cell [86]. Some studies about ruthenium complexes reviewed by Pessoa and Tomaz demonstrated that the presence of transferrin-complex adducts enhanced the anticancer activity of the complexes [87]. This highlights the importance of this protein in the mode of action. The Ru-transferrin adduct reaches the tumor cell, almost in a targeted way, since it is known that cancer cells have a higher need of iron and it has been proved that this causes an overexpression of transferrin receptor on the cell surface [88-90]. This approach led to the development of this kind of complexes (protein adducts) as a "Trojan horse" (Fig. **11**). The activation by reduction of Ru (III) to Ru (II) is the currently accepted hypothesis by which the complexes of Ru (III) act as a pro-drug (see Fig. **12**). It is believed that the complexes once released from the transport proteins in zones with low pressure of oxygen (acidic

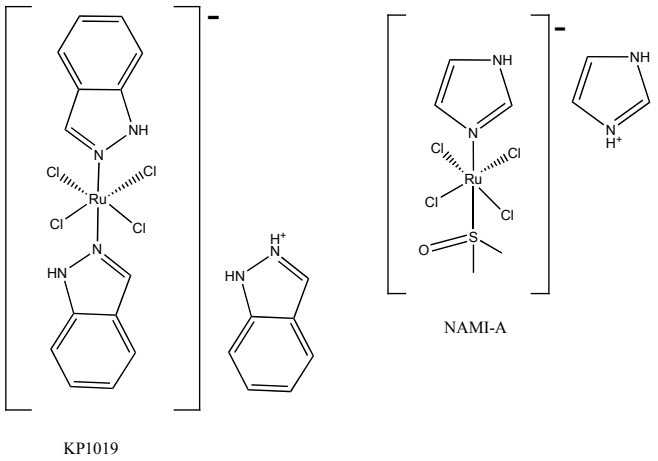


Fig. (10). Structure of KP1019 and NAMI-A.

environment) are reduced to Ru(II) complexes which are more reactive and ready to interact with proteins (imidazole nitrogen from histidine side chains) or nucleobases (nitrogen 7 in the purine bases) into the cell [82,91]. The mechanism of action for these complexes actually goes beyond the DNA cross-linking and adducts formation previously investigated [92]. There is some evidence that this kind of compounds also cause a disruption in the mitochondrial and cellular redox balance by producing reactive oxygen species (Fenton-like reaction) and GSH depletion. Different carried out experiments suggested that the addition of ROS scavengers reduce the cytotoxicity of these complexes [93-96]. However, the most accepted mechanisms of action based on KP1019 and NKP-1339, are redox balance disruption, cell cycle arrest on G_2/M, DNA synthesis blockage and finally apoptosis induced mitochondrial pathway [91]. As previously exposed, the ability to exchange ligands in the coordination sphere for the Ru complexes, and the possibility to induce modifications in their structure are the key factors to achieve a rational design of drugs and the establishment of structure activity relationship [97]. Since the mechanism of action of Ru (III) drugs is conservative in many compounds, the keystone in the investigation is more related to the characteristics of the ancillary ligands, aiming to improve pharmacokinetic behavior [98]. Another subfamily of the Ru coordination compounds is that composed of complexes containing at least one polypyridyl ligand (*e.g.* bipyridine or phenanthroline). The accepted mechanism of action of these drugs is the binding and cleaving of DNA inducing apoptosis in the cancer cells [99]. At normal physiological conditions, at least one of the polypyridyl ligands will remain inertly bound to the Ru center. This could be extended to the case in which every ligand in the complex is inert to hydrolysis reactions and therefore the coordination sphere of the metal remains saturated. In this last situation, the mechanism of the cytotoxicity should be different from that known for other metallopharmaceuticals (*i.e.* the activation by hydrolysis step is not viable).

3.2. Ru-Arene Complexes (Organometallic)

A second class of Ru related compounds, which have gained an important place in the research field of medicinal chemistry are the Ru-Arene complexes. The main concern about Ru (II) complexes is related to the stability and the ligand exchange rate. Nevertheless, Sadler and coworkers found out that arene moiety acts as a stabilizer of the Ru atom and increases the lipophilicity, which can increase the uptake by the cell enhancing the cytotoxic effect [100]. The Ru(II)-arene complexes have the general formula [(η6 - arene)Ru (X)(Y)(Z)] where X (*e.g.* Chloride) is a leaving group, and YZ are chelating ligands [101]. In general, the cy-

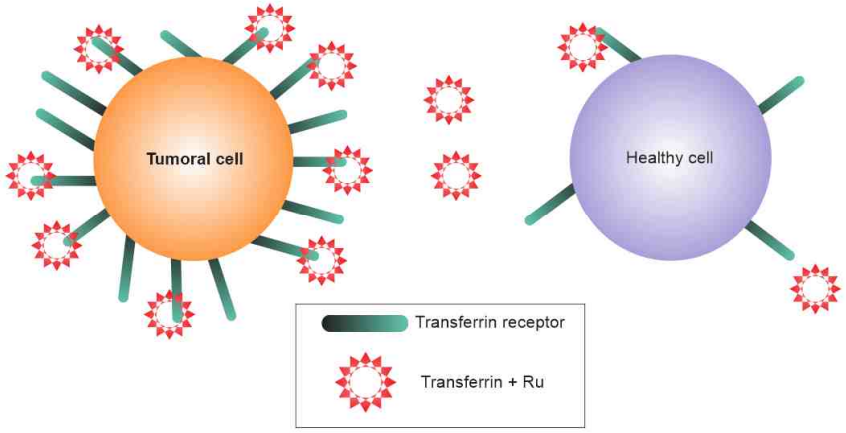


Fig. (11). Schematic representation of the overexpression of transferrin receptor on tumor cells and the possible delivery mechanism and recognition mediated by transferrin-Ru adduct.

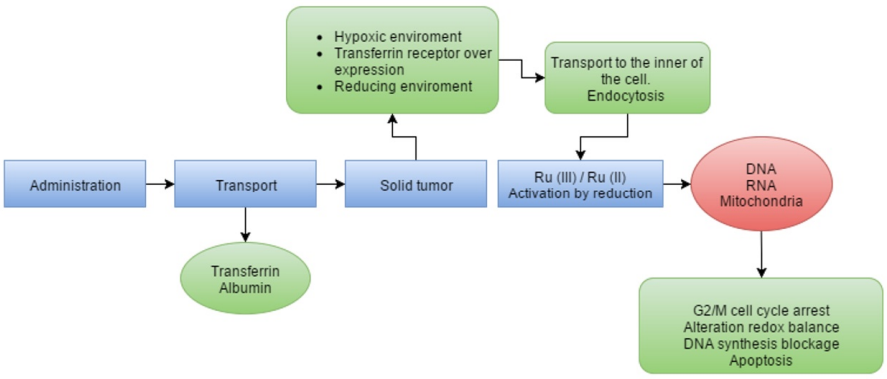


Fig. (12). Scheme of the potential mechanisms of action and transport.

totoxic effect increases in function of the size (lipophilic character) of the arene ligand, probably related to the capability to intercalate with the DNA. In this order, the bicyclic arenes showed higher rate of DNA damage compared with the single cycle arenes, and this effect is linked with the ability to form arene base π-π stacking (see Fig. **13**) [102-104]. The ligands occupying the position (Y)(Z), have an important role in the antitumor activity since it was demonstrated that monodentate ligands have lower activity compared with chelated ligands in the inclusion of ethylendiamine, which led to an increase in the biological activity even on cisplatin resistant cell lines [105,106]. Studies varying the chelating ligand with N-N, N-O and O-O groups revealed that the anticancer activity of complexes with N- N moiety was stronger than N-O and O-O [107, 108]. These complexes should be in an active form, capable of interacting with the molecular target (mainly attributed to DNA). The most described activation mechanism is the aquation, where the leaving group (chloride or another halide) is substituted by a water molecule. [(η6 - arene)Ru(**X**)(Y)(Z)] → [(η6 arene)Ru(***H*2O**)(Y)(Z)]. In the bloodstream the complex with halide is mostly kept in an inactive form due to high chloride extra cellular concentration (c.a. 100 mM). However, when the compound enters to the cell, the Cl^- concentration is about 4 mM which facilitates the replacement of the leaving group by the water molecule [104,105,109]. The rational design of complexes allows to set up the rate and extend of hydrolysis based on the properties of the arene, leaving the group and the chelate ligand. This is a useful tool to control the selectivity and half-life of the complex [110].

3.3. RAPTA Compounds

This class of compounds have a stabilization mechanism mediated by arene moiety, the family of ([Ru (η6-arene)(PTA)X2], PTA = 1,3,5-triaza-7-phosphaadamantane).

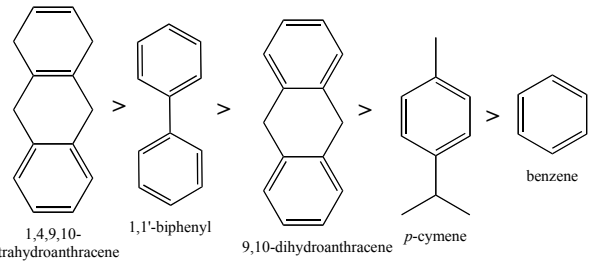


Fig. (13). Chemical structures of arene moiety sorted in decreasing order of anticancer activity.

Chemically these compounds are configured as follows: three Ru coordination positions are occupied by the arene ligand, a position is filled by the PTA (phosphine) ligand which gives it a hydrophilic (water soluble) character and two positions with labile ligands (mainly chloride) which are available for aquation activation (see Fig. **14**) [111]. These compounds have a low cytotoxic effect *in vitro*, but in further investigation it was found that they have a similar property to inhibit the metastasis process similar to the NAMI-A [112]. RAPTA-C suffers a rapid aquation driving to very high activation [109]. Modification of the core using bidentate ligands instead of Cl- leads to an increase in the stability, solubility and aquation rate (carbo-RAPTA and oxalo-RAPTA). In relation to the molecular target of these complexes, it is very interesting that RAPTA-C shows a selective pH dependent damage of DNA on acidic environment (pH< 7). In this

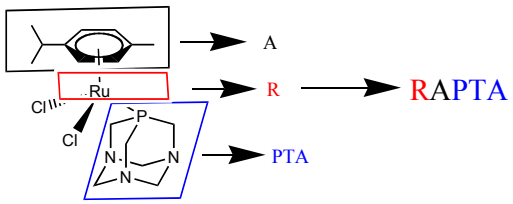


Fig. (14). Representation of the structure of RAPTA.

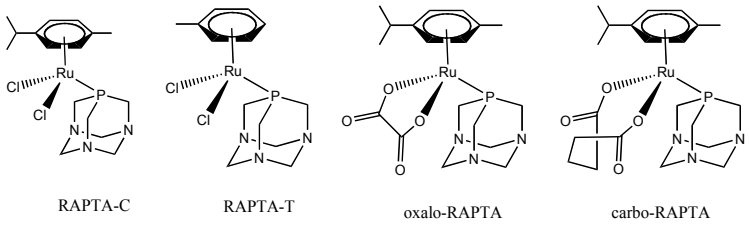


Fig. (15). Chemical structure of RAPTA derivatives.

order, scientific reports showed that DNA damage in tumor cells (acidic pH) is higher than in healthy cells (neutral pH). This effect can be attributed to the PTA ligand protonation at low pH,which is a more active form of the RAPTA-C compound [109]. The RAPTA complexes do not show selective *in vitro* binding to DNA, proteins and RNA [89]. But, this complex inhibited cell growth and division through G_2/M arrest and apoptosis induction [113]. In Fig. (**15**), the first members of the RAPTA series of compounds are displayed. Dyson and collaborators made modifications in the arene ring and the PTA moiety to evaluate the structure activity relationship,these changes increased the stability and led to control the aquation process and enhance the cytotoxic and antimetastic activity [111]. Nevertheless, Ru-Arene complexes overshadowed the investigations related to these complexes due to their better cytotoxic profile compared with the RAPTA family. Undoubtedly, the ruthenium complexes and compounds have proven be potential tools for the development of future therapeutics for cancer treatment. A development in Ru based drugs is an open door to a rational design where the flexibility to change the ligands led to find new cellular targets, enhancing the selectivity of the compounds. A great number of Ru compounds have been synthesized and their cytotoxic effects on several tumor cell lines have been demonstrated, despite the heterogeneity in their chemical structure. However, it should be noted that the effect of the lipophilic ligands, which in the most of the cases induced a strong anticancer effect on solid tumors, even with IC_{50} lower than those obtained with cisplatin. (see Table **4**). Nowadays the action mechanism of Ru metallodrugs is quite unclear, but scientific studies pointed out some targets recognized for the different compounds (see Fig. **16**) [83]. Table **4** shows the structure-activity on several tumor cell lines of different ruthenium complexes. [114-127].

3.4. Ru Complexes: Role in Photodynamic Therapy (PDT).

Among the practical applications of Ru complexes as alternative to the cancer treatment, beyond chemotherapy, photoactivated chemotherapy (PACT) and photodynamic therapy (PDT) have gained a notorious relevance in the last decade. According to SCOPUS® database in 2006, two documents related to Ru and photodynamic therapy were published, whilst in 2015, thirty-three scientific articles were published.

The photodynamic therapy (PDT) is the approach which mostly studied the cases of anticancer drugs activation through light which can be split into two categories: photoactivated chemotherapy (PACT) and photodynamic therapy (PDT).

The photodynamic therapy requires the presence of three agents: a photosensitizer (PS), light and molecular oxygen (O_2). The first one is a molecule with the ability to be excited by light in the range of 650 to 800 nm. The activation yields an electron displacement to higher energy levels with the subsequent energy release as fluorescence emission, phosphorescence or a non-radioactive decay, called intersystem crosslinking. [128-130]

The excited PS can be present as singlet state and /or triplet state, and produce two types of reactions. The **type I**: derived from an excited singlet state involves the oxidation or the reduction of the substrates or even the photosensitizer itself to produce superoxide, hydroxyl and peroxides radicals which can start the cascade of cytotoxic effects leading to the cellular death. The **type II:** involves the presence of the excited triplet state, produced by the intersystem crosslinking, and the PS in this state can react directly with molecular oxygen. This reaction gives two forms of oxygen singlet (which are highly reactive oxygen species) that can directly attack biomolecules as amino acid residues, unsaturated lipids and nucleobases. Most studies have pointed out that the cell death is mainly mediated by the last one mechanism [131]

The classic model of photosensitizers can be found in the tetrapyrrole ring (porphyrins, chlorins) which have been extensively studied in photochemistry. In this way, porphyrin derivatives were approved for human use in PDT for the treatment of several types of

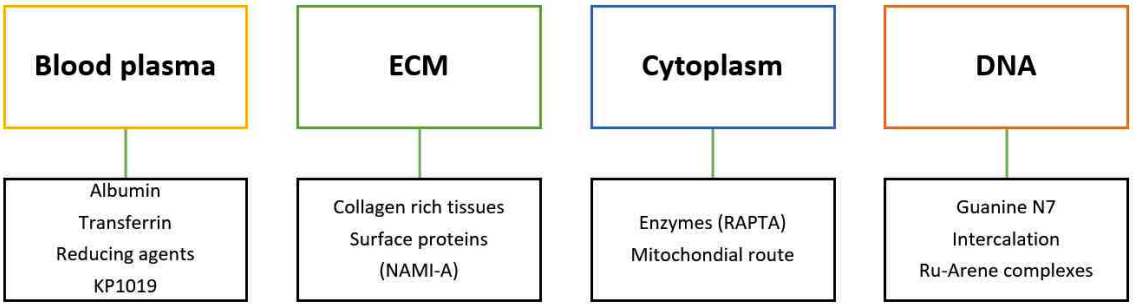
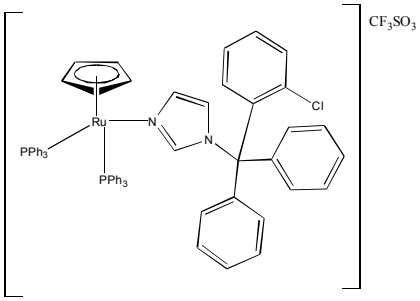
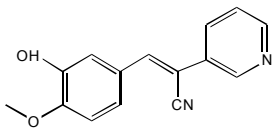
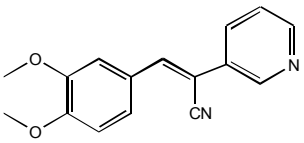
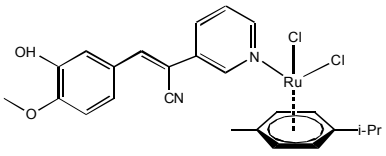
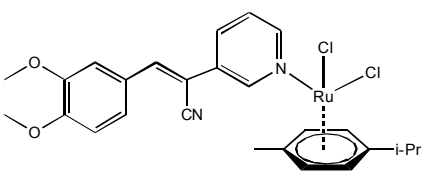
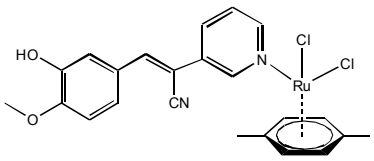
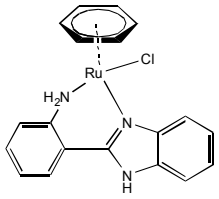
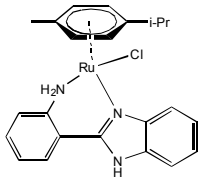
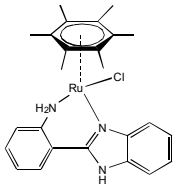
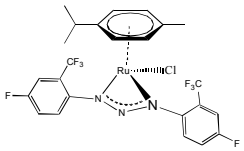
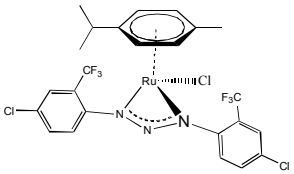
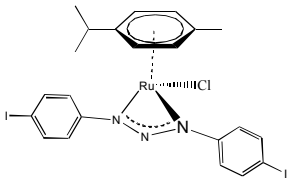
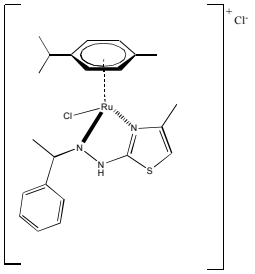


Fig. (16). Summary of the putative action mechanism of Ru compounds.

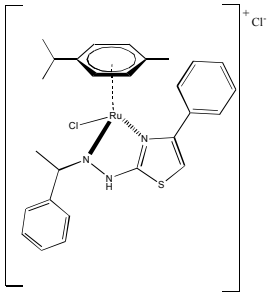
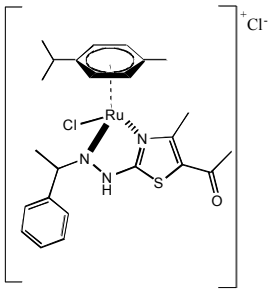
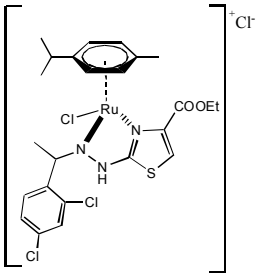
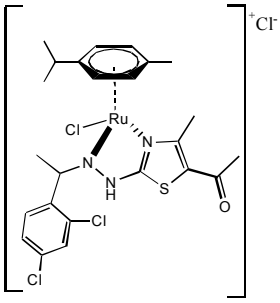
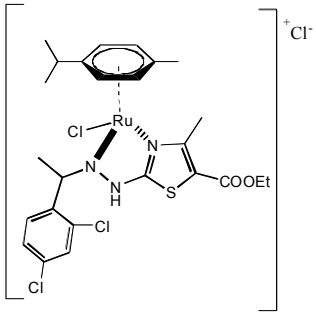
Table 4. Structure, cell line, IC50 values of different ruthenium complexes.

Structure	Cell line	IC_{50} (μM)	Reference
	A2780 MCF7 HeLa J774	0.54 ± 0.1 5.4 ± 2.0 3.6 ± 1.1 1.9 ± 0.1	[114]
1a 	518A2 518A2 HL-60 HL-60 Kb-V1/Vbl Kb-V1/Vbl MCF-7/Topo MCF-7/Topo	94 (24h) 16 ± 3.4 (48h) 11 ± 2.4 (24h) 9 ± 1.4 (48h) 9 ± 2.4 (24h) 6 ± 1.4 (48h) 50 (24h) 1.5 ± 0.3 (48h)	[115]
1b 	518A2 518A2 HL-60 HL-60 Kb-V1/Vbl Kb-V1/Vbl MCF-7/Topo MCF-7/Topo	45 ± 1.5 (24h) 50 ± 6.2 (48h) 33 ± 4.2 (24h) 32 ± 3.3 (48h) 46 ± 5.6 (24h) 20 ± 4.8 (48h) 48 ± 5.8 (24h) 11 ± 3.8 (48h)	[115]
2a 	518A2 518A2 HL-60 HL-60 Kb-V1/Vbl Kb-V1/Vbl MCF-7/Topo MCF-7/Topo	31 ± 2.3 (24h) 13 ± 4.2 (48h) 2 ± 1.1 (24h) 0.8 ± 0.25 (48h) 50 (24h) 3 ± 0.7 (48h) 50 (24h) 0.2 ± 0.14 (48h)	[115]
2b 	518A2 HL-60 Kb-V1/Vbl Kb-V1/Vbl MCF-7/Topo MCF-7/Topo	60 ± 1.7 (48h) 95 ± 5 (48h) 59 ± 3.1 (24h) 31 ± 4.7 (48h) 49 ± 3.9 (24h) 29 ± 3.4 (48h)	[115]

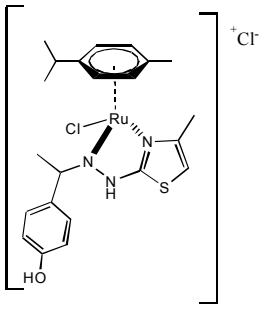
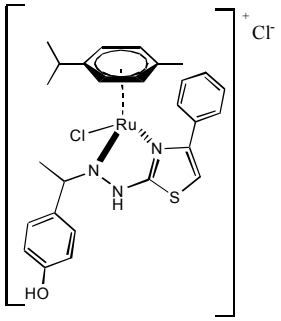
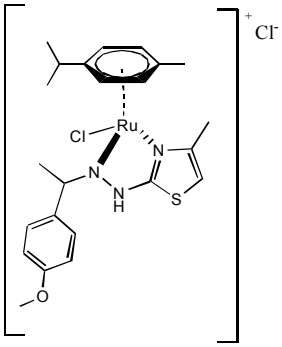
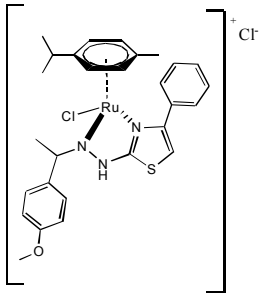
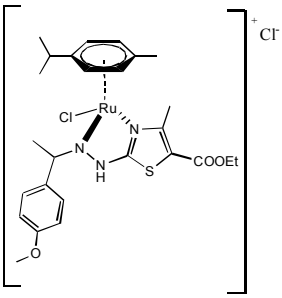
(Table 4) contd….

Structure	Cell line	IC_{50} (μM)	Reference
3a 	518A2 518A2 HL-60 HL-60 Kb-V1/Vbl Kb-V1/Vbl MCF-7/Topo MCF-7/Topo	3 ± 0.3 (24h) 2.2 ± 0.2 (48h) 1.3 ± 0.02 (24h) 1 ± 0.2 (48h) 20 ± 4.6 (24h) 7 ± 1.5 (48h) 75 ± 13.7 (24h) 7 ± 2.7 (48h)	[115]
	SiHa	37.1	[116]
	SiHa	16.91	[116]
	SiHa	11.11	[116]
	HeLa HEp-2 7T HCT 116 H460 MDA-MB-485	0.230 ± 0.053 0.226 ± 0.002 0.330 ± 0.037 0.249 ± 0.010 0.300 ± 0.033 0.297 ± 0.015	[117]
	HeLa HEp-2 7T HCT 116 H460 MDA-MB-485	0.103 ± 0.006 0.108 ± 0.015 0.109 ± 0.006 0.106 ± 0.009 0.117 ± 0.004 0.123 ± 0.018	[117]
	HeLa HEp-2 7T HCT 116 H460 MDA-MB-485	0.203 ± 0.025 0.180 ± 0.015 0.212 ± 0.020 0.180 ± 0.001 0.208 ± 0.018 0.329 ± 0.174	[117]
	HeLa A2780 A2780cisR HFL-1	7.42 ± 0.01 9.65 ± 0.01 12.95 ± 0.04 18.48 ± 0.02	[118]

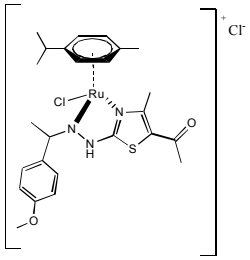
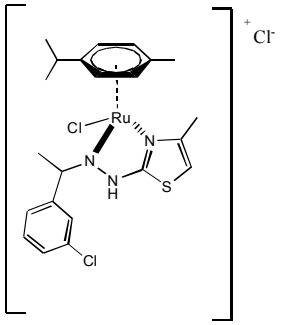
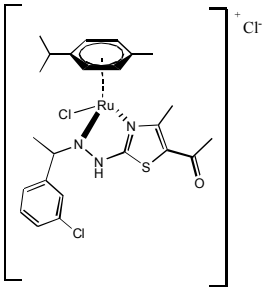
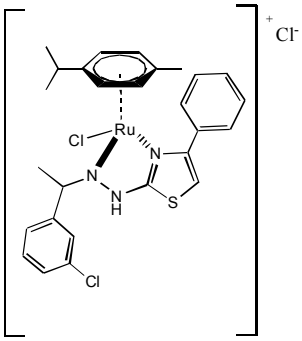
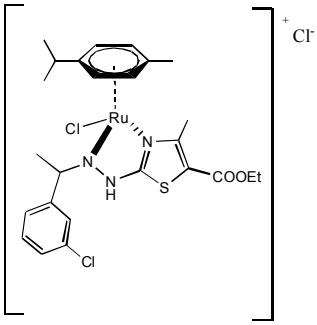
(Table 4) contd….

Structure	Cell line	IC_{50} (μM)	Reference
	HeLa A2780 A2780cisR HFL-1	2.55 ± 0.01 2.49 ± 0.02 0.93 ± 0.02 6.27 ± 0.01	[118]
	HeLa A2780 A2780cisR HFL-1	11.42 ± 0.02 6.99 ± 0.02 2.08 ± 0.01 6.43 ± 0.02	[118]
	HeLa A2780 A2780cisR HFL-1	3.65 ± 0.03 12.21 ± 0.03 12.62 ± 0.01 181 ± 0.89	[118]
	HeLa A2780 A2780cisR HFL-1	7.98 ± 0.01 50.08 ± 0.03 55.58 ± 0.03 47.33 ± 0.32	[118]
	HeLa A2780 A2780cisR HFL-1	0.84 ± 0.25 2.86 ± 0.01 6.06 ± 0.02 4.52 ± 0.01	[118]

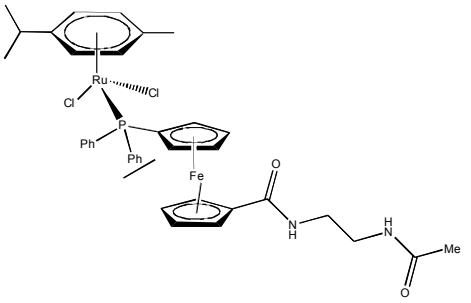
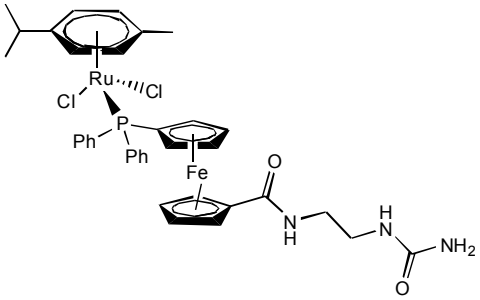
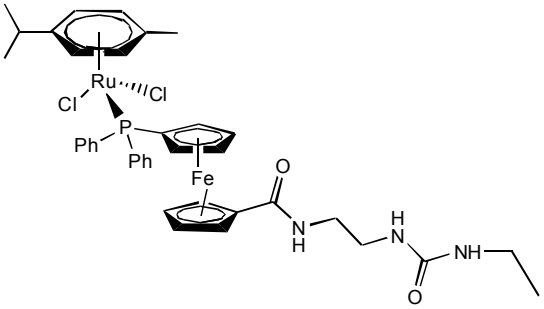
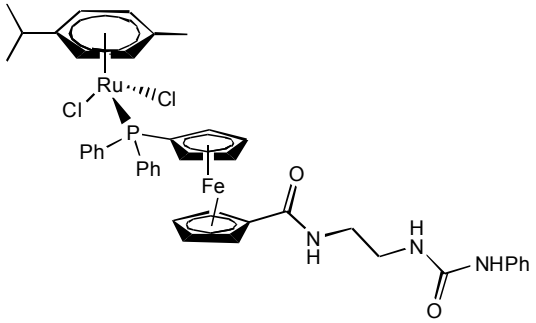
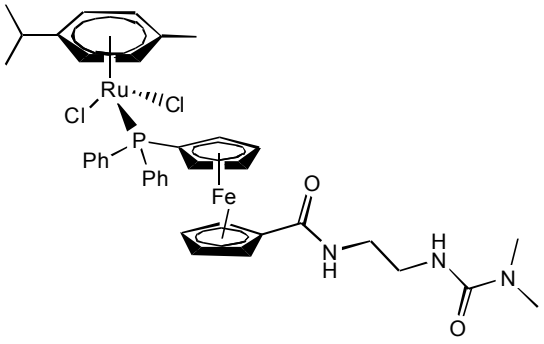
(Table 4) contd….

Structure	Cell line	IC_{50} (μM)	Reference
	HeLa A2780 A2780cisR HFL-1	19.40 ± 0.03 6.13 ± 0.25 6.19 ± 0.01 49.40 ± 0.31	[118]
	HeLa A2780 A2780cisR HFL-1	6.87 ± 0.23 5.25 ± 0.03 4.24 ± 0.01 7.30 ± 0.03	[118]
	HeLa A2780 A2780cisR HFL-1	7.95 ± 0.03 6.97 ± 0.33 6.80 ±0.05 6.47 ± 0.23	[118]
	HeLa A2780 A2780cisR HFL-1	2.26 ± 0.03 5.12 ± 0.03 1.33 ± 0.02 13.88 ± 0.03	[118]
	HeLa A2780 A2780cisR HFL-1	5.66 ± 0.01 9.47 ± 0.03 3.14 ± 0.01 4.27 ± 0.03	[118]

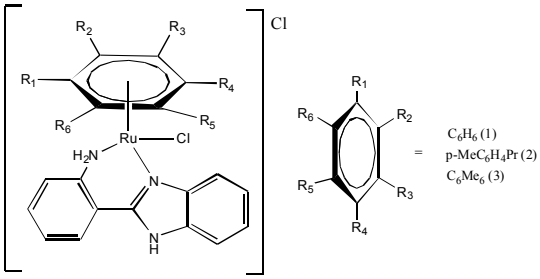
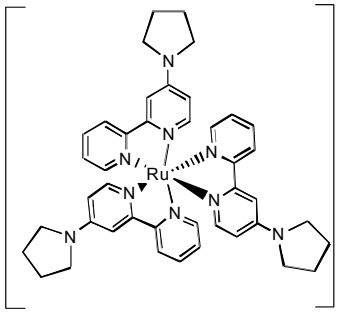
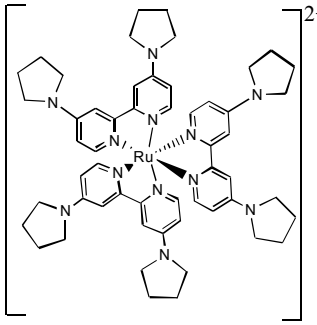
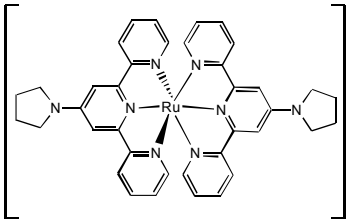
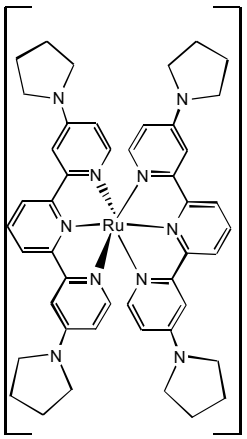
(Table 4) contd….

Structure	Cell line	IC_{50} (μM)	Reference
	HeLa A2780 A2780cisR HFL-1	7.88 ± 0.01 4.54 ± 0.01 2.25 ± 0.03 3.84 ± 0.01	[118]
	HeLa A2780 A2780cisR HFL-1	11.15 ± 0.01 6.13 ± 0.02 2.14 ± 0.04 6.32 ± 0.02	[118]
	HeLa A2780 A2780cisR HFL-1	2.69 ± 0.01 2.26 ± 0.03 2.14 ± 0.04 36.61 ± 0.48	[118]
	HeLa A2780 A2780cisR HFL-1	6.81 ± 0.03 7.70 ± 0.12 2.77 ± 0.01 13.69 ± 0.01	[118]
	HeLa A2780 A2780cisR HFL-1	1.72 ± 0.01 3.75 ± 0.02 3.76 ± 0.03 2.81 ± 0.03	[118]

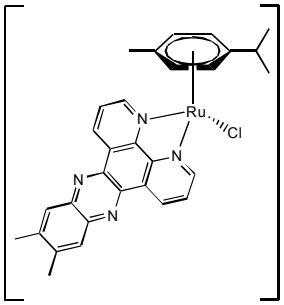
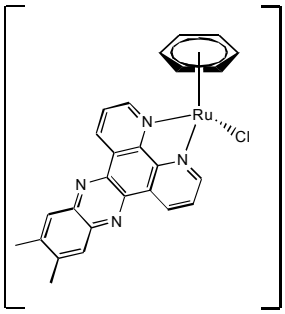
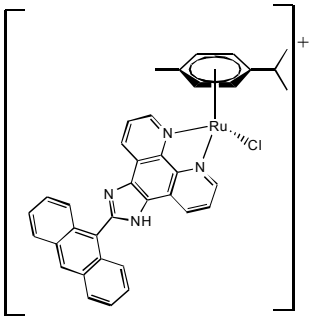
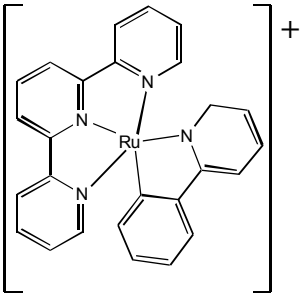
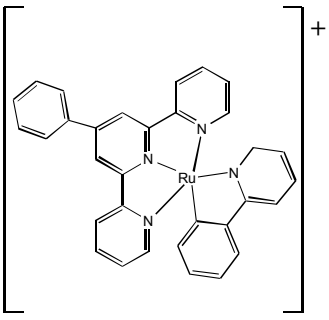
(Table 4) contd….

Structure	Cell line	IC_{50} (μM)	Reference
	A2780 A2780cisR HEK-293	113 ± 6 112 ± 16 232 ± 32	[119]
	A2780 A2780cisR HEK-293	195 ± 4 103 ± 10 247 ± 18	[119]
	A2780 A2780cisR HEK-293	132 ± 18 96 ± 6 90 ± 5	[119]
	A2780 A2780cisR HEK-293	19 ± 3 25 ± 1 20 ± 1	[119]
	A2780 A2780cisR HEK-293	69 ± 12 90 ± 7 61 ± 2	[119]

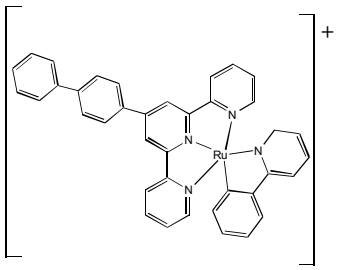
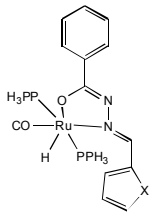
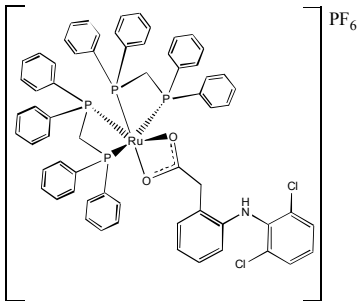
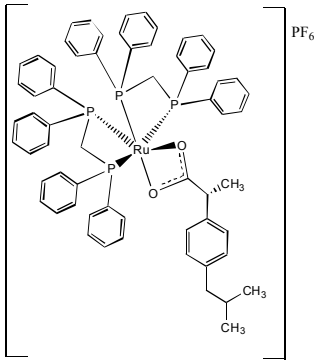
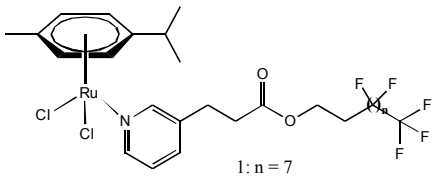
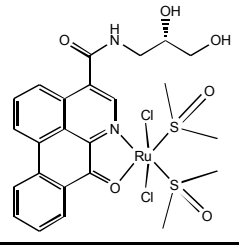
(Table 4) contd….

Structure	Cell line	IC_{50} (μM)	Reference
	SiHa	37.10 **(1)** 16.91 **(2)** 11.11 **(3)**	[120]
	CT26 A549	15.9 ± 2.4 21.0 ± 1.7	[121]
	CT26 A549	5.3 ± 1.1 3.8 ± 0.4	[121]
	CT26 A549	11.3 ± 2.0 13.4 ± 3.0	[121]
	CT26 A549	5.5 ± 0.90 6.5 ± 0.5	[121]

(Table 4) contd….

Structure	Cell line	IC_{50} (μM)	Reference
	A549 MDA-MB-231 HeLa MRC5	6.9 ± 2.9 0.7 ± 0.3 0.6 ± 0.5 0.4 ± 0.1	[122]
	A549 MDA-MB-231 HeLa MRC5	> 100 > 100 > 100 > 100	[122]
	A549 MDA-MB-231 HeLa MRC5	24.5 ± 0.1 3.7 ± 0.3 4.9 ± 0.1 3.2 ± 0.1	[122]
	HeLa Hep-G2 BEL-7402 A549 A549/CDDP LO-2	> 100 > 100 > 100 > 100 > 100 > 100	[123]
	HeLa Hep-G2 BEL-7402 A549 A549/CDDP LO-2	51.4 69.2 45.3 79.2 > 100 > 100	[123]

(Table 4) contd….

Structure	Cell line	IC_{50} (μM)	Reference
	HeLa Hep-G2 BEL-7402 A549 A549/CDDP LO-2	3.3 5.6 7.4 3.7 4.1 10.2	[123]
	HeLa MCF-7	21.0 ± 0.6 **(X=O)** 18.0 ± 0.5 **(X=S)** 6 ± 1 **(X=O)** 2 ± 1 **(X=S)**	[124]
	Hep-G2 MCF-7 MO59J GM07492A	7.6 ± 0.9 47 ± 6 8 ± 2 2 ± 1	[125]
	Hep-G2 MCF-7 MO59J GM07492A	5 ± 3 9 ± 3 6.5 ±0.2 6 ± 2	[125]
	SW480 LS174T	>200 **(1)** >200 **(2)** 41.4 ± 2.6 **(1)** >500 **(2)**	[126]
	BEL-7404 A549 MGC80-3 HeLa Hep-G2 BEL7402 HL-7702	7.4 ± 0.4 10.8 ± 0.2 10.4 ± 0.1 9.0 ± 0.6 16.2 ± 0.7 14.7 ± 0.3 69.2 ± 0.3	[127]

cancer, macular degeneration, *etc.* In the Fig. (**17**), the structure of porfirmer sodium (Photofrin®) is presented which is a Haematoporphyrin derivative, used in the treatment of bladder cancer and non-small cell lung carcinoma [132].

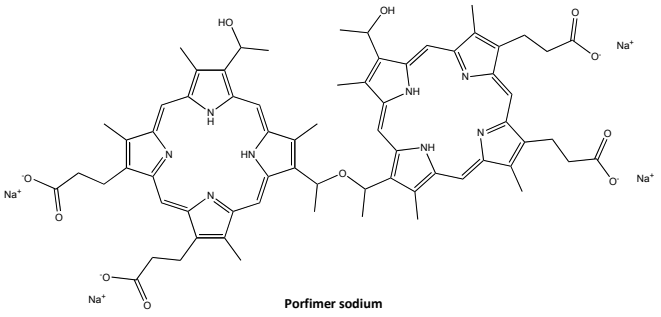


Fig. (17). Porfimer sodium, commercially known as Photofrin®. Drug currently approved by health organism for PDT.

As previously highlighted the ruthenium complexes have great potential in the medicinal chemistry as anticancer agents. Based on previously analysis, two approaches have been proposed in the development of the Ru related photosensitizer for PDT. The first one is the use of well-known porphyrin photosensitizers functionalized with ruthenium moieties (mostly organometallic) and the second one is to obtain ruthenium complexes that have photosensitizer behavior *per se* (polypyridyl complexes are the most studied).

3.5. Porphyrin Related PS

Schmitt *et al.* described that the addition of the Ru-arene moiety to tetrapyridylporphyrin ring increased the hydrophilicity and the uptake of the compounds in Me300 melanoma cell line. Besides, the effect on the cells was independent of the arene ligand and the photodynamic behavior was not affected by the incorporation of Ru (Fig. **18**) [133].

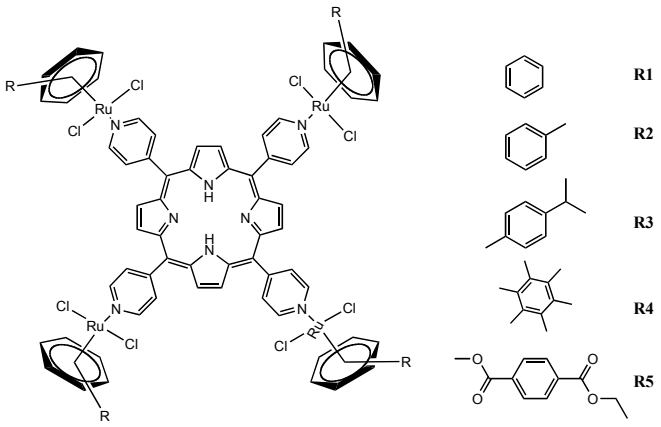


Fig. (18). Porphyrin substituted with Ru-arenes.

Later, Schmitt compared the effect of the arene ligand derivation in monopyridylporphyrin (mpp) and tetrapyridylporphyrin (tpp) compounds, and also studied the effect of the isomer used (3-pp and 4-pp) (Fig. **19**), in Me300 cell line. The cytotoxicity at 72h, without light stimulation revealed that monosubstituted porphyrins exerted a weak effect on cellular proliferation, while the tetrasubstituted analogues demonstrated an $IC_{50\ value}$ in the 10-20 μM range. This cytotoxic effect can be related to DNA synthesis inhibition. In relation to the photosensitizer behavior the isomer in the position 3 showed a better performance, since it needed low light radiation ($0.5J/cm^2$) and low concentrations (5μM) to decrease the cell viability significantly.In conclusion they propose a molecule tretracoordinated with Ru-arene in the position 3 of the tetrapyridylporphyrin moiety, as was proposed very interesting candidate for PDT[134].

Davia reported the synthesis of a compound porphyrin was functionalized in three positions with vainillin derivative, and the fourth position was coordinated with Ru- polypyridyl complexes. The results showed a high DNA binding rate for the compound and cell death at 10μM and activation through 60W tungsten bulb but importantly highlighted the lack of cytotoxic effects without light activation [135] (see Fig. **20**)

A Ru porphyrin (RuP) compound increased the cytotoxicity of AY27 cell line (rat bladder cancer) at 20 μM through necrosis pathway. However, there is not a big difference between dark toxicity and photoactivated toxicity with $15.6j/cm^2$ (17% and 14% cell viability compared to basal, respectively). Despite the generation of singlet oxygen the compound does not have good spectral properties to act as photosensitizer. Nevertheless, RuP has lower IC_{50} compared with cisplatin [136] (Fig. **21**).

Pernot *et al.* evaluated the anticancer *in vivo* effects of two arene ruthenium porphyrin compounds (Rut1 and Rut4, see Fig. **22**) on a mice model with ectopic human oral carcinoma xenograft. The results depicted that the presence of the Ru groups enhanced the photodynamic efficiency and Rut4 had a better performance. Associated with biodistribution high concentrations of Ru were found in the liver, kidney, spleen and plasma maybe due to the reticuloendothelial system elimination. Varying concentrations 1.45 and 1.50% of the administered dose were found in the tumor during 24 - 96h post injection. The subcellular distribution of the complex was found in the cytoplasm and not in the nucleus as expected for the Ru compounds [137].

The wide range of studies related to ruthenium chemistry found that some complexes have photo-

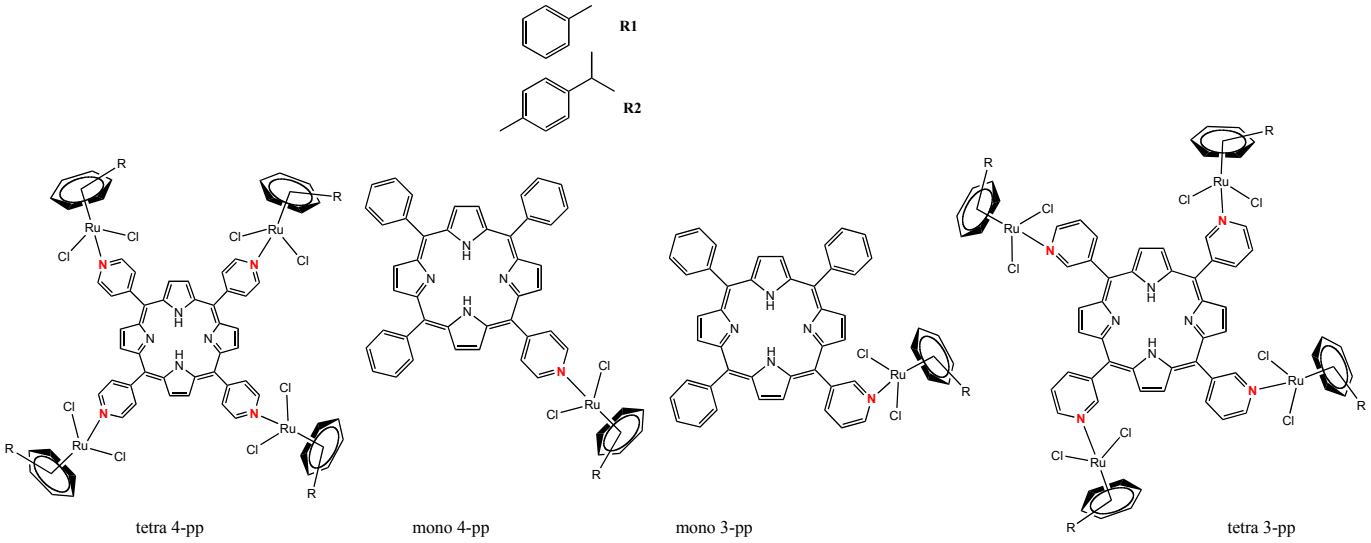


Fig. (19). Arene ligand derivation in monopyridylporphyrin (mpp) and tetrapyridylporphyrin (tpp) compounds.

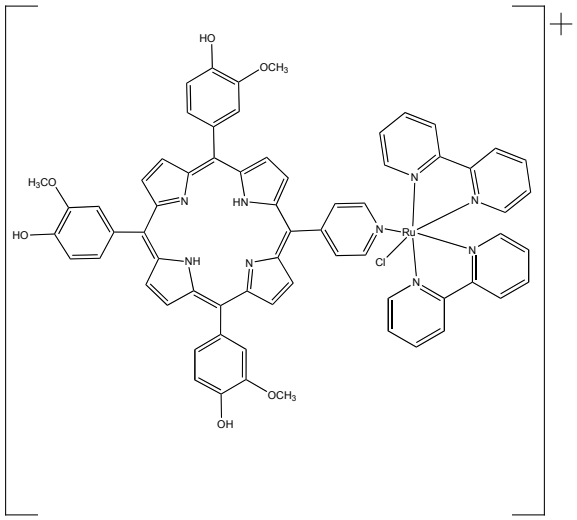


Fig. (20). Porphyrin functionalized with vanillin derivative and polypiridyl Ru complex.

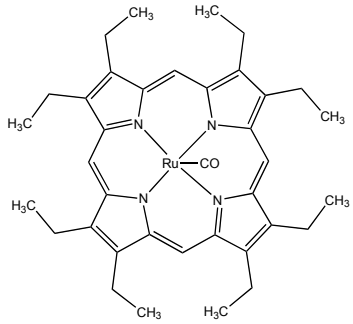


Fig. (21). Structure of Ru-Porphyrin.

physical properties which can be useful in PDT as photosensitizers. Different groups have been investigating with promising results.

Gasser and co-workers [138] reported a pool of six complexes of Ru(II), 2,2'-bipyridine and dipyrido[3,2-a:2',3'- c]phenazine (dppz). Substituents as NH_2, OMe, OAc, OH, CH_2OH and CH_2Cl were added on dppz moiety (Fig. **23**). The complexes showed a oxygen singlet quantum yield varying in the range of 50 - 94% and showed good stability in human plasma after 48h incubation. Cytotoxicity assays were carried out showing low effect on Hela (cervix cancer) and MRC-5 (normal phenotype) cell lines in dark (without light stimulation), however, when the cells were light-radiated (9.27 J/cm^2 at 420 nm), the IC_{50} value ranged between 2.0 and 20.5 μM, for the complexes 1,2,4 and 6. The complexes 1 and 2 induced a DNA photocleavage effect at 10, 30, and 50 μM concentration. This effect can be correlated with high binding affinity of the complexes by the DNA.

The design and synthesis of three highly charged ruthenium(II) polypyridyl complexes were reported by Chao, Gasser and co-workers. The complexes demonstrated high phototoxic effect on 2D and 3D Hela cells models. Complexes Ru1, Ru2 and Ru3 (Fig. **24**) had low dark toxicity with IC_{50} valu ranged between 300 - 400 μM in the monolayer and greater than 500 μM in the 3D model. After ethe photoactivation the IC_{50} values were 1.1, 2.2 and 2.9 μM in the monolayer system, and in the case of the spheroids for one photon PDT, the IC_{50} values were 10.1, 15.4, 21.2 μM and 1.9, 5.4 and 7.2 μM for two photon PDT. Besides, the results showed that the complexes were up taken *via* endocytosis and suffer lysosomal accumulation [139].

Glazer *et al.* reported the synthesis of two ruthenium complexes with bathophenanthroline and batho

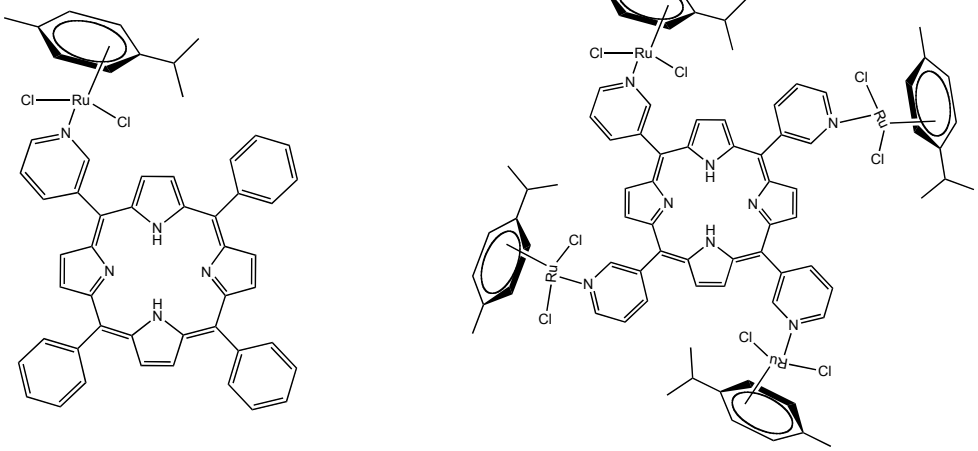


Fig. (22). Rut1 and Rut4 structures.

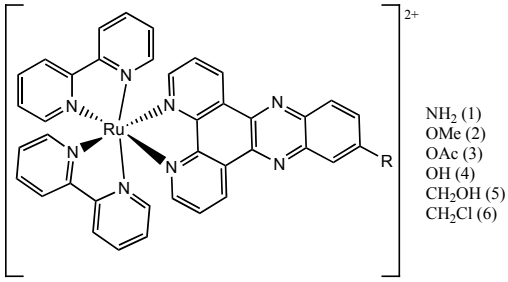


Fig. (23). Complexes of Ru(II) and 2,2'-bipyridine and dipyrido[3,2-a:2',3'- c]phenazine (dppz).

phenanthroline disulfonate (Fig. **25**) and the effects of these complexes on jurkat, HL60 and A549 cell lines. The compound with bathophenantroline induced dark cytotoxicity (IC_{50} ranged 0.62 to 3.15 μM) for three cell lines and even lower after irradiation with visible light. This complex was located at mitochondrial level, causing an impairment in the mitochondrial function leading to death mediated by necrosis.

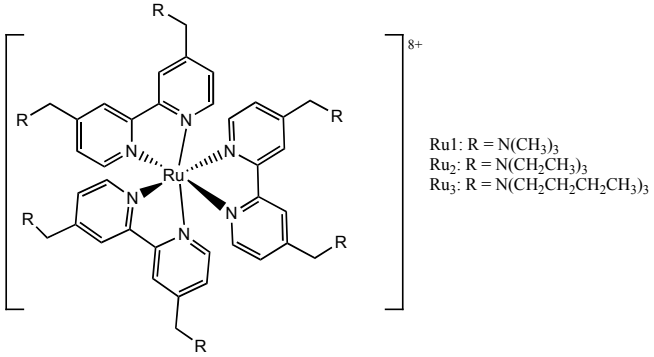


Fig. (24). Ru1, Ru2 and Ru3: two photon activated photosensitizer.

The sulfonated analogue had a very low dark toxicity (IC_{50} greater than 300 μM) while the photoactivation reduced the IC_{50} value to a range of 3.3 to 17.3 μM. The main mechanism of action observed after light stimulation was apoptosis. In addition, both complexes exerted photocleavage of the DNA [140].

On the other hand, Fig. (**26**) shows the summary of the main mechanism of action involved in the antitumor properties of Ru compounds using in the PDT therapies.

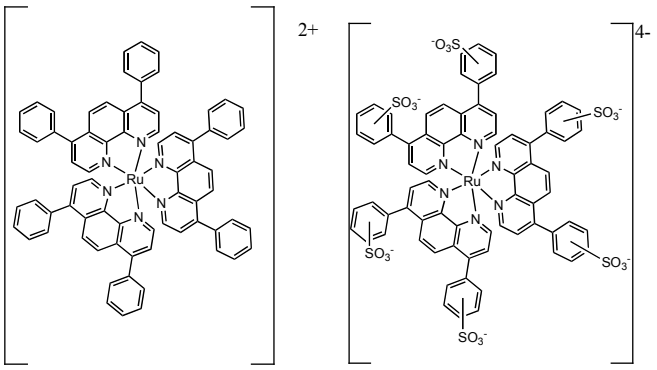


Fig. (25). Ru-bathophenanthroline and Ru-bathophenanthroline disulfonate complexes.

4. COPPER COMPLEXES

Copper is an essential element associated with biological pathways since it acts as cofactor in most aerobic organisms [141]. Copper-based complexes have been studied because it has been shown that endogenous metals are less toxic for normal cells than for cancer cells. Moreover, normal and tumor cells respond differently to copper and this is the major development of copper complexes having antiproliferative characteristics.

Even though copper level in the human organism is strictly controlled, high serum and tissue levels of Cu were found in many types of human cancers including

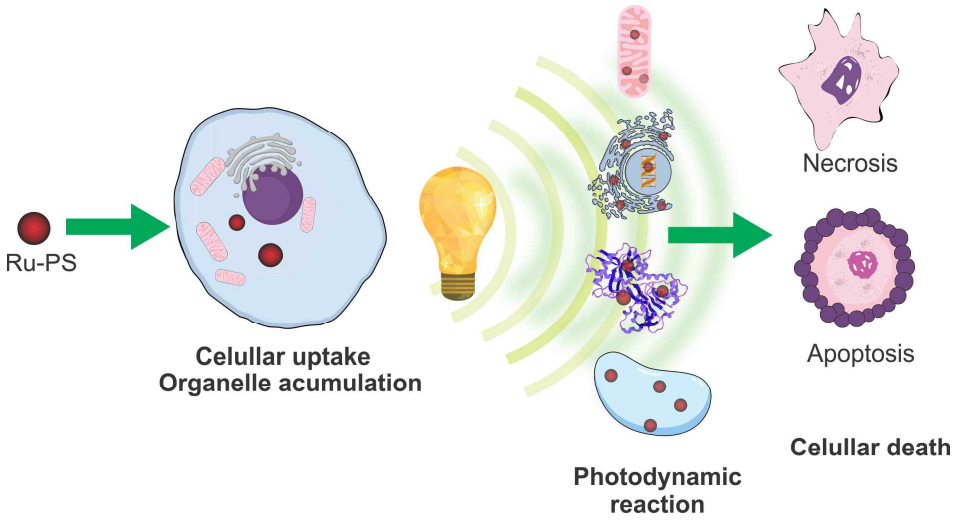


Fig. (26). Ruthenium compounds in the PDT anticancer therapies.

breast [142], prostate [143], colon [144], lung [145] and brain cancer [146]. The reasons for this phenomenon are connected to the important function that copper plays in angiogenesis [147], which is essential for growth, invasion and metastasis [148]. Therefore, it has been claimed that copper chelators may have high therapeutic value acting as antiangiogenic molecules [149].

Several antitumor studies have postulated copper complexes as anticancer or anticarcinogenic agents in *in vitro* and *in vivo* systems [150-152]. In particular, it has been demonstrated that copper thiosemicarbazones have antitumor activity [153] and developments in this area are still in progress [154,155]. In fact, novel copper thiosemicarbazone complexes showed cell growth inhibition, apoptosis induction and cell cycle arrest on cisplatin-resistant neuroblastoma cells [155].

It has been shown that a dimeric copper complex of the unsubstituted pyridoxal thiosemicarbazone induces apoptosis and arrest the cell cycle significantly on two human lymphocytes (CEM and U937 cell lines), but it is inactive on K562 human lymphoblasts *in vitro* and on TLX5 lymphoma *in vivo*. However, copper complexes with N-substituted pyridoxal thiosemicarbazones did not produce inhibition on cell proliferation on the aforementioned cell lines [156].

Isoflavones are a type of naturally occurring isoflavonoids which act as antioxidants because of their ability to trap singlet oxygen showing antitumor activity [157,158]. Copper(II) complexes showed moderate cytotoxicity against five human cancer cell lines, with IC_{50} values in the 10−50 μM range [159] pointing out a benefit of the complexation of these ligands, since the complexes showed a stronger inhibitory effects than free isoflavones or metal ions. Moreover, complexes provoked a significant G(2)/M phase arrest, which developed early apoptosis.

Hesperetin and apigenin are biologically active flavonoids which have been investigated as antitumor drugs against breast cancer and hepatoma cell lines [160]. Copper complexes with hesperetin and apigenin showed growth inhibition of SGC-7901 and HepG2 cell lines in comparison to the free ligands [161].

In a recent study, it was demonstrated that a Cu(II) complex with quercetin can cleavage plasmid DNA generating single and double DNA strand breaks, intercalate the DNA strand and increase the level of reactive oxygen species which produce oxidative DNA damage. Moreover, induction of apoptosis has been observed along with a decrease in the levels of survivin protein expression and increase in caspase-3 on A549 cells [162].

This study previously reported the biological actions of a copper(II) complex with santonic acid. The compound produced a cytotoxic effect on four cell lines (MC3T3-E1, UMR-106, Caco-2, TC7) displaying alteration in the nuclei and cytoplasm of treated cells [163].

Moreover, the lowering blood pressure drug valsartan coordinated with copper(II) through its carboxylate group giving rise to a binuclear compound, which showed antiproliferative activity toward two selected cell lines (normal MC3T3-E1 and tumor UMR-106) in the micromolar range, whereas valsartan itself had no effect up to 100 mM [164] (see Fig. **27** for a schematic representation of the complex).

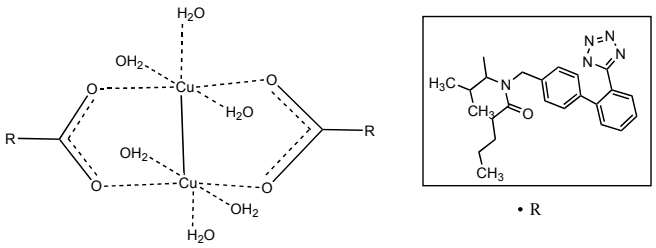


Fig. (27). Schematic representation of structure of copper(II) complex with valsartan.

Copper complexes have been demonstrated to show potential as cytotoxic drugs in *in vitro* assays, however, there is little information on the cellular mechanisms of action or the molecular targets implicated (see Fig. **28** for a schematic summary).

Previously, it has been shown that copper binds to DNA and interacts with DNA in a non-covalent way, through intercalation, electrostatic forces, and groove binding [6].

An example of the interaction of planar polycyclic containing copper compounds with DNA was shown for Casiopeinas ™. These mixed-chelate complexes carrying aromatic substituted diimine (phen or bipy and substituted analogs) showed antineoplastic properties (Fig. **29**) [165,166].

The authors described that Casiopeinas stacked between DNA bases produced a DNA breakup detected in either pUC19 plasmid or in HeLa cells [167,168]. Moreover, it has been demonstrated that only planar and unsubstituted ligands could intercalate into DNA

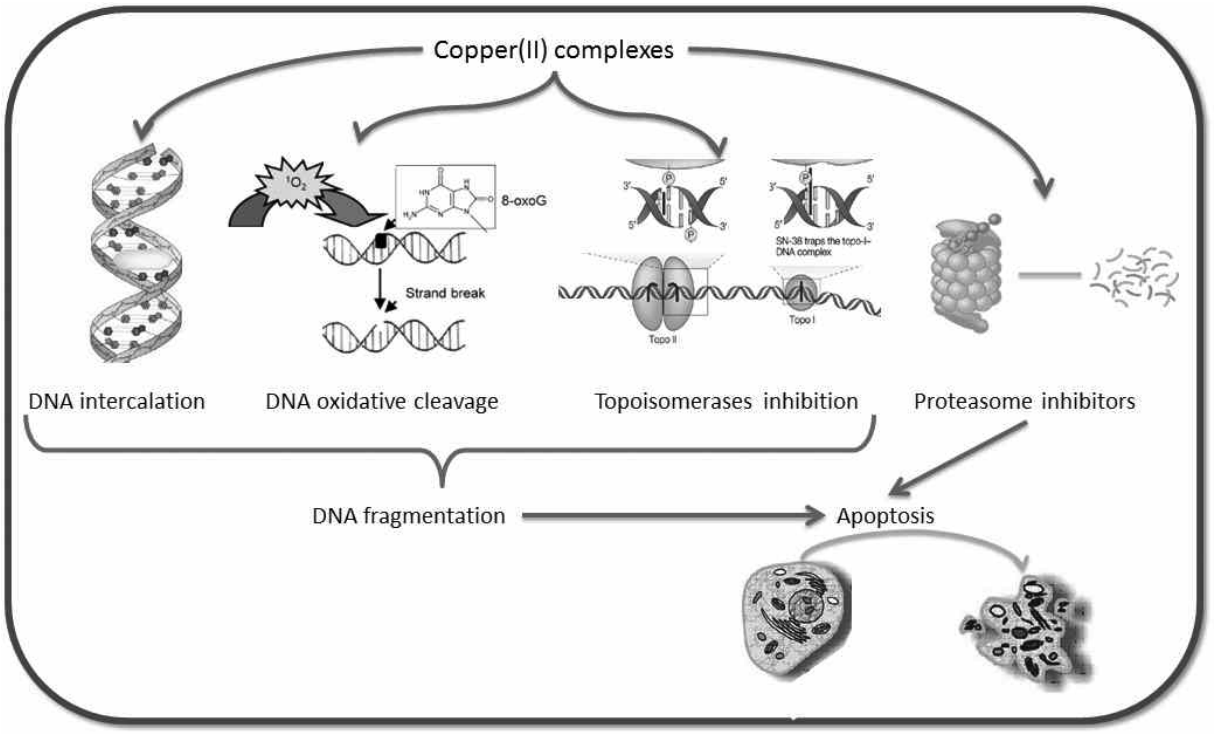
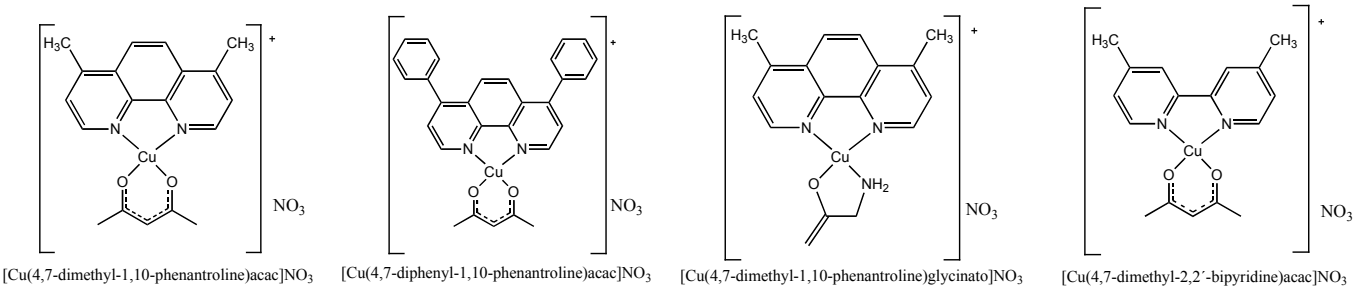


Fig. (28). Schematic representation of molecular targets involved in antitumor activity of copper complexes.



[Cu(4,7-dimethyl-1,10-phenantroline)acac]NO_3 [Cu(4,7-diphenyl-1,10-phenantroline)acac]NO_3 [Cu(4,7-dimethyl-1,10-phenantroline)glycinato]NO_3 [Cu(4,7-dimethyl-2,2′-bipyridine)acac]NO_3

Fig. (29). Schematic structures of CASIOPEINAS™ compounds.

[169-171] whereas nonplanar aromatic heterocyclic ligands did not show intercalation properties [172,173]. That is why physicochemical properties, such as the planarity play important roles in the mechanism of interaction with DNA [6]. On the other hand, Cu(II) complexes have been demonstrated to produce DNA oxidative cleavage due to their redox properties. Many copper complexes generated plasmid DNA cleavage through an oxidative pathway [170,174,175]. This phenomenon was observed through a Fenton-type reaction, where hydroxyl radicals [176] or different metal-based radicals were formed, producing oxidation and the breakup of DNA [177]. After DNA injury, many copper complexes trigger apoptotic cell death. Synthesis of proapoptotic or antiapoptotic proteins is deregulated as it was described for $Cu(N_9\text{-ABS})(phen)_2$] ($H_2N_9$-ABS = N-(9H-purin-6-yl)benzenesulfonamide) which induced a rise in p53 protein and a reduction of Bcl-2 expression in Caco-2 cells [178]. On the other hand, recent research has demonstrated that the inhibition of topoisomerases is another mechanism of action of copper(II) compounds. These enzymes regulate the overwinding or underwinding of DNA generating a transient DNA break which allows the DNA to be untangled [179].

Topoisomerase inhibitors have been postulated as important drugs for cancer chemotherapy treatments. A copper(II) complex of 2-furaldehyde oxime was found to injure topoisomerase activity by blocking the phosphorylation activation [180]. More recently, a copper salicylaldoxime, which effectively inhibited the L1210 leukemia cells, restrained topoisomerase dimmer formation with consequent induction of single-strand breaks in the DNA. A series of novel copper(II) complexes with quinolinone Schiff bases induced cytotoxicity and inhibition of DNA synthesis in HepG2 cells in a concentration and time-dependent fashion due to their topoisomerase inhibitory potential [181].

Copper complexes containing the planar heterocyclic phenanthroline showed that they can bind selectively to DNA by intercalation and electrostatic forces, and inhibit topoisomerase I, leading to apoptotic cell death in nasopharyngeal cancer cell line HK1 but not on normal NP69 cells [182].

Proteasome inhibition is another mechanism by which copper complexes exert their antitumor activity. The proteasome is a large multiprotein complex located in both the nucleus and the cytoplasm. It degrades wanted or damaged proteins which are tagged with ubiquitin molecules. The polyubiquitin chain is recognized by the proteasome, allowing it to degrade the tagged protein The proteasome is part of a major mechanism by which cells regulate many cellular functions, such as proliferation, apoptosis, angiogenesis, and metastasis formation [183]. An excellent anticancer strategy is targeting the ubiquitin−proteasome pathway, since cancer cells are more vulnerable to proteasome inhibition than normal cells [184], and currently, development of proteasome inhibitors is under exhaustive investigation. Several copper compounds have been investigated to selectively inhibit the proteasome *in vitro* and *in vivo* in human leukemia cells, however proteasome is not inhibited in non-transformed human natural killer cells under the same treatment [185]. Moreover, it has been established that certain types of organic ligands could bind to copper found inside the tumor cells and form proteasome inhibitors that lead to apoptosis [1]. Copper(II) complexes with asymmetric ligands containing the methylpyridin-amino-methylphenol moiety have been shown to inhibit the proteasome *in vitro* and *in vivo* [186]. These compounds were studied in prostate cancer cells resulting in an induction of apoptosis associated with a significant proteasome inhibition. It has been suggested that these copper complexes can cross over the cell membrane transporting the copper ion inside the cell [187]. It has been observed that ternary complexes binding with 1,10-phenanthroline reduce proteasome activity before induction of apoptosis in MDA-MB-231 human breast cancer cells, as well as in PC- 3 human prostate cancer cells [188]. The hypothesis is based on the fact that phenanthroline transports copper into the cancer cell, leading to direct proteasome inhibition or oxidation of the proteasome.

5. NEW PERSPECTIVES: DRUG DELIVERY SYSTEMS FOR ADMINISTERING ANTICANCER RU, V, CU BASED DRUGS

Certain pharmacological issues of drugs such as bioavailability, site specific targeting, degradation and solubility may be improved by the application of different drug delivery systems. Nanotechnology is expected to address some of the most important problems of conventional anticancer chemotherapy. Nanodrugs are promissory in cancer chemotherapy because of their selective access to cancer cells and tissues [189]. Low efficiency of transport processes of active species to biological targets has also improved because nanoestructures have been designed and developed. They act as vectors for metallodrug or simply as protectors of active species of the complexes for amplifying their activities and reducing their degradation [190, 191].

Numerous molecules and macromolecules have been employed as drug delivery systems for metallo-

drugs such as cyclodextrins, liposomes, lipid nanocapsules, proteins, carbon nanotubes, polymeric nanoparticles and ceramic materials [192].

Actually, copper complexes are being stabilized into nano-formulations for improving their biological activity. Copper complexes containing inorganic ligands ($Cu(NH_4)_2Cl_4$ and $CuCl_2$) were loaded on a functionalized titania to obtain drug delivery systems. They were tested as toxic agents on different cancer cell lines, as well as on cisplatin as a reference material. It was found that independently of the copper complex and also the cell line used, low concentrations of each copper compound were sufficient to kill almost 100 % of cancer cells. Both copper complexes alone as well as those loaded on TiO2 had higher toxic effect than cisplatin [193].

Moreover, it has been shown that multivalent copper(II)-conjugated phosphorus dendrimers and their mononuclear copper(II) complexes exerted potential antiproliferative properties against HCT116, MCF-7, OVCAR8 and U87 cell lines [194]. A direct association was found between the number of terminal moieties or the amount of copper complexed to the dendrimer and the growth inhibitory effect. These results demonstrated that the number of terminal moieties and Cu atoms increased cytotoxicity. Therefore, burdening copper complexes onto nanoestructures improved the anticancer properties. However, there have only been a few reports on the nano-functionalization of anticancer copper complexes so far, which strongly promoted the investigation of different copper complexes as anticancer agents in nano-formulations.

On the other hand, molecular vectors were designed to transport ruthenium complexes to cells with an additional protection against physiological degradation during long circulation times. Since despite their high effectiveness, they are poorly stable in physiological conditions and are present short half-life times in aqueous media [192]. Three amphiphilic nucleoside-based ruthenium complexes, ToThyRu, HoThyRu and DoHuRu were combined with a new organometallic ruthenium complex, named AziRu and stabilized in POPC phospholipid formulations, which were stable for months and showed high *in vitro* antiproliferative activity [195]. Another efficient drug delivery strategy, warranting high ruthenium content within the formulation, presented long half-life in physiological media and enhanced cell uptake. Amphiphilic ruthenium anionic complexes, based on the formation of stable nanoparticles with the cationic lipid 1,2-dioleyl-3-trimethylammoniumpropane chloride (DOTAP) showed IC_{50} values in the low μM range against MCF-7 and WiDr cells, thus, proving to be 10-20-fold more active than AziRu with a marked tendency to accumulate within, or in proximity of the nuclei (see Fig. **30** for a schematic representation) [196]. Moreover, Valente and co-workers [197] reported the synthesis of a D-glucose end-capped polylactide ruthenium cyclopenta-

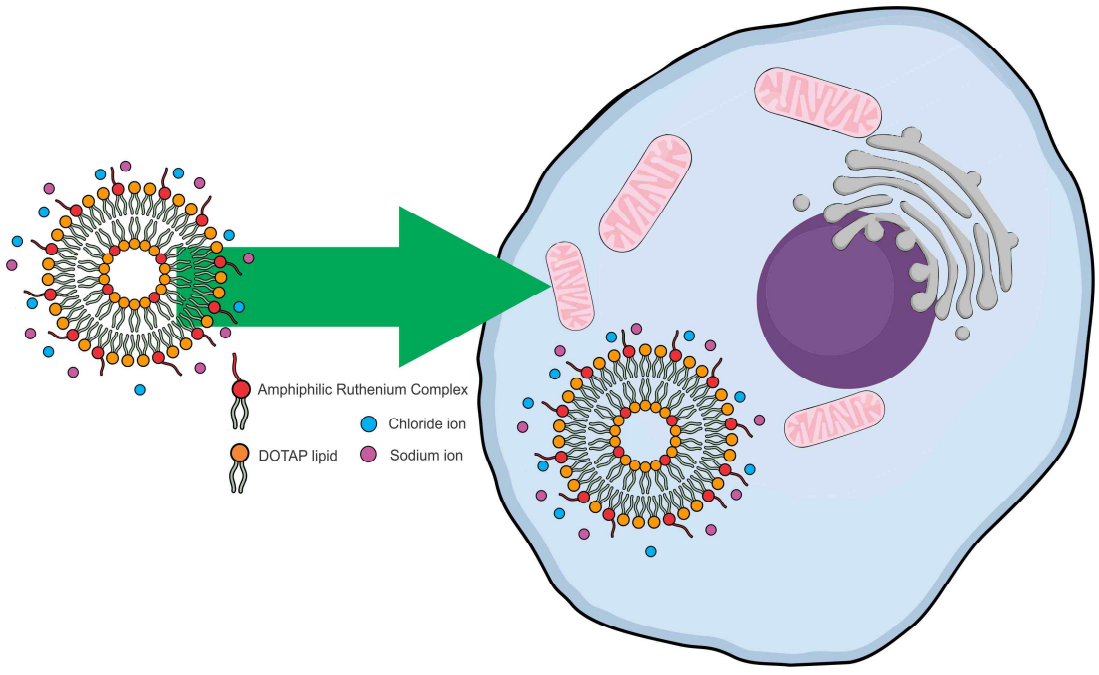
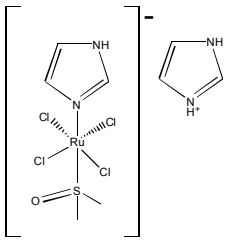
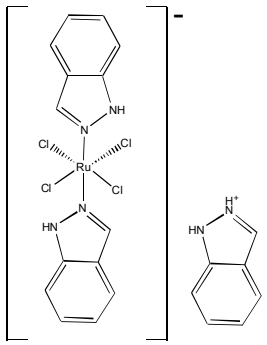
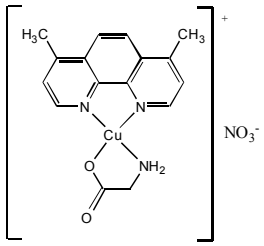
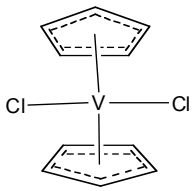
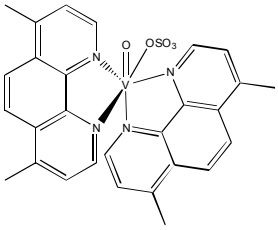


Fig. (30). Schematic graphic of accumulation whitin nuclei of amphiphilic ruthenium anionic complexes, based on the formation of stable nanoparticles with the cationic lipid 1,2-dioleyl-3-trimethylammoniumpropane chloride (DOTAP).

Table 5. The most prominent V, Cu and Ru compounds as antitumor agents.

Name	Structure	Biological data	References
Metal complexes under clinical investigation			
NAMI-A	 NAMI-A	In a phase I study it was concluded that NAMI-A can be safely administered as a three hour i.v. infusion at a dose of 300 mg/m2/day for five days, every three weeks. In this study, one patient (total: 24 patients) with non-small cell lung cancer showed stable disease.	[98]
KP1019	 KP1019	KP1019 showed promising results in a phase I clinical study. Five of six evaluable patients with solid tumours experienced stable disease. Only mild toxicities were observed and further investigation in phase II was suggested. In contrast to NAMI-A, KP-1019 was also active at the primary tumour site	[93, 95]
Casiopeína IIgly	 [Cu(4,7 -dimethyl-1,10-phenanthroline)glycinate]NO_3	CasII-gly prevents malignant cells to continue with their life cycle, by inhibiting estrogen-mediated G1/S cell cycle progression. The compound inhibited uncontrolled cell migration of cancer cells. Currently, *CasII-gly* is in the phase I clinical trials.	[165, 166]
Metal complexes under preclinical investigation			
$[VCp_2Cl_2]$		This metallodrug exhibiting a higher in vitro activity on several such us leukemias P388 and L1210, colon 38 and Lewis lung carcinomas, B16 melanoma, among others. In addition, in vivo studies revealed that vanadocene compounds show significant antitumor properties in MOLT-4 leukemia and human peripheral blood mononuclear cells	[33]
Metvan		METVAN induces apoptosis in human leukaemia cells, multiple myeloma cells and solid tumour cells derived from breast cancer, glioblastoma, ovarian, prostate and testicular cancer patients	[40]

dienyl complex (RuPMC), which displayed IC50 values in the micromolar range against human MCF7 and MDA-MB-231 breast and A2780 ovarian adenocarcinoma. Cellular distribution studies also showed that RuPMC was predominantly found in the nucleus and in the membrane. Encapsulation and delivery of cytotoxic ruthenium complexes within nanostructures have shown an advance in the enhance permeability and retention effect, along with improvements in the cytotoxic effects, decrease in the harmfulness and eva-sion of complex structure degradation. However, further studies still have to be performed for developing novel mechanisms of drug delivery structures.

Different drug delivery systems were described for vanadium compounds. Vanadocene dichloride could bind to transferrin and enhanced its anticancer activity in proliferating malignant cells that over-express transferrin receptors, indicating that transferrin plays an important role in the transport and targeted delivery of vanadocene dichloride into cancer cells [198]. An alternative delivery system for a complex of vanadium (IV) with aspirin (VOAspi) was developed using poly(beta-propiolactone) (PbetaPL) films by one of. UMR106 osteosarcoma cells were used as a model to evaluate the anticarcinogenic effects of the VOAspi released from the PbetaPPL film. VOAspi-PbetaPL film inhibited cell proliferation in a dose-response manner and induced formation of approximately half of the thiobarbituric acid reactive substances, and an index of lipid peroxidation compared to that with free VOAspi in solution [199].

CONCLUSION

The tremendous success of cisplatin, carboplatin and oxaliplatin in cancer chemotherapy provoked a great impulse of research in Inorganic Medicinal Chemistry. Scientific efforts have been specially devoted to solve problems such as side effects, and resistance of platinum therapy. Intensive research has been done to design and develop novel non platinum-based metal anti-cancer drugs (Table **5**). Different promising results have been reported in preclinical research for vanadium, ruthenium and copper complexes and several of these metal based compounds have been already investigated in clinical trials. In the preclinical stage, the main mechanisms of action involved with the antitumor properties of V, Ru and Cu compounds are: ROS generation, GSH/GSSG imbalance, cell cycle arrest, DNA fragmentation and apoptosis. Nevertheless, several compounds have attracted attention based on modes of action different from that of cisplatin (covalent binding to the DNA) whilst some metallocompounds interact with other specific molecular targets different from DNA (such as tyrosinkinases, proteasome, p53, etc). This research revealed new leadeing compounds for cancer therapy with well defined structure-activity relationships. In this order, the search of novel cellular targets including new inorganic and organic metal complexes is of great significance. Clinical research trials focused on copper and ruthenium compounds showed that the copper compound Cas-GlyII was entered in phase I due to the interesting antitumor profile found in pre-clinical studies. Besides, the ruthenium agent NAMI-A acts by its antimetastasis properties rather than by conventional cell growth inhibitory effects. In addition, KP1019 in contrast to NAMI-A activated the primary tumour site.

Currently, both Ruthenium complexes have completed phase II of clinical trials. Actually, clinical studies on vanadium, ruthenium and copper based drugs are ongoing and they will bring new candidates for modern chemotherapy in the next few years.

Besides, this research in this field tried to overcome the problems existing between the therapeutic action and the adverse side effects of V, Ru and Cu compounds. The balance towards the beneficial side is the crucial issue for the future use of these compounds as alternative therapies against cancer.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

The authors acknowledge the support from UNLP (11X/690), CONICET (PIP 1125) and ANPCyT (PICT 2014-2223). JFCV has a fellowship from CONICET, Argentina. ALDV, IEL and SBE are members of the Carrera del Investigador CONICET, Argentina.

REFERENCES

[1] Frezza, M.; Hindo, S.; Chen, D.; Davenport, A.; Schmitt, S.; Tomco, D.; Dou, Q.P. Novel metals and metal complexes as platforms for cancer therapy. *Curr. Pharm. Des.,* **2010**, *16*(16), 1813-1825.

[2] Orvig, C.; Abrams, M.J. Medicinal inorganic chemistry: introduction. *Chem. Rev.,* **1999**, *99*(9), 2201-2204.

[3] Thompson, K.H.; Orvig, C.; Sadler, P.J.; Abrams, M.J.; Murrer, B.A.; Farrell, N.; Thompson, K.H.; Bull, S.R. Metal complexes in medicinal chemistry: new vistas and challenges in drug design. *Dalton Trans.,* **2006**, *36*(6), 761-764.

[4] Evangelou, A.M. Vanadium in cancer treatment. *Crit. Rev. Oncol. Hematol.,* **2002**, *42*(3), 249-265.

[5] Bruijnincx, P.C.; Sadler, P.J. New trends for metal complexes with anticancer activity. *Curr. Opin. Chem. Biol.,* **2008**, *12*(2), 197-206.
[6] Santini, C.; Pellei, M.; Gandin, V.; Porchia, M.; Tisato, F.; Marzano, C. Advances in copper complexes as anticancer agents. *Chem. Rev.,* **2014**, *114*(1), 815-862.
[7] Hambley, T.W., Developing new metal-based therapeutics: challenges and opportunities. *Dalton Trans.,* **2007**, 4929-37.
[8] Jung, Y.; Lippard, S.J. Direct cellular responses to platinum-induced DNA damage. *Chem. Rev.,* **2007**, *107*(5), 1387-1407.
[9] Koepf-Maier, P.; Koepf, H. Non-platinum group metal antitumor agents. History, current status, and perspectives. *Chem. Rev.,* **1987**, *87*, 1137-1152.
[10] Ott, I.; Gust, R. Non platinum metal complexes as anticancer drugs. *Arch. Pharm. (Weinheim),* **2007**, *340*(3), 117-126.
[11] van Zutphen, S.; Reedijk, J. Targeting platinum anti-tumour drugs: Overview of strategies employed to reduce systemic toxicity. *Coord. Chem. Rev.,* **2005**, *249*, 2845-2853.
[12] Klein, C.A. Cancer. The metastasis cascade. *Science,* **2008**, *321*(5897), 1785-1787.
[13] Hanahan, D.; Weinberg, R.A. Hallmarks of cancer: the next generation. *Cell,* **2011**, *144*(5), 646-674.
[14] Korbecki, J.; Baranowska-Bosiacka, I.; Gutowska, I.; Chlubek, D. Biochemical and medical importance of vanadium compounds. *Acta Biochim. Pol.,* **2012**, *59*(2), 195-200.
[15] Navara, C.S.; Benyumov, A.; Vassilev, A.; Narla, R.K.; Ghosh, P.; Uckun, F.M. Vanadocenes as potent antiproliferative agents disrupting mitotic spindle formation in cancer cells. *Anticancer Drugs,* **2001**, *12*(4), 369-376.
[16] Rehder, D. *Vanadium*; Its Role for Humans, **2013**.
[17] Bishayee, A.; Waghray, A.; Patel, M.A.; Chatterjee, M. Vanadium in the detection, prevention and treatment of cancer: the in vivo evidence. *Cancer Lett.,* **2010**, *294*(1), 1-12.
[18] Sava, G.; Bergamo, A. Ruthenium-based compounds and tumour growth control (review). *Int. J. Oncol.,* **2000**, *17*(2), 353-365.
[19] Kostova, I. Ruthenium complexes as anticancer agents. *Curr. Med. Chem.,* **2006**, *13*(9), 1085-1107.
[20] Webb, M.I.; Walsby, C.J. Albumin binding and ligand-exchange processes of the Ru(III) anticancer agent NAMI-A and its bis-DMSO analogue determined by ENDOR spectroscopy. *Dalton Trans.,* **2015**, *44*(40), 17482-17493.
[21] Musumeci, D.; Rozza, L.; Merlino, A.; Paduano, L.; Marzo, T.; Massai, L.; Messori, L.; Montesarchio, D. Interaction of anticancer Ru(III) complexes with single stranded and duplex DNA model systems. *Dalton Trans.,* **2015**, *44*(31), 13914-13925.
[22] Wang, J.-Q.; Zhang, P.-Y.; Qian, C.; Hou, X.-J.; Ji, L.-N.; Chao, H. Mitochondria are the primary target in the induction of apoptosis by chiral ruthenium(II) polypyridyl complexes in cancer cells. *J. Biol. Inorg. Chem.,* **2014**, *19*(3), 335-348.
[23] Tisato, F.; Marzano, C.; Porchia, M.; Pellei, M.; Santini, C. Copper in diseases and treatments, and copper-based anticancer strategies. *Med. Res. Rev.,* **2010**, *30*(4), 708-749.
[24] Korfel, A.; Scheulen, M.E.; Schmoll, H.J.; Gründel, O.; Harstrick, A.; Knoche, M.; Fels, L.M.; Skorzec, M.; Bach, F.; Baumgart, J.; Sass, G.; Seeber, S.; Thiel, E.; Berdel, W.E. Phase I clinical and pharmacokinetic study of titanocene dichloride in adults with advanced solid tumors. *Clin. Cancer Res.,* **1998**, *4*(11), 2701-2708.
[25] Lümmen, G.; Sperling, H.; Luboldt, H.; Otto, T.; Rübben, H. Phase II trial of titanocene dichloride in advanced renal-cell carcinoma. *Cancer Chemother. Pharmacol.,* **1998**, *42*(5), 415-417.
[26] Cuffe, S.; Dowling, C.M.; Claffey, J.; Pampillón, C.; Hogan, M.; Fitzpatrick, J.M.; Carty, M.P.; Tacke, M.; Watson, R.W. Effects of titanocene dichloride derivatives on prostate cancer cells, specifically DNA damage-induced apoptosis. *Prostate,* **2011**, *71*(2), 111-124.
[27] Gómez-Ruiz, S.; Kaluderović, G.N.; Prashar, S.; Polo-Cerón, D.; Fajardo, M.; Zizak, Z.; Sabo, T.J.; Juranić, Z.D. Cytotoxic studies of substituted titanocene and ansa-titanocene anticancer drugs. *J. Inorg. Biochem.,* **2008**, *102*(8), 1558-1570.
[28] Olszewski, U.; Deally, A.; Tacke, M.; Hamilton, G. Alterations of phosphoproteins in NCI-H526 small cell lung cancer cells involved in cytotoxicity of cisplatin and titanocene Y. *Neoplasia,* **2012**, *14*(9), 813-822.
[29] Oberschmidt, O.; Hanauske, A.R.; Pampillón, C.; Sweeney, N.J.; Strohfeldt, K.; Tacke, M. Antiproliferative activity of Titanocene Y against tumor colony-forming units. *Anticancer Drugs,* **2007**, *18*(3), 317-321.
[30] Kater, L.; Claffey, J.; Hogan, M.; Jesse, P.; Kater, B.; Strauss, S.; Tacke, M.; Prokop, A. The role of the intrinsic FAS pathway in Titanocene Y apoptosis: The mechanism of overcoming multiple drug resistance in malignant leukemia cells. *Toxicol. In Vitro,* **2012**, *26*(1), 119-124.
[31] Vinklárek, J.; Pavlík, I.; Cernosek, Z. Behaviour of the Antitumor Agent Vanadocene Dichloride in Physiological and Therapeutic Media, Blood Plasma and Human Blood - An EPR Study. *Met. Based Drugs,* **1997**, *4*(4), 207-219.
[32] Harding, M.M.; Mokdsi, G. Antitumour metallocenes: structure-activity studies and interactions with biomolecules. *Curr. Med. Chem.,* **2000**, *7*(12), 1289-1303.
[33] Havelek, R.; Siman, P.; Cmielova, J.; Stoklasova, A.; Vavrova, J.; Vinklarek, J.; Knizek, J.; Rezacova, M. Differences in vanadocene dichloride and cisplatin effect on MOLT-4 leukemia and human peripheral blood mononuclear cells. *Med. Chem.,* **2012**, *8*(4), 615-621.
[34] Sanna, D.; Ugone, V.; Micera, G.; Pivetta, T.; Valletta, E.; Garribba, E. Speciation of the potential antitumor agent vanadocene dichloride in the blood plasma and model systems. *Inorg. Chem.,* **2015**, *54*(17), 8237-8250.
[35] Aubrecht, J.; Narla, R.K.; Ghosh, P.; Stanek, J.; Uckun, F.M. Molecular genotoxicity profiles of apoptosis-inducing vanadocene complexes. *Toxicol. Appl. Pharmacol.,* **1999**, *154*(3), 228-235.
[36] Ghosh, P.; D'Cruz, O.J.; Narla, R.K.; Uckun, F.M. Apoptosis-inducing vanadocene compounds against human testicular cancer. *Clin. Cancer Res.,* **2000**, *6*(4), 1536-1545.
[37] Ghosh, P.; Kotchevar, A.T.; DuMez, D.D.; Ghosh, S.; Peiterson, J.; Uckun, F.M. Synthesis and structure of bis(picyclopentadienyl)vanadium(IV) 1,10-phenanthroline and 2,2′-bipyridine compounds and their interactions with artificial membranes. *Inorg. Chem.,* **1999**, *38*(16), 3730-3737.
[38] Djordjevic, C. Antitumor activity of vanadium compounds. *Met. Ions Biol. Syst.,* **1995**, *31*, 595-616.
[39] Gleeson, B.; Claffey, J.; Hogan, M.; Müller-Bunz, H.; Wallis, D.; Tacke, M. Novel benzyl-substituted vanadocene anticancer drugs. *J. Organomet. Chem.,* **2009**, *694*, 1369-1374.
[40] Narla, R.K.; Dong, Y.; Klis, D.; Uckun, F.M. Bis(4,7-dimethyl-1,10-phenanthroline) sulfatooxovanadium(I.V.) as a novel antileukemic agent with matrix metalloproteinase inhibitory activity. *Clin. Cancer Res.,* **2001**, *7*(4), 1094-1101.
[41] Dong, Y.; Narla, R.K.; Sudbeck, E.; Uckun, F.M. Synthesis, X-ray structure, and anti-leukemic activity of oxovanadium(IV) complexes. *J. Inorg. Biochem.,* **2000**, *78*(4), 321-330.

[42] D'Cruz, O.J.; Uckun, F.M. Metvan: a novel oxovanadium(IV) complex with broad spectrum anticancer activity. *Expert Opin. Investig. Drugs,* **2002**, *11*(12), 1829-1836.

[43] Moridani, M.Y.; Pourahmad, J.; Bui, H.; Siraki, A.; O'Brien, P.J. Dietary flavonoid iron complexes as cytoprotective superoxide radical scavengers. *Free Radic. Biol. Med.,* **2003**, *34*, 243-253.

[44] Naso, L.; Ferrer, *E.G.*; Lezama, L.; Rojo, T.; Etcheverry, S.B.; Williams, P. Role of oxidative stress in the antitumoral action of a new vanadyl(IV) complex with the flavonoid chrysin in two osteoblast cell lines: relationship with the radical scavenger activity. *J. Biol. Inorg. Chem.,* **2010**, *15*(6), 889-902.

[45] Naso, L.G.; Ferrer, *E.G.*; Butenko, N.; Cavaco, I.; Lezama, L.; Rojo, T.; Etcheverry, S.B.; Williams, P.A. Antioxidant, DNA cleavage, and cellular effects of silibinin and a new oxovanadium(IV)/silibinin complex. *J. Biol. Inorg. Chem.,* **2011**, *16*(4), 653-668.

[46] Naso, L.G.; Lezama, L.; Rojo, T.; Etcheverry, S.B.; Valcarcel, M.; Roura, M.; Salado, C.; Ferrer, *E.G.*; Williams, P.A. Biological evaluation of morin and its new oxovanadium(IV) complex as antioxidant and specific anti-cancer agents. *Chem. Biol. Interact.,* **2013**, *206*(2), 289-301.

[47] Leon, I.E.; Di Virgilio, A.L.; Porro, V.; Muglia, C.I.; Naso, L.G.; Williams, P.A.; Bollati-Fogolin, M.; Etcheverry, S.B. Antitumor properties of a vanadyl(IV) complex with the flavonoid chrysin [VO(chrysin)2EtOH]2 in a human osteosarcoma model: the role of oxidative stress and apoptosis. *Dalton Trans.,* **2013**, *42*(33), 11868-11880.

[48] Leon, I.E.; Porro, V.; Di Virgilio, A.L.; Naso, L.G.; Williams, P.A.; Bollati-Fogolín, M.; Etcheverry, S.B. Antiproliferative and apoptosis-inducing activity of an oxidovanadium(IV) complex with the flavonoid silibinin against osteosarcoma cells. *J. Biol. Inorg. Chem.,* **2014**, *19*(1), 59-74.

[49] León, I.E.; Cadavid-Vargas, J.F.; Tiscornia, I.; Porro, V.; Castelli, S.; Katkar, P.; Desideri, A.; Bollati-Fogolin, M.; Etcheverry, S.B. Oxidovanadium(IV) complexes with chrysin and silibinin: anticancer activity and mechanisms of action in a human colon adenocarcinoma model. *J. Biol. Inorg. Chem.,* **2015**, *20*(7), 1175-1191.

[50] del Río, D.; Galindo, A.; Vicente, R.; Mealli, C.; Ienco, A.; Masi, D. Synthesis, molecular structure and properties of oxo-vanadium(iv) complexes containing the oxydiacetate ligand. *Dalt. Trans.,* **2003**, *9,* 1813-1820.

[51] Rivadeneira, J.; Virgilio, A.L.; Di, ; Barrio, D.A.; Muglia, C.I.; Bruzzone, L.; Etcheverry, S.B. Cytotoxicity of a Vanadyl (IV) Complex with a Multidentate Oxygen Donor in Osteoblast. *Cell Lines in Culture,* **2010**, 9-23.

[52] León, I.E.; Etcheverry, S.B.; Parajón-Costa, B.S.; Baran, E.J. Spectroscopic characterization of an oxovanadium(IV) complex of oxodiacetic acid and o-phenanthroline. Bioactivity on osteoblast-like cells in culture. *Biol. Trace Elem. Res.,* **2012**, *147*(1-3), 403-407.

[53] León, I.E.; Etcheverry, S.B.; Parajón-Costa, B.S.; Baran, E.J. Spectroscopic characterization of an oxovanadium(IV) complex of oxodiacetic acid and 2,2′-bipyridine. Bioactivity on osteoblast-like cells in culture. *J. Mex. Chem. Soc.,* **2013**, *57*, 175-179.

[54] León, I.E.; Butenko, N.; Di Virgilio, A.L.; Muglia, C.I.; Baran, E.J.; Cavaco, I.; Etcheverry, S.B. Vanadium and cancer treatment: antitumoral mechanisms of three oxidovanadium(IV) complexes on a human osteosarcoma cell line. *J. Inorg. Biochem.,* **2014**, *134*, 106-117.

[55] Yamaguchi, T.; Watanabe, S.; Matsumura, Y.; Tokuoka, Y.; Yokoyama, A. Oxovanadium complexes with quinoline and pyridinone ligands: syntheses of the complexes and effect of alkyl chains on their apoptosis-inducing activity in leukemia cells. *Bioorg. Med. Chem.,* **2012**, *20*(9), 3058-3064.

[56] Correia, I.; Adão, P.; Roy, S.; Wahba, M.; Matos, C.; Maurya, M.R.; Marques, F.; Pavan, F.R.; Leite, C.Q.; Avecilla, F.; Costa Pessoa, J. Hydroxyquinoline derived vanadium(IV and V) and copper(II) complexes as potential anti-tuberculosis and anti-tumor agents. *J. Inorg. Biochem.,* **2014**, *141*, 83-93.

[57] Strianese, M.; Basile, A.; Mazzone, A.; Morello, S.; Turco, M.C.; Pellecchia, C. Therapeutic potential of a pyridoxal-based vanadium(IV) complex showing selective cytotoxicity for cancer versus healthy cells. *J. Cell. Physiol.,* **2013**, *228*(11), 2202-2209.

[58] Lewis, N.A.; Liu, F.; Seymour, L.; Magnusen, A.; Erves, T.R.; Arca, J.F.; Beckford, F.A.; Venkatraman, R.; González-Sarrías, A.; Fronczek, F.R.; Vanderveer, D.G.; Seeram, N.P.; Liu, A.; Jarrett, W.L.; Holder, A.A. Synthesis, characterization, and preliminary in vitro studies of vanadium(IV) complexes with a Schiff base and thiosemicarbazones as mixed-ligands. *Eur. J. Inorg. Chem.,* **2012**, *2012*(4), 664-677.

[59] Balaji, B.; Balakrishnan, B.; Perumalla, S.; Karande, A.A.; Chakravarty, A.R. Photocytotoxic oxovanadium(IV) complexes of ferrocenyl-terpyridine and acetylacetonate derivatives. *Eur. J. Med. Chem.,* **2015**, *92*, 332-341.

[60] Fu, Y.; Wang, Q.; Yang, X.-G.; Yang, X.-D.; Wang, K. Vanadyl bisacetylacetonate induced G1/S cell cycle arrest *via* high-intensity ERK phosphorylation in HepG2 cells. *J. Biol. Inorg. Chem.,* **2008**, *13*(6), 1001-1009.

[61] Desoize, B. Metals and metal compounds in cancer treatment. *Anticancer Res.,* **2004**, *24*(3a), 1529-1544.

[62] León, I.E.; Etcheverry, S.B.; Parajón-Costa, B.S.; Baran, E.J. Bis(oxalato)dioxovanadate(V) and bis(oxalato)oxoperoxo-vanadate(V) complexes: spectroscopic characterization and biological activity. *Biol. Trace Elem. Res.,* **2013**, *155*(2), 295-300.

[63] Andrezálová, L.; Gbelcová, H.; Duračková, Z. DNA damage induction and antiproliferative activity of vanadium(V) oxido monoperoxido complex containing two bidentate heteroligands. *J. Trace Elem. Med. Biol.,* **2013**, *27*(1), 21-26.

[64] Sugiyama, H.; Matsugo, S.; Misu, H.; Takamura, T.; Kaneko, S.; Kanatani, Y.; Kaido, M.; Mihara, C.; Abeywardana, N.; Sakai, A.; Sato, K.; Miyashita, Y.; Kanamori, K. Regulation of the physiological effects of peroxidovanadium(V) complexes by the electronic nature of ligands. *J. Inorg. Biochem.,* **2013**, *121*, 66-76.

[65] Ishihara, Y.; Takeuchi, K.; Ito, F.; Shimamoto, N. Dual regulation of hepatocyte apoptosis by reactive oxygen species: Increases in transcriptional expression and decreases in proteasomal degradation of BimEL. *J. Cell. Physiol.,* **2011**, *226*(4), 1007-1016.

[66] Faneca, H.; Figueiredo, V.A.; Tomaz, I.; Gonçalves, G.; Avecilla, F.; Pedroso de Lima, M.C.; Geraldes, C.F.; Pessoa, J.C.; Castro, M.M. Vanadium compounds as therapeutic agents: some chemical and biochemical studies. *J. Inorg. Biochem.,* **2009**, *103*(4), 601-608.

[67] Gonçalves, G.; Tomaz, I.; Correia, I.; Veiros, L.F.; Castro, M.M.; Avecilla, F.; Palacio, L.; Maestro, M.; Kiss, T.; Jakusch, T.; Garcia, M.H.; Pessoa, J.C. A novel V(IV)O-pyrimidinone complex: synthesis, solution speciation and human serum protein binding. *Dalton Trans.,* **2013**, *42*(33), 11841-11861.

[68] Meshkini, A.; Yazdanparast, R. Chemosensitization of human leukemia K562 cells to taxol by a Vanadium-salen complex. *Exp. Mol. Pathol.,* **2010**, *89*(3), 334-342.

[69] Noblía, P.; Vieites, M.; Parajón-Costa, B.S.; Baran, E.J.; Cerecetto, H.; Draper, P.; González, M.; Piro, O.E.; Castellano, E.E.; Azqueta, A.; López de Ceráin, A.; Monge-Vega,

A.; Gambino, D. Vanadium(V) complexes with salicylaldehyde semicarbazone derivatives bearing in vitro anti-tumor activity toward kidney tumor cells (TK-10): crystal structure of [VVO2(5-bromosalicylaldehyde semicarbazone)]. *J. Inorg. Biochem.,* **2005**, *99*(2), 443-451.

[70] Rivadeneira, J.; Barrio, D.A.; Arrambide, G.; Gambino, D.; Bruzzone, L.; Etcheverry, S.B. Biological effects of a complex of vanadium(V) with salicylaldehyde semicarbazone in osteoblasts in culture: mechanism of action. *J. Inorg. Biochem.,* **2009**, *103*(4), 633-642.

[71] Rhule, J.T.; Hill, C.L.; Judd, D.A.; Schinazi, R.F. Polyoxometalates in medicine. *Chem. Rev.,* **1998**, *98*(1), 327-358.

[72] Hasenknopf, B. Polyoxometalates: introduction to a class of inorganic compounds and their biomedical applications. *Front. Biosci.,* **2005**, *10*, 275-287.

[73] Ishaque Khan, M.; Ayesh, S.; Doedens, R.J.; Yu, M.; O'Connor, C.J. Synthesis and characterization of a polyoxovanadate cluster representing a new topology. *Chem. Commun. (Camb.),* **2005**, (37), 4658-4660.

[74] Zhai, F.; Wang, X.; Li, D.; Zhang, H.; Li, R.; Song, L. Synthesis and biological evaluation of decavanadate Na4Co(H2O)6V10O28.18H2O. *Biomed. Pharmacother.* **2009**, *63,* 51-55.

[75] Li, Y.-T.; Zhu, C.-Y.; Wu, Z.-Y.; Jiang, M.; Yan, C.-W. Synthesis, crystal structures and anticancer activities of two decavanadate compounds. *Transit. Met. Chem.,* **2010**, *35*, 597-603.

[76] Nunes, G.G.; Bonatto, A.C.; de Albuquerque, C.G.; Barison, A.; Ribeiro, R.R.; Back, D.F.; Andrade, A.V.; de Sá, E.L.; Pedrosa, F. de O.; Soares, J.F.; de Souza, E.M. Synthesis, characterization and chemoprotective activity of polyoxovanadates against DNA alkylation. *J. Inorg. Biochem.,* **2012**, *108*, 36-46.

[77] Aureliano, M.; Gândara, R.M. Decavanadate effects in biological systems. *J. Inorg. Biochem.,* **2005**, *99*(5), 979-985.

[78] Aureliano, M.; Fraqueza, G.; Ohlin, C.A. Ion pumps as biological targets for decavanadate. *Dalton Trans.,* **2013**, *42*(33), 11770-11777.

[79] Treviño, S.; Sánchez-Lara, E.; Sarmiento-Ortega, V.E.; Sánchez-Lombardo, I.; Flores-Hernández, J.Á.; Pérez-Benítez, A.; Brambila-Colombres, E.; González-Vergara, E. Hypoglycemic, lipid-lowering and metabolic regulation activities of metforminium decavanadate (H2Metf)3 [V10O28]·8H2O using hypercaloric-induced carbohydrate and lipid deregulation in Wistar rats as biological model. *J. Inorg. Biochem.,* **2015**, *147*, 85-92.

[80] Hou, W.; Guo, J.; Wang, Z.; Xu, Y. Synthesis, structural characterization, and properties of two new polyoxovanadates based on decavanadate [V 10 O 28] 6−. *J. Coord. Chem.,* **2013**, *66*, 2434-2443.

[81] Franco, M.P.; Rüdiger, A.L.; Soares, J.F.; Nunes, G.G.; Hughes, D.L. Crystal structures of two deca-vanadates(V) with penta-aqua-manganese(II) pendant groups: (NMe4)2 [V10O28{{}Mn(H2O)5{}}2]{·}5H2O and [NH3C (CH2 OH)3]2[V10O28{{}Mn(H2O)5{}}2]{·}2H2O. *Acta Crystallogr. Sect. E. Crystallogr. Commun.,* **2015**, *71*, 146-150.

[82] Reisner, E.; Arion, V.B.; Keppler, B.K.; Pombeiro, A.J. Electron-transfer activated metal-based anticancer drugs. *Inorg. Chim. Acta,* **2008**, *361*, 1569-1583.

[83] Levina, A.; Mitra, A.; Lay, P.A. Recent developments in ruthenium anticancer drugs. *Metallomics,* **2009**, *1*(6), 458-470.

[84] Hartinger, C.G.; Jakupec, M.A.; Zorbas-Seifried, S.; Groessl, M.; Egger, A.; Berger, W.; Zorbas, H.; Dyson, P.J.; Keppler, B.K. KP1019, a new redox-active anticancer agent--preclinical development and results of a clinical phase I study in tumor patients. *Chem. Biodivers.,* **2008**, *5*(10), 2140-2155.

[85] Śpiewak, K.; Stochel, G.; Brindell, M. Influence of redox activation of NAMI-A on affinity to serum proteins: transferrin and albumin. *J. Coord. Chem.,* **2015**, *68*, 3181-3192.

[86] Jarosz, M.; Matczuk, M.; Pawlak, K.; Timerbaev, A.R. Molecular mass spectrometry in metallodrug development: a case of mapping transferrin-mediated transformations for a ruthenium(III) anticancer drug. *Anal. Chim. Acta,* **2014**, *851*, 72-77.

[87] Pessoa, J.C.; Tomaz, I. Transport of therapeutic vanadium and ruthenium complexes by blood plasma components. *Curr. Med. Chem.,* **2010**, *17*(31), 3701-3738.

[88] Guo, W.; Zheng, W.; Luo, Q.; Li, X.; Zhao, Y.; Xiong, S.; Wang, F. Transferrin serves as a mediator to deliver organometallic ruthenium(II) anticancer complexes into cells. *Inorg. Chem.,* **2013**, *52*(9), 5328-5338.

[89] Wee, H.A.; Dyson, P.J. Classical and non-classical ruthenium-based anticancer drugs: Towards targeted chemotherapy. *Eur. J. Inorg. Chem.,* **2006**, 4003-4018.

[90] Clarke, M.J.; Zhu, F.; Frasca, D.R. Non-platinum chemotherapeutic metallopharmaceuticals. *Chem. Rev.,* **1999**, *99*(9), 2511-2534.

[91] Trondl, R.; Heffeter, P.; Kowol, C.R.; Jakupec, M.a.; Berger, W.; Keppler, B.K. NKP-1339, the first ruthenium-based anticancer drug on the edge to clinical application. *Chem. Sci. (Camb.),* **2014**, *5*, 2925-2932.

[92] Stevens, S.K.; Strehle, A.P.; Miller, R.L.; Gammons, S.H.; Hoffman, K.J.; McCarty, J.T.; Miller, M.E.; Stultz, L.K.; Hanson, P.K. The anticancer ruthenium complex KP1019 induces DNA damage, leading to cell cycle delay and cell death in Saccharomyces cerevisiae. *Mol. Pharmacol.,* **2013**, *83*(1), 225-234.

[93] Kapitza, S.; Jakupec, M.A.; Uhl, M.; Keppler, B.K.; Marian, B. The heterocyclic ruthenium(III) complex KP1019 (FFC14A) causes DNA damage and oxidative stress in colorectal tumor cells. *Cancer Lett.,* **2005**, *226*(2), 115-121.

[94] Jungwirth, U.; Kowol, C.R.; Keppler, B.K.; Hartinger, C.G.; Berger, W.; Heffeter, P. Anticancer activity of metal complexes: involvement of redox processes. *Antioxid. Redox Signal.,* **2011**, *15*(4), 1085-1127.

[95] Schluga, P.; Hartinger, C.G.; Egger, A.; Reisner, E.; Galanski, M.; Jakupec, M. a.; Keppler, B.K. Redox behavior of tumor-inhibiting ruthenium(iii) complexes and effects of physiological reductants on their binding to GMP. *Dalt. Trans.,* **2006**, 1796.

[96] Zhang, C.X.; Lippard, S.J. New metal complexes as potential therapeutics. *Curr. Opin. Chem. Biol.,* **2003**, *7*(4), 481-489.

[97] Groessl, M.; Reisner, E.; Hartinger, C.G.; Eichinger, R.; Semenova, O.; Timerbaev, A.R.; Jakupec, M.A.; Arion, V.B.; Keppler, B.K., Structure−Activity Relationships for NAMI-A-type Complexes (HL)[trans -RuCl 4 L(S - dmso)ruthenate(III)] (L = Imidazole, indazole, 1,2,4-triazole, 4-amino-1,2,4-triazole, and 1-methyl-1,2,4-triazole): aquation, redox properties, protein binding, and anti. *J. Med. Chem.,* **2007**, *50*, 2185-2193.

[98] Leijen, S.; Burgers, S.A.; Baas, P.; Pluim, D.; Tibben, M.; van Werkhoven, E.; Alessio, E.; Sava, G.; Beijnen, J.H.; Schellens, J.H. Phase I/II study with ruthenium compound NAMI-A and gemcitabine in patients with non-small cell lung cancer after first line therapy. *Invest. New Drugs,* **2015**, *33*(1), 201-214.

[99] Grover, N.; Welch, T.W.; Fairley, T.A.; Cory, M.; Thorp, H.H. Covalent Binding of Aquaruthenium Complexes to DNA. *Inorg. Chem.,* **1994**, *33*, 3544-3548.

[100] Blunden, B.M.; Stenzel, M.H. Incorporating ruthenium into advanced drug delivery carriers - an innovative generation of chemotherapeutics. *J. Chem. Technol. Biotechnol.,* **2015**, *90*, 1177-1195.

[101] Bruijnincx, P.C.; Sadler, P.J. Controlling platinum, ruthenium and osmium reactivity for anticancer drug design. *Adv. Inorg. Chem.,* **2009**, *61*, 1-62.

[102] Allardyce, C.S.; Dorcier, A.; Scolaro, C.; Dyson, P.J. Development of organometallic (organo-transition metal) pharmaceuticals. *Appl. Organomet. Chem.,* **2005**, *19*, 1-10.

[103] Aird, R.E.; Cummings, J.; Ritchie, A.A.; Muir, M.; Morris, R.E.; Chen, H.; Sadler, P.J.; Jodrell, D.I. In vitro and in vivo activity and cross resistance profiles of novel ruthenium (II) organometallic arene complexes in human ovarian cancer. *Br. J. Cancer,* **2002**, *86*(10), 1652-1657.

[104] Chen, H.; Parkinson, J.A.; Morris, R.E.; Sadler, P.J. Highly selective binding of organometallic ruthenium ethylenediamine complexes to nucleic acids: novel recognition mechanisms. *J. Am. Chem. Soc.,* **2003**, *125*(1), 173-186.

[105] Ronconi, L.; Sadler, P.J. Using coordination chemistry to design new medicines. *Coord. Chem. Rev.,* **2007**, *251*, 1633-1648.

[106] Pizarro, A.M.; Habtemariam, A.; Sadler, P.J. Activation Mechanisms for Organometallic Anticancer Complexes. In: *Medicinal Organometallic Chemistry.* Jaouen, G.; Metzler-Nolte, N., Eds.; Springer Berlin Heidelberg, Berlin, **2010**, Vol. 32, pp. 21-56.

[107] Habtemariam, A.; Melchart, M.; Fernandez, R.; Parsons, S.; Oswald, I.D.; Parkin, A.; Fabbiani, F.P.; Davidson, J.E.; Dawson, A.; Aird, R.E.; Jodrell, D.I.; Sadler, P.J. Structure-activity relationships for cytotoxic ruthenium(II) arene complexes containing N,N-, N,O-, and O,O-chelating ligands. *J. Med. Chem.,* **2006**, *49*(23), 6858-6868.

[108] Fernández, R.; Melchart, M.; Habtemariam, A.; Parsons, S.; Sadler, P.J. Use of chelating ligands to tune the reactive site of half-sandwich ruthenium(II)-arene anticancer complexes. *Chemistry,* **2004**, *10*(20), 5173-5179.

[109] Scolaro, C.; Bergamo, A.; Brescacin, L.; Delfino, R.; Cocchietto, M.; Laurenczy, G.; Geldbach, T.J.; Sava, G.; Dyson, P.J. In vitro and in vivo evaluation of ruthenium(II)-arene PTA complexes. *J. Med. Chem.,* **2005**, *48*(12), 4161-4171.

[110] Wang, F.; Chen, H.; Parsons, S.; Oswald, I.D.; Davidson, J.E.; Sadler, P.J. Kinetics of aquation and anation of ruthenium(II) arene anticancer complexes, acidity and X-ray structures of aqua adducts. *Chemistry,* **2003**, *9*(23), 5810-5820.

[111] Murray, B.S.; Babak, M.V.; Hartinger, C.G.; Dyson, P.J. The development of RAPTA compounds for the treatment of tumors. *Coord. Chem. Rev.,* **2016**, *306*, 86-114.

[112] Weiss, A.; Berndsen, R.H.; Dubois, M.; Müller, C.; Schibli, R.; Griffioen, A.W.; Dyson, P.J.; Nowak-Sliwinska, P. In vivo anti-tumor activity of the organometallic ruthenium(ii)-arene complex [Ru(η 6 -p-cymene)Cl 2 (pta)] (RAPTA-C) in human ovarian and colorectal carcinomas. *Chem. Sci. (Camb.),* **2014**, *5*, 4742-4748.

[113] Chatterjee, S.; Kundu, S.; Bhattacharyya, A.; Hartinger, C.G.; Dyson, P.J. The ruthenium(II)-arene compound RAPTA-C induces apoptosis in EAC cells through mitochondrial and p53-JNK pathways. *J. Biol. Inorg. Chem.,* **2008**, *13*(7), 1149-1155.

[114] Rodríguez Arce, E.; Sarniguet, C.; Moraes, T.S.; Vieites, M.; Tomaz, A.I.; Medeiros, A.; Comini, M.A.; Varela, J.; Cerecetto, H.; González, M.; Marques, F.; García, M.H.; Otero, L.; Gambino, D. A new ruthenium cyclopentadienyl azole compound with activity on tumor cell lines and trypanosomatid parasites. *J. Coord. Chem.,* **2015**, *68*, 2923-2937.

[115] Biersack, B.; Zoldakova, M.; Effenberger, K.; Schobert, R. (Arene)Ru(II) complexes of epidermal growth factor receptor inhibiting tyrphostins with enhanced selectivity and cytotoxicity in cancer cells. *Eur. J. Med. Chem.,* **2010**, *45*(5), 1972-1975.

[116] Kumar, A.; Kumar, A.; Gupta, R.K.; Paitandi, R.P.; Singh, K.B.; Trigun, S.K.; Hundal, M.S.; Pandey, D.S. Cationic Ru(II), Rh(III) and Ir(III) complexes containing cyclic π-perimeter and 2-aminophenyl benzimidazole ligands: Synthesis, molecular structure, DNA and protein binding, cytotoxicity and anticancer activity. *J. Organomet. Chem.,* **2015**.

[117] Vajs, J.; Steiner, I.; Brozovic, A.; Pevec, A.; Ambriović-Ristov, A.; Matković, M.; Piantanida, I.; Urankar, D.; Osmak, M.; Košmrlj, J. The 1,3-diaryltriazenido(p-cymene) ruthenium(II) complexes with a high in vitro anticancer activity. *J. Inorg. Biochem.,* **2015**, *153*, 42-48.

[118] Grozav, A.; Balacescu, O.; Balacescu, L.; Cheminel, T.; Berindan-Neagoe, I.; Therrien, B. Synthesis, anticancer activity, and genome profiling of thiazolo arene ruthenium complexes. *J. Med. Chem.,* **2015**. acs.jmedchem. 5b00855

[119] Charvátová, H.; Riedel, T.; Císařová, I.; Dyson, P.J.; Štěpnička, P. (η6-Arene)ruthenium complexes with P-coordinated phosphinoferrocene amides bearing extended polar substituents at the amide nitrogen: Synthesis, characterization and cytotoxicity. *J. Organomet. Chem.,* **2016**, *802*, 21-26.

[120] Kumar, A.; Kumar, A.; Gupta, R.K.; Paitandi, R.P.; Singh, K.B.; Trigun, S.K.; Hundal, M.S.; Pandey, D.S. Cationic Ru(II), Rh(III) and Ir(III) complexes containing cyclic?? -perimeter and 2-aminophenyl benzimidazole ligands: Synthesis, molecular structure, DNA and protein binding, cytotoxicity and anticancer activity. *J. Organomet. Chem.,* **2016**, *801*, 68-79.

[121] Mazuryk, O.; Łomzik, M.; Martineau, D.; Beley, M.; Brindell, M.; Stochel, G.; Gros, P.C. Anticancer activity of ruthenium(II) polypyridine complexes bearing pyrrolidine substituents. *Inorg. Chim. Acta,* **2016**, *443*, 86-90.

[122] Nikolić, S.; Rangasamy, L.; Gligorijević, N.; Aranđelović, S.; Radulović, S.; Gasser, G.; Grgurić-Šipka, S. Synthesis, characterization and biological evaluation of novel Ru(II)-arene complexes containing intercalating ligands. *J. Inorg. Biochem.,* **2016**, *160*, 156-165.

[123] Huang, H.; Zhang, P.; Chen, Y.; Ji, L.; Chao, H. Labile ruthenium(ii) complexes with extended phenyl-substituted terpyridyl ligands: synthesis, aquation and anticancer evaluation. *Dalton Trans.,* **2015**, *44*(35), 15602-15610.

[124] Jayanthi, E.; Anusuya, M.; Bhuvanesh, N.S.; Khalil, K.A.; Dharmaraj, N. Synthesis and characterization of ruthenium(II) hydrazone complexes as anticancer chemotherapeutic agents: *in vitro* DNA/BSA protein binding and cytotoxicity assay. *J. Coord. Chem.,* **2015**, *68*(20), 1-15.

[125] Lopes, J.C.; Damasceno, J.L.; Oliveira, P.F.; Tavares, D.C.; Deflon, V.M.; Lopes, N.P.; Pivatto, M.; Batista, A.A.; Maia, P.I.; Von Poelhsitz, G. Ruthenium(II) complexes containing anti-inflammatory drugs as ligands: synthesis. Characterization and in vitro cytotoxicity activities on cancer cell lines. *J. Braz. Chem. Soc.,* **2015**, *26*, 1838-1847.

[126] Nowak-Sliwinska, P.; Clavel, C.M.; Păunescu, E.; te Winkel, M.T.; Griffioen, A.W.; Dyson, P.J. Antiangiogenic and anticancer properties of bifunctional ruthenium(ii)-p-cymene complexes: influence of pendant perfluorous chains. *Mol. Pharm.,* **2015**, *12*(8), 3089-3096.

[127] Chen, Z.F.; Qin, Q.P.; Qin, J.L.; Zhou, J.; Li, Y.L.; Li, N.; Liu, Y.C.; Liang, H. Water-soluble ruthenium(ii) complexes with chiral 4-(2,3-dihydroxypropyl)-formamide oxoaporphine (FOA): in vitro and in vivo anticancer activity by stabilization of G-quadruplex DNA, inhibition of telomerase

activity, and induction of tumor cell apoptosis. *J. Med. Chem.,* **2015**, *58*(11), 4771-4789.

[128] Mari, C.; Pierroz, V.; Ferrari, S.; Gasser, G. Combination of Ru(ii) complexes and light: new frontiers in cancer therapy. *Chem. Sci. (Camb.),* **2015**, *6*, 2660-2686.

[129] Abrahamse, H.; Hamblin, M.R.; Moan, J.; Peng, Q.; Agostinis, P.; Berg, K.; Cengel, K.A.; Rosilio, V. New photosensitizers for photodynamic therapy. *Biochem. J.,* **2016**, *473*(4), 347-364.

[130] Knoll, J.D.; Turro, C. Control and utilization of ruthenium and rhodium metal complex excited states for photoactivated cancer therapy. *Coord. Chem. Rev.,* **2015**, *282-283*, 110-126.

[131] Josefsen, L.B.; Boyle, R.W. Photodynamic therapy and the development of metal-based photosensitisers. *Met. Based Drugs,* **2008**, *2008*, 276109.

[132] Allison, R.R.; Downie, G.H.; Cuenca, R.; Hu, X.H.; Childs, C.J.; Sibata, C.H. Photosensitizers in clinical PDT. *Photodiagn. Photodyn. Ther.,* **2004**, *1*(1), 27-42.

[133] Schmitt, F.; Govindaswamy, P.; Süss-Fink, G.; Ang, W.H.; Dyson, P.J.; Juillerat-Jeanneret, L.; Therrien, B. Ruthenium porphyrin compounds for photodynamic therapy of cancer. *J. Med. Chem.,* **2008**, *51*(6), 1811-1816.

[134] Schmitt, F.; Govindaswamy, P.; Zava, O.; Süss-Fink, G.; Juillerat-Jeanneret, L.; Therrien, B. Combined arene ruthenium porphyrins as chemotherapeutics and photosensitizers for cancer therapy. *J. Biol. Inorg. Chem.,* **2009**, *14*(1), 101-109.

[135] Davia, K.; King, D.; Hong, Y.; Swavey, S. A porphyrin-ruthenium photosensitizer as a potential photodynamic therapy agent. *Inorg. Chem. Commun.,* **2008**, *11*, 584-586.

[136] Bogoeva, V.; Siksjø, M.; Sæterbø, K.G.; Melø, T.B.; Bjørkøy, A.; Lindgren, M.; Gederaas, O.A. Ruthenium porphyrin-induced photodamage in bladder cancer cells. *Photodiagn. Photodyn. Ther.,* **2016**, *14*, 9-17.

[137] Pernot, M.; Bastogne, T.; Barry, N.P.; Therrien, B.; Koellensperger, G.; Hann, S.; Reshetov, V.; Barberi-Heyob, M. Systems biology approach for in vivo photodynamic therapy optimization of ruthenium-porphyrin compounds. *J. Photochem. Photobiol. B,* **2012**, *117*, 80-89.

[138] Mari, C.; Pierroz, V.; Rubbiani, R.; Patra, M.; Hess, J.; Spingler, B.; Oehninger, L.; Schur, J.; Ott, I.; Salassa, L.; Ferrari, S.; Gasser, G. DNA intercalating Ru(II) polypyridyl complexes as effective photosensitizers in photodynamic therapy. *Chemistry,* **2014**, *20*(44), 14421-14436.

[139] Huang, H.; Yu, B.; Zhang, P.; Huang, J.; Chen, Y.; Gasser, G.; Ji, L.; Chao, H. Highly charged ruthenium(II) polypyridyl complexes as lysosome-localized photosensitizers for two-photon photodynamic therapy. *Angew. Chem. Int. Ed. Engl.,* **2015**, *54*(47), 14049-14052.

[140] Dickerson, M.; Sun, Y.; Howerton, B.; Glazer, E.C. Modifying charge and hydrophilicity of simple Ru(II) polypyridyl complexes radically alters biological activities: old complexes, surprising new tricks. *Inorg. Chem.,* **2014**, *53*(19), 10370-10377.

[141] Culotta, V. Cell biology of copper. *J. Biol. Inorg. Chem.,* **2010**, *15*(1), 1-2.

[142] Kuo, H.W.; Chen, S.F.; Wu, C.C.; Chen, D.R.; Lee, J.H. Serum and tissue trace elements in patients with breast cancer in Taiwan. *Biol. Trace Elem. Res.,* **2002**, *89*(1), 1-11.

[143] Gupte, A.; Mumper, R.J. Elevated copper and oxidative stress in cancer cells as a target for cancer treatment. *Cancer Treat. Rev.,* **2009**, *35*(1), 32-46.

[144] Nayak, S.B.; Bhat, V.R.; Upadhyay, D.; Udupa, S.L. Copper and ceruloplasmin status in serum of prostate and colon cancer patients. *Indian J. Physiol. Pharmacol.,* **2003**, *47*(1), 108-110.

[145] Díez, M.; Arroyo, M.; Cerdàn, F.J.; Muñoz, M.; Martin, M.A.; Balibrea, J.L. Serum and tissue trace metal levels in lung cancer. *Oncology,* **1989**, *46*(4), 230-234.

[146] Turecký, L.; Kalina, P.; Uhlíková, E.; Námerová, S.; Krizko, J. Serum ceruloplasmin and copper levels in patients with primary brain tumors. *Klin. Wochenschr.,* **1984**, *62*(4), 187-189.

[147] McAuslan, B.R.; Reilly, W. Endothelial cell phagokinesis in response to specific metal ions. *Exp. Cell Res.,* **1980**, *130*(1), 147-157.

[148] Chen, D.; Milacic, V.; Frezza, M.; Dou, Q.P. Metal complexes, their cellular targets and potential for cancer therapy. *Curr. Pharm. Des.,* **2009**, *15*(7), 777-791.

[149] Wei, H.; Zhang, W-J.; Leboeuf, R.; Frei, B. Copper induces--and copper chelation by tetrathiomolybdate inhibits--endothelial activation in vitro. *Redox Rep.,* **2014**, *19*(1), 40-48.

[150] Hancock, C.N.; Stockwin, L.H.; Han, B.; Divelbiss, R.D.; Jun, J.H.; Malhotra, S.V.; Hollingshead, M.G.; Newton, D.L. A copper chelate of thiosemicarbazone NSC 689534 induces oxidative/ER stress and inhibits tumor growth in vitro and in vivo. *Free Radic. Biol. Med.,* **2011**, *50*, 110-121.

[151] Bolos, C.A.; Chaviara, A.T.; Mourelatos, D.; Iakovidou, Z.; Mioglou, E.; Chrysogelou, E.; Papageorgiou, A. Synthesis, characterization, toxicity, cytogenetic and in vivo antitumor studies of 1,1-dithiolate Cu(II) complexes with di-, tri-, tetra- amines and 1,3-thiazoles. Structure-activity correlation. *Bioorg. Med. Chem.,* **2009**, *17*(8), 3142-3151.

[152] Carvallo-Chaigneau, F.; Trejo-Solís, C.; Gómez-Ruiz, C.; Rodríguez-Aguilera, E.; Macías-Rosales, L.; Cortés-Barberena, E.; Cedillo-Peláez, C.; Gracia-Mora, I.; Ruiz-Azuara, L.; Madrid-Marina, V.; Constantino-Casas, F. Casiopeina III-ia induces apoptosis in HCT-15 cells in vitro through caspase-dependent mechanisms and has antitumor effect in vivo. *Biometals,* **2008**, *21*(1), 17-28.

[153] Taylor, M.R.; Gabe, E.J.; Glusker, J.P.; Minkin, J.A.; Patterson, A.L. The crystal structures of compounds with antitumor activity. 2-keto-3-ethoxybutyraldehyde bis(thiosemicarbazone) and its cupric complex. *J. Am. Chem. Soc.,* **1966**, *88*(8), 1845-1846.

[154] Wolohan, P.; Yoo, J.; Welch, M.J.; Reichert, D.E. QSAR studies of copper azamacrocycles and thiosemicarbazones: MM3 parameter development and prediction of biological properties. *J. Med. Chem.,* **2005**, *48*(17), 5561-5569.

[155] Zhang, H.; Thomas, R.; Oupicky, D.; Peng, F. Synthesis and characterization of new copper thiosemicarbazone complexes with an ONNS quadridentate system: cell growth inhibition, S-phase cell cycle arrest and proapoptotic activities on cisplatin-resistant neuroblastoma cells. *J. Biol. Inorg. Chem.,* **2008**, *13*(1), 47-55.

[156] Belicchi Ferrari, M.; Bisceglie, F.; Pelosi, G.; Tarasconi, P.; Albertini, R.; Dall'Aglio, P.P.; Pinelli, S.; Bergamo, A.; Sava, G. Synthesis, characterization and biological activity of copper complexes with pyridoxal thiosemicarbazone derivatives. X-ray crystal structure of three dimeric complexes. *J. Inorg. Biochem.,* **2004**, *98*(2), 301-312.

[157] Privat, M.; Aubel, C.; Arnould, S.; Communal, Y.; Ferrara, M.; Bignon, Y-J. Breast cancer cell response to genistein is conditioned by BRCA1 mutations. *Biochem. Biophys. Res. Commun.,* **2009**, *379*(3), 785-789.

[158] Sánchez, Y.; Amrán, D.; de Blas, E.; Aller, P. Regulation of genistein-induced differentiation in human acute myeloid leukaemia cells (HL60, NB4) Protein kinase modulation and reactive oxygen species generation. *Biochem. Pharmacol.,* **2009**, *77*(3), 384-396.

[159] Chen, X.; Tang, L-J.; Sun, Y-N.; Qiu, P-H.; Liang, G. Syntheses, characterization and antitumor activities of transition

metal complexes with isoflavone. *J. Inorg. Biochem.,* **2010**, *104*(4), 379-384.

[160] Pereira, R.M.; Andrades, N.E.; Paulino, N.; Sawaya, A.C.; Eberlin, M.N.; Marcucci, M.C.; Favero, G.M.; Novak, E.M.; Bydlowski, S.P. Synthesis and characterization of a metal complex containing naringin and Cu, and its antioxidant, antimicrobial, antiinflammatory and tumor cell cytotoxicity. *Molecules,* **2007**, *12*(7), 1352-1366.

[161] Tan, M.; Zhu, J.; Pan, Y.; Chen, Z.; Liang, H.; Liu, H.; Wang, H. Synthesis, cytotoxic activity, and DNA binding properties of copper (II) complexes with hesperetin, naringenin, and apigenin. *Bioinorg. Chem. Appl.,* **2009**, *2009*, 347872.

[162] Tan, J.; Wang, B.; Zhu, L. DNA binding and oxidative DNA damage induced by a quercetin copper(II) complex: potential mechanism of its antitumor properties. *J. Biol. Inorg. Chem.,* **2009**, *14*(5), 727-739.

[163] Williams, P.A.; Zinczuk, J.; Barrio, D.A.; Piro, O.E.; Nascimento, O.R.; Etcheverry, S.B. Potential antitumoral properties of a new copper complex with santonic acid. *Bioorg. Med. Chem.,* **2008**, *16*(8), 4313-4322.

[164] Etcheverry, S.B.; Di Virgilio, A.L.; Nascimento, O.R.; Williams, P.A. Dinuclear copper(II) complexes with valsartan. Synthesis, characterization and cytotoxicity. *J. Inorg. Biochem.,* **2012**, *107*(1), 25-33.

[165] Alemón-Medina, R.; Breña-Valle, M.; Muñoz-Sánchez, J.L.; Gracia-Mora, M.I.; Ruiz-Azuara, L. Induction of oxidative damage by copper-based antineoplastic drugs (Casiopeínas). *Cancer Chemother. Pharmacol.,* **2007**, *60*(2), 219-228.

[166] Hernández-Esquivel, L.; Marín-Hernández, A.; Pavón, N.; Carvajal, K.; Moreno-Sánchez, R. Cardiotoxicity of copper-based antineoplastic drugs casiopeinas is related to inhibition of energy metabolism. *Toxicol. Appl. Pharmacol.,* **2006**, *212*(1), 79-88.

[167] Galindo-Murillo, R.; Hernandez-Lima, J.; González-Rendón, M.; Cortés-Guzmán, F.; Ruíz-Azuara, L.; Moreno-Esparza, R. π-Stacking between Casiopeinas® and DNA bases. *Phys. Chem. Chem. Phys.,* **2011**, *13*(32), 14510-14515.

[168] Serment-Guerrero, J.; Cano-Sanchez, P.; Reyes-Perez, E.; Velazquez-Garcia, F.; Bravo-Gomez, M.E.; Ruiz-Azuara, L. Genotoxicity of the copper antineoplastic coordination complexes casiopeinas. *Toxicol. In Vitro,* **2011**, *25*(7), 1376-1384.

[169] Lahiri, D.; Majumdar, R.; Mallick, D.; Goswami, T.K.; Dighe, R.R.; Chakravarty, A.R. Remarkable photocytotoxicity in hypoxic HeLa cells by a dipyridophenazine copper(II) Schiff base thiolate. *J. Inorg. Biochem.,* **2011**, *105*(8), 1086-1094.

[170] Buchtík, R.; Trávníček, Z.; Vančo, J.; Herchel, R.; Dvořák, Z. Synthesis, characterization, DNA interaction and cleavage, and in vitro cytotoxicity of copper(II) mixed-ligand complexes with 2-phenyl-3-hydroxy-4(1H)-quinolinone. *Dalton Trans.,* **2011**, *40*(37), 9404-9412.

[171] Jaividhya, P.; Dhivya, R.; Akbarsha, M.A.; Palaniandavar, M. Efficient DNA cleavage mediated by mononuclear mixed ligand copper(II) phenolate complexes: the role of co-ligand planarity on DNA binding and cleavage and anticancer activity. *J. Inorg. Biochem.,* **2012**, *114*, 94-105.

[172] Li, X.-W.; Zheng, Y.-J.; Li, Y.-T.; Wu, Z.-Y.; Yan, C.-W. Synthesis and structure of new bicopper(II) complexes bridged by N-(2- aminopropyl)-N'-(2-oxidophenyl) oxamide: the effects of terminal ligands on structures, anticancer activities and DNA-binding properties. *Eur. J. Med. Chem.,* **2011**, *46*(9), 3851-3857.

[173] Chen, Q.-Y.; Fu, H.-J.; Zhu, W.-H.; Qi, Y.; Ma, Z.-P.; Zhao, K.-D.; Gao, J. Interaction with DNA and different effect on the nucleus of cancer cells for copper(II) complexes of N-benzyl di(pyridylmethyl)amine. *Dalton Trans.,* **2011**, *40*(17), 4414-4420.

[174] Buchtík, R.; Trávníček, Z.; Vančo, J. In vitro cytotoxicity, DNA cleavage and SOD-mimic activity of copper(II) mixed-ligand quinolinonato complexes. *J. Inorg. Biochem.,* **2012**, *116*, 163-171.

[175] Arjmand, F.; Muddassir, M. A mechanistic approach for the DNA binding of chiral enantiomeric L- and D-tryptophan-derived metal complexes of 1,2-DACH: cleavage and antitumor activity. *Chirality,* **2011**, *23*(3), 250-259.

[176] Koppenol, W.H. The Haber-Weiss cycle--70 years later. *Redox Rep.,* **2001**, *6*(4), 229-234.

[177] Fleming, A.M.; Muller, J.G.; Ji, I.; Burrows, C.J. Characterization of 2′-deoxyguanosine oxidation products observed in the Fenton-like system Cu(II)/H2O2/reductant in nucleoside and oligodeoxynucleotide contexts. *Org. Biomol. Chem.,* **2011**, *9*(9), 3338-3348.

[178] García-Giménez, J.L.; González-Alvarez, M.; Liu-González, M.; Macías, B.; Borrás, J.; Alzuet, G. Toward the development of metal-based synthetic nucleases: DNA binding and oxidative DNA cleavage of a mixed copper(II) complex with N-(9H-purin-6-yl)benzenesulfonamide and 1,10-phenantroline. Antitumor activity in human Caco-2 cells and Jurkat T lymphocytes. Evaluation of p53 and Bcl-2 proteins in the apoptotic mechanism. *J. Inorg. Biochem.,* **2009**, *103*(6), 923-934.

[179] Qin, Y.; Meng, L.; Hu, C.; Duan, W.; Zuo, Z.; Lin, L.; Zhang, X.; Ding, J. Gambogic acid inhibits the catalytic activity of human topoisomerase IIalpha by binding to its ATPase domain. *Mol. Cancer Ther.,* **2007**, *6*(9), 2429-2440.

[180] Hall, I.H.; Taylor, K.; Miller, M.C., III; Dothan, X.; Khan, M.A.; Bouet, F.M. Cytotoxicity of copper complexes of 2-furaldehyde oxime derivatives in murine and human tissue cultured cell lines. *Anticancer Res.,* **1997**, *17*(4A), 2411-2418.

[181] Duff, B.; Thangella, V.R.; Creaven, B.S.; Walsh, M.; Egan, D.A. Anti-cancer activity and mutagenic potential of novel copper(II) quinolinone Schiff base complexes in hepatocarcinoma cells. *Eur. J. Pharmacol.,* **2012**, *689*(1-3), 45-55.

[182] Seng, H-L.; Wang, W-S.; Kong, S-M.; Alan Ong, H-K.; Win, Y-F.; Raja Abd Rahman, R.N.; Chikira, M.; Leong, W-K.; Ahmad, M.; Khoo, A.S.; Ng, C-H. Biological and cytoselective anticancer properties of copper(II)-polypyridyl complexes modulated by auxiliary methylated glycine ligand. *Biometals,* **2012**, *25*(5), 1061-1081.

[183] Dou, Q.P.; Smith, D.M.; Daniel, K.G.; Kazi, A. Interruption of tumor cell cycle progression through proteasome inhibition: implications for cancer therapy. *Prog. Cell Cycle Res.,* **2003**, *5*, 441-446.

[184] Drexler, H.C. Activation of the cell death program by inhibition of proteasome function. *Proc. Natl. Acad. Sci. USA,* **1997**, *94*(3), 855-860.

[185] Daniel, K.G.; Gupta, P.; Harbach, R.H.; Guida, W.C.; Dou, Q.P. Organic copper complexes as a new class of proteasome inhibitors and apoptosis inducers in human cancer cells. *Biochem. Pharmacol.,* **2004**, *67*(6), 1139-1151.

[186] Verani, C.N. Metal complexes as inhibitors of the 26S proteasome in tumor cells. *J. Inorg. Biochem.,* **2012**, *106*(1), 59-67.

[187] Hindo, S.S.; Frezza, M.; Tomco, D.; Heeg, M.J.; Hryhorczuk, L.; McGarvey, B.R.; Dou, Q.P.; Verani, C.N. Metals in anticancer therapy: copper(II) complexes as inhibitors of the 20S proteasome. *Eur. J. Med. Chem.,* **2009**, *44*(11), 4353-4361.

[188] Zhang, Z.; Bi, C.; Schmitt, S.M.; Fan, Y.; Dong, L.; Zuo, J.; Dou, Q.P. 1,10-Phenanthroline promotes copper complexes into tumor cells and induces apoptosis by inhibiting the pro-

teasome activity. *J. Biol. Inorg. Chem.,* **2012**, *17*(8), 1257-1267.

[189] Sutradhar, K.B.; Amin, M.L. Nanotechnology in cancer drug delivery and selective targeting. *ISRN Nanotechnol.,* **2014**, *2014*, 1-12.

[190] Sanchez-Cano, C.; Hannon, M.J.; Guo, Z.; Sadler, P.J.; Storr, T.; Thompson, K.H.; Orvig, C.; Discher, D.E. Novel and emerging approaches for the delivery of metallo-drugs. *Dalton Trans.,* **2009**, *38*(48), 10702-10711.

[191] Gómez-Ruiz, S. *Mol. Work*; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, **2012**, pp. 207-242.

[192] Wani, W.A.; Prashar, S.; Shreaz, S.; Gómez-Ruiz, S. Nanostructured materials functionalized with metal complexes: In search of alternatives for administering anticancer metallodrugs. *Coord. Chem. Rev.,* **2016**, *312*, 67-98.

[193] López, T.; Ortiz-Islas, E.; Guevara, P.; Rodríguez-Reinoso, F.; Gómez, E.; Cuevas, J.L.; Novaro, O. Release of copper complexes from a nanostructured sol–gel titania for cancer treatment. *J. Mater. Sci.,* **2015**, *50*, 2410-2421.

[194] El Brahmi, N.; El Kazzouli, S.; Mignani, S.M.; Essassi, M.; Aubert, G.; Laurent, R.; Caminade, A.-M.; Bousmina, M.M.; Cresteil, T.; Majoral, J.P. Original multivalent copper(II)-conjugated phosphorus dendrimers and corresponding mononuclear copper(II) complexes with antitumoral activities. *Mol. Pharm.,* **2013**, *10*(4), 1459-1464.

[195] Mangiapia, G.; D'Errico, G.; Simeone, L.; Irace, C.; Radulescu, A.; Di Pascale, A.; Colonna, A.; Montesarchio, D.; Paduano, L. Ruthenium-based complex nanocarriers for cancer therapy. *Biomaterials,* **2012**, *33*(14), 3770-3782.

[196] Mangiapia, G.; Vitiello, G.; Irace, C.; Santamaria, R.; Colonna, A.; Angelico, R.; Radulescu, A.; D'Errico, G.; Montesarchio, D.; Paduano, L. Anticancer cationic ruthenium nanovectors: from rational molecular design to cellular uptake and bioactivity. *Biomacromolecules,* **2013**, *14*(8), 2549-2560.

[197] Valente, A.; Garcia, M.H.; Marques, F.; Miao, Y.; Rousseau, C.; Zinck, P. First polymer "ruthenium-cyclopentadienyl" complex as potential anticancer agent. *J. Inorg. Biochem.,* **2013**, Vol. 127, .

[198] Zhang, Y.; Xiang, J.; Liu, Y.; Zhang, X.; Tang, Y. Constructing transferrin receptor targeted drug delivery system by using doxorubicin hydrochloride and vanadocene dichloride. *Bioorg. Med. Chem. Lett.,* **2011**, *21*(19), 5982-5986.

[199] Cortizo, M.S.; Alessandrini, J.L.; Etcheverr, S.B.; Cortizo, A.M. A vanadium/aspirin complex controlled release using a poly(beta-propiolactone) film. Effects on osteosarcoma cells. *J. Biomater. Sci. Polym. Ed.,* **2001**, *12*(9), 945-959.