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Organometallic Compounds in Cancer Therapy: Past Lessons and Future Directions

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Abstract: Over the past few years, modern medicinal chemistry has evolved towards providing us new and alternative chemotherapeutic compounds with high cytotoxicity towards tumor cells, alongside with reduced side effects in cancer patients. Organometallic compounds and their unique physic-chemical properties typically used in homogenous catalysis are now being translated as potential candidates for medical purposes. Their structural diversity, ligand exchange, redox and catalytic properties make them promising drug candidates for cancer therapy. Over the last decade this area has witnessed a steady growth and a few organometallic compounds have in fact already entered clinical trials, emphasizing its increasing importance and clinical relevance. Here we intend to stress out the different applications of organometallic compounds in medicine with emphasis on cancer therapy, as well as address setbacks regarding formulation issues, systemic toxicity and off-target effects. Advantages over classical coordination metal complexes, their nanovectorisation and specific molecular targets are also discussed.

Keywords: Cancer therapy, clinical trials, M-arenes, M-carbenes, M-carbonyls, metallocenes, nanovectorisation, organometallics compounds.

INTRODUCTION

Organometallic compounds are a type of coordination complexes containing at least one direct, covalent metal-carbon (M-C) bond [1]. Compounds containing metal to hydrogen bonds as well as some compounds containing non-metallic (metalloid such as boron, silicon, germanium, arsenic, and tellurium) elements bonded to carbon are sometimes included in this class of compounds [2]. The typical classes of organometallics, such as metallocenes, half-sandwich, carbene-, CO-, or π -ligands, widely used for catalysis mainly in petrochemical industry, have now also found application in medicinal chemistry [1, 3, 4].

Medicinal applications of transition metal-based organometallic compounds as new potential therapeutics against HIV, antibiotics [5], malaria [6], radiopharmaceuticals [7] and cancer have been described. However the the main scope of this review is to highlight the applications of organometallic compounds in cancer therapy. Applications of organometallic compounds are vast and well documented elsewhere [3, 7-14].

ORGANOMETALLICS IN CANCER THERAPY

The concentration of metals and their metabolism is tightly controlled and modulated by cells and tissues due to the intrinsic metals' reactivity in the biological setting. In fact, metal ions concentration is highly regulated inside cells and their unbalance is associated with several conditions and diseases, amongst which cancer. In this regard, it is understandable that metal-containing complexes are of high interest as potential anticancer agents since they may interfere with this delicate regulation. The discovery of cisplatin, cis-[Pt(II) (NH_3(2)Cl_2], constituted a landmark of the use of metal complexes in the fight against cancer and triggered the interest in platinum(II)- and other metal-containing complexes as novel anticancer agents. Despite current advances in cancer therapy, in 2012 there were 8.2 million deaths from cancer [15], and most currently used chemotherapeutic agents, like cisplatin, are highly toxic. This opens new avenues for the synthesis and characterization of novel pharmaceuticals with reduced toxicity and high therapeutic index.

Mode of Action: Metal Center Versus Ligands

Delineating the mechanism by which an organometallic compound exerts a particular effect in a complex environment, such as the cell, is not straightforward since there are multiple targets and multiple forms of targeting. Based on empirical studies, the mechanism of action of an organometallic compound can be described at least in one of seven cases: (1) direct binding of the metal fragment to the target; (2) the metal has a structural role and binding to the biological target occurs through non-covalent interactions; (3) the activity and assimilation is due to the organic ligands and the metal is a carrier for delivering them *in vivo;* (4) the metal center causes cell damage by production of reactive oxygen species (ROS) [16]; (5) the organometallic compound is photoactive and behaves as a photosensitizer [17]; (6) the metal is a radiation enhancer, or (7) the metal is radioactive [18].

It has been shown that the biological activity and mechanism of action of metal compounds may be regulated by the metal, its oxidation state, and ligands [17]. For instance, it is well accepted that for a great majority of metal compounds, the anticancer activity is associated to the binding of the metal center to target proteins and/or DNA. An exception for this mechanism of action are DNA-intercalating agents [1]. Nevertheless, even when the antitumor action is not directly associated to the metal center, the metal potentiates the tridimensional organization of the ligands around the metal and provides for the efficacy of the compounds [1]. One example is Ti(Z-C_5H_5)$_2$$Cl_2$, whose ligand is loss in water and contributes to the solvated Ti^{IV} ion binding to the iron transport protein transferrin, which in turn is selectively delivered to cancer cells that have a high iron requirement [5].

Other examples come from ferrocene and ruthenocene application. Certain ferrocifens show similar activities to that of

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Tamoxifen against breast cancer cell lines, and where the activity of the compound has been linked to the reversible redox behavior of the iron center. Although ruthenocene (ruthenium analogues of ferrocifen) show overall size and shape very similar to ferrocene, the electrochemistry is significantly different, with superior redox potential and no reversibility for the reaction of electron transfer [5]. This is yet another piece of evidence that similar metal ions or similar organometallic compounds do not correlate directly to similar antitumor activity.

Platinum and palladium compounds give more emphasis to ligands' role in anti-tumor activity. Platinum(II) and palladium(II) compounds have a similar coordination chemistry but there are some little differences with big impact in antitumor activity, namely the ligand-exchange kinetics. The hydrolysis of ligands in palladium complexes is approximately 100 times faster than the corresponding platinum complexes [19]. In these cases, Ruthenium complexes' activation occurs by dissociation of the chloride ligand ensuring that ruthenium is available to coordinate to DNA [20]. In this condition, chloride also favors the transport of the complex across the cell membrane, probably due to the concentration gradient of chloride involving the intracellular and extracellular milieu [20]. For clinical applications, medicinal chemistry usually focus on structurally rigid compounds with closed electron shells, such as Ru(II), Os(II) or Ir(III), and with stereochemically stable ligands [12].

Organometallics: Advantages over Classical Coordination Metal Complexes

The number of metal compounds in current clinical use for cancer therapy is extremely limited. Only three platinum coordination complexes (i.e. without Pt-C bonds), cisplatin (cis-diamminedichloridoplatinum(II)), carboplatin (cis-diammine(1,1-cyclobutanedicarboxylato)platinum(II)), and oxaliplatin are included in approximately 50-70% of therapeutic schemes used to treat cancer patients [5, 21]. Nedaplatin is a second cisplatin analogue but is not commonly used because there is no Phase III study of nedaplatin [22]. The platinum complexes use in clinical is restricted due to dose-dependent toxicity and resistance coupled to a narrow spectrum of activity, however, these complexes are considered as ''blockbusters'' in the pharmaceutical industry [5].

Another problem concerning these coordination complexes, as a consequence of the particular chemical structure, is that they offer little possibility for improvements to increase tumor specificity [23] in contrast to organometallic compounds that offer wide possibilities of structural diversity, ranging from linear to octahedral and even beyond. Moreover, and because the metal is usually relatively strongly bound to its carbon ligands, organic transformations on the periphery of the molecule are possible. This provides opportunities for structural modifications while keeping the metal and its ligands intact. This approach is best illustrated by the solid phase synthesis of organometal-peptide conjugates in the chapter "Biomedical Applications of Organometal-Peptide Conjugates" by Metzler-Nolte [24]. In addition, rational ligand design provides control over key kinetic properties, which one of the most described is hydrolysis rate of ligands. Organometallic compounds offer yet other advantages over "classical coordination metal complexes", i.e. their kinetic stability, lipophilicity and their metal atom usually in a low oxidation state [1].

The organometallic equivalent of cisplatin is the metallocene $Ti(Z\text{-}C_5H_5)_2Cl_2$, whose cytostatic action was revealed by H. Köpf and P. Köpf-Maier in 1979. However, $Ti(Z\text{-}C_5H_5)_2Cl_2$ is difficult to formulate for administration because of its ease of hydrolysis and formation of hydroxyl- and oxy-bridges species contributing to their abandonment in clinical trials [25]. These issues about formulation problems will be addressed later.

THERAPEUTIC ORGANOMETALLIC COMPOUNDS

Metallocenes, metal-arenes, metal-carbenes and CO- or π-ligands have increasing applications in medicinal organometallic chemistry. Below, the different classes of organometallic compounds are discussed and characterized, conveying simultaneously the medicinal properties of such compounds with notable applications in the treatment and diagnosis of cancer. The examples depicted pretend to elucidate molecular targets and mechanisms of action through biochemical and cell biology studies. Table **1** exhibits representative examples of the most recent organometallic complexes under investigation for their anti-tumor properties.

Metallocenes

According to IUPAC, metallocenes consist of organometallic coordination compounds in which one atom of a transition metal such as iron, ruthenium or osmium is bonded to, and only to, the face of cyclopentadienyl $[\eta^5\text{-}(C_5H_5)]$ ligands by π-bonds, which lie in parallel planes [26]. These bis-cyclopentadienyl complexes can be classified in two classes; Classical (I), and "bent" metallocenes (II). While classical metallocenes have cyclopentadienyl rings (Cp rings) parallel to each other, in "bent" metallocenes, as the name suggests, these are inclined at an angle to one another and present the possibility of additional ligands attached to the metal center [1, 27].

Ferrocenes

Although ferrocene itself revealed to be a particularly weak toxic compound and rather ineffective, ferrocenium salts $[Fe(\eta^5\text{-}C_5H_5)_2]^+$ with the electron richer transition metal iron as central atom, show inhibiting effect towards Ehrlich ascites tumor [28] and a variety of cancer cell lines [29]. The mechanism of action behind ferrocenium salt's antiproliferative effect was not well established. Recently, work on MCF7 and MCF10A cell lines revealed that the mechanism of toxicity seems to involve the generation of Reactive oxygen species (ROS) with MCF7 cells displaying greater toxicity than MCF10A cells. In fact ferrocenium, as a result of ferrocene oxidation within tumor cells presents an unpaired electron in one of non-bonding orbitals and, consequently, is considered a high stable free radical capable of inducing DNA oxidative damage and 8-oxoguanine as an initial product of guanine oxidation [30]. In an earlier report, Osela and co-workers had already demonstrated that "ferrocenium salts could generate hydroxyl radicals in physiological conditions, further proposing that the cytotoxic activity is not based on their direct linking to DNA but on their ability to generate oxygen active species which induce oxidative DNA damage" [31]. The utility of ferrocenyl derivatives against cancer became very popular molecules and were thoroughly investigated for their "bioorganometallic chemistry" and anti-proliferative purposes, with the development of different ferrocene bioconjugates (e.g. proteins, peptides, DNA, RNA, PNA) [32, 33]. A few examples of such conjugates include ferrocenylalkyl nucleobases against carcinoma 755, ferrocenyl derivatives of retinoids, ferrocene with DNA intercalators or ferrocenyl derivatives as inhibitors of topoisomerase II [33]. For instance, Wagner and co-workers evidenced that a particular borylated ferrocene is capable to penetrate the blood brain barrier, giving new hope for the treatment and possibly diagnosis of brain tumors using boron neutron capture therapy [34]. Kondapi and co-workers reported a series of ferrocene derivatives with inhibitory properties against topoisomerase IIα and β. Based on structure activity analysis, two compounds in particular, azalactone ferrocene and thiomorpholide amido methyl ferrocene, presented a significant effect against topoisomerase II activity consequently causing numerous genetic repercussions that resulted in neoplastic cell death [35]. Despite the numerous ferrocene derivatives described in the last decade probably the most renowned class of ferrocene antiproliferative compounds are those tailored with estrogen targeting motifs which selectively bind to typical hormone dependent tumors [5, 36]. It was thought that the activity of such

Table 1. Organometallic complexes evaluated for anti-tumor properties (2010-2014). Complexes discriminated by organometallic class, name, molecular formula, therapeutic indication, *IC50* values for tested cell lines and proposed mechanism of action.

Name	Molecular Formula - Ligand Type	Therapeutic Indication/ Tested Cell Lines	*IC50* Range (μM)[§]	Proposed Mechanism of Action	Refs.
Cyclopentadienyl Complexes					
Iridium(III) Cyclopentadienyl containing C,N-Chelating Ligands	$[(\eta^5\text{-Cp}^x)\text{Ir-}(C^\wedge N)Cl]$	Solid tumors- Chemotherapy/ (A2780)	0.7 - 6.5 (A2780)	Aqua-adduct formation Nucleobase binding	[82]
Iridium(III) Cyclopentadienyl Containing C,N or N,N-Chelating Ligands	$[(\eta^5\text{-Cp}^x)\text{Ir-}(XY)Cl]^{+/0}$	Solid tumors- Chemotherapy/ (A2780)	´0.4 - 0.7 (A2780)	Induction of ROS DNA fragmentation Protein synthesis inhibition	[83]
Naphthyl substituted titanocene dichloride	$[Ti(\eta^5\text{-}C_5H_5)\{\eta^5\text{-}C_5H_3R(CHR'(C_{10}H_7))\}Cl_2]$ - Naphthyl	Solid tumors- Chemotherapy/ (8505C; A549; A2780; DLD-1; FaDu)	45.1 - 194.5 (8505C) 53.3 - 191.7 (A549) 35.6 - 72.4 (A2780) 61.3 - 161.3 (DLD-1) 69 - 200 (FaDu)	(*a*)	[84]
Iron(II) cyclopentadienyl complexes containing imidazole based ligands	$[Fe(\eta^5\text{-Cp})(L)(P\text{-}P)][CF_3SO_3]$	Solid tumors- Chemotherapy/ (A2780; MCF7; HeLa)	0.2 - 3 (A2780) 0.8 - 3.1 (MCF7) 1.4 - 6.3 (HeLa)	(*a*)	[85]
Iron(II) cyclopentadienyl derivative complexes containing N-heteroaromatic ligands	$[Ru(\eta^5\text{-Cp})(P\text{-}P)(L)][X]$	Non-solid tumors- Chemotherapy/ (HL-60)	0.7 -5.0 (HL-60)	Apoptosis induction	[86]
Ruthenium(II) cyclopentadienyl complexes containing carbohydrate-derived ligands	$[(\eta^5\text{-}C_5H_5)Ru(PP)(L)]^+$	Solid tumors- Chemotherapy/ (HeLa)	2.6 - 10.6 (HeLa)	(*a*)	[87]
Ruthenium(II) water-soluble cytotoxic complex	[RuCp(mTPPMSNa)(2,2′-bipy)][CF_3SO_3]	Solid tumors- Chemotherapy/ (A2780; MCF7; MDA-MB-231; HT29; PC3; V79)	0.1 - 0.2 (A2780) 0.07 - 3.6 (MCF7) 0.28 - 0.29 () 18.1 (MDA-MB-231) 28.1 (HT29) 0.54 - 25.8 (PC3) 4.21 - 40 (V79)	HSA adducts Ubiquitin interactions	[88]
Metal-Arene Complexes					
Ruthenium (II) η^6-p-Cymene	$[(\eta^6\text{-p-cymene})_2Ru_2(Cl_2)_2]$-Quinolones	Solid tumors- Chemotherapy/ (A549; CH1; SW480)	>320 (A549) 18 - 320 (CH1) 225 - 320 (SW480)	HSA adducts 5'-GMP adducts	[89]
Ruthenium(II) arene - 1,2,3,4-tetrahydrisoquinoline amino alcohol ligands	$[(\eta^6\text{-p-cymene})_2Ru_2(Cl_2)_2]$-TIQ	Solid tumors- Chemotherapy/ (MCF-7; MDBK)	34 - 218 (MCF-7) 250 - 500 (MDBK)	DNA binding Caspase-3 activation	[90]
Multi-targeted organometallic ruthenium(II) arene	$[(\eta^6\text{-p-cymene})_2Ru_2(Cl_2)_2]$-ADP-ribose & PARP-1 inhibitors	Solid tumors- Chemotherapy/ (A549; A2780; HCT116; Hcc1937; MRC-5 N500)	85.1 - 500 (A549) 38.8 - 500 (A2780) 46.6 - 500 (Hcc1937) 143.0 - 500 (MRC-5 N500)	DNA binding Inhibition of transcription and PARP-1	[91]
Osmium(II) arene AFAP51	$[(\eta^6\text{-biphenyl})Os_2(\text{ethylenediamine})Cl]^+$	Solid tumors-Chemotherapy/ (MDA-MB-231; MCF-7; HBL-100)	48 (MDA-MB-231) 15 (MCF-7) 16 (HBL-100)	(*a*)	[92]
Pyridyl Ruthenium(II) arene	$[(\eta^6\text{-arene})Ru(N,N')(L)][PF_6]_2$	Solid tumors-Phototherapy/ (A2780)	7.4 - 92.0 (A2780)	Aqua-adduct formation Nucleobase binding	[93]
Ruthenium(II) arene complexes with PPh3moiety	$[(\eta^6\text{-arene})Ru(Cl)(PPh_3)(Lig\text{-}N)]$	Non-solid tumors- Chemotherapy/ (HL60)	5.2 - 202 (HL60)	Nucleobase binding	[94]
Osmium(II) arene complexes of 8-substituted indolo [3,2-c]quinolines	$[Os(\text{p-cymene})(Cl)(\mu\text{-}Cl)]_2$	Solid tumors-Chemotherapy/ (A549; SW480; CH1)	1.9 - 7.8 (A549) 0.4 - 2.3 (SW480) 0.2 - 1.3 (CH1)	DNA intercalation	[95]
Metal-Carbenes and other ligands					
Gold(I)− and Gold(III)−*N*-Heterocyclic Carbenes	$C_{52}H_{44}Au_2N_{12}P_2F_{12}$ $C_{26}H_{24}AuCl_2OF_6N_6P'$	Solid tumor- Chemotherapy/ (HCT 116; HepG2; A549; MCF7)	5.2 (HCT 116) 3.6 - 5.9 (HepG2) 3.7 - 5.1 (A549) 4.7 - 6.2 (MCF7)	Apoptosis induction Loss of ΔΨm Induction of ROS	[96]
Sulfonate- and ester-functionalized silver(I) *N*-heterocyclic carbene	$[Na_4(Im^{PrSO3})_2]AgCl$ $[Im^{AcEt}]AgCl$	Solid tumors- Chemotherapy/ (A549; HCT-15; MCF-7; A431; A375)	9.8 - 21.1 (A549) 11.2 - 21.1 (HCT-15) 14.6 - 25.6 (MCF-7) 8.2 - 19.3 (A431) 14.1 - 26.7 (A375)	TrxR inhibition Induction of ROS Apoptosis Induction (ASK-1)	[97]

Table 1. contd….

Name	Molecular Formula - Ligand Type	Therapeutic Indication/ Tested Cell Lines	*IC50* Range (μM)[§]	Proposed Mechanism of Action	Refs.
Metal-Carbenes and other ligands					
Caffeine-Based Gold(I) *N*–Heterocyclic Carbenes	[Au(caffein-2-ylidene)$_2$][BF_4]	Solid tumors- Chemotherapy/ (A2780; A2780/R; SKOV3; A549; HEK-293T)	0.5 - 28.4 (A2780) 17.1 - 49 (A2780/R) 07 - 62.7 (SKOV3) 5.9 - 100 (A549) 0.2 - 100 (HEK-293T)	Inhibition of PARP-1 DNA non-covalent binding	[98]
Novel Ruthenium(II) *N*–Heterocyclic Carbenes	[(η^6-p-cymene)$_2$Ru$_2$(Cl_2)$_2$]-NHC	Solid tumors- Chemotherapy/ (Caki-1; MCF-7)	13 - 500 (Caki-1) 2.4 - 500 (MCF-7)	(*a*)	[99]
N-Heterocyclic Carbene-Amine Pt(II)	[$PtLX_2$]-(NHC)	Solid and non-solid tumors- Chemotherapy/ (CCRF-CEM; NCI-H460)	0.6 - 2.7 (CCRF-CEM) 0.9 - 3.8 (NCI-H460)	DNA adducts	[100]
Re(I) tricarbonyl complex of 1,10-phenanthroline-5,6-dione	*fac*-[$Re(CO)_3$(phendione)Cl]	Solid tumors- Chemotherapy/ (T98G; PC3; MCF-7)	>50 (T98G) >50 (PC3) >50 (MCF-7)	PAF inhibition DNA binding	[101]
Molybdenum(II) Allyl Dicarbonyl Complexes	[Mo(allyl)$(CO)_2$(N-N)(py)]$^+$	Solid and non-solid tumors- Chemotherapy/ (HT-29; MCF-7; NALM-6)	1.8 - 27 (HT-29) 2.1 - 32 (MCF-7) 1.8 - 13 (NALM-6)	DNA fragmentation Apoptosis induction (Necrosis for NALM-6)	[102]

§ - IC_{50} range values represent the maximum difference between the activity of different ligands of the same organometallic core structure. Exposure times vary between each study.
a - Not available

compounds is linked to a reduction of the receptor's affinity to estrogen, similar to that described for the drug tamoxifen (typically applied against hormone dependent breast tumors [37]). Nevertheless, ferrocene derivatives showed to be also active against MDA-MB231 cell line which is negative for estrogen receptors, thus suggesting a different mode of action from tamoxifen. It is thought that extended π-system present in ferrocenes derivatives and absent from tamoxifen is of paramount importance for the mechanism of action of these compounds [5]. Another study from Jaouen and co-workers [38] focusing on prostate cancer therapy also took advantage of endogenous hormones and receptor-ligand specificity. These authors synthesized several ferrocenyl derivatives of testosterone and dihydrotestosterone (DHT) to block androgen-induced hormonal effect (that promotes malignant growth) by inducing the binding of the ferrocene like derivatives to the receptor with more affinity than its natural ligand, testosterone, and their anticancer activity was studied [38]. These ferrocenyl complexes possess a strong antiproliferative activity against hormone-independent prostate cancer cells [38]. Furthermore they reported that the ferrocenyl derivatives behaviour is most likely linked to the Fenton-type properties of ferrocene [38]. The concept of employing the unusual properties of ferrocene labelling to provide access to cytotoxic compounds was also demonstrated using steroids with an androstanic skeleton [38].

More recently, Ying Li research group reported novel ferrocenyl derivatives with anti-cancer effect in human lung cancer cells *in vitro*. In particular 7 novel 1-ferrocenyl-2-(5-phenyl-1H-1,2,4-triazol-3-ylthio) ethanone derivatives (Fig. **1**) were screened for their effects in A549 human lung cancer cells in an effort to determine their mechanisms of action. Results evidenced that compounds **5b**, **5d**, and **5e** (40 and 80 μmol/L) caused significant decrease of A549 cell viability, while the other 4 compounds had no effect on the cells. Furthermore compounds **5b**, **5d**, and **5e**, induced G1-phase arrest with an increase of the G1 population by over 20% for all the tested compounds. Even though, it was observed a decrease in cell viability for the compounds **5b**, **5d**, and **5e**, due to apoptosis or necrosis. Overall it was proposed that the mechanism of action underlying the antiproliferative activity, *via* inducing G1-phase arrest, it is interconnected to ROS/p38 MAP-kinase pathway [39].

Together these studies elegantly demonstrate the diversity of ferrocenes and their behaviour in different biological profiles and as potential anticancer drugs.

Titanocenes

Early transition metal cyclopentadienyl compounds, titanocene dichloride (Cp_2TiCl_2) also displayed promising results. In spite of a seemingly logical extension of cisplatin configuration, titanocene's mode of action is rather different [40]. A modified version of transmission electron microscopy (TEM) for the detection of intracellular Ti allowed to follow the intracellular fate of this metal. In fact, TEM imaging showed that initially there was an accumulation of Ti in the nuclei of human tumors xenografted under the skin of mice (after several titanocene dichloride doses) [41]. This evidence of Ti accumulation in the nuclei and the inhibition of DNA synthesis and transcription led to the initial proposition of DNA as the main target of this compound [41]. In another study the compound was found to undergo complete loss of the Cp rings under physiological conditions, in a sense that the organometallic species acts solely as a precursor to Ti^{IV} ions [5]. It was demonstrated that Ti^{IV} ions, due to a hard base character, show higher affinity to the negatively charged oxygen of the phosphate backbone of DNA, ultimately inducing cytotoxicity [5]. A recent computational analysis of titanocenes derivative, titanocene Y, suggests that the $Cp(R)_2Ti^{2+}$ di-cations bind to DNA phosphate group, nevertheless the study ignores presence or the interaction of proteins and assumes DNA as the molecular target [1]. Furthermore titanocene Y presented remarkable *in vitro* anti-tumor activity in the case of renal cell, ovarian, non-small cell lung and colon cancer [42] and *in vivo* xenografted Caki-1 tumors in mice [43], as well as in prostate cancer [44]. In addition to DNA, the protein kinase C, responsible for phosphorylating serine and threonine residues on proteins as well as topoisomerase II are also other potential targets [45].

In similar studies regarding titanocene derivatives, Ulrike Olszewski's group elegantly demonstrated that titanocene C presented the ability to induce cell cycle arrest at G1/0-S interphase in NCI-H526 small cell lung cancer pointing out helicases/ topoisomerases and HIST1H4 core histones as the main possible targets. Indeed, in HL-60 cell lines, titanocene Y showed a phenotype of no-cross-resistance to oxoplatin and cisplatin, while titanocene C

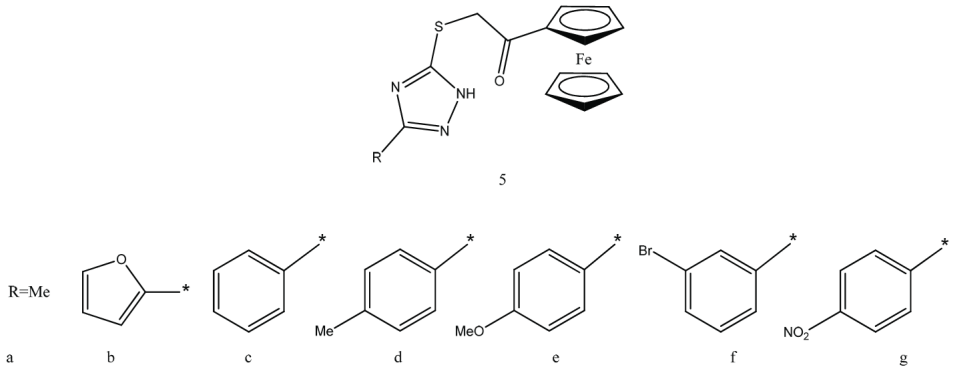

Fig. (1). Novel 1-ferrocenyl-2-(5-phenyl-1H-1,2,4-triazol-3-ylthio) ethanone derivatives (5a-g) [39].

presented lower cross-resistance to both drugs [46]. This combination of properties makes of titanocene Y a desirable anticancer drug candidate.

On a more pharmacodynamic and pharmacokinetic perspective titanocene Y was found to bind to serum albumin indicating that the biodistribution and transport of the metallocene to cancer cells occurred *via* protein binding [47]. Extrapolations of this biodistribution channel can be made for other titanocene derivatives.

New titanocene derivatives with high antiproliferative activity have been reported with a ethenyl-phenoxide or a benzyl group as substituent's of the cyclopentadienyl rings against MCF7 and SkBr3, two human breast cancer cell lines. Compounds **1**, **2** and **4** exhibited the strongest inhibitory effect. Nonetheless, the results seem to point out the need for a coordinating group, like a phenyl or ether, in order to stabilize the active species and generate a more cytotoxic complex [48].

Less Implemented Metallocenes

Other medically relevant metallocenes include zirconocene, vanadocene, niobocene and molybdocene [1, 49-53]. Through different modulations and functionalisation of the original structure, zirconocenes demonstrated good antiproliferative activities against several cancer cell lines, such as lung adenocarcinoma, head and neck tumor, anaplastic thyroid cancer, ovarian and colon carcinomas [54]. Nevertheless, these zirconocenes still need further optimization to be used in anticancer chemotherapy [54].

Also of particular importance is a series of molybdocene dichloride derivatives holding structure modification to yield more water soluble complexes [55]. These compounds seem to act *via* coordination to a series of biomolecules, the majority being proteins. In fact, several possible targets have been identified that support the observed antiproliferative activity, such as coordination to tubulin, interaction with human serum albumin, inhibition of protein kinase C and DNA cleavage [55]. In a recent study, Matthias Tacke group presented the synthesis and preliminary cytotoxicity studies of three molybdocene dichloride derivatives, modelled on Titanocene Y and Vanadocene Y that presented significant toxicity in Caki-1 cell line [56]. Emphasis was also given to the difference in the cytotoxic effects of substituting the metal center of these metallocenes with different transition metals, in this case molybdenum [56].

M-Arenes

Metal-arenes, also known as half-sandwich compounds or piano stool-complexes, consist of organometallic compounds that feature a facial planar organic compound, like benzene or cyclopentadienyl, forming the seat of the piano stool, and ligands which resemble the legs [57].

Ru Arenes

The ability of ruthenium complexes to bind to DNA has already been amply demonstrated [57, 58], however different reports suggest that the binding to DNA might not be as strong as once thought, and may even not be the primary target. In fact, under biological conditions, these complexes target a variety of biomolecules [1, 57]. Hébraud and co-workers studied the interactions between DNA and different ruthenium organometallic compounds showing that, when DNA is in excess, complexation involves intercalation of one of the organometallic ligands between the DNA base pairs; but when the organometallic compound is in excess relative to the DNA (high complexation ratio limit), a new mode of interaction is observed, in which the organometallic compound interacts weakly with DNA [59]. Moreover, it was shown that the weak interaction between DNA and organometallic compounds was more prone to occur when all DNA intercalation sites are occupied [59].

In addition to DNA, other macromolecular targets have been proposed for ruthenium organometallics. It was shown by electrospray ionization mass spectrometry method (ESI-MS) that ruthenium organometallic formed unambiguously stable protein adducts, with preferential binding of ruthenium metallo-fragments to surface histidines [60]. A particular study led by Paul J. Dyson [60] reported a series of novel glutathione-S-transferase (GST) inhibitors by conjugating ruthenium-arene complexes to ethacrynic acid. Enzyme kinetics and electrospray mass spectrometry experiments using GST P1-1 and its cysteine-modified mutant forms revealed that the complexes are effective enzyme inhibitors leading to a decrease in tumor growth. Furthermore, the inhibition constants *Ki* of the complexes on GST P1-1 were 3 to 4 times lower than ethacrynic acid revealing the involvement of ruthenium centers in the inhibition process [60]. Another exciting protein that was proposed to be targeted by ruthenium compounds is the multidrug resistance related P-Glycoprotein (Pgp). Some inhibitors of Pgp coordinated with ruthenium organometallics have in fact induced an even stronger protein inhibition compared to the Pgp inhibitors alone [1].

Others have showed that A2780 human ovarian cancer cells exposed to multinuclear arene ruthenium complexes (mono-, tetra- and octanuclear complexes) showed a close correlation between size of the compound and cell viability, monoruthenium compounds showing modest cytotoxicity and octanuclear compounds much higher [61]. A correlation between size and cytotoxicity had already been established in the same cancer cell line a few years earlier by Aird and co-workers, i.e. the activity increased with the size of the coordinated arene: benzene (Ben), p-cymene (Cym), biphenyl (Bip), dihydroanthracene (DHA), tetrahydroanthracene (THA) [62]. Two new Ru(II) complexes, [RuH(HL)$(PPh_3)_2$(CO)] and [RuH(HL) $(AsPh_3)_2$(CO(HL=2,20-bipyridine-5,50-dicarboxylic acid)] synthesized

and characterized by analytical and spectral methods by Karuppannan *et al.* [63] evidenced cytotoxic specificity towards a series of cancer cell lines including epithelial carcinoma cells (HEp-2), human liver carcinoma cells (HepG2) and mouse embryonic fibroblasts (NIH 3T3). In addition, antioxidant activity tests showed that the new complexes possess significant radical scavenging activity against free radicals outperforming standard antioxidants [63].

Several complexes of the type [Ru(η^6-p-cymene)L_2(pta)] (where PTA is 1,3,5-triaza-7-phosphaadamantane), called RAPTA complexes already evidenced to have moderate anticancer activity in various cell lines [64], however the specific mechanism of action by which they exert their cytotoxicity is still not very clear. In 2008 Casini studied a series of RAPTA compounds and evaluated them for their ability to inhibit thioredoxin reductase (TrxR) (cytosolic or mitochondrial) and cathepsin B, two possible targets for anticancer metallodrugs [65]. TrxR, which is known to pertain an active role in the redox balance in the cell, was found to be inhibited to larger extent when exposed to a specific RAPTA complex namely [(η^6-p-cymene)Ru(pta)(cbdca)] which possesses the same di-carboxylate ligand present in carboplatin, however cathepsin B is even more affected by the activity of such ruthenium arene complexes by comparison [45]. In other studies, the reactivity of RAPTA-C for instance within a solution containing ubiquitin, cytochrome c and superoxide dismutase showed that the later had high affinity towards ubiquitin and cytochrome c, but not superoxide dismutase. These results evidenced a particular compound selectivity thought to be linked to greater steric demand of this compound stemming from p-cymene ring and PTA ligand and hydrophobic interactions from the arene ring rather than electronic preferences. This behaviour clearly contrasts with cisplatin mode of action [66].

One might think that the main targets of ruthenium arene complexes are macromolecules but complexes of $Ru3^+$ are known to readily bind to nitric oxide (NO) and are being investigated as potential scavengers. NO is an important signalling molecule within the cell and slight changes to its regulation often leads to several diseases including cancer. Alterations in this small molecules' concentration might have a beneficial effect in the regression of some tumors and end up as potential new chemotherapeutic agents [45].

It is evident from these studies that there are distinct modes of action, depending on a complex array of variables, ranging from the tailoring and engineering of ruthenium complexes and respective ligands, to the biological environment as well as the different concentrations of the molecular intervenients at play.

M-Carbenes

Metal complexes harboring N-heterocyclic carbenes (NHC) ligands have been studied not only for their cytotoxicity against tumor cells but also for their antimicrobial properties [67]. It has been generally accepted that Au(I)−NHC complexes induce cell death by damaging mitochondrial function by targeting and inhibiting TrxR [68, 69]. Mitochondria play a central role in the onset of apoptosis, thus mitochondrial targeting is highly desirable in the design of novel pro-apoptotic anticancer complexes. Thereby, Au(I)-NHC complexes with anti-mitochondrial properties may take advantage of complex-metal-based reactivity with TrxR and/or mitochondrial swelling induced by their delocalized lipophilic cation (DLC) behavior [68, 69]. Reaction with TrxR may involve ligand loss as described for the interaction of gold-phosphole complex.

The stability of the Au−NHC bond provides more robust pharmacological properties when compared to other ligands. It has been demonstrated that $[Au(NHC)_2]^+$ complexes induce cancer death *via* p53 mediated apoptosis and by arresting cells in the S-phase. Additionally, Au(I)-NHC complexes with alkynyl and phosphine co-ligands demonstrated to be extremely cytotoxic in A2780 cell line (cisplatin-sensitive) [68, 69]. The high *in vitro* cytotoxicity of Au(I) complexes with substituted alkyne ligands may be due to the triphenylphosphine−gold(I) propargyl moiety [82].

Complexes with π-bonded alkynes also have *in vitro* anticancer activity. The compound Ir(η^5-C_5Me_5)(η^4-p-$C_6H_4Se_2$), containing a diselenobenzoquinone ligand, was also shown to have a cytotoxicity equivalent to that of cisplatin in human ovarian A2780 cancer cells [5].

NHC Ag(I) complexes have also been considered for their potential use as antimicrobial, antitumor and carbene transfer agents, showing their potential in the development of new transition metal-NHC systems. Functionalized Ag(I) mono-carbene complexes presented strong antiproliferative activity with equivalent *IC50s*. Most importantly, the similarities shared between these complexes and cisplatin are the linear C-Ag-C fragment (N-Pt-Cl in cisplatin) and the basicity of the Ag ion to bind cellular DNA of the affected cell to arrest cell multiplicity. Further studies on the stable Ag(I)-NHC complexes evidenced potent anticancer activity [70, 71]. Among the variety of Ag(I)-NHC screened for their antitumor activity some can be highlighted. Recently five Ag(I)-NHC based on 4,5-dichloroimidazolydene core have been evaluated against ovarian (OVCAR-3), breast (MB157) and HeLa cancerous cell lines. All complexes have clearly demonstrated higher antiproliferative activity against MB157 when compared to cisplatin. Moreover one of this Ag(I)-NHC, has presented better or similar anti proliferative activity than cisplatin or carboplatin against melanoma (A375), renal carcinoma (ACHN) and colon carcinoma (HT1376) [72].

Ag(I)-NHC like bromo-(1,3-dibenzyl-1,3-dihydro-2H-imidazol-2-ylidene) silver(I), bromo[1-(4-cyanobenzyl)-3-methyl-1,3-dihydro-2H-imidazol-2-ylidene]silver(I) and bromo[1-(4-cyanobenzyl)-3-methyl-1,3- dihydro-2H-benzimidazol-2-ylidene]silver(I) for instance, have also been evaluated. Reported *IC50s* for all three bromo Ag(I)-NHC on Caki-1 cells ranged from 27 to 34 μM, however these *in vitro* cytotoxicity revealed to be lower compared to cisplatin [73]. More detailed information on Ag(I)-NHC is reported elsewhere [72].

Multinuclear Ag(I)-NHC complexes, containing two or more metal centers in linear fashion and variable-length biogenic polyamines or aromatics as bridging linkers, constitute a new class of organometallic compounds of great potential and clinical importance. Due to their biocompatibility, Ag(I)-NHC complexes have demonstrated their use as metal-containing anticancer agents. Despite the well-established anticancer properties of several Ag (I)-NHC complexes, some disadvantages have been reported, such as precipitation in the biological environment, and higher toxicity values. Consequently new studies were performed with new Ag(I)-NHC complexes displaying potential anticancer activity. In recent studies it was reported that arene-bridged and non-bridged bis-carbene Ag(I)-complexes have anticancer activities towards HCT116 cell lines [70].

NHC copper complexes have also found a role as anti-cancer agents. Within a reductive environment, copper(II) originate copper(I) active species that may in turn react with intracellular oxygen or hydrogen peroxide to produce ROS and lead to cellular damage. Indeed, it was recently demonstrated that copper(I)-NHCs complexes are able to reach biological targets inside the cell causing havoc inside these cells, resulting in extensive cell death [68, 69].

NHC palladium complexes have also been used and a assume to exert their action *via* a mechanism similar to that of cisplatin, thus being able to target DNA in cancer cells leading to molecular damage and ultimately the death of cancer cells [67, 74] (see 5.).

M-Carbonyls

CO in gaseous form is difficult to handle however its therapeutic properties constitute a powerful asset when employed as potential drugs. Hence CO releasing molecules (CORMs) are developed to release CO in a controlled fashion in defined amounts at specific locations in the body [75]. Owing to the wide variety of CORMs these have are often found in the edge of medicinal inorganic chemistry for anticancer agents, among others like antimicrobial or antiviral, however these have not yet translated to the clinic [75].

Cell viability studies of new benzoheterocycle triosmium compounds employing MCF7 cell line, exhibited non-specific, acute cytotoxicity, probably due to accumulation on cell membranes by virtue of their amphiphilic character. *In vitro* studies presented telomerase enzymes as the prime target for such compounds, exhibiting good anti-telomerase activity on semi-purified enzyme in a cell-free assay [76].

More evidences on the potential of CORMs cytotoxicity come from new recent studies led by Lee's research group. These authors evaluated the *in vitro* cytotoxicity of several compounds of the $Os_3(CO)_{12}$-n Ln family showing selective activity towards estrogen receptor (ER)-independent (MDA-MB-231) when compared to ER-dependent cell lines (MCF-7) [77]. Even though the main molecular targets are still uncharacterized, apoptosis was suggested as the major mechanism for the observed antiproliferative activity. Despite positive results further studies are necessary for the development of a stable and liable organometallic CORM as an anticancer agent [77].

M-Alkyl/Aryls

M-Alkyls/Aryls are another particular subclass of metal - carbon compounds. M-Alkyls/Aryls feature a metal-carbon σ-bond and are usually actor ligands, although some alkyls behave simply as spectators [78]. Indeed, the antiproliferative activity of several organotin compounds has compared positively against common chemotherapy agents such as cisplatin [79-81]. In a recent study A. Silva and co-workers characterized the antiproliferative potential of an organotin(IV) coordination compound in human cancer cell lines. This organotin(IV) compound presented a high cytotoxic effect in colorectal and hepatocellular carcinoma cell lines, presenting *IC50s* of 0.238 μM (±0.011) and 0.199 μM (±0.003) respectively and a lower cytotoxicity in a healthy cell line of fibroblasts with an order of magnitude of 2.3 to 2.7 times lower. Furthermore induction of apoptosis was demonstrated through flow cytometry and Hoechst 3358 labelling as the main cell death mechanism [80]. Plus through proteomic approaches, microtubule stabilization proteins, TCTP and cofilin-1 might constitute possible molecular targets.

FROM THE BENCH TO BEDSIDE - TRANSLATIONAL DRUG DEVELOPMENT

In 2012 the Food and Drug Administration (FDA) approved 12 out of 33 new drugs to cancer therapy, the most productive therapeutic area in 2012 [103], amongst which some examples of organometallics that overcame the *in vitro*/*in vivo* phase studies. Titanocene dichloride entered clinical trials in 1993 and nephrotoxicity was identified as a problem, but the absence of bone marrow toxicity was promising and the organometallic went to clinical phase II trials [104]. However, improvements over other treatment regimens were not observed and the drug didn't succeed [5]. Various modified ansa systems have been synthesized and evaluated for anticancer activity. However, Kopf and co-workers discarded alkyl, alkenyl or aryl substituted ansa-titanocene complexes based on lower cytotoxicity [105]. One example of success is an organotechnetium. The first organometallic compound to enter clinical use was the 99mTc isonitrile complex $[TcI\{CNC(CH_3)_2CH_2OCH_3\}_6]^+$, with the commercial name Cardiolites®. Nowadays it is the most widely applied radiopharmaceutical in myocardial and other imaging applications [5]. The organometallic compound Photofrin® is another example of success. This marketed drug used in photodynamic therapy, builds its therapeutic effect on the fact that the drug molecules are activated by photo-irradiation [106]. At present, ferroquine, a ferrocene derivative, is the most advanced organometallic drug candidate nearly entering clinical phase III trials. However, it is planned to tackle malaria, based on the redox activity of iron [6].

A search at ClinicalTrials.gov, a registry and results database of publicly and privately supported clinical studies of human participants conducted around the world [107] with the term "organometallic AND cancer" retrieve no results. Despite the high anticancer activity of organometallic compounds only a few entered clinical trials [12]. Considering a broader search, like "metal anticancer drugs" in ClinicalTrials.gov we retrieved two Ruthenium(III) compounds, KP1019 and NAMI-A that completed phase I clinical trials and are currently in phase II trials [108]. NAMI-A was the one of the first ruthenium based anticancer drug to enter Phase I clinical trial [108, 109]. Twenty-four patients with various solid tumors including colorectal, lung, melanoma, ovarian and pancreatic cancers were treated with NAMI-A. Several toxic side effects were also observed including peripheral edema, alopecia, nausea, diarrhea, fatigue, anorexia, among others [108, 109]. KP1019 (trans-tetrachlorobis(indazole)ruthenate(III)) was the second ruthenium compound to enter Phase I clinical trial [110] demonstrating to be effective against several cancer cell lines, in particular against colorectal carcinoma (SW480 and HT29). The main mechanism of action seems to be due to apoptosis *via* the intrinsic mitochondrial pathway [111, 112]. In another study, eight patients with various advanced tumors including colorectal, endometrial, melanoma and bladder carcinomas were treated with KP1019 [113] and no significant side effects were observed [113]. Phase II clinical trials of KP1019 in patients suffering from advanced colorectal cancer is being planned [108, 111, 113]. NKP-1339 (KP1019 sodium salt) was capable to target tumor cells *via* albumin and transferrin binding followed by intracellular activation, inhibition of DNA synthesis and induction of apoptosis and G2/M arrest [114]. The limited toxic side effects observed in Phase I clinical trial of both KP1019 and NKP-1339 may turn out to be a major advantage towards effective use of these agents in cancer treatment [113, 114].

Complexes with iron, cobalt, gold, rhodium, iridium and osmium have shown potential anticancer activity and compounds with titanium, ruthenium, or gallium central atoms have already been evaluated in phase I and phase II trials [104]. As more and more research groups extend the use of organometallic compounds (structural diversity, possibility of ligand exchange, redox and catalytic properties) for medicinal purposes it is expectable that the number of clinical trials involving such compounds will increase exponentially.

SETBACKS REGARDING FORMULATION ISSUES - STABILITY AND SOLUBILITY HINDRANCES

Organometallic compounds' reactivity and stability is intimately connected to the physic-chemical properties of the different elements of the periodic table that comprises them, i.e. the nature of the organic ligands and the metal center to which these are attached will ultimately influence its overall stability, solubility and/or toxicity [115]. It could be said that the main disadvantages of organometallic compounds when considering their formulation are consequently deeply connected to poor hydrosolubility and hydrolytic instability. Solutions for these problems come from synthetically modifying the structure or finding new formulations with enhanced pharmokinetic and pharmacodynamic properties [105, 116-118]. So, it is easy to understand that hydrosolubility is a

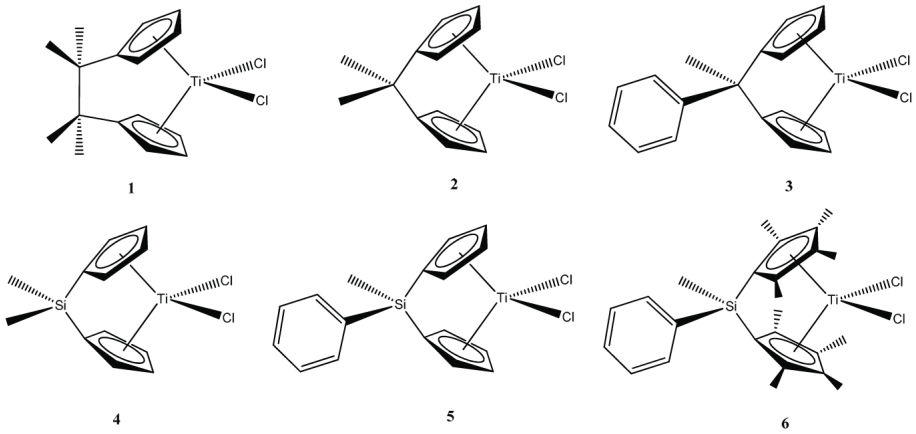

Fig. (2). Methyl- and phenyl-substituted carbon- and silicon-bridged ansa-titanocene [120].

prerequisite for metal-based drugs, as lack of control makes pharmacokinetic studies difficult, thus hindering, or, even excluding, progress towards the clinics. A prime example consists of titanocene dichloride removal from clinical trials phase II. Even though the main reason was due to lack of improvements over other chemo-therapeutic regimens, difficulties in formulation due to ease hydrolysis and ready formation of hydroxyl- and oxy-bridged species led to lower cytotoxic activity and hindered further developments [29]. Through oriented engineering of compound structure, ansa- type systems (ansa-metallocene derivatives) comprehend a plausible answer for improved aqueous solubility and stability [105, 118].

The bridging group is known to create a chelating effect that decreases the propensity for premature hydrolysis [29]. Ti(Z-$C_5H_5)_2Cl_2$ various modified ansa systems have already been evaluated for their antiproliferative activity evidencing meaningful activity against renal cancer cell line (LLC-PK). More significantly, the antiproliferative activity was considerably higher when compared to titanocene dichloride possibly because of a better stability [5]. In a new study dated from 2013, a series of six methyl- and phenyl-substituted carbon- and silicon-bridged ansa-titanocene complexes have also been tested on a panel of cancer cell lines (Fig. **2**). Melanoma A375 and B16, prostate cancer DU145 and LNCaP, and colon cancer HCT116, SW620 and CT26CL25 cancer cell lines were screened as to their sensibility to these synthesized ansa bridge compounds. Of the total six complexes, **2** to **5** evidenced to be less active when compared to the complexes **1** and **6**. The lack of activity seems to be due to a non-substitution of cyclopentadienyl rings, which increases the hydrophobic character and allows for the possibility of formation of micellar species decreasing the stability of the cyclopentadienyl bonds, leading to a higher degree of hydrolysis which may induce a lower cytotoxic activity. This phenomenon is counterbalanced in complex **1** by the incorporation of an ethylene ansa-bridge which increases the stability and the cytotoxicity. From a mechanistic point of view complexes **1** and **6** acted through inhibition of proliferation and subsequent induction of mitochondrial dependent apoptosis in colon cancer cell lines, HCT116 and SW620 [119].

A particular class that also benefitted from smart design in order to increase water solubility are the water-soluble ruthenium(II)-arene-PTA complexes, also known as RAPTA. The amphiphilic nature of the PTA ligand allows for these complexes to dissolve either in water or in organic solvents, a property considered to be fundamental in terms of formulation when considering the routes of administration of the drug candidate [120, 121]. The robustness of RAPTA compounds as an organometallic specie allows their modification. In fact these compounds solubility can be tuned in order to modify and improve their stability, plus their uptake and biomolecular interactions with their targets. The hydrophilicity of $[Ru(arene)Cl_2]_2$ complex was possible *via* the introduction of an alkyl sulfonate in the arene ligand [121].

Another notable example of organometallics extreme reactivity and rapid hydrolysis comes from palladium complexes. Ligand-exchange kinetics, the main key feature of these compounds that makes them interesting anticancer drug candidates is simultaneously a considerable problem when considering their stability and hydrosolubility issues. Strong coordinated nitrogen ligand and suitable leaving group has been suggested has a possible way to stabilize antitumor palladium anticancer drug candidates. If this group is reasonably non labile, the drug can maintain its structural integrity *in vivo* long enough to play an important role in physiological conditions [19].

Aqueous stability in other organometallic compounds might not be so easy to circumvent. For instance hydrosolubility of CORMs is in fact a major issue as quick release of the CO molecule after reaching the target tissue or organ might compromise the entire therapeutic effect of the compound [5]. Its high affinity towards hemoglobin might also complicate its bioavailability further hampering its therapeutic purposes [122]. First CORMs based on $[Mn_2(CO)_{10}]$ needed UV activation however the related mononuclear glycinato complex fac-$[Ru(CO)_3Cl(NH_2CH_2COO)]$ is more soluble in water and releases CO only under physiological conditions but the release of CO is still very fast and unspecific [19]. To overcome this problem, Steffen Romanski used enzyme-trigger to control the rate of CO release from acyloxybutadiene iron tricarbonyl complexes, while other researchers also achieved this goal by pH-dependent CO liberation, or photo-induced CO releasing [123]. CORMs utilization is still regarded as being very much in its infancy [122, 124], with several setbacks to determine their current value to medicine. The major problem is possibly how to set up a quality standard, because of all of the CORMs synthesized many of them will be absorbed in blood by plasma proteins, as well as present several forms in blood due to uncontrolled hydrolysis. A quality standard and a suitable pharmaceutical form are crucial for future perspectives regarding CORMs utilization in current medicine [123].

Nonetheless the imposed idea that all organometallic compounds are water and air sensitive is thus seen by this review as a general misconception in a sense that the vast majority of actual organic metallic compounds currently under investigation as potential drug candidates are carefully thought out (administration

routes, compound activation, etc.) and intelligently designed to avoid such hindrances under physiological conditions. In the end it all comes down to physic-chemical characteristics of the metal center in question as well as of the covalently bound ligands that determine the compounds stability and solubility.

NEED FOR IMPROVED VECTORISATION TO DECREASE TOXICITY. ADVENT OF NANOMEDICINE AND NANOENCAPSULATION

Nanotechnology derived structures, capable of delivery considerable amounts of drug to tumor cells in a targeted directed process, have been proposed and developed towards their use in cancer therapy. Several ligand-target therapeutic strategies are being developed to overcome current limitations of conventional chemotherapeutic drugs and providing additional tools for cancer therapy [125, 126]. Cancer nanotechnology offers a wealth of safety tools to treatment and diagnosis of cancer, such as targeted devices with unique therapeutic properties that, because of their small size, can pass biological barriers and deliver multiple therapeutic agents directly to cancer cells and adjacent tissues around tumor micro-environment [127, 130]. The working principle of a vector consists in using the affinity of certain substrates for metabolite receptors considering metabolic differences between normal and cancer cells, such as over-expression of receptors, thus enhancing selective recognition of cancer cells [127-131]. The use of nanovectorization systems provides for several advantages over traditional chemotherapy, in particular the capability to simultaneously hold multiple targeting moieties for several target molecules in cancer tissues and, also, the possibility to deliver much greater therapeutic payloads per target bypassing numerous biological barriers [127-129].

At present, there are only but a few nanomedicine products on the market, with the majority being pharmaceuticals that are formulated into nanosized structures to manipulate the pharmacodynamics, biodistribution and global effectiveness [130, 132]. Despite some reports on vectorisation of organometallic compounds, there are still no organometallics in nanomedicine market.

Cancer nanotechnology systems for compound vectorisation mainly include liposomes, metal nanoparticles, polymeric micelles, dendrimers, carbon nanotubes and quantum dots [133-136]. Most of these nanostructures may allow for the simultaneous diagnostics and drug-delivery, which convey a synergic efficacy in fighting tumors. Tumor targeting makes use of targeting ligands to facilitate the retention and cellular uptake of NPs *via* receptor-mediated endocytosis, even though accumulation at tumor site is largely determined by the particle physicochemical properties [136].

Nguyen *et al.* synthesized two organometallic triphenylethylene compounds 1, 2-di (4`-hydroxyphenyl) - 1-[4``-(2``-ferrocenyl-2``-oxoethoxy) phenyl] but-1-ene (DFO), and 1, 1-di (4`-hydroxyphenyl)-2-ferrocenylbut-1-ENE (Fc-diOH), with strong antiproliferative activity in breast cancer cells [138]. However they are insoluble in biological fluids. To overcome this obstacle the compounds Fc-diOH and DFO were incorporated in two types of stealth nanoparticles: PEG/PLA nanospheres and nanocapsules. Fc-diOH is an analogue of OH-Tam, where the tamoxifen β - aromatic benzyl ring has been replaced by the aromatic ferrocene moiety, and the amino side-chain has been replaced by a second hydroxyl group. FC-diOH has shown stronger cytotoxic compound to date (*IC50* ≈ 0.7 μM MCF-7; 0.44 μM MDA-MB231). In DFO the amino side-chain of OH-Tam has been altered into a carbonylferrocene. This compound shows lower cytotoxicity against tumor cells (*IC50* ≈10 μM MCF-7), but good affinity for the estrogen receptors [125].

Another example of organometallic compounds nano-encapsulated for cancer therapeutics is silver NHCs, namely silver fluorobenzoate that show good antiproliferative activities against human carcinomas [137].

The aqueous solubility of titanocenes (metallocene) with antitumor activities could also be enhanced by molecular encapsulation in cyclodextrins. A series of titanocenes were nanoencapsulated in α-, β-, and γ-cyclodextrin, this nanoparticle has also showed efficacy against tumor cells [138].

COMBINED THERAPY (PHOTOTHERAPY, PHOTO-INDUCED RELEASE)

In an effort to improve drug selectivity, it is possible to enhance the activity of a compound at the tumor site by applying external techniques or inducers. For example, topic application of ruthenium complexes (Fig. **3**) followed by photodynamic therapy, inhibited the vascular re-growth in the light treated area. This is one example of possible combined therapy, where the organometallic complex could mitigate some effects of other therapy (photodynamic therapy in this case) and in this way potentiate the treatment [139].

The time coordinate is important to determine the best effect. In the next example, the effect of the administration of the ruthenium complex is only seen during Photodynamic therapy. Indeed, porphyrin conjugates of Ru(II) complexes show increased cytotoxic activity to melanoma cells upon irradiation at 652 nm when compared to non-irradiated [17]. Photofrin®, the FDA approved organometallic compound drug for photodynamic therapy upon irradiation at 633 nm it produces reactive singlet oxygen species

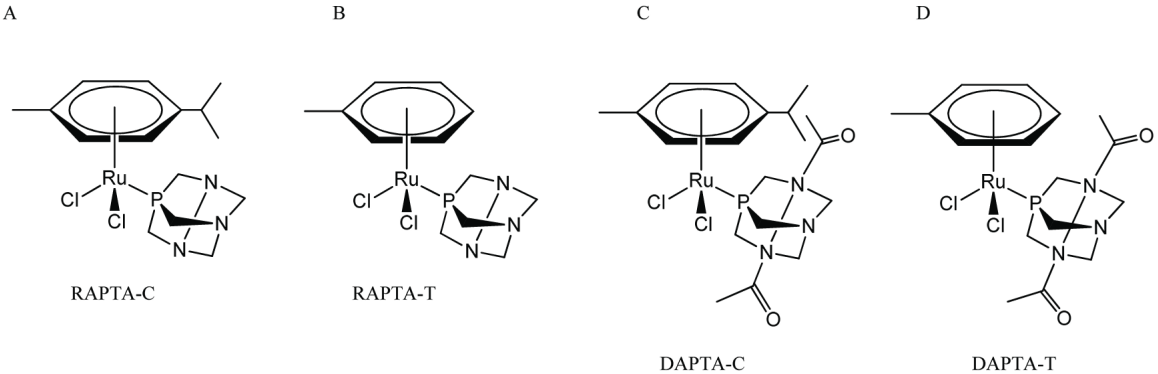

Fig. (3). Ruthenium-based complexes: (**A**) RAPTAC [Ru(η^6-p-cymene)Cl_2(PTA)], (**B**) RAPTA-T [Ru(η^6-toluene)Cl_2-(PTA)], (**C**) DAPTA-C [Ru(η^6-p-cymene)Cl2(DAPTA)], (**D**) DAPTA-T [Ru(η^6 toluene)Cl_2(DAPTA)], where PTA = 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane (PTA) and DAPTA = 3,7-diacetyl-1,3,7-triaza-5-phosphabicyclo[3.3.1]nonane [141].

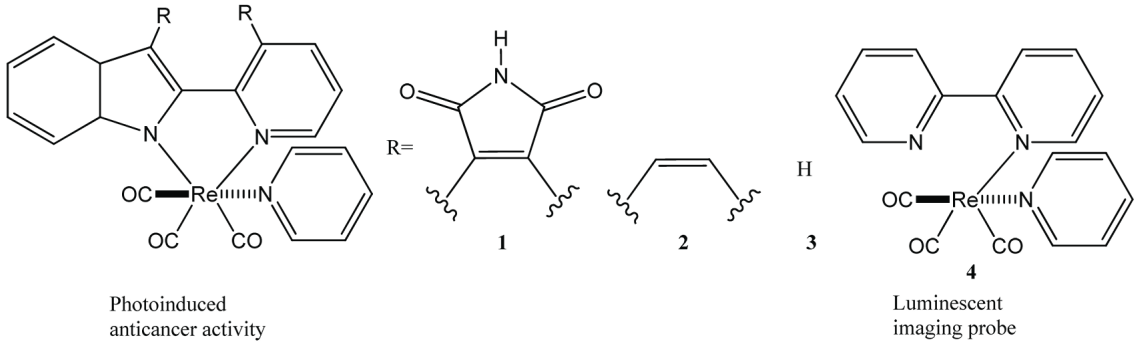

Fig. (4). From luminescent to light-induced anticancer Re complexes [143].

but with some side effects like skin sensitivity and hepatotoxicity [140].

One interesting example are derivatives of a nontoxic luminescent probe of rhenium applied for biological imaging that were discovered to have antitumor properties when irradiated at a suitable wavelength. Replacing just the 2,2'-bipyridine in **4** with 2-(2'-pyridyl)indolato and its derivatives (**1**-**3**) dramatically changes the physicochemical and biological properties of such rhenium(I) complexes (Fig. **4**) [141].

More organometallic compounds studied in photodynamic therapy include half-sandwich cyclopentadienyl complexes of iron and tungsten, (arene) ruthenium and related complexes, cobalt alkyl complexes, CO releasing molecules and bimetallic ferrocene conjugated transition metal complexes that are well described elsewhere [142]. Other therapies to improve tumor toxicity can be applied to organometallic compounds as shown by Collery and co-workers [143]. The authors combined three metals for the treatment of breast cancer MCF-7 tumor-bearing mice and determined the optimal doses. Two of them were organometallics, rhenium and gallium, and the other drug is the well-established cisplatin.

CONCLUSIONS AND FUTURE PERSPECTIVES

Organometallic chemistry importance is undeniable. Despite their presence in biological systems, it was not until the beginning of the XX century that organometallic compounds have been used in medicine, with particular interest in chemotherapy. Despite the increasing interest in organometallic compounds as potential anticancer agents, these are still overshadowed by the well-established cisplatin, which has been related to the compounds' instability and concerns about toxicity of many metals. The plethora of compounds and combinations possible *via* ingenious synthetic pathways has boosted the number of compounds that can be tested and used for the purpose of killing tumor cells. Careful assessment of their potential as chemotherapeutics is sometime a strenuous and hard task that needs pursuing since tumor cells are becoming resistance to conventional agents in use for decades. Increasing the cytotoxic selectivity and potency has been a trade mark for organometallic compounds reported to date. The biggest challenge nowadays is overcoming poor solubility in water and complex biologic fluids, cell internalization and reducing off-target effects. Some of these features have been addressed by formulation *via* nanotechnology based approaches.

The biggest change in drug development, particularly in the anticancer field, has been the move away from cytotoxic to molecularly targeted agents. In this context, nanomedicine brought new horizons to cancer therapy. Promising organometallic drug candidates can now be selectively delivered to tumor cells with minimum side effects. In the future, the crossing information between organometallic chemistry, biochemistry and nanomedicine could offer more possibilities to define possible cellular targets and drug-target interactions and in this way favoring translational drug development of organometallic compounds.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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