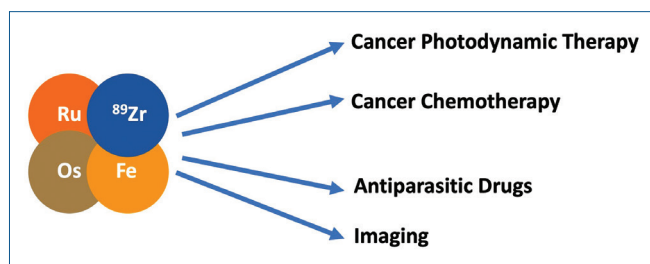




RECENT DEVELOPMENTS IN THE USE OF METAL COMPLEXES IN MEDICINAL CHEMISTRY

Metal complexes have long been used in medicine, but the field evolved significantly after the 1960s discovery of cisplatin, a key anticancer agent. This spurred growing interest in metal-based drugs. This mini-review highlights our group's past decade of research on designing radioimaging agents and developing new anticancer and antifungal candidates for more targeted therapies.



Compounds incorporating metals have been extensively used in medicine for many centuries for various purposes. But the most important breakthrough in this field coined as medicinal inorganic chemistry probably came with the discovery of cisplatin by Rosenberg *et al.* in the 1960's (Fig. 1a) [1]. Their experiment, at first not dedicated to finding new ways to fight cancer, generated the most used metal-based anticancer drug to date. Since then, and because cancer has for a long time been the second cause of death worldwide, the search for new anti-cancer drugs based on metal complexes has attracted a lot of attention. Cisplatin, which is still used in the clinic for the treatment of many cancers, has two major flaws:

- 1) it must be administered in high doses giving rise to severe side effects;
- 2) many cancer cells can develop resistance to the drug, making it much less efficient [2].

Therefore, one of the major focuses of the following decades after the discovery of cisplatin was put on developing new anticancer metal-based drugs with better activity at lower doses, and which could overcome the cisplatin resistance acquired by cancer cells. Thus, a new generation of Pt(II)-based anticancer drugs was developed, including carboplatin and oxaliplatin as the most notorious examples (Fig. 1a) [3]. Beyond Pt(II), other metals are used for therapeutic purposes. Notably, in the field of nuclear medicine, radioactive metals play an essential role in both diagnostic and therapy [4]. The field of application in which these radioisotopes can be used depends mostly on the type of particles emitted during the radioactive decay. Usually, radioisotopes such as Ga-68, Cu-64 or Zr-89, all positron emitters, are used for imaging purposes. For therapy, β^- emitters like Y-90 or Lu-177 or α emitters such as Ac-225 or Ra-223 are commonly used. ^{68}Ga -dotate, also called NetSpot, is clinically used for Positron Emission Spectroscopy (PET), a type of imaging technique largely used for diagnostic. On the other hand, Lutathera, a ^{177}Lu -DOTA complex, conjugated to somatostatin for targeting purposes, is used for the treatment of somatostatin receptor-positive neuroendocrine tumors (Fig. 1c). Altogether, nuclear medicine is

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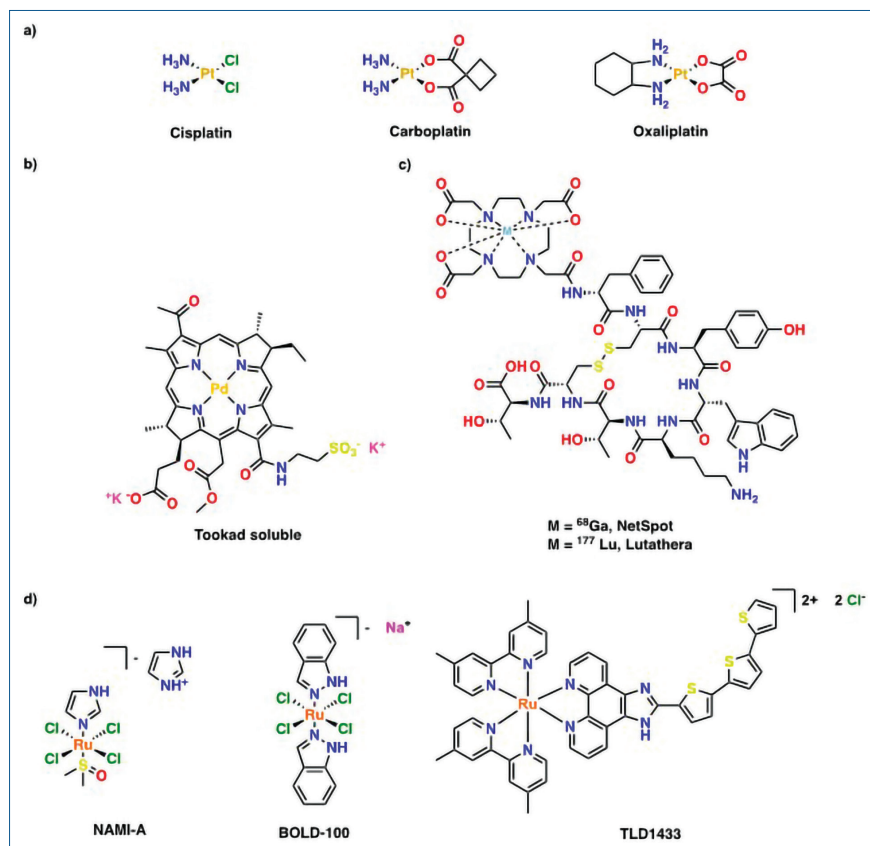


Fig. 1 - a) Clinically-approved platinum-based-anticancer drugs, b) TOOKAD-Soluble, a Pd(II) porphyrin approved for PDT, c) radionuclide bioconjugates approved for imaging ($M=^{68}\text{Ga}$) and therapy ($M=^{177}\text{Lu}$), d) Ru-based anticancer drug candidates which have reached clinical trials

the field in which most metal complexes are being used. Still in the imaging field, paramagnetic metals are widely used for Magnetic Nuclear Resonance (MRI), a routine diagnostic experiment. One of the first and most common metals used for MRI was Gd(III) and nowadays, over 500,000 people are receiving MRIs every day, which highlights the success of using metal complexes for medicine [5]. These examples show that metal complexes are already successfully prescribed for various applications. This observation motivated the field of medicinal inorganic chemistry to develop new generations of metallodrugs for diseases not yet efficiently diagnosed or treated, offering better pharmacokinetics and diminished side effects. Diagnostics plays a vital role in the treatment process of diseases, even before administration of the actual

drug. Our group has been active in the field of nuclear medicine with the goal of discovering imaging agents with enhanced pharmacokinetics. In particular, efforts have been devoted to find new chelators for ^{89}Zr , a known positron emitter that can be used for positron emission tomography (PET). As of now, the ligand used to chelate this radioactive isotope is desferrioxamine (DFO) (Fig. 2) [6]. This hexadentate ligand does not fill entirely the coordination sphere of ^{89}Zr , which can be prone to hydration by coordination of two aquo ligands on the remaining coordination sites for example. In 2014, in collaboration with the group of Prof. Thomas Mindt, we have described the synthesis of DFO*, an octadentate upgrade of the classic DFO, which could fill entirely the coordination sphere of ^{89}Zr , allowing better control of its stability [7]. In 2021, in an independent comparison study between different chelators of ^{89}Zr , Chomet *et al.* showed that DFO*

conjugated to the monoclonal antibody trastuzumab was the most suited for efficient tumor targeting imaging purposes [8]. With these highly encouraging results, the ^{89}Zr -DFO*-trastuzumab conjugate has now entered clinical trials for PET imaging of patients with breast or bladder cancer [9]. To circumvent the lack of activity encountered by treatments with cisplatin and its analogues due to the resistance mechanisms of cancer cells, alternative compounds incorporating different metals were investigated for their biological activity. No-

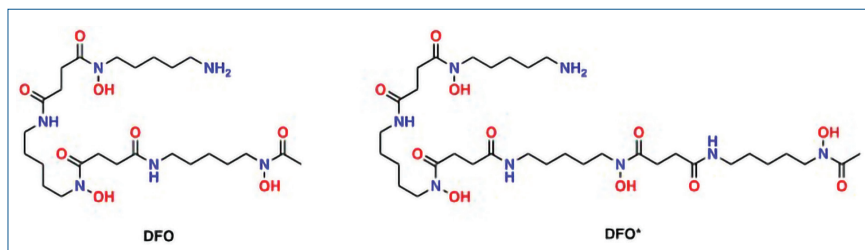


Fig. 2 - Ligands designed for the chelation of ^{89}Zr

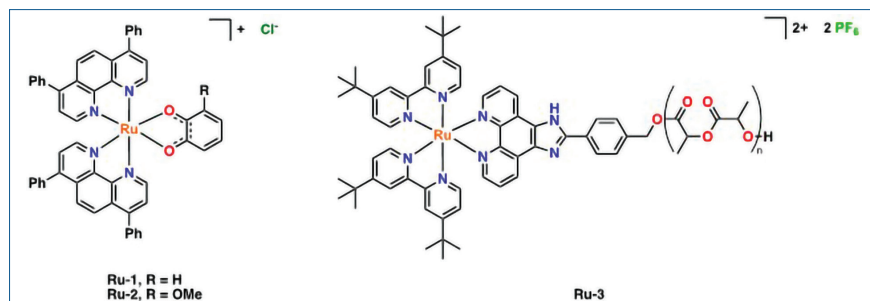


Fig. 3 - Ru(II) polypyridyl complexes used for non-PDT purposes, as alternatives to cisplatin

tably, ruthenium complexes such as the NAMI-A or KP1339 later renamed BOLD-100 both entered clinical trial, with BOLD-100 currently in Phase II trials in a combination therapy for the treatment of metastatic colon cancer (Fig. 1d) [10-12]. These results motivated us to design Ru-containing metallodrugs as alternatives for Pt-based chemotherapeutic agents. Thus, we synthesized **Ru-1**, a Ru(II) complex bearing a semi-quinonate (sq) ligand (Fig. 3) [13]. Semi-quinones are non-innocent compounds, and their redox properties had already been described as free molecules or as ligands on various metals prior to this study [14]. Nevertheless, coordination of the ligand to a bis-diphenylphenanthroline ruthenium(II) scaffold enabled a strong enhancement of its activity towards 2D cancer cells layers. These promising results led to its testing against 3D spheroids and ultimately in tumor bearing mice models. Overall, this complex showed great anticancer activity. In particular, against a cisplatin-resistant cell line, **Ru-1** exhibited a half maximal inhibitory concentration (IC_{50}) 36 times lower than cisplatin, making this drug a serious candidate as an alternative to platinum-based drugs. Investigation of the modes of action of the complex showed that it was able to induce damage by DNA metalation and mitochondrial disruption. Moreover, *in vivo* assays of **Ru-1** on two mice models showed reduction of tumor growth and prolongation of the mice's lives. Complete healing for one mouse in a group treated with the Ru complex at 10 mg/kg and two mice in the 15 mg/kg group was observed, demonstrating that compounds build around the **Ru-1** scaffold can be a promising alternative to Pt-based drugs. We then capitalized on the encouraging anticancer activity of the Ru-sq complex previously described

to improve its efficacy by derivatizing the sq ligand [15]. In a Structure-Activity Relationship (SAR) study, a series of functionalized sq ligands were used in coordination reactions to the same Ru(II) precursor than the one used for **Ru-1** complex to obtain the corresponding metal complexes (Fig. 3). When Electron Donating Groups (EDG) were added on the sq ligand, char-

acterization showed that only sq type complexes were obtained, whereas when the dioxo ligand was equipped with Electron Withdrawing Groups (EWG), the catecholato complex was obtained. These results attested that addition of simple moieties on the sq ligand can have drastic modifications on the electronic structure of the complexes. Upon biological evaluation, the metal complexes incorporating dioxo ligands in their sq form were significantly more toxic than their catecholato analogues. In particular, **Ru-2** displayed improved cytotoxicity in 2D cell layers, compared to the original **Ru-1**. Indeed, **Ru-2** was 6 times more toxic than **Ru-1** and 216 times more toxic than cisplatin in a cisplatin-resistant cell line. Moreover, **Ru-2** proved to be 8 times more toxic than doxorubicin, an anticancer DNA-intercalator, in a doxorubicin-resistant cell line. Also, the ability of these types of metal complexes to have multiple cell death modes of action was confirmed with the studies on **Ru-2**, showing that developing anticancer metal complexes as alternatives to clinically used drugs is possible.

A main concern appearing when designing new drugs, metal-based or not, are their behavior in a biological environment, namely their pharmacokinetics [16]. This includes water solubility, stability, and the efficiency of the payload delivery to the expected target. In this context, our group has also been working towards better delivery systems for their metal complexes. One way to tackle all the aforementioned challenges is to encapsulate the payload into a polymer matrix. This can be achieved by physical encapsulation, but this technique is accompanied with drawbacks, notably the burst release [17]. A more reliable solution is to create a covalent bond between the drug and



the polymer, enhancing the stability of the formed nanoparticles. In collaboration with the groups of Prof. Christophe Thomas and Didier Decaudin, we designed **Ru-3**, a Ru(II)-polypyridyl complex equipped with a phenanthroline-imidazole ligand for DNA intercalation-linked anticancer activity, which was functionalized with a benzylic alcohol (Fig. 3) [18]. This moiety can be used for Ring Opening Polymerization (ROP) with lactide monomers to form polymers directly incorporating the metal complex. After precipitation, the newly formed nanoparticles significantly improved the pharmacokinetic properties of the complex. A quantification of the drug's internalization showed a significantly higher uptake of the encapsulated complex, compared to the free complex. These results were confirmed when *in vivo* biodistribution assays were performed. As the free Ru complex accumulated significantly in the kidneys and the liver, the Ru-nanoparticle drug showed significantly reduced accumulation in these organs. Even more interestingly, an 18-fold difference between the accumulation of the free Ru complex and the nanoparticles in the tumor, in favor of the NPs was observed, showing that this encapsulation technique is promising for the encapsulation of various metal complexes.

In addition to classical techniques used for the treatment of cancers, or when surgery is not possible, Photodynamic Therapy (PDT) represents a viable complementary or even alternative therapeutic method. In PDT, a molecule called a Photosensitizer (PS) is administered to the patient. This molecule is able, after light irradiation at a specific wavelength, to be excited to a singlet state, before, after a so-called intersystem crossing (ISC), being transformed into a triplet state. This triplet state is extremely important because once the PS has reached its target (e.g., tumor), it can create cellular damage by two different pathways involving production of reactive oxygen species (ROS). In the type I mechanism, protons or electrons are transferred to the surrounding biological substrates, which results in the production of superoxide or hydroxyl radicals for example. In the type II mechanism, energy is transferred from the PS to molecular oxygen, resulting in the production of $^1\text{O}_2$. In both cases, the ROS produced will irre-

versibly damage the cellular content and lead to cell death [19]. Since the PS is activated by light, it is possible, with irradiation at the desired area with light, to target only the cancerous tissues, reducing efficiently the side effects caused by other chemotherapies. Recently, the metal-based photosensitizer **TOOKAD-Soluble**, has been approved for clinical use (Fig. 1b). Nevertheless, this palladium(II) porphyrin, like other tetrapyrrolic compounds, has major drawbacks, such as aggregation or photobleaching [20]. In parallel, to circumvent the problems encountered using tetrapyrrolic-based PSs, other types of metal complexes have been investigated as PSs. Ru(II) polypyridyl complexes, for example, are ideal candidates as for PDT PSs. They have appropriate photophysical properties, owing to the heavy-atom effect of the Ru(II) center allowing good ISC, and their cationic nature makes them more soluble in biological media than porphyrins. Recently, McFarland *et al.* have discovered **TLD-1433**, which has since then entered clinical trials to treat bladder cancer (Fig. 1d) [21].

In this context, our team has been working on the synthesis and biological applications of non-porphyrinic metal complexes for PDT (Fig. 4). One of the challenges faced when designing new PSs is to shift the absorption wavelength of the PS towards the red part of the visible spectrum, allowing deeper penetration of the light in human tissues. The aim is to treat more deep-seated or large tumors with less invasive techniques. The energy of the light used to irradiate the PS corresponds to the energy gap between the Highest Occupied Molecular Orbital and the Lowest Unoccupied Molecular Orbital (HOMO-LUMO). In metal complexes, this excitation corresponds to the transfer of an electron from the metal d orbitals to the ligand π^* orbitals, also called Metal-to-Ligand Charge Transfer (MLCT). Since this transition is in part ligand based, understanding how the variation of the coordination sphere of the metals changes the electronic properties of the complex is crucial to design PSs with optimal photophysical properties. To tackle this challenge, we described in 2020 the rational design of $[\text{Ru}(\text{phen})_2(\text{bpy})]^{2+}$ -based PSs for more efficient PDT (**Ru-4**) [22]. In this article, with the help of our colleague Dr. Ilaria Ciofini, TD-DFT

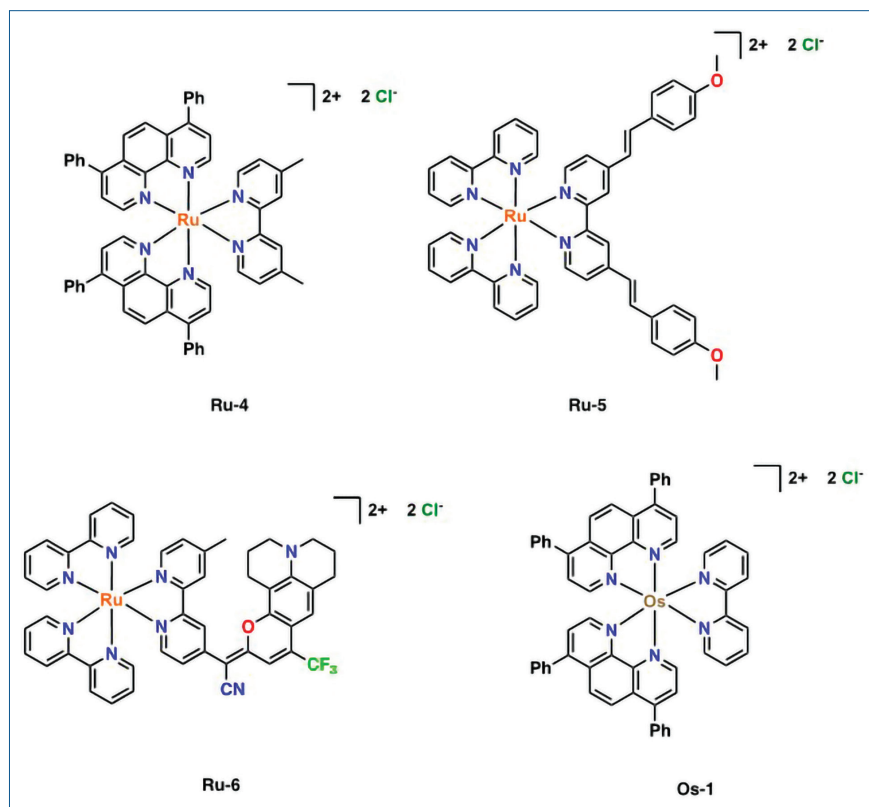


Fig. 4 - Ru(II) and Os(II) polypyridyl complexes studied in our group

(Time-Dependent Density Functional Theory) calculations of the HOMO-LUMO gap corresponding to the MLCT transition of a series of Ru polypyridyl compounds were carried out. These calculations showed that, depending on the substitution of the bpy ligand, the energy gap was modified, inducing a modification of the absorption spectra of the complexes. To confirm the theoretical results obtained, the complexes were then synthesized, and their photophysical and biological properties assessed, allowing to create guidelines for the synthesis of Ru(II)-based PSs with a significant red-shift, while keeping good stability and singlet oxygen production.

The same year, we described the design of a series of Ru(II) polypyridyl complexes, in which the bpy ligands were functionalized with substituted vinyl-styryl moieties acting as electron donating groups [23]. The improved electron donating ability of the ligands, compared to classical bpy ligands, caused a red-shift of the absorption spectrum of the complexes. Importantly, these complexes showed great two-photon (2P) absorption proper-

ties, which unlocks the possibility to irradiate the PSs in the near-infrared (NIR) region, thus improving the light penetration depth compared to the clinically used PSs. In particular, in collaboration with the group of Prof. Hui Chao, **Ru-5** showed extremely promising results *in vivo*, by eradicating a tumor in a multi-resistant mouse model. While continuing the work towards better Ru(II)-based PSs, our group also looked into the use of other heavy metals, which could be suitable for PDT, and which could lead to compounds with even better photophysical properties. Some complexes constructed around an Os(II) polypyridyl scaffold have recently been described in the literature for their use as PSs in the deeper region of the spectrum [24]. Thus, we worked on the synthesis of a series of Os(II) complexes based on the same **[M(DIP)₂(N-N)]**

scaffold used for the Ru(II) complexes described previously in the group (Fig. 4) [25]. These complexes showed absorption maxima in the visible region close to the one described for the analogue Ru(II) compounds, but contrary to the latter ones, the Os(II) complexes exhibited a panchromatic absorption tail ranging until after 700 nm. Having in hand complexes with such properties, PDT experiments were undertaken with irradiation wavelengths going up to 740 nm. Despite the low absorption intensity at this wavelength, **Os-1** showed remarkable cytotoxic activities against different cancer cell lines. The complex also showed good efficacy *in vivo*, making such compounds a valuable alternative to Ru(II)-based PSs for PDT.

Classic tetrapyrrolic-based PSs and most of the Ru(II)-based PSs are known to go through a type II PDT to exert their photocytotoxicity. The compounds rely heavily on the presence of molecular oxygen for the energy transfer reaction. This feature of the aforementioned PSs is problematic for the efficient treatment of tumors since tissues located deep inside it are not as vascularized as

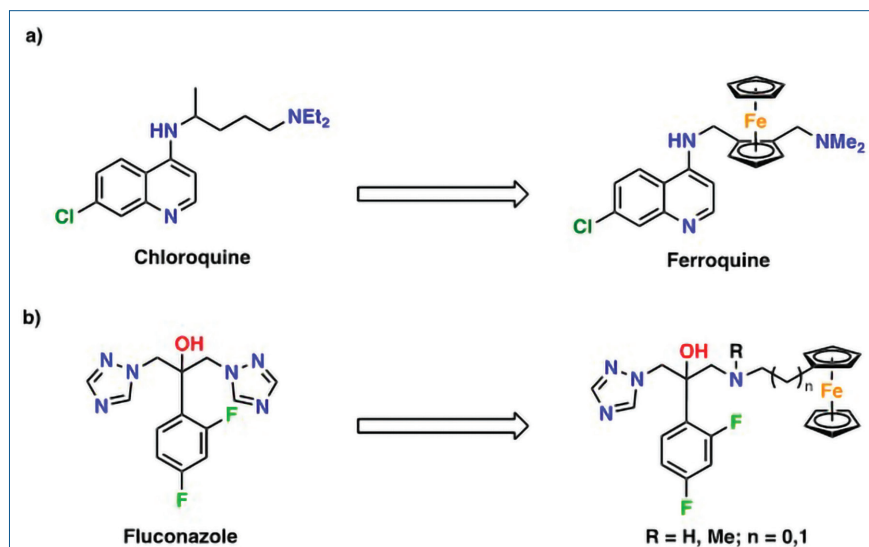


Fig. 5 - a) Chloroquine, an FDA approved antimalarial, and its metallic counterpart ferroquine, currently in clinical trials, b) fluconazole has been derivatized with a ferrocene moiety, on the same basis than chloroquine

the one on its outside part. Thus, these cells are much less oxygenated, making it harder for type II PSs to be effective [26]. In collaboration with the group of Vicente Marchán, we described the synthesis and biological applications of Ru(II)-based coumarin conjugates (Fig. 4) [27]. These complexes were constructed around a 2,2'-bipyridine ligand functionalized with a coumarin unit. This series of **coupy** ligands was used for coordination reactions to Ru(II) polypyridyl precursors. The Ru-**coupy** conjugates were able, upon light irradiation, to show formation of type I ROS, accompanied by an extremely strong phototoxicity *in vitro* against a mouse cancer cell line. These properties come with a lack of toxicity in the dark and high stability in biological media. Also, **Ru-6** showed promising results *in vivo* in mice models, making them very promising candidates to treat efficiently hypoxic tumors.

Our group has also been involved in the development of new generations of anti-infective drugs. Growing resistance to marketed anti-infective drugs has been observed, which makes the need to develop molecules bypassing them extremely urgent [28]. In the past years, ferrocene-derivatives of marketed drugs have been developed, with the aim of avoiding the resistance mechanisms involved in the inefficiency of the organic drugs. Ferrocene was first synthesized in the 1950's, and its first biological applications came in the 1960's.

This metallocene is an interesting drug candidate because of its high lipophilicity and, importantly, ferrocene can be, in some cellular organelles, oxidized to its ferrocenium form, which can lead to ROS production by Fenton reactions. The association of this sandwich Fe(II) complex with chloroquine led to the discovery of ferroquine, the lead ferrocene derivative now in Phase II clinical trials for the treatment of chloroquine resistant malaria strains (Fig. 5a). With this idea in mind, our group developed a series of ferrocene-based analogues of fluconazole, a marketed antifungal drug (Fig. 5b) [29]. These organo-metallic conjugates showed great

activity *in vivo* against different parasite types, and, importantly, with different modes of action than the ones described for the organic counterparts, which is very promising for the development of drugs active against resistant parasite types.

Conclusion

In the past decades, there has been growing concern in medicine due to the increasing resistance of cancers to existing drugs. Additionally, a lack of targeted activity of these drugs have created very often severe side effects to the patients. In this context, the urge to develop new generations of drugs to fight these diseases is evident. For many years, our group has been tackling these challenges. On one hand, we developed **Ru-1-2** as alternatives to cisplatin, which showed promising *in vivo* tumor reduction results. Additionally, these metal complexes were found to use different modes of action to kill the tumor, making them promising drug candidates to treat cisplatin-resistant tumors. With the chemical encapsulation of **Ru-3** in PLA polymers, we developed a platform for the higher and more targeted release of Ru complexes to the tumor site. Also, we have synthesized and tested **Ru-4-5-6** and **Os-1** for PDT, an important complementary technique to surgery and chemotherapy to help fight different types of localized diseases such as cancer. These complexes displayed a shift

of the irradiation wavelength in the deep-red region, better targeting of cancer cells, and improved pharmacokinetics.

An important milestone of our group has been definitively achieved with a ^{89}Zr complex reaching clinical trials for radioimaging purposes. Altogether, these achievements set the groundwork for future translational applications and the design of novel therapeutic platforms

Acknowledgements

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Recenti sviluppi nell’uso dei complessi metallici in chimica farmaceutica

I complessi metallici sono usati in medicina da tempo, ma il campo si è evoluto dopo la scoperta del cisplatino, un importante agente anticancro, negli anni Sessanta. Ciò ha stimolato l’interesse per i farmaci a base di metalli. In questa mini-review vengono presentati dieci anni di ricerche del gruppo su agenti radioimaging e nuovi candidati anticancro e antifungini mirati.