CHAPTER 1

GENERAL INTRODUCTION

The section on anticancer gold complexes is based on the review paper:
Dalton Trans. **2014**, *43*, 4209-4219

1/ Metal ions in medicine

Metal ions are naturally present in biological systems. Indeed, metals such as iron, calcium, copper, zinc, magnesium, sodium or potassium are well-known essential elements of human body homeostasis. However, beyond these elements, many more are also present in traces. Thus, even though some of them are generally considered as toxic, metals such as selenium or molybdenum present benefic effects when present at very low concentrations.[1, 2] Moreover, not only the element or its amount inside the cell has to be considered in biology, but also the coordination environment of the metals is crucial to determine the balance between beneficial and toxic effects. As an example, "free" copper ions are almost absent in the cytosol and are highly toxic. Copper is carried through the cytoplasm by chaperons such as Atox1 and delivered to its actual destination such as mitochondria where it is incorporated into metalloenzymes. One example of such enzyme is cytochrome c oxidase which has a Fe/Cu center in its active site for the conversion of O_2 to H_2O.[3] This highlights the importance of the coordination chemistry of metals in biological systems.

Within this frame, coordination chemistry appears as a mandatory tool to describe the interactions of metal ions in cells, as well as to develop new drugs based on metal ions. The field of medicinal metal-based chemistry started at the beginning of the XX^{th} century with the use of the arsenic-based compound Salvarsan for the treatment of syphilis.[4] Since then, this research area has been developed and metal-based compounds have appeared from anecdotic to widely used as treatments for several diseases as stomach ulcers (bismuth),[5] diabetes (vanadium),[6] rheumatoid arthritis (gold)[7] and cancer (platinum).[8] Another important set of applications of metal complexes in medicine is their use for diagnosis purposes such as for example contrast agent in Magnetic Resonance Imaging (MRI) (gadolinium-, manganese- or iron-based complexes)[9, 10] or as radio-pharmaceuticals (^{99m}technetium, ^{68}galium or ^{186}rhenium).[11, 12] A cartoon-picture of the current use of metal-based and inorganic compounds in the clinic is depicted in Figure 1.[13]

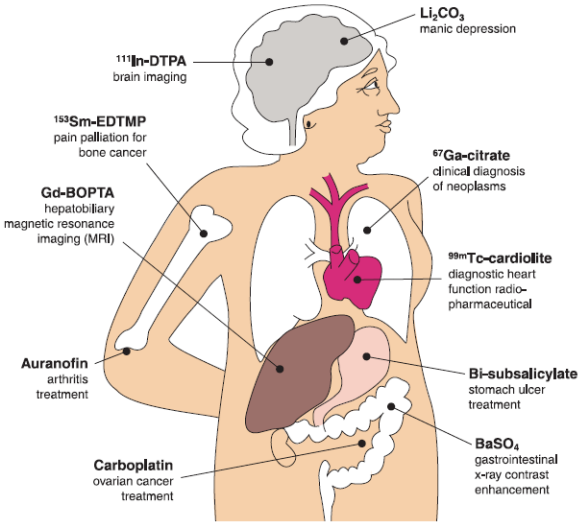


Figure 1: Overview of the use of metal-based drugs in medicine. Reproduced from ref. 13.

One of the main advantages of metal-based compounds is the high variability of possible structures and diversity of coordination geometries from linear two-coordinated, tetrahedral or square-planar, octahedral to even nine-coordinated for lanthanides as depicted in Figure 2.

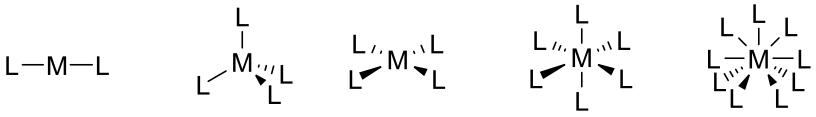


Figure 2: Examples of achievable geometries with metal-based compounds.

This leads to extremely diverse structures. Thus, several research groups exploited metal centers to build up unprecedented 3D-architectures enabling increasing the selectivity toward selected biological targets.[14, 15, 16, 17] Moreover, the particular redox chemistry of transition metals opens also opportunities to design compounds with tuned redox properties in biological systems. This chapter will present the state of the art of anticancer metallodrugs from the most studied and used, the platinum-based compounds, to continue with the recent advances dealing with other transition metals such as ruthenium, iron, titanium, copper and gold. I will also discuss the strategy of using polymetallic complexes. In each case, I will present different representative examples and discuss their pharmacological effects and possible mechanisms of action.

2/ Platinum-based anticancer drugs

2.1/ Pt(II) complexes

Since the end of the 60's and the discovery of the antiproliferative properties of the *cis*-diamminodichloridoplatinum(II) by Rosenberg[11], platinum(II) compounds have been widely used in clinics as chemotherapeutic agents (fig. 3). Nowadays, among the thousands of platinum(II) compounds synthesized and screened for their anticancer properties, only three have been worldwide approved: Cisplatin, Carboplatin and Oxaliplatin, and other three have been approved at least in one country: Nedaplatin in Japan, Lobaplatin in China and Heptaplatin in the Republic of Korea (fig. 3). [18]

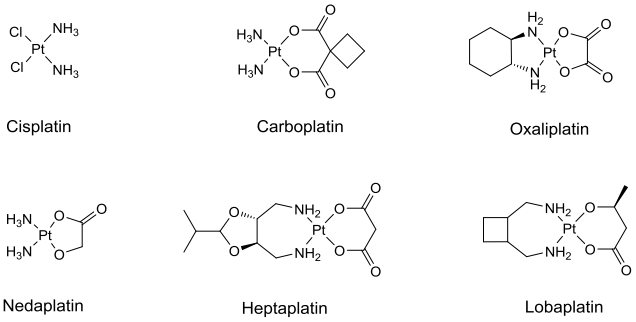


Figure 3: Platinum compounds that reached market in at least one country.

Cisplatin is widely used in treatment regimes in combination with different other drugs such as topoisomerase II inhibitors (doxorubicin, etoposide, mytomycin and epirubicin), mustards (cyclophosphamides, melphalan and ifosfamide), antimetabolites (gemcitabine, 5-fluorouracil and methotrexate), vinca alkaloids (vinblastine and vinorelbine) and taxols (paclitaxel).[19] It is currently applied to treat testicular cancer (for which the cure rate exceeds 90 % and rises almost 100 % for the early-stage diseases), ovarian (although it tends to be replaced by Carboplatin), bladder, melanoma, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), lymphomas and myelomas.[20, 21] However, in spite of their great clinical success, platinum compounds present severe side effects including nephrotoxicity, emetogenesis and neurotoxicity that limit the doses administrated to patients. While some side effects can be managed by combining with other drugs, neurotoxicity remains a significant dose-limiting toxic effect. Moreover, both acquired and intrinsic mechanisms of resistance limit their spectrum of action.[22] Understanding the cellular mechanism of Cisplatin-induced effects is thus a major goal in medicinal inorganic chemistry in order to

rationally design new compounds that overcome resistance mechanisms and present decreased side effects.

Concerning the mechanisms of action, Cisplatin remains in its intact dichlorido form in the blood stream due to the high extracellular chloride concentration (100 mM). Once it enters the cells, hydrolysis of one or both chlorido ligands to afford aqua complexes $[Pt(NH_3)_2Cl(OH_2)]^+$ or $[Pt(NH_3)_2(OH_2)_2]^{2+}$ will occur due to the lower chloride intracellular concentration (4-50 mM). These aqua species will then react with the biological targets.[23] DNA is recognized to be the main intracellular target of platinum-based drugs and Pt-nucleic acids interactions have been abundantly reviewed.[23, 24, 25, 26] Platinum(II) compounds interact with DNA *via* the coordination of the *cis*-$[Pt(NH_3)_2]$ fragment to the N^7 atom of purine bases (guanine and adenine) by displacing the aqua ligands to form mainly 1,2- *cis*(GG) and *cis*(AG), 1,3-intrastrand *cis*(GNG) crosslinks or interstrand crosslinks.[23] A cartoon-picture of the most relevant Cisplatin-DNA adducts is depicted in Figure 4.

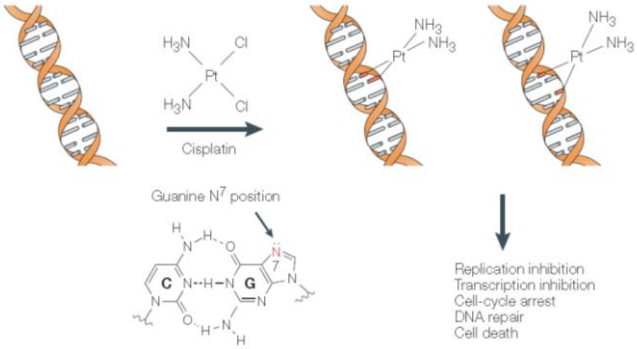


Figure 4: Mechanisms of interaction of Cisplatin with DNA. Reproduced from ref. 23.

Such crosslinks distort the structure of DNA duplex, notably by bending it significantly toward the major groove modifying the binding site of proteins. This bending can interfere with several transduction pathways including the p53 pathway, a protein responsible of induction of cell-cycle arrest or apoptosis in response to cellular stress and mutated in the majority of human tumors.[23] Moreover, it has been shown that Cisplatin can induce G2-arrest leading to cell death.[23, 24]

Beyond the interactions of platinum-based compounds with DNA described in the previous section, the reaction of this class of metallodrugs with proteins has appeared fundamental for the understanding of the biological properties of platinum-based compounds.[27] Indeed, interactions between proteins and platinum-based drugs are involved in numerous events including the regulation of metallodrug influx/efflux and detoxification.

The accumulation of Cisplatin is not completely understood and various mechanisms have been investigated and proposed, including passive diffusion, Na^+,K^+-ATPase, copper transporters (e.g. CTR1), and organic cation transporters (OCTs).[28] A cartoon-picture summarizing Cisplatin's tranporters is presented in Figure 5.

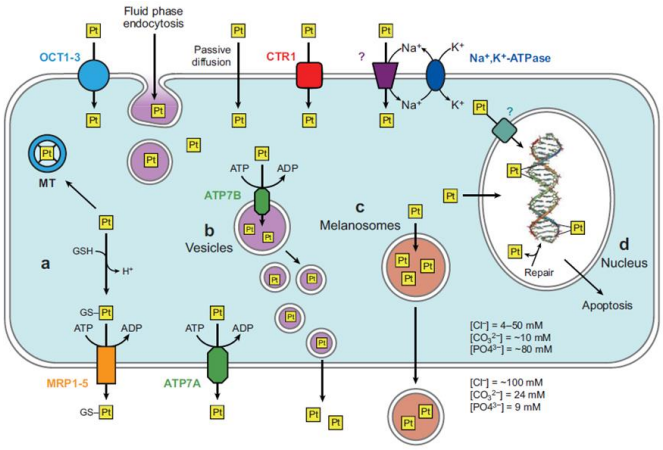


Figure 5: Proposed influx/efflux mechanisms of Cisplatin. Reproduced from ref. 28.

GSH and Atox1 are involved in the efflux mechanism of Cisplatin as well. Indeed, GSH, a tripeptide containing a cysteine residue, can coordinate heavy metals and transfer them to GS-X pumps eliminating GSH-conjugated species. That transporter family is known as multidrug resistance-associated proteins (MRPs) (fig. 5).[28] Atox1 is a chaperon involved in copper homeostasis by transferring it to the copper efflux transporters ATP7A and ATP7B. The crystal structure of an adduct Atox1-Cisplatin has been solved suggesting its involvement in the mechanism of excretion of Cisplatin (fig. 5).[27, 28] Metallothioneins (MTs) are a class of small proteins characterized by a high amount of cysteine residues (20 cysteines in a 61-68 amino acid protein) responsible for the detoxification of heavy metals including platinum by sequestrating them (fig. 5). Such interactions lead to inactivation of the drugs inducing mechanisms of resistance toward platinum-based compounds.[27] Moreover, zinc-finger proteins have been identified as possible target of platinum-based drugs by displacement of the zinc cation by platinum. Within this frame, Cisplatin has been demonstrated to inhibit the zinc-finger protein poly(ADP-ribose) polymerase 1 (PARP-1) involved in the DNA repair mechanism.[29]

2.2/ Pt(IV) complexes

Pt(IV) compounds present an octahedral geometry introducing two extra binding sites compared to Pt(II) complexes. Moreover, the kinetic inertness of Pt(IV) compounds toward reduction or ligand substitution makes them unlikely to react with biological nucleophiles. This increases their lifetime in blood and thus their chance to reach the tumor and enter the cells intact. It is hypothesized that the activity of Pt(IV) compounds follows the reduction of Pt(IV) to Pt(II) and the subsequent release of the two axial ligands leading to the biologically active Pt(II) species.

Within the various families of experimental Pt(IV) compounds developed so far, different drug design strategies were applied including: i) tuning the rate of reduction of the Pt(IV) center and the compound's lipophilicity to optimize the release of Pt(II) drug into the cell[30] and ii) making use of the two axial positions to couple Pt(II) moieties to bioactive ligands to allow "bifunctional" and targeted therapy.[31]

Satraplatin, a *trans*-diacetato-*cis*-dichlorido Pt(IV) compounds with a cyclohexylamine ligand (fig. 6) has been shown to induce cell death in Cisplatin-resistant cell lines including human ovarian, lung and prostate cancer cell lines.[18, 30] Satraplatin passed Phase I clinical trials in which myelosuppression and nausea appeared as dose limiting toxicities (DLTs). It reached Phase II clinical trial for the treatment of small-cells lung cancer and hormone refractory prostate cancer. It also gave interesting results in Phase III clinical trials in combination with prednisone on patients with refractory cancer (SPARC) reducing by 40 % the risk of cancer progression. However, it was rejected by the FDA due to lack of benefits in terms of overall survival. It is currently under Phase I, II and III clinical trials in combination with different drugs.

Some groups took advantage of the extracellular stability of the Pt(IV) metal center to build up targeted Cisplatin pro-drugs and "bifunctional" compounds bearing organic drugs. The main limitation of this approach is the intrinsic inertness of Pt(IV) complexes which renders the exchange of the axial ligands impossible and limitates the scope of ligands that can be introduced in these positions to the ones that can be incorporated during the oxidation step. Following this methodology, some *trans*-dihydroxido and *trans*-dichorido complexes were synthesized by oxidation with hydrogen peroxide or chlorine gas respectively.[32, 33] Keppler *et al.* reacted the dihydroxido complex with succinic anhydride to obtain complexes with free carboxylate groups available for further coupling with alcohols or amines.[34]

Dyson *et al.* developed a Cisplatin-based Pt(IV) complex incorporating two equivalents of ethacrynic acid (ethacraplatin, Fig. 6), a known inhibitor of the enzyme Glutathione-S-transferase which is supposed to be involved in the mechanism of resistance to Cisplatin. Indeed, ethacraplatin showed a faster toxicity against all tested Cisplatin-resistant cell lines compared to Cisplatin alone.[35]

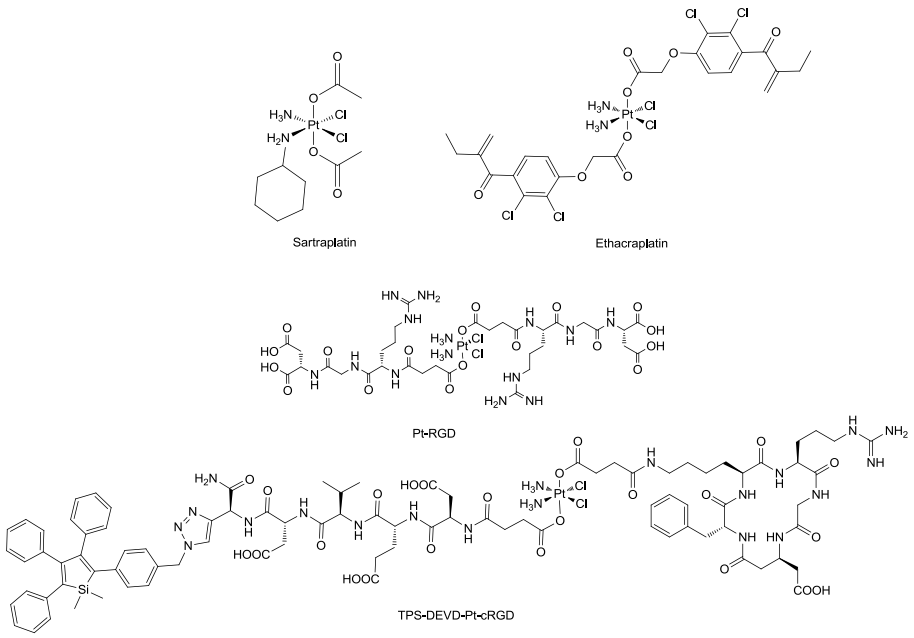


Figure 6: Examples of Pt(IV) complexes under investigation.

Lippard *et al.* developed a series of Pt(IV) complexes presenting different peptides for targeting $\alpha_V\beta_3/\alpha_V\beta_5$ integrins overexpressed in endothelial tumor cells.[35] An example (Pt-RGD) is presented in Figure 6. A recent work from Liu *et al.* reports the development of a fluorescent Pt(IV)-based targeted agent (TPS-DEVD-Pt-cRGD, Fig. 6).[37] In this compound cRGD moiety enables it to target cancer cells overexpressing integrin $\alpha_v\beta_3$ receptors. Moreover, the drug-induced apoptosis by *in cellulo* generated Cisplatin was detected monitoring the fluorescence of the tetraphenylsilole moiety (TPS) activated by caspase-3.

3/ Ruthenium-based compounds

Ruthenium is studied as potential anticancer agent since the early 80's following Clarke's work who showed that the coordination complex *fac*-$[(NH_3)_3RuCl_3]$ presented an anticancer activity in murine models.[38] In physiological environment, the most common oxidation states for ruthenium are +IV, +III and +II.[39] In addition, ruthenium presents a relative kinetic inertness with ligand exchange time in the range of minutes to a few days (corresponding to ligand exchange kinetic of platinum) associated with affinity for sulfur-, nitrogen- and oxygen-donor containing ligands.[40] As ruthenium belongs to the same column of transition metal as iron, it is isoelectronic to iron and can thus mimic its binding to

proteins like albumin and transferrin, which can facilitate its solubility and transportation through plasma.[38]

3.1/ Ru(III) coordination complexes

Among ruthenium-based compounds, coordination Ru(III) complexes are the only compounds to have reached clinical trials (fig. 7). Indeed, *trans*-[$RuCl_4$(Im)(*κ*-S-DMSO)][ImH] (NAMI-A) developed by Sava *et al.* and *trans*-[$RuCl_4(Ind)_2$] [IndH] (KP1019) developed by Keppler *et al.* entered clinical trials in 1999 and 2002 respectively.[41, 42]

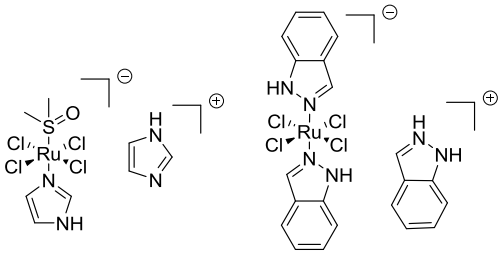


Figure 7: Chemical structure of NAMI-A and KP1019.

In spite of their high similarity, these two complexes present very different behavior in both *in vitro* and *in vivo* experiments: classically NAMI-A does not show cytotoxicity *in vitro*, whereas KP1019 shows marked antiproliferative activity against colorectal and colon carcinoma cell lines. Moreover, NAMI-A has been shown to act selectively against metastasis by inhibiting their proliferation and their development, when KP1019 has been proved to treat only primary tumors.[43] Such differences of behavior is attributed to very different kinetics of aquation and reduction to Ru(II).[44]

Since the pioneering studies on Ru(III) complexes, Clarke proposed the hypothesis of "activation by reduction", following the same principle as that of Pt(IV) derivatives, to explain the biological properties of such compounds: Ru(III) complexes are actually pro-drugs that require to be reduced to the active Ru(II) species by intracellular reductants.[43, 45] Due to their fast development and the lack of oxygen in the tumor environment, cancer cells overproduce energy *via* glycolysis resulting in an abnormally high level of lactic acid that creates a more reducing environment capable to convert Ru(III) into Ru(II). However this hypothesis has never been proved up to now and its relevance to explain biological properties observed *in vitro* and *in vivo* remains under debate.[46]

These complexes were originally designed following the instance of Cisplatin to interact with DNA but with the hope of a different mechanism of action to overcome

Cisplatin resistance mechanisms. Brabeck *et al.* showed that ruthenium-based compounds can actually interact with DNA.[47] In fact, although KP1019 may induce intrastrand adducts capable of stopping RNA synthesis *in vitro*, no real correlation between DNA binding and biological activity has been found yet. Conversely, for both NAMI-A and KP1019, it has been proved that their *in vivo* activity is due to interaction with other cellular components including proteins and enzymes. For example, NAMI-A's antimetastatic activity would be due to its interaction with collagen of the extracellular matrix,[48] as well as with intracellular proteases such as cathepsins[49] when KP1019 has been shown to be able to interact with proteins, including serum proteins albumin and transferrin. Moreover it can induce apoptosis through mitochondrial pathways by activation of caspase-3.[50]

3.2/ Ru(II) complexes

Based on the "activation by reduction" hypothesis suggesting that the actually effective species of ruthenium-based anticancer agent is at the +II oxidation state, several groups in the world started working on Ru(II)-based compounds which are active without the need of further reduction in a biological environment. Below we will present different families of Ru(II) complexes starting with the classical coordination compounds to continue with the organometallics. For each family I will focus on the most representative compounds and discuss their different mode of action.

3.2.1/ Ru(II) polypyridyl complexes

Ru(II) polypyridyl complexes have been known to efficiently interact with DNA.[47] In 2000, Reedijk *et al.* reported three isomers of dichlorido-bis(2-phenylazopyridine)ruthenium(II) complexes with very different anticancer activities *in vitro*: the α-[Ru(II)(azpy)$_2$Cl$_2$] (fig. 8) being almost ten times more efficient than Cisplatin.[51] This compound was shown to react with the nucleobase model 9-ethylguanine through coordination of the N^7 on the Ru center.[52] Analogues of this complex where the chlorides have been replaced by more stable toward hydrolysis dicarboxylate ligands showed a decreased activity but in any case no cross-resistance with Cisplatin or Carboplatin has been observed suggesting a different mode of action.[53] The replacement of the hydrolysable ligands by a third (N^N) chelating ligand of the bipy or azpy types resulted in the impossibility of direct binding between the metal center and DNA. Nonetheless, a reduced but still present anticancer activity was observed, suggesting that these compounds could trigger cell death through intercalative interaction with DNA.[54] Moreover, Gust *et al.* demonstrated that Ru(II) *tris*-polypyridyl bearing an extended aromatic surface ligand 4,5,9,16-tetraazadibenzo[a,c]naphtacene (fig. 8) with micromolar toxicity on cancer cells *in vitro* was active *via* a modification of the cell membrane function and cells adhesion properties.[55]

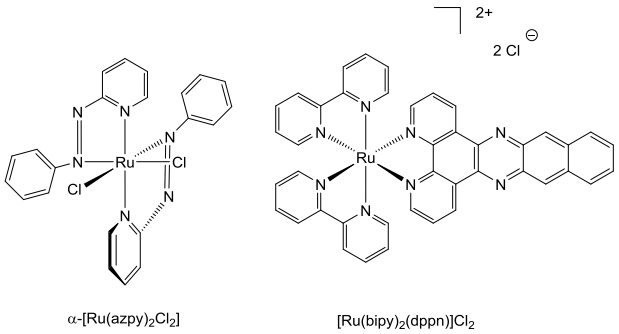


Figure 8: Chemical structures of two Ru(II) polypyridyl complexes with anticancer properties.

In the last years, Ru(II) polypyridyl complexes presenting extended aromatic systems have been proved to selectively and efficiently interact and stabilize the unusual DNA structure G-quadruplex.[56] G-quadruplexes are peculiar nucleic acid architectures adopted by guanine-rich DNA and RNA sequences, and their stability originate in the stacking of contiguous G-quartets (a planar and cyclic K^+-promoted association of four guanines in a Hoogsteen hydrogen-bonding arrangement) as presented in Figure 9.[57]

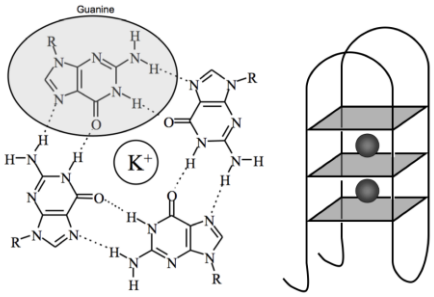


Figure 9: Scheme of a guanine quartet stabilized by the presence of a potassium cation (left) and an example of a G-quadruplex DNA structure in which planes represent quartets (right). Spheres represent K^+ ions.

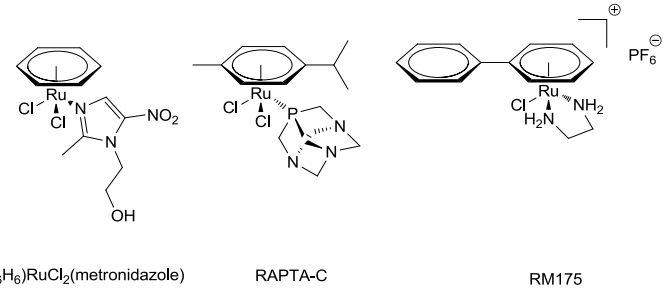
Quadruplexes are currently intensively studied since they are suspected to play important roles in key cellular events: quadruplex-forming DNA sequences are indeed found in several pharmacologically relevant areas such as eukaryotic telomeres,[58] promoter regions of identified oncogenes[59] or promotor regions of HIV-1.[60] Their stabilization by selective small molecules (also called G-quadruplex ligands)[61] is thus currently investigated as a mean to control key cellular events (telomere homeostasis and, beyond this, chromosomal stability, as

well as regulation of oncongenes expression, respectively). Moreover, accumulating evidence now points towards an increasing role of quadruplex ligands as DNA damaging agents.[62] This concept has been widely applied in the case of compounds that can target telomeric DNA.[63]

Finally it is worth mentioning that Ru(II) polypyridyl complexes possess interesting photophysical properties such as luminescence, photostability, as well as low metal to ligand charge transfer (MLCT) in the visible region avoiding the use of UV light and emission in the far-red region. Moreover, while MLCT-based luminescence is quenched in the free Ru(II) polypyridyl complexes, upon intercalation with DNA and overlapping of π systems between DNA bases an intense MLCT-based luminescence is displayed. That phenomenon called DNA "light switch" has been used to study interaction of such compounds with DNA.[64]

3.2.2/ Organometallic (η^6-arene)Ru(II) complexes

Tocher *et al.* showed in 1992 the ability of the organometallic moiety (η^6-arene)Ru(II)Cl_2 to enhance the cytotoxicity of the anticancer drug metronidazole [1-β-(hydroxyethyl)-2-methyl-5-nitroimidazole] (Fig. 10) upon coordination.[65] Following these results, the field was largely explored by Dyson *et al.* They used the fragment [(η^6-arene)Ru(II)Cl_2] coordinated by the water-soluble phosphane ligand PTA (PTA = 1,3,5-triaza-7-phosphaadamantane) to give the so-called RAPTA complexes.[66] The prototype RAPTA-C (arene = *p*-cymene) is depicted in Figure 10. Sadler *et al.* used ethylenediamine to chelate the fragment [(η^6-arene)Ru(II)Cl]$^+$ as in the case of the prototype compound RM175 (arene = biphenyl) (Figure 10).[67]



(η^6–C_6H_6)$RuCl_2$(metronidazole) RAPTA-C RM175

Figure 10: Chemical structure of pioneers (η^6-arene)Ru(II) complexes.

The obtained results were the starting point of a very large library of compounds in which the arene,[68] the chelating and phosphane ligands,[69] the hydrophobicity,[70] as well as the metal[71] have been changed to try to draw a structure-activity relationship. Even though

Dyson's and Sadler's compounds are very similar, both presenting the so-called "piano stool" geometry, they exert very different biological effects *in vitro* and *in vivo*.[72]

Indeed, RAPTA complexes show very low cytotoxicity *in vitro* against cancer cells but are highly active *in vivo* as antimetastatic agent being thus closer to NAMI-A in term of biological properties. RM175 is as efficient as Carboplatin (IC_{50} = 6 µM against A2780 cells), and its analog, where the more lipophilic ligand tetrahydroanthracene replaces biphenyl, is as toxic as Cisplatin (IC_{50} = 0.6 µM against A2780 cells) *in vitro*. These results were confirmed *in vivo* as RM175 reduced growth of A2780 xenograft twice more than Cisplatin for tolerated doses.[73] Moreover, it has been proved that these two types of arene-ruthenium compounds have different intracellular targets: the RAPTA family has been demonstrated to efficiently inhibit enzymes such as the cysteine protease cathepsin B, responsible for the degradation of the extracellular matrix, *via* interaction with Cys residues in the enzyme active sites.[74] RM175 interacts preferentially with DNA *via* non-covalent hydrophobic bonds with the arene ligand,[52] and through the substitution of the chloride ligand by N^7 of guanine residues.

Interestingly, arene Ru(II) complexes (fig. 11) have also been reported to selectively inhibit cancer cells growth by arrest in S-phase through stabilization of a G-quadruplex structure in an oncogene promotor area.[75]

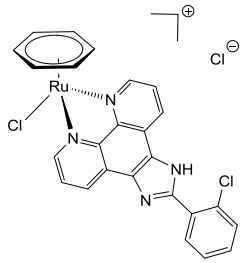


Figure 11: Example of arene Ru(II) polypyridyl complex reported for anticancer properties *via* G-quadruplex stabilization.

3.2.3/ Cyclometalated Ru(II) complexes

Another type of ruthenium(II) organometallic complexes designed for anticancer purposes are the cyclometalated compounds characterized by a covalent C-M (M = metal) bond and developed by Pfeffer *et al.* under the name RDC (Ruthenium Derived Compounds).[76] Among the various compounds of this family, RDC11 (fig. 12) has shown particularly interesting antiproliferative properties *in vitro* in several cancer cell lines.[77] Moreover, it has been shown that this compound could block the cell cycle in G_1 phase, a characteristic of cell death by apoptosis. RDCs induce apoptosis through activation of p53

similarly to Cisplatin. However, it was demonstrated that for RDCs, the induction of apoptosis is only partially p53-dependent which, in association with the lower propensity of RDCs to excretion by ATP7B than Cisplatin, could suggests that RDCs induce apoptosis through a different pathway.[77] Moreover, *in vivo* RDC11 has been shown to reduce tumor growth of B16F10 melanoma cells implanted in mice as efficiently as Cisplatin but with reduced side effects such as weight loss, liver, kidney and neurotoxicity.[78]

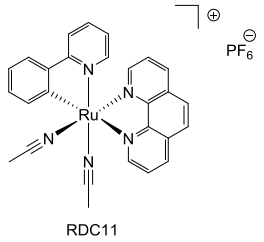


Figure 12: Chemical structure of the cycloruthenated complex RDC11.

3.2.3/ "Structural" organometallic Ru(II) complexes

This class of compounds developed by Meggers *et al.* relies on a very different design strategy: while metal-based drugs are usually engineered for the metal center to directly interact with the biological target, for instance by replacement of hydrolysable ligands, in these compounds the ruthenium center is completely inert toward ligand substitution and is just present to build up 3D structures unreachable by classical carbon chemistry.[46] As an example, compound DW1/2 (fig. 13) has been developed to mimic the natural product staurosporine, a potent inhibitor of protein kinases. This compound presents a metal-centered chirality and the S enantiomer (DW2) displays a better selectivity for one particular kinase, namely Pim-1 against which its efficacy is almost 100 times higher than staurosporine. Indeed this enantiomer mimics perfectly the interaction of the organic compound with the protein as shown by the crystal structure of Pim-1 with DW2.[79]

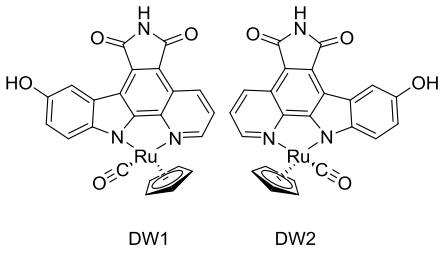


Figure 13: Chemical structure of two enantiomers of the half-sandwitch complex DW1/2.

The racemic mixture (DW1/2), which is an inhibitor of GSK3β kinase, was tested against a panel of human melanoma cells overexpressing this protein and showed an activity in the low micromolar range against all tested cell lines. DW1/2 induces apoptosis on melanoma *via* intrinsic mitochondrial pathway while it is poorly toxic against normal melanocytes.[80] It is worth mentioning that no interaction between the compound and DNA have been highlighted neither by coordination to nucleobases nor by intercalation into DNA duplex. This suggests that the anticancer activity of such compound is due to its ability to inhibit kinases and is thus independent to the direct involvement of the metallic center.[81]

4/ Iron-, titanium- and copper-based anticancer drugs

4.1/ Iron-based complexes

Chemotherapeutic treatment of breast cancer mainly consists in administrating selective estrogen receptor modulators (SERMs) with Tamoxifen (fig. 14) being one of the most potent compounds in clinic. Thus, the coupling of a SERM to a metal-based scaffold appeared to be an interesting way to enlarge the scope of treatable cancers by metallodrugs. This concept was successfully applied by Jaouen, Vessières and coworkers who replaced the β-aromatic cycle in Tamoxifen, or in the analogue bearing an hydroxyl group in position 4 of the α aromatic cycle (hydroxytamoxifen), with a ferrocene moiety affording ferrocifen or OH-ferrocifen, respectively (fig. 14).[82]

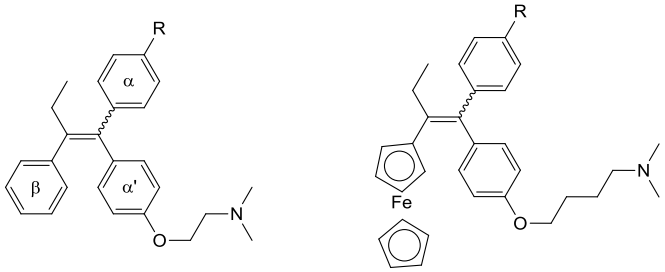


Figure 14: Chemical structure of the SERM tamoxifen and its ferrocene analog ferrocifen.

It is worth mentioning that ferrocene belongs to the metallocene family in which the metal center is coordinated to two cyclopentadienyl ligands (Cp). In ferrocene, the iron center is in the +II oxidation state making ferrocene a neutral entity. Moreover, ferrocene attracted interest in medicinal chemistry due to its chemical stability, low toxicity, broad range of derivatization as well as interesting redox properties.[83]

In vitro, OH-ferrocifen appeared to inhibit the proliferation of the estrogen receptor-positive cell line MCF-7 at 1 µM with the same efficiency as OH-tamoxifen. Moreover, upon addition of estradiol, an estrogen inducing proliferation of the estrogen receptor-positive cells, OH-ferrocifen maintained certain toxicity. This finding suggests that ferrocifens trigger their toxic effects both by the estrogen receptor (ER) inhibitor capacity of the organic part and by intrinsic cytotoxicity of the organometallic moiety.[82] This effect was confirmed in the screening of several cell lines, both ERα(+) and ERα(-). On ERα(+) cells, at a concentration of 0.1 µM, OH-ferrocifen presented an anti-hormone effect equivalent to OH-tamoxifen associated to a cytotoxic effect *via* accumulation of cells in S phase and ROS production. On ERα(-) cells, at a concentration of 1 µM, OH-ferrocifen still induced cytotoxicity while OH-tamoxifen appeared unefficient.[84]

To further investigate structure-activity relationships, different ferrocene-based analogues of Tamoxifen were synthetized bearing several modifications in the lead scaffold: *i.e.* the hydroxyl group was removed, the ethyl chain was replaced by an aromatic ring, the conjugation between ferrocene and the aromatic ring was cut and a cyclopentadienyl ligand was substituted by methyl groups.[85] These new derivatives were screened against hormone-dependent (Tamoxifen-sensitive) and hormone-independent (Tamoxifen-resistant) breast cancer cell lines. It appeared that some of the tested compounds presented cytotoxic effects even on the hormone-independent cell lines. Conjugation between the ferrocene and the aromatic rings as well as the presence of the ethyl chain on the same carbon as the ferrocene moiety have been shown to be necessary for the development of cytotoxicity in cells.

From a mechanistic point of view, various studies confirmed that the oxidation of ferrocifens to quinone methide is the crucial step to drug activation (fig. 15). In fact, the quinone methide is believed to be the metabolite which will further interact with biological targets. In this case, ferrocene can be thought to act as an intramolecular oxidation antenna.[85]

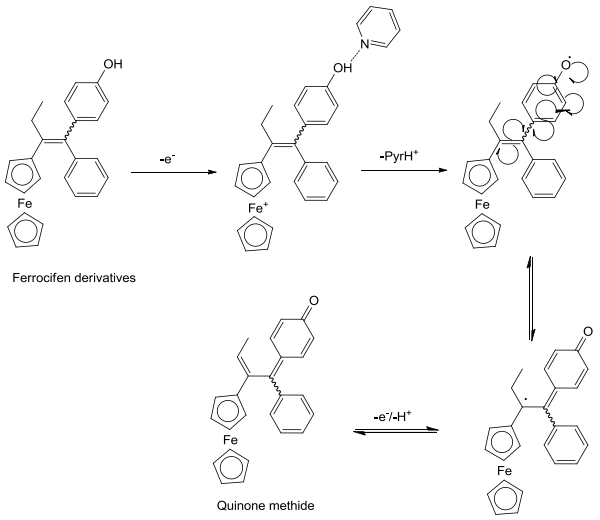


Figure 15: Mechanism of activation of ferrocifen in presence of a base and formation of quinone methide. Reproduced from Ref. 85.

4.2/ Titanium-based complexes

Titanocene dichloride (fig. 16) is an organometallic Ti(IV) complex that was tested as a possible anticancer agent in the 80's based on the fact that it presents two chlorido ligands in *cis* positions like Cisplatin.[86] The compound appeared to be active in cancer cells both *in vitro* and *in vivo* on xenografted human gastrointestinal and lung carcinomas.[87] Titanocene dichloride reached phase I clinical trials where nephrotoxicity and increase of creatinine and bilirubin were noticed. In phase II clinical trial, it was found to be inefficient against metastatic renal-cell carcinoma.[38] Titanium was shown to accumulate in nucleic acid rich areas where it acts by disrupting DNA synthesis, thus, highlighting DNA as a possible target *in vivo*.[88] Moreover, Sadler *et al.* showed that titanium-based compounds could interact with the iron-transporter protein transferrin which internalizes iron from blood to the cells *via* an endocytosis mechanism. This interaction is proposed to be responsible of titanium uptake and release inside the cells.[89] However, due to its very fast hydrolysis in physiological conditions, leading *in fine* to the insoluble TiO_2 species, titanocene dichloride didn't go further in the clinical trials even though some formulations to preserve the titanocene moiety were explored.[87, 88]

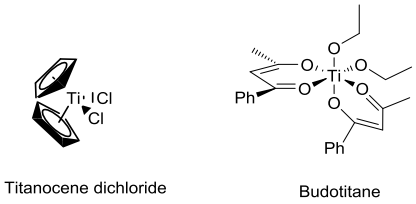


Figure 16: Chemical structure of titanium-based complexes that reached clinical trials.

Another example of titanium-based compound which reached clinical trial is budotitane (*cis*-$[(EtO)_2(bzac)_2Ti]$ where bzac = 1-phenylbutane-1,3-diketonate) (fig. 16). This coordination compound showed interesting results in colorectal cancer in animal models.[87] However, as for titanocene dichloride, hydrolysis of the metal-ligand bounds is the main limitation of budotitane. Some formulations of budotitane with glycerinepolyethylene-glycerolericinoleate and 1,3-propylene glycol yielded to an increased stability of budotitane in aqueous environment. However, the incapacity to well characterize the actually present species prevented budotitane to go further on drug development.[90]

Based on these results on titanocene dichloride, Tacke *et al.* developed different benzyl substituted titanocene analogues. One of them, the so-called titanocene Y bearing a 4-methoxybenzyl substituent presented an IC_{50} value 100-fold lower than titanocene dichloride on pig kidney carcinoma cells.[91] By replacing the two chlorido ligands by an oxalato ligand in titanocene Y (called oxali-titanocene Y), the same group succeeded in obtaining a 13-fold more toxic compound than titanocene Y on pig kidney carcinoma cells.[92] Moreover, oxali-titanocene Y also appeared almost 2-fold more toxic than Cisplatin on the same cell line.

More recently, Tshuva *et al.* described the use of tetradentate bis(phenolato)ligands to increase the stability of *cis*-diisopropoxy titanium complexes toward hydrolysis. Those coordination compounds showed toxicity in the same range as Cisplatin on ovarian and colon cancer cell lines *in vitro*.[93] Moreover, upon rigidification of the tetradentate ligand, Tshuva *et al.* synthetized *trans*-bisphenoxy titanium complexes with lower hydrolysis rate and cytotoxic activities against ovarian and colon cancer cell lines 10-times higher than Cisplatin or budotitane.[94]

4.3/ Copper-based complexes

Copper is a human endogenous metal ion and an essential element for aerobic organisms as catalytic cofactor involved in many biological pathways.[1] Aberrant levels of copper are linked with several pathologies such as Alzheimer's or Parkinson's diseases and Menkes' syndrome.[95] In the last years an increasing number of publications reported copper complexes, both in the +I and +II oxidation states, as efficient anticancer agents *in vitro.*

These complexes presented a very broad range of ligands due to the extremely diverse coordination chemistry of copper. Among the various ligand types screened for Cu(II) complexes we can report S-donor containing ligands such as thiosemicarbazones or dithiocarbamates, acac-based ligands (acac = acetylacetonate), Schiff's bases or bipyridine-like chelators. Some representative examples are depicted in Figure 17.[95]

The coordination of *N*-heterocyclic carbenes to Cu(I) appeared to create highly potent compounds with 10 to 100-fold higher cytotoxicity compared to Cisplatin.[96] While the majority of copper-based anticancer agents have been identified to interact with DNA upon intercalation or by cleavage of the strands upon generation of reactive oxygen species, some studies report copper compounds as efficient inhibitors of topoisomerase I and II and proteasome, thus extending the field of possible biological targets. Several compounds have been tested *in vivo* on Erlich ascites carcinoma, leukemia or breast cancer showing promising anticancer effects, but so far no defined copper-based complex has reached clinical trials.[95]

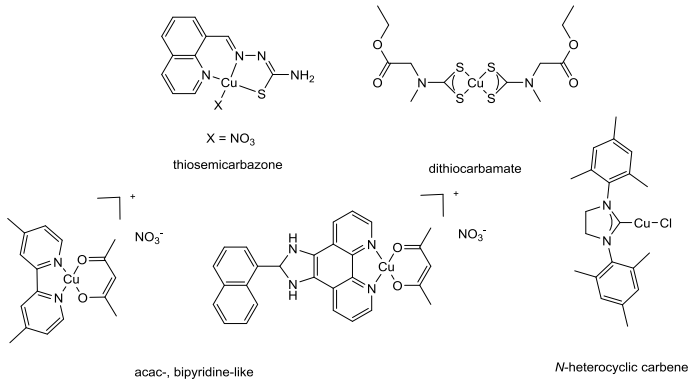


Figure 17: Examples of copper-based compounds studied as anticancer agents.

5/ Gold-based anticancer drugs

Pharmacologic properties of gold are known since the end of 19th century and gold compounds have been tested against different pathologies even though they are currently only used for the treatment of rheumatoid arthritis.[97] However, besides the anticancer application of gold compounds I will describe, it is worth mentioning that gold complexes are currently investigated as possible antimicrobial agents.[98] Gold presents a very broad spectrum of oxidation states from –I to +V, however, only the +I and +III oxidation states which are biologically relevant will be discussed below.[99] Au(I) has a d^{10} electronic configuration and it gives compounds with linear geometry. Au(III) has a d^8 electronic configuration and is thus isoelectronic to Pt(II) and presents a four coordinated square-planar

environment that makes Au(III) complexes interesting candidates for anticancer evaluation. However, due to the fairly reducing intracellular milieu, the tendency of Au(III) center to get reduced to Au(I) makes the choice of ligands very determining.[100]

In this section, I will describe various families of gold compounds including Au(I) and Au(III) complexes. For each oxidation state, I will provide examples of coordination compounds (with phosphane or *N*-donor ligands) as well as organogold (compounds presenting a Au-C bound) and discuss their mechanisms of action.

5.1/ Au(I) phosphane complexes

The first representative member of this family is Auranofin ((2,3,4,6-tetra-*O*-acetyl-1-(thio-*κ*S)-*β*-D-glucopyranosato)(triethylphosphane)gold(I)) (fig. 18), an orally administrated anti-arthritic agent, which has been reported by the end of the 70's to inhibit cancer cells proliferation and DNA, RNA and proteins syntheses *in vitro*.[101] *In vivo*, it has been shown to specifically increase the survival time of mice with P388 leukemia only when the treatment was given intraperitoneally.[102] Based on these promising results, Auranofin and more generally speaking Au(I) compounds bearing phosphane ligands have been extensively studied and information on their chemical and biological properties have been reviewed on a regular basis.[86, 103, 104, 105]

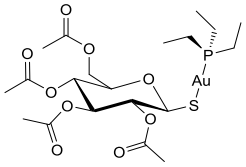


Figure 18: Chemical structure of Auranofin.

The intracellular mechanism of Auranofin has been extensively studied. Contrarily to Cisplatin, Auranofin is not reported to act by direct binding with DNA, but induces apoptosis *via* a mitochondria-related pathway.[101] It was shown to be a highly potent and selective inhibitor of the seleno-enzymes thioredoxin reductases (TrxR), both cytosolic and mitochondrial, with an IC_{50} on the nanomolar range.[106] Considering its overexpression in cancer cells, TrxR appeared as an interesting target for anticancer drugs. TrxRs as well as glutathione reductase (GR) are homodimeric enzymes whose role consists in reducing in a NADPH-dependent manner thioredoxin or glutathione, respectively. TrxR is responsible for the reduction of thioredoxin (Trx) which in turn reduces different proteins including ribonucleotide reductase (involved in conversion of ribonucleotide to desoxyribonucleotides for further synthesis of DNA) and peroxiredoxin (involved in the regulation of cellular level of hydrogen peroxide). [107] The thioredoxin system is depicted in Figure 19.

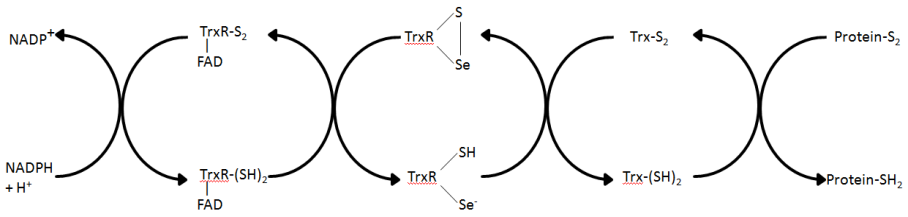


Figure 19: Representation of the electron transfer pathway mediated by the TrxR/Trx system.

Auranofin is supposed to interact preferentially with the selenocysteine residue of TrxRs considering the 1000-fold higher concentration required for the inhibition of glutathione reductase lacking the seleno-residue.[107] This inhibition of the thioredoxin reductase/thioredoxin systems induces mitochondrial membrane permeabilization enabling the release of cytochrome *c* to the cytosol which triggers apoptosis.[108] Moreover, inhibition of TrxR by Auranofin may allow overcoming the cross-resistance with Cisplatin in an ovarian cancer cell line *in vitro*.[109]

Beyond TrxR, Auranofin has been reported as potent inhibitor of the seleno-enzyme glutathione peroxidase responsible of the detoxification of hydrogen peroxyde[110] and more recently as inhibitor of the sulfur-containing enzyme gluthatione-S-transferase which is suspected to be involved in the mechanism of resistance to Cisplatin.[111] Structure-activity relationships studies on several Auranofin analogs, in which both the sugar and the phosphane ligands were varied, showed the great importance of the phosphane for the toxicity.[112] Moreover, a mass-spectrometry study carried out on the reaction between Auranofin, Et_3P-Au-Cl and cysteine or methylselenocysteine showed that, for both gold compounds, the same products $[Et_3P\text{-Au-cysteine}]^+$ and $[Et_3P\text{-Au-methylselenocysteine}]^+$ were formed, giving the opportunity to optimize Auranofin's properties by replacing the thio-*β*-D-glucose tetraacetate moiety.[113]

Other studies on complexes of general formula R_3P-Au(I)-Cl (R = alkyl or aryl) showed a positive correlation between the size of the substituents on the phosphorous atom and the cellular uptake and toxicity.[114] Various targets have been investigated including TrxR but also other proteins/enzymes relevant to cancer. Ph_3P-Au-Cl has been shown to efficiently inhibit *in vitro* cathepsin B, an enzyme belonging to the cysteine protease family implicated in inflammatory mechanisms.[115] The development of gold(I)-phosphole chloride (GoPI) (**1**, fig. 20) and it's thiosugar analogue (GoPI-sugar), the latter being more stable under physiological conditions, lead to highly efficient inhibitors of TrxR and GR accompanied by cytotoxic activities in the low micromolar range against glioblastoma or breast cancer cells *in vitro*.[116, 117]

Ott *et al.* associated the triethylphosphanegold(I) moiety with *N*-(*N',N'*-dimethylaminoethyl)-1,8-naphtalimide ligand, an anticancer agent acting through DNA intercalation of the naphtalimide core and interaction of the protonable amino group with phosphate of the DNA backbone.[118, 119] The resulting Au-Naphth-1 compound (**2**, fig. 20) revealed to be as toxic as Et_3P-Au-Cl *in vitro*, to inhibit TrxR to the same extent, but presented a higher uptake than Et_3P-Au-Cl and antiangiogenesis properties the authors attributed to the presence of the naphtalimide moiety.[118, 119]

Gimeno *et al.* reported the synthesis and the cytotoxic evaluation of Au(I) complexes bearing a diphenylphosphanoaminoheterocycle ligand associated to thiolate functionnalized pyridine or nucleic bases.[120] The compound [Au(TG){PPh_2NH(Htz)}] (TG = thioguanine) (**3**, fig. 20), appeared one order of magnitude more efficient than Auranofin against a cervical cancer cell line, whereas the reversed behavior was observed in case of a breast cancer cell line *in vitro*. Moreover, mechanistic studies demonstrated potent inhibition of TrxR (in the low or submicromolar range) associated with interactions with DNA. However, although the compounds interact with DNA, no structural change of DNA have been noticed which is in good agreement with the proposal of DNA not be being the main target of gold compounds.

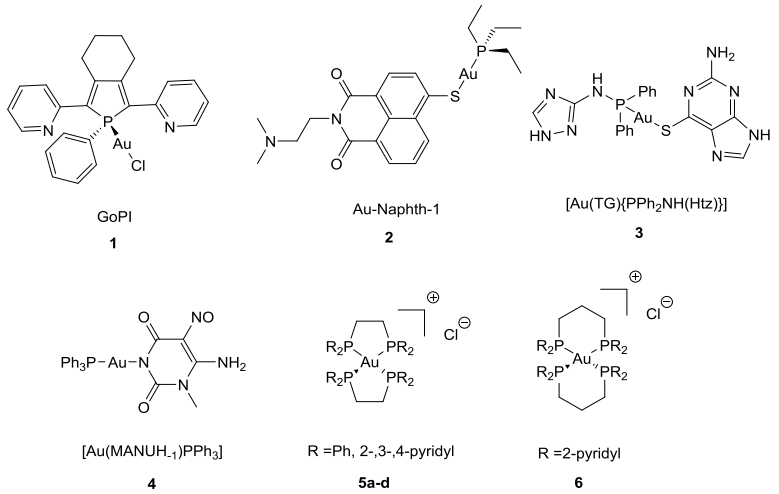


Figure 20: Examples of Au(I)-phosphane complexes investigated for anticancer properties.

In vivo, a triphenylphosphane-gold(I) complex bearing an uracil-based ligand ([Au($MANUH_{-1}$)PPh_3]) ($MANUH_{-1}$ = 6-amino-1-methyl-5-nitrosouracilato) (**4**, fig. 20) has been demonstrated to significantly reduce the growth of glioma tumor in mice. The treatment was associated with weak modification of serum parameters such as electrolytes, biomarkers of renal and hepatic functions and lipid profile, thus suggesting only very moderate side effects.[121]

In the early stage of development of Au(I)-based anticancer agents, Berners-Price *et al.* reported the use of a delocalized lipophilic cation (DLC) based on bis-(diarylylphosphano)ethane (**5a-d**, fig. 20). The tetrahedral bis-[bis(diphenylphosphano)ethane]gold(I) chloride (**5a**) has been shown to possess potent antitumor activity against a range of murine tumor model including leukemias, reticulum cell carcinoma, mammary adenocarcinoma and melanoma.[122] However due to severe hepatotoxicity, the development of this compound didn't go further.[123] The authors attributed this lack of selectivity to the high lipophilicity of the compound resulting in a non-specific binding to intracellular components. They showed that the higher selectivity resulted of an average balance between lipophilicity and hydrophilicity. By plotting the antitumor properties *in vitro vs* the lipophilicity of the studied compounds, the authors found a maximum of the antitumor properties *in vitro* for the compound with average lipophilicity. Moreover, *in vivo* on mice with murine subcutaneous colon 38 tumors, the compound with intermediate lipophilicity presented the highest activity on tumor growth with a tumor growth delay of 9 days, pointing out the importance of fine tuning these parameters for the development of new drug candidates.[124] Bis-[1,3-bis(dipyridylphosphano)propane]gold(I) chloride ($[Au(d2pypp)_2]Cl$) (**6** fig. 20) in which the spacer in between the two phosphanes is increased to give rise to 6-membered rings showed great selectivity for breast cancer cells compared to the healthy ones associated to an efficiency against malignant cells comparable to **5a**. The compound, as DLCs in general,[125] was shown to accumulate in mitochondria due to the negative mitochondrial membrane potential. Moreover, while the ethane-bridged compounds **5a-d** showed only poor interactions with thiols, **6** can efficiently inhibit activity of both purified and cellular Trx and TrxR. The authors attributed this different behavior to a more labile phosphane ligand enabling the coordination of sulfur- or selenium-donor atoms to the gold center.[126]

5.2/ Au(III) complexes with chelating *N*-donor ligands

The gold(III) complexes $[AuCl_3(Hpm)]$ and $[AuCl_2(pm)]$ (Hpm = 2-pyridylmethanol) were tested against a panel of cancer cell lines *in vitro* and showed comparable activities to Cisplatin and $NaAuCl_4$. The compounds were shown to interact closely with proteins such as albumin and transferrin but only weakly and reversibly with DNA. Although demonstrating the interest of gold(III) compounds, the substitution of the chlorido ligands by molecules of water at pH above 2 rendered them too instable in physiological environment for medicinal purposes.[127] Thus, several polydentate ligands were tested to stabilize the gold(III) center, such as ethylenediamine (en), phenanthroline (phen) (**19**), 2-phenylazopyridine (azpy) (**20**), diethylenetriamine (dien) (**21**), terpyridine (terpy) (**22**) or macrocycles (TACN and cyclam) (**23**).[128, 129, 130] Some of these complexes are depicted in Figure 21. The stability of some compounds was measured both by cyclic voltammetry and UV-Visible spectroscopy in the presence of reducing agents. It appeared

that polyamine complexes were particularly stable, with the complex **23** presenting the highest stability toward reduction (table 1), although the most toxic compounds on ovarian cancer cell lines appeared to be the least stable ones (table 1 **19** and **22**).[128]

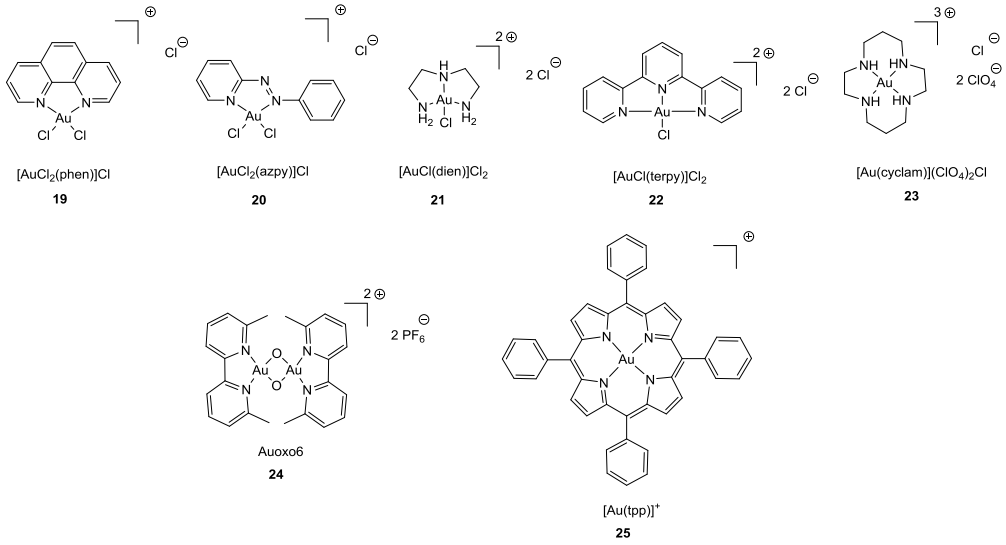


Figure 21: Exemples of Au(III) complexes with *N*-donor ligands tested as anticancer agents.

Table 1: Comparison between redox stability in aqueous solution at pH 7.4 and IC_{50} values on human ovarian cancer cells sensitive (A2780) and resistant to Cisplatin (A2780cisR) of some Au(III) complexes.

Complex	E_p (*vs* NHE*) (V)	IC_{50} A2780 (µM)	IC_{50} A2780cisR (µM)
23[a]	- 0.20	99.0	> 120.0
21[a]	+ 0.19	8.2 ± 0.93	18.7 ± 2.16
22[a]	+ 0.62	0.2	0.37 ± 0.032
19[a]	+ 0.80	3.8 ± 1.1	3.49 ± 0.91
$AuCl_4^-$	+ 0.55[a]	11[b]	17.7[b]

* normal hydrogen electrode [a] data from ref. 128 [b] data from ref. 127

The mechanism by which these compounds trigger their antiproliferative effect was carefully studied. Due to the isoelectronic structure of Au(III) cation compared to Pt(II), DNA was primarily envisaged as possible target. Even though it has been shown that Au(III) compounds can actually interact with nucleic acids through the coordination of their N-donor

atoms,[131] the interaction calf thymus DNA appeared to be weak and reversible and could not explain the cytotoxic properties of this class of compounds.[132]

Some dimeric dioxido-bridged Au_2 with bipyridine ligands have been developed by Cinellu *et al.* and are fairly stable at the +3 oxidation state under the dimeric form, with toxicities in the low micromolar range both against Cisplatin-sensitive and resistant ovarian cancer cell lines. The most cytotoxic one (**24**, fig. 21) has been demonstrated to tightly bind to calf thymus DNA. Indeed, inductively induced plasma optical emission spectroscopy (ICP-OES) measurements evidenced that more than 80 % of gold was still associated with DNA after ultrafiltration. Moreover, the reactivity of **24** with different proteins including human serum albumin (hSA), cytochrome *c* (cyt c) and bovine ubiquitin (Ubq) was investigated using spectrophotometric and mass spectrometry techniques. All experiments showed that **24** could react with protein through redox processes involving the breakdown of the dimeric structure as well as the release of the bipyridine ligand .[133]

Based on these results, the interactions with proteins appeared to be the main pathway by which such gold (III) complexes induce their toxic effects in cancer cells, explaining the low cross-resistance with Cisplatin.[134, 135] Among the various enzymes screened as possible target of gold(III) metallodrugs, some revealed to be of particular interest. Indeed, gold(III) are very potent inhibitors of TrxRs and can induce mitochondrial swelling even though in a lower extent than Auranofin.[106] More recent studies pointed out gold(III) as very powerful inhibitors of the zinc-finger enzyme PARP-1 compared to other metal-based drugs such as Ciplatin, NAMI-A or Auranofin in both purified enzyme and lysate.[29] Using mass spectrometry techniques, **19** was demonstrated to displace zinc cation from the zinc-finger domain upon loss of all ligands including the phenanthroline. More recently, **19** has been reported to selectively inhibit the water and glycerol membrane protein channels aquaporins.[136]

Finally, a unique family of Au(III) with *N*-donor ligands, is the Au(III) porphyrins' one, which is characterized by a very high stability upon reduction as assessed by cyclic voltammetry (E_p = -1.34 and -1.97 V *vs* NHE), but also in solution in the presence of cellular reductants such as glutathione. $[Au(tpp)]^+$ (tpp = *meso*-tetraphenylporphyrin) (**25**, fig. 21) has been tested against a large panel of human cancer cell lines including Cisplatin- and multi-drug resistant cell lines with several hundred folds higher efficiency.[137] *In vivo* data showed that **25** can reduce nasopharyngeal carcinoma tumor growth without body weight loss or liver injuries on mice. Mechanistic studies revealed that **25** triggers apoptosis through reduction of mitochondrial membrane potential and subsequent release of cytochrome c and apoptosis-activating factor.[138]

5.3/ Au(III) dithiocarbamate complexes

Gold(III) dithiocarbamate complexes developed by Fregona *et al.* are characterized by two symmetrical Au-S bounds giving rise to the stabilization of the +III oxidation state as

assessed by cyclic voltammetry (5 mV ≤ E_p(Au-dithiocarbamate) ≤ 125 mV compared to E_p = 1.29 V *vs* NHE for $KAuCl_4$) (fig. 22).

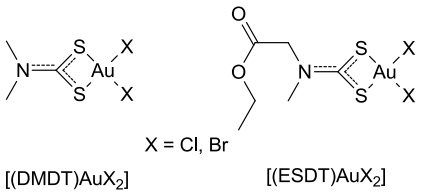


Figure 22: Chemical structures of the most studied dithiocarbamate-Au(III) complexes as anticancer agents.

These compounds appeared highly toxic against a panel of human cancer cell lines including leukemia, cervical adenocarcinoma, colon adenocarcinoma or malignant melanoma *in vitro*. Moreover, comparison between toxicity on Cisplatin-sensitive and -resistant cell lines showed no cross-resistance with Cisplatin suggesting a different mechanism of action.[139] Furthermore, *in vivo*, [(DMDT)$AuCl_2$] was proved to reduce prostate tumor xenografts in mice associated with good tolerance of the treatment.[140] Such compounds trigger apoptosis through a different cellular target compared to other gold-based metallodrugs. Indeed, it has been shown that dithiocarbamate compounds act through the inhibition of proteasome both *in vitro* and *in vivo*.[141]

5.4/ Au(I) *N*-heterocyclic carbene (NHC) complexes

As previously mentioned, much effort has been directed towards the application of gold complexes for targeting mitochondrial cell death pathways. Following the successful application of gold phosphane complexes as antitumor agents, Berners-Price *et al.* have synthesized a variety of organometallic cationic mononuclear gold(I) biscarbene complexes as potential chemotherapeutic agents (**7a-d** fig. 23).[142, 143, 144] The wingtip groups have been modified in order to adjust the lipophilic character of the complexes, a critical factor for targeting malignant cells. These gold organometallics display strong antimitochondrial effects, which can be attributed to both their cationic and lipophilic character. Very recently, a gold(I) biscarbene complex has been reported to decrease tumor size on mice bearing melanoma tumor without decreasing of body weight upon treatment.[145]

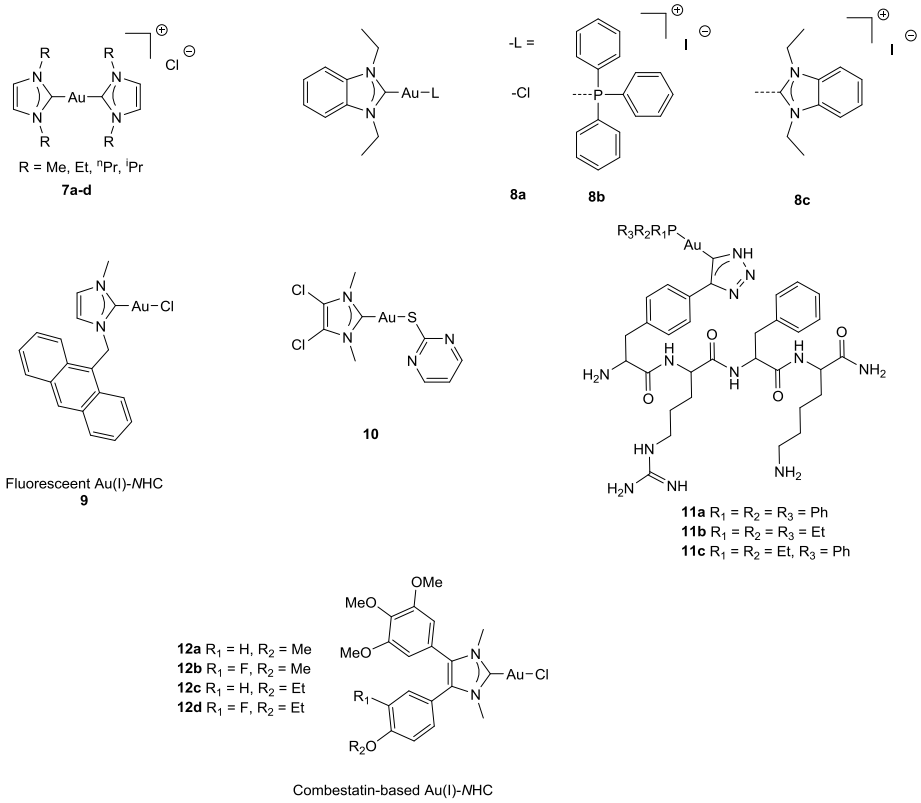


Figure 23: Structures of biologically active gold(I)-NHC complexes.

An important aspect of these organometallic complexes in regulating the biological activity is related to the presence of an ancillary ligand coordinated to gold, in addition to the Au-NHC bond. Rubbiani *et al* synthesized three NHC gold(I) complexes of the 1,3-benzimidazol-2-ylidene type (**8a-c** fig. 23) with different ligands (Cl, NHC, and PPh_3, respectively).[146] Initial DFT calculations revealed differences in bond dissociation energies (BDEs), which indicated an order of stability Cl < PPh_3 < NHC. Experimentally, the complexes have different reactivity regarding binding to albumin and inhibition of the target enzyme TrxR. The chlorido derivative was a strong and selective inhibitor of TrxR and showed an intensive binding to albumin similar to Auranofin. The cationic biscarbene complex with two NHC ligands exhibited the lowest inhibition of TrxR and had concomitantly the lowest albumin binding capacity. Finally, the triphenylphosphane derivative **8b** led to a strong inhibition of TrxR and also to an increased protein binding. Generally, TrxR was inhibited preferentially over structurally related enzymes (glutathione reductase GR and glutathione peroxidase GPx), and mass spectrometry studies with a

selenocysteine-containing model peptide indicated that covalent interactions with selenium are highly relevant for the molecular mechanism of drug action.

The cellular uptake, as well as the biodistribution of the three complexes, were also studied by atomic absorption spectroscopy.[146] Interestingly, the obtained results indicated that, for the derivatives **8a** and **8b**, which had the higher protein affinity, cellular gold levels in the presence of serum were decreased, suggesting that extracellular protein binding negatively influenced the compounds' uptake.

With the purpose of achieving metal compounds imaging in biological environments, a gold(I)-NHC complex bearing a fluorescent anthracenyl ligand (**9**) was recently synthesized and the cytotoxic effects of the compound were investigated *in vitro* on different human cell lines of normal and cancer cells (fig. 23).[147] The TrxR inhibition properties of this compound were also evaluated both directly on the purified enzyme and in cell extracts, comparing its ability to inhibit glutathione reductase, a pyridine-disulfide oxido-reductase maintaining glutathione in its reduced state. The effects of the new complex on the oxidation state of the thioredoxin system and peroxiredoxins were analysed.

In particular, considering mitochondrial peroxiredoxin 3 (Prx3), the redox state of the mitochondrial system after compound's administration was highlighted.[147] Peroxiredoxins (Prxs) are enzymes decomposing peroxides using a highly reactive cysteine thiolate in their active site. Prx3 is kept reduced by the mitochondrial thioredoxin system, thus playing a role in protecting mitochondria from H_2O_2 produced by the respiratory chain complexes.[148] Overall, the reported results indicate a correlation between the cytotoxicity of **9** and Trx/Prx3 oxidation *via* TrxR inhibition in cells.[147] Finally, fluorescence microscopy studies allowed visualizing the compound's uptake and biodistribution in cells, showing a larger diffusion in tumor cells compared to normal ones (fig. 24). A very recent study using a gold(I)-NHC complex bearing a coumarin moiety showed the same bioditribution in human prostate cancer cells PC3.[149]

Other studies confirmed that many gold(I)-NHC complexes with the 1,3-substituted imidazole-2-ylidene and benzimidazol-2-ylidene ligands of type NHC-Au-L (L = Cl or 2-mercapto-pyrimidine) (**10**, fig. 23) can potently inhibit both of the cytosolic and mitochondrial isoforms of the TrxR enzyme.[150] The compounds showed marked and selective TrxR inhibition properties in particular in cancer cell lines. Remarkably, the most selective TrxR inhibitors induced extensive oxidation of thioredoxins (Trxs), which was more relevant in cancer cells (A2780) than in HEK-293T cells. Trx oxidation might be induced either by inhibition of TrxR by gold(I)-NHC complexes or by a massive increment of H_2O_2. However, the latter has not been detected at least in the A2780 and HEK-293T cells, at variance with what has been observed for other Au(I)-NHC complexes.

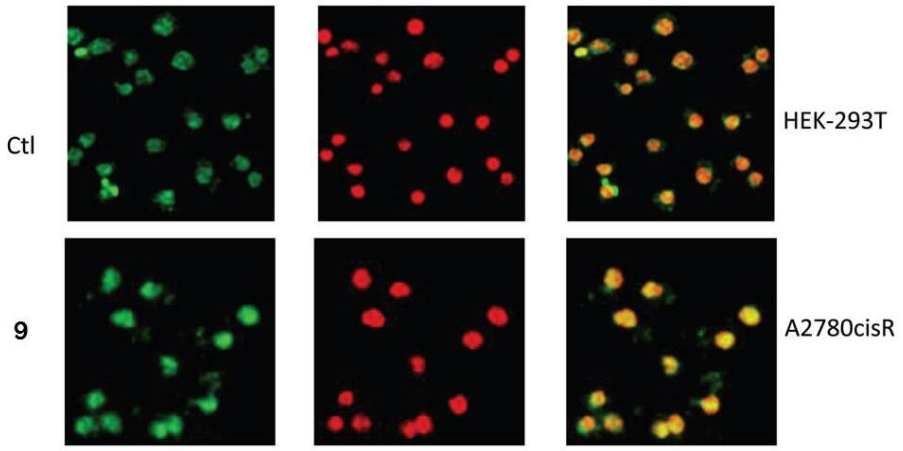


Figure 24: Visualization of the gold(I) NHC compound **9** using confocal microscopy in human embryonic kidney HEK-293T and human ovarian cancer Cisplatin resistant A2780cisR cells: a) fluorescence of the compound b) propidium iodide localization c) overlay. Images adapted from ref. 147.

In fact, it is worth mentioning that, among the several reported biologically active gold NHC complexes, distinct effects related to tumor cell proliferation inhibition have been described, including the increased formation of reactive oxygen species (ROS) or apoptosis induction.[151, 152, 153, 154] However, it should be noted that a general direct correlation between TrxR inhibition and cytotoxic effects of gold NHC complexes could not so far always be claimed.[155] This indicates that other mechanisms besides TrxR inhibition might contribute to their overall pharmacological profile. Very recently, Poly(ADP-ribose) polymerase 1 (PARP-1), an enzyme which is involved into DNA repair mechanism[156] and suspected to be involved into resistance to Cisplatin,[157] has been demonstrated as a credible target for some Au(I)-NHC complexes.[158] Moreover, some gold(I) *N*-heterocyclic carbene complexes have been proved to be efficient inhibitors of cysteine dependent protein tyrosine phosphatases (PTPs).[159] PTPs are an enzyme family that plays important roles in various cellular processes and signaling pathways and that are considered targets for diseases including cancer or autoimmune disorders.

The possibility to synthesize compounds with a high degree of structural diversity is certainly among the features that make gold NHC complexes extremely attractive in drug design, as exemplified by recently reported complexes with hetero-biscarbenes or peptide bioconjugates.[160, 161, 162, 163, 164] For example the peptide-Au-NHC compounds **11a-c** (fig. 23) hold great promise due to their resistance overcoming in p53 deficient tumor cells.[163] These complexes are also powerful inhibitors of TrxR, induce ROS formation and apoptosis, and trigger antimitochondrial effects.

The synthesis and cytotoxic properties of "bimodal" Au(I)-NHC complexes bearing a *N,N*-dimethyl-4,5-diarylimidazol-2-ylidene moiety, modeled on the naturally occurring vascular disrupting anticancer drug combretastatin A-4, have been recently reported (**12a-d,** fig. 23).[165] Notably, in this work the transport mechanisms of a few compounds have been investigated showing that compounds' uptake in 518A2 melanoma cells goes mainly *via* organic cation transporters (OCT-1/2) and copper transporters (Ctr1), similarly to Cisplatin.

5.3/ Gold alkynyl complexes

Gold alkynyl complexes are a type of organometallics containing a gold(I) central atom with one or more coordinated alkynyl ($R\text{-}C{\equiv}C^-$) ligands (see **13-18**, fig. 25).[166, 167] The alkynyl ligand is isoelectronic to cyanide ($N{\equiv}C^-$) or carbon monoxide (CO) and can be interpreted as a pseudohalido ligand. Their most intensively studied property is luminescence, which was initially described in 1993 and is thought to be related to intraligand π-π* or Au-C σ-π* transitions.[168] Despite the extended interest in these systems, biological properties related to metallodrug research are quite scarce.[169, 170, 171] Of note, gold(I) alkynyl derivatives containing the water-soluble 1,3,5-triaza-7-phosphaadamantane (PTA) and 3,7-diacetyl-1,3,7-triaza-5-phosphabicyclo[3.3.1]nonane (DAPTA) phosphane ligands (see examples **13-14** in fig. 25) are cytotoxic in cancer cells and their cellular uptake was confirmed by fluorescence microscopy using the luminescent properties of these organometallics.[172] As expected the complexes lack reactivity with DNA. Fluorescence microscopy also confirmed the cellular uptake of complexes containing fluorescent anthraquinone based ligands such as **15**.[173]

A recent study showed that mononuclear alkynyl gold complexes of the type alkynyl(triphenylphosphane)gold(I) (**16, 17**, fig. 25) exhibit a promising potential as future chemotherapeutics.[171] In fact, these compounds were able to trigger antiproliferative effects and are strong inhibitors of TrxR with a high selectivity over the related enzyme glutathione reductase. Moreover, effects against tumor cell metabolism and mitochondrial respiration were observed, as well as significant anti-angiogenic properties in zebrafish embryos.

Finally, phosphane-bridged dinuclear gold(I) alkynyl complexes such as **18** are scarcely active against TrxR but still cause strong antiproliferative effects accompanied by an efficient cellular accumulation.[174]

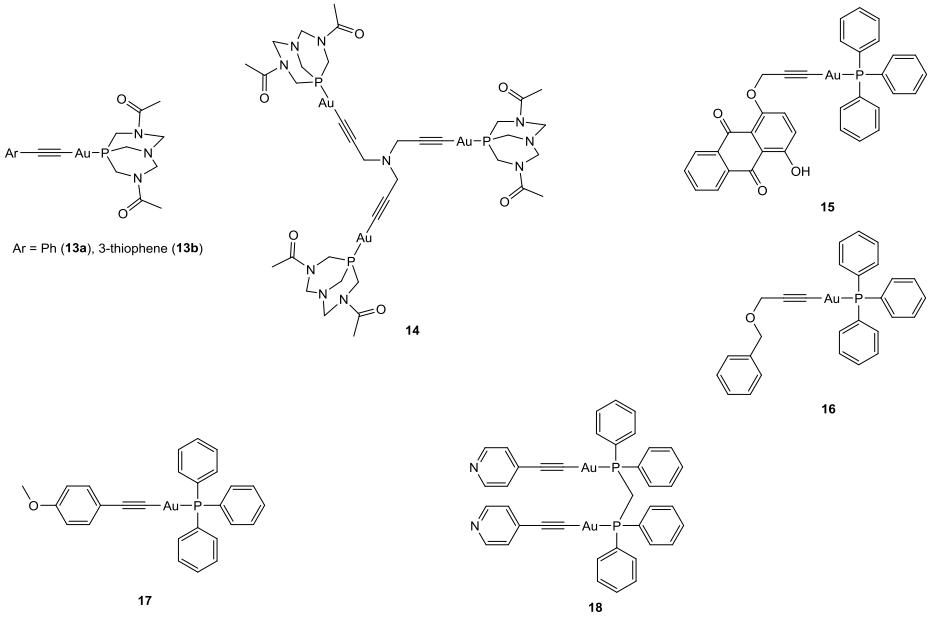


Figure 25: Structures of cytotoxic gold(I) alkynyl complexes.

5.6/ Cyclometalated Au(III) complexes with *C*,*N*-donor ligands

Cyclometallation reaction is the transition metal-mediated C-R bond activation of a wide range of organic molecules bearing donor atoms (D) such as N, O, P, S, and Se, and resulting in the formation of a chelate ring composed of a coordination D-M bond and a covalent M-C bond.[175] Cyclometalated complexes are generally characterized by redox and thermodynamic stability. Moreover steric and electronic properties can be easily tuned by modification of either the anionic cyclometalated or the ancillary ligands to afford compounds with enhanced lipophilic character.

A variety of cyclometalated gold(III) complexes of nitrogen-donor ligands have been synthesized so far, featuring both bidentate *C*,*N*- and tridentate *C*,*N*,*N*-, *C*,*N*,*C*- and *N*,*C*,*N*-donor ligands (fig. 26), with either five- or six-membered *C*,*N* rings. Below are presented those that have been mostly studied for their anticancer properties and interactions with biological targets.

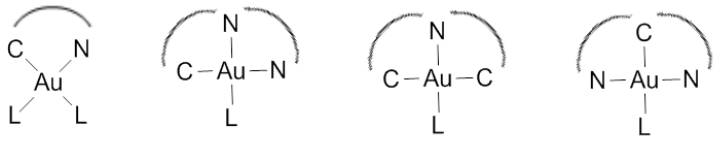


Figure 26: General representation of cyclometalated gold(III) complexes with *C,N*-donor ligands (L = ancillary ligand).

The discovery of the anti-tumor properties of the complex $[Au(damp)Cl_2]$,[176] (damp = 2-[(dimethylamino)methyl]phenyl), and its derivatives $[(damp)AuX_2]$ (X = SCN, OAc or X_2 = oxalato, malonato)[177] (**26,** fig. 27) by Parish and co-workers caused a renaissance of interest in gold(III) complexes as potential anticancer agents. These complexes displayed similar cytotoxicity to the one of Cisplatin against several human tumor cell lines, with the acetato and malonato complexes generally showing the best activities and selectivity *in vitro*, as well as moderate *in vivo* antitumor activity against human carcinoma xenografts.[176, 178] Complex **26** and its acetato and malonato analogues inhibit the cysteine proteases cathepsin B and K, with IC_{50} values of 0.6–1.36 μM and 1.3–3.3 μM, respectively,[179] and are also very potent inhibitors of TrxR.[180]

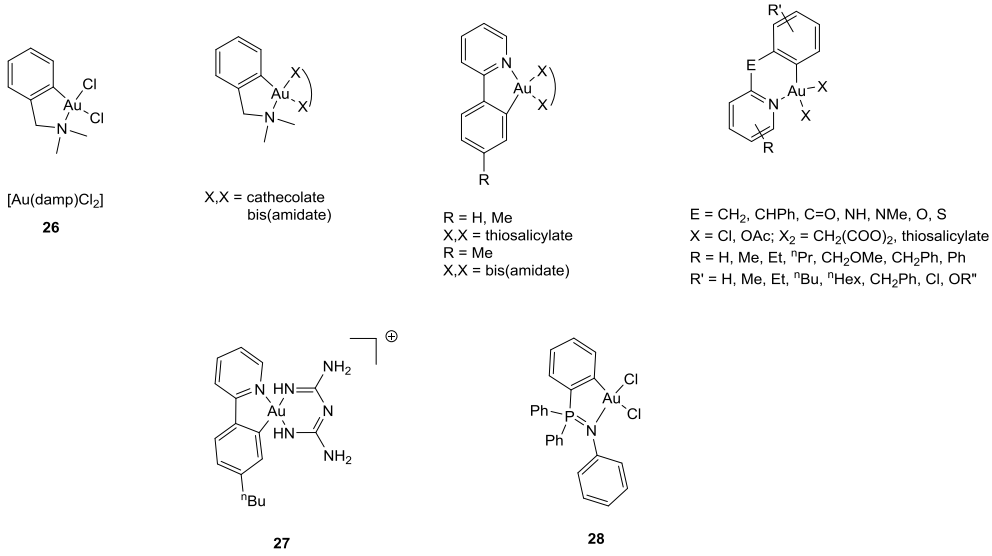


Figure 27: Representative examples of cytotoxic cycloaurated complexes of the type [(C,N)Au(X,X)].

Following these promising results, a series of square planar six-membered cycloaurated complexes based on a pyridinyl-phenyl linked backbone (fig. 27) was found to inhibit the cysteine proteases cathepsin B and K, with IC_{50} values very similar to those found for the five-membered cycloaurated complexes.[181] Five compounds of this series were evaluated for *in vitro* cytotoxicity against a panel of human tumor cell lines. In general, the leaving group had a pronounced effect on the cytotoxicity with the thiosalicylato compound being consistently more active than the chlorido compounds. Thus, the thiosalicylato compound was tested *in vivo* against the HT29 human colon tumor xenograft model, and a modest decrease in tumor growth was observed compared with the untreated control tumor. Various cyclometalated (C,N) gold(III) compounds, analogues of complex **26**, with one, two or three carbon-gold bonds were evaluated in another study.[180] In general, introduction of an increasing number of C-Au bonds makes the compounds more lipophilic, and thus facilitate intracellular uptake. However, the ability to interact with thiols or selenol groups of proteins appeared to be reduced. In addition, the damp derivative Au(damp)(C_6H_5)Cl, bearing two gold-carbon bonds, was found to be a potent inhibitor of TrxR (IC_{50} = 2.2 nM), nevertheless it is devoid of any anticancer activity against MCF-7 breast cancer and HT-29 colon cancer xenografts.[180]

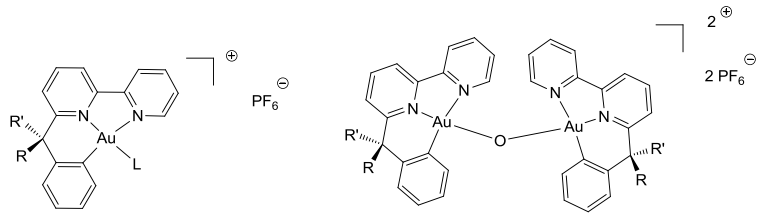
The water-soluble biguanide complex $[Au(R\text{-}C,N)(biguanide)]^+$ (R = 2-(4-*n*-butylphenyl)pyridine, **27** fig. 27), which combines the lipophilic character imparted by the cyclometalated 2-(4-*n*-butylphenyl)pyridine with the hydrophilic properties given by the H-bonding groups of the chelating biguanide, displays high toxicity against HeLa cells and low toxicity towards normal lung fibroblast CCD-19Lu cells.[182] Complex **27** was found to react rapidly with GSH to form gold-GSH adduct(s); it induces endoplasmic reticulum (ER) stress and ER swelling, up-regulates ER-stress markers such as CHOP and HSP70, causes partial S-phase cell cycle arrest in HeLa cells following apoptosis- and necrosis-independent cell death. Moreover, **27** showed anti-angiogenic effects at sub-cytotoxic concentrations.

The biological activities of the cationic organogold(III) complex containing the "pincer" iminophosphorane ligand (2-C_6H_4-PPh_2=NPh) (**28** fig. 27) and its derivatives obtained from substitution of the chlorides with chelating ligands such as thiosalicylate, catecholate and dithiocarbamates, have recently been reported.[183, 184, 185] The thiosalicylato and catecholato derivatives showed markedly higher anti-tumor activity versus P388 murine leukaemia cells compared to the parent chlorido complex.[183, 186] The cationic dithiocarbamate-substituted complexes were highly active against HeLa human cervical carcinoma and Jurkat-T acute lymphoblastic leukaemia cells, and exhibited low toxicity against normal T-lymphocytes.[178, 179] Mechanistic studies suggest that reactive oxygen species (ROS) production at the mitochondrial level is a critical step in the cytotoxic effect of these compounds.[185]

In 2002 Cinellu and Messori developed the cyclometalated hydroxido complex [($bipy^{dmb}$-H)Au(OH)][PF_6] ($bipy^{dmb}$ = 6-(1,1-dimethylbenzyl)-2,2'-bipyridine) (**29** fig. 28), stable under pseudo-physiological conditions and cytotoxic towards different ovarian cancer

cell lines sensitive and resistant to Cisplatin.[187] In a more recent study, the compound was tested against a panel of 12 cell lines and showed moderate antiproliferative effects, as well as scarce selectivity. Interestingly, the analogue of **29** containing a 2,6-xylidine ligand (**31**) was markedly more cytotoxic and had a high degree of selectivity against a panel of 36 human tumor cell lines. The obtained results were analyzed by *COMPARE* analysis and inhibition of mTOR, the proteasome, and/or DNA synthesis has been suggested as possible mechanism of action of such complex.[188]

The interaction of **29** with *calf-thymus* DNA has been proved to be weak and reversible, while the compound reacted with bovine serum albumin (BSA) most likely through coordination at the level of surface histidines.[189] Further studies of the interaction of complex **29** with model proteins showed that small amounts of metal-protein adducts are also formed with cytochrome *c* and lysozyme, in which the gold(III) center and the C,N,N pincer ligand are conserved.[190]



L = OH, R = R' = Me **29**
L = OH, R = H, R' = Me **30**
L = $NHC_6H_3Me_2$-2,6, R = R' =Me **31**

R = R' = Me **32**
R = H, R' = Me **33**

Figure 28: Representative mononuclear and dinuclear cyclometalated gold (C,N,N) complexes.

Recently, **29** was reported to form stable adducts with the copper chaperone Atox-1 containing gold in the oxidation state +1 as evidenced by high-resolution electrospray ionization mass spectrometry.[191] However, it must be noted that, under the applied experimental conditions – *i.e.* the reducing environment provided by DTT – the gold(III) center may be pre-reduced to gold(I) with simultaneous loss of the starting ligands. Of note, Atox-1 is an intracellular metallochaperone crucially involved in copper trafficking, and the above mentioned studies may suggest that gold compounds are able to interfere importantly with the intracellular copper trafficking system also *in vivo*.

Studies of the effects of cyclometalated gold(III) compounds **29** and **31** showed that they inhibit mitochondrial TrxR2 and disrupt mitochondrial function triggering mitochondrial swelling, although the effects are reduced compared to Auranofin. These results suggest that mitochondrial pathways are directly involved in the compounds' cytotoxic activity.[106, 192]

In order to investigate metal binding to TrxR, MALDI-ToF MS experiments on the intact TrxR1 enzyme provided evidence for extensive enzyme metallation by **31**, while experiments on trypsinized gold(III)-TrxR1 adducts identified a specific protein fragment – namely ^{236}IGEHMEEHGIK^{246} – bearing an attached gold(I) ion.[193] Independent mechanistic information on the system was derived from BIAM (biotin-conjugate iodoacetamide) assays capable of monitoring selective metal binding to cysteine and/or seleno-cysteine residues. In this type of assay, while the effects produced by Auranofin, used as a reference TrxR inhibitor, could be essentially ascribed to gold(I) coordination to the active site selenol, the effects caused by the various gold(III) compounds were better interpreted in terms of oxidative protein damage. Overall, these results point towards the possibility of protein binding by (C,N,N) gold(III) compounds to other sites than Cys or Sec, such Met and His residues.

Interestingly, the cationic dinuclear oxido complexes $[(N,N,C)_2Au_2(\mu\text{-}O)][PF_6]_2$ (with N,N,CH = 6-(1,1-dimethylbenzyl)-2,2'-bipyridine (**32**) or 6-(1-methylbenzyl)-2,2**'**-bipyridine (**33**)) (fig. 27) and their corresponding mononuclear hydroxido complexes were evaluated for cytotoxic actions against a representative panel of 12 human tumor cell lines. They showed moderate cytotoxicity towards the majority of them. Complex **33** was found to be particularly active against the human breast cancer 401NL cell line, while **32** showed poorly selective action. Most importantly, the dinuclear compounds appear to be very stable in the cellular environment, and possibly cause their still appreciable biological effects in the form of gold(III) species.[194] As a matter of fact, mass spectrometry studies of **32-33** with model proteins evidenced that the resulting gold-protein adducts still contain gold ions in the oxidation state +3 and that the multidentate ligands are retained,[194] at variance with what has been previously reported for other dinuclear and mononuclear gold(III) compounds bearing exclusively *N*-donor ligands.[133] This also implies that the reactivity with proteins facilitates the cleavage of the oxygen bridge and the conversion of the bimetallic complexes into monometallic species.

Substitution reactions of the chlorido ligand in complex [Au(C,N,C)Cl] **34** (HC,N,CH = 2,6-diphenylpyridine) afforded a series of cationic cycloaurated complexes, both mononuclear $[Au(C,N,C)L]^+$ (L = 1-methylimidazole, **34a**, pyridine, **34b**, triphenylphosphane, **34c**) and polynuclear $[Au_m(C,N,C)_mL]^{n+}$ [L = imidazolate (n = +1), $Ph_2P(CH_2)_pPPh_2$, n = +2, m = 2, p = 1-6; L = $Ph_2P(CH_2)_2PPh(CH_2)_2PPh_2$, n = +2, m = 3) (fig. 29).[195, 196] The mononuclear compounds, containing non-toxic *N*-donor ancillary ligands, e.g. **34a** and **34b**, were shown to exert anticancer potency comparable to that of Cisplatin, but they do not exhibit cross-resistance with Cisplatin against nasopharyngeal carcinoma, with resistance factors less than 1. Flow cytometry assays indicated that **34a** causes cell death by apoptosis and cell-cycle analysis revealed S-phase cell arrest. Interestingly, further studies showed that **34a** binds strongly by intercalation to *calf-thymus* DNA, and it also enhances G-quadruplex assembly, suggesting that it could act as an inhibitor of telomerase. Studies of human DNA topoisomerase inhibition indicated that **13a** inhibits the topoisomerase I

cleavage reaction since it prevents binding of the DNA substrate.[196]

The dinuclear complexes (fig. 29) resulted to be in general much more active than their mononuclear counterparts, with IC_{50} values from 0.055 to 4.3 μM toward cancer cells.[196] Their cytotoxicity correlates to that of the metal-free phosphane ligands (IC_{50} = 0.13–38.0 μM), with $[Au_2(C,N,C)_2(\mu\text{-dppp})]^{2+}$ **35** and the free dppp (dppp = 1,3-bis(diphenylphosphano)propane) being the most cytotoxic compounds in the series. Thus, the $[Au(C,N,C)]^+$ moiety was regarded as a vehicle to carry highly cytotoxic phosphane ligands to cancer cells. Moreover, the gold phosphane complexes, although very cytotoxic, were found to interact only weakly with DNA and did not cause cell cycle arrest.[196]

In vivo studies in rat bearing HCC orthografts showed that **35** causes significant inhibition in tumor growth (77% *vs.* vehicle control) and displays promising cytotoxicity towards cancer cells.[197] Transcriptomic and connectivity map analyses have revealed that the transcriptional profile of this compound is similar to those of inhibitors of thioredoxin reductase and inducers of ER stress. Indeed, **35** is a nanomolar inhibitor of purified TrxR1, and effective in the micromolar range when TrxR activity assays are performed on cell extracts. Notably, the compound has also been subjected to acute and sub-chronic toxicity evaluation in nude mice and in beagle dogs. From these studies, no severe and irreversible side-effects were observed in association with the consumption of the compound at its effective concentrations in these animal models. Moreover, the gold complex does not induce noticeable genotoxicity as indicated by the analysis of micronucleus formation in rat bone marrow cells treated with it.

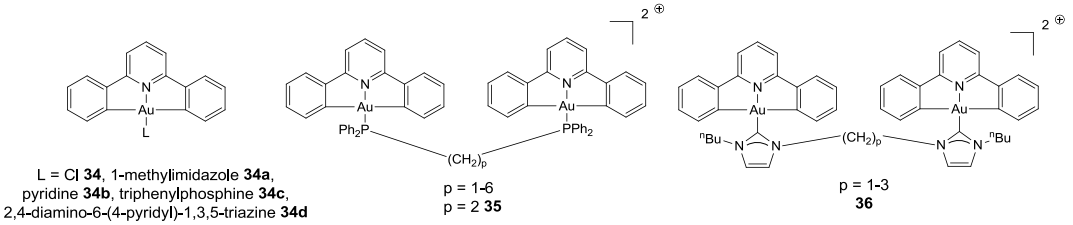


Figure 29: Representative mononuclear and dinuclear cyclometalated gold (C,N,C) complexes.

Replacing the phosphane by bridging carbene ligands (compounds **36**, fig. 29) resulted in the reduction of the cytotoxic properties of the compounds, as well as lack of selectivity towards non-cancer cells.

A first example of supramolecular polymers self-assembled from cyclometalated gold(III) (C,N,C)-type complexes was recently reported with possible applications in medicinal chemistry. In this study, the mononuclear complex $[Au(C,N,C)(4\text{-dpt})]^+$ **34d** was synthesized, containing the anti-angiogenic ligand 2,4-diamino-6-(4-pyridyl)-1,3,5-triazine

(4-dpt) able to form intermolecular hydrogen bonding and π-π interactions necessary to supramolecular complex formation.[198] The compound was found to self-assemble into a supramolecular polymer when dissolved in MeCN at a concentration of 20 mM at 323 K and then cooled at 298 K.[198]

The gold-polymer was cytotoxic towards B16 cancer cells, and to a lesser extent towards non-tumorigenic lung fibroblasts CCD-19Lu cells, after a prolonged time. It is worth mentioning that, to avoid any direct contact of the supramolecular polymer with the cells, double chambered transwell 24-well plates containing a semipermeable membrane at the bottom of the upper chamber were employed in the cytotoxicity assay. The authors proposed that sustainable release of the free 4-dpt ligand and the concomitant formation of adducts of the gold compound with GSH may account for its cytotoxicity. Taken together, these results suggest that the gold-polymer could have potential application in sustained delivery therapy with improved therapeutic efficiency and safety by reducing the frequency of drug administration and the dose of drug required. For example, the gold-polymer could encapsulate the gold(III) porphyrin complex [Au(TPP)]Cl (H_2TPP = meso-tetraphenylporphyrin),[198] thus providing the possibility to modulate its cytotoxicity *in vivo* and to reduce side toxicity through localized drug delivery.

6/Multinuclear metal compounds

Beyond replacement of the platinum metal center by other transition metals, another strategy used to achieve improved drug design consists in increasing the number of metal centers into a molecular entity to enhance the anticancer properties of the compound. This concept is called *multinuclearity*. As for mononuclear species, compounds can be divided into coordination and organometallic polynuclear complexes and will be presented in this order in the following section.

The first polymetallic complexes synthetized and used for anticancer purposes were built based on the scaffold of Cisplatin (**37** fig. 30).[199] These compounds were shown to have a higher affinity for DNA than Cisplatin leading to the development of different derivatives in which the *cis-trans* configuration, as well as the overall charge were varied to optimize the biological properties.[200] This led to the development of the trinuclear complex BBR3464 (fig. 30) which displayed higher toxicity on sensitive cell lines in comparison to Cisplatin associated to an ability to overcome Cisplatin-resistance on several cancer cell lines. This compound reached Phase II clinical trial where it showed a lack of activity on gastric and small cell lung cancer preventing any further development.[18]

Similarly, several ruthenium-based bimetallic complexes were developed on the basis of NAMI-A. An example of such compound is reported in Figure 30 (**38**). The bimetallic complexes revealed to have toxicity comparable with the parent compound

NAMI-A. Moreover, these complexes could reduce both the number and the size of metastasis in murine model *in vivo.*[201]

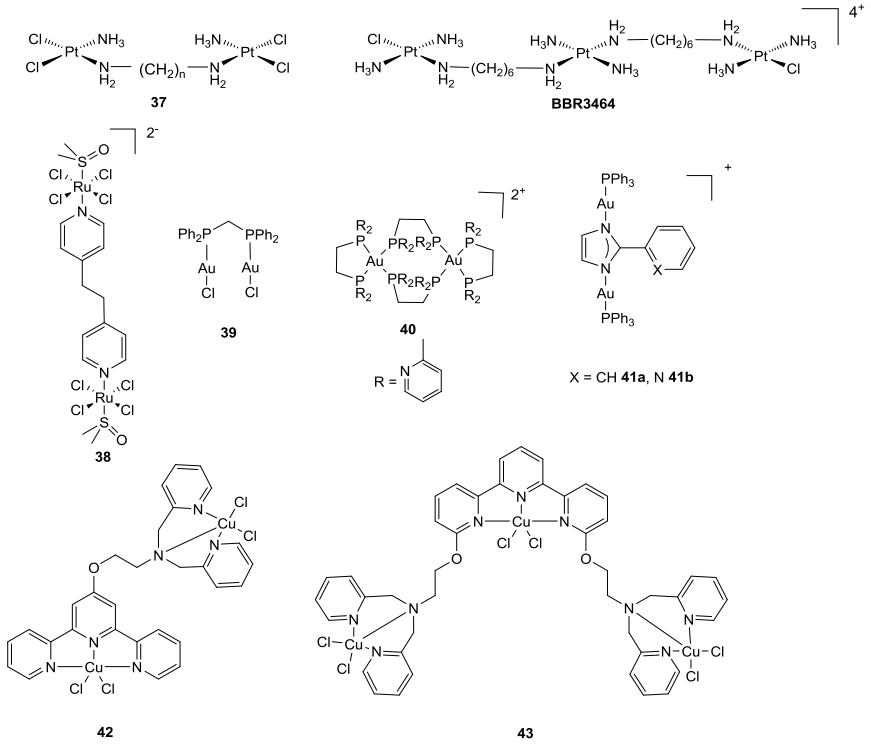


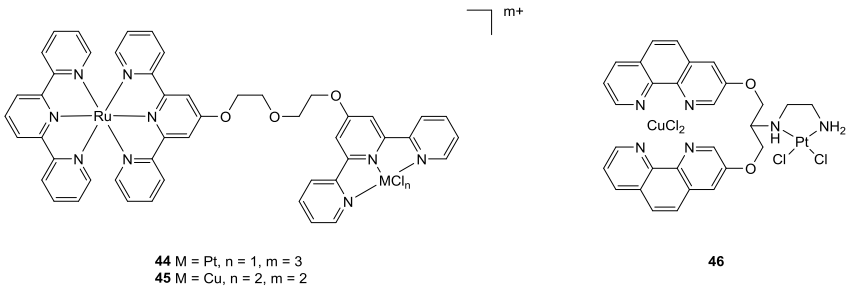
Figure 30: Examples of homopolynuclear coordination complexes studied as anticancer agents.

Gold in oxidation states +I and +III was also studied for the synthesis of polynuclear compounds. Examples of homobimetallic coordination complexes have already been described in section 5 (**24**, fig. 24). Dinuclear Au(I) complexes bearing polyphosphane ligands (**39**, **40**, fig. 30) appeared to be generally highly toxic both *in vitro* and *in vivo* on different murine tumor models.[200] Compound **39** has been reported by Che *et al.* to induce autophagy, a process associated with cytotoxicity mechanisms and consisting in the sequestration of cytoplasmic components into autophagosome for further degradation by lysosomes.[202] Other bimetallic gold(I) complexes based on the 2-arylimidazole scaffold (**41a-b**, fig. 30) were toxic in the low micromolar range against a panel of human cancer cell lines *in vitro*, with compound **41b** being able to efficiently inhibit PARP-1 in a Cisplatin-

sensitive ovarian cell line while being totally inactive on PARP-1 in the Cisplatin-resistant parent cell line.[203]

Vilar *et al.* reported the synthesis and biological investigation of copper-based di- and trinuclear complexes (**42**-**43**, fig. 30). [204] **42** has been shown to bind very efficiently to DNA with a 100-fold selectivity for G-quadruplex structures compared to duplex DNA structure. Complex **43** has also been reported of interacting with DNA, although with lower affinity than **42**, mainly *via* groove binding mode. **43** has been demonstrated to have a highly potent nuclease activity associated to higher toxicity than Cisplatin against different cancer cells but also in the same extent against healthy cells.[205]

Reedijk *et al.* synthesized different heteropolynuclear complexes as DNA binding and cleavage agents. They developed complexes based on the $Ru(terpy)_2$ moiety (terpy = 2,2':6',2''-terpyridine) coupled to [PtCl(terpy)] or [$CuCl_2$(terpy)] moieties (**44** and **45** fig. 31, respectively).



44 M = Pt, n = 1, m = 3
45 M = Cu, n = 2, m = 2

46

Figure 31: Examples of heteropolynuclear coordination complexes studied for interactions with DNA.

44 was shown to form adduct with DNA base model 9-ethylguanine by coordination of the N^7 to the platinum ion.[206] **45** and its analogues with higher numbers of metal centers appeared to be more efficient DNA cleavage agents than [$CuCl_2$(terpy)].[207] The authors attributed this increased potency to the presence of the inert ruthenium polypyridyl complex which favored the interaction with DNA *via* electrostatic interaction showing the possible associativity of the biological properties of each fragments. Another example of association of the properties of two metal-based fragments is presented in Figure 31 (**46**). Indeed, the Cisplatin-like fragment can preferentially bind on the GG DNA sequence placing the copper-based nuclease agent in the close proximity of that sequence. Thus, this bimetallic Pt/Cu complex can cleave DNA selectively around GG sequences.[208]

Several polynuclear organometallic complexes have also been developed. Keppler *et al.* prepared a series of dimeric ruthenium-based complexes with different linker length (**47,** fig. 32). The authors noticed an increase in toxicity *in vitro* against a panel of human

cancer cell lines including colon, ovarian, bladder, lung, breast, oesophagus and cervical cancer cells, following the increase of the size of the linker and the related compound's lipophilicity.[209] The compounds were shown to bind to transferrin but not to smaller proteins like ubiquitin or cytochrome *c*.

Interestingly, porphyrins were used to build up tetranuclear ruthenium complexes for possible "bimodal" applications in photodynamic therapy (PDT) and chemotherapy. Thus, for example using 5,10,15,20-tetra(4-pyridyl)porphyrin (4-tpp) as platform, the obtained tetrakis[(arene)RuCl] (**48**, fig. 32) showed moderate toxicity (IC_{50} = 50 µM for the most effective compound) against melanoma cells *in vitro* without irradiation.[210] However, upon irradiation at 652 nm, even at low doses (5 J/cm^2) photoactivation of the compound occurred resulting in an enhanced cytotoxicity.[210]

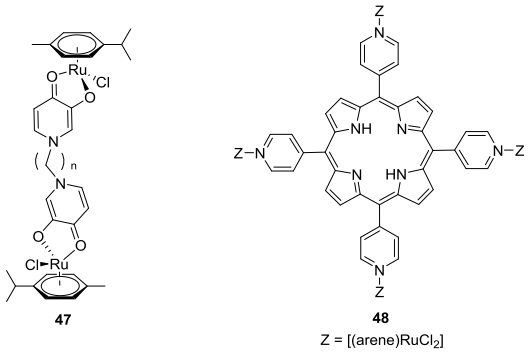


Figure 32: Examples of polynuclear organoruthenium complexes studied as anticancer agents.

Different supramolecular organometallic cages have been developed by Suss-Fink *et al.* to carry inside the cells insoluble compounds (fig. 33).[72]

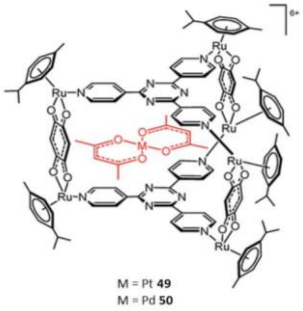
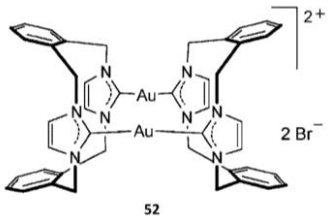


Figure 33: Ruthenium-based organometallic cage. Reproduced from ref. 72.

While platinum or palladium acetylacetonato complexes are not toxic against ovarian cancer cells *in vitro* due to their high hydrophobicity, by being incorporated into the water-soluble cage both encapsulated platinum and palladium compounds (**49**, **50** respectively) appeared more toxic than the empty cage. The palladium compound **50** appeared 20-fold more efficient than the empty cage (IC_{50} = 1 μM and 23 μM respectively).[72]

In the previous chapter I already described the biological activity of different cyclometalated Au(III) complexes (compounds **32**, **33** fig. 28 and **35**, **36** fig. 29). Here I will focus on the dinuclear Au(I)-NHC complexes developed by Berners-Price *et al.* An example of such compound is given in Figure 34.



52

Figure 34: Example of bimetallic Au(I)-NHC complexes. Reproduced from ref. 212.

52 was shown to induce mitochondrial swelling in isolated rat liver mitochondria, as well as mitochomdrial membrane permeabilzation in a Ca^{2+}-dependent pathway.[211] Moreover, it is worth mentioning that an analog of **52**, where an ortho-xylyl bridge has been replaced by a propyl bridge resulting in a shorter Au-Au distance, was observed into mouse macrophage cancer cells. The compound was shown to accumulate in lysosomes rather than in mitochondria.[212]

Heterometallic polynuclear organometallic complexes have been developed based on ferrocene and titanocene dichloride scaffolds. Süss-Fink *et al.* synthesized di- and trinuclear Ru/Fe and Ru/Fe/Ru on the type of compounds **53** and **54** (fig. 35). While the dinuclear compounds appeared moderately toxic against ovarian cancer cells both Cisplatin-sensitive and -resistant *in vitro* ($IC_{50} \approx 40$ μM), the trinuclear complexes appeared twice more efficient than their dinuclear analogs on both cell lines. Moreover, the equivalent toxicities on both sensitive and resistant cells suggest a different mechanism of action compared to Cisplatin.[213] Based on the ferrocene/mono- bisiminophosphorane platform Contel *et al.* developed polynuclear Au/Fe and Pd/Fe complexes (**55** and **56** fig. 35). Both **55** and **56** had been shown to have toxicity in the low micromolar range on all tested human cell lines including ovarian cancer (Cisplatin-sensitive and -resistant), breast cancer and healthy embryonic kidney cells for control. Both compounds appeared more toxic than their respective fragments. However, although both compounds have equivalent toxicities *in vitro*, they seemed to have different intracellular targets. Indeed **55** has been demonstrated to delay

plasmid DNA on electrophoresis experiment indicating DNA as potential target while **56** was shown to inhibit PARP-1 in the low micromolar range.[214]

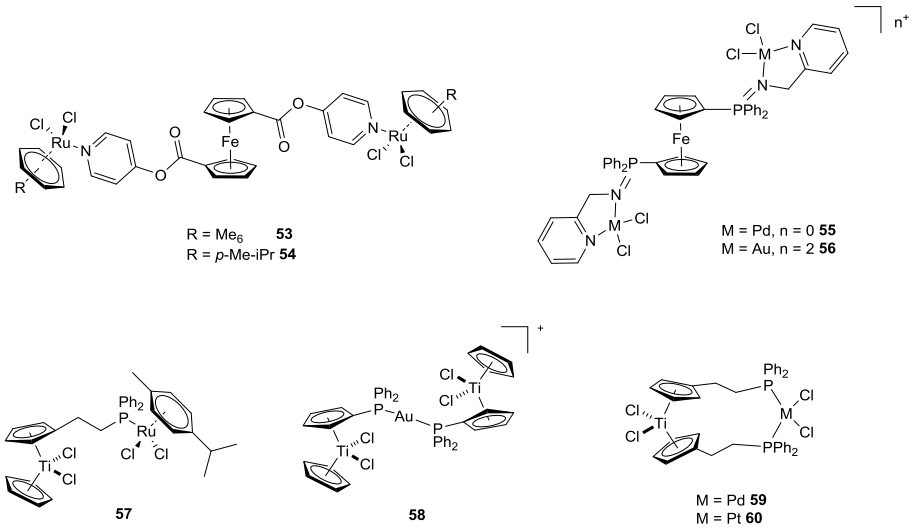


Figure 35: Examples of heteropolynuclear organometallic complexes studied anticancer purposes.

Le Gendre *et al.* developed different titanocene dichloride-based polynuclear complexes associating titanium with ruthenium and gold moieties (compounds **57** and **58**, respectively, Fig. 35). **57** revealed to be toxic in the low micromolar range against Cisplatin-sensitive and -resistant ovarian cancer cells *in vitro* (IC_{50} = 6.8 ± 1.3 μM and 4.5 ± 1.1 μM respectively) while both monometallic fragments: titanocene dichloride and RAPTA-C showed toxicities beyond 300 μM. Moreover, a mixture in a 1:1 ratio of these monometallic fragments gave toxicity beyond 200 μM evidencing the interest of polymetallic entities.[215] Such behavior was also noticed in the case of the trinuclear complex **58** (IC_{50} = 0.40 ± 0.15 μM and 0.41 ± 0.15 μM against sensitive and resistant ovarian cancer cells *in vitro*). It appeared also more toxic than the monometallic fragments, although in a lower extent due to the already high toxicity of the gold fragment. The higher measured toxicity for **58** was attributed to a higher intracellular uptake compared to its Ti/Au bimetallic analog as assessed by quantification of intracellular gold using inductively coupled plasma mass spectrometry (ICP-MS) techniques.[216]

Contel *et al.* developed Ti/Pd and Ti/Pt complexes (**59** and **60** fig. 34). While the platinum compound **60** appeared not toxic against human cervical and prostate cancer cells *in vitro*, the palladium analog **59** revealed to be highly toxic against the cervical cancer cell line. Both compounds were shown to interact with CT-DNA *via* direct covalent bonds.[217]

7/Aim and outline of the thesis

Within the field of Medicinal Inorganic Chemistry, the aim of this research project was the rational development of organometallic gold(I/III) complexes as possible anticancer agents. Thus, new series of gold compounds bearing *N*-heterocyclic carbene and (C,N) cyclometalated ligands were synthesized. The choice to focus on gold organometallics and not on coordination compounds was due to the fact that, in general, organometallic gold(I) and gold(III) complexes have higher stability compared to classical gold-based coordination complexes. Moreover, they are extremely suitable to design gold compounds acting as pro-drugs, but in which the redox properties and ligand exchange reactions can be modulated to achieve selective activation in diseased cells. In addition, the possibility to couple more than one metal center in the same molecule to obtain dinuclear gold or even heteronuclear compounds was explored.

Initially, I focused on the synthesis of new Au(I)-NHCs scaffolds and on the identification of their possible biological targets. To achieve this aim, I tested the reactivity of my compounds towards different biomolecules including DNA and selected proteins. Some of the compounds were also designed to contain fluorescent ligands to facilitate the study of their uptake and sub-cellular distribution *in cellulo.* Thus, **Chapter 2** focuses on the synthesis and chemical characterization of gold(I)-NHC complexes based on the natural product caffeine. Caffeine is a natural precursor for N-heterocyclic carbenes (NHCs) as it presents an imidazole ring, which is one of the most frequently used moieties for metal NHC synthesis. Moreover, caffeine and analogs has been recently reported as possible anticancer agents.[218] Thus, I expected to obtain "bifunctional" compounds combining the properties of both Au(I)-NHC complexes with the intrinsic biological properties of the caffeine scaffold. One of the compounds revealed to be selective against human ovarian cancer cells *in vitro*. Notably, in that study I identified a new possible biological target for Au(I)-NHC complexes, namely DNA G-quadruplex structures. The most promising compound out of the *in vitro* screening was also tested in rat healthy organs using an *ex vivo* technique developed in Pr. Groothuis's lab in Groningen. Specifically, I used the so-called Precision Cut Tissue Slices (PCTS) to evaluate compounds toxicity.[219]

In **chapter 3** I pursued the synthesis of bimetallic complexes. Thus, I explored the synthesis of homo- and heterobimetallic complexes based on the Au(I) NHC scaffold, ideal for functionalization *a posteriori.* In detail, this chapter presents two different synthetic strategies to reach late/late type bimetallic complexes either by coupling two metal-based fragments or by incorporating a free ligand on the Au(I)-NHC scaffold enabling the selective coordination of a second metal. I could also extend the family of reachable compounds by replacing the chloride ligand on gold(I) by a thio-β-D glucose tetraacetate group .

In **chapter 4**, gold(I)-NHC complexes, incorporating a fluorescent moiety to follow their uptake and distribution inside the cells, were devloped. Two different types of sugar

moieties (protected or not by acetate groups) were coupled to this scaffold to try to increase the uptake of the compounds possibly *via* GLUT-1 receptors. The various compounds were screened as possible inhibitors of the seleno-enzyme thioredoxin reductase (TrxR), a protein already reported as a target for gold-based metallodrugs (see Section 5), and promising results were obtained.

In **chapter 5**, I explored the chemistry of (C,N) cyclometalated Au(III) complexes. Thus, I synthetized and fully characterized Au(III) complexes with different phosphane- or sulfur-based ancillary ligands. The photophysical properties of the compound bearing a fluorescent coumarin moiety were investigated. The compounds were tested in a panel of cancer cell lines, as well as in a model of healthy cells, showing certain selectivity.

In **chapter 6**, a new series of coordination Au(I) complexes, bearing ligands based on an organic drug, and synthesized in the lab of Prof. M. A. Cinellu (Dept. of Chemistry, University of Sassari, Italy) was screened for their antiproliferative properties in a series of cancer and non-tumorigenic cells. In detail, the gold(I) complexes contained the ligand lansoprazole, currently used as proton pump inhibitor (PPI) for the treatment of gastric and duodenum ulcers. This specific ligand has been chosen because it was shown that pre-treatment with PPI increased uptake and efficacy of Cisplatin in tumors.[220] Therefore, with these compounds we explored the possibility of increasing potency of Au(I)-based drugs by designing ''bifunctional'' anticancer agents.

Finally, the data obtained in this thesis are summarized and discussed in **chapter 7**. Moreover, future directions conserning the use gold-based compounds in anticancer chemotherapy are also provided.

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