

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: <https://orca.cardiff.ac.uk/id/eprint/101633/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Jürgens, Sophie, E. Kühn, Fritz and Casini, Angela 2018. Cyclometalated complexes of platinum and gold with biological properties: state-of-the-art and future perspectives. *Current Medicinal Chemistry* 25 (4) , pp. 437-461.
10.2174/0929867324666170529125229

Publishers page: <http://dx.doi.org/10.2174/092986732466617052912522...>

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See <http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.



Outline

Abstract	Error! Bookmark not defined.
1. Introduction	4
1.1 Organometallic anticancer agents	6
2. Cyclometalated complexes	12
2.1 Pt(II) compounds	13
C [^] N complexes	13
C [^] N [^] N complexes	17
C [^] N [^] C complexes	19
C [^] N [^] S complexes	20
2.2 Au(III) compounds	22
C [^] N complexes	22
C [^] N [^] N complexes	23
C [^] N [^] C complexes	24
C [^] N [^] S complexes	27
3. Conclusions and Perspectives	29
Literature	31

ABSTRACT:

Background: The inherent problems accompanying chemotherapy necessitate the development of new anticancer approaches. The development of compounds that can disrupt cancerous cellular machinery by novel mechanisms, via interactions with proteins and non-canonical DNA structures (e.g. G-quadruplexes), as well as by alteration of the intracellular redox balance, is nowadays focus of intense research. In this context organometallic compounds of the noble metals Pt and Au have become prominent experimental therapeutic agents. This review provides an overview of the Pt(II) and Au(III) cyclometalated compounds with a chelating ring containing a strong C-M σ -bond to improve the stability of the compounds with respect to ligand exchange reactions and biological reduction. Furthermore, these properties can be easily tuned by modification of either the anionic cyclometalated or the ancillary ligands. Special focus has been set to C^N, C^N^C, C^N^N and C^N^S platinum(II) and gold(III) pincer complexes regarding their synthesis and biological modes of action as anticancer agents.

Methods: A structured search of both chemical and medicinal databases for peer-reviewed research literature has been conducted. The quality of retrieved papers was appraised using standard tools. The synthesis as well as the chemical and biological properties of the described compounds were carefully reviewed and described. The findings were outlined using a conceptual framework.

Results: In this review we included 155 papers, the majority originating from high-impact papers on the synthesis and biological modes of platinum(II) and gold(III) compounds. Among them, 17 papers were highlighted to give an introduction to the use of Pt and Au compounds with medicinal properties, mainly focussing on coordination compounds. The synthesis and medicinal properties of organometallic compounds of various metals (such as Fe, Ru, Ti) were outlined in 51 papers. These compounds included metallocenes, metallo-arenes, metallo-carbonyls, metallo-carbenes (e.g. *N*-heterocyclic carbenes), and alkynyl complexes. The C^N, C^N^C, C^N^N and C^N^S pincer complexes of platinum(II) (46 papers) and gold(III) (44 papers) were discussed concerning their synthesis, stability and advantages to develop therapeutic compounds. We strove to show the consistent development of C^N, C^N^C, C^N^N and C^N^S platinum(II) and gold(III) pincer complexes regarding their synthesis and biological modes from the early beginnings to the most recent findings.

Conclusion: This review supplies a profound overview of the development of organometallic compounds for medicinal purposes, setting special focus to the synthesis and stability of C^N, C^N^C, C^N^N and C^N^S pincer complexes of platinum(II) and gold(III) and their use as anticancer agents.

1. Introduction

The discovery of the cytostatic effect of *cis*-diamminedichloridoplatinum(II) (cisplatin, **1**) on *E. coli* by Rosenberg *et al.* in 1965 paved the way for the application of platinum based drugs in cancer therapy¹. Since the clinical approval of cisplatin in US in 1978, (and one year later in several European countries) another 25 cisplatin analogues have been approved for clinical trials. However, only carboplatin (**2**) and oxaliplatin (**3**) display pharmacological advantages compared to cisplatin and since their discovery have been marketed worldwide. The established mechanism of action for this family of chemotherapeutic agents involves their binding to nucleic acids after exchange of the chloride ligands with water molecules (or OH⁻ ligands) followed by direct coordination to DNA nucleobases. This reactivity with DNA leads to a stop of the transcription and translation of the cell DNA which results in cell death by apoptosis. Three other Pt(II) drugs have been additionally approved for single markets, namely nedaplatin in Japan, lobaplatin in China and heptaplatin in Korea².

Despite their clinical success, traditional Pt(II) based drugs for cancer therapy present major drawbacks, such as resistance, limited spectrum of action and severe side effects in patients, in part due to their non-selective binding to other intracellular components³. Thus, research has been focused on developing new inorganic drugs to circumvent these limitations and enable a more sophisticated, targeted approach towards cancer cells. In this context, gold complexes have been present in therapy for quite a while now, however their exploration as anticancer agents is more recent and has strongly increased throughout the last decade⁴. In fact, gold(I) and gold(III) complexes are an emerging class of metal complexes with potential antitumor properties alternative to cisplatin. This is mainly due to their outstanding cytotoxic properties exhibited through different antitumor mechanisms, specifically the selective inhibition of target proteins and enzymes.

As an example, auranofin (**4**) (Ridaura®) is a relatively simple Au(I) thiolate complex which is used in the clinic to treat severe rheumatoid arthritis. Early studies on the anticancer activity of auranofin revealed activity levels similar to cisplatin *in vitro*, which subsequently led to a large number of Au(I) complexes being evaluated for antiproliferative activity^{4b}. Initially approved as an antirheumatic agent in 1982 for worldwide clinical use, it recently passed phase II of clinical trials for the treatment of chromatic lymphocytic leukaemia (CLL), Small Lymphocytic Lymphoma (SLL) and Prolymphocytic Lymphoma (PLL)^{4b}.

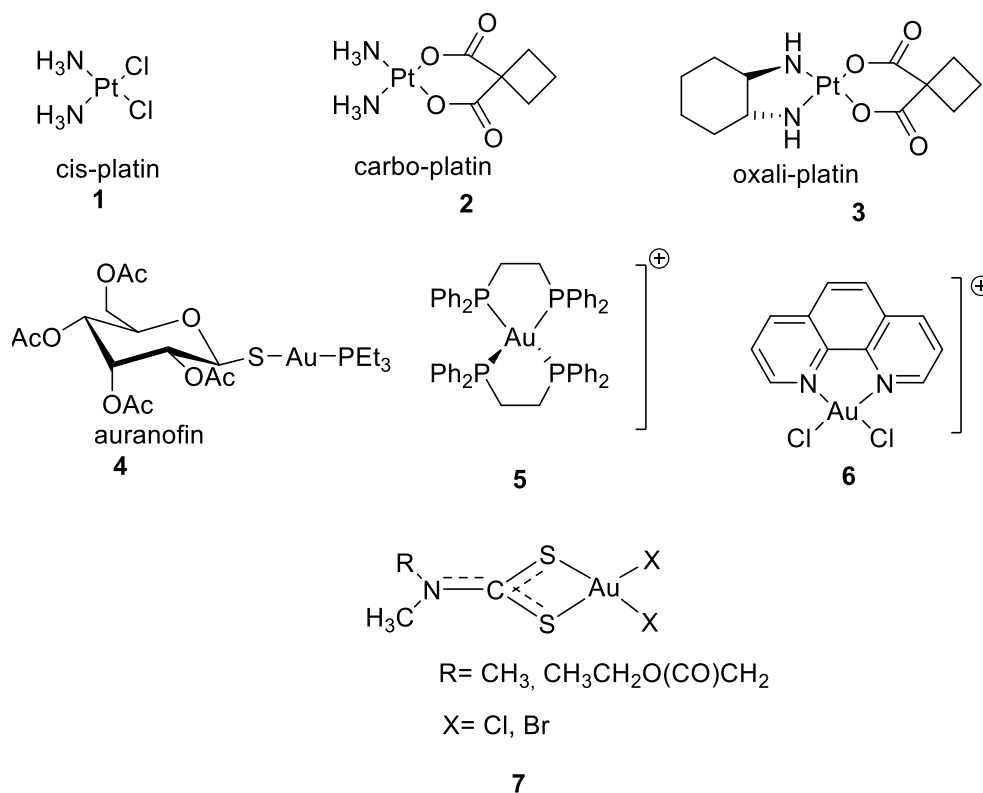


Figure 1. Prominent anticancer Pt(II) complexes and experimental cytotoxic Au(I) and Au(III) complexes.

Following the clinical success of auranofin, other Au(I) complexes featuring auxiliary phosphine ligands were synthesized by Berners-Price *et al.*,⁵ among which the bis[1,2-bis(diphenylphosphino)ethane]Au(I) chloride ([Au(dppe)₂]Cl) with chelating diphosphine ligands (**5**). Interestingly, **5** is more stable with respect to ligand exchange reactions than the linear complexes and less reactive towards thiols. [Au(dppe)₂]Cl has shown reproducible and significant *in vivo* antitumor activity in a range of murine models. However, due to severe hepatotoxicity of the compound, the studies were not continued⁶.

The modes of action of cytotoxic gold(I) compounds are still a matter of intense debate. However, there is now quite a wide consensus on the concept that their behaviour diverges profoundly from that of cisplatin and analogues, mainly grounded on a documented poor reactivity with double-helix DNA. On the other hand, there is good evidence that they often produce severe mitochondrial damage^{4a}. In this context, among the most studied and recognized targets for gold compounds, the seleno-enzyme thioredoxin reductase (TrxR) has been widely investigated⁷. Human TrxR contains a cysteine-selenocysteine redox pair at the C-terminal active site, and the solvent-accessible selenolate group constitutes a likely target for “soft metal ions such as gold.

Concerning gold(III) complexes, many families have been synthesized and the anticancer activity evaluated against numerous cancer cell lines *in vitro*. In most cases, the donor atoms stabilizing the Au(III) center are either Cl, Br, S or P⁸. As an example, the complex [Au(phen)Cl₂]Cl (**6**) contains a 1,10-phenanthroline as chelating N-donor moiety. Although displaying lower stabilities compared to other ligands, the phenanthroline chelated complex shows higher cytotoxicity towards

various cancer cell lines⁹. Recently, it was demonstrated that **6** is a functional selective inhibitor of the human water and glycerol channel aquaglyceroporin-3 (AQP3), possessing inhibitory effects on the proliferation of cells over-expressing this isoform¹⁰. Hence, **6** may produce a targeted therapeutic effect on carcinomas with enhanced AQP3 expression¹¹.

Pursuing the search of novel protein targets for anticancer gold compounds, some of us reported on the inhibitory effects of different cytotoxic gold-based complexes with phosphine or bipyridyl ligands, towards the zinc finger (ZF) enzyme poly(-adenosine diphosphate (ADP)-ribose) polymerase 1 (PARP-1)¹². Interestingly, Au(III) coordination complexes were among the most efficient in inhibiting PARP-1, at the nM level, followed by Au(I) compounds. Among the most recent reports, a new gold(III) complex bearing a 2-((2,2'-bipyridin)-5-yl)-1Hbenzimidazol-4-carboxamide ligand has been synthesized and characterized for its biological properties *in vitro*¹³. In addition to showing promising antiproliferative effects against human cancer cells, the compound potently and selectively inhibits the zinc finger protein PARP-1, with respect to the seleno-enzyme TrxR. The results hold promise for the design of novel gold-based anticancer agents disrupting PARP-1 function and to be used in combination therapies with DNA alkylating agents.

Interestingly, dithiocarbamate ligands were also described as efficient coordinating ligands for Au(III) ions¹⁴. Thus, Fregona *et al.* reported on the design of targeted Au(III) dithiocarbamate complexes with peptide-based ligands for carrier-mediated delivery of the compounds in cancer cells via peptide transporters¹⁵. Compounds of the type [Au(dpdtc) Cl₂] (dpdtc = dipeptidedithiocarbamate) (**7**) lead to reduced toxic and nephrotoxic side-effects in comparison to their analogues without the peptide moiety, while displaying increased tumor selectivity. Notably, several of these compounds resulted to be potent proteasome inhibitors.

Unfortunately, the majority of the cytotoxic Au(I)/Au(III) coordination compounds show limitations concerning stability in aqueous solution, and especially Au(III) complexes are easily reduced to Au(I) or Au(0), resulting in loss of activity and possible side effects. Therefore, research is nowadays dedicated to a larger extent to the design and synthesis of more stable derivatives, including organometallic compounds.

1.1 Organometallic anticancer agents

In recent years, organometallic compounds have become more popular and are now quite widespread as experimental anticancer agents. In fact, they combine the advantages of a classical inorganic system with the higher stability of an organic scaffold¹⁶. The organic ligands allow the introduction of stereospecificity which gives access to an even higher amount of structural possibilities. Furthermore "fine tuning" possibilities within the organic moiety regarding functional groups etc. is given, determining the physicochemical properties of the respective metal compound¹⁷. In fact, in organometallic complexes it is the metal-carbon (M-C) bond that endows these coordination compounds with peculiar features. On the one hand M-C bonds have high *trans* effects and *trans* influences, which affects the lability of bonds to other ligands (M-L) in the complex. Moreover, π -bonded aromatic arene and cyclopentadienyl ligands can act both as electron donors and π -acceptors. These ligands can therefore modify the donor/ acceptor behaviour (and reactivity) of other ligands in

the complex. Finally, by choosing specific targeting moieties, either incorporated into the ligand or attached to the metal center, targeting at specific cancer cells' receptors can be achieved.

Typical classes of organometallics include metallocenes, metallo-arenes, metallo-carbonyls, metallo-carbenes (e.g. *N*-heterocyclic carbenes), and alkynyl complexes, respectively¹⁸. Classically these compounds families have been widely applied in catalysis¹⁹. However, their medicinal use has been recently demonstrated. The utilized complexes show thereby a great variety, not only mononuclear compounds, but also multinuclear compounds have been synthesized and investigated. In this respect one has to distinguish between homonuclear and heteronuclear compounds. In Fig. 2, we provide an overview of some of the most representative structures of medicinally relevant organometallic complexes.

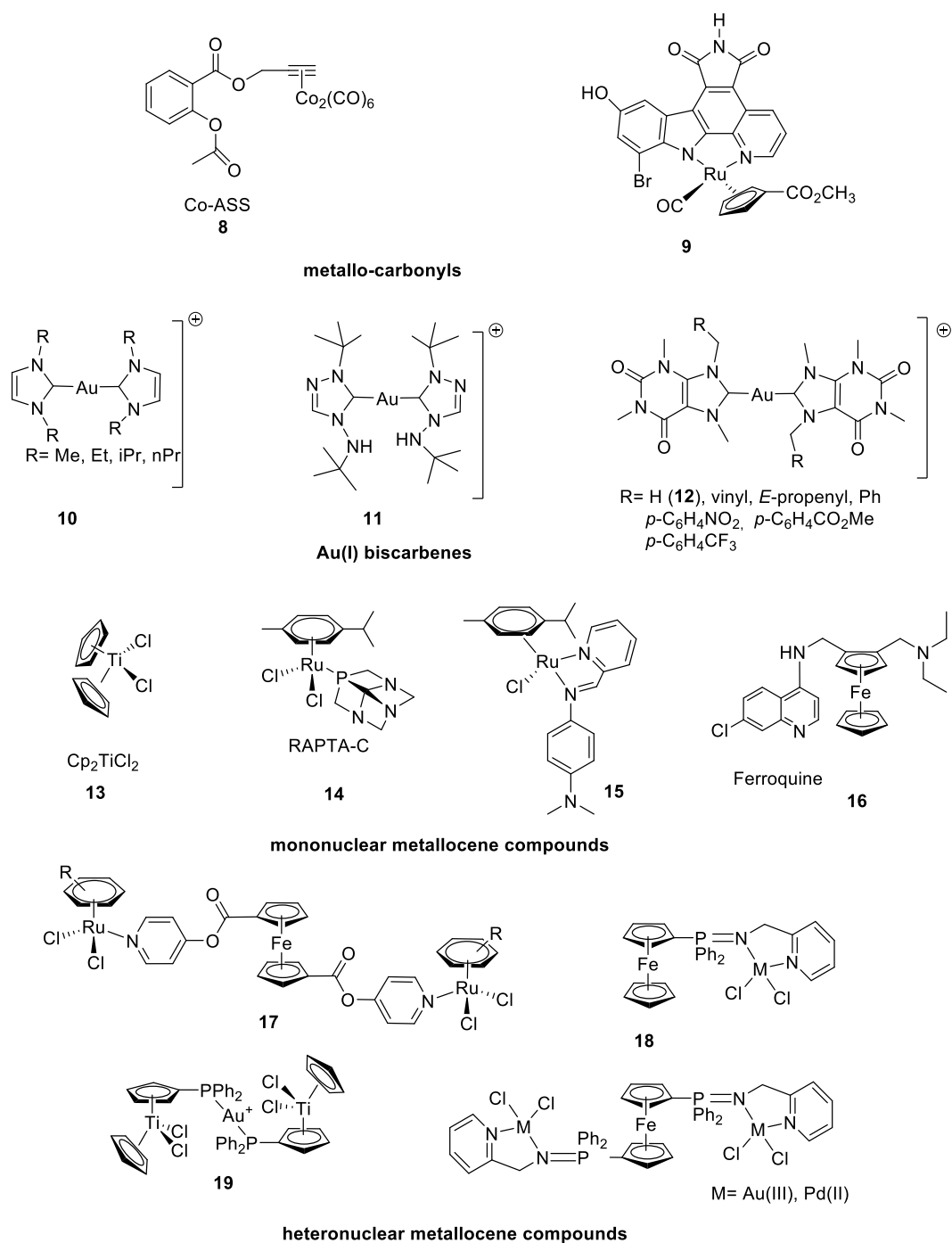


Figure 2. Organometallic complexes with medicinal properties.

To begin with, the biological properties of metallo-carbonyls have been studied for a number of metals, such as iron²⁰, ruthenium²¹ and manganese²². Carbonyl complexes offer the benefit of displaying high lipophilicity, leading to increased cellular uptake levels, which could be demonstrated in several studies²³. Among the various examples present in the literature, the alkyne hexacarbonyldicobalt Co₂(CO)₆ species Co-ASS (**8**) contains an acetylsalicylic acid (aspirin) moiety. Aspirin has been shown to reduce the recidivism risks in cancer patients, therefore making it interesting as a ligand in a combination therapy approach. Indeed, **8** was found to display good stabilities under physiological

conditions, to strongly inhibit both the COX-1 and COX-2 enzymes and to induce of apoptosis²³. Further recent studies with zebra fish embryos also indicate that **8** has anti-angiogenic properties, a trait which is not present with aspirin²⁴.

In contrast to modifying somewhat remote groups of organic inhibitors, in 2009 Meggers *et al.* have taken the concept of using metal fragments to occupy defined regions of 3D-space in enzyme active sites one step further, and synthesized a number of Ru(II) compounds as protein kinase inhibitors²⁵. The high selectivity displayed by these compounds is believed to be achieved by the rigid scaffold, leaving the metal as a pure structural motif. Complex **9** has therein shown promising activities against several cancer cell lines and in a melanoma spheroid model²⁶. Furthermore the CO ligands apparently play a significant role in the molecular interaction with biological targets, although the interactions need further systematic investigations^{18, 27}.

Organometallic compounds displaying *N*-heterocyclic carbene (NHC) ligands have also been investigated closely throughout the last decades, since they offer benefits such as easy accessibility from imidazolium salts, non-toxicity of the ligands and high complex stability²⁸. Steric and electronic effects contribute to the bonding of metals to NHC ligands. Whereas NHC complexes were initially considered as pure σ -donors, it is now commonly established that besides the NHC-to-metal $\sigma \rightarrow d$ donation also metal-to-NHC $d \rightarrow \pi^*$ and NHC-to-metal $\pi \rightarrow d$ donations contribute to the bonding. Moreover, saturation or aromaticity of the NHC ligand and the volume of attached side chains influence the stability and reactivity of the complexes. The first reports on biological activity of metal NHC compounds were published between 1996 and 1999 by Cetinkaya *et al.*, who described the antibacterial properties of ruthenium(II) and rhodium(I) NHC complexes²⁹.

Berners-Price *et al.* pioneered this field by synthesizing a series of linear, cationic Au(I) NHC complexes (**10**) with remarkable anticancer properties *in vitro*, and inducing mitochondrial damage. By varying the substituents at the N,N positions of the imidazolium precursor, the lipophilic properties could be finely tuned³⁰. Moreover, these compounds allow selective targeting of mitochondrial selenoproteins, such as TrxR. The antimitochondrial activity of cationic gold(I) NHC complexes could be related to their cationic and lipophilic character, which attributes the complex properties that are known from the class of delocalized lipophilic cations (DLCs). Notably, DLCs can selectively accumulate in the mitochondria of cancer cells driven by their enlarged mitochondrial membrane potential.

The effects of gold(I) NHC complexes on cell metabolism and their interference with pathways relevant for cancer cell proliferation have been studied for many derivatives³¹. In this area, Ott *et al.* reported on the synthesis of an Au(I) *N*-heterocyclic carbene **11**, utilizing an aminotriazole NHC carbene ligand,³² displaying good activity against various cancer cell lines and good to moderate inhibitory activity of TrxR. As 1,2,4-triazoles possess different electronic properties than the classical imidazolium salts, these complexes may show a different behaviour concerning donor abilities and physiochemical properties³³.

A further interesting linear, cationic Au(I) NHC complex series was reported by Casini and Picquet *et al* in 2014³⁴ featuring xanthine ligands, including caffeine, which naturally possess an imidazole ring and has recently been reported for its anticancer properties³⁵. Within this series, the bis-carbene complex ($[\text{Au}(\text{9-methylcaffeine-8-ylidene})_2]^+$ **12** showed selectivity *in vitro* against various

cancer cell lines with respect to non-tumorigenic ones, and most importantly was also demonstrated to be a selective G-quadruplex stabilizing agent. X-ray structural studies showed that the three molecules of compounds are bound to a G4 structure³⁶. G-quadruplexes (also known as G4) are nucleic acid sequences, rich in guanines, capable of forming a characteristic four-stranded fold. Interestingly, formation of quadruplexes causes a net decrease in the activity of the enzyme telomerase, which is responsible for maintaining the length of telomeres. Therefore, molecules that template the formation or stabilize the structure of G-quadruplex DNA might lead to development of new effective anticancer drugs based on selective telomerase inhibition³⁷.

Within the NHC family, also Pt(II) complexes have been described, including [(1,3-dibenzyl)imidazol-2-yl]platinum(II) carbene complexes with different spectator ligands (Cl⁻, dmsO, PPh₃)³⁸. Some of these compounds were shown to bind DNA predominantly by initiating its aggregation and precipitation to the effect of a G1 phase cell cycle arrest in melanoma cells.

Metallocene compounds, homonuclear as well as heteronuclear, have gained considerable interest in anticancer research throughout the last years. For example, titanocene dichloride (Cp₂TiCl₂) (**13**), was one of the early non-platinum based anticancer complexes that reached phase II of clinical trials³⁹. However, the clinical response was not significant enough to pursue it, especially since Cp₂TiCl₂ displays rather poor solubility and stability in water, leading to problems in drug formulation⁴⁰. Furthermore, the exact mode of action could not be elucidated so far⁴¹. Addressing the solubility and stability properties, various titanocene analogues have been synthesized. McGowan *et al.*⁴² demonstrated that by introducing amino groups to the bent metallocenes, improved hydrolytic stability could be achieved⁴³.

The organometallic arene complexes termed RAPTA are among the most investigated examples of Ru(II) half-sandwich complexes with antimetastatic and antiangiogenic properties⁴⁴. Consisting of a monodentate 1,3,5-triaza-7-phosphaadamantane (pta) ligand and a facial η^6 -arene ligand coordinated to Ru(II) in a so-called “piano-stool” conformation, RAPTA complexes display good stabilities under physiological conditions. Concerning the mode of action, aquation of the chloride ligands appears a prospective intracellular drug activation process⁴⁵. Although the exact target identification is still elusive, RAPTA compounds have been found to alter the expression, and thereby the activity, of key proteins involved in the regulation of the cell cycle and apoptosis⁴⁶. RAPTA-C (arene=cymene) (**14**) is the most representative example of this series⁴⁷. Similar to titanocene dichloride, a number of derivatives have been synthesized in order to stepwise alter the physicochemical properties⁴⁸.

Metal complexes can interfere in the cellular redox chemistry in several ways: directly through metal or ligand redox centers or indirectly by binding to biomolecules involved in cellular redox pathways. Within the metallocene family, Sadler *et al.* illustrated that organometallic ruthenium(II) and osmium(II) arene complexes and iridium(III) cyclopentadienyl complexes of the type [(arene/Cp^{xPh})M(N,N)Cl/I]ⁿ⁺ can achieve nanomolar potency toward cancer cells in combination with the redox modulator L-buthionine sulfoximine^{44b, 49}. A representative member of this series – the chlorido(iminopyridine)arene- ruthenium(II) complex [Ru(η^6 -*p*-cym)(*p*-Impy-NMe₂)Cl]⁺ (**15**) - is reported in Fig. 2. Thus, these complexes were proposed for possible use in combination therapy with redox modulators to increase their anticancer effects. These results highlights the importance of determining

not only the distribution of metal anticancer complexes in cells but also their speciation, the chemical form of the metal complex, including the oxidation state of the metal, the fate of the ligands, and dynamic processes such as efflux.

Ferrocene, a compact metallocene possessing stability in non-oxidating media, low toxicity, and reversible redox behaviour, has recently played an important role in bioorganometallics, as an antiparasitic or an antibacterial⁵⁰, and indeed as an antitumor agent⁵¹.

Examples of the vast and regularly increasing number of biologically active ferrocene compounds with antitumoral potential are presented in a recent review by Jaouen, Vessieres *et al.* and will not be treated in details here⁵². Overall, they illustrate the richness of the activity in this field, the variety of the structures brought into play and the diversity of possible mechanisms of activity.

Here, we selected to highlight the ferrocene containing complex ferroquine (**16**) structurally close to the antimalarial drug chloroquine⁵³. It shows similar activity compared to chloroquine but most importantly it is also active against chloroquine resistant malaria parasites⁵⁴. Since malaria resistance has become a critical issue in malaria-endemic countries, development of new, organometallic analogues is crucial. Ferroquine has completed phase IIb of clinical trials and is about to enter phase III⁵⁵.

Recently, there has been a strongly growing interest in the utilization of heteronuclear compounds as anticancer agents⁵⁶. This is due to the hypothesis that different metals within the same compound can either react in different pathways towards the targeting of cancer cells or improve the chemophysical properties of the overall scaffold. A greater challenge in this concept of *multinuclearity* consists in the combination of two (or more) different metal containing moieties, requiring a design of suitable ligands to coordinate selectively one metal and the other. Thus, a number of successful examples have been described in the literature, including ferrocene-based complexes. For example, in 2008 Dyson *et al.* reported on the synthesis of a ferrocenoyl pyridine arene ruthenium complex (**17**). Interestingly, this complex proved to be twice as active towards cancer cells, as their monomeric analogue⁵⁷. Contel *et al.* demonstrated the cytotoxic properties of a heterometallic complex series containing both a gold(III) and a palladium(II) metal center with iminophosphorane ligands (**18**), derived from ferrocenylphosphane⁵⁸. Especially the trimetallic derivatives have shown significant higher anticancer properties with respect to their corresponding monomeric analogues.

When other metal other than iron was used, Casini *et al.* reported in 2011 on the synthesis of the titanocene-gold trimetallic complex (**19**) showing 10-fold higher activity against cancer cells than the monomeric titanocene-phosphine and enhanced stability in aqueous solution⁵⁹. Interestingly, this complex also exhibits luminescent photophysical properties, originating from the bent metallocenes, making it suitable for distinctive uptake studies. The same group also reported on series of bimetallic Ti-Ru complexes based on a titanocene-phosphine backbone anchored to a Ru(II)-arene scaffold, which showed improved antiproliferative effects on cancer cells in comparison to their mononuclear Ti and Ru organometallic precursors⁶⁰.

Notably, the synthesis, characterization and stability studies of new titanocene complexes containing a methyl group and a carboxylate ligand (mba = S-C₆H₄-COO) bound to gold(I)-phosphane fragments through a thiolate group $[(\eta\text{-C}_5\text{H}_5)_2\text{TiMe}(\mu\text{-mba})\text{Au}(\text{PR}_3)]$ were recently reported⁶¹. Two compounds

were selected for further *in vivo* studies on mice based on their selectivity *in vitro* against renal cancer cell lines when compared to non-tumorigenic human kidney cell lines (HEK-293T and RPTC) and the favourable preliminary toxicity profile in C57BL/6 mice. Evaluation of Caki-1 xenografts in NOD. CB17-Prkdc SCID/J mice showed an impressive tumor reduction (67%) after treatment for 28 days (3 mg per kg b.w. every other day) with heterometallic compound $[(\eta\text{-C}_5\text{H}_5)_2\text{Ti}(\text{CH}_3)[\text{OC}(\text{O})\text{C}_6\text{H}_4\text{SAu}(\text{PPh}_3)]]$.

In the following section we will introduce the class of organometallic cyclometalated complexes, which constitutes the focus of this review. After a brief introduction on these chemical scaffolds, we will introduce the studies on cyclometalated Pt(II) and Au(III) compounds as experimental anticancer agents.

2. Cyclometalated complexes

Cyclometalation is a convenient method of stabilizing metals in different oxidation states. This is particularly useful in the case of Au(III) compounds which are otherwise prone to be reduced to their respective Au(I) species as well as to colloidal gold. In general, cyclometalation is defined as the metal mediated C-R bond activation of a cyclic organic ligand system. The chelating ring consists of a strong, covalent C-M σ bond and a coordination D-M bond. These ligands incorporate one or more donor atoms (such as O-, N-, P-, S- or -Se). Fig. 3 illustrates a general representation of the different classes of cyclometalated compounds. However, it is worth mentioning that a variety of processes other than cyclometalation have been developed in order to prepare metallacycles, especially oxidative addition involving C-X bond activation (X = F, Cl, Br, I etc.), and transmetalation. Similarly, metallacycles may be generated by elimination reactions, by cycloaddition and by hydrometalation.

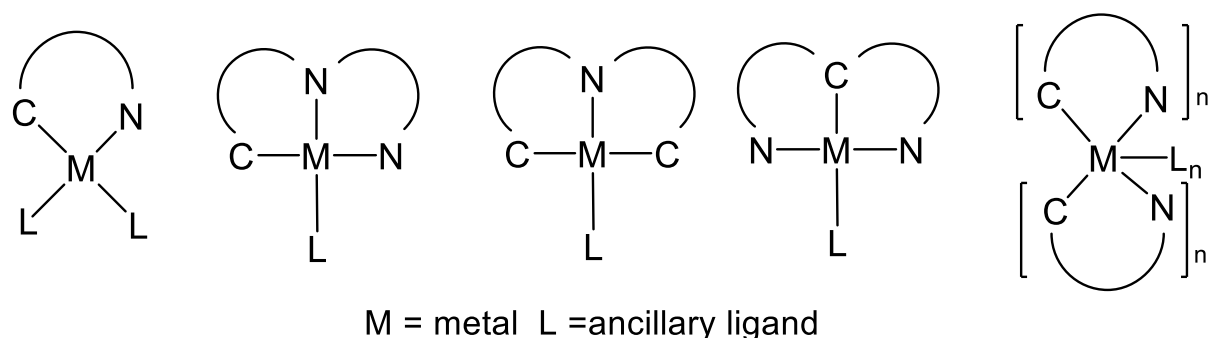


Figure 3. General representation of cyclometalated complexes.

In this review the main results obtained on the synthesis and biological activities of cyclometalated Pt(II) and Au(III) compounds are summarized. Numerous ligand systems utilizing different donor atoms have been reported¹⁸. In particular, cyclometalation reactions producing five-membered ring products proceed very easily, at room temperature with a variety of substrates, such as amines, imines, 2-phenylpyridines, benzo[*h*]quinones, other nitrogen donor ligand, oxygen-containing compounds, phosphorus and sulfur donors etc. Thus, a consistent number of reports include these families of complexes.

Since a complete overview of all ligand systems would go beyond the scope of this review, in the following chapters only C[^]N, C[^]N[^]C, C[^]N[^]N and C[^]N[^]S Pt(II) and Au(III) pincer complexes shall

be discussed, as these ligands show the highest stability in physiological environment and suitable chemico-physical properties for therapeutic applications. In addition, a number of studies report on the promising biological effects of these compounds and constitute the basis for future drug design.

2.1 Pt(II) compounds

Although the cisplatin analogues already in the clinic circumvented its severe side effects to some extent, still new platinum complexes with improved pharmacological and toxicological profiles would be highly valuable⁶². Therefore, research has been evolved towards finding new structural motifs for platinum(II) compounds, including those achievable in the cyclometalated scaffolds described below.

C^N complexes

Following the structural idea of cisplatin, a variety of bidentate Pt(II) compounds was synthesized in the early stage of the identification of biologically active cyclometalated anticancer complexes. The C^N ligand is kinetically rather inert and offers therefore higher stability than most other bidentate ligands. Given that most bidentate ligands are also relatively easy accessible, it is not surprising that Pt(II) C^N complexes make up a vast part of cyclometalated platinum complexes as experimental anticancer agents⁶³. As previously mentioned, cyclometalation reactions that give five-membered ring products are very favourable, since five-membered ring compounds display higher stability than most other ring sizes. The five-membered ring chelate effect also allows a higher selectivity and increased yields. Therefore, many of the reported C^N metal complexes are indeed five-membered ring complexes^{63a}.

The early stage bidentate C^N ligands mostly followed the structural design of Pt(II)(C^N)LX (L and X ancillary ligands) (Fig. 4). The group of Alonso *et al.* reported already in 1993 on the synthesis of a Pt(II) complex (**20**) utilizing a benzoylbenzylidenamine backbone and chlorido- or acetate-bridged labile ligand⁶⁴. The structural analogous Pd(II) complexes reported in this paper were synthesized via C-H activation of the respective ligand by Pd(OAc)₂. However, the standard orthoplatination method for the Pt complexes, using K₂PtCl₄ in MeOH as a precursor, only resulted in the formation of the coordination compound of 4-methoxyaniline, as these derivatives are not stable in aqueous media. The respective orthoplatinated compounds could only be achieved when using the dimeric precursor [Pt(μ-Cl)(η³-C₄H₇)₂]. This dimeric complex can increase its coordinative unsaturation level and is strong electrophilic. The respective acetate complex was obtained *via* reaction with AgOAc (see Fig. 4). Interestingly, these complexes were shown to exhibit significantly higher antiproliferative activity than their isostructural palladium compounds. By electrophoresis studies, the authors could demonstrate that these compounds are able to modify the plasmid DNA structure, since significant alteration in the gel electrophoretic mobility of plasmid DNA was observed upon binding of the metal complexes⁶⁴.

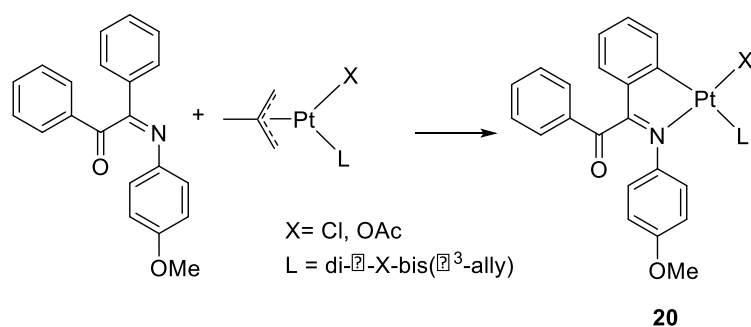


Figure 4. Chlorido- and aceto-bridged Pt(II) C^N complex as reported by Alonso *et al.*[65].

Another example for earlier Pt(II) C^N complexes is the 2-phenylpyridine Pt(II) complex **21** that was reported by Okuno *et al.* in 2001⁶⁵ (Fig. 5). In this case, the synthesis is straightforwardly carried out by reacting [PtCl₃(NH₃)]⁺ (substituting one chlorido ligand in cisplatin with NEt₄Cl) with 2-phenylpyridine in water. This compound was found to accumulate in the cisplatin-resistant mouse sarcoma 180 cell (S-180cisR) in line with its high cytotoxicity⁶⁶.

Hemmateenejad *et al.* introduced a chelating bisphosphine ligand to the platinum metal center (**22**)⁶⁷. The synthesis is carried out in a two-step mechanism, similar to complex **21**. First [Pt(dmsO)₂Cl₂] is reacted with 2-phenylpyridine; afterwards, the dmsO ligands are substituted with bis(diphenylphosphino)amine. The pentacoordinated compounds were found to have good proteasome-inhibitory activity and induced apoptosis *in vitro*. DNA binding studies in aqueous media confirmed that the complexes maintain their pentacoordinated configuration, acting as the pharmacologically active species⁶⁸. Interaction of the complexes with herring sperm DNA was investigated via fluorescence emission spectroscopy, suggesting that Pt(II) containing bisphosphine complexes with DNA binding capabilities can also target and inhibit the tumor proteasome⁶⁹.

In 2005, Che *et al.* reported a thiophene-containing complex series **23a-c** containing amino acid ligands⁷⁰. Interestingly, both protein binding affinity and cytotoxicity of **23a-c** are affected by the amino acid ligand. Thus, while **23a** has an IC₅₀ over 100 μ M in various carcinoma cell lines (non-toxic compound) and scarce HSA binding, complex **23b** exhibits marked cytotoxicity against cisplatin-resistant cells (e.g. human liver cancer cell line HepG2 and human lung cancer cell NCI-H460) as well as selectivity towards human serum albumin (HSA) via H-bonding interactions. Overall, the IC₅₀ values decrease in the order **23a** > **23c** > **23b**, mirroring a corresponding decrease in HSA binding affinity. Moreover, the compounds display luminescent properties which can be enhanced by protein binding, and that make them suitable as chemical probes for imaging.

Bautista *et al.* synthesized a series of Pt(II) compounds based on a 2-(dimethylaminomethyl)phenyl as the C^N backbone (**24-26**) (Fig. 5)⁷¹. These complexes are derived from N,C-chelating 2-(dimethylaminomethyl)phenyl (dmmba) and pentafluorophenyl groups in *cis*-[Pt(C₆F₅)₂(THF)₂], and all of them show up to 20-fold higher cytotoxicity against various cancer cell lines than cisplatin. By circular dichroism and electrophoretic mobility assays it could be observed that interactions of the complexes with the DNA alter the degree of super-helicity. With the aim of coupling bioactive ligand to organometallic compounds, the cationic complex **25** of structural formula [Pt(dmsO)(bpzm*)(1-Mecyt)]⁺ (with (1-Mecyt) = methyl-cytosine, (bpzm*) = bis(3,5-dimethylpyrazol-1-yl)methane) featured the model

nucleobase methyl-cytosine as a ligand⁷². This approach enables new ways of incorporating biologically important scaffolds into the complexes.

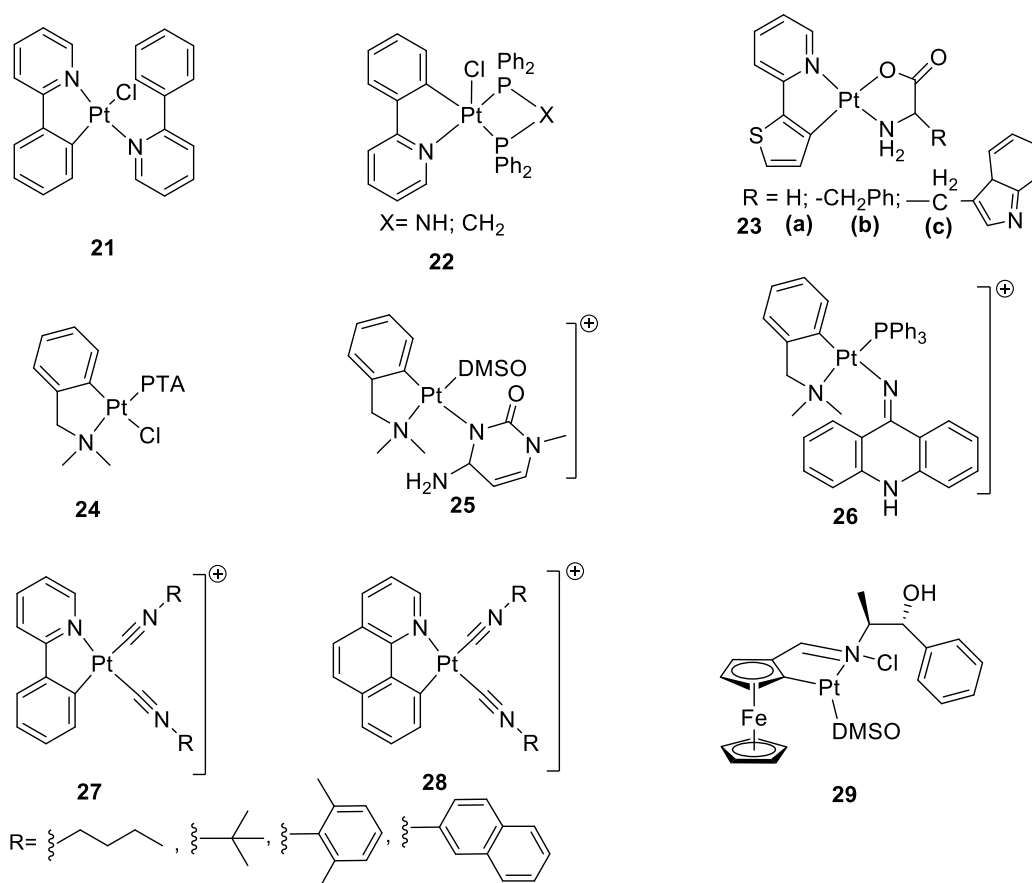


Figure 5. Pt(II) C^N complexes with cytotoxic properties.

Platinum coordination complexes with acridinylthiourea as a potent DNA intercalator have been reported before, acting through monofunctional platination of DNA nucleobases⁷³. Bautista, Laguna *et al.* introduced complex **26**, a Pt(II) C^N complex with an 9-aminoacridine ligand, as a DNA intercalator⁷⁴. The compound is luminescent in the solid state at room temperature, making it a suitable candidate for distinctive uptake studies. Complex **26** shows 20-fold higher toxicity against the leukaemia cancer cell line HL-60 than cisplatin. DNA adduct formation on plasmid DNA pBR322 was observed by circular dichroism and electrophoretic mobility as well.

Similar Pt(II) C^N complexes have also been reported to exert dual antitumor and antiangiogenic effects in cell lines *in vitro*⁷⁵; while two oxoisoaporphine Pt(II) C^N complexes were characterized as G-quadruplex stabilizers and shown to be able to induce apoptosis in cancer cells via inhibition of telomerase⁷⁶.

Topoisomerase inhibition as possible mechanism of anticancer action has been a subject of intensive research during the last years. The cytotoxicity of topoisomerase inhibitors has been assumed to result from the induction of enzyme-mediated DNA breaks⁷⁷. Complexes **27** and **28** of the general formula [Pt(phpy)(C≡NR)₂]⁺ (phpy = 2-phenylpyridine, R = 2-naphthyl) were introduced by

Che *et al.* in 2011 as effective topoisomerase II α inhibitors (Fig. 5)⁷⁸. By the displayed square-planar Pt(II) geometry within the C^N moiety these complexes were shown to act as major groove binder to the DNA. Using UV-Vis absorption and emission titration Che *et al.* could demonstrate that a stabilization of the covalent topoisomerase II α -DNA cleavage complex occurs with consequent induction of apoptosis.

Complex **29** represents a very recent field of Pt(II) C^N complexes, being tethered to a ferrocene moiety to give an heteronuclear compound⁷⁹. Due to the symmetry of the ferrocenyl moiety, two isomers exist, that differ in the planar chirality of the ferrocenyl ligand (*S_p* and *R_p*, respectively). The *R_p* isomer exhibits up to four times higher cytotoxic activity against various cell lines (such as colon cancer (HCT116) and breast cancer (MDA-MB-231)) than the *S_p* isomer, thus indicating that the orientation of the ferrocenyl unit in relation to the environment of the Pt(II) metal influences their cytotoxic activity.

Finally, an interesting C^N ligand system containing iminophosphoranes has been reported by Contel *et al.* in 2015⁸⁰, which lead to the formation of Pt(II)C^N and Au(III)C^N complexes (Figure 6) *via* transmetalation of the mercury precursor. Both complex types exhibit more pronounced cytotoxic activity than cisplatin against numerous cell lines (such as the ovarian cancer cell line A2780). Gold complex **31** induces mainly caspase-independent cell death, an effect described before for analogous cycloaurated iminophosphorane compounds⁸¹. The Pt(II) complex **30** is also able to activate alternative caspase-independent apoptosis mechanisms: experiments of DNA-drug interactions with plasmid DNA demonstrated that **30** induces the formation of left-handed helix of Z-form DNA through strong electrostatic interactions.

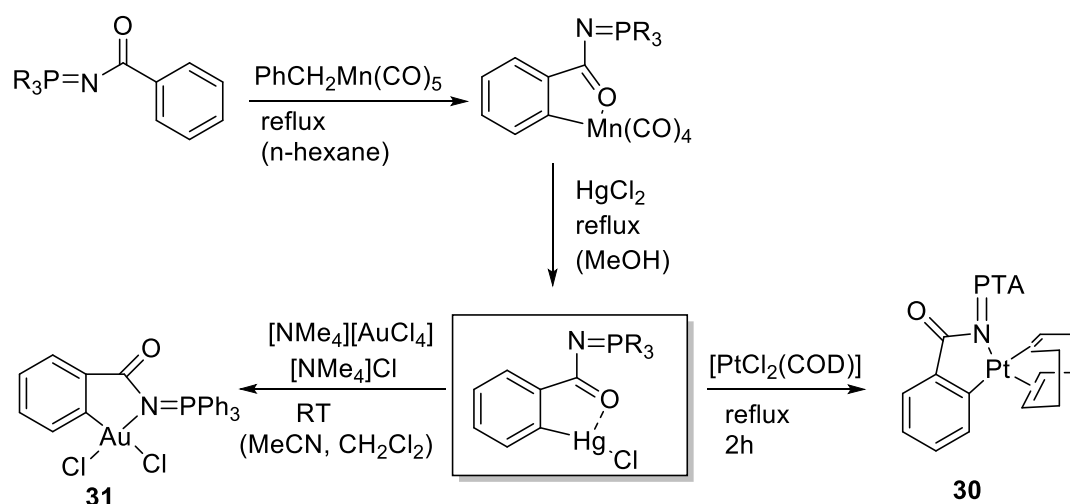


Figure 6. Synthesis of 30 and 31 [79].

Conventional chemotherapeutics, but also innovative precision anticancer compounds, are commonly perceived to target primarily the cancer cell compartment. However, recently it was discovered that some of these compounds can also exert immunomodulatory activities which might be exploited to synergistically enhance their anticancer effects. One specific phenomenon of the interplay between chemotherapy and the anticancer immune response is the so-called “immunogenic cell death” (ICD)⁸².

In this context, in 2015, Ang *et al.* evaluated the ICD activity of a library of thirteen Pt-based compounds, including cisplatin, oxaliplatin and carboplatin as well as their Pt(IV) prodrugs⁸³. Based on the fact that a critical step for ICD is the engulfment of dying cancer cells by dendritic cells, authors first screened the compounds with an *in vitro* phagocytosis assay. Interestingly, just one compound in the entire series – a C[^]N Pt(II) complex of 2-phenylpyridine bearing bis(NHC) ligands, previously synthesized by Che and co-workers⁸⁴ – was able to increase the tumor cell phagocytosis at low concentration. In the same study, the evaluation of the C[^]N Pt(II) complex was conducted and the obtained results showed that it fulfils the hallmarks of ICD, namely calreticulin exposure, ATP secretion, and extracellular HMGB1 release⁸³. Furthermore, they demonstrated that ER stress triggered by Pt-NHC was ROS-mediated, probably placing this drug in the type II ICD inducer class.

C[^]N[^]N complexes

Cyclometalated Pt(II) compounds featuring a C[^]N[^]N ligand represent an interesting family, since they have been shown not only to be able to covalently link to the nucleobases of the DNA, but also to function as metallointercalators by inserting between two adjacent DNA nucleobases through π - π stacking⁸⁵. As for the Pt(II) C[^]N type of compounds, the majority of the Pt(II) C[^]N[^]N complexes are also five-membered ring compounds, resulting from cyclometalation reactions^{63a}.

In 2010 Che *et al.* reported on the synthesis of Pt(II) C[^]N[^]N complex **32**, displaying an ancillary NHC ligand with differing N,N side chain lengths⁸⁶. The synthesis was carried out by reacting K₂PtCl₄ with the H-C[^]N[^]N ligand in acetonitrile under reflux. The carbene moiety was subsequently attached *via* deprotonation of the respective imidazolium precursor with KOtBu in a one pot-synthesis. Imidazolium salts with varying side-chain lengths were obtained by reacting methyl-imidazole with the respective alkyl halide. Binding of the NHC moiety to the Pt(II) center results in luminescence properties and significantly enhances the complex stability against ligand exchange reactions or biological reductions. The complexes in this series were shown to be stable against GSH reduction/substitution, which can be considered as an important feature for future drug design, since the elevated cellular GSH level has been linked to cisplatin-resistant cancer cells, probably through sequestration of cisplatin⁸⁷. The lipophilicity of the cationic complex **32** can be fine-tuned by varying the N,N chain length. Complex **32d** was thereby found to display more than 200-fold higher cytotoxic activity against cervical cancer cells (HeLa) than cisplatin. *Via* fluorescence microscopy it could be observed that complex **32d** preferably accumulates in cytoplasmic structures. Furthermore, the authors could demonstrate that **32d** has a synergistic effect with cisplatin *in vitro*. Even more notably, **32d** was capable of significantly inhibiting tumor growth in a nude mouse model.

Subsequently, the same group synthesized two N,N-bridged Pt(II) C[^]N[^]N complexes **33a** and **33b** with N-heterocyclic carbenes as ancillary ligands⁸⁶. Although showing higher cytotoxic activity than cisplatin, they displayed lower IC₅₀ values than their mononuclear analogues. Based on X-ray structures it was demonstrated that the short bridge between the two NHC moieties of **33a** confines the two Pt(II) C[^]N[^]N planes in close proximity rendering intra-molecular Pt–Pt interactions feasible.

In 2011, Biot *et al.* reported on a pyrazole Pt(II) C[^]N[^]C complex **34**⁸⁸. This complex was obtained by reacting the Pt(II) precursor *cis*-[PtCl₂(DMSO)₂] with 1-(2-dimethylaminoethyl)-3,5-diphenyl-1H-pyrazole in toluene under reflux conditions. This complex shows higher cytotoxic activity in lung

cancer cells (A549) and breast cancer cells (MDA-MB-231 and MCF-7) than cisplatin. However, it does not alter plasmid DNA mobility, suggesting a different mechanism of action.

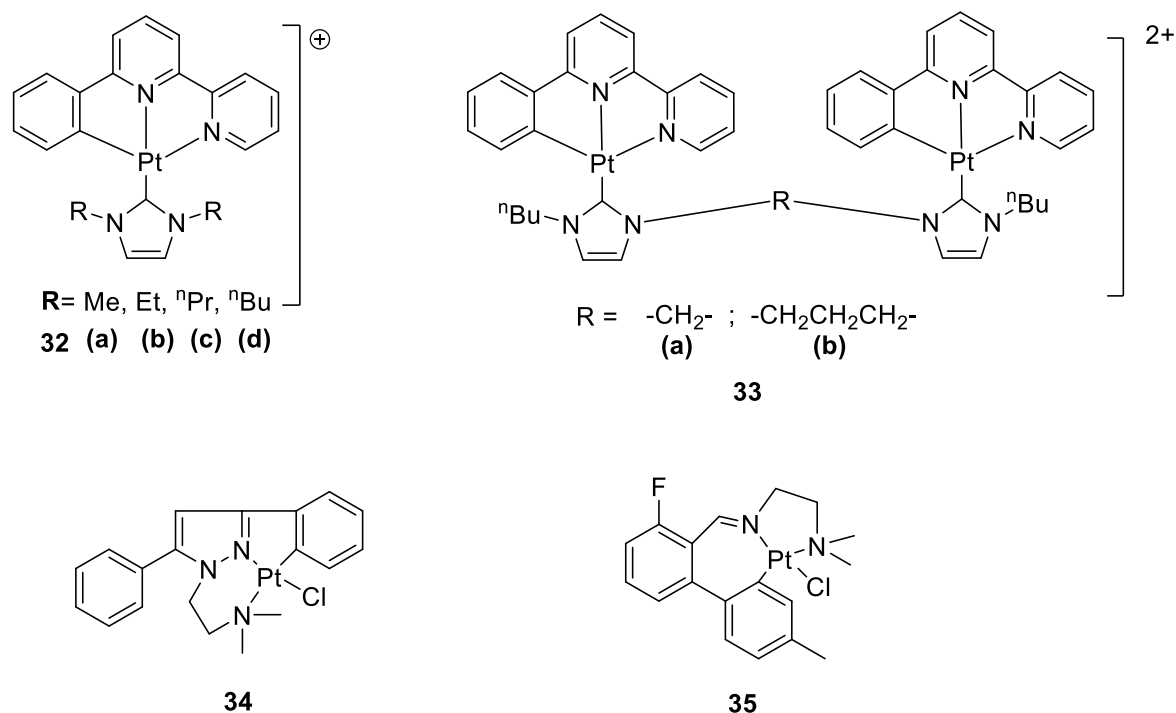


Figure 7. Recent Pt(II) C^NN systems with cytotoxic properties.

In 2012, the group of Cascante *et al.* reported on the synthesis of seven-membered Pt(II) C^NN complexes **35**⁸⁹ by reaction of the dimeric Pt(II) precursor [Pt(4-C₆H₄Me)₂(μ-SEt₂)₂] with the imine 2-F,6-ClC₆H₃CH=NCH₂CH₂NMe₂ in toluene under reflux. The reaction proceeds via a Pt(IV) intermediate, due to the intramolecular activation of one *ortho* C-Cl bond of the Pt(II) ligand. This novel framework complex shows higher cytotoxic activity in lung cancer cells (A549) and breast cancer cells (MDA-MB-231 and MCF-7) than cisplatin, being comparable in this respect to **34**. However, DNA electrophoretic mobility studies show that complex **35** modifies the DNA tertiary structure. Furthermore, induction of S-G₂/M arrest and apoptosis were also observed.

In 2015 Che *et al.* described the luminescent properties of a series of Pt(II) complexes of the general formula [Pt(C^NN)(C≡NR)]ClO₄⁹⁰. These pincer complexes act as metallo-intercalators and adducts with emission properties sensitive to the structure of nucleic acids. Various complexes were synthesized (Fig. 8), but only complex **36-1a** shows highly active inhibiting behaviour towards various cancer cell lines *in vitro* (SH-5YSY (neuroblastoma), NCI-H460 (non-small-cell lung carcinoma), SUNE1 (nasopharyngeal carcinoma)) and inhibited tumor growth in mouse model. This is one of the first examples of a metallo-intercalator that acts as an emission probe for nucleic acids, while having anti-cancer properties at the same time. The intercalation is carried out via the C^NN ligand plane being inserted in a parallel fashion to the DNA bases. As mentioned before, the emission occurs by π-stacking interactions between the intercalator and the double-stranded DNA nucleobases⁹¹.

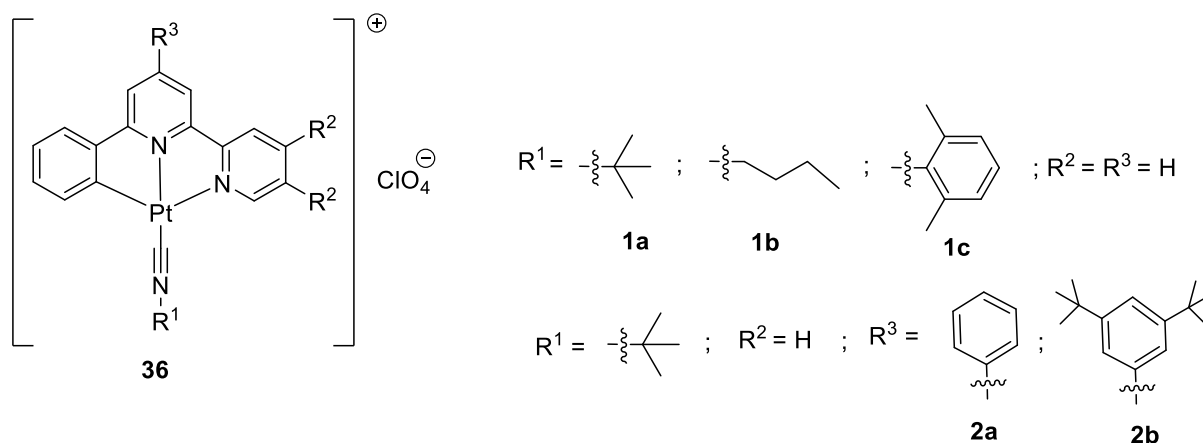


Figure 8. Luminescent Pt(II) C^NN complexes as reported by Che *et al.*[86]

The mode of action is believed to include cleavage-enhancement of the double-stranded DNA substrate, as indicated by the appearance of the 13-mer product. The stabilization of the topoisomerase-I-DNA complex, resulting in DNA damage, is thereby assumed to synergize the anticancer activity of **36-1a**. Fig. 9 depicts how **36-1a** selectively forms an emissive exciplex with double-stranded DNA. The difference observed in the emitted wavelength is indicative of differences between binding to dsRNA or dsDNA, respectively.

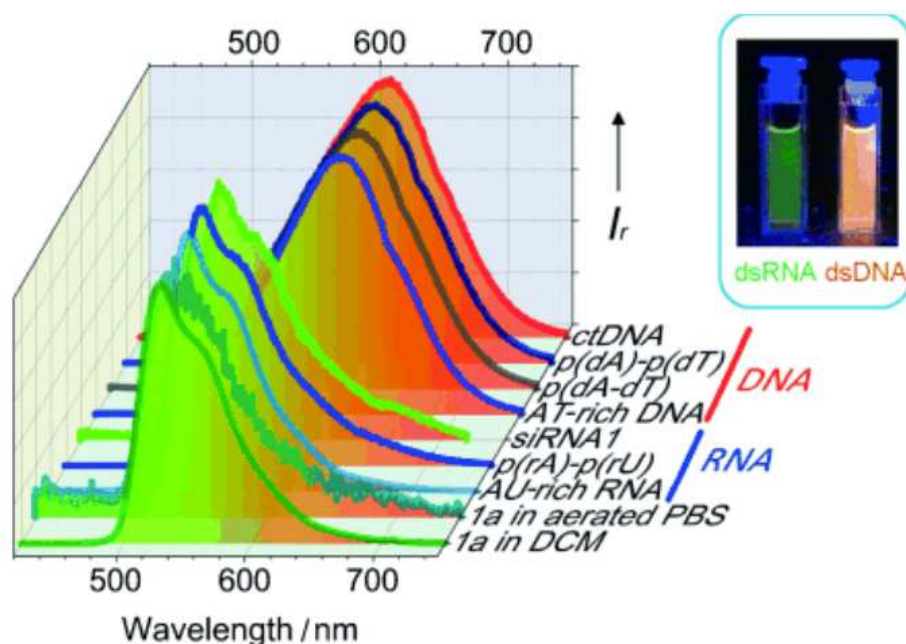


Figure 9. Emission spectrum of 35-1a with dsDNA and dsRNA [86]. Reprinted with permission of Wiley-VCH.

C^NA^C complexes

So far, only a handful of platinum(II) C^NA^C pincer complexes are known, which have been used mainly as photoluminescence emitters⁹² or as hydrovinylation catalysts⁹³.

The prominence of palladium(II) pincer complexes over their platinum(II) analogues is given by the fact that Pt(II) is one of the most kinetically inert metal ions known, making a double carboplatination more challenging than a single one, as observed for the C[^]N, C[^]N[^]N or C[^]N[^]S systems⁹⁴.

Thus, a unique Pt(II) C[^]N[^]C pincer carbene complex **37** was reported by Santra *et al.* in 2014 (Fig. 10)⁹⁵. The synthesis was carried out via transmetalation of the respective silver C[^]N[^]C complex with Ag₂O. The C[^]N[^]C pincer ligand was obtained by reacting 1-methylbenzimidazole and 2,6-bis(bromomethyl)pyridine under reflux in dioxane. Complex **37** displays modest activity against breast (MCF7), colon (HCT116) and lung (A549) cancer cell lines. This field certainly has potential for further expansion in the future.

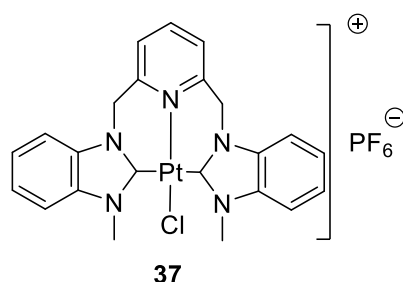


Figure 10. Pt(II) C[^]N[^]C pincer as reported by Santra *et al.* [91].

C[^]N[^]S complexes

Representative members of this family are reported in Fig. 11 below. Pt(II) C[^]N[^]S systems as anticancer agents were also pioneered by Alonso, Navarro-Ranninger *et al.* in 1998 by introducing complex **38**⁹⁶. This complex is obtained by reacting the Pt(II) precursor Pt₂Cl₄ in equimolar amounts with *p*-isopropylbenzaldehyde thiosemicarbazone in MeOH. This complex shows cytotoxic activity comparable to cisplatin against various human cancer and murine cell lines but also cytotoxic activity against cisplatin resistant cancer cell lines such as Pam-ras cells. DNA interaction studies suggest that complex **38** forms interhelical cross links with the DNA.

Following this initial work, Veith *et al.* presented a 2-acetylthiophenethiosemicarbazone (ATTSC) Pt(II) complex **39** in 2012⁹⁷. Interestingly, this complex is obtained when reacting K₂PtCl₄ with the Schiff base ATTSC in 1:2 ratio. For a K₂PtCl₄ to ATTSC ratio of 1:1 the tetrameric complex **40** is obtained with four platinum atoms displaying an almost square planar geometry, being coordinated intermolecularly to a sulfur atom of a neighbouring ligand. The crystal structures of these platinum complexes were solved and differentiated by single-crystal X-ray diffraction structure determinations. Both complexes show higher cytotoxic activity in colorectal (HT-29) and duodenal adenocarcinoma cells (HuTu-80) than cisplatin, with the tetrameric complex being slightly more active. This leads to the hypothesis that the tetrameric complex intercalates very well between the nucleobases of DNA tumor cells, causing greater conformational changes in the double helix of DNA, leading to apoptosis.

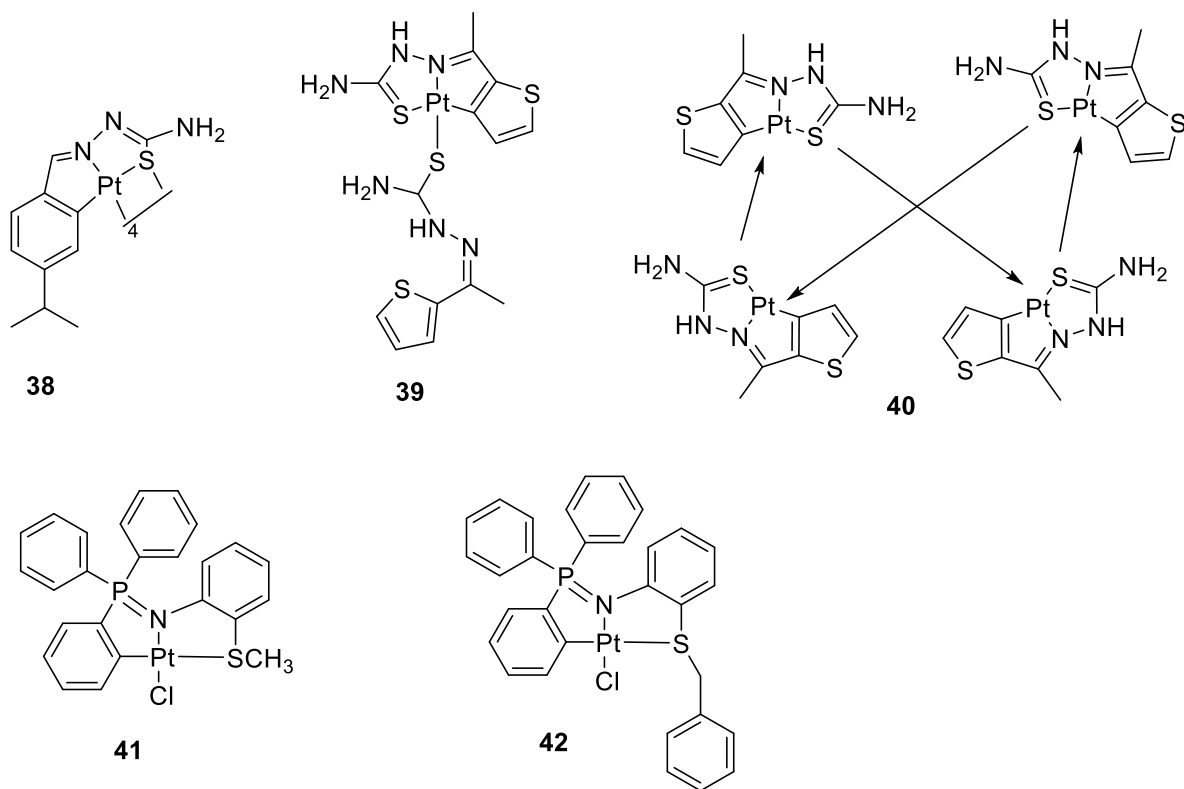


Figure 11. Recent Pt(II) C^NS complexes.

In 2016, Grévy *et al.* reported on the synthesis of non-symmetric Pt(II)C^NS complexes that include thioether functionalized iminophosphoranes (**41-42**)⁹⁸ (Fig. 11). These compounds were obtained by reacting the thioether moiety containing iminophosphorane $\text{PPh}_3=\text{NC}_6\text{H}_4\text{SR}$ [$\text{R} = \text{CH}_3$ (**41**), $\text{CH}_2\text{C}_6\text{H}_5$ (**42**)] with the Pt(II) precursor $[\text{Pt}(\text{SMe}_2)\text{Cl}_2]$ in refluxing dichloromethane. Preliminary cytotoxic studies show that both complexes have better cytotoxic activity than cisplatin against cervical (HeLa) and erythroleukemic (K562) cancer cell lines.

2.2 Au(III) compounds

Au(III) compounds as anticancer agents appeared a logical step forward from platinum(II)-based compounds, since gold(III) is isoelectronic (d^8) and isostructural to platinum(II) featuring the same square-planar geometry. However, at variance with Pt(II) complexes, numerous studies have shown that anticancer gold compounds have other biomolecules and biological pathways as possible targets⁹⁹. As Au(III) is easily reduced to its more stable Au(I)/Au(0) forms, Au(III) complexes are generally strongly oxidizing agents. Reductions can be easily driven by thiols groups of biological systems, making Au(III) potentially toxic¹⁰⁰. Therefore, Au(III) complexes were long believed not to be suitable for medicinal applications¹⁰¹. As a consequence, Pt(II) cyclometalated complexes for the treatment of cancer cells are (yet) more numerous. Cyclometalation, however, offers a convenient way for the stabilization of the Au(III) center and “fine tuning” of the physicochemical properties within the cyclometalated moiety.

C[^]N complexes

Representative members of this family are reported in Fig. 12 below. The class of Au(III) C[^]N complexes with cytotoxic properties was first introduced by Parish, Buckley *et al.* in 1996¹⁰². They synthesized a series of Au(III) complexes of the structure [(damp)AuX₂] with a 2[dimethylamino)methyl]-phenyl (damp) backbone (**43**). The compounds displayed cytotoxic activities comparable to cisplatin against various cancer cell lines, such as bladder (HT1376) and ovarian (CH1) cancer cells, with the malonato and acetato substituted complex being the most selective and active of the series *in vitro*, and showing moderate anticancer effects *in vivo*. Although the structure appears to be very similar to cisplatin, an alkaline elution study showed that the series does not cause interstrand DNA cross-links. Instead, both complexes lead to submicromolar inhibition of cysteine protease cathepsin B¹⁰³ and inhibition of TrxR¹⁰⁴.

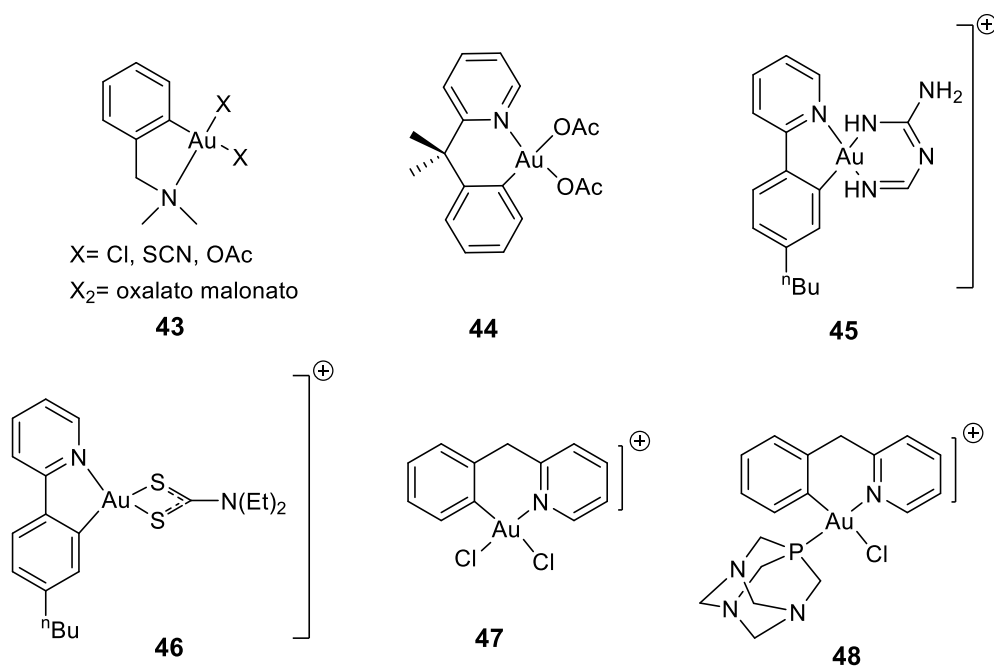


Figure 12. Au(III) C[^]N complexes with cytotoxic properties.

Messori *et al.* reported on the synthesis of complex **44** enabling a dimethylbenzyl-pyridine backbone in 2005¹⁰⁵. This complex proved to be stable under cellular reducing conditions; although the cytotoxic activity against ovarian cancer cell line A2780 is comparable to cisplatin, complex **44** displays significant cross-resistance, suggesting a different mechanism of action. It was demonstrated that **44** selectively inhibits TrxR activity, which is presumed to be caused by progressive oxidative damage of (seleno)cysteine residues of the active site of TrxR^{8a}. Other studies showed that the hydrolysis product of **44** is able to disrupt mitochondrial function and alter the glycolytic pathway in A2780 cancer cells, leading to apoptosis¹⁰⁶.

The cationic complex **45**, bearing a biguanide moiety, was synthesized by Che *et al.* in 2012¹⁰⁷. By introducing the polar biguanide ligand, a water-soluble complex was obtained that is able to form Au(III)-GSH complex detectable by ESI-MS. The complex displays higher toxicity than cisplatin against several cancer cell lines, such as cervical cancer (HeLa) cells. The toxicity of complex **45** is believed to be caused by swelling of the endoplasmic reticulum (ER), being supported by oligonucleotide microarray analysis and western blotting assays cells. Irreversible ER stress triggers apoptosis, with the activation of the canonical mitochondrial cell death pathway playing an essential role¹⁰⁸. A structural analogue (**46**) was reported by the same group, also utilizing a dimethylbenzyl-pyridine C[^]N backbone and a dithiocarbamate ligand¹⁰⁹. This complex selectively inhibits breast cancer cells (MCF-7) but is less toxic to non-tumorigenic immortalized liver cells (MIHA). Complex **46** was demonstrated to form adducts with cysteine-containing peptides and proteins (e.g. deubiquitinases) by ESI-MS experiments. Notably, Au(III) C[^]N complexes bearing a dithiocarbamate ligand had been previously proven by Contel *et al.* to cause mitochondria dysfunction induced by reactive oxygen species (ROS) and Bax/Bak activation¹¹⁰.

Synthesis of the 2-benzylpyridine derivative **47** was reported by Cinellu *et al.*¹¹¹ already in 1996 by reacting NaHAuCl₄ with 2-benzylpyridine in refluxing MeCN/H₂O overnight. In 2015 Casini, Cinellu *et al.* synthesized the structural analogue by replacing one chlorido ligand in the presence of excess KPF₆ with 1,3,5-triaza-7-phosphaadamantane (PTA)¹¹². PTA offers multiple benefits as a ligand, as it is non-toxic and increases the water solubility of the resulting complexes. The cytotoxic properties of complex **47** and **48** against various cancer cell lines such as ovarian adenocarcinoma (A2780), mammary carcinoma (MCF-7) and lung carcinoma (A549) were explored. Both complexes demonstrate good cytotoxic activity, with complex **48** displaying higher cytotoxic activity than **47**, which can probably be contributed to the increased water solubility induced by the PTA moiety and the overall positive charge of the compound. Among the possible mechanism of actions, potent inhibition of the zinc-finger protein PARP-1 is reported, which has been already demonstrated for several coordination gold(III) complexes with N-donor ligands¹¹³. Of note, organic compounds as PARP-1 inhibitors are currently in clinical trials for their selective cytotoxic properties, and their DNA damage repair (DDR) inhibiting abilities^{114 115}.

C[^]N[^]N complexes

In 2003, Cinellu and coworkers reported on the synthesis of the Au(III) complex **49** with a dimethylbenzyl-bipyridine C[^]N[^]N backbone (Fig. 13)¹¹⁶. By choosing a OH⁻ ligand in ancillary position

to the Au(III) center, good solubility of the complex under physiological conditions was obtained. Upon reaction with bovine serum albumin (BSA), tight metal–protein adducts were formed. It is believed that binding of complex **49** to BSA is achieved via histidine moieties on the protein surface. Interaction studies of organometallic complexes with serum albumin have attracted considerable interest during the last years¹¹⁷. Serum albumin is the most abundant plasma protein and serves many physiological functions, such as maintaining both the colloid osmotic blood pressure and – together with other compounds - the blood pH value. Furthermore, it is suspected that serum albumin protects cells against oxidative stress¹¹⁸. Complex **49** shows higher cytotoxic activity and selectivity against a series of 12 human tumor cell lines than cisplatin. Inhibition of biomolecular systems, such as mammalian target of rapamycin/rapamycin (mTOR), have been suggested as possible mechanism of action¹¹⁹.

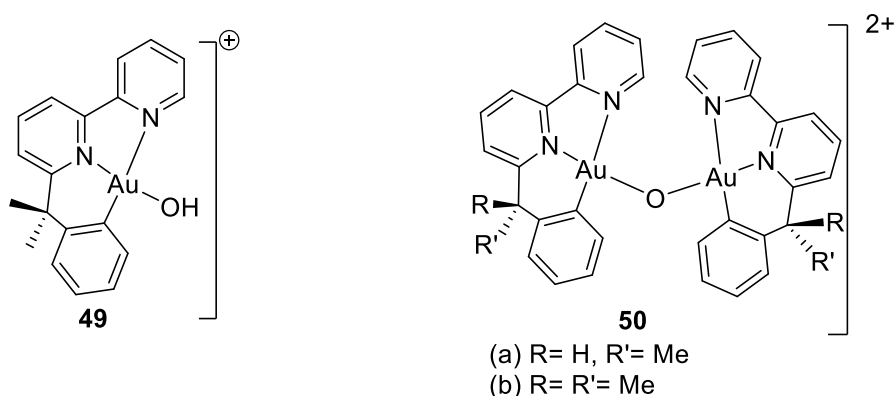


Figure 13. Mononuclear and dinuclear Au(III) C^NN complexes.

The same group reported on the dinuclear, oxo-bridged complex variants of general formula [(N^NC)₂Au₂(μ-O)][PF₆]₂ (**50**) (Fig. 13) and their cytotoxic properties in 2011¹¹⁶. Coordination oxo-bridged Au(III) N^N complexes had been previously reported before¹²⁰ (named “Auoxo” compounds) showing antiproliferative effects toward various human cancer cell lines. However, the latter compounds displayed stability issue of the Au(III) center in the reducing intercellular milieu, which were overcome introducing the strong Au-C bond in the organometallic analogues. Notably, these compounds reveal pronounced redox stability even in the presence of effective biological reductants such as ascorbic acid and glutathione. In comparison to complex **49**, complexes **50a-b** showed in general rather moderate cytotoxic activity against the series of 12 human tumor cell lines. However, **50a** proved to be particularly active against human breast cancer cells (401NL), while **50b** displayed only scarcely selective cytotoxic activity. Mass spectrometry studies with model proteins (hen egg white lysozyme and horse cytochrome c) indicate that upon reaction with proteins, complexes **50a-b** form monometallic adducts, preserving the Au(III) center and retaining the multidentate ligand. This also indicates that the complex-protein interaction facilitates the cleavage of the oxo-bridge and the conversion into the more active monometallic species.

C^NC complexes

The field of Au(III) C^NC complexes with anticancer properties have been closely examined by Che and coworkers (Fig. 14). As C^NC pincer ligands allow the highest stabilization of the Au(III) center among the herewith presented ligands, it is not surprising that this family, at variance with the case of Pt(II) complexes, makes up for the majority of the reported cyclometalated Au(III) compounds.

The Au(III) complex class utilizing the 2,6-diphenylpyridine as a C^NC ligand was reported by Che *et al.* already in 1998¹²¹ via reaction of K[AuCl₄] and [Hg(C^NCH)Cl] under reflux in acetonitrile. Substitution of the chlorido ligand of complex **51** leads to a number of cationic Au(III)C^NC complexes with different ancillary ligands (**52-54**). Complex **52** and **53**, containing non-toxic N-donor ligands, display cytotoxic activities similar to cisplatin against various cancer cell lines, such as HeLa cancer cells¹²². However, they do not show cross-resistance with cisplatin against nasopharyngeal (NPC) cancer cells. Gel-mobility shift assays and viscosity analysis show that **52** intercalates with DNA, causing DNA elongation. It has been reported that DNA intercalators enhance the assembly of G-quadruplexes. Consequently, native polyacrylamide gel electrophoresis (PAGE) was employed to examine the ability of **52** to intercalate with DNA and induce the formation of intramolecular G-quadruplexes from a model oligonucleotide¹²². This indicates that **52** behaves similarly to the classical [Pt(terpy)Cl]⁺ from the perspective of DNA intercalation and potential telomerase inhibition. In addition, by flow cytometry analysis in SUNE1 cells, it was demonstrated that **52** and **53** target cellular DNA *via* S-phase cell arrest, leading to apoptosis.

As reviewed by Sadler and Berners-Price already in 1987, phosphine containing compounds exhibit phosphine ligand-mediated cytotoxicity¹²³. However, due to their poor stability under physiological conditions and non-specific binding affinities towards various biomolecules, these compounds' application as anticancer compounds is hindered. By introducing the C^NC scaffold, significantly higher complex stabilities were obtained. For example, complex **54** and its dinuclear analogue **55** are soluble and stable in aqueous media¹²². These compounds neither change the melting temperature of calf-thymus DNA (ctDNA) significantly nor cause S-phase cell arrest, suggesting a different mode of action with respect to cisplatin. The dinuclear complexes of type **55** show higher cytotoxic activity against various cancer cell lines than their mononuclear counterparts. The highest cytotoxicities were displayed for n = 3, relating to the cytotoxicity of the free 1,2-bis(diphenylphosphino)propane (dppp) ligand. *In vivo* studies in rats with liver cancer (HCC) orthografts showed that the dppp derivative is a nanomolar inhibitor of TrxR1 and induces ER stress¹²⁴.

By replacing the PPh₃ moiety with a NHC ligand complex **56** and complex **57** - as the respective dinuclear compound - can be obtained⁸⁶. This results in a reduction of the cytotoxic activity and selectivity of the compounds, supporting the fact that indeed phosphine ligand-mediated cytotoxicity is crucial for complexes **54** and **55**. In this case, the mononuclear complex **56** displays higher cytotoxic activity than its dinuclear analogue **57**, being 167-fold more cytotoxic to non-small lung carcinoma cells (NCI-H460) than to normal lung fibroblast cells (CCD-19Lu). By DNA interaction studies with ctDNA it was demonstrated that complex **56** induces DNA strand breaks and subsequent cell death through the stabilization of TopoI-linked DNA¹²⁵.

A recent development in the field of Au(III) C^NC complexes is the discovery of supramolecular polymers, self-assembled from cyclometalated Au(III) C^NC complexes. In this framework, the mononuclear complex [Au(C,N,C)(4-dpt)]⁺ (4-dpt = 2,4-diamino-6-(4-pyridyl)-1,3,5-triazine) **58** was chosen by Che *et al.* due to the ability of the antiangiogenic 4-dpt ligand to form intramolecular hydrogen bonds and to establish π-π interactions¹²⁶. These factors proved to be essential for supramolecular complex formation by self-assembly in acetonitrile at ambient

temperature. The stability of **58** in phosphate-buffered saline in the absence and presence of the biological reductant glutathione (GSH) was examined. UV/Vis absorption spectrophotometry demonstrated that upon reaction with GSH, **58** shows an increased absorbance between approximately 260 and 380 nm. For incubation periods over 24 hours the 4-dpt ligand is released into the solution, and both $[\text{Au}(\text{C}^{\wedge}\text{N}^{\wedge}\text{C})\text{Cl}]$ and 4-dpt contribute to precipitation, as was demonstrated by ESI-MS and ^1H NMR. These results suggest that 4-dpt of complex **58** can be replaced by chloride ion in PBS, with formation of $[\text{Au}(\text{C}^{\wedge}\text{N}^{\wedge}\text{C})\text{Cl}]$ and release of 4-dpt. The log–log plot of the concentration-dependent specific viscosity of **58** in CH_3CN measured at 294 ± 1 K shows a significant increase in viscosity at concentrations above 6.3 g L^{-1} ; relating to typical characteristics of supramolecular polymer solutions. Via transmission electron (TEM) and scanning electron microscopy (SEM), partially aligned nanofibers with diameters and lengths of about 50 nm could be demonstrated for **58**. The significant viscosity at high concentration of **58** (20 mM) is contributed to the partial entanglement of these nanofibers. Complex **58** displays high cytotoxic activity towards murine cancer cell line B16 and non-tumorigenic lung fibroblast cells (CCD-19Lu). It was suggested that the sustained release of free 4-dpt ligand and simultaneous formation of Au(III)-glutathione adducts account for the observed cytotoxicity. GSH adduct formation was assessed via ESI-MS and UV-VIS for **58** in phosphate-buffered saline containing GSH (2 mM), showing significant cluster peaks for $[(\text{C}^{\wedge}\text{N}^{\wedge}\text{C})\text{Au}(\text{GSH})]^+$ and the dimeric species $[(\text{C}^{\wedge}\text{N}^{\wedge}\text{C})_2\text{Au}_2(\text{GSH})_2]^{2+}$. Furthermore, the nanofiber network of the polymer could be used to encapsulate other cytotoxic agents, thus enabling a localized drug delivery while reducing side toxicity.

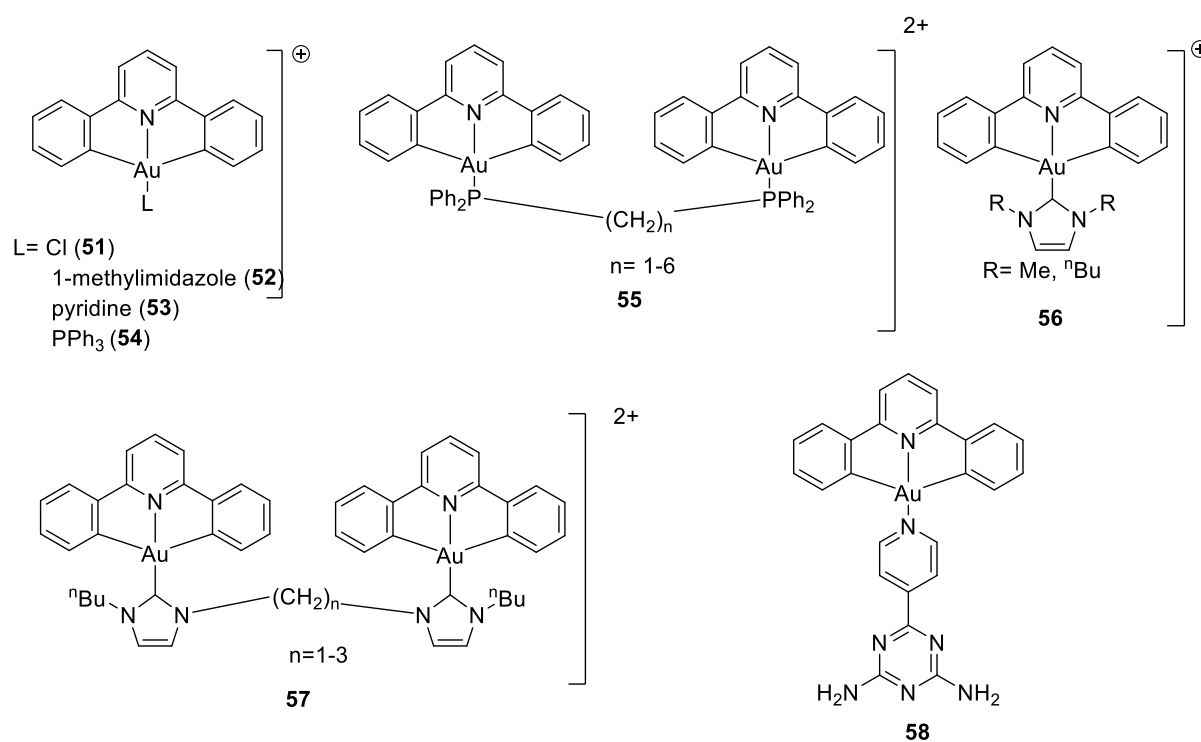


Figure 14. Representative Au(III) $\text{C}^{\wedge}\text{N}^{\wedge}\text{C}$ complexes synthesized by Che *et al.*

All Au(III) C[^]N[^]C follow a general synthesis route as depicted in Fig. 15. A direct C-H activation of the respective C[^]N[^]C ligand in the presence of a Au(III) precursor (usually KAuCl₄) is theoretically possible, but requires high temperatures, which can also result into decomposition of the ligand. As an alternative, a transmetalation pathway using organomercury(II) reagents is the most common synthesis strategy nowadays, as it allows milder reaction conditions and less formation of by-products occurs¹²⁷. In a first reaction step the 2,6-diphenylpyridine derivative is reacted with mercury(II) acetate, followed by metathesis with LiCl, yielding an organomercury(II) species. Subsequent transmetalation is achieved by reacting the organomercury(II) complex with KAuCl₄ in acetonitrile under reflux.

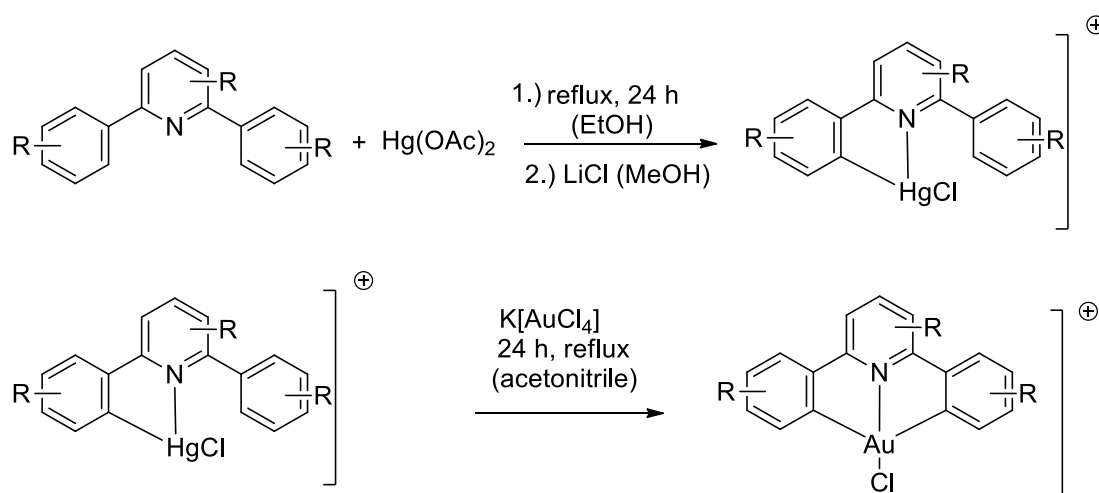


Figure 15. General complex formation of Au(III) C[^]N[^]C complexes via transmetalation.

C[^]N[^]S complexes

Bidentate 2-pyridyl carboxylate¹²⁸ and 2-pyridyl amidate complexes¹²⁹ of Au(III) have been reported to show both catalytic and biological activity. Tridentate analogues of bis(amidate), bis(carboxylate), and bis(iminothiolate) ligands as a C[^]N[^]S or S[^]C[^]S moiety, are reported for stabilizing other transition metals, mainly Pd(II)¹³⁰ and Ru(II)¹³¹. Surprisingly, these ligand classes have not been extended to Au(III) and there are, to the best of our knowledge, practically no reports of Au(III) C[^]N[^]S with anticancer properties. Some related “pseudo-pincer” compounds have been reported though (Fig. 16). For example, Sommer *et al.* synthesized the Au(III) complex **59** with a tridentate thiosemicarbazone ligand of the formula [Au(Hdamp-C¹)Cl(H₂pydoxmetsc)]Cl₂ (pydoxmetsc = pyridoxal methylthiosemicarbazone) in 1999¹³². This complex is involved in extended hydrogen bonding networks with counter ions and solvent molecules. The antiproliferative effect of **59** was tested on breast cancer cells (MCF-7) *in vitro* with promising cytotoxic results. In 2014, Bergman, Toste *et al.* reported on the Au(III) S[^]C[^]S complex **60** with bis(iminothiolate) ligands, which displays high stability in reducing environments and was recommended for further testing in various fields of application, including as anticancer agent¹³³.

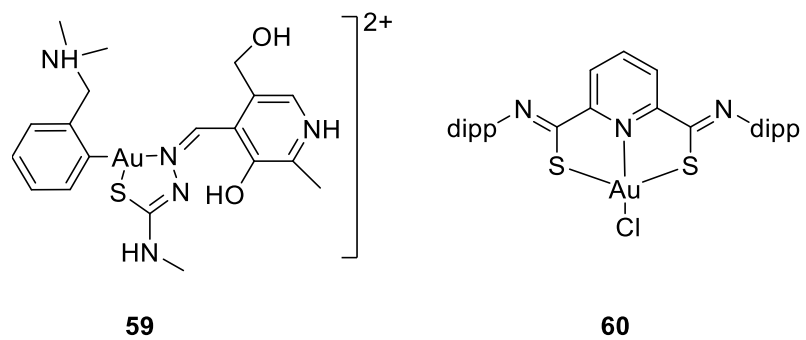


Figure 15. Au(III) pseudo-pincer and Au(III) S²N²S complexes.

3. Conclusions and Perspectives

In this review the use of organometallic compounds in medicinal chemistry is highlighted, with focus on cyclometalated Pt(II) and Au(III) C^N, C^N^N, C^N^C and C^N^S complexes. Cyclometalation offers multiple benefits, such as stabilization of the metal center (especially crucial for the easily reduced Au(III)) and fine-tuning of the redox properties by variations in the multidentate moiety. Overall, this allows the possibility of enhanced control of the physicochemical properties of the resulting complexes. Therefore, it is not surprising that research on cyclometalated Au(III) and Pt(II) complexes for anticancer treatment has significantly grown during the last years, with promising complexes being reported by numerous groups. As highlight in the field, Pt(II) C^N bis(NHC) complexes may be worth exploring for their ability to influence the immune system inducing ICD⁸³. In fact, one of the several ways in which chemotherapeutics engage a tumor-specific immune response is by triggering immunogenic cell death, whereby the dying cancer cells initiate a robust immune response, acting as an “anticancer vaccine”¹³⁴. Moreover, specifically concerning gold compounds, Au(III) C^N^C complexes have also been demonstrated to possess peculiar features as G-quadruplexes stabilizers,¹²² which could potentially be used either as therapeutic agents or (if luminescent) for imaging of such non-canonical DNA structures in cells.

The understanding of the mode of action of the various mentioned families of experimental organometallic anticancer compounds is one of the most vital questions when studying their cytotoxic properties. In fact, so far, only limited studies have been conducted, most commonly in cancer cells *in vitro*. In particular, it would be very interesting to compare the cytotoxic properties of Pt(II) and Au(III) analogues to highlight possible differences in the mechanisms of action.

Thus, in general, further exploration of the cellular uptake, subcellular distribution, as well as the fate of these metallodrugs (mono or poly-nuclear) inside cells is of major importance to get insights into their mechanism of action. Therefore, there is an increasing need for imaging methods that allow the direct mapping of the subcellular distribution of metal-based therapeutics. In this review, several complexes with luminescent properties have been described, which are highly relevant for uptake and cell distribution studies, and ultimately to provide insights into the compounds' pharmacological targets. Future optimization of cyclometalated scaffolds should include ligands and substituents enhancing their solubility in aqueous environment while maintaining a certain lipophilic character, to enhance their accumulation in cancer cells.

It is also worth mentioning that organometallic conjugates of receptor-targeting peptides are proposed as interesting candidates for novel targeted cancer therapies. To the best of our knowledge, this approach has not been applied to the delivery of cyclometalated Pt(II) and Au(III) complexes yet, and would be worth exploring *via* functionalization of the cyclometalated ligands.

An interesting new approach is the previously mentioned synthesis of supramolecular polymers self-assembled from cyclometalated Au(III) C^N^C complexes¹²⁶. The nanofiber network of the polymer could be used to encapsulate other cytotoxic agents, thus enabling a localized drug delivery while reducing toxicity for healthy tissue. This could also reduce the frequency of drug administration through a sustained-release of the cytotoxic compound.

Finally, there are still several very interesting cyclometalated Au(III) and Pt(II) complexes reported in the literature (such as highly luminescent Au(III) C^N^C complexes with a pyrazine moiety¹³⁵) that are

only applied in catalysis and have not been tested for their anticancer properties. It would be highly interesting to try to exploit them also for biological applications, for example as cytotoxic agents acting *via* a catalytic mechanism in cancer cells.

Acknowledgements

Authors thank Cardiff University for funding. A.C. acknowledges the support of the Technische Universität München – Institute for Advanced Study, funded by the German Excellence Initiative and the European Union Seventh Framework Programme under grant agreement n° 291763. S. J. is also thankful to TUM graduate school for funding.

Literature

1. Rosenberg, B.; Van Camp, L.; Krigas, T., Inhibition of Cell Division in *Escherichia coli* by Electrolysis Products from a Platinum Electrode. *Nature* **1965**, *205* (4972), 698-699.
2. (a) Apps, M. G.; Choi, E. H. Y.; Wheate, N. J., The state-of-play and future of platinum drugs. *Endocrine-Related Cancer* **2015**, *22* (4), R219-R233; (b) Wheate, N. J.; Walker, S.; Craig, G. E.; Oun, R., The status of platinum anticancer drugs in the clinic and in clinical trials. *Dalton Transactions* **2010**, *39* (35), 8113-8127.
3. Wang, D.; Lippard, S. J., Cellular processing of platinum anticancer drugs. *Nature reviews Drug discovery* **2005**, *4* (4), 307-320.
4. (a) Ott, I., On the medicinal chemistry of gold complexes as anticancer drugs. *Coordination Chemistry Reviews* **2009**, *253* (11), 1670-1681; (b) Nobili, S.; Mini, E.; Landini, I.; Gabbiani, C.; Casini, A.; Messori, L., Gold compounds as anticancer agents: chemistry, cellular pharmacology, and preclinical studies. *Medicinal Research Reviews* **2010**, *30* (3), 550-580.
5. McKeage, M. J.; Maharaj, L.; Berners-Price, S. J., Mechanisms of cytotoxicity and antitumor activity of gold(I) phosphine complexes: the possible role of mitochondria. *Coordination Chemistry Reviews* **2002**, *232* (1-2), 127-135.
6. McKeage, J. M.; Berners-Price, J. S.; Galettis, P.; Bowen, J. R.; Brouwer, W.; Ding, L.; Zhuang, L.; Baguley, C. B., Role of lipophilicity in determining cellular uptake and antitumour activity of gold phosphine complexes. *Cancer Chemotherapy and Pharmacology* **2000**, *46* (5), 343-350.
7. Bindoli, A.; Rigobello, M. P.; Scutari, G.; Gabbiani, C.; Casini, A.; Messori, L., Thioredoxin reductase: a target for gold compounds acting as potential anticancer drugs. *Coordination Chemistry Reviews* **2009**, *253* (11), 1692-1707.
8. (a) Zou, T.; Lum, C. T.; Lok, C.-N.; Zhang, J.-J.; Che, C.-M., Chemical biology of anticancer gold (III) and gold (I) complexes. *Chemical Society Reviews* **2015**, *44* (24), 8786-8801; (b) da Silva Maia, P. I.; Deflon, V. M.; Abram, U., Gold (III) complexes in medicinal chemistry. *Future medicinal chemistry* **2014**, *6* (13), 1515-1536.
9. Messori, L.; Abbate, F.; Marcon, G.; Orioli, P.; Fontani, M.; Mini, E.; Mazzei, T.; Carotti, S.; O'Connell, T.; Zanello, P., Gold(III) Complexes as Potential Antitumor Agents: Solution Chemistry and Cytotoxic Properties of Some Selected Gold(III) Compounds. *Journal of Medicinal Chemistry* **2000**, *43* (19), 3541-3548.
10. de Almeida, A.; Soveral, G.; Casini, A., Gold compounds as aquaporin inhibitors: new opportunities for therapy and imaging. *MedChemComm* **2014**, *5* (10), 1444-1453.
11. Serna, A.; Galan-Cobo, A.; Rodrigues, C.; Sanchez-Gomar, I.; Toledo-Aral, J. J.; Moura, T. F.; Casini, A.; Soveral, G.; Echevarria, M., Functional inhibition of aquaporin-3 with a gold-based compound induces blockage of cell proliferation. *Journal of cellular physiology* **2014**, *229* (11), 1787-801.
12. (a) Mendes, F.; Groessl, M.; Nazarov, A. A.; Tsybin, Y. O.; Sava, G.; Santos, I.; Dyson, P. J.; Casini, A., Metal-Based Inhibition of Poly (ADP-ribose) Polymerase—The Guardian Angel of DNA. *Journal of medicinal chemistry* **2011**, *54* (7), 2196-2206; (b) Serratrice, M.; Edafe, F.; Mendes, F.; Scopelliti, R.; Zakeeruddin, S. M.; Grätzel, M.; Santos, I.; Cinellu, M. A.; Casini, A., Cytotoxic gold compounds: synthesis, biological characterization and investigation of their inhibition properties of the zinc finger protein PARP-1. *Dalton Transactions* **2012**, *41* (11), 3287-3293.

13. Citta, A.; Scalcon, V.; Gobel, P.; Bertrand, B.; Wenzel, M.; Folda, A.; Rigobello, M. P.; Meggers, E.; Casini, A., Toward anticancer gold-based compounds targeting PARP-1: a new case study. *RSC Advances* **2016**, *6* (82), 79147-79152.
14. Schmitt, S. M.; Frezza, M.; Dou, Q. P., New applications of old metal-binding drugs in the treatment of human cancer. *Frontiers in bioscience (Scholar edition)* **2012**, *4*, 375.
15. Nardon, C.; Schmitt, S. M.; Yang, H.; Zuo, J.; Fregona, D.; Dou, Q. P., Gold (III)-dithiocarbamate peptidomimetics in the forefront of the targeted anticancer therapy: preclinical studies against human breast neoplasia. *PloS one* **2014**, *9* (1), e84248.
16. Gasser, G.; Ott, I.; Metzler-Nolte, N., Organometallic anticancer compounds. *Journal of medicinal chemistry* **2010**, *54* (1), 3-25.
17. Dyson, P. J., Systematic design of a targeted organometallic antitumour drug in pre-clinical development. *CHIMIA International Journal for Chemistry* **2007**, *61* (11), 698-703.
18. Gasser, G.; Ott, I.; Metzler-Nolte, N., Organometallic Anticancer Compounds. *Journal of Medicinal Chemistry* **2011**, *54* (1), 3-25.
19. (a) Herrmann, W. A., N-Heterocyclic Carbenes: A New Concept in Organometallic Catalysis. *Angewandte Chemie International Edition* **2002**, *41* (8), 1290-1309; (b) Herrmann, W. A.; Cornils, B., Organometallic Homogeneous Catalysis—Quo vadis? *Angewandte Chemie International Edition in English* **1997**, *36* (10), 1048-1067.
20. Schlawe, D.; Majdalani, A.; Velcicky, J.; Heßler, E.; Wieder, T.; Prokop, A.; Schmalz, H.-G., Iron-Containing Nucleoside Analogues with Pronounced Apoptosis-Inducing Activity. *Angewandte Chemie International Edition* **2004**, *43* (13), 1731-1734.
21. Reddy, V. D., Synthesis, characterization, and reactivity of a novel ruthenium carbonyl cluster containing tri-O-benzyl-d-glucal as a chiral carbohydrate ligand. *Journal of Organometallic Chemistry* **2006**, *691* (1–2), 27-34.
22. Nagel, C.; McLean, S.; Poole, R. K.; Braunschweig, H.; Kramer, T.; Schatzschneider, U., Introducing [Mn(CO)₃(tpa- κ 3N)]⁺ as a novel photoactivatable CO-releasing molecule with well-defined iCORM intermediates - synthesis, spectroscopy, and antibacterial activity. *Dalton Transactions* **2014**, *43* (26), 9986-9997.
23. (a) Ott, I.; Koch, T.; Shorafa, H.; Bai, Z.; Poeckel, D.; Steinhilber, D.; Gust, R., Synthesis, cytotoxicity, cellular uptake and influence on eicosanoid metabolism of cobalt-alkyne modified fructoses in comparison to auranofin and the cytotoxic COX inhibitor Co-ASS. *Organic & Biomolecular Chemistry* **2005**, *3* (12), 2282-2286; (b) Sergeant, C. D.; Ott, I.; Sniady, A.; Meneni, S.; Gust, R.; Rheingold, A. L.; Dembinski, R., Metallo-nucleosides: synthesis and biological evaluation of hexacarbonyl dicobalt 5-alkynyl-2[prime or minute]-deoxyuridines. *Organic & Biomolecular Chemistry* **2008**, *6* (1), 73-80.
24. Ott, I.; Kircher, B.; Bagowski, C. P.; Vlecken, D. H.; Ott, E. B.; Will, J.; Bendorf, K.; Sheldrick, W. S.; Gust, R., Modulation of the biological properties of aspirin by formation of a bioorganometallic derivative. *Angewandte Chemie International Edition* **2009**, *48* (6), 1160-1163.
25. Meggers, E., Targeting proteins with metal complexes. *Chemical Communications* **2009**, (9), 1001-1010.
26. Smalley, K. S. M.; Contractor, R.; Haass, N. K.; Kulp, A. N.; Atilla-Gokcumen, G. E.; Williams, D. S.; Bregman, H.; Flaherty, K. T.; Soengas, M. S.; Meggers, E.; Herlyn, M., An Organometallic Protein Kinase Inhibitor Pharmacologically Activates

- p53 and Induces Apoptosis in Human Melanoma Cells. *Cancer Research* **2007**, *67* (1), 209-217.
27. Franke, J. C.; Plötz, M.; Prokop, A.; Geilen, C. C.; Schmalz, H.-G.; Eberle, J., New caspase-independent but ROS-dependent apoptosis pathways are targeted in melanoma cells by an iron-containing cytosine analogue. *Biochemical Pharmacology* **2010**, *79* (4), 575-586.
28. (a) Teyssot, M.-L.; Jarrousse, A.-S.; Manin, M.; Chevy, A.; Roche, S.; Norre, F.; Beaudoin, C.; Morel, L.; Boyer, D.; Mahiou, R.; Gautier, A., Metal-NHC complexes: a survey of anti-cancer properties. *Dalton Transactions* **2009**, (35), 6894-6902; (b) Oehninger, L.; Rubbiani, R.; Ott, I., N-Heterocyclic carbene metal complexes in medicinal chemistry. *Dalton Transactions* **2013**, *42* (10), 3269-3284.
29. Cetinkaya, B.; Çetinkaya, E.; Kuecukbay, H.; Durmaz, R., Antimicrobial activity of carbene complexes of rhodium (I) and ruthenium (II). *ARZNEIMITTEL FORSCHUNG* **1996**, *46*, 821-823.
30. Hickey, J. L.; Ruhayel, R. A.; Barnard, P. J.; Baker, M. V.; Berners-Price, S. J.; Filipovska, A., Mitochondria-Targeted Chemotherapeutics: The Rational Design of Gold(I) N-Heterocyclic Carbene Complexes That Are Selectively Toxic to Cancer Cells and Target Protein Selenols in Preference to Thiols. *Journal of the American Chemical Society* **2008**, *130* (38), 12570-12571.
31. Cinellu, M.; Ott, I.; Casini, A., Gold Organometallics with Biological Properties. In *Bioorganometallic Chemistry: Applications in Drug Discovery, Biocatalysis, and Imaging*, First Edition ed.; Gérard Jaouen, M. S., Ed. Wiley-VCH Verlag GmbH & Co. KGaA: 2015; pp 117-139.
32. Serebryanskaya, T. V.; Zolotarev, A. A.; Ott, I., A novel aminotriazole-based NHC complex for the design of gold(i) anti-cancer agents: synthesis and biological evaluation. *MedChemComm* **2015**, *6* (6), 1186-1189.
33. Nelson, D. J.; Nolan, S. P., Quantifying and understanding the electronic properties of N-heterocyclic carbenes. *Chemical Society Reviews* **2013**, *42* (16), 6723-6753.
34. Bertrand, B.; Stefan, L.; Pirrotta, M.; Monchaud, D.; Bodio, E.; Richard, P.; Le Gendre, P.; Warmerdam, E.; de Jager, M. H.; Groothuis, G. M. M.; Picquet, M.; Casini, A., Caffeine-Based Gold(I) N-Heterocyclic Carbenes as Possible Anticancer Agents: Synthesis and Biological Properties. *Inorganic chemistry* **2014**, *53* (4), 2296-2303.
35. Daly, J. W., *Cell. Mol. Life Sci.* **2007**, *64*, 2153.
36. Bazzicalupi, C.; Ferraroni, M.; Papi, F.; Massai, L.; Bertrand, B.; Messori, L.; Gratteri, P.; Casini, A., Determinants for Tight and Selective Binding of a Medicinal Dicarbene Gold (I) Complex to a Telomeric DNA G-Quadruplex: a Joint ESI MS and XRD Investigation. *Angewandte Chemie International Edition* **2016**.
37. (a) Gonçalves, D. P.; Rodriguez, R.; Balasubramanian, S.; Sanders, J. K., Tetramethylpyridiniumporphyrazines—a new class of G-quadruplex inducing and stabilising ligands. *Chemical communications* **2006**, (45), 4685-4687; (b) Döchler, M., G-quadruplexes: targets and tools in anticancer drug design. *Journal of drug targeting* **2012**, *20* (5), 389-400.
38. Muenzner, J. K.; Rehm, T.; Biersack, B.; Casini, A.; de Graaf, I. A.; Worawutputtpong, P.; Noor, A.; Kempe, R.; Brabec, V.; Kasparkova, J.; Schobert, R., Adjusting the DNA interaction and anticancer activity of Pt (II) N-heterocyclic carbene complexes by steric shielding of the trans leaving group. *Journal of medicinal chemistry* **2015**, *58* (15), 6283-6292.
39. (a) Lummen, G.; Sperling, H.; Luboldt, H.; Otto, T.; Rubben, H., *Cancer Chemother. Pharmacol.* **1998**, *42*, 415-417; (b) Köpf-Maier, P., Antitumor activity of

- titanocene dichloride in xenografted human renal-cell carcinoma. *Anticancer Res* **1999**, *19* (1A), 493-504.
40. Mokdsi, G.; Harding, M. M., Water soluble, hydrolytically stable derivatives of the antitumor drug titanocene dichloride and binding studies with nucleotides. *Journal of Organometallic Chemistry* **1998**, *565* (1–2), 29-35.
41. (a) Top, S.; Kaloun, E. B.; Vessières, A.; Laiños, I.; Leclercq, G.; Jaouen, G., The first titanocenyl dichloride moiety vectorised by a selective estrogen receptor modulator (SERM). Synthesis and preliminary biochemical behaviour. *Journal of Organometallic Chemistry* **2002**, *643–644*, 350-356; (b) Mross, K.; Robben-Bathe, P.; Edler, L.; Baumgart, J.; Berdel, W. E.; Fiebig, H.; Unger, C., Phase I Clinical Trial of a Day-1, -3, -5 Every 3 Weeks Phase I Clinical Trial of Day-1, -3, -5 Every 3 Weeks Schedule with Titanocene Dichloride (MKT 5) in Patients with Advanced Cancer. (Phase I Study Group of the AIO of the German Cancer Society). *Onkologie* **2000**, *23* (6), 576-579.
42. Allen, O.; Croll, L.; Gott, A.; Knox, R.; McGowan, P., *Organometallics* **2004**, *23*, 288-292.
43. Tacke, M.; Allen, L. T.; Cuffe, L.; Gallagher, W. M.; Lou, Y.; Mendoza, O.; Müller-Bunz, H.; Rehmann, F.-J. K.; Sweeney, N., Novel titanocene anti-cancer drugs derived from fulvenes and titanium dichloride. *Journal of Organometallic Chemistry* **2004**, *689* (13), 2242-2249.
44. (a) Ang, W. H.; Casini, A.; Sava, G.; Dyson, P. J., Organometallic ruthenium-based antitumor compounds with novel modes of action. *Journal of Organometallic Chemistry* **2011**, *696* (5), 989-998; (b) Nowak-Sliwinska, P.; van Beijnum, J. R.; Casini, A.; Nazarov, A. A.; Wagnieres, G.; van den Bergh, H.; Dyson, P. J.; Griffioen, A. W., Organometallic ruthenium (II) arene compounds with antiangiogenic activity. *Journal of medicinal chemistry* **2011**, *54* (11), 3895-3902.
45. (a) Ang, W. H.; Daldini, E.; Scolaro, C.; Scopelliti, R.; Juillerat-Jeannerat, L.; Dyson, P. J., Development of organometallic ruthenium-arene anticancer drugs that resist hydrolysis. *Inorganic chemistry* **2006**, *45* (22), 9006-13; (b) Vock, C. A.; Renfrew, A. K.; Scopelliti, R.; Juillerat-Jeannerat, L.; Dyson, P. J., Influence of the Diketonato Ligand on the Cytotoxicities of [Ru(η^6 -p-cymene)(R2acac)(PTA)]⁺ Complexes (PTA = 1,3,5-triaza-7-phosphaadamantane). *European Journal of Inorganic Chemistry* **2008**, *2008* (10), 1661-1671.
46. Scolaro, C.; Bergamo, A.; Brescacin, L.; Delfino, R.; Cocchietto, M.; Laurenczy, G.; Geldbach, T. J.; Sava, G.; Dyson, P. J., In Vitro and in Vivo Evaluation of Ruthenium(II)–Arene PTA Complexes. *Journal of Medicinal Chemistry* **2005**, *48* (12), 4161-4171.
47. Bergamo, A.; Masi, A.; Dyson, P. J.; Sava, G., *Int. J. Oncol* **2008**, (33), 1281.
48. Chatterjee, S.; Kundu, S.; Bhattacharyya, A.; Hartinger, C. G.; Dyson, P. J., The ruthenium(II)–arene compound RAPTA-C induces apoptosis in EAC cells through mitochondrial and p53–JNK pathways. *JBIC Journal of Biological Inorganic Chemistry* **2008**, *13* (7), 1149-1155.
49. Romero-Canelón, I.; Pizarro, A. M.; Habtemariam, A.; Sadler, P. J., Contrasting cellular uptake pathways for chlorido and iodido iminopyridine ruthenium arene anticancer complexes. *Metallomics* **2012**, *4* (12), 1271-1279.
50. (a) Dubar, F.; Biot, C., On the Molecular Mechanisms of the Antimalarial Action of Ferroquine. In *Bioorganometallic Chemistry*, Wiley-VCH Verlag GmbH & Co. KGaA: 2014; pp 141-164; (b) Patra, M.; Gasser, G.; Metzler-Nolte, N., Small organometallic compounds as antibacterial agents. *Dalton transactions* **2012**, *41* (21), 6350-6358.

51. Hillard, E. A.; Vessières, A.; Jaouen, G., Ferrocene Functionalized Endocrine Modulators as Anticancer Agents. In *Medicinal Organometallic Chemistry*, Jaouen, G.; Metzler-Nolte, N., Eds. Springer Berlin Heidelberg: Berlin, Heidelberg, 2010; pp 81-117.
52. Jaouen, G.; Vessières, A.; Top, S., Ferrocifen type anti cancer drugs. *Chemical Society Reviews* **2015**, *44* (24), 8802-8817.
53. Ekengard, E.; Glans, L.; Cassells, I.; Fogeron, T.; Govender, P.; Stringer, T.; Chellan, P.; Lisensky, G. C.; Hersh, W. H.; Doverbratt, I.; Lidin, S.; de Kock, C.; Smith, P. J.; Smith, G. S.; Nordlander, E., Antimalarial activity of ruthenium(ii) and osmium(ii) arene complexes with mono- and bidentate chloroquine analogue ligands. *Dalton Transactions* **2015**, *44* (44), 19314-19329.
54. Wani, W. A.; Jameel, E.; Baig, U.; Mumtazuddin, S.; Hun, L. T., Ferroquine and its derivatives: New generation of antimalarial agents. *European Journal of Medicinal Chemistry* **2015**, *101*, 534-551.
55. (a) Dubar, F.; Egan, T. J.; Pradines, B.; Kuter, D.; Ncokazi, K. K.; Forge, D.; Paul, J.-F.; Pierrot, C.; Kalamou, H.; Khalife, J.; Buisine, E.; Rogier, C.; Vezin, H.; Forfar, I.; Slomianny, C.; Trivelli, X.; Kapishnikov, S.; Leiserowitz, L.; Dive, D.; Biot, C., The Antimalarial Ferroquine: Role of the Metal and Intramolecular Hydrogen Bond in Activity and Resistance. *ACS Chemical Biology* **2011**, *6* (3), 275-287; (b) Jaouen, G.; Vessieres, A.; Top, S., Ferrocifen type anti cancer drugs. *Chemical Society Reviews* **2015**, *44* (24), 8802-8817.
56. (a) Donzello, M. P.; Viola, E.; Ercolani, C.; Fu, Z.; Futur, D.; Kadish, K. M., Tetra-2,3-pyrazinoporphyrazines with Externally Appended Pyridine Rings. 12. New Heteropentannuclear Complexes Carrying Four Exocyclic Cis-platin-like Functionalities as Potential Bimodal (PDT/Cis-platin) Anticancer Agents. *Inorganic chemistry* **2012**, *51* (22), 12548-12559; (b) González-Pantoja, J. F.; Stern, M.; Jarzecki, A. A.; Royo, E.; Robles-Escajeda, E.; Varela-Ramírez, A.; Aguilera, R. J.; Contel, M., Titanocene–Phosphine Derivatives as Precursors to Cytotoxic Heterometallic TiAu₂ and TiM (M = Pd, Pt) Compounds. Studies of Their Interactions with DNA. *Inorganic chemistry* **2011**, *50* (21), 11099-11110.
57. Auzias, M.; Therrien, B.; Süß-Fink, G.; Štěpnička, P.; Ang, W. H.; Dyson, P. J., Ferrocenoyl Pyridine Arene Ruthenium Complexes with Anticancer Properties: Synthesis, Structure, Electrochemistry, and Cytotoxicity. *Inorganic chemistry* **2008**, *47* (2), 578-583.
58. Lease, N.; Vasilevski, V.; Carreira, M.; de Almeida, A.; Sanaú, M.; Hirva, P.; Casini, A.; Contel, M., potential anticancer heterometallic Fe–Au and Fe–Pd agents: initial mechanistic insights. *Journal of medicinal chemistry* **2013**, *56* (14), 5806-5818.
59. Wenzel, M.; Bertrand, B.; Eymin, M. J.; Comte, V.; Harvey, J. A.; Richard, P.; Groessl, M.; Zava, O.; Amrouche, H.; Harvey, P. D.; Le Gendre, P.; Picquet, M.; Casini, A., Multinuclear cytotoxic metallodrugs: physicochemical characterization and biological properties of novel heteronuclear gold-titanium complexes. *Inorg Chem* **2011**, *50* (19), 9472-80.
60. Pelletier, F.; Comte, V.; Massard, A.; Wenzel, M.; Toulot, S.; Richard, P.; Picquet, M.; Le Gendre, P.; Zava, O.; Edafe, F., Development of bimetallic titanocene– ruthenium– arene complexes as anticancer agents: relationships between structural and biological properties. *Journal of medicinal chemistry* **2010**, *53* (19), 6923-6933.
61. Fernández-Gallardo, J.; Elie, B. T.; Sadhukha, T.; Prabha, S.; Sanaú, M.; Rotenberg, S. A.; Ramos, J. W.; Contel, M., Heterometallic titanium–gold complexes inhibit renal cancer cells in vitro and in vivo. *Chemical Science* **2015**, *6* (9), 5269-5283.

62. (a) Hannon, M. J., Metal-based anticancer drugs: From a past anchored in platinum chemistry to a post-genomic future of diverse chemistry and biology. *Pure and Applied Chemistry* **2007**, *79* (12), 2243-2261; (b) Fricker, S. P., Metal based drugs: From serendipity to design. *Dalton Transactions* **2007**, (43), 4903-4917.
63. (a) Omae, I., Applications of five-membered ring products of cyclometalation reactions as anticancer agents. *Coordination Chemistry Reviews* **2014**, *280*, 84-95; (b) Cutillas, N.; Yellol, G. S.; de Haro, C.; Vicente, C.; Rodríguez, V.; Ruiz, J., Anticancer cyclometalated complexes of platinum group metals and gold. *Coordination Chemistry Reviews* **2013**, *257* (19–20), 2784-2797.
64. Navarro-Ranniger, C.; Lopez-Solera, I.; Perez, J. M.; Rodriguez, J.; Garcia-Ruano, J. L.; Raithby, P. R.; Masaguer, J.; Alonso, C., Analysis of two cycloplatinated compounds derived from N-(4-methoxyphenyl)-alpha-benzoylbenzylidenamine. Comparison of the activity of these compounds with other isostructural cyclopalladated compounds. *Journal of Medicinal Chemistry* **1993**, *36* (24), 3795-3801.
65. Okada, T.; El-Mehasseb, I. M.; Kodaka, M.; Tomohiro, T.; Okamoto, K.-i.; Okuno, H., Mononuclear Platinum(II) Complex with 2-Phenylpyridine Ligands Showing High Cytotoxicity against Mouse Sarcoma 180 Cells Acquiring High Cisplatin Resistance. *Journal of Medicinal Chemistry* **2001**, *44* (26), 4661-4667.
66. El-Mehasseb, I. M.; Kodaka, M.; Okada, T.; Tomohiro, T.; Okamoto, K.-i.; Okuno, H., Platinum (II) complex with cyclometalating 2-phenylpyridine ligand showing high cytotoxicity against cisplatin-resistant cell. *Journal of Inorganic Biochemistry* **2001**, *84* (1–2), 157-158.
67. Frezza, M.; Dou, Q. P.; Xiao, Y.; Samouei, H.; Rashidi, M.; Samari, F.; Hemmateenejad, B., In Vitro and In Vivo Antitumor Activities and DNA Binding Mode of Five Coordinated Cyclometalated Organoplatinum(II) Complexes Containing Biphosphine Ligands. *Journal of Medicinal Chemistry* **2011**, *54* (18), 6166-6176.
68. Samari, F.; Hemmateenejad, B.; Shamsipur, M.; Rashidi, M.; Samouei, H., Affinity of two novel five-coordinated anticancer Pt (II) complexes to human and bovine serum albumins: a spectroscopic approach. *Inorganic chemistry* **2012**, *51* (6), 3454-3464.
69. Xu, D.; Wang, X.; Ding, L., Spectroscopic studies on the interaction of γ -cyclodextrin–daunorubicin inclusion complex with herring sperm DNA. *Carbohydrate polymers* **2011**, *83* (3), 1257-1262.
70. Kit-Man Siu, P.; Ma, D.-L.; Che, C.-M., Luminescent cyclometalated platinum(ii) complexes with amino acid ligands for protein binding. *Chemical Communications* **2005**, (8), 1025-1027.
71. Ruiz, J.; Cutillas, N.; Vicente, C.; Villa, M. D.; López, G.; Lorenzo, J.; Avilés, F. X.; Moreno, V.; Bautista, D., New Palladium(II) and Platinum(II) Complexes with the Model Nucleobase 1-Methylcytosine: Antitumor Activity and Interactions with DNA. *Inorganic chemistry* **2005**, *44* (21), 7365-7376.
72. Ruiz, J.; Lorenzo, J.; Sanglas, L.; Cutillas, N.; Vicente, C.; Villa, M. D.; Avilés, F. X.; López, G.; Moreno, V.; Pérez, J.; Bautista, D., Palladium(II) and Platinum(II) Organometallic Complexes with the Model Nucleobase Anions of Thymine, Uracil, and Cytosine: Antitumor Activity and Interactions with DNA of the Platinum Compounds. *Inorganic chemistry* **2006**, *45* (16), 6347-6360.
73. Hess, S. M.; Mounce, A. M.; Sequeira, R. C.; Augustus, T. M.; Ackley, M. C.; Bierbach, U., Platinum-acridinylthiourea conjugates show cell line-specific cytotoxic enhancement in H460 lung carcinoma cells compared to cisplatin. *Cancer Chemotherapy and Pharmacology* **2005**, *56* (4), 337-343.

74. Ruiz, J.; Lorenzo, J.; Vicente, C.; López, G.; López-de-Luzuriaga, J. M.; Monge, M.; Avilés, F. X.; Bautista, D.; Moreno, V.; Laguna, A., New Palladium(II) and Platinum(II) Complexes with 9-Aminoacridine: Structures, Luminiscence, Theoretical Calculations, and Antitumor Activity. *Inorganic chemistry* **2008**, *47* (15), 6990-7001.
75. Zamora, A.; Pérez, S. A.; Rodríguez, V.; Janiak, C.; Yellol, G. S.; Ruiz, J., Dual antitumor and antiangiogenic activity of organoplatinum (II) complexes. *Journal of medicinal chemistry* **2015**, *58* (3), 1320-1336.
76. Chen, Z.-F.; Qin, Q.-P.; Qin, J.-L.; Liu, Y.-C.; Huang, K.-B.; Li, Y.-L.; Meng, T.; Zhang, G.-H.; Peng, Y.; Luo, X.-J., Stabilization of G-quadruplex DNA, inhibition of telomerase activity, and tumor cell apoptosis by organoplatinum (II) complexes with oxoisoaporphine. *Journal of medicinal chemistry* **2015**, *58* (5), 2159-2179.
77. (a) Pommier, Y., Topoisomerase I inhibitors: camptothecins and beyond. *Nature Reviews Cancer* **2006**, *6* (10), 789-802; (b) Sordet, O.; Khan, Q. A.; Kohn, K. W.; Pommier, Y., Apoptosis induced by topoisomerase inhibitors. *Current Medicinal Chemistry-Anti-Cancer Agents* **2003**, *3* (4), 271-290.
78. Liu, J.; Leung, C.-H.; Chow, A. L.-F.; Sun, R. W.-Y.; Yan, S.-C.; Che, C.-M., Cyclometalated platinum(ii) complexes as topoisomerase II[small alpha] poisons. *Chemical Communications* **2011**, *47* (2), 719-721.
79. Talancón, D.; López, C.; Font-Bardía, M.; Calvet, T.; Quirante, J.; Calvis, C.; Messeguer, R.; Cortés, R.; Cascante, M.; Baldomà, L.; Badia, J., Diastereomerically pure platinum(II) complexes as antitumoral agents.: The influence of the mode of binding $\{(N), (N,O)- \text{ or } (C,N)\}$ - of $(1S,2R)[(\eta^5-C_5H_5)Fe\{\eta^5-C_5H_4\}CHNCH(Me)CH(OH)C_6H_5\}]$ and the arrangement of the auxiliary ligands. *Journal of Inorganic Biochemistry* **2013**, *118*, 1-12.
80. Frik, M.; Fernández-Gallardo, J.; Gonzalo, O.; Mangas-Sanjuan, V.; González-Alvarez, M.; Serrano del Valle, A.; Hu, C.; González-Alvarez, I.; Bermejo, M.; Marzo, I.; Contel, M., Cyclometalated Iminophosphorane Gold(III) and Platinum(II) Complexes. A Highly Permeable Cationic Platinum(II) Compound with Promising Anticancer Properties. *Journal of Medicinal Chemistry* **2015**, *58* (15), 5825-5841.
81. Kilpin, K. J.; Henderson, W.; Nicholson, B. K., Synthesis and reactivity of gold (III) complexes containing cycloaurated iminophosphorane ligands. *Inorganica Chimica Acta* **2009**, *362* (10), 3669-3676.
82. Terenzi, A.; Pirker, C.; Keppler, B. K.; Berger, W., Anticancer metal drugs and immunogenic cell death. *Journal of Inorganic Biochemistry* **2016**.
83. Wong, D. Y. Q.; Ong, W. W. F.; Ang, W. H., Induction of immunogenic cell death by chemotherapeutic platinum complexes. *Angewandte Chemie* **2015**, *127* (22), 6583-6587.
84. Zou, T.; Lok, C.-N.; Fung, Y. M. E.; Che, C.-M., Luminescent organoplatinum(ii) complexes containing bis(N-heterocyclic carbene) ligands selectively target the endoplasmic reticulum and induce potent photo-toxicity. *Chemical Communications* **2013**, *49* (47), 5423-5425.
85. Hunter, C. A.; Sanders, J. K., The nature of. pi.-. pi. interactions. *Journal of the American Chemical Society* **1990**, *112* (14), 5525-5534.
86. Yan, J. J.; Chow, A. L.-F.; Leung, C.-H.; Sun, R. W.-Y.; Ma, D.-L.; Che, C.-M., Cyclometalated gold (III) complexes with N-heterocyclic carbene ligands as topoisomerase I poisons. *Chemical Communications* **2010**, *46* (22), 3893-3895.
87. (a) Chu, G., Cellular responses to cisplatin. The roles of DNA-binding proteins and DNA repair. *Journal of Biological Chemistry* **1994**, *269* (2), 787-790; (b) Perez, R., Cellular and molecular determinants of cisplatin resistance. *European Journal of Cancer* **1998**, *34* (10), 1535-1542; (c) Siddik, Z. H., Cisplatin: mode of cytotoxic action and molecular basis of resistance. *Oncogene* **2003**, *22* (47), 7265-7279.

88. Quirante, J.; Ruiz, D.; Gonzalez, A.; López, C.; Cascante, M.; Cortés, R.; Messeguer, R.; Calvis, C.; Baldomà, L.; Pascual, A.; Guérardel, Y.; Pradines, B.; Font-Bardía, M.; Calvet, T.; Biot, C., Platinum(II) and palladium(II) complexes with (N,N') and (C,N,N')- ligands derived from pyrazole as anticancer and antimalarial agents: Synthesis, characterization and in vitro activities. *Journal of Inorganic Biochemistry* **2011**, *105* (12), 1720-1728.
89. Cortés, R.; Crespo, M.; Davin, L.; Martín, R.; Quirante, J.; Ruiz, D.; Messeguer, R.; Calvis, C.; Baldomà, L.; Badia, J.; Font-Bardía, M.; Calvet, T.; Cascante, M., Seven-membered cycloplatinated complexes as a new family of anticancer agents. X-ray characterization and preliminary biological studies. *European Journal of Medicinal Chemistry* **2012**, *54*, 557-566.
90. Zou, T.; Liu, J.; Lum, C. T.; Ma, C.; Chan, R. C.-T.; Lok, C.-N.; Kwok, W.-M.; Che, C.-M., Luminescent Cyclometalated Platinum(II) Complex Forms Emissive Intercalating Adducts with Double-Stranded DNA and RNA: Differential Emissions and Anticancer Activities. *Angewandte Chemie International Edition* **2014**, *53* (38), 10119-10123.
91. (a) Saito, Y.; Kugenuma, K.; Tanaka, M.; Suzuki, A.; Saito, I., Fluorometric detection of adenine in target DNA by exciplex formation with fluorescent 8-arylethynylated deoxyguanosine. *Bioorganic & Medicinal Chemistry Letters* **2012**, *22* (11), 3723-3726; (b) Yamana, K.; Iwase, R.; Furutani, S.; Tsuchida, H.; Zako, H.; Yamaoka, T.; Murakami, A., 2'-Pyrene modified oligonucleotide provides a highly sensitive fluorescent probe of RNA. *Nucleic Acids Research* **1999**, *27* (11), 2387-2392.
92. (a) Tong, G. S. M.; Law, Y.-C.; Kui, S. C. F.; Zhu, N.; Leung, K. H.; Phillips, D. L.; Che, C.-M., Ligand-to-Ligand Charge-Transfer Transitions of Platinum(II) Complexes with Arylacetylide Ligands with Different Chain Lengths: Spectroscopic Characterization, Effect of Molecular Conformations, and Density Functional Theory Calculations. *Chemistry – A European Journal* **2010**, *16* (22), 6540-6554; (b) Lee, C.-S.; Sabiah, S.; Wang, J.-C.; Hwang, W.-S.; Lin, I. J. B., Water-Induced Changes of Photoluminescence of a Pincer-Type N-Heterocyclic Carbene Platinum(II) Complex. *Organometallics* **2010**, *29* (2), 286-289.
93. Serra, D.; Cao, P.; Cabrera, J.; Padilla, R.; Rominger, F.; Limbach, M., Development of Platinum(II) and -(IV) CNC Pincer Complexes and Their Application in a Hydrovinylation Reaction. *Organometallics* **2011**, *30* (7), 1885-1895.
94. Farrell, K.; Albrecht, M., Late Transition Metal Complexes with Pincer Ligands that Comprise N-heterocyclic Carbene Donor Sites. In *The Privileged Pincer-Metal Platform: Coordination Chemistry & Applications*, Koten, G. v.; Gossage, R. A., Eds. 2016; pp 85-87.
95. Dinda, J.; Adhikary, S. D.; Roymahapatra, G.; Nakka, K. K.; Santra, M. K., Synthesis, structure, electrochemistry and cytotoxicity studies of Ru(II) and Pt(II)-N-heterocyclic carbene complexes of CNC-pincer ligand. *Inorganica Chimica Acta* **2014**, *413*, 23-31.
96. Quiroga, A. G.; Pérez, J. M.; López-Solera, I.; Masaguer, J. R.; Luque, A.; Román, P.; Edwards, A.; Alonso, C.; Navarro-Ranninger, C., Novel Tetranuclear Orthometalated Complexes of Pd(II) and Pt(II) Derived from p-Isopropylbenzaldehyde Thiosemicarbazone with Cytotoxic Activity in cis-DDP Resistant Tumor Cell Lines. Interaction of These Complexes with DNA. *Journal of Medicinal Chemistry* **1998**, *41* (9), 1399-1408.
97. Ali, A. A.; Nimir, H.; Aktas, C.; Huch, V.; Rauch, U.; Schäfer, K.-H.; Veith, M., Organoplatinum(II) Complexes with 2-Acetylthiophene Thiosemicarbazone:

- Synthesis, Characterization, Crystal Structures, and in Vitro Antitumor Activity. *Organometallics* **2012**, *31* (6), 2256-2262.
98. Ramírez-Rave, S.; Ramírez-Apan, M. T.; Tlahuext, H.; Morales-Morales, D.; Toscano, R. A.; Grévy, J.-M., Non-symmetric CNS-Pt(II) pincer complexes including thioether functionalized iminophosphoranes. Evaluation of their in vitro anticancer activity. *Journal of Organometallic Chemistry* **2016**, *814*, 16-24.
99. Casini, A.; Messori, L., Molecular mechanisms and proposed targets for selected anticancer gold compounds. *Current topics in medicinal chemistry* **2011**, *11* (21), 2647-2660.
100. Messori, L.; Abbate, F.; Marcon, G.; Orioli, P.; Fontani, M.; Mini, E.; Mazzei, T.; Carotti, S.; O'Connell, T.; Zanello, P., Gold (III) complexes as potential antitumor agents: solution chemistry and cytotoxic properties of some selected gold (III) compounds. *Journal of Medicinal Chemistry* **2000**, *43* (19), 3541-3548.
101. Pricker, S. P., Medical uses of gold compounds: past, present and future. *Gold bulletin* **1996**, *29* (2), 53-60.
102. Buckley, R. G.; Elsome, A. M.; Fricker, S. P.; Henderson, G. R.; Theobald, B. R.; Parish, R. V.; Howe, B. P.; Kelland, L. R., Antitumor properties of some 2-[(dimethylamino) methyl] phenylgold (III) complexes. *Journal of medicinal chemistry* **1996**, *39* (26), 5208-5214.
103. Fricker, S. P.; Mosi, R. M.; Cameron, B. R.; Baird, I.; Zhu, Y.; Anastassov, V.; Cox, J.; Doyle, P. S.; Hansell, E.; Lau, G.; Langille, J.; Olsen, M.; Qin, L.; Skerlj, R.; Wong, R. S. Y.; Santucci, Z.; McKerrow, J. H., Metal compounds for the treatment of parasitic diseases. *Journal of Inorganic Biochemistry* **2008**, *102* (10), 1839-1845.
104. Engmann, L.; McNaughton, M.; Gajewska, M.; Kumar, S.; Birmingham, A.; G.Powis, *Anticancer drugs* **2006**, *17*, 539.
105. Coronello, M.; Mini, E.; Caciagli, B.; Cinellu, M. A.; Bindoli, A.; Gabbiani, C.; Messori, L., Mechanisms of Cytotoxicity of Selected Organogold(III) Compounds. *Journal of Medicinal Chemistry* **2005**, *48* (21), 6761-6765.
106. Gamberi, T.; Massai, L.; Magherini, F.; Landini, I.; Fiaschi, T.; Scaletti, F.; Gabbiani, C.; Bianchi, L.; Bini, L.; Nobili, S.; Perrone, G.; Mini, E.; Messori, L.; Modesti, A., Proteomic analysis of A2780/S ovarian cancer cell response to the cytotoxic organogold(III) compound Aubipyc. *Journal of Proteomics* **2014**, *103*, 103-120.
107. Zhang, J.-J.; Sun, R. W.-Y.; Che, C.-M., A dual cytotoxic and anti-angiogenic water-soluble gold (III) complex induces endoplasmic reticulum damage in HeLa cells. *Chemical Communications* **2012**, *48* (28), 3388-3390.
108. Urra, H.; Hetz, C., The ER in 4D: a novel stress pathway controlling endoplasmic reticulum membrane remodeling. *Cell Death Differ* **2012**, *19* (12), 1893-1895.
109. Che, C.-M.; Sun, R. W.-Y., Therapeutic applications of gold complexes: lipophilic gold (III) cations and gold (I) complexes for anti-cancer treatment. *Chemical Communications* **2011**, *47* (34), 9554-9560.
110. Shaik, N.; Martínez, A.; Augustin, I.; Giovinazzo, H.; Varela-Ramírez, A.; Sanaú, M.; Aguilera, R. J.; Contel, M., Synthesis of Apoptosis-Inducing Iminophosphorane Organogold(III) Complexes and Study of Their Interactions with Biomolecular Targets. *Inorganic Chemistry* **2009**, *48* (4), 1577-1587.
111. Cinellu, M. A.; Zucca, A.; Stoccoro, S.; Minghetti, G.; Manassero, M.; Sansoni, M., Synthesis and characterization of gold(III) adducts and cyclometallated derivatives with 6-benzyl- and 6-alkyl-2,2[prime or minute]-bipyridines. *Journal of the Chemical Society, Dalton Transactions* **1996**, (22), 4217-4225.

112. Bertrand, B.; Spreckelmeyer, S.; Bodio, E.; Cocco, F.; Picquet, M.; Richard, P.; Le Gendre, P.; Orvig, C.; Cinellu, M. A.; Casini, A., Exploring the potential of gold(III) cyclometallated compounds as cytotoxic agents: variations on the C^N theme. *Dalton Transactions* **2015**, 44 (26), 11911-11918.
113. (a) Mendes, F.; Groessel, M.; Nazarov, A. A.; Tsybin, Y. O.; Sava, G.; Santos, I.; Dyson, P. J.; Casini, A., Metal-Based Inhibition of Poly(ADP-ribose) Polymerase – The Guardian Angel of DNA. *Journal of Medicinal Chemistry* **2011**, 54 (7), 2196-2206; (b) Zhu, Y.; Cameron, B. R.; Mosi, R.; Anastassov, V.; Cox, J.; Qin, L.; Santucci, Z.; Metz, M.; Skerlj, R. T.; Fricker, S. P., Inhibition of the cathepsin cysteine proteases B and K by square-planar cycloaurated gold(III) compounds and investigation of their anti-cancer activity. *Journal of Inorganic Biochemistry* **2011**, 105 (5), 754-762.
114. Jackson, S. P.; Bartek, J., The DNA-damage response in human biology and disease. *Nature* **2009**, 461 (7267), 1071-1078.
115. Lord, C. J.; Ashworth, A., The DNA damage response and cancer therapy. *Nature* **2012**, 481 (7381), 287-294.
116. Gabbiani, C.; Casini, A.; Kelter, G.; Cocco, F.; Cinellu, M. A.; Fiebig, H. H.; Messori, L., Mechanistic studies on two dinuclear organogold(III) compounds showing appreciable antiproliferative properties and a high redox stability. *Metallomics* **2011**, 3 (12), 1318-1323.
117. (a) Kandagal, P.; Ashoka, S.; Seetharamappa, J.; Shaikh, S.; Jadegoud, Y.; Ijare, O., Study of the interaction of an anticancer drug with human and bovine serum albumin: spectroscopic approach. *Journal of pharmaceutical and biomedical analysis* **2006**, 41 (2), 393-399; (b) Ang, W. H.; Daldini, E.; Juillerat-Jeanneret, L.; Dyson, P. J., Strategy to tether organometallic ruthenium-arene anticancer compounds to recombinant human serum albumin. *Inorganic chemistry* **2007**, 46 (22), 9048-9050; (c) Divsalar, A.; Bagheri, M. J.; Saboury, A. A.; Mansoori-Torshizi, H.; Amani, M., Investigation on the interaction of newly designed anticancer Pd (II) complexes with different aliphatic tails and human serum albumin. *The Journal of Physical Chemistry B* **2009**, 113 (42), 14035-14042.
118. (a) Sitar, M. E.; Aydin, S.; Cakatay, U., Human serum albumin and its relation with oxidative stress. *Clin Lab* **2013**, 59 (9-10), 945-52; (b) Anraku, M.; Chuang, V. T. G.; Maruyama, T.; Otagiri, M., Redox properties of serum albumin. *Biochimica et Biophysica Acta (BBA)-General Subjects* **2013**, 1830 (12), 5465-5472.
119. Casini, A.; Kelter, G.; Gabbiani, C.; Cinellu, M. A.; Minghetti, G.; Fregona, D.; Fiebig, H.-H.; Messori, L., Chemistry, antiproliferative properties, tumor selectivity, and molecular mechanisms of novel gold(III) compounds for cancer treatment: a systematic study. *JBIC Journal of Biological Inorganic Chemistry* **2009**, 14 (7), 1139-1149.
120. Casini, A.; Cinellu, M. A.; Minghetti, G.; Gabbiani, C.; Coronello, M.; Mini, E.; Messori, L., Structural and Solution Chemistry, Antiproliferative Effects, and DNA and Protein Binding Properties of a Series of Dinuclear Gold(III) Compounds with Bipyridyl Ligands. *Journal of Medicinal Chemistry* **2006**, 49 (18), 5524-5531.
121. Wong, K.-H.; Cheung, K.-K.; Chan, M. C.-W.; Che, C.-M., Application of 2, 6-Diphenylpyridine as a Tridentate [Cv Nv C] Dianionic Ligand in Organogold (III) Chemistry. Structural and Spectroscopic Properties of Mono-and Binuclear Transmetalated Gold (III) Complexes. *Organometallics* **1998**, 17 (16), 3505-3511.
122. Li, C. K. L.; Sun, R. W. Y.; Kui, S. C. F.; Zhu, N.; Che, C. M., Anticancer cyclometalated [AuIII_m(C^λ N^λ C) m_L] n⁺ compounds: synthesis and cytotoxic properties. *Chemistry—A European Journal* **2006**, 12 (20), 5253-5266.

123. Berners-Price, S.; Sadler, P., Phosphines in medicine. *Chemistry in Britain* **1987**, 23, 541-4.
124. Castelli, S.; Vassallo, O.; Katkar, P.; Che, C.-M.; Sun, R. W.-Y.; Desideri, A., Inhibition of human DNA topoisomerase IB by a cyclometalated gold III compound: analysis on the different steps of the enzyme catalytic cycle. *Archives of biochemistry and biophysics* **2011**, 516 (2), 108-112.
125. Wai-Yin Sun, R.; Ma, D.-L.; Wong, E. L.-M.; Che, C.-M., Some uses of transition metal complexes as anti-cancer and anti-HIV agents. *Dalton Transactions* **2007**, (43), 4884-4892.
126. Zhang, J. J.; Lu, W.; Sun, R. W. Y.; Che, C. M., Organogold (III) supramolecular polymers for anticancer treatment. *Angewandte Chemie International Edition* **2012**, 51 (20), 4882-4886.
127. (a) Parish, R.; Wright, J. P.; Pritchard, R. G., Mercury (II) and gold (III) derivatives of 2-phenyl pyridines and 2-phenyl-4-(methylcarboxylato) quinoline. *Journal of Organometallic Chemistry* **2000**, 596 (1), 165-176; (b) Shi, Y.; Blum, S. A., Gold and Rhodium Transmetalation: Mechanistic Insights and Dual-Metal Reactivity. *Organometallics* **2011**, 30 (7), 1776-1779; (c) Herbst, A.; Bronner, C.; Dechambenoit, P.; Wenger, O. S., Gold Complexes with Tridentate Cyclometalating and NHC Ligands: A Search for New Photoluminescent Gold (III) Compounds. *Organometallics* **2013**, 32 (6), 1807-1814.
128. Hashmi, A. S. K.; Weyrauch, J. P.; Rudolph, M.; Kurpejović, E., Gold Catalysis: The Benefits of N and N,O Ligands. *Angewandte Chemie International Edition* **2004**, 43 (47), 6545-6547.
129. (a) Wilson, C. R.; Fagenson, A. M.; Ruangpradit, W.; Muller, M. T.; Munro, O. Q., Gold(III) Complexes of Pyridyl- and Isoquinolylamido Ligands: Structural, Spectroscopic, and Biological Studies of a New Class of Dual Topoisomerase I and II Inhibitors. *Inorganic chemistry* **2013**, 52 (14), 7889-7906; (b) Maiore, L.; Aragoni, M. C.; Deiana, C.; Cinellu, M. A.; Isaia, F.; Lippolis, V.; Pintus, A.; Serratrice, M.; Arca, M., Structure–Activity Relationships in Cytotoxic AuI/AuIII Complexes Derived from 2-(2'-Pyridyl) benzimidazole. *Inorganic chemistry* **2014**, 53 (8), 4068-4080.
130. Amoedo, A.; Graña, M.; Martínez, J.; Pereira, T.; López-Torres, M.; Fernández, A.; Fernández, J. J.; Vila, J. M., A Comparative Study of Cyclometallated Palladium (II) Compounds with Terdentate [C, N, S] Pincer Ligands– Crystal and Molecular Structure of [Pd {4-MeC₆H₃C (Me)= NNC (= S) NHMe}(PPh₃)] and [Pd {4-MeOC₆H₃C (H)= N [2-(SMe) C₆H₄]}(Cl)]. *European Journal of Inorganic Chemistry* **2002**, 2002 (3), 613-620.
131. (a) Pandiarajan, D.; Ramesh, R., Catalytic transfer hydrogenation of ketones by ruthenium (II) cyclometallated complex containing para-chloroacetophenone thiosemicarbazone. *Inorganic Chemistry Communications* **2011**, 14 (5), 686-689; (b) Bomben, P. G.; Robson, K. C.; Sedach, P. A.; Berlinguette, C. P., On the viability of cyclometalated Ru (II) complexes for light-harvesting applications. *Inorganic chemistry* **2009**, 48 (20), 9631-9643.
132. Abram, U.; Ortner, K.; Gust, R.; Sommer, K., Gold complexes with thiosemicarbazones: reactions of bi- and tridentate thiosemicarbazones with dichloro[2-(dimethylaminomethyl)phenyl-C₁N]gold(III), [Au(damp-C₁N)Cl₂]. *Journal of the Chemical Society, Dalton Transactions* **2000**, (5), 735-744.
133. Johnson, M. W.; DiPasquale, A. G.; Bergman, R. G.; Toste, F. D., Synthesis of Stable Gold(III) Pincer Complexes with Anionic Heteroatom Donors. *Organometallics* **2014**, 33 (16), 4169-4172.
134. Kroemer, G.; Galluzzi, L.; Kepp, O.; Zitvogel, L., Immunogenic cell death in cancer therapy. *Annual review of immunology* **2013**, 31, 51-72.

135. Fernandez-Cestau, J.; Bertrand, B.; Blaya, M.; Jones, G. A.; Penfold, T. J.; Bochmann, M., Synthesis and luminescence modulation of pyrazine-based gold (III) pincer complexes. *Chemical Communications* **2015**, 51 (93), 16629-16632.