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# Gold(III) Complexes for Biological Applications: an Overview

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## Abstract

Au(III) complexes have emerged as a particularly promising class of anticancer agents. The development of various types of ligands capable of stabilizing the Au(III) cation in physiological conditions (nitrogen-donor based, dithiocarbamate and cyclometalated ligands) has opened the way for the exploration of their mechanisms of action. At the same time, the bioconjugation of Au(III) complexes has emerged as a potential way for improving the selectivity of this class of compounds for cancer cells over healthy tissues.

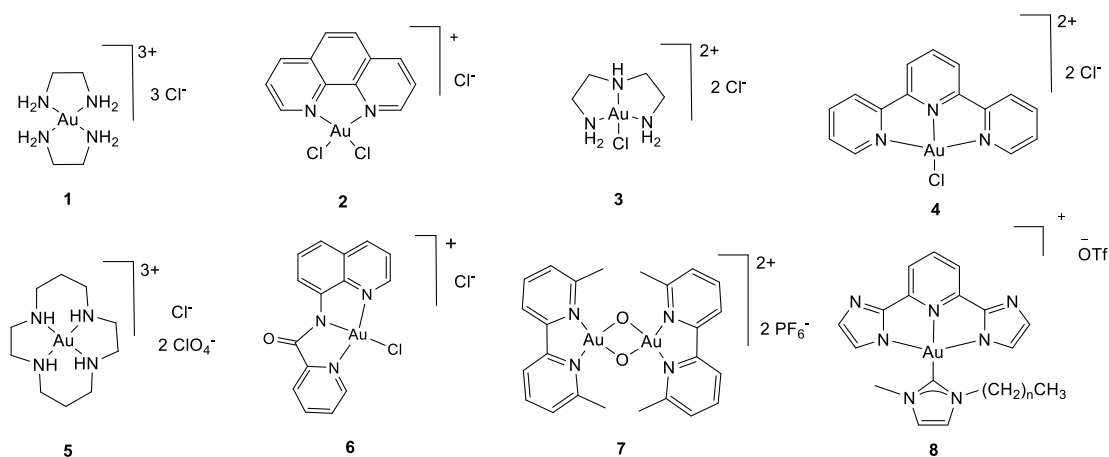
## Introduction

The discovery of the anticancer properties of *cis*-diamminedichloroplatinum(II) (cisplatin) in the 1960s marked the start of the modern era in the discovery and use of metallodrugs.<sup>[1]</sup> However, among the thousands of platinum-based compounds tested for anticancer applications, only two of them, carboplatin and oxaliplatin, have reached worldwide approval.<sup>[2]</sup> Those platinum-based drugs are highly effective chemotherapeutic agents and they are widely used for the treatment of various types of cancer, in particular head and neck, testicular and ovarian cancers.<sup>[3]</sup> However, despite their clinical success, platinum-based metallodrugs do not specifically target cancer cells but also affect other rapidly dividing cells, such as those found in bone marrow and the gastrointestinal tract. This causes severe side effects which limit the administrable dose to patients.<sup>[4]</sup> Other drawbacks include acquired or intrinsic drug resistance as well as a limited spectrum of action.<sup>[5]</sup> All of these factors have driven the interest towards the development of metallodrugs based on other transition metals.<sup>[6]</sup>

The pharmacologic properties of gold have been known since the end of 19<sup>th</sup> century, and gold compounds have been tested against different pathologies, even though they are currently only used for the treatment of rheumatoid arthritis.<sup>[7]</sup> Auranofin, (2,3,4,6-tetra-*O*-acetyl-1-(thio- $\kappa$ S)- $\beta$ -D-glucopyranosato)(triethylphosphine)gold(I), is an orally administrated anti-arthritis drug which has also been studied for its antiproliferative properties.<sup>[8]</sup> Based on those results, different Au(I) complexes bearing phosphine,<sup>[9]</sup> NHC,<sup>[10]</sup> alkynyl<sup>[11]</sup> and thiolate<sup>[12]</sup> ligands have been synthesized and tested as prospective anticancer drugs. Their mechanism of action has been investigated and Au(I) complexes are thought to trigger apoptosis *via* inhibition of selenium- and sulfur-containing enzymes such as thioredoxin reductase (TrxR), glutathione peroxidase, cysteine protease or glutathione-S-transferase.<sup>[13]</sup> Au(III) has a d<sup>8</sup> electronic configuration and is thus isoelectronic with Pt(II) and, like platinum, forms four-coordinated square-planar complexes, and it was therefore initially assumed that Au(III) would provide a good alternative for Pt(II) and show a similar mode of action as anti-cancer treatment. However, due to the reducing intracellular environment, the tendency of Au(III) centre to be reduced to Au(I) or to Au(0) particles makes the choice of ligands particularly relevant.<sup>[14]</sup> This review provides an overview and evaluation of the different classes of Au(III) complexes used as prospective anticancer drugs. The most recent evidence of their possible modes of actions will be discussed, along with current strategies aimed at increasing the selectivity of antiproliferative gold complexes towards their cellular targets.

## 1. Gold(III) complexes with chelating N-donor ligands

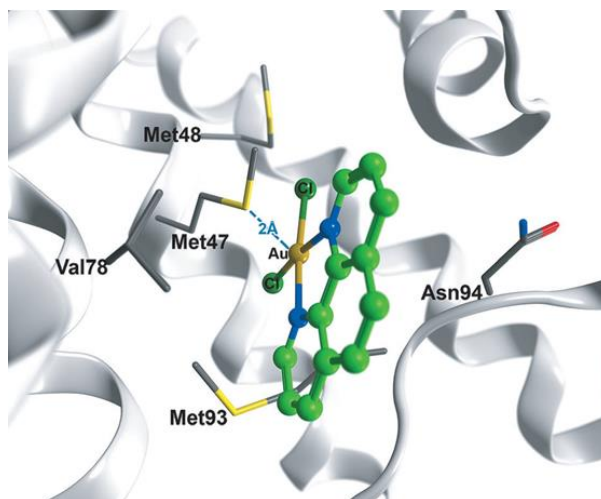
Some of the first gold(III) complexes examined for anticancer applications were pyridine-based complexes [AuCl<sub>3</sub>(Hpm)] and [AuCl<sub>2</sub>(pm)] (Hpm = 2-pyridylmethanol). They were tested *in vitro* against a panel of cancer cell lines and demonstrated comparable activities to cisplatin and NaAuCl<sub>4</sub>. The compounds were shown to interact closely with proteins such as albumin and transferrin but only weakly and reversibly with DNA.<sup>[15]</sup> While this shows that gold(III) compounds have potential, the facile substitution of the chloro ligands by water at pH above 2 rendered them too unstable in a physiological environment for medicinal purposes.<sup>[15]</sup> However, polydentate *N*-donor ligands successfully stabilize gold(III), such as ethylenediamine (en) (**1**), phenanthroline (phen) (**2**), diethylenetriamine (dien) (**3**), terpyridine (terpy) (**4**), macrocyclic ligands 1,4,8,11-tetraazacyclotetradecane (cyclam) (**5**) or aminoquinoline derivatives (**6**).<sup>[16,17]</sup> Some of these complexes are depicted in Figure 1.



**Figure 1:** Examples of Au(III) complexes with chelating *N*-donor ligands investigated for anticancer applications.

The most toxic compounds against the ovarian cancer cell lines A2780 appeared to be the least stable ones (**2** and **5**). Their activities were close to those of the respective ligands phen and terpy, which suggests that the toxicity of **2** and **5** may arise from the reduction of Au(III) and the subsequent release of the phen and terpy ligands.<sup>[16]</sup> The mechanism of action of those Au(III) complexes have been carefully investigated, starting with the potential interaction with DNA due to the electronic similarity with Pt(II). Although such interactions were shown to be possible, they are weak and reversible, thus ruling out DNA as primary target of that class of complexes.<sup>[18]</sup> However, it is possible to increase the interaction of Au(III) complexes with DNA by modifying the ligands. Indeed, complex **6**, with an aminoquinoline-based ligand, has been demonstrated to interact with DNA *via* an intercalation mechanism.<sup>[17]</sup>

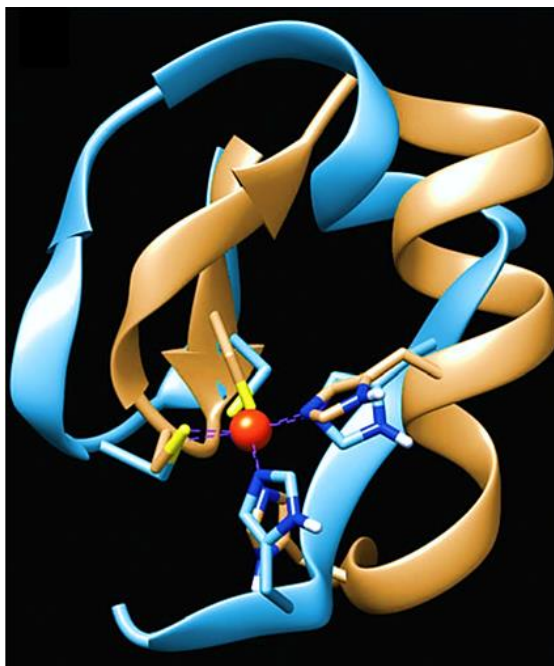
Complex **2** is a particularly good inhibitor of the water and glycerol channels aquaporins (AQPs).<sup>[19]</sup> Using homology models, a methionine residue (presenting a thioether functional group) inside the AQP7 channel and a cysteine residue (presenting a thiol functional group) have been identified as the possible interaction points of **2** with AQP7 (Figure 2) and AQP3, respectively.<sup>[20]</sup>



**Figure 2:** Molecular docking of complex **2** (green) inside the channel of human AQP7 (grey). Reproduced from ref. 20a with permission from the publisher. (authorization to be asked).

Recently, it has been demonstrated that the coordination of the cysteine residue to Au(III) complexes induces a conformational change of the protein, leading to the shrinkage of the channel and so is mostly responsible for its inhibition.<sup>[21]</sup> A more detailed theoretical study of the interaction of this class of Au(III) complexes with AQP3 suggests that this interaction occurs after the replacement of a chloride ligand by a hydroxide. The thus generated  $[(N^{\wedge}N)Au(OH)Cl]^+$  could form a non-covalent adduct with the outer part of AQP3, followed by the coordination of the deprotonated cysteine residue to the  $[(N^{\wedge}N)Au(OH)]^{2+}$  fragment. This complex is further stabilized by  $\pi$ -stacking interactions from the phenyl ring of a phenylalanine residue with the aromatic rings of the  $(N^{\wedge}N)$  ligand.<sup>[22]</sup>

The zinc-finger enzyme poly(ADP)ribose polymerase 1 (PARP-1) has been identified as another possible target of complex **2**. According to mass spectrometry measurements inhibition occurs *via* displacement of zinc upon loss of all ligands coordinated to gold.<sup>[23]</sup> When reacted with model peptides mimicking  $(Cys_2His_2)$  and  $(Cys_2HisCys)$  zinc-finger domains, **2** has been shown to displace  $Zn^{2+}$  with the same efficacy in both cases, leading to the formation of a “gold-finger” while maintaining the +III oxidation state. The change from the tetrahedral environment of Zn(II) to the square-planar geometry of Au(III) led to important conformational modifications (see Figure 3) which are potentially responsible for the inhibition of the enzyme by **2**.<sup>[24]</sup> Interestingly, the reaction of the  $(N^{\wedge}N^{\wedge}N)AuCl$  pincer complex **4** with a  $(Cys_4)$  zinc-finger domain led to fast reduction to Au(I), together with formation of a Cys-Au(I)-Cys moiety and a disulphide bridge.<sup>[25]</sup>



**Figure 3:** Overlay of the calculated structures of the (Cys<sub>2</sub>His<sub>2</sub>) zinc-finger domains in the presence of Zn(II) (brown) and Au(III) (blue) cations. Reproduced from ref. 24 with permission from the publisher. (authorization to be asked).

The dimeric oxo-bridged complex **9** is active in the low micromolar range against cisplatin-sensitive (A2780/S) and cisplatin-resistant (A2780/R) ovarian cancer cell lines. Using UV-visible spectroscopy, complex **9** has been shown to be quite stable in aqueous solution, although some aquation leading to the formation of the monomeric bis-hydroxo complex could occur. However, **9** is easily reduced by natural reductants like ascorbic acid or glutathione.<sup>[26]</sup> Against a panel of 36 cancer cell lines **9** had high activity with a good selectivity score. Using COMPARE analysis, the toxicity pattern of **9** was correlated with one of several histone deacetylase (HDAC) inhibitors.<sup>[27]</sup> Proteomic studies carried out on A2780 cells using 2D-differential gel electrophoresis (2D-DIGE) showed that upon treatment with **9** only a small fraction of the entire proteome presented a modified expression. Most of these proteins were involved in redox homeostasis, pointing to oxidative stress as the potential primary mechanism of action for **9**. Moreover, the close correlation of these results with those obtained with auranofin suggests that **9** might be reduced to a gold(I) species in the intracellular milieu.<sup>[28]</sup> This is in good agreement with data obtained by spectrophotometric and mass spectrometry techniques with different model proteins like human serum albumin (hSA), cytochrome c (cyt c) and ubiquitin (Ubq). In all cases, the interaction was found to occur *via* redox processes triggering the destruction of the dimeric architecture.<sup>[26]</sup>

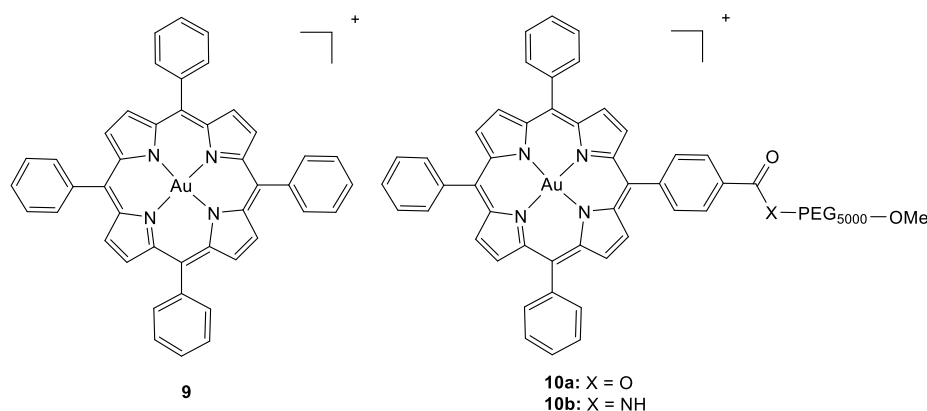
Complexes based on the 2,6-bis(benzimidazol-2-yl)pyridine (HN<sup>^</sup>N<sup>^</sup>NH) ligand (**8**, Figure 1), although perfectly stable in an aqueous medium, were quantitatively and quickly reduced by GSH with release of the HN<sup>^</sup>N<sup>^</sup>NH ligand and formation of the corresponding (GS)Au(I)-(NHC) complexes. The free HN<sup>^</sup>N<sup>^</sup>NH ligand shows strong fluorescence which is quenched on coordination to a gold(III) cation, so that ligand release can be used to monitor gold uptake into cells. Using fluorescence microscopy, the authors could indeed observe the characteristic blue fluorescence of free HN<sup>^</sup>N<sup>^</sup>NH after 10 min of incubation of HeLa cells with **8**, consistent with the uptake of **8** into the cells and its reduction to Au(I). The compound with the highest anticancer activity was the one bearing the longest alkyl chain (n = 15), with a cytotoxic activity in the low micromolar range associated with the highest intracellular gold uptake. Complex **8** reduced the size of HeLa xenografts in mice *in vivo*, making these compounds suitable as dual agents for anticancer and thiol sensing applications.<sup>[29]</sup>

## 2. Gold(III) complexes with porphyrin ligands

Gold(III) porphyrin complexes are a class of compounds characterized by very high redox stability ( $E_p = -1.00$  and  $-1.48$  V vs  $Cp_2Fe^{0/+1}$ ) in acetonitrile, but also by their stability in the presence of natural reductants such as glutathione. The tetraphenylporphyrin complex **9** (Figure 4) was tested against a panel of human cancer cell lines including drug-resistant variants, such as the multi-drug resistant human oral carcinoma (KB-V1) and cisplatin-resistant human nasopharyngeal carcinoma (CNE1). **9** showed sub-micromolar activity across the board, suggesting a different mechanism of action from cisplatin.<sup>[30]</sup> *In vivo* studies on mice showed that **9** reduced nasopharyngeal carcinoma tumour growth without body weight loss or liver injuries. The mechanism of action of **9** involves reduction of mitochondrial membrane potential, which triggers the release of cytochrome c and apoptosis-activating factor leading to apoptosis.<sup>[31]</sup> The heat-shock protein 60 (Hsp60), an important chaperone involved in the transport and folding of mitochondrial proteins, has been identified as a major molecular target of **9**. When replacing the Au(III) cation by a Pt(II) one or using a cyclometalated ligand led to a complete loss of the inhibitory activity, highlighting its dependence of the activity on both the Au(III) ion and the porphyrin ligand.<sup>[32]</sup>

The PEGylated complexes **10a/b** (Figure 4) have been shown to self-assemble in aqueous solution into core-shell micelles with diameters in the range of hundreds of nanometers. These structures accumulate faster and more selectively in the cancer cells than non-PEGylated analogues. This may potentially be due to the “enhanced permeability and retention” (EPR) effect (which refers to the higher permeability of cancer cells membranes as the result of their excessively fast development), although that effect is nowadays more and more debated.<sup>[33]</sup> In acidic conditions, which prevail in the intracellular space

between cancer cells in solid tumours, the ester function in complex **10a** can be cleaved within few hours, causing the release of the free Au(III) complex, while the amide in **10b** is much more stable. Thus both **10a**- and **10b**-based micelles can enter cancer cells easily, but the release of the active monomeric Au-porphyrin fragment is faster and more efficient for the ester **10a**. This causes **10a** to be much more cytotoxic than **10b**. Moreover, the self-assembly capacity of **10a** has been used to encapsulate and deliver the clinically-used anticancer drug doxorubicin into cancer cells, including into doxorubicin-resistant cells.<sup>[34]</sup>



**Figure 4:** Structures of the porphyrin-based gold complexes investigated for anticancer applications.

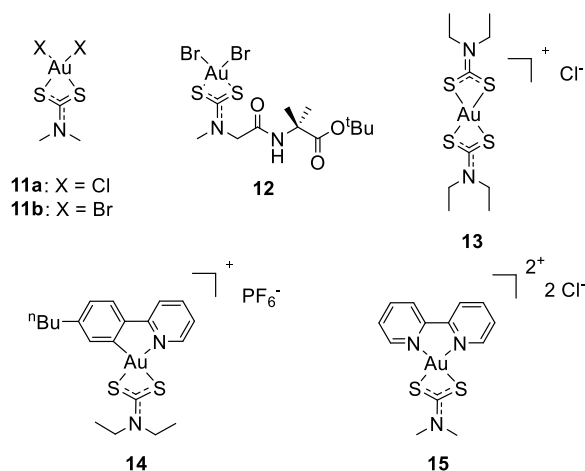
### 3. Gold(III) complexes with dithiocarbamate ligands

Gold(III) dithiocarbamate (DTC) complexes are characterized by two Au-S bonds which stabilize the +III oxidation state, as demonstrated by cyclic voltammetry:  $5 \text{ mV} \leq E_p(\text{AuDTC}) \leq 125 \text{ mV}$  ( $E_p$  = peak potential) compared to  $E_p = 1.29 \text{ V}$  vs SCE for  $\text{KAuX}_4$  ( $X = \text{Cl}, \text{Br}$ ) (Figure 5). Complexes **11a/b** (Figure 4) have been tested *in vitro* against a series of human cancer cell lines including leukemia, cervical adenocarcinoma, colon adenocarcinoma and malignant melanoma. They demonstrated cytotoxicities up to nanomolar levels against leukemia (HL60), lymphoma (Daudi) and melanoma (MeWo) cells. This high antiproliferative activity was accompanied by a lack of cross resistance with cisplatin, suggesting a different mode of action.<sup>[35]</sup> In prostate cancer (PC3) cells *in vitro*, **11a** has been demonstrated to trigger the overexpression of reactive oxygen species (ROS), to inhibit TrxR, and to induce apoptosis *via* mitochondrial membrane depolarization and activation of caspase-9.<sup>[36]</sup> Proteasome, an important actor of the protein degradation machinery in cells, has also been identified as a primary target of **11b** both *in vitro* and *in vivo*.<sup>[37]</sup>



In order to improve the selectivity of gold dithiocarbamate complexes, one strategy investigated involved the coupling of dipeptides to the  $\text{Au}(\text{DTC})\text{X}_2$  scaffold, to facilitate the internalization of the compounds *via* peptide transporters. To this end, dipeptides were introduced on the DTC ligand to give compounds of type **12** (Figure 5). Complex **12** demonstrated strong antiproliferative activity against a panel of five cancer cell lines expressing the peptide transporters PEPT1 and PEPT2.<sup>[38]</sup> Cationic  $[\text{Au}(\text{DTC})_2]^+$  complexes, e.g. **13**, have also been synthesized and tested. They exert very similar cytotoxic activities than the mono-DTC analogues **15**. A mass spectrometry study of the interaction of **13** and its mono-DTC analogue with sulfur-containing species revealed that in both cases the cationic  $[\text{Au}(\text{DTC})_2]^+$  was bound to cysteine or GSH. This suggests that in all cases the  $[\text{Au}(\text{DTC})_2]^+$  cation might be responsible for these interactions.<sup>[39]</sup>

Gold complexes containing cyclometalated 2-phenylpyridine or 2,2'-bipyridyl as well as DTC ligands (Figure 5, **14** and **15**) provide other types of coordinatively stable complexes.<sup>[40,41]</sup> Complex **14** effectively inhibits different deubiquitinases (DUBs). DUBs are proteases responsible for the cleavage of the bond between ubiquitin (Ub) and proteins. Ubiquitination of proteins serves as a “labelling” of proteins for their degradation by the proteasome. Thus, DUBs are involved in different cellular processes including cell cycle, chromatin remodelling or signalling pathways modified in cancer cells such as the ones involving p53 or transforming growth factor- $\beta$  (TGF- $\beta$ ).<sup>[42]</sup> Beyond the deubiquitinase inhibition activity, **14** triggers apoptosis and has anti-angiogenesis properties in breast cancer cells MCF-7.<sup>[40]</sup> Replacing a C<sup>N</sup> cyclometalated ligand by N<sup>N</sup> = 2,2'-bipyridyl leads to **15**, with a cytotoxicity in the sub-micromolar range. The anticancer activity of **15** is p53-independent and is apparently based on the mitochondrial apoptotic pathway.<sup>[41]</sup> This mechanism seems to be a common feature of the  $\text{Au}(\text{DTC})$  complexes.



**Figure 5:** Examples of Au(III) dithiocarbamate complexes investigated for anticancer applications.

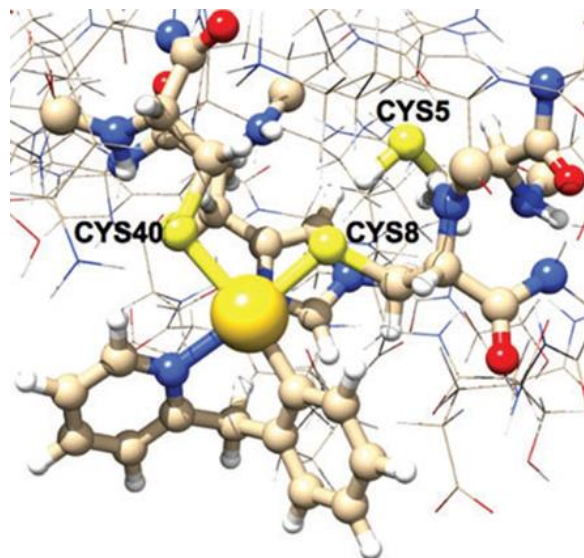
#### 4. Gold(III) complexes with cyclometalated ligands

##### *(C<sup>^</sup>N)Au(III) complexes*

Cyclometalated ligands are chelates with a covalent M-C and a coordinative M-E bond (E = N, O, P, S or Se) and are particularly successful in stabilizing Au(III) against reduction. C<sup>^</sup>N chelates are most widely used, alongside C<sup>^</sup>N<sup>^</sup>N, N<sup>^</sup>C<sup>^</sup>N and C<sup>^</sup>N<sup>^</sup>C pincer ligands.<sup>[43]</sup> The high stability of the cyclometalated Au(III) complexes has enabled the study of reaction intermediates,<sup>[44]</sup> fundamental organometallic reactions,<sup>[45]</sup> and development of photoemissive complexes.<sup>[46]</sup>

The first report of the use of cyclometalated Au(III) complexes as potential anticancer agents described complexes based on the (C<sup>^</sup>N) ligand 2-(N,N-dimethylamino)methylphenyl (complexes **16** and **17**, Figure 7).<sup>[47,48]</sup> Those complexes appeared more toxic than cisplatin on a panel of human cancer cells *in vitro*. The acetato and malonato derivatives (**16c** and **17b** respectively) had the most interesting antiproliferative activity and were able to overcome cross-resistance with cisplatin when tested on ovarian cancer cells sensitive and resistant to cisplatin (A2780 and A2780-R respectively).<sup>[48]</sup> When tested *in vivo* on mice bearing tumour xenografts, these complexes had an anti-tumour activity comparable to cisplatin.<sup>[47,48]</sup> These complexes have been demonstrated to be potent inhibitors of the cysteine protease cathepsin B.<sup>[49]</sup> The replacement of the N,N-dimethylamino moiety by pyridyl, to give **18** and **19**, offers a large palette of derivatization on both the phenyl and pyridine rings, and of the nature of the spacer between the rings (CH<sub>2</sub>, CR<sub>2</sub>, O, S, NH, NR, C=O). The thiosalicylato compound **19b** has been identified as a particularly potent inhibitor of cathepsin B and K, with *in vitro* antiproliferative activity in the low micromolar range against a panel of human cancer cell lines.<sup>[50]</sup>

The phosphine containing complex **18c** and its dichloro analogue **18a** were found to inhibit the zinc-finger enzyme PARP-1 at nanomolar levels.<sup>[51]</sup> Further computational and mass spectrometry investigations have highlighted the ability of **18a** to discriminate different zinc-finger domains and to have a higher affinity for the (CysCysHisCys) zinc-finger domain found in PARP-1, over the (CysCysHisHis) zinc-finger domain. Whereas the (N<sup>^</sup>N) complex **2** reacts with zinc-finger domains with the loss of the N<sup>^</sup>N ligand, the cyclometalated C<sup>^</sup>N complex **18a** maintains the (C<sup>^</sup>N)Au(III) motif upon reaction with the zinc-finger domain (Figure 6).<sup>[52]</sup> This may open the way to the development of highly selective PARP-1 inhibitors.



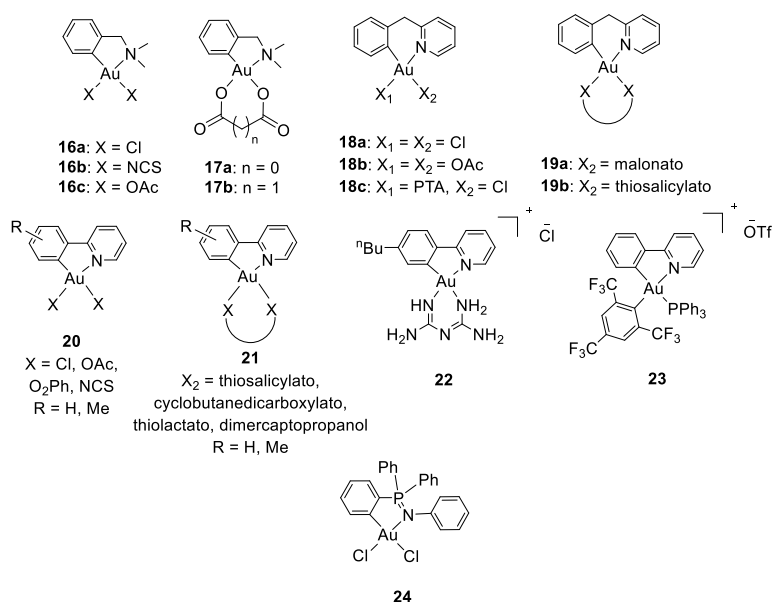
**Figure 6:** Calculated structure of the most stable isomer due to the reaction between the (Cys<sub>2</sub>HisCys) zinc-finger domain of PARP-1 and complex **18a**. Reproduced from ref. 52 with permission from the publisher. (authorization to be asked).

Uptake/efflux studies on precision-cut kidney slices using complex **18c** and cisplatin have demonstrated that both active and passive mechanisms are involved and that in the case of **18c** the efflux might occur predominantly through active processes. The major involvement of the organic cation transporters (OCTs) and the multidrug and toxin extrusion protein (MATE) transporters into the uptake mechanism of **18c** could be ruled out.<sup>[53]</sup>

The more rigid cyclometalated C<sup>^</sup>N ligand scaffold based on 2-phenylpyridine has also been explored to make anticancer organogold complexes with various ancillary ligands, including chloride, carboxylates, cyclobutanedicarboxylate, sulfur-containing ligands and phenyl derivatives (complexes **20** and **21**, Figure 7). These complexes demonstrated antiproliferative properties similar to cisplatin on human leukemia (MOLT-4) and mouse tumour (C2C12) cell lines, with the cyclobutanedicarboxylato complex being the most promising compound of that series.<sup>[54]</sup> Coupling the lipophilic nature of the (C<sup>^</sup>N)Au fragment to the hydrophilic character of the biguanide ligand as in **22** led to a compound displaying a high *in vitro* antiproliferative effect on HeLa cells associated with low toxicity towards normal lung fibroblasts. The compound reacts with GSH to form aggregates without reduction of the Au(III) center. Mechanistic studies on **22** pointed to endoplasmic reticulum (ER) swelling triggered by ER-stress induction, as evidenced by the up-regulation of ER-stress markers such as GRP78/Bip, CHOP and HSP70. **22** also displayed anti-angiogenic effects at sub-cytotoxic concentrations.<sup>[55]</sup> TrxR has also been identified

as a possible intracellular target of analogues of complex **23** (Figure 6). Moreover, **23** was shown to increase the amount of intracellular ROS in HeLa cells.<sup>[56]</sup>

Iminophosphorane have been used as cyclometalated (C<sup>^</sup>N) ligands to generate analogues of **24**. The chloro ligands can easily be substituted, e.g. by as dithiocarbamates or phosphines. These compounds appeared particularly efficient against acute lymphoblastic leukemia cells (Jurkat). They are potent TrxR inhibitors and trigger apoptosis *via* a mitochondria-related pathway involving ROS production and mitochondrial membrane depolarization.<sup>[57]</sup> The hydrophobic/hydrophilic balance of these systems can easily be tuned by using a more water-soluble phosphine such as the 1,3,5-triaza-7-phosphaadamantane (PTA).<sup>[58]</sup>



**Figure 7:** Examples of cyclometalated (C<sup>^</sup>N)Au(III) complexes.

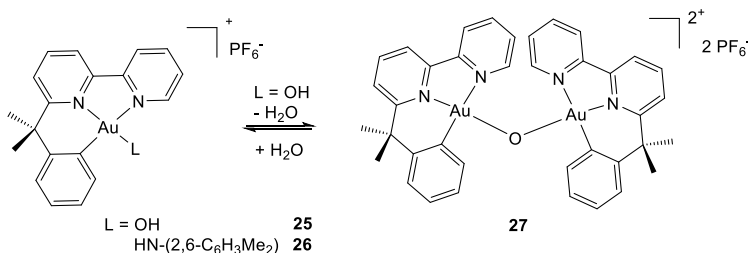
### (C<sup>^</sup>N<sup>^</sup>N)Au(III) complexes

Compound **25** (Figure 8) bearing a 6-(1,1-dimethylbenzyl)-2,2'-bipyridine ligand was shown to be stable toward reduction even in the presence of reductants like sodium ascorbate, while the (N<sup>^</sup>N)Au(III) (based on bipy or phen ligands, figure 1) were easily reduced under the same conditions.<sup>[16,59]</sup> **25** has been shown to be highly cytotoxic against A2780 ovarian cancer cells despite a weak interaction with DNA. Its mechanism of action is likely due to inhibition of enzymes *via* the direct interaction of the (C<sup>^</sup>N<sup>^</sup>N)Au(III) fragment with different proteins and enzymes, without reduction of the Au(III) cation. Such interactions have been described with bovin serum albumin (BSA),<sup>[60]</sup> cytochrome c, lysozyme<sup>[61]</sup>

and the enzyme thioredoxin reductase.<sup>[62]</sup> Using mass spectrometry techniques, the preferred interaction of **25** with selenocysteines over histidine, cysteine, methionine and glutamate has been highlighted, along with the weak interaction with 9-ethylguanine and guanine-based nucleotides. This correlates with TrxR being a major target of **25**.<sup>[63]</sup> Several lines of evidence point towards the involvement of the copper trafficking system into the uptake mechanism of gold complexes. Indeed, **34** has been shown to form stable adducts with the copper chaperone Atox-1 and to compete with the binding of copper(I).<sup>[64]</sup> **25** has also been identified as an inhibitor of the transmembrane transporter Na<sup>+</sup>/K<sup>+</sup>-ATPase<sup>[65]</sup> and the 20S proteasome sub-unit.<sup>[66]</sup> In order to observe the effects triggered by **25** on the expression of the proteins of a whole cell, proteomic analyses have been performed using 2D-DIGE methodologies. When comparing samples of A2780 ovarian cancer cells treated with **25** to samples of the untreated cells, the expressions of only few proteins were found to be modified (up- or down-regulated). These proteins could be identified by MALDI-TOF mass spectrometry techniques.<sup>[67]</sup> Most of the over-expressed proteins were shown to belong to the ‘stress response and chaperones’ functional class. The down-regulated proteins appeared to be involved in the glucose metabolism pathway and more generally speaking in energy production. Treatment with **25** therefore seems to trigger cellular stress *via* the down-regulation of the glucose metabolism pathway.

Complex **26** (Figure 8), the xylydine analogue of **25**, showed improved antiproliferative activity compared to **25** against A2780 and MCF-7 cells.<sup>[68]</sup> The COMPARE analysis of the results from a panel of 36 cell lines suggested the inhibition of mammalian target of rapamycin (mTOR), proteasome and DNA synthesis as potential mechanism of action.<sup>[27]</sup> TrxR has been identified as another potential target of **26** through the interaction with the cysteine and selenocysteine residues.<sup>[69]</sup>

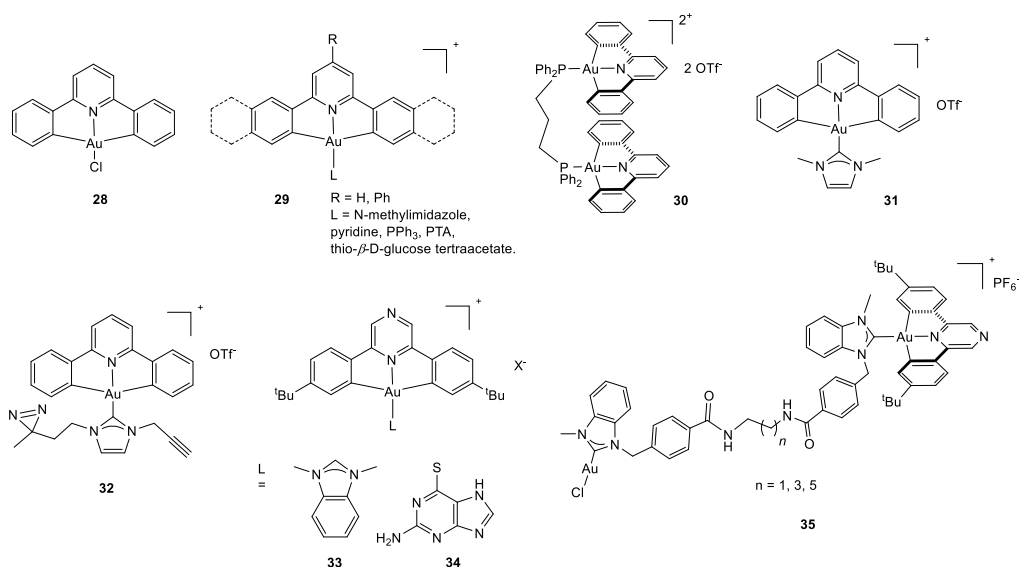
The dimeric oxo-bridged compound **27** (Figure 8) is reversibly hydrolysed to give two equivalents of **25**. The latter has been identified as the binding fragments with various proteins (lysozyme and cytochrome c).<sup>[70]</sup> In the same way, hydrolysis of **27** to **25** leads to the stabilization of the non-canonical G-quadruplex structure of DNA.<sup>[71]</sup>



**Figure 8:** Cyclometalated (C<sup>N</sup>N)Au(III) hydroxide and amide complexes.

**(C<sup>^</sup>N<sup>^</sup>C)Au(III) complexes**

Pincer complexes (C<sup>^</sup>N<sup>^</sup>C)Au(III)X (X = halide, N-, C- or P-donor) based on the 2,6-diphenylpyridine ligand, such as **28** (Figure 9), have shown promising anticancer properties *in vitro*.<sup>[72]</sup> This ligand platform is very stable and synthetically versatile and has opened the way to a large series of derivatives of type **29** with different ancillary ligands, including 1-methylimidazole, pyridine, triphenylphosphine, PTA and thio- $\beta$ -D-glucose tetraacetate.<sup>[72,73]</sup> Modifications of the pincer ligand has also been explored by benzo-annulation of the phenyl rings (as in 2,6-dinaphthylpyridine) or by introducing another aromatic substituent on the pyridine (2,4,6-triphenylpyridine), although these modifications do not seem to significantly modify the antiproliferative properties. On the other hand, changing the ancillary ligands X seems to have a major impact on the biological properties of the complexes. The *N*-methylimidazole in **29** (R =H) enhances the ability of the complex to intercalate with double-stranded DNA and to interact with G-quadruplex DNA, making DNA a potential target for that compound, whereas the phosphine-containing analogues interact only weakly with DNA. In the same way **29** (L = *N*-methylimidazole) blocks the cell cycle in the S-phase *i.e.* when the DNA has been replicated and so when the amount of DNA is the highest, while the dimeric complex **30** was unable to do so even after 48 h of incubation.<sup>[72]</sup>



**Figure 9:** Examples of cyclometalated (C<sup>^</sup>N<sup>^</sup>C)Au(III) complexes investigated for anticancer applications.

(C<sup>^</sup>N<sup>^</sup>C)Au complexes of bridging 1,3-bis(diphenylphosphino)propane (**30**, Figure 9) proved particularly effective *in vitro* against human hepatocellular carcinoma and human cervical epithelial carcinoma cells.<sup>[72,74]</sup> However, the cytotoxicity of these complexes appeared to be very close to that of the free diphosphine ligands themselves, which suggests that the complexes may simply act as carriers of the cytotoxic ligands. Based on that finding the more stable NHC analogues have also been tested and have shown much lower toxicity. Ligand lability therefore seems important for antiproliferative activity.<sup>[72,74]</sup> When tested *in vivo* on nude mice bearing hepatocellular carcinoma xenografts, **30** could reduce tumour growth more efficiently than cisplatin or doxorubicin, while no side-effects were observed. **30** has been shown to very effectively inhibit TrxR and induce ER stress leading to apoptosis.<sup>[74]</sup>

The [(C<sup>^</sup>N<sup>^</sup>C)Au(NHC)]<sup>+</sup> complex **31** inhibits topoisomerase 1 (Topo1), an enzyme responsible for the unwinding of chromosomal DNA, by stabilizing the cleavable topo1/DNA complex with higher efficacy than the known Topo1 poison comptotheclin. This stabilization of the cleavable topo1/DNA complex prevents the relegation after unwinding, leading to DNA strand breaks.<sup>[75]</sup> Complex **31** proved to be highly toxic against various human cancer cell lines *in vitro* and *in vivo* in nude mice bearing hepatocellular carcinoma xenografts (IC<sub>50</sub> ~ 0.2 to 1.2 μM).<sup>[75]</sup>

To gain a broader picture of the mechanism of action of this class of [(C<sup>^</sup>N<sup>^</sup>C)Au(NHC)]<sup>+</sup> complexes, **32** has been developed. This compound presents two complementary functional groups: a photolabile diazirine unit, and an alkynyl grafting function. When irradiated with UV light (λ = 365 nm), the diazirine releases N<sub>2</sub> and covalently binds with its closest neighbour, which will be the biomolecule with which it interacts. Then the alkynyl group was used to graft an azido-biotin fragment by the usual click-reaction, which enables the detection of **32**/protein conjugates with streptavidin-peroxidase.<sup>[76]</sup> Only six proteins were observed to be bound to **32**; these were identified as heat shock protein 60 (HSP60), vimentin (VIM), nucleoside diphosphate kinase A (NDKA), nucleophosmin (NPM), nuclease-sensitive element binding protein (Y box binding protein, YB-1), and peroxiredoxin 1 (PRDX1) which are all reported possible anti-cancer targets. When the same experiment was carried out with HeLa, NCI-H460 and HCT116 the same six proteins were identified. Proteomic analysis of HeLa cells highlighted the modulation of the eukaryotic initiation factor 2 (eIF2) signalling pathway involved in the synthesis of proteins upon treatment with **32**. Proteomic data are in good agreement with the identified targets since VIM, NPM and YB-1, three of the identified protein targets, are involved in eIF2 signalling pathway modulation.<sup>[76]</sup> Interestingly, proteins involved in protein synthesis have been similarly found to be down-

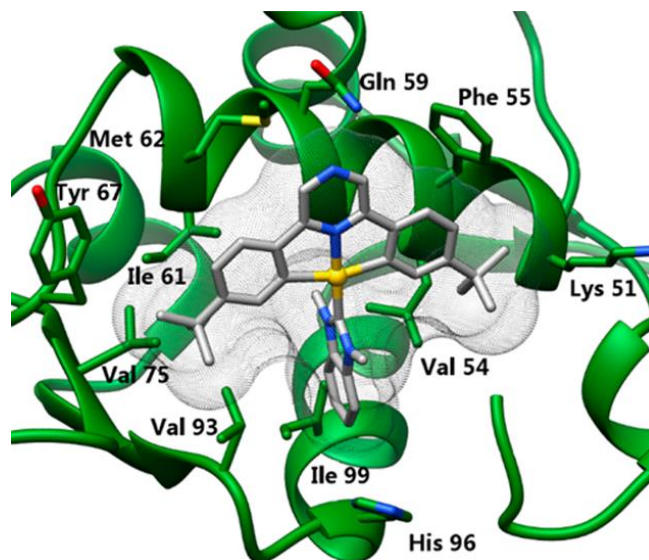
regulated upon treatment with the (C<sup>^</sup>N<sup>^</sup>N)Au(III) complex **25** (Figure 8), pointing toward a general mode of action for cyclometalated Au(III) complexes.<sup>[67]</sup>

Replacing the central pyridine ring by a pyrazine enhanced the photoemissive properties of the corresponding (C<sup>^</sup>N<sup>^</sup>C)Au(III) complexes and increases the tendency toward  $\pi$ -stacking and H-bonding.<sup>[77]</sup> This scaffold has been used to develop a library of complexes with various ancillary ligands including NHCs and the anti-cancer drug thioguanine (complexes **33** and **34**).<sup>[78]</sup> The cytotoxic profile of complex **34** was very similar to that of the free thioguanine, suggesting that, as was the case with **30**, the gold complex may act merely as the carrier of the antiproliferative agent.

The benzimidazol-2-ylidene complex **33** was found to be highly active *in vitro* against a panel of human cancer cell lines (IC<sub>50</sub> between 0.3 and 8  $\mu$ M). Interestingly, the caffeine-based analogue of **33** appeared 10 times less toxic on all studied cells lines. These differences in activity could be rationalized by reduced intracellular gold uptake, as measured by inductively coupled plasma-mass spectrometry (ICP-MS) techniques, which suggests that uptake occurs by passive diffusion across the cell membrane which is impeded by carbene ligands with very polar structural elements. The polarity and degree of functionalisation of the NHC ligands are therefore important for the anticancer properties.<sup>[78]</sup>

Compound **33** proved to be active also against cisplatin-resistant cell lines, and it could be shown that **33** is resistant to reduction or substitution by GSH over a period of several days. There are several potential modes of action for such a complex; for example, **33** is able to stabilize selectively both the G-quadruplex and the i-motif structures of DNA, compared to the canonical double helix DNA structure, and it disrupts the protein-protein interaction between MDM2 and p53.<sup>[78]</sup> Indeed, using biochemical and molecular docking techniques and saturation transfer difference NMR spectroscopy, **33** has been shown to interact with MDM2 on the p53 interaction site as depicted in Figure 10.





**Figure 10:** Representation of **33** (gray) docked in MDM2 (green). Reproduced from ref. 78 with permission from the publisher. (authorization to be asked).

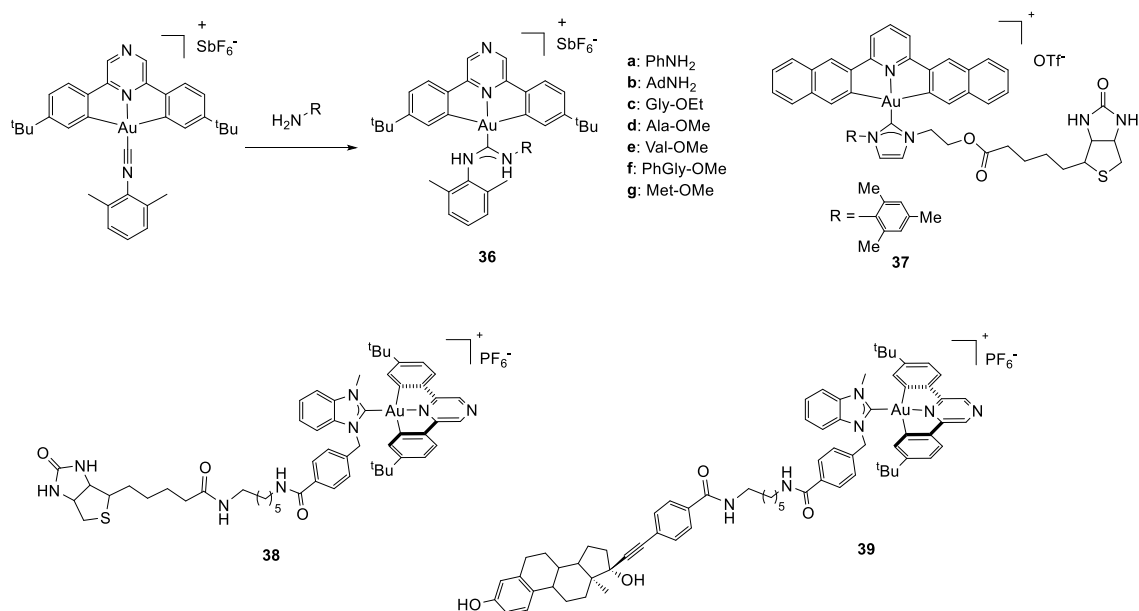
The concept of multinuclearity, *i.e.* the incorporation of several metal centres into a single molecule, has shown some promising results in terms of increasing the cytotoxicity of the compounds<sup>[79]</sup> or enabling their cellular localization (*i.e.* synthesis of theranostics) by incorporating a luminescent or radioactive metal.<sup>[80]</sup> Using an NHC ligand bearing a pentafluorophenol ester moiety has enabled a post-metalation introduction of a second metal complex with high yield and selectivity.<sup>[81]</sup> A similar strategy has been used to synthesize the series of homobimetallic Au(III)/Au(I) complexes **35** with different alkyl spacers between the two gold cations.<sup>[82]</sup> The complex with the longest linker showed activity only against the two breast cancer cell lines (MCF-7 and MDA-MB-231) but also showed a reduced activity against healthy fibroblasts. As for the monometallic complex **33** the differences in activity between the cell lines seem to be linked to differences in intracellular gold uptake. Compared to **33**, the presence of the NHC-AuCl moiety in **35** did not decrease the interaction of this compound with the G-quadruplex structure of DNA, which suggests that the Au(I) fragment does not prevent the recognition of the (C<sup>^</sup>N<sup>^</sup>C)Au(III) moiety by cellular targets.<sup>[82]</sup> On the contrary, although NHC-AuCl are not reported to stabilize the G-quadruplex structure of DNA,<sup>[83]</sup> the introduction of the Au(I) fragment increased the interaction of **35** with the G-quadruplex structure of DNA compared to the Au(III) complex with the pending amino linker.<sup>[82]</sup>

In order to enhance the selectivity of metal-based drugs toward cancer cells, a particular promising strategy appears to be the conjugation of the metal complex with a vector molecule which can be recognized by receptors and transporters that are over-expressed by cancer cells. This vector can be of different types: peptides, sugars, vitamins or aptamers.<sup>[84]</sup> However, the bioconjugation can be difficult to carry out and may lead to overall poor synthetic yields. This can limit the number of permutations that can be explored in terms of metal-based compounds, spacers and vectors. Thus, it is important to develop simple, high yielding and versatile bioconjugation methods. Reacting nucleophiles with an electrophilic isocyanide complex  $[(C^{\wedge}N^{\wedge}C)Au(C\equiv N-R)]^+$  leads to the formation of the corresponding acyclic carbene complexes of type **36**, as shown in Figure 11. The reaction has been tested with success with amines and amino esters to generate a series of complexes **36**.<sup>[85]</sup> The compounds were tested against a panel of cancer cells and showed higher activity than cisplatin against A549, MCF-7 and MDA-MB-231. However, these acyclic carbene complexes proved more sensitive to reduction by GSH than the related complex **33** bearing a cyclic carbene ligand. Since **36** did not induce overproduction of ROS, interference with the mitochondrial redox activity was ruled out as potential mechanism.

Attaching a biotin fragment to the  $[(C^{\wedge}N^{\wedge}C)Au(NHC)]^+$  backbone (complex **37**, Figure 11), enabled its binding to avidin. The natural photoluminescence of the **38**/avidin conjugate was enhanced by a factor of 45 in the presence of aggregated bovine serum albumin (BSA) among other proteins, while the presence of single stranded DNA among other DNA structures increased the luminescence intensity 30-fold, making it a selective detector for those biomolecules.<sup>[86]</sup> Both **37** and the **37**/avidin conjugate were more active than cisplatin against HeLa, HepG2 and MDA-MB-231 cells. The **37**/avidin conjugate was shown to trigger apoptosis without inducing generation of ROS.<sup>[86]</sup>

Following the methodology developed for the synthesis of the homobimetallic complexes **35**, the conjugation of biotin and 17 $\alpha$ -ethynylestradiol *via* different alkyl spacers has been achieved in high yields leading to complexes **38** and **39**, respectively.<sup>[82]</sup> Incubation of MCF-7 cells, expressing both biotin receptors (BR) and estrogen receptors (ER), with **38** and **39** led to a higher intracellular gold uptake compared to the non-conjugated complex **33**. Moreover, the amount of intracellular gold was reduced in cells that did not express the biotin and estrogen receptors. This suggests that the two biomolecules could selectively deliver the  $[(C^{\wedge}N^{\wedge}C)Au(NHC)]^+$  fragment to cancer cells provided these express the corresponding receptors. However, in spite of the increased metal uptake, the cytotoxic properties of **38** and **39** were reduced compared to the non-conjugated complex **33**. When tested against a potential intracellular target of the pharmacophore **33**, **38** and **39** showed no interaction with the DNA G-quadruplex structures. The results indicate that while vectors covalently bound to a  $[(C^{\wedge}N^{\wedge}C)Au(NHC)]^+$  backbone can increase the uptake of antiproliferative agents their also disturb the ability of the metal-

based pharmacophore to interact with its target resulting in an overall reduced activity.<sup>[80]</sup> Thus linking the vector to the  $[(C^{\wedge}N^{\wedge}C)Au(NHC)]^+$  backbone *via* a “self-immolative” spacer, so that the gold fragment can be liberated from the vector after entering the cell may open the way to highly effective and selective gold-based anticancer therapy.



**Figure 11:** Examples of cyclometalated  $(C^{\wedge}N^{\wedge}C)Au(III)$  complexes conjugated to biomolecules.

## Conclusion

Although gold in the oxidation state +III in a physiological medium is often easily reduced to gold(I) or gold nanoparticles, the development of chelating ligands with nitrogen donors, cyclometalated structures and dithiocarbamates has opened the way to enable Au(III) complexes to be used for chemotherapeutic purposes. The mechanisms by which these compounds are able to exert antiproliferative effects are slowly beginning to emerge. Different trends have been highlighted, although details remain very sketchy. Improving the selectivity of the compounds for cancer cells over healthy tissue in order to limit the side effects remains a major challenge, although some promising results have been obtained. More sophisticated synthetic approaches are required to fully utilise the potential of Au(III) complexes for the development of new generations of anticancer drugs.

## References

- [1] B. Rosenberg, L. Vancamp, T. Krigas, *Nature*, **1965**, *205*, 698-699.
- [2] N. J. Wheate, S. Walker, G. E. Craig, R. Oun *Dalton Trans.*, **2010**, *39*, 8113-8127.
- [3] T. W. Hambley, *J. Chem. Soc. Dalton Trans.*, **2001**, 2711-2718.
- [4] K. R. Barnes, S. J. Lippard, *Met. Ions Biol. Syst.*, **2004**, *42*, 143-177.
- [5] C. A. Rabik, M. E. Dolan, *Cancer Treat. Rev.*, **2007**, *33*, 9-23.
- [6] (a) A. Levina, A. Mitra, P. A. Lay, *Metallomics*, **2009**, *1*, 458-470. (b) G. Jaouen, A. Vessières, S. Top, *Chem. Soc. Rev.*, **2015**, *44*, 8802-8817. (c) C. Santini, M. Pellei, V. Gandin, M. Porchia, F. Tisato, C. Marzano, *Chem. Rev.*, **2013**, *114*, 815-862. (d) M. Cini, T. D. Bradshaw, S. Woodward, *Chem. Soc. Rev.*, **2017**, *46*, 1040-1051. (e) I. Ott, *Coord. Chem. Rev.*, **2009**, *253*, 1670-1681. (f) B. Bertrand, A. Casini, *Dalton Trans.*, **2014**, *43*, 4209-4219.
- [7] C. F. Shaw III, *Chem. Rev.*, **1999**, *99*, 2589-2600.
- [8] T. M. Simon, D. H. Kunishima, G. J. Vibert, A. Lorbe, *Cancer*, **1979**, *44*, 1965-1975.
- [9] (a) E. Viry, E. Battaglia, V. Deborde, T. Müller, R. Réau, E. Davioud-Charvet, D. Bagrel *ChemMedChem* **2008**, *3*, 1667-1670. (b) S. J. Berners-Price, C. K.; Mirabelli, R. K. Johnson, M. R. Mattern, F. L. McCabe, L. F. Faucette, C.-M. Sung, P. J. Sadler, S. T. Crooke, *Cancer Res.*, **1986**, *46*, 5486-5493. (c) O. Rackham, S. J. Nichols, P. J. Leedman, S. J. Berners-Price, Filipovska, A. *Biochem. Pharmacol.*, **2007**, *74*, 992-1002.
- [10] (a) A. Gautier, F. Cisnetti, *Metallomics*, **2012**, *4*, 23-32. (b) W. Liu, R. Gust, *Coord. Chem. Rev.*, **2016**, *329*, 191-213. (c) L. Oehninger, R. Rubbiani, I. Ott, *Dalton Trans.*, **2013**, *42*, 3269-3284. (d) S. J. Berners-Price, A. Filipovska, *Metallomics*, **2011**, *3*, 863-873.
- [11] (a) A. Meyer, C. P. Bagowski, M. Kokoschka, M. Stefanopoulou, H. Alborzinia, S. Can, D. H. Vlecken, W. S. Sheldrick, S. Wölfl, I. Ott, *Angew. Chem. Int. Ed.*, **2012**, *35*, 8895-8899. (b) R. G. Balasingham, C. F. Williams, H. J. Mottram, M. P. Coogan, S. J. A. Pope, *Organometallics*, **2012**, *31*, 5835-5843.
- [12] (a) L. Ortego, F. Cardoso, M. F. Fillat, A. Laguna, M. Meireles, M. D. Villacampa, M. C. Gimeno, *J. Inorg. Biochem.*, **2014**, *130*, 32-37. (b) B. Bertrand, A. de Almeida, E. P. M. van der Burgt, M. Picquet, A. Citta, A. Folda, M. P. Rigobello, P. Le Gendre, E. Bodio, A. Casini, *Eur. J. Inorg. Chem.*, **2014**, *27*, 4532-

4536. (c) E. Shuh, C. Pfluger, A. Citta, A. Folda, M. P. Rigobello, A. Bindoli, A. Casini, F. Mohr, *J. Med. Chem.*, **2012**, *55*, 5518-5528.

[13] (a) T. T. Zou, C. T. Lum, C. N. Lok, J. J. Zhang, C. M. Che, *Chem. Soc. Rev.*, **2015**, *44*, 8786—8801. (b) J. Chaudière, A. L. Tappel, *J. Inorg. Biochem.*, **1984**, *20*, 313-325. (c) A. De Luca, C. G. Hartinger, P. J. Dyson, M. Lo Bello, A. Casini, *J. Inorg. Biochem.*, **2013**, *119*, 38-42.

[14] L. Messori, G. Marcon, *Met. Ions Biol. Syst.*, **2004**, *42*, 385-424.

[15] P. Calamai, S. Carotti, A. Guerri, L. Messori, E. Mini, P. Orioli, G. P. Speroni, *J. Inorg. Biochem.*, **1997**, 103-109.

[16] L. Messori, F. Abbate, G. Marcon, P. Orioli, M. Fontani, E. Mini, T. Mazzei, S. Carotti, T. O'Connell, P. Zanello, *J. Med. Chem.*, **2000**, *43*, 3541-3548.

[17] T. Yang, C. Tu, J. Y. Zhang, L. P. Lin, X. M. Zhang, Q. Liu, J. Ding, Q. Xu, Z. Guo, *Dalton Trans.*, **2003**, 3419-3424.

[18] L. Messori, P. Orioli, C. Tempi, G. Marcon, *Biochem. Biophys. Res. Comm.*, **2001**, *281*, 352-360.

[19] A. P. Martins, A. Maronne, A. Ciancetta, A. G. Cobo, M. Echevarría, T. F. Moura, N. Re, A. Casini, G. Soveral, *PlosOne*, **2012**, *7*, e37435.

[20] (a) A. Madeira, A. de Almeida, C. de Graaf, M. Camps, A. Zorzano, T. F. Moura, A. Casini, G. Soveral, *ChemBioChem*, **2014**, *15*, 1487-1494. (b) A. P. Martins, A. Ciancetta, A. de Almeida, A. Marrone, N. Re, G. Soveral, A. Casini, *ChemMedChem*, **2013**, *8*, 1086 – 1092.

[21] A. de Almeida, A. F. Mósca, D. Wragg, M. Wenzel, P. Kavanagh, G. Barone, S. Leoni, G. Soveral, A. Casini, *Chem. Commun.*, **2017**, *53*, 3830-3833.

[22] V. Graziani, A. Marrone, N. Re, C. Coletti, J. A. Platts, A. Casini, *Chem. Eur. J.*, **2017**, *23*, 13802-13813.

[23] F. Mendes, M. Groessl, A. A. Nazarov, Y. O. Tsybin, G. Sava, I. Santos, P. J. Dyson, A. Casini, *J. Med. Chem.*, **2011**, *54*, 2196-2206.

[24] Ü. A. Laskay, C. Garino, Y. O. Tsybin, L. Salassa, A. Casini, *Chem. Commun.*, **2015**, *51*, 1612-1615.

- [25] A. Jacques, C. Lebrun, A. Casini, I. Kieffer, O. Proux, J.-M. Latour, O. S  n  que, *Inorg. Chem.* **2015**, *54*, 4104-4113.
- [26] A. Casini, M. A. Cinellu, G. Minghetti, C. Gabbiani, M. Coronello, E. Mini, L. Messori, *J. Med. Chem.*, **2006**, *49*, 5524-5531.
- [27] A. Casini, G. Kelter, C. Gabbiani, M. A. Cinellu, G. Minghetti, D. Fregona, H. H. Fiebig, L. Messori, *J. Biol. Inorg. Chem.*, **2009**, *14*, 1139-1149.
- [28] F. Magherini, A. Modesti, L. Bini, M. Puglia, I. Landini, S. Nobili, E. Mini, M. A. Cinellu, C. Gabbiani, L. Messori, *J. Biol. Inorg. Chem.*, **2010**, *15*, 573-582.
- [29] T. Zou, C. T. Lum, S. S.-Y. Chui, C.-M. Che, *Angew. Chem Int. Ed.*, **2013**, *52*, 2930-2933.
- [30] C. M. Che, R. W. Y. Sun, W. Y. Yu, C. B. Ko, N. Y. Zhu H. Z. Sun, *Chem. Commun.*, **2003**, 1718-1719.
- [31] C.-M. Che, R. W.-Y. Sun, *Chem. Commun.*, **2011**, *47*, 9554-9560.
- [32] D. Hu, Y. Liu, Y.-T. Lai, K.-C. Tong, Y.-M. Fung, C.-N. Lok, C.-M. Che, *Angew. Chem. Int. Ed.*, **2016**, *55*, 1387-1391.
- [33] J. W. Nichols, Y. H. Bae, *J. Control. Release*, **2014**, *190*, 451-464.
- [34] C. Y.-S. Chung, S.-K. Fung, K.-C. Tong, P.-K. Wan, C.-N. Lok, Y. Huang, T. Chen, C.-M. Che, *Chem. Sci.*, **2017**, *8*, 1942-1953.
- [35] L. Ronconi, L. Giovagnini, C. Marzano, F. Bettio, R. Graziani, G. Pilloni, D. Fregona, *Inorg. Chem.*, **2005**, *44*, 1867-1881.
- [36] L. Cattaruzza, D. Fregona, M. Mongiat, L. Ronconi, A. Fassina, A. Colombatti, D. Aldinucci, *Int. J. Cancer*, **2011**, *128*, 206-215.
- [37] V. Milacic, D. Chen, L. Ronconi, K. R. Landis-Piwowar, D. Fregona, Q. P. Dou, *Cancer Res.*, **2006**, *66*, 10478-10486.
- [38] M. N. Kouodom, L. Ronconi, M. Celegato, C. Nardon, L. Marchi  , Q. P. Dou, D. Aldinucci, F. Formaggio, D. Fregona, *J. Med. Chem.*, **2012**, *55*, 2212-2226.
- [39] M. Altaf, A. A. Isab, J. Van  o, Z. Dvoř  k, Z. Tr  vnick  k, H. Stoeckli-Evans, *RSC Adv.*, **2015**, *5*, 81599-81607.

- [40] J. J. Zhang, K. M. Ng, C. N. Lok, R. W. Y. Sun, C. M. Che, *Chem. Commun.*, **2013**, *49*, 5153-5155.
- [41] M. Altaf, M. Monim-ul-Mehboob, A.-N. Kawde, G. Corona, R. Larcher, M. Ogasawara, N. Casagrande, M. Celegato, C. Borghese, Z. H. Siddik, D. Aldinucci, A. A. Isab, *Oncotarget*, **2017**, *8*, 490-505.
- [42] J. M. Fraile, V. Quesada, D. Rodríguez, J. M. P. Freije, C. López-Otín, *Oncogene*, **2012**, *31*, 2373-2388.
- [43] R. Kumar, C. Nevado, *Angew. Chem. Int. Ed.* **2017**, *56*, 1994-2015.
- [44] (a) D.-A. Roşca, J. A. Wright, D. L. Hughes, M. Bochmann, *Nat. Commun.* **2013**, *4*, 2167-2173. (b) D.-A. Roşca, J. Fernandez-Cestau, J. Morris, J. A. Wright, M. Bochmann, *Sci. Adv.* **2015**, *1*:e1500761 (c) F. Rekhroukh, L. Estévez, C. Bijani, K. Miqueu, A. Amgoune, D. Bourissou, *Angew. Chem. Int. Ed.*, **2016**, *55*, 3414-3418.
- [45] M. Joost, A. Amgoune, D. Bourissou, *Angew. Chem. Int. Ed.*, **2015**, *54*, 15022-15045.
- [46] (a) C. Bronner, O. S. Wenger, *Dalton Trans.*, **2011**, *40*, 12409–12420. (b) C.-H. Lee, M.-C. Tang, Y.-C. Wong, M.-Y. Chan, V. W.-W. Yam, *J. Am. Chem. Soc.*, **2017**, *139*, 10539-10550. (c) W.-P. To, D.-L. Zhou, G. S. M. Tong, G. Cheng, C. Yang, C.-M. Che, *Angew. Chem. Int. Ed.*, **2017**, *56*, 14036 – 14041. (d) L. Currie, J. Fernandez-Cestau, L. Rocchigiani, B. Bertrand, S. J. Lancaster, D. L. Hughes, H. Duckworth, S. T. E. Jones, D. Credginton, T. J. Penfold, M. Bochmann, *Chem. Eur. J.* **2017**, *23*, 105-113.
- [47] R. V. Parish, B. P. Howe, J. P. Wright, J. Mack, R. G. Pritchard, R. G. Buckley, A. M. Elsome, S. P. Fricker, *Inorg. Chem.*, **1996**, *35*, 1659-1666.
- [48] R. G. Buckley, A. M. Elsome, S. P. Fricker, G. R. Henderson, B. R. C. Theobald, R. V. Parish, B. P. Howe, L. R. Kelland, *J. Med. Chem.*, **1996**, *39*, 5208-5214.
- [49] S.P. Fricker, R. M. Mosi, B. R. Cameron, I. Baird, Y. Zhu, V. Anastassov, J. Cox, P. S. Doyle, E. Hansell, G. Lau, J. Langille, M. Olsen, L. Qin, R. Skerlj, R. S. Y. Wong, Z. Santucci, J. H. McKerrow, J. *Inorg. Biochem.*, **2008**, *102*, 1839-1845.
- [50] Y. Zhu, B. R. Cameron, R. Mosi, V. Anastassov, J. Cox, L. Qin, Z. Santucci, M. Metz, R. T. Skerlj, S. P. Fricker, *J. Inorg. Biochem.*, **2011**, *105*, 754-762.

- [51] B. Bertrand, S. Spreckelmeyer, E. Bodio, F. Cocco, M. Picquet, P. Richard, P. Le Gendre, C. Orvig, M. A. Cinellu, A. Casini, *Dalton Trans.*, **2015**, *44*, 11911-11918.
- [52] M. N. Wenzel, S. M. Meier-Menches, T. L. Williams, E. Rämisch, G. Barone, A. Casini, *Chem. Commun.*, **2018**, *54*, 611-614.
- [53] S. Spreckelmeyer, N. Estrada-Ortiz, G. G. H. Prins, M. van der Zee, B. Gammelgaard, S. Stürup, I. A. M. de Graaf, G. M. M. Groothuis, A. Casini, *Metallomics*, **2017**, *9*, 1786-1795.
- [54] (a) D. Fan, C.-T. Yang, J. D. Ranford, P. F. Lee, J. J. Vittal, *Dalton Trans.*, **2003**, 2680-2685. (b) D. Fan, C.-T. Yang, J. D. Ranford, J. J. Vittal, P. F. Lee, *Dalton Trans.*, **2003**, 3376-3381.
- [55] J. J. Zhang, R. W. Y. Sun, C.-M. Che, *Chem. Commun.*, **2012**, *48*, 3388-3390.
- [56] R. Rubbiani, T. N. Zehnder, C. Mari, O. Blacque, K. Venkatesan, G. Gasser, *ChemMedChem*, **2014**, *9*, 2781-2790.
- [57] (a) N. Shaik, A. Martinez, I. Augustin, H. Giovinazzo, A. Varela-Ramirez, M. Sanau, R. J. Aguilera, M. Contel, *Inorg. Chem.*, **2009**, *48*, 1577-1587. (b) L. Vela, M. Contel, L. Palomera, G. Azaceta, I. Marzo, *J. Inorg. Biochem.*, **2011**, *105*, 1306-1313.
- [58] M. Frik, J. Fernández-Gallardo, O. Gonzalo, V. Mangas-Sanjuan, M. González-Alvarez, A. S. del Valle, C. Hu, I. González-Alvarez, M. Bermejo, I. Marzo, M. Contel, *J. Med. Chem.*, **2015**, *58*, 5825-5841.
- [59] G. Marcon, S. Carotti, M. Coronello, L. Messori, E. Mini, P. Orioli, T. Mazzei, M. A. Cinellu, G. Minghetti, *J. Med. Chem.*, **2002**, *45*, 1672-1677.
- [60] G. Marcon, L. Messori, P. Orioli, M. A. Cinellu, G. Minghetti, *Eur. J. Biochem.*, **2003**, *270*, 4655-4661.
- [61] C. Gabbiani, L. Massai, F. Scaletti, E. Michelucci, L. Maiore, M. A. Cinellu, L. Messori, *J. Biol. Inorg. Chem.*, **2012**, *17*, 1293-1302.
- [62] M. Coronello, E. Mini, B. Caciagli, M.A. Cinellu, A. Bindoli, C. Gabbiani, L. Messori, *J. Med. Chem.*, **2005**, *48*, 6761-6765.
- [63] S. M. Meir, C. Gerner, B. K. Keppler, M. A. Cinellu, A. Casini, *Inorg. Chem.*, **2016**, *55*, 4248-4259.



- [64] C. Gabbiani, F. Scaletti, L. Massai, E. Michelucci, M. A. Cinellu, L. Messori, *Chem. Commun.*, **2012**, 48, 11623-11625.
- [65] V. Petrović, S. Petrović, G. Joksić, J. Savić, M. Čolović, M. A. Cinellu, L. Massai, L. Messori, V. Vasić, *J. Inorg. Biochem.*, **2014**, 140, 228-235.
- [66] N. Micale, T. Schirmeister, R. Ettari, M. A. Cinellu, L. Maiore, M. Serratrice, C. Gabbiani, L. Massai, L. Messori, *J. Inorg. Biochem.*, **2014**, 141, 79-82.
- [67] T. Gamberi, L. Massai, F. Magheri, I. Landini, T. Fiaschi, F. Scaletti, C. Gabbiani, L. Bianchi, L. Bini, S. Nobili, G. Perrone, E. mini, L. Messori, A. Modesti, *J. Proteomics*, **2014**, 103, 103-120.
- [68] L. Messori, G. Marcon, M. A. Cinellu, M. Coronello, E. Mini, C. Gabbiani, P. Orioli, *Bioorg. Med. Chem.*, **2004**, 12, 6039-6043.
- [69] C. Gabbiani, G. Mastrobuoni, F. Sorrentino, B. Dani, M. P. Rigobello, A. Bindoli, M. A. Cinellu, G. Pieraccini, L. Messori, A. Casini, *Med. Chem. Commun.*, **2011**, 2, 50-54.
- [70] C. Gabbiani, A. Casini, G. Kelter, F. Cocco, M. A. Cinellu, H. H. Fiebig, L. Messori, *Metallomics*, **2011**, 3, 1318-1323.
- [71] P. Gratteri, L. Massai, E. Michelucci, R. Rigo, L. Messori, M. A. Cinellu, C. Musetti, C. Sissi, C. Bazzicalupi, *Dalton Trans.* **2015**, 44, 3633-3639.
- [72] K. L. Li, R. W. Y. Sun, S. C. F. Kui, N. Y. Zhu, C.-M. Che, *Chem. Eur. J.*, **2006**, 12, 5253-5266.
- [73] S. Jürgens, V. Scalcon, N. Estrada-Ortiz, A. Folda, F. Tonolo, C. Jandl, D. L. Browne, M. P. Rigobello, F. E. Kühn, A. Casini, *Bioorg. Med. Chem.*, **2017**, 25, 5452-5460.
- [74] R. W.-Y. Sun, C.-N. Lok, T. T.-H. Fong, C. K.-L. Li, Z. F. Yang, T. Zou, A. F.-M. Siu, C.-M. Che, *Chem. Sci.*, **2013**, 4, 1979-1988.
- [75] J. J. Yan, A. L.-F. Chow, C.-H. Leung, R. W.-Y. Sun, D.-L. Ma, C.-M. Che, *Chem. Commun.*, **2010**, 46, 3893-3895.
- [76] S. K. Fung, T. Zou, B. Cao, P.-Y. Lee, Y. M. E. Fung, D. Hu, C.-N. Lok, C.-M. Che, *Angew. Chem. Int. Ed.*, **2017**, 56, 3892-3896.
- [77] J. Fernandez-Cestau, B. Bertrand, M. Blaya, G. A. Jones, T. J. Penfold, M. Bochmann, *Chem. Commun.*, **2015**, 51, 16629-16632.

- [78] B. Bertrand, J. Fernandez-Cestau, J. Angulo, M. M. D. Cominetti, Z. A. E. Waller, M. Searcey, M. A. O'Connell, M. Bochmann, *Inorg. Chem.*, **2017**, *56*, 5728-5740.
- [79] (a) M. Wenzel, B. Bertrand, M.-J. Eymin, V. Comte, J. A. Harvey, P. Richard, M. Groessler, O. Zava, H. Amrouche, P. D. Harvey, P. Le Gendre, M. Picquet, A. Casini, *Inorg. Chem.*, **2011**, *50*, 9472–9480. (b) J. Fernández-Gallardo, B. T. Elie, T. Sadhukha, S. Prabha, M. Sanaffl, S. A. Rotenberg, J. W. Ramos, M. Contel, *Chem. Sci.*, **2015**, *6*, 5269–5283.
- [80] (a) B. Bertrand, P.-E. Doulain, C. Goze, E. Bodio, *Dalton Trans.*, **2016**, *45*, 13005-13011. (b) V. Fernández-Moreira, M. C. Gimeno, *Chem. Eur. J.* **2018**, DOI: 10.1002/chem.201705335.
- [81] (a) B. Bertrand, E. Bodio, P. Richard, M. Picquet, P. Le Gendre, A. Casini, *J. Organomet. Chem.*, **2015**, *775*, 124-129. (b) B. Bertrand, A. Citta, I. L. Franken, M. Picquet, A. Folda, V. Scalcon, M. P. Rigobello, P. Le Gendre, A. Casini, E. Bodio, *J. Biol. Inorg. Chem.*, **2015**, *20*, 1005-1020.
- [82] B. Bertrand, M. A. O'Connell, Z. A. E. Waller, M. Bochmann, *Chem. Eur. J.*, **2018**, DOI: 10.1002/chem.201705902.
- [83] B. Bertrand, L. Stefan, M. Pirrotta, D. Monchaud, E. Bodio, P. Richard, P. Le Gendre, E. Warmerdam, M. H. de Jager, G. M. Groothuis, M. Picquet, A. Casini, *Inorg. Chem.*, **2014**, *53*, 2296-2303.
- [84] (a) B. Albada, N. Metzler-Nolte, *Chem. Rev.*, **2016**, *116*, 11797– 11839. (b) M. Patra, T. C. Johnstone, K. Suntharalingam, S. J. Lippard, *Angew. Chem. Int. Ed.*, **2016**, *55*, 2550–2554. (c) G. Casi, D. Neri, *J. Med. Chem.*, **2015**, *58*, 875–8761. (d) W. Niu, X. Chen, W. Tan, A. S. Veige, *Angew. Chem. Int. Ed.*, **2016**, *55*, 8889–8893.
- [85] M. Williams, A. I. Green, J. Fernandez-Cestau, D. L. Hughes, M. A. O'Connell, M. Searcey, B. Bertrand, M. Bochmann, *Dalton Trans.*, **2017**, *46*, 13397-13408.
- [86] J. L.-L. Tsai, A. O.-Y. Chana C.-M. Che, *Chem. Commun.*, **2015**, *51*, 8547-8550.