Mechanistic Insights into Gold Organometallic Compounds and their Biomedical Applications

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Abstract: The application of gold in medicine can be traced back several thousand years and Au(I) compounds have been used in the treatment of rheumatoid arthritis since the last century. Recently research into gold-based drugs for a number of human diseases has seen a renaissance due to their markedly different modes of action with respect to the classical platinum chemotherapeutic compounds. Within this research area, organometallic gold complexes have been particularly explored, mainly due to their higher stability in physiological conditions guaranteed by the presence of a direct Au–C bond. Thus, a number of compounds have been tested for their uses as anticancer, antibacterial, antiprotozoal as well as anti-HIV agents. In this review a selection of the main results obtained on the synthesis, chemical properties and biological activities of two of the most explored families of organometallics – Au(I) N-heterocyclic carbenes (NHCs) and cyclometalated Au(III) compounds – are summarized. Their structure–activity relationships and modes of action at the cellular level are also discussed, which constitute the basis for future drug design.

Keywords: Biomedical applications · Gold organometallic compounds

1. Introduction

The discovery of the bacteriostatic properties of gold cyanide, K[Au(CN)$_2$], by Robert Koch in 1890 against tubercle bacillus was the beginning of the use of gold-based compounds in modern medicine. In the belief that rheumatoid arthritis was an atypical form of tuberculosis, the use of gold(I) salts was also proposed for the treatment of this disease. By the early 1930s, gold therapy (*chrysotherapy*) was discontinued as a treatment of tuberculosis based on its ineffectiveness; however, it is still widely used for the management of rheumatoid arthritis.

In recent years, gold compounds have found clinical relevance as anti-rheumatic drugs in the form of auranofin ([triethylphosphine(2,3,4,6-tetra-O-acetyl-β-1-D-(thiopyranosato-S)Au(I)]), Ridaura™), aurothioglucose (Solganol™) and aurothiomalate (Myochrisine™ or Aurolate™) (Fig. 1). The success of the platinum(II) complex cisplatin and derivatives in cancer chemotherapy stimulated interest in development of gold-based cytotoxic agents which has yielded extensive research into this field. Thus, the established antiarthritic drug auranofin was recently repurposed for its anticancer properties and is currently undergoing evaluation in three distinct US clinical trials.[1] To a lesser extent, gold compounds have also been evaluated as antimicrobial,[2] anti-malarial[3] and anti-HIV agents.[4]

Concerning Au(I) compounds, auranofin was initially found to be cytotoxic towards tumor cells *in vitro*, leading to the identification of other Au(I) phosphines with marked antitumor activity.[5] Among the various examples, bis-chelated Au(I) diphosphine complexes of the type [AuI(dppe)$_2$]Cl (dppe = bis(diphenylphosphino)ethane) were patented for therapeutic uses by Berners-Price *et al.* in 1986.[6] Despite their relatively good stability in physiological conditions, it was soon clear that more efforts should have been spent to modulate the reactivity of these Au(I) complexes for biomedical applications, including their selectivity to cancer cells.

Au(III) compounds as anticancer agents appeared to be 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, numerous studies have shown that anticancer gold compounds target other biomolecules and biological pathways with respect to Pt(II) complexes.[7] As Au(III) is easily reduced to more stable Au(I)/Au(0) species, Au(III) complexes are generally strongly oxidizing agents. Reductions can be easily driven by thiol groups of biological systems, making Au(III) potentially toxic.[8] Therefore, Au(III) complexes were long believed not to be suitable for medicinal applications.

In this context, organometallic chemistry offers a convenient way for the stabilization of the Au(I)/(III) center and 'fine-tuning' of the complexes' physiochemical properties *via* the establishment of a direct metal–carbon bond. Thus, different families of
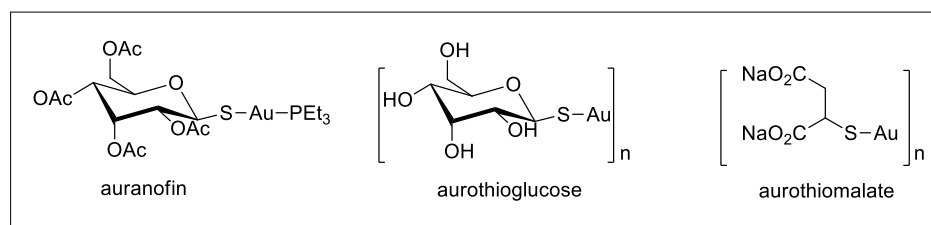

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Fig. 1. Structures of Au(I) anti-rheumatic drugs.

organometallic gold complexes, including N-heterocyclic carbenes (NHCs) and cyclometallated compounds, have been explored for biological applications. Moreover, also alkynyl gold(I) complexes were designed and tested for their anti-cancer properties, although so far they are much less represented.[9] Very recently, Laguna and co-workers reported on the synthesis of complex [Au(C≡CCH$_2$Spyridine)(PTA)] (PTA = 1,3,5-triaza-7-phosphaadamantane) which was tested for its anticancer activity *in vitro* and *in vivo*.[10] When this derivative is administered by intraperitoneal injection in athymic nude mice inoculated with HTC-116-luc2 (colon cancer) cells, it prolongs their survival and displays moderate inhibition of the tumor growth with no subsequent organ damage after treatment.

Metal–NHC compounds have been widely used for catalysis due to their strong σ-donor properties, which, together with the strength of the metal–carbon bond, make such compounds resistant towards oxidation and endow them with overall high chemical and thermal stability. Some of these properties made the research on the biological potential of metal–NHC complexes one of the most active areas within the field of bioorganometallic chemistry. In the past few years, over 100 reports and more than 350 new transition metal complexes containing NHC ligands as potential antitumor metallodrugs could be counted.[11]

Concerning cyclometalated complexes, numerous ligand systems utilizing different donor atoms have been described that were able to stabilize the Au(III) center.[12] Cyclometalation reactions producing five-membered ring products are very favorable, being performed at room temperature with a variety of substrates, such as amines, imines, 2-phenylpyridines, benzo[*h*]quinones, other nitrogen donor ligands, oxygen-containing compounds, phosphorus and sulfur donors *etc*. Thus, a consistent number of reports includes these families of complexes.[13]

Here, we will include representative examples of the above-mentioned families of NHC and cyclometalated gold complexes, focusing on those displaying most promising biological effects including as anticancer agents, as well as antibacterial and antiviral compounds. We refer the reader to more extensive reviews for deeper insights into the field.[11,14]

2. Gold(I) N-Heterocyclic Carbene Complexes

N-heterocyclic carbenes (NHCs) are defined as singlet-carbenes containing a divalent carbenic carbon with at least one nitrogen atom in the heterocycle.[13]

The development of stable metal NHC complexes has attracted increasing attention in research of metallopharmaceuticals. NHCs originated from the deprotonation of 1,3 di-substituted imidazolium salts, by appropriate external base, produce metal NHC complexes, stabilized through a 'push-pull' mechanism *via* synthetic routes with transmetalation being one of the most convenient, overcoming the difficulties of unstable free heterocyclic carbene isolation. Thus, the use of silver carbene complexes as carbene transfer reagents for the synthesis of other metal NHC complexes, including Au(I) NHCs, is very common.

Below, we will provide an overview of the most representative Au(I) NHC scaffolds for different therapeutic applications and will summarize the knowledge on their respective proposed modes of action. Since their discovery as antiproliferative agents, various experiments on cancer cells revealed a variety of effects of Au(I) NHCs on cellular metabolism, including strong increase of ROS formation and reduced mitochondrial activity, finally resulting in apoptotic cell death. Notably, most of these effects could be attributed to the strong and selective inhibition of the seleno-enzyme thioredoxin reductase (TrxR) by Au(I) NHC complexes.[15]

Thioredoxin reductases (EC 1.8.1.9) are homodimeric flavoproteins catalyzing the NADPH-dependent reduction of thioredoxin (Trx), which is the major protein disulfide reductase in cells.[16] TrxRs belong to the pyridine nucleotide-disulfide oxidoreductase family such as glutathione reductase, lipoamide dehydrogenase and trypanothione reductase. Thus, they form homodimers with each subunit featuring a redox-active disulfide bond and a bound FAD molecule.

Mammalian TrxRs possess a conserved -Cys-Val-Asn-Val-Gly-Cys- catalytic site, which is also found in human glutathione reductase (GR), located in the FAD-binding domain of the enzyme, and a NADPH binding site. Moreover, they contain a selenocysteine residue at the C-terminal active site that is crucial for catalysis[17] and that distinguishes them from GR or *E. coli* TrxR.[18] The redox active site is located on a flexible arm, solvent-exposed and reactive towards electrophilic agents,[19] constituting an ideal target for the development of selective enzyme inhibitors, also based on gold, preferentially binding selenol groups.[20] The thioredoxin system regulates crucial cell functions such as viability and proliferation.[21] Interestingly, TrxR is able to reduce different protein substrates and low molecular weight molecules, in addition to the natural substrate thioredoxin. Both TrxR and Trx appear overexpressed in certain cancer types,[22] leading to the assumption that the

thioredoxin system exerts a crucial role in tumor onset and progression. In fact, both enzymes are considered as likely 'druggable' targets for anticancer treatment.[23]

2.1 *Anticancer Agents*

Due to the presence of NHC ligands, gold(I) NHC complexes show high stability in physiological conditions and ease of derivatization, allowing adjustment of their physicochemical properties. A first study by Berners-Price *et al.* appeared in 2006, where the synthesis, characterization and antimitochondrial activity of a family of cationic, linear two-coordinate [(R$_2$Im)$_2$Au]$^+$ (Im = dialkylimidazol-2-ylidene) complexes of monodentate dialkylimidazol-2-ylidene ligands were reported, where the compounds' lipophilicity was fine-tuned by variation of the substituents (R) on the imidazolium salt precursor.[24] The results show correlation between the hydrophilic/lipophilic properties of the complexes and their ability to cause mitochondrial membrane permeabilization in isolated rat liver mitochondria.

The [(R$_2$Im)$_2$Au]$^+$ complexes were synthesized by reacting (Me$_2$S)AuCl with excess of the appropriate imidazol-2-ylidene, prepared *in situ* by deprotonation of the imidazolium salt with lithium bis(trimethylsilyl)amide (LiHMDS) in DMF at room temperature (Scheme 1). Of note, to influence the lipophilicity of the resulting Au-NHC complexes, the alkyl groups of the imidazolium salt were systematically varied.

Compounds **1·Br** and **3–6·Cl** induced dose-dependent (μM range), Ca^{2+}-sensitive mitochondrial swelling, with the most lipophilic compounds being the most active. Cyclosporin A, the benchmark inhibitor of the mitochondrial permeability transition pore, completely quenched the swelling induced by the gold complexes.

More recently, Ott and co-workers reported the Au(I) complex **7** (Fig. 2), based on an aminotriazole NHC ligand, *via* the Ag(I) oxide route.[25] Interestingly, the complex triggered cytotoxic effects in HT-29 and MDA-MB-231 cancer cells, and showed an effective and fast cellular accumulation. As other bis-NHCs, the compound showed only moderate inhibition of TrxR (IC$_{50}$ = 1.2 μM), in line with the idea that ligand exchange reactions at the Au(I) center should occur to allow direct binding to the protein's active site.

Gandin, Marzano and co-workers also developed the Au(I) 1,2,4-triazole based NHC complex **8** (Fig. 2) and assessed its cytotoxic properties in various human cancer cell lines, including cisplatin-sensitive and -resistant cells.[26] This mono-NHC Au(I) complex was less effective than cisplatin and the corresponding Ag(I)-
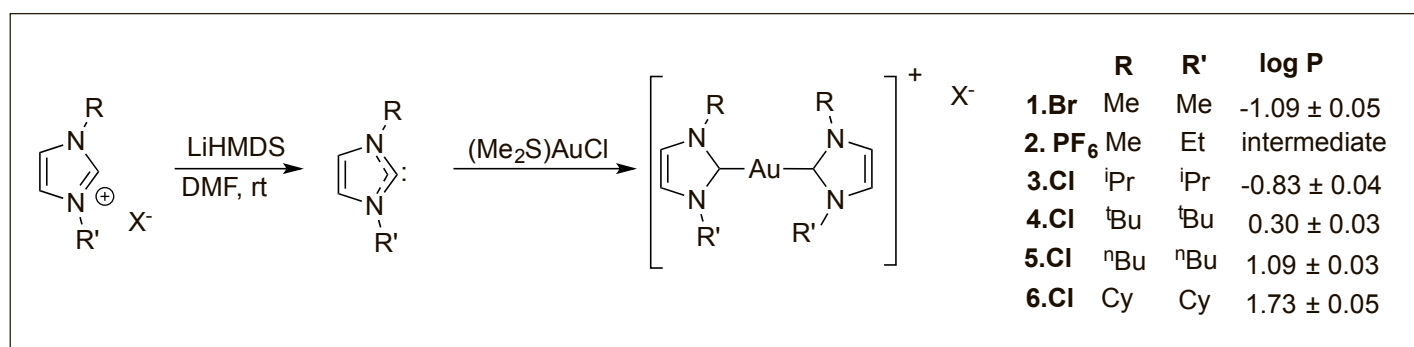
Scheme 1. Synthesis of Au(I) NHC complexes as reported in ref [24].

NHC and Cu(I)-NHC complexes against all tested cancer cell lines. In agreement with previous findings, TrxR was potently inhibited by **8**, with IC_{50} values in the nM range after 15 min of incubation.

Gold NHC complexes have recently been explored for their interactions with non-canonical nucleic acid structures. Within this framework, Casini *et al.* reported on a series of caffeine-based Au(I)-NHC complexes (**9a–h**, Fig. 2) bearing the methylated caffeine-2-ylidene ligand. The N7-substituted theophylline analogues have been synthesized and tested for antiproliferative activities in different cancerous and non-tumorigenic cell lines in comparison to 1,3-dimethylbenzimi dazol-2-ylidene derivatives.[26] Complex **9b** was almost two-fold more potent than cisplatin against A2780/R cells with low toxicity in non-cancerous HEK cells ($IC_{50} > 100$ μM). Notably, the compound was revealed to be scarcely toxic, also in healthy tissues *ex vivo*. Interestingly, further mechanistic studies showed that the highly selective antiproliferative properties of **9b** may be due to efficient and selective DNA G-quadruplex-binding. Of note, molecules that template the formation or stabilize the structure of G-quadruplex DNA (G4) might lead to development of new effective anticancer drugs based on selective telomerase inhibition. Concerning other possible target proteins, the reported studies showed that **9b** is only a modest PARP-1 inhibitor (*i.e.* a poor DDR impairing agent).[27]

Further X-ray diffraction studies on the adduct of the bis-carbene cationic complex [Au(9-methylcaffein-8-ylidene)₂]⁺ **9b** with a model of DNA G-quadruplex (G4, Tel 23 sequence (5'-TAGGG(TTAGGG)3-3')) showed that the compound is able to bind the G4 at two distinct sites. In detail, the gold compound showed possible binding at the 3'-3' (3'end-3'end) site, as well as at the 5'-5' (5'end-5'end) site.[28] The two binding domains, located in the 3'-3' and 5'-5' sites, host either one or two [Au(9-methylcaffein-8-ylidene)₂]⁺ molecules, respectively (Fig. 3). It is worth noting that this bis-carbene Au(I) complex is an ideal G4 stabilizer being: *i)*

planar, *ii)* prone to electrostatic interactions with negatively charged DNA due to its overall positive charge, and *iii)* having two caffeine ligands (guanine analogues) able to associate to guanine moieties in G4 *via* π-stacking interactions.

Following the success of the trinuclear Pt(II) compound BBR3464 in clinical trials,[29] the concept of *multinuclearity* has also been envisaged with Au(I) and Au(III)-based organometallic compounds. In most cases, the polynuclear compounds
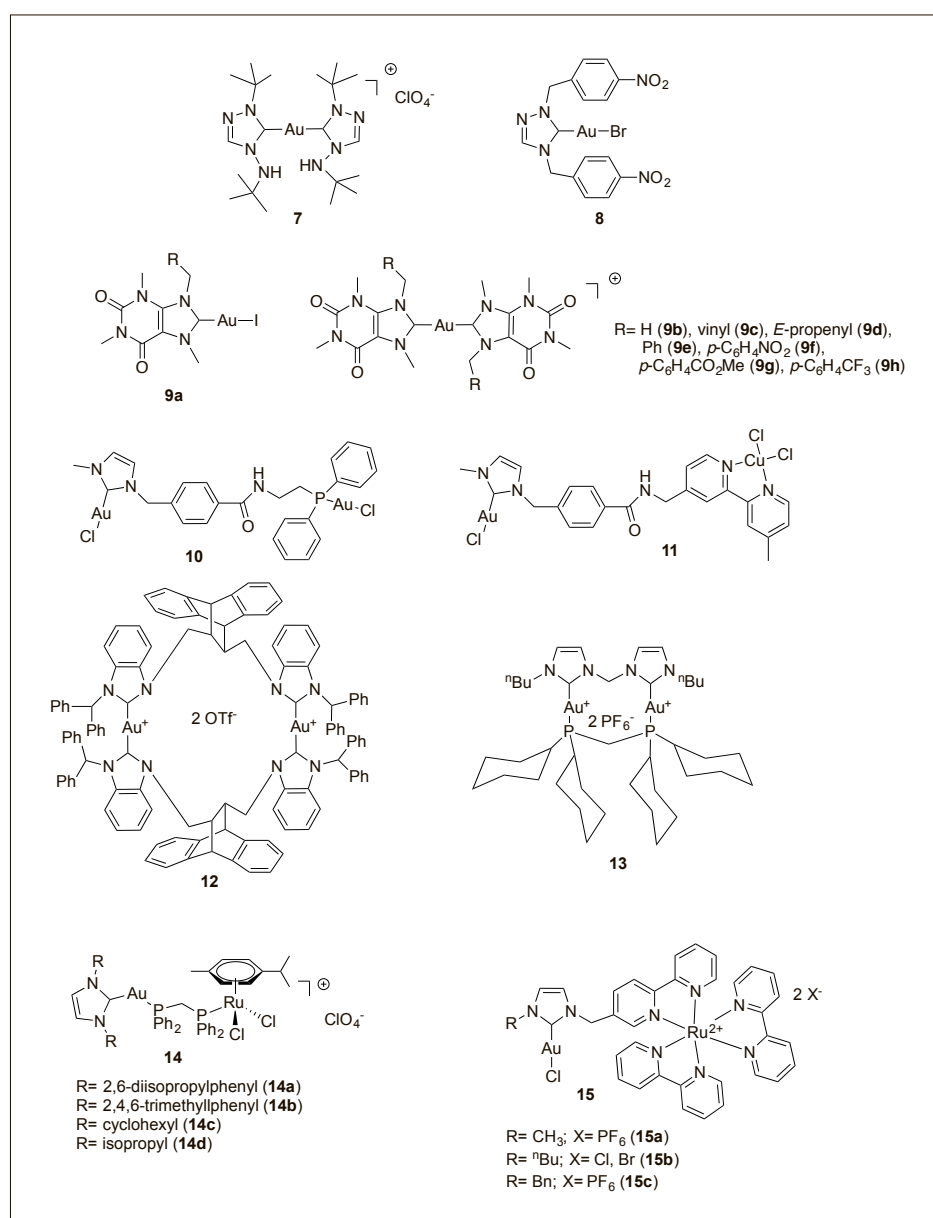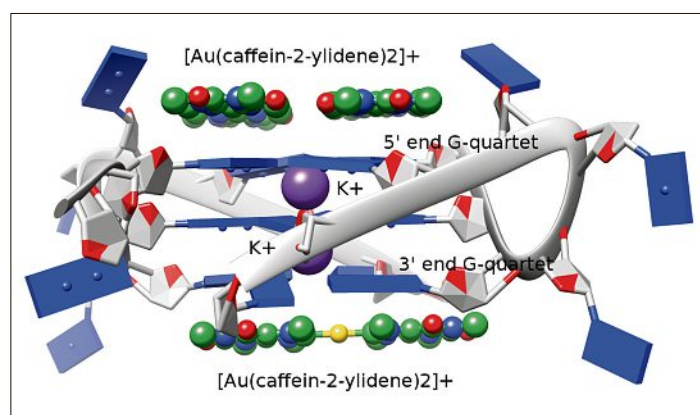

Fig. 2. Structure of anticancer Au(I) NHC complexes.

Fig. 3. Propeller organization of the Tel 23 sequence in the Tel 23: [Au(9-methylcaffein-8-ylidene)₂]⁺ adduct[28] (PDB accession code 5CCW).

were shown to be potent inhibitors of cancer cell proliferation with a significant improvement compared with their mono-nuclear parent complexes.

As an example, Le Gendre and co-workers recently prepared and structurally characterized five neutral or cationic Au(I)-NHC complexes *via* different methods.[30] Interestingly, some of them bear a penta-fluorophenolic ester group as a possible 'activable' moiety for further functional-ization. This group allowed an alkyl amine ligand or other Au(I)-phosphine complex (**10**, Fig. 2) to be tethered *via* microwave activation. The obtained compounds have shown promising antiproliferative effects in human ovarian cancer A2780 cells, with respect to non-tumorigenic ones (HEK-293T). In addition, the same authors also synthesized some homo- and hetero-bi-metallic complexes (Au(I)/Au(I) or Au(I)/Cu(II), Au(I)/Ru(II), respectively) based on the Au(I)–NHC scaffold carrying a pentaf-luorophenol ester moiety and another Au(I) phosphane complex or a bipyridine ligand bearing a pendant amine function (*e.g.* **11**, Fig. 2).[31] These complexes showed mod-erate anti-cancer properties against A2780, SKOV-3 and A549 cancer cell lines, with the Au-Cu complex **11** being among the most active derivatives. Some compounds in this series were potent TrxR inhibitors in the nanomolar range *in vitro* and appeared to be toxic against healthy liver tissue at concentrations in the low micromolar range *ex vivo*.

Due to the high activity of the Au(I)–bisNHC complexes and the fact that no chiral Au(I)–NHC complexes had been tested, Veige, Tan and co-workers developed the eighteen-membered chiral macrocyclic dicarbene-di-Au complex [(μ-diNHC)Au(I)]₂[OTF]₂ **12** (Fig. 2).[32] Interestingly, the enantiopure isomers of the ligand precursor provide access to the enantiopure versions of the binuclear Au(I) metallamacrocycles. Both isomers of **12** were characterized *via* different methods and displayed moderately cytotoxic to both healthy and cancerous lines without

marked selectivity. Moreover, **12** is capable of penetrating the cell membrane already after few hours incubation, as shown by confocal microscopy, and causes cell death *via* apoptosis, as evidenced by DNA gel electrophoresis.[32]

In 2014, Che *et al.* developed a binuclear Au(I) complex (**13**, Fig. 2) containing mixed diphosphine and bis-(NHC) bridging ligands.[33] The com-pound showed a favorable stability that guarantees efficient TrxR inhibition, while preventing inactivation by blood thiols. In addition, **13** demonstrated *in vitro* cytotoxic activity towards a panel of human cancer cells, including HeLa, MCF-7 (breast adenocarcinoma), SUNE1 (nasopharyngeal carcinoma), H1975 (lung adenocarcinoma), and B16F10 (mouse melanoma) cells, with IC₅₀ values up to 13.5-fold lower than those of cisplatin. Complex **13** also inhibited HeLa cell sphere formation *in vitro* which is an indication of the cytotoxic effects of the compound in cancer stem cell populations, and inhibited angiogenesis in tumor models *in vivo* (reduction of blood microvessels in the tumor tissue of mice bearing HeLa xenografts).[33] Besides, the complex significantly reduced tumor growth *in vivo,* in mice bearing HeLa xenograft as well as highly aggressive mouse B16F10 melanoma without causing mouse death or body weight loss. Furthermore, it did not show systemic anaphylaxis on guinea pigs and did not induce localized irritation on rabbits in toxicology studies.

Interestingly, within the scope of achieving heteronuclear cytotoxic organo-metallics, in 2016 Contel *et al.* described a simple and efficient synthetic method to obtain Au(I)–NHCs featuring a second ancillary ligand (such as phosphanes, phosphites, arsines and amines) starting from the chlorido precursor by abstraction of the chloride with silver perchlorate, and subsequent addition of the ancillary ligand.[34] This synthetic strategy has been successfully applied in the preparation of a family of cationic heterobimetallic Au(I)–

Ru(II) complexes (**14a–d**, Fig. 2). Of note, the new heterometallic compounds are soluble in DMSO/H₂O, DMSO/PBS or DMSO/medium (1:99) mixtures at micromolar concentrations, relevant for biological testing. The bimetallic com-plexes showed a synergistic effect and exhibited higher cytotoxicities than their monometallic counterparts against renal cancer Caki-1 and colon cancer HCT-116 cell lines. Importantly, **14a–d** are considerably less toxic to the non-tumorigenic human embryonic kidney cell line (HEK-293T) than cisplatin. In addition, no interaction between the heterobimetallic complexes and plasmid (pBR322) DNA was observed and complex **14a** was more efficient than the monometallic complex for the inhibition of TrxR in cancer cell extracts.[34]

Gornitzka and co-workers reported on the synthesis of three Au(I)–Ru(II) heterobimetallic complexes (**15a–c**, Fig. 2) combining an Au(I)–NHC unit and a Ru(bipy)₃ building block.[35] The compounds have been fully characterized and evaluated for *in vitro* cytotoxic, antileishmanial, and antimalarial activi-ties. The complexes were designed con-sidering the photophysical properties of the Ru polypyridyl moiety, and the known anticancer activities of both metallic units. Interestingly, even though all the complexes show characteristics very similar to those of Ru(bipy)₃ concerning luminescence properties, it should be noted that the luminescence quantum yield in water increases by about 30% in going from the mono-ruthenium complex (φ_{em} = 0.020) to the corresponding Au(I)–Ru(II) complex **15b** (φ_{em} = 0.026[35]). Moreover, the emission maximum of the ruthenium precursor (630 nm) is slightly red-shifted in comparison with that of **15b** (615 nm). This shift could be due to solvatochromic effects, as a charged imidazolium unit in the mononuclear Ru(II) complex is changed to a neutral carbene unit in the Au(I)–Ru(II) derivative. Furthermore, an Au(I)-chlorido moiety is coordinated by the carbene, also leading to strong changes in the molecule.

Complexes **15a–c** showed moderate anticancer properties very similar to those of Ru(bipy)₃ in human hepatocellular carcinoma (Hep3B) cells, as well as modest activities (IC₅₀ >10 μM) against *P. falciparum* FcB1-Colombia strain.[35] Further biological studies showed that the cytotoxic effects in tumor cells probably did not result *via* a DNA-related or an antimitochondrial mechanism. Moreover, the difference of 20 nm between the emission spectra of the Au(I)–Ru(II) and Ru(II) complexes allowed their uptake in cancer cells to be followed *via* fluorescence microscopy. The obtained results suggested that Au(I)–Ru(II) complexes are slowly

transformed into the corresponding Ru complexes by release of Au cations to the cells, leading to cell death.

As a most recent example of heteronuclear Au(I)–Fe(II) derivatives, four Au(I)–NHC complexes featuring 4-ferrocenyl-substituted imidazol-2-ylidene ligands were investigated for antiproliferative and antivascular properties.[36] The compounds were active against a panel of seven cancer cell lines, including multidrug-resistant ones, with low micromolar or nanomolar IC$_{50}$ (72 h) values, according to their lipophilicity and cellular uptake. Within this series, the two delocalized lipophilic cationic mono- and biscarbene complexes acted by increasing the reactive oxygen species in two ways: *via* a genuine ferrocene effect and by inhibiting the thioredoxin reductase.[36] Both complexes gave rise to a reorganization of the F-actin cytoskeleton in endothelial and melanoma cells, associated with a G1 phase cell cycle arrest and a retarded cell migration. They proved antiangiogenic in tube formation assays with endothelial cells and vascular-disruptive on real blood vessels in the chorioallantoic membrane of chicken eggs.[36] The cationic biscarbene complex was also tolerated well by mice where it led to a volume reduction of xenograft tumors (highly metastatic B16-F10 mouse melanoma cells) by up to 80%.

2.2 Antibacterial Agents

Concerning possible applications as antibacterial agents, the Youngs research group was the first to introduce NHCs as carrier ligands for Ag$^+$ in 2004 for antibacterial applications. However, over the years, much less attention has been paid to the development of other metal–NHC complexes as antibacterial agents in comparison to silver complexes.[37] In the same year, Cetinkaya *et al.* studied the antibacterial activity of six cationic Au(I) NHC complexes,[38] which included compounds **16**, **17** and **18** (Fig. 4), displaying promising antibacterial activity *in vitro* against a series of bacterial strains (minimum inhibitory concentrations, MIC, in the range 3.1–6.3 µg mL^{-1}). Remarkably,

the compounds' activity appeared to be strongly determined by the type of functional groups bound to the carrier ligands.

Afterwards, Ghosh *et al.* synthesized the compound [[1-benzyl-3-tert-butylimidazol-2-ylidene]AuCl] (**20**, Fig. 4) which inhibits the growth of Gram positive *B. subtilis* at a MIC of 15 ± 2.3 µM.[39] Interestingly, **20** is *ca.* two times more potent than its Ag(I) analogue **19** (Fig. 4). To unravel the mechanism of antimicrobial activity of **20**, the morphology of the *Bac. subtilis* cells was studied following 4 h incubation with 4 µM of **20**. The elongation of the *Bac. subtilis* cells grown in the presence of **20** was observed compared with the control cells, suggesting that the compound inhibits bacterial proliferation by blocking the cytokinesis step of cell division. More recently, other Au(I) NHC complexes with different N-substituents on the benzimidazole and bis(iminoacenaphthene) scaffolds showed only weak antibacterial activity.[40]

In 2012, Dinda *et al.* reported on the antibacterial activity of pyrazine functionalized pincer NHC–Ag(I) and NHC–Au(I) complexes against antibiotic-resistant human pathogens of both Gram-positive and Gram-negative bacteria, including their inhibitory effects on biofilm formation.[41] In this study, complete synthetic and structural studies of Au(I) and Ag(I) complexes of 2-(1-methylimidazolium)pyrimidinechloride, 2,6-bis(1-methylimidazol)pyrazinechloride and 2,6-bis(1-methyl imidazol)pyrazinehexafluorophosphate were reported. Among them, the [chloro[2,6-bis(1-methylimidazol)pyrazine]Ag(I)], **21** and [chloro[2,6-bis(1-methylimidazol)pyrazine]Au(I)], **22** complexes (Fig. 5) were observed to possess potent antimicrobial activity compared to several conventionally used antibiotics.[41] Biofilm formation by Gram-positive bacteria (*Streptococcus mutans*) and Gram-negative bacteria (*E. coli*) was also inhibited by **21** and **22** causing drastic damage to the bacterial cell wall and increasing membrane permeability. In addition, both compounds strongly bind to both Lys and Dap-Type peptidoglycan
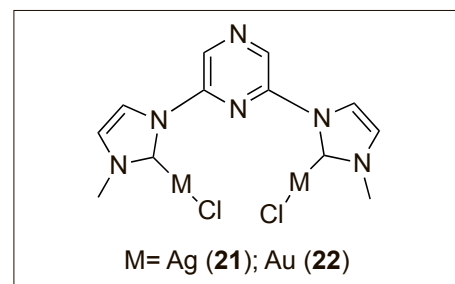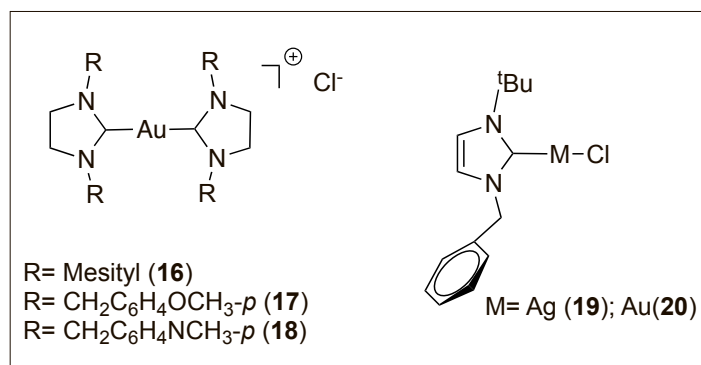

M= Ag (**21**); Au (**22**)

Fig. 5. Structures of dinuclear Au(I) and Ag(I) NHC complexes active as antibacterial agents.

layers, most likely inducing the observed damage of the bacterial cell wall.

Very recently, Ott and co-workers explored a new series of halogenated mono-NHC Au(I) complexes of general formula NHC-Au(I)-Cl.[42] The compounds showed promising activities in cancer cells, and the effective inhibition of mammalian and bacterial thioredoxin reductases was confirmed for all the reported derivatives. Interestingly, the determination of antibacterial effects against a panel of Gram-negative and Gram-positive strains demonstrated that all gold complexes displayed a high activity (low micromolar range) towards the Gram-positive bacteria, but they were substantially less active against the Gram-negative ones.[42] Such a clear and consistent selectivity for Gram-positive strains has not been reported for previously investigated gold–NHC complexes. Whereas some complexes of the type NHC-Au(I)-Cl had indeed triggered much stronger effects in Gram-positive bacteria,[40a] this was not the case for other structural derivatives like cationic biscarbene derivatives.[38b]

2.3 Antimalarial Agents

Malaria is an infectious disease caused by protozoan parasites of the genus *Plasmodium* and is still a major cause of illness and death in tropical countries.[43] Among the species that cause malaria (*P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi*) *P. falciparum* causes the most serious form of the disease. Malaria parasites are transmitted by the bite of an infected female mosquito of the Anopheles genus. Although numerous advances have been made in the treatment of this disease, about 3.2 billion people – nearly half the world's population – remain at risk of malaria. In 2015 alone, there were 214 million new cases of the disease and more than 400,000 malaria-related deaths.[44] Unfortunately, millions of people are still not accessing the health services necessary to prevent and treat this widespread disease.

In the treatment of malaria, several organic compounds have been successfully applied. As an example chloroquine (CQ)

Fig. 4. Structures of potent Au(I) and Ag(I) NHC complexes.

R= Mesityl (**16**)
R= CH$_2$C$_6$H$_4$OCH$_3$-*p* (**17**)
R= CH$_2$C$_6$H$_4$NCH$_3$-*p* (**18**)

M= Ag (**19**); Au(**20**)

is among the most widely used antimalarial drug. However, due to the emergence and spread of CQ-resistant *Plasmodium* parasites, the efficacy of CQ has greatly diminished. In this context, to obtain improved drugs able to overcome CQ resistance, numerous metal complexes, including gold(I) and gold(III) compounds, have been designed as potential antimalarial agents.[43,45]

Among the organometallics with antimalarial properties, the Au(I) complex featuring the ylideneamine functionalized heterocyclic ligand – [Au(NO₃)(1,3-ᵗBuIm-2-ylidene)] – was found to be much less active than CQ against the 3D7 *P. Falciparum* strain.[46] Similarly, among the series of dinuclear Au(I) complexes containing bis-NHC ligands, only complex **23** (Fig. 6) manifested moderate antiplasmodial activity (IC$_{50}$ *ca.* 15 μM) against the CQ-resistant *P. falciparum* strain FcM29-Cameroon, but was considerably less effective than CQ (IC$_{50}$ = 0.445 μM[47]). Other bis-carbene gold(I) complexes in this series showed no activity (IC$_{50}$ > 39 μM). Conversely, mononuclear gold(I) complexes containing nitrogen heterocyclic functionalized arms, namely quinoline (**24**) and bipyridine (**25**) moieties (Fig. 6) displayed significantly enhanced antiplasmodial activity (IC$_{50}$ values of 1.1 and 0.33 μM, respectively).[47]

Interestingly, in the same study, two gold(III) complexes **26** and **27** with coordinated bis-NHC ligands (Fig. 6) showed only moderate activity against *P. falciparum* strain FcM29 (IC$_{50}$ values of 9 and 13 μM, respectively).[47]

3. Cyclometalated Gold(III) 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 (D = donor atom). These ligands incorporate one or more donor atoms (such as O-, N-, P-, S- or Se-). However, it is worth mentioning that a variety of reaction pathways other than cyclometalation has been optimized to achieve 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.

Fig. 7 illustrates a general representation of the different classes of cyclometalated Au(III) compounds.

In the following sections, C^N, C^N^N and C^N^C cyclometalated Au(III) complexes will be discussed, and several studies report on their stability in physiological environment and suitable chemicophysical properties for therapeutic applications. We have decided to organize this section according to the different families, and within each section we will then summarize and highlight the compounds' biological properties and possible applications, mainly as anticancer agents.

3.1 C^N Complexes
The class of Au(III) C^N complexes with cytotoxic properties was first introduced by Parish *et al.* in 1996,[48] when a series of Au(III) complexes of the structure [(damp)AuX$_2$] with a 2[dimethylamino)methyl]-phenyl (damp) backbone (**28**) was synthesized and characterized (Fig. 8). The compounds displayed cytotoxic activities comparable to cisplatin against various cancer cell lines, 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*. Interestingly, while only minor interactions of the compounds with DNA could be observed, both complexes led to submicromolar inhibition of cysteine protease cathepsin B[49] as well as inhibition of TrxR.[50]

In 2005, Messori *et al.* reported on the synthesis of complex **29** featuring a dimethybenzyl-pyridine backbone (Fig. 8).[51] This complex proved to be stable under intercellular reducing conditions; although the cytotoxic activity against ovarian cancer cell line A2780 is comparable to cisplatin, complex **29** displays significant cross-resistance, suggesting a different mechanism of action. It was demonstrated that **29** selectively inhibits TrxR activity, which is hypothesized to be caused by binding to (seleno)cysteine residues in the active site of TrxR.[52] Further studies showed that the hydrolysis product of **29** is able to disrupt mitochondrial function and alter the glycolytic pathway in A2780 cancer cells, leading to apoptosis.[53]

The cationic complex **30**, bearing a bisguanide moiety, was synthesized by Che *et al.* in 2012 (Fig. 8).[54] By introducing the polar bisguanide ligand, a water-soluble complex was obtained that can form an 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 **30** is likely to be caused by swelling of the endoplasmic reticulum (ER), as suggested
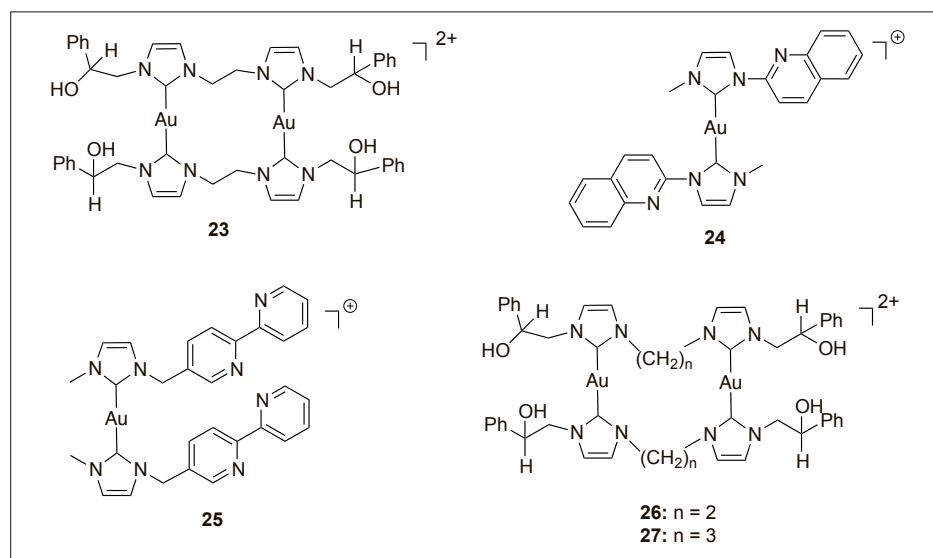

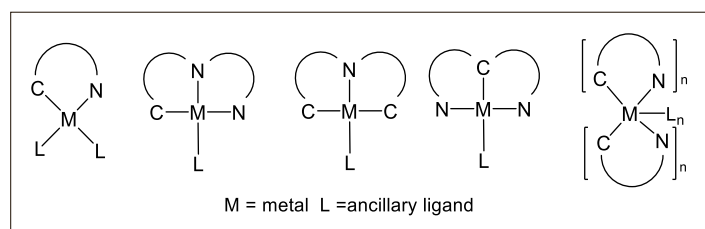Fig. 6. Antimalarial Au(I) and Au(III) complexes with N-heterocyclic carbenes.

Fig. 7. General representation of cyclometalated complexes.

M = metal L =ancillary ligand

by oligonucleotide microarray analysis and western blot assays. Irreversible ER stress triggers apoptosis, with the activation of the canonical mitochondrial cell death pathway playing an essential role.[55] A structural analogue (**31**) was reported by the same group, also utilizing a dimethybenzyl-pyridine C^N backbone and a dithiocarbamate ligand.[56] This complex selectively inhibits breast cancer cells (MCF-7) but is less toxic to non-tumorigenic immortalized liver cells (MIHA). Complex **31** 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 demonstrated by Contel *et al.* to cause mitochondrial dysfunction induced by reactive oxygen species (ROS) and Bax/Bak activation.[57]

Synthesis of the 2-benzylpyridine derivative **32** (Fig. 8) was reported by Cinellu *et al.*[58] already in 1996 by reacting NaHAuCl$_4$ with 2-benzylpyridine in refluxing MeCN/H$_2$O overnight. In 2015 Casini, Cinellu *et al.* synthesized the structural analogue by replacing one chlorido ligand in the presence of excess KPF$_6$ with 1,3,5-triaza-7-phosphaadamantane (PTA).[59] The cytotoxic properties of complex **32** and **33** 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 **33** displaying higher cytotoxic activity than **32**, which can probably be attributed to the increased water solubility induced by the PTA moiety, as well as the overall positive charge of the complex. Among the possible mechanism of actions, potent inhibition of the zinc-finger protein PARP-1 was reported, which has been already demonstrated for several coordination gold(III) complexes with N-donor ligands.[60]

3.3 C^N^N Complexes

In 2003, Cinellu and coworkers reported on the synthesis of the mononuclear Au(III) complex **34** with a dimethylbenzyl-bipyridine C^N^N backbone (Fig. 9).[61] 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 the plasma protein bovine serum albumin (BSA), tight metal–protein adducts were formed. It is believed that binding of complex **34** to BSA is achieved *via* histidine moieties on the protein surface. Complex **34** shows higher cytotoxic activity and selectivity against a series of 12 human tumor cell lines than cisplatin, and inhibition of biologically relevant pathways, such as mammalian target of rapamycin/rapamycin
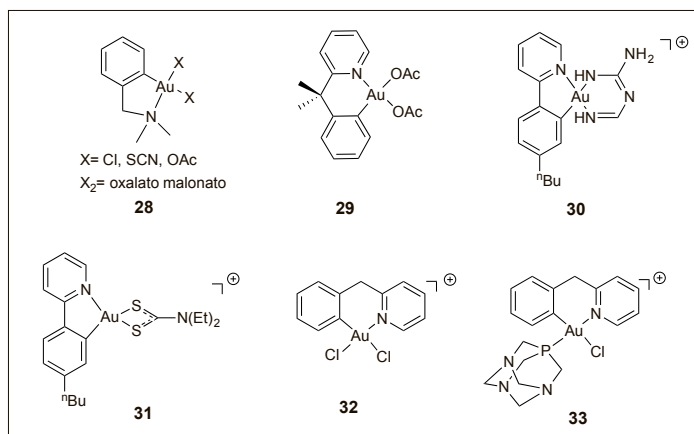

Fig. 8. Au(III) C^N complexes with cytotoxic properties.
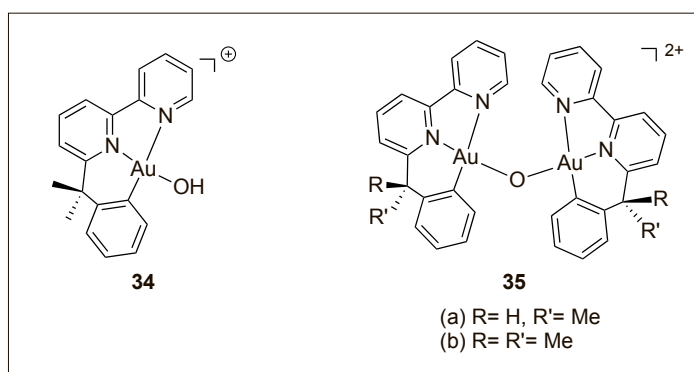

Fig. 9. Mononuclear and dinuclear Au(III) C^N^N complexes.

(mTOR), have been suggested as a possible mechanism of action.[62]

Following the above-mentioned trend of designing multinuclear complexes, the same group reported on the dinuclear, oxo-bridged complex variants of general formula [(N^N^C)$_2$Au$_2$(μ-O)][PF$_6$]$_2$ (**35**) (Fig. 9) and their cytotoxic properties in 2011.[61] Coordination oxo-bridged Au(III) N^N complexes had been previously reported[63] ('Auoxo' compounds) showing antiproliferative effects toward various human cancer cell lines. However, the latter compounds displayed the scarce stability of the Au(III) center in the reducing intercellular milieu, which was overcome by introducing the strong Au–C bond in the organometallic analogues. Notably, the latter reveal a marked redox stability even in the presence of effective biological reductants such as ascorbic acid and glutathione. In comparison to complex **34**, complexes **35a–b** showed in general rather moderate cytotoxic activity against the series of 12 human tumor cell lines. However, **35a** proved to be particularly active against human breast cancer cells (401NL), while **35b** displayed only scarcely selective cytotoxic activity. Mass spectrometry studies with model proteins (hen egg white lysozyme and horse heart cytochrome c) indicate that complexes **35a–b** form monometallic adducts with the proteins, preserving the Au(III) center and retaining the multidentate ligand. It appears

that the complex–protein interaction facilitates the cleavage of the oxo-bridge and conversion into the more reactive monometallic species.

3.4 C^N^C Complexes

Au(III) C^N^C complexes with anticancer properties have been explored by Che and coworkers (Fig. 10). As C^N^C pincer ligands allow the highest stabilization of the Au(III) center among the herewith presented ligands, it is not surprising that this family makes up the majority of the reported cyclometalated Au(III) compounds.

The Au(III) complex class utilizing the 2,6-diphenylpyridine as a C^N^C ligand was reported by Che *et al.* already in 1998[64] *via* reaction of K[AuCl$_4$] and [Hg(C^N^CH)Cl] under reflux in MeCN. Substitution of the chlorido ligand of complex **36** leads to a number of cationic Au(III)–C^N^C complexes with different ancillary ligands (**37–40**). Complex **37** and **38**, containing non-toxic N-donor ligands, display cytotoxic activities similar to cisplatin against various cancer cell lines, such as HeLa cancer cells.[65] However, they do not show cross-resistance with cisplatin against nasopharyngeal (NPC) cancer cells. Gel-mobility shift assays and viscosity analysis show that **37** intercalates with DNA, causing DNA elongation. It has been reported that DNA intercalators enhance the assembly

of G-quadruplexes. Consequently, the ability of compound **37** to intercalate with DNA and induce the formation of intramolecular G-quadruplexes from a model oligonucleotide, was examined by native polyacrylamide gel electrophoresis (PAGE),[65] showing that **37** behaves similarly to the classical DNA intercalator [Pt(terpy)Cl]+ and could potentially cause telomerase inhibition. In addition, by flow cytometry analysis in SUNE1 cells, it was demonstrated that **37** and **38** target cellular DNA *via* S-phase cell arrest, leading to apoptosis.

As observed in several studies, phosphine-containing compounds exhibit ligand-mediated cytotoxicity,[5,66] which, in addition to their poor stability under physiological conditions and non-specific binding affinities towards various biomolecules, prevent their application as anticancer drugs. By introducing the C^N^C scaffold, significantly higher complex stabilities were obtained. For example, complex **39** and its dinuclear analogue **40** are soluble and stable in aqueous media.[65] The dinuclear complexes of type **40** show higher antiproliferative activity against various cancer cell lines than their mononuclear counterparts, especially 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 stress, while being scarcely reactive with DNA.[67]

By replacing the PPh₃ moiety with a NHC ligand, complexes **41** and **42** – mono- and dinuclear compounds, respectively – can be obtained,[68] resulting in the reduction of the cytotoxic activity and selectivity of the compounds, supporting the fact that indeed phosphine ligand-mediated cytotoxicity is crucial for complexes **39** and **40**. In this case, the mononuclear complex **41** displays higher cytotoxic activity than its dinuclear analogue **42**, being 167-fold more cytotoxic to non-small lung carcinoma cells (NCI-H460) than to normal lung fibroblast cells (CCD-19Lu). DNA interaction studies showed that complex **41** induces DNA strand breaks and subsequent cell death through the stabilization of TopoI-linked DNA.[69]

Interestingly, a recent development in the field of Au(III) C^N^C complexes is the discovery of supramolecular polymers, self-assembled from cyclometalated Au(III) C^N^C 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) **43** (Fig. 10) 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 interactions.[70]
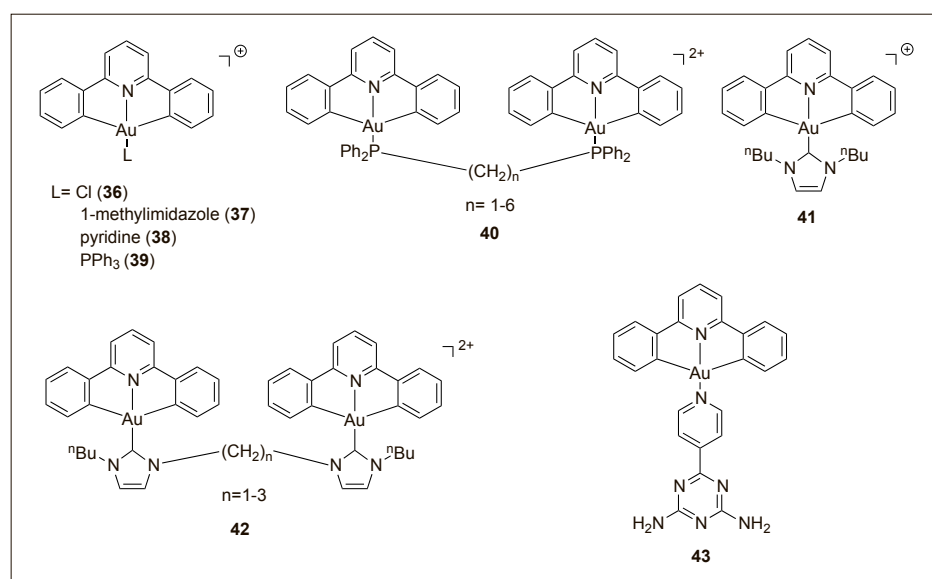

Fig. 10. Au(III) C^N^C complexes synthesized by Che *et al.*[70] with different types of ancillary ligands.

Via transmission electron (TEM) and scanning electron microscopy (SEM), partially aligned nanofibers with diameters and lengths of about 50 nm could be demonstrated for **43**. Complex **43** 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 **43** in phosphate-buffered saline containing GSH (2 mM), showing significant cluster peaks for [(C^N^C)Au(GSH)]+ and the dimeric species [(C^N^C)₂Au₂(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.

4. Conclusions and Perspectives

Nowadays, gold compounds constitute an important class of promising cytotoxic agents which could be optimized to target cancer cells, provided that adequate attention in disclosing the effective cellular and molecular mechanisms through which these compounds induce their antiproliferative effects will be dedicated. So far, a number of studies has shown that the redox metabolism and mitochondria are the likely cellular targets for several metal-based inorganic compounds, capable of inducing oxidative stress, such as the above-mentioned gold-based complexes. Within this framework, the selenoprotein thioredoxin reductase, playing a crucial role in maintaining the intracellular redox homeostasis, constitutes a major pharmacological target for gold compounds, with high affinity for protein selenol and thiol groups. Indeed, it is recognized that TrxR inhibition by gold compounds triggers severe mitochondrial deregulation eventually leading to cell apoptosis.

Certainly, physiological targets other than TrxR should be studied in more detail for organometallic gold complexes in the future. For example, therapeutically relevant zinc finger (ZF) proteins have recently been reported to be potently targeted by classical coordination metal compounds, including Au(I) and Au(III) complexes.[60a] While Au(I) NHC complexes were revealed to be only moderate inhibitors of these proteins,[27] cyclometallated Au(III) compounds may be better candidates. It is worth mentioning that the hypothesized mechanism of inhibition displacement of Zn²⁺ from the ZF peptide by gold ions leads to decreased protein activity, and to formation of the so-called 'gold-finger' (GF) domain.[71] So far, the reported results suggest that GF formation by Au(III) complexes leads to the strongest alteration of the native ZF structure responsible for protein inactivation.

Moreover, among the most intriguing protein targets for coordination Au(III) complexes, the aquaporins (AQPs) have been recently highlighted.[72] AQPs are membrane channels that facilitate the permeation of water and small solutes across membranes, driven by osmotic or solute gradients, with numerous roles in physiology. In fact, AQPs are essential proteins involved in crucial metabolic processes and expressed in several tissues. Most importantly, their selective inhibition by either small compounds or biologicals

constitute a realistic opportunity for therapeutic applications and/or methods for diagnosis and treatment of aquaporin related disorders, including cancer.[73]

In terms of possible chemical modifications, the use of carboxylic acid and alkyne functionalized Au(I)–NHC complexes for biomolecule conjugation is promising to develop organometallic gold scaffolds for biomedical applications. Similarly, the opportunity to derivatize the complexes with groups increasing their water solubility (*e.g.* COOH, OH, sugars, *etc.*) is worth exploring, although this may also limit their cellular uptake.

In general, 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 gain insight into their mechanism of action. Thus, there is an increasing need for imaging methods that allow the direct mapping of the subcellular distribution of metal-based therapeutics, while preserving important morphological information of the cell. The fluorescence properties with which certain metal complexes are endowed are certainly attractive to achieve their imaging in biological environments.[74] As an example, cytotoxic Au(I)–NHC complexes bearing a fluorescent anthracenyl ligand[75] or Au(I)–NHC[76] compounds bound to a coumarin moiety were designed and successfully studied for their cell uptake *via* fluorescence microscopy. Concerning fluorescent cyclometalated Au(III) complexes, some of the above mentioned complexes are endowed with peculiar photophysical properties.

Finally, there are still several very interesting cyclometalated Au(III) complexes reported in the literature, such as highly luminescent Au(III) C^N^C complexes bearing a pyrazine moiety,[77] 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.

In general, we are convinced that organometallic gold complexes hold great promise for the development of novel therapeutic agents and are worth exploring.

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