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Recent advances in gold–NHC complexes with biological properties

Malka Mora,[a] M. Concepción Gimeno *[b] and Renso Visbal *[ac]

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This tutorial review covers the recent advances made in the study of gold complexes containing N-heterocyclic carbene ligands with biological properties. The great stability, ease of modulation of the electronic properties and excellent σ-donating capacity displayed by NHCs allow gold–NHC derivatives to reach high stability in biological media and relatively good internalization into cells and for that they have emerged as excellent potential chemotherapeutics. The new gold–NHC derivatives show superior anticancer activity compared to other standards such as Cisplatin or Auranofin. In addition, the application of gold–NHC complexes in the treatment of other human diseases as antibacterial, antioxidant and antiparasitic agents is reviewed for the first time.

Key learning points

1. Conceptual introduction to the application of Au–NHC complexes as anti-bacterial and anti-cancer agents, and their mode of action.
2. A brief discussion of the very recent advances made in the development of new gold–NHC derivatives as anti-tumor agents.
3. An overview of the state-of-the-art in applying Au–NHC complexes to the treatment of important human diseases such as malaria, leishmaniasis and those caused by bacteria.
4. A summary of the first investigations of gold complexes containing N-heterocyclic carbene ligands as anti-oxidant agents.

Introduction

The discovery of Cisplatin has had a great impact on the clinical success of different anticancer metallopharmaceutical agents.[1] Although a great number of platinum-based metallodrugs have proved to be very effective against several tumors, they present various disadvantages such as the presence of undesirable side effects in cancer patients.[1] For this reason, many efforts have been focused on the exploration of other active metals and also on the design of organic ligands for the preparation of new antitumor drugs. Particularly, coinage metals (especially Ag and Au) have been found to be a special key for this goal, mostly due to their less harmful characteristics than many transition metals towards the human body. Additionally, gold has proven to be active in other different human diseases for example rheumatoid arthritis, malaria or AIDS.[2]

One of the biggest differences in the antitumor activity of Cisplatin and gold compounds lies in their mode of action. Cisplatin normally acts by direct interaction with DNA,[1] whereas the antiproliferative activity of gold complexes usually involves the inhibition of enzymes, especially those containing thiols. The strong binding affinity of gold with sulfur makes key enzymes such as thioredoxin reductase (TrxR), glutathione reductase and cysteine protease, which are overexpressed in cancer cells, potential targets for gold anticancer complexes.[3–6] TrxR has been established as an important target in the mechanism of action of gold complexes. There are two isoforms known for this enzyme, one which is located in the cytosol and another that is situated in the mitochondria. In both cases, TrxR is involved in the reduction of thioredoxin (Trx) to its dithiolic form, and the inhibition of this enzyme leads to apoptosis *via* the mitochondrial pathway.[3–6]

A very important aspect in the design of anticancer metal complexes is their stability under physiological conditions, which allows a better delivery and transport to tumor cells. For that reason, strong ligand–metal bonds are needed, and in this respect organometallic ligands such as N-heterocyclic carbenes (NHCs) play a key role. After the discovery of N-heterocyclic carbenes a huge number of organometallic compounds have been prepared with application in important

[a] *Departamento de Ciencias Naturales y Exactas, Universidad de la Costa, c/58 no. 55-66, 080002 Barranquilla, Colombia. E-mail: rvisbal4@cuc.edu.co*
[b] *Departamento de Química Inorgánica, Instituto de Síntesis Química y Catálisis Homogénea (ISQCH), CSIC-Universidad de Zaragoza, Pedro Cerbuna 12, 50009 Zaragoza, Spain. E-mail: gimeno@unizar.es*
[c] *Departamento de Gestión Industrial, Agroindustrial y Operaciones, c/58 no. 55-66, 080002 Barranquilla, Colombia*

research fields such as catalysis, materials science and medicine.[4,7] Coinage metals have not been an exception. Due to the strong sigma-donating properties and easy modulation of both the steric and electronic properties of NHCs, these ligands have attracted tremendous attention for the construction and design of a great variety of organometallic complexes.[7] In medicinal chemistry, N-heterocyclic carbenes have been revealed as very suitable scaffolds for being less toxic ligands than phosphines, easier to functionalize and because they can target relevant biomolecules in cancer progression.[8]

Many NHC gold(I) complexes with biological properties have been reported to date. As one of the two important representative examples, the seminal work by Berners-Price *et al.* described a family of lipophilic cationic Au(I)–NHC complexes **1–3** with excellent antitumor properties showing a particular selectivity towards mitochondrial selenoproteins, such as TrxR (Fig. 1).

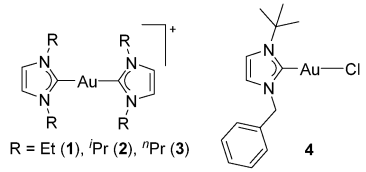


Fig. 1 Relevant Au(I)–NHC complexes with medicinal applications.

Interestingly, the cationic complexes selectively induced apoptosis in highly tumorigenic breast cancer cells but not in normal cells. This selective toxicity is the result of two-step ligand exchange reactions with cysteine (Cys) and selenocysteine (Sec) due to the release of the NHC ligands after incorporation into the mitochondria.[9,10]

The Ghosh research group reported the significant antimicrobial activities of the gold(I)–NHC complex **4** (Fig. 1) against *Bacillus subtilis* and *Escherichia coli* bacteria. Although this gold derivative did not show the potent anticancer activity of the bis-carbene analogous to palladium, it did inhibit bacterial growth, achieving over 80% inhibition of *B. subtilis* growth.[11]

The preparation of new gold derivatives is continuously growing, leading to a great number of papers reported year by year. Many reviews dealing with the anticancer properties of gold complexes have been reported, including the most recent ones by Liu and Gust or Che.[12–14] Here, we aim at compiling not only the very recent advances in the design of Au–NHC complexes with anticancer properties, but also all the gold species reported in the literature with other medicinal applications such as antibacterial, antimalarial, antioxidant, antileishmanial and antiparasitic.

Antitumor activity of gold–NHC complexes

After the discovery of Auranofin, gold medicinal chemistry has been significantly exploited, leading many research groups to

Malka Mora

Malka Mora was born in Cartagena de Indias, Colombia (1983). She obtained her degree in chemistry in 2008 from the University of Cartagena (Colombia), and earned an FPU grant from the Ministerio de España and obtained her PhD from the University of Zaragoza in the Department of Inorganic Chemistry under the supervision of Prof. Miguel Ángel Esteruelas and Prof. Ana M. López. In 2016, she joined the Universidad de la Costa (Colombia) in the Department of Natural and Exact Sciences as assistant professor. Her research focusses on the study of metal coordination complexes and their applications in catalysis, biochemistry and materials science.

M. Concepción Gimeno

M. Concepción Gimeno received her PhD from the University of Zaragoza. After her postdoctoral work with Prof. Stone at the University of Bristol, she joined the Institute of Chemical Synthesis and Homogeneous Catalysis (ISQCH, CSIC-University of Zaragoza), where she has been Professor from 2008. Her research interests focus on the synthesis of group 11 metal complexes with optical, medicinal or catalytic applications. She is the author of more than 240 scientific publications. She has been awarded with the IUPAC 2017 Distinguished Women in Chemistry or Chemical Engineering, the GEQO-Excellence in Organometallic Chemistry Research Award 2017 and RSEQ-Excellence Research Award in 2018.

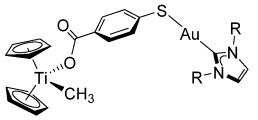
Renso Visbal

Renso Visbal was born in Cartagena de Indias, Colombia (1985). He obtained his degree in chemistry in 2008 from the University of Cartagena (Colombia), and received his MSc (2010) and PhD (2015) in chemistry from the University of Zaragoza under the supervision of Prof. M. Concepción Gimeno and Prof. Antonio Laguna. In 2015, he joined the Universidad de la Costa (Colombia) in the Department of Natural and Exact Sciences as assistant professor. His research focusses on the investigation of N-heterocyclic carbene copper, silver and gold complexes and their applications in luminescence, biochemistry and materials science.

the investigation of gold derivatives with new applications. Particularly, the use of gold compounds for the treatment of cancer has accelerated in the past few decades, and a huge number of studies addressing the antitumor properties have been reported. Recently, Contel and co-workers described a simple synthesis of cationic heterobimetallic Ru–Au anticancer agents (**5–8**), which exhibited high cytotoxic activity against Caki-1 (renal) and HCT 116 (colon) cancer cell lines as compared to Cisplatin (Fig. 2). These heterobimetallic species showed low toxicity to the non-tumorigenic HEK-293T (kidney) cell line and they were very effective against Caki-1 and HCT 116 cancer cell lines, in contrast to the mononuclear precursors (Au and Ru) and even Cisplatin. For example, complexes **5** and **7** displayed the half maximal inhibitory concentration (IC_{50}) values of 5.2 ± 0.9 and 3.8 ± 0.6 μM for Caki-1 cells, and 8.1 ± 1.8 and 6.4 ± 1.0 μM for HCT 116 cells, respectively, while the mononuclear derivatives reached over five times larger than these values.[15] Electrophoresis studies demonstrated that heterobimetallic complexes **5–8** did not interact significantly with DNA. These results are in accordance with those obtained for the mononuclear precursors of gold and ruthenium, which do not or weakly interact with DNA. Additionally, the activity of thioredoxin reductase was evaluated in Caki-1 cells treated with the mononuclear precursors and the heterobimetallic complex **5**. The experiments revealed a significant reduction of the activity of TrxR when using 5 μM of species **5** (63%) as compared to the gold (17%) and ruthenium (44%) precursors. The differences obtained from the anticancer activity and biomolecular interaction studies strongly suggest a synergistic effect for the heterobimetallic complexes in their *in vitro* activity against renal cancer cell lines.[15]

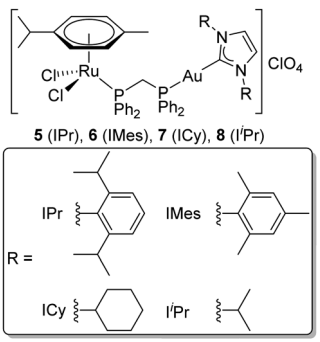
The same research group also prepared several titanocene–gold complexes containing N-heterocyclic carbene ligands (**9–12**) and evaluated their cytotoxic activity towards different cancer cell lines such as prostate (PC3 and DU145), Caki-1, colon (DLL1), triple negative breast (MDA-MB-231) and HEK-293T (Fig. 3).[16] The results revealed that the heterometallic compounds were considerably more toxic to the prostate and colon cancer cell lines than the titanocene derivative used as a control. Furthermore, all of the heterometallic complexes were more toxic to the tested cell lines (except the MDA-MB-231 cells) than the monometallic gold precursors, showing IC_{50} values between 13.9 ± 1.7 and 42.9 ± 5.8 μM after 72 h of incubation. Besides, all of the complexes displayed good selectivity towards the cancer cell lines (except the MDA-MB-231 cells). Particularly, compound **10** exhibited better selectivity to the HEK-293T cell line as compared to previously synthesized gold derivatives containing a phosphine ligand. Again, as observed in the previous work,[15] the heterobimetallic complexes did not show DNA interaction, but did display strong inhibition of the TrxR enzyme. This behavior was particularly observed in the activity of TrxR in the PC3 cancer cell line after treatment with complex **10** (5 μM) which presented a reduction up to 76%. This fact confirms that the inhibition of TrxR is involved in the cell death mechanism, and the heterobimetallic Au–M complexes are present playing a synergistic effect, which is crucial to reach such level of cytotoxicity. Moreover, the anti-invasive properties of complexes **9** and **10** were studied on prostate cancer PC3 cell lines. As compared to mononuclear precursors, heterometallic species showed better antimigratory properties in PC3 cells. This effect is very important in advanced tumors, where the increased cell migration is closely related to cancer cell invasion and metastasis.[16]

The anticancer gold–NHC species **13–15**, targeting antioxidant pathways, were reported by Arambula, Arumugan, and co-workers (Fig. 4). They conveniently incorporated a ferrocenyl group into the Au–NHC moiety, which displayed better antiproliferative properties against the A549 cancer cell line (lung) than ferrocene (reactive oxygen species(ROS) generator) or Au–NHC (TrxR inhibitor) alone. A good correlation with the amount of ferrocene contained within the complex was observed, showing that the species **15** displayed an IC_{50} value of 0.14 ± 0.03 μM, being 10 times more potent than Auranofin (1.67 ± 0.05 μM). The ICP-MS studies also showed that both Fe and Au were found in the cells following exposure to complex **15** at several concentrations, indicating that both fragments are stable enough to promote a good cellular uptake of the complex. Additionally, gold(I) bis-carbene species containing the ferrocene group (**13–15**) were evaluated for their ability to disrupt ROS regulation. The independent addition of complexes



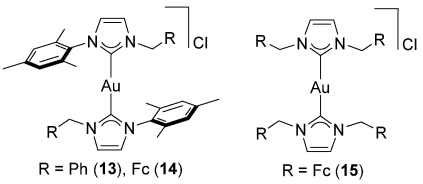
9 (SIPr), **10** (IPr), **11** (IMes), **12** (ICy)

Fig. 3 Cationic heterobimetallic Ru–Au derivatives with anticancer properties.



5 (IPr), **6** (IMes), **7** (ICy), **8** (I^{i}Pr)

Fig. 2 Cationic heterobimetallic Ru–Au derivatives with anticancer properties.



R = Ph (**13**), Fc (**14**) R = Fc (**15**)

Fig. 4 Gold(I) bis–NHC complexes containing a ferrocene group.

13–15 to A549 cancer cells resulted in an increase in ROS which was higher compared to the control samples but lower than that due to the Auranofin treatment. The exposure of A549 cells to L-buthionine-(*S*,*R*)-sulfoximine (BSO), which acts as a selective inhibitor of glutathione (GSH) synthesis, revealed that the cells with reduced levels of GSH showed increased levels of ROS after the addition of $[Au(NHC)_2]^+$ complexes (**13–15**). Also, the authors investigated the effects of the studied complexes on a possible thioredoxin pathway, finding that the thioredoxin reductase inhibition in cells independently treated with gold(I) bis-carbene species was significant, ranging from 55 to 60% inhibition, as compared to that found for Auranofin (70% inhibition). These results correlated very well with the cytotoxicity observed for complexes **13–15**.[17]

Gold(I) bis-carbenes (**16–17**) and gold(III)–NHC species (**18**) (see Fig. 5) were synthesized *via* the transmetallation route using silver(I)–NHC precursors by Haque and co-workers, and their cytotoxic activity was assessed toward different cancer cell lines (HCT 116, MCF-7 (breast), PC3 and U937 (leukemia)) and compared to that of tamoxifen and betulinic acid as standard drugs. After 72 h of incubation, the gold(III) complex (**18**) displayed the highest activity among all the series of complexes against HCT 116 cells, achieving an IC_{50} value of 0.05 ± 0.01 μM, which was 100 times more active than the standard used. However, none of the gold(I) derivatives showed such cytotoxicity, displaying moderate IC_{50} values between 23 and 50 μM. The gold(III) derivative (**18**) also displayed better cytotoxicity than the silver precursors, and the authors attributed this behavior to the steric and electronic effects of the carbene ligands as well as the nature of the metal ions. This trend was also found for the anticancer activity against MCF-7, PC3, and U937 cell lines, with complex **18** exhibiting the highest antiproliferative activity in the low-nanomolar level of 0.31 ± 0.02 and 0.34 ± 0.02 nM, and in the micromolar level of 0.19 ± 0.002 μM, respectively. Nevertheless, cationic gold(I) bis-carbene derivatives (**16** and **17**) were moderately active towards MCF-7 (7.62 ± 0.4 and 7.32 ± 0.8 μM) and U937 (4.7 ± 0.4 and 1.7 ± 0.06 μM) cancer cell lines, respectively, while a poor cytotoxicity was observed for complex **16** against PC3 cells achieving an IC_{50} value of 126.27 ± 1.7 μM. The good results obtained for the gold(III) derivative (**18**) were associated with the possible redox activity of this species, which can occur in the cellular environment and also with any biological activation *via* ligand detachment promoted by the presence of labile chloride ligands. Finally, in order to determine whether the gold compounds are cytotoxic towards normal cell lines, selective index studies were carried out. The selectivity index was determined from the ratio of the IC_{50} value obtained from normal cells and the IC_{50} value for cancer cells such as HCT 116, MCF-7, PC3 and U937. In addition, complexes **16–18** were treated with the human colorectal normal cell line (CCD-18Co) in order to determine the reactivity of the gold species toward normal cells. The results indicated that all of the complexes were found to be less cytotoxic against CCD-18Co cells, and overall, they showed activities that were 2–20 orders of magnitude lower than that of the standard. Again, the neutral gold(III) derivative **18** displayed a remarkable high selectivity toward all cancer cell lines compared with the other gold species.[18]

Complexes **19** and **20** were synthesized by Saha, Dinda, and co-workers, and their cytotoxic activity against several cancer cell lines such as HepG2 (liver), HCT 116, A549 and MCF-7 was investigated using the MTT assay (Fig. 6). In this study, the neutral gold(III) derivative (**20**) exhibited lower activity than the cationic bis-carbene species (**19**), with IC_{50} values ranging from 2.31 to 2.72 μM, and 0.94 to 1.28 μM, respectively. This behavior was associated with the reduction of Au(III) to Au(I) after interaction with intracellular thiols. With the aim of studying the induction of cell apoptosis, HepG2 cells were treated with complex **20**, and after 24 h of incubation, 58.4% of apoptotic cells were observed as compared to 0.1% in the control cells. In addition, subsequent studies showed that the gold(I) species may be inducing cell apoptosis *via* high expression of p53 and p21 along with nuclear translocation of p53. Finally, the role of **20** in ROS generation was also evaluated. The study revealed that treatment of HepG2 cells with compound **20** for 24 h produced an increase of the mean fluorescence intensity of dichlorofluorescein (DCF) from 567 to 12 659, indicating a shift in ROS generation from the control cells to treated cells (IC_{50} concentration). Additionally, a reduction in mitochondrial membrane potential ($\Delta\Psi_m$) with 62.6% cells indicating loss of $\Delta\Psi_m$ after treatment with complex **20** as compared to 4.3% of control cells was observed. Finally, complex **20** was found to be affecting the expression of mitochondrial proteins in HepG2 cells, producing a modification of the Bax/Bcl-2 ratio leading to an increase of the cytosolic cytochrome *c* and to a decrease of pro-caspase 9 and 3. These results together with those for the ROS generation and $\Delta\Psi_m$ confirm that in this case the cell death proceeds *via* the mitochondrial death pathway.[19]

Recently, Veige and co-workers described a simple strategy to selectively target CCRF-CEM leukemia cells while reducing side effects associated with the administration of gold compounds.

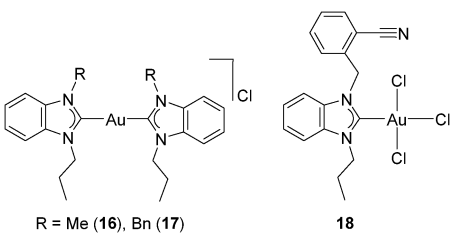


Fig. 5 Gold(I) bis–NHC complexes (**16** and **17**) and gold(III) species (**18**) with anticancer properties.

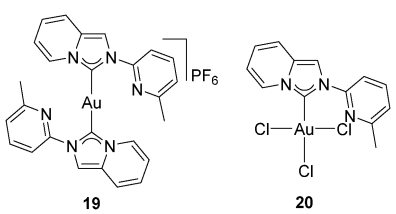


Fig. 6 Gold(I) and gold(III) complexes containing pyridine-substituted NHC ligands.

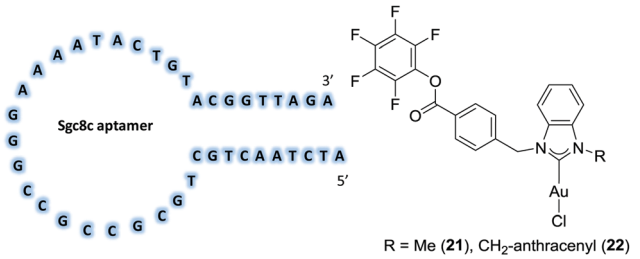


Fig. 7 N-heterocyclic carbene–gold(I) complexes used to design bioconjugates containing the sgc8c aptamer to selectively target leukemia cells.

In this study, two aptamer–[NHC–Au(I)–Cl] bioconjugates were conveniently designed from the gold(I)–NHC complexes (**21–22**) and the sgc8c aptamer depicted in Fig. 7. In both cases, the aptamer–drug conjugates (**sgc8c-21** and **sgc8c-22**) displayed a great increase in cytotoxicity (0.54 ± 0.85 and 2.39 ± 1.50 μM) as compared to complexes **21** (14.6 ± 1.40 μM) and **22** (31.1 ± 2.11 μM) alone. The authors attributed this effect to the high affinity of the aptamer conjugate for the leukemia cells and its facile access into the cell, as well as the hydrophilic nature of the gold compound (**21**) after conjugation to the aptamer. Interestingly, taking advantage of the anthracenyl group anchored to the nitrogen atom of the imidazole ring in **22**, the fate of the gold(I)–NHC fragment was monitored using fluorescence confocal microscopy. The results together with other MTS cell-proliferation assays indicated efficient cellular uptake of the Au(I)–NHC fragment into the cytoplasm and high selectivity towards CCRF-CEM cancer cells. This is in agreement with the results obtained from a comparative study using flow cytometry assays with the K562 and the CCRF-CEM cancer cell lines, demonstrating the great selectivity of the conjugate **sgc8c-22** for the latter. This can be rationalized considering that the conjugate **sgc8c-22** is able to recognize the PTK-7 protein expressed by CCRF-CEM leukemia cells, while K562 cells do not overexpress the related protein.[20]

Gold complexes **23–25** were synthesized *via* silver transmetallation of the corresponding Ag-precursors with [AuCl(SMe$_2$)] by Saturnino, Rosano, and co-workers (Fig. 8). The antitumor activity was investigated against human breast cancer cells (CMF-7) by using the MTT assay. The studies showed that the gold complexes **23** and **24** did not promote any significant reduction in growth. In contrast, the gold derivative **25** (1 μM) was 80 times more cytotoxic than Cisplatin (80.23 ± 5.3 μM). The treatment of MCF-7 cells with complex **25** revealed an increase in the levels of the proteolytic form of PARP (a well-recognized cellular substrate of mammalian caspases) as compared with the control. This result, together with those obtained from the TUNEL assay to assess DNA fragmentation, confirmed the role of apoptosis in cell growth inhibition induced by species **25**. In addition, the monitoring of a possible mitochondrial dysfunction using confocal microscopy revealed that compound **25** could be implied in the apoptotic cell death *via* mitochondrial damage.[21]

The P53 suppressor gene participates in apoptotic processes acting on multiple mitochondrial functions. In this context, the authors also evaluated the ability of compound **25** to regulate the P53–P21 expression, finding that the gold species causes a significant increase in the expression of the mentioned proteins. This fact led to the identification of the region within P53 responsible of the transactivation mediated by **25**, based on functional assays using p53-deleted constructs linking putative motifs for CTF-1/YY1, NF-Y, NFκB and Sp1-like proteins. The results revealed that the P53 transcription mediated by **25** depends on the transcription factor Sp1, which is implicated in different cellular processes such as cell differentiation, growth and apoptosis.[21]

Four gold(I)–NHC complexes containing 4-ferrocenyl substituted imidazole-2-ylidene ligands (**26–29**) were prepared and their anticancer properties against a panel of seven cancer cell lines were investigated (Fig. 9). The results are shown in Table 1. As observed, the neutral gold carbene complex **26** displayed a good cytotoxic activity in the low micromolar range (7–20 μM), while its analog featuring an *N*-ethyl group (**27**) exhibited better activity with IC_{50} values ranging from 0.2 to 4 μM. Additionally, the substitution of the chloride ligand by a triphenylphosphine (**28**) or a second NHC ligand (**29**) promoted the enhanced antiproliferative effect giving IC_{50} values in the lower submicromolar range. These results were associated with the better lipophilic character of the cationic species, which showed the highest cellular uptake in HCT-116 cancer cells. On the other hand, CCD18-Co fibroblasts were affected only when using higher concentrations of the gold–NHC derivatives. Moreover, neutral (**27**) and cationic ferrocenyl species (**28** and **29**) strongly inhibited the growth of HUVECs (human umbilical vein endothelial cells), which is in agreement with the antivascular activity observed for these complexes.[22]

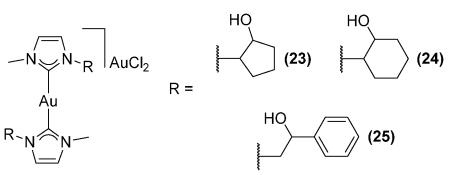


Fig. 8 Cationic gold(I)–NHC complexes featuring different lipophilic substituents.

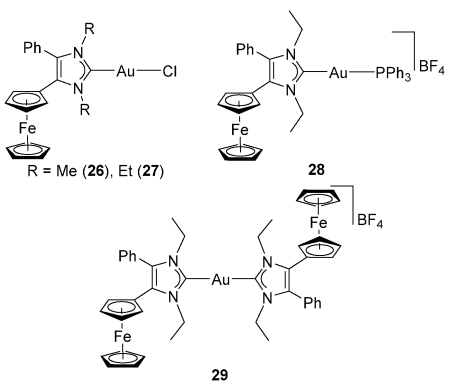


Fig. 9 Gold(I)–NHC complexes containing 4-ferrocenyl substituted imidazole-2-ylidene ligands.

Table 1 Inhibitory concentrations IC_{50} (μM) of complexes **26–29** obtained after treatment of cancer cells and non-malignant cells

Cell line[a]	26	27	28	29
518A2	11.3 ± 0.3	1.3 ± 0.1	0.20 ± 0.03	0.19 ± 0.02
Panc-1	6.7 ± 0.3	0.62 ± 0.01	0.14 ± 0.02	0.09 ± 0.00
MCF-7/Topo	7.1 ± 0.1	0.71 ± 0.02	0.60 ± 0.01	0.11 ± 0.00
KB-V1/Vbl	>50	13.3 ± 0.1	1.6 ± 0.4	1.5 ± 0.1
HCT-116	11.1 ± 0.3	0.21 ± 0.08	0.09 ± 0.01	0.06 ± 0.00
HT-29	10.8 ± 0.4	0.3 ± 0.08	0.16 ± 0.02	0.13 ± 0.01
DLD-1	19.5 ± 1.0	4.2 ± 1.4	1.2 ± 0.5	0.30 ± 0.11
CCD-18Co	>50	15.0 ± 0.9	8.9 ± 0.3	2.8 ± 0.1

[a] Human cancer cell lines: 518A2 (melanoma), Panc-1 (pancreatic ductular adenocarcinoma), MCF-7/Topo (breast adenocarcinoma), KB-V1/Vbl (cervix carcinoma), HT-29 and DLD-1 (colorectal adenocarcinoma).

The effects of gold complexes **28** and **29** on the cytoskeletal organization were also studied. The treatment of HUVECs and 518A2 cells with low concentrations of these compounds did not affect the polymerization of tubulin or the organization of microtubules in the cell. However, they induced a different reorganization of the actin cytoskeleton in both cell lines. Using propidium iodide staining and flow cytometry experiments the authors also demonstrated that the cationic complexes (**28** and **29**) are implied in the tumor cell cycle and cell migration processes. The treatment of 518A2 cells with small quantities of gold compounds (500 nM) reduced wound healing to approximately 70% (**28**) and even down to 40% (**29**), as compared to untreated cells.[22]

In order to better understand the implication of both mononuclear and dinuclear complexes in anticancer activity, several experiments to inhibit both TrxR and PARP 1 were carried out. In the first place, all of the complexes proved to be TrxR inhibitors. However, the triphenylphosphine derivative (**28**) was highlighted among all of the complexes, since it displayed a potent activity reaching an IC_{50} value of 0.6 ± 0.1 μM. This result was rationalized as a consequence of the facile substitution of the chloro (**27**) or phosphine (**28**) ligands, in contrast to **29**, in which the release of the NHC ligand is a little more difficult. On the other hand, the bis-carbene derivative **29** produced a greater inhibition of the PARP 1 enzyme (EC_{50} = 1.0 ± 0.4 μM) than complex **28** (EC_{50} = 2.0 ± 0.3 μM). These results are in agreement with those obtained for ROS generation in 518A2 melanoma cells. Again, complex **29** (500 nM) induced a 1.6 times higher level of ROS compared with untreated control cells. Nevertheless, although compound **26** almost did not affect the ROS generation at this concentration, when increasing the concentration up to 1 mM, both derivatives displayed similar ROS levels (180%). These observations pointed to the presence of two effects: one related to the presence of the ferrocenyl group increasing the ROS levels at low concentrations, and another one associated with the inhibition of the TrxR enzyme at high concentrations.[22]

Several neutral and cationic gold(I)–NHC complexes containing an acridine moiety and two biologically compatible ancillary ligands (**30–37**) were prepared by Gimeno and co-workers (Fig. 10). The acridine group, which is a chromophore ligand known by its properties as a DNA intercalator, was incorporated with two purposes: to prepare compounds with cytotoxic activity,

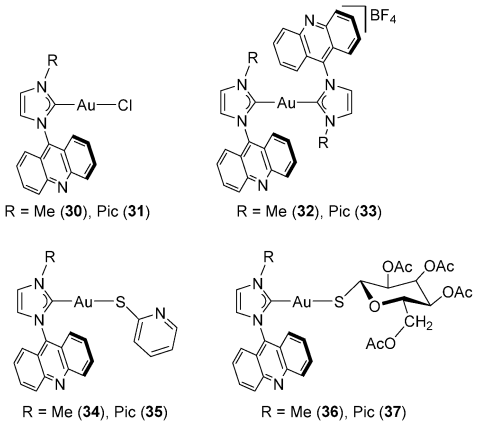


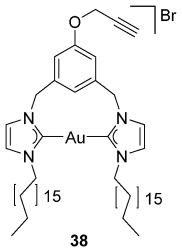
Fig. 10 Gold(I) N-heterocyclic carbene complexes bearing the acridine group.

and to use its optical properties to determine the biodistribution of the gold compounds in cells.[23]

The cytotoxic activity of the complexes was investigated against A549 and MiaPaca2 (pancreas) cancer cell lines and compared to that of Cisplatin. The results indicated that the neutral derivatives containing a chloride ligand (**30** and **31**) were the less effective on the series of both A549 ($IC_{50} \geq 50$ μM) and MiaPaca2 (IC_{50} = 22.8 ± 8.8 and 38.3 ± 2.6 μM) cells, respectively. However, the substitution of the chloride ligand by another N-heterocyclic carbene produced an enhancement of cytotoxicity, especially towards MiaPaca2 cells, reaching IC_{50} values of 6.9 ± 1.9 and 20.0 ± 1.6 μM, for **32** and **33**, respectively. Consequently, the best results were obtained when using bioactive molecules as ancillary ligands, as in **34–37**. In particular, the substitution of the chloride ligand by thiol–pyridine (**34** and **35**) increased the cytotoxicity against A549 and MiaPaca2 cancer cell lines, especially for the picolyl-substituted (**35**), whose IC_{50} values of 17.4 ± 4.5 and 7.5 ± 1.2 μM are considerably lower than those obtained for the chloride- or NHC-containing gold–NHC complexes. Additionally, the most effective of the complexes was the picolyl-substituted containing the tetra-*o*-acetyl-1-thio-β-D-glucopyranoside ligand, whose IC_{50} reached values of 13.0 ± 3.6 and 3.4 ± 0.8 μM for A549 and MiaPaca2 cancer cells, respectively.[23]

Finally, due to the intense emission displayed by all the gold(I)–NHC complexes, fluorescence confocal microscopy was used to ascertain the biodistribution of selected complexes in cells. Using concentrations half of their IC_{50} values, bis-carbene and thiolate-containing gold complexes were incubated with either MitoTracker or LisoTracker in order to co-localize the intracellular biodistribution. The results indicated that the complexes are not only located in the lysosomes, but also seem to have some nuclear permeability and accumulation in the nucleolus. The latter could be related to the assumed affinity of Au(I) to inhibit TrxR1 and Trx1.[23]

The *in vitro* anticancer activity of the gold(I)–NHC complex **38**, prepared from a bis-imidazolium-amphiphile salt, was evaluated toward two cancer cell lines such as HT-29 and MDA-MB-231 (Fig. 11). In this case, the incorporation of a gold



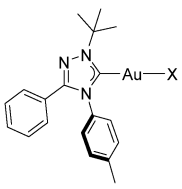
38

Fig. 11 Gold(I) bis–NHC complex **38** derived from a bis-imidazolium-amphiphile.

center in the corresponding imidazolium precursor did not show the expected improvement of cytotoxicity. The IC_{50} values found for both cancer cell lines (HT-29 and MDA-MB-231) were >10 and 7.3 ± 0.9 μM, which are very similar to those found for the bis-imidazolium salt precursor. The IC_{50} against HT-29 cells found for complex **38** is higher than the maximum concentration tested, due to its limited solubility in required conditions.[24]

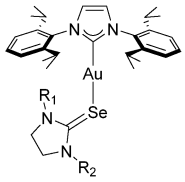
Two gold(I) complexes containing 1,2,4-triazole-based N-heterocyclic carbene ligands of the form (NHC)AuX (X = Br (**39**), I (**40**)) were synthesized by Růžička and co-workers (Fig. 12). Their antiproliferative activity against several cancer cell lines (HepG2, HL60 (leukemia), HeLa S3 (cervical) and CCRF-CEM) was evaluated and compared to that of Cisplatin and Auranofin. In general, all of the complexes displayed better cytotoxicity than Cisplatin but were less effective than Auranofin with IC_{50} values ranging from 1.54 ± 0.08 to 3.51 ± 0.17 μM (complex **39**) and 2.13 ± 0.18 to 7.73 ± 0.09 μM (complex **40**). After 72 h of incubation, all complexes tested on CCRF-CEM leukemic cells were found to be increasing the proportion of apoptotic cells compared to control cells. Particularly, complex **39** was found to be the most potent in apoptosis induction, suggesting based on the cell cycle effect analysis that the mechanism of anticancer activity of the tested derivatives may differ from that of Auranofin. Additionally, due to the stability and low solubility of the related compounds in physiological conditions, an improved formulation *via* liposomal encapsulation or chemical functionalization of the compounds can be used to increase their anticancer activity.[25]

The incorporation of several selenones to the $[IPr\text{-}Au]^+$ fragment for the preparation of complexes **41–45** has been carried out by Isab and co-workers (Fig. 13). The study of their antiproliferative properties has revealed that none of the complexes is more effective than Cisplatin against HCT15, A549 and MCF7 cancer cell lines, displaying IC_{50} values from 23 ± 4 to



X = Br (**39**), I (**40**)

Fig. 12 Gold(I) complexes containing 1,2,4-triazole-based N-heterocyclic carbene ligands.



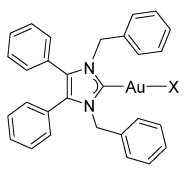
$R_1 = R_2 = H$ (**41**), $R_1 = H$, $R_2 = Et$ (**42**), $R_1 = H$, $R_2 = {}^nPr$ (**43**), $R_1 = R_2 = Me$ (**44**), $R_1 = R_2 = Et$ (**45**)

Fig. 13 Gold(I)–NHC complexes bearing selenones as co-ligands.

180 ± 2 μM. The authors stated that the poor activity observed with these complexes could be related to the strong binding of the IPr and selenone ligands and their great steric hindrance. Nevertheless, docking studies were performed in order to establish a possible interaction between gold(I)–NHC complexes and the TrxR enzyme. The results indicated that, except for species **42**, all of the complexes displayed negative scores, which suggested no affinity between gold derivatives and the target protein. Also, the interaction of the synthesized complexes with amino acids was explored. Interestingly, all of the gold compounds displayed strong interaction with tryptophan. They observed that the successive addition of small quantities of **42** into the tryptophan solution produced a significant reduction of the tryptophan oxidation peak current from +0.708 to +0.788 V.[26]

Two 1,3-dibenzyl-4,5-diphenyl-imidazol-2-ylidene gold(I) complexes containing a chloride (**46**) and 2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl-1′-thiolate (**47**) as ancillary ligands were prepared by Tacke and co-workers, and their antiproliferative properties were evaluated against a wide panel of leukemia, colon, melanoma, renal, lung, central nervous system (CNS), ovarian, prostate and breast cancer cell lines (Fig. 14). In general, all of the complexes displayed good growth inhibition towards a wide range of cancer cell lines, including the renal cells, reaching GI_{50} values of 1.78 and 1.95 μM for the chloride and thiolate derivatives, respectively. These results encouraged the authors to evaluate the maximum tolerable dose (MTD) in mice, finding MTD values of 10 and 7.5 mg kg^{-1} for NHC-AuCl and NHC-AuSR species, respectively. In the tumor xenograft experiments, the tumor volume growth reduction was significant until day 11 of treatment of caki-1 tumors with complexes **46** and **47**, but a slight increase of the volume was observed, reaching values around 0.16 cm^3. Additionally, the study of the inhibition of mammalian TrxR suggested (1.5 μM for **46** and 3.1 μM for **47**) an apoptotic induction through elevated oxidative stress.[27]

In a subsequent study, the same research group synthetized four analogues (**48–51**) and compared their cytotoxic activity



X = Cl (**46**), S-Gluc (**47**), S-Cy (**48**), SCN (**49**), NCO (**50**), N_3 (**51**)

Fig. 14 Gold(I)–NHC complexes with *in vitro* and *in vivo* anticancer activity.

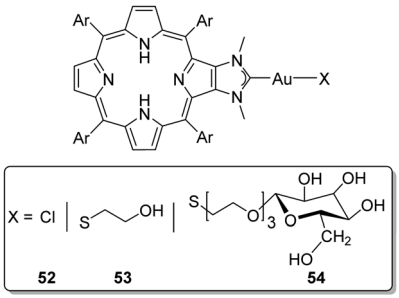
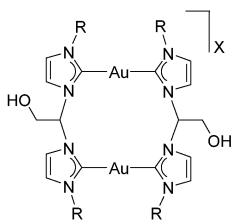


Fig. 15 Gold(I)–NHC complexes derived from porphyrins fused to imidazolium salts with photodynamic properties.

with those previously reported (Fig. 14). The four new derivatives showed similar anticancer properties to those observed for complexes **46** and **47**, except for **49**, which exhibited a superior cytotoxicity displaying GI_{50} values in the low micromolar range (0.29–1.95 μM). In general, all of the complexes displayed similar activity with average growth inhibition of 99%, 94% and 93% for **48**, **49** and **51**, respectively.[28]

Gary-Bobo, Richeter and co-workers conveniently used porphyrins fused to imidazolium salts as precursors to prepare Au(I)–NHC complexes **52–54** (Fig. 15). The presence of a porphyrin group allowed the investigation of their photodynamic properties, and subsequently, these gold derivatives were used as photosensitizers to kill MCF-7 breast cancer cells. The results indicated that the complexes increased their cytotoxicity after irradiation with UV light, promoting the Au–X bond cleavage. Complex **52** presented a higher photodynamic activity, probably because of an active endocytosis *via* mannose receptors. The generation of ROS in MCF-7 cells after laser irradiation in the absence or presence of mannose (10 mm) was also investigated. The results confirmed the implication of mannose receptors in the active endocytosis of derivative **52** in cancer cells. However, it remains unclear whether the phototoxicity is due to the photodynamic activity of porphyrin or the gold complex, which has lost its thiolate ligand upon irradiation.[29]

Dinuclear gold(I) N-heterocyclic carbene complexes with different wing-tip substituents were synthesized, and their antiproliferative properties were investigated against A549 and HepG2 cancer cell lines using the MTT assay (Fig. 16). The results showed that the complexes **55**, **56** and **58** did not present cytotoxic activity towards both cancer cells ($IC_{50} > 100$ μM), except for species **55**, which only displayed moderate anticancer



X = PF_6, R = Me (**55**), R = ^{i}Pr (**56**), Mes (**57**)
X = BPh_4, R = ^{i}Pr (**58**)

Fig. 16 Dinuclear gold(I) bis–NHC complexes with anticancer properties.

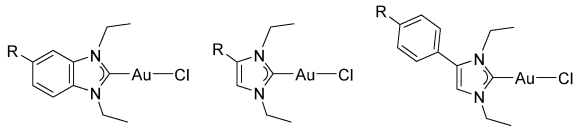


Fig. 17 Gold(I)–NHC complexes featuring anticancer and antibacterial properties.

effects (IC_{50} = 64.4 ± 6.2 μM) against HepG2 cells. Additionally, although complex **57** presented the best antiproliferative properties of the series with IC_{50} values of 42.2 ± 2.2 and 14.9 ± 0.9 μM for A549 and HepG2 cancer cells, respectively, its cytotoxicity was significantly lower than that observed for Auranofin and Cisplatin. The low solubility of the complexes in aqueous medium seems to be a possible explanation for these results.[30]

Several neutral chloride gold(I)–NHC complexes (**59–66**) were prepared and their biological properties investigated (Fig. 17). The antiproliferative properties of complexes **59–66** were evaluated in HT-29, MCF-7 and MDA-MB-231 cancer cell lines and non-tumor reference cell line (RC-124), and compared to that of Auranofin. In general, all of the complexes showed IC_{50} values in the range of 4–17 μM, affecting in the same manner non-tumor cells, which indicated that no bioselectivity for tumor cells was observed. Cellular uptake and protein binding studies revealed that the halide substituents of **65** and **66** might direct the complexes towards the proteins, whereas in the case of **64** cellular accumulation is the preferred route. In addition, the results of the investigations under serum-free conditions of the cellular uptake indicated that an intact Au–NHC fragment is required to accumulate large amounts of both gold and the NHC ligand in the cells. The inhibition of mammalian TrxR was also evaluated and compared to that found for Auranofin, which is known as a potent inhibitor of this enzyme in the nanomolar range (IC_{50} = 0.016 μM). The results indicated that all of the complexes were good inhibitors of TrxR displaying IC_{50} values ranging from 0.04 to 0.4 μM. This study also revealed a clear correlation between the incorporation of halogens in the backbone of the NHC ligand and the cytotoxic activity values found for complexes **60**, **61**, **63** and **66**. Finally, the authors investigated the apoptosis-inducing activity of complex **63** in two drug-resistant leukemia cells which overexpress drug efflux-transporter P-glycoprotein (P-pg). In this particular study, compound **63** induced apoptosis in both resistant cell lines, and such apoptosis was independent of Bcl-2 expression. Additionally, the gold levels found in both drug-resistant cell lines correlated very well with the induction of apoptosis. This fact indicates that the P-pg transporter is not involved in the transportation of the gold(I)–NHC complexes to the cell interior, and therefore, there must be another biological trafficking process implied in this case.[31]

Two pyrazine-based cyclometalated Au(III)–NHC complexes (**67** and **68**) were synthesized and the impact of the nature of the ancillary ligand on the biological properties was investigated (Fig. 18). The antiproliferative properties of both complexes were assayed against several cancer cell lines

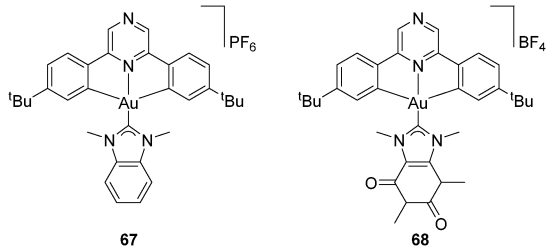


Fig. 18 Pyrazine-based cyclometalated gold(III)–NHC complexes with anticancer properties.

(HL60, MCF-7 A549, and MRC-5). Unlike caffeine-derived NHC complex **68**, which showed higher cytotoxicity than Cisplatin only in MCF-7 cells, complex **67** displayed excellent IC_{50} values in the low- to the submicromolar range (0.31 ± 0.15–7.8 ± 1.3 μM) in all cell lines. The cellular uptake experiments confirmed that the intracellular gold concentration of **67** was almost 11 times higher than that of the caffeine derivative (**68**). Additionally, the reactivity studies of complex **67** with glutathione suggested that this species is unlikely to trigger its antiproliferative effects *via* direct coordination of sulfur-donor-containing enzymes like TrxR or PARP-1. Finally, the study of DNA-binding properties indicated that both NHC-based compounds **67** and **68** interact in a dose-dependent manner with all of the DNA structures. Particularly, species **67** seemed to stabilize the telomeric i-motif structure of DNA even at physiological pH.[32]

Recently, several cyclometalated gold(III) complexes containing N-heterocyclic carbene ligands with varying alkyl chain length of the N-substituents (**69**–**74**) have been prepared and evaluated as anticancer agents in different cancer cell lines (HeLa, HCT116, and NCI-H460 (lung)) (Fig. 19). A clear trend was observed when increasing the alkyl chain length, which produced an enhancement of the cytotoxicity with IC_{50} values from 2.04 (**69**) to 0.36 (**72**) μM for HeLa cells. This trend was also observed for the other cancer cell lines.[33]

The relevance of this work resides in the target identification of anticancer gold complexes by the incorporation of a small photoaffinity diazirine group and a clickable alkyne moiety on NHC to generate complex **75** and also a benzophenone analogue **76** for comparison. These two gold(III)–NHC species also exhibited a potent cytotoxicity reaching IC_{50} values ranging from 0.23 to 0.68 μM. Among all of the complexes, derivative **72** presented the highest selective cytotoxicity against cancer cells, which was 30-fold less cytotoxic against MIHA (hepatocyte) cells. Additionally, complex **72** inhibited tumor growth by 71% without affecting

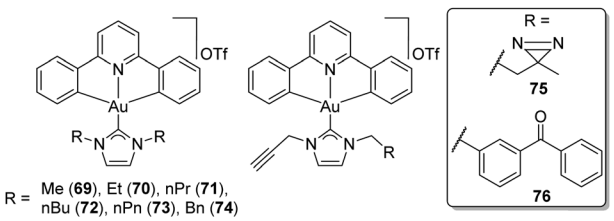


Fig. 19 Cyclometalated gold(I)–NHC complexes used for the specific identification in leukemia cells.

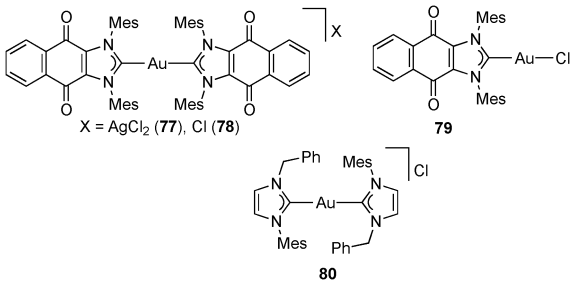


Fig. 20 1,4-Naphthoquinone fused gold(I)–NHC complexes with antioxidant properties.

survival after treatment of mice bearing xenografts of HeLa cancer (3 mg kg^{-1}). Finally, the photodynamic properties of complexes **75** and **76** allowed them to form covalent adducts with interacting proteins upon light irradiation, while the clickable alkyne moiety was used as a reporter (fluorophore) to determine the specific engagement of the gold(III) complexes with multiple cellular targets, including HSP60 (heat shock proteins), vimentin, nucleophosmin, and YB-1 (Y-box-binding protein 1).[33]

The design of systems to target the antioxidant pathway has been performed by Arumagam, Aranbula and coworkers with 1,4-naphthoquinone annulated N-heterocyclic carbene complexes **77**–**79** (Fig. 20).[34] As quinones can accentuate ROS production, the union between gold and NHC complexes bearing this quinone moiety could lead to a two-fold interruption of the antioxidant pathway *via* the ROS generation and inhibition of TrxR, which is an essential mediator of ROS homeostasis. The results were compared with controls of doxorubicin, a ROS enhancer, and complex **80**, which is a known good inhibitor of TrxR. Complex **77** produced a 27-fold increase in exogenous reactive oxygen species and TrxR inhibition, producing cell death *via* an apoptotic mechanism. This opens a new strategy in the design of new therapeutics by targeting key cancer pathways *via* multiple modes of action.

Recently, Ferraroni, Gratteri and co-workers investigated the binding mode of the cytotoxic gold(I)–bis-carbene species **81** (Fig. 21) to a telomeric DNA G-Quadruplex (Tel 23: 5′-TAGGG(TTAGGG)$_3$-3′). By means of ESI MS and X-ray diffraction analysis they observed that the bis-carbene compound (**81**) forms a supramolecular adduct in which the gold(I) species binds Tel 23 as an intact cation in different molar ratios. The XRD analysis also revealed two different binding modes from those found for typical host metal complexes. In this case, the results indicated that the bis-carbene binding is possible at the 3′–3′ site, as well as at the 5′–5′ site, interacting with G17 and G23 residues. They also found that only one caffeine unit per ligand molecule in **81**

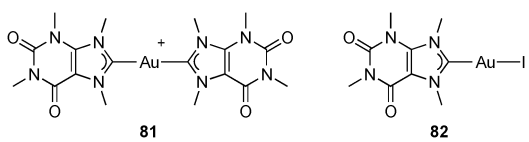


Fig. 21 Gold(I)–NHC complexes used for the study of DNA G-quadruplex binding.

interacts with the guanine residues, which may explain the greater selectivity of the gold(I) species for DNA G-quadruplex motifs over double-helix DNA.[35]

More recently, the related gold(I) binding mode mentioned above was studied by Leoni, Casini and coworkers for the first time by metadynamics. In this work, the interaction of complexes **81** and **82** with a human telomeric DNA G-quadruplex (hTelo) and an oncogene promoter sequence (C-KIT1) was theoretically addressed in order to get more insights into this ligand/target recognition process. The results were compared to those obtained from the gold complexes/G4 binding assays using fluorescence resonance energy transfer (FRET) DNA melting. From the metadynamics calculations, the authors found that the hTelo's trajectories with complex **81** displayed two possible binding sites. In one of these states with the lowest energy (*ca.* −37 kJ mol^{-1}) the gold(I) species is interacting with both adenine (A13) and guanine (G4 and G22) residues. On the other hand, in the higher energy state (*ca.* −14 kJ mol^{-1}) the bis-carbene motif (**81**) only interacts with the loop thymine (T11). A similar behavior was observed for NHC–Au(I)–NHC binding to C-KIT1, showing two different states, but this time associated with the interaction with a guanine (state I) by means of π-stacking interactions, and a second state in which the caffeine groups are interacting with both A1 and G6 residues. As expected, the monocarbene species (**82**) displayed a similar binding mode as observed for compound **81**; however, in accordance with the experimental results obtained from the DNA FRET melting profiles, the lack of a second caffeine ligand produced a lower Gibbs free-energy value.[36]

Antibacterial activity

Traditionally, silver is the metal of choice for antibacterial activity and it is known that the bacterial cell membrane is the primary target for Ag^+ ions. However medical applications are somehow limited by toxicity originated by the interaction between silver cations and blood cysteine. Consequently, gold complexes have emerged as possible antibacterial agents, and many complexes as Auranofin, its analogues and other species have shown excellent activities. In this regard gold–NHC complexes are more stable than the corresponding silver ones, thus facilitating better platforms for the design of new metallic drugs. Previous studies have shown that gold–NHC complexes inhibit bacterial proliferation by blocking cytokinesis.[11]

As mentioned above, gold–NHC complexes (**59–66**) depicted in Fig. 17 were used to evaluate their antibacterial effects in several Gram− (*A. baumannii*, *E. cloacae*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*) and Gram+ (*E. faecium* and *S. aureus*) bacteria and compared to Auranofin. In general, the Gram-negative bacteria presented high resistance to the treatment of gold(I) complexes **59–66**. However, the complexes were very effective against Gram-positive strains (*E. faecium* and *S. aureus*) exhibiting minimal inhibitory concentration (MIC) values ranging from 0.64 ± 0.14 to 12.51 ± 0.00 μM, which were lower than that obtained for the reference metallodrug. Additionally, all of the

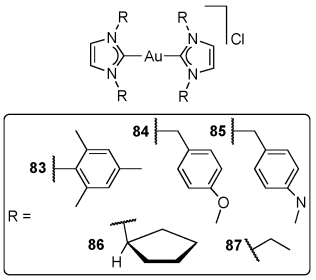


Fig. 22 Cationic gold(I)–NHC complexes with antimicrobial properties.

complexes proved to be very effective inhibitors against bacterial TrxR displaying an IC_{50} from 0.1 to 0.5 μM. The results obtained suggested that the incorporation of halogen substituents had a negative effect on the inhibition of *E. coli* TrxR.[31]

Several bis(NHC) gold derivatives (**83–87**) have been prepared and the antimicrobial studies performed (Fig. 22). The compounds are highly active towards Gram+ and Gram− bacteria. The authors found that better activities were achieved with mesityl or *para*-substituted benzyl derivatives on the NHC nitrogen atoms. Especially the *p*-methoxybenzyl species (**84**) showed excellent MIC values for the bacteria *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Enterococcus faecalis*, in contrast with the *p*-dimethylaminobenzyl derivative which is more active in the bacteria *Escherichia coli*. This allowed the conclusion that although the exact mechanism of action is unknown, it is clear that the substituents on the nitrogen atoms of the imidazole ring are responsible for the different activities in bacteria.[37]

Pyrazine functionalized Au(I)–NHC complexes were prepared and shown to be excellent antibacterial agents against human pathogens that are resistant to several antibiotics (Fig. 23). These complexes presented better antimicrobial activity than other reported complexes and commercial antibiotics. In this study, the neutral mononuclear complex **88** was more effective than the cationic bis-carbene species **89**, and again, both presented better antimicrobial properties against Gram-positive than Gram-negative bacteria. As observed from the flow cytometric and SEM analysis, the mechanism of action seems to be a strong interaction with the negatively charged bacterial cell membrane, by bonding to the Lys and Dap-Type peptidoglycan layers that causes extensive damage to the bacterial cell wall.[38]

The well-known gold drug Auranofin has shown important antimicrobial activity against bacteria and the reductive enzyme thioredoxin reductase has been identified as the main target.

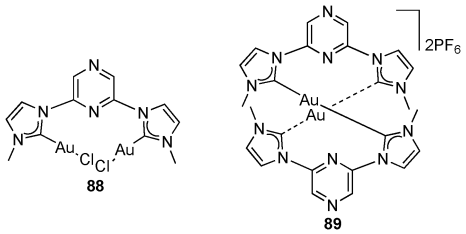


Fig. 23 Dinuclear Au(I)–NHC complexes as antibacterial agents.

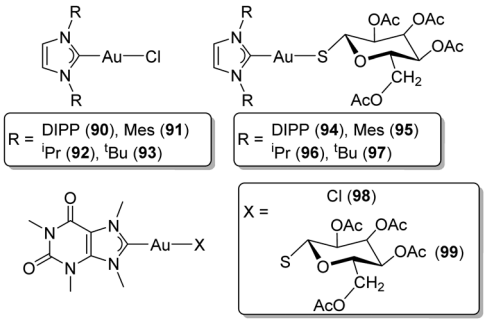


Fig. 24 NHC–gold Auranofin analogues with potent antiproliferative properties against bacteria *Helicobacter pylori*.

For that reason, several chloride and Auranofin analogues containing N-heterocyclic carbene ligands and caffeine derivatives (**90–99**) have been prepared and proved to inhibit or kill *Helicobacter pylori in vitro* (Fig. 24). The study showed that these gold–NHC compounds, especially *tert*-butyl and caffeine derivatives, inhibited the bacterial growth at 2 μM, which is in the same order as for Auranofin (MIC = 1.2 ± 0 μM). Moreover, all of the complexes displayed lower toxicity (20.7–58.0 μM) than Auranofin (3.0 ± 0 μM) against HEK-293 T cells (human embryonic kidney cells). Although the Au(I)–NHC derivatives could be well tolerated, further evaluation of the complexes *in vivo* is needed. Additionally, the authors showed a synergistic effect between Auranofin and the antibiotic amoxicillin, targeting both the enzyme TrxR and the cell wall synthesis, which opens new perspectives for the use of a combination therapy.[39]

A series of gold(I) complexes bearing N-heterocyclic carbenes and the steroid derivatives ethynylestradiol and ethisterone have been synthesized and their antibacterial activity evaluated (Fig. 25). Good activities of the gold species were found against *Staphylococcus aureus* and *Escherichia coli* bacteria. Unlike the gold(I)–NHC complexes, which inhibited bacterial growth, the steroid precursors did not present antibacterial activity alone. This is in accordance with the crucial role of the presence of a gold center in this system. In addition, the *in vivo* antimicrobial activity has been tested on *Galleria mellonella* larvae against *E. coli* showing the positive effect of the presence of the estradiol group, which greatly increases the activity.[40]

A series of biscarbene gold(I) complexes with variation in the substituents of one of the carbon atoms of the imidazole ring

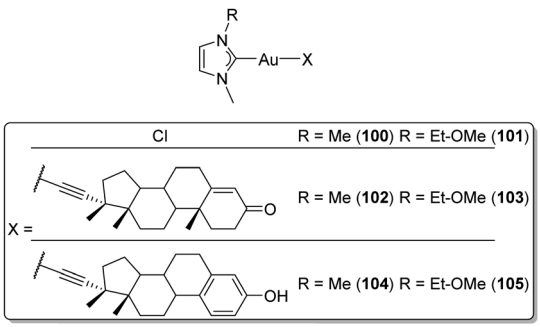


Fig. 25 Steroid-Au(I)–NHC complexes with antimicrobial properties.

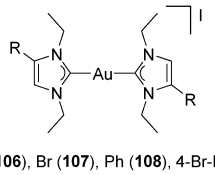


Fig. 26 Gold(I) bis-NHC complexes with antibacterial and anticancer properties.

have been reported (Fig. 26). The structure–activity relationship regarding the antibacterial activity, cytotoxicity and TrxR inhibition has been studied. The antibacterial effects were moderate and showed a preference for the Gram+ strains (**108** > **107** = **109** > **106**), suggesting a positive effect of the phenyl group. Particularly, complex **108** showed potent activity against methicillin-resistant *Staphylococcus aureus* (MRSA) (≈2 μM). In general, the inhibitory activity displayed by the gold complexes could be associated with moderate to low activities obtained from the experiments on the inhibition of mammalian and bacterial TrxRs. However, the complexes presented excellent antiproliferative activity in the MCF-7 cancer cell line, good cellular uptake, and inhibition of TrxR activity. Further studies are needed to separate or compare the cytotoxic and the antibacterial properties and for that more structure–activity relationship studies should be performed.[41]

Antioxidant activity

The *in vitro* antioxidant activity and the *in vitro* α-glucosidase, thymidine phosphorylase, β-glucuronidase and xanthine oxidase enzyme inhibition potentials for **110–118** complexes (Fig. 27) have been evaluated by Nolan and coworkers. The inhibitory activity of different derivatives towards α-glucosidase, thymidine phosphorylase and β-glucuronidase enzymes exhibited higher activity than the standard drugs (Table 2). Nevertheless, with the exception of the aurate salts **131** and **132**, the other compounds did not present antioxidant activity and were weak or inactive inhibitors of the xanthine oxidase enzyme. In general, the chloro complexes **116** and **117** were found to be the most potent

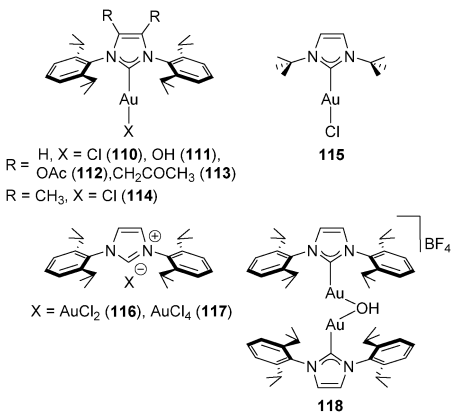


Fig. 27 Potent α-glucosidase, thymidine phosphorylase, and β-glucuronidase gold–NHC inhibitors.

Table 2 The *in vitro* α-glucosidase, thymidine phosphorylase and β-glucuronidase inhibition activity of complexes **110–126**

Compound	α-Glucosidase IC_{50} (μM)	Thymidine phosphorylase IC_{50} (μM)	β-Glucuronidase IC_{50} (μM)
110	36.6 ± 1.67	16.86 ± 0.7	2.60 ± 0.15
111	2.8 ± 0.51	—	0.14 ± 0.08
112	23.7 ± 2.42	13.63 ± 0.5	0.54 ± 0.03
113	3.9 ± 0.42	—	0.42 ± 0.12
114	91.5 ± 0.51	14.8 ± 0.9	0.66 ± 0.06
115	1.45 ± 0.05	3.4 ± 0.2	0.45 ± 0.16
116	0.8 ± 0.01	3.6 ± 0.2	7.9 ± 0.49
117	0.7 ± 0.01	3.2 ± 0.2	1.10 ± 0.05
118	2.6 ± 0.05	6.4 ± 0.2	0.27 ± 0.03
119	7.3 ± 0.23	—	21.5 ± 0.76
120	6.6 ± 0.11	—	1.03 ± 0.08
121	99 ± 0.95	—	17.7 ± 0.94
122	12.1 ± 0.13	—	9.28 ± 0.25
123	—	—	3.87 ± 0.06
124	1.5 ± 0.01	—	1.46 ± 0.06
125	—	—	1.28 ± 0.02
126	1.5 ± 0.1	—	2.27 ± 0.08
Acarbose	840 ± 1.73	—	—
7-Deazaxanthine	—	41 ± 1.46	—
D-Saccharic acid 1,4-lactone	—	—	45.75 ± 2.16

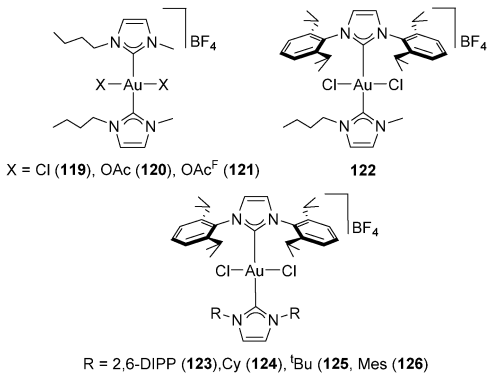


Fig. 28 Potent α-glucosidase and β-glucuronidase gold(I)/(III)–NHC inhibitors.

inhibitors of the series. The authors suggested that the presence of chloro ligands attached to the gold centre was essential for obtaining these results, although they did not clarify this statement.[42]

In a subsequent study, the inhibitory activity of the gold(III) derivatives (**119–126**) (Fig. 28) against α-glucosidase and β-glucuronidase enzymes was studied by the same research group. In general, the tested complexes showed higher activity than standard drugs (Table 2). On the other hand, they could observe that for α-glucosidase the most active complexes of the series were **124** and **126** (no clear trend in structure/activity relationship could be found) while for β-glucuronidase the compound containing acetate ligands (**120**) was found to be the most potent inhibitor.[43]

Antiparasitic activity

Cationic bis-carbene gold(I) derivatives (**127** and **128** in Fig. 29) exhibited a very high trypanocidal activity against *Trypanosoma*

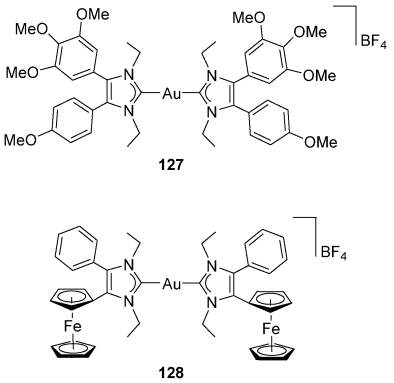


Fig. 29 Gold(I) bis-NHC complexes (**127** and **128**) with trypanocidal activity.

brucei parasites with IC_{50} values of 3 and 0.9 nM, being more potent than the well-known tubulin binders colchicine (IC_{50} = 1.4 μM) and vinblastine (IC_{50} = 0.1 μM). Additionally, these complexes presented index selectivity values (SI) of 77 and 148, respectively, which make them possible candidates for further *in vivo* testing of *T. b. brucei* models. The severe destruction of the cytoskeleton may be the mode of action of these species. On the other hand, a significant selectivity to the trypanosomes, in particular, for the ferrocene compound (**128**) was observed. High doses (10 μM) of this complex can inhibit the tubulin polymerization or cause destabilization of the intact microtubules.[44]

Antileishmanial activity

Leishmaniasis is a tropical disease caused by a parasite of the genus *Leishmania* and it is transmitted by the bite of an infected female phlebotomine sandfly. This parasite exists in two forms: the flagellated promastigote in the sandfly vector, and the amastigote in the mammalian host. Three types of clinical forms of leishmaniasis have been highlighted: visceral, cutaneous and mucocutaneous. These diseases may cause multiple lesions in the internal organs, skin, nose, throat, and mouth. At present, there are very few drugs to treat leishmaniasis, such as pentavalent antimonials, liposomal amphotericin B, pentamidine, paromomycin, and miltefosine. In addition, these drugs present several limitations including a very expensive cost and adverse effects on human health. In particular, reports describing gold complexes with antileishmanial activity are scarce. Here, we compile all the studies reported on the synthesis of gold–NHC species used in the treatment of leishmaniasis.

Hemmert and co-workers described the first examples of gold(I)–NHC derivatives with antileishmanial properties. They synthesized the mononuclear cationic and neutral gold(I) complexes containing functionalized quinolines (Fig. 30). All of the compounds were tested *in vitro* against the promastigote stage of *infantum*, and the most selective species were also assessed on the intracellular amastigote stage of *L. infantum*. The selectivity index (SI = CC_{50}/IC_{50}) values were obtained from the cytotoxic concentrations 50% (CC_{50}) for the murine J774A.1

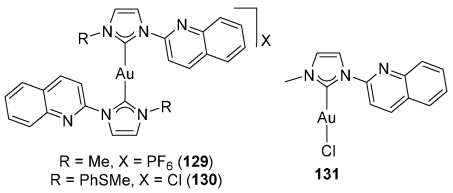


Fig. 30 The first cationic and neutral gold(I)–NHC derivatives containing functionalized quinolines with antileishmanial properties.

macrophage cell line. The two cationic Au(I) bis(NHC-quinoline) compounds **129** and **130** and the neutral complex **131** with IC_{50} values of 0.39 ± 0.14, 0.43 ± 0.26 and 1.53 ± 0.62 μM, respectively, presented the best selectivity index for promastigote (5.33, 3.02 and 6.19, respectively). Taking into account these results, the complexes were additionally evaluated with respect to the intracellular amastigote stage and, thereby the real antileishmanial potential of these species was determined. For the three derivatives, the antiproliferative activity against amastigote with IC_{50} values below 1 μM was observed, highlighting the neutral complex **131**, which presented an IC_{50} value of 0.96 ± 0.55 μM and a selectivity index near 10 (9.84). This complex displayed a promastigote IC_{50} value 38 times and 3 times higher than that of amphotericin B (IC_{50} = 0.04 ± 0.03 μM) and pentamidine (IC_{50} = 0.51 ± 0.28 μM) but 5.8 times lower than that of miltefosine (IC_{50} = 8.83 ± 2.77 μM). The amastigote IC_{50} value is 4.3 times lower than that of miltefosine. In general, the charge of the complex seems to be a relevant point for the lower selectivity observed for the cationic species as compared with the neutral derivative **131**.[45]

Very recently, the same research group carried out a complementary study for the evaluation of the antileismanial activity of several neutral gold(I)–NHC complexes.[37] Firstly, they prepared three more quinoline functionalized NHC–gold(I) complexes by the substitution of the methyl moiety in compound **131** by different aryl or alkyl groups (**132–134**). They also synthesized various gold(I)–NHC complexes containing the benzyl group (**135–137**), as depicted in Fig. 31. All of the neutral gold complexes studied were found to be cytotoxic against promastigote and axenic amastigote forms of *L. infantum*, exhibiting IC_{50} values ranging from 2.52 to 11.16 μM and from 0.19 to 2.17 μM, respectively. In particular, the authors found that in the quinoline containing gold(I)–NHC complexes, the nature of a second alkyl or aryl group did not show a significant effect on the selectivity towards antiamastigote activity. On the other hand, the benzyl analogues presented better results, especially for complex **136**, which displayed the best selectivity index for promastigote (3.09) of the series, possibly associated with the better stability due to the presence of the mesityl group. In accordance with this, complex **136** showed excellent results in terms of antiamastigote activity and selectivity, reaching values of 0.19 μM and 40.29, respectively, the latter being much higher than that obtained for the standard drug Amphotericin B (24.11).[46]

The *in vitro* antileishmanial properties of complexes **110–118**, and **119–126** have also been evaluated (Table 3).[42,43]

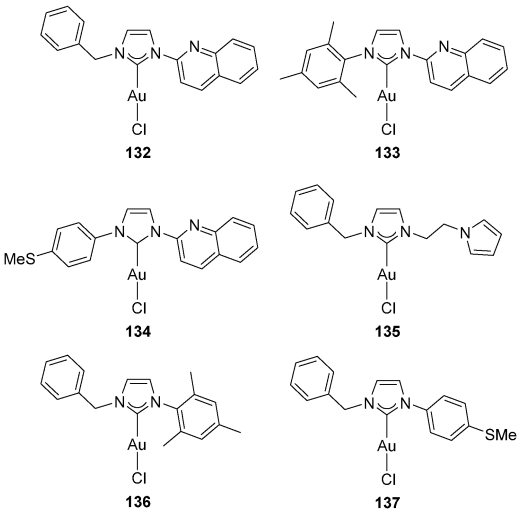


Fig. 31 Quinoline functionalized NHC–gold(I) complexes (**132–137**) evaluated as antileismanial agents.

Table 3 The *in vitro* antiprotozoal activity of complexes **110–126** against *Leishmania major* promastigotes

Compound	*Leishmania major* promastigotes IC50 (μM)
110	8.2 ± 0.24
111	8.94 ± 0.49
112	5.50 ± 0.93
113	5.36 ± 0.15
114	17.25 ± 0.847
115	8.38 ± 0.629
116	5.19 ± 0.471
117	4.60 ± 0.082
118	3.01 ± 0.29
Au(I) precursor of **119**	
119	0.37 ± 0.07
120	0.86 ± 0.2
121	0.35 ± 0.31
122	0.32 ± 0.04
123	0.12 ± 0.03
124	1.62 ± 0.02
125	0.33 ± 0.02
126	0.34 ± 0.12
Pentamidine	14.96 ± 0.264
Amphotericin B	0.13 ± 0.01

Although derivatives **110–118** displayed higher antileishmanial activity than the standard drug pentamidine (14.96 μM),[42] they displayed moderate activity as compared with the standard drug amphotericin B (0.13), resembling those obtained against *L. infantum* promastigotes by Hemmer and co-workers.[46] On the other hand, as observed for the cationic bis-carbene complexes described by Hemmer and co-workers,[45] the cationic gold(III)–NHC species (**119–126**) evaluated in this study also presented a significant decrease of the *in vitro* antileishmanial properties (0.12–0.86 μM).[43] It is noteworthy that other research studies have associated the potent antileishmanial activity observed for gold compounds with their ability to interact with DNA, leading to a drastic disorganization of the mitochondria.[47] In this particular case, the increased cytotoxicity of the synthesized compounds could be related to the presence of cationic species

or the formation of a more electrophilic gold(III) center. Nevertheless, because of the poor number of studies involving gold–NHC complexes as antileishmanial agents, it is rather difficult to establish a clear structure–activity relationship.

Antimalarial activity

Heterobimetallic Ru(II)–Au(I) complexes (**138–140**) were also tested for their biological activities against *Leishmania infantum* and *Plasmodium falciparum*, FcB1-Colombia strain (Fig. 32). In the first case, the compounds did not present relevant antileishmanial properties while they showed a moderate antiplasmodial activity with IC_{50} values of 25.5 ± 5.6 μM (**138**), 16.1 ± 2.2 μM (**139**) and 15.8 ± 4.1 μM (**140**).[47]

The *in vitro* antimalarial activity against the 3D7 strain of *Plasmodium falciparum* of the gold(I)–NHC complex **141** (Fig. 33) has been assessed. The results showed that this compound was much less active than the control drug (chloroquine) with IC_{50} values of 5.1 ± 1.1 and 0.0091 ± 0.00086 μM, respectively. In addition, when using concentrations 5–20 times higher than the corresponding IC_{50} values for the gold compound **141**, significant hemolysis was observed. This may be indicating that the antimalarial activity of the gold derivative could be associated with host cell membrane perturbations.[48]

The *in vitro* antimalarial activity against the chloroquine-resistant *P. falciparum* strain FcM29-Cameroon of two different families of complexes based on NHC ligands has been studied. The first family contains a series of mono- and dinuclear silver(I) and gold(I)/(III) compounds the dinuclear silver(I) species of which displayed the best antiplasmodial activities, but also strong hemolytic properties. This indicates the absence of specific activity of these derivatives against pathogens and thus, a cytotoxic activity. The second group of silver(I) and gold(I) complexes were obtained by means of different pharmacomodulations with the idea of increasing the antiplasmodial activity and diminishing their cytotoxic effect. The two mononuclear gold(I)–bis(NHC) derivatives (**129** (Fig. 30) and **142** (Fig. 34)) showed the best antiplasmodial properties in extemporaneous conditions, with IC_{50} values of 1.1 and 0.33 μM, respectively. The activity was almost the same after conservation of the stock solution in DMSO at 4 °C for 4–6 days (IC_{50} = 1.8 and 0.49 μM, respectively). It is noteworthy that for these complexes no hemolysis has been observed after modification. These results could be rationalized as a consequence of the lipophilicity, basicity, and structural features characteristic of these species, due to the presence of nitrogen containing heterocycles in the series of mononuclear gold(I) complexes.[49]

In a subsequent study, the same research group tested the *in vitro* cytotoxic activity of a series of cationic, neutral and anionic mononuclear gold(I) derivatives against *P. falciparum* strains and compared that to chloroquine and artemisinin, which are standard control drugs. The best results were obtained for the cationic compounds **144**, **145**, **146**, and **147**, (Fig. 35) with IC_{50} values of 2.1, 0.48, 0.21 and 0.32 μM, respectively, *vs.* 0.514 ± 0.02 μM (chloroquine) and 0.010 ± 0.7 μM (artemisinin).[50] This is consistent with preliminary studies on complexes **129** and **142**,[45,49] which showed that the antiplasmodial properties increase for cationic species, which could be associated with the better transportation of cationic molecules through the parasite cell membrane. Furthermore, the modulation of the steric properties of the NHC ligand has a crucial influence on the antiparasitic activity. As observed for complexes **143–146**, the protective effect of the ligand around the metal as well as the antiplasmodial properties increases from methyl (**143**: 22 μM) to mesityl (**146**: 0.21 μM). The increase of the steric hindrance avoids the release of the gold cation before getting its target. On the other hand, the authors mentioned that the lipophilic character of the complexes could allow them to cross the different parasite cell membranes more effectively. According to this, the highest lipophilic complex **146** is the most active against *P. falciparum*.[50]

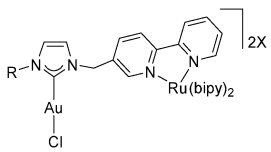


Fig. 32 Heterobimetallic Ru(II)–Au(I) derivatives (**138–140**) evaluated against *Leishmania infantum* and *Plasmodium falciparum*, FcB1-Colombia strain.

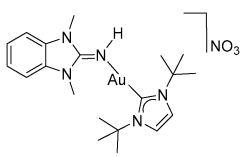


Fig. 33 NHC–gold(I) compound **141** tested against the 3D7 strain of *Plasmodium falciparum*.

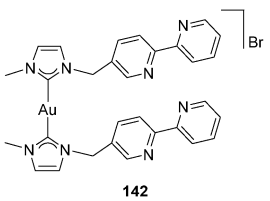


Fig. 34 Cationic gold(I)–bis(NHC) species **142** with antimalarial properties.

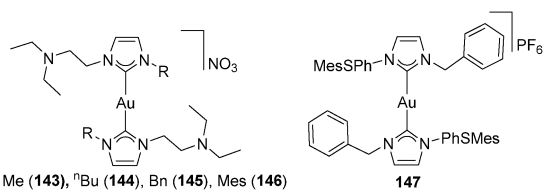


Fig. 35 Cationic compounds (**143–147**) with the best cytotoxic activity against *P. falciparum* strains.

Conclusions

N-Heterocyclic carbenes have become excellent scaffolds in medicinal chemistry. The ease of their modification allows a wide library of ligands to be prepared, which permits the tunability of the properties conferred to the metal complexes and gold in particular. Furthermore, the great stability of gold–NHC compounds in the biological media provides robustness towards ligand dissociation which may be key in the mechanism of action and pharmacological effect of these derivatives. Excellent antitumor activities have been achieved in both neutral and cationic gold–NHC complexes, compared with the reference Cisplatin or Auranofin. It has been noted that the substituents of the NHC imidazole ring are very important in the activity, for example ferrocenyl groups greatly enhance the anticancer effects. The use of bimetallic species with a synergistic effect between both moieties has been also revealed as a good approach to improve the activity. Cationic lipophilic gold(I) bis(carbenes) have been revealed to selectively accumulate in the mitochondria of cancer cells because of the elevated mitochondrial membrane potential. Additionally, it has been proved that targeting the antioxidant pathway by combination of ROS accentuators and TrxR inhibitors greatly enhances ROS production and cell death. Furthermore, several binding modes of some of these bis(carbene) species to G4 structures have been calculated by metadynamics, which highlights the great potential of bioinformatics in orienting drug design. Several targets have been identified, the inhibition of TrxR or ROS generation being the most important ones, because their lipophilic character modulates their preference to accumulate in mitochondria. Nevertheless, this cannot be seen as the only mechanism of action, and other intracellular targets have been considered, as other cellular proteins containing sulfur atoms such as ubiquitin-proteasome, tyrosine-kinases, and topoisomerase, for which strong bonding interactions with the gold centers exist, and also several DNA G-Quadruplexes have been identified. An advance that could help to understand the mechanism of action is the use of gold–NHC compounds bearing fluorophore groups, which allows knowing the localization inside the cell, as has been already proved in some complexes. Multiple targeting of known cancer pathways could also represent an interesting procedure for the synthesis of very efficient drugs. Additionally, bioinformatics could provide valuable information about binding interactions of drugs with biological molecules, and could contribute to drug design and development. Gold–NHC compounds show a superior antimicrobial activity in several bacterial strains and targeting of the TrxR and the cell wall synthesis has been observed. Other potential applications of the gold–NHC species as antioxidant and antiparasitic agents have emerged and could be relevant in diseases such as leishmaniasis where there is no optimal drug treatment. In conclusion, important advances have been made in gold–NHC compounds displaying a wide range of biological properties, and this could be relevant for future applications as metallodrugs. The efforts associated with this research field should focus on the development of a targeted therapy, with improved delivery and efficacy, together with a good *in vivo* antitumor activity and identification of the mechanism of action. This could lead to the entry of gold–NHC complexes in clinical trials, which is one of the challenges of future research.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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