Special topic

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Anticancer properties of gold complexes with biologically relevant ligands

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Abstract: The present review highlights our findings in the field of antitumor gold complexes bearing biologically relevant molecules, such as DNA-bases, amino acids or peptide derivatives. The results show that very active complexes are achieved with this sort of ligands in several cancer cells. In these compounds the gold center is bonded to these biological molecules mainly through a sulfur atom belonging to a cysteine moiety or to a thionicotinic moiety as result of the functionalization of the biological compounds, and additionally phosphines or *N*-heterocyclic carbenes are present as ancillary ligands. These robust compounds are stable in the biological media and can be transported to their targets without previous deactivation. The presence of these scaffolds represents a good approach to obtain complexes with improved biologically activity, better transport and biodistribution to cancer cells. Thioredoxin reductase (TrxR) has been shown as the main target for these complexes and in some cases, DNA interactions has been also observed.

Keywords: amino acids; antitumor compounds; Distinguished Women in Chemistry and Chemical Engineering; DNA-base derivatives; gold complexes; peptides.

Introduction

The medicinal chemistry of gold compounds has been explored throughout the history from the earliest civilizations. Gold has been used in the form of medicinal preparations or elixirs for different illness in countries like China, India, Egypt and later in Europe. The earliest therapeutic application of gold compounds dates from 2500 B.C. in China, where gold was used to treat furuncles, smallpox or skin ulcers and also was associated as a medicine to seek longevity [1]. Although gold medicines have historically been used for the treatment of a wide range of ailments, the use of gold in medicine with a more scientific basis began in the early 1920s when the gold compound $K[Au(CN)_2]$ was clinically tested for its in vitro bacteriostatic effect and employed by Robert Koch to kill mycobacteria, the bacteria that causes tuberculosis. Its toxicity in the treatment of the tuberculosis made that other gold species were used with this purpose, such as gold(I) thiolate complexes [2].

In the early 1930s, Jacques Forestier introduced the same thiolate complexes for the treatment of rheumatoid arthritis, which at that time was believed to be related to tuberculosis. These compounds have been utilized for a long time as the drug of choice for the treatment of rheumatoid arthritis. However, the undesired side-effects at high concentrations, mainly nephrotoxicity, gave rise to the search for less toxic gold compounds. Thus, a second generation of gold drugs evolved, Auranofin, [(tetra-O-acetyl-β-D-glucopyranosyl)-thio]

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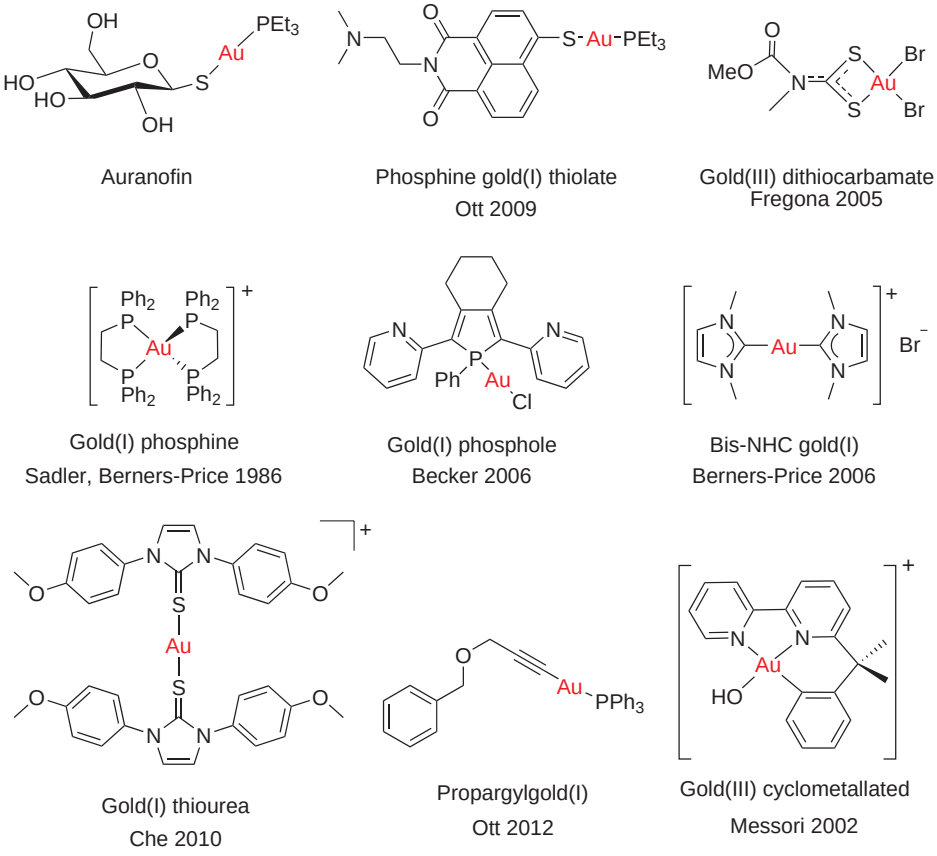


Fig. 1: Gold complexes with antitumor activity [15–22].

(triethylphosphine)-gold(I) (Fig. 1), which can be orally administrated, was designed and approved for the therapy of rheumatoid arthritis in 1985 [3].

The research carried out by Rosenberg and co-workers in the 1960s led to the discovery of the activity of cisplatin as chemotherapeutic drug for cancer treatment [4]. This opened a new world of opportunities for metal compounds as potential anticancer drugs. This fact, together with the immunodepressive and anti-inflammatory effects that some antitumor drugs produce, impulsed the research in the antitumor activity of auranofin and other related gold complexes. Nowadays, gold complexes have exhibited excellent antiproliferative activity in several tumor cell lines [5–10] and Auranofin itself is on clinical trials for the treatment of leukaemia cancer [11]. Additionally, mechanistic studies have showed that the interaction of gold complexes with DNA is not as strong as that found for platinum drugs, suggesting different pathways for the cytotoxicity. These studies conducted to the observation that relevant gold–protein interactions could be the potential biological targets responsible for the cytotoxic effects of gold compounds. Gold has a great tendency to bind sulfur donor ligands and, consequently, most of the thiol containing enzymes could be potential targets that are, in addition, overexpressed in cancer cells, thus providing potential anticancer targets for gold complex therapy. Mitochondria and pathways of oxidative phosphorylation are among the primary intracellular targets, being the inhibition of the seleno-enzyme thioredoxin reductase (TrxR), glutathione reductase, cathepsin cysteine proteases, deubiquitinases, cysteine proteases regulating the ubiquitin system or protein tyrosine phosphatases, the main responsible pathways for the cytotoxicity of gold compounds [12–14].

Stability of the gold compounds in the biological media is an important feature for the development of gold-based drugs, and for that reason complexes bearing strong ligand-metal bonds have been mainly chosen. Consequently, the archetypal sort of ligand which has been used to build biologically active gold compounds are thiolates [15], dithiocarbamates [16], phosphines or phospholes [17, 18], *N*-heterocyclic carbenes [19], thiourea [20], alkynyl [21], or cyclometallated ligands [22], for which many examples have been

reported in the last decades with excellent activity (see some examples in Fig. 1). Another important fact, in addition to the activity, is drug distribution and selectivity and for this reason ligands bearing biologically relevant molecules have been used with the purpose to improve these important aspects.

This review aims to cover the contributions of our group in the synthesis of anticancer gold complexes using relevant biological ligands, including thiolate complexes derived from DNA bases, which could be denominated as Auranofin analogues, or with amino acids or peptides, which are key molecules with an important role in many biological processes and additionally are overexpressed in cancer cells which, consequently, could increase the selectivity to target cancer cells.

Gold complexes with thiolate-based biomolecules

Thiolate metal complexes have received a great interest in different areas of research and special remark deserves their application in bioinorganic chemistry, mainly because of their presence in very diverse metalloproteins and metalloenzymes [23, 24], and in medicine as structural models for bioinorganic metallodrugs [25] or the use of gold thiolates in the treatment of arthritis [26].

Those complexes containing the P–Au–S unit have played an important role in the development of gold drugs because their structural similarity to Auranofin. Many efforts have been devoted to the synthesis of Auranofin analogues in the search for improved activity and selectivity of the final complexes. Special attention has received those thiolate ligands derived from molecules with intrinsic antitumor activity or arising from biological relevant molecules. The use of thiolate ligands derived from DNA bases such as thiouracile, thiocytoxine, thioguanine, etc. may represent a good approach to obtain complexes with improved biological activity, and with better transport and biodistribution in cancer cells. For this reason, we have reported several examples of gold complexes bearing this sort of molecules together with diverse ancillary ligands specially designed to improve properties such as the balance between lipophilicity and hydrophilicity as well as the cellular uptake.

As commented, the ancillary ligands bonded to gold are also important because they can provide the necessary stability in the biological media and can contribute to the final properties of the complexes. In this sense, aminophosphane is a family of ligands with the suitable characteristics to give access to metal complexes capable of forming hydrogen bonds, which is an important property in bioinorganic chemistry to produce more active compounds. With the main aim to develop biologically active compounds, a series of new gold(I) thiolate derivatives with different aminophosphanes as auxiliary ligands have been described. Thus, specifically 2-(diphenylphosphanylamino)pyridine (Ph_2PNHpy) and 3-(diphenylphosphanylamino)-1,2,4-triazole [$Ph_2PNH(Htz)$] were first synthetized and, then, used to prepare several mononuclear gold(I) compounds with thiolates starting from complexes **1** or **2** (Fig. 2) [27].

Complexes **3–6** were prepared by reaction between complex **1** and the respective thiol such as 2-mercaptopyridine (for **3**), 2-mercaptonicotinic acid (for **4**), 2-thiouracil (for **5**) or 2-thiocytosine (for **6**) in the presence of sodium carbonate or triethylamine. In contrast, complexes **7–9** were synthesized starting from **2** and the corresponding thiol such as 2-mercaptopyridine (for **7**), 2-thiocytosine (for **8**) or 6-thioguanine (for **9**) and trimethylamine.

The antitumor properties of complexes **1–9** were tested in vitro against two tumor human cell lines, HeLa (derived from cervical cancer) and MCF-7 (derived from breast cancer). Some of them showed excellent cytotoxic activity, especially derivatives with the PPh_2NHPy aminophosphine ligand (**3–6**, IC_{50} values from 1.08 to 1.34 μM) which showed excellent cytotoxic activity against MCF-7 cells, in comparison with complexes **7–9** (IC_{50} values from 2.89 to 17.38 μM) (Table 1) [28].

Complexes **3–6** also showed interesting cytotoxic activities against the HeLa cell line, especially complexes **3** and **4** (IC_{50} values of 1.70 and 0.91 μM, respectively), and complex **9** with a 0.34 μM for HeLa cell line. As control, we also investigated the antiproliferative effects of the metal-free ligands and chloride gold(I) derivatives **1** and **2** against the tumor cell line HeLa.

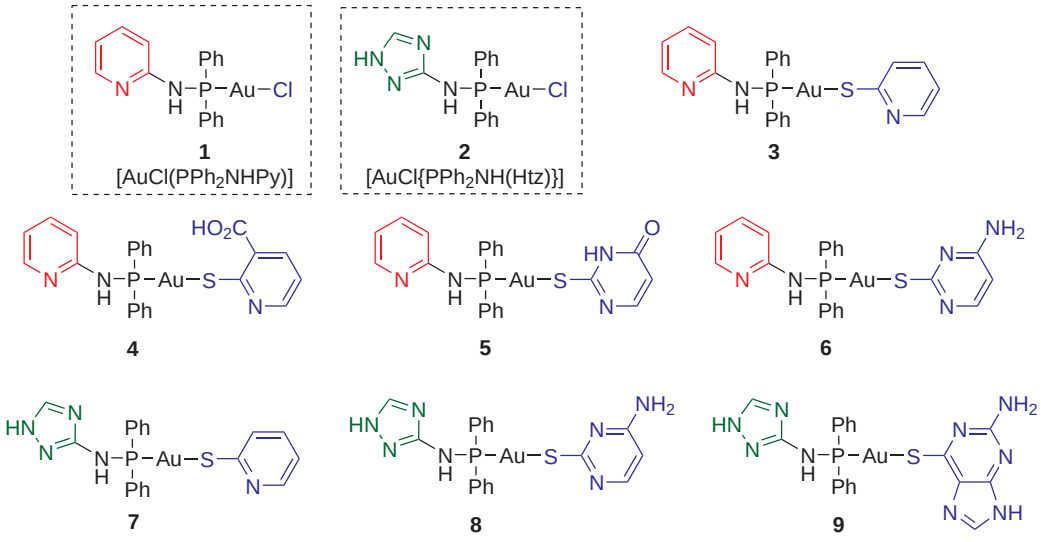


Fig. 2: Gold(I) complexes bearing thiolate functionalized DNA-bases and aminophosphane ligands.

Table 1: IC_{50} (48 h, μM) of complexes **1–9** against HeLa and MCF-7 cell lines.

	HeLa	MCF-7
Cisplatin	10.50 ± 1.57[a]	19.04 ± 1.51[a]
Auranofin	5.99 ± 0.01	1.11 ± 0.30[b]
[AuCl(PPh_2NHPy)] **1**	25.04 ± 0.1	
[AuCl{PPh_2NH(Htz)}] **2**	>50	
3	1.70 ± 0.05	1.17 ± 0.06
4	0.91 ± 0.16	1.14 ± 0.08
5	3.29 ± 0.13	1.34 ± 0.07
6	3.25 ± 0.02	1.08 ± 0.13
7	4.34 ± 0.03	5.55 ± 0.34
8	18.96 ± 3.09	2.89 ± 1.07
9	0.34 ± 0.08	17.38 ± 1.22

[a]C. Marzano, V. Gandin, M. Pellei, D. Colavito, G. Papini, G. G. Lobbia, E. D. Giudice, M. Porchia, F. Tisato, C. Santini. *J. Med. Chem.* **51**, 798 (2008). [b]I. Ott, T. Koch, H. Shorafa, Z. Bai, D. Poeckel, D. Steinhilber, R. Gust. *Org. Biomol. Chem.* **3**, 2282 (2005).

Interestingly, the ligands used in this study did not show activity, with the exception of 6-thioguanine with strong antiproliferative effects (IC_{50} value of 1.24 μM), only slightly weaker than that found for the corresponding gold complex **9** (IC_{50} value of 0.34 μM, Table 1). This ligand has been previously used as an anticancer agent and its biological activity has been previously investigated [29, 30]. Complex **1** bearing the aminophosphine and the chloro ligand also presented an IC_{50} value 8 times higher than the corresponding thiolate-complexes **3–6**.

Additionally, the interactions of complexes **3**, **5**, **7** and **9** with thioredoxin reductase in HeLa cells were studied, with the main objective to shed light on the mechanisms of action of these compounds. As shown in Table 2 the complexes exhibited a high inhibition of thioredoxin reductase activity.

Other potential biological targets were also studied. Thus, interaction of these thiolate gold(I) complexes with calf thymus CT-DNA in Tris buffer was analyzed by electronic absorption titration. The values of intrinsic binding constants Kb of the complexes with CT-DNA ranges between 2×10^6 M^{-1} and 7×10^6 M^{-1} which indicates that complexes **1–9** had a strong binding affinity for CT-DNA. This result is surprising because in the specific case of complex **2**, which did not show appreciable cytotoxic activity, it has a high intrinsic binding constant. Consequently, it is possible to propose, as in other gold(I) complexes reported in the literature, that the DNA is not the main target in spite of the binding interaction.

Table 2: Inhibitory effects on TrxR activity and antiproliferative effects for complexes **3**, **5**, **7** and **9** (IC_{50}) in HeLa cells.

	% Inhibition of TrxR	IC_{50}
3	77.4	1.70 ± 0.05
5	84.8	3.30 ± 0.13
7	54.8	4.30 ± 0.03
9	38.4	0.34 ± 0.08

Table 3: Antimicrobial activities evaluated as minimum inhibitory concentrations [μM] and minimum bactericidal concentrations [μM].

	E. faecalis		*S. aureus*		*E. coli*	
	MIC	MBC	MIC	MBC	MIC	MBC
1	10	30	>50	>50	_	
5	5	50	5	>50	>100	>100
6	5	20	5	>50	30	100
7	5	>50	40	50	50	>100
8	15	>50	30	>50	50	>100
9	5	30	30	>50	_	
Kanamacin	100	>100	>100		50	50

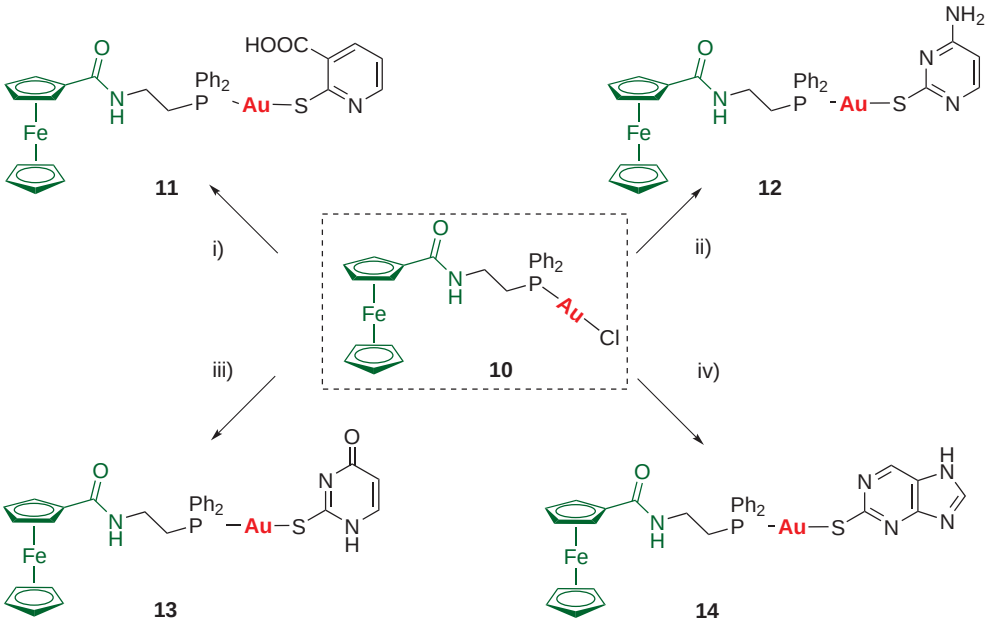
These complexes also exhibit antibacterial activity against *E. faecalis* ATCC 25923, *S. aureus* ATCC 29213 and *E. coli* TG1, which was evaluated by the Kirby–Bauer disk-diffusion test using the broad-spectrum antibiotic kanamicin as control (Table 3).

Based on the results shown in Table 3, it is possible to conclude that all complexes exhibited semiquantitative antimicrobial activities similar to controls. In contrast, complexes **2–4** did not present activity against any of the microorganisms tested. Since many of these microorganisms are resistant to one or more antibiotics, there is an increasing interest in the development of alternative forms of bacterial varying between 5 and 50 μm, interestingly these values suggest that some of our complexes could have potential applications in the impairment of the proliferation of *E. faecalis* and *S. aureus*. The activity of gold(I) complexes against the Gram-negative *E. coli* TG1 strain was lower, although complexes **6–8** still exhibit moderate bacteriostatic effect. This agrees with previous studies indicating that gold(I) complexes are, in general, more efficient against Gram-positive than against Gram-negative bacteria [31, 32]. The lower susceptibility could be attributed to the structural differences in the cell walls of those types of microorganisms. Although the analysis of the mechanism of action of these complexes in Gram-positive bacteria has not been performed, recent studies showed that the inhibition of thioredoxin reductase enzyme plays a very important role, especially in Auranofin itself which is a potent bacteria inhibitor [33]. This is in accordance with the higher inhibition also shown for cancer cells.

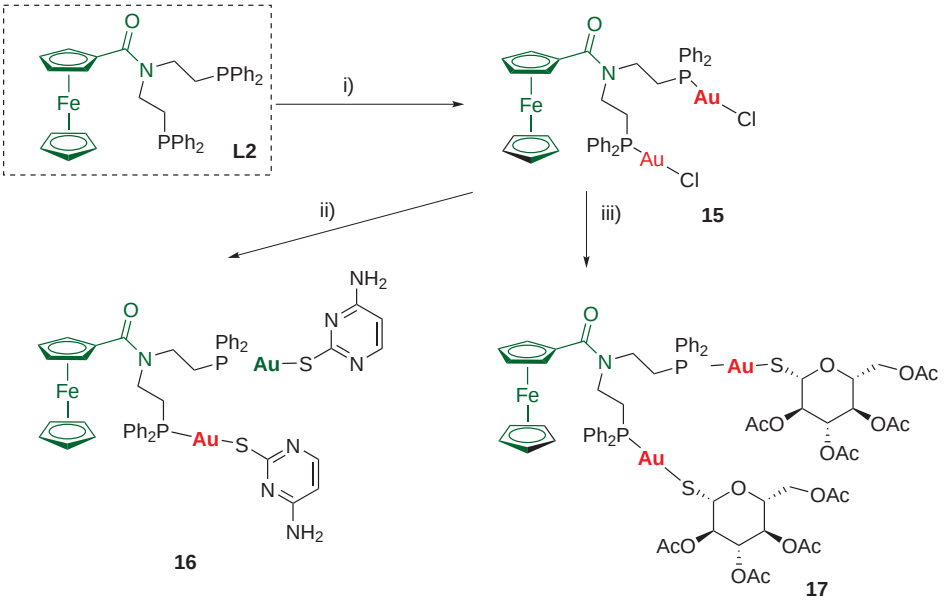
In a further study, we prepared ferrocenyl amide-phosphine ligands, since we envisioned their importance due to their ability to form hydrogen bonds and because of the presence of the ferrocenyl moiety, which has been used in medicine as an excellent scaffold to increase the biological activity of known commercial drugs [34]. The ancillary ligands used were prepared by reaction of chlorocarbonylferrocene with two aminophoshines, $H_2NCH_2CH_2PPh_2$ (**L1**) or $HN(CH_2CH_2PPh_2)_2$ (**L2**). The treatment of the precursor complex **10** bearing the monophosphine ligand **L1** with various thiols such as 2-mercaptonicotic acid, 2-thiocytosine, 2-thiouracil, and 6-mercaptopurine in the presence of K_2CO_3 produced the corresponding thiolate derivatives **11–14** (see Scheme 1) [35].

Similarly, the reaction of the gold species bearing the diphosphine **L2**, $[Au_2Cl_2\{(PPh_2CH_2CH_2)_2\text{-}NCOFc\}]$ (**15**), with thiols such as 2-thiocytosine or 2,3,4,6-tetra-6- acetyl-1-thiol-β-D-glucopyranosato in the presence of K_2CO_3 afforded complexes **16** or **17** (see Scheme 2).

Other gold(I) complexes with disubstituted ferrocenyl ligands represented in Fig. 3 were prepared for comparative purposes [36, 37]. The gold-chloride species further reacted with thiols such as 2-thiocytosine



Scheme 1: Synthesis of thiolate gold complexes **11–14** with the ligand $PPh_2CH_2CH_2NHCOFc$. (i) 2-mercaptonicotinic acid + K_2CO_3, (ii) 2-thiocytosine + K_2CO_3, (iii) 2-thiouracil + K_2CO_3, (iv) 2-mercaptopurine + K_2CO_3.

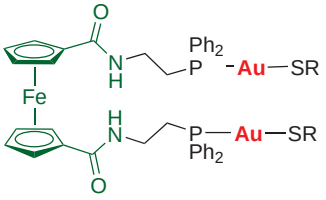


Scheme 2: Synthesis of gold complexes with the ligand $(PPh_2CH_2CH_2N)COFc$. (i) 2 [AuCl(tht)], (ii) 2-thiocytosine + K_2CO_3, (iii) 2,3,4,6-tetra-6-acetyl-1-thiol-β-D-glucopyranosato + K_2CO_3.

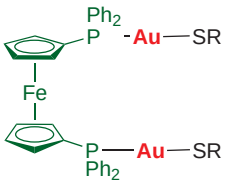
(for **19**), 2-thiouracil (for **21**), and 2,3,4,6-tetra-6-acetyl-1-thiol-β-D-glucopyranosato (for **18** and **20**) in the presence of K_2CO_3 to afford the thiolate gold species **18–21** (see Fig. 3).

The ferrocenyl-amide phosphine ligands and the gold complexes were tested for their antiproliferative effect against four tumor cell lines: the two murine cell lines NIH-3T3 (mouse embryonic fibroblasts) and PC-12 (pheochromocytoma of the rat adrenal medulla) and the two human cell lines A549 (adenocarcinomic human alveolar basal epithelial cells) and Hep-G2 (hepatocellular carcinoma).

All the ferrocenyl ligands used do not show any significant cytotoxic activity. However, the gold-chloride derivatives exhibit much better activity, with the smallest IC_{50} values for complex



SR = TG (**18**), thiocytosine (**19**)



SR = TG (**20**), thiouracil (**21**)

Fig. 3: Thiolate gold complexes prepared from disubstituted ferrocenyl ligands.

Table 4: IC_{50} (48 h, μM) of complexes **10–21** against NIH-3T3, PC-12, A549 and Hep-G2 cell lines.

Complex	NIH-3T3	PC-12	A549	Hep-G2
10	0.1	4.2	>100	68
11	0.4	2.1	12.6	7.8
12	0.8	2.4	95.1	>100
13	2.0	1.0	15.0	20.0
14	13.7		21.0	70.7
15	11.4		29.9	29.1
16	4.7		13.1	12.4
17	4.0		14.5	11.1
18	5.4		30.3	17.6
19	14.7		74.9	72.6
20	5.9		7.0	13.7
21	5.4		12.0	14.8

[AuCl($PPh_2CH_2CH_2NHCOFc$)] (**10**) in the murine cells, with a very low value for NIH-3T3 cells of 0.1 μM, and for the previously reported [Au_2Cl_2(μ-dppf)] which present IC_{50} values of 8.2 and 10.7 μM in the A549 and Hep-G2 cell lines, respectively (Table 4).

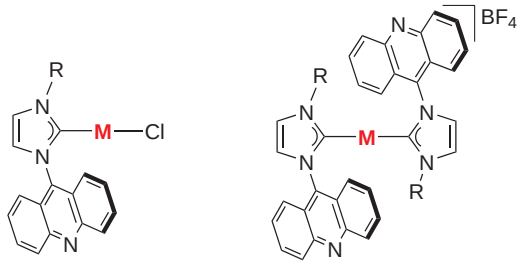
The cytotoxic activity of all the thiolate gold derivatives **11–21** with the different amido-phosphine ligands have been measured (Table 4). The general trend is that excellent IC_{50} values are found in the murine cell lines NIH-3T3 and PC-12 and moderate to good values are found in the human cell lines A549 and Hep-G2. This activity is much better than the corresponding of the chloro-gold derivatives with the same ancillary ligand and also than those found for Auranofin and Cisplatin. The structure-reactivity relationship (SAR) in these complexes shows that the most active are the gold thiolate complexes with the monosubstituted amidophosphine ligand.

N-heterocyclic carbene (NHC) complexes are receiving much attention during the last years and are emerging as a promising class of metallodrugs [38]. The strong σ-donating ability of NHCs is comparable to that of phosphines and this provides with strongly bonded NHC ligands to gold. This fact, together with the ease functionalization of NHC ligands, makes them interesting scaffolds to develop new gold drugs [39].

As the substituents in the NHC ligands are very important to modulate the biological properties of the final gold complexes, acridine-based NHC ligands were chosen for this aim as the acridine is a chromophore itself and it is also well-known to be a good DNA intercalator [40]. First, the synthesis of the NHC gold-chloride and bis(carbene) species **22–29** were accomplished by a transmetallation reaction using silver oxide and several examples represented in Fig. 4 [41].

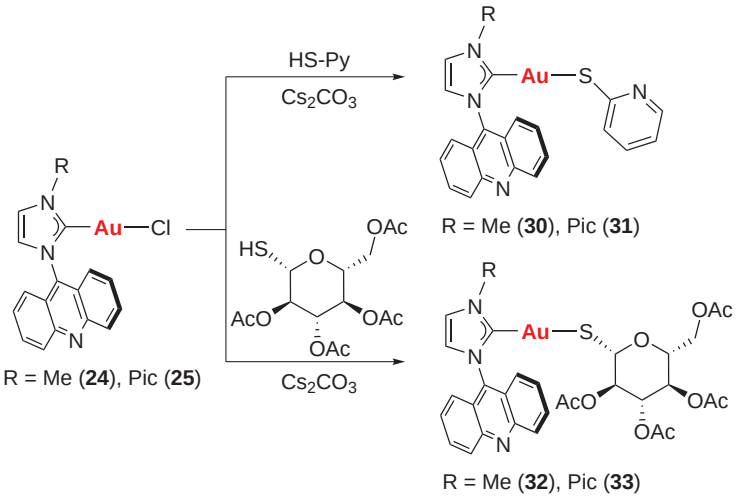
Additionally, the reaction of the NHC gold-chloride compounds with thiols produced the substitution of the chloride by the thiolate ligand (Scheme 3). We envisioned that the resulting thiolate acridine-NHC based Au(I) complexes containing thiopyridine or tetra-O-acetyl-1-thio-β-D-glucopyranoside could be promising candidates for biological studies (Table 5) [42].

All the complexes showed higher cytotoxicity towards MiaPaca2 cells than towards A549 cells. In general, Ag–NHC compounds seemed to be less cytotoxic than their Au–NHC analogs, following the same trend as previously found by Liu, Mao and coworkers for similar Au(I) and Ag(I)–NHC complexes [43]. Thiolate–Au–NHCs



R = Me (**22**), Pic (**23**), M = Ag(I) R = Me (**26**), Pic (**27**), M = Ag(I)
R = Me (**24**), Pic (**25**), M = Au(I) R = Me (**28**), Pic (**29**), M = Au(I)

Fig. 4: Ag(I) and Au(I) complexes **22–29** containing acridine-based NHC ligands.



R = Me (**24**), Pic (**25**)

Scheme 3: Synthesis of the thiolate NHC gold complexes.

Table 5: IC_{50} (mM) of complexes **22–33** (DMSO) and cisplatin (H_2O).

Complex	A549	MiaPaca2	Complex	A549	MiaPaca2
22	>50	44.4±10.9	**29**	41.9±4.2	20.0±1.6
23	>50	51.9±1.9	**30**	>50	28.4±2.5
24	>50	22.8±8.8	**31**	17.4±4.5	7.5±1.2
25	>50	38.3±2.6	**32**	19.4±2.3	2.8±0.8
26	>50	40.6±9.9	**33**	13.0±3.6	3.4±0.8
27	>50	14.3±4.3	Cis-Pt[a]	114.2±9.1	76.5±7.4
28	>50	6.9±1.9			

[a]IC_{50} value measured in the same experimental conditions as complexes **22–33**, i.e. incubation for 24 h at 37 °C.

(**30–33**) complexes, mimics of the well-known auranofin, exhibited the higher cytotoxic activity against both cell lines. Since complex **33** was found the most cytotoxic in the A549 cell line, its cell death mechanism was studied by a flow cytometry assay and the data suggested a programmed cell death mechanism (apoptotic mechanism).

Moreover, fluorescence cell microscopy was also used to know the cellular biodistribution of complex **27** in A549 cells, among others (Fig. 5). Superimposition images revealed a clear lysosomal localization as also found for some dinuclear gold(I)-NHC carbenes by Baker and Berners-Price in 2006 [44] as well as for the antiarthritic gold(I) thiomalate (Myocrisin) [45], in contrast to the expected mitochondrial pattern for gold(I) and silver(I) complexes [46, 47].

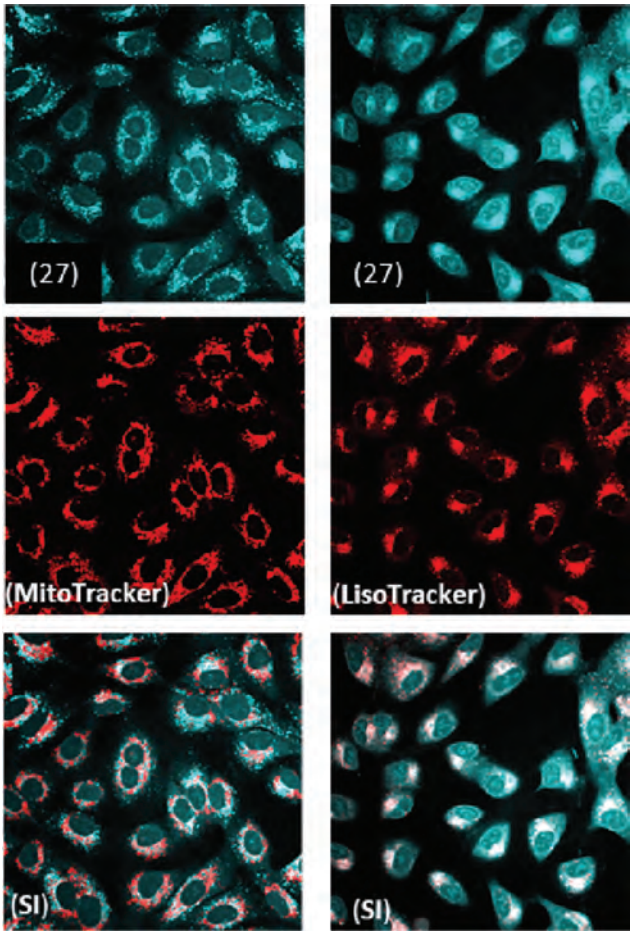


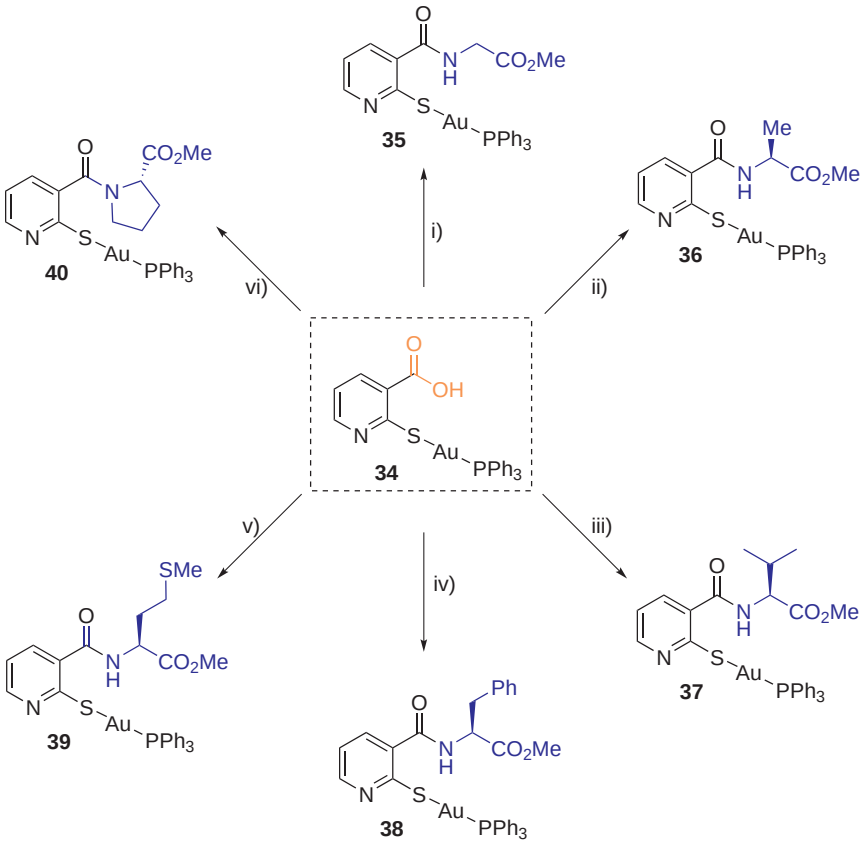
Fig. 5: Images of A549 cells incubated with complex **27** and MitoTracker (left column), **27** and LysoTracker (right column) **27** for 24 h (C = 1/2 of IC_{50}). Excitation at 405 nm: emission from **27** (blue emission) and at 577 nm: emission from either the LysoTracker or MitoTracker (red emission). SI: superimposition image of complex and internal dye.

The proofs also suggested that the complexes were not only located in the lysosomes, but also seem to have some nuclear permeability and accumulation in the nucleolus. Nuclear and nucleolar accumulation might be related to the affinity of Au(I) and Ag(I) to inhibit the TrxR1 and Trx1. Both enzymes are also present in the nuclear region especially in those stages of cellular stress promoted by the presence of a stress-inducing drug [48].

In order to shed some light on the cause of this nuclear and nucleolar localization, preliminary studies on DNA interaction studies were performed for **22** and **33** as representative complexes of neutral Ag(I) and Au(I) complexes, respectively. Based on the changes observed in the electrophoretic mobility, we proposed that the most cytotoxic complex **33** showed some interaction character with DNA. In contrast, complex **22** did not. This finding pointed towards the nature of different metal–NHC fragment as the origin of the nuclear affinity, which contrasted with the initial idea that the acridine skeleton might act as DNA intercalator.

Gold complexes with amino acids

Other interesting biological ligands are the amino acids, and beside the importance of this kind of molecules there are scarce examples of gold complexes with amino acid ligands and, consequently, their synthesis is still a challenge [49, 50]. We envisioned that if tumor cells over-express amino acid receptors, metal complexes with amino acid ligands could be more selective towards damaged cells. In order to achieve a better



Scheme 4: Synthesis of complexes **35–40**: (i) Glycine methyl ester, DIPEA, PyBOP; (ii) alanine methyl ester, DIPEA, PyBOP; (iii) valine methyl ester, DIPEA, PyBOP; (iv) phenylalanine methyl ester, DIPEA, PyBOP; (v) methionine methyl ester, DIPEA, PyBOP; (vi) proline methyl ester, DIPEA, PyBOP.

stability of the final complexes, the amino acids were modified with different functional groups to allow a better coordination of the gold center to them.

With this idea in mind, the design of gold(I) complexes with thiolates ligands bearing amino acid moieties seems to be an excellent approach to easily transport the gold complex to the biological target [51].

Therefore, we synthesized a series of gold(I) complexes starting from the nicotinic acid thiolate gold complex [Au(SpyCOOH)(PPh_3)] **34**, previously synthesized by Nomiya et al., who proved its antibacterial activity [52], by coupling of the acid moiety with several amino acid esters giving rise to complexes **35–40** (see Scheme 4) [53].

We further introduced different structural modifications taking complex **34** as a central core, in order to obtain new active derivatives and to establish a plausible structure–activity relationship (Fig. 6) [54].

The corresponding acid derivatives **41a–f** were obtained by hydrolysis of starting amino acid esters **35–40** using a mild base such as LiOH, in order to avoid racemization or decomposition of the gold compounds (Fig. 7).

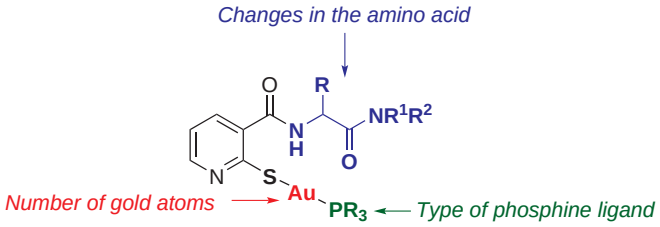
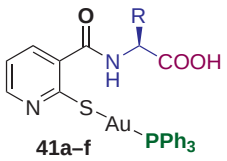
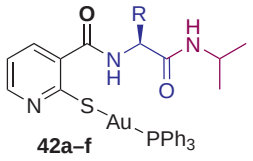


Fig. 6: Selected modifications introduced to establish SAR.



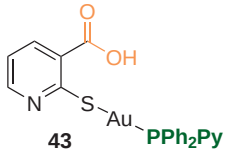
41a–f

Aa = Gly (**41a**), Ala (**41b**), Phe (**41c**), Val (**41d**), Met (**41e**), Pro (**41f**)



42a–f

Aa = Gly (**42a**), Ala (**42b**), Phe (**42c**), Val (**42d**), Met (**42e**), Pro (**42f**)



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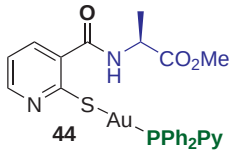

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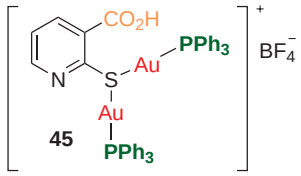


45

Fig. 7: Complexes prepared with the selected modifications.

We also performed the derivatization of amino acids **41a–f** into the secondary amides **42a–f** in a single step by coupling the amino acid derivatives with amines. Additionally, we synthesized complexes **43** and **44**, where the typical triphenylphosphine was substituted by diphenyl-2-pyridine phosphine and, finally, complex **45**, where two gold metal centers are brought together within the same sulfur donor atom. Interestingly, complex **45** could exhibit an increase in the cytotoxicity of the final complex and because it presents high lipophilicity that could help the complex to cross the cell membranes and/or accumulate in the mitochondria.

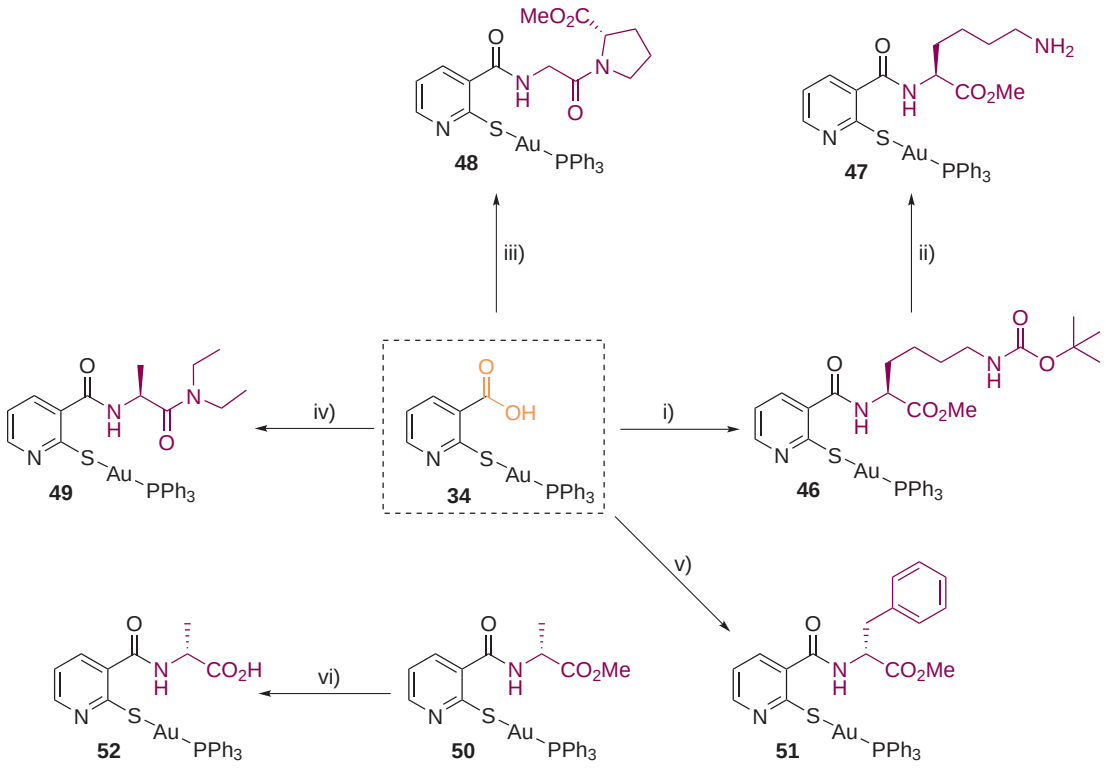
Other possible structural modifications were further introduced starting from acid **34**, where changes were based on the coupled amino acid, or in the chirality to afford the molecules depicted in Scheme 5. We believed that these variations could influence the activity and/or the mechanism of action of the resulting gold complexes.

All these complexes were evaluated against three different human tumor cell lines: A549 (lung carcinoma), Jurkat (T-cell leukaemia) and MiaPaca2 (pancreatic carcinoma). In general, the values obtained are considerably lower for these complexes in the most resistant cell lines, A549 or MiaPaca2, compared with cisplatin and present similar values in the Jurkat cell line. Importantly, the compounds showed some selectivity for leukaemia cells against non-tumor cells (Jurkat vs. R69) but this difference was not seen in the case of solid tumors.

The family of ester complexes (**35–40**) displayed better cytotoxicity than the precursor complex **34** in all the cellular lines, with IC_{50} values ranging from 2.4 to 12.8 μM (Table 6). Particularly, the proline derivative **40** was the most potent of this family of complexes, exhibiting excellent IC_{50} values in the three cancer lines, 2.4 ± 0.04 in Jurkat, 9.4 ± 0.19 in MiaPaca2 and 7.4 ± 0.34 μM in A549. The two families of acids **41** and amides **42** showed, in general, worse IC_{50} values than the corresponding esters.

The cytotoxicity values for the complexes with the structural modifications provided valuable information about the structure-reactivity relationship (Table 6). Thus, the effect of changing the phosphine ligand in complexes **43** and **44** was not significant, probably because both phosphines have similar electronic and steric properties. However, the coordination of an additional $AuPPh_3^+$ fragment afforded a more active species **45**. This corroborates the observation that the cytotoxic activity of the complex is mainly attributed to the gold(I) atom, and the ligands bonded to gold are important as carriers to reach the biological target. Additionally, the coupling of a different type of amino esters and the change in the quirality (complexes **46–52**) do not seem to really improve the cytotoxicity (Table 6).

Considering all this data, the better cytotoxicity was achieved by the compounds functionalized as esters, with two gold(I) atoms per molecule and with conformationally-restricted or rigid amino acids, as in the case of proline (Table 6). Following this structure-activity relationship observed, a new complex was prepared bearing the two gold centers and the functionalization with the proline ester (**53**) (Fig. 8). Compound **53** displayed excellent cytotoxic activity, with IC_{50} values of 4.1 ± 0.11 for A549, 1.2 ± 0.04 for MiaPaca2 and



Scheme 5: Synthesis of complexes **46–52:** (i) HCl · H-Lys(Boc)-OMe, PyBOP, DIPEA, MeCN, (ii) TFA–DCM (1:1 v/v), (iii) HCl · H-Gly-Pro-OMe, PyBOP, DIPEA, MeCN, (iv) HCl · H-Gly-OMe, PyBOP, DIPEA; LiOH, $KHSO_4$; $HNEt_2$, PyBOP, DIPEA; MeCN, (v) HCl-H-(D)-aa-OMe, PyBOP, DIPEA, MeCN, (vi) LiOH, $KHSO_4$.

0.9 ± 0.07 μM (Table 6). This compound is the most potent among all complexes prepared in these series, and it is significantly more active than the precursors **40** and **45** in all the cell lines.

Additionally, the cell death mechanism was studied in both A549 and Jurkat cells, showing that complex **40** induced non-apoptotic cell death in A549, while in Jurkat the cell death proceeded, both through apoptosis and necrosis. The generation of ROS was analyzed by flow cytometry after staining with 2-hydroxyethidium, showing higher levels of these radicals in Jurkat cells than in A549 cells. The ROS levels produced kept good correlation with the loss of mitochondria membrane potential. The data suggested that the damage to the mitochondria could lead to an increase in the production of ROS that, in final instance, triggers cell death. Additionally, the inhibition of TrxR assay revealed that complex **40** at concentrations near to IC_{50} values are potent inhibitors of this enzyme, by decreasing the enzyme activity at least in a 50 %. The inhibition of this enzyme would prevent the removal of ROS and may be the reason for the high levels of ROS observed in the previous assay.

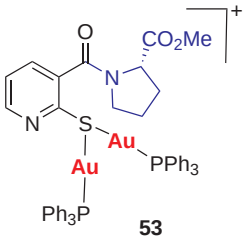
Following our previous works with the gold(I) complexes using the nicotinic acid thiolate with amino acid ligands [53], we decided to functionalized pyridine moieties with amino acids esters and to synthesize several gold(I) and gold(III) derivatives with two different phosphine fragments, PPh_3 and PPh_2py. The amino ester ligands bear then an aromatic amine as pyridine that coordinates metal fragments through the nitrogen atom, giving rise to all final metal complexes **55–63** (Scheme 6) [55].

After the synthesis, the in vitro cytotoxic activity of the ligands **L3–L5** and the metal complexes **55–63** was tested against four tumor human cell lines [A549 (human lung cancer), Hep-G2 (human liver cancer), HeLa (human cervix epithelial carcinoma), MCF-7 (breast cancer)] and one tumor mouse cell line [NIH-3T3 (mouse fibroblast)] (Table 7). The resulting values were also compared with those obtained for cisplatin. Both, the ligands and the gold(III) complexes showed no cytotoxic activity. In contrast, the gold(I) complexes displayed good IC_{50} values in vitro against the growth of the cancer cell lines tested. They are particularly

Table 6: IC_{50} (24 h, μM) of complexes **34–53** against A549, MiaPaca, Jurkat, 293T, R69 cell lines.

Complex	A549	MiaPaca2	Jurkat	293T	R69
Cisplatin	105±0.90	71±0.80	7.4±0.10	14.0±0.20	65±0.92
34	15.5±0.92	9.2±0.28	4.6±0.08	4.6±0.13	16±0.64
35	11.5±0.55	9.7±0.22	3.8±0.07	4.2±0.08	8.6±0.33
36	13.7±0.71	11.0±0.20	4.0±0.07	2.7±0.07	2.2±0.08
37	10.9±0.40	10.2±0.25	3.3±0.05	10.7±0.31	19.0±0.60
38	8.9±0.36	12.3±0.37	4.0±0.08	5.5±0.16	14.0±0.59
39	8.2±0.41	12.8±0.32	4.1±0.06	3.7±0.07	9.6±0.36
40	7.4±0.34	9.4±0.19	2.4±0.04	10.0±0.19	4.0±0.13
41a	14.7±0.88	8.2±0.13	7.6±0.11	35.2±0.53	25.9±1.04
41b	7.7±0.22	10.7±0.16	3.7±0.06	12.8±0.27	6.1±0.18
41c	14.7±0.20	12.3±0.32	4.3±0.08	11.3±0.29	6.5±0.25
41d	15.9±0.50	11.5±0.29	6.7±0.13	65.5±1.12	33.1±1.13
41e	14.1±0.55	14.5±0.26	3.6±0.06	49.3±1.53	28.4±1.16
41f	14.3±0.61	11.6±0.20	7.5±0.07	3.0±0.08	4.0±0.11
42a	28.3±1.02	27.2±0.65	3.9±0.06	14.4±0.42	4.9±0.16
42b	19.1±0.67	8.1±0.23	3.9±0.07	8.1±0.28	1.4±0.04
42c	14.4±0.60	12.5±0.32	3.8±0.06	7.6±0.26	5.2±0.15
42d	18.8±0.71	14.1±0.29	3.7±0.06	14.6±0.57	6.8±0.23
42e	19.4±0.62	15.2±0.33	5.3±0.11	13.6±0.40	3.0±0.09
42f	30.5±0.82	19.2±0.36	7.7±0.15	5.8±0.16	4.0±0.16
43	nt	18.2±0.51	5.2±0.07	14.0±0.28	4.9±0.18
44	15.7±0.66	17.4±0.48	3.8±0.04	12.0±0.19	3.2±0.13
45	6.9±0.21	7.5±0.19	3.3±0.04	7.8±0.19	1.8±0.05
46	8.3±0.39	13.1±0.26	3.4±0.06	3.5±0.11	2.8±0.08
47	32.5±1.24	29.3±0.70	36.5±0.77	>25	10.4±0.35
48	18.7±0.64	22.5±0.67	8.6±0.14	17.9±0.45	15.4±0.60
49	33.5±1.31	>50	nt	8.3±0.19	1.9±0.05
50	16.5±0.92	17.1±0.39	4.2±0.05	7.7±0.24	3.0±0.08
51	18.3±0.75	15.1±0.27	3.6±0.06	>25	1.2±0.02
52	>50	>50	nt	>25	10.7±0.29
53	4.1±0.11	1.2±0.04	0.9±0.07	4.5±0.14	0.8±0.02

nt, not tested.

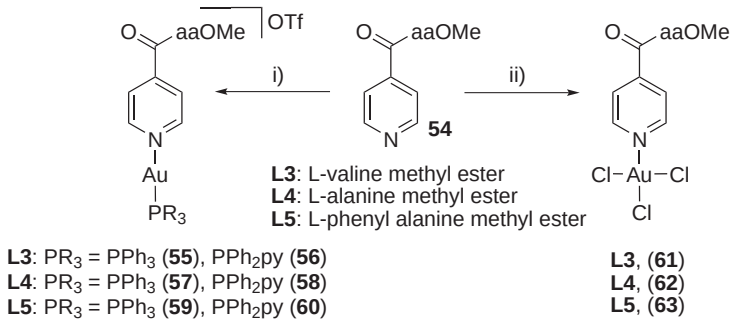


53

Fig. 8: Structure of complex **53**, synthesized following the best structure activity relationship.

effective against HeLa (IC_{50} values range between 4.33 and 11.71 μM) and MCF-7 (IC_{50} values range between 0.45 and 20.05 μM) cell lines, being **56** and **58**, bearing the PPh_2py ligand, and **59** with the PPh_3 phosphine and the phenyl alanine methyl ester the most potent agents against MCF-7 with IC_{50} values of 0.65, 1.78 and 0.45 μM, respectively.

In order to determine whether antiproliferation and cell death are associated with apoptosis, NIH-3T3 cells were exposed to complex **56** (Annexin V + FITC, PI) and analyzed by flow cytometry. These experiments showed that the mechanism by which this complex inhibits cell proliferation inducing cell death in NIH-3T3 cells is mainly apoptotic.



L3: PR_3 = PPh_3 (**55**), PPh_2py (**56**)
L4: PR_3 = PPh_3 (**57**), PPh_2py (**58**)
L5: PR_3 = PPh_3 (**59**), PPh_2py (**60**)

L3, (**61**)
L4, (**62**)
L5, (**63**)

Scheme 6: Synthesis of Au(I) complexes **55–60** and Au(III) complexes **61–63:** (i) [Au(OTf)(PR_3)] and (ii) $AuCl_3 \cdot nH_2O$.

Table 7: IC_{50} (48 h, μM) of complexes **55–63** and ligands **L3–L5** against A549, Hep-G2, NIH-3T3, HeLa and MCF-7 cell lines.

Complex	A549	Hep-G2	NIH-3T3	HeLa	MCF-7
L3	>50	>50	>50	>50	nt
L4	>50	>50	>50	>50	nt
L5	>50	>50	>50	>50	nt
55	24.56±0.34	nt	4.76±0.13	4.43±1.43	6.04±0.33
57	25.35±0.40	10.50±0.46	10.75±0.43	11.71±1.06	20.05±0.34
59	>50	25.95±0.16	3.71±0.64	10.66±0.33	0.45±0.20
56	10.21±0.60	10.18±0.41	13.41±1.37	4.33±0.46	0.65±0.34
58	25.22±0.59	10.81±0.78	10.31±0.37	5.58±0.86	1.78±0.05
60	25.35±0.44	10.85±0.54	6.43±1.55	6.93±0.50	10.14±0.69
61	>50	>50	>50	>50	nt
62	>50	>50	>50	>50	nt
63	>50	>50	>50	>50	nt

nt, not tested.

Continuing the studies with amino acids, and with the precedent that the proline amino acid ring provides higher activity to the compounds, probably because its more rigid conformation, we decided to center our research on the non-proteinogenic amino acid 4-mercaptoproline derivative **64** [56], which can be considered as a hybrid of proline and homocysteine. Therefore, we described the first synthesis of gold(I) bioconjugates with *N*-protected 4-mercaptoproline derivatives **65–69** (Fig. 9) [57].

Some structural modifications were performed on these compounds, such as the introduction of different phosphine ligands or a variable number of gold(I) atoms in the complex. Furthermore, the cytotoxicity (at

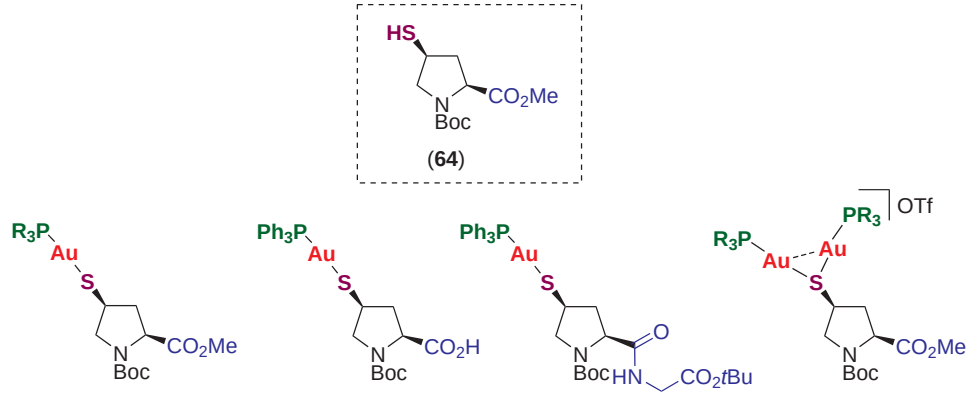


Fig. 9: Synthesis of gold complexes **65–69** with 4-mercaptoproline ester ligand derivatives.

Table 8: IC_{50} (24 h) of complexes **65–69** (μM).

Complex	A549	MiaPaca2	Jurkat
65	1.8±0.15	3.0±0.19	0.8±0.08
66	3.8±0.37	6.1±0.54	3.5±0.32
67	>25	>25	9.3±0.65
68	3.5±0.29	2.3±0.22	0.6±0.08
69	1.9±0.16	1.8±0.17	0.5±0.07
Cisplatin	114.2	76.5	10.8

24 h) of the synthesized complexes was studied in vitro against three different human tumor cell lines [A549, Jurkat and MiaPaca2] and their structure-reactivity relationship (SAR) was also explored (Table 8).

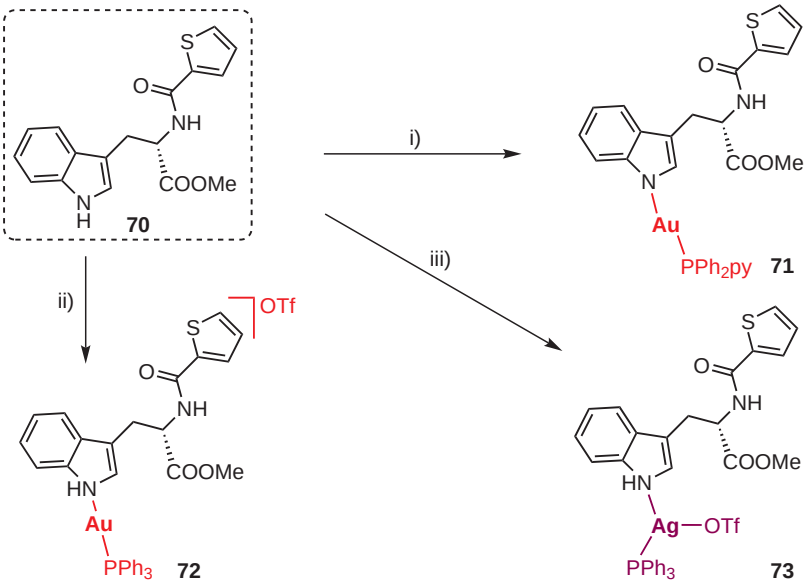
Based on the results shown in Table 8, all gold complexes exhibited strong cytotoxicity with IC_{50} values in the μM range (<10 μM). In general, the Jurkat cell line was the most sensitive, and the A549 and MiaPaca2 exhibited more resistance to our complexes. In all cases better activities were found in comparison with that achieved with cisplatin under the same conditions.

Analyzing the values of each complex, is possible to conclude that the cytotoxicity was mainly due to the gold atom. For instance, the presence of an additional $Au(PPh_3)^+$ fragment slightly improved the cytotoxicity of the new compound **69** in comparison with its mononuclear analog **66**. On the other hand, we thought that the thiolate ligands could be involved in diverse exchange reactions with other biomolecules in the body, and they could determine the transport or biodistribution of the resulting complexes in the cells.

More recently, we have prepared thiophene bioconjugate complexes **71–73** of Ag(I) and Au(I) and Au(III) with tryptophan methyl ester **70**, following the synthesis described in Scheme 7 [58].

Cytotoxicity studies towards several cancer lines such as A549, Hep-G2 or NIH-3T3 have been performed for the tryptophan derivatives **71–73** (Table 9). The thiophene bioconjugate **70** shows no activity whereas the gold (**71** and **72**) and silver (**73**) complexes exhibit moderate to good activities in two of the cell lines, with IC_{50} values going from 16±0.97 to 56±3.5 μM, and only the silver complex **73** is active in the A549 cell line (27±1.3 μM).

The bioorganometallic chemistry of ferrocene has aroused a great interest due to its potential biological applications [34, 59]. Few examples of bioconjugates of ferrocene with amino acids [60–62], peptides [63–66],



Scheme 7: Synthesis of metal complexes **71–73** with **70**. (i) [Au(acac)(PPh_2py)], (ii) [Au(OTf)(PPh_3)], (iii) [Ag(OTf)(PPh_3)].

Table 9: IC_{50} (48 h, μM) of complexes **70–73** against A549, Hep-G2 and NIH-3T3 cell lines.

Complex	A549	Hep-G2	NIH-3T3
70	>100	>100	>100
71	>100	49 ± 2.5	18 ± 0.92
72	>100	49 ± 2.8	16 ± 0.97
73	27 ± 1.3	56 ± 3.5	19 ± 1.2

proteins or DNA [67], among others, had been described and their potential applications in electrochemical sensor devices and immune assay reagents had been also explored [68, 69]. Ferrocene itself and its derivatives have shown cytotoxic and antianemic activity in the area of medicinal chemistry [70]. The ferrocenyl core is also found in the structure of some biologically active compounds such as antibiotic [71], anticancer [72, 73], or malaria drugs [74, 75] and it has been demonstrated that its presence could result in an increase of the activity of the final molecules.

In this context, several bioconjugates of ferrocene (**74–79**) were prepared with biologically relevant ligands such as amino acid esters and related species. Their synthetic procedure entailed the reaction of chlorocarbonyl ferrocene with the corresponding amino acid ester (histidine methyl ester, tryptophan methyl ester, methionine methyl ester and lysine ethyl ester) or histamine or prolinamide in the presence of NEt_3 (Fig. 10) [76].

Gold(I) and gold(III) derivatives **80–86** were prepared with some of the synthesized ferrocene bioconjugates **76**, **77** and **79** in order to test their biological activity (Fig. 11).

The ferrocene bioconjugates and the gold complexes **80–84** and **86** were tested for cytotoxicity against three tumor cell lines: two human cell lines, HeLa (derived from cervical cancer) and MCF-7 (derived from breast cancer), and one murine cell line, N1E-115 (derived from mouse sympathetic ganglion neurons of C-1300 mouse) (Table 10). The ferrocene bioconjugates did not exhibited significant antiproliferative activity in comparison with the gold complexes, which were more effective as cytotoxic agents.

These results displayed some interesting structure-reactivity relationships. In particular, the gold complex associated with methionine and PPh_3, complex **84**, showed a significant cytotoxicity in the two human cell lines. On the contrary, the gold(III) derivatives with pentafluorophenyl ligands showed larger IC_{50} values in HeLa and MCF-7 cells whereas in NIE-115 cells presents a smaller value (30 μM).

In order to study the mechanism of action of our complexes related with their antiproliferative activity, their interaction with the DNA (calf thymus DNA) was explored. It was found that there were not changes in

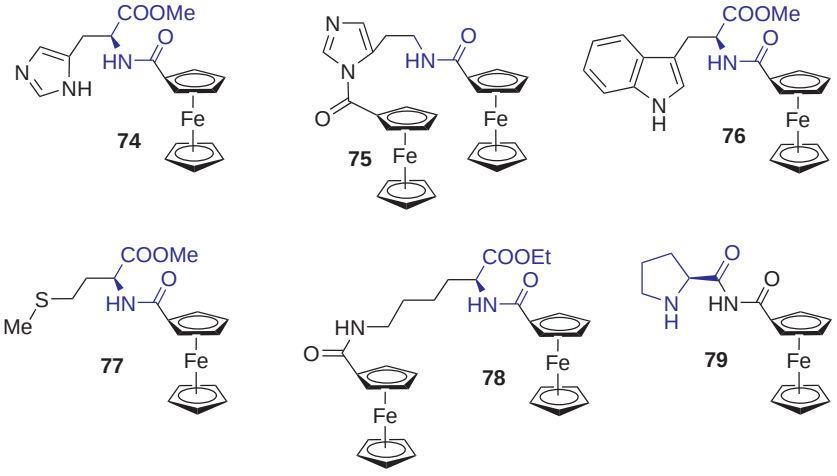
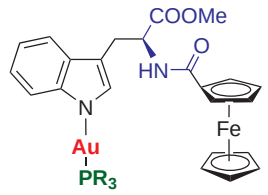
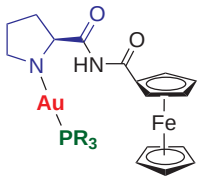


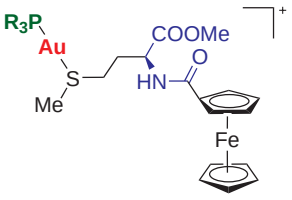
Fig. 10: Synthesis of starting ferrocenyl compounds **74–79**.



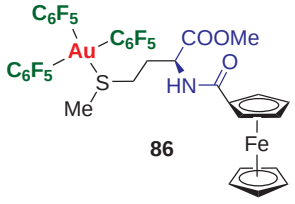
PR_3 = PPh_3 (**80**), PPh_2py (**81**)



PR_3 = PPh_3 (**82**), PPh_2py (**83**)



PR_3 = PPh_3 (**84**), PPh_2py (**85**)



86

Fig. 11: Gold complexes **80–86** with ferrocenyl-aminoacid ligands.

Table 10: IC_{50} values for complexes **80–84** and **86**.

Compounds	HeLa	MCF-7	N1E-115
80	32 ± 1.8	15 ± 1.2	27 ± 2.2
81	22 ± 1.9	45 ± 1.7	26 ± 1.6
82	28 ± 1.6	52 ± 1.2	54 ± 2.3
83	29 ± 1.3	32 ± 2.5	<10 ± 2.2
84	18 ± 1.4	15 ± 1.8	29 ± 1.7
86	87 ± 2.0	88 ± 2.2	31 ± 2.4

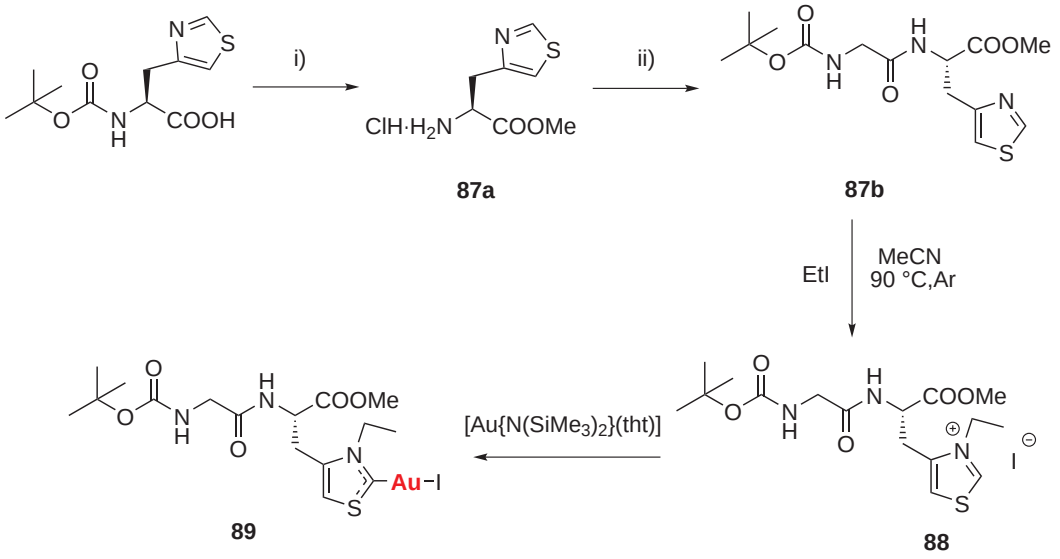
the specific viscosities upon the addition of compounds to a DNA solution, which confirmed that they do not act as DNA intercalators.

Gold complexes with peptides

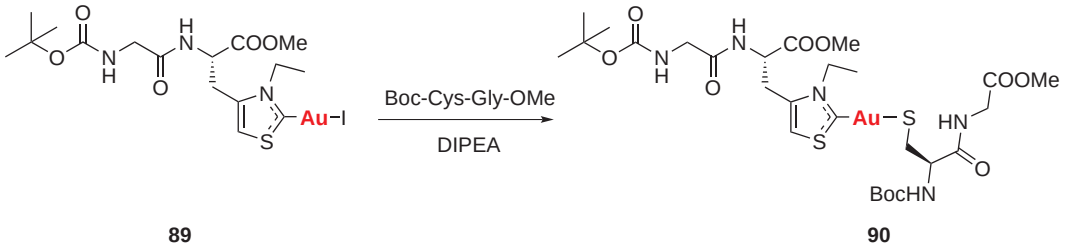
In spite of the great number of reported biologically active gold complexes, the works involving gold complexes bearing peptides are still scarce. The use of peptides on drug discovery is one of the most promising fields in the development of new medicines [77, 78]. Similarly to amino acids, peptides are also useful carriers to deliver the metal into the biological target and could enable the complex to penetrate into the cell. In fact, the use of peptide bioconjugates with metal complexes for targeting of tumor cells has gained considerable attention in the last years [79–82].

A major problem for cancer chemotherapeutics is crossing the cell membrane, and a strategy to overcome this is to make use of the peptide-based delivery systems, which can transport small peptides and peptide-like drugs to the cells. These peptide transporters have overexpressed receptors in some types of tumors, which could be a target for gold–peptide compounds, providing them with higher levels of internalization and subsequent selectivity [83]. In this context and with the aim of preparing more active and selective gold drugs, we proposed the formation of new gold(I) complexes with dipeptides.

We prepared a thiazolylalanine-containing peptide following the procedures depicted in Scheme 8 [84]. Alkylation of the dipeptide **87b** gave the thiazolium salt **88** and the corresponding gold(I) carbene **89**.



Scheme 8: Synthesis of the thiazolylalanine-containing peptide and the gold carbenes **87–89**. (i) $SOCl_2$, MeOH, (ii) Boc-Gly-OH, IBCF, NMM, THF.



Scheme 9: Synthesis of complex **90**.

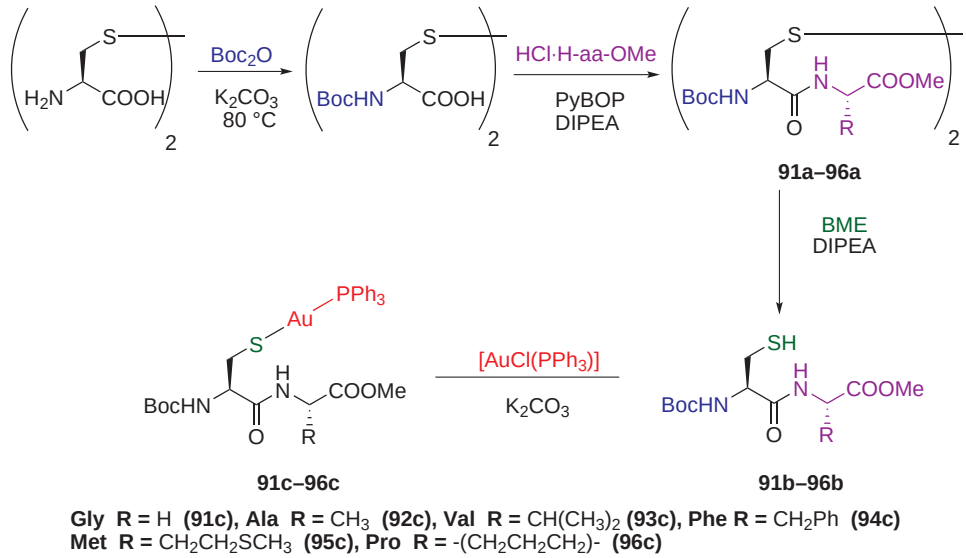
The iodide ligand can be substituted by a cysteine-containing dipeptide affording complex **90** (Scheme 9).

The cytotoxic activities of NSHC-gold(I) iodide **89** and bioconjugate **90** were assayed against three different human tumor cell lines: A549, Jurkat and MiaPaca2. Complex **89** displayed very good activity in vitro against all three tumor cell lines, with IC_{50} values of 0.4 ± 0.01, 16.6 ± 0.22 and 6.2 ± 0.1 μM, respectively. Surprisingly, compound **90** bearing a coordinated dipeptide showed much lower activity in these cancer cells. This may be due to the higher lability of the Au–I bond compared with the Au–S bond, or may simply be a consequence of the higher lipophilicity of **89**.

A family of cysteine-containing dipeptides **91b–96b** was prepared starting from cystine by coupling different amino acids and using several orthogonal protections. The reaction with $[AuCl(PPh_3)]$ in the presence of K_2CO_3 gave the corresponding gold complexes **91c–96c** (Scheme 10) [85]. In these molecules the gold center is coordinated directly to the dipeptide by the sulfur atom of the cysteine as thiolate.

In these gold(I) thiolate-dipeptide phosphine complexes different structural modifications such as change in the type of the amino protecting group (**98**), the type of phosphine (**97**), the number of gold fragments coordinated to the sulfur center (**100, 101**), or the use of non-proteinogenic conformationally-restricted amino acid ester in the peptide (**99**), were introduced in order to evaluate their influence in the biological activity of the final complexes. The modified structures are depicted in Fig. 12.

The cytotoxic activity in vitro of these complexes has been evaluated against different tumor human cell lines (A549, MiaPaca2 and Jurkat) (Table 11). The complexes exhibited excellent cytotoxic activity with IC_{50} values in the very low micromolar range, as low as 0.1 μM (Table 11). Interestingly, the best antipropiferative character was found when two $AuPPh_3^+$ fragments are coordinated to the Boc-Cys-Gly-OMe peptide, complex **100**, suggesting the importance of the polynuclearity within the cytotoxicity potential.



Scheme 10: Synthesis of cysteine-containing dipeptide gold complexes **91c–96c**.

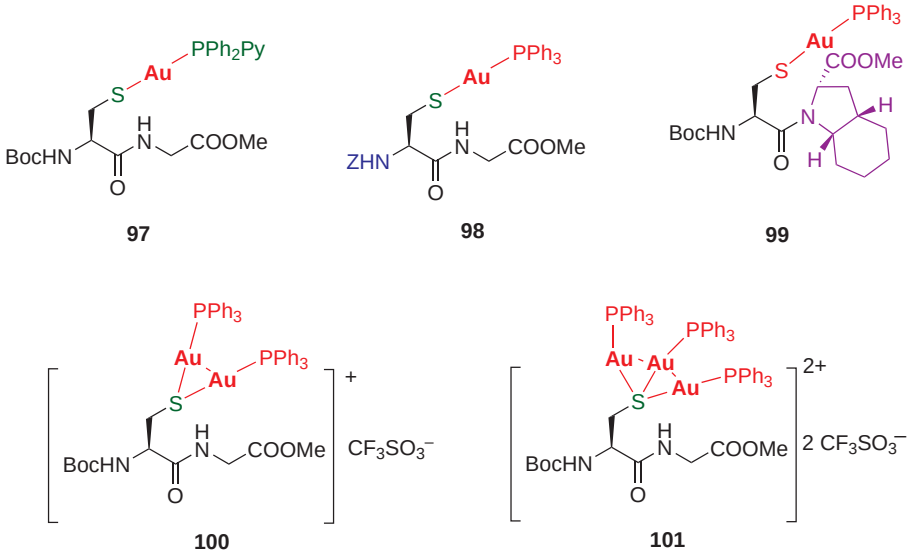


Fig. 12: Structural modifications performed giving rise to **97–101**.

Conclusions

The synthesis of antitumor gold complexes bearing relevant biomolecules has been accomplished by our research group. Gold compounds exhibit an extraordinary antiproliferative activity in several cancer cells and the ligands bonded to it are key for their transport through the bloodstream, to cross the cellular membrane and to reach its biological targets. For these reasons, ligands derived from biological molecules present in the body such as thiol-functionalized DNA bases or sugars, amino acid and peptide derivatives are typically used. Several families of complexes were prepared with these molecules and using different ancillary ligands such as aminophosphines, phosphines and *N*-heterocyclic carbene ligands. Excellent cytotoxic activity was found for most of the complexes in several cancer lines. In all the cases, the relation between the structure and the activity was sought, which conducted to the most effective species. In general, the

Table 11: IC_{50} (μM) (24 h), with standard deviations, of complexes against A549, MiaPaca2 and Jurkat.

Complex	A549	MiaPaca2	Jurkat
91c	1.5±0.2	2.0±0.2	0.9±0.1
92c	1.9±0.1	1.9±0.1	1.6±0.1
93c	2.3±0.1	3.0±0.1	2.2±0.1
94c	15.6±0.11	5.4±0.1	0.4±0.1
95c	4.8±0.1	1.8±0.1	1.7±0.1
96c	3.0±0.1	0.7±0.1	0.5±0.1
97	5.0±0.2	0.5±0.1	0.8±0.1
98	2.7±0.1	1.5±0.1	1.1±0.1
99	2.1±0.1	1.2±0.1	1.5±0.1
100	1.8±0.1	0.1±0.1	0.6±0.1
101	3.5±0.1	1.5±0.1	0.8±0.1

compounds bearing cysteine-containing dipeptides presented the highest activity in all the cancer cells. The structural modifications in which there were either a cyclic amino acid in the peptide or a second gold center, displayed the best structure-activity relationship. The gold complexes induced cellular death mainly through apoptosis or late apoptosis, although some necrosis process was also found. The studies performed showed that the mechanism of action could be derived from different processes. Inhibition of thioredoxin reductase, ROS production and in some cases interaction with DNA was observed, although other potential biological target could not be discarded.

Acronyms

A549	Adenocarcinomic human alveolar basal epithelial cells
aa	Amino acids
acac	Acetylacetonate
Ala	Alanine
ATCC	American Type Culture Collection
Boc	*tert*-Butoxycarbonyl
Cis-Pt	Cisplatin
CT-DNA	Deoxyribonucleic acid sodium salt from calf thymus
Cys	Cysteine
DIPEA	*N*,*N*-Diisopropylethylamine
DNA	Deoxyribonucleic acid
Dppf	1,1′-Bis(diphenylphosphino)ferrocene
E. coli	*Escherichia coli*
E. faecalis	*Enterococcus faecalis*
et	Ethyl
Fc	Ferrocene
FITC	Fluorescein isothiocyanate
Gly	Glycine
HeLa	Cervical cancer cell line (Henrietta Lacks)
Hep-G2	Hepatocellular carcinoma cells
Htz	1-H-1,2,4-Triazole
IBCF	Isobutyl chloroformate
IC_{50}	Half maximal inhibitory concentration
Jurkat	T-cell leukemia
Kb	Binding constant

MBC	Minimum Bactericidal Concentration
MCF7	Breast cancer cell line (Michigan Cancer Foundation-7)
Me	Methyl
Met	Methionine
MiaPaca2	Human pancreatic cancer cell line
MIC	Minimum Inhibitory Concentration
N1E-115	Mouse neuroblastoma cells
NHC	*N*-heterocyclic carbene
NIH-3T3	Mouse embryonic fibroblasts
NMM	*N*-methyl morpholine
NSHC	N,S-Heterocyclic Carbene
OMe	Metoxy
OTf	Triflate
PC-12	Pheochromocytoma of the rat adrenal medulla
Ph	Phenyl
[Ph_2PNH(Htz)]	3-(Diphenylphosphanylamino)-1,2,4-triazole
Ph_2PNHpy	2-(Diphenylphosphanylamino)pyridine
Phe	Phenylalanine
PI	Propidium iodide
Pic	Pycolyl
Pro	Proline
Py	Pyridine
PyBOP	benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
ROS	Reactive oxygen species
S. aureus	*Staphylococcus aureus*
SAR	Structure activity relationship
TG	Genotype
Tris buffer	2-Amino-2-(hydroxymethyl)propane-1,3-diol
Trx1	Cytosolic thioredoxin
TrxR	Thioredoxin reductase
TrxR1	Cytosolic thioredoxin reductase
Val	Valine
µM	Micromolar
Z	Carboxybenzyl

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