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Highly Cytotoxic Bioconjugated Gold(I) Complexes with Cysteine-Containing Dipeptides

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Abstract: Several gold(I) complexes with cysteine-containing dipeptides have been prepared starting from cystine by coupling different amino acids and using several orthogonal protections. The first step is the reaction of cystine, where the sulfur centre is protected as disulfide, with Boc_2O in order to protect the amino group, followed by coupling of an amino acid ester; finally the disulfide bridge is broken with mercaptoethanol to afford the dipeptide derivative. Further reaction with $[AuCl(PPh_3)]$ gives the gold-dipeptide-phosphine species. Starting from these formally gold(I) thiolate–dipeptide phosphine complexes with the general formula $[Au(SR)(PR_3)]$ different structural modifications, such as change in the type of the amino protecting group, the type of phosphine, the number of gold(I) atoms per molecule, or the use of a non-proteinogenic conformationally restricted amino acid ester, were introduced in order to evaluate their influence in the biological activity of the final complexes. The cytotoxic activity, in vitro, of these complexes was evaluated against different tumour human cell lines (A549, MiaPaca2 and Jurkat). The complexes show an outstanding cytotoxic activity with IC_{50} values in the very low micromolar range. Structure–activity relationship studies from the complexes open the possibility of designing more potent and promising gold(I) anticancer agents.

Introduction

Metal-based drugs are an important class of compounds in medicinal chemistry, employed clinically for the treatment of different diseases.[1] Cisplatin and the following generations of platinum-based drugs are among the most widely used chemotherapeutic agents.[2] However, their effectiveness is still restricted by several clinical problems, such as a limited spectrum of activity, development of resistance and undesired toxic side-effects. The use of different metal compounds with different biological properties and targets has emerged as an alternative strategy to overcome these limitations.

Gold compounds have been employed since the last century for the treatment of rheumatoid arthritis and in recent decades some gold(I) and gold(III) complexes with promising biological activities as anticancer, antimicrobial, fungicidal, anti-HIV, or in the treatment of asthma or parasitic diseases, among others, have been prepared.[3] In addition, the mechanistic studies show that in general DNA is not the main target and interactions with several enzimes,[4] and more specifically the selenoenzime thioredoxine reductase,[5] proteasome,[6] kinases,[7] among others have been reported as the modes that finally lead to apoptosis through a mitochondrial pathway.

Diverse approaches have been used to prepare gold(I) and gold(III) drugs. For gold(I) the synthesis has been focused on the use of auranofin analogues[8] or the coordination to gold of several ligands mainly phosphines,[9] and carbenes.[10] For gold(III), cyclometallated complexes or coordination to the metallic centre of bypiridine, dithiocarbamate or porphyrin ligands have been successfully employed in order to prepare new anticancer gold drugs.[11]

Surprisingly and in spite of the great number of reported gold complexes which show activity in biological systems, the number of gold species with biomolecules such as amino acids and peptides is very scarce. Peptide research on drug design and drug discovery is one of the most promising fields in the development of new drugs. Peptide sequences are constituents of larger proteins, where they are responsible for molecular recognition and biological activities.[12]

There are only a few reports of reactions of gold(I) and mainly gold(III) with amino acids such as cysteine and methionine.[13] Gold(I) peptides with the cysteine-modified neuropeptide encephalin[14] and by gold(I) azide cycloaddition reactions with alkynyl peptides have been reported by Metzler-Nolte and co-workers.[15] The latter type of compounds has shown to overcome cisplatin resistance in a p53-mutant cancer cell line. The crystal structure of a gold(I) complex with the human glu-

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tathione reductase[16] and also a gold protein with the $AuPEt_3$ fragment bonded to a histidine rest[17] have been reported, and could give interesting insights on the biological function of gold compounds. We have previously reported on the synthesis and functionalisation of gold(I)-phosphine-nicotinic acid thiolate with amino acids providing complexes with very good cytotoxic activity.[18] Additionally, gold(III) peptide derivatives with histidine residues[19] and dithiocarbamate functionalised dipeptides have also been described.[20]

In this context and with the aim of preparing more active and selective gold drugs, we propose the formation of gold(I) complexes with dipeptides. The introduction of peptides in the complexes might decrease the undesired toxic side-effects (they are biocompatible ligands) and they could serve as good carriers for the delivery of the gold atom to the biological target.[21] 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 over-expressed receptors in some types of tumours, which could be a target for gold–peptide compounds, providing them with higher levels of internalization and subsequent selectivity.[20]

Here we report on an efficient and general method for preparing dipeptides containing a cysteine moiety and different proteinogenic amino acids, using different orthogonal protections. The coordination of these dipeptides to a gold(I) phosphine fragment gives novel and stable gold–peptide bioconjugates, the cytotoxic activities of which were studied. Several structural modifications of these peptide–gold-phosphine complexes were performed in order to determine which factor could improve their activity. Consequently, changes in the phosphine ligand, the amino protecting group, the number of coordinated $AuPPh_3^+$ units, or other modifications, such as the introduction of a conformationally restricted non-proteinogenic amino acid (Oic) in the peptide were performed.

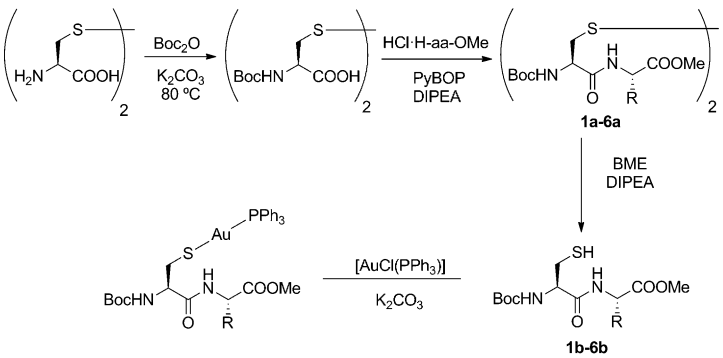
Cytotoxic activity in vitro against different tumour cell lines was analysed, showing outstanding cytotoxicity in all cases with IC_{50} values in the very low micromolar range.

Results and Discussion

Gold(I) dipeptide phosphine complexes

In our aim of preparing gold complexes with cysteine-containing dipeptides we thought of using as precursor the previously described complex, Ac-Cys($AuPPh_3$)-OH, in which there is an acid moiety that could couple with other amino acid esters, in a similar manner as we have reported earlier for the gold-phosphine complexes with the nicotinic acid thiolate.[18a] However, the preparation of the desired complexes was not possible by this route, probably because of solubility reasons. Then, a new synthetic strategy was selected, which consists of the use of commercially available cystine with different orthogonal protecting groups for each functional unit (amino, carboxylic acid and thiol) of the dipeptide.[22]

In the cystine the sulfur atom is already protected as disulfide, so the first step consisted of the protection of the amino group using Boc (*tert*-butoxycarbonyl) according to previously described conditions (Scheme 1). The success of the reaction was easily confirmed by ^1H NMR spectroscopy, and the spectrum shows the appearance of the signals corresponding to the Boc group at $\delta = 6.79$ (carbamate proton) and 1.36 ppm (alkyl protons). The following step was the coupling of the amino acid ester through the free carboxylic acid of (Boc-Cys-OH)$_2$ through standard solution peptide chemistry (employing PyBOP as activating agent and DIPEA as base), and subsequent flash chromatography yielded **1a**–**6a** derivatives in good yields. Six proteinogenic amino acid esters from glycine, alanine, valine, phenylalanine, methionine or proline were selected to prepare the dipeptide ligands. Each amino acid differs in the side chain group, which determine properties such as lipophilicity, polarity, reactivity or steric hindrance, and could have influence in the exchange reactions that are supposed to give the final complexes with other biomolecules in the organism,



Scheme 1. Synthesis of the gold(I) dipeptide complexes **1**–**6**.

affecting to the final activity, selectivity or biodistribution of the complex.

As common features, the ^1H and ^{13}C NMR spectra of compounds **1a**–**5a** show, in addition to all the expected resonances for the molecule, the signals belonging to the new amide bond in the range $\delta = 8.16$–7.40 ppm and $\delta = 170.5$–169.2 ppm, respectively. Compound **6a** appears as a mixture of rotamers (ratio 1:0.2) as a consequence of the conformationally restricted structure of proline, which possesses a five-membered ring (pyrrolidine) in its structure, giving a more complex spectrum. Selective reduction of the disulfides of **1a**–**6a** employing a strong reducing thiol (β-mercaptoethanol)[23] in soft basic media (DIPEA) afforded the desired dipeptide thiol ligands **1b**–**6b**. Six different dipeptides containing cysteine groups have been prepared according to the synthetic route show in Scheme 1: Boc-Cys-Gly-OMe (**1b**), Boc-Cys-Ala-OMe (**2b**), Boc-Cys-Val-OMe (**3b**), Boc-Cys-Phe-OMe (**4b**), Boc-Cys-Met-OMe (**5b**) and Boc-Cys-Pro-OMe (**6b**). The ^1H NMR spectra

present the resonance for the thiol group as a doublet of doublets or as a multiplet in the range $\delta = 1.46$-1.90 ppm, while the resonances corresponding to Cβ protons appear each one with a characteristic shape as doublet of doublet of doublets (diastereotopic protons). The IR spectra also present the absorption bands corresponding to the thiol group in the range 2562–2580 cm^{-1} as weak bands. The dipeptides **1b**–**6b** are valuable ligands that easily react with [AuCl(PPh_3)] employing K_2CO_3 as a base, to give the desired complexes **1**–**6** in high yields after chromatographic purification. Complexes **1**–**6**, just as all the complexes reported in this work, were fully characterized by ^1H, ^{13}C and ^{31}P NMR spectroscopy, IR spectroscopy and MS spectrometry. The ^1H NMR spectra show the resonances corresponding to the dipeptide and triphenylphosphine protons. The proton for the thiol group has disappeared, which agrees with coordination of the sulfur to gold affording the desired complexes. Remarkably, as a common feature, the resonances of the Cβ protons (diastereotopic protons) are simplified and each one appears as a doublet of doublets at approximately $\delta = 3.60$ and 3.25 ppm, strongly downfield shifted relative to the starting dipeptide. The $^{31}P\{^1H\}$ NMR spectra present a unique resonance for all of the complexes in the range $\delta =$ 36–38 ppm, which agrees with similar gold(I) complexes, with thiolates and phosphines as ligands previously reported, and is 3–5 ppm downfield shifted relative to the starting gold(I) complex. The $^{13}C\{^1H\}$ NMR APT spectra show the resonances for the dipeptide and triphenylphosphine moieties. Again, the signals belonging to Cβ appear downfield shifted after complex formation (in the range of 30–35 ppm). The assignment of the resonances was made with the ^1H-^{13}C HSQC and ^1H-^{13}C HMBC experiments. The IR spectra present, among others, the absorptions for the amide, ester and aromatic unit and also the disappearance of the absorptions for the thiol group. The mass spectra (HRMS ESI+) present for all the compounds the molecular peak $[M+Na]^+$ and $[M+H]^+$. The different gold(I) complexes with the dipeptides are shown in Figure 1.

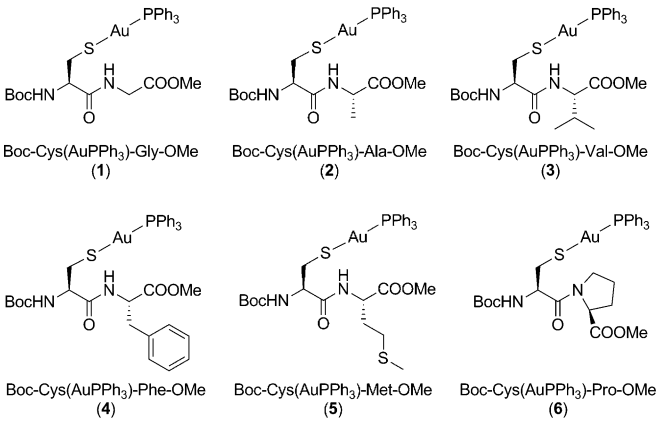


Figure 1. Gold(I) dipeptide complexes **1**–**6**.

Structural modifications

The structure of complexes **1**–**6** described above, allows the introduction of several structural modifications with the purpose of obtaining information on how the changes in the structure affect the biological properties of the complexes, and thus we can stablish a structure–activity relationship (SAR), and can obtain compounds with better activity, selectivity and pharmaceutical profile. The selected structural modifications performed were: 1) alteration of the phosphine (**7**), 2) alteration of the amino protecting group (**8**), 3) coupling of a non-proteinogenic conformationally restricted amino acid ester (**9**), and 4) alteration of the charge and number of gold atoms per molecule (**10**–**11**). All of these modifications could influence the polarity, electronic or steric properties of the complex, activity or selectivity, and alter the biodistribution or reactivity of the final complex towards other biomolecules in the organism. The different modifications carried out are summarized in Figure 2.

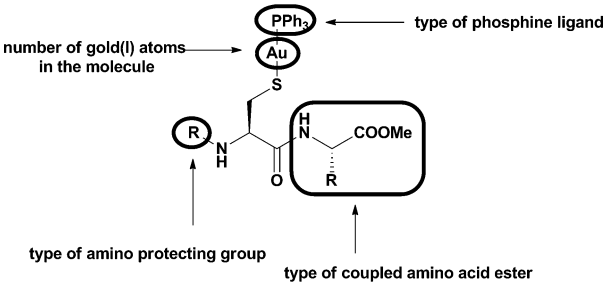
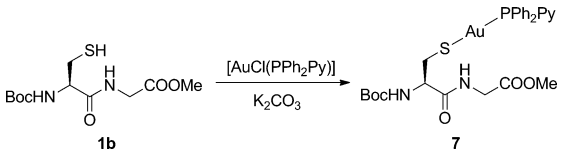


Figure 2. Structural modifications introduced in the complexes.

Preparation of the complex with a different phosphine ligand (7)

The phosphine PPh_2Py was selected to prepare the analogue of complex **1**. Because the type of phosphine coordinated to gold(I) has a great influence in the activity of the final complex, determining factors such as reactivity, behaviour in exchange reactions or biodistribution, the change for the related phosphine with a pyridine moiety PPh_2Py was employed as ancillary ligand, which could bind further to other metals, interact with other biomolecules or alter the lipophilicity of the complex. Treatment of Boc-Cys-Gly-OMe (**1b**) with [AuCl(PPh_2Py)] afforded complex **7** in high yield after chromatographic purification (Scheme 2).

The ^1H and $^{13}C\{^1H\}$ NMR spectra show the expected resonances from all the protons and carbon atoms, which are essentially the same as those observed for the analogous complex

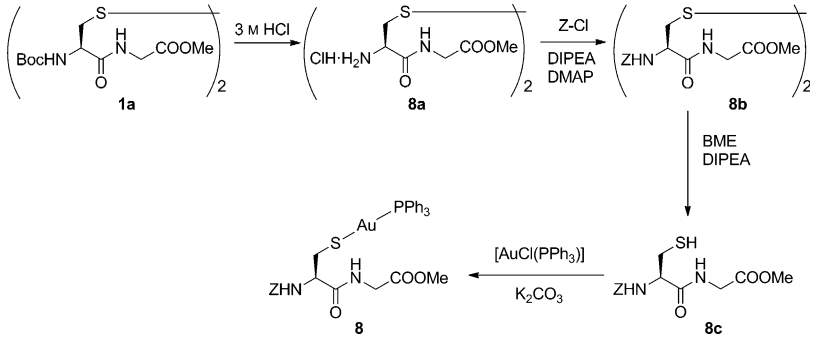


Scheme 2. Synthesis of complex **7**.

1, and will not be discussed further here. The only exceptions are the signals that belong to the pyridine, for which a correct assignment could be realized by 2D NMR spectroscopy (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC) since these resonances and those of the phenyl rings appear at similar chemical shift. Again, the ^{31}P{^{1}H} NMR spectra present a unique resonance corresponding to the phosphorus of the 2-diphenylphosphino pyridine ligand, the chemical shift is observed at $\delta = 37.0$ ppm, a value similar to that observed for **1**. IR and MS data agree with the proposed structure.

Preparation of the complex with different amino protecting group (8)

Boc (*tert*-butoxycarbonyl) was employed as the amino protecting group in the preparation of the complexes **1**–**6**. In this case, the Z (benzyloxycarbonyl) group was selected in order to evaluate how this change affects the biologic activity. Although in both cases the amino group is protected as a carbamate, the change introduced could affect the polarity or exchange reactions with other biomolecules in the organism, which would be important for the activity and selectivity of the drug. The [Au(Z-Cys-Gly-OMe)(PPh_3)] complex **8** was prepared following a similar synthetic route to that used in the preparation of complexes **1**–**6** (Scheme 3).



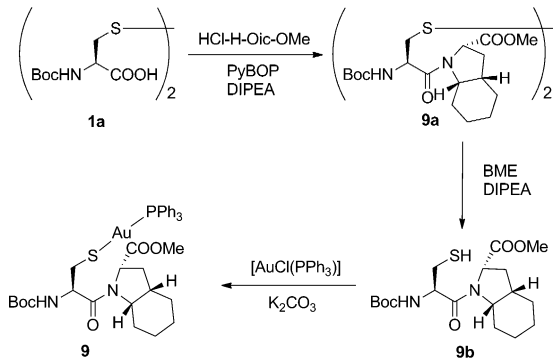
Scheme 3. Synthesis of complex **8**.

Again, orthogonal protection of the different functional groups in the molecule was necessary. In the first place, starting from the disulfide **1a**, cleavage of Boc amino protecting group was carried out employing standard conditions by treatment with 3 M HCl/AcOEt solution. Introduction of the Z protecting group was successfully achieved by treatment with a convenient source as Z-Cl, employing DIPEA as a base and DMAP as catalyst. The intermediate **8b** was obtained in moderate yield after chromatographic purification. Discussion of spectroscopic data (^{1}H and ^{13}C NMR spectra) is similar to that described previously for the analogue **1b** with the exception that there are signals belonging to the benzyl group instead of the *tert*-butyl group as can be found in the Supporting Information. Disulfide reduction was carried out in the same way as described above. Again, the ^{1}H NMR spectrum of dipeptide **8c** shows, as the most remarkable features, the appearance of the signal of the thiol group at $\delta = 1.70$ ppm as a doublet of doublets and the diastereotopic Cβ protons appears each one with the characteristic shape as a doublet of doublet of doublets. The IR spectrum clearly shows a band corresponding to the absorption of the SH group at 2579 cm^{-1}. The last step consisted of the deprotonation of the thiol and coordination of the corresponding thiolate to the gold(I) centre as described above to give the desired complex **8** in pure form and high yield. Complex **8** is also analogous to **1** and consequently in the spectroscopic data the same features are observed: the ^{1}H NMR spectrum shows the disappearance of the thiol proton and the Cβ protons resonance appears downfield shifted and with a more simplified shape. In the ^{13}C NMR spectrum the signal belonging to Cβ is also shifted downfield, and in the IR spectrum the thiol band also disappears after the formation of the complex, which agrees with the proposed structure.

Preparation of the complex with different coupled amino acid ester (9)

The type of amino acid ester has influence in the ability of the carrier to deliver the gold(I) to its target. In fact, in a previous work we observed that functionalisation with proline usually gives better cytotoxic complexes compared with other amino acids.[18b] In this case, we decided to couple to the cystine derivative the octahydroindole amino acid ester (Oic), a non-proteinogenic bicyclic proline analogue.[24] This amino acid has been employed in the design of peptides with interesting pharmaceutical profiles, mainly because conformationally restricted amino acids help to fix conformations and have influence in the interactions with receptors in the organism. Moreover, the dipeptides containing this moiety could present a higher resistance to hydrolysis by diverse enzymes. For all of these reasons, the preparation of a complex bearing this moiety is expected to improve the activity and selectivity. The synthetic route employed to prepare the complex **9** is shown in the Scheme 4.

As can be observed, this synthetic route is essentially the same as described previously for the preparation of the dipeptide complexes **1**–**6** and will not be further discussed here. In this case, the coupling of another amino ester type was carried out. Similar to complex **6** bearing the proline moiety, complex **9** appears as a mixture of rotamers in the NMR spectra. This complex possesses a high number of protons and carbon atoms, many of them showing similar chemical shifts. Nevertheless, all the signals could be correctly assigned by the realization of 2D NMR experiments (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC).

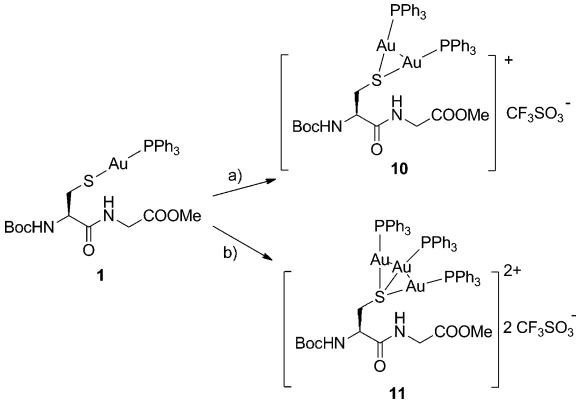


Scheme 4. Synthesis of complex **7**.

Preparation of the complexes with higher number of gold atoms per molecule (10 and 11)

Diverse studies indicate that in gold(I) complexes with thiolates and phosphines as ligands the cytotoxicity is due mainly to the metallic centre; whereas the phosphine ligand allows the gold(I) atom to cross the cell membrane, the thiolate takes part in exchange ligand reactions with other biomolecules. Keeping this in mind, it is logical to think that adding more gold(I) atoms per molecule will increase the cytotoxicity of the complex, as we observed previously.[18b] Moreover, there is a strong interest in the synthesis of lipophilic and cationic compounds which seem to be able to cross cell membranes and selectively accumulate in mitochondria,[25] just where it appears that gold(I) complexes exert their therapeutic effect, by inhibiting diverse enzymes as for example thioredoxin reductase (TrX). Moreover, further coordination of gold(I) atoms to sulfur could have a strong influence on the transference reactions of the metal to other biomolecules, determining its transport, biodistribution or enzyme-inhibition ability. For all of these reasons, we carried out the synthesis of the dinuclear (**10**) and trinuclear (**11**) cationic complexes derived from the mononuclear complex **1** by treatment with the high electrophilic complex [Au(OTf)(PPh_3)] generated in situ (Scheme 5).

Remarkably, the ^{1}H NMR spectrum of complex **11** appears as a mixture of rotamers in contrast with complexes **1** and **10**, probably due to more steric hindrance. In all cases we observed the expected resonances for the protons belonging to the dipeptide and triphenylphosphine. In the ^{1}H NMR spectra of complexes **10** and **11**, the carbamate and amide protons, and all the protons belonging to the dipeptide appear more downfield shifted than observed for complex **1**. Integration of aromatic protons corresponding to triphenylphosphine ligands also confirms the coordination of additional gold(I) triphenylphosphine moieties. The ^{19}F NMR spectra show a single signal at $\delta = -77.8$ ppm that belongs to the triflate counter anion in both cases. However, the most notable feature is the chemical shift for the phosphorus atoms in the ^{31}P NMR spectra, which appears at $\delta = 33.2$ ppm (dinuclear complex) and $\delta = 32.3$ ppm (trinuclear complex), that are strongly up-field shifted (4 and 5 ppm, respectively) compared with **1**. The mass spectra (ESI+) presents the molecular peak $[M]^+$.



Scheme 5. Synthesis of the dinuclear (**10**) and trinuclear (**11**) complexes derived from **1**. i) [Au(OTf)(PPh_3)], ii) 2 [Au(OTf)(PPh_3)].

Cytotoxic activity

The cytotoxicity of complexes **1**–**11** was tested against three different human tumour cell lines: Jurkat (T-cell leukaemia), MiaPaca2 (pancreatic carcinoma) and A549 (lung carcinoma), and compared to the results with cisplatin.

Compounds **1**–**11** are not soluble in water, but they are soluble in DMSO and in the DMSO/water mixtures used in the tests, which contain a small amount of DMSO. We did not observe any precipitation of the complexes or metallic gold while performing the tests. Their colourless [D_6]DMSO solutions are very stable at room temperature, as shown in the ^{1}H NMR spectra in which the signals remain the same for weeks. Cells were exposed to different concentrations of each compound for a total of 24 h. Using the colorimetric MTT viability assay (MTT = 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide), the IC_{50} values (final concentration $< 0.5\%$ DMSO) were calculated from dose-response curves obtained by non-linear regression analysis. IC_{50} values are concentrations of a drug required to inhibit tumour cell proliferation by 50%, compared to the control cells treated with DMSO alone. The IC_{50} values for complexes **1**–**11** are collected in Table 1. These values can be compared with those reported for cisplatin dissolved in water: IC_{50} at 24 h in A549, MiaPaca2 and Jurkat cells are 114.2, 76.5 and 10.8 μM, respectively.[26]

Table 1. IC_{50} [μM] (24 h), with standard deviations, of complexes against A549, MiaPaca2 and Jurkat cell lines.

Complex	A549	MiaPaca2	Jurkat
1	1.5±0.2	2.0±0.2	0.9±0.1
2	1.9±0.1	1.9±0.1	1.6±0.1
3	2.3±0.1	3.0±0.1	2.2±0.1
4	15.6±0.11	5.4±0.1	0.4±0.1
5	4.8±0.1	1.8±0.1	1.7±0.1
6	3.0±0.1	0.7±0.1	0.5±0.1
7	5.0±0.2	0.5±0.1	0.8±0.1
8	2.7±0.1	1.5±0.1	1.1±0.1
9	2.1±0.1	1.2±0.1	1.5±0.1
10	1.8±0.1	0.1±0.1	0.6±0.1
11	3.5±0.1	1.5±0.1	0.8±0.1

All the synthesized complexes were active against the different tumour cell lines in very low concentrations (low micromolar range). Complexes **1**–**11** exhibit excellent antiproliferative activities, with IC_{50} values ranging from 1.5 to 15.6 μM in A549 cells, 0.4 to 2.2 μM in Jurkat cells, and 0.1 to 5.4 μM in MiaPaca2. The Jurkat and MiaPaca2 cell lines were the most sensitive to our compounds, whereas A549 showed more resistance to the complexes.

The gold(I) dipeptide complexes (**1**–**6**) displayed very good cytotoxicity in all the tumour cell lines. The type of the proteinogenic amino ester coupled to cysteine has some influence on the cytotoxicity of the final complex: the complexes which incorporate glycine or proline in their structure showed the best IC_{50} values. The change of the type of phosphine ligand coordinated to gold(I) (complex **7**), the amino protecting group (complex **8**) or the amino acid ester coupled to cysteine (complex **9**) led to more potent complexes in the MiaPaca2 tumour cell line, although in the A549 resulted in a decrease of the cytotoxicity of the complex in comparison with the related complex **1**. The coordination of an additional $[AuPPh_3]^+$ fragment (complex **10**) to **1** has a strong influence in the cytotoxicity, mainly in the MiaPaca2 and Jurkat tumour cell lines. In particular, complex **10** was the most potent of all the series. Surprisingly, coordination of two additional $[AuPPh_3]^+$ fragments (complex **11**) to **1** did not improve the potency of the complex. Then, the use of a dipeptide containing glycine or proline, or the coordination of two gold(I) centres to the sulfur atom, giving a cationic complex, yielded the most potent cytotoxic complexes.

Comparison of the activity of this family of gold–peptide derivatives with the anticancer drug cisplatin, shows that these complexes displayed much more in vitro cytotoxic activity. In relation with other gold(I)–peptide species our complexes have much lower IC_{50} values, although comparison with the same cell lines is not possible. The activity is also higher than in the gold(I)–nicotinic acid thiolate functionalised with amino acids previously described by us.[14b] Nevertheless, the very low values obtained in these complexes in resistant cell lines such as A549 and MiaPaca2 measured after 24 h, represent outstanding values, which makes them very promising metallodrug candidates for continuing their evaluation and studying their mechanism of action.

Conclusions

A series of gold(I) complexes with cysteine-containing dipeptides have been prepared starting from cystine by coupling different amino acids, and using several orthogonal protections. In these molecules the gold centre is coordinated directly to the dipeptide, to the sulfur of the cysteine as thiolate. These novel derivatives with biologically relevant molecules could deliver the gold centre selectively to the tumour cells because they can act as peptidomimetics and target peptide delivery systems, which have over-expressed receptors in tumour cells.

In these formal gold(I) thiolate–dipeptide phosphine complexes, with the general formula $[Au(SR)(PR_3)]$, different structural modifications such as change in the type of the amino protecting group, the type of phosphine, the number of $AuPPh_3^+$ fragments coordinated to the sulfur centre, or the use of non-proteinogenic conformationally restricted amino acid ester in the peptide, were introduced in order to evaluate their influence in the biological activity of the final complexes. The cytotoxic activity, in vitro, of these complexes was evaluated against different tumour human cell lines (A549, MiaPaca2 and Jurkat). The complexes show excellent cytotoxic activity with IC_{50} values in the very low micromolar range (as low as 0.1 μM). The structural changes made to the parent compound led to the synthesis of the most effective compound in all the cell lines tested, which is the complex with two $AuPPh_3^+$ fragments coordinated to the Boc-Cys-Gly-OMe peptide.

Experimental Section

Instrumentation

C, H, and N analysis were carried out with a Perkin–Elmer 2400 microanalyzer. Mass spectra were recorded on a Bruker Esquire 3000 Plus, with the electrospray (ESI) technique and on a Bruker Microflex (MALDI-TOF). ^1H, $^{13}C\{H\}$, $^{31}P\{H\}$ and ^{19}F NMR, including 2D experiments, were recorded at room temperature on a Bruker Avance 400 spectrometer (^1H, 400 MHz, ^{13}C, 100.6 MHz) or on a Bruker Avance II 300 spectrometer (^1H, 300 MHz, ^{13}C, 75.5 MHz), with chemical shifts (δ, ppm) reported relative to the solvent peaks of the deuterated solvent.[21]

Starting materials

$[AuCl(PPh_3)]$ and $[AuCl(PPh_2Py)]$ were prepared according to published procedures.[27] All other reagents were commercially available. Solvents were used as received without purification or drying.

General procedure for coupling amino acid esters: synthesis of compounds 1a–6a and 9a (Procedure A)

To a solution of $(Boc\text{-}Cys\text{-}OH)_2$ (1 mmol) in anhydrous DMF (5 mL) was added the corresponding amino ester hydrochloride (2.4 mmol), PyBOP (2.2 mmol) and DIPEA (6.6 mmol). The mixture was stirred for 48 h under argon atmosphere at room temperature. Then, the resultant clear solution was diluted with AcOEt (100 mL) and washed with water (5×60 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered off and evaporated to dryness. The crude of the reaction was purified by column chromatography on silica gel using as eluent a mixture of ethyl acetate/hexane (1:1).

General procedure for reducing disulfides to thiols: synthesis of compounds 1b–6b, 8c and 9b (Procedure B)

To a solution of the corresponding disulfide $(Boc\text{-}Cys\text{-}aa\text{-}OMe)_2$ (1 mmol) in dry CH_2Cl_2 (50 mL), β-mercaptoethanol (4 mmol) and DIPEA (4 mmol) were added. The mixture was stirred for 48 h under argon atmosphere at room temperature. Then, the solvent was evaporated under reduced pressure and the crude of reaction redissolved in AcOEt (100 mL) and washed with an aqueous saturated solution of $KHSO_4$ (3×40 mL) and a saturated aqueous solution of NaCl (3×40 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered off and evaporated to dryness. The crude of the reaction was purified by column chromatography on silica gel using as eluent a mixture of acetone/hexane (1:1).

General procedure for the synthesis of gold(I) dipeptide complexes 1–9 (Procedure C)

To a solution of the corresponding dipeptide derivative Boc-Cys-aa-OMe (1 mmol) in acetone (10 mL) an excess of K_2CO_3 (2 mmol) was added. The mixture was stirred for 5 min. Then, [AuCl(PPh_3)] or [AuCl(PPh_2Py)] (1 mmol) in acetone (10 mL) was added and the reaction mixture was stirred at room temperature for 24 h. After this time, the reaction mixture was filtered over Celite and the clear solution was evaporated under reduced pressure. Complexes **1–9** were purified by column chromatography of silica gel using as eluent a mixture of AcOEt/hexane (1:1).

Detailed synthetic procedures for the individual compounds can be found in the Supporting Information.

Cell culture

Jurkat (leukaemia) and MiaPaca2 (pancreatic carcinoma) cell lines were maintained in RPMI 1640, while A549 (lung carcinoma) were grown in DMEM (Dulbecco's Modified Eagle's Medium). Both media were supplemented with 5% foetal bovine serum (FBS), 200 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin and 2 mM L-glutamine. Medium for A549 cells was also supplemented with 2.2 g L^{-1} Na_2CO_3, 100 μg mL^{-1} pyruvate and 5 mL non-essential amino acids (Invitrogen). Cultures were maintained in a humidified atmosphere of 95% air/5% CO_2 at 37 °C. Adherent cells were allowed to attach for 24 h prior to addition of compounds.

Cytotoxicity assay by MTT

The MTT assay was used to determine cell viability as an indicator for cell sensitivity to the complexes. Exponentially growing cells were seeded at a density of approximately 1×10^5 cells mL^{-1} for the adherent cell lines (A549, MiaPaca2) or 5×10^4 cells mL^{-1} (Jurkat), in a 96-well flat-bottomed microplate and 24 h later they were incubated for 24 h with the compounds. The complexes were dissolved in DMSO and tested in concentrations ranging from 0.1 to 25 μM and in quadruplicate. Cells were incubated with our compounds for 24 h at 37 °C. 10 μL of MTT (5 mg mL^{-1}) was added and plates were incubated for 1–3 h at 37 °C. Finally, 100 μL per well *i*PrOH (0.05 M HCl) was added. The optical density was measured at 490 nm using a 96-well multiscanner autoreader (ELISA). The IC_{50} was calculated by nonlinear regression analysis using Origin software (Origin Software, Electronic Arts, Redwood City, California, USA).

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