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ARTICLE

Bioactive Gold(I) Complexes with 4-Mercaptoproline Derivatives

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Unprecedented gold(I) bioconjugates bearing non-proteinogenic amino acid 4-mercaptoproline species as bioorganic ligands have been prepared. Firstly, the synthesis of Boc-Pro(SH)-OMe (**1**) has been accomplished by standard procedures. The subsequent reaction of **1** with [AuCl(PR_3)] gives complexes Boc-Pro(SAuPR_3)-OMe (PR_3 = PPh_3 (**2**), PPh_2Py (**3**)). Starting from complex **2** several structural modifications have been performed, in addition to the incorporation of a different phosphine in **3**, such as the formation of the acid Boc-Pro(SAuPPh_3)-OH (**4**), the synthesis of a dipeptide derivative by coupling the amino acid glycine *tert*-butyl ester Boc-Pro(SAuPPh_3)-Gly-O^{t}Bu (**5**), or the coordination of another gold phosphine fragment to the sulfur atom as in [Boc-Pro(SAuPPh_3)$_2$-OMe]OTf (**6**). The cytotoxic activity *in vitro* of these complexes has been evaluated against three different tumor human cell lines, A549 (lung carcinoma), Jurkat (T-cell leukaemia) and MiaPaca2 (pancreatic carcinoma). All the complexes displayed excellent cytotoxic activity with IC_{50} values in the low μM range and even in the nM range in some cases. Structure-Activity Relationships (SAR) observed from this family of complexes opens the possibility to design more potent and selective promising gold(I) anticancer agents.

Introduction

Metal-based drugs are currently successfully used in the chemotherapeutic treatment of certain types of cancer. For instance, cisplatin and other platinum-based drugs are clinically used in the treatment of testicular and ovarian cancer with a high therapeutic success,[1] meanwhile some ruthenium complexes are under clinical trials.[2] However, for platinum based drugs the effectiveness is still hindered by several clinical problems, such as a limited spectrum of activity, development of resistance and undesired toxicity,[3] which makes highly desirable the research of new metal complexes with better pharmacological profile, such as activity, selectivity and low side effects.

In this context, gold complexes occupy a relevant position and have been postulated as an interesting class of anticancer agents. Gold compounds have been traditionally used in medicine, and some gold(I) drugs, like auranofin, are being employed clinically in the treatment of rheumatoid arthritis since last century. However, in the last decades gold complexes have attracted great attention because of their excellent anticancer properties in vitro and in vivo, superior in many cases to cisplatin.[4] Consequently, a large number of gold(I) complexes bearing several types of ligands such as thiolates (auranofin analogues),[5] phosphines,[6] carbenes,[7] among others, have been reported. Mechanistic studies showed that these compounds exert their therapeutic effect over different targets than cisplatin,[8] thus opening up the way to the synthesis of new drugs, overcoming the aforementioned limitations.

In this context, amino acids and peptides are two important classes of biomolecules that form proteins and enzymes in the organism and display a wide range of biological activities.[9] They could also be employed as useful biocompatible ligands to deliver the gold(I) atom to its target. Moreover, due to the fact that tumor cells over-express amino acid receptors and have more requirements of nutrients, complexes with amino acids could be more selective to abnormal cells.[10]

Among all the proteinogenic amino acids, proline is the only one possessing a cyclic pyrrolidine moiety in its structure. This important fact determines the properties, structure, function and biological activities of the peptides and proteins that incorporate this fragment. An important proline derivative is 4-hydroxyproline which is found in the organism forming collagen, conjunctive and bone issue. The introduction of different functional groups at the 4 position has led to the amine, chloride or fluoride derivatives. The replacement of the hydroxide with the thiol group originates the non-proteinogenic amino acid 4-mercaptoproline, which can be considered as a hybrid of proline and homocysteine (Figure 1). This unusual amino acid puts together the properties of both amino acids that are the nucleophilic character and reducing ability of the thiol group and the conformational restrictions corresponding to proline. The employment of non-proteinogenic amino acids in the design of new compounds

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with better pharmacological properties has been extensively used. For instance, incorporation of conformationally restricted amino acids (cyclic or quaternary amino acids) often leads to the preparation of compounds with better hydrolytic resistance or receptor recognition.[11]

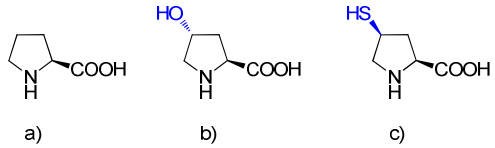


Fig 1. Structures of proline (a), 4-hydroxyproline (b), and 4-mercaptoproline (c).

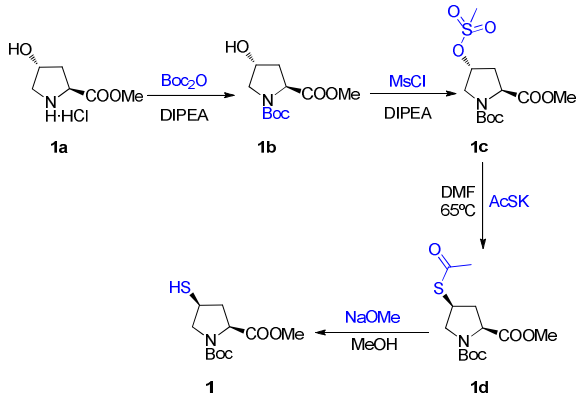
The synthesis of the first 4-mercaptoproline derivatives dates back to 1970,[12] and although some of the them showed interesting biological activities,[13] to the best of our knowledge only a nickel complex has been reported with a proline derivative substituted in 4 position with a sulfur fragment, but in any case the ligand is coordinated to the metal as thiolate.[14] Our research has been focusing on the synthesis of gold(I) derivatives with biologically-relevant ligands, and we have previously reported on the preparation of some gold(I) complexes with amino acids and peptides with interesting biological activities.[15]

Here we describe the synthesis of unprecedented gold(I) bioconjugates with *N*-protected 4-mercaptoproline ester derivatives. Several structural modifications, such as change of the type of phosphine ligand or change in the number of gold(I) atoms per molecule have been performed in order to establish some Structure-Activity Relationships (SAR) for this class of compounds. The cytotoxicity of the new complexes has been studied *in vitro* against three different human tumor cell lines. All the complexes exhibit strong cytotoxicity with IC_{50} values in the μM range, even in the nM in some cases.

Results and Discussion

Synthesis and Characterisation

The synthesis of the *N*-protected 4-mercaptoproline ester species **1**, used as starting material to prepare the gold complexes, was achieved following the synthetic route showed in Scheme 1. Firstly, the suitable 4-mercaptoproline protected ligand **1** was prepared starting from commercially available 4-hydroxyproline aminoester chlorohydrate **1a** in several steps. The introduction of the amino Boc (*tert*-butyloxycarbonyl) protecting group was performed according to standard methods using Boc_2O in the presence of DIPEA (N,N-diisopropilethylamine),[16] and the success of the reaction was confirmed by the appearance of a signal in the ^{1}H NMR spectrum of compound **1a** at δ = 1.41 ppm corresponding to the alkyl protons of the protecting group. The IR spectrum also shows the disappearance of the absorption band belonging to the amine, whereas the new carbamate absorption is observed at 1672 cm^{-1}. The hydroxyl group of compound **1b** can be derivatised to a methanesulfonate which is an excellent leaving group.[16c,17] The ^{1}H NMR spectrum of **1c** shows a new resonance as a singlet at δ = 3.05 ppm corresponding to the introduced methanesulfonate. This intermediate was reacted immediately with potassium thioacetate according to previously described conditions yielding the thioester derivative **1d**.[16] Note that displacement of the methanesulfonate by the thioacetate group is accompanied by inversion of configuration, which is characteristic of a SN_2 reaction type. The ^{1}H NMR spectrum clearly shows the disappearance of the signals belonging to the methanesulfonate and the appearance of a new singlet resonance at δ = 2.32 ppm corresponding to the thioacetate. The 4-mercaptoproline ester **1** was obtained by methanolysis of the thioester **1d**. As an important feature, the band of the thiol group can be observed as a doublet at δ = 1.76 ppm in the ^{1}H NMR spectrum. The IR spectrum also shows the absorption corresponding to the thiol group at 2554 cm^{-1}.



Scheme 1. Synthesis of the *N*-protected 4-mercaptoproline ester compound **1**.

This modified thio-proline molecule can be coordinated to a gold(I) centre by reaction with [AuCl(PPh_3)] in the presence of an excess of K_2CO_3 to give the desired complex **2** (see Scheme 2). The ^{1}H NMR spectrum of **2** shows all the expected resonances for the thioaminoester and triphenylphosphine ligands. Remarkably, the complex appears as a mixture of rotamers in a 1:0.3 ratio. The resonance belonging to the thiol disappears after complex formation, whereas the proton corresponding to C_γ appears strongly downfield shifted (around 0.5 ppm) compared to the starting thiol derivative. The protons of both methylene groups corresponding to carbons C_β and C_δ are diastereotopic, and appear at different chemical shifts due to the cyclic structure and the presence of two chiral centres in the molecule. In the $^{13}C\{^1H\}$ NMR spectrum all the signals belonging to the pyrrolidine ring appear strongly downfield shifted ($\Delta\delta \approx 5$ ppm) after coordination to gold(I) except in the case of C_γ. The $^{31}P\{^1H\}$ NMR spectrum shows a singlet at δ = 36.5 ppm characteristic of gold(I) complexes with thiolate and phosphine as ligands. The IR and MS data also confirms the expected stoichiometry. All the signals could be correctly assigned performing 2D NMR experiments.

In order to evaluate the effect of the phosphine ligand in the final cytotoxicity of the complex, we have replaced

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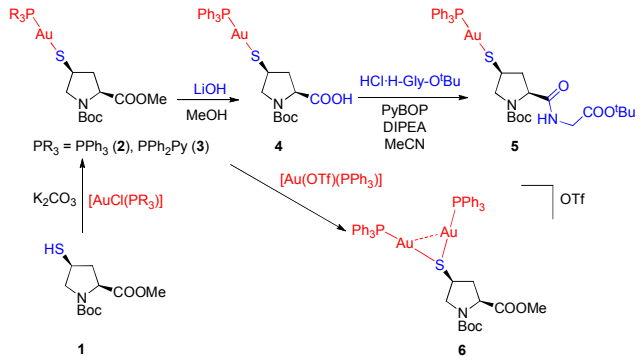
triphenylphosphine with 2-pyridyl diphenylphosphine. Complex **3** was prepared in a similar way to that described previously for complex **2**. In this case, [AuCl(PPh_2Py)] was employed as starting gold(I) complex. The ^{1}H and ^{13}C{^{1}H} NMR spectra are very similar with those of complex **2**, with the only exception of the signals belonging to the pyridyl ring of the phosphine ligands, that could be properly assigned by carrying out 2D NMR experiments. Although both phosphines possess similar electronic and sterical properties, they could shows differences in the lipophilicity character, and the pyridyl ring could interact or form hydrogen bonds with different biologically relevant molecules, thus influencing in the activity of the complex.



Scheme 2. Synthesis of gold(I) complexes from *N*-Boc protected mercaptoproline.

The preparation of the dipeptide ligand containing mercaptoproline and glycine *tert*-butyl ester by using a similar procedure to that described in Scheme 1 failed in the thioester acetate introduction. The introduction of the second amino acid ester group was accomplished with success, but the step of the nucleophilic substitution of the methanesulphonate group for the thioacetate produced a mixture of compounds, probably due to the higher steric hindrance of the dipeptide molecule compared to the amino ester **1c**, what prevented the SN_2 reaction. Therefore, we decided to follow a different route that starts from complex **2**, as is shown in Scheme 2. In the first step, basic hydrolysis of the amino ester moiety in complex **2**, employing LiOH as a base, gave the desired amino acid-containing complex **4**. Note that although the Au-S and Au-P bonds show high stability in basic media this type of complexes quickly decompose in acidic media (pH < 3) as we previously described. So during the reaction workup, careful acidification must be carried out. The spectroscopic data are very similar to complex **2**, with the notable exception of the disappearance of the methyl ester signals in the ^{1}H NMR spectrum. Again, complex **4** appears as a rotamers mixture in 1:1 ratio. In the ^{13}C{^{1}H} NMR spectrum, in addition to the disappearance of the methyl resonance, the new signal corresponding to the carboxylic acid is observed at δ = 177.5 ppm, strongly downfield-shifted (around 4.5 ppm) compared to the ester. The ^{31}P{^{1}H} NMR spectrum shows a single resonance similar to that observed for complex **2**. The IR spectrum clearly shows the disappearance of the ester band and the appearance of the bands corresponding to the carboxylic acid at 3300-3100 and 1694 cm^{-1}. The HRMS(ESI+) data also agree with the proposed structure.

The coupling of glycine *tert*-butyl ester through the free carboxylic acid of **4** gave the desired complex with the dipeptide **5**. The coupling reaction was carried out according to standard solution peptide synthesis techniques, using PyBOP (benzotriazolyloxy-tris[pyrrolidino]-phosphonium hexafluorophosphate) as activating agent and DIPEA as a base. Complex **5** was obtained in good yield and in pure form after chromatographic purification. The ^{1}H NMR spectrum shows all the expected resonances for the dipeptide and phosphine ligands. Complex **5** appears as a rotamers mixture (ratio 1:0.6). The new amide bond appears at δ = 6.81 and δ = 6.50 ppm for each rotamer. In the ^{13}C{^{1}H} NMR spectrum, the new amide bond appears at δ = 168.8 ppm, strongly upfield shifted (almost 10 ppm) compared to the starting free carboxylic acid. However, the signals of C_α and C_β appear downfield-shifted (3-4 ppm). In the IR spectrum, the disappearance of the carboxylic acid band and the appearance of the new amide bond at 1742 cm^{-1} are also observed. All the signals could be correctly assigned by the realisation of 2D NMR experiments.

Complexes with more than one gold(I) atom per molecule are expected to exhibit higher cytotoxic activity, as we previously have observed,[15] and in addition species with lipophilic cations have attracted a lot of attention due to their ability to cross cell membranes. For these reasons, we decided to synthesise the dinuclear cationic complex. The reaction of **2** with [Au(OTf)(PPh_3)] generated in situ afforded the desired dinuclear complex **6** in high yield and pure form after recrystallisation (Scheme 2). The ^{1}H NMR spectrum shows all the resonances expected for the molecule, slightly downfield shifted (around 0.2 ppm) compared to their analogues in complex **2**. In this case, the aromatic protons belonging to the triphenylphosphine integrate for 30H instead of 15H ppm because in this case two [Au(PPh_3)]$^+$ fragments coordinate to the thiolate ligand. In the ^{13}C{^{1}H} NMR spectrum, the most notable feature is the resonance corresponding to C_γ, that appears strongly downfield shifted (4 ppm) compared with complex **2**. Remarkably, the ^{31}P{^{1}H} NMR spectrum shows a single signal at δ = 34.2 ppm, strongly upfield shifted (around 3-4 ppm) compared to **2**, and characteristic for this type of dinuclear complexes. The HRMS(ESI+) shows [M]$^+$ = 1178.2186 m/z with the expected isotopic pattern and the IR spectrum also agree with the proposed structure.

Cytotoxic activity

The cytotoxic activity of complexes **2**-**6** was tested against three different human tumor cell lines: A549 (lung carcinoma), Jurkat (T-cell leukaemia) and MiaPaca2 (pancreatic carcinoma) and compared with that of cisplatin.

Compounds **2**-**6** 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

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performing the tests. Their colourless DMSO-d_6 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 **2**-**6** 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 μM, 76.5 μM and 10.8 μM, respectively.[18]

Table 1. IC_{50} (24 h) of complexes (**2-6**) against A549, MiaPaca2 and Jurkat (μM).

COMPLEX	A549	MiaPaca2	Jurkat
Boc-Pro(SAuPPh$_3$)-OMe (**2**)	1.8±0.15	3.0±0.19	0.8±0.08
Boc-Pro(SAuPPh$_2$Py)-OMe (**3**)	3.8±0.37	6.1±0.54	3.5±0.32
Boc-Pro(SAuPPh$_3$)-OH (4)	>25	>25	9.3±0.65
Boc-Pro(SAuPPh$_3$)-Gly-OtBu (**5**)	3.5±0.29	2.3±0.22	0.6±0.08
[Boc-Pro(SAuPPh$_3$)$_2$-OMe]OTf (**6**)	1.9±0.16	1.8±0.17	0.5±0.07
Cisplatin	114.2	76.5	10.8

Studies of the cytotoxic activity in many gold phosphine complexes revealed that cytotoxicity is mainly due to the metal atom, while the ancillary phosphine ligand stabilizes the gold metallic centre and allow membrane crossing. The role of thiolates in general and the thiolate amino acid ligand in particular is also important because these ligands take part in diverse exchange reactions with other biomolecules in the organism, which determine facts such as transport or biodistribution.

In general, the Jurkat cell line was the most sensitive, and the A549 and MiaPaca2 exhibit more resistance to our complexes. All the complexes displayed excellent cytotoxicity in all the cell lines, with IC_{50} values in the low micromolar range (< 25 μM) even in some cases in the nanomolar range with values as low as 0.5 μM.

The gold-amino ester conjugate **2** displayed excellent cytotoxicty much greater than cisplatin, over 100, 23 and 10 times folder in A549, MiaPaca2 and Jurkat cell lines, respectively. Therefore, the gold(I) complex with the 4-mercaptoproline ester constitutes a good candidate and an excellent start point for the design of new gold-peptide anticancer agents.

The change of triphenylphosphine by 2-pyridyl diphenylphosphine **3** as type of phosphine ligand used reduces by half the cytotoxic activity of the complex. Again, lipophilicity seems to be a key feature in the final cytotoxicity of the complex.

The complex with the 4-mercaptoproline acid **4** instead of the 4-mercaptoproline ester results in a drastic decrease of cytotoxicity. Then, the amino ester ligand plays an important role in the activity of the complex, and complexes with an ester instead of carboxylic acid moiety yield more active compounds. Most probably, the higher lipophilic character of the ester allows the complex to better penetrate into the cell, or another possibility is that the carboxylic acid moiety could further react with other biological molecules preventing the complex to reach their final targets.

To get a better insight in the relationship between lipophilicity and hydrophilicity in these complexes we have measured such relationship in terms of $logD_{7.4}$ (n-octanol/water partition coefficient under physiological conditions). Complexes **2** and **3** bearing the phosphine ligands PPh_3 and PPh_2py have a clear lipophilic character with log $logD_{7.4}$ values of 0.75 and 0.68, respectively, whereas the acid derivative **4** has a value of 0.41, which represent a slightly more balanced relationship between the lipophilic and hydrophilic character but still being mainly lipophilic.

The complex **5**, with a dipeptide containing 4-mercaptoproline, also exhibits excellent cytotoxic activity whit IC_{50} values comparable to those found for complex **2**. The effect of lengthen the peptide chain from one to two residues does not seem to have a critical influence in the cytotoxicity of the final complex, although the dipeptide complex is slightly more active in the MiaPaca2 and Jurkat cell lines, and slightly less active in A549 compared to the complex with the amino ester **2**. Finally, the introduction of an additional $[Au(PPh_3)]^+$ fragment gives excellent cytotoxic agents. Complex **6** is slightly more potent than its mononuclear analogue **2** in all cell lines. This complex is charged, highly lipophilic and with more gold(I) atoms per molecule.

Comparison of the bioactivity with related gold(I) phosphine complexes bearing sulfur ligands can be made. This complexes present better activity than the gold thionicotinic acid derivatives functionalised with amino acid esters[15c] and similar to those reported for gold cysteine containing dipeptides previously described by us.[15d] The importance of the P-Au-S unit is remarkable, as similar values were found in different cell lines for gold species with different sulfur ligands such as dithiocarbamates.[19]

From these results, we can extract some important Structure-Activity Relationships (SARs). Gold(I) complexes with thiolates and phosphines of this type are expected to be good cytotoxic agents. The functionalisation of the thiolate ligand with an ester moiety, lengthen the amino acid chain or coordinate additional gold(I) atoms seem to be suitable approaches in order to obtain excellent cytotoxic complexes. However, the change of the ester moiety or triphenylphosphine ligand by a carboxylic acid moiety or 2-pyridil diphenylphosphine ligands, respectively, is associated with a decrease in the potency of the complex.

Further design of new complexes following the SARs obtained from this work and further biological studies in order to obtain information about the mechanism and targets of our complexes are currently conducted in our laboratories.

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Conclusions

The synthesis of an *N*-protected mercaptoproline derivative has been developed and the first gold thiolate complexes have been prepared. The complex bearing a triphenylphosphine coordinated to the sulfur atom serves as precursors for the synthesis of other metal thiolates containing different 4-thioproline derivatives, including a 4-thioproline-glycine peptide. These are unprecedented examples of metal complexes coordinated to the 4-mercaptoproline derivatives in the form of thiolates.

In this formally gold(I) thiolate-amino acid or thiolate-dipeptide phosphine complexes with the general formula [Au(SR)(PR_3)] different structural modifications, such as change in the type of phosphine, the use of an ester, acid or amino acid in the carboxylic side chain, or the number of $AuPPh_3^+$ fragments coordinated to the sulfur centre were introduced, in order to evaluate their influence in the biological activity of the final complexes. The cytotoxic activity in vitro of these complexes has been evaluated against different tumor 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 0.5 μM. The structural changes performed in the parent compound have led to the synthesis of the most effective compound in all the cell lines, which is the complex with two $AuPPh_3^+$ fragments coordinated to the 4-mercaptoproline methyl ester ligand.

Experimental

Instrumentation. Mass spectra were recorded on a BRUKER ESQUIRE 3000 PLUS, with the electrospray (ESI) technique and on a BRUKER MICROFLEX (MALDI-TOF). ^{1}H, ^{13}C{^{1}H}, ^{31}P{^{1}H} and ^{19}F NMR, including 2D experiments, were recorded at room temperature on a BRUKER AVANCE 400 spectrometer (^{1}H, 400 MHz, ^{13}C{^{1}H}, 100.6 MHz) or on a BRUKER AVANCE II 300 spectrometer (^{1}H, 300 MHz, ^{13}C{^{1}H}, 75.5 MHz), with chemical shifts (δ, ppm) reported relative to the solvent peaks of the deuterated solvent.[20]

Starting Materials. [AuCl(PPh_3)] and [AuCl(PPh_2Py)] were prepared according to published procedures.[21] All other reagents were commercially available. Solvents were used as received without purification or drying.

Synthesis of Boc-Pro(OH)-OMe (1b). To a solution of HCl·H-Pro(OH)-OMe **1a** (1.090 g, 6 mmol) in CH_2Cl_2 (12 mL), Boc_2O (1.44 g, 6.6 mmol) and DIPEA (1.54 mL, 9 mmol) were added. The reaction mixture was stirred overnight at room temperature and, then, was washed with an aqueous saturated solution of NaCl (3x25 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered off and evaporated. The crude was purified by column chromatography of silicagel using as eluent a mixture of AcOEt/hexane (4:6) affording **1b** (yield = 79.6 %) as a colorless oil (1.170 g, 4.77 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz, δ (ppm), *J* (Hz)): 4.50 (m, 1H, $C_{\gamma,Pro}H_2$), 4.39 (t, 1H, *J* = 8.1, $C_{\alpha,Pro}H$), 3.73 (s, 3H, OCH_3), 3.56 (dd, diastereotopic protons, 2H, *J* = 11.7 and 4.2 and *J* = 30.9 and 4.2, $C_{\delta,,Pro}H_2$), 2.30 and 2.05 (m, diastereotopic protons, 2H, $C_{\beta,Pro}H_2$), 1.41 (s, 9H, $C_{Boc}H_3$). HRMS ESI(-) *m/z*: $[M+Na]^+$ = 268.1155 (calcd), 268.1153 (found). IR (cm^{-1}): 3500-3300 (br, *OH*), 1746 (s, *COOMe*), 1672 (s, *OCON*), 1155 and 1085 (s, *C-O*). TLC R_f: 0.4 (AcOEt/hexane 4:6).

Synthesis of Boc-Pro(OMs)-OMe (1c). To a solution of **1b** (1.170 g, 4.77 mmol) in dry CH_2Cl_2 (50 mL) under argon atmosphere, DIPEA (0.984 mL, 5.75 mmol) was added, and the mixture was stirred for 5 min. Then, MsCl (0.445 mL, 5.75 mmol) was added at 0 °C dropwise. The reaction mixture was stirred at room temperature for 2 h, and the solvent was evaporated, affording **1c** in nearly quantitative yield (1.542 g, 4.77 mmol), as an orange oil pure enough to be employed without further purification. ^{1}H NMR ($CDCl_3$, 300 MHz, δ (ppm), *J* (Hz)): rotamers mixture (ratio 1:0.7): 5.25 (m, 1H, $C_{\gamma,Pro}H_2$), 4.43 (td, 1H, *J* = 16.0 and 7.8, $C_{\alpha,Pro}H$), 3.82 (m, 2H, $C_{\delta,Pro}H_2$), 3.74 (s, 3H, OCH_3), 3.05 (s, 3H, CH_3SO_3), 2.61 and 2.26 (m, diastereotopic protons, 2H, $C_{\beta,Pro}H_2$), 1.46 and 1.42 (s, 9H, $C_{Boc}H_3$). HRMS ESI(+) *m/z*: $[M+Na]^+$ = 346.0930 (calcd), 346.0927 (found). IR (cm^{-1}): 1747 (s, *COOMe*), 1701 (s, *CON*), 1364 and 1159 (w, SO_3), 1159 (s, *C-O*). TLC R_f: 0.5 (AcOEt/hexane 3:7).

Synthesis of Boc-Pro(SAc)-OMe (1d). To a solution of **1c** (1.170 g, 4.77 mmol) in dry DMF (20 mL) under argon atmosphere, AcSK (0.953 g, 8.35 mmol) was added. The reaction mixture was stirred at 65 °C under 24 h. Then, the mixture was diluted with Et_2O (100 mL) and washed with water (3x50 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered off and evaporated, and the reaction crude was purified by column chromatography using as eluent a mixture of AcOEt/hexane (2:8) affording **1d** (yield = 70.2 %) as a brown oil (1.015 g, 3.35 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz, δ (ppm), *J* (Hz)): rotamers mixture (ratio 1:0.85): 4.33 (dt, 1H, *J* = 25.6 and 7.9, $C_{\alpha,Pro}H$), 3.97 (m, 1H, $C_{\gamma,Pro}H_2$), 3.96 and 3.34 (m, diastereotopic protons, 2H, $C_{\delta,Pro}H_2$), 3.47 (s, 3H, OCH_3), 2.70 and 1.96 (m, diastereotopic protons, 2H, $C_{\beta,Pro}H_2$), 2.32 (s, 3H, CH_3COS), 1.45 and 1.41 (s, 9H, $C_{Boc}H_3$). HRMS ESI(-) *m/z*: $[M+Na]^+$ = 326.1033 (calcd), 326.1051 (found). IR (cm^{-1}): 1750 (s, *COOMe*), 1691 (s, *OCON* and *CH$_3$COS*), 1154 (s, *C-O*). TLC R_f: 0.5 (AcOEt/hexane 3:7).

Synthesis of Boc-Pro(SH)-OMe (1). To a solution of **1d** (0.918 g, 3.03 mmol) in MeOH (30 mL), NaOMe (0.164 g, 3.03 mmol) was added. The reaction mixture was stirred at room temperature for 20 min. Then, the solvent was evaporated and the crude was dissolved in AcOEt (50 mL) and washed with water (3x50 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered off and evaporated, affording **1** (yield = 74.4 %) as a green oil (0.589 g, 2.25 mmol) pure enough to be employed without further purification. ^{1}H NMR ($CDCl_3$, 300 MHz, δ (ppm), *J* (Hz)): rotamers mixture (ratio 1:0.7): 4.22 (td, 1H, *J* = 21.3 and 8.0, $C_{\alpha,Pro}H$), 3.89 and 3.22 (m, diastereotopic protons, 2H, $C_{\delta,Pro}H_2$), 3.69 (s, 3H, OCH_3), 3.23 (m, 1H, $C_{\gamma,Pro}H_2$), 2.65 and 1.87 (m, diastereotopic protons, 2H, $C_{\beta,Pro}H_2$), 1.76 (d, 1H, *J* = 7.5, S*H*), 1.40 (B) and 1.35 (A) (s, 9H, $C_{Boc}H_3$). ^{13}C{^{1}H} NMR ($CDCl_3$, 300 MHz, δ (ppm), *J* (Hz)): rotamers mixture: 172.7 (A) and 172.5 (B) (*C*OOMe), 153.6 (B) and 152.9 (A) (CON_{Pro}), 80.4 (B) and 80.3 (A) (C, C_{Boc}), 58.9 (A) and 58.5 (B)

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($C_{\alpha,Pro}H$), 55.7 (B) and 55.2 (A) ($C_{\delta,Pro}H_2$), 52.1 (B) and 52.0 (A) (OCH_3), 41.1 (A) and 40.3 (B) ($C_{\beta,Pro}H_2$), 35.1 (B) and 34.4 (A) ($C_{\gamma,Pro}H$) and 28.2 (A) and 28.0 (B) ($C_{Boc}H_3$). HRMS ESI(+) *m/z*: $[M+Na]^+$ = 284.0927 (calcd), 284.0944 (found). IR(cm^{-1}): 2554 (w, SH), 1747 (s, *COOMe*), 1694 (s, *OCON*), 1154 (s, *C-O*).

Synthesis of Boc-Pro(SAuPPh$_3$)-OMe (2). To a solution of **1** (0.123 g, 0.47 mmol) in acetone (10 mL), K_2CO_3 (0.130 g, 0.94 mmol) and [AuCl(PPh_3)] (0.232 g, 0.47 mmol) were 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 evaporated under reduced pressure. Complex **2** was purified by column chromatography on silicagel using as eluent a mixture of acetone/hexane (3:7), affording pure complex **2** (0.334 g, 0.46 mmol) as an orange solid (Yield = 98.8 %). ^{1}H NMR ($CDCl_3$, 300 MHz, δ (ppm), *J* (Hz)): rotamers mixture (ratio 1:0.3): 7.48 (m, 15H, *Ar*), 4.20 (m, 1H, $C_{\alpha,Pro}H$), 4.01 and 3.34 (m, diastereotopic protons, 2H, $C_{\delta,Pro}H_2$), 3.79 (m, 1H, $C_{\gamma,Pro}H_2$), 3.71 (B) and 3.63 (A) (s, 3H, OCH_3), 2.77 and 1.96 (m, diastereotopic protons, 2H, $C_{\beta,Pro}H_2$), 1.41 (B) and 1.37 (A) (s, 9H, $C_{Boc}H_3$). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 300 MHz, δ (ppm), *J* (Hz)): rotamers mixture: 173.1 (A) and 172.9 (B) (*C*OOMe), 153.7 (B) and 152.9 (A) (CON_{Pro}), 134.0 (d, CH, *J* = 13.7, *C*2), 131.7 (d, CH, *J* = 2.3, *C*4), 129.1 (d, CH, *J* = 11.6, *C*3), 129.0 (d, C, *J* = 58.8, *C*1), 80.4 (B) and 79.7 (A) (C, C_{Boc}), 59.7 (A) and 59.1 (B) ($C_{\alpha,Pro}H$), 59.0 (B) and 58.5 (A) ($C_{\delta,Pro}H_2$), 51.8 (B) and 51.7 (A) (OCH_3), 45.5 (A) and 45.0 (B) ($C_{\beta,Pro}H_2$), 39.1 (B) and 38.6 (A) ($C_{\gamma,Pro}H$) and 28.3 (B) and 28.2 (A) ($C_{Boc}H_3$). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 300 MHz, δ (ppm), *J* (Hz)): 37.7. HRMS ESI(+) *m/z*: $[M+Na]^+$ = 742.1426 (calcd), 742.1455 (found). IR (cm^{-1}): 1746 (s, *COOMe*), 1693 (s, *OCON*), 1479 and 1435 (w, *Ar*), 1152 and 1099 (s, *C-O*), 746, 709 and 691 (w, *Ar*). TLC R_f: 0.5 (AcOEt/hexane 1:1).

Synthesis of Boc-Pro(SAuPPh$_2$Py)-OMe (3). The reaction of **1** (0.052 g, 0.2 mmol), K_2CO_3 (0.0552 g, 0.4 mmol) and [AuCl(PPh_2Py)] (0.991 g, 0.2 mmol) following a procedure similar to **2** afforded **3** (0.134 g, 0.19 mmol) as an orange solid (Yield = 96.3 %). ^{1}H NMR ($CDCl_3$, 300 MHz, δ (ppm), *J* (Hz)): rotamers mixture (ratio 1:0.5): 8.75 (d, 1H, *J* = 4.8, *H*1), 7.87 (t, 1H, *J* = 7.5, *H*4), 7.80-7.73 (m, 1H, *H*3), 7.67-7.62 and 7.50-7.40 (m, 10H, *Ar*), 7.38-7.33 (m, 1H, *H*2), 4.26-4.16 (m, 1H, $C_{\alpha,Pro}H$), 4.12-3,97 and 3.39-3.32 (m, diastereotopic protons, 2H, $C_{\delta,Pro}H_2$), 3.85-3.76 (m, 1H, $C_{\gamma,Pro}H_2$), 3.71 (B) and 3.64 (A) (s, 3H, OCH_3), 2.84-2.71 and 2.00-1.92 (m, diastereotopic protons, 2H, $C_{\beta,Pro}H_2$), 1.41 (B) and 1.37 (A) (s, 9H, $C_{Boc}H_3$). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 300 MHz, δ (ppm), *J* (Hz)): rotamers mixture: 173.2 (A) and 173.0 (B) (*C*OOMe), 154.4 (C, d, *J* = 80.8, *C*5), 152.9 (CON_{Pro}), 151.2 (CH, d, *J* = 15.4, *C*1), 136.6 (d, CH, *J* = 10.7, *C*3), 134.4 (d, CH, *J* = 13.7, *C*7), 131.8 (d, CH, *J* = 2.5, *C*9), 131.2 (d, CH, *J* = 32.0, *C*4), 129.0 (d, CH, *J* = 7.2, *C*8), 128.8 (d, C, *J* = 58.9, *C*6), 125.2 (d, CH, *J* = 2.4, *C*2), 80.5 (B) and 79.8 (A) (C, C_{Boc}), 59.7 (A) and 59.1 (B) ($C_{\alpha,Pro}H$), 58.5 (B) and 53.7 (A) ($C_{\delta,Pro}H_2$), 51.9 (B) and 51.7 (A) (OCH_3), 45.5 (A) and 45.1 (B) ($C_{\beta,Pro}H_2$), 39.1 (B) and 38.6 (A) ($C_{\gamma,Pro}H$) and 29.2 (B) and 28.3 (A) ($C_{Boc}H_3$). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 300 MHz, δ (ppm), *J* (Hz)): 35.7. HRMS ESI(+) *m/z*: $[M+H]^+$ = 721.1558 (calcd), 721.1725 (found). IR (cm^{-1}): 1748 (s, *COOMe*), 1694 (s, *OCON*), 1479 and 1436 (w, *Ar*), 1151 and 1099 (s, *C-O*), 746, 709 and 691 (w, *Ar*). TLC R_f: 0.5 (AcOEt/hexane 1:1).

Synthesis of Boc-Pro(SAuPPh$_3$)-OH (4). To a suspension of complex **2** (0.719 g, 1 mmol) in methanol (5 mL) was added LiOH (0.120 g, 5 mmol). The mixture was stirred at room temperature for 24 h. The reaction is followed by thin layer chromatography and is complete when the starting compound is strongly retained at the origin of the thin layer chromatography (R_f = 0 (acetone/hexane 1:1)). At this point, the methanol is evaporated and the product dissolved in water. The resulting white solution is acidified dropwise with a saturated solution of $KHSO_4$ until a slightly acid pH (3-4). Then, the solution was extracted three times with dichloromethane and the organic phase was dried over anhydrous $MgSO_4$, filtered off and evaporated to yield the desired complex **4** (yield = 91.2 %) as a green solid (0.644 g, 0.91 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz, δ (ppm), *J* (Hz)): rotamers mixture (ratio 1:1): 7.49 (m, 15H, *Ar*), 4.31 and 4.21 (m, 1H, $C_{\alpha,Pro}H$), 4.06 (A,B) and 3.38 (A) and 3.23 (B) (m, diastereotopic protons, 2H, $C_{\delta,Pro}H_2$,), 3.80 (m, 1H, $C_{\gamma,Pro}H_2$), 2.80 (A, B) and 2.31 (A) and 2.06 (B) (m, diastereotopic protons, 2H, $C_{\beta,Pro}H_2$), 1.44 (B) and 1.38 (A) (s, 9H, $C_{Boc}H_3$). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 300 MHz, δ (ppm), *J* (Hz)): rotamers mixture: 177.5 (*C*OOH), 155.3 (CON_{Pro}), 134.0 (d, CH, *J* = 13.7, *C*2), 131.8 (d, CH, *J* = 2.3, *C*4), 129.2 (d, CH, *J* = 11.6, *C*3), 129.0 (d, C, *J* = 58.7, *C*1), 81.0 and 80.3 (C, C_{Boc}), 59.7 (A) and 59.4 (B) ($C_{\alpha,Pro}H$), 58.6 ($C_{\delta,Pro}H_2$), 45.5 (B) and 44.3 (A) ($C_{\beta,Pro}H_2$), 38.9 ($C_{\gamma,Pro}H$) and 28.1 ($C_{Boc}H_3$). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 300 MHz, δ (ppm), *J* (Hz)): 36.3. HRMS ESI(-) *m/z*: $[M-H]^-$ = 704.1293 (calcd), 704.0564 (found). IR (cm^{-1}): 3300-3100 (br, *COOH*), 1709 (s, *OCON*), 1694 (s, *COOH*), 1479 and 1435 (w, *Ar*), 1153 and 1099 (s, *C-O*), 746, 710 and 691 (w, *Ar*).

Synthesis of Boc-Pro(SAuPPh$_3$)-Gly-O^{t}Bu (5). To a suspension of **4** (0.705 g, 1 mmol) in acetonitrile (5 mL) was added DIPEA (0.377 mL, 2.2 mmol). The mixture was stirred for 5 min at room temperature and then PyBOP added (0.624 g, 1.2 mmol). The resulting solution was stirred for an additional 45 min. To this solution at 0 °C was added dropwise a solution of HCl·H-Gly-O^{t}Bu (0.201 g, 1.2 mmol) and DIPEA (0.205 mL, 1.2 mmol) in acetonitrile (5 mL). After the addition the mixture was stirred for 48 h at room temperature. The acetonitrile was evaporated and dichloromethane (40 mL) was added. This solution was washed with a saturated $NaHCO_3$ solution in water (3 x 15 mL) and a saturated solution of NaCl (3 x 15 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 silicagel using as eluent a mixture of AcOEt/hexane (1:1), affording **5** (yield = 76.8 %) as a green oil (0.629 g, 0.77 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz, δ (ppm), *J* (Hz)): rotamers mixture (ratio 1:0.6): 7.46 (m, 15H, *Ar*), 6.87 (B) and 6.58 (A) (m, 1H, $CONH_{Gly}$), 4.12 (m, 1H, $C_{\alpha,Pro}H$), 4.12 and 3.25 (m, diastereotopic protons, 2H, $C_{\delta,Pro}H_2$,), 3.93 and 3.83 (dd and m, ABX system, 2H, *J* = 18.3 and 5.1, $C_{\alpha,Gly}H_2$), 3.72 (m, 1H, $C_{\gamma,Pro}H_2$), 2.76 and 2.10 (m, diastereotopic protons, 2H, $C_{\beta,Pro}H_2$,), 1.40 (s, 18H, $C_{Boc}H_3$). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 300 MHz, δ (ppm), *J* (Hz)): rotamers mixture: 172.5 (A) and 168.8 (B) (*C*OO^{t}Bu), 157.4 (A) and 154.0 (B) (CON_{Pro}), 134.0 (d, CH, *J* = 13.7, *C*2), 131.5 (d, CH, *J* = 2.4,

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*C*4), 129.7 (C, *C*1), 129.1 (d, CH, *J* = 11.5, *C*3), 81.9 (B) and 80.3 (A) (C, C_{Boc}), 62.1 ($C_{\alpha,Pro}H$), 59.3 (B) and 59.0 (A) ($C_{\delta,Pro}H_2$), 49.7 (B) and 46.0 (A) ($C_{\beta,Pro}H_2$), 42.0 ($C_{\alpha,Gly}H_2$), 38.7 (B) and 38.2 (A) ($C_{\gamma,Pro}H$) and 28.1 (B) and 27.9 (A) ($C_{Boc}H_3$). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 300 MHz, δ (ppm), *J* (Hz)): 38.3. HRMS ESI(+) *m/z*: $[M+H]^+$ = 819.2290 (calcd), 819.2311 (found). IR (cm^{-1}): 3320 (br, *CONH*), 1742 (s, *COOMe*), 1684 (s, *CONH*), 1479 and 1435 (w, *Ar*), 1151 and 1099 (s, *C-O*), 744 and 691 (w, *Ar*). TLC R_f: 0.5 (AcOEt/hexane 6:4).

Synthesis of [Boc-Pro(SAuPPh$_3$)$_2$-OMe]OTf (6). To a solution of complex **2** (0.072 g, 0.1 mmol) in CH_2Cl_2 (5 mL) was added [Au(OTf)(PPh_3)] (0.061 g, 0.1 mmol) freshly prepared. The mixture was stirred for 2 h at room temperature. Then, the yellow solution resultant was filtered on Celite. The solution was concentrated to ca. 5 mL and the addition of hexane (20 mL) afforded **6** (yield = 97.4 %) as a brown solid (0.126 g, 0.09 mmol). ^1H NMR ($CDCl_3$, 300 MHz, δ (ppm), *J* (Hz))**:** rotamers mixture (ratio 1:0.2): 7.46 (m, 30H, *Ar*), 4.59 (B) and 4.28 (A) (m, 1H, $C_{\alpha,Pro}H$), 4.59 (B) and 4.27 (A) (m, 1H, $C_{\gamma,Pro}$), 4.08 and 3.75 (m, diastereotopic protons, 2H, $C_{\delta,Pro}H_2$), 3.71 (B) and 3.66 (A) (s, 3H, O*C*H_3), 2.98 and 2.11 (A) and 2.87 and 2.47 (B) (m, 2H, $C_{\beta,Pro}H_2$) and 1.37 (s, 9H, $C_{Boc}H_3$). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 300 MHz, δ (ppm), *J* (Hz)): rotamers mixture: 172.8 (A) and 172.7 (B) (*C*OOMe), 166.4 (B) and 152.8 (A) (CON_{Pro}), 134.0 (d, CH, *J* = 13.6, *C*2), 132.5 (d, CH, *J* = 2.6, *C*4), 129.7 (d, CH, *J* = 11.9, *C*3), 127.6 (d, C, *J* = 61.2, *C*1), 114.4 (d, C, *J* = 305.6, *C*F_3), 80.8 (C, C_{Boc}), 58.9 (B) and 59.5 (A) ($C_{\alpha,Pro}H$), 58.0 (B) and 57.6 (A) ($C_{\delta,Pro}H_2$), 52.3 (B) and 52.2 (A) (O*C*H_3), 44.0 (A) and 43,3 (B) ($C_{\beta,Pro}H_2$), 42.4 (A) and 41.8 (B) ($C_{\gamma,Pro}H$) and 28.2 ($C_{Boc}H_3$). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 300 MHz, δ (ppm), *J* (Hz)): 34.2. ^{19}F NMR ($CDCl_3$, 300 MHz, δ (ppm), *J* (Hz)): -78.0. HRMS ESI(-) *m/z*: $[M]^+$ = 1178.2104 (calcd), 1178.2186 (found). IR (cm^{-1}): 1745 (s, *COOMe*), 1694 (s, *OCONH*), 1480 and 1436 (w, *Ar*), 1150 and 1100 (s, *C-O*), 744, 711 and 690 (w, *Ar*).

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% fetal bovine serum (FBS), 200 U/mL penicillin, 100 μg/mL streptomycin and 2 mM L-glutamine. Medium for A549 cells was also supplemented with 2.2 g/l Na_2CO_3, 100 μg/mL piruvate and 5 mL non-essential amino acids (Invitrogen). Cultures were maintained in a humidified atmosphere of 95% air/5% CO_2 at 37 ºC. 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 cells sensitivity to the complexes. Exponentially growing cells were seeded at a density of approximately $1x10^5$ cells/mL for the adherent cell lines (A549, MiaPaca2) or $5x10^4$ cells/mL (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 ºC. 10 μl of MTT (5 mg/mL) was added and plates were incubated for 1-3 h at 37 ºC. Finally, 100 μl/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 non-linear regression analysis using Origin software (Origin Software, Electronic Arts, Redwood City, California, USA).

Distribution Coefficients (log $D_{7.4}$)

The n-octanol-water coefficients of the complexes were determined as previously reported using a shake-flask method.[22] Buffered-saline distilled water (100 mL, phosphate buffer [PO_4^{3-}] = 10 μM, [NaCl] = 0.15 M, pH 7.4) and n-octanol (100 mL) were shaken together for 72 h to allow saturation of both phases. Approximately 1 mg of the complexes was dissolved in 5 mL of the aqueous phase and 5 mL of the organic phase were added, mixing for 10 minutes. The resultant emulsion was centrifuged to separate the phases. The concentration of the compounds in each phase was determined using UV absorbance spectroscopy. Log$D_{7.4}$ was defined as log{[compound$_{(organic)}$]/[compound$_{(aqueous)}$]}.

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References

1 (a) B. Rosenberg, L. VanCamp, J. E. Trosko and V. H. Monsour, *Nature,* 1969, **222**, 385; (b) *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*. Ed. B. Lippert, Wiley-VCH, Weinheim, 1999; (c) T. C. Johnstone, K. Suntharalingam and S. J. Lippard, *Chem. Rev.*, 2016, **116**, 3436.

2 (a) E. S. Antonarakis and A. Emadi, *Cancer Chemother. Pharmacol.,* 2010, **66**, 1; (b) A. Bergamo and G. Sava, *Dalton Trans.,* 2011, **40**, 7817.

3 L. Kelland, *Nat. Rev. Cancer,* 2007, **7**, 573.

4 (a) E. R. T. Tiekink, *Inflammopharmacology,* 2008, **16**, 138; (b) I. Ott, *Coord. Chem. Rev.,* 2009, **253**, 1670; (c) A. Casini and L. Messori, *Curr. Top. Med. Chem*., 2011, **11**, 2647; (d) B. Bertrand and A. Casini, *Dalton Trans.,* 2014, **43**, 4209; (e) C. M. Che and R. W. Y. Sun, *Chem. Commun.,* 2011, **47**, 9554; (f) T. Zou, C. T. Lum, C.-N. Lok, J.-J. Zhang and C. M. Che, *Chem. Soc. Rev.,* 2015, **44**, 8786; (g) E. M. Nagy, L. Ronconi, C. Nardon and D. Fregona, *Mini-Rev. Med. Chem.*, 2012, **12**, 1216.

5 See for example: (a) I. Ott, X. Quian, Y. Xu, V. Vlecker, I. J. Marques, D. Kubutat, J. Will, W. S. Sheldrick, P. Jesse, A. Prokop and C. P. Bagowski, *J. Med. Chem.,* 2009, **52**, 763; (b) E. Vergara, A. Casini, F. Sorrentino, O. Zava, E. Cerrada, M. P. Rigobello, A. Bindolli, M. Laguna and P. J. Dyson, *ChemMedChem.,* 2010, **5**, 96; (c) D. T. Hill, A. A. Isab, D. E. Griswold, M. J. DiMartino, E. D. Matz, A. L. Figueroa, J. E. Wawro, C. DeBrosse, C Reiff, R. C. Elder, B. Jones, J. W. Webb and C. F. Shaw III, *Inorg. Chem.,* 2010, **49**, 7663.

6 See for example: (a) S. Urig, K. Fritz-Wolf, R. Réau, C. Herold-Mende, K. Tóth, E. Davioud-Charvet and K. Becker, *Angew. Chem. Int. Ed.,* 2006, **45**, 1881; (b) C. Santini, M. Pellei, G.

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DOI: 10.1039/C6DT02000C

Papini, B. Morresi, R. Galassi, S. Ricci, F. Tisato, M. Porchia, M. P. Rigobello, V. Gandin and C. Marzano, *J. Inorg. Biochem.,* 2011, **105**, 232; (c) J. Fernández-Gallardo, B. T. Ellie, F. J. Sulzmaier, M. Sanau, J. W. Ramos and M. Contel, *Organometallics,* 2014, **33**, 6669.

7 See for example: (a) M. M. Jellicoe, S. J. Nichols, B. A. Callus, M. V. Baker, P. J. Barnard, S. J. Berners-Price, J. Whelan, G. C. Yeoh and A. Filipovska, *Carcinogenesis,* 2008, **29**, 1124; (b) E. Schuh, C. Pflüger, A. Citta, A. Folda, M. P. Rigobello, A. Bindoli, A. Casini and F. Mohr, *J. Med. Chem.,* 2012, **55**, 5518; (c) L. Oehninger, R. Rubbiani and I. Ott, *Dalton Trans.,* 2013, **42**, 3269; (d) W. Liu and R. Gust, *Chem. Soc. Rev.,* 2013, **42**, 755; (f) A. Citta, E. Schuh, F. Mohr, A. Folda, M. L. Massimino, A. Bindoli, A. Casini and M. P. Rigobello, *Metallomics* 2013, **5**, 1006; (g) F. Hackenberg, H. Muller-Bunz, R. Smith, W. Streciwilk, X. Zhu and M. Tacke, *Organometallics,* 2013, **32**, 5551.

8 (a) P. T. Barnard and S. J. Berners-Price, *Coord. Chem. Rev.,* 2007, **251**, 1889; (b) A. Casini, C. Hartinger, C. Gabbiani, E. Mini, P. J. Dyson, B. K. Keppler and L. Messori, *J. Inorg. Biochem.,* 2008, **102**, 564; (c) L. Dalla Via, C. Nardon and D. Fregona, *Future Med. Chem.,* 2012, **4**, 525; (d) M. Stallings-Mann, L. Jamieson, R. P. Regala, C. Weens, N. R. Murray and A. P. Fields, *Cancer Res.,* 2006, **66,** 1767.

9 Handbook of Biologically Active Peptides, A. J. Kastin, Ed., Elsevier, 2006.

10 A. F. A. Peacock, G. A. Bullen, L. A. Gethings, J. P. Williams, F. H. Kriel and J. Coates, *J. Inorg. Biochem.,* 2012, **117**, 298.

11 A. Flores-Ortega, A. I. Jiménez, C. Cativiela, R. Nussinov, C. Alemán and J. Casanovas, *J. Org. Chem.,* 2008, **73**, 3418.

12 (a) A. J. Verbiscar, and B. Witkop, *J. Org. Chem.,* 1970, **35**, 1924; (b) K. Plucinska, T. Kataoka, M. Yodo, W. L. Cody, J. X. He, C. Humblet, G. H. Lu, E. Lunney, T. C. Major, R. Panek, P. Schelkun, R. Skeean and G. Marshall, *J. Med. Chem.,* 1993, **36**, 1902; (c) V. Eswarakrishnan and L. Field, *J. Org. Chem.,* 1981, **46**, 4182.

13 (a) R. Correa, M. Del Río and M. De La Fuente, *Immunopharmacol.,* 1999, **44**, 281.; (b) D. J. Rawson, D. Brugier, A. Harrison, J. Hough, J. Newman, J. Otterburn, G. N. Maw, J. Price, L. R. Thompson, P. Turnpenny and A. N. Warren, *Bioorg. Med. Chem. Lett.,* 2011, **21**, 3771.

14 V. A. Soloshonok, N. Yu. Svistunova, V. P. Kukhar, V. I. Popov and N. Yu, Belokon, *Izvestiya Akademii Nauk, Seriya Khimicheskaya,* 1993, 786.

15 (a) A. Gutiérrez, J. Bernal, M. D. Villacampa, C. Cativiela, A. Laguna and M. C. Gimeno, *Inorg. Chem.,* 2013, **52**, 6473; (b) A. Gutiérrez, M. C. Gimeno, I. Marzo and N. Metzler-Nolte, *Eur. J. Inorg. Chem.*, 2014, 2512; (c) A. Gutiérrez, L. Gracia-Fleta, I. Marzo, C. Cativiela, A. Laguna and M. C. Gimeno, *Dalton Trans.,* 2014, **43**, 17054; (d) A. Gutiérrez, I. Marzo, C. Cativiela, A. Laguna and M. C. Gimeno, *Chem. Eur. J.,* 2015, **21**, 11088.

16 (a) X. Zhang, A. C. Schmitt and W. Jiang, *Tetrahedron Lett.,* 2001, **42**, 5335; (b) S. A. Kolodziej, G. V. Nikiforovich, R. Skeean, M. F. Lignon, V. Martínez and G. R. Marshall, *J. Med. Chem.,* 1995, **38**, 137; (c) A. Mollica, M. P. Paradisi, K. Varani, S. Spisani and G. Lucente, *Bioorg. Med. Chem.,* 2006, **14**, 2253.

17 A. Zhang and A. D. Schlüter, *Chem. Asian. J.*, 2007, **2**, 1540.

18 M. Frik, A. Martínez, B. T. Ellie, O. Gonzalo, D. Ramírez de Mingo, M. Sanau, R. Sánchez-Delgado, T. Sadhukha, S. Prabha, J. W. Ramos, I. Marzo and M. Contel, *J. Med. Chem.,* 2014, **57**, 9995.

19 (a) D. De Vos, S. Y. Ho and E. R. T. Tiekink, *Bioinorg. Chem. Appl.* 2004, **2**, 141; (b) F. K. Keter, I. A. Guzei, M. Nell, W. E. Van Zyl and J. Darkwa, *Inorg. Chem.*, 2014, **53**, 2058.

20 G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw and K. I. Goldberg, *Organometallics,* 2010, **29**, 2176.

21 (a) R. Usón and A. Laguna, *Inorg. Synth.,* 1982, **21**, 71; (b) N. W. Alcock, P. Moore, P. A. Lampe and K. F. Mok, *J. Chem. Soc. Dalton Trans.,* 1982, 207.

22 C. Wetzel, P. C. Kunz, M. U. Kassak, A. Hamacher, P. Böhler, W. Watjen, I. Ott, R. Rubiani and B. Springler, *Dalton Trans.*, 2011, **40**, 9212.

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Graphical Abstract

Bioactive Gold(I) Complexes with 4-Mercaptoproline Derivatives

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Unprecedented gold(I) bioconjugates bearing non-proteinogenic amino acid 4-mercaptoproline species as bioorganic ligands have been prepared. The complexes displayed excellent cytotoxic activity with IC_{50} values in the low μM range and even in the nM range.