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Structural and solution chemistry, protein binding and antiproliferative profiles of gold(I)/(III) complexes bearing the saccharinato ligand

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ABSTRACT

A series of new gold(I) and gold(III) complexes based on the saccharinate (sac) ligand, namely M[Au(***sac***)$_2$] (with M being Na^+, K^+ or NH_4^+), [(PTA)Au(***sac***)], K[Au(***sac***)$_3$Cl] and Na[Au(***sac***)$_4$], were synthesized and characterized, and some aspects of their biological profile investigated. Spectrophotometric analysis revealed that these gold compounds, upon dissolution in aqueous media, at physiological pH, manifest a rather favourable balance between stability and reactivity. Their reactions with the model proteins cytochrome c and lysozyme were monitored by mass spectrometry to predict their likely interactions with protein targets. In the case of disaccharinato gold(I) complexes, cytochrome c adducts bearing four coordinated gold(I) ions were preferentially formed in high yield. In contrast, [(PTA)Au(sac)] (PTA = 1,3,5-triaza-7-phosphaadamantane) turned out to be poorly effective, only producing a mono-metalated adduct in very low amount. In turn, the gold(III) saccharinate derivatives were less reactive than their gold(I) analogues: K[Au(sac)$_3$Cl] and Na[Au(sac)$_4$] caused moderate protein metalation, again with evidence of formation of tetragold adducts. Finally, the above mentioned gold compounds were challenged against the reference human tumor cell line A2780S and its cisplatin resistant subline A2780R and their respective cytotoxic profiles determined. [(PTA)Au(***sac***)] turned out to be highly cytotoxic whereas moderate cytotoxicities were observed for the gold(III) complexes and only modest activities for disaccharinato gold(I) complexes. The implications of these results are thoroughly discussed in the light of current knowledge on gold based drugs.

1. Introduction

Nowadays, gold compounds are drawing a great deal of attention within the *Metals in Medicine* community owing to their outstanding antiproliferative properties, and are being intensely investigated as a rich source of innovative cytotoxic drugs for cancer treatment [1–3]. Notably, during the last decade, a large number of gold compounds were synthesized and characterized; some of them showing very promising anticancer properties, both *in vitro* and *in vivo*. We like to remind here gold(III) porphyrins [4], gold(III) dithiocarbamates [5], some dinuclear gold(III) complexes with bipyridyl ligands [6], a few selected organogold(III) compounds [7–10]. Pairwise, a variety of gold (I) compounds manifested very attractive pharmacological profiles [11–17].

A major issue in the bioinorganic chemistry of gold(III)/(I) compounds, of potential use as anticancer agents, is the obtainment of gold species characterized by a sufficient degree of stability under physiological-like conditions and by the concomitant retention of a still appreciable reactivity toward biomolecular targets as it is usually required for metal-based *prodrugs*. These goals may be afforded through an appropriate selection of gold(III)/(I) ligands capable of tuning finely the reactivity of the metal center. In any case, a reasonable, sometimes difficult, compromise must be met between stability and reactivity. In addition, an acceptable solubility profile within aqueous solutions is typically required for optimal candidate drugs.

With this in mind we have prepared a few novel gold compounds, based on the presence of the saccharinate anion (**sac$^-$**) as a ligand, and analysed their solution behaviour within a standard buffer. Their reactions with cytochrome c and with lysozyme, used here as model proteins, were investigated in detail by ESI MS (electrospray ionization mass spectrometry) according to established protocols; their antiproliferative properties were specifically evaluated against A2780S/R human ovarian carcinoma cells.

The choice of saccharinate as a ligand was motivated by a number of reasons. First of all, sodium saccharinate is a compound widely used

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as a commercial sweetener with a very favourable safety profile [18]. As a consequence, its use could even decrease the toxicity of the gold centers for the human body and/or reduce the side effects of the drug. At the same time, saccharinate might confer to the resulting gold complexes an appreciable solubility in water. In addition, previous studies revealed that saccharinate acts as a relatively strong ligand in a variety of metal complexes so that such complexes may result reasonably stable within biological fluids [19]. Some relatively old studies described a variety of gold(III) and gold(I) complexes with imido ligands [20–22]. A few gold(I) and gold(III) complexes containing saccharinato ligands were reported more recently [22–26], one of which, the gold(I) derivative [(PEt$_3$)Au(sac)], displayed significant antiinflammatory activities *in vivo* [22].

Three different groups of compounds were specifically prepared for the present investigation: a few anionic disaccharinato gold(I) complexes, M[Au(sac)$_2$], **1**-M, (with M being Na^+, K^+ or NH_4^+); one neutral heteroleptic gold(I) complex, [(PTA)Au(sac)], **2**, (PTA = 1,3,5-triaza-7-phosphaadamantane); and two square planar gold(III) complexes, respectively K[Au(***sac***)$_3$Cl], **3**, and Na[Au(***sac***)$_4$], **4**. These novel compounds were characterized both in the solid state and in solution. Their reactivities with the model proteins horse heart cytochrome c (cyt c) and hen egg white lysozyme were subsequently assayed through ESI MS; their antiproliferative properties were measured against the cisplatin-sensitive or resistant human ovarian carcinoma cell lines (A2780S and A2780R). All the obtained results are comprehensively discussed in the frame of current knowledge on anticancer gold compounds.

2. Experimental section

2.1. General

All starting materials were used as received from commercial sources. [(PTA)$_2$Au]Cl [27] and M[Au(Sac)$_2$] [24] (M = Na, K, and NH_4) were prepared according to literature methods. Elemental analysis was performed with a Perkin–Elmer Elemental Analyser 240B by Mr. A. Canu (Dipartimento di Chimica, Università di Sassari). Conductivity measurements were performed with a Philips PW 9505 conductometer. Infrared spectra were recorded with a Jasco FTIR 480 Plus spectrophotometer using Nujol mulls. UV–Visible (UV–Vis) spectra were recorded on a Varian Cary 50 UV–Vis spectrophotometer. ^{1}H and ^{31}P{^{1}H} NMR spectra were recorded at room temperature (20 °C) with a Varian VXR 300 spectrometer operating at 300.0 and 121.4 MHz, respectively. Chemical shifts are given in ppm relative to internal TMS (Tetramethylsilane) (^{1}H) and external H_3PO_4 (^{31}P). Mass spectra were recorded in an LTQ-Orbitrap high-resolution mass spectrometer (Thermo, San Jose, CA, USA), equipped with a conventional ESI source.

2.2. Synthesis of [(PTA)Au(sac)] (2)

An aqueous suspension of **1**-Na (292.5 mg, 0.5 mmol) and [(PTA)$_2$Au]Cl (273.7 mg, 0.5 mmol) (80 mL) was stirred for 2 h at room temperature, with exclusion of light. Afterwards the white solid was filtered under vacuum and washed with H_2O, EtOH and Et_2O. Recrystallization from dichloromethane/diethyl ether gave **2** as a white solid. Yield 120.8 mg, 23 %; mp 221°–222 °C (dec.); (Found: C 29.66; H 2.60; N 10.18%. Calc. for $C_{13}H_{16}AuN_4O_3PS$: C 29.11; H 3.01, N 10.45%). IR (nujol), ν_{max}/cm^{-1}: 1678 (C = O), 1288 (SO_2 asym), 1253, 1165 (SO_2 sym), 1011, 971, 947. ^{1}H NMR (acetone-d$_6$): δ 7.8–7.9 (m, 4H, sac), δ 4.48 (d, 3H, *J* = 13 Hz, NC*H*$_2$N eq or ax), δ 4.59 (s, 6H, NC*H*$_2$P), δ 4.72 (d, 3H, *J* = 13 Hz, NC*H*$_2$N ax or eq). ^{31}P NMR (acetone-d$_6$): δ −32.85.

For comprehension: s = singlet, d = doublet, and m = multiplet.

2.3. Synthesis of K[Au(sac)$_3$Cl] (3)

A freshly prepared solution of K***sac*** (1.3273 g, 9.0 mmol) in 100 mL of water was added to an aqueous solution of $NaAuCl_4 \cdot 2H_2O$ (0.3979 g, 1.0 mmol) and refluxed for 1 h under stirring. During this time a yellow precipitate was formed. After cooling, the resulting suspension was filtered off and the solid washed with several amounts of cold H_2O, EtOH and Et_2O. Recrystallization from acetone/diethyl ether gave **3** as a yellow solid. Yield 0.5560 g, 68%; mp 259°–260 °C; (Found C 30.96; H 1.63; N 4.92%. Calc. for $C_{21}H_{12}AuClKN_3O_9S_3$: C 30.83; H 1.48; N 5.14%); $\Lambda_{MAX}/\Omega^{-1}cm^2mol^{-1}$: 210 (water), 80 (acetone); FT IR (nujol) n_{max}/cm^{-1}: 1701 (C = O), 1321 (SO_2 asym), 1241, 1177 (SO_2 sym), 970, 750, 675. ^{1}H NMR (acetone-d$_6$): δ 7.71–7.97 (12H, m).

2.4. Synthesis of Na[Au(Sac)$_4$]·2NaCl (4·2NaCl)

This compound was prepared as described above for **3**, substituting the equivalent molar quantity of Na***sac*** for K***sac***. Yield 0.7279 g, 69%; mp >270 °C; (Found C 31.69; H 1.75; N 5.36%. Calc. for $C_{28}H_{16}AuCl_2N_4Na_3O_{12}S_4$: C 31.56; H 1.51, N 5.26%). $\Lambda_{MAX}/\Omega^{-1}cm^2mol^{-1}$: 214 (water), 104 (acetone). FT IR (nujol), ν_{max}/cm^{-1}): 1703 (C = O), 1324 (SO_2 asym), 1244, 1180 (SO_2 sym), 971, 751, 675. ^{1}H NMR (acetone-d$_6$): δ 7.61–7.97 (16H, m).

2.5. X-ray data collection and structure determination

Crystals of compound **2** suitable for X-ray analysis were obtained by slow diffusion of Et_2O into a dichloromethane solution. After several days, clear needle-like crystals appeared. Data were collected on an Xcalibur Oxford Diffraction 4-circle diffractometer equipped with CCD area detector and with Mo-K$_\alpha$ radiation (λ = 0.71073) through the program CrysAlis CCD [28]. The program CrysAlis RED [29] was used to reduce the data and the absorption correction was applied through the program ABSPACK implemented in the CrysAlis suite. Direct methods run through Sir97 [30] were employed to solve the structure and the refinement was completed by full-matrix least squares against F^2 using all data (SHELX97 [31]). The aromatic ring of the saccharinate ligand was restrained on a regular hexagon. All the non-hydrogen atoms were refined anisotropically. All the hydrogen atoms were fixed in calculated positions and refined isotropically. The PARST [32] program was used to perform geometrical calculation and the program ORTEP-3 for windows [33] was employed for graphical plots. Crystallographic data and refinement parameters were reported in Table 1. CCDC reference number ....For crystallographic data in CIF or other electronic format see DOI:.....

Table 1
Crystallographic data and refinement parameters for compound 2.

[(TPA)Au(sac)]	
Empirical formula	$C_{13}H_{16}$ Au N O_3 P S
Formula weight	476.71
Temperature (K)	293
Wavelength (A)	0.71073
Crystal system, space group	Orthorhombic, C cc2
Unit cell dimensions (Å,°)	a = 17.057(2)
	b = 24.098(3)
	c = 7.7899(10)
Volume (Å^3)	3201.1((1)
Z, D_c (mg/cm^3)	8, 2.225
μ (mm^1)	9.438
F(000)	2048
Crystal size (mm)	0.2 × 0.05 × 0.02
θ range (°)	4.39 to 27.67
Reflections collected/unique	12,007/2940
Data/restraints/parameters	2940/1/196
Goodness-of-fit on F^2	0.878
Final R indices [I>2σ(I)]	RI = 0.0435, wR2 = 0.0885
R indices (all data)	RI = 0.0834, wR2 = 0.0952

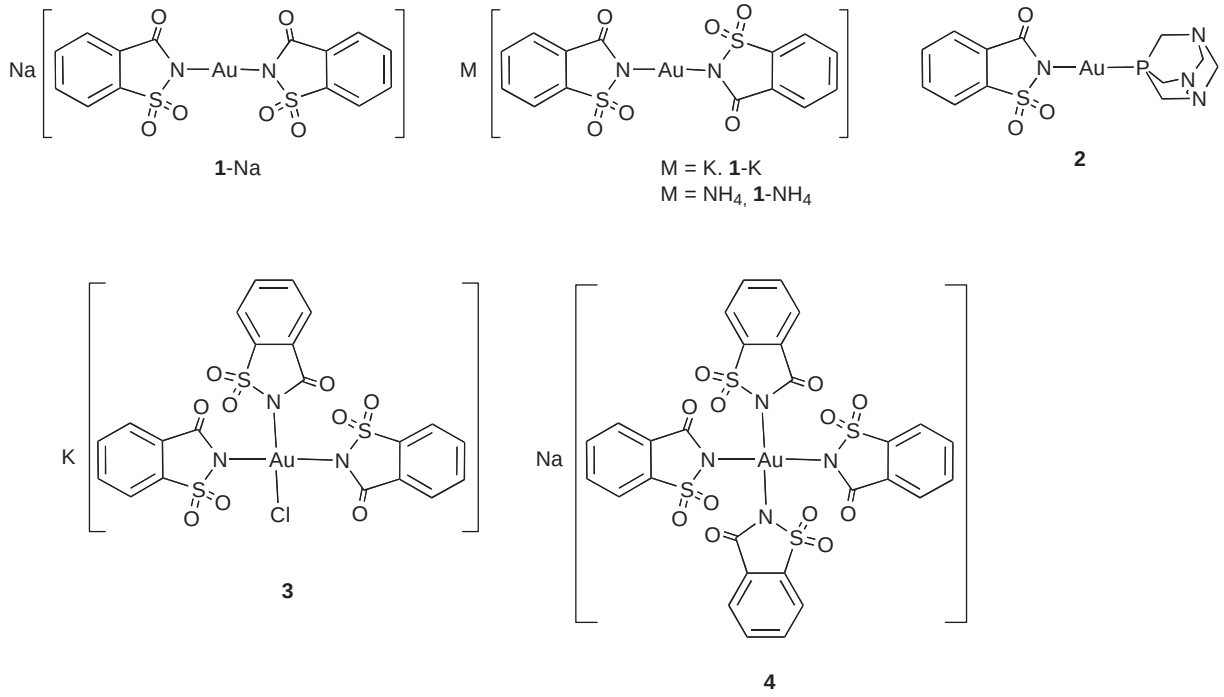


Fig. 1. Schematic drawing of the gold-saccharinato derivatives.

2.6. Solution chemistry

The solution chemistry of the above compounds was analysed by absorption UV–visible spectroscopy. UV–Vis absorption spectra of the gold compounds were recorded on a Varian Cary 50 spectrophotometer. Solutions of **1**-M-**4** (10^{-4} M) in 50 mM Phosphate buffer pH = 7.4 were monitored collecting the electronic spectra over 24 h at room temperature.

2.7. ESI mass spectrometry

Horse heart cytochrome c, and hen egg white lysozyme were purchased from Sigma (C7752 and L7651, respectively) and used as received. All samples were prepared in tetramethylammonium acetate buffer (TMAA) pH 7.4, with a protein concentration of 10^{-4} M, and a gold to protein molar ratio of 3:1. The reaction mixtures were incubated for 24 h at 37 °C, and the resulting products analysed by ESI MS. After a 20-fold dilution with MilliQ water, ESI MS spectra were recorded by direct introduction on an LTQ-Orbitrap high-resolution mass spectrometer (Thermo, San Jose, CA), equipped with a conventional ESI source. The working conditions were the following: spray voltage 3.1 kV, capillary voltage 45 V and capillary temperature 220 °C. The sheath and the auxiliary gases were set, respectively, at 17 (arbitrary units) and 1 (arbitrary units). For acquisition, Xcalibur 2.0 software (Thermo) was used and monoisotopic and average deconvoluted masses were obtained by using integrated Xtract tool.

2.8. Antiproliferative tests

Exponentially growing cells were seeded in 96-well microplates at a density of 5×10^3 cell/well and incubated with various compound concentrations. After designated incubation, the cells were fixed with 100 μL of ice-cold 10% trichloroacetic acid for 60 min at 4 °C, rinsed 6 times with water and air-dried. Fixed cells were stained with 50 μL of sulforhodamine B (SRB) solution (0.4% SRB/0.1% acetic acid), rinsed with 0.1% acetic acid and air-dried. At the end of the staining period, SRB was dissolved in 150 μL of 10 mM Tris–HCl solution (pH 10.5) for 10 min on a gyratory shaker. Optical density was read in a microplate reader interfaced with the software Microplate Manager/PV version 4.0 (Bio-Rad Laboratories, Milan, Italy) at 540 nm.

3. Results and discussion

3.1. Synthesis and structural characterization

The gold compounds that were prepared and considered for the present study are schematically represented in Fig. 1. They are: M[Au(***sac***)$_2$], **1**-M, (where M is Na^+, K^+, or NH_4^+), [(PTA)Au(***sac***)], **2**, K[Au(***sac***)$_3$Cl], **3**, and Na[Au(***sac***)$_4$], **4**. These compounds were prepared through simple synthetic procedures, as detailed in the experimental section, and then characterized through standard methods. The crystal structures of M[Au(***sac***)$_2$] complexes, **1**-M, were previously determined [22]. All three structures contain bis (saccharinato)aurate(I) anions with the saccharinato ligands N-η^1 bonded to the linearly two-coordinated gold(I) ion. Notably, a different orientation of the two saccharinato ligands was observed depending on the nature of the cation: in **1**-Na the ligand pair has the *cis* orientation (*i.e.* the carbonyl groups are on the same side), while in **1**-K and **1**-NH_4 the ligand pairs show a *trans* orientation (see Fig. 1). In any case, it can be easily predicted that due to free rotation about the N–Au bonds the various complexes will have identical structures in the solution. The structures of [(PTA)Au (sac)], **2**, and of K[Au(sac)$_3$Cl], **3**, have been solved in the frame of the present study. An ORTEP [34] view of compound **2** is shown in Fig. 2, with principal bond lengths and angles reported in the caption. The asymmetric unit contains one molecule of the complex. The gold(I) ion exhibits the usual linear coordination. Gold(I) is bound to the saccharinato ligand through the nitrogen atom N(1) (Au–N(1) 2.063(10) Å) and to the PTA moiety through the phosphorous atom P(1) (Au–P(1) 2.215(3) Å), the P(1)–Au(1)–N (1) angle being 172.1(3)°. Both bond lengths and angles are in agreement with the mean values obtained from a search in the Cambridge Structural Database (CSD) [35] (v. 5.30), retrieving 169 hits. In the crystal lattice a weak Au(1)–Au(1) interaction could be detected, the distance between the two metal ions measuring

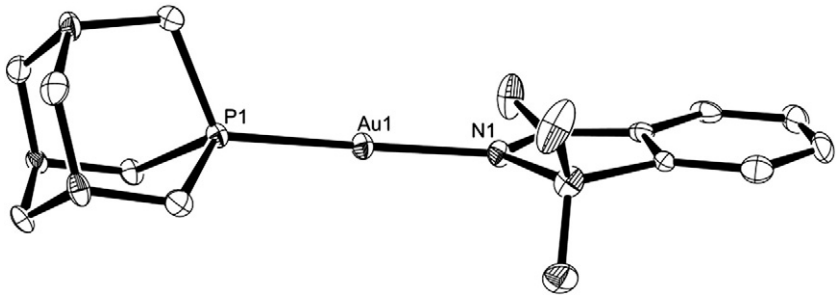


Fig. 2. ORTEP drawing of the complex [(PTA)Au(sac)], **2**. Thermal ellipsoids are shown at 20% probability. Selected bond distances (Å) and angles (deg): Au(1)–N(1) 2.063(10), Au(1)–P(1) 2.215(3) and N(1)–Au(1)–P(1) 172.1(3).

3.5298(6) Å (the molecule is reported by the symmetry operation $-x+1/2, -y+1/2, +z$). Moreover, a lone pair–π interaction may be pointed out: the contact entails the N(4) nitrogen atom and the aromatic ring of a molecule reported by the symmetry operation $-x+1/2, -y+1/2, +z+1$: the distance N(4)–Ct(1) [36] is 3.379 Å [Ct(1) is the geometric centroid of the aromatic ring C(2)–C(7)].

Unfortunately, due to severe twinning, the quality of the crystals and, thus, of the crystal structure of **3** is not very high. However, data are of sufficient quality as to determine, unambiguously, the type of gold(III) coordination. The chromophore of $K[Au(sac)_3Cl]$ is shown in Fig. 1S (ESI). It consists of a square planar gold(III) center coordinated to three nitrogen donors and one chloride. Detailed information on distances and angles could be extracted as well.

3.2. Solution chemistry

The solution behaviour of the study compounds was analysed by absorption spectroscopy. These compounds commonly manifest a rather modest solubility in water while being highly soluble in DMSO. In water, the solubility of complexes **1**-M ranges from 0.67 mg mL^{-1} for complex **1**-NH_4 to 1.25 mg mL^{-1} for complex **1**-Na, while it is 0.50 mg mL^{-1} for complex **2**. For both complexes **3** and **4** water solubility is about 0.45 mg mL^{-1}. Solutions suitable for spectrophotometric analysis were prepared by diluting DMSO mother solutions of the various complexes with the appropriate aqueous buffer. A reference buffer, made up of 10 mM sodium phosphate, pH 7.4, was used for all the experiments where not differently stated. The resulting samples were monitored over 24 h at 25 °C: spectra are shown in Fig. 3. These spectra reveal rather intense bands in the UV that are assigned to the saccharinato chromophore in accordance with previous studies [37].

Complexes **1**-M are characterized by an intense absorption band around 270 nm which manifests a slight blue shift during the first 5 h. After this time, an additional broad band centered at 550 nm, diagnostic of the formation of metallic gold, appears for **1-**Na and **1**-K, while for complex **1**-NH_4 a new absorption band is observed at 350 nm. Complex **2** does not exhibit well defined bands but, during the first 10 h, two broad absorption bands appear at 360 and 550 nm. The former is assigned to the PTA-Au fragment in analogy with previous observations.[1]

The various gold(III) complexes manifest some relevant time-dependent spectral changes: In the case of complex **3** the progressive disappearance of the visible band centered at 330 nm is observed, that may be ascribed, quite straightforwardly, to progressive aquation of the chloride ligand, in accordance with previous observations [38,39]. Indeed, it is well known that chloride, when ligated to a gold(III) center, generates charge transfer bands typically falling in the 310–330 nm spectral region [38]. The process is complete within *ca.* 3 h.

At variance, a low intensity band is observed for compound **4**, at 263 nm, whose intensity increases progressively, accompanied by a new broad band at about 320 nm.

The relevant spectral changes observed in all cases point out that the different compounds undergo progressive transformations over 24 h; nevertheless, they are sufficiently stable in the solution, at least within the first h after dissolution, and can be used as such for the *in vitro* cell tests.

3.3. Reactions with proteins

To gain more detailed insight into their likely interactions with proteins, the gold-saccharinate compounds **1-**M and **2**-**4** were reacted with horse heart cyt c (cyt c) and hen egg white lysozyme, HEWL, used here as models for globular proteins. Reactions were monitored through mass spectrometry methods in accordance with previous studies conducted in our laboratory [40]. Notably, cyt c was reacted with each of the six different compounds, at a fixed 3:1 molar ratio, and the resulting products analysed by ESI MS after 24 h incubation. Because of the strict requirements of the ESI MS measurements, the phosphate buffer had to be replaced by TMAA. Upon inspection of the resulting deconvoluted ESI MS spectra it emerges very clearly that, beyond the peak of native cyt c, centered at 12,358 Da, several additional peaks of higher mass are present, in the various samples, that may be straightforwardly assigned to gold drugs-cyt c adducts. In any case, relevant differences are evident in the metalation patterns produced by the six tested compounds (Fig. 4). Remarkably, reaction of cyt c with **1**-Na leads to the formation of a variety of gold protein adducts, in relatively large amounts, that correspond to cyt c bearing a variable number of bare gold(I) ions (from 1 to 4). It must be observed that the cyt c adduct bearing four attached gold(I) ions is by far the predominant species, implying occurrence of some cooperative effects in gold binding.

A similar reactivity was observed for **1**-K and **1**-NH_4, for which additional types of adducts containing the Au(***sac***) fragment are present in low yields. Compounds **3** and **4** induce a similar pattern of cyt c metalation, again with evidence of formation of a tetragold adduct, but the percentage of formed adducts is appreciably lower. Finally, we noticed that complex **2**, was almost completely unable to produce protein metalation. However, a peak of very low intensity could be detected corresponding to an adduct bearing a PTA-gold(I) fragment. In the case of lysozyme, lower levels of metalation were detected, yet characterized by more classical patterns of metalation. In the case of **1**-Na, **1**-NH_4 and **4**, bis- or mono-metalated adducts were identified, whereas no appreciable level of metalation was observed for **2** (Fig. 5).

[1] It is also observed in the UV spectrum of $[(PTA)_2Au][BF_4]$ under the same conditions.

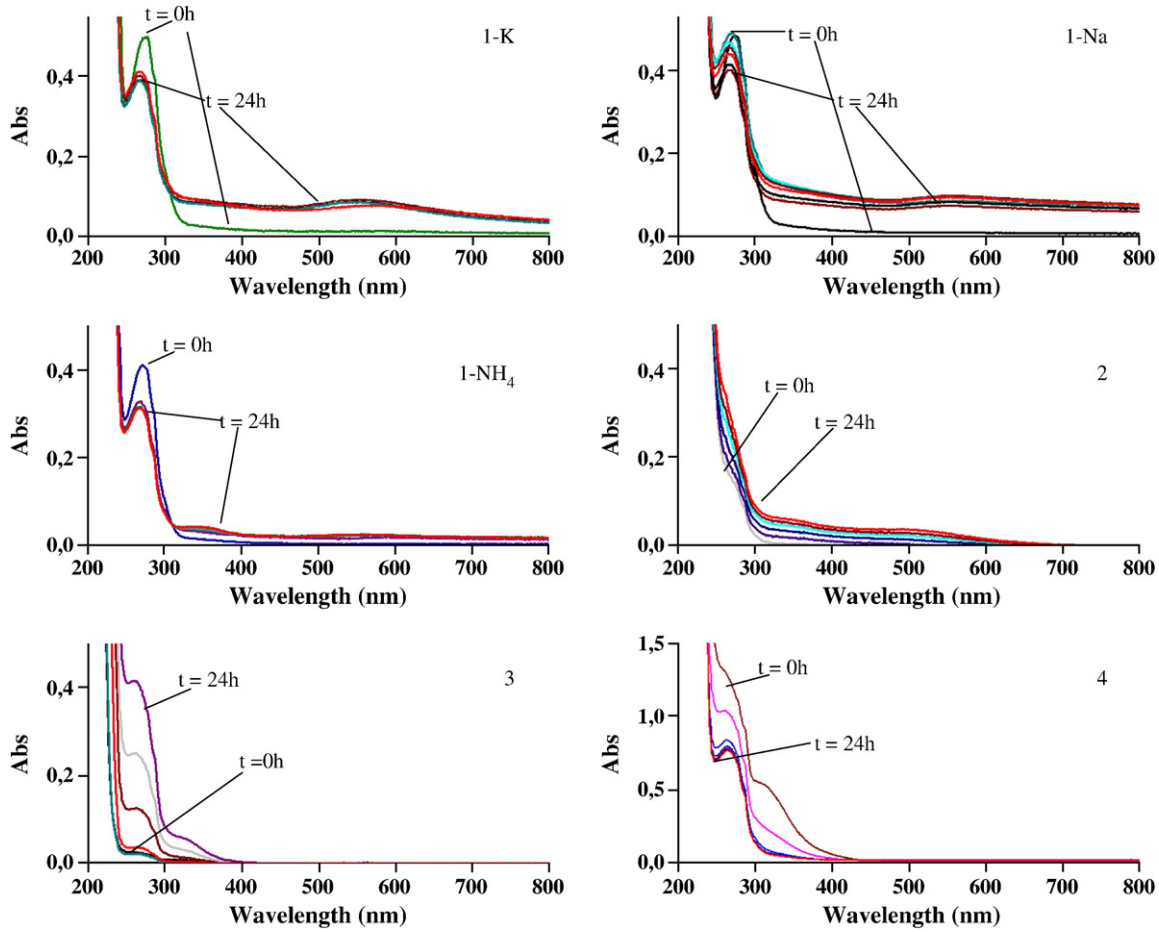


Fig. 3. Hydrolysis profiles of complexes **1**-M-**4** dissolved in phosphate buffer 10 mM pH 7.4. Spectra were recorded at different times over 24 h at room temperature.

3.4. Antiproliferative effects

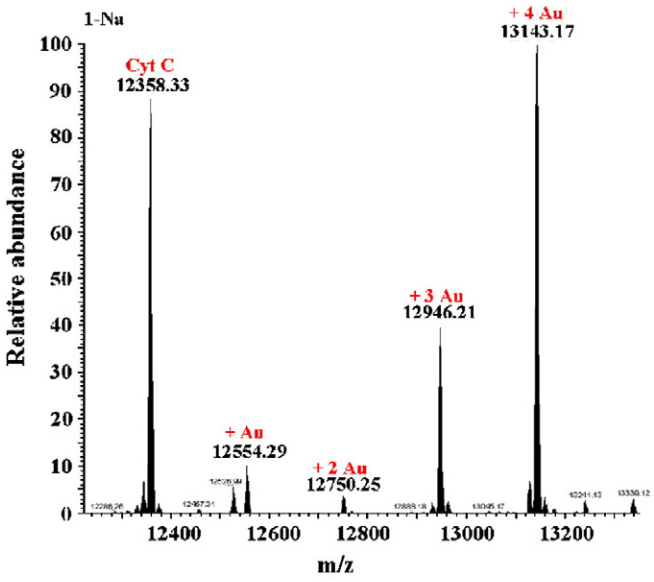
Afterward, the antiproliferative effects of the study compounds, expressed as IC_{50} values (drug concentrations required to inhibit cell growth by 50%), were determined against the human ovarian carcinoma A2780S cell line sensitive to cisplatin and its cisplatin-resistant counterpart A2780R. The compounds were diluted in DMSO as stock solutions. The cytotoxic activity was determined after a continuous exposure of 72 h. Inhibition of cell growth by the various compounds was monitored through the SRB assay. This assay was conducted in 96-well plates according to the procedure described by Skehan et al. [41]. In some cases (*i.e.* for the gold(III) compounds) the cytotoxic activity was slightly affected by the intrinsic cytotoxicity of DMSO but as the final DMSO concentration was always kept below 0.5%, its effect was generally modest and could be neglected. The IC_{50} values of cisplatin determined in both ovarian cancer cell lines are also reported (Table 2).

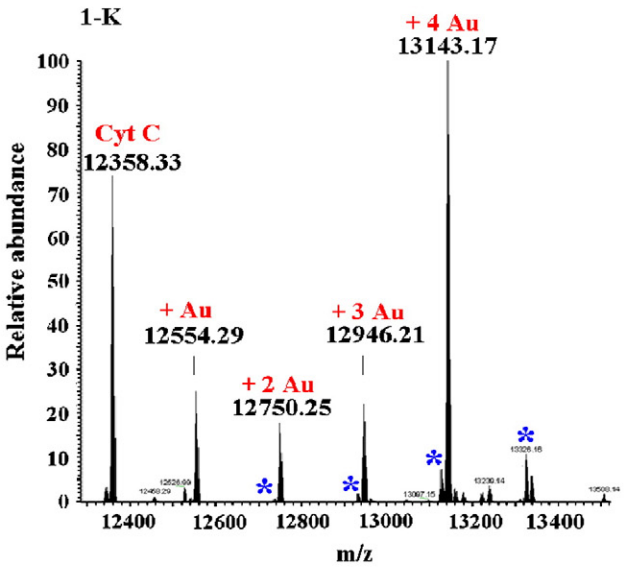
Among the study compounds, the mixed ligand gold(I) complex **2** showed the highest cytotoxic activity, although lower than that of cisplatin, with an IC_{50} value of 8.5 μM against the cisplatin-sensitive cell line and a cytotoxic activity against the resistant cell line comparable to that of cisplatin. The gold(I) disaccharinate complexes **1**-M showed a very low cytotoxic activity with an IC_{50} value of about 50 μM against both cell lines. Finally, the tetrasaccharinato gold(III) derivative **4** showed a cytotoxic effect comparable to that of cisplatin in the A2780R, with an IC_{50} value of 14.3 μM, while compound **3** was by far less active.

Two kinds of comments may be proposed here concerning these antiproliferative data.

i) Very interestingly, replacement of one saccharinate ligand with one PTA greatly enhances the cytotoxic activity of the resulting gold(I) derivative. This suggests a relevant role for the PTA ligand as a carrier of the cytotoxic gold(I) moiety. Most likely, this ligand, that offers a tight P donor to the gold(I) center, protects it from reactions with the several biologically occurring nucleophiles and from the consequent loss of activity. In a way, the situation of auranofin is somehow reproduced where the ligand acts as the leaving group while the phosphine remains bound to the gold(I) ion. The gold(I) phosphine cation is thus, most probably, the molecular species responsible for the observed biological actions [3,42].
ii) It is remarkable, that the tetrasaccharinato gold(III) species is found to be more active than the disaccharinato gold(I) analogues. The measured differences in cytotoxic activity may be traced to the concomitant occurrence of redox processes or to the reduced reactivity with proteins of $Na[Au(sac)_4]$ compared to $M[Au(sac)_2]$.

Fig. 4. Deconvoluted ESI MS spectral profiles for cytochrome c adducts with complexes 1-M-4, in TMAA pH 7.4 and after 24 h incubation at 37 °C. The initial gold/protein stoichiometry of each sample is 3:1. * means + Au(Sac).


1-Na
Cyt C
12358.33
+ Au
12554.29
+ 2 Au
12750.25
+ 3 Au
12946.21
+ 4 Au
13143.17
Relative abundance
m/z


1-K
Cyt C
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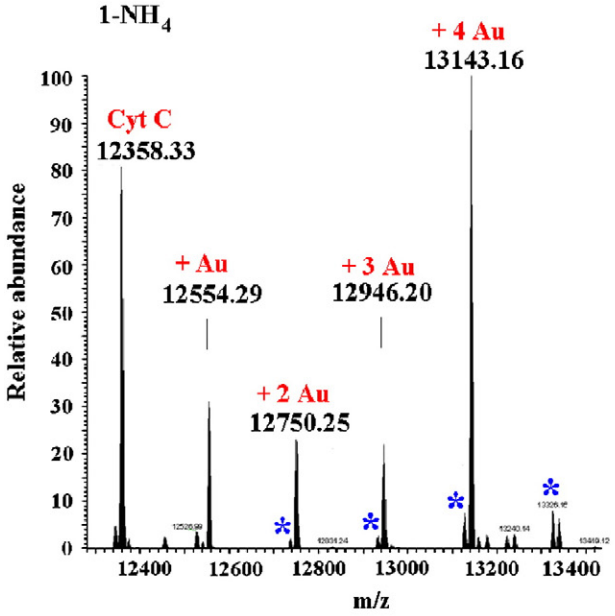
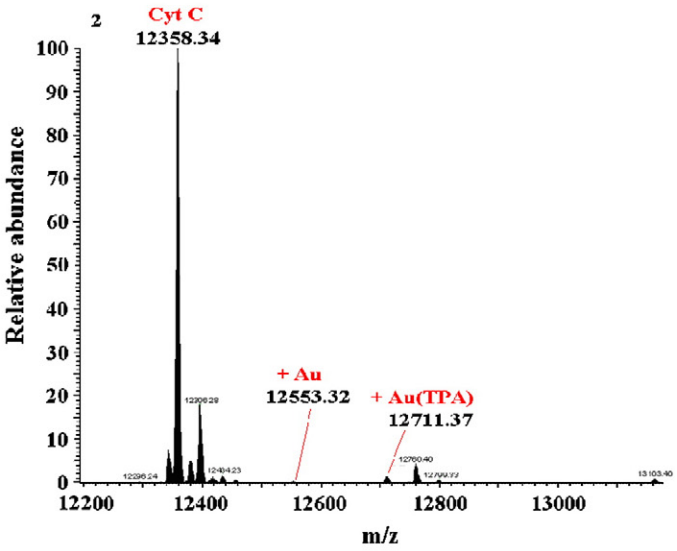
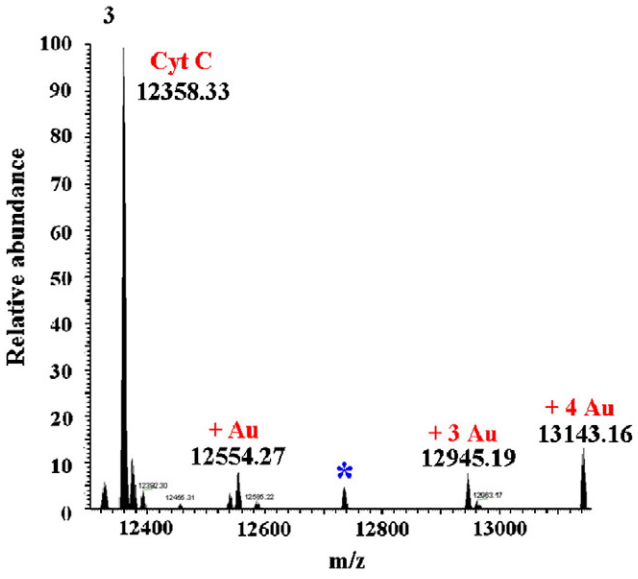
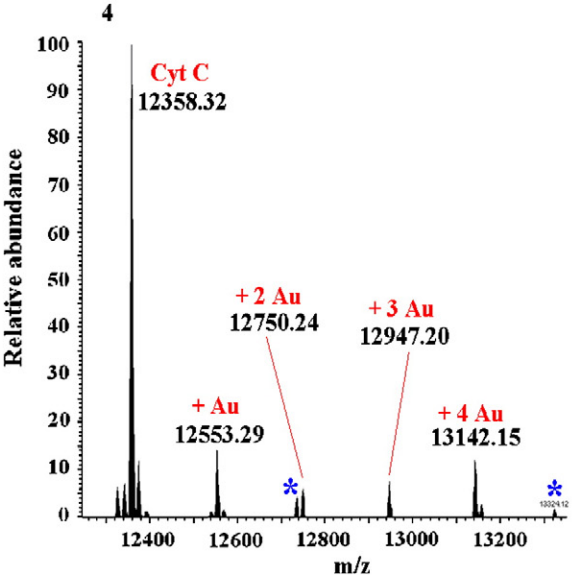
+ Au
12554.29
+ 2 Au
12750.25
+ 3 Au
12946.21
+ 4 Au
13143.17
Relative abundance
m/z


1-NH4
Cyt C
12358.33
+ Au
12554.29
+ 2 Au
12750.25
+ 3 Au
12946.20
+ 4 Au
13143.16
Relative abundance
m/z


2
Cyt C
12358.34
+ Au
12553.32
+ Au(TPA)
12711.37
Relative abundance
m/z


3
Cyt C
12358.33
+ Au
12554.27
+ 3 Au
12945.19
+ 4 Au
13143.16
Relative abundance
m/z


4
Cyt C
12358.32
+ 2 Au
12750.24
+ 3 Au
12947.20
+ Au
12553.29
+ 4 Au
13142.15
Relative abundance
m/z

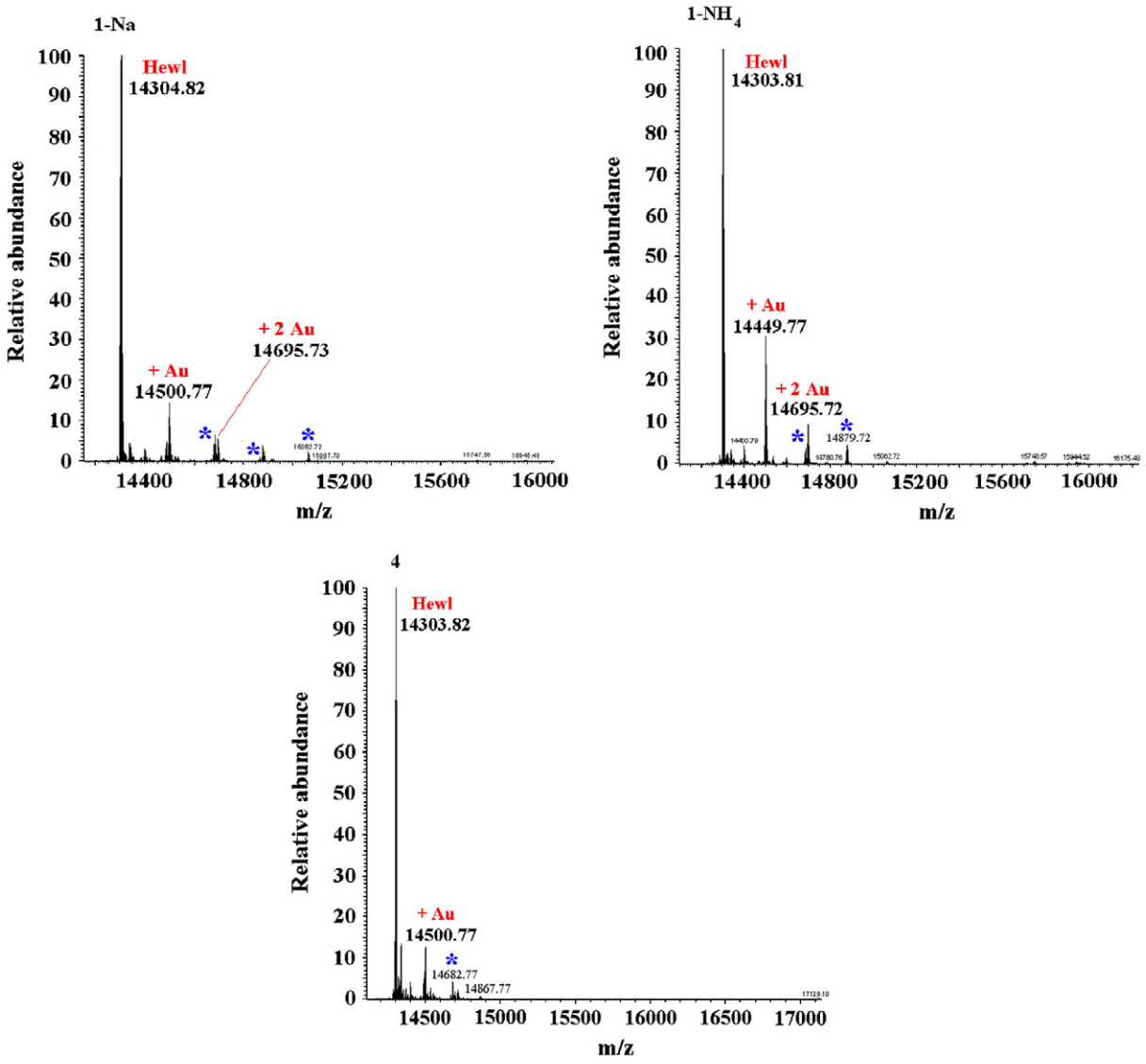


Fig. 5. Deconvoluted ESI MS spectral profiles for hen egg white lysozyme (Hewl) adducts with complexes **1**-Na, **1**-NH_4^+ and **4**, in TMAA pH 7.4 and after 24 h incubation at 37 °C. The initial gold/protein stoichiometry of each sample is 3:1. * means + Au(Sac).

4. Concluding remarks

Three different kinds of gold compounds, bearing distinct structural motifs, were prepared and characterized, based on the saccharinato ligand. Remarkably, these saccharinato gold complexes turned out to manifest, on the whole, a reasonable compromise between stability and reactivity and a still acceptable solubility in aqueous solutions. This confers them reactivity profiles that are quite appropriate for cytotoxic *prodrugs*, in analogy with established anticancer platinum complexes. Spectrophotometric studies revealed that all these compounds undergo some progressive transformations under physiological-like conditions. In particular, in the case of the chloro trisaccharinato gold(III) complex, the observed spectral changes are strongly suggestive of the release of the chloride ligand. Formation of some colloidal gold was detected for the disaccharinato gold(I) complexes.

The reactivity of these novel gold complexes with model proteins was carefully explored. Notably, ESI MS analysis revealed that all these compounds do react with cytochrome c, yet to largely different extents. Pronounced cyt c metalation and formation of a characteristic adduct bearing four gold(I) ions were afforded upon cyt c treatment with the disaccharinato gold(I) complexes. $K[Au(sac)_3Cl]$ revealed a far lower metalating efficiency; while [(PTA)Au(sac)] was nearly ineffective in metalating cyt c. More classical patterns of metalation were instead obtained in the case of lysozyme, with no evidence of a predominant tetragold adduct.

All six gold compounds were then challenged against the reference cisplatin-sensitive and resistant human tumor cell lines (A2780S and

Table 2
Drug sensitivity profiles of cisplatin-sensitive and -resistant human ovarian carcinoma cell lines (A2780S and A2780R) towards the study compounds. For comparison purposes the curves obtained with cisplatin are reported.

	IC50 (μM) ± SD	
Compound	A2780/S	A2780/R
1-Na	48.2 ± 0.1	54.0 ± 2.4
1-K	52.7 ± 2.4	44.2 ± 3.2
1-NH_4	52.4 ± 2.2	40.3 ± 3.7
2	8.5 ± 2.4	15.8 ± 0.2
3	23.6 ± 1.5	40.8 ± 1.8
4	14.9 ± 1.4	14.3 ± 0.9
Cisplatin	2.1 ± 0.3	16.1 ± 0.5

Results are the mean of at least three experiments; SD, standard deviation.

A2780R) and their antiproliferative potential measured. Interestingly, the mixed ligand gold(I) complex turned out to be the most active whereas a very moderate activity was found for the disaccharinato gold(I) complexes. Of the two gold(III) derivatives, only Na[Au(***sac***)$_4$] was found to be appreciably active.

As gold complexes, either +3 or +1, are known not to be very reactive toward DNA and nucleobases [43] it is very likely that the observed antiproliferative effects mainly arise from DNA-independent mechanisms and specifically from metalation of protein targets. Indeed, in recent times, a number of mechanisms were highlighted to account for the cytotoxic properties of gold(III,I) complexes involving, for instance, potent inhibition of the selenoenzyme thioredoxin reductase, proteasome inhibition or direct antimitochondrial effects. Gold compounds seem to act as suitable prodrugs; in fact, they first undergo chemical activation; then they produce/deliver metal containing species capable of metalating the final biomolecular targets.

Moreover, based on the above reported results, we can state that for the investigated saccharinato gold complexes reactivity with model proteins, in general, negatively correlates with the measured cytotoxic activities. As the mixed ligand complex PTA saccharinato gold(I) turned out to be the most cytotoxic it is suggested that the PTA ligand may act as a carrier and may protect the gold(I) center from *off target* reactions, ultimately leading to loss of activity. In addition, it is worthwhile highlighting the strict structural analogy existing between this latter compound and auranofin for which relevant antiproliferative effects *in vitro* were previously documented. It is also of interest to stress that the disaccharinato gold(I) complexes, though being moderately cytotoxic, behave as efficient protein metalating agents; this peculiar property might be exploited in the future even for different pharmacological purposes.

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