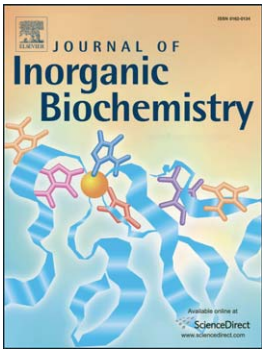

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Mono and dinuclear phosphinegold(I) sulfanylcarboxylates: influence of nuclearity and substitution of PPh_3 for PEt_3 on cytotoxicity

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ABSTRACT

Gold complexes of the type [Au(PEt_3)(H**x**spa)] were prepared by reacting triethylphosphinegold(I) chloride in ethanol/water (8:1) with the 3-(aryl)-2-sulfanylpropenoic acids H_2**x**spa [**x** = p = 3-phenyl-; f = 3-(2-furyl)-; t = 3-(2-thienyl)-; py = 3-(2-pyridyl); Clp = 3-(2-Chlorophenyl)-; -*o*-mp = 3-(2-methoxyphenyl)-; -*p*-mp = 3-(4-methoxyphenyl)-; -*o*-hp = 3-(2-hydroxyphenyl)-; -*p*-hp = 3-(4-hydroxyphenyl)-; -diBr-*o*-hp = 3-(3,5-dibromo-2-hidroxyphenyl-); spa = 2-sulfanylpropenoato] or 2-cyclopentylidene-2-sulfanylacetic acid (H_2cpa) and KOH in a 1:1:1 mole ratio. The compounds were characterized by IR spectroscopy and FAB mass spectrometry and by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy. The *in vitro* antitumor activity of these and of the previously described dinuclear [($AuPEt_3$)$_2$(**x**spa)] complexes against the HeLa-229, A2780 and A2780*cis* cell lines was determined and compared with those of the analogous PPh_3 complexes. The results show that the substitution of the PPh_3 ligand by PEt_3 is particularly effective in increasing the cytotoxicity of the dinuclear [($AuPR_3$)$_2$(**x**spa)] complexes, giving rise to compounds that are significantly more active than cisplatin against the aforementioned cell lines. In addition, and as a preliminary test for nephrotoxicity, the cytotoxicity of the most active compounds against the normal renal LCC-PK1 cell line was evaluated and compared with that of cisplatin.

Keywords: Gold(I) complexes; sulfanylpropenoic acids; 2-cyclopentylidene-2-sulfanylacetic acid; antitumor activities

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1. Introduction

A considerable number of new metal-based therapeutic agents, including antitumoral ones, are being prepared and biologically tested [1]; the search for effective alternatives to the widely used platinum-based anticancer drugs [2-4] is a particularly interesting and active field [5]. Recent examples of homo- [6,7] and heteronuclear [8,9] compounds are indicative of this activity.

Another metal with a great deal of potential included in this field is gold, which has been present in medicine since ancient history [10]. Interest has been considerably focused on Au(III) compounds [11-13] due to its structural and electronic similarity to the widely used cisplatin and cisplatin-related antitumor drugs. On the other hand, Au(I) complexes also exhibit cytotoxic activity against cells from several tumor cell lines, some of which are resistant to cisplatin, [12, 13]. This ability can be due to the different electronic/structural properties of these complexes, which lead them to interact with other biological targets such as proteins [14-16], and the thioredoxin reductase is now considered to be the most relevant [17-23].

Auranofin, triethylphosphine(2,3,4,6-tetra-o-acetyl-β,1-D-thiopyranosato-S)gold(I) is a well known antiarthritic drug that also shows significant cytotoxic activity [13, 24]; it is an interesting example of a group of Au(I) thiolate compounds, which contain the S-Au-P fragment and have also attracted interest as potential antitumor agents [12, 13]. The replacement of the thiolate ligand by other biological ligands is presumed to modulate the biological activity of these compounds, [10, 25] and consequently it is attractive to explore the activity of members of this family in which the S-Au bond has been stabilized. This stabilization can be achieved by endowing the thiolate ligand with other groups that are also capable of binding to the metal.

On this basis we have drawn up a concise compilation in which structural modifications could be related to changes in the *in vitro* antitumoral activity; thus, we have selected a number of sulfanylcarboxylic acids, R-CH-C(SH)-COOH, H_2**x**spa, and 2-cyclopentylidene-2-sulfanylacetic acid (H_2cpa) in order to prepare gold(I) complexes which could also contain a phosphine ligand. The sulfanylcarboxylic ligands (Scheme 1) present a wide spectrum of R groups which can modulate the hydrophilicity/lipophilicity of the complexes, a property of great importance for drug action [10, 25, 26]. The possibility of intra/intermolecular interactions and their associated structural effects is yet another point of interest for the selection of these groups. We initially prepared compounds of the type

[Au(PPh_3)(H**x**spa)] [27], which included an S-Au-P fragment, and maintained the COOH group protonated. These compounds were subsequently structurally modified by deprotonating this group; this was achieved by reacting them either with diisopropylamine or triethylamine, which affords compounds of the type [HQ][Au(PPh_3)(**x**spa)] and [HP][Au(PPh_3)(**x**spa)] (HQ = diisopropylammonium; HP = triethylammonium) [28] or with Au(PPh_3)Cl, thus including a new $AuPPh_3$ group to give dinuclear [($AuPPh_3$)$_2$(**x**spa)] compounds [29]. In addition, we have incorporated the $AgPPh_3$ fragment, thus preparing heteronuclear complexes containing Ag(I) and Au(I) centers [30, 31].

We now initiate the study of the effect that the substitution of the PPh_3 phosphine can have on the cytotoxicity of the complexes. This paper describes the synthesis, characterization and the study of the cytotoxic activity of complexes of the type [Au(PEt_3)(Hxspa)] against the human cervical carcinoma cell line HeLa-229 and the ovarian carcinoma cell line A2780 and its cisplatin-resistant mutant A2780*cis*. The activity of the previously described [($AuPEt_3$)$_2$(**x**spa)] complexes [32] was also investigated and the results obtained for both types of complexes were comparatively analyzed and contrasted with those of the equivalent PPh_3 complexes.

Scheme 1

In an attempt to get information about possible adverse effects limiting the potential therapeutic utility of these compounds, a preliminary study of toxicity on normal cells was also carried out. Thus, some of the compounds showing the best activity against the A2780*cis* cell line were selected to be tested on the normal epithelial renal LLC-PK1 cell line.

2. Experimental Section

2.1. Material and methods

2-Chlorobenzaldehyde, 2-methoxybenzaldehyde, 4-methoxybenzaldehyde, 2-hydroxybenzaldehyde, 4-hydroxybenzaldehyde, 3,5-dibromo-2-hydroxybenzaldehyde, 2-furancarboxaldehyde, 2-thiophenecarboxaldehyde, 2-pyridinecarboxaldehyde, cyclopentanone (all from Aldrich), benzaldehyde (Probus), rhodanine (Aldrich) and triethylphosphinegold(I) chloride (Aldrich) were used as supplied. The 3-(aryl)-2-sulfanylpropenoic acids H_2**x**spa [**x** = f = 3-(2-furyl)-; t = 3-(2-thienyl)-; p = 3-phenyl-; Clp =

3-(2-Chlorophenyl)-; -*o*-mp = 3-(2-methoxyphenyl)-; -*p*-mp = 3-(4-methoxyphenyl)-; *o*-hp = 3-(2-hydroxyphenyl)-; *p*-hp = 3-(4-hydroxyphenyl)-;-diBr-*o*-hp = 3-(3,5-dibromo-2-hydroxyphenyl-); py = 3-(2-pyridyl); spa = 2-sulfanylpropenoate] were prepared by condensation of the appropriate aldehyde with rhodanine [33], followed by hydrolysis in an alkaline medium and acidification with aqueous HCl [34-36]. For the preparation of 2-cyclopentyliden-2-sulfanylacetic acid (H_2cpa), cyclopentanone was used instead of an aldehyde.

Elemental analyses were performed with a Fisons 1108 microanalyser. Melting points were determined with a Büchi apparatus. Mass spectra were recorded using FAB (fast atom bombardment) (Micro mass Autospec spectrometer connected to a DS90 system, *m*-nitrobenzyl alcohol, Xe, 8 eV; *ca.* 1.28×10^{-15} J) and ESI-TOF (electrospray ionization time-of-flight) (positive-ion mode, Microtof® from Bruker Daltonics, methanol, capillary voltage 4500 V) ionization methods.

IR spectra (KBr pellets) were recorded on a Bruker IFS66V FT-IR spectrometer and are reported in the synthesis section using the following abbreviations: vs = very strong, s = strong, m = medium, w = weak, sh = shoulder, br = broad. ^{1}H and ^{13}C NMR spectra in solution were recorded in dmso-d_6 or $CDCl_3$ at room temperature on a Bruker AMX 300 operating at 300.14 and 75.40 MHz, respectively, using 5 mm o.d. tubes; chemical shifts are reported relative to TMS (tetramethylsilane) using the solvent signal (δ ^{1}H = 2.50 ppm; δ ^{13}C = 39.50 ppm or δ ^{1}H = 7.26 ppm; δ ^{13}C = 77.00 ppm) as reference. The ^{1}H-^{1}H COSY (correlated spectroscopy) NMR spectra and ^{1}H-^{13}C HMBC (heteronuclear multiple bond correlation) and HMQC (heteronuclear multiple quantun coherence) experiments were measured using a Varian Inova 400 spectrometer. ^{31}P NMR spectra were recorded at 202.46 MHz on a Bruker AMX 500 spectrometer using 5 mm o.d. tubes and are reported relative to external H_3PO_4 (85%). The following abbreviations were used as s = singlet, d = doublet, t = triplet, *p*st = *pseudo*triplet, m = multiplet, qn = quintet. ^{1}H, and ^{13}C NMR data were obtained from freshly prepared concentrated solutions.

2.2. X- ray crystallography

Data collection was carried out with a Bruker CCD Smart 1000 automatic diffractometer, 293 K, ω scan technique, Mokα radiation (λ = 0.71073 Å) and corrected for Lorentz and polarization effects [37]. MultiScan (SADABS) [38] correction was also

performed. The structure was solved by direct methods [39] and subsequent Fourier maps, and refined on F^2 by a full-matrix least-squares procedure using anisotropic displacement parameters [40]. Atomic scattering factors were taken from International Tables for X-ray Crystallography [41]. Molecular graphics were generated with MERCURY [42]. Although the X-ray experiment afforded the molecular connectivity in compound **9**, the lack of quality of the crystals and the disorder associated with the PEt_3 groups prevented a complete refinement of the model. Crystal data: Monoclinic, P2(1)/n, Unit cell dimensions: a = 20.416(4) Å; b = 13.055(3) Å; c = 20.490(4) Å; β = 102.393(4)º; V = 5333.9(19) $Å^3$; Z = 8; D (calculated) =1.603 Mg/m^3.

2.3. In vitro antitumor activity

2.3.1. Cell line and growth conditions

The human cervix carcinoma cell line HeLa-229 used in this study was kindly provided by Dra. Guadalupe Mengod (CSIC-IDIBAPS of Barcelona, Spain), the human ovarian cancer cell line A2780 and its cisplatin-resistant mutant A2780*cis* were both obtained from the European Collection of Cell Cultures through Sigma-Aldrich. The cells were grown in Dulbecco's Modified Eagle's medium (DMEM, HeLa-229, pH = 7.4) or RPMI 1640 medium (A2780, A2780*cis*) supplemented with 10% foetal calf serum (FCS) and 2 mM L-glutamine (pH = 7.4). Cells were maintained in continuous logarithmic culture in a humidified atmosphere of 5% CO_2 at 37 ºC and were harvested using trypsin-ethylenediaminetetraacetic acid. Pig proximal tubule LLC-PK1 cells were purchased from the American Tissue Culture Collection (ATCC) and were grown in Medium 199 supplemented with 3% foetal bovine serum (FBS) and 1.5 g/L $NaHCO_3$ (pH = 7.2) and maintained at 37 ºC in a 5% CO_2 atmosphere, with the medium replaced twice a week.

All media and supplements were purchased from Sigma-RBI, Spain.

2.3.2. In vitro chemosensitivity assay

The tumor cells were seeded into 96-well plates (Beckton-Dickinson, Spain) in a volume of 100 μL at a density of 4000 cells/well and were incubated for 4–6 h (HeLa-229) or 24 h (A2780, A2780*cis*) prior to dosage.

Solutions of the complexes in ethanol were added to the cells using the same concentration of ethanol per well (1%). After the appropriate incubation time, i.e. 48 h for HeLa-229 and 96 h for A2780 and A2780*cis*, the cells were fixed by adding 10 μL of 11%

glutaraldehyde per well for 15 min. The fixative was then removed and the wells were washed four times with distilled water. Cell biomass was determined by a crystal violet staining technique [43] and the optical density was measured at 595 nm with a Tecan Ultra Evolution microplate reader.

The renal LCC-PK1 cells were seeded in a 96-well microplate ($1{\cdot}10^4$ cells/well) in 100 µL of growth medium and the samples were maintained at 37 ºC in a 5% CO_2 atmosphere during 24 h. The growth medium was then replaced by fresh medium containing different concentrations of the compounds to be assayed and the samples were maintained at 37 ºC in a 5% CO_2 atmosphere for 48 h. After this time, cells were fixed to the plate with 10 µL of 11% glutaraldehyde for 15 min at room temperature and the medium was then removed and cells were washed 4 times with deionized water.

Cells were stained with 100 µL of a 0.1% crystal violet solution (0.1 g of crystal violet in 100 mL of 200 mM phosphoric acid, 200 mM formic acid and 200 mM MES (2-(*N*-morpholino)ethanesulfonic acid); pH = 6) for 15 min at room temperature. This solution was removed and the plate was washed four times with deionized water and dried. In order to enable homogenous coloration in all wells, 100 µL of 10% acetic acid were added to the wells and the plate was gently shaken at room temperature for 15 min. The crystal violet staining technique was also used and absorbance was read at 595 nm in a microplate reader (BioRad Microplate Reader model 680).

Each gold complex was tested using six or seven consecutive dilutions ranging from 50 µM to 0.025 µM. The effect of 1% ethanol on the cell viability (máx. 8%) was evaluated and corrected in all cases. The pH value remains essentially constant throughout the experiments.The compound concentration able to inhibit cell growth by 50% with respect to controls, IC_{50}, was then determined from semi-logarithmic dose-response sigmoid curves using GraphPad Prism Ver. 2.01 software (GraphPad Software Inc.).

The cytotoxicity of cisplatin (dissolved in water) was evaluated for comparison purposes under the same experimental conditions. All compounds were tested in three independent experiments with triplicate points. Results were expressed as mean±S.E. In order to test for differences between the appropriate complexes a Student´s T test was used ($p < 0.05$). The *in vitro* studies were performed at the Unit for the Evaluation of Pharmacological Activities of Chemical Compounds of the University of Santiago de Compostela.

2.4. Synthesis

The mononuclear complexes of the type [Au(PEt_3)(H**x**spa)] (**1**-**11**) were prepared by adding Au(PEt_3)Cl in a 1:1 mole ratio to a solution of the appropriate sulfanylcarboxylic acid and KOH in ethanol/water (8:1). After stirring at room temperature for one hour, the solvent was evaporated under vacuum and the oil formed was dissolved in chloroform, filtered and the solvent removed under vacuum.

The dinuclear complexes of the type [($AuPEt_3$)$_2$(**x**spa)] were prepared as previously described [32] by adding Au(PEt_3)Cl in a 2:1 mole ratio to a solution of the appropriate sulfanylcarboxylic acid and NaOH in methanol/water (8:1 v/v). After stirring and refluxing for 1 h, the methanol was evaporated and the crude product was dissolved in chloroform, filtered and the solvent removed.

[Au(PEt_3)(Hpspa)] (1). H_2pspa (0.077 g, 4.3 mmol), Au(PEt_3)Cl (0.150 g 4.3 mmol), ethanol (8 cm^3), KOH (0.024 g, 4.3 mmol), H_2O (1 cm^3), yellow oil. Yield: 82%. Anal. Found: C 36.5, H 4.6, S 6.2 %. Calc. for $C_{15}H_{22}O_2SPAu$: C 36.4, H 4.5, S 6.5 %. MS (FAB): the main metalated signals are at *m/z* (%) 977 (100), [($AuPEt_3$)$_3$S]$^+$; 808 (10), [($AuPEt_3$)$_2$pspa]$^+$; and 315 (56), [(PEt_3)Au]$^+$.IR (cm^{-1}): 1720vs, br, ν(C=O); 1454vs, 1413s, 1381s, ν(PEt_3). NMR (dmso-d_6): ^{1}H, δ 7.44 (s, 1H, C(3)H), 7.99 (d, 2H, C(5)H, C(9)H), 7.35 (*p*st, 2H, C(6)H, C(8)H), 7.22 (m, 1H, C(7)H), 1.80 (m, 6H, H(PCH_2)), 1.07 (m, 9H, H(PCH_3)); ^{13}C, δ 170.5 C(1), 127.2 C(2), 136.5 C(3), 134.4 C(4), 130.3 C(5) and C(9), 127.6 C(6) and C(8), 133.6 C(7), 17.3 (d, J(C-P) = 33.9 (PCH_2)), 9.0 (PCH_3),; ^{31}P {^{1}H}, δ 41.00(s). NMR ($CDCl_3$): ^{1}H, δ 7.94 (s, 1H, C(3)H), 8.08 (d, 2H, C(5)H, C(9)H), 7.35 (*p*st, 2H, C(6)H, C(8)H), 7.25 (m, 1H, C(7)H), 1.72 (m, 6H, H(PCH_2)), 1.07 (m, 9H, H(PCH_3)); ^{13}C, δ 169.5 C(1), 128.6 C(2), 139.1 C(3), 136.0 C(4), 130.9 C(5) and C(9), 127.6 C(6) and C(8), 128.3 C(7), 18.0 (d, J(C-P) = 33.9 (PCH_2)), 9.0 (PCH_3),; ^{31}P {^{1}H}, δ 34.9(s).

[Au(PEt_3)(Hfspa)] (2). H_2fspa (0.073 g, 4.3 mmol), Au(PEt_3)Cl (0.150 g 4.3 mmol), ethanol (8 cm^3), KOH (0.024 g, 4.3 mmol), H_2O (1 cm^3), brown oil. Yield: 80%. Anal. Found: C 31.9, H 4.2, S 6.6 %. Calc. for $C_{13}H_{20}O_3SPAu$: C 32.2, H 4.1, S 6.6 %. MS (FAB): the main metalated signals are at *m/z*(%) 977 (50), [($AuPEt_3$)$_3$S]$^+$; 799 (83), [($AuPEt_3$)$_2$fspa]$^+$; 484 (48), [M]$^+$; and 315 (100), [(PEt_3)Au]$^+$. IR (cm^{-1}): 1720vs, 1695vs, br, ν(C=O); 1456s, 1412s, 1381s ν(PEt_3). NMR (dmso-d_6): ^{1}H, δ 7.65 (s, 1H, C(3)H), 7.41 (d, 1H, C(5)H) 6.58 (t, 1H, C(6)H) 7.44 (d, 1H, C(7)H), 1.86 (qn, 6H, H(PCH_2)) 1.10 (t,12H, H(PCH_3)); ^{13}C, δ 170.5 C(1), 123.6 C(2), 132.7 C(3), 153.2 C(4), 114.1 C(5), 112.8 C(6), 143.2 C(7), 18.1 (d, J(C-P) = 34.5 (PCH_2)), 9.8 (PCH_3); ^{31}P {^{1}H}, δ 41.1 (s), 55.1 (s). NMR ($CDCl_3$): ^{1}H, δ 7.89 (s, 1H,

C(3)H), 7.68 (d, 1H, C(5)H) 6.52 (t, 1H, C(6)H) 7.45 (d, 1H, C(7)H), 1.78 (qn, 6H, H(PCH_2)) 1.12 (t,12H, H(PCH_3)); ^{13}C, δ 169.3 C(1), 125.6 C(2), 128.5 C(3), 152.9 C(4), 115.2 C(5), 111.9 C(6), 143.0 C(7), 18.0 (d, J(C-P) = 33.8 (PCH_2)), 8.9 (PCH_3); ^{31}P {^{1}H}, δ 36.6 (s), 55.1 (s).

[Au(PEt_3)(Htspa)] (3). H_2tspa (0.08 g, 4.3 mmol), Au(PEt_3)Cl (0.150 g 4.3 mmol), ethanol (8 cm^3), KOH (0.024 g, 4.3 mmol), H_2O (1 cm^3), yellow oil. Yield: 85%. Anal. Found: C 31.0, H 4.3, S 12.5 %. Calc. for $C_{13}H_{20}O_2S_2PAu$: C 31.2, H 4.0, S 12.8 %. MS (FAB): the main metalated signals are at *m/z* (%) 977 (13), $[(AuPEt_3)_3S]^+$; 815 (48), $[(AuPEt_3)_2tspa]^+$; 499 (11), $[M]^+$; and 315 (74), $[(PEt_3)Au]^+$. ESI-MS (+) peaks at *m/z* (%): 977 (6), $[(AuPEt_3)_3S]^+$; 815 (56), $[(AuPEt_3)_2(tspa)+H]^+$; 663 (14), $[(AuPEt_3)_2S+H]^+$; 523 (0.4), $[Au(PEt_3)(Htspa)+Na]^+$; 501 (0.1), $[Au(PEt_3)(Htspa)+H]^+$**;** 455 (3), $[Au(PEt_3)_2+Na-H]^+$; 433 (100), $[Au(PEt_3)_2]^+$; $[Au(PEt_3)]^+$ and 135 (4), $[OPEt_3+H]^+$. IR (cm^{-1}): 1721sh, 1707vs, br, ν(C=O); 1455s, 1413s, 1381m, ν(PEt_3). NMR (dmso-d_6): 7.88 (s, 1H, C(3)H), 7.40 (d, 1H, C(5)H) 7.10 (t, 1H, C(6)H), 7.57 (d, 1H, C(7)H), 1.83 (m, 6H, H(PCH_2)), 1.11 (m, 9H, H(PCH_3)); ^{13}C, δ 169.9 C(1), 129.9 C(2), 131.3 C(3), 140.1 C(4), 130.3 C(5), 126.1 C(6), 128.6 C(7), 17.3 (d, J(C-P) = 34.1 (PCH_2)), 8.9 (PCH_3); ^{31}P {^{1}H}, δ 38.3 (s), 52.2 (s). NMR ($CDCl_3$): ^{1}H, δ 8.27 (s, 1H, C(3)H), 7.45 (d, 1H, C(5)H) 7.10 (t, 1H, C(6)H), 7.49 (d, 1H, C(7)H), 1.76 (q, 6H, H(PCH_2)) 1.01 (t,12H, H(PCH_3)); ^{13}C, δ 169.0 C(1), 124.6 C(2), 134.6 C(3), 140.8 C(4), 132.8 C(5), 126.1 C(6), 129.2 C(7), 17.9 (d, J(C-P) = 34.2 (PCH_2)), 8.9 (PCH_3); ^{31}P {^{1}H}, δ 36.9 (s), 58.1 (s).

[Au(PEt_3)(H-*o*-pyspa)] (4). H_2-*o*-pyspa (0.078 g, 4.3 mmol), Au(PEt_3)Cl (0.150 g, 4.3 mmol), ethanol (8 cm^3), KOH (0.024 g, 4.3 mmol), H_2O (1 cm^3), orange oil. Yield: 87%. Anal. Found: C 33.6, H 4.0, N 2.6, S 6.2 %. Calc. for $C_{14}H_{21}O_2SNPAu$: C 33.9, H 4.3, N 2.8, S 6.5 %. MS (FAB): the main metalated signals are at *m/z* (%) 977 (100), $[(AuPEt_3)_3S]^+$; 810 (8) $[(AuPEt_3)_2$-o-pyspa$]^+$; 662 (3), $[(AuPEt_3)_2S]^+$; 495 (12), $[M]^+$; 433 (28), $[(PEt_3)_2Au]^+$ and 315 (34), $[(PEt_3)Au]^+$. IR (cm^{-1}): 1669s, ν (C═O); 1455vs, 1414m, 1361m, br, ν(PEt_3). NMR (dmso-d_6): ^{1}H, δ 7.45 (s, 1H, C(3)H), 8.52 (d, 1H, C(5)H), 7.74 (st, 1H, C(6)H), 7.12 (st, 1H, C(7)H), 8.60 (d, 1H, C(8)H), 1.84 (m, 6H, H(PCH_2)), 1.08 (m, 9H, H(PCH_3)); ^{13}C, δ 171.1 C(1), 142.2 C(2), 135.3 C(3), 156.4 C(4), 148.9 C(5), 132.1 C(6), 121.0 C(7), 124.2 C(8), 17.3 (d, J(C-P) = 34.1 (PCH_2)), 8.9 (PCH_3); ^{31}P {^{1}H}, δ 37.3 (s).

[Au(PEt_3)(HClpspa)] (5). H_2Clpspa (0.092 g, 4.3 mmol), Au(PEt_3)Cl (0.150 g, 4.3 mmol), ethanol (8 cm^3), KOH (0.024 g, 4.3 mmol), H_2O (1 cm^3), yellow oil. Yield: 89%. Anal. Found: C 33.9, H 4.0, S 5.8 %. Calc. for $C_{15}H_{21}O_2SClPAu$: C 34.1, H 4.0, S 6.0 %. MS

(FAB): the main metalated signals are at *m/z* (%) 977 (100), $[(AuPEt_3)_3S]^+$; 859 (15), $[(AuPEt_3)_2AuS]^+$; 433 (35), $[(PEt_3)_2Au]^+$ and 315 (30), $[(PEt_3)Au]^+$. IR (cm^{-1}): 1766vs, 1722 vs, br, ν(C═O); 1456vs, 1414vs, 1381vs, ν(PEt_3). NMR (dmso-d_6): ^1H, δ 12.50 (s, br, 1H, C(1)OH), 7.66 (s, 1H, C(3)H), 7.40 (d, 1H, C(6)H) 7.35 (*p*st, 1H, C(7)H), 7.19 (m, 1H, C(8)H), 8.10 (d, 1H, C(9)H), 1.85 (m, 6H, H(PCH_2)), 1.07 (m, 9H, H(PCH_3)). ^{13}C, δ 169.0 C(1), 126.5 C(2), 132.3 C(3), 135.2 C(4), 135.0 C(5), 128.7 C(6), 130.1 C(7), 128.4 C(8), 132.0 C(9), 17.5 (d, J(C-P) = 33.8 (PCH_2)), 9.1 (PCH_3); ^{31}P {^1H}, δ 34.7 (s); NMR ($CDCl_3$): ^1H, δ 8.08 (s, 1H, C(3)H), 7.36 (d, 1H, C(6)H), 7.45 (st, 1H, C(7)H), 7.14 (m, 1H, C(8)H), 8.45 (d, 1H, C(9)H), 1.76 (m, 6H, H(PCH_2)), 1.10 (m, 9H, H(PCH_3)); ^{13}C, δ 168.9 C(1), 125.7 C(2), 134.9 C(3), 135.6 C(4), 135.1 C(5), 129.2 C(6), 129.0 C(7), 128.5 C(8), 131.8 C(9), 18.0 (d, J(C-P) = 34.1 (PCH_2)), 9.0 (PCH_3); ^{31}P {^1H}, δ 36.4 (s).

[Au(PEt_3)(H-*o*-mpspa)] (6). H_2-*o*-mpspa (0.090 g, 4.32 mmol), Au(PEt_3)Cl (0.150 g, 4.3 mmol), ethanol (8 cm^3), KOH (0.024 g, 4.3 mmol), H_2O (1 cm^3), orange oil. Yield: 82%. Anal. Found: C 36.2, H 4.5, S 5.9 %. Calc. for $C_{16}H_{24}O_3SPAu$: C 36.6, H 4.6, S 6.1 %. MS (FAB): the main metalated signals are at *m/z* (%) 1154 (3), $[(AuPEt_3)_3$-*o*-mpspa$]^+$; 977 (68), $[(AuPEt_3)_3S]^+$; 839 (100), $[(AuPEt_3)_2$-o-mpspa$]^+$; 524 (3), $[M]^+$; 433 (20), $[(PEt_3)_2Au]^+$ and 315 (44), $[(PEt_3)Au]^+$. IR (cm^{-1}): 1724vs, 1674vs, br, ν(C═O); 1461vs, 1413vs, 1381s ν(PEt_3). NMR (dmso-d_6): ^1H, δ 12.08 (s, vbr, 1H, C(1)OH), 8.26 (s, 1H, C(3)H), 8.49 (d, 1H, C(6)H), 6.93 (st, 2H, C(7)H, C(9)H), 7.21 (st, 1H, C(8)H), 3.76 (s, 3H, OCH_3), 1.83 (q, 6H, H(PCH_2)) 1.01 (t,12H, H(PCH_3)); ^{13}C, δ 170.1 C(1), 119.2 C(2), 133.6 C(3), 125.3 C(4), 156.9 C(5), 110.3 C(6), 130.1 C(7), 128.2 C(8), 128.9 C(9), 55.3 C(OCH_3), 17.2 (d, J(C-P) = 34.1 (PCH_2)), 8.9 (PCH_3); ^{31}P {^1H}, δ 38.5 (s); NMR ($CDCl_3$): ^1H, δ 8.25 (s, 1H, C(3)H), 8.70 (d, 1H, C(6)H), 6.86 (st, 1H, C(7)H, C(9)H), 7.00 (st, 1H, C(8)H), 3.82 (s, 3H, OCH_3), 1.75 (q, 6H, H(PCH_2)) 1.16 (t,12H, H(PCH_3)).

[Au(PEt_3)(H-*p*-mpspa)] (7). H_2-*p*-mpspa (0.090 g, 4.32 mmol), Au(PEt_3)Cl (0.150 g, 4.3 mmol), ethanol (8 cm^3), KOH (0.024 g, 4.3 mmol), H_2O (1 cm^3), yellow oil. Yield: 85%. Anal. Found: C 36.8, H 4.8, S 6.3 %. Calc. for $C_{16}H_{24}O_3SPAu$: C 36.6, H 4.6, S 6.1 %. MS (FAB): the main metalated signals are at *m/z* (%) 977 (100), $[(AuPEt_3)_3S]^+$; 859 (5) $[(AuPEt_3)_2AuS]^+$; 839 (2), $[(AuPEt_3)_2$-*p*-mpspa$]^+$; 662 (4), $[(AuPEt_3)_2S]^+$; 433 (11), $[(PEt_3)_2Au]^+$ y 315 (18), $[PEt_3Au]^+$. IR (cm^{-1}): 1720vs, br, ν (C═O); 1456m, 14117m, 1380w, ν(PEt_3). NMR (dmso-d_6): ^1H, δ 12.00 (s, br, 1H, C(1)OH), 7.42 (s, 1H, C(3)H), 8.04 (d, 2H, C(5)H, C(9)H), 6.90 (d, 2H, C(6)H, C(8)H), 3.75 (s, 3H, OCH_3), 1.83 (q, 6H, H(PCH_2)) 1.07 (t,12H, H(PCH_3)); ^{13}C, δ 170.8 C(1), 129.3 C(2), 134.1 C(3), 130.9 C(4), 131.9 C(5) and

C(9), 113.1 C(6) and C(8), 158.5 C(7), 55.1 C(OCH_3), 17.3 (d, J(C-P) = 33.4 (PCH_2)), 9.0 (PCH_3); ^{31}P {^1H}, δ 41.5 (s), 54.8 (s).

[Au(PEt_3)(H-*o*-hpspa)] (8). H_2-*o*-hpspa (0.084 g, 4.3 mmol), Au(PEt_3)Cl (0.150 g, 4.3 mmol), ethanol (8 cm^3), KOH (0.024 g, 4.3 mmol), H_2O (1 cm^3), orange solid. Yield: 80%. Anal. Found: C 34.9, H 4.1, S 5.9 %. Calc. for $C_{15}H_{22}O_3SPAu$: C 35.3, H 4.3, S 6.3 %. MS (FAB): the main metalated signals are at *m/z* (%) 977 (100), $[(AuPEt_3)_3S]^+$; 825 (33), $[(AuPEt_3)_2$-*o*-hpspa$]^+$; 510 (12), $[M]^+$; 433 (22), $[(PEt_3)_2Au]^+$ and 315 (68), $[(PEt_3)Au]^+$. IR (cm^{-1}): 1725vs, 1716vs, 1689m, ν(C═O); 1451s, 1413m, 1383m, ν(PEt_3). NMR (dmso-d_6): ^1H, δ 12.20 (s, br, 1H, C(1)OH), 8.06 (s, 1H, C(3)H), 8.22 (s, 1H, C(5)OH), 7.07 (d, 1H, C(6)H), 7.27 (st, 1H, C(7)H), 6.72 (st, 1H, C(8)H), 7.50 (d, 1H, C(9)H), 1.89 (m, 6H, H(PCH_2)), 1.11 (m, 9H, H(PCH_3)); ^{13}C, δ 170.1 C(1), 123.3 C(2), 126.6 C(3), 125.1 C(4), 155.9 C(5), 116.3 C(6), 129.3 C(7), 118.0 C(8), 129.7 C(9), 18.2 (d, J(C-P) = 34.1 (PCH_2)), 10.0 (PCH_3); ^{31}P {^1H}, δ 35.1 (s).

[Au(PEt_3)(H-*p*-hpspa)] (9). H_2-*p*-hpspa (0.084 g, 4.3 mmol), Au(PEt_3)Cl (0.150 g, 4.3 mmol), ethanol (8 cm^3), KOH (0.024 g, 4.3 mmol), H_2O (1 cm^3), orange solid. Yield: 92%. Anal. Found: C 34.9, H 4.0, S 5.9 %. Calc. for $C_{15}H_{22}O3SPAu$: C 35.3, H 4.3, S 6.3 %. MS (FAB): the main metalated signals are at *m/z* (%) 977 (40), $[(AuPEt_3)_3S]^+$; 825 (50), $[(AuPEt_3)_2$-*p*-hpspa$]^+$; 510 (50), $[M]^+$; 433 (29), $[(PEt_3)_2Au]^+$ and 315 (100), $[(PEt_3)Au]^+$. ESI-MS (+) peaks at *m/z* (%): 977 (100), $[(AuPEt_3)_3S]^+$; 859 (20), $[(AuPEt_3)_2AuS]^+$; 825 (12), $[Au(PEt_3)_2$(H-*p*-hpspa+H$]^+$; 663 (7), $[(AuPEt_3)_2S+H]^+$; 433 (67), $[Au(PEt_3)_2]^+$; 315 (35), $[Au(PEt_3)]^+$ and 135 (1), $[OPEt_3+H]^+$. IR (cm^{-1}): 1700sh, 1687vs, ν(C═O); 1452s, 1413w, 1383s, ν(PEt_3). NMR (dmso-d_6): ^1H, δ 12.81 (s, vbr, 1H, C(1)OH), 7.41 (s, 1H, C(3)H), 7.94 (d, 2H, C(5)H, C(9)H), 6.73 (d, 2H, C(6)H, C(8)H), 9.74 (s, 1H, OH), 1.84 (m, 6H, H(PCH_2)), 1.04 (m, 9H, H(PCH_3)); ^{13}C, δ 171.0 C(1), 127.8 C(2), 129.6 C(3), 134.8 C(4), 132.1 C(5) and C(9), 114.6 C(6) and C(8), 157.2 C(7), 17.2 (d, J(C-P) = 34.1 (PCH_2)), 8.9 (PCH_3); ^{31}P {^1H}, δ 38.3 (s), 52.0 (s). Slow concentration of a chloroform solution afforded crystals suitable for X-ray diffractometry of formulae [Au(PEt_3)(H-*p*-hpspa)])·$OPEt_3$, **(9)**·$OPEt_3$.

[Au(PEt_3)(HdiBr-*o*-hpspa)] (10). H_2diBr-*o*-hpspa (0.151 g, 4.3 mmol), Au(PEt_3)Cl (0.150 g, 4.3 mmol), ethanol (8 cm^3), KOH (0.024 g, 4.3 mmol), H_2O (1 cm^3), yellow solid. Yield: 95%. Anal. Found: C 26.8, H 3.0, S 4.6%. Calc. for $C_{15}H_{20}O_3SBr_2PAu$: C 27.0, H 3.0, S 4.8 %. MS (FAB): the main metalated signals are at *m/z* (%) 982 (5) $[(AuPEt_3)_2$-diBr-*o*-hpspa$]^+$; 977 (100), $[(AuPEt_3)_3S]^+$; 662 (20), $[(AuPEt_3)_2S]^+$; 666 (3), $[M]^+$; 433 (46),

$[(PEt_3)_2Au]^+$ and 315 (83), $[(PEt_3)Au]^+$. IR (cm^{-1}): 1734vs, 1708m, ν(C═O); 1449m, 1414w, 1384w, ν(PEt_3). NMR ($CDCl_3$): ^1H, δ 13.00 (s, 1H, C(1)OH), 7.77 (s, 1H, C(3)H), 9.80 (s, 1H, C(5)OH), 7.87 (s, 1H, C(7)H), 7.66 (s, 1H, C(9)H), 1.82 (m, 6H, H(PCH_2)), 1.15 (m, 9H, H(PCH_3)); ^{13}C, δ 169.8 C(1), 123.1 C(2), 132.9 C(3), 127.2 C(4), 151.0 C(5), 110.5 C(6), 136.8 C(7), 113.6 C(8), 134.2 C(9), 18.7 (d, J(C-P) = 33.4 (PCH_2)), 9.7 (PCH_3); ^{31}P {^1H}, δ 35.1 (s).

[Au(PEt_3)(Hcpa)] (11). H_2cpa (0.067 g, 4.3 mmol), Au(PEt_3)Cl (0.150 g, 4.3 mmol), ethanol (8 cm^3), KOH (0.024 g, 4.3 mmol), H_2O (1 cm^3), brown oil. Yield: 85%. Anal. Found: C 33.2, H 5.0, S 6.5 %. Calc. for $C_{13}H_{24}O_2SPAu$: C 33.0, H 5.1, S 6.8 %. MS (FAB): the main metalated signals are at *m/z* (%) 977 (100), $[(AuPEt_3)_3S]^+$; 859 (11), $[(AuPEt_3)_2S]^+$; 786 (14), $[(AuPEt_3)_2cpa]^+$; 662 (6), $[(AuPEt_3)_2S]^+$; 472 (2), $[M]^+$; 433 (16), $[Au(PEt_3)_2]^+$ and 315 (28), $[AuPEt_3]^+$. IR (cm^{-1}): 1699vs, br, ν(C═O); 1452m, 1409m, 1383m, ν(PEt_3). NMR (dmso-d_6): ^1H, δ 3.50 (m, 2H, C(4)H_2), 1.60 (m, 2H, C(5)H_2), 1.60 (m, 2H, C(6)H_2), 3.50 (m, 2H, C(7)H_2), 1.87 (m, 6H, H(PCH_2)), 1.10 (m, 9H, H(PCH_3)); ^{13}C, δ 171.8 C(1), 122.6 C(2), 151.6 C(3), 36.4 C(4), 28.2 C(5), 26.3 C(6), 34.6 C(7), 18.1 (d, J(C-P) = 33.4 (PCH_2)), 8.9 (PCH_3); ^{31}P {^1H}, δ 38.5 (s).

3. Results and discussion

3.1. Synthesis and characterization

The complexes of the type [Au(PEt_3)(H**x**spa)] (**1**-**11**) were prepared by adding Au(PEt_3)Cl to a solution of the appropriate sulfanyl carboxylic acid and KOH (1:1:1 molar ratio) in ethanol/water. This solution was stirred for one hour, after this time the solvent was evaporated and the resulting oil was dissolved in chloroform. Complexes **8, 9** and **10** were obtained as solids but in the other cases the evaporation of the chloroform again afforded oily products in high yields. These oils were also obtained in several attempts using different solvents.

The FAB(+) spectra of these complexes show peaks due to the M^+, except for **1**, **5** and **7**, in which this peak was absent. Besides, the spectra show metalated peaks due to the cleavage of the Au-S and Au-P bonds; dimetalated and trimetallated species were also identified, as previously described for the equivalent triphenylphosphine derivatives [27].

*3.2. Crystal and molecular structure of [Au(PEt_3)(H-p-hpspa)]·$OPEt_3$, **(9)**·$OPEt_3$*

Attempts to obtain suitable crystals for an X-ray study by using different solvents were, in general, unsuccessful. However, in the case of [Au(PEt_3)(H-p-hpspa)] (**9**), a chloroform solution left standing after several weeks liberated an oily solid from which a crystal was selected by hand. Although suitable for X-ray diffraction, the crystal was of poor quality and had a weak diffraction power. It contained two Au(PEt_3)(H-*p*-hpspa) and two $OPEt_3$ molecules in the asymmetric unit. The poor quality of the crystal and the considerable disorder of the ethyl groups made a rigorous refinement of the structure difficult but the connectivity of the non-hydrogen atoms has been proved beyond any doubt and is shown in Figure 1 for the Au(PEt_3)(H-*p*-hpspa) unit. As seen in the figure, the Au atom is S-bonded (Au-S: ca. 2.30 Å) to the S atom of the sulfanylcarboxylate ligand and P-bonded (Au-P: ca. 2.25Å) to the P atom of the triethylphosphine ligand in a practically linear environment resembling the situation in the equivalent triphenylphosphine complexes [27]. The aforementioned limitations preclude a more detailed discussion of the bond distances and angles.

Figure 1

3.3. IR spectra

The IR spectra of these complexes, when compared with the corresponding spectra of their free ligands, show the absence of the ν(SH) band, which is present in the spectrum of the free ligands between 2590-2560 cm^{-1}, consistent with the deprotonation of this group and the S-coordination to the Au atom. The ν(C=O) band of the COOH group for the free ligands is located at 1670 (H_2pspa) , 1673 (H_2fspa), 1665 (H_2tspa) , 1627 (H_2-*o*-pyspa), 1683 (H_2Clpspa) , 1664 (H_2-*o*-mpspa), 1662 (H_2-*p*-mpspa) , 1682 (H_2-*o*-hpspa), 1686 (H_2-*p*-hpspa), 1682 (H_2-diBr-hpspa) and 1659 cm^{-1} (H_2cpa). These positions are compatible with the involvement of the C=O group in hydrogen bonds with the –OH fragment of a neighbour COOH group to form dimers, a usual symptom in carboxylic acids [44, 45]. The S-metalation of these acids with $AuPPh_3$ fragments does not significantly change the position of the ν(C=O) band in R-CS($AuPPh_3$)-COOH complexes, where the presence of the aforementioned interaction was identified by X-ray diffraction [27]; nevertheless, small changes in the position with respect to that in the free acid were detected and attributed to changes in the hydrogen bond pattern. The position of this band, however, significantly varies in the IR spectra of the complexes described here. For [Au(PEt_3)(H-*o*-pyspa)] (**4**) and [Au(PEt_3)(Hcpa)]

(**11**), the position of the ν(C=O) band is indicative of the presence of dimeric species, formed by the interaction of COOH groups, as occurs in the PPh_3 derivatives. On the contrary, in the IR of the complexes [Au(PEt_3)(Hpspa)] (**1**), [Au(PEt_3)(Htspa)] (**3**), [Au(PEt_3)(HClpspa)] (**5**), [Au(PEt_3)(H-*p*-mpspa)] (**7**) and [Au(PEt_3)(HdiBr-*o*-hspa)] (**10**) this band is located at wave numbers higher than 1700 cm^{-1}, shifting to positions found in complexes in which the –OH fragment of the COOH group is involved in an intramolecular hydrogen bond with the S atom, thus leading to monomeric units [46]. Similar monomeric R-CS($AuPEt_3$)-COOH units can be present here. Furthermore, in the IR spectra of [Au(PEt_3)(Hfspa)] (**2**), [Au(PEt_3)(H-*o*-mpspa)] (**6**), [Au(PEt_3)(H-*o*-hpspa)] (**8**) and [Au(PEt_3)(H-*p*-hpspa)] (**9**) we observe the presence of bands in these two positions, suggesting the coexistence of dimers and monomers. The fact that some of these bands are very broad suggests that the COOH groups can be involved in interactions of a different type and intensity.

3.4. Solution studies

In solution the compounds were studied by ^{1}H, ^{13}C and ^{31}P NMR. We tried to study the ^{1}H and ^{13}C spectra of all ligands and complexes in solvents of different donor character, like dmso-d_6 and $CDCl_3$, but the low solubility of some of them prevented the study. The signals were assigned on the basis of previous data for similar compounds [27, 32, 35] and, when necessary, ^{1}H-^{1}H COSY, ^{1}H-^{13}C gHSQC and gHMBC NMR experiments were carried out.

In the ^{1}H NMR spectra of the complexes, the broad signal that in the spectra of the free ligands is located between 4 and 5 ppm, assigned to the thiol group, was not present, according to the deprotonation of this group and the subsequent coordination of the S atom to the metal. This coordination also produces changes in the chemical shifts of the ligand protons, in particular, a shielding of the C(3)H signal, although in the complexes [Au(PEt_3)(H-*o*-hpspa)] (**8**), [Au(PEt_3)(Hfspa)] (**2**) and [Au(PEt_3)(H-*o*-pyspa)] (**4**) this signal is only slightly affected. In addition, the very broad signal at low field in the spectra of the ligands persists in that of the complexes, in accordance with the non-deprotonation of the COOH group. All the spectra show signals located around 1.80 and 1.05 ppm, attributed to the CH_2 and CH_3 groups of the PEt_3 ligand, respectively.

In the ^{13}C NMR spectra, the C signals of the ligands are also affected by the metal coordination. These changes are very significant in [Au(PEt_3)(H-*o*-pyspa)] (**4**), where C(2) and C(3) are shielded and deshielded more than 20 ppm respectively, as a consequence of the thione-thiol evolution when the ligand coordinates to the metal, and they are also indicative of the S-coordination. In the other complexes, coordination also produces changes, although

smaller, in the C(2), C(3), and C(4) positions, whereas the C(1) signals - with similar positions in dmso-d_6 and $CDCl_3$ - are located close to those previously found in the equivalent complexes [27], where the COOH group remains protonated. A singlet at 9 ppm and a doublet at 18 ppm, that are shifted slightly upfield with respect to $AuPEt_3Cl$, denote the presence of the phosphine ligand. [32, 47]

The $^{31}P\{^1H\}$ spectra consist of one singlet located between 34 and 42 ppm in dmso-d_6 - more shielded in $CDCl_3$ - that is attributed to the coordinated PEt_3. The chemical shift is similar to that previously found in complexes where S-Au-PEt_3 fragments are present [31, 47-52], where the ^{13}C chemical shifts and $^1J(^{13}C\text{-}^{31}P)$ values of the ethyl group are consistent with this connectivity [47, 48, 52]. When the spectra of the complexes were not immediately recorded, in some of them a very weak singlet appears between 50 and 60 ppm, which can be attributed [48, 53] to the presence of $OPEt_3$, formed after the decomposition of the complexes and further oxidation. No peak around -19 ppm that would be attributable to the free PEt_3 [47, 54] was observed, which implies that the phosphine oxidation process occurs very fast.

Keeping in mind that $OPEt_3$ was identified in the X-ray study of [Au(PEt_3)(H-*p*-hpspa)]·$OPEt_3$ (**9**)·$OPEt_3$, and that this compound was proposed as one of the metabolites formed in the biological mechanism of action of auranofin [47, 48, 50, 53, 55], the evolution with time of this compound towards the oxide formation was investigated by recording its ^{31}P NMR spectrum in dmso-d_6. At the same time, and due to the interest to compare the evolution of a compound of the type [Au(PEt_3)(HL)] with that of the equivalent [($AuPEt_3$)$_2$(L)], we also selected [Au(PEt_3)(Htspa)] (**3**) because the equivalent dinuclear complex was previously studied [32].

The ^{31}P spectra of a freshly prepared solution of both compounds show a very intense peak at 38 ppm attributable to the coordinated PEt_3 [32]. After a few minutes, a low intensity peak rises at 51.5 ppm; this signal is assigned to $OPEt_3$ [50, 53, 56, 57]. The amount of oxide present after 20 min, estimated from the integrated ^{31}P peaks, represents about 3% for compound **9**, and 5% for compound **3**, related to the coordinated PEt_3. Neither after 5 h nor 24 h, was a significant increase in the oxide formation detected and after 55 days the integral values were 8% for compound **9** and 5% for compound **3**. During the experiment, no signal at -19 ppm, attributable to the free PEt_3, was observed, meaning that the oxidation of the phosphine is very fast. Neither was any signal localized that could be assigned to the $[Au(PEt_3)_2]^+$ ion.

When the data for **3** are compared with those of the equivalent dinuclear complex, we found that the formation of the oxide is initially more significant for **3** (5% against 1.5% after

20 min) but the overall evolution with time seems to be less significant (5% against 10.8% after 55 days). In addition, a signal for $[Au(PEt_3)_2]^+$ was not identified for **3** but it was present in the spectra of the dinuclear complex [32]. The formation of this ion was, however, detected in the ESI/MS(+) electrospray spectrum (experimental part), where the remanence of the mononuclear complex is also evident together with the formation of dinuclear or even trinuclear species. This technique also visualizes the formation of $OPEt_3$, which contrasts with recent electrospray results obtained for Auranofin [58], a compound which also contains an S-Au-P fragment with PEt_3 as the P-donor ligand. It should be noted that the oxide was detected when Auranofin was incubated with glutathione or human serum albumin, both thiol ligands favouring the breaking of the Au-P bond and the subsequent formation of S-Au-S bonds. This additional support seems to be unnecessary in the present case.

3.5. Cytotoxic activity against tumor and normal cells

The human HeLa-229 cervix carcinoma cells and A2780 cells, together with its cisplatin-resistant mutant A2780*cis*, were used to analyze the effect that the replacement of the PPh_3 group with PEt_3 produces on the cytotoxicity of the previously studied mononuclear $[Au(PPh_3)(H\mathbf{x}spa)]$ complexes [27]. In addition, we have selected the equivalent dinuclear $[(AuPEt_3)_2(\mathbf{x}spa)]$ complexes. These complexes were previously [32] investigated *in vivo*, dissolved in water/polyethylene glycol (3:2) and intraperitoneally administered at well-tolerated doses of 2.5 and 5 mg/kg to Sprague-Dawley rats, showing significant anti-inflammatory activity against plantar edema induced by carrageenan. The election of these compounds enables us to compare their cytotoxic activity with those of the equivalent mononuclear $[Au(PEt_3)(H\mathbf{x}spa)]$ (**1**-**11**) and the dinuclear $[(AuPPh_3)_2(\mathbf{x}spa)]$ complexes [29], these latter containing the triphenylphosphine ligand.

The sulfanylcarboxylate ligands, as previously described [27], are inactive against these cell lines, except H_2tspa, which is slightly active against the HeLa culture cell. We have previously shown [29] the low activity of $Au(PPh_3)Cl$ against these three cell cultures [IC_{50}(μM) values of 25(1), 8.02(0.1) and 14(1) μM against HeLa, A2780 and A2780*cis*, respectively]; $Au(PEt_3)Cl$ was shown to be more active than the equivalent PPh_3 derivative, as the following IC_{50}(μM) values against the same lines show: $Au(PEt_3)Cl$: 1.7(0.06); 0.33(0.02); 1.8(0.06) μM.

Table S2 (Supplementary Information) shows the values of the IC_{50} parameter for the $[(AuPEt_3)_2(\mathbf{x}spa)]$ complexes against the HeLa cell line. The complexes of the type $[Au(PEt_3)(H\mathbf{x}spa)]$ (**1**-**11**) are scarcely active against these cells, showing very low values of

cellular growth inhibition and therefore the IC_{50} parameter was not determined. The 1:2 complexes are significantly active against this line, except in the cases of [$(AuPEt_3)_2$(diBr-*o*-hpspa)] and [$(AuPEt_3)_2$(*o*-pyspa], for which the low inhibition of the cellular growth precludes the determination of the IC_{50} value (note that the triphenylphosphine complexes of these two acids were the least active against this cell line when they were studied [29]). As seen in Figure 2, the incorporation of PEt_3 instead of PPh_3 increases the activity of the equivalent compounds, the effect being particularly relevant for the H_2fspa, H_2Clpspa and H_2-*p*-mpspa derivatives.

The [Au(PEt_3)(H**x**spa)] (**1**-**11**) complexes are more effective against the A2780 cell line than against the HeLa cell line - [Au(PEt_3)(H-*p*-mpspa)] (**7**) showed a low inhibition of the cellular growth and the IC_{50} parameter was not calculated. However, as graphically shown in Figure 3, only in the case of the H_2pspa, H_2fspa and H_2tspa derivatives were the PEt_3 derivatives more active than the PPh_3 ones. In addition, all of them are less active than other mononuclear gold(I) derivatives of a similar type, like [Au(PEt_3)(L)] {HL = menadione hydrogen bisulfite thiosemicarbazone [51]; 5-(hydroxymethyl)-8-methyl-3-thiol-7-azacoumarin [52]} and also than cisplatin. The situation is very different for the 1:2 complexes. As Figure 4 shows, except for the H_2-*o*-pyspa derivative, the introduction of a second $AuPEt_3$ fragment significantly improves the activity of the compounds, with IC_{50} values for these slightly better than those of cisplatin. All of these compounds are also more active than the equivalent PPh_3 derivatives, which are already significantly active.

Against the A2780*cis* cell line only four compounds of the type [Au(PEt_3)(H**x**spa)] (**1**-**11**), namely the H_2pspa, H_2fspa, H_2Clpspa and H_2-*o*-mpspa derivatives, have IC_{50} values revealing a better behaviour than cisplatin (although not better than the previously cited gold(I) complexes [51, 52] tested under the same conditions); furthermore, except for the H_2Clpspa derivative, they also have better values than the equivalent PPh_3 derivatives (Figure 5). No relevant increase in activity was obtained by substituting PPh_3 by PEt_3 in the other cases. With the exception of [$(AuPEt_3)_2$(*o*-pyspa)], that showed a low inhibition of the cellular growth, the situation is different for the compounds of the type [$(AuPEt_3)_2$(**x**spa)], all of which have IC_{50} values that are significantly better than the equivalent 1:1 complexes and the equivalent 1:2 PPh_3 complexes and they also display a behaviour that is better than that of cisplatin in all cases (Figure 6). The H_2diBr-*o*-hpspa derivative is the exception to this rule.

Whereas these promising activities are interesting, potential candidates to be used in clinic require an optimal ratio between the cancer killing dose and systemic toxicity [59]. A

preliminary study of this systemic toxicity can be made *in vitro* by comparing cytotoxicity against cancer cells and normal cells, the objective being a drug less toxic to normal cells than to tumor cells.

As nephrotoxicity is an adverse effect of cisplatin and a new drug should reduce this effect, the cell line selected was the normal epithelial renal LLC-PK1, the use of which showed to be a very effective method for the *in vitro* elucidation of the nephrotoxic mechanism caused by cisplatin [60]. Compounds showing some of the best values of activity against the A2780*cis* cell line were tested against this normal renal cell line and for comparison, cisplatin was evaluated under the same experimental conditions.

Table 2 shows the values of the IC_{50} parameter against this cell line. Here the pspa derivative shows a value which is not statistically significantly different to that of cisplatin whereas the other compounds are slightly more active. However, the higher activity of the gold complexes against the A2780 and A2780*cis* cell lines compared to cisplatin led to better values of the SI parameter (SI = selectivity index = IC_{50} for normal cells/IC_{50} for cancer cells). The values are different for both cell lines and it should be underlined that in the case of the A2780*cis* cell line, all the complexes show values (38.4, 7.0, 6.6 and 10.5 for the H_2pspa, H_2tspa, H_2-*o*-hpspa and H_2-*p*-hpspa, respectively) significantly better than the value found for cisplatin (1.4). These values are encouraging and qualify these compounds for further studies.

4. Conclusions

In conclusion, the screening of the cytotoxic activity of the triethylphosphine complexes against the HeLa-229, A2780 and A2780*cis* cell lines reveals that the [$(AuPEt_3)_2$(**x**spa)] compounds are highly effective, particularly against the A2780*cis* line, showing a high ability to circumvent cellular resistance to cisplatin. These compounds are the most active of a series of gold(I) complexes prepared to correlate structure and activity. The series was initiated with compounds of the type R-CH-C($SAuPPh_3$)-COOH, labelled as [$Au(PPh_3)$(H**x**spa)]; the reaction of these with diisopropylamine or triethylamine lead to compounds of the type [HQ][$Au(PPh_3)$(**x**spa)] and [HP][$Au(PPh_3)$(**x**spa)] (HQ = diisopropylammonium; HP = triethylammonium) which showed to be more active than the precursors with the COOH group protonated. The deprotonation of this group, incorporating a new $AuPPh_3$ group to give dinuclear [$(AuPPh_3)_2$(**x**spa)] compounds, produces a new increase in activity, which is again increased when these dinuclear complexes include PEt_3 instead of

PPh_3 as described in this paper. The screening of the cytotoxic activity against the normal renal LLC-PK1 cell line led to promising values of the selectivity index in the case of the A2780*cis* cell line, resistant to cisplatin.

Structural changes like deprotonation, metalation or changing the phosphine ligand, are useful to increase the activity of the compounds, reaching the maximum value for the $[(AuPEt_3)_2$**x**spa] species. From this accumulated experience, these dinuclear complexes seem to be the most promising compounds. The closeness of the IC_{50} values makes it difficult to identify one specific R-substituent on the sulfanylcarboxylate fragment responsible for the activity in this type of compounds. Additional studies including other cell lines can perhaps reveal new discriminatory aspects by which some specific compounds can be selected for further studies and thus increase the knowledge of the basis of its biological effects.

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Table 1

In vitro cytotoxicity against the A2780 and A2780*cis* cell line expressed as IC_{50} (μM) for the complexes [Au(PEt_3)(H**x**spa)] (**1**-**11**) and [($AuPEt_3$)$_2$(**x**spa)]. The resistance factor, RF, is expressed as IC_{50}(A2780cis)/IC_{50}(A2780).

Ligand (H_2**x**spa)	[Au(PEt_3)(H**x**spa)](**1**-**11**)			[($AuPEt_3$)$_2$(**x**spa)]		
	A2780	A2780cis	RF	A2780	A2780cis	RF
H_2pspa	2.3 (0.4)	1.3 (0.1)	0.56	0.19 (0.02)*	0.15 (0.03)*	0.79
H_2fspa	0.90 (0.07)	0.82 (0.04)	0.91	0.17 (0.02)*	0.36 (0.02)*	2.12
H_2tspa	1.3 (0.1)	3.06 (0.11)	2.35	0.064 (0.003)*	0.31 (0.02)*	4.84
H_2-*o*-pyspa	13(3)	6 (1)	0.46	11 (2)	-	-
H_2Clpspa	3.2 (0.5)	2.1 (0.2)	0.65	0.23 (0.02)*	0.44 (0.04)*	1.91
H_2-*o*-mpspa	4.6 (0.2)	1.2 (0.1)	0.26	0.20 (0.03)*	0.22 (0.02)*	1.10
H_2-*p*-mpspa	-	-	-	0.12 (0.02)	0.11 (0.02)	0.92
H_2-*o*-hpspa	6.5 (0.4)	9.7 (0.3)	1.49	0.34 (0.02)*	0.47 (0.05)*	1.38
H_2-*p*-hpspa	12 (4)	8.1 (0.3)	0.67	0.30 (0.02)*	0.38 (0.04)*	1.27
H_2-diBr-*o*-hpspa	13(2)	8.4 (0.5)	0.64	0.69 (0.05)*	3.58 (0.04)*	5.19
H_2cpa	17(4)	12 (2)	0.70	0.23 (0.02)*	0.75 (0.04)*	3.26
Cisplatin	0.44 (0.06)	3.6 (0.5)	8.18			

* Significant with respect to the 1:1 complexes ($P<0.05$)

Table 2

In vitro cytotoxicity against the LLC-PK1 cell line expressed as IC_{50} (μM) for [$(AuPEt_3)_2$ (**x**spa)] complexes.

Ligand (H_2**x**pspa)	[$(AuPEt_3)_2$(**x**spa)]
H_2pspa	5.76 (0.08)
H_2tspa	2.17 (0.03)
H_2-*o*-hpspa	3.11 (0.03)
H_2-*p*-hpspa	3.98 (0.08)
Cisplatin	5.13(0.39)

CAPTION TO THE FIGURES

Fig. 1. A view of the structure of the $Au(PEt_3)$(H-*p*-hpspa) (**9**) unit present in the crystal of $[Au(PEt_3)$(H-*p*-hpspa)]·$OPEt_3$, (**9**)·$OPEt_3$.

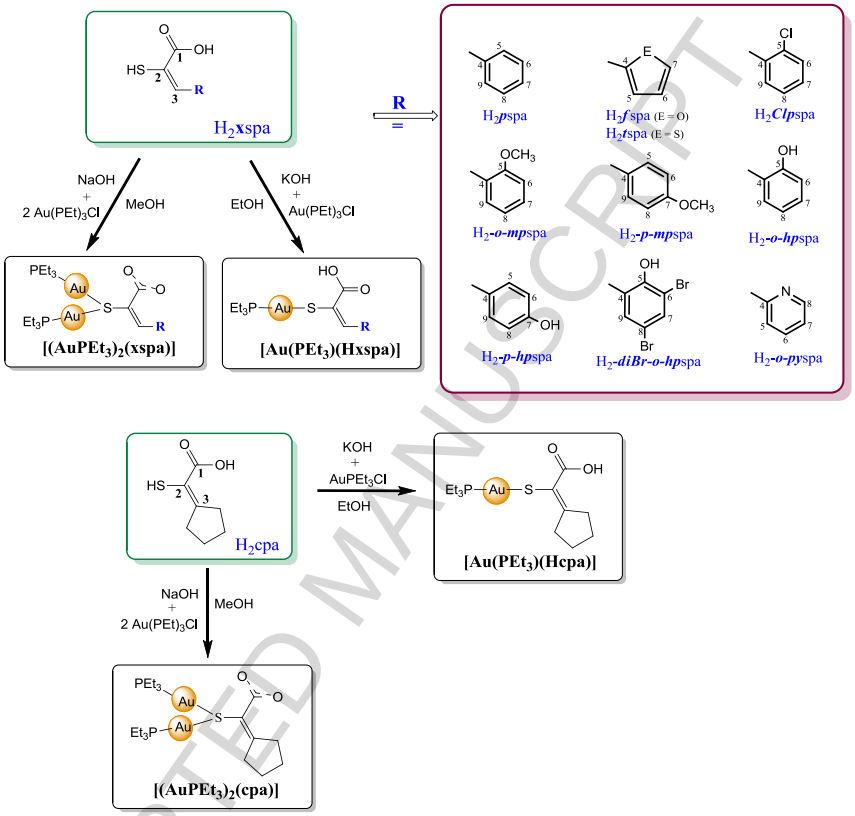
Fig. 2. The *in vitro* cytotoxicity of complexes $[(AuPPh_3)_2$(**x**spa)] and $[(AuPEt_3)_2$(**x**spa)] against the HeLa-229 cell line.

Fig. 3. The *in vitro* cytotoxicity of complexes $[Au(PPh_3)$(H**x**spa)] and $[Au(PEt_3)$(H**x**spa)] (**1-11**) against the A2780 cell line.

Fig. 4. The *in vitro* cytotoxicity of complexes $[(AuPPh_3)_2$(**x**spa)] and $[(AuPEt_3)_2$(**x**spa)] against the A2780 cell line.

Fig. 5. The *in vitro* cytotoxicity of complexes $[Au(PPh_3)$(H**x**spa)] and $[Au(PEt_3)$(H**x**spa)] (**1-11**) against the A2780*cis* cell line.

Fig. 6. The *in vitro* cytotoxicity of complexes $[(AuPPh_3)_2$(**x**spa)] and $[(AuPEt_3)_2$(**x**spa)] against the A2780*cis* cell line.



Scheme 1

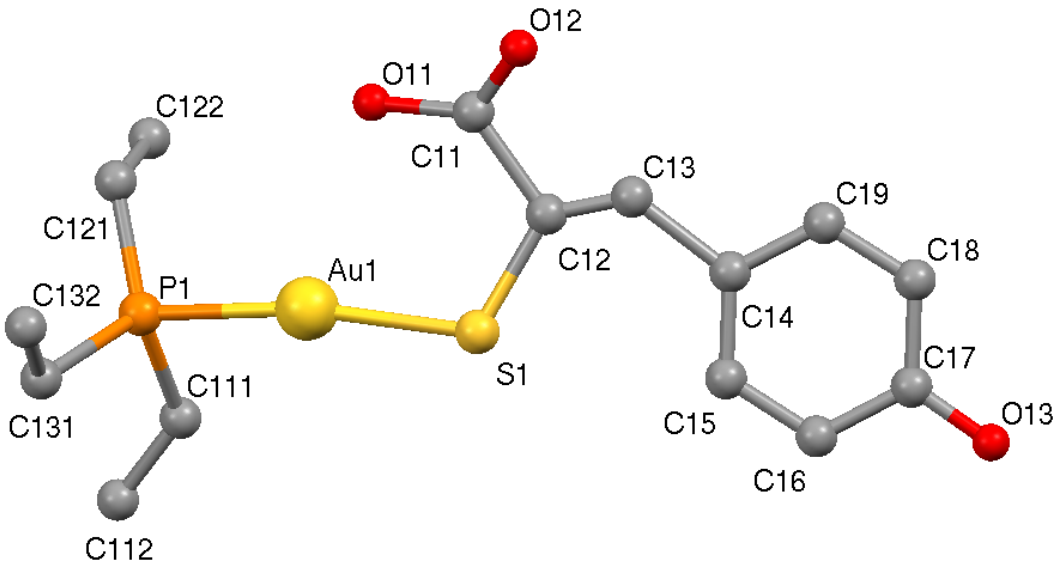


Fig. 1

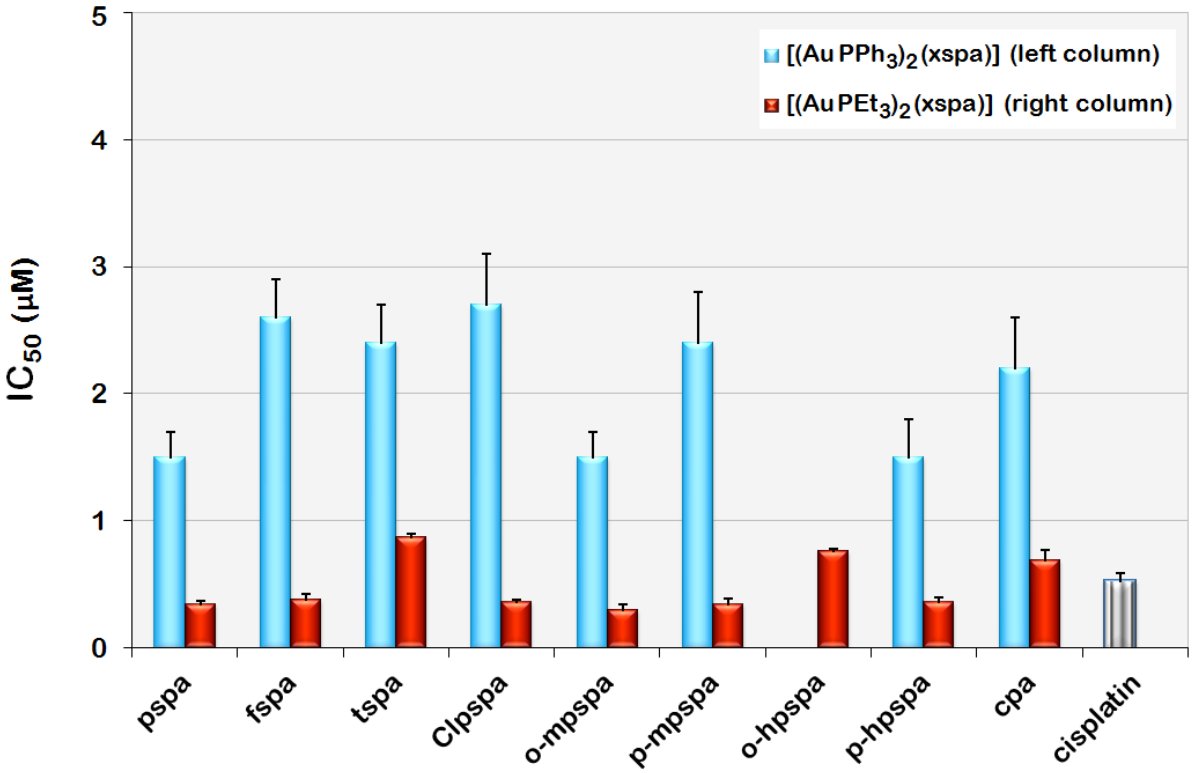


Fig. 2

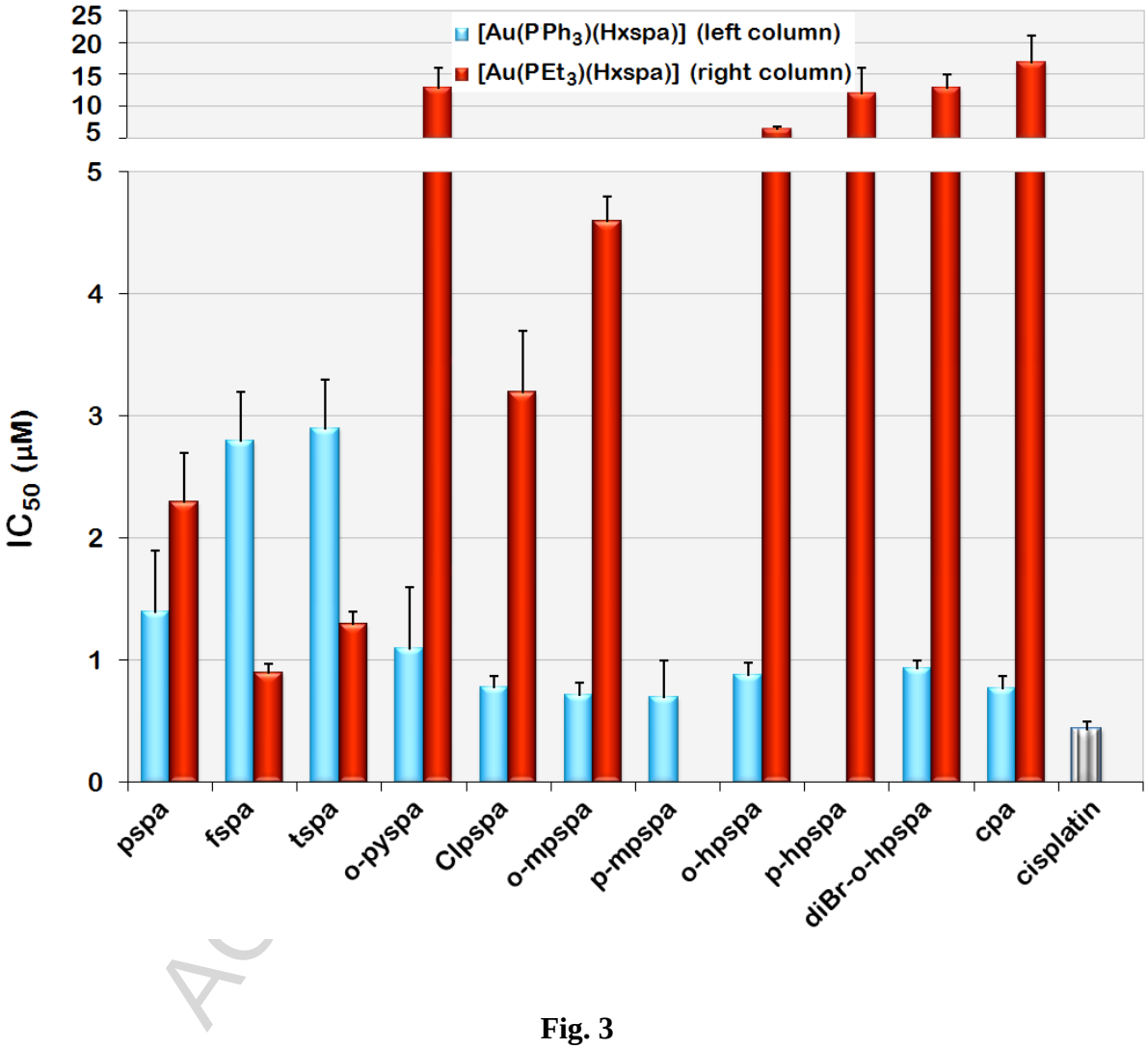


Fig. 3

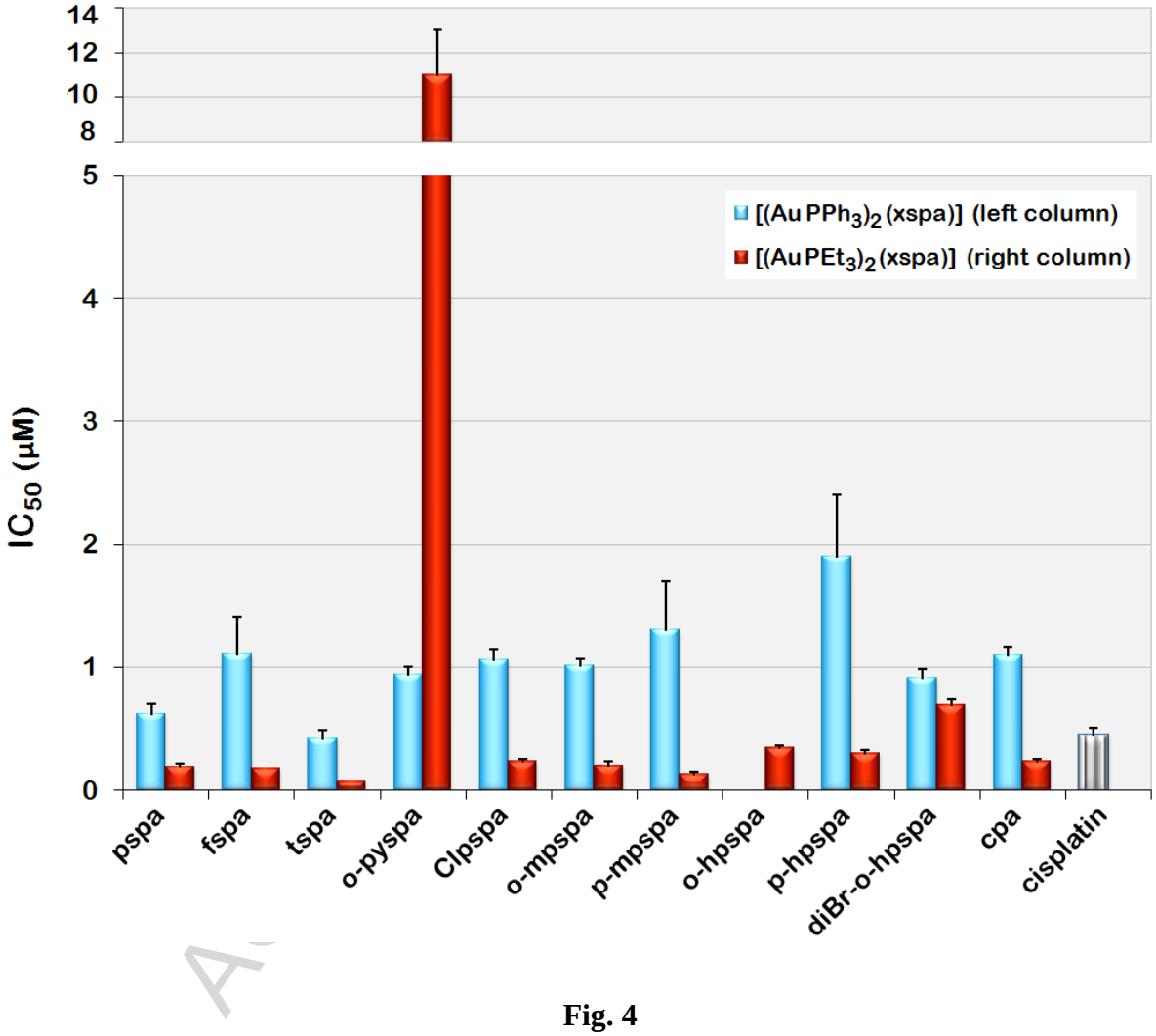


Fig. 4

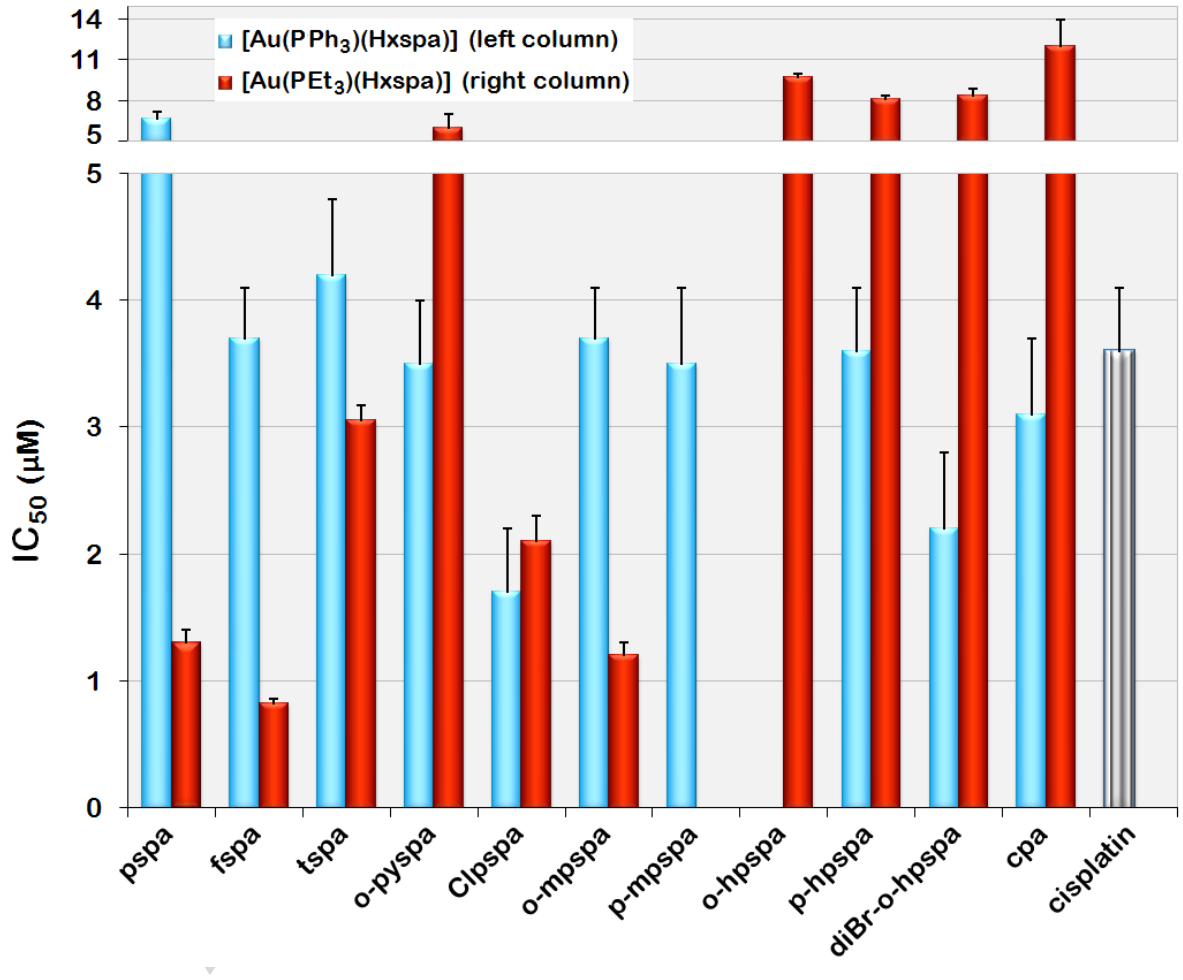


Fig. 5

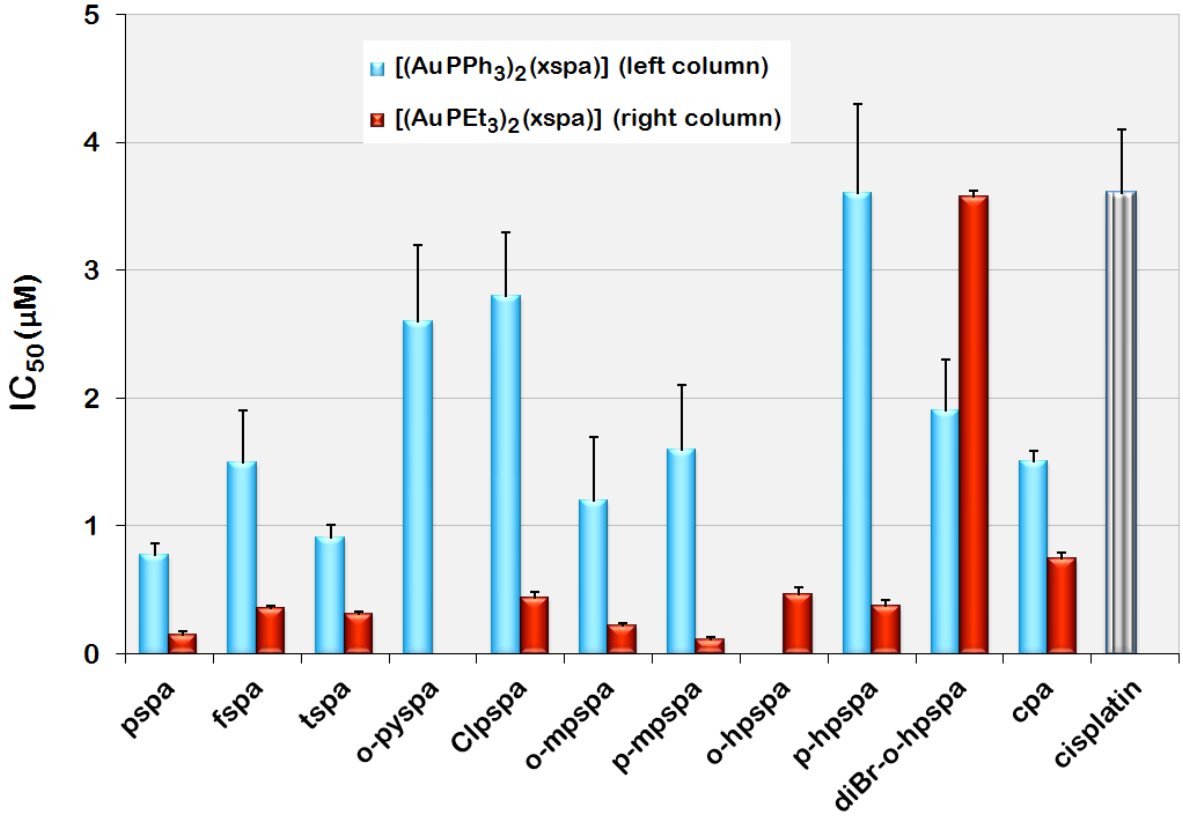
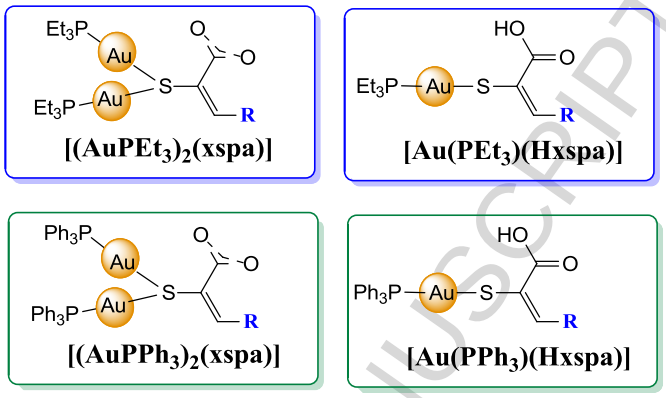


Fig. 6

Graphical Abstract



H_2xspa = 3-(aryl)-2-sulfanylpropenoic acids

Synopsis Graphical Abstract

Mono and dinuclear phosphinegold(I) sulfanylcarboxylates: influence of nuclearity and substitution of PPh_3 for PEt_3 on cytotoxicity.

Highlights

►Synthesis of new gold(I) complexes using sulfanylcarboxylates and triethylphosphine as ligands.

►Cytotoxic activity against cancer and normal cell lines.

►Influence of the substitution of triphenylphosphine by triethylphosphine on cytotoxicity.

►Influence of the incorporation of a second gold atom on cytotoxicity.