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Synthesis, structure and cytotoxicity studies of diisopropylammonium and triethylammonium salts of triphenylphosphinegold(I) sulfanylcarboxylates

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ABSTRACT

Compounds of the type [HQ][Au(PPh_3)(***x***spa)] and [HP][Au(PPh_3)(***x***spa)] {HQ = diisopropylammonium; HP = triethylammonium; H_2***x***spa = 3-*aryl*-2-sulfanylpropenoic acids [***x***: *p* = 3-phenyl-, *f* = 3-(2-furyl)-, *t* = 3-(2-thienyl)-, *-o-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-hydroxyphenyl]} were synthesized and characterized by IR and NMR (^{1}H, ^{13}C and ^{31}P) spectroscopy and by FAB mass spectrometry. The structures of [HQ][Au(PPh_3)(Clpspa)] and [HQ][Au(PPh_3)(*-o*-mpspa)] show that the crystal contains hydrogen-bonded diisopropylammonium cations and [Au(PPh_3)(***x***spa)]$^-$ anions. The anions in the two compounds have different structures, with the carboxylate group either coordinated or not coordinated to the gold atom, respectively. The *in vitro* antitumour activities against the HeLa-229, A2780 and A2780cis cell lines were determined for all complexes. The diisopropylammonium derivatives were generally found to be more active, in particular against the A2780cis cell line, and showed a high ability to circumvent the cellular resistance to cisplatin.

1. Introduction

Cisplatin is an effective antitumour agent that is widely used in the treatment of testicular, ovarian, bladder and a variety of other solid tumours. The clinical use of this drug, however, is limited by side effects such as neuro-, hepato- and nephrotoxicity. Besides, many tumours are intrinsically resistant to the drug and even those that are initially sensitive can develop an acquired resistance during treatment [1].

Whilst the mechanisms by which tumour cells become resistant to the drug are progressively being deciphered [2], new compounds of platinum and other metals are being prepared and biologically tested [3–5].

Among these materials, Au(I) coordination compounds exhibit cytotoxic properties toward several tumour lines and they are also effective against cells that are resistant to cisplatin [6,7]. A recent study [8] has shown that auranofin, triethylphosphine(2,3,4,6-tetra-O-acetyl-β-1-D-thiopyranosato-S)gold(I), a well known antiarthritic drug, induces apoptosis in cisplatin-resistant human ovarian cancer cells. The drug, which acts as a potent inhibitor of the homodimeric selenoprotein thioredoxin reductase, produces an alteration of the redox state of the cell and also creates the necessary conditions for augmented apoptosis; this phenomenon may be due to an increase in the permeability of the mitochondrial membranes, which in turn leads to a large release of proapoptotic factors.

The inhibition of this enzyme by auranofin has previously been reported *in vivo* in mice [9] and has also recently been reported for other gold(I) compounds [10–13]. These findings highlight new targets to increase the cytotoxic activity and to prevent the development of drug resistance [14]. At the same time, such studies reveal new applications for old drugs [15] and also give rise to renewed efforts to analyse the connection between mitochondria and apoptosis [16,17] and reinforce interest in gold compounds.

In a previous study [18] we aimed to obtain a small library in which the structural modification could be correlated with the antitumoural activity *in vitro*. In this respect we selected a number of sulfanylcarboxylic acids [R–CH=C(SH)–COOH, H_2***x***spa] and prepared R–CH=C(SAuPPh_3)–COOH, [Au(PPh_3)(H***x***spa)], complexes (hereafter referred to as H-complexes) [***x:*** *p* = 3-phenyl-, *f* = 3-(2-furyl)-, *t* = 3-(2-thienyl)-, *-o-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-hydroxyphenyl]. We also tested the antitumoural activity of these compounds against human cervix carcinoma and human ovarian carcinoma cell lines and found that the activity depends on the nature of the R substituent.

Bearing in mind that salt formation is a well-known technique to modify and optimize the properties and the activity of active pharmaceutical ingredients [19,20], we decided to deprotonate

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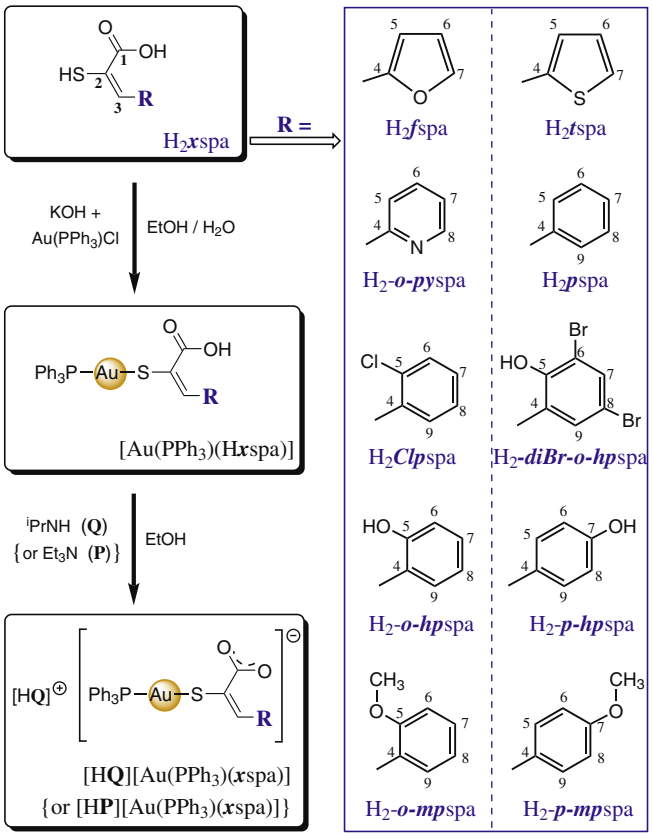
the COOH group of these previously prepared compounds and selected diisopropylamine [Q] and triethylamine [P] as bases to combine with the complexes (see Scheme 1). In this way, compounds of the type [HQ][Au(PPh$_3$)(***x***spa)] (abbreviated as HQ-complexes) and [HP][Au(PPh$_3$)(***x***spa)] (abbreviated as HP-complexes) were obtained and their cytotoxic activity was tested against the human cervical carcinoma cell line HeLa-229 and the human ovarian carcinoma cell lines A2780 and its cisplatin-resistant mutant A2780cis.

Two of the new complexes [HQ][Au(PPh$_3$)(Clpspa)] (**5**) and [Au(PPh$_3$)(-*o*-mpspa)] (**6**) were isolated as single crystals. The structures of these complexes were elucidated by X-ray crystallography and clear differences were observed. Whereas hydrogen bonding is present along with Au–S and Au–P bonds in **5**, the structure of **6** also shows the presence of a weak Au···O interaction. The complexes [HQ][Au(PPh$_3$)(pspa)] (**1**), [HQ][Au(PPh$_3$)(fspa)] (**2**) and [HQ][Au(PPh$_3$)(tspa)] (**3**) were reported previously [21].

2. Experimental

2.1. Materials and methods

Benzaldehyde (from Probus), 2-furancarboxaldehyde, 2-thiophenecarboxaldehyde, 2-pyridinecarboxaldehyde, 2-chlorobenzaldehyde, 2-methoxybenzaldehyde, 4-methoxybenzaldehyde, 2-hydroxybenzaldehyde, 4-hydroxybenzaldehyde, 3,5-dibromo-2-hydroxybenzaldehyde, rhodanine, triphenylphosphinegold(I) chloride, diisopropylamine and triethylamine (all from Aldrich) were all used as supplied. The 3-aryl-2-sulfanylpropenoic acids, H$_2$***x***spa, were prepared by condensation of the appropriate aldehyde with rhodanine, subsequent alkaline hydrolysis of the resulting 5-substituted rhodanine, and acidification with aqueous HCl [18,22–25].



Scheme 1.

Complexes of the type [Au(PPh$_3$)(H***x***spa)] were prepared by adding Au(PPh$_3$)Cl to a solution of the appropriate sulfanylcarboxylic acid and KOH in a 1:1:1 molar ratio in ethanol/water (4:1, v/v) [18].

Elemental analyses were performed with a Fisons 1108 microanalyser. Melting points were determined with a Büchi apparatus and are uncorrected. Mass spectra (MS) were recorded on a Kratos MS50TC spectrometer connected to a DS90 system and operating in FAB mode (*m*-nitrobenzyl alcohol, Xe, 8 eV; *ca.* 1.28×10^{-15} J); ions were identified by DS90 software and the data characterizing the metallated peaks were calculated using the isotope ^{197}Au. IR spectra (KBr pellets or Nujol mulls) were recorded on a Bruker IFS66 V FT-IR spectrophotometer 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 were recorded in dmso-d$_6$ and/or CDCl$_3$ at room temperature on a Bruker AMX 300 spectrometer operating at 300.14 and 75.40 MHz, respectively, using 5 mm o.d. tubes; chemical shifts are reported relative to TMS using the solvent signals (δ ^{1}H = 2.50 ppm; δ ^{13}C = 39.5 ppm for dmso-d$_6$ and δ ^{1}H = 7.26 ppm; δ ^{13}C = 77.0 ppm in CDCl$_3$) as reference. The splitting of proton resonances in the reported ^{1}H NMR spectra are defined as: s = singlet, d = doublet, t = triplet, m = multiplet, *pst* = pseudotriplet. ^{31}P NMR spectra were recorded in CDCl$_3$ or dmso-d$_6$ at 202.46 MHz on a Bruker AMX 500 spectrometer using 5 mm o.d. tubes and are reported relative to external neat H$_3$PO$_4$ (85%). All the physical measurements were carried out by the RIAIDT services of the University of Santiago de Compostela (USC).

2.2. Synthesis

The complexes were prepared by reacting the appropriate [Au(PPh$_3$)(H***x***spa)] with diisopropylamine or triethylamine in ethanol. After stirring at room temperature for 24 h the solvent was evaporated and the resulting solid was washed with water and vacuum dried. The complexes [HQ][Au(PPh$_3$)(pspa)] (**1**), [HQ][Au(PPh$_3$)(fspa)] (**2**) and [HQ][Au(PPh$_3$)(tspa)] (**3**) were prepared previously [21].

2.2.1. [HQ][Au(PPh$_3$)(-*o*-pyspa)] (***4***)

[Au(PPh$_3$)(H-*o*-pyspa)] (210 mg, 0.33 mmol), diisopropylamine (46 μL), ethanol (30 mL); 45% yield. Orange solid. M.p.: 70 °C. Anal.: found, C 51.6, H 4.8, S 4.1, N 3.7%. Calc. for C$_{32}$H$_{36}$N$_2$O$_2$PSAu, C 51.9, H 4.9, N 3.8, S 4.3%. MS (FAB): the main peaks for metallated fragments are at *m*/*z* 1409 (14%), [(AuPPh$_3$)$_3$S]$^+$; 1098 (4), [(AuPPh$_3$)$_2$-*o*-pyspa]$^+$; 721 (4), [Au(PPh$_3$)$_2$]$^+$; 640 (32), [(AuPPh$_3$)(H-*o*-pyspa)]$^+$ and 459 (34), [(AuPPh$_3$)]$^+$. IR (cm^{-1}): 1613 s, δ(NH$_2^+$); 1557 vs, ν_{as}(CO$_2^-$); 1341 s, ν_{sym}(CO$_2^-$); 1480 s, 1435 vs, ν(PPh$_3$). NMR (dmso-d$_6$) (ppm): ^{1}H, δ 6.40 (s, 1H, C(3)H), 8.48 (d, 1H, C(5)H), 7.70 (*pst*, 1H, C(6)H), 7.06 (t, 1H, C(7)H), 8.72 (d, 1H, C(8)H), 7.46–7.63 (m, 15H, H(PPh$_3$)), 1.14 (d, 12H, [HQ]CH$_3$), 3.19 (m, 2H, [HQ]CH); ^{13}C, δ 172.6 C(1), 135.5 C(2), 135.2 C(3), 157.2 C(4), 148.8 C(5), 130.0 C(6), 120.3 C(7), 123.6 C(8), 45.6 CH[HQ], 19.0 CH$_3$[HQ], 133.8 (d, C_o(Ph$_3$), J = 15.2), 129.3 (d, C_m(Ph$_3$), J = 10.7), 131.7 C_p(Ph$_3$); ^{31}P {^{1}H}, δ 40.5 (s).

2.2.2. [HQ][Au(PPh$_3$)(Clpspa)] (***5***)

[Au(PPh$_3$)(HClpspa)] (85 mg, 0.13 mmol), diisopropylamine (18 μL) in ethanol (15 mL); 65% yield. Yellow solid. M.p.: 92 °C. Anal.: found, C 51.0, H 4.3, S 4.0, N 1.6%. Calc. for C$_{33}$H$_{36}$NO$_2$PSClAu, C 51.2, H 4.7, S 4.1, N 1.8%. MS (FAB): the main peaks for metallated fragments are at *m*/*z* 1589 (6%), [(AuPPh$_3$)$_3$Clpspa]$^+$; 1409 (39), [(AuPPh$_3$)$_3$S]$^+$; 1131 (87), [(AuPPh$_3$)$_2$Clpspa]$^+$; 774 (2), [M]$^+$; 459 (100), [Au(PPh$_3$)]$^+$. IR (cm^{-1}): 1614 m, δ(NH$_2^+$); 1569 vs, ν_{as}(CO$_2^-$); 1332 s, ν_{sym}(CO$_2^-$); 1480 s, 1436 vs, ν(PPh$_3$). NMR (CDCl$_3$) (ppm): ^{1}H, δ 7.77 (s, 1H, C(3)H), 7.12 (d, 1H, C(6)H), 7.15 (*pst*, 1H, C(7)H), 6.88 (m, 1H, C(8)H), 8.44 (d, 1H, C(9)H), 7.38–7.50 (m,