

CYTOTOXICITY OF TRIORGANOPHOSPHINEGOLD(I) n-MERCAPTOBENZOATES, n = 2, 3 AND 4

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Abstract

The results of cytotoxicity trials against a panel of seven human cell lines for a series of triorganophosphinegold(I) 3- and 4-mercaptobenzoates are reported. While the new compounds show moderate to high toxicity, their potencies are inferior to those reported previously for their isomeric 2-mercaptobenzoate derivatives. The results therefore suggest a structure-activity relationship in that the 2-isomeric species are more active, particularly against the non-small cell lung cancer and renal cancer cell lines, results that may indicate some selectivity in their cytotoxic profile.

Introduction

Gold complexes are well known to possess significant anti-arthritic activity and several gold thiolates are currently used clinically in the treatment of that disease [1-3]. In addition to this biological profile, several gold(I), as well as gold(III) species, have proven to display cytotoxicity and anti-tumour activity as summarised in a recent survey [4]. In this context, one of the active classes of compounds features a linear P-Au-S entity as found in the anti-arthritic drug, auranofin (triethylphosphinegold(I) tetraacetatothioglucose). Our work in this area has focussed on making derivatives of auranofin, some of which display significant cytotoxicity and anti-tumour activity [5-8]. A difficulty encountered with these compounds has been their limited aqueous solubility. In order to overcome this limitation, thiols containing water-solubilising groups have been coordinated to the phosphinegold(I) entity. Amongst the thiols chosen for investigation was 2-mercaptobenzoic acid. These derivatives displayed moderate to high cytotoxicity profiles compared to standards such as cisplatin, doxorubicin, methotrexate, *etc.* [9]. In particular, significant cytotoxicity was found against the non-small cell lung cancer and renal cancer cell lines. As a continuation of work in this area, some 3- and 4-mercaptobenzoates have been prepared and subjected to screening for their cytotoxicity profiles. The results of this study are reported herein.

Results and Discussion

The triorganophosphinegold(I) n-mercaptobenzoates, R₃PAu(n-mbaH), have been synthesised using established procedures involving the metathesis of the triorganophosphinegold(I) chloride precursor with the appropriate n-mercaptobenzoic acid in the presence of base. Characterisation data are collected in the Experimental section. A number of the n-mercaptobenzoates compounds have been characterised crystallographically, by us [9 - 11] (*i.e.* n = 2 for R = Ph and Cy; n = 3 for R = Cy and Ph) and others [12] (*i.e.* n = 4 for R = Et).

The crystal structure of Cy₃PAu(4-mbaH) has been determined in the present study. A centrosymmetrically related pair of molecules is illustrated in Figure 1; selected geometric parameters are collected in the Figure caption. The gold atom exists in the expected linear geometry defined by the sulfur and phosphorus atoms so that the angle subtended at the gold atom is 175.06(4)°. The 4-mbaH ligand is essentially planar as seen in the magnitude of the O1/C1'/C1/C2 and O2/C1'/C1/C2 torsion angles of -174.3(4) and 5.0(7)°, respectively. Molecules associated via the familiar carboxylic acid dimer motif with the key parameters defining this association listed in the caption to Figure 1. The structure is in essential agreement with the Et₃PAu(4-mbaH) and o-tol₃PAu(4-mbaH) structures reported recently by Schmidbaur *et al.* [12].

A previous report [9] summarising the cytotoxicity of a series of triorganophosphine 2-mercaptobenzoates showed high levels of activity, in particular against the non-small cell lung cancer (H226) and renal cancer (A498) cell lines. These results are reproduced in Table 1. Subsequently, biological trials for some 3- and 4-mercaptobenzoate derivatives of R₃PAu, for R = Et, Cy & Ph, were also conducted.

As seen from Table 1, a general structure-activity relationship can be established in that the 2-mercaptobenzoate compounds are generally more active than their 3- and 4- isomeric counterparts. The most cytotoxic compound in the series investigated was the Et₃PAu(2-mbaH) compound which displayed a ID₅₀ value of 43 ng/ml against the IGROV cell line but, doxorubicin and methotrexate were more active. High activity against the H226 and A498 cell lines was only observed for the 2-mbaH series perhaps

indicating some selectivity in activity. It would be of some interest to ascertain whether this activity is maintained *in vivo* leading to anti-tumour activity.

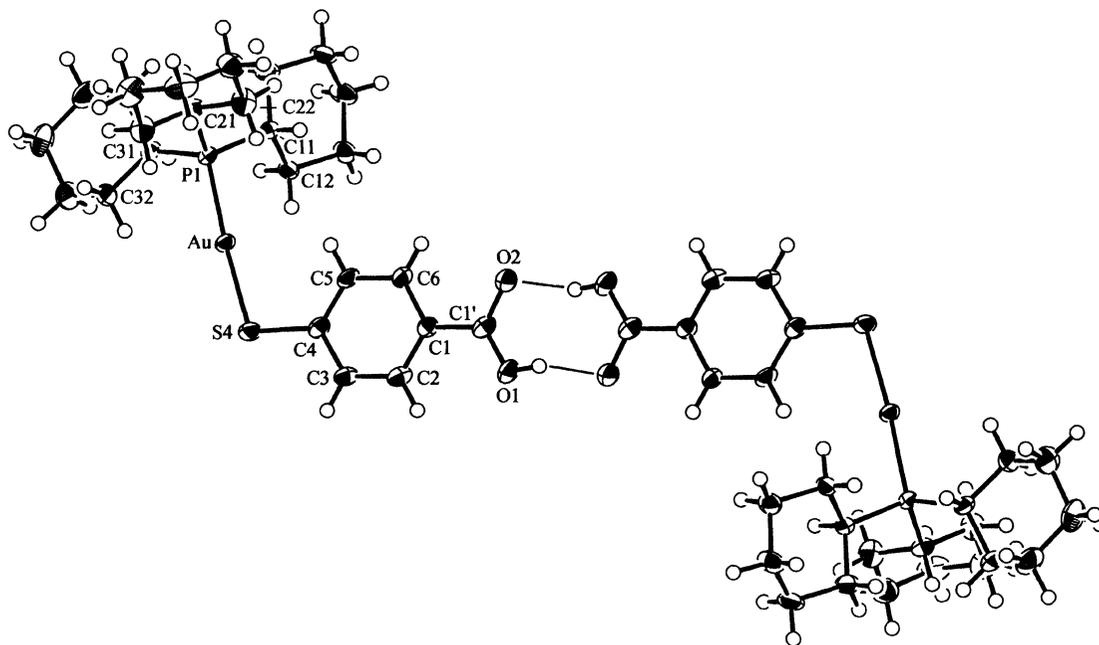


Figure 1. The centrosymmetric hydrogen-bonded dimer of $\text{Cy}_3\text{PAu}(4\text{-mbaH})$ (50% probability level). Selected bond distances and angles: Au-S4 2.308(2), Au-P1 2.273(1), S4-C4 1.759(5), C1'-O1 1.280(5), C1'-O2 1.250(5) Å; S4-Au-P1 175.06(4), Au-S4-C4 105.53(15)°. Parameters associated with the hydrogen bonding interaction are O1-H...O2ⁱ 1.81 Å, O1...O2ⁱ 2.628(5) Å and an angle subtended at H of 166°; symmetry operation -1-x, 1-y, 1-z.

Table 1. *In vitro* ID₅₀ values (ng/ml) for $\text{R}_3\text{PAu}(n\text{-mbaH})$, $n = 2, 3 \text{ \& } 4$ and R = Et, Cy & Ph, using the microculture sulforhodamine B (SRB) test as cell viability, and for the reference compounds: doxorubicin (DOX), cisplatin (CPT), 5-fluorouracil (5-FU), methotrexate (MTX) and etoposide (ETO)

R	n	Cell Line						
		MCF7	EVSA-T	WIDR	IGROV	M19MEL	A498	H226
Et	2 [9]	405	110	984	43	118	65	69
Cy	2 [9]	411	225	772	182	292	95	97
Ph	2 [9]	791	356	982	117	298	151	173
Cy	3	806	1046	949	283	403	697	848
Ph	3	1065	1287	957	280	797	1274	960
Et	4	1145	1197	1238	67	375	484	864
Cy	4	973	1353	976	338	592	743	923
Ph	4	858	940	553	148	322	348	586
standards								
DOX		8	11	10	16	60	90	199
CPT		422	967	699	558	169	2253	3269
5-FU		475	225	750	442	297	143	340
MTX		5	<3	18	23	7	37	2287
ETO		317	150	2594	505	580	1314	3934

Experimental

Preparations

The $R_3PAu(n\text{-mbaH})$ compounds were prepared by literature procedures established for the triorganophosphine 2-mercaptobenzoates [9]. Characterisation details for the $n = 3$, $R = \text{Cy}$ and Ph compounds are available in the literature [10]. During the course of the present study, the preparation and characterisation of the $n = 4$, $R = \text{Et}$ and Ph derivatives were reported [12]. Full details for the $n = 4$, $R = \text{Cy}$ compound are presented below as well as previously unreported spectroscopic details for the $n = 4$, $R = \text{Et}$ and Ph compounds.

$Cy_3PAu(4\text{-mbaH})$: Yield 71% of colourless crystals that decomposed above 250 °C. Crystals for the X-ray study were grown by the slow evaporation of a $CH_2Cl_2/EtOH$ (1/1) solution of the compound. C, 47.5; H, 6.1. $C_{25}H_{38}AuO_2PS$ requires C, 47.6; H, 6.1%. IR (KBr disk): $\nu(C=O)$ 1674(s) and $\nu(O-H)$ 2500 - 3200 (br) cm^{-1} . 1H NMR (d_6 -DMSO): δ 7.58 (d, 8.4 Hz, H2 of 4-mbaH); 7.42 (d, 8.4 Hz, H3 of 4-mbaH); 12.5 (br, CO_2H). $^{13}C\{^1H\}$ NMR (d_6 -DMSO): δ 124.9, 128.8, 131.0, 151.8, 167.2 (4-mbaH: C1, C2, C3, C4, CO_2); 32.4 (d, 28.3 Hz, $C\alpha$); 26.3 (d, 12.1 Hz, $C\beta$); 30.3 (Cy), 25.5 ppm ($C\delta$). ^{31}P NMR (d_6 -DMSO): δ 59.2 ppm.

$Et_3PAu(4\text{-mbaH})$: IR (KBr disk): $\nu(C=O)$ 1688(s) and $\nu(O-H)$ 2500 - 3200 (br) cm^{-1} . $^{13}C\{^1H\}$ NMR (d_6 -DMSO): δ 124.4, 128.8, 131.1, 151.9, 167.2 (4-mbaH: C1, C2, C3, C4, CO_2); 17.0 (d, 33.5 Hz, $C\alpha$); 9.0 ($C\beta$). ^{31}P NMR (d_6 -DMSO): δ 39.9 ppm.

$Ph_3PAu(4\text{-mbaH})$: IR (KBr disk): $\nu(C=O)$ 1669 (s) and $\nu(O-H)$ 2500 - 3200 (br) cm^{-1} . $^{13}C\{^1H\}$ NMR (d_6 -DMSO): δ 125.1, 129.1, 131.2, 150.8, 167.2 (4-mbaH: C1, C2, C3, C4, CO_2); 128.7 (d, 57.0 Hz, $C\alpha$); 133.7 (d, 13.7 Hz, $C\beta$); 129.6 (d, 11.5 Hz, Cy), 132.0 ppm (d, 2.3 Hz, $C\delta$). ^{31}P NMR (d_6 -DMSO): δ 38.7 ppm.

Biological screening

The following human tumour cell lines were used: MCF7 (breast cancer), EVSA-T (breast cancer), WIDR (colon cancer), IGROV (ovarian cancer), M19MEL (melanoma), A498 (renal cancer), and H226 (non-small cell lung cancer). Cell lines WIDR, M19 MEL, A498, IGROV and H226 belong to the currently used anti-cancer screening panel of the National Cancer Institute, USA [13]. The MCF7 cell line is estrogen receptor (ER)+/ progesterone receptor (PgR)+ and the cell line EVSA-T is (ER)-/(PgR)-.

Prior to the experiments, a mycoplasma test was carried out on all cell lines and found to be negative. All cell lines were maintained in a continuous logarithmic culture in RPMI 1640 medium with Hepes and phenol red. The medium was supplemented with 10% FCS, penicillin 100 IU/ml and streptomycin 100 $\mu g/ml$. The cells were mildly trypsinised for passage and for use in the experiments.

Chemicals

RPMI and FCS were obtained from Life Technologies (Paisley, Scotland). SRB, DMSO, penicillin and streptomycin were obtained from Sigma (St. Louis MO, USA), TCA and acetic acid from Baker BV (Deventer, NL) and PBS from NPBI BV (Emmer-Compascuum, NL).

Experimental Procedures

The test and reference compounds were dissolved to a concentration of 250000 ng/ml in full medium, by 20 fold dilution of a stock solution which contained 1 mg compound/200 μg after having been dissolved first in in DMSO solution. Cytotoxicity was estimated by the microculture sulforhodamine B (SRB) test [14].

Table 2. Crystal data for $Cy_3PAu(4\text{-mbaH})$

Empirical formula	$C_{25}H_{38}AuO_2PS$	Formula weight	630.57
Diffractometer	Rigaku AFC7R	Space group	monoclinic, $P2_1/c$
Unit cell dimensions	a 12.257(6) Å b 16.559(9) Å β 98.69(3) ° c 12.621(4) Å	Volume, Å ³	2532(2)
μ (Mo- $K\alpha$), cm^{-1}	59.94	Z	4
θ range, °	3.3 - 27.5	D_{calc} , $g\ cm^{-3}$	1.654
Reflections collected	5805	$F(000)$	1256
No. parameters refined	272	Temperature, K	173
wR (all data)	0.060	Reflections with $I > 2\sigma(I)$	4115
Weighting scheme	$w = [\sigma^2 + (0.0287P)^2]^{-1}$ where $P = (F_o^2 + 2F_c^2)/3$	R [$I > 2\sigma(I)$]	0.025
Diff. hole and peak, $e\text{Å}^{-3}$	-0.76 to 1.41	Goodness-of-fit on F^2	1.01
Programs	teXsan [16], DIRDIF [17], SHELXL-97 [18], ORTEPII [19]	CCDC deposition no.	181875

The experiment was started on day 0. On day 0, 150 μl of trypsinised tumour cells (1500-2000 cells/well) were plated in 96-wells flatbottom microtiter plates (Falcon 3072, BD). The plates were preincubated for 48 h at 37 °C, 8.5% CO_2 to allow the cells to adhere. On day 2, a three-fold dilution sequence of ten steps was made in full medium, starting with the 250000 ng/ml stock solution. Every dilution was used in quadruplicate by adding 50 μg to a column of four wells. This results in a highest concentration of 62500 ng/ml present in column 12. Column 2 was used for the blank. To column 1 PBS was added to diminish interfering evaporation. On day 7, the incubation was terminated by washing the plate twice with PBS. Subsequently the cells were fixed with 10% trichloroacetic acid in PBS and placed at

4 °C for one hour. After five washings with tap water, the cells were stained for at least 15 minutes with 0.4% SRB dissolved in 1% acetic acid. After staining the cells were washed with 1% acetic acid to remove the unbound stain. The plates were air-dried and the bound stain was dissolved in 150 µl 10 mM Tris-base. The absorbance was read at 540 nm using an automated microplate reader (Labsystems Multiskan MS). Data were used for construction of concentration-response curves and determination of the ID₅₀ value by use of Deltasoft 3 software.

Crystallography

A colourless crystal with dimensions 0.16 x 0.16 x 0.38 mm was used in the X-ray diffraction study. An empirical absorption correction was applied [15]. The maximum residual electron density peak was located in the vicinity of the gold atom.

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