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Alkynyl gold(I) phosphane complexes: Evaluation of structure–activity-relationships for the phosphane ligands, effects on key signaling proteins and preliminary in-vivo studies with a nanoformulated complex

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ABSTRACT

Gold alkynyl complexes with phosphane ligands of the type (alkynyl)Au(I)(phosphane) represent a group of bioorganometallics, which has only recently been evaluated biologically in more detail. Structure–activity-relationship studies regarding the residues of the phosphane ligand ($P(Ph)_3$, $P(2\text{-furyl})_3$, $P(DAPTA)_3$, $P(PTA)_3$, $P(Et)_3$, $P(Me)_3$) of complexes with an 4-ethynylanisole alkyne ligand revealed no strong differences concerning cytotoxicity. However, a relevant preference for the heteroatom free alkyl/aryl residues concerning inhibition of the target enzyme thioredoxin reductase was evident. Complex **1** with the triphenylphosphane ligand was selected for further studies, in which clear effects on cell morphology were monitored by time-lapse microscopy. Effects on cellular signaling were determined by ELISA microarrays and showed a significant induction of the phosphorylation of ERK1 (extracellular signal related kinase 1), ERK2 and HSP27 (heat shock protein 27) in HT-29 cells. Application of **1** in-vivo in a mouse xenograft model was found to be challenging due to the low solubility of the complex and required a formulation strategy based on a peanut oil nanoemulsion.

1. Introduction

Gold based therapeutics have a long tradition in medicine lasting from ancient times over alchemy into modern ages [1–6]. While gold and its salts have been used for hundreds to thousands of years, modern research has witnessed the development of more sophisticated bioactive complexes that contain several types of coordinated ligands (e.g. thiolates [7,8], phosphanes [9–11], porphyrines [12], dithiocarbamates [13,14], N-heterocyclic carbenes [15–18] or alkynes [19–25]) or heterobimetallic species [26,27]. Currently, Auranofin (see Fig. 1) and other gold(I) species are registered drugs for the treatment of rheumatoid arthritis and strong evidence for their efficacy against different diseases such as cancer or bacterial infections exists [2,28,29]. The renewed interest in gold based metallodrugs has led to increasing efforts in understanding their biochemical mechanisms of drug action and in the rational development of improved pharmacologically active compounds [5,6].

A single mode of action for all gold complexes unlikely exists, however, strong and selective inhibition of the enzyme thioredoxin reductase (TrxR) has been demonstrated for many gold species and might be in general of high relevance for the pharmacology of a large number of gold metallodrugs. Further important biochemical characteristics observed frequently with gold compounds include the inhibition of tumor cell proliferation, the induction of apoptosis, antimitochondrial effects or the increased formation of reactive oxygen species. However, stability of the ligands coordinated to gold is a critical issue and triggers a high demand for gold complexes with stably coordinated ligands. Enhanced

Abbreviations: DFT, density functional theory; ERK, extracellular signal related kinase; FAK, focal adhesion kinase; GSK, glycogen synthase kinase; HSP, heat shock protein; MAPK, mitogen activated protein kinase; TOR, target of rapamycin; TrxR, thioredoxin reductase.

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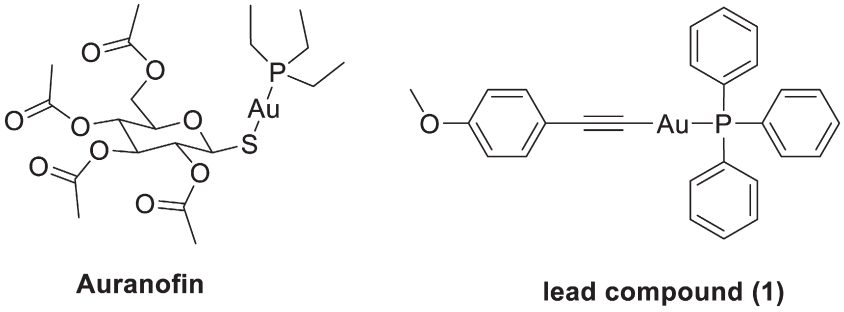


Fig. 1. The Au(I)(phosphane)thiolate complex Auranofin and the (alkynyl)Au(I)(phosphane) complex **1**.

stability might be reached by the use of carbene ligands with the formation of organometallic gold species.

In this context, we have recently reported on gold(I) complexes of the type (alkynyl)Au(I)(triphenylphosphane) that contain an anionic alkynyl group as well as a neutral phosphane ligand [19,30]. Such organometallic gold compounds promise an improved stability compared to the traditional gold drugs based on the relatively high bond dissociation energies around the gold center. Some of the studied complexes turned out to be very strong and selective inhibitors of TrxR, showed high antiproliferative activity in tumor cells, influenced key parameters of tumor cell metabolism, and triggered anti-angiogenic effects at non-toxic concentrations in zebrafish embryos [19].

Motivated by these encouraging biological properties, we selected a highly active complex of our previous report as a lead compound for further studies [19]. In the present study, the phosphane ligands were varied with the aim to establish possible structure–activity-relationships, and further biological properties were evaluated including effects on cellular signaling and in-vivo studies using a xenograft animal model.

1.1. Chemistry

Complexes **1**–**6** were prepared by reacting 4-ethynylanisole with the respective chloridogold(I)phosphane under basic conditions. The complexes were isolated and purified by filtration and washed as appropriate (Scheme 1).

Complex formation and identity was clearly confirmed by the absence of the terminal hydrogen signal of the alkyne, the presence of the M^+ signal in mass spectrometry, and singulet resonances in ^{31}P-NMR spectra. ^{13}C-NMR spectra were taken (with the exception of complex **4**), however, the very low signal intensities of the alkyne carbons did not allow a complete spectroscopic evaluation of these spectra. The high purities necessary for biological evaluation were confirmed by elemental analyses (deviations below 0.3% from the theoretical values).

Based on the biological screening described below, complex **1** was selected for further studies and in this context the synthesis procedure of **1** was stepwise improved resulting in a yield of 58%. The improved method for the synthesis of **1** is described in more detail in the Experimental section.

Density Functional Theory (DFT) at the RI-PBE-D3/def2-TZVPP COSMO level was used to calculate geometries of all complexes in vacuo and in water (Table 1). As example the calculated solution structure of **1** is shown in Fig. 2.

Table 1
Estimated bond dissociation energies (bond elongation of 5 Å) and ratios between C-Au and P-Au bonds.

Compound	C-Au	P-Au	C/P
	Kcal/mol	Kcal/mol	
1 (−Ph)	72.05	53.21	1.35
2 (−2-furyl)	74.02	48.00	1.54
3 (DAPTA)	72.71	50.38	1.44
4 (PTA)	71.03	51.97	1.37
5 (ethyl)	67.72	58.73	1.15
6 (methyl)	68.63	57.68	1.19

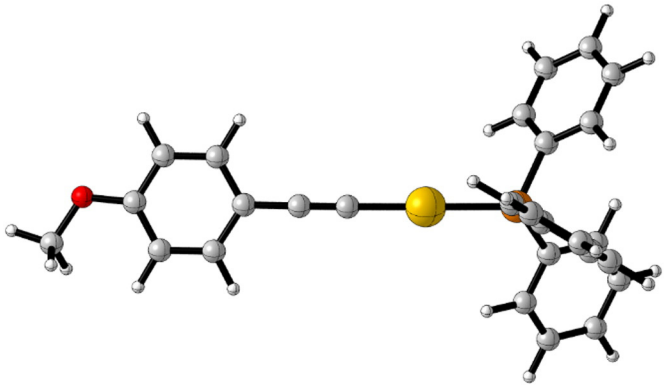
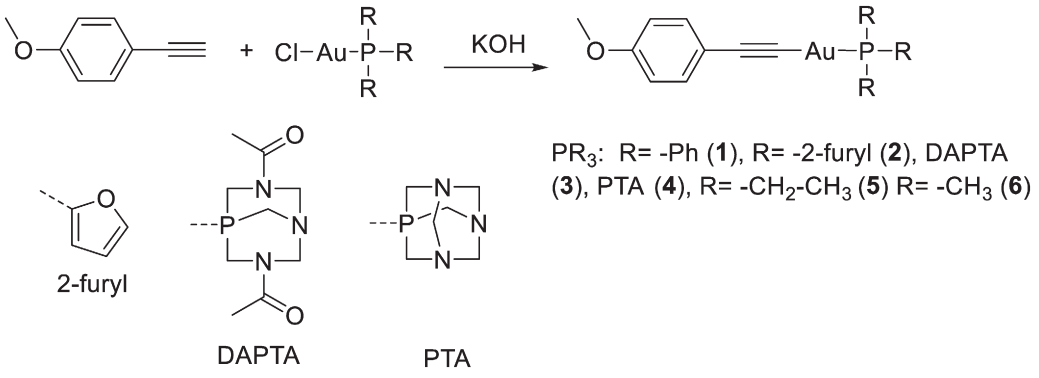


Fig. 2. Solution structure of complex 1 calculated by DFT.



Scheme 1. Synthesis of complexes **1**–**6**.

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