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New heteronuclear gold(I)–platinum(II) complexes with cytotoxic properties: Are two metals better than one?

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ABSTRACT

A series of mono- and heterodinuclear gold(I) and platinum(II) complexes with a new bipyridylamine-phosphine ligand have been synthesized and characterized. The X-ray structures of the ligand precursor 4-iodo-*N*,*N*-di(pyridin-2-yl)benzamide, and of one gold derivative are reported. All the complexes display antiproliferative properties in vitro in human cancer cells in the range of *cis*platin or higher, which appear to correlate with compounds' uptake. Interestingly, studies of the interactions of the compounds with models of DNA indicate different mechanisms of actions with respect to *cis*platin. The biological activity study of these complexes provides useful information about the interest of designing multimetallic complexes for enhanced cytotoxic properties.

1. Introduction

Platinum(II) complexes are well-established drugs in modern anticancer chemotherapy [1–3]. However, their effectiveness is still hindered by clinical problems, including acquired or intrinsic resistance, a limited spectrum of activity, and high toxicity leading to side effects. One of the strategies adopted to overcome these limitations finds its inspiration in metalloproteins [4] or even in bimetallic catalysis [5–7]. It consists in using multimetallic systems with the aim that the neighboring metals can cooperate. Interaction between metals in close proximity might indeed lead to novel modes of action and increase the overall cytotoxicity of the metallodrug [8–11]. Through the years, several polymetallic complexes were synthesized and tested and some noticeable results have been described. For example, polymetallic platinum complexes were reported to be more cytotoxic than monometallic ones, [9,12–16] and the polynuclear Pt(II) compound BBR3464 successfully entered in clinical trials, although it failed in phase II studies (Fig. 1) [17,18]. In the cytotoxic gold-based family, di- and trinuclear cyclometallated gold(III) complexes with bis- or tris(phosphine) ligands and phenyl-pyridines were shown to be potent inhibitors of cell proliferation of human tumor cells (Fig. 1—complex A) [19]. Notably, Therrien and coworkers also improved the selectivity of arene–ruthenium derivatives via the enhanced permeability and retention (EPR) effect designing supramolecular compounds [20].

Some studies also report on the design and synthesis of heterometallic systems [21–26]. The hypothesis is that the incorporation of two different cytotoxic metals in the same molecule may improve their activity as antitumor agents because of the interaction of the different metals with multiple biological targets or by the improved chemico-physical properties of the resulting hetero-polymetallic compound. Within this frame, Anderson et al. developed several platinum-ruthenium analogs of the antimetastatic Ru(III) complex NAMI-A as AH229 and achieved the potent inhibition of cell mobility (Fig. 1) [27,28]. Very recently, we and others have published on the synthesis and biological properties of titanocene-Au(I), titanocene-Ru(II) and titanocene-Pt(II) derivatives (Fig. 1—complex B) [29–31]. In general, a significant improvement of the cytotoxic properties for bimetallic complexes in comparison with a mixture of the two monometallic precursors was noticed, especially in the case of the Ti–Ru complexes [30]. Interestingly, trinuclear compounds such as $[[(\eta^5\text{-}C_5H_5)TiCl_2(\mu\text{-}\eta^5\text{-}C_5H_4PPh_2\text{-}\kappa P)]_2Au]PF_6$ showed an increased stability in aqueous solution with respect to the dinuclear derivatives, coupled to enhanced cell uptake and cytotoxicity [29].

On the basis of these results, we decided to examine a new heterobimetallic combination based on Au(I) and Pt(II) ions. Such a choice was dictated by the fact that gold and platinum complexes revealed to be among the most cytotoxic metallodrugs and that their putative intracellular targets are different. In fact, while platinum

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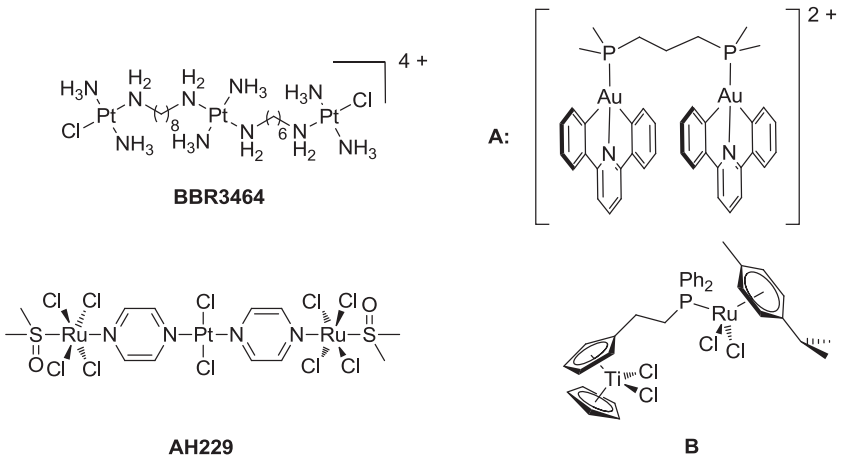

Fig. 1. Example of anticancer polymetallic complexes.

complexes are known for their high affinity of binding to nucleic acids, gold complexes react preferentially with protein targets [32,33].

To link together the two metals, we designed a bifunctional ligand that contains a dipyridylamine unit (DPA) similar to that first reported by Chitnis, [34,35] coupled to a diphenylphosphine at its end (Fig. 2). Thus, herein we report the synthesis of this dipyridylamine–phosphine ligand and its selective coordination with Au(I) and Pt(II). In addition, substitution of the chlorido ligand of gold with 1-thio-β-D-glucose tetraacetate allowed achieving a bimetallic complex that is somehow a derivative of the anti-arthritis and cytotoxic drug auranofin [11]. All the compounds have been tested for their antiproliferative activities in vitro in human cancer cell lines and non-tumorigenic cells.

2. Experimental section

2.1. General

Auranofin was purchased from Vinci-Biochem (Italy) and *cis*platin from Sigma–Aldrich. All solvents were dried and distilled under argon before use. All other reagents were commercially available and used as received. The analyses were performed at the " Plateforme d'Analyses Chimiques et de Synthèse Moléculaire de l'Université de Bourgogne". The identity and purity (≥95%) of the complexes were unambiguously established using high-resolution mass spectrometry and multinuclear NMR spectroscopy. The exact mass of the complexes were obtained on a Thermo LTQ Orbitrap XL ESI–MS (ElectroSpray Ionization Mass Spectrometry). ^{1}H (300.13, 500.13, or 600.23 MHz), ^{13}C (75.5, 125.8, or 150.9 MHz), ^{195}Pt (107.512 MHz) and ^{31}P (121.5, 202.5, or 242.9 MHz,) NMR spectra were recorded on Bruker 300 Avance III, Bruker 500 Avance III, or Bruker 600 Avance II spectrometers. Chemical shifts are quoted in parts per million (δ) relative to tetramethylsilane, TMS (^{1}H and ^{13}C), using the residual protonated solvent (^{1}H) or the deuterated solvent (^{13}C) as an internal standard. Alternatively, 85% H_3PO_4 (^{31}P) and K_2PtCl_4 in D_2O (^{195}Pt, −1617 ppm vs. Na_2PtCl_6) were used as external standards. The coupling constants are reported in Hertz. All aromatic positions: ortho, meta, para are defined using phosphorus as main substituent. Infrared spectra were recorded on a Bruker Vector 22 FT-IR spectrophotometer with the ATR technique or on a Bruker Vertex 70v.

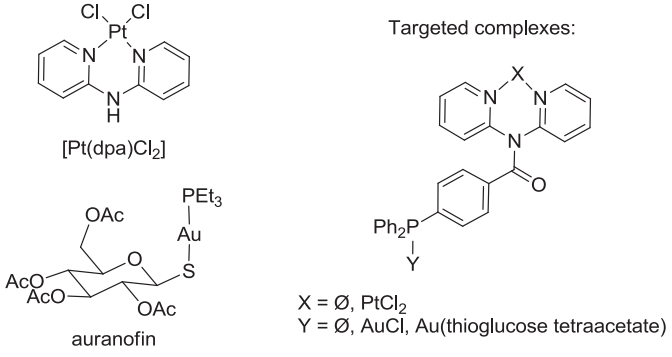

Fig. 2. Structure of the complexes discussed in this work.

2.2. Synthesis

2.2.1. 4-iodo-N,N-di(pyridin-2-yl)benzamide (c)

In a round-bottom flask under argon, protected from light and equipped with a reflux condenser, were introduced 1.053 g (3.960 mmol, 1 eq.) of 4-iodobenzoyl chloride, 677 mg (3.960 mmol, 1 eq.) of di(pyridin-2-yl)amine and 546 mg (3.960 mmol, 1 eq.) of potassium carbonate (K_2CO_3). Distilled acetonitrile (50 mL) was added, and the reaction mixture was stirred at 85 °C overnight. The resulting mixture was filtered while hot to remove the salts. During the cooling, colorless crystals grew up in the filtrate, which was evaporated under reduced pressure to obtain 4-iodo-*N*,*N*-di(pyridin-2-yl)benzamide c as a white powder; yield 1.460 g (3.641 mmol), 92%.

2.2.2. 4-(diphenylphosphino)-N,N-di(pyridin-2-yl)benzamide (1)

In a two neck round bottom flask under argon and equipped with a reflux condenser were introduced 1.364 g (3.402 mmol, 1 eq.) of 4-iodo-*N*,*N*-di(pyridin-2-yl)benzamide c and 1.53 mg (0.0068 mmol, 0.002 eq.) of $Pd(OAc)_2$ dissolved in 20 mL of distilled acetonitrile. 0.947 mL (6.804 mmol, 2 eq.) of triethylamine and 0.592 mL (3.402 mmol, 1 eq.) of diphenylphosphine were added, and the reaction mixture was stirred overnight at 85 °C. The solvent was evaporated, and the crude product purified by column chromatography (SiO_2, eluent: degassed methylene chloride/acetone 1:1) to yield 4-(diphenylphosphino)-*N*,*N*-di(pyridin-2-yl)benzamide 1 as a white meringue powder (1.535 g, 3.334 mmol, 98%).

2.2.3. [4-(diphenylphosphino)-N,N-di(pyridin-2-yl)benzamide-κP] AuCl (2)

In a Schlenk tube under argon were introduced 0.500 g (1.089 mmol, 1 eq.) of 4-(diphenylphosphino)-*N*,*N*-di(pyridin-2-yl)benzamide 1 and 0.350 g (1.089 mmol, 1 eq.) of AuCl(tht) (tht = tetrahydrothiophene). Degassed benzene (8 mL) was added, and the reaction mixture was stirred for 3 h at room temperature in the dark. The white precipitate was filtered under argon and dried to obtain the desired product 2 as a white powder (0.634 g, 0.916 mmol, 84%). Suitable crystals for X-ray diffraction were obtained from slow evaporation of a solution of 2 in methylene chloride.