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Synthesis and structural characterization of copper(I) complexes bearing N-methyl-1,3,5-triaza-7-phosphaadamantane (mPTA) Cytotoxic activity evaluation of a series of water soluble Cu(I) derivatives containing PTA, PTAH and mPTA ligands

Marina Porchia [a,*], Franco Benetollo [a], Fiorenzo Refosco [a], Francesco Tisato [a], Cristina Marzano [b], Valentina Gandin [b]

[a] ICIS-CNR, Corso Stati Uniti 4, 35127 Padova, Italy
[b] Dipartimento di Scienze Farmaceutiche, Università di Padova, via Marzolo 5, 35131 Padova, Italy

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ABSTRACT

New copper(I) complexes containing the water soluble N-methyl-1,3,5-triaza-7-phosphaadamantane (mPTA) phosphine have been synthesized by ligand-exchange reactions starting from $[Cu(CH_3CN)_4][BF_4]$ or $[Cu(CH_3CN)_4][PF_6]$ precursors and (mPTA)X (X = CF_3SO_3, I). Depending on the ligand counter ion, the hydrophilic $[Cu(mPTA)_4][(CF_3SO_3)_4(BF_4)]$ **3a** and $[Cu(mPTA)_4][(CF_3SO_3)_4(PF_6)]$ **3c** complexes or the iodine-coordinated $[Cu(mPTA)_3I]I_3$ **4** species were obtained respectively and fully characterized by spectroscopic methods. Single crystal structural characterization was undertaken for $[Cu(mPTA)_3I]I_3{\cdot}H_2O$, **4**$\cdot H_2O$, and $[Cu(mPTA)_4][(CF_3SO_3)_2(BF_4)_3]\cdot 0.25H_2O$, **3b**$\cdot 0.25H_2O$, the latter obtained by crystallization of $[Cu(mPTA)_4][(CF_3SO_3)_4(BF_4)]$ **3a**. The cytotoxicity of analogous tetrahedral homoleptic Cu(I) derivatives $[Cu(PTA)_4](BF_4)$ **1**, $[Cu(PTAH)_4][Cl_4(BF_4)]$ **2**, $[Cu(mPTA)_4][(CF_3SO_3)_4(BF_4)]$ **3a** and $[Cu(mPTA)_4][(CF_3SO_3)_4(PF_6)]$ **3c** was evaluated against a panel of several human tumor cell lines. All the complexes showed in vitro antitumor activity comparable to that of the reference metallodrug cisplatin. Tests performed on cisplatin sensitive and resistant cell lines showed that against human ovarian 2008/C13* cell line pair, the resistance factor of copper derivatives was roughly 7-fold lower than that of cisplatin, whereas against human cervix cancer A431/A431-Pt cell line pair it was about 2.5-fold lower. These results, confirming the circumvention of cisplatin resistance, support the hypothesis that phosphine copper(I) complexes follow different cytotoxic mechanisms than do platinum drugs.

1. Introduction

Since the discovery of the antitumor activity of cisplatin (*cis*-$[PtCl_2(NH_3)_2]$) for the treatment of several human tumors [1], thousands of platinum and other metal-based compounds have been tested for their potential antitumor properties in the last 40 years. A series of compounds showing encouraging perspectives were the phosphine complexes of group 11 metal ions [2]. Their biological properties were little explored until late 1970s when a thioglucose derivative of triethylphosphine gold(I) (auranofin) was found to possess antiarthritic activity [3] and subsequently shown to have in vivo antitumor activity in murine models, although only against P388 leukemia [4]. In an attempt to identify gold complexes with a wider spectrum of activity, Sadler and coworkers demonstrated the cytotoxicity of mono-cationic bis-diphosphine gold(I) compounds toward a panel of human tumor cell lines including B16 melanoma, P388 leukemia, and M5076 reticulum cell carcinoma [5]. In particular, it was found that $[Au(dppe)_2][Cl]$ (dppe = 1,2-bis(diphenylphosphino)ethane) was 10-fold more cytotoxic than dppe alone, suggesting, at that time, that metal ions could potentiate the cytotoxic properties of dppe [5]. In general, the higher drug tolerance profiles of gold-based agents compared to the severe toxic effects on normal tissues and/or the occurrence of inherited or acquired resistance induced by cisplatin have represented a hopeful prospect for the chemotherapeutic application of gold-based drugs. Despite this promising outlook, clinical trials in humans of gold compounds remain still elusive.

A rational extension of diphosphine gold(I) chemistry to the first row congener copper indicated that analogous copper(I) complexes of "CuP_4" stoichiometry could be efficiently prepared and

* Corresponding author. Fax: +39 049 8295951.
E-mail address: porchia@icis.cnr.it (M. Porchia).

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tested in vitro [6,7]. Among them, $[Cu(dppe)_2][Cl]$ and $[Cu(dppey)_2][Cl]$ (dppey = 1,2-bis(diphenylphosphino)ethylene) produced cytotoxic effects on cell metabolism comparable to those exhibited by Au(I) analogs, which were finally postulated to be the result of the uncoupling of mitochondrial oxidative phosphorylation [8]. However, the presence of several phenyl groups appended to the phosphorus donors in both $[Cu(dppey)_2][Cl]$ and $[Au(dppe)_2][Cl]$ species caused undesired nephrotoxicity [2] and cardiovascular toxicity [9] in animal models, respectively, thus precluding clinical trials in humans.

Aiming at the formation of less-toxic copper compounds, we have recently prepared a series of hydrophilic copper(I) derivatives including the water soluble tris(hydroxymethyl)phosphine (thp) ligand, either alone [10,11] or in combination with hydrophilic scorpionates [12]. These water soluble compounds were proved to be easier to handle during the in vitro tests and, more importantly, have shown cytotoxic activity against a large panel of human tumor cell lines belonging to a variety of tumor types, including cisplatin and multidrug resistant phenotypes [11–13]. Moreover, it has been found that the cytotoxic activity of these copper(I) complexes may be correlated to their ability to induce a non-apoptotic mechanism of cell death [11,12]. Such results, together with recent studies which reported that the antiproliferative action of auranofin is connected with the inhibition of the thioredoxin reductase leading to augmented apoptosis [14], corroborate the view that mechanisms of action different from the DNA damage induced by cisplatin could underlie the cytotoxic activity of phosphine Au(I) and Cu(I) drugs. In other words, other molecular targets, in addition to DNA, could be identified in order to inhibit cancer cell proliferation, thus offering further motivation for the design of novel metal-based anticancer drugs.

The water-soluble 1,3,5-triaza-7-phosphaadamantane (PTA) phosphine ligand and its derivatives are receiving growing attention in recent years [15–17]. The good water solubility of related transition metal PTA complexes makes possible their efficient application in aqueous phase catalysis [18–20]. The combination of PTA physico-chemical properties including water solubility, low steric hindrance (comparable to that of PMe_3) and resistance to oxidation in water, joint to the ability to reduce various metal ions, include this phosphine among those few ligands suitable for stabilization of Cu(I) in aqueous media. While a large variety of lipophilic Cu(I) phosphine complexes stable in classic organic media has already been reported [21] very few examples of water-soluble Cu(I) compounds stable to disproportionation in water are known. These comprise the polymeric $\{Na_5[Cu(TPPTS)_2]\cdot 5H_2O\}_n$ complex [22] (TPPTS = tris(*m*-sulfonatophenyl)phosphine), $[Cu(thp)_4][PF_6]$ (thp = tris(hydroxymethyl)phosphine), $[Cu(bhpe)_2][PF_6]$ (bhpe = bis[bis(hydroxymethyl)phoshino]ethane) [11], the nitrate and chloride salts $[Cu(PTA)_4][X]$ (X = NO_3, Cl) and $[Cu(HPTA)_4][NO_3]_5$ [23,24]. Other recent water soluble Cu(I) derivatives present a mixed coordination sphere with a monodentate tertiary phosphine and a tripodal scorpionate ligand [12,25–27].

Aim of our study was to prepare a series of water-soluble copper(I) compounds whose steric and electronic properties could be tuned by slight modifications of the ligands, in order to compare and to correlate their physico-chemical properties with a possible biological activity. The PTA phosphine and its N-protonated (PTAH) and N-methylated (mPTA) derivatives (Chart 1) proved to be an ideal series of ligands for this purpose, as they give rise to different charged complexes conferring indeed a good hydrosolubility.

In this study we report on the synthesis and characterization of new mPTA copper complexes, as well as the cytotoxic activity evaluation of stable water-soluble Cu(I) compounds containing PTA derivatives towards a panel of human tumor cell lines.

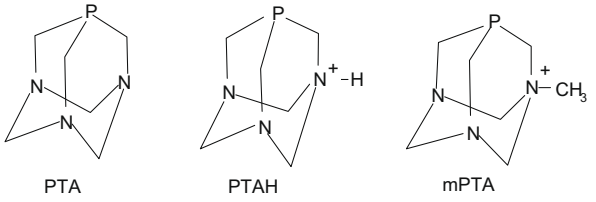

Chart 1. 1,3,5-Triaza-7-phosphaadamantane (PTA) ligand and its N-protonated (PTAH) and N-methylated (mPTA) derivatives.

2. Experimental

2.1. General procedures

All solvents and commercially available substances were of reagent grade and used without further purification. The Cu(I) starting compound $[Cu(CH_3CN)_4][BF_4]$ was prepared by reaction of $[Cu(H_2O)_6][BF_4]_2$ with metallic copper in acetonitrile whereas $[Cu(CH_3CN)_4][PF_6]$ was prepared from Cu_2O and HPF_6 according to Ref. [28]. The ligands PTA, (mPTA)I and (mPTA)(CF_3SO_3) were synthesized in accordance with published methods [16,17]. Elemental analyses were performed on a Carlo Erba 1106 Elemental Analyzer .^{1}H, ^{31}P, ^{13}C and ^{63}Cu NMR spectra were recorded on a Bruker AMX-300 instrument. The splitting of nuclear resonances in the reported NMR spectra is defined as s = singlet, d = doublet, q = quartet, m = multiplet, and bs = broad singlet. FT IR spectra were recorded on a Mattson 3030 Fourier transform spectrometer in the range 4000–400 cm^{-1} in KBr pellets. The intensity of reported IR signals is defined as w = weak, m = medium, s = strong, and vs = very strong. Mass spectra have been recorded by an electrospray LCQ ThermoFinnigan mass spectrometer.

2.2. Syntheses of the complexes

2.2.1. $[Cu(PTA)_4][BF_4]$ (**1**)

To an acetonitrile solution of $[Cu(CH_3CN)_4][BF_4]$ (136 mg, 0.43 mmol) an excess of PTA (320 mg, 2.03 mmol) was added at room temperature. The reaction mixture was stirred overnight and then filtered. The white residue was washed three times with chloroform and diethyl ether and then dried under vacuum. Yield: 90% ^{1}H NMR (D_2O, ppm): 4.06 (s, 6H, P–CH_2), 4.44–4.58 (*q*, 6H, N–CH_2). ^{31}P{H} NMR (D_2O, ppm): −80.5 (q, $^1J_{P-Cu}$ = 750 Hz). ^{13}C{H}-(D_2O, ppm): 71.5 (s, N–CH_2), 50.8 (s, P–CH_2). ^{63}Cu NMR (D_2O, ppm): 137.6 (quintet, $^1J_{Cu-P}$ = 750 Hz). Crystal of $[CuPTA_4]BF_4\cdot 6H_2O$, **1**$\cdot 6H_2O$, suitable for X-ray analysis were obtained from a acetonitrile/methanol solution. ESI-MS (electrospray ionization–mass spectrometry) (*m/z* assignment, % intensity): 377 ($[Cu(PTA)_2]^+$, 100), 220 ($[CuPTA]^+$, 5), 158 (PTA^+, 10). Anal. Calcd. for $[CuPTA_4]BF_4\cdot 3H_2O$ $CuP_4N_{12}C_{24}H_{48}BF_4\cdot 3H_2O$: C 34.60, H 6.53, N 20.18. Found: C 34.43, H 6.52, N 20.13.

2.2.2. $[Cu(PTAH)_4][Cl_4(BF_4)]$ (**2**)

Concentrated hydrochloric acid (37%, 0.17 mL, 2.04 mmol) was added to an aqueous solution of (**1**) (398 mg, 0.51 mmol) at room temperature. After removal of water the white residue was identified as (**2**). ^{1}H NMR (D_2O, ppm): 4.18 (s, 6H, P–CH_2), 4.88–4.74 (m, 6H, N–CH_2). ^{31}P{H} NMR (D_2O, ppm): −80.4 (bs). Anal. Calcd. for $CuP_4N_{12}C_{24}H_{52}Cl_4BF_4$: C 31.17, H 5.67, N 18.17. Found: C 31.50, H 5.55, N 18.30.

2.2.3. $[Cu(mPTA)_4][(CF_3SO_3)_4(BF_4)]$ (**3a**)

To an acetonitrile solution of (mPTA)(SO_3CF_3) (410 mg, 1.28 mmol) the Cu(I) precursor $[Cu(CH_3CN)_4][BF_4]$ (100 mg, 0.32 mmol) was added at room temperature. After 3 h stirring