Inorganica Chimica Acta 375 (2011) 114–121

Contents lists available at ScienceDirect

Inorganica Chimica Acta

journal homepage: www.elsevier.com/locate/ica

Synthesis, structural characterization and biological studies of novel mixed ligand Ag(I) complexes with triphenylphosphine and aspirin or salicylic acid

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ARTICLE INFO

Article history:
Received 21 January 2011
Received in revised form 16 April 2011
Accepted 20 April 2011
Available online 5 May 2011

Keywords:
Bioinorganic chemistry
Silver(I) complexes
Aspirin
Crystal structures
Cytotoxic activity
STD ^{1}H NMR

ABSTRACT

Two new mixed ligand silver(I) complexes of formulae {[Ag(tpp)$_3$(asp)](dmf)} (**1**) (aspH = o-acetylsalicylic acid and tpp = triphenylphosphine) and [Ag(tpp)$_2$(o-Hbza)] (**2**) (o-HbzaH = o-hydroxy-benzoic acid) were synthesized and characterized by elemental analyses, spectroscopic techniques and X-ray crystallography at ambient conditions. Three phosphorus and one carboxylic oxygen atoms from a de-protonated aspirin ligand in complex **1** and two phosphorus and two carboxylic oxygen atoms from a chelating o-Hbza anion in complex **2** form a tetrahedral geometry around Ag(I) ions in both complexes.

Complexes **1** and **2** and the silver(I) nitrate, tpp, aspNa and o-HbzaH were tested for their *in vitro* cytotoxic activity against leiomyosarcoma cells (LMS), human breast adenocarcinoma cells (MCF-7) and normal human fetal lung fibroblasts (MRC-5) cells with Thiazolyl Blue Tetrazolium Bromide (MTT) assay. For both cell lines **1** and **2** were found to be more active than cisplatin. Additionally, **1** and **2** exhibit lower activity on cell growth proliferation of MRC-5 cells. The type of LMS cell death caused by **1** and **2** were evaluated *in vitro* by use of flow cytometry assay. The results show that at concentrations of 1.5 and 1.9 μM of complex **1**, 44.1% and 69.4%, respectively of LMS cells undergo programmed cell death (apoptosis). When LMS cells were treated with 1.6 and 2.3 μM of **2**, LMS cells death was by 29.6% and 81.3%, respectively apoptotic. Finally, the influence of the complexes **1** and **2**, upon the catalytic peroxidation of linoleic acid to hydroperoxylinoleic acid by the enzyme lipoxygenase (LOX) was kinetically and theoretically studied. The binding of **1** and **2** towards LOX was also investigated by Saturation Transfer Difference (STD) ^{1}H NMR experiments.

1. Introduction

Aspirin (aspH) (o-acetyl-salicylic acid) was the first member of the class of drugs known as nonsteroidal, anti-inflammatory drugs (NSAIDs) discovered. Aspirin was introduced as an anti-pyretic, anti-inflammatory and analgesic drug at the end of nineteenth century, while today, is one of the most commonly drug in use, worldwide [1]. A number of epidemiological studies have indicated that long term aspirin/NSAID use is associated with 30–50% reduction in risk of colorectal cancer or adenomatous polyps or death from colorectal cancer [2a]. Other epidemiologic studies also found associations between aspirin/NSAID use and a lower death rate from cancers of the esophagus, stomach, breast, lung, prostate, urinary bladder and ovary [2b,c]. Salicylic acid (o-HbzaH) (o-hydroxybenzoic acid), is a precursor of aspirin, while it has been recently shown that organotin complexes of o-HbzaH possess strong antiproliferative activity [3]. The biomedical applications and uses of silver(I) complexes, on the other hand, are related to their antibacterial action [1b,4] which appears to involve interaction with DNA [4,5]. Recently, Ag(I) complexes have also been studied for their antitumor activity [5,6]. Despite the importance of aspirin only few structures of complexes are available up to now. These include the complexes of aspirin with calcium(II) [7a], copper(II) [7b–d], nickel(II) [7e], tin(IV) [7f], cadmium(II) [7g], zinc(II) [7h]. The silver(I) complex of salicylic acid, however, with formula {[Ag(o-Hbza)]$_2$} is already known [8].

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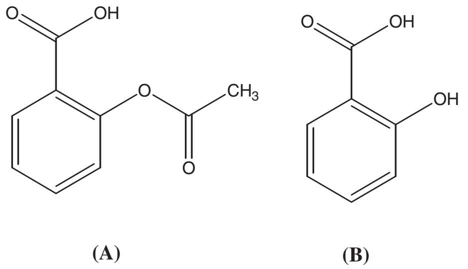
doi:10.1016/j.ica.2011.04.032



Scheme 1. Molecular formulae of aspH (A) and o-HbzaH (B).

Lipoxygenase (LOX) is an enzyme which catalyzes the oxidation of arachidonic acid to leukotrienes, in an essential mechanism for the cell life involving in inflammation mechanism [9a,b]. LOX inhibition is found to induce apoptosis [9c], while the lipid peroxides derived from fatty acids metabolism by LOX can regulate cellular proliferation [9d]. Thus, LOX inhibition provides a potential novel target for the treatment and chemoprevention for a number of different cancers.

In the course of our studies on metallotherapeutics [10], we have synthesized new silver(I) complexes of formulae {[Ag(tpp)$_3$(asp)](dmf)} (**1**) (aspH = o-acetylsalicylic acid (Scheme 1A) and tpp = triphenylphosphine) and [Ag(tpp)$_2$(o-Hbza)] (**2**) (o-HbzaH = o-hydroxy-benzoic acid (Scheme 1B). The complexes were characterized by elemental analyses, spectroscopic techniques and X-ray crystallography at ambient conditions. The anti-cancer cell screening results against LMS and MCF-7 showed that both complexes **1** and **2** are more active than cisplatin and less active against MRC-5 cells proliferation. These findings are compared with the results of the influence of **1** and **2**, on the catalytic peroxidation of linoleic acid to hydroperoxylinoleic acid by the enzyme LOX.

2. Results and discussion

2.1. General aspects

Crystals of the complexes **1** and **2** have been grown as follows: AgNO$_3$ reacts with the sodium salts of aspirin or o-hydroxy-benzoic and white precipitations are formed. Slow evaporation of DMF solutions of these precipitations in the presence of triphenylphosphine give crystals of **1** and **2**. The formula of the complexes was firstly deduced from its elemental analysis, m.p. and their spectroscopic data (see Section 4). The X-ray crystal structures of complexes were also determined. The crystals of the complexes are air stable when they store in darkness at room temperature. Complexes **1** and **2** are soluble in MeCN, CHCl$_3$, CH$_2$Cl$_2$ DMSO, DMF and CH$_3$OH. Since a dissociation of the Ag–P bonds have been observed for silver(I) complexes with phosphines and carboxylic acids in solution [11], the stability of the complexes **1** and **2** in DMSO solutions were tested by UV–Vis spectra and conductivity measurements. No any changes were observed between the initial UV spectrum and the corresponding one measured after 48 h for both complexes (Fig. S1). The period of 48 h for the stability testing of the complexes was chosen since biological experiments require 48 h of incubation with complexes. In order to assure that no any ionic species are also formed in DMSO or DMSO/water solutions, the molar conductance (Λ_m) values of the complexes **1** and **2** in DMSO solution (10^{-3} M) were determined (**1**: 15.0 (0 h), 15.2 (48 h), 15.0 (72 h) and **2**: 17.8 (0 h), 18.1 (48 h), 14.4 (72 h) Ω^{-1} cm^2 mol^{-1}). These values showed that the solutions of the complexes are not conducting, confirming their stability in DMSO or DMSO/water media. The molar conductance of the silver(I) nitrate complex with tetramethylethylenediamine [Ag((CH$_3$)$_2$NCH$_2$CH$_2$N(CH$_3$)$_2$)]NO$_3$ was 40 Ω^{-1} cm^2 mol^{-1}, in excellent agreement with values for fully dissociated 1:1 electrolytes, such as sodium nitrate, in DMSO [11b]. The corresponding molar conductivity of DMSO solutions, measured for the ionic AgX salts (X = NO_3^-, BF_4^-) with (1-benzyl-2-imidazolyl)diphenylphosphine [(BzIm)Ph$_2$P] are 302 and 320 Ω^{-1} cm^2 mol^{-1} [11c] indicating the formation of three ionic species in DMSO solution.

Attempts for the preparation of the initial sample by reacting AgNO$_3$ and aspirin in aqueous NH$_3$ 40% solution lead to the formation of the [Ag(o-Hbza)]$_2$ (**3**) complex, where a hydrolysis of the ester (aspirin) to its o-HbzaH processor has been occurred. The crystal structure of **3** was also determined (crystallized in $P2_1/c$ with a = 7.3973(2), b = 8.6899(2), c = 10.5388(3) Å, β = 107.2560(10)°) and is almost identical with those reported earlier [8a] ($P2_1/c$, a = 7.405(1), b = 8.826(2), c = 10.683(2) Å, b = 107.48(4)°).

2.2. Crystal and molecular structures of {[Ag(tpp)$_3$(asp)](dmf)} (1) and [Ag(tpp)$_2$(o-Hbza)] (**2**)

ORTEP diagrams of **1** and **2** along with their selected bond distances and angles are shown in Figs. 1 and 2.

In case of **1**, three phosphorus atoms from tpp ligands and one deprotonated carboxylic oxygen atom from aspirin form a tetrahedral geometry around Ag(I) ion. Two phosphorus atoms from tpp and two oxygen atoms from the deprotonated carboxylic group which chelates Ag(I) ion form the tetrahedral geometry around silver(I) in case of **2**. The average Ag–P bond distance in **1** is 2.53 Å (Ag1–P1 = 2.515(2), Ag1–P2 = 2.554(3), Ag1–P3 = 2.528(3)), while in **2** is 2.43 (Ag(1)–P(1) = 2.4030(6), Ag(1)–P(2) = 2.4589(7)). The corresponding bond distances observed in silver(I) mixed ligand complexes of phosphines and carboxylic acids are: 2.6026(8), 2.5441(7) and 2.5432(7) Å in (Ph$_3$P)$_3$AgOC(O)C$_2$F$_5$ [12a], 2.543(2), 2.563(2) and 2.546(3) Å in [Ag(2-sbaH)(PPh$_3$)$_3$] (2-sbaH$_2$ = 2-sulfobenzoic acid) [12b], 2.524(7), 2.545(8) and 2.503(8) Å in [Ag(dppe)(tfa)]$_n$ (dppe = diphenyl-phosphinethane, tfa = F$_3$CCO$_2$) [12c], 2.365(2) and 2.344(2) Å in [Ag$_2$(CH$_3$CO$_2$)$_2$(dppf)]$_2$ (dppf =

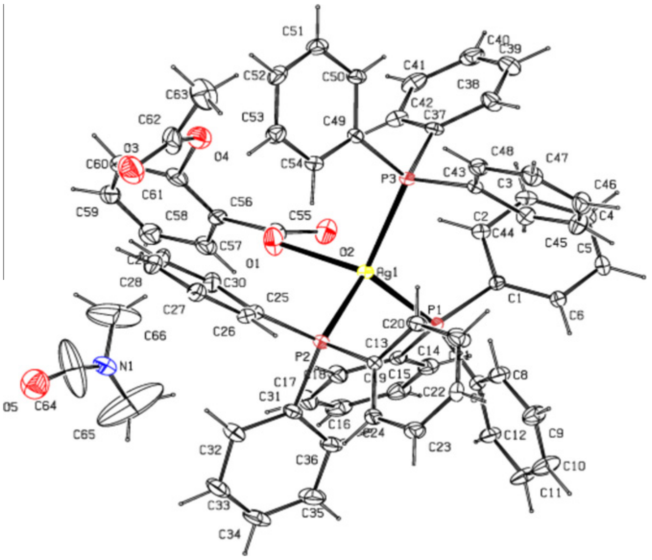


Fig. 1. ORTEP diagram together with labeling scheme of **1**. Thermal ellipsoids drawn at the 50% probability level. Selected bond lengths (Å) and angles [°]: Ag1–P1 = 2.515(2), Ag1–P2 = 2.554(3), Ag1–P3 = 2.528(3), Ag1–O1 = 2.395(10), O1–C55 = 1.144(18), O2–C55 = 1.287(17), O3–C62 = 1.16(2), O4–C61 = 1.388(17), O4–C62 = 1.303(18), P1–Ag1–P2 = 109.32(9), P1–Ag1–P3 = 115.37(8), P1–Ag1–O1 = 110.4(2), P2–Ag1–P3 = 117.09(8), P2–Ag1–O1 = 101.4(2),P3–Ag1–O1 = 101.9(3); solvated DMF: O5–C64 = 1.07(3), N1–C64 = 1.21(3).