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Silver(I) compounds of the anti-inflammatory agents salicylic acid and *p*-hydroxyl-benzoic acid which modulate cell function

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ABSTRACT

Silver nitrate reacts with salicylic acid ($salH_2$) or *p*-hydroxy-benzoic acid (p-$HbzaH_2$) and equimolar amount of NaOH to yield a white precipitations which are then treated with tri(*p*-tolyl)phosphine (tptp) or tri(*m*-tolyl)phosphine (tmtp) to yield the complexes $[Ag(tptp)_2(salH)]$ (**1**), $[Ag(tptp)_2(p\text{-}Hbza)]$ (**2**) and $[Ag(tmtp)_2(salH)]$ (**3**). Complexes **1** and **3** are also obtained when aspirin (aspH) is used. The acetic ester of salicylic acid is hydrolyzed to form the complexes **1** and **3**. However, when aspirin and tptp are used, a mixture of products was obtained which contains both **1** and an ionic complex of formula $\{[Ag(tptp)_4]^+[(salH)^-]\cdot[(CH_3)_2NCHO)]\cdot(H_2O)\}$ (**1a**). The complexes were characterized by m.p., e.a., mid-FT-IR, ^{1}H-,^{31}P-NMR, HRMS, UV–vis spectroscopic techniques and X-ray crystallography. Two phosphorus and one carboxylic oxygen atoms form a trigonal planar geometry around Ag(I) ions in complexes **1**–**3**. Complex **1a** consists of a $[Ag(tptp)_4]^+$ cation and a deprotonated $salH^-$ counter anion. The influence of **1**–**3** on the viability of MCF-7 (breast) and HeLa (cervix) adenocarcinoma cells, is evaluated. DNA binding tests indicate the ability of **1**–**3** to modify the activity of cells. The binding constants of **1**–**3** towards calf-thymus DNA, reveal stronger interaction of **2**. Changes in fluorescent emission light of ethidium bromide (EB) in the presence of DNA suggest intercalation or electrostatic interactions into DNA for **1** and **3**. Docking studies on DNA-complex interactions confirm the binding of **1**–**3** in the minor groove of B-DNA. Moreover, the influence of **1**–**3** on the peroxidation of linoleic acid to hydroperoxylinoleic acid by the enzyme lipoxygenase (LOX) was kinetically and theoretically studied.

1. Introduction

Salicylic acid ($SalH_2$) (or *o*-hydroxybenzoic acid), a non steroidal anti-inflammatory drug (NSAID) is a precursor of aspirin. Recently, it was shown that organotin [1], copper(II) [2] and silver(I) complexes of $salH_2$ possess strong antiproliferative activity against cancer cell lines [3–5]. The long-term use of salicylic acid or NSAIDs, is known to reduce colon cancer risk by 40–50% and may be preventative for lung, esophagus, and stomach cancers [2]. NSAIDs were found to activate the mechanism of apoptosis through 15-lipoxygenase-1 (15-LOX-1) inhibition [6,7], while 15-LOX-1 has been associated with the evolution of certain cancers [7]. Thus, the development of new lipoxygenase (LOX) inhibitors is a main research target in new drug designs. Moreover, salicylic acid is a versatile ligand, with two hard and strongly basic donor centers facilitating chelation and/or metal bridging for medium- to large-size cations [8]. The hydroxyl group of the salicylate ligand, on the other hand, may participate in intra- and/or intermolecular hydrogen bonding interaction leading to the formation of multidimensional assemblies [8].

Silver(I) complexes with carboxylic acids are active against adenocarcinoma cells. They wield antiproliferative effects by interacting with DNA and through binding with thiol groups of the proteins, hence differing from the behavior of several cytotoxic complexes of other metals [9]. Especially, 4-hydroxybenzoic acid (p-$HbzaH_2$) is an antioxidant agent used as an antimicrobial preservative in pharmaceuticals, cosmetics and foods [5]. Moreover, studies have indicated that p-$HbzaH_2$ itself possesses intrinsic estrogenic activity in human breast cancer cell lines [10]. Derivatives of 4-hydroxybenzoic acid were found to increase protein acetylation levels, arrest cell cycle progression and trigger apoptotic cell death, without affecting the viability of normal cells [11].

In the course of our studies on metallotherapeutics [1,4,5,9,12–15] we have synthesized two silver(I) complexes with the anti-inflammatory drug $salH_2$ or p-$HbzaH_2$ of formulae $[Ag(tpp)_2(salH)]$ and $[Ag(tpp)_2(p\text{-}Hbza)]$ where tpp is triphenylphosphine. These complexes were found to exhibit significant cytotoxic activity which is 5 folds higher against

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MCF-7 cells line than that of cisplatin. They induce apoptosis, while they bind to calf thymus (CT)-DNA strongly. Both [Ag(tpp)$_2$(salH)] and [Ag(tpp)$_2$(*p*-Hbza)] complexes inhibit, strongly, LOX activity.

It has been shown that high lipophilicity improves the activity of silver-phosphine complexes towards cancer cells [16,17]. The use of the salH$_2$, *p*-HbzaH$_2$, tptp and tmtp ligands for the preparation of mixed ligand silver(I) complexes is expected to increase the lipophlicity with simultaneous retention of the water solubility. Through a double mechanism: (i) the phosphine residues of complexes **1**–**3** are expected to retain the high solubility in organic media, and (ii) the position and the kind of the polar group (methoxy group) contained in salH$_2$ or *p*-HbzaH$_2$ can impact water solubility.

Here we report the synthesis of four silver(I) complexes with salH$_2$ or *p*-HbzaH$_2$ and tptp or tmtp (Fig. 1) of formula [Ag(tptp)$_2$(salH)] (**1**), {[Ag(tptp)$_2$(*p*-Hbza)] (**2**), [Ag(tmtp)$_2$(salH)] (**3**) and {[Ag(tptp)$_4$]$^+$[(salH)$^-$]·[(CH$_3$)$_2$NCHO)]·(H$_2$O)} (**1a**). Complexes **1**–**3** were characterized by e.a., vibrational spectroscopy (mid-FT-IR), ^{1}H-NMR, UV–vis spectroscopic techniques and X-ray crystallography. Our study also aims to develop new metallotherapeutics and therefore complexes **1**–**3** were investigated for their cytotoxic activity mechanism. Since cervical and breast cancers are considered to be the leading causes of death among women [18,19], compounds **1**–**3** were tested against two tumor adenocarcinoma cell lines; human breast adenocarcinoma (MCF-7) cells and human cervix adenocarcinoma (HeLa) cells. Compound **1a** was obtained in a mixture of products with **1** (Polymorph B) when aspirin and tptp were used for the preparation and therefore it is not evaluated for its biological activity. The use of particular ligands (salH$_2$ or *p*-HbzaH$_2$ and tptp or tmtp) for the preparation of mixed ligand silver(I) complexes is related to our target to form water soluble complexes which are also lipophilic, since the organic residues of complexes **1**–**3** are expected to retain the high solubility in organic media, while the position and the kind of the polar groups (hydroxyl groups) might lead to an increasing water solubility.

2. Results and discussion

2.1. General aspects

Crystals of **1**-**3** were grown by slow evaporation of DMF solutions. Complexes **1** (Polymorph A), **2** and **3** were formed from the reaction of triarylphosphines (tptp, tmtp) with the product derived from the reaction of AgNO$_3$ with the salH$_2$ or *p*-HbzaH$_2$ and equimolar amount of NaOH (Scheme 1).

The complexes **1** and **3** are also obtained from aspirin (aspH) by hydrolysis of the acetic ester to salicylic acid. However, in the case of aspirin and tptp, a mixture of products was obtained which contained both **1** (Polymorph B) and an ionic complex of formula {[Ag(tptp)$_4$]$^+$[(salH)$^-$]} (**1a**) (Scheme 1). The formulae of the complexes were firstly deduced from their spectroscopic data which were also used for the verification of the purity of the samples (see Experimental section). The X-ray crystal structures of complexes were also determined. The crystals of **1**–**3** are air stable when they are stored in darkness at room temperature.

2.2. Solid state studies

2.2.1. Crystal and molecular structures of [Ag(tptp)$_2$(salH)] (**1**), {[Ag(tptp)$_2$(*p*-Hbza)] (**2**), [Ag(tmtp)$_2$(salH)] (**3**) and {[Ag(tptp)$_4$]$^+$[(salH)$^-$]·[(CH$_3$)$_2$NCHO)]·(H$_2$O)} (**1a**)

ORTEP diagrams of **1**–**3** along with their selected bond distances and angles are shown in Figs. 2–5.

Two polymorphic forms of **1** (**1A** and **1B**) were obtained as results of different preparation procedures (from salH$_2$ or aspH respectively). Both forms are triclinic P-1, but the asymmetric part of form **1B** contains two symmetry-independent molecules while in **1A** there is only one molecule per asymmetric part. The two molecules in **1B** are quite similar geometrically.

Before discussing the coordination it would be useful to remark on the intramolecular hydrogen bonding. In cases where the hydrogen could be located in the difference Fourier map, its position was significantly moved to the center of O···O distance, as often happens in the strong hydrogen bonds, and the O···O separations are quite short. In such cases the hydrogen atom was left in the position found and refined as riding model (or freely). Table 1 lists the hydrogen bond data for the complexes. Fig. 6 shows one of the difference Fourier maps, calculated without hydrogen atom.

The coordination of the Ag ion in **1**–**3** can be described as trigonal, with two phosphorus and one carboxylic oxygen atom from salH$^-$ or *p*-HbzaH$^-$ anions forming a trigonal planar geometry. The average Ag–P bond distances are: 2.42 (**1**), 2.43 (**2**) and 2.41 (**3**) Å. The corresponding bond distances observed in silver(I) mixed ligand complexes of phosphines and carboxylic acids lie between 2.34 and 2.60 Å (av. 2.48 Å) [4,5,15,20–25]. The average Ag–O bond distances are: Ag-O10 = 2.415(5) (**1A**), Ag1-O43A = 2.405(2) (**1B^{I}**), Ag2-O92B = 2.409(2) (**1B^{II}**), Ag1-O34 = 2.3046(18) (**2**) and Ag1-O43A = 2.4308(19) (**3**) Å, There is always a weaker Ag···O′ interaction with the second carboxylic oxygen atom (probably of secondary nature, enforced by the geometrical constraints) and this causes a slight distortion of the Ag atom out of the plane defined by the P,P,O atoms. Interestingly one can observe the tendency of the COO group towards adopting almost bisecting position with respect to the P-Ag-P plane. The carbon carboxylic atom is very close to this plane and both oxygen atoms are in approximately equal distances on both sides of this plane. The tilt of the COO group causes then the significant distortion from the equivalence of Ag–O distances. Looking at Figs. 2–4 it might be noted that the closer Ag–O contact is not dependent on whether this oxygen atom is involved in strong intramolecular hydrogen bond or not. This might be further hypothesized, in the light of above facts that the coordination contact goes to the middle of delocalized (over COO fragment) electron density, analogous to the coordination to the π-bond systems.

In turn, to the cationic complex **1a** the silver ion is in very regular tetrahedral environment, the apexes of the tetrahedron are occupied by P atoms. Complex **1a** consists of a [Ag(tptp)$_4$]$^+$ cation and a deprotonated salH$^-$ counter anion. The geometry around the Ag(I) is tetrahedral in the case of **1a** (Fig. 5). Four P atoms from tptp ligands form a tetrahedron around Ag(I). The C–O bond lengths in the salH$^-$ moiety (1.252(4) Å and 1.260(4) Å) are almost equal confirming the anionic form of the

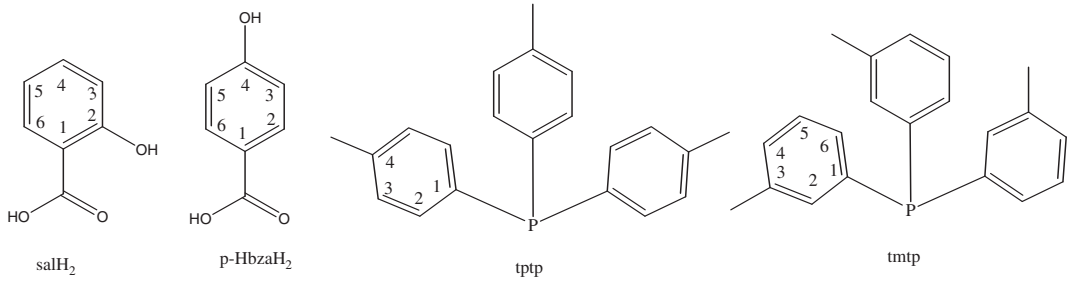

Fig. 1. Molecular diagrams of the ligands salH$_2$, *p*-HbzaH$_2$, tptp and tmtp used.