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Original article

A novel silver iodide metalo-drug: Experimental and computational modelling assessment of its interaction with intracellular DNA, lipoxygenase and glutathione

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ABSTRACT

The new mixed ligand silver(I) complex of formula $[AgI(TPP)_2(MBZT)]$ (**1**) was obtained by reacting 2-mercapto-benzothiazole (MBZT) with triphenylphosphine (TPP). The complex was characterized by m.p., vibrational spectroscopy (FT-IR), ^1H NMR, UV–vis, ESI-MS spectroscopic techniques and its structure was confirmed by X-ray crystallography. Mixed ligand complexes of silver(I) iodide with thiones and phosphines are very rare in the literature and to the best of our knowledge compound **1** is the first of this kind exhibiting significant biological effects.

Complex **1** was evaluated for its *in vitro* cytotoxic activity (cell viability) under irradiation with UV light and without irradiation against human cancer cell lines: MCF-7 (breast, ER positive), MDA-MB-231 (breast, ER negative), Caki-1 (renal), A549 (lung), OAW-42 (ovarian), HeLa (cervical) and additionally against the normal human lung cell line MRC-5 (normal human fetal lung fibroblast cells) and normal immortalized human mammary gland epithelial cell line (MTSV17) with SRB assay. The results showed that **1** mediates a strong cytotoxic response to the tested normal and cancer cell lines. It exhibits equal activity against MDA-MB-231 cells where estrogen receptors (ERs) are devoid with the one against MCF-7 where ERs are present. Molecular docking studies have shown that **1** is docked in the different pocket than that of the ERs modulators. The binding affinity of **1** towards the intracellular molecules DNA and lipoxygenase (LOX) was studied for the evaluation of the mechanism of its cytostasis. The binding constant (K_b) of **1** towards CT-DNA was calculated by UV–Vis and fluorescent spectra suggesting intercalation or electrostatic interactions of **1** into DNA. Docking studies on DNA-complex interactions confirm the binding of **1**. Moreover, the influence of complex **1** on the catalytic peroxidation of linoleic acid to hydroperoxylinoleic acid by the enzyme lipoxygenase (LOX) was kinetically and theoretically studied. In addition, since the deactivation of cisplatin caused by glutathione, seems to be an important determinant of its cytotoxic effects, the reaction of **1** with glutathione (GSH) was investigated by UV-absorption spectroscopy.

1. Introduction

Glutathione (GSH) is a ubiquitously expressed tripeptide that serves as the largest source of nonprotein thiol groups within the cell [1]. It also protects cells against reactive oxygen species (ROS) as well as against many toxins, mutagens, and drugs [2]. GSH plays an important role in multi-drug resistance either through its spontaneous reactions or through its function as a coenzyme in glutathione S-transferases (GST) [2]. Sulphur-donor nucleophiles, such as GSH and sulphur-containing amino acids, are known to interact with chemotherapeutics such as cisplatin [3]. This binding can prevent substantial quantities of cisplatin from reaching DNA in the nucleus and ultimately limits its efficacy but the of off-target platinum–protein interactions is not yet fully understood. These interactions with the HS-groups of GSH are restricted when the metal complexes have S-bonded ligands [4] such as thiones.

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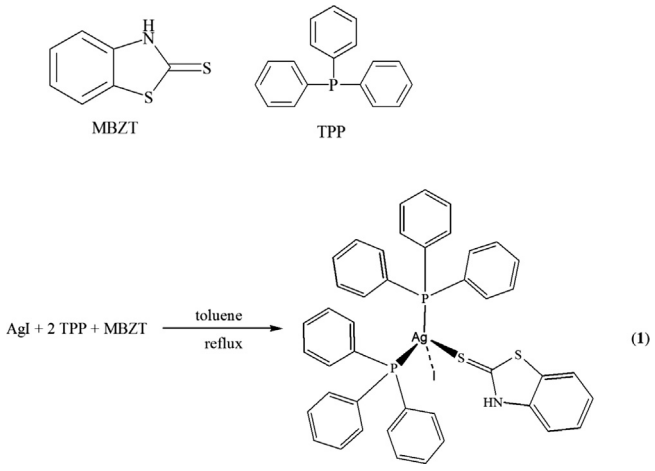
Heterocyclic-2-thiones establish an important class of N,S-donor ligands due to the relevance of their metal complexes in the biological systems [5]. Particularly, 2-mercaptobenzothiazole and its derivatives, are known to possess antimicrobial, anthelmintic, anti-inflammatory (by inhibiting COX and LOX enzymes), and anti-allergic activity [6]. Moreover, 2-mercaptobenzothiazole derivatives show antiproliferative activity against tumour cells [6]. Metal complexes of heterocyclic-2-thiones have been shown to have a variety of biochemical applications: as antifungal, antibacterial, antimicrobial, antibiotic, plant diseases, hepatitis, DNA cleavage [7]. Recently, silver(I) complexes have also been studied for their antitumour activity [8–11].

Breast cancer is a classical model of hormone dependent malignancy [12] and one of the most frequently diagnosed cancers [13]. Especially, estrogen receptors (ERs) are expressed in approximately 65% of human breast cancer, implying that this sex steroid plays an important role in the development and propagation of the disease. Estrogen probably acts as both a growth factor and a survival factor for breast cancer [14]. An antiestrogen drug for the treatment of all stages of breast cancer is tamoxifen. Besides its proven effectiveness, tamoxifen has an important drawback: the limited period of activity before resistance develops. Development of endocrine resistance is one reason that breast cancer is so frequent cause of death in women [15]. High levels of glutathione (GSH) have been found in many tumours that show different degrees of multidrug and/or radiation resistance, including melanoma, carcinoma of the bladder, lung cancer, colon cancer and breast tumour [16]. In breast tumours, the GSH concentration is twice than that more than twice that found in normal breast tissue, and GSH seems to be important in modulating the sensitivity of breast carcinomas to chemotherapeutic drugs [17].

Our studies aim in the development of new metallotherapeutics [9–11,18–20] which overcome the cell resistance while they can interact with ERs causing a selective inhibition of breast cancer cells. In the course of these studies we have synthesized a novel silver(I) iodide complex with the heterocyclic thioamide 2-mercapto-benzothiazole (MBZT) and triphenylphosphine (TPP) (Scheme 1). The complex was characterized by m.p., vibrational spectroscopy (FT-IR), ^{1}H NMR, UV–vis, ESI-MS spectroscopic techniques and X-ray crystallography. Complex **1** was tested for its *in vitro* cytotoxic activity in a panel of cancerous and non-cancerous cell lines. The binding affinity of **1** towards the intracellular molecules CT-DNA and lipoxygenase (LOX) was studied for the evaluation of the mechanism of cell death. The CT-DNA was chosen for this study since its double helix adopts the B form in its configuration [21a] and therefore can be used as a model of the cellular DNA which is also adopts the same configuration in solution, under physiological conditions (neutral pH, room temperature, about 200 mM NaCl) [21b]. The ability to bind to CT-DNA has been tested by Uv–Visible and fluorescence spectroscopies. Kinetics and theoretical studies have shown the influence of complex **1** on the catalytic peroxidation of linoleic acid to hydroperoxylinoleic acid by the enzyme lipoxygenase (LOX). The deactivation of **1** and cisplatin caused by glutathione is also evaluated in this present work.



Scheme 1. The ligands and the preparation reaction of the complex 1.

2. Results and discussion

2.1. General aspects

Complex **1** was synthesized by refluxing an toluene solution of silver(I) iodide, TPP and MBZT in 1:2:1 M ratio (Scheme 1).

Crystals of complex **1** are stable in air but were kept in darkness. Complex **1** was soluble in DMSO and acetone.

In order to exclude that a dissociation of the Ag–P bonds occurs in solution [22], the stability of the complex **1** in DMSO solutions was tested by UV–vis spectra, ^{1}H NMR and conductivity measurements. No changes were observed between the initial UV and ^{1}H NMR spectrum and the corresponding spectra when measured after 96 h (Figs. S1 and S2). In order to assure that no ionic species are formed simultaneously in DMSO solutions, the molar conductance (Λm) values of **1** in DMSO solution (10^{-3} M) were determined (**1**: 4.6 (0 h) and 5.4 (96 h) Ω^{-1} cm^2 mol^{-1}). The experiments were carried out at r.t. These values show that the solutions of the complex is not conducting, confirming their stability in DMSO or DMSO/water media. The molar conductance of the silver(I) nitrate in DMSO was 40 Ω^{-1} cm^2 mol^{-1}, in excellent agreement with values for fully dissociated 1:1 electrolytes [22b]. The period of at least 96 h for the stability testing of the complexes was chosen since biological experiments require 48 h incubation of the cell culture with complexes.

2.2. Vibrational spectroscopy

The vibrational thioamide bands I and II (mainly attributed to ν(C=N) and ν(C–N) vibrations), appear at 1486 and 1321 cm^{-1} in the IR spectrum of complex **1** (Fig. S3) and are shifted as compared to the corresponding ones of the free ligands MBZT (Fig. S4), which are observed at 1497 and 1320 cm^{-1} [19c]. Thioamide bands III–IV (mainly ν(C=S) and ν(C–S) vibrations), were observed at 1013-933 cm^{-1} in the spectrum of the free ligand [19c] and appear at 1012 and 997 cm^{-1} respectively in the spectrum of **1**. The shifts of 10 and 64 cm^{-1} observed in the thioamide bands I and IV are due to the coordination of MBZT towards silver(I) through the S donor atom. The band at 1094 cm^{-1} in the IR spectra of **1** is assigned to the antisymmetric vibrations of the ν(C–P) bond [11b]. Those at 493–513 cm^{-1} are assigned to the symmetric vibrations of the ν(C–P) bond [11b]. The corresponding ν(C–P) bands of the free triphenylphosphine ligand are found at 1089 cm^{-1} for the anti-symmetric vibration and at 491–513 cm^{-1} for the symmetric vibration (Fig. S5).

2.3. Crystal and molecular structure of {[AgI(MBZT)(TPP)$_2$]} (*1*)

Ortep diagram of **1** together with numbering scheme, bond distances and angles is shown in Fig. 1.

There are two symmetry-independent molecules (**A** and **B**) in the asymmetric part of the unit cell of **1**. Two P atoms from the TPP ligands, one S from MBZT and one I atom form the tetrahedral arrangement around the Ag ion. The two Ag–P bond lengths are