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New antibacterial, non-genotoxic materials, derived from the functionalization of the anti-thyroid drug methimazole with silver ions

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ABSTRACT

The new silver(I) compound $\{[AgBr(\mu_2\text{-S-MMI})(TPP))]_2\}$ (**1**) and the known one $[AgCl(TPP)_2(MMI)]$ (**2**) were obtained by refluxing toluene solutions of silver(I) halide with triphenylphosphine (TPP) and the anti-thyroid drug 2-mercapto-1-methyl-imidazole or methimazole (MMI). The complexes were characterized by m.p., vibrational spectroscopy (mid-FT-IR), ^1H, ^{31}P-NMR, UV–Vis spectroscopic techniques and X-ray crystallography. The antibacterial effect of **1** and **2** against the bacterial species *Pseudomonas aeruginosa* (PAO) and *Escherichia coli* was evaluated. Compound **1** exhibits comparable activity to the corresponding one of the silver nitrate which is an antibacterial drug in use. The *in vivo* genotoxicity of **1–2** by the mean of *Allium cepa* test shows no alterations in the mitotic index values due to the absence of chromosomal aberrations. The mechanism of action of the title compounds is evaluated. The DNA binding tests indicate the ability of the complexes **1–2** to modify the activity of the bacteria. The binding constants of **1–2** towards CT-DNA indicate interaction through opening of the hydrogen bonds of DNA. Docking studies on DNA-complexes interactions confirm the binding of both complexes **1–2** in the major groove of the CT-DNA. In conclusion the silver complex **1** is an anti-bacterial and non-genotoxic material, which can be applied to antibacterial drug in the future.

1. Introduction

The most commonly reported Gram-negative pathogens associated with nosocomial infection are *Escherichia coli* (*E. coli*) followed by *Pseudomonas aeruginosa* (*P. aeruginosa*) [1]. Silver ions, on the other hand, have been demonstrated to be exhibit high biocidal activity against bacteria, including *E. coli* and *P. aeruginosa* [2]. The Ag(I) saccharinate complexes, $[Ag_2(sac)_2(\mu\text{-dppm})H_2O]\cdot H_2O$ (where sac is saccharinate and dppm is 1,1-bis(diphenylphosphino)methane) shows high antibacterial activity against standard bacterial strains such as *Staphylococcus aureus*, *E. coli* and *Salmonella typhimurium*, and its activity was much higher than that of silver nitrate and silver sulfadiazine, as well as other well-known broad-spectrum antibitiotics such as ciprofloxacin and gentamicin [3]. Especially, silver compounds are antimicrobial agents in use for the treatment of wound burns, as coatings for catheters and endotracheal tubes, and as disinfectants [2]. Thus, the design and development of new anti-microbial agents against *P. aeruginosa* and *E. coli* is of great importance for the treatment of the nosocomial infections given the drug resistance which is developed by the microbes.

The antimicrobial activity of silver is related to its interaction with nucleic acids, especially, in the cytoplasm where it interacts with condensed DNA molecules, which they subsequently are losing their ability to replicate [4,5]. Moreover, silver ions interact with the cell membrane and/or they interfere with the electron transport system of the cell by reacting with the thiol (sulfhydryl) groups of proteins or with the key functional groups of enzymes [4,6]. Generally, Ag(I) causes (i) protein dysfunction by forming covalent bonds with S; (ii) produce reactive oxygen species (ROS) which results in the depletion of antioxidants reserves such as glutathione, within especially in the case of *E. coli* and (iii) they have been shown to impair membrane function [7].

The efficacy of a silver-drug as antibacterial agent is its bioavailability which must be slow and continuous for an appropriate time in the affected area [6]. The slow release of Ag(I) is closely related to the ancillary ligands chosen, which can play an important role in stabilizing the complexes, thus retaining the antibacterial effect over a longer period of time [6]. Tri-arylphosphines are among ancillary ligands, which are used to form stable complexes and to adjust their solubility in both

Abbreviations: CA, Chromosome aberrations; CBZ, 3-methyl-2-thioxo-4-imidazoline-1-carboxylate (carbimazole); CMBZT, 5-chloro-2-mercaptobenzothiazole; dppm, 1,1-bis(diphenylphosphino)methane; *E. coli*, *Escherichia coli*; H_2stsc, $\{(2\text{-HO-}C_6H_4)(H)C^2 = N^3\text{-}N^2H\text{-}C^1(=S)N^1H_2\}$; Hptsc, pyrrole-2-carbaldehyde thiosemicarbazone; MBZT, 2-mercaptobenzothiazole; MI, mitotic index %; MMI, 2-mercapto-1-methyl-imidazole or methimazole; MN, Micronucleus; MTZD, 2-mercaptothiazolidine; NA, Nuclear abnormalities; *P. aeruginosa*, *Pseudomonas aeruginosa*; PTU, 6-*n*-propyl-thiouracil; pySH, pyridine-2-thione; ROS, reactive oxygen species; sac, saccharinate; SAR, Structure Activity Relationship; TPP, triphenylphosphine.

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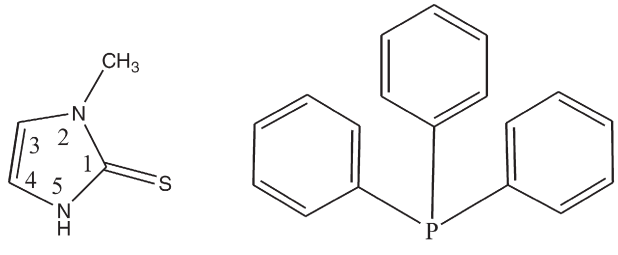
water and organic media. The silver nitrate on the other hand, becomes chemically consumed and it is rapidly inactivated through the formation of chemical complexes by chloride within few hours due to high solubility in water with the simultaneous very low lipophilicity [8].

Heterocyclic-2-thiones are important class of Y, S donor (Y = heteroatom) ligands because of the relevance of their metal complexes in the biological systems [9]. Metal derivatives of heterocyclic-2-thiones have shown a variety of biochemical applications, among this the antibacterial activity [9]. N-methyl-imidazole or methimazole (MMI) is an anti-thyroid drug, which contains the thioamide group (N–C–S) and is one of the most commonly employed anti-thyroid drug (with the 6-*n*-propyl-thiouracil (**PTU**) and 3-methyl-2-thioxo-4-imidazoline-1-carboxylate (carbimazole) (**CBZ**)) [10].

Recently, the mixed-ligand silver(I) complexes of formulae $[AgCl(TPP)_2(MTZD)]$, $\{[AgCl(TPP)_2(MBZT)]\cdot(MBZT)_2\cdot(toluene)\}$ and $[AgCl(TPP)_2(CMBZT)]$, (TPP = triphenylphosphine and MTZD = 2-mercaptothiazolidine, MBZT = 2-mercaptobenzothiazole or CMBZT = 5-chloro-2-mercaptobenzothiazole) were synthesized and fully characterized [5]. DNA binding tests indicate that the complex $[AgCl(TPP)_2(CMBZT)]$ interacts strongly with intercalation or electrostatic interactions with DNA. Moreover, the complex $[AgCl(TPP)_2(MTZD)]$ exhibits the strongest antibacterial activity against the bacterial strains *P. aeruginosa* and *E. coli*. Alterations, in the antimicrobial activity exhibited by these complexes were assigned in either to the variable resistance of the bacterial species against these complexes or differences in basal uptake rates and efflux rates, which are following the variations in the volumes of the Hirshfeld surfaces [5].

During our studies on the development of new metallotherapeutics [4–5,8,11–18] which would be able to overcome the cell resistance while they can still interact with intracellular components leading to cell inhibition, we have synthesized the silver(I) halide complexes with triphenylphosphine (TPP) and the heterocyclic thioamides: N-methyl-imidazole or methimazole (MMI) (Scheme 1) of formulae $\{[AgBr(\mu_2\text{-S-MMI})(TPP))]_2\}$ (**1**) and $[AgCl(TPP)_2(MMI)]$ (**2**) [19]. Although the $\{[Ag(\mu_2\text{-Br})(MMI)(TPP))]_2\}$ is reported by Lobana et.al [19] however, upon modification of the preparation conditions its geometrical isomer $\{[AgBr(\mu_2\text{-S-MMI})(TPP))]_2\}$ (**1**) is obtained. The complexes were characterized by m.p., vibrational spectroscopy (mid-FT-IR), ^1H NMR, UV–vis spectroscopic techniques and X-ray crystallography. Although, the crystal structure of **2** is identical with the previously reported [19], we proceeded with its refinement once again for comparison with that of **1**. Moreover, the used of toluene instead of acetonitrile afforded **2** reducing the reaction time from 24 h to 30 min. The binding affinity of **1–2** towards the CT-DNA was studied by UV–Visible spectroscopy for the evaluation of the mechanism of microbes' inhibition. The antibacterial effect of **1–2** against the bacterial species *P. aeruginosa* and *E. coli* is also evaluated. The *in vivo* genotoxicity of **1–2** was evaluated by the mean of *Allium cepa* test. The mechanism of action of the title compounds is evaluated by measuring their binding constancies towards CT-DNA.



MMI **TPP**

Scheme 1. Molecular diagrams of the ligands used.

2. Results and discussion

2.1. General aspects

Complexes **1–2** are readily synthesized by refluxing toluene suspensions of AgX (X = Br (1) or Cl (2)), TPP and MMI in 1:1:1 (**1**) or 1:2:1 (**2**) molar ratios for 3 (**1**) or 2 (**2**) hrs respectively (Scheme 2). Crystals of **2** were prepared previously by Lobana et al. [19]. According to the procedure followed in that case, the silver(I) chloride reacts with twofold amount of TPP in acetonitrile under 24 h stirring [19]. The white solid formed it reacts with MMI in chloroform and the product precipitated is crystallized from dichloromethane/acetonitrile

However, when AgBr is used under the same reaction conditions the dimmer $\{[Ag(\mu_2\text{-Br})(MMI)(TPP))]_2\}$ was isolated where the two Br atoms are acting as bridging atoms between the two monomeric units $\{[Ag(\mu_2\text{-Br})(MMI)(TPP))]$ (Scheme 2) [19]. In the contrary, in our case of $\{[AgBr(\mu_2\text{-S-MMI})(TPP))]_2\}$ (**1**) the two units are bridged by two sulfur atoms from MMI ligands (Scheme 2). Both structures are further stabilized by H[N]···Br hydrogen bonding interactions (Scheme 2).

Although the crystal structure of **2** is already known [18], the differences observed between the unit cells (**2:** space group: $P2_1/c$; a = 14.29170(10), b = 10.34660(10), c = 25.02640(10) Å, β = 91.53°, instead of (space group: $P2_1/c$; a = 14.3440(2), b = 10.236(2), c = 24.799(1) Å, β = 92.018(1)°). [19]) and the modification in the synthetic procedure prompt us to re-determine its crystal structure here. Compound **2** is synthesized, here, in high yield, in one step. The full re-refinement of its structure has been proceeding for comparison with the structures of **1**, while the geometrical data are also required for the docking studies (See computational part). Crystals of complexes **1–2** are stable in air but were kept in darkness. Complexes **1–2** were soluble in toluene and DMSO.

2.2. Solid state studies

2.2.1. Vibrational spectroscopy

The vibrational thioamide bands I and II, appear at 1574–1469 (**1**) and 1574–1455 (**2**) cm^{-1} in the IR spectra of the complexes **1–2** (Figs. S1–S2), which are attributed to the ν(C═N) and ν(C–N) vibrations mainly undergo shift as compared to the corresponding vibrational bands of the free ligands, which are observed at 1569–1460 cm^{-1} respectively [20]. Thioamide bands III–IV attributed to the ν(C═S) and ν(C–S) vibrations were observed at 1272–670 cm^{-1} respectively in the spectra of the free ligand and at 1282–670 (**1**) and 1083–675 (**2**) cm^{-1} respectively, in the spectra of complexes **1–2** [20]. The band at 1095 cm^{-1} in the IR spectra of **1–2** is assigned to the antisymmetric ν(C–P) vibrations and those at 507 (**1**) and 506 (**2**) cm^{-1} respectively to the symmetric ν(C–P) vibrations of **1–2**. The corresponding ν(C–P) bands of the free triphenylphosphine ligand are observed at 1088 cm^{-1} for the antisymmetric vibration and at 509 cm^{-1} for the symmetric vibration.

2.2.2. Crystal and molecular structures of $\{[AgBr(\mu_2\text{-S-MMI})(TPP))]_2\}$ (**1**) and the known one $[AgCl(TPP)_2(MMI)]$ (**2**)

Crystals of compounds **1–2**, suitable for single crystal X-ray analysis were grown by slow evaporation of the liquid remained after filtration of the initial crops of solid material from the reactions of silver(I) halides with the 2-mercapto-1-methyl-imidazole or methimazole (MMI) and triphenylphosphine (TPP) in a molar ratio of 1:1:1 (**1**) or 1:1:2 (**2**) respectively, in toluene. ORTEP diagrams of **1–2** along with their selected bond distances and angles are shown in Figs. 1–2.

Two isomeric complexes (**1A** and **1B**) are included in the unit cell of **1**. Complex **1** is di-nuclear with two silver ions under tetrahedral geometrical environment around each metal center consisting of one P from a TPP ligand, one Br and two μ_2-S atoms from MMI ligands (Fig. 1). Dimmer silver halide compounds of thiones, where the two Ag(I) ions

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