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SYNTHESIS OF Ag(I) AND Cu(I) COMPLEXES WITH 4-AMINO-5-METHYL-2H-1,2,4-TRIAZOLE-3(4H)-THIONE LIGAND AS THIOCARBOHYDRAZIDE DERIVATIVES AND THEIR ANTIMICROBIAL ACTIVITY

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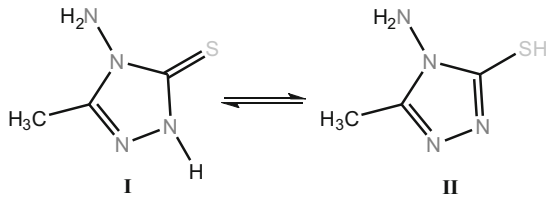
The antimicrobial activity of 4-amino-5-methyl-2H-1,2,4-triazole-3(4H)-thione (AMTT) and its silver(I) and copper(I) complexes has been investigated. Results show that MIC50 and MIC90 of the organic ligand against all test strains were higher than MIC50 and MIC90 of both complexes. The minimum MIC50 of the silver complex, copper complex, and the ligand against *Candida albicans* and *Aspergillus niger* were 5.6, 10.3, and 12.5 μg/mL, respectively. The MIC50 and MIC90 of the silver complex were lower than those of the copper complex against *Candida albicans, Aspergillus niger*, *Escherichia coli,* and *Pseudomonas aeruginosa*. At the same time, the MIC50 of the silver complex is higher than that of the copper complex against *Staphylococcus aureus.*

Keywords: Thiocarbohydrazide; N,S donor ligand; Ag (I) complex; Cu(I) complex; antimicrobial activity.

INTRODUCTION

1,2,4-Thiotriazines and 1,2,4-thiotriazoles are well known heterocyclic thiones derived from thiocarbohydrazide. Some of their derivatives exhibit biological activity and have been used for various purposes such as herbicides, neutral antibiotics, antibacterial agents, etc. [1 – 3]. The heterocyclic thiones exist in thione and thiol tautomeric forms. Therefore, there has been considerable interest in studying the coordination properties of both the neutral thione and the deprotonated thiol ligand and various binding modes to the metal atom, including S monodentate, N,S chelating, μ_n N,S bridging etc. [4, 5]. Metal complexes with thiosemicarbazone and thiocarbohydrazide derivatives possess a wide range of biological activity and are among the most potent antiviral, antitumor, and ant-inflammatory agents. Antitumor activity is one of the main findings for thiosemicarbazones and their metal complexes [6 – 8].

Because of the high tendency of silver(I) and copper(I) to form metal–sulfur bonds due to the Pearson principle, we are interested in studying the behavior of these ions towards 1,2,4-thiotriazine and 1,2,4-thiotriazole derivatives. In a series of studies, we have investigated the syntheses and crystal structures of several silver(I) and copper(I) complexes with these ligands [9 – 13]. In the present paper, we report the biological evaluation of Cu(I) and Ag(I) complexes with 4-amino-5-methyl-2H-1,2,4-triazole-3(4H)-thione (AMTT) (Scheme 1) derived from thiocarbohydrazide. Synthesis and characterization of $[Ag(AMTT)_2]NO_3$ and $[Cu(AMTT)]_nCl_n$



Scheme 1. Thionic (I) and thiolic (II) forms of 4-amino-5-methyl-2H-1,2,4-triazole-3(4H)-thione.

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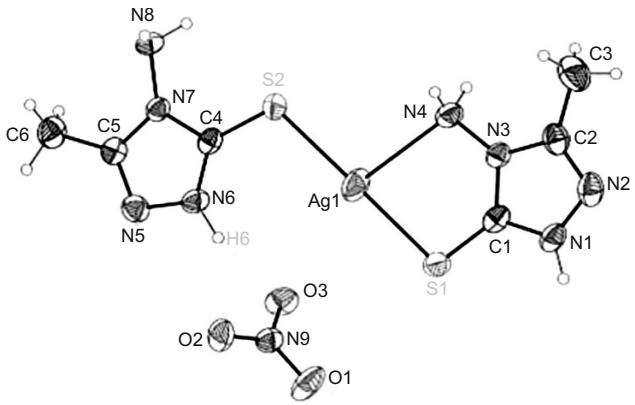

Fig. 1. Molecular structure of $[Ag(AMTT)_2]NO_3$ [29].

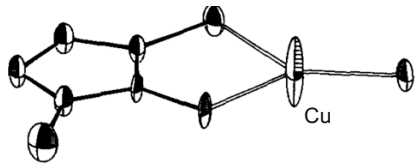

Fig. 2. Molecular structure of $[Cu(AMTT)]_nCl_n$ [30].

complexes have been reported by Yazdanbakhsh, et al. [14] and Cingi, et al. [15], respectively.

EXPERIMENTAL

General

All purchased chemicals were of reagent grade and used without further purification. IR spectra were recorded using Spectra Bruker Tensor 27 FTIR spectrometer (KBr pellets, nujol mulls, 4000 – 400 cm^{-1}). Elemental analyses were performed using a Costech ECS 4010 CHNS analyzer.

Synthesis of Complexes

4-Amino-5-methyl-2H-1,2,4-triazole-3(4H)-thione (AMTT) was prepared according to reported procedures [16] and $[Ag(AMTT)_2]NO_3$ and $[Cu(AMTT)]_nCl_n$ complexes were synthesized according to literature [14, 15].

$[Ag(AMTT)_2]NO_3$. $AgNO_3$ (0.17 g, 1 mmol) was added to a solution of AMTT (0.26 g, 2 mmol, 50 mL) in methanol and stirred for 2.5 h at room temperature. The solid crude was filtered. The filtrate was kept at 20°C, and colorless crystals of $[Ag(AMTT)_2]NO_3$ were separated. IR spectrum (cm^{-1}): 1585 sec (ν CN), 1474 sec (ν as NO_3), 1390 w (δ as NO_3), 1360 sh (δ s NH2), 1349 m (ν s NO3), 1089 m (ρ NH2), 1012 m (ρ CH3), 815 mw (δ NO3), 651 m (ρ NO3).

$[Cu(AMTT)]_nCl_n$. AMTT (0.66 g, 5 mmol) was added to a solution of 0.41 g (2.0 mmol) of $CuC1_2 \cdot 2H_2O$ in 60 mL of H_2O. The reaction mixture was stirred for 5 min at room temperature and then a solution of 5.5 M HCI was added dropwise until the dark solution turned to yellow-brown. The crystals of Cu complex were filtered and washed with ethanol. IR spectrum (KBr disc; ν, cm^{-1}): 3200 – 2900 (b) (ν NH_2 and NH), 1550 (ν CH), 2970 w (ν_{as} CH), 2920 w (ν_s CH), 1638 sec, 1352 sec, 1292 m (C band), 1204vs.

In Vitro Screening of Compounds for Antimicrobial Activity

To evaluate of antimicrobial activity of the ligand and its complexes, broth microdilution method was used in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Five standard strains including *Staphylococcus aureus* (*S. aureus*) ATCC 25923, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 25682, *Escherichia coli* (*E. coli*) ATCC 25922, *Candida albicans* (*C. albicans*) ATCC 10231, and *Aspergillus niger* (*A. niger*) ATCC 16888 obtained from Iranian Research Organization for Science and Technology. Fungal isolates were incubated on Sabouraud dextrose agar at 25°C, and bacterial strains were incubated on nutrient agar at 35°C for 48 hours. Then, microbial suspensions (adjusted at 2×10^4 cells/mL) were separately prepared in the RPMI1640 plus 2% glucose medium. In the next step, 100 µL of ligand and its complexes at serial concentrations were separately incubated with 100 µL of microbial suspension. Then, fungal and bacterial strains were incubated at 25°C and 35°C for 48 h, respectively. In this study, microbial suspensions not treated with ligand and its complexes were considered as negative control. In separate experiments, fungal strains were exposed to 2 µg/mL nystatin, and bacterial strains were treated with 1 µg/mL ceftriaxone. Finally, the optical density (OD) of each well was read at 405 nm by ELISA reader (NovinGostar, Iran), and minimum inhibitory concentrations (MICs) of the ligand and its complexes against different isolates were measured.

RESULTS AND DISCUSSION

As shown in Figs. 1 and 2, 4-amino-5-methyl-2H-1,2,4-triazole-3(4H)-thione exhibits different binding modes to Cu(I) and Ag(I) atoms. AMTT binds to Ag(I) as an S monodentate ligand and to Cu(I) as an N,S chelating ligand.

Antibacterial Activity

Table 1 shows the values of MIC50 and MIC90 of silver and copper complexes and the ligand against microbial strains. It can be seen that the MIC50 and MIC90 of the organic ligand against all strains was higher than MIC50 and MIC90 of both silver and copper complexes, which indicated the higher antimicrobial effect of complexes vs. organic ligand. It should be noted that the minimum MIC50 for the silver complex, copper complex, and the ligand against *C. albicans and A. niger* were 5.6 , 10.3 , and 12.5 µg/mL, respectively. It was shown than the MIC50 and MIC90 of the silver complex were smaller than those of the copper complex against *C. albicans, A. niger*, *E. coli,* and *P. aeruginosa*.

TABLE 1. MIC50 and MIC90 of the silver complex, copper complex, and ligand (AMTT) against Bacterial and Fungal Strains

Test microbe	Nitrogenous ligand		Ag complex		Cu complex	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Aspergillus niger	12.5	>200	5.6*	>90	10.3	>166
Candida albicans	12.5	>200	5.6*	41.5	10.3	>166
Escherichia coli	200	>200	22.5*	>90	41.5*	>166
Staphylococcus aureus	100	>200	>90	>90	20.7*	>166
Pseudomonas aeruginosa	100	>200	22.5*	>90	41.5*	>166

* $p < 0.05$ compared to the MIC50 of ligand, against similar strains.

Exceptionally, in case of *S. aureus,* the MIC50 of the silver complex was higher than that of the copper complex.

Therefore, the Ag complex has a higher antibacterial activity than the Cu complex and the ligand (AMTT) except the case of *S. aureus,* for which the MIC50 of the silver complex is higher than that of the Cu complex.

It can be concluded that the Ag complex has a higher antimicrobial activity than the Cu complex and the ligand (AMTT) against all microbial strains in this study and may be used as the disinfectant to remove different microbes on various surfaces.

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