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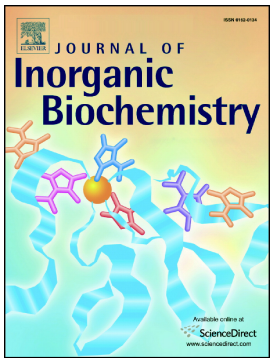

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Antibacterial activity of metal complexes based on cinnamaldehyde thiosemicarbazone analogues

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Short title: Antibacterial activity of cinnamaldehyde thiosemicarbazone analogues

Abstract

The development of microbial antibiotic resistance has become one of the biggest threats to global health and the search for new molecules active against resistant pathogenic strains is a challenge that must be tackled. In many cases nosocomial infections are caused by bacteria characterized by multi-drug resistance patterns (MDR) and by their ability to produce biofilms. These properties lead to the persistence of pathogens in the hospital environment. This paper reports the synthesis and characterization of three thiosemicarbazone derivatives based on a compound containing the cinnamaldehyde natural scaffold but possessing different logPow values. These molecules are then used as ligands to prepare complexes of the Cu(II) and Zn(II) ions. All these compounds, ligands and complexes, were screened *in vitro* on stains of *Escherichia coli* and *Klebsiella pneumoniae* for their antibacterial activity. Despite their molecular similarity they revealed variegated behaviors. Only two of them present interesting antimicrobial properties and have also been studied to verify their stability in solution. The compound with the lowest partition coefficient is the most promising. The MBC on *K. pneumoniae* and *E. coli* of these substances are very interesting and demonstrate that the use of metalloantibiotics is a promising device to fight antibiotic resistance.

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Keywords: thiosemicarbazone, zinc, copper, cinnamaldehyde, *Escherichia coli*, *Klebsiella pneumoniae*

1. Introduction

Antibiotic resistance today is one of the biggest threats to global health and food security and the development of new antibiotics or molecules that present activity against pathogenic strains is becoming more and more urgent. In many cases nosocomial infections are caused by bacteria characterized by multi-drug resistance patterns (MDR) and by the ability to produce biofilm, all this leading to the persistence of pathogens in the hospital environment. The WHO reports that very year in the European Union alone, about 25000 patients die for resistant bacterial infections acquired in hospitals. Also in the USA the cost for the management of hospital-acquired infections is extremely high, reaching US$ 28-34 billion [1] and, therefore, the interest in the development of alternative therapies and of new molecules effective in combating infections is increasing day by day.

Biofilm production is a survival strategy that bacteria use to maintain stable growth in stressful environmental conditions. A biofilm has been defined as "microbial communities that live together in a self-produced extracellular polymeric matrix consisting of exopolysaccharides (EPS), proteins and DNA adherent to abiotic surfaces"[2]. EPS confer structure complexity and a physical barrier that stops drugs from reaching their bacterial target and limits the action of disinfectants and detergents [3, 4]. At the same time it allows the exchange of genetic material among microorganisms, favoring the dissemination of genes and plasmids associated to the antibiotic resistance phenomenon. Biofilm formation ability by drug-resistance bacteria may result in an increased tolerance to toxic substances[3], and many biofilm producers show high resistance levels towards antibiotics[5-7]. In this perspective, studying and developing new molecules effective against biofilm producing bacteria is an important target in medicinal chemistry.

Transition metal complexes present characteristics that make them good candidates as antimicrobial agents. It should be noted that the properties required for metal complexes with biological properties are: an adequately high thermodynamic stability to transport the metal to the active site, a good hydrolytically stability, a proper molecular weight. In fact low molecular weight compounds with no charge and very low water solubility have the advantage to be able to cross biological membranes by passive diffusion[8]. Moreover, the ultimate goal of antimicrobials is the highest efficacy at the lowest dosage without allowing resistance. A renewed interest in metals as antimicrobial and biocidal agents is reflected in hopes that they may prevent resistance [9]. Interdisciplinary research is promoting not only our understanding of metal toxicity but also the design of metal-based compounds for use as antimicrobial agents and as alternatives to traditional antibiotics [10].

As representative of antimicrobial resistant species, we then selected two bacterial strains commonly found in the environment, usually commensal of the intestinal and respiratory districts, characterized

by the ability to form biofilm (*Escherichia coli*) and by the synthesis of Extended-Spectrum-Beta-Lactamases enzymes (*Klebsiella pneumoniae*), that confer them resistance to third generation cephalosporins. They are Gram-negative bacteria, part of the *Enterobacteriaceae* family. *E. coli* was an intestinal commensal part of the natural microflora of animals and humans, but it can also cause illnesses as gastroenteritis as well as extra-intestinal infections mainly at the urinary tract, at respiratory district, at gallbladder. Infections sometimes could evolve to septicaemia and meningitis. *E. coli* is a widespread bacteria and its transmission to humans or animals is mainly due to the ingestion of contaminated water or raw foods (oral-faecal route). *K. pneumoniae* is a commensal of intestinal and respiratory tract and can be responsible for pneumonia, infections of the urinary tract, of wound infections but it can cause also sepsis. Both bacteria are characterised by the presence of a capsule that they use to resist against phagocytosis.

Following our interest in the compehension of the biological activity of coordination compounds based on thiosemicarbazones [11-15], recently, antimicrobial activity of Cu(II) and Zn(II) complexes with aromatic N-substituted thiosemicarbazones have been reported [16]. In a previous study of ours on the antimycotic activity of a series of thiosemicarbazones, cinnamaldehyde thiosemicarbazone and its copper complex [15] showed a promising activity with no deleterious effects on human healthy cells. We have therefore embarked on a new research to verify if they also possess antibacterial properties. Three analogues with different logPow were synthesised to study the role of the polarity on their biological effects. Given the preliminary antimycotic activity shown by the bis(cinnamaldehydethiosemicarbazonato)copper(II) complex, we have also extended the synthesis to the other copper complexes and to the analogous zinc derivatives. We thus obtained a series of nine compounds which differ for the polarity of the ligand and the redox nature of the metal centre.

2. Experimental

2.1 Chemistry

2.1.1 Syntheses and characterizations

All reactants used were purchased form Sigma Aldrich. The ^{1}H-NMR spectra were recorded on a Bruker Anova spectrometer at 400 MHz. The FT-IR measurements were recorded on a Nicolet 5PC FTIR analysing products directly on the ATR accessory in the 4000–400 cm^{-1} range. The relative intensity of reported FT-IR signals are defined as s = strong, m = medium, and w = weak. Melting points were determined with a Gallenkamp instrument (Weiss-Gallenkamp). ESI-MS analysis were

performed using a Waters Acquity Ultraperformance ESI-MS spectrometer with Single Quadrupole Detector.

2.1.1.1 Preparation of the ligands

The desired thiosemicarbazones were obtained by mixing an equimolar amount of thiosemicarbazide with the proper aldehyde in absolute ethanol. The mixture was refluxed under stirring for 8 hours and left overnight at 0°C. The precipitate was filtered out, washed with cold ethanol and dried under vacuum.

(E)-cinnamaldehydethiosemicarbazone (1)

Thiosemicarbazide (0.34 g, 3.8 mmol), (E)-cinnamaldehyde (0.50 g, 3.8 mmol). White powder. Yield: 96%. Mp: 139 °C. FT-IR (cm^{-1}): 1533 (s), 1254 (w), 966 (m). 1H-NMR (δ, ppm; DMSO-d6): 6.87 (dd, J = 16.1, J' = 9.2 Hz, 1H), 7.04 (d, J = 16.1 Hz, 1H), 7.34 (t, J = 8.2 Hz, 1H), 7.39 (t, J = 8.2 Hz, 2H), 7.58 (d, J = 8.2 Hz, 2H), 7.61 (s, 1H), 7.92 (d, J = 9.2 Hz, 1H), 8.18 (s, 1H), 11.40 (s, 1H). ESI-MS (+) m/z calc. 206.07 found 206.09.

(E)-cinnamaldehyde-4,4-dimethyl-3-thiosemicarbazone (2)

4,4-Dimethyl-3-thiosemicarbazide (0.44 g, 3.8 mmol), (E)-cinnamaldehyde (0.50 g, 3.8 mmol). Orange powder. Yield: 79%. Mp: 139 °C. FT-IR (cm^{-1}): 1545 (m), 1325 (w), 981 (m). 1H-NMR (δ, ppm; DMSO-d6): 3.26 (s, 6H), 6.97 (m, 2H), 7.33 (t, J = 7.2 Hz, 1H), 7.40 (t, J = 7.2 Hz, 2H), 7. 61 (d, J = 7.2 Hz, 2H), 8.04 (dd, J = 6.1 Hz, J' = 2.5 Hz, 1H), 10.83 (s, 1H). ESI-MS (+) m/z calc. 234.33 found 204.57.

(E)-1-(4-methoxyphenyl)-1-pentene-3-one-3-thiosemicarbazone (3)

Thiosemicarbazide (0.10 g, 1.1 mmol), 1-(4 methoxyphenyl)-1-pentene-3-one (0.21 g, 1.1 mmol). Yellow crystals. Yield: 80%. Mp: 187 °C. FT-IR (cm^{-1}): 1577 (m), 1490 (w), 1242 (m). 1H-NMR (δ, ppm; DMSO-d6): 1.13 (t, J = 7.4 Hz, 3H), 2.56 (q, J = 7.4 Hz, 2H), 3.80 (s, 3H), 6.99 (d, J = 8.8 Hz, 2H), 7.10 (d, J = 16 Hz, 1H), 7.50 (d, J = 16 Hz, 1H), 7.59 (s, 1H), 7,74 (d, J = 8.8 Hz, 2H), 8.13 (s, 1H), 10.93 (s, 1H). ESI-MS (+) m/z calc. 264.36 found 264.09.

2.1.1.2 Preparation of the complexes

Synthetic approach used for the synthesis of copper(II) complexes

The appropriate ligand was mixed with copper(II) acetate monohydrate in ethanol with a metal to ligand ratio of 1 : 2. The mixture was left under stirring at room temperature for 2 hours. Usually, it was observed a change in the solution colour during the reaction. Finally, the solvent was removed under reduced pressure and the product was washed twice with diethylether, then dried under vacuum.

Bis-(E)-cinnamaldehydethiosemicarbazonate of Cu(II) (4)

(E)-Cinnamaldehydethiosemicarbazone (**1**) (0.10 g, 0.5 mmol), copper(II)acetate (0.05 g, 0.25 mmol). Brown powder. Yield: 60%. FT-IR (cm^{-1}): 1581 (s), 973 (s). ESI-MS (+) m/z calc. 471.09 found 472.16.

Bis-(E)-cinnamaldehyde-4,4-dimethyl-3-thiosemicarbazonate of Cu(II) (5)

(E)-cinnamaldehyde-4,4-dimethyl-3-thiosemicarbazone (**2**) (0.10 g, 0.4 mmol), copper(II)acetate (0.04 g, 0.2 mmol). Brown powder. Yield: 73%. FT-IR (cm^{-1}): 1507 (m), 1353 (w), 1137 (m). ESI-MS (+) m/z calc. 528.20 found 520.20.

(E)-1-(4-methoxyphenyl)-1-pentene-3-one-3-thiosemicarbazonate of Cu(II) (6)

(E)-1-(4-methoxyphenyl)-1-pentene-3-one-3-thiosemicarbazone (**3**) (0.10 g, 0.4 mmol), copper(II)acetate (0.04 g, 0.2 mmol). Brown powder. Yield: 87%. FT-IR (cm^{-1}): 1598 (m), 1477 (w). ESI-MS (+) m/z calc. 587.25 found 588.26.

Synthetic approach used for the synthesis of zinc(II) complexes

The appropriate ligand was mixed with zinc(II)acetate dihydrate in ethanol with a metal to ligand ratio of 1 : 2. The mixture was stirred at room temperature for 2 hours. The solutions changed colour gradually and the product precipitated during the reaction. Finally, the solid was filtered out, washed twice with diethylether, then dried under vacuum.

Bis-(E)-cinnamaldehydethiosemicarbazonate of Zn(II) (7)

(E)-Cinnamaldehydethiosemicarbazone (0.10 g, 0.5 mmol), zinc(II)acetate (0.05 g, 0.25 mmol). Yellow powder. Yield: 59%. FT-IR (cm^{-1}): 1631 (m), 1460 (s), 1185 (m), 963 (m). 1H-NMR (δ, ppm; DMSO-d6): 7.07 (d, J = 15.3, 1H), 7.13 (d, J = 15.3 Hz, 1H), 7.44 (m, 7H), 8.27 (d, J = 8.0 Hz, 1H). ESI- ESI-MS (+) m/z calc. 474.92 found 473.15.

Bis-(E)-cinnamaldehyde-4,4-dimethyl-3-thiosemicarbazonate of Zn(II) (8)

(E)-cinnamaldehyde-4,4-dimethyl-3-thiosemicarbazone (**2**) (0.10 g, 0.4 mmol), zinc(II)acetate (0.05 g, 0.2 mmol). Yellow powder. Yield: 71%. FT-IR (cm^{-1}): 1579 (m), 1432 (w). 1H-NMR (δ, ppm; DMSO-d6): 2.52 (s, 12H), 7.03 (dd, J = 15.7 Hz, J' = 9.7 Hz, 2H), 7.09 (d, J = 17.7 Hz, 2H), 7. 32 (m, 4H), 7.40 (m, 6H), 8.37 (d, J = 9.7 Hz, 2H). ESI-MS (+) m/z calc. 529.03 found 529.22.

(E)-1-(4-methoxyphenyl)-1-pentene-3-one-3-thiosemicarbazonate of Zn(II) (9)

(E)-1-(4-methoxyphenyl)-1-pentene-3-one-3-thiosemicarbazone (**3**) (0.10 g, 0.4 mmol), zinc(II)acetate (0.04 g, 0.2 mmol). Dark green powder. Yield: 87%. FT-IR (cm^{-1}): 1598 (m), 1477 (w). 1H-NMR (δ, ppm; DMSO-d6): 0.91 (t, J = 7.5 Hz, 6H), 2.73 (m, 2H), 2.91 (m, 2H), 3.78 (s, 6H), 6.82 (d, J = 16 Hz, 2H), 6.96 (d, J = 8.9, 4H), 7.05 (d, J = 16 Hz, 2H), 7.19 (s, 4H), 7.41 (d, J = 8.9 Hz, 4H). ESI-MS (+) m/z calc. 589.08 found 589.30.

2.1.2 Crystallographic data

The crystallographic data of compounds (**1**) and (**2**) were collected with a SMART APEX2 diffractometer with Bruker AXS CCD detector using Mo-Kα radiation and a graphite crystal monochromator [λ(Mo-Kα) 0.71073 Å]. The SAINT [17] software was used for integrating reflection intensities and scaling, and SADABS [18] for absorption correction. The structure was solved by direct methods using SHELXS[19] and refined by full-matrix least-squares on all F2 using SHELXL[20] implemented in the OLEX2 1.2 package [21]. All the non-hydrogen atoms in the molecules were refined anisotropically. The hydrogen atoms were partly found and partly placed in the ideal positions using riding models. The structure was solved by direct methods and difference Fourier synthesis using the SHELX suite of programs as implemented within the OLEX software. Thermal ellipsoid plots were generated using OLEX.

2.1.2.1 (E)-cinnamaldehyde-4,4-dimethyl-3-thiosemicarbazone (**2**)

The crystal system is orthorhombic, space group Pbca; cell parameters: a = 10.9446(5), b = 8.2168(4), c =27.9230(13)Å, ,V = 2511.1(2) $Å^3$. The asymmetric unit is formed by a single molecule of formula C12H15N3S1, Mr = 233.33, Z = 8, Dc = 1.234 g cm^{-3}, μ = 2.35 mm^{-1}, F (000) = 992. A semi-empirical absorption correction, based on multiple scanned equivalent re-flections, has been

carried out and gave 0.6069 < T < 0.7454. A total of 39304 reflections were collected up to a θ range of 32.3° (±16 h,±12 k, ±41 l), 4271 unique reflections (Rint = 0.11). R= 0.0636, wR2 = 0.1594. All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were placed in ideal positions and refined using riding models.

2.1.2.2 (E)-1-(4-methoxyphenyl)-1-pentene-3-one-3-thiosemicarbazone (**3**)

The crystal system is monoclinic, space group Cc; cell parameters: a = 11.364(8), b = 13.751(10) (2), c =8.695(6)Å, β = 91.824(14)°,V = 1358.2(17)Å^3. The asymmetric unit is formed by a single molecule of formula C13 H17 N3 O1 S1, Mr = 263.37, Z = 4, Dc = 1.288 g cm^{-3}, μ = 2.31 mm^{-1}, F (000) = 560. A semi-empirical absorption correction, based on multiple scanned equivalent reflections, has been carried out and gave 0.6063 < T < 0.7454. A total of 7034 reflections were collected up to a θ range of 26.69° (±14 h,±17 k, ±10 l), 2827 unique reflections (Rint = 0.07). R= 0.0507, wR2= 0.1135. All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were placed in ideal positions and refined using riding models.

CCDC1940625-1940626 contain the supplementary crystallographic data (http://www.ccdc.cam.ac.uk/data_request/cif; see also ESI).

2.1.3 Stability in solution

Due to their promising antibacterial activity, the stability of complexes **7** and **4** was evaluated in solution by monitoring their UV-vis absorption spectra with time. It was considered stable a complex which did not change significantly its profile in a 24 hours period incubated at 37°C. The solvent used was DMSO, the medium utilised to dissolve the samples for the biological tests. A 100 μM solution of complex in DMSO was prepared and the corresponding absorption spectra were recorded to identify the wavelength range to be used for the stability evaluation. For each complex three different wavelengths were identified: two associated with the absorption maxima and one from the baseline as a control. Once fixed these wavelengths, the corresponding absorbances were recorded every 15 minutes for 24 h and the values were plotted versus time to obtain the stability profiles. The temperature was fixed at 37°C and kept stable during the entire experiment.

2.1.4 Partition coefficient calculation

Log Pow for the ligands were calculated according to ref [22, 23].

2.2 Biology

2.2.1 Bacterial strains selection

Escherichia coli and *Klebsiella pneumoniae* were chosen as models to test the molecules. In this case we selected a common *E. coli*, non-pathogenic (phylogenetic group A), isolated from poultry meat in the laboratory of Food Inspection of Parma University characterized by the ability to produce biofilm and a strain of *K. pneumoniae* (ATCC 700603) for its ability to produce Extended-Spectrum-Beta-Lactamases (ESBL) encoded by SHV-18 gene.

Frozen stock bacteria were put into 2 mL of Buffered Peptone Water (BPW), and incubated for 2 h at 37°C. Then, an aliquot was seeded with a sterile calibrated loop on a generic agar plate (Tryptic Soy Agar, TSA). After an incubation at 37°C for 24 h, bacterial colonies were obtained which were used for the following experiments.

2.2.2 Study of the antimicrobial effect of the molecules

All the molecules were tested against both *E. coli* and *K. pneumoniae* for the definition of the Minimum Bactericidal Concentration (MBC) as described by the CLSI protocol (2006). MBC is the lowest concentration of a drug/broth dilution of antimicrobials that results in killing 99.9% of the bacteria being tested. MBC is determined by the micro-dilution method and it is measured by sub-culturing the broth dilutions that inhibit growth of a bacterial organism (i.e., those at or above the MIC- Minimal Inhibitory Concentration) determination onto generic fresh agar plates. The MBC is the lowest broth dilution of antimicrobial that prevents growth of the organism on the agar plate. Failure of the organism to grow on the plate implies that only nonviable organisms are present.

In the first step, we diluted the original molecules prepared in DMSO (10 mM)1:100 with sterile water. Then, in 96-well-plates we made serial two-fold dilutions in water from 50 µM to 24.4 NM (final concentration) and we added the bacterial solution at the concentration of 0.5 McFarland (~ $1.5x10^8$ cells/mL). In this way in each well the final volume is composed by 100 µL of molecules at different concentrations + 100 µL of bacterial solution. Micro-dilution plates were incubated at 37°C for 24 h. This part was repeated for each molecules and for both bacterial strains. Positive (bacterial solution) and negative (water and diluted molecules) controls were added in each plate.

The day after, 50 µL of each overnight cultures were seeded onto Tryptic Soy Agar (TSA) (a nonselective media that allow the growth of a wide variety of microorganisms) using a L-spatula in

double and plates were incubated overnight at 37°C. After 24 h plates were read and the MCB was defined following the scheme reported by CLSI (2006).

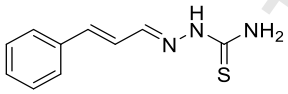
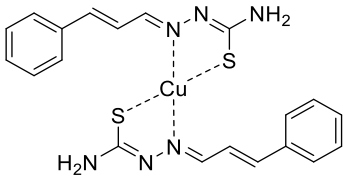
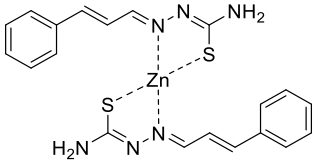
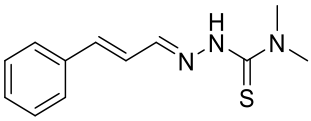
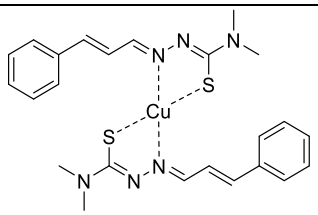
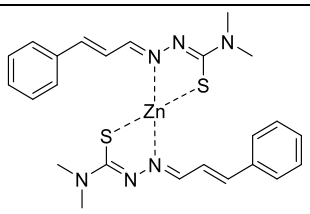
The first screening test using all nine molecules onto both bacterial strains was repeated twice. For the molecules that showed efficacy against both strains, we determined a MBC, a concentration value confirmed to be effective in reducing 99.9% of bacterial growth in at least three experiments.

The same method was used to evaluate if the single components of the molecules were active towards the two bacterial strains (metal ions, thiosemicarbazides, and aldehydes). In this way we may exclude, or not, that the bactericidal action is due to the single parts of each compound, if eventually it brakes, ant not to the compounds themselves.

3. Results and Discussion

3.1 Syntheses

(*E*)-cinnamaldehydethiosemicarbazone (**1**) was prepared according to a synthetic procedure previously described [24] and the other ligands were synthesized in a similar way. The compounds were obtained in good yields and with a good grade of purity as confirmed by TLC, NMR and X-Ray data. The metal complexes were obtained as described in the experimental section. A colour change of the solution containing the ligands and the copper ions was already significative of complexation. Spectroscopic and spectrometric data of the solid obtained confirmed that the isolated product was the desired one. Table 1 reports a scheme of the isolated products.

1 	**4** 	**7** 
2 	**5** 	**8** 

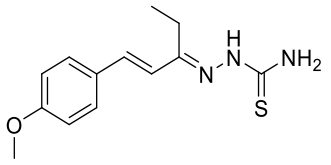
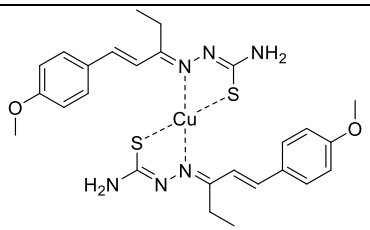
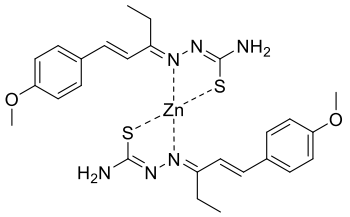
3		**6**		**9**	

Table **1**. Scheme of the isolated ligands (1-3), copper(II) (4-6) and zinc(II) (7-9) metal complexes.

3.2 Stability in solution

The spectroscopic results are reported in Figure **1**. In both complexes the stability profiles did not show significant changes during the 24 hours of the experiment. In detail, **7** showed a slight decrease of absorbance at 400 nm during the first 2 hours, however this effect did not last and the stability of the values from 2 to 24 hours ensured the stability of the complex.

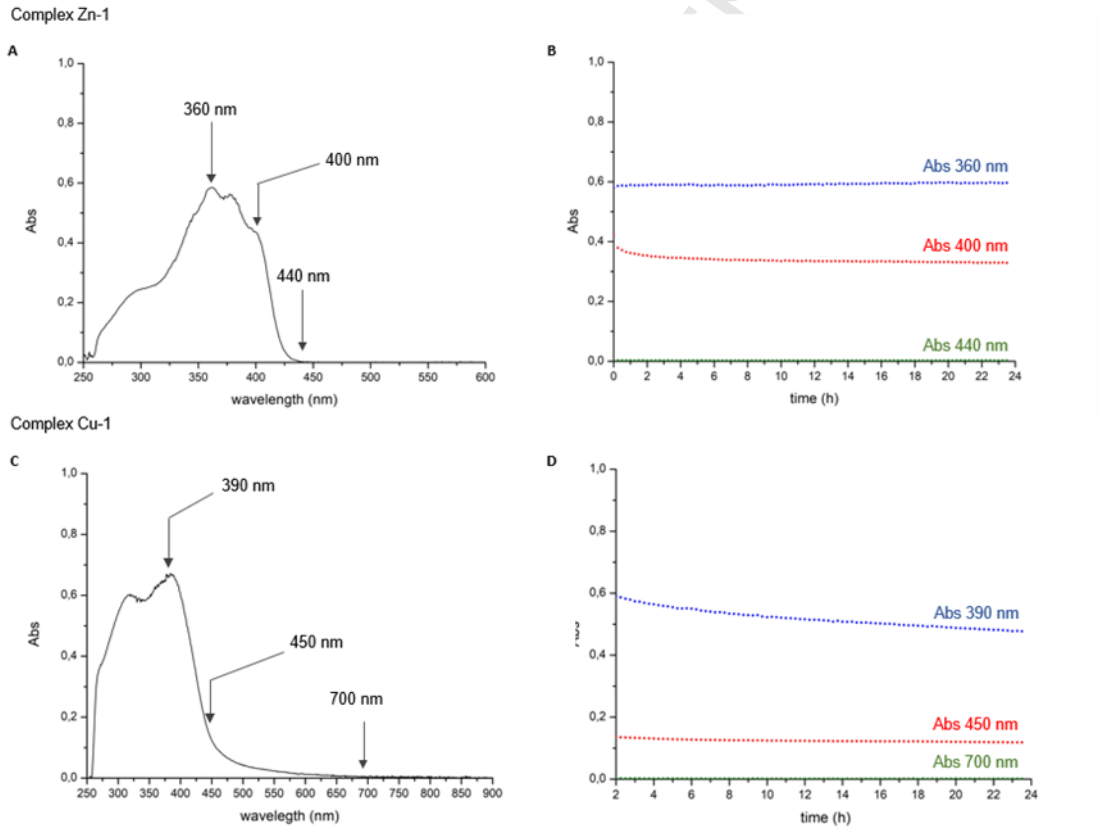


Figure 1. UV-visible spectra of compound **7** (box A) and **4** (box C) under study together with the analysis of absorbance variation for selected wavelength *vs* time (box B and D for compound **7** and **4** respectively)

Complex **4** underwent a lowering of the absorbance intensity at 390 nm, passing from 0.63 to 0.50. This effect, having a constant slope, can be assigned to small aggregations of the compound or to a progressive precipitation from the solution.

In both cases the absorbance of the baseline of the spectra remained stable on zero.

3.3 Crystal structures

Among the compounds presented in this paper, two of them and namely **2** and **3**, were obtained as single crystals of size suitable for an X-ray study and were structurally characterized for the first time.

3.3.1. X-ray structure of compound 2

The molecular structure of compound **2**, as determined by single-crystal X-ray crystallography, is reported in Fig. 2.

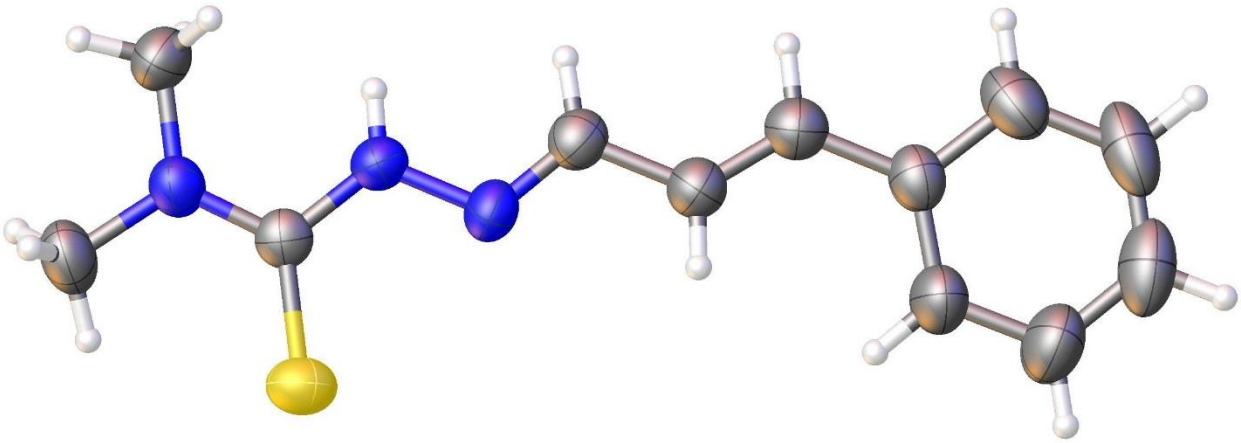
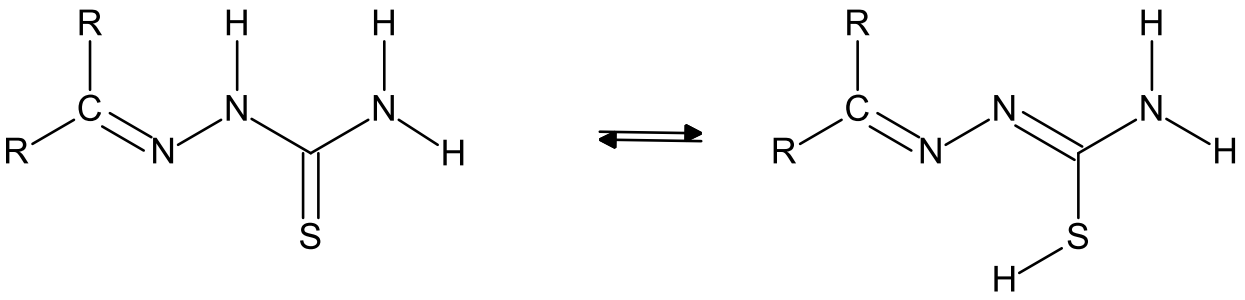


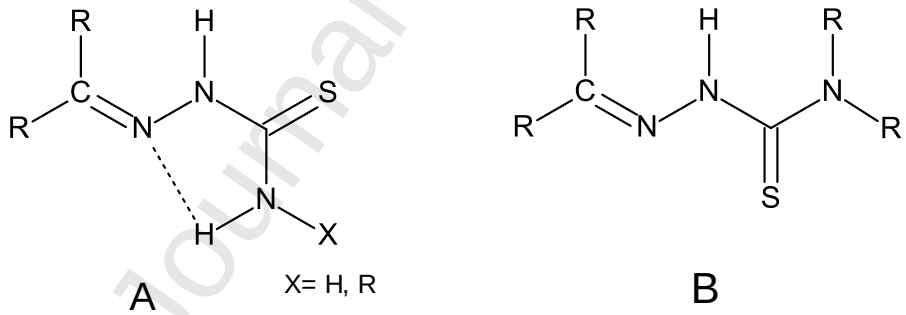
Figure 2. ORTEP representation of the asymmetric unit of compound **2**.

The molecule is overall planar, as expected for systems containing an extended system of conjugated double bonds. Since thiosemicarbazones are subject to a thione-thiol tautomerism phenomenon, this conjugation stabilises the thiol form and enhances the predisposition of these ligands to deprotonation, and consequently their acidity (scheme 1).



Scheme 1. Thiosemicarbazone thione-thiol tautomerism.

In the literature, the structures of very similar molecules are reported, which differ for the substituent on the terminal nitrogen, and namely the non substituted, the methyl, and the phenyl derivatives [24-26]. While all three structures known are superimposable, **2** presents a substantial difference in the fact that the terminal nitrogen and the sulfur are swapped. The three structures reported in the literature are in fact characterized by an intramolecular hydrogen bonding between the terminal amino group and the imino nitrogen (Scheme 2A), which is not possible in **2** since both terminal hydrogens are replaced by methyl groups (Scheme 2B).



Scheme 2. Effect of the terminal amino group substitution on the thiosemicarbazone configuration.

In addition, the presence of two methyls on the thiosemicarbazone terminal nitrogen, probably due to sterical hindrance, contributes to stabilize the molecule in a cis configuration of the NN-CS bond system (Scheme 2B). This pattern is consistently observed in all thiosemicarbazones found in the Cambridge Structural Database [27] with a doubly methylated terminal nitrogen.

As regards the geometrical parameters, in compound **2** the C-S distances of 1.68(2) is very close to the average value of non-deprotonated thiosemicarbazones found in the CSD [27]which is C=S = 1.69(2) Å. The N(hydrazine)-C(thione) bond distance of 1.336(2)Å is instead markedly shorter than

the average of 1.40(1)Å of the free thiosemicarbazones of the same set, suggesting a stronger double bond-like character.

The packing is characterized by a network of hydrogen bonds between the sulphur atom and the hydrazinic NH. This bond allows the formation of zigzag strands which in turn interact with each other through rather loose edge-to-face π-π interactions (Figure 1S).

3.3.2 X-ray structure of compound **3**

The molecular structure of compound **3**, as determined by single-crystal X-ray crystallography, is reported in Figure 3.

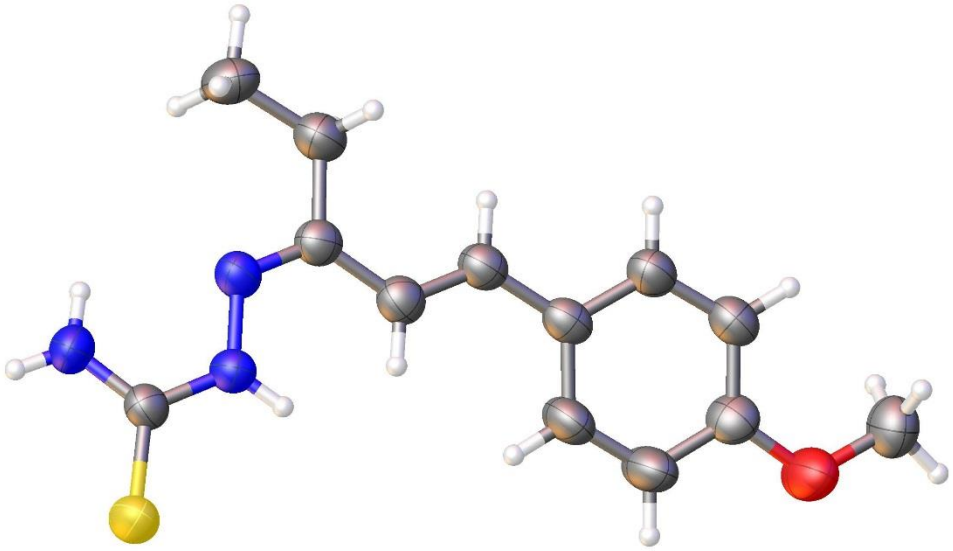


Figure 3. ORTEP representation of the asymmetric unit of compound **3.**

In this molecule, we find the common intramolecular hydrogen bond between the terminal amino group and the iminic nitrogen that characterizes the thiosemicarbazones with the non substituted terminal nitrogen and conferring the E configuration on to the NNCS atom system. The planarity, as can be inferred by the angle of 23.69° between the average plane of the aromatic ring and the plane of the thiourea fragment, is much less extended than in the previous molecule. Nevertheless, an extended conjugated double bond system is still present as can be seen from the bond lengths which fall in the range 1.305(6) to 1.516(8). The distortion from the plane is probably due to constraints

imposed by the packing in which the sulfur forms hydrogen bonds with the terminal NH group of an adjacent molecule and a looser one with the hydrazinic NH from another adjacent molecule.

3.4. Partition coefficient study

In order to verify if the difference in the biological activity among the three ligands could be related to an enhanced ability to diffuse in an apolar phase, the logP were calculated. The logP values found were 2.74 for (E)-cinnamaldehydethiosemicarbazone (1), 3.16 for (E)-cinnamaldehyde-4,4-dimethyl-3-thiosemicarbazone (2), and 3.11 for (E)-1-(4-methoxyphenyl)-1-pentene-3-one-3-thiosemicarbazone (**3**). Due to the difference between the logP of (**1**) compared to (**2**) and (**3**), it seems that a low octanol/water partition coefficient ratio can concur in the bactericidal activity of thiosemicarbazone metal complexes.

3.5 Biological tests

Among the nine molecules synthesized, only **4** and **7** showed an inhibitory or bactericidal activity against the strains under study. Compound **4** showed in particular its best activity against *E. coli* with a 8 μM MBC, while compound **7** showed its best activity against *K. pneumoniae* with a 14 μM MBC (Table 2).

Molecule	Bacterial strain	Concentration for MBC
4	***E. coli***	8 μM
	K. pneumoniae	30 μM
7	***E. coli***	25 μM
	K. pneumoniae	14 μM

Table 2. Minimum Bactericidal Concentration (MBC) of compounds **4** and **7** against *E. coli* and *K. pneumoniae* bacterial strains.

In order to understand the causes of the effectiveness of compounds **4** and **7**, the constituents of the thiosemicarbazones, aldehyde and thiosemicarbazide, were also tested but neither showed any bactericidal action. The results are really interesting and promising, in particular if compared with the activity shown by analogous compounds recently studied [16].

4. Conclusions

Thiosemicarbazones bearing the same aromatic scaffold with a different degree of polarity were synthesized and characterized together with their Cu(II) and Zn(II) metal complexes. From the biological studies, it results that among the compounds under scrutiny, the one with the lowest partition coefficient is the most promising. The investigations on the antibacterial activity show that the metal complexes and the ligands own an antibiotic activity stronger than that of the constituents. Moreover, the metal thiosemicarbazonates are stable in solution at least for 24 hours and this suggests that the observed biological activity can be ascribed to the non-dissociated metal complexes. The MBC found against *Klebsiella pneumoniae* and *Escherichia coli* are very interesting since they fall in the range 8 – 30 μM and therefore encourage an in-depth study to verify if they are compatible for use on human epithelial tissues.

Acknowledgements

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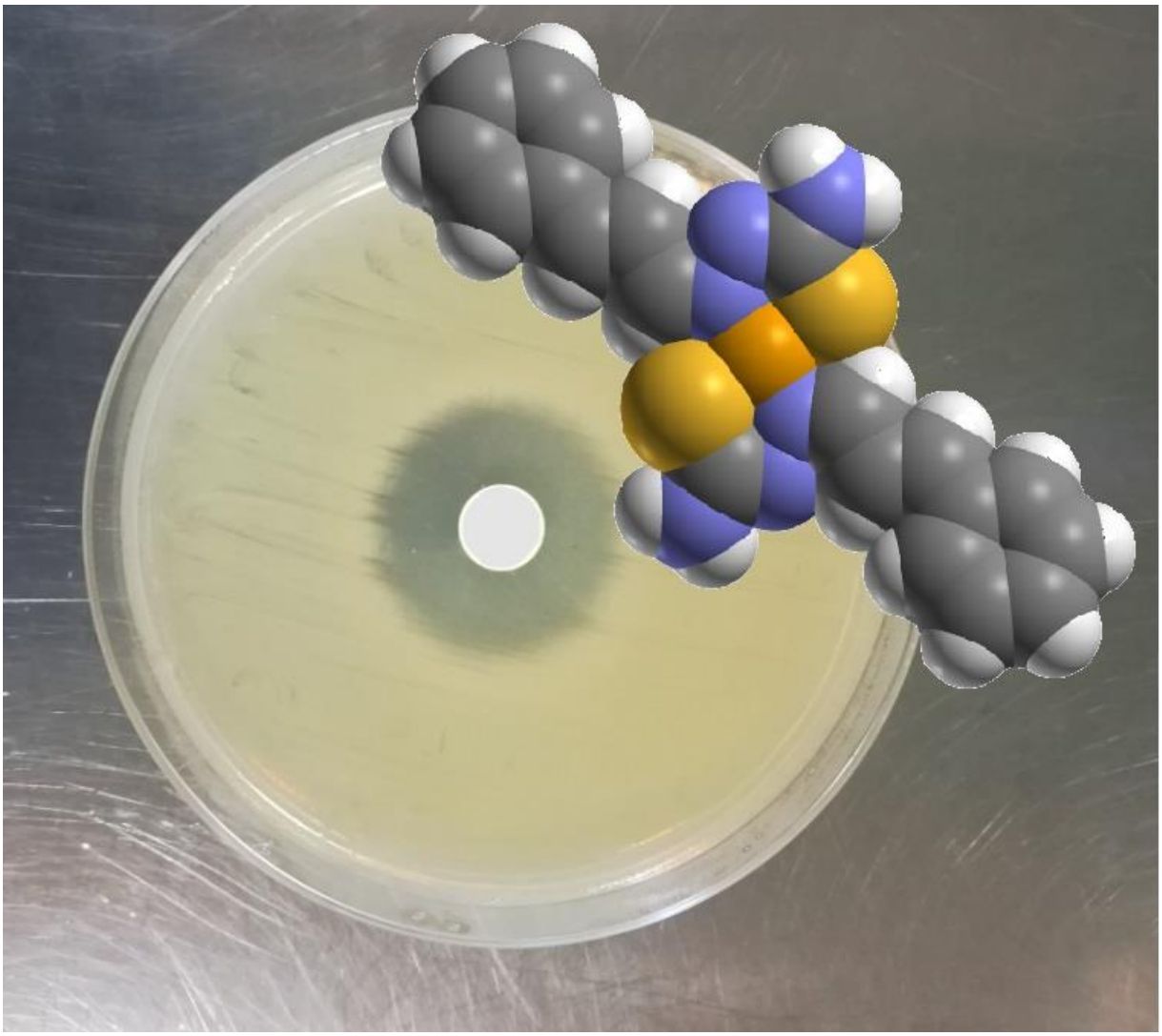
Conflicts of interest

There are no conflicts to declare.

Graphical abstract

Cinnamaldehyde thiosemicarbazone derivatives with different degree of octanol/water ratio were synthesized and characterized together with their Cu(II) and Zn(II) complexes. The compound with the lower partition coefficient is the most promising. The minimum bactericidal concentration found against *Klebsiella pneumoniae* and *Escherichia coli* fall in the range 8 – 30 μM.



Highlights

- Thiosemicarbazone metal complexes are good candidate as metalloantibiotics
- LogPow is a discriminating parameter for a good antimicrobial activity
- Cu(II) and Zn(II) cinnamaldehyde thiosemicarbazonate show a good stability in solution