Quim. Nova, Vol. 38, No. 6, 757-761, 2015 http://dx.doi.org/10.5935/0100-4042.20150075

Artigo

BIS(4-FLUOROPHENYLSULFONYLDITHIOCARBIMATO)ZINCATE(II) SALTS: NEW ANTIFUNGALS FOR THE CONTROL OF *Botrytis* BLIGHT

Alexandre A. Oliveira[a], Marcelo R. L. Oliveira[a,*], Mayura M. M. Rubinger[a], Elisa L. Piló[a], Daniele C. Menezes[a] and Laércio Zambolim[b]

[a]Departamento de Química, Universidade Federal de Viçosa, 36570-000 Viçosa – MG, Brasil

[b]Departamento de Fitopatologia, Universidade Federal de Viçosa, 36570-000 Viçosa – MG, Brasil

Recebido em 18/12/2014; aceito em 26/03/2015; publicado na web em 08/05/2015

Botrytis blight or gray mold is a highly destructive disease caused by *Botrytis* spp., that infects flowers, trees vegetables, fruit, especially grapevines and strawberry. Three new compounds with general formula $(A)_2[Zn(4\text{-}FC_6H_4SO_2N{=}CS_2)_2]$, where A = PPh_3CH_3 (**2a**), $PPh_3C_2H_5$ (**2b**), $PPh_3C_4H_9$ (**2c**), and the previously published compounds where A = PPh_4 (**2d**) and NBu_4 (**2e**), were synthesized by the reaction of 4-fluorophenylsulfonyldithiocarbimate potassium dihydrate and zinc(II) acetate dihydrate with the appropriate counter cations (A) halides. The new compounds were characterized by infrared, ^1H and ^{13}C NMR spectroscopies. All these salts inhibited the growth of *Botrytis cinerea*, with compounds **2c** and **2d** showing greater antifungal activity than zinc dimethyldithiocarbamate, the active principle of the fungicide Ziram. The bis(dithiocarbimate)zincate(II) salts are also active against the bacteria *Escherichia coli* and *Staphylococcus aureus*.

Keywords: dithiocarbimates; antimicrobial activity; *Botrytis cinerea*; *Escherichia coli*; *Staphylococcus aureus*.

INTRODUCTION

Several bis(dithiocarbamato)-zinc(II) complexes have been used as agrochemicals mainly due to their high efficiency in controlling plant fungal diseases, and relatively low toxicity.[1,2] However, there is a continuing need for replacement of active ingredients and formulations to meet the environmental requirements, as well as avoiding resistance mechanisms.[3] In these perspectives and due to the structural similarity with dithiocarbamates, we have been investigating the antifungal activity of salts containing anionic bis(*N*-R-sulfonyldithiocarbimato)zincate(II) complexes.[4-6] For example, it was observed that the compound tetrabutylammonium bis(4-fluorophenylsulfonyldithiocarbimato)zincate(II) (**2e**) was very active against *Colletotrichum gloeosporioides*, an important fungus that causes the plant disease known as anthracnose in fruit trees.[4]

Unlike the neutral dithiocarbamate analogues, the bis(dithiocarbimate)zincate(II) complexes are anionic species that potentially offer modulation of their activity by the use of active counter ions or by varying the solubility of the salts of the complexes through the use of different cations. The activity of some phosphonium halides against various fungi and bacteria species has been reported.[7] Thus, in this study we decided to evaluate the effect of different phosphonium cations on the antifungal activity of the anionic complex bis(4-fluorophenylsulfonyldithiocarbimate)zincate(II).

In this work, five compounds of general formula $(A)_2[Zn(4\text{-}FC_6H_4SO_2N{=}CS_2)_2]$ were prepared, where A = PPh_3CH_3 (**2a**), $PPh_3C_2H_5$ (**2b**), $PPh_3C_4H_9$ (**2c**), PPh_4 (**2d**) and NBu_4 (**2e**). The compounds **2d** and **2e** are described in the literature and both showed antifungal properties.[4,5] However, it was not possible to compare their effects on the fungal growth, for two different methodologies were employed.[4,5]

The investigation of the influence of the different cations on the biological activity of the dithiocarbimate-zinc complexes here reported was based on the growth of *Botrytis cinerea*, the causal agent of the fungal disease called gray mold or *Botrytis* blight on fruits, vegetables and ornamental plants.[8] For example, it is the most important post-harvest disease affecting strawberry, where the infected fruits rapidly take on a velvety, gray-brown coat of mycelium and spores. Large amounts of spores produced on each berry, when spread by air cause serious economic losses. In Brazil, there is a limited number of fungicides registered for the control of *B. Cinerea.* The most important classes belong to the benzimidazole and dicarboximide, for which there are reports of the occurrence of resistance of *Botrytis* spp.[9-13]

As the activities of some phosphonium halides against bacteria have been reported, we additionally investigated the activity of the synthesized compounds against *Escherichia coli* and *Staphylococcus aureus.* Both microorganisms are very commonly associated with food-borne illnesses.[7,14] *E. Coli* is usually harmless, being important part of the normal gut flora. Nevertheless, some strains are poisonous to humans and other mammals. The virulent strains cause diseases such as gram-negative pneumonia, gastroenteritis, urinary tract infections and septicemia.[15] Although anthropic contamination of food is the main source of *E. coli*, it is also found in agricultural plants.[16] *S. aureus* is also not necessarily pathogenic, being present in the skin and respiratory system of a number of healthy individuals. However, antibiotic-resistant strains are major pathogens in hospitals and long-term-care facilities.[17]

EXPERIMENTAL

Material and reagents

The solvents, carbon disulfide and potassium hydroxide purchased from Vetec were used without further purification. Other reagents were purchased from the trademarks: Aldrich (4-fluorobenzenesulfonamide), Vetec (zinc(II) acetate dihydrate), Alfa Aesar (tetraphenylphosphonium chloride, ethyltriphenylphosphonium chloride, 1-butyltriphenylphosphonium bromide, tetrabutylammonium bromide) and Spectrum (methyltriphenylphosphonium bromide). Uncorrected melting points were determined on a Mettler MQAPF-302 apparatus. Microanalyses for C, H and N were obtained from a Perkin-Elmer 2400 CHN elemental analyzer, and zinc was analyzed by atomic

*e-mail: marcelor@ufv.br

absorption with a Varian Spectra AA-200 spectrophotometer. The molar conductance (Λ_M) was measured in acetonitrile solutions at 25 ºC with a Conductivity Meter Jenway 4010. The IR spectra (4000-200 cm^{-1}) were recorded on a Perkin-Elmer FT-IR 1000 spectrophotometer employing the transmittance method and using CsI pellets. The ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded at 20 ºC on a Varian Mercury 300 spectrophotometer in dimethyl sulfoxide-d_6 with tetramethylsilane as internal standard.

Syntheses

The 4-fluorophenylsulfonyldithiocarbimate potassium dihydrate [$K_2L = K_2(4\text{-}FC_6H_4SO_2N{=}CS_2).2H_2O$] was prepared in dimethylformamide from 4-fluorobenzenesulfonamide in reaction with carbon disulfide and potassium hydroxide, and its formation was confirmed by IR and comparison with published data.[18] The bis(ditiocarbimate)-Zn(II) salts **2a**-**2e** were prepared as shown in Figure 1.

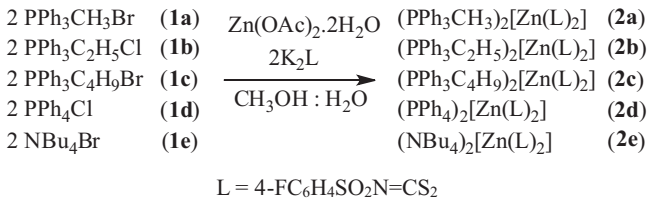

Figure 1. *Syntheses of the bis(ditiocarbimate)-Zn(II) salts*

A solution of $Zn(OAc)_2.2H_2O$ (1.0 mmol) in 1 mL of water was added to a solution of K_2L (2.0 mmol) in MeOH:H_2O 1:1 (10 mL). The reaction mixture was stirred for one hour at room temperature and then 2.0 mmol of the appropriate counter ion halide (PPh_3CH_3Br, $PPh_3C_2H_5Cl$, $PPh_3C_4H_9Br$, PPh_4Cl or NBu_4Br) previously solubilized in 2 mL of MeOH:H_2O (1:1) were added. The mixture was stirred for 30 minutes and the white solids obtained were filtered off, washed with distilled water, then drops of methanol followed by diethyl ether, and dried under reduced pressure for one day. The obtention of compounds **2d** and **2e** was confirmed by the comparison of their spectroscopic data and melting point values with published data.[4,5] The new compounds **2a**-**2c** were fully characterized by NMR and infrared spectroscopy, molar conductance, melting points, elemental analyses of C, H, N and Zn, as follows.

*$(PPh_3CH_3)_2[Zn(4\text{-}FC_6H_4SO_2N{=}CS_2)_2]$ (**2a**)*

Yield: 90%. M.p. 90.2 - 91.9 ºC. Anal. Calc. for $C_{52}H_{44}F_2N_2O_4P_2S_6Zn$: C, 55.83; H, 3.96; N, 2.50; Zn, 5.85. Found: C, 54.27; H, 4.08; N, 2.45; Zn, 5.81. IR (most important bands) (cm^{-1}): 3096, 3058 (υ =C-H); 2912 (υ C-H); 1374 (υ C=N); 1278 (υ_{as} SO_2); 1143 (υ_s SO_2); 942 (υ_{as} CS_2); 342 (υ ZnS). ^1H NMR (δ), *J* (Hz): 7.89 (s, 6H, H4'); 7.74 – 7.78 (m, 28H, H2', H3', H5', H6', H2, H6); 7.28 (t, 4H, H3, H5, $^3J_{H3,5\text{-}F}$ = 8.5); 3.15 (d, 6H, H7', $^2J_{H7'\text{-}P}$ = 14.6). ^{13}C NMR (δ), *J* (Hz): 207.4 (C7); 164.1 (d, C4, $^1J_{C4\text{–}F}$ = 248.7); 139.8 (d, C1, $^4J_{C1\text{-}F}$ = 2.7); 135.5 (d, C4', $^4J_{C4'\text{-}P}$ = 2.7); 133.9 (d, C3', C5', $^3J_{C3',5'\text{-}P}$ = 10.8); 130.8 (d, C2, C6 and C2', C6', $^2J_{C2',6'\text{-}P}$ = 12.6); 120.6 (d, C1', $^1J_{C1'\text{-}P}$ = 88.3); 115.7 (d, C3, C5, $^2J_{C3,5\text{-}F}$ = 22.3); 7.9 (d, C7', $^1J_{C7'\text{-}P}$ = 55.6). Λ_M (CH_3CN, S mol^{-1} cm^2): 238.

*$(PPh_3C_2H_5)_2[Zn(4\text{-}FC_6H_4SO_2N{=}CS_2)_2]$ (**2b**)*

Yield: 92%. M.p. 74.2 - 76.0 ºC. Anal. Calc. for $C_{54}H_{48}F_2N_2O_4P_2S_6Zn$: C, 56.56; H, 4.22; N, 2.44; Zn, 5.70. Found: C, 54.15; H, 4.13; N, 2.46; Zn, 5.51. IR (most important bands) (cm^{-1}): 3098, 3062 (υ =C-H); 2942, 2908 (υ C-H); 1373 (υ C=N), 1281 (υ_{as} SO_2), 1143 (υ_s SO_2), 940 (υ_{as} CS_2), 338 (υ ZnS). ^1H NMR (δ), *J* (Hz): 7.89 – 7.93 (m, 6H, H4'); 7.78 – 7.84 (m, 28H, H2', H3', H5', H6', H2, H6); 7.28 (t, 4H, H3, H5, $^3J_{H3,5\text{-}F}$ = 8.8); 3.53 – 3.65 (m, 4H, H7'); 1.22 (dt, 6H, H8', $^3J_{H8'\text{-}H7'}$ = 7.4; $^3J_{H8'\text{-}P}$ = 20.0). ^{13}C NMR (δ), *J* (Hz): 207.4 (C7); 164.1 (d, C4, $^1J_{C4\text{–}F}$ = 248.7); 139.8 (d, C1, $^4J_{C1\text{-}F}$ = 2.5); 135.6 (d, C4', $^4J_{C4'\text{-}P}$ = 2.4); 134.3 (d, C3', C5', $^3J_{C3',5'\text{-}P}$ = 10.0); 130.9 (d, C2', C6', $^2J_{C2',6'\text{-}P}$ = 12.5); 130.8 (d, C2, C6, $^3J_{C2,6\text{-}F}$ = 10.0); 118.9 (d, C1', $^1J_{C1'\text{-}P}$ = 85.6); 115.7 (d, C3, C5, $^2J_{C3,5\text{-}F}$ = 22.3); 15.1 (d, C7', $^1J_{C7'\text{-}P}$ = 51.4); 6.9 (d, C8', $^2J_{C8'\text{-}P}$ = 5.2). Λ_M (CH_3CN, S mol^{-1} cm^2): 238.

*$(PPh_3C_4H_9)_2[Zn(4\text{-}FC_6H_4SO_2N{=}CS_2)_2]$ (**2c**)*

Yield: 92%. M.p. 67.5 - 69.5 ºC. Anal. Calc. for $C_{58}H_{56}F_2N_2O_4P_2S_6Zn$: C, 57.92; H, 4.69; N, 2.33; Zn, 5.44. Found: C, 56.49; H, 4.66; N, 2.32; Zn, 5.30. IR (most important bands) (cm^{-1}): 3096, 3060 (υ =C-H); 2961, 2933, 2900, 2871 (υ C-H); 1373 (υ C=N), 1280 (υ_{as} SO_2), 1143 (υ_s SO_2), 940 (υ_{as} CS_2), 338 (υ ZnS). ^1H NMR (δ), *J* (Hz): 7.78 – 7.90 (m, 34H, H2', H3', H4', H5', H6', H2, H6); 7.28 (t, 4H, H3, H5, $^3J_{H3,5\text{-}F}$ = 7.5); 3.56 (bs, 4H, H7'); 1.49 (bs, 8H, H8', H9'); 0.89 (bs, 6H, H10'). ^{13}C NMR (δ), *J* (Hz): 207.2 (C7); 163.9 (d, C4, $^1J_{C4\text{–}F}$ = 248.5); 139.6 (d, C1, $^4J_{C1\text{-}F}$ = 2.6); 135.3 (d, C4', $^4J_{C4'\text{-}P}$ = 2.4); 134.0 (d, C3', C5', $^3J_{C3',5'\text{-}P}$ = 10.1); 130.7 (d, C2', C6', $^2J_{C2',6'\text{-}P}$ = 12.4); 130.6 (d, C2, C6, $^3J_{C2,6\text{-}F}$ = 10.0); 119.0 (d, C1', $^1J_{C1'\text{-}P}$ = 85.7); 115.5 (d, C3, C5, $^2J_{C3,5\text{-}F}$ = 22.3); 24.3 (d, C9', $^3J_{C9'\text{-}P}$ = 3.9); 23.6 (d, C8', $^2J_{C8'\text{-}P}$ = 17.4); 20.4 (d, C7', $^1J_{C7'\text{-}P}$ = 50.0); 13.7 (s, C10'). Λ_M (CH_3CN, S mol^{-1} cm^2): 228.

Biological assays

The antifungal activity of the zinc(II) compounds (**2a**-**2e**) was evaluated against *B. cinerea* by the *Poisoned food* technique.[19] The fungus was isolated from infected strawberry tissues with gray mold symptoms. The culture medium PDA (Potato Dextrose Agar) purchased from Himedia was previously sterilized in autoclave for 20 minutes at 121 ºC. Glassware and spatulas were sterilized at 140 ºC for 3.5 h. Discs of mycelia of the fungus (diameter of 6.62 mm) were placed on the center of Petri dishes containing 15 mL of the culture medium homogeneously mixed with the substances to be tested in different concentrations, dimethyl sulfoxide (DMSO) and Tween 80 (1% v/v each). Two independent tests were carried out to check the reproducibility of the experiment, in which each sample was prepared in four repetitions and the dishes were kept in the incubator chamber at 22 ºC for three days. The control (negative check treatment) was prepared with PDA, DMSO and Tween 80 only. The positive check treatment was prepared by the same procedure using bis(dimethydithiocarbamato)zinc(II) purchased from Aldrich, $Zn(DMDC)_2$, the active principle of the fungicide Ziram. The diameter of the fungus colony was measured with the aid of a digital caliper every 12 hours from the second day of incubation. The effects of the parent K_2L ligand, counter cations halides (**1a**-**1e**) and $Zn(OAc)_2.2H_2O$ were also tested under the same conditions. The percentages of inhibition were obtained after comparison with the control and the results were analyzed by polynomial regression using curves of dose employed (mM) versus percent inhibition results.

The activities of the salts (**1a**-**1e** and **2a**-**2e**) against the bacterial strains *E. coli* (ATCC 11229) and *S. aureus* (ATCC 25923) were also studied. The liquid cultures of the microorganisms were seeded aerobically at 37 ºC in Nutrient broth and the cultures were incubated at 37 °C for 24 h. The agar disk diffusion test was performed according to the Clinical and Laboratory Standards Institute guidelines (CLSI - Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard M02-A11, 2012). A 100 µL aliquot of overnight culture of the microorganisms corresponding to 0.5 turbidity on the McFarland scale (approximately 1.5×10^8 CFU or 1.5×10^8 bacteria per milliliter) was placed onto 10 mL of Nutrient agar. Suspensions

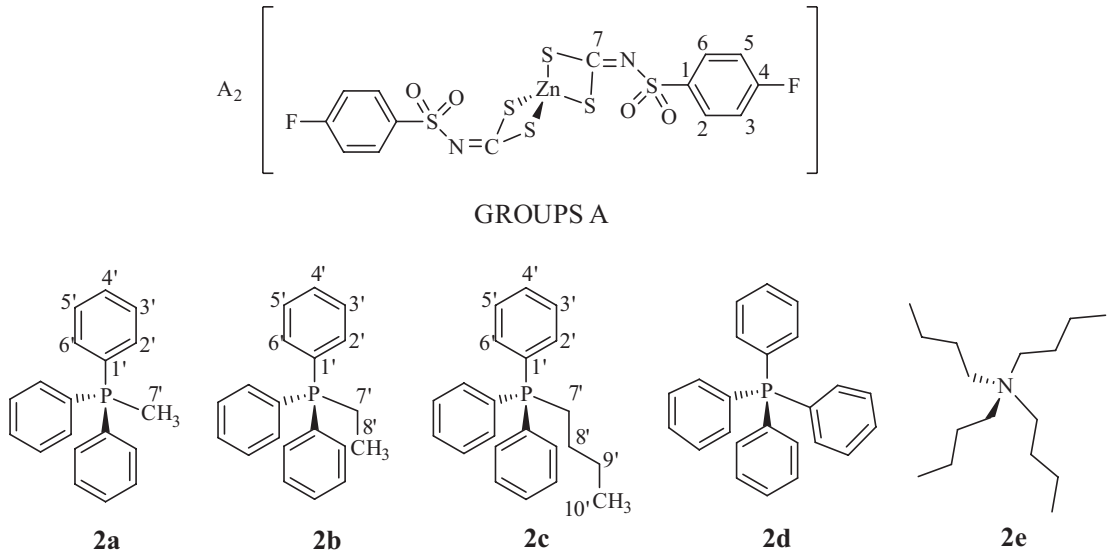

Figure 2. *Compounds* ***2a – 2e*** *structures and numbering of carbon atoms of* ***2a – 2c*** *for NMR attributions*

of the compounds (10 μL) in DMSO at 250 mmol L^{-1} were added to the paper discs (diameter of 0.5 cm). The commercial antibiotic agent norfloxacin was used as a positive control and DMSO as a negative control. The antimicrobial activities were evaluated by the presence or absence of inhibition zone around the disc and the measurements were expressed as the mean of triplicate evaluated in two independent experiments.

RESULTS AND DISCUSSION

Chemistry

Figure 2 shows the structures of compounds **2a**-**2e**. The spectroscopic data for **2d** and **2e** are in accordance with the previously published data for these compounds.[4,5] The new compounds **2a**-**2c** are white solids, stable under ambient conditions, insoluble in water, but soluble in dimethylsulfoxide, acetonitrile and chloroform. The elemental analyses of C, H, N and Zn were consistent with the proposed formulae. The molar conductance values at 10^{-3} mol L^{-1} in acetonitrile were in the range of 200-300 $Scm^2 mol^{-1}$, commonly attributed to 1:2 electrolytes.[20]

The υ(CN) band in the infrared spectrum of the free ligand (K_2L) is observed at 1259 cm^{-1} and a medium strong band assigned to the $\upsilon_{as}(CS_2)$ is observed at 977 cm^{-1}.[18] The υ(CN) band was shifted to higher wavenumber values in the spectra of **2a**-**2c** (*ca.* 1373 cm^{-1}), while the $\upsilon_{as}(CS_2)$ showed an opposite shift (to *ca.* 940 cm^{-1}). The observed shifts are consistent with the complexation of the dithiocarbimate group by two sulfur atoms.[4,5] The spectra of the complexes showed a medium-weak absorption band in the 300-350 cm^{-1} range assigned to the Zn-S stretching vibration, also indicating the coordination of the *gem*-disulfide ligand.[21]

The integration curves on the ^{1}H NMR spectra of **2a**-**2c** were consistent with a 2:1 proportion between the cations and the bis(dithiocarbimate)zincate(II) dianions. The ^{13}C NMR spectra of the salts **2a**-**2c** showed all the expected signals for the anionic complex with approximately the same chemical shifts already published for compounds **2d** and **2e**.[18] The C=N signal at ca. δ 207 was characteristic of bis(dithiocarbimate)zincate(II) complexes, being more shielded than the C=N of the free ligand (δ 225.3).[18] The aromatic carbon signals of the anionic complex were observed as doublets due to the ^{13}C-^{19}F coupling. The signal for C3 and C5 (δ *ca.* 115.7) was easily identified by the typical $J_{C\text{-}F}$ of 22 Hz.[18] The doublet for C2 and C6 (at *ca.* δ 130.5, $J_{C\text{-}F}$ =10 Hz) was very close to the signal of the carbon atoms C2' and C6' (at *ca.* δ 130.8, $J_{C\text{-}P}$ = 12.5 Hz) of the cations in the spectra of **2b** and **2c**. These signals were completely overlapped in the spectrum of **2a**. The remaining signals due to the phenyl rings of the cations in the spectra of **2a**-**2c** were similar, showing very close chemical shifts and approximately the same values of ^{13}C-^{31}P coupling constants. The main differences in the spectra of **2a**-**2c** were due to the aliphatic carbon chains attached to the phosphorus atom, the chemical shifts and ^{13}C-^{31}P coupling constants values decreasing with the distance from the phosphorus atom, as expected.[22,23]

Biological assays

The antifungal activities of the Zn(II) compounds (**2a**-**2e**) and of their parent counter cations halides (**1a** -**1e**) were evaluated against *B. cinerea*. The mycelial growth curves indicated that, except for the tetrabutylammonium bromide (**1e**), all tested compounds exhibit antifungal activity. The fungus colony growth showed a near-linear variation over time, regardless of the concentrations employed. As an example, Figure 3 presents the mycelial growth curves obtained for the compound **2d** in six different concentrations. From Figure 3 it is possible to note that the inhibitory activity is already observable

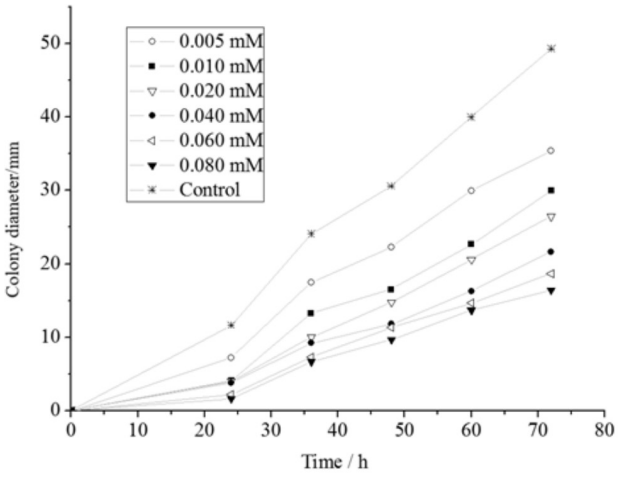

Figure 3. *Colony diameter of Botrytis cinerea over 3 days of incubation at 22 °C when treated with compound* ***2d*** *in different concentrations in comparison with the negative control (100% growth)*

from the first day of incubation, with increased growth differences over the time of the experiment.

The diameters of the colony after 72 hours of incubation were used to build the curves of dose-response for each compound, as exemplified in Figure 4 for compound **2d**. In all cases the second-degree polynomial model was the best fit for the experimental data, being observed correlation coefficients greater than 0.97.

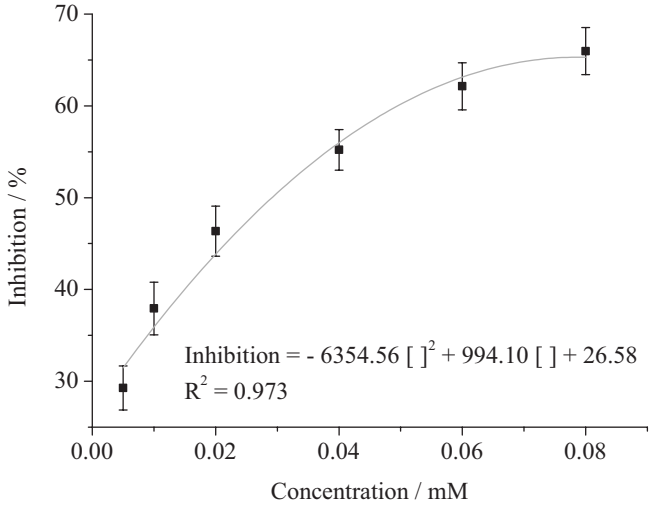

Figure 4. *Percent inhibition of the growth of the Botrytis cinerea colony after 3 days of incubation at 22 ºC when treated with different concentrations of* ***2d***

The concentrations of compounds **1a**-**1d** and **2a**-**2e** required to inhibit 50% in the radial growth of the colony relative to the negative control (IC_{50}) were obtained from the regression curves and the results are listed on Table 1. From the IC_{50} values it can be observed that the nature of the R group in R-triphenylphosphonium cations influences the biological activity of the halides **1a**-**1d**, following the decreasing order of activity: PPh_4Cl (**1d**) > $PPh_3C_4H_9Br$ (**1c**) > $PPh_3C_2H_5Cl$ (**1b**) > PPh_3CH_3Br (**1a**). The same trend was observed for the zinc anionic complexes salts **2a** – **2d** (**2d > 2c > 2b > 2a**). The biological activity of **1a**-**1d** is partly related to their ability to interact with the microbial cytoplasmic membranes, one site of action of cationic biocides.[7] These results clearly indicate that the chain lengths and molecular nature of the groups affect the antifungal activity of the phosphonium salts.

Table 1 shows that **2d** was the most active compound tested, being much more active than the bis(dimethyldithiocarbamato)zinc(II) (The pure active principle of Ziram) used as a positive control in the tests. It was also verified that NBu_4Br (**1e**) shows no inhibitory activity on mycelial growth up to 5 mM. Nevertheless, compound **2e** that contains the cation NBu_4^+ was active, presenting an IC_{50} of 1.243 mmol L^{-1}. Although the IC_{50} of **2e** was higher than the values calculated for compounds **2a**-**2d**, this value is important once it is related to the intrinsic activity of the anionic complex.

The precursor compounds K_2L and $Zn(OAc)_2$ were tested at the concentrations of 2.6 and 1.3 mmol L^{-1}, the respective molar equivalents of the ligands (L) and Zn(II) in compound **2e**, and close to the observed IC_{50} for **2e** (1.243 mmol L^{-1}). At these concentrations, K_2L and $Zn(OAc)_2$ presented inhibition percentage values of 38 and 20%, respectively, while **2e** inhibited 50%. These results showed that the bis(dithiocarbimate)zincate(II) chelate is considerably more toxic to the fungus when compared to the parent salts.

The activity of the free ligand K_2L, the phosphonium halides **1a**-**1e** and the complexes **2a**-**2e** were also tested against the gram-positive and gram-negative bacteria *S. aureus* and *E. Coli* (Table 2). Tetrabutylammonium bromide (**1e**) was the less active compound against *S. aureus* and showed no activity against *E. coli*. Thus the activity presented by the complex **2e** shows that the bis(dithiocarbimato)zincate(II) anion is at least partially responsible for the activity presented by the compounds **2a**-**2e** (Table 2). Norfloxacin, a broad-spectrum antibiotic active against both Gram-positive and Gram-negative bacteria, was used as a positive control.

It was also found that the phosphonium halides **1a**-**1d** presented larger inhibition zones than their bis(dithiocarbimato)zincate(II) salts **2a**-**2d**. Further, most of the tested compounds exhibited shorter inhibition zones than the antibiotic norfloxacin. This observation may be a consequence of the greater difficulty of diffusion of the less hydrophilic compounds in the aqueous matrix. Thus, these tests can be regarded only as qualitative results, which showed that the tested bis-dithiocarbimate-zinc(II) phosphonium salts are active against *S. aureus* and *E. coli*.

Table 1. *In vitro* antifungal activity of the bis(dithiocarbimate)-zinc(II) salts **2a**-**2e**, the respective countercations halides **1a**-**1e** and bis(dimethyldithiocarbamate)zinc(II) against *B. cinerea*

Compounds	IC_{50} / mM [a]
1a	1.943
1b	0.878
1c	0.318
1d	0.057
1e	-
2a	0.625
2b	0.356
2c	0.109
2d	0.030
2e	1.243
$Zn(DMDC)_2$ [b]	0.149

[a] Concentration needed to inhibit 50% of the growth of *B. cinerea* after 3 days of incubation at 22 ºC in comparison with the negative control; [b] Positive control: bis-dimethyldithiocarbamate-zinc(II).

Table 2. Means of inhibition zones diameters obtained in disk diffusion assays with the bis-ditiocarbimate-zinc(II) salts **2a**-**2e**, the respective countercations halides **1a**-**1e** and Norfloxacin against *S. aureus* and *E. coli*

Compounds	*S. aureus*	*E. coli*
1a	20.32 ± 1.70	20.88 ± 0.96
1b	21.92 ± 1.51	22.29 ± 1.75
1c	27.10 ± 0.87	28.17 ± 1.99
1d	32.22 ± 0.06	29.95 ± 1.16
1e	11.40 ± 1.02	7.15 [a]
2a	11.88 ± 1.56	13.68 ± 0.49
2b	13.20 ± 1.55	14.87 ± 2.02
2c	14.18 ± 0.20	12.84 ± 1.58
2d	18.82 ± 1.70	14.07 ± 0.70
2e	13.71 ± 0.48	16.31 ±1.08
Norfloxacin [b]	31.38 ± 0.45	29.12 ± 2.12

[a] Size of the paper disc: 7.15 mm; [b] Positive control.

CONCLUSIONS

Three new R-triphenylphosphonium salts of bis(4-fluorophenylsulfonyldithiocarbimato)zincate(II) (R = methyl (**2a**), ethyl (**2b**) and

butyl (**2c**)) were synthesized. The compounds were characterized by elemental analyses, conductivity molar measurements, in addition to IR, ^{1}H and ^{13}C NMR spectroscopies. The conductivity values and the ^{1}H NMR integration curves were consistent with a proportion of 2:1 for cation:anion. The shifted wavenumbers of the υ(CN) and $\upsilon_{as}(CS_2)$ vibrations compared those observed for the free ligand are in agreement with the chelation of the metal by the two sulphur atoms forming a ZnS_4 tetrahedral environment as observed for the tetraphenylphosphonium and tetrabutylammonium analogues **2d** and **2e**.[4,5]

Except for the tetrabutylammonium bromide (**1e**), the phosphonium halides (**1a**-**1d**) and the bis(dithiocarbimato)zincate(II) salts **2a**-**2e** inhibited the growth of *B. cinerea*. The compounds **1d**, **2c** and **2d** showed greater antifungal activity than the dithiocarbamate $Zn(DMDC)_2$, the active principle of the fungicide Ziram. It was proved that the nature of the cation affects the antifungal activity of the bis-dithiocarbimate-Zn(II) salts, with increased activity in this order: $NBu_4^+ < PPh_3CH_3^+ < PPh_3C_2H_5^+ < PPh_3C_4H_9^+ < PPh_4^+$. It is important to notice that the greater activity of **2d** is mainly due to the cation PPh_4^+. The counter cations halides **1a**-**1d** and the bis(dithiocarbimate) zincate(II) salts **2a**-**2e** were also active against *E. coli* and *S. aureus*. Thus the activity of the bis(dithiocarbimato)zincate(II) salts are worth of further investigation, especially due to their potential as antifungal agrochemicals.

SUPPLEMENTARY MATERIAL

IR and NMR spectra of the new compounds **2a**, **2b** e **2c** are available at http://quimicanova.sbq.org.br, in the form of PDF file, with free access.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Brazilian agencies CNPq, FAPEMIG and CAPES for financial support and student grants.

REFERENCES

1. Hogarth, D.; *Prog. Inorg. Chem.* **2005**, *53*, 71.
2. Gullino, M. L.; Tinvella, F.; Garibaldi, A.; Kemmitte, G. M.; Bacci, L.; Sheppard, B.; *Plant Dis.* **2010**, *94*, 1076.
3. Russell, P. E.; *Plant Pathol.* **2006**, *55*, 585.
4. Alves, L. C.; Rubinger, M. M. M.; Lindemann, R. H.; Perpetuo, G. J.; Janzack, J.; Miranda, L. D. L.; Zambolim, L.; Oliveira, M. R. L.; *J. Inorg. Biochem*. **2009**, *103*, 1045.
5. Amim, R. S.; Oliveira, M. R. L.; Janczak, K.; Rubinger, M. M. M.; Vieira, L. M. M.; Alves, L. C.; Zambolim, L.; *Polyhedron* **2011**, *30*, 683.
6. Bottega, F. C.; Oliveira, M. R. L.; Garcia, C. V.; Menezes, D. C.; Rubinger, M. M. M.; Zambolim, L.; *Quim. Nova* **2013**, *36*, 803.
7. Kanazawa, A.; Ikeda, T.; Endo, T.; *Antimicrob. Agents Chemother.* **1994**, *38*, 945.
8. Elad, Y.; Williamson, B.; Tudzynski, P.; Delen, N. In *Botrytis: Biology, Pathology and Control*; Elad, Y.; Williamson, B.; Tudzynski, P.; Delen, N., eds.; Springer: Dordrecht, 2004, pp.1-8.
9. Yourman, L. F.; Jeffers, S. N.; *Plant Dis.* **1999**, *83*, 569.
10. Leroux, P.; Chapeland, F.; Desbrosses, D.; Gredt, M.; *Crop Prot.* **1999**, *18*, 687.
11. Wedgea, D. E.; Smith, B. J.; Quebedeaux, J. P.; Constantin, R. J.; *Crop Prot.* **2007**, *26*, 1449.
12. Myresiotis, C. K.; Karaoglanidis, G. S.; Tzavella-Klonari, K.; *Plant Dis.* **2007**, *91*, 407.
13. Leroch, M.; Plesken, C.; Weber, R. W. S.; Kauff, F.; Scalliet, G.; Hahna, M.; *Appl. Environ. Microbiol.* **2013**, *79*, 159.
14. Mead, P. S.; Slutsker, L.; Dietz, V.; McCaig, L. F.; Bresee, J. S.; Shapiro, C.; Griffin, P. M.; Tauxe, R. V.; *Emerging Infect. Dis.* **1999**, *5*, 607.
15. Kaper, J. B.; Nataro, J. P.; Mobley, H. L. T.; *Nat. Rev. Microbiol.* **2004**, *2*, 123.
16. Méric, G.; Kemsley, E. K.; Falush, D.; Saggers, E. J.; Lucchini, S.; *Environ. Microbiol.* **2013**, *15*, 487.
17. Lowy, F. D.; *J. Clin. Invest.* **2003**, *111*, 1265.
18. Amim, R. S.; Oliveira, M. R. L.; Perpetuo, G. J.; Janczak, J.; Miranda, L. D. L.; Rubinger, M. M. M.; *Polyhedron* **2008**, *27*, 1891.
19. Singh, G.; Marimuthu, P.; Heluani, C. S.; Catalan, C. A. N.; *J. Agri. Food Chem.* **2006**, *54*, 174.
20. Geary, W. J.; *Coord. Chem. Rev*. **1971**, *7*, 81.
21. Nakamoto, K.; *Infrared and Raman Spectra of Inorganic and Coordination Compounds Part B*, 6th ed., Wiley: New York, 2009.
22. Castañeda, F.; Aliaga, C.; Acuña, C.; Silva, P.; Bunton, C. A.; *Phosphorus, Sulfur Silicon Relat. Elem.* **2008**, *183*, 1188.
23. Gray, G. A.; *J. Am. Chem. Soc.* **1973**, *95*, 7736.