Chemico-Biological Interactions 278 (2017) 152–161

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Synthesis, characterization and biological studies of a cobalt(III) complex of sulfathiazole

Ana Pontoriero[a], Natalia Mosconi[a], Laura Monti[a], Sebastián Bellú[a], Patricia A.M. Williams[b], Marcela Raimondi[c], Beatriz Lima[d], Gabriela Egly Feresin[d], Bibiana Nerli[a], Marcela Rizzotto[a,*]

[a] Instituto de Química Rosario (IQUIR, CONICET, UNR), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, 2000 Rosario, Argentina
[b] Centro de Química Inorgánica (CEQUINOR, CONICET, UNLP), Departamento de Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 47 y 115-C.C.962- (B1900AVV), 1900, La Plata, Argentina
[c] Área Microbiología, Facultad de Cs. Médicas, Universidad Nacional de Rosario, Santa Fe 3100, 2000, Rosario, Argentina
[d] IBT,-UNSJ, Avenida Libertador Gral, San Martín 1109, Oeste, San Juan, Argentina

ARTICLE INFO

Keywords:
Sulfadrug metal complex
BSA
Antimicrobial properties
Ames test
Allium test

ABSTRACT

The emergence of old and new antibiotic resistance created in the last decades revealed a substantial medical need for new classes of antimicrobial agents. The antimicrobial activity of sulfa drugs is often enhanced by complexation with metal ions, which is in concordance with the well-known importance of metal ions in biological systems. Besides, sulfonamides and its derivatives constitute an important class of drugs, with several types of pharmacological agents possessing antibacterial, anti-carbonic anhydrase, diuretic, hypoglycemic, antithyroid, antiviral and anticancer activities, among others.

The purpose of this work has been the obtainment, characterization and determination of biological properties (antibacterial, antifungal, mutagenicity and phytotoxicity) of a new Co(III)-sulfathiazole complex: Costz, besides of its interaction with bovine serum albumin (BSA). The reaction between sodium sulfathiazole (Nastz) and cobalt(II) chloride in the presence of H_2O_2 leads to a brown solid, $[Co^{III}(stz)_2OH(H_2O)_3]$, (Costz). The structure of this compound has been examined by means of elemental analyses, FT-IR, ^1H NMR, UV–Visible spectrometric methods and thermal studies. The Co(III) ion, which exhibits a distorted octahedral environment, could coordinate with the N thiazolic atom of sulfathiazolate. The complex quenched partially the native fluorescence of bovine serum albumin (BSA), suggesting a specific interaction with the protein. The Costz complex showed, *in vitro*, a moderate antifungal activity against *Aspergillus fumigatus* and *A. flavus*. As antibacterial, Costz displayed, *in vitro*, enhanced activity respective to the ligand against *Pseudomonas aeruginosa*. Costz did not show mutagenic properties with the Ames test. In the *Allium cepa* test the complex showed cytotoxic properties but not genotoxic ones. These results may be auspicious, however, further biological studies are needed to consider the complex Costz as a possible drug in the future.

1. Introduction

The emergence of old and new antibiotic resistance created in the last decades revealed a substantial medical need for new classes of antimicrobial agents [1]. Many biologically active compounds used as medicinal drugs possess modified pharmacological and toxicological profiles when are administered in the form of metal based compounds [2]. The synthesis of metal sulfanilamide compounds had received much attention due to the fact that sulfonamides were the first effective chemotherapeutic agents to be employed for the prevention and cure of bacterial infections in humans [3]. Besides, sulfonamides and its derivatives constitute an important class of drugs, with several types of pharmacological agents possessing antibacterial, anti-carbonic anhydrase, diuretic, hypoglycemic, antithyroid, antiviral and anticancer activity among others. At present they are the drugs of choice for the treatment of chancroid, nocardiosis and acute urinary tract infections caused by several microorganisms like *Escherichia coli, Proteus mirabilis*, and some others, and can be used combined with other drugs in the treatment of otitis, meningitis, toxoplasmosis, recurrent and chronic urinary tract infections and diarrhea, among other diseases [4], so, the synthesis of new sulfonamides and sulfonamide-metal complexes continues today [5]. The antimicrobial activity of sulfa drugs is often

* Corresponding author.
E-mail addresses: rizzotto@iquir-conicet.gov.ar, marcela.rizzotto@gmail.com (M. Rizzotto).

http://dx.doi.org/10.1016/j.cbi.2017.10.004
Received 23 June 2017; Received in revised form 22 September 2017; Accepted 3 October 2017
Available online 05 October 2017
0009-2797/

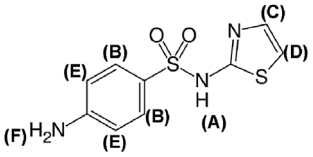

Fig. 1. Sulfathiazole (Hstz; as sodium salt: Nastz). Labels indicate the notation used for Hstz and their derivatives for ^{1}H NMR assignments.

enhanced by complexation with metal ions [6] which is in concordance with the well-known importance of metal ions in biological systems. Many complexes of Co(II) showing antimicrobial activity have been synthesized recently [7] even with sulfonamides as ligands [4,7e]. Co (II) complexes are the most studied, presumably due to their aqueous stability, availability, and ease of synthesis [8]. Among them, some examples of stable Co(III) complexes with antimicrobial activity have also been reported recently [9].

The purpose of this work has been the obtainment, characterization and determination of biological properties (antibacterial, antifungal, mutagenicity and phytotoxicity) of a new Co(III)-sulfathiazole complex: Costz, besides of its interaction with bovine serum albumin (BSA).

2. Material and methods

2.1. Materials

Sulfathiazole (Fig. 1), as sodium salt (Sigma, > 99%), cobalt(II) chloride hexahydrate (Merck, GR), and all other chemicals of commercially available reagent grade, were used as received. The related complex between Co(II) and sulfathiazole, ($[Co^{II}(stz)_2(H_2O)_4]$: Co(II) stz), previously obtained by us [10], was used for comparative purposes in several studies.

2.2. Preparative

$[Co^{III}(stz)_2OH(H_2O)_3]$: 3 mL of aqueous solution of cobaltous chloride containing 0.2450 g of $CoCl_2{\cdot}6H_2O$ (0.894 mmol) plus 2 mL of H_2O_2 10 vol (1.78 mmol) was added dropwise to 15 mL of stirring aqueous solution of sulfathiazole as sodium salt, Nastz, containing 0.5723 g of Nastz (2.06 mmol) at room temperature. The H_2O_2 solution in order to oxidize the Co(II) to Co(III) in the presence of sulfathiazolate was added in excess in order to impulse the completely oxidation of the Co(II). At first the resulting mixture became blue (because the initial formation of a Co(II)-sulfathiazolate complex) which immediately changed to brown with the formation of precipitate. After four days, the brown precipitate was centrifuged, washed several times with water added with H_2O_2 in order to avoid possible contamination with Co(II), filtered off and dried under vacuum. The non-affectation of sulfathiazole by hydrogen peroxide was checked by ^{1}H NMR. Yield: 0.3924 g (59.65%).

2.3. Physicochemical measurements

Elemental chemical analyses (C, H, N and S) were performed in a microanalyser Carlo Erba EA1108. The content of Co was determined by both complexometric back-titration with EDTA (ethylenediaminetetraacetic acid) [11] and atomic absorption spectroscopy with a double beam Perkin–Elmer spectrometer, model 3110 (Waltham, MA, USA). The content of Cl^- found in the mother liquor plus the washing waters, measured by Mohr's method [12] allows us to discard chloride in the complex structure. IR spectra of powdered samples were measured with a Bruker IFS 66 FTIR-spectrophotometer (Billerica, MA, USA) from 4000 to 400 cm^{-1}, using the KBr pellet technique. Raman spectra were measured with a Spex-Ramalog double monochromator spectrometer (Arlington, Tx, USA) using the 514.5 nm line of an Ar ion laser for excitation, over the region 200-2000 cm^{-1}. Thermogravimetric (TG) and differential thermal analysis (DTA) were performed on a Shimadzu system (models TG-50 and DTA-50 respectively) (Kioto, Japan), working in an oxygen flow (50 mL/min) and a heating rate of 10 °C/min. Sample quantities ranged between 10 and 20 mg Al_2O_3 were used as a DTA standard. Diffuse reflectance spectra were recorded between 400 and 900 nm with a Shimadzu UV-300 instrument, using MgO as an internal standard. ^{1}HNMR spectra in hexadeuteriodimethyl sulfoxide (DMSO-d_6) were obtained using a Bruker AC-300 E spectrometer at ambient probe temperature (*ca.* 25 °C). Proton chemical shifts were referenced to the central peak of DMSO-d_6 [^{1}H NMR, δ(DMSO) = 2.47 ppm]. Positive values of chemical shifts denote high frequency shifts with respect to standards. The monitoring of the reaction between Nastz and Co(II) in the presence of H_2O_2 was made in D_2O. The experience was also performed in DMSO-d_6 to observe the signals due to the protons of the amino group. The ^{1}H NMR spectrum of the solid Costz dissolved in DMSO (0.5 mL, 0.08 mol L^{-1}) showed broadening of all signals (Fig. S2 Supporting information). This fact, that could be attributed to the interaction of Co(III) with the sulfathiazolate moiety, also might be due to traces of Co(II) in the sample that hinders the interpretation; so, we decided to perform experiments in solution in which we followed by ^{1}HNMR the first moments of the interaction between Nastz and cobalt. Successive aliquots containing 1.56×10^{-9} mol of Co(II) (2 μL $CoCl_2{\cdot}6H_2O$, 7.82×10^{-4} mol L^{-1}) were added up to 0.5 mL of solution containing 4×10^{-5} mol of NaST and 4.4×10^{-5} mol of H_2O_2, into a NMR tube. ^{1}H NMR spectrum was taken immediately after each addition. For comparative purposes, ^{1}HNMR spectra of Nastz plus several aliquots (1; 2; 10; 30 μL) of Co(II) 7.21×10^{-4} mol L^{-1} were taken in D_2O without the addition of H_2O_2. The high molar ratio H_2O_2/Co(II) (≥1900) encourages the Co(II) to Co (III) oxidation: no signs of paramagnetism were observed in these experiences, and the high molar ratio ligand/metal (≥1700) promotes complex formation. To verify that the ligand was not affected by the hydrogen peroxide, the same experiment was performed with Nastz and H_2O_2 but without the addition of Co(II). We worked with very dilute solutions of Co(II) to avoid the appearance of solids into the NMR tube. TopSpin 2.0 and/or Origin 6.0 were used to graph the NMR spectra.

2.4. Binding with bovine serum albumin

2.0 mmol L^{-1} Costz complex solutions were prepared by dissolving 12.8 mg of its powder in 10.00 mL DMSO. 1500 μmol L^{-1} BSA stock solutions, based on their molecular weights of 67,000, were prepared by dissolving BSA in 0.1 mol L^{-1} pH 7.0 phosphate buffer solution (PBS). 2.0 mmol L^{-1} Nastz and $CoCl_2{\cdot}6H_2O$ solutions were prepared by dissolving, respectively, 5.5 mg and 4.8 mg of its crystal in 10.00 mL of distilled water. All other chemicals were of analytical reagent grade and were used as received. The measurements were made at room temperature, in a Jasco FP 770 spectrofluorometer (Oklahoma City, Oklahoma, USA), using a 1 × 1 cm thermostated quartz cuvette. In a typical fluorescence measurement, 30 μL of 1500 μmol L^{-1} BSA was added to 2.5 mL of PBS and the resulting solution was transferred to a 1.0 cm quartz cell. The Costz solution was then gradually added to the cell using a micropipette. The concentrations of the complex in the cell were ranged from 1.6 to 84.8 μmol L^{-1} and the total accumulated volume of complex solution was less than 100 μL. The corresponding fluorescence emission spectra were recorded, in the absence and presence of Costz, from 310 to 410 nm upon excitation wavelength at 300 nm. In order to compare, similar experiences were made by using Nastz and Co(II)stz, a related complex, instead of Costz. The UV-Vis absorbance spectra of PBS solutions with progressive addition of Costz and Nastz solutions were recorded from 290 to 350 nm in a Jasco model 530 double beam UV spectrophotometer. These titrations curves were made in order to correct the observed fluorescence from the inner filter effect. The relationship of fluorescence intensity and drug concentration was treated by means of the Scatchard model that assumes the presence of equivalent and non-cooperative binding sites. The

Scatchard equation [13] is given by (r/C) = K × n − K × r, where r is the ratio of the concentration of bound ligand to total available binding sites, C is the concentration of free ligand, and n is the number of binding sites *per* protein molecule. It was used to calculate the affinity constant (K) of a ligand with a protein.

2.5. Antibacterial assay

The following strains from the American Type Culture Collection (ATCC), Rockville, MD, USA, and from the Laboratory of Microbiology (LM, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina) were used for the antibacterial evaluation. Gram-positive Bacteria: *Staphylococcus aureus* methicillin-sensitive ATCC 29213, *S. aureus* methicillin-resistant ATCC 4330. Gram-negative Bacteria: *Escherichia coli* ATCC 25922, LM1-*E. coli*, LM2-*E. coli* and *Pseudomonas aeruginosa* ATCC 27853. Bacteria were grown on Müeller–Hinton agar medium. Preparation of inocula: cultures of less than 30 h-old were touched with a loop and transferred to sterile broth Müeller-Hinton. The broth was incubated at 37 °C until the growth reached a turbidity equal to or greater than that of 0.5 McFarland standard. The culture was adjusted with sterile physiological solution to give a final organism density of 5×10^5 colony-forming units/mL (CFU/mL) [14]. Assay: the antibacterial activity was evaluated with the agar dilution method using Müeller–Hinton agar medium for Gram (+) and Gram (−) bacteria [15]. Stock solutions of the ligand and its Co(III) complex in DMSO were diluted to give serial two-fold dilutions that were added to each medium resulting in concentrations ranging from 100 to 10 μg mL^{-1}. The final concentration of DMSO in the assay did not exceed 2%. The antimicrobial agent cefotaxime (Argentia Pharmaceutica) were included in the assays as positive control. Minimal Inhibitory Concentration (MIC) was defined as the lowest concentration of a compound showing no visible bacterial growth after incubation time (24 h) at 37 °C. Tests were done in triplicate. MICs ≤30 μg/mL were considered active.

2.6. Antifungal assays

The microorganisms used for the fungistatic evaluation were purchased from ATCC, or were clinical isolated from CEREMIC (identified with the capital letter C): Centro de Referencia en Micología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Suipacha 531-(2000)-Rosario, Argentina. Yeasts: *Candida albicans* ATCC10231, *C. tropicalis* C131, *Saccharomyces cerevisiae* ATCC9763, *Cryptococcus neoformans* ATCC32264; hialohyphomcetes: *Aspergillus fumigatus* ATCC26934, *A. flavus* ATCC9170, *A. niger* ATCC9029; dermatophytes: *Microsporum gypseum* C115, *Trichophyton rubrum* C113, *T. mentagrophytes* ATCC9972. The fungal strains were grown on Sabouraud-chloramphenicol agar slants for 48 h at 30 °C. The strains were maintained on slopes of Sabouraud-dextrose agar (SDA, Oxoid) and subcultured every 15 days to prevent pleomorphic transformations. Spore suspensions were obtained according to reported procedures [16] and adjusted to 1×10^3 spores with colony forming ability/mL. Assay: MIC of each compound was determined by using broth microdilution techniques according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2008, formerly National Committee for Clinical Laboratory Standards NCCLS) for yeasts (M27-A3) and for filamentous fungi (M38-A2). MIC values were determined in RPMI-1640 (Sigma, St Louis, Mo, USA) buffered to pH 7.0 with MOPS. Microtiter trays were incubated at 35 °C for yeasts and hialohyphomycetes and at 28-30 °C for dermatophyte strains in a moist, dark chamber, and MICs were visually recorded at 48 h for yeasts, and at a time according to the control fungus growth, for the rest of fungi. For the assay, stock solutions of pure compounds were two-fold diluted with RPMI from 250 to 0.98 μg mL^{-1} (final volume = 100 μL) and a final DMSO concentration ≤ 1%. A volume of 100 μL of inoculum suspension was added to each well with the exception of the sterility control where sterile water was added to the well instead. Endpoints (MIC) were defined as the lowest concentration of drug resulting in total inhibition of visual growth compared to the growth in the control wells containing no antifungal. Amphotericin B (Janssen Pharmaceutica, Belgium), Ketoconazole (Sigma Chem. Co. St Louis, MO, USA) and Terbinafine (Novartis, Bs. As., Argentina) were used as positive controls. MIC´s were confirmed by two replicates. MICs ≤250 μg/mL were considered active.

2.7. Mutagenicity assay (Ames test)

Mutagenicity activity was evaluated in a bacterial reverse mutation assay by the standard Ames test in the absence of S-9 mix, by using the *Salmonella typhimurium* histidine-requiring test with TA98 and TA100 strains [17], which together detect the 93% of the mutagens [18]. Diagnostic mutagens, including 4-Nitro-*o*-phenylenediamine (4NOPDA) for the TA98 strain and sodium azide, NaN_3 for the TA100 one, were prepared by dissolving them in DMSO and sterile water respectively, and served as positive control chemicals. Bacteria were aerobically grown at 37 °C in Oxoid nutrient broth Nº 2. The test was carried out by adding 0.2 mL of sterile 0.5 mmol L^{-1} histidine-biotin and 0.1 mL of the overnight bacterial culture (approximately 1×10^8 bacteria mL^{-1}) to 2.0 mL of molten top agar (45 °C). Doses from 10 to 100 μL of the tested solution were added to top agar tubes, which were then gently vortexed and subsequently transferred to plates with minimal glucose agar (30 mL/plate). After 48 h incubation time at 37 °C in darkness, the His^+ revertant colonies were manually counted. Culture medium: nutrient broth was prepared by dissolving 25 g of Oxoid nutrient broth Nº 2 in 1 L of water. Glucose minimal agar plate contained 1.5% agar, 0.02% $MgSO_4 \cdot 7H_2O$, 0.2% citric acid, 1% K_2HPO_4, 0.35% $NaHNH_4PO_4 \cdot 4H_2O$ and 2% glucose. Top agar contained 0.75% agar and 0.5% NaCl. In a typical experiment, 0.0100 g of Costz were dissolved in 3.00 mL of DMSO, giving a 8.99 mmol L^{-1} solution. The assayed doses were taken from this solution, generating dose-response curves by means of the standard plate assay. As sulfa-drugs have proven to be not mutagenic [19], the activity of the ligand was not tested by us. Triplicate plates were poured for each dose of Costz and the results were confirmed in three independent experiments.

2.8. Plant genotoxicity test (Allium cepa test)

For this test, which was carried out following standard procedures previously used [5c,20], equal sized young bulbs of common *Allium cepa* L. were used. Considering the antifungal MIC values, mother solution of Costz was prepared dissolving 0.100 g of the complex in 8.0 mL DMSO and commercial mineral water in sufficient quantity to 500.0 mL. Similarly, a mother 0.200 g L^{-1} solution of Nastz, the parent sulfonamide, was prepared. Aliquots from these solutions were taken out in order to carry out the experiments, in duplicate, with seven bulbs *per* dose. Onion bulbs were kept in mineral water for 48 h and then exposed to the Costz and Nastz solutions for 24 h. The roots were then fixed in 1:3 acetic acid-ethanol solution for 24 h, and finally stored in 70% ethanol. The roots growing in mineral water were used as a negative control, while the treatment with NaN_3 1.0×10^{-5} mol L^{-1} in mineral water represented a positive control. Length of roots (Δ of the each longest one *per* bulb) as index of toxicity and modifications in root consistency and shape (formation of tumors, hook roots, twisted roots) were observed as macroscopic parameters. Mitotic index (five slides, 1000 cells *per* slide) was the microscopic parameter to evaluate cellular division rate.

For the chromosome preparation and staining, root tips were hydrolyzed in 6 mol L^{-1} HCl at room temperature (25 °C, 10 min) before staining in Schiff's reagent (15 min). After the root caps were removed from well stained root tips, 1 mm of the meristematic zones was immersed in a drop of 2% orceine in 45% acetic acid (which carried out the staining of the chromosomes) on a clean slide and squashed into single cells. A light microscope was used with 640 × magnification for

observations. Changes in chromosome morphology were photographed under a light microscope (OLYMPUS BX40) with an OLYMPUS D-560 ZOOM photographic camera (Tokio, Japan).

2.9. Statistical analysis

One-way analysis of variance (ANOVA) was applied, followed by post hoc comparisons with the Student's *t*-test to estimate the significance of the differences between groups. Scatter plots were used to study at first the relationship between the variables, and linear regression to describe it [21]. Data were expressed as mean ± standard deviation (SD). A $p < 0.05$ was considered of statistical significance [22].

3. Results and discussion

3.1. General physicochemical characteristics

Elemental analyses of the brown powder gave satisfactory results for $[Co^{III}(stz)_2OH(H_2O)_3]$, where stz = sulfathiazolate. Found% (calcd.% for $CoC_{18}H_{23}N_6S_4O_8$): Co, 8.7 (9.2); C, 33.26 (33.39); H, 3.38 (3.63); N, 12.96 (13.16); S, 19.2 (20.1). Calculated molecular weight: 638.6 g/mol. The complex is only slightly soluble in water at room temperature and in HCl 1 M, but it is soluble in DMSO (8.11 mg mL^{-1}). It does not suffer decomposition in HCl 1 M or NaOH 1 M, different from its homologous complex between sulfathiazolate and Co(II), Co(II)stz [10].

3.2. Vibrational spectra

IR and Raman selected spectral data of the Costz complex and the ligand (as sulfathiazole and its sodium salt) are presented in Tables 1 and 2, respectively. The Raman spectrum of the Costz is very noisy, and merely the most intense bands can be observed in the spectral region 2000-400 cm^{-1}. Spectral data of Co(II)stz complex [10] are included for comparative purposes. In the 3600-3000 cm^{-1} region, broad bands appear for Nastz and Co-stz due to the presence of hydration water. The O-H (water and hydroxide) stretchings can be observed at 3589 and 3561 cm^{-1}, respectively (see Table 1 and Fig. S1). Similarly, this band is observed as a single band (stretching OH_{water}) at 3582 cm^{-1} in the Co(II)-ST complex [10]. Sulfathiazole (Hstz) shows characteristic NH_2 bands, corresponding to the asymmetric and symmetric stretchings at 3346 and 3320 cm^{-1}, respectively [23]. The third band that appears at 3280 cm^{-1} is assigned to the N-H stretching of the sulfonamide moiety. This band is not observed in the other compounds (Nastz and the sulfathiazole-cobalt complexes, presented in Table 1), probably due to deprotonation. A shift to higher energy of this group of bands can be seen. This fact is consistent with that observed for other metal-sulfathiazole complexes [24]. The band corresponding to the vibration of the thiazole ring is shifted from 1536 cm^{-1} in the ligand to *ca.* 1450 cm^{-1} in the other compounds, both in the IR and Raman spectra. In addition to the band of medium intensity at 1507 cm^{-1} in the Raman spectrum of Costz, a new strong band at 1539 cm^{-1} appears. Both bands can be assigned to the symmetric in-plane bending of the aromatic ring, δ_{ip} CCH_{arom}. The shift of the vibrational modes of the thiazole ring suggests the interaction of the metal ion to this group. The S-N stretching vibrations (sulfonamide) are located at lower energy in the IR spectrum of Hstz. These observations are indicative of the deprotonation of the N-sulfonamide atom. In the Raman spectra these bands appear with a very weak intensity. Besides, the band assigned to the N-H wagging at 732 cm^{-1} with medium intensity in the IR spectrum of Hstz disappears upon deprotonation and/or coordination. In all compounds the bands attributed to the SO_2 vibrations remain unaltered, suggesting no interaction of the $-SO_2-$ group with the metal ion. The increase in the intensity of the $\nu_s(SO_2)$ in the Raman spectra confirms this fact. C-S stretching is mainly not affected by coordination. According to the observed changes in the vibrational IR and Raman spectral data it is possible to suggest that the $N_{thiazolic}$ atom would be a coordination point to the Co(III) with sulfathiazolate, with a coordination sphere conformed by two $N_{thiazolic}$ atoms, three O-atoms of the water molecules and the O-atom of the OH-group. The coordination of Co(III) to the ligand would be similar to the one observed in the related complex of Co(II) with the same ligand (Co(II)stz). This conclusion is consistent with similar systems containing sulfathiazole and different metal ions [25] and with the related complexes of Co(II) with sulfathiazole [10] and phtalylsulfathiazole [20].

Table 1
Assignment of the vibrational FTIR spectra (frequency: ν, cm^{-1}) of sulfathiazole (Hstz), its sodium salt (Nastz) and its cobalt complexes (Co(II)stz [10] −in order to compare−) and Costz.

Hstz	Nastz	Co(II)stz (pink powder)	Co(II)stz (red crystals)	Costz	Assignments
	3589 m	3582 sh	masked	3589 sh 3561 sh	ν OH
3346 m	3492 s, 3418 s	3460 s, br	3468 m, 3443 m	3460 s, br	ν_{as} NH_2
3320 m	3336 s	3358 s, br	3386 sh, 3358 s	3363 s, br	ν_s NH_2
1575 s; 1536 vs	1445 vs	1450 s	1445 s	1529 m; 1442 s	Thiazole ring
1324 m	1321 m	1316 m	1318 m	1321 m	ν_{as} SO_2
1138 s	1131 s	1123 vs	1146 m	1136 vs	ν_s SO_2
920 s	958 s	968 m	962 m	937 m	ν $SN_{sulfonamide}$
732 m					ω NH
648 s; 633 m	648 sh; 633 m	651 m; 624 sh	648 m; 631 m	648 sh 631 m	ν (C-S)
573 s	569 m	577 m	572 m	574 s	ω SO_2
554 m	551 s	553 s	557 m	555 s	ω SO_2

vs: very strong; s: strong; m: medium; w: weak; br: broad.

Table 2
Assignment of the vibrational Raman spectra (frequency: υ, cm^{-1}) of sulfathiazole (Hstz), its sodium salt (Nastz), and its cobalt complexes (Co(II)stz −in order to compare−) and Costz.

Hstz	Nastz	Co(II)stz	Costz	Assignments
1594 vs	1599 m	1600 s	1594 sh	ν Ph
1496 w	1492 vs	1503 s	1539 vs; 1507 m	ν Ph
1527 m	1448 s	1431 s	1445s; 1423 s	ν C=N
1321 w	1320 w			ν_{as} (SO_2)
1130 vs	1125 m	1120 vs	1122 vs	ν_s (SO_2)
935 w	938 w	970 s	972 vw	ν $SN_{sulfonamide}$
648 s; 633 m	633 s	646 m	648 m	ν (C-S)

3.3. Thermogravimetric behavior

The thermal behavior of Hstz and Co-stz are shown in Fig. 2. The thermal degradation of Hstz proceeds in four different stages. The first decomposition process takes place between 10 and 296 °C with about 18.4% weight loss (DTA signals: 175 °C (endo, w) and 207 °C (endo, w). Upon further heating, the sample loses mass in two steps ($\Delta\omega$ = 8.6 and 6.1%) up to 425 °C, then the decomposition speeds up suddenly with a strong DTA signal at 514 °C (exo, s), giving volatile products. For Costz, thermal degradation occurs in several overlapping steps. The dehydration covers the range 10–225 °C with the elimination of four water molecules in two successive stages. In the first step three water molecules were eliminated (DTA signals, 59 °C (endo, vw), $\Delta\omega_{calcd}$ = 8.5%, $\Delta\omega_{exp}$ = 8.4%). In the second step, the OH group has been eliminated probably as a water molecule ($\Delta\omega_{calcd}$ = 2.9%, $\Delta\omega_{exp}$ = 3.0%). The last pyrolysis steps (225-1000 °C) involved two exothermic DTA signals located at 402 °C (lower intensity) and 448 °C (higher intensity). The

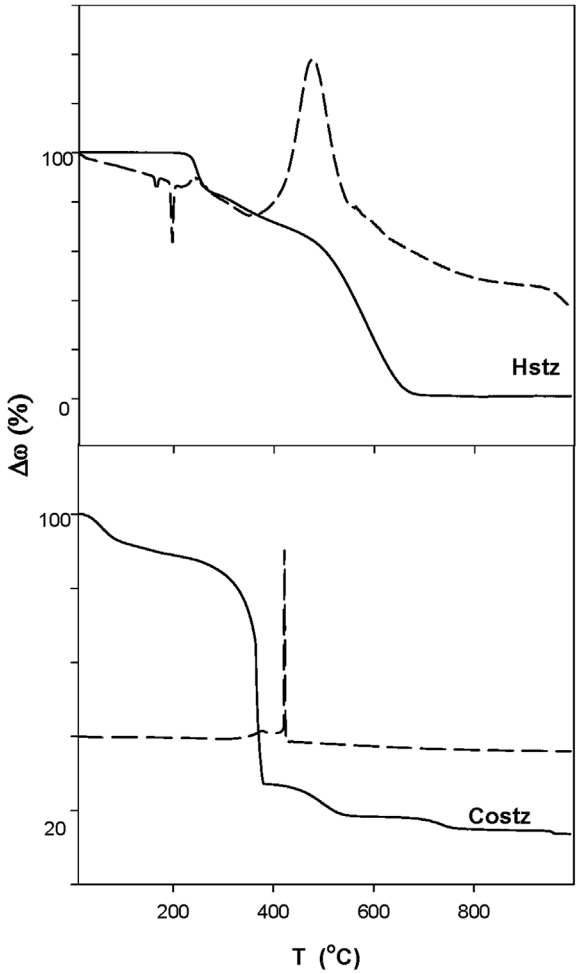

Fig. 2. TG (–) and DTA (——) traces for the thermal decomposition of sulfathiazole (Hstz) and Costz systems (O_2-flow = 60 cm^3 min^{-1}; heating rate = 10 °C min^{-1}). Plot Δ ϖ (%) vs T (°C).

final residue was characterized by IR spectroscopy as Co_2O_3. The total weight loss of 86.9% agrees with the theoretical value of (87%). The total stoichiometry of this process is proposed as follows:

φ, O_2

$[Co(stz)_2(OH)\ (H_2O)_3] \rightarrow Co_2O_3$ + other volatile products

3.4. Electronic spectra

Electronic spectra of complexes can provide valuable information on both, bond and structure, since the colors are closely related to the magnitude of the spacing between d-orbitals (e_g and t_{2g} or e and t_2 orbitals in octahedral and tetrahedral complexes, respectively), which depends on factors such as the geometry of the complex, the nature of the ligands present and the oxidation state of the central metal atom [26]. The diffuse reflectance spectra were measured in the 400-900 nm range. Two transitions are observed, at 600 and 470 nm. In the case of the Costz complex the metal ion has a low spin d^6 configuration ($^1A_{1g}$ ground term). The two transitions to the $^1T_{1g}$ and $^1T_{2g}$ states are observed at 600 and 470 nm, corresponding to octahedral low spin cobalt (III) complexes [27].

3.5. ^1H NMR spectra

3.5.1. Costz in DMSO-d_6

^{1}HNMR chemical shifts from the spectra of Costz in DMSO-d_6 are showed in Fig. S2 (Supporting Information). ^{1}HNMR spectrum of the Costz complex showed broadening of all signals with respect to the Nastz ones and large shifts for the thiazole ring when it is compared with free Nastz. Signals of the thiazole ring and the amine group move significantly downfield (Δ δ_H for H(C): +0.30; H(D): +0.24; and H(F), from the amine group: +0.36), meaning that the hydrogen is deshielded [28]. On comparison of the data observed with complexes between Ir(III) and nicotine, a large shift in the C(2)–H and C(6)–H were observed, in agreement with the fact that the interaction between protonated nicotine and Ir(III) is produced *via* the pyridine nitrogen [29] analogous to that observed with complexes of Ir(III) and polypyridyl ligands [30]. The signal of the amidic proton (H(A), δ = 12.39 ppm in Hstz) was absent in the ^{1}H NMR spectrum of the complex, likewise to the Nastz one, indicating that the sulfathiazole moiety is deprotonated in the complex [31]. These results are in agreement with those of the vibrational spectra.

3.5.2. Experiences of interactions in solution

3.5.2.1. Solvent: D_2O. The 1D ^{1}H NMR spectra of the ligand (Nastz) plus H_2O_2 in the presence and in the absence of 24 μL of Co(II) 7.82 × 10^{-4} mol L^{-1} are showed in Figs. S3–S5 (Supplementary information). The signals do not change the position, but the intensities of all of them are dramatically reduced upon Co(II) addition in the presence of H_2O_2, so much that difficult to integrate signals (Fig. S5). The spectra appear very different when hydrogen peroxide is not added, and the difference is already noticeable from the first additions of Co (II). With the addition of the first microliter of Co(II), proton signals C and D are shown broadened. With the addition of 30 μL, only protons signals B and E persist, very broadened and shifted downfield (Fig. S6).

3.5.2.2. Solvent: DMSO-d_6. 1D ^{1}H NMR spectra of the ligand (Nastz) plus 24 μL of Co(II) 7.82 × 10^{-4} mol L^{-1} in the presence of H_2O_2 are showed in Figs. S7–S12. In DMSO-d_6, in contrast what was observed in deuterated water, the intensity of the signals is unaffected, but changes in the chemical shift and width are observed. All signals showed downfield shifts, which were dependent of the different concentration of the metal ion. The higher changes of the spectral signals correspond to the protons of the thiazole ring and, to a lesser extent, to the protons of the amine group. Consistent with this, the protons B are the less affected. Although species in solution may not be exactly the same as in the solid state, previous experiences in our laboratory for the complex Co(II)-sulfathiazolate (homologous of the complex Costz) allowed us to suggest that the interaction between the thiazolic nitrogen and the Co (II) ion, detected in the crystal structure resolution by XRD [10] could remain in solution [32]. The experimental observations agree with the fact that the Co(III) cation, as hard acid, shows higher tendency to coordinate with the nitrogen atom (hard base) than with the sulfur one (soft base) [33]. Even if Co(III) may coordinate with S atoms, in general these are not belonging to heterocyclic derivatives [34–36]. These results let us suggest an interaction of Co(III) with the heterocyclic nitrogen atom, without discarding that there may be interaction also with the nitrogen of the amine group.

3.6. Binding with bovine serum albumin

Serum albumins are the most abundant proteins in plasma, and these play many physiological functions, such as the contribution to colloid osmotic blood pressure and to the maintenance of blood pH. The most outstanding property of albumins is their ability to reversibly bind to large variety of ligands, like pyridoxal phosphate, cystein, glutathione, Schiff base ligands, various metal ions such as Cu(II), Ni(II), Mn(II), Co(II), Hg(II), Zn(II), metal complexes and so on [37]. On the other hand, the binding of drugs to plasmic proteins, mainly albumin and α-glycoproteins, is one of the factors that affects the availability of drugs in the human body. The binding constant (K) is an important parameter which measures the extent of interaction and it is important

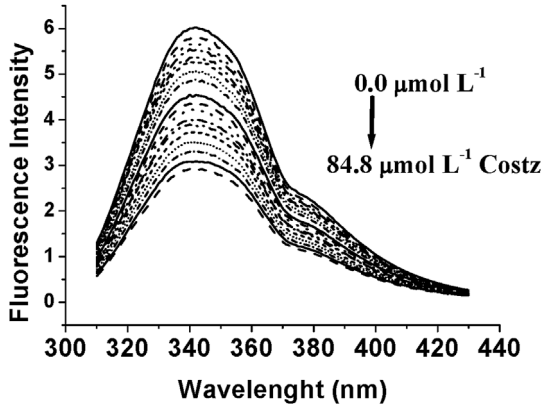

Fig. 3. Fluorescence spectra of BSA in the absence and presence of increasing quantities of Costz.

for the release of drugs attached to the protein [38]. All sulfonamides bind in different grades to plasmatic proteins, in special to albumin. The extension in which it occurs is determined by the hydrophobicity of the drug and by the pKa of the sulfa drug: at physiological pH, drugs of high pKa show low grade of binding to proteins, and *viceversa.* The pKa of sulfathiazole (7.1) is higher than another sulfa drugs pKa´s, for example: sulfaphenazole (5.91), sulfamethoxazole (5.81) and sulfisoxazole (4.62) [39]. In the present work we analyzed the interaction of BSA with the complex Co-stz by means of fluorescence spectroscopy. The interaction with the ligand (Nastz) and the related complex sulfathiazole-Co(II) were also studied. The maximum fluorescence intensity of the tryptophan and tyrosine residues is depicted on the bands at ca. 340 and 300 nm, respectively. If the small molecule can quench the fluorescence of the tryptophan residues, these tryptophan residues must be located in or near the binding position. The fluorescence spectra obtained with a constant concentration of BSA and increasing concentrations of Costz are shown in Fig. 3.

Results showed that the fluorescence intensity of BSA solution at 340 nm dropped regularly with the increasing concentrations of the studied drugs and the peak shapes did not change. The above results suggest an interaction between the drugs and BSA that results in the formation of non-fluorescent complexes, so the studied drugs should be located close to this tryptophan residue when they bind to the BSA. The native fluorescence of BSA is not zero for high concentrations of the ligands, so, it is possible to conclude that the ligands accede to only one of the two tryptophans of the BSA, which could be the Trp 214 one [40]. Fig. 4 A shows the titration curve of BSA with the ligands assayed; its saturation behavior is compatible with the presence of a specific interaction between the protein and the drugs. Linear Scatchard plots observed in Fig. 4 B confirm a binding model of n equivalent and independent sites [13]. Table 3 shows the K and n values obtained from the slope (K) and abscise intercept (n) of those plots. Analysis of K values suggests an interaction with BSA of similar affinity for both Nastz and Co(II)stz, while the affinity could be little high for the interaction with Costz, in agreement with K values recently informed for other Co(III) complexes [41].

Table 3
K and n values for the interaction of NaST and its cobalt complexes with BSA.

Ligand	Nastz	Co(II)stz	Costz
K × 10^{-5} (M^{-1})	1.40 ± 0.12	1.21 ± 0.08	2.60 ± 0.28
N	2.9 ± 0.1	2.30 ± 0.09	2.4 ± 0.2

K: binding constant; n: number of binding sites per protein molecule.

The BSA-binding constants of complexes of Co(II) complexes of the quinolone sparfloxacin in the absence or presence of the nitrogen-donor heterocyclic ligands 2,2′-bipyridine, 1,10-phenanthroline or 2,2′-bipyridylamine are in the range 1.96 × 10^5–1.08 × 10^6 M^{-1}; they are relatively high and may, thus, reveal the potential affinity of the complexes to bind to BSA in order to get transported towards their potential biological targets [7 h]. However, the constants of these complexes are significantly lower than the value of K ≈ 10^{15} M^{-1}, which is the binding constant of the strongest known non-covalent interaction of the avidin with diverse compounds [42b], thus, it may be consider the binding of the complexes to the albumins reversible and that there is high possibility of release upon arrival at the potential biotargets [42c]. So, the range of the binding constants of the sulfathiazole-cobalt complexes to BSA (K = 1.21 × 10^5 M^{-1} and 2.60 × 10^5 M^{-1} for Co(II) and Co(III) respectively) lie in an optimum range to suggest binding, transfer and release upon arrival at their targets [7h,42]. Besides, the number of sites for Co(II)stz and Costz are similar and differ slightly from that of Nastz.

3.7. Antibacterial properties

Emergence of resistance in bacterial strains has become one of the prime concerns of the 21st century [43]. It is well known that some drugs have greater activity when administered as metal complexes than as free organic compounds. The environment around the metal centre “as coordination geometry, number of coordinated ligands and their donor group” is the key factor to carry out specific physiological functions [44]. Thus, several metal complexes of different ligands, for example: Schiff bases [45], amines and quinolones [46], sulfanilamides [47] reported in recent times have showed antibacterial and/or antifungal activity, which, in many cases, improved the one of the ligands. Several cobalt(II) complexes recently reported have also showed antibacterial activity, which, in many cases, were better than the ligand ones [7,20] while a cobalt(III)-famotidine complex showed antibacterial and antifungal activity against representative bacteria: *E. coli*, *S. aureus* and *Micrococcus lysodeikticus* and the fungi: *A. niger* and *C. albicans*, better than the ligand alone [48]. Co(III) complexes of mixed ligand (phenanthroline/bipyridyl and benzoylhydrazones) exhibited

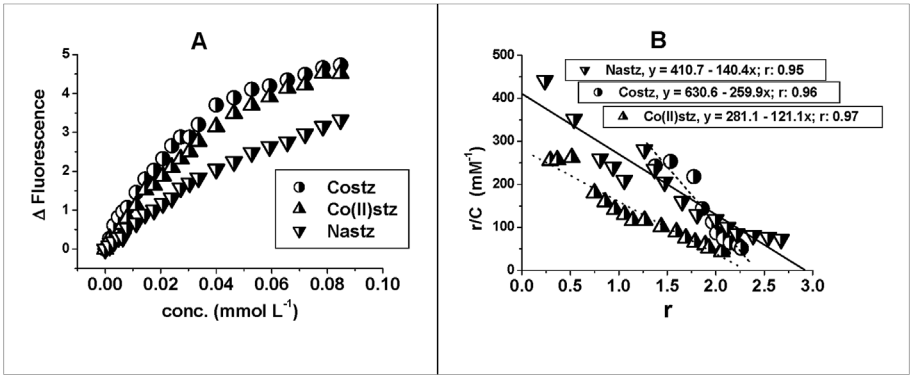

Fig. 4. Interaction of Costz, Co(II)stz and Nastz with BSA. **A.** Δ Fluorescence = Fluorescence (BSA) – Fluorescence (BSA + ligand); **B.** by the Scatchard equation. r: ratio of the concentration of bound ligand to total available binding sites, C: concentration of free ligand.

Table 4
MIC values in μg mL^{-1} of sodium sulfathiazole (Nastz) and the complex Costz acting against human pathogenic bacteria. MIC values of $CoCl_2{\cdot}6H_2O$, Co(II)stz and published MIC values of another related complexes (1-4) are given for comparison.

Compound Microorganism	Nastz	Costz	Co(II)stz[a]	Co(II)[b]	1[c]	2[d]	3[e]	4[f]	Cf.
Gram-positive Bacteria									
S. aureus methicillin-sensitive ATCC 29213	20.0	30.0	> 30.0	25.0			6.25		0.500
S. aureus methicillin-resistant ATCC 4330	20.0	30.0	> 30.0	25.0					0.500
S. aureus ATCC 6538	–	–	–	–	250 (500)[g]				
S. aureus clinical isolate (ref. [d]) or not specified	–	–	–	–	–	19.0–15.9 (4.4–7.2)[g]		0.250 (0.250)[g]	
Gram-negative Bacteria									
E. coli ATCC 25922	20.0	> 30.0	20.0	25.0	250 (500)[g]		50–200		0.500
LM_1-*E. coli*	25.0	> 30.0	25.0	25.0					5.000
LM_2-*E. coli*	20.0	> 30.0	25.0	25.0					0.500
E. coli clinical isolate (ref. [d]) or not specified						inactive-15.8 (8.8-inactive)[g]		0.031–0.062 (0.062)[g]	
P. aeruginosa ATCC 27853	> 30.0	30.0	20.0	25.0	250-500 (500-1000)[g]		200–400		7.500

MIC: Minimal Inhibitory Concentration. MICs ≤30 μg/mL were considered active. Standard drug: Cefotaxime (Cf.); ATCC: American Type Culture Collection; LM: Laboratory of Microbiology (Facultad de Ciencias Médicas, UNC, Mendoza, Argentina).

[a] Ref [20].
[b] Co(II): as $CoCl_2{\cdot}6H_2O$.
[c] Co(III) complexes of Schiff base ligands, ref. [9d].
[d] Co(II) complexes of azo group-containing Schiff base ligands, ref. [7f].
[e] Co(II) complexes of benzimidazole derivatives, ref. [7g].
[f] Co(II) complexes of the quinolone sparfloxacin, ref. [7h].
[g] In brackets: MIC of the respective free ligans.

good antimicrobial activity against *E. coli* [49]. The antimicrobial activity of the Co(III) complexes and the free ligands (two Schiff base ligands) exhibit antimicrobial properties and the Co(III) complexes show enhanced inhibitory activity compared with their parent ligand, but with elevated MIC values ([9d]). Other Co(II) with various ligands showed antibacterial activity good to moderate [7f; 7 g], whereas those of sparfloxacin (Hsf) showed lower values than the standard drug Cefotaxime, but generally not overcoming the activity of the free ligand Hsf [7 h]. Results of the antibacterial assay with Nastz and its Co(III) complex are showed in Table 4. MIC´s values of $CoCl_2{\cdot}6H_2O$ and related complexes are shown for comparative purposes. It was not possible to test aqueous Co(III) ion because of its instability [50].

Costz showed activity against the Gram positive tested bacteria and *P. aeruginosa* ATCC 27853 (MIC value 30 μg mL^{-1}), but not against the strains of *E. coli* assayed. The homologous complex Co(II)stz was active against all the Gram negative tested bacteria but not against the Gram positive ones. Both complexes were active against *P. aeruginosa* ATCC 27853, the only Nastz-resistant of the tested strain. It should be noted that *P. aeruginosa* is a leading nosocomial pathogen that may become multidrug resistant [51]. Chelation reduces the polarity of the metal atom and also increases the lipophilic nature of the central atom which subsequently favors its permeation through the lipid layer of the cell membrane [7a]. This fact could contribute to the action against *P. aeruginosa* ATCC 27853, in which the glycocalyx could act as a protective layer [52].

3.8. Antifungal properties

The increased incidence of invasive mycoses and the emerging problem of antifungal drug resistance have encouraged the search for new antifungal agents or effective combinations of existing drugs [53]. For similar complexes, activity appears to be directly proportional to the lability of the metalloelement-ligand bond [54]. Co(II) complexes of Schiff base derived from condensation of *o*-vanillin (3-methoxysalicylaldehyde) and sulfametrole [N^1-(4-methoxy-1,2,5-thiadiazole-3-yl)sulfanilamide] showed to be more potent antifungal compounds than the parent Schiff base ligand against *A. terreus* and *A. flavus* [55]. Complexes of silver and zinc sulfonamides (and some cerium derivatives) have successfully been used for the last 20 years for the prophylaxis and treatment of microbial and fungal burnt wound infections and they seem to constitute a valuable alternative for resolving the resistance problem [56]. Co(III) complexes of two tridentate Schiff base ligands showed better activity than the free ligand, but with MIC values that were considered not active by us (500 μg/mL) against *C. albicans* [9d]. Co(II) complexes of azo group-containing Schiff base [7f], benzimidazole derivatives [7 g] and pyrimidine derivative [7i] as ligands showed moderate to good antifungal activitiy against *C. albicans*. It is to be noted that in the latter the ligands were inactive. Results of the antifungal assay with the Costz complex and related complexes for comparison are showed in Table 5.

Two species of the *Aspergillus* genus tested (*A. fumigatus* and *A. flavus*) were susceptible to Costz, showing a similar behavior when compared to the related complex Co(II)stz [10]. Costz inhibits *A. flavus* with a better MIC value than the inorganic Co(II) ion, while the ligand Nastz does not produce any action. A particular effect of the complex itself, is then proved to be better than the sum of its components.

3.9. Mutagenic activity

The Ames *S. typhimurium* assay has been a classic bioassay to determine the potential mutagenicity of compounds since 1970s [17]. The results of the Ames test for Costz are shown in Table 6.

The reversion coefficient, R.C [17]. has been defined as follows: revertant number *per* tested plate/revertant number *per* control plate (spontaneous). I.e.: dividing the average revertants/plate of the tested substance by the spontaneous mutation rate. A non-statistical procedure has been established to evaluate the results of *Salmonella* experiments [17] In agreement with this procedure, a substance is considered a mutagen if it produces a reproducible, dose-related increase in the number of revertant colonies in one or more strains (i.e.: R.C. ≥ 2.0). A substance is considered a weak mutagen if it produces a reproducible, dose-related increase in the number of revertant colonies in one or more strains but the number of revertants is not double the background number of colonies. Although it is generally accepted that a negative result can be defined using 4-5 tester strains and strains TA98 and T-A100 are always considered necessary, a single strain is sufficient to demonstrate a mutagenic response [17]. The carcinogenicity and genotoxicity of metals like cadmium, chromium, cobalt and nickel strongly

Table 5
MIC values in μg mL^{-1} of sodium sulfathiazole (Nastz) and the complex Costz, acting against human opportunistic pathogenic fungi. MIC values of $CoCl_2{\cdot}6H_2O$, Co(II)stz and published MIC values of another related complexes (1-4) are given for comparison.

Compound	Nastz	Costz	Co(II)stz[a]	Co(II)[b]	1[c]	2[d]	3[e]	4[f]	Amp	Ket	Terb
Yeasts											
C. albicans ATCC 10231	> 250	> 250	> 250	250	500(1000)[g]				0.78	6.25	1.56
C. albicans clinical isolate					500(1000)[g]	19.0–15.9(8.814.4)[g]	12.5–400				
C. albicans ATCC 750								32(> 250)[g]			
C. tropicalis C131	> 250	> 250	> 250	250					1.56	6.25	0.78
S. cerevisiae ATCC 9763	> 250	> 250	> 250	250					0.78	3.12	3.12
C. neoformans ATCC 32264	> 250	> 250	> 250	125					0.78	1.56	0.39
Aspergillus											
A. fumigatus ATCC 26934	200	200	200	125					3.12	12.5	0.78
A. flavus ATCC 9170	> 250	100	100	250					0.78	6.25	0.78
A. niger ATCC 9029	> 250	> 250	> 250	250					0.78	6.25	1.56
Dermatophytes											
M. gypseum C 115	> 250	> 250	> 250	16					6.25	12.5	0.006
T. rubrum C 113	> 250	> 250	> 250	31.2					6.25	12.5	0.003
T. mentagrophytes ATCC 9972	> 250	> 250	> 250	31.2					6.25	12.5	0.006

MIC: Minimal Inhibitory Concentration. MICs ≤250 μg/mL were considered active. The MIC values of the standard drugs Amphotericin B (Amp), Ketoconazole (Ket) and Terbinafine (Terb) are in μg mL^{-1}. ATCC: American Type Culture Collection; C: from CEREMIC.
[a] Ref [10].
[b] Co(II): as $CoCl_2{\cdot}6H_2O$.
[c] Co(III) complexes of Schiff base ligands, ref. [9d].
[d] Co(II) complexes of azo group-containing Schiff base ligands, ref. [7f].
[e] Co(II) complexes of benzimidazole derivatives, ref. [7g].
[f] Co(II) complexes of pyrimidine derivative ligands, ref. [7i].
[g] In brackets: MIC of the respective free ligans.

depend on their chemical ligands (speciation) which modulate their bioavailability and reactivity with biochemical targets [57]. In this way, $CoCl_2$, non-mutagenic by itself, was found to be mutagenic in strains TA1537 and TA2637 of *S. typhimurium*, when combined to 4-substituted pyridines [58], and a series of hexacoordinate cobalt(III) compounds with substituted pyridines showed difference in the genetic activity that could be attributed to the influence of the ligands on the relative lability of the metal complex [59]. On the other hand, the cobaltous complex of sulfathiazole (Co(II)stz) did not show mutagenicity but a slight toxicity toward both strains in the tested range (5-500 μg/plate), more evident with the TA100 one [60]. Consistent with the analysis of the R.C. toward the generated dose-response curves by means of the standard plate assay, Costz showed non mutagenic effects to the used strains in the tested range.

3.10. Plant genotoxicity test (Allium cepa test)

The *Allium cepa* test, which is considered one of the most efficient approaches to assess toxic effects of environmental chemicals [61] was selected to evaluate the potential risks of the Costz complex. *Allium cepa* species has been frequently used to determine the cytotoxic and genotoxic effects of several substances, being considered the standard organism for quick tests, since it shows a high correlation with mammal test systems [62], although it can not replace studies with mammalian cell cultures. Another advantage of this test system is the presence of an oxidase enzyme system, which is useful for promutagen evaluations [63]. The results of phytotoxicity (as change of root length, expressed in cm) and mitotic index ‰ (MI) of Costz and Nastz tested for comparative purposes, evaluated with the *Allium cepa* test are shown in Table 7.

Phytotoxicity: The analysis by linear regression showed significative influence of the concentration of both sulfa drugs (Costz and Nastz) on the change of root length *Allium cepa* L. The Student´s *t*-test performed post ANOVA indicated that at the 0.05 level, the means of both mother solutions (Costz and Nastz) were significantly different from the negative control value (mineral water). Root degeneration was not observed in all the assayed range for any of the two substances.

Mitotic index: Inhibition of mitotic activities is often used for tracing cytotoxic substances. A MI decrease below 22% of the control causes lethal effects on test organisms, while a decrease below 50% (cytotoxic limit value) usually has sub-lethal effects [64]. On the other

Table 6
Mutagenic activity of the complex Co-stz with *S. typhimurium* TA98 and TA100 strains.

	TA98		TA100	
Dose μg/plate (nmol/plate)	Nº revertants/plate ± SD	R.C.[a] ± SD	Nº revertants/plate ± SD	R.C.[a] ± SD
0[b] (0)	24.00 ± 5.04	1.00	157.50 ± 16.87	1.00
33.2[c] (52.1)	23.143 ± 5.11	0.93 ± 0.11	147.375 ± 19.29	1.00 ± 0.05
99.6[c] (156.2)	21.43 ± 3.77	1.01 ± 0.03	137.125 ± 8.33	0.89 ± 0.08
166.0[c] (260.0)	23.625 ± 2.06	1.01 ± 0.09	146.50 ± 19.92	0.97 ± 0.17
232.4[c] (364.7)	20.75 ± 2.73	0.89 ± 0.05	140.22 ± 25.45	0.93 ± 0.20
332.0[c] (520.0)	20.43 ± 4.89	0.86 ± 0.02	121.80 ± 25.65	0.77 ± 0.15
4NOPDA, 1.25[d]	76.5 ± 0.5	3.19 ± 0.02	–	–
NaN_3, 0.15[d]	–	–	490 ± 2	3.11 ± 0.01

[a] R.C.: reversion coefficient = revertants with tested substance/spontaneous revertants.
[b] Negative control: without tested compounds, spontaneous revertants/plate.
[c] μg of Co-stz/plate.
[d] Positive controls with the respective diagnostic mutagens: 4-nitro-*o*-phenylenediamine (4NOPDA) for the TA98 strain and sodium azide, NaN3, for the TA100 strain. SD: standard deviation.

Table 7
Phytotoxicity (as change of root length, in cm: Δ ± SD) and mitotic index ‰ ± SD of Nastz and Costz evaluated with the *Allium cepa* test.

Allium test parameter	Phytotocicity		Mitotic Index ‰	
sulfa drug (mg/L)	Nastz	Costz	Nastz	Costz
0 (mineral water)[a]	1.72 ± 0.35		42.9 ± 11.8	
2.0	1.75 ± 0.20	1.64 ± 0.27	47.8 ± 8.5	48.0 ± 8.2
40.0	1.72 ± 0.30	1.53 ± 0.53	29.3 ± 4.3[d]	33.4 ± 7.5
80.0	1.83 ± 0.29	1.20 ± 0.53	36.0 ± 6.2	24.2 ± 4.1[d]
110.0	1.35 ± 0.38	1.20 ± 0.52	28.4 ± 11.3[d]	26.8 ± 6.3[d]
200.0	0.90 ± 0.36[d]	0.43 ± 0.22[d]	32.2 ± 7.1	21.8 ± 4.3[d]
Linear Regression[b]	y = 1.82–0.00375× r: -0.85; p: 0.03	y = 1.70–0.00625× r: -0.99; p < 0.0001	y = 36.07–0.032× r: -0.32; p: 0.53	y = 36.93–0.088× r: -0.77; p:0.07
Positive control[c]	2.90 ± 0.76		22.7 ± 4.9	

SD: standard deviation; r: Pearson's correlation coefficient.
[a] Negative control.
[b] y = a + bx; weight given by data sd error bars. p-value ≤ 0.05 was considered as statistically significant [22].
[c] Positive control: NaN_3 1.0 × 10^{-5} mol L^{-1}.
[d] Mean significantly different from the negative control mean (Student's *t*-test post ANOVA at the 0.05 level).

hand, the inhibition of division without the observation of chromosome aberrations is a promising result for anticancer therapy, as it leads first to block the development of cancer [65]. Although the linearization equation of mitotic index versus concentration of Costz gave a p value of = 0.07, which would indicate no association between the two variables, since p > 0.05, the t-Student test performed post ANOVA for Costz indicated that the MI means differs from the mean of the negative control from the concentration of 80 mg/L. Thus, the mean value of the mitotic index does not differ from the negative control at concentrations antibacterially active *in vitro* (40 mg/L), but they differ in the concentrations in which the complex is active *in vitro* as antifungal. Consequently, further biological studies would be necessary to consider the complex Costz as a possible drug in the future. Chromosome aberrations were not observed with both sulfa drug solutions.

4. Conclusion

The reaction between sulfathiazole and cobalt(II), in the presence of sufficient amount of H_2O_2, leads to a stable Co(III)-sulfathiazolate complex (Costz). Elemental and termogravimetric analysis are in agreement with the following minima formulae: $[Co^{III}(stz)_2OH(H_2O)_3]$. Spectrometric methods suggest a distorted octahedral environment for the Co(III) ion. The $N_{thiazolic}$ atom would be a coordination point to the Co(III) with sulfathiazolate. The complex quenched partially the native fluorescence of bovine serum albumin (BSA), suggesting a specific interaction with the protein. The Costz complex showed, *in vitro*, a moderate antifungal activity against *Aspergillus fumigatus* and *A. flavus*. As antibacterial, *in vitro*, Costz displayed enhanced activity respective to the ligand against *Pseudomonas aeruginosa*. Costz did not show mutagenic properties with the Ames test. In the *Allium cepa* test the complex showed cytotoxic properties but not genotoxic ones. These results may be auspicious, however, further biological studies are needed to consider the complex Costz as a possible drug in the future.

Acknowledgements

A.P. thanks the Argentinian Research Council (CONICET) for a doctoral scholarship, and Prats Foundation for financial support. P.A.M.W. is a research fellow of CICPBA, Argentina. M.R. is a research fellow of CIC-UNR and IQUIR, Argentina. This work was partially supported by CICITCA-Universidad Nacional de San Juan, Argentina, B.L. Have a fellowship from CONICET. G.E.F. is researcher from CONICET.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.cbi.2017.10.004.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cbi.2017.10.004.

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