Heteroleptic complexes of antifungal drugs with the silver ion[†]

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In spite of a large number of antibiotics and chemotherapeutics available for medical use, the emergence of old and new antibiotic resistance in the last decades revealed a substantial medical need for new classes of antimicrobial agents. In this paper we report the synthesis, characterization by elemental analysis, Fourier transform infrared and Nuclear magnetic resonance spectroscopies, and antifungal properties of two heteroleptic complexes of albendazole (albz) with Ag(I), using KSCN and o-phenanthroline (phen) as the second ligand, respectively. Both complexes showed a moderate antifungal activity with all the assayed fungi, mainly *Candida albicans* and *Candida tropicalis*, that showed no activity against albendazole. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords: albendazole; antifungal properties; heteroleptic complexes; silver complexes

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] Medicinal Inorganic Chemistry is a fairly recent offshoot of Bioinorganic Chemistry. The use of metals in medicine is not new.[2] Cisplatin may be considered the archetypal inorganic drug, as it contains not one atom of carbon.[3]

However, most metal-based pharmaceutical drugs are constructed today with carbon-based ligands. Thus, the importance of ligands in modifying the biological effects of metal-based drugs should be considered.[2] Medicinal Inorganic Chemistry is rich in metal and metalloid-based drugs, including Paul Erlich's organoarsenic compound for the treatment of syphilis, antiarthritic gold preparations, and diagnostic agents for magnetic resonance imaging (Gd, Mn, Fe) among others.[4]

Silver has the most outstanding properties among all metals with antimicrobial activity because of its higher toxicity to microorganisms and lower toxicity to mammalian cells.[5] The antimicrobial activities of both colloidal silver and silver ions have been known since ancient times. Indeed, early in the 20th century, the Food and Drug Administration approved the use of preparations containing silver for wound management.[6] However, and with the exception of a number of silver-containing preparations used in the management of burn patients,[7] the use of silver as an antimicrobial drug diminished after the introduction of penicillin and other antibiotics. Silver has well-characterized antimicrobial properties and Ag(I) ions are active against a wide range of bacteria, fungi, and viruses.[8] The pure metal is inactive; however, in the presence of moisture, silver readily ionizes to give silver cations, which show antimicrobial activity. Today, silver and silver nanoparticles, which have antifungal properties,[9] are used in healthcare, in the food industry and in domiciliary applications, and they are commonly found in hard surfaces, materials, textiles[10] and against indoor mould growth.[11]

Many heterocyclic compounds containing benzimidazoles, which are the nucleus of albendazole (Fig. 1) are a subject of interest to Medicinal Chemistry because of the broad spectrum of their biological and pharmaceutical properties.[12] The benzimidazole derivatives have been proved to be an important group of antifungals with systemic activity and are well-known for their pronounced ability to control a large number of fungal diseases.[13] As a consequence, during the last decade, the antibacterial and antifungal activities of benzimidazole derivatives have received much attention.[14]

Investigation of the biological activity of complex compounds is a field that has been developed over the years, because the antimicrobial activity of active drugs is often enhanced by complexation with metal ions and, on many occasions, mixed ligand complexes are synthesized.[15]

Recently, a series of novel compounds containing 1,4,7,10-tetraazacyclododecane and azoles were synthesized and characterized. Some complexes of Cu and Zn with these ligands

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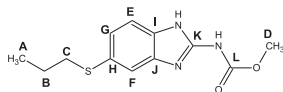 Instituto de Química Rosario, Suipacha 531, Rosario, Argentina

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Figure 1. Albendazole (albz), Methyl [5-(propylthio)-1*H*-benzoimidazol-2-yl]carbamate, $C_{12}H_{15}N_3O_2S$, molecular mass 265.333 g/mol, shown the notation used for albz and their derivatives for ^{13}C NMR spectroscopic assignments

displayed minimum inhibitory concentration (MIC) values from 2 to 512 µg/mL against *Candida albicans*, and MICs around 200 µg/mL against *Aspergillus fumigatus*.[16] The synthesis, structural characterization, antimicrobial and cytotoxic activities of these compounds were undertaken to contribute to a better understanding of the co-ordination behavior of these biological relevant compounds toward metal ions and their biological activity. Complexes of Ag(I) with benzimidazole derivatives[17] and complexes of albendazole with transition metal ions, but not Ag(I), have been informed in the literature .[18]

We report herein the synthesis, characterization by elemental analysis, Fourier transform infrared (FTIR) (4,000 to 400 cm^{-1}) and Nuclear magnetic resonance (NMR) (^{1}H NMR, 300.1 MHz; ^{13}C NMR, 50.3 MHz) spectroscopies, and the antifungal properties of two heteroleptic complexes of albendazole (albz) with Ag(I), using KSCN and *o*-phenanthroline (phen, Fig. 6) as the second ligand, respectively. The presence of a second or auxiliary ligand in a metal complex may have influence on the coordination of the metal ion with respect to the principal ligand (albendazole in our case) and on the biological properties of the complex. Thiocyanate ion, which in the human body is produced in the metabolism of cysteine and detoxification of cyanide, enters the body from the diet (such as cruciferous vegetables). SCN$^-$ functions in host defense as part of the secreted lactoperoxidase microbicidal pathway.[19] The thiocyanate ion (as KSCN) is widely used in the formation of metal complexes, either as principal ligand[20] or as a second one.[21] Phen forms strong complexes with most metal ions[22] and can act as a strong field bidentate ligand that forms very stable chelates with many transition metals of the first row.[23] This molecule has antibacterial, antifungal, and antiviral properties.[24] Phen is currently widely used in the formation of metal complexes.[25] Phen and substituted derivatives, both in the metal-free state and as ligands coordinated to transition metals, disturb the behavior of a wide variety of biological systems, by acting for example as a bactericidal,[26] or as an anticarcinogen agent among others.[27] Moreover, many metal complexes with antimicrobial activity contain phenanthroline.[28]

EXPERIMENTAL

Materials and methods

Chemistry

Albendazole (FNA grade, Parafarm > 99%), AgNO$_3$ (FNA grade, Parafarm >99%) and all other chemicals were commercially available and were used as received. Elemental chemical analyses (C, H, N, and S) were performed in a microanalyser Carlo Erba EA1108 (CARLO ERBA Reagents S.A. Italy). Ag assessments were performed by volumetric determination with potassium thiocyanate (USP29). Infrared (IR) spectra of powdered samples were measured with a Bruker IFS 66 FTIR-spectrophotometer (Bruker Optik GmbH, Ettlingen, Germany) from 4000 to 400 cm^{-1}, using the KBr pellet technique. ^{1}H NMR and ^{13}C NMR spectra of the

silver complexes (Ag–albz–SCN and Ag–albz–phen) and the corresponding ligands (albz and phen) were recorded with a Bruker Avance 300 NMR (Bruker BioSpin GmbH, Germany and Austria) spectrometer at ambient probe temperature (25 °C) with nominal operating frequencies of 300.1 and 50.3 MHz, respectively. All chemical shifts (δ) are quoted in parts per million (ppm). Chemical shifts (δ) are in ppm relative to the residual DMSO-d6 signals (2.50 and 39.5 ppm for ^{1}H and ^{13}C, respectively). Normal 2D techniques were used including Heteronuclear Single-Quantum Correlation and Heteronuclear Multiple Bond Correlation, for the assignment of the ^{13}C NMR signals.

Synthesis of the complexes: It was performed by mixing albz (dissolved in glacial acetic acid) with the second ligand and AgNO$_3$ (aq) in a 1:1:1 molar ratio.

[Ag–albz–SCN]: in a typical experience, 2 mL of aqueous solution of silver nitrate containing 0.1872 g (1.1 mmol) of AgNO$_3$ were added dropwise to 4 mL of stirring aqueous acetic solution containing 0.2654 g of albz (1 mmol) and 0.0987 g (1 mmol) of KSCN. Immediately, a white suspension was formed. The mixture was stirred in the absence of light for 20 min. Then, it was left to stand at room temperature, away from light. After 24 h, the mixture was filtered under vacuum, washed several times with acetic acid and then with water, and dried in the dark. Yield: 0.085 g (19.7%). Then another heteroleptic silver complex, [Ag–albz–phen], was synthesized following a similar technique, replacing KSCN by *o*-phenantroline (0.1802 g: 1 mmol). Yield: 0.109 g (28.6%). In order to compare some results, we obtained too the complexes Ag–albendazole, Ag–albz: AgC$_{12}$H$_{18}$N$_4$O$_5$S: [Ag–albz]]NO$_3$·H$_2$O,[29] and Ag- *o*-phenanthroline, Ag–phen: [Ag(phen)$_2$]$^{+[30]}$ from AgNO$_3$ and the respective ligands.

Biology

Antifungal assays: The microorganisms used for fungistatic evaluation were purchased from ATCC or were clinical isolates from CEREMIC (identified with the capital letter CCC), Centro de Referencia en Micología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Suipacha 531-(2000)-Rosario, Argentina. Yeasts: *C. albicans* ATCC10231, *C. tropicalis* CCC131, *Cryptococcus neoformans* ATCC32264; hialohyphomcetes: *A. flavus* ATCC9170, *A. fumigatus* ATCC26934, *A. niger* ATCC9029; dermatophytes: *Trichophyton mentagrophytes* ATCC9972, *T. rubrum* CCC113, *Microsporum gypseum* CCC115 were grown on Sabouraud-chloramphenicol agar slants for 48 h at 30 °C. For yeasts, cell suspensions in sterile distilled water were adjusted to give a final concentration of 1×10^3 viable yeast cells per mL.[31] For filamentous fungi, 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 guidelines of the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards) and adjusted to 1×10^3 spores colony-forming ability per milliliter. The MIC of each compound was determined by using broth microdilution techniques according to the guidelines of Clinical and Laboratory Standards Institute.[32] MIC values were determined in RPMI-1640 (Sigma, St Louis, MO, USA) buffered to pH 7.0 with 3-(N-morpholino) propanesulfonic acid (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 the fungi. For the assay,

stock solutions of pure compounds were two-fold diluted with Roswell Park Memorial Institute-1640 from 250 to 0.98 µg/mL (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, for which 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 relative to the growth in the control wells containing no antifungal. The minimum fungicidal concentration (MFC) was determined by plating a duplicate 5 mL from each clear well of MIC determinations onto a 150 mm SDA plate. After 48 h at 37 °C, MFCs were determined as the lowest concentration of each compound showing no growth in the plates. Both MIC and MFC were confirmed by two replicates. Despite the specialized international recommendations,[33] a variety of breakpoints is observed in the literature,[34] so we decided to take the value of 250 µg/mL as cut-off point for MIC's[21e,f,35] against all the analyzed fungi.

Amphotericin B (Janssen Pharmaceutica, Buenos Aires, Argentina), Ketoconazole (Sigma Chem. Co. St Louis, MO, USA), and Terbinafine (Novartis, Capital Federal, Argentina) were used as positive controls.

RESULTS AND DISCUSSION

Elemental analysis of the obtained powders gave satisfactory results for [Ag(albz)(SCN)], white solid, calculated MM: 431.3 g mol^{-1}, AgC$_{13}$H$_{15}$N$_4$O$_2$S$_2$; % exp. (calcd.): Ag, 24.0 (25.0); C, 35.7 (36.2); H, 3.5 (3.5); N, 12.5 (13.0); S, 15.3 (14.9) and [Ag$_2$(albz)(phen)$_3$] (NO$_3$)$_2$, pale yellow solid, calculated MM: 1145.7 g mol^{-1}, Ag$_2$C$_{48}$H$_{39}$N$_{11}$O$_8$S; % exp. (calcd.): Ag, 20.6 (18.8); C, 50.6 (50.3); H, 3.3 (3.4); N, 12.6 (13.4); S, 3.5 (2.8)

Vibrational Fourier transform infrared spectra

Fourier transform infrared spectra of albz and its heteroleptic silver complexes Ag–albz–SCN and Ag–albz–phen are shown in Figs 2 and 3. The IR spectrum of the homoleptic complex [Ag(albz)]NO$_3$·H$_2$O[29] was included for comparison. The main vibrational FTIR frequencies of such spectra are shown in Table 1.

Ag–albz–SCN complex

In the Ag–albz–SCN complex, the thiocyanate ion can coordinate to the metal through either the nitrogen or the sulfur atom. These two different modes of coordination are easily distinguishable by the infrared absorption frequencies of the thiocyanate radical. The free thiocyanate ion absorbs at about 750 and 2055 cm^{-1} (C–S and C–N stretching vibrations, respectively); these peaks are shifted to 780–860 and 2080–2095 cm^{-1}, respectively in the SCN-metal complex and 690–720 and 2100–2125 cm^{-1}, respectively in the NCS-metal complex.[36] The thiocyanate group in the complex Ag–albz–SCN absorbed at 770 cm^{-1} and 2084–2094 cm^{-1}, as would be expected if coordination occurred through the nitrogen atom of the thiocyanate ligand. No changes in the spectral vibrational bands corresponding to the C=O group were observed in this complex. The band assigned to the CNH bending at 1526 cm^{-1} shifted to 1548 cm^{-1}, and the amide 2 band assigned to CNH bending and C–N stretching at 1448 cm^{-1} became broader and weaker. Then, it can be inferred that the coordination of albz with the Ag(I) ion could be through the N$_{benzimidazole}$ atom, but not with the O atom of the carbonyl group.

Ag–albz–phen complex

The Infrared spectrum suggests a coordination of the silver ion with the C=O and the N$_{benzimidazole}$ of albz for Ag–albz–phen (similarly to Ag–albz). In the 3500–2000 cm^{-1} region the intensity of the vibrational bands of the complex diminishes. The NH stretching dissapeared suggesting coordination to the metal center. The broad absorption bands centered at 2665 cm^{-1} assigned to the H-bond of albz (NH$_{Bz}$···O=C) were not present in the upon coordination to the silver ion. The amide modes were also modified by metal coordination: Amide 1 is shifted to higher frequencies (from 1711 cm^{-1} to 1745 cm^{-1}), amide 2 splitted with lower intensity (from 1448 cm^{-1} to 1461–1425 cm^{-1}) and amide 3 is also shifted (from 1272 cm^{-1} to 1241 cm^{-1}). Considering that the vibrational modes of secondary aromatic amines appeared at higher frequencies than aliphatic ones (3450 cm^{-1} vs. 3360–3310 cm^{-1}), it seemed that the interaction occurred with the N atom of Benzimidazol, probably forming a chelate complex with Ag(I) through this atom and the C=O (carbonyl) group.

Moreover, the IR spectrum shows the presence of the nitrate in its anionic form (similarly to that of Ag–albz complex) at 1378 cm^{-1} with D$_{3h}$ symmetry, plus the typical bands of o-phenantroline slightly shifted to the blue because of coordination (Fig. 3 B) and located at 840 and 722 cm^{-1}.
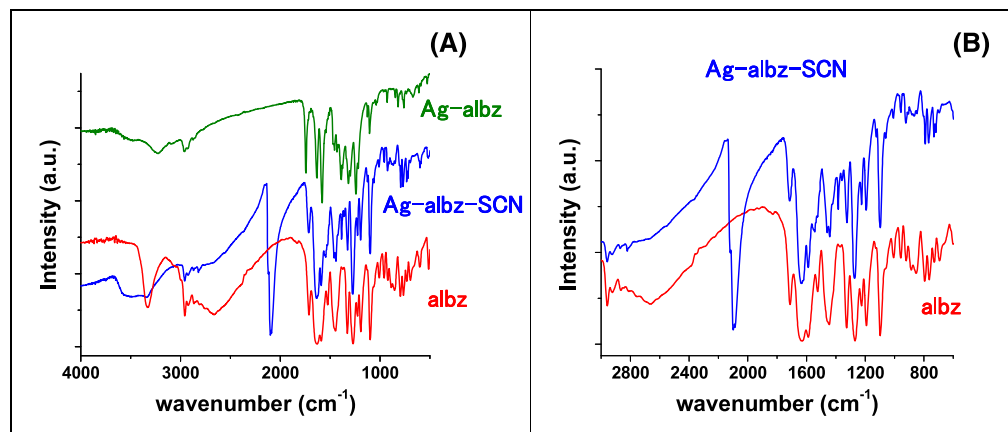

Figure 2. Fourier transform infrared spectra of: (A) albz (red), [Ag(albz)NO$_3$·H$_2$O] (olive) and [Ag(albz)SCN] (blue); (B) detail of albz and Ag–albz–SCN
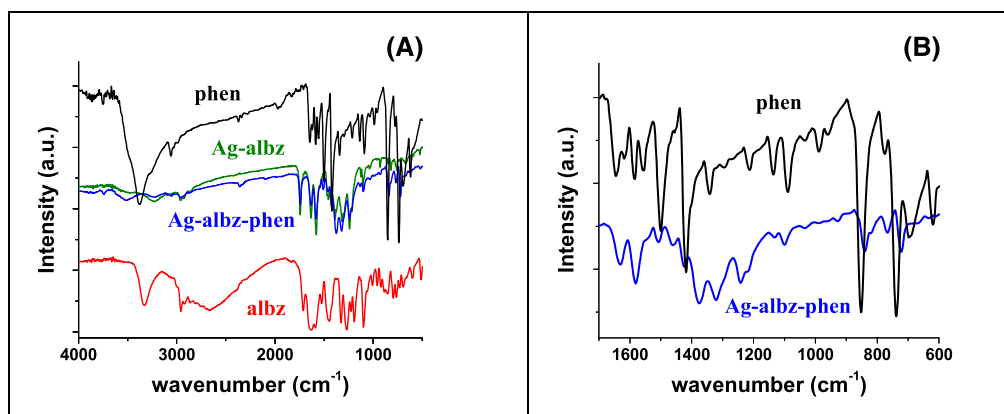
Figure 3. Fourier transform infrared spectra of: (A) albendazole (albz, red), o-phenantroline (phen, black), Ag–albz (olive) and Ag–albz–phen (blue); (B) detail of phen and Ag–albz–phen

Table 1. Assignment of the vibrational FTIR spectra (wavenumbers, cm^{-1}) of albendazole (albz) and its Ag(I) complexes (Ag–albz, Ag–albz–SCN and Ag–albz–phen)

albz	Ag–albz	Ag–albz–SCN	Ag–albz–phen	Assignment
	3489	3450 m, br	3513 w	ν O–H$_w$
3329 br m	3259 w	3352 m	3222 w	ν N–H
3070 sh	3060 vw		3073 vw	νiph C–H (arom)
3048 sh	3030vw		3055 vw	νooph C–H (arom)
2959 m	2966 w	2962 m	2959 w	δas CH3/CH2
2921 m	2928 w	2926 w	2929 w	δs CH3/CH2
2864 m	2877 vw	2867 w	2874 vw	ν C–H (aliph)
2665 br, m				H bonding (NH•••O=C)
		2094 vs		SCN
1711 m	1745 m	1716 w	1745 m	ν C=O, amide 1, ν C=O s, δ N–H, νC–N Amide I band
1631 br, vs	1634 m	1637 m	1634 m	ν C=N, δ NH Aromatic ring stretching
1588 vs	1582 vs	1589 w	1582 vs	ν C=C Aromatic CLNstretching
1526 m	1542 vw	1548 vw	1542 vw	δ CNH Amide II band/C–N stretching
			1508 w	
1448 s	1456 w, 1434 w	1456 m	1424 m, br	Amide 2, δ CNH, ν C–N CH$_2$ deformation
	1389 m	1383 m	1388 sh, 1374 s,br	ν_3 NO$_3$-D$_{3h}$
1326 s	1316 m	1326 m	1321 s, br	
1272 vs	1241 s	1273 s	1241 m	Amide 3? Ip- combination of δ NH, ν C–N Amide III band
1226 m	1218 m	1229 m	1218 sh	Amide IV band
1191 s	1127 w	1192 m	1132 w	νasC–O (ester) CH$_2$ wagging
1101 s	1105 m	1099 s	1100 m	νs C–O (ester) C–O stretching
694 m	694 sh	694 m	694 vw	νsC–S–C
670 sh	670 w	670 w	670 sh	

Band description: s, strong; m, medium; w, weak; vw, very weak; br, broad; sh, shoulder
FTIR, Fourier transform infrared.

The CSC symmetric stretching frequency at 694 cm^{-1} (medium) in the free albz is reduced in intensity (very weak) in the Ag–albz–phen and to a shoulder in Ag–albz. These observations suggest an evidence for S-coordination.[37] The CSC antisymmetric stretching of albz at 733 cm^{-1} is masked with the typical band phenanthroline in the Ag–albz–phen complex.[38]

Albendazole did not show deprotonation in none of the studied complexes, so, the thiocyanate and nitrate anions give the electroneutrality for the corresponding ones.

Nuclear magnetic resonance spectroscopy

Figures 4 and 5, and Table 2 show the ^{1}H and ^{13}C NMR data, respectively, for albz, Ag–albz–SCN and Ag–albz–phen. Ag–albz data were included for comparison. Figure 1 shows the notation used for albz and their derivatives for ^{13}C NMR spectroscopic assignments.

Data of ^{13}C NMR of phen in the complexes Ag–phen and Ag–albz–phen are showed on Table 3. Figure 6 shows the
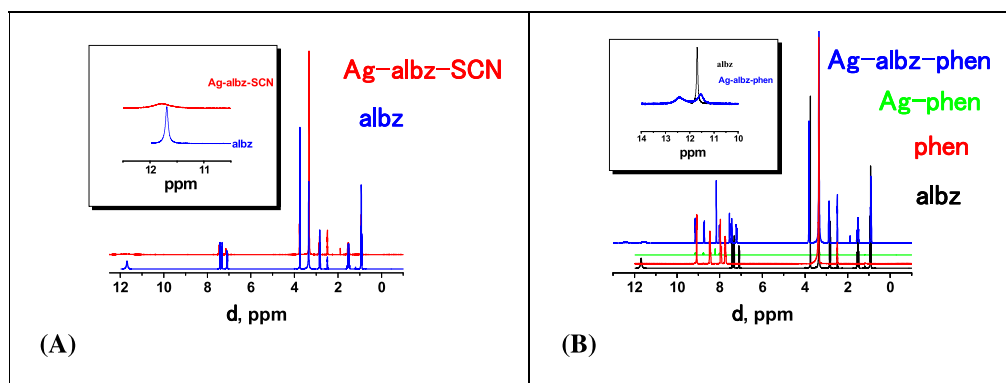
Figure 4. ^{1}H NMR spectra of: (A) albendazole (albz) and the Ag–albz–SCN complex; (B) albz, phen, and its Ag(I) complexes: Ag–albz–phen and Ag–phen for comparison in DMSO-*d6* (δ, ppm) In the insets: detail of the signals of the ionizable protons of albendazole
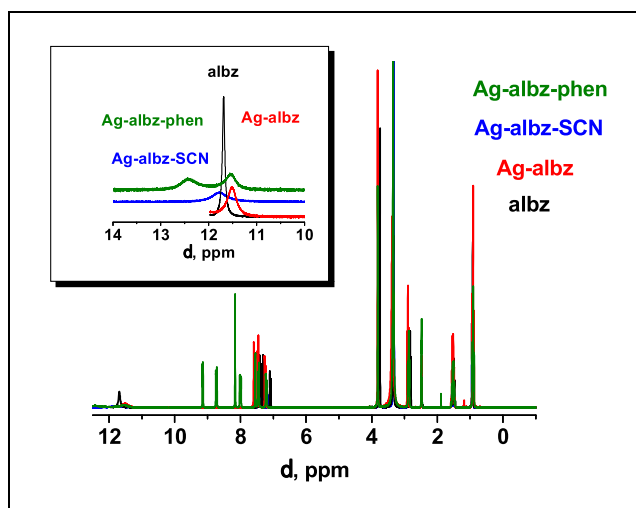

Figure 5. ^{1}H NMR spectra of albendazole (albz) and its homoleptic and heteroleptic Ag(I) complexes in DMSO-*d6* (δ, ppm). In the inset: detail of the signals of the ionizable protons of albz

notation used for phen and their Ag(I) complexes for ^{13}C NMR spectroscopic assignments.

^{1}H NMR spectra show that albendazole retains its two acidic protons in both heteroleptic complexes, similarly to the homoleptic one, Ag–albz. The signal of these protons broadens but remains (insets Figs 4 and 5), but it looks different for the Ag–albz–phen than for the others Ag(I)–albendazole complexes, leading to think that the silver–albendazole coordination in this complex may be different from that observed in the other ones.

^{13}C NMR signals of the albz carbons labeled E, F, I, and J disappear or were detected by means of Heteronuclear Single-Quantum Correlation technique (Ag–albz–SCN) in all the Ag–albz studied complexes. This fact could suggest the coordination of the Ag atom with the heterocyclic N atom. The signals of carbon "L" and carbon "C" are among the most affected in the Ag–albz and Ag–albz–phen complexes. These facts are in agreement with the coordination by the C=O group and by the S atom observed by IR.

Looking at phen and its Ag(I) complexes (Table 3), the chemical shifts on ^{13}C NMR spectra showed that the only ones carbons that

Table 2. ^{13}C NMR shift assignments of albendazole (albz) and its Ag(I) complexes (Ag–albz, Ag–albz–SCN and Ag–albz–phen) in DMSO-*d6* (δ, ppm)

Assignment	albz	Ag–albz	$\Delta\,\delta$: albz–(Ag–albz)	Ag–albz–SCN	$\Delta\,\delta$: alb –(Ag–albz–SCN)	Ag–albz–phen	$\Delta\,\delta$: alb –(Ag–albz–phen)
A	13.1	13.06	0.04	13.08	0.02	13.05	0.05
B	22.12	22.15	−0.03	22.08	0.04	22.1	0.02
C	36.39	36.88	−0.49	36.49	−0.1	36.71	−0.32
D	52.54	53.21	−0.67	52.79	−0.25	53.11	−0.57
E	114.23	—	—	114.37	−0.14	—	—
F	115.85	—	—	116.03	−0.18	—	—
G	124.1	125.12	−1.02	124.35	−0.25	124.83	−0.73
H	126.93	127.96	−1.03	127.76	−0.83	128.25	−1.32
I	135.54	—	—	—	—	—	—
J	136.84	—	—	—	—	—	—
K	147.86	148.16	−0.3	147.95	−0.09	—	—
L	154.81	153.58	1.23	154.14	0.67	153.67	1.14

Table 3. ^{13}C NMR shift assignments of *o*-phenanthroline (phen) and its Ag(I) complexes (Ag–phen and Ag–albz–phen) in DMSO-*d6* (δ, ppm)

Assignment	phen	Ag–phen	Δ δ: phen – (Ag–phen)	Ag–albz–phen	Δ δ: phen – (Ag–albz–phen)
A	123.26	125.01	−1.75	124.99	−1.73
B	126.62	127.28	−0.66	127.19	−0.57
C	128.40	129.07	−0.67	128.94	−0.54
D	136.14	138.49	−2.35	138.58	−2.44
E	145.49	142.15	3.34	141.68	3.81
F	149.90	151.44	−1.54	151.34	−1.44
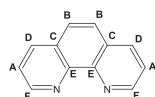
Figure 6. *o*-phenanthroline (phen), shown the notation used for phen and their derivatives for ^{13}C NMR spectroscopic assignments

are protected in both complexes (Ag–phen and Ag–albz–phen) are the labeled "E", and all the rest are deshielded. It seems that the involvement of the C phenanthroline is quite similar in both complexes, that is, it would not be to make a difference. It is comparable with that observed in other ternary complexes of phenanthroline.[39]

Coordination chemistry of the ion Ag(I)

In coordination chemistry, silver in the +1 oxidation state is found to adopt a wide variety of coordination geometries. From more than 3300 crystal structures containing silver ions coordinated to non-metal atoms, 24% are two-coordinate, 23% are three-coordinate and 44% are four-coordinate. The rest: 5% are five-coordinate, 4% are six-coordinate and minor of 1% are seven-coordinate and eight-coordinate. This variety is in part because of the lack of stereochemical preference that arises from a d10 configuration. With the exception of the higher coordination numbers, the least common geometry (< 2%) is square planar.[40]

Another factors also showed influence in coordinating the Ag (I) ion, such as the counterion[41] and the nature and/or the stoichiometry of the ligands, etc.[42]

Phen is a classic chelating bidentate ligand for transition metal ions that has played an important role in the development of coordination chemistry and still continues to be of considerable interest as versatile starting material for organic, inorganic and supramolecular chemistry. Phen is a rigid planar, hydrophobic, electron-poor heteroaromatic system whose nitrogen atoms are beautifully placed to act cooperatively in cation binding. These structural features determine its coordination ability toward metal ions.[43]

The heteroleptic Ag(I) complex of malonic acid (H_2mal) and phen, ([Ag_2(mal)(phen)$_3$]), of similar stoichiometry than Ag–albz–phen, showed anticancer[44] and antifungal properties.[45]

Some Ag(I) ternary complexes where one of the ligands is phen are curious because of the two different environments that surround the metal centers.[24b,46] Among these, some silver(I) dicarboxylate complexes containing 1,10-phenanthroline ligands, [Ag(phen)$_3$(OOC–(CH$_2$)y–COO)]·zH$_2$O   (y = 1–10;   z = 1–4),   whose

proposal structure exposes one silver center bonded to one molecule of phen and the another silver center bonded to two molecules of phen, both silver ions bridged by the dicarboxylate moiety.[47] A similar structure might be suggested for the complex Ag–albz–phen, acting the albz moiety as a bridge between the two silver centers, coordinating with one Ag(I) by mean of the N atom of benzimidazole and the O atom of the carbonyl group and with the another Ag(I) center by the S atom.

Antifungal evaluation

Minimum Inhibitory Concentration and minimum fungicidal concentration (MFC) of albendazole (albz) and its Ag(I) complexes: Ag–albz–SCN, Ag–albz–phen acting against human opportunistic pathogenic fungi are shown in Table 4. MIC's and MFC's of AgNO$_3$, the ligands albz and phen, and the Ag–phen complex were determined for comparison. Some silver complexes (2–4 in Table 4) were included from the literature for contrasting. Antifungal results of silver complexes of albz and its heteroleptic complexes with SCN⁻ or phen which MIC's and/or MFC's were better than the results of AgNO$_3$ or the ligands ones alone are in bold.

Silver, in its various forms, has high antimicrobial properties. The antifungal activity of silver nanoparticles against different strains of fungi was previously reported in the literature.[9,49] Recently, Ag (I) complexes that showed good antimicrobial activity were synthesized employing different ligands, such as saccharinate and tertiary monophosphanes,[50] sulfonamides,[21e,f] tryptophan[51] and pyridinedicarboxylate compounds[52] among others.[53]

Many benzimidazole drugs like antiparasitic thiabendazole, mebendazole, albendazole, antihistaminic norastemizole, and mizolastine, as well as antihypertensive telmisartan, etc. have been successfully developed and extensively used in clinic[54]; and azole derivatives coordinating with metal cation to form complexes always showed enhanced antimicrobial properties.[55] Besides, some silver complexes of imidazole showed increased antimicrobial activity when encapsulated into nanofibers.[56]

Several Ag(I) complexes of N-heterocyclic carbene that were prepared from benzimidazolium (Table 4, N° 3) presented MIC's from 400 to 50 µg/mL against *C. albicans* and 400 to 25 µg/mL against *C. tropicalis*.[34]

2-(Hydroxymethyl)benzimidazole complexed with Ag(I) (Table 4, N° 4) demonstrated considerable activity, especially against *C. albicans* (with MIC values 10–20 mg/L),[48] while a silver complex of 1,10-phenanthroline-5,6-dione, a phen derivative, showed high activity against several fungal lines. The complexation with the metal ion produced a clear enhancement in the activity.[57]

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Table 4. MIC/MFC values in µg/mL and (Ag(I), µM) of albendazole (albz) and its silver complexes: Ag–albz, Ag–albz–SCN, Ag–albz–phen acting against human opportunistic pathogenic fungi. AgNO₃, o-phenanthroline (phen) and complexes 1–4 were included for comparing

	albz	Ag-albz	Ag-albz–SCN	Ag-albz-phen	Amp	Ket	Terb	AgNO₃	phen	Ag-phen (1)	AgSCN (2)	(3)	(4)
C. albicans ATCC 10231	>250	31.25/ 62.5	31.25/ 125	**7.8/ 125**	0.78	6.25	1.56	6.36 (37.4 µM)/12.72	7.8/250	7.8/125	31.25/ 62.50	50-400	10-20
C. tropicalis C 131	>250	31.25/ 62.5	31.25/ 250	**7.8/ 125**	1.56	6.25	0.78	12.72 (74.9 µM)/25.44	7.8/>250	7.8/125	31.25/ 62.50	25-400	—
C. neoformans ATCC 32264	3.4/7.8	7.8/ 7.8	**0.5/ 3.9**	**3.4/ 3.4**	0.78	1.56	0.39	3.18 (18.7 µM) /12.72	3.4/7.8	3.4/3.4	15.60/ 62.50	—	—
A. fumigatus ATCC 26934	3.4/125	**3.4/ 7.8**	**15.6/ 31.25**	**15.6/ 31.25**	3.12	12.5	0.78	25.44 (149.7 µM) /25.44	7.8/125	15.6/31.25	31.25/ 62.50	—	—
A. flavus ATCC 9170	3.4/125	**3.4/ 7.8**	**7.8/ 15.6**	15.6/ 31.25	0.78	6.25	0.78	12.72 (74.9 µM)/12.72	7.8/125	15.6/31.25	31.25/ 125.0	—	—
A. niger ATCC 9029	3.4/125	**3.4/ 7.8**	**7.8/ 15.6**	15.6/ 31.25	0.78	6.25	1.56	25.44 (149.7 µM) /25.44	15.6/125	15.6/31.25	31.25/ 125.0	—	—
M. gypseum C 115	250/250	62.5/ 62.5	**7.8/ 7.8**	**7.8/ 15.6**	6.25	12.5	0.006	12.72 (74.9 µM)/12.72	7.8/7.8	7.8/7.8	62.50/ 62.50	—	—
T. rubrum C 113	250/250	62.5/ 62.5	**7.8/ 7.8**	7.8/ 7.8	6.25	12.5	0.003	6.36 (37.4 µM)/6.36	3.4/3.4	7.8/7.8	62.50/ 62.50	—	—
T. mentagrophytes ATCC 9972	125/125	62.5/ 62.5	**7.8/ 7.8**	7.8/ 7.8	6.25	12.5	0.006	12.72 (74.9 µM) /12.72	3.4/3.4	7.8/7.8	62.50/ 62.50	—	—

MIC, Minimal Inhibitory Concentration; MFC, Minimal Fungicidal Concentration; Amp, Amphotericin B; Ket, Ketoconazole; Terb, Terbinafine (MIC's in µg/mL). ATCC, American Type Culture Collection (Rockville, MD, USA); C, CEREMIC, Centro de Referencia Micológica, Facultad de Ciencias Bioquímicas y Farmacéuticas, Suipacha 531-(2000)-Rosario, Argentina. (1) Ag–phen, this work (2) AgSCN[21f] (3) Ag-NHC complexes[34] (4) [Ag(2-hydroxymethyl)benzimidazole)₂]NO₃[48] Remarkable results are in bolt.

From our work, both heteroleptic complexes (Ag–albz–SCN and Ag–albz–phen) showed a good antifungal effect against the studied fungi. They showed lower MIC's than albz against *C. albicans*, *C. tropicalis*, *M. gypseum*, *T. rubrum* and *T. mentagrophytes*. Besides, they showed similar or better activity than AgNO₃ against all the assayed fungi. In recent years, *Candida* spp. represent one of the most common pathogens which are responsible for fungal infections often causing hospital-acquired sepsis with an associated mortality rate of up to 40%,[58] so, these results are significant. Additionally, it is noteworthy that the MIC of Ag–albz–SCN against *C. neoformans* is lower than the MIC of the conventional antifungal agents Amphotericin B and Ketoconazole.

CONCLUSIONS

Two new heteroleptic complexes of Ag(I) and albz, with SCN⁻ and *o*-phenantroline as second ligands were obtained and characterized, showed interesting antifungal properties, so, it would be important as a future purpose to study their biological toxicity in order of their possible ability to act as medical drugs.

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