European Journal of Medicinal Chemistry 44 (2009) 1537–1544

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis, characterization and antimicrobial activity of Co(II), Zn(II) and Cd(II) complexes with *N*-benzyloxycarbonyl-*S*-phenylalanine

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ARTICLE INFO

Article history:
Received 30 May 2008
Received in revised form 8 July 2008
Accepted 15 July 2008
Available online 23 July 2008

Keywords:
Antimicrobial activity
Co(II), Zn(II), Cd(II) complexes
N-Benzyloxycarbonyl-*S*-phenylalanine
2D NMR

ABSTRACT

In this paper the first complexes of M^{2+} ions ($M^{2+} = Zn^{2+}$, Cd^{2+} and Co^{2+}) with *N*-benzyloxycarbonyl-*S*-phenylalaninato ligand (**1**–**3**) are described. The new complexes were characterized by means of elemental analysis, IR and UV–vis spectroscopy, molar conductivity measurements and ^{1}H, ^{13}C and ^{15}N NMR spectroscopy (1D and 2D). The Co(II) complex adopts an octahedral geometry, and the Zn(II) and Cd(II) complexes adopt a tetrahedral one. For the first time, the antimicrobial activity of *N*-benzyloxycarbonyl-*S*-phenylalaninato ligand (*N*-Boc-*S*-phe) and the complexes **1**–**3** was investigated against Gram-positive: *Micrococcus luteus, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis* and *Bacillus subtilis* and Gram-negative bacteria: *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and two strains of the yeast *Candida albicans*. It was shown that the complexes were effective against most strains. The best activity was detected against the yeast *C. albicans*.

1. Introduction

There is an increasing demand for the development of compounds having improved properties and which can be used against several different diseases, such as the treatment of an infection caused by a microorganism. Concerning bacterial diseases, antibiotic research at the industrial level has been focused on the identification of more refined variants of already existing drugs. Many antibiotic peptides with new mechanisms of action have been reported; most of those active against bacteria target the bacterial cell membrane by forming pores. However, hitherto none of these substances have been introduced into clinical use [1]. Despite the rapidity with which new chemotherapeutic agents are introduced, bacteria have shown a remarkable ability to develop resistance to these agents and the search for new drugs, such as metal complexes [2–7], is in progress.

There is also a pressing need for new antifungal agents because of the fast development of resistance of microorganisms to the state-of-the-art drugs currently used to treat different fungal infections. For this reason, the elaboration of new types of antifungal agents is presently a very real task. A promising field for this search is metal-based drugs [8–11]. Metal-based drugs have a different mode of action compared to the commonly used commercial polyene and azole antifungal drugs. Treatment of fungal cells with, for example, Cu(II) and Ag(I) complexes [2] resulted in a reduced amount of ergosterol in the cell membrane and a subsequent increase in its permeability.

Recently we commenced to investigate complexes with *N*-Boc amino acids [12,13]. It is interesting to note that *N*-benzyloxycarbonyl amino acids and their derivates were reported to be anticonvulsant, anti-inflammatory and anti-neoplastic agents [14–19]. *N*-Protected amino acids have abilities to function as cholecystokinin receptor antagonists [20] and derivates of *N*-Boc amino acids also show a good degree of inhibition of the gastric proton pump [21].

In spite of interesting biological activities, only a few complexes with *N*-Boc amino acids have hitherto been described [22–26]. As *N*-benzyloxycarbonylglycine has favorable membrane penetration properties [16,19], in our previous paper the preparation of neutral complexes of this ligand with various metal ions was described [12]. The antimicrobial activity of the obtained metal complexes was also determined and it was established that among the investigated strains, the Zn(II) and Co(II) complexes were selective, acting only against the yeast *Candida albicans*. In a further investigation, the complex of Zn(II) with *N*-benzyloxycarbonyl-*S*-alanine was synthesized and was shown to possess the same selectivity against *C. albicans* [13]. The small increase in lipophilicity obtained on going from *N*-benzyloxycarbonylglycine to *N*-benzyloxycarbonyl-*S*-alanine did not change the MIC value of this Zn(II) complex.

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doi:10.1016/j.ejmech.2008.07.017

In this study, new complex compounds of Co(II), Zn(II) and Cd(II) with *N*-benzyloxycarbonyl-*S*-phenylalanine were synthesized. As *N*-benzyloxycarbonyl-*S*-phenylalanine is more hydrophobic (and more lipophilic) than *N*-Boc-glycine and *N*-Boc-*S*-alanine, it was supposed that these complexes could have better antimicrobial activities than the previously investigated ones. The recently synthesized peptidomimetic compounds containing *N*-benzyloxycarbonyl-group as a part of their structure inhibited the growth of several investigated bacterial strains [1]. Because of this, the antibacterial activities of the newly synthesized complexes against eight bacteria were investigated. Since the previously described complexes were selective against *C. albicans*, the antifungal activities of the new complexes against two strains of *Candida* species were also studied.

2. Chemistry

2.1. Synthesis of [Zn(N-Boc-S-phe)$_2$(H$_2$O)$_2$] (**1**)

To a 50-cm^3 flask containing 0.200 g (0.70 mmol) of *N*-benzyloxycarbonyl-*S*-phenylalanine in 5 cm^3 of ethanol–water mixture (1:1) was added 0.045 g (0.33 mmol) of $ZnCl_2$ dissolved in a minimal volume of water. The flask was placed on a water bath at 45 °C and its content stirred with a magnetic stirrer. The pH value of the system was then adjusted to 6 with a sodium hydroxide solution. The obtained suspension was heated for 1 h under constant stirring. The reaction mixture was then filtered to remove the white precipitate and the obtained filtrate was left to stand at room temperature. After a few days, a white precipitate was obtained from the filtrate, which was separated by filtration and left standing at room temperature. The white powder, obtained in 38.6% (94.3 mg) yield, was soluble in DMSO but insoluble in water, methanol, ethanol and chloroform. Anal. calcd for $ZnC_{34}H_{36}N_2O_{10}$ or [Zn(*N*-Boc-*S*-phe)$_2$(H$_2$O)$_2$]: C, 58.49; H, 5.21; N, 4.02. Found: C, 58.68; H, 4.74; N, 4.09%. Mr = 698.13. Selected IR bands (cm^{-1}): 3340 (ν(N–H)), 1701 (ν(CO$_{amid}$)), 1626 (ν(COO$_{asym}$)), 1402 (ν(COO$_{sym}$)).

2.2. Synthesis of [Cd(N-Boc-S-phe)$_2$] (**2**)

The white cadmium(II) complex was obtained in the same manner as the Zn(II) complex, using $CdCl_2 \cdot 2.5H_2O$ as the cadmium source. Yield: 35.8% (88.85 mg). The complex was soluble in DMSO but insoluble in water, methanol, ethanol and chloroform. Anal. calcd for $CdC_{34}H_{32}O_8N_2$ or [Cd(*N*-Boc-*S*-phe)$_2$]: C, 57.59; H, 4.56; N, 3.95. Found: C, 57.48; H, 4.59; N, 4.00%. Mr = 709.09. Selected IR bands (cm^{-1}): 3338 (ν(N–H)), 1696 (ν(CO$_{amid}$)), 1533 (ν(COO$_{asym}$)), 1410 (ν(COO$_{sym}$)).

2.3. Synthesis of [Co(N-Boc-S-phe)$_2$(H$_2$O)$_2$] (**3**)

Pink crystals of the cobalt(II) complex (not suitable for X-ray analysis) were obtained in the same manner as complexes **1** and **2**, using $CoCl_2 \cdot 6H_2O$ as the cobalt source. Complex **3**, obtained in 12.9% (31.23 mg) yield, was soluble in DMSO, methanol, and ethanol but insoluble in water and chloroform. Anal. calcd for $CoC_{34}H_{36}N_2O_{10}$ or [Co(*N*-Boc-*S*-phe)$_2$(H$_2$O)$_2$]: C, 59.04; H, 5.27; N, 4.05. Found: C, 59.56; H, 5.27; N, 4.13%. Mr = 691.65. Selected IR bands (cm^{-1}): 3284 (ν(N–H)), 1680 (ν(CO$_{amid}$)), 1576 (ν(COO$_{asym}$)), 1404 (ν(COO$_{sym}$)).

3. Pharmacology

3.1. Microorganisms

The antimicrobial activity was evaluated using eight different laboratory control strains of bacteria, i.e., Gram-positive: *Micrococcus luteus* (ATCC 10240), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Enterococcus faecalis* (ATCC 29212) and *Bacillus subtilis* ATCC 6633, Gram-negative: *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (NCIMB 9111) and *Pseudomonas aeruginosa* (ATCC 27853), and two strains of yeast: *C. albicans* (ATCC 24433) and *C. albicans* (ATCC 10259).

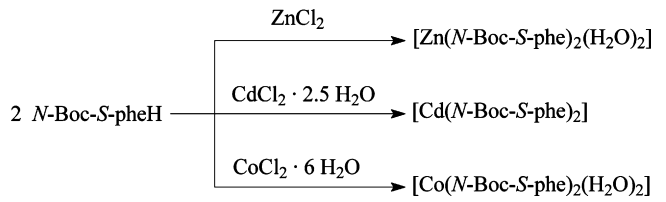
Active cultures for the experiments were prepared by transferring a loopful of cells from the stock into tubes that contained 10 ml of Mueller-Hinton broth (MHB) for the bacteria and Sabouraud dextrose broth (SDB) for the fungi. After incubation for 24 h at 37 °C and 25 °C, respectively, the cultures were diluted with fresh Mueller-Hinton and Sabouraud dextrose broth, respectively, in order to achieve optical densities corresponding to 2×10^6 colony forming units (CFU/ml) for the bacteria and 1×10^8 CFU/ml for the fungi and used as inoculums (NCIMB – National Collections of Industrial Food and Marine Bacteria; NCIMB Ltd, UK).

3.2. Agar diffusion method

Mueller-Hinton agar (MHA) and Sabouraud dextrose agar (SDA) (Institute for Immunology and Virology, Torlak, Belgrade) were used to test the sensitivity of the bacteria and the *C. albicans*, respectively. The MHA and SDA, sterilized and cooled to 45–50 °C, were distributed into sterile Petri dishes with a diameter of 9 cm (15 ml) to form a 4-mm thick layer. The plates were inoculated with previously prepared inoculums of bacteria or fungi in order to obtain semiconfluent growth. Prior to analysis, sterilized antibiotic discs (6 mm) were used, following the literature procedure (Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard NCCLS 1993). Fresh stock solutions of the metal salts, ligand and the complexes **1**–**3** were prepared in dimethylsulfoxide (DMSO) at a concentration of 1 mg/ml. The discs were impregnated with 20 μl of these solutions. To ensure that the solvent had no effect on microbial growth, a control test was performed with test medium supplemented with DMSO by the same procedures as employed in the experiments. The discs injected with the solutions were placed on the inoculated agar by pressing slightly and incubated at 37 °C (24 h) for the bacteria and 72 h at 26 °C for the yeast *C. albicans*. On each plate an appropriate reference antibiotic disc was applied depending on the test microorganism. The results were read by measuring the diameters of the inhibition zones in millimeters. In each case, triplicate tests were performed and the average was taken as the final reading. Ampicillin (10 μg/disc), amikacin (10 μg/disc) and nystatin (100 U/disc) served as the positive controls.

3.3. Determination of the minimal inhibitory concentration (MIC) and the minimal bactericidal or fungicidal concentration (MBC)

The broth microdilution method was used to determine the minimal inhibitory concentration (MIC) and the minimal bactericidal or fungicidal concentration (MBC) according to the National Committee for Clinical Laboratory Standards (NCCLS, 2001).



Scheme 1. Reaction of the simple metal salt with *N*-benzyloxycarbonyl-*S*-phenylalanine.

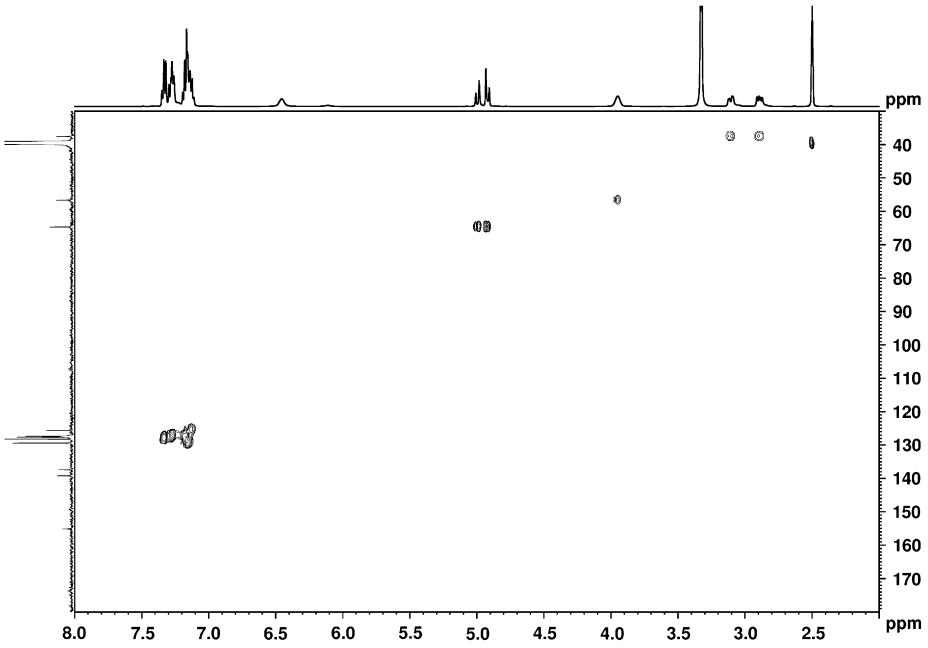


Fig. 1. (^{13}C–^{1}H) HSQC spectrum of complex **1**. Bruker 500 MHz, $c = 100$ mg/ml, DMSO-d_6, TMS as internal standard.

All tests were performed in a Mueller-Hinton broth for the bacterial strains and in a Sabouraud dextrose broth for the *C. albicans*. Overnight broth cultures of each strain were prepared and the final concentration in each well was adjusted to 2×10^6 CFU/ml for the bacteria and 2×10^5 CFU/ml for yeasts. Compounds were dissolved in 1% dimethylsulfoxide (DMSO) and then diluted to the highest concentration. Two-fold serial concentrations of the compounds were prepared (over the range 62.5–1000 μg/ml) in a 96-well microtiter plate. In the tests, triphenyltetrazolium chloride (TTC) (Aldrich Chemical Company Inc., USA) was also added to the culture medium as a growth indicator. The final concentration of TTC after inoculation was 0.05%. The microbial growth was determined by the absorbance at 600 nm using a universal microplate reader after incubation at 37 °C for 24 h for the bacteria, and at 26 °C for 48 h for the fungi. The MIC is defined as the lowest concentration of the compound at which the microorganism does not demonstrate visible growth.

To determine the MBC, broth was taken from each well (10 μl) and inoculated in Mueller-Hinton agar for 24 h at 37 °C for the bacterial strains and in Sabouraud dextrose agar for 48 h at 26 °C for the fungi. The MBC is defined as the lowest concentration of the tested compound at which the inoculated microorganisms were completely killed. All determinations were performed in triplicate and two positive growth controls were included.

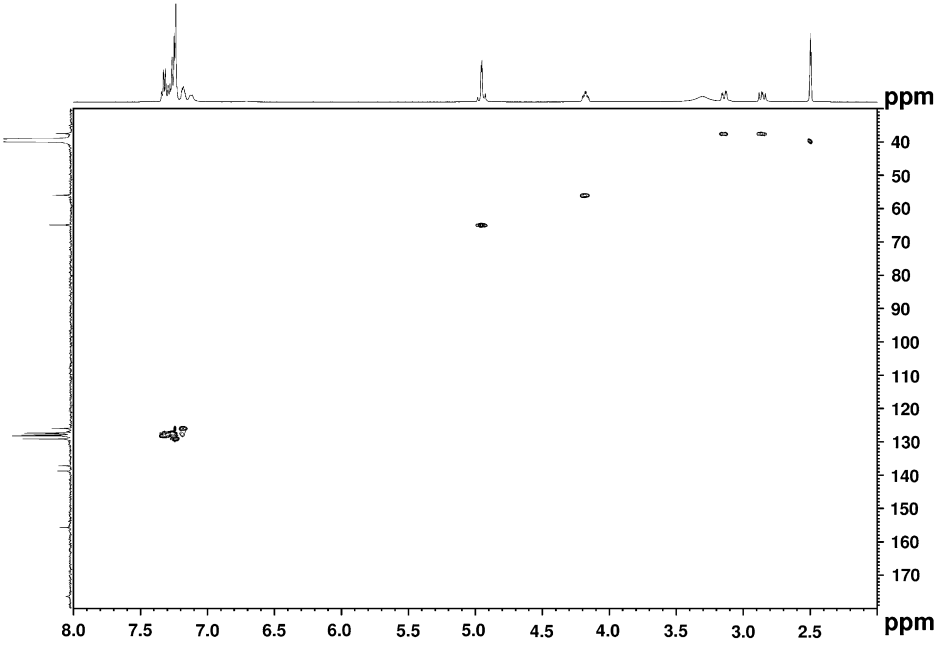


Fig. 2. (^{13}C–^{1}H) HSQC spectrum of complex **2**. Bruker 500 MHz, $c = 100$ mg/ml, DMSO-d_6, TMS as internal standard.

Table 1
^{1}H (DMSO-d_6) NMR data (500 MHz) of *N*-Boc-*S*-phe ligand (L) and complexes **1** and **2** (δ, multiplicity, *J* in Hz)

	H(1)	H(2)	H(3)	H(5)	H(10)a	H(10)b	H(7–9) + H(12–14)
L	12.70, br s	4.20, ddd, $J = 4.5$, $J = 10.5$, $J = 8.3$	7.60, d, $J = 8.3$	4.95, ABq, $\Delta\delta = 0.02$, $J = 12.9$	3.08, dd, $J = 4.5$, $J = 13.7$	2.87, dd, $J = 10.5$, $J = 13.7$	7.16–7.36, m
1		3.95, br s	6.45, br s	4.96, ABq, $\Delta\delta = 0.09$, $J = 12.7$	3.10, dd, $J = 3.7$, $J = 13.5$	2.89, dd, $J = 7.3$, $J = 13.5$	7.37–7.08, m
2		4.20, dt, $J \approx 9.0$, $J = 4.0$	7.12, br d, $J = 7.0$	4.95, ABq, $\Delta\delta = 0.02$, $J = 12.8$	3.14, dd, $J = 4.2$, $J = 13.7$, $J = 13.7$	2.86, dd, $J = 9.9$	7.15–7.36, m

4. Results and discussion

4.1. Synthesis

Syntheses were performed to obtain neutral complexes (because of their facilitated membrane transport), Scheme 1. The new complexes were synthesized via a simple reaction between $ZnCl_2$, $CdCl_2 \cdot 2.5H_2O$, or $CoCl_2 \cdot 6H_2O$ and *N*-benzyloxycarbonyl-*S*-phenylalanine in a 1:2 molar ratio in an ethanol–water mixture at pH = 5–6 (Scheme 1).

4.2. NMR spectroscopy

NMR spectral assignments and structural parameters for complexes **1** and **2** were obtained by the combined use of ^{1}H homonuclear spectroscopy (DQF-COSY) and heteronuclear proton-detected spectroscopy (2D HSQC, 2D HMBC), Figs. 1 and 2.

In the ^{1}H NMR spectra of complexes **1** and **2**, a signal for carboxylic proton was absent, indicating coordination through the carboxylic group (Table 1).

^{1}H NMR resonances' downfield shift is more prominent for CH- (complex **1**) and for the NH-proton resonances [27], Table 1.

The assumption that coordination through nitrogen atom does not occur was confirmed by ^{15}N NMR spectra. Namely, it is known that the attachment of the metal to nitrogen leads to an upfield shift of nitrogen-15 signal in comparison with the shift of the same signal in non-coordinated ligand [28,29]. The nitrogen resonates at δ 88.5 in the spectrum of *N*-benzyloxycarbonyl-*S*-phenylalanine, and at δ 90.9 and 90.7 in complexes **1** and **2**, respectively. A downfield shift ($\Delta\delta = 2.4$–2.2) was observed, indicating that nitrogen atom does not participate in the coordination. The changes in ^{15}N chemical shift upon coordination have been first observed in cobalt(III) complexes with amino acids by Juranić and co-authors [30]. Coordination of amino acids to cobalt(III) ion induces diamagnetic displacement of the amino acid ^{15}N resonance position by 24–42 ppm compared to those of the corresponding protonated amino acids.

The appropriate NH-proton resonates at δ 6.45 and 7.11 in the spectra of complexes **1** and **2**, respectively. In the spectrum of the ligand, the NH-proton resonates at δ 7.60. The significant upfield shift ($\Delta\delta = 1.15$ ppm) of the proton bound to nitrogen in the spectrum of Zn(II) complex is the result of diamagnetic shielding of the aromatic ring, which is concluded on the basis of analysis of the rotamer populations of the phenyl group. Namely, the mole fractions of the ***t***, ***g*** and ***h*** rotamers of *S*-phenylalaninato part of the *N*-Boc-*S*-phe ligand (Scheme 2) in complexes **1** and **2** were calculated on the basis of the vicinal coupling constants of the α and β protons (J_{AX} and J_{BX}), as in a previous study [31]. It was shown that NH$\cdots\pi$ interactions lead to a population increase of the ***h*** rotamer in the aromatic residue. If NH$\cdots\pi$ interactions are present in solution then the NMR spectrum should show an upfield chemical shift of the NH-proton that participates in this interaction [32]. The coupling constants and the calculated rotamer mole fractions of *S*-phenylalaninato side group in non-coordinated *N*-Boc-*S*-phe ligand and in complexes **1** and **2** are presented Table 2.

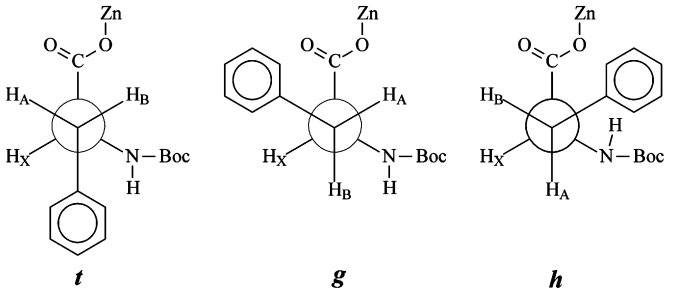
From Table 2, a significant increase of the rotamer ***h*** population (Scheme 2) is observed in the case of complex **1** in comparison to the population of rotamer ***h*** in the ligand and complex **2**. An increase of the rotamer ***h*** population mostly results from NH$\cdots\pi$ interactions in solution [31]. Thus, the significant upfield shift of the NH-proton in the NMR spectrum of complex **1** is probably the result of intramolecular NH$\cdots\pi$ interaction.

The NMR spectrum of complex **1** (Fig. 1) shows the presence of water molecules, which is in accordance with elemental analysis.

The complexes were also characterized by means of ^{13}C NMR spectroscopy (Table 3). The higher chemical shift of C(1) (Scheme 3) in complexes **1** and **2** in comparison with the chemical shift of this signal in non-coordinated *N*-benzyloxycarbonyl-*S*-phenylalanine, strongly suggests the coordination through the carboxylate groups. On the other hand, the insignificant change of the chemical shift of the carbamoyl carbon C(4) in complex **2**, or upfield chemical shift in NMR spectrum of complex **1**, indicates that the carbamoyl group does not participate in coordination.

It was shown that the ^{13}C NMR technique can provide useful information for the diagnosis of the binding modes of a carboxylate group [33,34]. Ama and Jasui were the first who observed differences in ^{13}C NMR shifts between monodentate and bidentate coordinated amino acids [35]. The magnitude of the Δcoord value (difference in the chemical shift of the carboxylic carbon resonance between the amino acid in neutral aqueous solution and the amino acid coordinated to cobalt(III) through carboxylic group) can be used to distinguish whether amino acid is monodentate or bidentate.

The higher downfield shift of the signal of the carboxylate C atom in the spectrum of complex **2** ($\Delta\delta_C = 3.2$ ppm) relative to the shift of the corresponding signal in the spectrum of complex **1** ($\Delta\delta_C = 0.5$ ppm) in comparison to the value of the chemical shift of this signal in the NMR spectrum of the free ligand, can be interpreted in terms of an increased polarization of the carbonyl bond, i.e., on the basis of $\Delta\delta_C$, it can be concluded that the coordination of



Scheme 2. Newman projections of the three staggered rotamers of *S*-phenylalaninato side group (as a part of *N*-Boc-*S*-phe ligand) in the Zn(II) complex (the β carbon is in the front and the α carbon is in the rear).

Table 2
Coupling constants and calculated rotamer mole fractions of *S*-phenylalaninato side groups in the *N*-Boc-*S*-pheH ligand and complexes **1** and **2**

	J_{AX}	J_{BX}	***t***	***g***	***h***
N-Boc-*S*-pheH	4.6	10.4	78	15	7
Complex **1**	4.2	7.2	43	10	47
Complex **2**	3.8	10.1	75	6	19

Table 3
^{13}C NMR chemical shifts (Bruker 500 MHz) in DMSO-d_6 solution for *N*-Boc-*S*-pheH (L) and complexes **1** and **2**

	C1	C2	C4	C5	C6	C10	C11	C(7,7′,8,8′,9) + C(12,12′,13,13′,14)
L	173.2	55.5	155.6	65.2	137.0	36.5	137.9	127.7–126.3 C(7,7′) 127.5 C(12,12′) 129.0
1	173.7	55.7	155.1	64.7	137.4	37.6	139.2	129.4–125.6 C(7,7′) 127.3 C(12,12′) 129.1
2	176.4	56.0	155.7	64.9	137.7	37.5	138.7	129.1–126.0 C(7,7′) 127.3 C(12,12′) 129.4

the carboxylic group is different in complexes **1** and **2**. The higher downfield shift of the carboxylate carbon in the ^{13}C NMR spectrum of complex **2** in comparison to that of the signal of the same atom in the spectrum of complex **1** is in agreement with a more positive C atom, which is expected in the case of the proposed bidentate coordination in complex **2** in comparison to the monodentate coordination assigned to **1** (Figs. 3 and 4) [35]. The higher deshielding effect of the carboxylate C atom in complex **2** can be explained in terms of a decrease in the electron density at the carbonyl carbon, which tends to contract the 2p orbitals and, consequently, causes r_{2p}^{-3} to increase.

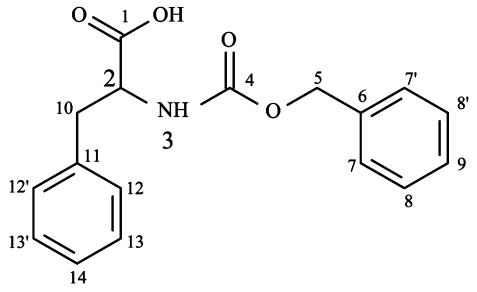
4.3. IR spectroscopy and molar conductivity data

On the basis of the differences in the frequencies (1600–1350 cm^{-1} region) of the asymmetric and symmetric skeletal vibrations of the carboxylic group in free ($\Delta\nu_{as-vs} = 186$ cm^{-1}) and the coordinated *N*-Boc-*S*-phe ligand in complexes **1**–**3**, it can be concluded that the modes of coordination of the carboxylic group in the complexes are different [36].

Namely, in the case of complex **1** ($\Delta\nu_{as-vs} = 224$ cm^{-1}), monodentate coordination of the carboxylic group is proposed.

Elemental analysis of complex **1** indicated that the complex crystallizes with water molecules (in accordance with its NMR spectrum). Its IR spectrum confirms that the new compound contains water molecules, which are coordinated to the zinc ions (bands at 3350 and 874 cm^{-1}) [36]. The molar conductivity data in DMSO solution (10^{-3} M) are in accordance with a neutral complex (34 Ω^{-1} cm^2 mol^{-1}) [37]. On the basis of ^{13}C NMR, IR, elemental analysis and molar conductivity data, tetrahedral geometry with two monodentate coordinated carboxylic groups and two coordinated water molecules around the Zn(II) ion was proposed (Fig. 3).

In the case of complex **2** ($\Delta\nu_{as-vs} = 123$ cm^{-1}), chelate bidentate coordination of the carboxylic group seems likely, i.e., changes of the coordination mode may be because of increasing ion size on going from Zn^{2+} to Cd^{2+}. Bidentate coordination of carboxylic



Scheme 3. *N*-Benzyloxycarbonyl-*S*-phenylalanine (*N*-Boc-*S*-pheH) with labeled atoms.

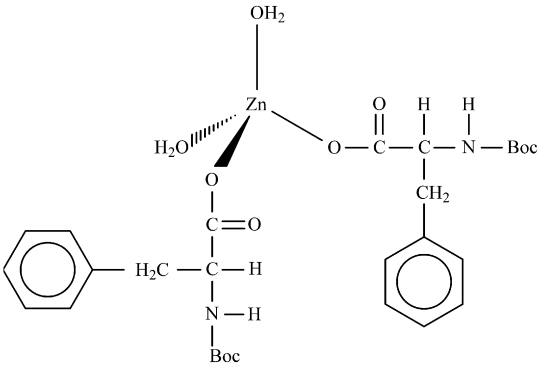


Fig. 3. Proposed structure of complex **1**.

group was proposed by means of ^{13}C NMR and IR data. In this way, NMR and IR spectroscopy provide complementary methods for investigating the binding modes of a carboxylate group.

Compound **2** crystallizes without water molecules (in agreement with elemental analysis, IR and NMR spectra).

The molar conductivity data of complex **2** in DMSO solution (10^{-3} M) are in accordance with a neutral complex (36 Ω^{-1} cm^2 mol^{-1}) [37]. Based on the IR and ^{13}C NMR experimental data, a tetrahedral geometry with two bidentate coordinated carboxylic groups of two *N*-Boc-*S*-phe ligands around the Cd(II) ion is proposed (Fig. 4).

In the case of complex **3** ($\Delta\nu_{as-vs} = 172$ cm^{-1}), chelate bidentate coordination of the carboxylate group is proposed. The results of the elemental analysis are in agreement with two molecules of water. The IR spectrum exhibited a broad band at 3284 cm^{-1}and a band at 832 cm^{-1}, assigned to the coordinated water molecules [38]. The Λ_M value of 38.3 Ω^{-1} cm^2 mol^{-1} (10^{-3} M solution) in CH_3OH at 25 °C implies the presence of a non-electrolyte species [37]. Based on the experimental data, an octahedral environment around the Co(II) ion is proposed (Fig. 5).

4.4. Electronic absorption spectrum and optical activity of complex **3**

The proposed octahedral geometry for the Co(II) complex (**3**) was confirmed by the electronic absorption spectrum. Namely, electronic absorption spectrum is in accordance with an octahedral $Co^{II}O_6$ chromophore. In the visible region, one asymmetric maximum at $\lambda = 557.2$ nm was observed ($^4A_{2g} \leftarrow {}^4T_{1g}(F)$ and $^4T_{1g}(P) \leftarrow {}^4T_{1g}(F)$ transitions) [39].

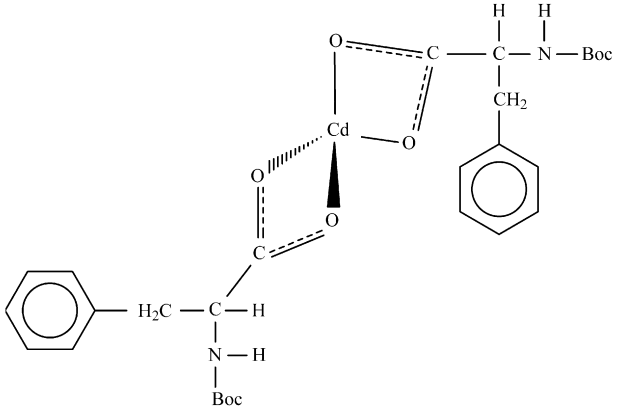


Fig. 4. Proposed structure of complex **2**.

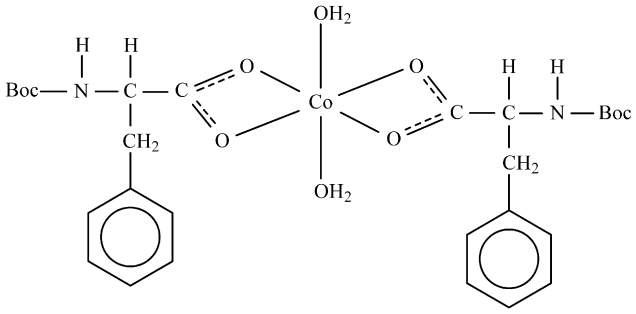


Fig. 5. Proposed structure of complex **3**.

Octahedral geometry for complex **3** was proposed, with two bidentate coordinated *N*-Boc-*S*-phe ligands bonded through oxygen atoms of the carboxylate groups and two coordinated water molecules. The water molecules in this geometry could be in the *trans* or *cis* position. *N*-Boc-*S*-phe ligand is very voluminous and it is supposed that the two water molecules would be in the *trans*-position. To check this supposition, the optical activity of complex **3** in DMSO solution was measured at $\lambda = 589.3$ nm. Namely, if two water molecules are in *trans*-position, such molecule would posses a plane of symmetry and would be optically inactive. The isomer with coordinated water molecules in *cis* position would be active. The measured optical activity (α) of complex **3** was zero (and, of course, the calculated $[\alpha]_D$ for a 10^{-3} M solution was zero). The proposed octahedral geometry with two water molecules in *trans*-position is in accordance with the structure of an analogous complex with *N*-Boc-glycine [12].

4.5. Antimicrobial activity

The results of the determination of the in vitro antimicrobial activities of the simple metal salt, *N*-Boc-*S*-phe ligand and the complexes **1**–**3** are presented in Tables 4–6.

Generally, if the zone of inhibition of the investigated strains caused by the newly synthesized complexes are compared the order is as follows: Cd(II) > Co(II) > Zn(II) [**2** > **3** > **1**]. Complex **2** exhibited the best activity, i.e., all the tested bacterial strains, except *E. faecalis*, were sensitive (Table 4). Among the Gram-positive bacteria, the zone of inhibition of the bacteria *B. subtilis* was comparable with that caused by the standard drugs amikacin and higher than for ampicillin. The Gram-negative bacteria (*K. pneumoniae* and *P. aeruginosa*) were less sensitive than the Gram-positive bacterial strains.

The best activity of complex **2** was observed against the yeast *C. albicans* and the zones of inhibition (26 and 35 mm, respectively) were higher than the zones of the standard drug nystatin (21 and 22.5 mm, respectively).

The MIC values of the tested compounds are presented in Table 5. Generally speaking, the investigated complexes exhibited better inhibitory activity against Gram-positive bacteria than against Gram-negative bacteria (**2** > **3** > **1**) (Table 5). The results of the broth microdilution method are in accordance with the agar diffusion method.

In all cases, the ligand was less active than the investigated complexes. Comparison of MIC values obtained for simple metal salts and complexes reveals that complexes **1** and **2** expressed 10-fold greater activity in comparison to simple metal salt, as well as for complex **3** in the case of bacteria *S. epidermidis*. Obviously, the process of chelation of *N*-benzyloxycarbonyl-*S*-phenylalaninato anions to a metal ion reduces the polarity and increases the lipophilic nature of central metal ion, which in turn favors its penetration through the lipid layer of the cell membrane of microorganisms [40]. The best inhibitory activity is obtained for yeast *Candida albicans* (the lowest MIC values).

The minimal bactericidal or fungicidal concentrations (MBC) are presented in Table 6. The obtained results are in accordance with the obtained MIC values.

It is interesting to compare the MIC values of the previously described complexes with *N*-benzyloxycarbonylglycine with those of the newly synthesized complexes. Namely, the MIC values for [Zn(*N*-Boc-gly)$_2$], [Cd(*N*-Boc-gly)$_2$(H$_2$O)$_2$]$_n$ and [Co(*N*-Boc-gly)$_2$(H$_2$O)$_2$] were 1.30, 1.11 and 0.97 mM, respectively [12]. The comparison of MIC values of Cd(II) complexes indicates that substitution of *N*-Boc-gly with *N*-Boc-*S*-phe ligand resulted in a more than 12-fold increase in the anti-*Candida* activity, from 1.11 to 0.09 mM. This increase in activity was also observed for complexes **1** and **3**. The increase in the lipophilicity of *N*-benzyloxycarbonyl-*S*-phenylalaninato ligand is probably the reason for the better penetration of the complexes with this ligand in comparison to the complexes with *N*-benzyloxy carbonylglycine.

In conclusion, it is interesting to note that hitherto investigated complexes with *N*-benzyloxycarbonyl-amino acids exhibited the best activity against the yeast *C. albicans* of the until now investigated bacterial and fungal strains. Complex **2** has an MIC value almost the same as that of the standard drug nystatin in the case of *C. albicans* ATCC 24433. Since *C. albicans* is the major fungal pathogen in humans (carried by over 50 % of the population), in this context further investigations in this field have sense.

5. Experimental protocols

5.1. Materials

All the employed reagents and solvents were of analytical grade. *N*-Boc-phenylalanine, and the metal salts were obtained from Aldrich and used without further purification.

Table 4
Antimicrobial activity (zone of inhibition in mm)

Microorganism	$ZnCl_2$	$CdCl_2 \cdot 2.5H_2O$	$CoCl_2 \cdot 6H_2O$	L	**1**	**2**	**3**	Amikacin	Ampicillin	Nystatin
M. luteus	0	31.0	9.0	0	0	22.0	0	31.0	33.0	n.t.
S. aureus	0	29.0	14.0	0	0	13.0	0	25.0	26.0	n.t.
S. epidermidis	0	27.0	0	8.0	0	25.5	0	33.0	12.0	n.t.
E. faecalis	0	0	18.0	0	0	0	15.0	23.0	16.0	n.t.
B. subtilis	11.0	35.0	13.0	0	0	30.0	0	32.0	15.0	n.t.
E. coli	6.0	8.0	0	0	0	10.0	0	30.0	18.0	n.t.
K. pneumonia	6.0	11.0	0	0	0	6.5	0	25.0	23.0	n.t.
P. aeruginosa	0	18.5	8.0	0	0	6.0	7.0	30.0	n.t.	n.t.
C. albicans (ATCC 10259)	10.0	25.0	14.0	0	0	26.0	13.0	n.t.	n.t.	21.0
C. albicans (ATCC 24433)	10.0	36.0	12.0	0	0	35.0	11.0	n.t.	n.t.	22.5

Concentration of metal salt, L and complexes **1**–**3**: 20 μg/disc. Ampicillin (10 μg/disc), amikacin (10 μg/disc) and nystatin (100 U/disc) served as a positive control; n.t.: not tested.

Table 5
Minimal inhibitory concentration (MIC in mM) evaluated for simple metal salt, ligand and complexes **1**–**3**

Microorganism	$ZnCl_2$	$CdCl_2 \cdot 2.5H_2O$	$CoCl_2 \cdot 6H_2O$	L	**1**	**2**	**3**	Amikacin	Ampicillin	Nystatin
M. luteus	>7.34	1.09	1.05	3.34	0.71	0.18	0.36	n.t.	0.001	n.t.
S. aureus	>7.34	1.09	1.05	3.34	0.71	0.18	0.36	n.t.	0.001	n.t.
S. epidermidis	>7.34	1.09	2.10	3.34	0.71	0.18	0.18	n.t.	0.003	n.t.
E. faecalis	>7.34	2.19	1.05	>3.34	>1.43	0.35	0.36	n.t.	0.003	n.t.
B. subtilis	3.67	1.09	1.05	1.67	0.36	0.18	0.72	n.t.	0.005	n.t.
E. coli	3.67	1.09	2.10	3.34	0.71	0.35	0.72	0.003	0.011	n.t.
K. pneumoniae	3.67	2.19	2.10	3.34	0.71	0.71	0.72	0.003	0.011	n.t.
P. aeruginosa	7.34	2.19	1.05	>3.34	1.43	0.71	0.36	0.006	0.023	n.t.
C. albicans (ATCC 10259)	3.67	0.27	0.5	0.83	0.36	0.09	0.18	n.t.	n.t.	0.03
C. albicans (ATCC 24433)	3.67	1.09	0.5	0.83	0.36	0.09	0.18	n.t.	n.t.	0.07

n.t.: Not tested.

Table 6
Minimal bactericidal or fungicidal concentration (in mM) evaluated for simple metal salt, ligand and complexes **1**–**3**

Microorganism	$ZnCl_2$	$CdCl_2 \cdot 2.5H_2O$	$CoCl_2 \cdot 6H_2O$	L	**1**	**2**	**3**	Amikacin	Ampicillin	Nystatin
M. luteus	>7.34	2.18	4.20	>3.34	1.44	0.36	1.44	n.t.	0.002	n.t.
S. aureus	>7.34	2.18	8.4	>3.34	1.44	0.36	1.44	n.t.	0.004	n.t.
S. epidermidis	>7.34	2.18	>4.20	>3.34	1.44	0.36	1.44	n.t.	0.006	n.t.
E. faecalis	>7.34	4.38	4.20	>3.34	>1.43	0.70	1.44	n.t.	0.006	n.t.
B. subtilis	7.34	2.18	4.20	3.34	0.72	0.36	1.44	n.t.	0.010	n.t.
E. coli	7.34	2.18	4.20	>3.34	1.42	0.70	1.44	0.006	0.022	n.t.
K. pneumoniae	7.34	4.38	4.20	>3.34	1.42	1.42	1.44	0.009	0.033	n.t.
P. aeruginosa	>7.34	4.38	4.20	>3.34	>1.43	>1.42	1.44	0.012	0.046	n.t.
C. albicans (ATCC 10259)	7.34	0.54	1.00	1.66	0.72	0.18	0.36	n.t.	n.t.	0.06
C. albicans (ATCC 24433)	7.34	2.18	1.00	1.66	0.72	0.18	0.72	n.t.	n.t.	0.14

n.t.: Not tested.

5.2. Measurements

Elemental analyses for C, H, N were performed on a Vario III CHNOS Elemental analyzer, Elementar Analysensysteme GmbH.

The solid state IR spectra were performed on a ThermoScientific Nicolet 6700 FT-IR Spectrometer.

The molar conductivities of complexes **1** and **2** in DMSO solution and of complex **3** in CH_3OH solution ($c = 10^{-3}$ mol/dm^3) were measured at room temperature on a Iskra Kranj MA 5961 conductivity meter.

The optical activity was measured using an Autopol IV, Automatic polarimeter (Rudolph Research Analytical).

The electronic absorption spectrum of complex **3** in DMSO solution was recorded on a GBC Cintra 40 UV–vis spectrophotometer.

^{1}H NMR (500 MHz) spectra were recorded using a Bruker Avance III 500 MHz spectrometer equipped with a broad band direct probe. All spectra were measured at 288 K in DMSO-d_6 solution. The chemical shifts (δ) are given in ppm. The internal standard was TMS. ^{13}C NMR (125 MHz) spectra were recorded using the same instrument in DMSO-d_6 solution. The solvent peak at 39.7 ppm was used to calibrate the scale of the chemical shifts. The external standard for ^{15}N spectra was a 0.1 M solution of urea (77.0 ppm). The NMR spectral assignments and structural parameters were obtained by combined use of ^{1}H homonuclear spectroscopy (2D DQF-COSY) and multinuclear proton-detected spectroscopy (2D HSQC, 2D HMBC).

Acknowledgement

This investigation was supported by the Ministry of Science of the Republic of Serbia, grant no. 142062.

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