Cobalt(II) and magnesium(II) complexes with 1,3-pdta-type of ligands: influence of an alkyl substituent at 1,3-propanediamine chain on the structural and antimicrobial properties of the complex

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ABSTRACT
To investigate how modification in the structure of 1,3-propanediamine chain of 1,3-pdta (1,3-propanediamine-\(N,N,N_0,N_0\)-tetraacetate) ligand affects the structural and biological properties of the corresponding metal complexes, two new octahedral complexes, [Co(H\(_2\)O)\(_5\)Co(2,2-diMe-1,3-pdta)]\(\cdot\)H\(_2\)O (1) and [Mg(H\(_2\)O)\(_5\)Mg(2,2-diMe-1,3-pdta)]\(\cdot\)1.5H\(_2\)O (2) (2,2-diMe-1,3-pdta = 2,2-dimethyl-1,3-propanediamine-\(N,N,N_0,N_0\)-tetraacetate), were synthesized and characterized by IR spectroscopy and single-crystal X-ray diffraction analysis. Additionally, UV-Vis and NMR spectroscopic methods were applied for the characterization of 1 and 2, respectively. Crystallographic data indicate that these complexes contain 2,2-diMe-1,3-pdta coordinated to the metal ion through 2 N and 4 O atoms forming [M(H\(_2\)O)\(_5\)M(2,2-diMe-1,3-pdta)] complex unit (M, M' = Co(II), Co(II) (1) and M, M' = Mg(II), Mg(II) (2)), which is composed of [M'(2,2-diMe-1,3-pdta)]\(^{2-}\) and [M(H\(_2\)O)\(_5\)O]\(^{2+}\) octahedra bridged by one of the axial carboxylate groups. The antimicrobial activities of 1 and 2 were evaluated against different bacteria and Candida spp., while their cytotoxic effect was tested on the normal human lung fibroblasts (MRC-5). The ability of 1 and 2 to inhibit formation of C. glabrata biofilms was also assessed. The obtained structural parameters and biological properties of the two complexes were compared to Co(II) and Mg(II) complexes with 1,3-pdta ligand.

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1. Introduction

Many metal(II) complexes with the hexadentate 1,3-pdta ligand (1,3-pdta = 1,3-propanediamine-\(N,N,N',N'\)-tetraacetate ion) have been prepared and characterized by single-crystal X-ray diffraction methods [1–13]. The X-ray results revealed that 1,3-pdta is able to form with the M(II) ions diverse types of structures ranging from discrete (or 0 D) complexes \([\text{M(H}_2\text{O)}_6]\)[\(\text{M}'(1,3\text{-pdta})\)]\(2\text{H}_2\text{O}\) (M(II), M'(II) = Mg, Cu [1]; Mn, Cu [2]; Mg, Ni [3]; Mg, Co [4]; Co, Co [4]; Mg, Mg [5]; Mg, Zn [6]; Zn, Zn [6]; Mg, Mg\(_{0.5}\)Mn\(_{0.5}\) [7] and Ni, Ni [8]) and \([\text{M(H}_2\text{O)}_6]\)[\(\text{M}'(1,3\text{-pdta})(\text{H}_2\text{O})\)]\(2\text{H}_2\text{O}\) (M(II), M'(II) = Mg, Cd [6] and Mg, Mn [7]) to 1 D \{[\text{Ca(H}_2\text{O)}_3\text{Ca}(1,3\text{-pdta})(\text{H}_2\text{O})\)]\(2\text{H}_2\text{O}\}_n [5], 2 D \{\text{Li}_2[\text{Co}(1,3\text{-pdta})(\text{H}_2\text{O)}_3]\text{H}_2\text{O}\}_n [9], \{\text{K}_2[\text{Ca}(1,3\text{-pdta})\text{H}_2\text{O}\text{CH}_3\text{OH}]\}_n [10] and 3 D \{[\text{M}_3(1,3\text{-pdta})(\text{H}_2\text{O})_6]\text{H}_2\text{O}\}_n (\text{M}(\text{II}) = \text{Sr} [11] and \text{Ba} [12]) and (\text{Sr}_2(1,3\text{-pdta})(\text{H}_2\text{O})_{3.5})_n [12] polymeric species.

The smaller M(II) ions of ionic radius \(r(\text{M})\) from 0.830 – 0.885 Å [14] form with the hexadentate 1,3-pdta ligand a series of isostructural crystals containing octahedral complexes of general formula \([\text{M(H}_2\text{O)}_6]\)[\(\text{M}'(1,3\text{-pdta})\)]\(2\text{H}_2\text{O}\) [1–8], while larger metal ions such as Mn(II) \((r(\text{Mn}) = 0.970 \text{ Å})\) and Cd(II) \((r(\text{Cd}) = 1.090 \text{ Å})\) form with this ligand another series of isostructural crystals built of pentagonal bipyramidal complexes, \([\text{M(H}_2\text{O)}_6]\)[\(\text{M}'(1,3\text{-pdta})(\text{H}_2\text{O})\)]\(2\text{H}_2\text{O}\) [6,7]. Besides in the abovementioned complexes of the octahedral and pentagonal bipyramidal geometry, the hexadentate coordination mode of the 1,3-pdta ligand has also been observed in the capped trigonal prismatic \{\text{K}_2[\text{Ca}(1,3\text{-pdta})\text{H}_2\text{O}\text{CH}_3\text{OH}]\}_n [10] and square antiprismatic \{[\text{Ca(H}_2\text{O)}_3\text{Ca}(1,3\text{-pdta})(\text{H}_2\text{O})]\text{H}_2\text{O}\}_n [5] complexes. However, for the isostructural \{\text{Sr}_2(1,3\text{-pdta})(\text{H}_2\text{O})_6\text{H}_2\text{O}\}_n [11] and \{[\text{Ba}_2(1,3\text{-pdta})(\text{H}_2\text{O})_6]\text{H}_2\text{O}\}_n complexes [12] with large metal(II) ions \((r(\text{Sr}) = 1.320 \text{ Å} \text{ and } r(\text{Ba}) = 1.490 \text{ Å})\) of tricapped trigonal prismatic geometry and for another strontium complex, \{\text{Sr}_2(1,3\text{-pdta})(\text{H}_2\text{O})_{3.5}\}_n [12], of coordination number (CN) nine, the bis-tridentate coordination mode of 1,3-pdta has been
established. The change of coordination mode of the M(II)-1,3-pdta system from hexadentate to bis-tridentate was due to the highly strained bonds about the chelating N atoms that appear in the hexadentate M(II)-1,3-pdta system with a large spherically symmetric metal(II) ion of high coordination number [5,11]. For M(II)-1,3-pdta complexes, the critical ionic radius was established to be that of the Mn(II) ion \( (r(Mn) = 0.970 \text{ Å}) \) [7].

The conformational behavior of the ligand backbone is expected to vary when the two hydrogen atoms of the central methylene group are replaced with two geminal methyl groups (Thorpe–Ingold effect) [15,16]. A simple aminopolycarboxylate ligand with the 2,2-dimethyl-1,3-propanediamine backbone is the 1,3-pdta analogue 2,2-dimethyl-1,3-propanediamine-N,N,N',N'-tetraacetate (2,2-diMe-1,3-pdta). We have recently synthesized this ligand and used it for the synthesis of seven-coordinate manganese(II) and cadmium(II) complexes of formula \( \{\text{Ba}[M(2,2-diMe-1,3-pdta)]-3H_2O\}_n \) (\( M = \text{Mn(II)} \) or \( \text{Cd(II)} \)) [17] and six-coordinate Ni(III) complex, \( \{\text{Mg(H}_2\text{O})_5\text{Ni(2,2-diMe-1,3-pdta)}\}-1.5\text{H}_2\text{O} \) [18], as well as isostructural Cr(III) and Co(III) complexes, \( \text{Na[Cr(2,2-diMe-1,3-pdta)]-3.75H}_2\text{O} \) and \( \text{Na[Co(2,2-diMe-1,3-pdta)]-3.88H}_2\text{O} \) [19]. Similar to the analogous M(II)-1,3-pdta complexes, the preferred mode of coordination of 2,2-diMe-1,3-pdta is hexadentate in both six-coordinate \( \{\text{Mg(H}_2\text{O})_5\text{Ni(2,2-diMe-1,3-pdta)}\}-1.5\text{H}_2\text{O} \) [18], \( \text{Na[Cr(2,2-diMe-1,3-pdta)]-3.75H}_2\text{O} \) and \( \text{Na[Co(2,2-diMe-1,3-pdta)]-3.88H}_2\text{O} \) [19] and seven-coordinate \( \{\text{Ba}[M(2,2-diMe-1,3-pdta)]-3H_2O\}_n \) (\( M = \text{Mn(II)} \) or \( \text{Cd(II)} \)) [16]) complexes.

Magnesium(II) is one of the most abundant divalent metal cations in both prokaryotic and eukaryotic cells [20]. Recently, new interest on this cation arose due to its antimicrobial properties. Thus, it was found that antibiotic activity was enhanced in the presence of Mg(II) ion [21]. This enhancement was explained by the fact that the presence of Mg(II) affects the curvature of the bacterial membrane, increasing the vulnerability of bacteria and efficiency of the antibiotic. Moreover, it was demonstrated that Mg(II) and Ca(II) ions disrupt model \textit{Staphylococcus aureus} membranes and kill stationary-phase \textit{S. aureus} cells, indicating their membrane activity [22]. More recently, it was shown that surfaces coated with magnesium or its compounds are more effective in prevention of bacteria adherence, as well as biofilm formation [23].

Cobalt is also an element of biological interest. Its biological role is mainly focused on its presence in the active center of vitamin B\textsubscript{12}, which indirectly regulates the synthesis of DNA. Many cobalt(II) complexes have been reported to show activity against different microbial species [24–26]. Thus, \textit{in vitro} antibacterial activity of cobalt(II) complexes with a series of amino acids was evaluated against different Gram-positive and Gram-negative bacteria. The complexes with leucine and histidine are more active than the parent free ligands, while a moderate activity was observed for complexes with methionine and phenylalanine. However, lower antibacterial activity was observed for cobalt(II) complexes with lysine and valine [24]. Mixed ligand complexes of cobalt(II) with different thiosemicarbazones and N-phthaloyl derivative of D,L-glycine, L-alanine and L-valine against various bacterial and fungal strains showed better antimicrobial activity than the parent ligands [25]. Recent study of antibacterial activity of cobalt(II) complexes with the quinolone antimicrobial agent enrofloxacin showed that these complexes were more active than free enrofloxacin [26]. A remarkable
antifungal activity against Candida albicans was observed for the coordination polymer 
of cobalt(II) with indole-3-carboxylic acid, while only moderate antibacterial activity 
was shown by complexes of this metal ion with oxydiacetate anions [27,28].

Herein, we present a synthesis of six-coordinate octahedral [Co(H2O)5Co(2,2-diMe-1,3-
pdta)]·H2O (1) and [Mg(H2O)5Mg(2,2-diMe-1,3-pdta)]·1.5H2O (2) complexes and their 
characterization by elemental analysis, infrared spectroscopy and single-crystal X-ray 
diffraction analysis. Electronic absorption spectroscopy has also been applied for the 
characterization of 1, while NMR (1H and 13C) spectroscopy was used for the characterization of 2. The strain of 1 and 2 has been analyzed and compared with that for the 
previously reported [Mg(H2O)5Ni(2,2-diMe-1,3-pdta)]·1.5H2O [18], as well as for 
[M(H2O)6][M'(1,3-pdta)]·2H2O (M(II), M'(II) = Co, Co [4] and Mg, Mg [5]) complexes. The 
antimicrobial activity of 1 and 2 and their 1,3-pdta analogues has been investigated 
against four bacterial species and six Candida spp. and compared with their cytotoxic 
effects on the normal human lung fibroblasts (MRC-5).

2. Experimental

2.1. Materials and measurements

2,2-Dimethyl-1,3-propanediamine and chloroacetic acid were obtained from Acros 
Organics. All other common chemicals were of reagent grade and used without 
purification.

All pH measurements were performed at room temperature using the pH meter 
S220 SevenCompact™ pH/Ion, Mettler Toledo, which was calibrated with buffer solutions 
of pH 4.01 and 7.00. Elemental analyses for carbon, hydrogen and nitrogen were 
performed by the Microanalytical Laboratory, Faculty of Chemistry, University of 
Belgrade. IR spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrom-
eter using KBr pellets from 4000 – 450 cm−1. UV-Vis spectra were recorded at room 
temperature from 1100 – 200 nm on a Shimadzu double-beam spectrophotometer 
after dissolving 1 in water immediately after its dissolution. The concentration of the 
solution used for this measurement was 5.0 × 10−2 M. The NMR spectra of 2 were 
recorded on a Varian Gemini 2000 spectrometer at 200 MHz (1H) and 50 MHz (13C) 
using standard Varian software. 5.0 mg of the complex was dissolved in 0.6 mL of D2O 
and transferred into a 5 mm NMR tube. TSP (sodium 3-(trimethylsilyl)propionate) was 
used as the internal reference. Chemical shifts, σ, are expressed in ppm (parts per mil-
lion) and scalar couplings, J, are reported in Hz (Hertz).

2.2. Synthesis of Ba2(2,2-diMe-1,3-pdta)-2H2O

Barium(II) salt of 2,2-diMe-1,3-H4pdta acid, Ba2(2,2-diMe-1,3-pdta)-2H2O, was used as a 
ligand for the preparation of cobalt(II) and magnesium(II) complexes. This ligand was 
prepared using a previously established method in our laboratory for the preparation 
of diaminopolycarboxylate acids and their barium(II) salts [1, 17–19, 29–32]. The purity 
of Ba2(2,2-diMe-1,3-pdta)-2H2O, obtained from the solution containing a mixture of 
2,2-dimethyl-1,3-propanediamine (0.2 mol, 20.4 g) and chloroacetic acid (0.91 mol, 
86.0 g) in aqueous NaOH solution (0.91 mol, 36.4 g in 80 mL H2O) after addition of
solution of BaCl₂·2H₂O (0.4 mol, 97.7 g in 180 mL H₂O) was checked by elemental analysis and IR spectroscopy. The obtained data of the product from the above mixture are in accord with those previously reported for Ba₂(2,2-diMe-1,3-pdta)·2H₂O [17].

2.3. Synthesis of [Co(H₂O)₅Co(2,2-diMe-1,3-pdta)]·H₂O (1)

CoSO₄·7H₂O (0.7028 g, 2.5 mmol) was dissolved in 15 mL of H₂O at 70 °C. Solid Ba₂(2,2-diMe-1,3-pdta)·2H₂O (2.5 mmol, 1.6024 g) was added and the reaction mixture was heated at 70 °C with stirring for 30 min. The precipitated BaSO₄ was removed by filtration. Solid CoSO₄·7H₂O (2.5 mmol, 0.7028 g) was added to the dark pink filtrate obtained after removing BaSO₄ and the mixture was stirred with heating for 20 min at 60 °C. Deposited BaSO₄ was filtered off and volume of the filtrate was reduced to 5 mL. After cooling at room temperature, this solution was mixed with 5 – 6 mL of ethanol and the obtained mixture was allowed to stand in a refrigerator for several days at 4 °C. The dark pink crystals of [Co(H₂O)₅Co(2,2-diMe-1,3-pdta)]·H₂O were filtered off, washed with ethanol and air-dried. Yield: 0.9317 g (67%). Anal. Calcd. for [Co(H₂O)₅Co(2,2-diMe-1,3-pdta)]·H₂O (1) = C₁₃H₃₀Co₂N₂O₁₄ (MW = 556.25): C, 28.07; H, 5.44; N, 5.04. Found: C, 27.78; H, 5.35; N, 5.17%. IR (KBr, ν, cm⁻¹): 3230br, 2970w, 2928w, 2864w, 1614vs, 1483w, 1463w, 1442m, 1396s, 1340m, 1324m, 1306m, 1089m, 972m, 916m, 878w, 802m, 729s, 635w. UV-Vis (H₂O, λ_max, nm): 1072.0 (ε = 7.6 M⁻¹cm⁻¹), 581.0 (sh; ε = 6.0 M⁻¹cm⁻¹), 501.0 (ε = 16.1 M⁻¹cm⁻¹), 485.0 (ε = 16.3 M⁻¹cm⁻¹), 466.0 (sh; ε = 15.4 M⁻¹cm⁻¹).

2.4. Synthesis of [Mg(H₂O)₅Mg(2,2-diMe-1,3-pdta)]·1.5H₂O (2)

MgSO₄·7H₂O (1.2324 g, 5 mmol) was dissolved in 40 mL of H₂O. Solid Ba₂(2,2-diMe-1,3-pdta)·2H₂O (2.5 mmol, 1.6024 g) was added and the reaction mixture was stirred with heating for 9 h at 90 °C. During this time, the volume of the reaction mixture was kept at 40 mL by adding distilled water. Deposited BaSO₄ was filtered off and the volume of filtrate was reduced to 5 mL. After one week of standing at room temperature, colorless crystals of [Mg(H₂O)₅Mg(2,2-diMe-1,3-pdta)]·1.5H₂O were filtered off, washed with ethanol and air-dried. Yield: 0.8804 g (71%). Anal. Calcd. for [Mg(H₂O)₅Mg(2,2-diMe-1,3-pdta)]·1.5H₂O (2) = C₁₃H₃₁Mg₂N₂O₁₄.₅ (MW = 496.02): C, 31.48; H, 6.30; N, 5.65. Found: C, 31.25; H, 6.19; N, 5.78%. IR (KBr, ν, cm⁻¹): 3383br, 2963w, 2926w, 1619v, 1444m, 1414m, 1393s, 1339m, 1325m, 1306w, 1246w, 1090m, 919m, 802m, 726m, 631w.¹H NMR (200 MHz, D₂O): δ = 1.02 (s, 2CH₃), 2.72 (s, 2CH₂ protons of 1,3-propanediamine ring), 3.35 (AA'BB', 4CH₄ protons of carboxylate rings) ppm.¹³C NMR (50 MHz, D₂O): δ = 34.49 (C6 and C7), 40.65 (C2), 67.79 (C4 and C4'), 72.73 (C1 and C3), 184.39 (C5 and C5') ppm.

2.5. Crystallographic data collection and refinement of the structures

Single crystals of 1 and 2 were selected and mounted on a loop with inert oil on a Stoe STADIVARI diffractometer. The crystals were kept at 250(2) K during data collection. Using Olex2 [33], the structures were solved with the ShelXT [34] structure
solution program using Intrinsic Phasing and refined with the ShelXL [35] refinement package using Least Squares minimization. Hydrogen atom positions were calculated geometrically and refined using the riding model. Crystal data and details of the structure determinations are given in Table S1. CCDC 2170842-2170843 contains the supplementary crystallographic data for this article. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via https://www.ccdc.cam.ac.uk/data_request/cif.

### 2.6. Assessment of antimicrobial properties

Minimal inhibitory concentration (MIC) values of the complexes and metal salts used for their synthesis were determined using standard broth microdilution assays, recommended by the National Committee for Clinical Laboratory Standards (M07-A8) for bacteria and Standards of European Committee on Antimicrobial Susceptibility Testing (v 7.3.1: Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts) for Candida spp. The tested bacterial strains included Staphylococcus aureus NCTC (National Collection of Type Cultures) 6571, Staphylococcus aureus ATCC (American Type Culture Collection) 43300 (MRSA), Pseudomonas aeruginosa NCTC 10662 and Klebsiella pneumoniae ATCC BAA 2146, while tested Candida strains included C. albicans ATCC 24433, C. albicans ATCC 10231, C. parapsilosis ATCC 22019, C. krusei ATCC 6258, C. glabrata ATCC 2001 and C. auris ATCC 21092. All analyzed compounds were dissolved in DMSO at a final concentration of 50 mM, and the highest tested concentration was 500 μM. The inoculums were 5 × 10^5 colony-forming units, cfu/mL, for bacteria and 1 × 10^5 cfu/mL for Candida species. The MIC value was recorded as the lowest concentration that completely inhibited the growth after 24 h at 37 °C using the Epoch Microplate Spectrophotometer, BioTek Instruments, Inc.

### 2.7. Anti-biofilm activity assessment on C. glabrata ATCC 2001

An anti-biofilm assay was conducted using the previously published methodology [36]. Briefly, the assay was carried out in 96-well round-bottom polystyrene microtiter plates. Cells were harvested from overnight grown cultures, washed twice with sterile phosphate-buffered saline (PBS; Sigma-Aldrich, Munich, Germany), and resuspended in RPMI 1640 medium (Sigma-Aldrich) containing 2% glucose (w/v) to give a final concentration of 1 × 10^5 cfu/mL. Candida suspension was incubated with compounds (concentrations started from MIC and lower, six dilutions in total) in 200 μL final volume per well for 48 h at 37 °C to allow biofilm formation. Biofilm growth was analyzed by crystal violet (CV) staining of adherent cells and the absorbance at 590.0 nm was read on an Epoch Microplate Spectrophotometer, BioTek Instruments, Inc.

### 2.8. Cytotoxicity

In vitro cytotoxicity was determined as an antiproliferative activity by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [37] on human lung
fibroblasts (MRC-5) obtained from American Type Culture Collection (ATCC). The cells, cultured in the complete RPMI 1640 medium as a monolayer (1 × 10^4 cells per well), were incubated with the investigated compounds at concentrations ranging from 1 to 250 μM, in a humidified atmosphere of 95% air and 5% CO₂ at 37 °C, and the cell viability was measured after 48 h. The extent of MTT reduction was measured spectrophotometrically at 540.0 nm using the Epoch Microplate Spectrophotometer, BioTek Instruments, Inc., and cell survival was expressed as a percentage of the control (DMSO treated cells) arbitrarily set to 100%. Cytotoxicity is expressed as the concentration of the compound inhibiting growth by 50% (IC₅₀).

3. Results and discussion

The analogue of diaminopolycarboxylate 1,3-propanediamine-N,N,N',N'-tetraacetate (1,3-pdta), 2,2-dimethyl-1,3-propanediamine-N,N,N',N'-tetraacetate (2,2-diMe-1,3-pdta) contains two methyl substituents at the central carbon of the 1,3-propanediamine chain. Recently, we have shown that this modification of 1,3-propanediamine chain reflects on the structural properties of the corresponding metal(II) complexes (metal(II) = Ni, Mn and Cd) [17,18].

As continuation of this study, herein we used barium(II) salt of 2,2-diMe-1,3-H₄pdta acid, Ba₂(2,2-diMe-1,3-pdta)₂H₂O, as a ligand for preparation of [Co(H₂O)₅Co(2,2-diMe-1,3-pdta)]·H₂O (1) and [Mg(H₂O)₅Mg(2,2-diMe-1,3-pdta)]·1.5H₂O (2). The reaction between CoSO₄·7H₂O (or MgSO₄·7H₂O) and Ba₂(2,2-diMe-1,3-pdta)·2H₂O in 2 : 1 molar ratio leads to formation of these complexes in good yields. Characterization of 1 and 2 was done by elemental analysis, infrared spectroscopy and single-crystal X-ray diffraction analysis. Additionally, electronic absorption spectroscopy has been applied for the characterization of 1, while NMR (¹H and ¹³C) spectroscopy was used for the characterization of 2.

3.1. Solid state studies

3.1.1. Crystal structures of 1 and 2

Complexes 1 and 2 crystallize in the same space group C2/c with almost the same lattice parameters. The structures of 1 and 2 are displayed in Figure 1. The bond lengths and angles for 1 and 2 are given in Table S2. The asymmetric unit of 1 consists of complex unit [Co(H₂O)₅Co(2,2-diMe-1,3-pdta)] and one non-coordinated water molecule. The asymmetric unit of 2 consists of complex unit [Mg(H₂O)₅Mg(2,2-diMe-1,3-pdta)] and one and a half non-coordinated water molecules. Similarly as in previously reported [Mg(H₂O)₅Ni(2,2-diMe-1,3-pdta)]·1.5H₂O complex [18], the complex units [M(H₂O)₅M'(2,2-diMe-1,3-pdta)] (M, M' = Co(II), Co(II) (1) and Mg(II), Mg(II) (2)) are
composed of \([\text{M}'(2,2\text{-diMe}-1,3\text{-pdta})]^2^-\) and \([\text{M}(\text{H}_2\text{O})_3\text{M}(2,2\text{-diMe}-1,3\text{-pdta})]\)^2+ octahedra bridged by one of the axial carboxylate groups. Bridging of the complex cation and anion is further supported by a hydrogen bond between one coordinated water molecule and an oxygen from bridging carboxylate group. Geometrical parameters describing hydrogen bond interactions are provided in Table S3.

To examine the impact of substitution of the hydrogen atoms with methyl groups from the central 1,3-propanediamine carbon of the ligand backbone on the strain of \(M\)-1,3-pdta system, we performed a strain analysis of related series of \([\text{M}'(1,3\text{-pdta})]^2^-\) and \([\text{M}'(2,2\text{-diMe}-1,3\text{-pdta})]^2^-\) \((\text{M}' = \text{Mg(II)}, \text{Co(II)} \text{and Ni(II)})\) complexes. The major contributions to strain are considered to be: 

(i) the octahedral angles around the metal ion, 
(ii) the ring angle sums of the various types of rings, 
(iii) the \(\text{M}–\text{O}–\text{C}\) bond angles, and 
(iv) the bond angles that the chelating nitrogen atom makes with its connectors.

The results of strain analysis are given in Table 1. In general, the \([\text{M}'(2,2\text{-diMe}-1,3\text{-pdta})]^2^-\) complexes show greater octahedral strain than the corresponding complexes of the 1,3-pdta ligand, as indicated by the calculated \(\sum \Delta(O_h)\) values for the series of Mg(II), Co(II) and Ni(II) complexes. As expected, the five-membered glycinate rings of the G type \((\text{girdle}, \text{or in-plane with respect to the diamine ring})\) are much more strained than those of the R type \((\text{relaxed}, \text{or out-of-plane with respect to the diamine ring})\). For the \([\text{M}'(1,3\text{-pdta})]^2^-\) complexes, the total deviation from the ideal chelate ring bond angle sum \((538.5°)\) is \(+1°\) and from \(-11°\) to \(-10°\) for the R and G type of the glycinate rings, respectively. For the \([\text{M}'(2,2\text{-diMe}-1,3\text{-pdta})]^2^-\) complexes, the total deviation from the ideal chelate ring bond angle sum is from \(-2°\) to \(-1°\) and from \(-14°\) to \(-13°\) for the R and G rings, respectively, indicating the greater strain in the corresponding glycinate rings of \([\text{M}'(2,2\text{-diMe}-1,3\text{-pdta})]^2^-\) system compared to that of \([\text{M}'(1,3\text{-pdta})]^2^-\). Important source of strain in this kind of chelates is bonding geometry made by the chelating nitrogen atoms. Each N atom makes four bonds with six ideally 109.5° bond angles. The total deviation about the chelating N atoms in M(II)-
1,3-pdta complexes is 11° for Ni(II) and Co(II) complexes and 13° for Mg(II), and for M(II)-2,2-diMe-1,3-pdta complexes, this deviation is 18° for Ni(II) and Co(II) and 21° for Mg(II) complex.

### 3.1.2. Infrared (IR) spectra of cobalt(II) and magnesium(II) complexes

IR carboxylate stretching frequencies for 1 and 2 are summarized in Table S4 and are compared with those for Co(II) [4] and Mg(II) [5] complexes with 1,3-pdta ligand. Both 1 and 2 show one very strong and sharp band at 1614 and 1618 cm⁻¹, respectively, indicating that all carboxylate groups of 2,2-diMe-1,3-pdta ligand in these complexes are coordinated [38]. These bands are almost identical in the shape and absorption intensity, and they do not show any tendency for splitting. However, in the same region, one very strong and broad band (1587 and 1597 cm⁻¹) with clear tendency for splitting on the lower energy side (1675 and 1687 cm⁻¹) was observed for cobalt(II) and magnesium(II) complexes with 1,3-pdta ligand, respectively (Table S4). The part of the IR spectra due to the symmetric stretching vibrations of the coordinated carboxylate groups for 1 and 2 is slightly complex compared to those for cobalt(II) and magnesium(II) complexes with 1,3-pdta. Based on the abovementioned facts, it can be concluded that the difference between carboxylate stretching frequencies of cobalt(II) and magnesium(II) complexes with 2,2-diMe-1,3-pdta and those for the complexes of these metal ions with 1,3-pdta might result from the presence of two methyl groups at the central carbon atom of 1,3-propanediamine ring of the 2,2-diMe-1,3-pdta complexes.

### 3.2. Solution studies

#### 3.2.1. NMR (¹H and ¹³C) spectroscopy of magnesium(II) complexes

The ¹H NMR spectra of magnesium(II) complexes with 2,2-diMe-1,3-pdta and 1,3-pdta ligands, [Mg(H₂O)₅Mg(2,2-diMe-1,3-pdta)]·1.5H₂O (2) and [Mg(H₂O)₆][Mg(1,3-
pdta]-2H2O [5], were recorded in D2O. The spectrum of 2 shows signals of one well-resolved AB pattern centered at 3.35 ppm, which belongs to the methylene protons from two pairs of equivalent R (out-of-plane) and G (in-plane) glycinate rings ($J = 16.76 \text{ Hz}$). This finding is opposite to those previously reported for the cobalt(III) complexes with hexadentate 2,2-diMe-1,3-pdta and 1,3-pdta ligands which showed two well-resolved AB patterns belonging to two pairs of nonequivalent R and G glycinate rings [19,39]. However, for the methylene glycinate protons of the previously synthesized [Mg(H2O)6][Mg(1,3-pdta)]·2H2O complex, only one very broad signal at 3.32 ppm was observed in the 1H NMR spectrum.

The 13C NMR spectra of 2 and [Mg(H2O)6][Mg(1,3-pdta)]·2H2O are shown in Figure S1 and corresponding numerical data are given in Table S5. The crystal structure of the latter complex was previously reported [5] and herein the synthesis of this complex was repeated to compare its 13C NMR spectrum with that for 2. In respect to the spectrum of [Mg(H2O)6][Mg(1,3-pdta)]·2H2O, all signals in that of 2 are shifted downfield. The largest shifting was observed for carbon atoms of 1,3-propanediamine ring (C1/C3 72.73 and 59.94, C2 40.65 and 25.52 ppm for 2,2-diMe-1,3-pdta and 1,3-pdta, respectively; see Figure S1 and Table S5), what is consequence of the presence of two methyl groups attached at the central carbon atom of 2,2-diMe-1,3-pdta ligand. Only one signal was observed for the methylene carbon of glycinate rings for both complexes. Only one signal for the methylene carbon of glycinate rings was also observed in the spectrum of [Co(2,2-diMe-1,3-pdta)]+ complex, while two signals for these carbon atoms appeared in the spectrum of [Co(1,3-pdta)]+ complex due to its two pairs of nonequivalent axial (R) and equatorial (G) glycinate rings [19]. Difference in the 13C NMR spectra between [Mg(1,3-pdta)]2− and [Co(1,3-pdta)]− could result from different size of Mg(II) and Co(III) ions.

### 3.2.2. Electronic absorption spectra of cobalt(II) complexes

The electronic absorption spectrum of 1 is compared with that previously reported for [Co(H2O)6][Co(1,3-pdta)]·2H2O [4] (Figure 2). The corresponding numerical data of these two complexes are compared with those for [Co(H2O)4Co(edta)]·2H2O, all having C2 symmetry and N2O4 chromophore of the ligand field, as well as with those for octahedral [Co(H2O)6]3+ complex [40] with $O_h$ symmetry assigned as $^4T_{1g} \rightarrow ^4T_{2g}$, $^4T_{1g} \rightarrow ^4A_{2g}$ and $^4T_{1g} \rightarrow ^4T_{1g}$ (P) (Table 2).

As can be seen from this table, the absorption spectrum of [Co(H2O)6]2+ exhibits lower multiplicity in comparison with those for [Co(H2O)4Co(edta)]·2H2O [27], [Co(H2O)6][Co(1,3-pdta)]·2H2O [4] and 1. However, the spectra of the latter two complexes showed lower band separation than that of [Co(H2O)4Co(edta)]·2H2O. This can be explained by the fact that the less strained [Co(H2O)6][Co(1,3-pdta)]·2H2O and 1, with flexible Co(II)-1,3-propanediamine six-membered ring, are much closer to the ideal octahedral configuration than [Co(H2O)4Co(edta)]·2H2O containing more strained five-membered Co(II)-ethylenediamine ring. However, as can be seen from Figure 2, the electronic absorption spectra of [Co(H2O)6][Co(1,3-pdta)]·2H2O [4] and 1 are slightly different in the shape of the second absorption band, whereas [Co(H2O)6][Co(1,3-pdta)]·2H2O showed higher tendency for the splitting of this band on the lower energy side (shoulder at 581.0 nm, Table 2). Also, clear band separation was observed for the second absorption maximum of 1 in comparison with that for
The differences in the electronic absorption spectra of \([\text{Co(1,3-pdta)}]_2\text{H}_2\text{O}\) and \([\text{Co(2,2-diMe-1,3-pdta)}]_2\text{H}_2\text{O}\) result from the presence of hexadentate 1,3-pdta and its 2,2-diMe-1,3-pdta structural analogue, respectively.

### 3.3. Antimicrobial properties of cobalt(II) and magnesium(II) complexes

There is a need for new antimicrobial compounds to combat the spread of antibiotic resistance. Metal complexes are currently used for treatment of different diseases, with many of them investigated as potential antimicrobial therapeutics \[41,42\]. In this study, the antimicrobial potentials of \(1\) and \(2\), the metal salts used for their synthesis and cobalt(II) and magnesium(II) complexes with 1,3-pdta ligand \[4,5\] were evaluated against two Gram-positive bacteria \(\text{Staphylococcus aureus}\) and methicillin-resistant \(S. \text{ aureus}\) (MRSA), two Gram-

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**Figure 2.** Electronic absorption spectra of \([\text{Co(H}_2\text{O)}_6][\text{Co(1,3-pdta)}]_2\text{H}_2\text{O}\) \[4\] and \([\text{Co(H}_2\text{O)}_5\text{Co(2,2-diMe-1,3-pdta)}]_2\text{H}_2\text{O}\) \(1\) complexes measured in water \((c = 5.0 \times 10^{-2} \text{ M})\).
negative bacteria *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, and six *Candida* spp., including two *C. albicans*, *C. parapsilosis*, *C. glabrata*, *C. krusei* and *C. auris* (Table 3). Overall, increased activity of complexes against a range of *Candida* spp., in comparison to the tested bacterial strains, was observed (Table 3). From all investigated complexes, 2 shows the most activity against *Candida* spp., with MIC of 3.9 μM. Also, this complex manifested 2-8 fold higher antifungal activity in comparison to [Mg(H$_2$O)$_6$][Mg(1,3-pdta)]·2H$_2$O, which might result from the presence of two methyl groups at the central carbon atom of 1,3-propanediamine ring of 2. This implies that structural modification of 1,3-propanedimine also reflects on the antimicrobial properties of the corresponding metal complexes. Furthermore, in respect to 1 and [Co(H$_2$O)$_6$][Co(1,3-pdta)]·2H$_2$O, both 2 and [Mg(H$_2$O)$_6$][Mg(1,3-pdta)]·2H$_2$O showed better antifungal activity, what can be a consequence of the presence of Mg(II) ion in the last two complexes [20–23].

Contrary to the presently reported results, chromium(III) and cobalt(III) complexes, Na[Cr(2,2-diMe-1,3-pdta)]·3.75H$_2$O and Na[Co(2,2-diMe-1,3-pdta)]·3.88H$_2$O, did not inhibit the growth of the tested microorganisms, even when the concentration of 500 μM was applied [19]. From these results, it can be concluded that the oxidation state of metal ion has an influence on the antimicrobial activity of the complexes with 2,2-diMe-1,3-pdta ligand. Thus, metal(II) complexes with this ligand show remarkable and selective antifungal activity, while the analogue metal(III) species penetrate through the microbial cell wall less effectively, resulting in a decrease of antimicrobial activity.

The cytotoxic effect of the investigated compounds against human lung fibroblasts (MRC-5) was assessed. Low toxicity is observed for cobalt(II) and magnesium(II) salts as well as 1, while complex 2 and 1,3-pdta complexes showed high toxic effects against MRC-5 cells (Table 3). This further implies that 1 with a positive value of selectivity index (a ratio between IC$_{50}$ and MIC values) against the tested *Candida* species, especially *C. glabrata*, could be considered as an antifungal therapeutic.

### 3.4. Anti-biofilm activity of cobalt(II) and magnesium(II) complexes

Antimicrobial resistance is especially connected with the presence of biofilms, which represent micro-structured consortia in which microbial cells are enclosed in a self-

| Strain                  | 1    | 2     | [Co(H$_2$O)$_6$][Co(1,3-pdta)]·2H$_2$O | [Mg(H$_2$O)$_6$][Mg(1,3-pdta)]·2H$_2$O | CoSO$_4$·7H$_2$O | MgSO$_4$·7H$_2$O |
|-------------------------|------|-------|--------------------------------------|---------------------------------------|----------------|----------------|
| *S. aureus* NCTC 6571   | >500 | >500  | >500                                 | >500                                  | >500           | >500           |
| *S. aureus* MRSA ATCC 43300 | >500 | >500  | >500                                 | >500                                  | >500           | >500           |
| *P. aeruginosa* NCTC 10662 | >500 | >500  | >500                                 | >500                                  | >500           | >500           |
| *K. pneumoniae*         | >500 | >500  | >500                                 | >500                                  | >500           | >500           |
| ATCC BAA2146            |      |       |                                      |                                       |                |                |
| *C. albicans* ATCC 24433 | 250  | 3.9   | 15.6                                 | 7.8                                   | >500           | 250            |
| *C. albicans* ATCC 10231 | 250  | 3.9   | >500                                 | 31.2                                  | >500           | 125            |
| *C. parapsilosis*       | 62.5 | 3.9   | >500                                 | 15.6                                  | >500           | 250            |
| ATCC 22019              |      |       |                                      |                                       |                |                |
| *C. krusei* ATCC 6258   | 31.2 | 3.9   | 61.5                                 | 15.6                                  | >500           | 62.5           |
| *C. glabrata* ATCC 2001  | 3.9  | 3.9   | 31.2                                 | 7.8                                   | >500           | 15.6           |
| *C. auris* ATCC 21092   | 150  | >500  | 250                                  | >500                                  | >500           | 500            |
| MRC-5 cells             | >250 | 2.6   | 1.2                                  | 2.4                                   | >250           | >250           |
produced extracellular polymeric matrix composed of carbohydrates, proteins and extracellular DNA. The concentration of microbial cells in biofilms is 100 – 1000 times higher than in planktonic phases, and in this form, microbes commonly show up to 1000 times higher resistance to clinically used antimicrobial agents [43,44].

The ability of 1 and 2, the metal salts used for their synthesis and cobalt(II) and magnesium(II) complexes with 1,3-pdta ligand [4,5] to inhibit the formation of C. glabrata ATCC 2001 biofilms was assessed (Figure 3). As can be seen from this figure, 2 shows a slightly higher efficiency in the anti-biofilm activity in comparison to 1. The highest inhibition percentage was 66% for 2 in a concentration of 0.98 μM and the comparable effect was reached from 3.9 to 1.95 μM, while for 1, it was 68% in a concentration of 3.9 μM (Figure 3a). In comparison to this, no inhibition of the biofilm formation was observed for [Mg(H2O)6][Mg(1,3-pdta)2H2O] complex at concentrations equal or lower to MIC values, while [Co(H2O)6][Co(1,3-pdta)2H2O] causes 45% biofilm inhibition at almost 10-fold higher concentration in comparison to 1 and 2 (31.2 μM; Figure 3b). From these results, it can be drawn that the introduction of two methyl
substituents at a central 1,3-propanediamine carbon atom significantly increases the inhibitory activity against microorganisms in both planktonic and biofilm forms.

4. Conclusion

We have demonstrated that coordination of 2,2-diMe-1,3-pdta ligand, which differs from 1,3-pdta by containing two methyl substituents at the central 1,3-propanediamine carbon atom, with Co(II) and Mg(II) ions leads to [M(H2O)5M’(2,2-diMe-1,3-pdta)] complexes (M, M’ = Co, Co (1) and Mg, Mg (2)), which differ in the number of free water molecules. Crystallographic analysis revealed that [M(H2O)5M’(2,2-diMe-1,3-pdta)] is composed of [M’(2,2-diMe-1,3-pdta)]2− and [M(H2O)5O]2+ octahedra bridged by one of the axial carbonylate groups. This complex unit is different from [M(H2O)6][M’(1,3-pdta)] obtained by coordination of Co(II) and Mg(II) ions with 1,3-pdta. Both hexadentate 2,2-diMe-1,3-pdta and 1,3-pdta ligands are very effective in stabilization of Co(II) in water solutions and in the presence of air. The strain analysis indicated by the calculated $\sum \Delta(O_n)$ values for a series of [M’(1,3-pdta)]2− and [M’(2,2-diMe-1,3-pdta)]2− complexes (M’ = Mg(II), Co(II) and Ni(II)) has shown that all 2,2-diMe-1,3-pdta complexes have greater octahedral strain than the corresponding 1,3-pdta complexes. The structural diversification between [M’(1,3-pdta)]2− and [M’(2,2-diMe-1,3-pdta)]2− was also confirmed by spectroscopic measurements for the corresponding cobalt(II) and magnesium(II) complexes. The structural difference between 1,3-pdta and 2,2-diMe-1,3-pdta complexes is attributed to the presence of two methyl groups at the central carbon atom of 1,3-propanediamine chain of the 2,2-diMe-1,3-pdta ligand. Complexes 1 and 2 showed increased activity against a range of Candida spp. in comparison to the tested bacterial strains, with most potent antifungal activity of 2. Also, this complex shows a slightly higher efficiency in the anti-biofilm activity in comparison to complex 1. Nevertheless, 1 has a positive value of selectivity index against the tested Candida species, especially C. glabrata, and could be further considered as potential antifungal agent.

Disclosure statement

No potential conflict of interest was reported by the authors.

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