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Novel methylene bridged ethylenediamine-type ligands: synthesis and spectral characterization

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Abstract: The synthesis of two new organic compounds, diisobutyl- and diisopentyl (*S*,*S*)-α[1],α[3]-bis(cyclohexylmethyl-1,3-imidazolidinediacetate is reported herein. The one-pot procedure was realized by the addition of the reducing agent and carbonyl compound into a methanolic solution of the parent compounds (isobutyl and isopentyl esters of (*S*,*S*)-α,α′-(1,2-ethanediyldiimino)bis[cyclohexanepropanoic acid] in appropriate stoichiometric ratios. The compounds were fully characterized by infrared, ESI-MS, 1D (^{1}H and ^{13}C) and 2D (COSY, HSQC and HMBC) NMR spectroscopy and elemental analysis. The spectral data confirmed the presence of the $–CH_2–$ group introduced between the nitrogen atoms of the ethylenediamine moiety, revealing a neutral form of the potential bidentate ligand.

Keywords: cyclohexyl derivatives; ethylenediamine; amine ligands; potential drugs.

INTRODUCTION

The chemistry of anticancer agents generally refers to pure organic species[1] and metal containing compounds.[2] After the promising discovery of cisplatin by Rosenberg in 1965,[3] the subsequent intensive research in the field of bioinorganic chemistry has resulted in the achievement of only slight progress.[4] Nowadays, the use of metal-based drugs is limited to cisplatin and its analogs, carboplatin and oxaliplatin.[5] It is also noteworthy to mention the Ru(III) complexes, NAMI-A (imidazolium *trans*-[tetrachloro(*S*-dimethyl sulfoxide)(1*H*-imidazole)ruthenate(III)]) and KP1019 (indazolium [*trans*-tetrachlorobis(1*H*-indazole)ruthenate(III)])[6] that have both entered phase II of clinical trials. Therefore, an ideal drug in terms of being extremely toxic and simultaneously highly selective is yet to be developed.

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Although a major part of pharmaceutical industry is based on organic and biologically derived species, the synthetic routes still precede over natural products.[7,8] The main reason is found in the opportunity to create a potential drug with the desired profile of biologic activity. Moreover, it is also possible to functionalize parent compounds by various structural modifications in order to utilize or expand their primary use. The structures of recently synthesized compounds with confirmed antitumor activity are given in Fig. S-1 of the Supplementary material to this paper.

Based on the above-mentioned concept, the synthesis and cytotoxic studies of (*S*,*S*)-α,α′-(1,2-ethanediyldiimino)bis[cyclohexanepropanoic acid] and (*S*,*S*)-α,α′-(1,3-propanediyldiimino)bis[cyclohexanepropanoic acid] joined with their corresponding esters and Pt(IV) and Ru(II) complexes were previously reported.[9–13] The obtained results demonstrated strong antitumor potential and, in some cases, even better activity compared to that of the conventional cisplatin. The mechanism of cytotoxic activity for (*S*,*S*)-α,α′-(1,2-ethanediyldiimino)bis[cyclohexanepropanoate] was particularly investigated, revealing induced apoptosis associated with oxidative stress, mitochondrial depolarization and nuclear translocation of the apoptosis-inducing factor.[14]

Since each study confirmed the positive influence of the variable length of the alkyl side chain on biological features (the longer the alkyl chain, the more active the compound),[11] two derivatives with the bulky alkyl chain of (*S*,*S*)-α,α′-(1,2-ethanediyldiimino)bis[cyclohexanepropanoic acid] were additionally synthesized and fully characterized. Hence, the synthesis and characterization of diisobutyl- and diisopentyl (*S*,*S*)-α[1],α[3]-bis(cyclohexylmethyl-1,3-imidazolidinediacetate are reported herein. The structures of these two compounds are given in Fig. 1.

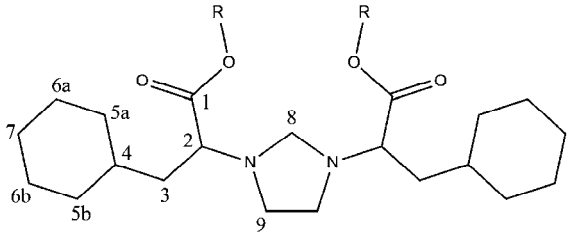

Fig. 1. The structure of the synthesized compounds, **C1** and **C2**.

EXPERIMENTAL

Reagents and instruments

The reagents and solvents were purchased from commercial suppliers and used without further purification. The precursor substances, isobutyl and isopentyl esters of (*S*,*S*)-α,α′-(1,2-ethanediyldiimino)bis[cyclohexanepropanoate] were synthesized starting from (*S*)-2-amino-

-3-cyclohexylpropanoic acid hydrochloride purchased from Senn Chemicals (Dielsdorf, Switzerland). The preparation method was previously described and published.[9,12]

Elemental analyses were performed on an Elemental Vario EL III microanalyzer. A Nicolet 6700 FT-IR spectrometer and the ATR technique were used for recording the infrared spectra. 1D (^{1}H and ^{13}C), 2D COSY (correlation spectroscopy) and 2D ^{1}H–^{13}C heteronuclear correlation spectra were recorded using a Bruker Avance III 500 spectrometer in $CDCl_3$ with TMS as the reference. The mass spectra were obtained with an Orbitrap LTQ XL instrument (Thermo Scientific, Bremen, Germany) in methanol.

Synthetic procedures

*Synthesis of the diisobutyl (*S,S*)-α^1,α^3-bis(cyclohexylmethyl-1,3-imidazolidinediacetate,* ***C1***. A suspension of the precursor, diisobutyl (*S,S*)-α,α′-(1,2-ethanediyldiimino)bis[cyclohexanepropanoate] dihydrochloride (0.2 g, 0.36 mmol) in methanol (10 mL) was mixed and heated up to 40 °C for 20 min on a steam bath until the mixture was well homogenized. The methylation mixture was made by dissolving sodium triacetoxyborohydride (0.23 g, 1.08 mmol) in methanol (10 mL) followed by the addition of 36 % aqueous formaldehyde (0.10 mL, 3.61 mmol). The obtained methylation solution was poured dropwise into the previously made suspension. In order to adjust the pH value to 4–5, glacial acetic acid (0.25 mL) was slowly added to the reaction mixture. The next portion of the same volume was added after 2 h and stirring was continued for the following 30 min. The whole reaction solution was washed out with diethyl ether (40 mL) followed by rinsing the ether extract with three equal portions of KOH solution (10 mL, 1 M) and a portion of brine (10 mL). The combined ether solutions were dried overnight using anhydrous K_2CO_3 and evaporated *in vacuo* to obtain a colorless oil.

*Synthesis of diisopentyl (*S,S*)-α^1,α^3-bis(cyclohexylmethyl-1,3-imidazolidinediacetate,* ***C2***. A suspension of the precursor, diisopentyl (*S,S*)- α,α′-(1,2-ethanediyldiimino)bis[cyclohexanepropanoate] dihydrochloride (0.2 g, 0.34 mmol) in methanol (10 mL) was mixed and heated at 40 °C for 20 min on a steam bath until the mixture was well homogenized. The methylation mixture was made by dissolving sodium triacetoxyborohydride (0.22 g, 1.03 mmol) in methanol (10 mL) followed by the addition of 36 % aqueous formaldehyde (0.09 mL, 3.44 mmol). The further procedure involved the same treatment as for **C1**.

The analytic and spectral data for **C1** and **C2** are given in the Supplementary material to this paper.

RESULTS AND DISCUSSION

Synthesis. The reductive methylation of diisobutyl- and diisopentyl esters of (*S,S*)-α,α′-(1,2-ethanediyldiimino)bis[cyclohexanepropanoic acid] was typical[15,16] with slight modifications. The precursor ester was well homogenized in methanol. The mixture for methylation containing 36 % aqueous formaldehyde and sodium triacetoxyborohydride dissolved in methanol was slowly added to the ligand solution, which was then stirred for the following 2 h. The pH value was adjusted using glacial acetic acid. The obtained solution was treated with appropriate amounts of diethyl ether, 1 M KOH and brine. After drying the ether solutions overnight, the compounds were obtained by evaporation *in vacuo*. The synthesized compounds were soluble in common organic solvents (ethanol, dimethyl

sulfoxide, diethyl ether, acetone, dichloromethane and chloroform) but insoluble in water.

Spectroscopic studies. The new compounds, **C1** and **C2**, were characterized by mass spectrometry, infrared spectroscopy and one- (^{1}H and ^{13}C) and two--dimensional homo and heteronuclear (^{1}H/^{1}H-COSY, HSQC and HMBC) NMR spectroscopy. Elemental analyses data were in a good agreement with the corresponding composition of the synthesized compounds.

The IR spectra of **C1** and **C2** showed strong bands at ≈1730 cm^{-1} assigned to the vibrations of the carbonyl group.[17] Two strong bands originating from asymmetric C–H stretching vibrations were found at ≈2930 and ≈2850 cm^{-1}. In addition, C–O stretching vibrations occurred around 1250 cm^{-1}, while the C–N group exhibited a weak absorption at around 1165 cm^{-1}.

The proposed structures of compounds **C1** and **C2** were also confirmed by their mass spectra, which indicated to a $[M+H]^+$ peak matched with the calculated molecular mass and proper isotope pattern. Furthermore, high intensities were also observed for peaks assigned to the $[M–CH_2+3H]^+$ fragment.

The ^{1}H-NMR spectrum of the products (given in the Supplementary material to this paper) indicated on the presence of cyclohexyl moiety arising in the area between 0.7–1.9 ppm with the exception of protons bonded to C5. Specifically, they appeared in a form of two separate sets of signals as their diastereotopic nature originates from the chiral C atom. Ethylenediamine protons were detected between 2.84 and 2.98 ppm in a form of two multiplets, as can be seen in Fig. 2,

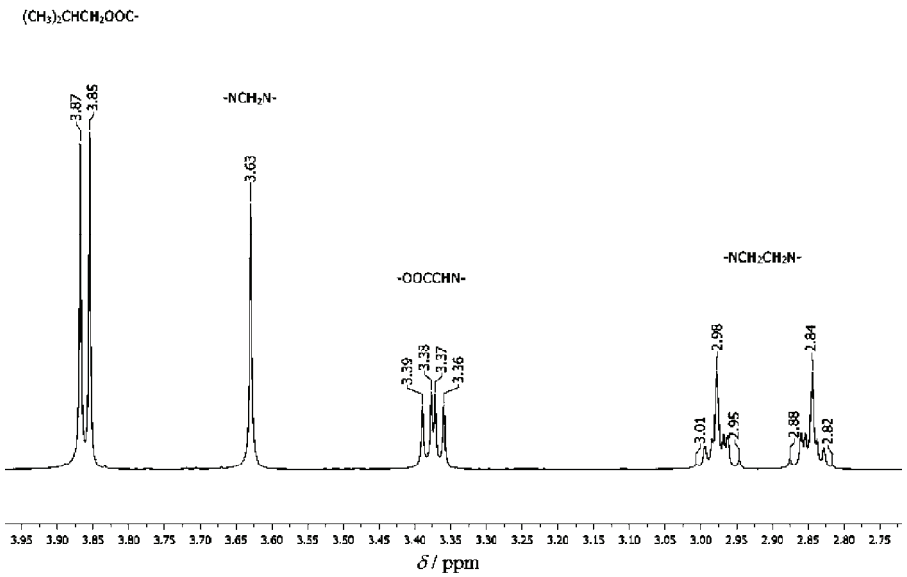

Fig. 2. ^{1}H-NMR spectrum of compound **C1** in the region 2.70–4.00 ppm recorded in $CDCl_3$.

which shows the ^{1}H-NMR spectrum of compound **C1** in the region 2.70–4.00 ppm. The multiplet located at ≈3.40 ppm was assigned to (ROOC)CH– protons. The presence of methylene bridged protons (–NCH_2N–) was confirmed in the form of a singlet (3.63 ppm) that showed negative correlation with the carbon resonance at 69.55 ppm in the edited HSQC spectrum. The same signal was correlated to carbons at 48.23 (–NCH_2CH_2N–) and 62.05 ppm (–OOCCHN–) in the HMBC spectrum, additionally confirming the newly formed imidazolidine ring. The ^{13}C-NMR spectra of **C1** and **C2**, the HSQC NMR spectrum of **C1** and the COSY spectrum of **C2** are given in the Supplementary material to this paper.

CONCLUSIONS

The primary structure of the compounds derived from cyclohexyl edda derivatives enables various structural modifications in ethylenediamine and alkyl chain moiety. In this sense, novel methylated forms of (*S*,*S*)-α,α′-(1,2-ethanediyldiimino)bis[cyclohexanepropanoic acid] which contained isobutyl and isopentyl groups were synthesized. The main reason for extending the alkyl side chain we found in the fact that more bulky molecules show significantly better antitumor activity which is planned to be investigated.

SUPPLEMENTARY MATERIAL

Analytic and spectral data for **C1** and **C2** and Figs. S-1–S-7 are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

НОВИ МЕТИЛОВАНИ ЛИГАНДИ ЕТИЛЕНДИАМИНСКОГ ТИПА: СИНТЕЗА И КАРАКТЕРИЗАЦИЈА

ЉИЉАНА Е. МИХАЈЛОВИЋ-ЛАЛИЋ, АЛЕКСАНДАР САВИЋ, ГАБРИЈЕЛА БРАЂАН, ТИБОР Ј. САБО и САЊА ГРГУРИЋ-ШИПКА

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Описана је синтеза два нова органска једињења, диизобутил- и диизопентил-(*S*,*S*)-α^1,α^3-бис(циклохексилметил-1,3-имидазолидиндиацетат, која су добијена у реакцији изобутил и изопентил естара (*S*,*S*)-α,α′-(1,2-етандиилдиимино)бис[циклохексанпропанске киселине] са погодним метилујућим агенсом и формалдехидом у одговарајућем стехиометријском односу. Синтетисана једињења су потпуно окарактерисана инфрацрвеном, ESI-MS, 1D (^{1}H и ^{13}C) and 2D (COSY, HSQC и HMBC) NMR спектроскопијом и елементалном анализом. Спектрални подаци су потврдили присуство –CH_2– групе уведене између два атома азота етилендиаминског дела, представљајући потенцијалне бидентатне лиганде.

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