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Synthesis, characterization, crystal structure, and antimicrobial studies of 2-morpholinoethyl-substituted benzimidazolium salts and their silver(I)-*N*-heterocyclic carbene complexes

Işıl Yıldırım[1] · Aydın Aktaş[1] · Duygu Barut Celepci[2] · Sevda Kırbağ[3] · Türkan Kutlu[1] · Yetkin Gök[1] · Muhittin Aygün[2]

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Abstract This article describes synthesis of *N*-morpholinoethylbenzimidazole (**1**), 2-morpholinoethyl-substituted benzimidazolium salts (NHC precursors, **2a**–**c**), and their Ag(I) *N*-heterocyclic carbene (NHC) complexes (**3a**–**c**). All compounds were characterized by ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR), Fourier Transform Infrared Spectroscopy (FTIR), and elemental analysis, and their antimicrobial effects examined. The molecular structure of the NHC precursor **2a**[1] was established by single-crystal X-ray diffraction analysis. Minimum Inhibition Concentration (MIC) values were determined to evaluate their antimicrobial activity against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* bacterial strains and *Candida albicans* fungal species. All tested samples were compared with silver nitrate. All the compounds exhibited strong antimicrobial activity.

Keywords *N*-Heterocyclic carbene · Ag–NHC complexes · Morpholine · Antimicrobial activity · Antibiotic

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✉ Işıl Yıldırım
isilyld@hotmail.com

[1] Department of Chemistry, Faculty of Science, Inonu University, 44280 Malatya, Turkey

[2] Department of Physics, Faculty of Science, Dokuz Eylül University, 35160 İzmir, Turkey

[3] Department of Biology, Faculty of Science, Fırat University, 23200 Elazığ, Turkey

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Introduction

Many silver(I) complexes containing nitrogen, oxygen, phosphorus, sulfur, and *N*-heterocyclic carbene (NHC) donor ligands have been evaluated as potential antifungal and antibacterial agents against different Gram-positive and Gram-negative bacteria. Many medicinal applications of Ag–NHC complexes are known, especially showing activity as antimicrobials [1–3]. Aromatic heterocyclic ligands (NHC) containing nitrogen atoms have attracted attention for synthesis of antimicrobial silver complexes [1–7]. NHC precursors are very important ligands in bioorganic and coordination chemistry and have found application in the rapidly developing field of metallosupramolecular chemistry [8, 9].

Although the antimicrobial activity of Ag compounds has been known since ancient times, the antimicrobial effects of 2-morpholinoethyl-substituted Ag(I)NHC compounds remain unknown. Therefore, we aimed to synthesize, characterize, and determine the crystal structure of a new series of 2-morpholinoethyl-substituted benzimidazole (**1**), benzimidazolium salts (**2a–c**), and their respective Ag(I)–NHC (**3a–c**) complexes. We also investigated their in vitro antimicrobial activity against Gram-positive bacterium *Staphylococcus aureus*, Gram-negative bacterium *Escherichia coli*, and *Candida albicans* fungal species.

Method

Reagents and instruments

The chemicals used in this work including 2-morpholinoethyl chloride, potassium hydroxide, 1-bromopropane, 2-bromopropane, 3-bromo-1-propanol, diethyl ether, dimethyl sulfoxide (DMSO), chloroform ($CHCl_3$), dichloromethane (DCM), dimethylformamide (DMF), silver oxide (Ag_2O), and argon gas as well as Schlenk flask etc. were purchased from Sigma Aldrich, abcr, and Merck. All other reagents were obtained commercially from Aldrich and used without further purification. Melting points were determined in glass capillaries under air using an Electrothermal-9200 melting point apparatus. FTIR spectra were recorded from KBr pellets in the range of 400–4000 cm^{-1} on an AT, UNICAM 1000 spectrometer. Proton (^1H) and carbon (^{13}C) NMR spectra were recorded using a Varian AS 300 Merkur spectrometer operating at 300 MHz (^1H) or 75 MHz (^{13}C) in $CDCl_3$ and DMSO-d_6 with tetramethylsilane as internal reference. The Inonu University Scientific and Technology Center performed elemental analyses. Gram-positive bacterium *Staphylococcus aureus* Cowan 1, Gram-negative bacterium *Escherichia coli* ATCC 25922, and *Candida albicans* FMC 17 fungal strains were used to evaluate antimicrobial activity. The strains were obtained from Fırat University Faculty of Science Department of Biology Microbiology Laboratory culture collections. As medium, Merck mark, Mueller–Hinton broth medium was used. Studies were performed in 96-well plates. The study was performed in Fırat University Department of Biology Microbiology Laboratory.

Synthesis of N-2-morpholinoethylbenzimidazole (***1***) This known compound was synthesized and characterized based on analysis of m.p., IR, ^{1}H and ^{13}C NMR, and micro analyses, showing results consistent with literature [10].

Synthesis of 1-isopropyl-3-(2-morpholinoethyl)benzimidazolium bromide (***2a***) Compound **2a** was synthesized from *N*-morpholinoethylbenzimidazole (1.16 g, 5 mmol) with 2-bromopropane (0.62 g, 5 mmol) in dry DMF (4 ml) using the Schlenk technique. Then, it was refluxed for 24 h at 80 °C in oil bath. Upon cooling to room temperature, dry ether was added to the reaction mixture. Then, the solvents were evaporated under vacuum to afford the product as white solid. The crude product was recrystallized from ethyl alcohol/diethyl ether (1:3) at room temperature. Yield: 1.38 g (78%). M.p.: 276–278 °C. $\nu_{(CN)}$: 1558.5 cm^{-1}. Anal. Cald. for $C_{16}H_{24}BrN_3O$: C: 54.24; H: 6.78; N: 11.86. Found C: 54.21; H: 6.74; N: 11.90. ^{1}H NMR (300 MHz, D_2O) in δ ppm: 1.61 (d, 6H, –CH(C***H***$_3$)$_2$, *J*: 9.0 Hz); 2.89 (s, 4H, –NC***H***$_2$CH$_2$O–); 3.25 (t, 2H, –NCH$_2$C***H***$_2$–NC$_4$H$_8$O, *J*: 6.0 Hz); 3.75 (t, 4H, –NCH$_2$C***H***$_2$O–, *J*: 4.5 Hz); 4.72 (t, 2H, –NC***H***$_2$CH$_2$–NC$_4$H$_8$O, *J*: 7.5 Hz); 4.96 (m, 1H, –NC***H***(CH$_3$)$_2$); 7.62–7.91 (m, 4H, Ar–***H***), 9.40 (s, 1H, 2-C***H***). ^{13}C NMR (300 MHz, D_2O) in δ ppm: 21.0 (–CH(***C***H$_3$)$_2$); 42.3 (–NC***H***$_2$CH$_2$O–); 51.1 (–NCH$_2$***C***H$_2$–NC$_4$H$_8$O); 52.3 (–N***C***H$_2$CH$_2$–NC$_4$H$_8$O); 55.1 (–N***C***H(CH$_3$)$_2$); 65.1 (–NCH$_2$***C***H$_2$O–); 112.8, 113.8, 126.8, 127.1, 130.9, 131.2 (Ar–***C***); 139.4 (2-***C***).

Synthesis of 1-isopropyl-3-(2-morpholinoethyl)benzimidazolium dibromide (***2a^1***) 2-Bromopropane for synthesis of **2a** compound was not commercially available and was synthesized by reaction of hydrogen bromide with isopropyl alcohol. **2a^1** compound was synthesized by reaction of 2-bromopropane synthesized by this method with *N*-(2-morpholinoethyl)benzimidazole. The spectroscopic results for the synthesized compound **2a^1** indicated presence of hydrogen bromide in the structure together with the salt structure. Finally, when the single crystal was obtained, the salt structure was completely uncovered. Compound **2a** was then resynthesized by reaction of 2-bromopropane with *N*-(2-morpholinoethyl)benzimidazole when 2-bromopropane was commercially available. Unfortunately, we could not obtain appropriate single crystals of compound **2a** for X-ray diffraction analysis.

The ^{1}H NMR spectrum of the resulting single crystals of **2a^1** was found to be different from that of the first compound: ^{1}H NMR (300 MHz, $CDCl_3$-d_6) in δ ppm: 2.55 (m, 6H, –CH(C***H***$_3$)$_2$); 2.89 (s, 4H, –NC***H***$_2$CH$_2$O–); 3.15 (s, 2H, –NCH$_2$C***H***$_2$–NC$_4$H$_8$O); 3.65 (s, 4H, –NCH$_2$C***H***$_2$O–); 4.41 (s, 2H, –NC***H***$_2$CH$_2$–NC$_4$H$_8$O); 4.84 (s, 1H, –NC***H***(CH$_3$)$_2$); 7.23–8.51 (m, 4H, Ar–***H***); 9.18 (s, 1H, 2-C***H***); 13.30 (s, 1H, –N–***H***–Br).

Synthesis of 1-(2-morpholinoethyl)-3-propylbenzimidazolium bromide (***2b***) Compound **2b** was synthesized in the same way as described for **2a**, but using 1-bromopropane (0.62 g, 5 mmol) instead of 2-bromopropane. Yield: 1.66 g (94 %). M.p.: 158–160 °C. $\nu_{(CN)}$: 1557.5 cm^{-1}. Anal. Cald. for $C_{16}H_{24}BrN_3O$: C: 54.24; H: 6.78; N: 11.86. Found: C: 54.38; H: 6.91; N: 11.94. ^{1}H NMR (300 MHz, $CDCl_3$) in δ ppm: 0.99 (t, 3H, –CH$_2$CH$_2$C***H***$_3$, *J*: 7.5 Hz); 2.06 (m, 2H, –CH$_2$C***H***$_2$CH$_3$); 2.57 (s, 4H, –NC***H***$_2$CH$_2$O–); 2.91 (t, 2H, –NC***H***$_2$CH$_2$–NC$_4$H$_8$O,

J: 6.0 Hz); 3.58 (s, 4H, –NCH_2CH_2O–); 4.53 (t, 2H, –$CH_2CH_2CH_3$, *J*: 7.5 Hz); 4.78 (t, 2H, –NCH_2CH_2–NC_4H_8O, *J*: 4.5 Hz); 7.59–7.80 (m, 4H, Ar–***H***); 11.10 (s, 1H, 2-C***H***). ^{13}C NMR (300 MHz, $CDCl_3$) in δ ppm: 11.0 (–$CH_2CH_2CH_3$); 22.7 (–$CH_2CH_2CH_3$); 44.1 (–NCH_2CH_2O–); 48.9 (–$CH_2CH_2CH_3$); 53.4 (–NCH_2CH_2–NC_4H_8O); 56.2 (–NCH_2CH_2–NC_4H_8O); 66.8 (–NCH_2CH_2O–); 113.1, 113.2, 127.1, 127.2, 131.1, 131.3 (Ar–***C***); 143.3 (2-C).

*Synthesis of 1-(3-hydroxypropyl)-3-(2-morpholinoethyl)benzimidazolium bromide (**2c**)* Compound **2c** was synthesized in the same way as described for **2a**, but using 3-bromo-1-propanol (0.7 g, 5 mmol) instead of 2-bromopropane. Yield: 1.40 g (75 %). M.p.: 238–240 °C. $\nu_{(CN)}$: 1562.8 cm^{-1}. Anal. Cald. for $C_{16}H_{24}BrN_3O_2$: C: 51.90; H: 6.53; N: 11.35. Found: C: 51.94; H: 6.45; N: 11.40. ^{1}H NMR (300 MHz, $CDCl_3$) in δ ppm: 2.13 (s, 2H, –$CH_2CH_2CH_2OH$); 2.58 (s, 4H, –NCH_2CH_2O–); 2.94 (s, 2H, –NCH_2CH_2–NC_4H_8O); 3.61 (s, 2H, –NCH_2CH_2–NC_4H_8O); 3.61 (s, 2H, –$NCH_2CH_2CH_2OH$); 3.61 (s, 2H, –$NCH_2CH_2CH_2OH$); 4.74 (s, 4H, –NCH_2CH_2O–); 5.20 (s, 1H, –$CH_2CH_2CH_2OH$); 7.58–7.76 (m, 4H, Ar–***H***); 10.94 (s, 1H, 2-C***H***). ^{13}C NMR (300 MHz, $CDCl_3$) in δ ppm: 31.0 (–$CH_2CH_2CH_2OH$); 44.1 (–NCH_2CH_2–NC_4H_8O); 44.5 (–NCH_2CH_2O–); 53.4 (–NCH_2CH_2–NC_4H_8O); 56.3 (–$NCH_2CH_2CH_2OH$); 56.9 (–$CH_2CH_2CH_2OH$); 66.6 (–NCH_2CH_2O–); 113.1, 113.3, 127.1, 131.0, 131.4 (Ar–***C***); 144.0 (2-***C***).

*Synthesis of bromo[1-isopropyl-3-(2-morpholinoethyl)benzimidazol-2-ylidene]silver(I) (**3a**)* Solution of 1-isopropyl-3-(2-morpholinoethyl)benzimidazolium bromide (0.5 g, 1.4 mmol), Ag_2O (0.16 g, 0.7 mmol), and activated 4-Å molecular sieves in DCM (20 mL) was stirred at room temperature for 24 h in the dark. The reaction mixture was filtered through Celite, and solvent removed under vacuum. The crude product was recrystallized from dichloromethane/hexane at room temperature. Yield: 0.59 g (91 %). M.p.: 188–190 °C. $\nu_{(CN)}$: 1478.1 cm^{-1}. Anal. Cald. for $C_{16}H_{23}AgBrN_3O$: C: 41.67; H: 5.03; N: 9.11. Found C: 41.75; H: 5.08; N: 9.18. ^{1}H NMR (300 MHz, DMSO-d_6) in δ ppm: 1.67 (d, 6H, –$CH(CH_3)_2$, *J*: 9.0 Hz); 2.43 (m, 4H, –NCH_2CH_2O–); 2.73 (t, 2H, –NCH_2CH_2–NC_4H_8O, *J*: 6.0 Hz); 3.55 (m, 4H, –NCH_2CH_2O–); 4.55 (t, 2H, –NCH_2CH_2–NC_4H_8O, *J*: 6.0 Hz); 5.09 (m, 1H, –$NCH(CH_3)_2$); 7.41–7.93 (m, 4H, Ar–***H***). ^{13}C NMR (300 MHz, DMSO-d_6) in δ ppm: 22.5 (–$CH(CH_3)_2$); 46.5 (–NCH_2CH_2O–); 53.5 (–NCH_2CH_2–NC_4H_8O); 53.6 (–$NCH(CH_3)_2$); 57.7 (–NCH_2CH_2–NC_4H_8O); 66.1 (–NCH_2CH_2O–); 112.2, 112.6, 123.7, 124.0, 132.2, 133.5 (Ar–***C***); 188.5 (2-***C***).

*Synthesis of bromo-[1-(2-morpholinoethyl)-3-propylbenzimidazol-2-ylidene] silver(I) (**3b**)* Compound **3b** was synthesized in the same way as described for **3a**, but using 1-(2-morpholinoethyl)-3-propylbenzimidazolium bromide (0.5 g, 1.4 mmol) instead of 1-isopropyl-3-(2-morpholinoethyl)benzimidazolium bromide. Yield: 0.49 g (75 %). M.p.: 259–261 °C. Anal. Cald. for $C_{16}H_{23}AgBrN_3O$: C: 41.67; H: 5.03; N: 9.11. Found: C: 41.73; H: 5.06; N: 9.16. $\nu_{(CN)}$: 1452.1 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) in δ ppm: 0.89 (t, 3H, –$CH_2CH_2CH_3$, *J*: 6.0 Hz); 1.89 (m, 2H, –$CH_2CH_2CH_3$); 2.44 (s, 4H, –NCH_2CH_2O–); 2.75 (t, 2H, –NCH_2CH_2–NC_4H_8O, *J*: 6.0 Hz); 3.53 (t, 4H, –NCH_2CH_2O–, *J*: 4.5 Hz); 4.46 (t, 2H, –$CH_2CH_2CH_3$, *J*: 6.0 Hz); 4.58 (t, 2H, –NCH_2CH_2–NC_4H_8O, *J*: 6.0 Hz);

7.42–7.83 (m, 4H, Ar–***H***). ^{13}C NMR (300 MHz, DMSO-d_6) in δ ppm: 11.1 (–CH_2CH_2***C***H_3); 23.2 (–CH_2***C***H_2CH_3); 45.9 (–***C***$H_2CH_2CH_3$); 49.9 (–NCH_2***C***H_2–NC_4H_8O); 53.6 (–N***C***H_2CH_2O–); 57.8 (–N***C***H_2CH_2–NC_4H_8O); 66.1 (–NCH_2***C***H_2O–); 112.0, 112.1, 123.8, 133.1, 133.3 (Ar–***C***); 189.4 (2-***C***).

*Synthesis of bromo-[1-(3-hydroxypropyl)-3-(2-morpholinoethyl)benzimidazol-2-ylidene] silver(I) (**3c**)* Compound **3c** was synthesized in the same way as described for **3a**, but using 1-(3-hydroxypropyl)-3-(2-morpholinoethyl)benzimidazolium bromide (0.52 g, 1.4 mmol) instead of 1-isopropyl-3-(2-morpholinoethyl)benzimidazolium bromide. Yield: 0.52 g (78 %). M.p.: 214–216 °C. Anal. Cald. for $C_{16}H_{23}AgBrN_3O_2$: C: 40.28; H: 4.86; N: 8.81. Found: C: 40.32; H: 4.89; N: 8.75. $\nu_{(CN)}$: 1443.6 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) in δ ppm: 2.00 (m, 2H, –CH_2C***H***$_2CH_2OH$); 2.44 (s, 4H, –NC***H***$_2CH_2O$–); 2.75 (t, 2H, –NCH_2C***H***$_2$–NC_4H_8O, *J*: 6.0 Hz); 3.42 (t, 2H, –NC***H***$_2CH_2CH_2OH$, *J*: 6.0 Hz); 3.53 (t, 4H, –NCH_2C***H***$_2O$–*J*: 6.0 Hz); 4.55 (m, 2H, –NCH_2CH_2C***H***$_2OH$); 4.75 (s, 1H, –$NCH_2CH_2CH_2O$***H***); 7.43–7.84 (m, 4H, Ar–***H***). ^{13}C NMR (300 MHz, DMSO-d_6) in δ ppm: 32.9 (–CH_2***C***H_2CH_2OH); 44.9 (–NCH_2***C***H_2–NC_4H_8O); 45.7 (–N***C***H_2CH_2O–); 53.5, 57.1 (–N***C***H_2CH_2–NC_4H_8O); 57.4 (–N***C***$H_2CH_2CH_2OH$); 57.8 (–NCH_2CH_2***C***H_2OH); 66.1 (–NCH_2***C***H_2O–); 112.0, 112.1, 113.6, 113.7, 123.8, 126.6, 133.1, 133.4 (Ar–*C*); 188.5 (2-*C*).

Crystal structure determination

Single-crystal X-ray diffraction data for **2a**[1] were collected at room temperature on a Rigaku-Oxford Xcalibur diffractometer with an Eos Charge Coupled Device (CCD) detector using graphite-monochromated Mo K_α radiation ($\lambda = 0.71073$ Å) with CrysAlis^{Pro} software [11]. Data reduction and analytical absorption correction were performed by CrysAlis^{Pro} program [12]. Utilizing OLEX2 [13], the structure was solved by direct methods with SHELXT [14] and refined by full-matrix least squares on F^2 in SHELXL [15]. Anisotropic thermal parameters were applied to all nonhydrogen atoms. Methyl H atoms (C–H = 0.96 Å), methylene H atoms (C–H = 0.97 Å), and aromatic H atoms (C–H = 0.93 Å) were positioned geometrically. Hydrogen atoms attached to carbon of benzimidazole ring and nitrogen were found in the difference map and refined freely.

Crystallographic details for 2a[1]

$C_{16}H_{25}Br_2N_3O$, $M = 435.21$, colorless prismatic, $0.48 \times 0.26 \times 0.19$ mm^3, orthorhombic, space group *Pbca*, $a = 13.0956(9)$ Å, $b = 12.1176(5)$ Å, $c = 22.8729(13)$ Å, $V = 3629.6(4)$ Å^3, $Z = 8$, $D_{calc} = 1.593$ g/cm^3, $F(000) = 1760$, $T = 294(2)$ K, 7272 reflections collected, 3425 unique ($R_{int} = 0.034$). Final goodness-of-fit = 1.001 $R_1 = 0.040$, $wR_2 = 0.069$, R indices based on 2346 reflections with $I \geq 2\sigma(I)$ (refinement on F^2). 216 parameters, 0 restrains, $\mu = 4.472$ mm^{-1}.

Antimicrobial activity

Microorganism culture preparation and inoculation

Bacterial strains *Staphylococcus aureus* Cowan 1 and *Escherichia coli* ATCC 25922 were inoculated in nutrient broth and incubated at 35 ± 1 °C for 24 h. Yeast *Candida albicans* FMC 17 was inoculated in yeast malt extract broth and incubated at 25 ± 1 °C for 48 h. After density adjustment according to McFarland standard (0.5), growing cultures in media were inoculated at 1 % in culture broth then kept in refrigerator at +4 °C. The number of microorganisms in 1 ml was calculated as 10^6/ml for bacteria and 10^4/ml for yeast.

Preparation of medium

Mueller–Hinton broth (5.5 g) was weighed and diluted to 250 ml, then the density was adjusted according to McFarland standard (0.5).

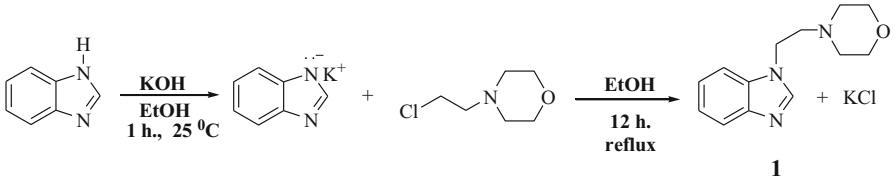
Minimal inhibitory concentration (MIC) method

Each synthesized compound or standard drug was diluted to 2000 µg/ml concentration as stock solution. DMSO solvent was used as diluent. Three separate 96-well plates were used (one for each type of microorganism), to which 100 µl medium was added. Then, 200 µl (i.e., 400 mg/ml) of stock solution was taken and added to the medium in the first well by pipette. From the first well, 200 µl was extracted and added to the second well, and so on to obtain dilution. Then, 100 µl culture was added to each plate. The final concentrations obtained were therefore 66.66, 44.4, 29.6, 19.75, 13.15. 8.75, and 5.85 µg/ml. Plates were incubated at 37 °C for 24 h, after which the microbial growth was observed for each each sample. The concentration with greatest turbidity on each plate was taken as an indication of the minimum inhibitory concentration. As positive control, $AgNO_3$ was used.

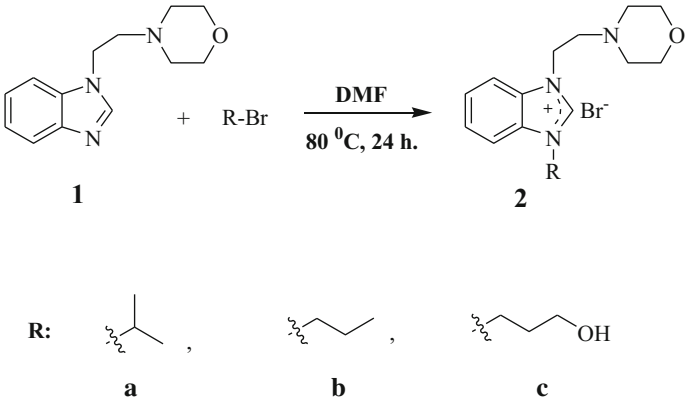
Results and discussion

Synthesis

Reaction of benzimidazole with 2-morpholinoethyl chloride resulted in formation of *N*-morpholinoethylbenzimidazole (**1**) (Scheme 1). The ^{1}H NMR spectrum of **1**



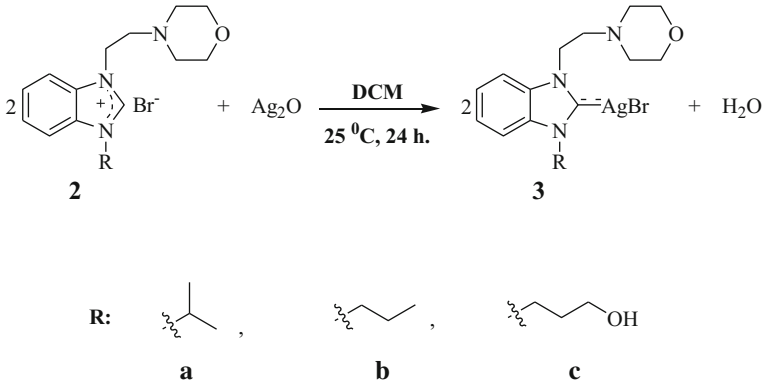
Scheme 1 Synthesis of *N*-morpholinoethylbenzimidazole (**1**)



Scheme 2 Synthesis of 2-morpholinoethyl-substituted NHC precursors (**2a–c**)

in $CDCl_3$ displayed the 2-CH proton at 7.97 ppm. The benzimidazolium salts **2a–c** were prepared by reaction of alkyl bromide (alkyl: *iso*-propyl, *n*-propyl, and 1-hydroxypropyl) with *N*-morpholinoethylbenzimidazole, respectively (Scheme 2). The reaction mixture was stirred in dimethylformamide overnight at 80 °C. After evaporation of solvent under vacuum, the oily product was washed with diethyl ether. The crude product was recrystallized from dichloromethane:diethyl ether (1:2) at room temperature. The compounds were obtained as white solid in very good yield (75–94 %). The ^{1}H NMR spectra of **2a–c** in $CDCl_3$ displayed the 2-CH protons at 9.40, 11.10, and 10.94 ppm, respectively. In the ^{13}C NMR spectra, the 2-C signals appeared at 139.4, 143.3, and 143.9 ppm for **2a**, **b**, and **c**, respectively.

Ag(I)–NHCs **3a–c** were prepared by reaction of Ag_2O with the benzimidazolium salts **2a–c** under stirring in dichloromethane for 24 h at room temperature in the dark (Scheme 3). The reaction mixture was then filtered through Celite, and the solvents were evaporated under vacuum to afford the product as white solid. The crude product was recrystallized from dichloromethane:diethyl ether (1:3) at room temperature. Absence of a downfield benzimidazolium proton (NC***H***N) signal and an upfield shift for all the signals were observed with respect to the ligand precursors in the ^{1}H NMR spectra of complexes **3a–c** in DMSO-d_6, indicating successful complex formation. In the ^{13}C NMR spectra of the silver complexes, resonances due to carbene carbon (N***C***N) atoms appeared as singlet peaks at 185.9, 189.4, and 188.5 ppm, respectively. These signals were shifted more toward the downfield region compared with the NHC precursors **2a–c**. This finding further demonstrates formation of the expected complexes. This signal shift is in accordance with literature reports [16–19].



Scheme 3 Synthesis of 2-morpholinoethyl-substituted Ag(I)NHC complexes (**3a**–**c**)

FTIR spectral studies

The IR spectrum for complex **2a** shows peaks at 500–600 cm^{-1} for C–Br stretching vibration, peak at 1200 cm^{-1} for C–N bond, peak at around 1400–1600 cm^{-1} for aromatic structure, and peak at 1558.5 cm^{-1} for benzimidazole C–N. Cross peaks for C=C and C=N groups are observed at 1600–1690 cm^{-1}. The peak at 2800 cm^{-1} corresponds to C–H, while the peak at around 3500–3700 cm^{-1} corresponds to NH stretching vibration. The IR spectrum for **2b** shows a peak at 600 cm^{-1} for C–Br stretching vibration, and peak at 1200 cm^{-1} for C–N bond. The peak observed at around 1300 cm^{-1} corresponds to C–H bending vibration, while the aromatic structure peak is seen at 1600 cm^{-1}, and the benzimidazole C–N peak at 1557.5 cm^{-1}. –N=C=N stretching vibration is seen at 2800 cm^{-1}. The peak at 2800 cm^{-1} corresponds to C–H. The peak at around 3500–3700 cm^{-1} corresponds to N–H stretching vibration. The IR spectrum for **2c** shows a peak for C–Br stretching vibration at 500–600 cm^{-1}, peak at 1200 cm^{-1} for C–N bond, peak at around 1400–1600 cm^{-1} for aromatic structure, and benzimidazole C–N peaks at around 1562.8 cm^{-1}. It also shows peaks between 1600 and 1690 cm^{-1} for C=C and C=N groups. The peak at 2800 cm^{-1} corresponds to C–H bonds, and the peak at 3500 cm^{-1} corresponds to N–H stretching vibration. The O–H stretching vibration peak at 3600 cm^{-1} corresponds to propanol group.

The IR spectrum for complex **3a** shows C–Br stretching vibration peak between 500 and 600 cm^{-1}, signals in the silver region of 720–850 cm^{-1}, peak at 1200 cm^{-1} for C–N bond, peak at around 1400–1600 cm^{-1} for aromatic structure, and benzimidazole C–N peaks at 1478.1 cm^{-1}. The peak at 2800 cm^{-1} corresponds to C–H, while the peak at around 3500–3700 cm^{-1} corresponds to N–H stretching vibration. The IR spectrum for complex **3b** shows a peak at 500–600 cm^{-1} for C–Br stretching vibration, signals at 720–850 cm^{-1} in the Ag region, and a peak at 1200 cm^{-1} for C–N bond. The peak at around 1300 cm^{-1} corresponds to C–H bending vibration, that at 1600 cm^{-1} to aromatic structure, and that at 1452.1 cm^{-1} to benzimidazole C–N. The peaks at around 2800 cm^{-1} correspond to C–H bond. The peaks in the vicinity of 3500 cm^{-1} correspond to N–H stretching vibration. The IR spectrum for complex **3c** shows peak at 500–600 cm^{-1} for C–Br stretching vibration, signals in the Ag region at 720–850 cm^{-1}, and a peak at 1200 cm^{-1} for C–N bond, while the peaks at 1400–1600 cm^{-1} correspond to the aromatic structure. Benzimidazole C–N peaks are seen at 1443.6 cm^{-1}. The peaks observed at 2800 cm^{-1} correspond to C–H bond. The peaks in the vicinity of 3500 cm^{-1} correspond to N–H stretching vibration. The peak at 3600 cm^{-1} corresponds to O–H stretching vibration.

Structural studies

Crystals suitable for determination of the molecular structure of $\mathbf{2a^1}$ by X-ray crystallography were obtained by slow diffusion of diethyl ether into saturated dichloromethane solution. The ORTEP [20] diagram for $\mathbf{2a^1}$ is depicted in Fig. 1a, and selected geometric parameters are presented in Table 1. The benzimidazole ring is almost planar with maximum deviation of 0.010(2) Å for atom C2, and links to the morpholine ring via $–CH_2–CH_2–$ bridge, in which the N2–C11–C12–N1 unit adopts a *gauche* conformation with torsion angle of 78.2(4)°. Also, the morpholine ring has chair conformation with puckering amplitude $q_2 = 0.019(2)$ Å, $\varphi_2 = 356.7(2)°$ [21]. Bond distances in the morpholine ring are comparable to literature [22–24]. The crystal structure of $\mathbf{2a^1}$ is stabilized by intermolecular N–H···Br and C–H···Br hydrogen bonds (Table 2, Fig. 1b). A strong hydrogen bond is observed between the acidic proton of the imidazolium cation and the bromide anion (Br1): The N–H···Br ionic bond angle is 170° with N–H and H···Br distance of 0.97 [25, 26] and 2.29 Å, respectively. Br2 connects the molecules as an acceptor to form an infinite One-Dimensional (1D) zigzag chain structure along *a*-axis.

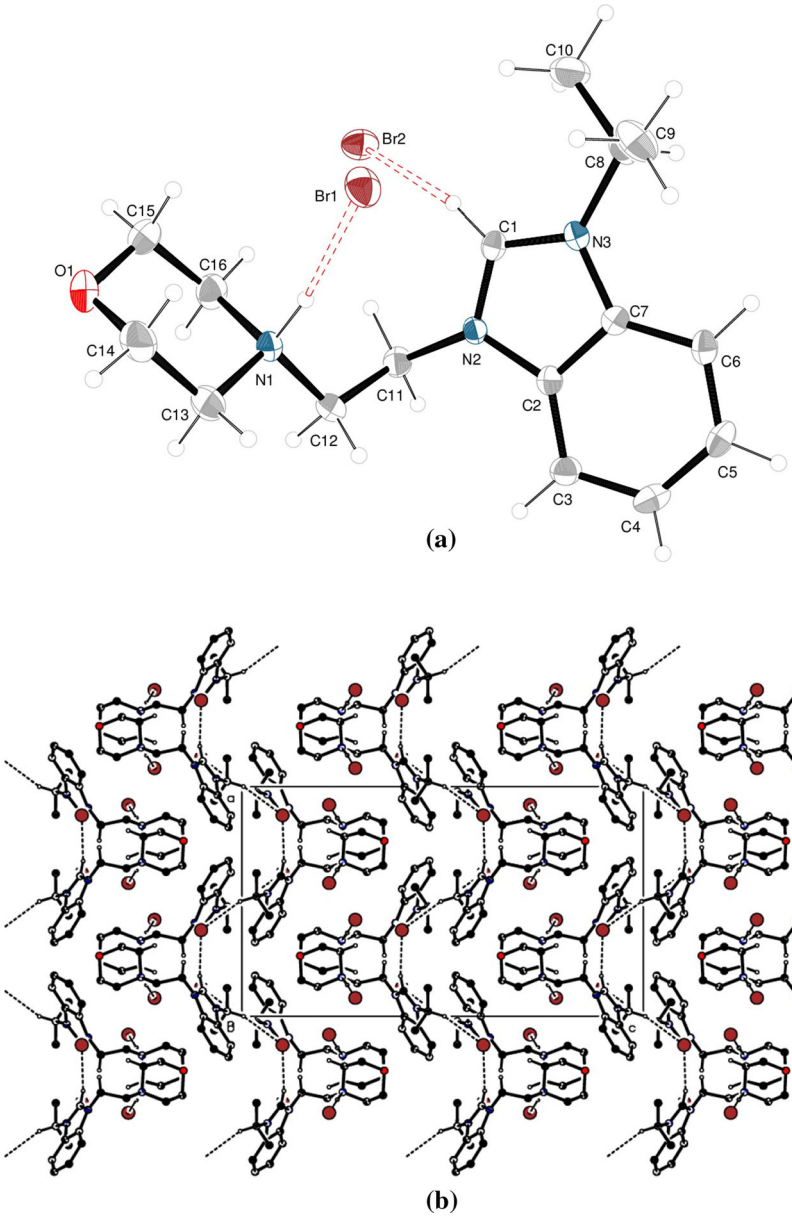


Fig. 1 **a** ORTEP view of **2a**[1] with 30 % probability level ellipsoids and **b** packing view (*b*-axis projection) of the compound

Table 1 Selected bond lengths (Å) and angles (°) for **2a**[1]

N1–C12	1.513(5)	N1–C13	1.497(5)
N1–C16	1.498(5)	N2–C1	1.340(4)
N2–C2	1.400(4)	N2–C11	1.468(4)
N3–C1	1.321(4)	N3–C7	1.401(4)
N3–C8	1.489(4)	O1–C14	1.411(5)
O1–C15	1.413(5)		
C1–N2–C11	125.1(3)	C1–N2–C2	108.1(3)
C2–N2–C11	126.3(3)	C1–N3–C8	126.9(3)
C13–N1–C12	109.1(3)	C16–N1–C12	113.5(3)
C16–N1–C13	109.1(3)		

Table 2 Hydrogen-bonding geometry (Å, °) for **2a**[1]

D–H···A	D–H	H···A	D···A	D–H···A
N1–H1···Br1	0.97	2.29	3.2515(2)	170
C1–H1A···Br2	0.93	2.66	3.4774(2)	147
C8–H8···Br2^{i}	0.98	2.84	3.7324(3)	151
C11–H11B···Br2	0.97	2.88	3.7688(3)	153
C16–H16B···Br2	0.97	2.92	3.8847(3)	177

Symmetry code (i): $-1/2 + x$, $1/2 - y$, $-z$

Antimicrobial activity

A study published in 2015 described synthesis of benzimidazole-based Ag(I)–NHC complexes and investigation of their antibacterial activity against Gram-positive bacterium *Staphylococcus aureus* (ATCC 12600) and Gram-negative bacterium *Escherichia coli* (ATCC 25922). It was concluded that these complexes inhibited both bacteria noticeably [27].

In their studies, Kalinowska-Lis et al. determined the antimicrobial activity of derivatives of benzimidazole Ag(I)–NHC complexes against three Gram-negative pathogens: *Pseudomonas aeruginosa* ATCC 15442, *Escherichia coli* ATCC 25922, and *Proteus hauseri* ATCC 1331 using the MIC method. They concluded that complex 5 was the most effective against *E. coli* with values of 21 μM, while complex 1 was least effective against *E. coli* with values of 145 μM. Moreover, complexes 2, 3, 4, and 5 were very effective when compared with $AgNO_3$ and Silver Sulfadiazine (AgSD). In addition, complex 2 was most effective against *P. hauseri* with values of 41 μM. Complex 5 exhibited the highest antibacterial activity against *P. aeruginosa* with values of 21 μM. Complexes 2, 4, and 5 were more active against *P. aeruginosa* when compared with $AgNO_3$ and AgSD as reference compounds [28].

A publication by Sarı and coworker reported the antimicrobial activity of novel benzimidazolium-based Ag(I)–NHC complexes. The results showed that, while complex 3 was especially effective against *E. coli*, *P. aeruginosa*, *S. aureus*, and *Enterococcus faecalis* with values of 50 μg/ml, it was most

effective against *C. albicans* and *C. tropicalis* with values of 25 μg/mL. Also, all complexes showed some influence at 25 μg/mL. Moreover, complex 6 had the same effect against *E. coli*, *P. aeruginosa*, *C. albicans*, and *C. tropicalis* with values of 50 μg/ml. In comparison with antibiotic controls, all their complexes exhibited antibacterial and antimicrobial activity, but complexes 3 and 6 were more effective than the others. Compound 3 was more effective against the yeast strains *C. albicans* and *C. tropicalis* than against the bacterial strains, while complex 5 exhibited same activity across all microorganisms with values of 100 μg/ml. Complexes 2 and 4 were the least effective against all microorganisms, with values of 200 μg/ml [29].

A study carried out in 2016 reported the antibacterial activity of derivatives of benzimidazole Ag(I)–NHC complexes (7–12). The results showed that these complexes exhibited low antibacterial activity (against Gram-negative and Gram-positive bacteria) compared with ampicillin antibiotic used as standard. Gram-negative bacterium (*E. coli*) showed increased sensitivity at high concentration. In particular, complexes 10 and 11 showed the highest activity with zone of inhibition of 11 mm. All the complexes had the same effect on *E. coli* at 3 μl concentration (zone of inhibition of 6 mm), while complex 9 showed the highest antimicrobial activity against Gram-positive bacterium (*S. aureus*) with zone of inhibition of 9 mm. Moreover, the MIC results showed that complex 9 exhibited high activity against *E. coli* and *S. aureus* with MIC level of 25 μg/ml. While complex 7 showed an effect at 25 μg/ml against *E. coli*, the value against *S. aureus* was 12.5 μg/ml. Complexes 10–12 exhibited the same effect (25 μg/ml MIC) against Gram-positive bacteria as well as against Gram-negative bacterial species (12.5 μg/ml MIC) [30].

In a previous work, the antimicrobial activity of derivatives of 5-fluoro-2-(substituted phenyl)-6-morpholino-1*H*-benzimidazole compounds (6–15) was investigated by diffusion method against *Bacillus subtilis*, *S. aureus*, *E. coli*, and *C. albicans*. In particular; 5-fluoro-2-(4-fluoro phenyl)-6-morpholino-1*H*-benzimidazole (compound 7) showed antifungal activity against *C. albicans* species with inhibition zone diameter of 17 mm, comparable to the standard fluconazole (18 mm). Additionally, 5-fluoro-2-(4-chlorophenyl)-6-morpholino-1*H*-benzimidazole (compound 11) and 5-fluoro-2-(3,4-dimethoxyphenyl)-6-morpholino-1*H*-benzimidazole (12) exhibited antibacterial activity with inhibition zone diameter of 10 mm and 12 mm, respectively, against *B. subtilis* [31].

In our study, the antimicrobial activity of the synthesized compounds (**1**, **2a–c**, **3a–c**) was estimated against one Gram-positive bacterial strain (*S. aureus*), one Gram-negative bacterial strain (*E. coli*), and one yeast (*C. albicans*) by evaluating the minimum inhibitory concentration (MIC, μg/ml) using an agar dilution procedure. All the synthesized compounds (**1**, **2a–c**, **3a–c**) exhibited antimicrobial activity against the fungus and bacteria when tested at concentrations of 66.6–5.85 μg/ml; the results are presented in Table 3. The tested complexes were found to be effective in inhibiting the growth of bacteria with

MIC values between 44.4 and 5.85 μg/ml. The starting compound *N*-morpholinoethylbenzimidazole (**1**) showed effective activity with MIC ranging from 13.15 to 19.75 μg/mL against the Gram-negative bacterial strain (*E. coli*), Gram-positive bacterial strain (*S. aureus*), and fungus (*C. albicans*). The 2-morpholinoethyl-substituted benzimidazolium salts (**2a–c**) showed effective activity with MIC ranging from 29.6 to 5.85 μg/ml against the Gram-negative bacterial strain (*E. coli*), Gram-positive bacterial strain (*S. aureus*), and fungus (*C. albicans*). Generally, the benzimidazolium salt **2c** was more effective than the benzimidazolium salts **2a**, **b**, which can be attributed to presence of hydroxypropyl group on the N-atom.

The 2-morpholinoethyl-substituted Ag(I)–NHC complexes (**3a–c**) showed effective activity with MIC ranging from 44.4 to 5.85 μg ml^{-1} against the tested bacterial strains and fungus. The silver carbene complexes **3a**, **c** showed greater antibacterial activity than the other complex **3b**. Among all the synthesized compounds, **2c** and **3c** were most effective against the Gram-positive bacterium (*S. aureus*) and Gram-negative bacterium (*E. coli*). All the synthesized compounds (**1**, **2a–c**, **3a–c**) exhibited antifungal activity with MIC ranging from 44.4 and 5.85 μg/ml. Compounds **1**, **2a**, **b**, **3a** exhibited the same activity against the fungus. Also, compounds **2c**, **3c** exhibited the same activity against the fungus. Synthesized compounds **1**, **2b**, **3a**, **b** showed high activity against the fungus *C. albicans*. The results of this study suggest that the alkyl groups, which contain bulky (isopropyl), electron-donating (propyl), and H-bonding (hydroxypropyl) substituents on the N-atom, may play an important role in the antimicrobial activity.

We have determined the antimicrobial activity of these new complexes, but the underlying mechanism and characteristics remain unclear. According to previous literature, it can be suggested that, as an antibacterial component, silver ions can inhibit cell division or cause structural abnormalities by damaging the cell membrane or harming cellular contents of the bacteria such as the cytoplasmic membrane or cytoplasmic contents. In addition, silver ions can interact especially with DNA [32, 33].

Table 3 Antimicrobial activity results (μg/ml) for **1**, NHC salts (**2a–c**), and Ag(I)-NHC complexes (**3a–c**)

	1	**2a**	**2b**	**2c**	**3a**	**3b**	**3c**	$AgNO_3$
Escherichia coli ATCC 25922	19.75	19.75	5.85	5.85	29.6	44.4	5.85	44.4
Staphylococcus aureus Cowan 1	13.15	13.15	13.15	5.85	19.75	29.6	5.85	44.4
Candida albicans FMC 17	19.75	19.75	19.75	29.6	19.75	44.4	29.6	44.4

Conclusions

The compounds synthesized in this work showed antimicrobial activity, having potential as antibiotics for treatment of bacterial infections. In the next phase of this work, we will investigate their anticancer effect. Further development of the results presented herein as well as future work by the authors and other researchers will provide basic information for scientific research.

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References

1. U. Kalinowska-Lis, A. Elczak, L. Chęcińska, K. Zawadzk, E. Patyna, K. Lisowska, J. Ochocki, Dalton Trans. **44**, 8178 (2015)
2. L. Ortego, J. Gonzalo-Asensio, A. Laguna, M.D. Villacampa, M.C. Gimeno, Inorg. Biochem. **146**, 19–27 (2015)
3. L. Kyros, C.N. Banti, N. Kourkoumelis, M. Kubicki, I. Sainis, S.K. Hadjikakou, J. Biol. Inorg. Chem. **19**(3), 449–464 (2014)
4. S. Patil, A. Deally, B. Gleeson, H. Muller-Bunz, F. Paradisi, M. Tacke, Appl. Organomet. Chem. **24**(11), 781–793 (2010)
5. M. Pellei, V. Gandin, M. Marinelli, C. Marzano, M. Yousufuddin, H.V.R. Dias, C. Santini, Inorg. Chem. **51**(18), 9873–9882 (2012)
6. D.C.F. Monteiro, R.M. Phillips, B.D. Crossley, J. Fielden, C.E. Willans, Dalton Trans. **41**, 3720–3725 (2012)
7. B. Glišić, L. Senerovic, P. Comba, H. Wadepohl, A. Veselinovic, D.R. Milivojevic, M.I. Djuran, J. Nikodinović-Runic, J. Inorg. Biochem. **155**, 115 (2015)
8. B. Glišić, B. Warżajtis, N.S. Radulović, U. Rychlewska, M.I. Djuran, Polyhedron **87**, 208–214 (2015)
9. B. Warżajtis, B. Glišić, N.S. Radulović, U. Rychlewska, M.I. Djuran, Polyhedron **79**, 221–228 (2014)
10. İ. Özdemir, N. Şahin, Y. Gök, S. Demir, B. Çetinkaya, J. Mol. Catal. A Chem. **234**, 181–185 (2005)
11. Agilent Technologies, *CrysAlis PRO and CrysAlis RED* (Yarnton, Oxfordshire England, 2002)
12. R.C. Clark, J.S. Reid, Acta Cryst. **A51**, 887–897 (1995)
13. O.V. Dolomanov, L.J. Bourhis, R.J. Gildea, J.A.K. Howard, H. Puschmann, Olex2: A complete structure solution, refinement and analysis program. J Appl. Cryst. **42**, 339–341 (2009)
14. G.M. Sheldrick, Acta Cryst. **A7**(1), 3–8 (2015)
15. G.Ö. Sheldrick, Acta Cryst. **A64**, 112–122 (2008)
16. A. Aktaş, S. Akkoç, Y. Gök, J. Coord. Chem. **66**, 2901–2909 (2013)
17. S. Akkoç, Y. Gök, J. Coord. Chem. **66**, 1396–1404 (2013)
18. Y. Gök, S. Akkoç, S. Albayrak, M. Akkurt, M.N. Tahir, Appl. Organometal. Chem. **28**, 244–251 (2014)
19. M.A. Iqbal, R.A. Haque, S.F. Nasri, A.A. Majid, M.B. Ahamed, E. Farsi, T. Fatima, J. Chem. Central **7**, 27 (2013)
20. L.J. Farrugia, J. Appl. Crystallogr. **45**, 849–854 (2012)
21. D. Cremer, J.A. Pople, J. Am. Chem. Soc. **97**, 1354–1358 (1975)
22. S. Türtekin, M. Akkurt, N. Şireci, H. Küçükbay, O. Büyükgüngör, Acta Cryst. **E60**, o817–o819 (2004)
23. M. Akkurt, S. Öztürk, N. Şireci, H. Küçükbay, O. Büyükgüngör, Acta Cryst. **E60**, o1185–o1187 (2004)

24. F.H. Allen, O. Kennard, D.G. Watson, L. Brammer, A.G. Orpen, R. Taylor, J. Chem. Soc. Perkin Trans. **1**, 1–19 (1987)
25. J.H. van den Hende, N.R. Nelson, Am. Chem. Soc. **89**(12), 2901–2905 (1967)
26. M. Mackay, D.C.K. Hodgkin, J. Chem. Soc. 3261–3267 (1955)
27. P.O. Aseknowo, R.A. Hague, M. Razalli, S. Budagumpi, Appl. Organometal. Chem. **29**, 126–137 (2015)
28. U. Kalinowska-Lis, A. Felczak, L. Checínska, I. Szabłowska-Gadomska, E. Patyna, M. Małecki, K. Lisowska, J. Ochocki, Molecules **21**(87), 1–14 (2016)
29. Y. Sarı, S. Akkoç, Y. Gök, V. Sifniotis, İ. Özdemir, S. Günal, V. Kayser. J Enzyme Inhib. Med. Chem. **31**(6), 1527–1530 (2016)
30. U.F.M. Haziz, R.A. Hague, A.A. Amirul, N. Shaheeda, M. Razali, Polyhedron **117**, 628–636 (2016)
31. C. Kuş, N. Altanlar Journal of Faculty of Pharmacy of Ankara. 35(4), 237–244 (2006)
32. S.H. Kim, L. Hyeong-Seon, R. Deok-Seon, C. Soo-Jae, L. Dong-Seok, Korean J. Microbiol. Biotechnol. **39**(1), 77–85 (2011)
33. Y. Yakabe, T. Sano, H. Ushio, T. Yasunaga, Chem. Lett. **4**, 373–376 (1980)