JOURNAL OF COORDINATION CHEMISTRY
2023, VOL. 76, NO. 1, 120–133
https://doi.org/10.1080/00958972.2022.2164191

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Novel benzimidazolium salts and their silver(I)-*N*-heterocyclic carbene complexes: synthesis, characterization and their biological properties

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ABSTRACT

This study was conducted to synthesize and characterize 1,3-dialkyl-5,6-dimethyl-benzimidazolium salts and their silver complexes. Four novel silver(I)-*N*-heterocyclic carbene complexes were synthesized and characterized by mass analyses, FT-IR and NMR spectroscopy. The biological capacity of the synthesized compounds was evaluated *in vitro* for their antimicrobial and antitumor activities. The minimum inhibitory concentration (MIC) of the 1,3-dialkyl-5,6-dimethyl-benzimidazolium salts and their complexes was determined for *E. coli*, *P. aeruginosa*, *S. aureus*, *C. glabrata* and *C. albicans in vitro* through BMD (Broth Microdilution). The results indicated that silver(I)-NHC complexes exhibit antimicrobial activity. In particular, complex **3c** presented a significant broad-spectrum antimicrobial activity. The anticancer properties of the synthesized compounds were evaluated against MCF-7, HCT116, SH-SY5Y and BEAS-2B cell lines. Anticancer activity measurements were carried out according to the Alamar Blue assay. Efficacy was established by comparison of the salts and silver compounds with *cisplatin*.

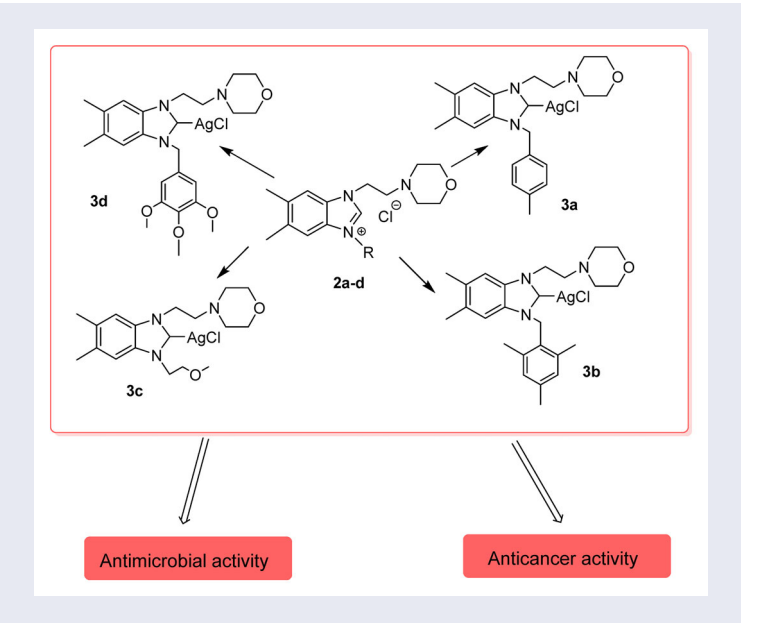
ARTICLE HISTORY
Received 16 September 2022
Accepted 17 December 2022

KEYWORDS
N-Heterocyclic carbene; benzimidazolium salts; morpholinoethyl; silver(I)-NHC complexes; antimicrobial and antitumor activities

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/00958972.2022.2164191.

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1. Introduction

The strong σ-donating properties of *N*-heterocyclic carbenes (NHCs) and the ability to tune their electronic and steric properties by varying the backbone and *N*-substituents have made them particularly useful as ligands since they form strong metal-carbon bonds with a variety of metals [1, 2]. Since the isolation of the first stable free NHC by Arduengo in 1991 [3], NHCs have emerged as active, robust and versatile ancillary ligands for several metal-mediated homogeneous catalytic reactions [4].

The use of benzimidazole dates several years back and plays a very vital role in a plethora of useful therapeutic activities [5, 6], like antioxidant [7], antiulcer [8], antihypertensive [9], analgesic, anti-inflammatory [10, 11], antiviral [12–14], antifungal [15], anticancer [16, 17], antibacterial [18, 19], anthelmintic [20, 21] and other biological activities.

The utilization of metallic silver compounds as antimicrobial agents has been known since the seventeenth and eighteenth centuries [22]. Silver(I)-*N*-heterocyclic carbene complexes have strong efficacy *in vitro* as well as *in vivo* and can be employed as strong antimicrobial agents [23]. In recent studies, silver complexes display less toxicity to the human body than other metal complexes, which has made them desirable antimicrobial substances [24].

The organic ligands, which silver salts have delighted in a long history as antimicrobial agents, have been demonstrated to display low poisonous quality for people [25]. Some studies have shown that Ag(I)-NHC complexes display *in vitro* anticancer activity against ovarian and breast cancer cells [26]. Also regarding the anticancer movement of silver edifices, Youngs *et al.* announced that Ag(I)-NHC buildings are dynamic against cancer lines [27, 28]. These results have encouraged further medicinal applications of NHC complexes of Ag(I) [29]. Recently, the mode of action and an *in vivo* model were published for the antibiotic lead compound [30, 31].

In this study, a new series of 2-morpholinoethyl-substituted 5,6-dimethyl-benzimidazolium salts (**2**) and their silver(I)-N-heterocyclic carbene complexes were synthesized and characterized. The biological capacity of the synthesized compounds was evaluated *in vitro* for their antimicrobial and antitumor activities.

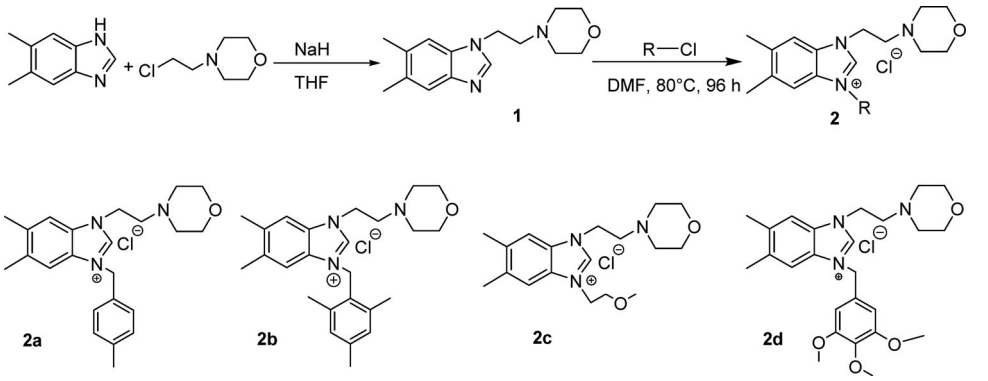
2. Experimental

2.1. General procedure

All reactions for the preparation of benzimidazolium salts and silver(I)-NHC complexes were carried out under argon in flame-dried glassware using standard Schlenk techniques. Chemicals were obtained from Sigma Aldrich and Fluka. Melting points were determined in glass capillaries under air with an Electrothermal-9200 melting point apparatus. FT-IR spectra were recorded as KBr pellets from 400 to 4000 cm^{-1} with an ATI UNICAM 1000 spectrometer. The mass analysis was determined using a Thermo Scientific Exactive Plus Benchtop Full-Scan Orbitrap Mass Spectrometer LC-MS/MS analyzer. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker Ascend^{TM} 400 Mercury spectrometer (Billerica, MA, USA) operating at 400 MHz (^{1}H), 100 MHz (^{13}C) in $CDCl_3$ with tetramethylsilane (TMS) as the internal reference. The NMR studies were carried out in high-quality 5-mm NMR tubes. The chemical shifts (δ) are reported in ppm relative to $CDCl_3$. Coupling constants (*J* values) are given in hertz. NMR multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, quint. = quintet, sex = sextet and m = multiplet signal. DS-11 FX + (UV, Blue, Red, Green) Spectrophotometer/Fluorometer, Allsheng AMR-100 Microplate Reader AC120 ∼ 240 V, 50/60 Hz plate reader, Daihan WIS 20 and 20 R Cooled Shaking Incubator, NF 800 Core brand Centrifuge tool used, 96-well plates, Eppendorf tubes, micropipette.

2.2. Synthesis of N-2-morpholinoethyl-5,6-dimethylbenzimidazole (1)

Suspension of NaH (1 mmol, 60% in mineral oil) in THF (50 mL) was put slowly (portion-wise) in a round-bottom flask (100 mL) that was already charged with benzimidazole (1 mmol). In complete addition, the reaction was stirred at ambient temperature for 1 h. The solution was then mixed with 4-(2-chloroethyl)morpholine (1.1 mmol) allowed by refluxing the mixture for 24 h. After this time, the reaction flask was cooled down. THF was evaporated in vacuum. The resulting gel formation was dissolved in DCM (30 mL) and the solution was filtered. It was concentrated up to 10 mL followed by the addition of ether (20 mL) for crystallization. The product was obtained as a crystalline solid (Scheme 1). Yield: 86%. ^{1}H NMR (400 MHz, $CDCl_3$), δ(ppm): 2.38 (s, 3H, $CH_3C_6H_2$), 2.40 (s, 3H, $CH_3C_6H_2$), 2.48 (m, 4H, NCH_2CH_2O), 2.75 (t, 2H, $J = 6.4$ Hz, NCH_2CH_2N), 3.69 (m, 4H, NCH_2CH_2O), 4.21 (t, 2H, $J = 6.4$ Hz, NCH_2CH_2N), 7.16 and 7.56 (m, 2H, $NC_6H_2N(CH_3)_2$), 7.87 (s, 1H, NCHN). ^{13}C NMR (100 MHz, $CDCl_3$), δ(ppm): 20.24 ($CH_3C_6H_2$), 20.62 ($CH_3C_6H_2$), 42.44 (NCH_2CH_2N), 53.76 (NCH_2CH_2O), 57.68 (NCH_2CH_2N), 66.91 (NCH_2CH_2O), 109.58, 120.40, 130.94, 131.96, 132.27 and 142.35 ($CH_2C_6H_4CH_3$), 142.62 (NCHN).



Scheme 1. Synthetic route for the preparation of 1,3-dialkyl-5,6-dimethyl-benzimidazolium salts, **2a–d**.

2.3. Preparation of 1,3-dialkyl-5,6-dimethyl-benzimidazolium chlorides (2a–d)

The 5,6-dimethylbenzimidazolium salts **2a**–**d** were synthesized using standard Schlenk techniques. *N*-2-Morpholinoethyl-5,6-dimethylbenzimidazole (1.03 g, 3.9 mmol) was dissolved in degassed DMF (10 mL) and alkyl chloride (0.56 g, 3.9 mmol) was added at room temperature. The reaction mixture was stirred at 80 °C for 96 h under argon. After completion of the reaction, the solvent was removed by vacuum and diethyl ether (10 mL) was added to obtain a white crystalline solid, which was filtered off. The solid was washed with diethyl ether (3 × 10 mL) and dried under a vacuum. The crude product was recrystallized from ethanol/diethyl ether mixture (1:3, v/v) at room temperature and, completely dried under vacuum.

2.3.1. 1-(2-morpholinoethyl)-3-(4-methylbenzyl)-5,6-dimethyl-benzimidazolium chloride, 2a

Yield: 96%; m. p. 234–235 °C; $\nu_{(CN)}$: 1563 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ(ppm): 2.31 (s, 3H, C**H**$_3$C$_6$H$_4$), 2.39 (s, 3H, C**H**$_3$C$_6$H$_2$), 2.42 (s, 3H, C**H**$_3$C$_6$H$_2$), 2.60 (m, 4H, NC**H**$_2$CH$_2$O), 2.93 (m, 2H, NC**H**$_2$CH$_2$N), 3.62 (m, 4H, NCH$_2$C**H**$_2$O), 4.74 (m, 2H, NCH$_2$C**H**$_2$N), 5.71 (s, 2H, NC**H**$_2$C$_6$H$_4$), 7.17, 7.35 and 7.48 (m, 6H, CH$_2$C$_6$**H**$_4$CH$_3$ and NC$_6$**H**$_2$N(CH$_3$)$_2$), 11.49 (s, 1H, NC**H**N). ^{13}C NMR (100 MHz, $CDCl_3$), δ(ppm): 20.68 (**C**H$_3$C$_6$H$_2$), 20.7 (**C**H$_3$C$_6$H$_2$), 21.2 (**C**H$_3$C$_6$H$_4$), 44.1 (N**C**H$_2$CH$_2$N), 50.8 **(**NCH$_2$**C**H$_2$N), 53.4 (N**C**H$_2$CH$_2$O), 56.1 (N**C**H$_2$C$_6$H$_4$), 66.8 (NCH$_2$**C**H$_2$O), 112.6, 113.3, 128.1, 129.5, 129.6, 129.9, 129.9, 130.2, 137.1 and 139.1 (CH$_2$**C**$_6$H$_4$CH$_3$ and N**C**$_6$H$_2$N (CH$_3$)$_2$), 143.1 (N**C**HN). HR-AM (H-ESI II) analysis calculated *(m/z)* for the cationic part of $[C_{23}H_{30}N_3O]^+$: 364.2383; found *(m/z)*: 364.2318.

2.3.2. 1-(2-morpholinoethyl)-3-(2.4.6-trimethylbenzyl)-5,6-dimethyl-benzimidazolium chloride, 2b

Yield: 85%; m. p. 222–223 °C; $\nu_{(CN)}$: 1556 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ(ppm): 2.31 (s, 3H, C**H**$_3$C$_6$H$_2$), 2.35 (s, 9H, C**H**$_3$C$_6$H$_2$), 2.42 (s, 3H, C**H**$_3$C$_6$H$_2$), 2.54 (m, 4H, NC**H**$_2$CH$_2$O), 2.83 (m, 2H, NC**H**$_2$CH$_2$N), 3.51 (m, 4H, NCH$_2$C**H**$_2$O), 4.79 (m, 2H, NCH$_2$C**H**$_2$N), 5.68 (s, 2H, NC**H**$_2$C$_6$H$_4$), 6.97, 7.16 and 7.49 (m, 4H, CH$_2$C$_6$**H**$_2$(CH$_3$)$_3$ and NC$_6$**H**$_2$N(CH$_3$)$_2$), 10.56 (s, 1H, NC**H**N). ^{13}C NMR (100 MHz, $CDCl_3$), δ(ppm): 20.3 (**C**H$_3$C$_6$H$_2$), 20.7 (**C**H$_3$C$_6$H$_2$), 20.8 (**C**H$_3$C$_6$H$_2$), 21.1 (**C**H$_3$C$_6$H$_2$), 43.8 (N**C**H$_2$CH$_2$N), 46.3 **(**NCH$_2$**C**H$_2$N), 53.3 (N**C**H$_2$CH$_2$O), 56.1

(N**C**$H_2C_6H_4$), 66.7 (NCH_2**C**H_2O), 112.6, 113.1, 125.4, 137.0, 137.1, 137.9 and 139.8 (CH_2**C**$_6H_2(CH_3)_3$ and N**C**$_6H_2$N (C$H_3)_2$), 142.7 (N**C**HN). HR-AM (H-ESI II) analysis calculated *(m/z)* for the cationic part of $[C_{25}H_{34}N_3O]^+$: 392.2702; found *(m/z)*: 392.2629.

2.3.3. 1-(2-morpholinoethyl)-3-(2-methoxyethyl)-5,6-dimethyl-benzimidazolium chloride, 2c

Yield: 45%; m. p. 168–169 °C; $\nu_{(CN)}$: 1564 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$), δ(ppm): 2.46 (s, 6H, C**H**$_3C_6H_2$), 2.62 (m, 4H, NC**H**$_2$CH_2O), 2.95 (t, 2H, $J = 5.9$ Hz, NC**H**$_2$CH_2N), 3.38 (s, 3H, C**H**$_3$O), 3.67 (m, 4H, NCH_2C**H**$_2$O), 3.96 (m, 2H, CH_3OC**H**$_2$CH_2), 4.69 (t, 2H, $J = 5.9$ Hz, NCH_2C**H**$_2$N), 4.74 (m, 2H, NC**H**$_2$CH_2O), 7.45 and 7.**5**1 (m, 2H, NC$_6$**H**$_2$N(C$H_3)_2$), 11.24 (s, 1H, N**C**HN). ^{13}C NMR (100 MHz, $CDCl_3$), δ(ppm): 20.7 (**C**$H_3C_6H_2$), 44.2 (N**C**H_2CH_2N), 47.4 (NCH_2**C**H_2N), 53.5 (N**C**H_2CH_2O), 56.2 (N**C**H_2CH_2O), 59.2 (**C**H_3O), 66.9 (NCH_2**C**H_2O), 70.4 (CH_2**C**H_2O), 112.3, 113.5, 129.6, 130.5 and 136.9 (N**C**$_6H_2$N (C$H_3)_2$), 143.1 (N**C**HN). HR-AM (H-ESI II) analysis calculated *(m/z)* for the cationic part of $[C_{18}H_{28}N_3O_2]^+$: 318.2182; found *(m/z)*: 318.2123.

2.3.4. 1-(2-morpholinoethyl)-3-(3,4,5-trimethoxylbenzyl)-5,6-dimethyl-benzimidazolium chloride, 2d

Yield: 92%; m. p. 223–225 °C;$\nu_{(CN)}$: 1591 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$), δ(ppm): 2.43 (s, 6H, C**H**$_3C_6H_2$), 2.60 (m, 4H, NC**H**$_2$CH_2O), 2.96 (m, 2H, NC**H**$_2$CH_2N), 3.59 (m, 4H, NCH_2C**H**$_2$O), 3.82 (s, 3H, C**H**$_3OC_6H_2$), 3.89 (s, 6H, C**H**$_3OC_6H_2$), 4.70 (m, 2H, NCH_2C**H**$_2$N), 5.67 (s, 2H, NC**H**$_2C_6H_4$), 6.88 and 7.47 (m, 4H, CH_2C_6**H**$_2(OCH_3)_3$ and NC$_6$**H**$_2$N(C$H_3)_2$), 11.51 (s, 1H, NC**H**N). ^{13}C NMR (100 MHz, $CDCl_3$), δ(ppm): 20.7 (**C**$H_3C_6H_2$), 20.8 (**C**$H_3C_6H_2$), 44.1 (N**C**H_2CH_2N), 51.3 (NCH_2**C**H_2N), 53.4 (N**C**H_2CH_2O), 56.1 (N**C**$H_2C_6H_4$), 56.7 (**C**H_3O), 60.8 (**C**H_3O), 66.8 (NCH_2**C**H_2O), 106.3, 112.7, 113.2, 128.6, 129.6, 129.9, 137.2 and 138.8 (CH_2**C**$_6H_2(OCH_3)_3$ and N**C**$_6H_2$N (C$H_3)_2$), 143.1 (NCHN), 153.8 (CH_2**C**$_6H_2(OCH_3)_3$). HR-AM (H-ESI II) analysis calculated *(m/z)* for the cationic part of $[C_{25}H_{34}N_3O_4]^+$: 440.2549; found *(m/z)*: 440.2467.

2.4. Preparation of silver(I)-NHC complexes

All reactions for preparation of the silver complexes were carried out under argon using standard Schlenk techniques. In a Schlenk flask equipped with a magnetic stirring bar, a solution of benzimidazolium salt (1.0 mmol), Ag_2O (1.5 mmol) in chloroform (10 mL) was stirred and heated for one week at 50 °C in dark conditions. The reaction mixture was filtered through celite and the solvent was removed under a vacuum. The crude product was recrystallized from chloroform/diethyl ether (1:3) at room temperature. The resulting white solid was isolated by filtration and dried in a vacuum.

2.4.1. Chloro[1-(2-morpholinoethyl)-3-(4-methylbenzyl)-5,6-dimethyl-benzimidazole-2-ylidene]silver(I), 3a

Yield: 47%; m. p. 270–271 °C; $\nu_{(CN)}$: 1562 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$), δ(ppm): 2.32 (s, 3H, C**H**$_3C_6H_4$), 2.38 (s, 3H, C**H**$_3C_6H_2$), 2.43 (s, 3H, C**H**$_3C_6H_2$), 2.53 (m, 4H, NC**H**$_2$CH_2O), 2.80 (m, 2H, NC**H**$_2$CH_2N), 3.70 (m, 4H, NCH_2C**H**$_2$O), 4.46 (m, 2H, NCH_2C**H**$_2$N), 5.50 (s, 2H, NC**H**$_2C_6H_4$), 7.10, 7.13, 7.23 and 7.32 (m, 6H, CH_2C_6**H**$_4CH_3$ and NC$_6$**H**$_2$N(C$H_3)_2$). ^{13}C NMR

(100 MHz, $CDCl_3$), δ(ppm): 20.5 (**C**$H_3C_6H_2$), 20.7 (**C**$H_3C_6H_4$), 40.8 (N**C**H_2CH_2N), 46.9 (**(**NCH_2**C**H_2N), 53.4 (N**C**H_2CH_2O), 58.0 (N**C**$H_2C_6H_4$), 66.9 (NCH_2**C**H_2O), 111.5, 112.3, 113.2, 127.0, 128.0, 129.7, 130.0, 132.1, 133.7, 137.0 and 138.3 (CH_2**C**$_6H_4CH_3$ and N**C**$_6H_2N(CH_3)_2$), Ag-$C_{carbene}$ did not appear. HR-AM (H-ESI II) analysis calculated *(m/z)* for the cationic part of $[M\text{-}AgCl]^+$: 364.2383; found *(m/z)*: 364.2322.

2.4.2. Chloro[1-(2-morpholinoethyl)-3-(2.4.6-trimethylbenzyl)-5,6-dimethyl-benzimidazole-2-ylidene]silver(I), 3b

Yield: 16%; m. p. 262–263 °C; $\nu_{(CN)}$: 1558 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$), δ(ppm): 0.85 (s, 3H, C**H**$_3C_6H_2$), 1.25 (s, 6H, C**H**$_3C_6H_2$), 2.23 (s, 6H, C**H**$_3C_6H_2$), 2.32 (m, 2H, NC**H**$_2CH_2N$), 2.39 (m, 4H, NC**H**$_2CH_2O$), 3.65 (m, 4H, NCH_2C**H**$_2O$), 4.40 (m, 2H, NCH_2C**H**$_2N$), 5.39 (s, 2H, NC**H**$_2C_6H_4$), 6.99, 7.08 and 7.22 (m, 4H, CH_2C_6**H**$_2(CH_3)_3$ and NC_6**H**$_2N(CH_3)_2$). ^{13}C NMR (100 MHz, $CDCl_3$), δ(ppm): 20.3 (**C**$H_3C_6H_2$), 20.4 (**C**$H_3C_6H_2$), 20.4 (**C**$H_3C_6H_2$), 20.5 (**C**$H_3C_6H_2$), 21.2 (**C**$H_3C_6H_2$), 46.2 (N**C**H_2CH_2N), 47.3 (**(**NCH_2**C**H_2N), 53.4 (N**C**H_2CH_2O), 54.0 (N**C**$H_2C_6H_4$), 66.9 (**(**NCH_2**C**H_2O), 111.49, 111.88, 112.54, 113.10, 125.32, 126.83, 127.96, 130.19, 130.28, 132.28, 132.87, 137.43, 137.98 and 139.61 (CH_2**C**$_6H_2(CH_3)_3$ and N**C**$_6H_2N(CH_3)_2$), Ag-$C_{carbene}$ did not appear. HR-AM (H-ESI II) analysis calculated *(m/z)* for the cationic part of $[C_{18}H_{28}N_3O_2]^+$: 392.2702; found *(m/z)*: 392.2631.

2.4.3. Chloro[1-(2-morpholinoethyl)-3-(2-methoxyethyl)-5,6-dimethyl-benzimidazole-2-ylidene]silver(I), 3c

Yield: 26%; m. p. 274–275 °C; $\nu_{(CN)}$: 1448 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$), δ(ppm): 2.41 (s, 6H, C**H**$_3C_6H_2$), 2.52 (m, 4H, NC**H**$_2CH_2O$), 2.78 (t, 2H, $J = 6.6$ Hz, NC**H**$_2CH_2N$), 3.31 (s, 3H, C**H**$_3O$), 3.71 (m, 4H, NCH_2C**H**$_2O$), 3.79 (t, 2H, $J = 5.3$ Hz, CH_3OCH_2**C**H_2), 4.43 (t, 2H, $J = 6.6$ Hz, NCH_2C**H**$_2N$), 4.49 (t, 2H, $J = 5.3$ Hz, NC**H**$_2CH_2O$), 7.22 and 7.31 (m, 2H, NC_6**H**$_2N(CH_3)_2$). ^{13}C NMR (100 MHz, $CDCl_3$), δ(ppm): 20.5 (**C**$H_3C_6H_2$), 47.0 (N**C**H_2CH_2N), 49.2 (NCH_2**C**H_2N), 53.4 (N**C**H_2CH_2O), 54.1 (N**C**H_2CH_2), 58.1 (**(**N**C**H_2CH_2O), 59.2 (**C**H_3O), 66.9 (**(**NCH_2**C**H_2O), 71.8 (CH_2**C**H_2O), 111.4, 112.3, 132.0, 132.9, 133.6, and 133.4 (N**C**$_6H_2N(CH_3)_2$), Ag-$C_{carbene}$ did not appear. HR-AM (H-ESI II) analysis calculated *(m/z)* for the cationic part of $[M\text{-}AgCl]^+$: 318.2182; found *(m/z)*: 318.2120.

2.4.4. Chloro[1-(2-morpholinoethyl)-3-(3,4,5-trimethoxylbenzyl)-5,6-dimethyl-benzimidazole-2-ylidene]silver(I), 3d

Yield: 35%; m. p. 223–224 °C; $\nu_{(CN)}$: 1585 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$), δ(ppm): 2.36 (s, 3H, C**H**$_3C_6H_2$), 2.40 (s, 3H, C**H**$_3C_6H_2$), 2.53 (m, 4H, NC**H**$_2CH_2O$), 2.80 (t, 2H, $J = 6.5$ Hz, NC**H**$_2CH_2N$), 3.69 (m, 4H, NCH_2C**H**$_2O$), 3.81 (s, 6H, C**H**$_3OC_6H_2$), 3.89 (s, 3H, C**H**$_3OC_6H_2$), 4.46 (t, 2H, $J = 6.4$ Hz, NCH_2C**H**$_2N$), 5.44 (s, 2H, NC**H**$_2C_6H_4$), 6.51, 7.18 and 7.25 (m, 4H, CH_2C_6**H**$_2(OCH_3)_3$ and NC_6**H**$_2N(CH_3)_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 20.4 (**C**$H_3C_6H_2$), 20.5 (**C**$H_3C_6H_2$), 47.0 (N**C**H_2CH_2N), 53.4 (**(**NCH_2**C**H_2N), 54.1 (N**C**H_2CH_2O), 56.7 (**C**H_3O), 58.2 (N**C**$H_2C_6H_4$), 60.9 (**C**H_3O), 66.7 (NCH_2**C**H_2O), 104.7, 106.3, 111.6, 112.1, 112.6, 113.1, 130.8, 132.3, 132.4, 133.8, 133.2 and 138.2 (CH_2**C**$_6H_2(OCH_3)_3$ and N**C**$_6H_2N(CH_3)_2$), 153.7 (CH_2**C**$_6H_2(OCH_3)_3$). Ag-$C_{carbene}$ did not appear.HR-AM (H-ESI II) analysis calculated *(m/z)* for the cationic part of $[M\text{-}AgCl]^+$: 440.2549; found *(m/z)*: 440.2467.

2.5. Antitumor activities of benzimidazolium salts and their silver complexes

2.5.1. Cell culture and incubation

In this work, the anticancer properties of samples were evaluated against MCF-7, HCT116, SHSY5Y, and BEAS-2B cell lines. All cells were allowed to grow in a DMEM medium supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin at 37 °C in a 5% CO_2 atmosphere. Cells that reached a confluence of 70–80% were chosen for plating purposes. The old medium was discarded and cells were washed several times with sterile PBS (pH = 7.4). Afterward, trypsin was added and distributed evenly onto cell surfaces. After the incubation with trypsin for 5 min at 37 °C, trypsin activity was inhibited by adding 2-fold volume of fresh media. The solution was gently triturated to obtain cell segregation. The obtained solution was centrifuged at 1000 rpm for 7 min, and then, the old medium was changed with 5 mL fresh medium. Cells were counted and diluted to get a final concentration of 1×10^5 cells/mL followed by seeding of the solution into wells (1×10^4 cells/well). Finally, plates containing the cells were incubated at 37 °C in a 5% CO_2 atmosphere for 24 h to allow cell attachment. Anticancer activity measurements were carried out according to the Alamar Blue assay.

2.5.2. The Alamar blue assay

While the cells were incubating, the test substance was diluted with fresh media to obtain the desired concentration from the stock. The old medium was aspirated out of the wells containing the cells, and 100 µl of the test substance was added to the wells. Afterward, plates were allowed to incubate for 24 h at 37 °C in 5% CO_2 for the treatment. After this period, 10 µL of Alamar Blue solution (Serva, Heidelberg, Germany) and 90 µL of fresh media were added to each well to give a final concentration of 10% Alamar blue followed by incubating at 37 °C for 4 h. The optical density was read on an ELISA reader at 570 nm and 600 nm. Cell viability percentages are determined by using the formula [((O2xA1) – (O1xA2))/((O2xP1) – (O1xP2))]x100. In this formula; O1 = molar extinction coefficient (E) of oxidized Alamar Blue at 570 nm, O2 = E of oxidized Alamar Blue at 600 nm, A1 = absorbance of test wells at 570 nm, A2 = absorbance of test wells at 600 nm, P1 = absorbance of positive growth control well (cells plus Alamar Blue but no test agent) at 570 nm, P2 = absorbance of positive growth control well (cells plus Alamar Blue but no test agent) at 600 nm. IC_{50} was calculated based on linear cell viability percentages.

2.6. Antimicrobial activity

Candida albicans (ATCC MYA-2876) and *Candida glabrata* (ATCC 2001), which are pathogenic yeast species, were used in antifungal tests, and for antimicrobial tests, *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC 27853) bacteria species were used. All types of bacteria and fungi used in the study were provided in the Laboratory of the Medical Biology and Genetics Department of Inonu University Faculty of Medicine (Battalgazi, Malatya, Turkey). Antifungal and antimicrobial MIC analyses were performed using the BMD (Broth Microdilution) test, as described in EUCAST EDef 7.3.2 for yeasts [32] and CLSI M07-A10 for bacteria [33] within different mediums mentioned in these documents.

Briefly, the stock solution of chemically synthesized powdered compounds (NHCs) to be used in antifungal and antimicrobial tests was prepared in 100% DMSO, and serial dilutions were made in flat bottom 96-well plates, in YPD (Yeast Peptone Dextrose) medium (2% peptone, 2% glucose, 1% yeast extract) pH 6,5 for yeasts [34] and LB (Luria-Bertani) broth medium (1% tryptone, 1% NaCl, 0.5% yeast extract, pH 7.0) for bacteria [35]. In sterile water, yeast ($1–5 \times 10^5$ CFU/mL) and bacteria (1×10^6 CFU/mL) cell solutions (inoculums) were prepared and added in equal volumes to 96-well plates containing different concentrations of the compounds to obtain the required cell density and concentrations of chemical compounds to be tested. After the cell solutions were added, the final concentrations of the compounds were between 0.8 and 800 mg/L, and the cell concentrations required for the test were $0.5–2.5 \times 10^5$ CFU/mL in yeasts and 5×10^5 CFU/mL in bacteria in the final step. Plates were incubated for 24 h at 37 °C for yeasts and 16–18 h at 37 °C for bacteria, and the MIC was determined spectrophotometrically at 530 nm after incubation in yeasts and analyzed by eyes for bacteria. The MIC value was measured as the lowest drug concentration causing at least 50% or more reduction in growth in yeasts compared to the control (no drug) cell group, and as the lowest drug concentration without visible growth in bacteria.

3. Results and discussion

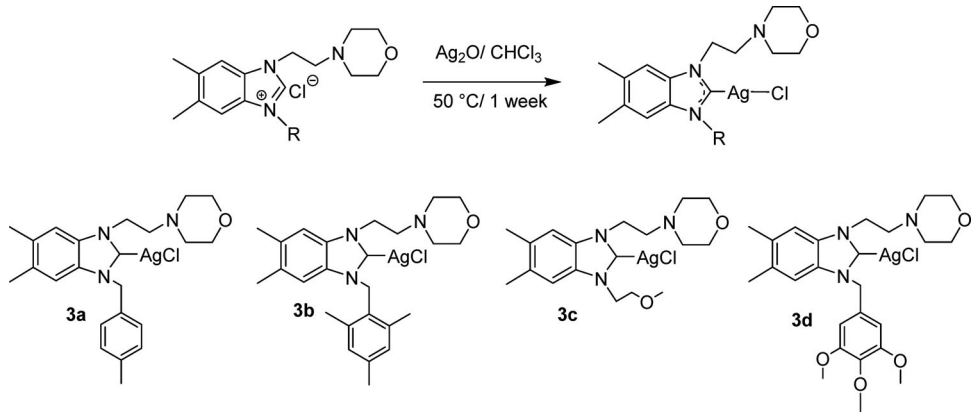
3.1. Chemistry

This article describes the synthesis of *N*-2-morpholinoethyl-5,6-dimethylbenzimidazole (**1**), 2-morpholinoethyl-substituted benzimidazolium salts (NHC precursors, **2a**–**d**) and their Ag(I)-NHC complexes (**3a**–**d**). The reaction of 5,6-dimethylbenzimidazole with 4-(2-chloroethyl) morpholine resulted in the formation of *N*-2-morpholinoethyl-5,6-dimethylbenzimidazole (**1**) (Scheme 1). The ^{1}H NMR spectrum of **1** in $CDCl_3$ displayed the NCHN proton at 7.87 ppm. In the ^{13}C NMR spectra, the NCHN signal appeared at 142.62 ppm.

The benzimidazolium salts **2a–d** were prepared by a reaction of alkyl chloride with N-2-morpholinoethyl-5,6-dimethylbenzimidazole, respectively (Scheme 1). The salts are air- and moisture-stable both in the solid state and in solution. The ^{1}H NMR spectra of **2a–d** in $CDCl_3$ displayed the NCHN protons at 11.49, 10.56, 11.24, and 11.51 ppm, respectively. In the ^{13}C NMR spectra, the NCHN signals appeared at 143.11, 142.70, 143.12, and 143.12 ppm for **2a**, **2b**, **2c,** and **2d**, respectively. These values are typical for NCHN protons of benzimidazolium salts [19].

Silver(I)-NHC complexes are generally accessible by reacting the basic Ag_2O with the benzimidazolium salts in dark conditions. Ag(I)-NHCs **3a**–**d** were prepared by reaction of Ag_2O with the benzimidazolium salts **2a**–**d** under stirring in chloroform for one week at 50 °C in the dark (Scheme 2).

In the ^{1}H NMR spectrum, the absence of the resonances for the acidic proton (NCHN) around 10–12 ppm showed the formation of expected Ag-NHC complexes. In the ^{13}C NMR spectra, the resonances for carbene carbons were not detected, which was also mentioned in the literature and given a reason for the fluxional behavior of the NHCs complexes [36]. The synthesized compounds are air-stable and soluble readily in dichloromethane and chloroform. The ^{1}H NMR spectra of **3a**–**3d** clearly show



Scheme 2. Synthesis of 1,3-dialkyl-5,6-dimethyl-benzimidazole-2-ylidene]silver(I), complexes **3a–d**.

Table 1. Physical and spectroscopic data for 1,3-dialkylbenzimidazolium salts **2a–d**.

Code	Chemical formula	Molecular weight	Melting point (°C)	^{1}H NMR (NC**H**N) (ppm)	^{13}C NMR (N**C**HN) (ppm)	IR $\nu_{(CN)}$ (cm^{-1})	Yield (%)
2a	$C_{23}H_{30}ClN_3O$	399.96	234.6–237.9	11.49	143.11	1564	96
2b	$C_{25}H_{34}ClN_3O$	428.02	222.7–225.3	10.56	142.70	1558	85
2c						$C_{18}H_{28}ClN_3O_2$	353.89
	168.2–171.4	11.24	143.12	1563	45		
2d						$C_{25}H_{34}ClN_3O_4$	476.01
	223.4–226.6	11.51	143.12	1562	92		

Table 2. Physical and spectroscopic data for Ag(I)–NHC complexes.

Code	Chemical formula	Molecular weight	Melting point (°C)	^{13}C-NMR (Ag-$\mathbf{C}_{carb}$) (ppm)	IR $\nu_{(CN)}$ (cm^{-1})	Yield (%)
3a	$C_{23}H_{29}AgClN_3O$	506.82	270.4–273.5	not observed	1443	47
3b	$C_{25}H_{33}AgClN_3O$	534.88	262.0–263.9	not observed	1453	37
3c	$C_{18}H_{27}AgClN_3O_2$	460.75	273.5–276.3	not observed	1448	2 6
3d	$C_{25}H_{33}AgClN_3O_4$	581.12	179.8–181.9	not observed	1422	35

that the carbenic protons of **2a–2d** were absent, indicating the successful deprotonation and formation of Ag(NHC) moiety. Likewise, in their ^{1}H-NMR spectrum, the absence of downfield resonance at $\delta = 10–12$ ppm suggested the successful formation of the Ag(NHC) moiety [37]. The physical and some spectroscopic data of 1,3-dialkylbenzimidazolium salts are summarized in Table 1.

All the new Ag(I)–NHC complexes **3a–d** depicted in Scheme 2 were obtained in good yield. The spectroscopic data of these complexes are consistent with the data observed in literature for other Ag(I)–NHC complexes [7, 18, 28, 38–40]. The formation of these complexes was also confirmed by mass spectrometry. The physical and some spectroscopic data of Ag(I)-NHC complexes are summarized in Table 2.

3.2. Anticancer activity

Since the first reports of the biological importance of NHC-silver(I) compounds by Youngs *et al.*, researchers have delved into designing a practical prescription for cancer

Table 3. IC_{50} values for NHC complexes (in μM), (mean ± SEM).

	IC_{50} (μM)			
Compound	BEAS-2B[a]	MCF-7[a]	HCT116[a]	SH-SY5Y[a]
2a	NA	373.04 ± 5.34	381.84 ± 7.21	517.84 ± 3.58
2b	94.05 ± 5.96	89.64 ± 4.44	82.21 ± 7.53	81.63 ± 4.46
2c	NA	NA	NA	NA
2d	NA	NA	NA	NA
3a	57.83 ± 6.26	46.33 ± 1.76	115.41 ± 7.35	44.94 ± 1.87
3b	43.85 ± 3.1	25.69 ± 0.56	48.24 ± 1.97	28.31 ± 3.19
3c	39.85 ± 6.9	21.93 ± 1.52	48.2 ± 0.49	34.21 ± 2.13
3d	81.02 ± 0.26	43.57 ± 4.37	76.53 ± 0.36	43.81 ± 1.22
cisplatin[b]	118.59 ± 4.90	76.31 ± 3.62	310.12 ± 3.09	152.12 ± 4.65

IC_{50} values are presented as mean ± SD of three independent experiments.
NA: Not Applicable ($IC_{50} > 800$ μM).
[a] Cell lines.
[b] Reference drug.

as well as bacterial infections utilizing silver. The anticancer properties of synthesized compounds were evaluated against MCF-7, HCT116, SH-SY5Y and BEAS-2B cell lines. The IC_{50} concentration for salts and Ag-NHC complexes was standing for the concentration that causes a 50% reduction in cell viability. Results are reported in Table 3.

The IC_{50} values of all compounds were calculated and compared with the anticancer drug *cisplatin*. Compounds **2c** and **2d** whose IC_{50} activities were examined did not show cytotoxic activity against all cell lines ($IC_{50} > 800$ μM). Although compound **2a** has a higher IC_{50} value than *cisplatin* against MCF-7, SH-SY5Y and HCT116 cell lines, it showed significant anticancer activity against this cell line, since it did not show any cytotoxic effect on healthy cells (BEAS-2B). Anticancer activity of compound **2b** on the MCF-7 cell line was not observed much different when compared with *cisplatin*. The IC_{50} value of the same compound was calculated lower in BEAS-2B, SH-SY5Y and HCT116 cell lines compared to *cisplatin*. The IC_{50} values of **3a**, **3b**, **3c** and **3d** were calculated to be lower in all cell lines when the anticancer drug *cisplatin* was compared.

When the IC_{50} values of the compounds against the BEAS-2B cell line were examined, compounds **2a**, **2c,** and **2d** did not show cytotoxic activity ($IC_{50} > 800$ μM). **2b**, **3a,** and **3d** have higher cytotoxicity activities against the BEAS-2B cell line compared to *cisplatin*. Compounds **3b** and **3c** showed the highest cytotoxic activity against BEAS-2B cells. When the IC_{50} values of the compounds were examined against the MCF-7 cell line, **2c** and **2d** did not show cytotoxic activity ($IC_{50} > 800$ μM). The IC_{50} values of **2a** and **2b** were higher than *cisplatin*. Compounds **3a** and **3d** also have higher anticancer activities against the MCF-7 cell line compared to *cisplatin*. Compounds **3b** and **3c** showed the highest activity in MCF-7 cells. When the IC_{50} values of the compounds were examined against the HCT116 cell line, **2c** and **2d** did not show cytotoxic activity ($IC_{50} > 800$ μM). Compound **2a** had higher IC_{50} values than *cisplatin*. Compounds **2b**, **3a** and **3d** also have higher anticancer activities against the HCT116 cell line compared to *cisplatin*. Compounds **3b** and **3c** showed the highest activity in HCT116 cells. When the IC_{50} values of the compounds were examined against the SH-SY5Y cell line, **2c** and **2d** did not show cytotoxic activity ($IC_{50} > 800$ μM). Compound **2a** had higher IC_{50} values than *cisplatin*. Compounds **2b**, **3a** and **3d** also have higher anticancer activity against the SH-SY5Y cell line compared to *cisplatin*. Compounds **3b** and **3c** showed the highest activity against SH-SY5Y cells.

Heterocyclic scaffolds such as morpholines and indoles have been immensely explored as potential drugs against cancer [41]. It was observed that several morpholines [42], benzimidazole compounds [43, 44] and silver(I)-NHC complexes [45, 46] have shown anticancer activity. Also, several imidazole-based compounds are available as clinical drugs to treat various types of cancers with high therapeutic potency and have revealed huge progress in medicinal chemistry [47, 48]. In addition, benzimidazoles have been studied extensively and are reported to be very potent cytotoxic agents against various cancer cell lines [49].

In 2021, Yamani *et al.* synthesized a series of novel pyrazole-benzimidazole derivatives 56(a–x) and screened them for anticancer potentials. Compound 56q exhibited excellent selectivity and high activity against FGFR (1–3). Due to its favorable pharmacokinetic profile, low toxicity and potent antitumor activity *in vivo*, compound 56q is currently under evaluation in phase I clinical trial [50].

Recently, a publication by Sagam *et al.* reported the anticancer activity of new morpholine-benzimidazole-pyrazole hybrids against three human cancer cell lines, breast (MCF7), prostate (PC3) and lung (A549). The *in vitro* anticancer potency of all synthesized compounds (sixteen compounds) revealed that five compounds were more active [51].

3.3. Antimicrobial activity

The antimicrobial activities of the benzimidazolium salts and their complexes were determined by using the broth microdilution procedure and were tested with different concentrations of the compounds. The minimum inhibitory concentrations (MICs) of salts and silver complexes against bacteria, fungus and reference drugs are summarized in Table 4. Ampicillin and Tetracycline were used as standard drugs for bacterial strains and Amphotericin B and Voriconazole for fungal strains. The MIC was determined as the lowest concentration at which the compound prevented visible growth against *E. coli*, *P. aeruginosa*, *S. aureus*, *C. glabrata* and *C. albicans*.

Table 4. Antifungal and antibacterial MIC (μg/mL) values of the synthesized salts and the Ag–NHC complexes.

	MIC (μg/mL)				
	Yeasts		Gram negative bacteria		Gram positive bacteria
Compound	*C. albicans*	*C. glabrata*	*E. coli*	*P. aeruginosa*	*S. aureus*
2a	NA	NA	NA	NA	NA
2b	200	800	NA	NA	NA
2c	NA	NA	NA	NA	NA
2d	NA	NA	NA	NA	NA
3a	200	NA	100	100	100
3b	100	200	100	100	100
3c	100	100	50	50	100
3d	200	200	100	100	100
Ampicillin	–	–	12,5	400	3,12
Tetracycline	–	–	0.78	12,5	0.2
Amphotericin B	0,05	0,1	–	–	–
Voriconazole	0,4	0,4		–	–

NA: Not Active.

A study carried out in 2017 reported the antimicrobial activity of benzimidazolium salts and their Ag(I)–NHC complexes. The 2-morpholinoethyl-substituted benzimidazolium salts (**2a**–**d**) 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*).

Recently, a publication by Lasmari *et al.* reported the antimicrobial activity of novel benzimidazolium salts and their Ag(I)–NHC complexes. The results showed that all Ag-NHC complexes proved a good activity with MIC values of 6.25–25 μg/mL against both Gram-positive and Gram-negative bacteria [39].

Among all the synthesized benzimidazolium salts, only compound **2b** has an antifungal activity with MIC values of 200 and 800 μg/mL against *C. albicans* and *C. glabrata*, respectively. All the silver complexes presented antimicrobial activity. However, complex **3c** was more effective than the other complexes. Compound **3c** was more effective against the Gram-negative bacteria (*E. coli* and *P. aeruginosa*) than against the other strains. Furthermore, all silver complexes were more active against *P. aeruginosa* than the standard drug Ampicillin. The antimicrobial activity of **3a** and **3d** was the least effective, having similar activities across all microorganisms. Consequently, the results indicate that the Ag-NHC complexes are more active than corresponding benzimidazolium salts.

Previous research has shown that the silver–ligand bond plays a more important role in antibacterial activity than other factors like the degree of polymerization, chirality, or solubility of these complexes [24]. However, according to various studies, the differences in the activities between these dinuclear and mononuclear complexes varied depending on the type of cancer lines or bacteria/fungi tested [52].

4. Conclusion

A series of 2-morpholinoethyl-substituted benzimidazolium salts and their silver complexes have been prepared; their structures were confirmed by ^{1}H NMR, ^{13}C NMR, IR and elemental analysis. The silver complex **3c** showed the best antimicrobial activity of the other complexes tested. Although the mechanism of antimicrobial activity is not known, it was found that the antimicrobial activity of the silver carbene complexes against different kinds of bacteria and fungi varies with the nature of the ligand. In addition, the silver(I)-NHC complexes showed significant antitumor activity against MCF-7, SH-SY5Y and HCT116 cell lines, in particular, complexes **3b** and **3c** that presented the highest activity.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was supported by İnönü University Research Fund (İÜ-BAP: FBG-2021-2562). We are grateful to MESRS and DGRST (Algeria) for the scholarship with İnönü University, Malatya, Türkiye.

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