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RESEARCH ARTICLE

In vitro antimicrobial studies of new benzimidazolium salts and silver *N*-heterocyclic carbene complexes

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Abstract

A series of new benzimidazolium salts (**1a**–**g**) were synthesized from the reaction of 1-(4-vinylbenzyl)benzimidazole with various alkyl halides. These salts were used to synthesize silver *N*-heterocyclic carbene (Ag-NHC) complexes (**2a**–**f**). The thirteen compounds were characterized by FT-IR, NMR (^{1}H and ^{13}C) spectroscopic methods and an elemental analysis technique. These selected candidates were tested for their *in vitro* antimicrobial activities. Antibacterial and antifungal results indicated that the new salts, and particularly their silver complexes, were found to be strongly effective against seven Gram (−) bacterial strains, three Gram (+) bacterial strains and one yeast (*Candida albicans*).

Keywords

Antimicrobial activity, benzimidazolium salt, *N*-heterocyclic carbene, silver complex

History

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Introduction

Heterocyclic compounds such as benzimidazole, imidazole, triazole, benzodiazepine and indazole are very important for both pharmacological and industrial applications[1–4]. Different pharmacological and biological properties of many hetero-aromatic compounds, which are stable, safe, and have biological activity, have been investigated for these two significant applications by different research groups[5–12]. The rapid development of microbial resistance to currently used antibiotics has become a serious healthcare problem[13,14]. Hence, there is an immediate need for research on novel antimicrobial agents with potent activity against resistant microorganisms[13,15]. The benzimidazole ring has a significant heterocyclic pharmacophore in drug discovery[16]. In particular, this nucleus is a constituent of vitamin-B12[17]. Benzimidazoles are regarded as a promising class of bioactive heterocyclic compounds, which have attracted considerable interest in medicinal chemistry because of their various range of biological activities including antiviral, anticancer[16,18,19], antimicrobial[1,16,18–20], anti-inflammatory[16,19], anthelmintic, antihistaminic, proton pump inhibitor, antioxidant, antihypertensive and anticoagulant activities[16]. The aim of this study was to investigate the *in vitro* antimicrobial activity of new synthesized benzimidazolium salts (**1a**–**f**) and Ag-NHC complexes (**2a**–**g**), which were formed *in situ* deprotonation with silver oxide of **1a**–**f**. The structures of these compounds were characterized by spectroscopic and analytical methods. All of them were more effective against both Gram (−) and Gram (+) bacteria compared to yeast. In general, silver complexes showed better results than NHC precursors.

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Experimental

General considerations

All reactions for the synthesis of 1-(4-vinylbenzyl) substituted benzimidazolium salts (**1a**–**g**) and their silver complexes (**2a**–**f**) were made under argon. Chemical reagents and solvents were purchased from Sigma-Aldrich (Interlab, St Charles, MO) and Merck (Darmstadt, Germany). NMR (^{1}H and ^{13}C NMR) studies were performed in $CDCl_3$ and DMSO-d_6. The ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded with a Bruker AC300P FT spectrophotometer (Coventry, UK). Chemical shifts (δ) were given in ppm relative to tetramethylsilane as an internal reference. Coupling constants (J) were given in hertz (Hz). ^{1}H NMR signals were labeled as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), pentet (p), hextet (hex) or heptet (hept). Melting points (m.p.) were measured in glass capillary tubes using an Electrothermal-9200 melting point device. Elemental analyses were performed with the CHNS-932 LECO apparatus (Isomass Scientific Inc., Calgary, Canada). The FT-IR spectra of the synthesized new compounds were recorded in the 450–4000 cm^{-1} region using a Shimadzu FT-IR 8400 spectrophotometer (Shimadzu Middle East & Africa FZE, Japan).

Synthesis of new compounds

*General preparation of 1-(4-vinylbenzyl)-3-alkylbenzimidazolium salts, (**1**)*

The synthesis of benzimidazolium salts were prepared according to literature[1,21]. 1-(4-Vinylbenzyl)benzimidazole was synthesized from 4-vinylbenzyl chloride and benzimidazole in ethyl alcohol.

Alkyl halides (1 mmol) were added slowly to a solution of 1-(4-vinylbenzyl)benzimidazole (1 mmol) in *N*,*N*-dimethylformamide (DMF) (4 ml) at 25 °C and the mixture was mixed at 80 °C for 12 h. The formed salt was washed with diethyl ether (3 × 15 ml) and dried under vacuum. The product was crystallized from ethyl alcohol/diethyl ether mixture (2:1) at room temperature.

1-(4-Vinylbenzyl)-3-ethylbenzimidazolium chloride, ***1a***

Yield: 85%, m.p.: 280–281 °C, $IR_{(CN)}$: 1561.0 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6), δ: 1.59 (t, 3H, CH_2CH_3, $J = 7.2$ Hz); 4.57 (q, 2H, CH_2CH_3, $J = 7.2$ Hz); 5.28 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 10.2$ Hz); 5.85 (s, 2H, $CH_2C_6H_4CH{=}CH_2$); 5.88 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 17.7$ Hz); 6.73 (m, 1H, $CH_2C_6H_4CH{=}CH_2$); 7.52–8.13 (m, 8H, Ar–*H*); 10.46 (s, 1H, NC*H*N). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 14.6 and 42.6 (CH_2CH_3); 49.9 ($CH_2C_6H_4CH{=}CH_2$); 114.3, 114.6, 115.5, 126.9, 127.1, 129.2, 131.6, 134.0, 134.1, 136.4, 137.9 and 141.5 (Ar–*C* and $CH_2C_6H_4CH{=}CH_2$); 142.8 (2-*C*H). Anal. calc. for $C_{18}H_{19}N_2Cl$ (298.81 g/mol): C, 72.35%; H, 6.41%; N, 9.37%. Found: C, 72.50%; H, 6.30%; N, 9.41%.

1-(4-Vinylbenzyl)-3-butylbenzimidazolium chloride, ***1b***

Yield: 78%, m.p.: 141–142 °C, $IR_{(CN)}$: 1556.2 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6), δ: 0.94 (t, 3H, $CH_2CH_2CH_2CH_3$, $J = 7.2$ Hz); 1.35 (hex., 2H, $CH_2CH_2CH_2CH_3$, $J = 7.2$ Hz); 1.93 (p, 2H, $CH_2CH_2CH_2CH_3$, $J = 7.2$ Hz); 4.55 (t, 2H, $CH_2CH_2CH_2CH_3$, $J = 6.9$ Hz); 5.27 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 11.1$ Hz); 5.82 (s, 2H, $CH_2C_6H_4CH{=}CH_2$); 5.89 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 19.8$ Hz); 6.71 (m, 1H, $CH_2C_6H_4CH{=}CH_2$); 7.38–7.99 (m, 8H, Ar–*H*); 10.35 (s, 1H, NC*H*N). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 13.9, 19.6, 30.9 and 47.0 ($CH_2CH_2CH_2CH_3$); 50.1 ($CH_2C_6H_4CH{=}CH_2$); 114.4, 115.7, 127.1, 129.2, 131.3, 131.8, 134.0, 136.4 and 137.9 (Ar–*C* and $CH_2C_6H_4CH{=}CH_2$); 143.0 (2-*C*H). Anal. calc. for $C_{20}H_{23}N_2Cl$ (326.86 g/mol): C, 73.49%; H, 7.09%; N, 8.57%. Found: C, 73.55%; H, 7.17%; N, 8.52%.

1-(4-Vinylbenzyl)-3–(2-methoxyethyl)benzimidazolium chloride, ***1c***

Yield: 83%, m.p.: >355 °C, $IR_{(CN)}$: 1557.9 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6), δ: 2.52 (s, 3H, $CH_2CH_2OCH_3$); 3.81 (t, 2H, $CH_2CH_2OCH_3$, $J = 4.8$ Hz); 4.75 (t, 2H, $CH_2CH_2OCH_3$, $J = 4.8$ Hz); 5.29 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 10.8$ Hz); 5.83 (s, 2H, $CH_2C_6H_4CH{=}CH_2$); 5.85 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 18.0$ Hz); 6.73 (m, 1H, $CH_2C_6H_4CH{=}CH_2$); 7.51–8.14 (m, 8H, Ar–*H*); 10.05 (s, 1H, NC*H*N). Anal. calc. for $C_{19}H_{21}N_2OCl$ (328.84 g/mol): C, 69.40%; H, 6.44%; N, 8.52%. Found: C, 69.29%; H, 6.55%; N, 8.56%.

1-(4-Vinylbenzyl)-3-(2-ethoxyethyl)benzimidazolium chloride, ***1d***

Yield: 88%, m.p.: 127–128 °C, $IR_{(CN)}$: 1562.7 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6), δ: 1.07 (t, 3H, $CH_2CH_2OCH_2CH_3$, $J = 4.2$ Hz); 3.47 (q, 2H, $CH_2CH_2OCH_2CH_3$, $J = 4.2$ Hz); 3.95 (t, 2H, $CH_2CH_2OCH_2CH_3$, $J = 4.8$ Hz); 4.86 (t, 2H, $CH_2CH_2OCH_2CH_3$, $J = 4.8$ Hz); 5.25 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 11.1$ Hz); 5.70 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 17.7$ Hz); 5.87 (s, 2H, $CH_2C_6H_4CH{=}CH_2$); 6.63 (m, 1H, $CH_2C_6H_4CH{=}CH_2$); 7.28–7.99 (m, 8H, Ar–*H*); 11.41 (s, 1H, NC*H*N). Anal. Calc. for $C_{20}H_{23}N_2OCl$ (342.86 g/mol): C, 70.06%; H, 6.76%; N, 8.17%. Found: C, 70.15%; H, 6.67%; N, 8.21%.

1–(4-vinylbenzyl)-3-isopropylbenzimidazolium chloride, ***1e***

Yield: 78%, m.p.: >355 °C, $IR_{(CN)}$: 1554.8 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6), δ: 1.65 [d, 6H, $CH(CH_3)_2$, $J = 6.3$ Hz]; 5.04 [hept., 1H, $CH(CH_3)_2$, $J = 6.9$ Hz]; 5.28 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 10.8$ Hz); 5.73 (s, 2H, $CH_2C_6H_4CH{=}CH_2$); 5.83 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 17.4$ Hz); 6.71 (m, 1H, $CH_2C_6H_4CH{=}CH_2$); 7.25–8.46 (m, 8H, Ar–*H*); 10.05 (s, 1H, NC*H*N). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 21.9 and 50.3 [$CH(CH_3)_2$]; 51.3 ($CH_2C_6H_4CH{=}CH_2$); 114.3, 114.6, 115.8, 127.1, 127.3, 128.9, 131.2, 131.4, 133.8, 136.3 and 137.9 (Ar–*C* and $CH_2C_6H_4CH{=}CH_2$); 141.2 (NCHN). Anal. calc. for $C_{19}H_{21}N_2Cl$ (312.84 g/mol): C, 72.95%; H, 6.77%; N, 8.95%. Found: C, 72.86%; H, 6.81%; N, 8.99%.

1-(4-Vinylbenzyl)-3-(3,4,5-trimethoxybenzyl)benzimidazolium chloride, ***1f***

Yield: 90%, m.p.: 176–177 °C, $IR_{(CN)}$: 1557.5 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$), δ: 3.80 [s, 3H, $CH_2C_6H_2(OCH_3)_3$_3,4,5]; 3.86 [s, 6H, $CH_2C_6H_2(OCH_3)_3$_3,4,5]; 5.26 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 11.1$ Hz); 5.96 [s, 2H, $CH_2C_6H_2(OCH_3)_3$_3,4,5]; 5.87 (s, 2H, $CH_2C_6H_4CH{=}CH_2$); 5.72 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 18.3$ Hz); 6.63 (m, 1H, $CH_2C_6H_4CH{=}CH_2$); 6.87 [s, 2H, $CH_2C_6H_2(OCH_3)_3$_3,4,5]; 7.29–7.69 (m, 8H, Ar–*H*); 12.05 (s, 1H, NC*H*N). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 56.7 and 60.8 [$CH_2C_6H_2(OCH_3)_3$_3,4,5]; 51.4 ($CH_2C_6H_4CH{=}CH_2$); 51.8 [$CH_2C_6H_2(OCH_3)_3$_3,4,5]; 105.9, 113.7, 115.3, 127.1, 128.3, 129.5, 131.2, 131.3, 131.4, 132.1, 135.8, 138.4, 138.5 and 153.9 (Ar–*C* and $CH_2C_6H_4CH{=}CH_2$); 143.9 (NCHN). Anal. calc. for $C_{26}H_{27}N_2O_3Cl$ (450.96 g/mol): C, 69.25%; H, 6.03%; N, 6.21%. Found: C, 69.38%; H, 6.21%; N, 6.19%.

1-(4-Vinylbenzyl)-3-(2,3,4,5,6-pentamethylbenzyl)benzimidazolium chloride, ***1 g***

Yield: 84%, m.p.: >330 °C, $IR_{(CN)}$: 1557.7 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6), δ: 2.24 [s, 6H, $CH_2C_6(CH_3)_5$_3,5]; 2.26 [s, 6H, $CH_2C_6(CH_3)_5$_2,6]; 2.28 [s, 3H, $CH_2C_6(CH_3)_5$_4]; 5.29 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 10.5$ Hz); 5.73 [s, 2H, $CH_2C_6(CH_3)_5$_2,3,4,5,6]; 5.77 (s, 2H, $CH_2C_6H_4CH{=}CH_2$); 5.85 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 17.7$ Hz); 6.71 (m, 1H, $CH_2C_6H_4CH{=}CH_2$); 7.41–8.24 (m, 8H, Ar–*H*); 9.45 (s, 1H, NC*H*N). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 16.9, 17.2 and 17.5 [$CH_2C_6(CH_3)_5$_2,3,4,5,6]; 46.9 [$CH_2C_6(CH_3)_5$_2,3,4,5,6]; 50.0 ($CH_2C_6H_4CH{=}CH_2$); 114.5, 115.7, 126.2, 127.1, 127.3, 127.4, 128.7, 129.2, 131.5, 132.0, 133.5, 134.4, 136.4, 136.5 and 137.8 (Ar–*C* and $CH_2C_6H_4CH{=}CH_2$); 141.8 (NCHN). Anal. calc. for $C_{28}H_{31}N_2Cl$ (431.01 g/mol): C, 78.03%; H, 7.25%; N, 6.50%. Found: C, 78.12%; H, 7.40%; N, 6.44%.

General preparation of 1-(4-vinylbenzyl) substituted Ag-NHC complexes, ***2***

New Ag-NHC complexes were prepared according to literature[1,5,22,23]. Silver oxide (1 mmol), benzimidazolium salt (2 mmol) and activated molecular sieves in CH_2Cl_2 (25 ml) were stirred at 25 °C for 1 day in dark conditions. After the reaction finished, the mixture was filtered through celite. The solvent in the medium was aspirated under reduced pressure. The synthesized compound was crystallized from a dichloromethane/diethyl ether (2:1) mixture at room temperature.

[1-(4-Vinylbenzyl)-3-ethylbenzimidazol-2-ylidene]chlorosilver(I), ***2a***

Yield: 68%, m.p.: 131–132 °C, $IR_{(CN)}$: 1396.6 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6), δ: 1.44 (t, 3H, CH_2CH_3, $J = 7.2$ Hz); 4.52 (q, 2H, CH_2CH_3, $J = 7.2$ Hz); 5.24 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 10.8$ Hz); 5.71 (s, 2H, $CH_2C_6H_4CH{=}CH_2$); 5.72 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 15.3$ Hz); 6.68 (m, 1H, $CH_2C_6H_4CH{=}CH_2$); 7.33–7.47 (m, 8H, Ar–*H*). ^{13}C NMR

(75 MHz, DMSO-d_6), δ: 16.4 and 44.4 (CH_2CH_3); 52.1 ($CH_2C_6H_4CH{=}CH_2$); 112.5, 112.8, 115.3, 124.5, 126.9, 128.2, 133.4, 133.7, 136.3, 136.4, 136.5 and 137.4 (Ar–*C* and $CH_2C_6H_4CH{=}CH_2$); 188.3 (*C*–Ag). Anal. calc. for $C_{18}H_{18}N_2AgCl$ (405.67 g/mol): C, 53.29%; H, 4.47%; N, 6.91%. Found: C, 53.18%; H, 4.59%; N, 6.86%.

[1-(4-Vinylbenzyl)-3-butylbenzimidazol-2-ylidene]chlorosilver(I), ***2b***

Yield: 64%, m.p.: 132–133 °C, $IR_{(CN)}$: 1395.7 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6), δ: 0.89 (t, 3H, $CH_2CH_2CH_2CH_3$, $J = 7.2$ Hz); 1.31 (hex., 2H, $CH_2CH_2CH_2CH_3$, $J = 7.2$ Hz); 1.84 (p, 2H, $CH_2CH_2CH_2CH_3$, $J = 7.2$ Hz); 4.49 (t, 2H, $CH_2CH_2CH_2CH_3$, $J = 7.2$ Hz); 5.22 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 10.8$ Hz); 5.72 (s, 2H, $CH_2C_6H_4CH{=}CH_2$); 5.79 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 18.2$ Hz); 6.68 (m, 1H, $CH_2C_6H_4CH{=}CH_2$); 7.32–7.46 (m, 8H, Ar–*H*). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 14.1, 19.9, 32.4 and 48.9 ($CH_2CH_2CH_2CH_3$); 52.0 ($CH_2C_6H_4CH{=}CH_2$); 112.6, 112.8, 116.2, 124.5, 126.3, 128.1, 133.7, 133.8, 136.4, 136.5 and 137.4 (Ar–*C* and $CH_2C_6H_4CH{=}CH_2$); 188.9 (*C*–Ag). Anal. calc. for $C_{20}H_{22}N_2AgCl$ (433.72 g/mol): C, 55.38%; H, 5.11%; N, 6.46%. Found: C, 55.54%; H, 5.29%; N, 6.40%.

[1-(4-Vinylbenzyl)-3-(2-methoxyethyl)benzimidazol-2-ylidene]chlorosilver(I), ***2c***

Yield: 71%, m.p.: 134–135 °C, $IR_{(CN)}$: 1394.5 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6), δ: 3.42 (s, 3H, $CH_2CH_2OCH_3$); 3.43 (t, 2H, $CH_2CH_2OCH_3$, $J = 4.8$ Hz); 4.64 (t, 2H, $CH_2CH_2OCH_3$, $J = 4.8$ Hz); 5.23 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 11.1$ Hz); 5.69 (s, 2H, $CH_2C_6H_4CH{=}CH_2$); 5.76 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 17.7$ Hz); 6.67 (m, 1H, $CH_2C_6H_4CH{=}CH_2$); 7.14–7.81 (m, 8H, Ar–*H*). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 49.0, 52.0 and 71.2 ($CH_2CH_2OCH_3$); 58.8 ($CH_2C_6H_4CH{=}CH_2$); 112.6, 112.9, 115.4, 124.6, 126.9, 128.1, 133.5, 134.3, 136.3, 136.4 and 137.4 (Ar–*C* and $CH_2C_6H_4CH{=}CH_2$); 191.4 (*C*–Ag). Anal. calc. for $C_{19}H_{20}N_2OAgCl$ (435.7 g/mol): C, 52.38%; H, 4.63%; N, 6.43%. Found: C, 52.27%; H, 4.55%; N, 6.47%.

[1-(4-Vinylbenzyl)-3–(2-ethoxyethyl)benzimidazol-2-ylidene]chlorosilver(I), ***2d***

Yield: 63%, m.p.: 148–149 °C, $IR_{(CN)}$: 1455.0 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6), δ: 0.99 (t, 3H, $CH_2CH_2OCH_2CH_3$, $J = 6.9$ Hz); 3.39 (q, 2H, $CH_2CH_2OCH_2CH_3$, $J = 6.9$ Hz); 3.79 (t, 2H, $CH_2CH_2OCH_2CH_3$, $J = 4.2$ Hz); 4.66 (t, 2H, $CH_2CH_2OCH_2CH_3$, $J = 4.2$ Hz); 5.24 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 10.8$ Hz); 5.73 (s, 2H, $CH_2C_6H_4CH{=}CH_2$); 5.79 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 16.8$ Hz); 6.68 (m, 1H, $CH_2C_6H_4CH{=}CH_2$); 7.33–7.85 (m, 8H, Ar–*H*). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 15.4, 49.2, 52.0 and 66.1 ($CH_2CH_2OCH_2CH_3$); 68.9 ($CH_2C_6H_4CH{=}CH_2$); 112.7, 112.9, 115.3, 124.4, 124.5, 126.9, 127.0, 128.1, 133.5, 133.8, 134.3, 136.4, 136.5 and 137.4 (Ar–*C* and $CH_2C_6H_4CH{=}CH_2$); 189.3 (*C*–Ag). Anal. calc. for $C_{20}H_{22}N_2OAgCl$ (449.72 g/mol): C, 53.41%; H, 4.93%; N, 6.23%. Found: C, 53.59%; H, 4.81%; N, 6.29%.

[1-(4-Vinylbenzyl)-3-(3,4,5-trimethoxybenzyl)benzimidazol-2-ylidene]chlorosilver(I), ***2e***

Yield: 59%, m.p.: 225–226 °C, $IR_{(CN)}$: 1392.1 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6), δ: 3.61 [s, 3H, $CH_2C_6H_2(OCH_3)_3$–*4*]; 3.79 [s, 6H, $CH_2C_6H_2(OCH_3)_3$–*3,5*]; 5.24 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 11.1$ Hz); 5.65 [s, 2H, $CH_2C_6H_2(OCH_3)_3$–3,4,5]; 5.75 (s, 2H, $CH_2C_6H_4CH{=}CH_2$); 5.79 (d, 1H, $CH_2C_6H_4CH{=}CH_2$, $J = 18.3$ Hz); 6.72 (m, 1H, $CH_2C_6H_4CH{=}CH_2$); 6.79 [s, 2H, $CH_2C_6H_2(OCH_3)_3$–3,4,5]; 7.34–7.89 (m, 8H, Ar–*H*). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 56.3 and 60.5 [$CH_2C_6H_2(OCH_3)_3$–3,4,5]; 52.1 ($CH_2C_6H_4CH{=}CH_2$); 52.4 [$CH_2C_6H_2(OCH_3)_3$–3,4,5]; 105.9, 112.9, 115.3, 124.6, 126.9, 127.0, 128.1, 132.0, 133.7, 133.8, 133.9, 136.3, 136.4, 137.4, 137.5 and 153.5 (Ar–*C* and –*CH*=*CH*$_2$); 189.3 (*C*-Ag). Anal. calc. for $C_{26}H_{26}N_2O_3AgCl$ (557.82 g/mol): C, 55.98%; H, 4.70%; N, 5.02%. Found: C, 55.87%; H, 4.81%; N, 5.05%.

[1-(4-Vinylbenzyl)-3-(2,3,4,5,6-pentamethylbenzyl)benzimidazol-2-ylidene]chlorosilver(I), ***2f***

Yield: 55%, m.p.: 289–290 °C, $IR_{(CN)}$: 1395.0 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6), δ: 2.15–2.49 [m, 15H, $NCH_2C_6(CH_3)_5$–*2,3,4,5,6*]; 3.35 [s, 2H, $NCH_2C_6(CH_3)$–2, 3,4,5,6]; 5.59 (s, 2H, $CH_2C_6H_4CH{=}CH_2$); 5.22–6.73 (m, 3H, $CH_2C_6H_4CH{=}CH_2$); 7.23–8.23 (m, 8H, Ar–*H*). Anal. calc. for $C_{28}H_{30}N_2AgCl$ (537.87 g/mol): C, 62.52%; H, 5.62%; N, 5.21%. Found: C, 62.64%; H, 5.75%; N, 5.17%.

Evaluation of antibacterial and antifungal activity

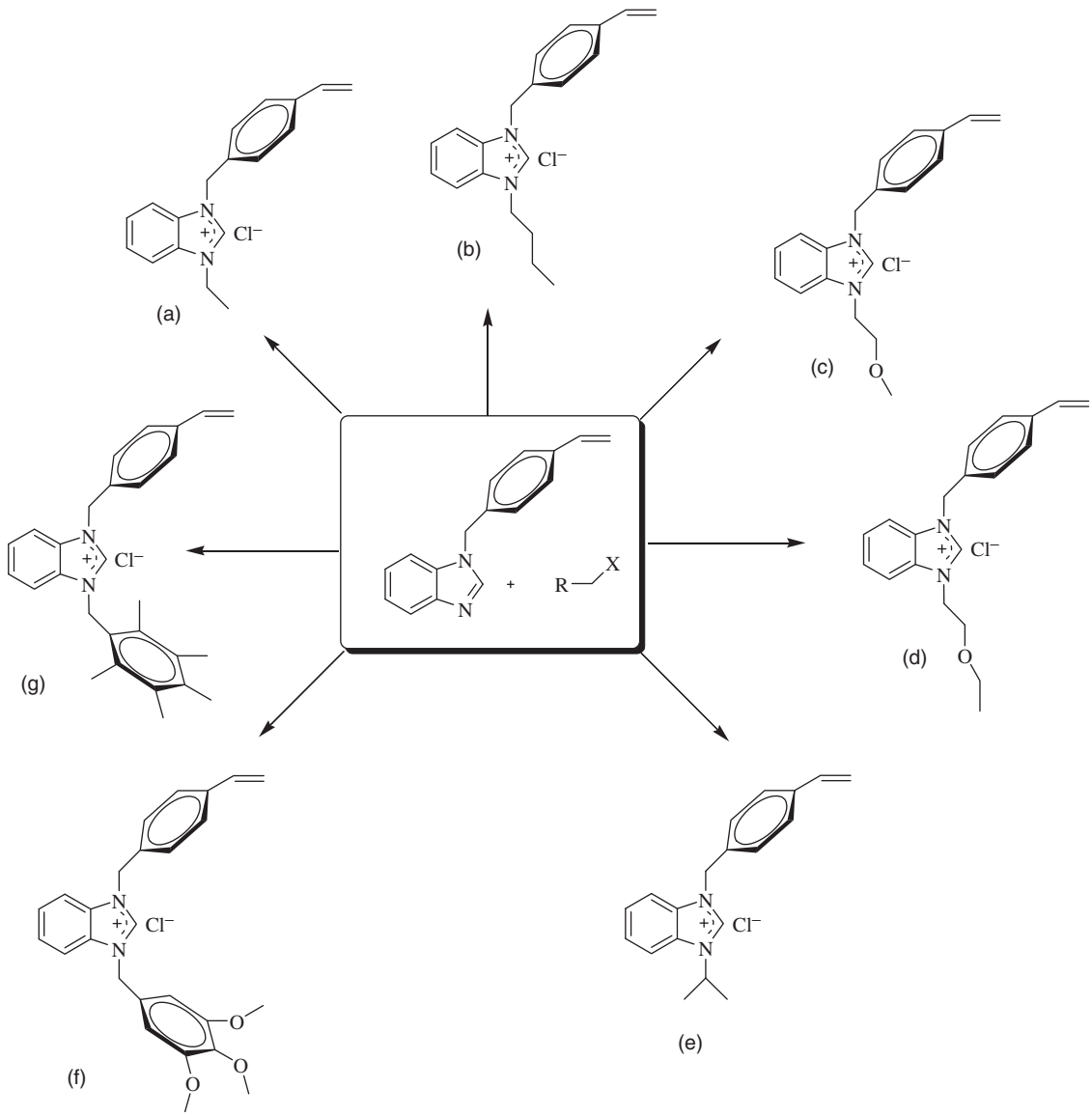
The following 11 microorganisms, consisting of 10 bacteria and 1 yeast, were studied: *Aeromonas hydrophila* (ATCC 7965), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (FMC 5), *Proteus mirabilis* (BC 3624), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (NRRLE 4463), *Yersinia enterocolitica* (ATCC 1501), *Bacillus cereus* (FMC 19), *Listeria monocytogenes* (1/2B), *Staphylococcus aureus* (ATCC 29213) and *Candida albicans* (ATCC 1223).

The antimicrobial activities of 13 compounds (**1a–g**; **2a–f**) were obtained *in vitro* using the agar-well diffusion method[19]. The stock solutions (10 mg/ml^{-1}) of the compounds were prepared by dissolving them in dimethyl sulfoxide (DMSO). All samples were sterilized through a 0.2-μm membrane filter. *Y. enterocolitica* and *C. albicans* were grown in nutrient broths and malt extract at room temperature (25 °C) for 18 h, respectively. The other bacteria were grown in nutrient broth at 35 °C for 18 h and then suspensions were adjusted to 0.5 McFarland standard turbidity. Two hundred and fifty microliters (250 μl) of each microorganism was added to a flask containing 25 ml of sterile Mueller Hinton agar or malt extract agar at 45 °C and poured into Petri dishes (9 cm diameter). Then, the agars were allowed to solidify at 4 °C for 1 h. Holes were made in the agar using sterile cork borers (Ø = 6 mm). The solutions of compounds (50 μl) were applied to the holes and DMSO was used as a control. *C. albicans* and *Y. enterocolitica* were incubated at room temperature for 14–24 h in the inverted position. The other microorganisms were incubated at 35 °C for 18–24 h. At the end of the period, the inhibition zones which formed on the medium were measured in millimeters (mm). Tetracycline (10 mg/ml) (Sigma T3258–56) and natamycin (30 mg/ml) (Delvocid DMS) standard antibiotics were used as positive controls.

Results and discussion

Synthesis of N-heterocyclic carbene precursors and their silver complexes

The 1-(4-vinylbenzyl)-3-alkylbenzimidazolium salts (**1a–g**) as NHC-precursors, which are stable against light, air and humidity both in the hard form and in the solution state, were obtained by quaternization of 1-(4-vinylbenzyl)benzimidazole with various alkyl halides in DMF (Scheme 1). These salts were soluble in polar solvents such as dimethylsulfoxide, ethyl alcohol and dimethyl formamide but were not soluble in common slightly polar or nonpolar solvents like diethyl ether and hexane. The structures of **1a–g** were verified using spectroscopic and



Scheme 1. Synthesis of 1-(4-vinylbenzyl) substituted new benzimidazolium salts.

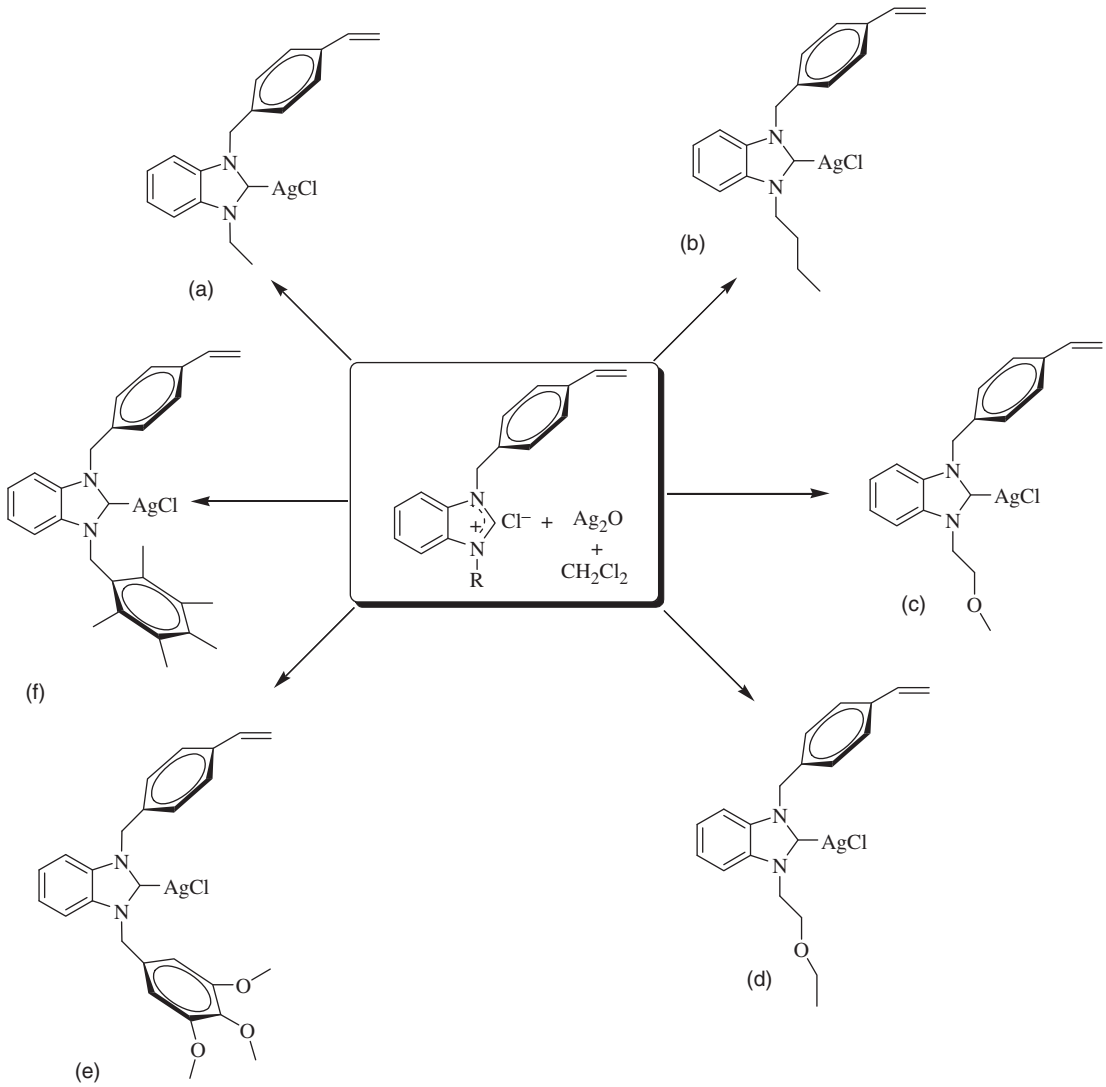
elemental analysis techniques. The ^{1}H NMR spectra of the benzimidazolium salts, which were collected in $CDCl_3$ and DMSO-d_6 over the scan range δ 0–12 ppm, further confirmed the designated structures; the resonances for NC*H*N were seen as a sharp singlet at δ 10.46, 10.35, 10.05, 11.41, 10.05, 12.05 and 9.45 ppm for **1a–g**, respectively.

^{13}C NMR chemical shifts were consistent with the suggested structures. The imino carbon resonances were observed as a typical singlet at 142.8, 143.0, 141.2, 143.9 and 141.8 ppm for benzimidazolium salts (**1a**, **1b**, **1e–g**), respectively. The N*CH*N proton and carbon signals of benzimidazolium salts were obtained in similar field with our previous studies[1,21,24]. The FT-IR data clearly indicated the presence of $\nu_{(CN)}$ at 1561.0, 1556.2, 1557.9, 1562.7, 1554.8, 1557.5 and 1557.7 cm^{-1} for the benzimidazolium salts (**1a–g**), respectively.

The complexes (**2a–f**) were synthesized by treatment of benzimidazolium salts with Ag_2O in CH_2Cl_2 (Scheme 2). To form of the Ag-NHC complexes, the reaction mixture was mixed for 24 h at 25 °C in dark conditions. In the ^{1}H NMR spectra, the lack of a downfield NC*H*N signal shows the successful formation of Ag-NHC complexes. The signal of benzylic proton (N–CH_2–Ar) belonging to Ag-NHC complexes was observed as a sharp singlet signal at around δ 5 ppm. These resonance values are very similar with resonance values of corresponding salts. The structures of the supposed Ag-NHC compounds were also verified with ^{13}C NMR, because the carbene carbon resonance is shifted downfield, such as at 188.3, 188.9, 191.4 189.3 and 189.3 ppm for new complexes **2a–g**. This signal shift is in accordance with literature reports[1,25,26]. Carbene carbon signal shifted downfield only. Other carbon signals of Ag-NHC complexes observed were similar to benzimidazolium salts. The FT-IR data clearly indicated the presence of $\nu_{(CN)}$ at 1396.6, 1395.7, 1394.5, 1455.0, 1392.1 and 1395.0 cm^{-1} for the Ag-NHC complexes (**2a–f**), respectively. Unfortunately, an appropriate single crystal from these synthesized complexes for X-ray diffraction was not obtained. However, in a previous study, we had obtained the single crystals of similar silver complexes in order to prove the coordination bond between the silver and carbon atoms[1,27].

Evaluation of the antimicrobial activity

The antimicrobial activity of the synthesized compounds was determined against three Gram (+) bacterial strains (*B. subtilis*, *L. monocytogenes*, *S. aureus*), seven Gram (−) bacterial strains (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, *S. typhimurium*, *Y. enterocolitica*) and one yeast (*C. albicans*) by agar diffusion assay. The results are given in Table 1. No inhibition zone was seen in the control (DMSO). All of the compounds demonstrated



Scheme 2. Synthesis of 1-(4-vinylbenzyl) substituted new Ag-NHC complexes.

Table 1. Antimicrobial activities of compounds **1a–g**, **2a–f** and reference drug against the tested microorganisms.

	Compounds													
Organisms	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**	Tetracycline (10 mg/ml)
Gram (−)														
A. hydrophila	9.0	10.0	9.0	11.0	–	11.0	17.0	15.0	17.0	14.0	17.0	17.0	16.0	22.0
E. coli	10.0	11.0	10.0	11.0	–	10.0	11.0	17.0	16.0	15.0	15.0	17.0	17.0	21.0
K. pneumoniae	9.0	12.0	9.0	12.0	–	12.0	18.0	16.0	17.0	17.0	16.0	18.0	17.0	23.0
P. mirabilis	10.0	14.0	9.0	11.0	–	12.0	17.0	14.0	14.0	11.0	12.0	13.0	14.0	24.0
P. aeruginosa	8.0	9.0	–	11.0	–	10.0	10.0	18.0	17.0	17.0	18.0	16.0	17.0	21.0
S. typhimurium	9.0	10.0	8.0	10.0	–	11.0	10.0	15.0	16.0	16.0	17.0	15.0	16.0	16.0
Y. enterocolitica	7.0	8.0	7.0	7.0	–	8.0	10.0	16.0	18.0	14.0	12.0	16.0	17.0	27.0
Gram (+)														
B. cereus	10.0	12.0	11.0	11.0	–	13.0	17.0	17.0	16.0	15.0	14.0	17.0	16.0	26.0
L. monocytogenes	11.0	14.0	11.0	12.0	–	14.0	17.0	16.0	16.0	17.0	17.0	16.0	17.0	21.0
S. aureus	10.0	12.0	10.0	11.0	–	11.0	17.0	15.0	17.0	16.0	15.0	18.0	19.0	23.0
Yeast														Natamycin (30 mg ml^{-1})
C. albicans	7.0	8.0	9.0	8.0	–	8.0	10.0	13.0	14.0	13.0	12.0	12.0	15.0	24.0

Notes: Inhibition zones include diameter of hole (6 mm). Sample amount 50 µl. −, not active.

potent antimicrobial activity against the microorganisms tested in this study. Generally, the silver complexes **2a–f** were more effective than benzimidazolium salts **1a–g**. Compound **1g** showed the most activity among the synthesized salts, which may be attributed to the presence of the aromatic group. The alkyl group which contains an electron-donating substituent may have contributed to the antibacterial activity of **1a–e** compounds. The antibacterial activity of the silver complexes was almost similar to that of the standard drug, tetracycline. In the case of silver complexes, their antimicrobial activities were similar to each

other. However, it may be said that **2d** was slightly less effective than the other silver complexes. Also, it was seen that *Y. enterocolitica* was more resistant to the salts among the Gram (−) bacterial strains. Silver complexes (12–15 mm, inhibition zones) were more potent antifungals than the salts (7–10 mm, inhibition zones). The **1b** salt was more effective against *P. mirabilis* and *L. monocytogenes* than against other organisms. The **2a** and **2d** complexes showed the highest activity against *P. aeruginosa*. Compared to all synthesized compounds, the **2f** complex was most effective against the Gram-positive bacteria (*S. aureus*) and yeast (*C. albicans*). Many studies have shown that benzimidazole derivatives are effective against different strains of microorganisms. 5,6-Dimethylbenzimidazole is a constituent of naturally occurring vitamin B_{12}. For this reason, benzimidazole derivatives have attracted the attention of researchers. Although vitamin B_{12} is able to induce the growth of bacteria, benzimidazole and some of its derivatives inhibit bacterial growth. Owing to the structural similarity to purine, the antibacterial ability of benzimidazole is explained by its competition with purines resulting in inhibition of the synthesis of bacterial nucleic acids and proteins[15].

Conclusion

In this study, seven benzimidazolium salts (**1a**–**g**) and six Ag-NHC complexes (**2a**–**f**) were synthesized and their structures were characterized by NMR, IR and elemental analysis. They were screened for their *in vitro* antimicrobial activities. These compounds displayed good antimicrobial potential. The biological activity results indicated that the silver complexes were found to have more potent antimicrobial properties than their salts. Thus, these newly synthesized compounds may be used as antimicrobial agents in the food and pharmaceutical industries. It is believed that these compounds will form the basis for the synthesis of pharmacologically important drugs.

Declaration of interest

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