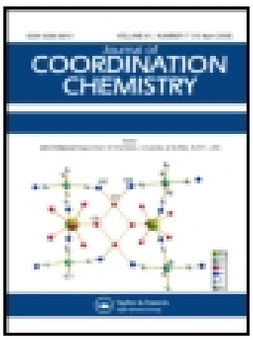

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Non-symmetrically *p*-nitrobenzyl- and *p*-cyanobenzyl-substituted *N*-heterocyclic carbene-silver(I) complexes: Synthesis, characterization and antibacterial studies

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Various nitro/nitrile-functionalized benzimidazol-2-ylidene carbene complexes of silver(I) (**7a-d** and **11a-d**) were synthesized by combination of 1-allyl/1-isopropyl/1-sec-butyl/1-isopentyl-3-(nitro/cyano-benzyl)-3*H*-benzimidazol-1-ium hexafluorophosphate (**6a-d** and **10a-d**) with silver(I) oxide in acetonitrile. The compounds were characterized by ^{1}H, ^{13}C NMR, FT-IR, mass spectrometry, and elemental analysis. Additionally, the *in vitro* antibacterial activity of the *N*-heterocyclic carbene (NHC) precursors (**6a-d** and **10a-d**) and their corresponding NHC-silver(I) complexes (**7a-d** and **11a-d**) were investigated against Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli* using the qualitative Kirby-Bauer disk diffusion method. All the NHC-silver(I) complexes exhibited medium-to-high antibacterial activity with areas of clearance ranging from 12 mm to 21 mm, while the NHC precursors were inactive against both strains of bacteria.

Keywords: NHCs; Silver complexes; Synthesis; Antibacterial; *Staphylococcus aureus*; *Escherichia coli*

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1. Introduction

N-Heterocyclic carbenes (NHCs) were first isolated when Arduengo documented a deprotonation of 1,3-bis(adamantyl)imidazolium chloride [1]. Ever since, NHCs experienced an exponential increase in research and applications. Researchers have developed a variety of convenient routes to prepare NHC-metal complexes with transition metals [2, 3]. NHC-metal complexes are known particularly as catalysts [4-8] for many chemical transformations; later discoveries revealed that NHC-silver and gold complexes can be used in medicinal applications [9-12].

Silver compounds have been used for medicinal applications for more than a hundred years for water purification, wound care antiseptics and infections. Silver nitrate was commonly used as an antimicrobial compound in the 17^{th} and 18^{th} centuries, before the discovery of penicillin. It was not until bacteria resistance to other antibiotics were reported that silver compounds regained importance. Antibacterial activity of silver compounds is attributed to soluble silver ions, Ag^{+}. Silver metal and insoluble silver salts, such as silver chloride, do not display antibacterial activity. The only side-effect of long-term silver treatment is the unpleasant color change (to grey or blue) of patients' skin. The effect called Argyria occurs due to irreversible formation and deposition of silver sulfide in dermis and eyes.

Silver sulfadiazine (figure 1) reintroduced silver compounds as antimicrobials in 1968 and has remained an effective topical-burn treatment [13, 14]. Soon after the discovery of *N*-heterocyclic carbenes, silver complexes with these ligands were synthesized for antimicrobial applications [15]. Research groups have reported the antibacterial activities of various *p*-nitrobenzyl- and *p*-cyanobenzyl-substituted NHC-silver(I) complexes against Gram-positive bacteria *Staphylococcus aureus* and the Gram-negative bacteria *Escherichia coli* [16-25]. All the reported NHC-silver complexes are light- and water-stable and can be synthesized in high yield with high purity from inexpensive commercially available starting materials and show medium-to-high antibacterial activities. These positive outcomes encouraged our research efforts on diverse *p*-nitrobenzyl- and *p*-cyanobenzyl-substituted NHC-silver complexes as potential antibacterial drug candidates.

We have focused on antibacterial studies of complexes [26]. Within this manuscript, we present the synthesis, characterization and preliminary *in vitro* antibacterial studies of a series of eight new non-symmetrically *p*-nitrobenzyl-substituted and *p*-cyanobenzyl-substituted NHC-silver(I) complexes. These compounds were tested on the Gram-positive bacteria

Staphylococcus aureus and the Gram-negative bacteria *Escherichia coli*. During the course of these studies, compounds **10b** and **11b** were reported by other researchers [17].

2. Experimental

2.1. *General considerations*

All reactions were carried out under aerobic conditions in oven-dried glassware with magnetic stirring. Benzimidazole, *p*-nitrobenzyl bromide, *p*-cyanobenzylbromide, allyl bromide, isopropyl bromide, 2-bromobutane, isopentyl bromide, potassium hexafluorophosphate, silver(I) oxide and potassium hydroxide were procured commercially from Sigma-Aldrich chemical company and used without purification. Heating was accomplished by either a heating mantle or silicone oil bath. Reactions were monitored by thin-layer chromatography (TLC) performed on 0.25 mm Merck TLC silica gel plates using UV light as a visualizing agent. Concentration under vacuum refers to the removal of volatile solvent using a rotary evaporator attached to a dry diaphragm pump (10-15 mm Hg) followed by pumping to a constant weight with an oil pump (<300 mTorr). ^{1}H NMR spectra were recorded at 500 MHz and are reported relative to DMSO-d_6 (δ 2.50 ppm). ^{1}H NMR coupling constants (J) are reported in Hertz (Hz) and multiplicities are indicated as follows: s (singlet), d (doublet), t (triplet), m (multiplet). Proton-decoupled ^{13}C NMR spectra were recorded at 125 MHz and reported relative to DMSO-d_6 (δ 39.5 ppm). IR spectra were recorded on an Alpha-P Bruker FT-IR spectrometer. Elemental analyses of compounds were done on a Euro Vector S.P.A, Euro EA 3000 CHNS elemental analyzer. Liquid chromatography mass spectra (LCMS) were recorded on an Agilent technologies quadrupole LC-MS system.

2.2. *Synthesis*

General experimental procedure for synthesis of 1-alkyl-3-(4-nitrobenzyl)benzimidazolium bromides (**5a-d**). Benzimidazole (**1**) (1.18 g, 10.0 mmol) and potassium hydroxide (0.84 g, 15.0 mmol) were stirred for 2 h in minimum DMSO at 100 °C. The temperature of the reaction mixture was reduced to 40 °C and alkyl bromide (10.0 mmol) was added in one portion and stirring was continued for a further 2 h. Afterwards the reaction mixture was cooled to room temperature and ice-cold water (200 mL) was added. The precipitate was separated by decantation, washed with water and dried under vacuum to get 1-alkylbenzimidazoles (**3a-d**).

The obtained 1-alkylbenzimidazoles (**3a-d**) were used in the next step without purification. The mixture of 4-nitrobenzyl bromide (**4**) (2.16 g, 10.0 mmol) and 1-alkylbenzimidazole (10.0 mmol) was stirred in dioxane (60 mL) at 100-110 °C for 24 h. Then the reaction mixture was cooled to room temperature. The precipitate was filtered, washed with fresh dioxane (30 mL) and dried under vacuum to get pure 1-alkyl-3-(4-nitrobenzyl)benzimidazolium bromides (**5a-d**).

1-Allyl-3-(4-nitrobenzyl)benzimidazolium bromide (**5a**)**.** Compound **5a** was prepared from allyl bromide (**2a**) (0.86 g, 10.0 mmol) and 1-allylbenzimidazole (**3a**) (1.58 g, 10.0 mmol) according to the general procedure. Light yellow solid. Yield: 70%.

1-Isopropyl-3-(4-nitrobenzyl)benzimidazolium bromide (**5b**)**.** Compound **5b** was prepared from isopropyl bromide (**2b**) (0.93 g, 10.0 mmol) and 1-isopropylbenzimidazole (**3b**) (1.60 g, 10.0 mmol), according to the general procedure. White solid. Yield: 76%.

1-(Sec-butyl)-3-(4-nitrobenzyl)benzimidazolium bromide (**5c**)**.** Compound **5c** was prepared from 2-bromobutane (**2c**) (1.08 g, 10.0 mmol) and 1-(sec-butyl)benzimidazole (**3c**) (1.74 g, 10.0 mmol), according to the general procedure. White solid. Yield: 80%.

1-Isopentyl-3-(4-nitrobenzyl)benzimidazolium bromide (**5d**)**.** Compound **5d** was prepared from 1-isopentyl bromide (**2d**) (1.19 g, 10.0mmol) and 1-isopentylbenzimidazole (**3d)** (1.88 g, 10.0 mmol), according to the general procedure. Yellow solid. Yield: 86%.

General experimental procedure for synthesis of 1-alkyl-3-(4-nitrobenzyl)benzimidazolium hexafluorophosphates (**6a-d**)**.** The bromide salts (**5a-d**) (10.0 mmol) were directly converted into their hexafluorophosphates counterpart by metathesis using KPF_6 (2.76 g, 15.0 mmol) in a mixture of methanol (45 mL) and water (5 mL). The resultant mixture was stirred for 4 h at room temperature and poured into water (200 mL). The separated precipitate was filtered and washed with water (100 mL) and dried under vacuum to obtain **6a-d** in high purity.

1-Allyl-3-(4-nitrobenzyl)benzimidazolium hexafluorophosphate (**6a**)**.** Compound **6a** was prepared from bromide salt (**5a**) (3.74 g, 10.0 mmol), according to the general procedure. White solid. Yield: 68%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 10.10 (s, 1H, NCHN), 8.26-8.28 (m, 2H, $CH_{Nitrobenzyl}$), 7.90-7.70 (m, 5H, $CH_{Nitrobenzyl}$+$CH_{Benzimid}$), 7.11-7.13 (m, 1H, $CH_{Benzimid}$), 6.33-6.35 (m, 1H, CH), 5.95-5.99 (m, 2H, CH_2), 5.20-2.22 (m, 2H, CH_2), 5.45-5.49 (m, 1H, CH), 5.25-5.20 (m, 1H, CH). ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm): 147.6, 142.9, 141.2, 131.2,

130.4, 129.4, 129.3, 127.3, 127.1, 123.9, 120.2, 113.9, 113.8 ($NCN+C_{Benzimid}+C_{Nitrobenzyl}+CH+CH_2$), 49.3 ($CH_2$), 49.1 ($CH_2$). Mass data (*m*/*z*, LC-MS): 295.0 [M^+-PF_6]. Anal. Calcd for $C_{17}H_{16}F_6N_3O_2P$: C, 46.48; H, 3.67; N, 9.57. Found: C, 46.45; H, 3.58; N, 9.80. IR (KBr, cm^{-1}) ν: 3145, 3020, 2944, 2850, 2759, 1640, 1550, 1365, 832.

1-Isopropyl-3-(4-nitrobenzyl)benzimidazolium hexafluorophosphate (**6b**)**.** Compound **6b** was prepared from bromide salt (**5b**) (3.76 g, 10.0 mmol), according to general the procedure. White solid. Yield: 75%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 10.00 (s, 1H, NCHN), 8.26 (d, *J* = 8.5 Hz, 2H, $CH_{Nitrobenzyl}$), 8.17 (d, *J* = 8.0 Hz, 1H, $CH_{Benzimid}$), 7.87 (d, *J* = 8.0 Hz, 1H, $CH_{Benzimid}$), 7.77 (d, *J* = 8.5 Hz, 2H, $CH_{Nitrobenzyl}$), 7.63-7.71 (m, 2H, $CH_{Benzimid}$), 5.93 (s, 2H, CH_2), 5.07-5.12 (m, 1H, CH), 1.68 (d, *J* = 6.5 Hz, 6H, CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm): 147.6, 141.5, 141.4, 131.0, 130.8, 129.3, 126.9, 126.7, 124.0, 114.3, 113.7 ($NCN+C_{Benzimid}+C_{Nitrobenzyl}$), 50.9 (CH), 49.2($CH_2$), 21.4 ($CH_3$). Mass data (*m*/*z*, LC-MS): 297.0 [M^+-PF_6]. Anal. Calcd for $C_{17}H_{18}F_6N_3O_2P$: C, 46.27; H, 4.11; N, 9.52. Found: C, 46.21; H, 4.10; N, 9.49. IR (KBr, cm^{-1}) ν: 3140, 3019, 3112, 2941, 2883, 2849, 2760, 1645, 1557, 1360, 831.

1-(Sec-butyl)-3-(4-nitrobenzyl)benzimidazolium hexafluorophosphate (**6c**)**.** Compound **6c** was prepared from **5c** (3.90 g, 10 mmol), according to the general procedure. White solid. Yield: 80%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 10.06 (s, 1H, NCHN), 8.27 (d, *J* = 8.5 Hz, 2H, $CH_{Nitrobenzyl}$), 8.19 (d, *J* = 7.5 Hz, 1H, $CH_{Benzimid}$), 7.91 (d, *J* = 7.5 Hz, 1H, $CH_{Benzimid}$), 7.76 (d, *J* = 8.5 Hz, 2H, $CH_{Nitrobenzyl}$), 7.64-7.71 (m, 2H, $CH_{Benzimid}$), 5.94 (s, 2H, CH_2), 4.91-4.97 (m, 1H, CH), 1.96-2.11 (m, 2H, CH_2), 1.68 (d, *J* = 6.5 Hz, 3H, CH_3), 0.90 (t, *J* = 7.5 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm): 147.6, 141.7, 141.4, 131.0, 129.5, 129.3, 127.0, 126.8, 124.0, 114.3, 113.8 ($NCN+C_{Benzimid}+C_{Nitrobenzyl}$), 56.2 (CH), 49.3 ($CH_3$), 28.4 ($CH_2$), 19.2 ($CH_3$), 10.0 ($CH_3$). Mass data (*m*/*z*, LC-MS): 311.0 [M^+-PF_6]. Anal. Calcd for $C_{18}H_{20}F_6N_3O_2P$: C, 47.48; H, 4.43; N, 9.23. Found: C, 47.45; H, 4.38; N, 9.21. IR (KBr, cm^{-1}) ν: 3139, 3021, 2942, 2880, 2850, 2761, 1641, 1555, 1363, 830.

1-Isopentyl-3-(4-nitrobenzyl)benzimidazolium hexafluorophosphate (**6d**)**.** Compound **6d** was prepared from **5d** (4.04 g, 10.0 mmol), according to the general procedure. White solid. Yield: 85%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 9.91 (s, 1H, NCHN), 8.21-8.24 (m, 2H,$CH_{Benzimid}$), 8.08 (d, *J* = 8.0 Hz, 1H, $CH_{Nitrobenzyl}$), 7.86 (d, *J* = 8.0 Hz, 1H, $CH_{Nitrobenzyl}$), 7.70 (d, *J* = 8.5 Hz, 2H, $CH_{Nitrobenzyl}$), 7.59-7.67 (m, 2H, $CH_{Benzimid}$), 5.89 (s, 2H, CH_2), 4.49 (t, *J* =

7.5 Hz, 2H, CH_2), 1.80-1.85 (m, 2H, CH_2), 1.61-1.68 (m, 1H, CH), 0.94 (d, J = 6.5 Hz, 6H, CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm): 147.6, 142.8, 141.4, 131.3, 130.9, 129.4, 126.9, 126.8, 124.0, 114.0, 113.7 (NCN+$C_{Benzimid}$+$C_{Nitrobenzyl}$), 49.1 (CH_2), 45.4 (CH_2), 36.9 (CH_2), 25.2 (CH), 22.1 (CH_3). Mass data (*m*/*z*, LC-MS): 325.0 [M^+-PF_6]. Anal. Calcd for $C_{19}H_{22}F_6N_3O_2P$: C, 48.62; H, 4.72; N, 8.95. Found: C, 48.57; H, 4.70; N, 8.91. IR (KBr, cm^{-1}) ν: 3140, 3015, 2944, 2885, 2849, 2760, 1640, 1547, 1350, 834.

General experimental procedure for synthesis of bis-(1-alkyl-3-(4-nitrobenzyl)benzimidazolium)-silver(I) hexafluorophosphates (7a-d). 1-Alkyl-3-(4-nitrobenzyl)benzimidazolium hexafluorophosphate (5.0 mmol) was dissolved in acetonitrile (80 mL) and silver(I) oxide (2.31 g, 10.0 mmol) was added. The mixture was stirred at 45 °C for 1 d. Light was excluded by covering the flask with aluminum foil. The reaction mixture was filtered through a pad of Celite and the resultant filtrate was concentrated to 5 mL under vacuum. Fine grey precipitate was afforded by addition of diethylether (20 mL) to the filtrate. The precipitate was filtered, washed with diethylether (20 mL) and dried by suction at room temperature for 30 min to get pure (**7a-d**).

Bis-(1-allyl-3-(4-nitrobenzyl)benzimidazolium)-silver(I) hexafluorophosphate (7a). Compound **7a** was prepared from 1-allyl-3-(4-nitrobenzyl)benzimidazolium hexafluorophosphate (**6a**) (2.19 g, 5.0 mmol), according to the general procedure. Grey solid. Yield: 67%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 8.12-8.14 (m, 2H, $CH_{Nitrobenzyl}$), 7.71-7.72 (m, 2H, $CH_{Nitrobenzyl}$), 7.46-7.53 (m, 10H, $CH_{Nitrobenzyl}$+$CH_{Benzimid}$), 7.05-7.06 (m, 2H, $CH_{Benzimid}$), 6.22-6.25 (m, 2H, CH), 5.92-5.96 (m, 8H, CH_2), 5.17-5.30 (m, 4H, CH_2). ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm): 190.3, 147.0, 143.8, 133.7, 132.6, 129.6, 128.2, 128.1, 124.83, 123.8, 118.1, 112.5, 112.3 (NCN+$C_{Benzimid}$+$C_{Nitrobenzyl}$+CH+CH_2), 50.9 (CH_2), 50.4 (CH_2). Mass data (*m*/*z*, LC-MS): 696.0[$M]^+$. Anal. Calcd for $C_{34}H_{32}AgF_6N_6O_4P$: C, 48.53; H, 3.83; N, 9.90; Ag, 12.82. Found: C, 48.52; H, 3.80; N, 9.89; Ag, 12.79. IR (KBr, cm^{-1}) ν: 3142, 3019, 2938, 2848, 2760, 1828, 1641, 1551, 1350, 1242, 830.

Bis-(1-isopropyl-3-(4-nitrobenzyl)benzimidazolium)-silver(I) hexafluorophosphate (7b). Compound **7b** was prepared from 1-isopropyl-3-(4-nitrobenzyl)benzimidazolium hexafluorophosphate (**6b**) (2.20 g, 5.0 mmol), according to the general procedure. Grey solid.

Yield: 71%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 8.13 (d, *J* = 9.0 Hz, 4H, $CH_{Nitrobenzyl}$), 7.98 (d, *J* = 8.0 Hz, 2H, $CH_{Nitrobenzyl}$), 7.66 (d, *J* = 8.0 Hz, 2H, $CH_{Nitrobenzyl}$), 7.39-7.47 (m, 8H, $CH_{Benzimid}$), 5.92 (s, 4H, CH_2), 5.09-5.15 (m, 2H, CH), 1.68 (d, *J* = 7.0 Hz, 12H, CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm): 188.0, 147.0, 144.0, 133.6, 132.5, 128.0, 124.4, 124.1, 123.8, 113.0, 112.3 (NCN+$C_{Benzimid}$+$C_{Nitrobenzyl}$), 52.4 (CH), 51.2 (CH_2), 22.4 (CH_3). Mass data (*m*/*z*, LC-MS): 700.0 $[M]^+$. Anal. Calcd for $C_{34}H_{36}AgF_6N_6O_4P$: C, 48.30; H, 4.29; N, 9.94; Ag, 12.76. Found: C, 48.29; H, 4.30; N, 9.92; Ag, 12.68. IR (KBr, cm^{-1}) ν: 3143, 3023, 2941, 2880, 2841, 2757, 1826, 1637, 1551, 1363, 1267, 832.

Bis-(1-(*sec*-butyl)-3-(4-nitrobenzyl)benzimidazolium)-silver(I) hexafluorophosphate (7c). Compound **7c** was prepared from 1-(*sec*-butyl)-3-(4-nitrobenzyl)benzimidazolium hexafluorophosphate (**6c**) (2.27 g, 5.0 mmol), according to the general procedure. Grey solid. Yield: 77%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 8.14 (d, *J* = 9.0 Hz, 4H, $CH_{Nitrobenzyl}$), 7.97 (d, *J* = 7.5 Hz, 2H, $CH_{Nitrobenzyl}$), 7.67 (d, *J* = 7.5 Hz, 2H, $CH_{Nitrobenzyl}$), 7.39-7.47 (m, 8H, $CH_{Benzimid}$), 5.93 (s, 4H, CH_2), 4.80-4.89 (m, 2H, CH), 1.96-2.03 (m, 4H, CH_2), 1.67 (d, *J* = 7.0 Hz, 6H, CH_3), 0.75 (t, *J* = 7.5 Hz, 6H, CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm): 188.2, 147.0, 144.1, 133.6, 132.9, 127.8, 124.4, 124.2, 123.9, 112.9, 112.3 (NCN+$C_{Benzimid}$+$C_{Nitrobenzyl}$), 58.1 (CH), 51.1 (CH_3), 29.0 (CH_2), 20.8 (CH_3), 10.9 (CH_3). Mass data (*m*/*z*, LC-MS): 730.0 $[M]^+$. Anal. Calcd for $C_{36}H_{40}AgF_6N_6O_4P$: C, 49.50; H, 4.62; N, 9.62; Ag, 12.35. Found: C, 49.48; H, 4.63; N, 9.59; Ag, 12.32. IR (KBr, cm^{-1}) ν: 3142, 3039, 2881, 2849, 2760, 1853, 1649, 1551, 1350, 1245, 830.

Bis-(1-isopentyl-3-(4-nitrobenzyl)benzimidazolium)-silver(I) hexafluorophosphate (7d). Compound **7d** was prepared from 1-isopentyl-3-(4-nitrobenzyl)benzimidazolium hexafluorophosphate (**6d**) (2.34 g, 5.0 mmol), according to the general procedure. Grey solid. Yield: 84%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 8.17 (d, *J* = 8.5 Hz, 4H, $CH_{Nitrobenzyl}$), 7.83 (d, *J* = 8.0 Hz, 2H, $CH_{Nitrobenzyl}$), 7.63 (d, *J* = 8.0 Hz, 2H, $CH_{Nitrobenzyl}$), 7.38-7.50 (m, 8H, $CH_{Benzimid}$), 5.91 (s, 4H, CH_2), 4.51 (t, *J* = 7.5 Hz, 4H, CH_2), 1.68-1.76 (m, 4H, CH_2), 1.56-1.64 (m, 2H, CH), 0.91 (d, *J* = 6.5 Hz, 12H, CH_3), 0.75 (t, *J* = 7.5 Hz, 6H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm): 189.7, 171.5, 147.1, 144.1, 133.3, 133.2, 128.2, 124.2, 123.9, 112.3, 112.2 (NCN+$C_{Benzimid}$+$C_{Nitrobenzyl}$), 51.0 (CH_2), 47.3 (CH_2), 38.9 (CH_2), 25.3 (CH), 22.4 (CH_3). Mass data (*m*/*z*, LC-MS): 758.0 $[M]^+$. Anal. Calcd for $C_{38}H_{44}AgF_6N_6O_4P$: C, 50.62; H, 4.92; N, 9.32;

Ag, 11.96. Found: C, 50.61; H, 4.90; N, 9.31; Ag, 11.93. IR (KBr, cm^{-1}) ν: 3142, 3041, 29445, 2883, 2850, 2759, 1878, 1638, 1550, 1348, 1244, 832.

General experimental procedure for synthesis of 1-alkyl-3-(4-cyanobenzyl)benzimidazolium bromides (9a-d). The mixture of 4-cyanobenzyl bromide (**8**) (1.96 g, 10.0 mmol) and 1-alkylbenzimidazole (10.0 mmol) was stirred in dioxane (60 mL) at 100-110 °C for 24 h. Then the reaction mixture was cooled to room temperature. The isolated precipitate was filtered, washed with fresh dioxane (30 mL) and dried under vacuum to get pure 1-alkyl-3-(4-cyanobenzyl)benzimidazolium bromides (**9a-d**).

1-Allyl-3-(4-cyanobenzyl)benzimidazolium bromide (9a). Compound **9a** was prepared from allyl bromide (**2a**) (0.86 g, 10.0 mmol) and 1-allylbenzimidazole (**3a**) (1.58 g, 10.0 mmol), according to the general procedure. Light yellow solid. Yield: 72%.

1-Isopropyl-3-(4-cyanobenzyl)benzimidazolium bromide (9b). Compound **9b** was prepared from isopropyl bromide (**2b**) (0.93 g, 10.0 mmol) and 1-isopropylbenzimidazole (**3b**) (1.60 g, 10.0 mmol), according to the general procedure. White semi solid. Yield: 74%.

1-(Sec-butyl)-3-(4-nitrobenzyl)benzimidazolium bromide (9c). Compound **9c** was prepared from 1-bromobutane (**2c**) (1.08 g, 10.0 mmol) and 1-(*sec*-butyl)benzimidazole (**3c**) (1.74 g, 10.0 mmol), according to general procedure. White solid. Yield: 83%.

1-Isopentyl-3-(4-cyanobenzyl)benzimidazoliumbromide (9d). Compound **9d** was prepared from 1-isopentyl bromide (**2d**) (1.19 g, 10.0 mmol) and 1-isopentylbenzimidazole (**3d**) (1.88 g, 10.0 mmol), according to the general procedure. White solid. Yield: 89%.

General experimental procedure for synthesis of 1-alkyl-3-(4-cyanobenzyl)benzimidazolium hexafluorophosphates (10a-d). The bromide salts (**9a-d**) (10.0 mmol) were directly converted into their hexafluorophosphate counterpart by metathesis using KPF_6 (2.76 g, 15.0 mmol) in a mixture of methanol (45 mL) and water (5 mL). The resultant mixture was stirred for 4 h at room temperature and poured into water (200 mL). The separated white precipitate was filtered and washed with water (100 mL) and dried under vacuum to obtain (**10a-d**) in high purity.

1-Allyl-3-(4-cyanobenzyl)benzimidazolium hexafluorophosphate (**10a**)**.** Compound **10a** was prepared from **9a** (3.54 g, 10.0 mmol), following the general procedure. Light yellow solid. Yield: 76%. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.08 (s, 1H, NCHN), 7.89-7.92 (m, 3H, $CH_{Cyanobenzyl}$), 7.67-7.74 (m, 4H, $CH_{Benzimid}$), 7.10-7.12 (m, 1H, $CH_{Cyanobenzyl}$), 6.31-6.34 (m, 1H, CH), 5.89-5.92 (m, 2H, CH_2), 5.90 (s, 2H, CH_2), 5.50-5.41 (m, 1H, CH), 5.20-5.18 (m, 1H, CH). ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm): 142.8, 139.2, 132.8, 131.2, 130.3, 129.3, 129.0, 127.2, 127.1, 120.2, 118.4, 113.9, 113.7, 111.4 (NCN+ $C_{Benzimid}$+$C_{Cyanobenzyl}$+CH+CH_2), 49.5 (CH_2), 49.2 (CH_2). Mass data (*m*/*z*, LC-MS): 275.0 [M^+-PF_6]. Anal. Calcd for $C_{18}H_{16}F_6N_3P$: C, 51.56; H, 3.85; N, 10.02. Found: C, 51.53; H, 3.88; N, 10.00. IR (KBr, cm^{-1}) ν: 3144, 3015, 2944, 2848, 2760, 2251, 1645, 838.

1-Isopropyl-3-(4-cyanobenzyl)benzimidazolium hexafluorophosphate (**10b**)**.** Compound **10b** was prepared from **9b** (3.56 g, 10.0 mmol), following the general procedure. White solid. Yield: 78%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 10.01 (s, 1H, NCHN), 8.16 (d, *J* = 8.0 Hz, 1H, $CH_{Cyanobenzyl}$), 7.90 (d, *J* = 8.0 Hz, 2H, $CH_{Cyanobenzyl}$), 7.85 (d, *J* = 8.0 Hz, 1H, $CH_{Cyanobenzyl}$), 7.63-7.71 (m, 4H, $CH_{Benzimid}$), 5.86 (s, 2H, CH_2), 5.05-5.11 (m, 1H, CH), 1.67 (d, *J* = 6.5 Hz, 6H, CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm): 141.5, 139.5, 132.8, 131.0, 130.8, 128.9, 126.9, 126.7, 118.4, 114.3, 113.7 111.4 (NCN+CN+$C_{Benzimid}$+$C_{Cyanobenzyl}$), 50.9 (CH), 49.2 (CH_2), 21.4 (CH_3). Mass data (*m*/*z*, LC-MS): 277.0 [M^+-PF_6]. Anal. Calcd for $C_{18}H_{18}F_6N_3P$: C, 51.31; H, 4.31; N, 9.97. Found: C, 51.28; H, 4.34; N, 9.93. IR (KBr, cm^{-1}) ν: 3145, 3113, 3022, 2943, 2843, 2759, 2249, 1640, 835.

1-(*Sec*-butyl)-3-(4-cyanobenzyl)benzimidazolium hexafluorophosphate (**10c**)**.** Compound **10c** was prepared from **9c** (3.70 g, 10.0 mmol), following the general procedure. White solid. Yield: 84%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 10.04 (s, 1H, NCHN), 8.18 (d, *J* = 8.0 Hz, 1H, $CH_{Cyanobenzyl}$), 7.89-7.94 (m, 3H, $CH_{Cyanobenzyl}$), 7.64-7.72 (m, 4H, $CH_{Benzimid}$), 5.91 (s, 2H, CH_2), 4.90-4.94 (m, 1H, CH), 2.00-2.04 (m, 2H, CH_2), 1.67 (d, *J* = 6.5 Hz, 3H, CH_3), 0.89 (t, *J* = 7.5 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm): 143.5, 141.6, 139.4, 132.9, 130.9, 127.0, 126.9, 126.7, 118.4, 114.3, 113.8, 111.47 (NCN+CN+$C_{Benzimid}$+$C_{Cyanobenzyl}$), 56.2 (CH), 49.5 (CH_2), 28.3 (CH_2), 19.2 (CH_3), 10.0 (CH_3). Mass data (*m*/*z*, LC-MS): 291.0 [M^+-PF_6]. Anal. Calcd for $C_{19}H_{20}F_6N_3P$: C, 52.42; H, 4.63; N, 9.65. Found: C, 52.40; H, 4.67; N, 9.68. IR (KBr, cm^{-1}) ν: 3143, 3024, 2942, 2847, 2758, 2236, 1641, 840.

1-Isopentyl-3-(4-cyanobenzyl)benzimidazolium hexafluorophosphate (**10d**)**.** Compound **10d** was prepared from **9d** (3.84 g, 10.0 mmol), following the general procedure. White solid. Yield: 89%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 9.92 (s, 1H, NCHN), 8.12 (d, *J* = 8.0 Hz, 1H, $CH_{Cyanobenzyl}$), 7.88-7.92 (m, 3H, $CH_{Cyanobenzyl}$), 7.64-7.71 (m, 4H, $CH_{Benzimid}$), 5.86 (s, 2H, CH_2), 4.52 (t,*J*= 7.5 Hz, 2H, CH_2), 1.84-1.89 (m, 2H, CH_2), 1.65-1.71 (m, 1H, CH), 0.98 (d, *J* = 6.5 Hz, 6H, CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm): 142.7, 139.5, 132.9, 131.4, 130.9, 129.0, 126.9, 126.8, 118.4, 114.0, 113.7, 111.5 (NCN+CN+$C_{Benzimid}$+$C_{Cyanobenzyl}$), 49.4 (CH_2), 45.4 (CH_2), 36.9 (CH_2), 25.2 (CH), 22.2 (CH_3). Mass data (*m*/*z*, LC-MS): 305.0 [M^+-PF_6]. Anal. Calcd for $C_{20}H_{22}F_6N_3P$: C, 53.46; H, 4.93; N, 9.35. Found: C, 53.41; H, 4.92; N, 9.33. IR (KBr, cm^{-1}) ν: 3144, 3033, 2940, 2845, 2758, 2243, 1641, 835.

General experimental procedure for synthesis of bis-(1-alkyl-3-(4-cyanobenzyl)benzimidazolium)-silver(I) hexafluorophosphates (**11a-d**). 1-Alkyl-3-(4-cyanobenzyl)benzimidazolium hexafluorophosphate (5.0 mmol) was dissolved in acetonitrile (80 mL) and silver(I) oxide (2.31 g, 10.0 mmol) was added. The mixture was stirred at room temperature for 2 d. Light was excluded by covering the flask by aluminum foil. The reaction mixture was filtered through a pad of Celite, and the resulted filtrate was concentrated to 5 mL under vacuum. Addition of diethylether (20 mL) gave grey precipitate, which was isolated by filtration, washed with diethylether (20 mL) and dried under vacuum to obtain pure (**11a-d**).

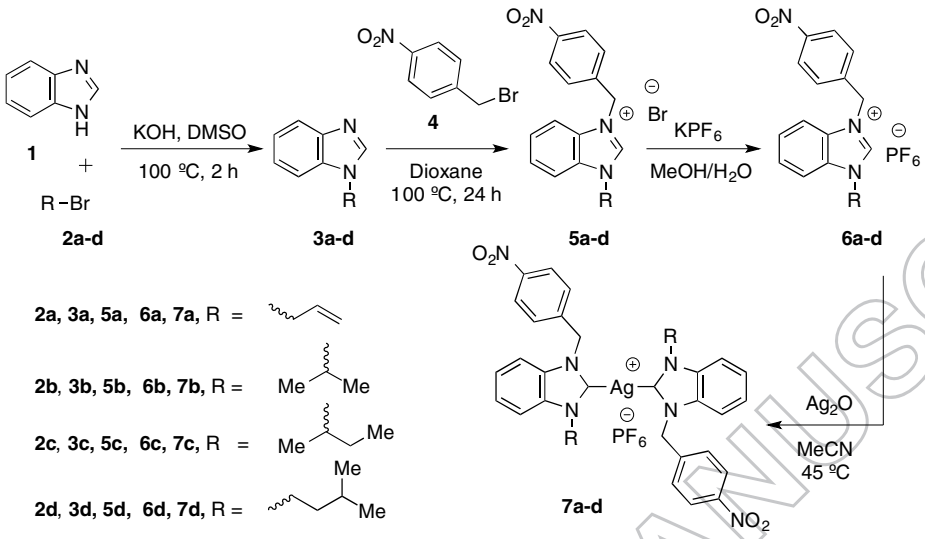
Bis-(1-allyl-3-(4-cyanobenzyl)benzimidazolium)-silver(I) hexafluorophosphate (**11a**). Compound **11a** was prepared from 1-allyl-3-(4-cyanobenzyl)benzimidazolium hexafluorophosphate (**10a**) (2.09 g, 5.0 mmol), following the general procedure. Grey solid. Yield: 76%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 7.78-7.80 (m, 2H, $CH_{Cyanobenzyl}$), 7.71-7.72 (m, 2H, $CH_{Cyanobenzyl}$), 7.44-7.53 (m, 10H, $CH_{Cyanobenzyl}$+$CH_{Benzimid}$), 7.03-7.05 (m, 2H, $CH_{Benzimid}$), 6.20-6.23 (m, 2H, CH), 5.86-5.90 (m, 8H, CH_2), 5.16-5.30 (m, 4H, CH_2). ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm): 190.2, 141.8, 133.7, 132.6, 129.5, 127.9, 124.8, 124.7, 124.6, 118.4, 118.1, 112.5, 112.3, 110.8 (NCN+ $C_{Benzimid}$+$C_{Cyanobenzyl}$+CH+CH_2), 51.2 (CH_2), 51.1 (CH_2). Mass data (*m*/*z*, LC-MS): 632.0 $[M]^+$. Anal. Calcd for $C_{36}H_{32}AgF_6N_6P$: C, 53.95; H, 4.02; N, 10.49; Ag, 13.46. Found: C, 53.92; H, 4.00; N, 10.45; Ag, 13.41. IR (KBr, cm^{-1}) ν: 3143, 3020, 2939, 2845, 2759, 2244, 1843, 1643, 1244, 833.

Bis-(1-isopropyl-3-(4-cyanobenzyl)benzimidazolium)-silver(I) hexafluorophosphate (11b). Compound **11b** was prepared from 1-isopropyl-3-(4-cyanobenzyl)benzimidazolium hexafluorophosphate (**10b**) (2.10 g, 5.0 mmol), following the general procedure. Grey solid. Yield: 79%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 7.97-7.98 (m, 2H, $CH_{Cyanobenzyl}$), 7.77-7.83 (m, 4H, $CH_{Cyanobenzyl}$), 7.66-7.67 (m, 2H, $CH_{Cyanobenzyl}$), 7.39-7.44 (m, 8H, $CH_{Benzimid}$), 5.80-5.86 (m, 4H, CH_2), 5.07-5.09 (m, 2H, CH), 1.65 (d, J = 76.5 Hz, 12H, CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm): 187.8, 167.3, 142.0, 133.6, 132.4, 127.7, 126.8, 124.1, 118.4, 113.0, 112.2, 110.7 (NCN+CN+$C_{Benzimid}$+$C_{Cyanobenzyl}$), 52.3 (CH), 51.4 (CH_2), 22.3 (CH_3). Mass data (*m*/*z*, LC-MS): 660.0 $[M]^+$. Anal. Calcd for $C_{36}H_{36}AgF_6N_6P$: C, 53.68; H, 4.50; N, 10.43; Ag, 13.39. Found: C, 53.69; H, 4.51; N, 10.42; Ag, 13.35. IR (KBr, cm^{-1}) ν: 3142, 3056, 2941, 2847, 2761, 2260, 1863, 1644, 1272, 840.

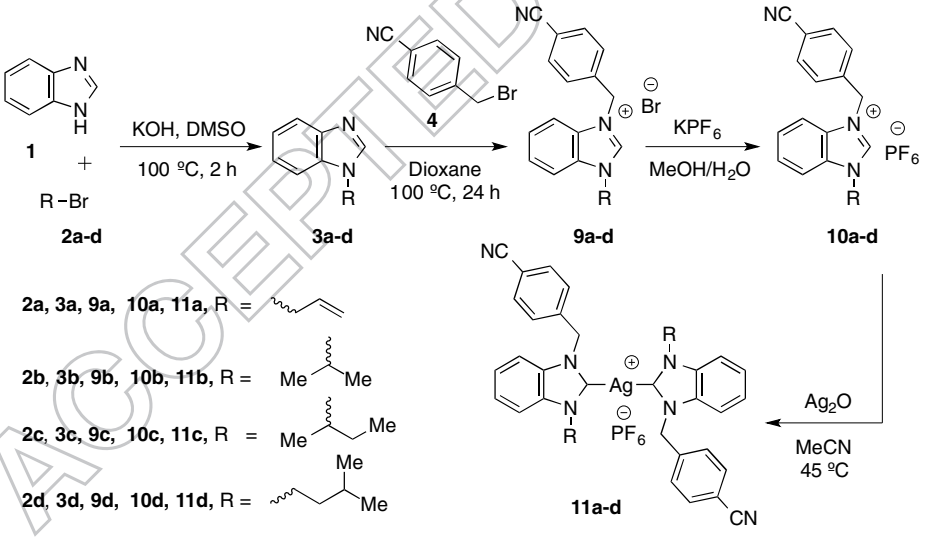
Bis-(1-(*sec*-butyl)-3-(4-cyanobenzyl)benzimidazolium)-silver(I) hexafluorophosphate (11c). Compound **11c** was prepared from 1-(*sec*-butyl)-3-(4-cyanobenzyl)benzimidazolium hexafluorophosphate (**10c**) (2.17 g, 5.0 mmol) according to the general procedure. Grey solid. Yield: 85%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 7.92-7.99 (m, 4H, $CH_{Cyanobenzyl}$), 7.62-7.85 (m, 4H, $CH_{Cyanobenzyl}$), 7.28-7.49 (m, 8H,$CH_{Benzimid}$), 5.90 (s, 4H, CH_2), 4.74-4.77 (m, 2H, CH), 2.06-2.10 (m, 4H, CH_2), 1.61-1.63 (m, 6H, CH_3), 0.66-0.71 (m, 6H, CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm): 171.5, 167.3, 142.0, 134.0, 132.7, 127.9, 127.0, 124.0, 118.4, 112.9, 112.3, 110.8 (NCN+CN+$C_{Benzimid}$+$C_{Cyanobenzyl}$), 58.1 (CH), 51.3 (CH_2), 28.9 (CH_2), 20.8 (CH_3), 10.8 (CH_3). Mass data (*m*/*z*, LC-MS): 690.0 $[M]^+$. Anal. Calcd for $C_{38}H_{40}AgF_6N_6P$: C, 54.75; H, 4.84; N, 10.08; Ag, 12.94. Found: C, 54.77; H, 4.83; N, Ag, 12.91, 10.01. IR (KBr, cm^{-1}) ν: 3141, 3042, 2942, 2850, 2783, 2241, 1844, 1639, 838.

Bis-(1-isopentyl-3-(4-cyanobenzyl)benzimidazolium)-silver(I) hexafluorophosphate (11d). Compound **11d** was prepared from 1-isopentyl-3-(4-nitrobenzyl)benzimidazolium hexafluorophosphate (**10d**) (2.24 g, 5.0 mmol), according to general the procedure. Grey solid. Yield: 90%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 7.84 (d, J = 8.5 Hz, 2H, $CH_{Cyanobenzyl}$), 7.79 (d, J = 8.5 Hz, 4H, $CH_{Cyanobenzyl}$), 7.64 (d, J = 8.0 Hz, 2H, $CH_{Cyanobenzyl}$), 7.39-7.48 (m, 8H, $CH_{Benzimid}$), 5.87 (s, 4H, CH_2), 4.51 (t, J = 7.5 Hz, 4H, CH_2), 1.72-1.76 (m, 4H, CH_2), 1.57-1.63 (m, 2H, CH), 0.91 (d, J = 6.5 Hz, 12H, CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm): 189.6, 142.1, 133.3, 132.9, 132.7, 129.0, 127.8, 124.3, 118.4, 112.3, 112.2, 110.8 (NCN+CN+$C_{Benzimid}$+$C_{Cyanobenzyl}$), 51.2 (CH_2), 47.3 (CH_2), 38.9 (CH_2), 25.3 (CH), 22.3 (CH_3).

Mass data (*m/z*, LC-MS): 718.0 $[M]^+$. Anal. Calcd for $C_{40}H_{44}AgF_6N_6P$: C, 55.76; H, 5.15; N, 9.75; Ag, 12.52. Found: C, 55.75; H, 5.10; N, 9.73; Ag, 12.50. IR (KBr, cm^{-1}) ν: 3140, 3046, 2945, 2842, 2760, 2255, 1885, 1641, 1226, 830.



Scheme 1. General reaction scheme for synthesis of non-symmetrically *p*-nitrobenzyl-substituted NHC precursors (**6a-d**) and their corresponding NHC-silver(I) complexes (**7a-d**).



Scheme 2. General reaction scheme for synthesis of non-symmetrically *p*-cyanobenzyl-substituted NHC precursors (**10a-d**) and their corresponding NHC-silver(I) complexes (**11a-d**).

2.3. *Antibacterial studies*

Initial *in vitro* antibacterial activity of non-symmetrically *p*-nitrobenzyl-substituted and *p*-cyanobenzyl-substituted NHCs and their corresponding NHC-silver(I) complexes were screened simultaneously with silver(I) oxide against two bacterial strains. The tested organisms included *S. aureus* as a Gram-positive bacteria and *E. coli* as Gram-negative bacteria.

To assess the biological activity of **6a-d**, **7a-d**, **10a-d**, **11a-d** and silver(I) oxide, the Kirby-Bauer disk diffusion method was applied [27]. All bacteria were individually cultured from a single colony in sterile HB medium [28] overnight at 37 °C (orbital shaker incubator). All the work carried out was performed under sterile conditions.

A 10 mL stock solution of each compound was prepared at 800 µg/mL. For each strain, 70 µL of culture were spread evenly on agar-MH medium. Five 5-mm-diameter paper discs were placed at equal distances in each plate. First one being control, the remaining discs were loaded with 3 µL, 6 µL, 9 µL and 12 µL of stock solution. The plates were covered and placed in an incubator at 37 °C for 24 h. The plates were then removed and the zone of clearance (defined as the diameter of inhibited bacterial growth around the filter paper) for each sample was measured in millimeters.

3. Results and discussion

3.1. *Synthesis and characterization*

The synthetic pathway for non-symmetrically *p*-nitrobenzyl-substituted and *p*-cyanobenzyl-substituted *N*-heterocyclic carbene-silver(I) complexes described in this work is outlined in schemes 1 and 2. 1-Allylbenzimidazole (**3a**), 1-isopropylbenzimidazole (**3b**), 1-(*sec*-butyl)benzimidazole (**3c**) and 1-isopentylbenzimidazole (**3d**) were prepared by stirring benzimidazole (**1**) with one equivalent of alkyl halides [allyl bromide (**2a**), isopropyl bromide (**2b**), 2-bromobutane (**2c**) and isopentyl bromide (**2d**)] in the presence of potassium hydroxide as a base in DMSO at 100-110 °C for 4 h. The bromide salts (**5a-d** and **9a-d**) were prepared by reacting one equivalent of alkyl halides [4-nitrobenzyl bromide (**4**) and 4-cyanobenzyl bromide (**8**)] with 1-allylbenzimidazole (**3a**), 1-isopropylbenzimidazole (**3b**), 1-(*sec*-butyl)benzimidazole (**3c**) and 1-isopentylbenzimidazole (**3d**)] in dioxane at 100-110 °C for 24 h, with yields fluctuating between 71%-89%. All the benzimidazolium hexafluorophosphate salts (**6a-d** and **10a-d**) were prepared by stirring bromide salts (**5a-d** and **9a-d**) with KPF_6 in a mixture of

methanol and water at room temperature for 4 h as air and moisture stable white powders, with moderate to good yields (68%-89%). The synthesized benzimidazolium hexafluorophosphate salts (**6a-d** and **10a-d**) were insoluble in common organic solvents such as hexane, pentane, diethylether and toluene and soluble in DMSO, DMF, acetonitrile and acetone.

Structures of the benzimidazolium hexafluorophosphate salts (**6a-d** and **10a-d**) were confirmed by ^{1}H, ^{13}C NMR, FT-IR, MS spectrometry, and elemental analyses. ^{1}H NMR spectra of the NHC precursors (**6a**-**d** and **10a**-**d**) show a characteristic downfield shift in the range δ = 10.10-9.91 ppm for the NCHN proton, indicating formation of benzimidazolium hexafluorophosphate salts (**6a-d** and **10a-d**) [29-31]. In the FT-IR spectra of NHC precursors (**6a-d**) stretching vibrational bands of nitro were observed as medium-intensity bands at 1557-1547 and 1365-1350 cm^{-1}. The FT-IR spectra of **10a-d** exhibit a strong band at ~2250 cm^{-1} assigned to ν(C≡N). IR spectra of all the NHC precursors (**6a-d** and **10a-d**) displayed two sharp bands of medium intensities at 3145-3123 and 2944-2941 cm^{-1}, attributed to the aliphatic and aromatic ν(C–H) stretching vibrations. Additionally, the benzimidazolium hexafluorophosphate salts (**6a-d** and **10a-d**) identities have been confirmed by a base peak for the $[M^{+}\text{-}PF_6]$ fragments in their positive mode LRMS mass spectra. The results obtained from elemental analyses are in agreement with the proposed structures of the benzimidazolium hexafluorophosphate salts (**6a-d** and **10a-d**).

The NHC-silver(I) complexes (**7a-d** and **11a-d**) were synthesized by reaction of hexafluorophosphate salts (**6a-d** and **10a-d**) with two equivalents of silver(I) oxide in acetonitrile. The reaction mixture was stirred for 2 d at room temperature in the dark to afford the NHC-silver(I) complexes as grey solids in good yields (scheme 2).

The NHC-silver(I) complexes (**7a-d** and **11a-d**) were characterized by ^{1}H, ^{13}C NMR, FT-IR and mass spectrometry, and elemental analyses. The absence of a downfield NCHN signal in the ^{1}H NMR spectra for **7a-d** and **11a-d** indicates complex formation [25-33]. However, the resonances of aromatic, benzylic methylene and aliphatic protons of NHC-silver(I) complexes (**7a-d** and **11a-d**) were observed with no or negligible changes from their respective benzimidazolium hexafluorophosphate salts (**6a**-**d** and **10a**-**d**). The ^{13}C NMR resonances of the carbene carbons in **7a-d** and **11a-d** occur in the δ range of 190.3-171.5 ppm. These signals are shifted downfield compared to the corresponding precursor **6a-d** and **10a-d** carbene carbon resonances at 147.6-141.5 ppm which further supports the formation of NHC-silver(I)

complexes. In FT-IR spectra of NHC-silver(I) complexes (**7a-d**) stretching vibrational bands of nitro were observed as medium intensity bands at 1551-1550 and 1363-1348 cm^{-1}. FT-IR spectra of NHC-silver(I) complexes (**11a-d**) exhibit a strong band at ~2250 cm^{-1} assigned to ν(C≡N). There is no difference in the ν(C≡N) stretching frequency of NHC-silver(I) complexes and their respective benimidazolium salts, suggesting non-involvement of nitrile in coordination. The FT-IR spectra of **7a-d** and **11a-d** displayed two sharp bands of medium intensity at 3142-3140 and 2944-2939 cm^{-1}, attributed to the aliphatic and aromatic ν(C–H), respectively. These spectral assignments are consistent with reported compounds [28, 30]. LRMS mass spectra of the eight NHC-silver(I) complexes (**7a-d** and **11a-d**) have been recorded and show molecular ion peaks $[M]^+$ corresponding to the mass of the cationic complexes. Results obtained from the elemental analyses are in agreement with the proposed structures of **7a-d** and **11a-d**.

3.2. *Biological evaluation*

Preliminary *in vitro* antibacterial assay for the benzimidazolium hexafluorophosphate salts (**6a-6d** and **10a-10d**) and their corresponding NHC-silver(I) complexes (**7a-d** and **11a-d**) were carried out by Kirby-Bauer disc diffusion against *S. aureus*, a gram-positive species and *E. coli*, a gram-negative species. The observed *in vitro* antibacterial activities of **6a-d**, **10a-d**, **7a-d** and **11a-d** are tabulated in table 1. Silver(I) oxide used to prepare the complexes and the solvent DMSO used to prepare the stock solutions played no role in growth inhibition on the same bacteria as previously reported [34].

Benzimidazolium hexafluorophosphate salts (**6a-d** and **10a-d**) were inactive against both bacterial strains. But, upon complexation with silver, **7a-d** and **11a-d** exhibited moderate to good antibacterial activity with the diameter of the zone of inhibition ranging from 12 mm to 21 mm for *S. aureus* and 13 mm to 20 mm for *E. coli*, indicating that silver is the active center and responsible for the biological potential. Also, despite screening of different alkyl substitutions on nitrogen of the benzimidazole core for antibacterial activity, irrespective of the functionalization (nitro and nitrile), none of the salts exhibited difference on the bioactivity. This proves that neither the difference in functionalization, nor the nature of the alkyl substituent of the heterocyclic moiety plays a significant role toward antibacterial potential, as both functionalizations display comparable results. Also, as the range of activity is comparable for both species, it can be established that the complexes are efficient antibacterial agents

irrespective of the cell wall physiology of the bacteria. Hence, it can be stated that the *N*-substitutions alone cannot bring up the desired potential. The synergic effect of both the NHC and silver is responsible for the overall behavior of the complex. The study also provides proof that *N*-heterocyclic moiety has a huge role in the biological behavior when compared to non-NHC based silver(I) complexes possessing biologically relevant moiety [35-38].

4. Conclusions and outlook

Several new non-symmetrically *p*-nitrobenzyl- and *p*-cyanobenzyl-substituted NHC-silver(I) complexes (**7a-d** and **11a-d**) were synthesized through reaction of non-symmetrically *p*-nitrobenzyl- and *p*-cyanobenzyl-substituted benzimidazolium hexafluorophosphate salts (**6a-d** and **10a-d**) with silver(I) oxide. The complexes displayed high antibacterial activity compared to their NHC precursors against both Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. coli*. It is also clear that, as the complex concentration increases, the antibacterial activity also increases. Furthermore, enhanced antibacterial activity is linked to stable silver(I) complexes that slowly release Ag^+ ions by decomposition. Summarizing, it can be said that the NHC-silver(I) complexes are easily accessible from inexpensive starting materials; give moderate-to-high yields and the synthesis does not require harsh conditions and is not excessively sensitive towards air and moisture.

Further work is underway to improve these values by performing formulation experiments to improve solubility of these NHC-silver(I) complexes, which should allow for *in vivo* testing in the nearby future. These new NHC-silver(I) complexes could be used for transmetallation reactions, opening additional research venues.

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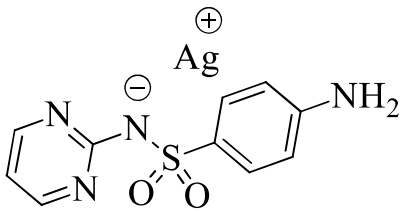

Figure 1. Structure of silver sulfadiazine.

Table 1. Area of clearance for **6a-d**, **7a-d**, **10a-d** and **11a-d** in mm.

Compound (#)	Concentration (nM)	*S. aureus* (Gram positive) Zone of inhibition (mm)	*E. coli* (Gram negative) Zone of inhibition (mm)
6a	5.46	6±1	6±1
	10.92	6±1	6±1
	16.38	6±1	6±1
	21.85	6±1	6±1
6b	5.43	6±1	6±1
	10.87	6±1	6±1
	16.31	6±1	6±1
	21.75	6±1	6±1
6c	5.27	6±1	6±1
	10.54	6±1	6±1
	15.81	6±1	6±1
	21.08	6±1	6±1
6d	5.11	6±1	6±1
	10.22	6±1	6±1
	15.33	6±1	6±1
	20.45	6±1	6±1
7a	2.85	16±1	15±1
	5.71	17±1	16±1
	8.57	19±1	18±1
	11.43	20±1	19±1
7b	2.84	16±1	15±1
	5.69	17±1	16±1
	8.53	18±1	17±1
	11.38	20±1	19±1
7c	2.75	16±1	15±1
	5.50	17±1	16±1
	8.26	20±1	19±1
	11.01	20±1	19±1
7d	2.66	12±1	11±1
	5.33	14±1	13±1
	8.00	14±1	15±1
	10.67	16±1	15±1

10a	5.72	6±1	6±1
	11.44	6±1	6±1
	17.17	6±1	6±1
	22.89	6±1	6±1
10b	5.69	6±1	6±1
	11.39	6±1	6±1
	17.08	6±1	6±1
	22.78	6±1	6±1
10c	5.51	6±1	6±1
	11.02	6±1	6±1
	16.53	6±1	6±1
	22.05	6±1	6±1
10d	5.34	6±1	6±1
	10.68	6±1	6±1
	16.02	6±1	6±1
	21.36	6±1	6±1
11a	3.00	17±1	16±1
	6.00	18±1	17±1
	9.00	20±1	19±1
	12.00	21±1	20±1
11b	2.98	17±1	16±1
	5.97	18±1	17±1
	8.96	19±1	18±1
	11.94	21±1	20±1
11c	2.88	17±1	16±1
	5.77	18±1	17±1
	8.65	20±1	20±1
	11.54	20±1	20±1
11d	2.79	15±1	13±1
	5.58	16±1	14±1
	8.37	17±1	16±1
	11.16	18±1	16±1