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Synthesis, cytotoxicity and antibacterial studies of symmetrically and non-symmetrically benzyl- or *p*-cyanobenzyl-substituted *N*-Heterocyclic carbene–silver complexes

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From the reaction of 1H-imidazole (1a), 4,5-dichloro-1H-imidazole (1b) and 1H-benzimidazole (1c) with *p*-cyanobenzyl bromide (2), symmetrically substituted *N*-heterocyclic carbene (NHC) [(3a–c)] precursors, 1-methylimidazole (5a), 4,5-dichloro-1-methylimidazole (5b) and 1-methylbenzimidazole (5c) with benzyl bromide (6), non-symmetrically substituted *N*-heterocyclic carbene (NHC) [(7a–c)] precursors were synthesized. These NHC–precursors were then reacted with silver(I) acetate to yield the NHC-silver complexes [1,3-bis(4-cyanobenzyl)imidazole-2-ylidene] silver(I) acetate (4a), [4,5-dichloro-1,3-bis(4-cyanobenzyl)imidazole-2-ylidene] silver(I) acetate (4b), [1,3-bis(4-cyanobenzyl)benzimidazole-2-ylidene] silver(I) acetate (4c), (1-methyl-3-benzylimidazole-2-ylidene) silver(I) acetate (8a), (4,5-dichloro-1-methyl-3-benzylimidazole-2-ylidene) silver(I) acetate (8b) and (1-methyl-3-benzylbenzimidazole-2-ylidene) silver(I) acetate (8c) respectively. The four NHC-precursors 3a–c, 7c and four NHC–silver complexes 4a–c and 8c were characterized by single crystal X-ray diffraction. The preliminary antibacterial activity of all the compounds was studied against Gram-negative bacteria *Escherichia coli*, and Gram-positive bacteria *Staphylococcus aureus* using the qualitative Kirby-Bauer disc-diffusion method. All NHC–silver complexes exhibited medium to high antibacterial activity with areas of clearance ranging from 4 to 12 mm at the highest amount used, while the NHC-precursors showed significantly lower activity. In addition, all NHC–silver complexes underwent preliminary cytotoxicity tests on the human renal-cancer cell line Caki-1 and showed medium to high cytotoxicity with IC_{50} values ranging from 53 (±8) to 3.2 (±0.6) μM.

Keywords: anticancer drugs; antibacterial drugs; silver acetate; NHC; Caki-1; *Staphylococcus aureus*; *Escherichia coli*

Introduction

N-Heterocyclic carbenes (NHCs) are cyclic carbenes that are usually derived from the deprotonation of imidazolium salts. Discovered by Öfele[1] and Wanzlick[2] in 1968 and isolated in the free state by Arduengo[3] in 1991, *N*-heterocyclic carbenes have become extremely popular supporting ligands in organometallic chemistry and homogeneous catalysis.[4–6] More recently, NHCs have found an application in NHC–silver complexes exhibiting antimicrobial activity, in particular for the possible treatment of cystic fibrosis and chronic lung infections[7–9] and possibly even in the treatment of cancer.[10]

A literature survey revealed that the silver(I) complexes of phosphines, carboxylates, thio groups and thioamides, tripodal thioglycosides and the natural product coumarin show a high level of anticancer activity against a variety of different cell lines.[11–18] Youngs and co-workers have recently reported anticancer activity of *N*-heterocyclic carbene–silver complexes derived from 4,5-dichloro-1H-imidazole against the human cancer cell lines OVCAR-3 (ovarian), MB157 (breast) and HeLa (cervical).[10] These silver complexes have been shown to be very stable and can be synthesized efficiently. Very recently, we reported the anticancer and antibacterial activity of *p*-methoxybenzyl-substituted and benzyl-substituted *N*-heterocyclic carbene–silver complexes.[19] All the reported NHC–silver complexes are light- and water-stable and can be synthesized in high yield and in high purity from cheap commercially available starting materials.

Keeping this in mind and in continuation of our studies on the stability and solubility of *N*-heterocyclic carbene–silver complexes and the search for new potential anticancer and antibacterial agents, here we present the synthesis, preliminary cytotoxicity and antibacterial studies of a series of six novel symmetrically substituted and non-symmetrically substituted NHC–silver acetate derivatives. These compounds were tested on the human cancerous renal-cell line Caki-1 as well as on the Gram-positive bacteria *Staphylococcus aureus* and the Gram-negative bacteria *Escherichia coli*. In particular, even though still

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on qualitative screening, the antibacterial activity has significantly improved with respect to our previously reported compounds.[19]

Experimental

General Conditions

All the solvents used were of analytical grade and were used without further purification. 1H-Imidazole, 4,5-dichloro-1H-imidazole, 1H-benzimidazole, 1-methylimidazole, 1-methylbenzimidazole, *p*-cyanobenzyl bromide, benzyl bromide, silver acetate, methyl iodide and K_2CO_3 were purchased from Sigma-Aldrich Chemical Company. NMR spectra were measured on a Varian 400 MHz spectrometer. Chemical shifts are reported in ppm and are referenced to TMS. IR spectra were recorded on a Perkin Elmer Paragon 1000 FT-IR spectrometer employing a KBr disc. UV–vis spectra were recorded on a Unicam UV4 spectrometer. Electron spray mass spectrometry (MS) was performed on a quadrupole tandem mass spectrometer (Quattro Micro, Micromass/Water's Corp., USA), using solutions made up in 50% dichloromethane and 50% methanol. MS spectra were obtained in the ES+ (electron spray positive ionization) mode for compounds **3a–c, 4a–c, 7a–c and 8a–c**. CHN analysis was done with an Exeter Analytical CE-440 Elemental Analyser. Ag was estimated by spectrophotometry (atomic absorption spectra 55B Varian), while Cl and Br were determined in mercurimetric titrations. X-ray diffraction data for compounds **3a–c, 4a–c, 7c** and **8c** were collected using Mo–K_α radiation and a Bruker Smart APEX CCD area detector diffractometer. A full sphere of reciprocal space was scanned by phi-omega scans. Pseudo-empirical absorption correction based on redundant reflections was performed by the program SADABS.[20] The structures were solved by direct methods using SHELXS-97[21] and refined by full matrix least-squares on F^2 for all data using SHELXL-97.[21] In **3a** all hydrogen atoms were located in the difference Fourier map and allowed to refine freely. The same applies to the 'carbenic' hydrogen atom in **3c.** In **4c** the O–H bond distances in the water molecules were restrained to be 0.84 Å, and the thermal displacement parameters of the water protons were fixed to be 1.5 times the equivalent thermal displacement parameter of the oxygen atom. In **7c** the hydrogen atoms of the water molecule could not be detected. All other hydrogen atoms were added at calculated positions and refined using a riding model. Their isotropic temperature factors were fixed to 1.2 times (1.5 times for methyl groups) the equivalent isotropic displacement parameters of the parent carbon atom. Anisotropic thermal displacement parameters were used for all non-hydrogen atoms. Further details about the data collection are listed in Table 1, as well as reliability factors. Further details are available free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif under the CCDC numbers 764 016, 764 017, 764 018, 764 019, 764 020, 764 021, 764 022 and 764 023 for **3a, 3b, 7c, 3c, 4c, 4a, 4b** and **8c** respectively. Suitable crystals of **3a–c, 4a–c, 7c** and **8c** for X-ray studies were grown from the slow evaporation of a saturated CH_2Cl_2 and methanol solutions respectively at room temperature.

Synthesis

The synthesis of 4,5-dichloro-1-methylimidazole (**5b**) was carried out according to the literature procedure[7] where as 1-methyl-3-benzylimidazolium bromide (**7a**) and 1-methyl-3-benzylbenzimidazolium bromide (**7c**) were carried out according to our new and milder procedures instead of using literature procedures.[22–24]

*Synthesis of 1,3-bis(4-cyanobenzyl)imidazolium bromide (**3a**)*

1H-Imidazole (0.39 g, 5.84 mmol) and K_2CO_3 (1.21 g, 8.76 mmol) were stirred for 15 min in 30 ml of acetonitrile. 4-Cyanobenzylbromide (2.28 g, 11.7 mmol) was added in one portion and stirring was continued at room temperature for further 3 days. After the solvent was removed under reduced pressure, 75 ml of water was added. The precipitate was filtered out and was washed with diethyl ether to yield (1.49 g, 3.93 mmol, 67.4% yield) **3a** as white powder and dried *in vacuo*.

^{1}H NMR (δ ppm DMSO-d_6, 400 MHz): 9.48 (s, 1H, NCHN), 7.91 (d, J = 8.2 Hz, 4H, CH_{Benzyl}), 7.88 (d, J = 1.4 Hz, 2H, CH_{Imid}), 7.60 (d, J = 8.2 Hz, 4H, CH_{Benzyl}), 5.57 (s, 4H, CH_2). ^{13}C NMR (δ ppm DMSO-d_6, 100 MHz, proton decoupled): 140.4, 137.5, 133.3, 129.6, 123.6, 118.8, 111.9 (NCN + CN + C_{Imid} + C_{Benzyl}), 51.9 (CH_2). IR absorptions (KBr, cm^{-1}): 3436 (w), 3133 (w), 3063 (m), 2982 (s), 2851 (w), 2227 (s), 1609 (m), 1568 (s), 1506 (w), 1412 (m), 1356 (w), 1209 (m), 1161 (s), 1019 (w), 858 (m), 824 (w), 783 (s), 635 (m), 557 (m). UV–vis (CH_3OH, nm): λ 207 (ε 13 692), λ 225 (ε 9946), λ 268 (ε 4828). MS (*m/z*, QMS-MS/MS): 299.43 [M^+–Br]. Microanalysis calculated for $C_{19}H_{15}N_4Br$ (379.26): calcd–C, 60.17%; H, 3.98%; N, 14.77%; Br, 21.06%; found: C, 59.87%; H, 4.01%; N, 14.60%; Br, 21.19%.

*Synthesis of 4,5-Dichloro-1,3-bis(4-cyanobenzyl)imidazolium bromide (**3b**)*

4,5-Dichloro-1H-imidazole (0.79 g, 5.84 mmol) and K_2CO_3 (1.21 g, 8.76 mmol) were stirred for 15 min in 30 ml of acetonitrile. 4-Cyanobenzylbromide (1.14 g, 5.84 mmol) was added in one portion and stirring was continued at room temperature for further 2 days. After the solvent was removed under reduced pressure 75 ml of water were added. The aqueous phase was extracted with CH_2Cl_2 (4 × 20 ml). Organic phases were combined and dried over magnesium sulfate. The residue was obtained after solvent removal under reduced pressure. The resulting residue was dissolved in CH_3CN and another portion of 4-cyanobenzylbromide (1.14 g, 5.84 mmol) was added. The reaction mixture was heated under reflux for 6 days. The light yellow coloured precipitate formed was filtered off and washed several times with diethyl ether and dried *in vacuo* to yield (0.75 g, 1.67 mmol, 28.7% yield) **3b**.

^{1}H NMR (δ ppm DMSO-d_6, 400 MHz): 9.60 (s, 1H, NCHN), 7.95 (d, J = 8.2 Hz, 4H, CH_{Benzyl}), 7.62 (d, J = 8.2 Hz, 4H, CH_{Benzyl}), 5.63 (s, 4H, CH_2). ^{13}C NMR (δ ppm DMSO-d_6, 100 MHz, proton decoupled): 138.5, 137.9, 133.3, 129.4, 119.9, 118.8, 112.0 (NCN + CN + CCl + C_{Benzyl}), 51.4 (CH_2). IR absorptions (KBr, cm^{-1}): 3442 (w), 2913 (s), 2234 (s), 1577 (m), 1546 (m), 1507 (w), 1449 (w), 1420 (s), 1340 (m), 1198 (w), 1144 (w), 1023 (w), 871 (w), 821 (s), 617 (w), 552 (m). UV–vis (CH_3OH, nm): λ 209 (ε 22 871), λ 230 (ε 25 134), λ 279 (ε 9688). MS (*m/z*, QMS-MS/MS): 368.29 [M^+–Br]. Microanalysis calculated for $C_{19}H_{13}N_4Cl_2Br$ (448.14): calcd–C, 50.92%; H, 2.92%; N, 12.50%; Br, 17.82%; Cl, 15.82%; found–C, 50.43%; H, 2.73%; N, 12.35%; Br, 17.20%; Cl, 15.64%.

*Synthesis of 1,3-bis(4-cyanobenzyl)benzimidazolium bromide (**3c**)*

1H-Benzimidazole (0.68 g, 5.84 mmol) and K_2CO_3 (1.21 g, 8.76 mmol) were stirred for 15 min in 30 ml of acetonitrile. 4-Cyanobenzylbromide (2.28 g, 11.68 mmol) was added in one portion and stirring was continued at room temperature for further 3 days. After the solvent was removed under reduced pressure 75 ml of water was added. The precipitate was filtered, washed

Table 1. Crystal data and structure refinement for **3a–c, 4a–c, 7c** and **8c**

Identification code	**3a**	**3b**	**3c**	**4a**	**4b**	**4c**	**7c**	**8c**
Empirical formula	$C_{19}H_{15}N_4Br$	$C_{19}H_{13}N_4Cl_2Br$	$C_{23}H_{17}N_4Br$	$C_{22}H_{21}N_4O_3Ag$	$C_{22}H_{19}N_4O_3Cl_2Ag$	$C_{25}H_{21}N_4O_3Ag$	$C_{15}H_{17}N_2OBr$	$C_{17}H_{17}N_2O_2Ag$
Molecular formula	$[C_{19}H_{15}N_4]^+[Br]^-$	$[C_{19}H_{13}N_4Cl_2]^+[Br]^-$	$[C_{23}H_{17}N_4]^+[Br]^-$	$C_{21}H_{17}N_4O_2Ag \times CH_4O$	$C_{21}H_{15}N_4O_2Cl_2Ag \times CH_4O$	$C_{25}H_{19}N_4O_2Ag \times H_2O$	$C_{15}H_{15}N_2Br \times H_2O$	$C_{17}H_{17}N_2O_2Ag$
Formula weight	379.26	448.14	429.32	497.30	566.18	533.33	638.44	389.20
Crystal system	Monoclinic	Monoclinic	Monoclinic	Monoclinic	Triclinic	Monoclinic	Monoclinic	Monoclinic
Space group	$P2_1/n$ (#14)	$P2_1$ (#4)	$P2_1$ (#4)	$P2_1/c$ (#14)	P–1 (#2)	$P2_1/c$ (#14)	C 2/c (#15)	$P2_1$ (#4)
Unit cell dimensions	$a = 11.7757(7)$ Å	$a = 6.2458(6)$ Å	$a = 4.7770(3)$ Å	$a = 14.8000(16)$ Å	$a = 8.7884(7)$ Å	$a = 4.6333(5)$ Å	$a = 17.5945(13)$ Å	$a = 4.7138(10)$ Å
	$b = 12.7513(8)$ Å	$b = 11.9794(11)$ Å	$b = 14.8455(10)$ Å	$b = 9.3859(10)$ Å	$b = 10.2087(8)$ Å	$b = 22.201(3)$ Å	$b = 17.8622(13)$ Å	$b = 11.289(2)$ Å
	$c = 11.8902(7)$ Å	$c = 12.6329(12)$ Å	$c = 13.7200(9)$ Å	$c = 15.5648(16)$ Å	$c = 14.2024(11)$ Å	$c = 24.371(3)$ Å	$c = 11.0149(8)$ Å	$c = 14.232(3)$ Å
	$\alpha = 90^\circ$	$\alpha = 90^\circ$	$\alpha = 90^\circ$	$\alpha = 90^\circ$	$\alpha = 108.111(1)^\circ$	$\alpha = 90^\circ$	$\alpha = 90^\circ$	$\alpha = 90^\circ$
	$\beta = 109.117(1)^\circ$	$\beta = 93.589(2)^\circ$	$\beta = 91.803(1)^\circ$	$\beta = 109.293(2)^\circ$	$\beta = 102.782(2)^\circ$	$\beta = 91.027(3)^\circ$	$\beta = 123.980(1)^\circ$	$\beta = 92.264(4)^\circ$
	$\gamma = 90^\circ$	$\gamma = 90^\circ$	$\gamma = 90^\circ$	$\gamma = 90^\circ$	$\gamma = 101.196(1)^\circ$	$\gamma = 90^\circ$	$\gamma = 90^\circ$	$\gamma = 90^\circ$
Volume	1686.92(18) Å^3	943.35(15) Å^3	972.50(11) Å^3	2040.7(4) Å^3	1132.55(15) Å^3	2506.4(5) Å^3	2870.6(4) Å^3	756.8(3) Å^3
Z	4	2	2	4	2	4	8	2
Density (calculated)	1.493 mg m^{-3}	1.578 mg m^{-3}	1.466 mg m^{-3}	1.619 mg m^{-3}	1.660 mg m^{-3}	1.413 mg m^{-3}	1.487 mg m^{-3}	1.708 mg m^{-3}
Absorption coefficient	2.444 mm^{-1}	2.472 mm^{-1}	2.129 mm^{-1}	1.020 mm^{-1}	1.158 mm^{-1}	0.836 mm^{-1}	2.858 mm^{-1}	1.340 mm^{-1}
F(000)	768	448	436	1008	568	1080	1312	392
Crystal size	0.60 × 0.60 × 0.40 mm^3	0.80 × 0.60 × 0.05 mm^3	0.80 × 0.15 × 0.05 mm^3	0.40 × 0.30 × 0.03 mm^3	0.40 × 0.20 × 0.05 mm^3	0.80 × 0.03 × 0.02 mm^3	1.20 × 0.40 × 0.30 mm^3	0.50 × 0.20 × 0.18 mm^3
Theta range for data collection	2.11–28.29°	2.35–30.50°	2.74–30.52°	2.58–26.50°	2.48–28.29°	1.91–24.18°	2.79–27.16°	2.30–28.35°
Index ranges	$-15 \le h \le 15$, $-16 \le k \le 16$, $-15 \le l \le 15$	$-8 \le h \le 8$, $-17 \le k \le 16$, $-17 \le l \le 17$	$-6 \le h \le 6$, $-20 \le k \le 21$, $-19 \le l \le 19$	$-18 \le h \le 18$, $-11 \le k \le 11$, $-19 \le l \le 19$	$-11 \le h \le 11$, $-13 \le k \le 13$, $-18 \le l \le 18$	$-5 \le h \le 5$, $-25 \le k \le 25$, $-27 \le l \le 28$	$-22 \le h \le 22$, $-22 \le k \le 22$, $-14 \le l \le 14$	$-6 \le h \le 6$, $-15 \le k \le 15$, $-18 \le l \le 18$
Reflections collected	16 889	10 886	22 705	17 392	23 121	16 499	13 303	7567
Independent reflections	4177 [*R*(int) = 0.0202]	5549 [*R*(int) = 0.0339]	5872 [*R*(int) = 0.0318]	4200 [*R*(int) = 0.0353]	5613 [*R*(int) = 0.0225]	4002 [*R*(int) = 0.0396]	3168 [*R*(int) = 0.0279]	3695 [*R*(int) = 0.0249]
Completeness to θ_{max}	99.7%	99.7%	99.7%	99.6%	99.6%	99.3%	99.5%	99.2%
Maximum and minimum Transmission	0.4415 and 0.3218	0.8864 and 0.2788	0.9010 and 0.5496	0.9701 and 0.7336	0.9444 and 0.7057	0.9835 and 0.7334	0.4813 and 0.1943	0.7945 and 0.4264
Data/restraints/ parameters	4177/0/277	5549/1/235	5872/1/257	4200/0/274	5613/0/292	4002/2/305	3168/0/185	3695/1/201
Goodness-of-fit on F^2	1.056	1.090	1.042	1.162	1.065	1.171	1.108	1.032
Final *R* indices [$I > 2\sigma(I)$]	$R_1 = 0.0255$, $wR_2 = 0.0651$	$R_1 = 0.0519$, $wR_2 = 0.1309$	$R_1 = 0.0304$, $wR_2 = 0.0710$	$R_1 = 0.0440$, $wR_2 = 0.0992$	$R_1 = 0.0277$, $wR_2 = 0.0684$	$R_1 = 0.0649$, $wR_2 = 0.1432$	$R_1 = 0.0633$, $wR_2 = 0.1303$	$R_1 = 0.0369$, $wR_2 = 0.0931$
R indices (all data)	$R_1 = 0.0298$, $wR_2 = 0.0668$	$R_1 = 0.0547$, $wR_2 = 0.1332$	$R_1 = 0.0328$, $wR_2 = 0.0720$	$R_1 = 0.0510$, $wR_2 = 0.1020$	$R_1 = 0.0303$, $wR_2 = 0.0698$	$R_1 = 0.0761$, $wR_2 = 0.1479$	$R_1 = 0.07320$ $wR_2 = 0.1357$	$R_1 = 0.0384$, $wR_2 = 0.0943$
Largest difference peak and hole	0.557 and −0.207 e Å^{-3}	0.576 and −0.955 e Å^{-3}	0.725 and −0.223 e Å^{-3}	1.653 and −0.730 e Å^{-3}	1.045 and −0.236 e Å^{-3}	1.329 and −1.245 e Å^{-3}	2.431 and −3.146 e Å^{-3}	1.738 and −1.048 e Å^{-3}

with diethyl ether and dried in suction at room temperature for 4 h to yield (1.52 g, 3.54 mmol, 61.1% yield) **3c** as white solid.

^{1}H NMR (δ ppm DMSO-d_6, 400 MHz): 10.06 (s, 1H, NCHN), 7.92–7.89 (m, 6H, $CH_{Benzimid}$ + CH_{Benzyl}), 7.70 (d, J = 8.3 Hz, 4H, CH_{Benzyl}), 7.64–7.60 (m, 2H, CH_{Benzyl}), 5.90 (s, 4H, CH_2). ^{13}C NMR (δ ppm DMSO-d_6, 100 MHz, proton decoupled): 143.9, 139.7, 133.3, 131.5, 129.6, 127.4, 118.8, 114.4, 111.9 (NCN + CN + $C_{Benzimid}$ + C_{Benzyl}), 49.9 (CH_2). IR absorptions (KBr, cm^{-1}): 2966 (w), 2230 (s), 1609 (w), 1562 (s), 1477 (m), 1417 (s), 1374 (m), 1198 (m), 1129 (m), 1025 (m), 841 (w), 822 (m), 785 (m), 754 (s), 684 (m), 613 (w). UV–vis (CH_3OH, nm): λ 224 (ε 32 267), λ 230 (ε 32 882), λ 269 (ε 11 682), λ 280 (ε 8339). MS (*m/z*, QMS-MS/MS): 349.42 [M$^+$—Br]. Microanalysis calculated for $C_{23}H_{17}N_4Br$ (429.32): calcd–C, 64.34%; H, 3.99%; N, 13.05%; Br, 18.61%; found–C, 63.95%; H, 3.97%; N, 12.92%; Br, 18.40%.

*Synthesis of (1,3-bis(4-cyanobenzyl)imidazole-2-ylidene) silver(I) acetate (**4a**)*

1,3-bis(4-cyanobenzyl)imidazolium bromide (0.379 g, 1.00 mmol) was dissolved in methanol (40 ml) and silver acetate (0.333 g, 2.00 mmol) was added. The mixture was stirred at reflux for 1 day. The pale yellow precipitate, presumably AgBr, was filtered and a clear solution was obtained. The volatile components were removed *in vacuo* to produce an off-white sticky solid. The solid was washed with diethyl ether and dried under reduced pressure for 1 h to yield a white solid (0.250 g, 0.537 mmol, 53.7% yield) **4a**.

^{1}H NMR (δ ppm DMSO-d_6, 400 MHz): 7.82 (d, J = 8.2 Hz, 4H, CH_{Benzyl}), 7.60 (s, 2H, CH_{Imid}), 7.49 (d, J = 8.2 Hz, 4H, CH_{Benzyl}), 5.43 (s, 4H, CH_2), 1.80 (s, 3H, CH_3). ^{13}C NMR (δ ppm DMSO-d_6, 100 MHz, proton decoupled): 179.8 (NCN), 175.0 (C═O), 143.0, 133.1, 128.9, 123.3, 118.9, 111.2 (CN + C_{Imid} + C_{Benzyl}), 54.1 (CH_2), 23.3 (CH_3). IR absorptions (KBr, cm^{-1}): 3399 (w), 3082 (w), 2977 (w), 2360 (s), 2228 (s), 1576 (s), 1506 (w), 1411 (s), 1352 (w), 1239 (m), 1160 (m), 1103 (w), 1019 (m), 822 (m), 787 (w), 647 (w), 549 (s). UV–vis (CH_3OH, nm): λ 210 (ε 14 792), λ 230 (ε 11 802), λ 268 (ε 5045). MS (*m/z*, QMS-MS/MS): 406.32 [M$^+$-CH_3OH-O_2CCH_3]. Microanalysis calculated for $C_{22}H_{21}N_4O_3Ag$ (497.30): calcd–C, 53.13%; H, 4.25%; N, 11.26%; Ag, 21.69%; found–C, 52.90%; H, 4.10%; N, 10.45%; Ag, 21.04%.

*Synthesis of (4,5-dichloro-1,3-bis(4-cyanobenzyl)imidazole-2-ylidene) silver(I) acetate (**4b**)*

4,5-Dichloro-1,3-bis(4-cyanobenzyl)imidazolium bromide (0.448 g, 1.00 mmol) was dissolved in methanol (40 ml) and silver acetate (0.333 g, 2.00 mmol) was added. The mixture was stirred at room temperature for 1 day. The pale yellow precipitate, presumably silver bromide, was filtered and discarded. The volume of the reaction mixture was reduced under reduced pressure. The residue was washed with pentane and diethyl ether and dried under reduced pressure to yield a white solid (0.425 g, 0.794 mmol, 79.4% yield) **4b**.

^{1}H NMR (δ ppm DMSO-d_6, 400 MHz): 7.83 (d, J = 8.2 Hz, 4H, CH_{Benzyl}), 7.46 (d, J = 8.1 Hz, 4H, CH_{Benzyl}), 5.59 (s, 4H, CH_2), 1.71 (s, 3H, CH_3). ^{13}C NMR (δ ppm DMSO-d_6, 100 MHz, proton decoupled): 185.6 (NCN), 175.3 (C═O), 141.2, 133.1, 128.4, 118.8, 118.1, 111.4 (CN + CCl + C_{Benzyl}), 53.5 (CH_2), 24.1(CH_3). IR absorptions (KBr, cm^{-1}): 3433 (s), 2360 (s), 2229 (s), 1578 (s), 1507 (w), 1389 (s), 1338 (w), 1210 (m), 1120 (m), 1019 (m), 820 (s), 547 (m). UV–vis (CH_3OH, nm): λ 207 (ε 20 921), λ 228 (ε 14 765), λ 274 (ε 5795). MS (*m/z*, QMS-MS/MS): 475.19 [M$^+$ CH_3OH-O_2CCH_3]. Microanalysis calculated for $C_{22}H_{19}N_4O_3Cl_2Ag$ (566.18): calcd–C, 46.67%; H, 3.38%; N, 9.89%; Cl, 12.52%; Ag, 19.05%; found–C, 46.52%; H, 2.91%; N, 9.93%; Cl, 12.38%; Ag, 18.92%.

*Synthesis of (1,3-bis(4-cyanobenzyl)benzimidazole-2-ylidene) silver(I) acetate (**4c**)*

1,3-bis(4-Cyanobenzyl)benzimidazolium bromide (0.429 g, 1.00 mmol) was dissolved in methanol (40 ml) and silver acetate (0.333 g, 2.00 mmol) was added. The mixture was stirred at room temperature for 1 day. The pale yellow precipitate, presumably AgBr, was filtered and a clear solution was obtained. The volatile components were removed *in vacuo* to produce a white sticky solid. The solid was washed with pentane and diethyl ether and dried under reduced pressure for 2 h to yield a white solid (0.400 g, 0.776 mmol, 77.6% yield) **4c**.

^{1}H NMR ^{1}H NMR (δ ppm DMSO-d_6, 400 MHz): 7.77 (d, J = 8.0 Hz, 4H, CH_{Benzyl}), 7.67 (dd, J = 6.0, 3.1 Hz, 2H, $CH_{Benzimid}$), 7.49 (d, J = 7.7 Hz, 4H, CH_{Benzyl}), 7.37 (dd, J = 6.1, 3.0 Hz, 2H, $CH_{Benzimid}$), 5.86 (s, 4H, CH_2), 1.74 (s, 3H, CH_3). ^{13}C NMR (δ ppm DMSO-d_6, 100 MHz, proton decoupled): 180.0 (NCN), 175.2 (C═O), 142.2, 133.8, 133.1, 128.5, 124.8, 118.8, 112.8, 111.2 (CN + $C_{Benzimid}$ + C_{Benzyl}), 51.83 (CH_2), 23.97 (CH_3). IR absorptions (KBr, cm^{-1}): 3441 (s), 3060 (w), 2924 (w), 2360 (m), 2230 (s), 1576 (s), 1477 (w), 1400 (s), 1352 (w), 1261 (w), 1182 (w), 1100 (w), 1020 (m), 819 (m), 798 (m), 745 (m), 547 (w).

UV–vis (CH_3OH, nm): λ 229 (ε 13 200), λ 275 (ε 6289), λ 284 (ε 5313). MS (*m/z*, QMS-MS/MS): 456.28 [M$^+$-H_2O-O_2CCH_3]. Microanalysis calculated for $C_{25}H_{21}N_4O_3Ag$ (533.33): calcd–C, 56.30%; H, 3.96%; N, 10.50%; Ag, 20.22%; found–C, 57.50%; H, 3.85%; N, 10.53%; Ag, 20.78%.

*Synthesis of 1-methyl-3-benzylimidazolium bromide (**7a**)*

Benzyl bromide (11.89 ml, 100 mmol) was added in one portion to a stirred suspension of 1-methylimidazole (3.96 ml, 50.0 mmol) in 150 ml of toluene. The mixture was stirred for 24 h at room temperature. The toluene was decanted and the colourless semisolid mass was washed two times with pentane and three times with diethyl ether. Compound **7a** (11.5 g, 45.4 mmol, 90.9% yield) was obtained as a colourless wax after drying under reduced pressure.

^{1}H NMR (δ ppm $CDCl_3$, 400 MHz): 9.52 (s, 1H, NCHN), 7.19 (s, 1H, CH_{Imid}), 7.13 (s, 1H, CH_{Imid}), 7.05–6.98 (m, 2H, CH_{Benzyl}), 6.80–6.74 (m, 3H, CH_{Benzyl}), 5.09 (s, 2H, CH_2), 3.46 (s, 3H, N-CH_3). ^{13}C NMR (δ ppm $CDCl_3$, 100 MHz, proton decoupled): 136.0, 133.2, 128.7, 128.4, 127.7, 123.5, 121.8 (NCN + C_{Imid} + C_{Benzyl}), 52.3 (CH_2), 36.2 (N-CH_3). IR absorptions (KBr, cm^{-1}): 3422 (w), 3142 (w), 3081 (w), 1627 (m), 1572 (s), 1497 (s), 1455 (s), 1335 (w), 1295 (w), 1161 (s), 1083 (m), 1029 (m), 855 (w), 822 (m), 722 (s), 662 (m), 569 (w), 464 (w). UV–vis (CH_3OH, nm): λ 213 (ε 6852), λ 259 (ε 2003). MS (*m/z*, QMS-MS/MS): 173.40 [M$^+$-Br]. Microanalysis calculated for $C_{11}H_{13}N_2Br$ (253.14): calcd–C, 52.19%; H, 5.17%; N, 11.06%; Br, 31.56%; found–C, 51.93%; H, 4.98%; N, 10.98%; Br, 31.68%.

*Synthesis of 4,5-dichloro-1-methylimidazole (**5b**)*

4,5-Dichloroimidazole (1.23 g, 9.00 mmol) and potassium hydroxide (2.24 g, 40.0 mmol) were stirred in acetonitrile (50 ml) for 2 h at room temperature. The excess potassium hydroxide was filtered from the solution and iodomethane (0.562 ml, 9.00 mmol) was added. The reaction mixture was stirred at room temperature

for 24 h. The volatile components were removed, and the crude product was re-dissolved in dichloromethane. The solid, presumably KI, was filtered and discarded, and the volatile components were removed *in vacuo* to yield a yellow crystalline solid (1.32 g, 8.72 mmol, 97.0% yield) (**5b**).

^{1}H NMR (δ ppm $CDCl_3$, 400 MHz): 7.36 (s, 1H, NCHN), 3.61 (s, 3H, N-CH_3). ^{13}C NMR (δ ppm $CDCl_3$, 100 MHz, proton decoupled): 134.6 (NCN), 125.5, 113.7 (CCl), 32.5 (N-CH_3). IR absorptions (KBr, cm^{-1}): 3437 (m), 3099 (s), 2953 (w), 1663 (w), 1521 (s), 1494 (s), 1463 (w), 1362 (m), 1262 (s), 1209 (m), 1126 (s), 988 (s), 835 (m), 721 (m), 666 (s), 623 (m), 540 (m). UV–vis (CH_3OH, nm): λ 225 (ε 4371), λ 275 (ε 933). MS (*m/z*, QMS-MS/MS): 150.92 [M^+]. Microanalysis calculated for $C_4H_4N_2Cl_2$ (150.99): calcd–C, 31.81%; H, 2.67%; N, 18.55%; Cl, 46.95%; found–C, 31.37%; H, 2.59%; N, 18.05%; Cl, 46.40%.

*Synthesis of 4,5-dichloro-1-methyl-3-benzylimidazolium bromide (**7b**)*

Benzyl bromide (2.37 ml, 20.0 mmol) was added in one portion to a stirred suspension of 4,5-dichloro-1-methylimidazole (1.50 g, 10.0 mmol) in 40 ml of toluene. The mixture was stirred for 48 h at room temperature. Afterwards the solvent was removed under reduced pressure. The resulting yellow residue was saturated with diethyl ether to get a light yellow precipitate, which was filtered, washed with diethyl ether and finally dried *in vacuo* in order to give compound **7b** (2.05 g, 6.36 mmol, 63.7% yield).

^{1}H NMR (δ ppm $CDCl_3$, 400 MHz): 11.29 (s, 1H, NCHN), 7.56 (d, J = 6.2 Hz, 2H, CH_{Benzyl}), 7.47–7.34 (m, 3H, CH_{Benzyl}), 5.64 (s, 2H, CH_2), 4.08 (s, 3H, N-CH_3). ^{13}C NMR (δ ppm $CDCl_3$, 100 MHz, proton decoupled): 138.0 (NCN), 131.6, 129.6, 129.4, 128.9 (C_{Benzyl}), 119.9, 118.9 (CCl), 52.6 (CH_2), 35.6 (N-CH_3). IR absorptions (KBr, cm^{-1}): 3410 (s), 3363 (s), 3121 (w), 3068 (m), 2918 (m), 2865 (w), 1629 (w), 1583 (m), 1560 (m), 1455 (s), 1346 (w), 1261 (w), 1160 (s), 1096 (w), 1029 (w), 801 (w), 732 (m), 703 (m), 621 (w). UV–vis (CH_3OH, nm): λ 228 (ε 4894), λ 274 (ε 2665). MS (*m/z*, QMS-MS/MS): 242.13 [M^+-Br]. Microanalysis calculated for $C_{11}H_{11}N_2Cl_2Br$ (322.03): calcd–C, 41.02%; H, 3.44%; N, 8.69; Br, 24.81%; Cl, 22.01%; found–C, 41.78%; H, 3.43%; N, 8.57%; Br, 24.37%; Cl, 21.79%.

*Synthesis of 1-methyl-3-benzylbenzimidazolium bromide (**7c**)*

Benzyl bromide (2.37 ml, 20.0 mmol) was added in one portion to a stirred suspension of 1-methylbenzimidazole (1.32 g, 10.0 mmol) in 40 ml of toluene. The mixture was stirred for 4 days at room temperature. Afterwards the solvent was removed under reduced pressure. The resultant residue was washed four times with diethyl ether and dried *in vacuo* to yield (2.75 g, 9.06 mmol, 90.7% yield) a colourless solid **7c**.

^{1}H NMR (δ ppm $CDCl_3$, 400 MHz): 11.38 (s, 1H, NCHN), 7.76 (d, J = 8.2 Hz, 1H, $CH_{Benzimid}$), 7.66–7.53 (m, 5H, CH_{Benzyl}), 7.38–7.30 (m, 3H, $CH_{Benzimid}$), 5.89 (s, 2H, CH_2), 4.29 (s, 3H, N-CH_3). ^{13}C NMR (δ ppm $CDCl_3$, 100 MHz, proton decoupled): 142.0, 131.7, 131.1, 129.9, 128.3, 128.1, 127.3, 126.2, 124.2, 112.7, 112.0 (NCN + $C_{Benzimid}$ + C_{Benzyl}), 50.3 (CH_2), 33.0 (CH_3). IR absorptions (KBr, cm^{-1}): 3410 (s), 3131 (w), 3065 (m), 2965 (w), 1562 (s), 1490 (w), 1458 (m), 1424 (w), 1384 (m), 1348 (w), 1277 (w), 1195 (m), 1094 (w), 1018 (w), 874 (w), 757 (s), 713 (m), 659 (w). UV–vis (CH_3OH, nm): λ 222 (ε 7831), λ 250 (ε 6382), λ 269 (ε 6270). MS (*m/z*, QMS-MS/MS): 223.41 [M^+-Br-H_2O]. Microanalysis calculated for $C_{15}H_{17}N_2BrO$ (321.21): calcd–C, 56.09%; H, 5.33%; N, 8.72%; Br, 24.88%; found–C, 56.31%; H, 5.08%; N, 8.98%; Br, 24.15%.

*Synthesis of (1-methyl-3-benzylimidazole-2-ylidene) silver(I) acetate (**8a**)*

1-Methyl-3-benzylimidazolium bromide (0.253 g, 1.00 mmol) was dissolved in dichloromethane (40 ml), silver acetate (0.333 g, 2.00 mmol) was added, and the mixture was refluxed for 2 d. The precipitate, presumably AgBr, was filtered and a clear solution was obtained. The volatile components were removed *in vacuo* to produce an off-white sticky solid. The solid was washed with pentane and dried under reduced pressure to yield (0.080 g, 0.235 mmol, 23.6% yield) **8a**.

^{1}H NMR (δ ppm $CDCl_3$, 400 MHz): 7.42–7.24 (m, 5H, CH_{Benzyl}), 6.97 (d, J = 1.7 Hz, 1H, CH_{Imid}), 6.92 (d, J = 1.7 Hz, 1H, CH_{Imid}), 5.27 (s, 2H, CH_2), 3.85 (s, 3H, N-CH_3), 2.07 (s, 3H, $COCH_3$). ^{13}C NMR (δ ppm $CDCl_3$, 125 MHz, proton decoupled): 179.9 (NCN), 177.9 (C=O), 135.5, 129.0, 128.5, 127.9, 122.5, 121.0 (C_{Benzyl} + C_{Imid}), 55.7 (CH_2), 38.8 (N-CH_3), 22.5 ($COCH_3$). IR absorption (KBr, cm^{-1}): 3382 (w), 3135 (w), 3096 (w), 1579 (s), 1406 (s), 1158 (s), 1082 (w), 1018 (m), 919 (m), 819 (w), 721 (s), 646 (m), 621 (w), 463 (w). UV–vis (CH_3OH, nm): λ 219 (ε 4075), λ 230 (ε 3008), λ 269 (ε 1878). MS (*m/z*, QMS-MS/MS): 280.10 [M^+-O_2CCH_3]. Microanalysis calculated for $C_{13}H_{15}N_2O_2Ag$ (339.14): calcd–C, 46.04%; H, 4.45%; N, 8.26%; Ag, 31.80%; found–C, 45.86%; H, 5.01%; N, 8.31%; Ag, 30.39%.

*Synthesis of (4,5-dichloro-1-methyl-3-benzylimidazole-2-ylidene) silver(I) acetate (**8b**)*

4,5-Dichloro-1-methyl-3-benzylimidazolium bromide (0.253 g, 1.00 mmol) was dissolved in CH_2Cl_2 (40 ml) and silver acetate (0.333 g, 2.00 mmol) was added. The mixture was stirred at room temperature for 1 day. The yellow precipitate of AgBr was filtered and a clear solution was obtained. The volatile components were removed *in vacuo* to produce an off-white sticky solid. The solid was washed with diethyl ether and dried under reduced pressure to yield (0.150 g, 0.367 mmol, 36.8% yield) **8b** as a white solid.

^{1}H NMR (δ ppm $CDCl_3$, 400 MHz): 7.47–7.01 (m, 5H, CH_{Benzyl}), 5.25 (s, 2H, CH_2), 3.76 (s, 3H, N-CH_3), 1.99 (s, 3H, $COCH_3$). ^{13}C NMR (δ ppm $CDCl_3$, 100 MHz, proton decoupled): 180.1 (NCN), 179.0 (C=O), 134.1, 129.0, 128.7, 127.7, 118.2, 117.4 (CCl + C_{Benzyl}), 54.5 (CH_2), 38.0 (N-CH_3), 22.6 ($COCH_3$). IR absorptions (KBr, cm^{-1}): 3443 (s), 3064 (w), 1575 (s), 1405 (m), 1338 (w), 1261 (w), 1130 (w), 1097 (w), 1022 (w), 800 (w), 746 (w), 704 (m), 468 (w). UV–vis (CH_3OH, nm): λ 216 (ε 13 422), λ 241 (ε 8362), λ 280 (ε 4414). MS (*m/z*, QMS-MS/MS): 348.22 [M^+-O_2CCH_3]. Microanalysis calculated for $C_{13}H_{13}N_2O_2Cl_2Ag$ (408.03): calcd–C, 38.26%; H, 3.21%; N, 6.86%; Cl, 17.37%; Ag, 26.43%; found–C, 37.85%; H, 3.13%; N, 6.66%; Cl, 17.25%; Ag, 26.21%.

*Synthesis of (1-methyl-3-benzylbenzimidazole-2-ylidene) silver(I) acetate (**8c**)*

1-Methyl-3-benzylbenzimidazolium bromide (0.322 g, 1.00 mmol) was dissolved in dichloromethane (50 ml) and silver acetate (0.333 g, 2.00 mmol) was added. The mixture was stirred at room temperature for 1 day. The yellow precipitate, presumably AgBr, was filtered and discarded. The volume of the reaction mixture was reduced under pressure to 5 ml. Pentane (200 ml) was added and the fine white precipitate was filtered out, washed with diethyl ether and dried *in vacuo* to yield a white solid (0.260 g, 0.668 mmol, 66.8% yield) **8c**.

^{1}H NMR (δ ppm $CDCl_3$, 400 MHz): 7.59–7.09 (m, 9H, $CH_{Benzimid}$ + CH_{Benzyl}), 5.61 (s, 2H, CH_2), 4.08 (s, 3H, N-CH_3), 2.11 (s, 3H, $COCH_3$). ^{13}C NMR (δ ppm $CDCl_3$, 125 MHz, proton decoupled): 180.0 (NCN),

179.2 (C═O), 134.9, 134.6, 133.5, 129.0, 128.4, 127.2, 124.1, 111.9, 111.2 ($C_{Benzimid}$ + C_{Benzyl}), 53.4 (CH_2), 35.9 (N-CH_3), 22.7 ($COCH_3$). IR absorptions (KBr, cm^{-1}): 3433 (w), 1669 (w), 1580 (s), 1445 (w), 1403 (m), 1345 (w), 1262 (w), 1193 (w), 1094 (w), 1024 (w), 806 (w), 747 (s), 703 (m), 649 (w). UV–vis (CH_3OH, nm): λ 206 (ε 21 306), λ 274 (ε 3421), λ 285 (ε 4640). MS (*m/z*, QMS-MS/MS): 330.18 [M^+-O_2CCH_3]. Microanalysis calculated for $C_{17}H_{17}N_2O_2Ag$ (389.20): calcd–C, 52.46%; H, 4.40%; N, 7.19%; Ag, 27.71%; found–C, 51.92%; H, 4.36%; N, 7.01%; Ag, 27.40%.

Antibacterial studies

Qualitative antibacterial activity of symmetrically substituted and non-symmetrically substituted *N*-heterocyclic carbenes and their corresponding silver complexes were screened against two bacterial strains. The test organisms included *Staphylococcus aureus* (SA) (NCTC 7447) as a Gram-positive bacteria and *Escherichia coli* as Gram-negative bacteria.

To assess the biological activity of compounds **3a–c, 4a–c, 7a–c** and **8a–c**, the Kirby–Bauer disc-diffusion method was applied.[25] All bacteria were individually cultured from a single colony in sterile LB medium[26] overnight at 37 °C (orbital shaker incubator). All the work carried out was performed under sterile conditions.

For each strain, 70 µl of culture were spread evenly on agar-LB medium. Four 5 mm diameter paper discs were placed evenly separated on each plate. Two stock solutions (90 : 10 DMSO : H_2O) of every compound were prepared at 2.3 µM and 4.6 µM to be able to test the effect of different concentrations. Each plate was then tested with 5 and 7 µl of 2.3 µM solution and 5 and 10 µl for the 4.6 µM 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 distance between the edge of the filter paper disc and the beginning of the bacterial growth) for each sample was measured in millimetres and are summarized in Tables 3 and 4.

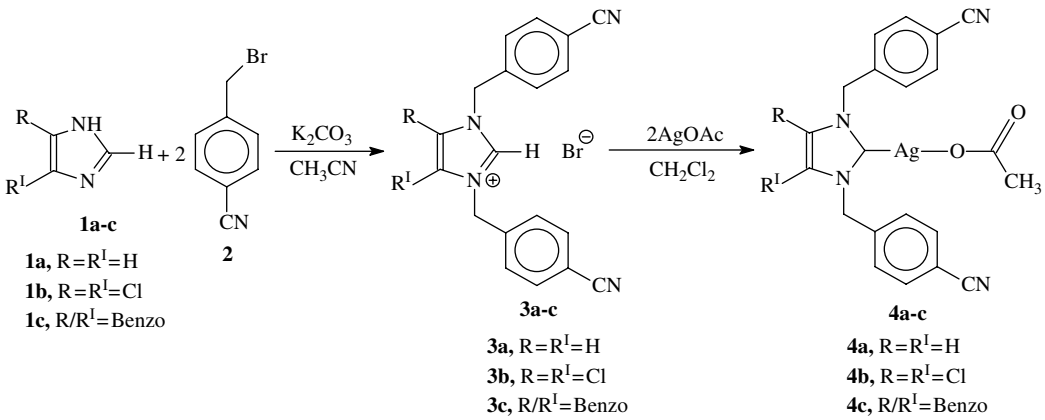
Cytotoxicity Studies

Preliminary *in vitro* cell tests were performed on the human cancerous renal cell line Caki-1 in order to compare the cytotoxicity of the compounds presented in this paper. These cell lines were chosen based on their regular and long-lasting growth behaviour, which is similar to that shown in kidney carcinoma cells. They were obtained from the ATCC (American Tissue Cell Culture Collection) and maintained in Dulbecco's modified Eagle medium containing 10% (v/v) fetal calf serum, 1% (v/v) penicillin streptomycin and 1% (v/v) L-glutamine. Cells were seeded in 96-well plates containing 200 µl microtitre wells at a density of 5000 cells per 200 µl of medium and were incubated at 37 °C for 24 h to allow for exponential growth. Then the compounds used for the testing were dissolved in the minimal amount of DMSO (dimethylsulfoxide) possible and diluted with medium to obtain stock solutions of 5×10^{-4} M in concentration and less than 0.7% of DMSO. The cells were then treated with varying concentrations of the compounds and incubated for 48 h at 37 °C. Then, the solutions were removed from the wells and the cells were washed with PBS (phosphate buffer solution) and fresh medium was added to the wells. Following a recovery period of 24 h incubation at 37 °C, individual wells were treated with a 200 µl of a solution of MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] in medium. The solution consisted of 30 mg of MTT in 30 ml of medium. The cells were incubated for 3 h at 37 °C. The medium was then removed and the purple formazan crystals were dissolved in 200 µl DMSO per well. A Wallac Victor (Multilabel HTS Counter) Plate Reader was used to measure absorbance at 540 nm. Cell viability was expressed as a percentage of the absorbance recorded for control wells. The values used for the dose response curves represent the values obtained from four consistent MTT-based assays for each compound tested.

Results and Discussion

Synthesis

The synthetic route for symmetrically substituted and non-symmetrically substituted *N*-heterocyclic carbenes as ligand precursors and their corresponding silver complexes described in this work is given in Schemes 1–3. We did not follow the synthetic procedure for the non-symmetrically substituted NHCs precursors, 1-methyl-3-benzylimidazolium bromide (**7a**), and 1-methyl-3-benzylbenzimidazolium bromide (**7c**),[22–24] but synthesized them according to new and milder procedures. On the other hand, the synthesis of 4,5-dichloro-1-methylimidazole (**5b**) was carried out according to a literature procedure.[7] The symmetrically substituted NHC precursors 1,3-bis(4-cyanobenzyl)imidazolium bromide (**3a**) and 1,3-bis(4-cyanobenzyl)benzimidazolium bromide (**3c**) were prepared by stirring 1H-imidazole (**1a**) and 1H-benzimidazole



1a-c

1a, R=R^I=H
1b, R=R^I=Cl
1c, R/R^I=Benzo

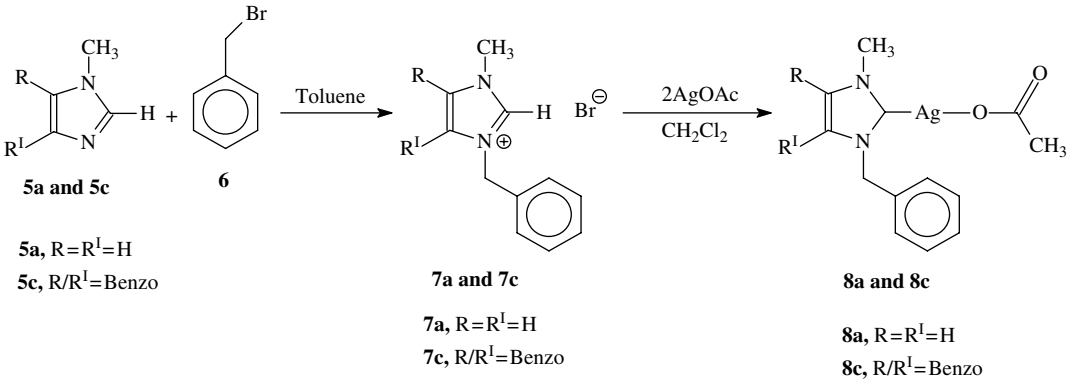
3a-c

3a, R=R^I=H
3b, R=R^I=Cl
3c, R/R^I=Benzo

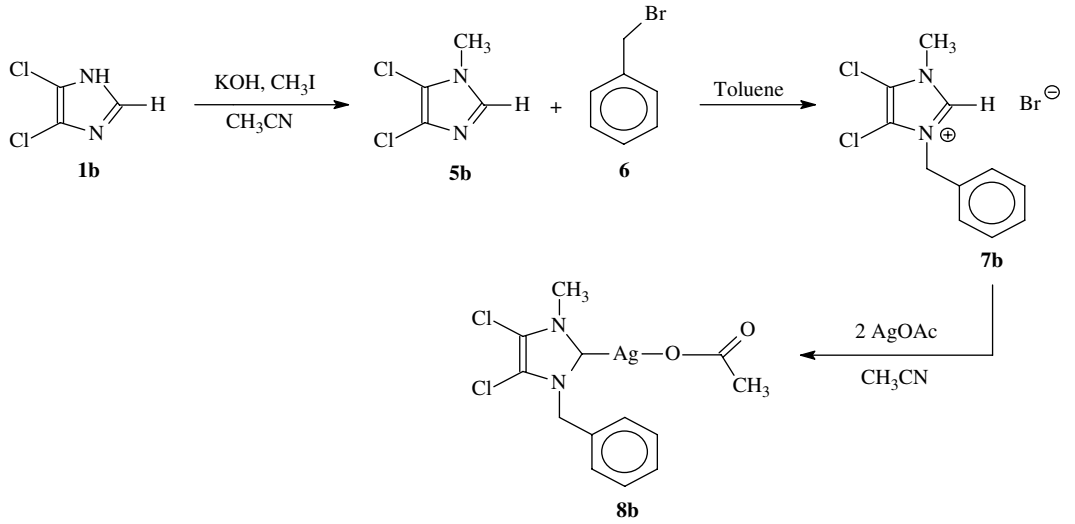
4a-c

4a, R=R^I=H
4b, R=R^I=Cl
4c, R/R^I=Benzo

Scheme 1. General reaction scheme for the synthesis of symmetrically substituted *N*-heterocyclic carbenes (**3a–c**) and their corresponding *N*-heterocyclic carbene–silver complexes (**4a–c**).



Scheme 2. General reaction scheme for the synthesis of non-symmetrically substituted *N*-heterocyclic carbenes (**7a** and **7c**) and their corresponding *N*-heterocyclic silver–**c**arbene complexes (**8a** and **8c**).



Scheme 3. General reaction scheme for the synthesis of non-symmetrically substituted *N*-heterocyclic carbene (**7b**) and its *N*-heterocyclic carbene–silver complex (**8b**).

(**1c**) with 2 equivalents of *p*-cyanobenzyl bromide (**2**) in the presence of K_2CO_3 as a base in CH_3CN at room temperature for 3 days with 67 and 61% yields respectively. 4,5-Dichloro-1,3-bis(4-cyanobenzyl)imidazolium bromide (**3b**) was prepared by heating 4,5-dichloro-1H-imidazole (**1b**) with 2 equivalents of *p*-cyanobenzyl bromide (**2**) in the presence of K_2CO_3 as a base in CH_3CN for 6 d with a yield of 29%. The non-symmetrically substituted NHC precursors 1-methyl-3-benzylimidazolium bromide (**7a**) and 1-methyl-3-benzylbenzimidazolium bromide (**7c**) were prepared by stirring 1-methylimidazole (**5a**) and 1-methylbenzimidazole (**5c**) with benzyl bromide (**6**) in toluene at room temperature for 2–4 days with 91 and 90% yields respectively. 4,5-Dichloro-1-methylimidazole (**5b**) is formed in 98% yield from the deprotonation of 4,5-dichloroimidazole (**1b**) with potassium hydroxide and subsequent methylation with iodomethane in acetonitrile. 4,5-Dichloro-1-methyl-3-benzylimidazolium bromide (**7b**) is formed in 64% yield by the reaction of 4,5-dichloro-1-methylimidazole (**5b**) with benzyl bromide in toluene.

The NHC precursors were fully characterized by ^{1}H, ^{13}C NMR, IR, UV–visible, mass spectroscopy and elemental analysis. In addition, the solid-state structure of the NHC precursors **3a–c** and **7c** was determined by single crystal X-ray diffraction. The ^{1}H NMR spectra of all precursors **3a–c** and **7a–c** show a characteristic downfield shift in the range δ = 9.48–11.38 ppm for the NCHN proton attributable to the positive charge of the molecule.[27,28] Additionally, their identities were also confirmed by a base peak for the [M^+—Br] fragments in their positive mode ESI mass spectra.

The NHC–silver complexes [1,3-bis(4–**c**yanobenzyl)imidazole-2-ylidene] silver(I) acetate (**4a**), [4,5-dichloro-1,3-bis(4-cyanobenzyl)imidazole-2-ylidene] silver(I) acetate (**4b**), [1,3-bis(4-cyanobenzyl)benzimidazole-2-ylidene] silver(I) acetate (**4c**), (1-methyl-3-benzylimidazole-2-ylidene) silver(I) acetate (**8a**), (4,5-dichloro-1-methyl-3-benzylimidazole-2-ylidene) silver(I) acetate (**8b**) and (1-methyl-3-benzylbenzimidazole-2-ylidene) silver(I) acetate (**8c**) were synthesized by the reaction of **3a–c** and **7a–c** with 2 equivalents of silver acetate in dichloromethane/methanol. The reaction mixture was stirred for 1–2 days at room temperature or refluxed for 2–4 days to afford the NHC–silver acetate complexes as off-white solid in 23–79% yield. The complexes were fully characterized by ^{1}H, ^{13}C NMR, IR, UV–visible, mass spectroscopy and elemental analysis. Furthermore, the solid-state structures of **4a–c** and **8c** were analysed by single crystal X-ray diffraction. The absence of a downfield NCHN signal and presence of new signals at 2.11–1.71 ppm for the acetate protons in all the ^{1}H NMR spectra for **4a–c** and **8a–c**; however, indicates a successful complex formation. The ^{13}C NMR resonances of the carbene carbon atoms in

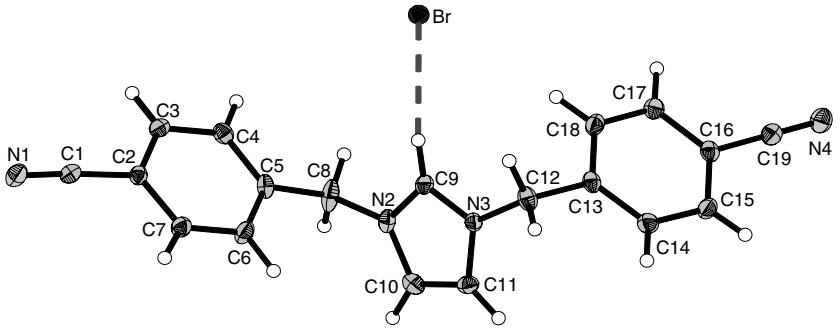


Figure 1. X-Ray diffraction structure of **3a**; molecule; thermal ellipsoids are drawn on the 50% probability level.

complexes **4a–c** and **8a–c** occur in the range δ 185.6–179.8 ppm, respectively. These signals are shifted downfield compared with the corresponding precursors of **3a–c** and **7a–c** carbene carbons' resonance at the range 143.9–136.0 ppm, respectively, which further demonstrates the formation of expected NHC–silver acetate complexes. Also the appearance of the ^{13}C NMR resonances for the carbonyl and methyl carbons of the acetate group of complexes **4a–c** and **8a–c** in the range 175.0–179.9 and 22.5–24.1 ppm respectively showed the formation of the NHC–silver complexes.[7,9] Furthermore, positive mode ESI mass spectra of all six NHC–silver complexes (**4a–c** and **8a–c**) are dominated by [$M^+ - O_2CCH_3$] fragment peaks arising from the loss of one acetate ligand.

Structural Discussion

The crystal structures of the compounds **3a–c, 4a–c, 7c** and **8c** were determined. The molecular structures of the compounds **3a–c, 4a–c, 7c** and **8c** are depicted in Figs 1–9. The crystal data and refinement details for all eight compounds are given in Table 1, whereas selected bond lengths and bond angles are displayed in Table 2.

The X-ray structures of compounds **3a–c** and **7c** reveal that the molecules are nonplanar. In the five-membered ring (NCNCC) of compounds **3a–c** and **7c**, the bond distances and angles are in good agreement with the bond distances found in the similar compounds 1-(2,4,6-trimethylphenyl)-3-(*N*-phenylacetamido)imidazolium chloride, 1,3-dimethyl-4,5-dichloroimidazolium iodide and 1,3- diisopropylbenzimidazolium bromide reported in the literature (see Table 2).[7,29,30] In compounds **3a–c** there is an absence of any lattice-held water molecules or organic solvent molecules in the unit cells of the determined structures. Compound **7c** has one lattice held water molecule in the unit cell of the determined structure.

Also in all the silver complexes reported here, bond lengths and angles in and directly around the NHC core agree very well among each other and with literature data.[31–35] Comparing precursor **3a–c** and **7c** with the corresponding complexes **4a–c** and **8c** (see Table 2), one finds a slight increase of both the C(9)–N distances. NHC silver complexes **4a–c** and **8c** are mononuclear complexes. Compounds **4a** and **4b** have lattice-held methanol molecules where as compound **4c** has lattice held water molecule. In the X-ray structure of **4a–c** and **8c** the acetate moiety acts as a monodentate ligand.

Biological Evaluation

The biological activities of the NHC-precursors and their corresponding NHC–silver complexes are summarized in Figs 10–13.

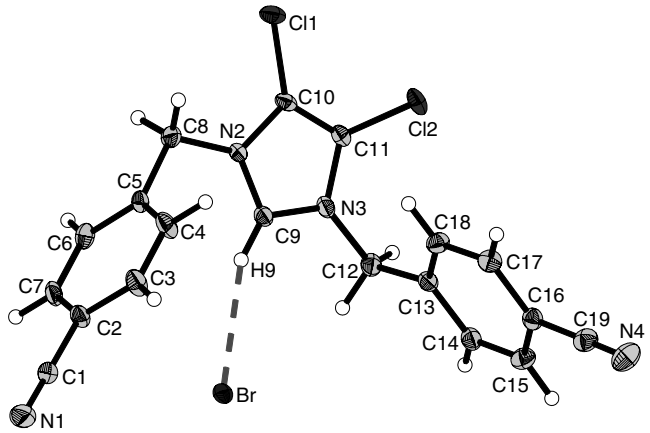


Figure 2. X-Ray diffraction structure of **3b**; molecule; thermal ellipsoids are drawn on the 50% probability level.

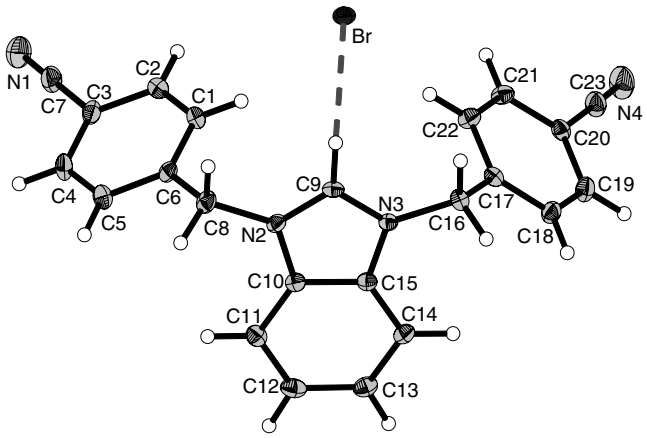


Figure 3. X-Ray diffraction structure of **3c**; molecule; thermal ellipsoids are drawn on the 50% probability level.

The results are displayed in Tables 3 and 4. With respect to our previously tested compounds,[19] the concentration of the stock solutions is reduced 4-fold, hence the amount of compound used in each test is significantly less, indicating a stronger biological activity. Minimal antibacterial activity was observed for compounds **3a–c** and **7a–c** against both Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli* using the Kirby–Bauer disc diffusion method. Improved activity was observed with compounds **4a, 4b** and **8a** against Gram-positive

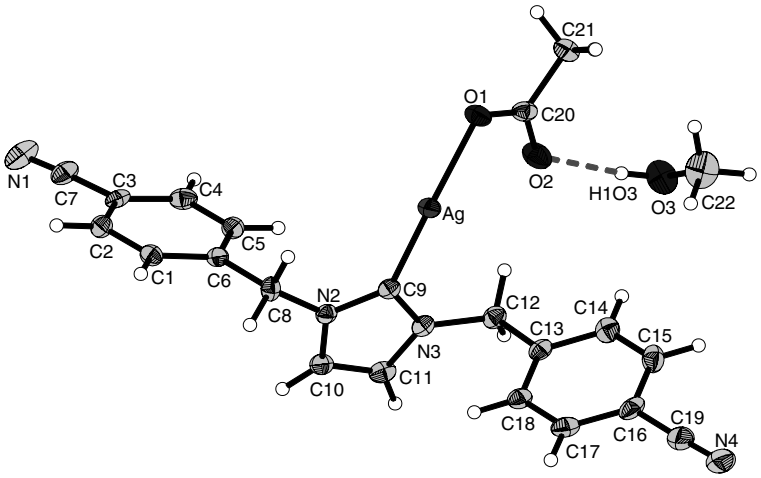


Figure 4. X-Ray diffraction structure of **4a**; molecule; thermal ellipsoids are drawn on the 50% probability level.

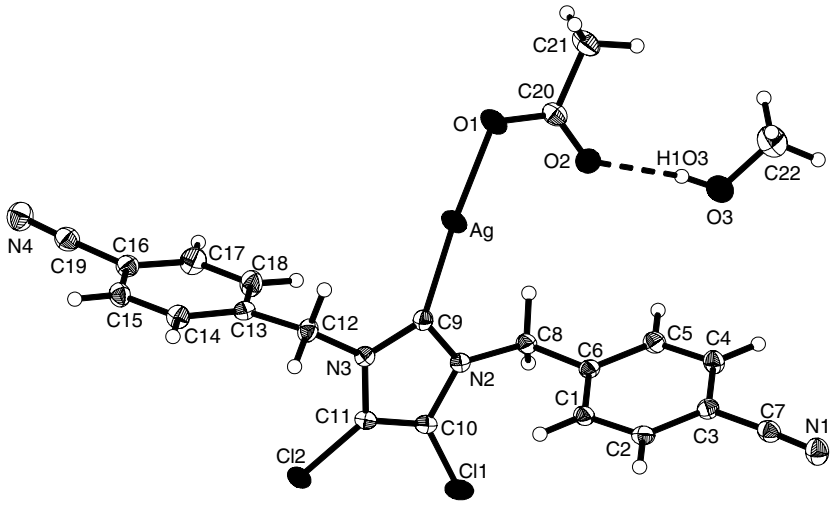


Figure 5. X-Ray diffraction structure of **4b**; molecule; thermal ellipsoids are drawn on the 50% probability level.

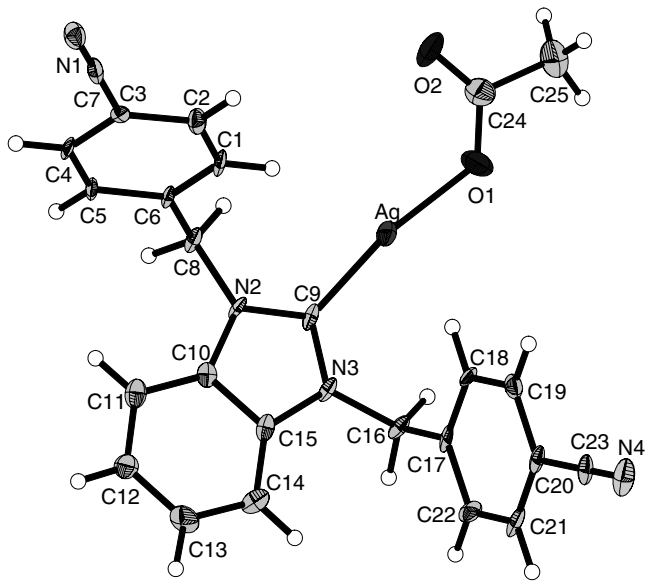


Figure 6. X-Ray diffraction structure of **4c**; molecule; thermal ellipsoids are drawn on the 50% probability level.

bacteria *Staphylococcus aureus* while **4c, 8b** and **8c** compounds have shown the best activity so far. In case of Gram-negative bacteria *Escherichia coli* **4a, 4b, 8a** and **8b** compounds showed moderate activity while the best result was obtained with **4c** and **8c.** The NHC–silver complexes **4a, 4b** and **8a** showed medium antibacterial activity with areas of clearance 4–5 mm against both Gram-positive bacteria *Staphylococcus aureus* and Gram negative-bacteria *Escherichia coli* whereas the NHC–silver complexes **4c** and **8c** showed highest antibacterial activity with areas of clearance 9–12 mm against both Gram-positive bacteria *Staphylococcus aureus* and Gram negative-bacteria *Escherichia coli* at the highest amount (0.46 µmol) used (see Tables 3 and 4). Thus the NHC–silver complexes **4c** and **8c** are the most promising antibacterial drug candidates in this paper. The metal salt (silver acetate) 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.[19,36]

Thus, on the basis of the above observation it can be stated that (i) the NHC–silver complexes **4a–c** and **8a–c** are more active against both Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli* than the free NHC-precursors **3a–c** and **7a–c**. (ii) It was concluded that, as

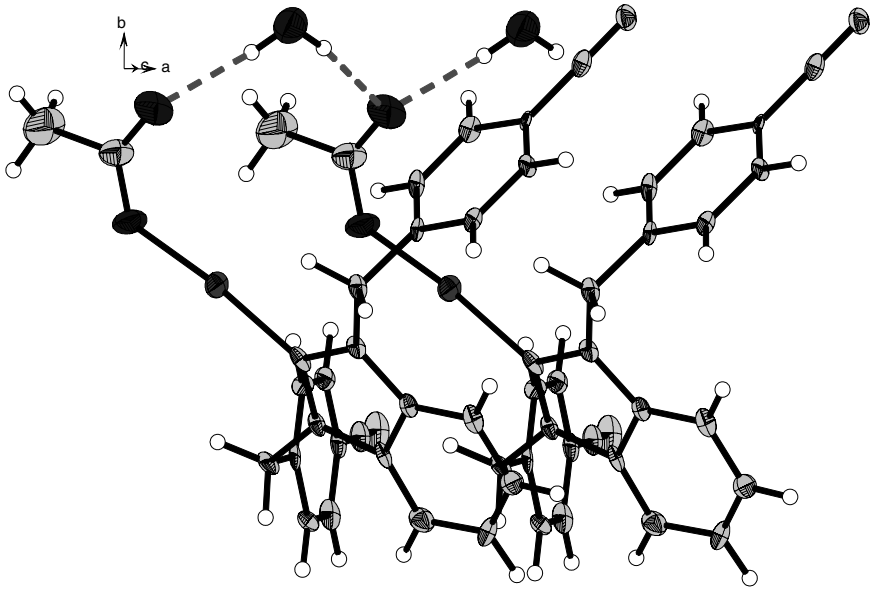


Figure 7. Hydrogen bonded chain of **4c** running along [1 0 0].

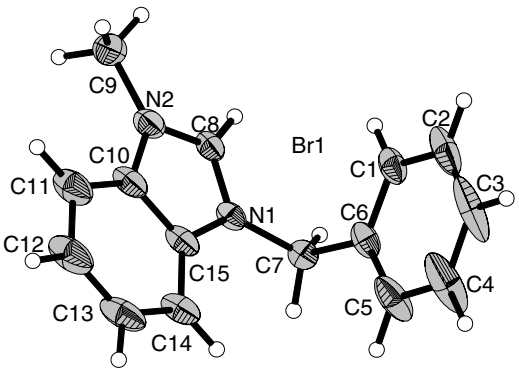


Figure 8. X-Ray diffraction structure of **7c**; molecule; thermal ellipsoids are drawn on the 50% probability level.

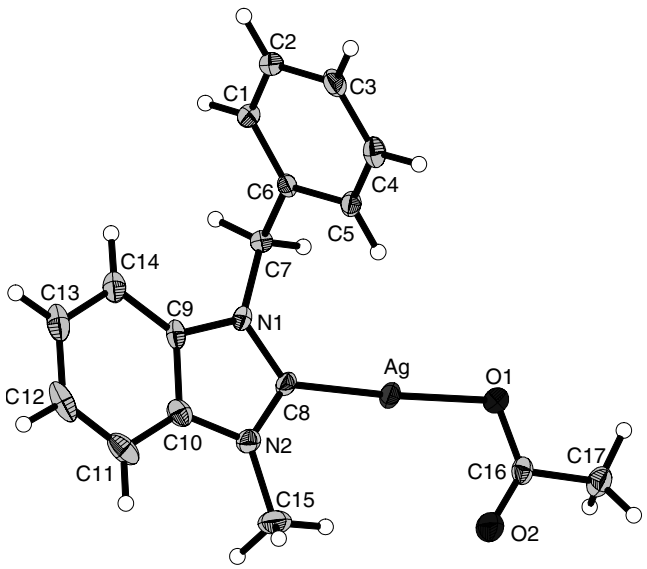


Figure 9. X-Ray diffraction structure of **8c**; molecule; thermal ellipsoids are drawn on the 50% probability level.

the NHC-precursors and NHC–silver complexes concentration increases, the antimicrobial activity becomes higher; and (iii) it was also observed that, compared with the NHC-precursors the NHC–silver complexes exhibited an approximately 6-fold increase in magnitude of antibacterial activity (see Figs 10–13), which is due to the synergistic effect of the increased lipophilicity of the complexes. Chelation decreases the polarity of the metal ion, which further leads to the enhanced lipophilicity of the complex. Since the microorganism cell wall is surrounded by a lipid membrane which favours the passage of lipid soluble materials, increased lipophilicity allows the penetration of complex into and through the membrane and deactivates the active enzyme sites of the microorganisms.[37]

In comparison with the known reported NHC-precursors and NHC–silver complexes from the literature,[19] the NHC-precursors (**3a–c** and **7a–c**) and their corresponding NHC–silver complexes (**4a–c** and **8a–c**) have shown high antibacterial activity.

Cytotoxicity Studies

The *in vitro* cytotoxicity of compounds **4a–c** and **8a–c** were determined by MTT-based assays[38] involving a 48 h drug

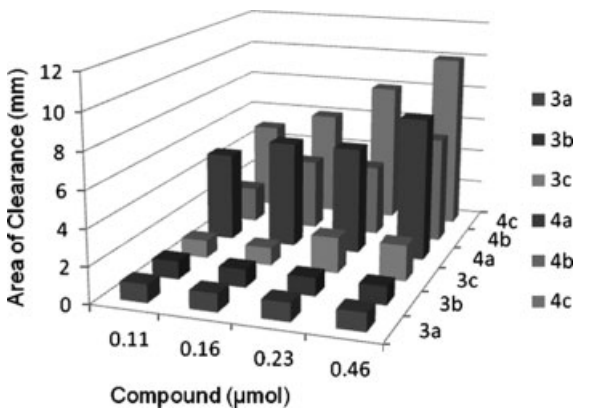


Figure 10. Area of clearance on *Staphylococcus aureus* (Gram +ve) by **3a–c** and **4a–c**.

Table 2. Selected bond lengths (Å) and angles (deg) for compounds **3a–c, 4a–c, 7c** and **8c**

Bond lengths (Å)	**3a**	**3b**	**3c**	**4a**	**4b**	**4c**	Bond lengths (Å)	**7c**	**8c**
N(2)–C(9)	1.326(2)	1.335(5)	1.336(2)	1.357(5)	1.354(2)	1.355(7)	N(1)–C(8)	1.323(6)	1.359(5)
N(2)–C(10)	1.380(2)	1.378(5)	1.393(2)	1.375(5)	1.379(2)	1.400(8)	C(8)–N(2)	1.329(6)	1.349(5)
C(9)–N(3)	1.3275(19)	1.342(5)	1.328(2)	1.351(5)	1.357(2)	1.349(8)	N(2)–C(10)	1.393(6)	1.399(6)
N(3)–C(11)	1.3813(19)	1.382(5)		1.373(5)	1.378(2)		C(10)–C(15)	1.380(7)	
C(10)–C(11)	1.351(2)	1.353(5)		1.339(6)	1.348(2)		N(1)–C(15)	1.394(5)	
N(3)–C(15)			1.394(2)			1.400(8)	C(9)–C(10)		1.378(6)
C(10)–C(15)			1.398(3)			1.397(8)	N(1)–C(9)		1.396(5)
C(10)–Cl(1)		1.691(4)			1.6864(18)		Ag–C(8)		2.048(4)
C(11)–Cl(2)		1.688(4)			1.6987(18)		Ag–O(1)		2.097(3)
Ag–C(9)				2.077(4)	2.0625(17)	2.073(6)	O(1)–C(16)		1.278(5)
Ag–O(1)				2.164(3)	2.1181(14)	2.085(5)	O(2)–C(16)		1.232(5)
O(1)–C(20)				1.269(5)	1.269(2)		C(16)–C(17)		1.525(6)
O(2)–C(20)				1.246(5)	1.244(2)				
C(20)–C(21)				1.513(6)	1.512(3)				
O(1)–C(24)						1.253(9)			
O(2)–C(24)						1.214(9)			
C(24)–C(25)						1.532(11)			
Bond angles (deg)							*Bond angles (deg)*		
N(2)–C(9)–N(3)	108.04(13)	108.2(3)	110.28(15)	103.7(3)	104.65(14)	106.1(5)	N(1)–C(8)–N(2)	110.1(4)	105.7(3)
C(9)–N(2)–C(10)	109.16(13)	108.6(3)	108.25(15)	111.2(3)	111.12(14)	111.0(5)	C(8)–N(2)–C(10)	108.2(4)	111.1(3)
C(9)–N(3)–C(11)	109.25(13)	108.9(3)		111.7(3)	110.58(14)		C(15)–C(10)–N(2)	106.6(4)	
C(10)–C(11)–N(3)	106.61(14)	106.6(3)		106.5(4)	107.21(15)		C(10)–C(15)–N(1)	106.5(4)	
C(11)–C(10)–N(2)	106.93(14)	107.8(3)		107.0(4)	106.43(15)		C(8)–N(1)–C(15)	108.5(4)	
C(9)–N(3)–C(15)			108.64(15)			111.3(5)	C(9)–C(10)–N(2)		106.0(4)
N(3)–C(15)–C(10)			106.25(15)			105.7(5)	C(10)–C(9)–N(1)		106.5(4)
N(2)–C(10)–C(15)			106.58(15)			105.9(5)	C(8)–N(1)–C(9)		110.7(3)
C(9)–Ag–O(1)				163.36(13)	174.90(6)	170.1(2)	C(8)–Ag–O(1)		176.82(14)
C(20)–O(1)–Ag				105.6(3)	111.65(12)		C(16)–O(1)–Ag		110.2(3)
C(24)–O(1)–Ag						128.3(5)			

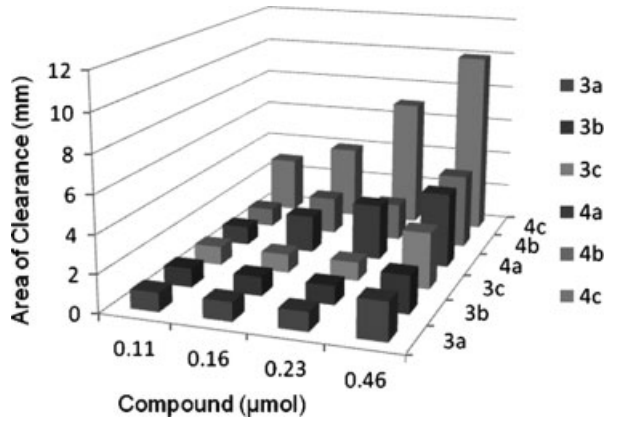


Figure 11. Area of clearance on *Escherichia coli* (Gram −ve) by **3a–c** and **4a–c**.

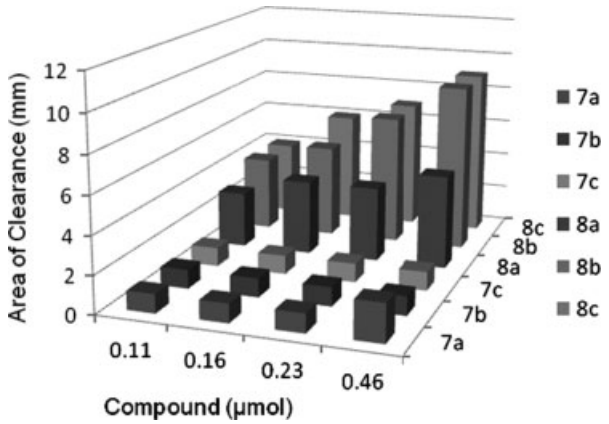


Figure 12. Area of clearance on *Staphylococcus aureus* (Gram +ve) by **7a–c** and **8a–c**.

exposure period, followed by 24 h of recovery time. Compounds were tested for their activity on the human cancerous renal-cell line Caki-1 and the results are shown in Figs 14 and 15, respectively. Symmetrically substituted NHC–silver acetate complexes **4a–c**, which contain 1H-imidazole, 4,5-dichloro-1H-imidazole and 1H-benzimidazole groups, have IC_{50} values of 29 (±5), 30 (±7) and 53 (±8) µM respectively. Compounds **4a** and **4b** show a very similar IC_{50} values and a 2-fold increase in magnitude when compared with **4c** and, in comparison with cisplatin (IC_{50} value = 3.3 µM), the IC_{50} values for the three compounds are not impressive. Non-symmetrically substituted NHC–silver acetate complexes **8a–c**, which also contain 1-methylimidazole, 4,5-dichloro-1-methylimidazole and 1-methylbenzimidazole groups, exhibit IC_{50} values of 3.2 (±0.6), 24 (±7) and 34 (±3) µM respectively. Compound **8a** is the most promising one in this paper because of its lowest IC_{50} value; **8a** exhibits an approximately 8-

Table 3. Area of clearance for compounds **3a–c** and **4a–c** in mm

Compounds		*Staphylococcus aureus* (Gram + ve)	*Escherichia coli* (Gram −ve)
3a (mm)	0.11 µmol (44.3 µg)	1	1
	0.16 µmol (62.0 µg)	1	1
	0.23 µmol (88.6 µg)	1	1
	0.46 µmol (177.2 µg)	1	2
3b (mm)	0.11 µmol (51.2 µg)	1	1
	0.16 µmol (71.7 µg)	1	1
	0.23 µmol (104.7 µg)	1	1
	0.46 µmol (209.5 µg)	1	2
3c (mm)	0.11 µmol (50.1 µg)	1	1
	0.16 µmol (70.1 µg)	1	1
	0.23 µmol (100.2 µg)	2	1
	0.46 µmol (200.5 µg)	2	3
4a (mm)	0.11 µmol (54.3 µg)	5	1
	0.16 µmol (76.1 µg)	6	2
	0.23 µmol (108.7 µg)	6	3
	0.46 µmol (217.5 µg)	8	4
4b (mm)	0.11 µmol (62.5 µg)	2	1
	0.16 µmol (87.5 µg)	4	2
	0.23 µmol (125.0 µg)	4	2
	0.46 µmol (250.0 µg)	6	4
4c (mm)	0.11 µmol (60.1 µg)	5	3
	0.16 µmol (84.2 µg)	6	4
	0.23 µmol (120.3 µg)	8	7
	0.46 µmol (240.7 µg)	10	10

Table 4. Area of clearance for compounds **7a–c** and **8a–c** in mm

Compounds		*Staphylococcus aureus* (Gram +ve)	*Escherichia coli* (Gram −ve)
7a (mm)	0.11 µmol (29.5 µg)	1	1
	0.16 µmol (41.3 µg)	1	1
	0.23 µmol (59.0 µg)	1	1
	0.46 µmol (118.0 µg)	2	2
7b (mm)	0.11 µmol (37.5 µg)	1	1
	0.16 µmol (52.5 µg)	1	1
	0.23 µmol (75.0 µg)	1	1
	0.46 µmol (150.0 µg)	1	2
7c (mm)	0.11 µmol (35.25 µg)	1	1
	0.16 µmol (49.35 µg)	1	2
	0.23 µmol (70.5 µg)	1	2
	0.46 µmol (141.0 µg)	1	3
8a (mm)	0.11 µmol (39.5 µg)	3	3
	0.16 µmol (55.3 µg)	4	4
	0.23 µmol (79.0 µg)	4	5
	0.46 µmol (158.0 µg)	5	6
8b (mm)	0.11 µmol (47.5 µg)	4	3
	0.16 µmol (66.5 µg)	5	4
	0.23 µmol (95.0 µg)	7	5
	0.46 µmol (190.0 µg)	9	6
8c (mm)	0.11 µmol (45.2 µg)	4	5
	0.16 µmol (63.3 µg)	6	8
	0.23 µmol (90.5 µg)	7	10
	0.46 µmol (181.0 µg)	9	12

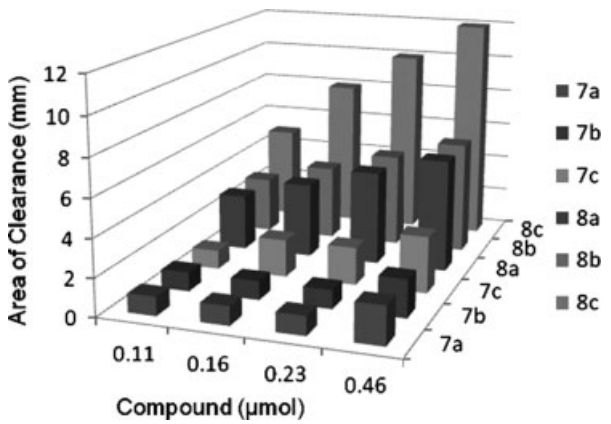


Figure 13. Area of clearance on *Escherichia coli* (Gram −ve) by **7a–c** and **8a–c**.

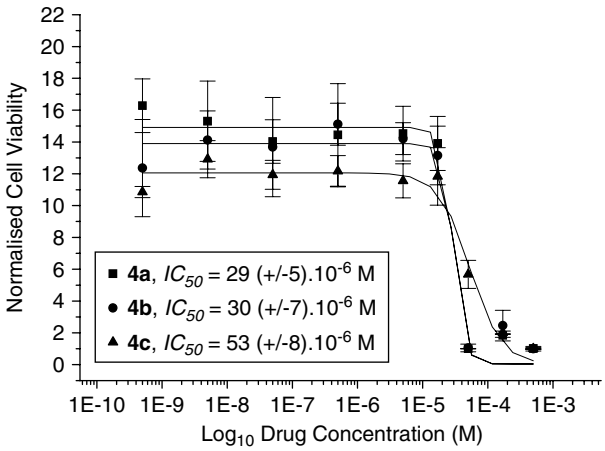


Figure 14. Cytotoxicity curves from typical MTT assays showing the effect of compounds **4a–c** on the viability of Caki-1 cells (**4a** and **4b** have overlapping response curves around their IC_{50} concentrations).

and 11-fold increase in cytotoxicity when compared with **8b** and **8c** and ranges therefore in the cytotoxicity class of platinum-based drugs.

Symmetrically and non-symmetrically substituted NHC–silver acetate complexes show very similar IC_{50} values; both compound classes are easily soluble in DMSO and all compounds are stable in saline solution for 24 h with respect to silver chloride or silver precipitation. It was also observed that, compared with known reported NHC–silver complexes from the literature,[10,19] the NHC–silver complexes (**4a–c** and **8a–c**) have almost the same cytotoxic activity.

Conclusions and Outlook

A series of six novel symmetrically substituted and non-symmetrically substituted *N*-heterocyclic carbene–silver acetate derivatives (**4a–c** and **8a–c**) were synthesized through the reaction of appropriately symmetrically substituted and non-symmetrically substituted *N*-heterocyclic carbenes (**3a–c** and **7a–c**) with silver acetate. Almost all the complexes have shown high antibacterial activity compared with the precursors and it is also clear that, as the precursors and complexes concentration

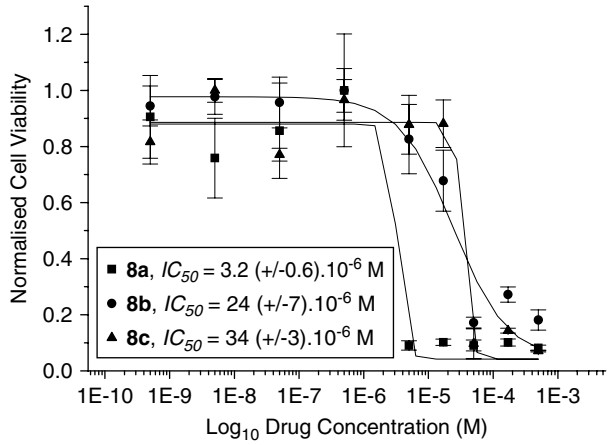


Figure 15. Cytotoxicity curves from typical MTT assays showing the effect of compounds **8a–c** on the viability of Caki-1 cells.

increases, the antibacterial activity becomes higher. While screening this novel series we have noted a marked improvement in antibacterial activity with respect to the compounds previously reported.[19] We were able to significantly reduce the substrate concentration in the stock solution (from 18.4 μM previously utilized down to 4.6 μM, 4-fold) and achieve a greater area of clearance in many cases, e.g. from 7 mm with complexes [4,5-dichloro-1,3-bis(4-methoxybenzyl)imidazole-2-ylidene]silver(I)acetate and (1,3-dibenzylimidazole-2-ylidene)silver(I)acetate[19] to 12 mm with compound **8c** when tested against *E. Coli* for example. We are currently looking at the possibility of determining MIC_{50} values for the best compounds. Complexes **4a–c** and **8a–c** yielded antitumoral IC_{50} values of 29 (±5), 30 (±7), 53 (±8), 3.2 (±0.6), 24 (±7) and 34 (±3) μM respectively on the Caki-1 cell line. The complex **8a**, however, gave a superior IC_{50} value of 3.2 (±0.6) μM. Further work is currently underway in order to improve these values by performing formulation experiments to improve solubility of these NHC–silver acetate complexes, which should allow for *in vivo* testing of **8a** in the nearby future.

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