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Research Article

Novel Nonsymmetrically *p*-Benzyl-Substituted (Benz)imidazole *N*-Heterocyclic Carbene-Silver(I) Acetate Complexes: Synthesis and Biological Evaluation

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Nonsymmetrically substituted *N*-heterocyclic carbene (NHC) precursors **1a–d** and **3a–d** were synthesised by first reacting 1H-(benz)imidazole with *p*-cyanobenzyl bromide to give 4-(1H-imidazole-1-ylmethyl)benzonitrile (**1**) and 4-(1H-benzimidazole-1-ylmethyl)benzonitrile (**3**) and afterwards introducing benzyl bromide, 1-(bromomethyl)-4-methylbenzene, 1-(bromomethyl)-4-methoxybenzene, and methyl 4-(bromomethyl)benzoate. The NHC-silver(I) acetate complexes (1-benzyl-3-(4-cyanobenzyl)-2,3-dihydro-1H-imidazole-2-ylidene) silver(I) acetate (**2a**), (1-(4-cyanobenzyl)-3-(4-methylbenzyl)-2,3-dihydro-1H-imidazole-2-ylidene) silver(I) acetate (**2b**), (1-(4-cyanobenzyl)-3-[4-(methoxycarbonyl)benzyl]-2,3-dihydro-1H-imidazole-2-ylidene) silver(I) acetate (**2c**), (1-benzyl-3-(4-cyanobenzyl)-2,3-dihydro-1H-benzimidazole-2-ylidene) silver(I) acetate (**4a**), (1-(4-cyanobenzyl)-3-(4-methylbenzyl)-2,3-dihydro-1H-benzimidazole-2-ylidene) silver(I) acetate (**4b**), (1-(4-cyanobenzyl)-3-(4-methoxybenzyl)-2,3-dihydro-1H-benzimidazole-2-ylidene) silver(I) acetate (**4c**), and (1-(4-cyanobenzyl)-3-[4-(methoxycarbonyl)benzyl]-2,3-dihydro-1H-benzimidazole-2-ylidene) silver(I) acetate (**4d**) were yielded by reacting these NHC precursors with silver(I) acetate. The silver(I) acetate complex **4b** was characterised by single crystal X-ray diffraction. Preliminary *in vitro* antibacterial studies against the Gram-positive bacteria *Staphylococcus aureus* and the Gram-negative bacteria *Escherichia coli*, using the Kirby-Bauer disc diffusion method, were carried out on the seven NHC-silver(I) acetate complexes **2a–c** and **4a–d**. Also the IC_{50} values of these seven complexes were determined by an MTT-based assay against the human renal cancer cell line Caki-1. The complexes **2a–c** and **4a–c** revealed the following IC_{50} values, respectively, 25 (±1), 15 (±2), 5.4 (±0.8), 16 (±2), 7.1 (±1), 20 (±4), and 14 (±1) μM.

1. Introduction

N-Heterocyclic carbenes (NHCs) are versatile ligands in silver complexes exhibiting antimicrobial activity, in particular for the possible treatment of pulmonary infections accompanying cystic fibrosis (CF) and chronic lung infections [1–3] and maybe in the treatment of cancer [4]. Youngs' research group have reported antimicrobial activity of NHC-silver complexes derived from 1H-imidazole, 4,5-dichloro-1H-imidazole and xanthines against a panel of highly resistant pathogens recovered from the respiratory tract of cystic fibrosis (CF) patients [1, 3, 5]. Another important contribution by the Ghosh research group led to the synthesis and antimicrobial evaluation of NHC-silver complexes derived from 1-benzyl-3-tert-butylimidazole [6]. In addition, *in vitro* and murine *in vivo* efficacy and toxicity studies of nebulised methylated caffeine-silver(I) complex (SCC1), for treatment of pulmonary infections [7]. Recently, a larger number of known compounds were evaluated as potential antibiotics by Roland et al. [8].

In addition, silver complexes have been reported to have anticancer activity *in vitro*. Egan has reported that silver complexes of coumarin derivatives possess anticancer activity against certain types of cancer [9]. Zhu et al. has

reported that silver carboxylate dimers possess anticancer activity against human carcinoma cells [10]. Liu et al. has shown phosphine complexes of silver to be active anticancer agents, even against cisplatin-resistant cell lines [11]. Youngs and coworkers have reported anticancer activity of NHC-silver complexes derived from 4,5-dichloro-1H-imidazole against the human cancer cell lines OVCAR-3 (ovarian), MB157 (breast), and HeLa (cervical) [4]. We have reported the anticancer (CAKI-1, renal) and antibacterial (*E. coli*, *S. aureus*) activity of benzyl-substituted *N*-heterocyclic carbene-silver [12–17] and carbene-gold complexes [18, 19].

Within this paper we present a new series of non-symmetrically *p*-benzyl-substituted *N*-heterocyclic carbene-silver acetate complexes derived from imidazole and benzimidazole, their synthesis, cytotoxicity, and antibacterial studies.

2. Experimental

2.1. General. All reactions were carried out under aerobic conditions. All silver(I) acetate reactions were carried out under exclusion of light. 1H-Imidazole, 1H-benzimidazole, benzyl bromide, 4-methylbenzyl bromide, methyl 4-(bromomethyl)benzoate, 4-cyanobenzyl bromide, silver(I) acetate, and K_2CO_3 were procured commercially from Sigma-Aldrich Chemical Company and were used without further purification. IR spectra were recorded on a Perkin-Elmer Paragon 1000 FT-IR spectrometer employing a KBr disc. NMR spectra were measured either on a Varian 300 MHz or 400 MHz spectrometer. All chemical shifts are reported in ppm and referenced to TMS. ESI MS was performed on a quadrupole tandem mass spectrometer (Quattro Micro, Micromass/Water's Corp., USA), using solutions in 100% MeOH. MS spectra were obtained in the ES+ (electron spray positive ionisation) mode for all compounds. CHN Analysis was carried out in an Exeter Analytical CE-440 elemental analyzer. Crystal Data was collected using an Agilent Technologies (former Oxford Diffraction) SuperNova diffractometer fitted with an Atlas detector. **4b** was measured with Mo-Kα (0.71073 Å) at 100 K. A four times redundant dataset was collected, assuming that the Friedel pairs are not equivalent. An analytical absorption correction based on the shape of the crystal was performed [20]. The structure was 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]. Hydrogen atoms were added at calculated positions and refined using a riding model. Their isotropic thermal displacement parameters were fixed to 1.2 (1.5 for methyl groups) times the equivalent ones of the parent atom. Anisotropic thermal displacement parameters were used for all nonhydrogen atoms. A suitable crystal of **4b** was grown in a saturated solution of chloroform with slow infusion of pentane. Further details about the data collection are listed in Table 1.

CCDC 853680 (for **4b**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/cgi-bin/catreq.cgi/.

2.2. Synthesis

4-(1H-Imidazole-1-ylmethyl)benzonitrile (1). 1H-Imidazole (1.478 g, 22.03 mmol), 4-(bromomethyl)benzonitrile (4.319 g, 22.03 mmol), and K_2CO_3 (4.560 g, 33.00 mmol) were stirred in CH_3CN at room temperature for 2 d. After the solvent was removed under reduced pressure, water (30 mL) was added. The aqueous phase was extracted with CH_2Cl_2 (3x, 20 mL), and the combined organic phases were dried with magnesium sulfate. After removing the solvent under reduced pressure, the product (**1**) was yielded (3.511 g, 19.14 mmol, 87% yield) as a white powder.

^{1}H NMR (δ ppm, $CDCl_3$, 300 MHz): 7.65 (d, J = 7.7, 2H, $CH_{cyanobenzyl}$), 7.56 (s, 1H, CH_{imid}), 7.21(d, J = 7.2, 2H, $CH_{cyanobenzyl}$), 7.12 (d, 1H, CH_{imid}), 6.89 (s, 1H, NCHN), 5.20 (s, 2H, CH_2).

^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz, proton decoupled): 139.6, 134.4, 132.9, 128.4, 128.2, 118.7, 113.9, 111.9 (NCHN, CN, CH_{imid}), 50.2 (CH_2).

IR absorptions (KBr, cm^{-1}): 3427 (m), 3132 (s), 3063 (m), 2981 (s), 2852 (s), 2226 (s), 1608 (m), 1567 (s), 1411 (s), 1355 (m), 1209 (s), 1161 (s), 769 (m), 634 (m), 550 (m).

MS (*m/z*, QMS-MS/MS): 182.07 [M$^+$-H].

Microanalysis calculated for $C_{11}H_9N_3$ (183.21): calcd.: C, 72.11%; H, 4.95%; N, 22.94%; found: C, 72.03%; H, 5.05%; N, 22.92%.

1-Benzyl-3-(4-cyanobenzyl)-2,3-dihydro-1H-imidazole-2-ylium Bromide (1a). 4-(1H-Imidazole-1-ylmethyl) benzonitrile (**1**) (0.500 g, 2.73 mmol) and 1.5 equivalents of benzyl bromide (0.701 g, 4.10 mmol) were dissolved in toluene (30 mL) and heated under reflux for 6 h. After removing the solvent under reduced pressure, the obtained sticky white product was washed with pentane (2x, 10 mL), diethylether (3x, 5 mL), and THF (2x, 10 mL) to yield a white powder (0.512 g, 1.44 mmol, 53% yield).

^{1}H NMR (δ ppm, $CDCl_3$, 300 MHz): 11.36 (s, 1H, NCHN), 7.71 (bs, 4H, $CH_{cyanobenzyl}$), 7.44 (s, 5H, CH_{benzyl}), 7.08 (s, 1H, $CH_{imidazole}$), 7.04 (s, 1H, $CH_{imidazole}$), 5.78 (s, 2H, CH_2), 5.48 (s, 2H, CH_2).

^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz, proton decoupled): 137.9 (NCHN), 137.8, 133.2, 132.1, 129.9, 129.8, 129.7, 129.0, 121.8, 121.7, 117.9, 113.6 (CN + C_{imid} + $C_{cyanobenzyl}$ + C_{benzyl}), 53.9, 52.6 (CH_2).

IR absorptions (KBr, cm^{-1}): 3461 (m), 3130 (s), 3058 (s), 2980 (s), 2920 (s), 2226 (s), 1608 (m), 1567 (s), 1411 (m), 1355 (m), 1209 (s), 1160 (s), 770 (s), 634 (m), 549 (m).

MS (*m/z*, QMS-MS/MS): 274.12 [M$^+$-Br].

Microanalysis calculated for $C_{18}H_{16}BrN_3$ (354.24): calcd.: C, 61.03%; H, 4.55%; N, 11.86%; Found: C, 60.96%; H, 4.52%, N, 11.68%.

1-(4-Cyanobenzyl)-3-(4-methylbenzyl)-2,3-dihydro-1H-imidazole-2-ylium Bromide (1b). 4-(1H-Imidazole-1-ylmethyl)benzonitrile (**1**) (0.500 g, 2.73 mmol) and 1.5 equivalents of 1-(bromomethyl)-4-methylbenzene (0.759 g, 4.10 mmol) were dissolved in toluene (30 mL) and heated

Table 1: Crystal data and structure refinement for **4b**.

		4b
Empirical formula		$C_{25}H_{22}N_3O_2Ag$
Formula weight [g/mol]		504.33
Crystal system		Monoclinic
Space group		$P2_1/n$ (#14)
Unit cell dimensions [Å]	a	7.6287(2)
	b	12.4073(3)
	c	23.0001(6)
	β	94.496(3)°
Volume [Å^3]		2170.30(10)
Z		4
Density [mg/m^3] (calc.)		1.543
Absorption coefficient [mm^{-1}]		0.956
F(000)		1024
Crystal size [mm^3]		0.2663 × 0.1227 × 0.1080
Theta range for data collection		3.32 to 26.42°.
Index ranges		$-9 \le h \le 9$
		$-15 \le k \le 15$
		$-28 \le l \le 28$
Reflections collected		35074
Independent reflections		4454 [R(int) = 0.0421]
Completeness to θ_{max}		99.6%
Max. and min. transmission		0.913 and 0.808
Data/restraints/parameters		4454/0/287
Goodness of fit in F^2		1.086
Final R indices ($I > 2\sigma(I)$)		R1 = 0.0238
		wR2 = 0.0512
R indices (all data)		R1 = 0.0301
		wR2 = 0.0543
Largest diff. peak and hole		0.871 and 0.323 e·Å^{-3}

under reflux for 6 h. After removing the solvent under reduced pressure, the obtained sticky white product was washed with pentane (2x, 10 mL), diethylether (3x, 5 mL), and THF (2x, 10 mL) to yield a white powder (0.784 g, 2.13 mmol, 78% yield).

^{1}H NMR (δ ppm, $CDCl_3$, 300 MHz): 10.74 (s, 1H, NCHN), 7.76 (d, J = 8.2 Hz, 2H, $CH_{cyanobenzyl}$), 7.64 (d, J = 8.2 Hz, 2H, $CH_{cyanobenzyl}$), 7.52 (s, 1H, $CH_{imidazole}$), 7.35–7.26 (m, 2H, CH_{imid}, $CH_{methylbenzyl}$), 7.18 (d, J = 7.6 Hz, 3H, CH_{imid}, $CH_{methylbenzyl}$), 5.82 (s, 2H, CH_2), 5.45 (s, 2H, CH_2), 2.33 (s, 3H, CH_3).

^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz, proton decoupled): 139.8(NCHN), 138.3, 136.9, 133.0, 130.2, 129.9, 129.4, 128.9, 122.5, 121.9, 118.1, 113.2 (CN + C_{imid} + $C_{cyanobenzyl}$ + $C_{methylbenzyl}$), 53.4, 52.3 (CH_2), 21.2 (CH_3).

IR absorptions (KBr, cm^{-1}): 3455 (m), 3130 (s), 3052 (s), 2981 (s), 2852 (s), 2227 (s), 1609 (m), 1566 (s), 1411 (m), 1209 (m), 1160 (s), 769 (s), 635 (m), 551 (s).

MS (*m/z*, QMS-MS/MS): 288.11 [M$^+$-Br].

Microanalysis calculated for $C_{19}H_{18}BrN_3$ (368.27): calcd.: C, 61.97%; H, 4.93%; N, 11.41%. Found: C, 62.34%; H, 5.01%; N, 11.21%.

1-(4-Cyanobenzyl)-3-(4-methoxybenzyl)-2,3-dihydro-1H-imidazole-2-ylium Bromide (1c). 4-(1H-Imidazole-1-ylmethyl)benzonitrile (**1**) (0.500 g, 2.73 mmol) and 1.5 equivalents of 1-(bromomethyl)-4-methoxybenzene (0.59 mL, 4.1 mmol) were dissolved in toluene (30 mL) and heated under reflux for 6 h. After removing the solvent under reduced pressure, the obtained sticky white product was washed with pentane (2x, 10 mL), diethylether (3x, 5 mL), and THF (2x, 10 mL) to yield a white powder (1.038 g, 2.70 mmol, 99% yield).

^{1}H NMR (δ ppm, $CDCl_3$, 300 MHz): 11.24 (s, 1H, NCHN), 7.69 (d, J = 9.2 Hz, 4H, $CH_{cyanobenzyl}$), 7.50–7.17 (m, 4H, $CH_{methoxybenzyl}$), 6.92 (s, 2H, $CH_{imidazole}$), 5.81 (s, 2H, CH_2), 5.42 (s, 2H, CH_2), 3.80 (s, 3H, CH_3).

^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz, proton decoupled): 138.3(NCHN), 137.0, 133.0, 130.7, 129.8, 129.3, 124.3, 122.2, 121.8, 121.5, 118.1, 114.9, 113.2 (CN + C_{imid} + $C_{cyanobenzyl}$ + $C_{methylbenzyl}$), 55.4 (OCH_3), 53.3, 52.4 (CH_2).

IR absorptions (KBr, cm^{-1}): 3414 (m), 3059 (s), 2986 (s), 2964 (s), 2223 (s), 1610 (m), 1553 (s), 1516 (s), 1257 (m), 1146 (s), 1032 (m), 846 (m), 629 (s), 562 (m).

MS (*m/z*, QMS-MS/MS): 304.06 [M$^+$-Br].

Microanalysis calculated for $C_{19}H_{18}BrN_3O$ (384.27): calcd.: C, 59.39%; H, 4.72%; N, 10.94%. Found: C, 59.01%; H, 4.80%; N, 10.47%.

1-(4-Cyanobenzyl)-3-[4-(methoxycarbonyl)benzyl]-2,3-dihydro-1H-imidazole-2-ylium Bromide (1d). 4-(1H-Imidazole-1-ylmethyl)benzonitrile (**1**) (0.500 g, 2.73 mmol) and 1.5 equivalents of methyl 4-(bromomethyl)benzoate (0.939 g, 4.10 mmol) were dissolved in toluene (30 mL) and heated under reflux for 6 h. After removing the solvent under reduced pressure, the obtained sticky white product was washed with pentane (2x, 10 mL), diethylether (3x, 5 mL), and THF (2x, 10 mL) to yield a white powder (0.461 g, 1.12 mmol, 41% yield).

^{1}H NMR (δ ppm, $CDCl_3$, 300 MHz):

^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz, proton decoupled): 160.1 (C=O), 142.6 (NCHN), 137.9, 137.2, 133.1, 131.3, 130.6, 130.0, 129.9, 129.0, 122.4, 122.2, 117.9, 113.4 (C_{imid} + $C_{cyanobenzyl}$ + $C_{methoxycarbonylbenzyl}$), 55.1, 52.2 ($CH_2$), 32.2 ($CH_3$).

IR absorptions (KBr, cm^{-1}): 3403 (m), 3132 (s), 3062 (s), 2983 (s), 2851 (s), 2226 (s), 1718 (m), 1609 (m), 1566 (s), 1412 (s), 1286 (s), 1161 (s), 769 (s), 635 (m), 556 (s).

MS (*m/z*, QMS-MS/MS): 332.09 [M$^+$-Br].

Microanalysis calculated for $C_{20}H_{18}BrN_3O_2$ (412.28): calcd.: C, 58.26%; H, 4.40%; N, 10.19%; found: C, 59.01%; H, 4.68%; N, 10.89%.

(1-Benzyl-3-(4-cyanobenzyl)-2,3-dihydro-1H-imidazole-2-ylidene) Silver(I) Acetate (2a). 1-Benzyl-3-(4-cyanobenzyl)-2,3-dihydro-1H-imidazole-2-ylium bromide (**1a**) (0.499 g, 1.41 mmol) and 2 equivalents of silver(I) acetate (0.471 g, 2.82 mmol) were dissolved in 30 mL of methanol/dichloromethane (1 : 1) and stirred in darkness at room temperature for 4 d. After filtering off the AgBr byproduct, the solvent was reduced to 3 mL and by adding dropwise 10 mL of pentane a white powder precipitated. The solvent was decanted and the white precipitate was washed with pentane (2x, 10 mL) and diethylether (3x, 10 mL) to yield a white powder (0.422 g, 0.96 mmol, 68% yield).

^{1}H NMR (δ ppm, $CDCl_3$, 300 MHz): 7.70 (m, 5H, $CH_{cyanobenzyl}$, CH_{benzyl}), 7.39 (m, 4H, $CH_{cyanobenzyl}$, CH_{benzyl}), 7.01 (d, 2H, CH_{imid}), 5.40 (s, 4H, CH_2), 2.05 (s, 3H, $CH_{3\ acetate}$).

^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz, proton decoupled): 188.1 (NCN), 166.1 (C=O), 144.1, 137.5, 136.9, 133.1, 131.2, 130.6, 129.1, 128.2, 127.5, 117.9 ($C_{cyanobenzyl}$ + C_{imid} + C_{benzyl}), 52.4, 51.2 (CH_2), 21.7 ($CH_{3\ acetate}$).

IR absorptions (KBr, cm^{-1}): 3410 (s), 3154 (s), 2230 (s), 1564 (s), 1411 (s), 1237 (m), 777 (m), 672 (w), 557 (m).

MS (*m/z*, QMS-MS/MS): 382.23 [M$^+$-$OCOCH_3$].

Microanalysis calculated for $C_{20}H_{18}AgN_3O_2$ (440.24): calcd.: C, 54.56%; H, 4.12%; N, 9.54%; found: C, 55.01%; H, 4.24%; N, 9.07%.

(1-(4-Cyanobenzyl)-3-(4-methylbenzyl)-2,3-dihydro-1H-imidazole-2-ylidene) Silver(I) Acetate (2b). 1-(4-Cyanobenzyl)-3-(4-methylbenzyl)-2,3-dihydro-1H-imidazole-2-ylium bromide (**1b**) (0.501 g, 1.36 mmol) and 2 equivalents of silver(I) acetate (0.451 g, 2.72 mmol) were dissolved in 30 mL of methanol/dichloromethane (1 : 1) and stirred in darkness at room temperature for 4 d. After filtering off the AgBr byproduct, the solvent was reduced to 3 mL, and by adding dropwise 10 mL of pentane a white powder precipitated. The solvent was decanted, and the white precipitate was washed with pentane (2x, 10 mL) and diethylether (3x, 10 mL) to yield a white powder (0.296 g, 0.650 mmol, 48% yield).

^{1}H NMR (δ ppm, $CDCl_3$, 300 MHz): 7.70 (m, 4H, $CH_{cyanobenzyl}$, $CH_{methylbenzyl}$), 7.40 (m, 4H, $CH_{cyanobenzyl}$, $CH_{methylbenzyl}$), 7.01 (d, 2H, CH_{imid}), 5.40 (d, 4H, CH_2), 1.91 (d, 6H, $CH_{3\ acetate}$, $CH_{3\ methylbenzyl}$).

^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz, proton decoupled): 178.4 (NCN), 176.5 (C=O), 140.4, 138.0, 133.2, 132.9, 129.5, 128.4, 121.9, 121.3, 118.0, 112.8, 110.0 (CN + C_{imid} + $C_{cyanobenzyl}$ + $C_{methylbenzyl}$), 55.2, 52.7 (CH_2), 30.9 ($CH_{3\ methylbenzyl}$), 23.3 ($CH_{3\ actetate}$).

IR absorptions (KBr, cm^{-1}): 3391 (s), 3053 (m), 2227 (s), 1567 (s), 1410 (s), 1238 (m), 1158 (m), 771 (s), 690 (s), 624 (m).

MS (*m/z*, QMS-MS/MS): 392.23 [M$^+$-$OCOCH_3$].

Microanalysis calculated for $C_{21}H_{20}AgN_3O_2$ (454.27): calcd.: C, 55.52%; H, 4.44%; N, 9.25%; found: C, 55.48%; H, 4.62%; N, 9.09%.

(1-(4-Cyanobenzyl)-3-[4-(methoxycarbonyl)benzyl]-2,3-dihydro-1H-imidazole-2-ylidene) Silver(I) Acetate (2c). 1-(4-Cyanobenzyl)-3-[4-(methoxycarbonyl)benzyl]-2,3-dihydro-1H-imidazole-2-ylium bromide (**1d**) (0.449 g, 1.09 mmol) and 2 equivalents of silver(I) acetate (0.363 g, 2.18 mmol) were dissolved in 30 mL of methanol/dichloromethane (1 : 1) and stirred in darkness at room temperature for 3d. After filtering off the AgBr byproduct, the solvent was reduced to 3 mL, and by adding dropwise 10 mL of pentane a white powder precipitated. The solvent was decanted, and the white precipitate was washed with pentane (2x, 10 mL) and diethylether (3x, 10 mL) to yield a white powder (0.309 g, 0.620 mmol, 57% yield).

^{1}H NMR (δ ppm, $CDCl_3$, 300 MHz): 7.68 (d, 4H, $CH_{cyanobenzyl}$, $CH_{methoxycarbonylbenzyl}$), 7.40 (d, 4H, $CH_{cyanobenzyl}$, $CH_{methoxycarbonylbenzyl}$), 7.01 (s, 2H, CH_{imid}), 5.75 (d, 2H, CH_2), 5.72 (d, 2H, CH_2), 3.91 (s, 3H, $CH_{3\ methoxycarbonylbenzyl}$), 2.07 (s, 3H, $CH_{3\ acetate}$).

^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz, proton decoupled): 178.3 (NCN), 156.2 (C=O), 140.2, 133.0, 130.5, 128.5, 122.0, 118.0, 112.9, 110.0, 55.4, 55.3 (CH_2), 30.9 ($OCH_{3\ methoxycarbonylbenzyl}$), 22.4 ($OCH_{3\ acetate}$).

IR absorptions (KBr, cm^{-1}): 3417 (s), 3080 (s), 2229 (s), 1720 (m), 1566 (s), 1412 (s), 1281 (w), 656 (s).

MS (*m/z*, QMS-MS/MS): 440.23 [M$^+$-$OCOCH_3$].

Microanalysis calculated for $C_{22}H_{20}AgN_3O_4$ (498.28): calcd.: C, 53.03%; H, 4.05%; N, 8.43%; found: C, 52.66%; H, 4.12%, N, 8.01%.

4-(1H-Benzimidazole-1-ylmethyl)benzonitrile (3). 1H-Benzimidazole (1.488 g, 12.70 mmol), 4-(bromomethyl)benzonitrile (2.489 g, 12.70 mmol), and K_2CO_3 (2.633 g, 19.05 mmol) were stirred in CH_3CN at room temperature for 2 d. After the solvent was removed under reduced pressure, water (30 mL) was added. The aqueous phase was extracted with CH_2Cl_2 (3x, 20 mL), and the combined organic phases were dried with magnesium sulfate. After removing the solvent under reduced pressure, the product (**3**) was yielded as a white powder (2.637 g, 11.81 mmol, 93% yield).

^{1}H NMR (δ ppm, $CDCl_3$, 300 MHz): 7.98 (s, 1H, NCHN), 7.86 (d, 1H, $CH_{benzimid}$), 7.64 (d, 2H, $CH_{cyanobenzyl}$), 7.24 (m, 5H, $CH_{cyanobenzyl}$, $CH_{benzimid}$), 5.45 (s, 2H, CH_2).

^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz, proton decoupled): 138.2, 137.9, 134.6, 134.2, 128.9, 128.1, 120.3, 119.1, 118.4, 113.7 (NCHN, CN, $C_{benzimid}$), 47.3 (CH_2).

IR absorptions (KBr, cm^{-1}): 3427 (m), 3060 (m), 2222 (s), 1615 (m), 1490 (s), 1457 (s), 1364 (m), 1284 (s), 1259 (s), 1167 (s), 761 (m), 745 (m), 556 (s).

MS (*m/z*, QMS-MS/MS): 232.09 [M$^+$-H].

Micro analysis calculated for $C_{15}H_{11}N_3$ (223.27): calcd.: C, 77.23%, H, 4.75%; N, 18.01%; found: C, 76.47%; H, 4.73%; N, 17.77%.

1-Benzyl-3-(4-cyanobenzyl)-2,3-dihydro-1H-benzimidazole-2-ylium Bromide (3a). 4-(1H-Benzimidazole-1-ylmethyl) benzonitrile (**3**) (0.500 g, 2.24 mmol) and 1.5 equivalents of benzyl bromide (0.575 g, 3.36 mmol) were dissolved in toluene (30 mL) and heated under reflux for 6 h. After removing the solvent under reduced pressure, the obtained sticky white product was washed with pentane (2x, 10 mL), diethylether (3x, 5 mL), and THF (2x, 10 mL) to yield a white powder (0.624 g, 1.54 mmol, 69% yield).

^{1}H NMR (δ ppm, $CDCl_3$, 300 MHz): 12.02 (s, 1H, NCHN), 7.73 (d, J = 8.0 Hz, 2H, $CH_{cyanobenzyl}$), 7.65 (d, J = 8.0 Hz, 2H, $CH_{cyanobenzyl}$), 7.53 (d, J = 10.9 Hz, 6H, $CH_{benzimidazole}$, CH_{benzyl}), 7.39 (d, J = 5.1 Hz, 3H, $CH_{benzimid}$, CH_{benzyl}), 6.10 (s, 2H), 5.83 (s, 2H).

^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz, proton decoupled): 143.7 (NCHN), 137.7, 133.1, 132.1, 131.2, 129.5, 129.1, 128.3, 127.4, 117.9, 113.9, 113.3 (CN + $C_{benzimid}$ + $C_{cyanobenzyl}$ + C_{benzyl}), 51.9, 50.7 (CH_2).

IR absorptions (KBr, cm^{-1}): 3451 (s), 3384 (s), 3121 (s), 3035 (s), 2877 (s), 2228 (s), 1607 (m), 1554 (s), 1454 (s), 1184 (s), 1020 (m), 770 (s), 622 (m).

MS (*m/z*, QMS-MS/MS): 324.14 [M$^+$-Br].

Microanalysis calculated for $C_{22}H_{18}BrN_3$ (404.30): calcd.: C, 65.36%; H, 4.49%; N, 10.39%; found: C, 65.40%; H, 4.40%, N, 10.21%.

1-(4-Cyanobenzyl)-3-(4-methylbenzyl)-2,3-dihydro-1H-benzimidazole-2-ylium bromide (3b). 4-(1H-Benzimidazole-1-ylmethyl)benzonitrile (**3**) (0.500 g, 2.24 mmol) and 1.5 equivalents of 1-(bromomethyl)-4-methylbenzene (0.622 g, 3.36 mmol) were dissolved in toluene (30 mL) and heated under reflux for 6 h. After removing the solvent under reduced pressure, the obtained sticky white product was washed with pentane (2x, 10 mL), diethylether (3x, 5 mL), and THF (2x, 10 mL) to yield a white powder (0.787 g, 1.88 mmol, 84% yield).

^{1}H NMR (δ ppm, $CDCl_3$, 300 MHz): 11.94 (s, 1H, NCHN), 7.72 (d, J = 8.2 Hz, 2H, $CH_{cyanobenzyl}$), 7.64 (d, J = 8.2 Hz, 2H, $CH_{cyanobenzyl}$), 7.53 (d, J = 16.2 Hz, 4H, $CH_{benzimidazole}$), 7.39 (d, J = 8.0 Hz, 2H, $CH_{methylbenzyl}$), 7.18 (d, J = 8.0 Hz, 2H, $CH_{methylbenzyl}$), 6.10 (s, 2H, CH_2), 5.77 (s, 2H, CH_2), 2.33 (s, 3H, CH_3).

^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz, proton decoupled): 143.5 (NCHN), 139.5, 137.8, 133.0, 131.2, 130.1, 129.1, 128.3, 127.3, 118.0, 114.0, 113.4, 113.1 143.7, 137.7, 133.1, 132.1, 131.2, 129.5, 129.1, 128.3, 127.4, 117.9, 113.9, 113.3 (CN + $C_{benzimid}$ + $C_{cyanobenzyl}$ + $C_{methylbenzyl}$), 51.8, 50.7 (CH_2), 21.2 (CH_3).

IR absorptions (KBr, cm^{-1}): 3465 (s), 3397 (s), 3127 (s), 3032 (s), 2968 (s), 2885 (s), 2230 (s), 1608 (m), 1561 (s), 1416 (m), 1372 (s), 1191 (s), 1019 (m), 761 (s), 613 (m), 554 (m).

MS (*m/z*, QMS-MS/MS): 338.13 [M$^+$-Br].

Microanalysis calculated for $C_{23}H_{20}BrN_3$ (418.33): calcd.: C, 66.04%; H, 4.82%; N, 10.04%; found: C, 65.99%; H, 4.79%, N, 10.04%.

1-(4-Cyanobenzyl)-3-(4-methoxybenzyl)-2,3-dihydro-1H-benzimidazole-2-ylium Bromide (3c). 4-(1H-Benzimidazole-1-ylmethyl)benzonitrile (**3**) (0.500 g, 2.24 mmol) and 1.5 equivalents of 1-(bromomethyl)-4-methoxybenzene (0.484 mL, 3.36 mmol) were dissolved in toluene (30 mL) and heated under reflux for 6 h. After removing the solvent under reduced pressure, the obtained sticky white product was washed with pentane (2x, 10 mL), diethylether (3x, 5 mL), and THF (2x, 10 mL) to yield a white powder (0.651 g, 1.50 mmol, 67% yield).

^{1}H NMR (δ ppm, $CDCl_3$, 300 MHz): 11.91 (s, 1H, NCHN), 7.72 (d, J = 8.2 Hz, 2H, $CH_{cyanobenzyl}$), 7.63 (d, J = 8.2 Hz, 2H, $CH_{cyanobenzyl}$), 7.60–7.41 (m, 6H, $CH_{benzimid}$, $CH_{methoxybenzyl}$), 6.89 (d, J = 8.6 Hz, 2H, $CH_{methoxybenzyl}$), 6.09 (s, 2H, CH_2), 5.75 (s, 2H, CH_2), 3.77 (s, 3H, CH_3).

^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz, proton decoupled): 144.3 (NCHN), 137.9, 133.1, 131.2, 129.9, 129.1, 127.3, 124.2, 117.9, 114.8, 113.9, 113.2 (CN + $C_{benzimid}$ + $C_{cyanobenzyl}$ + $C_{methoxybenzyl}$), 55.34 (OCH_3), 51.5, 50.6 (CH_2).

IR absorptions (KBr, cm^{-1}): 3420 (s), 3122 (s), 3035 (s), 2968 (s), 2840 (s), 2229 (s), 1792 (w), 1609 (s), 1555 (s), 1514 (s), 1459 (s), 1370 (s), 1254 (s), 1178 (s), 1112 (s), 1013 (s), 763 (s), 613 (s).

MS (*m/z*, QMS-MS/MS): 355.08 [M$^+$-Br].

Microanalysis calculated for $C_{23}H_{20}BrN_3O$ (434.33): calcd.: C, 63.60%; H: 4.64%; N, 9.67%; found: C, 63.41%; H, 4.70%; N, 9.69%.

1-(4-Cyanobenzyl)-3-[4-(methoxycarbonyl)benzyl]-2,3-dihydro-1H-benzimidazole-2-ylium Bromide (3d). 4-(1H-Benzimidazole-1-ylmethyl)benzonitrile (**3**) (0.500 g,

2.24 mmol) and 1.5 equivalents of methyl 4-(bromomethyl) benzoate (0.770 g, 3.36 mmol) were dissolved in toluene (30 mL) and heated under reflux for 6 h. After removing the solvent under reduced pressure, the obtained sticky white product was washed with pentane (2x, 10 mL), diethylether (3x, 5 mL), and THF (2x, 10 mL) to yield a white powder (0.735 g, 1.59 mmol, 71% yield).

^{1}H NMR (δ ppm, $CDCl_3$, 300 MHz): 12.09 (s, 1H, NCHN), 8.03 (d, J = 8.2 Hz, 2H, $CH_{cyanobenzyl}$), 7.72 (d, J = 8.2 Hz, 2H, $CH_{cyanobenzyl}$), 7.66 (d, J = 8.2 Hz, 2H, $CH_{benzimidazole}$), 7.58 (d, J = 8.2 Hz, 2H, $CH_{benzimidazole}$), 7.51 (d, J = 5.5 Hz, 4H, $CH_{methoxycarbonylbenzyl}$), 6.07 (s, 2H, CH_2), 5.96 (s, 2H, CH_2), 3.91 (s, 3H, CH_3).

^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz, proton decoupled): 166.20 (C=O), 143.88 (NCHN), 139.76, 139.48, 133.32, 131.50, 130.17, 129.56, 129.03, 127.42, 118.86, 114.45, 114.39, 111.93 (CN + $C_{benzimid}$ + $C_{cyanobenzyl}$ + $C_{methoxycarbonylbenzyl}$), 52.75 (OCH3), 50.14, 49.96 ($CH_{2\ cyanobenzyl}$ + $CH_{2\ methoxycarbonylbenzyl}$).

IR absorptions (KBr, cm^{-1}): 3433 (s), 2963 (s), 2228 (s), 1717 (s), 1560 (m), 1435 (m), 1416 (m), 1283 (s), 1189 (m), 1107 (m), 748 (m), 601 (m).

MS (*m/z*, QMS-MS/MS): 382.05 [M$^+$-Br].

Microanalysis calculated for $C_{24}H_{20}BrN_3O_2$ (462.34): calcd.: C, 62.35%; H, 4.36%; N, 9.09%; found: C, 61.90%; H, 4.14%; N, 9.10%.

(1-Benzyl-3-(4-cyanobenzyl)-2,3-dihydro-1H-benzimidazole-2-ylidene) Silver(I) Acetate (4a). 1-Benzyl-3-(4-cyanobenzyl)-2,3-dihydro-1H-benzimidazole-2-ylium bromide (**3a**) (0.150 g, 0.370 mmol) and 2 equivalents of silver(I) acetate (0.124 g, 0.740 mmol) were dissolved in 30 mL of dichloromethane and stirred in darkness at room temperature for 3 d. After filtering off the AgBr byproduct, the solvent was reduced to 3 mL, and by adding dropwise 10 mL of pentane a white powder precipitated. The solvent was decanted, and the white precipitate was washed with pentane (2x, 10 mL) and diethylether (3x, 10 mL) to yield a white powder (0.094 g, 0.192 mmol, 52% yield).

^{1}H NMR (δ ppm, $CDCl_3$, 300 MHz): 7.64 (d, 2H, $CH_{cyanobenzyl}$), 7.30 (m, 11H, $CH_{cyanobenzyl}$, $CH_{benzimid}$, CH_{benzyl}), 5.74 (s, 2H, CH_2), 5.66 (s, 2H, CH_2), 2.09 (s, 3H, $CH_{3\ acetate}$).

^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz, proton decoupled): 189.21 (NCN), 165.20 (C=O), 142.29, 136.61, 133.19, 129.23, 128.74, 128.50, 128.00, 124.63, 118.95, 113.02, 112.72, 111.28, 109.99 ($C_{cyanobenzyl}$, $C_{benzimid}$, C_{benzyl}), 52.56, 51.85 (CH_2), 23.82 ($CH_{3\ acetate}$).

IR absorptions (KBr, cm^{-1}): 3476–3364 (m), 3037-2922 (s), 2229 (m), 1607 (s), 1562 (s), 1390 (s), 1335 (m), 740 (s), 657 (s), 614 (s), 552 (m), 454 (m).

MS (*m/z*, QMS-MS/MS): 430.05 [M$^+$-$OCOCH_3$].

Microanalysis calculated for $C_{24}H_{20}AgN_3O_2$ (490.30): calcd.: C, 58.79%; H, 4.11%; N, 8.57%; found: C, 58.71%; H, 4.03%; N, 8.69%.

(1-(4-Cyanobenzyl)-3-(4-methylbenzyl)-2,3-dihydro-1H-benzimidazole-2-ylidene) Silver(I) Acetate (4b). 1-(4-Cyanobenzyl)-3-(4-methylbenzyl)-2,3-dihydro-1H-benzimidazole-2-ylium bromide (**3b**) (0.498 g, 1.19 mmol) and 2 equivalents of silver(I) acetate (0.399 g, 2.39 mmol) were dissolved in 30 mL of dichloromethane and stirred in darkness at room temperature for 3d. After filtering off the AgBr byproduct, the solvent was reduced to 3 mL, and by adding dropwise 10 mL of pentane a white powder precipitated. The solvent was decanted, and the white precipitate was washed with pentane (2x, 10 mL) and diethylether (3x, 10 mL) to yield a white powder (0.462 g, 0.916 mmol, 77% yield).

^{1}H NMR (δ ppm, $CDCl_3$, 300 MHz): 7.25 (m, 12H, $CH_{cyanobenzyl}$, $CH_{methylbenzyl}$, $CH_{benzimi}$), 5.73 (d, 2H, CH_2), 5.61 (d, 2H, CH_2), 2.32 (s, 3H, $CH_{3\ methylbenzyl}$), 2.08 (s, 3H, $CH_{3\ acetate}$).

^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz, proton decoupled): 179.1 (NCN), 138.5, 133.9, 133.7, 132.9, 131.7, 131.7, 129.8, 127.9, 127.3, 124.5, 118.2, 112.4, 111.6 ($C_{cyanobenzyl}$, $C_{benzimid}$, $C_{methylbenzyl}$), 55.5, 52.8 (CH_2), 22.8, 21.1 ($CH_{3\ methylbenzyl}$, $CH_{3\ actetate}$).

IR absorptions (KBr, cm^{-1}): 3417 (m), 3030 (s), 2229 (m), 1571 (s), 1391 (s), 796 (m), 743 (m), 668 (m), 612 (m)

MS (*m/z*, QMS-MS/MS): 445.95 [M$^+$-$OCOCH_3$].

Microanalysis calculated for $C_{25}H_{22}AgN_3O_2$ (504.33): calcd.: C, 59.54%; H, 4.40%; N, 8.33%; found: C, 60.04%; H, 4.38%, N, 8.37%.

(1-(4-Cyanobenzyl)-3-(4-methoxybenzyl)-2,3-dihydro-1H-benzimidazole-2-ylidene) Silver(I) Acetate (4c). 1-(4-Cyanobenzyl)-3-(4-methoxybenzyl)-2,3-dihydro-1H-benzimidazole-2-ylium bromide (**3c**) (0.499 g, 1.15 mmol) and 2 equivalents of silver(I) acetate (0.383 g, 2.30 mmol) were dissolved in 30 mL of dichloromethane and stirred in darkness at room temperature for 2 d. After filtering off the AgBr byproduct, the solvent was reduced to 3 mL, and by adding dropwise 10 mL of pentane a white powder precipitated. The solvent was decanted, and the white precipitate was washed with pentane (2x, 10 mL) and diethylether (3x, 10 mL) to yield a white powder (0.418 g, 0.805 mmol, 70% yield).

^{1}H NMR (δ ppm, $CDCl_3$, 300 MHz): 7.25 (m, 12H, $CH_{cyanobenzyl}$, $CH_{methoxybenzyl}$, $CH_{benzimi}$), 5.74 (d, 2H, CH_2), 5.58 (d, 2H, CH_2), 3.78 (s, 3H, CH_3), 2.07 (s, 3H, $OCH_{3\ acetate}$).

^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz, proton decoupled): 178.8 (NCN), 159.8 (C=O), 133.9, 133.7, 132.9, 128.8, 127.9, 124.4, 118.1, 114.5, 112.4, 111.5 ($C_{cyanobenzyl}$, $C_{benzimid}$, $C_{methoxybenzyl}$), 55.3, 53.2 (CH_2), 30.9 ($CH_{3\ methoxybenzyl}$), 22.9 ($CH_{3\ acetate}$).

IR absorptions (KBr, cm^{-1}): 3390 (m), 2929 (s) [C–H], 2228 (s), 1608 (s), 1571 (s), 1513 (s), 1393 (s), 1249 (s), 1176 (s), 1116 (w), 1024 (s), 799 (s), 745 (s), 538 (s).

MS (*m/z*, QMS-MS/MS): 461.97 [M$^+$-$OCOCH_3$].

Microanalysis calculated for $C_{25}H_{22}AgN_3O_3$ (520.33): calcd.: C, 57.71%; H, 4.26%; N, 8.08%; found: C, 57.72%; H, 4.18%; N, 8.27%.

(1-(4-Cyanobenzyl)-3-[4-(methoxycarbonyl)benzyl]-2,3-dihydro-1H-benzimidazole-2-ylidene) Silver(I) Acetate (4d). 1-(4-Cyanobenzyl)-3-[4-(methoxycarbonyl)benzyl]-2,3-dihydro-1H-benzimidazole-2-ylium bromide (**3d**) (0.499 g, 1.08 mmol) and 2 equivalents of silver(I) acetate (0.360 g, 2.16 mmol) were dissolved in 30 mL of dichloromethane and stirred in darkness at room temperature for 2 d. After filtering off the AgBr byproduct, the solvent was reduced to 3 mL, and by adding dropwise 10 mL of pentane a white powder precipitated. The solvent was decanted, and the white precipitate was washed with pentane (2x, 10 mL) and diethylether (3x, 10 mL) to yield a white powder (0.361 g, 0.658 mmol, 61% yield).

^{1}H NMR (δ ppm, $CDCl_3$, 300 MHz): 7.39 (m, 12H, $CH_{cyanobenzyl}$, $CH_{methoxycarbonylbenzyl}$, $CH_{benzimi}$), 5.74 (d, 2H, CH_2), 5.72 (d, 2H, CH_2), 3.90 (s, 3H, CH_3), 2.08 (s, 3H, $OCH_{3\ acetate}$).

^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz, proton decoupled: 179.3 (NCN), 166.3 (C=O), 140.0, 139.4, 133.8, 133.7, 133.0, 130.4, 127.9, 127.2, 124.8, 118.1, 112.7, 112.2, 111.8 ($C_{cyanobenzyl}$, $C_{benzimid}$, $C_{methoxycarbonylbenzyl}$), 53.4, 53.0 ($CH_2$), 52.3 ($CH_{3methoxycarbonylbenzyl}$), 22.6 ($CH_{3actetate}$).

IR absorptions (KBr, cm^{-1}): 3422 (m), 2952 (s), 2229 (m), 1715 (s), 1573 (s), 1394 (s), 1283 (s), 1106 (m), 1018 (w), 739 (m).

MS (*m/z*, QMS-MS/MS): 490.07 [M^+-$OCOCH_3$].

Microanalysis calculated for $C_{26}H_{22}AgN_3O_4$ (548.34): calcd.: C, 56.95%; H, 4.04%; N, 7.66%; found: C, 56.77%; H, 4.03%; N, 7.48%.

2.3. Antibacterial Studies. The silver(I) acetate complexes were screened in preliminary *in vitro* antibacterial tests against two bacterial strains. The test organisms included *Staphylococcus aureus* (SA) (NCTC 7447) as a Gram-positive bacteria and *Escherichia coli* (*E. coli*) as Gram-negative bacteria.

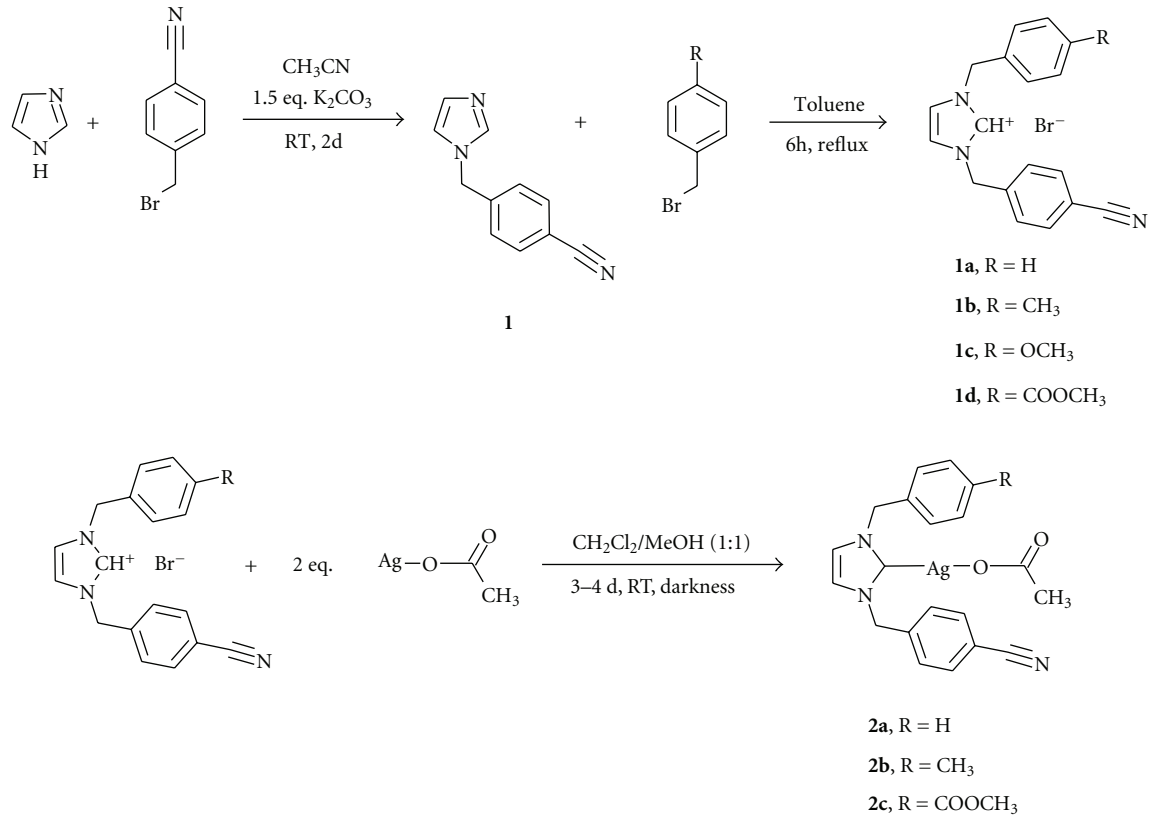
To assess the biological activity of compounds **2a–c** and **4a–d**, the qualitative Kirby-Bauer disk-diffusion method was applied [22]. All bacteria were individually cultured from a single colony in sterile LB medium [23] overnight at 37°C in an orbital shaker incubator. All the work carried out was performed under sterile conditions.

For each strain, 70 μL of culture was spread evenly on agar-LB medium. Four 5 mm diameter Whatman paper discs were placed evenly separated on each plate. Two stock solutions (9 : 1 DMSO : H_2O) of every compound were prepared at 2.2 μM and 4.4 μM to be able to test the effect of different concentrations. Each plate was then tested with 5 μL and 7 μL of 2.2 μM solution and 5 μL and 10 μL for the 4.4 μM solution. The plates were covered and placed in an incubator at 37°C for 24 h. The plates were then removed and the area of clearance, which is defined as the distance between the edge of the filter paper disc and the beginning of the bacterial growth, was measured for each sample in mm.

2.4. 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. This cell line was chosen based on its regular and long-lasting growth behaviour, which is similar to the one shown in kidney carcinoma cells. The cells were obtained from the ATCC (American Tissue Cell Culture Collection) and maintained in Dulbecco's Modified Eagle medium containing 10% (v/v) FCS (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 5,000 cells/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 200 μL of a solution of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) in medium. The solution consisted of 22 mg of MTT in 40 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. For all tests, cells with low passage numbers were used. 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.

3. Results and Discussion

In Schemes 1 and 2, the synthetic routes for the asymmetric substituted *N*-heterocyclic carbene ligand precursors as well as their corresponding silver(I) acetate complexes are given. The initial precursors 4-(1H-imidazole-1-ylmethyl)benzonitrile (**1**) and 4-(1H-benzimidazole-1-ylmethyl)benzonitrile (**3**) were prepared by stirring 1H-imidazole and 1H-benzimidazole with 4-(bromomethyl)benzonitrile in acetonitrile and K_2CO_3 at room temperature for 2d with 69 and 93% yields, respectively. 1-Benzyl-3-(4-cyanobenzyl)-2,3-dihydro-1H-imidazole-2-ylium bromide (**1a**), 1-(4-cyanobenzyl)-3-(4-methylbenzyl)-2,3-dihydro-1H-imidazole-2-ylium bromide (**1b**), 1-(4-cyanobenzyl)-3-(4-methoxybenzyl)-2,3-dihydro-1H-imidazole-2-ylium bromide (**1c**), and 1-(4-cyanobenzyl)-3-[4-(methoxycarbonyl)benzyl]-2,3-dihydro-1H-imidazole-2-ylium bromide (**1d**) were synthesised by stirring (**1**) with benzyl bromide, 1-(bromomethyl)-4-methylbenzene, 1-(bromomethyl)-4-methoxybenzene, and 4-(bromomethyl)benzoate in toluene under reflux for 6 h to yield the *N*-heterocyclic carbene ligand precursors in 53, 78, 99, and 41%, respectively. The benzimidazole containing



Scheme 1: General reaction scheme for the synthesis of imidazole containing asymmetric substituted NHC precursors **1a–d** and their corresponding NHC-silver(I) acetate complexes **2a–c**.

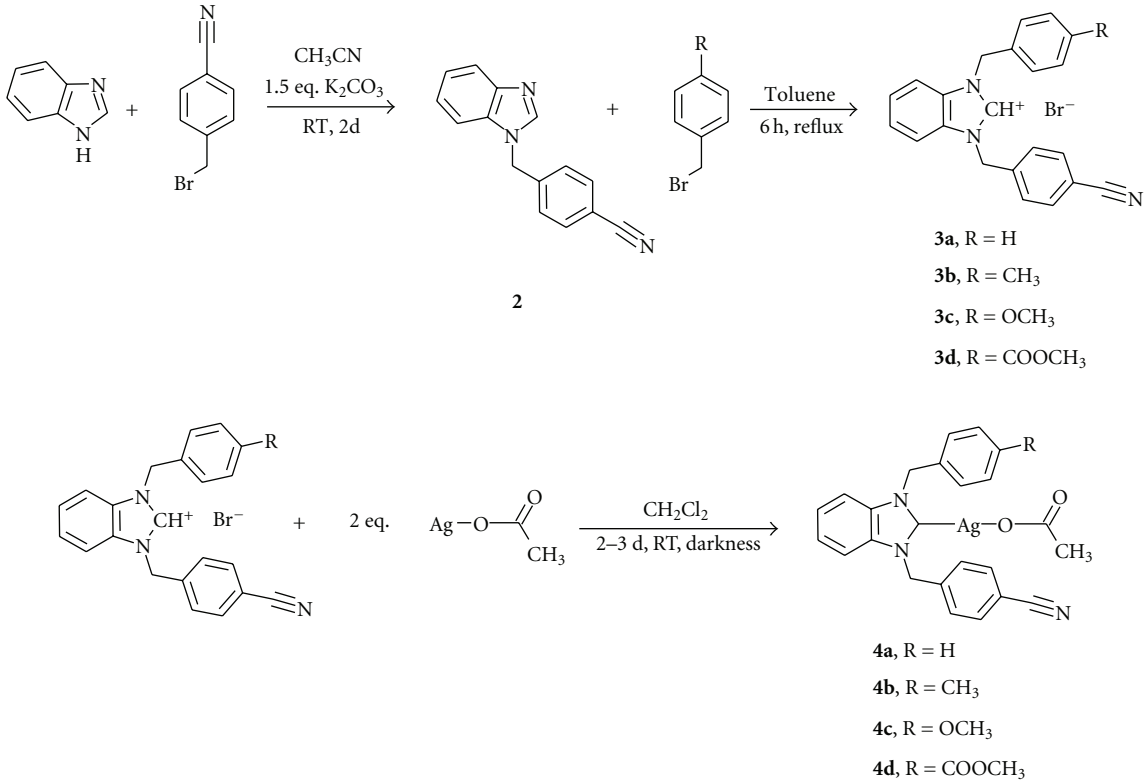
precursors 1-benzyl-3-(4-cyanobenzyl)-2,3-dihydro-1H-benzimidazole-2-ylium bromide (**3a**), 1-(4-cyanobenzyl)-3-(4-methylbenzyl)-2,3-dihydro-1H-benzimidazole-2-ylium bromide (**3b**), 1-(4-cyanobenzyl)-3-(4-methoxybenzyl)-2,3-dihydro-1H-benzimidazole-2-ylium bromide (**3c**), and 1-(4-cyanobenzyl)-3-[4-(methoxycarbonyl)benzyl]-2,3-dihydro-1H-benzimidazole-2-ylium bromide (**3d**) followed the same reaction route to give the respective yields of 69, 84, 67, and 71%.

In the absence of light, the silver(I) acetate complexes (1-benzyl-3-(4-cyanobenzyl)-2,3-dihydro-1H-imidazole-2-ylidene) silver(I) acetate (**2a**), (1-(4-cyanobenzyl)-3-(4-methylbenzyl)-2,3-dihydro-1H-imidazole-2-ylidene) silver(I) acetate (**2b**), and (1-(4-cyanobenzyl)-3-[4-(methoxycarbonyl)benzyl]-2,3-dihydro-1H-imidazole-2-ylidene)silver(I) acetate (**2c**) were prepared by stirring the respective precursor with 2 equivalents of silver(I) acetate in a mixture of dichloromethane and methanol (1 : 1) at room temperature for 3-4 d. The silver complexes were obtained in 68, 48, and 57% yield respectively. The synthesis of the asymmetric benzimidazole silver(I) acetate complexes was carried out under the same conditions but in dichloromethane and with shorter reaction times of 2-3d to give the silver(I) acetate complexes (1-benzyl-3-(4-cyanobenzyl)-2,3-dihydro-1H-benzim idazole-2-ylidene) silver(I) acetate (**4a**), (1-(4-cyanobenzyl)-3-(4-methylbenzyl)-2,3-dihydro-1H-benzimidazole-2-ylidene) silver(I) acetate (**4b**), (1-(4-cyanobenzyl)-3-(4-methoxybenzyl)-2,3-dihydro-1H-benzimidazole-2-yl-id-e-ne) silver(I) acetate (**4c**), and (1-(4-cyanobenzyl)-3-[4-(methoxycarbonyl)benzyl]-2,3-dihydro-1H-benzimidazole-2-ylidene) silver(I) acetate (**4d**) in 52, 77, 70, and 61% yields.

The asymmetric substituted *N*-heterocyclic carbene ligand precursors as well as the silver(I) acetate complexes were fully characterized by spectral (^{1}H NMR, ^{13}C NMR, IR, mass) and elemental analysis studies.

Furthermore, the solid state structure of the silver(I) acetate complex **4b** was determined by single crystal X-ray diffraction.

Due to the positive charge of the molecule, the ^{1}H NMR spectra of all NHC precursors **1a–d** and **3a–d** show a characteristic downfield shift in the range δ = 10.74–12.09 ppm for the NCHN proton [23–25]. In addition, their identities have also been confirmed by a base peak for the [M$^+$-Br] fragments in their positive mode ESI mass spectra. A successful formation of the complexes **2a–c** and **4a–d** is indicated by the absence of a downfield NCHN signal and presence of new signals at 2.09–1.91 ppm for the acetate protons in all the ^{1}H NMR spectra. The ^{13}C NMR resonances of the carbene carbon atoms in complexes **2a–c** and **4a–d** occur in the range δ 188.1–178.3 ppm respectively. These signals are shifted downfield compared to the corresponding



Scheme 2: General reaction scheme for the synthesis of benzimidazole containing asymmetric substituted NHC precursors **3a–d** and their corresponding NHC-silver(I) acetate complexes **4a–d**.

precursors of **1a–d** and **3a–d** carbene carbons resonance at the range 144.3–137.9 ppm, respectively, which further demonstrates the formation of expected NHC-silver(I) acetate complexes. Also the appearance of the ^{13}C NMR resonances for the carbonyl and methyl carbons of the acetate group of complexes **2a–c** and **4a–d** in the range 176.5–156.2 and 23.3–21.1 ppm, respectively, showed the formation of the NHC-silver(I) acetate complexes [1, 3]. Furthermore, positive-mode ESI mass spectra of all six NHC-silver(I) acetate complexes (**2a–c, 4a–d**) are dominated by [M^+-O_2CCH_3] fragment peaks arising from the loss of one acetate ligand.

3.1. Structural Discussion. The crystal of **4b** was formed in a saturated solution of chloroform with slow infusion of pentane. It crystallized in the monoclinic space group $P2_1/n$ (no. 14) in absence of any solvent molecules. The crystal data and refinements are found in Table 1, whereas selected bond lengths and bond angles are compiled in Table 2. The X-ray structure shows that the benzimidazole ring is planar and the bond lengths and angles in the five-membered imidazole ring (NCNCC) are in good agreement with those in similar compounds reported earlier by our group [13–16]. Complex **4b** crystallises in two different species, which are shown in Figures 1 and 2.

The major species (95%, Figure 1) shows a nearly linear bond angle of 170.76 (7)° at the two-coordinated silver atom. The bond distance Ag(1)–C(9) of 2.060 (2) Å agrees very well with previously reported examples of this compound class [13–17] and shows that the silver is strongly bonded to the carbene. The Ag(1)–O(1) bond length of 2.1088 (14) Å is quite short compared to the ones our group previously reported but is still within range of the corresponding ones in similar molecules (see e.g., [26, 27]). This short distance suggests a predominantly covalent character of the Ag(1)–O(1) bond.

In comparison to this, the minor species (5%, Figure 2), where the silver atom is bonded to both oxygen atoms of the acetate group, the bond lengths to the oxygen atoms are much longer (Ag(2)–O(1): 2.408(6) Å, and Ag(2)–O(2): 2.554(5) Å). Furthermore the bond angle C(9)–Ag(2)–O(1) is no longer linear with 135.9(4)° and the C(9)–Ag(2)–O(2) angle is found to be 164.9(3)°. This bonding pattern is characteristic for a predominantly ionic coordination of the acetate to the silver. Both of these modes have been observed earlier (see e.g., [17]). Having both of them in the same structure suggests a rather small energy difference between the two forms.

3.2. Biological Evaluation

3.2.1. Antibacterial Testing. Using the Kirby-Bauer disk diffusion method, the *in vitro* antibacterial activity of the NHC-silver(I) acetate complexes was tested and summarised in Figures 3 and 4. The metal salt (silver(I) acetate) used

Table 2: Selected bond lengths [Å] and angles [°] for **4b**.

Bond length [Å]			Bond angle [°]		
	4b (95% species)	**4b** (5% species)		**4b** (95% species)	**4b** (5% species)
Ag(1)–C(9)	2.060(2)		C(9)–Ag(1) –O(1)	170.76(7)	
Ag(1)–O(1)	2.1088(14)		C(9)–Ag(2)–O(1)		135.9(4)
Ag(2)–C(9)		2.074(5)	C(9)–Ag(2)–O(2)		164.9(3)
Ag(2)–O(1)		2.408(6)	O(1)–Ag(2)–O(2)		53.05(11)
Ag(2)–O(2)		2.554(5)	N(3)–C(9)–N(2)	105.98(17)	
N(2)–C(9)	1.350(3)		N(3)–C(9)–Ag(1)	127.63(14)	
N(2)–C(10)	1.395(2)		N(2)–C(9)–Ag(1)	125.82(14)	
C(9)–N(3)	1.348(2)		N(3)–C(9)–Ag(2)		118.7(2)
C(10)–C(15)	1.388(3)		N(2)–C(9)–Ag(2)		133.79(19)
C(15)–N(3)	1.391(3)		C(15)–C(10)–N(2)	105.82(17)	
O(1)–C(24)	1.276(3)		C(10)–C(15)–N(3)	106.11(17)	
O(2)–C(24)	1.237(2)		C(9)–N(3)–C(15)	111.09(16)	
C(24)–C(25)	1.511(3)		C(9)–N(2)–C(10)	110.96(16)	
			C(24)–O(1)–Ag(1)	109.21(12)	
			C(24)–O(1)–Ag(2)		93.9(2)
			C(24)–O(2)–Ag(2)		88.1(2)
			O(2)–C(24)–O(1)		124.18(19)
			O(2)–C(24)–C(25)		119.62(19)
			O(1)–C(24)–C(25)		116.19(18)

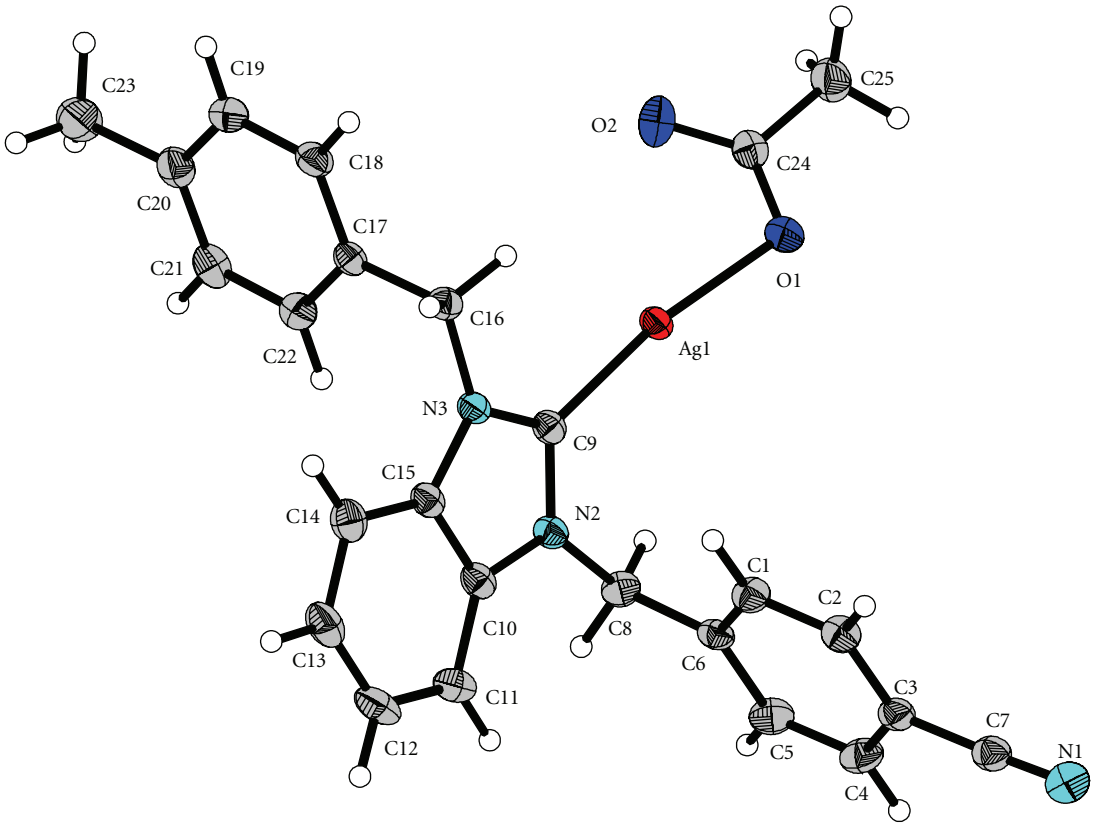


Figure 1: X-ray diffraction structure of **4b** showing the major occupied Ag position; thermal ellipsoids are drawn on the 50% probability level.

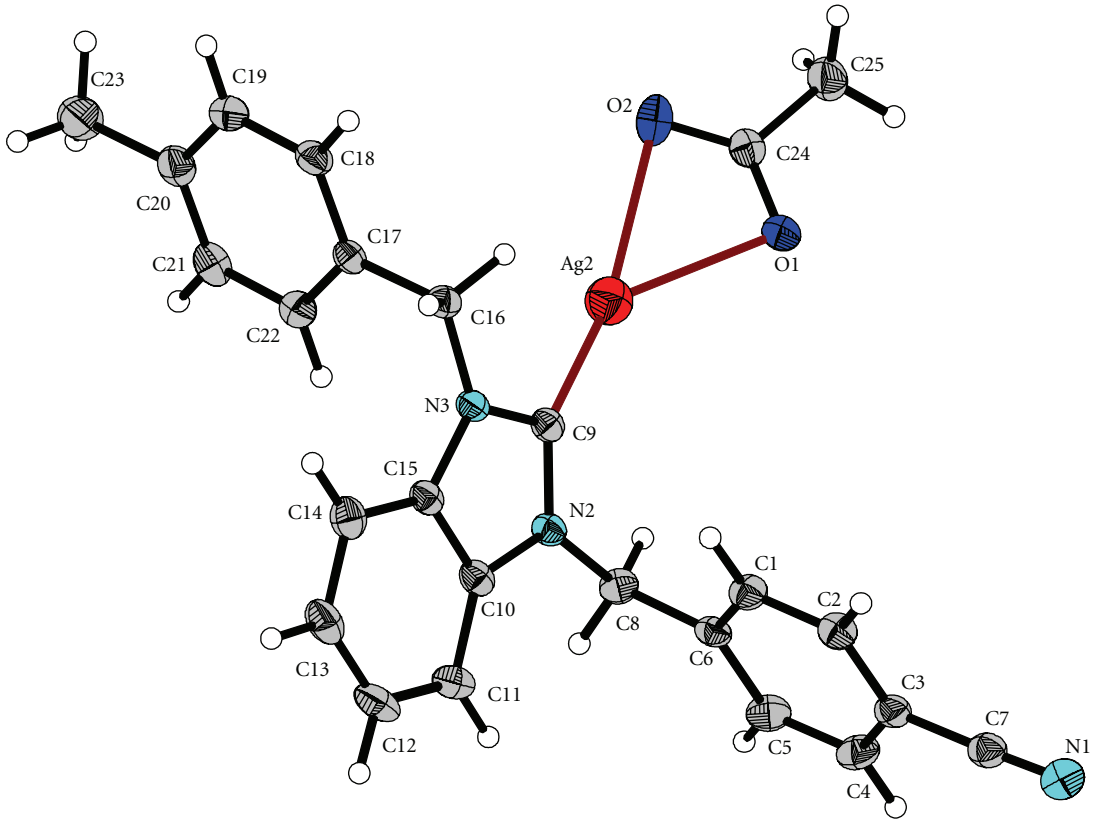


Figure 2: X-ray diffraction structure of **4b** showing the minor occupied Ag position; thermal ellipsoids are drawn on the 50% probability level, Ag2 with fixed radius.

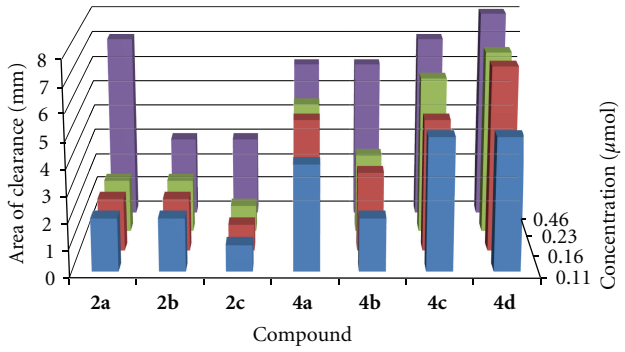


Figure 3: Area of clearance on *Staphylococcus aureus* (Gram +ve) by **2a–c** and **4a–d**.

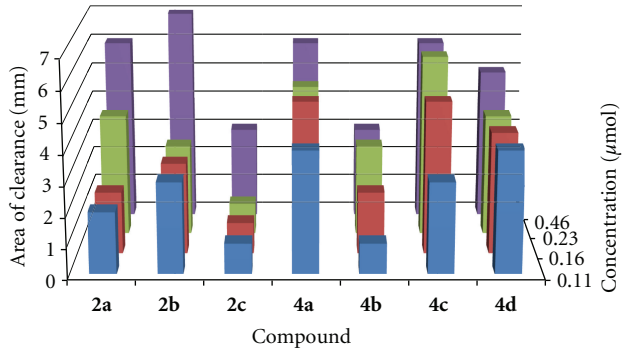


Figure 4: Area of clearance on *Escherichia coli* (Gram −ve) by **2a–c** and **4a–d**.

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 [17, 28].

Compounds **2b** and **2c** showed almost no antibacterial activity against the Gram-positive bacteria *Staphylococcus aureus,* and compound **4d** showed the best activity against this bacterial strain, but, in comparison to previous reported NHC-silver(I) acetate complexes, the activity is more in a medium range. Hereby, an area of clearance of 0 mm is considered as no activity, areas of 1–4 mm as low, 5–9 as medium, and areas of clearance ≥10 mm as high activity. Low antibacterial activity was observed for complexes **2c** and **4b** against the Gram-negative bacteria *Escherichia coli* and all other complexes (**2a**, **2b**, **4a**, **4c**, and **4d**) exhibited only medium activity against this bacteria strain.

3.2.2. Cytotoxicity Studies. Our interest focuses on the probable difference in activity due to the influence of different lipophilicity in the two compound classes. The *in vitro*

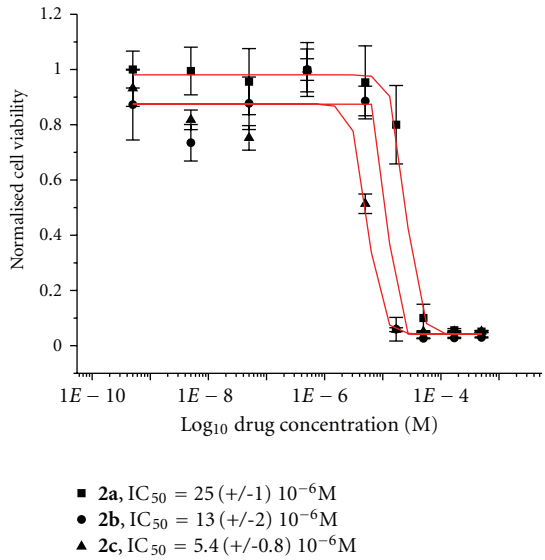


Figure 5: Cytotoxicity curves from typical MTT assays showing the effect of compounds **2a–c** on the viability of Caki-1 cells.

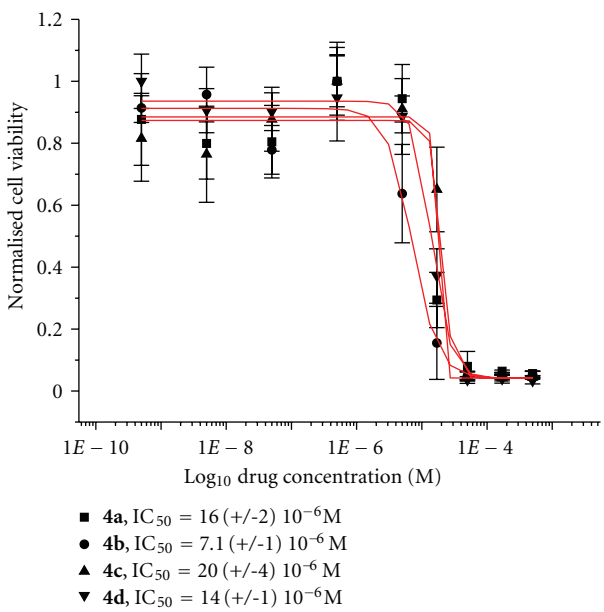


Figure 6: Cytotoxicity curves from typical MTT assays showing the effect of compounds **4a–d** on the viability of Caki-1 cells.

anticancer activity of the asymmetric substituted NHC-silver(I) acetate complexes was tested in an MTT-based assay against the human renal cancer cell line Caki-1. In this test a 48 h drug exposure period was followed by a 24 h recovery period, and the log dose response curves for complexes **2a–c** and **4a–d** are shown in Figures 5 and 6, respectively. The IC_{50} values of the imidazole containing complexes are **2a**: 25 (±1), **2b**: 15 (±2), **2c**: 5.4 (±0.8) μM. Slightly less activity was observed for the more lipophilic NHC-silver(I) acetate complexes **4a–d**, which gave IC_{50} values of 16 (±2), 7.1 (±1), 20 (±4), 14 (±1) μM, respectively. Compounds **2c** and **4b** show the highest cytotoxic activities with single-digit micromolar IC_{50} values.

It has been shown that there is no difference between the cytotoxic activities of the two compound classes, since all compounds show a good level of activity. The solubility of all compounds in DMSO was good, and they are stable in saline solution with respect to silver chloride precipitation.

4. Conclusion and Outlook

In summary, a series of seven nonsymmetrically *p*-cyanobenzyl-substituted NHC-silver(I) acetate complexes **2a–c** and **4a–d** were synthesised by reacting appropriate nonsymmetrically *p*-cyanobenzyl-substituted *N*-heterocyclic carbenes with silver(I) acetate. The preliminary antibacterial activity of the NHC-silver(I) acetate complexes was tested *in vitro* against two bacterial strains, where complex **4d** showed superior activity against *Staphylococcus aureus* and complex **2b** against *Escherichia coli*. Against the renal cancer cell line Caki-1, the NHC-silver(I) acetate complexes **2a–c** and **4a–d** yielded IC_{50} values of 25 (±1), 15 (±2), 5.4 (±0.8), 16 (±2), 7.1 (±1), 20 (±4), and 14 (±1) μM, respectively. The complex **2c**, however, gave a superior IC_{50} value of 5.4 (±0.8) μM. Further work is currently underway to improve these results by varying the substituents on the imidazole ring in order to enhance stability and on the benzimidazole nitrogen atoms in order to improve biological activity. Overall, this should lead to enhanced solubility, stability, and activity in biological media and enable *in vivo* testing of a NHC-silver(I) acetate in the nearby future.

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