DOI: 10.1002/zaac.201000395

Synthesis, Cytotoxicity and Antibacterial Studies of Novel Symmetrically and Non-Symmetrically *p*-Nitrobenzyl-Substituted N-Heterocyclic Carbene–Silver(I) Acetate Complexes

Siddappa Patil,[a] Anthony Deally,[a] Brendan Gleeson,[a] Frauke Hackenberg,[a] Helge Müller-Bunz,[a] Francesca Paradisi,[a] and Matthias Tacke*[a]

Keywords: Antitumour agents; Antibacterial drugs; Silver; N-heterocyclic carbenes; Caki-1

Abstract. From the reaction of 1H-imidazole (**1a**), 4,5-dichloro-1H-imidazole (**1b**), 1H-benzimidazole (**1c**), 1-methylimidazole (**1d**), 4,5-dichloro-1-methylimidazole (**1e**) and 1-methylbenzimidazole (**1f**) with *p*-nitrobenzyl bromide (**2**), symmetrically and non-symmetrically *p*-nitrobenzyl-substituted N-heterocyclic carbene (NHC) [(**3a**–**f**)] precursors were synthesised. These NHC-precursors were then reacted with silver(I) acetate to yield the NHC-silver acetate complexes [1,3-bis(4-nitrobenzyl)imidazol-2-ylidene]silver(I) acetate (**4a**), [4,5-dichloro-1,3-bis(4-nitrobenzyl)imidazol-2-ylidene]silver(I) acetate (**4b**), [1,3-bis(4-nitrobenzyl)benzimidazol-2-ylidene]silver(I) acetate (**4c**), [1-methyl-3-(4-nitrobenzyl)imidazole-2-ylidene] silver(I) acetate (**4d**), [4,5-dichloro-1-methyl-3-(4-nitrobenzyl)imidazole-2-ylidene] silver(I) acetate (**4e**) and [1-methyl-3-(4-nitrobenzyl)benzimidazole-2-ylidene] silver(I) acetate (**4f**), respectively. The two NHC–silver(I) acetate complexes **4a** and **4e** were characterised by single-crystal X-ray diffraction. All compounds studied in this work were preliminary screened for their antimicrobial activities in vitro against Gram-positive bacteria *Staphylococcus aureus,* and Gram-negative bacteria *Escherichia coli* using the qualitative Kirby–Bauer disk-diffusion method. All NHC–silver(I) acetate complexes exhibited medium to high antibacterial activity with areas of clearance ranging from 3 to 7 mm at the highest amount used, whereas the NHC-precursors showed significantly lower activity. In addition, NHC–silver(I) acetate complexes **4a**–**f** had their preliminary cytotoxicity tests on the human renal-cancer cell line Caki-1 and showed medium to high cytotoxicity with IC_{50} values ranging from 15 (+/–1) to 27 (+/–2) μM.

Introduction

The biomedical applications of metal complexes based on N-heterocyclic carbene (NHC)[1–5] are just beginning to unfold, despite such complexes being phenomenally successful in homogeneous catalysis.[6–8] N-Heterocyclic carbene complexes of silver are commonplace in the organometallic literature. The interest in NHC–silver complexes is largely due to their ease of synthesis and their ability to serve as useful to other NHC–metal complexes by NHC transfer reactions.[9] In addition, their diverse properties in bonding and structure and potential applications in medicine,[10–15] nanomaterials,[16] liquid crystals[17] and organic catalysis[18] also contribute to the attraction of NHC–silver complexes.

Silver compounds have been used for medicinal applications for more than hundred years. It was targeted for water purification, wound care antiseptics and infections. Silver nitrate was commonly used as antimicrobial compound since the 17th and 18th century before it lost relevance with the discovery of penicillin. Only when first resistances were reported, silver compounds regained importance. Antibacterial activity of silver compounds is attributed to soluble silver ions, Ag^+. Elementary silver as well as insoluble silver salt precipitates, such as silver chloride, do not reveal antibacterial activity. The only side effect of long term silver treatment is the more or less unpleasant colour change to grey or blue skin of patients. The effect called Argyria occurs due to irreversible formation and deposition of silver sulfide in dermis and eyes.

The introduction of silver sulfadiazine (silvadine) as an effective antimicrobial agent was reported by *Fox* in 1968.[19] High antimicrobial activity and minimal side effects of silver sulfadiazine have made it a very convenient therapy for the treatment of infections in burns over the past four decades.[20–23] Silver–carbene complexes, in particular those of N-heterocyclic carbenes, have gained a significant amount of interest in the past few years.[9,18] The first use of silver NHCs as antimicrobial agents was reported by *Youngs* et al. in 2004.[24] In this report, two silver(I) complexes [silver(I) 2,6-bis(ethanolimidazolemethyl)pyridine hydroxide and silver(I) 2,6-bis(propanolimidazolemethyl)pyridine hydroxide] showed better antimicrobial activity than $AgNO_3$ against the microorganisms, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Another important contribution by the *Ghosh* research group led to the synthesis and antimicrobial evaluation of NHC–silver complexes derived from 1-benzyl-3-

* Prof. Dr. M. Tacke
E-Mail: matthias.tacke@ucd.ie
[a] Conway Institute of Biomolecular and Biomedical Research
Centre for Synthesis and Chemical Biology (CSCB)
UCD School of Chemistry and Chemical Biology
University College Dublin
Belfield, Dublin 4, Ireland

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tert-butylimidazole.[25] Recently, *Gürbüz* and colleagues have shown that the new imidazolidin-2-ylidene silver complexes displayed effective antimicrobial activity against a series of bacteria and fungi.[26]

Cancer is a widely spread disease that remains an important public health problem throughout Europe. According to International Agency for Research on Cancer, nearly 2.9 million new cases of cancer and more than 1.7 million cancer deaths were reported in the Europe in 2004.[27] The number of new cases diagnosed each year even increased by 300,000 in 2006.[28] For some sorts of cancer still no treatment exists or the treatment implies harsh conditions for the patients. Namely the treatment for the four most mortal cancer forms – lung, colorectal, breast and stomach cancer-need to make quick and significant progress.[27] This situation calls for the development of milder and more specific anticancer drugs.

The introduction of cisplatin into clinics in 1978 started a broad search for other cytotoxic metal complexes. Despite the resounding success of cisplatin and closely related platinum antitumor agents, the movement of other transition metal anticancer drugs towards the clinic has been exceptionally slow.[29–31] In general, metallocene dichlorides (Cp_2MCl_2) with M = Ti, V, Nb and Mo show remarkable antitumor activity,[32,33] and titanocene dichloride, Cp_2MCl_2, has especially become a very promising candidate as an anticancer drug. Cp_2MCl_2 shows medium-high cytotoxicity in vitro and a high efficacy in the animal models. Unfortunately, the efficacy in Phase II clinical trials in patients with metastatic renal-cell carcinoma[34] or metastatic breast cancer[35] was too low to be pursued. Further substitution of the cyclopentadienyl ligand led to bis[(*p*-methoxybenzyl) cyclopentadienyl] titanium(IV) dichloride (Titanocene Y), that showed to be more effective in the treatment against xenografted CAKI-1 tumours in mice than that of cisplatin.[36]

Recently, carbene–silver complexes have been reported to have anticancer activity in vitro. *Young* and co-workers 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).[14] These silver complexes were shown to be very stable and can be synthesized efficiently. We have recently reported the anticancer and antibacterial activity of symmetrically and non-symmetrically *p*-methoxybenzyl-, *p*-cyanobenzyl- or benzyl-substituted N-heterocyclic carbene–silver complexes. All the reported NHC–silver complexes have shown medium to high anticancer and antibacterial activity.[37,38] This encourages further research on N-heterocyclic carbene–silver complexes as cytotoxic drug candidates.

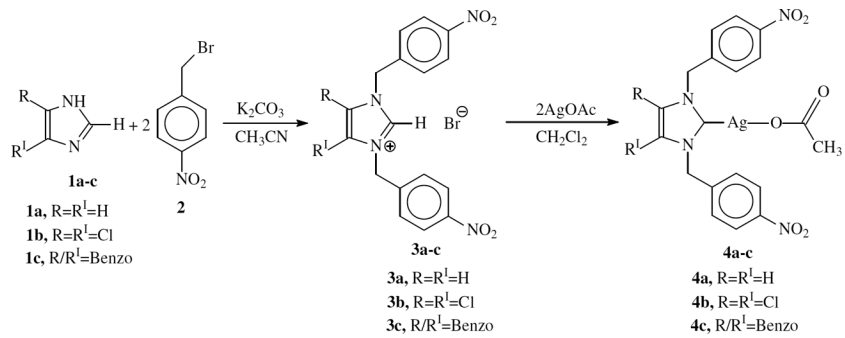
Within this paper, we present the synthesis, preliminary cytotoxicity and antimicrobial activity of a series of six novel symmetrically and non-symmetrically *p*-nitrobenzyl-substituted NHC–silver acetate derivatives. These compounds were tested on the human cancerous renal-cell line Caki-1 as well as on the Gram-positive bacteria *Staphylococcus aureus* and the Gram-negative bacteria *Escherichia coli*.

Results and Discussion

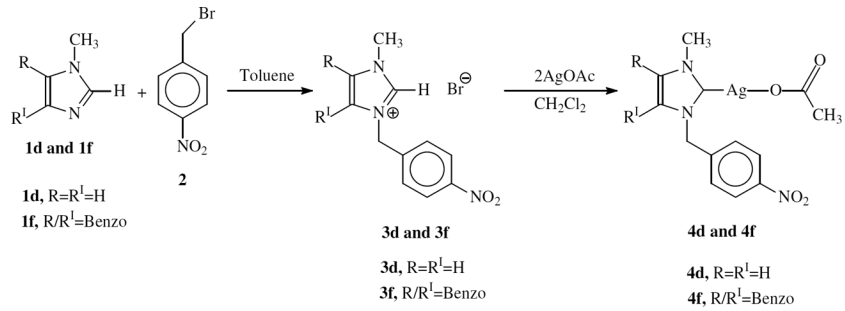
Synthesis

The synthetic route for symmetrically and non-symmetrically *p*-nitrobenzyl-substituted N-heterocyclic carbenes as ligand precursors and their corresponding silver(I) acetate complexes described in this work is given in Scheme 1, Scheme 2 and Scheme 3, respectively. The symmetrically substituted NHC precursors 1,3-bis(4-nitrobenzyl)imidazolium bromide (**3a**) and 1,3-bis(4-nitrobenzyl)benzimidazolium bromide (**3c**) were prepared by stirring 1H-imidazole (**1a**) and 1H-benzimidazole (**1c**) with 2 equivalents of *p*-nitrobenzyl bromide (**2**) in the presence of K_2CO_3 as a base in CH_3CN at room temperature for 3 d with 93 % and 81 % yields respectively. 4,5-Dichloro-1,3-bis(4-nitrobenzyl)imidazolium bromide (**3b**) was prepared by heating 4,5-dichloro-1H-imidazole (**1b**) with 2 equivalents of *p*-nitrobenzyl bromide (**2**) in the presence of K_2CO_3 as a base in CH_3CN for 6 d with a yield of 79 %. The non-symmetrically substituted NHC precursors 1-methyl-3-(4-nitrobenzyl)imidazolium bromide (**3d**) and 1-methyl-3-(4-nitrobenzyl)benzimidazolium bromide (**3f**) were prepared by stirring 1-methylimidazole (**1d**) and 1-methylbenzimidazole (**1f**) with *p*-nitrobenzyl bromide (**2**) in toluene at room temperature for 2 d with 67 % and 80 % yields respectively. 4,5-Dichloro-1-methylimidazole (**1e**) is formed in 97 % 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-(4-nitrobenzyl)imidazolium bromide (**3e**) is formed in 82 % yield by the reaction of 4,5-dichloro-1-methylimidazole (**5b**) with *p*-nitrobenzyl bromide (**2**) in toluene. The NHC precursors were fully characterised by ^1H, ^{13}C NMR, IR, UV/Vis spectroscopy, mass spectrometry and elemental analysis. The ^1H NMR spectra of all precursors **3a**–**f** show a characteristic downfield shift in the range 9.46–10.87 ppm for the NCHN proton attributable to the positive charge of the molecule.[37,38] Additionally, their identities have also been confirmed by a base peak for the $[M^+ - Br]$ fragments in their positive mode ESI mass spectra.

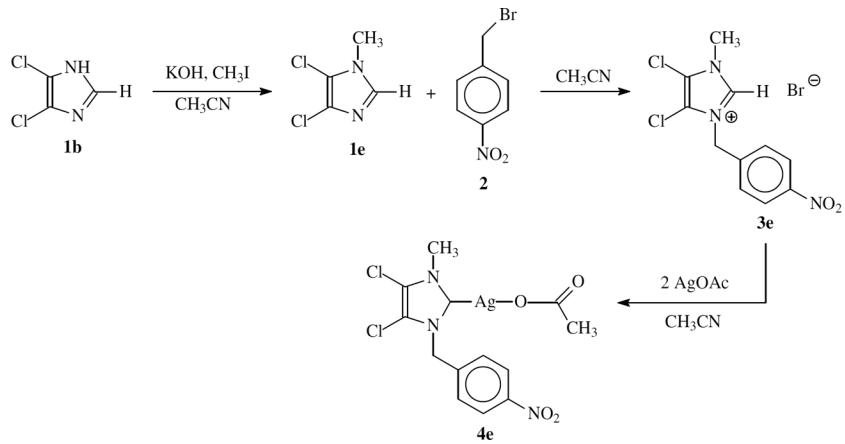
The NHC-silver complexes [1,3-bis(4-nitrobenzyl)imidazol-2-ylidene]silver(I) acetate (**4a**), [4,5-dichloro-1,3-bis(4-nitrobenzyl)imidazol-2-ylidene]silver(I) acetate (**4b**), [1,3-bis(4-nitrobenzyl)benzimidazol-2-ylidene]silver(I) acetate (**4c**), 1-methyl-3-(4-nitrobenzyl)imidazole-2-ylidene) silver(I) acetate (**4d**), (4,5-dichloro-1-methyl-3-(4-nitrobenzyl)imidazole-2-ylidene) silver(I) acetate (**4e**) and (1-methyl-3-(4-nitrobenzyl)benzimidazole-2-ylidene)silver(I) acetate (**4f**) were synthesized by the reaction of **3a**–**f** with 2 equivalents of silver(I) acetate in dichloromethane/methanol. The reaction mixture was stirred for 2–3 d at room temperature to afford the NHC–silver(I) acetate complexes as off white solids in 53 % to 79 % yield. The complexes were fully characterised by ^1H, ^{13}C NMR, IR, UV/Vis spectroscopy, mass spectrometry and elemental analysis. Furthermore, the solid-state structures of **4a** and **4e** were analysed by single-crystal X-ray diffraction. The absence of a downfield NCHN signal and presence of new signals at δ = 2.09–1.76 ppm for the acetate protons in all the ^1H NMR spectra for **4a**–**f**, however, indicates a successful



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



Scheme 2. General reaction scheme for the synthesis of non-symmetrically *p*-nitrobenzyl-substituted *N*-heterocyclic carbenes (**3d** and **3f**) and their corresponding *N*-heterocyclic carbene–silver(I) acetate complexes (**4d** and **4f**).



Scheme 3. General reaction scheme for the synthesis of non-symmetrically *p*-nitrobenzyl-substituted *N*-heterocyclic carbene (**3e**) and its *N*-heterocyclic carbene–silver(I) acetate complex (**4e**).

complex formation. The ^{13}C NMR resonances of the carbene carbon atoms in complexes **4a**–**f** occur in the range 183.3–179.0 ppm respectively. These signals are shifted downfield compared to the corresponding precursors of **3a**–**f** carbene carbons resonance at the range 130.0–141.8 ppm, respectively, which further demonstrates the formation of expected NHC–silver(I) acetate complexes. Also the appearance of the ^{13}C NMR spectroscopic resonances for the carbonyl and methyl carbon atoms of the acetate group of complexes **4a**–**f** in the range 163.9–177.4 and 22.0–24.2 ppm respectively showed the formation of the NHC–silver(I) acetate complexes.[1,12,37,38] Furthermore, positive mode ESI mass spectra of all six NHC-silver(I) acetate complexes (**4a**–**f**) are dominated by [M^+–O_2CCH_3] fragment peaks arising from the loss of one acetate ligand.

Structural Discussion

Suitable crystals for X-ray crystallography to determine the molecular structure of **4a** and **4e** were grown from saturated

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dichloromethane solutions with slow infusion of pentane. **4a** crystallised with four molecules in the unit cell, whilst **4e** crystallised with two molecules in the unit cell. Compounds **4a** and **4e** crystallised in the monoclinic space group *C*2/*c* (#15) and triclinic space group $P\bar{1}$ (#2). In compounds **4a** and **4e** there is an absence of any lattice held water molecules or organic solvent molecules in the unit cells of the determined structures. The molecular structures of the compounds **4a** and **4e** are shown in Figure 1 and Figure 2. The crystal data and refinement details for two compounds are tabulated in Table 1, whereas selected bond lengths and bond angles are compiled in Table 2.

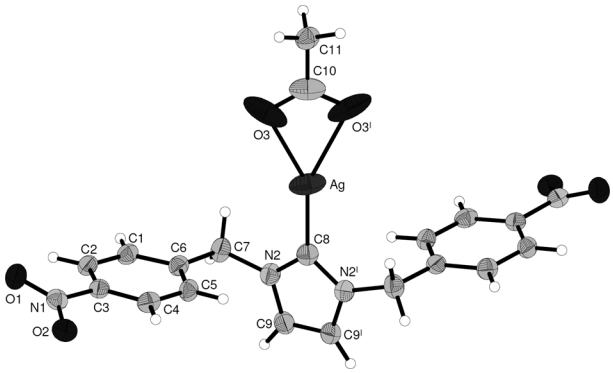

Figure 1. X-ray diffraction structure of **4a**; molecule; thermal ellipsoids are drawn on the 50 % probability level; symmetry operations: I 1 –*x*, *y*, 1.5–*z*.

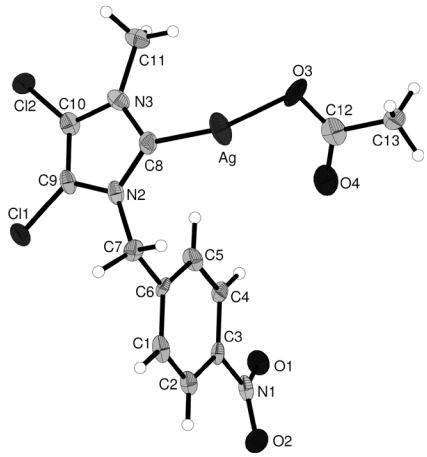

Figure 2. X-ray diffraction structure of **4e**; molecule; thermal ellipsoids are drawn on the 50 % probability level.

The NHC–silver(I) acetate complexes **4a** and **4e** are mononuclear complexes. In the NHC–silver(I) acetate complexes **4b** and **4e** reported here, the bond lengths and angles in and directly around the NHC core agree very well among each other and with literature data.[37–39] In **4a** (Figure 1), the second oxygen atom of the acetate is 2.69(1) Å away from the silver which is in our opinion still too far to call it a bond. **4e** (Figure 2), however, lies on a twofold axis which makes both Ag–O distances equal. The thermal ellipsoids of the oxygen atoms, the silver atom and C(10) suggest that the silver atom and acetate group might be disordered across the twofold axis, making the two Ag–O distances slightly different. An attempt to refine this disorder was unsuccessful as the split positions were too close to each other to be refined independently.

Biological Evaluation

The in vitro antibacterial activities of symmetrically and non-symmetrically *p*-nitrobenzyl-substituted *N*-heterocyclic carbenes and their corresponding silver complexes were tested using the qualitative Kirby–Bauer disk diffusion method against the chosen Gram-positive bacteria *Staphylococcus aureus* (NCTC 7447), and Gram-negative bacteria *Escherichia coli.* The metal salt (silver acetate) used to prepare the complexes and the solvent (DMSO) induced very little growth inhibition on the same bacteria as previously reported.[37,40] The antibacterial activities of the NHC-precursors and their corresponding NHC–silver complexes are summarized in Figure 3, Figure 4, Figure 5, and Figure 6. The results are tabulated in Table 3 and Table 4, respectively.

The results indicate that all the NHC-precursors **3a,b** and **3d**–**f** (except **3c**) show a weak antibacterial activity against the both Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli*, whereas NHC-precursor **3c** has exhibited minimal antibacterial activity with an area of clearance of 3 mm at 0.43 μmol. Medium antibacterial activity was observed for all NHC-silver(I) acetate complexes **4a**–**f** against *Escherichia coli* but high antibacterial activity was observed towards *Staphylococcus aureus* with an area of clearance of 7 mm at 0.43 μmol.

The previously synthesized NHC–silver(I) acetate complexes showed an activity of up to 12 mm area of clearance at a concentration of 0.46 μmol for (1-benzyl-3-methylbenzimidazole-2-ylidene) silver(I) acetate.[38] Thus, the presented NHC–silver(I) acetate complexes **4a**–**f** do not belong to the most promising drug candidates of the NHC–silver series. Possibly, the nitro group on the benzyl ligand negatively affected the lipophilicity of these compounds. The compounds exhibit antimicrobial activity when they are capable of penetrating into and through the lipid membrane of microorganisms, once inside they are able to interfere with cell metabolism by enzymatic inhibition, for example. Consequently, higher lipophilicity enhances antibacterial activity. For example, complexes **4a**–**f** revealed increased antibacterial activity compared to their precursors **3a**–**f** because NHC-silver acetate complexes are more lipophilic than polar NHC-precursors.

Cytotoxicity Studies

We are interested in the different types of NHC-silver(I) acetate complexes as possible anti-cancer drugs. Therefore, the in vitro cytotoxicity of symmetrically and non-symmetrically *p*-nitrobenzyl-substituted NHC-silver(I) acetate complexes **4a**–**f** was evaluated by MTT-based assays[41] on the human cancerous renal-cell line Caki-1. This test involves a 48 h drug exposure period, followed by a 24 h recovery time. The log dose

Table 1. Crystal data and structure refinement for **4a** and **4e**.

Identification code	**4a**	**4e**
Empirical formula	$C_{19}H_{17}N_4O_6Ag$	$C_{13}H_{12}N_3O_4Cl_2Ag$
Molecular formula	$C_{19}H_{17}N_4O_6Ag$	$C_{13}H_{12}N_3O_4Cl_2Ag$
Formula weight	505.24	453.03
Crystal system	monoclinic	triclinic
Space group	*C*2/*c* (#15)	$P\bar{1}$ (#2)
Unit cell dimensions	*a* = 19.8284(5) Å	*a* = 7.8488(7) Å
	b = 12.8367(3) Å	*b* = 10.476(1) Å
	c = 7.5132(2) Å	*c* = 10.7467(8) Å
	α = 90°	*α* = 65.757(8)°
	β = 93.235(2)°	*β* = 76.373(7)°
	γ = 90°	*γ* = 79.596(8)°
Volume	1909.30(8) Å^3	779.52(12) Å^3
Z	4	2
Density (calculated)	1.758 Mg·m^{-3}	1.930 Mg·m^{-3}
Absorption coefficient	8.890 mm^{-1}	13.750 mm^{-1}
F(000)	1016	448
Crystal size	0.2167 × 0.0269 × 0.0178 mm	0.1321 × 0.0323 × 0.0193 mm
Theta range for data collection	4.10 to 76.97°	4.59 to 62.49°
Index ranges	$-24 \le h \le 24$	$-9 \le h \le 9$
	$-16 \le k \le 16$	$-12 \le k \le 11$
	$-9 \le l \le 9$	$-12 \le l \le 12$
Reflections collected	19665	8806
Independent reflections	2009 [*R*(int) = 0.0388]	2446 [*R*(int) = 0.0411]
Completeness to θ_{max}	99.5 %	98.1 %
Max. and min. Transmission	0.862 and 0.403	0.811 and 0.395
Data / restraints / parameters	2009 / 0 / 139	2446 / 0 / 210
Goodness-of-fit on F^2	1.149	1.182
Final *R* indices [$I > 2\sigma(I)$]	*R*1 = 0.0482, *wR*2 = 0.1062	*R*1 = 0.0718, *wR*2 = 0.1475
R indices (all data)	*R*1 = 0.0501, *wR*2 = 0.1077	*R*1 = 0.0758, *wR*2 = 0.1504
Largest diff. peak and hole	0.971 and –2.371 e·Å^{-3}	5.012 and –3.241 e·Å^{-3}

Table 2. Selected bond lengths /Å and angles /° for compounds **4a** and **4e**.

Bond Lengths /Å	**4a**	**4e**	Bond Angles /°	**4a**	**4e**
N(2)–C(8)	1.351(5)	1.362(12)	N(2)#1–C(8)–N(2)	104.5(5)	
N(2)–C(9)	1.386(5)	1.388(12)	C(8)–N(2)–C(9)	111.1(3)	111.4(8)
C(9)–C(9)#1	1.340(8)		C(9)#1–C(9)–N(2)	106.7(2)	
Ag–C(8)	2.065(6)	2.040(9)	N(2)–C(8)–Ag	127.8(2)	
Ag–O(3)	2.438(5)	2.173(7)	C(8)–Ag–O(3)	153.14(10)	166.8(3)
O(3)–C(10)	1.258(5)		O(3)–Ag–O(3)#1	53.7(2)	
C(10)–C(11)	1.489(9)		C(10)–O(3)–Ag	92.0(4)	
C(8)–N(3)		1.379(13)	O(3)–C(10)–O(3)#1	122.3(7)	
C(10)–N(3)		1.389(12)	O(3)–C(10)–C(11)	118.9(4)	
C(9)–C(10)		1.355(13)	N(2)–C(8)–N(3)		104.1(8)
			C(8)–N(3)–C(10)		110.6(8)
			C(9)–C(10)–N(3)		107.0(8)
			C(10)–C(9)–N(2)		106.8(8)
			C(12)–O(3)–Ag		103.4(7)
			O(3)–C(12)–O(4)		124.2(10)
			O(3)–C(12)–C(13)		117.7(10)
			O(4)–C(12)–C(13)		118.1(10)

response curve for NHC–silver(I) acetate complexes **4a**–**f** can be viewed in Figure 7 and Figure 8, respectively.

Symmetrically *p*-nitrobenzyl-substituted NHC–silver(I) acetate complexes **4a**–**c**, which contains 1H-imidazole, 4,5-dichloro-1H-imidazole and 1H-benzimidazole groups, has IC_{50} values 27 (+/–2), 15 (+/–1) and 22 (+/–2) μM, respectively. Compound **4b** is the most promising candidate in this paper because of lowest IC_{50} value and has an approximately twofold and 1.5-fold increase in magnitude when compared with compounds **4a** and **4c** respectively. In comparison with cisplatin (IC_{50} value = 3.3 μM), **4b** has an approximately fivefold decrease in magnitude. Non-symmetrically *p*-nitrobenzyl-substituted NHC-silver(I) acetate complexes **4d**–**f**, which also contains 1-methylimidazole, 4,5-dichloro-1-methylimidazole and 1-methylbenzimidazole groups, has IC_{50} values 20 (+/–1), 17 (+/–1) and 16 (+/–9) μM, respectively. Compounds **4d**–**f** show

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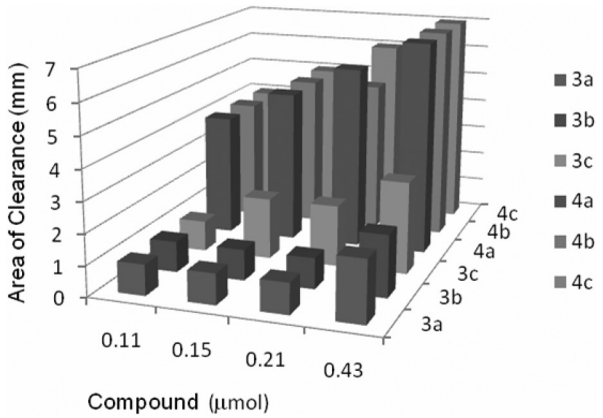

Figure 3. Area of clearance on *Staphylococcus aureus* (Gram +ve) by **3a**–**c** and **4a**–**c**.

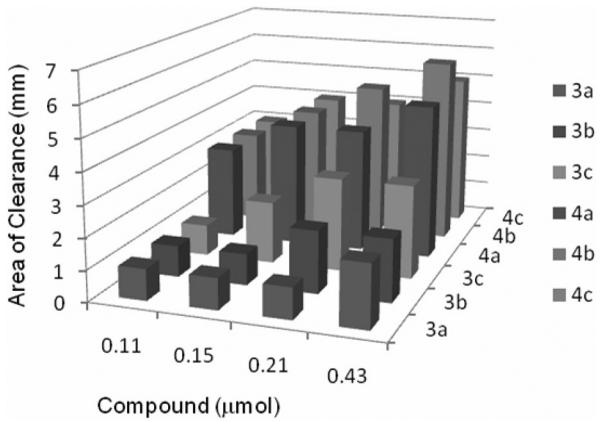

Figure 4. Area of clearance on *Escherichia coli* (Gram -ve) by **3a**–**c** and **4a**–**c**.

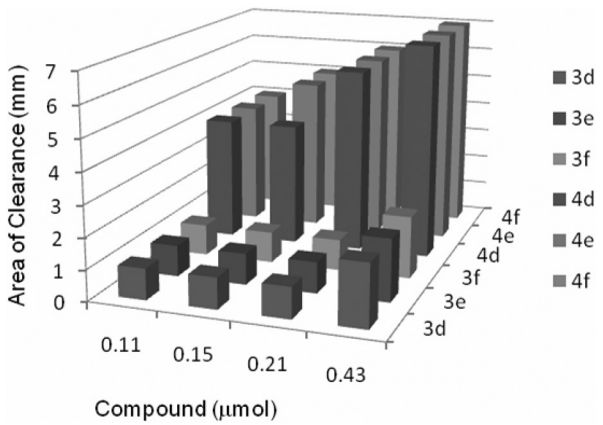

Figure 5. Area of clearance on *Staphylococcus aureus* (Gram +ve) by **3d**–**f** and **4d**–**f**.

a very similar IC_{50} value and in comparison with cisplatin (IC_{50} value = 3.3 μM) the IC_{50} values for the three compounds are not impressive.

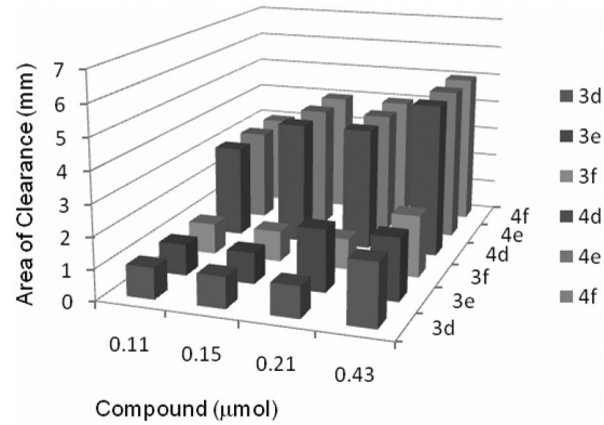

Figure 6. Area of clearance on *Escherichia coli* (Gram -ve) by **3d**–**f** and **4d**–**f**.

Table 3. Area of clearance for compounds **3a**–**f** in mm.

Compounds		Staphylococcus aureus (Gram + ve)	Escherichia coli (Gram – ve)
3a /mm	0.11 μmol (45.0 μg)	1	1
	0.15 μmol (63.0 μg)	1	1
	0.21 μmol (90.0 μg)	1	1
	0.43 μmol (180.0 μg)	2	2
3b /mm	0.11 μmol (52.0 μg)	1	1
	0.15 μmol (72.8 μg)	1	1
	0.21 μmol (104.5 μg)	1	2
	0.43 μmol (209.0 μg)	2	2
3c /mm	0.11 μmol (50.0 μg)	1	1
	0.15 μmol (70.0 μg)	2	2
	0.21 μmol (100.5 μg)	2	3
	0.43 μmol (201.0 μg)	3	3
3d /mm	0.11 μmol (32.0 μg)	1	1
	0.15 μmol (44.8 μg)	1	1
	0.21 μmol (64.0 μg)	1	1
	0.43 μmol (128.0 μg)	2	2
3e /mm	0.11 μmol (39.2 μg)	1	1
	0.15 μmol (54.9 μg)	1	1
	0.21 μmol (78.5 μg)	1	2
	0.43 μmol (157.0 μg)	2	2
3f /mm	0.11 μmol (37.2 μg)	1	1
	0.15 μmol (52.1 μg)	1	1
	0.21 μmol (74.5 μg)	1	1
	0.43 μmol (149.0 μg)	2	2

Symmetrically and non-symmetrically *p*-nitrobenzyl-substituted NHC-silver(I) acetate complexes show almost similar IC_{50} values; both compound classes are easily soluble in DMSO and all compounds are stable in saline solution with respect to silver chloride precipitation. It was also observed that, compared to known reported NHC-silver complexes from the literature,[37,38] the NHC-silver complexes (**4a**–**f**) have almost same cytotoxic activity.

Conclusions and Outlook

In summary, a series of six new symmetrically and non-symmetrically *p*-nitrobenzyl-substituted NHC–silver(I) acetate complexes **4a**–**f** were synthesised through the reaction of ap-

Table 4. Area of clearance for compounds **4a**–**f** in mm.

Compounds	Staphylococcus aureus (Gram + ve)	Escherichia coli (Gram – ve)
4a /mm 0.11 μmol (54.2 μg)	4	3
0.15 μmol (75.9 μg)	5	4
0.21 μmol (108.5 μg)	6	4
0.43 μmol (217.0 μg)	7	5
4b /mm 0.11 μmol (62.5 μg)	4	3
0.15 μmol (87.5 μg)	5	4
0.21 μmol (125.0 μg)	5	5
0.43 μmol (250.0 μg)	7	6
4c /mm 0.11 μmol (59.5 μg)	4	3
0.15 μmol (83.3 μg)	5	4
0.21 μmol (119.0 μg)	6	4
0.43 μmol (238.0 μg)	7	5
4d /mm 0.11 μmol (41.2 μg)	4	3
0.15 μmol (57.7 μg)	4	4
0.21 μmol (82.5 μg)	6	4
0.43 μmol (165.0 μg)	7	5
4e /mm 0.11 μmol (48.5 μg)	4	3
0.15 μmol (67.9 μg)	5	4
0.21 μmol (97.0 μg)	6	4
0.43 μmol (194.0 μg)	7	5
4f /mm 0.11 μmol (46.5 μg)	4	3
0.15 μmol (65.1 μg)	5	4
0.21 μmol (93.0 μg)	6	4
0.43 μmol (186.0 μg)	7	5

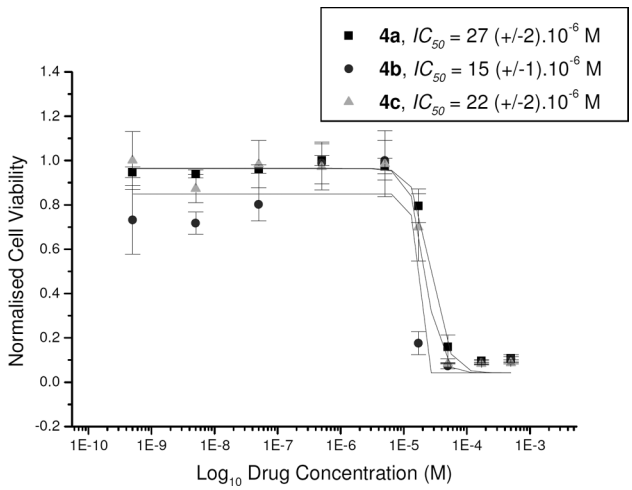

Figure 7. Cytotoxicity curves from typical MTT assays showing the effect of compounds **4a**–**c** on the viability of Caki-1 cells.

propriately symmetrically and non-symmetrically *p*-nitrobenzyl-substituted N-heterocyclic carbenes **3a**–**f** with silver(I) acetate. The preliminary antibacterial activity of the NHC-precursors and their corresponding NHC–silver(I) acetate complexes were tested in vitro against two microbial strains. All the complexes have shown high antibacterial activity compared to the precursors and it is also clear that, as the complexes concentration increases, the antibacterial activity becomes higher. NHC–silver(I) acetate complexes **4a**–**f** yielded antitumor IC_{50} values of 27 (+/–2), 15 (+/–1), 22 (+/–2), 20 (+/–1) 17 (+/–1) and 16 (+/–9) μM, respectively, on the Caki-1 cell line. The complex **4b** however gave a superior IC_{50} value of 15 (+/–1) μM. Further work is currently underway in order

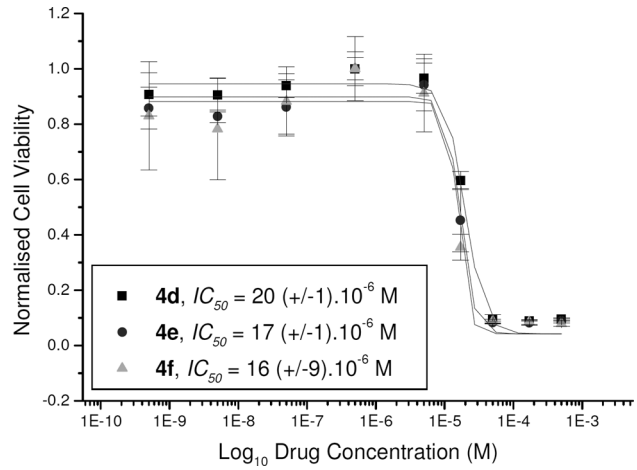

Figure 8. Cytotoxicity curves from typical MTT assays showing the effect of compounds **4d**–**f** on the viability of Caki-1 cells.

to improve these values by performing formulation experiments to improve solubility of these NHC–silver(I) acetate complexes, which should allow for in vivo testing of **4b** in the nearby future.

Experimental Section

Materials and Measurements

All reagents were of analytical grade; they were purchased and used without further purification. 1*H*-Imidazole, 4,5-dichloro-1*H*-imidazole, 1*H*-benzimidazole, 1-methylimidazole, 1-methylbenzimidazole, *p*-nitrobenzyl bromide, silver acetate, methyl iodide and K_2CO_3 were procured commercially from Sigma–Aldrich Chemical Company and were used without further purification. NMR spectra were measured with a Varian 400 MHz spectrometer. Chemical shifts are reported in ppm and are referenced to TMS. IR spectra were recorded with a Perkin–Elmer Paragon 1000 FT-IR Spectrometer employing a KBr disc. UV/Vis spectra were recorded with a Unicam UV4 Spectrometer. Electron spray mass spectrometry (MS) was performed with 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 ionisation) mode for compounds **1e, 3a**–**f** and **4a**–**f**. CHN analysis was done with an Exeter Analytical CE-440 Elemental Analyser. Silver was estimated by spectrophotometric methods (Atomic absorption spectra 55B Varian), whereas chlorine and bromine were determined in mercurimetric titrations. Crystal data were collected using an Oxford Diffraction SuperNova diffractometer fitted with an Atlas detector. Compounds **4a** was measured with Mo-K_α (0.71073 Å), and **4e** with Cu-K_α (1.54184 Å). **4a** and **4e** were collected at 100K. An at least twice 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.[42] The structures were solved by direct methods using SHELXS-97[43] and refined by full-matrix least-squares on F^2 for all data using SHELXL-97.[43] All 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 carbon atom the hydrogen atom is attached to. Anisotropic thermal displacement parameters were used for all non-hydrogen atoms. Suita-

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ble crystals of **4a** and **4e** were grown in a saturated dichloromethane solution with slow infusion of pentane. Further details about the data collection are listed in Table 1, as well as reliability factors.

CCDC-798552 (for **4a**), and -798553 (for **4e**), respectively, contain 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/data_request/cif.

Syntheses

1,3-Bis(4-nitrobenzyl)imidazolium bromide (3a): A mixture of 1H-imidazole (0.136 g, 2.00 mmol) and K_2CO_3 (0.414 g, 3.00 mmol) was stirred for 15 min in acetonitrile (30 mL). 4-Nitrobenzyl bromide (0.864 g, 4.00 mmol) was added in one portion and stirring was continued at room temperature for further 2 d. After the solvent was removed under reduced pressure, water (50 mL) was added. The precipitate was filtered off, washed with diethyl ether and dried in suction at room temperature for 7 h to yield the product **3a** (0.780 g, 1.86 mmol, 93.0 % yield) as brown solid.

^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 9.46 (s, 1 H, NCHN), 8.28 (d, J = 8.8 Hz, 4 H, $CH_{Nitrobenzyl}$), 7.89 (d, J = 1.6 Hz, 2 H, CH_{Imid}), 7.67 (d, J = 8.8 Hz, 4 H, $CH_{Nitrobenzyl}$), 5.62 (s, 4 H, CH_2). **^{13}C NMR** ([D$_6$]DMSO, 100 MHz, proton decoupled): δ = 139.6, 138.6, 130.0, 124.5, 123.1, 121.7 (NCN+C_{Imid}+ $C_{Nitrobenzyl}$), 52.2 (CH_2). **IR** (KBr): 3427 (m), 3016 (m), 1605 (w), 1518 (s), 1347 (s), 1214 (w), 1151 (m), 1108 (m), 1018 (w), 858 (m), 807 (m), 756 (w), 729 (m), 619 (m) cm^{-1}. **UV/Vis** (CH_3OH, nm): λ 259 (ε 10475), λ 368 (ε 2022), λ 443 (ε 1566). **MS** (*m*/*z*, QMS-MS/MS): 339.1 [M$^+$-Br]. **Micro Analysis** Calculated for $C_{17}H_{15}N_4O_4Br$ (419.23): Calcd.: C, 48.70 %; H, 3.61 %; N, 13.36 %; Br, 19.06 %; Found: C, 49.00 %; H, 3.39 %; N, 13.05 %; Br, 19.15 %.

4,5-Dichloro-1,3-bis(4-nitrobenzyl)imidazolium bromide (3b): 4,5-Dichloro-1H-imidazole (0.273 g, 2.00 mmol) and K_2CO_3 (0.414 g, 3.00 mmol) were stirred for 15 min in acetonitrile (30 mL). 4-Nitrobenzylbromide (0.432 g, 2.00 mmol) was added in one portion and stirring was continued at room temperature for further 2 d. After the solvent was removed under reduced pressure, water (50 mL) was added. The aqueous phase was extracted with CH_2Cl_2 (4 × 20 mL). Organic phases were combined and dried with magnesium sulfate. The residue was obtained after solvent removal under reduced pressure. The resulting residue was dissolved in acetonitrile and another portion of 4-nitrobenzylbromide (0.432 g, 2.00 mmol) was added. The reaction mixture was heated under reflux for 4 d. The light yellow coloured precipitate formed was filtered off and washed three times with diethyl ether and dried in vacuoto yield the product **3b** (0.750 g, 1.53 mmol, 76.8 % yield).

^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 9.70 (s, 1 H, NCHN), 8.29 (d, J = 8.7 Hz, 4 H, $CH_{Nitrobenzyl}$), 7.71 (d, J = 8.5 Hz, 4 H, $CH_{Nitrobenzyl}$), 5.71 (s, 4 H, CH_2). **^{13}C NMR** ([D$_6$]DMSO, 100 MHz, proton decoupled): δ = 148.1, 140.3, 138.0, 129.9, 124.4, 119.9 (NCN+CCl+ $C_{Nitrobenzyl}$), 51.2 (CH_2). **IR** (KBr): 2978 (m), 1609 (m), 1580 (m), 1530 (s), 1353 (s), 1203 (w), 1146 (m), 1020 (w), 884 (w), 853 (m), 798 (w), 738 (m), 697 (w), 614 (w) cm^{-1}. **UV/Vis** (CH_3OH, nm): λ 255 (ε 10753), λ 366 (ε 2272). **MS** (*m*/*z*, QMS-MS/MS): 408.9 [M$^+$-Br]. **Micro Analysis** Calculated for $C_{17}H_{13}N_4O_4Cl_2Br$ (488.12): Calcd.: C, 41.83 %; H, 2.68 %; N, 11.48 %; Cl, 14.53 %; Br, 16.37 %; Found: C, 41.68 %; H, 2.38 %; N, 11.21 %; Cl, 14.40 %; Br, 16.58 %.

1,3-Bis(4-nitrobenzyl)benzimidazolium bromide (3c): 1H-Benzimidazole (0.236 g, 2.00 mmol) and K_2CO_3 (0.414 g, 3.00 mmol) were stirred for 30 min in acetonitrile (30 mL) before 4-nitrobenzylbromide (0.864 g, 4.00 mmol) was added in one portion. The mixture was stirred at room temperature for 8 h. After the solvent was removed under reduced pressure, water (50 mL) was added. The precipitate was filtered off, washed with pentane and diethyl ether. The pink crystalline powder was dried for 4 h under vacuum to yield the product **3c** (0.765 g, 1.63 mmol, 81.5 % yield).

^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 10.09 (s, 1 H, NCHN), 8.27 (d, J = 8.0 Hz, 4 H, $CH_{Nitrobenzyl}$), 7.91 (dd, J = 6.3, 3.1 Hz, 2 H, $CH_{Benzimid}$), 7.77 (d, J = 8.0 Hz, 4 H, $CH_{Nitrobenzyl}$), 7.63 (dd, J = 6.3, 3.1 Hz, 2 H, $CH_{Benzimid}$), 5.98 (s, 4 H, CH_2). **^{13}C NMR** ([D$_6$]DMSO, 100 MHz, proton decoupled): δ = 148.0, 144.1, 141.5, 131.5, 129.9, 127.5, 124.4, 114.4 (NCN+$C_{Benzimid}$+$C_{Nitrobenzyl}$), 49.7 (CH_2). **IR** (KBr): 3391 (s), 3031 (w), 1607 (m), 1565 (m), 1523 (s), 1435 (w), 1349 (s), 1200 (w), 1109 (m), 1029 (w), 802 (w), 763 (s), 722 (m), 594 (w), 424 (w) cm^{-1}. **UV/Vis** (CH_3OH, nm): λ 264 (ε 11443), λ 366 (ε 2104). **MS** (*m*/*z*, QMS-MS/MS): 389.2 [M$^+$-Br]. **Micro Analysis** Calculated for $C_{21}H_{17}N_4O_4Br$ (469.29): Calcd.: C, 53.75 %; H, 3.65 %; N, 11.94 %; Br, 17.03 %; Found: C, 53.55 %; H, 3.52 %; N, 12.00 %; Br, 17.14 %.

[1,3-Bis(4-nitrobenzyl)imidazol-2-ylidene]silver(I) acetate (4a): 1,3-Bis(4-nitrobenzyl)imidazolium bromide (0.419 g, 1.00 mmol) was dissolved in dichloromethane (40 mL), silver(I) acetate (0.333 g, 2.00 mmol) was added, and the mixture was stirred at room temperature for 2 d. The yellow precipitate, presumably silver bromide, was filtered off, and a clear solution was obtained. The volatile components were removed in vacuo to produce a colourless sticky solid. The solid was washed with diethyl ether (3 × 10 mL) and dried under reduced pressure for 2 h to yield **4a** (0.400 g, 0.79 mmol, 79.17 % yield) as a colourless solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.27–8.20 (m, 4 H, $CH_{Nitrobenzyl}$), 7.51–7.44 (m, 4 H, $CH_{Nitrobenzyl}$), 7.06 (s, 2 H, CH_{Imid}), 5.46 (s, 4 H, CH_2), 2.09 (s, 3 H, $COCH_3$). **^{13}C NMR** ($CDCl_3$, 100 MHz, proton decoupled): δ = 179.0 (NCN), 172.1 (C=O), 148.1, 141.9, 128.6, 124.4, 122.0 (C_{Imid}+$C_{Nitrobenzyl}$), 55.1 (CH_2), 22.2 ($COCH_3$). **IR** (KBr): 3436 (s), 2928 (w), 1577 (m), 1518 (s), 1413 (m), 1348 (s), 1246 (w), 1107 (m), 804 (m), 734 (m), 669 (w), 467 (m) cm^{-1}. **UV/Vis** (CH_3OH, nm): λ 229 (ε 9292), λ 255 (ε 9033), λ 363 (ε 2306). **MS** (*m*/*z*, QMS-MS/MS): 446.1 [M$^+$-O_2CCH_3]. **Micro Analysis** Calculated for $C_{19}H_{17}N_4O_6Ag$ (505.23): Calcd.: C, 45.17 %; H, 3.39 %; N, 11.09 %; Ag, 21.35 %; Found: C, 44.99 %; H, 3.41 %; N, 11.21 %; Ag, 21.43 %.

[4,5-Dichloro-1,3-bis(4-nitrobenzyl)imidazol-2-ylidene]silver(I) acetate (4b): 4,5-Dichloro-1,3-bis(4-nitrobenzyl)imidazolium bromide (0.488 g, 1.00 mmol) and silver(I) acetate (0.333 g, 2.00 mmol) were mixed in methanol (50 mL) and stirred at room temperature for 2 d. The yellow precipitate, presumably silver bromide, was filtered off 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 diethyl ether and dried under reduced pressure for 3 h to yield **4b** (0.350 g, 0.60 mmol, 60.96 % yield) as a colourless solid.

^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 8.20 (d, J = 8.6 Hz, 4 H, $CH_{Nitrobenzyl}$), 7.53 (d, J = 8.3 Hz, 4 H, $CH_{Nitrobenzyl}$), 5.65 (s, 4 H, CH_2), 1.79 (s, 3 H, $COCH_3$). **^{13}C NMR** ([D$_6$]DMSO, 100 MHz, proton decoupled): δ = 183.3 (NCN), 175.2 (C=O), 147.4, 143.1, 128.6, 124.3, 118.1 (CCl+ $C_{Nitrobenzyl}$), 53.3 (CH_2), 24.2 ($COCH_3$). **IR** (KBr): 3427 (s), 1572 (s), 1409 (s), 1347 (m), 1108 (w), 1016 (m), 797 (w), 735 (w), 650 (w) cm^{-1}. **UV/Vis** (CH_3OH, nm): λ 256 (ε 18567), λ

359 (ε 2520). **MS** (*m*/*z*, QMS-MS/MS): 515.1 [M^+-O_2CCH_3]. **Micro Analysis** Calculated for $C_{19}H_{15}N_4O_6Cl_2Ag$ (574.12): Calcd.: C, 39.75 %; H, 2.63 %; N, 9.76 %; Cl, 12.35 %; Ag, 18.79 %; Found: C, 39.54 %; H, 2.71 %; N, 9.57 %; Cl, 12.21 %; Ag, 18.81 %.

[1,3-Bis(4-nitrobenzyl)benzimidazol-2-ylidene]silver(I) acetate (4c): 1,3-Bis(4-nitrobenzyl)benzimidazolium bromide (0.469 g, 1.00 mmol) was dissolved in dichloromethane (40 mL). Silver(I) acetate (0.333 g, 2.00 mmol) was added and the mixture was stirred at room temperature for 2 d. The yellow precipitate, presumably silver bromide, was filtered off and the solution was concentrated to 5 mL. Pentane (100 mL) was added and the desired product was filtered off as a colourless precipitate. The solid was stirred in diethyl ether (50 mL) for 30 minutes to remove the acetic acid and the product **4c** (0.375 g, 0.67 mmol, 67.53 % yield) was collected by vacuum filtration.

^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 8.18 (d, J = 8.0 Hz, 4 H, $CH_{Nitrobenzyl}$), 7.68 (dd, J = 6.0, 3.0 Hz, 2 H, $CH_{Benzimid}$), 7.62 (d, J = 8.0 Hz, 4 H, $CH_{Nitrobenzyl}$), 7.37 (dd, J = 6.0, 3.0 Hz, 2 H, $CH_{Benzimid}$), 5.94 (s, 4 H, CH_2), 1.76 (s, 3 H, $COCH_3$). **^{13}C NMR** ([D$_6$]DMSO, 100 MHz, proton decoupled): δ = 190.5 (NCN), 176.0(C=O), 147.6, 144.1, 133.8, 129.0, 124.8, 124.3, 112.8 ($C_{Benzimid}$+ $C_{Nitrobenzyl}$), 51.7 (CH_2), 23.6 ($COCH_3$). **IR** (KBr): 3441 (m), 2926 (w), 1575 (s), 1521 (s), 1438 (w), 1402 (m), 1347 (s), 1185 (w), 1108 (m), 735 (m) cm^{-1}. **UV/Vis** (CH_3OH, nm): λ 268 (ε 16956), λ 281(ε 14625), λ 361(ε 2549). **MS** (*m*/*z*, QMS-MS/MS): 496.1 [M^+-O_2CCH_3]. **Micro Analysis** Calculated for $C_{23}H_{19}N_4O_6Ag$ (555.29): Calcd.: C, 49.75 %; H, 3.45 %; N, 10.09 %; Ag, 19.43 %; Found: C, 49.14 %; H, 3.23 %; N, 10.00 %; Ag, 19.15 %.

1-Methyl-3-(4-nitrobenzyl)imidazolium bromide (3d): To a solution of 1-methylimidazole (0.396 g, 5.00 mmol) in toluene (30 mL), 4-nitrobenzylbromide (1.08 g, 5.00 mmol) was added; the resulting solution was stirred for 2 d at room temperature. Afterwards, the solvent was removed under reduced pressure. The resultant residue was first washed with pentane and afterwards with diethyl ether. Compound **3d** (1.00 g, 3.35 mmol, 67.0 % yield) was obtained as a colourless solid after drying under reduced pressure.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 10.87 (s, 1 H, NCHN), 8.24 (d, J = 8.8 Hz, 2 H, $CH_{Nitrobenzyl}$), 7.79 (d, J = 8.8 Hz, 2 H, $CH_{Nitrobenzyl}$), 7.31 (s, 1 H, CH_{Imid}), 7.23 (s, 1 H, CH_{Imid}), 5.89 (s, 2 H, CH_2), 4.08 (s, 3 H, N-CH_3). **^{13}C NMR** ([D$_6$]DMSO, 100 MHz, proton decoupled): δ = 139.6, 138.6, 130.0, 124.5, 123.1, 121.7, 109.9 (NCN+C_{Imid}+$C_{Nitrobenzyl}$), 52.2 (CH_2), 36.9 (N-CH_3). **IR** (KBr): 3362 (s), 3011 (s), 1608 (w), 1758 (w), 1581 (s), 1450 (w), 1352 (s), 1168 (s), 1105 (m), 1016 (w), 858 (m), 805 (m), 771 (w), 723 (s), 657 (w), 624 (m), 478 (w) cm^{-1}. **UV/Vis** (CH_3OH, nm): λ 254 (ε 7702), λ 366 (ε 1297). **MS** (*m*/*z*, QMS-MS/MS): 218.1 [M^+-Br]. **Micro Analysis** Calculated for $C_{11}H_{12}N_3BrO_2$ (298.14): Calcd.: C, 44.31 %; H, 4.06 %; N, 14.09 %; Br, 26.80 %; Found: C, 44.40 %; H, 4.00 %; N, 13.90 %; Br, 26.25 %.

4,5-Dichloro-1-methylimidazole (1e): 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 off 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 off 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) (**1e**).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.36 (s, 1 H, NCHN), 3.61 (s, 3 H, N-CH_3). **^{13}C NMR** ($CDCl_3$, 100 MHz, proton decoupled): δ = 134.6 (NCN), 125.5, 113.7 (CCl), 32.5 (N-CH_3). **IR** (KBr): 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) cm^{-1}. **UV/Vis** (CH_3OH, nm): λ 225 (ε 4371), λ 275 (ε 933). **MS** (*m*/*z*, QMS-MS/MS): 150.9 [M^+]. **Micro Analysis** 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 %.

4,5-Dichloro-1-methyl-3-(4-nitrobenzyl)imidazolium bromide (3e): To a solution of 4,5-Dichloro-1-methylimidazole (0.754 g, 5.00 mmol) in acetonitrile (40 mL), 4-nitrobenzylbromide (1.08 g, 5.00 mmol) was added; the resulting solution was stirred for 1 h at room temperature and heated for 2 d at 85 °C. 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 off, washed with diethyl ether and finally dried in vacuoin order to give compound **3e** (1.50 g, 4.08 mmol, 81.73 % yield).

^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 9.60 (s, 1 H, NCHN), 8.27 (d, J = 8.7 Hz, 2 H, $CH_{Nitrobenzyl}$), 7.67 (d, J = 8.6 Hz, 2 H, $CH_{Nitrobenzyl}$), 5.71 (s, 2 H, CH_2), 3.86 (s, 3 H, N-CH_3). **^{13}C NMR** ([D$_6$]DMSO, 100 MHz, proton decoupled): δ = 148.1, 140.6, 137.6, 129.7, 124.3 (NCN+$C_{Nitrobenzyl}$), 120.5, 118.6 (CCl), 50.8 (CH_2), 35.6 (N-CH_3). **IR** (KBr): 3363 (s), 3063 (m), 2949 (w), 1519 (s), 1449 (m), 1349 (s), 1156 (m), 846 (m), 613 (m), 480 (w) cm^{-1}. **UV/Vis** (CH_3OH, nm): λ 229 (ε 8669), λ 255 (ε 8562), λ 368 (ε 1685). **MS** (*m*/*z*, QMS-MS/MS): 287.0 [M^+-Br]. **Micro Analysis** Calculated for $C_{11}H_{10}N_3Cl_2BrO_2$ (367.03): Calcd.: C, 36.00 %; H, 2.75 %; N, 11.45 %; Cl, 19.32 %; Br, 21.77 %; Found: C, 36.05 %; H, 2.65 %; N, 11.05 %; Cl, 18.99 %; Br, 21.82 %.

1-Methyl-3-(4-nitrobenzyl)benzimidazolium bromide (3f): 4-Nitrobenzylbromide (1.08 g, 5.00 mmol) was added to a solution of 1-methylbenzimidazole (0.660 g, 5.00 mmol) in toluene (45 mL). The reaction mixture was stirred at room temperature for 2 d. Afterwards, the solvent was removed under reduced pressure. The resulting residue was first washed with pentane and afterwards with diethyl ether. Compound **3f** (1.40 g, 4.02 mmol, 80.41 % yield) was obtained as a colourless solid after drying under reduced pressure.

^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 9.91 (s, 1 H, NCHN), 8.26 (d, J = 8.7 Hz, 2 H, $CH_{Benzimid}$), 8.06 (d, J = 7.6 Hz, 1H $CH_{Nitrobenzyl}$), 7.90 (d, J = 7.6 Hz, 1 H, $CH_{Nitrobenzyl}$), 7.81–7.57 (m, 4 H, $CH_{Benzimid}$+ $CH_{Nitrobenzyl}$), 5.98 (s, 2 H, CH_2), 4.13 (s, 3 H, N-CH_3). **^{13}C NMR** ([D$_6$]DMSO, 100 MHz, proton decoupled): δ = 148.0, 143.8, 141.8, 132.5, 131.1, 129.8, 127.2, 127.1, 124.3, 114.2, 113.9 (NCN+$C_{Benzimid}$+$C_{Nitrobenzyl}$), 49.3 (CH_2), 33.9 (N-CH_3). **IR** (KBr): 3410 (m), 3108 (w), 2963 (s), 1607 (w), 1564 (m), 1518 (s), 1487 (m), 1412 (w), 1346 (s), 1280 (w), 1201 (w), 1103 (m), 1016 (m), 856 (m), 803 (m), 757 (s), 706 (s), 603 (m), 476 (w), 428 (m) cm^{-1}. **UV/Vis** (CH_3OH, nm): λ 240 (ε 11331), λ 265 (ε 13667). **MS** (*m*/*z*, QMS-MS/MS): 268.1 [M^+-Br]. **Micro Analysis** Calculated for $C_{15}H_{14}N_3O_2Br$ (348.19): Calcd.: C, 51.74 %; H, 4.05 %; N, 12.07 %; Br, 22.95 %; Found: C, 51.01 %; H, 3.92 %; N, 12.15 %; Br, 23.00 %.

(1-Methyl-3-(4-nitrobenzyl)imidazole-2-ylidene) silver(I) acetate (4d): 1-Methyl-3-(4-nitrobenzyl)imidazolium bromide (0.596 g, 2.00 mmol) was dissolved in dichloromethane (50 mL). Silver(I) acetate (0.667 g, 4.00 mmol) was added and the mixture was stirred for 3 d at room temperature. The precipitate, presumably silver bromide, was filtered off and the solution was concentrated to 2 mL. Diethyl ether was added and the product was crystallised in the freezer over-

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night. The resulting colourless solid was filtered off and dried on a vacuum line to yield the clean, dry product **4d** (0.410 g, 1.06 mmol, 53.36 % yield).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.16 (d, J = 8.7 Hz, 2 H, $CH_{Nitrobenzyl}$), 7.49 (d, J = 8.7 Hz, 2 H, $CH_{Nitrobenzyl}$), 7.15 (d, J = 2.0 Hz, 2 H, CH_{Imid}), 5.44 (s, 2 H, CH_2), 3.87 (s, 3 H, N-CH_3), 2.01 (s, 3 H, $COCH_3$). **^{13}C NMR** ($CDCl_3$, 100 MHz, proton decoupled): δ = 180.1 (NCN), 177.4 (C=O), 147.6, 143.0, 128.5, 124.0, 123.1, 121.5 (C_{Imid}+$C_{Nitrobenzyl}$), 54.4 (CH_2), 38.7 (N-CH_3), 22.7 ($COCH_3$). **IR** (KBr): 3402 (m), 1571 (s), 1518 (s), 1409 (s), 1348 (s), 1233 (m), 1162 (s), 1108 (m), 1016 (m), 923 (m), 858 (m), 804 (m), 734 (w), 653 (w), 478 (w) cm^{-1}. **UV/Vis** (CH_3OH, nm): λ 260 (ε 11302), λ 356 (ε 1879). **MS** (*m*/*z*, QMS-MS/MS): 325.1 [M^+-O_2CCH_3]. **Micro Analysis** Calculated for $C_{13}H_{14}N_3O_4Ag$ (384.14): Calcd.: C, 40.65 %; H, 3.67 %; N, 10.94 %; Ag, 28.08 %; Found: C, 40.47 %; H, 3.82 %; N, 10.77 %; Ag, 28.00 %.

(4,5-Dichloro-1-methyl-3-(4-nitrobenzyl)imidazole-2-ylidene) silver(I) acetate (4e): A mixture of 4,5-dichloro-1-methyl-3-(4-nitrobenzyl)imidazolium bromide (0.367 g, 1.00 mmol) and silver(I) acetate (0.333 g, 2.00 mmol) in dichloromethane (50 mL) was stirred for 2 d at room temperature. The yellow silver bromide suspension was filtered off to give a colourless solution. The volatile components were removed in vacuo to produce a colourless sticky solid. The solid was first washed with pentane and afterwards with diethyl ether and dried under reduced pressure for 2 h to yield **4e** (0.350 g, 0.77 mmol, 77.25 % yield) as a colourless solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.23 (d, J = 8.7 Hz, 2 H, $CH_{Nitrobenzyl}$), 7.53 (d, J = 8.7 Hz, 2 H, $CH_{Nitrobenzyl}$), 5.46 (s, 2 H, CH_2), 3.87 (s, 3 H, N-CH_3), 2.09 (s, 3 H, $COCH_3$). **^{13}C NMR** ($CDCl_3$, 100 MHz, proton decoupled): δ = 180.9 (NCN), 172.4 (C=O), 148.1, 140.9, 128.6, 124.3, 118.8, 117.3 (C_{Imid}+$C_{Nitrobenzyl}$), 53.6 (CH_2), 38.1 (N-CH_3), 22.6 ($COCH_3$). **IR** (KBr): 3419 (m), 2946 (w), 1581 (s), 1528 (s), 1406 (m), 1347 (s), 1270 (w), 1195 (w), 1110 (m), 1015 (w), 842 (m), 806 (m), 739 (w), 707 (m), 567 (w), 484 (w) cm^{-1}. **UV/Vis** (CH_3OH, nm): λ 259 (ε 9170), λ 367 (ε 1975). **MS** (*m*/*z*, QMS-MS/MS): 393.9 [M^+-O_2CCH_3]. **Micro Analysis** Calculated for $C_{13}H_{12}N_3O_4Cl_2Ag$ (453.03): Calcd.: C, 34.47 %; H, 2.67 %; N, 9.28 %; Cl, 15.65 %; Ag, 23.81 %; Found: C, 34.32 %; H, 2.53 %; N, 9.01 %; Cl, 15.34 %; Ag, 23.64 %.

(1-Methyl-3-(4-nitrobenzyl)benzimidazole-2-ylidene)silver(I) acetate (4f): 1-Methyl-3-(4-nitrobenzyl)benzimidazolium bromide (0.348 g, 1.00 mmol) was dissolved in dichloromethane (60 mL), and silver acetate (0.333 g, 2.00 mmol) was added. The mixture was stirred at room temperature for 2 d. The yellow precipitate, presumably silver bromide, was filtered off and discarded. The volume of the reaction mixture was reduced under pressure to 5 mL. Pentane (100 mL) was added and the fine colourless precipitate **4f** (0.325 g, 0.74 mmol, 74.85 % yield) was filtered off and washed with diethyl ether (75 mL).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.19 (d, J = 8.7 Hz, 2 H, $CH_{Benzimid}$), 7.58–7.21 (m, 6 H, $CH_{Benzimid}$+ $CH_{Nitrobenzyl}$), 5.74 (s, 2 H, CH_2), 4.10 (s, 3 H, N-CH_3), 2.08 (s, 3 H, $COCH_3$). **^{13}C NMR** ($CDCl_3$, 100 MHz, proton decoupled): δ = 179.0 (NCN), 163.9 (C=O), 147.9, 142.0, 134.6, 133.3, 128.0, 124.6, 124.6, 124.2, 111.5, 111.4 ($C_{Benzimid}$+$C_{Nitrobenzyl}$), 52.4 (CH_2), 36.0 (N-CH_3), 22.6 ($COCH_3$). **IR** (KBr): 3432 (s), 1564 (s), 1460 (s), 1397 (m), 1290 (s), 1106 (w), 1019 (w), 747 (m), 670 (w) cm^{-1}. **UV/Vis** (CH_3OH, nm): λ 269 (ε 9561), λ 364 (ε 2131). **MS** (*m*/*z*, QMS-MS/MS): 375.9 [M^+-O_2CCH_3]. **Micro Analysis** Calculated for $C_{17}H_{16}N_3O_4Ag$ (434.19): Calcd.: C, 47.03 %; H, 3.71 %; N, 9.68 %; Ag, 24.84 %; Found: C, 46.98 %; H, 3.53 %; N, 9.76 %; Ag, 24.51 %.

Antibacterial Studies

Preliminary in vitro antibacterial activity of symmetrically and non-symmetrically *p*-nitrobenzyl-substituted *N*-heterocyclic carbenes as ligand precursors and their corresponding silver(I) acetate complexes were screened 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 **3a**–**f** and **4a**–**f**, the qualitative Kirby–Bauer disk-diffusion method was applied. All bacteria were individually cultured from a single colony in sterile LB medium 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 Whatman paper discs were placed evenly separated on each plate. Two stock solutions (90:10 DMSO:H_2O) of every compound were prepared at 2.1 μM and 4.3 μM to be able to test the effect of different concentrations. Each plate was tested with 5 μL and 7 μL of 2.1 μM solution and 5 μL and 10 μL for the 4.3 μM solution. The plates were covered and placed in an incubator at 37 °C for 24 h. The plates were afterwards removed and the area 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.

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 the one 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) 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. Afterwards, 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 afterwards treated with varying concentrations of the compounds and incubated for 48 h at 37 °C. Subsequently, 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 solution of MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) in medium (200 μL) (30 mg MTT in 30 mL medium). The cells were incubated for 3 h at 37 °C. Afterwards, the medium was removed and the purple formazan crystals were dissolved in DMSO (200 μL 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.

Acknowledgement

The authors thank the *Irish Research Council for Science Engineering and Technology* (IRCSET) for funding through a postdoctoral fellowship for Dr. Siddappa Patil.

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Received: November 1, 2010
Published Online: January 27, 2011

