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Synthesis, Cytotoxicity and Antibacterial Studies of *p*-Methoxybenzyl-Substituted and Benzyl-Substituted N-Heterocyclic Carbene–Silver Complexes

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p-Methoxybenzyl-substituted and benzyl-substituted N-heterocyclic carbene (NHC) [(**3a–c**) and (**6a–c**)] precursors were synthesised from the reaction of 1*H*-imidazole (**1a**), 4,5-dichloro-1*H*-imidazole (**1b**), and 1*H*-benzimidazole (**1c**) with *p*-methoxybenzyl bromide (**2**) and benzyl bromide (**5**). These NHC precursors were then treated with silver(I) acetate to yield the NHC–silver complexes [1,3-bis(4-methoxybenzyl)-imidazol-2-ylidene]silver(I) acetate (**4a**), [4,5-dichloro-1,3-bis(4-methoxybenzyl)imidazol-2-ylidene]silver(I) acetate (**4b**), [1,3-bis(4-methoxybenzyl)benzimidazol-2-ylidene]-silver(I) acetate (**4c**), (1,3-dibenzylimidazol-2-ylidene)silver(I) acetate (**7a**), (1,3-dibenzyl-4,5-dichloroimidazol-2-ylidene)-silver(I) acetate (**7b**), and (1,3-dibenzylbenzimidazol-2-ylidene)silver(I) acetate (**7c**), respectively. The NHC precursor **3c**, four NHC–silver complexes **4c** and **7a–c** were characterised by single-crystal X-ray diffraction method. The preliminary antibacterial activity of all the compounds was studied against Gram-negative bacteria *Escherichia coli*, and Gram-positive bacteria *Staphylococcus aureus* using the Kirby–Bauer disk-diffusion method. Almost all the NHC–silver complexes have shown high antibacterial activity compared to the NHC precursors. In addition, the NHC–silver complexes had their cytotoxicity investigated through MTT-based preliminary in vitro testing on the Caki-1 cell lines in order to determine their IC_{50} values. NHC–silver complexes **4a–c** and **7a–c** were found to have IC_{50} values of 7.3 (+/–6), 12.7 (+/–3), 25.2 (+/–5), 2.5 (+/–3), 10.8 (+/–4) and 12.5 (+/–4) μM respectively on the Caki-1 cell line.

Introduction

N-Heterocyclic carbenes (NHCs), which are usually derived from imidazolium halides, are electronically stabilised cyclic carbene ligands unfolding a new chapter of organometallic chemistry.[1,2] Quite quickly NHCs have been developed into promising "spectator" ligands for catalysis, but more recently NHCs found an application in NHC–silver complexes exhibiting antimicrobial activity, in particular for the possible treatment of cystic fibrosis (CF) and chronic lung infections[3–5] and maybe in the treatment of cancer.[6]

This possible anticancer activity attracted our interest, since we investigate novel organometallic anticancer drugs. By starting from fulvenes via reductive dimerisation with titanium dichloride, carbolithiation or hydridolithiation of the fulvene followed by transmetallation with titanium tetrachloride in the latter two cases these syntheses lead to *anti*-proliferative titanocene derivatives.[7] Hydridolithiation of 6-anisylfulvene and subsequent reaction with $TiCl_4$ makes bis[(*p*-methoxybenzyl)cyclopentadienyl]titanium(IV) dichloride (Titanocene **Y**)[8] available, which exhibits an IC_{50} value of 21 μM when tested on the LLC-PK cell line, while the corresponding Vanadocene **Y**[9] shows an improved IC_{50} value of 3.0 μM. Because this *p*-methoxybenzyl-substitution proved so successful in the case of the metallocene chemistry, it will now be applied to NHC–silver complexes.

Within this paper, we present the synthesis, preliminary cytotoxicity and antibacterial studies of a series of six new *p*-methoxybenzyl-substituted and benzyl-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

Syntheses

The synthetic pathway for *p*-methoxybenzyl-substituted and benzyl-substituted N-heterocyclic carbenes as ligand precursors and their corresponding silver complexes described in this work is outlined in Schemes 1, 2 and 3. We

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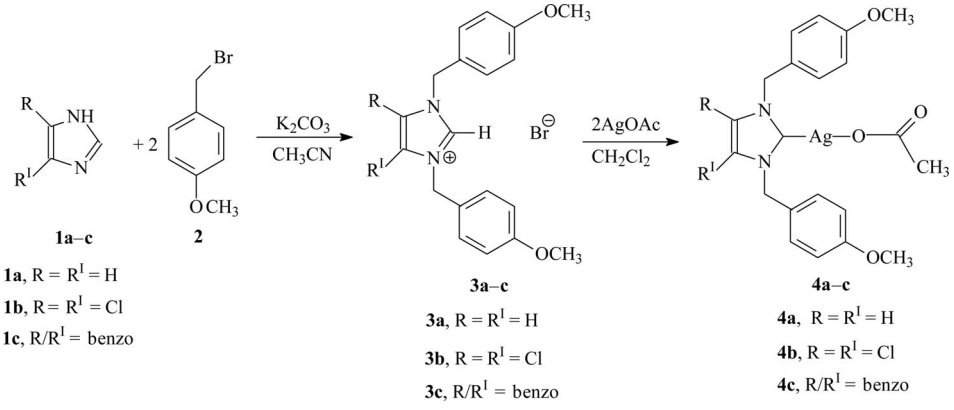
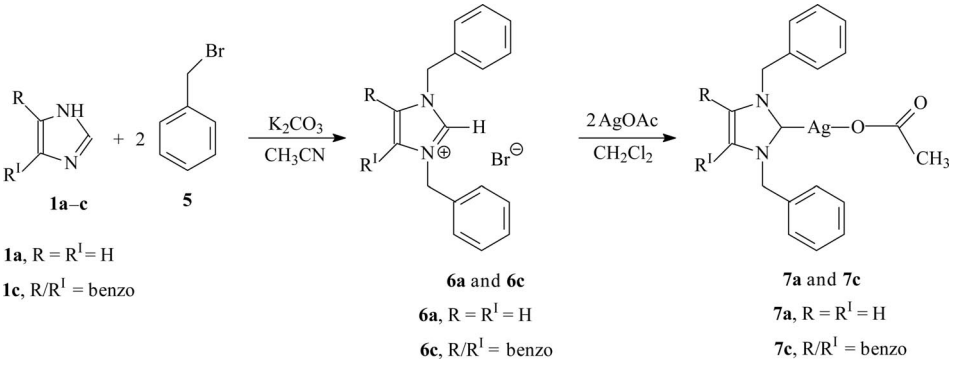
did not follow the synthetic procedure of 1,3-dibenzylimidazolium bromide (**6a**), and 1,3-dibenzylbenzimidazolium bromide (**6c**), published in literature[10–12] instead we synthesised according to our new and milder procedure. On the other hand, the synthesis of 1,3-dibenzyl-4,5-dichloroimidazolium bromide (**6b**) was carried out according to a published literature procedure.[13] The NHCs precursors 1,3-bis(*p*-methoxybenzyl)imidazolium bromide (**3a**), 4,5-dichloro-1,3-bis(*p*-methoxybenzyl)imidazolium bromide (**3b**), 1,3-bis(*p*-methoxybenzyl)benzimidazolium bromide (**3c**), 1,3-dibenzylimidazolium bromide (**6a**) and 1,3-dibenzylbenzimidazolium bromide (**6c**) were prepared by stirring 1*H*-imidazole (**1a**), 4,5-dichloro-1*H*-imidazole (**1b**), and 1*H*-benzimidazole (**1c**) with 2 equiv. of alkyl halides [*p*-methoxybenzyl bromide (**2**) and benzyl bromide (**5**)] in the presence of K_2CO_3 as a base in CH_3CN at room temperature for 2–3 d with moderate to good 40% to 91% yields. 4,5-Dichloro-1-benzylimidazole (**5′**) was prepared in a straightforward way by deprotonation of 4,5-dichloroimidazole with K_2CO_3 in CH_3CN and further alkylation with benzyl bromide. Subsequent benzylation of the obtained product in toluene with another equivalent of the benzyl bromide yielded 69.0% of the 1,3-dibenzyl-4,5-dichloroimidazolium bromide (**6b**) as an air stable white powder. All the precursors were fully characterized by ^1H, ^{13}C NMR, IR, UV/Vis, mass spectroscopy, and elemental analysis. Additionally the solid-state structure of **3c** was determined by single-crystal X-ray diffraction method.

The ^1H NMR spectra of all precursors **3a**–**c** and **6a**–**c** show a characteristic downfield shift in the range δ = 10.12–11.85 ppm for the NCHN proton attributable to the positive charge of the molecule.[14–17] In addition, their identities have also been confirmed by a base peak for the [M^+ – Br] fragments in their positive mode ESI mass spectra.

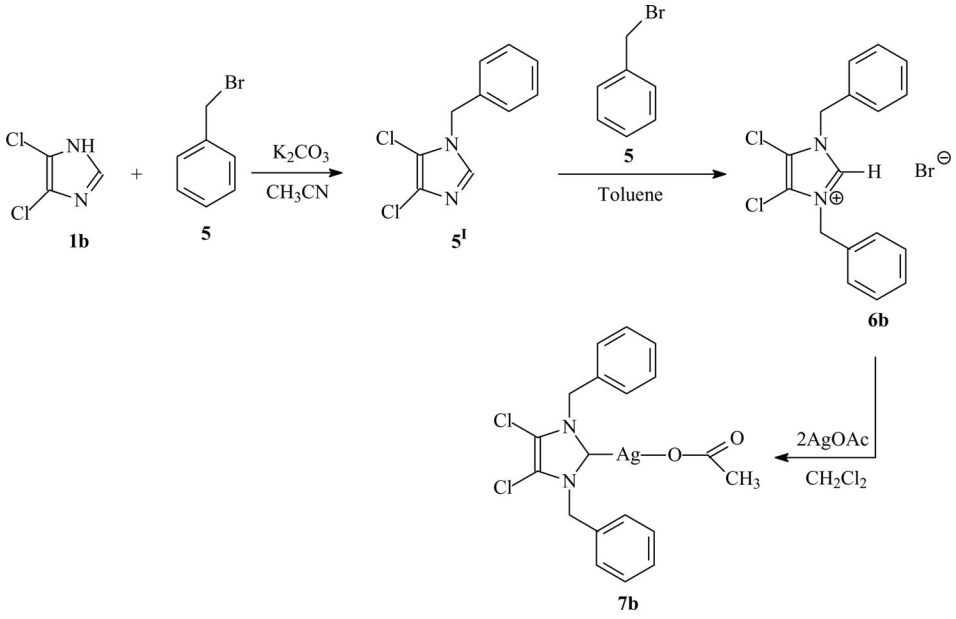
The NHC–silver complexes [1,3-bis(4-methoxybenzyl)-imidazol-2-ylidene]silver(I) acetate (**4a**), [4,5-dichloro-1,3-bis(4-methoxybenzyl)imidazol-2-ylidene]silver(I) acetate (**4b**), [1,3-bis(4-methoxybenzyl)benzimidazol-2-ylidene]silver(I) acetate (**4c**), (1,3-dibenzylimidazol-2-ylidene)silver(I) acetate (**7a**), (1,3-dibenzyl-4,5-dichloroimidazol-2-ylidene)-silver(I) acetate (**7b**), and (1,3-dibenzylbenzimidazol-2-ylidene)silver(I) acetate (**7c**) were synthesized by the reaction of **3a**–**c** and **6a**–**c** with 2 equiv. of silver acetate in CH_2Cl_2. The reaction mixture was stirred for 1–2 d at room temperature in dark to afford the NHC–silver acetate complexes as off-white solid in 62% to 87% yield. The complexes were fully characterized by ^1H, ^{13}C NMR, IR, UV/Vis, mass spectroscopy, and elemental analysis. Furthermore, the solid-state structures of **4c** and **7a**–**c** were analysed by single-crystal X-ray diffraction method. The absence of a downfield



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



Scheme 2. General reaction scheme for the synthesis of benzyl-substituted N-heterocyclic carbenes **6a** and **6c** and their corresponding N-heterocyclic silver-carbene complexes **7a** and **7c**.



Scheme 3. General reaction scheme for the synthesis of benzyl-substituted N-heterocyclic carbene **6b** and its N-heterocyclic carbene–silver complex **7b**.

NCHN signals and presence of new signals at 2.08–1.79 ppm for the acetate protons in all the ^{1}H NMR spectra for **4a**–**c** and **7a**–**c**, however, O_2CCH_3 indicates a successful complex formation. The ^{13}C NMR resonances of the carbene carbon atoms in complexes **4a**–**c** and **7a**–**c** occur in the range δ = 178.9–175.1 ppm respectively. These signals are shifted downfield compared to the corresponding precursors of **3a**–**c** and **6a**–**c** carbene carbon atoms resonance at the range 130.8–143.1 ppm, respectively, which further demonstrates the formation of expected NHC–silver acetate complexes. Also appearance of the ^{13}C NMR resonances for the carbonyl and methyl carbon atoms of the acetate group of complexes **4a**–**c** and **7a**–**c** in the range 168.6–166.1 and 23.7–21.0 ppm, respectively, showed the formation of the NHC–silver complexes.[3,5] Furthermore, positive mode ESI mass spectra of all six NHC–silver complexes (**4a**–**c** and **7a**–**c**) are dominated by [M^+ – O_2CCH_3] fragment peaks arising from the loss of one acetate ligand.

Structural Discussion

The crystal structures of the compounds **3c**, **4c**, and **7a**–**c** have been determined. The molecular structures of the

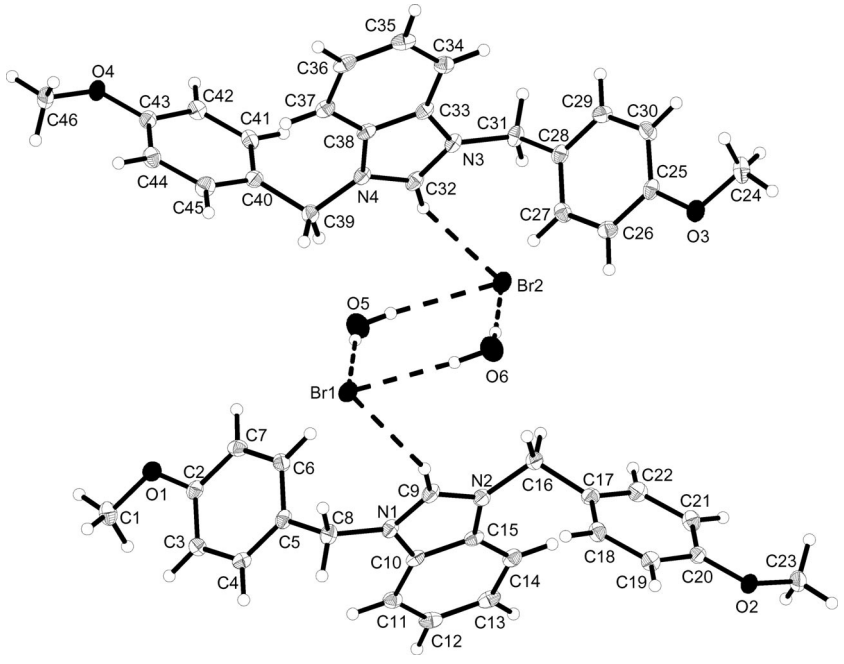


Figure 1. X-ray diffraction structure of **3c**; hydrogen bond linked moiety; thermal ellipsoids are drawn on the 50% probability level.

compounds **3c**, **4c**, and **7a**–**c** are depicted in Figures 1, 2, 3, 4, 5, and 6. The crystal data and refinement details for all five compounds are found in Table 1, whereas selected bond lengths and bond angles are displayed in Table 2.

The X-ray structure of **3c** reveals that the benzimidazolium core is planar. In the five-membered ring (NCNCC), the bond lengths and angles are in good agreement with the bond lengths found in the similar compound 1,3-diisopropylbenzimidazolium bromide reported in literature (see Table 2).[18] Hydrogen bonds connect two cations, two anions and two water molecules to isolated dimeric moieties (Figure 1).

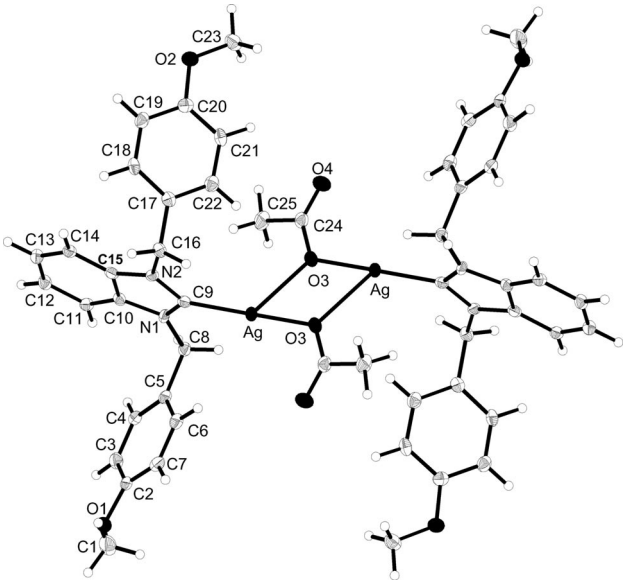


Figure 2. X-ray diffraction structure of **4c**; molecule; thermal ellipsoids are drawn on the 50% probability level.

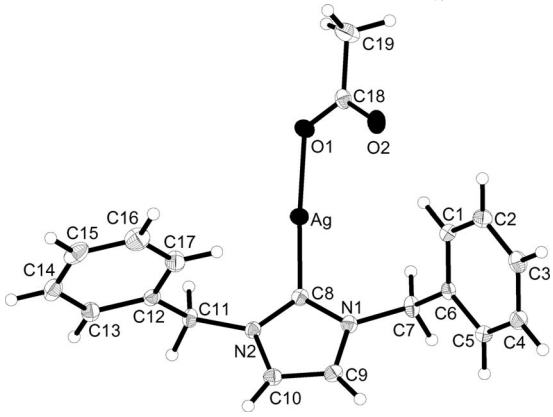


Figure 4. X-ray diffraction structure of **7a**; molecule; thermal ellipsoids are drawn on the 50% probability level.

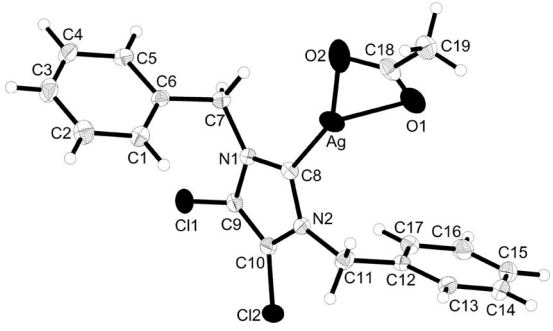


Figure 5. X-ray diffraction structure of **7b**; molecule; thermal ellipsoids are drawn on the 50% probability level.

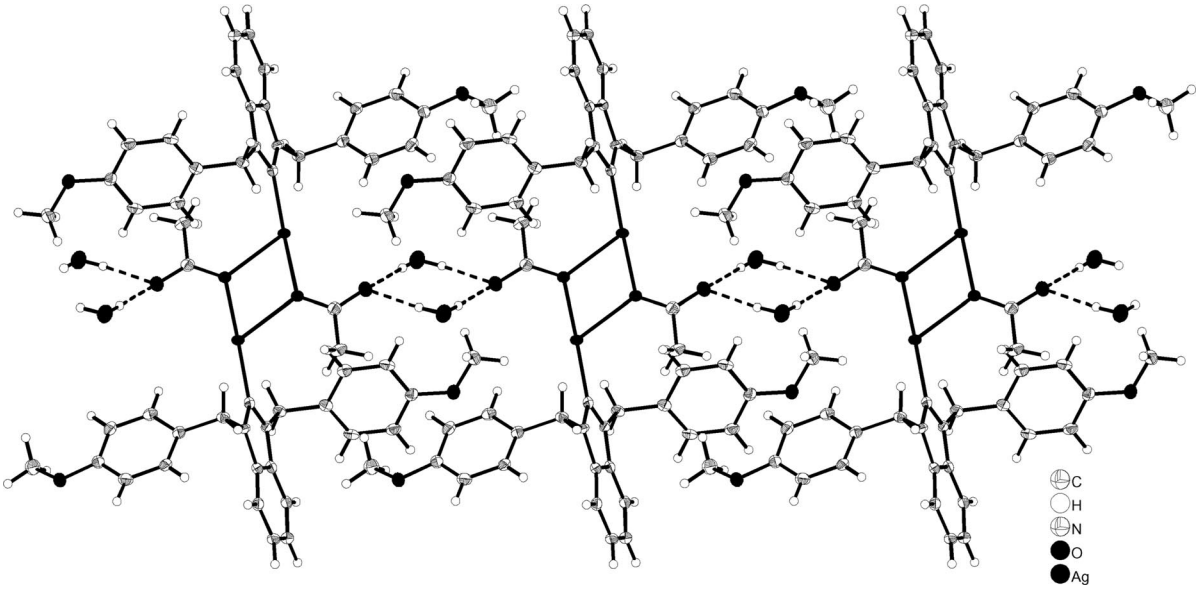


Figure 3. Hydrogen-bonded chain of **4c** running along [1 1 0].

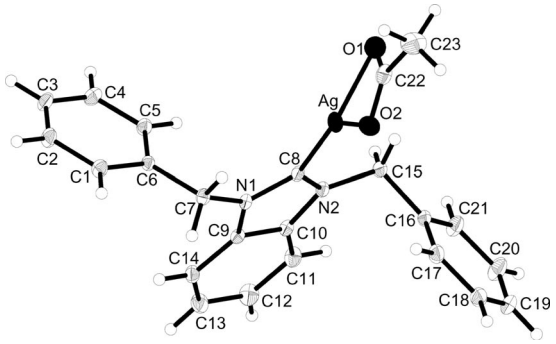


Figure 6. X-ray diffraction structure of **7c**; molecule; thermal ellipsoids are drawn on the 50% probability level.

Also, in all the silver complexes reported here, bond lengths and angles in and directly around the NHC core agree very well among each other and with literature data.[19–23] Comparing precursor **3c** with the corresponding complex **4c** (see Table 2), one finds a slight increase of both the C(9)–N distances. The biggest difference between the silver complexes reported here is the behaviour of the acetate groups: In **4c**, one oxygen of the anion connects strongly to one silver and weakly to another, resulting in a binuclear species around an inversion centre [with an Ag–Ag distance of 3.81(1) Å] (Figure 2). The second oxygen is 3.2 Å away from the silver atom, clearly too far for any interaction. Instead, it acts as a hydrogen-bond acceptor for two adjacent water molecules. The remaining proton of each water molecule acts as hydrogen-bond donor to another complex dimer. That leads to hydrogen-bonded chains as depicted in Figure 3. The compounds **7a** to **7c** are mononuclear complexes. In **7a** (Figure 4), the second oxygen atom of the acetate is now 2.81 Å away from the silver, which is significantly closer than in **4c** but in our opinion still too far to call it a bond. In **7b** (Figure 5) and **7c** (Figure 6), however, O(2) is close enough to the silver atom to do so (Table 2). This is further supported by the significant deviation of the C(8)–Ag–O(1) angle from 180° (Table 2).

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

Identification code	**3c**	**4c**	**7a**	**7b**	**7c**
Empirical formula	$C_{23}H_{25}BrN_2O_3$	$C_{50}H_{54}Ag_2N_4O_{10}$	$C_{19}H_{19}AgN_2O_2$	$C_{19}H_{17}AgCl_2N_2O_2$	$C_{23}H_{21}AgN_2O_2$
Molecular formula	$[C_{23}H_{23}N_2O_2]^+$ $[Br]^-$· H_2O	$C_{50}H_{50}Ag_2N_4O_8$· $2H_2O$	$C_{19}H_{19}AgN_2O_2$	$C_{19}H_{17}AgCl_2N_2O_2$	$C_{23}H_{21}AgN_2O_2$
Formula weight	457.36	1086.71	415.23	484.12	465.29
Crystal system	monoclinic	triclinic	monoclinic	monoclinic	triclinic
Space group	$P2_1/c$ (#14)	$P\bar{1}$ (#2)	$C2/c$ (#15)	$P2_1/c$ (#14)	$P\bar{1}$ (#2)
Unit cell dimensions	a = 7.2090(5) Å	a = 10.6490(13) Å	a = 19.8904(15) Å	a = 7.4711(8) Å	a = 8.0255(8) Å
	b = 22.4949(16) Å	b = 10.7944(14) Å	b = 10.8442(8) Å	b = 21.285(2) Å	b = 11.1776(11) Å
	c = 25.7455(18) Å	c = 11.7540(15) Å	c = 15.8836(12) Å	c = 12.4037(13) Å	c = 11.8084(11) Å
	α = 90°	α = 96.122(3)°	α = 90°	α = 90°	α = 85.681(2)°
	β = 95.146(2)°	β = 99.845(3)°	β = 90.791(1)°	β = 102.327(2)°	β = 81.621(2)°
	γ = 90°	γ = 118.625(3)°	γ = 90°	γ = 90°	γ = 73.819(2)°
Volume	4158.2(5) Å^3	1140.4(2) Å^3	3425.7(4) Å^3	1927.0(4) Å^3	1005.79(17) Å^3
Z	8	1	8	4	2
Density (calculated)	1.461 Mg/m^3	1.582 Mg/m^3	1.610 Mg/m^3	1.669 Mg/m^3	1.536 Mg/m^3
Absorption coefficient	2.004 mm^{-1}	0.924 mm^{-1}	1.190 mm^{-1}	1.339 mm^{-1}	1.022 mm^{-1}
F(000)	1888	556	1680	968	472
Crystal size	0.60 × 0.20 × 0.15 mm	0.60 × 0.50 × 0.30 mm	0.80 × 0.60 × 0.10 mm	0.50 × 0.10 × 0.05 mm	0.40 × 0.30 × 0.20 mm
Theta range (data coll.)	1.81 to 26.40°	1.80 to 26.45°	2.05 to 29.00°	1.91 to 24.10°	1.74 to 26.38°
Index ranges	$-9 \le h \le 8$	$-13 \le h \le 13$	$-27 \le h \le 27$	$-8 \le h \le 8$	$-10 \le h \le 10$
	$-28 \le k \le 28$	$-13 \le k \le 13$	$-14 \le k \le 14$	$-24 \le k \le 24$	$-13 \le k \le 13$
	$-32 \le l \le 32$	$-14 \le l \le 14$	$-21 \le l \le 21$	$-14 \le l \le 14$	$-14 \le l \le 14$
Reflections collected	36494	10305	35864	14050	17951
Independent reflections	8466	4685	4552	3064	4112
R(int)	0.0312	0.0404	0.0229	0.0199	0.0203
Completeness to $\theta_{max.}$	99.4%	99.5%	99.9%	99.9%	99.9%
Max./min. transmission	0.7531/0.4885	0.7691/0.5975	0.8903/0.6467	0.9361/0.7583	0.8216/0.7261
Data/restraints/param.	8466/0/551	4685/2/307	4552/0/218	3064/0/237	4112/0/256
Goodness-of-fit on F^2	1.054	1.005	1.062	1.048	1.215
Final R indices	R_1 = 0.0322	R_1 = 0.0415	R_1 = 0.0211	R_1 = 0.0411	R_1 = 0.0418
[$I > 2\sigma(I)$]	wR_2 = 0.0777	wR_2 = 0.0969	wR_2 = 0.0535	wR_2 = 0.0895	wR_2 = 0.0922
R indices	R_1 = 0.0400	R_1 = 0.0504	R_1 = 0.0223	R_1 = 0.0474	R_1 = 0.0455
(all data)	wR_2 = 0.0809	wR_2 = 0.1017	wR_2 = 0.0542	wR_2 = 0.0934	wR_2 = 0.0952
Largest diff. peak/hole	0.578/–0.237 e Å^{-3}	1.336/–0.425 e Å^{-3}	0.622/–0.402 e Å^{-3}	0.823/–1.047 e Å^{-3}	1.698/–2.355 e Å^{-3}

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

Bond lengths [Å]	**3c**	**4c**	Bond lengths [Å]	**7a**	**7b**	**7c**
N(1)–C(9)	1.328(3)	1.348(4)	C(8)–N(2)	1.353(2)	1.349(5)	1.355(4)
N(1)–C(10)	1.395(2)	1.392(4)	N(1)–C(8)	1.356(2)	1.347(5)	1.356(4)
C(9)–N(2)	1.328(3)	1.360(4)	N(1)–C(9)	1.387(2)	1.376(5)	1.393(4)
N(2)–C(15)	1.394(2)	1.388(4)	N(2)–C(10)	1.388(2)	1.372(5)	1.393(4)
C(10)–C(15)	1.393(3)	1.384(5)	C(9)–C(10)	1.352(2)	1.331(5)	1.380(5)
C(9)–Ag		2.072(3)	C(8)–Ag	2.065(1)	2.064(4)	2.059(3)
Ag–O(3)		2.612(3)	Ag–O(1)	2.131(1)	2.277(4)	2.250(3)
Ag–O(3)#1		2.121(2)	Ag–O(2)		2.502(5)	2.475(3)
			O(1)–C(18)	1.282(2)	1.238(6)	
			O(2)–C(18)	1.244(2)	1.225(6)	
			C(9)–Cl(1)		1.688(4)	
			C(10)–Cl(2)		1.691(4)	
			O(1)–C(22)			1.252(5)
			O(2)–C(22)			1.225(5)
Bond angles [°]	**3c**	**4c**	**Bond angles [°]**	**7a**	**7b**	**7c**
N(1)–C(9)–N(2)	110.4(2)	105.5(3)	N(2)–C(8)–N(1)	104.3(1)	105.0(3)	105.8(3)
C(9)–N(1)–C(10)	108.4(2)	111.1(3)	C(8)–N(1)–C(9)	111.5(1)	110.2(3)	110.7(3)
C(9)–N(2)–C(15)	108.3(2)	111.2(3)	C(8)–N(2)–C(10)	111.3(1)	110.6(3)	110.9(3)
C(10)–C(15)–N(2)	106.5(2)	105.9(3)	C(10)–C(9)–N(1)	106.3(1)	107.3(3)	106.5(3)
C(15)–C(10)–N(1)	106.3(2)	106.3(3)	C(9)–C(10)–N(2)	106.6(1)	106.9(3)	106.1(3)
C(9)–Ag–O(3)		112.9(1)	C(8)–Ag–O(1)	172.76(5)	150.3(2)	164.2(1)
C(9)–Ag–O(3)#1		170.1(1)	C(8)–Ag–O(2)		155.2(1)	141.6(1)
O(3)#1–Ag–O(3)		73.1(1)	C(18)–O(1)–Ag	107.64(9)	97.2(3)	
			O(1)–Ag–O(2)		53.4(1)	54.1(1)
			C(18)–O(2)–Ag		86.8(4)	
			O(2)–C(18)–O(1)		122.3(5)	
			C(22)–O(1)–Ag			97.2(3)
			C(22)–O(2)–Ag			87.3(2)
			O(2)–C(22)–O(1)			121.4(4)

[a] Symmetry operations: #1 $-x + 1, -y + 1, -z + 1$.

Biological Evaluation

The biological activities of the silver acetate, NHC precursors and their corresponding silver complexes are summarized in Figures 7, 8, 9, and 10. Almost no antibacterial activity was observed for silver acetate and compound **7c** against the both Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli* using the Kirby–Bauer disk-diffusion method. Low to medium activity was observed with the compounds **3a**, **3b**, **4c**, **6b** and **7b** against Gram-positive bacteria *Staphylococcus aureus* whereas the **3c**, **4a**, **4b**, **6a**, **6c** and **7a** compounds have shown high activity. In case of Gram-negative bacteria *Escherichia coli* **3b**, **3c**, **4b**, **4c**, **6a**, **6b** and **6c** compounds showed medium activity whereas the **3a**, **4a**, **7a** and **7b** compounds showed high activity. The solvent used to prepare the stock solutions (DMSO) played no role in growth inhibition on the same bacteria as previously reported.[24]

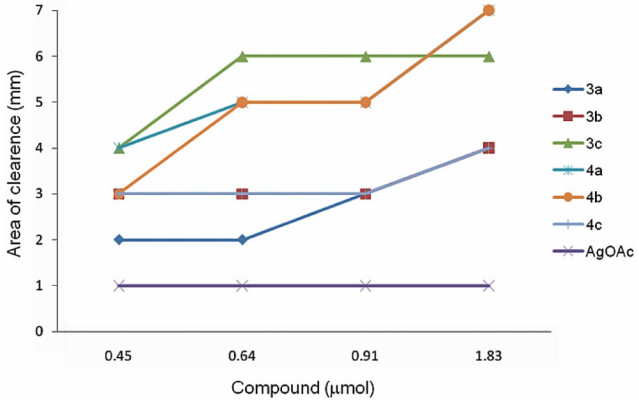


Figure 7. Area of clearance on *Staphylococcus aureus* (Gram +ve) by **3a**–**c**, **4a**–**c** and AgOAc.

Thus, on the basis of the above observation it can be stated that (i) the complexes **4c** and **7c** are less active against the both Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli* than the free precursors **3c** and **6c**. This is probably due to the poor solubility of the complexes **4c** and **7c** in DMSO. (ii) On comparison with the complexes **4c** and **7c**, the complexes **4a**, **4b**, **7a** and **7b** have shown enhanced activity. The enhanced activity of the complexes **4a**, **4b**, **7a** and **7b** is due to the fact that easy solubility in DMSO. (iii) It was concluded that, as the precursors and complexes concentration increases, the antimicrobial activity becomes higher and (iv) It was observed that, compared to the precursors and silver acetate, the complexes exhibited enhanced antibacterial activity, which is due to the synergistic effect that increases the lipophilicity of the complexes. Chelation decreases the polarity of the metal ion, which further leads to the enhanced lipophilicities of the complex. Because the microorganism cell is surrounded by a lipid membrane, which favours the passage of lipid soluble materials, increased lipophilicities allows the

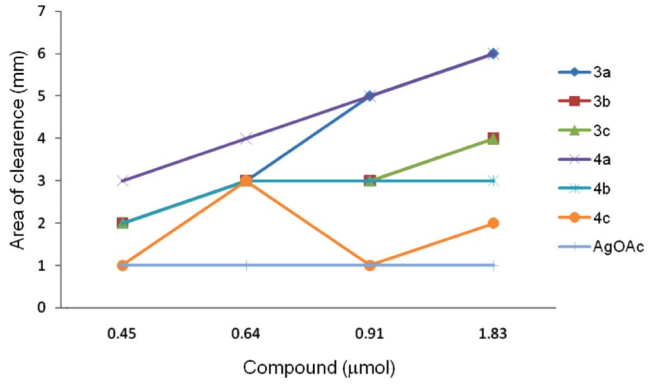


Figure 8. Area of clearance on *Escherichia coli* (Gram -ve) by **3a**–**c**, **4a**–**c** and AgOAc.

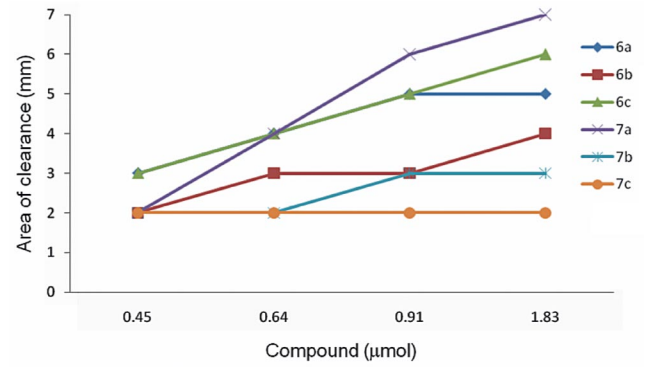


Figure 9. Area of clearance on *Staphylococcus aureus* (Gram +ve) by **6a**–**c** and **7a**–**c**.

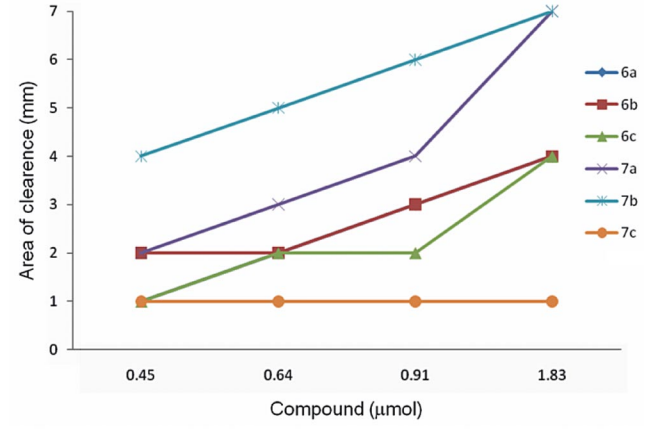


Figure 10. Area of clearance on *Escherichia coli* (Gram -ve) by **6a**–**c** and **7a**–**c**.

penetration of complex into and through the membrane and deactivates the active enzyme sites of the microorganisms.[25]

In comparison with the known reported NHC precursors and NHC–silver complexes from the literature[3,5] the NHC precursors **3a**–**c** and **6a**–**c** and their corresponding NHC–silver complexes **4a**–**c** and **7a**–**c** have almost same antibacterial activity.

Cytotoxicity Studies

The in vitro cytotoxicity of compounds **4a**–**c** and **7a**–**c** were determined by MTT-based assays[26] involving a 48 h drug-exposure period, followed by 24 h of recovery time. Compounds were tested for their activity on the human cancerous renal-cell line Caki-1 and the results are shown in Figure 11 and 12, respectively. *p*-Methoxybenzyl-substituted NHC–silver acetate complexes **4a**–**c**, which contains 1*H*-imidazole, 4,5-dichloro-1*H*-imidazole and 1*H*-benzimidazole groups, has IC_{50} values 7.3, 12.7 and 25.2 μm, respectively. Compound **4a** has an approximately twofold and threefold increase in magnitude when compared with **4b** and **4c**, respectively, and in comparison to cisplatin (IC_{50} value = 3.3 μM) it represents an approximately twofold decrease in magnitude. Benzyl-substituted NHC–silver acetate complexes **7a**–**c**, which also contains 1*H*-imidazole, 4,5-dichloro-1*H*-imidazole and 1*H*-benzimidazole groups, has IC_{50} values of 2.5, 10.8 and 12.5 μM, respectively. Compound **7a** is the most promising candidate in this paper because of highest IC_{50} value and has an approximately threefold and fourfold increase in magnitude when compared with **7b** and **7c**, respectively. In comparison with cisplatin, **7a** has an approximately equal in magnitude in terms of the IC_{50} value.

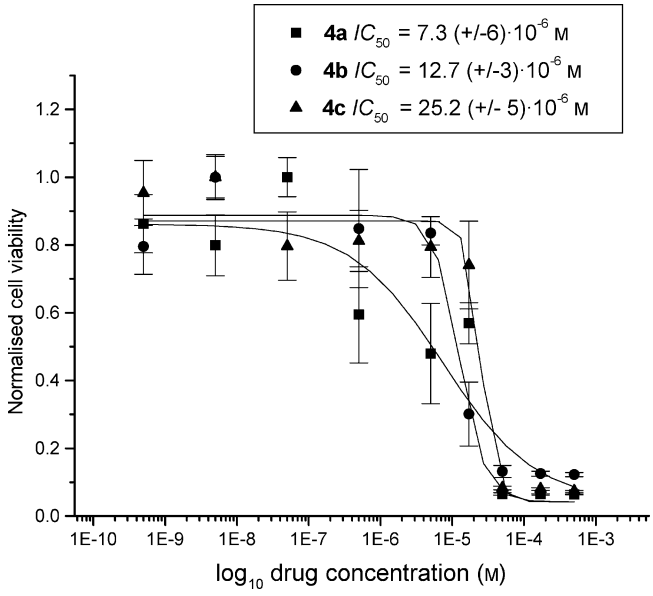


Figure 11. Cytotoxicity curves from typical MTT assays showing the effect of compounds **4a**–**c** on the viability of Caki-1 cells.

In comparison to *p*-methoxybenzyl-substituted NHC–silver acetate complexes to benzyl-substituted NHC–silver acetate complexes IC_{50} values are almost same in magnitude because of their solubility factor. Both *p*-methoxybenzyl-substituted NHC–silver acetate complexes and benzyl-substituted NHC–silver acetate complexes are easily soluble in DMSO solvent 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,[6] the NHC–silver complexes **4a**–**c** and **7a**–**c** have almost same cytotoxicity activity.

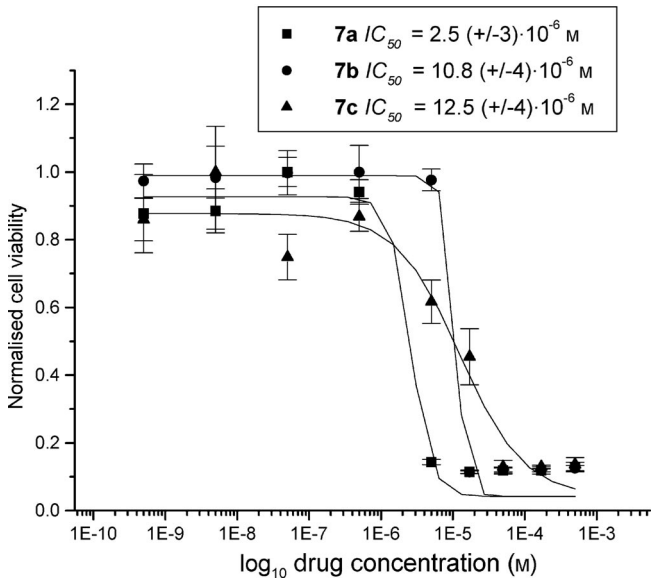


Figure 12. Cytotoxicity curves from typical MTT assays showing the effect of compounds **7a**–**c** on the viability of Caki-1 cells.

Conclusions and Outlook

A series of six new *p*-methoxybenzyl-substituted and benzyl-substituted N-heterocyclic carbene silver acetate derivatives **4a**–**c** and **7a**–**c** were synthesised through the reaction of appropriately *p*-methoxybenzyl-substituted and benzyl-substituted N-heterocyclic carbenes **3a**–**c** and **6a**–**c** with silver acetate. Almost all the complexes have shown high antibacterial activity compared to the precursors and it is also clear that, as the precursors and complexes concentration increases, the antibacterial activity becomes higher. Complexes **4a**–**c** and **7a**–**c** yielded antitumor IC_{50} values of 7.3, 12.7, 25.2, 2.5, 10.8 and 12.5 μM, respectively, on the Caki-1 cell line. The complex **7a** however gave a superior IC_{50} value of 2.5 μM. Further work is currently underway in order to improve these values by performing formulation experiments to improve solubility of these NHC–silver acetate complexes, which should allow for in vivo testing of **7a** in the nearby future.

Experimental Section

General: If not indicated otherwise, all manipulations were carried out without taking precautions to exclude air and moisture. All solvents were used as received. 1*H*-Imidazole, 4,5-dichloro-1*H*-imidazole, 1*H*-benzimidazole, *p*-methoxybenzyl bromide, benzyl bromide, silver acetate 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 **3a**–**c**, **4a**–**c**, **6a**–**c** and **7a**–**c**. CHN analysis was done with an Exeter Analytical CE-440 Elemental Analyser. Ag was estimated by spectrophotometric method (Atomic absorption spectra 55B Varian), while Cl and Br were determined in mercurimetric titrations. X-ray diffraction data for compounds **3c**, **4c** and **7a**–**c** were collected using Mo-K_α radiation and a Bruker SMART APEX CCD area detector diffractometer. A full sphere of reciprocal space was scanned by phi-omega scans. Pseudo-empirical absorption correction based on redundant reflections was performed by the program SADABS.[27] The structures were solved by direct methods using SHELXS-97[28] and refined by full-matrix least-squares on F^2 for all data using SHELXL-97.[28] Hydrogen atoms involved in hydrogen bonding were located in the difference Fourier map and allowed to refine freely. In **4c** the O–H bond lengths in the water molecules were restrained to be 0.84 Å, and the thermal displacement parameters of the water protons were fixed to be 1.5 times the equivalent thermal displacement parameter of the oxygen atom. All other hydrogen atoms were added at calculated positions and refined using a riding model. Their isotropic temperature factors were fixed to 1.2 times (1.5 times for methyl groups) the equivalent isotropic displacement parameters of the parent carbon atom. Anisotropic thermal displacement parameters were used for all non-hydrogen atoms. Suitable crystals of **3c**, **4c** and **7a**–**c** for X-ray studies were grown from the slow evaporation of acetone, CH_2Cl_2 and methanol solutions respectively at room temperature.

CCDC-742733 (for **3c**), -742734 (for **4c**), -742735 (for **7a**), -742736 (for **7b**) and -742737 (**7c**) respectively, contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Syntheses: The synthesis of 1,3-dibenzyl-4,5-dichloroimidazolium bromide (**6b**) was carried out accordingly to literature procedure[13] where as the syntheses of 1,3-dibenzylimidazolium bromide (**6a**) and 1,3-dibenzylbenzimidazolium bromide (**6c**) were carried out according to our new procedure instead of literature procedures.[10–12]

1,3-Bis(4-methoxybenzyl)imidazolium Bromide (3a): 1*H*-Imidazole (0.79 g, 11.68 mmol) and K_2CO_3 (2.42 g, 17.52 mmol) were stirred for 15 min in 60 mL of dry acetonitrile. *p*-Methoxybenzyl bromide (3.40 mL, 23.36 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 140 mL of water were added. The aqueous phase was extracted with CH_2Cl_2 (4 × 40 mL). Organic phases were combined and dried with magnesium sulfate. The solvent was evaporated off under reduced pressure. The crude product was washed first with pentane and then with ether. The resulted white solid dried under vacuum to yield (1.85 g, 4.75 mmol, 40.9% yield) **3a**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 10.77 (s, 1 H, NCHN), 7.42 (d, J = 8.6 Hz, 4 H, CH_{Benzyl}), 7.14 (d, J = 1.5 Hz, 2 H, CH_{Imid}), 6.90 (d, J = 8.7 Hz, 4 H, CH_{Benzyl}), 5.47 (s, 4 H, CH_2), 3.79 (s, 6 H, OCH_3) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, proton-decoupled): δ = 159.4, 135.9, 129.7, 123.5, 120.3, 113.8 (NCN, C_{Imid}, C_{Benzyl}), 54.3 (OCH_3), 52.0 (CH_2) ppm. IR absorptions (KBr): $\tilde{\nu}$ = 3100 (w), 3080 (w), 2998 (m), 2969 (w), 2934 (w), 2838 (m), 1612 (m), 1558 (m), 1515 (s), 1463 (m), 1439 (w), 1305 (m), 1253 (s), 1183 (m), 1151 (s), 1034 (m), 860 (w), 821 (w) cm^{-1}. UV/Vis (CH_3OH): λ (ε) = 204 (22507), 228 (23746), 274 (3654), 281 (3482) nm. MS (*m*/*z*, QMS-MS/MS): 309.42 [M$^+$ – Br]. $C_{19}H_{21}BrN_2O_2$ (389.29): calcd. C 58.62, H 5.44, N 7.20, Br 20.53; found C 57.94, H 5.42, N 7.03, Br 19.82.

4,5-Dichloro-1,3-bis(4-methoxybenzyl)imidazolium Bromide (3b): 4,5-Dichloro-1*H*-imidazole (1.59 g, 11.68 mmol) and K_2CO_3 (2.42 g, 17.52 mmol) were stirred for 15 min in 60 mL of dry acetonitrile. *p*-Methoxybenzyl bromide (3.40 mL, 23.36 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 140 mL of water were added. The aqueous phase was extracted with CH_2Cl_2 (4 × 40 mL). Organic phases were combined and dried with magnesium sulfate. The solvent was evaporated off under reduced pressure. The resulted yellow resude was saturated with diethyl ether (10 mL) to get a fine yellow solid (2.56 g, 5.58 mmol, 47.8% yield) **3b**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 11.77 (s, 1 H, NCHN), 7.55 (d, J = 8.5 Hz, 4 H, CH_{Benzyl}), 6.91 (d, J = 8.5 Hz, 4 H, CH_{Benzyl}), 5.53 (s, 4 H, CH_2), 3.79 (s, 6 H, OCH_3) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, proton-decoupled): δ = 160.6, 130.8, 129.0, 123.4, 118.9, 114.8 (NCN,CCl,C_{Benzyl}), 55.3 (OCH_3), 52.2 (CH_2) ppm. IR absorptions (KBr): $\tilde{\nu}$ = 3020 (m), 2965 (w), 2940 (w), 2861 (w), 1614 (m), 1584 (m), 1546 (m), 1518 (s), 1458 (m), 1427 (m), 1403 (w), 1338 (m), 1308 (m), 1294 (m), 1255 (s), 1179 (m), 1143 (m), 1029 (m), 942 (w), 815 (m) cm^{-1}. UV/Vis (CH_3OH): λ (ε) = 202 (23016), 228 (17075), 272 (2381), 283 (1891) nm. MS (*m*/*z*, QMS-MS/MS): 378.28 [M^+ – Br]. $C_{19}H_{19}BrCl_2N_2O_2$ (458.18): calcd. C 49.81, H 4.18, N 6.11, Br 17.44, Cl 15.48; found C 51.28, H 4.25, N 6.25, Br 16.89, Cl 16.85.

1,3-Bis(4-methoxybenzyl)benzimidazolium Bromide (3c): 1*H*-Benzimidazole (1.37 g, 11.68 mmol) and K_2CO_3 (2.42 g, 17.52 mmol) were stirred for 15 min in 60 mL of dry acetonitrile. *p*-Methoxybenzyl bromide (3.40 mL, 23.36 mmol) was added in one portion and stirring was continued at room temperature for further 3 d. After the solvent was removed under reduced pressure 150 mL of water were added. The precipitate was filtered, washed with diethyl ether (3 × 20 mL) and dried in suction at room temperature for 4 h. A fine white powder was obtained and recrystallised from acetone to give white crystalline product (2.25 g, 4.91 mmol, 42.9% yield) **3c**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 11.85 (s, 1 H, NCHN), 7.60–7.57 (m, 2 H, $CH_{Benzimid}$), 7.51–7.49 (m, 6 H, $CH_{Benzimid}$, CH_{Benzyl}), 6.89 (d, J = 8.4 Hz, 4 H, CH_{Benzyl}), 5.78 (s, 4 H, CH_2), 3.78 (s, 6 H, OCH_3) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, proton-decoupled): δ = 159.2, 141.8, 130.32, 129.0, 126.0, 123.4, 113.7, 112.7 (NCN, $C_{Benzimid}$, C_{Benzyl}), 54.3 (OCH_3), 50.1 (CH_2) ppm. IR absorptions (KBr): $\tilde{\nu}$ = 3114 (w), 3059 (w), 2966 (w), 2834 (w), 1611 (m), 1564 (s), 1513 (s), 1477 (w), 1441 (m), 1401 (m), 1342 (w), 1303 (m), 1253 (s), 1176 (m), 1027 (m), 924 (w), 822 (m), 799 (m), 743 (m), 709 (w) cm^{-1}. UV/Vis (CH_3OH): λ (ε) = 202 (23982), 226 (13108), 276 (4639), 278 (4554) ppm. MS (*m*/*z*, QMS-MS/MS): 359.90 [M^+ – Br – H_2O]. $C_{23}H_{25}BrN_2O_3$ (457.37): calcd. C 60.40, H 5.51, N 6.13, Br 17.47; found C 61.89, H 5.30, N 6.08, Br 17.71.

[1,3-Bis(4-methoxybenzyl)imidazol-2-ylidene]silver(I) Acetate (4a): 1,3-Bis(4-methoxybenzyl)imidazolium bromide (0.77 g, 2.0 mmol) was dissolved in CH_2Cl_2 (60 mL) and silver acetate (0.66 g, 4 mmol) was added. The mixture was stirred at room temperature for 2 d. Light was excluded by covering the flask with aluminium foil. The yellow precipitate of AgBr was filtered and discarded. The volume of the reaction mixture was reduced under reduced pressure to 5 mL. Hexane (30 mL) was added and kept in refrigerator for one week. The fine white precipitate was filtered and washed with 20 mL of hexane and dried under reduced pressure to yield a off white solid (0.83 g, 1.74 mmol, 87.4% yield) **4a**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.24 (d, J = 8.6 Hz, 4 H, CH_{Benzyl}), 6.91–6.85 (m, 6 H, CH_{Imid}, CH_{Benzyl}), 5.21 (s, 4 H, CH_2), 3.79 (s, 6 H, OCH_3), 2.08 (s, 3 H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, proton-decoupled): δ = 177.8 (NCN), 167.9 (C=O), 159.6, 129.5, 127.5, 121.0, 114.4 (C_{Imid}, C_{Benzyl}), 55.4 (OCH_3), 55.2 (CH_2), 22.6 (CH_3) ppm. IR absorptions (KBr): $\tilde{\nu}$ = 3123 (w), 3006 (w), 2963 (w), 2836 (m), 1611 (m), 1560 (s), 1514 (s), 1440 (w), 1410 (s), 1345 (w), 1305 (m), 1252 (s), 1178 (m), 1145 (m), 1030 (m), 962 (w), 925 (w), 823 (m), 769 (m) cm^{-1}. UV/Vis (CH_3OH): λ (ε) = 204 (25860), 229 (20349), 275 (2679), 282 (2341) nm. MS (*m*/*z*, QMS-MS/MS): 416.23 [M^+ – O_2CCH_3]. $C_{21}H_{23}AgN_2O_4$ (475.28): calcd. C 53.06, H 4.88, N 5.89, Ag 22.69; found C 52.63, H 4.92, N 5.60, Ag 19.28.

[4,5-Dichloro-1,3-bis(4-methoxybenzyl)imidazol-2-ylidene]silver(I) Acetate (4b): 4,5-Dichloro-1,3-bis(4-methoxybenzyl)imidazolium bromide (0.91 g, 2.0 mmol) was dissolved in CH_2Cl_2 (60 mL) and silver acetate (0.66 g, 4.0 mmol) was added. The mixture was stirred at room temperature for 2 d. Light was excluded by covering the flask by aluminium foil. The yellow silver bromide suspension was filtered to give a light yellow colored solution. The volatile components were removed in vacuo to produce a light yellow sticky solid. The sticky solid was washed with hexane (3 × 20 mL) and dried under reduced pressure for 4 h to yield a light yellow crystalline solid (0.79 g, 1.45 mmol, 72.7% yield) **4b**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.34 (d, J = 8.7 Hz, 4 H, CH_{Benzyl}), 6.88 (d, J = 8.7 Hz, 4 H, CH_{Benzyl}), 5.28 (s, 4 H, CH_2), 3.79 (s, 6 H, OCH_3), 2.07 (s, 3 H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, proton-decoupled): δ = 176.1 (NCN), 167.6 (C=O), 158.9, 128.5, 125.2, 116.7, 113.4 (CCl, C_{Benzyl}), 54.3 (OCH_3), 53.3 (CH_2), 21.0 (CH_3) ppm. IR absorptions (KBr): $\tilde{\nu}$ = 2999 (w), 2960 (w), 2935 (w), 2837 (w), 1611 (m), 1582 (m), 1513 (s), 1439 (m), 1406 (w), 1385 (w), 1332 (m), 1305 (m), 1253 (s), 1177 (m), 1114 (m), 1032 (m), 920 (w), 810 (m), 718 (w) cm^{-1}. UV/Vis (CH_3OH): λ (ε) = 204 (34418), 228 (30891), 276 (4252), 286 (2753) nm. MS (*m*/*z*, QMS-MS/MS): 485.24 [M^+ – O_2CCH_3]. $C_{21}H_{21}AgCl_2N_2O_4$ (544.21): calcd. C 46.34, H 3.89, N 5.14, Cl 13.02, Ag 19.82; found C 46.87, H 4.06, N 4.96, Cl 13.14, Ag 19.56.

[1,3-Bis(4-methoxybenzyl)benzimidazol-2-ylidene]silver(I) Acetate (4c): A mixture of 1,3-bis(4-methoxybenzyl)benzimidazolium bromide (0.91 g, 2.0 mmol) and silver acetate (0.66 g, 4.0 mmol) in CH_2Cl_2 (60 mL) was stirred at room temperature for 3 d. Light was excluded by covering the flask by Al foil. The reaction mixture was filtered to remove a yellow precipitate presumably AgBr and the volatile components were removed in vacuo to yield a white solid (0.86 g, 1.58 mmol, 79.6% yield) **4c**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.30–7.28 (m, 2 H, $CH_{Benzimid}$), 7.23–7.18 (m, 6 H, $CH_{Benzimid}$, CH_{Benzyl}), 6.77 (d, J = 8.4 Hz, 4 H, CH_{Benzyl}), 5.48 (s, 4 H, CH_2), 3.69 (s, 6 H, OCH_3), 2.03 (s, 3 H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, proton-decoupled): δ = 178.9 (NCN), 168.3 (C=O), 159.6, 133.9, 128.8, 127.0, 124.0, 114.4, 112.0 ($C_{Benzimid}$, C_{Benzyl}), 55.2 (OCH_3), 53.1 (CH_2), 22.7 (CH_3) ppm. IR absorptions (KBr): $\tilde{\nu}$ = 3448 (s), 2955 (m), 2834 (w), 1614 (m), 1557 (m), 1516 (s), 1464 (m), 1439 (w), 1384 (w), 1301 (m), 1259 (s), 1181 (m), 1114 (w), 1092 (w), 1029 (m), 914 (w), 879 (w), 809 (m), 754 (m), 701 (w) cm^{-1}. UV/Vis (CH_3OH): λ (ε) = 207 (28063), 223 (28049), 277 (11104), 285 (9306) nm. MS (*m*/*z*, QMS-MS/MS): 466.76 [M^+ – H_2O – O_2CCH_3]. $C_{25}H_{27}AgN_2O_5$ (543.36): calcd. C 55.25, H 5.01, N 5.15, Ag 19.85; found C 55.77, H 5.08, N 5.01, Ag 20.28.

1,3-Dibenzylimidazolium Bromide (6a): 1*H*-Imidazole (0.79 g, 11.7 mmol) and K_2CO_3 (2.42 g, 17.5 mmol) were stirred for 15 min in 60 mL of acetonitrile. Benzyl bromide (2.77 mL, 23.36 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 140 mL of water were added. The aqueous phase was extracted with CH_2Cl_2 (4 × 40 mL). Organic phases were combined and dried with magnesium sulfate. The solvent was evaporated off under reduced pressure. The white solid was washed with diethyl ether to remove unreacted 1-benzylimidazole and dried in

vacuo to yield **6a** (3.50 g, 10.62 mmol, 91.0% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 10.79 (s, 1 H, NCHN), 7.49–7.45 (m, 4 H, CH_{Benzyl}), 7.38–7.35 (m, 6 H, CH_{Benzyl}), 7.26 (d, J = 1.5 Hz, 2 H, CH_{Imid}), 5.56 (s, 4 H, CH_2) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, proton-decoupled): δ = 137.0, 132.7, 129.5, 129.4, 129.0, 121.8 (NCN, C_{Imid}, C_{Benzyl}), 53.4 (CH_2) ppm. IR absorptions (KBr): $\tilde{\nu}$ = 3453 (s), 3124 (w), 3068 (m), 2929 (w), 2847 (w), 1628 (w), 1559 (s), 1496 (m), 1456 (s), 1383 (w), 1261 (w), 1208 (w), 1150 (s), 1078 (w), 1029 (w), 874 (w), 822 (w), 720 (s) cm^{-1}. UV/Vis (CH_3OH): λ (ε) = 204 (20788), 268 (14373) nm. MS (*m*/*z*, QMS-MS/MS): 249.47 [M^+ – Br]. $C_{17}H_{17}BrN_2$ (329.26): calcd. C 62.02, H 5.20, N 8.51, Br 24.26; found C 62.25, H 5.29, N 9.16, Br 22.21.

4,5-Dichloro-1-benzylimidazole (5′): 4,5-Dichloro-1*H*-imidazole (0.80 g, 5.84 mmol) and K_2CO_3 (1.21 g, 8.76 mmol) were stirred for 10 min in 30 mL of acetonitrile. Benzyl bromide (0.69 mL, 5.84 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 70 mL of water were added. The aqueous phase was extracted with CH_2Cl_2 (4 × 20 mL). Organic phases were combined and dried with sodium sulfate. **5′** was obtained as orange solid after solvent removal under reduced pressure (1.24 g, 5.46 mmol, 93.0% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.40–7.33 (m, 4 H, NCHN, CH_{Benzyl}), 7.19–7.17 (m, 2 H, CH_{Benzyl}), 5.08 (s, 2 H, CH_2) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, proton-decoupled): δ = 134.4, 134.3, 129.1, 128.6, 127.3, 126.4, 113.8 (NCN, C_{Benzyl}, CCl), 49.7 (CH_2) ppm. IR absorptions (KBr): $\tilde{\nu}$ = 3122 (m), 3032 (w), 2962 (w), 1519 (s), 1496 (w), 1484 (s), 1456 (m), 1437 (s), 1389 (m), 1346 (m), 1254 (s), 1180 (m), 1115 (m), 976 (m), 807 (s), 722 (s) cm^{-1}. UV/Vis (CH_3OH): λ (ε) = 208 (9035), 227 (3280), 258 (413), 260 (399), 269 (352) nm. MS (*m*/*z*, QMS-MS/MS): 227.37 [M + H^+]. $C_{10}H_8Cl_2N_2$ (226.01): calcd. C 52.89, H 3.55, N 12.34, Cl 31.22; found C 51.40, H 3.83, N 11.36, Cl 30.79.

1,3-Dibenzyl-4,5-dichloroimidazolium Bromide (6b): Benzyl bromide (0.79 mL, 6.6 mmol) was added in one portion to a stirred suspension of 1-benzyl-4,5-dichloroimidazole (0.50 g, 2.2 mmol) in 5 mL of toluene. The mixture was stirred for 40 h at room temperature. Afterwards the solvent was removed under reduced pressure. The residue was washed with THF (4 × 5 mL) and the title compound **6b** was isolated as a white solid (0.609 g, 1.52 mmol, 69.0% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 11.79 (s, 1 H, NCHN), 7.57–7.55 (m, 4 H, CH_{Benzyl}), 7.41–7.34 (m, 6 H, CH_{Benzyl}), 5.66 (s, 4 H, CH_2) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, proton-decoupled): δ = 137.8, 131.5, 129.6, 129.4, 128.9, 119.3 (NCN, C_{Benzyl}, CCl), 52.7 (CH_2) ppm. IR absorptions (KBr): $\tilde{\nu}$ = 3098 (m), 3012 (s), 2861 (m), 1585 (s), 1550 (s), 1498 (s), 1456 (s), 1405 (m), 1343 (s), 1305 (m), 1205 (s), 1180 (s), 1146 (s), 1077 (m), 1031 (m), 901 (w), 768 (s), 731 (s), 710 (s) cm^{-1}. UV/Vis (CH_3OH): λ (ε) = 204 (24368), 261 (428), 268 (234) nm. MS (*m*/*z*, QMS-MS/MS): 318.22 [M^+ – Br]. $C_{17}H_{15}BrCl_2N_2$ (398.12): calcd. C 51.29, H 3.80, N 7.04, Br 20.07, Cl 17.81; found C 51.10, H 3.68, N 6.95, Br 20.54, Cl 18.03.

1,3-Dibenzylbenzimidazolium Bromide (6c): 1*H*-Benzimidazole (1.37 g, 11.68 mmol) and K_2CO_3 (2.42 g, 17.52 mmol) were stirred for 15 min in 60 mL of acetonitrile. Benzyl bromide (2.77 mL, 23.36 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 150 mL of water were added. The white precipitate was filtered, washed with diethyl ether (2 × 25 mL) to remove unreacted 1-benzylbenzimidazole and dried in suction at room temperature for 4 h to afforded the product (3.20 g, 8.43 mmol, 72.2% yield) **6c**. ^{1}H NMR ([D_6]DMSO, 400 MHz): δ = 10.12 (s, 1 H, NCHN), 7.97–7.93 (m, 2 H, $CH_{Benzimid}$), 7.63–7.59 (m, 2 H, $CH_{Benzimid}$), 7.53–7.51 (m, 4 H, CH_{Benzyl}), 7.43–7.34 (m, 6 H, CH_{Benzyl}), 5.79 (s, 4 H, CH_2) ppm. ^{13}C NMR ([D_6]DMSO, 125 MHz, proton-decoupled): δ = 143.1, 134.3, 131.5, 129.4, 129.2, 128.7, 127.2, 114.4 (NCN, $C_{Benzimid}$, C_{Benzyl}), 50.4 (CH_2) ppm. IR absorptions (KBr): $\tilde{\nu}$ = 3452 (s), 3123 (w), 3057 (m), 2924 (m), 2853 (w), 1605 (m), 1558 (s), 1476 (m), 1457 (s), 1427 (s), 1373 (s), 1337 (w), 1190 (m), 1081 (w), 1016 (m), 913 (w), 822 (w), 756 (s), 739 (m), 703 (s) cm^{-1}. UV/Vis (CH_3OH): λ (ε) = 207 (26158), 256 (6902), 262 (7042), 270 (7469), 278 (6021) nm. MS (*m*/*z*, QMS-MS/MS): 299.49 [M^+ – Br]. $C_{21}H_{19}BrN_2$ (379.29): calcd. C 66.50, H 5.05, N 7.39, Br 21.07; found C 65.92, H 5.03, N 6.79, Br 19.74.

(1,3-Dibenzylimidazol-2-ylidene)silver(I) Acetate (7a): 1,3-Dibenzylimidazolium bromide (0.329 g, 1.0 mmol) was dissolved in CH_2Cl_2 (50 mL) and silver acetate (0.333 g, 2.0 mmol) was added. The mixture was stirred at room temperature in the absence of light for 1 d. The yellow silver bromide suspension was filtered to give a colorless solution. The volume of the reaction was concentrated in vacuo. Addition of diethyl ether gave an off-white precipitate which was isolated by filtration, washed with diethyl ether and dried in vacuo to yield **7a** (0.300 g, 0.72 mmol, 72.2% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.39–7.27 (m, 10 H, CH_{Benzyl}), 6.91 (s, 2 H, CH_{Imid}), 5.30 (s, 4 H, CH_2), 2.08 (s, 3 H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz, proton-decoupled): δ = 177.0 (NCN), 166.6 (C=O), 134.4, 128.0, 127.6, 126.2, 120.3 (C_{Imid}, C_{Benzyl}), 54.9 (CH_2), 21.7 (CH_3) ppm. IR absorptions (KBr): $\tilde{\nu}$ = 3428 (s), 3092 (w), 3030 (w), 2965 (w), 2930 (w), 1637 (w), 1560 (s), 1496 (m), 1454 (m), 1413 (s), 1350 (w), 1262 (w), 1229 (w), 1150 (m), 1078 (m), 924 (w), 880 (w), 806 (w), 728 (s), 709 (w) cm^{-1}. UV/Vis (CH_3OH): λ (ε) = 205 (27199), 209 (27265), 258 (3043), 269 (2988) nm. MS (*m*/*z*, QMS-MS/MS): 356.22 [M^+ – O_2CCH_3]. $C_{19}H_{19}AgN_2O_2$ (415.27): calcd. C 54.95, H 4.61, N 6.74, Ag 25.97; found C 54.51, H 4.68, N 6.52, Ag 26.20.

(1,3-Dibenzyl-4,5-dichloroimidazol-2-ylidene)silver(I) Acetate (7b): 1,3-Dibenzyl-4,5-dichloroimidazolium bromide (0.199 g, 0.5 mmol) was dissolved in CH_2Cl_2 (25 mL) and silver acetate (0.166 g, 1.0 mmol) was added. The mixture was stirred at room temperature in the dark for 1 d. The yellow silver bromide suspension was filtered to give a colorless solution. The volatile components were removed in vacuo to produce off white sticky solid. The solid was first washed with hexane and then with diethyl ether and dried under reduced pressure for 2 h to yield (0.152 g, 0.31 mmol, 62.7% yield) **7b**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.39–7.32 (m, 10 H, CH_{Benzyl}), 5.38 (s, 4 H, CH_2), 2.06 (s, 3 H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, proton-decoupled): δ = 177.5 (NCN), 168.6 (C=O), 134.1, 129.1, 128.8, 127.7, 118.0 (C_{Benzyl}, CCl), 54.8 (CH_2), 22.0 (CH_3) ppm. IR absorptions (KBr): $\tilde{\nu}$ = 3424 (s), 3063 (w), 3029 (w), 2960 (w), 2926 (w), 2855 (w), 1556 (s), 1496 (m), 1454 (w), 1411 (s), 1345 (m), 1260 (m), 1207 (m), 1078 (w), 1028 (w), 928 (w), 804 (w), 744 (w), 714 (w) cm^{-1}. UV/Vis (CH_3OH): λ (ε) = 207 (29415), 246 (9196), 286 (2907) nm. MS (*m*/*z*, QMS-MS/MS): 425.23 [M^+ – O_2CCH_3]. $C_{19}H_{17}AgCl_2N_2O_2$ (484.15): calcd. C 47.13, H 3.54, N 5.78, Cl 14.64, Ag 22.28; found C 46.26, H 3.61, N 5.66, Cl 14.50, Ag 21.54.

(1,3-Dibenzylbenzimidazol-2-ylidene)silver(I) Acetate (7c): 1,3-Dibenzylbenzimidazolium bromide (0.189 g, 0.50 mmol) was dissolved in CH_2Cl_2 (25 mL) and silver acetate (0.166 g, 1.0 mmol) was added. The mixture was stirred at room temperature in the dark for 1 d. The yellow silver bromide suspension was filtered to give a colorless solution. The volatile components were removed in vacuo to produce off-white sticky solid. The solid was washed with hexane and dried under reduced pressure for 1 h to yield (0.153 g, 0.32 mmol, 65.7% yield) **7c**. ^{1}H NMR ([D_6]DMSO, 400 MHz): δ

= 7.69–7.65 (m, 2 H, $CH_{Benzimid}$), 7.41 (d, J = 7.0 Hz, 4 H, $CH_{Benzimid}$, CH_{Benzyl}), 7.35–7.24 (m, 8 H, CH_{Benzyl}), 5.73 (s, 4 H, CH_2), 1.79 (s, 3 H, CH_3) ppm. ^{13}C NMR ([D_6]DMSO, 100 MHz, proton-decoupled): δ = 175.7 (NCN), 166.1 (C=O), 136.6, 133.8, 129.2, 128.4, 127.9, 124.4, 112.8 ($C_{Benzimid}$, C_{Benzyl}), 52.4 (CH_2), 23.7 (CH_3) ppm. IR absorptions (KBr): $\tilde{\nu}$ = 3434 (s), 3063 (w), 2925 (w), 1565 (s), 1496 (m), 1477 (m), 1401 (s), 1261 (w), 1185 (w), 1096 (w), 1078 (w), 1026 (m), 925 (w), 821 (w), 746 (s), 705 (m) cm^{-1}. UV/Vis (CH_3OH): λ (ε) = 207 (30176), 247 (5368), 276 (7706), 285 (7598). MS (*m/z*, QMS-MS/MS): 406.28 [M^+ – O_2CCH_3]. $C_{23}H_{21}AgN_2O_2$ (465.33): calcd. C 59.36, H 4.55, N 6.02, Ag 23.18; found C 59.19, H 4.58, N 5.91, Ag 23.21.

Antibacterial Studies: Preliminary antibacterial activity of *p*-methoxybenzyl-substituted and benzyl-substituted N-heterocyclic carbenes and their corresponding silver complexes were screened simultaneously with silver acetate against two bacterial strains. The test organisms included *Staphylococcus aureus* (SA) (NCTC 7447) as a gram-positive bacteria and *Escherichia coli* as gram-negative bacteria.

To assess the biological activity of compounds **3a**–**c**, **4a**–**c**, **6a**–**c**, **7a**–**c** and silver acetate, the Kirby–Bauer disk-diffusion method was applied.[29] All bacteria were individually cultured from a single colony in sterile LB medium[30] overnight at 37 °C (orbital shaker incubator). All the work carried out was performed under sterile conditions.

For each strain, 70 μL of culture were spread evenly on agar-LB medium. Four 5 mm diameter paper discs were placed evenly separated on each plate. Two stock solutions (90:10 DMSO:H_2O) of every compound was prepared at 9.2 μM and 18.4 μM to be able to test the effect of different concentrations. Each plate was then tested with 5 μL and 7 μL of a 9.2 μM solution and 5 μL and 10 μL for the 18.4 μM solution. The plates were covered and placed in an incubator at 37 °C for 24 h. The plates were then removed and the zone of clearance (defined as the diameter of inhibited bacterial growth around the filter paper) 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. Then the compounds used for the testing were dissolved in the minimal amount of DMSO (dimethyl sulfoxide) possible and diluted with medium to obtain stock solutions of 5×10^{-4} M in concentration and less than 0.7% of DMSO. The cells were then treated with varying concentrations of the compounds and incubated for 48 h at 37 °C. Then, the solutions were removed from the wells and the cells were washed with PBS (phosphate buffer solution) and fresh medium was added to the wells. Following a recovery period of 24 h incubation at 37 °C, individual wells were treated with a 200 μL of a solution of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] in medium. The solution consisted of 30 mg of MTT in 30 mL of medium. The cells were incubated for 3 h at 37 °C. The medium was then removed and the purple formazan crystals were dissolved in 200 μL DMSO per well. A Wallac Victor (Multilabel HTS Counter) Plate Reader was used to measure absorbance at 540 nm. Cell viability was expressed as a percentage of the absorbance recorded for control wells. The values used for the dose response curves represent the values obtained from four consistent MTT-based assays for each compound tested.

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