Synthesis, Cytotoxicity and Antibacterial Studies of Novel Symmetrically and Nonsymmetrically 4-(Methoxycarbonyl)benzyl-Substituted *N*-Heterocyclic Carbene – Silver Acetate Complexes

by **Siddappa Patil**, **Karolin Dietrich**, **Anthony Deally**, **Brendan Gleeson**, **Helge Müller-Bunz**, **Francesca Paradisi**, and **Matthias Tacke***

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 (e-mail: matthias.tacke@ucd.ie)

From the reaction of 1*H*-imidazole (**1a**), 4,5-dichloro-1*H*-imidazole (**1b**), 1*H*-benzimidazole (**1c**), 1-methyl-1*H*-imidazole (**1d**), and 1-methyl-1*H*-benzimidazole (**1f**) with methyl 4-(bromomethyl)benzoate (**2**), symmetrically and nonsymmetrically 4-(methoxycarbonyl)benzyl-substituted *N*-heterocyclic carbene (NHC) precursors, **3a**–**3f**, were synthesized. These NHC precursors were then reacted with silver(I) acetate (AgOAc) to yield the NHC – silver acetate complexes (acetato-κO){1,3-bis[4-(methoxycarbonyl)benzyl]imidazol-2-ylidene}silver (**4a**), (acetato-κO){4,5-dichloro-1,3-bis[4-(methoxycarbonyl)benzyl]-2,3-dihydro-1*H*-imidazol-2-yl}silver (**4b**), (acetato-κO){1,3-bis[4-(methoxycarbonyl)benzyl]-2,3-dihydro-1*H*-benzimidazol-2-yl}silver (**4c**), (acetato-κO){1-[4-(methoxycarbonyl)benzyl]-3-methyl-2,3-dihydro-1*H*-imidazol-2-yl}silver (**4d**), (acetato-κ*O*){4,5-dichloro-1-[4-(methoxycarbonyl)benzyl]-3-methyl-2,3-dihydro-1*H*-imidazol-2-yl}silver (**4e**), and (acetato-κO){1-[4-(methoxycarbonyl)benzyl]-3-methyl-2,3-dihydro-1*H*-benzimidazol-2-yl}silver (**4f**), respectively. The three NHC – AgOAc complexes **4a**, **4c**, and **4d** were characterized by single-crystal X-ray diffraction. All compounds studied in this work were preliminarily screened for their antimicrobial activities *in vitro* against *Gram*-positive bacteria *Staphylococcus aureus*, and *Gram*-negative bacteria *Escherichia coli* using the qualitative disk-diffusion method. All NHC – AgOAc complexes exhibited weak-to-medium antibacterial activity with areas of clearance ranging from 4 to 7 mm at the highest amount used, while the NHC precursors showed significantly lower activity. In addition, NHC – AgOAc complexes **4a** and **4b**, and **4d**–**4f** exhibited in preliminary cytotoxicity tests on the human renal-cancer cell line Caki-1 medium-to-high cytotoxicities with IC_{50} values ranging from 3.3 ± 0.4 to 68.3 ± 1 μM.

Introduction. – *N*-Heterocyclic carbenes (NHCs) were introduced to organometallic and inorganic chemistry by the synthesis and isolation of chromium and mercury NHC complexes by *Öfele* [1], and *Wanzlick* and *Schönherr* [2] in 1968. The subsequent synthesis and characterization of numerous carbene–metal complexes by *Lappert* and co-workers was a significant contribution to this area of chemistry [3]. NHCs have become an important area of research after the isolation of the first stable carbene by *Arduengo et al.* in 1991 [4]. In recent years, researchers have developed a variety of convenient routes to prepare NHC complexes of main group and transition metals [5][6]. Metal NHC complexes have been known particularly as catalysts [7–11] for various chemical transformations; later discoveries revealed that silver and gold derivatives of NHCs can be used in medicinal applications [12–15].

The antimicrobial activity of elemental silver and silver salts has been known for centuries [16][17]. In many ancient societies, drinking H_2O was purified and stored

using elemental silver [16]. The introduction of 2% $AgNO_3$ solution to prevent the bacterial eye infection, *ophthalmia neonatorum*, in newborn babies was reported in 1881 [18]. The use of silver has also been reported for a variety of medicinal applications such as for treatment of wounds and infections [19][20]. The introduction of silver sulfadiazine (silvadine) as an effective antimicrobial agent was reported by *Fox* in 1968 [21]. 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 [19][22–24]. Silver–carbene complexes, in particular those of NHCs, have attracted a significant amount of interest in the past few years [25][26]. The first use of silver NHCs as antimicrobial agents was reported by *Youngs* and co-workers in 2004 [12]. In this report, two Ag^I complexes (silver(I)–2,6-bis(ethanol-imidazolemethyl)pyridine hydroxide and silver(I)–2,6-bis(propanolimidazoleme-thyl)pyridine hydroxide) showed better antimicrobial activity than $AgNO_3$ against the microorganisms *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa.* Another important contribution by *Ghosh* and co-workers led to the synthesis and antimicrobial evaluation of NHC–silver complexes derived from 1-benzyl-3-(*tert*-butyl)-1*H*-imidazole [27]. Very recently, *Gürbüz* and co-workers have shown that the new imidazolidin-2-ylidene–silver complexes displayed effective antimicrobial activity against a series of bacteria and fungi [28].

In the chemotherapeutic treatment of cancer, the discovery of the cytotoxic activity of cisplatin by *Rosenberg et al.* induced a new research field of organometallic complexes as drug candidates [29]. Cisplatin was accepted as drug in 1978 and proved to be especially successful against testicular and bladder cancer, where the probability of healing increased by 90% [30]. The drug did not only successfully treat 30,000 patients per year but also satisfied the pharmaceutical industry with the highest turnover for a cytostatic drug in the US in 1983. Second-generation cytostatic drug carboplatin with less side effects was introduced to the UK market in 1990 [31]. In a simplified mechanistic explanation, the Pt complex binds to the DNA strings of the cancer cell and prohibits further DNA replication and, therefore, further tumor growth.

Soon, other transition metal complexes were considered as possible anticancer drugs. Especially treatments for cancer types that did not react to cisplatin treatment were required. Promising candidates were the titanium complexes titanocene dichloride [30], which showed *in vitro* activity against breast, lung, and intestinal carcinoma but failed in clinical trials phase II [32], and also its successor budotitane (*cis*-diethoxybis(1-phenylbutane-1,3-dionato)titanium(IV)), which also failed to go beyond phase I after excellent preclinical results [33]. Further substitution of the cyclopentadienyl ligand led to bis[(*p*-methoxybenzyl)cyclopentadienyl]titanium(IV) dichloride (Titanocene Y), which was more effective in the treatment of xenografted CAKI-1 tumors in mice than cisplatin [34].

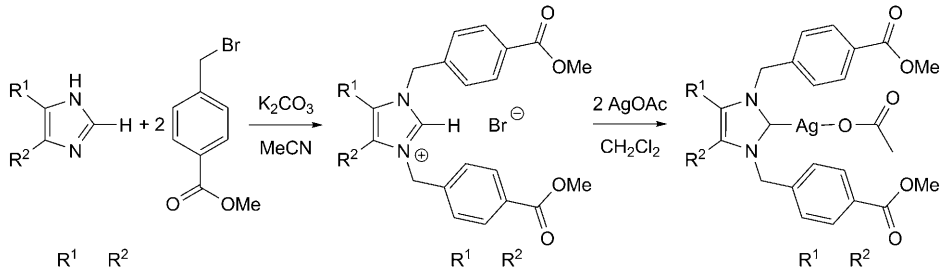
Recently, Ag complexes have been reported to have anticancer activity *in vitro*. *Egan* and co-workers have reported that Ag complexes of coumarin derivatives possess anticancer activity against certain types of cancer [35]. *Zhu et al.* has reported that silver carboxylate dimers possess anticancer activity against human carcinoma cells [36]. *McKeage* and co-workers have shown phosphine complexes of silver to be active anticancer agents, even against cisplatin-resistant cell lines [37]. *Youngs* and co-workers have reported anticancer activities of NHC–silver complexes derived from

4,5-dichloro-1*H*-imidazole against the human cancer cell lines OVCAR-3 (ovarian), MB157 (breast), and HeLa (cervical) [38]. These Ag complexes have been shown to be very stable and can be synthesized efficiently. We have recently reported the anticancer and antibacterial activities of symmetrically *p*-methoxybenzyl-substituted and benzyl-substituted NHC–silver complexes. All the reported NHC–silver complexes have shown medium-to-high anticancer and antibacterial activities [39]. This encourages further research on NHC–silver complexes as cytotoxic drug candidates.

Within this article, we present a new series of symmetrically and nonsymmetrically 4-(methoxycarbonyl)benzyl-substituted NHC–silver acetate complexes, their synthesis, cytotoxicity, and antibacterial studies.

Results and Discussion. – *Synthesis of* **3a**–**3f** *and* **4a**–**4f**. The synthetic route for symmetrically and nonsymmetrically 4-(methoxycarbonyl)benzyl-substituted NHCs as ligand precursors and their corresponding Ag complexes described in this work is given in *Schemes 1–3*. The symmetrically substituted NHC precursors 1,3-bis[4-(methoxycarbonyl)benzyl]-1*H*-imidazol-3-ium bromide (**3a**), 4,5-dichloro-1,3-bis[4-(methoxycarbonyl)benzyl]-1*H*-imidazol-3-ium bromide (**3b**), and 1,3-bis[4-(methoxycarbonyl)-

Scheme 1. *General Reaction Scheme for the Synthesis of Symmetrically Substituted* N-*Heterocyclic Carbenes* **3a**–**3c** *and Their Corresponding* N-*Heterocyclic Carbene–AgOAc Complexes* **4a**–**4c**



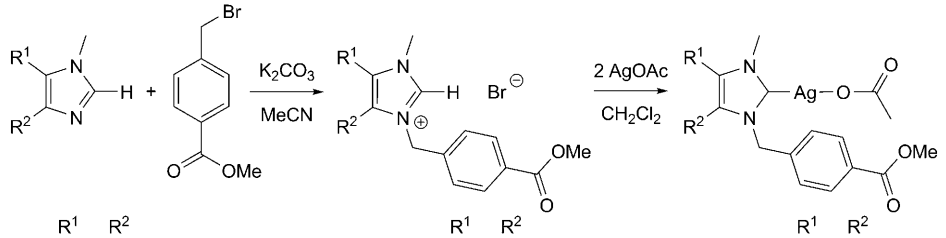
	R^1	R^2
1a	H	H
1b	Cl	Cl
1c	Benzo	

2

	R^1	R^2
3a	H	H
3b	Cl	Cl
3c	Benzo	

	R^1	R^2
4a	H	H
4b	Cl	Cl
4c	Benzo	

Scheme 2. *General Reaction Scheme for the Synthesis of Nonsymmetrically Substituted* N-*Heterocyclic Carbenes* **3d** *and* **3f** *and Their Corresponding* N-*Heterocyclic Carbene–AgOAc Complexes* **4d** *and* **4f**



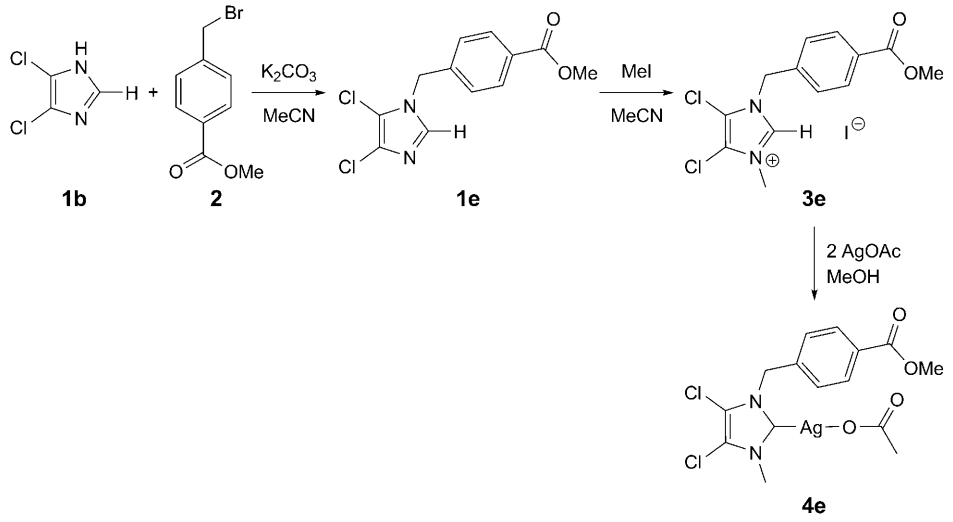
	R^1	R^2
1d	H	H
1f	Benzo	

2

	R^1	R^2
3d	H	H
3f	Benzo	

	R^1	R^2
4d	H	H
4f	Benzo	

Scheme 3. *General Reaction Scheme for the Synthesis of Nonsymmetrically Substituted* N*-Heterocyclic Carbene* **3e** *and Its* N*-Heterocyclic Carbene–AgOAc Complex* **4e**



benzyl]-1*H*-3,1-benzimidazol-3-ium bromide (**3c**) were prepared by stirring 1*H*-imidazole (**1a**), 4,5-dichloro-1*H*-imidazole (**1b**), and 1*H*-benzimidazole (**1c**) with 2 equiv. of methyl 4-(bromomethyl)benzoate (**2**) in the presence of K_2CO_3 as a base in MeCN at room temperature or refluxed for 3 d with 73, 19 and 44 yields, respectively (*Scheme 1*). The nonsymmetrically substituted NHC precursors 3-[4-(methoxycarbonyl)benzyl]-1-methyl-1*H*-imidazol-3-ium bromide (**3d**) and 3-[4-(methoxycarbonyl)-benzyl]-1-methyl-1*H*-3,1-benzimidazol-3-ium bromide (**3f**) were prepared by stirring 1-methyl-1*H*-imidazole (**1d**) and 1-methyl-1*H*-benzimidazole (**1f**) with methyl 4-(bromomethyl)benzoate (**2**) in toluene at room temperature for 3–4 d with 82 and 79% yields, respectively (*Scheme 2*). Methyl 4-[(4,5-dichloro-1*H*-imidazol-1-yl)methyl]benzoate (**1e**) was formed in 83% yield from the deprotonation of 4,5-dichloro-1*H*-imidazole (**1b**) with K_2CO_3 and subsequent alkylation with methyl 4-(bromomethyl)benzoate (**2**) in MeCN (*Scheme 3*). 4,5-Dichloro-1-[4-(methoxycarbonyl)benzyl]-3-methyl-1*H*-imidazol-3-ium iodide (**3e**) was prepared by heating **1e** with MeI in MeCN for 1 d with a yield of 65%.

The NHC precursors were fully characterized by UV, IR, and ^{1}H- and ^{13}C-NMR spectroscopy, mass spectrometry, as well as elemental analysis. The ^{1}H-NMR spectra of all precursors **3a**–**3f** show a characteristic downfield shift in the range of δ(H) 9.55–12.01 for H–C(2) attributable to the positive charge of the molecule [40][41]. Additionally, their identities have also been confirmed by a base peak for the $[M - Br]^+$ fragments in ESI mass spectra.

The NHC–AgOAc complexes (acetato-κO){1,3-bis[4-(methoxycarbonyl)benzyl]-2,3-dihydro-1*H*-imidazol-2-yl}silver (**4a**), (acetato-κO){4,5-dichloro-1,3-bis[4-(methoxycarbonyl)benzyl]-2,3-dihydro-1*H*-imidazol-2-yl}silver (**4b**), (acetato-κO){1,3-bis[4-(methoxycarbonyl)benzyl]-2,3-dihydro-1*H*-benzimidazol-2-yl}silver (**4c**), (acetato-κO){1-[4-(methoxycarbonyl)benzyl]-3-methyl-2,3-dihydro-1*H*-imidazol-2-yl}silver

(**4d**), (acetato-κO){4,5-dichloro-1-[4-(methoxycarbonyl)benzyl]-3-methyl-2,3-dihydro-1*H*-imidazol-2-yl}silver (**4e**), and (acetato-κO){1-[4-(methoxycarbonyl)benzyl]-3-methyl-2,3-dihydro-1*H*-benzimidazol-2-yl}silver (**4f**) were synthesized by the reaction of **3a**–**3f** with 2 equiv. of AgOAc in CH_2Cl_2. The mixture was stirred for 2–4 d at room temperature or refluxed for 2–4 d to afford the NHC–AgOAc complexes as off white solids in 58–89% yield. The complexes were fully characterized by UV/VIS, IR, and ^{1}H- and ^{13}C-NMR spectroscopy, mass spectrometry, as well as elemental analysis. Furthermore, the solid-state structures of **4a**, **4c**, and **4d** were analyzed by single crystal X-ray diffraction. The absence of a downfield shift for H–C(2) signal and presence of new signals at 3.89–1.99 ppm for the acetate H-atoms in all the ^{1}H-NMR spectra for **4a**–**4f**, however, indicates a successful complex formation. The ^{13}C-NMR resonances of the carbene C-atoms in complexes **4a**–**4f** appear in the range of δ(C) 182.4–179.9. These signals are shifted downfield compared to those of the corresponding precursors **3a**–**3f** (δ(C) 137.8–131.2), which further evidences the formation of expected NHC–AgOAc complexes. Also the appearance of the ^{13}C-NMR resonances for the CO and Me C-atoms of the AcO group of complexes **4a**–**4f** in the range of δ(C) 179.4–177.3 and 22.9–15.2, respectively, indicated the formation of the NHC–AgOAc complexes [42][43]. Furthermore, the ESI mass spectra of **4a**–**4f** are dominated by $[M - AcO]^+$ fragment peaks arising from the loss of one AcO ligand.

Structural Discussion. The crystal structures of the NHC–AgOAc complexes **4a**, **4c**, and **4d** have been determined. The molecular structures of **4a**, **4c**, and **4d** are shown in *Figs. 1–4*. The crystal data and refinement details for all three complexes are listed in *Table 1*, whereas selected bond lengths and bond angles are compiled in *Table 2*. Suitable crystals for X-ray crystallography to determine the molecular structure of **4a** and **4c** were grown from the slow evaporation of a saturated MeOH solution, while crystals of **4d** were formed in a saturated CH_2Cl_2 solution with slow infusion of pentane. The complexes **4a**, **4c**, and **4d** crystallized in the monoclinic space group *C*2/*c* (#15), *Cc* (#9), and $P2_1$ (#4), respectively. In **4a**, a H_2O molecule placed on the twofold axis links two complexes by H-bonding, forming isolated moieties of $(\mathbf{4a})_2 \times H_2O$. The crystals of the complexes **4c** and **4d** contain no solvent.

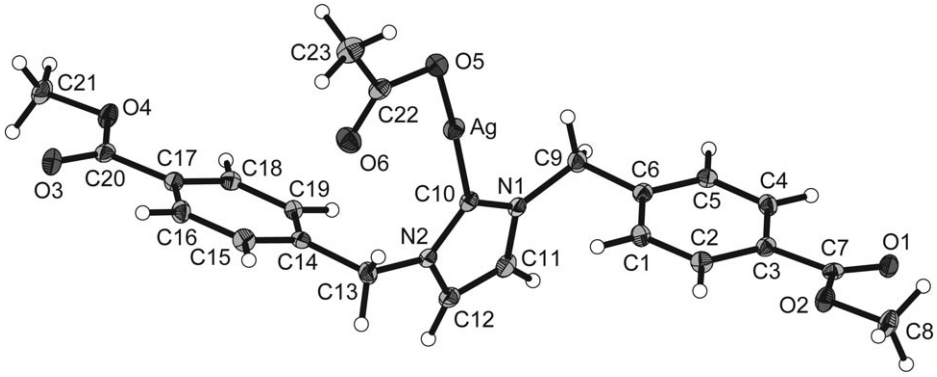


Fig. 1. *X-Ray diffraction structure of* **4a** (thermal ellipsoids are drawn at the 50% probability level)

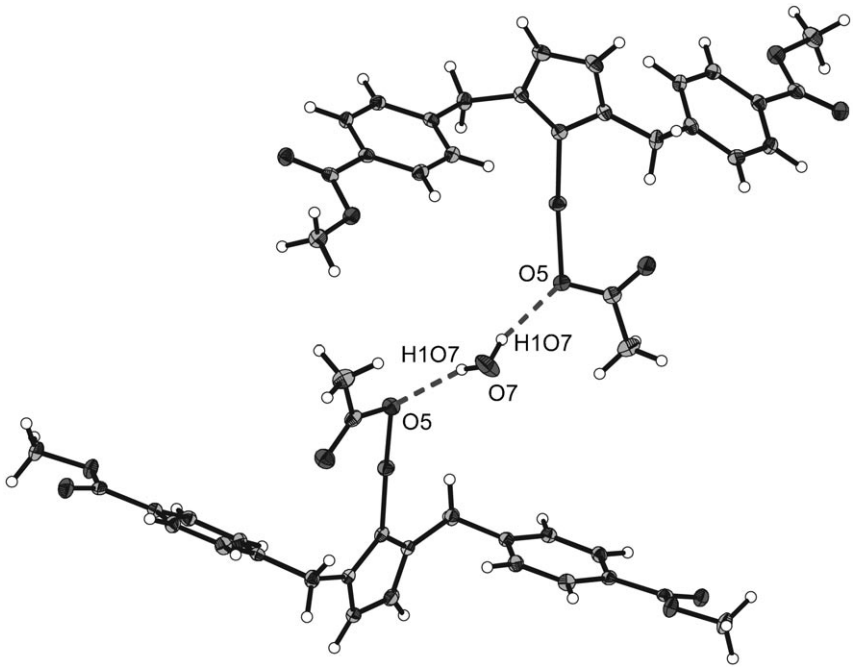


Fig. 2. *H-Bonded moiety of* **4a** (thermal ellipsoids are drawn at the 50% probability level)

The NHC–AgOAc complexes **4a**, **4c**, and **4d** are mononuclear complexes. In the complexes **4a**, **4c**, and **4d** reported here, the bond lengths and angles in and directly around the NHC core agree very well among each other and with literature data [39][44]. In the X-ray structure of **4a**, **4c**, and **4d**, the AgOAc moiety acts as a monodentate ligand. In **4a**, **4c**, and **4d**, Ag is two-coordinated in an approximately linear fashion.

Biological Evaluation. Symmetrically and nonsymmetrically 4-(methoxycarbonyl)-benzyl-substituted NHCs and their corresponding Ag complexes were preliminarily screened for their antibacterial activities *in vitro* against the selected *Gram*-positive bacteria *Staphylococcus aureus* (NCTC 7447) and *Gram*-negative bacteria *Escherichia coli* using the qualitative disk-diffusion method. The results of the antibacterial activities of NHC precursors and their corresponding NHC–AgOAc complexes are given in *Tables 3* and *4*, respectively. The metal salt (AgOAc) used to prepare the complexes and the solvent (DMSO) played no role in growth inhibition on the same bacteria as previously reported [39][45].

Almost no antibacterial activity was observed for NHC precursors **3a**–**3f** against both *Gram*-positive bacteria *Staphylococcus aureus* and *Gram*-negative bacteria *Escherichia coli.* The NHC–AgOAc complexes **4a**–**4c** were less soluble in DMSO (1.0–1.2 mg in 100 µl instead of 2.1–2.5 mg in 100 µl) and, therefore, showed minimal antibacterial activity towards both *Gram*-positive bacteria *Staphylococcus aureus* and *Gram*-negative bacteria *Escherichia coli.* Weak antibacterial activity was observed for NHC–AgOAc complexes **4d**–**4f** against *Gram*-negative bacteria *Escherichia coli*, but

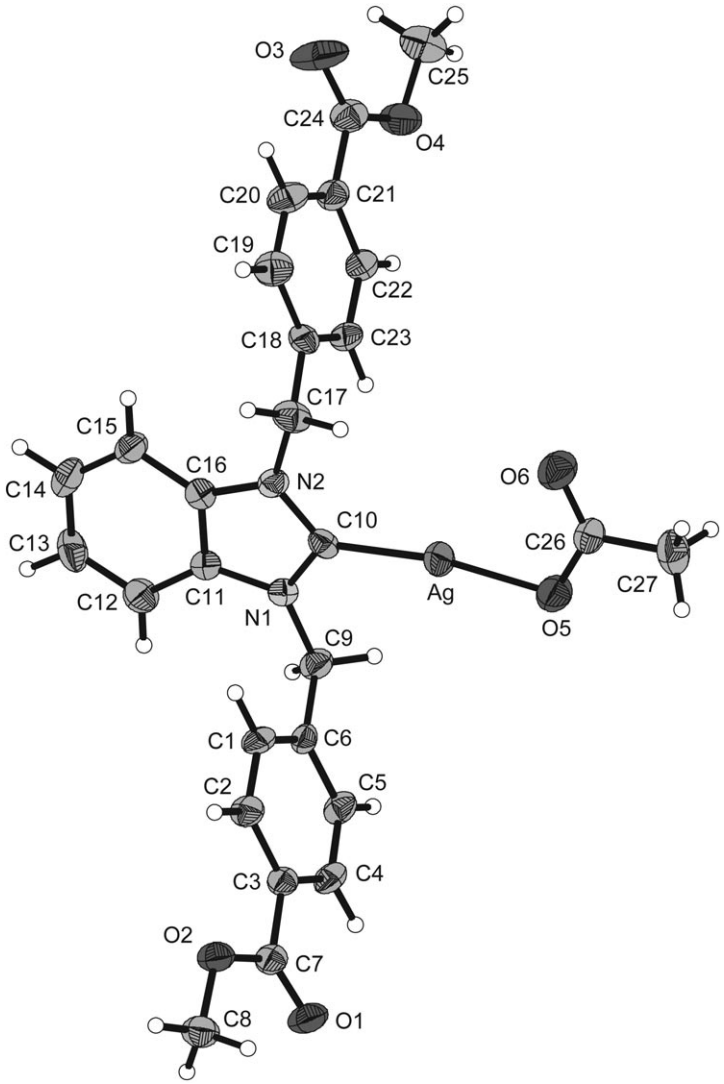


Fig. 3. *X-Ray diffraction structure of* **4c** (with 50% probability ellipsoids)

medium antibacterial activity was observed against *Gram*-positive bacteria *Staphylococcus aureus* with an area of clearance of 7 mm at 0.40 μmol.

The previously synthesized NHC – AgOAc complexes in our laboratory showed an activity of up to 12 mm area of clearance at a concentration of 0.46 μmol for (1-benzyl-3-methylbenzimidazol-2-ylidene)silver(I) acetate [46]. Thus, the presented NHC – AgOAc complexes **4a** – **4f** do not belong to the most promising drug candidates of the NHC – Ag series. Possibly, the methyl ester group on the benzyl ligand did not sufficiently increase the lipophilicity of these compounds. The compounds exhibit

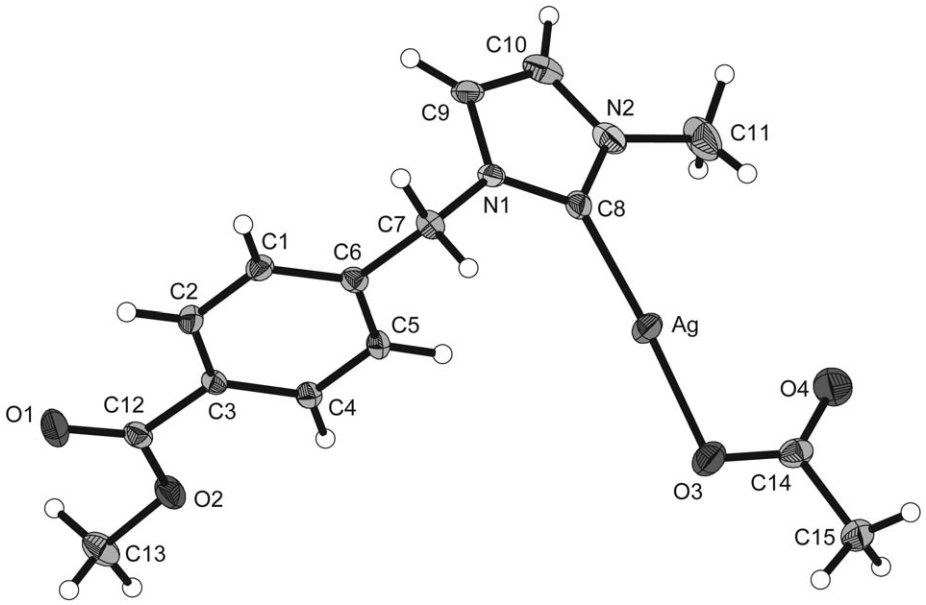


Fig. 4. *X-Ray diffraction structure of* **4d** (with 50% probability ellipsoids)

antimicrobial activity, when they are capable of penetrating into and through the lipid membrane of microorganisms. Inside, they can prohibit active enzyme sites of the microorganisms. Consequently, higher lipophilicity enhances antibacterial activity. For example, NHC–AgOAc complexes **4a**–**4f** revealed increased antibacterial activity compared to NHC precursors **3a**–**3f**, because **4a**–**4f** are more lipophilic than polar NHC precursors.

In some previous complexes, the polarity of the Ag ion was even more reduced by chelation. The AcO moiety coordinated *via* two O-atoms to the Ag centre in a bidentate fashion leading to highly active complexes [39]. Despite the lack of this coordination, the presented complexes **4a**–**4f** showed a moderate antibacterial activity.

Cytotoxicity Studies. The different types of NHC–AgOAc complexes are possible anticancer drug candidates. Therefore, the *in vitro* cytotoxicities of symmetrically and nonsymmetrically 4-(methoxycarbonyl)benzyl-substituted complexes **4a** and **4b**, and **4d**–**4f** were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT)-based assays [47] 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-response curves for **4a** and **4b**, and **4d**–**4f** are displayed in *Fig. 5* and *6*, respectively.

Two symmetrically substituted NHC–AgOAc complexes **4a** and **4b**, which contain 1*H*-imidazole and 4,5-dichloro-1*H*-imidazole groups exhibit IC_{50} values 13.5 ± 2 and 68.3 ± 1 μM, respectively. The third symmetrically substituted complex **4c**, which contains 1*H*-benzimidazole group, was not completely soluble in DMSO and thus not reliable for anticancer-activity studies. Compound **4a** has an approximately fivefold increase in magnitude when compared with **4b**, and, in comparison to cisplatin (IC_{50}

Table 1. *Crystal Data and Structure Refinement for* **4a**, **4c**, *and* **4d**

	4a	**4c**	**4d**
Empirical formula	$C_{46}H_{48}Ag_2N_4O_{13}$	$C_{27}H_{25}AgN_2O_6$	$C_{15}H_{17}AgN_2O_4$
Molecular formula	$(C_{23}H_{23}AgN_2O_6)_2 \times H_2O$	$C_{27}H_{25}AgN_2O_6$	$C_{15}H_{17}AgN_2O_4$
Formula weight	1080.62	581.36	397.18
Crystal system	Monoclinic	Monoclinic	Monoclinic
Space group	*C*2/*c* (#15)	*Cc* (#9)	$P2_1$ (#4)
Unit cell dimensions [Å] *a*	34.431(2)	23.9033(5)	4.6273(1)
b	7.6357(2)	14.4382(2)	14.8477(3)
c	19.534(1)	7.3241(2)	11.1590(2)
α	90°	90°	90°
β	121.446(8)°	105.684(2)°	96.543(2)°
γ	90°	90°	90°
Volume [Å^3]	4381.3(4)	2433.58(9)	761.68(3)
Z	4	4	2
Density [Mg/m^3] (calc.)	1.638	1.587	1.732
Absorption coefficient [mm^{-1}]	7.779	0.874	1.342
F(000)	2200	1184	400
Crystal size [mm^3]	0.3492 × 0.2741 × 0.0259	0.1567 × 0.1053 × 0.0842	0.2526 × 0.1212 × 0.0873
Theta range for data collection	4.53 to 67.01°	3.33 to 29.45°	3.30 to 33.01°
Index ranges	$-36 \le h \le 40$	$-32 \le h \le 26$	$-7 \le h \le 6$
	$-8 \le h \le 9$	$-19 \le k \le 19$	$-22 \le k \le 21$
	$-22 \le l \le 23$	$-10 \le l \le 9$	$-16 \le l \le 16$
Reflections collected	13955	11822	18906
Independent reflections	3854 (R(int) = 0.0317)	4581 (R(int) = 0.0316)	5185 (R(int) = 0.0284)
Completeness to θ_{max}	98.8%	99.5%	99.4%
Max. and min. transmission	0.897 and 0.452	0.937 and 0.911	0.898 and 0.771
Data/restraints/parameters	3854/1/300	4581/2/329	5185/1/202
Goodness-of-fit on F^2	1.080	1.040	1.091
Final *R* indices ($I > 2\sigma(I)$)	$R_1 = 0.0224$,	$R_1 = 0.0277$,	$R_1 = 0.0329$,
	$wR_2 = 0.0605$	$wR_2 = 0.0693$	$wR_2 = 0.0846$
R Indices (all data)	$R_1 = 0.0237$,	$R_1 = 0.0309$,	$R_1 = 0.0364$,
	$wR_2 = 0.0613$	$wR_2 = 0.0705$	$wR_2 = 0.0858$
Largest diff. peak and hole	0.423 and −0.656 e Å^{-3}	0.903 and −0.468 e Å^{-3}	3.478 and −0.724 e Å^{-3}

value 3.3 μM), it represents an *ca.* 20-fold decrease in magnitude. The nonsymmetrically substituted complexes **4d**–**4f**, which also contain 1-methyl-1*H*-imidazole, methyl 4-[(4,5-dichloro-1*H*-imidazol-1-yl)methyl]benzoate, and 1-methyl-1*H*-benzimidazole groups, have IC_{50} values 3.3 ± 0.4, 9.4 ± 1, and 13.2 ± 1 μM, respectively. Compound **4d** turned out to be the most promising candidate in this study because of the highest IC_{50} value and has an approximately three- and fourfold increase in magnitude when compared with **4e** and **4f**, respectively. Compared with cisplatin (IC_{50} 3.3 μM), **4d** has an approximately equal activity in magnitude in terms of the IC_{50} value.

Compared to symmetrically substituted complexes **4a** and **4b**, the nonsymmetrically substituted complexes **4d**–**4f** have shown almost high cytotoxic activities because of their solubility factor. Symmetrically substituted complexes are less soluble in DMSO compared to nonsymmetrically substituted complexes. The complexes **4a** and **4b**, and **4d**–**4f** are stable in saline solution with respect to AgCl precipitation. It was also

Table 2. *Selected Bond Lengths* [Å] *and Angles* [°] *for Compounds* **4a**, **4c**, *and* **4d**

Bond Lengths [Å]	**4a**	**4c**	**4d**	Bond Angles [°]	**4a**	**4c**	**4d**
N(1)–C(10)	1.353(3)	1.340(4)		N(1)–C(10)–N(2)	104.39(17)	105.8(2)	
C(10)–N(2)	1.354(3)	1.368(4)	1.377(5)	C(10)–N(2)–C(12)	111.18(16)		
N(1)–C(11)	1.383(2)	1.388(3)		C(10)–N(1)–C(11)	111.28(16)	111.2(2)	
C(12)–N(2)	1.380(3)			C(12)–C(11)–N(1)	106.40(17)		
C(11)–C(12)	1.350(3)			C(11)–C(12)–N(2)	106.75(17)		
Ag–C(10)	2.067(2)	2.055(3)		C(10)–Ag–O(5)	171.80(6)	171.10(10)	
Ag–O(5)	2.1557(14)	2.093(2)		O(6)–C(22)–O(5)	123.32(19)		
O(5)–C(22)	1.290(2)			C(10)–N(2)–C(16)		110.7(2)	
O(6)–C(22)	1.236(3)			N(1)–C(11)–C(16)		106.1(2)	
C(22)–C(23)	1.509(3)			N(2)–C(16)–C(11)		106.2(2)	
C(16)–N(2)		1.378(4)		O(6)–C(26)–O(5)		122.2(3)	
C(11)–C(16)		1.391(5)		N(2)–C(8)–N(1)			104.1(2)
O(5)–C(26)		1.273(4)		C(8)–N(2)–C(10)			111.5(2)
O(6)–C(26)		1.244(4)		C(8)–N(1)–C(9)			111.7(2)
C(26)–C(27)		1.503(4)		C(9)–C(10)–N(2)			107.0(3)
N(1)–C(8)			1.354(3)	C(10)–C(9)–N(1)			105.6(3)
C(8)–N(2)			1.352(4)	C(8)–Ag–O(3)			176.10(10)
N(1)–C(9)			1.389(4)	O(4)–C(14)–O(3)			125.0(3)
C(9)–C(10)			1.357(4)				
Ag–C(8)			2.067(3)				
Ag–O(3)			2.137(2)				
O(3)–C(14)			1.269(4)				
O(4)–C(14)			1.236(4)				
C(14)–C(15)			1.519(4)				

observed that, compared to known NHC–AgOAc complexes [39][46], the complexes **4a** and **4b**, and **4d**–**4f** have almost the same cytotoxicity.

Conclusions and Outlook. – In summary, we have synthesized a series of six new symmetrically and nonsymmetrically 4-(methoxycarbonyl)benzyl-substituted *N*-heterocyclic carbene–silver acetate complexes **4a**–**4f** through the reaction of appropriately symmetrically and nonsymmetrically substituted *N*-heterocyclic carbenes **3a**–**3f** with AgOAc. The antibacterial activities of the all compounds were tested *in vitro* against both *Gram*-positive bacteria *Staphylococcus aureus* and *Gram*-negative bacteria *Escherichia coli.* Almost all the complexes have shown high antibacterial activity compared to the precursors, and it is also clear that, as the precursor and complex concentration increases, the antibacterial activity becomes higher. NHC–AgOAc complexes **4a** and **4b**, and **4d**–**4f** exhibited antitumor IC_{50} values of 13.5 ± 2, 68.3 ± 1, 3.3 ± 0.4, 9.4 ± 1, and 13.2 ± 1 μM, respectively, against the Caki-1 cell line. The most promising compound in this study, **4d**, is in the cytotoxicity range of cisplatin against Caki-1, indicating its high potential as an anticancer drug. Further work is currently underway in order to improve these values by performing formulation experiments to increase solubility of these complexes, which should allow for *in vivo* testing of **4d** in the nearby future.

Table 3. *Area of Clearance* [mm] *in Antibacterial Activity of Compounds* **3a**–**3f**

		Staphylococcus aureus (*Gram*-pos.)	*Escherichia coli* (*Gram*-neg.)
3a	0.10 μmol (45.2 μg)	0	0
	0.14 μmol (63.8 μg)	0	0
	0.20 μmol (91.2 μg)	0	0
	0.41 μmol (182.5 μg)	1	1
3b	0.10 μmol (52.7 μg)	0	1
	0.14 μmol (73.7 μg)	1	1
	0.20 μmol (105.4 μg)	1	1
	0.41 μmol (210.8 μg)	2	2
3c	0.10 μmol (50.7 μg)	0	1
	0.14 μmol (71.0 μg)	0	2
	0.20 μmol (101.5 μg)	1	2
	0.41 μmol (203.1 μg)	3	3
3d	0.10 μmol (31.8 μg)	0	0
	0.14 μmol (44.6 μg)	0	0
	0.20 μmol (63.7 μg)	0	0
	0.41 μmol (127.5 μg)	0	0
3e	0.10 μmol (43.7 μg)	0	0
	0.14 μmol (61.2 μg)	1	0
	0.20 μmol (87.5 μg)	1	0
	0.41 μmol 175.0 μg)	1	0
3f	0.10 μmol (45.8 μg)	1	0
	0.14 μmol (64.1 μg)	1	0
	0.20 μmol (91.6 μg)	1	0
	0.41 μmol (183.3 μg)	1	0

Experimental Part

General. All the solvents used were of anal. grade and were used without further purification. *1*H-*Imidazole* (**1a**), *4,5-dichloro-1*H-*imidazole* (**1b**), *1*H-*benzimidazole* (**1c**), *1-methyl-1*H-*imidazole* (**1d**), *1-methyl-1*H-*benzimidazole* (**1f**), *methyl 4-(bromomethyl)benzoate* (**2**), AgOAc, MeI, and K_2CO_3 were purchased from *Sigma–Aldrich Chemical Company* and were used without further purification. UV/VIS Spectra: *Unicam UV4* spectrometer; λ (ε) in nm. IR Spectra: *Perkin-Elmer Paragon 1000* FT-IR; KBr disc; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Varian 400* MHz spectrometer; chemical shifts δ in ppm referenced to TMS, *J* in Hz. ESI-MS: quadrupole tandem mass spectrometer (*Quattro Micro*, *Micromass*/*Water's Corp.*, USA), using solns. made up in 50% CH_2Cl_2 and 50% MeOH; *m*/*z* (rel.). ESI-MS (pos.-ion) for **1e**, **3a**–**3f**, and **4a**–**4f**. CHN Analysis: *Exeter Analytical CE-440* elemental analyser. Ag was estimated by spectrophotometric method (atomic absorption spectra *55B Varian*), while Cl, Br, and I were determined by mercurimetric titrations. Crystal data were collected using an *Oxford Diffraction SuperNova* diffractometer fitted with an *Atlas* detector. Complex **4a**: with CuK_α (1.54184 Å); **4c** and **4d**: with MoK_α (0.71073 Å); **4c** was collected at 200 K, **4a** and **4c** at 100 K. An at least complete data set was collected, assuming that the pairs are not equivalent. An analytical absorption correction based on the shape of the crystal was performed [48]. The structures were solved by direct methods using SHELXS-97 [49] and refined by full-matrix least-squares on F^2 for all data using SHELXL-97 [49]. The H-atoms of the H_2O molecules in **4a** were located in the difference *Fourier* map. Their thermal displacement parameters were fixed to 1.5 times the equivalent one of the parent O-atom. O–H Bond lengths were restrained to be 0.84 Å using DFIX. All other H-atoms were added at calculated positions and refined using a riding model. Their isotropic temp. factors were fixed to 1.2 times (1.5 times for Me groups) the equivalent

Table 4. *Area of Clearance* [mm] *in Antibacterial Activity of Compounds* **4a**–**4f**

		Staphylococcus aureus (*Gram*-pos.)	*Escherichia coli* (*Gram*-neg.)
4a	0.10 μmol (54.4 μg)	3	3
	0.14 μmol (76.2 μg)	4	4
	0.20 μmol (108.9 μg)	4	4
	0.41 μmol (217.8 μg)	5	5
4b	0.10 μmol (62.5 μg)	3	3
	0.14 μmol (87.5 μg)	4	4
	0.20 μmol (125.0 μg)	4	4
	0.41 μmol (250.0 μg)	4	4
4c	0.10 μmol (59.5 μg)	3	3
	0.14 μmol (83.4 μg)	3	3
	0.20 μmol (119.1 μg)	3	4
	0.41 μmol (238.3 μg)	4	4
4d	0.10 μmol (40.7 μg)	3	3
	0.14 μmol (56.9 μg)	4	4
	0.20 μmol (81.4 μg)	5	4
	0.41 μmol (162.8 μg)	7	6
4e	0.10 μmol (47.7 μg)	3	3
	0.14 μmol (66.8 μg)	4	4
	0.20 μmol (95.5 μg)	5	4
	0.41 μmol (191.0 μg)	7	6
4f	0.10 μmol (45.8 μg)	3	2
	0.14 μmol (64.1 μg)	4	3
	0.20 μmol (91.6 μg)	4	3
	0.41 μmol (183.3 μg)	7	4

isotropic displacement parameters of the C-atom to which the H-atom is attached. Anisotropic thermal displacement parameters were used for all non-H-atoms. Suitable crystals of **4a** and **4c** were formed from the slow evaporation of a sat. MeOH soln., while crystals of **4d** were grown in a sat. CH_2Cl_2 soln. with slow infusion of pentane. Further details about the data collection, as well as reliability factors, are listed in *Table 1*.

CCDC-788649 (for **4a**), -788650 (for **4c**), and -788651 (for **4d**) contain the supplementary crystallographic data. These data can be obtained free of charge from the *Cambridge Crystallographic Data Centre via* www.ccdc.cam.ac.uk/data_request/cif.

*1,3-Bis[4-(methoxycarbonyl)benzyl]-1*H*-imidazol-3-ium Bromide* (**3a**). Compound **1a** (0.136 g, 2.00 mmol) and K_2CO_3 (0.415 g, 3.00 mmol) were stirred for 15 min in 100 ml of MeCN. Compound **2** (0.916 g, 4.00 mmol) was added in one portion, and stirring was continued at r.t. for further 2 d. After the solvent was removed under reduced pressure, 150 ml of H_2O were added, and the mixture was stirred for 30 min. The white solid was filtered, washed with Et_2O (3×20 ml), and dried in suction.

The obtained methyl 4-(1*H*-imidazol-1-ylmethyl)benzoate (0.623 g, 2.88 mmol) and **2** (0.398 g, 2.88 mmol) were refluxed in 70 ml of MeCN for 1 d. The solvent was removed under reduced pressure. After drying for 2 h under vacuum, **3a** was obtained (0.749 g, 2.11 mmol, 73%). White crystalline powder. UV/VIS (MeOH): 230 (10495), 276 (1835). IR: 1716*s*, 1614*m*, 1550*w*, 1439*m*, 1385*m*, 1318*w*, 1282*s*, 1185*m*, 1150*m*, 1106*s*, 1022*m*, 874*s*, 734*s*. ^{1}H-NMR ($CDCl_3$): 9.55 (*s*, NCHN); 8.32 (*d*, $J = 8.0$, 4 arom. H); 7.96 (*s*, 2 CH=); 7.79 (*d*, $J = 8.0$, 4 arom. H); 5.80 (*s*, 2 CH_2); 4.16 (*s*, 2 MeO). ^{13}C-NMR ($CDCl_3$): 166.3 (C=O); 138.7, 136.7, 130.8, 130.0, 128.3, 123.0 (NCN, CH=, arom. C); 52.3 (MeO); 51.4 (CH_2). MS (QMS-MS/MS): 365.15 ($[M - Br]^+$). Anal. calc. for $C_{21}H_{21}BrN_2O_4$ (445.31): C 56.64, H 4.75, N 6.29, Br 17.94; found: C 56.03, H 4.78, N 5.79, Br 17.28.

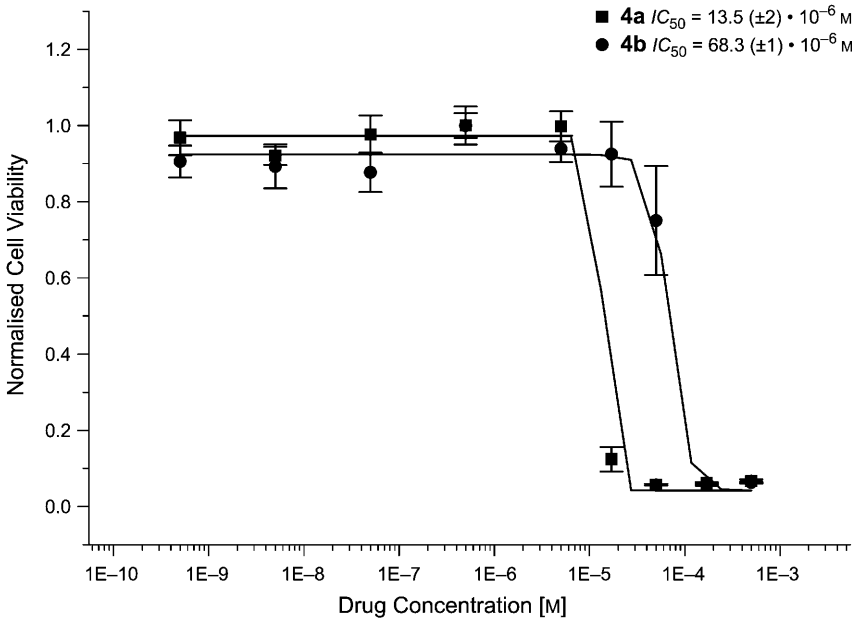


Fig. 5. *Cytotoxicity curves from typical MTT assays showing the effect of compounds* **4a** and **4b** *on the viability of Caki-1 cells*

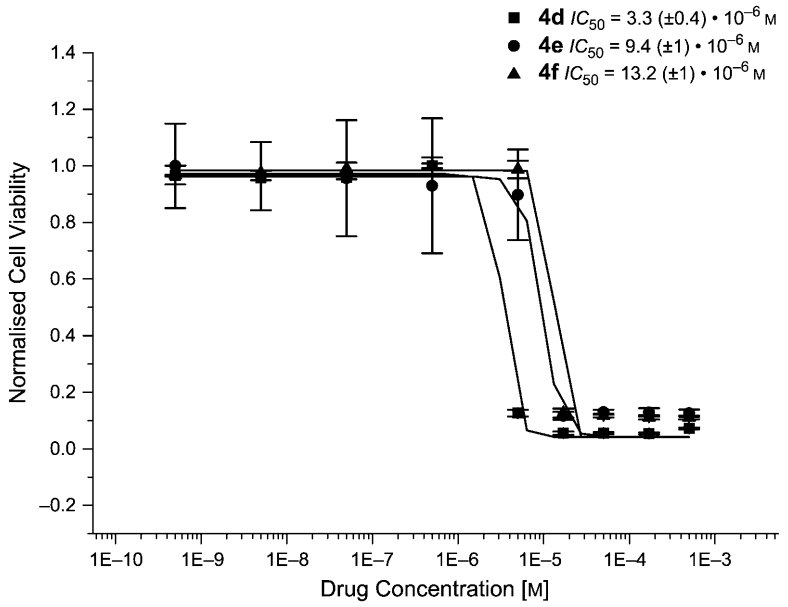


Fig. 6. *Cytotoxicity curves from typical MTT assays showing the effect of compounds* **4d**–**4f** *on the viability of Caki-1 cells*

*4,5-Dichloro-1,3-bis[4-(methoxycarbonyl)benzyl]-1*H-*imidazol-3-ium Bromide* (**3b**). Compound **1b** (598 mg, 4.37 mmol) and K_2CO_3 (905 mg, 6.55 mmol) were stirred for 15 min in 200 ml of MeCN. Compound **2** (2.00 g, 8.73 mmol) was added, and the mixture was stirred at r.t. for 4 d. The yellow suspension was filtered; **2** (520 mg, 2.27 mmol) was added again, and the mixture was refluxed for 4 d. The solvent was removed under reduced pressure, washed with H_2O (100 ml), Et_2O (50 ml), and toluene (2 × 100 ml): **3b** (0.420 g, 0.817 mmol, 19%). White crystalline powder. UV/VIS (MeOH): 275 (6874), 285 (5800). IR: 1724*s*, 1614*s*, 1583*m*, 1551*w*, 1440*m*, 1278*s*, 1109*m*, 1017*s*, 759*m*. ^{1}H-NMR ($CDCl_3$): 12.01 (*s*, NCHN); 8.05 (*d*, *J* = 8.4, 4 arom. H); 7.62 (*d*, *J* = 8.4, 4 arom. H); 5.75 (*s*, 2 CH_2); 3.92 (*s*, 2 MeO). ^{13}C-NMR ($CDCl_3$): 166.2 (C=O); 138.3, 137.8, 130.4, 130.2, 128.9, 119.9 (NCN, CCl, arom. C); 52.8 (MeO); 51.6 (CH_2). MS (QMS-MS/MS): 434.95 ($[M - Br]^+$). Anal. calc. for $C_{21}H_{19}BrCl_2N_2O_4$ (514.20): C 49.05, H 3.72, N 5.45, Br 15.54, Cl 13.79; found: C 49.16, H 3.42, N 5.31, Br 15.3, Cl 15.98.

*1,3-Bis[4-(methoxycarbonyl)benzyl]-1*H-*benzimidazol-3-ium Bromide* (**3c**). Compound **1c** (516 mg, 4.37 mmol) and K_2CO_3 (905 mg, 6.55 mmol) were stirred for 15 min in 230 ml of MeCN before **2** (2.00 g, 8.73 mmol) was added in one portion. The mixture was stirred at r.t. for 5 d. After the solvent was removed under reduced pressure, 300 ml of H_2O were added. The precipitate was filtered, and washed with Et_2O and MeCN. The white crystalline powder was dried for 4 h under vacuum to yield **3c** (0.960 g, 1.94 mmol, 44%). UV/VIS (MeOH): 270 (2852), 280 (2542). IR: 1719*s*, 1709*s*, 1553*s*, 1431*m*, 1287*s*, 1189*m*, 1152*m*, 1107*m*, 736*m*, 612*m*. ^{1}H-NMR ($CDCl_3$): 11.99 (*s*, NCHN); 8.04 (*d*, *J* = 8.4, C_6H_4–COOMe); 7.58 (*d*, *J* = 8.4, C_6H_4–COOMe); 7.51 (*m*, 4 CH=); 6.00 (*s*, 2 CH_2); 3.90 (*s*, 2 MeO). ^{13}C-NMR ($CDCl_3$): 166.1 (C=O); 137.1, 131.2, 131.1, 130.6, 130.0, 128.2, 127.4, 113.6 (NCN, CH=, C_6H_4–COOMe); 52.3 (MeO); 51.2 (CH_2). MS (QMS-MS/MS): 415.08 ($[M - Br]^+$). Anal. calc. for $C_{25}H_{23}BrN_2O_4$ (495.37): C 60.62, H 4.68, N 5.66, Br 16.13; found: C 60.42, H 4.78, N, 5.42, Br 16.25.

*(Acetato-*κO*){1,3-bis[4-(methoxycarbonyl)benzyl]-2,3-dihydro-1*H-*imidazol-2-yl}silver* (**4a**). Compound **3a** (94 mg, 0.212 mmol) and AgOAc (76 mg, 0.450 mmol) were refluxed in CH_2Cl_2 (70 ml) for 1 d. The flask was covered with aluminium foil to avoid light exposure. The colourless soln. was filtered, and the solvent was reduced under vacuum to 4 ml. Et_2O (50 ml) was added to the colourless sticky oily liquid, and the mixture was stirred for 1 h. The solvent was removed with a syringe, and the resulting solid compound was dried for 4 h under reduced pressure: **4a** (0.090 mg, 0.169 mmol, 79%). White crystalline powder. UV/VIS (MeOH): 230 (10153), 276 (1942). IR: 1719*s*, 1612*m*, 1584*m*, 1433*m*, 1416*m*, 1283*s*, 1178*m*, 1016*m*, 799*s*, 676*w*. ^{1}H-NMR ($CDCl_3$): 8.04 (*d*, *J* = 8.3, 4 arom. H); 7.34 (*d*, *J* = 8.3, 4 arom. H); 6.96 (*s*, 2 CH=); 5.39 (*s*, 2 CH_2); 3.92 (*s*, 2 MeO); 2.08 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 180.2 (NCN); 178.9 (C=O); 166.4 (C=O); 140.1, 130.6, 130.4, 127.8, 121.7 (CH=, arom. C); 55.5 (MeO); 52.3 (CH_2); 22.7 (Me). MS (QMS-MS/MS): 472.27 ($[M - AcO]^+$). Anal. calc. for $C_{23}H_{23}AgN_2O_6$ (531.31): C 51.99, H 4.36, N 5.27, Ag 20.30; found: C 51.90, H 4.32, N 5.04, Ag 19.98.

*(Acetato-*κO*){4,5-dichloro-1,3-bis[4-(methoxycarbonyl)benzyl]-2,3-dihydro-1*H-*imidazol-2-yl}silver* (**4b**). Compound **3b** (400 mg, 0.778 mmol) was stirred for 10 min in CH_2Cl_2 (100 ml). AgOAc (260 mg, 1.56 mmol) was added, and the suspension was stirred at r.t. for 5 d. The flask was covered with aluminium foil to avoid light exposure. The solvent was removed under reduced pressure, and the white grayish powder was partly dissolved in $CHCl_3$ (300 ml) to obtain better solubility. The resulting suspension was filtered over *Celite* to remove AgBr. The solvent of the colourless soln. was removed under reduced pressure. The residue was dried for 2 h: **4b** (0.371 g, 0.618 mmol, 79%). White crystalline powder. UV/VIS (MeOH): 230 (16784), 282 (4083). IR: 1720*s*, 1614*m*, 1581*m*, 1512*s*, 1436*s*, 1293*s*, 1183*m*, 1019*m*, 753*m*. ^{1}H-NMR ($CDCl_3$): 8.00 (*d*, *J* = 7.7, 4 arom. H); 7.35 (*d*, *J* = 7.7, 4 arom. H); 5.45 (*s*, 2 CH_2); 3.92 (*s*, 2 MeO); 2.03 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 179.9 (NCN); 177.3 (C=O); 165.3 (C=O); 138.0, 129.6, 129.4, 126.5, 117.2 (CCl, arom. C); 53.3 (CH_2); 51.3 (MeO); 21.6 (Me). MS (QMS-MS/MS): 541.16 ($[M - AcO]^+$). Anal. calc. for $C_{23}H_{21}AgCl_2N_2O_6$ (600.20): C 46.03, H 3.53, N 4.67, Cl 11.81, Ag 17.97; found: C 46.25, H 3.51, N, 4.58, Cl 11.64, Ag 17.82.

*(Acetato-*κO*){1,3-bis[4-(methoxycarbonyl)benzyl]-2,3-dihydro-1*H-*benzimidazol-2-yl}silver* (**4c**). **3c** (103 mg, 0.208 mmol) and AgOAc (69.2 mg, 0.415 mmol) were refluxed in CH_2Cl_2 (50 ml) for 2 d. The flask was covered with aluminium foil to avoid light exposure. The suspension was cooled to r.t., and charcoal was added to bind AgBr. A colourless soln. was obtained after filtering over *Celite*. The solvent was removed under vacuum: **4c** (0.070 g, 0.120 mmol, 58%). Beige crystalline powder. UV/VIS (MeOH): 230 (16787), 275 (7423), 285 (6805). IR: 1719*s*, 1613*m*, 1575*m*, 1481*m*, 1434*m*, 1396*m*, 1016*m*,

743*m*, 672*w*. ^{1}H-NMR ($CDCl_3$): 7.92 (*d*, *J* = 8.1, 4 C_6H_4–COOMe); 7.28 (*d*, *J* = 8.1, 4 C_6H_4–COOMe); 7.20 (*s*, 4 CH=); 5.66 (*s*, 2 CH_2); 3.83 (*s*, 2 MeO); 1.99 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 180.4 (NCN); 179.4 (C=O); 166.3 (C=O); 139.6, 133.8, 130.5, 130.4, 127.1, 124.6, 112.1 (CH=, C_6H_4–COOMe); 53.3 (MeO); 52.2 (CH_2); 22.7 (Me). MS (QMS-MS/MS): 522.33 ($[M - AcO]^+$). Anal. calc. for $C_{27}H_{25}AgN_2O_6$ (581.37): C 55.78, H 4.33, N 4.82, Ag 18.55; found: C 55.25, H 4.44, N 4.43, Ag 18.33.

*3-[4-(Methoxycarbonyl)benzyl]-1-methyl-1*H*-imidazol-3-ium Bromide* (**3d**). Compound **2** (0.458 g, 2 mmol) was added to a soln. of **1d** (0.158 g, 2 mmol) in toluene (40 ml). The mixture was stirred at r.t. for 4 d. Afterwards, the solvent was removed under reduced pressure. The resultant residue was first washed with pentane and then with Et_2O. Compound **3d** (0.510 g, 1.63 mmol, 82%) was obtained after drying under reduced pressure. White solid. UV/VIS (MeOH): 230 (98352), 275 (4031), 367 (1341). IR: 3450*s*, 3138*w*, 3054*m*, 1712*s*, 1613*m*, 1442*m*, 1291*s*, 1157*m*, 856*w*, 764*m*, 621*m*, 477*w*, 356*w*. ^{1}H-NMR ($CDCl_3$): 10.44 (*s*, NCHN); 8.00 (*d*, *J* = 8.3, 2 arom. H); 7.62 (*d*, *J* = 8.2, 2 arom. H); 7.55–7.50 (*m*, 2 CH=); 5.75 (*s*, CH_2); 4.08 (*s*, MeN); 3.90 (*s*, MeO). ^{13}C-NMR ($CDCl_3$): 166.2 (C=O); 137.9, 137.5, 131.0, 130.4, 128.9, 123.6, 122.1 (NCN, CH=, arom. C); 52.6 (MeO); 52.3 (CH_2); 36.8 (MeN). MS (QMS-MS/MS): 231.2 ($[M - Br]^+$). Anal. calc. for $C_{13}H_{15}BrN_2O_2$ (311.17): C 50.18, H 4.86, N 9.00, Br 25.68; found: C 49.98 H 5.00, N 8.97, Br 25.45.

*Methyl 4-[(4,5-Dichloro-1*H*-imidazol-1-yl)methyl]benzoate* (**1e**). Compound **1b** (0.273 g, 2 mmol) and K_2CO_3 (0.414 g, 3 mmol) were stirred for 30 min in 40 ml of MeCN. Compound **2** (0.458 g, 2 mmol) was added in one portion, and stirring was continued at r.t. for further 4 d. After the solvent was removed under reduced pressure, 70 ml of H_2O were added. The aq. phase was extracted with CH_2Cl_2 (4 × 25 ml). Org. phases were combined and dried ($MgSO_4$). Compound **1e** (0.475 g, 1.66 mmol, 83%) was obtained after solvent removal under reduced pressure. White solid. UV/VIS (MeOH): 227 (13078), 275 (4956). IR: 3431*w*, 3121*w*, 2954*w*, 1714*s*, 1611*w*, 1481*m*, 1428*m*, 1287*s*, 1180*m*, 1112*m*, 976*w*, 804*w*, 734*m*, 664*w*, 536*w*, 484*w*. ^{1}H-NMR ($CDCl_3$): 8.05 (*d*, *J* = 8.3, 2 arom. H); 7.43 (*s*, NCHN); 7.22 (*d*, *J* = 8.5, 2 arom. H); 5.15 (*s*, CH_2); 3.92 (*s*, MeO). ^{13}C-NMR ($CDCl_3$): 166.3 (C=O); 139.2, 134.4, 130.5, 130.4, 127.0, 126.7, 113.6 (NCN, CCl, arom. C); 52.2 (MeO); 49.3 (CH_2). MS (QMS-MS/MS): 285.0 (M^+). Anal. calc. for $C_{12}H_{10}Cl_2N_2O_2$ (285.13): C 50.55, H 3.54, N 9.82, Cl 24.87; found: C 50.43, H 3.27, N 9.53, Cl 24.98.

*4,5-Dichloro-1-[4-(methoxycarbonyl)benzyl]-3-methyl-1*H*-imidazol-3-ium Iodide* (**3e**). MeI (0.560 ml, 9.00 mmol) was added in one portion to a stirred suspension of **1e** (0.285 g, 1 mmol) in 40 ml of MeCN. The mixture was heated under reflux for 2 d. Afterwards, the solvent was removed under reduced pressure. The resultant residue was first washed with pentane and then with Et_2O. Compound **3e** (0.280 g, 0.655 mmol, 65%) was obtained after drying under reduced pressure. Yellow solid. UV/VIS (MeOH): 225 (14904), 278 (6014). IR: 3424*m*, 3031*s*, 1715*s*, 1581*w*, 1436*w*, 1278*s*, 1194*w*, 1105*m*, 1021*w*, 964*w*, 877*w*, 764*m*, 710*m*, 601*w*, 482*w*. ^{1}H-NMR ($CDCl_3$): 10.89 (*s*, NCHN); 8.05 (*d*, *J* = 8.3, 2 arom. H); 7.64 (*d*, *J* = 8.3, 2 arom. H); 5.72 (*s*, CH_2); 4.06 (*s*, MeN); 3.91 (*s*, MeO). ^{13}C-NMR ($CDCl_3$): 166.1 (C=O); 137.1, 135.8, 131.2, 130.5, 128.9, 120.5, 119.3 (NCN, CCl, arom. C); 52.3 (MeO); 52.2 (CH_2); 36.1 (MeN). MS (QMS-MS/MS): 300.1 ($[M - I]^+$). Anal. calc. for $C_{13}H_{13}Cl_2IN_2O_2$ (427.06): C 36.56, H 3.07, N 6.56, Cl 16.60, I 29.72; found: C 36.34, H 3.12, N 6.42, Cl 16.57, I 29.69.

*3-[4-(Methoxycarbonyl)benzyl]-1-methyl-1*H*-3,1-benzimidazol-3-ium Bromide* (**3f**). Compound **2** (0.458 g, 2 mmol) was added to a soln. of **1f** (0.264 g, 2 mmol) in toluene (40 ml). The mixture was stirred at r.t. for 4 d. Afterwards, the solvent was removed under reduced pressure. The resultant residue was first washed with pentane and then with Et_2O. Compound **3f** (0.575 g, 1.59 mmol, 79%) was obtained after drying under reduced pressure. White solid. UV/VIS (MeOH): 230 (9238), 270 (6544), 368 (1588). IR: 3421*w*, 3016*w*, 2951*m*, 1710*s*, 1612*w*, 1565*m*, 1426*m*, 1289*s*, 1191*w*, 1112*m*, 1019*w*, 759*s*, 687*w*, 601*w*, 467*w*. ^{1}H-NMR ($CDCl_3$): 11.71 (*s*, NCHN); 8.01 (*d*, *J* = 8.3, 2 CH=); 7.71 (*d*, *J* = 8.3, CH=); 7.67–7.51 (*m*, 1 CH=, C_6H_4–COOMe); 6.03 (*s*, CH_2); 4.30 (*s*, MeN); 3.89 (*s*, MeO). ^{13}C-NMR ($CDCl_3$): 166.1 (C=O); 143.7, 137.3, 132.1, 130.9, 130.9, 130.5, 128.2, 127.3, 127.3, 113.5, 112.8 (NCN, CH=, C_6H_4–COOMe); 52.2 (MeO); 50.9 (CH_2); 33.9 (MeN). MS (QMS-MS/MS): 281.1 ($[M - Br]^+$). Anal. calc. for $C_{17}H_{17}BrN_2O_2$ (361.23): C 56.52, H 4.74, N 7.75, Br 22.12; found: C 56.34, H 4.73, N 7.66, Br 22.18.

*(Acetato-*κ*O){1-[4-(methoxycarbonyl)benzyl]-3-methyl-2,3-dihydro-1*H*-imidazol-2-yl}silver* (**4d**). Compound **3d** (0.311 g, 1.00 mmol) was dissolved in CH_2Cl_2 (35 ml), AgOAc (0.333 g, 2.00 mmol) was added, and the mixture was stirred at r.t. for 2 d. The yellow precipitate, presumably AgBr, was filtered, and a clear soln. was obtained. The volatile components were removed *in vacuo* to produce a white sticky

solid. The solid was washed with Et_2O (3 × 10 ml) and dried under reduced pressure for 2 h to yield **4d** (0.300 g, 0.755 mmol, 75%). White solid. UV/VIS (MeOH): 239 (12230), 277 (5445), 362 (1841). IR: 3087*w*, 2950*w*, 1711*s*, 1610*m*, 1578*s*, 1405*s*, 1312*w*, 1284*s*, 1185*m*, 1162*m*, 1111*s*, 1018*m*, 920*w*, 743*m*, 732*w*, 647, 620*w*. ^{1}H-NMR ($CDCl_3$): 8.02 (*d*, *J* = 8.3, 2 arom. H); 7.32 (*d*, *J* = 8.2, 2 arom. H); 7.01–6.93 (*m*, 2 CH=); 5.35 (*s*, CH_2); 3.91 (*s*, MeN); 3.87 (*s*, MeO); 2.08 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 180.5 (NCN); 179.1 (C=O); 166.3 (C=O); 140.3, 130.4, 130.3, 127.7, 122.8, 121.0 (CH=, arom. C); 55.3 (MeO); 52.2 (CH_2); 38.8 (MeN); 22.9 (Me). MS (QMS-MS/MS): 338.2 ([*M* – AcO]$^+$). Anal. calc. for $C_{15}H_{17}AgN_2O_4$ (397.17): C 45.36, H 4.31, N 7.05, Ag 27.16; found: C 44.99, H 3.95, N 7.01, Ag 26.96.

*(Acetato-κO){4,5-dichloro-1-[4-(methoxycarbonyl)benzyl]-3-methyl-2,3-dihydro-1*H*-imidazol-2-yl}silver* (**4e**). Compound **3e** (0.427 g, 1.00 mmol) was dissolved in CH_2Cl_2 (40 ml), and AgOAc (0.333 g, 2.00 mmol) was added. The mixture was stirred at r.t. for 2 d. The yellow AgBr suspension was filtered to give a light yellow coloured soln. The volatile components were removed *in vacuo* to produce a light yellow sticky solid. The sticky solid was washed with pentane (3 × 10 ml) and dried under reduced pressure for 4 h to yield **4e** (0.405 g, 0.868 mmol, 87%). Colourless solid. UV/VIS (MeOH): 230 (14964), 279 (6144). IR: 3436*m*, 2956*w*, 1717*s*, 1615*w*, 1576*s*, 1428*m*, 1384*m*, 1282*s*, 1180*w*, 1109m, 1018*w*, 801*w*, 757*w*, 668*w*. ^{1}H-NMR ($CDCl_3$): 8.03 (*d*, *J* = 8.3, 2 arom. H); 7.39 (*d*, *J* = 8.3, 2 arom. H); 5.40 (*s*, CH_2); 3.91 (*s*, MeN); 3.87 (*s*, MeO); 2.09 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 180.5 (NCN); 179.3 (C=O); 166.3 (C=O); 138.9, 130.6, 130.3, 127.6, 118.5, 117.5 (CCl, arom. C); 54.2 (MeO); 52.2 (CH_2); 38.1(MeN); 15.2 (Me). MS (QMS-MS/MS): 407.0 ([*M* – AcO]$^+$). Anal. calc. for $C_{15}H_{15}AgCl_2N_2O_4$ (466.06): C 38.66, H 3.24, N 6.01, Cl 15.21, Ag 23.14; found: C 38.42, H 3.52, N 5.97, Cl 15.13, Ag 23.10.

*(Acetato-κO){1-[4-(methoxycarbonyl)benzyl]-3-methyl-2,3-dihydro-1*H*-benzimidazol-2-yl}silver* (**4f**). Compound **3f** (0.361 g, 1.00 mmol) was dissolved in CH_2Cl_2 (35 ml), and AgOAc (0.333 g, 1.00 mmol) was added. The mixture was stirred at r.t. for 2 d. The yellow AgBr suspension was filtered to give a colourless soln. The volatile components were removed *in vacuo* to produce off white sticky solid. The solid was first washed with pentane and then with Et_2O, and dried under reduced pressure for 2 h to yield **4f** (0.400 g, 0.894 mmol, 89%). Colourless solid. UV/VIS (MeOH): 230 (12177), 275 (8183), 285 (7285). IR: 3447*m*, 1717*s*, 1613*w*, 1576*s*, 1434*w*, 1392*w*, 1347*w*, 1285*s*, 1707*m*, 1019*w*, 745*s*, 668*m*. ^{1}H-NMR ($CDCl_3$): 7.99 (*d*, *J* = 8.3, 2 CH=); 7.58–7.13 (*m*, CH=, C_6H_4–COOMe); 5.67 (*s*, CH_2); 4.10 (*s*, MeN); 3.89 (*s*, MeO); 2.09 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 182.4 (NCN); 179.1 (C=O); 166.3 (C=O); 139.8, 134.6, 133.4, 130.3, 130.3, 127.1, 124.3, 124.3, 111.7, 111.3 (CH=, C_6H_4–COOMe); 53.1 (MeO); 52.2 (CH_2); 35.9 (MeN); 22.7 (Me). MS (QMS-MS/MS): 388.1 ([*M* – AcO]$^+$). Anal. calc. for $C_{19}H_{19}AgN_2O_4$ (447.23): C 51.03, H 4.28, N 6.26, Ag 24.12; found: C 50.97, H 4.18, N, 6.34, Ag 24.13.

Antibacterial Studies. In vitro antibacterial activity of symmetrically and nonsymmetrically 4-(methoxycarbonyl)benzyl-substituted *N*-heterocyclic carbenes and their corresponding Ag complexes were screened preliminarily 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**–**3f** and **4a**–**4f**, the qualitative *Kirby–Bauer* disk-diffusion method was applied. All bacteria were individually cultured from a single colony in sterile LB (lysogeny broth) medium overnight at 37° (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 solns. (DMSO/H_2O 90 : 10) of every compound were prepared at 2.0 and 4.1 µM to be able to test the effect of different concentrations. Each plate was then tested with 5 µl and 7 µl of 2.0 µM soln., and 5 µl and 10 µl for the 4.1 µM soln. The plates were covered and placed in an incubator at 37° for 24 h. The plates were then 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 mm (see *Tables 3* and *4*).

Cytotoxicity Studies. Preliminary *in vitro* cell tests were performed on the human cancerous renal cell line Caki-1 in order to compare the cytotoxicity of the compounds presented in this paper. These cell lines were chosen based on their regular and long-lasting growth behaviour, which is similar to 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 (DME) 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 5000 cells/200 µl of medium and were incubated at 37° for 24 h to allow for exponential growth. Then, the compounds used for the testing were dissolved in the minimal amount of DMSO possible and diluted with medium to obtain stock solns. 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°. Then, the solns. were removed from the wells, and the cells were washed with PBS (phosphate buffer soln.), and fresh medium was added to the wells. Following a recovery period of 24-h incubation at 37°, individual wells were treated with 200 µl of a soln. of MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) in medium. The soln. consisted of 30 mg of MTT in 30 ml of medium. The cells were incubated for 3 h at 37°. The medium was then removed, and the purple formazan crystals were dissolved in 200 µl of 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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