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Synthesis, characterization, and antimicrobial activity of silver carbene complexes derived from 4,5,6,7-tetrachlorobenzimidazole against antibiotic resistant bacteria[†]

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Abstract

Silver *N*-heterocyclic carbene complexes have been shown to have great potential as antimicrobial agents, affecting a wide spectrum of both Gram-positive and Gram-negative bacteria. A new series of three silver carbene complexes (SCCs) based on 4,5,6,7-tetrachlorobenzimidazole has been synthesized, characterized, and tested against a panel of clinical strains of bacteria. The imidazolium salts and their precursors were characterized by elemental analysis, mass spectrometry, ¹H and ¹³C NMR spectroscopy, and single crystal X-ray diffraction. The silver carbene complexes, **SCC32**, **SCC33**, and **SCC34** were characterized by elemental analysis, ¹H and ¹³C NMR spectroscopy, and single crystal X-ray diffraction. These complexes proved highly efficacious with minimum inhibitory concentrations (MICs) ranging from 0.25 to 6 μg mL⁻¹. Overall, the complexes were effective against highly resistant bacteria strains, such as methicillin-resistant *Staphylococcus aureus* (MRSA), weaponizable bacteria, such as *Yersinia pestis*, and pathogens found within the lungs of cystic fibrosis patients, such as *Pseudomonas aeruginosa*, *Alcaligenes xylosoxidans*, and *Burkholderia gladioli*. **SCC33** and **SCC34** also showed clinically relevant activity against a silver-resistant strain of *Escherichia coli* based on MIC testing.

Introduction

Silver has been used throughout history for its antimicrobial properties. Silver coins were once placed into containers to prevent contamination of the water, and some surgical textbooks recommended silver being included with a surgeon's tools to treat ulcers and abscesses.^{1–3} Silver nitrate was used in the 19th century to promote wound healing on burn victims.⁴ This practice continued with Fox's advent of silver sulfadiazine in 1965, which is still used in burn wards worldwide.^{5,6} Recently, silver has been used in several different applications. Catheters, surgical instruments, and surgical masks have been embedded with silver to prevent nosocomial infections.^{7–9} Sutures and wound dressings have also been

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embedded with silver, allowing for a 50–60% reduction in infections.^{10,11} Silver itself has no known use within the body and is rapidly excreted, making it attractive as an internalized therapeutic agent.²

Widespread use of silver has led to bacterial resistance to silver.¹² This has been observed in burn wards using silver salts and in hospitals that utilize silver-coated catheters.^{13,14} Expression of resistance is erratic in its occurrence; however several different clinical strains have been isolated.¹⁵ The plasmid pMG101 has been shown to bestow resistance to several heavy metals including the silver ion, and when introduced into *E. coli* allows for growth in >0.6 mM solution of silver ions.^{16,17} In addition, the plasmid confers resistance to several organic-based antibiotics, including ampicillin, chloramphenicol, tetracycline, streptomycin, and sulphonamides, making it the standard model for antibacterial resistance.¹⁸

N-Heterocyclic carbene (NHC) studies first began with the metallation of imidazol-2-ylidenes by Wanzlick and Öfele in 1968.^{19,20} Stemming from this research, Arduengo discovered and isolated the first stable free carbene in 1991, inspiring a plethora of research.²¹ Since then, NHCs have proven to be versatile ligands in both transition metal and main group coordination chemistry.^{22,23} The first silver carbene complex was synthesized by Arduengo in 1993 for use in catalysis.²⁴ Arduengo's method required harsh conditions and often led to decomposition of the metal complex. To remedy this problem, Wang and Lin developed a synthetic method that generated the carbene *in situ* by using a silver salt as a base.²² Silver carbene complexes (SCCs) are currently being explored due to their high efficacy against antibiotic-resistant bacteria, biosafety level 3 bacteria, and various types of cancer.^{25–29}

Two main traits are prevalent in the previous studies of the SCCs. Modification of the substituents on the ligand can improve the efficacy of the drugs by aiding in drug transport across the cell membrane, increasing the viable dose, or enabling targeting of the drug.³⁰ Stability of the SCCs has been improved by the use of electron withdrawing groups at the 4 and 5 positions of the imidazolin-2-ylidene ligand.²⁷ Following these trends, we have synthesized three new silver NHC complexes expanding upon these two traits. Concurrent with our research, Özdemir and Tacke have used benzimidazole to form antimicrobial NHC complexes with silver.^{31,32} The benzene ring of benzimidazole allows for the addition of two more of the sigma-withdrawing pi-donating chlorine atoms than imidazole; increasing the stability of the NHCs. Herein, we report the synthesis of three SCCs based upon 4,5,6,7-tetrachlorobenzimidazole. These three SCCs have been successfully synthesized, characterized, and tested for their antimicrobial activity.

Results and discussion

Synthesis of new SCCs

4,5,6,7-Tetrachlorobenzimidazole (**1**) was synthesized based on a literature procedure.³³ Benzimidazole was dissolved in a mixture of nitric and hydrochloric acids and allowed to reflux for 36 h to produce **1**. Synthesis of (1,3-dimethyl-4,5,6,7-tetrachlorobenzimidazole-2-ylidene)silver(I) acetate (**SCC32**) was performed as shown in Scheme 1. The imidazolium salt was prepared *via* methylation of **1** with excess iodomethane to give 1,3-dimethyl-4,5,6,7-tetrachlorobenzimidazole iodide (**2**).²⁸ The reaction of **2** with silver acetate gave the water-insoluble **SCC32** in a 76% yield. (1-Hydroxyethyl-3-methyl-4,5,6,7-tetrachlorobenzimidazole-2-ylidene)silver(I) acetate (**SCC33**) was synthesized in a similar fashion (Scheme 2). Deprotonation of **1** with potassium hydroxide followed by the addition of 2-iodoethanol yielded 1-Hydroxyethyl-4,5,6,7-tetrachlorobenzimidazole (**3**). Methylation of **3** with excess iodomethane and subsequent reaction with two equivalents of silver acetate gave **SCC33** in a 90% yield (Scheme 2). This addition of the hydroxyethyl group allowed

for increased water solubility. Synthesis of the lipophilic compound (1-methylnaphthyl-3-methyl-4,5,6,7-tetrachlorobenzimidazole-2-ylidene)silver(I) acetate (**SCC34**) is illustrated in Scheme 3. Compound **1** was deprotonated with potassium carbonate and reacted with one equivalent of 2-(bromomethyl) naphthalene to produce 1-methylnaphthyl-4,5,6,7-tetrachlorobenzimidazole (**5**). Compound **5** was methylated with methyl trifluoromethanesulfonate under an inert atmosphere and combined with tetra-*n*-butylammonium iodide to form **6**. This was subsequently reacted with two equivalents of silver acetate to form **SCC34** in a 37% yield.

For all compounds, ^1H and ^{13}C NMR spectroscopy was used to confirm the formation of the imidazolium cation and the SCC. The loss of the resonance of the imidazolium cation proton from the ^1H NMR spectra of **2**, **4**, and **6** (occurring at 9.89, 9.94, and 9.46 ppm, respectively) was the most informative change in the spectra of all three silver complexes. The most informative change in the ^{13}C NMR spectra was a shift of the imidazolium cation carbon resonance from around 148 ppm to around 190 ppm, signifying the formation of the silver complexes. All silver complexes were also verified by single crystal X-ray diffraction. Single crystals of some of the SCC precursors were obtained and the X-ray crystallographic data is available in the ESI.[†]

Single crystals of **SCC32**, **SCC33**, and **SCC34** suitable for X-ray diffraction were obtained from the slow evaporation of a concentrated sample in chloroform, dry methylene chloride, and methanol, respectively (Fig. 1–3). The asymmetric unit of these contain one SCC. Additionally, the asymmetric unit of **SCC33** contains a disordered solvent molecule, which forms a network of weak hydrogen bonding with three molecules of **SCC33**. The asymmetric unit of **SCC34** also contains a solvent molecule; however the solvent interacts with only two molecules of **SCC34**.

All bond lengths and angles between the metal center to the carbene carbon and oxygen atoms of the acetate are consistent with previously reported silver carbene complexes.^{25,26} Both **SCC33** and **SCC34** show short Ag–Ag contacts (3.25 Å and 3.26 Å, respectively), however this is not observed in **SCC32**. Additionally, while the metal center–acetate bond length of **SCC32** was consistent with previously reported structures, it was found to be longer than that of **SCC33** and **SCC34** (2.155 Å compared to 2.104 Å and 2.122 Å). The lack of the Ag–Ag contact appears to be the reason for this increase in bond length.

***In vitro* efficacy determination of new SCCs**

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of **SCC32**, **SCC33**, and **SCC34** against strains of *Pseudomonas aeruginosa*, *Alcaligenes xylosoxidans*, *Stenotrophomonas maltophilia*, *Staphylococcus aureus*, *Yersinia pestis*, *Burkholderia gladioli*, *Burkholderia multivorans*, and *Escherichia coli* species were determined by broth microdilution (Table 1). The MIC₉₀ of **SCC32**, **SCC33**, and **SCC34** were 1 $\mu\text{g mL}^{-1}$, 2 $\mu\text{g mL}^{-1}$, and 4 $\mu\text{g mL}^{-1}$, respectively, for non-silver-resistant organisms tested. The MIC for the J53 strain of *E. coli* lacking the silver-resistant plasmid was 0.5 $\mu\text{g mL}^{-1}$ for both **SCC32** and **SCC33** and 2 $\mu\text{g mL}^{-1}$ for **SCC34**. In contrast, the MIC of **SCC32**, **SCC33**, and **SCC34** for J53 containing the silver-resistant plasmid, pMG101, was >20, 8, and 10 $\mu\text{g mL}^{-1}$, respectively. Therefore, the antimicrobial activity of the SCCs can be primarily attributed to the silver functionality. Following the determination of the MICs, the minimum bactericidal concentrations for each of the SCCs against the panel of bacteria was determined (Table 1). With the exception of the silver-resistant *E. coli* strain, all three SCCs appeared to be bactericidal for all of the strains tested. The bactericidal concentrations for all three SCCs were observed to be $\leq 6 \mu\text{g mL}^{-1}$, thus indicating that the SCCs are capable of killing numerous bacterial strains at clinically achievable

concentrations. These compounds show comparable or enhanced activity when compared to previously reported silver complexes tested against the same clinical strains.^{26,28,34} Interestingly, the growth of the silver-resistant *E. coli* J53+pMG101 strain was found to be inhibited by **SCC33** and **SCC34**, but not by **SCC32**, thus indicating that the substituents present on the 4,5,6,7-tetrachlorobenzimidazole carrier molecule may play an important synergistic role and may contribute to the antimicrobial activity of the therapeutic molecule. Furthermore, **SCC34** was found to be bactericidal against the same strain. Subsequent testing showed the imidazolium cation precursors had no activity against the *E. coli* J53+pMG101 strain, though **6** showed some activity against the two MRSA strains. The primary structural difference between the two SCCs is the presence of the methylnaphthyl substituent on the carrier molecule for **SCC34** as compared to the hydroxyethyl substituent in the case of **SCC33**. These results point towards structure-dependent antimicrobial efficacy of the SCCs. Furthermore, the results of **SCC34** against the J53+pMG101 strain compare favorably to those of **SCC23** (Fig. 4), currently the most effective SCC against the J53+pMG101 strain (with MIC values of 10 and 6 $\mu\text{g mL}^{-1}$ respectively).³⁴ This suggests that the efficacy against the silver-resistant J53+pMG101 strain is a result of the presence of methylnaphthyl substituents on the imidazolium carriers.

Experimental

General considerations

All reactions were carried out under aerobic conditions unless otherwise specified. Benzimidazole, 2-iodoethanol, iodomethane, methyl trifluoromethanesulfonate, tetra-n-butylammonium iodide, and silver acetate were purchased from Alfa Aesar. 2-(Bromomethyl)naphthalene was purchased from Waterstone Technologies. All solvents were purchased from Fisher Scientific. All reagents were used as received without further purification. All solvents were used as received with the exception of the dry methylene chloride used in the synthesis of **6**, which was collected using a PurSolv solvent purification system by Innovative Technologies. Melting points were obtained on a MelTemp apparatus. ¹H and ¹³C NMR spectra were collected on a Varian 500 MHz instrument with all spectra referenced to deuterated solvents. Mass spectrometry was performed by the University of Akron mass spectrometry lab. Elemental Analysis was performed by the University of Illinois at Urbana-Champaign. FTIR data was collected on a Thermo Scientific Nicolet iS5 Infrared Spectrometer. Crystals of the compounds were coated in paratone oil, mounted on a CryoLoop and placed on a goniometer under a stream of nitrogen. Crystal structure data sets were collected on a Bruker APEX CCD diffractometer with graphite-monochromated Mo K α radiation ($\lambda = 0.71073 \text{ \AA}$) or a Bruker APEX II Duo CCD system equipped with a Cu ImuS micro-focus source ($\lambda = 1.54178 \text{ \AA}$). The unit cell was determined by using reflections from three different orientations. The data was integrated using SAINT.^{35,36} An empirical absorption correction and other corrections were applied to the data using multi-scan SADABS.³⁵ Structure solution, refinement, and modelling were accomplished by using the Bruker SHELXTL package.^{36,37} The structure was determined by full-matrix least-squares refinement of F^2 and the selection of the appropriate atoms from the generated difference map. Hydrogen atom positions were calculated and $U_{\text{iso}}(H)$ values were fixed according to a riding model.

Synthesis of 4,5,6,7-tetrachlorobenzimidazole (1)—Benzimidazole (5.0 g, 42 mmol) was dissolved into 1.2 L of concentrated hydrochloric acid. Concentrated nitric acid (600 mL) was added and the solution was refluxed for 36 h. After cooling to room temperature, a light yellow precipitate formed. This precipitate was collected and recrystallized from cold ethanol twice for purification, leaving 9.6 g of a white powder (90% yield). Mp: 321–324 °C. Found: C, 32.9; H, 0.5; N, 10.6 Calc. for C₇H₂Cl₄N₂: C, 32.85; H, 0.8; N, 10.95%. FTIR

(KBr, cm^{-1}) $\nu(\text{CN})$ 1249, $\nu(\text{CCl})$ 620. ^1H NMR (500 MHz, DMSO-d_6) 7.79 (1H, s, NH) $^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, DMSO-d_6) 156.2 (NCHN), 143.5 (Ar–Cl), 118.6 (Ar–Cl), 117.6 (Ar). MS: m/z = 256.8 (theor. for $\text{C}_7\text{H}_3\text{Cl}_4\text{N}_2^+$ = 256.905).

Crystals suitable for single crystal X-ray diffraction were grown from a concentrated ethanol solution. Crystal data for **1**: $\text{C}_7\text{H}_2\text{Cl}_4\text{N}_2$, M_r = 255.91, triclinic, a = 7.4984(19) Å, b = 15.060(4) Å, c = 16.612(4) Å, α = 108.611(4)°, β = 94.721(4)°, γ = 90.265(4)°, V = 1770.9(8) Å³, T = 100(2) K, space group $P\bar{1}$, Z = 8, $\mu(\text{Mo K}\alpha)$ = 1.280 mm^{-1} , 13 921 reflections measured, 7025 independent reflections (R_{int} = 0.0275). The final R_1 values were 0.0377 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1055 ($I > 2\sigma(I)$). The final R_1 values were 0.0449 (all data). The final $wR(F^2)$ values were 0.1227 (all data). The goodness of fit on F^2 was 1.084.

Synthesis of 1,3-dimethyl-4,5,6,7-tetrachlorobenzimidazolium iodide (2)—

Compound **1** (1 g, 4 mmol) was dissolved into 15 mL of DMF and potassium carbonate (1.1 g, 7.7 mmol) was added. The mixture was allowed to stir for 30 min to ensure deprotonation, and the excess base was filtered off. Iodomethane (4.0 mL, 43 mmol) was added and the reaction was heated at 80 °C overnight. The volatile components were removed *via* rotary evaporation and the resulting solid was washed with water to yield a yellow solid (0.70 g, 41% yield). Mp: 242–244 °C. Found: C, 26.3; H, 1.5; N, 6.5. Calc. for $\text{C}_9\text{H}_7\text{Cl}_4\text{N}_2\text{I}$: C, 26.2; H, 1.7; N, 6.8%. FTIR (KBr, cm^{-1}) $\nu(\text{CN})$ 1270, $\nu(\text{CCl})$ 666. ^1H NMR (500 MHz, DMSO-d_6) 9.89 (1H, s, NCHN), 4.28 (6H, s, CH_3). $^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, DMSO-d_6) 148.6 (NCN), 130.9 (Ar–Cl), 129.6 (Ar–Cl), 119.1 (Ar), 37.7 (CH_3). MS: m/z = 284.8 (theor. for $\text{C}_9\text{H}_7\text{Cl}_4\text{N}_2^+$ = 284.9).

Synthesis of (1,3-dimethyl-4,5,6,7-tetrachlorobenzimidazole-2-ylidene)silver(I) acetate (SCC32)—

Compound **2** (0.61 g, 1.5 mmol) was dissolved into 40 mL of methylene chloride. Silver acetate (0.49 g, 3.0 mmol) was added, and the reaction was stirred for 30 min. A yellow precipitate, presumed to be silver iodide, was filtered off, and the solvent was removed *via* rotary evaporation to give an off-white product (0.51 g, 76% yield). Mp: 253–256 °C. Found: C, 29.2; H, 1.8; N, 6.1. Calc. for $\text{C}_9\text{H}_7\text{Cl}_4\text{N}_2\text{I}$: C, 26.2; H, 1.7; N, 6.8%. FTIR (KBr, cm^{-1}) $\nu(\text{C=O})$ 17.19, $\nu(\text{CN})$ 1272, $\nu(\text{CCl})$ 616. ^1H NMR (500 MHz, DMSO-d_6) 4.31 (6H, s, NCH_3), 1.81 (3H, s, CH_3). $^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, DMSO-d_6) 179.2 (NCN), 172.6 (COO), 135.2 (Ar–Cl), 131.5 (Ar–Cl), 116.8 (Ar–Cl), 21.5 (CH_3). Crystals suitable for single crystal X-ray diffraction were grown from a concentrated chloroform solution.

Crystal data for **SCC32**: $\text{C}_{11}\text{H}_9\text{Cl}_4\text{N}_2\text{O}_2\text{AgI}$, M_r = 450.87, monoclinic, a = 8.404(2) Å, b = 5.5910(16) Å, c = 15.250(5) Å, β = 101.844(4)°, V = 701.3(4) Å³, T = 100(2) K, space group $P2$ (1), Z = 2, $\mu(\text{Mo K}\alpha)$ = 2.198 mm^{-1} , 5411 reflections measured, 2767 independent reflections (R_{int} = 0.0427). The final R_1 values were 0.0367 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0809 ($I > 2\sigma(I)$). The final R_1 values were 0.0415 (all data). The final $wR(F^2)$ values were 0.0833 (all data). The flack parameter was 0.02(4). The goodness of fit on F^2 was 0.999.

Synthesis of 1-hydroxyethyl-4,5,6,7-tetrachlorobenzimidazole (3)—

Compound **1** (1.7 g, 6.5 mmol) was placed in a Chemglass screw cap pressure tube and dissolved in 10 mL of THF. Potassium hydroxide (0.44 g, 7.8 mmol) was added and the mixture was heated to 90 °C for 30 min. 2-Iodoethanol (2 mL, 25.6 mmol) was added, the pressure tube was closed, and the reaction was heated at 90 °C for 72 h. The reaction was cooled and a white precipitate, presumed to be potassium iodide, was filtered off and the remaining solvent was removed *via* rotary evaporation. The resulting solid was washed with cold ethanol and then benzene to produce a white powder (1.46 g, 75% yield). Mp: 254–255 °C. Found: C, 35.9;

H, 1.7; N, 8.9 Calc. for $C_9H_6Cl_4N_2O_1$: C, 36.0; H, 2.0; N, 9.3%. FTIR (KBr, cm^{-1}) $\nu(OH)$ 3294, $\nu(CN)$ 1307 and 1064, $\nu(CCl)$ 671. 1H NMR (500 MHz, DMSO- d_6) 8.40 (1H, s, NCHN), 4.98 (1H, t, OH), 4.55 (2H, t, CH_2), 3.75 (2H, td, CH_2). $^{13}C\{^1H\}$ NMR (125 MHz, DMSO- d_6) 149.4 (NCHN), 141.3 (Ar-Cl), 129.7 (Ar-Cl), 125.4 (Ar-Cl), 124.1 (Ar-Cl), 122.4 (Ar), 114.9 (Ar), 60.3 (CH_2), 48.8 (CH_2). MS: m/z = 300.9 (theor. for $C_9H_6Cl_4N_2O_1^+$ = 299.97).

Synthesis of 1-hydroxyethyl-3-methyl-4,5,6,7-tetrachlorobenzimidazolium

iodide (4)—Compound **3** (1.0 g, 3.6 mmol) was dissolved in 17 mL of DMF. Iodomethane (1.00 mL, 10.8 mmol) was added and the solution was heated at 90 °C for 20 h. The solution was then cooled and a light yellow precipitate was collected *via* filtration and then immediately washed with cold ethanol to give a light yellow powder (1.59 g, 62% yield). Mp: 218–220 °C. Found: C, 27.1; H, 1.9; N, 6.1 Calc. for $C_{10}H_9Cl_4N_2O_1I_1$: C, 27.2; H, 2.05; N, 6.3%. FTIR (KBr, cm^{-1}) $\nu(OH)$ 3304, $\nu(CN)$ 1305 and 1075, $\nu(CCl)$ 673. 1H NMR (500 MHz, DMSO- d_6) 9.94 (1H, s, NCHN), 5.17 (1H, t, OH), 4.79 (2H, t, CH_2), 4.33 (3H, s, CH_3), 3.85 (2H, t, CH_2). $^{13}C\{^1H\}$ NMR (125 MHz, DMSO- d_6) 148.1 (NCN), 130.7 (Ar-Cl), 130.5 (Ar-Cl), 129.5 (Ar-Cl), 128.5 (Ar-Cl), 118.7 (Ar), 118.2 (Ar), 58.9 (CH_2), 52.1 (CH_2), 37.3 (CH_3). MS: m/z = 312.9 (theor. for $C_7H_3Cl_4N_2^+$ = 312.9).

Crystals suitable for X-ray diffraction were grown from a concentrated chloroform solution. Crystal data for **4**: $C_{10}H_9Cl_4N_2O_1I_1$, M_r = 441.89, monoclinic, a = 9.2405(9) Å, b = 6.9153(7) Å, c = 22.244(2) Å, β = 99.331(2)°, V = 1402.6(2) Å³, T = 100(2) K, space group $P2(1)/c$, Z = 4, $\mu(Mo K\alpha)$ = 3.033 mm⁻¹, 11 737 reflections measured, 3346 independent reflections (R_{int} = 0.0311). The final R_1 values were 0.0257 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0630 ($I > 2\sigma(I)$). The final R_1 values were 0.0276 (all data). The final $wR(F^2)$ values were 0.0639 (all data). The goodness of fit on F^2 was 1.087.

Synthesis of (1-hydroxyethyl-3-methyl-4,5,6,7-tetrachlorobenzimidazole-2-ylidene) silver(I) acetate (SCC33)

—Compound **4** (0.16 g, 0.37 mmol) was dissolved in hot ethanol. The solution was cooled to room temperature, silver acetate (0.13 g, 0.75 mmol) was added and the reaction was stirred for 1 h. A yellow precipitate, presumed to be silver iodide, was filtered off and the volatiles were removed *via* rotary evaporation to give a white solid (0.16 g, 90% yield). Mp: 210–212 °C. Found: C, 30.3; H, 2.1; N, 5.7 Calc. for $C_{12}H_{11}Cl_4N_2O_3Ag_1$: C, 30.0; H, 2.3; N, 5.8%. FTIR (KBr, cm^{-1}) $\nu(C=O)$ 1707, $\nu(CN)$ 1324 and 1081, $\nu(CCl)$ 679. 1H NMR (500 MHz, DMSO- d_6) 8.40 (1H, s, NCHN), 4.98 (1H, t, OH), 4.55 (2H, t, CH_2), 3.75 (2H, dt, CH_2). $^{13}C\{^1H\}$ NMR (125 MHz, DMSO- d_6) 197.0 (NCN), 131.6 (Ar-Cl), 130.9 (Ar-Cl), 128.1 (Ar-Cl), 127.8 (Ar-Cl), 116.8 (Ar), 116.2 (Ar), 60.9 (CH_2), 53.3 (CH_2), 23.3 (CH_3).

Crystals suitable for single crystal X-ray diffraction were grown from a concentrated dry methylene chloride solution. Crystal data for **SCC33**: $C_{13}H_{13}Cl_6N_2O_3Ag_1$, M_r = 565.82, monoclinic, a = 8.616(3) Å, b = 13.028(5) Å, c = 17.176(6) Å, β = 92.684(5)°, V = 1925.9(12) Å³, T = 100(2) K, space group $P2(1)/c$, Z = 4, $\mu(Mo K\alpha)$ = 1.951 mm⁻¹, 14 692 reflections measured, 3909 independent reflections (R_{int} = 0.0908). The final R_1 values were 0.0528 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1296 ($I > 2\sigma(I)$). The final R_1 values were 0.0655 (all data). The final $wR(F^2)$ values were 0.1389 (all data). The goodness of fit on F^2 was 1.015.

Synthesis of 1-methylnaphthyl-4,5,6,7-tetrachlorobenzimidazole (5)

—Compound **1** (0.5 g, 2 mmol) was dissolved into 3 mL of DMF. Potassium carbonate (0.30 g, 2.2 mmol) was added and the mixture was stirred for 30 min to ensure deprotonation. 2-(Bromomethyl)naphthalene (0.44 g, 2.0 mmol) was then added and the reaction was heated at 80 °C for 1 h. The precipitate was then filtered off and the volatiles were removed *via*

rotary evaporation. The resulting yellow solid was washed with methanol to yield a white powder (0.43 g, 55% yield). Mp: 175–177 °C. Found: C, 54.4; H, 2.3; N, 7.0. Calc. for $C_{18}H_{10}Cl_4N_2$: C, 54.6; H, 2.5; N, 7.1%. FTIR (KBr, cm^{-1}) $\nu(CN)$ 1307 and 1104, $\nu(CCl)$ 665. 1H NMR (500 MHz, $DMSO-d_6$) 8.78 (1H, s, NCHN), 7.90 (3H, m, Ar), 7.50 (2H, m, Ar), 7.47 (1H, d, Ar), 7.33 (1H, d, Ar), 5.97 (2H, s, CH_2). $^{13}C\{^1H\}$ NMR (125 MHz, $DMSO-d_6$) 149.8 (NCN), 141.9 (Ar–Cl), 135.5 (Ar–Cl), 133.3 (Ar–Cl), 132.8 (Ar–Cl), 130.2 (Ar), 128.9 (Ar), 128.2 (Ar), 127.9 (Ar), 126.8 (Ar), 126.6 (Ar), 126.3 (Ar), 125.2 (Ar), 125.1 (Ar), 124.7 (Ar), 123.2 (NC), 115.5 (NC), 49.9 (CH_2).

Crystals suitable for single crystal X-ray diffraction were grown from a concentrated chloroform solution. Crystal data for **5**: $C_{18}H_{10}Cl_4N_2$, $M_r = 396.08$, triclinic, $a = 4.979(5)$ Å, $b = 12.523(13)$ Å, $c = 13.184(13)$ Å, $\alpha = 84.139(16)^\circ$, $\beta = 87.926(16)^\circ$, $\gamma = 83.410(16)^\circ$, $V = 812.1(14)$ Å³, $T = 100(2)$ K, space group $P\bar{1}$, $Z = 2$, $\mu(Mo\ K\alpha) = 0.730\ mm^{-1}$, 6080 reflections measured, 3170 independent reflections ($R_{int} = 0.0531$). The final R_1 values were 0.0596 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1484 ($I > 2\sigma(I)$). The final R_1 values were 0.0830 (all data). The final $wR(F^2)$ values were 0.1622 (all data). The goodness of fit on F^2 was 0.937.

Synthesis of 1-methylnaphthyl-3-methyl-4,5,6,7-tetrachlorobenzimidazolium iodide (6)—Synthesis of **6** was conducted under anaerobic conditions. Compound **5** (0.77 g, 2.0 mmol) was dissolved into 30 mL of dry methylene chloride combined with methyl trifluoromethanesulfonate (0.25 mL, 2.3 mmol). The resulting mixture was refluxed for 20 h, during which time a precipitate formed. The precipitate was collected, washed with benzene, and redissolved in methanol that contained tetra-*n*-butylammonium iodide (0.59 g, 1.5 mmol). A white solid that precipitated out was then collected and washed with cold ethanol to yield a white powder (0.54 g, 50% yield). Mp: 187–189 °C. Found: C, 42.4; H, 2.2; N, 5.0. Calc. for $C_{19}H_{13}Cl_4N_2I$: C, 42.4; H, 2.4; N, 5.2%. FTIR (KBr, cm^{-1}) $\nu(CN)$ 1305 and 1104, $\nu(CCl)$ 669. 1H NMR (500 MHz, $DMSO-d_6$) 9.46 (1H, s, NCHN), 7.98 (4H, m, Ar), 7.57 (3H, m, Ar), 5.66 (2H, s, NCH_2), 3.84 (3H, s, NCH_3). $^{13}C\{^1H\}$ NMR (125 MHz, $DMSO-d_6$) 148.3 (NCN), 132.7 (Ar–Cl), 132.6 (Ar–Cl), 131.8 (Ar–Cl), 130.8 (Ar–Cl), 129.8 (Ar), 128.6 (Ar), 128.5 (Ar), 127.7 (Ar), 127.6 (Ar), 126.6 (Ar), 125.9 (Ar), 124.7 (Ar), 52.2 (CH_2), 37.7 (CH_3). MS: $m/z = 408.8$ (theor. for $C_{19}H_{13}Cl_4N_2^+ = 408.98$).

Crystals suitable for single crystal X-ray diffraction were grown from a concentrated solution of chloroform. Crystal data for **6**: $C_{19}H_{13}Cl_4N_2I$, $M_r = 538.01$, monoclinic, $a = 24.500(3)$ Å, $b = 12.9443(12)$ Å, $c = 14.1288(13)$ Å, $\beta = 117.003(2)^\circ$, $V = 3992.3(7)$ Å³, $T = 100(2)$ K, space group $C2/c$, $Z = 8$, $\mu(Mo\ K\alpha) = 2.146\ mm^{-1}$, 17 009 reflections measured, 4569 independent reflections ($R_{int} = 0.0396$). The final R_1 values were 0.0330 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0798 ($I > 2\sigma(I)$). The final R_1 values were 0.0404 (all data). The final $wR(F^2)$ values were 0.0850 (all data). The goodness of fit on F^2 was 1.048.

Synthesis of (1-methylnaphthyl-3-methyl-4,5,6,7-tetrachlorobenzimidazole-2-ylidene) silver(I) acetate (SCC34)—Compound **6** (0.54 g, 1.0 mmol) was dissolved into 40 mL of hot methanol and silver acetate (0.33 g, 2.0 mmol) was added. The reaction was stirred for 1 h at room temperature. A yellow solid, presumed to be silver iodide, was filtered off and the volatiles were removed *via* rotary evaporation to obtain a white powder (0.21 g, 37% yield). Mp: 197–198 °C. Found: C, 43.75; H, 3.0; N, 4.7. Calc. for $C_{21}H_{15}Cl_4N_2O_2Ag$: C, 43.7; H, 2.6; N, 4.85%. FTIR (KBr, cm^{-1}) $\nu(C=O)$ 1714, $\nu(CN)$ 1272 and 1033, $\nu(CCl)$ 671. 1H NMR (500 MHz, $DMSO-d_6$) 7.79 (4H, m, Ar), 7.34 (3H, m, Ar), 6.191 (2H, s, CH_2), 4.38 (3H, s, CH_3), 1.78 (3H, s, CH_3). $^{13}C\{^1H\}$ NMR (125 MHz, $DMSO-d_6$) 197.5 (NCN), 175.6 (COO), 134.8 (Ar–Cl), 132.8 (Ar–Cl), 132.3 (Ar–Cl), 131.9 (Ar–Cl), 130.8 (Ar), 128.4 (Ar), 128.3 (Ar), 128.3 (Ar), 128.2 (Ar), 128.1 (Ar), 127.7 (Ar), 127.5 (Ar), 54.0 (CH_2), 23.3 (CH_3), 15.1 (CH_3).

Crystals suitable for single crystal X-ray diffraction were grown from a concentrated methanol solution. Crystal data for **SCC34**: $C_{22}H_{19}Cl_4N_2O_3Ag$, $M_r = 609.06$, triclinic, $a = 10.1480(4) \text{ \AA}$, $b = 11.5407(4) \text{ \AA}$, $c = 12.1076(5) \text{ \AA}$, $\alpha = 106.806(1)^\circ$, $\beta = 105.315(1)^\circ$, $\gamma = 111.769(1)^\circ$, $V = 1145.37(8) \text{ \AA}^3$, $T = 100(2) \text{ K}$, space group $P\bar{1}$, $Z = 2$, $\mu(\text{Cu K}\alpha) = 11.596 \text{ mm}^{-1}$, 7216 reflections measured, 3486 independent reflections ($R_{\text{int}} = 0.0227$). The final R_1 values were 0.0239 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0662 ($I > 2\sigma(I)$). The final R_1 values were 0.0243 (all data). The final $wR(F^2)$ values were 0.0667 (all data). The goodness of fit on F^2 was 1.323.

Bacteria

The laboratory strain PAO1-V was provided by Dr Maynard Olson (University of Washington, Seattle). The mucoid clinical isolate of *P. aeruginosa* PA M57-15 was provided by Dr Thomas Ferkol (Washington University, St. Louis, MO). The PA HP3, PA RR05, PA LF05, AX RE05, AX 22, SM AH08, SA EH06, SA LL06, YP1-1CO92-LCR-, BG 80, and BM 54 strains were cultured from the sputum of cystic fibrosis patients at St. Louis Children's Hospital. The silver-sensitive and silver-resistant *E. coli* strains, J53 and J53+pMG101 respectively, were provided by Dr Simon Silver (University of Chicago, Chicago, IL). The J53 strain is known to be sensitive to killing by silver cations and serves as a positive control. In contrast, the J53+pMG101 strain is a J53 derivative that harbors the pMG101 plasmid originally conferring silver resistance to a burn ward isolate of *Salmonella* and serves as a negative control. All bacterial strains were maintained as glycerol stocks at -80°C .

In vitro antimicrobial activity

Minimum inhibitory concentrations were determined by broth microdilution method as previously described by a standard Clinical and Laboratory Standards Institute (CLSI) protocol.³⁸ Briefly, bacteria were streaked from frozen glycerol stocks onto TSA or blood agar plates and incubated overnight at 37°C . Colonies from the fresh plates were suspended in the CLSI standard M–H broth to an optical density at 650 nm (OD_{650}) of 0.2 and grown at 37°C in a shaking incubator at 200 rpm to an OD_{650} of 0.4, which corresponds to $\sim 5 \times 10^8$ colony forming units (CFU) mL^{-1} . The bacteria were diluted in the broth to a concentration of 10^5 in 100 μL , which was added to triplicate wells of a 96-well plate, containing 100 μL of **SCC32**, **SCC33** or **SCC34** solution at various concentrations. The dilutions for SCCs were prepared from 1 mg mL^{-1} stocks in DMSO with the final solution composition being 95% sterile water and 5% DMSO by volume. Solutions containing 95% sterile water and 5% DMSO but no SCCs were used as the appropriate control. The final concentrations tested were 0.125, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 15, and 20 $\mu\text{g mL}^{-1}$. The plates were incubated overnight at 37°C . The MIC was the lowest of these concentrations, at which each of the triplicate wells in each 96-well plate was clear after 16–24 h incubation. Each triplicate measurement was performed at least in duplicate for a minimum of six separate measurements. The MBC of various SCCs was determined by plating the wells with growth inhibition on TSA or blood agar plates and noting the lowest concentration that resulted in no growth after an overnight incubation at 37°C .

Conclusions

The data presented show a large increase in activity against bacteria in comparison to previously reported benzimidazole-based silver carbene complexes. Like previously reported SCCs that contain electron withdrawing groups, all new SCCs showed single-digit $\mu\text{g mL}^{-1}$ concentration MICs *in vitro*. The addition of a hydroxyethyl substituent allowed for relative water solubility, providing a method of treatment that does not require any carrier for the drug. **SCC33** inhibited growth of the silver-resistant J53+pMG101 *E. coli*

strain, while **SCC34** showed bactericidal activity against silver-resistant bacteria. This demonstrates the ability of the imidazolium cation carrier to enhance the antimicrobial activity of the silver cation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
Thermal ellipsoid plot of **SCC32** with thermal ellipsoids drawn at 50% probability.
Hydrogen atoms were removed for clarity.

Fig. 2.
Thermal ellipsoid plot of **SCC33** with thermal ellipsoids drawn at 50% probability.
Hydrogen atoms and solvent methylene chloride molecule were removed for clarity.

Fig. 3.
Thermal ellipsoid plot of **SCC34** with thermal ellipsoids drawn at 50% probability.
Hydrogen atoms and solvent methanol molecule were removed for clarity.

Fig. 4.
Schematic representation of SCC23.

Scheme 1.
Synthesis of **SCC32**.

Scheme 2.
Synthesis of **SCC33**.

Scheme 3.
Synthesis of **SCC34**.

Table 1
MIC/MBC of tetrachlorobenzimidazole-based SCCs against clinical isolate bacterial strains

Bacterial species isolate	SCC32 ($\mu\text{g mL}^{-1}$)		SCC33 ($\mu\text{g mL}^{-1}$)		SCC34 ($\mu\text{g mL}^{-1}$)	
	MIC	MBC	MIC	MBC	MIC	MBC
<i>Pseudomonas aeruginosa</i>						
PA 01-V	0.5	6	2	2	2	4
PA M57-15	1	2	1	4	0.5	2
PA RR05	0.5	1	0.5	1	0.5	2
PA HP3	0.25	1	2	6	0.5	6
PA LF05	0.5	2	0.5	1	1	2
<i>Alcaligenes xylosoxidans</i>						
AX 22	0.5	2	0.5	2	1	4
AX RE05	0.25	0.5	0.5	1	1	1
<i>Stenotrophomonas maltophilia</i>						
SM AH08	0.5	1	0.5	6	4	6
<i>Methicillin-resistant Staphylococcus aureus</i> (MRSA)						
SA LL06	1	6	1	2	4	6
SA EH06	1	6	1	4	2	6
<i>Yersinia pestis</i>						
YPI-CO92-LCR-	1	1	0.5	1	1	1
<i>Burkholderia gladioli</i>						
BG 80	1	4	2	4	1	2
<i>Burkholderia multivorans</i>						
BM 54	0.25	0.5	2	4	4	6
<i>Escherichia coli</i>						
J53	0.5	1	0.5	1	2	2
J53+ipMG101	>20	>20	8	>20	10	15