

Research Article

Novel Silver(I) Bis-Benzimidazole Complexes: Design, Synthesis, and *In Vitro* Antibacterial EvaluationGhanem Shaker Aljebouri^{1*}¹Department of Chemistry, College of Science, University of Baghdad, Baghdad, 10071, Iraq.*Corresponding author: ghanem.shaker@sc.uobaghdad.edu.iq

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Abstract

A series of novel (Ag(I)-NHC) complexes, Cn1–Cn4, was synthesized from bis-benzimidazolium ligands Ln1–Ln4 bearing benzyl, propargyl, cyanomethyl, and tert-butyl ester substituents, and then assessed for antibacterial activity. The ligands were prepared through N-alkylation of the corresponding bis-benzimidazole precursor in refluxing 1,4-dioxane. Treatment with Ag₂O in acetonitrile at room temperature produced the Ag(I)-NHC complexes. FTIR and ¹H, ¹³C NMR spectroscopy confirmed ligand formation and coordination to Ag(I); notably, the benzimidazolium C2–H resonance disappeared, while Ag–C(NHC) signals appeared at δ 182.35–184.45 ppm. Antibacterial activity was tested against four types of selected microorganism, using the (MIC) assay. Each complex showed measurable activity. Cn4 was the most potent overall, with MIC values of 6.25, 3.12, 6.25, and 12.5 μmol/L against the four strains, respectively. Antibacterial activity ranked Cn4 > Cn1 ≈ Cn3 > Cn2, pointing to a clear effect of the N-substituent on biological performance. Against the tested Gram-positive strains, Cn4 was more active than amoxicillin; amoxicillin, however, showed greater activity against both Gram-negative strains. These results point to bis-benzimidazole-derived Ag(I)-NHC complexes especially Cn4 as promising antibacterial candidates, while also showing how substituent modification can tune their biological activity.

1. Introduction

Antimicrobial Resistance (AMR) condition in which the microorganisms that cause various diseases develop the ability to resist the commonly used antibiotics employed to treat such diseases [1] has become a serious threat to public health. It is associated with higher death rates, rising medical expenses, and a diminished response to antimicrobial therapy [2]. The problem is driven partly by antibiotic misuse and partly by the limited supply of new drugs to replace antimicrobial agents that have lost their effectiveness [3]. As germs resistant to multiple treatments become more common, pharmaceutical and medical researchers are searching for antimicrobial drugs that are more potent and less toxic. If AMR remains unchecked, morbidity, mortality, and healthcare expenditures may all increase. Many organic substances, such as heterocyclic compounds, are currently among the most sought-after chemical components in the design of new drugs or the synthesis of novel compounds being investigated to combat antimicrobial resistance [4–7]. Several examples illustrate how these contribute to the development of antibacterial agents. Metal-NHC compounds, however, have mainly been used in catalytic chemistry [8, 9]. More recently, NHC ligands have shown promise as carrier molecules for anticancer drugs [10]. The growing number of published reviews reflects continued interest in the potential of NHC-metal complexes [11, 12]. Silver (I)-NHC complexes may assist in combating bacterial and cancerous infections, and research into their antimicrobial and anticancer potential has increased in recent years [13, 14]. For an effective antimicrobial Ag(I)-NHC

complex, the rate of Ag^+ dissociation in affected regions must be limited. Activity depends on the structure of the NHC ligand [15]; hydrophobic substituents and greater steric bulk on the imidazole ring, for example, can delay the release of silver ions [16]. Even with these reported benefits, the relationships among ligand structure, $\text{Ag(I)}\text{-NHC}$ complex formation, and antibacterial performance have not yet been studied sufficiently. In the present work, four bis-benzimidazolium ligands (Ln1-Ln4), each bearing a different N-substituent, were synthesized and then converted into the corresponding silver(I)-NHCs complexes (Cn1-Cn4). FTIR and multinuclear NMR spectroscopy were used to characterize the synthesized compounds. The $\text{Ag(I)}\text{-NHC}$ complexes were then tested against selected bacterial strains for antibacterial activity, followed by a structure activity relationship analysis examining how the N-substituents affected that activity.

2. Experimental section

The bis-benzimidazole precursor served as the starting material for synthesizing the bis-benzimidazolium ligands. N-alkylation was carried out using benzyl bromide, propargyl bromide, bromoacetonitrile, and tert-butyl bromoacetate. The reactions used 1,4-dioxane and acetonitrile (CH_3CN) as solvents, and silver(I) oxide (Ag_2O) was used to prepare the corresponding silver(I)-NHCs. Biological activity (antibacterial activity) was tested against selected microorganism strains, with amoxicillin serving as the reference antibacterial agent. FTIR spectra were collected over the range of $[400\text{-}4000]\text{ cm}^{-1}$. The ^{13}C and ^1H NMR spectra were measured at 100 and 400 MHz, respectively, with DMSO-d_6 as the solvent. Chemical shifts are given as δ (ppm) relative to the appropriate reference. Melting points were recorded with a suitable melting-point apparatus and reported in $^\circ\text{C}$.

2.1. Synthesis of Bis-Benzimidazolium Ligands (Ln1-Ln4)

The bis-benzimidazole ligands Ln1-Ln4 were synthesized by N-alkylation of the corresponding bis-benzimidazole precursor. Briefly, the bis-benzimidazole compound was dissolved in 1,4-dioxane, and the appropriate alkyl bromide (R-Br) was added to the reaction mixture [17]. The mixture of reactants was heated under reflux with continuous stirring for 40–44 h. Benzyl bromide, 1-propyl bromide, bromoacetonitrile, and tert-butyl-2-bromoacetate were used as the alkylating agents to afford Ln1 , Ln2 , Ln3 , and Ln4 , respectively. Upon completion of the reaction, its progress was checked by (TLC) technique. The mixture solution was allowed to cool to room temperature, and the resulting solid was then collected by filtration. It was washed with a suitable solvent to remove unreacted materials and the obtained final product was dried under reduced pressure, giving the corresponding bis-benzimidazolium bromide ligands Ln1-Ln4 .

Ligand (Ln1): FTIR (cm^{-1}): 2984 and 2879, corresponding to the stretching vibrations of C–H bonds; 1174, assigned to the C–N stretching vibration of the benzimidazole ring. Yield of the reaction: 78%. M.P.: $187\text{-}189\text{ }^\circ\text{C}$, ^1H NMR data was measured at 400 MHz using DMSO-d_6 as the solvent δ 9.87(s, 2H, NCHN), 7.75–7.41 (m, 18H, Ar–H, aromatic protons), 5.59 (s, 4H, -N- CH_2 -phenyl), 4.47 (t, J = 6.6 Hz, 4H, N- CH_2 -butyl), 1.79–1.71(m, 4H, (- CH_2 -), β - CH_2 protons of the N-butyl group); ^{13}C NMR data was measured at 100 MHz using DMSO-d_6 as the solvent δ 141.68 (2C, NCHN), 137.24, 135.28, 133.57, 130.21, 129.25, 128.74, 127.14, 124.69, 120.27, 118.38 (24C, Ar-C), 50.34 (2C, (-N- CH_2 -ph), 47.22 (N- CH_2 -butyl), 25.57 (the beta-methylene carbons of butyl chain).

Ligand (Ln2): FTIR (cm^{-1}): 3287, corresponding to the C–H stretching vibration of the propargyl group; 2947 and 2858, corresponding to the stretching vibrations of C–H bonds; 2146, assigned to the $\text{C}\equiv\text{C}$ stretching vibration of the alkyne triple bond; 1169, assigned to the C–N stretching vibration of the benzimidazole ring. Yield of the reaction: 69%. M.P.: $174\text{-}178\text{ }^\circ\text{C}$, ^1H NMR data was measured at 400 MHz using DMSO-d_6 as the solvent δ 9.84 (s, 2H, NCHN), 7.81–7.44 (m, 8H, Ar-H, aromatic protons), 5.47 (s, 4H, -N- CH_2 -propargyl), 4.58 (t, J = 6.6 Hz, 4H, N- CH_2 -butyl), 3.62 (s, 2H, - $\text{C}\equiv\text{C-H}$, propargyl group), 1.83–1.75(m, 4H, (- CH_2 -), β - CH_2 protons of the N-butyl group); ^{13}C NMR data was measured at 100 MHz using DMSO-d_6 as the solvent δ 142.24(2C, NCHN), 133.57, 130.21, 129.17, 127.87, 125.28, 112.47 (12C, Ar-C), 82.08(2C, - $\text{C}\equiv\text{C-H}$, the carbon directly attached to the N- CH_2 group), 72.49 (2C, $\text{C}\equiv\text{C-H}$, propargyl group), 46.14 (N- CH_2 -butyl), 24.89 (the beta-methylene carbons of butyl chain).

Ligand (Ln3): FTIR (cm^{-1}): 2928 and 2858, corresponding to the stretching vibrations of C–H bonds; 2238, assigned to the $\text{C}\equiv\text{N}$ stretching vibration of the nitrile moiety; 1184, assigned to the C–N stretching vibration of the benzimidazole ring. Yield of the reaction: 74%. M.P.: $169\text{-}171\text{ }^\circ\text{C}$, ^1H NMR data was measured at 400 MHz using DMSO-d_6 as the solvent δ 9.88 (s, 2H, NCHN), 8.04–7.52 (m, 8H, Ar-H, aromatic protons), 5.63 (s, 4H, -N- CH_2 - $\text{C}\equiv\text{N}$, nitrile moiety), 4.54 (t, J = 6.6 Hz, 4H, N- CH_2 -butyl), 1.81–1.74(m, 4H, (- CH_2 -), β - CH_2 protons of the N-butyl group); ^{13}C NMR data was measured at 100 MHz using DMSO-d_6 as the solvent δ 142.87 (2C, NCHN), 134.74, 131.74, 129.48, 127.08, 124.65, 112.38 (12C, Ar-C), 114.25 (2C, - $\text{C}\equiv\text{N}$), 47.74 (N- CH_2 -butyl), 39.57 (2C, 2(N- CH_2 -nitrile moiety), 24.57 (the beta-methylene carbons of butyl chain).

Ligand (Ln4): FTIR (cm^{-1}): 2978 and 2868, corresponding to the stretching vibrations of C-H bonds; 1731, assigned to the C=O stretching vibration; 1168, assigned to the C-N stretching vibration of the benzimidazole ring. Yield of the reaction: 68%. M.P.: $195\text{-}197\text{ }^\circ\text{C}$, ^1H NMR data was measured at 400 MHz using DMSO-d_6 as the solvent δ 9.83(s, 2H, NCHN), 8.11–7.55 (m, 8H, Ar-H, aromatic protons), 5.67 (s, 4H, -N- CH_2 -ester), 4.57 (t, J = 6.6 Hz, 4H, N- CH_2 -butyl), 1.80–1.72(m, 4H, (- CH_2 -), β - CH_2 protons of the N-butyl group), 1.31 (s, 18H, - $\text{C}(\text{CH}_3)_3$); ^{13}C NMR data was measured at 100 MHz using DMSO-d_6 as the solvent δ 142.38(2C, NCHN), 135.74, 133.57, 131.22, 128.41, 125.68, 114.87(12C, Ar-C), 81.19 (2C, - $\text{C}(\text{CH}_3)_3$), 52.04 (2C, 2(N- CH_2 -carboxylate), 47.57 (N- CH_2 -butyl), 28.54 (6C, $(\text{CH}_3)_3$), 24.57 (β -methylene carbons of butyl chain).

2.2. Synthesis of Silver(I)-NHCs Complexes (Cn1-Cn4)

The silver(I) complexes Cn1-Cn4 were synthesized from the corresponding bis-benzimidazolium bromide ligands Ln1-Ln4 , respectively. In a typical procedure, the appropriate ligand (Ln1 , Ln2 , Ln3 , or Ln4) was dissolved in acetonitrile (CH_3CN), followed by the addition of Ag_2O [18]. The solution mixture was stirred for 48 hrs at $25\text{ }^\circ\text{C}$ under protection of light. Upon completion of the reaction, the end product was washed with a suitable solvent to remove unreacted materials and dried under reduced pressure, then solid formed was collected by filtration, giving the corresponding $\text{Ag(I)}\text{-NHC}$ complexes (Cn1-Cn4).

Complex (Cn1): FTIR (cm^{-1}): 2979 and 2844, corresponding to the stretching vibrations of C–H bonds; 1164, assigned to the C–N stretching vibration of the benzimidazole ring. Yield of the reaction: 70%. M.P.: $211\text{-}213\text{ }^\circ\text{C}$, ^1H NMR data was measured at 400 MHz using DMSO-d_6 as the solvent δ 7.84–7.46 (m, 18H, Ar-H, aromatic protons), 5.64 (s, 4H, -N- CH_2 -benzyl), 4.56 (t, J = 6.6 Hz, 4H, N- CH_2 -butyl),

1.81-1.73(m, 4H, (-CH₂-), β -CH₂ protons of the N-butyl group); ¹³C NMR data was measured at 100 MHz using DMSO-d₆ as the solvent δ 182.35(2C, C-Ag), 138.24, 136.47, 134.92, 131.85, 130.14, 129.58, 127.35, 124.73, 121.28, 116.59 (12C, Ar-C), 53.74 (2C, (-N-CH₂-ph), 49.29 (N-CH₂-butyl), 24.11 (β -CH₂ protons of the N-butyl group).

Complex (Cn2): FTIR (cm⁻¹): 3291, corresponding to the C–H stretching vibration of the propargyl group; 2957 and 2891, corresponding to the stretching vibrations of C–H bonds; 2134, assigned to the C $\equiv$ C stretching vibration of the alkyne triple bond; 1154, assigned to the C–N stretching vibration of the benzimidazole ring, Yield of the reaction: 66%. M.P.: 190-192°C, ¹H NMR data was measured at 400 MHz using DMSO-d₆ as the solvent δ 7.93–7.51 (m, 8H, Ar-H, aromatic protons), 5.56 (s, 4H, -N-CH₂-propargyl), 4.64 (t, J = 6.6 Hz, 4H, N-CH₂-butyl), 3.61 (s, 2H, -C $\equiv$ C-H, propargyl group), 1.86-1.78(m, 4H, (-CH₂-), β -CH₂ protons of the N-butyl group); ¹³C NMR data was measured at 100 MHz using DMSO-d₆ as the solvent δ 183.57 (2C, C-Ag), 134.85, 133.24, 131.07, 128.92, 124.78, 114.47 (12C, Ar-C), 83.15 (2C, -C $\equiv$ C-H, the carbon directly attached to the N-CH₂ group), 73.18 (2C, $\equiv$ C-H), 47.48 (N-CH₂-butyl), 25.19 (β -CH₂ protons of the N-butyl group).

Complex (Cn3): FTIR (cm⁻¹): 2977 and 2865, corresponding to the stretching vibrations of C–H bonds; 2251, assigned to the C $\equiv$ N stretching vibration; 1175, assigned to the C–N stretching vibration of the benzimidazole ring, Yield of the reaction: 67%. M.P.: 234-236°C, ¹H NMR data was measured at 400 MHz using DMSO-d₆ as the solvent δ 8.12–7.55 (m, 8H, Ar-H, aromatic protons), 5.71 (s, 4H, -N-CH₂-C $\equiv$ N, nitrile moiety), 4.62 (t, J = 6.6 Hz, 4H, N-CH₂-butyl), 1.84-1.77(m, 4H, (-CH₂-), β -CH₂ protons of the N-butyl group); ¹³C NMR data was measured at 100 MHz using DMSO-d₆ as the solvent δ 182.95 (2C, C-Ag), 135.98, 133.78, 131.41, 127.27, 124.34, 113.47 (12C, Ar-C), 116.74 (2C, -C $\equiv$ N, nitrile moiety), 49.13 (N-CH₂-butyl), 41.45 (2C, 2(N-CH₂-nitrile), 24.62 (β -CH₂ protons of the N-butyl group).

Complex (Cn4): FTIR (cm⁻¹): 2947 and 2882, corresponding to the stretching vibrations of C–H bonds; 1737, assigned to the C=O stretching vibration of ester; 1157, assigned to the C–N stretching vibration of the benzimidazole ring, Yield of the reaction: 64%. M.P.: 241-243°C, ¹H NMR data was measured at 400 MHz, DMSO-d₆ δ 8.16–7.57 (m, 8H, Ar-H, aromatic protons), 5.78 (s, 4H, -N-CH₂-ester), 4.64 (t, J = 6.6 Hz, 4H, N-CH₂-butyl), 1.84-1.76(m, 4H, (-CH₂-), β -CH₂ protons of the N-butyl group), 1.32 (s, 18H, -C(CH₃)₃); ¹³C NMR data was measured at 100 MHz, DMSO-d₆ δ 184.45 (2C, C-Ag), 136.24, 132.98, 130.68, 127.78, 124.68, 116.38 (12C, Ar-C), 82.01 (2C, -C(CH₃)₃), 52.87 (2C, 2(N-CH₂-carboxylate), 47.57 (N-CH₂-butyl), 28.14 (6C, (CH₃)₃), 24.82 (β -CH₂ protons of the N-butyl group).

2.3. Antibacterial Activity

The antibacterial activity of the synthesized silver(I) complexes Cn1–Cn4 was assessed against selected bacterial strains through the (MIC) assay [19]. Staphylococcus aureus and Enterococcus faecalis represented the Gram-positive group; Pseudomonas aeruginosa and Escherichia coli represented the Gram-negative group. After culturing the bacteria under appropriate conditions, serial dilutions of each silver(I) complex were prepared to evaluate its inhibitory effect. The MIC was defined as the lowest concentration of each complex that completely inhibited visible bacterial growth after incubation. The obtained MIC values were recorded and compared among the synthesized complexes to assess the influence of the ligand structure and silver(I) coordination on antibacterial activity. The results demonstrated the antibacterial potential of the synthesized silver(I) complexes against both types of the bacterial strains.

3. Results and Discussion

3.1. Synthesis and Characterization of Bis-Benzimidazolium Ligands (Ln1–Ln4)

The bis-benzimidazolium bromide ligands Ln1–Ln4 were obtained by N-alkylating the corresponding bis-benzimidazole precursor with different alkylating agents in refluxing 1,4-dioxane. Benzyl bromide, propargyl bromide, bromoacetonitrile, and tert-butyl bromoacetate

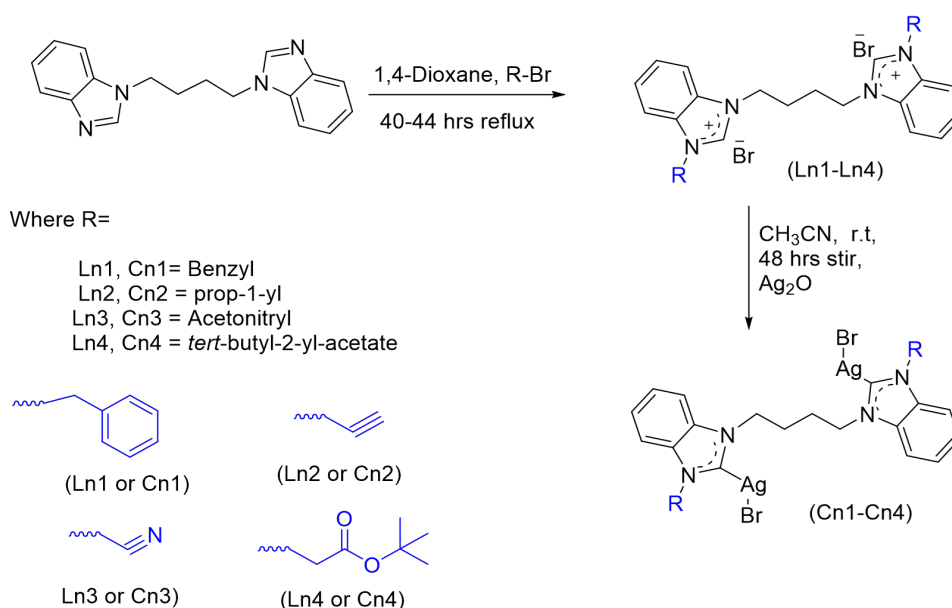


Figure 1: Synthetic Route to Bis-Benzimidazolium Ligands (Ln1–Ln4) and Their Corresponding Ag(I)–NHC Complexes (Cn1–Cn4)

introduced benzyl, propargyl, nitrile, and ester functionalities, respectively, giving a structurally varied ligand series. Prolonged heating was necessary, with reaction times of 40–44 h, consistent with the relatively high nucleophilicity requirements of benzimidazole nitrogen atoms during quaternization. Isolated yields of 68 to 78% show that the N-alkylation strategy was generally effective and reproducible Figure 1.

3.2. Synthesis and Characterization of Benzimidazolium salt ligands (Ln1–Ln4)

The formation of bis-benzimidazolium salts Ln1–Ln4 was supported by FTIR and multinuclear NMR spectroscopy. A characteristic downfield singlet at δ 9.83–9.88 ppm in the ^1H NMR spectra, integrating for two protons, was assigned to the benzimidazolium C2-H (NCHN) protons and confirmed the presence of two benzimidazolium units in each ligand. The corresponding ^{13}C NMR resonances at δ 141.68–142.87 ppm further supported formation of the benzimidazolium framework. The diagnostic signals of the respective N-substituents confirmed successful N-alkylation. For Ln1, the benzyl N–CH₂ resonance appeared at δ 5.59 ppm in the ^1H NMR spectrum and at δ 50.34 ppm in the ^{13}C NMR spectrum. Ln2 showed the characteristic propargyl signals at δ 5.47 and 3.62 ppm (^1H NMR) and δ 82.08 and 72.49 ppm (^{13}C NMR), together with a $\nu(\text{C}\equiv\text{C})$ band at 2146 cm^{-1} . For Ln3, the nitrile functionality was confirmed by the $\nu(\text{C}\equiv\text{N})$ band at 2238 cm^{-1} , the N–CH₂–CN resonance at δ 5.63 ppm, and the nitrile carbon at δ 114.25 ppm. Ln4 exhibited the characteristic ester carbonyl absorption at 1731 cm^{-1} , a tert-butyl singlet at δ 1.31 ppm integrating for 18H, and corresponding ^{13}C NMR signals at δ 81.19 and 28.54 ppm. The FTIR spectra also showed benzimidazole C–N stretching bands at 1168–1184 cm^{-1} , along with aliphatic C–H absorptions at 2858–2984 cm^{-1} . In Ln2, Ln3, and Ln4, the characteristic alkyne, nitrile, and ester absorptions, respectively, were retained, indicating that the pendant functional groups remained intact during N-alkylation. Their melting points ranged from 169–197 °C, consistent with an ionic bis-benzimidazolium bromide character. The variation in melting points likely reflects differences in substituent polarity, molecular packing, and intermolecular interactions.

3.3. Synthesis and Characterization of Silver(I)–NHC Complexes (Cn1–Cn4)

The silver(I)–NHC complexes Cn1–Cn4 were prepared from the corresponding bis-benzimidazolium bromide ligands Ln1–Ln4 with Ag₂O in acetonitrile. The reaction was carried out at room temperature for 48 h under light-protected conditions Figure 1. Ag₂O deprotonates the acidic benzimidazolium C2–H sites, after which the resulting NHC centers coordinate to Ag(I). Light protection was used to minimize the photoreduction of Ag(I) to metallic silver. The Ag(I)–NHC complexes were identified through complementary FTIR and multinuclear NMR data. In the ^1H NMR spectra of Cn1–Cn4, the benzimidazolium NCHN proton resonance at $\delta \approx 9.8$ ppm was no longer present, providing a key diagnostic indication of complex formation. At the same time, the ^{13}C NMR spectra showed Ag–C(NHC) resonances at δ 182.35, 183.57, 182.95, and 184.45 ppm for Cn1–Cn4, respectively, consistent with the formation of metal–carbene bonds. Because these signals fall within a narrow range, the carbene centers appear to experience broadly similar electronic environments, with the small differences likely arising from the nature of the N-substituents. The characteristic functional groups of the ligands remained unchanged upon metalation. In Cn1, the benzyl N–CH₂ resonance was still present at δ 5.64 ppm. The propargyl group in Cn2 was also retained, as shown by the alkyne proton at δ 3.61 ppm, the alkyne carbon signals at δ 83.15 and 73.18 ppm, and the $\nu(\text{C}\equiv\text{C})$ band at 2134 cm^{-1} . For Cn3, the nitrile carbon appeared at δ 116.74 ppm, together with an N–CH₂–CN resonance at δ 5.71 ppm and a $\nu(\text{C}\equiv\text{N})$ band at 2251 cm^{-1} . Cn4 preserved its tert-butyl ester as well: the $\nu(\text{C}=\text{O})$ absorption was observed at 1737 cm^{-1} , the tert-butyl group produced ^{13}C NMR signals at δ 82.01 and 28.14 ppm, and the N–CH₂–carboxylate resonance occurred at δ 52.87 ppm. These results indicate that metalation occurred selectively at the benzimidazolium carbene-forming site, without any detectable change to the pendant functional groups. The FTIR spectra of Cn1–Cn4 continued to show the benzimidazole C–N stretching bands at 1154–1175 cm^{-1} , together with the aliphatic C–H absorptions. The characteristic bands of the alkyne in Cn2, the nitrile in Cn3, and the ester in Cn4 were also retained, indicating that these substituents remained structurally intact after Ag(I) coordination. Complex formation coincided with higher melting points. Taken together, the disappearance of the benzimidazolium C2–H resonance, the emergence of Ag–C(NHC) signals at $\delta \approx 182$ –184 ppm, and the retained diagnostic FTIR and NMR features of the pendant substituents support the formation of the silver(I)–NHC complexes Cn1–Cn4.

3.4. Biological Activity

The synthesized silver(I)–NHC complexes Cn1–Cn4 were evaluated against selected bacteria strain. As shown in Table 1, all four complexes displayed measurable antibacterial activity, but their potency differed according to both the bacterial species and the N-substituent. Cn4 performed best overall, with MIC values of 6.25 ± 0.2 , 3.12 ± 0.1 , 6.25 ± 0.2 , and 12.5 ± 0.3 $\mu\text{mol/L}$ against *E. faecalis*, *S. aureus*, *E. coli*, and *P. aeruginosa*, respectively. The overall activity followed the order Cn4 > Cn1 $\approx$ Cn3 > Cn2. Cn4's greater activity may be related to its tert-butyl ester substituents. Its polar carbonyl groups and hydrophobic tert-butyl moieties may work together to promote interactions with bacterial membranes and cellular uptake. The coordinated Ag(I) centers may contribute as well, both through their intrinsic antimicrobial properties and by changing the physicochemical and cellular behavior of the NHC framework. Cn1 and Cn3 showed similar intermediate activity, while Cn2 was the least active, especially against Gram-negative bacteria. The benzyl groups of Cn1 may strengthen membrane interactions by increasing hydrophobicity. Cn3, with its cyanomethyl groups, offers a different balance between polarity and hydrophobicity. The propargyl substituents in Cn2, by comparison, seem less suited to antibacterial activity. Overall, the results show that changing the NHC N-substituents substantially affects the antibacterial performance of the Ag(I) complexes. Among the microorganisms tested, *S. aureus* showed the greatest susceptibility to all four complexes (MIC = 3.12–12.5 $\mu\text{mol/L}$). *P. aeruginosa*, by contrast, was the least susceptible (MIC = 12.5–50 $\mu\text{mol/L}$), possibly because Gram-negative bacteria have an additional permeability barrier and intrinsic resistance mechanisms, particularly in *P. aeruginosa*. Compared with amoxicillin, Cn4 was more active against both Gram-positive strains. Its MIC values were approximately 2.9-fold lower for *E. faecalis* and 6.7-fold lower for *S. aureus*. Amoxicillin, however, showed greater activity against *E. coli*, with an MIC of 3 ± 0.2 $\mu\text{mol/L}$ compared with 6.25 ± 0.2 $\mu\text{mol/L}$ for Cn4, and against *P. aeruginosa*, at 10 ± 0.2 $\mu\text{mol/L}$ versus 12.5 ± 0.3 $\mu\text{mol/L}$. Cn1 and Cn3 likewise outperformed amoxicillin against *E. faecalis* and *S. aureus*, while Cn2 was less active against every strain tested. Overall, Cn4 emerged as the most promising antibacterial candidate in this series. The findings also show that the N-substituent strongly influences the antibacterial activity of the bis-benzimidazole-derived Ag(I)–NHC complexes. Further mechanistic work is needed to determine how membrane disruption, Ag(I) release, oxidative stress, and interactions with bacterial biomolecular targets each contribute.

Table 1: Minimum inhibitory concentrations (MICs) of silver(I)–NHC complexes Cn1–Cn4 against selected bacterial strains

Compound	Enterococcus faecalis (Gram +)	Staphylococcus aureus (Gram +)	Escherichia coli (Gram -)	Pseudomonas aeruginosa (Gram -)
Cn1	12.5 ± 0.3	6.25 ± 0.2	12.5 ± 0.4	25 ± 0.6
Cn2	25 ± 0.5	12.5 ± 0.3	25 ± 0.5	50 ± 0.8
Cn3	12.5 ± 0.3	6.25 ± 0.2	12.5 ± 0.3	25 ± 0.5
Cn4	6.25 ± 0.2	3.12 ± 0.1	6.25 ± 0.2	12.5 ± 0.3
Amoxicillin	18 ± 0.5	21 ± 0.5	3 ± 0.2	10 ± 0.2

3.5. Structure Activity Relationship

The antibacterial activity of the silver(I)–NHC complexes varied with the N-substituent on the benzimidazolium framework. Of the synthesized compounds, Cn4 was the most active overall, showing MIC values of 6.25, 3.12, 6.25, and 12.5 $\mu\text{mol/L}$ against *E. faecalis*, *S. aureus*, *E. coli*, and *P. aeruginosa*, respectively. Its greater activity may reflect the combined influence of the ester functionality and tert-butyl group, which can affect the complex's polarity and hydrophobicity, as well as its interactions with bacterial cells. Cn1, which carries a benzyl substituent, showed moderate activity, while Cn3, with a cyanomethyl group, had a similar antibacterial profile. Cn2 performed the least effectively against the tested strains; it contains a propargyl substituent. Overall, activity followed the order Cn4 > Cn1 $\approx$ Cn3 > Cn2, suggesting that a relatively balanced combination of hydrophobic and polar characteristics may support antibacterial activity. The complexes were generally more active against Gram-positive strains, especially *S. aureus*, than against Gram-negative bacteria. Differences in bacterial cell-envelope structure and permeability may account for this pattern. Overall activity likely depends on both the antibacterial contribution of the Ag(I)–NHC center and the physicochemical characteristics of the N-substituents. Still, these SAR findings indicate a correlation, not a definitive mechanism, and further work on cellular uptake and antibacterial action is needed.

4. Conclusions

A series of bis-benzimidazolium bromide ligands (Ln1–Ln4) and the corresponding silver(I)–NHCs (Cn1–Cn4) were successfully synthesized and characterized using FTIR and multinuclear NMR spectroscopy. Complex formation was supported by the disappearance of the benzimidazolium C2–H resonance and the appearance of Ag–C(NHC) signals. Every synthesized complex showed measurable antibacterial activity against both Gram-positive and Gram-negative bacteria, although Cn4 was the most potent overall. Activity followed the order Cn4 > Cn1 $\approx$ Cn3 > Cn2, showing that altering the N-substituent can substantially influence the biological performance of the Ag(I)–NHC framework. The effect was most pronounced for Cn4 against *S. aureus* and *E. faecalis*, whereas the Gram-negative strains showed lower susceptibility, with *P. aeruginosa* being particularly resistant. Of the compounds examined, Cn4 provides the most promising starting point for further development of bis-benzimidazole-derived Ag(I)–NHC complexes as potential antibacterial agents.

Article Information

Data Availability: The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest: The authors declare that they have no conflict of interest.

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