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Topology control in nitrile-functionalized silver(I)–N-heterocyclic carbene complexes: Synthesis, molecular structures, and *in vitro* anticancer studies

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ABSTRACT

A series of nitrile-functionalized N-heterocyclic carbene (NHC) proligands based on *N*-methyl-*N*′-benzonitrile and *N,N*′-dibenzonitrile imidazolium salts, **1–4**, has been developed and investigated. Salts were obtained by the reaction of 2-bromomethylbenzonitrile with 1*H*-imidazole/1-methylimidazole in dioxane/ethanol at refluxing temperature. Following deprotonation, salts form mononuclear bis-NHC silver(I) complexes (**6–8**) with half an equivalent of silver(I) oxide. A dimeric silver(I) structure **5** was observed under similar conditions when NHC proligand **2** was treated with one equivalent of silver(I) oxide, however, similar complexation was not found in both the symmetrically substituted proligands **3** and **4**. Compounds **2**, **5** and **6** were additionally characterized by single crystal X-ray diffraction method. The dinuclear complex **5** is a symmetrical structure, with the two NHC ligands in identical chemical environment. Each silver center in **5** is coordinated by a carbene carbon atom of one ligand and nitrile nitrogen atom of another ligand unit. Preliminary *in vitro* anticancer studies against the human derived colorectal (HCT 116) cancer cell line using MTT-based assay method, were carried out on the reported compounds. The imidazolium proligand **3** and silver(I) complexes **5–8** revealed the following IC_{50} values, respectively, 5.0 ± 0.3, 1.7 ± 0.4, 6.0 ± 0.7, 1.7 ± 0.2 and 27.2 ± 1.1 μM.

1. Introduction

N-heterocyclic carbenes (NHCs) are neutral azole based derivatives carrying a lone pair of electron on carbene carbon atom. NHCs are stronger σ-donors and weaker π-acceptor ligands compared to phosphines, due to which these can form stronger bonds with varieties of hard and soft metal ions results in highly stable carbene complexes [1]. The period since the fall 1990s has seen a tremendous growth in the use of NHCs to prepare diverse carbene complexes with most of the metal ions/atoms for various applications [2]. Further, the steric and electronic properties of NHCs can be expediently tuned by changing the appropriate substituents and backbones at both the nitrogen atoms [3,4]. Non-functionalized NHCs are potentially monodentate ligands, which can give rise to complexes with enhanced stability compared to phosphines. However, donor-functionalized NHCs are normally bi- or poydentate chelates in nature and can give rise to complexes with even more enhanced stability through their chelation [5].

Today, NHCs become a spectacular class of ligands display a surprisingly expanded collections of applications that extends to nearly all facets of C–C couplings, olefin oligo/polymerizations and metathesis, C–H bond activations, and in contemporary bioorganometallic chemistry as anticancer and antimicrobial agents [6]. One of the main advances in the bioorganometallic chemistry of NHCs has been a move away from structure-defined anticancer activity determination, where relatively little is understood about the nature of metal ion/atom, influence of steric and electronic factors on solubility/interaction of carbene complex in biofluids and influence of the metal coordination environment on the anticancer activity. For many metal-carbene complexes meant for biological activity it has proved possible to design and synthesize the complex to an extent that selective cell death can be achieved [7]. In this aspect, functionalized Ag(I)–NHC complexes play a prominent role in bioorganometallic chemistry as anticancer agents against various human derived cancer lines. NHC ligands would stabilize the Ag(I) and allow it to be delivered for antimicrobial therapeutics. In practice, caffeine derived Ag–NHC complex used as an antimicrobial agent, named SCC1 and trademarked Silvamist®. The *in vitro* and *in vivo* studies using SCC1 against tobramycin-resistant bacteria *Burkholderia dolosa* found it to be an effective compound through nebulization into the lungs of mice [8]. These antimicrobial properties of SCC1, its analogues SCC8 and SCC-benzoate (Chart 1) are the driving force to the current state of many research

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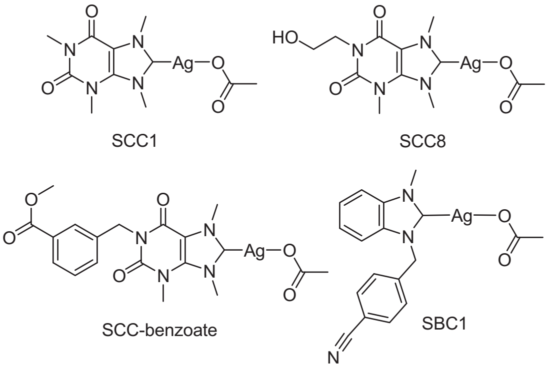


Chart 1. Ag(I)–NHC complexes as antimicrobial/anticancer agents.

groups working on bioorganometallic chemistry of NHC Ag(I) complexes. On the other hand, a nitrile-functionalized anticancer drug candidate SBC1 was evaluated for its potential *in vitro* and *in vivo* against many human derived cancer cell lines, which showed potent activity [9].

While chemists have employed different strategies in the design and synthesis of novel Ag and Au–NHC-based anticancer agents; however, their exact mechanisms of action still remain relatively less explored [10]. These include the more selective delivery and activation of metal-based drugs/prodrugs to the infected sites and the discovery of novel non-covalent interactions with the DNA of infected cell. Recently, we have reported a series of mono- and bis-carbene Ag(I) complexes of imidazole based NHC ligands and their proven anticancer potential against different human cancer lines [11,12]. Interestingly, increased anticancer potentials of complexes were observed when the central azole is functionalized. We are emphasizing this comparison between non-functionalized and functionalized compounds where efforts have been made to identify their biomolecular target especially in the cancer infected cell and their mode of action. This article covers imidazole derived Ag(I)–NHC complexes, their design, synthesis, anticancer potential and their comparison in more detail than our recent papers.

2. Experimental

2.1. General considerations

All chemicals and solvents were obtained from commercial sources and used as received without further purification. Imidazole, 2-bromomethylbenzonitrile, 1-methylimidazole, potassium hexafluorophosphate, 5-fluorouracil (standard used in anticancer activity), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide for the MTT assay were purchased from Sigma–Aldrich. Synthesis and spectral characterization of the bromide and hexafluorophosphate salts **3** and **4**, and silver complex **8** have been reported in our earlier papers [13]. NMR spectra were recorded in d_6-DMSO using Bruker 500 MHz Ascend and Bruker Avance 300 MHz spectrometers. Chemical shifts (δ) are described in ppm from TMS as internal reference. Signals are labeled as singlet (s), doublet (d), triplet (t) and multiplet (m). The melting points of the reported compounds were assessed by using a Stuart Scientific SMP-1 (UK) instrument. Elemental analysis was carried out by Perkin-Elmer series II, 2400 microanalyzer. The single crystal X-ray diffraction data of the compounds were collected using a Bruker SMART APEX2 CCD area-detector diffractometer. Calculations, structure refinement, molecular graphics and the material for publication were performed using the SHELXTL and PLATON software packages.

2.2. Syntheses

2.2.1. Synthesis of 3-(2-cyanobenzyl)-1-methylimidazolium hexafluorophosphate (2)

A mixture of 2-bromomethylbenzonitrile (0.91 g, 4.6 mmol) and 1-methylimidazole (0.38 g, 4.6 mmol) was refluxed in 1,4-dioxane (30 mL) for 20 h. The resulted solution was cooled using ice bath to get thick brown oil and the solvent was removed by decantation. The product was then washed with fresh 1,4-diaoxane (3 × 3 mL) and so obtained bromide salt **1** was directly converted into its hexafluorophosphate counterpart by salt metathesis reaction using KPF_6 (0.83 g, 4.5 mmol) in methanol (25 mL). Resultant mixture was stirred for 3 h and was left to stand overnight. White precipitate thus formed was filtered, washed with distilled water (3 × 5 mL) to remove unreacted KPF_6, and air dried. Colourless blocks were obtained by slow evaporation of the salt solution in acetonitrile at ambient temperature. Yield: 78.6%; M.P: 150.0–151.0 °C. ^1H NMR (500 MHz, d_6-DMSO): δ 3.90 (3H, s, *N*-CH_3), 5.69 (2H, s, benzonitrilic-CH_2), 7.47 (1H, d, Ar–H, *J* = 8.0 Hz), 7.64 (1H, t, Ar–H, *J* = 7.25 Hz), 7.77 (2H, t, Ar–H, *J* = 6.75 Hz), 7.98 (2H, d, imidazolium H4′ and H5′, *J* = 9.0 Hz), and 9.18 (1H, s, imidazolium H2′). $^{13}C\{^1H\}$NMR (125 MHz, d_6-DMSO): δ 35.9 (*N*-CH_3), 50.3 (benzonitrilic-CH_2), 111.0 (benzonitrile-ArC), 116.8 (nitrile-CN), 122.7, 124.2 (imidazolium C4′ and C5′), 129.2, 129.6, 133.59, 134.0 (benzonitrile-ArC), 137.3 (benzonitrile-ArC) and 137.4 (imidazolium C2′). FTIR (KBr disc) cm^{-1}: 3152, 3121 ν(C–H), 2223 ν(C≡N), 1637, 1015 ν(C=N, imidazole). *Anal.* Calc. for $C_{12}H_{12}N_3F_6P_1$: C, 42.0; H, 3.5; N, 12.2. Found: C, 42.0; H, 2.8; N, 12.1%.

2.2.2. Synthesis of bis-[3-(2-Cyanobenzyl)-1-methylimidazolium silver(I)] bishexafluorophosphate (5)

A solution of the imidazolium salt **2** (0.4 g, 1.2 mmol) and Ag_2O (0.27 g, 1.2 mmol) in acetonitrile (30 mL) was stirred at room temperature for 12 h in glassware wrapped with aluminium foil to exclude light. A solution with black suspension was obtained. The black suspension was filtered through a pad of celite and the filtrate was evaporated to dryness under reduced pressure to afford complex **5** as grey solid. This solid was further recrystallized with acetonitrile and left to dry at ambient temperature. Crystals suitable for single crystal X-ray diffraction studies were obtained by slow diffusion of diethyl ether into the complex solution in acetonitrile at room temperature. Yield: 68.6%; M.P: 236.5–237.2 °C. ^1H NMR (300 MHz, d_6-DMSO): δ 3.77 (3H, s, *N*-CH_3), 5.53 (2H, s, benzonitrilic-CH_2), 7.27 (1H, d, Ar–H, *J* = 8.8 Hz), 7.50 (2H, t, Ar–H, *J* = 6.5 Hz), 7.68 (1H, t, Ar–H, *J* = 6.8 Hz), and 7.87 (2H, d, imidazolium H4′ and H5′, *J* = 9.3 Hz). $^{13}C\{^1H\}$NMR (75 MHz, d_6-DMSO): δ 38.7 (*N*-CH_3), 52.9 (benzonitrilic-CH_2), 111.4 (benzonitrile-ArC), 118.2 (nitrile-CN), 123.0, 123.5 (imidazolium C4′ and C5′), 129.8, 130.0, 134.3, 134.5 (benzonitrile-ArC), 141.1 (benzonitrile-ArC) and 181.7 (C2′–Ag). FTIR (KBr disc) cm^{-1}: 3147, 3115 ν(C–H), 2269 ν(C≡N), 1666, 1042 ν(C=N, imidazole). *Anal.* Calc. for $C_{24}H_{22}Ag_2N_6F_{12}P_2$: C, 32.0; H, 2.5; N, 9.3. Found: C, 32.4; H, 1.8; N, 9.5%.

2.2.3. Synthesis of 3-(2-cyanobenzyl)-1-methylimidazolium silver(I) hexafluorophosphate (6)

This compound was prepared in a manner analogous to that for **5**, only with aforementioned salt **2** (0.60 g, 1.8 mmol) was added to the acetonitrile solution (30 mL) of Ag_2O (0.20 g, 0.9 mmol) and the mixture was stirred for 12 h to yield complex **6** as grey solid. Yield: 75.4%; M.P: 176.4–178.0 °C. ^1H NMR (500 MHz, d_6-DMSO): δ 3.77 (3H, s, *N*-CH_3), 5.53 (2H, s, benzonitrilic-CH_2), 7.28 (1H, d, Ar–H, *J* = 8.0 Hz), 7.50 (2H, t, Ar–H, *J* = 7.3 Hz), 7.67 (1H, t, Ar–H, *J* = 6.3 Hz), and 7.85 (2H, d, imidazolium H4′ and H5′, *J* = 9.0 Hz). $^{13}C\{^1H\}$NMR (125 MHz, d_6-DMSO): δ 38.3 (*N*-CH_3), 52.1 (benzonitrilic-CH_2), 110.6 (benzonitrile-ArC), 117.2 (nitrile-CN), 122.6, 123.3 (imidazolium C4′ and C5′), 128.7, 128.9, 133.4, 133.7

(benzonitrile-ArC), 140.2 (benzonitrile-ArC) and 181.0 (C2′–Ag). FTIR (KBr disc) cm^{-1}: 3145, 3112 ν(C–H), 2225 ν(C≡N), 1667, 1051 ν(C═N, imidazole). *Anal.* Calc. for $C_{24}H_{22}Ag_1N_6F_6P_1$: C, 44.5; H, 3.4; N, 13.0. Found: C, 44.0; H, 3.3; N, 12.3%.

2.2.4. Synthesis of 1,3-bis (2-cyanobenzyl)imidazolium silver(I) bromide (7)

A solution of the imidazolium salt **3** (0.62 g, 1.6 mmol) and Ag_2O (0.18 g, 0.8 mmol) in dichloromethane (30 mL) was stirred at room temperature for 12 h in glassware wrapped with aluminium foil to exclude light. A clear solution with black suspension was obtained. The black suspension was filtered through a pad of celite and the filtrate was evaporated to dryness under reduced pressure to give grey solid. Yield: 72.1%; M.P: 242.3–243.0 °C. ^1H NMR (500 MHz, d_6-DMSO): δ 5.47 (2H, s, benzonitrilic-CH_2), 7.23 (1H, s, Ar–H), 7.47 (1H, t, Ar–H, $J = 7.8$ Hz), 7.53 (1H, s, Ar–H), 7.61 (1H, t, Ar–H, $J = 7.8$ Hz), and 7.79 (1H, d, imidazolium H4′ and H5′, $J = 7.5$ Hz). $^{13}C\{^1H\}$NMR (125 MHz, d_6-DMSO): δ 52.4 (benzonitrilic-CH_2), 110.6 (benzonitrile-ArC), 117.1 (nitrile-CN), 123.0 (imidazolium C4′), 128.6, 129.0, 133.5, 133.7, 139.7(benzonitrile-ArC), 158.0 (benzonitrile-ArC) and 181.5, 183.0 (C2′–Ag). FTIR (KBr disc) cm^{-1}: 3015, 2944 ν(C–H), 2225 ν(C≡N), 1649, 1048 ν(C═N, imidazole). *Anal.* Calc. for $C_{38}H_{28}Ag_1N_8Br_1$: C, 58.1; H, 3.6; N, 14.3. Found: C, 58.0; H, 3.9; N, 13.8%.

2.3. Determination of anticancer activity

2.3.1. Cell culture

Initially, HCT 116 cells were allowed to grow under optimal incubator conditions. Cells that have reached a confluence of 70–80% were chosen for cell plating purposes. The old medium was carefully aspirated out of the plate. Next, cells were washed 2–3 times using sterile phosphate buffered saline (PBS) with (pH 7.4). PBS was completely discarded after washing and then trypsin was added and distributed evenly onto cell surfaces. Cells were incubated at 37 °C in 5% CO_2 for 1 min. Then, the flasks containing the cells were gently tapped to aid cell segregation and observed under inverted microscope (if cell segregation is not satisfying, the cells will be incubated for another minute). Trypsin activity was inhibited by adding 5 mL of fresh complete media of 10% fetal bovine serum (FBS). Cells were counted and diluted to get a final concentration of 2.5×10^5 cells per mL, and inoculated into wells (100 μL cells per well). Finally, plates containing the cells were incubated at 37 °C with an internal atmosphere of 5% CO_2.

2.3.2. MTT assay

Cancer cells (100 mL cells per well, 1.5×10^5 cells per mL) were inoculated in wells of microtitre plate. Then the plate is incubated in a CO_2 incubator overnight in order to allow for cell attachment. 100 mL of test complexes were added into each well containing the cells. Test complexes were diluted with media into the desired concentrations from the stock. The plates were incubated at 37 °C withan internal atmosphere of 5% CO_2 for 72 hours. A 20 mL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] reagent was added into each well and incubated again for 4 h. After this, 50 mL of MTT lysis solution (DMSO) was added into the wells. The plates were further incubated for 5 min in a CO_2 incubator. Finally, plates were read at 570 and 620 nm wavelengths using a standard ELISA microplate reader (Ascent Multiskan). Data were recorded and analyzed for the assessment of the effects of test complexes on cell viability and growth inhibition. The percentage of growth inhibition was calculated from the optical density (O.D.) that was obtained from MTT assay. The formula used for the calculation of growth inhibition is carried out using Eq. (1).

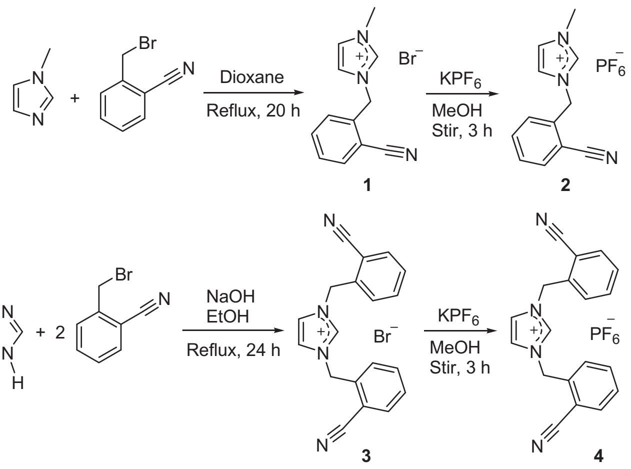
$$\%\ \text{of growth inhibition} = \frac{[\text{O.D.(control)} - \text{O.D.(survived)}]}{\text{O.D.(control)}} \times 100$$

3. Results and discussion

3.1. Syntheses

The synthetic sequence for the preparation of NHC proligands **1–4** is initiated by the N-alkylation of 1-methylimidazole or 1H-imidazole by adopting established literature procedures [13]. Halide salt **1** was synthesized by the reaction of 1-methylimidazole with 2-bromomethylbenzonitrile in 1,4-dioxane at refluxing temperature afforded the desired imidazolium salt after 20 h. Subsequently, the halide salt **1** was directly converted to its hexafluorophosphate counterpart **2** by metathesis reaction using KPF_6 in methanol under the conditions described in Scheme 1. Conversely, in the preparation of symmetrically substituted imidazolium salt **3**, two equivalents of 2-bromomethylbenzonitrile was treated with imidazole under basic conditions in ethanol at refluxing temperature for one day. Further, hexafluorophosphate counterpart **4** of salt **3** was prepared by metathesis reaction using KPF_6 in methanol. Analytically pure compounds **1–4** were obtained for both halide and hexafluorophosphate salts with excellent yields.

It is well recognized that functionalized azolium salts act as proficient ligand systems in the preparation of transition metal-carbene complexes showing versatile coordination modes. This is even more pertinent in the case of nitrile-functionalized salts, as it has an additional coordinating site leading to facile complex formation. For this reason, we elected the variable preparative methods to control the topology of the silver(I) complexes from this new nitrile-functionalized NHC proligands. Gratifyingly, metalation involving reaction of the proligand **2** with half an equivalent of silver(I) oxide in acetonitrile at mild conditions afforded the mono-metallic bis-NHC complex **6**. In another attempt, the same salt was treated with one equivalent of silver(I) oxide by keeping all other reaction conditions same to check the coordinating competition between nitrile nitrogen and carbene carbon atoms. Interestingly, a binuclear silver(I) complex **5** was obtained in almost quantitative yield, showing chelation of both the aforementioned atoms to silver(I) centers. At optimized conditions, symmetrically substituted proligads **3** and **4** were treated with half an equivalent of silver(I) oxide afforded the corresponding mononuclear bis-NHC



Scheme 1. Synthetic route to imidazolium halide and hexafluorophosphate salts **1–4**.

silver(I) complexes **7** and **8**, respectively as shown in Scheme 2. However, under previously optimized and various other conditions, the formation of either binuclear or polymeric silver(I) complexes by the reaction of symmetrically substituted proligands **3** and **4** with one equivalent of silver(I) oxide proved to be unsuccessful. The topology of the complexes has an important effect on their activity which intern depends on stability to air and moisture, light and well-defined structures [14]. This new family of nitrile-functionalized silver(I)–NHC complexes demonstrate the bench-stable, well-defined structures suitable for anticancer applications. The new NHC proligands and the corresponding silver(I)–carbene complexes of both the mononuclear and binuclear forms were consistent with their elemental analysis data.

3.2. Spectral characterizations

The NMR spectroscopic characterization of both imidazolium salts and their silver(I) carbene complexes was assisted by a combination of ^{1}H and ^{13}C NMR spectroscopic analyses. Data from both spectroscopic investigations done for all compounds **1–8**, was collected using a dimethylsulphoxide-d_6 solution. The ^{1}H NMR spectra of the salts **2–4** displayed a distinguished singlet in the region δ 9.18–9.8 ascribable to the resonance of imidazole C2 protons [15]. After the successful formation of the corresponding silver(I)–carbene complexes, the peaks associated with C2 (acidic imidazolium protons) of imidazolium salts **2–4** completely disappeared in the ^{1}H NMR spectra of complexes **5–8**. On the other hand, in the ^{13}C NMR spectra of the salts **2–4**, a signal appeared at the most down filed region around δ 137 corresponding to the carbene carbon resonance, however, the same is shifted in the ^{13}C NMR spectra of complexes downfield to δ 181.7, 181.0 and 183.0, respectively, confirming the successful formation of carbene complexes.

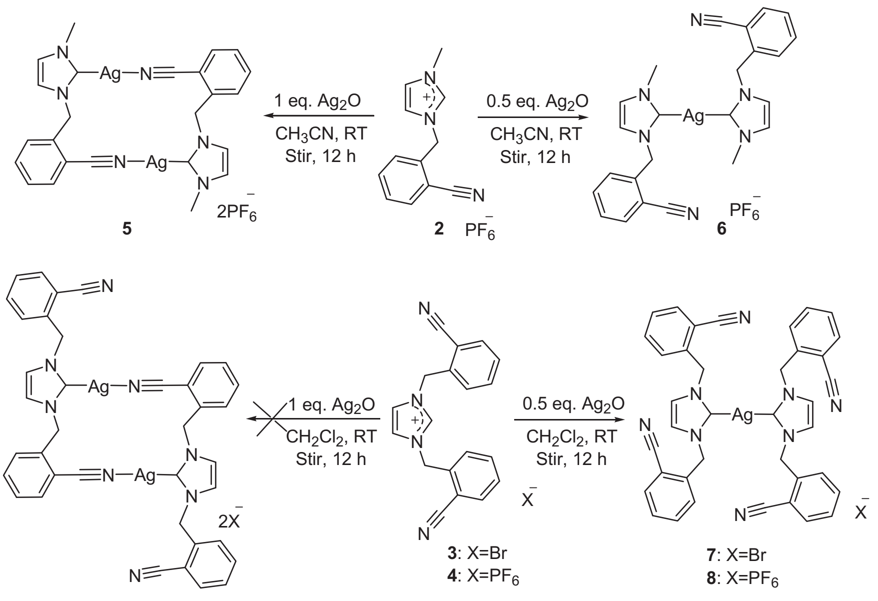
Analysis of silver(I) complex **5** by ^{1}H NMR is indicative of a very symmetric system, showing only one set of proton resonances for both NHC systems and displaying no restrained rotation of benzyl group [16]. Complex **7** gives rise to two doublet resonances in the ^{13}C NMR spectrum at δ 181.5 and 183.0, which are attributable to the corresponding carbene carbon resonances. This data stand in contrast to the singlet signal reported for the complexes **5**, **6** and **8** in the same region. This is due to the presence of ^{13}C–^{109}Ag and ^{13}C–^{107}Ag coupling of ^{1}J(C–^{109}Ag) 197 and ^{1}J(C–^{107}Ag) 183 Hz, respectively. This anomaly suggests the absence of any NHC-exchange phenomena at the Ag centers, which is consistent with the previous reports [17].

In the IR spectrum of the complex **5**, the ν(C≡N) stretching frequency upon coordination to the silver(I) center shifted to higher value, 2269 cm^{-1}, in comparison to that of the imidazolium salt **2** (2223 cm^{-1}). Markedly, there is no difference in the ν(C≡N) stretching frequency of monometallic silver(I) complexes and their respective imidazolium salts, suggesting the non-involvement of nitrile group in the coordination. This is a key index to check the coordination behavior of NHC ligands having multiple coordination sites potentially available for hosting two or more metal centers. Notably, there are two bands assigned to the ν(C–N) and ν(C=N) stretching frequencies for each compound, due to the presence of the two different CN modules. Both the bands shifted to the higher values in the range 1649–1667 and 1042–1051 cm^{-1} in comparison to that of the imidazolium salt **2** (1637 and 1015 cm^{-1}), suggesting the successful complex formation. Further, the IR spectrum of all reported compounds displayed two sharp bands of medium intensities in the range 3015–3112 and 2944–3145 cm^{-1}, attributed to the aliphatic and aromatic ν(C–H) stretching vibrations. These spectral assignments are consistent with the similar reported compounds available in the literature [18].

3.3. Single crystal X-ray crystallography

Imidazolium salt **2** and its bi- as well as mononuclear silver(I) complexes **5** and **6** were additionally characterized by single crystal X-ray diffraction technique. Crystal refinement data, and selected bond distances and bond angles of compounds **2**, **5** and **6** are tabulated in Tables 1–4, respectively. Single crystals of hexafluorophosphate salt **2** suitable for X-ray diffraction analysis were grown from its acetonitrile solution, while single crystals of the silver(I) complexes **5** and **6** were obtained by laying diethyl ether onto their acetonitrile solution at room temperature.

Salt **2** crystallizes in the triclinic space group $P\bar{1}$ possessing an imidazolium cationic repeating unit and a hexafluorophosphate anion in an asymmetric unit. As shown in Fig. 1, the nitrile-module is pointing away from the carbene carbon unit, and planes of both imidazole and benzyl benzene rings are almost perpendicular to



Scheme 2. Synthetic route to silver(I)–carbene complexes **5–8**.

Table 1
Crystal data and structure refinement details for compounds **2**, **5**, and **6**.

	2	**5**	**6**
Formula	$C_{12}H_{12}N_3F_6P$	$C_{24}H_{22}Ag_2N_6F_{12}P_2$	$C_{24}H_{22}AgN_6F_6P$
Formula weight	343.22	900.16	636.68
Crystal system	triclinic	monoclinic	orthorhombic
Space group	P-1 (No. 2)	*P*21/*c* (No. 14)	*Pnna* (No. 52)
a (Å)	8.2968(1)	10.5366(2)	8.1836(1)
b (Å)	8.4500(1)	13.3055(2)	25.8048(4)
c (Å)	11.6925(2)	11.8843(2)	12.0793(2)
α (°)	101.338(1)	90.00	90.00
β (°)	99.163(1)	119.320(1)	90.00
γ (°)	116.450(1)	90.00	90.00
V (Å^3)	690.580(19)	1452.68(5)	2550.86(7)
Z	2	2	4
D_{calc} (g/cm^3)	1.651	2.058	1.658
μ (Mo Kα/mm)	0.267	1.565	0.919
$F(000)$	348	880	1276
Crystal size (mm)	0.20 × 0.24 × 0.34	0.17 × 0.25 × 0.44	0.24 × 0.32 × 0.42
T (K)	293	293	293
Radiation (Å)	Mo Kα 0.71073	Mo Kα 0.71073	Mo Kα 0.71073
θ Minimum–maximum (°)	1.9–32.7	2.2–32.7	1.6–32.7
Tot.; Uniq. Data	13974, 4988	17948, 5310	32836, 4672
R_{int}	0.021	0.023	0.023
Nref; Npar	4988, 200	5310, 209	4672, 194
R, wR_2, S	0.0408, 0.1175, 1.06	0.0233, 0.0605, 1.05	0.0251, 0.0642, 1.08

Table 2
Selected bond lengths (Å) and angles (°) for **2**.

Bond distance (Å)			
N2–C1	1.331(2)	C10–C11	1.444(2)
N3–C1	1.3342(18)	N1–C11	1.147(2)
N2–C12	1.4670(18)	N2–C2	1.381(2)
N3–C4	1.4785(18)	N3–C3	1.380(2)
C4–C5	1.509(2)	P1–F1	1.5883(11)
C5–C10	1.4034(19)	P1–F5	1.6001(12)
C5–C6	1.394(2)		
Bond angle (°)			
N2–C1–N3	108.69(14)	C4–C5–C6	120.40(12)
C1–N2–C2	108.69(12)	N1–C11–C10	178.94(16)
C1–N3–C3	108.47(12)	C4–C5–C10	120.92(14)
C1–N3–C4	125.79(13)	C5–C10–C11	119.64(14)
N3–C4–C5	110.76(11)	C1–N2–C12	125.45(13)

Table 4
Selected bond lengths (Å) and angles (°) for **6**.

Bond distance (Å)			
Ag1–C1	2.090(11)	C4–C5	1.5137(17)
N2–C4	1.464(16)	N1–C2	1.3834(16)
N2–C1	1.358(15)	N2–C3	1.3864(15)
N3–C11	1.149(19)	C5–C10	1.3974(17)
N1–C1	1.355(16)	C2–C3	1.3493(19)
C11–C10	1.440(18)	P1–F1	1.6101(8)
N1–C12	1.4586(17)		
Bond angle (°)			
C1–Ag1–C1A	175.0(4)	Ag1–C1–N2	129.3(8)
N1–C1–N2	104.1(9)	Ag1–C1–N1	126.6(8)
C1–N1–C2	111.4(10)	C1–N2–C3	111.4(10)
N2–C4–C5	111.9(10)	N3–C11–C10	179.4(15)

Table 3
Selected bond lengths (Å) and angles (°) for **5**.

Bond distance (Å)			
Ag1–C1	2.070(17)	N2–C4	1.469(19)
Ag1–Ag1A	3.634(0)	N1–C1	1.353(19)
C4–C5	1.512(2)	N1–C12	1.465(2)
Ag1–N3A	2.096(16)	N1–C2	1.383(2)
N2–C1	1.356(2)	N2–C3	1.3846(18)
C10–C11	1.437(2)	C5–C10	1.4053(19)
N3–C11	1.140(2)	P1–F1	1.5871(13)
Bond angle (°)			
N3A–Ag1–C1	168.6(5)	N3–C11–C10	178.2(17)
Ag1–C1–N2	132.2(10)	Ag1–C1–N1	123.2(12)
C1–N2–C4	124.6(13)	C1–N2–C3	110.9(12)
Ag1–N3–C11	160.6(12)	C5–C10–C11	120.4(14)
C1–N1–C2	111.4(14)		

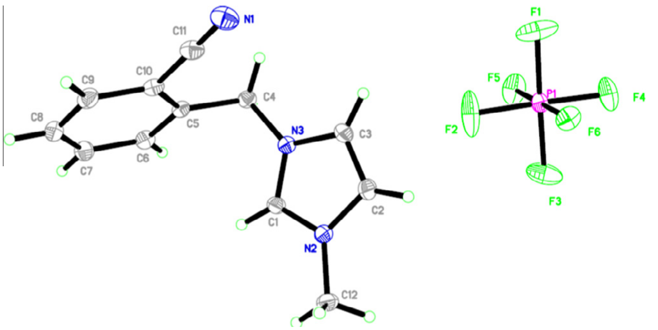


Fig. 1. ORTEP diagram of imidazolium salt **2** with thermal ellipsoid drawn at 50% probability.

each other. The bond angle at nitrile carbon is almost linear (178.93(16)°) with $C\equiv N$ bond distance of 1.1464(19) Å, which is consistent with the similar structures [19]. The dihedral angle between the benzene and imidazole rings and the internal bond angle at N2–C1–N3 center are 110.76(10) and 108.68(11)°, respectively. In the crystal structure, the imidazolium cations and hexafluorophosphate anions are connected by C–H- - -F (2.549(3) Å) and C–H- - -N (3.086(10) Å) hydrogen bonding interactions into a three-dimensional framework.

Although a large number of Ag–NHC complexes have been structurally characterized, only a few binuclear complexes stabilized by both, a NHC and an N-donor module were reported. Binuclear complex **5** crystallizes in the monoclinic space group *P*21(*c*).

A perspective view of the structure of complex **5** is depicted in Fig. 2. The asymmetric unit of the complex consists of one half of the complex cation having only one silver(I) center and one hexafluorophosphate anion. In this dimeric complex structure, the nitrile moiety of one ligand cooperatively binds the second silver(I) center through the nitrogen like a N-bonded dimer, and the second coordination of the silver(I) centers is furnished by the carbene carbon atoms. The silver(I) centers exist in an almost linear coordination geometry with C1–Ag1–N3 bond angles 168.57(5)° formed by carbene carbon and nitrile–nitrogen coordination. This deviation linearity is due to the coordination of nitrile–nitrogen atom, whose plane was almost perpendicular to the plan of imidazole ring in the ligand system. The dimeric silver(I) structures also have an important consequence for the moderate to strong Ag–Ag interactions [20]. For this complex, the Ag–Ag distance measured is much longer than those observed for the analogues NHC–Ag–Ag–NHC dimers, evidencing no Ag–Ag interactions. On the other hand, few strange interactions between silver(I) and fluoride atoms is observed with the interaction bond distance in the range 3.0722(13)–3.3773(12) Å. In the extended crystal structure of **5**, like imidazolium salt **2**, the complex cations and hexafluorophosphate anions are connected via C–H- - -F (2.529(12) Å), and C–H- - -N (3.072(5) Å) hydrogen bonding interactions.

Single crystal X-ray analysis of silver(I) complex **6** is indicative of a mononuclear bis-NHC silver(I) complex crystallized in the orthorhombic space group *Pnna*, showing one half of the complex cation and one hexafluorophosphate anion in the asymmetric unit. A perspective view of the complex **6** is shown in Fig. 3. In the complex cation of **6**, the silver(I) center is coordinated by two carbene carbon atoms, without showing any nitrile-silver(I) interactions. Both the imidazolium rings are orientated at a torsion angle 23.06(10)°, forming almost linear C1–Ag–C1A bond angle of 175.03(6)° [21]. Two of the fluorine atoms of the general-position hexafluorophosphate anion are disordered over two sets of sites. In the extended complex structure, the silver(I) complex cations and hexafluorophosphate anions linked together into three-dimensional networks via C–H- - -F (3.282(10) Å), and C–H- - -N (2.854(6) Å) hydrogen bonding interactions. In the complex structures of **5** and **6**, compared with the NHC proligand **2**, the internal bond angle values at carbene carbon centers decreased to 104.51(12) and 104.08(10)°, respectively from 108.68(11)° (salt **2**), as a result of the coordination of carbene carbon atoms with the silver centers. Similar observations are found with complexes having analogues ligand architectures [22].

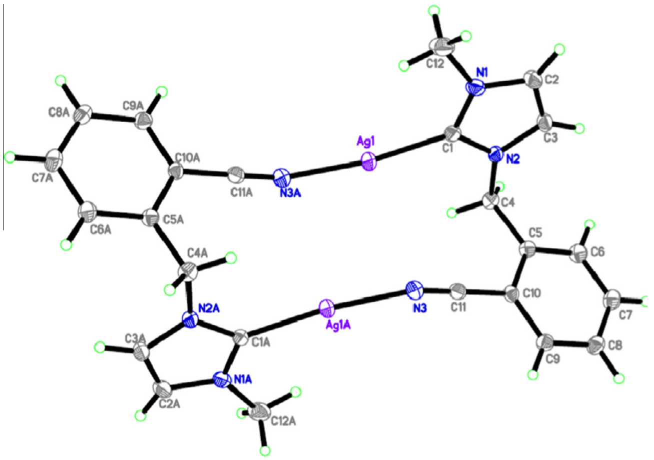


Fig. 2. ORTEP diagram of binuclear silver(I) carbene – complex **5** with thermal ellipsoid drawn at 50% probability. Hexafluorophosphate anions are removed for clarity.

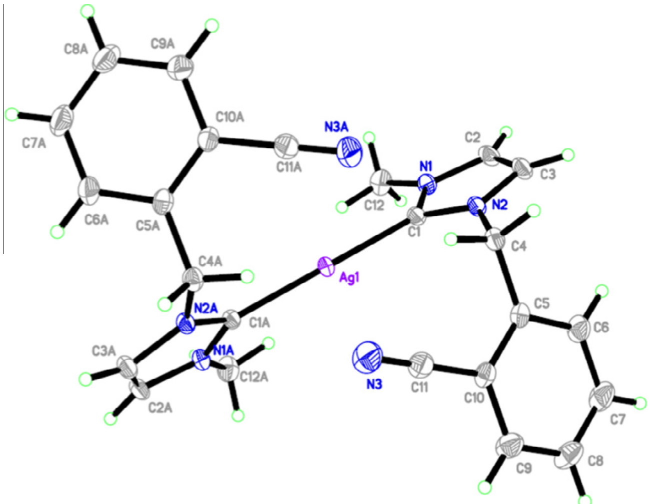


Fig. 3. ORTEP diagram of mononuclear silver(I) carbene – complex **6** with thermal ellipsoid drawn at 50% probability. Hexafluorophosphate anion is removed for clarity.

3.4. Anticancer activity

Several transition metal complexes with NHCs have been recently studied for various biological applications [23], and some of them show strong anticancer potentials. So far, most of the structurally characterized silver(I)–carbene complexes can be classified into categories of NHC–Ag–NHC, NHC–Ag–acetate, NHC–Ag–halides, and NHC–Ag–Ag–halide [24]. The NHC–Ag–Y (Y is coordinating group other than halides/acetate) type complexes are relatively rare; only a few binuclear silver(I) complexes have been reported for catalytic purposes [25]. However, to our knowledge, silver(I)–NHC complexes of NHC–Ag–Y type have not been reported along with their anticancer studies, thus, complex **5** represents the first example of a binuclear silver(I)–carbene compound supported by a functionalized NHC ligand.

Since we have demonstrated [26] that the nitrile-functionalized benzimidazole-based silver(I)–carbene complexes are favorable candidates to bind and/or cleave DNA in the presence and absence of an oxidizing agent, we were also interested in studying analogues complexes for anticancer potentials. Since silver(I)–NHC complexes of imidazole derived ligands are promising anticancer agents [7a], we capitalized on this by inserting additional coordinating nitrile arm for the formation of bis-carbene and carbene–nitrile coordinated silver(I) complexes which do show potential anticancer activity. For the present study, the *in vitro* cytotoxicity of both, imidazolium salts **1–4** and their silver(I) complexes **5–8**, was determined using MTT-based assay. Compounds **1–8** were tested for their activity against the HCT 116 cell line at different concentration levels using 5-fluorouracil as standard. Figs. 4 and 5 show the effect of test compound concentration on the HCT 116 cell line after 24 h of incubation time. Imidazolium salts **1**, **2** and **4** were found inactive against tested cancer cell line. Treatment with these compounds did not show any toxic effect on proliferation of HCT 116 cells as the IC_{50} value was found more than 200 μM. The cell growth did not get affected and cellular morphology was almost similar to that of untreated cells. However, action of salt **3** on HCT 116 cells evidenced strong cytotoxic nature with IC_{50} value of 5.0 ± 0.3 μM as the antiproliferative effect was similar to that of the standard reference 5-FU. The viability of the cells was severely affected, as it showed all the treated cells lost their viable characteristic features.

The mononuclear silver(I) complex **6** against HCT 116 (IC_{50} = 6.0 ± 0.7 μM) is clearly less effective than binuclear silver(I)

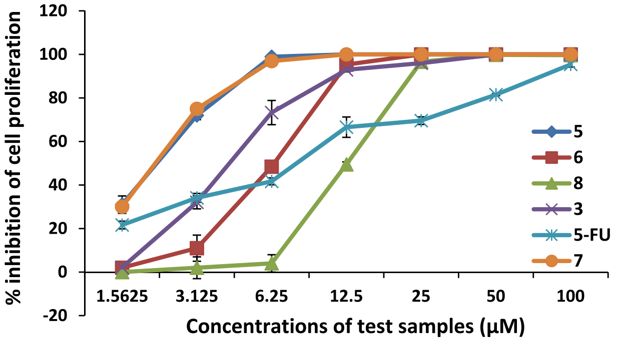


Fig. 4. MTT assay results of imidazolium salt **3** and silver(I)–carbene complexes **5–8** vs. the HCT 116 cell lines.

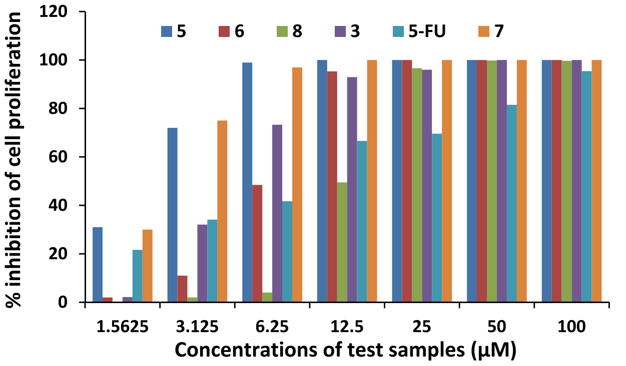


Fig. 5. Effects of increasing amounts of imidazolium salt **3** and silver(I)–carbene complexes **5–8** on the percentage inhibition of cell proliferation.

complex **5** bearing bidentate NHC ligand (IC_{50} = 1.7 ± 0.4 µM). This may be explained by the binuclear nature of the complex which obviously has double silver(I) concentration than monuclear complex, and the bidentate nature of the ligand rendering the complex **5** more stable than complex **6**. Thus, it is possible that increased stability of binuclear complex **5** results in a slower release of silver(I) ions ended with the higher activity. HCT 116 cells treated with these complexes exhibited more pronounced anti-proliferative activity than the standard reference drug 5-FU. Complexes **5** and **6** have similar mode of action on tested cancer cell line as both of them affected almost all cells. Except few dead cells, only debris can be seen in the photomicrograph as shown in Fig. 6. Finally, to check the effect of counterions on the anti-proliferative activity of the compounds, we evaluated the complexes **7** and **8** for their potential against the same cell line. Complexes **7** and **8** have bromide and hexafluorophosphate counterions, respectively, this leads to different complex stability despite having same chelating effect by NHC ligands. Complex **8** having bulkier counterion, however, is less cytotoxic with IC_{50} value of 27.2 ± 1.1 µM than the bromide complex **7** (IC_{50} = 1.7 ± 0.2 µM), which is nearly 17 times more potent against HCT 116 cell line. The cells treated with complex **7** showed marked signs of cytotoxicity caused by the compound, which affected the viable characteristic features. In contrast, complex **8** is more stable than complex **7** as is having bulkier hexafluorophosphate conterion, hence, it is possible that this complex can become too stable and doesn't release adequate silver(I) at the required site, therefore becomes less efficient over the drug-exposure period. Anticancer potential of these complexes are comparable with the similar silver(I)–carbene complexes of the type NHC–Ag–halide/acetate [27].

4. Conclusion

In summary, we report the nitrile-functionalized imidazole-based silver(I)–carbene complexes and their anticancer potential toward HCT 116 cancer cell line. Nitrile-functionalized NHC proligands with multiple coordination sites potentially available for hosting two silver(I) ions were reacted with silver(I) oxide at mild reaction conditions. Depending on the molar ratio of silver(I) oxide employed, the stepwise formation of the resultant mononuclear and binuclear silver(I) complexes proved possible. Synthetic routes employed to obtain both NHC proligands and their silver(I) complexes were modular and high yielding. The obtained compounds were tested for their potential anticancer activity; showing binuclear silver(I) complex is more potential than mononuclear complex as well as NHC proligands. The promise of the nitrile-functionalized NHC proligands binding to two silver(I) centers through simple synthetic routes; efforts toward other transition metal-carbene complexes are currently underway.

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Appendix A. Supplementary material

CCDC 945717, 945718 and 945719 contains the supplementary crystallographic data for 2, 5 and 6, respectively. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Supplementary

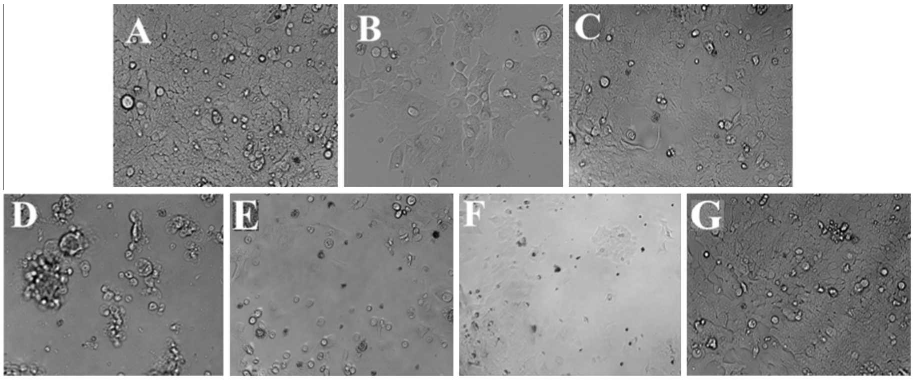

Fig. 6. Images of control HCT116 cells and cells treated with imidazolium salt **3** and silver(I)–carbene complexes **5–8** after 72 h of incubation. (A: control HCT116 cells, B: cells treated with 5FU, C: cells treated with salt **3**, D: cells treated with complex **5**, E: cells treated with complex **6**, F: cells treated with complex **7**, G: cells treated with complex **8**).

data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ica.2013.11.011.

References

[1] (a) E.A.B. Kantchev, C.J. Orien, M.G. Organ, Angew. Chem., Int. Ed. 46 (2007) 2768;
(b) J.C. Garrison, W.J. Youngs, Chem. Rev. 105 (2005) 3978.

[2] (a) W.A. Herrmann, C. Kocher, Angew. Chem., Int. Ed. Engl. 36 (1997) 2162;
(b) D. Bourissou, O. Guerret, P. Francüois, F.P. Gabbai, G. Bertrand, Chem. Rev. 100 (2000) 39;
(c) B. Bildstein, M. Malaun, H. Kopacka, K. Wurst, M. Mitterbock, K.-H. Ongania, G. Opromolla, P. Zanello, Organometallics 18 (1999) 4325;
(d) H.M.J. Wang, C.Y.L. Chen, I.J.B. Lin, Organometallics 18 (1999) 1216.

[3] (a) K.J. Cavell, D.S. McGuinness, Coord. Chem. Rev. 248 (2004) 671;
(b) E. Peris, R.H. Crabtree, Coord. Chem. Rev. 248 (2004) 2239.

[4] (a) S. Diez-Gonzalez, S.P. Nolan, Coord. Chem. Rev. 251 (2007) 874;
(b) C.M. Crudden, D.P. Allen, Coord. Chem. Rev. 248 (2004) 2247.

[5] (a) D. Yuan, H.V. Huynh, Molecules 17 (2012) 2491;
(b) S. Budagumpi, R.A. Haque, A.W. Salman, M.Z. Ghdhayeb, Inorg. Chim. Acta 392 (2012) 61.

[6] (a) S. Budagumpi, R.A. Haque, A.W. Salman, Coord. Chem. Rev. 256 (2012) 1787;
(b) S. Budagumpi, K.-H. Kim, I. Kim, Coord. Chem. Rev. 255 (2011) 2785;
(c) S. Patil, A. Deally, B. Gleeson, H. Müller-Bunz, F. Paradisi, M. Tacke, Appl. Organomet. Chem. 24 (2010) 781;
(d) S. Ray, R. Mohan, J.K. Singh, M.K. Samantaray, M.M. Shaikh, D. Panda, P. Ghosh, J. Am. Chem. Soc. 129 (2007) 15042.

[7] (a) S. Budagumpi, R.A. Haque, S. Endud, G.U. Rehman, A.W. Salman, Eur. J. Inorg. Chem. (2013) 4367;
(b) G. Gasser, I. Ott, N. Metzler-Nolte, J. Med. Chem. 54 (2011) 3.

[8] (a) C.L. Cannon, L.A. Hogue, R.K. Vajravelu, G.H. Capps, A. Ibricevic, K.M. Hindi, A. Kascatan-Nebioglu, M.J. Walter, S.L. Brody, W.J. Youngs, Antimicrob. Agents Chemother. 53 (2009) 3285;
(b) W.J. Youngs, A.R. Knapp, P.O. Wagers, C.A. Tessier, Dalton Trans. 41 (2012) 327.

[9] I. Fichtner, J. Cinatl, M. Michaelis, L.C. Sanders, R. Hilger, B.N. Kennedy, A.L. Reynolds, F. Hackenberg, G. Lally, S.J. Quinn, I. McRae, M. Tacke, Lett. Drug Des. Discovery 9 (2012) 815.

[10] (a) A. Kascatan-Nebioglu, M.J. Panzner, C.A. Tessier, C.L. Cannon, W.J. Youngs, Coord. Chem. Rev. 251 (2007) 884;
(b) D.A. Medvetz, K.M. Hindi, M.J. Panzner, A.J. Ditto, Y.H. Yun, W.J. Youngs, Met. Based Drugs (2008), http://dx.doi.org/10.1155/2008/384010.

[11] R.A. Haque, M.Z. Ghdhayeb, A.W. Salman, S. Budagumpi, M.B. Khadeer, Inorg. Chem. Commun. 22 (2012) 113;
R.A. Haque, M.Z. Ghdhayeb, S. Budagumpi, A.W. Salman, M.B. Khadeer, Inorg. Chim. Acta 394 (2013) 519.

[12] M.A. Iqbal, R.A. Haque, M.B. Khadeer, Med. Chem. Res. (2012), http://dx.doi.org/10.1007/s00044-012-0240-6.

[13] (a) R.A. Haque, S.A. Ahmed, Z.H. Zetty, M. Hemamalini, H.-Kun Fun, Acta Crystallogr., Sect E 67 (2011) o3462;
(b) R.A. Haque, H.Z. Zulikha, M.Z. Ghdhayeb, S. Budagumpi, A.W. Salman, Heteroat. Chem. 23 (2012) 486.

[14] W. Liu, R. Gust, Chem. Soc. Rev. 42 (2013) 755.

[15] S. Patil, A. Deally, B. Gleeson, H. Müller-Bunz, F. Paradisi, M. Tacke, Metallomics 3 (2011) 74.

[16] S. Patil, J. Claffey, A. Deally, M. Hogan, B. Gleeson, L.M. Menéndez, Eur. J. Inorg. Chem. (2010) 1020.

[17] (a) M.V. Baker, D.H. Brown, R.A. Haque, B.W. Skelton, A.H. White, Dalton Trans. (2004) 3756;
(b) H.M.J. Wang, I.J.B. Lin, Organometallics 17 (1998) 972;
(c) F.E. Hahn, C. Radloff, T. Pape, A. Hepp, Chem. Eur. J. 14 (2008) 10900.

[18] W.N.O. Wylie, A.J. Lough, R.H. Morris, Organometallics 29 (2010) 570.

[19] R.A. Haque, Z.H. Zetty, A.W. Salman, H.-K. Fun, C.W. Ooi, Acta Crystallogr., Sect E 68 (2012) o489.

[20] M.A. Iqbal, R.A. Haque, S. Budagumpi, M.B. Khadeer, Inorg. Chem. Commun. 28 (2013) 64;
(b) V.J. Catalano, M.A. Malwitz, Inorg. Chem. 42 (2003) 5483;
(c) V.J. Catalano, A.O. Etogo, J. Organomet. Chem. 690 (2005) 6041;
(d) K. Singh, J.R. Long, P. Stavropoulos, J. Am. Chem. Soc. 119 (1997) 2942.

[21] R.A. Haque, M.A. Iqbal, S. Budagumpi, M.B. Khadeer, Appl. Organomet. Chem. 27 (2013) 214;
(b) R.A. Haque, A. Washeel, S.G. Teoh, M. Hemamalini, H.-K. Fun, Acta Crystallogr., Sect E 66 (2010) m1286.

[22] (a) A.W. Salman, R.A. Haque, S. Budagumpi, H.Z. Zulikha, Polyhedron 49 (2013) 200;
(b) F. Hackenberg, G. Lally, H. Müller-Bunz, F. Paradisi, D. Quaglia, W. Streciwilk, M. Tacke, Inorg. Chim. Acta 395 (2013) 135.

[23] L. Oehninger, R. Rubbiani, I. Ott, Dalton Trans. 42 (2013) 3269.

[24] (a) I.J.B. Lin, C.S. Vasam, Coord. Chem. Rev. 251 (2007) 642;
(b) I.J.B. Lin, C.S. Vasam, Inorg. Chem. 25 (2004) 75;
(c) R.A. Haque, S.F. Nasri, M.A. Iqbal, J. Coord. Chem. (2013), http://dx.doi.org/10.1080/00958972.2013.813492.

[25] H. Guernon, C.Y. Legault, Organometallics (2013) 2–9. http://dx.doi.org/10.1021/om400078f.

[26] R.A. Haque, S. Budagumpi, S.Y. Choo, M.K. Choong, B.E. Lokesh, K. Sudesh, Appl. Organomet. Chem. 26 (2012) 689.

[27] (a) M.A. Sharkey, J.P. O'Gara, S.V. Gordon, F. Hackenberg, C. Healy, F. Paradisi, S. Patil, B. Schaible, M. Tacke, Antibiotics 1 (2012) 25;
(b) W. Liu, K. Bensdorf, A. Hagenbach, U. Abram, B. Niu, A. Mariappan, R. Gust, Eur. J. Med. Chem. 46 (2011) 5927.