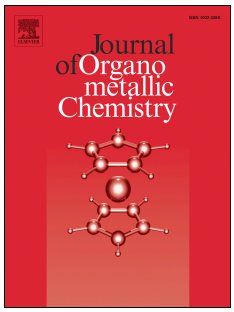
Accepted Manuscript



Synthesis, characterization and anti-proliferative activity of propylene linked bis-benzimidazolium salts and their respective dinuclear Silver(I)-*N*-heterocyclic carbene complexes

Sunusi Y. Hussaini, Rosenani A. Haque, Patrick O. Asekunowo, A.M.S. Abdul Majid, M. Taleb Agha, Mohd. R. Razali

PII: S0022-328X(17)30238-3

DOI: 10.1016/j.jorganchem.2017.04.011

Reference: JOM 19898

To appear in: *Journal of Organometallic Chemistry*

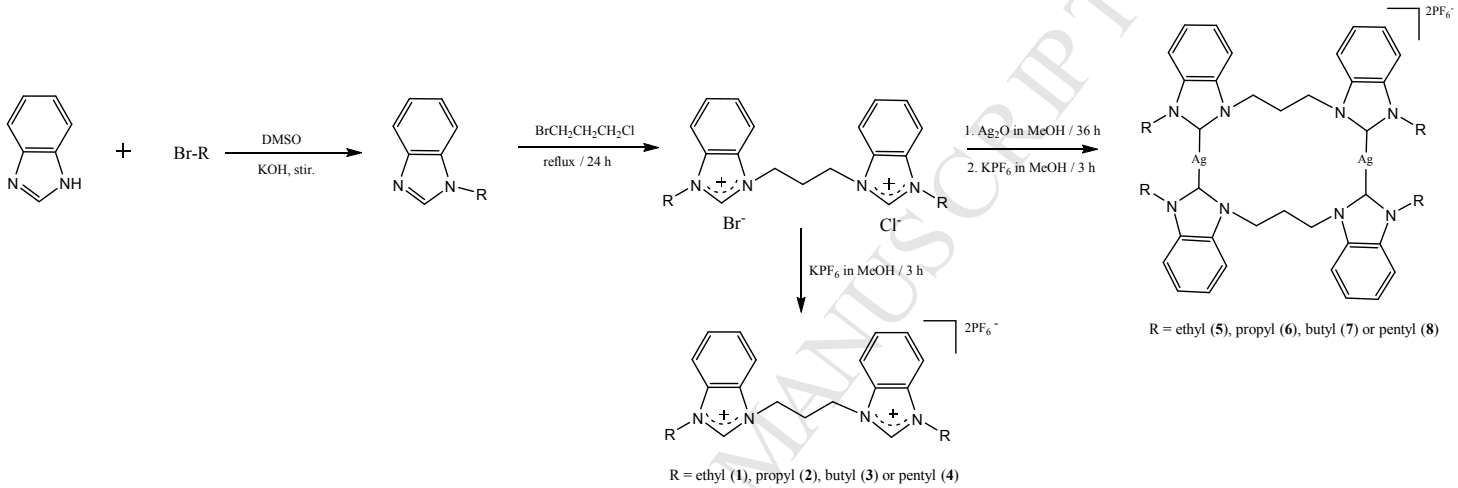
Received Date: 9 March 2017

Revised Date: 10 April 2017

Accepted Date: 12 April 2017

Please cite this article as: S.Y. Hussaini, R.A. Haque, P.O. Asekunowo, A.M.S. Abdul Majid, M. Taleb Agha, M.R. Razali, Synthesis, characterization and anti-proliferative activity of propylene linked bis-benzimidazolium salts and their respective dinuclear Silver(I)-*N*-heterocyclic carbene complexes, *Journal of Organometallic Chemistry* (2017), doi: 10.1016/j.jorganchem.2017.04.011.

Graphical Abstract



R = ethyl (**1**), propyl (**2**), butyl (**3**) or pentyl (**4**)

R = ethyl (**5**), propyl (**6**), butyl (**7**) or pentyl (**8**)

Synthesis, characterization and anti-proliferative activity of propylene linked bis-benzimidazolium salts and their respective dinuclear Silver(I)-*N*-heterocyclic carbene complexes

Sunusi Y. Hussaini,[a,c] Rosenani A. Haque,[a] Patrick O. Asekunowo,[a] A.M.S. Abdul Majid,[b] Taleb Agha M.[b] and Mohd. R. Razali[a‡]

[a]School of Chemical Sciences, Universiti Sains Malaysia, Malaysia.

[b]EMAN Research and Testing Laboratory, School of Pharmacy, Universiti Sains Malaysia, Malaysia.

[c]Department of Chemistry, Kano University of Science and Technology Wudil, Kano, Nigeria.

‡Correspondence email: mohd.rizal@usm.my

Abstract

A new series of propylene linked bis-benzimidazolium salts, **1-4** were reacted with Ag_2O to facilitate the formation of their respective dinuclear Ag(I)-*N*-heterocyclic carbene (NHC) complexes, **5-8** respectively. All compounds were fully characterized by elemental analyses, ^{1}H-NMR, ^{13}C-NMR and FTIR spectroscopy techniques. The molecular structures of compounds **1** and **6** were elucidated through single crystal X-ray diffraction analyses. Both salts and complexes were tested for anti-cancer potential against breast cancer (MCF-7) cell line. The IC_{50} values are in the range of 7±1-18±3 µM show that the complexes **5-8** possess moderate antiproliferative effect while their respective salts **1-4** are found to be inactive against MCF-7 cell line.

Keywords: Dinuclear Ag(I)-NHC complexes; Anti-cancer activity; *N*-heterocyclic carbene.

1. Introduction

N-heterocyclic carbenes (NHCs) are generally originated from the so-called persistent carbenes which are stabilized by two π-donor nitrogen to formed stable compounds of divalent carbon carbene atom [1]. The NHCs are known as a strong σ-donating and weak π-accepting when connected with metal ions [2-4]. Metal-NHC complexes are accessible by different routes, but the most common route is deprotonation of azolium precursors with simple metal salt or coordination compounds (neutral, cation and anion) in the presence of strong base, such as potassium tert-butoxide [5]. From literature, synthesized metal-NHC complexes adopt different structural diversity have been investigated for various applications due to their high and ease of derivatization. These thus allow the metal-NHC complexes to be explored for transmetalation, catalysis and drugs developments [6-9].

The newly emerging interest of medicinal and transmetalation applications of Ag(I)-NHC complexes led an extensive investigations due to their ease of synthesis, ease of handling and stability towards air and moisture. These properties influenced the highly abundance of Ag(I)-NHC in literature that concentrated in synthesis and applications in bioorganometallic as well as transmetalation reactions [8, 10, 11]. The biological applications of Ag(I)-NHC complexes are primarily based on the silver element and its salts. The ionic silver and silver salts compounds were used as antimicrobial agents and used to prevent infections in wound dressing treatment [12,13]. Meanwhile, the Ag(I)-NHC complexes show high effect against a different type of cancer cell line due to high stability and slow release of silver ions from Ag(I)-NHC complexes to the affected area over a period of time; eventually the cytoplasm constituents leak and cause the death of cells [14].

The higher nuclearity of Ag(I)-NHC complexes with multidentate benz/imidazolium-based NHC ligands are postulated to be more stable compared with mononuclear Ag(I)-NHCs complexes of monodentate ligands [15]. The later complexes are also documented to be more active and betterment biological applications in which two Ag(I) ions are slowly released for better activity in high nuclearity Ag(I) complexes [16]. Furthermore, the multidentate ligands precursor can also be anionic tethered NHCs which able to compensate the charges of the coordinated metal [17]. Bis-benzi/imidazolium salts bearing numerous spacers or linkers between the two benz/imidazolium moieties are linked, for examples by alkylene [18], xylylene [19] or pyridyl [20]. These spacers provide an extra stability to their respective dinuclear Ag(I)-NHC complexes in which the scaffolds formed behaving as an agents in biological and medicinal applications [21-23]. Several works were reported on synthesis of linked bis-benz/imidazolium salts and their respective dinuclear Ag(I)-NHC complexes with potential activities against various types of cancer cell lines and showed effective results in their activity due to the present of two Ag(I) ions [24]. Herein, our main focus is to develop the benzimidazole-based dinuclear Ag(I)-NHC complexes of propylene linker with various alkyl group as substituents to investigate their efficacy of anti-proliferative differences against breast cancer cell line (MCF-7).

2. Experimental

2.1 Material and Measurements

All chemicals and solvents were used as received. The NMR spectra were recorded on Bruker 500 MHz spectrometer at ambient temperature in either DMSO-d_6 or acetonitrile-d_3 using TMS as an internal standard. FT-IR spectra were recorded on Perkin Elmer 200 system

spectrometer. Elemental analysis was carried out on Perkin Elmer Series II, 2400 micro-analyzer. Melting points were assessed using Stuart Scientific SMP-1 (UK) instrument.

2.2 Syntheses

2.2.1. Synthesis of 1,1-propylenebis(3-ethylbenzimidazolium)bis(hexafluorophosphate) (1)

The mixture of benzimidazole (1.00 g, 8.46 mmol) and KOH (0.95 g, 17.00 mmol) in DMSO (30 mL) was stirred for 30 minutes at room temperature.1-bromoethane (0.93 g, 8.46 mmol) was added dropwise and the mixture was stirred at room temperature for 3 h. The solution mixture was then poured into water (250 mL) and extracted with chloroform (4 x 25 mL). The product was filtered through four plies of Whatman filter papers and clear solution of *N*-ethylbenzimidazole was obtained. The mixture of *N*-ethylbenzimidazole (1.50 g, 10.30 mmol) and 1-bromo-3-chloropropane (0.81 g, 5.20 mmol) in acetonitrile (30 mL) was refluxed at 80 ^{O}C for 24 h and then the solvent was evaporated under reduced pressure to yield a white crystalline solid of 1,1-propylenebis(3-ethylbenzimidazolium)bromidechloride salt which was converted to its hexafluorophophate salt by metathesis using KPF_6 (0.95 g, 5.20 mmol) in methanol (20 mL). The mixture was stirred at room temperature for 3 h, which was then solvent was removed under reduced pressure. The obtained white powder was washed with distilled water (3 x10 mL). The product was recrystallized from acetonitrile by ether diffusion. Yield: 2.19 g (67%), mp: 183-186 ^{o}C. ^{1}H NMR (500 MHz, DMSO-d_6): 1.58 (6H, t, *J* = 7.5 Hz, 2 x **CH_3**); 2.66 (2H, m, NCH_2**CH_2**CH_2N); 4.52 (4H, q, 2 x N**CH_2**-CH_3); 4.67 (4H, t, *J* =7.0 Hz, 2 x N**CH_2**CH_2); 7.74 (2H, m, Ar-H); 8.11(8H, m, 2 x Ar-H); 9.73 (2H, s, 2 x N**CH**N). ^{13}C {^{1}H} NMR (125 MHz, DMSO-d_6): 13.98 (CH_3), 27.93 (NCH_2**CH_2**CH_2N); 42.13 (N**CH_2**CH_3); 43.92 (N**CH_2**CH_2); 113.66, 126.63, 130.98, 131.12

(benzimidazole **Ar-C**); 141.88 (N**C**H**C**N). FTIR (KBr disc) in cm^{-1}, 3168, 3117 (C-H_{arom}); 2984, 2889 (C-H_{alph}); 1577 (C=N); 1290 (C-N). Anal. Calc. for $C_{21}H_{26}F_{12}N_4P_2$: C, 40.39; H, 4.17; N, 8.97%. Found: C, 40.35; H, 3.93; N, 8.98 %

2.2.2. Synthesis of 1,1-propylenebis(3-propylbenzimidazolium)bis(hexafluorophosphate) (2)

The procedure is similar to that in 2.2.1 but using 1-bromopropane (1.05 g, 8.46 mmol) instead of 1-bromoethane. Yield:1.62 g (80%), mp: 185-188 ^oC. ^1H NMR (500 MHz, DMSO-d_6): 0.97 (6H, t, *J* = 7.0 Hz, 2 x **CH_3**); 1.96 (4H, m, 2 x **CH_2**CH_3); 2.64 (2H, m, NCH_2**CH_2**CH_2N); 4.46 (4H, t, *J* = 7.5 Hz, 2 x N**CH_2**-R); 4.66 (4H, t, *J* =7.5 Hz, 2 x N**CH_2**CH_2); 7.74 (2H, m, Ar-H); 8.15 (8H, m, 2 x Ar-H) 9.75 (2H, s, 2 x N**C**H**C**N). ^{13}C {^1H} NMR (125 MHz, DMSO-d_6): 10.68 (CH_3); 22.02 (CH_2**CH_2**CH_3); 27.95 (NCH_2**CH_2**CH_2N); 43.96 (N**CH_2**-R); 48.17 (N**CH_2**CH_2); 113.77, 126.62, 131.09, 131.19 (benzimidazole **Ar-C**); 142.17 (N**C**H**C**N). FTIR (KBr disc) in cm^{-1}, 3162, 3094 (C-H_{arom}); 2974, 2885 (C-H_{alph}); 1574 (C=N); 1265 (C-N). Anal. Calc. for $C_{23}H_{30}F_{12}N_4P_2$: C, 42.34; H, 4.60; N, 8.59%. Found: C, 42.25; H, 4.56; N, 8.57%

2.2.3. Synthesis of 1,1-propylenebis(3-butylbenzimidazolium)bis(hexafluorophosphate) (3)

The procedure is similar to that in 2.2.1 but using 1-bromobutane (1.16 g, 8.46 mmol) instead of 1-bromoethane Yield: 2.13 g (77%), mp: 187-189 ^oC. ^1H NMR (500 MHz, DMSO-d_6) 0.96 (6H, t, *J* = 7.5 Hz, 2 x **CH_3**); 1.40 (4H, m, 2 x **CH_2**CH_3); 1.91 (4H, m, 2 x **CH_2**CH_2CH_3); 2.63 (2H, m, NCH_2**CH_2**CH_2N); 4.50 (4H, t, *J* = 7.5 Hz, 2 x N**CH_2**-R); 4.67 (4H, t, *J* = 7.5 Hz, 2 x N**CH_2**CH_2); 7.74 (4H, m,2 x Ar-H); 8.13 (4H, m, 2 x Ar-H); 9.81 (2H, s, 2 x N**C**H**C**N). ^{13}C {^1H} NMR (125 MHz, DMSO-d_6): 13.36 (**CH_3**); 19.07 (**CH_2**CH_3); 27.94 (**CH_2**CH_2CH_3); 30.53 (NCH_2**CH_2**CH_2N); 43.95 (N**CH_2**-R); 46.50 (N**CH_2**CH_2); 113.54,

126.61, 131.09, 131.16 (benzimidazole **Ar-C**); 142.13 (N**C**H**C**N). FTIR (KBr disc) in cm^{-1}, 3160, 3103 (C-H_{arom}); 2964, 2939 (C-H_{alph}); 1570 (C=N), 1298 (C-N). Anal. Calc. for $C_{25}H_{34}F_{12}N_4P_2$: C, 44.12; H, 5.00; N, 8.24%. Found: C, 43.67; H, 4.58; N, 8.22%

2.2.4. Synthesis of 1,1-propylenebis(3-pentylbenzimidazolium)bis(hexafluorophosphate) (4)

The procedure is similar to that in 2.2.1 but using 1-bromopentane (1.28 g, 8.46 mmol) instead of 1-bromoethane. Yield: 2.24 g (79%), mp: 190-192 ^{o}C. ^{1}H NMR (500 MHz, DMSO-d_6): 0.90 (6H, t, *J* = 7.0 Hz, 2 x **CH_3**); 1.36 (8H, m, 4 x **CH_2**); 1.93 (4H, m, 2 x NCH_2**CH_2**); 2.64 (2H, m, NCH_2**CH_2**CH_2N); 4.64 (4H, t, *J* = 7.5 Hz, 2 x N**CH_2**-R); 4.66 (4H, t, *J* = 7.0 Hz, 2 x N**CH_2**CH_2); 7.74 (2H, m, 2 x Ar-H); 8.13 (2H, m, 2 x Ar-H); 9.74 (2H, s, 2 x N**C**H**C**N). ^{13}C {^{1}H} NMR (125 MHz, DMSO-d_6): 13.70 (**CH_3**); 21.58 (**CH_2**CH_3); 27.85 (**CH_2**CH_2CH_3); 27.93 (NCH_2**CH_2**); 28.20 (NCH_2**CH_2**CH_2N); 43.95 (N**CH_2**-R); 46.71(N**CH_2**CH_2); 113.53, 126.61, 131.07, 131.16 (benzimidazole **Ar-C**); 142.11 (N**C**H**C**N). FTIR (KBr disc) in cm^{-1}, 3160, 3102 (C-H_{arom}); 2965, 2937 (C-H_{alph}); 1568 (C=N), 1265 (C-N). Anal. Calc. for $C_{27}H_{38}F_{12}N_4P_2$: C, 45.77; H, 5.37; N, 7.91 %. Found: C, 45.55; H, 4.97; N, 7.87 %.

2.2.5. Synthesis of 1,1-propylenebis(3-ethylbenzimidazol-2-ylidene)disilver(I) bis(hexafluorophosphate) (5)

Compound **1** (0.50 g, 1.60 mmol) was dissolved in methanol (50 mL) along with Ag_2O (0.76 g, 3.30 mmol) with exclusion of light by covering using aluminum foil. The mixture was stirred for 36 h at room temperature and then filtered. The obtained clear solution was converted to its hexafluorophosphate by presence of KPF_6 (0.60 g, 3.20 mmol) in a mixture of methanol/water (40 mL). The white precipitate appeared was collected and washed with distill

water (3 x 5 mL) to facilitate the formation of grey powder after air-dried. Product of grey powder was further recrystallized from acetonitrile to give white crystalline solid. Yield: 0.64 g (35%). mp: 211-213 ^{o}C. ^{1}H NMR (500 MHz, acetonitrile-d_3): 1.41 (6H, t, J = 7.5 Hz, 2 x **CH_3**); 2.76 (2H, m, NCH_2**CH_2**CH_2N); 4.52 (4H, q, 2 x N**CH_2**-CH_3); 4.81 (4H, t, J = 7.0 Hz 2 x N**CH_2**CH_2); 7.45 (2H, m, 2 x Ar-H); 8.13 (8H, m, 2 x Ar-H). ^{13}C {^{1}H} NMR (125 MHz, acetonitrile-d_3): 14.96 (**CH_3**), 28.27 (NCH_2**CH_2**CH_2N); 46.08 (N**CH_2**CH_3); 56.87 (N**CH_2**CH_2); 110.95, 123.11, 131.72, 132.05 (benzimidazole **Ar-C**); 187.05 (C-Ag). FTIR (KBr disc) in cm^{-1}, 3624, 3406 (C-H_{arom}); 2990, 2880 (C-H_{alph}); 1465 (C=N); 1168 (C-N); 750 (P-F). Anal. Calc. for $C_{42}H_{48}Ag_2F_{12}N_8P_2$: C, 41.39; H, 4.10; N, 9.01%. Found: C, 41.32; H, 4.11; N, 8.93 %.

2.2.6 Synthesis of 1,1-propylenebis(3-propylbenzimidazol-2-ylidene)disilver(I) bis(hexafluorophosphate) (6)

The procedure is similar to that in **2.2.5** but using compound **2** (0.50 g, 1.60 mmol) instead of compound **1** to obtain **6** as white crystalline solid. Single crystals appropriate for single crystal X-Ray diffraction studies were obtained by diffusion of diethyl ether into the salt solution in acetonitrile at room temperature. Yield: 1.02 g (52%) mp: 218-220 ^{O}C . ^{1}H NMR (500 MHz, acetonitrile-d_3): 0.86 (6H, t, J = 7.0 Hz, 2 x **CH_3**); 1.87 (4H, m, 2 x **CH_2**CH_3); 2.94 (2H, m, NCH_2**CH_2**CH_2N); 4.34 (4H, t, J = 7.5 Hz, 2 x N**CH_2**-R); 4.75 (4H, t, J = 6.5 Hz 2 x N**CH_2**CH_2); 7.48 (8H, m, Ar-H); 7.67 (8H, m, 2 x Ar-H). ^{13}C{^{1}H} NMR (125 MHz, acetonitrile-d_3): 12.58 (CH_3); 19.52 (CH_2**CH_2**CH_3); 30.35 (NCH_2**CH_2**CH_2N); 50.81 (N**CH_2**-R); 57.57 (N**CH_2**CH_2); 118.59, 132.92, 133.06, 133.39 (benzimidazole **Ar-C**); 187.74 (Ag-**C,**d). FTIR (KBr disc) in cm^{-1}, 3056 (C-H_{arom}); 2969, 2942 (C-H_{alph}); 1481(C=N); 1182

(C-N); 749 (P-F). Anal. Calc. for $C_{46}H_{56}Ag_2F_{12}N_4P_2$: C, 45.04; H, 4.57; N, 9.13%. Found: C, 44.93; H, 4.49; N, 9.18%

2.2.7 Synthesis of 1,1-propylenebis(3-butylbenzimidazol-2-ylidene)disilver(I) bis(hexafluorophosphate) (7)

The procedure is similar to that in **2.2.5** but using compound **3** (0.50 g, 1.50 mmol) instead of compound **1** to obtain **7** as white crystalline solid. Yield: 0.97g (51%) mp: 219-223 ^{o}C. ^{1}H NMR (500 MHz, acetonitrile-d_3): 0.79 (6H, t, *J* = 7.5 Hz, 2 x **CH_3**); 0.99 (4H, m, 2 x **CH_2**CH_3); 1.41 (4H, m, 2 x **CH_2**CH_2CH_3); 2.82 (2H, m, NCH_2**CH_2**CH_2N); 4.56 (4H, t, *J* = 7.0 Hz, 2 x N**CH_2**-R); 4.72 (4H, t, *J* = 7.0 Hz, 2 x N**CH_2**CH_2); 7.52 (4H, m, 2 x Ar-H); 7.71 (4H, m, 2 x Ar-H). ^{13}C {^{1}H} NMR (125 MHz, acetonitrile-d_3): 12.58 (**CH_3**); 19.52 (**CH_2**CH_3); 29.63 (**CH_2**CH_2CH_3); 30.52 (NCH_2**CH_2**CH_2N); 50.81 (N**CH_2**-R); 57.57 (N**CH_2**CH_2); 111.83, 118.59, 132.64, 133.39 (benzimidazole **Ar-C**); 188.37 (**C**-Ag). FTIR (KBr disc) in cm^{-1}, 3061 (C-H_{arom}); 2961, 2874 (C-H_{alph}); 1481 (C=N), 1182 (C-N); 747 (P-F). Anal. Calc. for $C_{50}H_{64}Ag_2F_{12}N_8P_2$: C, 46.80; H, 4.99; N, 8.74%. Found: C, 47.54; H, 4.89; N, 8.56%.

2.2.8. Synthesis of 1,1-propylenebis(3-pentylbenzimidazol-2-ylidene)disilver(I) bis(hexafluorophosphate) (8)

The procedure is similar to that in **2.2.5** but using compound **4** (0.50 g, 1.40 mmol) instead of compound **1** to obtain **8** as white crystalline solid. Yield: 0.89g (48%) mp: 220-223 ^{o}C. ^{1}H NMR (500 MHz, acetonitrile-d_3): 0.72 (6H, t, *J* = 7.0 Hz, 2 x **CH_3**); 1.21 (8H, m, 4 x **CH_2**); 1.82 (4H, m, 2 x NCH_2**CH_2**); 2.95 (2H, m, NCH_2**CH_2**CH_2N); 4.29 (4H, t, *J* = 7.5 Hz, 2 x N**CH_2**-R); 4.69 (4H, t, *J* = 7.0 Hz, 2 x N**CH_2**CH_2); 7.52 (2H, m, 2 x Ar-H); 7.71 (2H, m, 2 x Ar-H). ^{13}C {^{1}H} NMR (125 MHz, acetonitrile-d_3): 12.90 (**CH_3**); 21.78 (**CH_2**CH_3); 27.95

(**CH$_2$**CH$_2$CH$_3$); 28.29 (NCH$_2$**CH$_2$**); 29.61 (NCH$_2$**CH$_2$**CH$_2$N); 48.96 (N**CH$_2$**-R); 49.04 (N**CH$_2$**CH$_2$); 111.66, 124.21, 132.75, 133.49 (benzimidazole **Ar-C**); 187.46 (**C**-Ag,d). FTIR (KBr disc) in cm^{-1}, 3431 (C-H$_{arom}$); 2956, 2861 (C-H$_{alph}$); 1466 (C=N), 1118 (C-N); 743 (P-F). Anal. Calc. for $C_{54}H_{72}Ag_2F_{12}N_8P_2$: C, 48.44; H, 5.38; N, 8.37%. Found: C, 48.77; H, 5.13; N, 8.10%.

2.3 Crystal structure determination

Single crystal X-ray diffraction data for **1** and **6** were collected on APEXII Duo CCD area-detector diffractometer operating at 50 kV and 30 mA using Mo Kα radiation (λ = 0.71073 Å) at 297 K. Data collection and reduction were performed using the APEX2 and SAINT software. The SADABS software was used for absorption correction. Solution was obtained by direct methods using SHELXS-2014 followed by successive refinements using full matrix least squares method against F^2 using SHELXL-2014 [25]. All non-hydrogen atoms were refined anisotropically whereas hydrogen atoms were refined isotropically. In complex **6**, the propyl chains are disordered over two positions and were refined with 50:50 occupancies. The PF_6 molecules in the lattice in compound **6** are also disordered over two positions of equal weight. Crystal data and structure refinement details are tabulated in Table 1.

Table 1: Crystal data and structure refinement details of **1** and **6**.

	1	**6**
Formula	$C_{21}H_{25}F_{12}N_4P_2$	$C_{46}H_{56}Ag_2F_{12}N_8P_2$
Formula weight	623.39	1226.67
Crystal system	Monoclinic	Triclinic
Space group	*C*2/*c*	*P*-1
a(Å)	20.2339(18)	13.0807(10)
b(Å)	9.2233(7)	13.1888(10)
c(Å)	13.7804(10)	17.0316(13)
$\alpha(^o)$	90(1)	97.903(3)
$\beta(^o)$	94.138(2)	104.192(3)
$\gamma(^o)$	90(1)	93.580(3)
$V(Å^3)$	2565.0(4)	2807.4(4)
Z	4	2
D_c	1.614	1.451
$F_{(000)}$	1268	1240
Tot.; Uniq. Data	69514, 3154	92749, 12867
R_{int}	0.031	0.074
N_{ref}, N_{par}	3154, 177	12867, 866
R, wR_2, *S*	0.0380, 0.1006, 1.05	0.0761, 0.2636, 1.07

2.4 In Vitro Anticancer Activity

2.4.1 Preparation of cell culture

The MCF-7 cells were developed under optimal incubator conditions. Cells with the confluence of 70 – 80% were selected for cell plating purposes. The cells were washed using sterile phosphate buffered saline (PBS) (pH 7.4) three times. After washing the PBS was

completely separated, than trypsin was added and distributed evenly onto cell surfaces. The cells were incubated at 37 ^{o}C in 5% CO_2 for a minute. The flasks containing the cells were gently tapped to aid cell segregation and observed under an inverted microscope. Trypsin activity was inhibited by adding 5mL of fresh complete media (10% PBS). Cells were counted and diluted to final concentration of 2.5 x 10^5 cells/mL and inoculated the cells were incubated at 37 ^{o}C with an internal atmosphere of 5% CO_2.

2.4.2 MTT Assay

Measurement of cell viability using the MTT assay was performed in 96-well plate using the human breast cancer cell line (MCF-7). MTT reagent was freshly prepared at 5 mg in sterile PBS, filtered through a 0.2 μm syringe filter, and further diluted in fresh culture medium to obtain a final concentration of 500 μg/mL. The 1 × 104 cells in 100 μL media were seeded in 96-wells plate, after 24 h the cells attached to the wells. Samples were added to each well within 100 μL media at concentrations of 100 μg/mL. The old culture medium was aspirated carefully using a vacuum pump, and 200 μL of culture medium containing MTT reagent were added and incubated for 3–5 h. At the end of the incubation, the supernatant was aspirated carefully and the water-insoluble formazan salt was solubilized in 200 μL of DMSO per well. After 10 minutes of incubation at 37 °C, optical density (OD) of the violet color was measured using a microplate reader (Thermolab Systems 354, Finland) at a primary wavelength of 570 nm and a reference wavelength of 650 nm. The percentage of viable cells at each concentration was calculated using the equation:

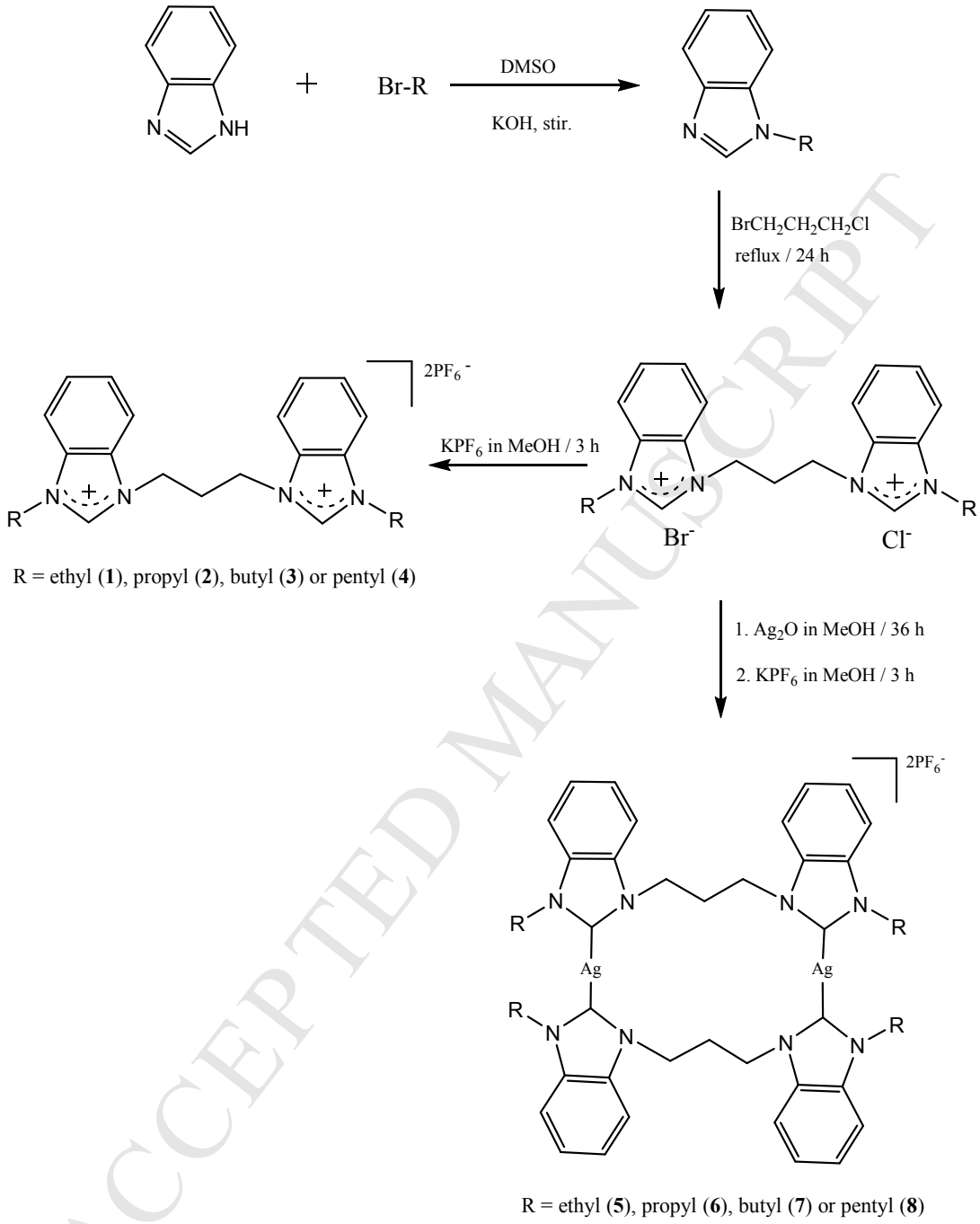
$$\% \text{ cell inhibition} = [1-((\text{Abs Sample} - \text{Abs Blank}) / (\text{Abs Control} - \text{Abs Blank}))] \times 100$$

where Abs Sample is the absorbance of treated wells, Abs Blank is the absorbance of cell free wells and Abs Control is the absorbance of treated wells with DMSO (1% v/v). Percent cell inhibition was plotted against the concentrations of the sample to calculate this value.

3.0 Results and discussion

3.1 Synthesis

The bis-(benzimidazolium) salts (**1-4**) possessing propylene linker were prepared from benzimidazole by stepwise alkylation reaction in the presence of KOH in DMSO for 3h at room temperature in which the *N*-alkylbenzimidazole was obtained [26]. The *N*-alkylbenzimidazole was refluxed with 1-bromo-3-chloropropane in 2:1 whereas after the completion, the solvent was evaporated and the solid formed washed with diethyl ether and dried at ambient temperature. The bromidechloride ion salts obtained were subsequently converted to PF_6^- ions (Scheme 1); the later ionic compounds are known to be more stable in air and moisture condition which is used for further analysis and anticancer activity. All dinuclear Ag(I)-NHC complexes (**5-8**) were synthesized via *in situ* deprotonation reaction between their respective salt and Ag_2O in 1:2 molar ratio where Ag_2O behaving as base to deprotanate the acidic proton of carbene in the salts. The dinuclear Ag(I)-NHC complexes were obtained in a good yield and the complexes were undergo metathesis reaction by added the methanolic solution of KPF_6 (Scheme 1) [27-29].



R = ethyl (**1**), propyl (**2**), butyl (**3**) or pentyl (**4**)

R = ethyl (**5**), propyl (**6**), butyl (**7**) or pentyl (**8**)

Scheme 1: Synthesis of bis-(benzimidazolium) salts and their respective dinuclear Ag(I)-NHC complexes.

3.2 FT-IR Analysis

The FT-IR spectra of salts and their respective Ag(I) complexes can be used as preliminary studies of successful synthesis[30]. The intensity bands can be seen as medium intensity around 3000-3650 cm^{-1} is assigned for ν(C-H_{arom}) while the bands at 2800-2900cm^{-1} are corresponding for ν(C-H_{alph}) in both salts and their respective complexes. A sharp and strong intensity bands in the range of 1560-1580 cm^{-1} as demonstrated in all salts indicating the presence of ν(C=N) vibrations that attributed to the benzimidazolium ring. Upon the complexation with silver(I) ions, the aforementioned bands are shifted to the range of 1460-1490 cm^{-1} and this observation occurs in all complexes [31].

3.3 ^{1}H- and ^{13}C- NMR analysis

The formation of bis(benzimidazolium) salts and their respective dinuclear Ag(I)-NHC complexes in each step was traced by ^{1}H- and ^{13}C-NMR analysis over the scan range of δ 0-12 and δ 0-200 ppm respectively. The ^{1}H-NMR spectra shows a sharp singlet peak at region of δ 9.73 to 9.81 ppm ascribed to the benzimidazolium rings (NC*H*N) acidic proton, in accord with our previous reports [32, 33]. The formation of complexes was confirmed by completely disappearance of acidic (NC*H*N) proton in all complexes [34]. All other peaks remain the same with no other significant changes for both salts and complexes; peaks resonance between δ 7.40 to 8.20 ppm was assigned to benzimidazolium ring protons while the peaks resonance range from δ 4.46 to 4.67 ppm and δ 27.60 to 2.95 ppm are assigned to CH_2-N and CCH_2C (propylene bridge) protons respectively. The presence of peaks from δ 0.60 to 1.95 ppm are assigned to CH_2CH_3 protons [35].

The ^{13}C NMR spectra were further used to evaluate the characteristic of salts and Ag(I)-NHC complexes. The spectra of salts displayed peaks at range of δ 140-142 ppm ascribed to the benzimidazole ring carbon (NC*H*N) [36]. However, upon complexation with Ag(I), the characteristic of parent doublet peak at the range of δ 187-188 ppm are observed for C-Ag bonds with the average coupling constant of 187.7 Hz [37, 38]. All other peaks remain the same with no other significant changes for both salts and complexes; peaks resonance between δ 110.0 to 135.0 ppm was assigned to benzimidazolium ring carbon, the peaks range from δ 43.0 to 58.0 ppm and the peaks in the region of δ 27.0 to 30.0 ppm are assigned to CH_2-N and CCH_2C (propylene bridge) respectively.

3.4 Crystal Structures

Compound **1** crystallizes in monoclinic space group *C*2/*c* with half of the molecule in the asymmetric unit (Figure 1). The presence of two hexafluorophosphate anions in the lattice thus balances the charge of the molecule. The N1-C1-N2 bond angle in this compound being 110.49(12)^o, agreeable with those reported for similar type of ligand [38-40].

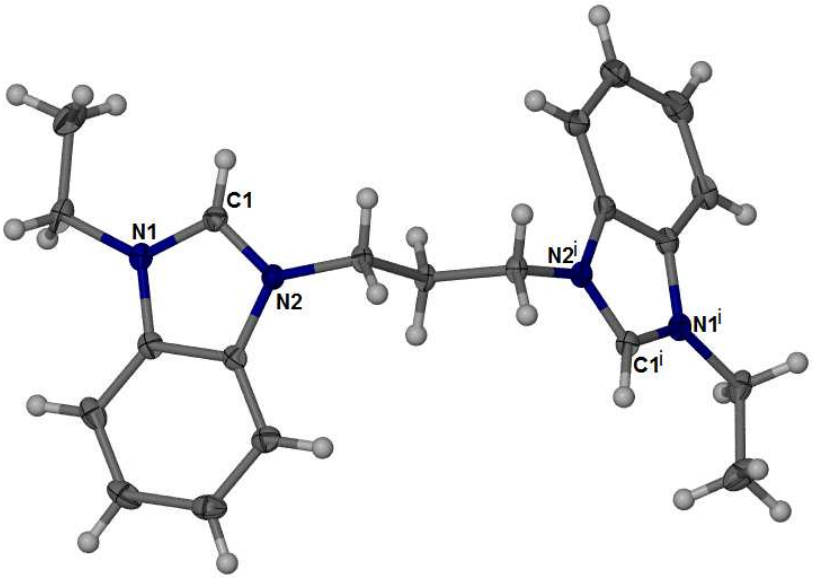
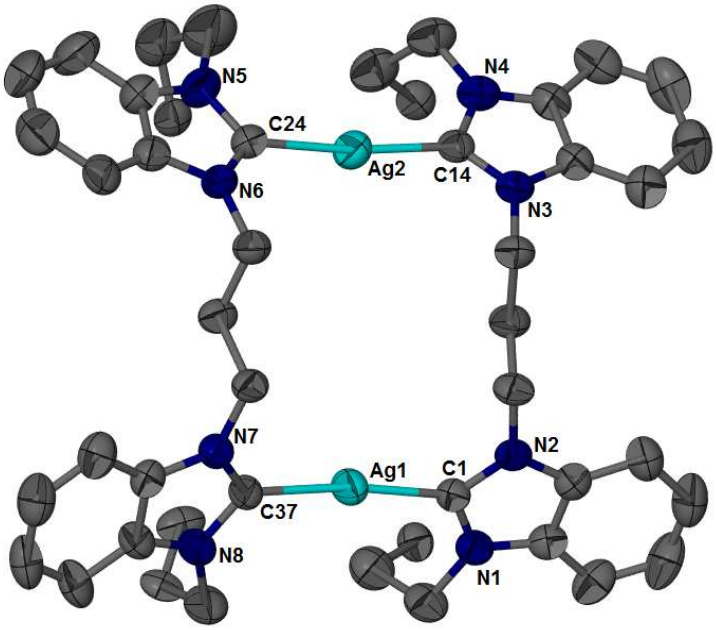


Figure 1: Structure of salt **1** with the ellipsoids shown at 50% probability. The hexafluorophosphate anions in the lattice omitted for clarity. Symmetry element used: ^{i}= -x, y, ½-z.

Complex **6** crystalline in triclinic space group *P*-1 with the entire molecule in the asymmetric unit (Figure 2(a)). The metal complex is balanced by the presence of two hexafluorophosphate anions. The two Ag(I) ions are bridged by two bis-NHC ligand that display a μ-κ^1(C)Ag:κ^1(C`)Ag` bridging mode. Both Ag(I) anions display a distorted linear geometry with the angle of C1-Ag1-C37 and C14-Ag2-C24 is 173.2(3)^o and 173.9(3)^o, respectively (Table 2). The bond distance between the Ag(I) ions and the carbene carbon is in the range of 2.088(6) – 2.095(7) Å, and agreeable with those reported before [41]. The structure of this complex is somewhat similar to that reported complex having similar ligand architecture, however with the allyl group attached to the nitrogen atom [41], which leads to the similar molecular arrangement. The face-to-face π-π supramolecular interactions are observed to play an important role in

stabilizing the crystal structure of **6**. Among them is the interaction between two adjacent benzimidazolium rings with the separation being 3.416(5) Å (Figure 2(b)).

(a)



(b)

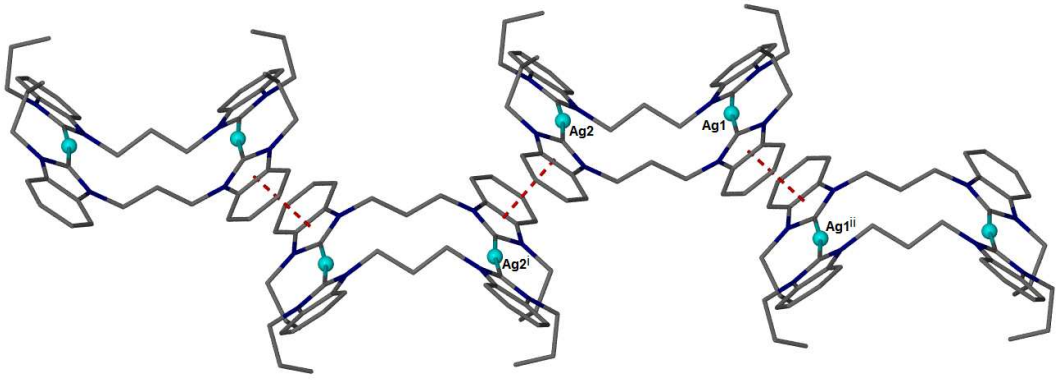


Figure 2: (a) Structure of complex **6** with the ellipsoids shown at 50% probability. Hydrogen atoms and hexafluorophosphate anions in the lattice omitted for clarity. (b) The face-

to-face π-π interactions between adjacent dinuclear complexes in crystal structure of **6**. Symmetry elements used: [i]= 1-x, 2-y, 1-z; [ii]= -x, 1-y, 1-z.

Table 2: Selected bond lengths [Å] and angles [°] for **6**.

Ag1-C1	2.088(6)	Ag1-C24	2.095(7)
Ag1-C14	2.090(7)	Ag1-C37	2.095(6)
C1-Ag1-C37	173.2(2)	N3-C14-N4	105.8(6)
C14-Ag2-C24	173.9(2)	N5-C24-N6	106.5(6)
N1-C1-N2	105.9(5)	N7-C37-N8	105.9(6)

3.4 In vitro anticancer study

Preliminary screen of proligands **1-4** and their respective dinuclear Ag(I)-NHC complexes, **5-8**, respectively were evaluated using MTT assay on cancer cell line, MCF-7. The comparative studies of dinuclear Ag(I)-NHC complexes and their respective proligands indicates that complexes exhibited good inhibition against MCF-7, while their corresponding proligands showed no inhibition. The antiproliferative potencies of the test complexes shows clearly that all complexes (**5-8**) inhibited the proliferation of MCF-7 cells in a dose-dependent manner parallel to standard drug used in this study, Tamoxifen (Figure 3). All complexes exhibited almost similar IC_{50} in the range of 7±1 to 18±3 μM (Table 3). However, complex **6** appeared to have enhanced cytotoxicity effect more than complexes **5** and **8**, while complex **7** is less active compared with the aforementioned complexes. In overall, all new complexes are comparable to

or less potent than standard drug (IC_{50} = 11±2 µM, Tamoxifen). The present results are consistent with other reported studies in the literature having linked bis-benzimidazole based dinuclear Ag(I)-NHC complexes against various cancer cell line [41-42].

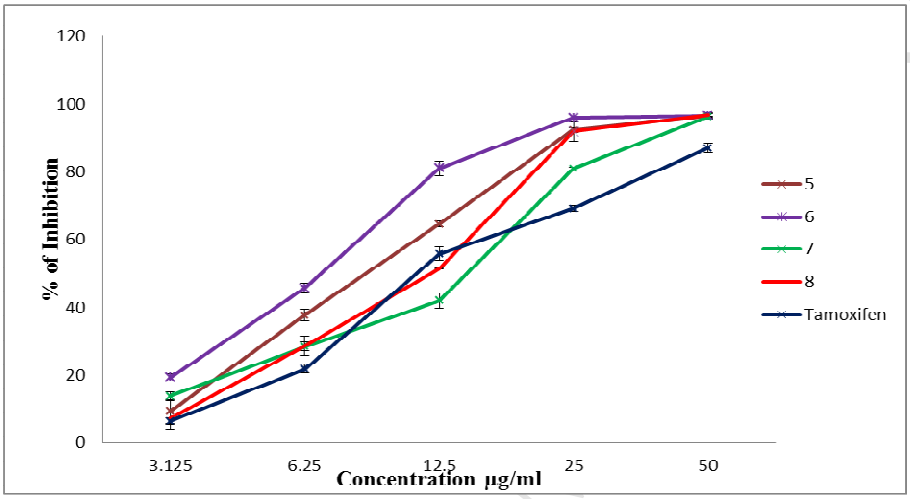


Figure 3. Dose-dependent antiproliferative effect of dinuclear Ag(I)–NHC complexes (**5-8**) on breast cancer (MCF-7) cell line.

Table 3. The IC_{50} values of tested complexes against MCF-7 cell line.

Complex	IC_{50}(µM) MCF-7	SD±
5	9	1
6	7	1
7	18	3

8	11	2
Tamoxifen	11	2

Different Ag(I) complexes have been studied with high efficacy in their biological potential [43-44], and reported mechanism reaction studies of these complexes indicates that available Ag^+ released might bind and interact with the proteins involved in cell wall and finally disrupt the cell functions [45]. This study suggested that incorporation of Ag(I)-NHC bond plays a vital role in antiproliferative effects due to their strong σ-donor and weak π-accepting of NHC ligands [46]. Furthermore, the activity of Ag^+ ions are varies depends on structure, ionization, solubility and stability of Ag(I)-NHC complexes [47]. However, both propylene linker and alkyl carbon chain length substituents enhances the stability of complexes by improving the lipophilicity of complexes. This stabilities then alleviate the transport of Ag^+ into the cell membrane and subsequently into the cell organelles where Ag^+ may possibly impart the toxicity effect by inhibiting cellular respiration and metabolism of biomolecules.

4.0 Conclusions

In conclusion, a series of new propylene linked bis-benzimidazolium salts and their respective dinuclear Ag(I)-NHC complexes were synthesized and characterized by FTIR, ^{1}H-NMR, ^{13}C-NMR and elemental analyses. Single crystal X-ray diffraction study of dinuclear Ag(I) complexes reveals that the Ag(I) ions display a distorted linear geometry with significant π-π interactions stabilized the entire crystal packing. All compounds were screened for their

possible cytotoxicity on breast cancer, MCF-7 and the tested compounds show dose-dependent cytotoxicity against breast cancer. From the anticancer results its shows that the dinuclear Ag(I)-NHC complexes are active while their respective salts are not active.

5.0 Supplementary material

Crystallographic data for the structure in this work has been deposited at the Cambridge Crystallographic Data center, 1527934 and 1527935 for **1** and **6**, respectively. Copy of these material can be obtained from the Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK (Fax: +44 1223 336033; E-mail: deposit@ccdc.cam.ac.uk/deposit).

6.0 Acknowledgements

The author thanks Universiti Sains Malaysia for the RUI Grant (1001/PKimia/811346). Sunusi Y. Hussaini thanks Kano University of Science and Technology, Wudil, Nigerian for the study sponsorship.

7.0 References

[1] D. Bourissou, O. Guerret, F. P. Gabbai, G. Bertrand, Chem. Rev. 100 (2000) 39-91.

[2] H. G. Raubenheimer, S. Cronje, Chem. Soc. Rev. 37 (2008) 1998-2011.

[3] W. A. Herrmann, Angew. Chemie. Int. Ed. 41(2002) 1290-1309.

[4] A. J. Arduengo, H. V. R. Dias, R. L. Hariow, M. Kline, J. Am. Chem. Soc. 114 (1992) 5530.

[5] P. L. Arnold, S. Pearson, Coord. Chem. Rev. 251 (2007) 596-609.

[6] M. L. Teyssot, A. S. Jarrousse, M. Manin, A. Chevry, S. Roche, F. Norre, C. Beaudoin, L. Morel, D. Boyer, R. Mahiou, A. Gautier, Dalton Trans. 35 (2009) 6894-6902.

[7] S. Budagumpi, R. A. Haque, A. W. Salman, Coord. Chem. Rev. 256 (2012) 1787-1830.

[8] Y. A. Wanniarachchi, M. A. Khan, L. M. Slaughter, Organometallics. 23 (2004) 5881-5884.

[9] D. J. Nielsen, K. J. Cavell, B. W. Skelton, A. H. White, Inorg. Chim. Acta. 327 (2002) 116-125.

[10] J. C. Garrison, W. J. Youngs, Chem. Rev. 105 (2005) 3978-4008.

[11] M. V. Baker, D. H. Brown, R. A. Haque, B. W. Skelton, A. H. White, J. Inclusion Phenom. and Macrocyclic Chem. 65 (2009) 97-109.

[12] K. M Hindi, M. J. Panzner, C. A. Tessier, C. L. Cannon, W. J. Youngs, Chem. Rev. 109 (2009) 3859-3884.

[13] S. Budagumpi, R. A. Haque, S. Endud, G. U. Rehman, A. W. Salman, Eur. Inorg Chem. 2013 (2013) 4367-4388.

[14] R. A. Haque, P. O. Asekunowo, M. R. Razali, Trans. Met. Chem. 39 (2014) 281-290.

[15] A. Poethig, T. Strassner, Organometallics 30 (2011) 6674-6684.

[16] S. Ray, R. Mohan, J. K. Singh, M. K. Samantaray, M. M. Shaikh, D. Panda, P. Ghosh, J. Am. Chem. Soc. 129 (2007) 15042-15053.

[17] S. T. Liddle, I. S. Edworthy, P. L. Arnold, Chem. Soc. Rev. 36 (2007) 1732-1744.

[18] U. F. M. Haziz, R. A. Haque, A. A. Amirul, N. Shaheeda, M. R. Razali, Polyhedron 117 (2016) 628-636.

[19] Y. Du, T. Huiling, D. Hong, S. Yanhui, C. Changsheng, P. Guangsheng, J. of Chem. Research 40 (2016) 735-739.

[20] R. A. Haque, H. Z. Zulikha, M. Z Ghdhayeb, S. Badugumpi, A. W. Salman, Heteroatom Chem. 23 (2012) 486-497.

[21] R. A. Haque, P. O. Asekunowo, S. Budagumpi, Inorg. Chem. Commun. 47 (2014) 56-59.

[22] R. A. Haque, M. A Iqbal, S. Badugumpi, M. B. K. Ahamed, A. M. S. Abdul Majid, N. Hasanudin, Appl. Organomet. Chem. 27 (2013) 214-223.

[23] R. A. Haque, N. Hasanudin, M. A. Iqbal, A. Ahmad, S. Hashim, A. M. S. Abdul Majid, M. B. K. Ahamed, J. Coord. Chem. 66 (2013) 3211-3228.

[24] W. J. Youngs, A. R. Knapp, P. O. Wagers, C. A. Tessier, Dalton Trans. 41 (2012) 327-336.

[25] G. M. Sheldrick, Acta Cryst. C. 71 (2015) 3-8.

[26] J. A. Mata, A. R. Chianese, J. R. Miecznikowski, M. Poyatos, E. Peris, J. W. Faller, R. H. Crabtree, Organometallics 23 (2004) 1253-1263.

[27] R. A. Haque, S. A. Ahmed, Z. H. Zetty, M. Hemamalini, H. K. Fun, Acta Crystallogr. Sect. E 67 (2011) o3462-o3462.

[28] I. J. B. Lin, C. S. Vasam, Coord. Chem. Rev. 251 (2007) 642-670.

[29] M. Asif, M. A. Iqbal, M. A. Hussein, C. E. Oon, R. A. Haque, M. B. K. Ahamed, A. S. Abdul Majid, A.M.S. Abdul Majid, Eur. J. Med. Chem. 108 (2016) 177-187.

[30] M. A. Iqbal, R. A. Haque, S. Badugumpi, M. B. K. Ahaned, A. M. S. Abdul Majid, Inorg. Chem. Commun. 28 (2013) 64-69.

[31] R. A. Haque, M. A Iqbal, P. O. Asekunowo, A. M. S. Abdul Majid, M. B. K. Ahamed, M. I. Umar, S. S. Al-Rawi, F. S. R. Al-Suede, Med. Chem. Research 22 (2013) 4663-4676.

[32] M. A Iqbal, R. A. Haque, S. F. Nasri, A. M. S. Abdul Majid, M. B. K. Ahamed, E. Farsi, T. Fatima, Chem. Centr. J. 7 (2013) 1-17.

[33] R. A. Haque P. O. Asekunowo, M. R. Razali, F. Mohamad, Heteroatom Chem. 25 (2014) 194-204.

[34] P. O. Asekunowo, R. A. Haque, J. Coord. Chem. 67 (2014) 3649-3663.

[35] M. A Iqbal, R. A. Haque, M. B. K. Ahamed, A. M. S. Abdul Majid, S. S. Al-Rawi, Med. Chem. Research 22 (2013) 2455-2466.

[36] M. A. Iqbal, R. A. Haque, H. K. Fun, T. S Chia, Acta Crystallogr Sect E 68 (2012) o466-o467.

[37] H. V. Huynh,C. H. Yeo, Y. X. Chew, Organometallics 29 (2010) 1479-1486.

[38] P. O. Asekunowo, R. A. Haque, M. R. Razali, Trans. Met. Chem. 40 (2015) 79-88.

[39] T. Fatima, ,R. A. Haque, M. R. Razali, A. Ahmad, M. A Iqbal, M. Asif, M. B. K. Ahamed, A. M. S. Abdul Majid, J. Coord. Chem. 69 (2016) 3367-3383.

[40] P. O. Asekunowo, R. A. Haque, M. R. Razali, S. Budagumpi, Appl. Organometal. Chem. 29 (2015) 126-137.

[41] R. A. Haque, S. F. Nasri, M. A. Iqbal, Journal of Coordination Chemistry 66 (2013) 2679-2692.

[42] N. I. Sulaiman, N. R. Salimin, R. A. Haque, M. A. Iqbal, S. W. Ng, M. R. Razali. Polyhedron 97 (2015) 188-196.

[43] K. Nomiya, A. Yoshizawa, K. Tsukagoshi, N. C. Kasuga, S. Hirakawa, J. Watanabe, J. Inorg. Biochem. 98 (2004) 46-60.

[44] A. S. Kazachenko, A. V. Laegler, O. V. Peryanova, Y. A. Vstavskaya, Pharm. Chem. J. 34 (2000) 257-258.

[45] S. J. Tan, Y. K. Yan, P. P. F. Lee, K. H. Lim, Future Med. Chem. 2 (2010) 1591-1608.

[46] W. Liu, R. Gust, Chem. Soc. Rev. 42 (2013) 755-773.

[47] A. Kascatan-Nebioglu, M. J. Panzner, C. A. Tessier, C. L. Cannon, W. J. Youngs, Coord. Chem. Rev. 251 (2007) 884-895.