Bis-N-heterocyclic carbene silver(I) and palladium(II) complexes: Efficient antiproliferative agents against breast cancer cells

Rosenani A. Haque, Noorhafizah Hasanudin, Mouayed A. Hussein, Safa A. Ahamed, and Muhammad Adnan Iqbal

School of Chemical Sciences, Universiti Sains Malaysia, Minden, Malaysia; University of Basrah, College of Science, Department of Chemistry, Basra, Iraq; Department of Chemistry, College of Education, University of Samarra, Iraq; Department of Chemistry, University of Agriculture, Faisalabad, Pakistan; Community College, University of Agriculture, Faisalabad, Pakistan

ABSTRACT
A new Pd(II)-N-heterocyclic carbene (NHC) complex 2 was derived from a binuclear Ag(I)-NHC complex 1. 1H NMR spectra of complexes showed uniquely different characteristic behavior of bridging methylene protons than compared with the ligand. X-ray crystallography showed that palladium at the center of bis-NHC units in 2 was found in square-planar geometry. Ligand and complexes were tested against breast cancer cells (MCF-7). The ligand did not show considerable antiproliferative activity (IC50 181.0 ± 3.0 μM) whereas complexes showed significant activity (1: 0.5 ± 0.1 μM; 2: 2.3 ± 0.2 μM) compared with the standard drug, tamoxifen (2.4 ± 0.2 μM).

Introduction
Pd(II)-N-heterocyclic carbene (NHC) complexes were first reported by Hermann et al. as catalyst in the Heck coupling of aryl halides with n-butyl acrylate to afford high yield product. The first Pd(II)-NHC complex used in the Heck reaction as catalyst is a bis-NHC Pd(II) complex.[2]

Later on, the interest of Pd(II)-NHC complexes in catalysis fields grown, especially in cross-coupling chemistry.[13–9] A number of Pd(II)-NHC complexes have been applied as catalysts in various organic syntheses due to their stability towards air and moisture, low-to-moderate cost and the availability of palladium in the stable and variable oxidation states.[6,10,11] Specifically, chelated-diNHC palladium(II) complexes have shown extreme stability in the presence of heat and moisture leading to notable catalytic properties.[12–15]

For long Pd(II)-NHC complexes could not get attention in medicinal chemistry until recently when few Pd(II)-NHC complexes showed an attractive anticancer potential against various types of cancer cell lines. In 2007, Ray et al. synthesized and studied the efficiency of Pd(II)-NHC complexes (a and b) against HCT116 (colon adenocarcinoma), HeLa (cervical cancer), and MCF-7 (breast cancer) and the results were found to be considerably stronger than cisplatin. Recently, Pd(II)-NHC complexes (c and d) were also tested against HCT116 cancer cell lines and showed significant anticancer activity.[17] Figure 1 shows examples of Pd(II)-NHC complexes (a–e) tested for their biological properties. Very few of Pd(II)-NHC complexes have been tested for their anticancer potential so far.[17–23] However, Pd(II) coordination complexes, other than NHC chemistry, have a number of recent examples in anticancer study.[24–28] The advantage of using NHCs over other coordination ligands is that the NHCs release metal ions at slower rate due to their electron-withdrawing effects and hence they remain more effective in catalytic as well as biological applications.[29–31]

According to the World Health Organization, there were 7.6 million deaths due to cancer in 2008 and this number is likely to increase to 13.1 million by the year 2030.[32–34] For some types of cancer, there is still no treatment that exists, or treatment has indicated severe side effects. The four types of cancer that require quick and significant research development are lung, colorectal, breast, and stomach.[35] Breast cancer is the first most common type of cancer in women and the second most frequent cause of deaths.[34] It can be seen that all the marketing drugs are organic in nature. However, due to the recent developments in metal-based anticancer drugs (lobaplatin, nedaplatin, carboplatin), their marketing has drifted to interest in metal-based anticancer drugs[36]

In the current report, a bis-N-heterocyclic carbene palladium(II) complex 2 was synthesized from a dinuclear Ag(I)-NHC complex 1 (Scheme 1) and all the synthesized compounds (L, 1, and 2) were tested against breast cancer cell lines (MCF-7).

Experimental
Materials and instrumentation
The melting point of title compound was determined using a Stuart Scientific SMP1 melting point apparatus. NMR spectra...
\(^1\text{H} \) and \(^{13}\text{C}\) were recorded on a Bruker spectrophotometer 500 MHz NMR machine. Tetramethylsilane was used as an internal standard and the samples were prepared in deuterated dimethyl sulfoxide (DMSO-\(d_6\)). Elemental analysis was carried out using a Perkin Elmer 2400 series-11 CHN analyzer. X-ray crystallographic data were recorded on a Bruker SMART APEXII CCD area-detector diffractometer using graphite monochromated Mo K\(\alpha\) radiation (\(\lambda = 0.71073 \text{ Å}\)) at 100 K. The data were collected and reduced using APEX2 and SAINT programs. The molecular structures of each of the compounds were solved using the SHELXS-97 program package, and refined using the SHELXL-97 program. All non-hydrogen atoms were anisotropically refined. The molecular graphics were created using SHELXTL. All chemicals, including

**Figure 1.** Pd(II)-NHC complexes tested for their biological properties.

\[ \text{Scheme 1.} \text{ Synthesis of disilver(I)-NHC complex 1 and palladium(II)-NHC complex 2 from ligand L.} \]
thiosemicarbazide, aldehydes, and solvents, were purchased from Sigma-Aldrich.

DMEM and RPMI 1640 growth media were purchased from ScienCell (USA). Heat inactivated fetal bovine serum (HIFBS) and trypsin was obtained from GIBCO (UK). Phosphate-buffered saline (PBS), penicillin/streptomycin (PS) solution, MTT reagent, and the reference standard, tamoxifen, was purchased from Sigma-Aldrich (Germany). All the other chemicals used in this assay were of analytical grade.

**Synthesis**

Ligand L and Complex 1 were synthesized according to the reported procedure and their characteristic patterns were verified as reported in the literature. The palladium(II) complex 2 was synthesized as described subsequently.

**Synthesis of di(1',1''-(3'-methyl)benzylimidazolium)-3,3'-methylenepalladium(II) dihexafluorophosphate**

Pd(COD)Cl2 (0.09 g, 0.32 mmol) was added into acetonitrile solution containing silver(I)-NHC complex 1 (0.4 g, 0.32 mmol) in dark condition and the mixture was allowed to stir for 24 h, followed by filtration giving a pale yellow clear filtrate. The solvent was removed in vacuo to yield a pale yellow powder. Crystal suitable for X-ray analysis was obtained by slow evaporation of the complex solution in dioxane at ambient temperature. Yield: 0.09 g (24.32%), 220–221°C. 1H NMR (500 MHz, d6-DMSO, δ ppm) 4.38 (4H, d, J = 15.5 Hz, benzylic-trans), 4.50 (4H, d, J = 16 Hz, benzylic-cis), 5.63 (2H, d, J = 13 Hz, Nimid-CH2-Nimid), 6.01 (2H, d, J = 13.5 Hz, Nimid-CH2-Nimid), 6.59 (4H, d, J = 8.0 Hz), 6.80 (4H, d, J = 2.0 Hz, imidazolium H5), 7.24 (4H, d, J = 2.0 Hz, imidazolium H4'), 6.59 (4H, d, J = 8.0 Hz, Ar-H), 6.96 (4H, m, Ar-H), 7.07 (4H, m, Ar-H), 7.18 (4H, d, J = 6.5 Hz, Ar-H); 13C NMR (125 MHz, d6-DMSO, δ ppm): 51.5 (4 × Nimid-C=Ar-benzyl), 62.9 (4 × Nimid-C=Nimid-methylene), 122.7, 123.6 (imidazolium C5- and C4'), 110.1 (C-CN), 116.7 (CN), 128.2, 129.0, 133.5, 133.6, 138.5 (Ar-C), 169.7 (C=O). Anal. Calcd. for C46H36N12PdF12P2: C, 47.91; H, 3.15; N, 14.58%. Found: C, 47.90, H, 2.35, N, 15.62%.

**Characterization by NMR**

Biimidazolium ligand L, its binuclear Ag(I)-NHC complex 1 and Pd(II)-NHC complex 2 were characterized by NMR spectrophotometer for 1H and 13C nuclei. The characteristic patterns of L and 1 were found as reported previously.

The most downfield singlet at 9.45 δ ppm appeared for the NCHN proton of imidazolium ring, which disappeared in the 1H NMR spectra of 1 and 2 due to its replacement by metal ions (Ag and Pd). See Figures S1–S3. Furthermore, the benzene and imidazolium ring protons appeared in the range 6.6–8.0 δ ppm in L and 1–2. Figure 2 shows uniquely different patterns of these aromatic range protons in all the three compounds. For example, in L, the benzene-H appeared as four types of chemical shifts in the range 7.5–8.0 δ ppm, one sandwiched between imidazolium protons (H5'/H4'), whereas in complexes these patterns changed (Figure 2). In complex 1, two whereas in complex 2, three of these aromatic protons were found sandwiched by imidazolium protons (Figure 2). This phenomenon indicates that the presence and variation of bonded metal ions to NHCs may also affect the electronic distribution at terminal aromatic rings, which has been rarely reported.

More interesting characteristic features were observed for benzyl (–CH2–) and methylene (–CH2–N) protons. For example, 1H NMR of 1 showed methylene protons depressed compared with the same protons in L (a singlet), whereas in 2 the signals corresponding to the benzyl and methylene protons appeared as two sets of doublets (Figure 2). The depressed methylene proton signals in 1 may be due to the Ag–Ag metal interaction as reported previously. However, the appearance of two sets of doublets, one for methylene protons (2) and the other one for benzyl protons (1) can be tentatively assigned to the endo benzyl and methylene protons, and those are closer to the metal center and build deshielding effects due to the palladium ion. However, the more upfield doublets are corresponding to the exo benzyl and methylene protons. The appearance of two benzyl doublets indicates that the complex has two planes of symmetry in solution. In 13C NMR spectra, the signal for Ag–C carbene appeared as two sets of doublets ca. at δ 182 ppm, whereas for Pd–C carbene for complex 2 appeared as singlet at δ 169.7 ppm.

**In vitro anticancer studies**

**Cell lines and antiproliferative assay**

Human breast cancer cells (MCF-7) were purchased from American Type Culture Collection (Rockville, MD, USA). MCF-7 cells were maintained in DMEM medium (GIBCO, USA). The effect of 2 on cell proliferation was evaluated using MTT assay according to our previously reported method.

DMSO (0.1%) was used as negative control, whereas tamoxifen was used as standard reference drugs.

**Results and discussion**

**Synthesis**

Scheme 1 shows the synthesis of the binuclear silver(I)-NHC complex 1 and respective palladium(II)-NHC complex 2 using NHC precursor L.

**Crystallographic study**

The crystals of 2 suitable for X-ray diffraction studies were obtained by slow evaporation of the complex solution in 1,4-dioxane at ambient temperature. The molecular structure is depicted in Figure 3. The complex crystallized in the monoclinic crystal system.
Figure 2. Selected regions of the $^1$H NMR (500 MHz) for L, silver complex 1 and the corresponding palladium complex 2 in $d_6$-DMSO.

Figure 3. Molecular structure of bis-NHC palladium (II) complex 2 drawn at 50% probability. Solvent molecules have been omitted for the clarity of the picture.
with the P21/c space group. The structural analysis showed that it is a mononuclear Pd(II) complex, chelated by two units of ligands. The complex consists of one Pd(II) cation coordinated with two moieties of the ligand in a square-planar geometry and containing two hexafluorophosphate anions. Two solvent molecules (1,4-dioxane) were also found packed with the main molecule during crystallization process. The average Pd-C carbene bond distance is 2.02 Å. This value is in the typical range and comparable with those in literature.[43,46] Each chelated unit created a six membered ring upon coordination with Pd(II) with bite angle C1-Pd1-C13 = 84.79(9)° and C24-Pd1-C36 = 82.79(9)°.[47] These values are comparable with previous reports and have the Pd(II) center adopting a square-planar coordination geometry.[48] However, the dihedral angles for N2-C12-N3 and N7-C35-N6 are 109.59(6)° and 108.79(5)°, respectively. Furthermore, the internal imidazole ring angles (NCN) were found at around 103.8(19)° and 104.5(2)° for N1-C1-N2 and N3-C13-N4, respectively. These angles (NCN) are in the similar range as compared to the dinuclear Ag(I)-NHC complex but about 4° smaller than that of respective ligand, see Haque et al.[38] for a comparison. Interestingly, the NHC-Pd-NHC bond angles were found deviated from linearity (176.3°), which is different from a previously reported Pd(II)-NHC complex having

| Table 1. Crystal data and structure refinement details for complex 2. |
|---------------------------------|--------------------------------------------------|
| Empirical formula              | C_{46}H_{36}N_{12}Pd, 1.5(C_{4}H_{8}O_{2}), 2(F_{6}P) |
| Formula weight                 | 1285.36                                          |
| Crystal system                 | Monoclinic                                       |
| Space group                    | P21/c (No. 14)                                   |
| a, b, c (Å)                    | 12.1787 (15), 16.1821 (2), 14.2623 (14)          |
| α, β, γ (°)                    | 90, 122.437 (7), 90                              |
| V (Å³), Z                      | 2372.2 (5), 2                                   |
| D (calcld.) (g/cm³)            | 1.768                                            |
| Abs coeff (mm⁻¹)               | 0.990                                            |
| F(000)                         | 1256                                             |
| Crystal size (mm)              | 0.20×0.26×0.27                                   |
| Temperature (K)                | 100                                              |
| Radiation (Å)                  | MoKα 0.71073                                     |
| θ Min-max (°)                  | 2.1, 32.5                                        |
| Dataset                        | −18: 18; −24: 24; −20: 21                        |
| Tot; uniq. data                | 56227, 8580                                      |
| R(int)                         | 0.051                                            |
| Nref; Npar                     | 8580, 334                                        |
| R, wR, S                       | 0.0358, 0.0970, 1.09                              |

**Figure 4.** Shows dose dependent chart of standard drug, ligand L and complexes 1 and 2. Standard deviation (n = 3).

**Figure 5.** Shows the photomicrographs of MCF-7 cell line (middle) before the application of test compounds, after application of standard drug tamoxifen (right) and complex 2 (left).
similar structure\textsuperscript{[48]} and a Ni(II)-NHC complex.\textsuperscript{[46]} One of the solvent molecules (1,4-dioxane) and one PF$_6$ anionic unit were found disorder. Please see the ORTEP diagram of Figure S4 for 2 along with solvent molecules. The crystal packing shows the cationic and anionic components are connected via C–H⋯F and C–N⋯H hydrogen bonding in three-dimensional network (Supplementary file, Figure S5). Crystallographic data of 2 are presented in Table 1. Selected bond distances and angles are presented in Table S1.

**In vitro anticancer study**

The synthesized compounds L, 1, and 2 were tested against the breast cancer cell line (MCF-7), using MTT assay, which showed a dose dependent cytotoxic behavior (Figure 4). Figure 5 depicts the selected photomicrographs of the test compounds.

Photomicroscopic image of MCF-7 cells treated with the vehicle (0.1% DMSO) group showed enormous growth of the cells in a confluent layer whereas treatment with tamoxifen (standard) showed significant (p < 0.01) inhibition in cell proliferation with IC$_{50}$ = 2.4 ± 0.2 µM. The treated cells showed severely affected morphology. The ligand (L) was found almost inactive (IC$_{50}$ = 181.0 ± 3.0 µM), however silver complex 1 was found to be the most active (IC$_{50}$ = 0.5 ± 0.1 µM) among all the test compounds. The palladium complex 2 showed cytotoxicity (IC$_{50}$ = 2.5 ± 0.2 µM) nearly equal to the standard drug (tamoxifen, IC$_{50}$ = 2.4 ± 0.1 µM). Literature shows that so far Pd(II)-NHC complexes a and e (Figure 2) have been tested against MCF-7 cell line showing IC$_{50}$ values 1.0 and 6.5 µM, respectively. In comparison with the reported Pd(II)-NHC complexes a and e, it can be noticed that 2 is better than e and a close rival of a. Furthermore, comparing the cytotoxicity of Ag(I)-NHC complex 1 with the reported Ag(I)-NHC complexes G–J (Figure 6),\textsuperscript{[49–54]} 1 also showed better cytotoxicity. It can be seen from Figure 6 that all the reported complexes (G–J) have IC$_{50}$ values in the range 2.4–17 µM, whereas 1 showed IC$_{50}$ value 0.5 µM. The enhanced cytotoxicity of 1 might be due the presence of terminal benzonitrile groups. The other considerable factor might be the presence of two silver centers (e.g., G) instead of one (e.g., H–J). Some recent reports highlights that the dinuclear silver(I)-NHC complexes show better biological significance than compared to mononuclear due to increased metal centers in a molecule.\textsuperscript{[55–59]}

**Conclusions**

A new Pd(II)-NHC complex 2 was derived from a binuclear Ag (I)-NHC complex 1 and its molecular structure was crystallographically elucidated. Ligand L and complexes 1–2 were tested against the human breast cancer cell line (MCF-7) where 1 showed the best results; however, 2 depicted cytotoxicity parallel to the standard drug (tamoxifen). Furthermore, on comparing the cytotoxicity of 2 with that of reported Pd(II)-NHC complexes, it remained a potential metal based anticancer compound.

**Acknowledgments**

The article was compiled and communicated by Muhammad Adnan Iqbal.
Funding

Noorhafizah Hasanudin thanks Universiti Sains Malaysia for financial support during her MSc. Muhammad Adnan Iqbal thanks Universiti Sains Malaysia for postdoctoral fellowship ion research.

References

1. Herrmann, W. A.; Reisinger, C.-P.; Spiegler, M. J. Organomet. Chem. 1998, 557, 93–96.
2. Beller, M.; Fischer, H.; Kühlein, K.; Reisinger, C.-P.; Herrmann, W. J. Organomet. Chem. 1996, 520, 257–259.
3. O’Brien, C. J.; Kanchev, E. A. B.; Valente, C.; Hadei, N.; Chass, G. A.; Lough, A.; Hopkinson, A. C.; Organ, M. G. Chem. Eur. J. 2006, 12, 4743–4748.
4. Valente, C.; Calimisz, S.; Hoi, K. H.; Mallik, D.; Sayah, M.; Organ, M. G. Angew. Chem. Int. Ed. 2012, 51, 3314–3332.
5. Christmann, U.; Villar, R. Angew. Chem. Int. Ed. 2004, 44, 366–374.
6. Fortman, G. C.; Nolan, S. P. Chem. Soc. Rev. 2011, 40, 5151–5159.
7. Chartoire, A.; Frogneux, X.; Nolan, S. P. Adv. Synth. Catal. 2012, 354, 1897–1901.
8. Godoy, F.; Segarra, C.; Poyatos, M.; Peris, E. Organometallics 2011, 30, 684–688.
9. Hashim, A. S. K.; Lothschuittiz, C.; Böobling, C.; Rominger, F. Organometallics 2011, 30, 2411–2417.
10. Herrmann, W. A.; Köoeecher, C. Angew. Chem. Int. Ed. Engl. 1997, 36, 2162–2187.
11. Yang, C.-C.; Lin, P.-S.; Liu, F.-C.; Lin, I. J. B.; Lee, G.-H.; Peng, S.-M. J. Organomet. Chem. 2010, 699, 9599–9597.
12. Budagumpi, S.; Haque, R. A.; Salman, A. W. Coord. Chem. Rev. 2012, 59, 285–290.
13. Nan, G.; Qin, Y.; Wei, Z. Synth. React. Inorg. Met.-Org. Nano-Met. Chem. 2012, 42, 1577–1830.
14. Saito, S.; Saika, M.; Yamashiki, R.; Asumaya, I.; Masu, H. Organometallics 2011, 30, 1366–1373.
15. Sluiter, S. N.; Warsink, M.; Lutz, C. J. Dalton Trans. 2013, 42, 7365–7372.
16. Ray, S.; Mohan, R.; Singh, J. K.; Samantaray, M. K.; Shaikh, M. M.; Panda, D.; Ghosh, P. J. Am. Chem. Soc. 2007, 129, 15042–15053.
17. Haque, R. A.; Salman, A. W.; Budagumpi, S.; Abdullah, A. A.; Majid, A. M. A. Metallolements 2013, 5, 760–769.
18. Wang, C.-H.; Shihi, W.-C.; Chang, H. C.; Kuo, Y.-Y.; Hung, W.-C.; Ong, T.-G.; Li, W.-J. J. Med. Chem. 2011, 54, 5245–5249.
19. Skander, M.; Retailleau, P.; Bourrié, B.; Schio, L.; Mailliet, P.; Marine, M. J. Med. Chem. 2010, 53, 2146–2154.
20. Mata, J. A.; Poyatos, M. Curr. Org. Chem. 2011, 15, 3309–3324.
21. Sun, R. W.-Y.; Chow, A. L.-F.; Li, X.-H.; Yan, J. J.; Chui, S. S.-Y.; Che, C.-M. Chem. Sci. 2011, 2, 728–736.
22. Carreira, M.; Calvo-Sanjuáin, R.; Sanaúa, M.; Marzo, I.; Contel, M. Organometallics 2012, 31, 5772–5781.
23. Teysnot, M.-L.; Jarrousse, A.-S.; Cherry, A.; De Haze, A.; Beaudoin, C.; Manin, M.; Nolan, S. P.; Diez-Gonzalez, S.; Morel, ; Gautier, L. A. Chem. Eur. J. 2009, 15, 314–318.
24. Tetteh, S.; Dodoo, D. K.; Apiah-Opong, R.; Tuffour, I. Transit. Met. Chem. 2014, 39, 667–674.
25. Tanaka, M.; Katoaka, H.; Yano, S.; Ohi, H.; Kawamoto, K.; Shibahara, T.; Mizoshita, T.; Mori, Y.; Tanida, S.; Kamiya, T.; Joh, T. BMC Cancer 2013, 13, 237.
26. Islam, M. A.-A. A.; Sheikh, M. C.; Alam, M. S.; Zangrando, E.; Alam, M. A.; Taraldar, M. T. H.; Miyatake, R. Transit. Met. Chem. 2014, 39, 141–149.
27. Ramadon, R. M.; Al-Raddady, N. S. React. Inorg. Met.-Org. Nano-Met. Chem. 2015, 45, 1183–1190.
28. Wang, L.-W.; Liu, S.-Y.; Wang, J.-J.; Peng, W.; Li, S.-H.; Zhou, G.-Q.; Qin, X.-Y.; Wang, S.-X.; Zhang, J.-C. Synth. React. Inorg. Met.-Org. Nano-Met. Chem. 2015, 45.