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In vitro cytotoxic activities of new silver and PEPPSI palladium *N*-heterocyclic carbene complexes derived from benzimidazolium salts

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ABSTRACT

New benzimidazolium salts (**2**, **3**) and their silver (**4**, **5**) and palladium (**6**, **7**) *N*-heterocyclic carbene (Ag- and Pd-NHC) complexes were designed, synthesized and structurally characterized by NMR (^{1}H and ^{13}C), IR, HRMS and UV–Vis spectroscopic methods. All the synthesized compounds were tested in human colon cancer (DLD-1), breast cancer (MDA-MB-231) and embryonic kidney (HEK 293T) (non-cancerous) cell lines for *in vitro* cytotoxicity. Compound **4** specifically showed low IC_{50} values for these cell lines (IC_{50} values 12.41 ± 2.89, 11.98 ± 2.52 and 4.21 ± 1.74 μmol/L in DLD-1, MDA-MB-231 and HEK 293T cell lines, respectively). The Pd-NHC complexes (**6**, **7**) did not exhibit cytotoxic activity (IC_{50} values >200 in DLD-1, MDA-MB-231, HEK 293T cell lines). Therefore, Ag-NHC complexes can be further investigated as potential anticancer agents.

1. Introduction

Cancer is the leading cause of death in the 21st century. Many studies, therefore, have centered on finding the most effective drugs in order to prevent or cure this worldwide illness. In the last five decades, scientists have shown a growing interest in metallotherapeutic drugs and metal based diagnostic agents [1]. Platinum based complexes such as cis-platin, nedaplatin, oxaliplatin, carboplatin and lobaplatin, have been developed for antitumor therapy [2] which have shown success but carry severe and even toxic side effects such as neurotoxicity, nephrotoxicity, hematological and gastrointestinal toxicities. Hence, the search is ongoing for new drug candidates. To this end, derivatives of different non-platinum metals such as ruthenium, silver, titanium, iron, palladium, cobalt, gold, copper, zinc, nickel and gallium complexes have been synthesized and their anticancer activities have been investigated and tested for anticancer activity [3–14].

Six compounds were prepared and characterized as new cancer drug candidates and their anticancer properties were investigated. The *in vitro* cytotoxicity of these compounds was determined against human breast cancer (MDA-MB-231) and colon cancer (DLD-1) cell lines, and non-cancerous kidney cell line (HEK 293T) by MTT assay. The benzimidazolium salts (IC_{50} is around 131 μM for **2** and 18 μM for **3**), and silver complex (IC_{50} is around 12 μM for **4**) showed increased potency to inhibit cancer cell growth compared to **P**yridine **E**nhanced **P**recatalyst **P**reparation **S**tabilization and **I**nitiation (PEPPSI) Pd-NHC complexes (>200 for **6** and **7**) against DLD-1 and MDA-MB-231 cancer cell lines, respectively. While compounds **2**–**4** exhibited high cytotoxicity against HEK 293T, the PEPPSI complexes showed none.

2. Materials and methods section

2.1. Reagents and solvents

The necessary reagents and solvents for the synthesis of new compounds were purchased from Merck (Darmstadt, Germany), Sigma–Aldrich (Interlab A.S., USA) or Scharlau (Barcelona, Spain) chemical companies.

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2.2. Cell culture

DLD-1, MDA-MB-231 cancer cell lines and HEK 293T non-cancerous cell line were donated by Dr. Binh Pham and Fanfan Zhou (The University of Sydney). Dulbecco's Modified Eagle's Medium (DMEM), Fetal Bovine Serum (FBS), Glutamax, trypsin–EDTA, and Phosphate Buffered Saline (PBS) were bought from Lonza (Arch Chemicals, Inc., North Sydney, Australia), Gibco (Life technologies, USA) or Medicago (Astral Scientific, Sweden). Busulfan, cis-diammineplatinum (II) dichloride and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Fluka, Nalgene and Sigma (Sigma–Aldrich, USA). Hoechst 33258, pentahydrate (bis-benzimide) (Molecular Probes by Life Technologies, Australia) and propidium iodide (PI) stains were bought from ThermoFisher Scientific. Tissue culture flasks and 96-well plates were purchased from Jet Biofil (Pathtech, China).

2.3. General methods

The benzimidazolium salts (**2**, **3**) and Ag-NHC (**4**, **5**) compounds were prepared under argon gas atmosphere. At the same time, PEPPSI Pd-NHC (**6**, **7**) complexes were synthesized in ambient conditions. Nuclear magnetic resonance (NMR) experiments were conducted using a Bruker 300 or 400 MHz Ultra Shield NMR. Hertz (Hz) for coupling constants (J) and ppm relative to TMS for chemical shifts (δ) were given. Absorbance studies were carried out on a Shimadzu Pharmaspec UV-1700 spectrophotometer (Shimadzu Australasia, Rydalmere, Australia). An electrothermal-9200 melting point apparatus was used for determining the melting points. A Shimadzu Fourier Transform Infrared (FT-IR) 8400 spectrophotometer was used for taking the FT-IR spectra. High Resolution Mass Spectrometry (HRMS) was performed on a Bruker Apex Qe 7T Fourier Transform ion cyclotron resonance mass spectrometer equipped with an ESI/MALDI dual source in positive ion ESI mode. An Agilent IncuCyte zoom system (Millennium, Sydney, Australia) was utilized for following the morphology of the cells. Cell counting was conducted using an Invitrogen Countess automated cell counter.

2.4. Synthesis of benzimidazolium salts, *2* and *3*

Synthesis of benzimidazolium salts was performed according to our previous studies [15,16]. The desired products were purified with crystallization in ethylalcohol-diethylether solvents mixture.

2.4.1. 1-(2-Methylbenzonitrile)-3-benzylbenzimidazolium bromide, *2*

Compound **2** (404.3 g/mol) was prepared from 1-benzylbenzimidazole (1.15 g, 1 mmol) and 2-(bromomethyl)benzonitrile (1.08 g, 1 mmol) at 80 °C for 24 h. Yield: 95%, m.p.: 235–236 °C, color: white. IR: 1554.5 (CN); 2152.4 (C≡N); 2933.5, 2964.4 and 3022.2 cm^{-1} (C–H). ^{1}H NMR (400.13 MHz, DMSO-d_6, 298 K), δ: 5.72 (s, 2 H, NCH_2C$_6$H$_5$); 6.08 [s, 2 H, NCH_2C$_6$H$_4$(CN)-2]; 7.20–8.08 (m, 13 H, Ar-*H*); 10.08 (s, 1 H, NC*H*N). ^{13}C NMR (400.13 MHz, DMSO-d_6, 298 K), δ: 49.07 (N*C*H$_2$C$_6$H$_5$); 50.56 [N*C*H$_2$C$_6$H$_4$(CN)-2]; 111.36, 114.29, 114.68, 117.44, 127.41, 127.73, 128.81, 129.22, 129.43, 130.06, 131.43, 131.83, 134.24, 134.31, 134.44, 134.50, 137.32, 137.41 and 143.99 (Ar–*C*; *C*≡N); 144.76 (N*C*HN). HRMS [L-Br]$^+$ Calc. for $C_{22}H_{18}N_3$: 324.4. Found *m*/*z*: 324.14.

2.4.2. 1-(2-Methylbenzonitrile)-3-(2,3,4,5,6-pentamethylbenzyl) benzimidazolium chloride, *3*

Compound **3** (429.98 g/mol) was prepared from 1-(2-methylbenzonitrile)benzimidazole (0.95 g, 1 mmol) and 2,3,4,5,6-pentamethylbenzyl chloride (0.8 g, 1 mmol) at 80 °C for 24 h. Yield: 80%, m.p.: 215–216 °C, color: white. IR: 1554.5 (CN); 2212.2 (C≡N); 2904.6 and 3014.5 cm^{-1} (C–H). ^{1}H NMR (300.13 MHz, DMSO-d_6, 298 K), δ: 2.22 and 2.25 [s, 15 H, NCH$_2$C$_6$(CH_3)$_5$-2,3,4,5,6]; 5.77 [s, 2 H, NCH_2C$_6$(CH$_3$)$_5$-2,3,4,5,6]; 5.99 [s, 2 H, NCH_2-C$_6$H$_4$(CN)-2]; 7.38–8.31 (m, 8 H, Ar-*H*); 9.25 (s, 1 H, NC*H*N). ^{13}C NMR (300.13 MHz, DMSO-d_6, 298 K), δ: 16.39, 16.67 and 16.96 [NCH$_2$C$_6$(*C*H$_3$)$_5$-2,3,4,5,6]; 46.56 [N*C*H$_2$C$_6$(CH$_3$)$_5$-2,3,4,5,6]; 48.54 [N*C*H$_2$C$_6$H$_4$(CN)-2]; 110.30, 113.63, 114.27, 116.82, 125.29, 126.88, 127.23, 128.46, 129.31, 131.37, 131.62, 132.96, 133.79, 133.91, 133.95, 136.36 and 137.27 (Ar-*C*; *C*≡N); 142.19 (N*C*HN).

2.5. Synthesis of Ag-NHC complexes, *4* and *5*

Ag-NHC complexes were synthesized according to our previous studies from the benzimidazolium salt (2 mmol), silver oxide (1 mmol) and molecular elect (ME) (5–10 beads) in dried dichloromethane (10 mL) at room temperature for 18 h using a Schleng line under argon gas atmosphere [17,18]. White colored products were purified with crystallization.

2.5.1. [1-(2-methylbenzonitrile)-3-benzylbenzimidazol-2-ylidene] bromosilver (I), *4*

Compound **4** (511.16 g/mol) was prepared from **2** (0.5 g, 2 mmol) and silver oxide (0.144 g, 1 mmol) in CH_2Cl_2. Yield: 14%, m.p.: 267–270 °C, color: white. IR: 1458.4 (CN); 2167.8 (C≡N); 3058.9 cm^{-1} (C–H). ^{1}H NMR (400.13 MHz, DMSO-d_6, 298 K), δ: 5.74 (s, 2 H, NCH_2C$_6$H$_5$); 5.96 [s, 2 H, NCH_2C$_6$H$_4$(CN)-2]; 7.15–7.90 (m, 13 H, Ar-*H*). ^{13}C NMR (400.13 MHz, DMSO-d_6, 298 K), δ: 50.62 (N*C*H$_2$C$_6$H$_5$); 52.56 [N*C*H$_2$C$_6$H$_4$(CN)-2]; 110.98, 112.68, 113.07, 117.65, 124.84, 125.08, 127.83, 128.40, 128.51, 129.23, 129.34, 133.70, 134.06, 134.16, 134.23, 134.29, 136.56 and 139.95 (Ar-*C*; *C*≡N); 191.67 (N*C*N). HRMS [M−Br]$^+$ Calc. for $C_{22}H_{17}N_3Ag$: 430.05. Found *m*/*z*: 430.04.

2.5.2. [1-(2-Methylbenzonitrile)-3-(2,3,4,5,6-pentamethylbenzyl) benzimidazol-2-ylidene] chlorosilver (I), *5*

Compound **5** (536.84 g/mol) was prepared from **3** (0.46 g, 2 mmol) and silver oxide (0.124 g, 1 mmol). Yield: 20%, m.p.: 217–218 °C, color: bright white. IR: 1479.3 (CN); 2225.7 (C≡N); 2920.0 and 2964.4 cm^{-1} (C–H). ^{1}H NMR (400.13 MHz, DMSO-d_6, 298 K), δ: 2.14 [s, 15 H, NCH$_2$C$_6$(CH_3)$_5$-2,3,4,5,6]; 5.60 [s, 2 H, NCH_2-C$_6$(CH$_3$)$_5$-2,3,4,5,6]; 6.13 [s, 2 H, NCH_2C$_6$H$_4$(CN)-2]; 7.40–8.31 (m, 8 H, Ar-*H*). ^{13}C NMR (400.13 MHz, DMSO-d_6, 298 K), δ: 17.28, 17.37 and 17.48 [NCH$_2$C$_6$(*C*H$_3$)$_5$-2,3,4,5,6]; 47.51 [N*C*H$_2$C$_6$(CH$_3$)$_5$-2,3,4,5,6]; 51.36 [N*C*H$_2$C$_6$H$_4$(CN)-2]; 110.68, 112.54, 112.71, 117.47, 124.71, 125.01, 127.76, 129.25, 133.47, 134.08, 134.19, 134.63 and 135.92 (Ar-*C*; *C*≡N).

2.6. Synthesis of PEPPSI Pd-NHC complexes, *6* and *7*

Compounds **6** and **7** were prepared according to the literature from benzimidazolium salt (1 mmol), $PdCl_2$ (1 mmol), K_2CO_3 (5 mmol) and 3-chloropyridine (3 mL) at 80 °C for 16 h [19]. The products were purified via crystallization method.

2.6.1. [1-(2-Methylbenzonitrile)-3-benzylbenzimidazol-2-ylidene]-N-(3-chloropyridine)dichloropalladium (II), *6*

Compound **6** (614.26 g/mol) was synthesized from **2** (0.2 g, 1 mmol), $PdCl_2$ (0.088 g, 1 mmol), K_2CO_3 (0.34 g, 5 mmol) and 3-chloropyridine (3 mL). Yield: 62%, m.p.: 208–209 °C, color: yellow. IR: 1413.7 (CN); 2131.2 (C≡N); 2970.2, 3022.2 and 3066.6 cm^{-1} (C–H). ^{1}H NMR (400.13 MHz, $CDCl_3$, 298 K), δ: 6.25 (s, 2 H, NCH_2C$_6$H$_5$); 6.48 [s, 2 H, NCH_2C$_6$H$_4$(CN)-2]; 6.96–9.05 (m, 17 H, Ar-*H*). ^{13}C NMR (400.13 MHz, $CDCl_3$, 298 K), δ: 50.70 (NCH$_2$C$_6$H$_5$); 53.97 [NCH$_2$C$_6$H$_4$(CN)-2]; 110.86, 111.14, 111.83, 117.29, 123.81, 124.94, 128.05, 128.11, 128.77, 128.98, 129.48, 129.60, 132.71, 133.03, 133.50, 133.58, 134.39, 134.53, 138.19, 138.51, 150.04,