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(54) Title: PALLADIUM (II) COMPLEXES CONTAINING N-HETEROCYCLIC CARBENE LIGAND, SYNTHESIS, AND APPLICATIONS IN CANCER TREATMENT

(57) Abstract: Palladium (II) N-heterocyclic carbene (NHC) complexes which are stable in the presence of biological thiols. A representative complex, [Pd (C^NN') (N, N'-nBu₂NHC)] (CF₃SO₃) (Pd1d, HC^NN' = 6-phenyl-2, 2'-bipyridine, N, N'-nBu₂NHC = N, N'-di-n-butylimidazolium), displays potent killing activity toward cancer cell lines but is less cytotoxic toward normal human fibroblast cell lines.



PALLADIUM(II) COMPLEXES CONTAINING N-HETEROCYCLIC CARBENE LIGAND,
SYNTHESIS, AND APPLICATIONS IN CANCER TREATMENT

Technical Field

Described herein are palladium(II) N-heterocyclic carbene (NHC) complexes which are stable in the presence of biological thiols that display potent killing activity toward cancer cell lines.

Background

There has been a growing interest in expanding the horizon of applications of palladium complexes such as in functional molecular materials and organic electronics besides catalysis. In this regard, palladium(II) complexes have recently been examined as potential chemotherapeutic agents against cancer. The anti-proliferative properties of palladium complexes have been reported since 1980s. Most of the reported palladium(II) complexes showed some DNA binding properties. Yet, only a few palladium(II) complexes demonstrated good *in vivo* anti-cancer activity. The main challenge for the development of anti-cancer palladium(II) complexes is their undesirably high reactivity toward sulfur-containing bio-molecules such as glutathione (GSH) and ease of substitution reactions under physiological conditions, leading to low *in vivo* stability and multiple speciation that complicates the potential therapeutic applications.

Summary

Metal complexes which inhibit activities of enzymes such as histone deacetylase, telomerase, topoisomerase, thioredoxin reductase and protein kinases can be effective for treatment of cancers, including those with drug-resistance. Described herein are physiologically stable anti-cancer metal complexes advantageous in minimizing multiple speciation that affects biological activities and pharmacokinetic properties. The advantages are achieved, in part, by introducing strong metal-carbon bonds and multidentate ligands serving as effective ways to suppress demetalation of the metal complexes. N-Heterocyclic carbene (NHC) is strong σ -donor ligand that can form stable metal-carbene bond with most transition metal ions. In addition, NHC itself is relatively non-toxic and versatile structures of NHC can be easily prepared.

Together with the improved stability provided by the chelating effect of tridentate pincer ligands, described herein are a series of anti-cancer cyclometalated palladium(II) complexes containing NHC ligands, $[\text{Pd}(\text{C}^{\wedge}\text{N}^{\wedge}\text{N})(\text{NHC})]^+$, which are stable in the presence of biological thiols. The palladium(II) NHC complexes display *in vitro* cytotoxicities, and *in vivo* anti-cancer activities. Proteomics and subsequent biochemical assays revealed that the complexes induce mitochondrial dysfunction and inhibit the cancer promoting epidermal growth factor receptor pathway.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a series of chemical structures of cyclometalated palladium(II) complexes containing N-heterocyclic carbene (NHC) ligands as anti-cancer drugs can be exemplified as follows.

Figure 2 shows cellular uptake of palladium as quantified by ICP-MS. HeLa, NCI-H1650 and normal CCD-19Lu cells treated with the palladium complexes (5 μ M) for 3 h.

Figure 3 shows a) Keynode network analysis of proteomic data obtained from HeLa cells treated with **Pd1d** indicated that **Pd1d** acts on EGFR/Grb2 pathway. b–d) Inhibitory effect of **Pd1d** on EGFR signaling. Cancer cells were treated with indicated concentrations of **Pd1d** for 2 h. After EGFR stimulation with EGF (50 ng/mL) for 15 min, cells were harvested and lysed, and the activation/phosphorylation of EGFR (pEGFR) and its downstream protein kinase (pERK) were analyzed by Western blotting. b) HeLa cervical cancer cells, c) NCI-H460 lung cancer cells, d) HCC-827 lung cancer cells.

Figure 4 shows a) Average tumor volumes of NCI-H460 xenografts bearing mice treated with solvent or **Pd1d** through intraperitoneal injection of 8 repeated doses for 17 days. Error bars represent standard deviations, n = 5, * denote p<0.05 compared to solvent control. b) Body weight of mice in different groups

Figure 5 shows proposed anti-cancer mechanisms of action of $[\text{Pd}(\text{C}^{\wedge}\text{N}^{\wedge}\text{N})(\text{NHC})]^+$.

Figure S1 shows 500 MHz ^1H - ^1H COSY NMR spectrum of Pd1b in CD_3CN at 298 K: a) ^1H - ^1H COSY NMR spectrum. b) ^1H - ^1H NOESY NMR spectrum. c) ^1H NMR spectrum.

Figure S2 shows Perspective views of the X-ray crystal structures of the cation of Pd1a (left) and Pd1c (right). Hydrogen atoms are not shown.

Figure S3 shows a) Pd1d (50 μ M) in CH_3CN (solid line), CH_2Cl_2 (dashed line) and CH_3OH (dotted line) at 298 K. b) UV-vis absorption spectrum of Pd1a–Pd1d (50 μ M) in DMSO:PBS (1:19).

Figure S4 shows UV-vis absorption spectrum of a) Pd1a, $\text{C}^{\wedge}\text{N}^{\wedge}\text{N}$ ligand, $[\text{Pd}(\text{C}^{\wedge}\text{N}^{\wedge}\text{N})\text{Cl}]$ and $(\text{CH}_3)_2\text{NHC}$ ligand (50 μ M) in CH_3CN at 298 K and b) Pd1a and Pd2a (50 μ M) in CH_3CN .

Figure S5 shows a) ^1H NMR spectra (400 MHz, 298 or 310 K) of Pd1b (2 mM) and GSH (20 mM) in D_2O :DMSO (9:1 v/v) solution mixture after mixing for 1 h and 24 h. No

significant changes of the signals of Pd1b were found. b) ESI-MS-TOF spectrum of a reaction mixture of Pd1a with GSH at 1:10 ratio (reaction time: 1 h and 24 h).

Figure S6 shows a) ^1H NMR spectra (400 MHz, 298 K) of Pdiso (2 mM) and GSH (20 mM) in D_2O (150 mM ammonium bicarbonate solution):DMSO (9:1, v/v) solution mixture just after mixing. b) ^{31}P NMR spectra (400 MHz, 298 K) of PdPPh_3 (4 mM) and GSH (16 mM) in D_2O (150 mM ammonium bicarbonate solution):DMSO (9:1, v/v) solution mixture just after mixing.

Figure S7 shows a) ESI-MS-TOF spectrum of *trans*- $[\text{Pd}(\text{NHC})_2\text{Cl}_2]$ in CH_3OH /ammonium bicarbonate buffer (50 mM) (1:9, v/v). b) ESI-MS-TOF spectrum of a reaction mixture of *trans*- $[\text{Pd}(\text{NHC})_2\text{Cl}_2]$ with GSH at 1:100 ratio in CH_3OH /ammonium bicarbonate buffer (50 mM) (1:9, v/v). (reaction time: 1 h).

Figure S8 shows a) Representative LC-MS chromatograms and b) mass spectra of (top) cell extract of HeLa cells treated with Pd1a (10 μM), (middle) Pd1a (10 μM) in cell culture medium for 48 h, and (bottom) Pd1a (10 μM) in CH_3CN .

Figure S9 shows Unbound palladium content after incubation of different palladium complexes with human serum albumin (HSA) for 2 h based on ICP-MS analysis.

Figure S10 shows Emission spectral changes of HSA (3 μM , in Tris-HCl buffer (0.05 M Tris, 0.1 M NaCl, pH 7.40)) with increasing concentration of Pd1a, Pd1d and Pd2a. The **concentration of the compounds was varied from 0.0 to 56.61 μM . $\lambda_{\text{ex}} = 280 \text{ nm}$.**

Figure S11 shows Caspase-3 and caspase-9 activities in HeLa cells treated with or without Pd1a and Pd1d (0.5 μM) at different time points.

Figure S12 shows The expression and cleavage of PARP in HeLa cells after treatment with Pd1a or Pd1d (0.5 μM) for 0, 12, 24, or 48 h.

Figure S13 shows UV-vis spectral change of Pd1d in PBS:DMSO (9:1, v/v) with increasing **concentration of ctDNA**. Inset: $[\text{DNA}]/\epsilon_{\text{ap}}$ vs $[\text{DNA}]$. Absorbance was monitored at 323 nm.

Figure S14 shows (Left) Gel electrophoresis of 123 bp DNA ladder (50 μM base pairs) in 1.5 % agarose gel showing the mobility of the DNA in the absence (first lane), or the presence of Pd1a–Pd1d (forth to seventh lane) and ethidium bromide (EB, second and third lane) in a 1:1 or 1:10 molar ratio. (Right) Gel mobility shift assay showing the mobility of the DNA in the absence (first and last lane), or the presence of PdPPh_3 and Pdiso in a 1:1 or 1:10 molar ratio (third to sixth lane) and ethidium bromide (EB, second) in a 1:1 molar ratio

Figure S15 shows Assay on DNA double strand break after incubation of HeLa cells with $[\text{Pd}(\text{C}^{\wedge}\text{N}^{\wedge}\text{N})(\text{NHC})]^+$ (1 μM). Foci formation indicated by arrows in the image of treated sample showing that cisplatin can induce DNA DSBs. The HeLa cells were also stained with DAPI, which displayed blue fluorescence for visualization of nucleus.

Figure. S16 shows Effect of $[\text{Pd}(\text{CNN})(\text{NHC})]^+$ complexes (2.5 μM) or CCCP (2.5 μM) or vehicle control on mitochondrial potential of HeLa cells after incubation for 2 h. a) Untreated cells showing orange fluorescence owing to the strong J-aggregate of JC-1. b) CCCP treated cells showing green fluorescence due to low $\Delta\Psi_m$, resulting in cytosolic accumulation of monomeric JC-1. c and d) Cells treated with Pd1a and Pd1d (2.5 μM) respectively.

Figure S17 shows The cytotoxicity profile of a) Pd1a or b) Pd1d in HeLa cells pre-treated with or without the thiol anti-oxidant *N*-acetylcysteine (NAC).

Figure S18 shows Positive MS/MS spectrum of $[\text{C}_{27}\text{H}_{31}\text{N}_4\text{Pd}]^+$ ion at m/z 517.2 with a collision energy at 37 V in a) tumor b) blood with Pd1d (2 mg/kg) through intraperitoneal injection of 8 repeated doses for 17 days.

Figure S19 shows a) Average tumor volumes of HeLa xenografts bearing mice treated with solvent or Pd1d through intraperitoneal injection of 7 repeated doses for 13 days. Error bars represent standard deviations, $n = 5$, * denote $p < 0.05$ compared to solvent control. b) Body weight of mice in different groups. c) Photos showing mice bearing HeLa xenografts after treatment with solvent or Pd1d through intraperitoneal injection of 7 repeated doses for day 13.

Figure S20 shows *In vitro* anti-angiogenic property of $[\text{Pd}(\text{C}^{\wedge}\text{N}^{\wedge}\text{N})(\text{NHC})]^+$ complexes on MS1 cells. a) The anti-angiogenic activity of Pd1a and Pd1d (0.25 or 0.5 μM) as revealed by the tube-formation assay after 1 h treatment. b) Wound healing assay on MS1 cells. (i) After 48 h, cells migrated to the wounded area. (ii) No significant inhibitory effect incubation with Pd1a at 0.5 μM . (iii) Migration was inhibited by incubation with Pd1a at 0.5 μM . Pictures taken at 100 $\times$ magnification.

Figure S21 to S28 shows characterization of the palladium(II)-carbene complexes.

Detailed Description

Terms not specifically defined herein should be given the meanings that would be given to them by one of skill in the art in light of the disclosure and the context. As used in the specification, however, unless specified to the contrary, the following terms have the meaning indicated and the following conventions are adhered to.

It is to be understood that unless otherwise indicated this invention is not limited to specific reactants, reaction conditions, or the like, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a catalyst" or "a complex" encompasses a combination or mixture of different catalysts or complexes as well as a single catalyst or complex, reference to "a substituent" includes a single substituent as well as two or more substituents that may or may not be the same, and the like.

In this specification and in the claims that follow, reference will be made to a number of terms, which shall be defined to have the following meanings:

The term "alkyl" as used herein refers to a linear, branched, or cyclic saturated hydrocarbon group typically although not necessarily containing 1 to about 20 carbon atoms, preferably 1 to about 12 carbon atoms, 1 to 6 carbon atoms, such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, t-butyl, octyl, decyl, and the like, as well as cycloalkyl groups such as cyclopentyl, cyclohexyl and the like. Generally, although again not necessarily, term "cycloalkyl" intends a cyclic alkyl group, typically having 4 to 8, preferably 5 to 7, carbon atoms. The term "substituted alkyl" refers to alkyl substituted with one or more substituent groups, and the terms "heteroatom-containing alkyl" and "heteroalkyl" refer to alkyl in which at least one carbon atom is replaced with a heteroatom. If not otherwise indicated, the term "alkyl" includes linear, branched, cyclic, unsubstituted, substituted, and/or heteroatom-containing alkyl.

The term "alkane" as used herein refers to a linear, branched, or cyclic saturated hydrocarbon typically although not necessarily containing 1 to about 20 carbon atoms, preferably 1 to about 12 carbon atoms, 1 to 6 carbon atoms, such as methane, ethane, propane, butane, octane, decane, and the like.

The term "alkoxy" as used herein intends an alkyl group bound through a single, terminal ether linkage; that is, an "alkoxy" group maybe represented as -O-alkyl where alkyl is as defined above, such as methoxy, ethoxy, propoxy, butoxy, octoxy, decoxy, and the like.

The term "aromatic ring" as used herein and unless otherwise specified, refers to a single aromatic ring or multiple aromatic rings that are fused together, directly linked, or indirectly linked (such that the different aromatic rings are bound to a common group such as a methylene or ethylene moiety). Preferred aromatic ring contain 5 to 20 carbon atoms, and particularly preferred aromatic ring contain 5 to 14 carbon atoms. Exemplary aromatic ring contains one aromatic ring or two fused or linked

aromatic rings, e.g., benzene, naphthalene, thiophene, benzothiophene, anthracene, pyrene, furan, pyrimidine, pyrrole, pyridine, fluorene, carbozole, carborane, isoquinoline, 1-isoquinoline, 2-quinoline, and the like. "Substituted aromatic ring" refers to an aromatic ring moiety substituted with one or more substituent groups.

By "substituted", as alluded to in some of the aforementioned definitions, is meant that, at least one hydrogen atom bound to a carbon (or other) atom is replaced with one or more non-hydrogen substituents. Examples of such substituents include, without limitation: functional groups such as halo, hydroxyl, sulfhydryl, C1-C20 alkoxy, C2-C20 alkenyloxy, C2-C20 alkynyloxy, C5-C20 aryloxy, C6-C20 aralkyloxy, C6-C20 alkaryloxy, acyl (including C2-C20 alkylcarbonyl (-CO-alkyl) and C6-C20 arylcarbonyl (-CO-aryl)), acyloxy (-O-acyl, including C2-C20 alkylcarbonyloxy (-O-CO-alkyl) and C6-C20 arylcarbonyloxy (-O-CO-aryl)), C2-C20 alkoxycarbonyl (-CO-O-alkyl), C6-C20 aryloxycarbonyl (-CO-O-aryl), halocarbonyl (-CO-X where X is halo), C2-C20 alkylcarbonato (-O-(CO)-O-alkyl), C6-C20 arylcarbonato (-O-(CO)-O-aryl), carboxy (-COOH), carboxylato (-COO-), carbamoyl (-CO-NH₂), mono-(C1-C20 alkyl)-substituted carbamoyl (-CO-NH(C1-C20 alkyl)), di-(C1-C20 alkyl)-substituted carbamoyl (-CO-N(C1-C20 alkyl)₂), mono-(C5-C20 aryl)-substituted carbamoyl (-CO-NH-aryl), di-(C5-C20 aryl)-substituted carbamoyl (-CO-N(C5-C20 aryl)₂), di-N-(C1-C20 alkyl), N-(C5-C20 aryl)-substituted carbamoyl, thiocarbamoyl (-CS-NH₂), mono-(C1-C20 alkyl)-substituted thiocarbamoyl (-CO-NH(C1-C20 alkyl)), di-(C1-C20 alkyl)-substituted thiocarbamoyl (-CO-N(C1-C20 alkyl)₂), mono-(C5-C20 aryl)-substituted thiocarbamoyl (-CO-NH-aryl), di-(C5-C20 aryl)-substituted thiocarbamoyl (-CO-N(C5-C20 aryl)₂), di-N-(C1-C20 alkyl), N-(C5-C20 aryl)-substituted thiocarbamoyl, carbamido (-NH-(CO)-NH₂), cyano (-C≡N), cyanato (-O-C≡N), thiocyanato (-S-C≡N), isocyano (-N≡C-), formyl (-CO-H), thioformyl (-CS-H), amino (-NH₂), mono-(C1-C20 alkyl)-substituted amino, di-(C1-C20 alkyl)-substituted amino, mono-(C5-C20 aryl)-substituted amino, di-(C5-C20 aryl)-substituted amino, C2-C20 alkylamido (-NH-(CO)-alkyl), C6-C20 arylamido (-NH-(CO)-aryl), imino (-CR=NH where R = hydrogen, C1-C20 alkyl, C5-C20 aryl, C6-C20 alkaryl, C6-C20 aralkyl, etc.), C2-C20 alkylimino (-CR=N(alkyl), where R = hydrogen, C1-C20 alkyl, C5-C20 aryl, C6-C20 alkaryl, C6-C20 aralkyl, etc.), arylimino (-CR=N(aryl), where R = hydrogen, C1-C20 alkyl, C5-C20 aryl, C6-C20 alkaryl, C6-C20 aralkyl, etc.), nitro (-NO₂), nitroso (-NO), sulfo (-SO₂-OH), sulfonato (-SO₂-O-), C1-C20 alkylsulfanyl (-S-alkyl; also termed "alkylthio"), C5-C20 arylsulfanyl (-S-aryl; also termed "arylthio"),

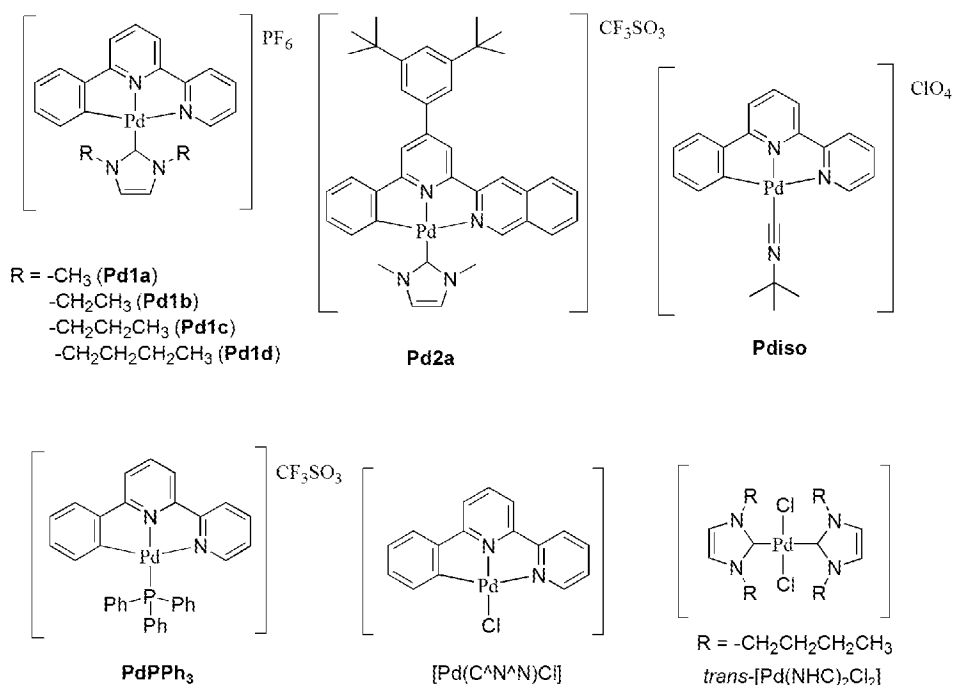
C1-C20 alkylidithio

(-S-S-alkyl), C5-C20 arylidithio (-S-S-aryl), C1-C20 alkylsulfinyl (-SO-alkyl), C5-C20 arylsulfinyl (-SO-aryl), C1-C20 alkylsulfonyl (-SO₂-alkyl), C5-C20 arylsulfonyl (-SO₂-aryl), boryl (-BH₂), borono (-B(OH)₂), boronato (-B(OR)₂ where R is alkyl or other hydrocarbyl), phosphono (-P(O)(OH)₂), phosphonato (-P(O)(O-)₂), phosphinato (-P(O)(O-)), phospho (-PO₂) phosphino (-PH₂), silyl (-SiR₃ wherein R is hydrogen or hydrocarbyl), and silyloxy (-O-silyl); and the hydrocarbyl moieties C1-C20 alkyl (preferably C1-C12 alkyl, more preferably C1-C6 alkyl), C2-C20 alkenyl (preferably C2-C12 alkenyl, more preferably C2-C6 alkenyl), C2-C20 alkynyl (preferably C2-C12 alkynyl, more preferably C2-C6 alkynyl), C5-C20 aryl (preferably C5-C14 aryl), C6-C20 alkaryl (preferably C6-C16 alkaryl), and C6-C20 aralkyl (preferably C6-C16 aralkyl).

In addition, the aforementioned functional groups may, if a particular group permits, be further substituted with one or more additional functional groups or with one or more hydrocarbyl moieties such as those specifically enumerated above. Analogously, the above-mentioned hydrocarbyl moieties may be further substituted with one or more functional groups or additional hydrocarbyl moieties such as those specifically enumerated.

The "pharmaceutically acceptable anion" as used herein and unless otherwise specified, may be a chloro, bromo, iodo, tartrate, hydrogen tartrate, succinate, maleate, fumarate, sulfate, hydrogen sulfate or methylsulfate anion.

A series of chemical structures of cyclometalated palladium(II) complexes containing N-heterocyclic carbene (NHC) ligands as anti-cancer drugs can be exemplified as follows.



N-Heterocyclic carbene (NHC) ligand is a strong σ -donor ligand that can form stable metal-C bond and can be prepared easily and is non-toxic. Complexes can contain tridentate CNN ligand to improve the stability. Pdiso, PdPPh₃, [Pd(C^N^N)Cl] and *trans*-[Pd(NHC)₂Cl₂] were synthesized to compare with [Pd(C^N^N)(NHC)]⁺ in terms of the physiological stability and cytotoxicities.

Cyclometalated palladium(II) complexes containing NHC ligands with different alkyl chain length (**Pd1a–Pd1d** Figure 1) were prepared. In general, a mixture of [Pd(C^N^N)Cl] and the corresponding NHC ligand in the presence of KOtBu was heated under reflux for 24 h to give pure palladium(II) NHC complexes after purification by column chromatography and re-crystallization. The complexes were characterized by FAB-MS, ¹H NMR, ¹⁹F NMR and ³¹P NMR spectroscopies, and elemental analyses (Supporting Information). Other palladium(II) complexes, **Pd2a**, **PdPPh₃**, **Pdiso**, *trans*-[Pd(NHC)₂Cl₂] and [Pd(C^N^N)Cl], were also prepared for comparative study. Crystals of **Pd1a** and **Pd1c** suitable for X-ray crystallography were obtained by diffusion of pentane or diethyl ether into dichloromethane solutions. Perspective views of the crystal structures are shown in Figure S2. The Pd–C_{carbene} distances of **Pd1a** and **Pd1c** are 1.995 Å. Crystallographic and structural refinement data of **Pd1a** and **Pd1c** and selected bond angles/distances are summarized in Table S1–S6 in the Supporting Information.

Table 1 below shows *in vitro* cytotoxic IC₅₀ values (μM, 72 h) of the palladium(II) complexes and cisplatin toward human cell lines of lung cancer (NCI-H1650 and NCI-H460), breast cancer (MDA-MB-231), cervical cancer (HeLa), ovarian cancer (A2780) and its cisplatin resistant clone (A2780cis), and normal lung fibroblast (CCD-19Lu).

Complex	NCI-H1650	NCI-H460	MDA-MB-231	HeLa	A2780cis	A2780	CCD-19Lu
	IC ₅₀ [μ]						
Pd1a	2.5 $\pm$ 0.2	2.1 $\pm$ 0.2	0.9 $\pm$ 0.05	1.8 $\pm$ 0.1	2.2 $\pm$ 0.2	1.7 $\pm$ 0.2	32.1 $\pm$ 1.1
Pd1b	1.2 $\pm$ 0.09	1.1 $\pm$ 0.1	0.9 $\pm$ 0.06	1.4 $\pm$ 0.2	1.8 $\pm$ 0.2	1.5 $\pm$ 0.2	22.1 $\pm$ 1.9
Pd1c	0.5 $\pm$ 0.03	0.4 $\pm$ 0.05	0.8 $\pm$ 0.05	0.5 $\pm$ 0.05	1.2 $\pm$ 0.1	1.6 $\pm$ 0.1	15.4 $\pm$ 1.1
Pd1d	0.09 $\pm$ 0.01	0.08 $\pm$ 0.01	0.5 $\pm$ 0.06	0.1 $\pm$ 0.01	0.5 $\pm$ 0.05	0.2 $\pm$ 0.03	11.8 $\pm$ 1.2
Pd2a	0.9 $\pm$ 0.1	1.1 $\pm$ 0.09	0.4 $\pm$ 0.04	0.4 $\pm$ 0.03	2.5 $\pm$ 0.2	1.3 $\pm$ 0.2	8.9 $\pm$ 0.7
<i>trans</i> -[Pd(NHC) ₂ Cl ₂]	>100	>100	>100	>100	>100	>100	>100
[Pd(C [^] N [^] N)Cl]	>100	>100	>100	>100	>100	>100	>100
PdCl ₂	>100	>100	>100	>100	>100	>100	>100
Pdiso	>50	>50	>50	>50	>50	>50	>50
PdPPh₃	14.5 $\pm$ 1.2	15.2 $\pm$ 1.4	18.2 $\pm$ 1.7	11.2 $\pm$ 1.0	2.2 $\pm$ 0.1	3.2 $\pm$ 0.3	25.5 $\pm$ 2.1
cisplatin	15.5 $\pm$ 0.9	9.5 $\pm$ 0.8	20.5 $\pm$ 1.5	12.1 $\pm$ 1.1	30.5 $\pm$ 3.1	1.5 $\pm$ 0.1	95.5 $\pm$ 8.1

The UV-visible absorption data and spectra of the [Pd(C[^]N[^]N)(NHC)]⁺ complexes are depicted in Table S7 and Figure S3 and S4. Complexes **Pd1a–Pd1d** show strong absorption bands in the region of 233–330 nm and a weaker absorption at *ca.* 383 nm, which are attributable to the intraligand (IL) charge transfer transitions.

The stability of the palladium(II) complexes in the presence of biologically important thiols was examined. **Pd1b** (2 mM) in aqueous buffer solution (150 mM ammonium bicarbonate)/DMSO (4:1, v/v) mixture was stable in the presence of 10-fold excess of GSH (20 mM) at 40 °C and revealed no significant changes of the signals of **Pd1b** in the ¹H NMR spectrum for 24 h. (Figure S5). In contrast, upon addition of excess GSH (20 mM) to **Pdiso** (2 mM) or **PdPPh₃** (4 mM), the ¹H NMR signals in aromatic region (6–8.5 ppm) of **Pdiso** were different from those obtained without GSH (Figure S6a), while the ³¹P NMR signal of **PdPPh₃** revealed a significant change in chemical shift from 40 ppm to -8 ppm (Figure S6b). These findings suggest that, unlike the [Pd(C[^]N[^]N)(NHC)]⁺ complexes, palladium(II) phosphine and isocyanide complexes undergo reactions readily with GSH. The solution stability of [Pd(C[^]N[^]N)(NHC)]⁺ in the presence of GSH was further examined by electrospray ionization mass spectrometry (ESI-MS). A mixture of **Pd1a** (0.2 mM) and 100-fold excess of GSH (20 mM) in aqueous buffer solution (50 mM ammonium bicarbonate, pH 7.6) was freshly prepared and the mass spectrum was recorded (Figure S5b). The peak at *m/z* = 433.3, corresponding to [b-Pd1a-PF₆]⁺, was still observed after 24 h of incubation with GSH. In contrast, *trans*-[Pd(NHC)₂Cl₂] (0.2 mM) was found to react readily with GSH (20 mM) in pH 7.6 buffer solution, as revealed by the vanishing of the ion peak of *trans*-[Pd(NHC)₂Cl₂] (*m/z* = 503.4) and the emergence of [Pd(NHC)₂(GSH)]⁺ adduct peak (*m/z* = 772.3) upon incubation with GSH for 1 h (Figure S7). In addition, the *in vitro* stability of **Pd1a** was also studied by LC-MS. No new peaks were found in the LC-MS chromatograms obtained from incubation of cell culture medium or HeLa cell extract with **Pd1a** (10 μ M) for 48 h (Figure S8). Furthermore, [Pd(C[^]N[^]N)(NHC)]⁺ complexes were at least 5-fold less inhibitory toward thio-dependent enzyme thioredoxin reductase compared to the thiol reactive **Pdiso** and **PdPPh₃** (Table S8). We also investigated the reaction of **Pd1d** with

large excess of non-thiol biological reductants, ascorbic acid. The absorption spectra of **Pd1d** did not have significant changes and ascorbic acid oxidation, as determined by monitoring the absorbance at 265 nm, was not obvious up to 24 h.

As serum albumin is known to strongly bind with and hence lower the bioavailability of drug molecules, the level of free palladium(II) complexes in the presence of human serum albumin (HSA) was examined by measuring the amount of unbound palladium in the solution mixture of the complexes (2 μM) and HSA (60 μM) by inductively coupled plasma mass spectrometry (ICP-MS) (Figure S9). It was found that more than 60 % of **Pd1a** and 55 % of **Pd1d** were left unbound after a 2-h incubation, while less than 16 % of unbound palladium was found for other palladium(II) complexes. This suggests that $[\text{Pd}(\text{C}^{\wedge}\text{N}^{\wedge}\text{N})(\text{NHC})]^+$ complexes have weaker interaction with HSA compared to other palladium(II) complexes. By protein fluorescence titration experiments, the binding constants of **Pd1a**, **Pd1d** and **Pd2a** with HSA were determined to be $(2.5 \pm 0.1) \times 10^5$, $(4.7 \pm 0.3) \times 10^4$ and $(1.8 \pm 0.2) \times 10^6 \text{ M}^{-1}$, respectively (Figure S10).

The *in vitro* cytotoxicities of the palladium(II) complexes toward different human cancer cell lines including a cervical epithelial cancer (HeLa), lung cancer (NCI-H1650 and NCI-H460), an aggressive triple-negative breast cancer (MDA-MB-231), and an ovarian cancer (A2780) and its cisplatin resistant clone (A2780cis) were investigated (Table 1). All the $[\text{Pd}(\text{C}^{\wedge}\text{N}^{\wedge}\text{N})(\text{NHC})]^+$ complexes were found to display promising anti-proliferative activity toward the cancer cell lines with IC_{50} values of 0.09–2.5 μM , which were up to 172-fold more cytotoxic than cisplatin. The $[\text{Pd}(\text{C}^{\wedge}\text{N}^{\wedge}\text{N})(\text{NHC})]^+$ complexes displayed similar cytotoxicities in both A2780 and A2780cis while cisplatin is relatively less cytotoxic (~20-fold) towards the cisplatin-resistant A2780cis than the A2780. Notably, $[\text{Pd}(\text{C}^{\wedge}\text{N}^{\wedge}\text{N})(\text{NHC})]^+$ complexes were less cytotoxic toward a human normal lung fibroblast cell line (CCD-19Lu), e.g. **Pd1d** showed an IC_{50} value of 11.8 μM toward CCD-19Lu, which was about 140-fold higher than that toward NCI-H1650 cells. On the other hand, other palladium(II) complexes were found to show much lower cytotoxicity toward the cancer cells. This may be attributable to the aforementioned poor stability of these complexes in cellular conditions (Figure S6 and S7) and their more significant binding with serum proteins (Figure S9).

The involvement of apoptosis in the cytotoxic action of the $[\text{Pd}(\text{C}^{\wedge}\text{N}^{\wedge}\text{N})(\text{NHC})]^+$ complexes on cancer cells was examined. HeLa cells treated with **Pd1a** or **Pd1d** (0.5 μM) showed dramatic increase in the cell population with reduced DNA content (sub-G1 phase in flow cytometry analysis) (Table S9), increases in the enzymatic activities of caspase-3 and caspase-9 (Figure S11) and cleavage of PARP-1 (Figure S12), signifying induction of apoptosis.

The cellular uptake of $[\text{Pd}(\text{C}^{\wedge}\text{N}^{\wedge}\text{N})(\text{NHC})]^+$ and other palladium(II) complexes was investigated by measuring palladium metal content in cell lysates using ICP-MS (Figure 2). Cancer cells (HeLa and NCI-H1650) were found to show 2-fold higher cellular uptake of $[\text{Pd}(\text{C}^{\wedge}\text{N}^{\wedge}\text{N})(\text{NHC})]^+$ complexes as compared to the normal CCD-19Lu cells. On the other

hand, **PdPPh₃**, **Pdiso**, *trans*-[Pd(NHC)₂Cl₂] and [Pd(C[^]N[^]N)Cl] all displayed much lower uptake into cancer and normal cells compared to that of [Pd(C[^]N[^]N)(NHC)]⁺ complexes (with up to 50-fold difference) (Table 1) in the design of palladium medicines for cancer treatment.

The binding constants, K_b , for **Pd1a**, **Pd1d** and **Pd2a** with calf-thymus DNA (ct DNA) determined from UV-visible absorption data and Scatchard plots were found to be $(14.2 \pm 0.8) \times 10^3$, $(8.5 \pm 0.6) \times 10^3$ and $(9.5 \pm 0.7) \times 10^3 \text{ M}^{-1}$, respectively (Figure S13). The K_b values of **Pd1a**, **Pd1d** and **Pd2a** are markedly lower than those of other palladium(II) complexes in the literature ($K_b = 10^{4-5} \text{ M}^{-1}$),^[4c, 9] and also the typical DNA intercalator ethidium bromide ($K_b = 10^7$) by up to several orders of magnitude.^[4] In a gel mobility shift assay, DNA ladder (123 bp) mixed with [Pd(C[^]N[^]N)(NHC)]⁺ at molar concentration equivalent to that of base pairs did not show retarded mobility (Figure S14). To examine whether [Pd(C[^]N[^]N)(NHC)]⁺ complexes trigger DNA damage such as double strand break (DSB) inside the cells, fluorescence microscopic examination of phosphorylated Histone 2AX (**γH2AX**) was performed. **γH2AX is an established marker for DSB, forming foci at the site of DNA breaks and appearing as punctate staining in the nucleus in immunofluorescence staining experiments.**^[10] It was found that HeLa cells displayed **γH2AX foci after treatment with cisplatin for 24 h but not with the [Pd(C[^]N[^]N)(NHC)]⁺ complexes** (Figure S15). These results suggest that DNA is unlikely to be a major molecular target of [Pd(C[^]N[^]N)(NHC)]⁺.

As mitochondria is known to play key roles in activating cell apoptosis, the effect of **Pd1a** and **Pd1d** on mitochondria was investigated by a fluorescent probe of mitochondrial membrane potential, JC-1. HeLa cells treated with **Pd1a** or **Pd1d** (2.5 μM) for 2 h followed by staining with JC-1 displayed green fluorescence, while orange fluorescence was observed in HeLa cells treated with solvent control (Figure S16). The green fluorescence from cells treated with [Pd(C[^]N[^]N)(NHC)]⁺ indicates disruption of the mitochondrial membrane potential by the complexes that leads to cytosolic accumulation of green-emissive monomeric JC-1. To assess whether production of reactive oxygen species (ROS), which may be augmented owing to mitochondrial dysfunction, is involved in the [Pd(C[^]N[^]N)(NHC)]⁺-induced cytotoxicity, HeLa cells were treated with **Pd1a** or **Pd1d** in the presence of excess cell permeable anti-oxidant N-acetylcysteine (NAC) and no significant change in cell viability was found (Figure S17). These data are also consistent with the *in vitro* stability of the [Pd(C[^]N[^]N)(NHC)]⁺ in the presence of thiols.

A proteomic analysis of changes in protein expression mediated by **Pd1d** was performed using HPLC-LTQ-Orbitrap MS followed by computational pathway analysis. The lists of proteins (Table S10) whose expressions were altered by **Pd1d** were uploaded to ExPlainTM which is connected to the BIOBASE database for upstream searching of keynodes for pathway and keynode analysis. Through the pathway analysis, it was found that 24 and 56 regulated pathways were identified from the HeLa cells treated with **Pd1d** for 2 and 10 h, respectively (Table S11). Many of these pathways were associated with

apoptotic cell death and angiogenesis. Furthermore, the Keynode network analysis^[11] deduced that **Pd1d** acts on an upstream signaling pathway involving the growth factor receptor-bound protein 2 (Grb2) that is a key adaptor protein of diversified cancer-promoting growth factor receptors such as epidermal growth factor receptor (EGFR) (Figure 3a). To verify the effect of **Pd1d** on EGFR signaling pathway as implied by proteomic analysis, the EGF stimulated phosphorylation of EGFR and its downstream protein kinase ERK1/2 in HeLa cells were studied by Western blotting experiment (Figure 3b). Incubation of HeLa cells with **Pd1d** for 2 h led to significant decreases in phosphorylation of EGFR and ERK1/2 in a dose-dependent manner, suggesting that **Pd1d** inhibits EGFR signaling. Similar EGFR inhibition was also demonstrated in two lung cancer cell lines (NCI-H460 and HCC-827) under the same experimental conditions (Figure 3c & 3d).

The *in vivo* anti-cancer activity of **Pd1d** was examined. Treatment of nude mice bearing xenografts of NCI-H460 cancer cells with **Pd1d** at 1 mg/kg and 2 mg/kg through intraperitoneal injection of 8 repeated doses for 17 days was found to inhibit tumor growth by 32 % ($p < 0.05$) and 61 % ($p < 0.05$), respectively, as compared to those treated with solvent control (Figure 4a). Importantly, no mouse death or significant body weight loss was found after the treatment with **Pd1d** at these dosages (Figure 4b). At the end of the experiments (two days after the last compound administration), mice were sacrificed and the *in vivo* stability of **Pd1d** was studied by LC-MS/MS. Intact cation of **Pd1d** was found in tumor ($0.048 \pm 0.015 \mu\text{g/g}$ tissue) and blood plasma ($9 \pm 3 \text{ nM}$) (Figure S18). The *in vivo* anti-cancer activity of **Pd1d** was also examined in nude mice bearing xenografts of HeLa cancer cells with **Pd1d** at 1 mg/kg and 3 mg/kg through intraperitoneal injection of 7 repeated doses for 13 days. It was found to inhibit tumor growth by 40 % ($p < 0.05$) and 55 % ($p < 0.05$), respectively, as compared to those treated with solvent control (Figure S19). Furthermore, the anti-cancer properties of $[\text{Pd}(\text{C}^{\wedge}\text{N}^{\wedge}\text{N})(\text{NHC})]^+$ complexes in terms of their anti-angiogenic activities were investigated. Incubation of mouse endothelial cells (MS1) with **Pd1a** and **Pd1d** at sub-cytotoxic concentrations (0.25 and 0.5 M) resulted in distortion of the endothelial tube formation in Matrigel (Figure S20) and the cell migration in wound healing assay (Figure S21).

In summary, $[\text{Pd}(\text{C}^{\wedge}\text{N}^{\wedge}\text{N})(\text{NHC})]^+$ complexes demonstrate high stability *in vitro* and in the presence of physiological thiols. Eventually the complexes show potent *in vitro* cytotoxicities against cancer cells as well as effective *in vivo* anti-cancer activities toward tumor xenograft in nude mice with no observable toxicity. The mechanism of anti-cancer action of the complexes, as investigated by proteomics analysis and biochemical assays, involved induction of mitochondrial dysfunction and inhibition of EGFR signaling pathway in association with apoptotic cell death of the

cancer cells (Figure 5). This highlights the promising prospect of using NHC and pincer type cyclometalated ligands in the development of palladium(II) complexes for anti-cancer treatment.

The examples, as shown in the Supporting Information, illustrate embodiments for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

With respect to any figure or numerical range for a given characteristic, a figure or a parameter from one range may be combined with another figure or a parameter from a different range for the same characteristic to generate a numerical range.

Other than in the operating examples, or where otherwise indicated, all numbers, values and/or expressions referring to quantities of ingredients, reaction conditions, etc., used in the specification and claims are to be understood as modified in all instances by the term "about."

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

The following Supporting Information as referred to above is incorporated herein by reference.

Supporting Information: Anti-cancer Cyclometalated Palladium(II) Complexes with N-Heterocyclic Carbene Ligands Exhibit High Stability and Cytotoxicity *In Vitro* and Suppress Tumor Growth *In Vivo* (Tommy Tsz-Him Fong, Chun-Nam Lok, Clive Yik-Sham Chung, Yi-Man Eva Fung, Pui-Keong Chow, Pui-Ki Wan, and Chi-Ming Che*)

Materials and methods

Analytical grade organic solvents were used in all experiments unless otherwise stated. $\text{Pd}(\text{C}^{\wedge}\text{N}^{\wedge}\text{N})\text{Cl}$ [$\text{HC}^{\wedge}\text{N}^{\wedge}\text{N}$ = 6-phenyl-2,2'-bipyridine],^[1] 1,3-dimethyl-1*H*-imidazol-3-ium bromide, 1,3-diethyl-1*H*-imidazol-3-ium bromide, 1,3-dipropyl-1*H*-imidazol-3-ium bromide, 1,3-dibutyl-1*H*-imidazol-3-ium bromide were synthesized according to the literature procedure.^[2] $\text{Pd}(\text{R}-\text{C}^{\wedge}\text{N}^{\wedge}\text{N}-\text{R}')\text{Cl}$ ($\text{R}-\text{C}^{\wedge}\text{N}^{\wedge}\text{N}-\text{R}'$ = 3-(6'-aryl-2'-pyridinyl)isoquinoline)^[3] and $[\text{Pd}(\text{C}^{\wedge}\text{N}^{\wedge}\text{N})(\text{PPh}_3)](\text{CF}_3\text{SO}_3)$ (**PdPPh₃**)^[1] have been reported previously. UV/vis spectra were recorded on a Perkin-Elmer Lambda 19 UV/vis spectrophotometer. For MTT and protein assays, the absorbance was quantified using Perkin-Elmer Fusion Reader (Packard BioScience Company). Fast Atom Bombardment (FAB) mass spectra were obtained on a Finnigan Mat 95 mass spectrometer. ¹H NMR and ¹³C NMR spectra were obtained on DPX 300, 400 M Bruker FT-NMR spectrometers relative to the signal of tetramethylsilane. Elemental analysis was performed by the Institute of Chemistry at the Chinese Academy of Science, Beijing. Other chemicals unless otherwise stated were purchased from Sigma-Aldrich Co.

Single crystals X-ray diffraction

Single crystals of complexes **Pd1a** and **Pd1c** that were suitable for X-ray diffraction studies were grown by diffusing pentane or Et₂O into a concentrated solution of the complexes in dichloromethane. The X-ray diffraction data were collected on a Bruker X8 Proteum diffractometer. The crystal was kept at 100 K during data collection. The diffraction images were interpreted and the diffraction intensities were integrated by using the program SAINT. Multi-scan SADABS was applied for absorption correction. By using Olex2,^[4] the structure was solved with the ShelXS^[5] structure solution program using direct methods and refined with the XL^[5] refinement package using least squares minimization. The positions of the H atoms were calculated on the basis of the riding mode with thermal parameters equal to 1.2 times that of the associated C atoms and these positions participated in the calculation of the final R indices. In the final stage of least-squares refinement, all non-hydrogen atoms were refined anisotropically. Crystallographic parameters are summarized in Table S1. CCDC 1448613-1448614 contain the supplementary crystallographic data for this paper. The data can be obtained free of charge from The Cambridge Crystallographic Data Center via www.ccdc.cam.ac.uk/data_request/cif

Synthesis and characterization of the palladium(II)-carbene complexes

Complex Pd1a. A mixture of [Pd(C[^]N[^]N)Cl] (80.0 mg, 0.215 mmol), 1,3-dimethyl-1*H*-imidazol-3-ium bromide (25.1 mg, 0.258 mmol) and KO^tBu (29.0 mg, 0.258 mmol) in 30 mL dimethylformamide were heated to reflux for 24 h. Excess ammonium hexafluorophosphate was added and the reaction mixture was further refluxed for 1 h. After cooling to room temperature, DMF was removed by vacuum distillation and the residue was washed with water and hence diethyl ether. Light yellow product was obtained after silica-gel chromatography separation with CH₂Cl₂ and CH₃CN (13:1, v/v) as eluent. Yield: 50 %. ¹H NMR (400 MHz, CD₃CN, 25 °C): 8.00 (d, *J* = 8.0 Hz, 1 H), 7.83–7.92 (m, 3 H), 7.74 (m, 1 H), 7.63 (d, *J* = 8.0 Hz, 1 H), 7.40 (d, *J* = 8.1 Hz, 1 H), 7.27 (m, 1 H), 7.06 (s, 2 H), 6.88 (t, *J* = 7.6 Hz, 1 H), 6.75 (t, *J* = 7.5 Hz, 1 H), 6.07 (d, *J* = 7.5 Hz, 1 H), 3.65 (s, 6 H); ³¹P NMR (161 MHz, CD₃CN): -144.53; ¹⁹F NMR (376 MHz, CD₃CN): -73.86; positive FAB-MS: *m/z* 433 [M – PF₆]⁺; Elemental Analysis: calcd for C₂₁H₁₉F₆N₄PPd: C, 43.58; H, 3.31; N, 9.68; found: C, 43.72; H, 3.32; N, 9.66. See Figure S21.

Complex Pd1b. The procedure is similar to that for **Pd1a** except 1,3-diethyl-1*H*-imidazol-3-ium bromide was used. Yield: 45 %. ¹H NMR (300 MHz, CDCl₃, 25 °C): 8.26 (d, *J* = 8.1 Hz, 1 H), 8.10–8.14 (m, 1 H), 8.06–8.09 (m, 2 H), 7.96 (d, *J* = 8.0 Hz, 1 H), 7.71 (d, *J* = 8.1 Hz, 1 H), 7.59–7.62 (m, 1 H), 7.53 (d, *J* = 7.7 Hz, 1 H), 7.26 (s, 2 H), 7.12 (t, *J* = 7.6 Hz, 1 H), 6.99 (t, *J* = 7.5 Hz, 1 H), 6.29 (d, *J* = 7.5 Hz, 1 H), 4.34–4.46 (m, 4 H, -CH₂-), 1.45 (t, *J* = 7.4 Hz, 6 H, CH₃); ³¹P NMR (400 MHz, CD₃CN): -144.24; ¹⁹F NMR (376 MHz, CD₃CN): -73.25; Positive FAB-MS: *m/z* 461 [M – PF₆]⁺; Elemental Analysis, calcd for C₂₃H₂₃F₆N₄PPd: C, 45.45; H, 3.98; N, 9.22; found: 45.10, H 3.88, N 9.00. See Figure S22.

Complex Pd1c. The procedure is similar to that for **Pd1a** except

1,3-propyl-1*H*-imidazol-3-ium bromide was used. Yield: 48 %. ^1H NMR (400 MHz, CDCl_3 , 25 °C): 8.29 (d, $J = 8.3$ Hz, 1 H), 7.98–8.17 (m, 4 H), 7.73 (d, $J = 7.9$ Hz, 1 H), 7.58–7.62 (m, 1 H), 7.53 (d, $J = 7.6$ Hz, 1 H), 7.27 (s, 2 H), 7.12 (t, $J = 7.4$ Hz, 1 H), 6.99 (t, $J = 7.5$ Hz, 1 H), 6.31 (d, $J = 7.5$ Hz, 1 H), 4.20–4.39 (m, -N-CH₂- on -*n*Pr, 4 H), 1.89 (sextet, $J = 7.3$ Hz, 4 H, -CH₂- on -*n*Pr), 0.87 (t, $J = 7.3$ Hz, 6 H, -CH₃ on -*n*Pr); ^{31}P NMR (161 MHz, CD_3CN): -144.76; ^{19}F NMR (376 MHz, CD_3CN): -73.23; Positive FAB-MS: m/z 489 $[\text{M} - \text{PF}_6]^+$; Elemental Analysis, calcd for $\text{C}_{25}\text{H}_{27}\text{F}_6\text{N}_4\text{PPd} \cdot (\text{H}_2\text{O})_{0.5}$: C, 46.63; H, 4.38; N, 8.70; found: 46.50, H 4.26, N 8.49. See Figure S23.

Complex Pd1d. The procedure is similar to that for **Pd1a** except 1,3-dibutyl-1*H*-imidazol-3-ium bromide was used. Yield: 52 %. ^1H NMR (400 MHz, CDCl_3 , 25 °C): 8.46 (d, $J = 8.0$ Hz, 1 H), 8.02–8.23 (m, 4 H), 7.71 (d, $J = 7.9$ Hz, 1 H), 7.63–7.64 (m, 1 H), 7.53 (d, $J = 7.6$ Hz, 1 H), 7.27 (s, 2 H), 7.14 (t, $J = 7.4$ Hz, 1 H), 6.99 (t, $J = 7.5$ Hz, 1 H), 6.30 (d, $J = 7.0$ Hz, 1 H), 4.25–4.42 (m, 4 H, -N-CH₂- on -*n*Bu), 1.84 (sextet, -CH₂- on -*n*Bu, $J = 7.1$ Hz, 4 H), 1.28 (sextet, 4H, $J = 7.6$ Hz, -CH₂- on -*n*Bu), 0.80 (t, $J = 7.3$ Hz, 6 H, -CH₃ on -*n*Bu); ^{31}P NMR (161 MHz, CD_3CN): -144.14; ^{19}F NMR (376 MHz, CD_3CN): -73.66; Positive FAB-MS: m/z 517 $[\text{M} - \text{PF}_6]^+$; Elemental Analysis, calcd for $\text{C}_{27}\text{H}_{31}\text{F}_6\text{N}_4\text{PPd}$: C, 48.92; H, 4.71; N, 8.45; found: 48.76, H 4.86, N 8.38. See Figure S24.

Complex Pd2a. The procedure is similar to that for **Pd1a** except π -extended $\text{Pd}(\text{R}-\text{C}^{\wedge}\text{N}^{\wedge}\text{N}-\text{R}')\text{Cl}$ was used. Yield: 45 %. ^1H NMR (300 MHz, CDCl_3 , 25 °C): 8.93 (s, 1 H), 8.52 (s, 1 H), 8.41 (d, $J = 7.8$ Hz, 1 H), 8.0–8.06 (m, 2 H), 7.87–7.92 (m, 1 H), 7.74–7.83 (m, 2 H), 7.65 (d, $J = 7.8$ Hz, 2 H), 7.54 (s, 2 H), 7.27 (s, 2 H), 7.18 (t, $J = 7.4$ Hz, 1 H), 7.05 (t, $J = 7.4$ Hz, 1 H), 6.36 (d, $J = 7.1$ Hz, 1 H), 4.04 (s, 6 H), 1.24–1.67 (s, 18 H); positive FAB-MS: m/z 671 $[\text{M} - \text{CF}_3\text{SO}_3]^+$; Elemental Analysis, calcd for $\text{C}_{40}\text{H}_{41}\text{F}_3\text{N}_4\text{O}_3\text{PdS} \cdot 2\text{H}_2\text{O}$: C, 56.04; H, 5.29; N, 6.54; found: 55.95, H 5.07, N 6.78. See Figure S25.

Complex Pdiso. To a yellow suspension of $[\text{Pd}(\text{C}^{\wedge}\text{N}^{\wedge}\text{N})\text{Cl}]$ (0.10 g, 0.22 mmol) in $\text{CH}_3\text{CN}:\text{CHCl}_3$ (1:1, v/v; 20 mL) at room temperature was added excess *tert*-butylisocyanide (0.5 mL, 4.4 mmol). Upon stirring, the color of the mixture changed to pale yellow after 30 min. Addition of excess LiClO_4 in acetonitrile followed by diethyl ether yielded a white precipitate, which was collected and dried. Recrystallization by slow diffusion of diethyl ether into an acetonitrile solution of the crude product afforded white crystals: yield 0.12 g, 91 %. ^1H NMR (400 MHz, $\text{DMSO}-d_6$, 25 °C): 8.66 (s, 1 H), 8.57 (d, $J = 7.8$ Hz, 1 H), 8.29–8.36 (m, 3 H), 8.14 (d, $J = 6.8$ Hz, 1 H), 7.82 (d, $J = 7.6$ Hz, 2 H), 7.21–7.27 (m, 3 H), 1.70 (s, 9 H); positive FAB-MS: m/z 378 $[\text{M}^+ - \text{C}\equiv\text{NtBu} + \text{CH}_3\text{CN}]$; Elemental Analysis, calcd for $\text{C}_{21}\text{H}_{20}\text{ClN}_3\text{O}_4\text{Pd}$: C, 48.48; H, 3.87; N, 8.08; found: C, 48.62; H, 3.69; N, 8.18. See Figure S26.

***trans*-[Pd(NHC)₂(Cl)₂].** It was synthesized according to a modification of a procedure reported in the literature.^[6] 1,3-Dibutyl-1*H*-imidazol-2-ium bromide (0.274 g, 1.26 mmol) and Ag_2O (0.146 g, 0.63 mmol) in dichloromethane (30 mL) were stirred at room temperature for 4 h. The solution was then filtered by celite, and the

filtrate was evaporated under reduced pressure. [1,3-Dibutyl-1*H*-imidazol-2-ylidene]AgCl was crystallized from the acetonitrile solution of the crude product by slow-evaporation technique. Yield = 0.117 g (29 %); ^1H NMR (400 MHz, CDCl_3): 7.23 (s, 2 H), 4.17 (t, $J = 7.1$ Hz, 4 H), 1.84 (pentet, $J = 7.2$ Hz, 4 H), 1.36 (sextet, $J = 7.3$ Hz, 4 H), 0.95 (t, $J = 7.3$ Hz, 6 H). The *trans*-[Pd(NHC) $_2$ (Cl) $_2$] was then synthesized by heating the acetonitrile solution (15 mL) of [1,3-dibutyl-1*H*-imidazol-2-ylidene]AgCl (0.117 g, 0.36 mmol) and Pd(COD)Cl $_2$ (47.4 mg, 0.17 mmol) under reflux for 6 h. The volume of solution mixture was reduced to ~1 mL, and the light yellow crystal of *trans*-[Pd(NHC) $_2$ (Cl) $_2$] was obtained by slow evaporation of the acetonitrile solution. Yield = 30 mg (32.8 %); ^1H NMR (400 MHz, CDCl_3): 6.82 (t, $J = 3.9$ Hz, 4 H), 4.49 (m, 8 H), 2.08 (m, 8 H), 1.47 (m, 8 H), 1.01 (t, $J = 6.3$ Hz, 12 H); $^{13}\text{C}\{^1\text{H}\}$ NMR (150 MHz, CDCl_3): 169.1, 120.5, 50.7, 33.0, 20.1, 13.8; Positive ESI-MS: $m/z = 503.4$ [$\text{M} - \text{Cl}$] $^+$; Elemental Analysis, calcd for $\text{C}_{22}\text{H}_{40}\text{Cl}_2\text{N}_4\text{Pd}$: C, 49.12; H, 7.50; N, 10.42; found: C, 48.72; H, 7.89; N, 10.28. See Figure S27 and S28.

Stability analysis

Solution stabilities of the palladium(II) complexes were investigated by ESI-MS, ^{31}P NMR, and ^1H NMR. The aqueous solution with 10 % DMSO containing the complexes (2 mM) and GSH (20 mM) was analyzed by ESI-MS, ^{31}P NMR, and ^1H NMR to examine the formation of complex-GSH adduct.

The stability of **Pd1a** was also examined in HeLa cells using LC/MS. **Pd1a** (1 μM) was added to the HeLa cells for 2 h, 8 h, 24 h, and 48 h. HeLa cells were washed with PBS twice and 500 μL of miliQ water was added and incubated for 10 min at room temperature. It was then diluted by 20-fold with acetonitrile. Solutions were then centrifuged at 15000 rpm for 15 min at 4°C. The supernatant was kept and dried. Acetonitrile (200 μL) was added and centrifuged again and the supernatant was then transferred to UPLC vials for LC/MS analysis.

Interaction with human serum albumin

The binding with human serum albumin (HSA) was determined by a modified procedure.^[7] In general, a 280 mg amount of HSA was dissolved in 7 mL of minimal essential medium. Pd(C $^{\wedge}$ N $^{\wedge}$ N)Cl and *trans*-[Pd(NHC) $_2$ (Cl) $_2$] was added to the HSA-containing medium with final concentration of 2 μM and the mixture was shaken at 50 rpm. After 2 h, 100 μL of the solution was taken, and treated with 500 μL of cold (-20 °C) acetone, and stored at -20 °C for 30 min to allow precipitation of the protein fraction. Afterwards, the solution was centrifuged at 13000 rpm for 1 min at 4 °C. The supernatant was taken and the protein precipitates were treated with 500 μL of 60 % HNO_3 (*) at 60-70 °C for 2 h and at room temperature for 12 h. The final volume was adjusted to 10 mL for inductively coupled plasma-mass spectrometry (ICP-MS) analysis. The total palladium content was measured by digesting the solutions of palladium and HSA mixture without precipitation. The percentage of bound palladium was determined as the palladium contents in precipitated proteins divided by the total palladium contents.

(*) **Caution:** Concentrated HNO_3 is highly reactive with acetone, causing explosion.

The acetone should be evaporated before adding HNO₃, and the digestion needs to be handled carefully!

Cell culture

All cell lines were obtained from American Type Culture Collection (ATCC). Bronchioalveolar carcinoma (NCI-H1650), breast adenocarcinoma (MDA-MB-231), cervical epithelial carcinoma (HeLa), and normal lung fibroblast (CCD-19Lu) were maintained in Eagle's minimum essential medium. All cell culture media were supplemented with 10 % (v/v) fetal bovine serum, L-glutamine (2 mM) and penicillin/streptomycin (100 U/mL). Cells were incubated in 5 % CO₂ humidified air atmosphere at 37 °C and subcultured when 80 % confluence was reached.

Cytotoxicity assay

The cytotoxic properties of the complexes were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.^[8] Cells were seeded in 96-well plates and incubated overnight prior to be examined. All of the complexes were dissolved in DMSO. The concentration of the complexes was calculated according to the elemental composition of the complexes determined by the elemental analyses. Media in the presence of the tested complexes were added and serially diluted to various concentrations (i.e. from 50 μM to 0.2 μM), and media containing cisplatin were used as the positive control. The maximum concentration of DMSO in media did not exceed 0.1 % (v/v). The cells were incubated for 72 h and followed by the addition of MTT solution. The cells were further incubated for 3 h and solubilization buffer (100 μL, 10 % SDS in 0.01 M HCl) was subsequently added. The absorption intensities at 580 nm in each well were measured by a Perkin-Elmer FusionTM α-FP plate reader. The IC₅₀ values of the complexes (concentrations at which could inhibit cellular growth by 50 % compared to the negative control) were determined from the plots of the cell viability percentage versus the complex concentration. For each set of data, at least three independent experiments have been done.

Cellular uptake measurement

Cellular uptake experiments were conducted according to the literature method with some modifications. The cells were seeded at a density of 1×10^6 cells/dish in 6-cm culture dishes. After 24 h incubation, cells were left untreated or treated with the metal complexes. Cells were harvested after 24 and 48 h, trypsinized and washed four times with cold PBS. Milli-Q water (500 μL) was added and monolayer cells were scraped off from the culture dishes. All samples (300 μL) were digested in 70 % HNO₃ (500 μL) at 70 °C for 2 h, which were then diluted 1:100 in water for ICP-MS analysis.

***In vivo* tumor growth inhibition experiment**

Female BALB/cAnN-nu (nude) mice, 4–7 week old, were purchased from Charles River Laboratories (Wilmington, MA). Mice were maintained under specific

pathogen-free conditions. All animal experiments were conducted under the guidelines approved by the Committee on the Use of Live Animals in Teaching and Research of the University of Hong Kong. To establish tumors, 5×10^6 HeLa cells suspended in PBS (100 μ L) were injected into the right and left back flanks of each mouse respectively by subcutaneous injection. The mice were divided into different groups with a solvent control and drug treated groups after the tumor volume had reached about 30 mm³ (2–3 days after tumor inoculation). Mice were treated with the compounds or solvent control according to the body weight once every 2–3 days by intraperitoneal injection until the mice were sacrificed. Tumor sizes were measured once every 2–3 days using a digital caliper and tumor volumes were calculated by the formula $V = ab^2 \times 0.52$, where a and b were the longest and the shortest diameters of the tumor. The volume of PET diluent injected was kept $\leq 10 \mu$ L per injection in all mice, and mice in all group received the same amount of PET diluent.

Western blotting analysis

HeLa cells (5×10^5) were cultured on 60 mm dish and incubated overnight before experiments. HeLa cells were treated with **Pd1d** for various concentrations (0.5, 2 and 4 μ M). After 2 h incubation, cells were harvested and lysed using the lysis buffer (150 mM NaCl, 100 mM Tris-HCl pH 7.4, 10 % glycerol, 1 % Triton X-100, 10 mM NaF, 5 mM sodium pyrophosphate, 5 mM sodium orthovanadate, 0.1 % SDS) with protease inhibitor cocktail. The cellular protein content was quantified by the Protein Assay (Bio-Rad). Total protein extracts (40 μ g) were loaded onto 12.5 % SDS-polyacrylamide gel, separated by electrophoresis followed by transfer of proteins from the gel to polyvinylidene fluoride (PVDF) membranes. After the transfer of protein, the membrane was then blocked with 3 % BSA in TBST buffer and incubated with corresponding primary antibodies at 4 °C overnight. Primary antibodies of PARP (1:1500), cleaved PARP (1:1500), EGFR (1:1000), pEGFR (1:1000), ERK 1/2 (1:1500), pERK 1/2 (1:1500), GAPDH (1:2000) and Actin (1:2000) were obtained from Cell Signaling Technology. After washing, the membrane was incubated with secondary antibody conjugated with horseradish peroxidase (1:5000) for 90 min. The immunoreactive signals were detected using enhanced chemiluminescence kit (GE healthcare) following the procedures given in the user manual. Equal loading of each lane was confirmed by the intensity of β -actin.

Immunofluorescence staining

Cells (2×10^4) were seeded on glass coverslips and treated with freshly prepared palladium(II) complexes (1 μ M) or cisplatin (10 μ M) following overnight culture at 37 °C, 5 % CO₂. Briefly, for γ H2AX staining, cells were fixed in 4 % paraformaldehyde/PBS (15 min, RT), permeabilized with 0.3 % Triton X-100/PBS (15 min, on ice), blocked in 0.2 % BSA/PBS (1 h, RT), stained for γ H2AX (Cell Signaling, #2577L, 1:100 dilution, at 4 °C, 2 h), and labeled with secondary antibodies (Invitrogen, Alexa Fluor® 568 dye, 1:200 dilution, 2 h, RT). For DNA staining, cells were stained with DAPI (1:400 dilution, at 4 °C, 15 min). Coverslips were then visualized using a fluorescence microscope (ZEISS).

Purified TrxR enzyme assay

Recombinant rat TrxR1 (ICMO Corp, Sweden; 1 nM) was reduced with NADPH (0.2 mM) and then incubated with the metal complexes (1 nM to 1 μ M) for 30 min in a 100 mM potassium phosphate buffer, pH 7.4 and 1 mM EDTA. The enzyme activities (initial rates of increases in O.D._{412 nm}) were measured using 3 mM DTNB.

Fluorescence quenching experiment

Fluorescence quenching experiment was followed with a reported procedure.^[5] In brief, a solution of HSA (3 μ M, in Tris-HCl buffer (0.05 M Tris, 0.1 M NaCl, pH 7.40)) was excited at 280 nm and the emission spectrum was recorded. Then the solution was added with the aliquots of stock solution of the complex (5 mM/10 mM) and the emission spectra were recorded at the same excitation wavelength after equilibration for 1 min per aliquot until saturation point was almost reached. The binding constant was determined by applying the following equation

$$\log[(I_0-I)/I] = \log K + n \log [Q]$$

where I_0 and I are the fluorescence intensity of HSA without and with complex, respectively; $[Q]$ is complex concentration. Plot of $\log[(I_0-I)/I]$ versus $\log [Q]$ gave the y-intercept equaling to $\log K$, and the binding constant can be obtained accordingly.

Absorption-titration experiment

Absorption titration experiment was performed according to a reported procedure.^[4] The absorption spectra of a solution of the complex in PBS (with 5 % DMSO) was recorded and the solution was added with the aliquots of stock solution of ctDNA (2.63 mM) and the absorption spectra were recorded after equilibration for 1 min per aliquot until saturation point was almost reached. The binding constant was determined by applying the Scatchard equation:

$$[DNA]/\Delta\epsilon_{ap} = [DNA]/\Delta\epsilon + 1/(\Delta\epsilon \times K_b),$$

where $\Delta\epsilon_{ap} = |\epsilon_A - \epsilon_F|$ where $\epsilon_A = \text{Abs}/[\text{complex}]$, and $\Delta\epsilon = |\epsilon_B - \epsilon_F|$ where ϵ_B and ϵ_F correspond to the extinction coefficients of the DNA-bound and -unbound complex, respectively. Plot of $[DNA]/\Delta\epsilon_{ap}$ versus $[DNA]$ gave a slop equaling to $1/\Delta\epsilon$ and a y intercept equaling to $1/(\Delta\epsilon \times K_b)$, and K was obtained from the ratio of the slop to the y-intercept.

Measurement of mitochondrial membrane potential

HeLa cells (2×10^4 cells/mL) were seeded in glass-bottomed dishes with supplemented culture medium and incubated for 24 h and then were treated with 2.5 μ M or 5 μ M of the complexes or vehicle control for 2 h in a CO₂ incubator at 37 °C. JC-1 (5 μ M) staining solution was then added to the cells and incubation was continued for 30 min. After washing with serum free culture medium twice, the cells were visualized using a fluorescence microscopy. For positive control, carbonyl cyanide 3-chlorophenylhydrazone (CCCP, 50 μ M) and JC-1 (5 μ M) were co-incubated with HeLa cells for 30 min and examined with fluorescence microscope. The fluorescence intensity for both J-aggregates and monomeric forms of JC-1 were measured with a 96-well plate reader (J-aggregates: excitation/emission = 535/580 nm; JC-1 monomers: excitation/emission = 485/530 nm).

Proteomic analysis

Sample preparation

HeLa cells were treated with Pd1d at 0.5 μ M concentration for 2 and 10 h in a CO₂ incubator at 37 °C. Equal amount of DMSO were added to HeLa cells as a positive control. The cells were then washed with cold PBS twice and the cells were lysed with a urea lysis buffer (20 mM Tris-HCl, 8 M urea, protein phosphatase inhibitor cocktail, pH 8.0). The cells were then scraped in 1.5 mL Eppendorf tubes and spin down at 10,000 rpm for 15 min at 4 °C. The supernatant was kept and protein concentration was then measured. 50 μ g of protein were then precipitated by adding 5 $\times$ volume of ice-cold acetone (storage at -20 °C). The samples were mixed and kept at -20 °C for 30 min followed by centrifugation at 13,000 rpm for 20 min at 4 °C. The solvents were discarded and the pellets were dried by SpeedVac (Thermo Fisher Scientific). The dried samples were re-suspended with 25 μ L of Suspension Buffer (100 mM Tris, 8 M urea, pH 8.5) and DTT was added to make a final concentration of 5 mM to denature the samples at 60 °C. After denaturing for 20 min, iodoacetamide was added to make a final concentration of 25 mM. Then the samples were kept in the dark for 30 min at 25 °C, 100 mM Tris (pH 8.5) was added to dilute the concentration of urea to 1 M, and 0.5 μ g trypsin was added to each reaction mixture, and followed by digestion for overnight at 37 °C. The reaction mixtures were then acidified by addition of 10 μ L formic acid to stop the proteolysis. After centrifugation at 14,000 rpm for 15 min, the supernatants were transferred to new tubes (to be frozen at -80 °C for long term storage). The resulting peptides were desalted and enriched by StageTips. For each sample, three biological replicates were prepared. The samples were re-dissolved with H₂O (containing 0.1 % formic acid, v/v) for subsequent HPLC-MS/MS analysis.

HPLC-MS/MS analysis

MS analysis was performed with a LTQ Orbitrap Velos Orbitrap MS (Thermo) connected online with a HPLC. The analytical column was a self-packed PicoTip® column (360 μ m outer diameter, 75 μ m inner diameter, 15 μ m tip, New Objective) packed with 10 cm length of C18 material (ODS-A C18 5- μ m beads, YMC) with a high-pressure injection pump (Next Advance). The mobile phases of HPLC are A (0.1 % formic acid in HPLC grade H₂O, volume percentage) and B (0.1 % formic acid in HPLC grade acetonitrile, volume percentage). Three micrograms of sample was loaded onto the analytical column by the auto-sampler and rinsed with 2 % B for 6 min and subsequently eluted with a linear gradient B from 2 % to 40 % for 120 min. For the MS analysis, LTQ-Orbitrap Velos mass spectrometer (Thermo Fisher Scientific) was operated in a data-dependent mode cycling through a high-resolution (6000 at 400 m/z) full scan MS1 (300–2000 m/z) in Orbitrap followed by CID MS2 scans in LTQ on the 20 most abundant ions from the immediate preceding full scan. The selected ions were isolated with a 2-Da mass window and put into an exclusion list for 60 seconds after they were first selected for CID.

Proteins identification and quantification

The raw data were directly used for protein identification and quantification using MaxQuant (Version 1.5.0.25). The data were searched against IPI human database (Version 3.87), in which trypsin specificity was used with up to two missed cleavages allowed. Methionine oxidation was set as a variable modification, and iodoacetamide

derivative of cysteine was set as a fixed modification. Default settings were used for mass tolerance for MS1 and MS2. The false discovery rate (FDR) was determined by searching against a reverse database and kept FDR at 1 %.

Signaling pathway analysis

Lists of quantified proteins (shown as their Protein IDs) were uploaded to the ExPlain™ tool (version 3.1, BIOBASE) and converted to genes (shown as their Gene Symbols) for further signaling pathway analysis. Details of procedure for pathway analysis have been described previously.^[9,10]

Intracellular reactive oxygen species (ROS) measurement

The production of intracellular ROS was measured in the HeLa cell line using the oxidation-sensitive fluorescent dye dichlorodihydrofluorescein acetate (DCF-DA). HeLa cells were exposed to 0.5 μ M of complexes for 1–2 h prior to staining with DCF. An increase in green fluorescence intensity was used to quantify the generation of intracellular ROS. After adding DCF at a final concentration of 5 μ M to the culture medium, the cells were incubated at 37 °C for an additional 1 h, harvested, washed with PBS, and the fluorescent signal was measured immediately with a plate reader (excitation/emission = 440/530 nm).

Caspase activities

The activities of caspase-3 and caspase-9 were determined by caspase substrates Ac-DEVD-AMC and Ac-LHED-AMC, respectively. Briefly, cells were collected by scrapping and pelleted by centrifugation. Cell lysis buffer was then added (0.5 ml/10 cm plate) to cell pellet and homogenized. The cell lysates were clarified by centrifugation. 200 μ l of fluorescent substrate solution (50 μ M) and 25 μ l lysate solution were mixed in a black microplate. The plate was incubated at 37°C. RFU was read on a fluorescence plate reader with excitation at 380 nm and emission at 460 nm. The protein concentrations of the lysates were measured by Biorad protein assays.

Cell cycle arrest

HeLa cells (2×10^5) were treated with complexes (0.5 μ M) for 12, 24 and 48 h. Cells were collected by trypsinization and fixed in 70 % ethanol at -20 °C for 30 min. After fixation, cells were washed twice with PBS and treated with RNase A (0.1 mg/mL) for 1 h at 37 °C. Cells were then stained with propidium iodide (10 μ g/mL). The fluorescence signals were manipulated with FACSCalibur flow cytometry (BD Biosciences). 10,000 ungated events were acquired for each sample. Populations of cells at different phases of cell cycle were analyzed with CellQuest Pro software.

Tube-formation assay

By using the In Vitro Angiogenesis Kit, 10 $\times$ diluent buffer and ECMatrix™ solution were mixed in a ratio of 1:9. The mixture (50 μ L) was transferred to each well of a 96-well plate. The matrix solution was incubated at 37 °C to allow polymerization. After 1 h, MS1 cells (50,000) premixed with different concentrations of **Pd1a/Pd1d**

(0.25 or 0.5 μ M) in 100 μ L of DMEM medium were added to the top of the polymerized matrix. After incubation at 37 °C for 8 h, tube formation was examined under an inverted light microscope at 100 $\times$ magnification and was quantified using the Sigma Scan Pro. software. The percentage of inhibition was calculated based on the distance measured relative to the untreated control.

Wound-healing assay

MS1 cells were cultured on 60-mm culture dishes at a density of 2×10^6 cells/dish. After 24 h, a single wound was created in the middle of the cell monolayer by gently removing the attached cells with a sterile plastic pipette tip. The cells were washed twice with PBS and solutions of different concentrations of **Pd1a/Pd1d** (0.25 or 0.5 μ M) in 5 mL of DMEM were added. After incubation at 37 °C for 8 h, migration of the cells into the wound was observed under an inverted microscope.

Quantification of Pd1d in mouse tumors and plasma using LC-MS/MS

Nude mice bearing xenografts of NCI-H460 cancer cells were treated with **Pd1d** at 2 mg/kg through intraperitoneal injection of 8 repeated doses for 17 days. The mice were subjected to cardiac puncture. Blood (about 600–800 μ L) collected was added with Na₂EDTA (20 mg/mL) in a ratio of 10:1 to prevent coagulation, centrifuged (3500 rpm for 15 min at 4 °C) and plasma were transferred to a centrifuge tube. The mouse tumors were harvested and dissected into pieces and weighed. Each of weighed tissues was homogenized in 1xPBS (1:3, w/v) by tissue homogenizer. Both plasma and homogenized tissue samples were stored at -80 °C until they were analyzed.

Chromatographic and mass spectrometric conditions

The analysis was performed on a Waters ACQUITYTM UPLC system and a Waters Q-TOF PremierTM mass spectrometer with an electrospray ionization source (Micromass MS Technologies). The data acquisition and analysis were performed using Waters MassLynx version 4.1. Separation was achieved by a Waters ACQUITYTM BEH C₁₈ column (100 $\times$ 2.1 mm i.d., 1.7 μ M) connected to a Waters ACQUITYTM BEH C₁₈ guard column (5 $\times$ 2.1 mm i.d., 1.7 μ M) with gradient elution system consisted of 0.1 % formic acid in water (A) and 0.1 % formic acid in acetonitrile (B). Separation was achieved by using: 5–55 % B from 0 to 3 min; 55–90 % B from 3 to 15 min; 90–100 % B from 15 to 25 min; 100 % B from 25 to 30 min and returned to initial conditions and equilibrated for 3 minutes. The flow rate was 0.4 mL/min and the injection volume was 2 μ L.

The mass spectrometer was operated in positive mode and the conditions were optimized as follows: source temperature, 120 °C; desolvation temperature, 500 °C; nebulization gas flow rate, 800 L/h; cone gas flow rate, 20 L/h; capillary voltage, 3 kV; sampling cone voltage, 25 V. The data were collected into two separate data channels with the instrument spending 0.5 s on data acquisition for each channel and a 0.1 s inter-channel delay. The TOF scan range was from 100 to 1000 Da. The optimized fragmentation transition for quantification of **Pd1d** was m/z 517.2 $\rightarrow$ 337.0 with a collision energy of 37 eV.

Standard and sample preparation

Stock solution (2 μM) of each analyte was prepared using methanol. They were subjected to serial dilution to give concentrations of **Pd1d** at: 0.005, 0.01, 0.05, and 0.1 μM . A simple and rapid protein precipitation method was used for sample preparation. A volume of 100 μL from each concentration of each analyte was transferred to a centrifuge tube and evaporated to dryness by a concentrator. A blank plasma/tumor homogenate (100 μL) was added and followed by acetonitrile (300 μL) and the mixture was vortexed for 10 s and centrifuged (15000 rpm) for 15 min. The supernatant was collected and evaporated to dryness by a concentrator. Methanol (100 μL) was added and then vortexed again for 10 s and centrifuged (15000 rpm) for 15 min. The supernatant (2 μL) was subject to LC-MS analysis. Analyte-treated plasma and tumor were prepared in a similar manner.

Supporting Tables

Table S1. Crystal data of complex **Pd1a**.

Complex	Pd1a
Empirical formula	$\text{C}_{21}\text{H}_{19}\text{F}_6\text{N}_4\text{PPd}$
Formula weight	578.28
Temperature/K	100
Crystal system	Triclinic
Space group	P-1
a/Å	11.865(2)
b/Å	13.505(3)
c/Å	14.862(3)
$\alpha/^\circ$	84.748(4)
$\beta/^\circ$	69.465(4)
$\gamma/^\circ$	73.759(4)
Volume/Å ³	2141.1(7)
Z	4
$\rho_{\text{calc}}/\text{g cm}^{-3}$	1.794
μ/mm^{-1}	8.338
F(000)	1151.0
Crystal size/mm ³	$0.4 \times 0.3 \times 0.2$
Radiation	CuKα ($\lambda = 1.54178$)
2 θ range for data collection/ $^\circ$	6.36 to 132.14
Index ranges	$-13 \leq h \leq 14$, $-15 \leq k \leq 15$, $-17 \leq l \leq 17$
Reflections collected	25385
Independent reflections	7257 [$R_{\text{int}} = 0.0588$, $R_{\text{sigma}} = 0.0503$]
Data/restraints/parameters	7257/0/599
Goodness-of-fit on F^2	1.044
Final R indexes [$I \geq 2\sigma(I)$]	$R_1 = 0.0441$, $wR_2 = 0.1209$
Final R indexes [all data]	$R_1 = 0.0446$, $wR_2 = 0.1215$
Largest diff. peak/hole / e Å ⁻³	1.42/-0.96

Table S2. Crystal Data of complex **Pd1c**.

Complex	Pd1c
Empirical formula	C ₂₅ H ₂₇ F ₆ N ₄ PPd
Formula weight	634.87
Temperature/K	100.0
Crystal system	tetragonal
Space group	I-4
a/Å	19.703(2)
b/Å	19.703(2)
c/Å	14.3173(16)
$\alpha/^\circ$	90
$\beta/^\circ$	90
$\gamma/^\circ$	90
Volume/Å ³	5558.2(13)
Z	8
$\rho_{\text{calc}}/\text{g}/\text{cm}^3$	1.517
μ/mm^{-1}	6.481
F(000)	2560.0
Crystal size/mm ³	0.2 × 0.2 × 0.2
Radiation	CuKα ($\lambda = 1.54178$)
2 θ range for data collection/ $^\circ$	6.344 to 133.708
Index ranges	-19 $\leq h \leq$ 23, -21 $\leq k \leq$ 23, -17 $\leq l \leq$ 12
Reflections collected	27804
Independent reflections	4840 [$R_{\text{int}} = 0.0658$, $R_{\text{sigma}} = 0.0459$]
Data/restraints/parameters	4840/54/358
Goodness-of-fit on F^2	1.085
Final R indexes [$I \geq 2\sigma(I)$]	$R_1 = 0.0387$, $wR_2 = 0.1107$
Final R indexes [all data]	$R_1 = 0.0387$, $wR_2 = 0.1108$
Largest diff. peak/hole / e Å ⁻³	1.32/-0.37
Flack parameter	-0.010(6)

Table S3. Bond distances of **Pd1a**.

Atom	Atom	Distance [Å]	Atom	Atom	Distance [Å]
Pd1	N1	1.988(3)	N7	C41	1.448(5)
Pd1	N2	2.154(3)	N7	C38	1.361(5)
Pd1	C17	1.995(4)	N7	C39	1.383(5)
Pd1	C1	1.982(4)	N5	C28	1.348(5)
N1	C7	1.359(5)	N5	C32	1.346(5)
N1	C11	1.341(5)	N8	C40	1.380(5)
N2	C12	1.358(5)	N8	C38	1.354(5)
N2	C16	1.346(5)	N8	C42	1.466(5)
N4	C17	1.355(5)	C22	C27	1.424(6)
N4	C21	1.461(5)	C22	C23	1.389(6)
N4	C19	1.378(5)	C33	C32	1.487(6)
N3	C17	1.351(5)	C33	C34	1.374(6)
N3	C18	1.384(5)	C27	C28	1.479(6)
N3	C20	1.454(6)	C27	C26	1.391(6)
C2	C1	1.404(6)	C28	C29	1.394(6)
C2	C3	1.386(6)	C31	C32	1.385(6)
C13	C12	1.384(6)	C31	C30	1.392(6)
C13	C14	1.378(6)	C37	C36	1.384(6)
C1	C6	1.424(6)	C36	C35	1.382(7)
C12	C11	1.488(6)	C40	C39	1.337(7)
C14	C15	1.381(6)	C26	C25	1.383(6)
C7	C8	1.389(6)	C29	C30	1.381(6)
C7	C6	1.465(6)	C35	C34	1.383(6)
C4	C5	1.388(6)	C23	C24	1.396(6)
C4	C3	1.394(6)	C25	C24	1.396(7)
C9	C10	1.388(6)	P1	F5	1.586(3)
C9	C8	1.387(6)	P1	F3	1.591(3)
C18	C19	1.336(6)	P1	F1	1.595(3)
C10	C11	1.381(6)	P1	F6	1.599(3)
C5	C6	1.399(6)	P1	F4	1.574(3)
C16	C15	1.392(6)	P1	F2	1.590(3)
Pd2	N6	2.129(3)	P2	F12	1.590(3)
Pd2	N5	1.986(3)	P2	F8	1.570(3)
Pd2	C22	2.015(4)	P2	F9	1.617(4)
Pd2	C38	1.996(4)	P2	F11	1.593(3)
N6	C33	1.366(5)	P2	F10	1.572(4)
N6	C37	1.337(5)	P2	F7	1.562(4)

Table S4. Bond distances of **Pd1c**.

Atom	Atom	Distance [Å]	Atom	Atom	Distance [Å]
Pd1	C16	2.009(6)	C13	C14	1.377(11)
Pd1	N1	1.990(5)	C7	C6	1.389(8)
Pd1	N2	2.142(5)	C3	C4	1.388(9)
Pd1	C17	1.995(6)	C14	C15	1.401(10)
P1	F3	1.607(6)	C4	C5	1.392(9)
P1	F4	1.602(6)	C5	C6	1.481(8)
P1	F5	1.611(6)	F1A	P1A	1.56(3)
P1	F6	1.586(7)	F2A	P1A	1.56(2)
P1	F2	1.592(7)	N3	C17	1.347(8)
P1	F1	1.620(7)	N3	C18	1.373(10)
C16	C11	1.422(9)	N3	C20	1.469(9)
C16	C15	1.380(9)	N4	C19	1.372(10)
N1	C10	1.342(8)	N4	C17	1.356(8)
N1	C6	1.337(8)	N4	C23	1.461(8)
N2	C5	1.365(8)	C19	C18	1.324(12)
N2	C1	1.340(9)	C22	C21	1.514(10)
C11	C12	1.400(9)	C25	C24	1.518(10)
C11	C10	1.482(8)	C23	C24	1.508(10)
C12	C13	1.388(10)	C21	C20	1.515(10)
C2	C3	1.386(10)	F6A	P1A	1.55(2)
C2	C1	1.393(10)	F5A	P1A	1.56(2)
C10	C9	1.390(9)	F4A	P1A	1.56(2)
C8	C7	1.384(9)	F3A	P1A	1.56(2)
C8	C9	1.396(9)			

Table S5. Bond angles of **Pd1a**.

Atom	Atom	Atom	Angle [°]	Atom	Atom	Atom	Angle [°]
N1	Pd1	N2	77.86(13)	C40	N8	C42	124.6(3)
N1	Pd1	C17	174.32(14)	C38	N8	C40	111.0(3)
C17	Pd1	N2	107.01(14)	C38	N8	C42	124.3(3)
C1	Pd1	N1	82.02(15)	C27	C22	Pd2	111.6(3)
C1	Pd1	N2	159.86(14)	C23	C22	Pd2	131.2(3)
C1	Pd1	C17	93.04(15)	C23	C22	C27	117.2(4)
C7	N1	Pd1	117.1(3)	N6	C33	C32	115.4(3)
C11	N1	Pd1	120.1(3)	N6	C33	C34	121.7(4)
C11	N1	C7	122.5(3)	C34	C33	C32	122.9(4)
C12	N2	Pd1	113.0(3)	C22	C27	C28	116.3(4)
C16	N2	Pd1	128.7(3)	C26	C27	C22	121.0(4)
C16	N2	C12	118.3(4)	C26	C27	C28	122.8(4)
C17	N4	C21	124.1(3)	N5	C28	C27	112.4(3)
C17	N4	C19	111.3(3)	N5	C28	C29	119.2(4)
C19	N4	C21	124.5(3)	C29	C28	C27	128.4(4)
C17	N3	C18	111.2(3)	C32	C31	C30	118.1(4)
C17	N3	C20	124.2(3)	N5	C32	C33	113.4(4)
C18	N3	C20	124.5(3)	N5	C32	C31	120.3(4)
N4	C17	Pd1	126.1(3)	C31	C32	C33	126.3(4)
N3	C17	Pd1	129.6(3)	N6	C37	C36	122.4(4)
N3	C17	N4	104.0(3)	C35	C36	C37	118.8(4)
C3	C2	C1	122.3(4)	C39	C40	N8	107.1(4)
C14	C13	C12	119.6(4)	C25	C26	C27	120.6(4)
C2	C1	Pd1	131.2(3)	N7	C38	Pd2	128.1(3)
C2	C1	C6	116.2(4)	N8	C38	Pd2	127.5(3)
C6	C1	Pd1	112.6(3)	N8	C38	N7	104.2(3)
N2	C12	C13	121.7(4)	C30	C29	C28	119.0(4)
N2	C12	C11	115.2(3)	C36	C35	C34	119.3(4)
C13	C12	C11	123.1(4)	C40	C39	N7	106.9(4)
C13	C14	C15	119.4(4)	C22	C23	C24	121.7(4)
N1	C7	C8	119.1(4)	C26	C25	C24	119.3(4)
N1	C7	C6	112.2(3)	C23	C24	C25	120.2(4)
C8	C7	C6	128.6(4)	C29	C30	C31	120.9(4)
C5	C4	C3	119.1(4)	C33	C34	C35	119.3(4)
C8	C9	C10	121.1(4)	F5	P1	F3	89.99(17)
C19	C18	N3	106.7(3)	F5	P1	F1	89.81(16)
C11	C10	C9	118.3(4)	F5	P1	F6	179.1(2)
C4	C5	C6	120.5(4)	F5	P1	F2	91.5(2)
N1	C11	C12	113.7(3)	F3	P1	F1	178.0(2)
N1	C11	C10	120.3(4)	F3	P1	F6	90.11(17)
C10	C11	C12	126.0(4)	F1	P1	F6	90.12(16)

C9	C8	C7	118.6(4)	F4	P1	F5	89.46(19)
C1	C6	C7	116.0(4)	F4	P1	F3	90.3(2)
C5	C6	C1	121.4(4)	F4	P1	F1	91.7(2)
C5	C6	C7	122.5(4)	F4	P1	F6	89.7(2)
N2	C16	C15	122.5(4)	F4	P1	F2	178.7(2)
C2	C3	C4	120.6(4)	F2	P1	F3	90.6(2)
C14	C15	C16	118.6(4)	F2	P1	F1	87.4(2)
C18	C19	N4	106.8(3)	F2	P1	F6	89.4(2)
N5	Pd2	N6	78.61(13)	F12	P2	F9	88.23(19)
N5	Pd2	C22	81.94(15)	F12	P2	F11	178.8(3)
N5	Pd2	C38	177.13(14)	F8	P2	F12	91.04(16)
C22	Pd2	N6	160.55(15)	F8	P2	F9	86.2(2)
C38	Pd2	N6	103.67(14)	F8	P2	F11	89.13(19)
C38	Pd2	C22	95.78(16)	F8	P2	F10	175.0(3)
C33	N6	Pd2	112.8(3)	F11	P2	F9	90.6(3)
C37	N6	Pd2	128.5(3)	F10	P2	F12	89.90(17)
C37	N6	C33	118.5(4)	F10	P2	F9	88.9(3)
C38	N7	C41	124.5(3)	F10	P2	F11	89.8(2)
C38	N7	C39	110.8(3)	F7	P2	F12	91.2(2)
C39	N7	C41	124.7(3)	F7	P2	F8	90.9(3)
C28	N5	Pd2	117.8(3)	F7	P2	F9	177.0(3)
C32	N5	Pd2	119.7(3)	F7	P2	F11	90.0(3)
C32	N5	C28	122.5(3)	F7	P2	F10	94.0(3)

Table S6. Bond angles of **Pd1c**.

Atom	Atom	Atom	Angle [°]	Atom	Atom	Atom	Angle [°]
C16	Pd1	N2	160.7(2)	C13	C14	C15	120.8(6)
N1	Pd1	C16	82.2(2)	C16	C15	C14	120.8(6)
N1	Pd1	N2	78.6(2)	C3	C4	C5	119.5(6)
N1	Pd1	C17	175.6(2)	N2	C5	C4	121.1(6)
C17	Pd1	C16	93.9(2)	N2	C5	C6	116.0(5)
C17	Pd1	N2	105.2(2)	C4	C5	C6	122.9(5)
F3	P1	F5	178.0(5)	N1	C6	C7	120.1(5)
F3	P1	F1	89.4(4)	N1	C6	C5	113.8(5)
F4	P1	F3	88.9(4)	C7	C6	C5	126.1(5)
F4	P1	F5	89.1(4)	C10	C9	C8	118.1(5)
F4	P1	F1	89.9(4)	C17	N3	C18	110.6(6)
F5	P1	F1	90.5(4)	C17	N3	C20	125.4(5)
F6	P1	F3	88.9(5)	C18	N3	C20	124.0(6)
F6	P1	F4	177.8(5)	C19	N4	C23	125.4(6)
F6	P1	F5	93.1(5)	C17	N4	C19	109.9(6)
F6	P1	F2	89.7(4)	C17	N4	C23	124.5(6)
F6	P1	F1	90.2(4)	C18	C19	N4	107.7(7)
F2	P1	F3	90.9(4)	N3	C17	Pd1	129.3(5)
F2	P1	F4	90.2(4)	N3	C17	N4	104.8(5)
F2	P1	F5	89.2(4)	N4	C17	Pd1	125.3(4)
F2	P1	F1	179.7(5)	N4	C23	C24	112.8(6)
C11	C16	Pd1	111.3(4)	C22	C21	C20	111.8(6)
C15	C16	Pd1	130.7(5)	C19	C18	N3	106.9(7)
C15	C16	C11	118.0(6)	N3	C20	C21	112.9(5)
C10	N1	Pd1	117.6(4)	C23	C24	C25	111.8(6)
C6	N1	Pd1	119.5(4)	N2	C1	C2	121.9(6)
C6	N1	C10	122.8(5)	F1A	P1A	F2A	179.3(15)
C5	N2	Pd1	112.0(4)	F1A	P1A	F5A	88.4(15)
C1	N2	Pd1	128.7(5)	F6A	P1A	F1A	89.2(15)
C1	N2	C5	119.3(6)	F6A	P1A	F2A	91.1(15)
C16	C11	C10	116.6(5)	F6A	P1A	F5A	92(2)
C12	C11	C16	121.0(6)	F6A	P1A	F4A	177(2)
C12	C11	C10	122.4(5)	F6A	P1A	F3A	90(2)
C13	C12	C11	119.4(6)	F5A	P1A	F2A	91.0(15)
C3	C2	C1	119.2(6)	F4A	P1A	F1A	89.0(15)
N1	C10	C11	112.2(5)	F4A	P1A	F2A	90.6(15)
N1	C10	C9	119.8(5)	F4A	P1A	F5A	85.4(19)
C9	C10	C11	128.0(5)	F4A	P1A	F3A	92(2)
C7	C8	C9	120.8(6)	F3A	P1A	F1A	88.2(15)
C14	C13	C12	120.1(6)	F3A	P1A	F2A	92.4(15)
C8	C7	C6	118.3(5)	F3A	P1A	F5A	176(2)
C2	C3	C4	119.0(6)				

Table S7. UV-visible absorption data of the palladium(II) complexes.

Complex	$\lambda_{\max}/\text{nm}$ ($\epsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$)
Pd1a	251 (sh, 23100), 270 (sh, 18100), 312 (9300), 332 (sh, 6500), 383 (sh, 1600)
Pd1b	251 (sh, 25600), 270 (sh, 19800), 312 (10200), 332 (sh, 7000), 383 (sh, 1650)
Pd1c	251 (sh, 26700), 270 (sh, 21300), 312 (10700), 332 (sh, 7300), 383 (sh, 1600)
Pd1d	251 (sh, 25100), 270 (sh, 19700), 312 (10300), 332 (sh, 7400), 383 (sh, 1700)
Pd2a	254 (sh, 35400), 292 (sh, 32200), 347 (10800), 365 (sh, 9200), 400 (sh, 3350)
Pdiso	233 (sh, 66000), 266 (sh, 16800), 320 (8650), 386 (2800)

Table S8. Inhibition of purified TrxR by palladium(II) **Pd1a–Pd1d**, **Pdiso**, **PdPPh₃**, *trans*-[Pd(NHC)₂Cl₂], [Pd(C[^]N[^]N)Cl] and auranofin.

Complex	IC ₅₀ (nM)
Pd1a	350±20.2
Pd1b	382±21.1
Pd1c	351±25.8
Pd1d	321±29.8
Pdiso	41.0±3.12
PdPPh₃	21.2±1.43
<i>trans</i> -[Pd(NHC) ₂ Cl ₂]	418±28.1
[Pd(C [^] N [^] N)Cl]	310±25.1
auranofin	14±0.53

Table S9. The percentages of cells in each phase of the cell cycle after **Pd1a** and **Pd1d** (0.5 μM) treatment at different time points.

Complex	Treatment time [h]	Sub-G1 [%]	G0/G1 [%]	S [%]	G2/M [%]
Control	12	1.8±0.1	65.7±5.8	11.2±1.1	21.0±1.7
Pd1d	12	4.2±0.3	58.9±5.3	14.7±1.1	21.6±1.8
Control	24	1.7±0.2	59.9±4.9	17.0±1.5	21.4±1.8
Pd1a	24	2.6±0.2	58.0±5.6	17.5±1.5	22.1±2.1
Pd1d	24	22.7±2.1	38.5±3.2	14.2±1.2	23.7±1.9
Control	48	8.0±0.7	68.4±6.1	8.2±0.7	15.3±1.2
Pd1a	48	46.0±3.8	29.3±1.2	4.4±0.3	19.5±2.0

References

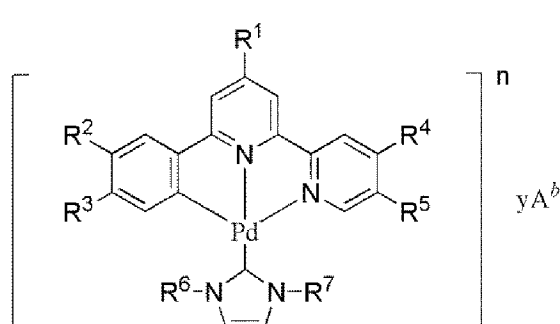
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Claims

What is claimed is:

1. A pharmaceutical composition for treatment of cancer comprising a cyclometalated N-heterocyclic carbene complex having the formula I:



or a pharmaceutically acceptable salt thereof, wherein
 R^1 , R^2 , R^3 , R^4 , and R^5 are each independently alkyl or $-H$;
 R^6 and R^7 are independently an alkyl chain;
 A is a OSO_2CF_3 , Cl , PF_6 or a pharmaceutically acceptable anion;
 n is +1;
 b is -1; and
 y is 1.

2. A pharmaceutical composition of I of claim 1, wherein,
 R^1 , R^2 , R^3 , R^4 , and R^5 are each $-H$;
 R^6 and R^7 is a C_2 alkyl chain;
 A is a OSO_2CF_3 , Cl , PF_6 or a pharmaceutically acceptable anion;
 n is +1;
 b is -1; and
 y is 1.

3. A pharmaceutical composition of I of claim 1, wherein,
 R^1 , R^2 , R^3 , R^4 , and R^5 are each $-H$;
 R^6 and R^7 is a C_3 alkyl chain;
 A is a OSO_2CF_3 , Cl , PF_6 or a pharmaceutically acceptable anion;
 n is +1;
 b is -1; and
 y is 1.

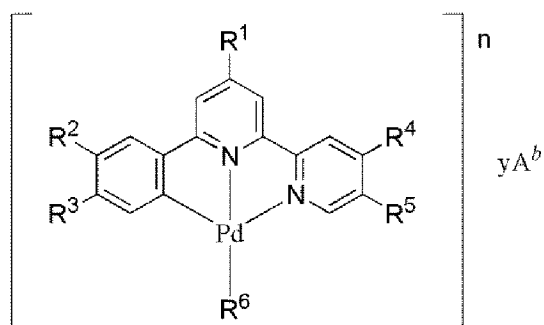
4. A pharmaceutical composition of I of claim 1, wherein,
 R^1 , R^2 , R^3 , R^4 , and R^5 are each $-H$;
 R^6 and R^7 is a C_4 alkyl chain;
 A is a OSO_2CF_3 , Cl , PF_6 or a pharmaceutically acceptable anion;

n is +1;
b is -1; and
y is 1.

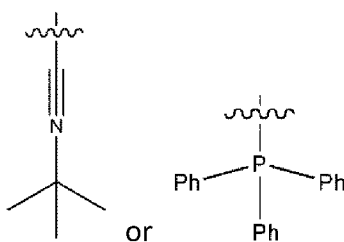
5. A pharmaceutical composition of I of claim 1, wherein,
 R^1 , R^2 , R^3 , R^4 , and R^5 are each $-H$;
 R^6 and R^7 is a combination of C_1 to C_4 alkyl chain;
A is a OSO_2CF_3 , Cl, PF_6 or a pharmaceutically acceptable anion;
n is +1;
b is -1; and
y is 1.

6. A pharmaceutical composition for treatment of cancer comprising a cyclometalated complex having the formula II:

II

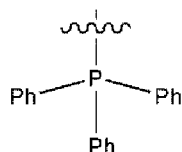


R^1 , R^2 , R^3 , R^4 , and R^5 are each independently alkyl or $-H$;
 R^6 is



A is a OSO_2CF_3 , Cl, ClO_4 or a pharmaceutically acceptable anion;
n is +1;
b is -1; and
y is 1.

7. A pharmaceutical composition of II of claim 5, wherein,
 R^1 , R^2 , R^3 , R^4 , and R^5 are each $-H$;
 R^6 is



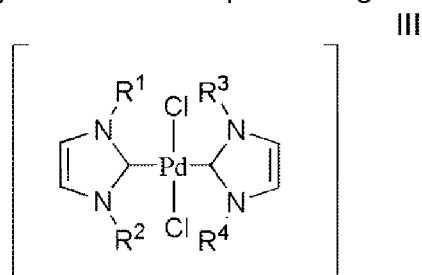
A is a OSO_2CF_3 , Cl or a pharmaceutically acceptable anion;

n is +1;

b is -1; and

y is 1.

8. A pharmaceutical composition for treatment of cancer comprising a cyclometalated N-heterocyclic carbene complex having the formula III:



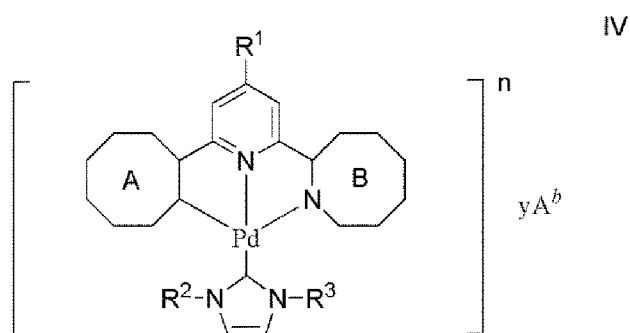
or a pharmaceutically acceptable salt thereof, wherein

R^1 , R^2 , R^3 , and R^4 are each independently an alkyl chain.

9. A pharmaceutical composition of III of claim 7, wherein

R^1 , R^2 , R^3 , and R^4 are each C_2 alkyl chain, C_3 alkyl chain or C_4 alkyl chain.

10. A pharmaceutical composition for treatment of cancer comprising a cyclometalated N-heterocyclic carbene complex having the formula IV:



or a pharmaceutically acceptable salt thereof, wherein

R^1 is substituent containing any aromatic ring/substituted aromatic ring not limited to benzene, naphthalene, thiophene, benzothiophene, anthracene, pyrene, furan, pyrimidine, pyrrole, pyridine, fluorene, carbozole, carborane; or any alkoxy chain not limited to methoxy, ethoxy; or substituent containing any saturated hydrocarbon chain not limit to alkane, ethane, propane, butane;

R^2 and R^3 are independently a C_1 - C_4 alkyl chain;

A is a OSO_2CF_3 , Cl, PF_6 or a pharmaceutically accepted anion;

n is +1;

b is -1;

y is 1;

Ring A is any aromatic ring/substituted aromatic ring providing an anionic carbon donor to the palladium(II) ion; it is not limited to benzene, naphthalene, thiophene, benzothiophene, anthracene, pyrene, furan, pyrimidine, pyrrole, pyridine, fluorene, carbozole, carborane; and

Ring B is any aromatic ring/substituted aromatic ring with a nitrogen coordinating to palladium(II) ion; it is not limited to pyrimidine, pyridine, isoquinoline, 1-isoquinoline, 2-quinoline.

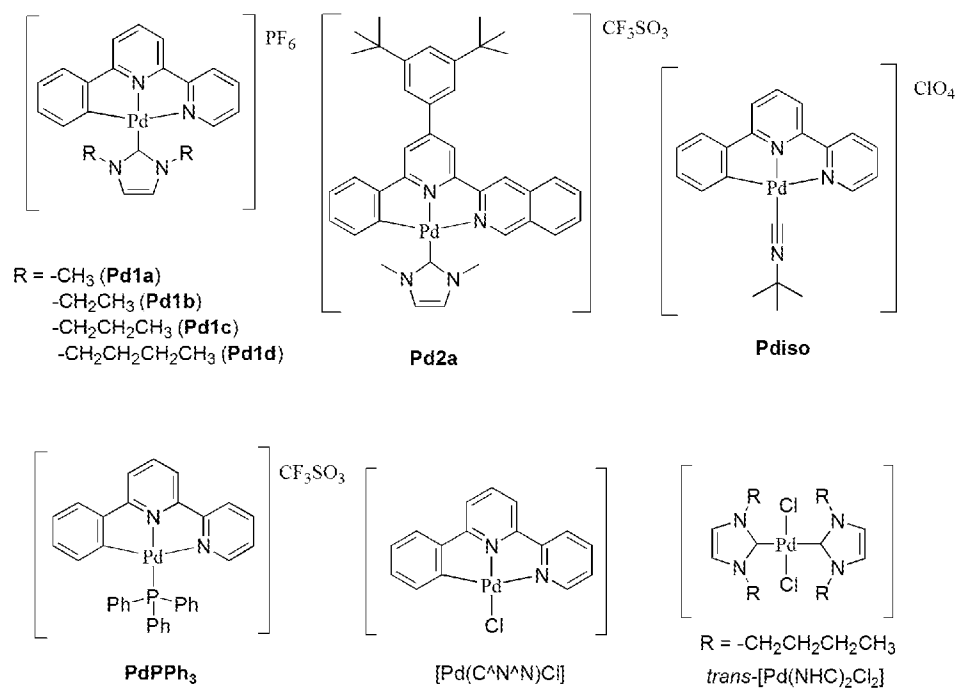


Figure 1

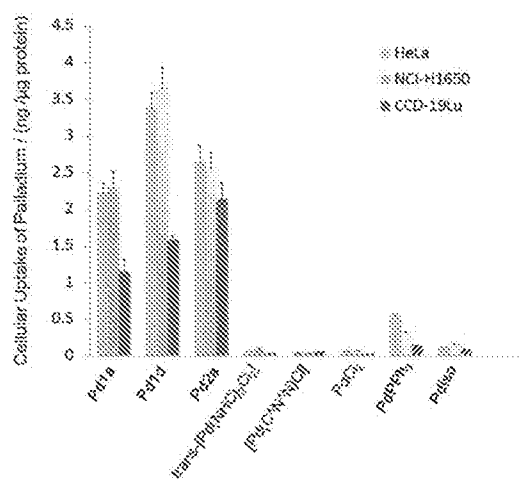


Figure 2

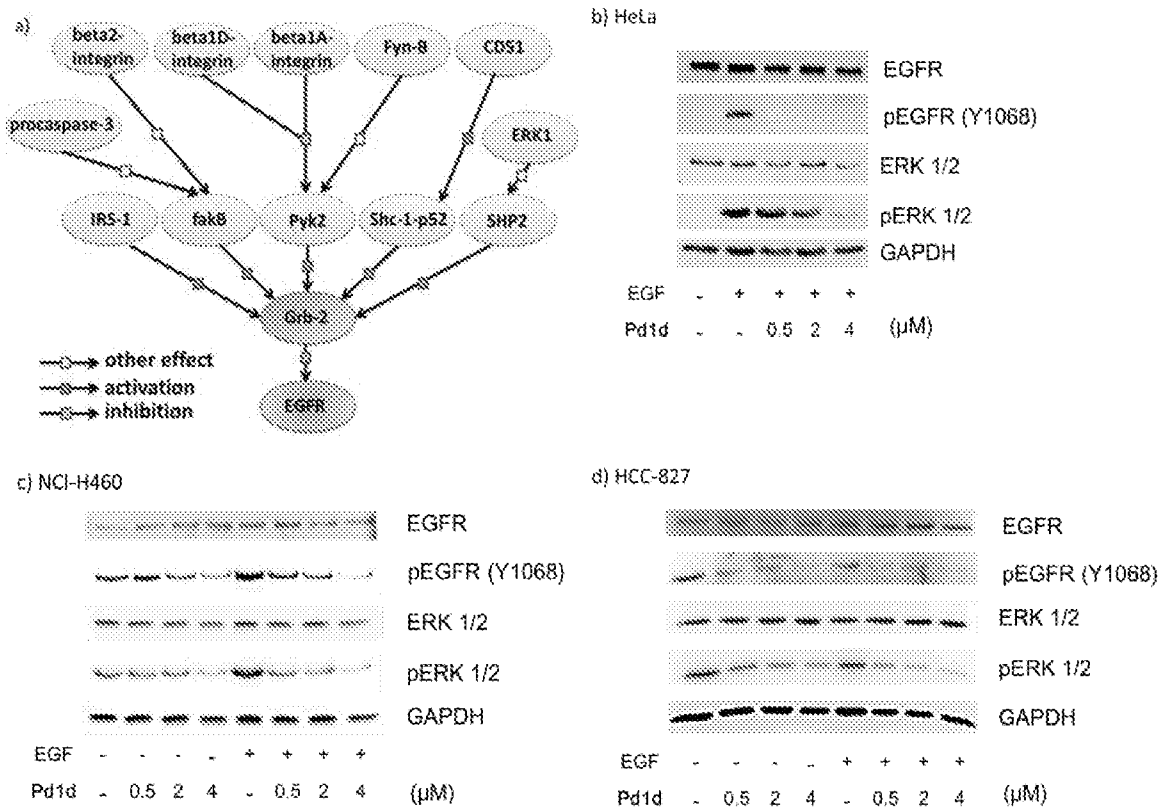


Figure 3

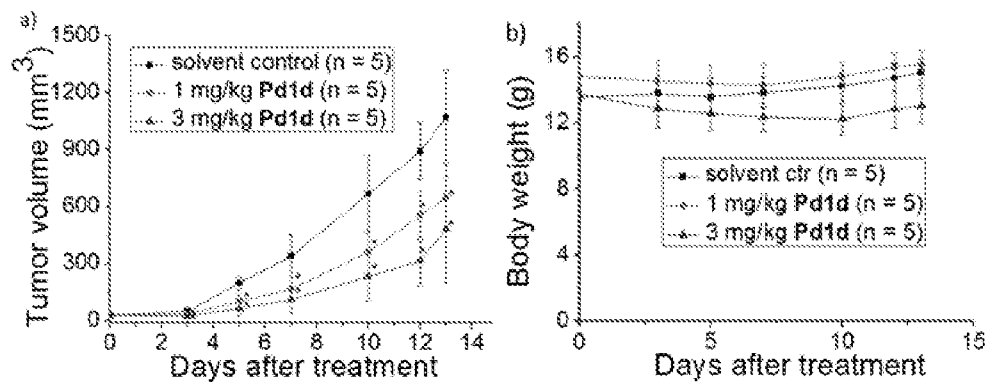


Figure 4

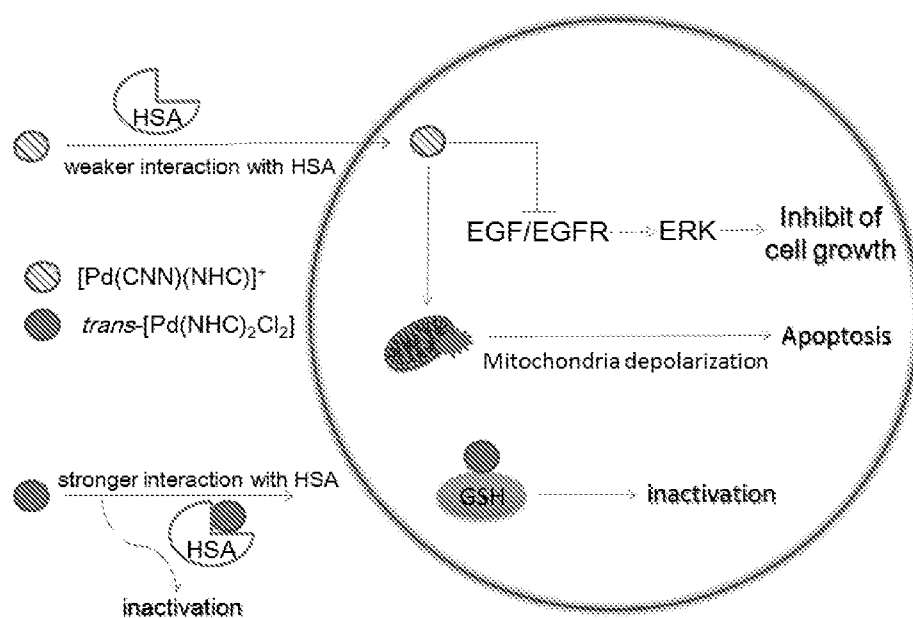
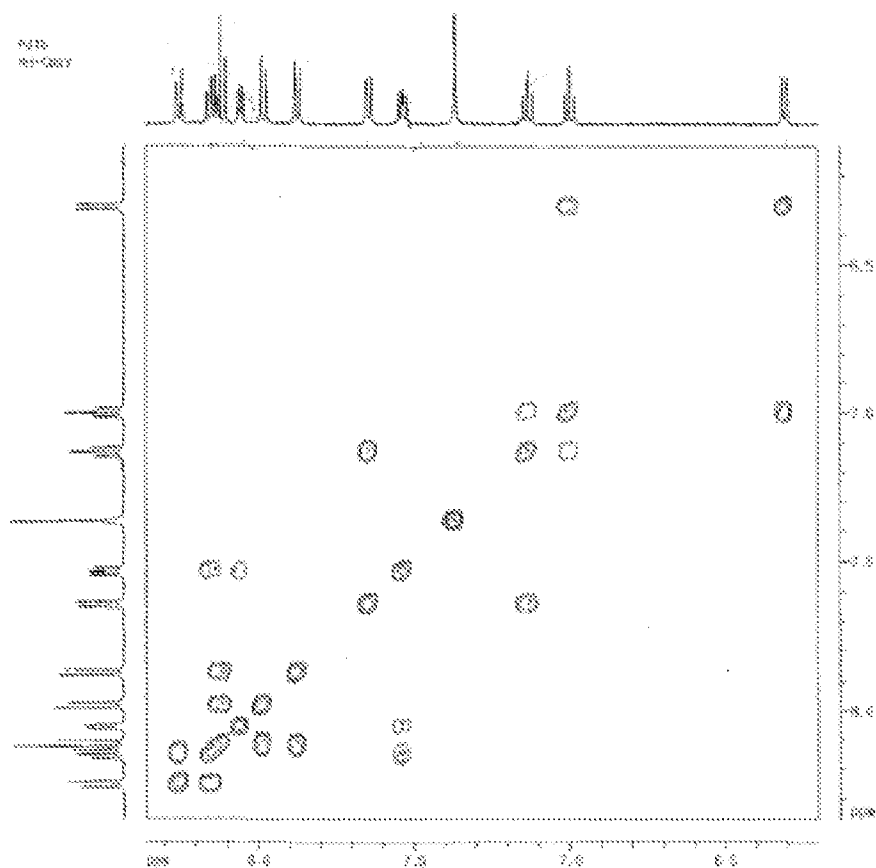


Figure 5

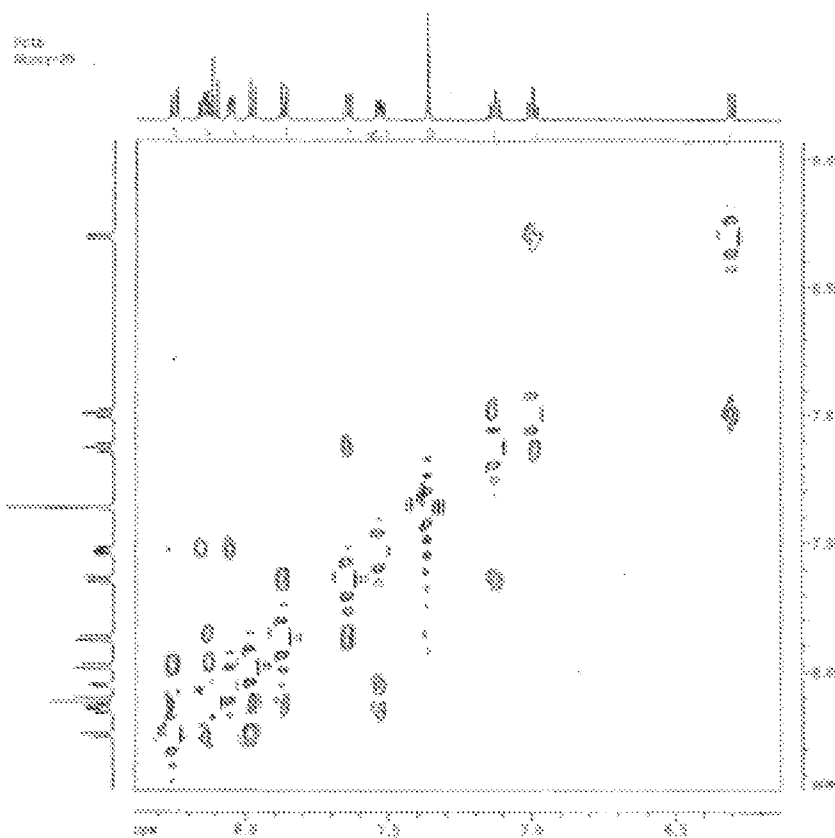
Supporting Figures

Figure S1.

a)



b)



c)

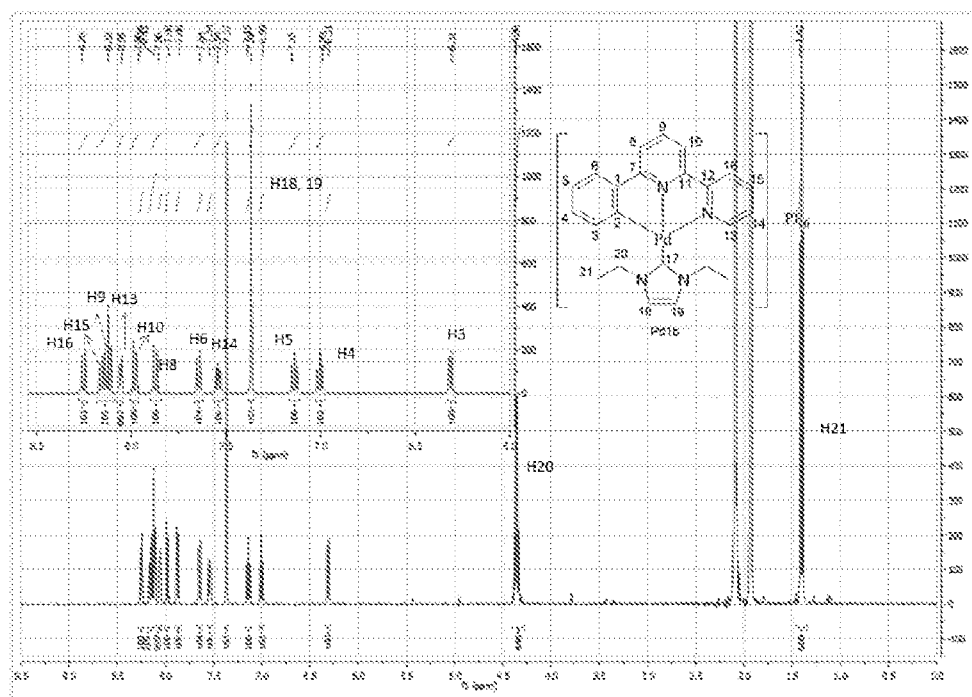


Figure S2

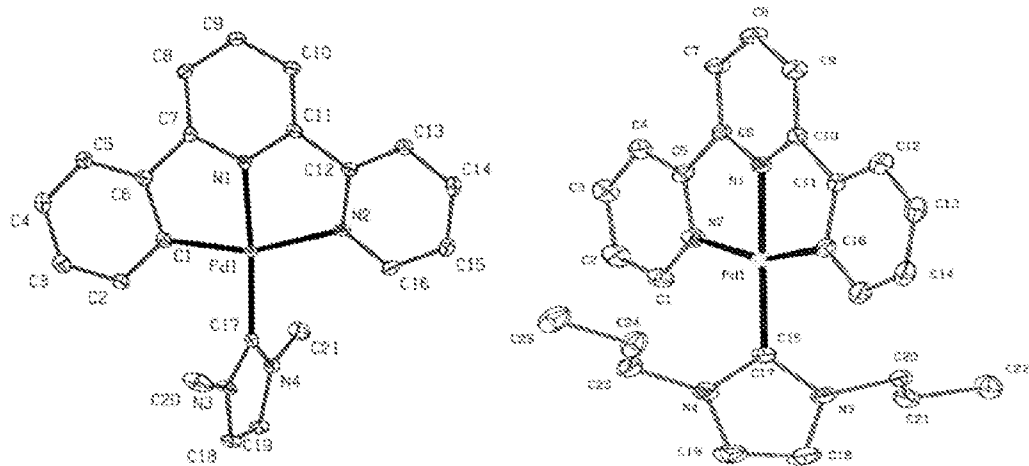


Figure S3

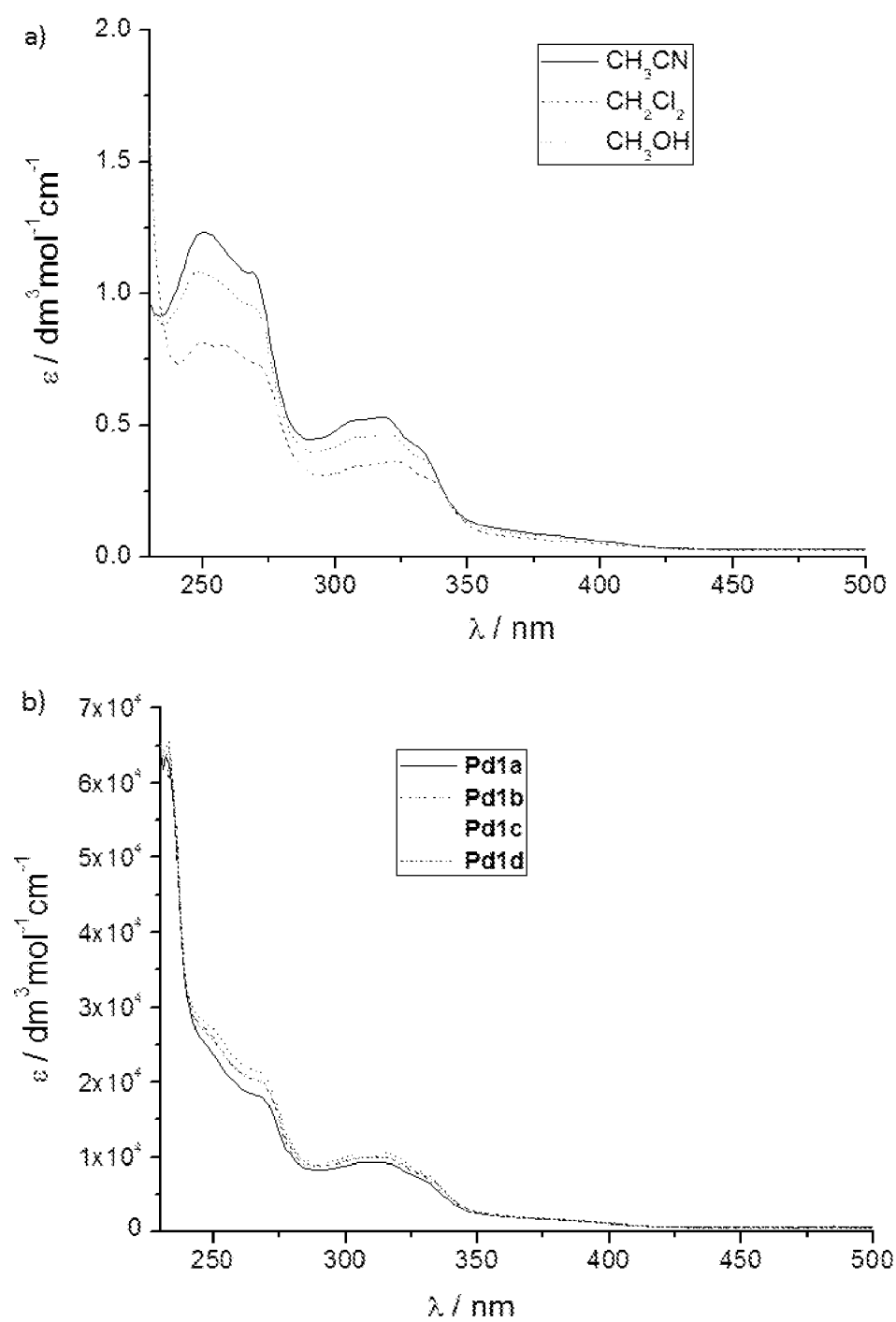


Figure S4

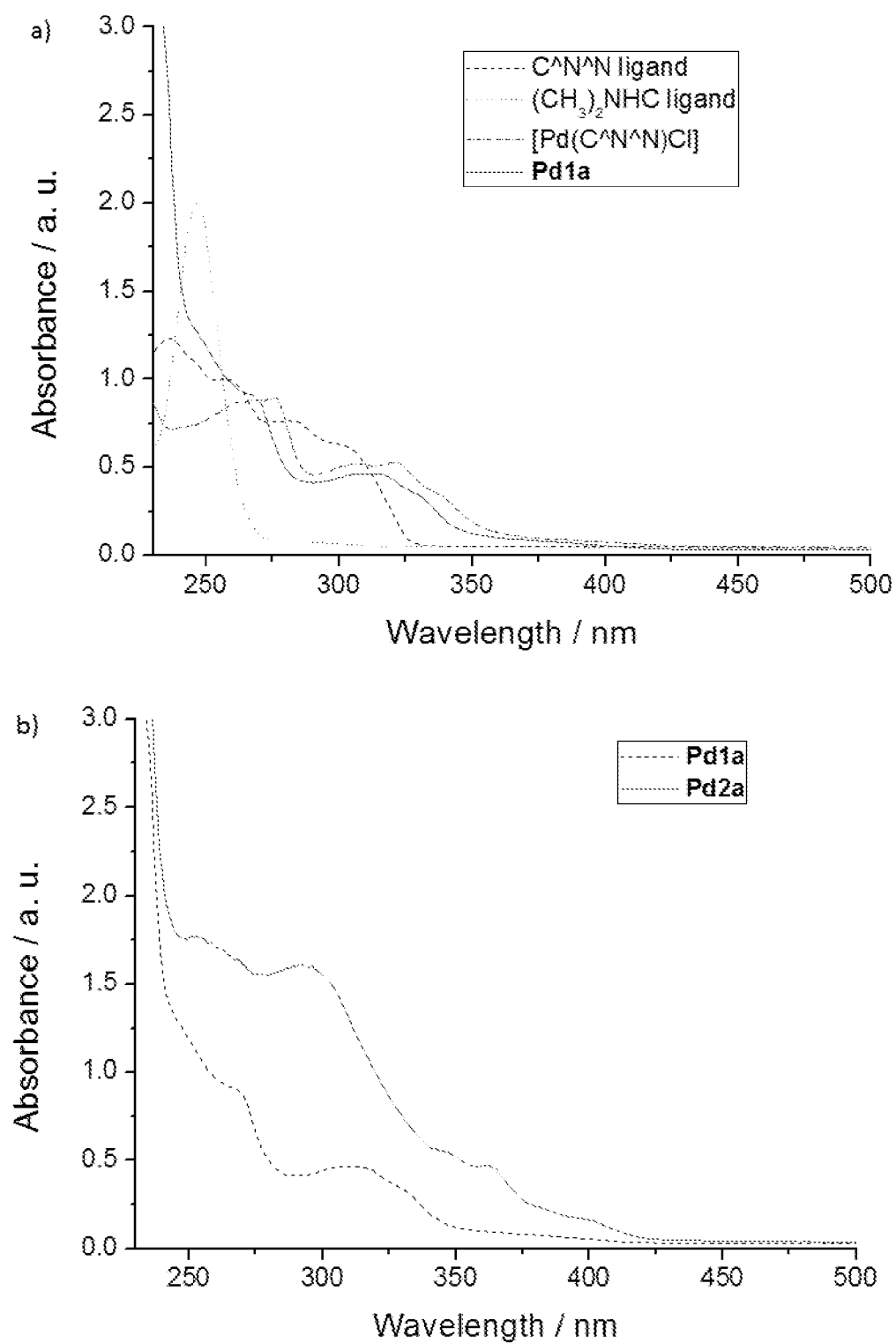
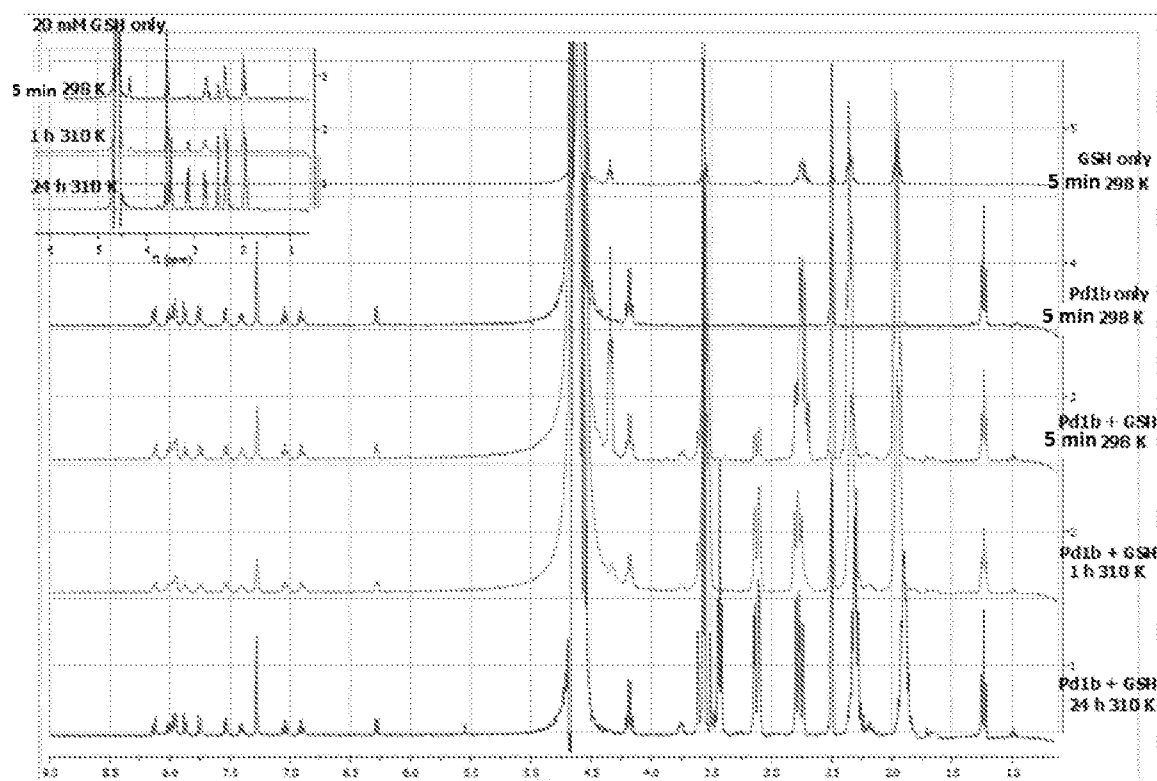


Figure S5

a)



b)

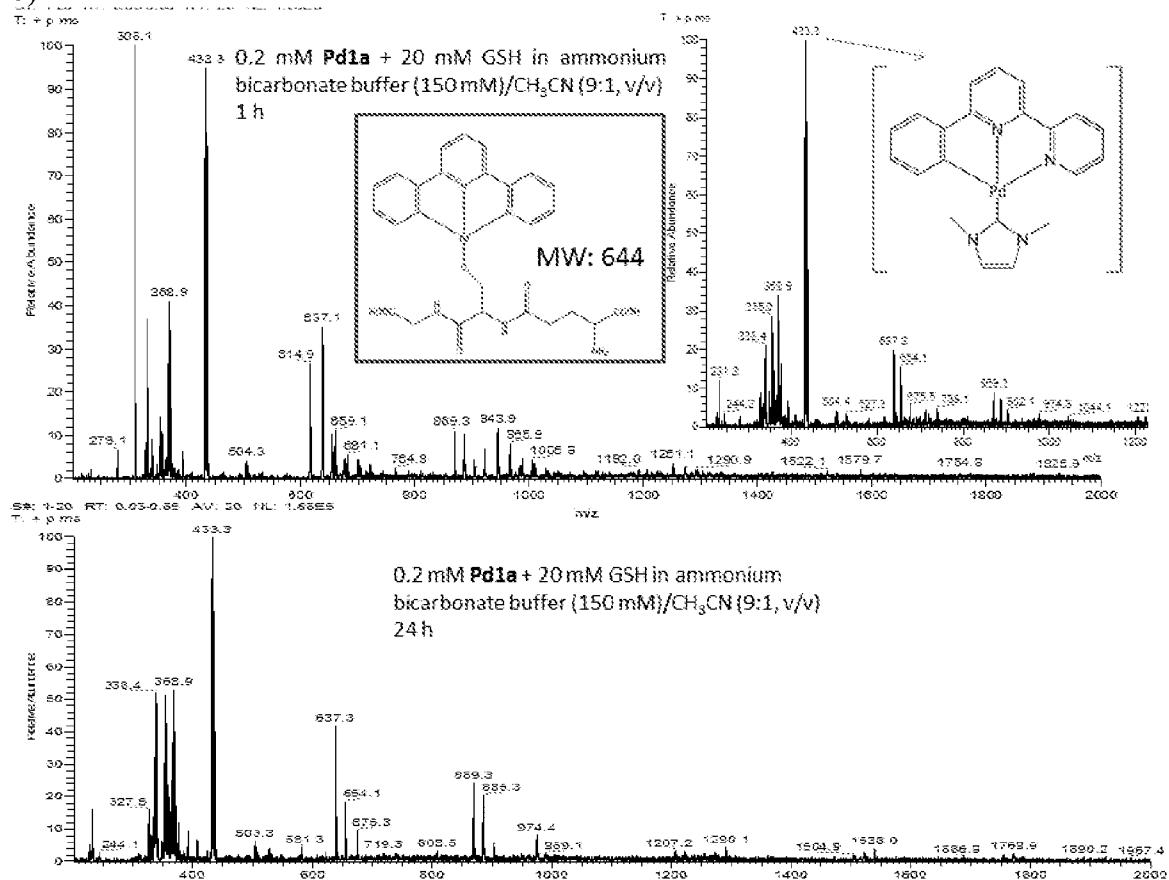


Figure S6

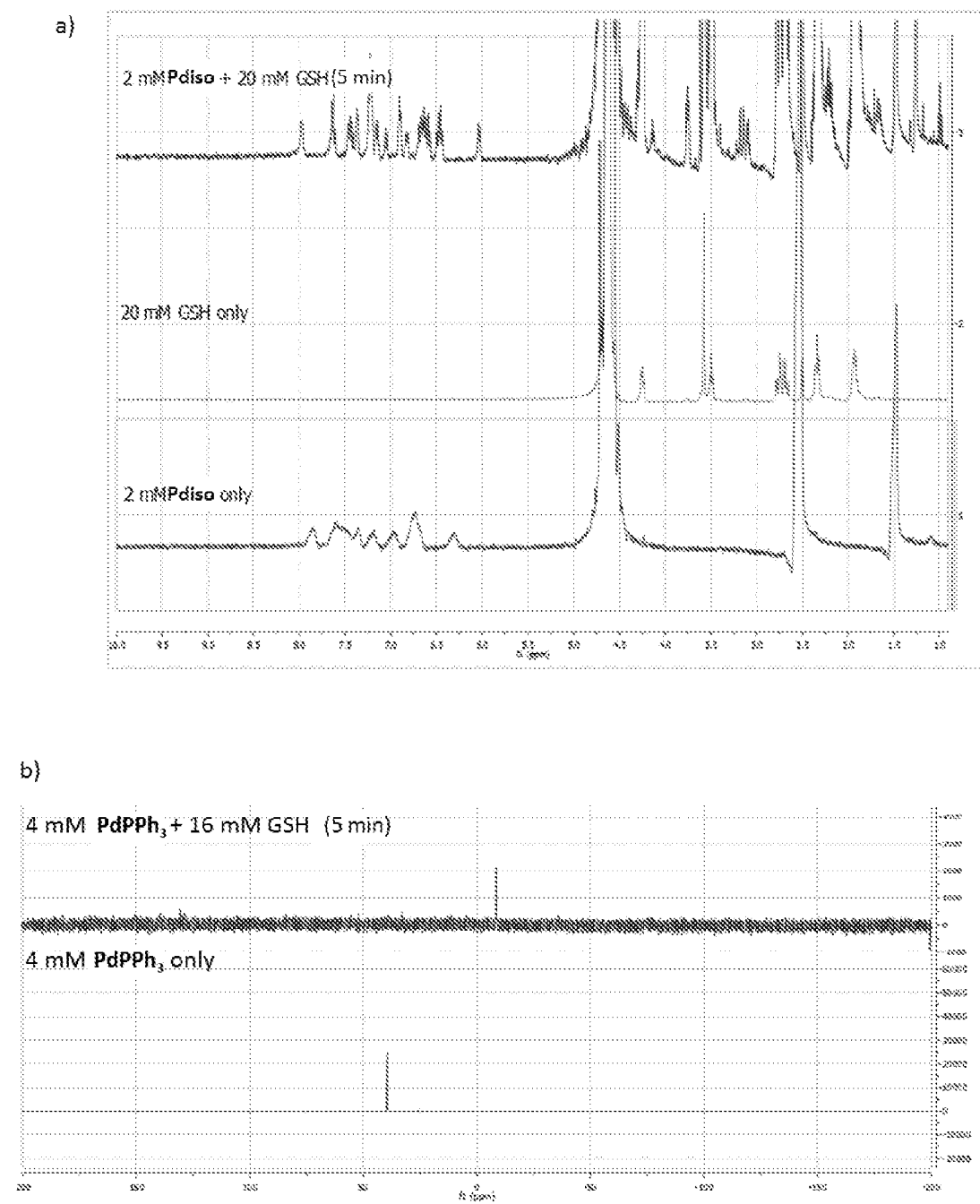
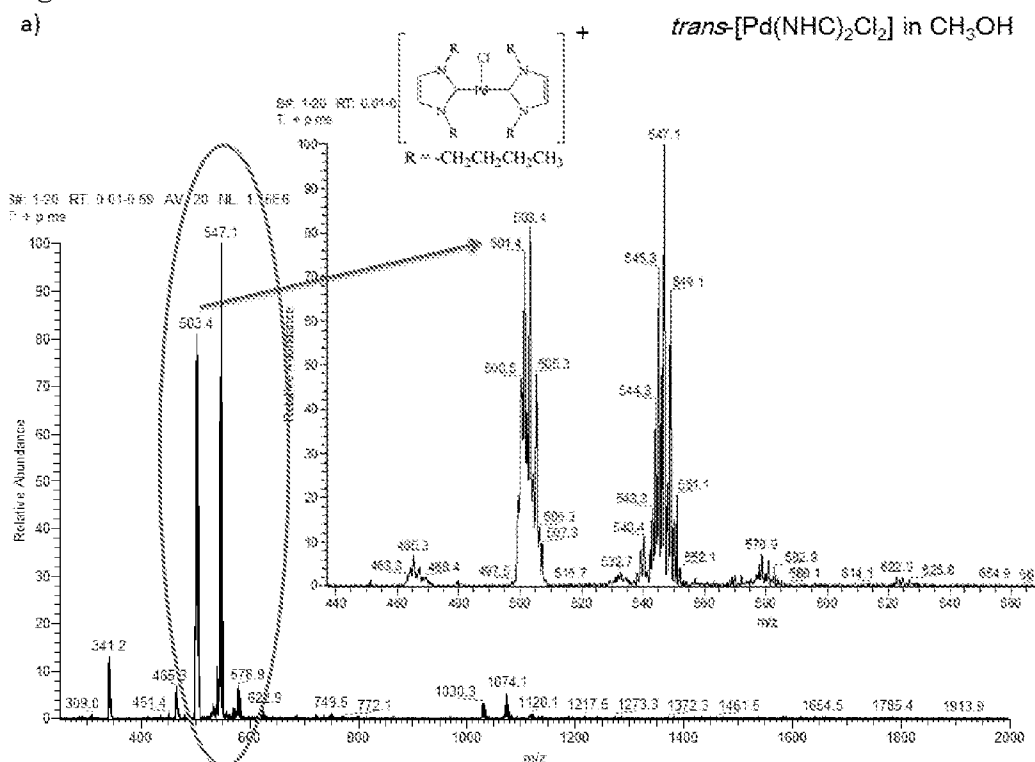


Figure S7



b) 0.2 mM $\text{trans-[Pd(NHC)}_2\text{Cl}_2\text{]} + 20$ mM GSH
in $\text{CH}_3\text{OH/ammonium bicarbonate buffer (50 mM)}$ (1:9, v/v)

1. Both signals at m/z 503
and 547 vanished

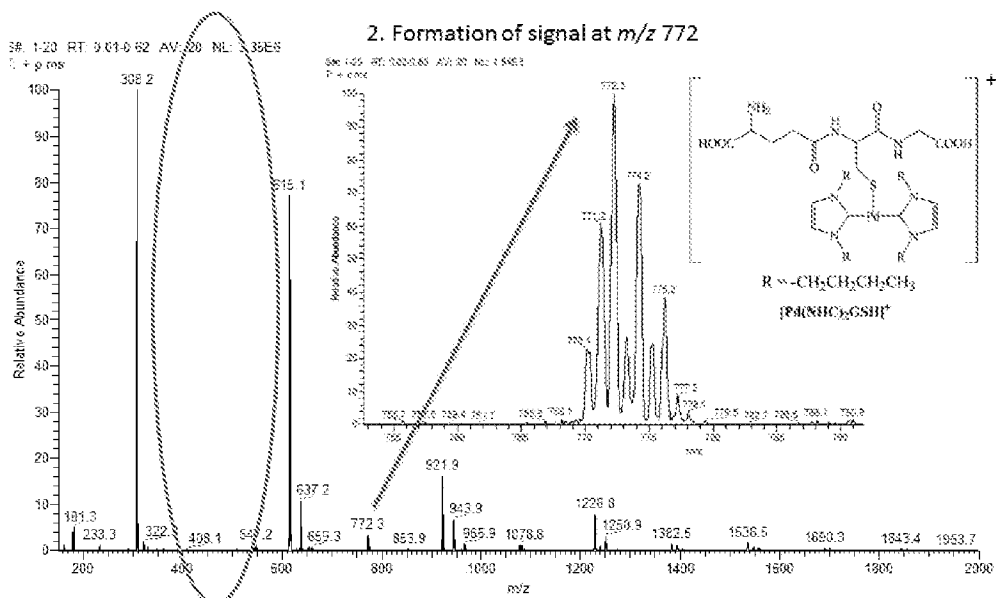
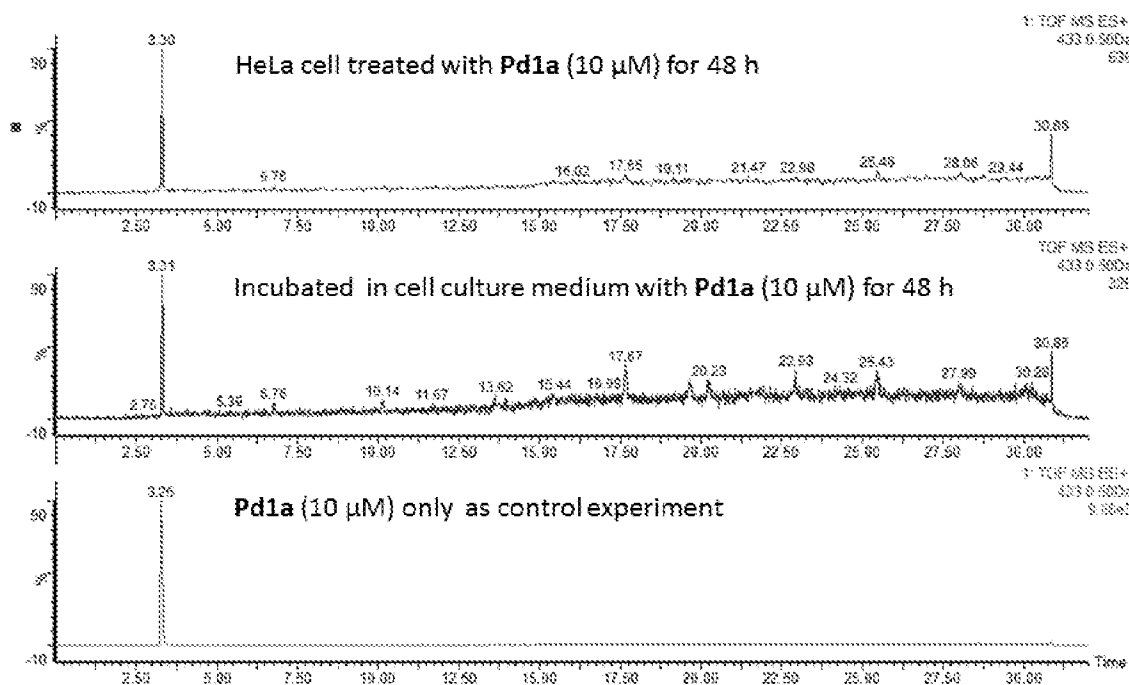


Figure S8

a)



b)

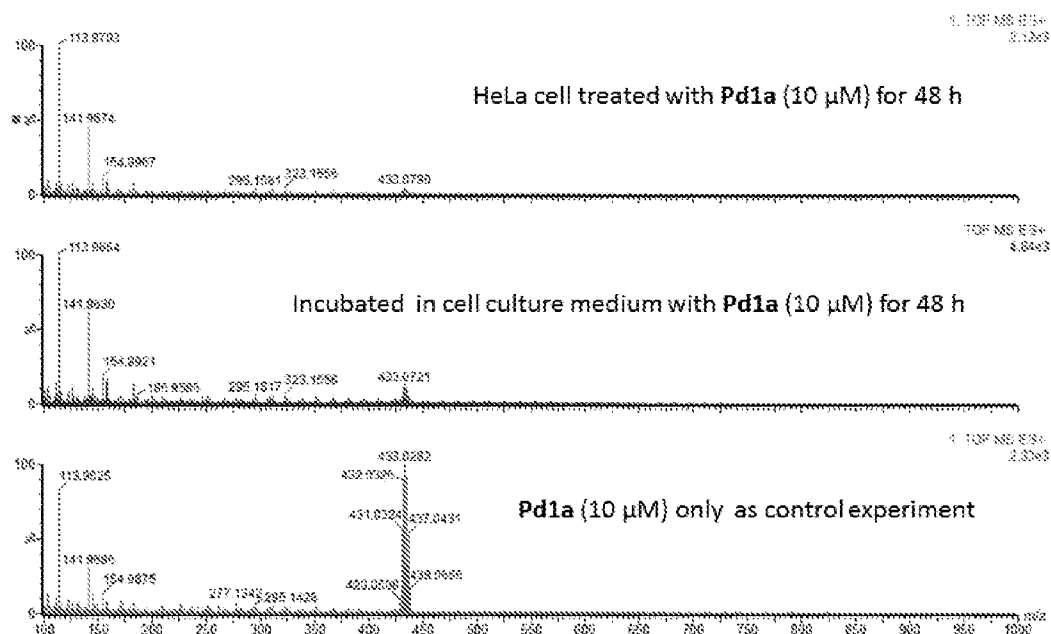


Figure S9

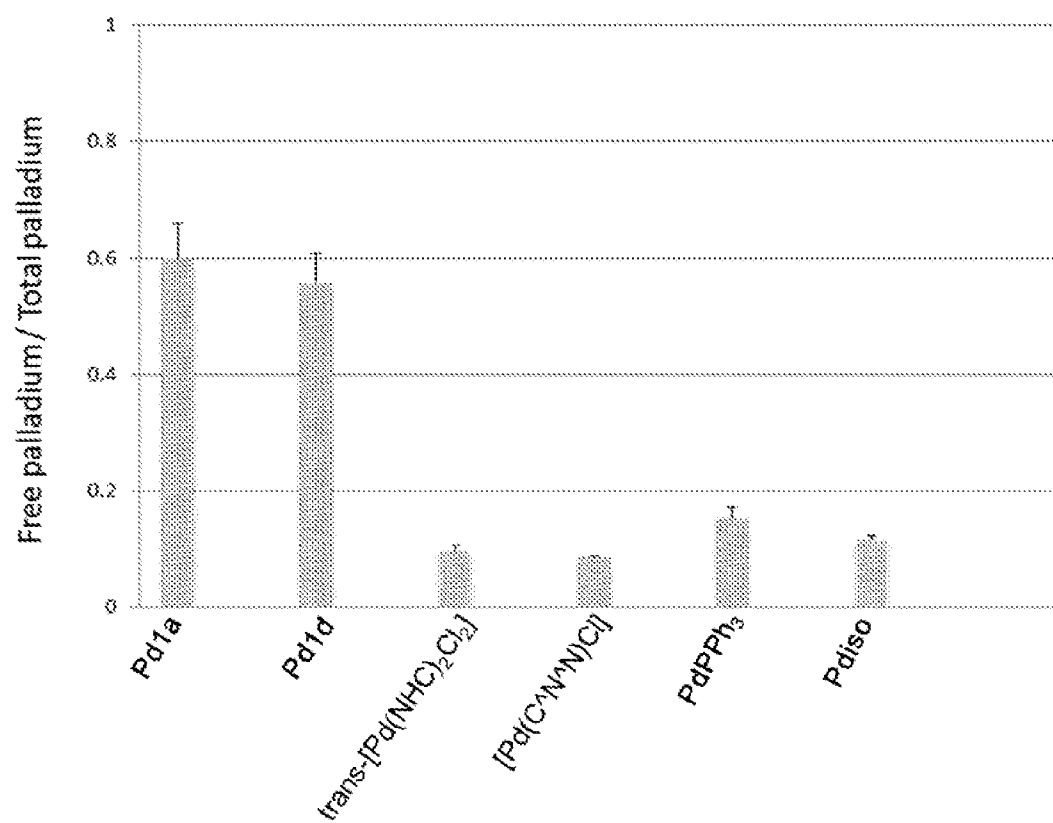


Figure S10

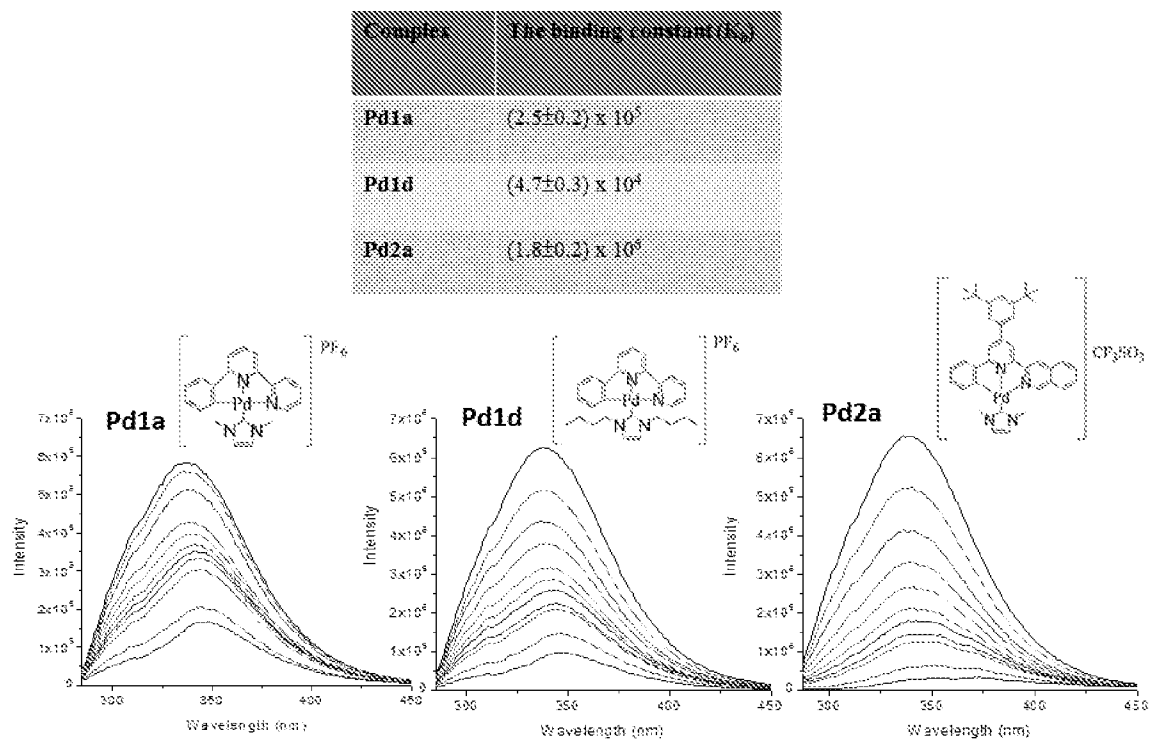


Figure S11

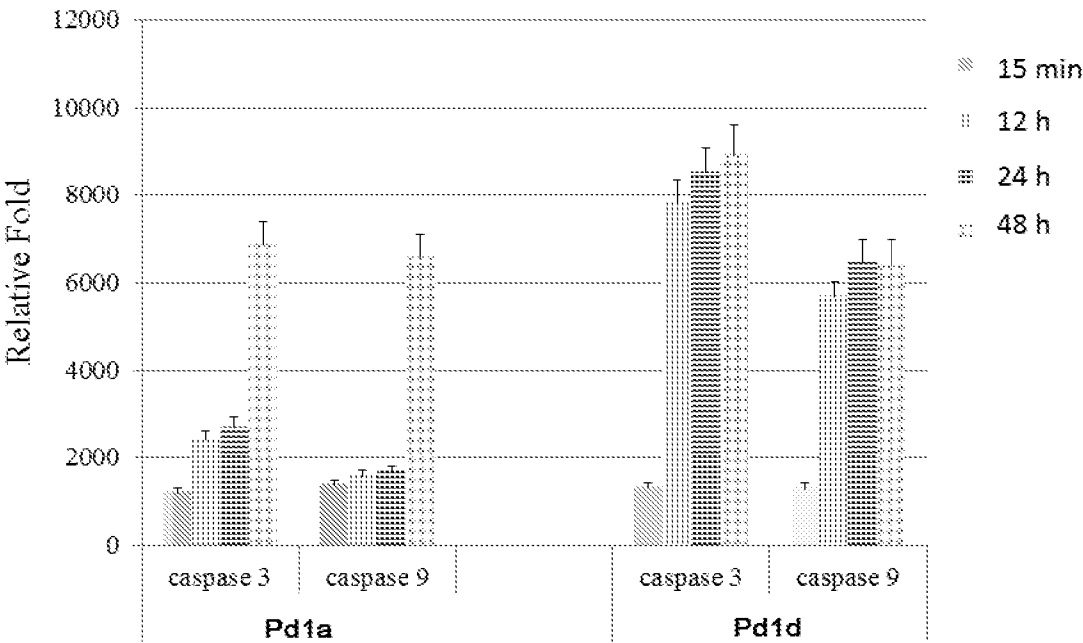


Figure S13

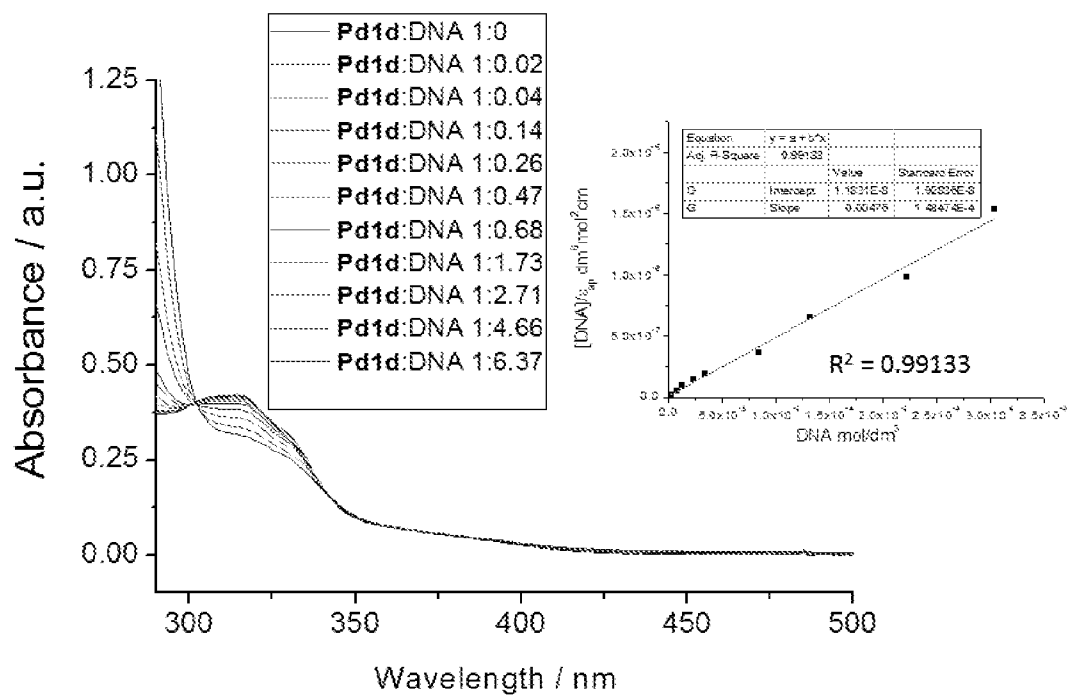


Figure S14

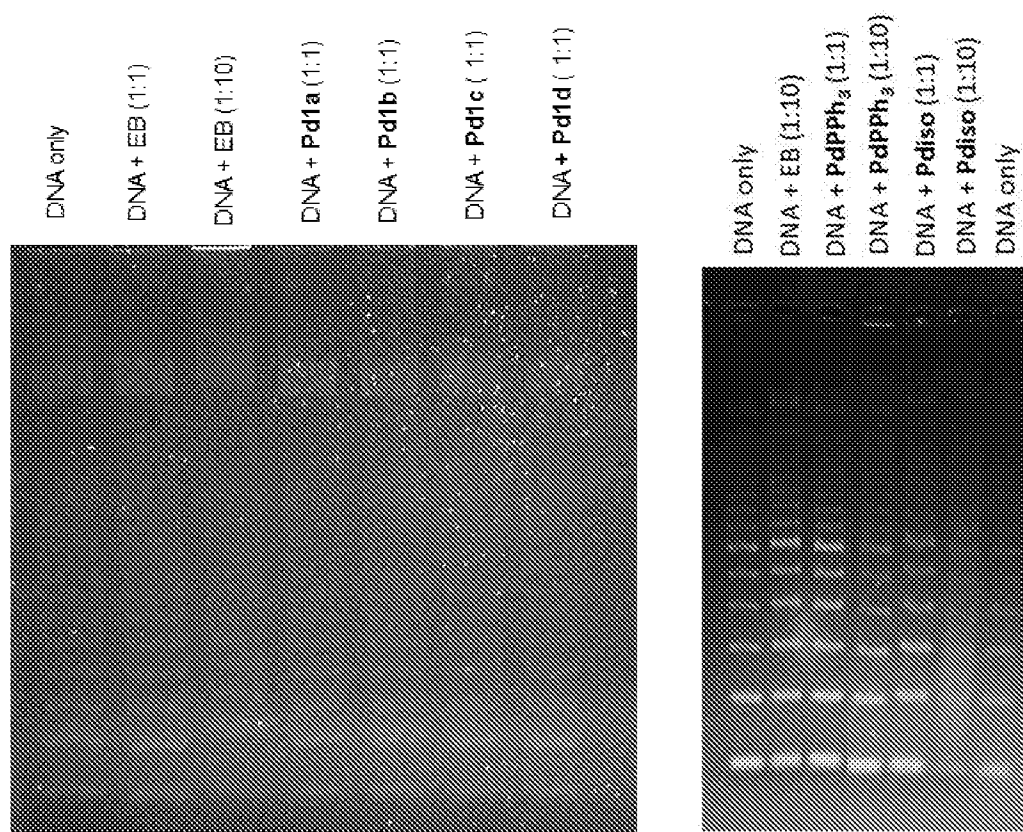


Figure S15

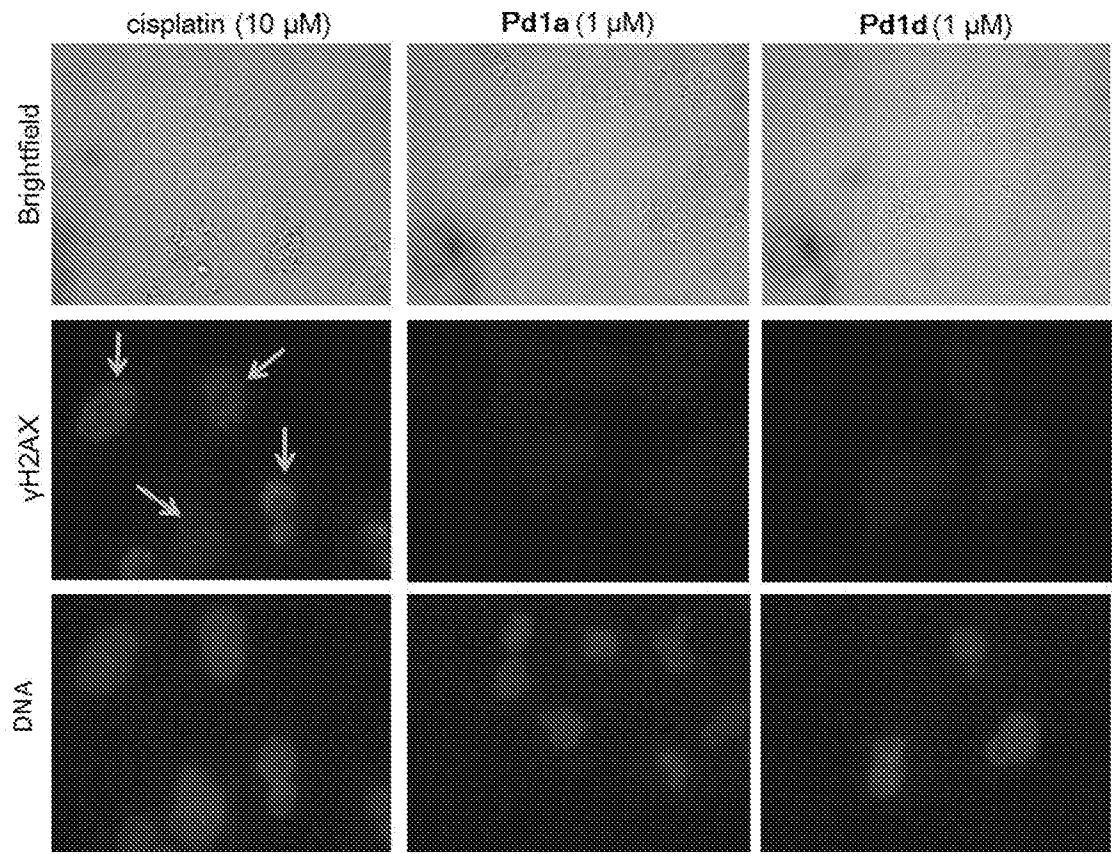


Figure. S16

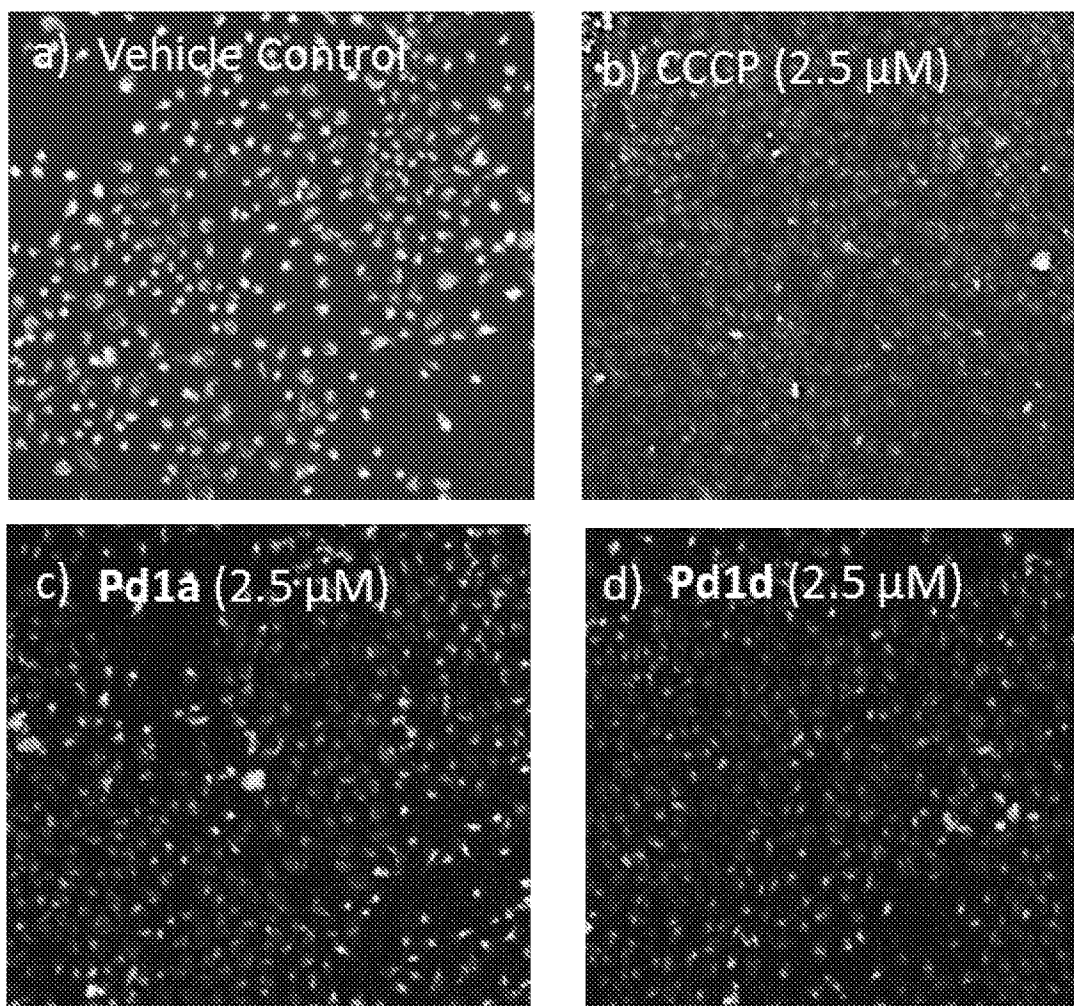


Figure S17

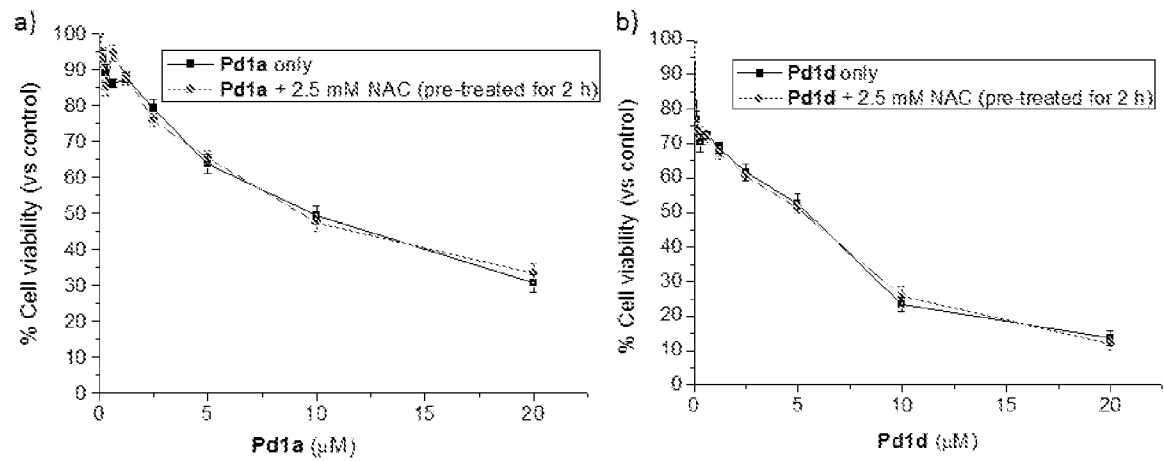


Figure S18

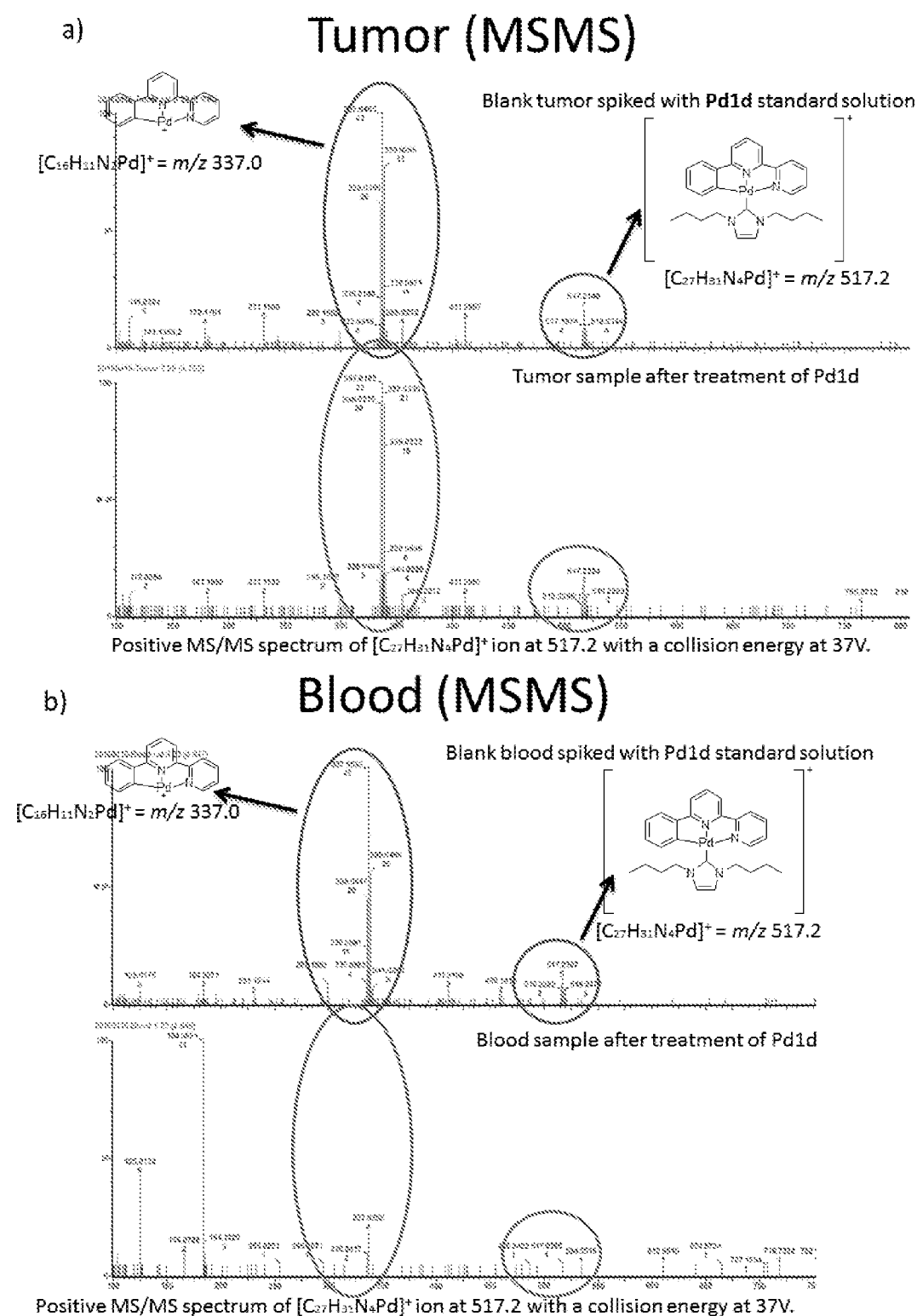


Figure S19

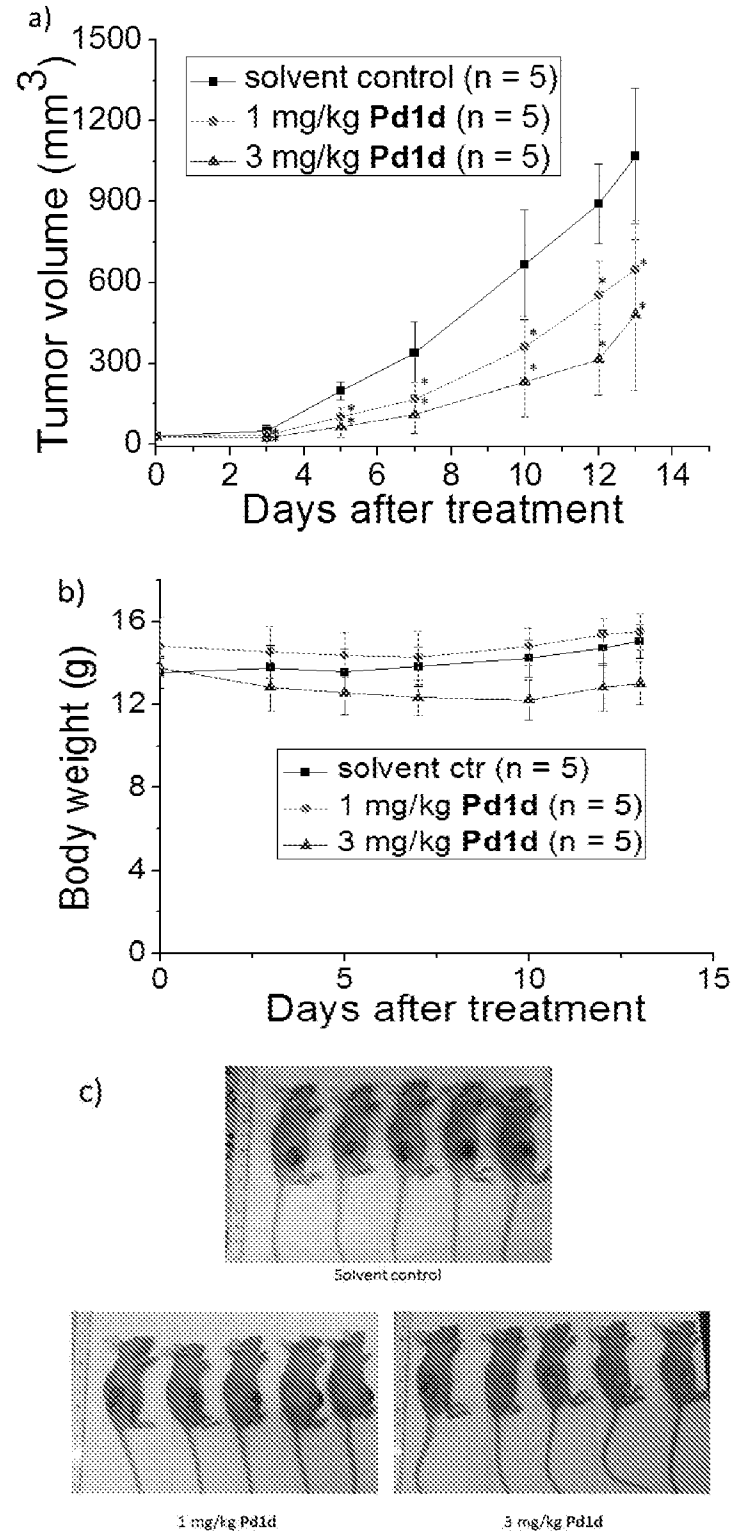
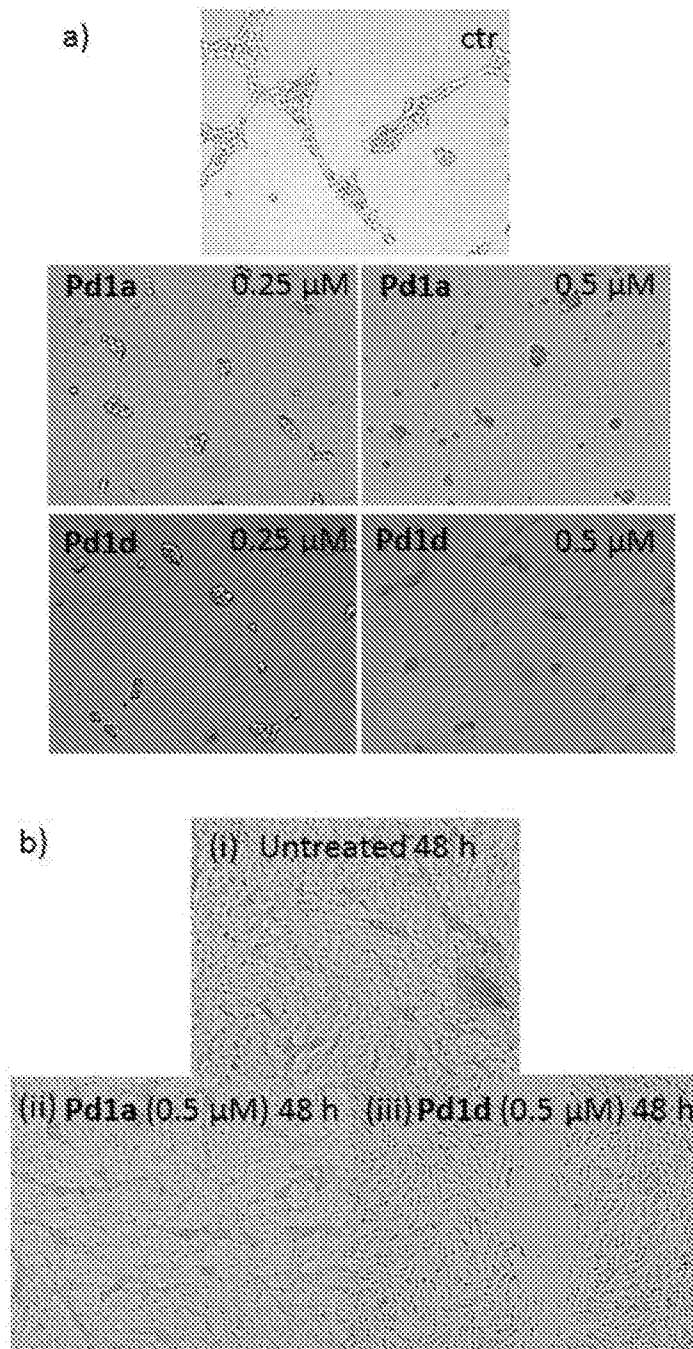


Figure S20



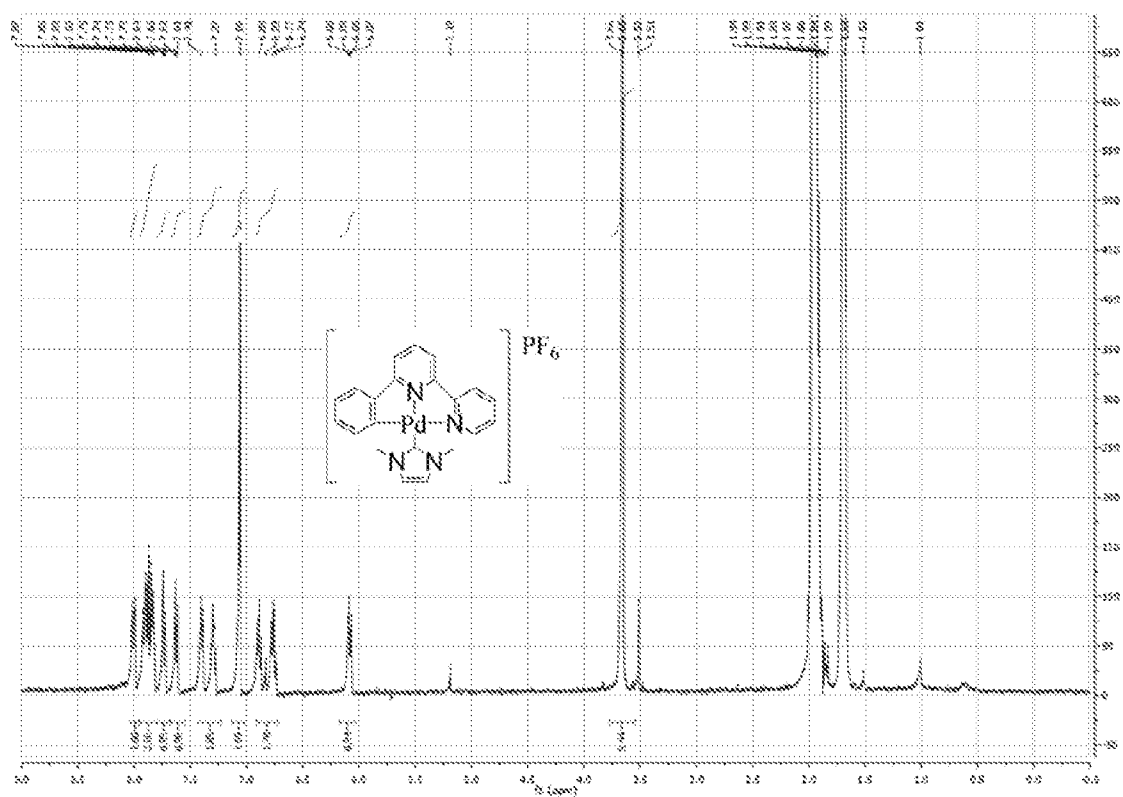


Figure S21

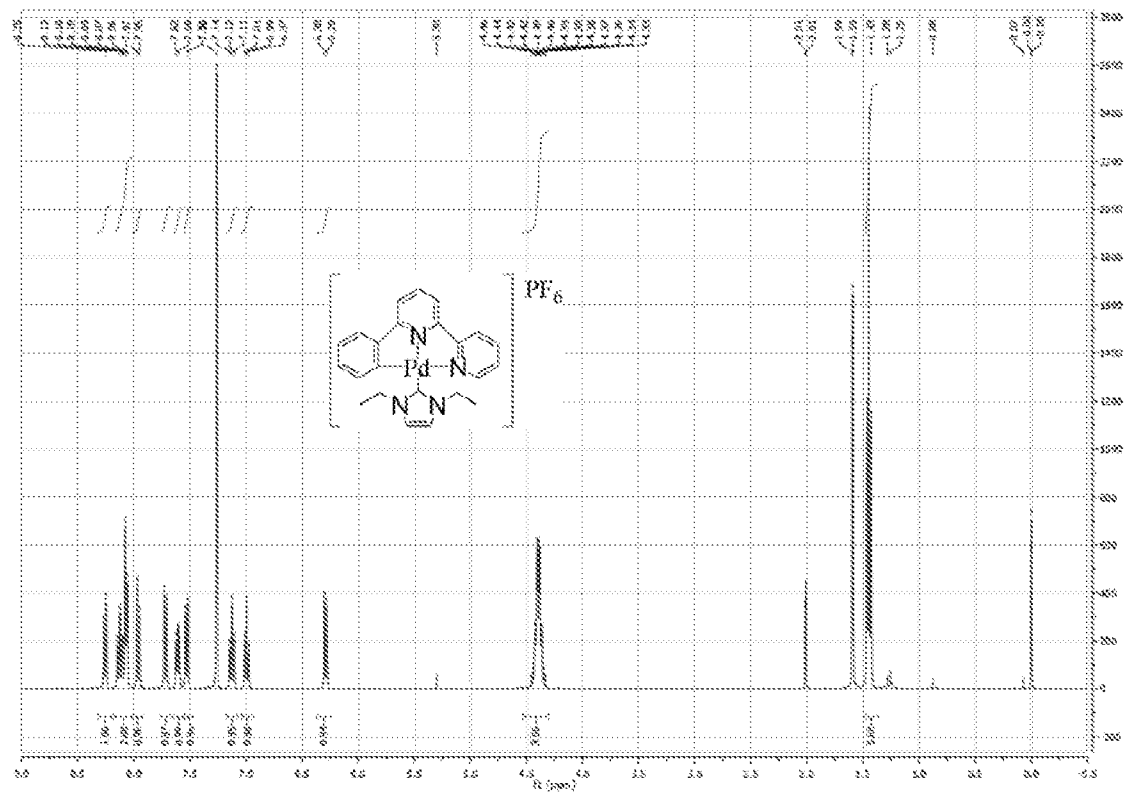


Figure S22

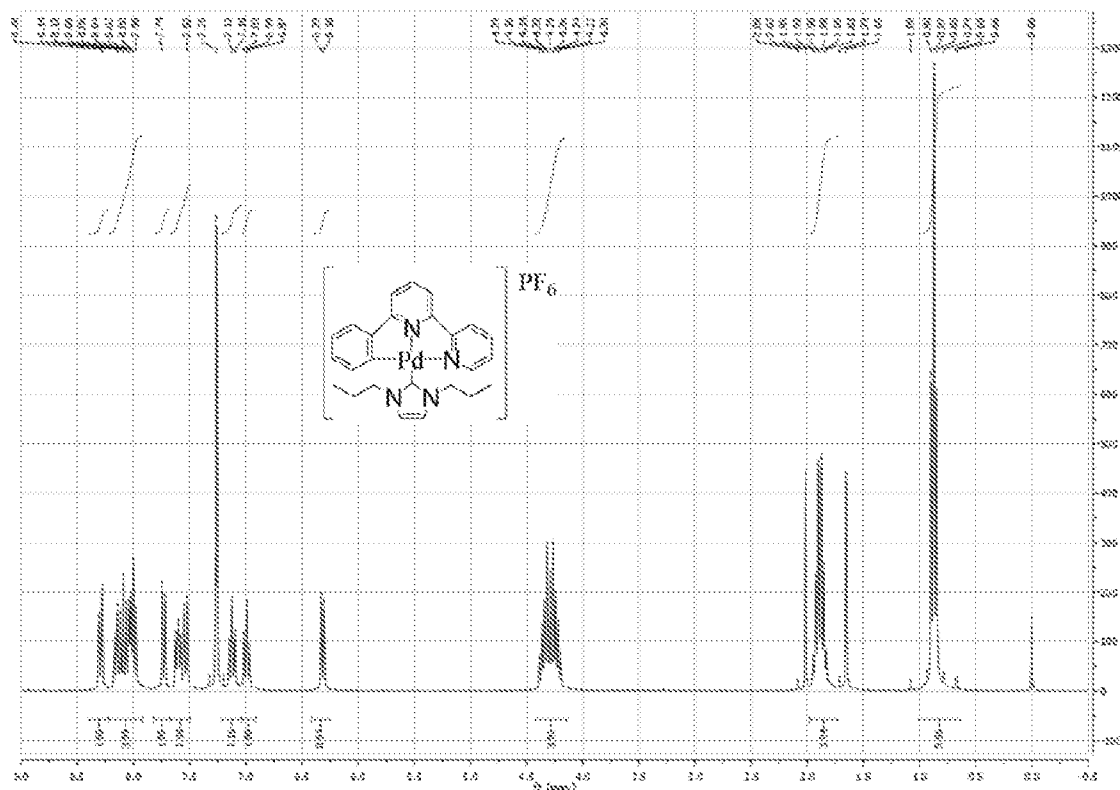


Figure S23

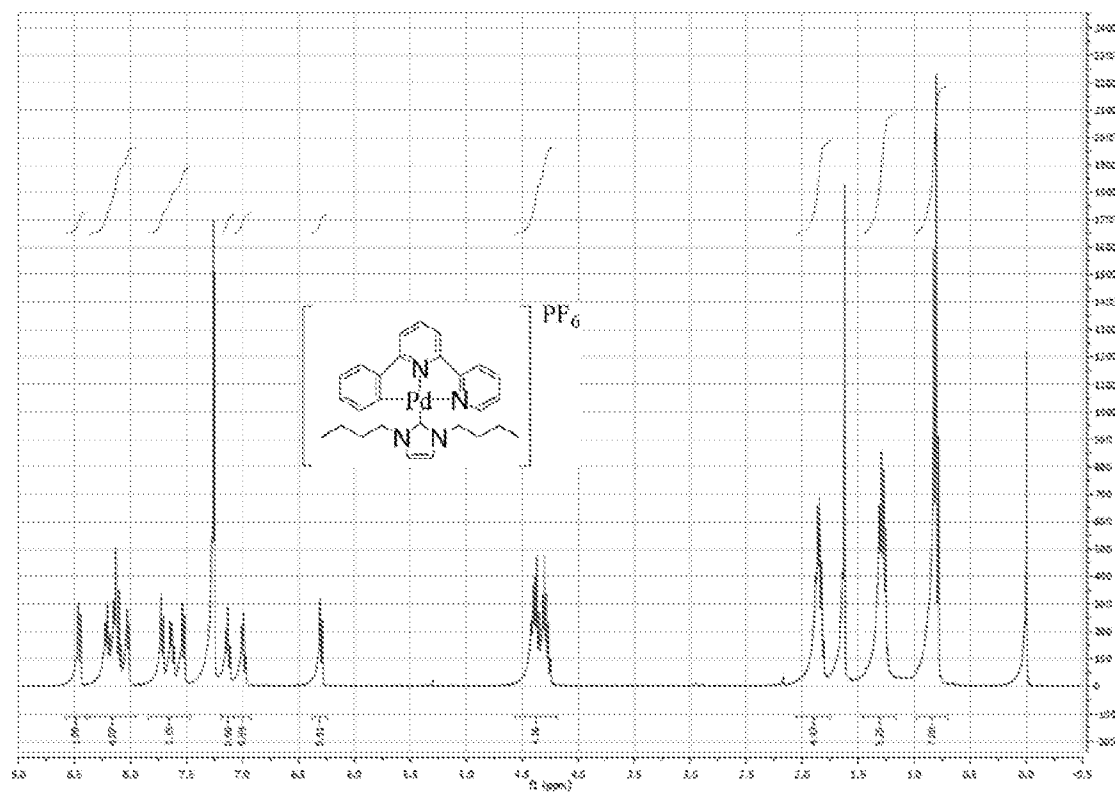


Figure S24

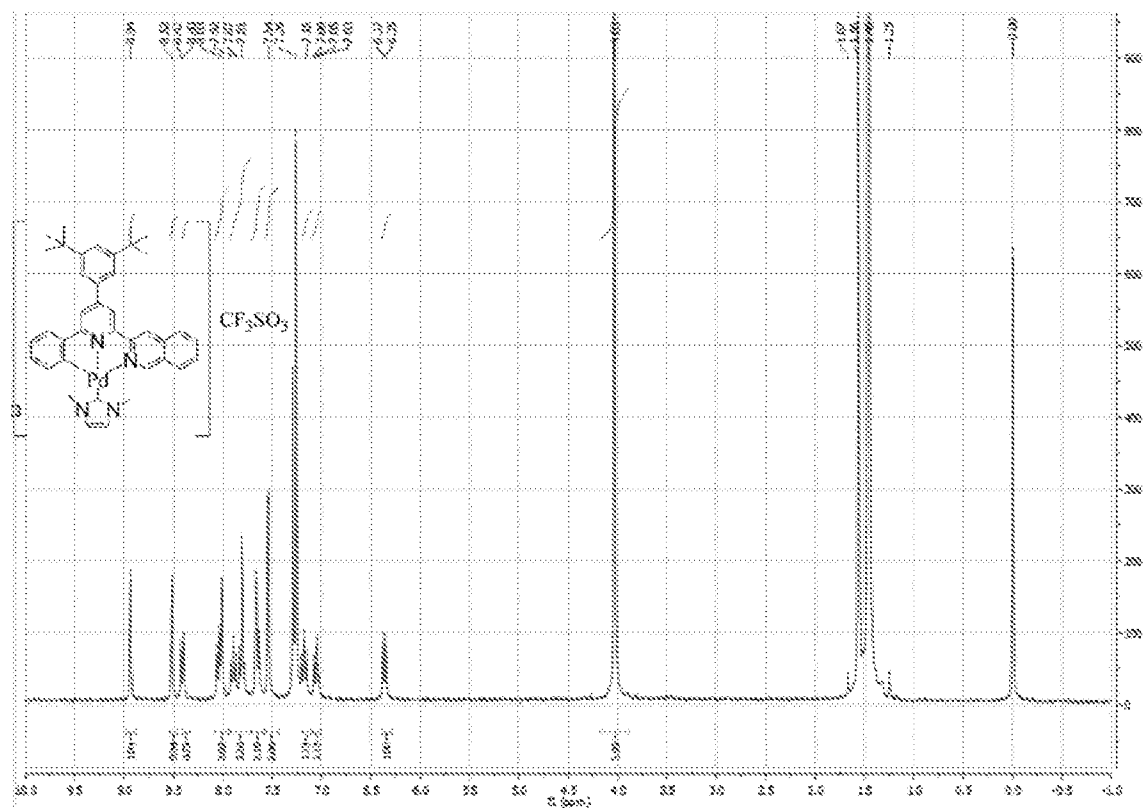


Figure S25

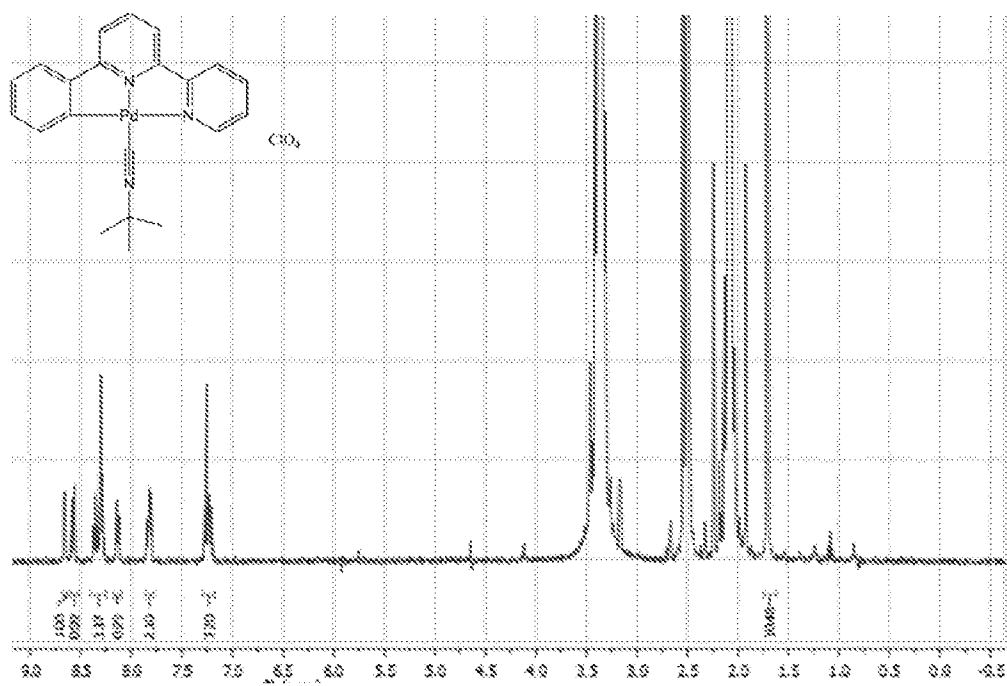


Figure S26

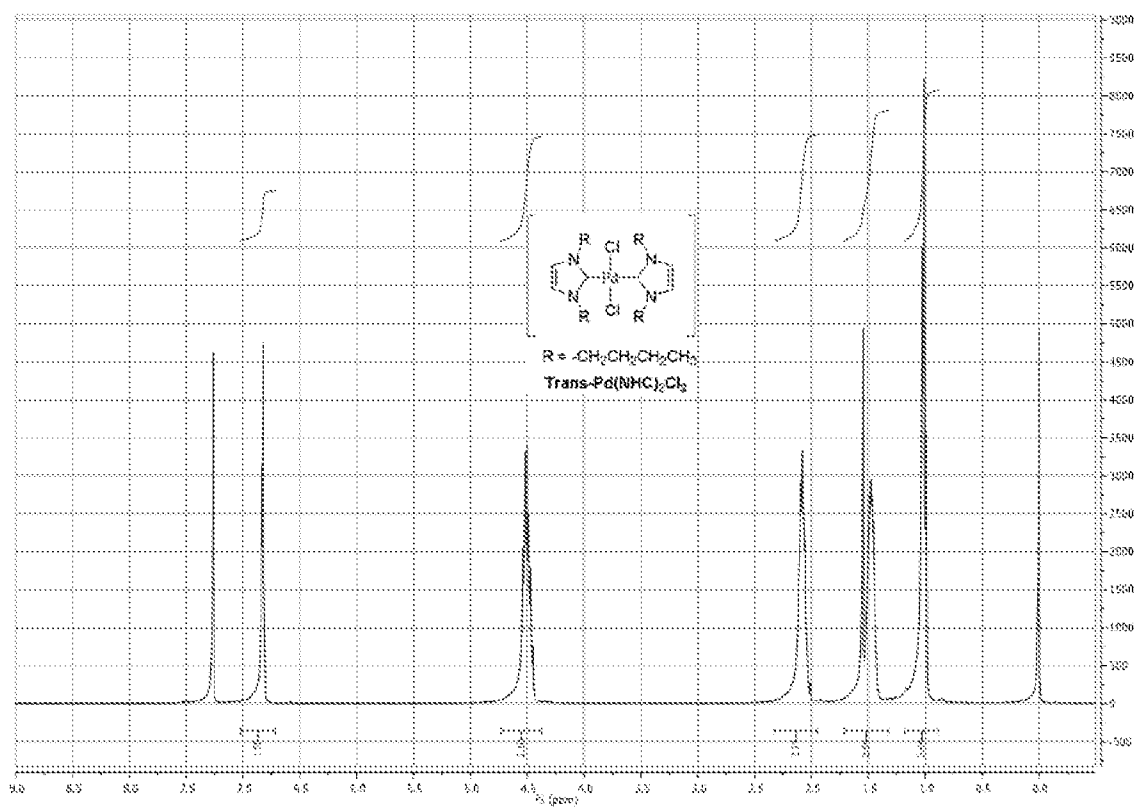


Figure S27

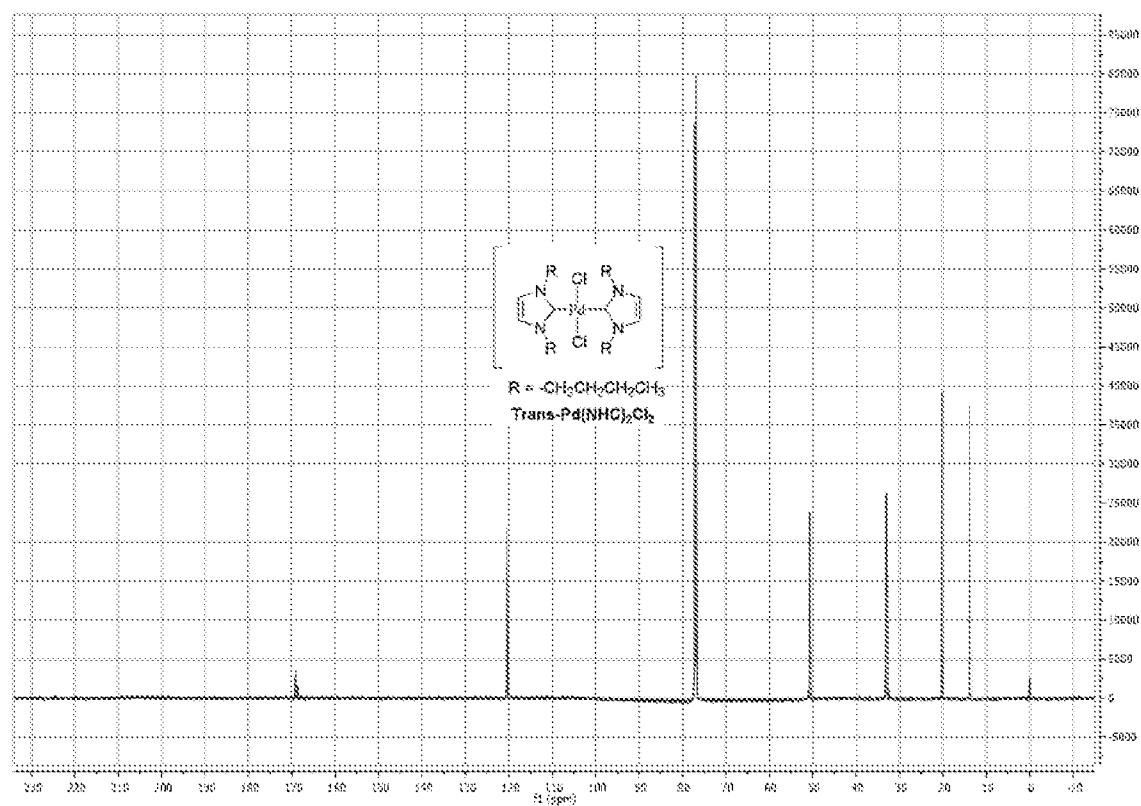


Figure S28

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2017/095174

A. CLASSIFICATION OF SUBJECT MATTER C07F 17/02(2006.01)i; C07F 17/00(2006.01)i; A61K 31/435(2006.01)i; A61K 31/41(2006.01)i; A61K 31/015(2006.01)i; A61P 35/00(2006.01)i According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) A61K,A61P,C07F Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Data base: WPI, EPODOC, CNPAT, Chinese patent full text search system, EMBASE,CNKI Search terms:cyclometal+, heterocyclic,carbene,pd,palladium,cancer,tumour, tumor,cancino+,UNIV HONG KONG,fibroblast		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	WO 2016206583 A1 (UNIV HONG KONG) 29 December 2016 (2016-12-29) the whole document	1-10
A	CN 102741262 A (UNIV HONG KONG) 17 October 2012 (2012-10-17) claims 1-35	1-10
A	TW 201602117 A (UNIV NAT CHANGHUA EDUCATION) 16 January 2016 (2016-01-16) the whole document	1-10
A	US 2015194616 A1 (LI G, LI J) 09 July 2015 (2015-07-09) the whole document	1-10
A	LEE, Jingyi et al. ""Palladium complexes with tridentate N-heterocyclic carbene ligands:selective "normal" and "abnormal" bindings and their anticancer activities"" <i>Organometallics</i> , Vol. 34, No. 17, 28 August 2015 (2015-08-28), ISSN: 0276-7333, the abstract	1-10
☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 08 September 2017		Date of mailing of the international search report 16 October 2017
Name and mailing address of the ISA/CN STATE INTELLECTUAL PROPERTY OFFICE OF THE P.R.CHINA 6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088 China Facsimile No. (86-10)62019451		Authorized officer ZHANG,Ting Telephone No. (86-10)62411123

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2017/095174

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
WO	2016206583	A1	29 December 2016	US	2016369354	A1	22 December 2016
CN	102741262	A	17 October 2012	CN	102741262	B	27 August 2014
				WO	2011050574	A1	05 May 2011
				EP	2493896	B1	26 April 2017
				EP	2493896	A1	05 September 2012
				US	8530659	B2	10 September 2013
				US	2011098264	A1	28 April 2011
TW	201602117	A	16 January 2016	TW	523861	B1	01 March 2016
US	2015194616	A1	09 July 2015	None			