Supporting Information

Antimicrobial efficacy of a hemilabile Pt(II)-NHC compound against drug-resistant *S. aureus*

Mandeep Kaur, [§a] Ritesh Thakare, [§b] Arindom Bhattacherya, [§a] Prem Anand Murugan, [d] Grace Kaul, [b,c] Manjulika Shukla, [b] Alok Kr. Singh, [b,e] Saravanan Matheshwaran, *[d] Sidharth Chopra, *[b,c] and Jitendra K. Bera *[a]

[a] Department of Chemistry and Centre for Environmental Sciences and Engineering, Indian Institute of Technology Kanpur, Kanpur 208016, India

E-mail: jbera@iitk.ac.in

[b] Department of Molecular Microbiology and Immunology, CSIR-Central Drug Research Institute, Lucknow-226031, India

E-mail: skchopra007@gmail.com

[c] Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India

[d] Department of Biological Sciences and Bioengineering, Centre for Environmental Sciences and Engineering, and Mehta Family Centre for Engineering in Medicine, Indian Institute of Technology Kanpur, Kanpur 208016, India

E-mail: saran@iitk.ac.in

[e] Current Affiliation: Amity Institute of Biotechnology, Amity University Noida Campus, Sector-125, Noida - 201313 (U.P.)

Table of Contents

Experimental Procedures

1.1 General Procedures

All reactions with metal complexes were carried out under nitrogen atmosphere using standard Schlenk-vessel and vacuum line techniques. The crystallized compounds were powdered, washed several times with dry petroleum ether, and dried in vacuum for at least 48 h prior to elemental analyses. ^{1}H NMR spectra were obtained on JEOL JNM-LA 500MHz spectrometer. ^{1}H NMR chemical shifts were referenced to the residual hydrogen signal of the deuterated solvents. The chemical shift is given as dimensionless δ values and is frequency referenced relative to TMS for ^{1}H and ^{13}C NMR spectroscopy. Elemental analyses were performed on a Thermoquest EA1110 CHNS/O analyser. GC-MS experiment was performed on an Agilent 7890A GC and 5975C MS system. ESI-MS were recorded on a Waters Micro mass Quattro Micro triple-quadruplet mass spectrometer. For FESEM analysis the samples were gold-coated (about 10 nm) using a gold sputtering unit and observed using a ZEISS Gemini SEM – Field-emission Scanning Electron Microscope.

Materials: Solvents were dried by conventional methods, distilled over nitrogen, and deoxygenated prior to use. $K_2[PtCl_4]$ was purchased from Arora Matthey, India. $[PtCl_2(COD)]$ was prepared according to the literature procedure.[1]

1.2 Synthesis and Characterisation

Synthesis of [L^{1}H]Br and [L^{2}H]Br: 2-bromopyridine (0.48 mL, 5 mmol), imidazole (0.51 g, 7.5 mmol), KOH (0.56 g, 10 mmol), and Cu_2O (72 mg, 0.5 mmol) was placed in a round-bottom flask and 20 mL of DMSO was added. The reaction mixture was stirred at 110 °C for 48 h. After the mixture was cooled to ambient temperature, water was added, and the aqueous phase was extracted with ethyl acetate (4 × 25 mL). The combined organic layers were dried over Na_2SO_4. The crude product was purified by column chromatography over silica with EtOAc as eluent hexane/ethyl acetate (98:2) to afford the desired product. The final product was quaternized with 4-bromobutene and 4-bromobutane led to the formation of [L^{1}H]Br and [L^{2}H]Br respectively. The counter anion of [L^{1}H]Br was exchanged with PF_6 by adding KPF_6 (40 mg, 0.216 mmol) to a solution of [L^{1}H]Br (60 mg, 0.216 mmol) in acetonitrile, and the mixture was stirred at room temperature for 1 h. The removal of acetonitrile under vacuum followed by the addition of diethyl ether gave [L^{1}H]PF_6 as a white solid. Similar procedure followed for [L^{2}H]PF_6. **[L^{1}H]Br**. ^{1}H NMR (400 MHz, $CDCl_3$, 294 K): δ 9.29 (s, 1H), 8.51 (d, *J* = 3.68, 1H), 8.14 (t, *J* = 2.06 Hz, 1H), 8.00 (dt, *J* = 7.78 Hz, 1.84 Hz, 1H), 7.83 (d, *J* = 8.24 Hz, 1H), 7.48 (m, 2H), 7.24 (s, 1H), 5.77 (m, 1H), 5.10 (dt, *J* = 13.84 Hz, *J* = 1.21 Hz, 2H), 4.42 (t, *J* = 6.84 Hz, 2H), 2.67 (q, *J* = 7.53 Hz, 2H). ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$, 294 K): δ 149.3, 145.5, 140.6, 133.0, 132.1, 125.4, 123.0, 120.0, 119.3, 113.9, 49.9, 34.1. Analytically Calculated for $C_{12}H_{14}N_3Br$: C, 51.6; H, 5.05; N, 15.05 Found: C, 51.4; H, 5.01; N, 15.02. **[L^{2}H]Br**: ^{1}H NMR (400 MHz, $CDCl_3$, 294 K): δ 9.28 (s, 1H), 8.49 (d, *J* = 4.12, 1H), 8.14(s, 1H), 7.97 (t, *J* = 7.58 Hz, 1H), 7.80 (d, *J* = 8.24 Hz, 1H), 7.50 (s, 1H), 7.45 (m, 1H), 4.43 (t, *J* = 7.8 Hz, 2H), 1.91 (qn, *J* = 7.8 Hz, 2H), 0.937 (t, *J* = 7.32 Hz, 3H). ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$, 294 K): δ 149.2, 145.8, 140.5, 132.9, 125.3,

123.1, 119.4, 113.8, 50.6, 31.8, 19.3, 13.2. Analytically Calculated for $C_{12}H_{13}N_3Br$: C, 51.23; H, 5.73; N, 14.94 Found: C, 51.20; H, 5.71; N, 14.82.

Synthesis of [Pt(L^1)Cl]PF$_6$ (1): A solution of [L^{1}H]PF$_6$ (60 mg, 0.17 mmol) in dichloromethane (20 mL) was treated with Ag_2O (46 mg, 0.20 mmol), and the suspension was stirred for 4 h at room temperature under the exclusion of light and in N_2 atmosphere. After 4 h, [$PtCl_2$(COD)] (64.5 mg, 0.17 mmol) was added, and the solution was further stirred for 12 h at room temperature. The mixture was subsequently filtered over a pad of celite and the solution was concentrated under reduced pressure and 10 mL hexane was added while stirring to get a light yellow precipitate. The precipitate was washed with hexane (3 × 10 mL) and dried under vacuum. X-ray quality crystals were grown by layering hexane onto a dichloromethane solution of **1** inside an 8 mm o.d. vacuum-sealed glass tube. Yield: 50 mg (70%). ^{1}H NMR (500 MHz, CD_3CN, 294 K): δ 9.13 (dt, *J* = 19 Hz, *J* = 6.3 Hz, 1H), 8.41 (dt, *J* = 7.75 Hz, *J* = 1.7 Hz, 1H), 7.90 (d, *J* = 8.05 Hz, 1H), 7.76 (d, *J* = 2.3 Hz, 1H), 7.72 (t, *J* = 6.3 Hz 1H), 7.31 (d, *J* = 2.3 Hz, 1H), 5.71 (m, 1H), 5.23 (d, *J* = 7.45 Hz, 1H), 4.89 (d, *J* = 15.5 Hz, 1H), 4.43 (t, *J* = 13.8 Hz, 1H), 4.24 (td, *J* = 15 Hz, *J* = 3.2 Hz 1H), 3.02 (m, 1H), 2.77 (m, 1H); ^{13}C{^{1}H} NMR (125 MHz, CD_3CN, 294 K): δ 186.6, 151.7, 148.2, 146.0, 144.9, 124.3, 116.4, 94.0, 70.3, 43.8, 31.3. ESI-MS, *m/z*: 429.0521 [**1**-PF_6]$^+$. Analytically Calculated for $C_{12}H_{13}N_3ClPtPF_6$: C, 25.08; H, 2.28; N, 7.32. Found: C, 24.75; H, 2.16; N, 7.20.

Synthesis of [Pt(L^2)(COD)](PF$_6$)$_2$ (2): The reaction of [L^{2}H]PF$_6$ (60 mg, 0.17 mmol), Ag_2O (46 mg, 0.20 mmol) and [$PtCl_2$(COD)] (64.5 mg, 0.17 mmol) in acetonitrile (20 mL) was carried out by following a procedure similar to that described for the synthesis of **1**. X-ray quality crystals were grown by layering hexane onto a dichloromethane solution of **2** inside an 8 mm o.d. vacuum-sealed glass tube. Yield: 80 mg (85%). ^{1}H NMR (400 MHz, DMSO-D_6, 294 K) δ 8.60 (d, *J* = 4.6 Hz, 1H), 8.48 (m, 1H), 8.17 (dt, *J* = 17.2, 8.2 Hz, 1H), 8.00 (m, 1H), 7.96 (m, 1H), 7.61-7.58 (dd, *J* = 7.6, 4.7 Hz, 1H), 5.49 (t, 2H), 4.24 (t, *J* = 7.1 Hz, 4H), 2.24-2.18 (m, 8H), 1.84 – 1.80 (m, 2H), 1.30 – 1.26 (m, 3H), 0.87 (td, *J* = 7.3, 3.3 Hz, 4H). ^{13}C{^{1}H} NMR (125 MHz, DMSO−d_6, 294 K): δ 149.1, 146.3, 140.5, 134.8, 128.3, 125.1, 123.3, 119.1, 114.4, 114.0, 101.2, 49.0, 31.0, 30.3, 27.5, 27.3, 18.7, 13.2. ESI-MS, *m/z*: 504.1932 [**2**-2PF_6+H]$^+$. Analytically Calculated for $C_{20}H_{27}N_3PtP_2F_{12}$: C, 40.5; H, 4.54; N, 7.01. Found: C, 39.98; H, 4.50; N, 6.99.

Synthesis of [Pt(L^2)Cl$_2$] (3): A suspension of [L^{2}H]Br (80 mg, 0.23 mmol), NaOAc (21 mg, 0.271 mmol), and K_2PtCl_4 (87.9 mg, 0.23 mmol) in acetonitrile (20 mL) was refluxed for 12 h. The mixture was cooled to room temperature and filtered over a pad of celite. The resultant yellow solution was concentrated under reduced pressure and 10 mL hexane was added while stirring to get the precipitate. The precipitate was washed with hexane (3 × 10 mL) and dried under vacuum. X-ray quality crystals of compound **3** were obtained by layering hexane onto a mixed dichloromethane/acetonitrile (5/1) solution of the yellow precipitate inside an 8 mm o.d. vacuum–sealed glass tube. Yield: 65 mg (70%). ^{1}H NMR (400 MHz, DMSO-D_6, 294 K) δ 9.50 (t, *J* = 5.0 Hz, 1H), 8.33 – 8.29 (m, 2H), 8.03 (dd, *J* = 8.5, 4.4 Hz, 1H), 7.59 (t, *J* = 2.4 Hz, 1H), 7.54 – 7.49 (m, 1H), 4.57 (dt, *J* = 19.3, 7.5 Hz, 2H), 1.74 (p, *J* = 7.6 Hz, 2H), 1.28 (m, 2H), 0.87 (t, *J* = 7.3 Hz, 3H); ^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6, 294 K): δ 151.9, 147.5, 146.5, 141.6, 124.1, 122.1, 115.9, 111.6, 47.9, 32.3, 18.5, 13.0. ESI-MS, *m/z*: 396.0912 [**3**-2Cl]$^+$.

1.3 X-Ray Data Collections and Refinements for 1, 2 and 3

Single crystal X-ray structural studies were performed on a CCD Bruker SMART APEX diffractometer equipped with an Oxford Instruments low–temperature attachment. Data were collected at 100(2) K using graphite–monochromatic Mo–Kα radiation (λ_α = 0.71073 Å). The frames were indexed, integrated and scaled using SMART and SAINT software packages,[2] and the data were corrected for absorption using the SADABS program.[3] The structures were solved and refined using SHELX suite of programs [4] while additional crystallographic calculations were performed for compound **1** by the "SQUEEZE" option in PLATON.[5] The crystallographic figures have been generated using Diamond 3 software [6] (50% probability of thermal ellipsoids). Additional crystallographic calculations were performed. The hydrogen atoms were included into geometrically calculated positions in the final stages of the refinement and were refined according to 'riding model'. Crystallographic data and pertinent refinement parameters for **1**, **2**, and **3** are presented in Table S1**.**

Table S1. Crystallographic Data and Pertinent Refinement Parameters for **1**, **2** and **3**

	1	**2.CH_2Cl_2**	**3**
Empirical formula	$C_{12}H_{13}ClF_6N_3PPt$	$C_{21}H_{29}Cl_2F_{12}N_3P_2Pt$	$C_{12}H_{15}Cl_2N_3Pt_{0.83}$
Formula Weight	574.76	879.40	433.85
Crystal System	Triclinic	Triclinic	Monoclinic
Space Group	*P–1*	*P–1*	*C2/c*
a (Å)	6.6487(5)	13.328(2)	13.7494(9)
b (Å)	11.9212(8)	14.393(2)	11.5541(8)
c (Å)	12.4491(8)	15.469(2)	17.3694(11)

α (deg)	111.4410(10)	72.190(4)	90
β (deg)	98.2170(10)	89.773(4)	97.787(2)
γ (deg)	102.0040(10)	85.898(4)	90
V (Å^3)	871.99(10)	2817.4(7)	2733.9(3)
Z	2	4	8
ρ_{calcd} (g cm^{-3})	2.189	2.073	2.108
μ (mm^{-1})	8.349	5.383	8.897
F(000)	540	1704	1653
Reflections			
Collected	10329	9828	16048
Independent	4323	9828	2429
Observed [I >2σ (I)]	3773	8200	2230
No. of variables	217	731	164
GOF	1.055	1.015	1.081
R_{int}	0.0352	0.0456	0.0443
Final R indices [I > 2σ(I)]^a	R1 = 0.0321	R1 = 0.0461	R1 = 0.0318
	wR2 = 0.0663	wR2= 0.0931	wR2 = 0.0820
R indices (all data)^a	R1 = 0.0405	R1 = 0.0619	R1 = 0.0359
	wR2 = 0.0687	R1 = 0.0979	wR2 = 0.0841
CCDC numbers	2181712	2181713	2181714

$^a R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ with $F_o^2 > 2\sigma(F_o^2)$. $wR_2 = [\Sigma w(|F_o^2| - |F_c^2|)^2/\Sigma|F_o^2|^2]^{1/2}$

2 Biological studies

2.1 Bacterial Strains

Compounds **1, 2** and **3** were screened against a bacterial panel consisting of ESKAPE pathogens namely *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* BAA-1705, *Acinetobacter baumannii* BAA-1605, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 and *Enterococcus* sp. The panel was further expanded to drug-resistant clinical *S. aureus* strains, including strains resistant to vancomycin. These strains were procured from BEI/NARSA/ATCC (Biodefense and Emerging Infections Research Resources Repository/Network on Antimicrobial Resistance in *Staphylococcus aureus*/American Type Culture Collection, USA) and routinely cultivated on Mueller–Hinton Agar (MHA). Before starting the experiment, a single colony was picked from MHA plate, inoculated in Mueller–Hinton cation supplemented broth (CA–MHBII) and incubated overnight at 37°C with shaking for 18–24 h to get the starter culture.

2.2 Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was carried out according to CLSI guidelines for broth microdilution assay.[7] 10 mg/mL stock solutions of test compounds were prepared in dimethyl sulfoxide (DMSO). Bacterial cultures were inoculated in CA–MHBII and optical density (OD) of cultures were measured at 600 nm wavelength, followed by dilution to achieve ~10^5–10^6 cfu/mL. The compounds were tested, ranging from 64–0.5 mg/L in two-fold serial dilutions, and the bacterial suspension was added to each well containing the test compound along with appropriate controls. The plates were incubated at 37°C for 18–24 h, following which the growth was enumerated and MIC was identified. The MIC is defined as the lowest compound concentration where there is no visible growth. For each compound, MIC determinations were carried out independently three times using duplicate samples.

2.3 Cell Cytotoxicity Assay

Cell toxicity was performed against Vero cell using MTT assay.[8] The cells/well around ~10^3 were seeded in 96 well plates and incubated at 37°C with a 5% CO_2 atmosphere. After 24 h, **1** was added, ranging from 100-12.5 mg/L and incubated for 72 h at 37°C

with a 5% CO_2 atmosphere. After the incubation was over, MTT was added at 5 mg/L in each well, incubated at 37°C for further 4 h, residual medium was discarded, and 0.1 mL of DMSO was added to solubilize the formazan crystals and OD was taken at 540 nm for calculation of CC_{50}. The CC_{50} is defined as the lowest concentration of compound which leads to a 50% reduction in cell viability. Doxorubicin was used as positive control and each experiment was repeated in triplicate.

Table S2. Cytotoxicity (mg/L) against Vero cells and selectivity indexes of **1**, **2** and **3**

Compounds	MIC (mg/L) against *S. aureus* ATCC 29213	CC_{50} (mg/L) against Vero cells (ATCC CCL-81)	Selectivity Index (CC_{50}/MIC)
1	0.25	>40	>160
2	2	40	20
3	2	40	20

2.4 Bacterial Time-Kill Assay

The presence or absence of bactericidal activity was assessed by time-kill method as described earlier.[9] Briefly, *S. aureus* ATCC 29213 was diluted ~10^5 cfu/mL in CA-MHBII and treated with 1x and 10x of MIC of **1** and vancomycin, incubated at 37°C with shaking for 24 h. 0.1 mL samples were collected at time intervals of 0, 1, 6 and 24 h, serially diluted in PBS and plated on MHA followed by incubation at 37°C for 18-20 h. The time-kill curves were constructed by counting colonies from plates and plotting cfu/mL of surviving bacteria at each time point in presence and absence of compound. Each experiment was repeated three times in duplicate and the mean data was plotted.

2.5 Drug interaction of 1 with FDA Approved Drugs

Interaction of **1** with FDA approved drugs was tested by checkerboard method.[10] Serial two-fold dilutions of each drug were freshly prepared prior to testing. Compound **1** was two-fold diluted along the abscissa ranging from 8–0.0008 mg/L (12 dilutions) while the antibiotics were serially diluted along the ordinate in 96 well microtiter plate. 95 µL of ~10^6 CFU/mL was added to each well and plates were incubated at 37°C for 24 h. After incubation, ΣFICs (fractional inhibitory concentrations) were calculated as follows: ΣFIC = FIC A + FIC B, where FIC A is MIC of drug A in combination/MIC of drug A alone and FIC B is MIC of drug B in combination/MIC of drug B alone. The combination is considered synergistic when ∑FIC is ≤0.5, indifferent when ∑FIC is >0.5 to 4, and antagonistic when ∑FIC is >4 (12).

Table S3. Interaction of **1** with FDA approved drugs

Drug target	Name of the drug	MIC (mg/L) of 1 in presence of drug	MIC (mg/L) of drug in presence of 1	FIC A	FIC B	ΣFIC	Inference
		'A'	'B'				
Cell wall	Ceftazidime	0.25	16	0.5	1	1.5	No synergy
	Vancomycin	0.25	1	0.5	1	1.5	No synergy
	Daptomycin	0.25	0.5	0.5	0.5	1	No synergy
	Meropenem	0.25	0.125	0.5	0.25	0.75	No synergy
Protein synthesis (30S Ribosome)	Gentamicin	0.125	0.125	0.25	0.25	0.5	Synergy
	Minocycline	0.0075	0.125	0.015	0.25	0.265	Synergy
Protein synthesis (50S Ribosome)	Linezolid	0.25	0.5	0.5	0.25	0.75	No synergy

DNA	Levofloxacin	0.25	0.125	0.5	0.5	1	No synergy
RNA	Rifampicin	0.25	0.0075	0.5	1	1.5	No synergy

2.6 Determination of Post Antibiotic Effect (PAE)

To determine PAE of **1**, overnight culture of *S. aureus* ATCC 29213 was diluted in CAMHBII ~10^6 CFU/mL and exposed to 1x and 5x MIC of vancomycin, levofloxacin, **1** and incubated at 37°C for 1 h. Following the incubation period, the culture was centrifuged and washed two times with pre-warmed CAMHBII to remove any traces of antibiotics. Finally, cells were resuspended in drug-free MHBII and incubated further at 37°C. Samples were taken after every 1 h, serially diluted and plated on TSA for enumeration of CFU. The PAE was calculated as PAE = T – C; where T is referred to the difference in time required for 1 $\log_{10}$ increase in cfu versus cfu observed immediately after removal of drug and C in a similarly treated drug free control.[11]

Table S4. *In vitro* post-antibiotic effect (PAE) of **1** and control antibiotics

Treatments	**Time required for 1 $\log_{10}$ CFU/mL increase (h)**	**PAE (h)**
Untreated *S. aureus* ATCC 29213	2	-
1 1x MIC	~5	~3
1 10x MIC	~5	~3
Auranofin 1x MIC	~2	0
Auranofin 10x MIC	~5	~3
Vancomycin 1x MIC	~3	~1
Vancomycin 10x MIC	~4	~2

2.7 Evaluation of activity against preformed *Staphylococcal* biofilm

Briefly, *S. aureus* ATCC 29213 was cultured overnight in TSB supplemented with 1% glucose at 37°C with shaking. The overnight culture was diluted 1:100, 0.2 mL were transferred into 96 well polystyrene flat-bottom tissue culture plates, the lids were sealed with adhesive and incubated for 48 h at 37°C to allow biofilm formation. Subsequently, media was decanted, plates were rinsed gently with 1x PBS (pH 7.2) to remove the planktonic bacteria and plates were refilled with media containing different drug concentrations and incubated for 24 h at 37°C. Post drug treatment, media was once again decanted, and wells were rinsed with thrice with 1x PBS. The biofilm was fixed at 60°C for 1 h and stained by 0.06% crystal violet for 10 min. The wells were rinsed with PBS and bound crystal violet was diluted by 30% acetic acid (0.2 mL each) and quantified by measuring absorbance at 600 nm.[12]

2.8 Field Emission Scanning Electron Microscopy (FESEM) Analysis

An overnight culture of *S. aureus* ATCC 29213 was diluted 1:100 in TSB with 1% glucose. The *S. aureus* ATCC 29213 was tested at 1x and 8x of MIC of **1**. For each concentration, 4 mL of bacterial suspension was supplemented with desired amount of compound and added into individual wells in a 6–well plate. A cover slip (~10 ×10 mm) was kept in middle of each well. The plate was incubated at 37°C for 48 h to allow formation of biofilm on coverslips. The slide was rinsed three times with 1x PBS to remove planktonic cells and incubated with fresh media for 24 h at 37°C with above-mentioned drug concentrations. After incubation, coverslips were rinsed three times again with 1x PBS, followed by desiccation for 3 h. The samples were gold-coated (about 10 nm) using a gold sputtering unit and observed using a ZEISS Gemini SEM-Field Emission Scanning Electron Microscope.

2.9 *In vivo* efficacy in Murine Neutropenic Thigh Infection Model

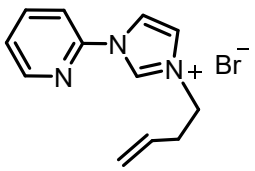
For *in vivo* evaluation of the antibacterial activity of **1**, balb/c mice weighing ~18–20 gm were rendered neutropenic by intraperitoneally (IP) administered cyclophosphamide injections (100 mg/Kg of body weight) given 24 h and 1 h before infection.[9] Following induction of neutropenia, thigh of mice was infected with ~10^9 cfu of *S. aureus* ATCC 29213. Post infection (3 h), **1** (10 mg/Kg) and vancomycin (25 mg/Kg) body weight, were injected IP into mice, twice at an interval of 3 h between injections. Control animals were administered saline in same volume and frequency as those receiving treatment. After 24 h, mice were sacrificed, thigh

tissue was collected, weighed and homogenized in 5 mL of saline. The homogenate was serially diluted and plated on MHA plates for cfu determination. After incubation for 18–24 h at 37°C, cfu were enumerated and the data was averaged across three experiments.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 6.0 software (GraphPad Software, La Jolla, CA, USA). Comparison between three or more groups was analyzed using one–way ANOVA, with post–hoc Tukey's multiple comparisons test. p–values of <0.05 were considered to be significant.

3. Spectroscopy data of [L¹H]Br, [L²H]Br, 1, 2 and 3



[L¹H]Br

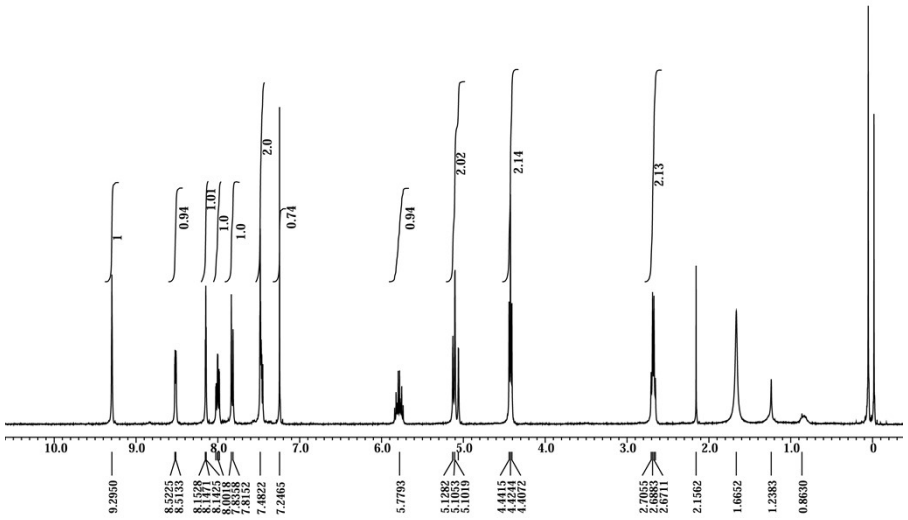


Figure S1. ¹H NMR of **[L¹H]Br** in $CDCl_3$.

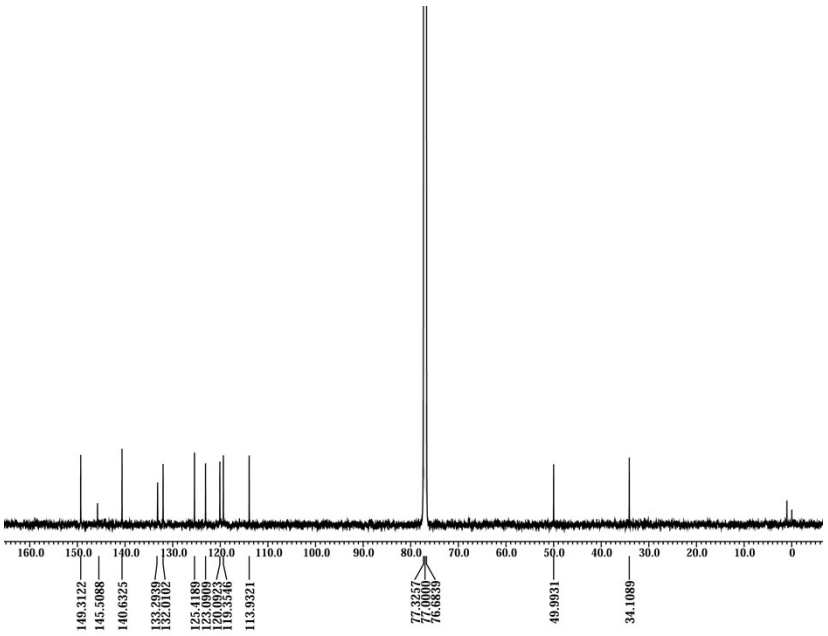
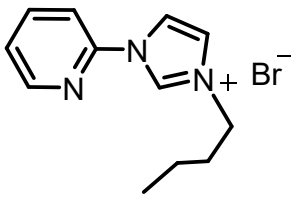


Figure S2. ¹³C NMR of **[L¹H]Br** in $CDCl_3$.



[L²H]Br

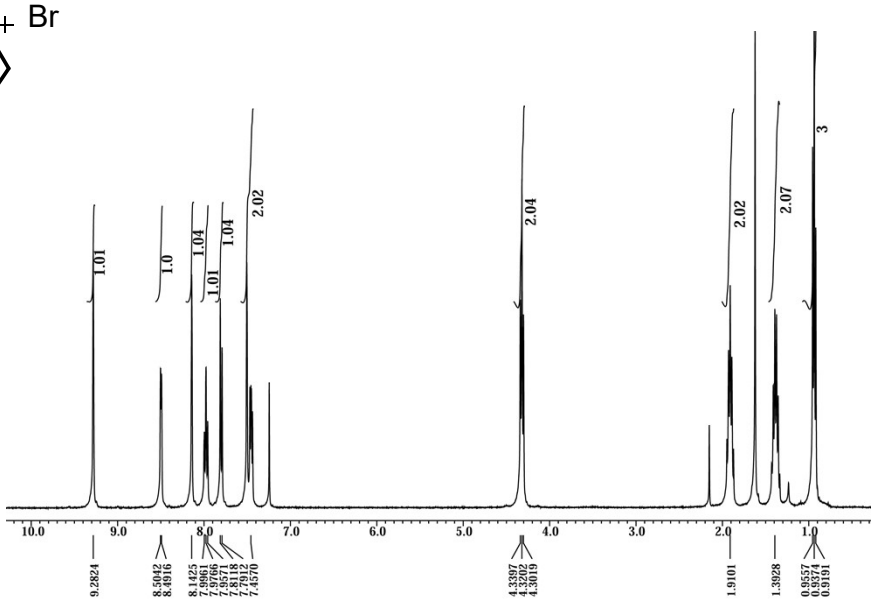


Figure S3. ^{1}H NMR of **[L²H]Br** in $CDCl_3$.

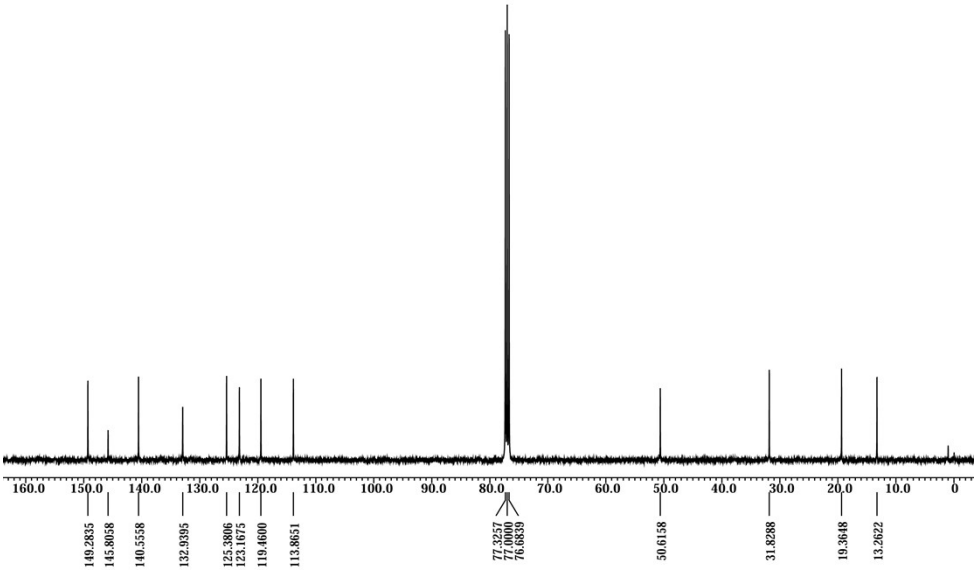


Figure S4. ^{13}C NMR of **[L²H]Br** in $CDCl_3$.

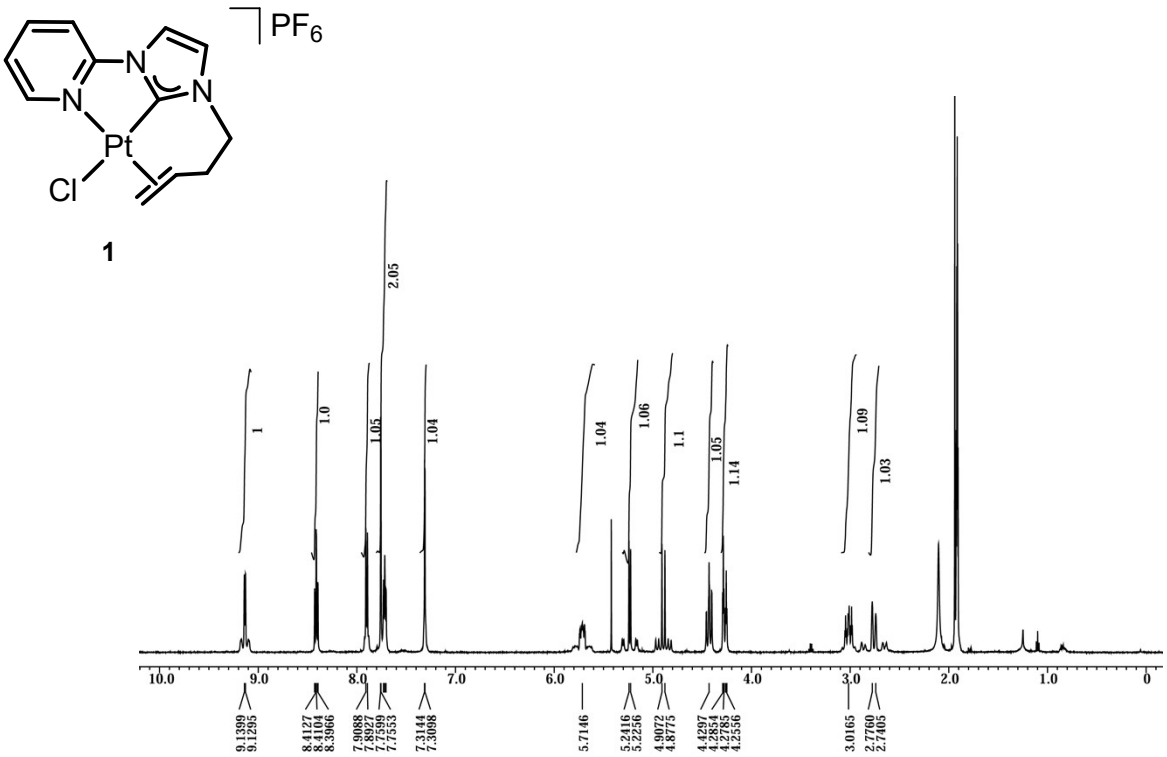


Figure S5. ^{1}H NMR of **1** in CD_3CN.

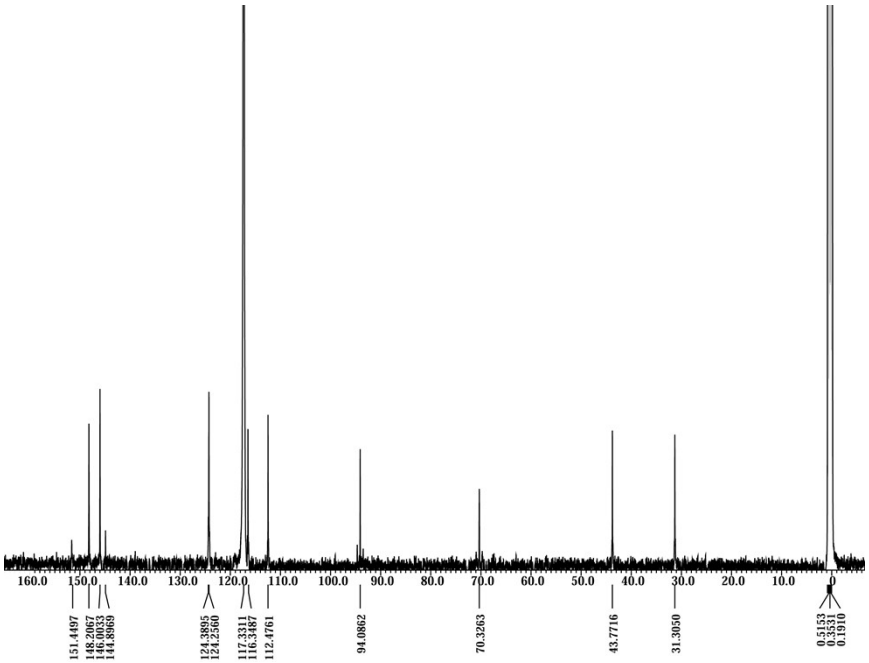


Figure S6. ^{13}C NMR of **1** in CD_3CN.

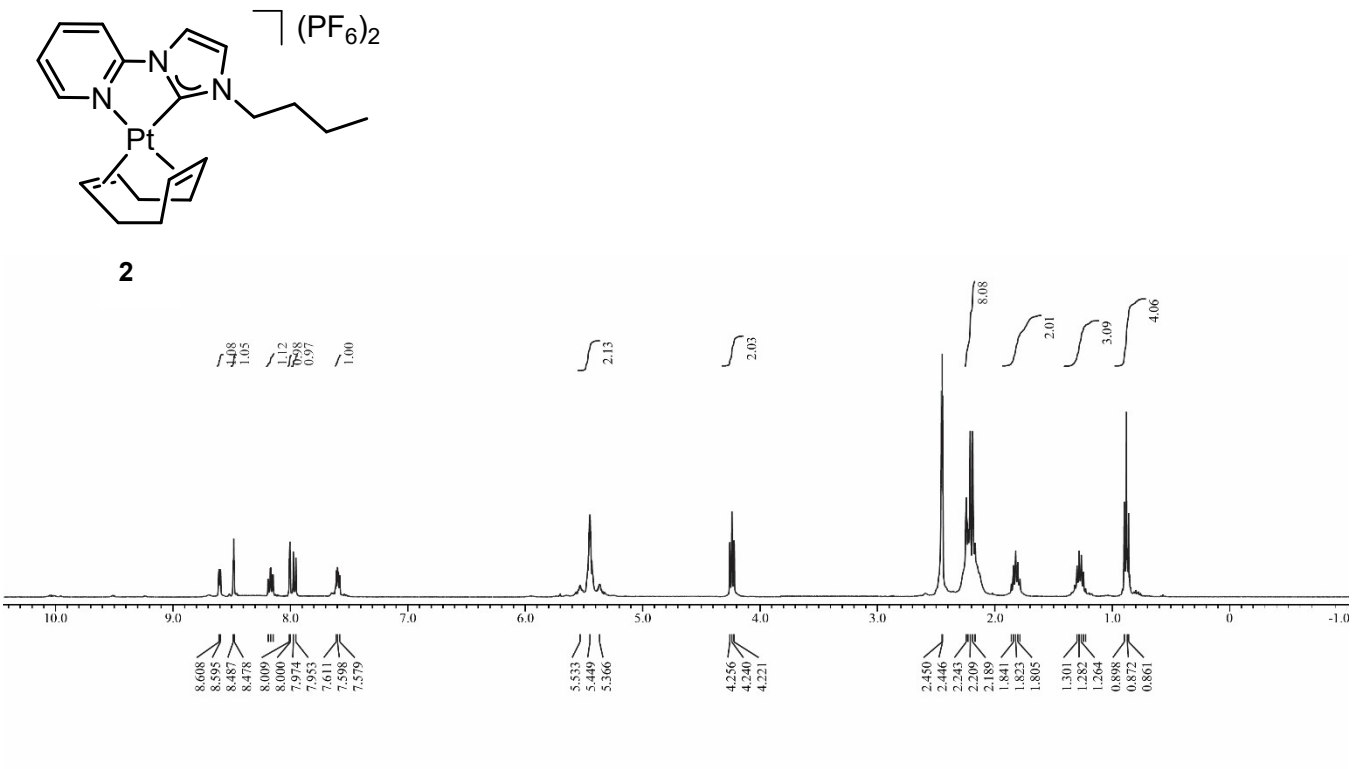


Figure S7. ^{1}H NMR of **2** in DMSO-d_6.

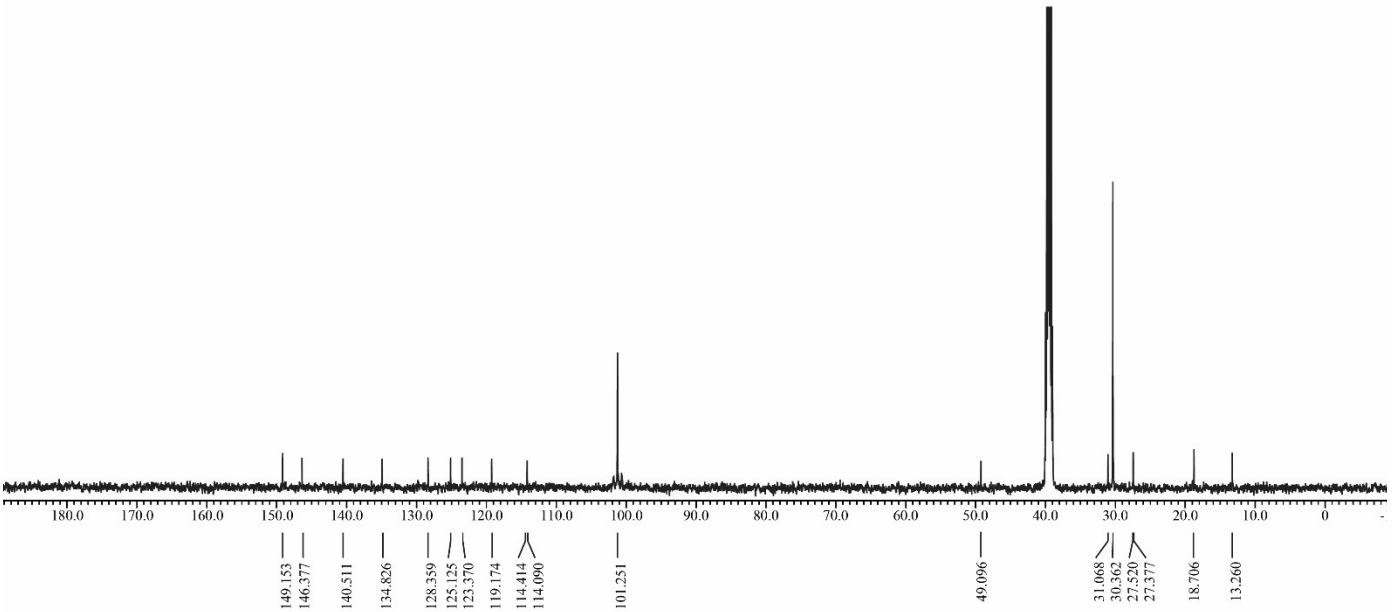


Figure S8. ^{13}C NMR of **2** in DMSO−d_6.

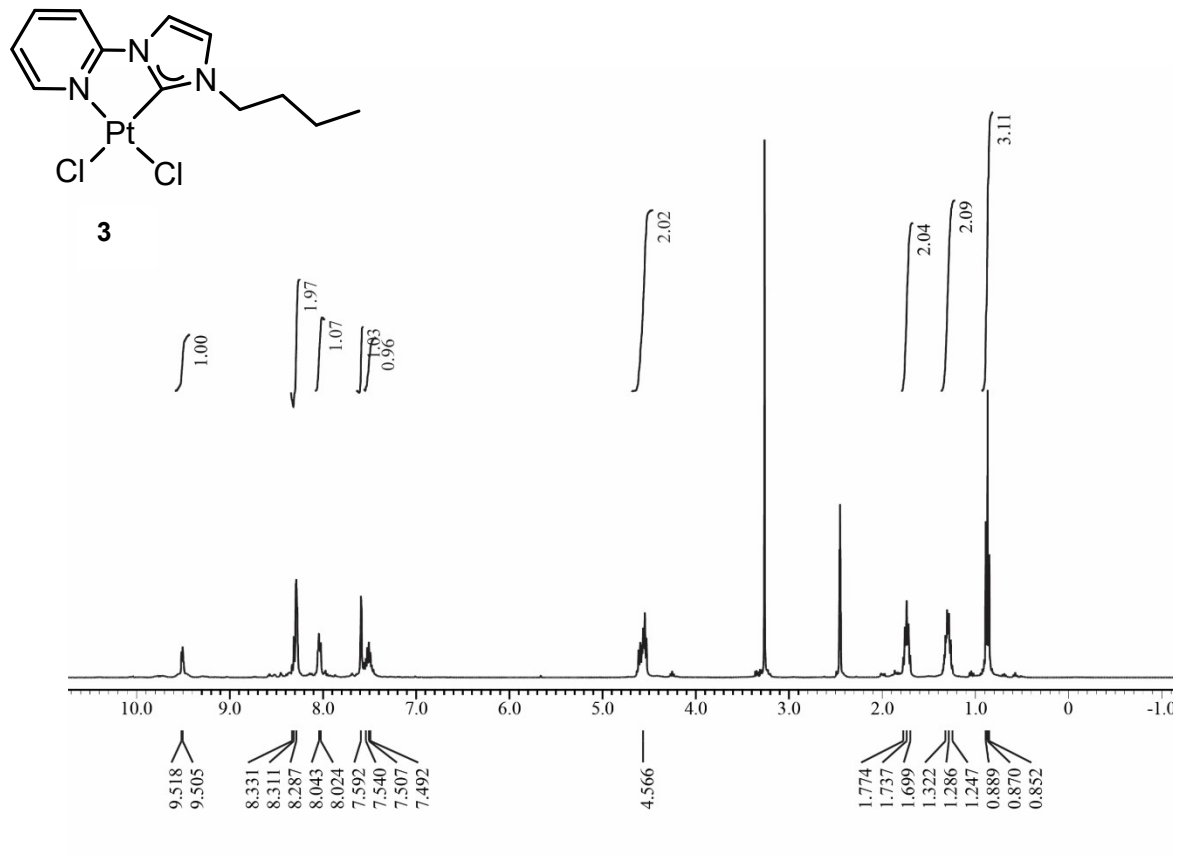


Figure S9. ^{1}H NMR of **3** in DMSO-d_6.

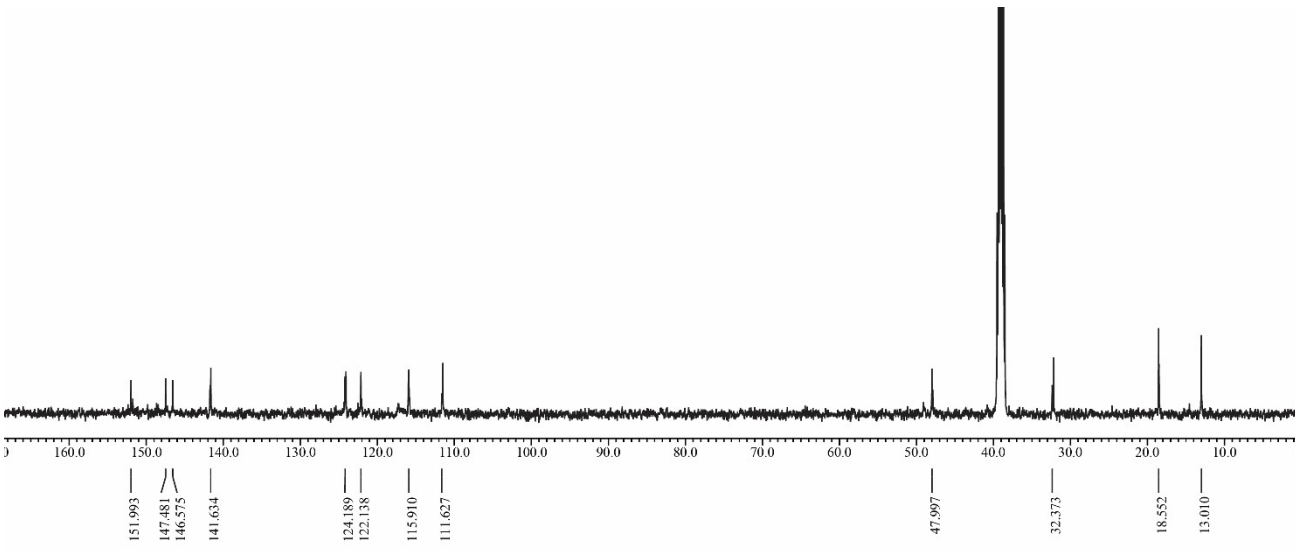


Figure S10. ^{13}C NMR of **3** in DMSO-d_6.

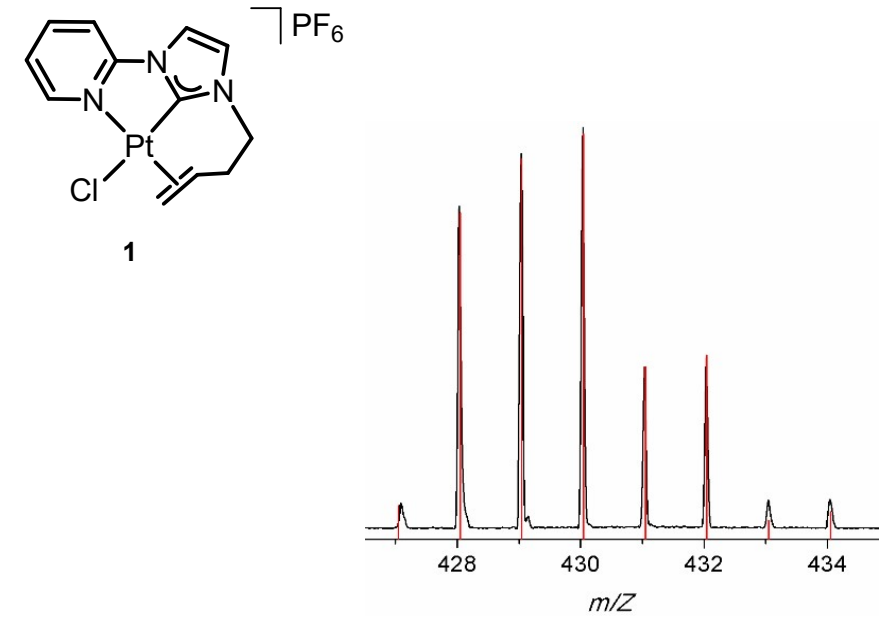


Figure S11. Simulated (red line) and experimental (black line) for [**1** – PF_6]$^+$.

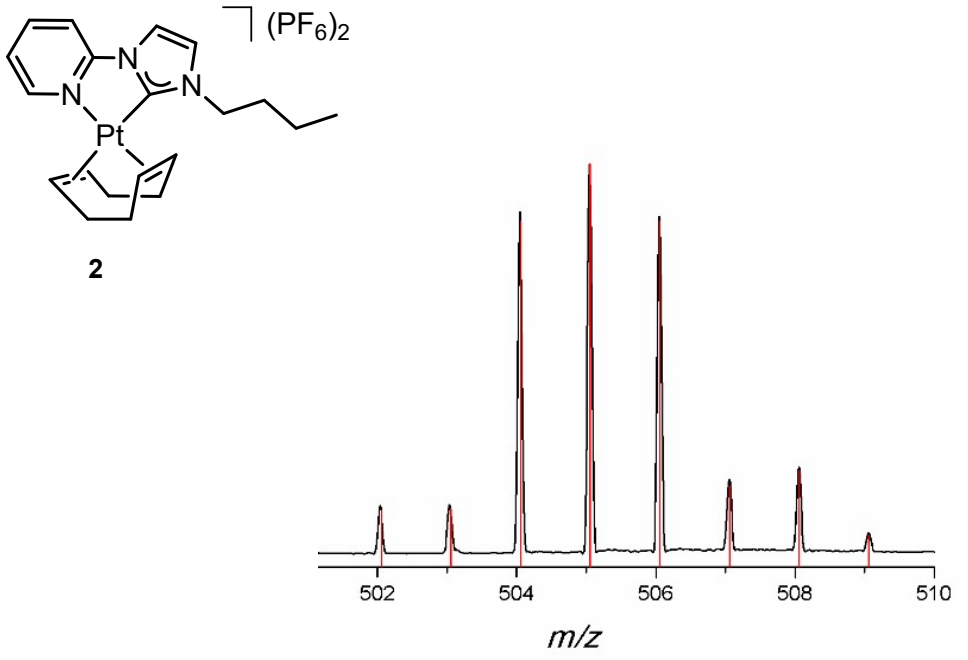


Figure S12. Simulated (red line) and experimental (black line) for [**2** – 2PF_6+H]$^+$.

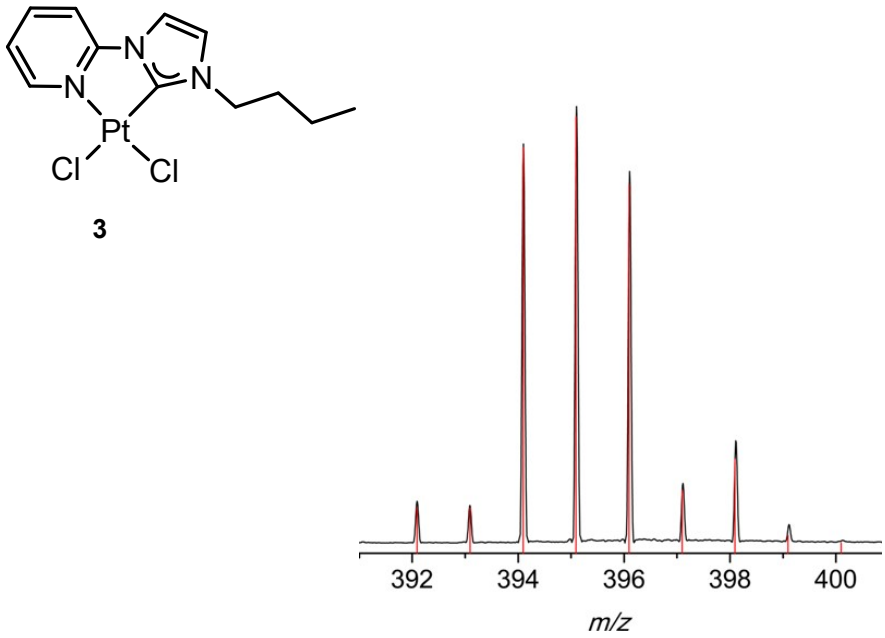


Figure S13. Simulated (red line) and experimental (black line) for [**3**−2Cl]$^{+}$

References

[1] E. Costa, P. G. Pringle, M. Ravetz, R. Puddephatt, *J. Inorg. Synth.***1996,** *31*, 284.
[2] SAINT + Software for CCD Diffractometers; Bruker AXS; Madison, W. I. **2000**.
[3] Sheldrick, G. M. *SADABS Program for Correction of Area Detector Data*; University of Göttingen: Göttingen, Germany, **1999**
[4] (a) *SHELXTL* Package v. 6.10; Bruker AXS; Madison, W. I. **2000**. (b) Sheldrick, G. M. *SHELXS–86 and SHELXL–97*; University of Göttingen: Göttingen, Germany, **1997**.
[5] Spek, A. L. *PLATON*; University of Utrecht: Netherlands, **2001**.
[6] Bradenburg, K. *Diamond, version 3.1eCrystal Impact GbR*; Bonn, Germany, **2005**
[7] CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. CLSI document M100-S21. Wayne, PA: Clinical and Laboratory Standards Institute; **2012.**
[8] P. R. Twentyman, M. Luscombe, *Br. J. Cancer* **1987**, *56*, 279–285.
[9] R. Thakare, A. K. Singh, S. Das, N. Vasudevan, G. R. Jachak, D. S. Reddy, A. Dasgupta, S. Chopra, *Int. J. Antimicrob. Agents* **2017**, *50*, 389–392.
[10] F. C. Odds, *J. Antimicrob. Chemother.* **2003**, *52*, 1.
[11] M. T. E. Suller, D. Lloyd, *J. Appl. Microbiol.* **2002**, *92*, 866–872.
[12] S. M. Kwasny, T. J. Opperman, *Curr. Protoc. Pharmacol.* **2010**, *50*, 13A.8.1-13A.8.23.

Author Contributions

[§]M. K, R.T & A.B equally contributed in this work