Novel Metal(II) Arene 2-Pyridinecarbothioamides: A Rationale to Orally Active Organometallic Anticancer Agents

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Experimental

Materials

All reactions were carried out in dry solvents under an inert atmosphere. Chemicals obtained from commercial suppliers were used as received and were of analytical grade. Methanol and dichloromethane were dried using standard procedures. OsO_4 (99.8%) and $RuCl_3 \cdot 3H_2O$ (40.4%) were purchased from Johnson Matthey, ubiquitin (bovine erythrocytes) and cytochrome-C from Sigma, α-terpinene and 4-fluoroaniline from Acros, L-histidine (His), 2-picoline, aniline and sodium sulfide nonahydrate from Merck, $N_2H_4 \cdot 2HCl$, 5'-deoxyguanosine monophosphate (5'-dGMP) and L-cysteine (Cys) from Fluka, 4-morpholinoaniline from Fisher and L-methionine (Met), 4-aminophenol, 2,4,6-trimethylaniline, 4-aminobenzophenone and sulfur from Sigma-Aldrich. The solvents for ESI-MS studies were methanol (VWR Int., HiPerSolv CHROMANORM), formic acid (Fluka) and milliQ water (18.2 MΩ, Synergy 185 UV Ultrapure Water System, Millipore, France). The dimers bis[dichlorido(η^6-*p*-cymene)ruthenium(II)],[1, 2] and bis[dichlorido(η^6-*p*-cymene)osmium(II)],[3] and the ligands *N*-phenyl- (**1**),[4] *N*-(4-hydroxyphenyl)- (**2**),[5] *N*-(4-fluorophenyl)- (**3**)[6] and *N*-(2,4,6-trimethylphenyl)-2-pyridinecarbothioamide (**4**)[7] were synthesized by adapting literature procedures.

Instrumentation

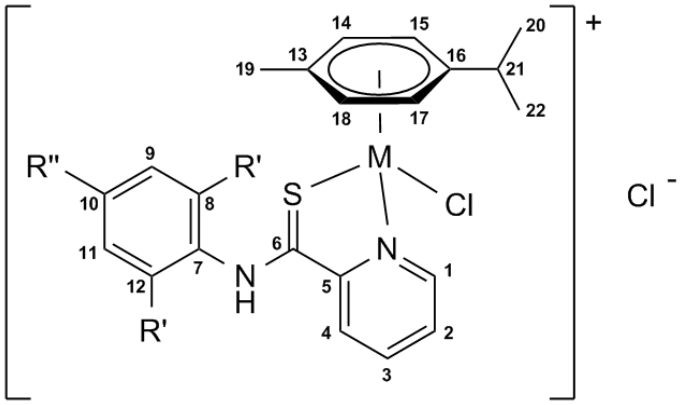
^1H and $^{13}C\{^1H\}$ NMR spectra were recorded at 25 °C on a Bruker FT NMR spectrometer Avance III 500 MHz at 500.10 (^1H) and 125.75 MHz ($^{13}C\{^1H\}$) and 2D NMR data were collected in a gradient-enhanced mode. Protons were numbered according to crystal structure numbering (see Figure 1). Elemental analysis was carried out on a Perkin–Elmer 2400 CHN Elemental Analyzer by the Laboratory for Elemental Analysis, Faculty of Chemistry, University of Vienna. UV–vis experiments were performed on a

temperature-controlled Perkin-Elmer Lambda 650 spectrophotometer using a Peltier element. ESI mass spectra were recorded on a Bruker AmaZon SL ion trap mass spectrometer (Bruker Daltonics GmbH, Bremen, Germany) by direct infusion at a flow rate of 3–4 µL/min. The following parameters were employed: capillary –3.5 kV, gas flow 6 psi, dry gas 6 L/min, dry temperature 180–200 °C, end plate offset –500 V and RF 69–71%. The spectra were recorded and processed using ESI Compass 1.3 and Data Analysis 4.0 software (both Bruker Daltonics GmbH, Bremen, Germany). Protein samples were additionally analyzed on a MaXis UHR ESI time-of-flight mass spectrometer (Bruker Daltonics, Bremen, Germany) employing the following parameters: capillary –4.5 kV, gas flow 8 psi, dry gas 6 L/min, dry temperature 150 °C, 400 Vpp funnel RF, 4 eV quadrupole ion energy and 100 µs transfer time. Samples were diluted to 2 µM using water/methanol/formic acid (50 : 50 : 0.2) and injected by direct infusion into the mass spectrometer at a flow rate of 3 µL/min. Spectra were recorded in positive ion mode over 0.5 min and averaged. The Data Analysis 4.0 software package (Bruker Daltonics, Bremen, Germany) was used for processing and maximum entropy deconvolution (automatic data point spacing and 30000 instrument resolving power).

X-ray diffraction measurements of single crystals were carried out on a Bruker X8 APEX II CCD diffractometer at 100 K (**2B**) and 200 K (**3A**). The crystals were positioned at 35 mm from the detector and the following data collection parameters were used: 1236 frames for 30 sec over 1° for **2B** and 788 frames for 10 s over 1° for **3A**. The data was processed using the SAINT Plus software package.[8] Crystal data, data collection parameters, and structure refinement details are given in Table S1, bond lengths and angles in Table S2. The structures were solved by direct methods and refined by full-matrix least-squares techniques. Non-hydrogen atoms were refined with anisotropic displacement parameters. H atoms were inserted at calculated positions and refined with a riding model. SHELX software programs were used for solving the structures and refinement.[9]

Synthesis

General procedure for the synthesis of *N*-substituted 2-pyridinecarbothioamides. The method of Klingele and Brooker[7] was adapted. In brief, a mixture of *N*-substituted aniline (25 mmol), sulfur (75 mmol) and sodium sulfide (0.5 mol%) was refluxed in 2-picoline (15 mL) for 48 h at 135 °C. The reaction mixture was cooled to room temperature and the solvent was evaporated under high vacuum. The residue was dissolved in dichloromethane, filtered through a pad of silica gel and washed with additional dichloromethane (100 mL). The solvent was removed under reduced pressure. After recrystallization in hot methanol, the product was filtered and dried.



NMR numbering scheme used for the metal(II) arene 2-pyridinecarbothioamides.

***N*-(4-Morpholinophenyl)-2-pyridinecarbothioamide (5).** 4-Morpholinoaniline (4.46 g, 25 mmol), sulfur (2.41 g, 75 mmol) and sodium sulfite (0.13 g, 0.5 mol%) were refluxed in 2-picoline (15 mL). After work-up and recrystallization from hot methanol, the orange product was filtered and dried. Yield: 5.00 g (88%). Elemental analysis found: C, 63.89; H, 5.88; N, 14.03; S, 10.95, calculated for $C_{16}H_{17}N_3OS$: C, 64.19; H, 5.72; N, 14.04; S, 10.71. ^{1}H NMR (500.10 MHz, $CDCl_3$, 25 °C): δ = 11.97 (s, 1H, -N*H*), 8.80 (d, $^3J_{(H3,H4)}$ = 8 Hz, 1H, H-4), 8.54 (d, $^3J_{(H1,H2)}$ = 5 Hz, 1H, H-1), 8.00 (d, $^3J_{(H8,H9)/(H11,H12)}$ = 9 Hz, 2H, H-9/H-11), 7.87 (td,

$^{3}J_{(H2,H3)/(H3,H4)}$ = 7.5 Hz, $^{4}J_{(H1,H3)}$ = 2 Hz, 1H, H-3), 7.45 (ddd, $^{3}J_{(H2,H3)}$ = 7.5 Hz, $^{3}J_{(H1,H2)}$ = 5 Hz, $^{4}J_{(H1,H2)/(H2,H3)}$ = 1 Hz, 1H, H-2), 7.00 (d, $^{3}J_{(H8,H9)/(H11,H12)}$ = 7 Hz, 2H, H-8/H-12), 3.89 (t, $^{3}J_{(H'1,H'2)/(H'3,H'4)}$ = 5 Hz, 4H, H'-2/H'-3), 3.22 (t, $^{3}J_{(H'1,H'2)/(H'3,H'4)}$ = 5 Hz, 4H, H'-1/H'-4) ppm. $^{13}C\{^{1}H\}$ NMR (125.75 MHz, $CDCl_3$, 25 °C): δ = 186.56 (C-6), 151.59 (C-5), 148.38 (C-10), 146.50 (C-1), 137.43 (C-3), 125.83 (C-2), 124.76 (C-4), 123.86 (C-9/C-11), 115.71 (C-8/C-12), 66.64 (C'-2/C'-3), 49.39 (C'-1/C'-4) ppm. MS (ESI^+): *m/z* 300.04 $[M + H]^+$ (m_{ex} = 300.11).

***N*-(4-Benzoylphenyl)-2-pyridinecarbothioamide (6).** 4-Aminobenzoylphenone (4.93 g, 25 mmol), sulfur (2.41 g, 75 mmol) and sodium sulfite (0.13 g, 0.5 mol%) were refluxed in 2-picoline (15 mL). After work-up and recrystallization from hot methanol, the yellow crystals were filtered and dried. Yield: 3.50 g (77%). Elemental analysis found: C, 71.53; H, 4.77; N, 8.84, calculated for $C_{19}H_{14}N_2OS$: C, 71.67; H, 4.43; N, 8.80. ^{1}H NMR (500.10 MHz, $CDCl_3$, 25 °C): δ = 12.33 (s, 1H, -N*H*), 8.80 (d, $^{3}J_{(H3,H4)}$ = 8 Hz, 1H, H-4), 8.58 (d, $^{3}J_{(H1,H2)}$ = 5 Hz, 1H, H-1), 8.31 (d, $^{3}J_{(H8,H9)/(H10,H11)}$ = 8.5 Hz, 2H, H-9/H-11), 7.94 (d, $^{3}J_{(H8,H9)/(H10,H11)}$ = 8.5 Hz, 2H, H-8/H-12), 7.92 (t, $^{3}J_{(H2,H3)/(H3,H4)}$ = 6.5 Hz, 1H, H-3), 7.82 (d, $^{3}J_{(H'3,H'4)/(H'6,H'7)}$ = 7.5 Hz, 2H, H'-3/H'-7), 7.60 (t, $^{3}J_{(H'4,H'5)/(H'5,H'6)}$ = 7.5 Hz, 1H, H'-5), 7.53 (t, $^{3}J_{(H1,H2)/(H2,H3)}$ = 5 Hz, 1H, H-2), 7.50 (t, $^{3}J_{(H'3,H'4)/(H'6,H'7)}$ = 7.5 Hz, 2H, H'-4/H'-6) ppm. $^{13}C\{^{1}H\}$ NMR (125.75 MHz, $CDCl_3$, 25 °C): δ = 195.42 (C'-1), 188.05 (C-6), 151.10 (C-5), 146.18 (C-1), 142.28 (C-7), 138.04 (C-3), 137.65 (C-10), 135.04 (C'-2), 132.38 (C'-5), 131.21 (C-8/C-12), 129.95 (C'-3/C'-7), 128.33 (C'-4/C'-6), 126.34 (C-2), 125.12 (C-4), 121.61 (C-9/C-11) ppm. MS (ESI^+): *m/z* 319.02 $[M + H]^+$ (m_{ex} = 319.09).

General procedure for the synthesis of [chlorido(η^6-p-cymene)(*N*-substituted 2-pyridinecarbothioamide)ruthenium(II)] chloride complexes. *N*-Substituted 2-pyridinecarbothioamide (2 eq.) was dissolved in dry methanol (20 mL) and heated to 40 °C under argon atmosphere. The ruthenium

dimer [Ru(η^6-p-cymene)Cl_2]$_2$ (1 eq.) was added under argon atmosphere and the reaction mixture was stirred for 4–18 h at 40 °C. The reaction mixture turned deep red upon addition of the dimer. The solvent was evaporated under reduced pressure and the solid residue was dissolved in dichloromethane and filtered. Hexane was added for precipitation. The pure product was obtained after filtration under suction and drying under vacuum at 40 °C.

[Chlorido(η^6-p-cymene)(*N*-phenyl-2-pyridinecarbothioamide)ruthenium(II)] chloride (1A). The compound was prepared following the general procedure using *N*-phenyl-2-pyridinecarbothioamide (70 mg, 0.326 mmol) and [Ru(η^6-p-cymene)Cl_2]$_2$ (100 mg, 0.163 mmol). The reaction time was 4 h. After precipitation with hexane, the solvent was decanted and the product was dried under reduced pressure to give a red solid. Yield: 152 mg (90%). Elemental analysis found: C, 49.09; H, 4.94; N, 5.17; S, 5.61, calculated for $C_{22}H_{24}Cl_2N_2SRu \cdot H_2O$: C, 49.07; H, 4.87; N, 5.20; S, 5.95. ^{1}H NMR (500.10 MHz, $CDCl_3$, 25 °C): δ = 14.57 (s, 1H, -N*H*), 9.73 (d, $^3J_{(H3,H4)}$ = 8 Hz, 1H, H-4), 9.40 (d, $^3J_{(H1,H2)}$ = 5 Hz, 1H, H-1), 8.15 (t, $^3J_{(H2,H3)/(H3,H4)}$ = 7.5 Hz, 1H, H-3), 7.89 (d, $^3J_{(H8,H9)/(H11,H12)}$ = 8 Hz, 2H, H-8/H-12), 7.60 (t, $^3J_{(H1,H2)/(H2,H3)}$ = 6 Hz, 1H, H-2), 7.50 (t, $^3J_{(H8,H9)/(H11,H12)}$ = 8 Hz, 2H, H-9/H-11), 7.40 (t, $^3J_{(H9,H10)/(H10,H11)}$ = 8 Hz, 1H, H-10), 5.72 (d, $^3J_{(H14,H15)}$ = 6 Hz, 1H, H-15), 5.62 (d, $^3J_{(H17,H18)}$ = 6 Hz, 1H, H-18), 5.58 (d, $^3J_{(H17,H18)}$ = 6 Hz, 1H, H-17), 5.42 d, $^3J_{(H14,H15)}$ = 6 Hz, 1H, H-14), 2.78 (sept, $^3J_{(H20,H21)/(H21,H22)}$ = 7 Hz, 1H, H-21), 2.22 (s, 3H, H-19), 1.23 (d, $^3J_{(H20,H21)}$ = 7 Hz, 3H, H-20), 1.17 (d, $^3J_{(H21,H22)}$ = 7 Hz, 3H, H-22) ppm. ^{13}C{^{1}H} NMR (125.75 MHz, $CDCl_3$, 25 °C): δ = 190.83 (C-6), 157.43 (C-1), 153.82 (C-5), 140.16 (C-3), 137.64 (C-7), 129.25 (C-9/C-11), 129.06 (C-2), 128.80 (C-10), 127.80 (C-4), 125.48 (C-8/C-12), 106.61 (C-16), 102.90 (C-13), 87.58 (C-15), 87.13 (C-18), 84.76 (C-17), 84.06 (C-14), 31.04 (C-21), 22.63 (C-20), 21.90 (C-22), 18.73 (C-19) ppm. MS (ESI$^+$): *m/z* 448.88 $[M - Cl - H]^+$ (m_{ex} = 448.57).

[Chlorido(η^6-p-cymene)(*N*-{4-hydroxyphenyl}-2-pyridinecarbothioamide)ruthenium(II)] chloride (2A). The compound was prepared following the general procedure using *N*-(4-hydroxyphenyl)-2-pyridinecarbothioamide (76 mg, 0.326 mmol) and [Ru(η^6-p-cymene)Cl_2]$_2$ (100 mg, 0.163 mmol). The reaction time was 18 h. A red microcrystalline product was obtained after filtration. Yield: 71 mg (41%). Elemental analysis found: C, 47.54; H, 4.80; N, 4.89; S, 5.44, calculated for $C_{22}H_{24}Cl_2N_2OSRu \cdot H_2O$: C, 47.65; H, 4.73; N, 5.05; S, 5.78. ^{1}H NMR (500.10 MHz, d_4-MeOD, 25 °C): δ = 9.66 (d, $^3J_{(H1,H2)}$ = 5.5 Hz, 1H, H-1), 8.40 (d, $^3J_{(H3,H4)}$ = 8 Hz, 1H, H-4), 8.28 (t, $^3J_{(H2,H3)/(H3,H4)}$ = 7.5 Hz, 1H, H-3), 7.84 (t, $^3J_{(H1,H2)/(H2,H3)}$ = 6 Hz, 1H, H-2), 7.46 (d, $^3J_{(H8,H9)/(H11,H12)}$ = 8.5 Hz, 2H, H-9/H-11), 6.96 (d, $^3J_{(H8,H9)/(H11,H12)}$ = 8.5 Hz, 2H, H-8/H-12), 6.04 (d, $^3J_{(H14,H15)}$ = 6 Hz, 1H, H-15), 5.94 (d, $^3J_{(H17,H18)}$ = 6 Hz, 1H, H-18), 5.90 (d, $^3J_{(H17,H18)}$ = 6 Hz, 1H, H-17), 5.63 (d, $^3J_{(H14,H15)}$ = 6 Hz, 1H, H-14), 2.75 (sept, $^3J_{(H20,H21)/(H21,H22)}$ = 7 Hz, 1H, H-21), 2.21 (s, 3H, H-19), 1.21 (d, $^3J_{(H20,H21)}$ = 7 Hz, 3H, H-20), 1.14 (d, $^3J_{(H21,H22)}$ = 7 Hz, 3H, H-22) ppm. ^{13}C{^{1}H} NMR (125.75 MHz, d_4-MeOD, 25 °C): δ = 192.92 (C-6), 160.17 (C-1), 159.62 (C-10), 154.84 (C-5), 141.15 (C-3), 130.70 (C-2), 130.47 (C-7), 127.69 (C-9/C-11), 124.85 (C-4), 117.24 (C-8/C-12), 107.29 (C-16), 105.50 (C-13), 89.27 (C-15), 89.24 (C-18), 86.68 (C-17), 84.92 (C-14), 32.43 (C-21), 22.96 (C-20), 21.95 (C-22), 18.85 (C-19) ppm. MS (ESI$^+$): *m/z* 464.86 $[M - Cl - H]^+$ (m_{ex} = 464.57).

[Chlorido(η^6-p-cymene)(*N*-{4-fluorophenyl}-2-pyridinecarbothioamide)ruthenium(II)] chloride (3A). The compound was prepared following the general procedure using *N*-(4-fluorophenyl)-2-pyridinecarbothioamide (100 mg, 0.431 mmol) and [Ru(η^6-p-cymene)Cl_2]$_2$ (132 mg, 0.215 mmol). The reaction time was 4 h. A dark red solid was obtained after filtration. Yield: 210 mg (90%). Elemental analysis found: C, 47.71; H, 4.38; N, 5.30; S, 6.10, calculated for $C_{22}H_{23}Cl_2N_2FSRu \cdot H_2O$: C, 47.48; H, 4.53; N, 5.04; S, 5.75. ^{1}H NMR (500.10 MHz, d_4-MeOD, 25 °C): δ = 9.68 (d, $^3J_{(H1,H2)}$ = 5 Hz, 1H, H-1), 8.47 (d, $^3J_{(H3,H4)}$ = 8 Hz, 1H, H-4), 8.30 (t, $^3J_{(H2,H3)/(H3,H4)}$ = 7.5 Hz, 1H, H-3), 7.86 (t, $^3J_{(H1,H2)/(H2,H3)}$ = 6.5 Hz, 1H, H-

2), 7.63 (m, 2H, H-9/H-11), 7.34 (t, $^{3}J_{(H7,H8)/(H11,H12)}$ = 8 Hz, 2H, H-8/H-12), 6.05 (d, $^{3}J_{(H14,H15)}$ = 5.5 Hz, 1H, H-15), 5.94 (d, $^{3}J_{(H17,H18)}$ = 5.5 Hz, 1H, H-18), 5.92 (d, $^{3}J_{(H17,H18)}$ = 5.5 Hz, 1H, H-17), 5.65 (d, $^{3}J_{(H14,H15)}$ = 5.5 Hz, 1H, H-14), 2.75 (sept, $^{3}J_{(H20,H21)/(H21,H22)}$ = 6.5 Hz, 1H, H-21), 2.21 (s, 3H, H-19), 1.21 (d, $^{3}J_{(H20,H21)}$ = 6.5 Hz, 3H, H-20), 1.14 (d, $^{3}J_{(H21,H22)}$ = 6.5 Hz, 3H, H-22) ppm. $^{13}C\{^{1}H\}$ NMR (125.75 MHz, d_4-MeOD, 25 °C): δ = 194.06 (C-6), 163.71 (C-10), 160.20 (C-1), 154.84 (C-5), 141.17 (C-3), 135.73 (C-7), 130.89 (C-2), 128.79 (C-9/C-11), 125.16 (C-4), 117.76 (C-8/C-12), 107.46 (C-16), 105.48 (C-13), 89.32 (C-15), 89.21 (C-18), 86.73 (C-17), 85.08 (C-14), 32.43 (C-21), 22.96 (C-20), 21.97 (C-22), 18.86 (C-19) ppm. MS (ESI^{+}): *m/z* 466.88 $[M - Cl - H]^{+}$ (m_{ex} = 466.57).

[Chlorido(η^{6}-p-cymene)(*N*-{2,4,6-trimethylphenyl}-2-pyridinecarbothioamide)ruthenium(II)] chloride (4A). The compound was prepared following the general procedure using *N*-(2,4,6-trimethylphenyl)-2-pyridinecarbothioamide (83 mg, 0.326 mmol) and $[Ru(\eta^{6}\text{-p-cymene})Cl_2]_2$ (100 mg, 0.163 mmol). The reaction time was 4 h. An intense red powder was obtained after filtration. Yield: 154 mg (84%). Elemental analysis found: C, 51.97; H, 5.25; N, 4.92; S, 5.59, calculated for $C_{25}H_{30}Cl_2N_2SRu{\cdot}H_2O$: C, 51.72; H, 5.56; N, 4.83; S, 5.52. ^{1}H NMR (500.10 MHz, d_4-MeOD, 25 °C): δ = 9.71 (d, $^{3}J_{(H1,H2)}$ = 5.5 Hz, 1H, H-1), 8.47 (d, $^{3}J_{(H3,H4)}$ = 5.5 Hz, 1H, H-4), 8.34 (t, $^{3}J_{(H2,H3)/(H3,H4)}$ = 8 Hz, 1H, H-3), 7.88 (t, $^{3}J_{(H1,H2)/(H2,H3)}$ = 6 Hz, 1H, H-2), 7.12 (s, 1H, H-9), 7.07 (s, 1H, H-11), 6.05 (d, $^{3}J_{(H14,H15)}$ = 6 Hz, 1H, H-15), 5.99 (d, $^{3}J_{(H17,H18)}$ = 6 Hz, 1H, H-18), 5.91 (d, $^{3}J_{(H17,H18)}$ = 6 Hz, 1H, H-17), 5.56 (d, $^{3}J_{(H14,H15)}$ = 6 Hz, 1H, H-14), 2.73 (sept, $^{3}J_{(H20,H21)/(H21,H22)}$ = 7 Hz, 1H, H-21), 2.36 (s, 3H, C_{ar}-CH_3), 2.23 (s, 3H, C_{ar}-CH_3), 2.23 (s, 3H, H-19), 2.14 (s, 3H, C_{ar}-CH_3), 1.19 (d, $^{3}J_{(H20,H21)}$ = 7 Hz, 3H, H-20), 1.11 (d, $^{3}J_{(H21,H22)}$ = 7 Hz, 3H, H-22) ppm. $^{13}C\{^{1}H\}$ NMR (125.75 MHz, d_4-MeOD, 25 °C): δ = 193.76 (C-6), 160.44 (C-1), 153.66 (C-5), 141.40 (C-3), 141.13 (C-7), 136.00 (C_{ar}-CH_3), 135.35 (C_{ar}-CH_3), 133.92 (C_{ar}-CH_3), 131.08 (C-2), 131.03 (C-9), 130.79 (C-11), 124.84 (C-4), 107.22 (C-16), 106.28 (C-13), 89.65 (C-18), 88.84 (C-15), 87.07 (C-17), 83.97 (C-

14), 32.47 (C-21), 22.95 (C-20), 22.04 (C-22), 21.18 (C_{ar}-CH_3), 18.99 (C-19), 17.83 (C_{ar}-CH_3), 17.64 (C_{ar}-CH_3) ppm. MS (ESI^+): *m/z* 490.91 $[M - Cl - H]^+$ (m_{ex} = 490.65).

[Chlorido(η^6-p-cymene)(*N*-{4-morpholinophenyl}-2-pyridinecarbothioamide)ruthenium(II)] chloride (5A). The compound was prepared following the general procedure using *N*-(4-morpholinoylphenyl)-2-pyridinecarbothioamide (98 mg, 0.326 mmol) and $[Ru(\eta^6\text{-p-cymene})Cl_2]_2$ (100 mg, 0.163 mmol). The reaction time was 18 h. A dark red solid was obtained after filtration. Yield: 145 mg (73%). Elemental analysis found: C, 49.90; H, 5.69; N, 6.69; S, 4.71, calculated for $C_{26}H_{31}Cl_2N_3OSRu \cdot 1.25H_2O$: C, 49.72; H, 5.38; N, 6.69; S, 5.09. ^{1}H NMR (500.10 MHz, $CDCl_3$, 25 °C): δ = 14.37 (s, 1H, -N*H*), 9.60 (brs, 1H, H-4), 9.38 (brs, 1H, H-1), 8.09 (brs, 1H, H-3), 7.93 (brs, 2H, H-9/H-11), 7.57 (brs, 1H, H-2), 7.05 (brs, 2H, H-8/H-11), 5.71 (d, $^3J_{(H14,H15)}$ = 5.5 Hz, 1H, H-15), 5.60 (brs, 1H, H-17), 5.57 (brs, 1H, H-18), 5.41 (d, $^3J_{(H14,H15)}$ = 5.5 Hz, 1H, H-14), 3.91 (brs, 4H, H'-2/H'-3), 3.27 (brs, 4H, H'-1/H'-4), 2.78 (sept, $^3J_{(H20,H21)/(H21,H22)}$ = 6.5 Hz, 1H, H-21), 2.20 (s, 3H, H-19), 1.22 (d, $^3J_{(H20,H21)}$ = 6.5 Hz, 3H, H-20), 1.15 (d, $^3J_{(H21,H22)}$ = 6.5 Hz, 3H, H-22) ppm. $^{13}C\{^1H\}$ NMR (125.75 MHz, $CDCl_3$, 25 °C): δ = 157.31 (C-1), 154.16 (C-5), 139.90 (C-3), 128.71 (C-2), 127.40 (C-4), 126.37 (C-9/C-11), 116.00 (C-8/C-12), 106.37 (C-16), 102.74 (C-13), 87.63 (C-15), 86.97 (C-17), 84.62 (C-18), 83.99 (C-14), 66.19 (C'-2/C'-3), 49.51 (C'-1/C'-4), 31.01 (C-21), 22.66 (C-20), 21.86 (C-22), 18.71 (C-19) ppm. MS (ESI^+): *m/z* 533.97 $[M - Cl - H]^+$ (m_{ex} = 533.67).

[Chlorido(η^6-p-cymene)(*N*-{4-benzoylphenyl}-2-pyridinecarbothioamide)ruthenium(II)] chloride (6A). The compound was prepared following the general procedure using *N*-(4-benzoylphenyl)-2-pyridinecarbothioamide (104 mg, 0.326 mmol) and $[Ru(\eta^6\text{-p-cymene})Cl_2]_2$ (100 mg, 0.163 mmol). The reaction time was 6 h. Violet-red crystals were obtained after filtration. Yield: 106 mg (50%). Elemental

analysis found: C, 53.91; H, 4.48; N, 4.36; S, 4.81, calculated for $C_{29}H_{28}Cl_2N_2OSRu \cdot H_2O$: C, 54.20; H, 4.71; N, 4.36; S, 4.99. ^{1}H NMR (500.10 MHz, d_4-MeOD, 25 °C): δ = 9.71 (d, $^3J_{(H1,H2)}$ = 5 Hz, 1H, H-1), 8.54 (d, $^3J_{(H3,H4)}$ = 8 Hz, 1H, H-4), 8.34 (t, $^3J_{(H2,H3)/(H3,H4)}$ = 7.5 Hz, 1H, H-3), 7.99 (d, $^3J_{(H8,H9)/(H11/H12)}$ = 8.5 Hz, 2H, H-9/H-11), 7.89 (t, $^3J_{(H1,H2)/(H2,H3)}$ = 6.5 Hz, 1H, H-2), 7.86 (d, $^3J_{(H8,H9)/(H11/H12)}$ = 8.5 Hz, 2H, H-8/H-12), 7.83 (d, $^3J_{(H‘3,H‘4)/(H‘6/H‘7)}$ = 7.5 Hz, 2H, H‘-3/H‘-7), 7.70 (t, $^3J_{(H‘4,H‘5)/(H‘5,H‘6)}$ = 7.5 Hz, 1H, H‘-5), 7.58 (d, $^3J_{(H‘3,H‘4)/(H‘6/H‘7)}$ = 7.5 Hz, 2H, H‘-4/H‘-6), 6.11 (d, $^3J_{(H14,H15)}$ = 6 Hz, 1H, H-15), 5.99 (d, $^3J_{(H17,H18)}$ = 6 Hz, 1H, H-18), 5.97 (d, $^3J_{(H17,H18)}$ = 6 Hz, 1H, H-17), 5.70 (d, $^3J_{(H14,H15)}$ = 6 Hz, 1H, H-14), 2.77 (sept, $^3J_{(H20,H21)/(H21,H22)}$ = 7 Hz, 1H, H-21), 2.23 (s, 3H, H-19), 1.22 (d, $^3J_{(H20,H21)}$ = 7 Hz, 3H, H-20), 1.15 (d, $^3J_{(H21,H22)}$ = 7 Hz, 3H, H-22) ppm. ^{13}C{^{1}H} NMR (125.75 MHz, d_4-MeOD, 25 °C): δ = 197.03 (C’-1), 194.39 (C-6), 160.31 (C-1), 154.85 (C-5), 142.76 (C-7), 141.24 (C-3), 138.94 (C-10), 138.44 (C’-2), 134.23 (C’-5), 132.50 (C-9/C-11), 131.06 (C-2, C’-3/C’-7), 129.76 (C’-4/C’-6), 126.27 (C-8/C-12), 125.43 (C-4), 107.80 (C-16), 105.76 (C-13), 89.41 (C-15/C-17), 86.99 (C-18), 85.27 (C-14), 32.48 (C-21), 22.98 (C-20), 22.00 (C-22), 18.89 (C-19) ppm. MS (ESI$^+$): *m/z* 552.92 $[M - Cl - H]^+$ (m_{ex} = 552.67).

General procedure for the synthesis of [chlorido(η^6-p-cymene){*N*-substituted 2-pyridinecarbothioamide}osmium(II)]$^+$ complexes. *N*-Substituted 2-pyridinecarbothioamide (2 eq.) was dissolved in dry methanol (20 mL) and heated to 40 °C under argon atmosphere. The osmium dimer $[Os(\eta^6\text{-p-cymene})Cl_2]_2$ (1 eq.) was added under argon atmosphere and the reaction mixture was stirred for 4–5 h at 40 °C. The reaction mixture turned deep red upon addition of the osmium dimer. The solvent was evaporated under reduced pressure and the solid residue was redissolved in dichloromethane and filtered. Hexane was added for precipitation in the fridge. The product was obtained after filtration and drying under vacuum at 40 °C.

[Chlorido(η^6-p-cymene)(*N*-phenyl-2-pyridinecarbothioamide)osmium(II)] chloride (1B). The compound was prepared following the general procedure using *N*-phenyl-2-pyridinecarbothioamide (54 mg, 0.253 mmol) and [Os(η^6-p-cymene)Cl_2]$_2$ (100 mg, 0.127 mmol). The reaction mixture was stirred for 5 h at 40 °C. After work-up and precipitation with hexane, the solvent was decanted and the product was dried *in vacuo* to yield a deep red solid. Yield: 87 mg (57%). Elemental analysis, found: C, 42.41; H, 4.02; N, 4.73; S, 4.70, calculated for $C_{22}H_{24}Cl_2N_2SOs \cdot H_2O$: C, 42.03; H, 4.17; N, 4.46; S, 5.09. ^{1}H NMR (500.10 MHz, $CDCl_3$, 25 °C): δ = 14.32 (s, 1H, -N*H*), 9.85 (d, $^3J_{(H3,H4)}$ = 8 Hz, 1H, H-4), 9.32 (d, $^3J_{(H1,H2)}$ = 5 Hz, 1H, H-1), 8.13 (t, $^3J_{(H2,H3)/(H3,H4)}$ = 7.5 Hz, 1H, H-3), 7.92 (d, $^3J_{(H8,H9)/(H11,H12)}$ = 8 Hz, 2H, H-8/H-12), 7.60 (t, $^3J_{(H1,H2)/(H2,H3)}$ = 6 Hz, 1H, H-2), 7.53 (t, $^3J_{(H8,H9)/(H11,H12)}$ = 8 Hz, 2H, H-9/H-11), 7.41 (t, $^3J_{(H9,H10)/(H10,H11)}$ = 8 Hz, 1H, H-10), 5.92 (d, $^3J_{(H14,H15)}$ = 5.5 Hz, 1H, H-15), 5.83 (d, $^3J_{(H17,H18)}$ = 5.5 Hz, 1H, H-17), 5.81 (d, $^3J_{(H17,H18)}$ = 5.5 Hz, 1H, H-18), 5.62 (d, $^3J_{(H14,H15)}$ = 5.5 Hz, 1H, H-14), 2.71 (sept, $^3J_{(H20,H21)/(H21,H22)}$ = 7 Hz, 1H, H-21), 2.32 (s, 3H, H-19), 1.24 (d, $^3J_{(H20,H21)}$ = 7 Hz, 3H, H-20), 1.15 (d, $^3J_{(H20,H22)}$ = 7 Hz, 3H, H-22) ppm. ^{13}C{^{1}H} NMR (125.75 MHz, $CDCl_3$, 25 °C): δ = 193.52 (C-6), 159.15 (C-1), 153.81 (C-5), 140.43 (C-3), 137.57 (C-7), 130.59 (C-2), 129.27 (C-9/C-11), 128.74 (C-10), 128.33 (C-4), 125.59 (C-8/C-12), 97.71 (C-16), 95.99 (C-13), 79.66 (C-15), 79.19 (C-16), 76.85 (C-17), 74.66 (C-14), 31.16 (C-21), 23.04 (C-20), 22.16 (C-22), 18.69 (C-19) ppm. MS (ESI$^+$): *m/z* 539.01 $[M - Cl - H]^+$ (m_{ex} = 539.12).

[Chlorido(η^6-p-cymene)(*N*-{4-hydroxyphenyl}-2-pyridinecarbothioamide)osmium(II)] chloride (2B). The compound was prepared following the general procedure using *N*-(4-hydroxyphenyl)-2-pyridinecarbothioamide (58 mg, 0.253 mmol) and [Os(η^6-p-cymene)Cl_2]$_2$ (100 mg, 0.127 mmol). The reaction time was 4 h and the product was obtained as a dark red crystalline solid. Yield: 75 mg (47%). Elemental analysis, found: C, 41.49; H, 3.76; N, 4.45; S, 4.80; O, 3.52, calculated for $C_{22}H_{24}Cl_2N_2OSOs \cdot 0.5H_2O$: C, 41.63; H, 3.97; N, 4.41; S, 5.05; O, 3.78. ^{1}H NMR (500.10 MHz, d_4-MeOD,

25 °C): δ = 9.57 (d, $^{3}J_{(H1,H2)}$ = 5 Hz, 1H, H-1), 8.47 (d, $^{3}J_{(H3,H4)}$ = 8 Hz, 1H, H-4), 8.26 (t, $^{3}J_{(H2,H3)/(H3,H4)}$ = 7.5 Hz, 1H, H-3), 7.79 (t, $^{3}J_{(H1,H2)/(H2,H3)}$ = 7.5 Hz, 1H, H-2), 7.46 (d, $^{3}J_{(H8,H9)/(H11,H12)}$ = 9 Hz, 2H, H-9/H-11), 6.96 (d, $^{3}J_{(H8,H9)/(H11,H12)}$ = 9 Hz, 2H, H-8/H-12), 6.21 (d, $^{3}J_{(H14,H15)}$ = 5.5 Hz, 1H, H-15), 6.11 (d, $^{3}J_{(H17,H18)}$ = 5.5 Hz, 1H, H-17), 6.06 (d, $^{3}J_{(H17,H18)}$ = 5.5 Hz, 1H, H-18), 5.80 (d, $^{3}J_{(H14,H15)}$ = 5.5 Hz, 1H, H-14), 2.65 (sept, $^{3}J_{(H20,H21)/(H21,H22)}$ = 7 Hz, 1H, H-21), 2.28 (s, 3H, H-19), 1.20 (d, $^{3}J_{(H20,H21)}$ = 7 Hz, 3H, H-20), 1.09 (d, $^{3}J_{(H21,H22)}$ = 7 Hz, 3H, H-22) ppm. $^{13}C\{^{1}H\}$ NMR (125.75 MHz, d_4-MeOD, 25 °C): δ = 196.25 (C-6), 161.26 (C-1), 159.57 (C-10), 154.97 (C-5), 141.23 (C-3), 131.70 (C-2), 130.40 (C-7), 127.71 (C-9/C-11), 125.21 (C-4), 117.27 (C-8/C-12), 98.74 (C-16), 98.69 (C-13), 81.38 (C-15), 81.01 (C-18), 78.55 (C-17), 75.42 (C-14), 32.54 (C-21), 23.35 (C-20), 22.19 (C-22), 18.72 (C-19) ppm. MS (ESI^{+}): *m/z* 555.05 $[M - Cl - H]^{+}$ (m_{ex} = 555.11).

[Chlorido(η^{6}-p-cymene)(*N*-{4-fluorophenyl}-2-pyridinecarbothioamide)osmium(II)] chloride (3B). The compound was prepared following the general procedure using *N*-(4-fluorophenyl)-2-pyridinecarbothioamide (59 mg, 0.253 mmol) and $[Os(\eta^{6}\text{-p-cymene})Cl_2]_2$ (100 mg, 0.127 mmol). The reaction time was 4 h and the product was obtained as a dark red solid. Yield: 117 mg (74%). Elemental analysis, found: C, 40.94; H, 3.81; N, 4.33; S, 4.75, calculated for $C_{22}H_{23}Cl_2FN_2SOs \cdot H_2O$: C, 40.93; H, 3.90; N, 4.34; S, 4.97. ^{1}H NMR (500.10 MHz, $CDCl_3$, 25 °C): δ = 14.36 (s, 1H, -N*H*), 9.80 (d, $^{3}J_{(H3,H4)}$ = 7.5 Hz, 1H, H-4), 9.29 (brs, 1H, H-1), 8.14 (br t, $^{3}J_{(H2,H3)/(H3,H4)}$ = 8 Hz, 1H, H-3), 7.90 (t, $^{3}J_{(H8,H9)/(H11,H12)}$ = 6.5 Hz, 2H, H-9/H-11), 7.60 (brs, 1H, H-2), 7.18 (t, $^{3}J_{(H8,H9)/(H11,H12)}$ = 6.5 Hz, 2H, H-8/H-12), 5.90 (brs, 1H, H-15), 5.79 (brs, 1H, H-17), 5.77 (brs, 1H, H-18), 5.60 (brs, 1H, H-14), 2.70 (sept, $^{3}J_{(H20,H21)/(H21,H22)}$ = 6.5 Hz, 1H, H-21), 2.31 (s, 3H, H-21), 1.22 (d, $^{3}J_{(H20,H21)}$ = 7 Hz, 3H, H-20), 1.14 (d, $^{3}J_{(H21,H22)}$ = 7 Hz, 3H, H-22) ppm. $^{13}C\{^{1}H\}$ NMR (125.75 MHz, $CDCl_3$, 25 °C): δ = 193.73 (C-6), 162.05 (C-10), 158.88 (C-1), 153.77 (C-5), 140.59 (C-3), 133.49 (C-7), 130.60 (C-2), 128.37 (C-4), 127.75 (C-9/C-11), 116.29 (C-8/C-12), 97.84

(C-16), 96.00 (C-13), 79.70 (C-15), 79.08 (C-18), 76.85 (C-17), 74, 78 (C-14), 31.19 (C-21), 23.06 (C-20), 22.20 (C-22), 18.76 (C-19) ppm. MS (ESI^+): *m/z* 557.08 $[M - Cl - H]^+$ (m_{ex} = 557.11).

[Chlorido(η^6-p-cymene)(*N*-{2,4,6-trimethylphenyl}-2-pyridinecarbothioamide)osmium(II)] chloride (4B). The compound was prepared following the general procedure using *N*-(2,4,6-trimethylphenyl)-2-pyridinecarbothioamide (66 mg, 0.258 mmol) and $[Os(\eta^6\text{-p-cymene})Cl_2]_2$ (102 mg, 0.129 mmol). The reaction time was 4 h and the product was obtained as a deep violet powder. Yield: 127 mg (76%). Elemental analysis, found: C, 45.15; H, 4.57; N, 4.30; S, 4.73, calculated for $C_{25}H_{30}Cl_2N_2SOs{\cdot}0.5H_2O$: C, 45.44; H, 4.73; N, 4.24; S, 4.85. ^{1}H NMR (500.10 MHz, $CDCl_3$, 25 °C): δ = 14.19 (s, 1H, -N*H*), 10.05 (brs, 1H, H-4), 9.35 (brs, 1H, H-1), 8.15 (brs, 1H, H-3), 7.56 (brs, 1H, H-2), 7.02 (s, 1H, H-9), 6.97 (s, 1H, H-11), 5.86 (s, 1H, H-15), 5.80 (brs, 2H, H-17/H-18), 5.46 (s, 1H, H-14), 2.64 (sept, $^3J_{(H20,H21)/(H21,H22)}$ = 7.5 Hz, 1H, H-21), 2.34 (s, 3H, H-19), 2.32 (s, 3H, C_{ar}-C*H*$_3$), 2.31 (s, 3H, C_{ar}-C*H*$_3$), 2.24 (s, 3H, C(10)-C*H*$_3$), 1.18 (d, $^3J_{(H20,H21)}$ = 7 Hz, 3H, H-20), 1.07 (d, $^3J_{(H21,H22)}$ = 7 Hz, 3H, H-22) ppm. $^{13}C\{^1H\}$ NMR (125.75 MHz, $CDCl_3$, 25 °C): δ = 195.38 (C-6), 159.45 (C-1), 152.92 (C-5), 140.61 (C-3), 139.05 (C-7), 134.90 (C_{ar}), 133.98 (C_{ar}), 133.13 (C_{ar}), 130.74 (C-2), 129.91 (C-11), 129.57 (C-9), 128.20 (C-4), 97.61 (C-16), 96.82 (C-13), 79.88 (C-18), 78.97 (C-15), 77.25 (C-17), 72.41 (C-14), 31.28 (C-21), 23.09 (C-20), 22.14 (C-22), 21.19 (*C*H$_3$), 18.81 (*C*H$_3$), 18.25 (C-19), 18.18 (*C*H$_3$) ppm. MS (ESI^+): *m/z* 581.08 $[M - Cl - H]^+$ (m_{ex} = 581.17), *m/z* 616.98 $[M]^+$ (m_{ex} = 617.14).

[Chlorido(η^6-p-cymene)(*N*-{4-morpholinophenyl}-2-pyridinecarbothioamide)osmium(II)] chloride (5B). The compound was prepared following the general procedure using *N*-(4-morpholinophenyl)-2-pyridinecarbothioamide (76 mg, 0.253 mmol) and $[Os(\eta^6\text{-p-cymene})Cl_2]_2$ (100 mg, 0.127 mmol). The reaction time was 4 h and the product was obtained as a black microcrystalline solid. Yield: 130 mg (74%).

Elemental analysis, found: C, 43.66; H, 4.58; N, 5.86; S, 4.26, calculated for $C_{26}H_{31}Cl_2N_3OSOs \cdot H_2O$: C, 43.75; H, 4.66; N, 5.89; S, 4.48. ^{1}H NMR (500.10 MHz, $CDCl_3$, 25 °C): δ = 14.41 (s, 1H, -N*H*), 9.75 (brs, 1H, H-4), 9.21 (brs, 1H, H-1), 8.11 (brs, 1H, H-3), 7.97 (d, $^3J_{(H8,H9)/(H11,H12)}$ = 8 Hz, 2H, H-9/H-11), 7.51 (brs, 1H, H-2), 7.26 (brs, 2H, H-8/H-12), 5.89 (d, $^3J_{(H14,H15)}$ = 5 Hz, 1H, H-15), 5.76 (brs, 1H, H-17), 5.73 (brs, 1H, H-18), 5.59 (brs, 1H, H-14), 4.01 (brs, 4H, H'-2/H'-3), 3.34 (brs, 4H, H'-1/H'-4), 2.70 (sept, $^3J_{(H20,H21)/(H21,H22)}$ = 7 Hz, 1H, H-21), 2.29 (s, 3H, H-19), 1.23 (d, $^3J_{(H20,H21)}$ = 7 Hz, 1H, H-20), 1.14 (d, $^3J_{(H21,H22)}$ = 7 Hz, 1H, H-22) ppm. ^{13}C{^{1}H} NMR (125.75 MHz, $CDCl_3$, 25 °C): δ = 157.90 (C-1), 154.10 (C-5), 147.99 (C-7), 140.16 (C-3), 129.58 (C-2), 127.77 (C-4), 126.54 (C-9/C-11), 116.97 (C-8/C-12), 97.57 (C-16), 95.65 (C-13), 79.63 (C-15), 78.46 (C-18), 76.75 (C-17), 74.51 (C-14), 65.92 (C'-2/C'-3), 50.25 (C'-1/C'-4), 31.12 (C-21), 23.03 (C-20), 22.11 (C-22), 18.57 (C-19) ppm. MS (ESI$^+$): *m/z* 624.04 $[M - Cl - H]^+$ (m_{ex} = 624.17).

[Chlorido(η^6-p-cymene)(*N*-{4-benzoylphenyl}-2-pyridinecarbothioamide)osmium(II)] chloride (6B). The compound was prepared following the general procedure using *N*-(4-morpholinophenyl)-2-pyridinecarbothioamide (81 mg, 0.253 mmol) and $[Os(\eta^6\text{-p-cymene})Cl_2]_2$ (100 mg, 0.127 mmol). The reaction time was 4 h and the product was obtained as black crystals. Yield: 150 mg (83%). Elemental analysis, found: C, 47.82; H, 3.80; N, 3.96; S, 4.16, calculated for $C_{29}H_{28}Cl_2N_2OSOs \cdot 0.5H_2O$: C, 48.13; H, 4.04; N, 3.87; S, 4.42. ^{1}H NMR (500.10 MHz, $CDCl_3$, 25 °C): δ = 14.67 (s, 1H, -N*H*), 9.88 (d, $^3J_{(H3,H4)}$ = 8 Hz, 1H, H-4), 9.28 (d, $^3J_{(H1,H2)}$ = 5 Hz, 1H, H-1), 8.16 (t, $^3J_{(H2,H3)/(H3,H4)}$ = 8 Hz, 1H, H-3), 8.09 (d, $^3J_{(H8,H9)/(H11,H12)}$ = 7.5 Hz, 2H, H-9/H-11), 7.93 (d, $^3J_{(H8,H9)/(H11,H12)}$ = 7.5 Hz, 2H, H-8/H-12), 7.83 (d, $^3J_{(H'3,H'4)/(H'6,H'7)}$ = 7 Hz, 2H, H'-3/H'-7), 7.62 (m, 1H, H-2), 7.60 (m, 1H, H'-5), 7.51 (t, $^3J_{(H'3,H'4)/(H'4,H'5)}$ = 8 Hz, 2H, H'-4/H'-6), 5.91 (d, $^3J_{(H14,H15)}$ = 5.5 Hz, 1H, H-15), 5.81 (d, $^3J_{(H17,H18)}$ = 5.5 Hz, 1H, H-18), 5.77 (d, $^3J_{(H17,H18)}$ = 5.5 Hz, 1H, H-17), 5.62 (d, $^3J_{(H14,H15)}$ = 5.5 Hz, 1H, H-14), 2.71 (sept, $^3J_{(H20,H21)/(H21,H22)}$ = 7.5

Hz, 1H, H-21), 2.31 (s. 3H, H-19), 1.23 (d, $^{3}J_{(H20,H21)}$ = 7 Hz, 3H, H-20), 1.15 (d, $^{3}J_{(H20,H21)}$ = 7 Hz, 3H, H-22) ppm. $^{13}C\{^{1}H\}$ NMR (125.75 MHz, $CDCl_3$, 25 °C): δ = 195.60 (C'-1), 194.36 (C-6), 158.19 (C-1), 153.84 (C-5), 140.99 (C-7), 140.34 (C-3), 137.34 (C-10), 137.08 (C'-2), 132.78 (C-2), 130.98 (C-8/C-12), 130.13 (C'-5), 130.11 (C'-3/C'-7), 128.44 (C'-4/C'-6), 128.32 (C-4), 125.29 (C-9/C-11), 98.12 (C-16), 96.14 (C-13), 79.71 (C-15), 78.71 (C-17), 76.65 (C-18), 74.82 (C-14), 31.18 (C-21), 23.02 (C-20), 22.13 (C-22), 18.61 (C-19) ppm. MS (ESI^{+}): *m/z* 643.02 $[M - Cl - H]^{+}$ (m_{ex} = 643.15).

Methods

Hydrolysis experiments

Compounds **1A** and **1B** (1–5 mM) were investigated on their hydrolysis behavior. The compounds were dissolved in a mixture of D_2O/H_2O (90/10) or in 104 mM NaCl solution in D_2O/H_2O (90/10), and the samples were analyzed by ^1H NMR spectroscopy by suppressing the water signal. Following a preparation time of *ca.* 10 min, spectra were recorded every 10 min for 14 h using 32 scans/spectrum. UV-vis experiments were used to verify the NMR experiments. For this purpose, solutions of **1A** and **1B** in H_2O or in 104 mM NaCl solution were prepared at 20–50 µM concentrations and UV-vis spectra were recorded every 20 min for 24 h after a preparation time of 15 min.

Lipophilicity measurements

The lipophilicity of compounds **1A–6B** was determined using HPLC methods,[10, 11] following OECD guidelines.[12] The HPLC system (TM100, Dionex) was equipped with a reversed-phased column (Zorbax Eclipse Plus C18, Agilent, 5µm pore size, 4.6 µm inner diameter and 250 mm column length) that was thermostatted at 25 °C and a UV detector (UVD 170U, Dionex). Potassium iodide (0.1 mM) was used as an internal standard for the determination of the column dead-time. For delineating the lipophilicity, the capacity factors of each compound (250 µM) were measured at three different methanol : water ratios using isocratic methods and 0.5 % formic acid. Measurements were carried out in duplicate and fitted to the equation $log\ k = S \cdot \varphi + log\ k_w$, where $log\ k$ is the logarithmic capacity factor, S the slope, φ the organic solvent concentration and $log\ k_w$ the intercept at zero organic solvent concentration. Capacity factors were only considered if detected within the working limits of $-0.5 < log\ k < 1.5$, where the mentioned linear relationship is valid.[10] The corresponding correlation factors were all found at $R^2 > 0.9979$. The quotient of

the intercept and the slope gives the chromatographic lipophilicity index $\varphi_0 = -log\ k_w/S$, which shows a better correlation with lipophilicity than extrapolated $log\ k_w$ values.[13] The index φ_0 is compound specific and gives the concentration of organic solvent needed to obtain a retention time that is exactly the two-fold column dead-time, *i.e.* $log\ k = 0$.

Interaction with biomolecules and stability in hydrochloric acid

The stability of **1A** and **1B** in the presence of biological nucleophiles and in hydrochloric acid was investigated by electrospray ionization mass spectrometry (ESI-MS). Compound **1A** and **1B** were dissolved in aqueous solution and incubated in equimolar ratios with Cys, His, Met, ub, cyt or 5'-dGMP at 37 °C. Spectra were recorded up to 7 d. The samples containing amino acids or 5'-dGMP were diluted with methanol, whereas protein samples were diluted with water : methanol : formic acid (50 : 50 : 0.1) prior to direct infusion into the MS. Furthermore, both **1A** and **1B** (200 µM) were dissolved in 60 mM HCl (pH = 1.2) and incubated at 37 °C. Spectra were recorded after 1, 3 and 19 h at final concentrations of 10 µM. For comparison purposes, **2A** and **2B** were also incubated with Cys and Met and spectra were recorded after 1, 3 and 19 h at final concentrations of 10 µM.

Cytotoxicity in cancer cell lines

Cell lines and culture conditions. CH1 cells (adenocarcinoma of the ovary, human) were provided by Lloyd R. Kelland (CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, U.K). SW480 (adenocarcinoma of the colon, human) and A549 (non-small cell lung cancer, human) cells were from Brigitte Marian (Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Austria). All cell culture reagents were purchased from Sigma-Aldrich. Cells were grown in 75 cm^2 culture flasks (Starlab) as adherent monolayer cultures in complete culture medium, *i.e.* Eagle's minimal essential

medium (MEM) supplemented with 10% heat-inactivated fetal calf serum, 1 mM sodium pyruvate, 4 mM L-glutamine, and 1% non-essential amino acids (from 100× ready-to-use stock) without antibiotics. Cultures were maintained at 37 °C in a humidified atmosphere containing 95% air and 5% CO_2.

MTT assay conditions. Cytotoxicity was determined by the colorimetric MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide, purchased from Fluka) microculture assay. For this purpose, cells were harvested from culture flasks by trypsinization and seeded in 100 µL/well aliquots of complete culture medium into 96-well microculture plates (Starlab). Cell densities of 1.5×10^3 cells/well (CH1), 2.5×10^3 cells/well (SW480) and 4×10^3 cells/well (A549) were chosen in order to ensure exponential growth of untreated controls throughout the experiment. For 24 h, cells were allowed to settle and resume exponential growth. The test compounds were dissolved in DMSO, serially diluted in complete culture medium (such that the DMSO content in actual test solutions did not exceed 0.5%) and added in 100 µL/well aliquots for an exposure time of 96 hours. At the end of exposure, the medium was replaced with 100 µL/well RPMI1640 culture medium (supplemented with 10% heat-inactivated fetal calf serum) plus 20 µL/well MTT solution in phosphate-buffered saline (5 mg/ml). After incubation for 4 h, the supernatants were removed, and the formazan crystals formed by vital cells were dissolved in 150 µL DMSO per well. Optical densities at 550 nm were measured with a microplate reader (Tecan Spectra Classic), using a reference wavelength of 690 nm to correct for unspecific absorption. The quantity of vital cells was expressed in terms of T/C values by comparison to untreated control microcultures, and 50% inhibitory concentrations (IC_{50}) were calculated from concentration-effect curves by interpolation. Evaluation is based on means from at least three independent experiments, each comprising at least three replicates per concentration level.

Adduct formation on the nucleosome core particle

NCP crystals were produced and stabilized in harvest buffer (37 mM $MnCl_2$, 40 mM KCl, 20 mM K-cacodylate [pH 6.0], 24% 2-methyl-2,4-pentanediol and 2% trehalose) as previously described.[14, 15] The 37 mM $MnCl_2$ buffer component was subsequently eliminated by gradual replacement with 10 mM $MgSO_4$ followed by thorough rinsing of crystals with the $MgSO_4$-containing buffer to remove any residual $MnCl_2$.[16] The crystal structures reported here stems from 44–48 hour incubation of crystals with 1 mM **1A**, **3A**, **1B** or **3B** included in the buffer. Single crystal X-ray diffraction data were recorded as described previously[15] at beam line X06DA of the Swiss Light Source (Paul Scherrer Institute, Villigen, Switzerland) using a Mar225 CCD detector and an X-ray wavelength of 1.14 Å (NCP-**1B**, NCP-**3B**) or 1.50 Å (NCP-**1A**, NCP-**3A**). Data were processed with MOSFLM[17] and SCALA from the CCP4 package.[18] The native 2.5 Å resolution NCP145 model (*pdb* code 3REH)[19] was used for initial structure solution by molecular replacement. Structural refinement and model building were carried out with routines from the CCP4 suite.[18] Restraint parameters for the adducts were based on the small molecule crystal structure of **2B** reported here. Data collection and structure refinement statistics are given in Table S3.

Atomic coordinates and structure factors have been deposited in the RCSB Protein Data Bank under accession codes X, Y, Z and Q. Graphic figures were prepared with PyMOL (DeLano Scientific LLC, San Carlos, CA, USA).

Table S1. X-ray diffraction parameters for the measurement of single crystals of **3A** and **2B**[a].

compound	**3A**	**2B**
CCDC N°	902335	902334
chemical formula	$C_{22}H_{23}Cl_2FN_2RuS \cdot C_3H_6O$	$C_{22}H_{24}Cl_2N_2OOsS$
M (g mol^{-1})	596.53	625.59
temperature (K)	200(2)	100(2)
crystal size (mm)	0.20 × 0.10 × 0.06	0.20 × 0.10 × 0.08
crystal color, habit	red, block	red, block
crystal system	monoclinic	monoclinic
space group	P21n	P21/c
a (Å)	14.0346(9)	14.4797(14)
b (Å)	8.6278(5)	11.9305(11)
c (Å)	22.7026(13)	13.1126(13)
V (Å^3)	2749.0(3)	2239.8(4)
β (deg)	90.353(3)	98.591(3)
Z	4	4
D_c (g cm^{-3})	1.441	1.855
μ (mm^{-1})	0.87	6.041
F(000)	1216.0	1216.0
Θ range (deg)	2.91 to 30.18	2.60 to 30.20
h range	-19/19	-20/20
k range	-11/12	-16/16
l range	-31/32	-18/18
no. unique refls.	7964	6623
no. parameters	301	266
R_{int}	0.082	0.057
R_1 (obs.)	0.0469	0.0425
wR_2 (all data)	0.1031	0.0547
S	0.98	1.031

[a] $R_1 = \Sigma||F_o| - |F_c||/\Sigma w|F_o|$, [b] $wR_2 = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$, [c] $S = \{\Sigma[w(F_o^2 - F_c^2)^2]/(n-p)\}]^{1/2}$, where n is the number of reflections and p is the total number of parameters refined

Table S2. Selected bond lengths (Å), angles (°) and torsion angles (°) of **3A** and **2B**.

Bond Lengths (Å)	**3A (M = Ru)**	**2B (M = Os)**
M–S	2.3414(9)	2.3468(8)
M–N1	2.095(3)	2.105(2)
M–Cl	2.3924(4)	2.3987(9)
M–centroid	1.687(3)	1.682(3)
Bond Angles (°)	**3A**	**2B**
S–M–N1	81.28(8)	80.89(7)
S–M–Cl	89.81(3)	88.04(3)
N1–M–Cl	83.68(8)	82.41(8)
Torsion Angles (°)	**3A**	**2B**
C6–N2–C7–C8	52.7(5)	74.8(4)
N1–C5–C6–S	15.9(4)	4.1(4)

Table S3. Data collection and refinement statistics for NCP treated with **1A**, **3A**, **1B** and **3B**.

	NCP–1A	**NCP–3A**	**NCP–1B**	**NCP–3B**
Data collection*				
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions				
a (Å)	106.80	106.48	106.71	106.78
b (Å)	109.81	109.82	109.93	109.73
c (Å)	182.38	181.39	181.84	181.91
Resolution (Å)	2.58–60.8 (2.58–2.72)	2.87–60.5 (2.87–3.03)	2.38–60.6 (2.38–2.51)	2.41–58.6 (2.41–2.54)
R_{merge} (%)	6.8 (47.8)	11.7 (32.9)	4.1 (45.9)	7.5 (40.4)
$I / \sigma I$	15.8 (2.3)	8.2 (2.0)	21.3 (2.1)	12.8 (2.0)
Completeness (%)	84.2 (46.5)	99.7 (98.5)	82.6 (39.3)	96.5 (81.6)
Redundancy	6.4 (4.2)	6.4 (3.5)	6.1 (3.0)	6.1 (2.9)
Refinement				
Resolution (Å)	2.58–60.8	2.87–60.5	2.38–60.6	2.41–58.6
No. reflections	55962	48147	69745	78440
R_{work} / R_{free} (%)	25.2 / 27.3	23.7 / 28.0	25.2 / 28.0	26.5 / 27.5
No. atoms	12063	12063	12119	12122
Protein	6086	6086	6086	6086
DNA	5939	5939	5939	5939
Solvent	16	16	16	16
Adduct	22	22	78	81
B-factors (Å^2)	77	72	76	75
Protein	51	45	47	48
DNA	105	99	104	102
Solvent	74	85	78	75
Adduct	123	109	119	117
R.m.s. deviations				
Bond lengths (Å)	0.008	0.010	0.009	0.009
Bond angles (°)	1.30	1.50	1.34	1.28

* Values in parentheses are for the highest-resolution shell.

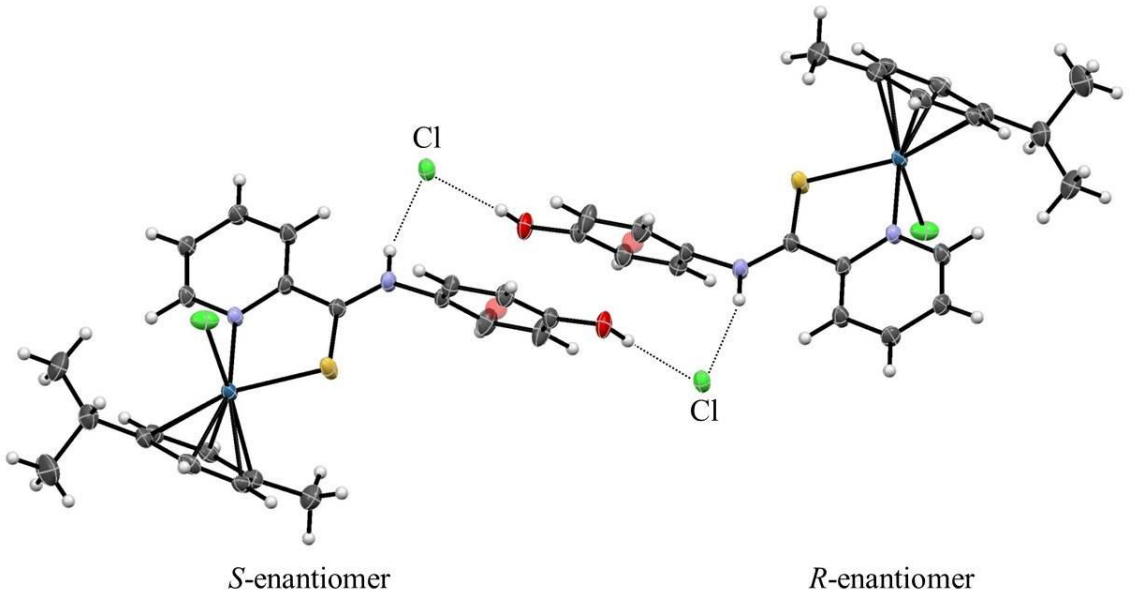


Figure S1. The hydrogen bonding network of two independent molecules in the crystal structure lattice of **2B** is shown, featuring both stereoisomers in a 1 : 1 ratio. The phenol rings are aligned in a parallel offset fashion.

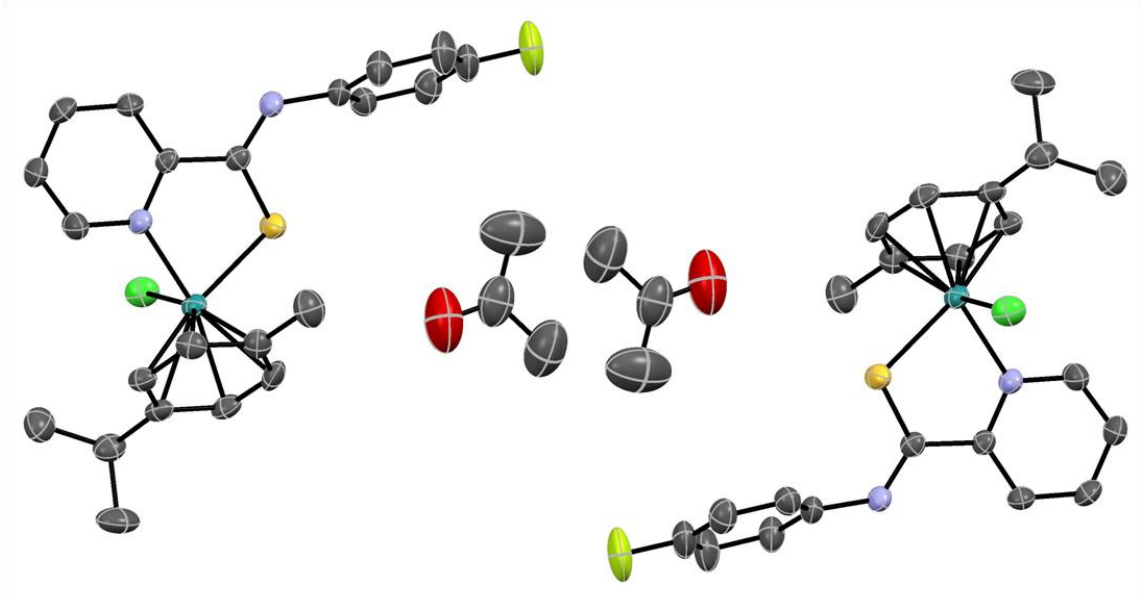


Figure S2. The S- (left) and R-enantiomers (right) in the crystal structure lattice of **3A** with two co-crystallized acetone molecules. Hydrogen atoms and counter anions are omitted for clarity.

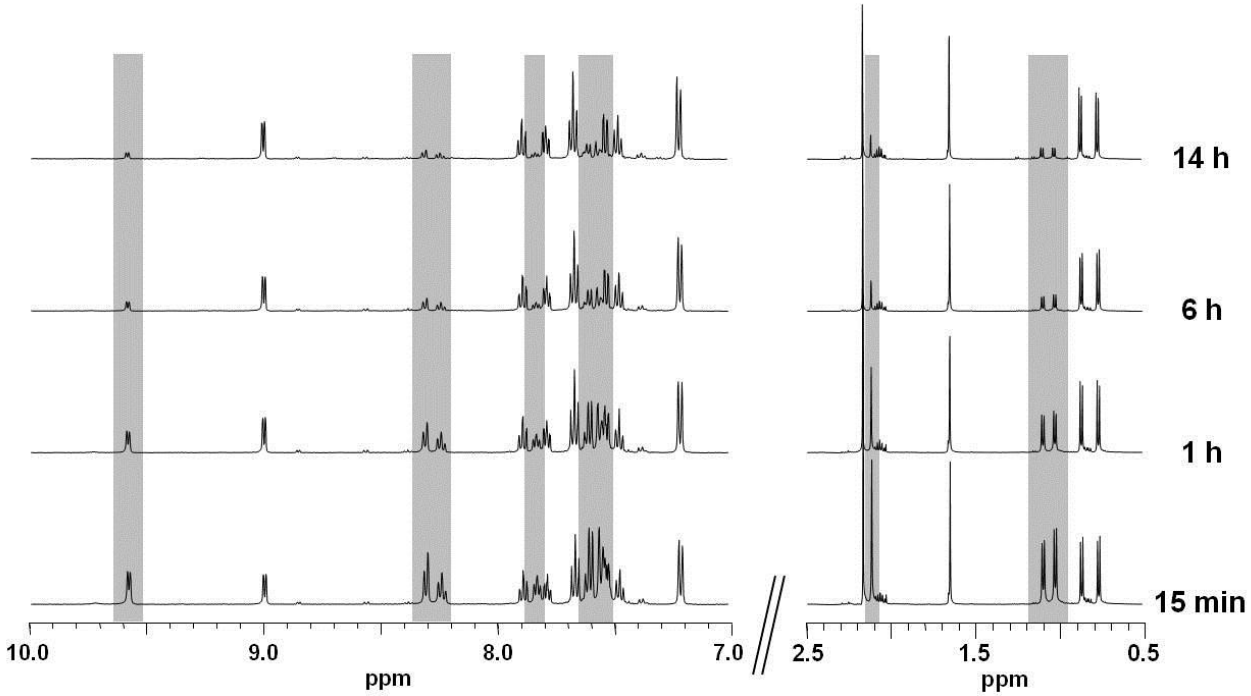


Figure S3. Low- and high-field regions of the NMR experiments monitoring the hydrolysis of **1A** in 104 mM NaCl aqueous solution. The highlighted peaks were assigned to the chlorido species.

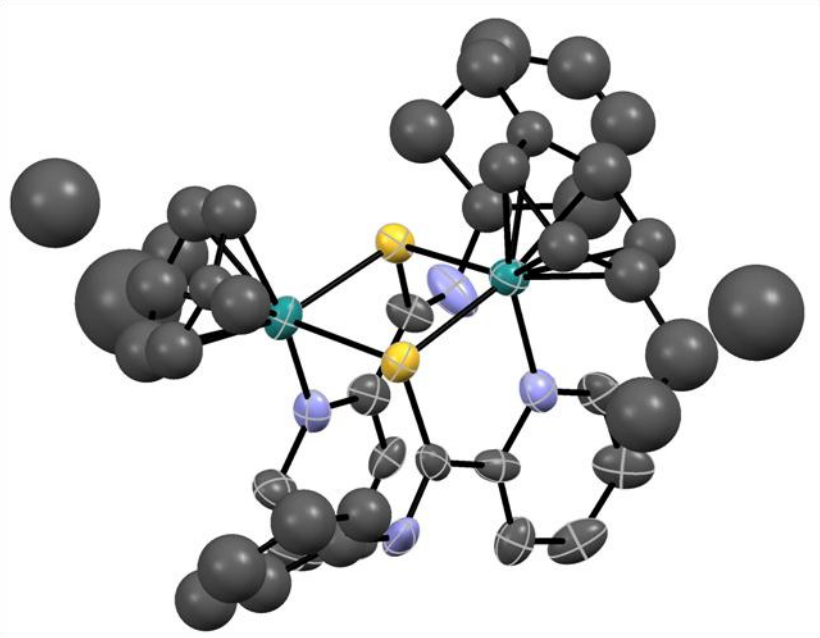
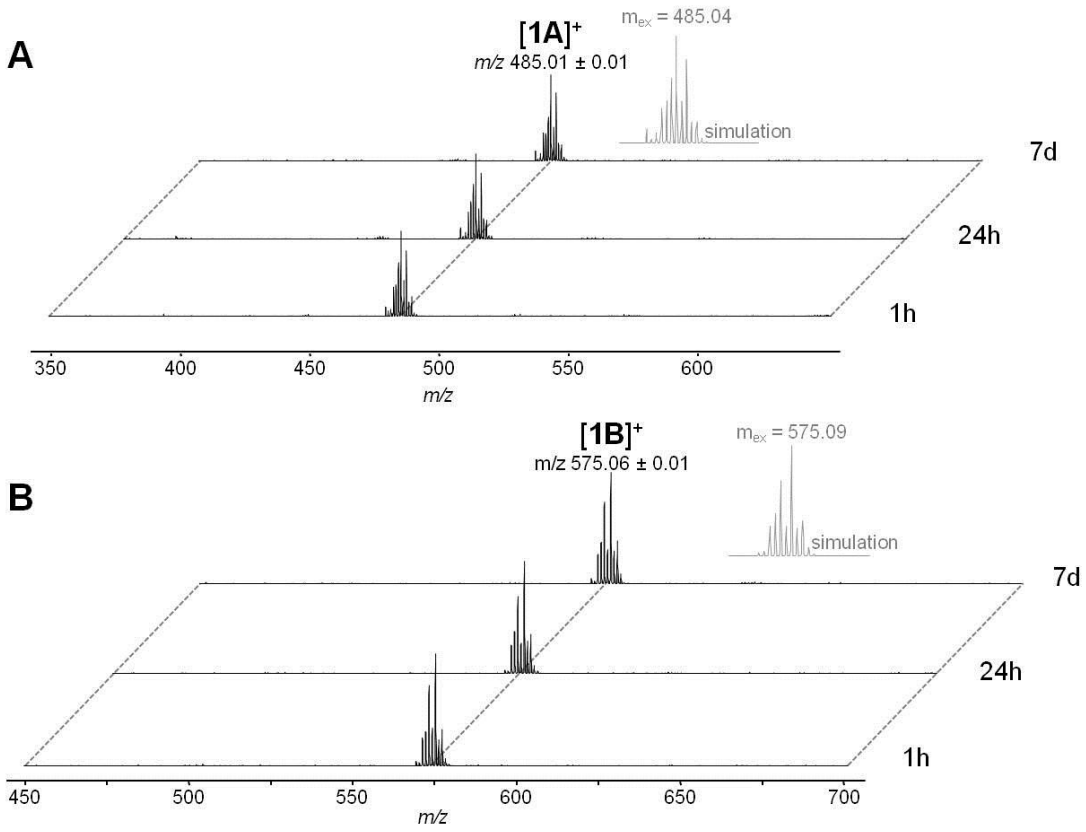


Figure S4. The crystal structure of the 2Ru-2S dimer (top) of **1A** obtained from basic aqueous solution is shown. The hydrogen atoms and counter ions as well as solvent molecules are omitted for clarity.

1



2

3 **Figure S5**. Time-dependent stability determined for **1A** (A) and **1B** (B) in HCl (60 mM, pH 1.2) by ESI-MS. The

4 compounds do not hydrolyze and are stable over the entire incubation period.

5

6

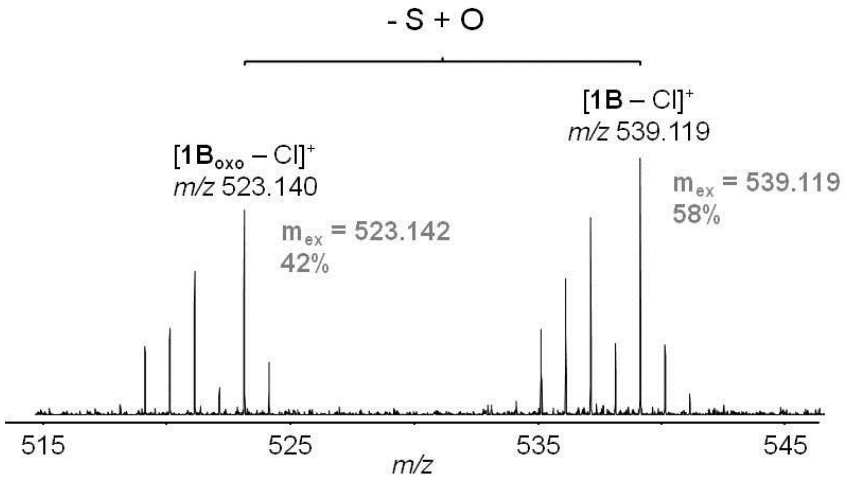


Figure S6. High resolution ESI-TOF mass spectrum of **1B** and its associated S→O exchange. The mass accuracy of the oxo-species is 4 ppm. Stock solutions of **1B** in DMSO were prepared, which inhibits dimer formation.

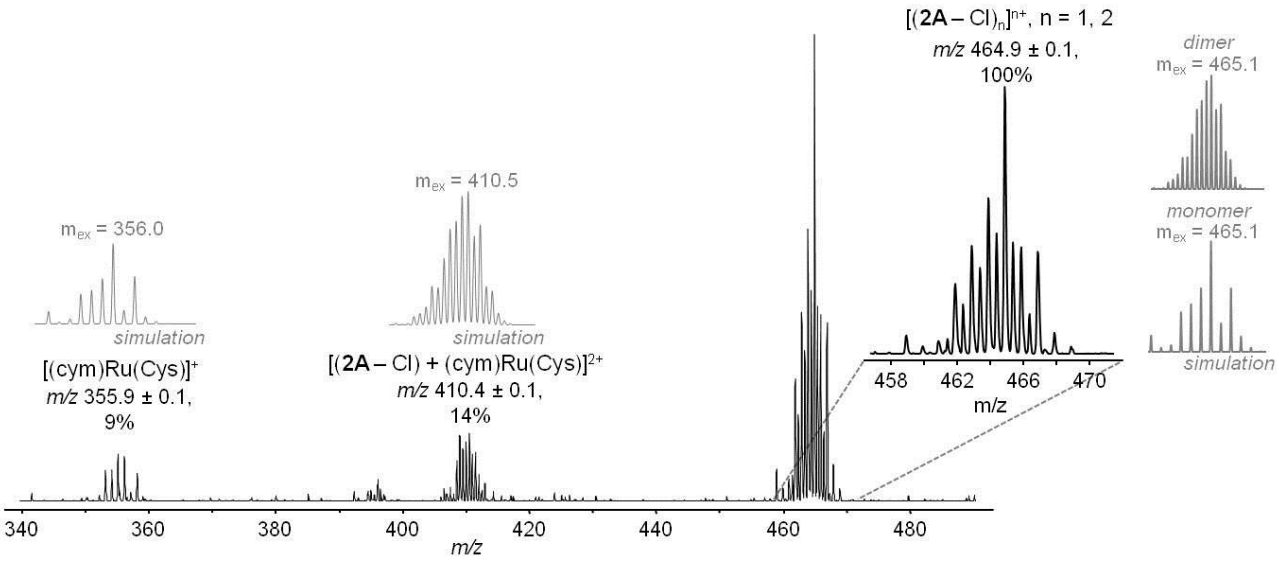


Figure S7. ESI IT mass spectrum of the reaction between **2A** and Cys after 24 h. Adduct formation with Cys is characterized by ligand cleavage.

Table S4. The pre-calculated molecular properties of the chlorido complexes are listed for the quantitative estimate of drug-likeness (QED): MW (molecular weight), LogD (distribution coefficient), HBA (hydrogen bond acceptor), HBD (hydrogen bond donor), PSA (polar surface area), ROTB (rotatable bonds), AROM (number of aromatic rings), ALERTS (number of structural alerts).[20] The LogD was calculated from φ_0 according to ref. 13. PSA was calculated using ChemBio3D 12.0 software (CambridgeSoft). In fact, Bickerton *et al.* used a calculated LogP (octanol-water coefficient) in their report. However, LogD was employed for calculating QED in the present study since the organometallics are charged. Calculation of the weighted QED for maximum information content (QED_w^{mo}) was carried out according to ref. 20.

Compound	MW	LogD	HBA	HBD	PSA	ROTB	AROM	ALERTS
1A	485.03	-0.52	2	1	15.27	3	3	0
2A	501.03	-1.13	2	2	23.67	3	3	0
3A	503.02	-0.38	3	1	15.27	3	3	0
4A	527.11	2.9	2	1	15.27	3	3	0
5A	570.13	-0.13	4	1	27.74	4	3	0
1B	574.19	-0.35	2	1	15.27	3	3	0
2B	590.19	-1.03	2	2	23.67	3	3	0
3B	592.18	-0.21	3	1	15.27	3	3	0
4B	616.27	3.15	2	1	15.27	3	3	0
5B	659.29	-0.06	4	1	27.74	4	3	0

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