Electronic supplementary materials *Mendeleev Commun.*, 2022, **32**, 186–188

Ru^{II} and Ru^{III} complexes with imidazole ligands containing (benzyloxy)pyridinone moiety

Anna A. Fateeva, Ilya A. Shutkov, Dmitrii M. Mazur, Olga N. Kovaleva,
Elena R. Milaeva and Alexey A. Nazarov

Materials and Methods

Unless stated otherwise, all the reagents used in this study were obtained from the commercial sources (Aldrich, ABCR). The starting compounds 5-benzyloxy-2-methyl-4*H*-pyran-4-one, 3-benzyloxy-2-methyl-4*H*-pyran-4-one, 3-benzyloxy-2-ethyl-4*H*-pyran-4-one,[S1] $[(\eta^6\text{-}p\text{-cymene})RuCl_2]_2$,[S2] $Na[Ru(DMSO)_2Cl_4]$[S3] were synthesized as reported. NMR spectra were recorded on a Bruker Avance III (^{1}H: 400 MHz); chemical shifts (δ) are given in ppm relative to TMS, coupling constants (J) – in Hz. The solvent signals were used as references ($CDCl_3$: δ_H = 7.26 ppm). C, H, and N elemental analyses were carried out on a Euro EA 3028HT CHNS/O analyzer. Mass spectra were obtained on amaZon SL (Bruker, Bremen, Germany) spectrometer equipped with electrospray ionization (ESI) source; MeOH was used as a solvent.

5-Benzyloxy-1-[3-(1*H*-imidazol-1-yl)propyl]-2-methylpyridin-4(1*H*)-one (1a)

1-(3-Aminopropyl)imidazole (2.85 mL; 23.9 mmol) was added to a solution of 5-benzyloxy-2-methyl-4*H*-pyran-4-one (3.18 g; 14.7 mmol) in the 1:1 mixture of ethanol and water (80 mL). The solution was adjusted to pH 13-14 by addition of 2 M sodium hydroxide solution. The mixture was refluxed for 12 hours. The solvent volume was reduced to minimum and water (50 mL) was added to the residue which was washed with diethyl ether (2 x 40 mL). The product was filtered and dried *in vacuo* to give 3.1 g (67%) of brown solid. NMR ^{1}H ($CDCl_3$) δ= 7.40-7.22(m, 6H, H_{Ar}), 7.09(s, 1H, H_{Im}), 6.82(s, 1H, H_{Im}), 6.74(s, 1H, H_{Ar}), 6.27(s, 1H, H_{Im}), 5.13(s, 2H, $OC\underline{H}_2$), 3.81(t, 2H, J=6.8 Hz, $NC\underline{H}_2$), 3.66-3.58 (m, 2H, $NC\underline{H}_2$), 2.11(s, 3H, $C\underline{H}_3$), 2.07-1.98(m, 2H, $C\underline{H}_2$). Elem. anal. calc. (%) for ($C_{19}H_{21}N_3O_2*1.5H_2O$): C 68.04, H 6.71, N 12.53, found: C 67.77, H 6.48, N 12.76.

3-Benzyloxy-1-[3-(1*H*-imidazol-1-yl)propyl]-2-methylpyridin-4(1*H*)-one (1b)

Compound **1b** was prepared similar to **1a** starting from 1-(3-aminopropyl)imidazole (4.50 mL; 37.7 mmol) and 3-benzyloxy-2-methyl-4*H*-pyran-4-one (5.00 g; 23.1 mmol). The crude product was isolated by column chromatography on silica gel eluting with 5:1 $CHCl_3$/MeOH mixture. The fractions containing the product were combined, and 25% acetic acid (4.0 mL) was added. The pure product was extracted with dichloromethane (5 x 10 mL). The organic phases were combined, and the solvent was removed under reduced pressure, the product was dried *in vacuo* to give 3.7 g (51%) of a brown oil. NMR ^{1}H ($CDCl_3$) δ= 7.46(s, 1H, H_{Im}), 7.40-7.28(m, 5H, H_{Ar}), 7.12(s, 1H, H_{Im}), 7.06(d, 1H, J=7.6 Hz, H_{Ar}), 6.89(s, 1H, H_{Im}), 6.40(d, 1H, J=7.6 Hz, H_{Ar}), 5.22(s, 2H, O-C$\underline{H}_2$), 3.96(t, 2H, J=6.7 Hz, N-C$\underline{H}_2$), 3.73-3.66(m, 2H, N-C$\underline{H}_2$), 2.16-2.07(m, 2H, C$\underline{H}_2$), 1.97(s, 3H, C$\underline{H}_3$). Elem. anal. calc. (%) for ($C_{19}H_{21}N_3O_2$*0.45CH_2Cl_2): C 64.60, H 6.10, N 11.62, found: C 64.73, H 6.47, N 11.73.

3-Benzyloxy-2-ethyl-1-[3-(1*H*-imidazol-1-yl)propyl]pyridin-4(1*H*)-one (1c)

Compound **1c** was prepared similar to **1a** starting from 1-(3-aminopropyl)imidazole (4.50 mL; 37.7 mmol) and 3-benzyloxy-2-ethyl-4*H*-pyran-4-one (5.00 g; 21.7 mmol). Yield 3,5 g (49%) of brown oil. NMR ^{1}H ($CDCl_3$) δ= 7.47(s, 1H, H_{Im}), 7.43-7.28(m, 5H, H_{Ar}), 7.12(s, 1H, H_{Im}), 7.08(d, 1H, J=7.6 Hz, H_{Ar}), 6.90(s, 1H, H_{Im}), 6.41(d, 1H, J=7.6 Hz, H_{Ar}), 5.27(s, 2H, OC$\underline{H}_2$), 3.98(t, 2H, J=6.7 Hz, NC$\underline{H}_2$), 3.76-3.68 (m, 2H, NC$\underline{H}_2$), 2.43(q, 2H, J=7.5 Hz, C$\underline{H}_2$$CH_3$), 2.21-2.11(m, 2H, CH_2), 0.94(t, J=7.5 Hz, CH_2C$\underline{H}_3$). Elem. anal. calc. (%) for ($C_{20}H_{23}N_3O_2$*0.25CH_2Cl_2): C 68.46, H 6.65, N 11.85, found: C 68.82, H 6.53, N 12.18.

Sodium tetrachloro{5-benzyloxy-1-[3-(1*H*-imidazol-1-yl)propyl]-2-methylpyridin-4(1*H*)-one}bis(dimethyl sulfoxide)ruthenate(III)-*S,N* (2a)

A solution of compound **1a** (97 mg; 0.3 mmol) in acetone (10.0 mL) was added to a solution of Na[Ru(DMSO)$_2$Cl$_4$] (127 mg; 0.3 mmol) in acetone (5.0 mL), and the reaction mixture was stirred for 12 h. The precipitated product was isolated by centrifugation and dried *in vacuo* to yield 161 mg (89%) of red powder. T(dec.) = 163-164°C. MS-ESI: m/z: 645[M-Na$^+$]$^-$. Calc. (%) for ($C_{21}H_{27}Cl_4N_3O_3RuSNa$): C 37.79, H 4.08, N 6.30, found: C 37.43, H 3.95, N 6.24.

Sodium tetrachloro{3-benzyloxy-1-[3-(1*H*-imidazol-1-yl)propyl]-2-methylpyridin-4(1*H*)-one}bis(dimethyl sulfoxide)ruthenate(III)-*S,N* (2b)

Complex **2b** was prepared similarly to **2a** starting from of **1b** (97 mg; 0.3 mmol) and Na[Ru(DMSO)$_2$Cl$_4$] (127 mg; 0.3 mmol). Yield: 171 mg (94%) of red powder. T(dec.) = 176-178°C, MS-ESI: m/z:645[M-Na$^+$]$^-$. Elem. anal. calc. (%) for ($C_{21}H_{27}Cl_4N_3O_3RuSNa$*1.1$CHCl_3$): C 33.23, H 3.55, N 5.26, found: C 33.00, H 3.06, N 5.43.

Sodium tetrachloro{3-benzyloxy-2-ethyl-1-[3-(1*H*-imidazol-1-yl)propyl]pyridin-4(1*H*)-one}-bis(dimethyl sulfoxide)ruthenate(III)-*S,N* (2c)

Complex **2c** was prepared similar to **2a** starting from of **1c** (102 mg; 0.3 mmol) and Na[Ru(DMSO)$_2$Cl$_4$] (127 mg; 0.3 mmol). Yield: 158 mg (89%) of red powder. T(dec.) = 182-184°C MS-ESI: m/z: 659[M-Na$^+$]$^-$. Elem. anal. calc. (%) for ($C_{22}H_{29}Cl_4N_3O_3RuSNa$*0.9$CH_2Cl_2$): C 36.29, H 4.10, N 5.54, found: C 36.14, H 4.47, N 5.73.

(η^6-*p*-Cymene){5-benzyloxy-1-[3-(1*H*-imidazol-1-yl)propyl]-2-methylpyridin-4(1*H*)-one}-ruthenium(II)-*N* dichloride (3a)

A solution of [(η^6-*p*-cymene)$RuCl_2]_2$ (142 mg; 0.5 mmol) in acetone (10 mL) was added to a solution of **1a** (150 mg; 0.5 mmol) in acetone (10 mL), and the mixture was stirred for 12 h. The solvent was removed under reduced pressure, the solid residue was washed with ether, and dried *in vacuo* to give 248 mg (82%) of dark orange powder. T(dec.) = 170-172°C. NMR ^1H ($CDCl_3$) δ= 7.95(s, 1H, H_{Im}), 7.47-7.27(m, 5H, H_{Ar}), 7.22(s, 1H, H_{Ar}), 7.10(s, 1H, H_{Im}), 6.76(s, 1H, H_{Ar}), 6.24(s, 1H, H_{Im}), 5.42(d, 2H, J=6.1 Hz, H_{Cym}), 5.24(d, 2H, J=6.1 Hz, H_{Cym}), 5.14(s, 2H, $OC\underline{H}_2$), 3.68(t, 2H, J=7.7 Hz, $NC\underline{H}_2$), 3.61(t, 2H, J=7.5 Hz, $NC\underline{H}_2$), 2.98-2.85(m, 1H, $(CH_3)_2C\underline{H}$), 2.13(s, 3H, $C\underline{H}_{3(Cym)}$), 1.86-1.76 (m, 2H, $CH_2C\underline{H}_2CH_2$), 1.31-1.22(m, 9H, $CH(C\underline{H}_3)_2$, $C\underline{H}_3$). Calc. (%) for ($C_{29}H_{35}Cl_2N_3O_2Ru$*1.5H_2O): C 53.05, H 5.83, N 6.40, found: C 53.00, H 6.15, N 6.34.

(η^6-*p*-Cymene){3-benzyloxy-1-[3-(1*H*-imidazol-1-yl)propyl]-2-methylpyridin-4(1*H*)-one}-ruthenium(II)-*N* dichloride (3b)

Compound **3b** was prepared similar to **3a** from of **1b** (142 mg; 0.5 mmol) and [(η^6-*p*-cymene)$RuCl_2]_2$ (142 mg; 0.5 mmol). Yield: 250 mg (83%) of red powder. T(dec.) = 165-168°C. NMR ^1H ($CDCl_3$) δ= 8.00(s, 1H, H_{Im}), 7.42-7.25(m, 7H, H_{Ar}), 7.22(s, 1H, H_{Im}), 6.79(s, 1H, H_{Im}), 5.45(d, 1H, J=76.1 Hz, H_{Cym}), 5.28(d, 2H, J=6.0 Hz, H_{Cym}), 5.17(s, 2H, $OC\underline{H}_2$), 3.75-3.61(m, 4H, $NC\underline{H}_2$), 2.96-2.87 (m, 1H, $(CH_3)_2C\underline{H}$), 2.14(s, 3H, $C\underline{H}_{3(Cym)}$), 1.99(s, 3H, $C\underline{H}_3$), 1.85-1.75(m, 2H, $CH_2C\underline{H}_2CH_2$), 1.28(d, 6H, J=6.8 Hz $(C\underline{H}_3)_2CH$). Elem. anal. calc. (%) for ($C_{29}H_{35}Cl_2N_3O_2Ru$*1.5H_2O): C 53.05, H 5.83, N 6.40, found: C 53.01, H 6.20, N 5.91.

(η^6-*p*-Cymene){ 3-benzyloxy-2-ethyl-1-[3-(1*H*-imidazol-1-yl)propyl]-pyridin-4(1*H*)-one}-ruthenium(II)-*N* dichloride (3c)

Compound **3c** was prepared similar to **3a** from of **1c** (136 mg; 0.4 mmol) and [(η^6-*p*-cymene)$RuCl_2]_2$ (114 mg; 0.5 mmol). Yield: 223 mg (76%) of dark red powder. T(dec.) = 180-182°C. NMR ^1H ($CDCl_3$) δ= 7.98(s, 1H, H_{Im}), 7.44-7.27(m, 7H, H_{Ar}), 7.23(s, 1H, H_{Im}), 6.79(s, 1H, H_{Im}), 5.45(d, 1H, J=6.1 Hz, H_{Cym}), 5.27(d, 2H, J=6.1 Hz, H_{Cym}), 5.24(s, 2H, $OC\underline{H}_2$), 3.77(t, 2H, J=7.3 Hz, $NC\underline{H}_2$), 3.66(t, 2H, J=7.8 Hz, $NC\underline{H}_2$), 3.00-2.87 (m, 1H, $(CH_3)_2C\underline{H}$), 2.49(q, 2H, J=7.6 Hz, $C\underline{H}_2CH_3$), 2.14(s, 3H, $C\underline{H}_{3(Cym)}$), 1.92-1.81(m, 2H, $CH_2C\underline{H}_2CH_2$), 1.29(d, 6H, J=7.0 Hz $(C\underline{H}_3)_2CH$), 0.95(t, 3H, J=7.5Hz, $CH_2C\underline{H}_3$). Elem. anal. calc. (%) for ($C_{30}H_{37}Cl_2N_3O_2Ru$*H2O): C 54.46, H 5.94, N 6.35, found: C 54.63, H 6.36, N 6.30.

(η^6-*p*-Cymene){5-benzyloxy-1-[3-(1*H*-imidazol-1-yl)propyl]-2-methylpyridin-4(1*H*)-one}-ruthenium(II)-*N* oxalate (4a)

Silver oxalate (95 mg; 0.3 mmol) was added to a suspension of [(η^6-*p*-cymene)$RuCl_2$]$_2$ (96 mg; 0.2 mmol) in water (60.0 mL), and the mixture was stirred for 12 h. The precipitate was filtered off, and the filtrate was concentrated under reduced pressure. A solution of **1a** (100 mg; 0.3 mmol) in minimum methanol was added to the above residue dissolved in methanol (17 mL), and the mixture was stirred for 12 h. The solvent was removed under reduced pressure. The product was putified by column chromatography on silica gel using methanol as eluent. The solvent was removed under reduced pressure, and the product was dried *in vacuo* to yield 115 mg (56%) of red powder. T(dec.) = 133-135°C. NMR ^{1}H ($CDCl_3$) δ= 7.77(s, 1H, H_{Im}), 7.45-7.20(m, 6H, H_{Ar}), 6.94(s, 1H, H_{Im}), 6.76(s, 1H, H_{Im}), 6.19(s, 1H, 6.32 H_{Ar}), 5.47(d, 2H, J=6.4 Hz, H_{Cym}), 5.31(d, 2H, J=6.2 Hz, H_{Cym}), 5.09(s, 2H, $OC\underline{H}_2$), 3.74(t, 2H, J=7.6 Hz, $NC\underline{H}_2$), 3.66(t, 2H, J=7.6 Hz, $NC\underline{H}_2$), 2.77-2.64(m, 1H, $(CH_3)_2C\underline{H}$), 2.11(s, 3H, $C\underline{H}_{3(Cym)}$), 2.07(s, 3H, $C\underline{H}_3$), 1.83-1.72 (m, 2H, $CH_2C\underline{H}_2CH_2$), 1.23(d, 6H, J=7.0 Hz, $(C\underline{H}_3)_2CH$). Elem. anal. calc. (%) for ($C_{31}H_{35}N_3O_6Ru$*CH_3OH): C 56.63, H 5.79, N 6.19, found: C 56.56, H 5.30, N 6.22.

(η^6-*p*-Cymene){3-benzyloxy-1-[3-(1*H*-imidazol-1-yl)propyl]-2-methylpyridin-4(1*H*)-one}-ruthenium(II)-*N* oxalate (4b)

Compound **4b** was prepared similar to **4a** from of silver oxalate (95 mg; 0.3 mmol), [(η^6-*p*-cymene)$RuCl_2$]$_2$ (96 mg; 0.2 mmol) and **1b** (100 mg; 0.3 mmol). Yield: 126 mg (62%) of red powder. T(dec.) = 142-144°C. NMR ^{1}H ($CDCl_3$) δ= 7.68(s, 1H, H_{Im}), 7.44-7.27(m, 6H, H_{Ar}), 6.93(s, 1H, H_{Im}), 6.76(s, 1H, H_{Im}), 6.32(d, 1H, J=7.5 Hz, H_{Ar}), 5.53(d, 2H, J=6.1 Hz, H_{Cym}), 5.37(d, 2H, J=6.2 Hz, H_{Cym}), 5.15(s, 2H, $OC\underline{H}_2$), 3.81-3.66(m, 4H, $NC\underline{H}_2$), 2.81-2.70 (m, 1H, $(CH_3)_2C\underline{H}$), 2.13(s, 3H, $C\underline{H}_{3(Cym)}$), 2.02(s, 3H, $C\underline{H}_3$), 1.85-1.74 (m, 2H, $CH_2C\underline{H}_2CH_2$), 1.26(d, 6H, J=7.0 Hz, $(C\underline{H}_3)_2CH$). Elem. anal. calc. (%) for ($C_{31}H_{35}N_3O_6Ru$*$2CH_3OH$): C 55.76, H 6.10, N 5.91, found: C 55.75, H 6.41, N 5.90.

(η^6-*p*-Cymene){3-benzyloxy-2-ethyl-1-[3-(1*H*-imidazol-1-yl)propyl]pyridin-4(1*H*)-one}-ruthenium(II)-*N* oxalate (4c)

Compound **4c** was prepared similar to **4a** starting from of silver oxalate (95 mg; 0.3 mmol), [(η^6-*p*-cymene)$RuCl_2$]$_2$ (96 mg; 0.2 mmol) and **1c** (101 mg; 0.3 mmol). Yield: 106 mg (53%) of red powder. T(dec.) = 155-157°C. NMR ^{1}H ($CDCl_3$) δ= 7.68(s, 1H, H_{Im}), 7.46-7.27(m, 6H, H_{Ar}), 6.93(s, 1H, H_{Im}), 6.78(s, 1H, H_{Im}), 6.34(d, 1H, J=7.5 Hz, H_{Ar}), 5.53(d, 2H, J=6.2 Hz, H_{Cym}), 5.37(d, 2H, J=6.2 Hz, H_{Cym}), 5.23(s, 2H, $OC\underline{H}_2$), 3.80(t, 4H, J=7.7 Hz, $NC\underline{H}_2$), 2.82-2.70 (m, 1H, $(CH_3)_2C\underline{H}$), 2.53(q, 2H, J=7.6Hz, $C\underline{H}_2CH_3$), 2.14(s, 3H, $C\underline{H}_{3(Cym)}$), 1.92-1.79 (m, 2H, $CH_2C\underline{H}_2CH_2$), 1.26(d, 6H, J=6.8 Hz $(C\underline{H}_3)_2CH$), 0.94(t, 3H, J=7.5 Hz, $CH_2C\underline{H}_3$). Elem. anal. calc. (%) for ($C_{32}H_{37}N_3O_6Ru$*$1.5CH_3OH$): C 56.76, H 6.11, N 5.93, found: C 56.40, H 5.81, N 5.81.

Cell lines and culture conditions

The MCF7, A549, HCT116 and WI38 cell lines were cultured in Dulbecco modified Eagle’s medium (DMEM; Gibco, UK) with 10% fetal bovine serum (Gibco, USA) and antibiotics (PanEco, Russia) in 5% CO_2, 37ºC. The compounds were pre-dissolved at 40 mM in dimethyl sulfoxide (DMSO) and added to the cell culture at the required concentration with maximum DMSO content of 0.5 v/v%. Cells in 96-well plates (7×10^3 cells/well) were treated with various concentrations of compounds and cisplatin at 37ºC for 72 h. Cell viability was determined using the MTT assay as follows: cells were incubated at 37ºC for 50 min with 100 µL of 5 mg/mL solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma-Aldrich, St. Louis, USA) in cell culture medium. The supernatant was discarded, and the formazan was dissolved in 100 µL of DMSO. The optical density of the solution was measured at 570 nm on a multiwell plate reader (Multiskan FC, Thermo Fisher Scientifics, USA). The percentage of viable (i.e., MTT converting) cells was calculated from the absorbance of untreated cells (100%). Each experiment was repeated three times, each concentration was tested in three replicates.

NMR spectra of compounds

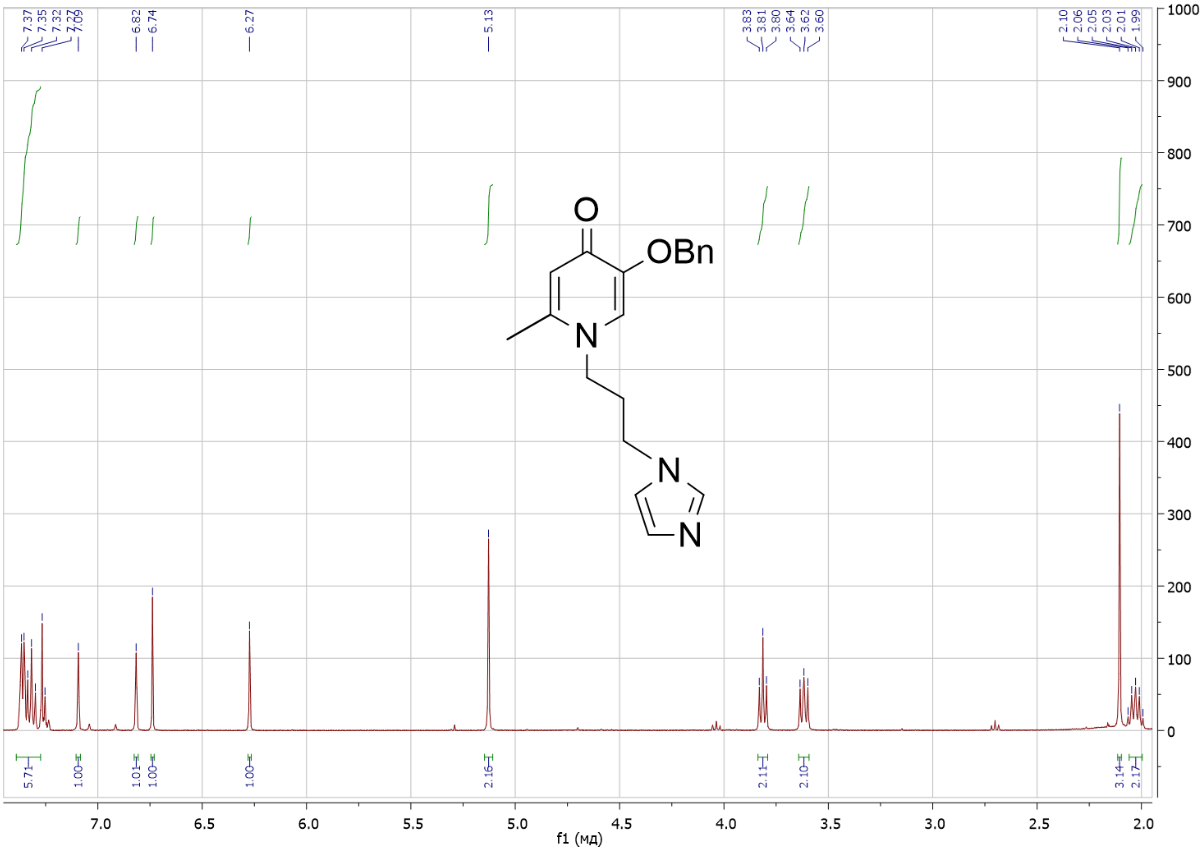

Figure S1. ^{1}H NMR spectra of compound **1a**

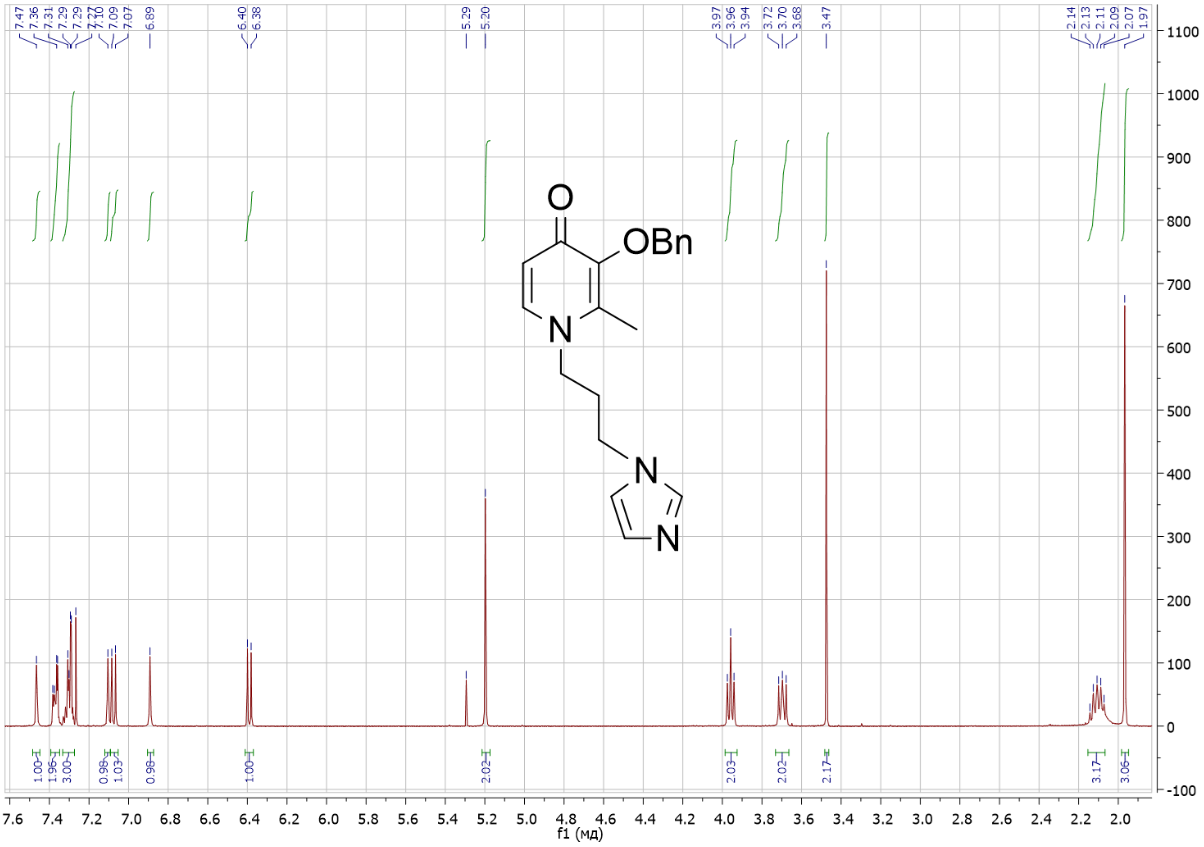

Figure S2. ^{1}H NMR spectra of compound **1b**

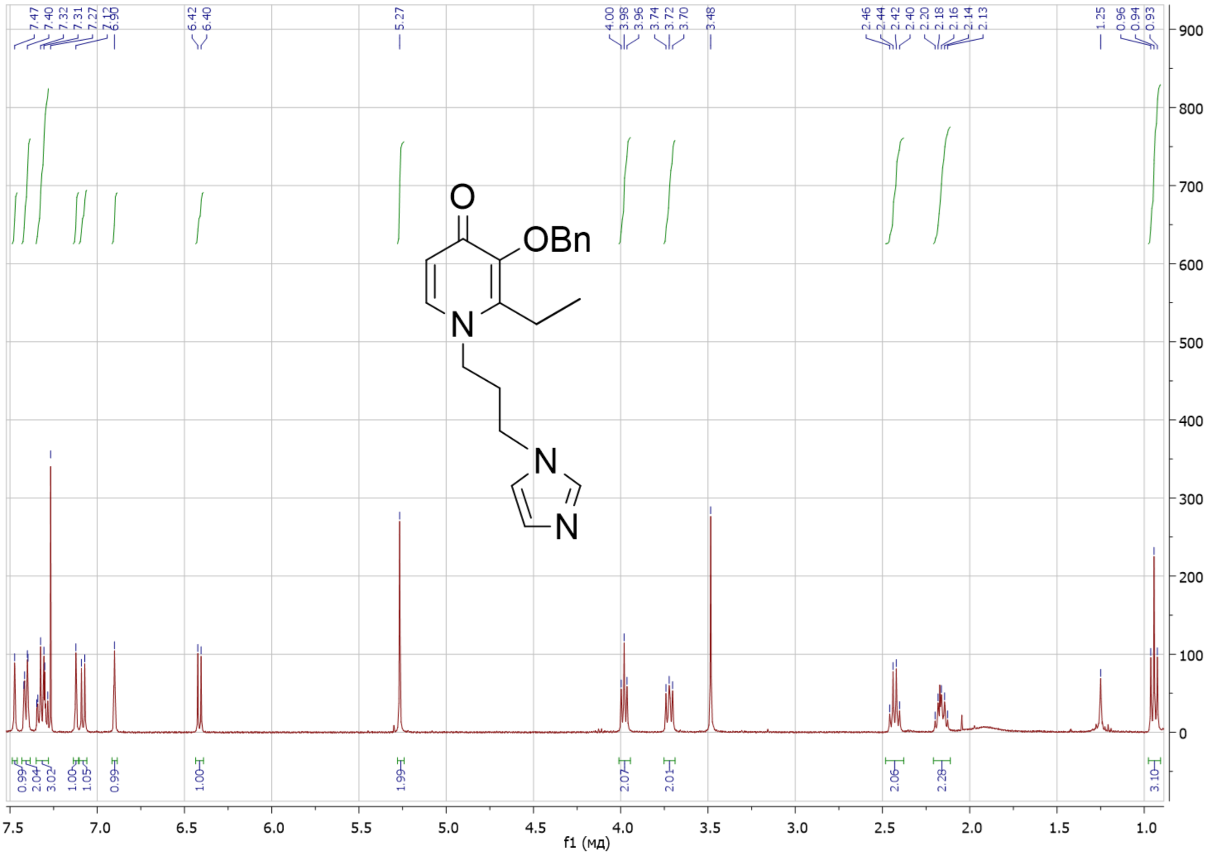

Figure S3. ^{1}H NMR spectra of compound **1c**

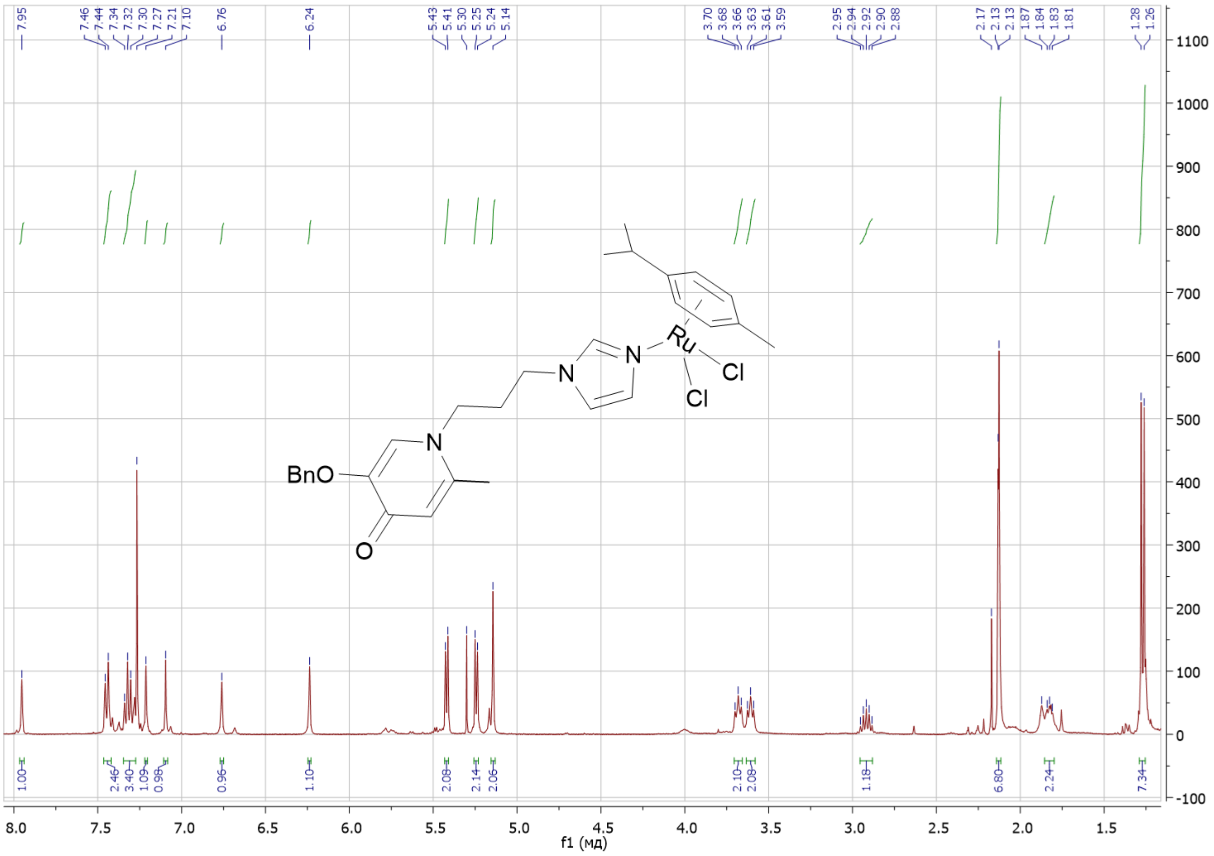

Figure S4. ^{1}H NMR spectra of compound **3a**

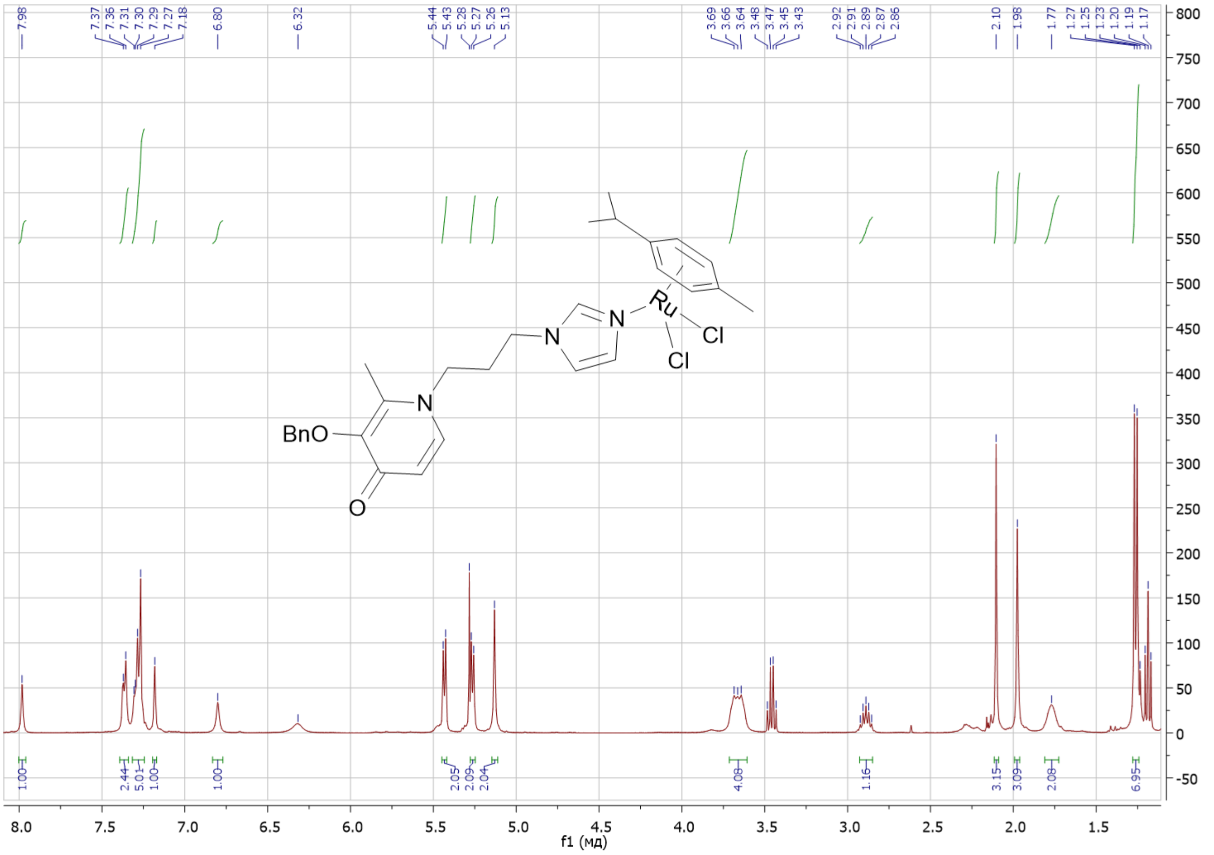

Figure S5. ^{1}H NMR spectra of compound **3b**

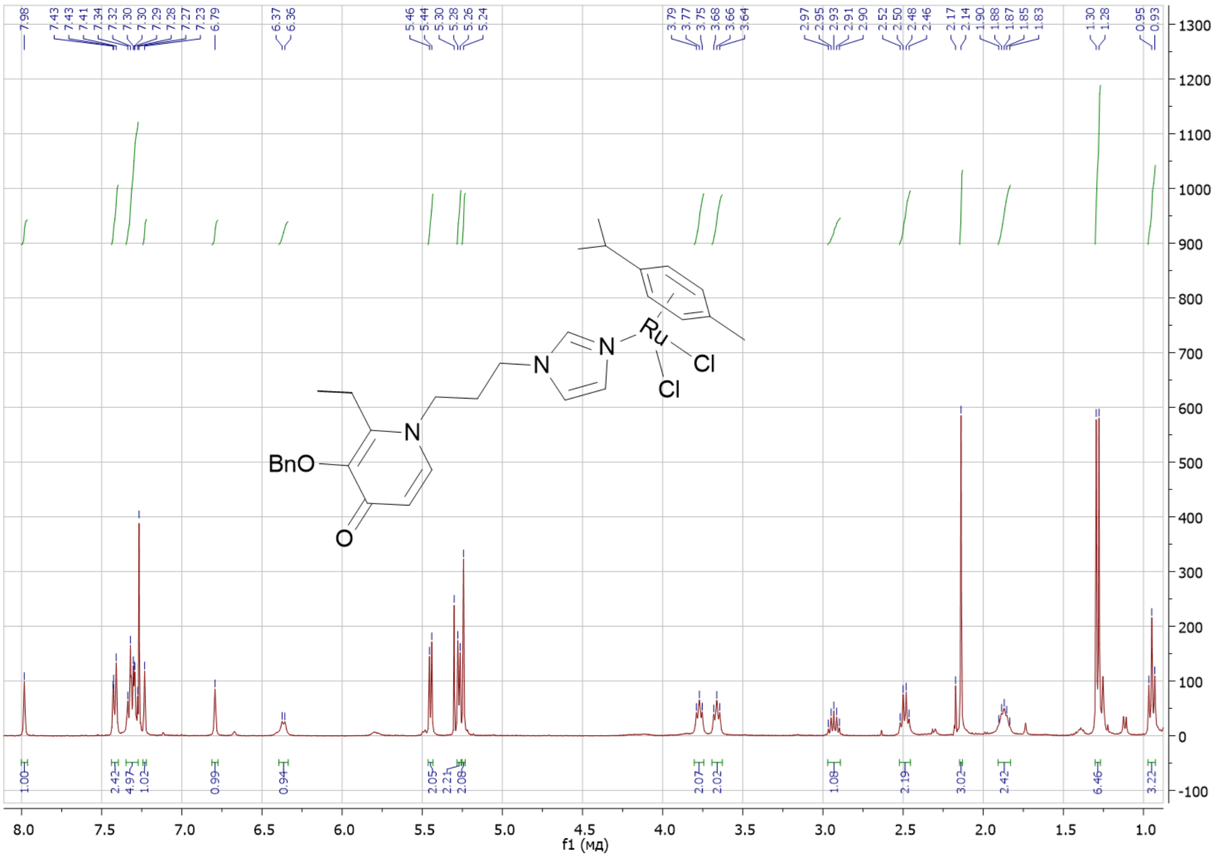

Figure S6. ^{1}H NMR spectra of compound **3c**

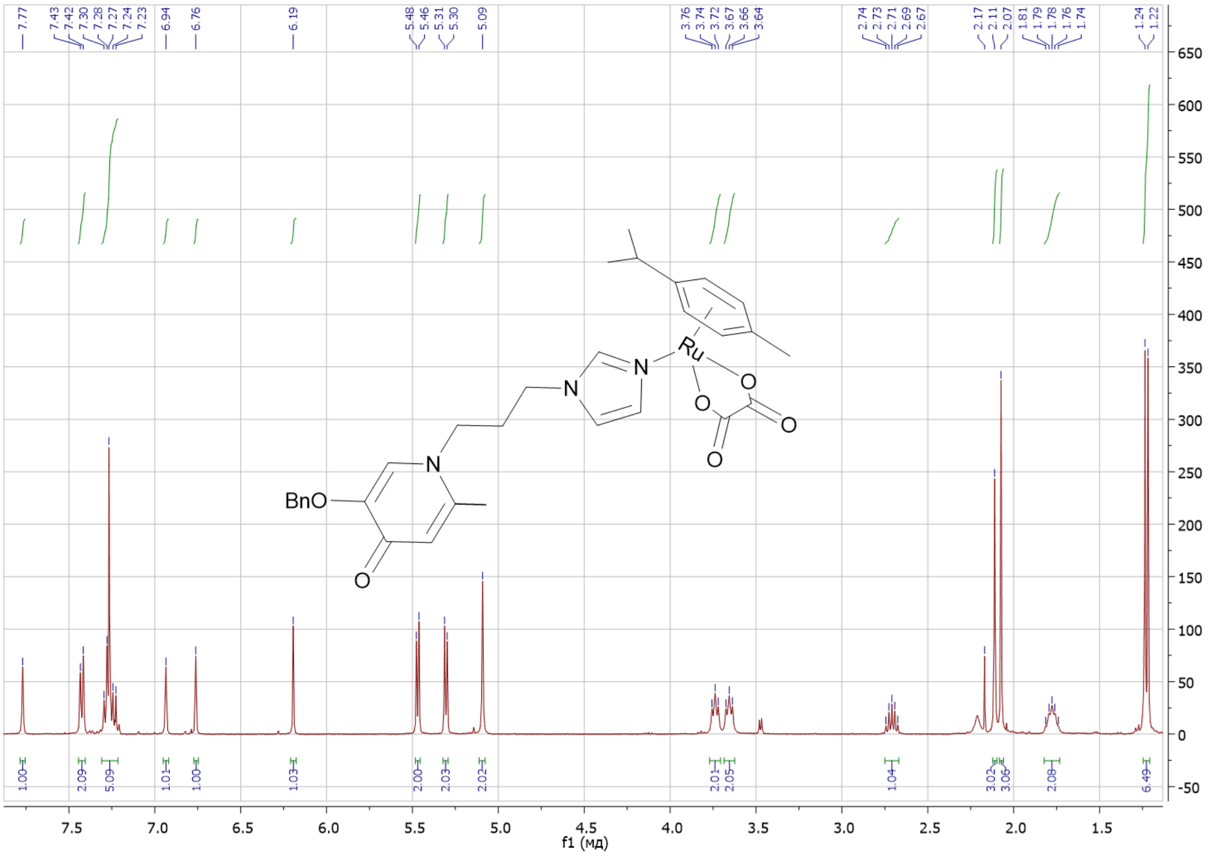

Figure S7. ^{1}H NMR spectra of compound **4a**

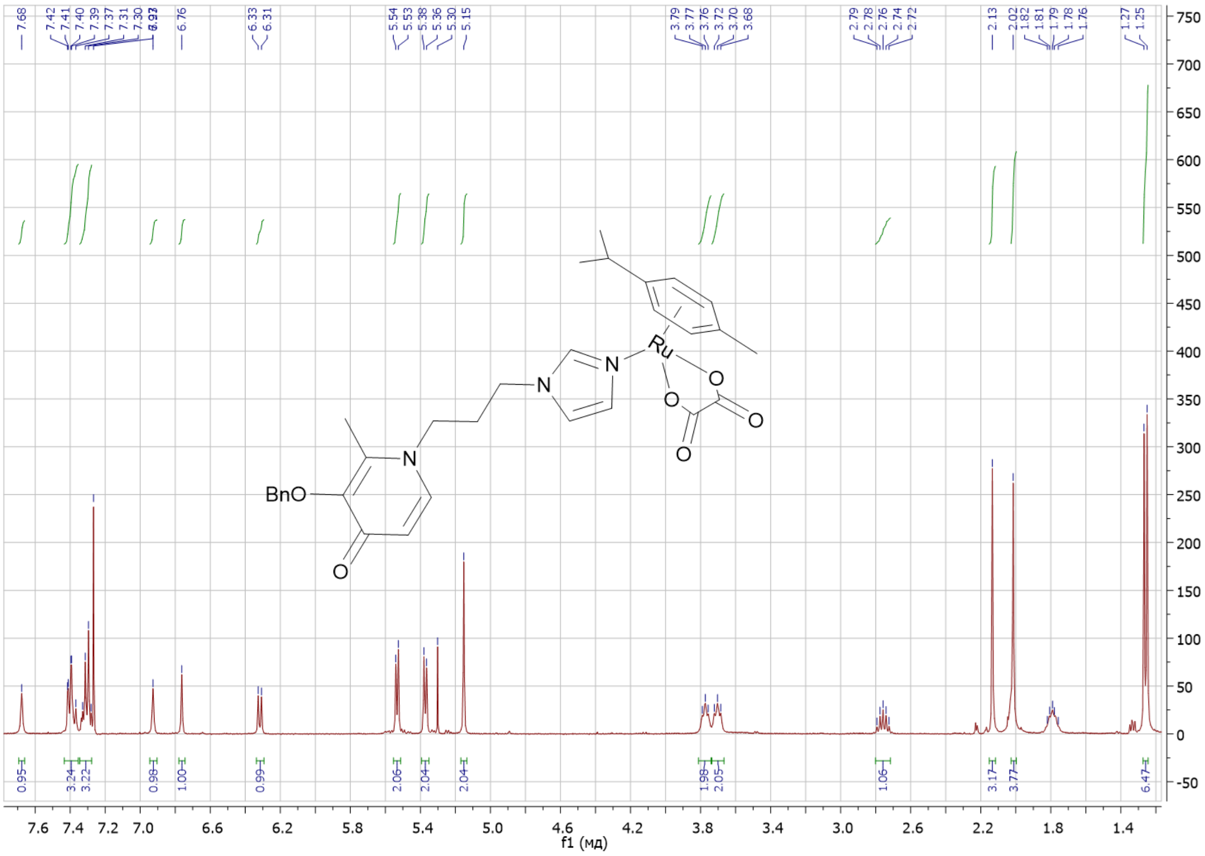

Figure S8. ^{1}H NMR spectra of compound **4b**

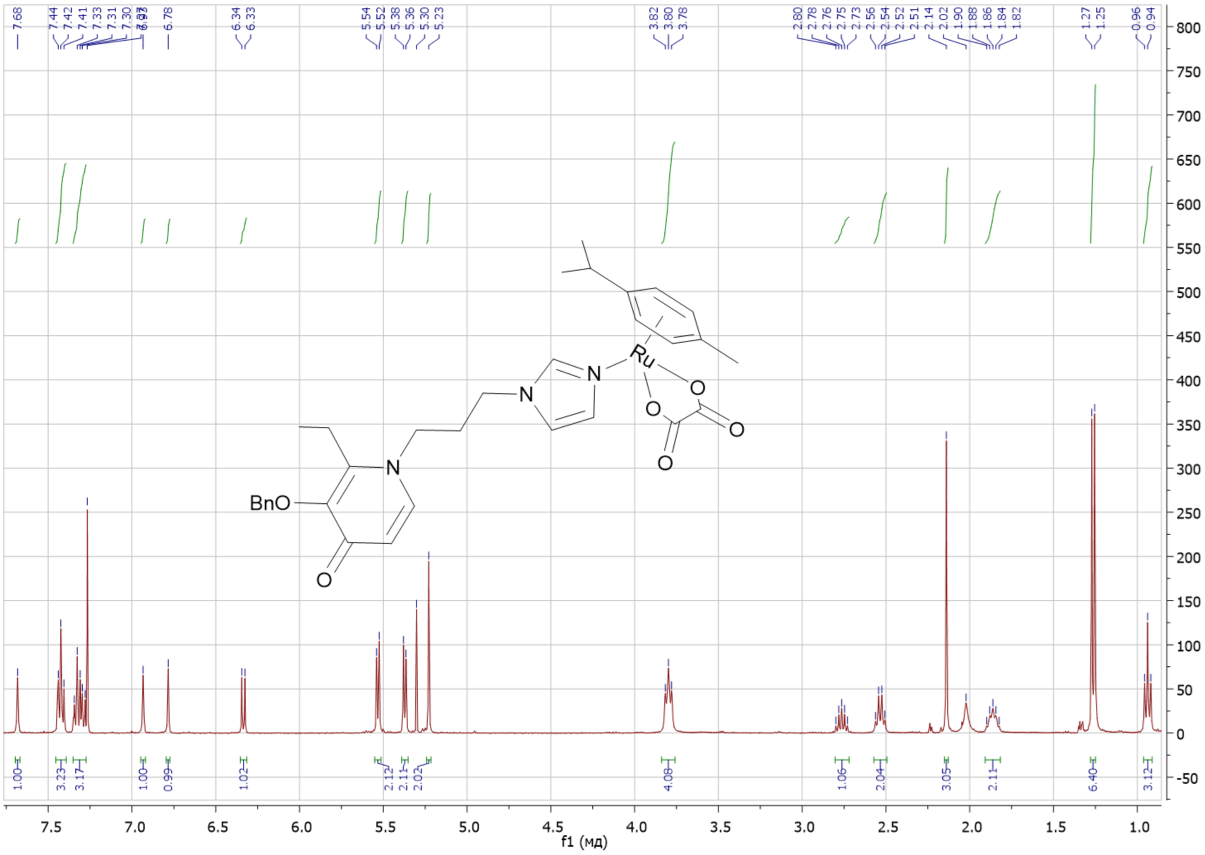

Figure S9. ^{1}H NMR spectra of compound **4c**

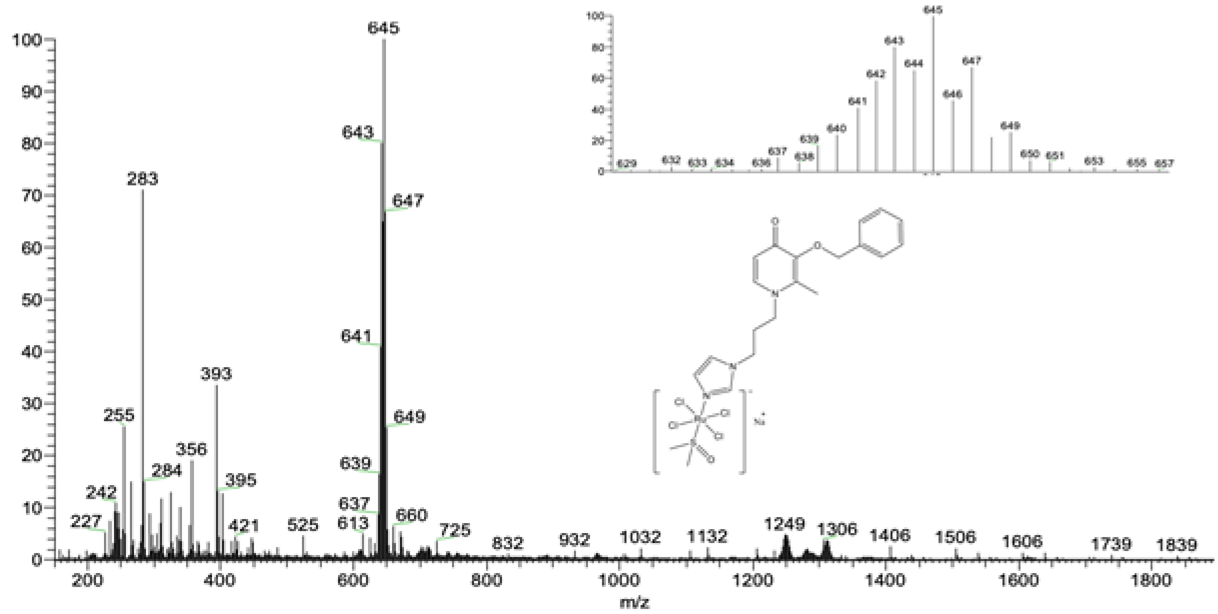

Figure S10 Mass spectrum ESI-MS of complex Ru^{III} **2b**

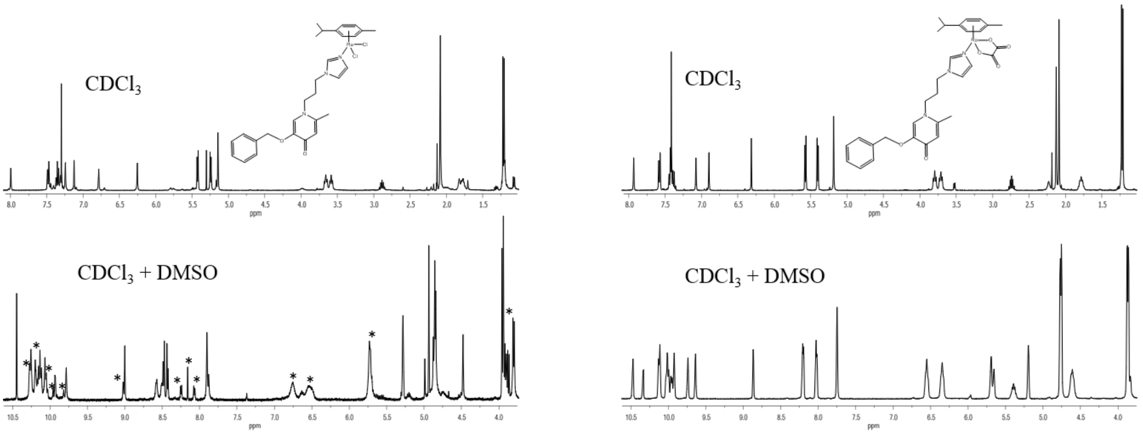

Figure S11. Behaviour of Ru^{II} complexes **3a** and **4a** in the presence of DMSO.

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