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Rhodium(III) dihalido complexes: the effect of ligand substitution and halido coordination on increasing cancer cell potency

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Bioinorganic chemistry; Cytotoxicity; Isomers; Picolinamide ligands; Rhodium(III) complexes

Abstract

This work presents the synthesis of eight new rhodium(III) dihalido complexes, $[RhX_2(L)(LH)]$ (where X = Cl or I), which incorporate two bidentate *N*-(3-halidophenyl)picolinamide ligands. The ligands have different binding modes in the complexes, whereby one is neutral and bound *via N,N* (LH) coordination, whilst the other is anionic and bound *via N,O* (L) coordination. The solid state and solution studies confirm multiple isomers are present when X = Cl, however, after a halide exchange with potassium iodide (X = I) the complexes exist exclusively as single stable *trans* isomers. NMR studies reveal the Rh(III) *trans* diiodido complexes remain stable in aqueous

solution with no ligand exchange reported over 96 h. Chemosensitivity data against a range of cancer cell lines show two cytotoxic complexes, where L = *N*-(3-bromophenyl)picolinamide ligand. The results have been compared to the analogous Ru(III) complexes, and overall highlight the Rh(III) *trans* diiodido complex to be ~78x more cytotoxic than the analogous Rh(III) dichlorido complex, unlike the Ru(III) complexes which are equitoxic against all cell lines. Additionally, the Rh(III) *trans* diiodido complex is more selective towards cancerous cells, with selectivity index (SI) values > 25-fold higher than cisplatin against colorectal carcinoma.

Introduction

Cisplatin, *cis*-[$Pt(NH_3)_2Cl_2$] (**CDDP**), and its platinum derivatives are still the most frequently used transition metal complexes in the treatment of various cancer types.[1] However, the corresponding *trans* isomer, transplatin (t*rans*-[$Pt(NH_3)_2Cl_2$]), remains therapeutically inactive.[2,3] It has been suggested that the orientation of the *cis* chlorides is key for therapeutic activity, whereby **CDDP** forms ~80% of intra-strand DNA crosslinks, whilst transplatin forms mono-adducts and inter-strand DNA crosslinks.[4–7] In 2019 Quiroga et al. reported a series of aliphatic amine Pt(II) complexes, and highlighted the *cis* dichlorido complexes have similar modes of action to cisplatin, whilst the *trans* diiodido complexes exhibit different modes of action and activate the cytoplasmic protein BID, suggesting a pro-apoptotic cascade.[8,9] Generally, Pt-based therapeutics have poor cancer cell selectivity, which is associated with many adverse side-effects, and has led chemotherapy research towards new non Pt-based alternatives such as Ru, Os, Rh and Ir complexes,[10–12] in an attempt to overcome some of these selectivity issues. In particular, organometallic complexes have been prominent, with many compounds exhibiting

high potency towards cancerous cells, whilst remaining non-toxic towards normal cell types, allowing for a more targeted therapy and reduction in patient side effects.[13]

The anti-tumor activity of Rh(II) coordination compounds were explored over 40 years ago, with the dinuclear Rh complex $[(CH_3COO)_4(H_2O)_2Rh_2]$ (**Figure 1A**), showing good anti-tumour activity against the Ehrlich ascites, sarcoma 180 and P388 lymphocytic leukemia but low activity against L1201 and B16 melanoma.[14] A variety of Rh(III) analogues of the well-known Ru(III) anti-cancer complexes NAMI-A and KP1019 have shown contrasting activities, whereby *mer,cis*-$[RhCl_3(Me_2SO)(Im)_2]$ (**Figure 1B**), Na[*trans*-$[RhCl_4(Me_2SO)(Im)]$ (**Figure 1C**) and (ImH)*trans*-$[RhCl_4(Im)_2]$ (Im = imidazole) (**Figure 1D**) were all found to be inactive against human ovarian carcinoma (A2780),[15] with IC_{50} values of > 200 μM, > 100 μM and > 1000 μM, respectively.[15] In contrast, complexes such as *mer,cis*-$[RhCl_3(Me_2SO)_2L]$ (L = Im or NH_3 **Figure 1E**), have shown significant cytotoxicity against A2780 cells with IC_{50} values of 1.5 ± 0.4 μM (L = Im) and 15.6 ± 2 μM (L = NH_3).[15] Such Rh complexes were shown to inhibit the growth of primary MCa mammary tumors implanted in metastatic cancer cells in the lungs. Rhodium(III) complexes of the type *mer*-$[RhCl_3(Me_2SO)(pp)]$ (pp = 2,2′-bipyridine (byp) or benzo[i]dipyrido[3,2-a:2′,3′-c]phenazine (dppn), **Figure 1F**) are stable in chloroform in light for 24 h but exhibit slow isomerization to *mer/fac* in polar solutions under the same conditions.[16] The rate and extent of isomerization was dependent on the size of the polypyridyl ligand, and highlighted that the presence of multiple isomers can be problematic. These isomers can have significantly different cytotoxicity values, and yet determining the active species– though essential for future drug development – has proven difficult.[17,18]

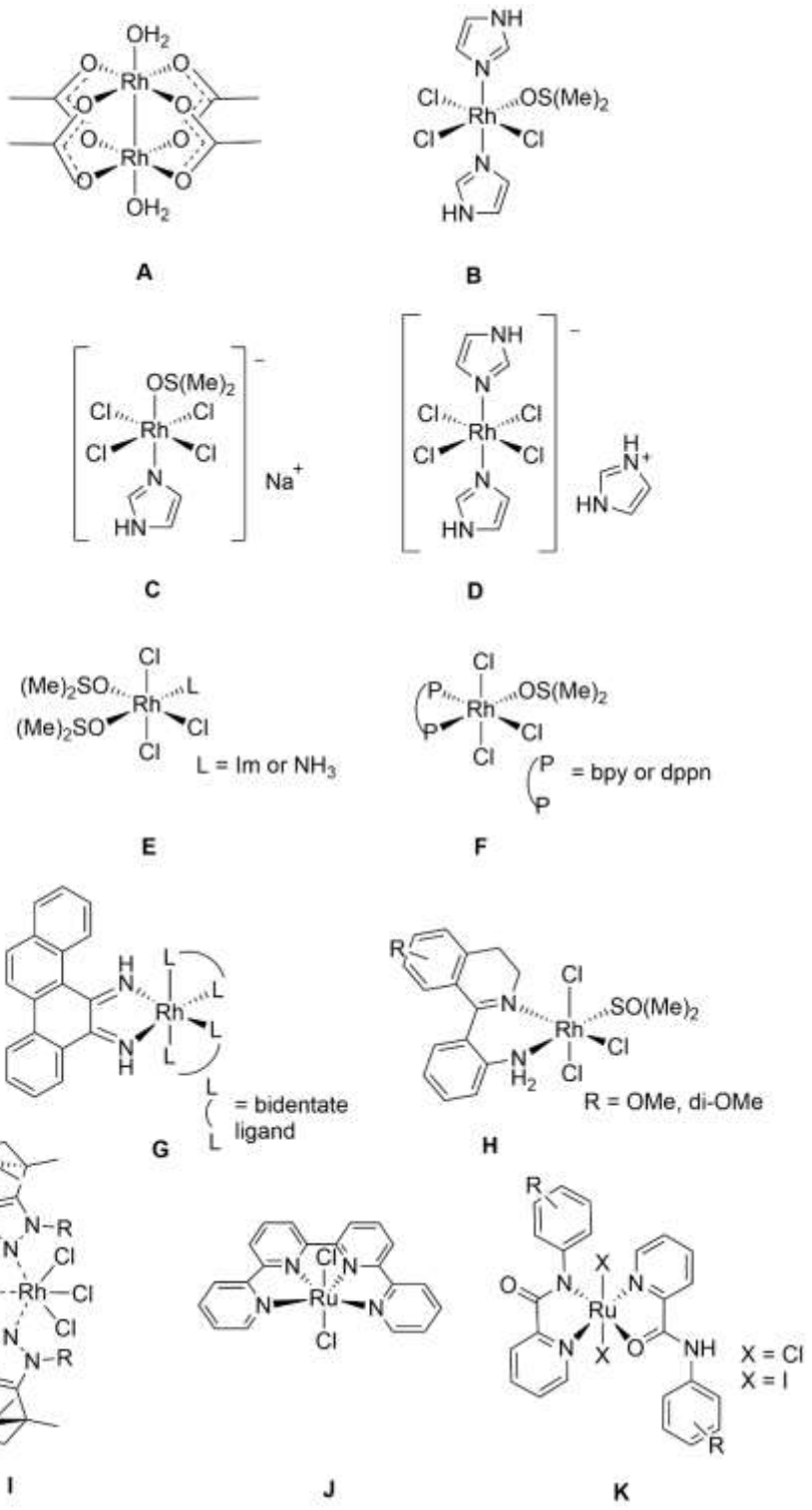


Figure 1. Selected rhodium and ruthenium complexes which have been screened for their cytotoxic potential.

The majority of Rh(III) coordination complexes are based on polypyridyl ligands, and this was in part due to their potential DNA binding properties, redox properties, hydrophobic nature and their conjugated systems, which can be useful in photoactivate therapy (e.g. PDT) in the treatment of cancer.[19,20] Barton and co-workers have conducted extensive research on Rh(III) metallo-insertors (e.g. **Figure 1G**), which bind DNA mismatches, disrupt DNA synthesis, and have low micromolar cytotoxicity values. These complexes are more potent in cells which were unable to repair DNA mismatches, and this highlights their significance in the treatment of cancer.[21–23] When comparing the work to Ru(II) polypyridyl complexes, it was shown that complexes with ancillary ligands in a *cis* arrangement can form covalent bonds to biomolecules, however, bioactive Ru(II) *trans* polypyridyl complexes are not well explored.[24]

In 2019, Khan et al. reported Rh(III) isoquinoline complexes (**Figure 1H**) which accumulated in the mitochondria and induced apoptosis *via* mitochondrial membrane damage,[25] and highlighted their potential different modes of action to the Pt(II) based therapeutics. Petrović et al. reported new Rh(III) complexes of the type $[RhCl_3(L)]$, with the incorporation of camphor-derived bis(pyrazolylpyridine) ligands (**Figure 1I**), and show their binding to bio-molecules, whereby the binding weakens following the order: guanosine monophosphate (5'-GMP) > glutathione (GSH) > methionine (L-Me).[26] The complexes also had good affinity for calf thymus-DNA (ct-DNA) and bovine serum albumin (BSA), again showing potentially different modes of action for Rh(III) complexes. To date the majority of Rh anti-cancer research has been focused on arene-based compounds, and there are relatively few reports on the anti-cancer properties of coordination Rh(III) complexes of the type $[RhX_2L_2]$.

Alongside other research groups, we have been interested in answering the question "does geometry matter in the design of anticancer complexes?"[24] Wachter et al. reported that geometry

plays a very important role, and showed a Ru(II) *trans* complex (**Figure 1J**) which exhibited higher *in vitro* anti-cancer activity and was significantly superior to that of the analogous *cis* complex. Complementary to this work, in 2017 we highlighted Ru(III) dihalido picolinamide complexes (**Figure 1K**), which exist as multiple isomers, and are dependent on the nature of the ancillary halide (X = Cl or I).[27] The Ru(III) dichlorido complexes existed as a range of different isomers, whilst the Ru(III) diiodido complexes yielded single stable *trans* isomers. In particular, the *N*-(3-bromophenyl)picolinamide substituted Ru(III) diiodido complex exhibited nanomolar potency against several cancer cell lines. Overall, the diiodido complexes were ~13x more cytotoxic than the analogues chloride complexes, highlighting potentially different modes of action, similar to the Pt(II) work reported by Quiroga et al.[8]

Since we have already reported the potential of using picolinamide ligands to increase cytotoxicity of metal complexes,[27–29] we provide further insight into the biological cytotoxicity and the stability of the *cis* and *trans* dihalido complexes, by reporting eight new *N*-((3-halidophenyl)picolinamide)rhodium(III) dihalido complexes, $[RhX_2(L)(LH]$, where X = chlorido (**1-4**) or iodido (**5-8**). The complexes are fully characterized and we report seven new crystal structures. Alongside SC-XRD, the solid state data from PXRD measurements indicate a "mixture" of isomers for the dichlorido complexes **1-4**, but single *trans* isomers for the diiodido complexes **5-8**. The stability of the complexes in solvent and phosphate buffer solution (PBS) were assessed, and confirms the *trans* diiodido complexes remain stable towards ligand exchange. The library of complexes have been screened against a range of human cell lines, and results show the Rh(III) *trans* diiodido complex (where L/LH = *N*-(3-bromophenyl)picolinamide), exhibits high cytotoxicity which is ~78x more active than the

analogous dichlorido complex, and is >25x more selective than cisplatin against human colorectal cancer.

Experimental

General: All complexes were synthesized using aerobic reaction conditions. Chemicals were obtained from Sigma-Aldrich Chemical Co., Acros Organics, Alfa Aesar and Strem Chemical Co., and, unless otherwise stated, and were used as supplied. General preparation and characterization data by NMR spectroscopy, FTIR, ES+MS and microanalysis values were obtained for complexes **1-8**.

Instrumentation: All NMR spectra were recorded on a Bruker DPX 300, Bruker 400 Ultrashield Plus or a Bruker Ascend 500 spectrometer. Elemental analyses were acquired by Ms. Tanya Marinko-Covell at the Microanalytical Service (University of Leeds) and Mr. Stephen Boyer at the Elemental Analysis Service (London Metropolitan University). Mass Spectra were recorded on a Bruker maXis impact mass spectrometer or on a Micromass ZMD spectrometer with electrospray ionization and photoiodide array analyzer at the University of Leeds. Infrared spectra were obtained using a Platinum ATR Spectrometer on a crystal plate with samples analyzed using OPUS software.

X-ray crystallographic analysis*:* A suitable single crystal was selected and immersed in an inert oil. The crystal was then mounted on a glass capillary and attached to a goniometer head on a Bruker X8 Apex diffractometer (**1, 3Δ, 4a, 4b, 6** and **7**) or a XtaLAB Synergy Dualflex HyPix (**3Λ)** using graphite monochromated Mo-Kα radiation (λ = 0.71073 Å) or Agilent SuperNova X-ray diffractometer fitted with an Atlas area detector and a kappa-geometry 4-circle goniometer, using graphite monochromated Mo-Kα radiation (λ = 0.71073 Å), using 1.0° ϕ-rotation frames. The crystal was cooled to 100-120 K by an Oxford Cryostream low temperature device.[30] The full

data set was recorded and the images processed using APEX2[31] or CrysAlis Pro software.[32] Structure solution by direct methods was achieved through the use of SHELXS programs,[33] and the structural model refined by full matrix least squares on F^2 using SHELXL.[33] Molecular graphics were plotted using Mercury.[34] Editing of CIFs and construction of tables and bond lengths and angles was achieved using PLATON[35] or Olex2 programs.[36] Unless otherwise stated, hydrogen atoms were placed using idealized geometric positions (with free rotation for methyl groups), allowed to move in a "riding model" along with the atoms to which they are attached, and refined isotropically.

Chemosensitivity Assays: Cell viability assays were conducted using human cell lines: colorectal carcinomas – *p53*-wildtype and *p53*-null (HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$), lung carcinoma (A549), melanoma (FM55), pancreatic carcinoma (MIA PaCa-2), ovarian carcinomas – cisplatin sensitive and cisplatin resistant (A2780 and A2780cisR), breast adenocarcinoma (MCF-7) and normal epithelial retinal (ARPE-19). All cell lines were routinely maintained as monolayer cultures in appropriate complete medium, and maintained in either T-25 or T-75 flasks at 37 °C and 5% CO_2. A549, MIA PaCa-2 and ARPE-19 cells were cultured in high glucose DMEM-F12 medium containing 10% fetal calf serum, whilst HCT116 $p53^{+/+}$, HCT116 $p53^{-/-}$, A2780, A2780cisR and MCF-7 were cultured in RPMI-1640 supplemented with 10% fetal calf serum, all medium was supplemented with sodium pyruvate (1 mM) and L-glutamine (2 mM). Prior to chemosensitivity studies, cell monolayers were passaged using Trypsin-EDTA and diluted to a concentration of 1 x 10^4 cells/mL. All assays were conducted using 96-well plates, in which 100 μL of the cell suspension was added to each well and incubated for 24 h at 37 °C and 5% CO_2 (column 1 contains just media to serve as a blank). All complexes were made fresh using stock solutions of dimethyl sulfoxide (DMSO) at 100 mM. After 24 h, 100 μL of drug dilutions in media

were added to the plates in columns 3-12 (column 2 contains 100% cells to serve as a control), and then incubated for a further 96 h (24 h or 72 h) at 37°C and 5% CO_2. After 96 h, 20 µL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 5 mg/mL) was added to each well and incubated for 3 h at 37°C and 5% CO_2. All solutions were then removed via pipette and 150 µL of DMSO added. Each well was mixed using a pipette and the absorbance of each well was measured at 540 nm using a ThermoFisher Multiskan FC spectrophotometer microplate reader. Results were plotted on a logarithmic scale, and the half maximal inhibitory concentration (IC_{50}) determined from duplicate of triplicate repeats, and reported as an $IC_{50} \pm$ Standard Deviation (SD).

Data Analysis: Statistical analysis of the results was conducted using Student's t-test, for $p < 0.05$ being considered as significant, and $p < 0.01$ as very significant.

N-(3-X-phenyl)picolinamide Ligand Preparation (X = F, Cl, Br or I): The ligands have been previously reported,[27,29] and were prepared using the same synthetic route, which is a modification of published procedures.[37,38] Functionalized aniline (25 mmol, 1 eq.) was added to a solution of pyridine-2-carboxylic acid (25 mmol, 1 eq.) in pyridine (15 mL) and warmed to 50°C for 15 min. To this mixture, triphenylphosphite (25 mmol, 1 eq.) was added and heated to 110°C for 18 h, yielding orange solutions. Addition of water (100 mL) yielded white solids to which dichloromethane (40 mL) was added and the organic layer separated. The aqueous layer was extracted with 1:1 (v/v) aqueous HCl (3 x 100 mL). To neutralize the extract, sodium bicarbonate was added until pH 7. The brown solids were isolated by filtration then washed with distilled water. After recrystallization from methanol, washing with water and drying *in vacuo*, the ligands are isolated as pale brown solids or needle-like crystals. ^{1}H NMR was conducted on all ligands to confirm their successful synthesis.

Rhodium dichloride complexes, [$RhCl_2(L)(LH]$: *N*-(3-halidophenyl)picolinamide ligand (0.80 mmol, 2 eq.) was added to a solution of $RhCl_3 \cdot 3H_2O$ (0.40 mmol, 1 eq.) in ethanol (30 mL), followed by triethylamine (0.40 mmol, 1 eq.). The solution was heated under reflux for 2 h, yielding a yellow-orange solution. Pentane was added to precipitate the complexes as yellow solids which were filtered, washed with pentane, dried *in vacuo* and recrystallized via vapor diffusion in methanol/pentane to yield analytically pure products. All yields were calculated before recrystallization.

[$(C_{12}H_8FN_2O)(C_{12}H_9FN_2O)RhCl_2$] (1): Yield: 0.183 g, 0.23 mmol, 49%; ***ES+MS (CH_3OH, m/z):*** *Anal. Calc for $C_{24}H_{17}N_4O_2F_2Cl_2Rh$:* 605.2*; Anal. Found:* 605.0 $[M]^+$; ***Elemental Analysis:*** *Anal. Calc.:* C 47.6; H 2.8; N 9.3, Cl 11.7%; *Anal. Found:* C 47.3; H 3.0; N 8.9; Cl 11.4%; ***FTIR (cm^{-1}):*** 3330 (b), 3079 (w), 1563 (s), 1486 (m), 1475 (m), 1401 (m), 1349 (w), 1305 (w), 1264 (m), 1189 (w), 1120 (w), 1030 (w), 971 (w), 907 (w), 865 (m), 761 (s), 706 (m), 676 (s), 593 (w), 518 (m), 457 (m), 448 (w); ***Major isomer: ^{1}H NMR (d_4-MeOD, 300.13 MHz, 300K):*** δ 9.81 (d, 1H, $^3J(^1H\text{-}^1H)$ = 5.9 Hz), 9.38 (d, 1H, $^3J(^1H\text{-}^1H)$ = 5.5 Hz), 8.35 (d, 1H, $^3J(^1H\text{-}^1H)$ = 7.2 Hz), 8.30 (dd, 1H, $^3J(^1H\text{-}^1H)$ = 7.7 Hz, $^4J(^1H\text{-}^1H)$ = 1.5 Hz), 8.19 (m, 2H), 7.90 (ddd, $^3J(^1H\text{-}^1H)$ = 7.6 Hz, $^3J(^1H\text{-}^1H)$ = 5.8 Hz, $^4J(^1H\text{-}^1H)$ = 1.6 Hz), 7.81 (t, $^3J(^1H\text{-}^1H)$ = 6.5 Hz), 7.36 (m, 3H), 7.03 (m, 2H), 6.78 (m, 3H); ***$^{13}C\{^1H\}$ NMR (d_4-MeOD, 75.47 MHz, 300 K):*** δ 178.4 (Q, C=O), 174.6 (Q, C=O), 169.7 (CH), 164.4 (Q), 158.8 (Q), 157.3 (Q), 156.1 (Q), 148.9 (CH), 146.2 (CH), 145.0 (CH), 132.9 (CH), 131.5 (CH), 128.4 (CH), 125.2 (CH), 119.2 (CH), 118.8 (CH), 118.3 (CH), 114.8 (CH), 113.8 (CH), 113.4 (CH), 107.2 (C-F), 106.7 (CH), 106.0 (C-F), 97.7 (CH); ***Minor isomer: ^{1}H NMR (d_4-MeOD, 75.47 MHz, 300K):*** δ 9.69 (d, 1H, $^3J(^1H\text{-}^1H)$ = 5 Hz), 9.32 (d, 1H, $^3J(^1H\text{-}^1H)$ = 5.2 Hz), 8.59 (m, 2H), 7.59 (br. m, 9H), 7.19 (d, 2H, $^3J(^1H\text{-}^1H)$ = 6 Hz), 6.70 (br. m, 1H); ***$^{13}C\{^1H\}$ NMR (d_4-MeOD, 75.47 MHz, 300 K):*** δ 195.8 (Q, C=O), 190.3 (Q, C=O), 187.5 (Q), 180.7 (Q),

180.4 (CH), 177.1 (Q), 172.4 (Q), 170.8 (CH), 166.1 (CH), 165.3 (CH), 159.6 (CH), 155.8 (CH), 151.9 (CH), 146.3 (CH), 141.3 (CH), 139.6 (CH), 129.6 (CH), 124.2 (CH), 117.4 (CH), 111.9 (Q, C-F), 109.2 (Q, C-F), 105.1 (CH), 94.1 (CH), 90.9 (CH).

[($C_{12}H_8ClN_2O$)($C_{12}H_9ClN_2O$)$RhCl_2$] (2): Yield: 0.185 g, 0.29 mmol, 62%. ***ES+MS (CH_3OH, m/z):*** *Anal. Calc for $C_{24}H_{17}N_4O_2Cl_4Rh$:* 638.1*; Anal. Found:* 636.9 $[M-H]^+$; ***Elemental Analysis:*** *Anal. Calc. (with 1 molecule of EtOH):* C 45.6; H 3.4; N 8.2; Cl 20.7%. *Anal. Found:* C 44.9; H 3.0; N 8.0; Cl 20.5%; ***FTIR (cm^{-1}):*** 3287 (b), 3086 (w), 1563 (s), 1479 (m), 1428 (w), 1357 (w), 1305 (w), 1297 (w), 1189 (w), 1155 (w), 1098 (w), 1059 (w), 974 (w), 886 (m), 763 (m), 676 (s), 603 (w), 516 (w), 447 (m); ***Major isomer: ^{1}H NMR (d_4-MeOD, 300.13 MHz, 300K):*** δ 9.69 (d, 1H, $^3J(^1H\text{-}^1H)$ = 5.7 Hz), 9.29 (d, 1H, $^3J(^1H\text{-}^1H)$ = 5.5 Hz), 8.31 (d, 1H, $^3J(^1H\text{-}^1H)$ = 7.9 Hz), 8.21 (td, 2H, $^3J(^1H\text{-}^1H)$ = 7.6 Hz, $^4J(^1H\text{-}^1H)$ = 1.3 Hz), 8.14 (m, 1H), 8.09 (m, 2H), 7.80 (m, 3H), 7.50 (s, 1H), 7.41 (s, 1H), 6.80 (m, 3H); ***$^{13}C\{^1H\}$ NMR (d_4-MeOD, 75.47 MHz, 300 K):*** δ 195.4 (Q, C=O), 194.2 (Q, C=O), 190.4 (Q), 185.7 (Q), 185.5 (Q), 179.9 (Q), 178.5 (CH), 173.7 (CH), 167.5 (CH), 157.4 (CH), 154.1 (CH), 151.3 (CH), 141.5 (CH), 137.8 (CH), 135.1 (CH), 131.2 (CH), 128.9 (CH), 128.1 (CH), 126.7 (CH), 124.1 (CH), 119.7 (Q, C-Cl), 117.5 (Q, C-Cl); ***Minor isomer: ^{1}H NMR (d_4-MeOD, 300.13 MHz, 300K):*** δ 9.65 (d, 1H, $^3J(^1H\text{-}^1H)$= 5.1 Hz), 9.46 (d, 1H, $^3J(^1H\text{-}^1H)$ = 5.6 Hz), 8.66 (d, 1H, $^3J(^1H\text{-}^1H)$= 7.6 Hz), 8.50 (t, 1H, $^3J(^1H\text{-}^1H)$ = 7.0 Hz), 8.39 (t, 1H, $^3J(^1H\text{-}^1H)$= 8.4 Hz), 8.01 (d, 1H, $^3J(^1H\text{-}^1H)$ = 7.6 Hz), 7.93 (m, 2H), 7.86 (s, 1H), 7.75 (s, 1H), 7.45 (m, 1H), 7.30 (br. m, 2H), 7.09 (br. m, 2H), 6.97 (br. m, 1H); ***$^{13}C\{^1H\}$ NMR (d_4-MeOD, 75.47 MHz, 300 K):*** δ 194.7 (Q, C=O), 193.3 (Q, C=O), 188.9 (CH), 184.3 (Q), 181.7 (Q), 175.1 (Q), 174.1 (Q), 168.3 (CH), 165.5 (CH), 163.5 (CH), 160.0 (CH), 154.8 (CH), 153.2 (CH), 148.2 (CH), 140.2 (CH), 133.2 (CH), 129.7 (CH), 125.9 (CH), 123.9 (CH), 114.7 (Q, C-Cl), 110.7 (Q, C-Cl), 107.3 (CH).

[($C_{12}H_8BrN_2O$)($C_{12}H_9BrN_2O$)$RhCl_2$] (3): Yield: 0.183 g, 0.25 mmol, 50%. ***ES+MS (CH_3OH, m/z):*** *Anal. Calc for* $C_{24}H_{17}N_4O_2Br_2Cl_2Rh$*:* 727.0; *Anal. Found:* 726.8 $[M\text{-}H]^+$; ***Elemental Analysis:*** *Anal. Calc.:* C 39.7; H 2.4; N 7.7%. *Anal. Found:* C 39.4; H 2.4; N 7.5%; ***FTIR (cm^{-1})*** **3a:** 2997 (w), 2915 (w), 2759 (b), 1543 (s), 1467 (m), 1427 (w), 1338 (m), 1297 (w), 1256 (m), 1146 (m), 1058 (m), 969 (m), 928 (w), 860 (m), 778 (m), 751 (m), 682 (s), 593 (w), 567 (w), 512 (m), 484 (m), 437 (m); **3b:** 3189 (w), 3052 (b), 2980 (w), 1618 (m), 1590 (m), 1562 (s), 1469 (m), 1420 (w), 1389 (w), 1344 (w), 1297 (w), 1229 (m), 1153 (w), 1085 (w), 1058 (w), 983 (m), 907 (w), 860 (m), 758 (s), 672 (s), 600 (w), 553 (w), 512 (m), 470 (w), 430 (w); **3c:** 3380 (b), 3209 (b), 3066 (w), 1618 (s), 1598 (s), 1568 (s), 1466 (m), 1433 (w), 1413 (w), 1390 (w), 1340 (w), 1311 (w), 1260 (w), 1151 (w), 1062 (w), 1025 (w), 999 (w), 966 (w), 922 (w), 896 (w), 860 (m), 776 (s), 758 (s), 723 (w), 677 (s), 601 (w), 561 (w), 548 (w), 498 (w), 475 (m), 435 (m), 415 (w); ***Major isomer: ^{1}H NMR (d_6-$(CH_3)_2CO$, 300.13 MHz, 300K):*** δ 9.85 (d, 1H, $^3J(^1H\text{-}^1H)$ = 5.6 Hz), 9.39 (d, 1H, $^3J(^1H\text{-}^1H)$ = 5.6 Hz), 8.57 (d, 1H, $^3J(^1H\text{-}^1H)$ = 7.8 Hz), 8.29 (dtd, 2H, $^3J(^1H\text{-}^1H)$ = 10.4 Hz, $^3J(^1H\text{-}^1H)$ = 7.8 Hz, $^4J(^1H\text{-}^1H)$ = 1.5 Hz), 8.14 (m, 1H), 7.95 (ddd, 1H, $^3J(^1H\text{-}^1H)$ = 7.8 Hz, $^3J(^1H\text{-}^1H)$ = 5.5 Hz, $^4J(^1H\text{-}^1H)$ = 1.2 Hz), 7.90 (ddd, 1H, $^3J(^1H\text{-}^1H)$ = 7.6 Hz, $^3J(^1H\text{-}^1H)$ = 5.7 Hz, $^4J(^1H\text{-}^1H)$ = 1.6 Hz), 7.83 (t, 1H, $^3J(^1H\text{-}^1H)$ = 2.0 Hz), 7.64 (ddd, 1H, $^3J(^1H\text{-}^1H)$ = 8.1 Hz, $^3J(^1H\text{-}^1H)$ = 2.1 Hz, $^4J(^1H\text{-}^1H)$ =0.9 Hz), 7.45 (ddd, 1H, $^3J(^1H\text{-}^1H)$ = 8.0 Hz, $^3J(^1H\text{-}^1H)$ = 1.9 Hz, $^4J(^1H\text{-}^1H)$ = 1.0 Hz), 7.34 (t, 1H, $^3J(^1H\text{-}^1H)$ = 8.1 Hz), 7.18 (t, 1H, $^3J(^1H\text{-}^1H)$ = 1.9 Hz), 7.01 (m, 2H), 6.86 (m, 1H); ***$^{13}C\{^1H\}$ NMR (d_6-$(CH_3)_2CO$, 75.47 MHz, 300 K):*** δ 199.2 (Q, C=O), 197.2 (CH), 194.1 (Q, C=O), 188.6 (Q), 168.7 (Q), 153.3 (CH), 152.7 (Q), 151.7 (Q), 139.6 (CH), 139.4 (CH), 130.8 (CH), 130.5 (CH), 130.1 (CH), 129.9 (CH), 129.6 (CH), 129.2 (CH), 126.9 (CH), 126.3 (CH), 125.5 (CH), 125.3 (CH), 121.5 (CH), 121.2 (Q, C-Br), 120.8 (Q, C-Br); ***Minor isomer 1: ^{1}H NMR (d_6-$(CH_3)_2CO$, 300.13 MHz, 300K):*** δ 9.46 (d, 1H, $^3J(^1H\text{-}^1H)$= 5.6 Hz), 8.91 (d, 1H,

$^{3}J(^{1}H\text{-}^{1}H)$ = 5.2 Hz), 8.04 (dd, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 3.0 Hz, $^{4}J(^{1}H\text{-}^{1}H)$ = 1.4 Hz), 7.8 (d, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 1.9 Hz), 7.75 (m, 2H), 7.73 (m, 1H), 7.68 (ddd, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 7.5 Hz, $^{3}J(^{1}H\text{-}^{1}H)$ = 5.7 Hz, $^{4}J(^{1}H\text{-}^{1}H)$ = 1.5 H), 7.14 (t, 2H, $^{3}J(^{1}H\text{-}^{1}H)$ = 1.9 Hz), 7.12 (m, 1H), 7.05 (m, 2H), 6.97 (s, 1H), 6.93 (s, 1H), 6.91 (m, 1H); ***$^{13}C\{^{1}H\}$ NMR (d_6-$(CH_3)_2CO$, 75.47 MHz, 300 K):*** δ 209.0 (Q, C=O), 201.1 (Q, C=O), 195.4 (CH), 192.2 (CH), 161.2 (CH), 159.5 (Q), 156.8 (Q), 155.7 (CH), 149.7 (Q), 146.1 (Q), 143.6 (CH), 141.6 (CH), 138.5 (CH), 136.2 (CH), 134.0 (CH), 133.3 (CH), 132.7 (CH), 131.7 (CH), 127.2 (CH), 123.6 (CH), 120.7 (Q, C-Br), 120.3 (CH) 118.4 (Q, C-Br), 115.4 (CH); ***Minor isomer 2: ^{1}H NMR (d_6-$(CH_3)_2CO$, 300.13 MHz, 300K):*** δ 9.70 (d, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 5.4 Hz), 9.50 (d, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 5.8 Hz), 8.72 (d, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 8.6 Hz), 8.39 (m, 1H), 8.09 (m, 1H), 8.05 (dd, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 3.1 Hz, $^{4}J(^{1}H\text{-}^{1}H)$ = 1.4 Hz), 8.02 (dd, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 3.1 Hz, $^{4}J(^{1}H\text{-}^{1}H)$ = 1.4 Hz), 7.99 (d, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 7.3 Hz), 7.86 (m, 1H), 7.78 (d, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 1.5 Hz), 7.55 (m, 1H), 7.51 (m, 1H), 7.39 (t, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 1.9(x2) Hz), 7.37 (t, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 2.0 Hz), 7.15 (m, 1H), 7.07 (dd, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 1.9 Hz, $^{4}J(^{1}H\text{-}^{1}H)$ = 1.1 Hz); ***$^{13}C\{^{1}H\}$ NMR (d_6-$(CH_3)_2CO$, 75.47 MHz, 300 K):*** δ 203.2 (Q, C=O), 196.5 (Q, C=O), 190.4 (CH), 186.6 (CH), 183.6 (Q), 156.1 (Q), 154.0 (CH), 147 (Q), 145.7 (CH), 143.3 (Q), 137.8 (CH), 135.8 (CH), 134.5 (CH), 131.1 (CH), 127.9 (CH), 125.0 (CH), 119.3 (CH), 117.6 (CH), 116.5 (CH), 115.7 (CH), 114.3 (Q, C-Br), 112.8 (CH), 110.9 (Q, C-Br), 109.3 (CH).

$[(C_{12}H_8IN_2O)(C_{12}H_9IN_2O)RhCl_2]$ (4): Yield: 0.294 g, 0.36 mmol, 73%. ***ES+MS (CH_3OH, m/z):*** *Anal. Calc for $C_{24}H_{17}N_4O_2Cl_2I_2Rh$:* 821.0; *Anal Found:* 820.8 $[M\text{-}H]^{+}$; ***Elemental Analysis:*** *Anal. Calc. (with 0.5 molecules of H_2O):* C 34.4; H 2.3; N 6.7%. *Anal. Found:* C 34.0; H 2.2; N 6.3%; ***FTIR (cm^{-1}):*** 3236 (b), 3091 (w), 1564 (s), 1469 (m), 1418 (w), 1302 (w), 1270 (w), 1155 (w), 1063 (w), 1028 (w), 995 (w), 860 (w), 764 (m), 681 (s), 601 (w), 515 (w), 437 (w); ***Major isomer: ^{1}H NMR (d_4-MeOD, 300.13 MHz, 300K):*** δ 9.83 (d, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 5.6 Hz), 9.42 (d,

1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 5.2 Hz), 8.43 (d, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 8.7 Hz), 8.35 (d, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 8.7 Hz), 8.28 (td, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 8.0 Hz, $^{4}J(^{1}H\text{-}^{1}H)$ = 1.0 Hz), 8.01 (dd, 2H, $^{3}J(^{1}H\text{-}^{1}H)$ = 8.4 Hz, $^{3}J(^{1}H\text{-}^{1}H)$ = 1.5 Hz), 7.94 (m, 1H), 7.79 (m, 1H), 7.71 (m, 2H), 7.37 (s, 1H), 7.19 (m, 3H), 6.83 (s, 1H); ***$^{13}C\{^{1}H\}$ NMR (d_4-MeOD, 75.47 MHz, 300 K):*** δ 192.0 (Q, C=O), 190.2 (CH), 188.50 (Q, C=O), 173.6 (CH), 158.0 (Q), 155.7 (Q), 154.7 (CH), 141.6 (Q), 139.7 (Q), 137.6 (CH), 137.4 (CH), 135.8 (CH), 135.3 (CH), 134.7 (CH), 133.1 (CH), 132.3 (CH), 131.6 (CH), 130.1 (CH), 126.4 (CH), 124.9 (CH), 121.4 (Q, C-I), 118.6 (Q, C-I), 112.9 (CH); ***Minor isomer: ^{1}H NMR (d_4-MeOD, 300.13 MHz, 300K):*** δ 9.16 (d, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 5.3 Hz), 9.03 (d, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 5.2 Hz), 8.29 (dd, 2H, $^{3}J(^{1}H\text{-}^{1}H)$ = 7.8 Hz, $^{4}J(^{1}H\text{-}^{1}H)$ = 1.6 Hz), 8.08 (d, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 8.3 Hz), 7.81 (m, 2H), 7.73 (d, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 6.2 Hz), 7.69 (br. m, 3H), 7.45 (s, 1H), 7.32 (d, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 7.1 Hz), 7.21 (d, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 7.7 Hz), 7.00 (s, 1H), 6.95 (br. m, 1H); ***$^{13}C\{^{1}H\}$ NMR (d_4-MeOD, 75.47 MHz, 300 K):*** δ 178.6 (Q, C=O), 178.3 (CH), 177.6 (Q, C=O), 176.1 (Q), 174.6 (Q), 157.6 (CH), 144.3 (Q), 143.5 (Q), 140.4 (CH), 139.3 (CH), 139.0 (CH), 136.2 (CH), 136.1 (CH), 135.2 (CH), 133.7 (CH), 132.8 (CH), 127.0 (CH), 126.3 (CH), 125.5 (CH), 122.8 (CH), 122.0 (CH), 117.8 (Q, C-I), 117.6 (CH), 116.0 (Q, C-I).

Rhodium diiodide complexes, [RhI_2(L)(LH)]: *N*-(3-halidophenyl)picolinamide ligand (1.20 mmol, 2 eq.) was added to a solution of $RhCl_3 \cdot 3H_2O$ (0.60 mmol, 1 eq.) in ethanol (30 mL), followed by triethylamine (0.60 mmol, 1 eq.). The solution was heated under reflux for 2 h, yielding a yellow-orange solution. Excess KI (6.0 mmol, 5 eq.) was added to the solution and reflux continued for 18 h. The dark brown solids were filtered, washed with ethanol, dried *in vacuo* and recrystallized via vapor diffusion in dimethylformamide/diethyl ether to yield analytically pure products. All yields were calculated before recrystallization.

[($C_{12}H_8FN_2O$)($C_{12}H_9FN_2O$)RhI_2] (5): Yield: 0.160 g, 0.19 mmol, 45%. ***ES+MS (DMF, m/z):*** *Anal. Calc for $C_{24}H_{17}N_4O_2F_2I_2Rh$ (with 1 molecule of CH_3OH):* 820.2; *Anal. Found:* 820.8 $[M]^+$; ***Elemental Analysis:*** *Anal. Calc. (with 0.33 molecules of DMF and H_2O):* C 36.2; H 2.9; N 7.8%. *Anal. Found:* C 36.2; H 2.6; N 7.7%; *FTIR (cm^{-1}):* 3054 (b), 2865 (w), 1567 (s), 1471 (m), 1427 (w), 1343 (m), 1305 (m), 1258 (m), 1155 (m), 1074 (m), 981 (w), 875 (m), 760 (s), 676 (m), 613 (w), 497 (m), 440 (m); ***^{1}H NMR (d_4-MeOD, 300.13 MHz, 300K):*** δ 9.62 (d, 1H, $^3J(^1H\text{-}^1H)$ = 5.6 Hz), 8.45 (d, 1H, $^3J(^1H\text{-}^1H)$ = 4.9 Hz), 7.77 (td, 1H, $^3J(^1H\text{-}^1H)$ = 7.8 Hz), 7.71 (m, 1H), 7.66 (m, 1H), 7.42 (dd, 1H, $^3J(^1H\text{-}^1H)$ = 8.2 Hz, $^3J(^1H\text{-}^1H)$ = 1.4 Hz), 7.36 (m, 1H), 7.29 (ddd, 1H, $^3J(^1H\text{-}^1H)$ = 7.4 Hz, $^3J(^1H\text{-}^1H)$ = 5.8 Hz, $^4J(^1H\text{-}^1H)$ = 2.0 Hz), 7.00 (s, 1H), 6.87 (t, 1H, $^3J(^1H\text{-}^1H)$ = 7.1 Hz), 6.7 (m, 5H), 6.6 (s, 1H); ***$^{13}C\{^1H\}$ NMR (d_4-MeOD, 75.47 MHz, 300 K):*** δ 194.2 (Q, C=O), 192.50 (Q, C=O), 191.6 (CH), 174.2 (CH), 172.8 (Q), 167.9 (Q), 159.9 (Q), 157.6 (Q), 147.6 (CH), 147.1 (CH), 135.7 (CH), 133.4 (CH), 132.3 (CH), 130.5 (CH), 129.1 (CH), 128.7 (CH), 128.1 (CH), 127.0 (CH), 125.3 (CH), 122.0 (CH), 118.8 (Q, C-Cl), 117.5 (CH), 117.3 (CH), 116.5 (Q, C-Cl).

[($C_{12}H_8ClN_2O$)($C_{12}H_9ClN_2O$)RhI_2] (6): Yield: 0.124 g, 0.15 mmol, 36%. *ES+MS (DMF, m/z): Anal. Calc for $C_{24}H_{17}N_4O_2F_2I_2Rh$:* 788.1; *Anal. Found:* 788.2 $[M]^+$; ***Elemental Analysis:*** *Anal. Calc*: C 36.6; H 2.2; N 7.1%. *Anal. Found:* C 36.3; H 2.4; N 7.3%; *FTIR (cm^{-1}):* 3066 (b), 2939 (w), 1590 (m), 1579 (s), 1483 (m), 1444 (m), 1405 (m), 1357 (m), 1307 (m), 1265 (m), 1183 (m), 1157 (m), 1125 (m), 1062 (m), 988 (w), 965 (m), 869 (m), 796 (m), 767 (s), 750 (s), 705 (m), 675 (s); ***^{1}H NMR (d_6-acetone, 400.13 MHz, 300 K):*** δ 9.40 (br. d, 1H, 8.73 $^3J(^1H\text{–}^1H)$ = 5.6 Hz), 8.73 (d, 1H, $^3J(^1H\text{–}^1H)$ = 8.4 Hz), 8.28 (br, t, 1H, $^3J(^1H\text{–}^1H)$= 7.6 Hz), 8.08 (d, 1H, $^3J(^1H\text{–}^1H)$ = 7.8 Hz), 7.89 (m, 1H), 7.71-7.56 (m, 5H), 7.51 (br, s, 1H), 7.50-7.46 (m, 1H), 7.36 (m, 1H), 7.45-7.41 (m, 1H), 7.00 (s, 1H), 7.24 (br. d, 1H, $^3J(^1H\text{–}^1H)$ = 5.5 Hz), 7.14 (m, 1H); ***$^{13}C\{^1H\}$ NMR (d_6-***

acetone, 75.47 MHz, 300 K): δ 190.6 (Q, C=O), 189.90 (Q, C=O), 187.2 (CH), 166.4 (CH), 163.0 (Q), 160.0 (Q), 159.9 (Q), 150.8 (Q), 148.3 (CH), 139.6 (CH), 136.5 (CH), 134.0 (CH), 132.3 (CH), 130.7 (CH), 128.1 (CH), 127.0 (CH), 126.9 (CH), 125.6 (CH), 124.1 (CH), 122.9 (CH), 119.4 (CH), 118.8 (Q, C −Cl), 117.9 (CH).

[($C_{12}H_8BrN_2O$)($C_{12}H_9BrN_2O$)RhI_2] (7): Yield: 0.156 g, 0.170 mmol, 36%. ***ES+MS (DMF, m/z):*** *Anal. Calc for $C_{24}H_{17}N_4O_2Br_2I_2Rh$:* 909.9; *Anal Found:* 910.7 $[MH]^+$; ***Elemental Analysis:*** *Anal. Calc. (0.5 molecules of KI):* C 27.2; H 1.6; N 5.2%. *Anal. Found:* C 27.2; H 1.7; N 5.0%; ***FTIR (cm^{-1}):*** 3044 (b), 2919 (w), 1610 (s), 1544 (s), 1468 (m), 1430 (w), 1334 (m), 1298 (m), 1263 (m), 1148 (m), 1059 (w), 996 (w), 924 (w), 862 (m), 783 (m), 778 (m), 678 (s), 600 (w), 562 (w), 516 (m), 514 (m), 437 (m); ***^{1}H NMR (d_4-MeOD, 300.13 MHz, 300K):*** *δ* 9.96 (d, 1H, $^3J(^1H\text{-}^1H)$ = 5.3 Hz), 9.62 (d, 1H, $^3J(^1H\text{-}^1H)$ = 5.0 Hz), 8.41 (d, 1H, $^3J(^1H\text{-}^1H)$ = 7.6 Hz), 8.09 (m, 1H), 7.92 (d, 1H, $^3J(^1H\text{-}^1H)$ = 7.9 Hz), 7.73 (td, 1H, $^3J(^1H\text{-}^1H)$ = 7.6 Hz, $^4J(^1H\text{-}^1H)$ = 1.8 Hz), 7.42 (m, 2H), 7.31 (dd, 1H, $^3J(^1H\text{-}^1H)$ = 6.5 Hz, $^4J(^1H\text{-}^1H)$ = 4.8 Hz), 7.18 (t, 1H, $^3J(^1H\text{-}^1H)$ = 8.5 Hz), 7.01 (m, 2H), 6.91 (d, 1H, $^3J(^1H\text{-}^1H)$ = 8.3 Hz), 6.87 (m, 2H), 6.76 (s, 1H); ***$^{13}C\{^1H\}$ NMR (d_4-MeOD, 75.47 MHz, 300 K):*** *δ* 193.7 (Q, C=O), 192.3 (CH), 192.0 (Q, C=O), 188.3 (Q), 179.2 (CH), 173.4 (Q), 163.0 (Q), 147.9 (Q), 142.5 (CH), 138.9 (CH), 138.3 (CH), 136.8 (CH), 130.8 (CH), 130.2 (CH), 129.8 (CH), 128.9 (CH), 128.2 (CH), 126.7 (CH), 124.2 (CH), 123.4 (CH), 122.0 (Q, C-Br), 117.4 (CH), 116.6 (Q, C-Br).

[($C_{12}H_8IN_2O$)($C_{12}H_9IN_2O$)RhI_2] (8): Yield: 0.234 g, 0.234 mmol, 41%. ***ES+MS (DMF, m/z):*** *Anal. Calc for $C_{24}H_{17}N_4O_2I_4Rh$:* 1003.9; *Anal. Found:* 1004.7 $[MH]^+$; ***Elemental Analysis:*** *Anal. Calc.:* C 28.7; H 1.7; N 5.6%. *Anal. Found:* C 28.6; H 1.8; N 5.6%; ***FTIR (cm^{-1}):*** 2978 (b), 2668 (w), 1614 (s), 1584 (m), 1556 (w), 1465 (m), 1392 (m), 1293 (m), 1261 (m), 1150 (m), 1091 (s), 1055 (s), 1030 (s) 995 (s), 901 (s), 855 (m), 759 (m), 716 (m), 680 (s), 654 (m), 637 (m), 622 (s),

598 (s), 554 (s), 508 (s), 459 (s); ***^{1}H NMR (d_6-DMSO, 300.13 MHz, 300K):*** δ 9.65 (br. d, 1H, $^{3}J(^{1}H\text{-}^{1}H)$ = 5.0 Hz), 7.92 (m, 2H), 7.89 (d, 2H, $^{3}J(^{1}H\text{-}^{1}H)$ = 10 Hz), 7.61 (d, 2H, $^{3}J(^{1}H\text{-}^{1}H)$ = 5 Hz) 7.52 (td, 2H, $^{3}J(^{1}H\text{-}^{1}H)$ = 5 Hz, $^{4}J(^{1}H\text{-}^{1}H)$ = 2.5 Hz), 7.47 (s, 2H), 7.17 (t, 2H, $^{3}J(^{1}H\text{-}^{1}H)$ = 10 Hz), 7.02 (m, 2H), 6.75 (t, 2H) ***$^{13}C\{^{1}H\}$ NMR (d_6-DMSO, 75.47 MHz, 300 K):*** δ 167.9 (Q, C=O), 159.3 (Q), 146.9 (Q), 139.3 (CH), 138.2 (CH), 135.6 (CH), 129.6 (CH), 127.2 (CH), 127.1 (CH), 125.2 (CH), 124.9 (CH), 93.9 (Q, C-I).

Stability studies of Complex 3 (water inclusion): Complex **3** (3.6 mg, 4.95 mmol) was made up to a 7.0 mM concentration with 0.7 mL d_6-DMSO:PBS (80:20 *v/v*) and an NMR recorded at various time points over a period of 96 h.

Stability studies of Complex 7 (water inclusion): Complex **7** (4.5 mg, 4.95 mmol) was made up to a 7.0 mM concentration with 0.7 mL d_3-MeCN:PBS (80:20 *v/v*) and an NMR recorded at various time points over a period of 96 h.

Ligand exchange with Complex 3 (water inclusion): Complex **3** (3.6 mg, 4.95 mmol) was treated with one equivalent of *N*-(3-chlorophenyl)picolinamide ligand (L′) (1.2 mg, 4.95 mmol) and made up to a 7.0 mM concentration with 0.7 mL d_6-DMSO:PBS (80:20 *v/v*). NMR spectra were recorded at various time points over a period of 96 h.

Ligand exchange with Complex 7 (water inclusion): Complex **7** (4.5 mg, 4.95 mmol) was treated with one equivalent of *N*-(3-chlorophenyl)picolinamide ligand (L′) (1.2 mg, 4.95 mmol) and made up to a 7.0 mM concentration with 0.7 mL of d_3-MeCN:PBS (80:20 *v/v*). NMR spectra were recorded at various time points over a period of 96 h.

Ligand exchange with Complexes 1 and 4 (water exclusion): Each complex (3.6 mg, 4.95 mmol) was treated with one equivalent of *N*-(X-phenyl)picolinamide ligand (X = 3-fluoro or 3-

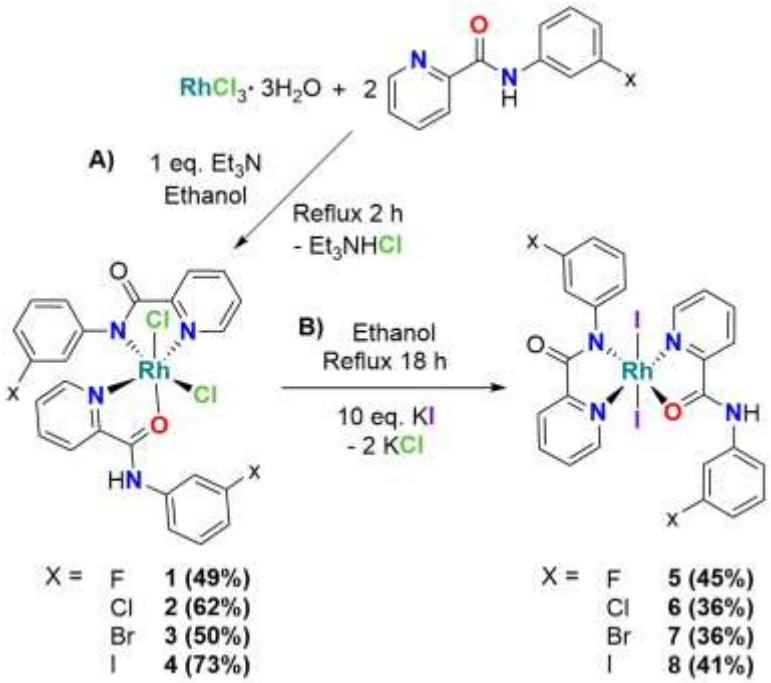
iodo) (1.2 mg, 4.95 mmol) and made up to a 7.0 mM concentration with either 0.7 mL $CDCl_3$ or 0.7 mL d_6-DMSO. NMR spectra were recorded after approximately 10 mins.

Ligand exchange with Complexes 5 and 8 (water exclusion): Each complex (4.5 mg, 4.95 mmol) was treated with one equivalent of *N*-(X-phenyl)picolinamide ligand (X = 3-fluoro or 3-iodo) (1.2 mg, 4.95 mmol) and made up to a 7.0 mM concentration with either 0.7 mL $CDCl_3$ or 0.7 mL d_6-DMSO. NMR spectra were recorded after approximately 10 mins.

Results and Discussion

The *N*-(3-halidophenyl)picolinamide ligands were prepared by modifications of previously reported methods.[27,37,38] The Rh(III) dichlorido complexes, $[RhCl_2(L)(LH]$ **1-4**, were prepared by heating to reflux, $RhCl_3 \cdot 3H_2O$ (1 eq.) with a functionalized *N*-(3-halidophenyl)picolinamide ligand (2 eq.) and triethylamine (1 eq.), for 2 h in ethanol (**Scheme 1A**). The Rh(III) diiodido complexes, $[RhI_2(L)(LH]$ **5-8**, were synthesized using a halide-exchange reaction and heating to reflux the dichlorido complexes **1-4** and excess KI, in ethanol for 18 h (**Scheme 1B**).[27] All complexes were obtained as analytically pure compounds in moderate yields (36-73%) and have

been fully characterized using FTIR, NMR, ES-MS, elemental analysis, PXRD and single X-ray diffraction where possible.



Scheme 1. Synthesis of **A)** Rh(III) dichlorido complexes [$RhCl_2$(L)(LH] (**1-4**); **B)** Rh(III) diiodido complexes [RhI_2(L)(LH] (**5-8**).

Single Crystal X-ray Diffraction (SC-XRD):

[$RhCl_2$(L)(LH)]: Single crystal X-ray diffraction (SC-XRD) was obtained for complexes **1**, **3Δ, 3Λ** and **4a** and **4b** (**Figure 2**) by vapor diffusion of pentane/methanol. Complex **1** crystallized in an orthorhombic cell and structural solution was performed in space group $P2_12_12_1$. Complex **3** crystallized as orange plates, however, when the reaction was repeated the enantiomer of complex **3** was obtained (**Figure 3**). Both structures, **3Δ** and **3Λ**, crystallized in a monoclinic cell and structural solutions were performed in space group *Cc*. Upon taking the Δ enantiomer and re-dissolving it in methanol, the complex crystallizes as the Λ enantiomer, showing these structures are labile and fluxional. Although SC-XRD was only obtained on the orange plates, three sets of

different colored crystal morphologies were also observed for complex **3**, however, on a second attempted synthesis only two morphologies were obtained (**Figure S5**). SC-XRD confirmed complex **3** (**Δ** and **Λ**) to be the *cis(Cl)-trans(N,N)-cis(N,O)* isomer (**Figure 2**). Within the sample vial of complex **4**, two different crystal types were also observed, red blocks(**4a**) and orange plates (**4b**). SC-XRD was obtained for both of these crystals, and confirmed both to be in a monoclinic cell and structural solutions were performed in $P2_1/c$ and $P2_1/n$, respectively. The two crystals of complex **4** were found to be different structural isomers: *cis(Cl)-trans(N,N)-cis(N,O)* (**4a**) and *trans(Cl)-trans(N,N)-trans(N,O)* (**4b**) (**Figure 2**). The X-ray crystallographic data and bond angles are stated in **Table S1** and **Table S2**, respectively.

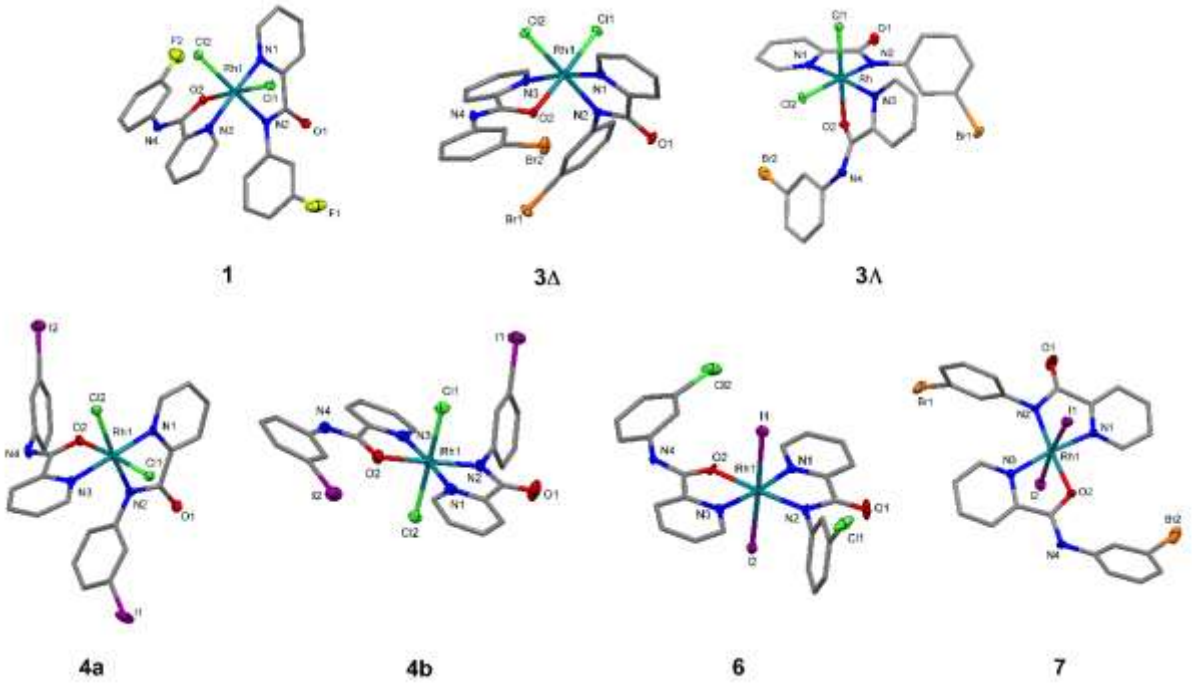


Figure 2. Molecular structures of rhodium dichlorido compounds **1**, **3Δ, 3Λ**, **4a** and **4b**, and rhodium diiodido compounds **6** and **7**. Hydrogen atoms and solvent molecules are omitted for

clarity and displacement ellipsoids are at the 50% probability level (shown only for the heteroatoms).

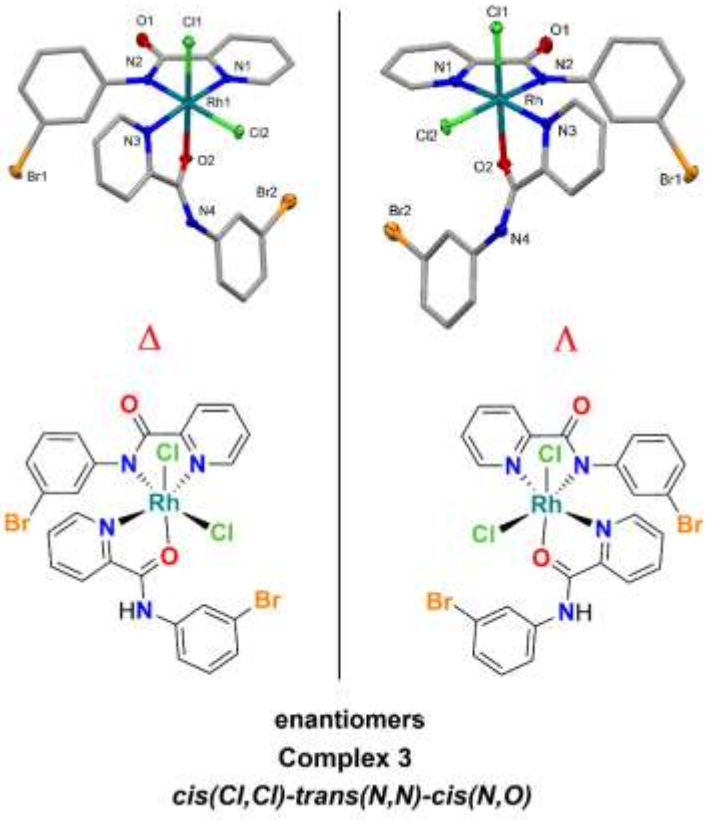


Figure 3. Chemical structures (SC-XRD and Chemdraw) of complexes **3Δ** and **3Λ.**

[RhI₂(L)(LH]: SC-XRD were obtained for complexes **6** and **7** (**Figure 2**) by vapor diffusion of diethyl ether/DMF. Both complexes crystallized in a monoclinic cell, with structural solutions performed in space group *Cc*. Only one crystal morphology was observed for these Rh(III) diiodido complexes, and both were identified to be the *trans(X)-trans(N,N)-trans(N,O)* isomer. The X-ray crystallographic data and bond angles are stated in **Table S3** and **Table S4**, respectively.

In all cases, the ligands have different binding modes to the metal center, whereby one ligand is neutral and bound *N,N* (LH) and the other ligand is anionic and bound *N,O* (L). Both the dichlorido and diiodido complexes have slightly distorted bond angles suggesting a *pseudo* octahedral geometry (**Table S2** and **Table S4**), with all bond lengths as expected for Rh(III) compounds (**Table 1**).

Table 1. Selected bond lengths (Å) for compounds **1**, **3 (Δ and Λ)**, **4a**, **4b**, **6** and **7**, with s.u.s shown in parenthesis.

Bond Lengths (Å)	**X = Cl**					**X = I**	
	1	**3 (Δ)**	**3 (Λ)**	**4a**	**4a**	**6**	**7**
Rh(1)-X(1)	2.3168(15)	2.3078(10)	2.3115(9)	2.3134(13)	2.3358(18)	2.6636(12)	2.6776(7)
Rh(1)-X(2)	2.3568(13)	2.3636(10)	2.3665(8)	2.3558(14)	2.3408(17)	2.6631(12)	2.6730(7)
Rh(1)-N(1)	2.017(4)	2.021(3)	2.028(3)	2.030(4)	2.022(6)	2.008(10)	2.040(6)
Rh(1)-N(2)	2.022(4)	2.011(3)	2.009(3)	2.018(4)	2.021(6)	2.014(19)	2.007(5)
Rh(1)-N(3)	2.030(5)	2.027(3)	2.034(3)	2.031(4)	2.072(6)	2.035(10)	2.089(5)
Rh(1)-O(2)	2.043(4)	2.051(3)	2.050(2)	2.050(3)	2.077(5)	2.066(9)	2.073(4)

FTIR Spectroscopy: Several of the Rh(III) dichlorido complexes crystallize in different fractions, with different colors and morphologies. The first synthetic attempt and recrystallization of complex **3**, yielded red needles (*cis(Cl)-trans(N,N)-cis(N,O*), **3Δ**, **Figure 2-3**), orange blocks **3b** and yellow microcrystals **3c**. The different fractions were well-separated and collected for further characterization. The FTIR spectra show the presence of the different

fractions (**Figure S1**), highlighting a weak NH stretch at 3033 cm^{-1} (**3Δ**), 3057 cm^{-1} (**3b**) and 3065 cm^{-1} (**3c**), and the CO stretch (1613-1543 cm^{-1}) begins to separate into asymmetric and symmetric stretches, however, this is only observed in fractions **3b** and **3c**.

Powder X-ray diffraction (PXRD): PXRD was obtained for the bulk samples of both complexes **3** and **7** (**Figure S2a-b** respectively), and were compared to the simulated *cis* and *trans* spectra. Complex **3** was also compared to the single crystals of the *cis(X,X)-trans(N,N)-cis(N,O)* (**3Δ**) isomer (**Figure 2-3**). We were unable to confirm the major isomer from PXRD alone, as the PXRD pattern shows a mix of different isomers. However, PXRD for complex **7** shows only the expected single *trans* isomer which was observed in single crystal XRD, and gives further evidence that the bulk sample consists of one isomer.

NMR Spectroscopy: The ^{1}H NMR spectra of the complexes do not often show the NH,[39] which is usually due to the use of polar solvents which are required for solubility. This often highlights the uncertainty of ligand binding in solution. On the first synthetic attempt of complex **3**, several isomers were observed in the ^{1}H NMR in d_3-acetone, which were proposed to be **3Δ**, **3b**, and **3c** (**Figure S3**). The different morphologies were separated out and each ^{1}H NMR obtained, showing **3c** to have additional weak resonances, suggesting yet another isomer may be present within this solution. The ratio of the possible isomers determined from the integrals of the ^{1}H NMR spectra show 1:0.2 for complex **3Δ** and 1:0.2:0.1 for complex **3c**. Variable temperature NMR (213 K – 313 K) was also conducted on complex **3c**, and highlighted the appearance of new resonances at low temperature, and provides additional confirmation for multiple isomers in solution (**Figure S4**). On cooling the sample, a shift for the *ortho* pyridine hydrogens (Ha and

Ha', see **Figure S3**) to a higher frequency was observed, whilst the remaining pyridine protons shift to lower frequencies.

A second attempt was made to synthesize and recrystallize complex **3**, however, this gave rise to a dark orange/red product that on recrystallization from pentane/methanol vapor diffusion, gave orange plates and a yellow microcrystalline material (**Figure S5**). The crude ^{1}H NMR has four resonances for the C-H *ortho* to py-N, Ha/Ha', whereby the resonances at 9.44 pm (d) and 8.66 ppm (d) are attributed to the yellow microcrystalline material and the resonances at 9.87 pm (d) and 9.44 ppm (d), the orange plates (**Figure S6**). The resonances in the yellow material are ~1 ppm apart, and we believe this to be from the non-symmetrical *cis(Cl,Cl)-cis(N,N)-cis(N,O)* isomer, which appears to be the major product and has inequivalent protons. The SC-XRD of the orange plates confirmed the *cis(Cl,Cl)-trans(N,N)-cis(N,O)* arrangement, however, this is not the major product.[40] It should also only have one set (or two very close overlapping) resonances for this type of ligand arrangement, as the protons are almost equivalent. Therefore, we are unable to confirm if the additional resonances observed are due to the enantiomer which we have also crystallized out (**3Λ**), or if it is the *cis(Cl,Cl)-cis(N,N)-trans(N,O)* arrangement. The ^{1}H NMR of complex **3** was also conducted in $CDCl_3$ (**Figure S7**) so we could observe the NH resonances, and only two were present at 11.66 ppm (br. s, major) and 12.06 (br. s, minor). The major NH resonance is assigned to the *cis(Cl,Cl)-cis(N,N)-cis(N,O)* arrangement, whilst the minor NH resonance cannot be observed in the NMR of the orange crystals, possibly due to its poor solubility $CDCl_3$. These results prove how unpredictable the isomerization is for the dichlorido complexes, and how difficult it would be to determine the active species. This reinforces our drive to focus on the single stable *trans* diiodido complexes.

The stability of the Rh(III) dichlorido complexes were assessed in DMSO:PBS (phosphate buffer solution) (80:20 *v/v*). In these studies we could not use >20% of PBS, as the complexes have poor solubility at higher water content (**Table S7**). Complex **3** (**Figure S14**) shows very few changes, with small new resonances at 8.3 ppm and 7.0-7.3 ppm, however, this species could not be identified, and could either be another isomer or an aqua species. Importantly, the majority of the complex remains unaffected in these conditions, and the complex is stable over 96 h. Due to poorer solubility in DMSO, the stability of the Rh(III) diiodido complexes were assessed in MeCN:PBS (80:20 *v/v*), and as with complex **3**, complex **7** shows small new resonances appearing over time, but again the majority of the complex remains unaffected over 96 h (**Figure S15**).

Stability studies with respect to ligand exchange were conducted with complexes **1**, **4**, **5** and **8**, and assessed by ^{1}H NMR spectroscopy. A bulk mixture of each complex was treated with one equivalent of a different *meta*-substituted picolinamide ligand L′, and whilst the NMR for the diiodido complexes shows only free L′ in $CDCl_3$ and d_6-DMSO, the NMR for the dichlorido complexes shows possible dissociation of L and a potentially new mixed ligand complex with incorporation of L′ (**Figures S9-S13**). This is similar to our previously reported work on the ligand exchange of titanium coordination compounds and further suggests these complexes are fluxional and possible ligand rearrangement occurs in solution.[41]

To further assess this exchange, NMR reactions were conducted with the inclusion of water, and again, due to poor solubility of all complexes, reactions could only be conducted in <20% of water (**Table S7**). Complexes **3** and **7** in DMSO:PBS (80:20 *v/v*) and MeCN:PBS (80:20 *v/v*) respectively (~7.0 mM), were treated with one equivalent of *N*-(3-chlorophenyl)picolinamide ligand (L′) and the exchange monitored over 96 h. The ^{1}H NMR spectra of the dichlorido

complex **3** shows the NH resonance for the 3'-ClH ligand at 10.8 ppm, which over time exchanges to the 3'-BrH ligand at 10.7 ppm. It is unclear whether this is a mixed ligand complex or a complete exchange of the 3'-BrH ligands from complex **3** (**Figure S16-S17**), however, it does confirm the complexes instability towards ligand exchange. When assessing the same ligand exchange with complex **7** in MeCN:PBS, the NH from the 3'-ClH ligand appears at 10.3 ppm and does not change over the 96 h (**Figure S18-S19**), giving clear evidence that the diiodido complexes are not only stable with the inclusion of water over 96 h, but they remain stable towards ligand exchange.

Chemosensitivity Studies:

96 h incubation: MTT assays were conducted to determine the *in vitro* cell viability of complexes **1-8** and cisplatin (**CDDP**). Initially, the complexes were screened against the isogenic human colorectal carcinomas; HCT116 *p53*-wildtype (HCT116 $p53^{+/+}$), and HCT116 *p53*-null (HCT116 $p53^{-/-}$), human lung carcinoma (A549), human melanoma (FM55) and human pancreas carcinoma (MIA PaCa-2), and the values are shown in **Table 2**. After a 96 h incubation period, only the *N*-(3-bromophenyl)picolinamide Rh(III) diiodido complex **7** exhibited any cytotoxicity, with values ranging from 1.28 ± 0.09 μM (HCT116 $p53^{+/+}$) to 4.65 ± 0.04 μM (FM55), and has similar cytotoxicity to **CDDP** ($p > 0.05$). The analogous Rh dichlorido complex **3** is non-cytotoxic against these cell lines, therefore, cytotoxicity increases by up to 80x (HCT116 $p53^{+/+}$) when converting from the dichlorido to diiodido complex. Complexes **1**, **2**, **4-6** and **8** are non-toxic towards all cell lines tested at the tested threshold ($IC_{50} > 100$ μM), therefore, the data has been eliminated from **Table 2**. To the best of our knowledge, complex **7** is the first reported Rh(III) *trans* diiodido coordination compound to have significantly increased cytotoxicity, and remain stable in solution. To further understand the extent of this reactivity and selectivity of

complexes **3** and **7**, the results were compared to the ruthenium analogues **3-Ru** and **7-Ru** (**Figure 4**).[27]

Table 2. IC_{50} values/μM (± SD) for compounds **3**, **7**, **3-Ru**, **7-Ru** and **CDDP** against a range of human cell lines, after a 96 hour incubation period. Selectivity Index (SI) are shown in parenthesis. Complexes **1**, **2**, **4-6** and **8** have IC_{50} values > 100 μM and are eliminated from the table.

	HCT116 *p53*$^{+/+}$	HCT116 *p53*$^{-/-}$	A549	FM55	MIA PaCa-2	A2780	A2780cisR	MCF-7	ARPE-19
3	>100	>100	>100	>100	>100	4.4 ± 0.6 (22.9*)	9.0 ± 0.6 (11.2*)	36.5 ± 0.6 (2.7*)	>100
7	1.28 ± 0.09 (26.4)	1.53 ± 0.04 (23.9)	4.3 ± 0.3 (7.7)	4.65 ± 0.04 (7.0)	2.3 ± 0.1 (14.2)	6.0 ± 0.4 (5.5)	9.2 ± 0.6 (3.6)	2.2 ± 0.2 (16.3)	33 ± 2
3-Ru	1.96 ± 0.08	1.7 ± 0.1 (0.7)	3.3 ± 0.1 (0.4)	4.8 ± 0.2 (0.2)	2.20 ± 0.09 (0.5)	1.3 ± 0.07 (0.9)	4.4 ± 0.5 (0.3)	1.2 ± 0.2 (1.1)	1.19 ± 0.05
7-Ru	1.31 ± 0.08 (1.8)	1.71 ± 0.02 (1.4)	4.1 ± 0.3 (0.6)	4.63 ± 0.04 (0.5)	1.74 ± 0.09 (1.4)	17 ± 1 (0.1)	15 ± 1 (0.2)	1.8 ± 0.4 (1.1)	2.43 ± 0.02
CDDP	1.5 ± 0.1 (4.0)	3.54 ± 0.07 (1.7)	3.0 ± 0.1 (2.0)	6.1 ± 0.3 (1.0)	3.6 ± 0.7 (1.7)	1.3 ± 0.1 (4.5)	14 ± 1 (0.4)	1.5 ± 0.2 (4.0)	6 ± 1

n.d. results were not determined for these complexes and cell lines; * denotes the minimum selectivity indices (SI), as at least one of the IC_{50} values is > 100 μM

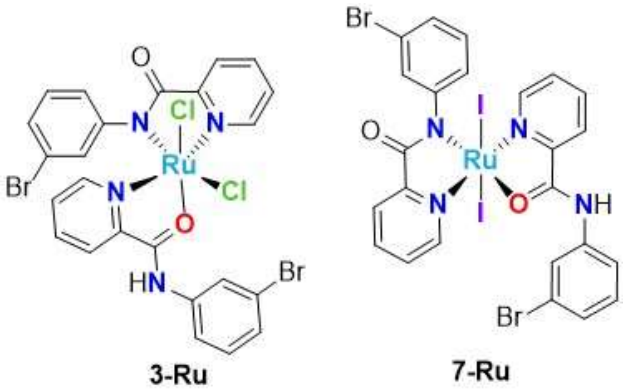


Figure 4. Ruthenium analogues **3-Ru** and **7-Ru,** which were synthesized and tested to compare cell results with complexes **3** and **7**.

The results for complexes **3-Ru** and **7-Ru** (**Table 2**) show that both of these Ru(III) complexes are cytotoxic towards all tested cell lines, and the values are not statistically different ($p > 0.05$) between each different cell line. It was previously highlighted that such Ru complexes increase in cytotoxicity when switching from the dichlorido to the diiodido.[27] The Ru(III) dichlorido complex (**3-Ru**) has high cytotoxicity against all cell lines, whereas the rhodium dichlorido complex **3** is non-toxic in almost all cell lines. When comparing Rh(III) to Ru(III), a 57-fold increase in cytotoxicity is observed against HCT116 *p53*$^{-/-}$, however, the selectivity of the complexes decreases. Complexes **3**, **7**, **3-Ru** and **7-Ru** were further screened against human ovarian carcinomas: cisplatin-sensitive (A2780) and cisplatin-resistant (A2780cisR), and human breast adenocarcinoma (MCF-7) (**Table 2** and **Figure S20**). Due to their lack of cytotoxicity towards the previous cell lines, the Rh(III) complexes **1**, **2**, **4-6** and **8** were eliminated from the additional screening.

Against MCF-7, complexes **7**, **3-Ru** and **7-Ru**, all exhibit similar potency, and the values are similar to those observed in the previous cell screening. Complex **3**, which was previously non-toxic ($IC_{50} > 100$ μM), now exhibits a moderate IC_{50} value of 36.5 ± 0.6 μM. When analyzing

the results against the human ovarian cell lines, a reverse in cytotoxicity was observed, and the diiodido complexes **7** and **7-Ru** exhibit up to an 18-fold decrease in cytotoxicity, when comparing their cytotoxicity against A2780 with HCT116 $p53^{+/+}$. Whereas the dichlorido complexes either remain cytotoxic (**3-Ru**), or increase by up to 22-fold (**3**, A2780 *cf.* HCT116 $p53^{+/+}$). This highlights the potential of such Rh(III) dichlorido complexes in targeting ovarian carcinomas

Selectivity Index (SI): In order to assess the potential of these compounds to target cancerous cells over normal cells, all complexes were screened for 96 h against human retinal epithelial cells (ARPE-19). The results are shown in **Table 2** and as with the other screening results, complexes **1-2**, **4-6** and **8** were all non-toxic against this cell line (IC_{50} > 100 μM) and so the results have been eliminated from **Table 2**. Complex **3** shows no cytotoxicity towards this cell line, however, the diiodido analogue **7** is moderately potent, with an IC_{50} = 33 ± 1 μM. Complex **7** remains 5.5x less cytotoxic than **CDDP** (IC_{50} = 6 ± 1 μM), highlighting a potentially different mode of action. The analogous Ru(III) complexes, **3-Ru** and **7-Ru**, exhibit high potency towards normal cells, with IC_{50} values 2.5-5x higher than **CDDP**.

Using the IC_{50} values against the normal cell line, divided by the IC_{50} value for each cancerous cell line, the selectivity indices (SI) were calculated. SI values > 1 indicate an increase in selectivity towards cancer cells over normal cells, and these values are given in the parenthesis of **Table 2** and are graphically presented in **Figure 5**. Complexes **3-Ru** and **7-Ru** have high potency towards all cell lines, and they exhibit low to no selectivity, with SI values ranging between 0.1-1.1. Complex **3** is only cytotoxic towards three of the cell lines, and is non-toxic towards the normal cell line, therefore it has high selectivity, specifically against A2780 (SI = 22.7*, see **Table 2** footnote). Complex **7** has a moderate cytotoxicity towards normal cells (IC_{50}

= 33 ± 2 μM), and has an increased selectivity towards the majority of the cancer cell lines, with high selectivity towards HCT116 *p53*$^{+/+}$ (SI = 25.5), HCT116 *p53*$^{-/-}$ (SI = 21.4) and MCF-7 (SI = 16.3).

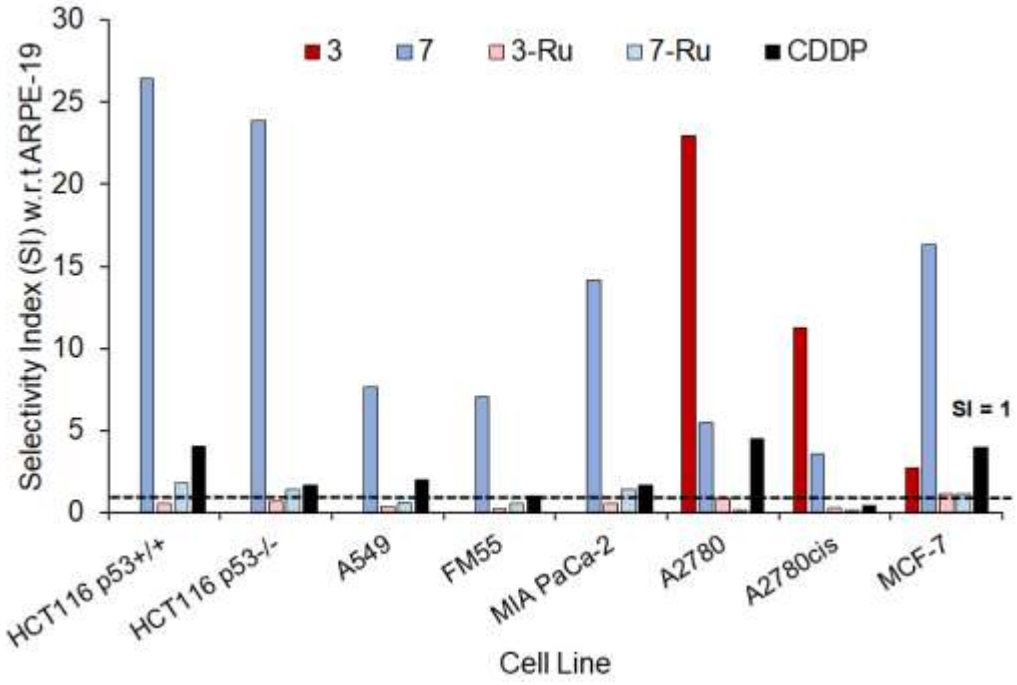


Figure 5. Selectivity indices (SI) for complexes **3**, **7**, **3-Ru**, **7-Ru** and **CDDP**. SI > 1 shows selectivity for the cancer cell lines, SI = 1 show equitoxicity (dotted line), and SI < 1 shows selectivity for the normal cell line ARPE-19.

24 h and 72 h incubation (+ recovery period): Over 50% of colorectal cancers are well known to have inactive or mutated *p53* genes.[42] The *p53*-null cell line HCT116 *p53*$^{-/-}$ can be considered a more representative example of advanced colorectal cancers, therefore, identifying compounds which are selectively active against this cell line could be a significant step forward towards cancer targeting. In light of this, we screened complexes **7**, **3-Ru** and **7-Ru** against the isogenic colorectal cell lines HCT116 *p53*$^{+/+}$ (*p53*-wildtype) and HCT116 *p53*$^{-/-}$ (*p53*-null) after

incubation periods of 24 and 72 h (**Figure 6a** and **Table S5**). The results show that the ruthenium analogues, **3-Ru** and **7-Ru**, are cytotoxic towards both cells after short incubation times, but remain cytotoxic towards normal cells (ARPE-19). These complexes are equitoxic against both HCT116 cell lines, with selectivity factors (SF) ranging from 0.3 – 3.2 (**Figure 6b** and **Table S6**). Interestingly, the Rh(III) diiodido complex **7** is non-toxic against HCT116 *p53*$^{-/-}$ at 24 h and 72 h (IC_{50} > 100 μM), and requires the longer incubation periods to become cytotoxic. After 24 h, complex **7** is cytotoxic against HCT116 *p53*$^{+/+}$ and is >44-fold more active than **CDDP**, with an IC_{50} value of 1.7 ± 0.1 μM (IC_{50} **CDDP** = 77 ± 2 μM). This complex has no selectivity towards HCT116 *p53*$^{-/-}$, with SF values ranging from 0.02 – 0.90 (*cf.* 0.4 – 0.8 **CDDP**). However, it is significantly selective towards the HCT116 *p53*$^{+/+}$, with SF values >57 and >75, after incubation periods of 24 h and 72 h respectively (*cf.* 1.3-1.4 **CDDP**). Importantly, after 24 h, complex **7** shows only very moderate cytotoxicity towards normal cells with an IC_{50} value of 27 ± 1 μM, meaning it retains selectivity (SI > 21) even after short incubation periods. This is contrary to **CDDP**, which is more cytotoxic towards normal cells (SI = 0.5). As the results show varying degrees of cytotoxicity towards both isogenic colorectal cell lines, a conclusion cannot be drawn on the *p53*-dependence, however, we have highlighted that unlike cisplatin, complex **7** is significantly selective in treating HCT116 *p53*$^{+/+}$ after 24 h.

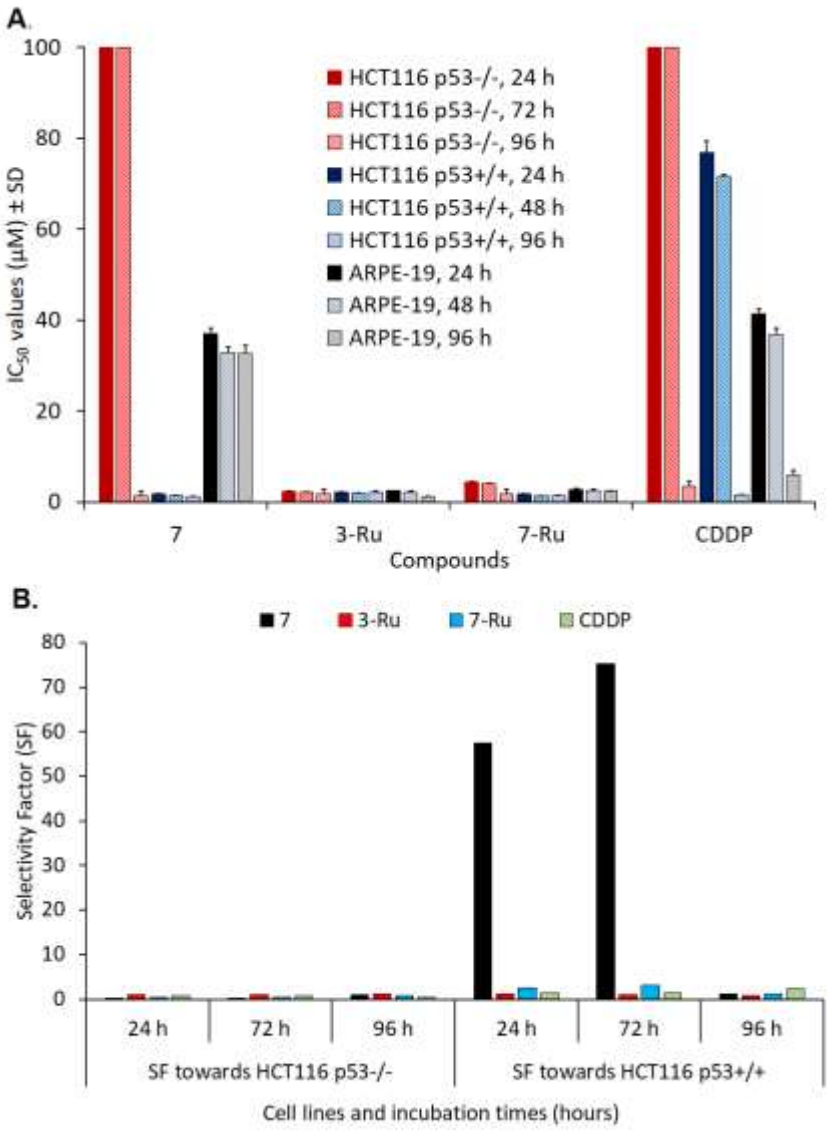


Figure 6. a) IC_{50} values (μM) complexes **7**, **3-Ru**, **7-Ru** and **CDDP** when screened against HCT116 *p53*$^{-/-}$, HCT116 *p53*$^{+/+}$ and ARPE-19, after 24 h, 72 h and 96 h incubation periods; **b)** Selectivity Factors (SF) for complexes **7**, **3-Ru**, **7-Ru** and **CDDP** when screened against HCT116 *p53*$^{-/-}$ and HCT116 *p53*$^{+/+}$.

Conclusion

A library of *N*-(3-halidophenyl)picolinamide rhodium dihalido complexes (**1-8**) is presented, with single crystal X-ray diffraction for seven new complexes. Solid state and solution studies

confirm the dichlorido complexes (**1-4**) exist as multiple isomers, whilst the diiodido complexes (**5-8**) exist as single and stable *trans* isomers. We report that the Rh(III) dichlorido complexes are fluxional in solution, with new resonances appearing when exchange studies are conducted with a different functionalized ligand. However, the Rh(III) diiodido complexes remain stable in solution and show no ligand exchange over a period of 96 h. These results complement the solid state PXRD studies, which also confirm the stability of the Rh(III) diiodido complexes.

The library of complexes was screened against a range of cell lines, and the *N*-(3-bromophenyl)picolinamide rhodium(III) complexes (**3** and **7**) exhibit moderate to high *in vitro* cytotoxicity. The Rh(III) *trans* diiodido complex **7** has the highest activity, with IC_{50} values ranging from 1.28 ± 0.09 μM (HCT116 $p53^{+/+}$) to 4.65 ± 0.04 μM (FM55), and has similar cytotoxicity to **CDDP** ($p > 0.05$). In contrast, the analogues Rh(III) dichlorido complex **3** was not cytotoxic against most cell lines ($IC_{50} > 100$ μM), however, it exhibited increased cytotoxicity by up to 22-fold against the human ovarian carcinoma cell line, A2780.

When comparing these cytotoxicity values with the Ru(III) analogues (**3-Ru** and **7-Ru**), complex **7** exhibited similar cytotoxicity, but has significantly higher selectivity towards cancerous cells (SI = 3.6 – 26.4) when compared to its Ru(III) analogue (SI = 0.1 – 1.8). Cytotoxicity screening after 24 and 72 h show complexes **3-Ru** and **7-Ru** are cytotoxic towards cancerous and normal cell lines, whereas complex **7** is non-toxic against the colon cancer cell line HCT116 $p53^{-/-}$ at both 24 h and 72 h, and requires longer incubation periods to become cytotoxic. Complex **7** remains cytotoxic against HCT116 $p53^{+/+}$ and is 44-fold more active than cisplatin and retains its high selectivity (SI > 21). As the cytotoxicity values vary against these isogenic cell lines, a conclusion cannot be drawn on the *p53*-dependence. Overall, these results highlight that Rh(III) *trans* diiodido complexes have the potential to be cancer selective and

cancer specific, as they outperform cisplatin, however, ligand modifications are now necessary to increase the effectiveness of these and other *trans* drugs. These complexes exists as single stable isomers, which is crucial for drug design, as the active species can be more easily determined. The isomers of these complexes possibly have different modes of action, and our future work aims to underpin their cellular uptake and mechanistic pathways.

ASSOCIATED CONTENT

Supporting Information. The experimental details, protocols and single X-ray diffraction data are provided in the Supporting Information.

Instructions for Authors.

The following files are available free of charge.

brief description (file type, i.e., PDF)

brief description (file type, i.e., PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS

A2780, cisplatin-sensitive human ovarian carcinoma; A2780cisR, cisplatin-resistant human ovarian carcinoma; A549, human lung carcinoma; ARPE-19, normal human retinal epithelial; BSA, bovine se rum albumin; CCDP, cisplatin; ct, calf thymus; DMSO, dimethylsulfoxide; DNA, deoxyribonucleic acid; ES-MS, electrospray mass spectrometry; FM55, human melanoma; FTIR, Fourier transform infrared; dppn, 1,8-bis(diphenylphosphino)naphthalene; GMP, guanosine monophosphate; GSH, glutathione; h, hour; HCT116 $p53^{+/+}$, human colorectal carcinoma *p53*-wildtype; HCT116 $p53^{-/-}$; human colorectal carcinoma *p53*-null; IC_{50}, half maximal inhibitory concentration; Im, imidazole; MCF-7, human breast adenocarcinoma; MeCN, acetonitrile; MIA PaCa-2, human pancreatic carcinoma; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NMR, nuclear magnetic resonance; PBS, phosphate buffer

solution; PXRD, powder X-ray diffraction; SC-XRD, single crystal X-ray diffraction; SF, selectivity factor; SI, selectivity index.

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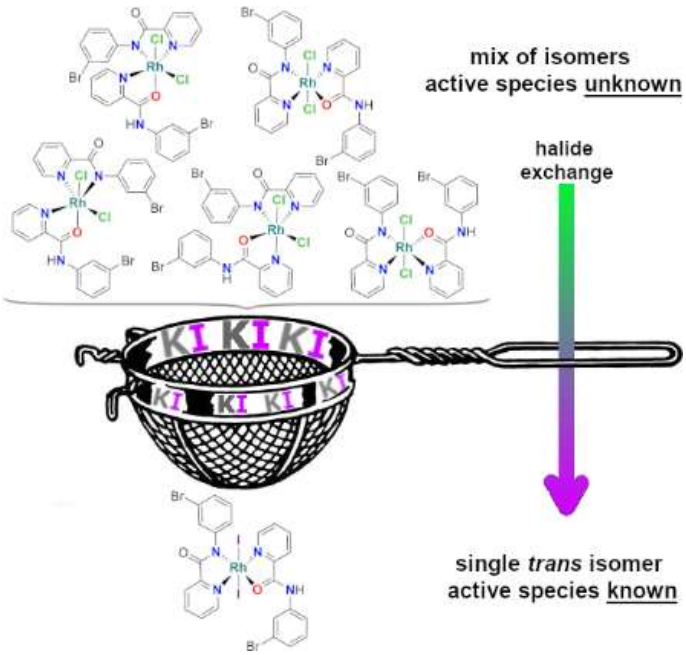

Importance of Isomers: Upon changing the ancillary halide ligands from dichlorido to diiodido in rhodium(III) picolinamide complexes, single stable *trans* isomers can be isolated from a mix of different isomers. These *trans* isomers remain stable in solution and are not fluxional to ligand exchange. Importantly, the complexes exhibit increased cytotoxicity and selectivity towards cancer cells, with selectivity >25 that of cisplatin.