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Ligand-Controlled Reactivity and Cytotoxicity of Cyclometalated Rhodium(III) Complexes

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Abstract: We report the synthesis, characterisation and cytotoxicity of six cyclometalated rhodium(III) complexes \([\text{Cp}^X\text{Rh(C}^\text{N})\text{Z}]^{0/+}\), in which \(\text{Cp}^X = \text{Cp}^*, \text{Cp}^{\text{ph}}, \text{or Cp}^{\text{biph}}, \text{C}^\text{N} = \text{benzo}[h]\text{quinoline}, \text{and Z} = \text{chloride or pyridine. Three x-ray crystal structures showing the expected \text{“piano-stool” configurations have been determined. The chlorido complexes hydrolysed faster in aqueous solution, also reacted preferentially with 9-ethyl guanine or glutathione compared to their pyridine analogues. The 1-biphenyl-2,3,4,5-,tetramethylcyclopentadienyl complex [Cp}^{\text{biph}}\text{Rh(benzo-hquinoline)Cl}] (3a) was the most efficient catalyst in coenzyme reduced nicotinamide adenine dinucleotide (NADH) oxidation to NAD}^+ \text{ and induced an elevated level of reactive oxygen species (ROS) in A549 human lung cancer cells. The pyridine complex [Cp}^{\text{biph}}\text{Rh(benzo[h]quinoline)py}]^+ (3b) was the most potent against A549 lung and A2780 ovarian cancer cell lines, being 5-fold more active than cisplatin towards A549 cells, and acted as a ROS scavenger. This work highlights a ligand-controlled strategy to modulate the reactivity and cytotoxicity of cyclometalated rhodium anticancer complexes.}
INTRODUCTION
The approval of cisplatin as an anticancer drug in the late 1970s has not only led to new generations of platinum drugs in clinical use (carboplatin and oxaliplatin) or trials, but also to the search for a new era of transition metal-based anticancer agents.[1-4] Group 9 metals iridium and rhodium have attracted much attention although are less widely studied than platinum.[5-8] Ir$^\text{III}$ and Rh$^\text{III}$ complexes with low spin d$^6$ configurations are usually considered to be kinetically inert, however, recent studies have revealed that their reactivity toward biological targets can be adjusted by the rational selection of the surrounding ligands directly coordinated to the metal.[6,9] Kinetically stable iridium/rhodium pyridocarbazole complexes have been synthesized by Meggers and co-workers as potent enzyme inhibitors,[10-13] and Sheldrick and co-workers have reported various pentamethyl-cyclopentadienyl (Cp*) iridium/rhodium complexes with chelating polypyridine ligands as potent anticancer agents.[14] More recently, attention has turned to cyclometalated iridium and rhodium complexes in which five-membered chelate rings contain a strong M-C σ bond.[15,16] Nevertheless, research on cyclometalated rhodium complexes has largely focused on non-cyclopentadienyl cyclometalated rhodium complexes that target DNA, enzyme or protein-protein interfaces.[5,9,17-20] Reported cyclopentadienyl rhodium anticancer complexes to date, have mainly contained N$^\text{N}$,[21,22] N$^\text{S}$,[23] N$^\text{O}$,[24-26] or O$^\text{O}$[22,27,28] chelating ligands.

Our recent work has demonstrated that replacing the N$^\text{N}$ coordinating ligand 2,2$'$-bipyridine or 1,10-phenanthroline with C$^\text{N}$ chelating 2-phenylpyridine or benzo[h]quinoline in pentamethyl-cyclopentadienyl (Cp*) iridium complexes, not only enhances nucleobase binding and lipophilicity, but also switches on anticancer activity towards human A2780 ovarian cancer cells.[29,30] Moreover, the chelated iridium(III) biphenyl-tetramethyl-cyclopentadienyl complex [Cp$^{\text{bip}}$Ir(phenylpyridine)(pyridine)]$^+$ can utilize NADH as a hydride source to transfer hydride electrons to oxygen, generating hydrogen peroxide and reactive oxygen species in cancer cells to trigger cell death.[31] Recently, we have described rhodium anticancer complexes [Cp$^X$Rh(N$^\text{N}'$)Cl]+ (Cp$^X$ = Cp*, Cp$^{\text{ph}}$, or Cp$^{\text{bip}}$) which can be effective transfer hydrogenation catalysts inducing reductive stress when co-administrated with non-toxic does of sodium formate as the hydride donor in cancer cells.[32] Intriguingly, the Cp$^X$ ring in these catalytic [Cp$^X$Rh(N$^\text{N}'$)Cl]+$^+$ (N$^\text{N}'$ is the bipyridine, dimethylbipyridine, or phenanthroline) complexes can be readily activated by deprotonation and undergo
rapid deuteration in aqueous media, so providing a novel activation pathway for half-sandwich Rh(III) complexes.[33] Based on these interesting discoveries, we have extended our studies to include cyclometalated cyclopentadienyl rhodium anticancer complexes with potential catalytic properties.

Herein, six cyclometalated rhodium complexes (Scheme 1) bearing different cyclopentadienyl CpX rings with C^N chelating ligand benzo[h]quinoline have been synthesized and fully characterized by ^1H NMR, ^13C NMR, high resolution ESI-MS and elemental analysis. Five novel complexes 2a, 3a, 1b-3b are reported, and the X-ray crystal structures of complexes 2a, 3a and 1b-PF_6 have been determined. Complexes 1a-3a contain chloride, while complexes 1b-3b feature pyridine as the monodentate ligand. Their aqueous chemistry including hydrolysis, binding to nucleobase 9-ethylguanine (9-EtG) or abundant cellular thiol glutathione (GSH, γ-L-Glu-L-Cys-Gly), as well as catalytic activity in the oxidation of coenzyme NADH are evaluated and compared. Cytotoxicity has been screened against human A549 lung and A2780 ovarian cancer cell lines. Cellular Rh accumulation, ROS induction and apoptosis in A549 cancer cells induced by complexes 3a and 3b at equipotent concentrations have been investigated to elucidate their possible mechanism of action.

Scheme 1. Structures of the six cyclometalated rhodium(III) benzo[h]quinoline complexes studied in this work.
RESULTS AND DISCUSSION

Synthesis and Characterization

The synthesis of the rhodium dimers \([\text{Cp}^X\text{Rh}(\mu-\text{Cl})\text{Cl}]_2\) \((\text{Cp}^X = \text{Cp}^{\text{ph}}, \text{Cp}^{\text{biph}})\) followed the reported procedure.\(^{32,34}\) The rhodium chloride complexes 1a-3a were synthesized via C-H activation of benzo[h]quinoline by the rhodium dimer aided by sodium acetate in dichloromethane at ambient temperature as reported previously.\(^{35}\) In contrast, pyridine complexes 1b-3b were obtained in good yields through the reaction of the corresponding chloride complexes with silver nitrate and then with excess pyridine (Scheme 2). All complexes were characterized by \(^1\text{H}\) and \(^{13}\text{C}\) NMR, elemental analysis and high resolution ESI-MS.

![Scheme 2](image)

**Scheme 2.** Synthesis route for rhodium complexes containing chloride (1a-3a) and pyridine (1b-3b) ligands.

X-ray crystal structures

Crystal structures of \([\text{Cp}^{\text{ph}}\text{Rh}(\text{benzo}[h]\text{quinoline})\text{Cl}]\) \((2a)\), \([\text{Cp}^{\text{biph}}\text{Rh}(\text{benzo}[h]\text{quinoline})\text{Cl}]\) \((3a)\) and \([\text{Cp}^\prime\text{Rh}(\text{benzo}[h]\text{quinoline})\text{pyridine}]\text{PF}_6\) \((1b\cdot\text{PF}_6)\) with PF$_6^-$ as the counter anion were determined and are shown in Figure 1 with the atom numbering scheme. Crystallographic data are listed in Table S1, and selected bond lengths and angles in Table S2. All structures adopt the familiar “piano-stool” geometry. The distances between Rh and the centroid of the \(\eta^5\)-cyclopentadienyl ring are within 1.827-1.832 Å. The length of the Rh-C (quinoline) bond in 2a, 3a, 1b-PF$_6$ \([2.050(3), 2.0458(15), 2.058(2), \text{respectively}]\) is significantly shorter than the Rh-N (quinoline) bond length \([2.082(3), 2.1030(14), 2.1068(18), \text{respectively}]\).
Figure 1. X-ray structures with atom numbering of (a) [Cp^phRh(benzo[h]quinoline)Cl] (2a); (b) [Cp^biphRh(benzo[h]quinoline)Cl] (3a); (c) [Cp^Rh(benzo[h]quinoline)-pyridine]PF_6 (1b·PF_6), drawn with thermal ellipsoids at 50% probability. Hydrogen atoms and counterions have been omitted for clarity.

Aqueous solution chemistry

The hydrolysis of these complexes was monitored by ^1H NMR (Figures 2, S1, S2) as well as UV-vis spectroscopy at 310 K in methanol/water. Methanol was used to ensure the sufficient solubility of the complexes in water. All the chloride complexes showed faster hydrolysis than their pyridine counterparts. Their hydrolysis rates and half-lives of hydrolysis (listed in Table 1) were determined by fitting the UV-vis absorption changes versus time to pseudo-first-order kinetics (Figure S6). With the extension of Cp^* to Cp^ph, to Cp^biph, the hydrolysis half-lives of the chloride complexes become shorter, while the hydrolysis half-lives of the pyridine complexes become longer. The hydrolysis extent over 24 h for chloride complexes 1a-3a was 43%-60% at equilibrium based on integration of ^1H NMR peaks. In addition, after 24 h, an additional set of ^1H NMR peaks was visible for complexes 1a-3a which can be attributed to release of the benzo[h]quinoline ligand (ca. 25%), as characterized by ^1H NMR, ^1H-^1H COSY and confirmed by ESI-MS with m/z 180.13 (calculated m/z 180.08) (Figure S7-S9). In contrast, after 24 h at 310 K, no apparent change was observed for millimolar solutions of the pyridine complexes 1b-3b in d_4-MeOD/D_2O (1/9-1/2 v/v) by ^1H NMR (Figure S3-S5), although the hydrolysis of micromolar solutions of complexes 1b-3b could be observed by UV-vis spectroscopy at 310 K (Figure S10).

When 104 mM NaCl (to mimic the chloride concentration in blood plasma), was added to the 24 h equilibrium hydrolysis solution of complexes 1a (Figure 2c) and 2a (Figure S1c), peaks for the aqua adducts disappeared and those for the parent
chloride complexes increased in intensity, confirming that hydrolysis of the chloride complexes is reversible.

**Figure 2.** 400 MHz $^1$H NMR spectra of [Cp*Rh(benzo[h]quinoline)Cl] (1a) (ca. 0.5 mM) in d$_4$-MeOH/D$_2$O (v/v 4/1) after (a) 15 min; (b) 24 h; and (c) 10 min after addition of NaCl (104 mM). After 15 min, 60% of the parent complex 1a (■) hydrolysed to its aqua adduct [Cp*Rh(benzo[h]quinoline)D$_2$O]$^+$ (●). After 24 h, one third of the aqua adduct [Cp*Rh(benzo[h]quinoline)D$_2$O]$^+$ had decomposed into the free chelated ligand and [Cp*Rh(D$_2$O)$_3$]$^{2+}$ (▲). After addition of NaCl, all the aqua adducts were converted into 1a, with the released ligand remaining unbound and the [Cp*Rh(D$_2$O)$_3$]$^{2+}$ reacting with Cl$^-$ to form [Cp*RhCl$_2$]$_2$ (▲).

**Table 1.** Hydrolysis data for complexes 1a-3a and 1b-3b at 310 K.

| Complex | Cp$^X$ | Extent$^a$ | $k$$^b$ (min$^{-1}$) | $t_{1/2}$$^b$ (min) |
|---------|--------|------------|----------------------|---------------------|
| 1a      | Cp$^*$ | 43%        | 0.014 ± 0.002        | 49.7                |
| 2a      | Cp$^{ph}$ | 50%        | 0.026 ± 0.002        | 26.8                |
| 3a      | Cp$^{bph}$ | 60%        | 0.138 ± 0.016        | 5.0                 |
| 1b      | Cp$^*$ | 0%         | 0.019 ± 0.001        | 36.0                |
| 2b      | Cp$^{ph}$ | 0%         | 0.010 ± 0.001        | 70.0                |
| 3b      | Cp$^{bph}$ | 0%         | 0.007 ± 0.001        | 95.9                |

[a]: Determined by $^1$H NMR in d$_4$-MeOH/D$_2$O. [b]: At equilibrium, determined by UV-vis spectroscopy in MeOH/H$_2$O.
**Interaction with 9-EtG**

Reactions between chloride/pyridine complexes and nucleobase 9-ethylguanine (9-EtG) were studied by $^1$H NMR spectroscopy. Equimolar 9-EtG was added to an equilibrium solution of [Cp*Rh(benzo[h]quinoline)Cl] (1a) in $d_4$-MeOD/D$_2$O (4/1, v/v, pH* 7.4) and the $^1$H NMR spectra showed that ca. 90% of 1a had rapidly formed adducts with 9-EtG (within 20 min) based on the integral of the Cp* protons of 1a (δ 1.71 ppm), aqua adducts of 1a (1.68 ppm) and 9-EtG adducts (δ 1.66 ppm) (Figure 3). The new singlet peak for H8 of the bound 9-EtG appeared at 7.39 ppm, shifted by 0.37 ppm to high field relative to that of free 9-EtG. The ESI-MS of the final NMR solution showed a major peak at m/z 595.3, assignable as the adduct of 1a with 9-EtG [Cp*(Rh-9-EtG)(benzo[h]quinoline)]$^+$ (calcd m/z 595.1). Meanwhile, in the presence of 4 mM NaCl, 1a still showed a high affinity for 9-EtG with ca. 90% 1a binding to 9-EtG (Figure S11) at 310 K. Adducts of 2a and 3a with 9-EtG were also characterised by $^1$H NMR (Figure S12) and ESI-MS (Table S3).

By contrast, a $^1$H NMR study of the pyridine complex [Cp*Rh(benzo[h]quinoline)py]NO$_3$ (1b) with equimolar 9-EtG in $d_4$-MeOD/D$_2$O (1/4, v/v, pH* 7.4) showed that only 30% of complex 1b formed an adduct with 9-EtG after 24 h at 310 K (Figure 4) based on the integral of the Cp* methyl protons at 1.57 ppm (1b) and 1.56 ppm (9-EtG adduct with 1b). This new adduct of complex 1b with 9-EtG was also confirmed by ESI-MS by the peak at m/z 595.3 (calcd m/z 595.1).

**Figure 3.** 300 MHz $^1$H NMR spectra of (a) equilibrium solution of ca. 1.0 mM complex [Cp*Rh(benzo[h]quinoline)Cl] (1a) in $d_4$-MeOD/D$_2$O (4/1 v/v, pH* 7.4), containing both the chloride complex 1a (●) and its aqua adduct (●). (b) 20 min after addition of equimolar 9-EtG, ca. 90% of 1a had formed an adduct (▲) with 9-EtG.
Figure 4. 300 MHz $^1$H NMR spectrum of ca. 2.6 mM complex [Cp*Rh(benzo[h]quinoline)py]$^+$ (1b) (■) in $d_4$-MeOD/D$_2$O (v/v 1/4, pH* 7.4) with equimolar 9-EtG after 24 h at 310 K. 30% of complex 1b formed an adduct (■) with 9-EtG.

**NADH oxidation**

NADH is a crucial coenzyme in numerous biological catalytic processes. Previously, we have found that cyclopentadienyl iridium complexes bearing N^N coordinating ligands can accept the hydride from NADH and induce the reduction of protons to H$_2$,[36] and quinones to semiquinones.[37] The 1-biphenyl-2,3,4,5,-tetramethylcyclopentadienyl (Cp$^{biph}$) iridium complex with the C^N chelating 2-phenylpyridine can also transfer hydride from NADH to oxygen to produce the reactive oxygen species (ROS) hydrogen peroxide.[31] Therefore, the time dependence of reactions between rhodium complexes (0.8 µM) and NADH (75-144 µM) was studied over 24 h in 1.6% MeOH/98.4% phosphate buffer (5 mM, pH 7.4) by UV-vis spectroscopy at 310 K (Figure S13, S14). The reactions proceeded via first-order kinetics (Figure S15).

The turnover number (TON) and turnover frequency (TOF) were determined based on the decrease in absorption of NADH at 339 nm due to conversion of NADH to NAD$^+$. In reactions with NADH at a higher concentration (144 µM), pyridine complexes 1b-3b showed a much lower TOF than their respective chloride analogues 1a-3a (Table 2). This might due to the decrease in hydrolysis of pyridine complexes compared to chloride analogues, as hydrolysis is believed to be the first step to interact with NADH.[31] In the presence of 75 µM or 117 µM NADH, respectively, complex 3a bearing a Cp$^{biph}$ ring showed a ca. 2.3 x or ca. 2.6 x higher TOF than the other chloride complexes 1a and 2a. In contrast, in the presence of 144 µM NADH, pyridine complex 1b, which has the fastest hydrolysis rate among all these pyridine complexes, exhibited the highest TON and TOF. Furthermore, hydrogen peroxide was detected.
(appearance of blue colour on Quantofix test sticks) in the reaction mixture of complex 3a (ca. 1 mM) with 3.5 mol equiv. NADH in MeOH/H$_2$O (1/1 v/v) after 24 h at 310 K (Figure S16).

Table 2. TONs and TOFs for rhodium complexes (0.8 µM) in the catalytic oxidation of NADH to NAD$^+$ in 1.6% MeOH/98.4% phosphate buffer (5 mM, pH 7.4) over 24 h at 310 K.

| Complex | NADH (µM) | TON | TOF (h$^{-1}$) |
|---------|-----------|-----|----------------|
| 1a      | 75        | 13  | 1.16 ± 0.06    |
|         | 117       | 49  | 3.35 ± 0.11    |
| 2a      | 75        | 14  | 1.10 ± 0.08    |
|         | 117       | 51  | 3.38 ± 0.15    |
| 3a      | 75        | 32  | 2.85 ± 0.12    |
|         | 117       | 58  | 7.58 ± 0.20    |
| 1b      | 144       | 72  | 2.85 ± 0.06    |
| 2b      | 144       | 35  | 1.63 ± 0.02    |
| 3b      | 144       | 49  | 1.73 ± 0.04    |

Sodium formate as hydride donor

Inspired by the ability of half-sandwich rhodium(III) complexes with N^N chelating ligands to act as transfer hydrogenation catalysts using sodium formate as an hydride source and convert pyruvate to lactate,$^{[32]}$ the ability of complex [Cp*Rh(benzo[h]quinoline)Cl] (1a) to reduce pyruvate to lactate was investigated. Upon addition of 10 mol equiv. sodium formate to a solution of 1a in d$_4$-MeOD/H$_2$O (99/1 v/v), the colour turned from orange to red. In the hydride region, a doublet appeared at -12.26 ppm ($J^1$H$^{-103}$Rh) = 32 Hz, Figure S17) which can be assigned to the hydride complex [Cp*Rh(H)(benzo[h]quinoline)] in accord with the reported $J$ value$^{[38]}$ for the same Rh-H species. Meanwhile, a triplet at -19.34 ppm ($J$ = 24 Hz) was also observed in Figure S17. The $J$ value of this triplet is characteristic of hydride bridging two Rh(III) centres,$^{[39]}$ suggesting a possible nucleophilic attack of the Rh-H
bond on the labile coordination site occupied by MeOD or H₂O on another Rh molecule. Subsequently, when 5 mol equiv. pyruvate was added to this Rh-hydride solution, ca.17% of pyruvate (δ 2.36 ppm, singlet) was reduced to lactate (δ 1.32 ppm, doublet) based on ¹H NMR peak integrals over 24 h at 310 K (Figure S18).

Reactivity with GSH

The abundant cellular tripeptide glutathione (GSH) is a detoxification agent and ROS scavenger. Reactions between complex [Cp⁰Rh(benzo[h]quinoline)Cl] (3a)/pyridine analogue [Cp⁰Rh(benzo[h]quinoline)py]⁺ (3b) and GSH were investigated. LC-MS analysis of 3b with 2 mol equiv. GSH in MeOH/H₂O (1/9 v/v) revealed that most of the parent complex 3b remained intact within the first 30 min, but after 18 h, 3b was converted into dinuclear [(Cp⁰Rh)₂(µ-SG)₃]⁺ adducts (Figure S19). However, under the same conditions, the chloride complex 3a formed [Cp⁰Rh(SG)+H]⁺ as detected by ESI-MS with m/z 683.6 (calcd 683.6) as soon as it was mixed with GSH. These differences in reactions with GSH may have a significant effect on the anticancer activity of these two complexes.

Cytotoxicity

The anticancer activity of rhodium complexes against human A549 lung and A2870 ovarian cancer cell lines was evaluated in vitro using the SRB assay after 24 h treatment and subsequent 72 h of cell recovery. The detailed half maximal inhibitory concentration (IC₅₀) values are listed in Table S4 and shown in Figure 5. Compared to cisplatin (CDDP) as the positive control, chloride complexes 1a-3a and pyridine complexes 1b-2b all showed antiproliferative activity towards these two cell lines. In particular, the pyridine complex [Cp⁰Rh(benzo[h]quinoline)py]NO₃ (3b) exhibited the highest activity among all screened rhodium complexes, ca. 5 x greater potency than cisplatin against A549 lung cancer cells.

From the study of cyclopentadienyl iridium complexes with the C^N chelating ligand 2-phenylpyridine, the extension of Cp⁰ (from Cp* to Cp⁰ to Cp⁰⁰) greatly enhanced the anticancer activity perhaps due to the increased hydrophobicity facilitating passage through the cell membrane or to the extended phenyl or biphenyl ring which can intercalate between DNA bases.[40] Among the Cp⁰ analogues, Cp⁰⁰ capped complexes 3a and 3b were the most hydrophobic and exhibited the highest potency as expected. Moreover, pyridine complex 3b was ca. 2 x and ca. 10 x more
potent than its chloride analogue 3a towards A2780 and A549 cancer cells, respectively (A2780: IC$_{50}$ 0.88 µM for 3b and 1.60 µM for 3a; A549: IC$_{50}$ 0.74 µM for 3b and 7.69 µM for 3a). Therefore, despite the replacement of the chloride with pyridine decreasing hydrolysis, inhibiting adduct formation with 9-EtG/GSH and lowering the catalytic TOF, the in vitro anticancer activity of pyridine complexes was higher than that of chloride analogues, in line with the behaviour of cyclometalated iridium pyridine complexes which showed slower hydrolysis/less interaction with biomolecules, but higher potency than their chloride analogues.[31]

**Figure 5.** Anticancer activity of rhodium complexes toward human A549 lung and A2780 ovarian cancer cells in comparison with cisplatin (CDDP). The values of half maximal growth inhibitory concentration (IC$_{50}$) are determined by SRB assay and given as the mean ± standard deviations for three independent experiments. Stock solutions of tested complexes were prepared with 0.5% DMSO and the drug-treatment period was 24 h.

**Cellular Rh Accumulation**

Complexes [Cp$^{biph}$Rh(benzo[h]quinoline)Cl] (3a) and [Cp$^{biph}$Rh(benzo[h]quinoline)-py]NO$_3$ (3b) bearing the Cp$^{biph}$ ring, were the most potent candidates among the chloride family 1a-3a and pyridine family 1b-3b. However, complex 3b is positively charged and 10 x more potent than neutral complex 3a toward A549 cancer cells (Figure 5). Thus, to elucidate their different anticancer activities, the cellular accumulation of rhodium in A549 human lung cancer cells after 24 h treatment with complexes 3a and 3b at equipotent concentrations of 0.5 x and 1 x IC$_{50}$ at 310 K was quantified by ICP-MS as shown in Figure 6 (values in Table S5). From Figure 6, the
accumulation of Rh in the cells treated with complex 3a or 3b increased in a concentration-dependent manner. After 24 h the cellular Rh accumulation of chloride complex 3a was ca. 49-fold (at 0.5 x IC50) and ca. 59 fold (at 1 x IC50) higher than that of complex 3b. This significant difference correlates with the higher hydrophobicity of neutral complex 3a compared to the positive complex 3b, in contrast to the reported cyclometalated iridium complex [Cp^biphIr(2-phenylpyridine)py]^+ which was found to have 20-fold higher Ir accumulation than the neutral iridium complex [Cp^biphIr(2-phenylpyridine)Cl] in A2780 cancer cells.^[31]

**Figure 6.** Accumulation of Rh (ng/million cells) in A549 human lung cancer cells after 24 h treatment with chloride complex 3a or pyridine complex 3b at concentrations of 0.5 x IC50 and 1 x IC50. The values represent mean ± standard deviations for three independent samples. The asterisk denotes the p-values obtained by comparing each dataset with the negative control (untreated cells) using a t-test, *p < 0.05, **p < 0.01.

**ROS induction in A549 cancer cells**

The chloride complexes 1a-3a exhibited higher catalytic efficiency than pyridine analogues in the oxidation of NADH to NAD^+ with production of hydrogen peroxide. We investigated whether these catalytic chloride complexes can induce a high level of reactive oxygen species (ROS) in A549 human lung cancer cells after 24 h treatment with complexes [Cp^biphRh(benzo[h]quinoline)Cl] (3a) and [Cp^biphRh(benzo[h]quinoline) -py]NO_3 (3b) at concentrations of IC50 or 2 x IC50 by flow cytometry fluorescence analysis using a total ROS/Superoxide detection kit (Figure 7, details in Table S6). This assay not only allowed determination of the level of total ROS consisting of H_2O_2,
peroxy and hydroxyl radicals, peroxynitrite, and NO species, but also the level of superoxide (SO). After 24 h drug treatment, a concentration-dependent ROS level modulation indicated by the fluorescence signals of FL1 (indicating the total ROS level) and FL2 (indicating the SO level) channels was observed: in cells treated with complex 3a, the ROS+/SO+ cell population increased significantly from 18 ± 2 % at IC50 concentration to 47 ± 2 % at 2 x IC50 concentration of complex. In contrast, for cells treated with complex 3b, the cell population producing ROS decreased significantly from 60 ± 2 % (ROS+/SO-)/22 ± 2 % (ROS+/SO+) by IC50 concentration treatment to 38 ± 0.1 (ROS+/SO-)/10 ± 0.3 (ROS+/SO+) by 2 x IC50 concentration treatment, combined with an increase in the quadrant of ROS-/SO- from 17 ± 0.2 % to 51 ± 0.2 (Figure 7, Table S6). This observation suggested opposing mechanisms of action for the two complexes: complex 3a inducing ROS formation, complex 3b acting as a ROS scavenger.

**Figure 7.** Flow cytometry plots of total ROS and superoxide production after 24 h treatment with 1× and 2× IC50 concentrations of complexes (a) 3a and (b) 3b. Cell population of total ROS and superoxide production after 24 h treatment with 1× and 2× IC50 concentrations of complexes (c) 3a and (d) 3b. The values represent mean ±
standard deviations for three independent samples. The asterisk denotes the $p$-values obtained by comparing each dataset with the negative control (untreated cells) using a t-test, $^*p < 0.05$, $^{**}p < 0.01$, $^{*^3}p > 0.05$.

Apoptosis assay

Apoptosis is well recognized as a distinct cell death mechanism in tumours responding to anticancer therapies.[41] To investigate whether apoptosis is involved in the anticancer activity, A549 cells were treated with [Cp$bip$Rh(benzo[h]quinoline)Cl] (3a) and [Cp$bip$Rh(benzo[h]quinoline)py]NO$_3$ (3b) at their equipotent concentrations of IC$_{50}$ and 2 x IC$_{50}$ for 24 h, then stained with FITC labelled-annexin V/propidium iodide (PI) and analysed by flow cytometry. This assay determined cell populations as early apoptosis (annexin V+/PI-, stained by annexin V only), late apoptosis (annexin V+/PI+, stained by annexin V and PI), necrosis (annexin V-/PI+, stained by PI only), and viable (annexin V-/PI-, unstained by annexin V and PI). No obvious induction of apoptosis by complex 3a or 3b was observed at their equipotent concentrations (Figure S20). However, there was a significant increase in necrotic cell population induced by the treatment with complex 3b at 2 x IC$_{50}$ concentration (Table S7).

CONCLUSIONS

In summary, we have described the synthesis and characterization of novel cyclopentadienyl C^N chelated Rh$^{III}$ anticancer complexes. The reactivity and cytotoxicity of these complexes can be reasonably modulated by selection of the monodentate ligand as chloride or pyridine. The hydrolysis rate of chloride complexes increases in the order Cp$^*$ < Cp$^{ph}$ < Cp$bip$, showing that incorporation of the extended Cp$^X$ ring confers more labile kinetics on the monodentate chloride ligand. On the contrary, when the chloride is replaced with pyridine, the rate of hydrolysis is slowed down by orders of magnitude and decreases in the order Cp$bip$ > Cp$^{ph}$ > Cp$^*$. This difference in hydrolysis kinetics for the chloride and pyridine complexes leads to the differences in reactivity, and subsequent differences in cytotoxicity.

The pyridine complex 3b reacts more slowly with glutathione than the chloride analogue, resulting in less deactivation and an order of magnitude greater potency towards lung cancer cells. Meanwhile, complex 3b accumulates to a much lesser extent than the chloride analogue in cancer cells at equipotent IC$_{50}$ concentrations,
indicating that complex 3b requires a lower dose than 3a to achieve the same therapeutic potency. Significantly, the rhodium complexes studied here exhibit a different mechanism of action from cisplatin, which acts through interaction with DNA. The chloride complex 3a shows the greatest catalytic efficiency in NADH oxidation and induces a remarkable increase in the level of ROS in lung cancer cells. Whereas the pyridine complex 3b can act as a ROS scavenger, which is distinct from the related pyridine iridium complex [Cp\textsuperscript{biph}Ir(2-phenylpyridine)py]\textsuperscript{+} which induces a higher level of ROS in ovarian cancer cells.\textsuperscript{[31]} Although the reason of the ROS scavenging activity is not clear, pyridine complex 3b is the first example of an organometallic rhodium complex with such a behaviour. Cyclometalated rhodium complexes such as these may provide a new generation of transition metal-based chemotherapeutic agents and are worthy of further investigation.

**EXPERIMENTAL SECTION**

**Materials**
Rhodium(III) trichloride hydrate was purchased from Precious Metals Online (PMO Pty Ltd.) and used as received. [Cp\textsuperscript{*}Rh(µ-Cl)Cl]\textsubscript{2}, benzo[h]quinoline, silver nitrate, sodium formate, sodium pyruvate, β-nicotinamide adenine dinucleotide reduced disodium salt (NADH), and celite were purchased from Sigma-Aldrich. MeOD-d\textsubscript{4}, D\textsubscript{2}O, and CDCl\textsubscript{3}-d\textsubscript{1} for NMR spectroscopy and Quantofix® peroxides test sticks (1-100 mg/L range) were purchased from Sigma-Aldrich. Reduced glutathione was obtained from Alfa Aesar. Disodium hydrogen phosphate dihydrate, disodium hydrogen phosphate dodecahydrate, anhydrous sodium acetate, HPLC grade solvents (water and acetonitrile) with added trifluoroacetic acid with analytic grade, anhydrous DCM/pyridine and laboratory grade solvents used in syntheses were purchased from fisher scientific. A549 human lung and A2780 human ovarian cancer cell lines were purchased from the European Collection of Animal Cell Culture (ECACC, Salisbury, U.K.). Roswell Park Memorial Institute (RPMI-1640) medium, and phosphate-buffered saline (PBS) were purchased from PAA Laboratories GmbH. The total ROS/superoxide detection kit was purchased from Enzo Life Sciences. The apoptosis detection regents were purchased from Abcam.

**Synthesis**
The dimeric rhodium precursors [Cp\textsuperscript{ph}Rh(µ-Cl)Cl]\textsubscript{2}, [Cp\textsuperscript{biph}Rh(µ-Cl)Cl]\textsubscript{2} and complex [Cp’Rh(benzo[h]quinoline)Cl] (1a) were synthesized according to the literature.\textsuperscript{[32,34,35]}
Synthesis of \([\text{Cp}^\text{x} \text{Rh(benzo[h]quinoline)}\text{Cl}](2a \text{ and } 3a)\)

**General procedure:** \([\text{Cp}^\text{x} \text{Rh(µ-Cl)}\text{Cl}])_2\) (0.12 mmol) with benzo[h]quinoline (0.36 mmol) and anhydrous sodium acetate (0.98 mmol) in 20 ml anhydrous DCM was stirred under nitrogen for 48 h. Then the solution was filtered through celite and purified by 10 g silica cartridge on Biotage (methanol/dichloromethane, 5/95 v/v). Then column-purified powder was recrystallized from dichloromethane/diethyl ether at 277 K to give a red crystalline solid.

\([\text{Cp}^\text{ph} \text{Rh(benzo[h]quinoline)}\text{Cl}]) (2a): 43 \text{ mg, yield 70\%; } ^1\text{H NMR} (500 MHz, CDCl}_3, 298 \text{ K}): \delta 8.69 (d, J = 5.1 Hz, 1H), 8.14 (d, J = 8.0 Hz, 1H), 7.97 (d, J = 6.7 Hz, 1H), 7.79 (d, J = 8.7 Hz, 1H), 7.62-7.53 (m, 5H), 7.43-7.3 (m, 4H), 1.85 (s, 3H), 1.78 (s, 3H), 1.75 (s, 3H), 1.45 (s, 3H);

\(^{13}\text{C NMR} (125 MHz, CDCl}_3, 298 \text{ K}): \delta 175.18 (J_{C-Rh} = 33.8 \text{ Hz}), 155.32, 149.49, 140.54, 135.64, 133.83, 133.54, 131.99, 130.89, 129.71, 128.79, 127.77, 127.21, 123.13, 121.53, 121.32, 107.6, 100.31, 95.87, 94.64, 88.79, 10.84, 10.48, 9.54, 9.22.\ HR-MS (ESI): m/z calcd for \([\text{Cp}^\text{ph} \text{Rh(benzo[h]quinoline)}]+: 478.1037;\) found: 478.1032; elemental analysis calcd (%) for C\text{28}H\text{25}ClRhN: C, 65.45; H, 4.90; N, 2.73; found: C, 64.55; H, 4.86; N, 2.69.

\([\text{Cp}^\text{bip} \text{Rh(benzo[h]quinoline)}\text{Cl}]) (3a): 41 \text{ mg, yield 58\%; } ^1\text{H NMR} (500 MHz, CDCl}_3, 298 \text{ K}): \delta 8.73 (d, J = 5.1 Hz, 1H), 8.14 (d, J = 7.9 Hz, 1H), 8.00 (d, J = 6.9 Hz, 1H), 7.80 (d, J = 8.7 Hz, 1H), 7.68-7.55 (m, 9H), 7.48 (t, J = 7.6 Hz, 2H), 7.40-7.34 (m, 2H), 1.89 (s, 3H), 1.80 (s, 3H), 1.77 (s, 3H), 1.50 (s, 3H);

\(^{13}\text{C NMR} (125 MHz, CDCl}_3, 298 \text{ K}): \delta 175.14 (J_{C-Rh} = 33.8 \text{ Hz}), 155.30, 149.51, 140.59, 140.54, 140.31, 135.66, 133.83, 133.53, 131.26, 131.02, 130.20, 129.71, 129.04, 127.65, 127.36, 127.21, 127.11, 123.14, 121.59, 121.34, 107.56, 100.39, 95.44, 94.73, 88.91, 10.91, 10.53, 9.54, 9.23.\ HR-MS (ESI): m/z calcd for \([\text{Cp}^\text{bip} \text{Rh(benzo[h]quinoline)}]\): 554.1350; found: 554.1353; elemental analysis calcd (%) for C\text{34}H\text{29}ClRhN: C, 69.22; H, 4.95; N, 2.37; found: C, 69.31; H, 4.96; N, 2.17.

**Synthesis of \([\text{Cp}^\text{x} \text{Rh(benzo[h]quinoline)}\text{py}])\text{NO}_3 (1b-3b)\)

**General procedure:** \([\text{Cp}^\text{x} \text{Rh(benzo[h]quinoline)}\text{Cl}]) (0.05 mmol) was stirred with silver nitrate (0.05 mmol) in 10 ml methanol for 1.5 h. Then the solution was centrifuged and pyridine (0.5 mmol) was added to the upper clear layer. The colour changed from orange to bright yellow, and the solution was stirred 24 h. Then the solution was concentrated, and product recrystallized from diethyl ether giving a yellow solid which was dried under vacuum.
[Cp’Rh(benzo[h]quinoline)py]NO₃ (1b): 18 mg, yield 65%; ¹H NMR (500 MHz, d₄-MeOD, 298 K): δ 9.44 (d, J = 5.0 Hz, 1H), 8.54 (d, J = 7.4 Hz, 1H), 8.50 (d, J = 5.6 Hz, 2H), 8.35 (d, J = 7.2 Hz, 1H), 7.91-7.81 (m, 3H), 7.75-7.71 (m, 3H), 7.62 (t, Jᵣ = 6.8 Hz, Jₑ = 7.3 Hz, 2H), 1.65 (s, 15H); ¹³C NMR (125 MHz, d₄-MeOD, 298 K): δ 175.14 (Jₑ-Rh = 35.0 Hz), 155.99, 154.20, 151.45, 141.71, 140.14, 138.82, 135.17, 134.29, 131.98, 130.77,128.77, 127.51, 124.94, 124.35, 123.77, 98.62, 9.04. HR-MS (ESI): m/z calcd for [Cp’Rh(benzo[h]quinoline)pyridine]⁺ 495.1302, found 495.1297; elemental analysis calcd (%) for C₂₈H₂₈RhN₃O₃: C, 60.33; H, 5.06; N, 7.54; found: C, 59.65; H, 4.98; N, 7.50.

[CpʰRh(benzo[h]quinoline)py]NO₃ (2b): 23 mg, yield 74%; ¹H NMR (500 MHz, d₄-MeOD, 298 K): δ 9.22 (d, J = 5.0 Hz, 1H), 8.53-8.50 (m, 3H), 8.27 (d, J = 6.8 Hz, 1H), 7.91 (d, J = 8.7 Hz, 1H), 7.82-7.74 (m, 5H), 7.30 (t, Jᵣ = 6.7 Hz, Jₑ = 7.4 Hz, 2H), 7.26 (d, J = 7.5 Hz, 1H), 7.16 (t, Jᵣ = 7.8 Hz, Jₑ = 7.6 Hz, 2H), 6.90 (d, J = 7.3 Hz, 2H), 1.90 (s, 3H), 1.76 (s, 3H), 1.71 (s, 3H), 1.68 (s, 3H); ¹³C NMR (125 MHz, d₄-MeOD, 298 K): δ 175.69 (Jₑ-Rh = 33.8 Hz), 155.82, 154.29, 151.54, 141.54, 140.40, 138.98, 135.38, 134.38, 132.14,131.63, 131.02,130.85, 129.63, 129.43, 129.02, 127.72, 125.10, 124.27, 124.06, 105.56, 102.90, 102.08, 98.47, 93.07, 10.52, 10.35, 9.00. HR-MS (ESI): m/z calcd for [CpʰRh(benzo[h]quinoline)pyridine]⁺ 557.1459, found 557.1458; elemental analysis calcd (%) for C₃₃H₃₈RhN₃O₃: C, 63.98; H, 4.88; N, 6.78; found: C, 63.91; H, 4.84; N, 6.76.

[CpʰbiphRh(benzo[h]quinoline)py]NO₃ (3b): 22 mg, yield 63%; ¹H NMR (500 MHz, d₄-MeOD, 298 K): δ 9.24 (d, J = 5.1 Hz, 1H), 8.54 (d, J = 5.6 Hz, 2H), 8.51 (d, J = 8.0 Hz, 1H), 8.30 (d, J = 7.0 Hz, 1H), 7.91 (d, J = 6.7 Hz, 1H), 7.83-7.74 (m, 5H), 7.53 (d, J = 7.4 Hz, 2H), 7.43 (d, J = 8.2 Hz, 2H), 7.39 (t, Jᵣ = 7.4 Hz, Jₑ = 7.7 Hz, 2H), 7.33-7.28 (m, 3H), 6.99 (d, J = 8.2 Hz, 2H), 1.93 (s, 3H), 1.77 (s, 3H), 1.73 (s, 3H), 1.71 (s, 3H); ¹³C NMR (125 MHz, d₄-MeOD, 298 K): δ 175.67 (Jₑ-Rh = 33.8 Hz), 155.81,154.29, 151.59, 142.40, 141.54, 141.21,140.40, 139.00, 135.38, 134.40, 132.16, 131.55, 130.84, 130.57, 129.97, 129.02, 128.79, 149.49, 140.54, 135.64, 133.83, 133.54, 131.99, 130.89, 129.71, 128.79, 128.05, 127.82, 127.73, 126.12, 125.13, 124.35, 124.07, 105.50, 102.72, 101.93, 98.28, 93.41, 10.57, 10.43, 9.02, 9.01. HR-MS (ESI): m/z calcd for [CpʰbiphRh(benzo[h]quinoline)pyridine]⁺ 633.1772; found 633.1768; elemental analysis calcd (%) for C₃₉H₃₄RhN₃O₃: C, 67.34; H, 4.93; N, 6.04; found: C, 67.25; H, 4.86; N, 5.95.

Methods and Instrumentation
**X-ray Crystallography:** Suitable crystals were selected and mounted on a glass fibre with Fomblin oil and placed on an Xcalibur Gemini diffractometer with a Ruby CCD area detector. The crystals were kept at 150(2) K except crystal of complex 3a at 100(2) K during data collection. Using Olex2,[42] all the structures were solved with the ShelXT[43] structure solution program using direct methods and refined with the ShelXL[44] refinement package using least squares minimization. X-ray crystallographic data for complexes 2a, 3a, and 1b-PF₆ have been deposited in the Cambridge Crystallographic Data Centre under the accession numbers CCDC 1952984, 1952985, 1952986, respectively. X-ray crystallographic data in CIF format are available from the Cambridge Crystallographic Data Centre (http://www.ccdc.cam.ac.uk/).

**NMR Spectroscopy:** ¹H and ¹³C NMR spectroscopy and ¹H-¹H gs (gradient selected) COSY(correlation spectroscopy), ¹H-¹³C HMQC (heteronuclear multiple quantum coherence) and HMBC (heteronuclear multiple bond coherence) were acquired at 298 K (unless stated otherwise) on Bruker Advance 300 MHz, Bruker Advance III HD 500 MHz or 600 MHz NMR spectrometers. ¹H NMR chemical shifts were internally referenced to CHCl₃ (7.26 ppm) for chloroform-d₄ and (CHD₂)OD (3.31 ppm) for methanol-d₄. ¹³C NMR chemical shifts were internally referenced to CDCl₃ (77.16 ppm) for chloroform-d₄, and CD₃OD (49.00 ppm) for methanol-d₄. The data were processed using Mestronova and Topspin (version 2.1 Bruker UK Ltd.).

**Electrospray Mass Spectrometry:** Electrospray ionization mass spectra (ESI-MS) were obtained by preparing the samples in methanol into a Bruker Esquire 2000 spectrometer. The mass spectra were recorded with a scan range of m/z either 50-500 or 400-1000 for positive ion mode. HR-MS analysis was carried with a Bruker MaXis plus Q-TOF mass spectrometer equipped with electrospray ionisation source. The mass spectrometer was operated in electrospray positive ion mode with a scan range 50-2,400 m/z.

**Elemental Analysis:** CHN elemental analyses were carried out on a CE-440 elemental analyzer by Warwick Analytical (UK) Ltd.

**pH measurements:** pH or pH* (pH meter reading without correction for effect of deuterium on glass electrode) values of samples in H₂O or D₂O were measured at ca. 298 K, using a HATCH minilab pocket pH meter with ISFET (silicon chip non-glass pH sensor) sensor, calibrated with buffer solutions included of pH 4, 7, and 10.
**UV-Vis spectroscopy:** A Cary 300 UV-Vis recording spectrophotometer was used with 1 cm path-length quartz cuvettes (3.0 mL) and a PTP1 Peltier temperature controller. Experiments were carried out at 310 K from 800 to 200 nm in 0.5 nm intervals unless stated otherwise. Spectra were recorded using UV Winlab software and analysed using Origin 2018.

**Reactions with NADH:** The time dependence of reactions of rhodium complexes (0.8 μM) with NADH (75-144 μM) were studied over 24 h in 1.6% MeOH-98.4% phosphate buffer (5 mM, pH 7.4) by UV-vis spectroscopy at 310 K. The concentration of NADH was obtained using the extinction coefficient ε(339 nm) = 6,220 M⁻¹cm⁻¹.[31] TON was calculated from the difference in NADH concentration divided by the concentration of metal complex catalyst over 24 h. TOF was calculated from the linear fitting of TON versus time (h) by Origin 2018.

**ICP-OES:** ICP-OES analysis was carried out on a PerkinElmer Optima 5300 DV series Optical Emission Spectrophotometer. The Rhodium Specpure® plasma standard (Alfa Aesar, 1000 pm in 10% HCl) was diluted with ca. 3.6% HNO₃ Milli-Q water to provide freshly prepared calibrants at concentrations of 700, 600, 500, 400, 300, 200, 100, 50, and 0 ppb, which were adjusted to match the sample matrix by standard addition of sodium chloride (TraceSELECT®). Total dissolved solids did not exceed 0.2% w/v. Data were acquired and processed using WinLab32 V3.4.1 for Windows.

**ICP-MS:** The whole cell pellet was digested in 72% nitric acid at 353 K overnight. Samples after digestion were diluted to give a final concentration of ca. 3.6% nitric acid and analysed using an ICP-MS Agilent Technologies 7500 series in no-gas/helium mode. ICP-MS TOP and Offline Data Analysis (ChemStation version B.03.05) by Agilent Technologies were used to process the data. Biological triplicates were produced for each sample and statistical significance was calculated (Welch’s unpaired t-test).

**High-Performance Liquid Chromatography (HPLC)/LC-MS:** Reverse phase HPLC (RP-HPLC) was performed on a HP 1100 Series HPLC System (Agilent) using a ZORBAX Eclipse Plus C-18 column (250 x 4.6 mm, 5 μm). Eluents: A-water (0.1% v/v TFA), B-acetonitrile (0.1% v/v TFA). Solvent gradient method: 0-30 min, 10%-80% B; 30-40 min, 80% B; 40-41 min, 80%-10% B; 40-55 min, 10% B. A flow rate of 1 mL min⁻¹ was used with detection wavelength at 254 nm. LC-MS was carried out on a Bruker Amazon X connected online to an Agilent 1260 HPLC with detection wavelength 254 nm. The mobile phases and the solvent gradient method were the same as HPLC.
**Cell Culture:** A549 human lung cell line and A2780 human ovarian cell line were grown in RPMI-1640 with L-glutamine supplemented with 10% v/v fetal bovine serum, and 1% v/v penicillin/streptomycin. All cells were grown as adherent monolayers at 310 K with 5% CO₂ humidified, and were passaged at ca. 80% confluency.

**Cell Viability Assay:** Briefly, 5000 cells were seeded per well in 96-well plates. The cells were pre-incubated in drug-free media at 310 K for 48 h before adding different concentrations of test compounds. Stock solutions of the complexes were prepared in DMSO (<0.5%)/cell culture medium and the concentration of each metal complex in the stock solution was determined by ICP-OES. The drug exposure period was 24 h. After this, supernatants were removed by suction and each well was washed with 100 μL PBS. The cells were allowed a further 72 h recovery in free fresh medium at 310 K. SRB assay was used to determine cell viability.[45] Absorbance measurements of the solubilized dye (on a BioRad iMark microplate reader using a 470 nm filter) allowed the determination of viable treated cells compared to untreated controls. IC₅₀ values were determined as duplicates of triplicates in two independent sets of experiments and their standard deviations were calculated.

**Cellular Rh Accumulation:** The accumulation of Rh in A549 human lung carcinoma cells was determined by ICP-MS (Inductively Coupled Plasma Mass Spectroscopy). 1×10⁶ cells were seeded into a 100 mm Petri dish and incubated in drug-free media for 24 h at 310 K with 5% CO₂. For a further 24 h, the cells were incubated in media containing 0.5× and 1 × IC₅₀ concentrated complex 3a or 3b. Following this, cells were washed, detached using trypsin-EDTA, resuspended in fresh media, counted and centrifuged. The cell pellets were digested as ICP-MS protocol for Rh analysis.

**ROS Determination:** 5×10⁴ A549 human lung carcinoma cells were seeded per well in a 6-well plate and incubated for 24 h at 310 K. 1× or 2× IC₅₀ concentrated complex 3a or 3b was added to the cells and incubated for a further 24 h. After which, total ROS and SO concentrations were analysed by flow cytometry using the total ROS/superoxide detection kit by Enzo Life Sciences. Briefly, the cells were enzymatically detached using trypsin-EDTA, washed in PBS, resuspended in staining solution, prepared according to manufacturer’s instructions (25 nM concentration of both dyes, total ROS and SO) and incubated at room temperature for 30 min. Samples were then analysed on the Beckton Dickinson LSRII using Beckton Dickinson FACSDIVA software. Data were processed using flowjo V10 for Windows. At all times,
samples were kept under dark conditions to avoid light-induced ROS production. Welch’s t-tests were carried out to establish statistical significance of the variations.

**Apoptosis Assay:** 5×10^4 A549 human lung carcinoma cells were seeded per well in a 6-well plate and incubated for 24 h at 310 K. 1× or 2× IC\textsubscript{50} concentrated complex 3a or 3b was added to the cells and incubated for a further 24 h. After which, the cells were enzymatically detached using trypsin-EDTA, washed in PBS, resuspended in staining solution (FITC Annexin V diluted 1 in 100 and 1 µg/mL PI) and incubated at room temperature for 30 min. Samples were then analysed on the Beckton Dickinson LSRII using Beckton Dickinson FACSDIVA software. Data was processed using flowjo V10 for Windows. Welch’s t-tests were carried out to establish statistical significance of the variations.

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**Keywords:** Anticancer; Cyclometalated; Cyclopentadienyl; Cytotoxicity; Rhodium; Reactive Oxygen Species

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Key Topic: **Rhodium, Anticancer**

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The chemical reactivity and cytotoxicity of such organo-rhodium compounds can be controlled by the nature of the monodentate ligand (chloride or pyridine). Compared to the pyridine analogues, the chloride compounds show faster hydrolysis and higher catalysis efficiency. However, the pyridine compounds exhibit higher cytotoxicity (lower IC\textsubscript{50} values) with lower cellular Rh accumulation. More interestingly, the pyridine complexes appear to act as ROS scavengers, while the chloride analogues induce ROS elevation in cancer cells.