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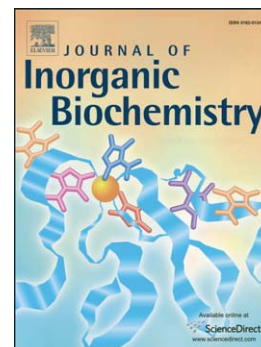
Half-sandwich rhodium(III) transfer hydrogenation catalysts: Reduction of NAD^+ and pyruvate, and antiproliferative activity

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**Half-sandwich rhodium(III) transfer hydrogenation
catalysts: reduction of NAD⁺ and pyruvate, and
antiproliferative activity**

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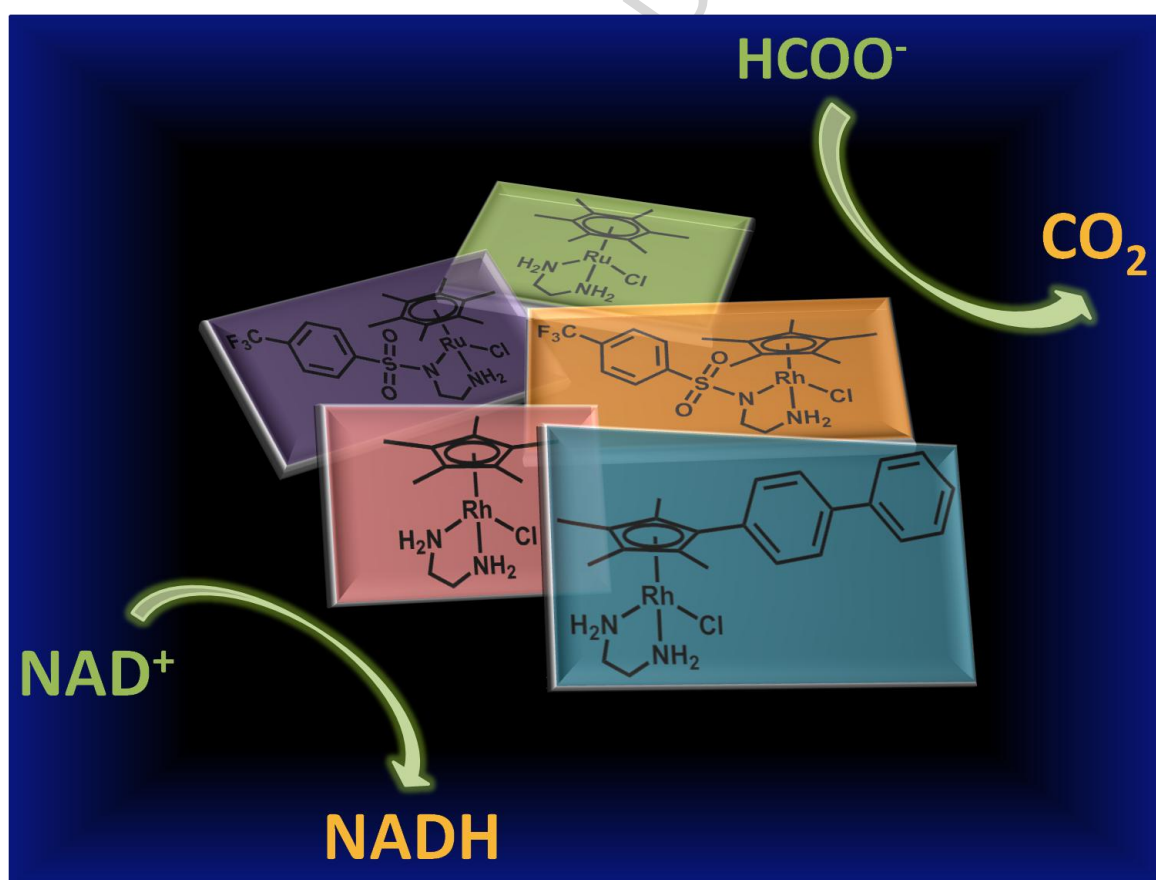
KEYWORDS: NADH, NAD⁺, pyruvate, transfer-hydrogenation, anticancer, organo-rhodium.

ABSTRACT: Organometallic complexes have the potential to behave as catalytic drugs. We investigate here Rh(III) complexes of general formula $[(Cp^x)Rh(N,N')(Cl)]$, where N,N' is ethylenediamine (en), 2,2'-bipyridine (bpy), phenanthroline (phen) or *N*-(2-aminoethyl)-4-(trifluoromethyl)benzenesulfonamide (TfEn), and Cp^x is pentamethylcyclopentadienyl (Cp^*), 1-phenyl-2,3,4,5-tetramethylcyclopentadienyl (Cp^{xPh}) or 1-biphenyl-2,3,4,5-tetramethylcyclopentadienyl (Cp^{xPhPh}). These complexes can reduce NAD^+ to NADH using formate as an hydride source under biologically-relevant conditions. The catalytic activity decreased in the order of *N,N*-chelated ligand $bpy > phen > en$ with Cp^* as the η^5 -donor. The en complexes (**1-3**) became more active with extension to the Cp^x ring, whereas the activity of the phen (**7-9**) and bpy (**4-6**) compounds decreased. $Cp^*Rh(bpy)Cl]^+$ (**4**) showed the highest catalytic activity, with a TOF of $37.4 \pm 2 \text{ h}^{-1}$. Fast hydrolysis of the chlorido complexes **1-10** was observed by 1H NMR ($< 10 \text{ min}$ at 310 K). The pK_a^* values for the aqua adducts were determined to be *ca.* 8-10. Complexes **1-9** also catalysed the reduction of pyruvate to lactate using formate as the hydride donor. The efficiency of the transfer hydrogenation reactions was highly dependent on the nature of the chelating ligand and the Cp^x ring. Competition reactions between NAD^+ and pyruvate for reduction by formate catalysed by **4** showed a preference for reduction of NAD^+ . The antiproliferative activity of complex **3** towards A2780 human ovarian cancer cells increased by up to 50% when administered in combination with non-toxic doses of formate, suggesting that transfer hydrogenation can induce reductive stress in cancer cells.

- **Graphical abstract (synopsis)**

We report for the first time half-sandwich Rh(III) complexes as catalytic anticancer agents capable of reducing nicotinamide adenine dinucleotide (NAD^+) and pyruvate in the presence of a hydride source (formate). The complexes display low-moderate antiproliferative activity towards cancer cells. However, a significant increase in activity upon coadministration with formate can be achieved.

Graphical abstract (figure attached)



1. Introduction

Transfer hydrogenation reactions for the reduction of ketones, imines or C=C double bonds have been intensively studied in recent years [1-4]. Precious metal complexes containing Ru, Rh or Ir are often effective as transfer hydrogenation catalysts, and half-sandwich organometallic complexes in particular have achieved high conversions, turnover frequencies (TOF) and enantio-selectivities [1-4]. Ruthenium(II) catalysts are often the most efficient, partly due to their slower rate of ligand exchange compared to Rh(III) or Ir(III), which, in many cases, provides higher chemo- and enantio-selectivity [1-3,5-7]. Although rhodium catalysts can be more active, this is often accompanied by a loss of chemo- and enantio-selectivity. Nevertheless, catalytic activity and enantio-selectivity depend on the choice of appropriate chiral ligands, on the substrate, the hydride donor, and on the reaction conditions [1-3,5-7].

There is current interest in metal-based complexes capable of catalysing reactions in cells [8-11]. Metallodrugs with catalytic properties can potentially be administered in smaller doses consequently leading to lower toxicity [8-11]. Transfer hydrogenation reactions have attracted much attention for the reduction of molecules inside cells [8-11]. Organo-Ru(II) and -Ir(III) complexes can catalyse hydride transfer reactions under biologically-relevant conditions (pH 7.2, 310 K, aqueous media) [12-17]. For example, $[(\eta^6\text{-arene})\text{Ru}(\text{bipyrimidine})\text{Cl}]^+$ or $[(\text{Cp}^{\text{X}}\text{Ir}(\text{phenanthroline})\text{Cl})^+$ where Cp^{X} is pentamethylcyclopentadienyl (Cp^*), 1-phenyl-2,3,4,5-tetramethylcyclopentadienyl (Cp^{xPh}) or 1-biphenyl-2,3,4,5-tetramethylcyclopentadienyl (Cp^{xPhPh}) can utilise nicotinamide adenine dinucleotide reduced (NADH) to transfer hydride and reduce biomolecules such as pyruvate [13,14]. The reverse transfer of hydride to nicotinamide adenine dinucleotide (NAD^+) can also be achieved using formate as an hydride source, $[(\eta^6\text{-arene})\text{Ru}(\text{ethylenediamine})\text{Cl}]^+$ and $[(\text{arene})\text{Ru}(N\text{-(2-aminoethyl)-sulfonamide})\text{Cl}]^+$ where arene = *p*-cymene (*p*-cym), benzene

(bn) or hexamethyl benzene (hmb) and biphenyl (bip) can regenerate NADH from NAD^+ [12,15,16].

Our recent work suggests that transfer hydrogenation catalysed by organometallic complexes can be achieved inside cells [12,13]. For example, the Ir(III) complex $[(\text{Cp}^{\text{xphph}})\text{Ir}(\text{phenylpyridine})\text{py}]^+$, where py is pyridine, can utilize NADH as a biological hydride donor, to generate an iridium-hydride complex. The hydrido complex is able to transfer hydride to molecular oxygen, increasing the levels of hydrogen peroxide and reactive oxygen species in cancer cells [13]. Also, the Ru(II) complex $[(p\text{-cym})\text{Ru}(\text{TsEn})\text{Cl}]$ where TsEn is *N*-(2-aminoethyl)-4-toluene sulfonamide reduces the levels of NAD^+ in A2780 ovarian cancer cells when co-administered with sodium formate, potentiating the anti-cancer activity of the complex [12].

The aim of the present work was to synthesise a series of Rh(III) Cp^* catalysts which can carry out transfer hydrogenation reactions in cells. Half-sandwich complexes of the type $[(\text{Cp}^{\text{x}}\text{Rh}(N,N')\text{Cl})^{\text{n}+}]$, where N,N' = ethylenediamine (en), 2,2'-bipyridine (bpy), 2,2'-dimethylbipyridine (mbpy) and phenanthroline (phen) or were synthesised and fully characterized (Figure 1). The activity of these Rh(III) complexes for the reduction of NAD^+ in the presence of formate was compared with their Ru(II) analogues $[(\eta^6\text{-arene})\text{Ru}(\text{en})\text{Cl}]$ and $[(\eta^6\text{-arene})\text{Ru}(\text{TsEn})\text{Cl}]$. We also studied the reduction of pyruvate to lactate *via* hydride transfer from formate, a process carried out naturally *in vivo* by the enzyme lactate dehydrogenase and the coenzyme NADH. Finally, the antiproliferative activity of the complexes towards cancer cells in the presence of excess formate was also investigated.

2. Experimental

2.1 Materials.

Rhodium(III) trichloride hydrate was purchased from Precious Metals Online (PMO Pty Ltd.) and used as received. Ethylenediamine (en) was purchased from Sigma-Aldrich and freshly distilled prior to use. The ligands 3-phenyl-1,2,4,5-tetramethyl-1,3-cyclopentadienyl (Cp^{xPh}) and 3-biphenyl-1,2,4,5-tetramethyl-1,3-cyclopentadienyl (Cp^{xPhPh}) were synthesised following the methods in the literature [18,19]. The Rh(III)-arene precursor dimers $[(\text{Cp}^{\text{x}})\text{RhCl}_2]_2$ were prepared following literature methods [20], as was the ligand N-(2-aminoethyl)-4-(trifluoromethyl)benzenesulfonamide (TfEnH) [15]. 2,2'-Bipyridine, 4,4'-dimethyl-2,2'-dipyridine, 1,10-phenanthroline, 4-bromo-biphenyl, 1,6 M n-butyllithium in hexane, phenyllithium in ether (1.8 mM), 2,4-pentamethylcyclopentadiene, 2,3,4,5-tetramethyl-2-cyclopentanone were obtained from Sigma-Aldrich. Magnesium sulphate, ammonium hexafluorophosphate, silver nitrate, potassium hydroxide, sodium chloride, hydrochloric acid were obtained from Fisher Scientific. Sodium formate, perchloric acid, β -nicotinamide adenine dinucleotide hydrate (NAD^+), β -nicotinamide adenine dinucleotide reduced disodium salt (NADH) and sodium pyruvate were purchased from Sigma-Aldrich. DMSO- d_6 , MeOD- d_4 , D_2O , $(\text{CD}_3)_2\text{CO}-d_6$ and CDCl_3 for NMR spectroscopy were purchased from Sigma-Aldrich and Cambridge Isotope Labs Inc. Non-dried solvents used in syntheses were obtained from Fisher Scientific and Prolabo.

2.2 NMR Spectroscopy.

^1H NMR spectra were acquired in 5 mm NMR tubes at 298 K or 310 K on Bruker AV-400 or Bruker AV III 600 spectrometers. Data processing was carried out using XWIN-NMR version 3.6 (Bruker U.K. Ltd). ^1H NMR chemical shifts were internally referenced to TMS via 1,4-dioxane in D_2O ($\delta = 3.75$), residual DMSO ($\delta = 2.52$ ppm) or CHCl_3 ($\delta = 7.26$ ppm). 1D spectra were recorded using standard pulse sequences. Typically, data were acquired with

16 transients into 32 k data points over a spectral width of 14 ppm and for the kinetic experiments, 32 transient into 32 k data points over a spectral width of 30 ppm using a relaxation delay of 2 s.

2.3 *pH* measurements.*

pH* values (pH meter reading without correction for the effect of deuterium on glass electrode) were measured at ambient temperature using a minilab IQ125 pH meter equipped with a ISFET silicon chip pH sensor and referenced in KCl gel. The electrode was calibrated with Aldrich buffer solutions of pH 4, 7 and 10. pH* values were adjusted with KOH or HClO₄ solutions in D₂O.

2.4 *Elemental analysis.*

Elemental analyses were performed by Warwick Analytical Service using an Exeter Analytical elemental analyzer (CE440).

2.5 *Electrospray ionization mass spectrometry (ESI-MS).*

Positive ion electrospray mass spectra were obtained on a Bruker Daltonics Esquire 2000 ion trap mass spectrometer. All samples were prepared in methanol (100%). Data were processed using Data-Analysis version 3.3 (Bruker Daltonics).

2.6 *Synthesis*

[(Cp*)RhCl₂]₂. Hydrated rhodium(III) trichloride (500 mg, 2.12 mmol) was reacted with 2,4-pentamethylcyclopentadiene (302 mg, 2.22 mmol) dissolved in dry methanol (50 mL) and heated to reflux (343 K) under a nitrogen atmosphere for 48 h. The dark red precipitate was filtered off and washed with ether (3 x 20 mL) to give a dark red powder. The crude product was then recrystallised from methanol. Yield: 410.6 mg (62.7 %). ¹H NMR (400 MHz, DMSO-*d*₆): δ_H 1.627 (s, 10H).

[(Cp^{xPh})RhCl₂]₂. [(Cp^{xPh})RhCl₂]₂ was synthesised following the same procedure described for [(Cp*)RhCl₂]₂ using hydrated Rh(III)Cl₃ (500.7 mg, 1.05 mmol) and HCp^{xPh} (532.5 mg, 2.68

mmol). The red precipitate was recrystallised from methanol. Yield: 592.7 mg (75.47 %). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ_{H} 7.67 (m, 4H), 7.43 (m, 6H), 1.71 (s, 4H), 1.67 (m, 4H).

$[(\text{Cp}^{\text{xPhPh}})\text{RhCl}_2]_2$. $[(\text{Cp}^{\text{xPhPh}})\text{RhCl}_2]_2$ was synthesised following the procedure described for $[(\text{Cp}^*)\text{RhCl}_2]_2$ using hydrated Rh(III)Cl_3 (992.6 mg, 2.1 mmol) and $\text{HCp}^{\text{xPhPh}}$ (960.5 mg, 4.4 mmol). The red-orange precipitate obtained was recrystallised from methanol. Yield: 613.4 mg (33.2 %). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ_{H} 7.75 (m, 12H), 7.49 (t, 4H, $J = 7.4$ Hz), 7.40 (t, 2H, $J = 7.4$ Hz), 1.73 (s, 24H).

General Synthesis of complexes 1-9. The Rh(III) dimer was placed in a round-bottom flask to which dry dichloromethane was added. Upon addition of the corresponding ligand (2.5 mol. equiv.), the reaction was stirred overnight at ambient temperature, after which the solvent was removed on the rotary evaporator to afford a crude powder. The crude product was re-dissolved in methanol and filtered. Excess ammonium hexafluorophosphate was then added and the solution stored in the freezer. The resulting product was collected by filtration and recrystallised from acetone or methanol.

$[(\text{Cp}^*)\text{Rh(en)Cl}]\text{PF}_6$ (1). $[(\text{Cp}^*)\text{RhCl}_2]_2$ (51.3 mg, 83.0 μmol), ethylenediamine (9.88 mg, 0.16 mmol, 11 μl). Bright yellow crystals were collected. Yield: 42.3 mg (78.3 %). ^1H NMR (400 MHz, CDCl_3): δ_{H} 5.91 (s, 2H), 3.05 (s, 2H), 2.88 (s, 2H), 2.74 (s, 2H), 1.90 (s, 15H). Anal: Calc for $\text{C}_{12}\text{H}_{23}\text{ClF}_6\text{N}_2\text{PRh}$ C: 27.91, H: 4.61, N: 6.21; Found C: 28.11, H: 4.81, N: 5.95. ESI-MS: Calc for $\text{C}_{12}\text{H}_{23}\text{ClN}_2\text{O}_2\text{Rh (M)}^+$ 333.0 m/z found 333.0 m/z .

$[(\text{Cp}^{\text{xPh}})\text{Rh(en)Cl}]\text{PF}_6$ (2). $[(\text{Cp}^{\text{xPh}})\text{RhCl}_2]_2$ (50.5 mg, 68.0 μmol), ethylenediamine (8.99 mg, 0.15 mmol, 10 μl). Recrystallisation from methanol resulted in bright yellow crystals. Yield: 31.7 mg (59.0 %). ^1H NMR (400 MHz, CDCl_3): δ_{H} 7.49 (m, 5H), 5.59 (s, 2H), 3.11 (s, 2H), 3.02 (s, 2H), 2.76 (s, 2H), 2.05 (s, 6H), 1.91 (s, 6H). Anal: Calc for $\text{C}_{17}\text{H}_{25}\text{ClF}_6\text{N}_2\text{PRh}$ C: 37.76, H: 4.66, N: 5.18; Found C: 37.02, H: 4.67, N: 5.26. ESI-MS: Calc for $\text{C}_{17}\text{H}_{25}\text{ClN}_2\text{Rh (M)}^+$ 395.0 m/z found 395.0 m/z .

[(Cp^{xPhPh})Rh(en)Cl]PF₆ (3). [(Cp^{xPhPh})RhCl₂]₂ (51.6 mg, 57.6 μmol), ethylenediamine (7.11 mg, 0.12 mmol, 8 μl). Recrystallisation from methanol resulted in bright yellow crystals. Yield: 47.7 mg (54.2 %) ¹H NMR (400 MHz, CDCl₃): δ_H 7.69 (d, 2H, J = 8.4 Hz), 7.60 (m, 4H), 5.49 (t, 2H, J = 7.5 Hz), 7.41 (t, 1H, J = 7.5), 5.59 (s, 2H), 3.14 (s, 2H), 3.03 (s, 2H), 2.79 (s, 2H), 2.07 (s, 6H), 1.97 (s, 6H). Anal: Calc for C₂₃H₂₉ClF₆N₂PRh C: 44.79, H: 4.74, N: 4.54; Found C: 43.98, H: 4.65, N: 4.54. ESI-MS: Calc for C₂₃H₂₉ClN₂Rh (M)⁺ 471.1 m/z, found 471.1 m/z.

[(Cp*)Rh(bpy)Cl]PF₆ (4). [(Cp*)RhCl₂]₂ (100.1 mg, 16.2 mmol), 2,2'-bipyridine (64.7 mg, 41.4 mmol) Recrystallization from acetone resulted in bright orange crystals. Yield: 126.6 mg (67.9 %). ¹H NMR (400 MHz, Acetone-*d*₆): δ_H 9.14 (d, 2H, J = 5.7 Hz), 8.67 (d, 2H, J = 8.1 Hz), 8.38 (t, 2H, J = 8.0 Hz), 7.95 (dd, 2H, J₁ = 8.0 Hz, J₂ = 5.7 Hz), 1.82 (s, 15H). Anal: Calc for C₂₀H₂₃ClF₆N₂PRh C: 41.8, H: 4.03, N: 4.87; Found C: 41.85, H: 3.97, N: 4.84. ESI-MS: Calc for C₂₀H₂₃ClN₂Rh (M)⁺ 430.1 m/z found 430.0 m/z.

[(Cp^{xPh})Rh(bpy)Cl]PF₆ (5). [(Cp^{xPh})RhCl₂]₂ (101.3 mg, 13.6 mmol, 2,2'-bipyridine (56.0 mg, 35.9 mmol). Recrystallization from acetone resulted in bright orange crystals. Yield: 113.5 mg (65.2 %). ¹H NMR (400 MHz, Acetone-*d*₆): δ_H 8.85 (d, 2H, J = 5.5 Hz), 8.70 (d, 2H, J = 8.1 Hz), 8.37 (t, 2H, J = 8.4 Hz), 7.85 (t, 2H, J = 6.4 Hz), 7.76 (d, 2H, J = 6.6 Hz), 7.60 (m, 3H), 1.92 (s, 6H), 1.85 (s, 6H). Anal: Calc for C₂₅H₂₅ClF₆N₂PRh + acetone C: 48.40, H: 4.50, N: 4.03; Found C: 49.89, H: 4.22, N: 3.93. ESI-MS: Calc for C₂₅H₂₅ClN₂Rh (M)⁺ 490.1 m/z found 490.0 m/z.

[(Cp^{xPhPh})Rh(bpy)Cl]PF₆ (6). [(Cp^{xPhPh})RhCl₂]₂ (100.8 mg, 11.2 mmol) 2,2'-bipyridine (45.2 mg, 28.9 mmol). Recrystallization from acetone resulted in bright orange crystals. Yield: 87.2 mg (54.3 %) ¹H NMR (400 MHz, Acetone-*d*₆): δ_H 8.91 (d, 2H, J = 8.2 Hz), 8.89 (d, 2H, J = 8.4 Hz), 8.35 (t, 2H, J = 8.0 Hz), 7.87 (m, 6H), 7.79 (d, 2H, J = 7.6 Hz), 7.54 (t, 2H, J = 7.6 Hz), 7.45 (t, 1H, J = 7.6 Hz), 1.95 (s, 6H), 1.90 (s, 6H). Anal: Calc for

$C_{31}H_{29}ClF_6N_2PRh$ C: 52.23, H: 4.10, N: 3.93; Found C: 52.73, H: 4.31, N: 3.70. ESI-MS: Calc for $C_{31}H_{29}ClN_2Rh$ (M)⁺ 567.1 m/z, found 567.0 m/z.

[(Cp*)Rh(phen)Cl]PF₆ (7). [(Cp*)RhCl₂]₂ (100.2 mg, 16.2 mmol), 1,10-phenanthroline (80.78 mg, 40.7 mmol). Recrystallization from acetone resulted in bright orange crystals. Yield: 138.7 mg (71.4 %). ¹H NMR (400 MHz, CDCl₃): δ_H 9.43 (d, 2H, J = 5.2 Hz), 8.71 (d, 2H, 8.1 Hz), 8.29 (dd, 2H, J₁ = 8.2 Hz, J₂ = 5.2 Hz), 8.1 (s, 2H), 1.89 (s, 15H). Anal: Calc for $C_{22}H_{23}ClF_6N_2PRh$ C: 44.13, H: 3.87, N: 4.68; Found C: 44.13, H: 3.79, N: 4.62. ESI-MS: Calc for $C_{22}H_{23}ClN_2Rh$ (M)⁺ 453.1 m/z found 453.0 m/z.

[(Cp^{xPh})Rh(phen)Cl]PF₆ (8). [(Cp^{xPh})RhCl₂]₂ (124.0 mg, 16.7 mmol), 1,10-phenanthroline (83.6 mg, 42.1 mmol). Recrystallization from acetone resulted in bright red crystals. Yield: 130.3 mg (59.0 %). ¹H NMR (400 MHz, CDCl₃): δ_H 9.08 (d, 2H, J = 5.2 Hz), 8.69 (d, 2H, J = 8.2 Hz), 8.12 (m, 4H), 7.78 (d, 2H, J = 7.6 Hz), 7.61 (m, 3H), 2.06 (s, 6H), 1.85 (s, 6H). Anal: Calc for $C_{27}H_{25}ClF_6N_2PRh + MeOH$ C: 48.54, H: 4.22, N: 4.04; Found C: 47.68, H: 4.09, N: 4.17. ESI-MS: Calc for $C_{27}H_{25}ClN_2Rh$ (M)⁺ 515.1 m/z found 515.0 m/z.

[(Cp^{xPhPh})Rh(phen)Cl]PF₆ (9). [(Cp^{xPhPh})RhCl₂]₂ (100.7 mg, 11.2 mmol), 1,10-phenanthroline (55.8 mg, 28.2 mmol). Yield: 109.8 mg (66.3 %). ¹H NMR (400 MHz, CDCl₃): δ_H 9.11 (d, 2H, J = 5.2 Hz), 8.69 (d, 2H, J = 8.1 Hz), 8.13 (dd, 2H, J₁ = 8.1 Hz, J₂ = 5.2 Hz), 8.12 (s, 2H), 7.86 (d, 2H, J = 8.2 Hz), 7.82 (d, 2H, J = 8.2 Hz), 7.70 (d, 2H J = 7.4 Hz), 7.53 (t, 2H, J = 7.4), 7.45 (t, 1H, J = 7.4 Hz), 2.06 (s, 6H), 1.88 (s, 6H). Anal: Calc for $C_{23}H_{29}ClF_6N_2PRh + MeOH$ C: 53.11, H: 4.33, N: 3.64; Found C: 54.37, H: 4.34, N: 3.49. ESI-MS: Calc for $C_{23}H_{29}ClN_2Rh$ (M)⁺ 591.1 m/z, found 591.1 m/z.

[(Cp*)Rh(TfEn)Cl] (10). [(Cp*)RhCl₂]₂ (49.7 mg, 80.4 μmol) and *N*-(2-aminoethyl)-4-(trifluoromethyl)benzenesulfonamide (TfEnH) (43.5 mg, 162.2 μmol) were dissolved in dichloromethane (25 mL) and triethylamine (45 μL, 320.5 μmol) was added. The reaction was then stirred under nitrogen atmosphere overnight. The solution was placed in a separating

funnel and washed with brine, the organic layer separated and dried over MgSO_4 and filtered. The solution was concentrated *in vacuo* and the product obtained recrystallised from methanol to afford an orange powder. Yield: 40.3 mg (46.4 %). ^1H NMR (400 MHz, CDCl_3): δ_{H} 8.00 (d, 2H, $J = 8.1$ Hz), 7.54 (d, 2H, $J = 8.1$ Hz), 3.19 (s, 2H), 2.60 (s, 3H), 1.71 (s, 15H). Anal: Calc for $\text{C}_{19}\text{H}_{25}\text{ClF}_3\text{N}_2\text{O}_2\text{RhS}$ C: 42.20, H: 4.66, N: 5.18; Found C: 41.92, H: 4.36, N: 5.01. ESI-MS: Calc. for $\text{C}_{19}\text{H}_{25}\text{ClF}_3\text{N}_2\text{O}_2\text{RhS}$ ($\text{M}-\text{Cl}$) $^+$ 505.0 m/z , found 505.0 m/z .

2.7 Aqueous solution chemistry

Solutions of complexes **1-10** (1.4 mM, in 20 % MeOD/ 80 % D_2O) were prepared and ^1H NMR spectra at 310 K were recorded at time 0 (<10 min) and 24 h. The samples were then incubated at 310 K.

Aqueous solutions of the chlorido complexes **1-10** (1.4 mM, in 20 % MeOD/ 80 % D_2O) were treated with silver nitrate (0.95 mol. equiv.) and stirred overnight at room temperature. ^1H NMR spectra were recorded after filtration of the samples through celite to remove the silver chloride formed.

2.8 pK_a determination for aqua species

Solutions of complexes **1-4**, **7** and **10** (1.4 mM, D_2O) were prepared and treated with 0.95 mol equiv. of silver nitrate. The reaction mixture was then filtered through celite to obtain the corresponding aqua adducts. Changes in the chemical shifts of the methyl protons of the Cp^x ligand in the aqua adducts with the pH^* (pH meter reading) over a range from 2 to 12 were followed by ^1H NMR spectroscopy. Solutions of KOH or HClO_4 in D_2O were used to adjust the pH^* . ^1H -NMR spectra were recorded at 298 K on a Bruker AV III 600 spectrometer. The data were fitted to the Henderson–Hasselbalch equation using Origin 7.5.

2.9 Reduction of pyruvate and NAD⁺

Complexes **1-3** and **10** were dissolved in D₂O (1.4 mM, 4 mL) in a glass vial. Complexes **1-9** were prepared in MeOD/D₂O (3:2 v/v) (1.4 mM, 4 mL) in a glass vial and treated with 0.95 mol. equiv. of silver nitrate.

Aqueous solutions of sodium formate (25 mol equiv.) and substrate (2-9 mol equiv, substrate = NAD⁺ or pyruvate) in D₂O were also prepared and incubated at 310 K. In a typical experiment, an aliquot of 200 µL of the complex, formate and substrate solutions were added to a 5 mm NMR tube. The pH* of the solution mixture was adjusted to 7.2 ± 0.2 bringing the total volume to 0.635 mL ¹H NMR spectra were recorded at 310 K every 162 s until the completion of the reaction.

Molar ratios of substrate and product were determined by integrating the peaks for NAD⁺, pyruvate, lactate and 1,4-NADH. The turnover number (TON) for the reaction was calculated as follows:

$$\text{TON} = \frac{I_x}{I_x + I_y} \frac{[\text{substrate}]_0}{[\text{Catalyst}]}$$

where I_n is the integral of the signal at n ppm. x = 6.96 (NADH) or 1.32 ppm (lactate). y = 9.33 (NAD⁺) or 2.36 ppm (pyruvate). [substrate]₀ is the concentration of NAD⁺ or pyruvate at the start of the reaction.

2.10 Mechanistic studies

A set of four experiments was performed to compare the characteristics of the catalytic cycle of Rh(III) complexes **1** and **10** (1.4 mM, D₂O) with their Ru(II) analogues.

The turnover frequencies for the reduction reaction of NAD⁺ using complexes **1** or **10** and formate (25 mol equiv.) were determined following the procedure described above. The reaction was studied using different concentrations of NAD⁺ (1, 2 and 5 mol equiv. for complex **10**; 5, 7.5 and 10 mol equiv. for complex **1**).

A second series of experiments using different concentrations of sodium formate (5, 10, 25, 50 and 100 mol equiv. for complex **1**; 10, 25, 50, 100 and 500 mol equiv. for complex **10**) and a constant concentration of NAD^+ (5 mol equiv.) was also performed.

The optimum pH range for the catalytic process was studied in a series of experiments. Each experiment was performed at a different pH^* over a range from 6 to 10. The pH^* of the reaction was adjusted using solutions of KOH or HClO_4 in D_2O .

Transfer hydrogenation reactions using 1,4-NADH (400 μL , 2.8 mM) as a hydride source (400 μL , 2.8 mM) in D_2O by complex **1** and **10** were studied by ^1H NMR for a period of 10 h. The pH^* of the reaction mixture was adjusted to 7.2 ± 0.2 , and the experiment performed at 310 K.

2.11 Competition studies.

Complexes **1** (1.4 mM) was dissolved in $\text{CD}_3\text{OD}/\text{D}_2\text{O}$ (3:2 v/v, 4 mL) in a glass vial. Aqueous solutions of sodium formate (100 mol equiv.), NAD^+ (4.5 mol equiv.) and pyruvate (4.5 mol equiv.) in D_2O were also prepared and incubated at 310 K. In a typical experiment, an aliquot of 200 μL of complex, 50 μL of formate and 150 μL of pyruvate and NAD^+ were added to a 5 mm NMR tube. The pH^* of the solution mixture was adjusted to 7.2 ± 0.2 bringing the total volume to 0.635 mL. ^1H NMR spectra were recorded at 310 K every 162 s until the completion of the reaction.

2.12 Antiproliferative activity and cell viability modulation assays.

The antiproliferative activities of complexes **1-10** in A2780 ovarian cancer cells were determined. Briefly, 96-well plates were used to seed 5000 cells per well. The plates were pre-incubated in drug-free media at 310 K for 48 h before addition of various concentrations of the compounds. The drug exposure period was 24 h, after which, the supernatant was removed by suction and each well washed with PBS. A further 48 h was allowed for the cells to recover in drug-free medium at 310 K. The sulforhodamine B (SRB) colorimetric assay

was used to determine cell viability [21]. IC_{50} values, as the concentration which causes 50% growth inhibition, were determined as duplicates of triplicates in two independent sets of experiments and their standard deviations were calculated.

The data were analysed using Origin 8.5. IC_{50} values were obtained from plots of the percentage survival of cells versus the logarithm of the concentration expressed in millimolar units and fitted to a sigmoidal curve. IC_{50} values for cisplatin were determined in each well-plate as a validation.

Cell viability modulation assays were carried out in A2780 ovarian cancer cells. These experiments were performed as described above for IC_{50} determinations with the following experimental modifications. A fixed concentration of complexes was used, 150 μ M. Co-administration of the complex with three different concentrations of sodium formate (0.5, 1 and 2 mM) was studied. Both solutions (complex and sodium formate) were added to each well independently, but within 5 min of each other. Cell viability percentages were determined as duplicates of triplicates in two independent sets of experiments and their standard deviations were calculated.

Stock solutions of the Rh(III) complexes were freshly prepared for every experiment in 5% DMSO and a mixture 0.9% saline : medium (1:1 v/v). The stock solution was further diluted using RPMI-1640 to achieve working concentrations. The final concentration of DMSO was between 0.5 and 0.1 % v/v. Metal concentrations were determined by ICP-MS.

3. Results

Rh(III) complexes **1-9** were synthesised (Figure 1) using a similar procedure. Typically, the ligand (2 - 2.5 mol equiv), was added to a dichloromethane solution of the rhodium dimer, $[(Cp^x)RhCl_2]_2$ and the reaction mixture stirred at ambient temperature. The details for individual reactions are described in the experimental section. Complex **10** was synthesised by reacting the dimer with the TfEnH ligand in dichloromethane, and in the presence of triethylamine (4 mol equiv) at ambient temperature overnight.

The complexes were characterised by elemental analysis, NMR spectroscopy and mass spectrometry. The x-ray crystal structures of complexes **3-6**, **8** and **9** were determined and will be reported elsewhere [22].

3.1 Aqueous solution chemistry.

Aquation of complexes **1-10** (1.4 mM in D₂O) at 310 K was followed by ¹H NMR over a period of 24 h. For complexes **1-3** and **10**, only one set of peaks (aqua adducts) was observed. For complex **4-9**, two sets of peaks were observed and assigned as the chlorido and the aqua species. Peaks for aqua species were assigned by comparison of the ¹H spectra of **1-10** in D₂O and the products of reactions between **1-10** and silver nitrate. After 24 h incubation (310 K), no apparent changes were observed by ¹H NMR. Compounds **6** and **9** gave rise to precipitates after 24 h and ¹H NMR spectra could not be recorded. The extent of hydrolysis is shown in 0.

Changes in the ¹H NMR chemical shifts of the methyl groups of cyclopentadienyl protons from the aqua adducts of complexes **1-4**, **7** and **10** were followed over the pH* range from 2 to 12. The data were fitted to the Henderson–Hasselbalch equation. The pK_a* values for complexes **1-4**, **7** and **10** are shown in 0 (Figure 2).

3.2 Transfer hydrogenation reactions with NAD^+

Catalytic conversion of NAD^+ to NADH using complexes **1-10** and sodium formate (hydride source) was followed by ^1H NMR spectroscopy. In a typical experiment, 200 μL of complex (1.4 mM, D_2O or $\text{MeOD}/\text{D}_2\text{O}$ 3:2 v/v), sodium formate (35 mM, D_2O) and NAD^+ (2.8-12.6 mM, D_2O) were mixed in a 5 mm NMR tube, final ratio 1:25:2-9, complex : sodium formate : NAD^+ . The pH^* of the reaction mixture was adjusted to 7.2 ± 0.2 . ^1H NMR spectra at 310 K were recorded every 162 s until completion of the reaction. The turnover frequency (TOF) for the reactions were determined as described in the experimental section (0).

The turnover frequencies for complexes **1-3** in D_2O , increased in the order $\text{Cp}^* < \text{Cp}^{\text{xPh}} < \text{Cp}^{\text{xPhPh}}$ (0). The most active complex was $[(\text{Cp}^{\text{xPhPh}})\text{Rh}(\text{en})\text{Cl}]^+$ (**3**), with a TOF of 24.19 h^{-1} . The reaction was regioselective giving 1,4-NADH exclusively. On changing the ethylenediamine (en) chelating ligand (complex **1**) to *N*-(2-aminoethyl)-4-(trifluoromethyl)benzenesulfonamide (TfEn) (complex **10**), the catalytic activity decreased (0). Furthermore, a decrease in the regioselectivity of the reaction was also observed with 7.5% of 1,6-NADH being produced.

Complex **1** was less active than complex **2** in the reduction of NAD^+ in 20% $\text{MeOD}/80\%$ D_2O , following the same trend as observed for the reaction in D_2O . However, the catalytic activity of the complexes **1** and **2** in 20% MeOD was 2x times higher than that in D_2O .

Complexes **4-9** were also studied for the reduction of NAD^+ in 20% $\text{MeOD}/80\%$ D_2O . Complexes containing Cp^* (**4** and **7**) were more active than those containing Cp^{xPh} (**5** and **7**), which, in turn, were more active than Cp^{xPhPh} complexes (**6** and **8**), (0). The reactions of complexes **4-9** were largely regioselective, giving only 1 to 10% of 1,6-NADH.

3.2.1 Effect of NAD^+ and sodium formate concentration on the catalysis rate.

Transfer hydrogenation reactions using complex **1**, sodium formate and varying concentrations of NAD^+ were studied (ratio of 1:25:X, X = 5, 7.5 and 10 mol equiv of

NAD⁺). The turnover frequency (7.5 h⁻¹) remained the same with increasing concentrations of NAD⁺. For complex **10**, the TOF (4.12 h⁻¹) was unaffected by the concentration of NAD⁺ (X= 1, 2 or 5 mol equiv).

A second series of experiments on the reduction of NAD⁺, varying the concentration of sodium formate, was performed (complex **1**, sodium formate and NAD⁺ in the ratio 1:X:5, X = 5, 10, 25, 50 or 100 mol equiv). A notable increase in the catalytic activity was observed with increase of the hydride source (sodium formate), (Figure 3). From the plot of TOF vs formate concentration, typical Michaelis-Menten behaviour can be observed. From the reciprocal of the TOF vs formate concentration, a maximum turnover frequency (TOF_{max}) of 41.49 h⁻¹ and a Michaelis constant (K_M) of 54.16 mM were calculated.

The TOF_{max} (37.24 h⁻¹) and K_M (108.37 mM) for complex [(Cp*)Rh(TfEn)Cl] (**10**) were also determined by performing a series of experiments with different concentrations of sodium formate (10, 25, 50, 100 or 500 mol equiv.), Figure 3.

3.2.2 pH dependence

The pH* dependence of the catalytic reaction (from pH* 4 to 10) was also investigated *via* a series of experiments using complex **1**, sodium formate and NAD⁺ (ratio 1:25:6, respectively) in D₂O. A dependence on pH* was observed. The highest TOF (7.5 h⁻¹) was achieved at pH* 7.3 ± 0.1, however the turnover frequencies between pH* 6 - 8 are similar. A decrease in the catalytic activity was observed when the pH* is higher than 9 (Figure 4).

3.2.3 Transfer hydrogenation reactions using NADH as a hydride donor

NADH has previously been shown to be able to act as a hydride donor [14]. As a consequence, experiments in which complex **1-3** and **10** were reacted with NADH were performed, however, no hydride transfer from NADH was observed over a period of 10 h.

3.3 Transfer hydrogenation reactions with pyruvate

Lactate formation *via* hydride transfer from formate to pyruvate catalysed by complexes **1-9** was followed by ^1H NMR. In a typical experiment, 200 μL of complex (1.4 mM, D_2O or $\text{MeOD}/\text{D}_2\text{O}$ 3:2 v/v), formate (35 mM, D_2O) and pyruvate (7 mM, D_2O) were mixed in a 5 mm NMR tube, final ratio 1:25:5, complex : formate : pyruvate. ^1H NMR spectra at 310 K were recorded every 162 s until completion of the reaction.

Molar ratios of pyruvate and lactate were determined by integrating the signals of pyruvate (3H, singlet, 2.36 ppm) and of lactate (3H, singlet, 1.32 ppm), Figure 5. The activity of the complexes towards reduction of pyruvate to lactate is dependent on the nature of both the Cp^{X} ring and the N,N' chelating ligand. Extended Cp^{XPhPh} rings result in an increase in the catalytic activity for complexes **1** and **2**. However, there was a decrease in the TOF when the N,N' -chelated ligand is 2,2'-bipyridine (bpy) or phenanthroline (phen) (Figure 6).

3.4 Competition for the reduction between NAD^+ and pyruvate.

Transfer hydrogenation reactions with complex $[(\text{Cp}^*)\text{Rh}(\text{bpy})\text{Cl}]^+$ (**4**), sodium formate, and both NAD^+ and pyruvate as hydride acceptors were performed. For these experiments, 4.5 mol equiv of NAD^+ and pyruvate were reduced using 25 mol equiv. of formate.

The reduction of both NAD^+ and pyruvate by complex **4** was observed, but the rate of reduction was slower for both substrates (Figure 7). In addition, pyruvate was reduced only when the reduction of NAD^+ was almost complete.

3.5 Cancer cell growth inhibition.

The IC_{50} values for Rh(III) complexes **1- 10** in A2780 human ovarian cancer cells were determined (0). Complexes **1-3** and **10** were inactive up to the maximum concentration tested (100 μM). Complexes **4-9** were moderately active with IC_{50} values between 14 and 65 μM .

The percentage cell survival of A2780 cells after incubation with complexes **1-10** and varying concentrations of sodium formate (0, 0.5, 1, 2 mM) was determined (Figure 8). A significant increase of the antiproliferative activity of the Rh(III) complexes upon addition of formate is evident. The largest increase was observed for complex $[(Cp^{xPhPh})Rh(en)Cl]^+$ (**3**), with a decrease in cell survival of up to 50%.

4. Discussion

Ruthenium(II), rhodium (III) and Iridium (III) half sandwich complexes have previously been reported to catalyse the reduction of NAD^+ *via* transfer hydrogenation using sodium formate as a hydride source [22,23]. In the previous reports, Rh(III) complexes generally displayed higher turnover frequencies (TOF) than Ir(III), while Ru(II) complexes have the lowest catalytic activity [16,24-27]. However, the reported catalytic reactions were usually carried out under non-physiological conditions: high temperatures, very high concentrations of formate, $pH \neq 7.4$ or with high concentrations of non-aqueous solvents [16,24-27].

The complexes selected for the present work have all been studied for the reduction of NAD^+ under non physiological conditions: $[(Cp^*)Rh(bpy)Cl]^+$ (**4**), developed by Steckhan and Fish [27,28], $[(Cp^*)Rh(phen)Cl]^+$ (**7**), published by Süss-Fink [26], $[(Cp^*)Rh(en)Cl]^+$ (**1**), rhodium analogue of $[(p-cym)Ru(en)Cl]^+$ studied in our group [16], and $[(Cp^*)Rh(TfEn)Cl]^+$ (**10**).

Noyori-type catalysts such as $[(Cp^*)Rh(TfEn)Cl]$ (**10**) are some of the more successful catalysts for transfer hydrogenation reactions [3]. Reduction of NAD^+ using catalysts has been recently studied successfully by several groups [24,29,30].

In our previous work, we observed that the nature of arene and Cp rings can have a significant effect on the catalytic properties of half-sandwich metal complexes. For example, iridium complexes containing Cp^x with extended aromatic substituents had improved

catalytic activity for the regeneration of NAD^+ [14]. With this in mind, complexes containing Cp^{xPh} and Cp^{xPhPh} rings were studied in the present work.

4.1 Hydrolysis and acidity of aqua adducts

Previous studies with related compounds have shown that the $16^{\text{e-}}$ species or the aqua adducts of the complexes are the active catalysts in transfer hydrogenation reactions [2,31]. However, in aqueous media the presence of water and pH has been shown to play a critical role [2,31]. Aquation of the complexes **1-10** in D_2O (1.4 mM) was confirmed by comparing the ^1H NMR spectra of D_2O solutions of the complexes and solutions obtained after removal of the chloride ligand by reaction with AgNO_3 to precipitate AgCl . Complexes **1-10** hydrolysed rapidly at 310 K, reaching equilibrium by the time the first ^1H NMR spectrum was recorded (< 10 min). Consequently the turnover frequency for transfer hydrogenation is not affected by the use of the chlorido complex instead of the aqua adduct.

Complete conversion of the chlorido complexes **1-3** and **10** to their aqua adducts was observed. However, complexes **4-9** reached equilibrium with 30-60% formation of the aqua species (0). This lower conversion would not be expected to affect the catalytic activity since the hydrolysis is fast, and the formation of hydrido species will shift the equilibria towards aqua adduct formation.

pH^* titrations to determine the pK_a of the aqua adducts of complexes **1-4**, **7** and **10** were carried out so that the nature of the complexes at pH 7.2 (aqua or hydroxido complexes) could be determined. The formation of hydroxido adduct would be expected to hamper the catalytic reaction since hydroxido ligands bind more tightly than the aqua ligands. The pK_a^* values of the aqua adducts determined by NMR pH^* titrations, are all > 9 (0). These complexes will therefore exist largely as the aqua complexes at pH values close to 7.4.

4.2 Transfer hydrogenation reactions with NAD^+

4.2.1 Comparison of Ru(II) and Rh(III)

First, we compared the catalytic activity for the reduction of NAD^+ by $[(\text{Cp}^*)\text{Rh}(\text{en})\text{Cl}]^+$ (**1**) and $[(\text{Cp}^*)\text{Rh}(\text{TfEn})\text{Cl}]$ (**13**) with the arene-Ru(II) analogues we studied previously $[(\text{hmb})\text{Ru}(\text{en})\text{Cl}]^+$, $[(p\text{-cym})\text{Ru}(\text{TfEn})\text{Cl}]$ and $[(\text{bn})\text{Ru}(\text{TfEn})\text{Cl}]$, (**0**) [16,24].

The catalytic activity of the Rh(III) complexes was expected to be higher than that of their Ru(II) analogues based on the literature. [16,24-27] Complex **1** was up to 8.5x times more active than $[(\text{hmb})\text{Ru}(\text{en})\text{Cl}]^+$, (**0**). However, complex **10** had TOF values in the same range as those of the Ru analogues $[(\eta^6\text{-arene})\text{Ru}(\text{TfEn})\text{Cl}]$ ($\text{TOF}_{p\text{-cym}} = 3.1 \text{ h}^{-1}$, $\text{TOF}_{\text{bn}} = 6.6 \text{ h}^{-1}$) [24].

The TOF of complex **10** was 2x times lower than complex **1** (**0**). This decrease in catalytic activity could be due to steric hindrance generated by the sulphonamide group. Despite the fact that half-sandwich complexes containing *N*-(2-aminoethyl)-sulfonamides are known to display very high catalytic activity for hydride transfer reactions [32,33] [3,34,35], our results are perhaps not surprising, since low catalytic activities have been previously reported with Noyori-type catalysts for the reduction of NAD^+ compared with other Rh(III) catalysts [30].

Next a series of ethylenediamine-Rh(III) complexes containing extended Cp^x ligands was studied. A trend was observed in which the presence of a more electron-withdrawing Cp^x ring gives higher catalytic activity (**0**). Accordingly, the complex $[(\text{Cp}^{\text{xPhPh}})\text{Rh}(\text{en})\text{Cl}]^+$ shows the highest activity followed by $[(\text{Cp}^{\text{xPh}})\text{Rh}(\text{en})\text{Cl}]^+$ and, in turn, both are more active than $[(\text{Cp}^*)\text{Rh}(\text{en})\text{Cl}]^+$. With **3**, an improvement in the TOF of 24x was achieved, compared with the ruthenium analogue $[(\text{hmb})\text{Ru}(\text{en})\text{Cl}]^+$. This increase in activity can be attributed to the effect of a less electron-rich Cp ring. The more acidic metal centre may facilitate the

coordination of negatively-charged formate, which can then undergo β -elimination to generate Rh-H and CO₂.

In order to compare the reactivity of Cp-Rh(III) with their arene-Ru(II) analogues, a set of experiments was performed using complexes **1** and **10**: dependence of the reaction rate on the NAD⁺ and formate concentrations, reaction with NADH, and optimum pH* of the reaction.

For reactions of [(Cp*)Rh(en)Cl]⁺ (**1**) and [(Cp*)Rh(TfEn)Cl] (**10**) and varying concentrations of NAD⁺, no significant alterations in the reaction rate were observed (TOF = 7.5 and 4.1 h⁻¹, respectively). The unchanged turnover frequency implies that the reaction rate does not depend on the NAD⁺ concentration. However, when the experiments were performed with increasing concentrations of the hydride source (sodium formate), the reaction rate increased, suggesting that formate is involved in the rate-determining step (Figure 3). This behaviour is similar to that of [(Cp*)Rh(bpy)Cl]⁺ previously observed by Fish *et al.* [36], with the immobilised tethered Cp*Rh complex (Rh(III)-TsDPEN) where TsDPEN is (N-[(R,2R)-2-amino-1,2-diphenylethyl]-4-toluene sulfonamide) studied by Hollmann *et al.* [30] and with [[(η^6 -arene)Ru(en)Cl]⁺ and [[(η^6 -arene)Ru(TfEn)Cl] studied in our laboratory [16,24]. Plotting the turnover frequency against formate concentration shows a typical Michaelis-Menten behaviour. The maximum turnover frequency calculated from the double reciprocal of TON vs formate concentration for complex **1** is TOF_{max} = 41.49 h⁻¹, ca. 28 x higher than that of [(hmb)Ru(en)Cl]⁺ (TOF_{max}=1.46 h⁻¹) [16].

The TOF_{max} for complex **10** was 37.2 h⁻¹, only ca. 5x higher than that of [(*p*-cym)Ru(TsEn)Cl] (TOF_{max}=6.9 h⁻¹) [24]. At high concentrations of hydride (Sodium formate), the complexes containing TfEn ligands are 5x times more active than their ruthenium analogues. However, complex [(Cp*)Rh(TsEn)Cl] (**10**) is still less active than the ethylenediamine complex [(Cp*)Rh(en)Cl]⁺ (**1**).

The Michaelis constant for complex **10** (K_M = 108.4 mM) indicates a weaker affinity for formate compared to complexes [(Cp*)Rh(en)Cl]⁺ (K_M = 41.5 mM), [(hmb)Ru(en)Cl]⁺ (K_M =

58 mM), [16] and [(*p*-cym)Ru(TsEn)Cl] (42.1 mM) [24]. Compared with [(Cp*)Rh(bpy)H₂O]²⁺ (K_M 140 mM) [36], complex **10** show stronger affinity for formate. This implies that the low catalytic activity when using 25 mol equiv. of formate is due to reduced affinity for formate, but the catalytic activity increases markedly in formate-saturated solutions.

Some iridium and ruthenium complexes such as [(Cp*)Ir(phen)Cl]⁺, [(Cp*)Ir(phenylpyridine)Cl] and [(*p*-cym)Ru(bipyrimidine)Cl] have been shown to oxidise NADH through transfer hydrogenation [13,14,17,37]. In such processes, the NADH acts as a natural hydride donor, and the metal catalyses hydride transfer from NADH to other substrates such as quinones, pyruvate or oxygen [13,14,37]. In this study, we investigated the possibility of oxidising NADH using the rhodium(III) complexes **1** and **10**. No oxidation of NADH occurred after 12 h at 310 K and pH 7.2 ± 0.2. In contrast to the above mentioned compounds, the lack of reactivity with NADH is similar to the ruthenium complex [(*p*-cym)Ru(TfEn)Cl]. These experiments emphasise the critical influence of the ligands in half-sandwich complexes on the reactivity of the complex.

The last experiment performed was to determine the optimum pH* for the reduction of NAD⁺. In line with our aim of applying the catalytic reduction of NAD⁺ in biological systems, we worked at pH 7.2. However, it is interesting to note the effect of pH on the reaction rate. Surprisingly, the maximum activity for complex **1** was observed at pH* 7.3 (Figure 3) which is close to physiological pH. Furthermore, slight variations were observed over the pH range 5 to 9. At pH* > 9, the concentration of OH⁻ inhibits the reaction due to the formation of the more inert Rh(III) hydroxido adduct. This effect of pH was also observed for the ruthenium systems [2,24].

4.2.2 Reduction of NAD⁺

In order to select the best catalyst candidate to work with, 3 well-known half sandwich Rh(III) compounds were studied for their catalytic activity towards the reduction of NAD⁺.

We also studied the effect of extended phenylation of Cp^{X} rings on their TOF. These reactions were performed in 20% methanol and 80% water to aid solubility.

As we observed previously, there is an increase on the TOF for ethylenediamine-containing-complexes when using extended rings. Interestingly, there is a 2-fold increase in the TOF due to the effect of 20% methanol. This effect was observed previously for the ruthenium(II) complexes $[(\text{arene})\text{Ru}(\text{TfEn})\text{Cl}]$ [24].

The TOF of complexes **4-9** for the reduction of NAD^+ decreases when using Cp^{xPh} or Cp^{xPhPh} as ligands (0). Those results are surprising, since the trend is opposite to that for complexes **1-3**. These results may be attributable to steric factors due to the extended Cp ring and the size of the chelating ligand (bipyridine and phenanthroline compared to ethylenediamine).

The catalytic activity for the reduction of NAD^+ is also dependent on the N,N' chelating ligand. For complexes containing Cp^* , the turnover frequencies increase in the order $\text{en} < \text{phen} < \text{bpy}$, whereas for complexes containing Cp^{xPh} or Cp^{xPhPh} , the TOFs increase in the order $\text{phen} < \text{bpy} < \text{en}$ (0). Higher activity ($\text{TOF} = 37.4 \pm 2 \text{ h}^{-1}$) was obtained with $[\text{Cp}^*\text{Rh}(\text{bpy})\text{Cl}]^+$ (**4**). However, the catalytic activity for the reduction of NAD^+ ethylenediamine complex $[(\text{Cp}^{\text{xPh}})\text{Rh}(\text{en})\text{Cl}]^+$ (**2**) is in the same range as that obtained with complex $[\text{Cp}^*\text{Rh}(\text{bpy})\text{Cl}]^+$ (**4**).

4.3 Reactions with pyruvate

We initially studied the reduction of NAD^+ , but it is well known that this type of catalyst can also be used for the reduction of other molecules. Previously, the possibility of reducing biomolecules such as pyruvate was reported, using $[(\text{Cp}^*)\text{Ir}(\text{phen})\text{Cl}]^+$ as a catalyst [14]. Therefore, we studied the catalytic reduction of pyruvate using complexes **1-9**.

Complexes **1** and **2** reduce pyruvate in the presence of sodium formate, and complete conversion to lactate was achieved. Complexes containing the Cp^* ring were less active than those containing an extended cyclopentadienyl ring (Figure 6). Similar to the reduction of

NAD⁺, when using bipyridine (**4-6**) and phenanthroline (**7-9**) as chelating ligands, the catalytic activity decreases with extended Cp rings. Interestingly, the catalytic activity of compounds **6** and **9** was extremely low, and after 8 h less than 30% conversion was achieved. The complexes containing Cp^{XPhPh} were not able to reduce pyruvate effectively before decomposition, and the reactions cannot be considered as catalytic (Figure 6).

The low catalytic activity of complexes **1-9** for the reduction of pyruvate was expected since both, pyruvate and formate, contain negatively-charged carboxylate groups which can compete with hydride for binding to Rh(III).

4.4 Competition for the reduction of NAD⁺ and pyruvate.

Competition experiments were performed using complex [(Cp*)Rh(bpy)Cl]⁺ (**4**). For these experiments, 4.5 mol equiv. of NAD⁺ and pyruvate were reduced using 25 mol equiv. of formate. The experiment showed a clear preference of complex **4** for the reduction of NAD⁺. NAD⁺ was reduced completely but at a slight lower rate than when the reaction was performed without pyruvate (Figure 7), attributable to competitive binding of formate and pyruvate.

Pyruvate was reduced only after the levels of NAD⁺ became very low. The reduction of pyruvate in the presence of NAD⁺ was extremely slow compared with the reduction of pyruvate on its own, with 50% reduction of pyruvate to lactate after 2 h 15 min.

4.5 Cancer cell growth inhibition

The antiproliferative activity of complexes **1-10** in A2780 human ovarian cancer cells was studied (0). IC₅₀ values for complexes **1-3** and **10** were higher than 100 μM (inactive), while complexes **4-9** showed moderate activity, with IC₅₀ values between 14 and 65 μM. Higher activities were obtained with the phenanthroline series (IC₅₀ (**7**) = 17.8 ± 0.6 μM, (**9**) = 14.68 ± 0.08 μM). Interestingly, in all cases lower IC₅₀ values were obtained when using the Cp^{XPhPh} capping ligand, perhaps due to an increase in hydrophobicity and increased

accumulation of the metal complex in cells [38,39], or due to the intercalation of the extended aromatic unit between nucleobases of DNA [32,33].

Cell survival with complexes **1-10** upon addition of formate at concentrations of 0.5, 1 and 2 mM was also determined (Figure 8). The complexes show enhanced activity in combination with formate. Formate alone had no effect on cell viability. It seems reasonable to propose that co-administration of the complexes with formate gives rise to catalytic transfer hydrogenation reactions in the A2780 human ovarian cancer cells, although perhaps with few turnovers in view of the variety of nucleophiles in the cell which might terminate the reactions.

In our previous work we have shown that transfer hydrogenation catalysts such as $[(\text{bip})\text{Ru}(\text{en})\text{Cl}]^+$ or $[(p\text{-cym})\text{Ru}(\text{TfEn})\text{Cl}]$ together with formate can reduce the levels of nicotinamide adenine dinucleotide (NAD^+) in cells [12]. NAD^+ is an important co-enzyme involved in maintaining the redox balance, the Kerb's cycle and other metabolic pathways such as synthesis of ADP-ribose, ADP-ribose polymers and cyclic ADP-ribose which are crucial for genome stability, DNA repair, and maintenance of calcium homeostasis [40-45]. Changes in the cellular redox status play an important role in cell death. In particular, cancer cells, might be more sensitive to redox variations, since they are under constant oxidative stress due to high production of reactive oxygen species (ROS) [40-45].

Despite the higher catalytic activity towards the reduction of NAD^+ of the Rh(III) complexes **1-10** compared with the Ru(II) complexes studied previously [16,24], the enhancement in antiproliferative activity of the Rh(III) complexes is not as high as for their Ru(II) analogues [12]. The increase in catalytic activity for the reduction of NAD^+ does not correlate with an increase in antiproliferative activity when the drugs are co-administered with sodium formate (Figure 8b). The inconsistency between the catalytic activity and effects on cells might be due to various factors. The poisoning of the catalyst [46], for example. Ward *et al* have studied the possibility of synthesizing Ir(III) Noyori-type catalysts capable of reducing

NAD^+ , quinones and ketones [29,47]. In order to reduce poisoning due to sulfur-containing molecules, such as glutathione, the compounds were conjugated to biotin-streptavidine [47]. The artificial metalloenzyme was able to carry out transfer hydrogenation reactions and reduce, to a certain extent, the effect of poisoning due to glutathione [47].

The anticancer activity of the compounds may be due in part not only to the reduction of NAD^+ but also other biomolecules. For example, in this work we have demonstrated that complexes **1-5** and **7-8** can reduce pyruvate, while compounds **6** and **9** cannot. Pyruvate is an essential component of cellular energy pathways (Krebs' cycle) and of special importance in cancer cells. It has been previously observed that cancer cells consume high levels of glucose and release lactate and carbon dioxide (Warburg effect) [48,49]. This behaviour is linked to the malfunctioning of mitochondria. Cancer cells have a high rate of consumption of glucose (glycolysis) due to the need to generate nutrients such as nucleotides and aminoacids. However, a high rate of glycolysis, requires high levels of NAD^+ . Cancer cells regenerate NAD^+ at a very fast rate by reducing pyruvate to lactate (Figure 9) [48,49].

In the presence of the Rh(III) catalysts **1-5** and **7-8**, the pool of pyruvate in cells may be reduced due to conversion to lactate. As a consequence, the regeneration of NAD^+ and the anaerobic glycolysis in cancer cells will be disrupted. The reduction of pyruvate, in combination with the reduction of NAD^+ will affect not only the redox regulatory system of the cell, but also the generation of ATP (energy) and formation of various nutrients necessary for cell growth and reproduction.

5. Conclusions

During the last decade, there has been increasing interest in developing metal-based catalysts which are effective in cells. Such technology might be useful in areas such as protein labelling, imaging, and for the treatment of diseases. For example, Chen and Meggers have shown that metal compounds such as $\text{Pd}(\text{dba})_2$ or $[\text{CpRu}(\text{QA})(\eta^3\text{-allyl})]\text{PF}_6$ (QA = 2-quinolinecarboxylate) can be used to remove protector groups from N-protected anticancer

drugs [50,51]. Cowan et al. have shown that Cu(II)/Ni(II)-ATCUN compounds can decompose RNA in hepatitis or HIV [52,53]. Also organo-ruthenium compounds can oxidise glutathione catalytically [54].

In the current work, we have studied the possibility that the Rh(III) complexes $[(Cp^x)Rh(N,N')(Cl)]$ may be more active catalysts than their Ru(II) arene analogues. Four series of compounds which can reduce NAD^+ to NADH using formate as an hydride source, have been investigated under biologically-relevant conditions (aqueous solution, pH = 7.2, 310 K). The catalytic activity decreased in the order of *N,N*-chelated ligand $bpy > phen > en$ with Cp^* as the η^5 -donor. However, while the ethylenediamine-containing compounds (**1-3**) became more active with extension to the Cp^x ring, we observed a decrease in catalytic activity for *N,N*-chelated phenanthroline- (**7-9**) and bipyridine- (**4-6**) compounds, perhaps due to an increase in steric hindrance. The complex $[Cp^*Rh(bpy)Cl]^+$ (**4**) showed the highest catalytic activity towards the reduction of NAD^+ , with a TOF of $37.4 \pm 2 \text{ h}^{-1}$.

The catalytic activity of complexes $[Cp^xRh(en)Cl]^+$ (**1-3**) (7.50, 19.65 and 24.19 h^{-1} , respectively) is up to two orders of magnitude higher than for their Ru(II) analogues (TOF $[(hmb)Ru(en)Cl]^+ = 0.85 \text{ h}^{-1}$). Interestingly, Noyori type Rh(III) compounds such as **10** with a turnover frequency of 4.12 h^{-1} , display no marked improvement compared to the Ru(II) arene analogues (c.f. 6.62 h^{-1} for $[(bn)Ru(TfEn)Cl]$).

Mechanistic studies on these Rh(III) complexes were carried out in order to investigate the catalytic cycle. Fast hydrolysis of the chlorido complexes **1-10** was observed by 1H NMR ($< 10 \text{ min}$ at 310 K). Compounds **1-3** and **10** gave quantitative conversion to aqua adducts, while complexes **4-9** (at millimolar concentrations) reached equilibrium with a 30-60% of the aqua species present. The pK_a^* values determined for the aqua adducts of *ca.* 8-10 indicate that the Rh(III) complexes are likely to be present in their aqua forms around pH 7 rather than forming less reactive hydroxido species. Complexes **1** and **10** showed a dependence of the

reaction rate on formate concentration, but not on NAD^+ concentration. No reaction between complexes **1-4** and 1,4-NADH was detected after 10 h. Optimum pH* for the reaction with complex **1** was 7.3 ± 0.1 , close to physiological pH. These results indicate that these Rh(III) Cp^x compounds exhibit similar behaviour to their Ru(II) arene analogues.

The maximum turnover frequencies of Rh(III) complexes **1** and **10** of 41.5 and 37.2 h^{-1} , respectively, at high concentrations of formate are significant improvements in catalytic activity compared to the Ru(II) analogues (6.9 h^{-1}), even for complex $[(\text{Cp}^*)\text{Rh}(\text{TfEn})\text{Cl}]$ (**10**). This improvement in the maximum turnover frequency might be expected to translate in significant enhancements in antiproliferative activity towards cancer cells when the complexes are used in combination with high concentrations of formate.

We also demonstrated that some Rh(III) complexes, notably **1-5**, **7** and **8**, can catalyse the reduction of pyruvate to lactate using formate as the hydride donor. Such reactions might occur in cells. However complexes **6** and **9** with very low TOFs are not effective catalysts. The transfer hydrogenation reactions were shown to be greatly affected by the chelating ligand and the capping Cp^x ring. The catalytic activity of ethylenediamine compounds (**1-3**) increases for extended Cp^x rings, while the bipyridine and phenanthroline compounds (**4-9**) show the opposite trend. Studies of competition reactions between NAD^+ and pyruvate for reduction by formate catalysed by complex **4** suggested a clear preference for the reduction of NAD^+ , although, some lactate was still formed.

The antiproliferative activity of the Rh(III) complexes towards A2780 human ovarian cancer cells increased by up to 50% (for complex **3**) when administered in combination with formate. However, the improvement in the activity of these Rh(III) complexes induced by formate is much lower than in the case of Ru(II) complexes. It is possible that Rh(III) centres, being more reactive than Ru(II), are more easily poisoned in the complicated mixture of biomolecules present in culture media and in cells.

Abbreviations

Cp*, pentamethylcyclopentadienyl; Cp^{xPh}, 1-phenyl-2,3,4,5-tetramethylcyclopentadienyl; Cp^{xPhPh}, 1-biphenyl-2,3,4,5-tetramethylcyclopentadienyl; *p*-cym, *p*-cymene; bn, benzene; hmb, hexamethylbenzene; bip, biphenyl; bpy, 2,2'-bipyridine; phen, phenanthroline; TOF, turnover frequency; TON, turnover number; NAD⁺, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; en, ethylenediamine; TfEn, N-(2-aminoethyl)-4-(trifluoromethyl)benzenesulfonamide; TsEn, N-(2-aminoethyl)-4-toluene sulfonamide and TsDPEN, (N-[(R,2R)-2-amino-1,2-diphenylethyl]-4-toluene sulfonamide)

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Tables

Table 1. Hydrolysis of complexes **1 – 10** and pK_a^* values for their aqua adducts.

Complex	Hydrolysis ¹ (%)	pK_a^{*2}
1	100	9.35 ± 0.06
2	100	9.48 ± 0.06
3	100	9.35 ± 0.02
4	56	8.84 ± 0.08
5	38	
6	49	
7	59	8.86 ± 0.06
8	39	
9	48	
10	100	>10

¹ In 20 % MeOD/ 80% D₂O (v/v) at 310 K; the reactions reached equilibrium by the time the first spectrum was recorded <10 min.

² in D₂O at 298 K; - not determined

Table 2. Turnover frequencies (TOF) for the reduction of NAD^+ by complexes **1-6** and **10-13**. The reactions were carried out either in D_2O or 20% MeOD/ 80% D_2O (v/v) using 6-9 mol equiv NAD^+ and 25 mol equiv. formate. pH^* was adjusted to 7.2 ± 0.2 and the spectra recorded at 310 K.

Complex	Cp^x	N,N'	TOF (h^{-1})	TOF (h^{-1})
			D_2O	MeOD/ D_2O
1	Cp^*	en	7.50 ± 0.06	17.98 ± 0.01
2	Cp^{xPh}	en	19.65 ± 0.01	35.5 ± 0.9
3	Cp^{xPhPh}	en	24 ± 1	-
4	Cp^*	bpy	-	37 ± 2
5	Cp^{xPh}	bpy	-	27.7 ± 0.7
6	Cp^{xPhPh}	phen	-	16.4 ± 0.2
7	Cp^*	phen	-	28.0 ± 0.7
8	Cp^{xPh}	phen	-	18.6 ± 0.5
9	Cp^{xPhPh}	phen	-	8.2 ± 0.3
10	Cp^*	TfEn	4.12 ± 0.01	-

Table 3. IC₅₀ values for complexes **1-10** in A2780 human ovarian cancer cells.

Complex	Cp ^x	N,N'	IC ₅₀ (μ M)
1	Cp [*]	en	>100
2	Cp ^{XPh}	en	>100
3	Cp ^{XPhPh}	en	100
4	Cp [*]	bpy	64 $\pm$ 1
5	Cp ^{XPh}	bpy	-
6	Cp ^{XPhPh}	bpy	36.07 $\pm$ 0.07
7	Cp [*]	phen	17.8 $\pm$ 0.6
8	Cp ^{XPh}	phen	57 $\pm$ 1
9	Cp ^{XPhPh}	phen	14.68 $\pm$ 0.05
10	Cp [*]	TfEn	>100

Table 4. Comparison of the catalytic cycles of ruthenium and rhodium complexes.

	[(p-cym)Ru(TsEn)Cl]	[(Cp*)Rh(TfEn)Cl] (10)	[(hmb)Ru(en)Cl] ⁺	[(Cp*)Rh(en)Cl] (1)
Hydrolysis	Very fast ^a	Very fast	Moderate ^b	Very fast
% aqua adduct (24 h)	100 ^a	100	32 ^b	100
pKa	9.71 ^a	>10	ca. 8 ^c	9.31
TOF (h ⁻¹)	1.58 ^a	4.12 ± 0.01	0.85 ^d	7.50 ± 0.06
[NAD ⁺] dependence	No ^a	No	No ^d	No
TOF _{max} (h ⁻¹)	6.4 ^a	37.2	1.46 ^d	41.5
K _M (mM)	27.8 ^a	108.4	58 ^d	41.5
Optimum pH*	7.2 ± 1 ^a	ND	ND	7.3 ± 0.01
Reaction with 1,4 NADH	No ^e	No	ND	No

^a ref [12], ^b ref [55], ^c ref [56], ^d ref [16]

Figures

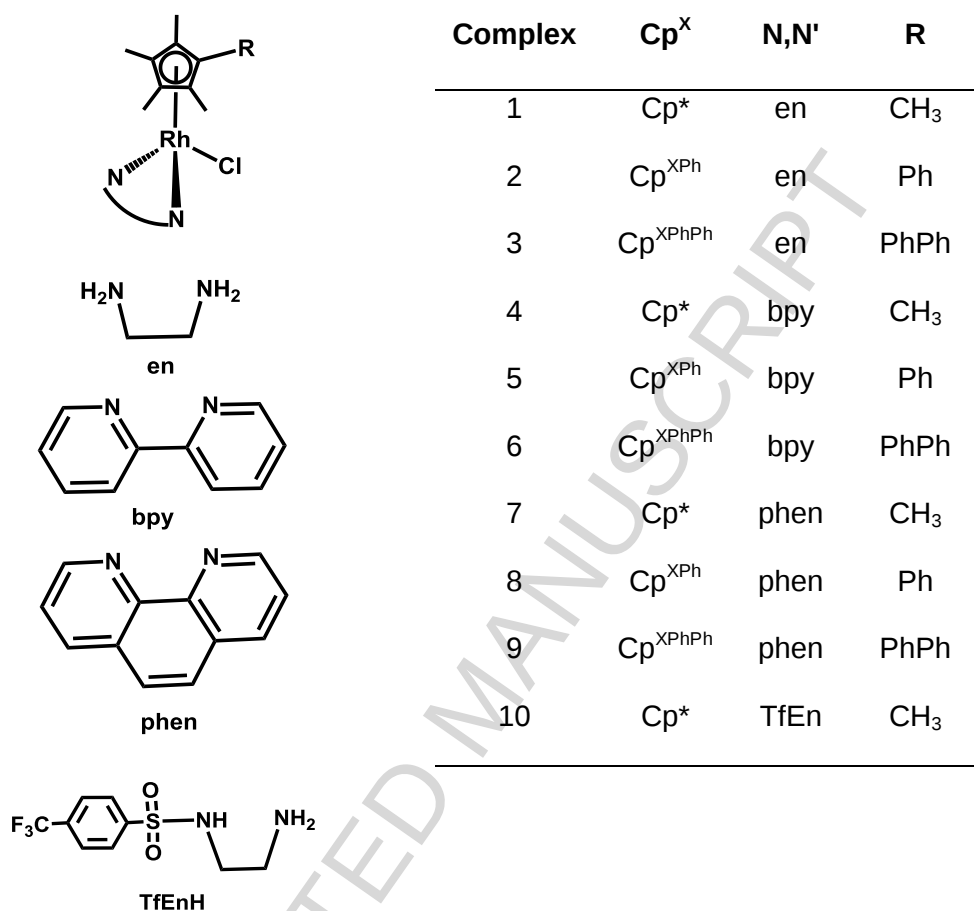
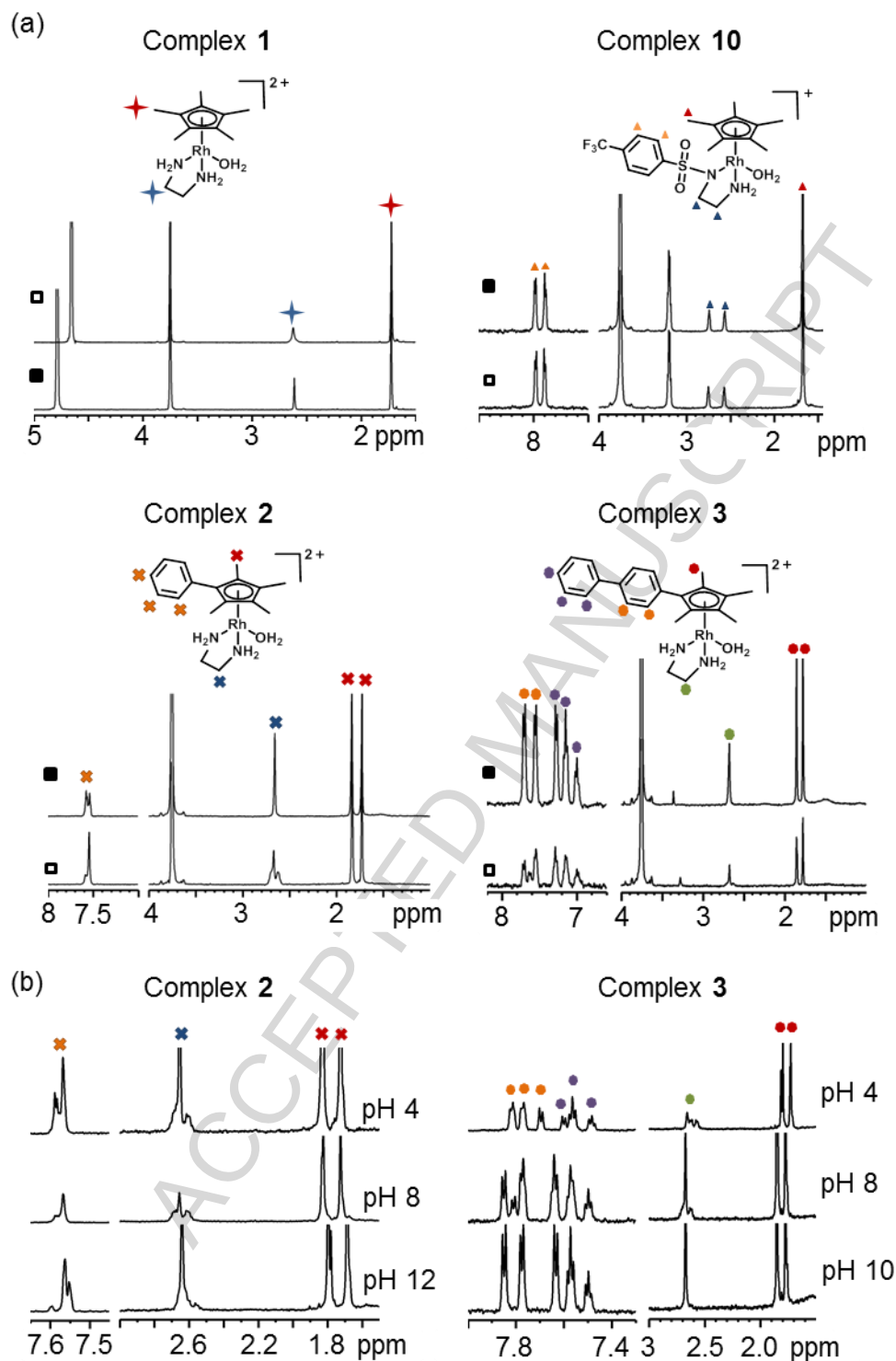


Figure 1. Rh(III) complexes studied in this work, for the reduction of NAD⁺ by transfer hydrogenation using sodium formate as a hydride source.



^1H NMR spectra of complexes **1-3** and **10** in D_2O (■) and their aqua adducts generated by reaction of complexes **1-3** and **10** with silver nitrate (●), (a). The NMR spectra for complexes **2** and **3** display different pattern for the compound and the compound in presence of silver nitrate, however this is due to a change of the pH. ^1H NMR of **2** and **3** at different pH, (b).

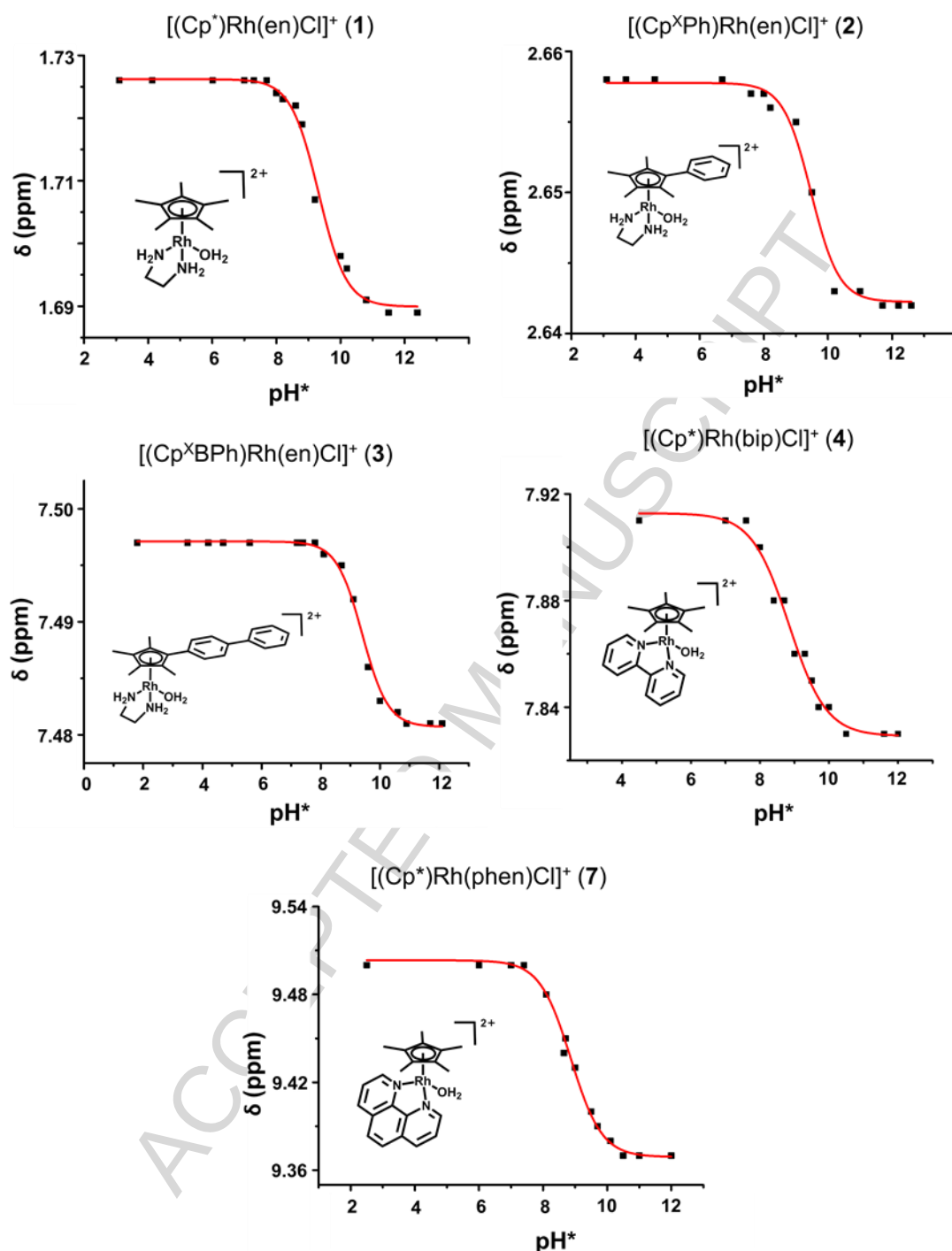


Figure 2. Dependence of ^1H NMR chemical shifts of complexes 1-4 and 6 on pH^* . The red lines represent computer fits to the Henderson-Hasselbalch equation with the pK_a values in Table 1.

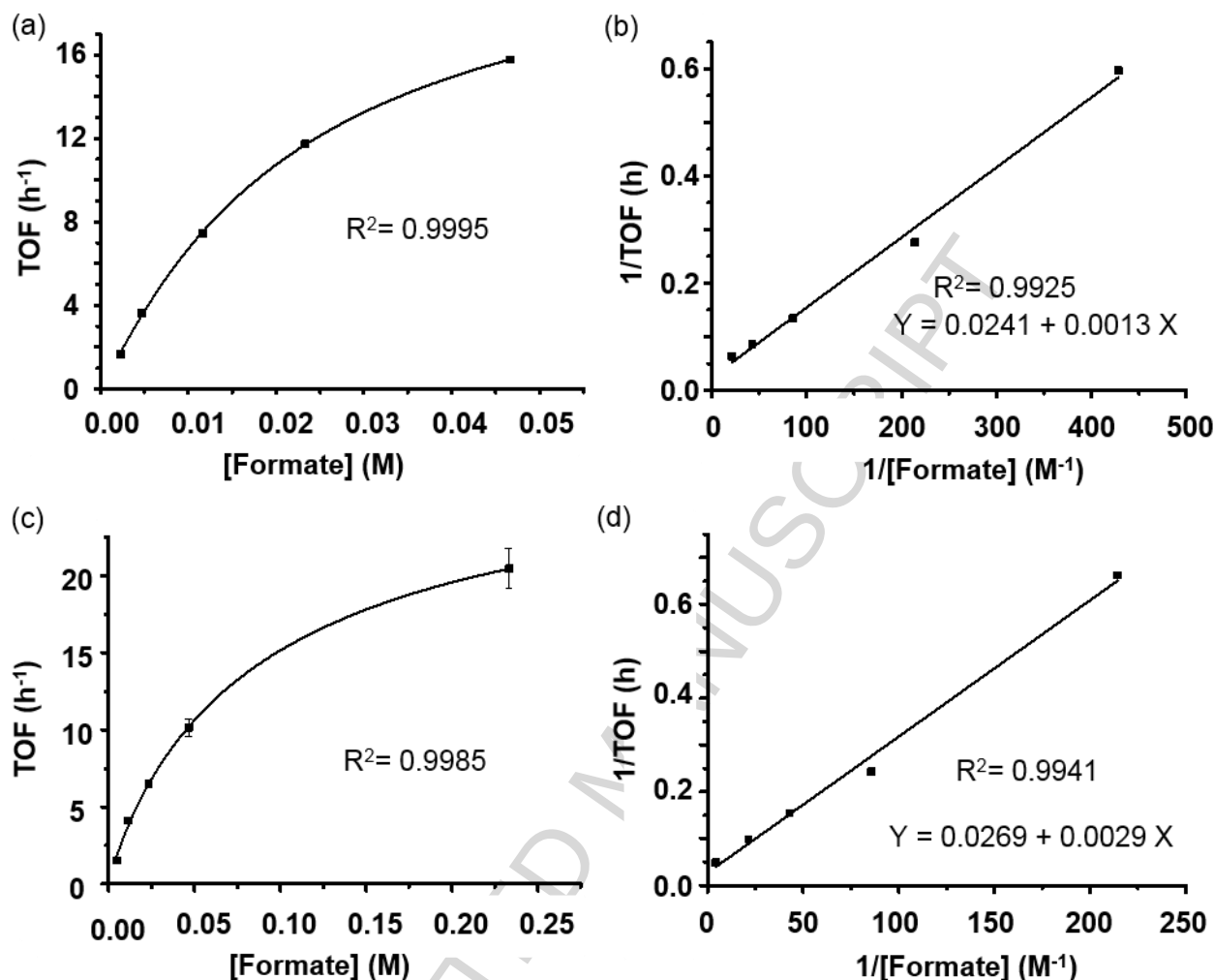


Figure 3. (a) Plot of the TOF versus formate concentration (5, 10, 25, 50 or 100 mol equiv), for the reduction of NAD⁺ catalyzed by complex [(Cp*)Rh(en)Cl]⁺ (**1**) at pH* 7.2 and 310 K. A typical Michaelis-Menten behaviour is observed. (b) Plot of the reciprocal of the TOF against formate concentration, for catalysis by complex **1**. For Michaelis-type kinetics, $\text{TOF}^{-1} = (K_M / \text{TOF}_{\text{max}})(1/[S]) + (1/\text{TOF}_{\text{max}})$. Michaelis constant ($K_M = 54.16 \text{ mM}$) and the maximum turnover frequency ($\text{TOF}_{\text{max}} = 41.49 \text{ h}^{-1}$) were determined. (c) Plot of the TOF against the formate concentration (5, 10, 25, 50 or 100 mol equiv), $\text{TOF} = \text{TOF}_{\text{max}} [S]/(K_M + [S])$ by complex [(Cp*)Rh(TfEn)Cl]⁺ (**10**) at pH* 7.2 and temperature 310 K. (d) Plot of the reciprocal of the TOF against formate concentration, for catalysis by complex **10**. For Michaelis-type kinetics, $\text{TOF}^{-1} = (K_M / \text{TOF}_{\text{max}})(1/[S]) + (1/\text{TOF}_{\text{max}})$. Michaelis constant ($K_M = 108.37 \text{ mM}$) and the maximum turnover frequency ($\text{TOF}_{\text{max}} = 37.24 \text{ h}^{-1}$) were determined.

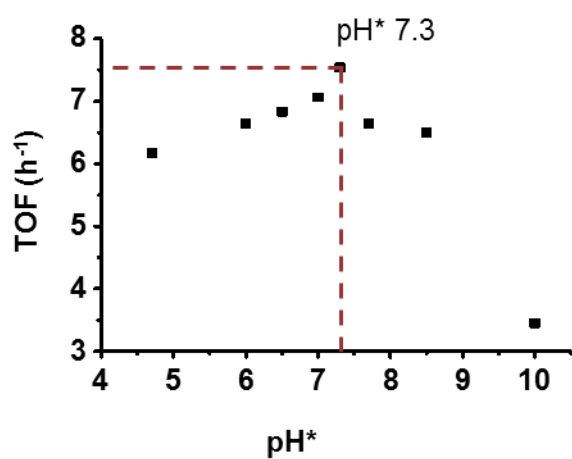


Figure 4. Dependence of turnover frequency on pH* for the reduction of NAD⁺ by complex 1 using formate as a hydride source (mol. ratio 5:1:25, respectively, 310 K in D₂O). Maximum turnover frequency achieved at pH* 7.3 ± 0.1.

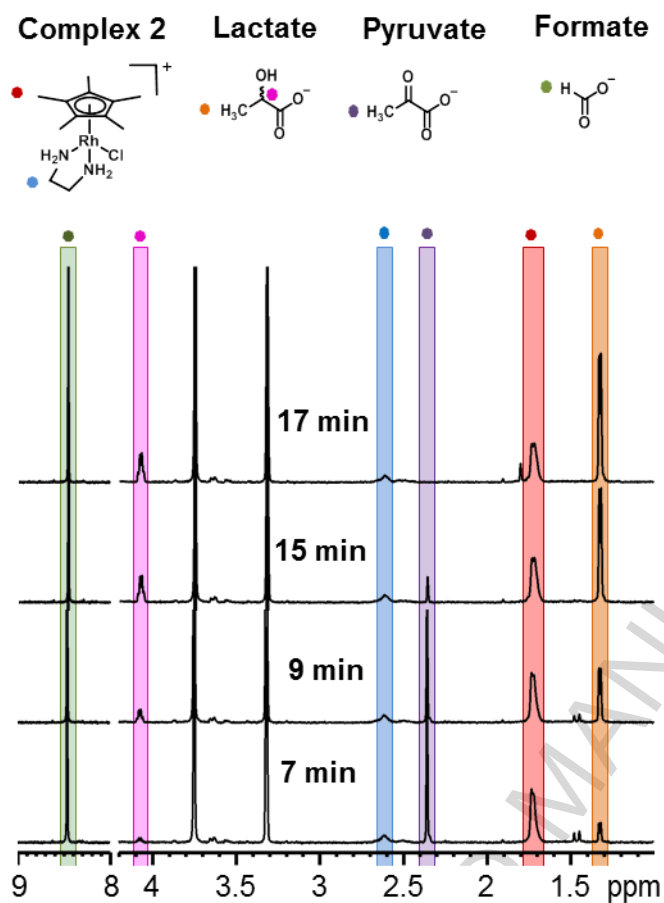


Figure 5. ^1H NMR spectra for the reaction of complex 2, formate and pyruvate in molar ratios of 1:25:6, respectively, in 20% MeOD / 80% D₂O (v/v) at 310K and pH* 7.2 ± 0.2.

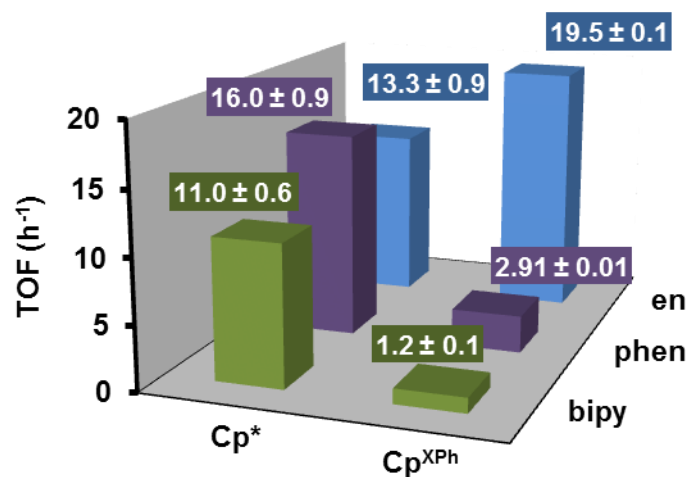


Figure 6. Dependence of the turnover frequencies for the reduction of pyruvate to lactate on the Cp^x ring substituent (Ph) and chelated ligand (bipy, phen or en) for complexes **1**, **2**, **4**, **5**, **7** and **8**. The reactions were carried out in 20% MeOD/ 80% D₂O (v/v) using 6-9 mol equiv. pyruvate and 25 mol equiv. formate. The pH* was adjusted to 7.2 ± 0.2 and the NMR spectra recorded at 310 K.

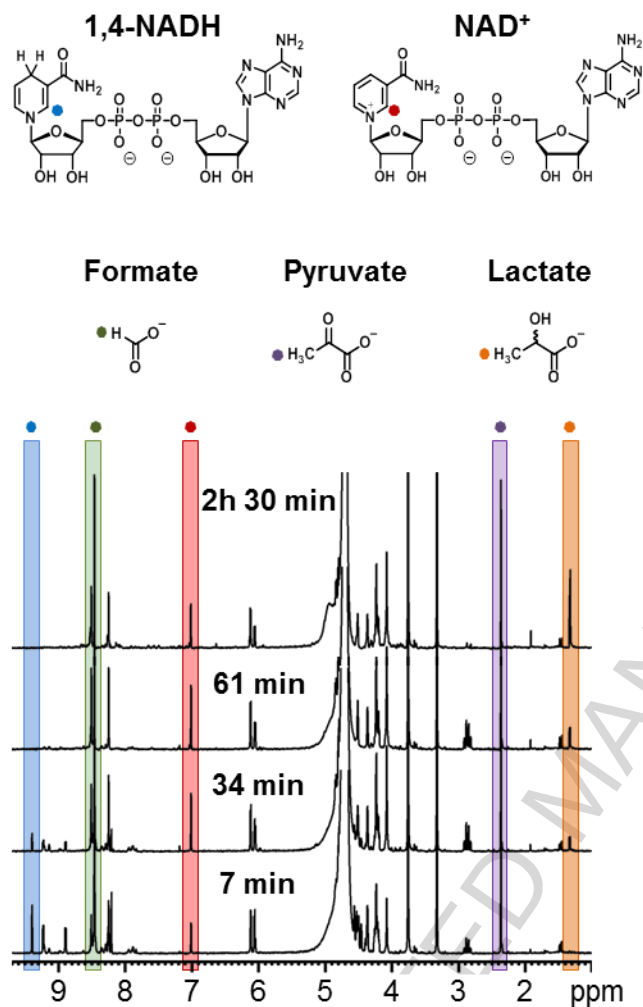
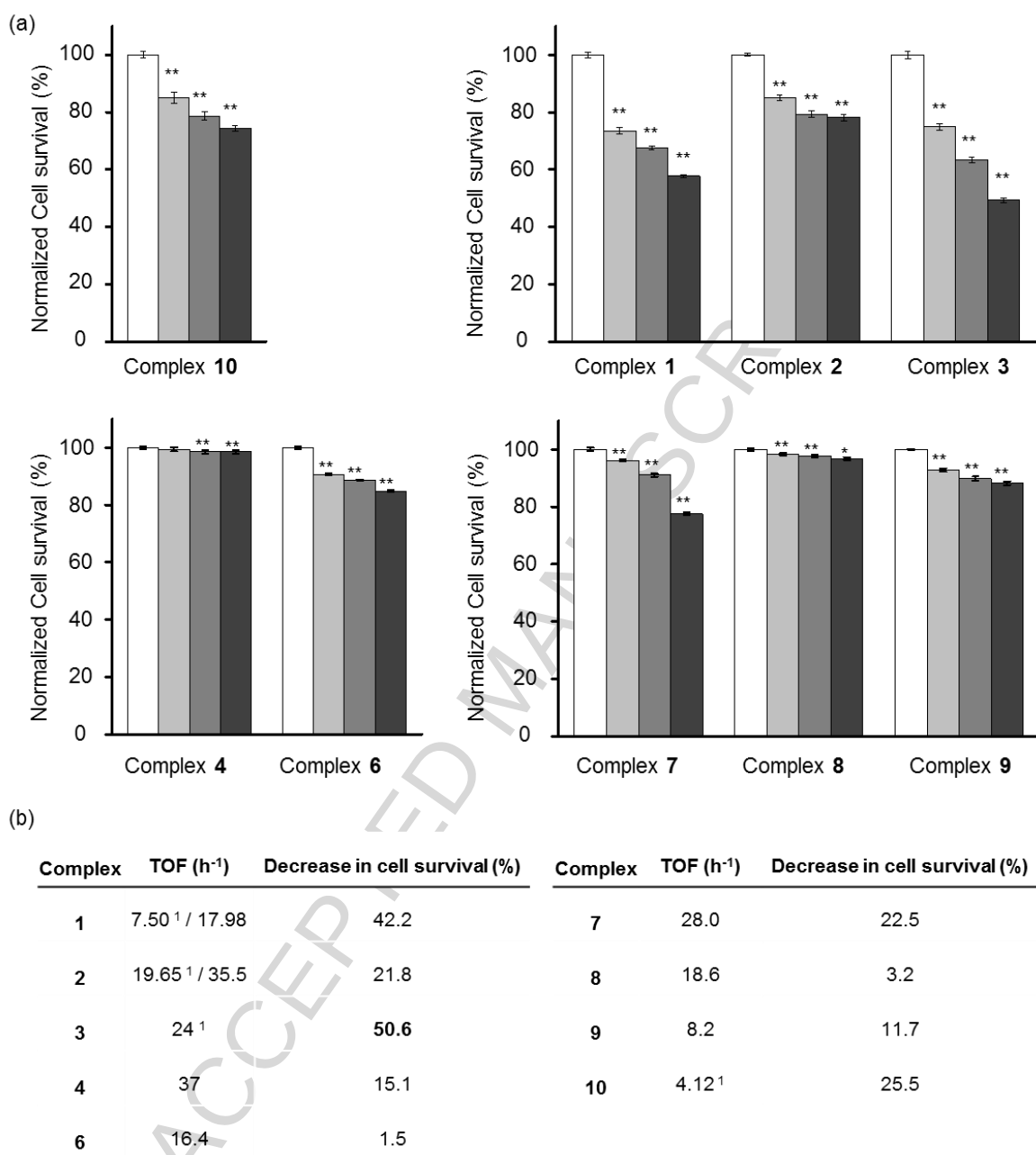


Figure 7. ¹H NMR spectra for the reaction of complex 4, formate and NAD⁺ and pyruvate in molar ratios of 1:25:4.5:4.5, respectively, in 20% MeOD / 80% D₂O (v/v) at 310K and pH* 7.2 ± 0.2.



¹ TOF measured in D₂O

Figure 8. (a) Survival of A2780 human ovarian cancer cells when treated with complexes **1-4** and **6-10** (concentration = 1/3 IC₅₀ for **4-9**, 150 μM for **1-3** and **10**) and varying concentrations of sodium formate (key to shading of bars: 0 (white), 0.5, 1 or 2 (black) mM). * $P < 0.05$, ** $P < 0.01$. Cell survival was normalized to the % survival of cells treated only with Rh(III) complex. (b) Table showing the % decrease in cell survival and the TOF for the reduction of NAD⁺ (in 20 % MeOD/ 80 % D₂O, pH 7.2 $\pm$ 0.2, 310 K) for complexes **1-4** and **6-10**.

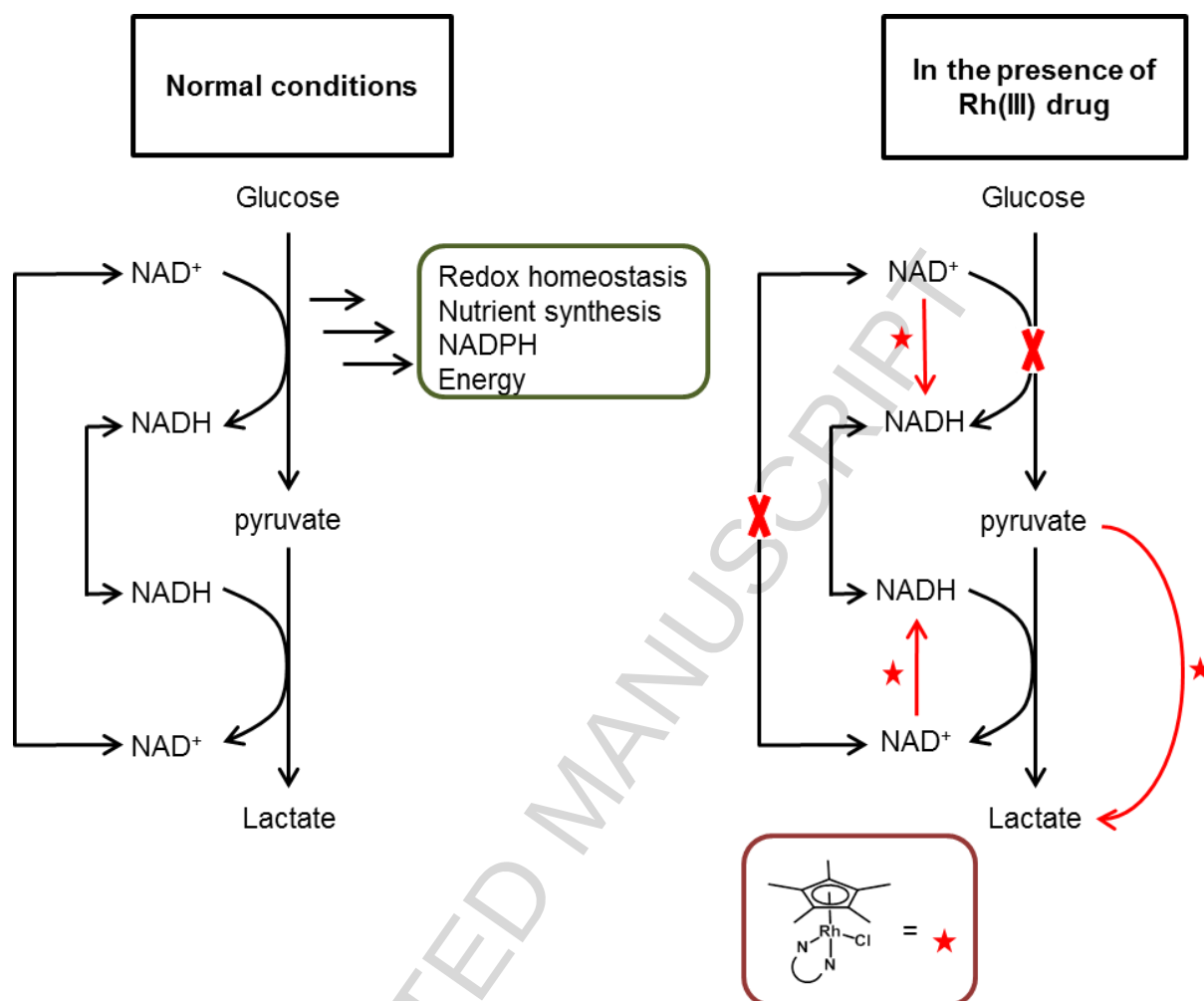


Figure 9. Anaerobic glycolysis and role of pyruvate in cancer cells under normal conditions (left) and possible effects of due to the presence of Rh(II) complexes co-administered with sodium formate.

Highlights

- We prepared half-sandwich (Cp^x)Rh complexes; Cp^x is pentamethylcyclopentadienyl or related ligands
- These Rh(III) catalysts can reduce coenzyme NAD^+ to NADH
- Catalytic activity of $[(\text{Cp}^x)\text{Rh}(N,N')(\text{Cl})]$ depends on Cp^x and N,N-chelated ligands
- Complexes hydrolyse rapidly (min) and can reduce pyruvate to lactate
- Formate increases activity towards human ovarian cancer cells by up to 50%