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Octahedral Co(III) Salen Complexes: The Role of Peripheral Ligand Electronics on Axial Ligand Release upon Reduction

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Draft

Abstract:

A series of octahedral Co^{III} salen complexes (where salen represents a N_2O_2 bis-Schiff-base bis-phenolate framework) were prepared with axial imidazole ligating groups. When using 1-methylimidazole (1-MeIm) axial ligands, the $\text{Co}^{\text{III}} / \text{Co}^{\text{II}}$ reduction potential could be altered by 220 mV via variation of the electron-donating ability of the *para*-ring substituents ($\text{R} = \text{H}$ (**1**), OMe (**2**), tBu (**3**), Br (**4**), NO_2 (**5**), and CF_3 (**6**)). In addition, the irreversibility of the reduction process suggested substantial geometrical changes and axial ligand exchange upon reduction to the more labile Co^{II} oxidation state. Installing an imidazole-coumarin conjugate as the axial ligands resulted in fluorescence quenching when bound to the Co^{III} centre ($\text{R} = \text{H}$ (**7**), OMe (**8**), and CF_3 (**9**)). The redox properties and fluorescence increase upon ligand release for **7-9** were studied under reducing conditions, and in the presence of excess competing ligand (1-MeIm). It was determined that the Lewis acidity of the Co^{III} centre was the dominant factor in controlling axial ligand exchange for this series of complexes.

1. Introduction:

There is an increasing interest in metal-based drugs for diagnostic and therapeutic applications, due to the need to increase both potency and selectivity.¹⁻⁵ The ability to vary the coordination number, geometry, redox states, and overall charge provide major design advantages to metal-based over organic-based drugs. In addition, the ligand(s) play a key role in determining the pharmacokinetic properties of metal-containing drugs, allowing for tuning of the complex to increase efficacy.⁶ Metal complexes have been extensively studied as anticancer agents, with the development of Cisplatin playing an instrumental role in bringing medicinal inorganic chemistry into the mainstream.⁷ In certain cases, the ligand(s) can also be active drugs, and complexation to a metal complex can provide a means for selective delivery and/or activation.⁸ Metal complexes are well-suited as tumour-selective drug delivery agents, and can be activated by environmental factors such as pH,⁹⁻¹⁰ redox,¹¹ and light.¹²⁻¹³ The work herein focuses on the utilization of redox changes for metal complex activation.

A number of transition metal ions exhibit changes in geometrical preference and/or lability in different oxidation states, and these features have been exploited in the design of metal complexes that undergo selective activation in environments with different redox states. In the case of cancer, solid tumours exhibit a reduced O₂ supply, which in combination with elevated levels of reducing agents and reductase enzymes results in a hypoxic microenvironment.¹⁴⁻¹⁶ A number of metal-based pro-drugs have been developed to target hypoxic tumour tissues, including complexes of Pt^{IV}, Ru^{III}, and Co^{III} (**Figure 1**).^{8,11,17} Reduction of the Pt^{IV} complex Satraplatin (**Figure 1A**) produces the activated Pt^{II} species. Reduction of the Ru^{III} complex NKP1339 (**Figure 1B**)¹⁸ to Ru^{II} is hypothesized as one mode of activation for this important class of anticancer agents,¹⁹ however, recent studies suggest that the Ru^{III} oxidation

state may be predominant *in vivo*.²⁰⁻²¹ Both Satraplatin and NKP1339 have entered clinical trials. The Co^{III} complex of a nitrogen mustard (**Figure 1C**) exhibited a 20-fold increase in cytotoxicity under hypoxic conditions;²² Co^{III} is kinetically inert whereas Co^{II} is quite labile and undergoes rapid ligand exchange with water.²³

Figure 1. Representative redox-activated anticancer drugs including (A) the Pt^{IV} complex Satraplatin, (B) the Ru^{III} complex NKP1339, (C) a Co^{III}-nitrogen mustard conjugate, and (D) Co^{III} salen complexes investigated in this work, where R = H, OMe, tBu, Br, NO₂, or CF₃, and L are imidazole ligands.

Bioreductive Co^{III} pro-drugs of a number of antitumour agents have been developed, including nitrogen mustards,²² matrix metalloproteinase inhibitors,²⁴ and curcumin.²⁵ A key feature of these complexes, and other bioreductive pro-drugs, is the ability to tune the reduction potential so that activation occurs selectively under hypoxic conditions in a tumour.²⁶ The approximate cellular reduction potential is estimated to be between -420 to -150 mV (vs. NHE),²⁷ and the reduction potential of a tumour is usually at the lower end of this range.²⁸ Previously, we showed that it is possible to tune the reduction potential of metal salen (salen represents an N₂O₂ bis-Schiff-base bis-phenolate framework., **Figure 1D**) complexes over a large range via alteration of the *para*-ring substituents,²⁹⁻³² and we have now extended this work to a series of octahedral Co^{III} salen complexes (**Figure 1D**). It was hypothesized that bioreduction to Co^{II} would lead to significant geometrical rearrangement and axial ligand exchange (loss of L in **Figure 1D**), thus providing a selective release mechanism for cytotoxic ligands in the axial position. In addition, a number of reports have demonstrated the cytotoxicity of Co^{II} salen complexes, a product of the reduction process.³³⁻³⁴ Herein, we detail the synthesis and electrochemical properties of a series of octahedral Co^{III} salen complexes, and preliminary

studies on axial ligand exchange under reducing conditions.

2. Experimental:

2.1 General Considerations: All reagents and solvents used for synthesis were from commercial sources and used as received. Literature methods were used or adapted to prepare the salen ($\text{H}_2\text{Sal}^{\text{R}}$) ligands ($\text{R} = \text{H}, \text{OMe}, ^t\text{Bu}, \text{H}, \text{Br}, \text{CF}_3, \text{NO}_2$)^{31,35-36} and $\text{Co}^{\text{II}}(\text{Sal}^{\text{R}})$ complexes ($\text{R} = \text{H}, \text{OMe}, ^t\text{Bu}, \text{Br}, \text{CF}_3, \text{NO}_2$).³¹ 4-((1*H*-imidazol-1-yl) methyl)-7-methoxy-2*H*-chromen-2-one (hereafter named Coumarin) was prepared according to a literature method.³⁷ **Perchlorate salts are potentially explosive and in this work only small quantities were used in all syntheses.** ¹H NMR spectra were recorded on Bruker AV-400 or AV-500 instruments. Mass spectra (positive ion) were obtained on an Agilent 6210 TOF ESI-MS instrument. Elemental analyses (C, H, N) were performed by Mr. Paul Mulyk at Simon Fraser University on a Carlo Erba EA1110 CHN elemental analyzer. Cyclic voltammetry (CV) was performed on a PAR-263A potentiometer, equipped with a glassy carbon working electrode and a platinum counter electrode, and a BASi MF-2062 non-aqueous electrode kit was used to make a silver/silver nitrate reference electrode with $^n\text{Bu}_4\text{NClO}_4$ (0.1 M) solutions in DMF. Ferrocene was used as an internal standard. Absorption spectra were collected using a Varian CARY 5000 UV-Visible-Near IR spectrophotometer. Measurements were performed in single-beam mode with baseline correction using the same quartz cuvette (1 cm path length) in all experiments. The excitation and emission spectra were collected using a Photon Technology International (PTI) 814 photomultiplier detection system. Signals were collected at 90° to the incident excitation beam. The maximum emission of the Coumarin solution was used as a reference. The photophysical data were processed with the Felix32 system. The kinetic studies of Coumarin ligand release

over 12 h were performed at 25 °C under constant agitation, and each well in the 96-well plate was excited at 327 nm and emission was collected at 400 nm using a Synergy 4 fluorescence plate reader from BioTek. Each condition was measured in triplicate.

2.2 Synthesis:

[Co^{III}(Sal^H)(1-MeIm)₂](ClO₄) (**1**): To Co^{II}(Sal^H) (0.1 g, 0.3 mmol) in a MeOH/DCM solution (25/25 mL) was added 3 eq. of 1-methylimidazole (1-MeIm) (0.076 g, 0.920 mmol). The solution was stirred at 298 K for 2 h and turned a dark red colour. NaClO₄ (0.23 g, 1.80 mmol) in MeOH (2 mL) was then added, and stirring in the air at 298 K was continued for 1 day. Slow evaporation of the solvent afforded dark red crystals after 1 day that were collected by filtration, washed with cold MeOH, and dried in *vacuo*. Yield: 0.14 g, 79%. ¹H NMR (400 MHz, MeOD) δ 8.20 (s, 2H), 7.46 (s, 2H), 7.32 – 7.25 (m, 4H), 7.18 (d, *J* = 8.3 Hz, 2H), 6.94 (t, *J* = 1.6 Hz, 2H), 6.69 (t, *J* = 1.5 Hz, 2H), 6.63 – 6.57 (m, 2H), 4.06 (s, 4H), 3.60 (s, 6H). ¹³C NMR (101 MHz, MeOD) δ 168.71, 164.95, 138.94, 135.24, 134.51, 127.65, 122.77, 118.35, 115.21, 56.97, 33.61. ESI(+)-MS *m/z* (relative intensity) = 489.15 ([Co^{III}(Sal^H)(1-MeIm)₂]⁺). Elemental analysis calculated for [Co^{III}(Sal^H)(1-MeIm)₂](ClO₄) (C₂₄H₂₆N₆ClO₆Co) C: 48.95%, H: 4.45%, N: 14.27%, Found C: 48.71%, H: 4.65%, N: 14.11%.

[Co^{III}(Sal^{OMe})(1-MeIm)₂](ClO₄) (**2**): This complex was synthesized following the same procedure as for compound **1**, combining Co^{II}(Sal^{OMe}) (0.100 g, 0.260 mmol), 1-MeIm (0.064 g, 0.780 mmol) and NaClO₄ (0.19 g, 1.56 mmol). X-ray quality crystals were isolated by slow evaporation of a MeOH solution. Yield: 0.163 g, 97%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.18 (s, 2H), 7.54 (t, *J* = 1.5 Hz, 2H), 7.08 (t, *J* = 1.6 Hz, 2H), 6.99 (d, *J* = 9.1 Hz, 2H), 6.93 (dd, *J* = 9.2,

3.1 Hz, 2H), 6.79 (d, $J = 3.1$ Hz, 2H), 6.64 (t, $J = 1.5$ Hz, 2H), 4.00 (s, 4H), 3.65 (s, 6H), 3.57 (s, 6H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 167.90, 160.42, 148.20, 138.67, 127.42, 124.81, 123.02, 121.97, 116.30, 114.66, 56.85, 55.45, 34.47. Elemental analysis calculated for $[\text{Co}^{\text{III}}(\text{Sal}^{\text{OMe}})(1\text{-MeIm})_2]\text{ClO}_4$ ($\text{C}_{26}\text{H}_{30}\text{N}_6\text{ClO}_8\text{Co}$) C: 48.12%, H: 4.66%, N: 12.95%, Found C: 48.30%, H: 4.96%, N: 12.76%.

$[\text{Co}^{\text{III}}(\text{Sal}^{\text{tBu}})(1\text{-MeIm})_2]\text{ClO}_4$ (**3**): This complex was synthesized following the same procedure as for compound **1**, combining $\text{Co}^{\text{II}}(\text{Sal}^{\text{tBu}})$ (0.063 g, 0.145 mmol), 1-MeIm (0.036 g, 0.435 mmol) and NaClO_4 (0.100 g, 0.869 mmol). Yield: 0.06 g, 90%. ^1H NMR (500 MHz, DMSO- d_6) δ 8.21 (s, 2H), 7.57 (d, $J = 1.5$ Hz, 2H), 7.30 (dd, $J = 8.9, 2.7$ Hz, 2H), 7.19 (d, $J = 2.7$ Hz, 2H), 7.07 (t, $J = 1.6$ Hz, 2H), 7.01 (d, $J = 8.9$ Hz, 2H), 6.66 (t, $J = 1.5$ Hz, 2H), 4.04 (s, 4H), 3.57 (s, 6H), 1.21 (s, 18H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 168.60, 163.44, 138.63, 135.75, 132.94, 129.93, 127.42, 122.04, 121.98, 116.66, 56.73, 34.51, 33.29, 31.14. ESI(+)-MS m/z (relative intensity) = 601.27 ($[\text{Co}^{\text{III}}(\text{Sal}^{\text{tBu}})(1\text{-MeIm})_2]^+$). Elemental analysis calculated for $[\text{Co}^{\text{III}}(\text{Sal}^{\text{tBu}})(1\text{-MeIm})_2]\text{ClO}_4 \cdot 2\text{H}_2\text{O} \cdot 2\text{CH}_2\text{Cl}_2$ ($\text{C}_{34}\text{H}_{50}\text{N}_6\text{Cl}_5\text{O}_8\text{Co}$) C: 45.02%, H: 5.56%, N: 9.27%, Found C: 44.43%, H: 5.32%, N: 9.74%.

$[\text{Co}^{\text{III}}(\text{Sal}^{\text{Br}})(1\text{-MeIm})_2]\text{ClO}_4$ (**4**): This complex was synthesized following the same procedure as for compound **1**, combining $\text{Co}^{\text{II}}(\text{Sal}^{\text{Br}})$ (0.100 g, 0.207 mmol), 1-MeIm (0.050 g, 0.621 mmol) and NaClO_4 (0.15 g, 1.24 mmol). Yield: 0.12 g, 78%. ^1H NMR (500 MHz, DMSO- d_6) δ 8.19 (s, 2H), 7.56 (s, 2H), 7.46 (d, $J = 2.7$ Hz, 2H), 7.27 (dd, $J = 9.1, 2.7$ Hz, 2H), 7.09 (d, $J = 1.6$ Hz, 2H), 6.98 (d, $J = 9.1$ Hz, 2H), 6.61 (d, $J = 1.5$ Hz, 2H), 3.99 (s, 4H), 3.56 (s, 6H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 168.05, 164.37, 138.86, 137.02, 135.83, 127.31, 124.57, 122.30, 119.64,

104.17, 57.06, 34.56. ESI(+)-MS m/z (relative intensity) = 646.97 ($[\text{Co}^{\text{III}}(\text{Sal}^{\text{Br}})(1\text{-MeIm})_2]^+$). $[\text{Co}^{\text{III}}(\text{Sal}^{\text{Br}})(1\text{-MeIm})_2]\text{ClO}_4 \cdot 0.5\text{CH}_2\text{Cl}_2$ ($\text{C}_{24.5}\text{H}_{25}\text{N}_6\text{Cl}_2\text{O}_6\text{CoBr}_2$) C: 37.29%, H: 3.19%, N: 10.65%, Found C: 37.16%, H: 3.59%, N: 10.65%.

$[\text{Co}^{\text{III}}(\text{Sal}^{\text{NO}_2})(1\text{-MeIm})_2]\text{ClO}_4$ (**5**): This complex was synthesized following the same procedure as for compound **1**, combining $\text{Co}^{\text{II}}(\text{Sal}^{\text{NO}_2})$ (0.100 g, 0.240 mmol), 1-MeIm (0.060 g, 0.722 mmol) and NaClO_4 (0.176 g, 1.440 mmol). Yield: 0.11 g, 79%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.48 (s, 2H), 8.46 (d, $J = 2.7$ Hz, 2H), 8.07 (dd, $J = 9.3, 2.8$ Hz, 2H), 7.68 (s, 2H), 7.23 – 7.14 (m, 4H), 6.67 (s, 2H), 4.12 (s, 4H), 3.59 (s, 6H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 170.58, 169.56, 139.14, 135.62, 132.54, 128.91, 127.12, 122.89, 122.77, 117.59, 57.41, 34.67. ESI(+)-MS m/z (relative intensity) = 579.11 ($[\text{Co}^{\text{III}}(\text{salen}^{\text{NO}_2})(1\text{-MeIm})_2]^+$). Elemental analysis calculated for $[\text{Co}^{\text{III}}(\text{Sal}^{\text{NO}_2})(1\text{-MeIm})_2]\text{ClO}_4$ ($\text{C}_{24}\text{H}_{24}\text{N}_8\text{ClO}_{10}\text{Co}$) C: 42.46%, H: 3.56%, N: 16.51%, Found C: 42.87%, H: 3.68%, N: 16.04%.

$[\text{Co}^{\text{III}}(\text{Sal}^{\text{CF}_3})(1\text{-MeIm})_2]\text{ClO}_4$ (**6**): To a stirring solution of $\text{H}_2\text{Sal}^{\text{CF}_3}$ (0.03 g, 0.0742 mmol) (in Et_2O) under N_2 , $\text{Co}(\text{OAc})_2 \cdot 4\text{H}_2\text{O}$ (0.018 g, 0.0742 mmol) in MeOH was added. The mixture was stirred for 20 min and then 3 eq. of 1-MeIm (0.018 g, 0.223 mmol) and NaClO_4 (0.176 g, 1.440 mmol) were added to the reaction. The solution was then stirred under an aerobic atmosphere overnight and then concentrated under low pressure causing a precipitate to form. A dark red powder was collected by filtration, washed with cold methanol, and dried *in vacuo*. Yield: 0.04 g, 71%. X-ray quality crystals were isolated by slow evaporation from MeOH. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.38 (s, 2H), 7.75 (d, $J = 2.4$ Hz, 2H), 7.64 (s, 2H), 7.48 (dd, $J = 9.1, 2.6$ Hz, 2H), 7.19 (d, $J = 8.9$ Hz, 2H), 7.14 (t, $J = 1.6$ Hz, 2H), 6.66 (t, $J = 1.6$ Hz, 2H), 4.07 (s, 4H), 3.59

(s, 6H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 169.47, 168.19, 139.45, 130.93, 127.73, 126.50, 123.69, 122.98, 118.05, 115.24, 114.91, 57.62, 35.08. ^{19}F NMR (500 MHz, CDCl_3) δ -59.50 (s). ESI(+)-MS m/z (relative intensity) = 625.14 ($[\text{Co}^{\text{III}}(\text{Sal}^{\text{CF}_3})(1\text{-MeIm})_2]^+$). Elemental analysis calculated for $[\text{Co}^{\text{III}}(\text{Sal}^{\text{CF}_3})(1\text{-MeIm})_2]\text{ClO}_4 \cdot 2\text{H}_2\text{O}$ ($\text{C}_{26}\text{H}_{28}\text{N}_6\text{ClO}_8\text{Co}$) C: 41.04%, H: 3.71%, N: 11.04%, Found C: 40.60%, H: 3.37%, N: 11.47%.

$[\text{Co}^{\text{III}}(\text{Sal}^{\text{H}})(\text{Coumarin})_2]\text{Cl}$ (7): Three drops of NEt_3 was added to a solution of $\text{H}_2\text{Sal}^{\text{H}}$ (0.123 g, 0.378 mmol in DCM) in a round-bottom flask under N_2 . CoCl_2 (0.050 g, 0.385 mmol in MeOH) was added into the flask, which was then purged with N_2 for 20 min. Then 4-((1*H*-imidazol-1-yl)methyl)-7-methoxy-2*H*-chromen-2-one (Coumarin)³⁷ (0.213 g, 0.831 mmol) was added to the reaction. The solution was stirred in air overnight. The solvent was then removed *in vacuo*, and a brown solid was obtained. The solid was washed with EtOAc and filtered, and then the crude product was further purified by size exclusion chromatography using a Sephadex[®] column (LH-20; MeOH eluent). Yield: 0.28 g, 83%. ^1H NMR (400 MHz, MeOD) δ 8.21 (s, 1H), 7.74 (t, J = 1.5 Hz, 1H), 7.42 (d, J = 8.8 Hz, 1H), 7.27-7.18 (m, 2H), 7.14-7.08 (m, 2H), 6.85 (d, J = 2.5 Hz, 1H), 6.83-6.78 (m, 2H), 6.58-6.53 (m, 1H), 5.40 (d, J = 1.4 Hz, 2H), 5.27 (d, J = 1.3 Hz, 1H), 4.08 (s, 2H), 3.85 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 170.42, 166.26, 164.89, 162.26, 156.74, 151.99, 141.09, 136.96, 135.86, 129.65, 125.99, 123.83, 122.94, 119.86, 117.03, 113.94, 110.73, 111.69, 111.40, 102.11, 58.37, 56.51. ESI (+)-MS m/z (relative intensity) = 837.21 ($[\text{Co}^{\text{III}}(\text{Sal}^{\text{H}})(\text{Coumarin})_2]^+$). Elemental analysis calculated for $[\text{Co}^{\text{III}}(\text{Sal}^{\text{H}})(\text{Coumarin})_2]\text{Cl} \cdot \text{CH}_2\text{Cl}_2$ ($\text{C}_{45}\text{H}_{40}\text{N}_6\text{Cl}_3\text{O}_8\text{Co}$) C: 56.41%, H: 4.21%, N: 8.77%, Found C: 56.19%, H: 4.03%, N: 9.26%.

[Co^{III}(Sal^{OMe})(Coumarin)₂]Cl (**8**): The complex was synthesized following the same procedure as for **7**, combining H₂Sal^{OMe} (0.13 g, 0.385 mmol), CoCl₂ (0.05 g, 0.385 mmol) and Coumarin (0.22 g, 0.847 mmol). Yield: 0.35 g, 97%. ¹H NMR (400 MHz, MeOD) δ 8.19 (s, 1H), 7.73 (t, *J* = 1.4 Hz, 1H), 7.45 (d, *J* = 9.0 Hz, 1H), 7.14 (t, *J* = 1.7 Hz, 1H), 7.07 (d, *J* = 9.2 Hz, 1H), 6.96 (dd, *J* = 9.2, 3.1 Hz, 1H), 6.91 (d, *J* = 2.5 Hz, 1H), 6.85 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.82 (t, *J* = 1.5 Hz, 1H), 6.77 (d, *J* = 3.2 Hz, 1H), 5.43 (d, *J* = 1.3 Hz, 2H), 5.38-5.34 (m, 1H), 4.09 (s, 2H), 3.89 (s, 3H), 3.73 (s, 3H). ¹³C NMR (101 MHz, MeOD) δ 169.90, 164.89, 162.27, 161.02, 157.26, 156.76, 152.07, 151.31, 141.05, 129.61, 126.98, 125.98, 124.48, 122.93, 118.50, 115.77, 113.92, 111.69, 110.86, 102.11, 58.35, 56.50, 56.27. ESI (+)-MS *m/z* (relative intensity) = 897.23 ([Co^{III}(Sal^{OMe})(Coumarin)₂]⁺). Elemental analysis calculated for [Co^{III}(Sal^{OMe})(Coumarin)₂]Cl•1CH₂Cl₂•0.5H₂O (C₄₇H₄₅N₆Cl₃O_{10.5}Co) C: 54.96%, H: 4.42%, N: 8.18%, Found C: 54.82%, H: 3.87%, N: 8.88%.

[Co^{III}(Sal^{CF3})(Coumarin)₂]Cl (**9**): The complex was synthesized following the same procedure as for **7**, combining H₂Sal^{CF3} (0.071 g, 0.176 mmol), CoCl₂ (0.023 g, 0.176 mmol) and Coumarin (0.090 g, 0.351 mmol). Yield: 94%. ¹H NMR (400 MHz, MeOD) δ 8.37 (s, 1H), 7.86 (t, *J* = 1.4 Hz, 1H), 7.71-7.67 (m, 1H), 7.50 (d, *J* = 8.8 Hz, 2H), 7.27 (d, *J* = 9.0 Hz, 1H), 7.19 (t, *J* = 1.7 Hz, 1H), 6.91 (d, *J* = 2.5 Hz, 1H), 6.89 – 6.83 (m, 2H), 5.47 (d, *J* = 1.4 Hz, 2H), 5.31 (s, 1H), 4.20 (s, 2H), 3.89 (s, 3H). ¹⁹F NMR (400 MHz, MeOD) δ -62.95 (s). ¹³C NMR (101 MHz, MeOD) δ 170.82, 168.64, 164.95, 162.26, 156.77, 152.17, 141.22, 134.98, 132.69, 129.51, 125.95, 124.57, 123.48, 119.34, 113.99, 111.64, 110.49, 102.05, 68.86, 58.68, 56.49, 47.95, 9.22. ESI (+)-MS *m/z* (relative intensity) = 973.11 ([Co^{III}(Sal^{CF3})(Coumarin)₂]⁺). Elemental analysis calculated for [Co^{III}(Sal^{CF3})(Coumarin)₂]Cl•0.5CH₂Cl₂•3H₂O (C_{46.5}H₄₃N₆Cl₂O₁₁CoF₆) C:

50.51%, H: 3.92%, N: 7.60%, Found C: 50.48%, H: 4.53%, N: 7.54%.

2.3 X-ray Structure Determination: Single crystal X-ray crystallographic analyses of selected $[\text{Co}^{\text{III}}(\text{Sal}^{\text{R}})(1\text{-MeIm})_2]\text{ClO}_4$ ($\text{R} = \text{H}$ (**1**), OMe (**2**), and CF_3 (**6**)) complexes were performed on a Bruker X8 APEX II diffractometer and each crystal was mounted on a 150 μm MiTeGen glass fibre. For **1**, data were collected to a maximum 2θ value of 57.2° , in a series of ϕ and ω in 0.50° widths with 10.0 s exposures. The crystal-to-detector distance was 50 mm. For **2**, data collection was completed with TRIUMPH graphite monochromated $\text{Mo K}\alpha$ radiation. Data were collected to a maximum 2θ value of 61.0° , in a series of ϕ and ω in 0.50° widths with 30.0 s exposures. The crystal-to-detector distance was 50 mm. For **6**, data collection was performed with graphite monochromated $\text{Cu K}\alpha$ radiation. Data were collected at 149 ± 2 K to a maximum 2θ value of 133.3° , in a series of ϕ and ω in 0.80° widths with 10.0 s exposures. The crystal-to-detector distance was 40 mm. The structures were solved by intrinsic phasing (SIR92)³⁸ and refined by least-squares procedures using SHELXL-2013.³⁹ Single-crystal X-ray crystallographic analysis of a red crystal of **9** was performed by the SCrALS (Service Crystallography at Advanced Light Source) program at the Small-Crystal Crystallography Beamline 11.3.1 at the Advanced Light Source (ALS), Lawrence Berkeley National Laboratory using synchrotron radiation tuned to $\lambda = 0.7749$ Å. Intensity data were collected at 296 K on a D8 goniostat equipped with a Bruker APEXII CCD detector at Beamline 11.3.1. For data collection, frames were measured for a duration of 1 s at 0.3° intervals of ω with a maximum 2θ value of 60° . The data frames were collected using the program APEX2 and processed using the program SAINT within APEX2. The data were corrected for absorption and beam corrections based on the multiscan technique as implemented in SADABS. The structure was solved by the intrinsic phasing method⁴⁰ and

subsequent refinements were performed using ShelXle.⁴¹ For all structures non-hydrogen atoms were refined anisotropically. All C–H hydrogen atoms were placed in calculated positions but were not refined. A summary of the crystal data and experimental parameters for the structure determinations are given in **Table 2.1**. Crystallographic data has been deposited at CCDC with numbers 1545820-1545823. All crystal structure plots were produced using ORTEP-3⁴² and rendered with POV-Ray (v.3.6.2).⁴³⁻⁴⁴

Table 1. Selected crystallographic data for $[\text{Co}^{\text{III}}(\text{Sal}^{\text{R}})(\text{L})_2][\text{X}]$ complexes.

$[\text{Co}^{\text{III}}(\text{Sal}^{\text{R}})(\text{L})_2]\text{X}$	R = H (1) L = 1-Melm, X = ClO_4^-	R = OMe (2) L = 1-Melm, X = ClO_4^-	R = CF_3 (6) L = 1-Melm, X = ClO_4^-	R = CF_3 (9) L = Coumarin, X = Cl^-
Formula	$\text{C}_{24}\text{H}_{26}\text{ClN}_6\text{O}_6\text{Co}$	$\text{C}_{26}\text{H}_{30}\text{ClN}_6\text{O}_8\text{Co}$	$\text{C}_{26}\text{H}_{24}\text{ClN}_6\text{O}_6\text{F}_6\text{Co}$	$\text{C}_{46}\text{H}_{36}\text{ClN}_6\text{O}_8\text{F}_6\text{Co}$
Formula weight	588.89	648.94	724.89	1027.20
space group	P 21 21 21	P-1	P-1	P-1
a (Å)	12.4129(7)	8.4249(4)	10.9036(5)	10.5080(7)
b (Å)	13.1381(8)	11.8610(6)	11.0510(5)	14.4981(10)
c (Å)	15.6039(9)	15.0154(8)	14.3468(7)	16.3735(11)
α (°)	90	74.3740(10)	106.780(4)	78.449(2)
β (°)	90	81.6870(10)	96.598(3)	87.379(2)
γ (°)	90	76.8910(10)	105.318(3)	69.154(2)
V [Å ³]	2544.7(3)	1401.76(12)	1561.74	2283.1(3)
Z, D_{calc} [g/cm ³]	4	2	2	2
T (K)	296(2)	150(2)	149(2)	273(2)
ρ_{calcd} (g cm ⁻³)	1.537	1.537	1.542	1.494
λ (Å)	0.71073	0.71073	1.54178	0.7749
μ (cm ⁻¹)	0.832	0.768	5.921	0.654
R indices ^a with $I > 2\sigma(I)$ (data)	0.0545	0.0285	0.0733	0.0903
wR ₂	0.1477	0.0731	0.1904	0.2629
R ₁	0.0660	0.0373	0.0879	0.1041
Goodness-of-fits on F ²	1.027	1.028	1.063	1.024

^aGoodness-of-fits on F

3. Results and Discussion:

3.1 Synthesis and Characterization of $[\text{Co}^{\text{III}}(\text{Sal}^{\text{R}})(1\text{-MeIm})_2][\text{ClO}_4]$ (1-6**):** We initially chose to target the synthesis of a series of octahedral $[\text{Co}^{\text{III}}(\text{Sal}^{\text{R}})(\text{L})_2]^+$ complexes with neutral axial ligands, and 1-methylimidazole (1-MeIm) was chosen as a representative ligand due to a number of recent reports detailing the synthesis of octahedral $[\text{Co}^{\text{III}}(\text{Sal}^{\text{R}})(\text{L})_2]^+$ complexes with imidazole donors.⁴⁵⁻⁴⁷ The syntheses of $[\text{Co}^{\text{III}}(\text{Sal}^{\text{R}})(1\text{-MeIm})_2][\text{ClO}_4]$ (**1-6**) are detailed in **Scheme 1**, employing R groups of differing electron-donating ability. For compounds **1-5** the synthesis proceeded from isolated square planar $\text{Co}^{\text{II}}\text{Sal}^{\text{R}}$ complexes to afford the Co^{III} products in moderate yield via reaction with 3 eq. of 1-MeIm under aerated conditions. Interestingly, the $\text{Co}^{\text{II}}\text{Sal}^{\text{CF}_3}$ precursor was difficult to isolate, likely due to the increased Lewis acidity of the Co^{II} centre combined with the enhanced solubility of this complex. Thus, the synthesis of $[\text{Co}^{\text{III}}(\text{Sal}^{\text{CF}_3})(1\text{-MeIm})_2][\text{ClO}_4]$ (**6**) was completed using a one-pot procedure initially reacting $\text{H}_2\text{Sal}^{\text{CF}_3}$ with Co^{II} , and then subsequent addition of 1-MeIm (**Scheme 1**). The resulting complexes **1-6** were characterized by a number of methods including ^1H and ^{13}C NMR, MS, elemental analysis, X-ray crystallography for **1**, **2**, and **6**.

Scheme 1. Synthesis of $\text{Co}^{\text{III}}\text{Sal}^{\text{R}}$ complexes with 1-methylimidazole (1-MeIm) as axial ligands. (Path A) 3 eq. 1-MeIm, NaClO_4 MeOH/ CH_2Cl_2 , 12 h, 298 K. (Path B) 1 eq. CoCl_2 , N_2 , and then 3 eq. 1-MeIm, NaClO_4 , MeOH/ CH_2Cl_2 , 12 h, 298 K. See experimental section for further details.

The solid state structures of **1**, **2**, and **6** exhibit a distorted octahedral N_4O_2 coordination sphere around Co from the Sal^{R} ligand in the equatorial plane and the two axial 1-MeIm ligands. The molecular structures of **1** and **2** are presented in the Supporting Information (**Figures S1-S2**), with the molecular structure of **6** shown in **Figure 2**. The metrical parameters reported in

this work (**Table 2**) are in agreement with published solid state structures of $\text{Co}^{\text{III}}\text{Sal}^{\text{R}}$ complexes with imidazole axial ligands.⁴⁵⁻⁴⁷

Figure 2. ORTEP plot of $[\text{Co}^{\text{III}}(\text{Sal}^{\text{CF}_3})(1\text{-MeIm})_2][\text{ClO}_4]$ (**6**) (50 % probability) using POV-Ray, excluding hydrogen atoms, ClO_4^- counterion, and MeOH solvent. See Table 2 for metrical parameters.

Table 2. Selected interatomic distances [$\text{\AA}$] and angles [deg] for $[\text{Co}^{\text{III}}(\text{Sal}^{\text{R}})(1\text{-MeIm})_2][\text{ClO}_4]$ complexes **1**, **2**, and **6**.

$[\text{Co}^{\text{III}}(\text{salen}^{\text{R}})(1\text{-MeIm})_2]\text{ClO}_4$	R = H (1)	R = OMe (2)	R = CF_3 (6)
Co1 – O1	1.893(3)	1.893(9)	1.897(3)
Co1 – O2	1.888(4)	1.907(9)	1.908(3)
Co1 – N1	1.894(4)	1.897(10)	1.895(4)
Co1 – N2	1.906(5)	1.893(10)	1.894(4)
Co1 – N3	1.968(4)	1.943(10)	1.949(4)
Co1 – N5	1.938(4)	1.949(10)	1.955(4)
O2 – Co1 – N5	88.5(2)	88.69(4)	90.0(15)
N1 – Co1 – N5	89.2(2)	92.05(4)	91.9(17)
N2 – Co1 – N5	91.0(2)	89.42(4)	90.7(17)
O1 – Co1 – N5	89.7(2)	89.27(4)	88.6(15)
O2 – Co1 – N3	90.1(2)	87.30(4)	89.6(16)
N1 – Co1 – N3	92.2(2)	91.99(4)	88.6(17)
N2 – Co1 – N3	90.1(2)	91.62(4)	92.4(18)
O1 – Co1 – N3	89.2(2)	89.71(4)	88.3(15)

The solid state data allows for investigation of the influence of the *para*-ring substituents on the coordination sphere bond lengths. Analysis of X-ray distances for square planar $\text{Cu}^{\text{II}}\text{Sal}^{\text{R}}$ derivatives have shown a small but significant decrease in Cu-O bond lengths ($\text{CF}_3 > \text{tBu} > \text{OMe}$) as the electron donating ability of the *para*-ring substituent is increased.⁴⁸⁻⁵⁰ The same trend in Ni-O bond lengths is also observed for square planar $\text{Ni}^{\text{II}}\text{Sal}^{\text{R}}$ complexes.^{32,48-49} However, comparison of the Co-O distances for each of the complexes (**Table 2**) does not show a statistically significant change across the three complexes. In addition, no obvious trend is apparent in the Co-N distances, either equatorial or axial, in the three complexes. Thus, the

electronic effect of the *para*-ring substituents do not significantly influence the coordination sphere bond lengths for octahedral $\text{Co}^{\text{III}}\text{Sal}^{\text{R}}$ complexes.

The stability of complexes **1-6** were investigated by ^1H NMR in 2% $\text{DMSO}-d_6$ in deuterated phosphate-buffered saline (PBS) solution over 24 h at 37 °C. At 24 h minor signals attributable to free 1-MeIm appear for **1-4**, indicating ca. 10% ligand exchange based on integrated peak intensity (See **Figures S3-S8**). Thus axial ligand exchange occurs relatively slowly for **1-4** under the test conditions. Interestingly, for the $\text{R} = \text{NO}_2$ (**5**) and $\text{R} = \text{CF}_3$ (**6**) derivatives no ligand exchange is observed at 24 h by ^1H NMR, indicating that the complexes with electron-withdrawing *para*-ring substituents are more stable to axial ligand exchange. The increased stability is likely due to the enhanced Lewis acidity of the Co^{III} centre as a result of the electron-withdrawing effect of these *para*-ring substituents.³¹

3.2 Electrochemistry of $[\text{Co}^{\text{III}}(\text{Sal}^{\text{R}})(1\text{-MeIm})_2][\text{ClO}_4]$ (1-6**):** The electrochemical properties of **1-6** were assessed via cyclic voltammetry (CV) to better understand how the different *para*-substituents shift the $\text{Co}^{\text{III}} / \text{Co}^{\text{II}}$ reduction potential. As shown in **Figure 3** and **Table 3** there is a large value of ΔE_p (peak-to-peak difference, $\Delta E_p = |E_a - E_c|$) and a different current response for the reduction (E_c) and oxidation (E_a) processes. These results support an irreversible electrochemical process for **1-6**, and thus reduction (E_c) suggests significant geometrical re-arrangement, including axial ligand loss to afford a $\text{Co}^{\text{II}}\text{Sal}^{\text{R}}$ complex. Subsequent re-oxidation of $\text{Co}^{\text{II}}\text{Sal}^{\text{R}}$ (E_a) is consistent with independent electrochemical analysis of the $\text{Co}^{\text{II}}\text{Sal}^{\text{R}}$ complexes (**Figure S9**), as the E_a values are consistent with those reported in **Table 3**.

Figure 3. Cyclic voltammetry of $[\text{Co}^{\text{III}}(\text{Sal}^{\text{R}})(1\text{-MeIm})_2][\text{ClO}_4]$ (**1-6**). Conditions: 1 mM complex, 0.1 M $n\text{Bu}_4\text{NClO}_4$, scan rate 100 mV s^{-1} , DMF, 298 K. Arrow signifies scan direction. Inset: Hammett plot of the $E_{1/2}$ value of $[\text{Co}^{\text{III}}(\text{Sal}^{\text{R}})(1\text{-MeIm})_2][\text{ClO}_4]$ (**1-6**) versus σ_{para} of the *para*-ring substituents.

Table 3. Electrochemical data (mV) for $[\text{Co}^{\text{III}}(\text{Sal}^{\text{R}})(1\text{-MeIm})_2][\text{ClO}_4]$ (**1-6**) versus NHE.

$[\text{Co}(\text{salen}^{\text{R}})(1\text{-MeIm})_2]\text{ClO}_4$	E_{c}	E_{a}	$E_{1/2}$	$\Delta E_{\text{p}}^{\text{a}}$
OMe (2)	-540	260	-140	800
$t\text{Bu}$ (3)	-570	320	-125	890
H (1)	-490	290	-100	780
Br (4)	-400	300	-50	700
CF_3 (6)	-420	470	25	790
NO_2 (5)	-230	390	80	620

^aPeak-to-peak differences ($\Delta E_{\text{p}} = |E_{\text{a}} - E_{\text{c}}|$). Peak-to-peak difference for the Fc^+/Fc couple at 298 K is 130 mV

The trend in reduction potential ($E_{1/2}$) for **1-6** follows the relative electronic properties of the phenolate *para*-substituents since the donating *para*-ring substituents afford a lower $\text{Co}^{\text{III}} / \text{Co}^{\text{II}}$ reduction potential due to the increased electron density at the Co centre. Overall, using the different *para*-ring substituents allows for tuning of the $E_{1/2}$ value from -140 mV to 80 mV (220 mV difference), and E_{c} value from -570 mV to -230 mV (340 mV difference). Plotting the $E_{1/2}$ values against the Hammett substituent constants (σ_{para})⁵¹⁻⁵² as shown in **Figure 3** (Inset) demonstrates that the reduction potential is predominantly affected by the donating ability of the *para*-ring substituents. These promising results show that it is possible to significantly alter the $\text{Co}^{\text{III}} / \text{Co}^{\text{II}}$ reduction potential and, in addition, reduction leads to significant geometrical rearrangement and axial ligand loss.

3.3 Synthesis and Characterization of $[\text{Co}^{\text{III}}(\text{Sal}^{\text{R}})(\text{Coumarin})_2][\text{Cl}]$ (7-9**):** The synthesis of **7-9** followed closely to that of **6**, using a one-pot procedure to first synthesize the $\text{Co}^{\text{II}}\text{Sal}^{\text{R}}$ complex *in situ*, and then oxidation in the presence of 2 eq. of the Coumarin ligand (**Scheme 2**). The

Coumarin ligand was chosen as a reporter due to its known fluorescence properties, and was coupled to the imidazole moiety for coordination to Co. Encouragingly, upon binding to the Co^{III} centre the Coumarin fluorescence is quenched (see below). Use of a quenched fluorophore allows for the rational optimization of ligand release under physiological conditions, and monitoring of release under hypoxic conditions, and in cells. We initially focused on complexes incorporating the ClO₄⁻ counterion, however, the resulting products were difficult to purify and exhibited limited solubility in water. Employing CoCl₂ as the starting material, and without addition of NaClO₄, we were able to isolate **7-9** in moderate yields (**Scheme 2**). These three complexes span a large range of electron-donating abilities (OMe, H, and CF₃), thus affording a representative series of complexes for further analysis.

Scheme 2. Synthesis of Co^{III}Sal^R complexes with Coumarin-imidazole conjugates (Coumarin) as axial ligands. Conditions: 1 eq. CoCl₂, MeOH/CH₂Cl₂ under N₂, then 3 eq. Coumarin, 12 h, 298 K. See experimental section for further details.

The solid state structure of **9** is shown in **Figure 4**, and exhibits a distorted octahedral N₄O₂ coordination sphere at Co with the Sal^{CF₃} ligand in the equatorial plane and the two axial Coumarin ligands. The pattern of first-coordination sphere bond lengths for **9** is similar to that reported for complexes **1**, **2**, and **6**, reflecting the common ligating groups.

Figure 4. ORTEP plot of [Co^{III}(Sal^{CF₃})(Coumarin)₂][Cl] (**9**) (50 % probability) using POV-Ray, excluding hydrogen atoms and disordered Cl counterion. Note significant disorder is present for one coumarin and the CF₃ substituents. Selected interatomic distances [Å] and angles [deg]: Co1-O1 1.901(2), Co1-O2 1.887(3), Co1-N1 1.891(2), Co1-N2 1.895(4), Co1-N3 1.946(4), Co1-N5 2.018(3); angles: O2-Co1-N5 88.4(12), N1-Co1-N5 92.7(4), N2-Co1-N5 94.3(3), O1-Co1-N5 86.2(12), O2-Co1-N3 89.2(17), N1-Co1-N3 89.8(18), N2-Co1-N3 91.7(18), O1-Co1-N3 87.8(16), O1-Co1-N2 179.5(18), O2-Co1-N1 179.0(18).

The stability of the $[\text{Co}^{\text{III}}(\text{Sal}^{\text{R}})(\text{Coumarin})_2][\text{Cl}]$ (**7-9**) complexes was investigated by ^1H NMR in 50% $\text{DMSO}-d_6$ in D_2O over 72 h at 37 °C. The increased amount of DMSO used here in comparison to the ^1H NMR stability studies for **1-6** was necessary due to the lower aqueous solubility of the Coumarin derivatives. In each case, compounds **7-9** displayed no obvious sign of decomposition over the 72 h period (**Figures S11-S13**).

3.4 Electrochemistry of $[\text{Co}^{\text{III}}(\text{Sal}^{\text{R}})(\text{Coumarin})_2][\text{Cl}]$ (**7-9**): The electrochemical properties of **7-9** were assessed via CV to determine reduction potentials and possibility of axial ligand loss upon reduction. As shown in **Figure 5** and **Table 4**, complexes **7-9** exhibit a large value of ΔE_p and a different current response for the reduction (E_c) and oxidation (E_a) processes. These results suggest a similar electrochemical response for **7-9** in comparison to **1-6** consistent with significant geometrical re-arrangement, including axial ligand loss upon reduction. The $E_{1/2}$ values follow a trend consistent with the relative electron-donating ability of the *para*-ring substituents ($\text{OMe} > \text{H} > \text{CF}_3$). Interestingly, the $E_{1/2}$ values are ca. 80 mV more positive in comparison to the $[\text{Co}^{\text{III}}(\text{Sal}^{\text{R}})(1\text{-MeIm})_2][\text{ClO}_4]$ derivatives with the same Sal^{R} ligands. The shift indicates that the $E_{1/2}$ value depends slightly on the axial ligand and the counterion in this experiment.

Figure 5. Cyclic voltammetry of $[\text{Co}^{\text{III}}(\text{Sal}^{\text{R}})(\text{Coumarin})_2][\text{Cl}]$ (**7-9**). Conditions: 1 mM complex, 0.1 M $n\text{Bu}_4\text{NClO}_4$, scan rate 100 mV s^{-1} , DMF, 298 K. Arrow signifies scan direction.

Table 4. Electrochemical data (mV) for $[\text{Co}^{\text{III}}(\text{Sal}^{\text{R}})(\text{Coumarin})_2][\text{Cl}]$ (**7-9**) versus NHE.

$[\text{Co}^{\text{III}}(\text{salen}^{\text{R}})(\text{Coumarin})_2]\text{Cl}$	E_{c}	E_{a}	$E_{1/2}$	$\Delta E_{\text{p}}^{\text{a}}$
R = OMe (8)	-390	270	-60	660
R = H (7)	-310	270	-20	580
R = CF ₃ (9)	-280	370	45	650

^aPeak-to-peak differences ($\Delta E_{\text{p}} = |E_{\text{a}} - E_{\text{c}}|$). Peak-to-peak difference for the Fc^+/Fc couple at 298 K is 120 mV.

3.5 Emission studies of $[\text{Co}^{\text{III}}(\text{Sal}^{\text{R}})(\text{Coumarin})_2][\text{Cl}]$ (7-9**):** We next assessed the use of fluorescence to monitor the rate of ligand loss from compounds **7-9** upon reduction in solution. The photophysical properties of the Coumarin ligand were first investigated, with an optimal excitation wavelength (λ_{ex}) of 327 nm and emission wavelength (λ_{em}) of 400 nm (**Figure S14**). Upon binding to the Co^{III} centre the Coumarin ligand emission is quenched (**Figure 6**), likely due to excited state energy-transfer.⁵³⁻⁵⁵ A fluorescence signal is observed however for the R=H (**7**, ca. 3% of concentration-matched Coumarin signal) and R = OMe (**8**, ca. 14% of concentration-matched Coumarin signal) analogues upon dissolution, likely due to partial axial ligand exchange in the solvent medium. Interestingly, essentially no fluorescence signal is observed for the R = CF₃ derivative (**9**, ca. 0.4% of concentration-matched Coumarin signal).

Figure 6. Emission spectra of Coumarin (dash-dot), **7** (black line), **8** (grey dashed), and **9** (dotted). Conditions: 2% DMSO in PBS (0.1 M), 298 K, $\lambda_{\text{ex}} = 327$ nm.

The ligand exchange processes of **7-9** were studied in the presence of excess dithionite ($\text{Na}_2\text{S}_2\text{O}_4$). The reduction potential for dithionite ($E_{1/2} = -660$ mV vs. NHE)⁵⁶ is considerably lower than **7-9** (**Table 4**), and thus we expected this reagent to reduce the Co^{III} complexes. As this reagent readily reacts with O_2 in solution, solutions were degassed (purging with N_2) before a 10-fold excess of $\text{Na}_2\text{S}_2\text{O}_4$ was added to ensure reduction of **7-9**.⁵⁶⁻⁵⁸ We then compared the

change in fluorescence of **7-9** in solution in the presence of excess $\text{Na}_2\text{S}_2\text{O}_4$, excess 1-MeIm, and finally without any additives.

The change in fluorescence of **7** ($3\text{ }\mu\text{M}$) over a 12 h period was measured: (i) on its own in solution, (ii) with $30\text{ }\mu\text{M}$ dithionite, and (iii) with $30\text{ }\mu\text{M}$ 1-MeIm (**Figure 7**). Coumarin fluorescence was used as the control, and was shown to be unaffected by either $\text{Na}_2\text{S}_2\text{O}_4$, or 1-MeIm. Over a period of 12 h compound **7** exhibited a ca. 60 % increase (relative to Coumarin control) in fluorescence in the presence of excess $\text{Na}_2\text{S}_2\text{O}_4$. In the presence of excess 1-MeIm, a ca. 44 % increase in fluorescence is observed, indicating a significant amount of axial ligand exchange. However, a higher fluorescence response is observed under reducing conditions. In the absence of both reducing agent and 1-MeIm, the complex also released Coumarin (ca. 27 % of Coumarin control), suggesting ligand exchange in the buffer solution.

Figure 7. Fluorescence analysis of solutions of **7** ($3\text{ }\mu\text{M}$) in the presence of $30\text{ }\mu\text{M}$ $\text{Na}_2\text{S}_2\text{O}_4$ (black squares), $30\text{ }\mu\text{M}$ 1-MeIm (black triangles), and without additives (black circles) over a period of 12 h. The Coumarin standard ($6\text{ }\mu\text{M}$) is shown as grey circles. Conditions: 2% DMSO in PBS (0.1 M), 298 K, $\lambda_{\text{ex}} = 327\text{ nm}$, $\lambda_{\text{em}} = 400\text{ nm}$.

The change in fluorescence of **8** over a 12 h period under the same conditions is shown in **Figure 8**. The overall response to $\text{Na}_2\text{S}_2\text{O}_4$ (ca. 67 % of Coumarin control), excess 1-MeIm (ca. 44 % of Coumarin control), and buffer (ca. 26 % of control) are similar to that described for compound **7**. However, the fluorescence increases occur in the first 2 h, and from 2 – 12 h the fluorescence readings remain relatively constant.

Figure 8. Fluorescence analysis of solutions of **8** (3 μM) in the presence of 30 μM $\text{Na}_2\text{S}_2\text{O}_4$ (black squares), 30 μM 1-MeIm (black triangles), and without additives (black circles) over a period of 12 h. The Coumarin standard (6 μM) is shown as grey circles. Conditions: 2% DMSO in PBS (0.1 M), 298 K, $\lambda_{\text{ex}} = 327$ nm, $\lambda_{\text{em}} = 400$ nm.

As shown in **Figure 9**, complex **9** exhibited a minimal increase in fluorescence under the test conditions over 12 h. An expanded view of the fluorescence plot shows that while small, the fluorescence intensity increase is greater in the presence of $\text{Na}_2\text{S}_2\text{O}_4$ in comparison to 1-MeIm and buffer alone. Indeed, the fluorescence intensity of **9** in buffer is the same as that in the presence of excess 1-MeIm, indicating minimal ligand exchange for **9**.

Figure 9. Fluorescence analysis of solutions of **9** (3 μM) in the presence of 30 μM $\text{Na}_2\text{S}_2\text{O}_4$ (black squares), 30 μM 1-MeIm (black triangles), and without additives (black circles) over a period of 12 h. The Coumarin standard (6 μM) is shown as grey circles. Conditions: 2% DMSO in PBS (0.1 M), 298 K, $\lambda_{\text{ex}} = 327$ nm, $\lambda_{\text{em}} = 400$ nm. Inset: Expanded view of the fluorescence increase in the presence of 30 μM $\text{Na}_2\text{S}_2\text{O}_4$ (black squares), 30 μM 1-MeIm (black triangles).

The rate of axial ligand exchange (without added reductant) for **7-9** can be rationalized by the effect of the *para*-ring substituents on the Lewis acidity at the metal centre. The electron-withdrawing $\text{R} = \text{CF}_3$ substituent is expected to increase the Lewis acidity at the metal centre to the greatest extent ($\text{CF}_3 > \text{H} > \text{OMe}$), resulting in increased bond strength between the Co^{III} centre and the axial ligands. This is reflected in the rate of exchange of the Co^{III} complexes in the presence of 1-MeIm ($\text{OMe} > \text{H} > \text{CF}_3$) as shown in **Figures 7-9**. These results are consistent with a literature report⁵⁹ that demonstrated increased resistance to ligand exchange (via reduced enzyme inhibition) for $\text{Co N}_3\text{N}'\text{ethylenebis}(\text{acetylacetonate})$ complexes when electron-withdrawing groups are installed on the ligand. Interestingly, the observed rates ($\text{OMe} > \text{H} > \text{CF}_3$) of reduction in the presence of excess $\text{Na}_2\text{S}_2\text{O}_4$ are opposite to that expected based on the reduction potentials (**Table 4**), however the potential window is relatively narrow for the three

derivatives (-0.06 to 0.45 V vs. NHE). The reduction processes studied in this work are relatively slow (timescale of h), which agrees with reports of similar Co complexes in the literature.^{10,25,60-}

⁶² Due to the slow reduction process, and reaction conditions (in air), in which Na₂S₂O₄ decomposes, the observed fluorescence increase is likely a combination of both reduction and axial ligand exchange.

4. Summary:

In this work we have studied the electrochemical properties and axial ligand exchange of a series of octahedral Co^{III} salen (Sal^R) complexes. We first focused on tuning the Co^{III} / Co^{II} reduction potential of **1-6** using a series of Sal^R ligands with *para*-ring substituents of different electron-donating abilities (H, OMe, tBu, Br, NO₂, CF₃). Using peripheral ligand electronics we were able to tune the Co^{III} / Co^{II} reduction potential in the range of -140 to 80 mV vs. NHE. While at the edge of the generally-accepted potential window in cells (-420 to -150 mV vs. NHE),²⁷ our results suggest that it will be possible to tune the Co^{III} / Co^{II} reduction potential further into a physiologically-relevant range by employing ring substituents of enhanced electron-donating ability (such as NMe₂).²⁹⁻³² In addition, using 1-MeIm axial ligands, we showed that reduction to Co^{II} results in significant geometrical re-organization and axial ligand exchange. By installing fluorescent coumarin-imidazole conjugate (Coumarin) axial ligands, we explored the electrochemical properties and axial ligand exchange in a smaller series of octahedral Co^{III} complexes **7-9** (H, OMe, CF₃). Upon binding to Co^{III}, the Coumarin fluorescence was quenched, allowing for a sensitive read-out of axial ligand exchange upon release. Our results show that both **7** (R = H), and **8** (R = OMe) exhibit substantial ligand exchange in the presence of either excess reductant or competing ligands, with the latter compound reacting much more quickly. Interestingly, compound **9** did not exhibit a substantial fluorescence change under any

conditions, even though the $\text{Co}^{\text{III}} / \text{Co}^{\text{II}}$ reduction potential suggests the most significant driving force for reduction. Overall, the current results suggest that the dominant factor influencing axial ligand exchange is the Lewis acidity at the Co centre, and not the $\text{Co}^{\text{III}} / \text{Co}^{\text{II}}$ reduction potential. Future work will focus on decoupling these competing processes for this series of complexes.

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602x145mm (96 x 96 DPI)

Draft

527x248mm (96 x 96 DPI)

A **Satraplatin****B** **NKP1339****C** **Co^{III} mustard****D** **This work**

454x359mm (96 x 96 DPI)

116x82mm (96 x 96 DPI)

112x124mm (150 x 150 DPI)

162x114mm (96 x 96 DPI)

104x117mm (150 x 150 DPI)

124x130mm (300 x 300 DPI)

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