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Cobalt complexes with tripodal ligands: implications for the design of drug chaperones†

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Extensive research is currently being conducted into metal complexes that can selectively deliver cytotoxins to hypoxic regions in tumours. The development of pharmacologically suitable agents requires an understanding of appropriate ligand–metal systems for chaperoning cytotoxins. In this study, cobalt complexes with tripodal tren (tris-(2-aminoethyl)amine) and tpa (tris-(2-pyridylmethyl)amine) ligands were prepared with ancillary hydroxamic acid, β-diketone and catechol ligands and several parameters, including: pK_a, reduction potential and cytotoxicity were investigated. Fluorescence studies demonstrated that only tpa complexes with β-diketones showed any reduction by ascorbate *in situ* and similarly, cellular cytotoxicity results demonstrated that ligation to cobalt masked the cytotoxicity of the ancillary groups in all complexes except the tpa diketone derivative $[Co(naac)tpa](ClO_4)_2$ (naac = 1-methyl-3-(2-naphthyl)-propane-1,3-dione). Additionally, it was shown that the hydroxamic acid complexes could be isolated in both the hydroxamate and hydroximate form and the pK_a values (5.3–8.5) reveal that the reversible protonation/deprotonation of the complexes occurs at physiologically relevant pHs. These results have clear implications for the future design of prodrugs using cobalt moieties as chaperones, providing a basis for the design of cobalt complexes that are both more readily reduced and more readily taken up by cells in hypoxic and acidic environments.

Introduction

The remodelling of the vascular system that occurs under hypoxia is intrinsically linked to the progression of many diseases including stroke, ischemic heart disease, peripheral vascular disease, anaerobic infection and, importantly, cancer.[1] Indeed, hypoxia occurs to a variable extent in most tumours.[2] Its prominence in cancer is attributable to interrupted, or absent, endothelial linings and the impaired vascularisation that occur as a result of uncontrolled cell proliferation.[3]

Hypoxia in tumours is generally associated with a poor treatment prognosis. In chemotherapy, hypoxic cells are less susceptible to traditional drugs because of the growth arrested state,[4] limited drug penetration[5] and induced resistance mechanisms of such cells.[6] Similarly, radiotherapy agents are far less effective in regions of hypoxia,[7] mainly because oxygen is required to convert the initial free radicals formed by ionisation into DNA strand breaks.[8]

However, while tumoural hypoxia has been seen as an impediment to therapy, it has been suggested that it could be exploited as a target for selective activation of prodrugs. One of the difficulties associated with chemotherapy is that cancer cells, unlike bacteria and viruses, do not have molecular targets that are completely foreign to the host which has limited the usefulness of some chemotherapeutic agents due to toxic side-effects.[4] For this reason differences between tumours and healthy tissue such as pH,[9] tumour-specific enzymes,[10] tumour-specific antigens,[4,11] hypoxia[12] and combinations of these[13] have been explored as potential targets.

3-Aminobenzotriazine-1,4-di-*N*-oxide or tirapazamine (TPZ) is one of the most well known biologically active aromatic *N*-oxides and has shown one of the largest differential toxicities between hypoxic and normoxic cells for any hypoxia-activated prodrug, with hypoxic cell response (HCR) values between 50 and 300 for a variety of different cell lines.[14] Other hypoxia-selective drugs include aliphatic *N*-oxides,[15] quinones,[16] nitroaromatics,[17] and metal complexes.[18] Cobalt complexes are of particular interest as hypoxia selective prodrugs because of the marked difference in lability between the higher (III) and lower (II) oxidation states of cobalt. Ware and co-workers have examined a number of cobalt complexes bearing acetylacetone-derived ancillary ligands with monodentate,[19] bidentate[20] and tridentate nitrogen mustards.[21] A number of different nitrogen mustard complexes with alternate ancillary ligands including

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dialkyldithiocarbamate,[22] tropolonate,[23] carbonato,[18] oxalate groups[18] as well as cyclen derivatives with hydroxyquinolines[24] and tpa complexes with hydroxamic acids have been investigated.[25]

At this stage however, it is not known whether the biologically relevant behaviour of these complexes is exclusively limited to certain ligand systems and the relationships between charge, pK_a, lipophilicity and reduction potential are just being un-ravelled.[26,27] The purpose of this study is to examine a variety of cobalt complexes with tripodal ligands and different bidentate oxygen co-ligands to ascertain which systems are likely to be the most biologically effective.

Experimental

Materials

All solvents used were laboratory grade and were used without further purification unless otherwise stated. $CoCl_2 \cdot 6H_2O$ and ethane-1,2-diamine were obtained from Merck. Hydrogen per-oxide (30%, v/v) was obtained from Ajax chemicals. All other chemicals were purchased from Sigma Aldrich. C25 Sephadex (bead size 40–120 μM) was used for cation exchange chromato-graphy (column dimensions are given as diameter × height).

Caution! Many of the procedures involved the preparation of perchlorate salts of metal complexes. While no issues were experienced with these compounds, such compounds should also be handled with care as they are potentially hazardous. Par-ticular care was taken with the tris(2-methylpyridyl)amine per-chlorate (tpa·nHClO$_4$) ligand as organic perchlorate molecules are potentially explosive.

DLD-1 human colon carcinoma cells were obtained from ATCC and used within 6 months of resuscitation. Cells were maintained in Advanced DMEM (Invitrogen) and supplemented with 2% FBS and 2 mM glutamine in a humidified environment at 37 °C and 5% CO_2.

Instrumentation

Microanalysis (C, H, N) was conducted by Chemical & Micro-Analytical Services Pty Ltd or the Campbell Microanalytical Laboratory, Department of Chemistry at the University of Otago.

^{1}H and ^{13}C NMR spectra were collected at 300 K on a Bruker 300 MHz spectrometer using commercially available deuterated solvents. TSP (sodium (3-(trimethylsilyl)propionate)) was used as an internal reference in D_2O. In all other solvents isotopic impurities were used as internal reference signals.[28]

The mass spectrometry was performed using Electro-Spray Ionisation on a Finnigan LCQ-8 spectrometer.

UV-visible measurements were performed on a Cary 1E UV-visible spectrophotometer using a 1 cm × 1 cm quartz cuvette. Solutions for pH titrations for hydroxamic acid derivatives were performed at approximately 1 mM in DMF–water (1 : 1) with 20 mM buffer (citrate/phosphate/triethylamine).

Fluorescence measurements were performed on a LS 50 B luminescence spectrometer using a 1 cm × 1 cm silica cuvette. Scans were run as 2.0×10^{-5} M solutions in methanol–water (1 : 1) at 100 nm min^{-1}. The relative fluorescence intensities were calculated by comparing the areas under the emission spectra. Emission spectra for naphthalene hydroxamic acid and catechol compounds were run between 300 and 500 nm with excitation at 280 nm, while β-diketone complexes were run at 380–600 nm with excitation at 350 nm.

Ascorbic acid reduction studies were performed on 1.0×10^{-5} M solutions of complexes in phosphate-buffered (0.1 mM) methanol–water (1 : 1) over 24 hours after the addition of ascorbic acid (20 equivalents). Complexes used in the reduction study were: [Co(cat)tpa]·ClO$_4$, [Co(cat)tren]·ClO$_4$, [Co(nha-H)-tpa]·ClO$_4$, [Co(nha-H)tren]·ClO$_4$, [Co(naac)tpa]·(ClO$_4$)$_2$ and [Co(naac)tren]·(ClO$_4$)$_2$. The equipment and set up used was the same as described for the fluorescence experimentation. The pH of solution in all cases was 7.0.

Electrochemistry was performed on a BAS100B instrument using a glassy carbon working electrode, a silver/silver chloride reference electrode and a platinum auxiliary electrode. Scans were performed at a scan rate of 100 mV s^{-1} unless otherwise stated and the solutions were degassed with argon for at least ten minutes prior to scanning. All experiments were performed at room temperature and were iR compensated. Scans done in DMF and DMF–water (4 : 1) were performed with 0.1 M tetra-butylammonium perchlorate (TBAP) as the supporting electro-lyte and the ferrocene/ferrocenium couple was used as an internal reference. Aqueous scans were performed with sodium perchlorate (0.1 M) as the supporting electrolyte and were refer-enced to the Ag/AgCl couple.

Crystallography

Data were collected to approximately 56° 2θ using either a Bruker SMART 1000 diffractometer employing graphite-mono-chromated Mo-Kα radiation generated from a sealed tube (0.71073 Å)[29] or a Bruker–Nonius APEX2-X8-FR591 diffracto-meter employing graphite-monochromated Mo-Kα radiation generated from a rotating anode (0.71073 Å).[30] Data integration and reduction were undertaken with either teXsan,[31] or SAINT and XPREP.[29,30] Subsequent computations were carried out using either teXsan[31] or the WinGX-32 graphical user inter-face.[32] Structures were solved by direct methods using SHELXS-86[34] or SIR97.[54] Multi-scan empirical absorption cor-rections, when applied, were applied to the data set using the program SADABS.[33] Data were refined and extended with SHELXL-93 or SHELXL-97.[34] In general, non-hydrogen atoms with occupancies greater than 0.5 were refined anisotropically. Carbon-bound hydrogen atoms were included in idealised pos-itions and refined using a riding model. Oxygen and nitrogen bound hydrogen atoms were first located in the difference Fourier map before refinement with bond length restraints. Where these hydrogen atoms could not be located, they were not modelled. Disorder was modelled using standard crystallo-graphic methods including constraints and restraints where necessary. Structural data are summarised in Table 1 and specific details (where necessary) pertaining to the refinement is given below.

[Co(naac)tren]·0.5H$_2$O·2ClO$_4$. Two of the perchlorate anions in the structure are disordered and modelled each over two posi-tions with occupancies of 0.875 and 0.125 respectively.

Table 1 Crystallographic data

Compound	[Co(naac)tren]· 0.5H$_2$O·2ClO$_4$	[Co(bha)tren]· 2ClO$_4$	[Co(aha)tren]· 2ClO$_4$	[Co(bzac)tren]· 0.6I·1.4ClO$_4$	[Co(acac)tren]· 2H$_2$O·2Cl	[Co(nha)tren]· 2ClO$_4$
Formula of refinement model	C$_{80}$H$_{121}$Cl$_8$Co$_4$ N$_{16}$O$_{42.50}$	C$_{13}$H$_{24}$Cl$_2$ CoN$_5$O$_{10}$	C$_8$H$_{22}$Cl$_2$ CoN$_5$O$_{10}$	C$_{16}$H$_{27}$Cl$_{1.4}$ CoI$_{0.6}$N$_4$O$_{7.6}$	C$_{11}$H$_{25}$Cl$_2$ CoN$_4$O$_4$	C$_{18}$H$_{28}$Cl$_2$ CoN$_5$O$_{10}$
Molecular weight	2506.25	540.20	478.13	581.72	407.18	604.28
Crystal system	Monoclinic	Orthorhombic	Triclinic	Orthorhombic	Triclinic	Monoclinic
Space group	$P2_1/c$	$P2_12_12_1$	$P\bar{1}$	$P2_12_12_1$	$P1$	$P2_1/n$
a (Å)	13.3978(8)	9.1320(6)	8.455(3)	10.139(2)	7.779(3)	8.2091(3)
b (Å)	28.7543(18)	13.5840(9)	9.447(3)	14.370(3)	7.864(3)	16.7587(7)
c (Å)	26.4339(17)	16.711(1)	12.232(4)	15.118(3)	8.045(3)	17.1486(7)
α (°)			98.584(6)		100.627(6)	
β (°)	93.677(4)		101.007(6)		106.855(6)	93.560(2)
γ (°)			112.488(6)		102.071(6)	
V (Å^3)	10162.5(11)	2073.0(2)	859.2(5)	2202.7(8)	444.5(3)	2354.65(16)
D_c (g cm^{-3})	1.638	1.731	1.848	1.754	1.521	1.705
Z	4	4	2	4	1	4
Crystal size (mm)	0.28 × 0.20 × 0.20	0.2 × 0.2 × 0.1	0.2 × 0.2 × 0.02	0.32 × 0.19 × 0.15	0.339 × 0.255 × 0.129	0.30 × 0.28 × 0.25
Crystal colour	Red	Red	Red	Red	Red	Red
Crystal habit	Block	Prism	Plate	Prism	Plate	Block
Temperature (K)	150(2)	273(2)	273(2)	150(2)	150(2)	150(2)
λ(MoKα)	0.71073	0.71073	0.71073	0.71073	0.71073	0.71073
μ (mm^{-1})	0.951	1.149	1.372	1.838	1.286	1.022
T(Empirical)$_{min,max}$	0.766, 0.827	0.8046, 0.9015	0.283766, 1.000000	0.649, 0.759	N/A	0.6688, 0.7457
$2\theta_{max}$ (°)	63.60	56.02	55.88	56.74	55.90	60.00
hkl range	−19 to 18, −42 to 42, −39 to 39	−12 to 12, 0 to 17, 0 to 22	−11 to 10, −12 to 12, 0 to 16	−13 to 13, −18 to 18, −20 to 19	−10 to 10, −10 to 10, −10 to 10	−11 to 11, −23 to 23, −24 to 23
N_{obs} (N_{var})	218 559 (1507)	4887 (280)	2975 (235)	22 247 (308)	4102 (212)	38 930 (325)
N_{ind} (R_{merge})	34 567 (0.0320)	2802 (N/A)	3992 (N/A)	5347 (0.0383)	3778 (0.0640)	6851 (0.0477)
$N_{obs} - (I > 2\sigma(I))$	23 279	4569	2097	4869	3772	5628
$R_1 - (I > 2\sigma(I))$, w$R_2 -$ (all)	0.0346, 0.1054	0.0425, 0.1219	0.0720, N/A	0.0308, 0.0777	0.0557, 0.1505	0.0335, 0.1215
GoF	1.038	1.048	2.510	1.051	1.054	1.132
Residual extrema/e$^-$ Å^{-3}	−0.480, 0.628	−0.372, 1.082	−1.26, 1.48	−0.331, 0.609	−0.940, 0.769	−0.619, 0.648

Compound	[Co(Clacac)tpa]· 0.5H$_2$O·2ClO$_4$	[Co(nha-H)tpa]· EtOH·2H$_2$O·ClO$_4$	[Co(bzac)tpa]· 2ClO$_4$,	[Co(cat)tpa]· ClO$_4$	[Co(naac)tpa]· 2ClO$_4$	[Co(acac)tpa]· H$_2$O·2ClO$_4$
Formula of refinement model	C$_{46}$H$_{50}$Cl$_6$ Co$_2$N$_8$O$_{21}$	C$_3$H$_{30}$ ClCoN$_5$O$_9$	C$_{28}$H$_{27}$Cl$_2$ CoN$_4$O$_{10}$	C$_{56}$H$_{48}$Cl$_2$ Co$_2$N$_8$O$_{12}$	C$_{32}$H$_{29}$Cl$_2$ CoN$_4$O$_{10}$	C$_{23}$H$_{27}$Cl$_2$ CoN$_4$O$_{11}$
Molecular weight	1381.50	710.98	709.37	1213.78	759.42	665.32
Crystal system	Monoclinic	Orthorhombic	Monoclinic	Monoclinic	Monoclinic	Triclinic
Space group	Cc	$P2_12_12$	$P2_1/n$	$P2_1/c$	$P2_1/n$	$P2_1/c$
a (Å)	17.281(6)	14.729(5)	12.011(2)	21.553(3)	12.836(5)	15.9070(8)
b (Å)	17.298(6)	23.560(8)	18.474(3)	10.8220(14)	17.758(7)	8.9780(4)
c (Å)	18.867(6)	9.935(3)	13.884(3)	22.239(3)	14.707(6)	19.1400(9)
α (°)						
β (°)	90.172(7)		106.166(6)	93.066(9)	104.636(7)	97.115(3)
γ (°)						
V (Å^3)	5640(3)	3447.4(19)	2958.9(9)	5179.7(12)	3244(2)	2712.4(2)
D_c (g cm^{-3})	1.627	1.370	1.592	1.556	1.555	1.629
Z	4	4	4	4	4	4
Crystal size (mm)	0.46 × 0.31 × 0.25	0.50 × 0.26 × 0.24	0.28 × 0.20 × 0.13	0.2 × 0.15 × 0.10	0.345 × 0.267 × 0.198	0.30 × 0.25 × 0.20
Crystal colour	Red	Green	Red	Green	Red	Red
Crystal habit	Prism	Prism	Prism	Plate	Prism	Block
Temperature (K)	150(2)	150(2)	90(2)	150(2)	150(2)	90(2)
λ(MoKα)	0.71073	0.71073	0.71073	0.71073	0.71073	0.71073
μ (mm^{-1})	0.957	0.633	0.826	0.818	0.759	0.897
T(Empirical)$_{min,max}$	0.670, 0.787	0.853, 1.000	0.6793, 0.7458	0.6688, 0.7457	0.737, 1.00	0.6640, 0.7460
$2\theta_{max}$ (°)	56.70	56.60	57.44	50.00	56.16	60.10
hkl range	−23 to 22, −22 to 23, −25 to 24	−19 to 19, −31 to 31, −13 to 13	−16 to 16, −24 to 24, −18 to 18	−25 to 25, −12 to 12, −26 to 26	−16 to 16, −23 to 22, −19 to 19	−22 to 22, −12 to 12, −26 to 26
N_{obs} (N_{var})	27 035 (835)	34 314 (352)	26 163 (407)	52 547 (721)	31 644 (443)	30 220 (378)
N_{ind} (R_{merge})	13 195 (0.0736)	8350 (0.0273)	7646 (0.0711)	9100 (0.1921)	7718 (0.0813)	7922 (0.0536)
$N_{obs} - (I > 2\sigma(I))$	12 072	7494	5192	4288	5676	6203
$R_1 - (I > 2\sigma(I))$, w$R_2 -$ (all)	0.0836, 0.2292	0.0656, 0.1651	0.0516, 0.1453	0.0635, 0.1303	0.0407, 0.0995	0.0420, 0.1189
GoF	1.014	1.393	1.033	0.937	1.205	1.028
Residual extrema/e$^-$ Å^{-3}	−0.589, 0.756	−0.557, 0.980	−0.524, 0.905	−0.647, 0.744	−0.459, 0.663	−0.727, 1.113

[Co(acac)tren]·2H$_2$O·2Cl. This data set proved to be twinned by a two-fold rotation about *c*. Accordingly no absorption correction was applied. The water-hydrogen atoms could not be located in the difference Fourier map and were not modelled.

[Co(Clacac)tpa]·0.5H$_2$O·2ClO$_4$. The structure crystallised in the monoclinic space group *Cc* with *β* very close to 90° (90.172(7)°) and *a* and *b* approximately equal, proving to be a twin emulating tetragonal symmetry with an additional general twining component. The twinning was accounted for by the use of the appropriate twin law[35] resulting in a significant decrease in the *R* factor. In addition, one of the two complexes in the asymmetric unit is rotationally disordered and was modelled over two positions with occupancies of 0.75 and 0.25. The thermal parameters of the lower occupancy component were refined isotropically with a shared value. Two of the perchlorate anions are also modelled over two positions with a total occupancy of 1 each. The water-hydrogen atoms could not be located in the difference Fourier map and were not modelled.

[Co(nha-H)tpa]·EtOH·2H$_2$O·ClO$_4$. The napthalene group, water solvents and perchlorate anions are all disordered.

[Co(cat)tpa]·ClO$_4$. One of the perchlorate anions is disordered and modelled over two positions.

Cytotoxicity studies

IC$_{50}$ values were determined by seeding 1×10^5 cells per mL into each well of a 96-well plate and incubating under standard culturing conditions. Compounds were prepared as 5 mM solutions in 10% DMSO except [Co(naac)tpa](ClO$_4$)$_2$ and [Co-(naac)tren](ClO$_4$)$_2$ which were prepared as 5 mM solutions in 20% DMSO. naacH was prepared as a 13 mM solution in 75% DMSO, and catH$_2$ was prepared as a 5 mM solution in 50% DMSO solution prior to the assay. Concentrated drug samples were prepared to ensure the solvent systems were not toxic to the DLD-1 cells. All solutions were then filtered through a 0.25 mm Millex Syringe Filters (Millipore). The dose volume of the compound solutions was kept below 40 µL and diluted in culture medium to be added to triplicate wells, spanning a 4-log range of final concentrations. The cells were then incubated for 72 hours before 1.0 mM MTT was added to each well and further incubated for 4 hours. The media was removed and 150 µL of DMSO was added to each well. The absorbance of each well was then measured at 600 nm after shaking for 1 min in a Victor^{3}V microplate reader (Perkin-Elmer). IC$_{50}$ values were determined as the compound concentration that reduced the absorbance to 50% of that in untreated control wells.

Synthesis

[CoCl$_2$tren]Cl·H$_2$O,[36] [CoCO$_3$tren]ClO$_4$·0.5H$_2$O,[36] tpa·nHClO$_4$,[37] [Co(NO$_2$)$_2$tpa]ClO$_4$,[37] [CoCl$_2$tpa]ClO$_4$,[38] [Co(aha-H)tpa]ClO$_4$,[25] [Co(bha-H)tpa]ClO$_4$[25] and 1-methyl-3-(2-naphthyl)propane-1,3-dione (naacH),[39] were all synthesised using published experimental procedures. Methyl-2-(naphthalen-1-yl)acetate was prepared from 2-(naphthalen-1-yl)acetic acid using standard esterification techniques. A schematic diagram of the ligands used in this study is shown in Fig. 1.
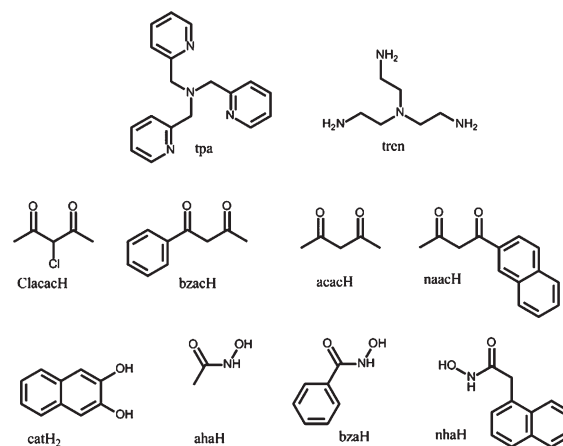

Fig. 1 Ligands employed in this study.

[Co(acac)tpa](ClO$_4$)$_2$. Acetylacetone (acacH) (1.4 mmol, 140 mg, 136 µL) was added to a dispersion of [CoCl$_2$tpa]ClO$_4$ (520 mg, 1.0 mmol) in methanol (5.0 mL). After 5 minutes, triethylamine (1.2 mmol, 120 mg, 170 µL) was added and the solution was refluxed for 1 hour at 50 °C. The solution was cooled on an ice bath, diethyl ether (25 mL) was added and the precipitate collected. The crude product was dispersed in an aqueous NaClO$_4$ solution (0.10 M, 5.0 mL) and acetone added until the complete dissolution of the product. Slow evaporation of this solution yielded red square crystals of [Co(acac)tpa](ClO$_4$)$_2$ (Yield: 52%, 337 mg, 0.52 mmol). Crystals suitable for X-ray analysis were taken directly from this mixture. ^{1}H NMR (DMSO): *δ* 9.14 (d, H, pyridyl), 8.24 (d, 2H, pyridyl), 8.16 (t, 2H, pyridyl), 7.99 (t, H, pyridyl), 7.79 (d, 2H, pyridyl), 7.74 (t, H, pyridyl), 7.60 (t, 2H, pyridyl), 7.40 (d, H, pyridyl), 5.88 (s, 1H, γ-hydrogen of acac), 5.54 (half of AB system, 2H, CH$_2$N), 5.27 (s, 2H, CH$_2$N), 5.03 (half of AB system, 2H, CH$_2$N), 2.75 (s, 3H, CH$_3$), 2.00 (s, 3H, CH$_3$). ^{13}C NMR (DMSO): *δ* 191.84, 191.64, 163.26, 162.22, 148.84, 147.09, 141.58, 140.94, 126.52, 125.97, 124.73, 122.24, 99.62, 69.78, 68.435, 27.33, 26.24. Mass spec (ESI positive ion): *m/z*: 547 ([Co(acac)tpa](ClO$_4$)$^+$), 224 ([Co(acac)tpa]$^{2+}$). *Anal.* Required for CoH$_{25}$C$_{23}$N$_4$O$_{10}$Cl$_2$: C, 42.67, H, 3.89, N, 8.66%. Found: C, 42.63, H, 3.91, N, 8.71%.

[Co(Clacac)tpa](ClO$_4$)$_2$. [Co(Clacac)tpa](ClO$_4$)$_2$ (ClacacH = 3-chloro-2,4,-pentanedione) was synthesised in an analogous manner to [Co(acac)tpa](ClO$_4$)$_2$ (Yield: 60%, 408 mg, 0.60 mmol). Slow evaporation of a solution of the product yielded red square crystals of [Co(Clacac)tpa](ClO$_4$)$_2$ suitable for X-ray analysis. ^{1}H NMR (DMSO): *δ* 9.12 (d, H, pyridyl), 8.26 (d, 2H, pyridyl), 8.19 (t, 2H, pyridyl), 8.01 (t, H, pyridyl), 7.82 (d, 2H, pyridyl), 7.74 (t, H, pyridyl), 7.63 (t, 2H, pyridyl), 7.39 (d, H, pyridyl), 5.60 (half of AB system, 2H, CH$_2$N), 5.25 (s, 2H, CH$_2$N), 5.02 (half of AB system, 2H, CH$_2$N), 2.99 (s, 3H, CH$_3$), 2.27 (s, 3H, CH$_3$). ^{13}C NMR (DMSO): *δ* 189.45, 162.99, 162.78, 149.51, 146.76, 141.46, 140.77, 126.45, 125.82, 124.59, 122.08, 106.11, 69.58, 68.20, 28.14, 27.22. Mass spec (ESI positive ion) *m/z*: 581 ([Co(Clacac)tpa](ClO$_4$)$^+$), 241 ([Co(Clacac)tpa]$^{2+}$). *Anal.* Required for CoH$_{24}$C$_{23}$N$_4$O$_{10}$Cl$_3$: C, 40.52, H, 3.55, N, 8.22%. Found: C, 40.41, H, 3.61, N, 8.25%.

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[Co(bzac)tpa](ClO$_4$)$_2$. 1-Phenyl-1,3-butanedione (bzacH) (1.4 mmol, 227 mg) was added to a dispersion of [CoCl$_2$tpa]-ClO$_4$ (520 mg, 1.0 mmol) in methanol (5.0 mL). After 5 minutes, triethylamine (1.2 mmol, 121 mg, 170 μL) was added and the solution was refluxed for 1 hour at 60 °C. The solution was cooled on an ice bath and the precipitate was collected. The crude product was washed several times with diethyl ether before being dispersed in an aqueous NaClO$_4$ solution (0.10 M, 5.0 mL). Acetone was added until complete dissolution of the product was achieved. Slow evaporation of this solution yielded red needles of [Co(bzac)tpa](ClO$_4$)$_2$ (Yield: 35%, 248 mg, 0.35 mmol). ^{1}H NMR (DMSO): δ 9.20 (d, H, pyridyl), 8.33 (d, 2H, pyridyl), 8.14 (t, 2H, pyridyl), 8.03 (t, H, pyridyl), 7.87 (d, 2H, phenyl), 7.81–7.77 (m, 3H, pyridyl), 7.63–7.54 (m, 3H, (2H pyridyl, H phenyl), 7.49–7.40 (m, 3H, (2H phenyl, 1H pyridyl)) 5.60 (half of AB system, 2H, CH$_2$N), 5.34 (s, 2H, CH$_2$N), 5.14 (half of AB system, 2H, CH$_2$N), 2.93 (s, 3H, CH$_3$). ^{13}C NMR (DMSO): δ 194.44, 181.52, 163.35, 163.12, 149.78, 147.10, 141.60, 141.04, 134.32, 133.29, 128.85, 127.72, 126.62, 126.01, 124.96, 122.31, 96.60, 70.08, 68.60, 28.10. Mass spec (ESI positive ion): m/z: 609 ([Co(bzac)tpa](ClO$_4$)$^+$); 255 ([Co(bzac)-tpa]$^{2+}$). *Anal*. Required for CoH$_{27}$C$_{28}$N$_4$O$_{10}$Cl$_2$: C, 47.41, H, 3.84, N, 7.90%. Found: C, 47.37, H, 3.96, N, 7.88%.

[Co(naac)tpa](ClO$_4$)$_2$. [Co(naac)tpa](ClO$_4$)$_2$ was synthesised in an analogous manner to [Co(bzac)tpa](ClO$_4$)$_2$ (Yield: 42%, 319 mg, 0.42 mmol). Slow evaporation of an acetone–water solution containing the product yielded crystals suitable for X-ray analysis. ^{1}H NMR (DMSO): δ 9.24 (d, H, pyridyl), 8.52 (s, H, naphthyl), 8.37 (d, 2H, pyridyl), 8.17–7.82 (m, 10H, (6H pyridyl, 4H naphthyl)) 7.65–7.57 (m, 4H, (2H pyridyl, 2H naphthyl)), 7.46 (d, H, pyridyl) 6.92 (s, 1H, γ-hydrogen of acac), 5.72 (half of AB system, 2H, CH$_2$N), 5.38 (s, 2H, CH$_2$N), 5.21 (half of AB system, 2H, CH$_2$N), 2.99 (3H, s, CH$_3$). ^{13}C NMR (DMSO): δ 194.23, 181.45, 163.36, 163.18, 149.58, 147.14, 141.60, 141.05, 134.72, 131.97, 131.67, 129.58, 128.94, 128.76, 128.43, 127.41, 126.95, 126.62, 126.01, 125.00, 123.58, 122.31, 96.96, 70.09, 69.00, 28.20. Mass spec. (ESI positive ion): m/z: 659 ([Co(naac)tpa](ClO$_4$)$^+$); 280 ([Co(naac)tpa]$^{2+}$). *Anal*. Required for CoH$_{29}$C$_{32}$N$_4$O$_{10}$Cl$_2$: C, 50.61, H, 3.85, N, 7.38%. Found: C, 50.68, H, 3.91, N, 7.43%.

[Co(acac)tren]Cl$_2$·2H$_2$O. [Co(acac)tren]Cl$_2$·2H$_2$O was prepared by a modification of the procedure used for synthesising [Co(acac)tren]I$_2$ by Nakano *et al*.[40] [CoCl$_2$tren]Cl·H$_2$O (1.0 g, 3.0 mmol) was dissolved in water (20 mL) and acacH (480 mg, 495 μL, 4.8 mmol) added. After 10 minutes an aqueous sodium hydroxide solution (1.0 M, 5.0 mL) was added and the solution was stirred for 3 hours at 60 °C. After the mixture was cooled to room temperature, water (75 mL) was added and the solution was loaded onto a Sephadex column (Na$^+$ form, 3.5 × 20 cm). The column was washed with water and 0.01 M NaCl and the product was eluted with 0.3 M NaCl. Once the excess salt was removed (with considerable product loss), the compound was dissolved in a minimal amount of water, and deep red crystals of [Co(acac)tren]Cl$_2$·2H$_2$O were precipitated upon the slow diffusion of ethanol (Yield: 48%, 616 mg 1.5 mmol). Crystals suitable for X-ray analysis were taken directly from this crop. ^{1}H NMR (D$_2$O): δ 5.78 (s, 1H, γ-hydrogen of acac), 3.62 (td,

2H, tren), 3.44 (td, 2H, tren), 3.30 (t, 2H, tren), 3.14 (dd, 2H, tren), 2.97 (t, 2H, tren), 2.79 (dd, 2H, tren), 2.23, (s, 3H, CH$_3$ of acac), 2.14, (s, 3H, CH$_3$ of acac). ^{13}C NMR (D$_2$O): δ 195.45, 193.63, 101.58, 63.78, 63.32, 47.44, 45.87, 29.43, 28.95. Mass spec (ESI positive ion): m/z: 152 ([Co(acac)tren^{2+}). *Anal*. Required for CoH$_{29}$C$_{11}$N$_4$O$_4$Cl$_2$: C, 32.13, H, 7.11, N, 13.63%. Found: C, 32.07, H, 7.07, N, 13.70%.

[Co(bzac)tren]I$_2$. [Co(bzac)tren]I$_2$ was prepared by an adaptation of the procedure used for synthesising [Co(acac)tren]I$_2$ by Nakano *et al*.[40] A mixture of [CoCO$_3$tren]ClO$_4$·0.5H$_2$O (1.0 g, 2.7 mmol) in 2.5 M HCl (4 mL) and stirred for half an hour until effervescence had ceased. BzacH (620 mg, 3.9 mmol) was added to this solution followed by an aqueous sodium hydroxide solution (0.50 M, 16 mL) and the mixture was stirred at 80 °C for 3 hours. The solution was cooled to room temperature, filtered, and solid KI was added to the filtrate until the precipitation of orange/red [Co(bzac)tren]I$_2$ was achieved. The product was washed once with ethanol and several times with diethyl ether before being recrystallised from water (Yield: 51%, 930 mg, 1.5 mmol). Crystals suitable for X-ray analysis were formed by slow evaporation of the filtrate. ^{1}H NMR (D$_2$O): δ 7.99 (d, 2H, phenyl), 7.66 (t, H, phenyl), 7.55 (t, 2H, phenyl), 6.50 (s, 1H, γ-hydrogen of acac), 3.75, (td, 2H, tren), 3.50 (td, 2H, tren), 3.35 (t, 2H, tren), 3.16 (dd, 2H, tren), 2.97 (t, 2H, tren), 2.89 (dd, 2H, tren) 2.30 (s, 3H, CH$_3$ of acac) ^{13}C NMR (D$_2$O): δ 197.86, 184.60, 138.83, 135.50, 131.62, 130.25, 98.67, 64.25, 63.53, 47.52, 45.98, 29.77. Mass spec (ESI positive ion): m/z: 183 ([Co(bzac)tren]$^{2+}$). *Anal*. Required for CoH$_{27}$C$_{16}$N$_4$O$_2$I$_2$: C, 30.99, H, 4.39, N, 9.04%. Found: C, 31.03, H, 4.42, N, 9.02%. [Co(bzac)tren](ClO$_4$)$_2$ could also be prepared by a small modification of the above synthesis by addition of dilute perchloric acid (0.1 M) instead of solid KI. The NMR was as described for [Co(acacphen)tren]I$_2$. *Anal*. Required for CoH$_{27}$C$_{16}$N$_4$O$_{10}$Cl$_2$: C, 34.00, H, 4.81, N, 9.91%. Found: C, 34.02, H, 4.81, N, 9.72%.

[Co(naac)tren](ClO$_4$)$_2$. NaacH (1.0 g, 5.0 mmol) was added to a dispersion of [CoCl$_2$tren]Cl·H$_2$O (1.0 g, 3.0 mmol) in MeOH (40 mL). The solution was stirred for five minutes before triethylamine (4.5 mmol) was added and the solution was refluxed for 48 hours. The solution was dried over a nitrogen line, water (100 mL) was added and the solution was filtered. The filtrate was then loaded onto a Sephadex column, washed with water, and the major product was eluted with 0.30 M NaClO$_4$. Slow evaporation of the eluent solution caused the precipitation of red crystals of [Co(naac)tren](ClO$_4$)$_2$. (Yield: 25%, 461 mg, 0.75 mmol) ^{1}H NMR (D$_2$O): δ 8.56 (s, 1H, naphthyl), 8.12–8.03 (m, 4H, naphthyl), 7.75–7.65 (m, 2H, naphthyl), 6.67 (s, 1H, γ-hydrogen of acac), 3.86 (td, 2H, tren), 3.56 (td, 2H, tren), 3.39 (t, 2H, tren), 3.19 (dd, 2H, tren), 3.03 (t, 2H, tren), 2.95 (dd, 2H, tren), 2.32 (s, 3H, CH$_3$). ^{13}C NMR (D$_2$O): δ 197.79, 184.46, 137.74, 136.36, 135.15, 132.14, 131.46, 131.32, 131.13, 130.63, 130.11, 126.60, 98.99, 64.35, 63.56, 47.56, 46.00, 29.80. Mass spec (ESI positive ion): m/z: 515 ([Co(naac)tren](ClO$_4$)$^+$); 208 ([Co(naac)tren]$^{2+}$). *Anal*. Required for CoH$_{29}$C$_{20}$N$_4$O$_{10}$Cl$_2$: C, 39.04, H, 4.75, N, 9.11%. Found: C, 38.94, H, 4.79, N, 8.98%.

[Co(aha)tren](ClO$_4$)$_2$·H$_2$O. Aqueous sodium hydroxide (1.0 M, 3.5 mL) was added dropwise slowly to a solution of acetohydroxamic acid (ahaH) (285 mg, 3.8 mmol) and [CoCl$_2$-tren]Cl·H$_2$O (1.0 g, 3.0 mmol) in water (30 mL) and the mixture stirred at 70 °C for 6 hours. The solution was cooled, the solvent removed over nitrogen and ethanol (20 mL) added to the solid residue. The solution was filtered and a saturated solution of sodium perchlorate in ethanol was added to the filtrate. The resulting precipitate was collected, washed with ethanol, isopropanol and diethyl ether (Yield: 48%, 688 mg, 1.4 mmol). Crystals were grown from an ethanol–water solution of the product. ^{1}H NMR (D$_2$O): δ 3.45–3.30 (m, 4H, tren), 3.23–3.15 (m, 4H, tren), 3.00–2.92 (m, 4H, tren), 2.05 (s, 3H, CH$_3$). ^{13}C NMR (D$_2$O): δ 173.13, 65.66, 62.97, 47.24, 46.21, 16.97. Mass spec (ESI positive ion): m/z: 277 ([Co(aha-H)tren]$^+$). *Anal.* Required for CoH$_{24}$C$_8$N$_5$O$_{11}$Cl$_2$: C, 19.37, H, 4.65, N, 14.01%. Found: C, 19.37, H, 4.88, N, 14.12%.

[Co(aha-H)tren](ClO$_4$). [Co(aha-H)tren](ClO$_4$) was synthesised by dissolving [Co(aha)tren](ClO$_4$)$_2$·H$_2$O (200 mg, 0.42 mmol) in ethanol (5 mL) and adding one and a half equivalents of sodium hydroxide (1 M, 1.5 equivalents). The solution was allowed to sit in a fridge overnight, over which time violet crystals of [Co(aha-H)tren](ClO$_4$) precipitated out of solution (Yield: 52%, 82 mg, 0.22 mmol). NMR experiments performed in DMSO confirmed the removal of the N*H* proton on the hydroxamic acid. The complex crystallised as two isomers in approximately 3 : 2 ratio. Major isomer: ^{1}H NMR (D$_2$O): δ 3.64–2.85 (m, 12 H, tren), δ 1.88 (s, 3H, CH$_3$). ^{13}C NMR (D$_2$O): δ 168.15, 62.85, 59.12, 44.51, 43.90, 14.51. Minor isomer: ^{1}H NMR (D$_2$O): 3.64–2.85 (m, 12 H, tren), 1.82 (s, 3H, CH$_3$). ^{13}C NMR (D$_2$O): 167.46, 62.68, 60.01, 44.12, 43.69, 14.19. Mass spec (ESI positive ion): m/z: 277 ([Co(aha-H)tren]$^+$).

[Co(bha-H)tren](ClO$_4$). [Co(bha-H)tren](ClO$_4$) was synthesised by an adaptation of the procedure used for [Co(aha)tren]-(ClO$_4$)$_2$ but with aqueous sodium hydroxide (1.0 M, 10 mL), benzohydroxamic acid (bhaH) (520 mg, 3.8 mmol) and [CoCl$_2$-tren]Cl·H$_2$O (1.0 g, 3.0 mmol) in water (30 mL). [Co(bha-H)tren]-(ClO$_4$) was collected as a purple solid (Yield: 64%, 840 mg, 1.9 mmol). The complex crystallised as two isomers in a ratio approximately 3 : 2. Major isomer: ^{1}H NMR (D$_2$O): δ 7.92 (d, 2H, phenyl), 7.70 (m, 3H, phenyl), 3.91 (m, 2H, tren), 3.64–2.85 (m, 10 H, tren). ^{13}C NMR (D$_2$O): δ 128.65, 127.44, 126.10, 124.00, 59.51, 56.25, 42.32, 41.58. Minor isomer: ^{1}H NMR (D$_2$O): δ 7.75 (d, 2H, phenyl, 7.70 (m, 3H, phenyl), 3.55 (m, 2H, tren) 3.64–2.85 (m, 10 H, tren). ^{13}C NMR (D$_2$O): δ 128.62, 127.44, 126.31, 124.19, 59.99, 57.44, 42.18, 41.01. The carbon resonances for the C=O quaternary carbon were not observed in either isomer. Mass spec (ESI positive ion): m/z: 340 ([Co(bha-H)tren]$^+$) *Anal.* Required for C$_{13}$H$_{23}$ClCoN$_5$O$_6$: C, 35.51, H, 5.27, N, 15.93%. Found: C, 35.42, H, 5.27, N, 15.80%.

[Co(bha)tren](ClO$_4$)$_2$. [Co(bha)tren](ClO$_4$)$_2$ was synthesised by dispersing [Co(bha-H)tren](ClO$_4$) (200 mg, 0.46 mmol) in water (5.0 mL) and adding 1.1 equivalents of perchloric acid (0.1 M). The solution was allowed to sit in a fridge overnight, over which time violet crystals of [Co(bha)tren](ClO$_4$) precipitated (Yield: 55%, 135 mg, 0.25 mmol). NMR experiments

performed in DMSO confirmed the appearance of the N*H* proton on the hydroxamic acid at approximately 12.0 ppm. ^{1}H NMR (D$_2$O): δ 7.78 (t, 2H, phenyl), 7.68 (t, H, phenyl), 7.57 (t, 2H, phenyl), 3.48 (m, 4H, tren), 3.25 (m, 4H, tren), 3.03 (m, 4H, tren). ^{13}C NMR (D$_2$O): δ 169.78, 133.05, 129.46, 127.09, 126.30, 63.30, 60.00, 44.86, 43.84. Mass spec (ESI positive ion): m/z: 439 ([Co(bha)tren]ClO$_4^+$); 340 ([Co(bha-H)tren]$^+$); 170 ([Co(bha)tren]$^{2+}$).

2-(Naphthalen-1-yl)hydroxamic acid. A suspension of hydroxylammonium chloride (10 g, 0.14 mol) in MeOH (10 mL) was added dropwise to a solution of KOH (12 g, 0.21 mol) in water (20 mL), with the temperature kept below 5 °C using an ice bath. The solution was filtered and the filtrate was added to a solution of methyl-2-(naphthalen-1-yl)acetate (3.0 g, 15 mmol) in methanol (10 mL) and stirred for 4 hours at 5 °C. The volume was reduced to 20 mL over a stream of nitrogen before concentrated HCl was added dropwise until the formation of nhaH was apparent as a white precipitate. This was collected at the pump, washed with generous amounts of water and diethyl ether, and dried under vacuum (Yield: 78%, 2.75 g, 11.7 mmol). ^{1}H NMR (MeOD): δ 8.04 (d, H, naphthyl), 7.73–7.62 (m, 2H, naphthyl), 7.55–7.42 (m, 4H, naphthyl), 3.91 (s, 2H, CH$_2$). ^{13}C NMR (MeOD): δ 170.86, 135.30, 133.74, 132.25, 129.66, 128.87, 127.25, 126.77, 126.50, 124.73, 38.11. Mass spec (ESI positive ion): m/z: 224 ([nhaH + Na]$^+$). Mass spec (ESI negative ion): m/z: 200 ([nha]$^-$). *Anal.* Required for H$_{11}$C$_{12}$NO$_2$: C, 71.62, H, 5.51, N, 6.96%. Found: C, 71.74, H, 5.61, N, 6.94%.

[Co(nha-H)tpa]ClO$_4$·H$_2$O. Aqueous sodium hydroxide (1.0 M, 2.5 mL) was added dropwise to a dispersion of [CoCl$_2$tpa]ClO$_4$ (520 mg, 1 mmol) and nhaH (240 mg, 1.2 mmol) in methanol (40 mL). After 1 hour the solution was reduced in volume to approximately 30 mL, water was added (200 mL), the was solution filtered and the filtrate was loaded onto a Sephadex column (Na$^+$ form, 3.5 × 20 cm). The column was washed with water and the major green band eluted with 0.1 M NaCl. The eluent was reduced in volume over a nitrogen line, a few drops of saturated NaClO$_4$ solution were added and the solution was left to stand in the refrigerator overnight. The resulting precipitate of [Co(nha-H)tpa]ClO$_4$·H$_2$O was collected, washed with water and diethyl ether and air dried (Yield: 30%, 200 mg, 0.3 mmol). The complex crystallised as two isomers in 1 : 1 ratio. Crystals suitable for X-ray diffraction were prepared by slow evaporation of an ethanol–water (95 : 5) solution containing the product. ^{1}H NMR (DMSO): δ 9.07 (d, H, pyridyl), 9.02 (d, H, pyridyl), 8.48 (d, 2H, pyridyl), 8.39 (d, H, pyridyl), 7.99–7.74 (m, 10H), 7.56–7.26 (m, 19H), 6.97 (t, 3H) 6.56 (d, H), 6.46 (d, H, pyridyl), 5.19–4.88 (m, 12H, CH$_2$N), 4.06 (s, 2H, CH$_2$CONH) 3.58 (s, 2H, CH$_2$CONH). ^{13}C NMR (DMSO): δ 162.98, 162.48, 162.24, 161.62, 161.49, 149.34, 149.25, 149.15, 147.63, 139.65, 139.50, 139.30, 135.43, 134.90, 133.29, 132.25, 132.06, 131.35, 128.24, 128.00, 127.44, 126.78, 126.01, 125.54, 125.36, 125.20, 125.06, 124.90, 124.74, 124.19, 123.89, 123.05, 122.89, 121.27, 68.91, 68.54, 68.04, 67.72, 32.81, 31.76. Mass spec (ESI positive ion): m/z: 548 ([Co(nha-H)tpa]$^+$). *Anal.* Required for CoH$_{29}$C$_{30}$N$_5$O$_7$Cl: C, 54.10, H, 4.39, N, 10.52%. Found: C, 53.98, H, 4.47, N, 10.50%.

[Co(nha)tpa](ClO₄)₂. [Co(nha)tpa](ClO₄)₂ was synthesised by dispersing [Co(nha-H)tpa](ClO₄) (200 mg, 0.31 mmol) in ethanol–water (4 : 1, 5 mL) and adding 1.1 equivalents of perchloric acid (0.1 M). The volume was reduced until the desired complex first precipitated, at which point the solution was allowed to sit in a fridge overnight, over which time violet crystals of [Co(nha)tren](ClO₄) precipitated out of solution (Yield: 75%, 173 mg, 0.23 mmol). The complex crystallised as two isomers in 5 : 2 ratio. NMR experiments performed in DMSO confirmed the appearance of the N*H* proton on the hydroxamic acid at approximately 12.5 ppm. Major isomer: ^{1}H NMR (DMSO): δ 13.20 (s, N*H*), 8.60 (d, H, pyridyl), 8.45 (d, 2H, pyridyl), 8.10–7.20 (m, 16H, pyridyl and naphthyl), 5.19–4.95 (m, 6H, C*H₂*N), 3.96 (s, 2H, C*H₂*CONH). Minor Isomer: ^{1}H NMR (DMSO): 13.20 (s, N*H*), 8.85 (d, H, pyridyl), 8.45 (d, 2H, pyridyl), 8.10–7.20 (m, 16H, pyridyl and naphthyl), 5.19–4.95 (m, 6H, C*H₂*N), 4.50 (s, 2H, C*H₂*CONH). ^{13}C NMR (D₂O): δ 170.45, 170.40, 163.98, 163.24, 162.88, 159.85, 149.30, 148.91, 148.75, 148.28, 141.50, 141.12, 141.00, 139.84, 133.49, 133.45, 131.82, 131.39, 131.01, 131.40, 128.88, 128.03, 126.98, 126.90, 126.81, 126.74, 126.69, 126.61, 126.51, 126.39, 126.19, 126.08, 125.54, 124.92, 124.30, 123.67, 122.60, 69.81, 69.65, 69.24, 69.18, 32.85, 31.96. Mass spec (ESI positive ion): m/z 548 ([Co(nha)tpa]$^+$); 274 ([Co(nha)tpa]$^{2+}$).

[Co(nha-H)tren]ClO₄·H₂O. [Co(nha-H)tren]ClO₄·H₂O was prepared using a derivation of the synthesis of [Co(nha-H)tpa]-ClO₄·H₂O described above. Aqueous sodium hydroxide (1.0 M, 6.0 mL) was added dropwise to a dispersion of [CoCl₂tren]-Cl·H₂O (660 mg, 2 mmol) and nhaH (500 mg, 2.5 mmol) in methanol (150 mL). After 24 hours the solution was reduced in volume to approx 30 mL, water (100 mL) was added, the solution was filtered and the filtrate was loaded onto a Sephadex column (Na$^+$ form, 3.5 × 20 cm). The column was washed with water and the major maroon band was eluted with 0.2 M NaClO₄. Small crystals of [Co(nha-H)tren]ClO₄·H₂O formed upon slow evaporation of the eluent. (Yield: 42%, 436 mg, 0.84 mmol) ^{1}H NMR (D₂O): δ 8.13 (d, 1H, naphthyl), 8.00 (d, 1H, naphthyl), 7.92 (d, 1H, naphthyl), 7.67–7.47 (m, 4H, naphthyl), 4.01 (s, 2H, C*H₂*CONH), 3.64–2.85 (m, 12H, tren). ^{13}C NMR (D₂O): δ 171.70, 136.39, 136.01, 134.51, 131.70, 130.54, 129.40, 129.19, 128.71, 127.10, 126.28, 65.09, 62.56, 47.09, 45.87, 35.35. Mass spec (ESI positive ion): m/z: 404 ([Co-(nha-H)tren]$^+$). *Anal.* Required for CoH₂₉C₁₈N₅O₇Cl: C, 41.43, H, 5.60, N, 13.42%. Found: C, 41.41, H, 5.75, N, 13.41%.

[Co(nha)tren](ClO₄)₂. [Co(nha)tren](ClO₄)₂ was synthesised by dispersing [Co(nha-H)tren](ClO₄) (200 mg, 0.39 mmol) in ethanol (5.0 mL) and adding one equivalent of perchloric acid (0.10 M). The volume was reduced until the desired complex first precipitated, at which point the solution was allowed to sit in a fridge overnight, over which time red crystals of [Co(nha)-tren](ClO₄)₂ precipitated out of solution (Yield: 68%, 163 mg, 0.27 mmol). The complex crystallised as two isomers in a 1 : 1 ratio. NMR experiments performed in DMSO confirmed the appearance of the N*H* proton on the hydroxamic acid at approximately 13.1 ppm. ^{1}H NMR (D₂O): δ 8.28 (d, 1H, naphthyl), 8.10–7.91 (m, 4H, napthyl), 7.72–7.50 (m, 9H, napthyl), 4.27 (s, 2H, C*H₂*), 4.21 (s, 2H, C*H₂*), 3.41–2.75 (m, 24H, tren).

^{13}C NMR (D₂O): δ 174.14, 172.32, 136.09, 135.99, 134.01, 133.83, 131.97, 131.93, 131.50, 131.33, 131.04, 130.91, 130.85, 129.41, 129.10, 128.89, 128.29, 128.20, 126.34, 125.48, 65.08, 64.23, 62.41, 61.60, 46.75, 46.09, 45.97, 45.87, 34.85, 34.79. Mass spec (ESI positive ion): m/z: No ions observed.

[Co(cat)tpa]ClO₄. 2,3-Napthyldiol (catH₂) (1.2 mmol, 190 mg) was added to a dispersion of [CoCl₂tpa]ClO₄ (520 mg, 1.0 mmol) in methanol (5.0 mL). After 5 minutes, triethylamine (2.4 mmol, 242 mg, 340 μL) was added and the solution was refluxed for 1 hour at 60 °C. The solution was cooled on an ice bath, diethyl ether added and the precipitate was collected. The crude product was washed several times with diethyl ether before being dispersed in an aqueous NaClO₄ solution (0.10 M, 5.0 mL). Acetone was added until complete dissolution of the product was achieved. Slow evaporation of this solution yielded green needles of [Co(cat)tpa]ClO₄ (Yield: 82%, 500 mg, 0.82 mmol). Crystals suitable for X-ray diffraction were taken directly from this mixture. ^{1}H NMR (DMSO): δ 9.31 (d, H, pyridyl), 8.43 (d, 2H, pyridyl), 8.00 (m, 3H, pyridyl, napthyl), 7.70 (m, 3H, pyridyl, naphthyl), 7.47 (t, 2H, pyridyl), 7.40 (t, 2H, pyridyl), 7.25 (s, H, napthyl), 7.17 (d, H, pyridyl), 6.90, (m, 2H, naphthyl), 6.47 (s, H, naphthyl), 5.43, (d, 2H, C*H₂*), 5.15 (m, 4H, CH₂ × 2). ^{13}C NMR (DMSO): δ 163.91, 163.83, 163.74, 165.02, 151.71, 149.49, 141.20, 141.82, 129.75, 129.54, 126.59, 126.28, 125.01, 124.89, 124.58, 122.47, 121.28, 121.26, 109.77, 108.92, 69.97, 69.48. Mass spec (ESI positive ion): m/z: 507 ([Co(cat)tpa]$^+$). *Anal.* Required for C₂₈H₂₄ClCoN₄O₆: C, 55.41, H, 3.99, N, 9.23%. Found: C, 55.36, H, 4.03, N, 9.21%.

[Co(cat)tren]ClO₄·1.5H₂O. catH₂ (1.2 mmol, 190 mg) in MeOH (20 mL) was added to a solution of [CoCl₂tren]Cl (330 mg, 1.0 mmol) in water (5 mL). After 5 minutes, triethyl-amine (2.4 mmol, 242 mg, 340 μL) was added and the solution was stirred at room temperature. After 24 hours the solution was reduced in volume to approx 5 mL, water (100 mL) was added, the solution was filtered and the filtrate was loaded onto a Sepha-dex column (Na$^+$ form, 3.5 × 20 cm). The column was washed with water and the major maroon band was eluted with 0.20 M NaClO₄. Small crystals of [Co(cat)tren]ClO₄·H₂O formed upon slow evaporation of the eluent. (Yield: 36%, 130 mg, 0.36 mmol). ^{1}H NMR (DMSO): δ 7.30 (m, 2H, naphthyl), 6.81, (m, 3H, naphthyl) 6.65 (s, H, naphthyl), 4.99 (s, 2H, amine), 4.45 (s, 2H, amine), 3.98 (s, 2H, amine), 2.80 (m, 12H, tren). ^{13}C NMR (DMSO): δ 165.9, 163.3, 128.9, 128.6, 123.4, 123.3 119.9, 119.4, 108.3, 107.0, 61.1, 58.7, 43.9, 42.8. Mass spec (ESI positive ion): m/z: 362 ([Co(cat)tren]$^+$). *Anal.* Required for C₁₆H₂₇ClCoN₄O₇.₅: C, 39.24, H, 5.56, N, 11.44%. Found: C, 39.17, H, 5.50, N, 11.46%.

Results and discussion

Crystal structures

The bond lengths and angles observed in the solid-state structures of the complexes (Table 2) are commensurate with those of similar compounds described in the literature.[21,25,41,42] ORTEP[43] representations of [Co(naac)tpa](ClO₄)₂ and [Co(naac)tren]-(ClO₄)₂ are shown in Fig. 2. For all the tpa complexes with the exception of [Co(Clacac)tpa](ClO₄)₂, the bond distance from the

Table 2 Bond length (Å) comparison between the cobalt-tren and cobalt-tpa complexes with different ancillary ligands

Tren complexes

Structure	Co–N(1)	Co–N(2)	Co–N(3)	Co–(N4)	Co–O(1)	Co–O(2)
[Co(naac)tren](ClO$_4$)$_2$	1.9502(11)	1.9380(11)	1.9620(12)	1.9509(11)	1.8901(9)	1.8918(9)
	1.9462(11)	1.9465(11)	1.9656(12)	1.9557(11)	1.8850(9)	1.8909(9)
	1.9467(11)	1.9477(11)	1.9659(12)	1.9562(11)	1.8840(9)	1.8877(9)
	1.9482(11)	1.9396(11)	1.9594(12)	1.9516(11)	1.8906(9)	1.8905(9)
[Co(bha)tren](ClO$_4$)$_2$	1.925(3)	1.940(3)	1.962(3)	1.949(3)	1.894(2)	1.903(2)
[Co(aha)tren](ClO$_4$)$_2$	1.936(4)	1.945(4)	1.964(4)	1.958(4)	1.906(4)	1.899(3)
[Co(bzac)tren](ClO$_4$)$_2$	1.942(7)	1.942(7)	1.956(8)	1.954(7)	1.879(6)	1.882(6)
[Co(acac)tren](ClO$_4$)$_2$	1.927(6)	1.937(6)	1.961(10)	1.974(10)	1.899(6)	1.902(5)
[Co(nha-H)tren]ClO$_4$	1.9389(16)	1.9521(16)	1.9668(16)	1.9669(16)	1.9006(14)	1.9029(13)

Tpa complexes

Structure	Co–N(1)	Co–N(2)	Co–N(3)	Co–(N4)	Co–O(1)	Co–O(2)
[Co(Clacac)tpa](ClO$_4$)$_2$	1.928(11)	1.837(9)	1.916(8)	1.945(8)	1.894(7)	1.933(8)
	1.82(2)	1.73(2)	1.908(7)	1.945(7)	1.94(2)	2.06(2)
[Co(nha-H)tpa](ClO$_4$)	1.956(3)	1.939(3)	1.918(3)	1.921(3)	1.854(2)	1.867(3)
[Co(bzac)tpa](ClO$_4$)$_2$	1.944(2)	1.935(2)	1.928(3)	1.929(3)	1.872(2)	1.886(2)
[Co(cat)tpa](ClO$_4$)	1.946(5)	1.916(4)	1.915(5)	1.927(5)	1.892(4)	1.897(4)
	1.958(5)	1.918(4)	1.919(5)	1.926(5)	1.872(4)	1.891(4)
[Co(naac)tpa](ClO$_4$)$_2$	1.9421(17)	1.9296(18)	1.9211(19)	1.9316(19)	1.8739(15)	1.8857(15)
[Co(acac)tpa](ClO$_4$)$_2$	1.9510(17)	1.9304(17)	1.9240(18)	1.9281(18)	1.8847(14)	1.9027(14)
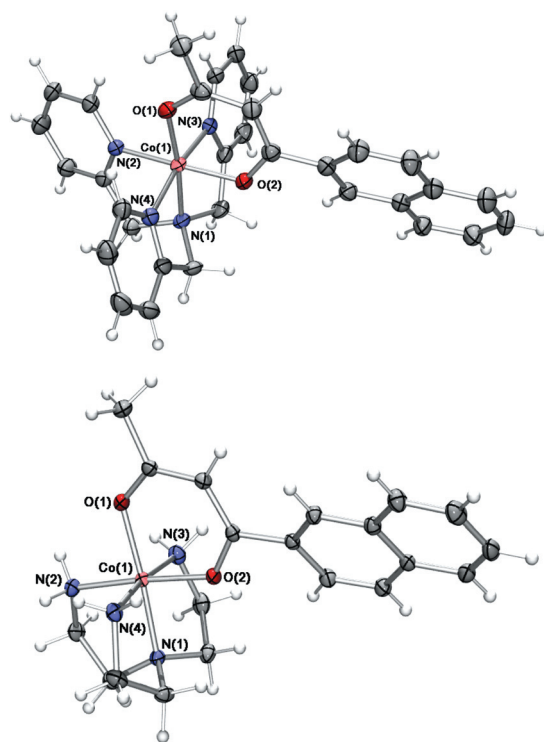
Fig. 2 ORTEP representations of [Co(naac)tpa](ClO$_4$)$_2$ (top) and [Co(naac)tren](ClO$_4$)$_2$ (bottom).

tertiary amine to the metal centre is longer (1.9421(17)–1.958(5) Å) than the Co–N lengths associated with the pyridyl moieties (1.915(5)–1.939(3) Å). In contrast, the Co–N bond lengths of the tertiary and equatorial amines of the tren ligands (1.925(3)–1.9521(16) Å) are generally shorter than the Co–N lengths for the axial amines (1.9509(11)–1.9669(16) Å). Crystal packing is generally dominated by hydrogen bonding in the tren-containing species and π–π interactions in the tpa-containing complexes.

NMR studies indicate that the unsymmetric β-diketone derivatives synthesised here exist as only one isomeric form in solution despite two isomers being possible. The crystallographic data suggests in all cases that the bulkier β-diketone substituent is *cis* to the tertiary amine of the tripodal ligand. While this would be expected to be the more sterically favoured isomer for the tpa derivatives, this reasoning can not be extended to the tren derivatives, where the steric preference between the *cis* and *trans* isomers is unlikely to be of significance.

In contrast to the β-diketone derivatives, the NMR data of several of the hydroxamic acid complexes showed that more than one isomeric form is present in solution. While the Co–O lengths for the β-diketone moieties are similar in the tpa and tren derivatives, the substitution of tpa for tren affects these bond lengths for the hydroxamic acid complexes.

In these derivatives, the Co–O bond lengths are uniformly longer in the tren hydroxamic acid complexes (1.894(2)–1.906(4) Å) than in the tpa hydroxamic acid structures described here (1.854(2)–1.867(3) Å). The other bond lengths are commensurate with previously observed structures, and the carbonyl C=O bond lengths observed in the solid state[25] support the hydroximate/hydroxamate assignments made from NMR, MS and elemental data. The CIFs can be found in the ESI.†

pK_a studies

The absorption, excitation and emission spectra of nhaH have been previously described.[44] While the absorption spectra are unchanged on varying pH, the fluorescence of nhaH was eliminated when the pH was raised (Fig. 3). These results suggest the pK_a of the hydroxamic acid is approximately 9.0, which is within the range observed for other hydroxamic acids.[45]

For the complexes studied here, both the hydroxamate (monoanionic) and hydroximate (dianionic) forms could be isolated. Interconversion between the two is associated with addition or removal respectively of the N–H proton which gives rise to a distinctive colour change that can be followed by UV-vis
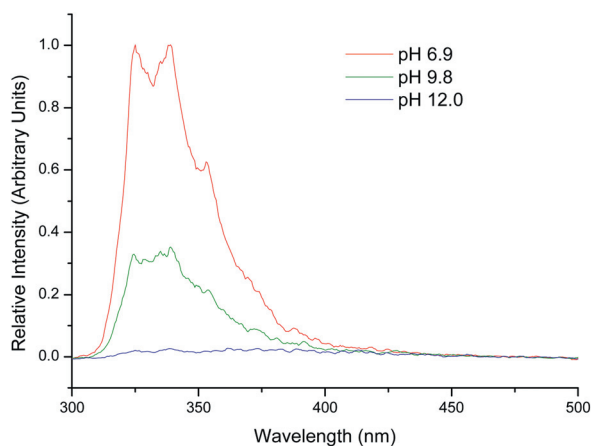
Fig. 3 Comparison between the fluorescence emission of nhaH at different pHs. Studies were performed in methanol–water (1 : 1) with excitation at 280 nm.
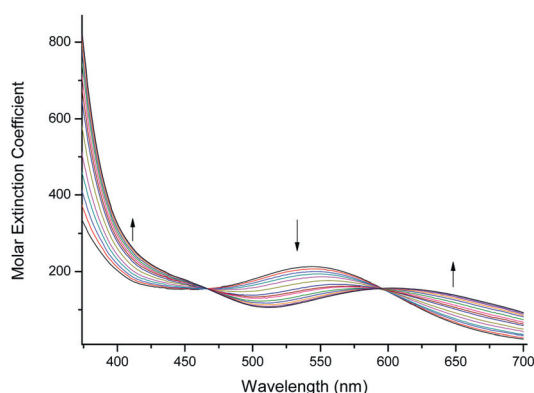

Fig. 4 UV-vis spectra of [Co(bha)tpa](ClO$_4$)$_2$ as the pH is increased from 2.0 to 8.0. Studies were performed at approximately 1 mM in DMF–water (1 : 1) with 20 mM buffer (citrate/phosphate/triethylamine).
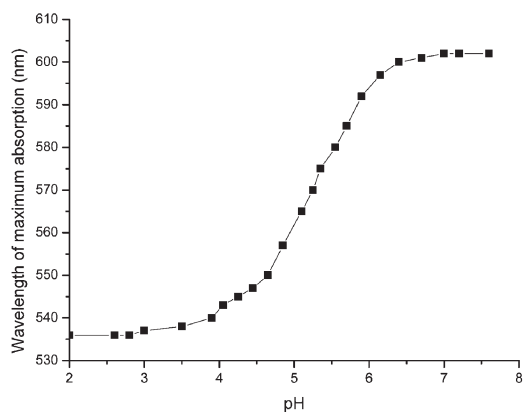

Fig. 5 pH titration curve (pH *vs.* λ_{max}) for [Co(bha)tpa](ClO$_4$)$_2$. Studies were performed at approximately 1 mM in DMF–water (1 : 1) with 20 mM buffer (citrate/phosphate/triethylamine).

spectrometry (Fig. 4 and 5). As shown in Table 3, the pK_a values for the cobalt-tpa complexes are significantly lower than for the equivalent cobalt-tren complexes and the aromatic hydroxamic

Table 3 pK_a values obtained for hydroxamic acids and their complexes

Ligand	Free ligand	Cobalt-tren complex	Cobalt-tpa complex
ahaH	9.4[46]	8.5	7.0
bhaH	8.8[46]	7.7	5.3
nhaH	9.1	7.8	6.2

Table 4 Reduction potentials of cobalt complexes. Scans performed in DMF and 80% DMF are referenced to Fc/Fc$^+$. Those performed in H$_2$O are referenced to Ag/AgCl

Compound	DMF E_{pc}	E_{pa}	DMF E_{pc}	(80%) E_{pa}	H$_2$O E_{pc}	E_{pa}
[Co(acac)tpa](ClO$_4$)$_2$	−605	−465	−600	−455	—	—
[Co(Clacac)tpa](ClO$_4$)$_2$	−520	−315	−550	−380	—	—
[Co(bzac)tpa](ClO$_4$)$_2$	−560	−415	−570	−400	—	—
[Co(naac)tpa](ClO$_4$)$_2$	−560	−420	−570	−400	—	—
[Co(nha)tpa](ClO$_4$)$_2$	−740	—^{a}	−670	—	—	—
[Co(nha-H)tpa](ClO$_4$)	−1385	—	−840	—	—	—
[Co(bha)tpa](ClO$_4$)$_2$	−720	—	nr^b	—	nr	—
[Co(bha-H)tpa](ClO$_4$)	−1350	—	−850	—	−770	—
[Co(aha)tpa](ClO$_4$)$_2$	−800	—	−680	—	−520	—
[Co(aha-H)tpa](ClO$_4$)$_2$	−1400	—	−880	—	−835	—
[Co(cat)tpa](ClO$_4$)	−1120	−910	−1040	—	—	—
[Co(acac)tren](ClO$_4$)$_2$	−978	—	−940	—	−685	—
[Co(bzac)tren](ClO$_4$)$_2$	−960	—	−910	—	−510	—
[Co(naac)tren](ClO$_4$)$_2$	−952	—	−910	—	−450	—
[Co(nha)tren](ClO$_4$)$_2$	−1150	—	−1040	—	−570	—
[Co(nha-H)tren](ClO$_4$)	−1650	—	−1380	—	−860	—
[Co(bha)tren](ClO$_4$)$_2$	−1130	—	−970	—	−530	—
[Co(bha-H)tren](ClO$_4$)	−1630	—	−1370	—	−830	—
[Co(aha)tren](ClO$_4$)$_2$	−1210	—	−1050	—	−620	—
[Co(aha-H)tren](ClO$_4$)	−1720	—	−1400	—	−930	—
[Co(cat)tren](ClO$_4$)	−1420	—	−1240	—	−780	—

^{a} '—' indicates no peak observed. ^{b} 'nr' indicates results were not reproducible.

acids (5.3) had significantly lower pK_a values than for the aliphatic aha derivatives (6.2 and 7.0 for [Co(aha)tpa](ClO$_4$)$_2$, and [Co(nha)tpa](ClO$_4$)$_2$ respectively).

The shift from hydroxamate to hydroximate not only changes the formal charge of the complex, but also greatly influences the reduction potential (discussed further below). With the correct choice of ligand and complex charge, this can be potentially exploited to incorporate two methods of tumour targeting in the one complex, and studies along these lines have begun.[27]

Electrochemistry

The electrochemistry results for all compounds are shown in Table 4. The reduction potentials of all tren compounds are significantly more negative than for the comparable tpa complexes of the same ancillary ligands subtype. This stems from the differences in bonding of the pyridyl and amine nitrogen donors with the metal centres. While amines can only undergo σ interactions with metal centres, pyridyl rings are capable of acting as π-acceptors[47] and the relationship between π-acceptor capabilities and reduction potential is well established.[48]

The greater irreversibility observed for the tren complexes is also related to their more negative reduction potentials (Fig. 6). Previous studies have qualitatively revealed that an increased rate
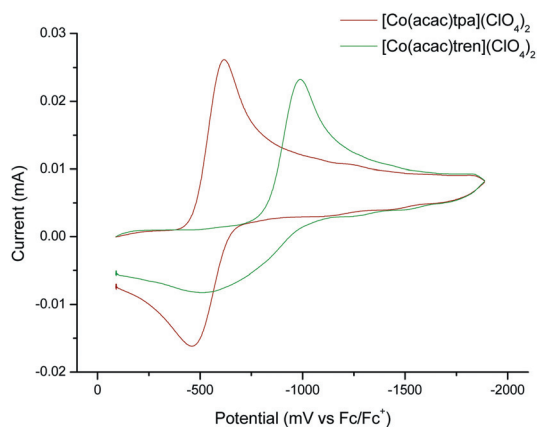
Fig. 6 Comparison between the cyclic voltammograms of [Co(acac)-tpa](ClO$_4$)$_2$ and [Co(acac)tren](ClO$_4$)$_2$. Scans were performed in DMF at 100 mV s^{-1}.

Table 5 IC$_{50}$ (μM) of the compounds used in this study in human DLD-1 colon carcinoma cells

Compound	Normoxia	Hypoxia
tren	>200	>200
tpa	5 ± 0.3	5 ± 0.2
nhaH	>200	>200
naacH	60 ± 1.6	74 ± 3
catH$_2$	127 ± 7	113 ± 4
[CoCl$_2$tren]ClO$_4$	>200	>200
[CoCl$_2$tpa]ClO$_4$	>200	>200
[Co(nha-H)tren]ClO$_4$	>200	>200
[Co(nha-H)tpa]ClO$_4$	>200	>200
[Co(naac)tren](ClO$_4$)$_2$	>200	>200
[Co(naac)tpa](ClO$_4$)$_2$	66 ± 3	74 ± 4
[Co(cat)tren]ClO$_4$	>200	>200
[Co(cat)tpa]ClO$_4$	>200	>200

of solvolysis and thus hindrance of the reoxidation wave occurs with decreased reduction potential.[49] In addition, the π back-bonding interactions between the metal centre and tpa potentially result in decreased ligand lability,[50] which may contribute to the greater reversibility observed in the reduction of the tpa complexes.

Within a particular tripodal ligand class, the reduction potentials of the complexes corresponded strongly with the ancillary oxygen ligand in the order β-diketone > hydroxamate > catechol > hydroximate. This is consistent with the doubly deprotonated ligands conferring a greater electron density onto the metal centres than the comparable monoanionic oxygen bidentates.

The presence of an electron-withdrawing chloride group in the [Co(Clacac)tpa](ClO$_4$)$_2$ derivative made the reduction potential significantly more positive. This is commensurate with previous studies of cobalt(III) β-diketonato complexes which found direct correlations between reduction potentials and the Hammett σ parameters of the substituents on the β-diketone ligands.[51]

The pH of the solutions used for the electrochemistry scans also influenced the cyclic voltammetry results, particularly for the cobalt–hydroxamic acid complexes. Increasing the pH above the pK_a values of the hydroxamate complexes resulted in deprotonation and hence formation of the hydroximate *in situ*. This corresponded with a shift to the more negative reduction potentials associated with the doubly deprotonated (hydroximate) ligands. The reduction potentials of cobalt–hydroximate complexes prepared *in situ* by this manner were indistinguishable from those of freshly prepared cobalt–hydroximate solutions. For the catechol and diketone derivatives, no shift in reduction potential was observed with altered pH. However, an increase in the current magnitude in the anodic return wave was observed at higher pH values, presumably because high pH maintains the free ancillary ligand that forms under reduction in a deprotonated form, which facilitates reoxidation of the cobalt complexes.

Reduction with ascorbic acid

As the naphthyl moieties are fluorescent, all the complexes containing naphthyl derivatives were tested for reduction with ascorbate by fluorimetry (full details in experimental data).

For all cobalt complexes the fluorescence intensity was less than 1% of that observed for the equivalent concentration of free ligand, which could be related to deprotonation of the ligands upon binding to cobalt, or more directly to the presence of the cobalt which has previously been reported to quench fluorescence by electron or energy exchange mechanisms.[52]

When ten equivalents of ascorbic acid were added to the solution and the fluorescence was measured at a variety of time points over 24 hours, only [Co(naac)tpa](ClO$_4$)$_2$ demonstrated any increase in fluorescence after 24 hours, which is consistent with the reduction potentials for most of the complexes being too negative to allow reduction by ascorbate. In contrast, the [Co(naac)tpa](ClO$_4$)$_2$ solution showed a steady increase in fluorescence such that after 24 hours it was approximately 40% of the intensity of the comparable reference solution of the ligand which is consistent with reduction and ligand dissociation under these conditions.

Cytotoxicity

As with the fluorescence work, all naphthyl derived ligands and their associated compounds were tested for cytotoxicity. None of the complexes or their ligands were particularly cytotoxic (Table 5) with the exception of the free tpa ligand. Similar to what has previously been observed, the cytotoxicity of the free tpa ligand was far greater than that of any of the tpa complexes,[53] which suggests that the toxicity of the tpa ligand is masked by coordination to a metal centre, and this is maintained in biological environments. The naacH free ligand was shown to be mildly cytotoxic under both hypoxic and normoxic conditions (IC$_{50}$ 74 ± 3 and 60 ± 1.6 μM respectively) and there was no statistically significant difference between the cytotoxicity of the free ligand and the cytotoxicity of the respective tpa complex [Co(naac)tpa](ClO$_4$)$_2$ (IC$_{50}$ 74 ± 4 and 66 ± 3 μM under hypoxic or normoxic condition respectively). In contrast, [Co(naac)tren]-(ClO$_4$)$_2$ has far lower cytotoxicity than free naacH under both hypoxic and normoxic conditions. The above results indicated that the cytotoxicity of the naacH ligand is masked upon binding to the cobalt-tren derivative while the behaviour of the cobalt-tpa derivative is indistinguishable from that of free ligand in solution. This is despite both compounds showing no

degradation by NMR over 48 h in aqueous solution. Unfortunately, the [Co(naac)tpa](ClO$_4$)$_2$ complex did not show any hypoxic selectivity and it was not possible to ascertain whether any of the complexes are hypoxia selective due to their low cytotoxicity.

Conclusions

Cobalt complexes that are selectively reduced under hypoxic conditions have been investigated as chaperones for prodrugs. In this study, a clear correlation between reduction potential, ligand dissociation under ascorbic acid challenge, and cytotoxicity was observed. Specifically, the free naacH ligand and [Co(naac)tpa]-(ClO$_4$)$_2$ show comparable cytotoxicities, both under hypoxic and normoxic conditions, while all other complexes demonstrate higher IC$_{50}$ values for the cobalt complexes when compared to their respective free ligands. The cyotoxicity data supports the notion that [Co(naac)tpa](ClO$_4$)$_2$ quickly dissociates and loses its naacH ligand in solution while [Co(naac)tren](ClO$_4$)$_2$, and the other complexes investigated do not. This is commensurate with [Co(naac)tpa](ClO$_4$)$_2$ having a significantly less negative reduction potential (-560 mV E_{pc} in DMF vs. F$_c^+$/F$_c$) than any of the other complexes examined in cytotoxicity studies (-970 to -1650 mV in DMF vs. F$_c^+$/F$_c$), as well as being the only complex to display appreciable reduction by ascorbic acid over 24 hours. Together, these results support the correlation between reduction potential and ligand dissociation.

The ability to manipulate formal complex charge to influence biological behaviour has also been investigated for cobalt complexes.[26,27] The intrinsic acid properties of the hydroxamic acid make them potentially useful for this purpose. The results of this study demonstrate that the pK_a of the hydroxamate–hydroximate transition can be influenced by the ancillary ligand as well as the substituents on the hydroxamic acid and that the reduction potential is also affected. As such, cobalt hydroxamic acid complexes can potentially target tumours by two distinct mechanisms. In the first instance, protonation of the hydroxamic acid changes the formal charge of the cobalt complex and thus also changes its cellular uptake characteristics, as has previously been shown in 3D cell culture by Yamamoto.[27] Additionally, the protonation of hydroxamic acid allows reduction of the cobalt metal centre to occur at significantly more positive potentials and thus reduction is considerably more facile under acidic conditions. The combination of these two elements suggest that cobalt hydroxamic acid complexes can be designed such that under the acidic and hypoxic conditions found in many tumours; the hydroxamic acid is primarily in the hydroxamate form, the cobalt complex formally neutral and the reduction potential relatively positive, all of which favour high uptake and/or ligand dissociation. In contrast, the same complexes under the more alkaline, normoxic conditions of normal tissue would exist with the hydroxamic acid primarily in hydroximate form, the cobalt complex formally negatively charged and the reduction potential more negative, favouring low cellular uptake and lower ligand dissociation. The combination of these two modes of selectively can thus be exploited to significantly reduce toxic side-effects and, as such, development of novel cobalt complexes based on these criteria has begun.

Abbreviations

acacH	acetylacetone
ahaH	acetohydroxamic acid
bhaH	benzohydroxamic acid
bzacH	1-phenyl-1,3-butanedione
ClacacH	3-chloro-2,4,-pentanedione
haH	generic hydroxamic acid
HCR	hypoxic cell response
naacH	1-methyl-3-(2-naphthyl)propane-1,3-dione
nhaH	1-napthylacetohydroxamic acid
TAP	tumour activated prodrug
tpa	tris-(2-pyridylmethyl)amine
TPZ	tirapazamine
tren	tris-(2-aminoethyl)amine
trp	generic tripodal ligand

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