Bioorganic & Medicinal Chemistry 19 (2011) 4861–4867

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

N-alkylated cyclen cobalt(III) complexes of 1-(chloromethyl)-3-(5,6,7-trimethoxyindol-2-ylcarbonyl)-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinolin-5-ol DNA alkylating agent as hypoxia-activated prodrugs

Guo-Liang Lu [a], Ralph J. Stevenson [a], John Yu-Chih Chang [b], Penelope J. Brothers [b], David C. Ware [b], William R. Wilson [a], William A. Denny [a,*], Moana Tercel [a]

[a] Auckland Cancer Society Research Centre, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand
[b] School of Chemical Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand

ARTICLE INFO

Article history:
Received 17 May 2011
Revised 21 June 2011
Accepted 26 June 2011
Available online 1 July 2011

Keywords:
Cancer therapy
Hypoxia
Prodrug
Cobalt(III) complex
Pyrrolo[3,2-*f*]quinolin-5-ol
Cyclen
Cytotoxicity

ABSTRACT

A series of cobalt complexes of the potent DNA minor groove alkylator 1-(chloromethyl)-3-(5,6,7-trimethoxyindol-2-ylcarbonyl)-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]quinolin-5-ol (*seco*-6-azaCBI-TMI) were prepared from a series of N-substituted cyclen ligands. The final N-substituted complexes carried formal overall charges ranging from +2 to −2 and showed limited improvements in solubility. They showed similar stabilities to that of the complex with the unsubstituted cyclen ligand, and large but variable attenuation of the cytotoxicity of the free alkylator (2–30-fold), compared to 150-fold for the unsubstituted ligand. However, they had oxic/hypoxic ratios (2–22-fold) comparable to that of the unsubstituted cyclen complex (**5**).

1. Introduction

The occurrence of hypoxia in solid tumors is increasingly recognized as a consistent tumor-specific condition that could be exploited for the selective activation of prodrugs.[1] One design for such prodrugs, able to undergo selective bioreductive activation in hypoxic tumor cells, is transition metal complexes of DNA alkylating agents. The lability of cobalt(III) complexes with nitrogen-based ligands is dramatically increased on one-electron reduction of the metal, resulting in release of the coordinated ligands,[2] and this has been exploited in the design of complexes with nitrogen mustards (e.g., **1**)[3,4] and matrix metalloproteinase inhibitors (e.g., **2**).[5] We have also explored this approach in the context of cobalt(III) complexes of 8-hydroxyquinolines (8-QOHs) (e.g., **3**),[6,7] and of the related but much more cytotoxic (*seco*-6-azaCBI-TMI) minor groove alkylators (e.g., **4**).[8] While earlier complexes such as **1**, with bidentate auxiliary ligands, showed less than ideal stability,[3] those such as **3** with the tetradentate 1,4,7,10-tetraazacyclododecane (cyclen) auxiliary ligand were stable in high-density cell cultures,[6] and efficiently masked the cytotoxicity of the ligand in the complex (Fig. 1). The corresponding Co(III)/cyclen complex (**5**) of the racemic deprotonated pyrrolo[3,2-*f*]quinolinato ligand (**4**$^-$) was also much more stable than the corresponding free effector **4** in solution and deactivated it by about 150-fold against SKOV3 human ovarian cancer cells in culture.[9] The hypoxic cell selectivity (aerobic cell IC_{50}/hypoxic cell IC_{50}) was shown to be only about 20-fold, but surprisingly this ratio was not increased in A549 human non-small-cell cancer cells over-expressing cytochrome P450 reductase,[8] which is known to be the case with most classes of bioreductive drugs.[10] In previous work we explored the use of tertiary amine-substituted solubilizing side chains attached to the toxin, but this resulted in a 20–30-fold loss of potency compared to a neutral side chain example.[9]

In the present work we explore a series of Co(III)/cyclen complexes of **4** using a variety of N-alkylated cyclen auxiliary ligands. The aims were to determine the effects of such N-alkylation on complex stability (and thus on suppression of the effector cytotoxicity), and to utilize them to attach the solubilizing functions to the

Abbreviations: 8-QOH, 8-hydroxyquinoline; Bn, benzyl; CBI, *seco*-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one; Cbz, benzyloxycarbonyl; cyclen, 1,4,7,10-tetraazacyclododecane; DIPEA, diisopropylethylamine; HCR, hypoxic cytotoxicity ratio; HT29, human colon carcinoma cell line; HOTf, triflic acid; P/E, prodrug IC_{50}/effector IC_{50} ratio; SKOV3, human ovarian cancer cell line; TEA, triethylamine; TMI, 5,6,7-trimethoxyindole.

* Corresponding author. Tel.: +64 9 3737599; fax: +64 9 3737502.
E-mail address: b.denny@auckland.ac.nz (W.A. Denny).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.06.076

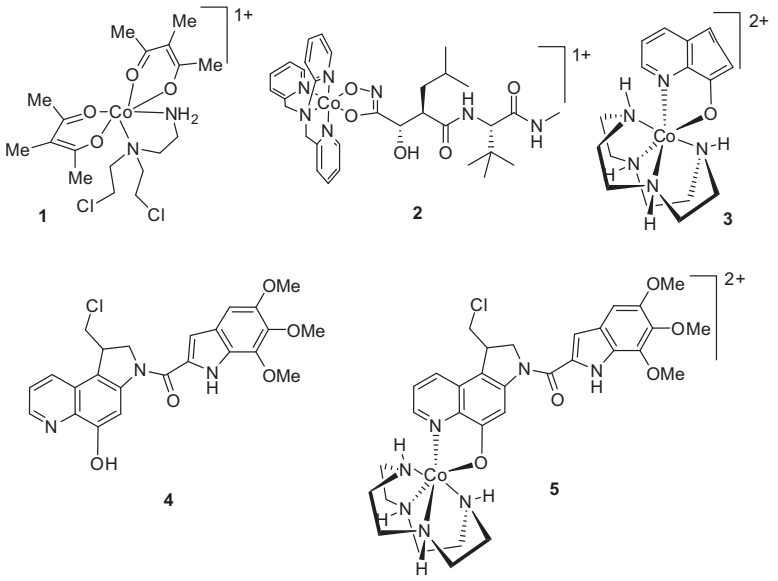


Figure 1. Structures of Co(III) complexes and ligand **4**.

auxiliary ligands. Another motivation was to explore the effect of varying the complex net charge on their biology, and whether anionic side chains, by substantially lowering overall lipophilicity, might suppress cellular uptake and hence decrease cytotoxicity of the complexes further, which would be desirable in the context of them as radiolytically-activated prodrugs.[9]

2. Results and discussion

2.1. Chemistry

2.1.1. Ligand syntheses

The syntheses of the substituted cyclen ligands, **L6**, **HL8**, **H_2L9**, and **H_4L10**, used for the syntheses of complexes **6**, **8–10**, respectively (Fig. 2) have been previously described.[7,11] The preparation of the new cyclen derivative **H_2L7** (for complex **7**) is shown in Scheme 1. Reaction of the known[12] 2a,4a,6a,8a-decahydrotetraazacyclopent[*fg*]acenaphthylene (**12**) with the phosphonate[13] **13** gave a good yield (85%) of the mono N-substituted acenaphthylene **14** using one equivalent of iodide **13**, which was deprotected with hydrazine hydrate to give the N-substituted cyclen phosphonate ester **15** as a gum (Scheme 1). This was converted directly to the crystalline tetrahydrobromide salt of the corresponding phosphonic acid ligand, **H_2L7·4HBr**.

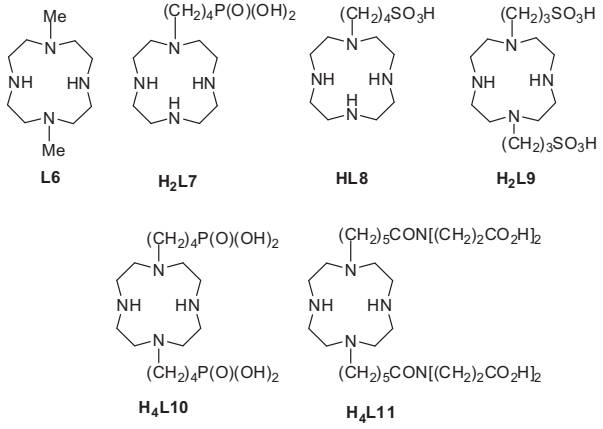
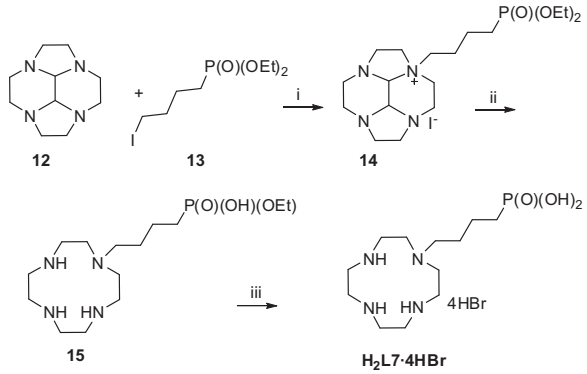


Figure 2. Structures of N-substituted cyclen ligands.

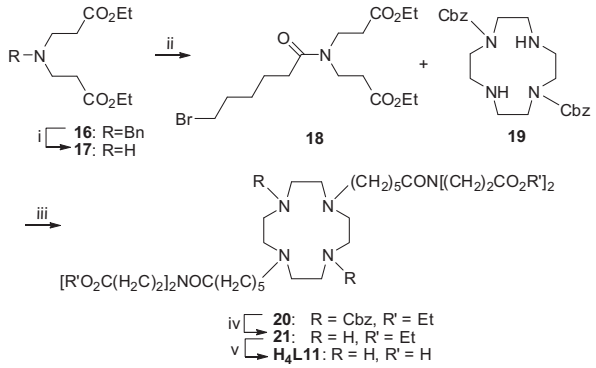


Scheme 1. Synthesis of cyclen **H_2L7**. Reagents and conditions: (i) toluene, 60 °C, four days; (ii) $H_2NNH_2{\cdot}H_2O$, 100 °C, 25 h (N_2); (iii) HBr, AcOH, 40 °C, four days.

The disubstituted cyclen ligand **H_4L11** (for complex **11**) was prepared (Scheme 2) by debenzylation of the known[14] diester **16** with ammonium formate over palladium to give the known[15] diester **17**, which was coupled with 6-bromohexanoyl chloride to give the bromodiester **18** in 81% yield. Reaction of this with the known[16] *N,N*-di(benzyloxycarbonyl)cyclen **19** in DMF/K_2CO_3 gave the *tetra*-N-substituted cyclen **20**, which was purified by gradient elution flash chromatography in 47% yield from contaminating mono-alkylated product (27%). Stepwise removal of the CBz groups (H_2/5% Pd-C) followed by saponification of the esters (1 M NaOH in EtOH) gave the desired ligand **H_4L11** in quantitative yield.

2.1.2. Cobalt(III) complexes

The synthesis of complex **5** has been previously described.[9] The syntheses of the new Co(III) cyclen complexes (**6–11**) in Table 1 were guided by the synthetic procedures reported previously[7] for the corresponding 8-hydroxyquinolinato (8-QO) complexes. These procedures were developed with the properties of the pyrrolo[3,2-*f*]quinolin-5-ol effector **4** in mind and utilize reaction conditions that are compatible with the reactive nature of this compound. The much lower solubility of **4** relative to 8-QOH was a notable difference for these syntheses relative to those for the model 8-QOH complexes, requiring longer reaction times with the concomitant possibility of hydrolysis of the reactive chloromethyl group. Complex **10** was prepared from chlorocobalt precursor complex



Scheme 2. Synthesis of ligand **H_4L11**. Reagents and conditions: (i) HCO_2NH_4, 10%Pd-C, EtOH reflux, 2 h; (ii) 6-bromohexanoyl chloride, NEt_3, CH_2Cl_2, 20 °C, 2 h; (iii) K_2CO_3, DMF, 60 °C, 15 h; (iv) H_2/Pd-C, EtOH, 20 °C, 15 h; (v) NaOH, EtOH, 20 °C, 15 h.

[Co(**H_3L10**)Cl_2] prepared in the model study[7] and similar precursors [Co(**L6**)Cl_2]Cl and [Co(**H_4L11**)Cl_2]Cl were developed for **L6** and **H_4L11**. Reaction of each of these with **4** in MeOH with added base, usually pyridine, gave the target complexes [Co(**L6**)(**4**)]Cl_2 (**6**), [Co(**H_2L10**)(**4**)] (**10**) and [Co(**H_4L11**)(**4**)]Cl_2 (**11**). Complexes [Co(**HL7**)(**4**)](OTf) (**7**) and [Co(**L8**)(**4**)](OTf) (**8**) were prepared using the method developed for the model complex [Co(**L8**)(8QO)](OTf)[7] utilizing the triflato precursors [Co(**H_2L7**)$(OTf)_2$)]OTf and [Co(**HL8**)$(OTf)_2$](OTf) with **4**. Complex [Co(**L9**)(**4**)] (**9**) was prepared in one step from *trans*-[Co$(py)_4Cl_2$](NO_3)[17] with **H_2L9·4HCl** and **4**. For each route complexation of **4** was the last step and in general methanol was the most suitable solvent for this reaction with addition of an amine base to deprotonate the phenol in **4**. The final products were purified by chromatography on Sephadex LH-20 resin. The salts of non-coordinating bases such as TEA or DIPEA were often co-eluted with the products and were then difficult to remove. The weaker base pyridine was much superior, since the formed pyridinium salt separated cleanly from the product band, with no adsorption to the column. Ligands **L6** and the tetracarboxylic acid **H_4L11** coordinate as neutral ligands in **6** and **11**, whereas each phosphonato or sulfonato side chain in **7–10** is monoanionic, and the overall charges (determined by HRMS) on the complexes reflect the zwitterionic formulation arising from the anionic side chain(s) and positively charged cobalt(III) center. Some complexes were mixtures of syn/anti diastereomers, as shown previously for related 8-QOH complexes.[7]

2.2. Physicochemical properties and in vitro cytotoxicity

The physicochemical properties of the various cobalt/cyclen complexes of the pyrrolo[3,2-*f*]quinoline (**4**) are shown in Table 1.While, as noted previously, the free effector **4** is relatively unstable in aqueous solution, converting initially to the corresponding spirocyclized compound,[8] the Co(III) complexes of **4** with N-alkylated cyclens (**6–11**) were much more stable in culture medium, and compared well with that of the complex with the unsubstituted cyclen (**5**), showing that alkylation of the secondary amines of the cyclen does not impair complex stability. As expected, the doubly charged complexes **5**, **6** and **11** have much better solubility (~100-fold) than the free effector **4**, as do the complexes **7** and **8**, where an anionic side chain off the cyclen has lowered the overall charge to +1. Complexes **9** and **10**, with two monoanionic side chains, are formally neutral, with **10**, bearing the phosphonate groups, showing a 10-fold drop in solubility. Complex **11** has a formal overall net charge of −2, although pK_a calculations (ACD/pKDB v7.70, ACD Labs, Toronto, Ontario, Canada M5C) show the second pK_a in the RCONH$(CH_2CH_2CO_2H)_2$ side chain to be about 4.8, suggesting that the side chain will not be fully ionized at cellular pH.

A study of the electrochemistry of the complexes was attempted, but was not possible due to their insufficient solubility and the occurrence of other redox reactions, possibly involving the effector, that complicate the cyclic voltammograms. Reduction potentials were determined previously[7] for a series of related complexes of *N*-alkyl or *N,N*-dialkylcyclens with 8-QOH rather than the *seco*-6-azaCBI-TMI as the ligand. These indicated that the addition of each *N*-alkyl substituent to the cyclen resulted in about a 100 mV increase in reduction potential, but that varying the nature of the alkyl unit had little effect, and that the dialkylcyclen complexes showed less reversible one-electron reduction.

The complexes were evaluated for cytotoxicity under both oxic and hypoxic conditions in two human tumor cell lines, SKOV3 ovarian carcinoma and HT29 colon carcinoma (Table 1). Under aerobic conditions the different cobalt(III) complexes provided attenuation of the cytotoxicity of **4**, but this was highly variable; in SKOV3, P/E (prodrug/effector) ratios range from 146 to 3, and in HT29 P/E ratios are from 110 to 2. The original unsubstituted cyclen complex **5** gave the highest ratios in both cell lines, with the dimethyl cyclen analog **6** being next. The complexes with a net +1 charge (**7** and **8**) were about 10-fold more potent as oxic cytotoxins in SKOV3 cells, while the charge neutral complexes (**9** and **10**) were about twofold more potent again.

As shown previously,[9] the free effector **4** is an equipotent cytotoxin in both aerobic and hypoxic cells, with hypoxic cytotoxicity ratios (HCR = IC_{50}[oxic]/IC_{50}[hypoxic]) in both cell lines of ~1. However, the cobalt complexes show distinct hypoxic selectivity. The most selective compounds were again those containing the simple cyclens **5** and **6** (HCRs about 20), with the +1 net charge compounds **7** and **8** about half as selective and the remainder of the complexes showing more modest hypoxic selectivity. The cytotoxicities of all the complexes were remarkably similar under hypoxia, and with the exception of the unsubstituted cyclen **5** were essentially the same as for the free effector, consistent with efficient bioreductive activation under hypoxia.

3. Conclusions

The results show that the aqueous solubilities and stabilities of these cobalt complexes are not significantly changed by substitution of the NH groups on **5** by quite large alkyl groups. Varying the formal net charge on the complex from +1 to −2 (**7–11**) also had little effect on HCRs. Possible reasons for this may be that the complexes are too lipophilic overall, if the full formal side chain negative charges are not developed (especially with **11**). The compounds are presumed to enter cells by passive diffusion, in which case the overall lipophilicity of the complexes is the major determinant. An alternative explanation is that the complexes are reduced by outer-membrane nitroreductases, but while these are known[18] we do not know what role (if any) they play here. Nevertheless the complexes appear to be efficiently reduced, with potencies under hypoxia close to that of the free drug. The more modest HCRs seen for the *N,N*-dialkylcyclen analogs may be related to the less reversible reductive behavior noted previously[7] for analogoau complexes with 8-HQOH.

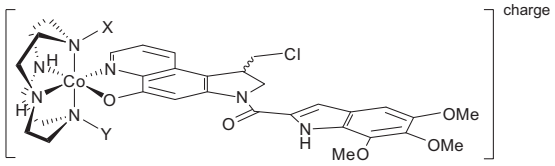
The relatively limited suppression of effector toxicity by this approach precluded the evaluation of the anionic complexes as radiolytically-activated prodrugs.

4. Experimental

4.1. Chemistry

Chemicals and solvents were purchased from commercial sources and were used as received unless specified otherwise.

Table 1
Physicochemical and biological properties of cobalt complexes



No.	Compound	Formal charge	Sol.[a] (mM)	Stab.[b] (%)	IC_{50} (nM)[c]							
					SKOV3				HT29			
					Oxic	P/E[d]	Hypoxic	HCR[e]	Oxic	P/E	Hypoxic	HCR
4			0.04		0.26 ± 0.03	—	0.40 ± 0.04	0.69 ± 0.08	0.63 ± 0.15	—	0.69 ± 0.05	0.90 ± 0.18
5	X = Y = H	+2	3.0	46	38 ± 14	146	3.7 ± 1.5	18 ± 8	69 ± 27	110	5.6 ± 1.7	13 ± 3
6	X = Y = Me	+2	>4.7	81	7.9 ± 0.1	30	0.40 ± 0.07	20 ± 3	14 ± 4	22	0.67 ± 0.09	22 ± 9
7	X = $(CH_2)_4P(O)(OH)(O^-)$, Y = H	+1	4.8	39	4.5 ± 0.3	17	0.91 ± 0.19	6.1 ± 1.3	1.43 ± 0.04	2.3	0.28 ± 0.07	6.5 ± 1.7
8	X = $(CH_2)_4SO_3^-$, Y = H	+1	>6.6	56	2.96 ± 0.75	11	0.53 ± 0.04	5.2 ± 1.2	1.08 ± 0.25	1.7	0.12 ± 0.02	11.5 ± 2.8
9	X = Y = $(CH_2)_3SO_3^-$	0	4.4	52	0.98 ± 0.04	3.8	0.25 ± 0.02	3.9 ± 0.2	1.6 ± 0.1	2.5	0.56 ± 0.04	2.8 ± 0.2
10	X = Y = $(CH_2)_4P(O)(OH)(O^-)$	0	0.37	54	0.74 ± 0.26	2.8	0.22 ± 0.02	2.2 ± 0.1	1.2 ± 0.3	1.9	0.39 ± 0.08	3.0 ± 0.9
11	X = Y = $(CH_2)_5CON\text{-}[(CH_2)_2CO_2^-]_2$	−2	>5	58	2.26 ± 0.42	8.7	0.50 ± 0.06	5.3 ± 1.6	2.4 ± 1.9	3.8	0.41 ± 0.20	2.9 ± 1.2

[a] Solubility in culture medium at 20 °C.
[b] Stability (% remaining after 24 h at 37 °C (see text).
[c] IC_{50} for inhibition of cell proliferation following 4 h drug exposure. Values are means, and errors are ranges for two experiments (compounds **6** and **9**) or SEM for 3–6 determinations.
[d] IC_{50} ratios = IC_{50} prodrug/IC_{50} effector (compound **4**).
[e] HCR (hypoxic cytotoxicity ratio) is the ratio oxic IC_{50}/hypoxic IC_{50}.

Deionised MilliQ water was utilized where mentioned. Gel permeation chromatography was performed either on Sephadex G10 gel or Sephadex LH-20 gel in water or in methanol. Amersham Bioscience supplied all resins. Analytical grade solvents were used in all chromatography. High resolution fast atom bombardment mass spectra (FAB^+) were recorded on a VG 70-SE mass spectrometer. Spectra were measured in *m*-nitrobenzyl alcohol as the matrix under argon and were referenced to polyethyleneglycol. Low resolution mass spectra ($APCI^+$) were run on a Surveyor MSQ mass spectrometer using a methanol solution of the samples. Microanalyses (C, H, N and S) were carried out at the Campbell Microanalysis Laboratory, University of Otago. ^1H and ^{13}C NMR spectra were recorded on a Bruker DRX-400 or a Bruker Avance-300 spectrometer at 400 and 100 MHz or 300 and 75 MHz, respectively. Spectra recorded in $CDCl_3$, D_2O, CD_3CN, CD_3OD, and DMSO-d_6 were referenced to TMS (tetramethylsilane), TSP-d_4 (3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt), or their respective residual solvent peaks.

4.1.1. 4-(1,4,7,10-Tetraazacyclododecan-1-yl)butylphosphonic acid tetrahydrobromide (H_2L7·4HBr) (Scheme 1)

A mixture of diethyl 4-bromobutylphosphonate[19] (2.62 g, 9.59 mmol) and NaI (2.88 g, 19.21 mmol) in acetone (30 mL) was allowed to reflux for 1 h before all volatile components were removed by evaporation. The residue was extracted with CH_2Cl_2 and filtered through Celite to give diethyl 4-iodobutylphosphonate (**13**) as a pale yellow oil (3.05 g, 99%), which had identical ^1H NMR characteristics to those reported in the literature.[13] MS ($APCI^+$): *m/z* 321.1 (M+1). Calcd for $C_8H_{18}IO_3P$: M^+ 320.0.

A solution of **13** (3.00 g, 9.37 mmol) in toluene (10 mL) was added to a solution of decahydro-2*a*,4*a*,6*a*,8*a*-tetraazacyclopent[*fg*]acenaphthylene[12] (**12**) (1.82 g, 9.37 mmol) in toluene (10 mL). The mixture was heated at 60 °C for four days and the resultant precipitate was filtered and washed with toluene to give diethyl 4-(4,7,10-triaza-1-azoniatricyclo[8.2.2.2^{4,7}]hexadec-1-yl)butylphosphonate iodide (**14**) (4.11 g, 85%) as a white powder: ^1H NMR (400 MHz, D_2O) δ 1.34 (t, J = 7.1 Hz, 6H), 1.65–1.76 (m, 2H), 1.88–2.10 (m, 4H), 2.44–2.58 (m, 2H), 2.77–2.97 (m, 6H), 3.17–3.33 (m, 4H), 3.39–3.46 (m, 1H), 3.50–3.55 (m, 1H), 3.59–3.69 (m, 2H), 3.75–3.83 (m, 2H), 3.87–3.93 (m, 2H), 4.09–4.22 (m, 4H). ^{13}C NMR (100 MHz, D_2O) δ 16.19 (d, J_{PC} = 5.6 Hz), 19.42 (d, J_{PC} = 6.0 Hz), 23.84 (d, J_{PC} = 16.8 Hz), 23.85 (d, J_{PC} = 138.9 Hz), 44.15 (s), 48.12 (s), 48.27 (s), 48.67 (s), 48.91 (s), 51.77 (s), 57.54 (s), 58.17 (s), 62.64 (s), 63.93 (d, J_{PC} = 6.4 Hz), 72.21 (s), 84.27 (s). MS ($APCI^+$): *m/z* 515.7 (M+1), 387.5 (M−I). Calcd for $C_{18}H_{36}IN_4O_3P$: M^+ 514.2.

A solution of **14** (3.10 g, 6.03 mmol) in hydrazine hydrate (30 mL) was heated at 100 °C for 25 h under nitrogen, before the excess hydrazine hydrate was removed under reduced pressure. The resultant residue was recrystallized from EtOH to give ethyl hydrogen 4-(1,4,7,10-tetraazacyclododecan-1-yl)butylphosphonate (**15**) (2.75 g) as a white gum (hygroscopic, wet, and used directly in the next step): ^1H NMR (400 MHz, D_2O) δ 1.24 (t, J = 7.1 Hz, 3H), 1.46–1.69 (m, 6H), 2.78–2.82 (m, 2H), 2.96–3.12 (m, 16H), 3.85–3.92 (m, 2H) (exchangeable NH and OH not observed). ^{13}C NMR (100 MHz, D_2O) δ 16.51 (d, J_{PC} = 6.0 Hz), 21.18 (d, J_{PC} = 6.4 Hz), 25.68 (d, J_{PC} = 15.2 Hz), 26.34 (d, J_{PC} = 134.4 Hz), 42.45 (s), 43.04 (s), 44.33 (s), 49.75 (s), 53.08 (s), 60.90 (d, J_{PC} = 5.4 Hz). MS ($APCI^+$): *m/z* 337.3 (M+1). Calcd for $C_{14}H_{33}N_4O_3P$: M^+ 336.2.

A solution of the crude **15** obtained above (2.75 g, 6.03 mmol) was treated with 30% HBr in AcOH (80 mL) at 40 °C for four days after which the volatile components were removed under reduced pressure. The resulting residue was recrystallized (2×) from MeOH/Et_2O to give 4-(1,4,7,10-tetraazacyclododecan-1-yl)butylphosphonic acid tetrahydrobromide (**H_2L7·4HBr**) (3.49 g, 92% over two steps) as a brown solid: ^1H NMR (400 MHz, D_2O) δ 1.55–1.66 (m, 2H), 1.70–1.85 (m, 4H), 2.95 (t, J = 7.5 Hz, 2H), 3.12–3.23 (m, 16H) (exchangeable NH and OH not observed). ^{13}C NMR (100 MHz, D_2O) δ 20.14 (d, J_{PC} = 6.7 Hz), 24.48 (d, J_{PC} = 14.3 Hz), 26.02 (d, J_{PC} = 134.6 Hz), 42.72 (s), 43.53 (s), 44.03 (s), 50.33 (s), 54.30 (s). ^{31}P NMR (162 MHz, D_2O, decoupled) δ 30.17. MS ($APCI^+$): *m/z* 309.3 (M+1). Calcd for $C_{12}H_{29}N_4O_3P$: M^+ 308.2.

4.1.2. Bis-1,7-[3,3′-(6-(1,4,7,10-tetraazacyclododecan-1-yl))] (hexanoylazanediyl)dipropanoic acid (H_4L11) (Scheme 2)

Diethyl 3,3′-(benzylazanediyl)dipropanoate (**16**) was made according to the literature method[14] in a yield of 91.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (t, J = 7.1 Hz, 6H), 2.45 (t, J = 7.1 Hz, 4H), 2.81 (t, J = 7.1 Hz, 4H), 3.60 (s, 2H), 4.10 (q, J = 7.1 Hz, 4H), 7.22–7.29 (m, 5H). MS (APCI$^+$): m/z 308.2 (M+1). Calcd for $C_{17}H_{25}NO_4$: M^+ 307.2.

A mixture of **16** (4.88 g, 15.9 mmol), HCO_2NH_4 (10.0 g, 159 mmol) and 10% Pd-C catalyst (2.44 g) in EtOH (ca. 350 mL) was heated at reflux and stirred for 2 h before all the volatiles were removed under reduced pressure. The residue was dissolved in CH_2Cl_2, filtered through Celite, and evaporated to give diethyl 3,3′-azanediyldipropanoate (**17**) as a pale yellow liquid (3.30 g, 96%), which had identical ^{1}H NMR characteristics to those reported in the literature.[13] MS (APCI$^+$): m/z 218.1 (M+1). Calcd for $C_{10}H_{19}NO_4$: M^+ 217.1.

Oxalyl chloride (3.92 mL, 46.33 mmol) was added dropwise to a stirred solution of 6-bromohexanoic acid (4.39 g, 22.50 mmol) and DMF (ca. 0.2 mL) in dry CH_2Cl_2 (50 mL), and the reaction was then stirred overnight. All the volatiles were removed under reduced pressure, and the resulting oil was pumped under high vacuum. The crude 6-bromohexanoyl chloride was dissolved in CH_2Cl_2 (40 mL) and added portionwise by syringe into a solution of **17** (3.20 g, 14.73 mmol) and NEt_3 (10.45 ml, 75.00 mmol) in CH_2Cl_2 (25 mL). LC–MS was used to monitor the reaction. After 30 mL of the acid chloride solution was added, LC–MS showed most of the diethyl 3,3′-azanediyldipropanoate had reacted. The mixture was stirred for a further 2 h before being washed with aqueous $NaHCO_3$, brine, dried over anhydrous Na_2SO_4 and filtered. Solvent was removed and the crude product was subjected to flash chromatography (4 × 20 cm silica gel; EtOAc/petroleum ether; 1:3 to 1:1, gradient elution) to give diethyl 3,3′-(6-bromohexanoylazanediyl)dipropanoate (**18**) as a colorless oil (4.73 g, 81%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24–1.29 (m, 6H), 1.44–1.52 (m, 2H), 1.62–1.70 (m, 2H), 1.85–1.92 (m, 2H), 2.35 (t, J = 7.4 Hz, 2H), 2.55–2.61 (m, 4H), 3.42 (t, J = 6.8 Hz, 2H), 3.58 (t, J = 7.0 Hz, 2H), 3.64 (t, J = 7.3 Hz, 2H), 4.10–4.18 (m, 4H). MS (APCI$^+$): m/z 394.3/396.3 (1:1, M+1). Calcd for $C_{16}H_{28}{}^{79/81}BrNO_5$: M^+ 393.1/395.1.

A mixture of **18** (830 mg, 2.11 mmol), dibenzyl 1,4,7,10-tetraazacyclododecane-1,7-dicarboxylate (**19**)[16] (440 mg, 1.00 mmol), and K_2CO_3 (552 mg, 4.00 mmol) in DMF (10 mL) was stirred at room temperature for 7 h and then at 60 °C for 15 h. The mixture was poured into water and extracted with Et_2O (×4). The extracts were combined and washed with water, brine, dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue obtained was subjected to flash chromatography (silica gel: NEt_3/MeOH/CH_2Cl_2: 0.25:2.5:100 to 0.5:5:100, gradient elution) to give dibenzyl 4,10-bis(6-(bis(3-ethoxy-3-oxopropyl)amino)-6-oxohexyl)-1,4,7,10-tetraazacyclododecane-1,7-dicarboxylate (**20**) (506 mg, 47%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23–1.28 (m, 16H), 1.41 (br, 4H), 1.58 (br, 4H), 2.29–2.42 (br, 8H), 2.54–2.65 (m, 16H), 3.42 (br, 8H), 3.55–3.64 (m, 8H), 4.10–4.17 (m, 8H), 5.12 (s, 4H), 7.27–7.35 (m, 10H). MS (APCI$^+$): m/z 1068.0 (M+1). Calcd for $C_{56}H_{86}N_6O_{14}$: M^+ 1066.6. Further elution with NEt_3/MeOH/CH_2Cl_2 (1:10:100) gave another colorless oil (202 mg, 27%), which appeared by NMR and MS to be the mono-alkylated product: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23–1.60 (m, 16H), 2.25–2.76 (m, 12H), 3.40–3.65 (m, 12H), 4.10–4.16 (m, 4H), 45.12–5.17 (m, 4H), 7.28–7.34 (m, 10H), 11.0 (br, 1H). MS (APCI$^+$): m/z 754.3 (M+1). Calcd for $C_{40}H_{59}N_5O_9$: M^+ 753.4.

A mixture of **20** (155 mg, 0.14 mmol) and 5% Pd-C (80 mg) in EtOH (5 mL) was hydrogenated under 60 psi of hydrogen gas for 15 h. Solvent was removed and the resulting residue was taken up in CH_2Cl_2 and the catalyst was filtered off. Removal of the solvent gave 1,7-bis(6-(bis(3-ethoxy-3-oxopropyl)amino)-6-oxohexyl)-1,4,7,10-tetraazacyclododecane (**21**) (100 mg, 86%) as a colorless viscous oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24–1.36 (m, 16H), 1.45–1.53 (m, 4H), 1.60–1.67 (m, 4H), 2.35 (t, J = 7.4 Hz, 4H), 2.52–2.62 (m, 12H), 2.75 (br, 8H), 2.86 (br, 8H), 3.57 (t, J = 7.0 Hz, 4H), 3.65 (t, J = 7.2 Hz, 4H), 4.10–4.18 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) single peaks at δ 14.01, 24.80, 26.66, 26.75, 32.67, 33.87, 41.99, 44.00, 47.15, 51.31, 56.24, 60.37, 60.74, 170.80, 171.77, 172.77. MS (APCI$^+$): m/z 799.6 (M+1). Calcd for $C_{40}H_{74}N_6O_{10}$: M^+ 798.5.

A solution of **21** (100 mg, 0.12 mmol) and NaOH (1 mL, 1.0 mol L^{-1}) in EtOH (5 mL) was stirred for 15 h. The mixture was neutralized with HCl (1 mL, 1.0 mol L^{-1}) to pH 5–6. All volatiles were removed and the residue was extracted with EtOH and the solid (NaCl) was filtered off. Removal of solvent gave 3,3′,3″,3‴-(6,6′-(1,4,7,10-tetraazacyclododecane-1,7-diyl)bis(hexanoyl)bis(azanediyl))tetrapropanoic acid (**H_4L11**) (90 mg, 100%) as a pale yellow solid: ^{1}H NMR (400 MHz, D_2O) δ 1.31–1.35 (m, 4H), 1.59–1.63 (m, 8H), 2.45 (t, J = 7.4 Hz, 4H), 2.61 (t, J = 7.0 Hz, 4H), 2.67 (t, J = 7.2 Hz, 4H), 2.78–2.82 (br, 4H), 3.02–3.23 (br, 16H), 3.61 (t, J = 7.0 Hz, 4H), 3.71 (t, J = 7.0 Hz, 4H) (exchangeable NH and COOH not observed). ^{13}C NMR (100 MHz, D_2O) single peaks at δ 24.26, 25.18, 26.47, 32.86, 32.97, 34.01, 42.60, 42.71, 44.95, 49.48, 53.29, 176.46, 176.87. HRMS (FAB, +ve): m/z 687.43045 [M+H]$^+$. Calcd for $C_{32}H_{59}N_6O_{10}$: 687.42927.

4.2. Cobalt(III) complexes

4.2.1. 1-(Chloromethyl)-3-[(5,6,7-trimethoxyindol-2-yl) carbonyl]-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]-5-quinolinolato (1,4,7,10-tetraazacyclododecane-1,7-dimethyl cobalt(III) triflate [Co(L6)(4)]Cl_2 (6)

A mixture of *trans*-[Co(py)$_4$Cl$_2$]Cl·6H$_2$O[17] (118 mg, 0.2 mmol) and 1,7-dimethyl-1,4,7,10-tetraazacyclododecane[11] (**L6**) (40 mg, 0.2 mmol) in MeOH (10 mL) was stirred at 50 °C overnight (~15 h) to give a dark blue solution, which was concentrated and loaded on a LH-20 Sephadex column. MeOH was used to elute the blue band, giving, after removal of the solvent, [Co(**L6**)Cl$_2$]Cl (75 mg, 100%) as a dark blue solid which was used directly: ^{1}H NMR (400 MHz, DMSO-d_6): 2.19–2.24 (m, 2H), 2.33–2.38 (m, 2H), 2.60 (s, 6H), 2.60–2.67 (m, 2H), 2.80–2.92 (m, 4H), 3.00–3.08 (m, 2H), 3.42–3.58 (m, 4H), 6.88 (br, NH), 7.77 (br, NH).

A mixture of [Co(**L6**)Cl$_2$]Cl (55 mg, 0.15 mmol), **4** (77 mg, 0.16 mmol), DIPEA (0.032 mL, 0.18 mmol) and MeOH (5 mL) was stirred at 45 °C overnight (~18 h). All volatiles were removed, and the residue was redissolved in MeOH and filtered through Celite. The filtrate was concentrated and purified by Sephadex LH-20 column chromatography (twice) using MeOH as eluant to give [Co(**L6**)(**4**)]Cl$_2$ (**6**) (84 mg, 70%) as a greenish brown solid. HPLC purity: 96% (45% + 51%, two isomers). ^{1}H NMR (400 MHz, D_2O): δ 1.57/1.67 (2s, 3H), 1.58/1.70 (2s, 3H), 2.84–3.44 (m, 12H), 3.67–3.77 (m, 2H), 3.83/3.85 (2s, 3H), 3.84/3.86 (2s, 3H), 3.89–3.94 (m, 1H), 4.02 (s, 3H), 4.16 (br, 1H), 4.28–4.57 (m, 5H), 7.00–7.06 (m, 2H), 7.74–7.85 (m, 1H), 8.05 (br, 1H), 8.35–8.56 (m, 2H) (exchangeable NH not observed). Anal. Calcd for $C_{34}H_{45}Cl_3CoN_7O_5{\cdot}3H_2O$: C, 47.98; H, 6.04; N, 11.52. Found: C, 47.80; H, 6.51; N, 11.35.

4.2.2. 1-(Chloromethyl)-3-[(5,6,7-trimethoxyindol-2-yl) carbonyl]-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]-5-quinolinolato (1,4,7,10-tetraazacyclododecane-1-butane-4-phosphonato) cobalt(III) triflate [Co(HL7)(4)](OTf) (7)

A solution of $Na_3[Co(CO_3)_3]{\cdot}3H_2O$[20] (290 mg, 0.80 mmol) and **H_2L7** (253 mg, 0.40 mmol) in water (50 mL) was stirred at 60 °C overnight for 21 h to give a purple solution. The resulting precipitate was filtered through Celite and the filtrate was concentrated

and loaded onto a SP Sephadex C-25 ion exchange column. Water was used to flush the column and a purple fraction was collected and desalted (Sephadex LH-20 column with MeOH as eluant) to give [Co(**HL7**)(CO_3)] (165 mg, 92%) as a purple solid, which was used directly: ^{1}H NMR (400 MHz, D_2O) δ 1.50 (br, 4H), 1.72–1.88 (m, 2H), 2.59–2.75 (m, 2H), 2.76–3.08 (m, 14H), 3.38–3.42 (m, 2H) (exchangeable NH and OH not observed). ^{13}C NMR (100 MHz, D_2O) δ 22.38 (d, J_{PC} = 3.3 Hz), 23.12 (d, J_{PC} = 16.0 Hz), ~27–28 (br), 47.88 (s), 47.95 (s), 49.35 (s), 50.01 (s), 53.53 (s), 55.06 (s), 56.04 (s), 58.14 (s), 63.23 (s), 167.46 (s) ^{31}P NMR (162 MHz, D_2O, decoupled) δ 22.98.

Triflic acid (3 mL) was added to cooled (0 °C) [Co(**HL7**)(CO_3)] (145 mg, 0.32 mmol) and the mixture was warmed to room temperature and then heated at 60 °C for 2 h. Et_2O was added to precipitate the product which was triturated and washed with Et_2O to give [Co(**H_2L7**)($OTf)_2$]OTf (250 mg, 95%) as a pink solid, which was used directly: ^{1}H NMR (400 MHz, D_2O) δ 1.50–1.70 (m, 2H), 1.75–1.85 (m, 2H), 1.98–2.08 (m, 2H), 2.46–2.64 (m, 4H), 2.77–2.86 (m, 1H), 2.91–2.95 (m, 1H), 3.00–3.34 (m, 10H), 3.59–3.64 (m, 1H), 3.81–3.86 (m, 1H) (exchangeable NH and OH not observed). ^{13}C NMR (100 MHz, D_2O) δ 20.82 (d, J_{PC} = 4.5 Hz), 23.14 (d, J_{PC} = 16.0 Hz), 26.92 (d, J_{PC} = 135.2 Hz), 48.58 (s), 48.71 (s), 50.07 (s), 50.22 (s), 54.03 (s), 57.45 (s), 58.97 (s), 59.21 (s), 63.42 (s), 120.15 (q, J_{FC} = 317.0 Hz). ^{31}P NMR (162 MHz, D_2O, decoupled) δ 28.24.

A mixture of [Co(**H_2L7**)($OTf)_2$]OTf (241 mg, 0.30 mmol), **4** (115 mg, 0.24 mmol) and pyridine (0.3 mL) in MeOH (20 mL) was stirred at 50 °C for three days. All volatiles were removed and the residue was dissolved in MeOH and filtered through Celite. The filtrate was concentrated and purified by chromatography twice on a Sephadex LH-20 column eluted with MeOH to give [Co(**HL7**)(**4**)](OTf) (**7**) (133 mg, 46%) as a green solid. Purity was 95% by HPLC and the product consisted of two isomers according to its ^{1}H NMR. ^{1}H NMR (400 MHz, D_2O): two isomers (approximately 1:1) δ 0.80–1.30 (m, 4H), 1.40–1.70 (m, 4H), 2.25–2.35 (m, 1H), 2.47–2.56 (m, 2H), 2.66–3.00 (m, 6H), 3.05–3.45 (m, 6H), 3.52–3.76 (m, 3H), 3.83/3.85 (2s, 6H, 2MeO), 3.97/4.07 (2s, 3H, MeO), 4.08–4.19 (m, 1H), 4.30–4.70 (m, 2H), 6.98/7.03 (2s, 1H), 7.05/7.10 (2s, 1H), 7.60/7.80 (2dd, J = 8.5, 5.3 Hz, 1H), 7.96 (br, 1H), 8.36/8.50 (2d, J = 8.5 Hz, 1H), 8.77/8.90 (2s, J = 5.3 Hz, 1H) (exchangeable NH and OH not observed). ^{31}P NMR (162 MHz, D_2O): δ 24.42. ^{19}F NMR (376 MHz, D_2O): δ −78.89. HRMS (FAB, +ve): 832.23854/834.23901 $[M]^+$, calcd for $C_{36}H_{49}{}^{35/37}ClCoN_7O_8P$: 832.24008/834.23713. Anal. Calcd for $C_{37}H_{49}ClCoF_3N_7O_{11}PS{\cdot}5H_2O$: C, 41.44; H, 5.55; N, 9.14; S, 2.99. Found: C, 41.57; H, 5.27; N, 9.23; S, 3.03.

4.2.3. 1-(Chloromethyl)-3-[(5,6,7-trimethoxyindol-2-yl) carbonyl]-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]-5-quinolinolato (1,4,7,10-tetraazacyclododecane-1-butane-4-sulfonate) cobalt(III) chloride [Co(L8)(4)](OTf) (8)

A mixture of [Co(**HL8**)($OTf)_2$](OTf)[7] (0.122 g, 0.15 mmol), **4** (0.070 g, 0.15 mmol) and pyridine (0.075 mL, 0.75 mmol) in MeOH (15 mL) was stirred under nitrogen at 50 °C for 45 h to give a green solution with some precipitate. The mixture was concentrated to a volume about 5 mL and cooled in freezer to give more precipitate. The precipitate was filtered, redissolved in warm MeOH and chromatographed on a Sephadex LH-20 gel filtration column using MeOH as eluant. The first major green band was collected and reduced to dryness to give the product [Co(**L8**)(**4**)](OTf) (**8**) (78.0 mg, 53%) as green solid: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.97, 1.29, 1.46, 2.06 (m, 16H, CH_2), 2.28, 2.38, 2.67–3.01, 3.24, 3.46 (m, 32H, NCH_2), 3.80, 3.82 (s, 12H, OCH_3), 3.94 (m, 8H, CH_2Cl, OCH_3), 4.03 (m, 2H, CH_2Cl), 4.21, 4.30 (m, 2H, CHCH$_2$Cl), 4.39, 4.51 (d, 2H, CONCH_2, $^2J_{HH}$ = 10.8 Hz), 4.72, 4.94 (t, 2H, CONCH_2, $^2J_{HH}$ = 10.8 Hz), 5.96, 6.64, 6.78 (br s, 4H, NH), 6.96 (s, 2H, indole CH), 7.08 (d, 2H, indole CH, $^4J_{HH}$ = 2.0 Hz), 7.55 (br s, 2H, NH), 7.81 (m, 2H, quinoline CH), 8.05 (s, 2H, quinoline CH), 8.67 (d, 2H, quinoline CH, $^3J_{HH}$ = 8.8 Hz), 9.12 (d, 2H, quinoline CH, $^3J_{HH}$ = 4.4 Hz), 11.50 (m, 2H, indole NH). HRMS (FAB, +ve): *m/z* 832.22941/834.23030 $[M]^+$, calcd for $CoC_{36}H_{48}{}^{35/37}ClN_7O_8S$: 832.23056/834.22761.

4.2.4. 1-(Chloromethyl)-3-[(5,6,7-trimethoxyindol-2-yl) carbonyl]-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]-5-quinolinolato (1,4,7,10-tetraazacyclododecane-1,7-dipropane-3,3′-disulfonate)cobalt(III) [Co(L9)(4)] (9)

A mixture of *trans*-[$Co(py)_4Cl_2$]NO_3 (0.035 g, 0.069 mmol), **H_2L9·4HCl**[7] (0.041 g, 0.073 mmol), and **4** (0.032 g, 0.068 mmol) were added into a flask pre-purged with nitrogen followed by absolute EtOH (10 mL) with triethylamine (0.053 g, 0.524 mmol). The mixture was heated under nitrogen at 50 °C with stirring for 14 days until all the suspension was of dark green color and none of the yellow/white five remained. The greenish precipitate of [Co(**L9**)(**4**)] (**9**) (57 mg, 88%) was filtered off and washed first with cold EtOH (5 mL), then with cold Et_2O (10 mL), and dried in a vacuum desiccator: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.07 (m, 2H, NCH_2(CH_2)$_2SO_3^-$), 1.49 (m, 2H, NCH$_2$CH_2CH$_2SO_3^-$), 1.66 (m, 4H, NCH$_2$CH_2CH$_2SO_3^-$, C$H_2SO_3^-$), 1.91 (m, 2H, C$H_2SO_3^-$), 2.29 (m, 2H, NCH_2(CH_2)$_2SO_3^-$), 2.54, 2.59 (m, 4H, CH_2N(CH_2)$_3SO_3^-$), 2.72 (m, 2H, HNCH_2), 2.90 (m, 2H, CH_2N(CH_2)$_3SO_3^-$), 2.94 (m, 2H, HNCH_2), 3.11 (m, 2H, NCH_2(CH_2)$_2SO_3^-$), 3.35, 3.61 (m, 4H, HNCH_2), 3.80, 3.82 (s, 6H, OCH_3), 3.88 (m, 1H, CH_2Cl), 3.95 (s, 3H, OCH_3), 3.98 (m, 1H, CH_2Cl), 4.34 (m, 1H, CHCH$_2$Cl), 4.43 (d, 1H, CONCH_2, $^2J_{HH}$ = 10.7 Hz), 4.83 (t, 1H, CONCH_2, $^2J_{HH}$ = 10.3 Hz), 6.21 (br s, 1H, NH), 6.97, 7.06 (s, 2H, indole CH), 7.79 (br s, 1H, NH), 7.83 (m, 1H, quinoline CH), 8.16 (s, 1H, quinoline CH), 8.67 (d, 1H, quinoline CH, $^3J_{HH}$ = 8.6 Hz), 9.29 (d, 1H, quinoline CH, $^3J_{HH}$ = 4.4 Hz), 11.40 (s, 1H, indole NH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 18.1 (NCH$_2$CH_2CH$_2SO_3^-$), 39.7 (CHCH$_2$Cl), 47.2 (HNCH_2), 48.1 (C$H_2SO_3^-$, CH_2Cl), 49.1 (HNCH_2), 55.5 (ONCH_2), 55.9 (OCH_3), 56.6 (NCH_2(CH_2)$_2SO_3^-$), 58.2 (CH_2N(CH_2)$_3SO_3^-$), 60.9, 61.0 (OCH_3), 62.0 (CH_2N(CH_2)$_3SO_3^-$), 98.0, 106.2 (indole CH), 106.9 (quinoline CH), 111.4 (quinoline quaternary C), 123.0 (indole quaternary C), 123.9 (quinoline CH), 125.3 (quinoline quaternary C), 125.8, 130.9 (indole quaternary C), 135.4 (quinoline CH), 139.0, 139.7 (indole quaternary C), 142.9, 145.7 (quinoline quaternary C), 148.3 (quinoline CH), 149.1 (indole quaternary C), 160.8 (CO), 166.1 (quinoline quaternary C). Analysis ($CoC_{38}H_{51}ClN_7O_{11}S_2{\cdot}4H_2O$) Calcd: C, 45.08; H, 5.87; N, 9.68; Cl, 3.50. Found: C, 45.02; H, 5.83; N, 9.80; Cl, 3.21. HRMS (FAB, +ve): *m/z* 940.21836/942.21650 $[M+H]^+$, calcd for $CoC_{38}H_{51}{}^{35/37}ClN_7O_{11}S_2$: 940.21868/942.21573.

4.2.5. 1-(Chloromethyl)-3-[(5,6,7-trimethoxyindol-2-yl) carbonyl]-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]-5-quinolinolato (1,4,7,10-tetraazacyclododecane-1,7-dibutane-4,4′-diphosphonate)cobalt(III) [Co(H_3L10)(4)] (10)

A suspension of **4** (0.024 g, 0.052 mmol) in MeOH (10 mL) and pyridine (0.022 g, 0.283 mmol) was added to a suspension of [Co(**H_2L10**)Cl_2][7] (0.030 g) in MeOH (10 mL), and the mixture was stirred under nitrogen at 50 °C for seven days to give a green solution and green precipitate. The precipitate was filtered off and retained. The filtrate was concentrated to about 2 mL under reduced pressure, and filtered to remove a yellow precipitate. The green-yellow filtrate was chromatographed on a Sephadex LH-20 gel filtration column, eluting with MeOH. The first major green band was collected and was reduced to dryness to give a green crystalline solid. This was combined with the green reaction precipitate to give [Co(**H_2L10**)(**4**)] (**10**) (24.0 mg, 48%): ^{1}H NMR (CD_3OD, 400 MHz): δ 1.02–1.45, 1.64 (m, 15H, side chain CH_2), 2.38 (m, 2H, side chain CH_2, macrocycle CH_2), 2.64, 2.74, 2.86, 3.09, 3.24, 3.45 (m, 15H, macrocycle CH_2), 3.85 (m, 1H, CH_2Cl), 3.90, 3.91 (s, 6H, OCH_3), 3.98 (dd, 1H, CH_2Cl, $^2J_{HH}$ = 11.4 Hz, $^3J_{HH}$ = 3.8 Hz), 4.07 (s, 3H, OCH_3), 4.32 (m, 1H, CHCH$_2$Cl), 4.71 (dd, 1H, CONCH_2, $^2J_{HH}$ = 10.9 Hz, $^4J_{HH}$ = 2.2 Hz), 4.87 (dd, 1H, CONCH_2, $^2J_{HH}$ = 10.8 Hz, $^3J_{HH}$ = 8.9 Hz), 6.99, 7.15 (s, 2H, in-

dole C*H*), 7.92 (dd, 1H, quinoline C*H*, $^3J_{HH}$ = 5.2 Hz, $^3J_{HH}$ = 8.5 Hz), 8.40 (s, 1H, quinoline C*H*), 8.66 (d, 1H, quinoline C*H*, $^3J_{HH}$ = 7.9 Hz), 9.20 (d, 1H, quinoline C*H*, $^3J_{HH}$ = 4.6 Hz) (exchangeable NH and OH not observed). HRMS (FAB, +ve): *m/z* 968.26535/970.27300 $[M+H]^+$, calculated for $CoC_{40}H_{58}{}^{35/37}ClN_7O_{11}P_2$: 968.26901/970.26606.

4.2.6. 1-(Chloromethyl)-3-[(5,6,7-trimethoxyindol-2-yl) carbonyl]-2,3-dihydro-1*H*-pyrrolo[3,2-*f*]-5-quinolinolato 3,3′,3″,3‴ -(6,6′-(1,4,7,10-tetraazacyclododecane-1,7-diyl) bis(hexanoyl)bis(azanediyl))tetrapropanoic acid cobalt(III) dichloride, [Co(**H$_4$L11**)(**4**)]Cl$_2$ (**11**)

A mixture of *trans*-$[Co(py)_4Cl_2]Cl{\cdot}6H_2O$ (100 mg, 0.17 mmol) and **H$_4$L11** (128 mg, 0.19 mmol) in MeOH (10 mL) was stirred at 45 °C for 29 h after which all the volatiles were removed. The resulting residue was purified by Sephadex LH-20 column (×2) using MeOH as eluant to give [Co(**H$_4$L11**)Cl$_2$]Cl (90 mg, 62%) as a blue solid, which was used directly: ^{1}H NMR (400 MHz, CD_3OD): 1.29–1.47 (m, 4H), 1.61–1.73 (m, 8H), 2.41–2.47 (m, 4H), 2.58–2.64 (m, 8H), 2.78–2.86 (m, 2H), 2.94–3.04 (m, 4H), 3.13–3.18 (m, 2H), 3.25–3.45 (m, 12H), 3.61 (br, 4H), 3.67–3.81 (m, 4H) (exchangeable NH and COOH not observed). ^{13}C NMR (101 MHz, D_2O): single peaks at 23.55, 26.26, 28.13, 33.93, 49.62, 51.02, 52.22, 60.44, 62.51, 64.79, 66.08 and a broad peak around 176.85.

A mixture of [Co(**H$_4$L11**)Cl$_2$]Cl (90 mg 0.11 mmol), **4** (0.12 mmol) and pyridine (86 μL, 1.06 mmol) in MeOH (30 mL) was stirred at 45–50 °C for seven days. All volatiles were removed. The residue was redissolved in MeOH and the resulting solution was filtered through Celite. The filtrate was concentrated and purified by LH-20 column twice using MeOH as eluant to give a green solid, which was further purified by preparative HPLC (Synergi Max-RP C12 column, using a gradient of 10–60% aqueous ammonium formate (0.045 M, pH 3.45) in 90% acetonitrile/water as mobile phase at a flow rate of 15 mL/min) to give [Co(**H$_4$L11**)(**4**)]Cl$_2$ (**11**) (23 mg, 19%) as a green solid. HPLC purity was 99%, with a ~2:1 ratio of isomers according to NMR: ^{1}H NMR (400 MHz, D_2O): 0.70–1.70 (m, 12H), 2.05–2.15 (m, 4H), 2.30–2.50 (m, 8H), 2.65–2.75 (m, 2H), 2.80–2.95 (m, 2H), 3.00–3.60 (m, 14H), 3.70–3.80 (m, 3H), 3.98/4.00 (2s, 6H), 4.10/4.12 (2s, 3H), 4.35–4.45 (m, 2H), 4.50–5.00 (m, 10H), 7.14–7.28 (m, 2H), 7.94–8.03 (m, 1H), 8.20–8.39 (br, 1H), 8.64–8.69 (m, 1H), 9.12–9.18 (m, 1H) (exchangeable NH and COOH not observed). FAB-MS: *m/z* found 1210 $[M-H]^+$ (the peak was too small to get accurate mass); calcd for $C_{56}H_{78}{}^{35}ClCoN_9O_{15}$: 1210.4638.

4.3. Compound physicochemical properties

4.3.1. Stability

Compounds were dissolved in αMEM culture medium containing 5% fetal calf serum, typically at ~30 μM. Solutions were incubated at room temperature or 37 °C and sampled at intervals for HPLC analysis. The HPLC system comprised an Agilent MSD LC/MS with diode array absorbance and positive mode electrospray ionization detectors as previously described.[8]

4.3.2. Solubility

Compound solubilities in αMEM culture medium containing 5% fetal calf serum were determined as described previously.[9]

4.4. Cytotoxicity in cell culture

The cytotoxicity of the effectors and their metal complexes was determined by assessing inhibition of cell proliferation following 4 h exposure under oxic and hypoxic conditions, using attached cells in 96 well plates, as described previously.[8] Compounds were formulated in DMSO and diluted to <1% DMSO in the cultures.

Acknowledgments

The authors are grateful to Maruta Boyd, Sisira Kumara, Karin Tan and Jane Botting for technical assistance. This work was funded by the Cancer Society of New Zealand and the Foundation for Research, Science and Technology, New Zealand (Grant no. UOAX0211).

References and notes

1. Brown, J. M.; Wilson, W. R. *Nat. Rev. Cancer* **2004**, *4*, 437.
2. Simic, M.; Lilie, J. *J. Am. Chem. Soc.* **1974**, *96*, 291.
3. Ware, D. C.; Palmer, B. D.; Wilson, W. R.; Denny, W. A. *J. Med. Chem.* **1993**, *36*, 1839.
4. Gambino, D. *Curr. Med. Chem.* **2010**, *17*, 3616.
5. Failes, T. W.; Cullinane, C.; Diakos, C. I.; Yamamoto, N.; Lyons, J. G.; Hambley, T. W. *Chem. Eur. J.* **2007**, *13*, 2974.
6. Ahn, G.-O.; Ware, D. C.; Denny, W. A.; Wilson, W. R. *Radiat. Res.* **2004**, *162*, 315.
7. Chang, J. Y.-C.; Stevenson, R. J.; Lu, L. G.; Brothers, P. J.; Clark, G. R.; Denny, W. A.; Ware, D. C. *Dalton Trans.* **2010**, *39*, 11535.
8. Ahn, G.-O.; Botting, K. J.; Patterson, A. V.; Ware, D. C.; Tercel, M.; Wilson, W. R. *Biochem. Pharmacol.* **2006**, *71*, 1683.
9. Milbank, J. B. J.; Stevenson, R. J.; Ware, D. C.; Chang, J. Y. C.; Tercel, M.; Ahn, G.-O.; Wilson, W. R.; Denny, W. A. *J. Med. Chem.* **2009**, *52*, 6822.
10. Wilson, W. R.; Hicks, K. O.; Pullen, S. M.; Ferry, D. M.; Helsby, N. A.; Patterson, A. V. *Radiat. Res.* **2007**, *167*, 625.
11. Rohovec, J.; Gyepes, R.; Císařová, I.; Rudovský, J.; Lukeš, I. *Tetrahedron Lett.* **2000**, *41*, 1249.
12. Weisman, G. R.; Ho, S. C. H.; Johnson, V. *Tetrahedron Lett.* **1980**, *21*, 335.
13. Kim, C. U.; Luh, B. Y.; Misco, P. F.; Bronson, J. J.; Hitchcock, M. J. M.; Ghazzouli, I.; Martin, J. C. *J. Med. Chem.* **1990**, *33*, 1207.
14. Vafina, G. F.; Yakhina, G. R.; Khakimova, T. V.; Spirikhin, L. V.; Galin, F. Z.; Yunusov, M. S. *Russ. J. Org. Chem.* **2003**, *39*, 49.
15. Cicchi, S.; Bonanni, M.; Cardona, F.; Revuelta, J.; Goti, A. *Org. Lett.* **2003**, *5*, 1773.
16. Kovacs, Z.; Sherry, A. D. *Synthesis* **1997**, *7*, 759.
17. Christine, N. E.; Cecil, F. W. *J. Chem. Soc., Dalton Trans.* **1980**, 2405.
18. Moreno, S. N.; Mason, R. P.; Docampo, R. *J. Biol. Chem.* **1984**, *259*, 6298.
19. García-Barradas, O.; Juaristi, E. *Tetrahedron: Asymmetry* **1997**, *8*, 1511.
20. Bauer, H. F.; Drinkard, W. C.; Thompson, R. J. *Inorg. Synth.* **1966**, *8*, 202.