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References

Experimental Details

Materials and Methods. All synthetic procedures were performed under normal atmospheric conditions. ^1H NMR spectra were recorded on a BrukerAvance 400 MHz Ultrashield NMR spectrometer. ^1H NMR spectra were referenced internally to residual solvent peaks, and chemical shifts are expressed relative to tetramethylsilane, SiMe_4 ($\delta = 0$ ppm). High resolution electron spray ionisation mass spectra were recorded on a BrukerDaltonics Esquire 3000 spectrometer by Dr. Lisa Haigh (Imperial College London). Fourier transform infrared (FTIR) spectra were recorded with an IRAffinity-1S Shimadzu spectrophotometer. Elemental analysis was performed commercially by London Metropolitan University. *trans*-Dichloro(cyclam)-cobalt(III) chloride, **2** was prepared according to a previously reported protocol.^[1] For all biophysical and cellular studies, a mM range stock solution of **1** in DMSO was initially prepared. The stock solution was diluted in the appropriate biological solution to the working concentration(s). The cobalt concentration of the stock solution was determined by inductively coupled plasma mass spectrometry (ICP-MS, PerkinElmer NexION 350D).

Synthesis of Co(1,4,8,11-tetraazacyclotetradecane)(naproxen)₂ (1). Naproxen (50 mg, 0.2 mmol) was suspended in MeOH (50 mL) dried with Na_2SO_4 , and to this *trans*-dichloro(cyclam)-cobalt(III) chloride, **2** (30 mg, 0.08 mmol) in MeOH (2.5ml) was added. Ag_2O (100 mg, 0.43 mmol) was added to the resulting green solution, and the mixture was stirred for 2 days. Excess Ag_2O and AgCl were removed by filtration and the solvent was removed. The resulting residue was washed with water, dissolved in acetone and filtered to yield the crude product. An aqueous solution of NaPF_6 was added to the crude product dissolved in a minimum amount of DMSO. The resulting precipitate was collected and washed with water and diethyl ether. The product was isolated as a light purple solid (28 mg, 42%); ^1H NMR (400MHz, DMSO-d_6): δ_{H} 8.17 (br s, 2H), 7.71 (dd, 4H), 7.53 (s, 2H), 7.25 (s, 2H), 7.20 (dd, 2H), 7.13 (dd, 2H), 7.01 (br s, 2H), 3.86 (s, 6H), 3.48 (q, 2H), 2.65-1.29 (m, 20H), 1.24 (d, 6H); ^{13}C NMR (400MHz, DMSO-d_6): δ_{C} 186.25, 157.30, 138.45, 133.36, 129.24, 128.81, 127.04, 126.91, 126.08, 119.13, 106.06, 55.55, 52.17, 52.00, 48.71, 46.35, 46.22, 26.73, 18.49; IR (solid, cm^{-1}): 3266, 2969, 2944, 2882, 1585, 1462, 1372, 1340, 1323, 1268, 1215, 1164, 1071, 1026, 929, 840, 823, 813, 669, 557; HR ESI-MS Calcd. for $\text{C}_{38}\text{H}_{50}\text{CoN}_4\text{O}_6$ $[\text{M-PF}_6]^+$: 717.7231 a.m.u. Found $[\text{M-PF}_6]^+$: 717.3070 a.m.u.; Anal. Calcd. for **1**, $\text{C}_{38}\text{H}_{50}\text{CoN}_4\text{O}_6\text{PF}_6$: C, 52.90; H, 5.84; N, 6.49. Found: C, 52.83; H, 5.79; N, 6.50.

X-ray Single Crystal Diffraction Analysis. Standard procedures were used to mount the crystal on a Gemini diffractometer with graphite-monochromated Mo $\text{K}\alpha$ radiation ($\lambda = 0.71073$ Å) at 293 K. The crystal structure was solved using direct methods in SHELXS and refined by full-matrix least-squares routines, based on F^2 , using the SHELXL program.^[2] All the H atoms were placed in geometrically idealised positions and constrained to ride on their parent atoms. The structure has been deposited with the Cambridge Crystallographic Data Centre (CCDC 148346). This information can be obtained free of charge from www.ccdc.cam.ac.uk/data_request/cif.

DNA Cleavage Studies. Plasmid DNA (pUC19) was purchased from Invitrogen. The DNA cleavage activity of **1** and **2** was determined by monitoring the conversion of supercoiled plasmid DNA (form I) to nicked circular DNA (form II) and linear DNA (form III) in Tris-HCl buffer (5 mM, pH 7.4), using agarose-gel electrophoresis. To probe the effect of compound concentration on cleavage, solutions containing DNA (100 ng) and the test compound (0-50 μM), with a total reaction volume of 20 μL , were incubated at 37 °C for 24 h. To determine the oxidative cleavage mechanism, solutions containing DNA (100 ng), the

test compound (15 μM), and various radical scavengers (10 mM or 40 mM of KI, DMSO, $t\text{BuOH}$, and NaN_3), with a total reaction volume of 20 μL , were incubated at 37 $^\circ\text{C}$ for 24 h. Reactions were also conducted in the absence of oxygen, and in the presence of 10 equivalents of ascorbic acid or glutathione. For the T4 DNA ligase assay,^[3] solutions containing DNA (100 ng), the test compound (15 μM), and the T4 DNA ligase (2 units, in ligation buffer) with a total reaction volume of 20 μL , were incubated at 37 $^\circ\text{C}$ for 2 h. After incubation, loading buffer (5 μL , containing 0.25% bromophenol blue, 0.25% xylene cyanol and 60% glycerol) was added and reaction mixtures were immediately loaded onto a 1% agarose gel containing ethidium bromide (1.0 mg mL^{-1}). The DNA fragments were separated by applying 60 V for 2 h in Tris-acetate EDTA (TAE) buffer. The DNA bands were analysed under UV light using a Fujifilm Image Reader LAS-3000.

Measurement of Water-Octanol Partition Coefficient (Log P). The log P value of **1** was determined using the shake-flask method and UV-Vis spectroscopy. The octanol used in this experiment was pre-saturated with water. An aqueous solution of **1** (500 μL , 100 μM) was incubated with the pre-saturated octanol (500 μL) in a 1.5 mL tube. The tube was shaken at room temperature for 48 h. The two phases were separated by centrifugation and **1** content in each phase was determined by UV-Vis spectroscopy.

Cell Lines and Cell Culture Conditions. The human mammary epithelial cell lines, HMLER and HMLER-shEcad were kindly donated by Prof. R. A. Weinberg (Whitehead Institute, MIT).^[4] HMLER and HMLER-shEcad cells were maintained in Mammary Epithelial Cell Growth Medium (MEGM) with supplements and growth factors (BPE, hydrocortisone, hEGF, insulin, and gentamicin/amphotericin-B). The cells were grown at 310 K in a humidified atmosphere containing 5% CO_2 .

Cytotoxicity MTT assay. The colourimetric MTT assay was used to determine the toxicity of **1**, **2**, naproxen, and salinomycin. HMLER or HMLER-shEcad cells (5×10^3) were seeded in each well of a 96-well plate. After incubating the cells overnight, various concentrations of the compounds (0.0004-100 μM), were added and incubated for 72 h (total volume 200 μL). Stock solutions of the compounds were prepared as 10 mM solutions in DMSO and diluted using media. The final concentration of DMSO in each well was 0.5% and this amount was present in the untreated control as well. After 72 h, the medium was removed, 200 μL of a 0.4 mg/mL solution of MTT in MEGM was added to each well, and the plate was incubated for an additional 4 h. The MEGM/MTT mixture was aspirated and 200 μL of DMSO was added to dissolve the resulting purple formazan crystals. The absorbance of the solutions in each well was read at 550 nm. Absorbance values were normalized to DMSO-containing control wells and plotted as concentration of test compound versus % cell viability. IC_{50} values were interpolated from the resulting dose dependent curves. The reported IC_{50} values are the average of three independent experiments, each consisting of six replicates per concentration level (overall $n = 18$).

Tumorsphere Formation and Viability Assay. HMLER-shEcad cells (5×10^3) were plated in ultralow-attachment 96-well plates (Corning) and incubated in MEGM supplemented with B27 (Invitrogen), 20 ng/mL EGF, and 4 $\mu\text{g/mL}$ heparin (Sigma) for 5 days. Studies were also conducted in the presence of **1**, **2**, naproxen, salinomycin, vinorelbine, cisplatin, and paclitaxel (0-66 μM). Mammospheres treated with **1**, **2**, naproxen, and salinomycin (at their respective IC_{20} values, 5 days) were counted and imaged using an inverted microscope. The viability of the mammospheres was determined by addition of a resazurin-based reagent, TOX8 (Sigma). After incubation for 16 h, the fluorescence of the solutions was read at 590

nm ($\lambda_{\text{ex}} = 560$ nm). Fluorescence values were normalized to DMSO-containing controls and plotted as concentration of test compound versus % mammospheres viability. IC₅₀ values were interpolated from the resulting dose dependent curves. Identical studies were also conducted in the presence of cobalt chloride (5 μ M). The reported IC₅₀ values are the average of two independent experiments, each consisting of five replicates per concentration level (overall n = 10).

Cellular Uptake. To measure the cellular uptake of **1** and **2**, *ca.* 1 million HMLER-shEcad cells were treated with 0.5 μ M of **1** and **2** at 37 °C for 12 h. Then the media was removed, the cells were washed with PBS solution (2 mL $\times$ 3), harvested, and centrifuged. The cellular pellet was suspended in an appropriate volume of PBS to obtain a homogeneous cell suspension (1 mL). The suspension was divided into two. One part was used to determine the cobalt content in the whole cell and other was used for nuclear, cytoplasmic, and membrane analysis. The Thermo Scientific NE-PER Nuclear and Cytoplasmic Extraction Kit were used to extract and separate the nuclear, cytoplasmic, and membrane fractions. The fractions were dissolved in 65% HNO₃ overnight (250 μ L final volume). The sample were then diluted 5-fold with water and analysed using ICP-MS (PerkinElmer NexION 350D). Cobalt levels are expressed as Co (ppb) per million cells. Results are presented as the mean of five determinations for each data point.

RNAi Signatures.^[5] The cobalt(III)-cyclam complex, **1** was dosed to achieve an LD80-90 in *E μ -Myc^{p19arf/-}* cells by propidium iodide exclusion as determined by flow cytometry after 48 h incubation. GFP enrichment/depletion was then determined by flow cytometry at 72 h. Linkage ratios (LR) and p-values were generated as described previously. All flow cytometry was conducted using a FACScan flow cytometer (BD Biosciences).

GFP Competition assays. *E μ -Myc^{p19arf/-}* lymphoma cells were infected with GFP-tagged shRNAs such that 15-25% of the population were GFP positive. An eighth of a million cells in 250 μ L B-cell media (BCM) were then seeded into 24-well plates. For wells that would remain untreated as a control, only 1/16th of a million cells were seeded. Next, 250 μ L of media containing the active agent was added to the cells. After 24 h, 300 μ L of cells from untreated wells are removed and replaced by 300 μ L fresh BCM. All wells then received 500 μ L BCM before being placed by in the incubator for another 24 h. At 48 h, cells transduced with the control vector, MLS, were checked for viability via flow cytometry on a FACScan flow cytometer (BD Biosciences) using propidium iodide as a live/dead marker.

Immunoblotting Analysis. HMLER-shEcad cells (5 $\times$ 10⁵ cells) were incubated with **1** (various concentrations, 0.125-0.5 and 0.1-0.4 μ M) for 72 h at 37 °C. Cells were washed with PBS, scraped into SDS-PAGE loading buffer (64 mM Tris-HCl (pH6.8)/ 9.6% glycerol/ 2% SDS/ 5% β -mercaptoethanol/ 0.01% Bromophenol Blue), and incubated at 95 °C for 10 min. Whole cell lysates were resolved by 4-20 % sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE; 200 V for 25 min) followed by electro transfer to polyvinylidene difluoride membrane, PVDF (350 mA for 1 h). Membranes were blocked in 5% (w/v) non-fat milk in PBST (PBS/0.1% Tween 20) and incubated with the appropriate primary antibodies (Cell Signalling Technology). After incubation with horseradish peroxidase-conjugated secondary antibodies (Cell Signalling Technology), immune complexes were detected with the ECL detection reagent (BioRad) and analyzed using a chemiluminescence imager (Amersham Imager 600).

COX Inhibition Enzyme Immunoassay (EIA). The cyclooxygenase-2 (COX-2) inhibitory properties of **1** and naproxen was determined using the “COX (ovine) Inhibitor Screening Assay (Cayman Chemicals).” The assay was performed according to the manufacturer’s instructions. Inhibitory activity was determined at 0.5 μ M and 5 μ M for **1** and naproxen. Absorption was recorded using a microplate reader at 412 nm.

Flow cytometry. HMLER-shEcad cells were seeded in 6-well plates (at a density of 5×10^5 cells/ mL) and the cells were allowed to attach overnight. The cells were treated with lipopolysaccharide (LPS) (2.5 μ M for 24 h), and then treated with **1** (0.5-2 μ M), **2** (20 μ M), and naproxen (20 μ M) and incubated for a further 48 h. The cells were then harvested by trypsinization, fixed with 4% paraformaldehyde (at 37 °C for 10 min), permeabilized with ice-cold methanol (for 30 min), and suspended in PBS (200 μ L). The Alexa Fluor® 488 nm labelled anti-COX-2 antibody (5 μ L) was then added to the cell suspension and incubated in the dark for 1 hr. The cells were then washed with PBS (1 mL) and analysed using a FACSCanto II flow cytometer (BD Biosciences) (10,000 events per sample were acquired). The FL1 channel was used to assess COX-2 expression. Cell populations were analysed using the FlowJo software (Tree Star).

Figure S1. ¹H NMR spectrum in DMSO-d₆ of the cobalt(III)-cyclam complex, **1**.

Figure S2. ¹³C NMR spectrum in DMSO-d₆ of the cobalt(III)-cyclam complex, **1**.

Figure S3. Crystal structure of **1** (ellipsoid thermal probability was drawn at the level of 30%; the hexafluorophosphate anion and hydrogen atoms were omitted for clarity). The structure has been deposited with the Cambridge Crystallographic Data Centre (CCDC 148346). This information can be obtained free of charge from www.ccdc.cam.ac.uk/data_request/cif.

Table 1. Crystallographic data for **1**.

1	
formula	C ₃₈ H ₅₀ CoF ₆ N ₄ O ₆ P
<i>F</i> _w	862.72
crystal system	Monoclinic
space group	<i>P</i> 2(1)
<i>a</i> , Å	8.2998(15)
<i>b</i> , Å	11.7067(17)
<i>c</i> , Å	21.027(3)
<i>α</i> , deg.	90
<i>β</i> , deg.	91.860(14)
<i>γ</i> , deg.	90
<i>V</i> , Å ³	2042.0(6)
<i>Z</i>	2
<i>D</i> _{calcd} , Mg/m ³	1.403
Reflections collected	8569
Reflections independent (<i>R</i> _{int})	6057 (0.0314)
Goodness-of-fit on <i>F</i> ²	1.031
<i>R</i> (<i>I</i> > 2σ <i>I</i>)	0.0702, 0.1648

Table 2. Selected bond lengths (Å) and angles (°) of **1**.

Co(1)-O(2)	1.904(5)	Co(1)-O(3)	1.911(5)
Co(1)-N(4)	1.959(8)	Co(1)-N(3)	1.967(7)
Co(1)-N(1)	1.968(7)	Co(1)-N(2)	1.978(9)
O(2)-Co(1)-O(3)	178.1(3)	O(2)-Co(1)-N(4)	91.9(3)
O(3)-Co(1)-N(4)	89.9(3)	O(2)-Co(1)-N(3)	95.3(3)
O(3)-Co(1)-N(3)	85.7(3)	N(4)-Co(1)-N(3)	85.4(3)
O(2)-Co(1)-N(1)	84.2(3)	O(3)-Co(1)-N(1)	94.9(3)
N(3)-Co(1)-N(1)	178.9(4)	O(2)-Co(1)-N(2)	87.4(3)
O(3)-Co(1)-N(2)	90.8(3)	N(4)-Co(1)-N(2)	179.2(4)
N(3)-Co(1)-N(2)	94.8(4)	N(1)-Co(1)-N(2)	86.2(4)

Figure S4. Concentration-dependent DNA cleavage by **1** and **2** after a 24 h incubation period. a) Lane 1: DNA only, Lane 2-5: DNA + 2, 5, 10, and 15 μ M of **1**; b) Lane 1: DNA only, Lane 2-6: DNA + 2, 5, 10, and 15 μ M of **2**.

Figure S5. Concentration-dependent DNA cleavage by naproxen after a 24 h incubation period. Lane 1: DNA only, Lane 2-5: DNA + 2, 5, 10, 20, and 50 μ M of naproxen.

Figure S6. Effect of ascorbic acid or glutathione on **1**- and **2**- mediated DNA cleavage after a 24 h incubation period. a) Lane 1: DNA only, Lane 2-5: DNA + 2, 5, 10, and 15 μ M of **1** with 10 equivalents of ascorbic acid; b) Lane 1: DNA only, Lane 2-5: DNA + 2, 5, 10, and 15 μ M of **2** with 10 equivalents of ascorbic acid. c) Lane 1: DNA only, Lane 2-6: DNA + 2, 5, 10, 15, and 20 μ M of **1** with 10 equivalents of glutathione.

Figure S7. Inhibition of **1**-mediated DNA cleavage by NaN_3 , KI, DMSO or $t\text{BuOH}$, and in the absence of oxygen after a 24 h incubation period. a) Lane 1: DNA only, Lane 2: DNA + **1** (15 μ M), Lane 3-6: DNA + **1** (15 μ M) + NaN_3 , KI, DMSO or $t\text{BuOH}$. b) Lane 1: DNA only in the absence of oxygen, Lane 2-6: DNA + 2, 5, 10, 15, and 20 μ M of **1** in the absence of oxygen.

Figure S8. Re-ligation by T4 DNA ligase following **1**-mediated DNA cleavage. Lane 1: DNA only, Lane 2: DNA + **1** (15 μ M), Lane 3: DNA + **1** (15 μ M) + T4 DNA ligase.

Figure S9. UV-Vis spectrum of **1** (30 μM) in PBS over the course of 24 h at 37 °C.

Figure S10. UV-Vis spectrum of **1** (50 μM) in 0.9% NaCl solution over the course of 24 h at 37 °C.

Figure 11. UV-Vis spectrum of **1** (50 μM) in Tris-HCl buffer (5 mM, pH 7.4) in the presence of ascorbic acid (0.5 mM) over the course of 24 h at 37 $^{\circ}\text{C}$.

Figure S12. UV-Vis spectrum of **1** (50 μM) in 0.9% NaCl solution in the presence of ascorbic acid (0.5 mM) over the course of 24 h at 37 $^{\circ}\text{C}$.

Figure S13. UV-Vis spectrum of naproxen (50 μ M) in 0.9% NaCl solution at 37 $^{\circ}$ C.

Figure S14. Full ESI-TOF mass spectrum (negative mode) of **1** (50 μ M) in 0.9% NaCl solution, in the presence of ascorbic acid (0.5 mM) after 72 h.

Figure S15. A) Full ESI-TOF mass spectrum (positive mode) of **1** (50 μM) in 0.9% NaCl solution, in the presence of ascorbic acid (0.5 mM) after 72 h. B) Part of the ESI-TOF mass spectrum (positive mode, 150-290 m/z) of **1** (50 μM) in 0.9% NaCl solution, in the presence of ascorbic acid (0.5 mM) after 72 h.

Figure S16. UV-vis spectrum of **1** (50 μM) in MEGM cell media over the course of 24 h at 37 $^{\circ}\text{C}$.

Figure S17. Representative dose-response curves for the treatment of HMLER-shEcad cells with **1**, **2**, and naproxen, after 72 h incubation.

Figure S18. Representative dose-response curves for the treatment of HMLER cells with **1**, **2**, and naproxen, after 72 h incubation.

Figure S19. Representative dose-response curves for the treatment of HMLER and HMLER-shEcad cells with salinomycin, after 72 h incubation.

Figure S20. Representative bright-field images ($\times 10$) of HMLER-shEcad mammospheres in the A) absence and presence of B) **1**, C) **2**, D) naproxen, and E) salinomycin (at their respective IC₂₀ values, 5 days).

Figure S21. Representative dose-response curves for the treatment of HMLER-shEcad mammospheres with **1**, **2**, naproxen, paclitaxel, vinorelbine, cisplatin, and salinomycin after 5 days incubation.

Table S3. IC₅₀ values of **2**, naproxen, vinorelbine, cisplatin, and paclitaxel against HMLER-shEcad mammospheres determined after 5 days incubation (mean of three independent experiments $\pm$ SD).

Compound	Mammosphere IC ₅₀ [μ M]
2	> 66
naproxen	> 66
vinorelbine	22.20 $\pm$ 0.29
cisplatin	8.24 $\pm$ 0.08
paclitaxel	> 66

Figure S22. Representative dose-response curves for the treatment of HMLER-shEcad mammospheres with **1** after 5 days incubation in the presence and absence of CoCl₂ (5 μ M).

Figure S23. Quantification of mammosphere formation with HMLER-shEcad cells untreated and treated with **1** (at the IC₂₀ value, 5 days) in the presence of cobalt chloride (5 μ M). Error bars represent standard deviations and Student *t* test, * = $p < 0.05$.

Figure S24. Representative bright-field images ($\times 10$) of HMLER-shEcad mammospheres supplemented with cobalt chloride ($5 \mu\text{M}$) in the A) absence and B) presence of **1** (at the IC_{20} value, 5 days).

Figure S25. RNAi signatures derived from the treatment of $\text{E}\mu\text{-Mycp}^{19\text{arf-/-}}$ lymphoma cells with **1**, puromycin, and actinomycin D at the LD80–90 concentration.

Figure S26. Immunoblotting analysis of proteins related to the DNA damage and apoptosis pathways. Protein expression in HMLER-shEcad cells following treatment with **1** (0.125, 0.25, and 0.5 μM) after 72 h incubation. Whole cell lysates were resolved by SDS-PAGE and analyzed by immunoblotting against γH2AX , phos-CHK2, cleaved caspase 3, cleaved caspase 7, and $\beta\text{-actin}$ (loading control).

Figure S27. Cyclooxygenase-2 (COX-2) inhibitory properties of **1** (0.5 and 5 μ M) and naproxen (0.5 and 5 μ M). Error bars represent standard deviations and Student *t test*, * = $p < 0.05$, ** = $p < 0.01$.

Figure S28. A) Representative histograms displaying the green fluorescence emitted by anti-COX-2 Alexa Fluor 488 nm antibody-stained HMLER-shEcad cells treated with LPS (2.5 μ M) for 24 h (red) followed by 48 h in media containing **1** (0.5 - 2 μ M, blue, green, and orange), B) Representative histograms displaying the green fluorescence emitted by anti-COX-2 Alexa Fluor 488 nm antibody-stained HMLER-shEcad cells treated with LPS (2.5 μ M) for 24 h (red) followed by 48 h in media containing naproxen (20 μ M, blue) and **2** (20 μ M, green).

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