

Supporting Information for

Anti-cancer stem cell cobalt(III)-polypyridyl complexes containing salicylic acid

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References

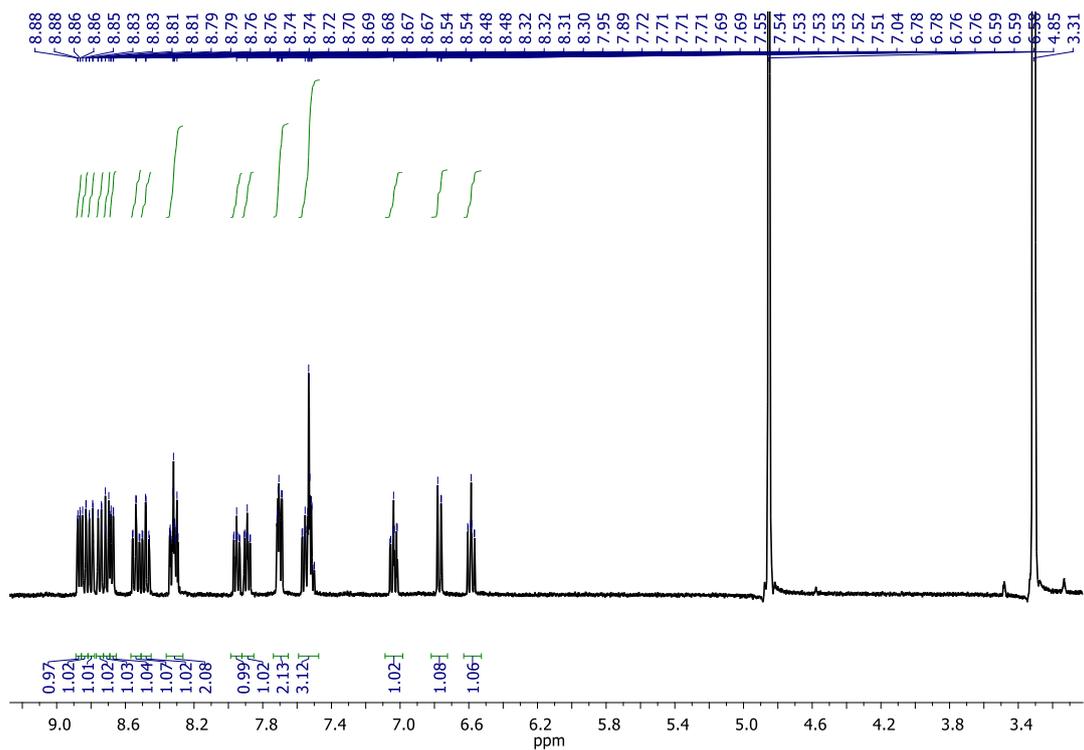


Figure S1. ^1H NMR spectrum of **4** in CD_3OD .

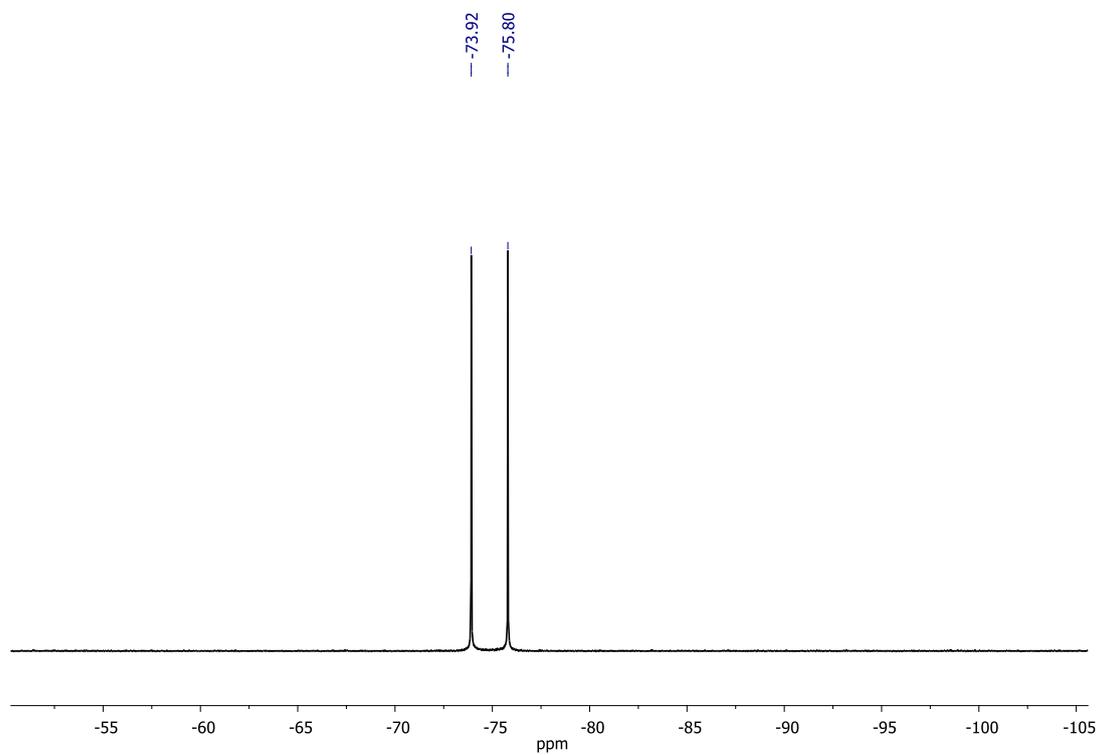


Figure S2. $^{19}\text{F}\{^1\text{H}\}$ NMR spectrum of **4** in CD_3OD .

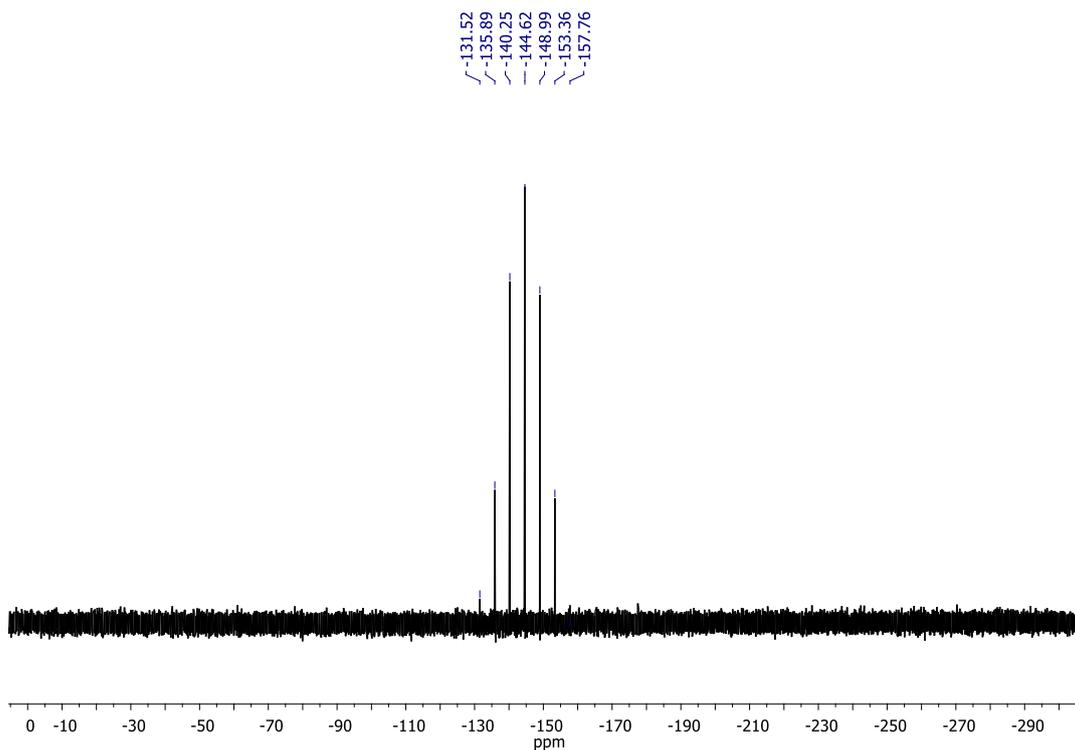


Figure S3. $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum of **4** in CD_3OD .

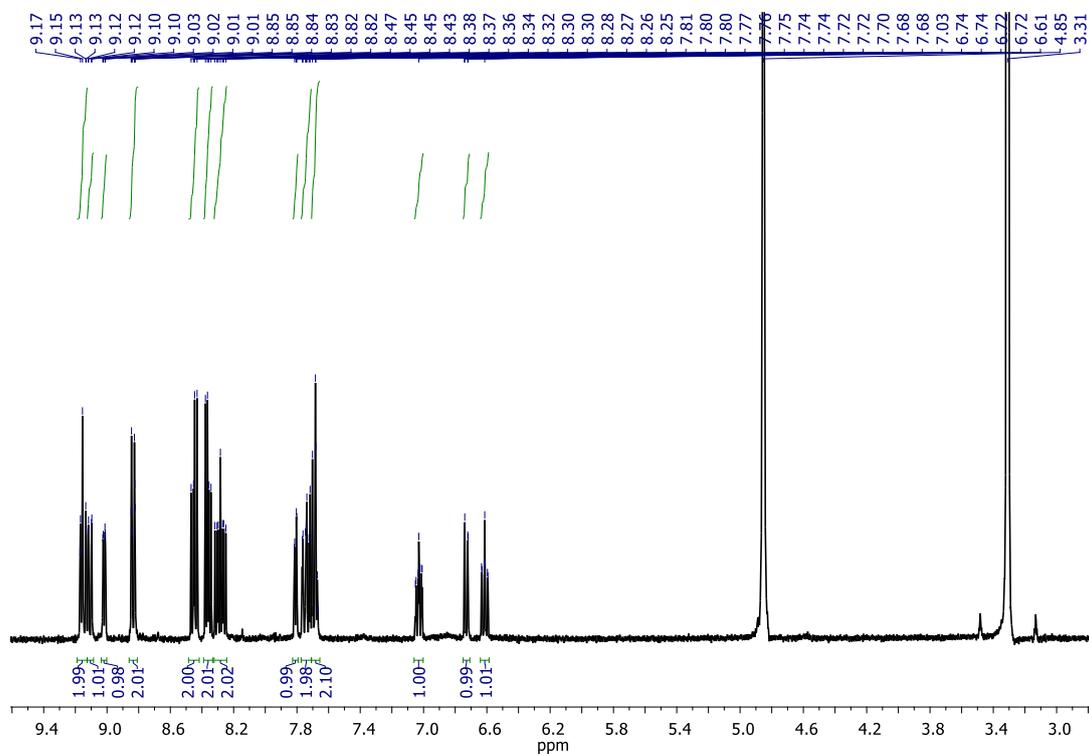


Figure S4. ^1H NMR spectrum of **5** in CD_3OD .

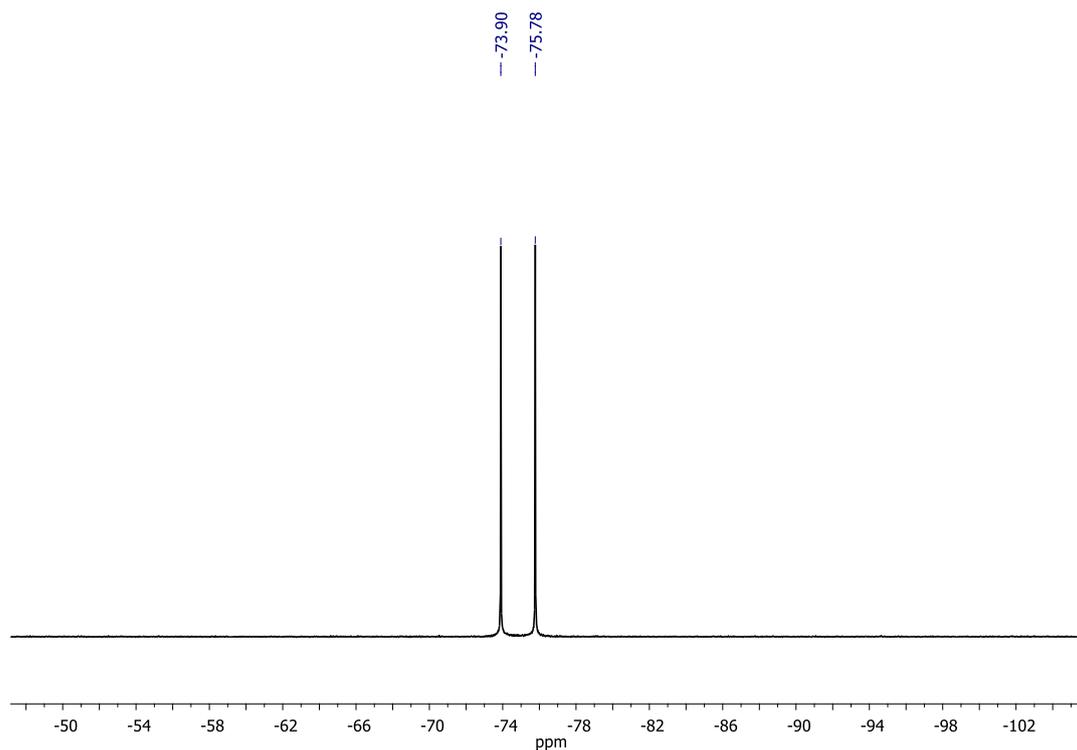


Figure S5. $^{19}\text{F}\{^1\text{H}\}$ NMR spectrum of **5** in CD_3OD .

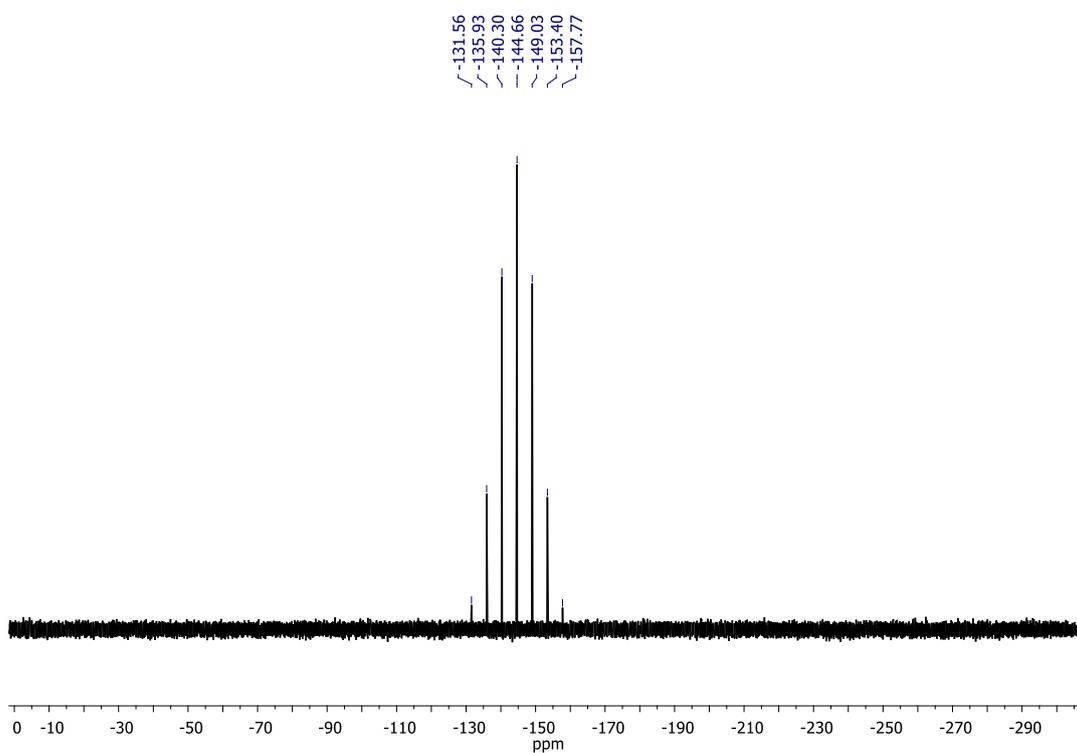


Figure S6. $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum of **5** in CD_3OD .

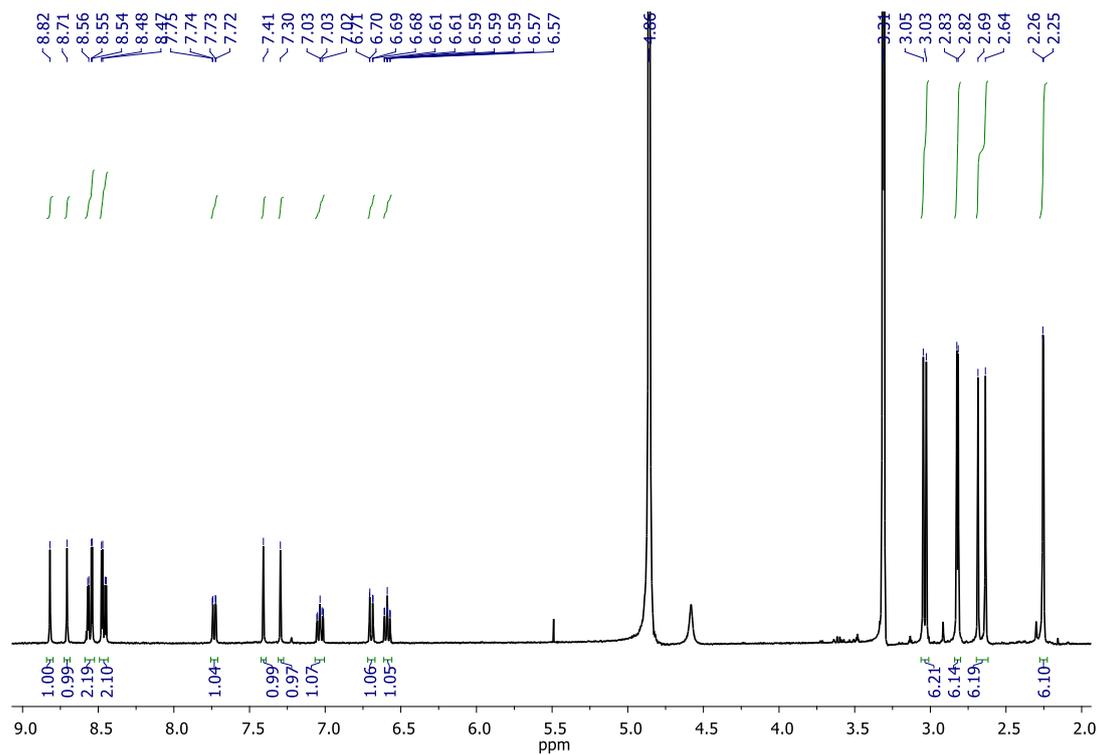


Figure S7. ^1H NMR spectrum of **6** in CD_3OD .

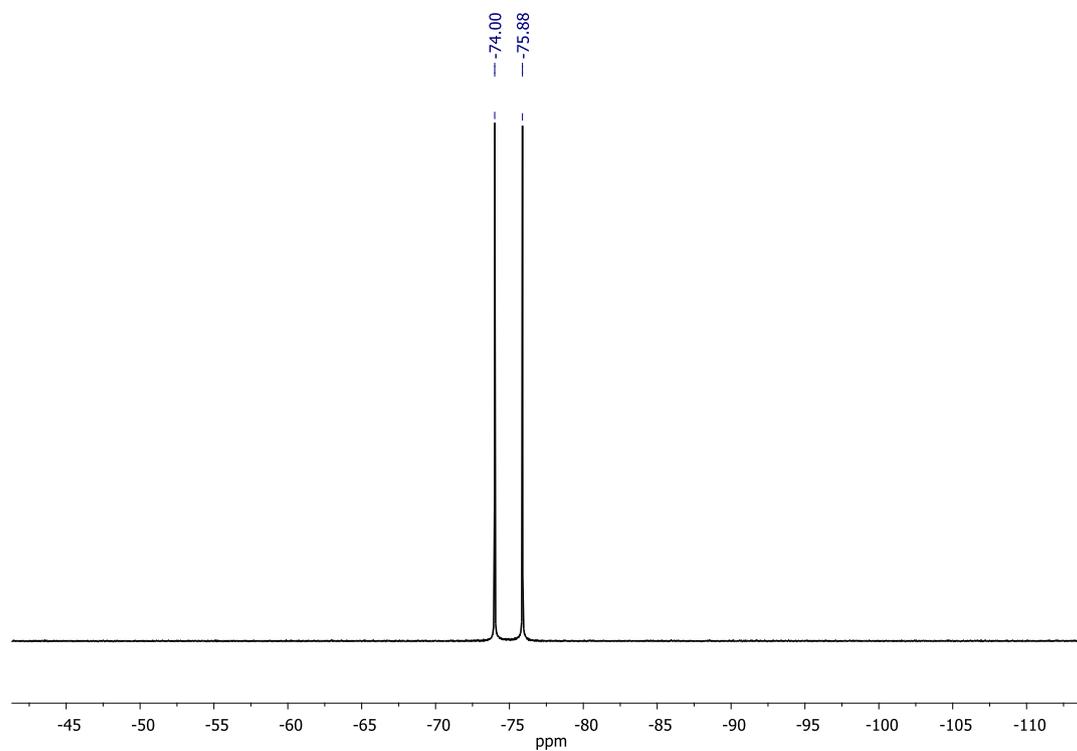


Figure S8. $^{19}\text{F}\{^1\text{H}\}$ NMR spectrum of **6** in CD_3OD .

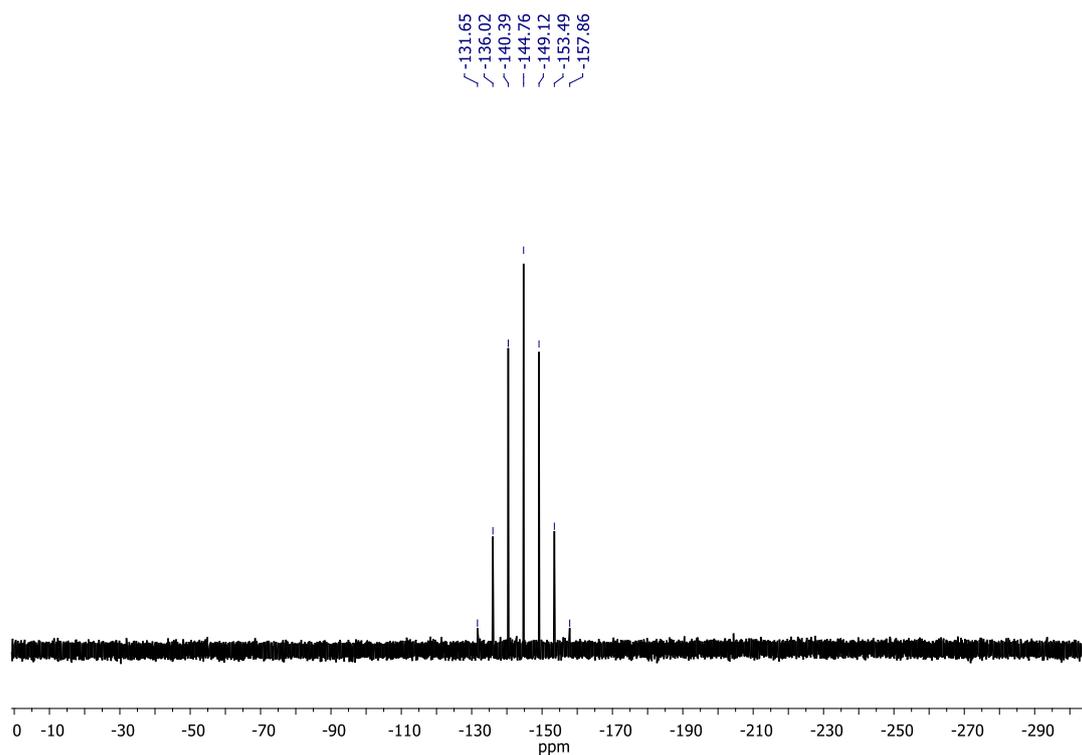


Figure S9. $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum of **6** in CD_3OD .

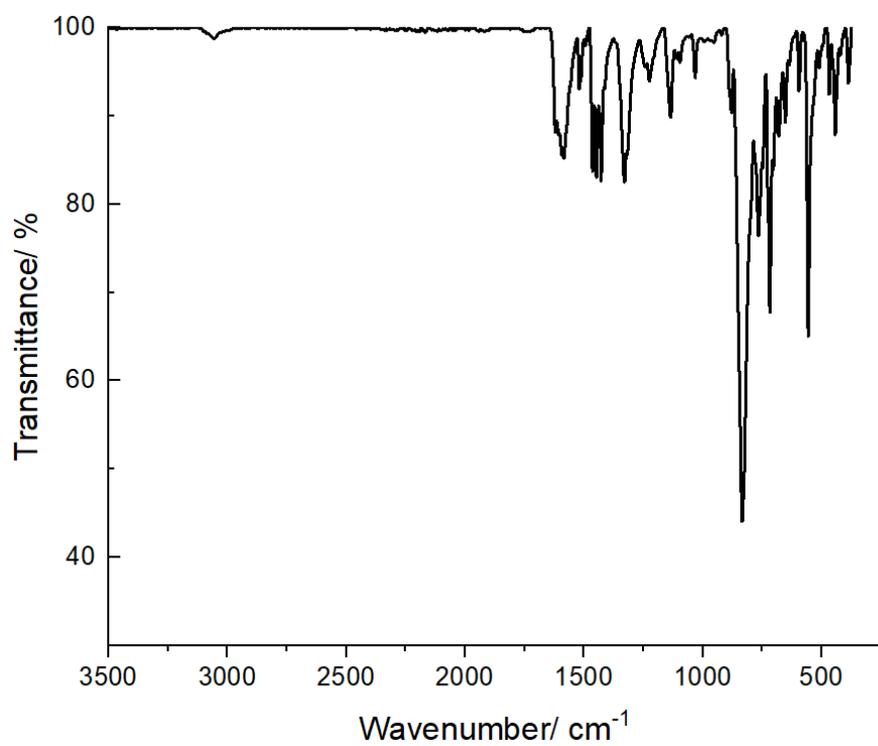


Figure S10. ATR-FTIR spectrum of **4** in the solid form.

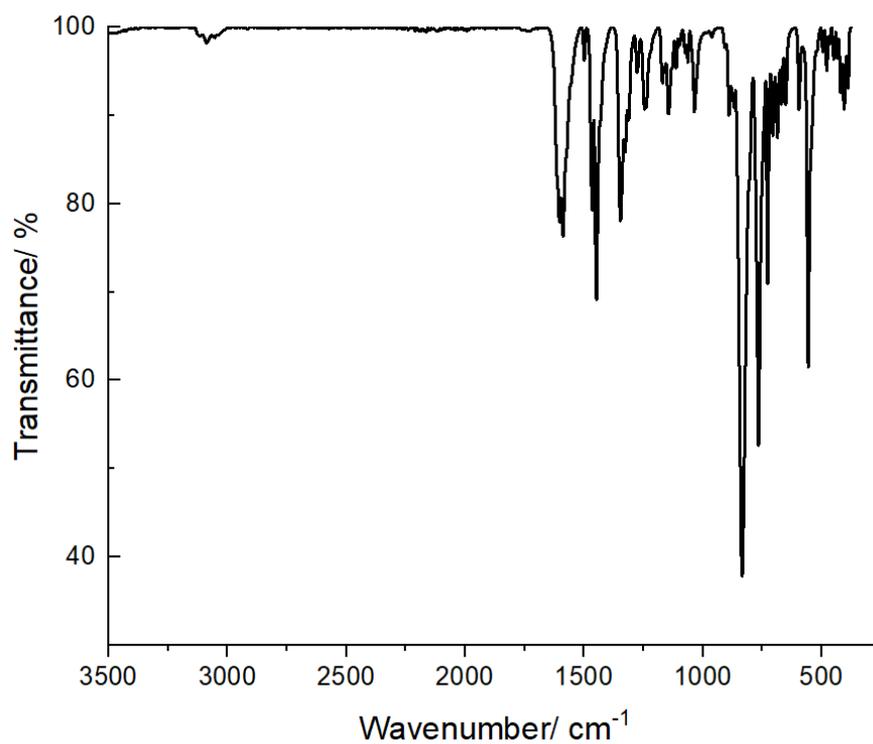


Figure S11. ATR-FTIR spectrum of **5** in the solid form.

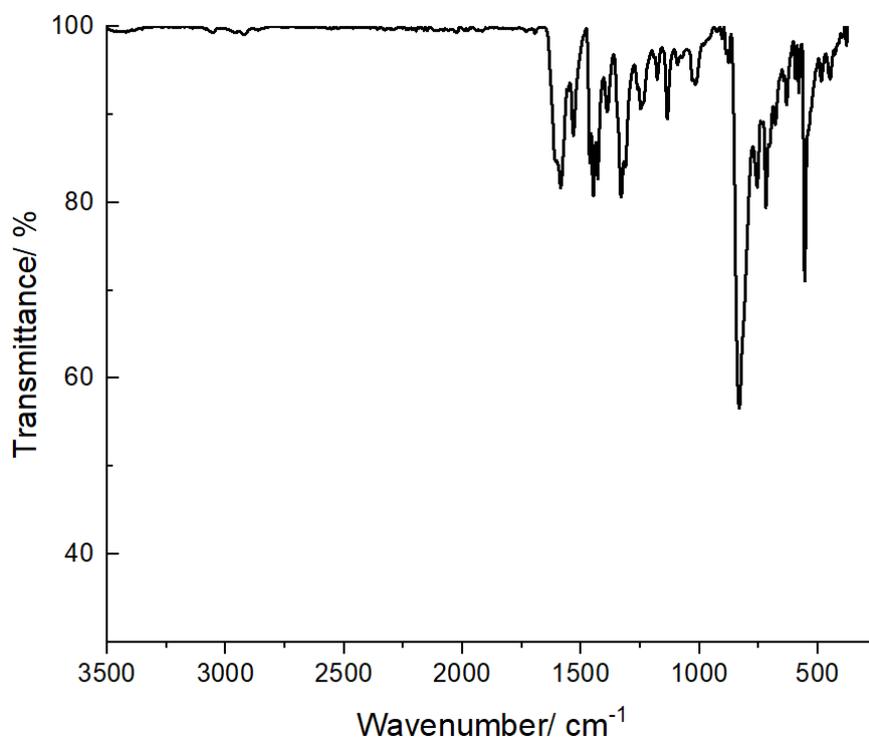


Figure S12. ATR-FTIR spectrum of **6** in the solid form.

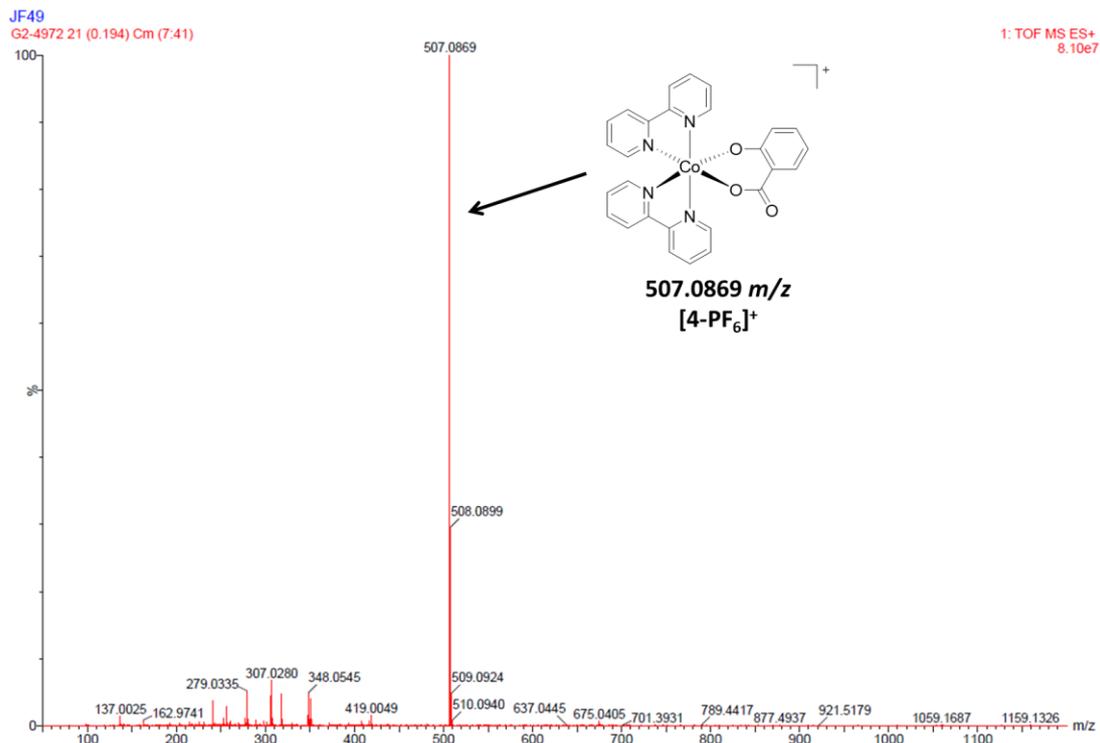


Figure S13. High resolution ESI mass spectrum (positive mode) of **4**.

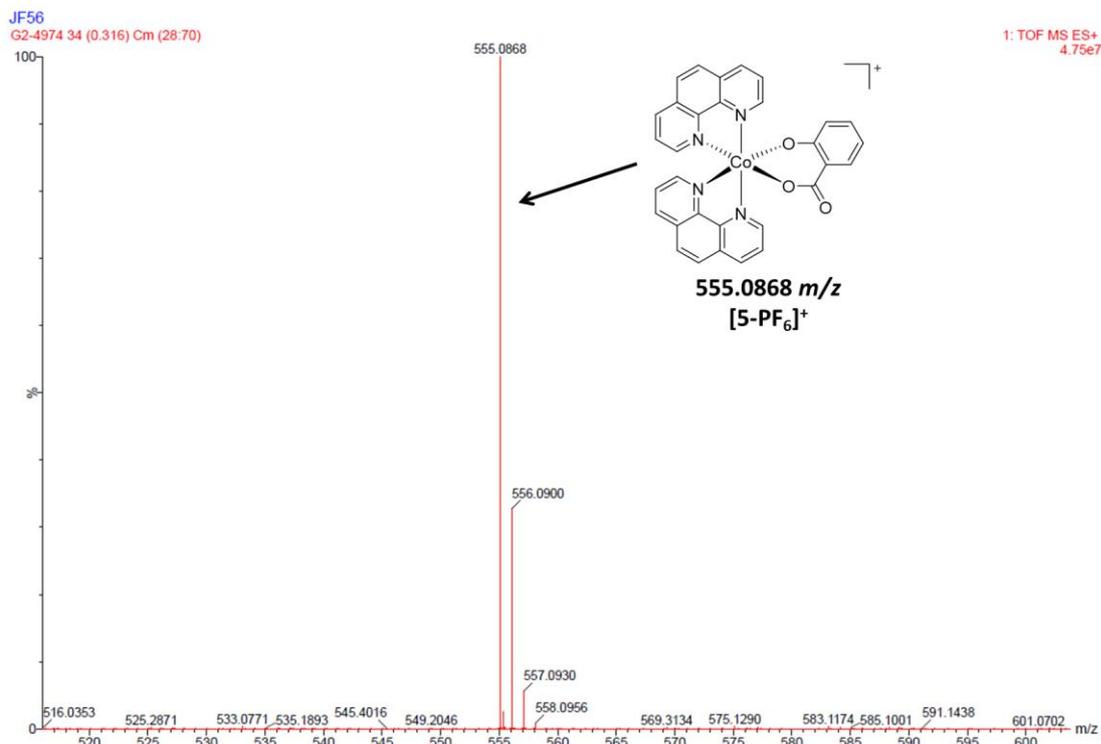


Figure S14. High resolution ESI mass spectrum (positive mode) of **5**.

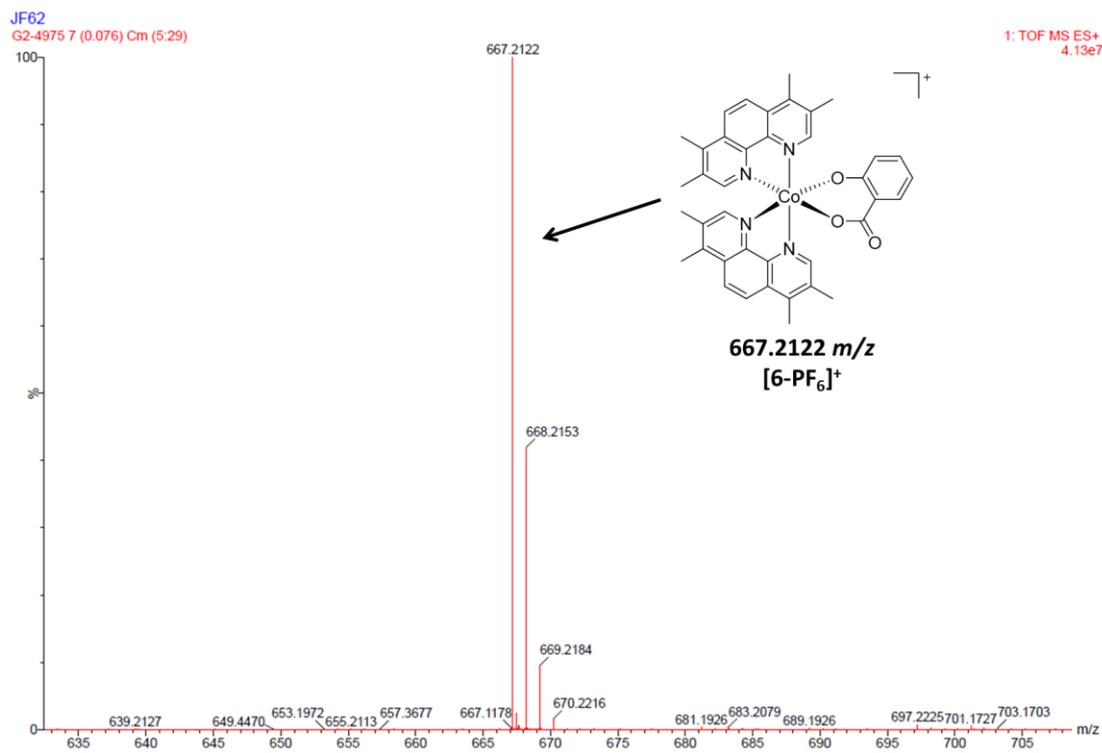


Figure S15. High resolution ESI mass spectrum (positive mode) of **6**.

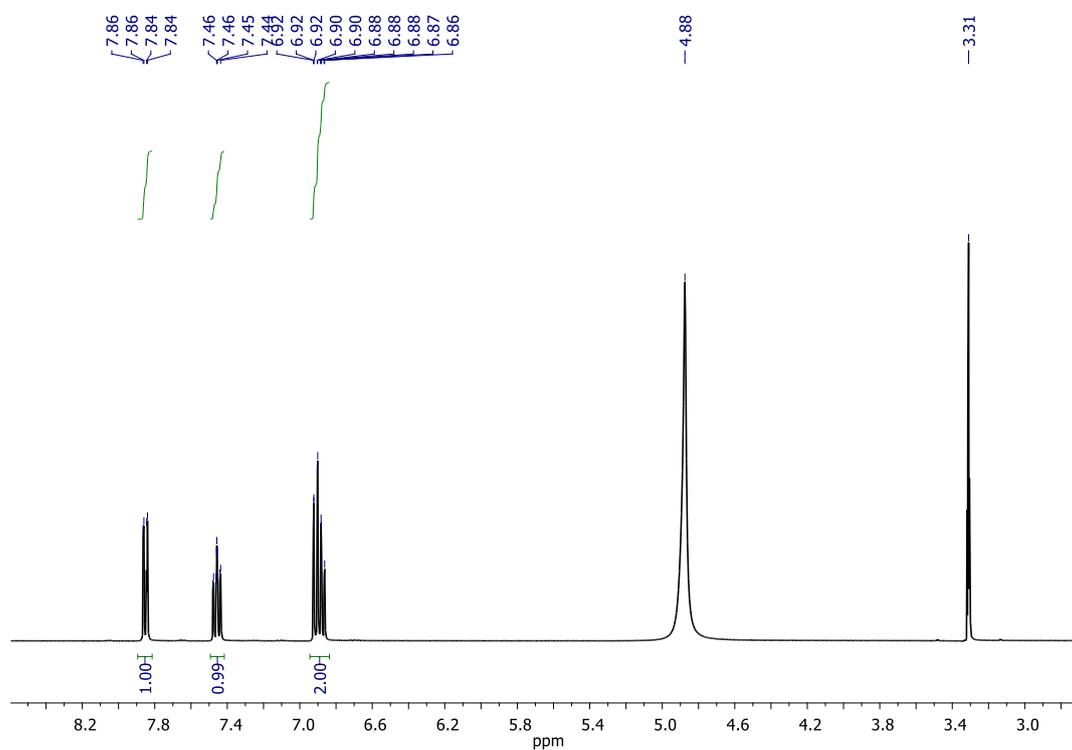
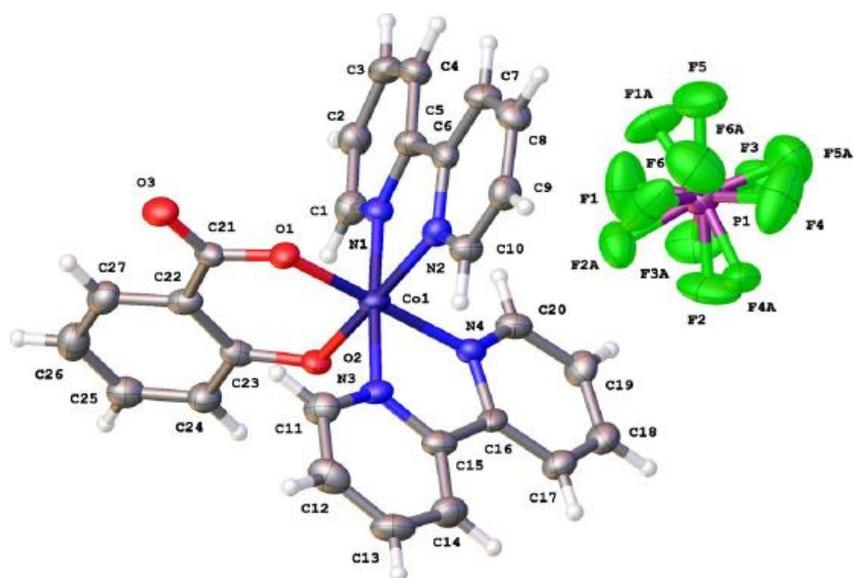


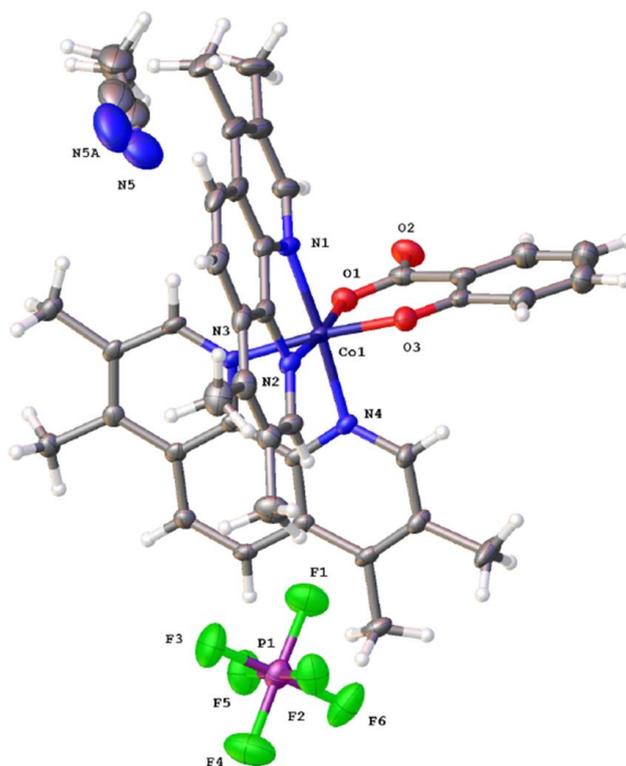
Figure S16. ¹H NMR spectrum of salicylic acid in CD₃OD.

Table S1. Crystallographic data for cobalt(III) complexes **4** and **6**.

Metal complex	4	6
CCDC No.	2363763	2363764
formula	C ₂₇ H ₂₀ CoF ₆ N ₄ O ₃ P	C ₃₉ H ₃₆ CoF ₆ N ₄ O ₃ P·CH ₃ CN
<i>F</i> _w	652.37	853.67
Crystal system	monoclinic	triclinic
Space group	P2 ₁ /n	P-1
<i>a</i> , Å	12.8883(10)	11.7523(5)
<i>b</i> , Å	14.6645(13)	11.8069(5)
<i>c</i> , Å	14.7855(12)	15.3027(7)
<i>α</i> , deg.	90	111.089(2)
<i>β</i> , deg.	114.553(5)	102.681(2)
<i>γ</i> , deg.	90	96.806(2)
<i>V</i> , Å ³	2541.8(4)	1886.79(15)
<i>Z</i>	4	2
<i>D</i> _{calcd} , Mg/m ³	1.705	1.503
2 <i>θ</i> / deg.	7.674 to 144.638	7.898 to 145.228
Reflections collected	22898	30983
Independent reflections	4883	7305
Goodness-of-fit on <i>F</i> ²	1.035	1.064
<i>R</i> ₁ , <i>wR</i> ₂ [<i>I</i> ≥ 2σ (<i>I</i>)]	0.0728, 0.1576	0.0599, 0.1724
<i>R</i> ₁ , <i>wR</i> ₂ [all data]	0.1344, 0.1897	0.0721, 0.1848

Table S2. Selected bond lengths (Å) and angles (°) for cobalt(III) complex **4**.

Co(1)-O(1)	1.878(4)	Co(1)-N(2)	1.943(5)
Co(1)-O(2)	1.856(4)	Co(1)-N(3)	1.925(5)
Co(1)-N(1)	1.925(5)	Co(1)-N(4)	1.940(5)
O(1)-Co(1)-N(1)	89.05(18)	O(2)-Co(1)-N(4)	87.23(18)
O(1)-Co(1)-N(2)	85.03(18)	N(1)-Co(1)-N(2)	83.3(2)
O(1)-Co(1)-N(3)	93.23(19)	N(1)-Co(1)-N(3)	177.0(2)
O(1)-Co(1)-N(4)	174.9(2)	N(1)-Co(1)-N(4)	94.76(19)
O(2)-Co(1)-O(1)	96.07(17)	N(3)-Co(1)-N(2)	95.0(2)
O(2)-Co(1)-N(1)	92.56(19)	N(3)-Co(1)-N(4)	82.9(2)
O(2)-Co(1)-N(2)	175.71(19)	N(4)-Co(1)-N(2)	92.0(2)
O(2)-Co(1)-N(3)	89.10(19)		

Table S3. Selected bond lengths (Å) and angles (°) for cobalt(III) complex **6**.

Co(1)-O(1)	1.877(2)	Co(1)-N(2)	1.942(3)
Co(1)-O(3)	1.866(2)	Co(1)-N(3)	1.955(2)
Co(1)-N(1)	1.936(2)	Co(1)-N(4)	1.933(2)
O(1)-Co(1)-N(1)	91.24(11)	O(3)-Co(1)-N(4)	92.50(10)
O(1)-Co(1)-N(2)	174.33(9)	N(1)-Co(1)-N(2)	83.73(11)
O(1)-Co(1)-N(3)	87.87(10)	N(1)-Co(1)-N(3)	93.08(10)
O(1)-Co(1)-N(4)	90.50(10)	N(2)-Co(1)-N(3)	89.78(10)
O(3)-Co(1)-O(1)	96.49(10)	N(4)-Co(1)-N(1)	175.92(10)
O(3)-Co(1)-N(1)	90.96(10)	N(4)-Co(1)-N(2)	94.35(10)
O(3)-Co(1)-N(2)	86.23(10)	N(4)-Co(1)-N(3)	83.30(10)
O(3)-Co(1)-N(3)	173.99(9)		

Table S4. Experimentally determined LogP values for the cobalt(III) complexes **4-6**.

Cobalt(III) complex	LogP value
4	-1.57 ± 0.01
5	-1.21 ± 0.03
6	-0.02 ± 0.01

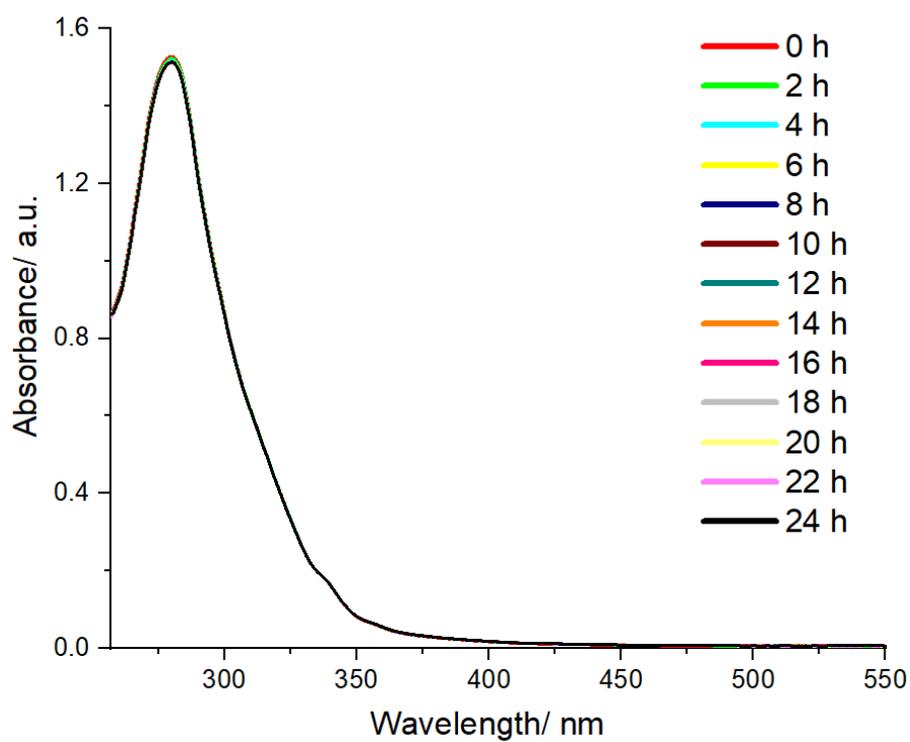


Figure S17. UV-Vis spectra of **6** (25 μM) in DMSO over the course of 24 h at 37 °C.

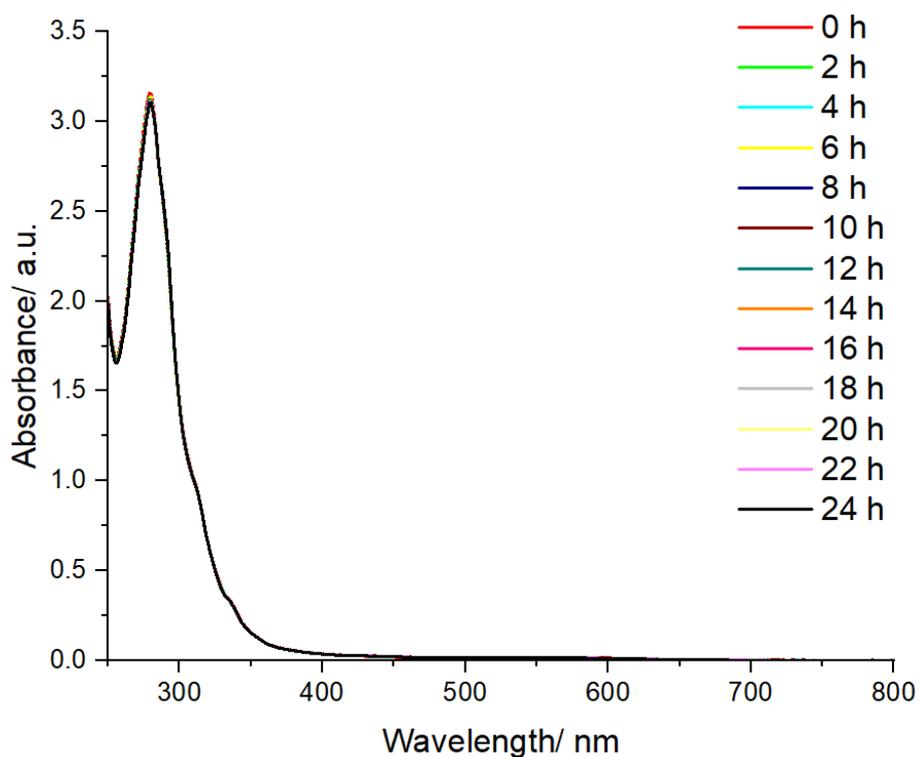


Figure S18. UV-Vis spectra of **6** (50 μM) in H₂O:DMSO (4:1) over the course of 24 h at 37 °C.

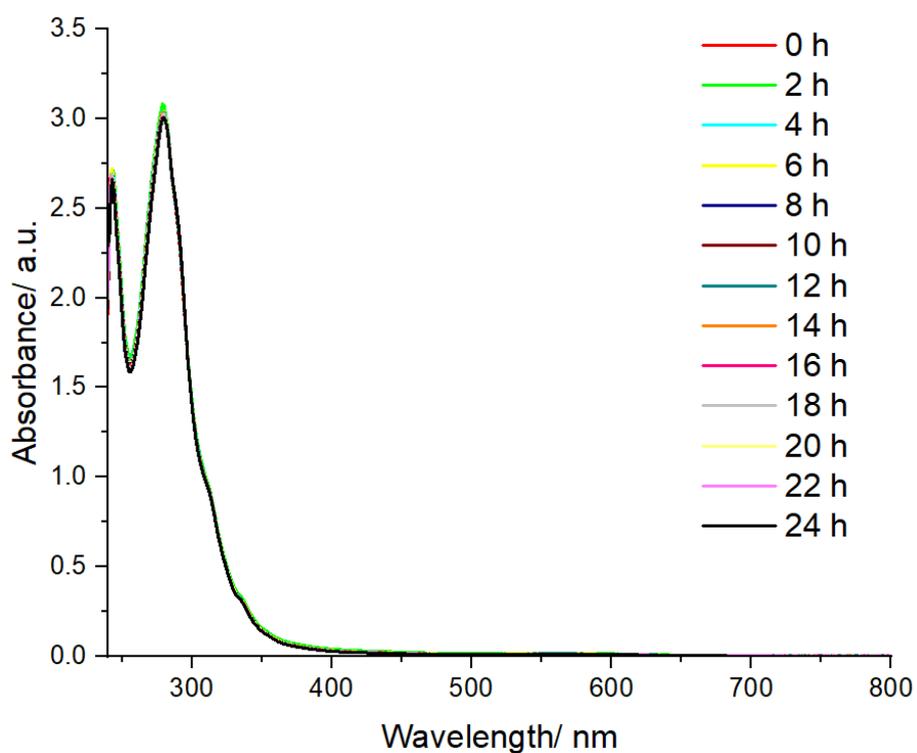


Figure S19. UV-Vis spectra of **6** (50 μM) in PBS:DMSO (4:1) over the course of 24 h at 37 °C.

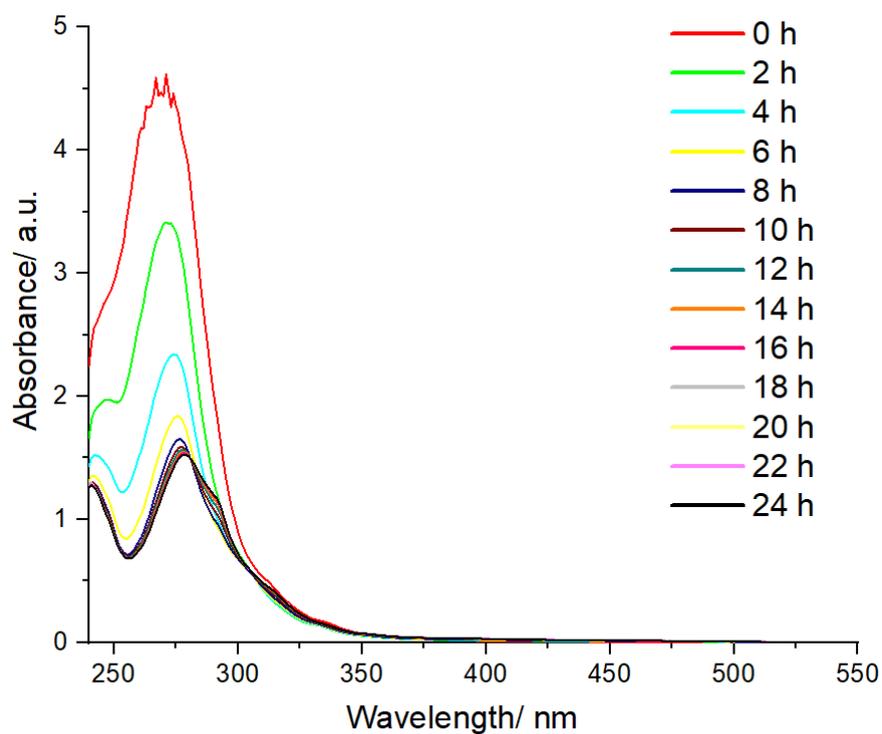


Figure S20. UV-Vis spectra of **6** (25 μM) in PBS:DMSO (4:1) in the presence of ascorbic acid (250 μM) over the course of 24 h at 37 $^{\circ}\text{C}$.

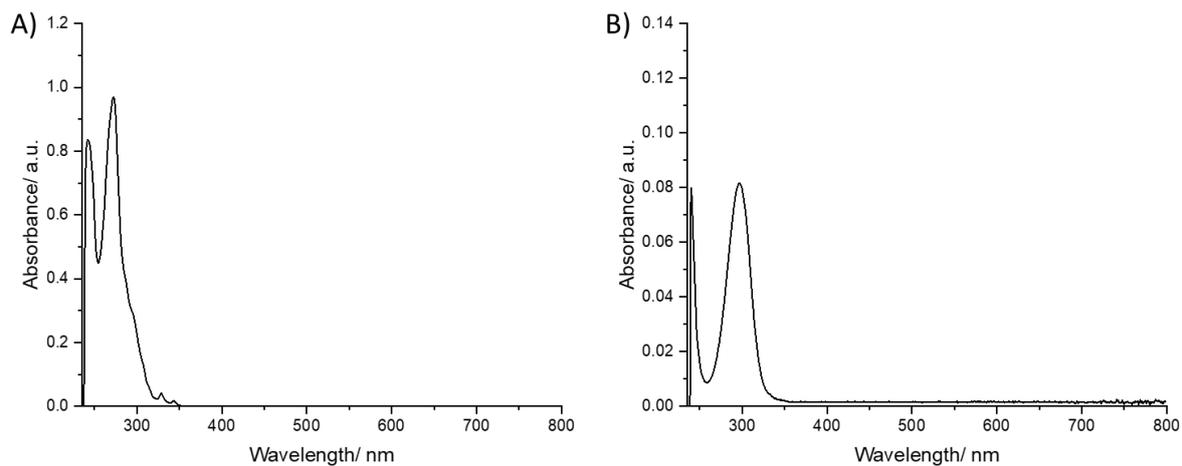


Figure S21. UV-Vis spectrum of (A) 3,4,7,8-tetramethyl-1,10-phenanthroline (25 μM) and (B) salicylic acid (25 μM) in PBS:DMSO (4:1).

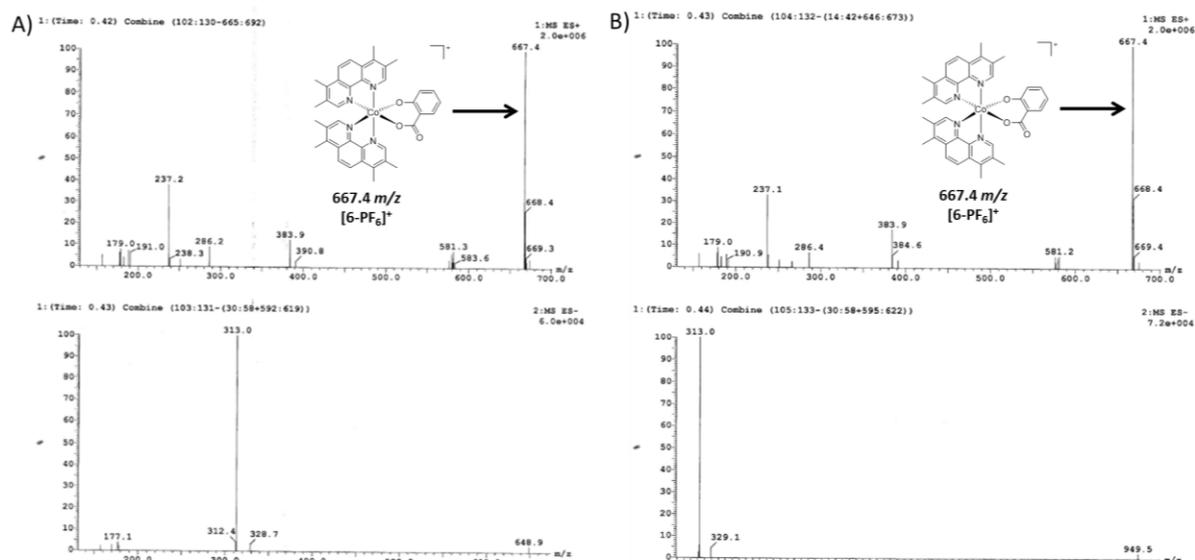


Figure S22. ESI mass spectra (positive and negative mode) of **6** (40 μM) in $\text{H}_2\text{O}:\text{DMSO}$ (10:1) (A) before and (B) after incubation for 24 h at 37 $^\circ\text{C}$.

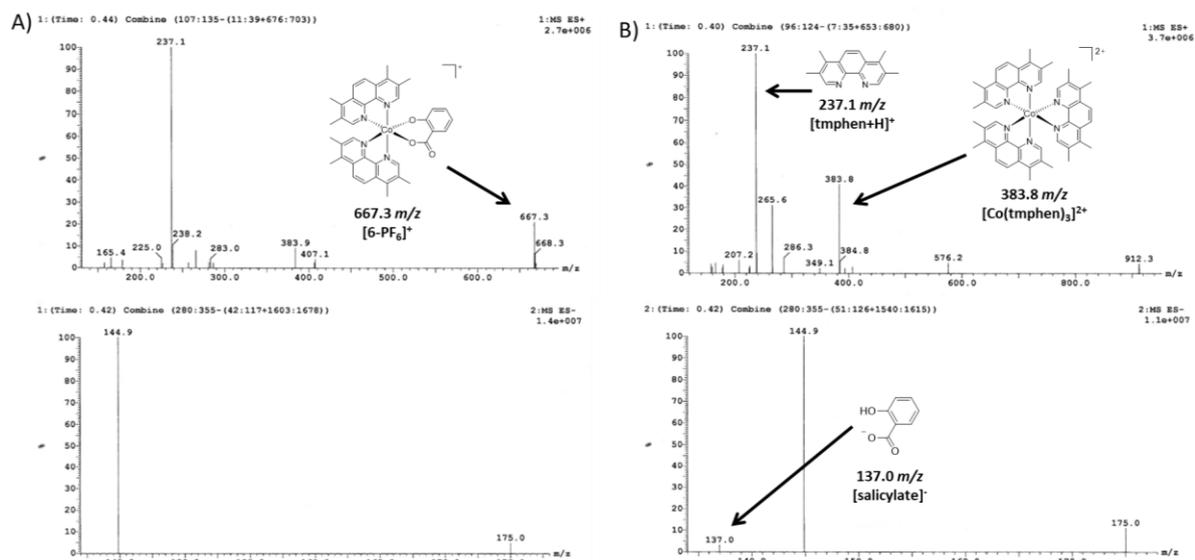


Figure S23. ESI mass spectra (positive and negative mode) of **6** (40 μM) in $\text{H}_2\text{O}:\text{DMSO}$ (10:1) in the presence of ascorbic acid (400 μM) (A) before and (B) after incubation for 24 h at 37 $^\circ\text{C}$.

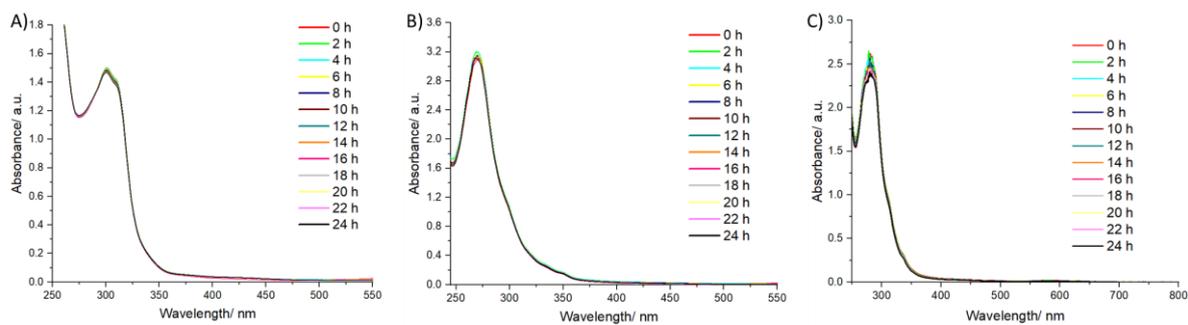


Figure S24. UV-Vis spectra of (A) **4** (50 μ M), (B) **5** (50 μ M), and (C) **6** (50 μ M) in MEGM:DMSO (4:1) over the course of 24 h at 37 $^{\circ}$ C.

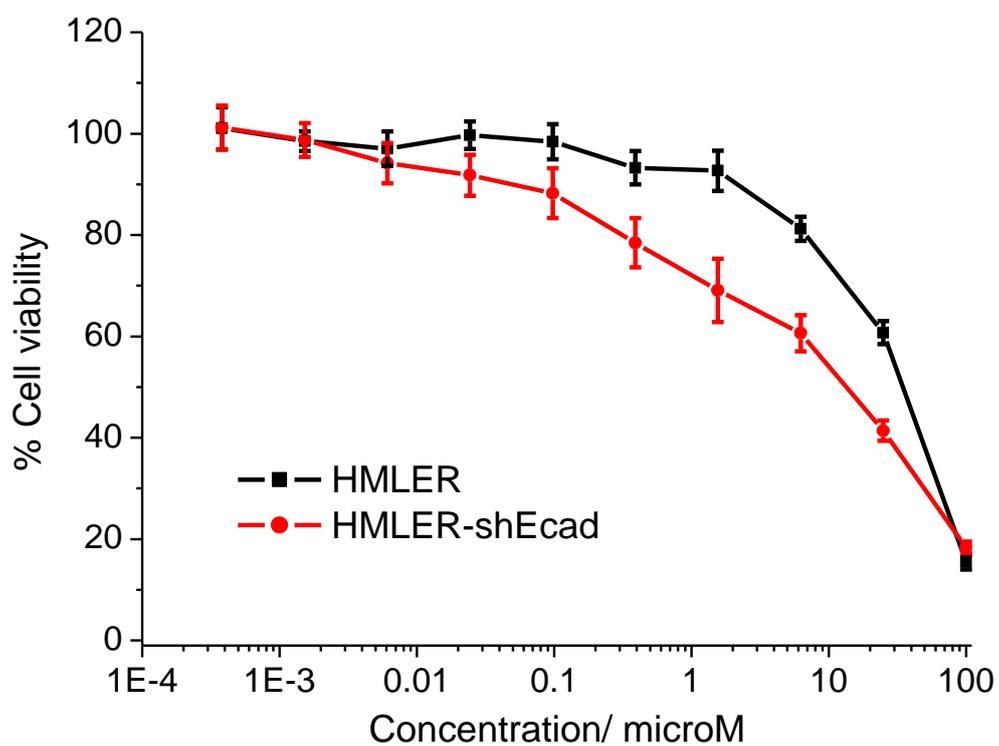


Figure S25. Representative dose-response curves for the treatment of HMLER and HMLER-shEcad cells with **4** after 72 h incubation.

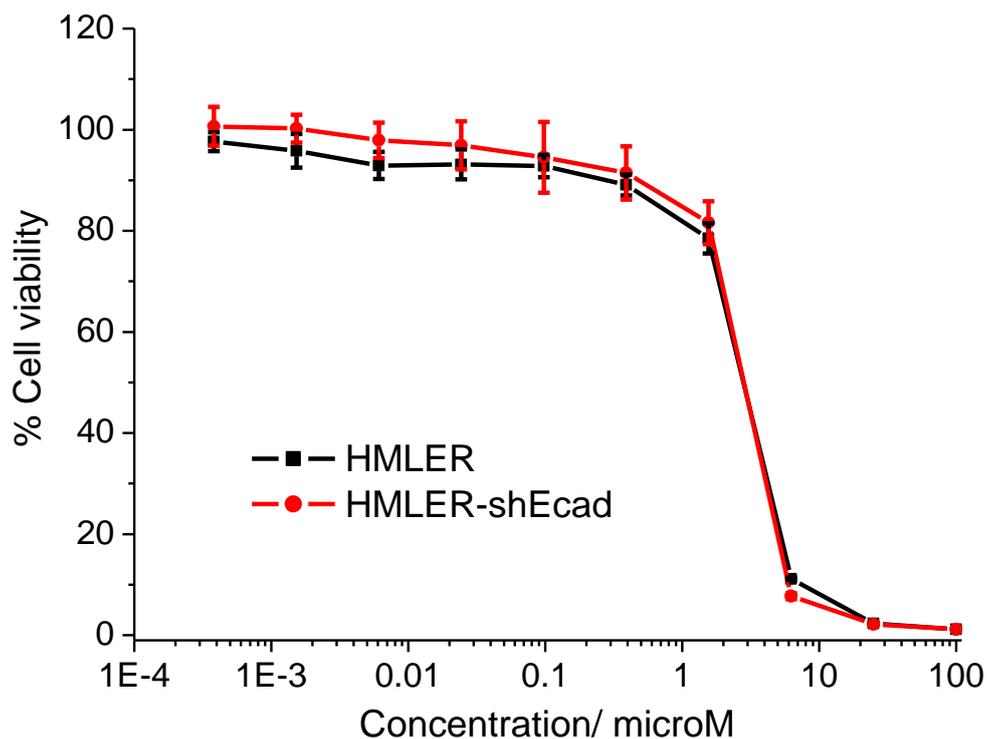


Figure S26. Representative dose-response curves for the treatment of HMLER and HMLER-shEcad cells with **5** after 72 h incubation.

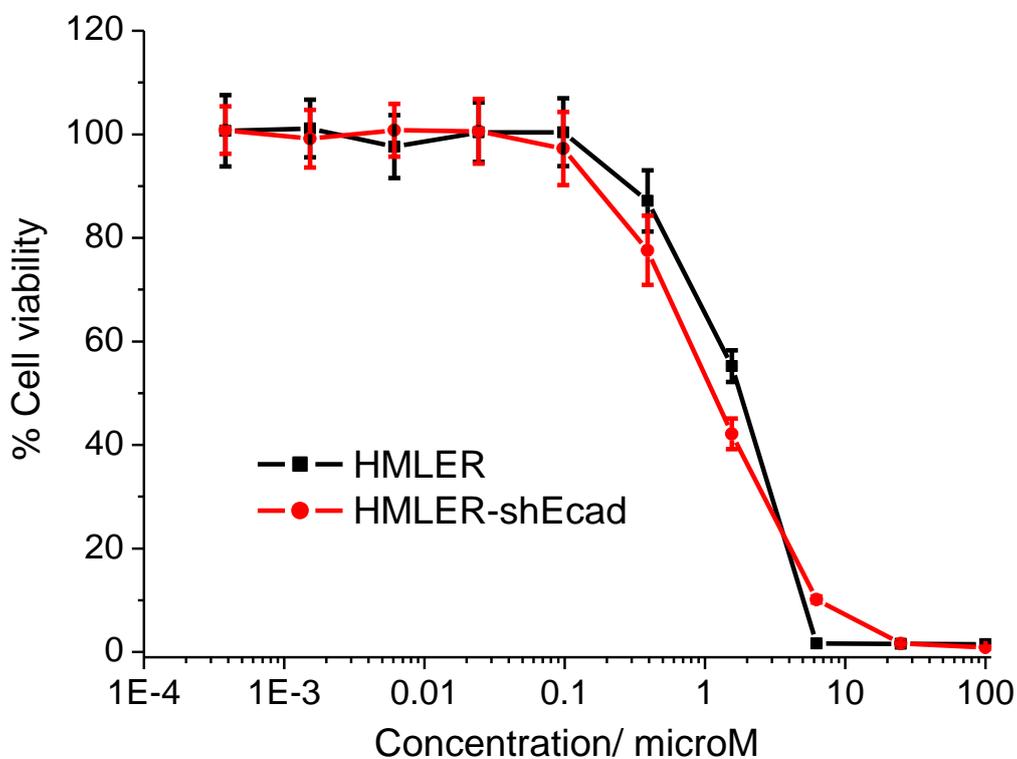


Figure S27. Representative dose-response curves for the treatment of HMLER and HMLER-shEcad cells with **6** after 72 h incubation.

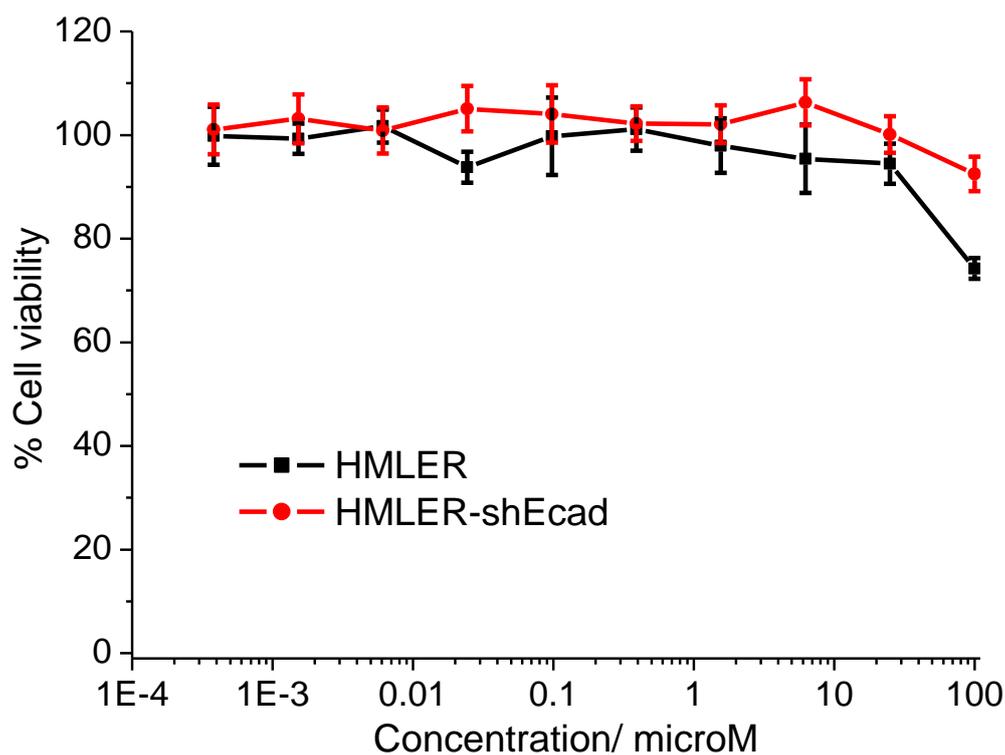


Figure S28. Representative dose-response curves for the treatment of HMLER and HMLER-shEcad cells with salicylic acid after 72 h incubation.

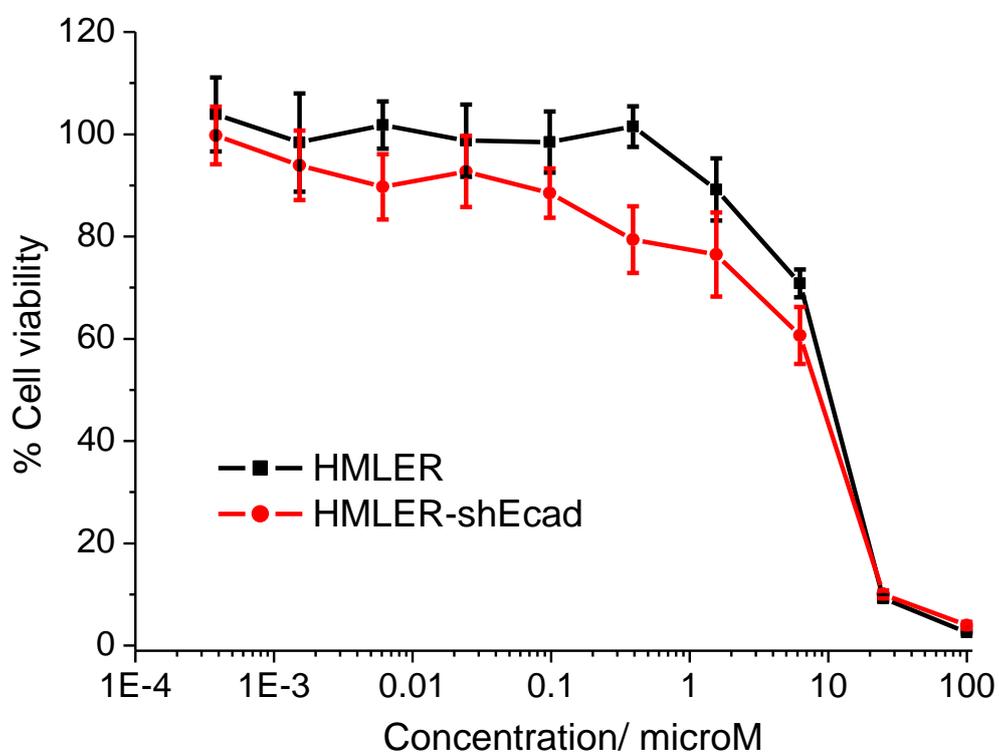


Figure S29. Representative dose-response curves for the treatment of HMLER and HMLER-shEcad cells with **1b** after 72 h incubation.

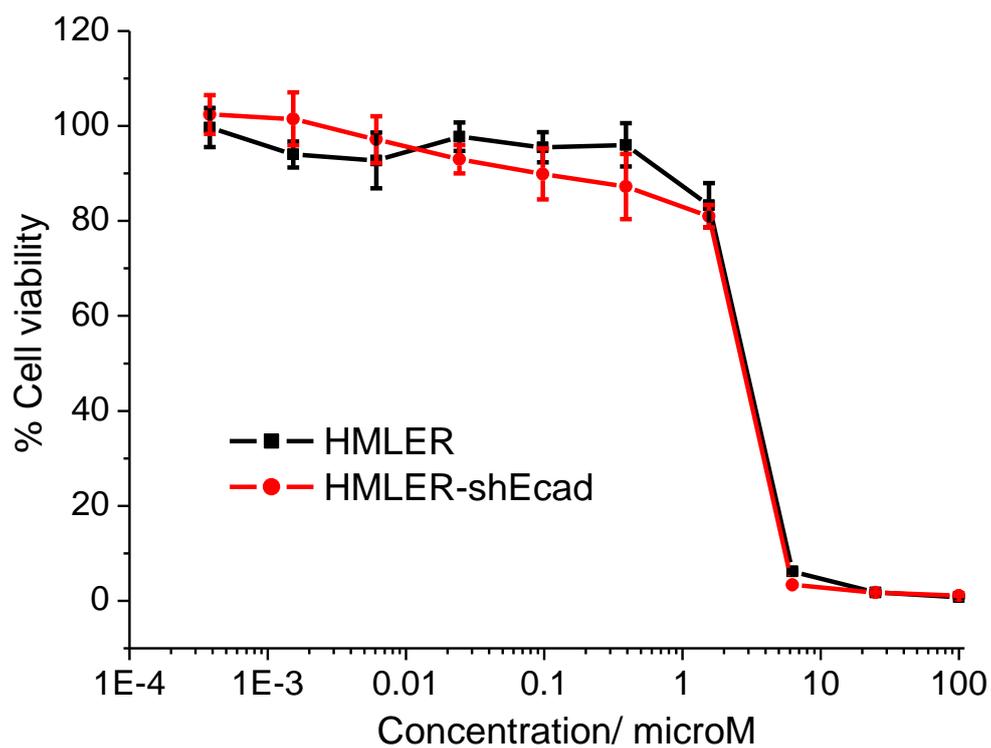


Figure S30. Representative dose-response curves for the treatment of HMLER and HMLER-shEcad cells with **2b** after 72 h incubation.

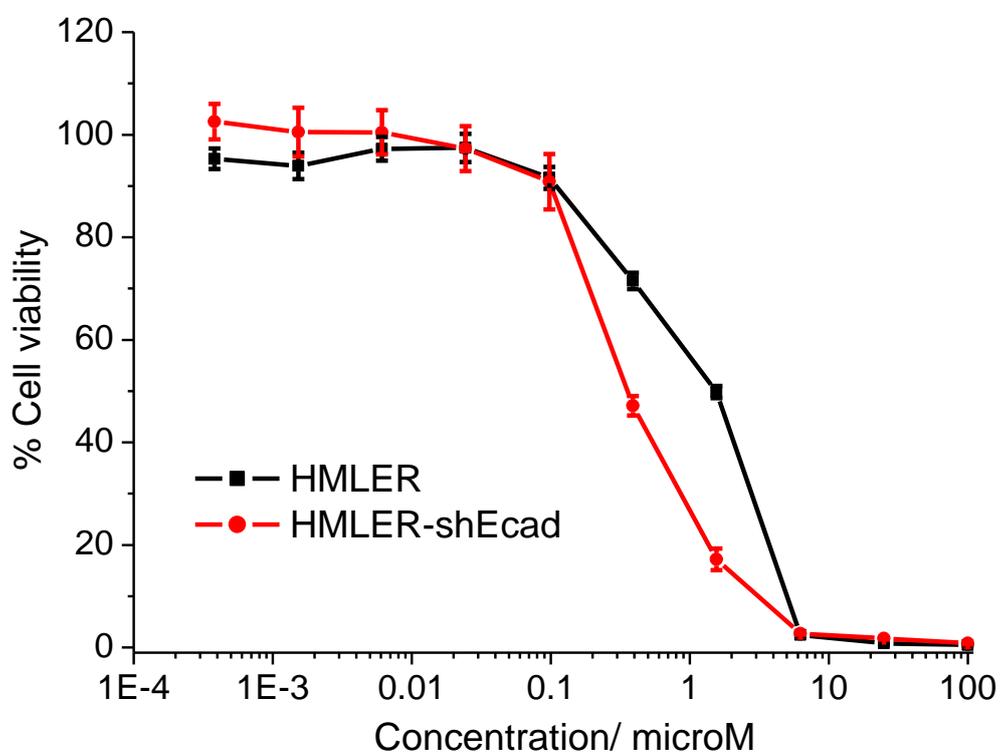


Figure S31. Representative dose-response curves for the treatment of HMLER and HMLER-shEcad cells with **3b** after 72 h incubation.

Table S5. IC₅₀ values of salicylic acid and **1b-3b** against HMLER and HMLER-shEcad cells. Determined after 72 h incubation (mean of three independent experiments ± SD).

Test compounds(s)	HMLER [μM]	HMLER-shEcad [μM]
salicylic acid	> 100	> 100
1b	9.99 ± 0.11	8.33 ± 0.88
2b	2.85 ± 0.16	2.72 ± 0.01
3b	1.55 ± 0.04	0.36 ± 0.001

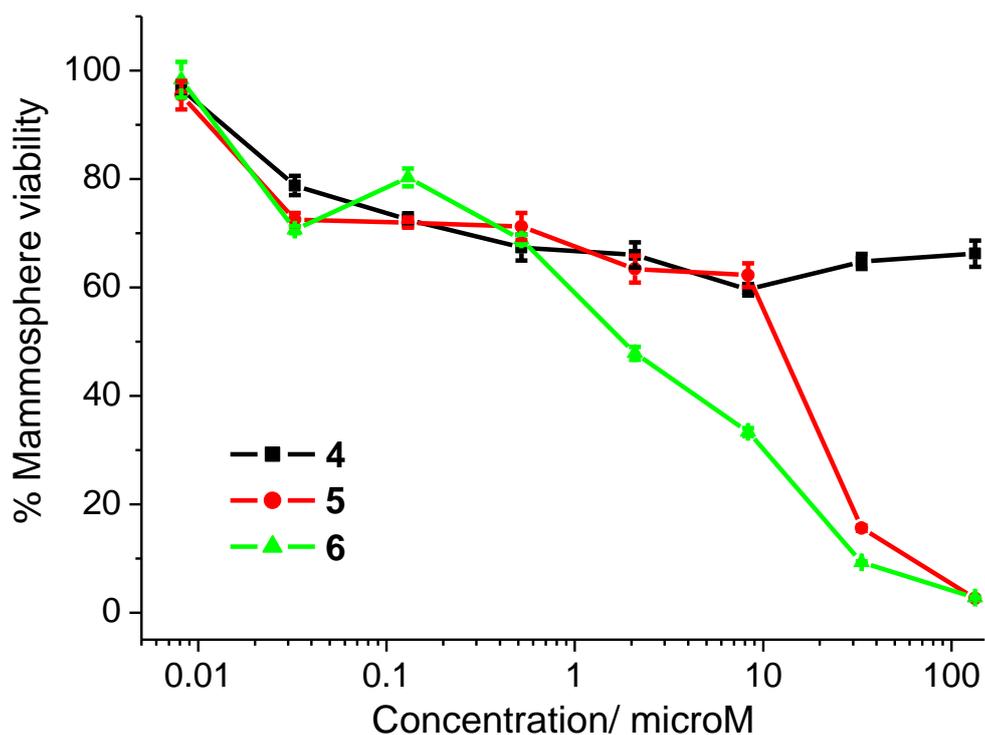


Figure S32. Representative dose-response curves for the treatment of HMLER-shEcad mammospheres with **4-6** after 5 days incubation.

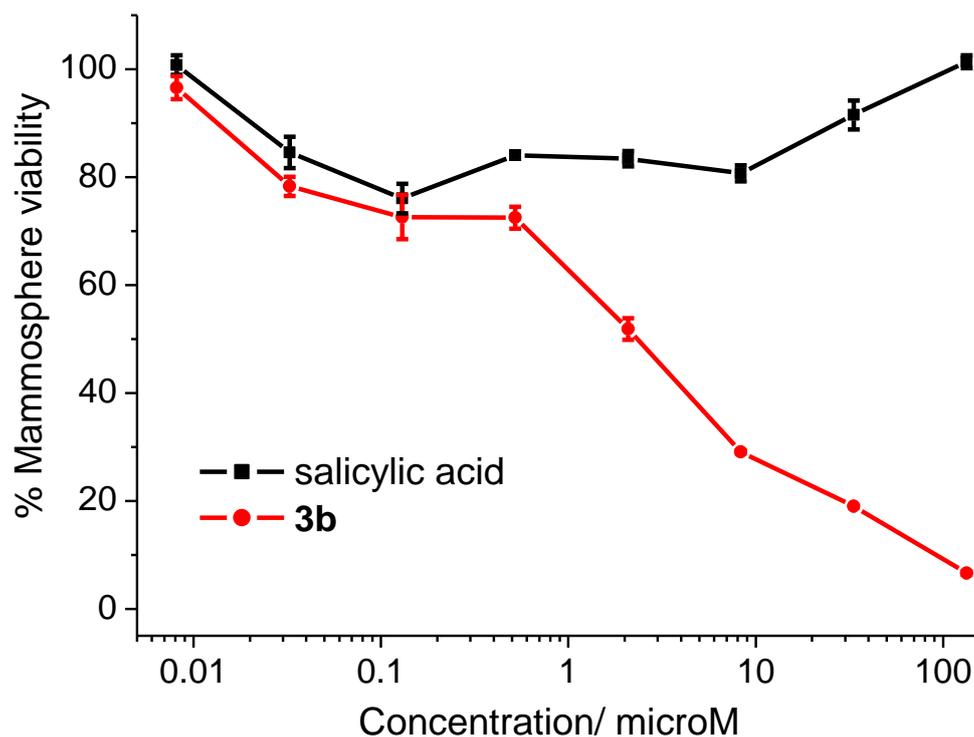


Figure S33. Representative dose-response curves for the treatment of HMLER-shEcad mammospheres with salicylic acid and **3b** after 5 days incubation.

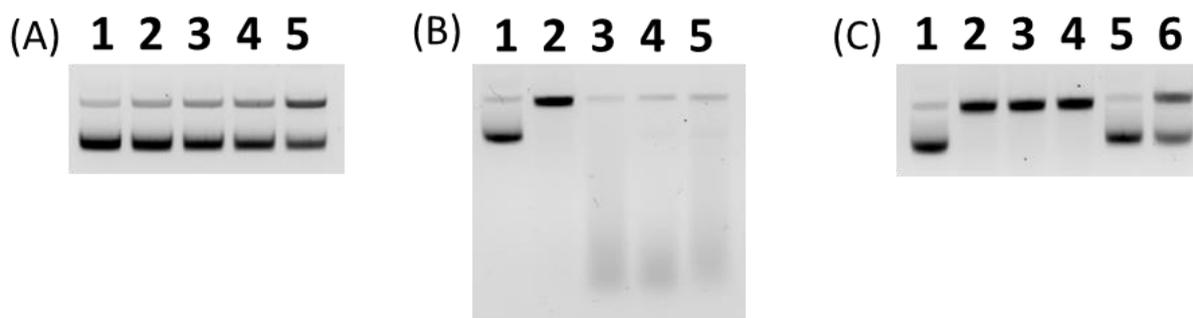


Figure S34. (A) Concentration-dependent DNA cleavage by **6** after 24 h incubation, Lane 1: DNA only, Lane 2-5: DNA + 10, 20, 50 or 100 μM of **6**. (B) Effect of ascorbic acid on **6**-mediated DNA cleavage after 24 h incubation, Lane 1: DNA only, 2-5: DNA + 10, 20, 50 or 100 μM of **3** with 10 equivalents of ascorbic acid. (C) Inhibition of **6**-mediated DNA cleavage by ROS scavengers after 24 h incubation. a) Lane 1: DNA only, Lane 2: DNA + **6** (10 μM) with 10 equivalents of ascorbic acid, Lane 3-6: DNA + **6** (10 μM) with 10 equivalents of ascorbic acid + *t*BuOH (10 mM), DMSO (10 mM), KI (40 mM) or NaN_3 (40 mM).

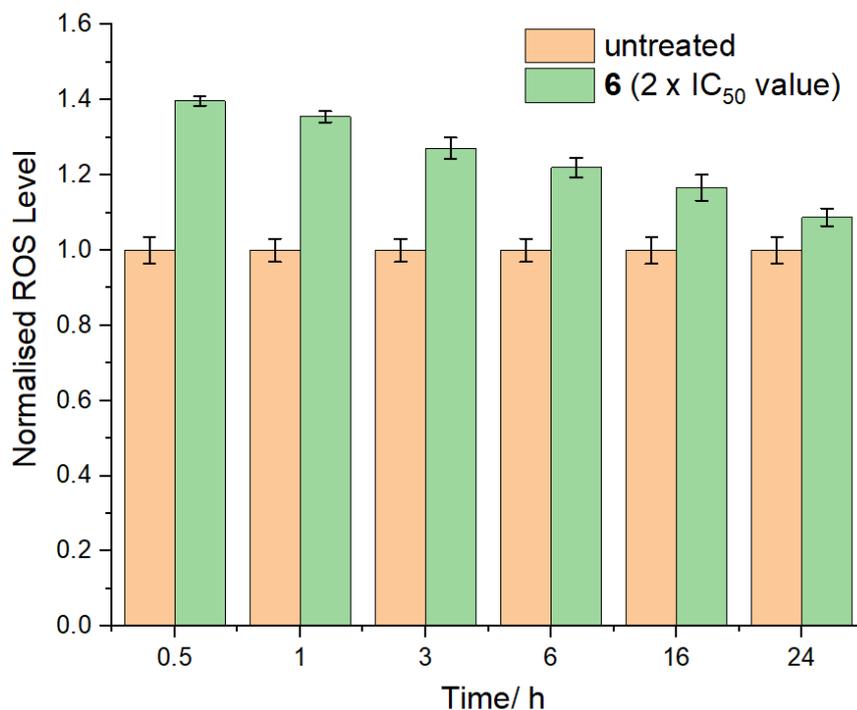


Figure S35. Normalised ROS activity in untreated HMLER-shEcad cells and HMLER-shEcad cells treated with **6** ($2 \times IC_{50}$ value for 0.5, 1, 3, 6, 16, and 24 h).

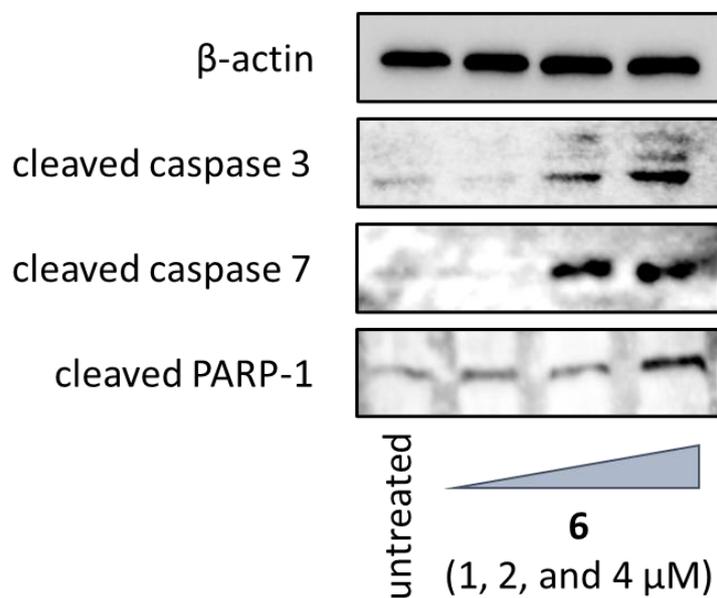


Figure S36. Immunoblotting analysis of proteins related to the apoptosis pathway. Protein expression in HMLER-shEcad cells following treatment with **6** (1-4 μ M) for 48 h.

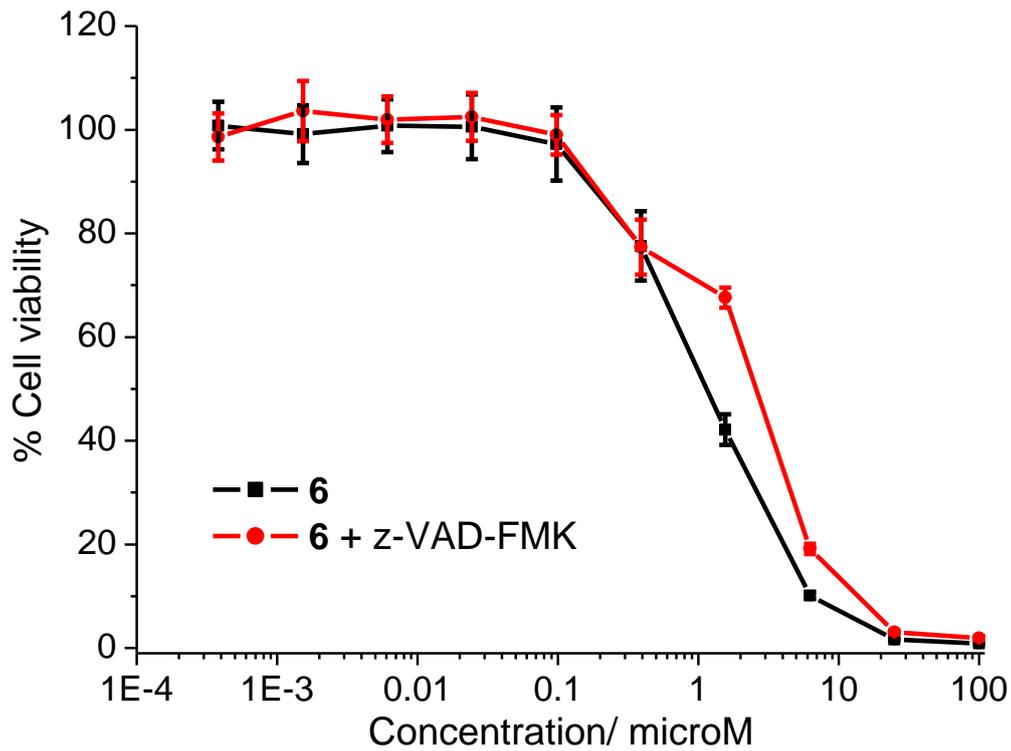


Figure S37. Representative dose-response curves for the treatment of HMLER-shEcad cells with **6** alone and in the presence of z-VAD-FMK (5 μM) after 72 h incubation.

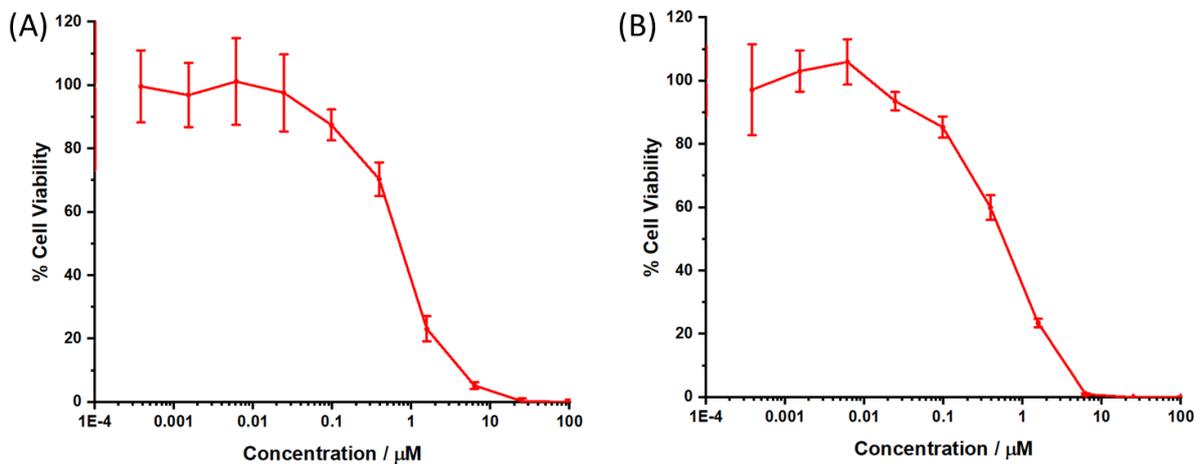


Figure S38. Representative dose-response curves for the treatment of HMLER-shEcad cells with **6** alone and in the presence of (A) IM-54 (10 μM) or (B) necrostatin-1 (20 μM) after 72 h incubation.

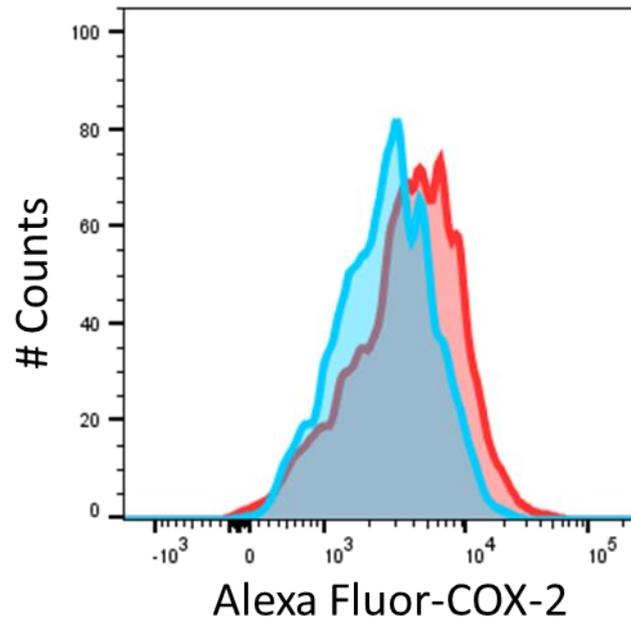


Figure S39. Representative histograms displaying the green fluorescence emitted by anti-COX-2 Alexa Fluor 488 nm antibody-stained HMLER-shEcad cells treated with LPS ($2.5 \mu\text{M}$) for 24 h followed by 48 h in fresh media (red) or media containing salicylic acid ($40 \mu\text{M}$, blue).