

Supplementary Information for:

Antivascular and anticancer activity of dihalogenated A-ring analogues of combretastatin A-4

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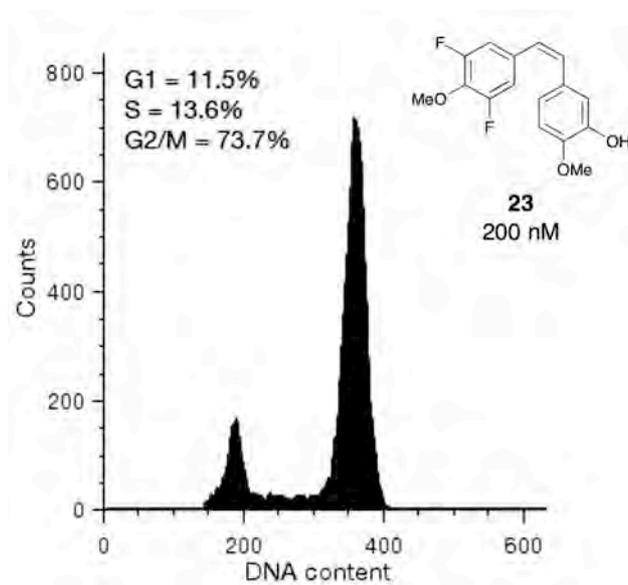
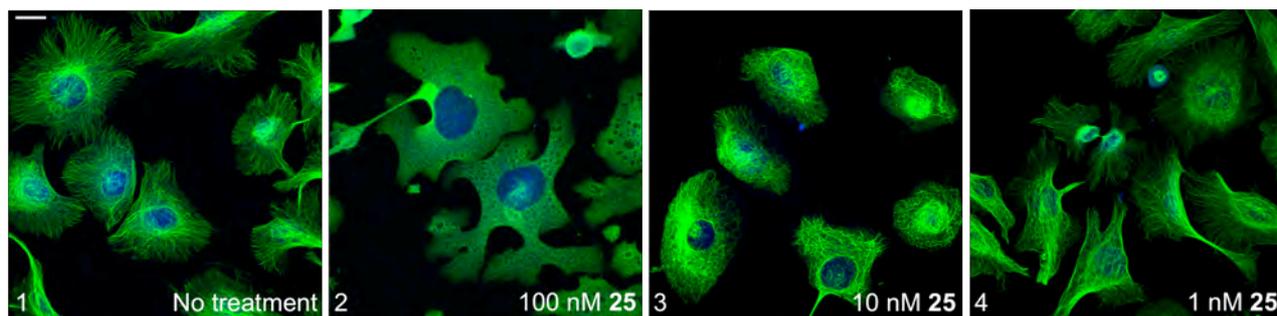


Fig. S1 FACS analysis of ovarian carcinoma SK-OV-3 cells stained with propidium iodide after 24 h exposure to control for compound **23** at 200 nM.

(a)



(b)

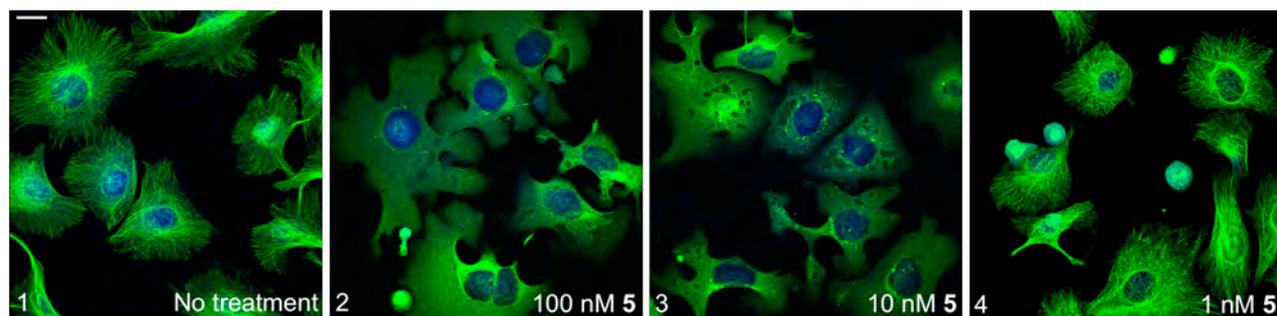
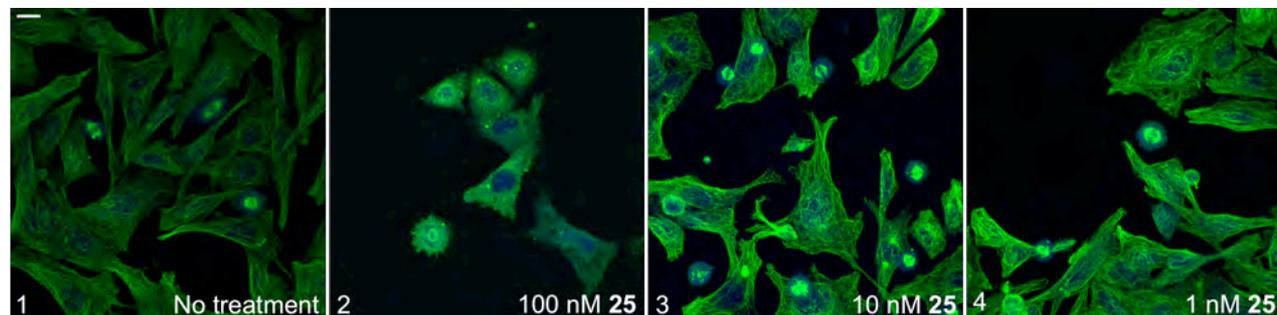


Fig. S2 Confocal images of ovarian carcinoma (SK-OV-3) cells. **(a)** Treatment with **25**. **(b)** Treatment with CA4 (**5**). Staining: anti-tubulin, Dm1a (green), DNA, DAPI (blue). Representative images shown, scale bar 20 μm .

(a)



(b)

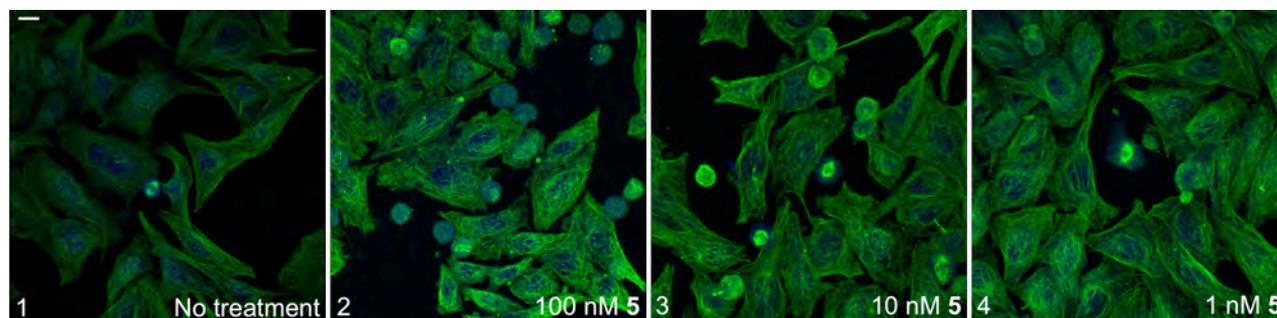


Fig. S3 Confocal images of taxol-resistant ovarian carcinoma (SK-OV-3TR) cells. **(a)** Treatment with **25**. **(b)** Treatment with CA4 (**5**). Staining: anti-tubulin, Dm1a (green), DNA, DAPI (blue). Representative images shown, scale bar 10 μm .

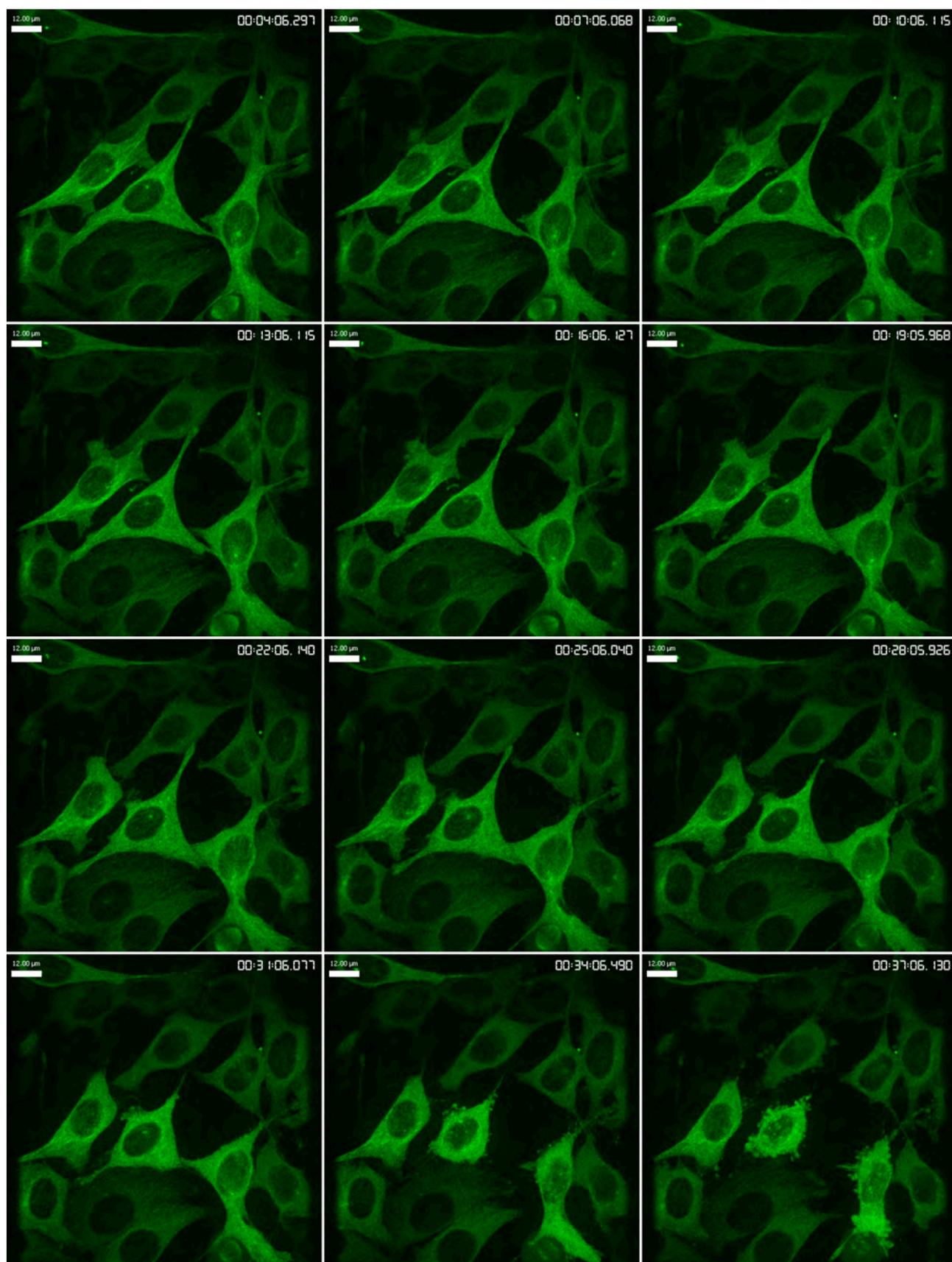


Fig. S4 GFP-tubulin HeLa (cervical cancer) cells after treatment with **25** at 100 nM. Images taken at 3-minute intervals immediately after drug addition, scale bar 12 μ m.

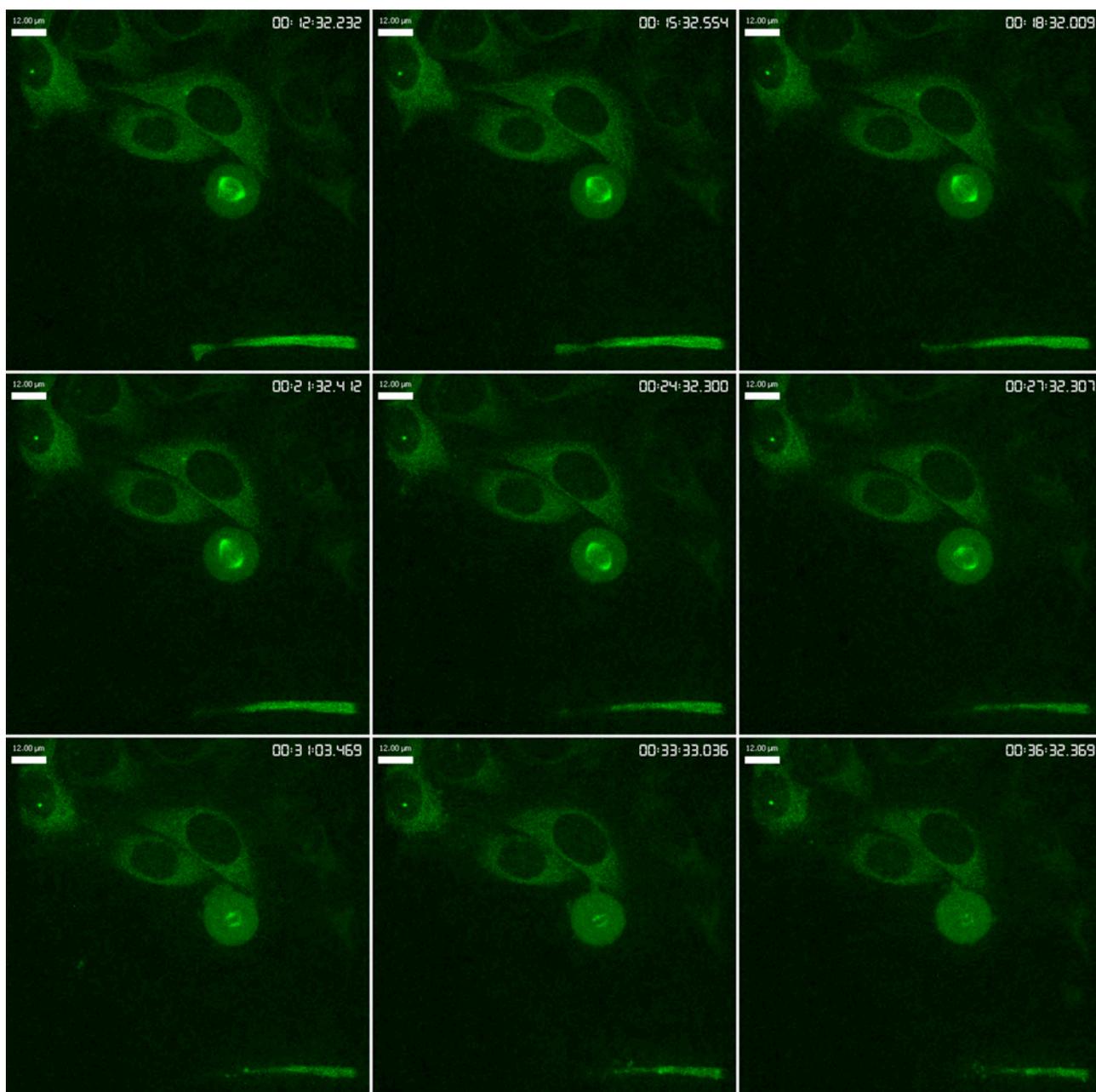
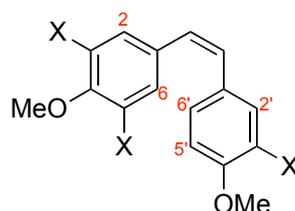


Fig. S5 GFP-tubulin HeLa (cervical cancer) cells after treatment with **25** at 100 nM. Images taken at 3-minute intervals immediately after drug addition, scale bar 12 μ m.

General Experimental Procedures

All non-aqueous reactions were conducted in oven-dried (200 °C) glassware under an inert atmosphere of dry argon unless otherwise stated. Solvents were purified and dried using standard methods as required: THF and Et₂O by distillation from calcium hydride and lithium aluminium hydride respectively; CH₂Cl₂, toluene and acetonitrile by distillation from calcium hydride. All other solvents were used as supplied unless otherwise stated. All reagents were used as supplied or purified using standard procedures. Flash column chromatography was performed with silica gel 60, particle size 40-63 nm under pressure, using gradient elutions. All solvents used for chromatographic purification were distilled prior to use. Hexanes refers to petroleum ether distillate (b.p. 40–60 °C). Analytical TLC was performed using silica gel 60 F₂₅₄ pre-coated glass backed plates and visualised by ultraviolet radiation (254 nm), potassium permanganate or acidic ceric ammonium molybdate. ¹H NMR spectra were recorded on a Bruker DPX-400 (400 MHz) spectrometer. Chemical shifts are reported in ppm with the solvent resonance resulting from incomplete deuteration as the internal standard (CDCl₃: 7.26 ppm). Data are reported as follows: chemical shift δ/ppm (integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet or combinations thereof), coupling constants, and assignment. ¹³C NMR spectra were recorded on a Bruker DPX-400 (100 MHz) spectrometer with complete proton decoupling. Chemical shifts are reported in ppm with the solvent resonance as the internal standard (¹³CDCl₃: 77.0 ppm, t). High resolution mass spectrometry (HRMS) was performed on a Waters Micromass LCT spectrometer using electrospray ionisation and Micromass MS software. Infrared spectra were recorded as thin films on a Perkin-Elmer Spectrum One FT-IR spectrometer and selected absorbances only are reported (s = strong, m = medium, w = weak, br = broad). Elemental analyses were performed by the Microanalysis Service at the Department of Chemistry, University of Cambridge,

Data is labelled according to the general structure shown below. When only one aromatic ring is present the dashed nomenclature is dropped.



4-Hydroxy-3,5-diiodobenzaldehyde¹ (**15**)

To a stirred solution of 4-hydroxybenzaldehyde (**12**) (3.51 g, 28.8 mmol), sodium periodate (6.16 g, 28.8 mmol), and sodium chloride (3.36 g, 57.5 mmol) in acetic acid/water (9:1, 100 mL) at rt was added potassium iodide (9.55 g, 57.5 g) in portions. After 96 h, EtOAc (500 mL) and 10% sodium thiosulfate solution (500 mL) were added. The organic layer was separated and the aqueous phase was extracted with EtOAc (500 mL). The combined organic layers were washed with 10% sodium thiosulfate solution (500 mL), brine (500 mL), dried over MgSO₄, and the excess solvent was removed *in vacuo* to give **15** (10.5 g, 28.1 mmol, 98%) as a white solid. R_f (30% EtOAc/Petrol) 0.34. Found: C, 22.20; H, 1.10. C₈H₆Br₂O₂ requires C, 22.49%; H 1.08%. ν_{max} (film)/cm⁻¹ 3181 (br), 1665 (s), 1563 (m), 1456 (m), 1095 (s). δ_H (CDCl₃) 6.28 (1H, br s, OH), 8.20 (2H, s, ArH), 9.74 (1H, s, CHO). δ_C (d₆-DMSO) 86.4, 132.1, 140.6, 160.6, 189.1.

3,5-Dichloro-4-methoxybenzaldehyde² (**9**)

To a solution of 4-hydroxybenzaldehyde (**12**) (122 mg, 1 mmol) in CH₂Cl₂ (3 mL) at rt in a 2-5 mL vial was added sulfonyl chloride (0.810 mL, 10 mmol). The vial was sealed and heated under microwave irradiation at 80 °C for 10 h. The reaction vessel was vented, after which a white precipitate formed. The mixture was concentrated *in vacuo* to yield a white solid (187 mg). To a solution of this intermediate (180 mg) in acetone (5 mL) at room temperature was added K₂CO₃ (193 mg, 1.4 mmol). After 30 min, iodomethane (117 μL, 1.88 mmol) was added and the reaction mixture was heated under reflux for 4 h. Upon cooling to room temperature, the solvent was removed *in vacuo*, the residue was partitioned between water (20 mL) and EtOAc (20 mL) and the organic layer separated. The aqueous phase was extracted with EtOAc (20 mL × 3), the combined organic layers were dried over MgSO₄ and excess solvent was removed *in vacuo*. The residue was purified by flash column chromatography (5–10% EtOAc in hexanes) to give **9** (141 mg, 0.688 mmol, 69% over 2 steps) as a white solid. R_f (10% EtOAc/Petrol) 0.45. Found C, 46.78; H, 2.93. C₈H₆Cl₂O₂ requires C, 46.86%; H 2.95%. ν_{max} (film)/cm⁻¹ 2856 (w), 1691 (s), 1586 (m), 1556 (m), 1266 (s). δ_H (CDCl₃) 3.97 (3H, s, OCH₃), 7.82 (2H, s, ArH), 9.86 (1H, s, CHO). δ_C (CDCl₃) 61.0, 130.0, 130.6, 133.1, 157.3, 188.6.

3,5-Dibromo-4-methoxybenzaldehyde³ (**10**)

A suspension of 3,5-dibromo-4-hydroxybenzaldehyde (**14**) (5.00 g, 17.90 mmol) and potassium carbonate (3.70 g, 26.80 mmol) in acetone (50 mL) was stirred for 30 min at rt. Iodomethane (1.67 mL, 26.8 mmol) was added and the reaction mixture was heated to 65 °C for 4 h. When cool, the solvent was removed *in vacuo* and the residue was taken up in EtOAc (50 mL) and H₂O (50 mL). This was extracted with EtOAc (3 × 100 mL), and the combined organic extracts were washed with brine (50 mL), dried over MgSO₄ and the excess solvent was removed *in vacuo* to give **10** (4.55 g, 15.50 mmol, 86%) as a pale yellow solid, which was used without further purification. R_f (20% EtOAc in hexanes) 0.52. Found: C, 32.75; H, 2.03; Br, 53.88. C₈H₆Br₂O₂ requires C, 32.69%; H, 2.06%; Br, 54.37%. ν_{max}(film)/cm⁻¹ 3060 (w), 2952 (w), 2852 (w), 1686 (s), 1581 (w), 1547 (m), 1469 (m), 1413 (m), 1367 (m), 1260 (s), 1205 (m), 1187 (m), 1067 (w), 980 (s), 921 (m), 876(m), 781 (m), 744 (m), 730 (s). δ_H (CDCl₃) 3.97 (3H, s, OCH₃), 8.03 (2H, s, ArH), 9.86 (1H, s, CHO). δ_C (CDCl₃) 60.9, 119.3, 134.0, 134.3, 159.2, 188.4.

3,5-Diiodo-4-methoxybenzaldehyde (**11**)

To a stirred solution of **15** (4 g, 10.7 mmol) in acetone (60 mL) at rt was added K₂CO₃ (2.21 g, 16.0 mmol). After 30 mins, iodomethane (1.33 mL, 21.4 mmol) was added and the reaction mixture was heated at reflux for 3.5 h after which, a further portion of iodomethane (1.33 mL, 21.4 mmol) was added. After 1.5 h, the mixture was cooled to rt, the solvent removed *in vacuo*, the residue was partitioned between water (250 mL) and EtOAc (150 mL) and the organic layer separated. The aqueous phase was extracted with EtOAc (150 mL × 3) combined organic layers were dried over MgSO₄ and excess solvent was removed *in vacuo*. The residue was purified by flash column chromatography (5-10% EtOAc/Petrol) to give **11** (3.75 g, 9.67 mmol, 90%) as a white solid. R_f (10% EtOAc in hexanes) 0.69. Found: C, 24.44; H, 1.56. C₈H₆I₂O₂ requires C, 24.77%; H, 1.56%. ν_{max}(film)/cm⁻¹ 2938 (w), 2859 (w), 1689 (s), 1673 (s), 1536 (s), 1184 (s). δ_H (CDCl₃) 3.88 (3H, s, OCH₃), 8.22 (2H, s, ArH), 9.77 (1H, s, CHO). δ_C (CDCl₃) 60.8, 91.2, 135.2, 141.0, 163.5, 187.8. m/z (ESI+) calculated for C₈H₆I₂O₂Na [M+Na]⁺ 410.8349, found 410.8343.

*3-(tert-Butyldimethylsilyloxy)-4-methoxybenzaldehyde (TBDMS-isovanillin)*⁴

Prepared according to literature procedure⁴ to give title compound (8.45 g, 31.70 mmol, 97%) as a pale yellow oil. R_f (40% EtOAc in hexanes) 0.71. ν_{\max} (film)/cm⁻¹ 2931 (w), 1689 (s), 1595 (m), 1579 (m), 1508 (s), 1442 (m), 1433 (m), 1275 (s), 1131 (s), 1025 (w), 994 (w), 837 (s), 781 (s). δ_H (CDCl₃) 0.17 (6H, s, SiCH₃), 1.00 (9H, s, SiC(CH₃)₃), 3.89 (3H, s, OCH₃), 6.95 (1H, d, J 8.3 Hz, Ar-H4), 7.36 (1H, d, J 2.0 Hz, Ar-H2), 7.47 (1H, dd, J 8.3, 2.0 Hz, Ar-H6), 9.82 (1H, s, CHO). δ_C (CDCl₃) -4.6, 18.5, 25.7, 55.6, 111.2, 120.1, 126.2, 130.3, 145.6, 156.6, 190.9. m/z (ESI+) calculated for C₁₄H₂₃O₃Si [M+H]⁺ 267.1416, found 267.1463.

*(3-((tert-Butyldimethylsilyl)oxy)-4-methoxybenzyl)triphenylphosphonium bromide*⁵ (**16**)

Prepared from TBDMS-isovanillin according to literature procedure^{4,5} to give **16** (80% over 3 steps) as a white solid. δ_H (CDCl₃) -0.10 (6H, s, Si(CH₃)₂), 0.78 (9H, s, SiC(CH₃)₃), 3.66 (3H, s, OCH₃), 5.03 (2H, d, J 13.6 Hz), 6.28 (1H, s, Ar-H2), 6.55 (1H, d, J 8.3 Hz), 6.72 (1H, d, J 8.3 Hz), 7.60 (15H, m, PArH), 7.70 (4H, m, PArH). δ_C (CDCl₃) -4.8, 18.3, 25.6, 30.4 (d, J_{CP} 46.9 Hz), 55.4, 112.3, 117.4, 118.3, 122.9 (d, J_{CP} 4.8 Hz), 125.7 (d, J_{CP} 6.4 Hz), 130.1 (d, J_{CP} 12.4 Hz), 134.2 (d, J_{CP} 9.7 Hz), 135.0 (d, J_{CP} 2.8 Hz), 145.0 (d, J_{CP} 3.3 Hz), 151.3 (d, J_{CP} 3.7 Hz). m/z (ESI+) calculated for C₃₂H₃₈O₂PSi [M-Br]⁺ 513.2379, found 513.2386.

General Procedure for Wittig Reactions

To a suspension of (3-((tert-butyldimethylsilyl)oxy)-4-methoxybenzyl)triphenylphosphonium bromide (**16**) (1.50 mmol) in dry THF (10 mL) at -20 °C was added *n*-butyllithium (1.80 mmol of 2.5 M solution in hexanes) dropwise and the solution allowed to warm to rt. Aldehyde (1.25 mmol) in dry THF (5 mL) was added dropwise and the solution stirred for 20 min. Ice (10 mL) was added and the solution was extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed with brine (10 mL), dried over MgSO₄, and the excess solvent was removed *in vacuo* to give a residue upon which ¹H NMR was then performed to ascertain the (*E*):(*Z*) ratio.

*(Z)-tert-Butyl(2-methoxy-5-(3,4,5-trimethoxystyryl)phenoxydimethylsilane*⁶ ((*Z*)-**17**)

Prepared following the general procedure using 3,4,5-trimethoxybenzaldehyde (**7**). Crude (*E*):(*Z*) 53:47. Purified by flash column chromatography (0–10% EtOAc in hexanes) to give (*Z*)-**17** (228 mg, 0.53 mmol, 35%) as a colourless oil, (*E*)-**17** (261 mg, 0.61 mmol, 40%) as a white solid, and a mixture of isomers (11 mg, 0.02 mmol, 2%).

(*Z*)-**17**: R_f (15% EtOAc in hexanes) 0.39. ν_{\max} (film)/cm⁻¹ 2930 (w), 1580 (m), 1507 (s), 1272 (s), 1248 (s), 1126 (s), 838 (s), 781 (s). δ_H (CDCl₃) 0.06 (6H, s, SiCH₃), 0.93 (9H, s, SiC(CH₃)₃), 3.70 (6H, s, OCH₃), 3.78 (3H, s, OCH₃), 3.83 (3H, s, OCH₃), 6.42 (1H, d, J 12.0 Hz, *cis* CH=CH), 6.47 (1H, d, J 12.0 Hz, *cis* CH=CH), 6.50 (2H, s, Ar-H2,H6), 6.74 (1H, d, J 8.3 Hz, Ar-H5'), 6.79 (1H, d, J 1.9 Hz, Ar-H2'), 6.85 (1H, dd, J 8.4, 1.9 Hz, Ar-H4'). δ_C (CDCl₃) -4.2, 18.9, 26.2, 55.9, 56.6, 61.4, 103.8, 112.5, 119.0, 120.9, 127.0, 128.4, 130.9, 133.8, 138.2, 145.6, 151.3, 153.8. m/z (ESI+) calculated for C₂₄H₃₆O₆Si [M+H]⁺ 431.2254, found 431.2271.

(Z)-*tert*-Butyl(5-(3,5-difluoro-4-methoxystyryl)-2-methoxyphenoxy)dimethylsilane ((*Z*)-**18**)

Prepared following the general procedure using 3,5-difluoro-4-methoxybenzaldehyde (**8**). Crude (*E*):(*Z*) 53:47. Purified by flash column chromatography (0.25% Et₂O in hexanes) to give (*Z*)-**18** (389 mg, 0.96 mmol, 37%) as a colourless oil, (*E*)-**18** (377 mg, 0.93 mmol, 36%) as a white solid, and a mixture of isomers (117 mg, 0.29 mmol, 11%).

(*Z*)-**18**: R_f (10% Et₂O in hexanes) 0.46. ν_{\max} (film)/cm⁻¹ 2955 (w), 2931 (w), 1601 (w), 1571 (m), 1508 (s), 1429 (s), 1030 (s). δ_{H} (CDCl₃) 0.08 (6H, s, SiCH₃), 0.94 (9H, s, SiC(CH₃)₃), 3.80 (3H, s, OCH₃), 3.97 (3H, t, *J*_{H-F} 0.94 Hz, OCH₃), 6.30 (1H, d, *J* 12.1 Hz, *cis* CH=CH), 6.52 (1H, d, *J* 12.1 Hz, *cis* CH=CH), 6.77 (5H, m, ArH). δ_{C} (CDCl₃) -4.8, 18.3, 25.6, 55.4, 61.8 (t, *J*_{CF} 3.1 Hz), 111.8, 126.3 (dd, *J*_{CF} 16.6, 6.5 Hz), 121.1, 122.7, 126.3 (t, *J*_{CF} 2.3 Hz), 129.1, 131.3, 132.7 (t, *J*_{CF} 9.3), 135.1 (t, *J*_{CF} 14.4 Hz), 144.7, 150.7, 155.4 (dd, *J*_{CF} 248, 6.5 Hz). *m/z* (ESI+) calculated for C₂₂H₂₉O₃F₂Si [M+H]⁺ 407.1854, found 407.1859.

(E,Z)-*tert*-Butyl(5-(3,5-dichloro-4-methoxystyryl)-2-methoxyphenoxy)dimethylsilane ((*E,Z*)-**19**)

Prepared following the general procedure using 3,5-dichloro-4-methoxybenzaldehyde (**9**). Crude (*E*):(*Z*) 57:43. Purified by flash column chromatography (0–5% Et₂O in hexanes) to give a mixture of (*Z*)-**19** and (*E*)-**19** (261 mg, 0.59 mmol, 90%) as a white residue. R_f (15% EtOAc in hexanes) 0.68. ν_{\max} (film)/cm⁻¹ 2932 (m), 1728 (w), 1509 (s), 1281 (s), 1131 (m), 997 (m), 841 (s). *m/z* (ESI+) calculated for C₂₂H₂₈Cl₂O₃Si [M+H]⁺ 439.1263, found 439.1273.

(*Z*)-**19** (integrates to 43%): δ_{H} (CDCl₃) 0.07 (6H, s, SiCH₃), 0.93 (9H, s, SiC(CH₃)₃), 3.80 (3H, s, OCH₃), 3.88 (3H, s, OCH₃), 6.30 (1H, d, *J* 12.1 Hz, *cis* CH=CH), 6.53 (1H, d, *J* 12.1 Hz, *cis* CH=CH), 6.71 (1H, d, *J* 1.9 Hz, Ar-H2'), 6.76 (1H, d, *J* 8.3 Hz, Ar-H5'), 6.80 (1H, dd, *J* 8.3, 1.9 Hz, Ar-H6'), 7.26 (2H, s, Ar-H2,H6).

(*E*)-**19** (integrates to 57%): δ_{H} (CDCl₃) 0.21 (6H, s, SiCH₃), 1.05 (9H, s, SiC(CH₃)₃), 3.83 (3H, s, OCH₃), 3.92 (3H, s, OCH₃), 6.74 (1H, d, *J* 16.2 Hz, *trans* CH=CH), 6.84 (1H, d, *J* 8.3 Hz, Ar-H5'), 6.92 (1H, d, *J* 16.2 Hz, *trans* CH=CH), 7.01 (1H, d, *J* 2.0 Hz, Ar-H2'), 7.04 (1H, dd, *J* 8.3, 2.1 Hz, Ar-H6'), 7.40 (2H, s, Ar-H2,H6).

(E,Z)-*tert*-Butyl(5-(3,5-dibromo-4-methoxystyryl)-2-methoxyphenoxy)dimethylsilane ((*E,Z*)-**20**)

Prepared following the general procedure using 3,5-dibromo-4-methoxybenzaldehyde (**10**) (413 mg, 1.40 mmol). Crude (*E*):(*Z*) 60:40. Purified by flash column chromatography (0–5% EtOAc in hexanes) to give a mixture of (*Z*)-**20** and (*E*)-**20** (709 mg, 1.34 mmol, 96%) as a white residue. R_f (15% EtOAc in hexanes) 0.60. ν_{\max} (film)/cm⁻¹ 2930 (w), 1509 (m), 1474 (s), 1422 (m), 1262 (s), 997 (m), 851 (m), 740 (m). *m/z* (ESI+) calculated for C₂₂H₂₉Br₂O₃Si [M+H]⁺ 529.0232, found 529.0205.

(*Z*)-**20** (integrates to 40%): δ_{H} (CDCl₃) 0.08 (6H, s, SiCH₃), 0.93 (9H, s, SiC(CH₃)₃), 3.83 (3H, s, OCH₃), 3.89 (3H, s, OCH₃), 6.31 (1H, d, *J* 12.0 Hz, *cis* CH=CH), 6.53 (1H, d, *J* 12.0 Hz, *cis* CH=CH), 6.78 (1H, d, *J* 2.0 Hz, Ar-H2'), 6.82 (1H, dd, *J* 8.7, 2.0 Hz, Ar-H6'), 6.83 (1H, d, *J* 8.4 Hz, Ar-H5'), 7.38 (2H, d, *J* 0.6 Hz, Ar-H2,H6).

(*E*)-**20** (integrates to 60%): δ_{H} (CDCl₃) 0.18 (6H, s, SiCH₃), 1.02 (9H, s, SiC(CH₃)₃), 3.80 (3H, s, OCH₃), 3.87 (3H, s, OCH₃), 6.72 (1H, d, *J* 16.0 Hz, *trans* CH=CH), 6.84 (1H, d, *J* 8.4 Hz, Ar-H5'), 6.91 (1H, d, *J* 16.2 Hz, *trans* CH=CH), 7.01 (1H, d, *J* 2.1 Hz, Ar-H2'), 7.03 (1H, dd, *J* 8.3, 2.1 Hz, Ar-H6'), 7.61 (2H, s, Ar-H2,H6).

(E,Z)-*tert*-Butyl(5-(3,5-diiodo-4-methoxystyryl)-2-methoxyphenoxy)dimethylsilane⁷ ((*E,Z*)-**21**)

Prepared following general procedure using 3,5-diiodo-4-methoxybenzaldehyde (**11**) (490 mg, 1.26 mmol). Crude (*E*):(*Z*) 58:42. Purified by flash column chromatography (0–5% EtOAc in hexanes) to give a mixture of (*Z*)-**21** and (*E*)-**21** (760 mg, 1.22 mmol, 97%) as a white residue. R_f (15% EtOAc in hexanes) 0.66. ν_{\max} (film)/ cm^{-1} 2928 (w), 1506 (s), 1461 (s), 1274 (s), 1248 (s), 1251 (s), 1230 (s), 1131 (s), 992 (s), 838 (s), 781 (s). m/z (ESI+) calculated $\text{C}_{22}\text{H}_{28}\text{I}_2\text{O}_3\text{SiNa}$ $[\text{M}+\text{Na}]^+$ 644.9789, found 644.9777.

(*Z*)-**21** (integrates to 42%): δ_{H} (CDCl_3) 0.08 (6H, s, SiCH_3), 0.94 (9H, s, $\text{SiC}(\text{CH}_3)_3$), 3.80 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 6.27 (1H, d, J 12.0 Hz, *cis* CH=CH), 6.50 (1H, d, J 12.0 Hz, *cis* CH=CH), 6.69 (1H, d, J 1.9 Hz, Ar-H2'), 6.75 (1H, d, J 8.3 Hz, Ar-H5'), 6.79 (1H, dd, J 8.3, 1.9 Hz, Ar-H6'), 7.63 (2H, s, Ar-H2,H6).

(*E*)-**21** (integrates to 58%): δ_{H} (CDCl_3) 0.18 (6H, s, SiCH_3), 1.02 (9H, s, $\text{SiC}(\text{CH}_3)_3$), 3.82 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 6.68 (1H, d, J 16.2 Hz, *trans* CH=CH), 6.82 (1H, d, J 8.2 Hz, Ar-H5'), 6.88 (1H, d, J 16.2 Hz, *trans* CH=CH), 6.99 (1H, d, J 2.0 Hz), 7.02 (1H, dd, J 8.2, 2.1 Hz), 7.86 (2H, s, Ar-H2,H6)

Triphenyl(3,4,5-trimethoxybenzyl)phosphonium bromide⁵ (**22**)

Prepared from 3,4,5-trimethoxybenzaldehyde (**7**) according to literature procedure⁸ to give **22** (73% over 3 steps). δ_{H} (CDCl_3) 3.53 (6H, s, OCH_3), 3.77 (3H, s, OCH_3), 5.39 (2H, d, J_{PH} 14.0 Hz, CH_2P), 6.46 (2H, d, J_{PH} 2.6 Hz, ArH), 7.64 (6H, m, PArH), 7.77 (9H, m, PArH). δ_{C} (CDCl_3) 30.9 (d, J_{CP} 45.9 Hz), 56.3, 60.9, 109.0 (d, J_{CP} 5.6 Hz), 118.0 (d, J_{CP} 85.1 Hz), 122.5 (d, J_{CP} 9.0 Hz), 130.0 (d, J_{CP} 12.4 Hz), 134.7 (d, J_{CP} 9.6 Hz), 134.8 (d, J_{CP} 3.1 Hz), 137.7 (d, J_{CP} 4.5 Hz), 153.0 (d, J_{CP} 3.8 Hz).

General Procedure for Deprotection using TBAF

To a solution of TBDMS-protected stilbene (1.00 mmol) in dry THF (30 mL) was added tetrabutylammonium fluoride (1.30 mmol of 1 M solution in THF) dropwise and stirred for 30 min. Ice/water (50 mL) was added and the solution was extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with brine (30 mL), dried over MgSO_4 , and the excess solvent was removed *in vacuo* to give a yellow residue.

(*Z*)-2-Methoxy-5-(3,4,5-trimethoxystyryl)phenol⁶ ((*Z*)-**5**)

Prepared following the general procedure using (*Z*)-*tert*-butyl(2-methoxy-5-(3,4,5-trimethoxystyryl)phenoxy)dimethylsilane (**17**) (208 mg, 0.48 mmol). Purified by flash column chromatography (20% EtOAc in hexanes) to give (*Z*)-**5** as a colourless oil (111 mg, 0.35 mmol, 73%). R_f (25% EtOAc in hexanes) 0.19. ν_{\max} (film)/ cm^{-1} 3402 (br), 2938 (w), 1578 (s), 1505 (s), 1270 (s), 1233 (s), 1118 (s), 1002 (s). δ_{H} (CDCl_3) 3.70 (6H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 5.73 (1H, br s, OH), 6.42 (1H, d, J 12.2 Hz, *cis* CH=CH), 6.47 (1H, d, J 12.2 Hz, *cis* CH=CH), 6.53 (2H, s, Ar-H2,H6), 6.73 (1H, d, J 8.3 Hz, Ar-H5'), 6.80 (1H, dd, J 8.4, 2.0 Hz, Ar-H6'), 6.92 (1H, d, J 2.0 Hz, Ar-H2'). δ_{C} (CDCl_3) 41.0, 56.0, 60.9, 106.2, 110.5, 115.1, 121.1, 129.0, 129.5, 130.7, 132.7, 145.4, 145.9, 152.9. m/z (ESI+) calculated for $\text{C}_{18}\text{H}_{21}\text{O}_5$ $[\text{M}+\text{H}]^+$ 317.1389, found 317.1393.

(Z)-5-(3,5-Difluoro-4-methoxystyryl)-2-methoxyphenol ((*Z*)-**23**)

Prepared following the general procedure using (*Z*)-*tert*-butyl(5-(3,5-difluoro-4-methoxystyryl)-2-methoxyphenoxy)dimethylsilane (**18**) (189 mg, 0.47 mmol). Purified by flash column chromatography (5% EtOAc in hexanes) to give (*Z*)-**23** as a colourless oil (125 mg, 0.43 mmol, 92%). R_f (10% EtOAc in hexanes) 0.20. ν_{\max} (film)/ cm^{-1} 3530 (br), 2938 (w), 1617 (w), 1572 (m), 1508 (s), 1439 (s), 1026 (s). δ_{H} (CDCl_3) 3.88 (3H, s, OCH_3), 3.98 (3H, br s, OCH_3), 5.57 (1H, s, OH), 6.31 (1H, d, J 12.1, *cis* CH=CH), 6.52 (1H, d, J 12.1 Hz, *cis* CH=CH), 6.74 (2H, s, Ar-H₂,H₆), 6.80 (3H, m, Ar-H₂',H₅'H₆'). δ_{C} (CDCl_3) 55.9, 61.8 (t, J 3.3 Hz), 110.4, 112.5 (dd, J 16.6, 6.5), 114.7, 120.8, 126.6 (t, J 2.3 Hz), 129.6, 131.1), 132.4 (t, J 9.1 Hz), 135.2 (t, J 14.2 Hz), 145.3, 146.0, 155.3 (dd, J 247, 6.5 Hz). m/z (ESI+) calculated for $\text{C}_{16}\text{H}_{15}\text{O}_3\text{F}_2$ [$\text{M}+\text{H}$]⁺ 293.0989, found 293.1000.

(Z)-5-(3,5-Dichloro-4-methoxystyryl)-2-methoxyphenol ((*Z*)-**24**)

Prepared following the general procedure using (*E,Z*)-*tert*-butyl(5-(3,5-dichloro-4-methoxystyryl)-2-methoxyphenoxy)dimethylsilane (**19**) (258 mg, 0.59 mmol). Purified by flash column chromatography (0–5% EtOAc in hexanes) to give (*Z*)-**24** (79 mg, 0.24 mmol, 41%) as a colourless oil, (*E*)-**24** (90 mg, 0.28 mmol, 47%) as a white solid, and a mixture of isomers (24 mg, 0.07 mmol, 12%).

(*Z*)-**24**: R_f (15% EtOAc in hexanes) 0.27. ν_{\max} (film)/ cm^{-1} 3525 (br), 2935 (w), 1582 (w), 1542 (w), 1507 (s), 1477 (m), 1261 (s), 1122 (s), 993 (s), 806 (s). δ_{H} (CDCl_3) 3.89 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 5.53 (1H, s, OH), 6.31 (1H, d, J 12.1 Hz, *cis* CH=CH), 6.53 (1H, d, J 12.1 Hz, *cis* CH=CH), 6.74 (2H, merged, Ar-H₅',H₆'), 6.82 (1H, d, J 0.8 Hz, Ar-H₂'), 7.19 (2H, s, Ar-H₂,H₆). δ_{C} (CDCl_3) 56.0, 60.8, 110.6, 114.9, 121.0, 126.0, 129.0, 129.1, 129.6, 131.7, 135.0, 142.1, 145.5, 146.2. m/z (ESI+) calculated for $\text{C}_{16}\text{H}_{15}\text{O}_3\text{Cl}_2$ [$\text{M}+\text{H}$]⁺ 325.0398, found 325.0411.

(Z)-5-(3,5-Dibromo-4-methoxystyryl)-2-methoxyphenol ((*Z*)-**25**)

Prepared following the general procedure using (*E,Z*)-*tert*-butyl(5-(3,5-dibromo-4-methoxystyryl)-2-methoxyphenoxy)dimethylsilane (**20**) (174 mg, 0.33 mmol). Purified by flash column chromatography (0–5% EtOAc in hexanes) to give (*Z*)-**25** (49 mg, 0.12 mmol, 36%) as a colourless oil, (*E*)-**25** (59 mg, 0.14 mmol, 43%) as a white solid, and a mixture of isomers (4 mg, 0.01 mmol, 3%).

(*Z*)-**25**: R_f (15% EtOAc in hexanes) 0.25. ν_{\max} (film)/ cm^{-1} 3523 (w, br), 2930 (w), 1583 (w), 1509 (s), 1472 (m), 1422 (m), 1268 (s), 1124 (m), 998 (m), 764 (s). δ_{H} (CDCl_3) 3.87 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 5.81 (1H, s, OH), 6.29 (1H, d, J 12.1 Hz, *cis* CH=CH), 6.51 (1H, d, J 12.1 Hz, *cis* CH=CH), 6.71 (1H, dd, J 8.8, 2.0 Hz, Ar-H₆'), 6.74 (1H, d, J 8.0 Hz, Ar-H₅'), 6.80 (1H, d, J 1.6 Hz, Ar-H₂'), 7.49 (2H, d, J 0.5 Hz, Ar-H₂,H₆). δ_{C} (CDCl_3) 56.0, 60.7, 110.6, 115.0, 117.8, 121.0, 125.5, 129.4, 131.8, 132.9, 136.2, 145.6, 146.4, 152.8. m/z (ESI+) calculated for $\text{C}_{16}\text{H}_{15}\text{Br}_2\text{O}_3$ [$\text{M}+\text{H}$]⁺ 414.9367, found 414.9351.

(Z)-5-(3,5-Diiodo-4-methoxystyryl)-2-methoxyphenol⁷ ((*Z*)-**26**)

Prepared following the general procedure using (*E,Z*)-*tert*-butyl(5-(3,5-diiodo-4-methoxystyryl)-2-methoxyphenoxy)dimethylsilane (**21**) (700 mg, 1.12 mmol). Purified by flash column chromatography (0–5% EtOAc in hexanes) to give (*Z*)-**26** (209 mg, 0.41 mmol, 37%) as a colourless oil and (*E*)-**26** (308 mg, 0.61 mmol, 54%) as a white solid.

(Z)-**26**: R_f (15% EtOAc in hexanes) 0.22. ν_{\max} (film)/ cm^{-1} 3427 (br), 2934 (w), 1729 (w), 1580 (w), 1506 (s), 1267 (s), 1245 (s), 1120 (s), 991 (s). δ_{H} (CDCl_3) 3.85 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 5.50 (1H, s, OH), 6.27 (1H, d, J 12.1 Hz, *cis* CH=CH), 6.50 (1H, d, J 12.1 Hz, *cis* CH=CH), 6.74 (2H, merged, Ar-H5', H6'), 6.82 (1H, d, J 0.9 Hz, Ar-H2'), 7.66 (2H, s, Ar-H2, H6). δ_{C} (CDCl_3) 56.1, 60.8, 90.0, 110.6, 115.0, 121.0, 125.1, 130.0, 131.6, 137.4, 140.0, 145.5, 146.3, 157.6. m/z (ESI+) calculated for $\text{C}_{16}\text{H}_{15}\text{I}_2\text{O}_3$ [M+H]⁺ 508.9111, found 508.9124.

Biological Materials

Dulbecco's phosphate buffered saline (PBS), dimethyl sulphoxide (DMSO), methanol, trypan blue solution (0.4%), RPMI-1640, Dm1a anti- α -tubulin antibody, propidium iodide, RNase A and Hoescht stain were purchased from Sigma, UK. Trypsin / EDTA solution, trypsin neutralizing solution (TNS), foetal bovine serum (FBS), Endothelial Basal Medium-2 and Single Quot[®] Kit supplements, Dulbecco's Modified Eagle Medium (DMEM), and Leibovitz's medium were purchased from Lonza Group Ltd., UK. Vectashield mounting medium and biotinylated ULEX europaeus agglutinin were purchased from Vector Laboratories, UK. Alexa Fluor[®] 488-coupled antibodies were purchased from Molecular Probes, UK. Mouse anti-smooth muscle actin, mouse anti-human fibroblast, mouse anti-human CD31, biotinylated goat anti-mouse IgG antibodies and streptavidin-FITC conjugate were purchased from Invitrogen, UK. The CellTiter 96[®] AQueous One Solution Reagent was purchased from Promega, UK.

Cervical cancer (HeLa) and GFP-tubulin expressing HeLa cells were grown from stocks provided by the research group of Dr Fanni Gergely at the Cancer Research UK Cambridge Research Institute (CRI). Ovarian cancer cells (SKOV3 and SKOV3-TR⁹) were grown from stocks provided by the research group of Dr James Brenton, Cancer Research UK Cambridge Research Institute (CRI). Human peripheral blood mononuclear cells (PBMCs) were donated by Chantal Cho, Department of Pharmacology, University of Cambridge.

Human umbilical vein endothelial cells (HUVECs) were isolated from umbilical cords following elective caesarean sections performed in the Rosie Hospital, Addenbrookes, Cambridge. This study was approved by the Local Research Ethics Committee and all patients gave written informed consent.

Culture of Cancer Cell Lines

Cancer cell lines were cultured using standard methods that are briefly described below. HeLa cells were grown in DMEM supplemented with 10% FBS, glutamine and penicillin/streptomycin. HeLa cells expressing GFP-tubulin were cultured in the same medium with selection pressure maintained by the addition of G418 (400 $\mu\text{g}/\text{mL}$). SK-OV-3 cells were cultured in RPMI-1640 supplemented with 10% FBS, glutamine and penicillin/streptomycin, and SK-OV-3TR cells were cultured in the same medium containing paclitaxel (300 nM). Media changes were performed after 48 h. HeLa cells were incubated at 37 °C in humidified air with 6% CO_2 , SK-OV-3 cells were incubated at 37 °C in humidified air with 5% CO_2 .

Stocks of cell lines were grown throughout the course of this work and passaged when necessary. A description of the subculture process is described. Cells were subcultured at 80-90% confluence. This was achieved by aspirating the medium, washing with PBS (7 mL) and addition of trypsin / EDTA solution (3 mL). Flasks were incubated for 5 min then sharply tapped to ease cell detachment. Serum-containing medium (3 mL) was added to neutralise the trypsin, and an appropriate amount added to a T75 flask

depending on required cell density. Medium was added to give 10 mL total volume and the flask was returned to the incubator.

HUVEC Isolation and Culture

Umbilical cords were received in a solution of PBS containing 0.25 $\mu\text{g}/\text{mL}$ amphotericin B and were used within 12 h of delivery. The following procedure is a slight modification from that reported.¹⁰ Cords were removed from solution, washed with PBS and inspected for holes and clamp marks. Damaged areas were removed to leave a section of 10–15 cm in length. A blunt needle was then inserted and clamped into the umbilical vein, and the vein flushed with pre-warmed PBS (20 mL) using a syringe. A blunt needle was inserted and clamped into the opposite end of the vein, and the vein washed further with PBS (20 mL) by passing between two syringes. The PBS was removed and collagenase (0.25% w/v in 10 mL DMEM, filtered through 0.45 and 0.2 μm syringe filters) was added to the vein using two syringes. The cord was incubated at 37 °C submerged in PBS for 15 min. Every 3 min the collagenase solution was moved through the vein using the syringes. The cord was removed and collagenase solution collected in a 50 mL tube. The vein was rinsed with DMEM (30 mL) and the washings collected in the tube, and then centrifuged at 1200 rpm, 6 min, 25 °C. The media was aspirated and the cells resuspended in pre-warmed EGM-2 (1 mL) containing gentamicin/amphotericin-B (0.1%) which was added to a T25 flask (previously coated with 1% gelatin solution at 37 °C for 30 min). The flask was incubated at 37 °C in humidified air with 5% CO₂, with the media changed after 24 h.

HUVECs were grown in Endothelial Cell Growth Medium-2® (EGM-2), which consists of Endothelial Basal Cell Medium (EBM-2) supplemented by hydrocortisone, human Epidermal Growth Factor (hEGF), 2 % FBS, Vascular Endothelial Growth Factor (VEGF), human Fibroblast Growth Factor-basic (hFGF-b), R³-Insulin-like Growth Factor-1 (R³-IGF-1), ascorbic acid and heparin. Media changes were performed after 48 h. Culture flasks were incubated at 37 °C in humidified air with 5% CO₂. This media was additionally supplemented with gentamicin for the first 24 h after HUVEC isolation.

HUVEC isolates were grown from isolation to passage 3 (P3) and these cells were frozen and used in subsequent assays. A description of the culture process is described, though larger flasks and volumes were used for later passages. Cells were subcultured at 70-80% confluence. The media was aspirated, the cells washed with PBS (3 mL) and trypsin / EDTA solution was added (1 mL). The flask was incubated at 37 °C for 3 min and then sharply tapped to ease cell detachment. TNS (2 mL) was added and the detached cells collected in a 15 mL tube. The flask was rinsed with PBS (3 mL) and all the cells recovered by centrifugation (1200 rpm, 6 min, 25 °C). The supernatant was removed and the cells were resuspended in EGM-2 (6 mL). This solution was added to a T75 flask containing media (4 mL) and this was returned to the incubator.

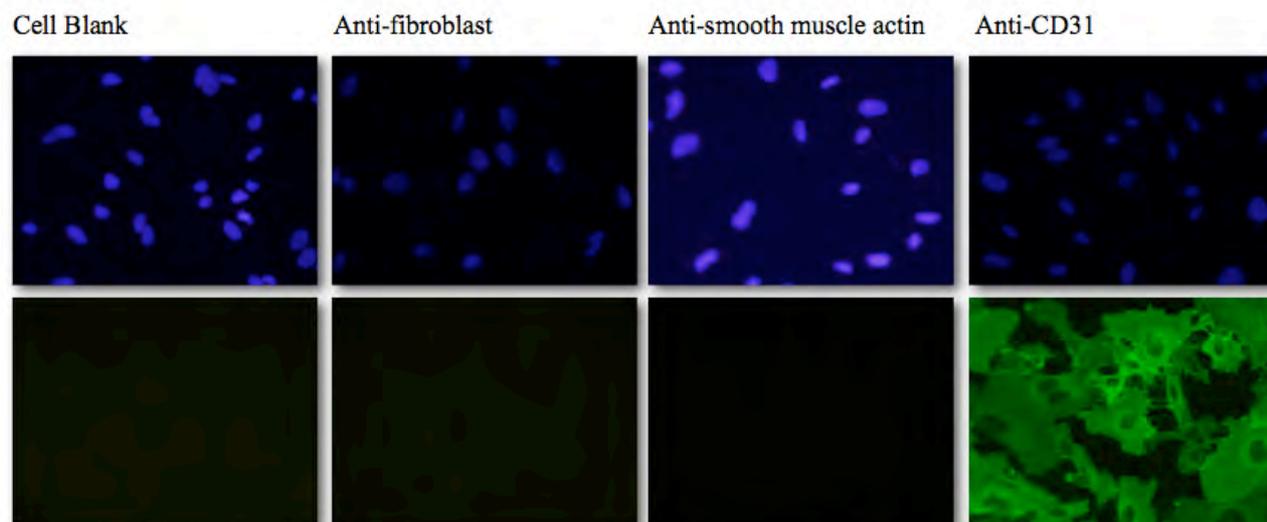
Cells were frozen at P3 at 2.5×10^5 cells per tube using the following procedure. Cells were harvested as described above and finally resuspended in EGM-2 (1 mL). An aliquot of cell suspension (10 μL) was removed and diluted with PBS (140 μL). This was diluted with trypan blue (1:1), and 10 μL of this was counted using a haemocytometer. The cell suspension was then diluted as required with FBS (final concentration 90%), and then DMSO was added slowly (final concentration 10%) to give 2.5×10^5 cells per 0.5 mL of solution. The suspension was gently mixed and aliquoted into freezing tubes (0.5 mL) which were transferred to freezing containers and placed in an –80 °C freezer for 24 h. The tubes were then transferred to cryostorage in liquid nitrogen and were removed for assays as required.

Immunocytochemistry of HUVECs

An aliquot of EGM-2 containing 1.6×10^5 cells was removed prior to freezing and diluted to 4.2 mL with EGM-2. 500 μL of this suspension was added to each well of an 8-well chamber slide (cell density 2×10^4 cells/well) and slide was incubated at 37 °C for 24–48 h (until 60% confluent). The medium was aspirated, and the cells fixed in 1:1 MeOH/acetone at 4 °C for 20 min. A solution of 10% rabbit serum in PBS (50 μL) was added to each well and incubated in a humidified chamber at room temperature for 20 min. The solution was removed, and primary antibody solutions (50 μL per well of dilution in 10% FBS in PBS) were added. The slide was incubated at room temperature in a humidified chamber for 1 h. The antibody solutions were removed and slides were washed with PBS (3 x 5 min). Secondary antibody solutions (50 μL per well of dilution in 10% FBS in PBS) were added. The slides were incubated at room temperature in a humidified chamber for 1 h. The antibody solutions were removed and the slides were washed with PBS (3 x 5 min). Streptavidin-FITC conjugate (50 μL of a 1:100 dilution in 10% FBS in PBS) was added to each well and the slides were incubated at room temperature in a humidified chamber for 1 h in the dark. The streptavidin-FITC conjugate solution was removed and the slides washed with PBS (4 x 5 min) and air-dried in the dark. One drop of Vectashield + DAPI mount was added to each well and a coverslip was placed on the slide. The slides were viewed using a Leica DMRB microscope at 100x magnification and images were recorded using an Olympus DP71.

The number of cells staining positive for fibroblast and smooth muscle actin per chamber were counted to give an estimate of cell purity (Table S1), representative images shown (Fig. S6). The staining for fibroblast and smooth muscle cells gave very few positive cells across all the isolates (< 0.1% indicates none were seen on the slide), whereas in the positive placental controls very bright staining can be observed.

HUVECs



Placental controls

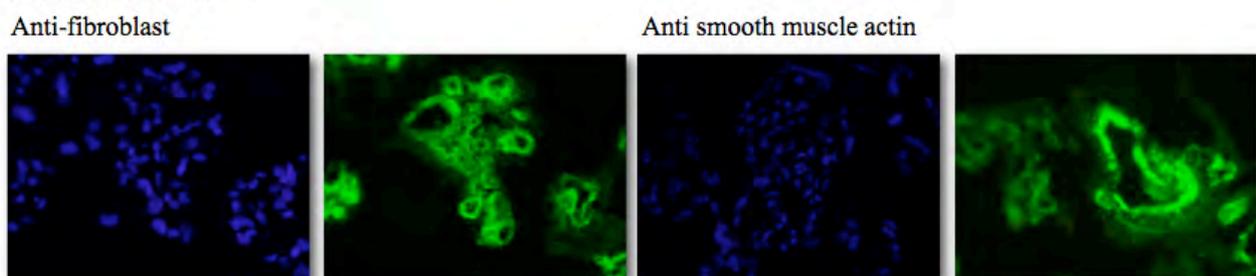


Fig. S6 Representative images for HUVEC immunocytochemistry. Staining: DNA, DAPI, blue; streptavidin/FITC, green, magnification 100 \times .

HUVEC Isolate	Tubes Frozen (2.5×10^5 cells per tube)	Purity
1	28	<0.1% SMA, fibroblast
2	44	<0.1% SMA, 0.1% fibroblast
3	22	<0.1% SMA, fibroblast
4	19	0.1% SMA, <0.1% fibroblast

Table S1. HUVEC isolates, the number of cells obtained and their purity as determined by immunocytochemistry.

xCELLigence Assay on HUVECs

HUVECs were seeded at 7.5×10^3 cells per well in EGM-2 (40 μ L) into an E-plate 96 after determination of background impedance using EGM-2 (50 μ L), and incubated for 5 h in the Real-Time Cell Analyzer (RTCA) station, recording impedance every 10 min. Drug compound was added in EGM-2 (10 μ L, 10 \times concentrations to give appropriate 1 \times plate concentrations), and the plate returned to the RTCA. Impedance

was recorded every 40 s for 3 h, then every 30 min for 37 h. Data was obtained as cell index values, which account for background impedance. These values were normalised using the cell index value recorded immediately prior to drug addition. Dose-response curves at the required timepoint were generated and used to determine EC₅₀ values, which were calculated using a variable slope dose-response curve with Hill slope constrained to > -2.0 using GraphPad Prism.

MTS Assay on HUVECs

HUVECs were thawed, resuspended in EGM-2 medium and centrifuged (1200 rpm, 3 min, 25 °C). The medium was aspirated and the cells resuspended in fresh medium (1 mL) before cell counting (trypan blue exclusion, 1:1 dilution). The cell suspension was diluted and cells seeded into the inner 60 wells of 96-well plates (90 µL, density 7.5×10^3 cells per well), with the outer wells filled with PBS (90 µL) to prevent evaporation. Cells were incubated for 5 h (37 °C, 5% CO₂), before addition of drug compound in EGM-2 medium (10 µL, 10× concentrations to give appropriate 1× plate concentrations). The drug compounds were dissolved as a 0.1 M solution in DMSO, and then diluted with EGM-2 to give the appropriate concentrations. The final plate concentration of DMSO for the highest concentration of drug compound tested (0.1%) was used as a control. After drug addition, the plates were returned to the incubator for 48 h before a visual inspection of cells was performed, followed by the addition of CellTiter 96[®] AQueous One Solution Reagent (20 µL) to each well, taking care to minimise the exposure of the reagent and plates to light. The plates were then incubated (37 °C, 5% CO₂) for a further 2 h, before the absorbance at 492 nm was measured.

MTS Assay on Cancer Cell Lines

An example procedure for the MTS assay on HeLa cells is described below, but is equally applicable to SKOV3 or SKOV3-TR cells, although it must be noted that SKOV3-TR cells were not seeded with the addition of paclitaxel. A 10 cm plate of 70–90% confluent HeLa cells was aspirated, washed with PBS (7 mL), trypsin/EDTA solution was added (3 mL) and the cells were incubated for 5 min. The trypsin was neutralised with DMEM (7.5 mL), and 500 µL of this suspension was submitted for cell counting using a ViCell XR. All cells plated had greater than 98% viability.

The cell suspension was then diluted and cells seeded into the inner 60 wells of 96-well plates (90 µL, density 7×10^3 cells per well) with the outer wells filled with PBS (90 µL) to prevent evaporation. Cells were incubated for 24 h (37 °C, 5% CO₂) before the addition of drug compound in DMEM medium (10 µL, 10× concentrations to give appropriate 1× plate concentrations). The drug compounds were dissolved as a 0.1M solution in DMSO, and then diluted with DMEM to give the appropriate concentrations. The final plate concentration of DMSO for the highest concentration of drug compound tested (0.1%) was used as a control, along with colchicine at a final concentration of 10 and 100 µM. After drug addition, the plates were returned to the incubator for 48 h before a visual inspection of cells was performed, followed by the addition of CellTiter 96[®] AQueous One Solution Reagent (20 µL) to each well, taking care to minimise the exposure of the reagent and plates to light. The plates were then incubated for a further 2 h, before the absorbance at 492 nm was measured using a Tecan Spectrophotometer.

Each plate in the MTS assays had drug concentrations repeated in quadruplicate and the assay itself was repeated on three separate occasions. The data is expressed as a percentage of the 0.1% DMSO control with the errors recorded as the standard deviation of the quadruplicate results.

MTS Assay on Human PBMCs

Human PBMCs were thawed, suspended in RPMI-1640 media supplemented with 10% FBS and centrifuged (1200 rpm, 3 min, 25 °C). The supernatant was removed, and the cells were resuspended in media. After cell counting (trypan blue exclusion, 1:1 dilution), the cell suspension was diluted and the cells were seeded into the central 60 wells of 96-well plates (90 μ L, density of 7–10 \times 10⁴ cells per well). The outer wells were filled with PBS (90 μ L) to prevent evaporation. Drug compound in RPMI-1640 + 10% FBS was added (10 μ L, 10 \times concentrations to give appropriate 1 \times plate concentrations), The final plate concentration of DMSO for the highest concentration of drug compound tested (0.1%) was used as a control, and the plate was incubated for 48 h. MTS reagent was added, and the plates incubated for 2 h with light exclusion, and the absorbance at 492 nm measured. The assay was repeated for three PBMC populations, and no loss in cell viability was observed for all concentrations tested (up to 10 μ M).

FACS Analysis of SK-OV-3 Cells

Cells were subcultured into a T₂₅ flask (5 \times 10⁵ cells, 3mL media) and grown for 24 h. Fresh media containing required concentration of drug/control was added (3 mL) and the cells were incubated with drug for a further 24 h. The supernatant media was collected, and combined with a PBS wash (5 mL). Trypsin (1 mL) was added and cells incubated for 5 min. The trypsin was neutralised with media (2 mL) and this was combined with the supernatant and a further PBS wash (5 mL). The cell suspension was centrifuged (1000 rpm, 6 min), the supernatant was removed, and the cell pellet was resuspended in PBS (5 mL). This was centrifuged (1000 rpm, 6 min), and the supernatant was removed. The cell pellet was resuspended in PBS (0.5 mL), and this suspension was carefully added to ice cold 70% ethanol solution (4.5 mL). The cells were fixed for a minimum of 2 h, before centrifuging (1000 rpm, 5 min). The supernatant was removed and the cells resuspended in PBS (5 mL). The cells were washed *via* two centrifuging and resuspension cycles, and were finally resuspended in 1 mL of a solution of DNase-free RNase A (20 μ g/mL) and propidium iodide (20 μ g/mL) in 0.1% (v/v) Triton X-100 in PBS. Cells were incubated at rt for 30 min in the dark. Cell fluorescence was determined using a FACSCalibur (BDBiosciences), gating for mononuclear cells. Data was exported and analysed using FlowJo (v9.0.2 for Mac, Tree Star, Inc., Oregon, USA, www.flowjo.com).

Cell Fixation and Antibody Staining for Confocal Microscopy

Cells subcultured (SK-OV-3TR cells grown without paclitaxel for at least 16 h) onto a plate containing Thermanox coverslips coated with sodium metasilicate and sodium hexasilicate. When the cells were approximately 50% confluent, the coverslips were removed and placed into the inner 8 wells of a 24-well plate containing medium (450 μ L), with the outer wells containing PBS (500 μ L) to maintain plate humidity. Drug solution in medium (50 μ L, 10 \times concentrations to give appropriate 1 \times plate concentrations) was then added along with a blank (50 μ L of medium). Plates were incubated overnight (16 h). The media was then aspirated, washed with PBS (500 μ L per well) followed by fixation in freshly diluted 3% formaldehyde solution in PBS (500 μ L) followed by incubation at 37 °C for 10 min. After aspiration cells were permeabilised with PBS-T (0.1% Triton in PBS, 500 μ L) for 5 min, and then incubated at 37 °C with blocking solution (10% bovine serum albumin (BSA) in PBS (500 μ L)) for 5 min. This was removed and Dm1a (1 in 1000 in blocking solution, 500 μ L) was added and incubated at 37 °C for 2 h. The primary antibody solution was removed and stored at –80 °C for re-use. Cells were then washed 3 times (5 min at 37 °C) with PBS-T (500 μ L). The appropriate Alexa Fluor[®] 488-coupled secondary antibody was then added as

a solution in BSA in PBS (1 in 1000 in blocking solution, 500 μL) and the plate returned to the incubator for a further 2 h ensuring minimal light exposure. Cells washed 3 times (5 min at 37 $^{\circ}\text{C}$) with PBS-T (500 μL), with a final wash in water (500 μL). The coverslips inverted onto microscope slides with mounting medium containing Hoescht stain (30 μL). The slides were allowed to dry at rt overnight and then stored at 4 $^{\circ}\text{C}$ until they were viewed using a Nikon Eclipse TE200-S confocal microscope.

Live Microscopy on GFP-tubulin HeLa Cells

HeLa cells expressing GFP-tubulin were seeded into a 35 mm plate containing a glass lens in the base with G418 (400 $\mu\text{g}/\text{mL}$). At approximately 40% confluence, the media was removed and replaced with pre-warmed Leibovitz's medium (low CO_2 , no phenol red) and incubated for 30 min. The plate was transferred to a preheated chamber attached to a Nikon Eclipse TE200-S microscope fitted with spinning disk sample protection.

Investigation of low drug concentrations (10 nM) and blank control was performed under 491 nm laser irradiation. A Z-stack was obtained with 1 μm spacing on 10 fields every 10 min over 3 h, with a brightfield image obtained every 10 timepoints. Gain was set at 1 and sensitivity set to maximum for both laser and brightfield.

Investigation of the higher drug concentrations (1 μM and 100 nM) was performed under the same conditions, except for the Z-stack timings. These were obtained with 1 μm spacing on 10 fields every 4 min over 1 h, with a brightfield image obtained every 10 timepoints. Data was processed using Volocity (v4.4.0, PerkinElmer, Massachusetts, USA, www.improvision.com).

References

1. L. Emmanuvel, R. K. Shukla, A. Sudalai, S. Gurunath and S. Sivaram, *Tetrahedron Lett.*, 2006, **47**, 4793-4796.
2. E. J. de Jong, J. A. Field, H.-E. Spinnler, J. B. P. A. Wijnberg and J. A. M. de Bont, *Appl. Environ. Microbiol.*, 1994, **60**, 264-270.
3. S. M. Johnson, S. Connelly, I. A. Wilson and J. W. Kelly, *J. Med. Chem.*, 2008, **51**, 6348-6358.
4. G. R. Pettit, S. B. Singh, G. M. Cragg, *J. Org. Chem.*, 1985, **50**, 3404-3406.
5. S. B. Singh, G. R. Pettit, *J. Org. Chem.*, 1989, **54**, 4105-4114.
6. G.R. Pettit, S.B. Singh, M.R. Boyd, E. Hamel, R.K. Pettit, J.M. Schmidt, F. Hogan, *J. Med. Chem.*, 1995, **38**, 1666-1672.
7. *US Pat.*, 7 507 851, 2009.
8. Y. Kong, J. Grembecka, M. C. Edler, E. Hamel, S. L. Mooberry, M. Sabat, J. Rieger, M. L. Brown, *Chem. & Biol.*, 2005, **12**, 1007-1014.
9. A. A. Ahmed, A. D. Mills, A. E. K. Ibrahim, J. Temple, C. Blenkiron, M. Vias, C. E. Massie, N. G. Iyer, A. McGeoch, R. Crawford, B. Nicke, J. Downward, C. Swanton, S. D. Bell, H. M. Earl, R. A. Laskey, C. Caldas, J. Brenton, *Cancer Cell*, 2007, **12**, 514-527.
10. E.A. Jaffe, R.L. Nachman, C.G. Becker, C.R. Minick, *J. Clin. Invest.*, 1973, **52**, 2745-2756.

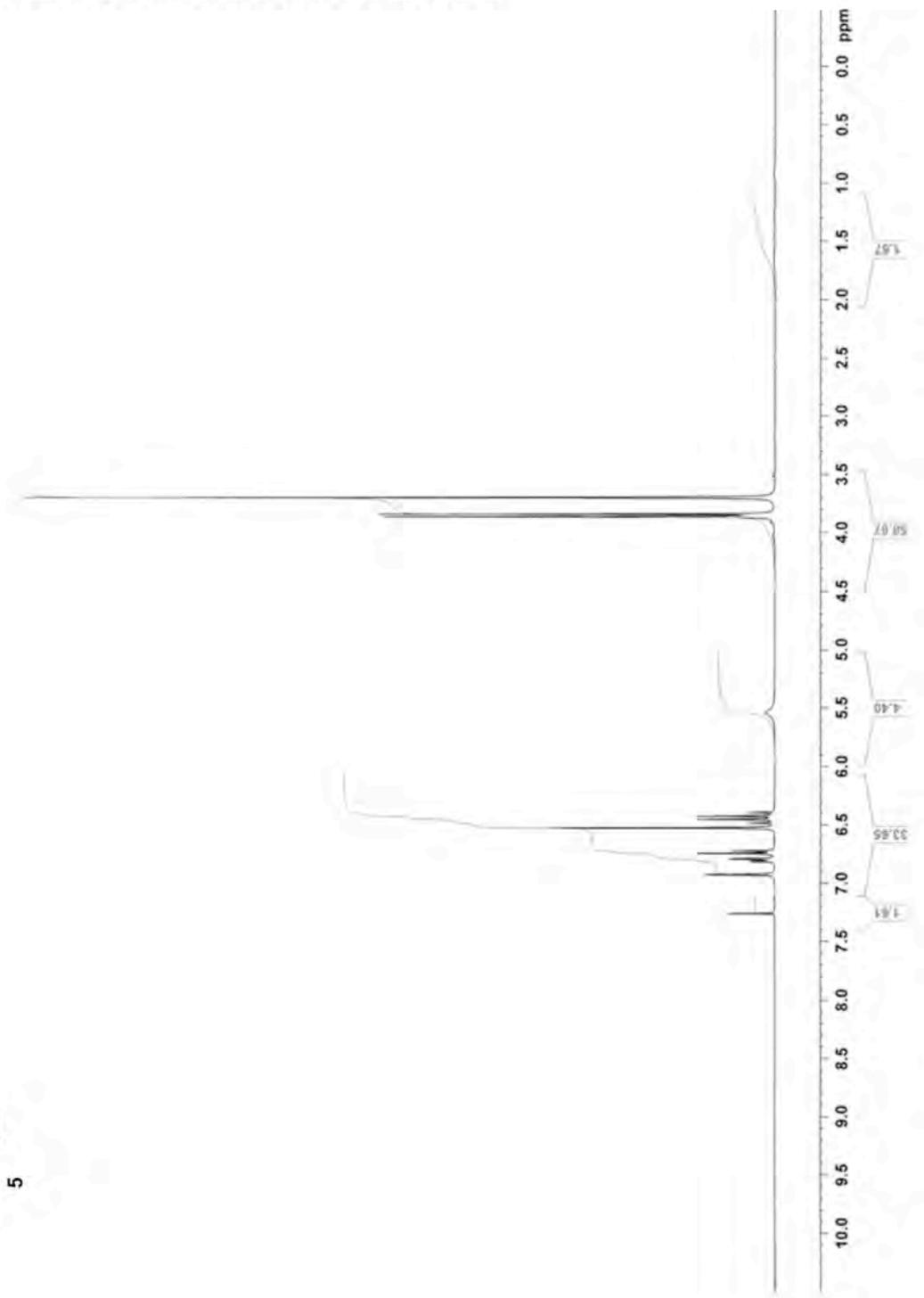
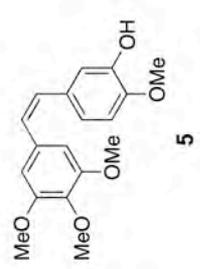


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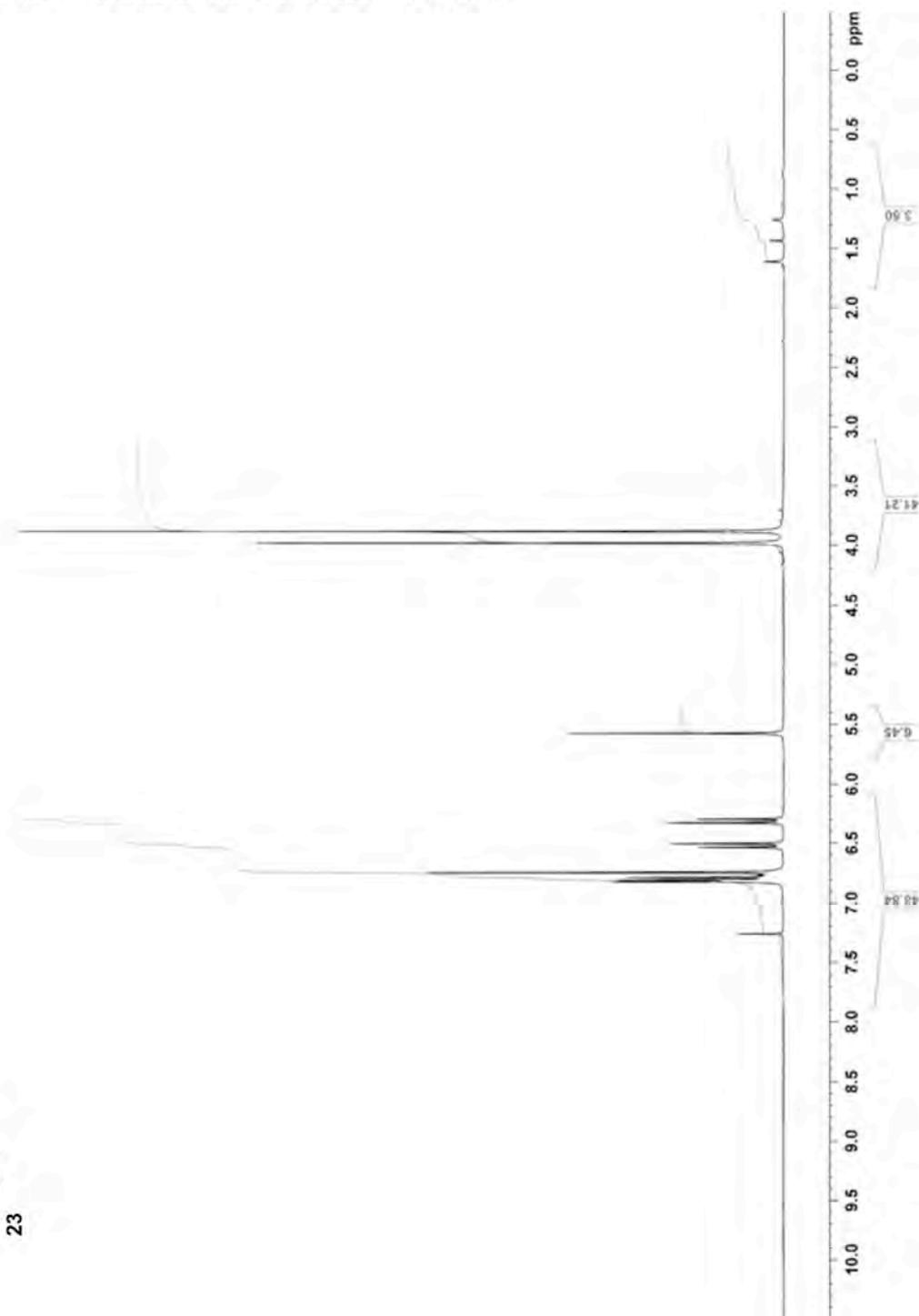
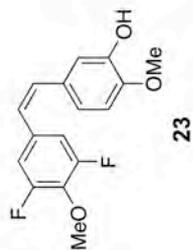


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RG 64
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D1 1.0000000 sec
TD0 1

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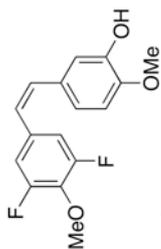
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13C NMR

13C NMR

13C NMR

13C NMR



23



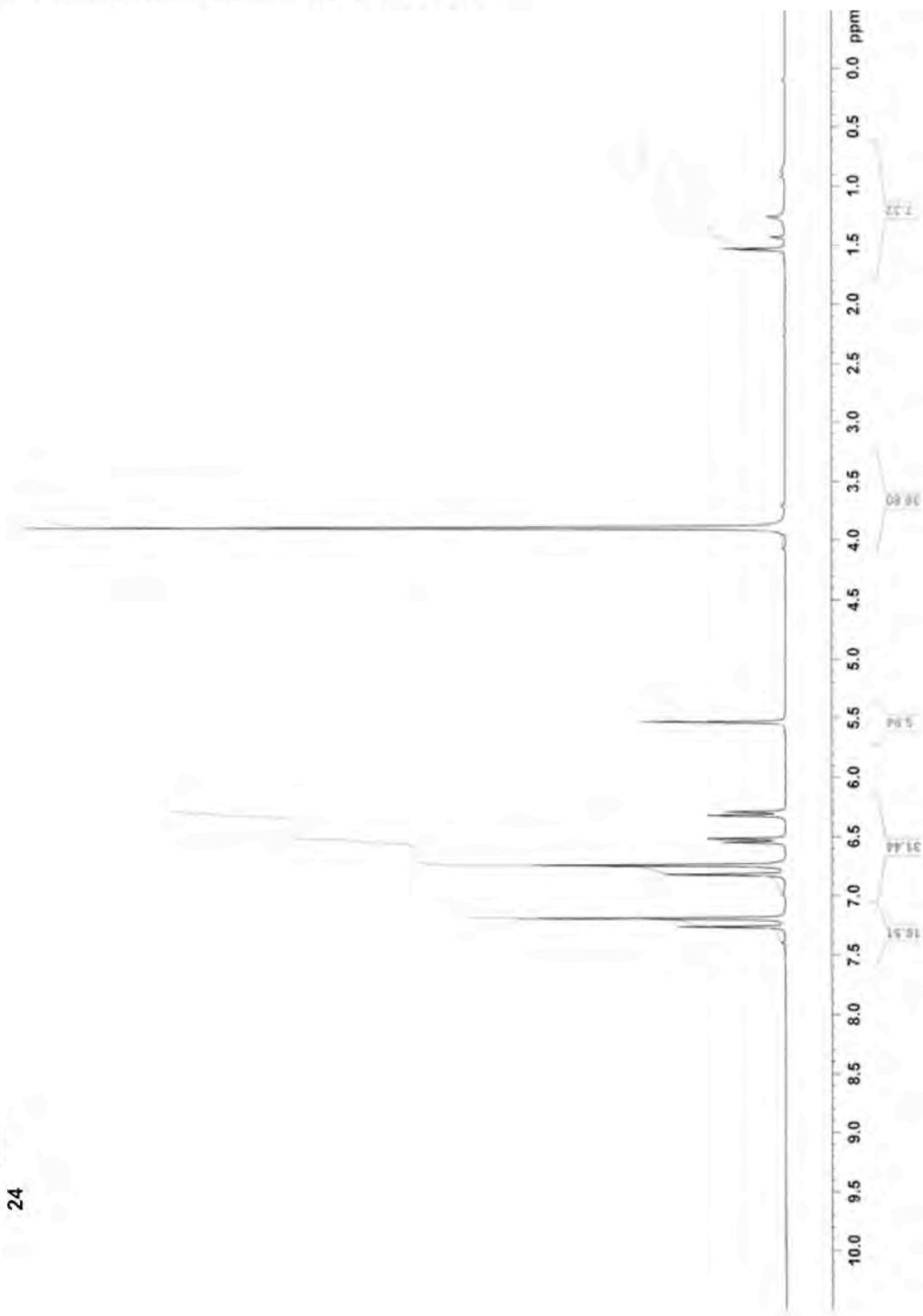
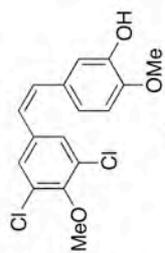


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SOLVENT CDCl3
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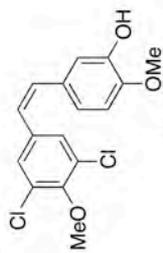
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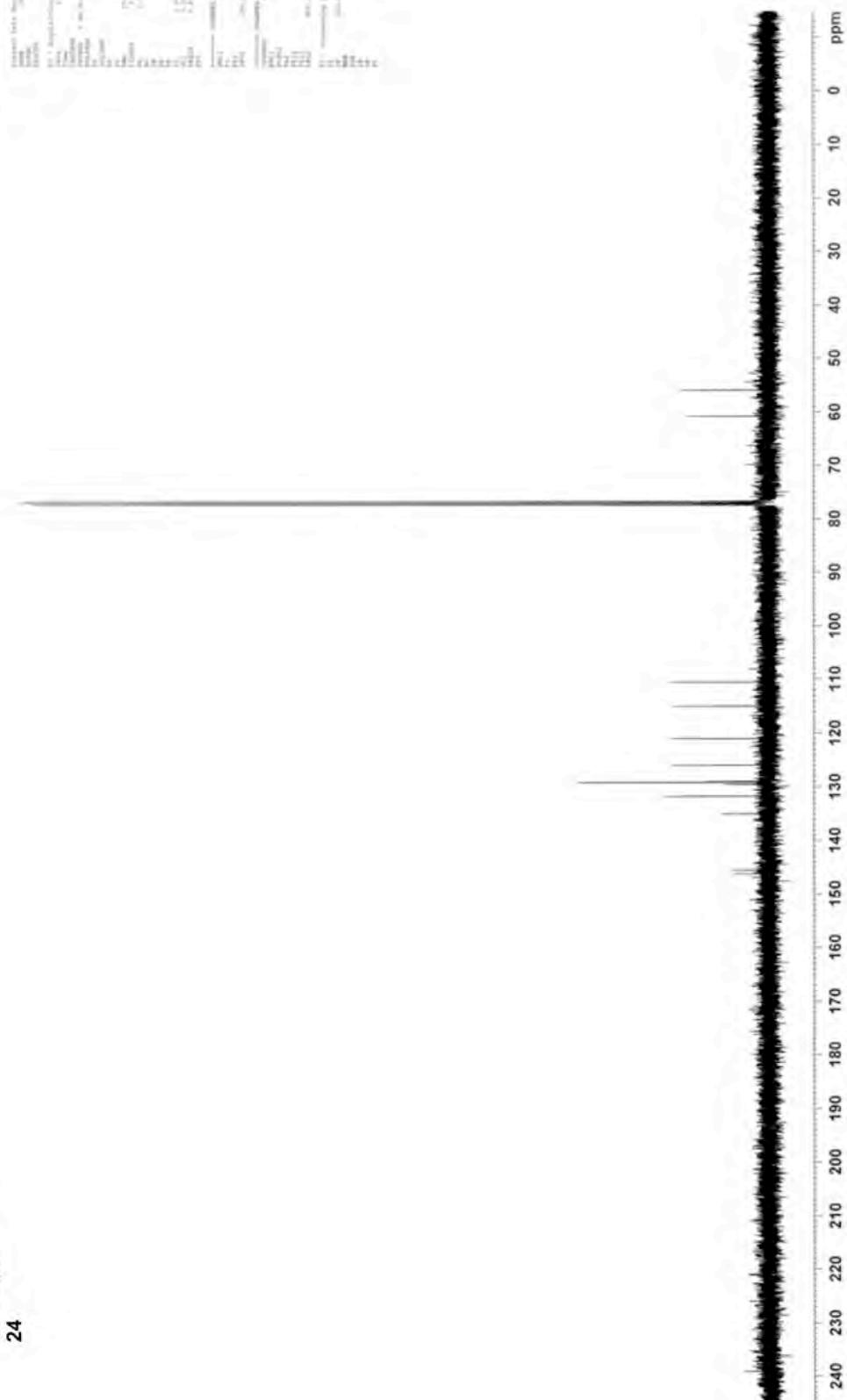




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24



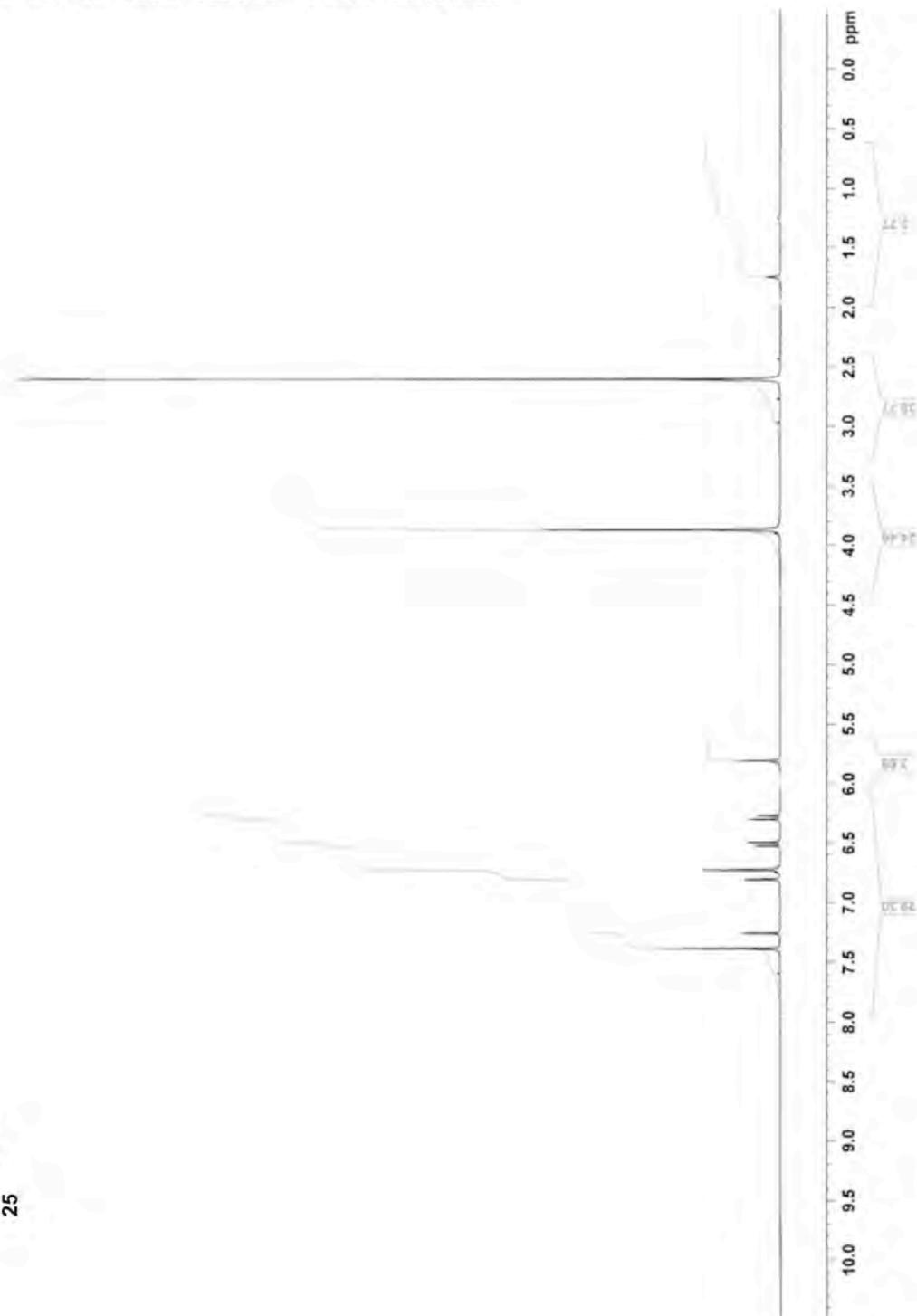
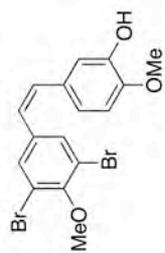


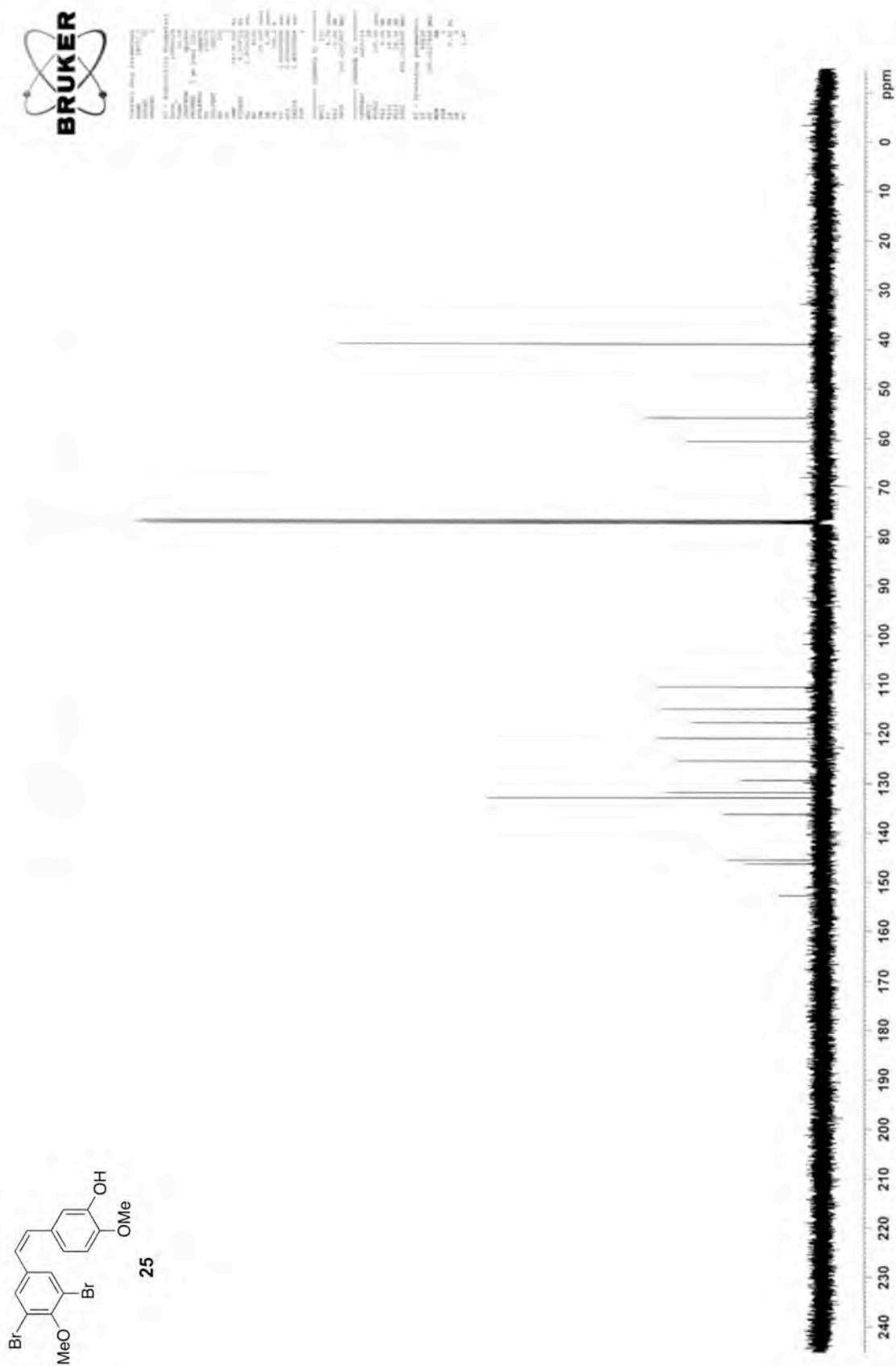
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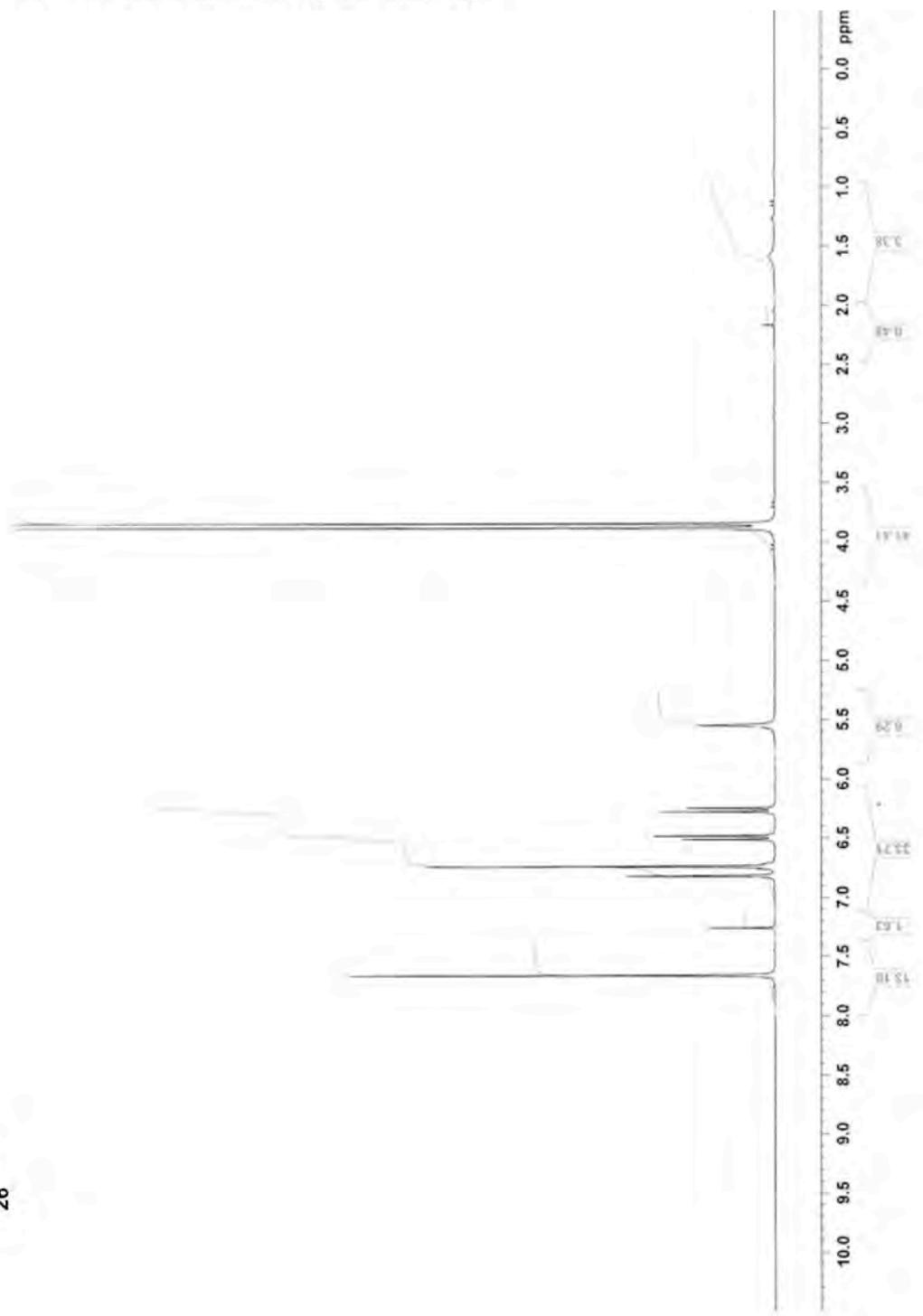
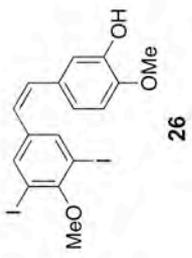


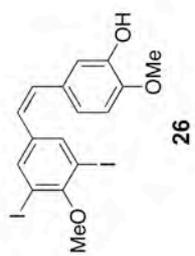
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F2 - Processing parameters
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