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Chemistry & Drug Discovery

Supporting Information

Cis-Stilbene Derived Furopyranones Show Potent Antiproliferative Activity by Inducing G2/M Arrest

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Contents:

Synthesis and Characterization of Compounds

General. Chemicals, solvents, and reagents for reactions were from Acros, Aldrich, or Fluka, and were of the highest quality available. Solvents for extraction and chromatography were of technical grade and distilled prior to use. Thin layer chromatography (TLC): silica-gel 60 F_{254} glass plates (Merck); visualisation by UV and/or by dipping in a solution of anisaldehyde (2%) and conc. H_2SO_4 (3%) in EtOH followed by heating. Flash column chromatography (CC): Silica 60 A C.C 40-63 µm (SDS, France) at low pressure. Melting points were determined in open capillaries using a *Büchi Melting Point B-545* apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR: Bruker AVANCE 300, d values in ppm (solvent signals as internal standards), J [Hz]. Infrared spectra were recorded on an *OMNILAB Jasco FT/IR-460 Plus* spectrophotometer with a *Specac MK II Golden Gate™ Single Reflection ATR System*. EI-MS / EI-MSHR: Micromass Autospec Q (*Waters / Micromass*), Ionization mode: electron impact, Ionization energy: 70 eV, Sample inlet: solids probe, Acceleration voltage: 8 kV, Mass resolving power: >1000 (10% valley), Calibration: External calibration using perfluorokerosene (PFK). The mass accuracy is on the order of ± 2 ppm. ESI-MSHR: Applied Biosystems / Sciex QSTAR Pulsar (hybrid quadrupole time-of-flight mass spectrometer), Ion source: nanoelectrospray, Injection: glass needle; sample volume: 1-4 µl; flow rate: 10-30 nl/min, Needle potential: 700 to 900 V (both polarities), Curtain gas: nitrogen, External calibration with caesium iodide and reserpine (positive ion mode), mass accuracy is better than ± 5 ppm. HPLC: Bio-Tek Kontron Instruments, HPLC 545V Diode Array Detector, System 525; HPLC-Cartridge from MERCK (Germany), LiChrospher® Si 60 (10 µm), LiChroCART® 250-10; Software: Galaxie Chromatography Data System, Version 1.7.4.5, from Varran; Gradient: Ethyl acetate / hexane (40:60 ? 100:0); UV detection: ?=255-320 nm; all compounds had a retention time in between 13 and 18 min.

General method A (esterification). The corresponding carboxylic acids (1.5 eq.) were dissolved in 1,2-dichloroethane under an argon atmosphere. The reaction mixture was cooled to 0°C and triethylamine (1.6 eq.) was added followed by dropwise addition of pivaloyl chloride (1.5 eq.). The solution was stirred for 30 minutes. Cinnamyl alcohol (1 eq.) or 3-methyl-2-buten-1-ol (1 eq.), dissolved in 1,2-dichloroethane, was added slowly to the stirred solution followed by 4-

dimethylaminopyridine (0.2 eq.). The ice bath was removed and the reaction mixture was allowed to stir for 1 - 2.5 hours. After addition of cold sat. $NaHCO_3$-solution, the reaction mixture was extracted with diethyl ether. The organic phase was dried (Na_2SO_4) and filtered. After concentration, the crude product was purified by flash chromatography and dried under high vacuum.

General method B (*hetero*-Diels-Alder reaction). The corresponding *a,ß*-unsaturated *?*-ketoeseters were dissolved in *o*-xylene and refluxed for the indicated time. The solution was poured on saturated aqueous $NaHCO_3$-solution, extracted with ethyl acetate and washed with brine. The organic phase was dried (Na_2SO_4) and filtered. After concentration, the crude product was purified by flash chromatography and dried under high vacuum. Where possible, the obtained compounds were further recrystallised from the indicated solvents. The ratio of the *cis*/*trans*-isomers was determined by ^{1}H-NMR.

5,6-Bis-(4-fluoro-phenyl)-3-hydroxymethyl-2-phenyl-3,4-dihydro-2*H*-pyran-4-carboxylic acid benzylamide (5a). 2-Hydroxypyridine (28.5 mg, 0.30 mmol) and benzylamine (0.16 ml, 1.50 mmol) were added to a suspension of **3d** (60 mg, 0.15 mmol) in toluene (2.5 ml). The mixture was stirred and refluxed over night. At room temperature the yellow solution was poured onto a saturated NH_4Cl-solution and extracted with ethyl acetate. The combined organic phases were dried (Na_2SO_4) and concentrated. Flash chromatography (ethyl acetate/hexane, 2:3) gave **5a** (36 mg, 47%) as a white solid. For X-ray analysis the compound was recrystallised in MeOH. Mp (MeOH): 207°C. TLC (ethyl acetate/hexane, 2:3): R_f 0.24. ^{1}H-NMR (300 MHz, DMSO): d 2.42 (m, 1H), 3.02 (m, 1H), 3.17 (m, 1H), 3.89 (d, *J* = 5.46 Hz, 1H), 3.95 (dd, *J* = 5.46 Hz, 15.64 Hz, 1H), 4.36 (dd, *J* = 6.78 Hz, 15.64 Hz, 1H), 4.47 (t, *J* = 4.62 Hz, 1H), 5.54 (d, *J* = 10.36 Hz, 1H), 6.72-6.75 (m, 2H), 6.94-7.18 (m, 11H), 7.33-7.43 (m, 5H), 8.34 (t, *J* = 5.75 Hz, 1H). EI-MS *m/z* (%): 511 ($[C_{32}H_{27}F_2NO_3]^+$, 15), 493 (46), 123 (96), 117 (100), 91 (97).EI-MSHR: Calc. mass ($C_{32}H_{27}F_2NO_3$) = 511.195901, found: 511.195280.

5,6-Bis-(4-fluoro-phenyl)-3-hydroxymethyl-2-phenyl-3,4-dihydro-2*H*-pyran-4-carboxylic acid butylamide (5b). 2-Hydroxypyridine (18.3 mg, 0.19 mmol) and *n*-butylamine (0.10 ml, 0.96 mmol) were added to a suspension of **3d** (39 mg, 0.10

mmol) in toluene (1.5 ml). The mixture was stirred and refluxed over night. At room temperature the yellow solution was poured onto a saturated NH_4Cl-solution and extracted with ethyl acetate. The combined organic phases were dried (Na_2SO_4) and concentrated. Flash chromatography (ethyl acetate/hexane, 2:3) gave **5b** (36 mg, 47%) as a white solid. TLC (ethyl acetate/hexane, 2:3): R_f 0.22. ^{1}H-NMR (300 MHz, DMSO): d 0.68 (t, *J* = 7.25 Hz, 3H), 0.84-0.97 (m, 2H), 1.04-1.13 (m, 2H), 2.39 (m, 1H), 2.77 (m, 1H), 2.95-3.18 (m, 3H), 3.74 (d, *J* = 5.46 Hz, 1H), 4.41 (t, *J* = 4.71 Hz, 1H), 5.51 (d, *J* = 10.36 Hz, 1H), 6.93-7.02 (m, 4H), 7.04-7.17 (m, 4H), 7.33-7.42 (m, 5H), 7.72 (t, *J* = 5.93 Hz, 1H). EI-MS *m/z* (%): 477 ($[C_{29}H_{29}F_2NO_3]^+$, 18), 459 (74), 123 (100), 117 (100), 95 (57), 91 (52). EI-MSHR: Calc. mass ($C_{29}H_{29}F_2NO_3$) = 477.211551, found: 477.211360.

5,6-Bis-(4-fluoro-phenyl)-3-hydroxymethyl-2-phenyl-3,4-dihydro-2H-pyran-4-carboxylic acid isobutylamide (5c). 2-Hydroxypyridine (50 mg, 0.53 mmol) and isobutylamine (0.25 ml, 2.5 mmol) were added to a suspension of **3d** (102 mg, 0.25 mmol) in toluene (3 ml). The mixture was stirred and refluxed for 3 h. At room temperature the yellow solution was poured onto a saturated NH_4Cl-solution and extracted with ethyl acetate. The combined organic phases were dried (Na_2SO_4) and concentrated. Flash chromatography (ethyl acetate/hexane, 2:3) gave **5c** (60 mg, 52%) as a white foam. TLC (ethyl acetate/hexane, 2:3): R_f 0.21. ^{1}H-NMR (300 MHz, $CDCl_3$): d 7.40-7.33 (m, 5H), 7.23-7.16 (m, 2H), 7.10-7.03 (m, 2H), 6.93-6.78 (m, 4H), 6.10 (t, *J* = 6.03 Hz, 1H), 5.08 (d, *J* = 10.74 Hz, 1H), 3.88 (d, *J* = 5.84 Hz, 1H), 3.38-3.24 (m, 2H), 3.11-2.97 (m, 3H), 2.65 (m, 1H), 1.60 (m, 1H), 0.75 (d, *J* = 6.78 Hz, 3H), 0.70 (d, *J* = 6.78 Hz, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$): 172.1, 162.6 (d, 1C, $^1J_{CF}$=249.00 Hz), 161.6 (d, 1C, $^1J_{CF}$=247.14 Hz), 152.2, 138.4, 135.5 (d, 1C, $^4J_{CF}$=3.12 Hz), 131.8 (d, 2C, $^3J_{CF}$=8.11 Hz), 131.3 (d, 2C, $^3J_{CF}$= 7.49Hz), 128.9, 128.8, 127.3, 115.7 (d, 2C, $^2J_{CF}$= 21.22 Hz), 114.9 (d, 2C, $^2J_{CF}$= 21.85 Hz), 107.6, 77.7, 61.4, 47.8, 47.3, 44.7, 29.8, 28.6, 20.0, 19.9. EI-MS m/z (%): 477 ($[C_{29}H_{29}F_2NO_3]^+$, 33), 459 (57), 269 (40), 123 (95), 117 (100), 105 (67), 91 (60), 57 (29).

5,6-Bis-(4-fluoro-phenyl)-3-hydroxymethyl-2-phenyl-3,4-dihydro-2H-pyran-4-carboxylic acid propylamide (5d). 2-Hydroxypyridine (50 mg, 0.53 mmol) and propylamine (0.21 ml, 2.5 mmol) were added to a suspension of **3d** (99 mg, 0.25

mmol) in toluene (3 ml). The mixture was stirred and refluxed for 5 h. At room temperature the yellow solution was poured onto a saturated NH_4Cl-solution and extracted with ethyl acetate. The combined organic phases were dried (Na_2SO_4) and concentrated. Flash chromatography (ethyl acetate/hexane, 2:3) gave **5d** (98 mg, 84%) as a white foam. TLC (ethyl acetate/hexane, 2:3): R_f 0.30. ^{1}H-NMR (300 MHz, DMSO): d 7.74 (t, *J* = 5.75 Hz, 1H), 7.43-7.33 (m, 5H), 7.15-7.05 (m, 4H), 7.01-6.93 (m, 4H), 5.50 (d, *J* = 10.36 Hz, 1H), 4.43 (t, *J* = 4.71 Hz, 1H), 3.74 (d, *J* = 5.46 Hz, 1H), 3.18-3.10 (m, 1H), 3.01-2.90 (m, 2H), 2.83-2.72 (m, 1H), 2.45-2.34 (m, 1H), 1.14 (m, 2H), 0.56 (t, *J* = 7.44 Hz, 3H). ^{13}C-NMR (75 MHz, DMSO): 170.9, 161.4 (d, 1C, $^1J_{CF}$=245.89 Hz), 160.65 (d, 1C, $^1J_{CF}$=242.77 Hz), 149.4, 139.6, 135.9 (d, 1C, $^4J_{CF}$=3.12 Hz), 132.3 (d, 1C, $^4J_{CF}$=3.12 Hz), 131.9 (d, 2C, $^3J_{CF}$=7.49 Hz), 131.4 (d, 2C, $^3J_{CF}$= 8.11 Hz), 128.4, 128.2, 127.3, 114.6 (d, 2C, $^2J_{CF}$= 21.22 Hz), 114.4 (d, 2C, $^2J_{CF}$= 21.84 Hz), 109.8, 76.5, 59.5, 45.9, 44.3, 40.3, 22.0, 11.0. ESI-MS positive mode: *m/z* $[C_{28}H_{27}F_2NO_3 + H]^+$ = 464.4.

4-(4-Nitro-phenyl)-4-oxo-but-2-enoic acid 3-phenyl-allyl ester (8a). *General method A:* Cinnamyl alcohol (404 mg, 3.01 mmol), 4-(4-nitro-phenyl)-4-oxo-but-2-enoic acid (1.00 g, 4.52 mmol), triethylamine (0.65 ml, 4.67 mmol), pivaloyl chloride (0.54 ml, 4.40 mmol), DMAP (63 mg, 0.51 mmol), reaction time 2h. Flash chromatography (ethyl acetate/hexane, 1:4 ? 1:2 ? 1:0) gave **8a** (642 mg, 63%) as a orange amorphous solid. TLC (ethyl acetate/Hexane, 1:1): R_f 0.79. ^{1}H-NMR (300 MHz, $CDCl_3$): d 4.92 (d, *J* = 6.59 Hz, 2H), 6.34 (dt, *J* = 15.82 Hz, 6.59 Hz, 1H), 6.73 (d, *J* = 15.82 Hz, 1H), 6.98 (d, *J* = 15.64 Hz, 1H), 7.28 – 7.43 (m, 5H), 7.90 (d, *J* = 15.45 Hz, 1H), 8.15 (d, *J* = 9.04 Hz, 2H), 8.36 (d, *J* = 9.04 Hz, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$): d 66.44 (t), 122.33 (d), 124.30 (d, 2C), 126.88 (d, 2C), 128.56 (d), 128.88 (d, 2C), 130.02 (d, 2C), 134.15 (d), 135.50 (d), 135.68 (d), 136.08 (s), 141.18 (s), 150.85 (s), 165.05 (s), 188.27 (s).EI MS *m/z* (%): 337 ($[C_{19}H_{15}NO_5]^+$, 7), 205 (40), 150 (37), 133 (100), 117 (63), 115 (82), 105 (69), 91 (39), 76 (37). IR (cm^{-1}): 1708, 1668 (C=O), 1523 (N=O).

4-(3-Nitro-phenyl)-4-oxo-but-2-enoic acid 3-phenyl-allyl ester (8b). *General method A:* Cinnamyl alcohol (202 mg, 1.51 mmol), 4-(3-nitro-phenyl)-4-oxo-but-2-enoic acid (500 mg, 2.26 mmol), triethylamine (0.33 ml, 2.34 mmol), pivaloyl chloride (0.27 ml, 2.20 mmol), DMAP (31 mg, 0.26 mmol) reaction time 1.5 h. Flash

chromatography (ethyl acetate/hexane, 1:2) gave **8b** (339 mg, 67%) as a yellow oil. TLC (ethyl acetate/hexane, 1:2): R_f 0.50. ^{1}H-NMR (300 MHz, $CDCl_3$): d 4.93 (d, *J* = 6.59 Hz, 2H), 6.35 (dt, *J* = 15.82 Hz, 6.59 Hz, 1H), 6.74 (d, *J* = 15.82 Hz, 1H), 7.01 (d, *J* = 15.45 Hz, 1H), 7.28 – 7.44 (m, 5H), 7.74 (t, *J* = 8.01 Hz, 1H), 7.94 (d, *J* = 15.64 Hz, 1H), 8.33 (d, *J* = 7.96 Hz, 1H), 8.48 (d, *J* = 8.29 Hz, 1H), 8.82 (s, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): d 66.42 (t), 122.38 (d), 123.81 (d), 126.89 (d, 2C), 128.20 (d), 128.53 (d), 128.86 (d, 2C), 130.43 (d), 134.18 (d), 134.41 (d), 135.35 (d), 135.46 (d), 136.11 (s), 138.00 (s), 148.79 (s), 165.05 (s), 187.51 (s). EI MS *m/z* (%): 337 ($[C_{19}H_{15}NO_5]^+$, 7), 205 (44), 150 (42), 133 (100), 117 (70), 115 (83), 105 (65), 91 (43), 76 (47). IR (cm^{-1}): 1723, 1674 (C=O), 1529 (N=O).

4-Oxo-4-phenyl-but-2-enoic acid 3-phenyl-allyl ester (8c). *General method A:* Cinnamyl alcohol (508 mg, 3.78 mmol), 3-benzoylacrylic acid (1.00 g, 5.67 mmol), triethylamine (0.82 ml, 5.87 mmol), pivaloyl chloride (0.68 ml, 5.52 mmol), DMAP (79 mg, 0.64 mmol), reaction time 2 h. Flash chromatography (ethyl acetate/hexane, 1:6) gave **8c** (883 mg, 80%) as a orange oil. TLC (ethyl acetate/hexane, 1:2): R_f 0.69. ^{1}H-NMR (300 MHz, $CDCl_3$): d 4.91 (d, *J* = 6.50 Hz, 2H), 6.34 (dt, *J* = 15.89 Hz, 6.50 Hz, 1H), 6.73 (d, *J* = 16.01 Hz, 1H), 6.93 (d, *J* = 15.64 Hz, 1H), 7.28 – 7.44 (m, 5H), 7.52 (t, *J* = 7.44 Hz, 2H), 7.63 (t, *J* = 7.35 Hz, 1H), 7.95 (d, *J* = 15.64 Hz, 1H), 8.01 (d, *J* = 8.38 Hz, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$): d 66.14 (t), 122.63 (d), 126.87 (d, 2C), 128.44 (d, 2C), 128.84 (d), 129.07 (d, 2C), 129.08 (d, 2C), 132.45 (d), 134.06 (d), 135.13 (d), 136.19 (s), 136.76 (s), 136.95 (d), 165.54 (s), 189.65 (s). EI MS *m/z* (%): 292 ($[C_{19}H_{16}O_3]^+$, 79), 160 (100), 131 (54), 115 (61), 105 (97), 77 (64). IR (cm^{-1}): 1709, 1672 (C=O).

4-(4-Nitro-phenyl)-4-oxo-but-2-enoic acid 3-methyl-but-2-enyl ester (8d). *General method A:* 3-Methyl-2-buten-1-ol (0.30 ml, 3.01 mmol), 4-(4-nitro-phenyl)-4-oxo-but-2-enoic acid (1.00 g, 4.52 mmol), triethylamine (0.65 ml, 4.67 mmol), pivaloyl chloride (0.54 ml, 4.40 mmol), DMAP (63 mg, 0.51 mmol), reaction time 1 h. Flash chromatography (ethyl acetate/hexane, 1:4 ? 1:3) gave **8d** (509 mg, 58%) as a yellow amorphous solid. TLC (ethyl acetate/hexane, 1:2): R_f 0.71. ^{1}H-NMR (300 MHz, $CDCl_3$): d 1.76 (s, 3H), 1.79 (s, 3H), 4.75 (d, *J* = 7.35 Hz, 2H), 5.41 (t, *J* = 7.35 Hz, 1H), 6.93 (d, *J* = 15.64 Hz, 1H), 7.85 (d, *J* = 15.64 Hz, 1H), 8.14 (d, *J* = 8.85 Hz, 2H), 8.36 (d, *J* = 9.04 Hz, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$): d 18.27 (q), 25.98 (q), 62.71 (t),

118.00 (d), 124.26 (d, 2C), 129.99 (d, 2C), 134.51 (d), 135.38 (d), 140.48 (s), 141.26 (s), 150.82 (s), 165.28 (s), 188.39 (s). EI MS *m/z* (%): 289 ($[C_{15}H_{15}NO_5]^+$, 2), 205 (78), 150 (66), 104 (61), 85 (100), 76 (67), 69 (81), 53 (57), 41 (90). IR (cm^{-1}): 1721, 1665 (C=O), 1525 (N=O).

4-(3-Nitro-phenyl)-4-oxo-but-2-enoic acid 3-methyl-but-2-enyl ester (8e). *General method A:* 3-Methyl-2-buten-1-ol (0.12 ml, 1.21 mmol), 4-(3-nitro-phenyl)-4-oxo-but-2-enoic acid (400 mg, 1.81 mmol), triethylamine (0.26 ml, 1.87 mmol), pivaloyl chloride (0.22 ml, 1.76 mmol), DMAP (25 mg, 0.20 mmol), reaction time 2 h. Flash chromatography (ethyl acetate/hexane, 1:2) gave **8e** (241 mg, 69%) as a yellow oil. TLC (ethyl acetate/hexane, 1:2): R_f 0.60. ^{1}H-NMR (300 MHz, $CDCl_3$): d 1.76 (s, 3H), 1.79 (s, 3H), 4.76 (d, *J* = 7.35 Hz, 2H), 5.41 (t, *J* = 7.35 Hz, 1H), 6.96 (d, *J* = 15.64 Hz, 1H), 7.74 (t, *J* = 8.01 Hz, 1H), 7.89 (d, *J* = 15.45 Hz, 1H), 8.32 (d, *J* = 8.01 Hz, 1H), 8.48 (d, *J* = 8.20 Hz, 1H), 8.81 (s, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): d 18.28 (q), 25.98 (q), 62.71 (t), 118.02 (d), 123.80 (d), 128.14 (d), 130.40 (d), 134.40 (d), 134.55 (d), 135.02 (d), 138.06 (s), 140.45 (s), 148.79 (s), 165.28 (s), 187.63 (s). EI MS *m/z* (%): 289 ($[C_{15}H_{15}NO_5]^+$, 2), 205 (78), 150 (81), 104 (58), 85 (100), 76 (78), 69 (82), 53 (61), 41 (91). IR (cm^{-1}): 1712, 1674 (C=O), 1532 (N=O).

4-Oxo-4-phenyl-but-2-enoic acid 3-methyl-but-2-enyl ester (8f). *General method A:* 3-Methyl-2-buten-1-ol (0.38 ml, 3.78 mmol), 3-benzoylacrylic acid (1.00 g, 5.67 mmol), triethylamine (0.82 ml, 5.87 mmol), pivaloyl chloride (0.68 ml, 5.52 mmol), DMAP (79 mg, 0.64 mmol), reaction time 1.5 h Flash chromatography (ethyl acetate/hexane, 1:7) gave **8f** (849 mg, 92%) as a orange oil. TLC (ethyl acetate/hexane, 1:2): R_f 0.67. ^{1}H-NMR (300 MHz, $CDCl_3$): d 1.75 (s, 3H), 1.79 (s, 3H), 4.74 (d, *J* = 7.35 Hz, 2H), 5.41 (t, *J* = 7.35 Hz, 1H), 6.88 (d, *J* = 15.64 Hz, 1H), 7.51 (t, *J* = 7.63 Hz, 2H), 7.62 (t, *J* = 7.35 Hz, 1H), 7.90 (d, *J* = 15.45 Hz, 1H), 7.99 (d, *J* = 6.97 Hz, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$): d 189.77 (s), 165.79 (s), 140.14 (s), 136.81 (s), 136.61 (d), 133.99 (d), 132.80 (d), 129.04 (d, 4C), 118.20 (d), 62.43 (t), 25.97 (q), 18.27 (q). EI MS *m/z* (%): 244 ($[C_{15}H_{16}O_3]^+$, 4), 160 (69), 105 (36), 68 (45), 44 (100), 41 (38). IR (cm^{-1}): 1718, 1671 (C=O).

6-(4-Nitro-phenyl)-4-phenyl-3a,7a-dihydro-3*H*,4*H*-furo[3,4-*c*]pyran-1-one (9a). *General Method B:* Ester **8a** (600 mg, 1.78 mmol) was treated according to the

general method in *o*-xylene (30 ml) for 22 h. Flash chromatography ($CHCl_3$/Et_2O/hexane 1:1:1) yielded **9a** (330 mg, 55%) as a yellow foam [ratio of *cis/trans* (92:8)]. Pure *cis*-**9a** (yield = 55%): TLC ($CHCl_3$/ethyl acetate/hexane, 1:1:1): R_f 0.30. ^{1}H-NMR (300 MHz, $CDCl_3$): d 2.95 (m, 1H), 3.52 (dd, *J* = 7.72 Hz, 5.09 Hz, 1H), 4.14 (dd, *J* = 10.17 Hz, 0.94 Hz, 1H), 4.35 (dd, *J* = 10.17 Hz, 6.41 Hz, 1H), 4.56 (d, *J* = 10.93 Hz, 1H), 6.02 (d, *J* = 4.90 Hz, 1H), 7.42 – 7.52 (m, 5H), 7.76 (d, *J* = 9.04 Hz, 2H), 8.17 (d, *J* = 9.04 Hz, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$): d 175.76 (s), 152.70 (s), 148.04 (s), 140.19 (s), 137.62 (s), 129.58 (d), 129.26 (d, 2C), 127.90 (d, 2C), 125.74 (d, 2C), 123.79 (d, 2C), 96.52 (d), 78.59 (d), 67.98 (t), 39.92 (d), 38.64 (d). EI MS *m/z* (%): 337 ($[C_{19}H_{15}NO_5]^+$, 15), 150 (79), 143 (100), 142 (57), 128 (57), 115 (65), 105 (46), 91 (49), 77 (39). IR (cm^{-1}): 1770 (C=O); 1514, 1342 (N=O). Mp (MeOH): 149°C. ESI-MSHR positive mode: Calc. mass m/z (*cis*-$C_{19}H_{16}NO_5$) = 338.1028, found: 338.1022. HPLC purity: 97.1%.

6-(3-Nitro-phenyl)-4-phenyl-3a,7a-dihydro-3*H*,4*H*-furo[3,4-*c*]pyran-1-one (9b). *General Method B:* Ester **8b** (149 mg, 0.44 mmol) was treated according to the general method in *o*-xylene (15 ml) for 24 h. Flash chromatography (ethyl acetate/hexane, 1:3 ? 1:2 ? 1:0) yielded **9b** (69 mg 46%) as a foam [ratio of *cis/trans* (42:58)]. Pure *cis*-**9b** (yield = 35%) was separated by flash chromatography ($CHCl_3$/ethyl acetate/hexane, 1:1:1): TLC (ethyl acetate/hexane, 1:2): R_f 0.30. ^{1}H-NMR (300 MHz, $CDCl_3$): d 2.95 (m, 1H), 3.51 (dd, *J* = 7.72 Hz, 4.90 Hz, 1H), 4.14 (dd, *J* = 10.17 Hz, 1.13 Hz, 1H), 4.35 (dd, *J* = 10.08 Hz, 6.31 Hz, 1H), 4.57 (d, *J* = 10.93 Hz, 1H), 5.97 (d, *J* = 4.90 Hz, 1H), 7.43 – 7.53 (m, 6H), 7.92 (d, *J* = 8.10 Hz, 1H), 8.17 (d, *J* = 8.23 Hz, 1H), 8.44 (s, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): d 175.91 (s), 152.55 (s), 148.60 (s), 137.63 (s), 136.07 (s), 130.85 (d), 129.55 (d), 129.49 (d), 129.27 (d, 2C), 127.92 (d, 2C), 123.70 (d), 120.12 (d), 94.99 (d), 78.61 (d), 68.00 (t), 39.03 (d), 38.50 (d). EI MS *m/z* (%): 337 ($[C_{19}H_{15}NO_5]^+$, 13), 150 (97), 143 (100), 142 (92), 133 (45), 128 (61), 115 (74), 105 (47), 104 (38), 91 (42), 76 (46). IR (cm^{-1}): 1770 (C=O); 1525, 1346 (N=O). Mp (MeOH): 126°C. ESI-MSHR positive mode: Calc. mass m/z (*cis*-$C_{19}H_{16}NO_5$) = 338.1028, found: 338.1018.

4,6-Diphenyl-3a,7a-dihydro-3*H*,4*H*-furo[3,4-*c*]pyran-1-one (9c). *General Method B:* Ester **8c** (785 mg, 2.69 mmol) was treated according to the general method in *o*-xylene (45 ml) for 46 h. Flash chromatography (toluene/Et_2O/hexane, 8:1:3) yielded

cis-**9c** and *trans*-**9c** (307 mg 39%) as a light yellow solid [ratio of *cis/trans* (37:63)]. Pure *cis*-**9c** (yield = 15%) was separated by flash chromatography (toluene/ethyl acetate/hexane, 8:1:3): TLC (*cis*, toluene/Et_2O/hexane, 8:1:3): R_f 0.30. ^{1}H-NMR (*cis*, 300 MHz, $CDCl_3$): d 2.91 (m, 1H), 3.45 (dd, *J* = 7.72 Hz, 4.78 Hz, 1H), 4.13 (dd, *J* = 10.11 Hz, 1.29 Hz, 1H), 4.32 (dd, *J* = 9.93 Hz, 6.25 Hz, 1H), 4.56 (d, *J* = 10.66 Hz, 1H), 5.81 (d, *J* = 4.78 Hz, 1H), 7.30 – 7.35 (m, 3H), 7.43 – 7.47 (m, 5H), 7.59 – 7.63 (m, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$): d 176.50 (s), 154.58 (s), 138.29 (s), 134.32 (s), 129.22 (d), 129.09 (d, 3C), 128.43 (d, 2C), 127.88 (d, 2C), 125.12 (d, 2C), 92.32 (d), 78.11 (d), 68.01 (t), 39.17 (d), 38.42 (d). EI MS *m/z* (%): 292 ($[C_{19}H_{16}O_3]^+$, 13), 244 (30), 115 (31), 105 (100), 77 (67). IR (cm^{-1}): 1761 (C=O). Mp (*cis*, MeOH): 147°C. EI-MSHR: Calc. mass (*cis*-$C_{19}H_{16}O_3$) = 292.109945, found: 292.109950. HPLC purity (*cis*-**9c**): 96.0%. Pure *trans*-**9c** (yield = 11%) was separated by flash chromatography ($CHCl_3$/ethyl acetate/hexane, 1:1:3): TLC (*trans*, $CHCl_3$/Et_2O/hexane, 1:1:3): R_f 0.22. ^{1}H-NMR (*trans*, 300 MHz, $CDCl_3$): d 2.84 (m, 1H), 3.41 (dd, *J* = 13.56 Hz, 2.26 Hz, 1H), 4.16 (m, 2H), 5.39 (d, *J* = 10.55 Hz, 1H), 5.81 (d, *J* = 2.26 Hz, 1H), 7.32 – 7.46 (m, 8H), 7.62 – 7.66 (m, 2H).

4,4-Dimethyl-6-(4-nitro-phenyl)-3a,7a-dihydro-3*H*,4*H*-furo[3,4-*c*]pyran-1-one (9d). *General Method B:* Ester **8d** (437 mg, 1.51 mmol) was treated according to the general method in *o*-xylene (25 ml) for 22 h. Flash chromatography ($CHCl_3$/Et_2O/hexane, 1:1:2) yielded **9d** (289 mg, 66%) as a yellow foam [ratio of *cis/trans* (36:64)]. Pure *cis*-**9d** (yield = 20%) was separated by flash chromatography (toluene/hexane/ethyl acetate, 8:2:1): TLC (toluene/hexane/Et_2O, 8:2:1): R_f (*cis*) 0.40. ^{1}H-NMR (*cis*, 300 MHz, $CDCl_3$): d 1.37 (s, 3H), 1.40 (s, 3H), 2.85 (q, *J* = 8.58 Hz, 1H), 3.37 (dd, *J* = 8.27 Hz, 3.49 Hz, 1H), 4.22 (t, *J* = 9.19 Hz, 1H), 4.46 (t, *J* = 8.64 Hz, 1H), 5.61 (d, *J* = 3.68 Hz, 1H), 7.73 (d, *J* = 9.19 Hz, 2H), 8.19 (d, *J* = 8.82 Hz, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$): d 175.84 (s), 150.01 (s), 147.97 (s), 141.00 (s), 125.73 (d, 2C), 123.74 (d, 2C), 94.26 (d), 74.39 (s), 68.11 (t), 43.12 (d), 37.68 (d), 25.25 (q), 24.61 (q). EI MS *m/z* (%): 289 ($[C_{15}H_{15}NO_5]^+$, 60), 245 (100), 230 (41), 216 (54), 202 (71), 150 (66), 120 (69), 95 (73), 76 (40), 44 (52), 41 (62). IR (cm^{-1}): 1760 (C=O); 1508, 1350 (N=O). Mp (*cis*, MeOH): 132°C. ESI-MSHR positive mode: Calc. mass m/z (*cis*-$C_{15}H_{16}NO_5$) = 290.1028, found: 290.1025. HPLC purity (*cis*-**9d**): 98.1%. Impure *trans*-**9c** [yield = 10% → ratio of *cis/trans* (34:66)]. TLC (toluene/hexane/Et_2O, 8:2:1): R_f (*trans*) 0.47. ^{1}H-NMR (*trans*, 300 MHz, $CDCl_3$): d

1.38 (s, 3H), 1.55 (s, 3H), 2.61 (m, 1H), 3.15 (dd, *J* = 14.13 Hz, 2.26 Hz, 1H), 4.05 (dd, *J* = 11.68 Hz, 8.29 Hz, 1H), 4.43 (dd, *J* = 8.29 Hz, 6.41 Hz, 1H), 5.82 (d, *J* = 2.26 Hz, 1H), 7.74 (d, *J* = 9.04 Hz, 2H), 8.19 (d, *J* = 9.04 Hz, 2H).

4,4-Dimethyl-6-(3-nitro-phenyl)-3a,7a-dihydro-3*H*,4*H*-furo[3,4-*c*]pyran-1-one (9e). *General Method B:* Ester **8e** (183 mg, 0.63 mmol) was treated according to the general method in *o*-xylene (18 ml) for 18 h. Flash chromatography (ethyl acetate/hexane, 1:3 ? 1:0) yielded **9e** (80 mg, 44%) as a light yellow foam [ratio of *cis/trans* (97:3)]. Pure *cis*-**9e** (yield = 44%): TLC (ethyl acetate/hexane, 1:2): R_f 0.30. ^{1}H-NMR (300 MHz, $CDCl_3$): d 1.39 (s, 3H), 1.41 (s, 3H), 2.85 (m, 1H), 3.37 (dd, *J* = 8.48 Hz, 3.58 Hz, 1H), 4.23 (t, *J* = 9.23 Hz, 1H), 4.46 (dd, *J* = 9.23 Hz, 8.10 Hz, 1H), 5.56 (d, *J* = 3.58 Hz, 1H), 7.52 (t, *J* = 8.10 Hz, 1H), 7.89 (d, *J* = 8.15 Hz, 1H), 8.18 (d, *J* = 8.15 Hz, 1H), 8.42 (s, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): d 175.98 (s), 149.83 (s), 148.62 (s), 136.82 (s), 130.79 (d), 129.45 (d), 123.67 (d), 120.08 (d), 92.70 (d), 74.45 (s), 68.11 (t), 43.28 (d), 37.57 (d), 25.27 (q), 24.71 (q). EI MS *m/z* (%): 289 ($[C_{15}H_{15}NO_5]^+$, 59), 245 (78), 216 (48), 202 (55), 150 (100), 104 (42), 95 (49), 76 (55), 68 (42), 41 (55). IR (cm^{-1}): 1767 (C=O); 1526, 1338 (N=O). Mp (MeOH): 156°C. EI-MSHR: Calc. mass (*cis*-$C_{15}H_{15}NO_5$) = 289.095023, found: 289.095000. HPLC purity: 92.6%.

4,4-Dimethyl-6-phenyl-3a,7a-dihydro-3*H*,4*H*-furo[3,4-*c*]pyran-1-one (9f). *General Method B:* Ester **8f** (767 mg, 3.14 mmol) was treated according to the general method in *o*-xylene (44 ml) for 28.5 h. Flash chromatography (toluene/Et_2O/hexane, 8:1:3) yielded *cis*-**9f** and *trans*-**9f** (562 mg, 73%) as a foam [ratio of *cis/trans* (34:66)]. Pure *cis*-**9f** (yield = 7%) was separated by flash chromatography ($CHCl_3$/ethyl acetate/hexane, 1:1:3): TLC (*cis*, $CHCl_3$/Et_2O/hexane, 1:1:3): R_f 0.18. ^{1}H-NMR (*cis*, 300 MHz, $CDCl_3$): d 1.35 (s, 3H), 1.39 (s, 3H), 2.81 (q, *J* = 8.58 Hz, 1H), 3.32 (dd, *J* = 8.27 Hz, 3.49 Hz, 1H), 4.25 (t, *J* = 9.19 Hz, 1H), 4.42 (t, *J* = 8.46 Hz, 1H), 5.38 (d, *J* = 2.94 Hz, 1H), 7.33 – 7.37 (m, 3H), 7.56 – 7.59 (m, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$): d 176.70 (s), 151.99 (s), 135.20 (s), 129.08 (d), 128.46 (d, 2C), 125.16 (d, 2C), 90.20 (d), 73.59 (s), 68.19 (t), 43.52 (d), 37.66 (d), 25.11 (q), 25.04 (q). EI MS *m/z* (%): 244 ($[C_{15}H_{16}O_3]^+$, 40), 105 (91), 77 (45), 44 (85), 40 (100). IR (cm^{-1}): 1759 (C=O). Mp (*cis*, MeOH): 96°C. EI-MSHR: Calc. mass (*cis*-$C_{15}H_{16}O_3$) = 244.109945, found: 244.110090. HPLC purity (*cis*-**9f**): 94.3%. Pure *trans*-**9f** (yield = 28%) was separated

by flash chromatography (toluene/hexane/ethyl acetate, 8:3:1): TLC (*trans*, toluene / hexane/Et_2O, 8:3:1): R_f 0.28. ^{1}H-NMR (*trans*, 300 MHz, $CDCl_3$): d 1.35 (s, 3H), 1.52 (s, 3H), 2.55 – 2.65 (m, 1H), 3.12 (dd, *J* = 14.22 Hz, 2.17 Hz, 1H), 4.03 (dd, *J* = 11.68 Hz, 8.29 Hz, 1H), 4.40 (dd, *J* = 8.20 Hz, 6.69 Hz, 1H), 5.61 (d, *J* = 1.88 Hz, 1H), 7.30 – 7.37 (m, 3H), 7.57 – 7.60 (m, 2H). EI-MSHR: Calc. mass (*trans*-$C_{15}H_{16}O_3$) = 244.109945, found: 244.109920. HPLC purity (*trans*-**9f**): 87.1% (major impurity is *cis*-**9f,** 8.2%).

Cellular Assays

Description of the assay: Cells are seeded into 96-well plates (3 x 10^3 cells per well) and grown over night in an incubator. Cells are then treated for 72 h with the indicated concentrations of compound. Effect of inhibitors on viability and onset of apoptosis is assessed by the YO-PRO-1 assay as described.[1] Briefly, after the treatment period of 72 h with compounds, a 25 µL aliquot of a solution containing 100 mM sodium citrate, pH 4.0, 134 mM sodium chloride and 12.5 µM YO-PRO-1 dye (YO-PRO-1 iodide, #Y3603, Molecular Probes) is directly added to the 100 µL medium in the wells of the 96-well plate to a final dye concentration of 2.5 µM. The plate is incubated for 10 min at ambient temperature in the dark. The uptake of the YO-PRO-1 dye into cells is assessed by a first measurement using a Cytofluor II fluorescence plate reader (PerSeptive Biosystems; instrument settings: excitation 485/20nm, emission 530/25nm, gain 75). After the first reading, 25 µL of lysis buffer consisting of 20 mM sodium citrate, pH 4.0, 26.8 mM sodium chloride, 0.4 % NP40, 20 mM EDTA and 20 mM is added to each well. Upon completion of cell lysis after incubation for 30 min at room temperature, the total amount of YO-PRO-1 bound to DNA is determined by a second measurement using the Cytofluor II fluorescence plate reader with the identical setting as described above.

Data evaluation: The raw data obtained with the Cytofluor II fluorescence plate reader are transferred as 96-well-matrix to an EXCEL-template. EXCEL-routines are used for calculation of means and standard deviations of the triplicates. The percentage of apoptotic cells is calculated using the formula:

[(values of the first reading)/(values of the second reading)]x100 = % apoptotic cells.

To determine the anti-proliferative effect of a compound, the corresponding value of the second reading representing totally bound YO-PRO-1 dye is expressed as percentage of the value of the control cells set as 100 %. IC_{50} values are then calculated from dose response curves according to the following formula, considering the region around 50 % inhibition to be a straight line (half-logarithmic plot):

$$10[\log C_1 + (50\% - I_1)*(\log C_2 - \log C_1)/(I_2 - I_1)] = IC_{50}$$

where: C_1 = concentration resulting in inhibition just below 50 %

C_2 = concentration resulting in inhibition just above 50 %

I_1 = % inhibition measured at C_1

I_2 = % inhibition measured at C_2

Cell cycle analysis

Cell cycle stages were analyzed by laser-scanning cytometry (LSC, CompuCyte, Cambridge, MA).[2] Briefly, KB31 and A549 cells (3.0×10^5 cells) were plated out in 100 mm dishes and grown over night. After treatment for 24h with compounds **3c** (Figures S1 and S2) and **3d** (Figure S3) using the indicated concentrations, cells were collected, fixed using ice-cold 70% ethanol and stained with propidium iodide (PI) following standard protocols.

Figure S1: DNA profile of KB31 cells treated with compound 3c.

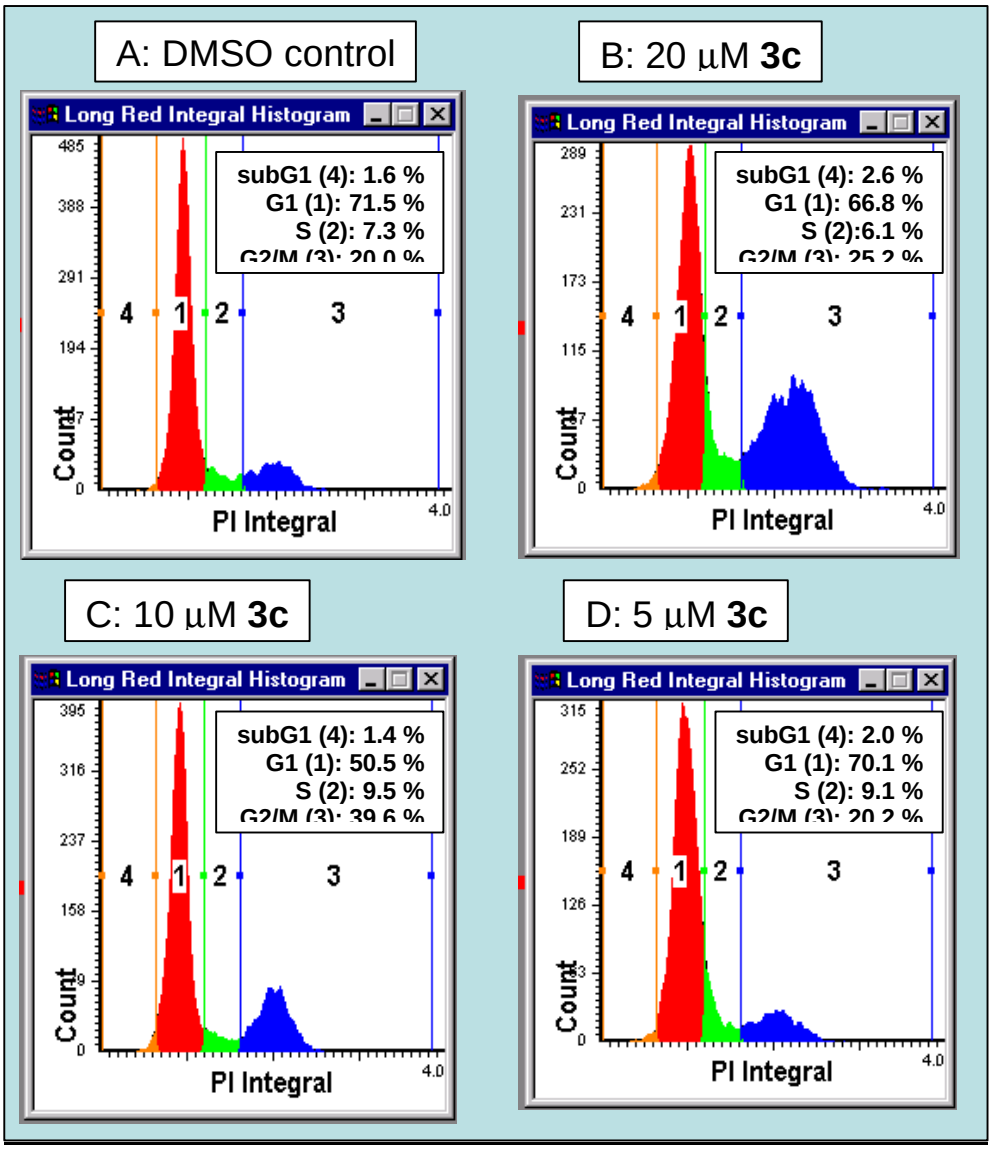


Figure S2: DNA profile of A549 cells treated with compound 3c.

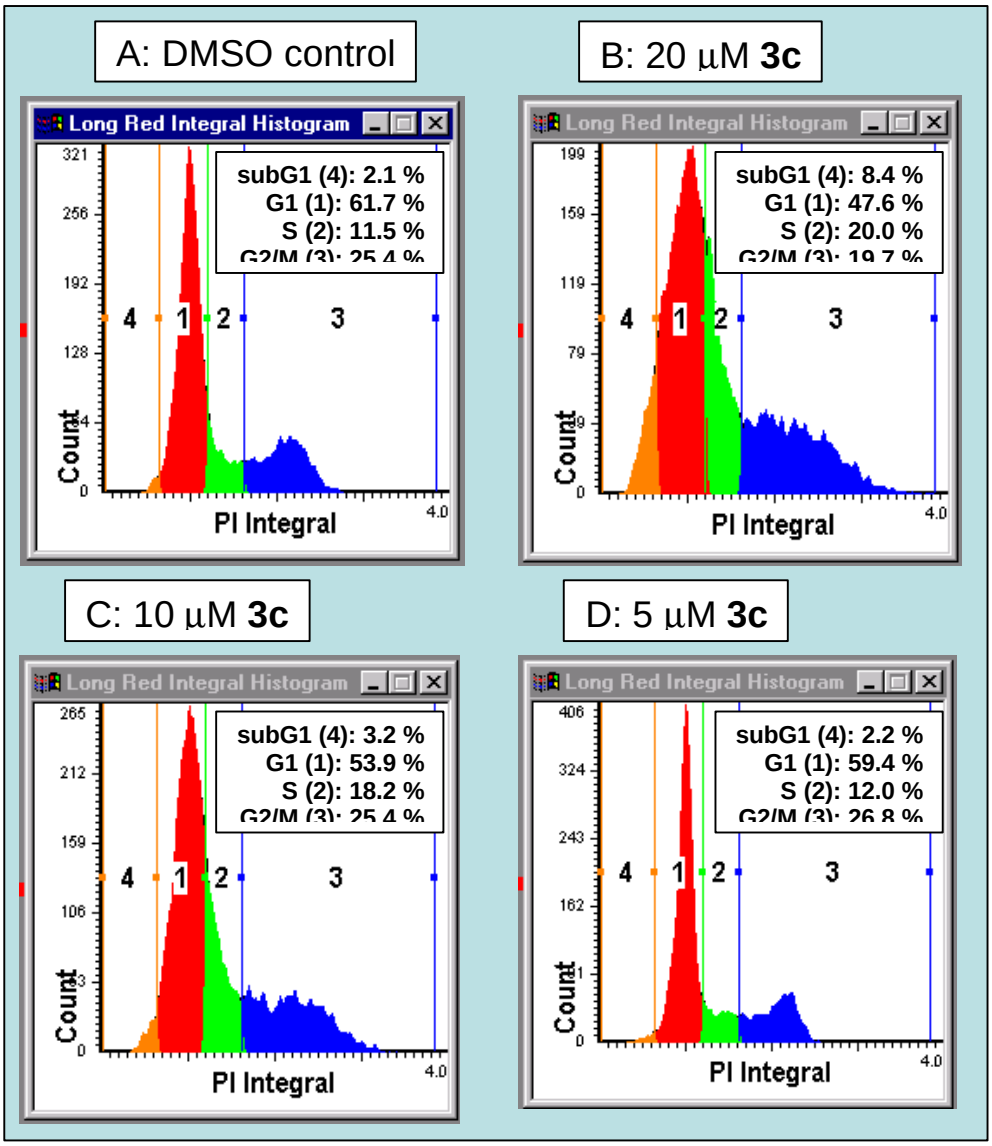


Figure S3: DNA profile of A549 cells treated with compound 3d.

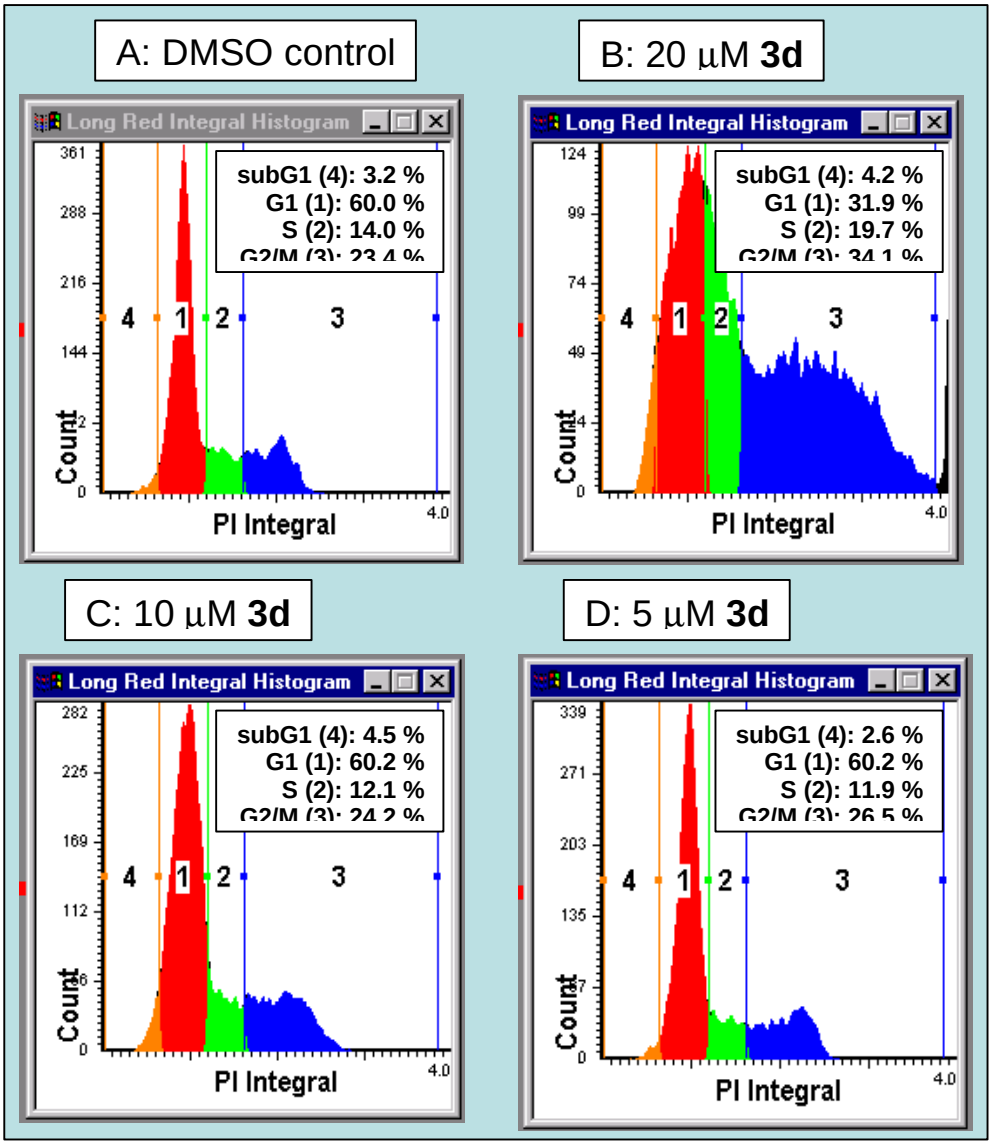
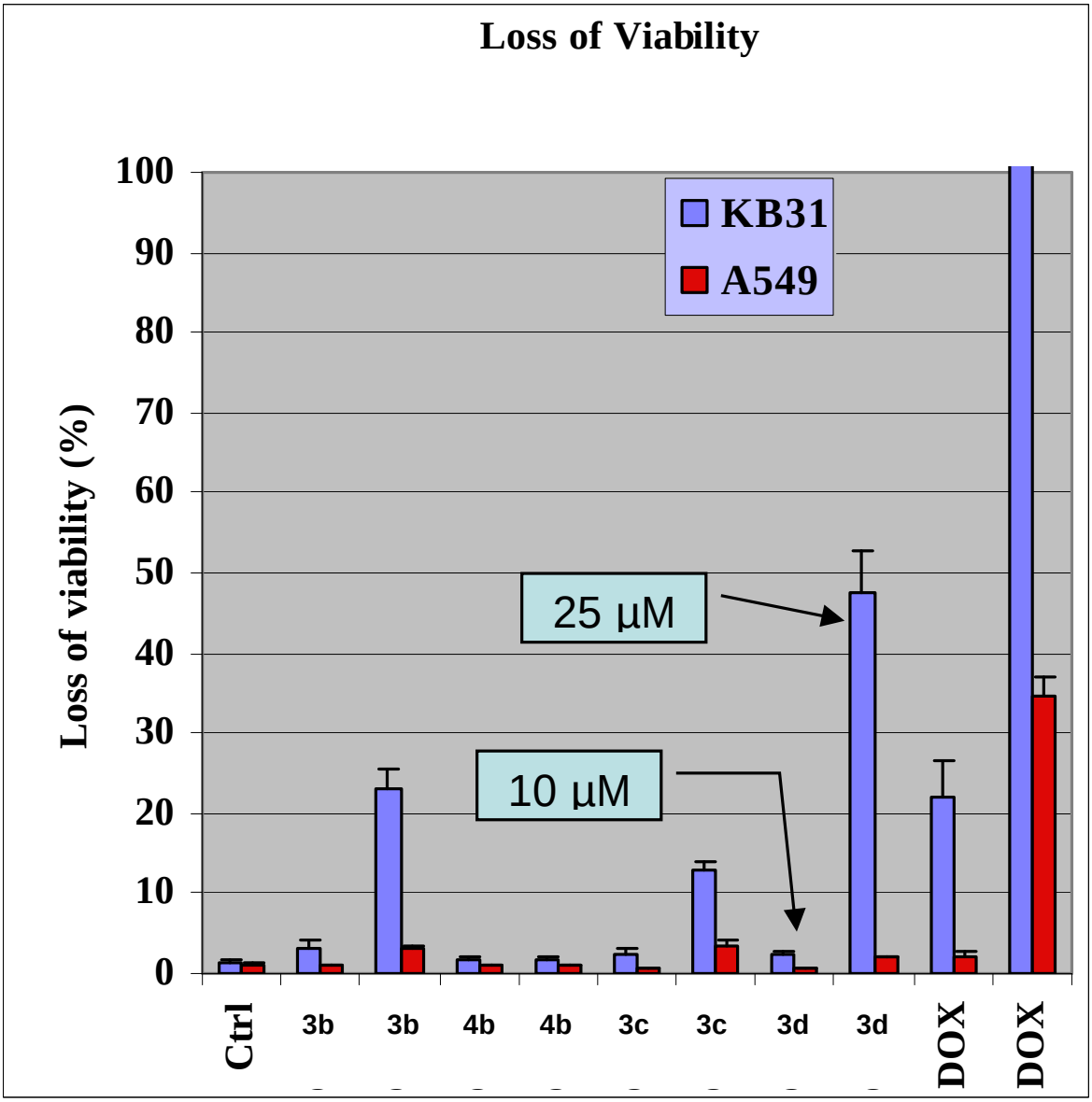


Figure S4: Viability Tests of the Synthesised Compounds in A549 and KB31 Cells. Each compound was tested at two different concentrations (10 µM and 25 µM); Data represent the mean of triplicate determinations of two independent experiments. In the control sample (Ctrl) 0.1 % DMSO was present during the assay. In addition, a sample was treated with 5 µM doxorubicin (DOX) to demonstrate induction of apoptosis and loss of viability.



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[2] E. Luther, L. Kamentsky, M. Henriksen, E. Holden, *Methods Cell Biol.*, **2004,** *75,* 185-218.