Induction of apoptosis by cyclobutanones and derived polycyclic γ-lactones: A preliminary analysis of antiproliferative activity

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Supporting Information
**General Information**

Starting materials and reagents were purchased from commercial suppliers and used after further purification (crystallization/ distillation). Bruker (400 MHz) and JEOL AL-300 FT (300 MHz) NMR spectrometers were used to record $^1$H and $^{13}$C NMR spectra. Tetramethylsilane (TMS) was used as internal standard; chemical shifts (δ) are reported in ppm and coupling constants (J) are reported in Hertz (Hz). IR spectra were recorded with Shimadzu FT-IR-8400S as thin film between KBr discs. High resolution mass spectra were recorded on a bruker microTOF using ESI-TOF method. Roswell Park Memorial Institute medium (RPMI-1640), Fetal bovine serum (FBS), Penicillin, Streptomycin, L-glutamine, fluorescent probe 2',7'-dichlorodihydro-fluorescein diacetate (H2DCF-DA) and Triton X-100 were obtained from Sigma-Aldrich Corp. (St. Louis, MO, USA). Propidium iodide (PI) was purchased from Invitrogen Life Technologies (Carlsbad, CA). The remaining reagents were of analytical grade.

**General procedure for synthesis of cyclobutanones (7a-f)**

$\beta$-Ionone derived chalcones (4a-f, 500 mg) were dissolved in aqueous methanol (1 % v/v) and taken in an immersion well type Pyrex glass, water cooled photoreactor [23,24]. The solutions were purged with dry nitrogen (O$_2$ free) for at least 15 min prior to irradiation. The irradiation was carried out with a 125 Watt medium pressure Hg arc placed coaxially inside the reactor and N$_2$ was continuously bubbled during irradiation. At the end of reaction (Tlc), solvent was removed from photolysates under reduced pressure using an Eyela rotary evaporator and mixture of the obtained products exo compounds 6a-f and cyclobutanones 7a-f were adsorb on silica gel (5g) and stirred in chloroform (30ml) for 3 h. After completion of reaction (tlc) silica gel was filtered off and washed with chloroform. Obtained cyclobutanones (7a-f) were purified by column chromatography over silica gel (Acme Synthetic
Chemicals, Mumbai, India, 60-120 mesh, 15 g, columns packed in hexane) and characterized by detailed spectroscopic analysis.

**General Procedure for Synthesis of polycyclic γ-lactones (8a-f)**

A solution of 50 mg of compounds 7a-f in 8 ml of methanol was added to a solution of H\(_2\)O\(_2\) (30 %, 304 mg, 16.5-17 moles) and formic acid (80 %, 316 mg, 34 moles). Reaction mixture was stirred vigorously and progress of the reaction was monitored by Tlc [23,24]. At the end of reaction (3 h), solvent was distilled off under reduced pressure and reaction mixture was extracted with DCM (CH\(_2\)Cl\(_2\)). The extracts were washed with water and dried over anhydrous sodium sulphate. The crude mixture was subjected to column chromatography over silica gel (Acme Synthetic Chemicals Mumbai, India, 60-120 mesh, 20 g, column packed in hexane), using hexane-chloroform (gradient) as eluant, to obtain pure 8a-f.

**Spectroscopic data of compounds 7a-f and 8a-f**

**5-o-Chlorophenyl-7,11,11-trimethyl-tricyclo[5.4.0.3\(\_\)6]undec-1-ene-4-one (7a)**

Colorless solid (375 mg, yield 75 %); m.p. 98-99 °C (hexane); IR (KBr): \(\nu_{\text{max}} = 1778\) (C=O), 1593, 1473, 1371, 1083, 1035, 837 cm\(^{-1}\); \(^{1}\)H NMR (300 MHz, CDCl\(_3\)): \(\delta = 7.59-7.12\) (m, 4H, Ar-Hs), 5.43 (d, 1H, \(J = 2.1\) Hz, C2-H), 4.75 (dd, 1H, \(J = 6.3\) Hz & \(J = 3.9\) Hz, C5-H), 4.16-4.00 (m, 1H, C3-H), 2.82 (t, 1H, \(J = 6.3\) Hz, C6-H), 1.82-1.73 (m, 2H, -CH\(_2\)), 1.69-1.42 (m, 4H, 2 \times -CH\(_2\)), 1.27 (s, 3H, -CH\(_3\)), 1.17 (s, 3H, -CH\(_3\)), 1.14 (s, 3H, -CH\(_3\)); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta = 205.4\) (C=O), 159.9 (C\(_1\)), 134.5 (arom. q), 133.8 (arom. q), 130.1 (arom. CH), 129.9 (arom. CH), 128.4 (arom. CH), 127.0 (arom. CH), 117.4 (C2), 67.4 (C5), 61.6 (C3), 49.9 (C11), 48.3 (C6), 40.4 (C10), 34.7 (C7), 32.6 (C8), 31.4 (CH\(_3\)), 28.7 (CH\(_3\)), 28.4 (CH\(_3\)), 18.1 (C9); HR-MS (TOF, ESI): \(m/z\) calcd for C\(_{20}\)H\(_{23}\)OCl + [H]: 315.1554; found: 315.1502 \([\text{M} + \text{H}]^+\).

**5-o-Fluorophenyl-7,11,11-trimethyl-tricyclo[5.4.0.3\(\_\)6]undec-1-ene-4-one (7b)**

3
Colorless semisolid (350 mg, yield 70%); IR (KBr): $\nu_{\text{max}} = 1766$ (C=O), 1510, 1228, 838 cm$^{-1}$; 

$^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.11-6.85 (m, 4H, Ar-H), 5.34 (d, 1H, $J = 1.8$ Hz, C2-H), 4.43 (dd, 1H, $J = 6.3$ & 2.4 Hz, C5-H), 3.98-3.93 (m, 1H, C3-H), 2.55 (t, 1H, $J = 6.3$ Hz, C6-H), 1.86-1.79 (m, 2H, -CH$_2$), 1.60-1.52 (m, 4H, 2 x -CH$_2$), 1.31 (s, 3H, -CH$_3$), 1.17 (s, 3H, -CH$_3$), 1.14 (s, 3H, -CH$_3$); 

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 203.5 (C=O), 159.7 (C1), 136.4 (arom. CH) 134.8 (arom. CH), 132.3 (arom. q), 130.3 (arom. CH), 128.7 (arom. q), 128.7 (arom. CH), 117.7 (C2), 66.9 (C5), 61.8 (C3), 49.8 (C11), 48.7 (C6), 40.5 (C10), 34.7 (C7), 33.0 (C8), 31.5 (-CH$_3$), 28.8 (-CH$_3$), 28.5 (-CH$_3$), 18.9 (C$_9$); 

HR-MS (TOF, ESI): $m/z$: calcd for C$_{20}$H$_{23}$OF + [K]: 338.1510; found: 338.1574 [M + K]$^+$. 

5-o-Bromophenyl-7,11,11-trimethyl-tricyclo[5.4.0.0$^{3,6}$]undec-1-ene-4-one (7c)

Colorless solid (350 mg, yield 70%); m.p. 88-89 °C (hexane); IR (KBr): $\nu_{\text{max}} = 1772$ (C=O), 1471, 1217, 1085, 908 cm$^{-1}$; 

$^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.02 (d, 1H, $J = 8.1$ Hz, Ar-H), 4.93 (brs, 1H, C2-H), 4.33 (dd, 1H, $J = 6.6$ Hz & 2.4 Hz, C5-H), 3.62-3.60 (m, 1H, C3-H), 2.32 (t, 1H, $J = 6.6$ Hz, C6-H), 1.42-1.18 (overlapping multiplets, 6H, 3 x -CH$_2$ ), 0.74 (s, 3H, -CH$_3$), 0.65 (s, 3H, -CH$_3$), 0.35 (s, 3H, -CH$_3$); 

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 205.1 (C=O), 159.9 (C1), 136.2 (arom. q), 133.2 (arom. CH), 130.0 (arom. CH), 128.7 (arom. CH), 127.6 (arom. CH), 124.3 (arom. q), 117.4 (C2), 67.4 (C5), 63.6 (C3) 50.0 (C11), 48.5 (C6), 40.5 (C10), 34.7 (C7), 32.7 (C8), 31.4 (CH$_3$), 29.7 (CH$_3$), 28.7 (CH$_3$), 18.8 (C9); 

HR-MS (TOF, ESI): $m/z$: calcd for C$_{20}$H$_{23}$OBr: 359.1084; found: 359.1010 [M]$^+$. 

5-o-Anisyl-7,11,11-trimethyl-tricyclo[5.4.0.0$^{3,6}$]undec-1-ene-4-one (7d)

Colorless solid (350 mg, yield 70%); m.p. 96-97 °C (hexane); IR (KBr): $\nu_{\text{max}} = 1774$ (C=O), 1521, 1473, 1308, 1247, 1182, 837 cm$^{-1}$; 

$^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.28-7.15 (m, 1H, Ar-H), 6.99 (d, 1H, $J = 7.5$ Hz, Ar-H), 6.86-6.81 (m, 2H, Ar-Hs), 5.41 (d, 1H, $J = 1.8$ Hz, C2-H), 4.40 (dd, 1H, $J = 6.3$ Hz & 3.0 Hz, C5-H), 4.12-4.08 (m, 1H, C3-H), 3.81 (s, 3H, -OCH$_3$), 2.73 (t, 1H, $J = 6.3$ Hz, C6-H),
1.78-1.48 (m, 6H, 3 × -CH₂), 1.22 (s, 3H, -CH₃), 1.17 (s, 3H, -CH₃), 1.15 (s, 3H, -CH₃); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta \) 205.5 (C=O), 159.9 (C1), 134.5 (arom. q), 133.8 (arom. q), 130.1 (arom. CH), 129.9 (arom. CH), 128.4 (arom. CH), 127.0 (arom. CH), 117.4 (C2), 67.4 (C5), 61.6 (C3), 58.8 (-OCH₃), 49.9 (C11), 48.3 (C6), 40.4 (C10), 34.7 (C7), 32.6 (C8), 31.4 (CH₃), 28.7 (CH₃), 28.4 (CH₃), 18.8 (C9); HR-MS (TOF, ESI): \( \text{m/z: calcd for } C_{21}H_{26}O_{2} + [\text{Na}] = 333.1754; \) found: 333.1806 [M + Na].

5-o-Tolyl-7,11,11-trimethyl-tricyclo[5.4.0.0\(^3\)\(^6\)]undec-1-ene-4-one (7e)

Colorless solid (350 mg, yield 70 %); m.p. 78-79 °C (hexane); IR (KBr): \( v_{\text{max}} = 1770 \) (C=O), 1490, 1458, 1373, 1217, 1081, 1005, 977, 837 cm\(^{-1}\); \(^{1}H\) NMR (300 MHz, CDCl\(_3\)): \(\delta \) 7.25-7.06 (m, 4H, Ar-Hs), 5.46 (d, 1H, \(J = 2.1 \) Hz, C2-H), 4.62 (dd, 1H, \(J = 6.3 \) Hz & 2.7 Hz, C5-H), 4.10 - 4.04 (m, 1H, C3-H), 2.89 (t, 1H, \(J = 6.3 \) Hz, C6-H), 2.33 (s, 3H, Ph-CH₃), 1.76-1.26 (m, 6H, 3 × -CH₂), 1.31 (s, 3H, -CH₃), 1.17 (s, 3H, -CH₃), 1.14 (s, 3H, -CH₃); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta \) 206.4 (C=O), 159.8 (C1), 136.4 (arom. q), 134.6 (arom. q), 130.6 (arom. CH), 127.3 (arom. CH), 126.9 (arom. CH), 126.0 (arom. CH), 117.9 (C2), 66.6 (C5), 61.7 (C3), 49.9 (C11), 46.6 (C6), 40.5 (C10), 34.7 (C7), 32.7 (C8), 31.4 (CH₃), 28.8 (CH₃), 28.1 (CH₃), 20.2 (CH₃), 18.7 (C9); HR-MS (TOF, ESI): \( \text{m/z: calcd for } C_{21}H_{26}O + [\text{Na} + \text{H}] = 318.3068; \) found: 318.3005 [M + Na + H].

5-(3,4-Dichloro)phenyl-7,11,11-trimethyl-tricyclo[5.4.0.0\(^3\)\(^6\)]undec-1-ene-4-one (7f)

Colorless solid (375 mg, yield 75 %); m.p. 102-103 °C (hexane); IR (KBr): \( v_{\text{max}} = 1776 \) (C=O), 1587, 1558, 1473, 1384, 1103, 1049, 867 cm\(^{-1}\); \(^{1}H\) NMR (300 MHz, CDCl\(_3\)): \(\delta \) 7.52 (s, 1H, Ar-H), 7.32 (d, 1H, \(J = 8.4 \) Hz, Ar-H), 7.25 (d, 1H, \(J = 8.4 \) Hz, Ar-H), 5.60 (brs, 1H, C2-H), 4.92 (dd, 1H, \(J = 6.6 \) Hz & 2.4 Hz, C5-H), 4.35-4.28 (m, 1H, C3-H), 2.95 (t, 1H, \(J = 6.6 \) Hz, C6-H), 1.89-1.42 (m, 6H, 3 × -CH₂), 1.32 (s, 3H, -CH₃), 1.13 (s, 3H, -CH₃), 1.11 (s, 3H, -CH₃); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta \) 204.5 (C=O), 159.9 (C1), 151.1 (q. arom), 136.5 (q. arom), 135.1 (q. arom), 130.8 (arom. CH), 129.5
(arom. CH), 123.9 (arom. CH), 117.3 (C2), 67.5 (C5), 60.9 (C3), 49.9 (C11), 48.3 (C6), 40.0 (C10), 34.2 (C7), 32.6 (C8), 31.7 (CH3), 31.3 (CH3), 26.0 (CH3), 18.7 (C9); HR-MS (TOF, ESI): m/z: calcd for C20H22Cl2O: 349.1154; found: 349.1115 [M]+.

3-(o-Chlorophenyl)-3b,7,7-trimethyl-3,3a,4,5,6,7,8a-octahydro-1H-indeno[1,2-c]furan-2-one (8a)

Colorless solid (40 mg, yield 80 %); m.p. 108-109 °C (hexane); IR (KBr): v_{max} = 1770 (C=O), 1463, 1338, 1217, 1197, 1039, 908, 757 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)): δ 7.59-7.53 (m, 2H, Ar-Hs), 7.32-7.09 (m, 2H, Ar-Hs), 5.64 (d, 1H, J = 6.3 Hz, C3-H), 5.37 (brs, 1H, C8-H), 3.69 (dist.d, 1H, J = 6.0 Hz, C8a-H), 2.89 (t, 1H, J = 6.3 Hz, C3a-H), 1.78-1.37 (m, 6H, 3×CH\(_2\)), 1.24 (s, 3H, -CH\(_3\)), 1.19 (s, 3H, -CH\(_3\)), 1.15 (s, 3H, -CH\(_3\)); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz): δ 177.4 (C=O), 160.5 (C7a), 136.6 (arom. q), 133.1 (arom. q), 130.3 (arom. CH), 129.3 (arom. CH), 128.5 (arom. CH), 127.3 (arom. CH), 115.1 (C8), 85.7 (C3), 79.3 (C7), 58.6 (C8a), 51.0 (C3a), 40.6 (C6), 34.6 (C3b), 34.4 (C4), 31.3 (CH\(_3\)), 28.4 (CH\(_3\)), 28.2 (CH\(_3\)), 18.5 (C5); HR-MS (TOF, ESI): m/z: calcd for C\(_{20}\)H\(_{23}\)O\(_2\)Cl + [H]: 331.1465; found: 331.1471 [M+H]+.

3-(o-Fluorophenyl)-3b,7,7-trimethyl-3,3a,4,5,6,7,8a-octahydro-1H-indeno[1,2-c]furan-2-one (8b)

Colorless solid (40 mg, yield 80 %); m.p. 113-115°C; IR (KBr): v_{max} = 1782 (C=O), 1608, 1514, 1438, 1217, 1016, 838, 757 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)): δ 7.37-7.00 (m, 4H, Ar-Hs), 5.30 (d, 1H, J = 6.3 Hz, C3-H), 5.18 (brs, 1H, C8-H), 3.78-3.70 (m, 1H, C8a-H), 2.73 (t, 1H, J = 6.3 Hz, C3a-H), 1.64-1.37 (m, 6H, 3xCH\(_2\)), 1.22 (s, 3H, CH\(_3\)), 1.17 (s, 3H, CH\(_3\)), 1.14 (s, 3H, CH\(_3\)); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz): δ 176.4 (C=O), 163.9 (arom. q), 161.4 (C7a), 160.2 (arom. CH), 134.9 (arom. q), 130.2 (arom. CH), 129.2 (arom. CH), 128.0 (arom. CH), 115.2 (C8), 81.6 (C3), 59.7 (C8a), 50.8 (C3a), 49.7 (C7), 40.3 (C6), 34.3 (C3b), 34.0 (C4), 31.1 (CH\(_3\)), 28.6 (CH\(_3\)), 27.9 (CH\(_3\)), 18.6 (C5); HR-MS (TOF, ESI): m/z: calcd for C\(_{20}\)H\(_{23}\)O\(_2\)F + [Na]: 337.1534; found: 337.1581 [M+Na]+.
3-(o-Bromophenyl)-3b,7,7-trimethyl-3,3a,3b,4,5,6,7,8a-octahydro-1H-indeno[1,2-c]furan-2-one (8c)

Colorless solid (42.5 mg, yield 85 %); m.p. 128-129 °C; IR (KBr): \( \nu_{\text{max}} = 1772 \) (C=O), 1471, 1375, 1217, 1014, 981, 759 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 300 MHz): \( \delta \) 7.60 (d, 1H, \( J = 7.8 \) Hz, Ar-H), 7.26-7.18 (m, 3H, Ar-Hs), 5.68 (d, 1H, \( J = 6.3 \) Hz, C3-H), 5.38 (brs, 1H, C8-H), 4.12 (dist.d, 1H, \( J = 7.2 \) Hz, C8a-H), 2.95 (t, 1H, \( J = 6.3 \) Hz, C3a-H), 1.78-1.44 (m, 6H, 3 \( \times \) CH\(_2\)), 1.28 (s, 3H, -CH\(_3\)), 1.25 (s, 3H, -CH\(_3\)), 1.18 (s, 3H, -CH\(_3\)); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \( \delta \) 177.3 (C=O), 160.5 (C7a), 138.1 (arom. q), 133.6 (arom. CH), 130.2 (arom. CH), 128.8 (arom. CH), 127.9 (arom. CH), 123.2 (arom. q), 115.0 (C8), 81.2 (C3), 58.8 (C8a), 50.0 (C3a), 49.6 (C7), 40.7 (C6), 34.6 (C3b), 34.4 (C4), 31.3 (CH\(_3\)), 28.3 (CH\(_3\)), 27.9 (CH\(_3\)), 18.9 (C5); HR-MS (TOF, ESI): \( m/z \): calcd for C\(_{20}\)H\(_{23}\)O\(_2\)Br: 375.0955; found: 375.0970 [M]+.

3-(o-Methoxyphenyl)-3b,7,7-trimethyl-3,3a,3b,4,5,6,7,8a-octahydro-1H-indeno[1,2-c]furan-2-one (8d)

Colorless solid (42.5 mg, yield 85 %); m.p. 104-105 °C (hexane); IR (KBr): \( \nu_{\text{max}} = 1760 \) (C=O), 1602, 1494, 1375, 1247, 1199, 1163, 1029, 977, 756 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 300 MHz): \( \delta \) 7.34-7.25 (m, 2H, Ar-Hs), 6.98-6.90 (m, 2H, Ar-Hs), 5.36 (d, 1H, \( J = 6.9 \) Hz, C3-H), 5.48 (d, 1H, \( J = 1.8 \) Hz, C8-H), 3.89 (s, 3H, -OCH\(_3\)), 3.73 (dd, 1H, \( J = 6.9 \) & 1.8 Hz, C8a-H), 2.93 (t, 1H, \( J = 6.9 \) Hz, C3a-H), 1.80-1.69 (m, 2H, -CH\(_2\)), 1.59-1.39 (m, 4H, 2\( \times \)-CH\(_2\)), 1.22 (s, 3H, -CH\(_3\)), 1.17 (s, 3H, -CH\(_3\)), 1.14 (s, 3H, -CH\(_3\)); \(^{13}\)C NMR (CDCl\(_3\), 75 MHz): \( \delta \) 177.8 (C=O), 160.2 (C7a), 157.0 (arom. q), 129.8 (arom. CH), 128.0 (arom. CH), 127.7 (arom. q), 120.6 (arom. CH), 115.6 (C8), 111.2 (arom. CH), 78.9 (C3), 58.2 (C8a), 55.5 (-OCH\(_3\)), 50.4 (C3a), 50.0 (C7), 40.6 (C6), 34.5 (C3b), 33.9 (C4), 31.3 (CH\(_3\)), 28.5 (CH\(_3\)), 28.2 (CH\(_3\)), 18.9 (C5); HR-MS (TOF, ESI): \( m/z \): calcd for C\(_{21}\)H\(_{26}\)O\(_3\) + [H]: 327.1936; found: 327.1981 [M + H]⁺.
3-(o-Methylphenyl)-3b,7,7-trimethyl-3a,3b,4,5,6,7,8a-octahydro-1H-indeno[1,2-c]furan-2-one (8e)

Colorless solid (40 mg, yield 80 %); m.p. 131-132 °C; IR (KBr): $v_{\text{max}} = 1772$ (C=O), 1638, 1589, 1460, 1385, 1354, 1273, 1225, 1200, 1180, 1134, 1018, 1007, 982, 833, 818, 756 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.25-7.10 (m, 4H, Ar-Hs), 5.39 (dist.d, 2H, $J = 6.9$ Hz, C3-H & C8-H), 3.72 (dist.d, 1H, $J = 6.9$ Hz, C8a-H), 3.03 (t, 1H, $J = 7.8$ Hz, C3-H), 2.41 (s, 3H, -CH$_3$), 1.77-1.72 (m, 2H, -CH$_2$), 1.55-1.39 (m, 4H, 2 x -CH$_2$), 1.23 (s, 3H, -CH$_3$), 1.18 (s, 3H, -CH$_3$), 1.15 (s, 3H, -CH$_3$); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 176.7 (C=O), 159.9 (C7a), 137.9 (arom. q), 133.4 (arom. q), 131.0 (arom. CH), 128.1 (arom. CH), 126.5 (arom. CH), 123.5 (arom. CH), 115.4 (C8), 74.6 (C3), 61.8 (C3a), 56.0 (C8a), 44.3 (C7), 40.8 (C6), 33.4 (C3b), 33.0 (C4), 29.7 (CH$_3$), 28.0 (CH$_3$), 23.6 (CH$_3$), 19.3 (Ar-CH$_3$), 18.3 (C5); HR-MS (TOF, ESI): $m/z$: calcd for C$_{21}$H$_{26}$O$_2$: 311.1975; found: 311.2007 [M + H]$^+$.  

3-(3,5-Dichlorophenyl)-3b,7,7-trimethyl-3a,3b,4,5,6,7,8a-octahydro-1H-indeno[1,2-c]furan-2-one (8f)  

Colorless semisolid (40 mg, yield 85 %); IR (CHCl$_3$): $v_{\text{max}} = 1772$ (C=O), 1593, 1493, 1385, 1270, 1181, 1092, 986, 835, 735 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.43 (s, 1H, Ar-H), 7.30-7.26 (m, 2H, Ar-Hs), 5.64 (d, 1H, $J = 6.9$ Hz, C3-H), 5.36 (d, 1H, $J = 1.5$ Hz, C8-H), 3.75 (dd, 1H, $J = 6.8$ & 1.5 Hz, C8a-H), 2.90 (t, 1H, $J = 6.9$ Hz, C3a-H), 1.77-1.71 (m, 2H, -CH$_2$), 1.62-1.34 (m, 4H, 2 x -CH$_2$), 1.22 (s, 3H, -CH$_3$), 1.17 (s, 3H, -CH$_3$), 1.14 (s, 3H, -CH$_3$); $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta$ 177.0 (C=O), 160.5 (C7a), 135.3 (arom. q), 135.2 (arom. q), 133.9 (arom. q), 130.2 (arom. CH), 129.6 (arom. CH), 127.6 (arom. CH), 115.0 (C8), 78.6 (C3), 58.7 (C8a), 50.0 (C3a), 49.9 (C7), 40.6 (C6), 34.6 (C3b), 34.2 (C4), 31.3 (CH$_3$), 29.7 (CH$_3$), 28.3(CH$_3$), 18.8 (C5); HR-MS (TOF, ESI): $m/z$: calcd for C$_{20}$H$_{22}$O$_2$Cl$_2$: 365.1028; found: 365.1071 [M]$^+$.  

8
Pharmacology evaluation

Cytotoxic activity

The Sulforhodamine B (SRB) assay is a colorimetric assay used for cytotoxic screening to assess cell growth inhibition (Skehan et al.,).\textsuperscript{32} Cells are cultured in a 96 well tissue culture plate and the cell growth which depends upon the rate of multiplication is measured indirectly by the intensity of the color of the dye which is directly proportional to the number of cells present. The human cancer cell lines (procured from N.C.I., Frederick, U.S.A.) were grown in tissue culture flasks in complete growth medium (RPMI-1640 medium with 2 mM glutamine, pH 7.4, supplemented with 10 % fetal calf serum, 100 µg/ml streptomycin and 100 units/ml penicillin) in a carbon dioxide incubator (37 °C, 5 % CO\textsubscript{2}, 90 % RH). The cells at sub-confluent stage were harvested from the flask by treatment with trypsin [0.05 % in PBS (pH 7.4) containing 0.02 % EDTA]. Cells with viability of more than 98 % as determined by trypan blue exclusion were used. For determination of cytotoxicity the cell suspension of 1 x 10\textsuperscript{5} cells/ml was prepared in complete growth medium. Stock solutions (2 x 10\textsuperscript{-2} M) of synthesized compounds 7a-f and 8a-f were prepared in DMSO. The stock solutions were serially diluted with complete growth medium containing 50 µg/ml of gentamycin to obtain working test solutions of required concentrations. \textit{In vitro} cytotoxicity against five human cancer cell lines of different tissues was determined using 96-well tissue culture plates. The 100 µl of cell suspension was added to each well of the 96-well tissue culture plate. The cells were allowed to grow in carbon dioxide incubator (37 °C, 5 % CO\textsubscript{2}, 90 % RH) for 24 h. Test materials in complete growth medium (100 µl) were added after 24 h of incubation to the wells containing cell suspension. The plates were further incubated for 48 hours in a carbon dioxide incubator. The cell growth was stopped by gently layering trichloroacetic acid (50 %, 50 µl) on top of the medium in all the wells. The plates were incubated at 4 °C for 1 h to fix the cells attached to the bottom of the wells. The liquid of all the wells
was gently pipette out and discarded. The plates were washed five times with distilled water to remove trichloroacetic acid, growth medium, low molecular weight metabolites, serum proteins etc. The plates were stained with sulforhodamine B dye (0.4 % in 1 % acetic acid, 100 µl) for 30 minutes. The plates were washed five times with 1% acetic acid and then air-dried. The adsorbed dye was dissolved in Tris-HCl buffer (100 µl, 0.01 M, pH 10.4) and plates were gently stirred for 10 min on a mechanical stirrer. The optical density (OD) was recorded on ELISA reader at 540 nm. The cell growth was determined by subtracting mean OD value of respective blank from the mean OD value of experimental set. Considering the growth in absence of any test material as 100 %, percent growth inhibition in presence of test material was calculated. IC$_{50}$ values were determined by non-linear regression analysis using Graph Pad Software (2236 Avenida de la Playa La Jolla, CA 92037, USA).

Confocal microscopy

PC-3 cells (1×10$^6$) in 1 ml of culture medium were transferred into 24 well plates and grown to confluence at 37 °C with 5% CO$_2$ for 24 h. The cultures were then exposed to compounds 7d and 8d at its IC$_{50}$ value and incubated for another 12 h. After incubation, the medium was aspirated with 1 ml culture medium without serum and then stained with DAPI (10 mM) for 1 h at 37°C. The confocal fluorescence images of the cells treated with compounds 7d and 8d were scanned on a Nikon eclipse TiE inverted fluorescence microscope equipped with a Nikon AiR laser scanning confocal microscope system (Nikon Corp., Japan) equipped with NIS element software viewer.

Scanning electron microscopy (SEM)

For SEM, PC-3 cells were incubated in compounds 7d and 8d for 12 h and sedimented at 1800 rpm for 10 min. Cell pellets were fixed immediately with 2.5 % glutaraldehyde in 0.1M phosphate buffer (pH 7.2) at 4 °C for 1 h, post-fixed with 1 % OsO$_4$ for 1 h in the same buffer, dehydrated with graded ethanol solutions and dried in a critical point drier covered with a 9-nm gold film by flash evaporation.
of carbon and gold in a Quorum (Q150RES) attached to vacuum pump EDWARDS model RV3. Specimens were then examined with an EVO LS 10 scanning electron microscope (Carl Zeiss, Germany).

**Flow-cytometric analysis of nuclear DNA**

Cells were plated at $1 \times 10^6$ cells/dish in 24 well plates and allowed to adhering for 6 h; these were allowed to grow both in the presence of and in the absence of compounds 7d and 8d. After 12 h of treatment, the cells were harvested from plate by collecting trypsinized cells together with floating cells in the medium. For each condition, a volume of the cell suspension corresponding to $1 \times 10^6$ cells was centrifuged and the resultant cell pellet was resuspended in ice-cold phosphate buffer saline (PBS, 1.0 ml). Cells were fixed in ice-cold 70 % ethanol and stained with propidium iodide (PI). These cells were analyzed in FL-2 and FL-3 channel by BD Accuri C6 flow cytometry (BD Biosciences Immunocytometry Systems, San Jose, CA). DNA content histograms and cell cycle phase distributions were modelled from at least 30,000 single events.

**Spectrofluorimetric determination of mitochondrial membrane potential**

Mitochondrial membrane potential was estimated by spectrofluorimetry using Rhodamine-123 dye. The PC-3 cells ($1 \times 10^6$) were incubated in the presence and absence of the compound 7d and 8d at concentrations 5, 10 and 15 µM for 12 h at 37 °C in 5 % CO$_2$ incubator in 24 well plate and then washed with phosphate buffered saline (PBS). After treatment period, Rhodamine-123 (10 µm/ml) was added to each well for 1 h in serum free media. Samples were measured directly in a multipurpose plate reader (Biotek synergy HT) at the excitation and emission wavelengths of 480 nm and 530 nm, respectively.

**Spectrofluorimetric estimation of ROS level**
The PC-3 cells (1×10⁶) were incubated with DCFH-DA (25 µM) for 30 min at 37°C and then washed with phosphate buffered saline (PBS). Stained cells were treated with compounds 7d and 8d at concentration 5, 10 and 15 µM for 24 h, washed and analyzed using spectrofluorimeter at the excitation and emission wavelengths of 480 nm and 530 nm, respectively.

**Annexin V-FITC apoptosis assay**

To determine the extent of apoptotic and necrotic cell death, cells were stained with annexin V conjugated with FITC and PI using the annexin V-FITC apoptosis detection kit (Miltneyi Biotech), according to the manufacturer’s protocols. Annexin V has a high affinity for phosphatidylserine exposed on the outer membrane of apoptotic cells, while PI is transported into the late-stage apoptotic/necrotic cells with disrupted cell membranes. The CHO cells (1x10⁶) were treated with compounds 7d and 8d at concentration 10 µM for 24 h. The cells were centrifuged at 2000 rpm for 8 min at 4 °C and washed with annexin V-FITC binding buffer (10 mM HEPES, 140 mM NaCl and 2.5 mM CaCl₂·2H₂O; pH 7.4). Again after centrifuging at 2000 rpm at 4 °C, the cell pellets were dissolved in annexin V-FITC binding buffer containing annexin V-FITC and propidium iodide. After 15 min incubation in dark at room temperature flow cytometric analysis was done. Then, viable (annexin V-negative, PI-negative), early apoptotic (annexin V-positive, PI-negative), late apoptotic (annexin V-positive, PI-positive) and necrotic (annexin V-negative, PI-positive) cells were detected by flow cytometry (Accuri C6 flow cytometer; Becton–Dickinson)
$^1$H NMR of compound 8d

$^{13}$C NMR of compound 8d
$^1$H NMR of compound 7a

$^{13}$C NMR of compound 7a