## Electronic Supporting Information

**Design, synthesis and antiproliferative activity of urocanic-chalcone hybrid derivatives**

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Experimental

General Experimental

Chemicals, solvents and reagents used are commercially available and were used without further purification. PE refers to petroleum ether, bp 40-60 °C. TLCs were carried out on Merck Aluminium backed TLC plates Silica Gel 60 F254 and viewed using UV light of wavelength 254 nm and then stained with potassium permanganate. Merck Silica Gel (0.040-0.063 mm) was used for column chromatography. Compounds were loaded as an oil, CH₂Cl₂ solution or dry loaded by adsorption onto silica.

Melting points were obtained using a Reichert-Jung heated-stage microscope. Infrared spectra were recorded on a Perkin-Elmer Spectrum RXI FT-IR system and all values are recorded in cm⁻¹.

NMR spectra were obtained on Varian Mercury VX (400 MHz) or Bruker Avance III (400 MHz) spectrometers. The chemical shifts are recorded in parts per million (ppm) with reference to tetramethylsilane. The coupling constants J are quoted to the nearest 0.5 Hz and are not corrected. The multiplicities are assigned as a singlet (s), doublet (d), triplet (t), doublet of doublets (dd) and multiplet (m). Mass spectra and high resolution mass spectra were obtained on a micrOTOF™ from Bruker Daltonics (Bremen, Germany) coupled with an electrospray source (ESI-TOF) using an autosampler in an Agilent 1100 LC system. Data was processed using external calibration with the Bruker Daltonics software, DataAnalysis™ as part of the overall hardware control software, Compass 1.1™.

HPLC

Analytical RP-HPLC was performed on a Dionex HPLC system equipped with a Dionex Acclaim 3 µm C-18 (150 × 4.6 mm) column with a flow rate of 1 mL/min. with detection at 214 nm and 254 nm shown (pages S30-S41). Mobile phase A was 0.1% TFA in H₂O and mobile phase B was 0.1% TFA in MeCN. The gradient was T = 0 min., B = 5%; T = 10 min., B = 95%; T = 15 min., B = 95%; T = 15.1 min., B = 5%; T = 18.1 min., B = 5%.
MTS cell proliferation assay

1: Human cancer cell lines HT29, MDA-MB-231 and LNCaP were supplied by Cancer Research UK. They were maintained in DMEM with high glucose (4.5 g/L) and L-glutamine, supplemented with penicillin 100 U/mL, streptomycin 100 µg/mL and foetal bovine serum at 10% for HT29 and MDA-MB-231, and 20% for LNCaP. FEK-4 primary human skin fibroblasts were a gift from Prof. Rex M. Tyrrell (University of Bath) and were maintained in MEM supplemented with L-glutamine, supplemented with penicillin 100 U/mL, streptomycin 100 µg/mL and 15% foetal bovine serum. All reagents supplied by Invitrogen. Cells were maintained in 75 cm² tissue culture flasks (Nunc) with a weekly 1:10 split.

2: For the MTS assay, seed densities of 500, 1000, 1500 and 2000 cells per well in 50 µL were used for HT29, MDA-MB-231, FEK-4 and LNCaP cell lines respectively. The seed densities had been determined previously to give an acceptable optical density value after 3 days incubation.

3: Plates were incubated at 37 °C, in humidified 5% CO₂ in air for 2-4 hours.

4: Test agents were prepared at 100 × final concentration in DMSO (Sigma), diluted 1 in 50 in culture medium and 50 µl added to the appropriate wells, to give a final volume of 100 µl.

5: Quadruplicate samples were run as follows:
- Culture medium only (background)
- Cells only
- Cells + 1% DMSO
- Cells + test compound

6: Plates were incubated at 37 °C, in humidified 5% CO₂ in air for 3 days. This exposure time appears to be adequate to demonstrate anti-proliferative activity, and is routinely used by other workers.

7: The MTS reagent was added, 20 µl per well. This is Promega Cell Titer® Aqueous One Solution Cell Proliferation Assay.

8: Plates were incubated at 37 °C, in humidified 5% CO₂ in air, for colour development.

9: Optical density readings at 490nm were taken at 1-4 hours.

10: Because the culture medium gives a high OD₄₉₀ this was subtracted from all other OD₄₉₀ values prior to calculation of mean and s.d.

11: Means and standard deviations were calculated from background corrected OD₄₉₀ values.

12: IC₅₀ values were calculated using the pharmacology function in SigmaPlot 8 (SPSS Inc). Each assay was repeated on three separate occasions, except A3 FEK-4 (twice) and C3-H2 FEK-4 and Doxorubicin (once), and average IC₅₀ values with standard deviations determined. Doxorubicin was used as a positive control.

Note: This assay is based upon the development of a coloured metabolite from viable cells. Therefore the inhibition of colour development by an active agent does not distinguish between inhibition of cell metabolism ie cytostasis and reduction in cell number ie cytotoxicity. Nevertheless, this assay provides a very quick and easy first approach for screening test compounds.
General Methods

Method i (A1, B1, C1)

Following the procedure reported, except using 1 equivalent LiOH·H₂O, LiOH·H₂O (2.5 mmol) was added to rapidly stirred solution of acetophenone (2.5 mmol) in EtOH (2 mL) at 30 °C open to the atmosphere for 10 min. resulting in a rapid colour change from colourless to yellow. The aldehyde (2.5 mmol) was then added and stirring continued for 6 h resulting in a gradual colour change from yellow to orange. After 6 h the solvent was removed under reduced pressure and distilled water (5 mL) added followed by 1.5M HCl(aq) (5 mL) to the remaining residue. The product was extracted with EtOAc (3 × 20 mL), the organic layers were combined and washed with saturated brine solution (20 mL). The organic fraction was dried (Na₂SO₄), filtered and solvent removed under reduced pressure to give a yellow solid. The solid was purified by column chromatography with silica gel using PE:EtOAc 6:4 to afford the desired chalcone.

\[(E)-1-(4-methoxyphenyl)-3-(1H-pyrrol-2-yl)prop-2-en-1-one \text{ (A1)}\]

Following Method i on a 5 mmol scale, the product A1 was obtained as a yellow solid (0.49 g, 43%).

\textbf{Mp} 170-172 °C (EtOAc/heptane);

\textbf{IR} \nu_{\text{max}}(\text{film})/\text{cm}^{-1} 3050, 1649 and 1594;

\textbf{¹H NMR} \delta_{\text{H}}(400 MHz; CDCl₃) 3.87 (3 H, s, OCH₃), 6.31-6.33 (1 H, m, pyrrole CH), 6.65-6.70 (1 H, m, pyrrole CH), 6.95 (2 H, d, J 9.0 Hz, Ar CH), 6.94-6.96 (1 H, m, pyrrole CH), 7.16 (1 H, d, J 15.0 Hz, COCH=CH), 7.73 (1 H, d, J 15.0 Hz, COCH=CH), 7.99 (2 H, d, J 8.5 Hz, Ar CH) and 8.95 (1 H, br s, pyrrole NH);

\textbf{¹³C NMR} \delta_{\text{c}}(100MHz; CDCl₃) 55.46 (OCH₃), 111.4 (pyrrole CH), 113.8 (pyrrole CH), 114.7 (Ar CH), 115.7 (pyrrole CH), 122.3 (Ar CH), 129.5 (Cq), 130.5 (HC=CH), 131.6 (Cq), 133.8 (HC=CH), 163.2 (Cq) and 188.7 (C=O);

\textbf{MS} \text{m/z (ES⁺)} Found 228.1025 (MH⁺) and 250.0846 (MNa⁺). \text{C}_{14}\text{H}_{14}\text{NO}_2 \text{(MH⁺)} requires 228.1025 and \text{C}_{14}\text{H}_{13}\text{NO}_2\text{Na} \text{(MNa⁺)} requires 250.0844.
Following **Method i**, the product **B1** was obtained as a yellow solid (0.34 g, 53%).

**Mp** 80-81 °C (EtOAc/heptane);

**IR** ν\textsubscript{max}(film)/cm\textsuperscript{-1} 3458, 1651 and 1584;

\( ^1\text{H} \text{ NMR} \) δ\textsubscript{H}(400 MHz; CDCl\textsubscript{3}) 3.91 (3 H, s, OCH\textsubscript{3}), 3.92 (3 H, s, OCH\textsubscript{3}), 6.31-6.33 (1 H, m, pyrrole CH), 6.69-6.71 (1 H, m, pyrrole CH), 6.85 (1 H, d, J 8.0, Ar CH), 6.95-6.98 (1 H, d, J 15.5 Hz, Ar CH), 6.71 (1 H, dd, J 1.5 and 8.5 Hz, Ar CH), 7.21 (1 H, d, J 15.5 Hz, COCH=CH) and 9.25 (1 H, br s, pyrrole NH);

\( ^{13}\text{C} \text{ NMR} \) δ\textsubscript{C}(100MHz; CDCl\textsubscript{3}) 56.0 (OCH\textsubscript{3}), 110.1 (pyrrole CH), 111.0 (pyrrole CH), 111.4 (pyrrole CH), 115.0 (Ar CH), 115.4 (Ar CH), 122.6 (Ar CH), 122.9 (HC=CH), 129.5 (Cq), 131.8 (Cq), 134.0 (HC=CH), 149.2 (Cq), 153.0 (Cq) and 188.7 (C=O);

**MS** m/z (ES\textsuperscript{+}) Found 258.1135 (MH\textsuperscript{+}) and 280.0949 (MNa\textsuperscript{+}). C\textsubscript{15}H\textsubscript{16}NO\textsubscript{3} (MH\textsuperscript{+}) requires 258.1130 and C\textsubscript{15}H\textsubscript{15}NO\textsubscript{3}Na (MNa\textsuperscript{+}) requires 280.0950.

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Following **Method i**, the product **C1** was obtained as a yellow solid (0.53 g, 74%).

**Mp** 104-106 °C (EtOAc/heptane);

**IR** ν\textsubscript{max}(film)/cm\textsuperscript{-1} 3457, 1654 and 1575;

\( ^1\text{H} \text{ NMR} \) δ\textsubscript{H}(400 MHz; DMSO) 3.76 (3 H, s, OCH\textsubscript{3}), 3.90 (6 H, s, OCH\textsubscript{3}), 6.22-6.24 (1 H, m, pyrrole CH), 6.74-6.75 (1 H, m, pyrrole CH), 7.15-7.16 (1 H, m, pyrrole CH), 7.35 (2 H, s, Ar CH), 7.54 (1 H, d, J 15.0 Hz, COCH=CH) and 11.71 (1 H, br s, pyrrole NH);

\( ^{13}\text{C} \text{ NMR} \) δ\textsubscript{C}(100MHz; DMSO) 56.1 (OCH\textsubscript{3}), 60.2 (OCH\textsubscript{3}), 105.7 (Ar CH), 110.6 (pyrrole CH), 114.4 (pyrrole CH), 116.4 (pyrrole CH), 124.1 (HC=CH) 129.2 (Cq), 133.7 (Cq), 134.1 (HC=CH), 141.5 (Cq), 152.9 (Cq) and 187.1 (C=O);

**MS** m/z (ES\textsuperscript{+}) Found 288.1241 (MH\textsuperscript{+}) and 310.1061 (MNa\textsuperscript{+}). C\textsubscript{16}H\textsubscript{18}NO\textsubscript{4} (MH\textsuperscript{+}) requires 288.1236 and C\textsubscript{16}H\textsubscript{17}NO\textsubscript{4}Na (MNa\textsuperscript{+}) requires 310.1055.
Method ii (A2, B2, C2)

Following the procedure reported,\(^3\) acetophenone (5 mmol), the aldehyde (5 mmol) and NaOH (7 mmol) was added to a porcelain mortar and ground using a porcelain pestle at room temperature (20 °C) for 5 min. resulting in the formation of a viscous yellow paste. The paste was then purified by column chromatography with silica gel using PE:EtOAc 6:4 solvent system to afford the desired chalcone.

\(\text{(E)}\)-1-(4-methoxyphenyl)-3-(1-methyl-1\(H\)-pyrrol-2-yl)prop-2-en-1-one (A2)

Following Method ii, the product A2 was obtained as a yellow solid (0.76 g, 63%).

**Mp** 101-103 °C (EtOAc/heptane);

**IR** \(\nu_{\max}(\text{film})/\text{cm}^{-1}\) 1654 and 1575;

**\(^1\text{H NMR}\)** \(\delta_{H}(400 \text{ MHz}; \text{CDCl}_3)\) 3.74 (3 H, s, pyrrole CH\(_3\)), 3.87 (OCH\(_3\)), 6.20-6.22 (1 H, m, pyrrole CH), 6.79-6.80 (1 H, m, pyrrole CH), 6.82-6.83 (1 H, m, pyrrole CH), 6.96 (2 H, d, J 9.0 Hz, Ar CH), 7.31 (1 H, d, J 15.0 Hz, COCH=CH), 7.79 (1 H, d, J 15.0 Hz, COCH=CH) and 8.02 (2 H, d, J 9.0 Hz, Ar CH);

**\(^{13}\text{C NMR}\)** \(\delta_c(100\text{MHz}; \text{CDCl}_3)\) 34.3 (pyrrole CH\(_3\)), 55.4 (OCH\(_3\)), 109.5 (pyrrole CH), 111.9 (pyrrole CH), 113.7 (Ar CH), 116.5 (pyrrole CH), 127.4 (HC=CH), 130.3 (Cq), 130.4 (Ar CH), 131.4 (HC=CH), 131.5 (Cq), 163.0 (Cq) and 188.2 (C=O);

**MS** m/z \((\text{ES}^+)\) Found 242.1191 (MH\(^+\)) and 264.1007 (MNa\(^+\)). \(\text{C}_{15}\text{H}_{16}\text{NO}_2\) (MH\(^+\)) requires 242.1181 and \(\text{C}_{15}\text{H}_{15}\text{NO}_2\text{Na}\) (MNa\(^+\)) requires 264.1001.
(E)-1-(3,4-dimethoxyphenyl)-3-(1-methyl-1H-pyrrol-2-yl)prop-2-en-1-one (B2)

Following Method ii, the product B2 was obtained as a yellow solid (1.07 g, 79%).

Mp 126-126 °C (EtOAc/heptane);

IR $\nu_{\text{max}}$(film)/cm$^{-1}$ 1647, 1597 and 1573;

$^1$H NMR $\delta$(400 MHz; CDCl$_3$) 3.75 (3 H, s, pyrrole CH$_3$), 3.94 (3 H, s, OCH$_3$), 3.95 (3 H, s, OCH$_3$), 6.19-6.21 (1 H, m, pyrrole CH), 6.78-6.79 (1 H, m, pyrrole CH), 6.82-6.83 (1 H, m, pyrrole CH), 6.90 (1 H, d, $J$ 8.5 Hz, Ar CH), 7.30 (1 H, d, $J$ 15.5 Hz, COCH=CH), 7.61 (1 H, d, $J$ 2.0 Hz, Ar CH), 7.64 (1 H, dd, $J$ 2.0 and 8.0 Hz, Ar CH) and 7.79 (1 H, d, $J$ 15.0 Hz, COCH=CH);

$^{13}$C NMR $\delta$(100MHz; CDCl$_3$) 34.3 (pyrrole CH$_3$), 55.9 (OCH$_3$), 56.0 (OCH$_3$), 109.6 (pyrrole CH), 109.9 (pyrrole CH), 110.6 (pyrrole CH), 111.9 (Ar CH), 116.3 (Ar CH), 122.4 (HC=CH), 127.5 (Ar CH) 130.3 (HC=CH), 131.4 (Cq), 131.7 (Cq), 149.0 (Cq), 152.8 (Cq) and 188.0 (C=O);

MS $m/z$ (ES$^+$) Found 272.1273 (MH$^+$) and 294.1094 (MNa$^+$). C$_{16}$H$_{18}$NO$_3$ (MH$^+$) requires 272.1287 and C$_{16}$H$_{17}$NO$_3$Na (MNa$^+$) requires 294.1106.

(E)-3-(1-methyl-1H-pyrrol-2-yl)-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (C2)

Following Method ii, the product C2 was obtained as an orange oil (1.25 g, 83%).

IR $\nu_{\text{max}}$(film)/cm$^{-1}$ 1647 and 1568;

$^1$H NMR $\delta$(400 MHz; CDCl$_3$) 3.77 (3 H, s, pyrrole CH$_3$), 3.92 (3 H, s, OCH$_3$), 3.94 (6 H, s, OCH$_3$), 6.21-6.24 (1 H, m, pyrrole CH), 6.81-6.83 (1 H, m, pyrrole CH), 6.85-6.87 (1 H, m, pyrrole CH), 7.21 (1 H, d, $J$ 15.0 Hz, COCH=CH), 7.26 (2 H, s, Ar CH) and 7.80 (1 H, d, $J$ 15.0 Hz, COCH=CH);

$^{13}$C NMR $\delta$(100MHz; CDCl$_3$) 34.3 (pyrrole CH$_3$), 56.3 (OCH$_3$), 56.3 (OCH$_3$), 105.7 (Ar CH), 109.7 (pyrrole CH), 112.2 (pyrrole CH), 116.4 (pyrrole CH), 127.8 (HC=CH), 130.2 (Cq), 132.1 (HC=CH), 134.1 (Cq), 153.0 (Cq), 153.0 (Cq) and 188.7 (C=O);

MS $m/z$ (ES$^+$) Found 302.1371 (MH$^+$) and 324.1192 (MNa$^+$). C$_{17}$H$_{20}$NO$_4$ (MH$^+$) requires 302.1392 and C$_{17}$H$_{19}$NO$_4$Na (MNa$^+$) requires 324.1212.
Method iii (A3, B3, C3)

Following the procedure reported,\(^4\) except using 2 equivalents of BF\(_3\cdot\)OEt\(_2\), BF\(_3\cdot\)OEt\(_2\) (5 mmol) was added dropwise under dry conditions to a rapidly stirred solution of acetophenone (2.5 mmol) and aldehyde (2.5 mmol) in dry dioxane (2 mL) under N\(_2\) at 25 °C. The solution was heated to 75 °C for 6 h and the reaction followed by TLC. The reaction was cooled and quenched by addition of EtOAc (100 mL) and distilled water (100 mL) and the aqueous fractions extracted with EtOAc (3 × 50 mL). 2M NaOH (50 mL) was added to the aqueous layer and gently heated at 50 °C with magnetic stirring for 30 min., resulting in a slight colour change and formation of a black precipitate. The aqueous layer was extracted with EtOAc (3 × 50 mL) and the organic layers were combined and washed with saturated brine solution (50 mL) and dried using Na\(_2\)SO\(_4\). The solvent was filtered and removed under reduced pressure to produce a yellow/orange solid/oil which was purified by column chromatography with silica gel using CH\(_2\)Cl\(_2\)::MeOH 9:1 solvent system to afford the desired chalcone.

\((E)\)-3-(1H-imidazol-5-yl)-1-(4-methoxyphenyl)prop-2-en-1-one (A3)

Following Method iii, the product A3 was obtained as an orange solid (0.30 g, 53%).

\textbf{Mp} 173-175 °C (EtOAc/heptane);
\textbf{IR} \nu_{\text{max}}(\text{film})/\text{cm}^{-1} 3458, 1660 and 1604;
\textbf{\(^1\)H NMR} \delta_{\text{H}}(400 \text{ MHz; DMSO}) 3.85 (3 H, s, OCH\(_3\)), 7.08 (2 H, d, J 9.0 Hz, Ar CH), 7.63 (1 H, d, J 15.0 Hz, COCH=CH), 7.67 (1 H, d, J 15.5 Hz, COCH=CH), 7.64 (1 H, s, Im CH), 7.85 (1 H, s, Im CH), 8.03 (2 H, d, J 9.0 Hz, Ar CH) and 12.56 (1 H, br s, Im NH);
\textbf{\(^{13}\)C NMR} \delta_{\text{C}}(100\text{MHz; DMSO}) 55.5 (OCH\(_3\)), 114.0, 117.8, 130.4 (Ar CH, Im CH and HC=CH), 130.8 (Cq), 162.8 (Cq), 162.9 (Cq) and 187.2 (C=O);
\textbf{MS} \text{m/z (ES\textsuperscript{+})} \text{Found 229.0978 (MH}\textsuperscript{+})\text{. C}_{13}\text{H}_{13}\text{N}_{2}\text{O}_{2} (\text{MH}\textsuperscript{+}) \text{requires 229.0977.}
(E)-1-(3,4-dimethoxyphenyl)-3-(1H-imidazol-5-yl)prop-2-en-1-one (B3)

Following Method iii, the product B3 was obtained as a pale yellow solid (0.48 g, 74%).

Mp 170-171 °C (THF/heptane);

IR ν_{max}(film)/cm^{-1} 3457, 1659 and 1605;

^1H NMR δ_{H}(400 MHz; DMSO) 3.85 (3 H, s, OCH$_3$), 3.86 (3 H, s, OCH$_3$), 7.10 (1 H, d, J 8.5 Hz, Ar CH), 7.54 (1 H, d, J 2.0 Hz, Ar CH), 7.64 (1 H, d, J 15.0 Hz, COCH=CH), 7.64 (1 H, s, Im CH), 7.68 (1 H, d, J 15.5 Hz, COCH=CH), 7.73 (1 H, dd, J 2.0 and 8.5 Hz, Ar CH), 7.86 (1 H, s, Im CH) and 12.30 (1 H, br s, Im NH);

^13C NMR δ_{c}(100MHz; DMSO) 55.5 (OCH$_3$), 55.7 (OCH$_3$), 110.5 (Ar CH), 110.9 (Ar CH), 117.7 (Im CH), 122.6 (Ar CH), 130.9 (Cq), 135.0 (HC=CH) 135.6 (HC=CH), 138.0 (Im CH) 148.8 (Cq), 152.9 (Cq) and 187.2 (C=O);

MS m/z (ES') Found 259.1082 (MH$^+$) and 281.0897 (MNa$^+$). C$_{14}$H$_{15}$N$_{2}$O$_{3}$ (MH$^+$) requires 259.1083 and C$_{14}$H$_{14}$N$_{2}$O$_{3}$Na (MNa$^+$) requires 281.0902.

(E)-3-(1H-imidazol-5-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (C3)

Following Method iii, the product C3 was obtained as an orange solid (0.53 g, 74%).

Mp 174-176 °C (EtOAc/heptane);

IR ν_{max}(film)/cm^{-1} 3456, 1661 and 1581;

^1H NMR δ_{H}(400 MHz; CDCl$_3$) 3.85 (6 H, s, OCH$_3$), 3.89 (3 H, s, OCH$_3$), 7.26 (2 H, Ar CH), 7.38 (1 H, s, Im CH), 7.69 (1 H, d, J 15.5 Hz, COCH=CH), 7.77 (1 H, s, Im CH), 7.77 (1 H, d, J 15.0 Hz, COCH=CH) and 8.17 (1 H, br s, Im NH);

^13C NMR δ_{c}(100MHz; CDCl$_3$) 56.2 (OCH$_3$), 60.9 (OCH$_3$), 105.9 (Ar CH), 119.3 (Im CH), 123.4 (Im CH), 133.4 (Cq), 134.6 (HC=CH), 135.9 (Cq), 137.2 (HC=CH), 142.3 (Cq), 153.0 (Cq) and 189.1 (C=O);

MS m/z (ES') Found 289.1183 (MH$^+$). C$_{15}$H$_{17}$N$_{2}$O$_{4}$ (MH$^+$) requires 289.1188.
(E)-3-(1-methyl-1H-imidazol-5-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (C4)

Following Method iii, the product C4 was obtained as an orange oil (0.41 g, 54%).

IR $\nu_{\text{max}}$ (film)/cm$^{-1}$ 1657, 1591 and 1579;

$^1$H NMR $\delta$ (400 MHz; CDCl$_3$) 3.78 (3 H, s, Im CH$_3$), 3.94 (3 H, s, OCH$_3$), 3.95 (6 H, s, OCH$_3$), 7.25 (2 H, s, Ar CH), 7.37 (1 H, d, $J$ 15.5 Hz, COCH=CH), 7.57 (1 H, s, Im CH), 7.65 (1 H, s, Im CH) and 7.69 (1 H, d, $J$ 15.0 Hz, COCH=CH);

$^{13}$C NMR $\delta_c$ (100 MHz; CDCl$_3$) 32.1 (Im CH$_3$), 56.4 (OCH$_3$), 61.0 (OCH$_3$), 105.9 (Ar CH), 119.6 (Im CH), 129.1 (Im CH), 129.6 (Cq), 132.3 (HC=CH), 133.3 (Cq), 141.1 (HC=CH), 142.6 (Cq), 153.1 (Cq) and 188.2 (C=O);

MS m/z (ES$^+$) Found 303.1337 (MH$^+$) and 325.1150 (MNa$^+$). C$_{16}$H$_{19}$N$_2$O$_4$ (MH$^+$) requires 303.1345 and C$_{16}$H$_{18}$N$_2$O$_4$Na (MNa$^+$) requires 325.1164.

(E)-3-(1H-imidazol-2-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (C6)

Following Method iii on a 5 mmol scale, the product C6 was obtained as a yellow solid (0.55 g, 38%).

M$\text{p}$ 198-201 ºC (EtOAc/heptane);

IR $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3439, 1661, 1607 and 1582;

$^1$H NMR $\delta$ (400 MHz; CDCl$_3$) 3.89 (6 H, s, OCH$_3$), 3.93 (3 H, s, OCH$_3$), 7.26-7.30 (4 H, m, Ar CH and Im CH), 7.75 (1 H, d, $J$ 15.0 Hz, COCH=CH) and 7.86 (1 H, d, $J$ 15.0 Hz, COCH=CH);

$^{13}$C NMR $\delta_c$ (100MHz; CDCl$_3$) 56.3 (OCH$_3$), 61.0 (OCH$_3$), 106.1 (Ar CH and Im CH), 122.4 (HC=CH), 131.0 (HC=CH), 132.8 (Cq), 142.8 (Cq), 143.8 (Cq), 153.2 (Cq) and 188.7 (C=O);

MS m/z (ES$^+$) Found 289.1184 (MH$^+$) and 311.0998 (MNa$^+$). C$_{15}$H$_{17}$N$_2$O$_4$ (MH$^+$) requires 289.1188 and C$_{15}$H$_{17}$N$_2$O$_4$Na (MNa$^+$) requires 311.1008.
Method iv (C3-H₂)

3-(1H-imidazol-5-yl)-(3,4,5-trimethoxyphenyl)propan-1-one (C3-H₂)

The chalcone C3 (100 mg, 0.347 mmol) was added to a stirred solution of 10wt% Pd/C (20 mg) in MeOH (4 mL) under 1 atm of H₂ and stirring continued at 25 °C for 19 h. The reaction was then quenched with EtOAc (50 mL) and washed through celite with distilled water, the organic layer was extracted with EtOAc (3 × 50 mL) and the organic layers were combined and washed with saturated brine solution (50 mL). The organic fraction was dried (Na₂SO₄), filtered and solvent removed under reduced pressure to give the product C3-H₂ as a pale yellow oil (0.057 g, 57%) without the need for further purification.

IR ν max(film)/cm⁻¹ 3454, 1678, 1586 and 1505;

¹H NMR δ(H(400 MHz; CDCl₃) 3.05 (2 H, t, J 7.0 Hz, CH₂), 3.35 (2 H, t, J 7.0 Hz, CH₂), 3.89 (6 H, s, OCH₃), 3.90 (3 H, s, OCH₃), 6.85 (1 H, s, Im CH), 7.21 (2 H, s, Ar CH), 7.45 (1 H, br s, Im NH) and 7.65 (1 H, s, Im CH);

¹³C NMR δ(c(100MHz; CDCl₃) 20.5 (CH₂), 38.2 (CH₂), 56.3 (OCH₃), 60.9 (OCH₃), 105.5 (Ar CH), 118.2 (Im CH), 131.9 (Cq), 134.2 (Im CH), 135.1 (Cq), 142.7 (Cq), 153.0 (Cq) and 198.7 (C=O);

MS m/z (ES⁺) Found 291.1350 (MH⁺) and 313.1162 (MNa⁺). C₁₅H₁₉N₂O₄ (MH⁺) requires 291.1345 and (MNa⁺) C₁₅H₁₈N₂O₄Na requires 313.1164.
Method v (C5)

\((E)-3-(1\text{-}methyl\text{-}1\text{-}H\text{-}imidazol\text{-}4\text{-}yl})\text{-}1\text{-}(3\text{,}4\text{,}5\text{-}trimethoxyphenyl)prop\text{-}2\text{-}en\text{-}1\text{-}one (C5)\)

Following the procedure reported,\(^5\) except cooled to 0 °C, NaH (60% dispersion in mineral oil, 1.5 mmol) was added to a stirred solution of the chalcone (1.0 mmol) in DMF (5 mL) at 0 °C followed by dropwise addition of MeI (1.5 mmol) and the reaction was kept at 0 °C and followed by TLC until the disappearance of the chalcone starting material. The reaction was quenched with the addition of EtOAc (50 mL) and H\(_2\)O (50 mL), the organic layer separated and the aqueous fraction extracted with EtOAc (2 × 50 mL). The organic fractions were combined and washed with saturated brine solution (20 mL). The organic fraction was dried (Na\(_2\)SO\(_4\)), filtered and solvent removed under reduced pressure. Crude \(^1\)H NMR revealed the presence of C4, in addition to the product C5 in a ratio of 25:75 (C4:C5). The mixture was purified by column chromatography with silica gel using CH\(_2\)Cl\(_2\):IPA solvent system increasing from 0% to 12% IPA in 1% increments of 200 mL to afford the desired product C5 as an orange oil (0.11g, 36%).

\(R_f\) (12% IPA in CH\(_2\)Cl\(_2\)) = 0.63 (C5), 0.47 (C4);

\(\text{IR} \ \nu_{\text{max}}(\text{film})/\text{cm}^{-1} 1659, 1603 \text{ and } 1580;\)

\(^1\)H NMR \(\delta_H(400 \text{ MHz; CDCl}_3) 3.71 \text{ (3 H, s, Im CH}_3\text{), } 3.90 \text{ (3 H, s, OCH}_3\text{), } 3.92 \text{ (6 H, s, OCH}_3\text{), } 7.15 \text{ (1 H, s, Im CH), } 7.33 \text{ (2 H, s, Ar CH), } 7.49 \text{ (1 H, s, Im CH) and 7.70 (2 H, s, COCH=CH); NB The peak at } \delta_H 7.70 \text{ ppm can vary depending on the concentarion of the sample and can appear as two doublets;}\)

\(^1\)H NMR – Diluted \(\delta_H(400 \text{ MHz; CDCl}_3) 3.73 \text{ (3 H, s, Im CH}_3\text{), } 3.92 \text{ (3 H, s, OCH}_3\text{), } 3.94 \text{ (6 H, s, OCH}_3\text{), } 7.17 \text{ (1 H, s, Im CH), } 7.34 \text{ (2 H, s, Ar CH), } 7.50 \text{ (1 H, s, Im CH), } 7.69 \text{ (1 H, d, J 15.0 Hz, COCH=CH) and 7.74 (1 H, d, J 15.0 Hz, COCH=CH);}\)

\(^13\)C NMR \(\delta_c(100 \text{MHz; CDCl}_3) 33.8 \text{ (Im CH}_3\text{), } 56.4 \text{ (OCH}_3\text{), } 60.9 \text{ (OCH}_3\text{), } 106.0 \text{ (Ar CH), } 119.6 \text{ (Im CH) 124.1 (Im CH), } 133.6 \text{ (Cq), } 135.1 \text{ (HC=CH), } 138.0 \text{ (Cq), } 139.1 \text{ (HC=CH), } 142.3 \text{ (Cq), } 153.1 \text{ (Cq) and 188.9 (C=O);}\)

\(\text{MS} \ m/z (\text{ES}) \text{ Found } 303.1354 (\text{MH}^+) \text{ and } 325.1166 (\text{MNa}^+). \ C_{16}H_{19}N_2O_4 (\text{MH}^+) \text{ requires } 303.1345 \text{ and } C_{16}H_{18}N_2O_4Na (\text{MNa}^+) \text{ requires } 325.1164.\)
Method vi (C7)

\((E)-3-(1\text{-methyl-1H-imidazol-2-yl})-1-(3,4,5\text{-trimethoxyphenyl})\text{prop-2-en-1-one} (C7)\)

\[
\begin{array}{c}
\text{MeO} \quad \text{O} \quad \text{O} \\
\text{OCH}_3 \quad \text{OCH}_3 \quad \text{OCH}_3 \\
\text{Im} \quad \text{Im} \quad \text{Cq} \\
\text{Ar} \quad \text{Ar} \quad \text{Cq} \\
\end{array}
\]

The chalcone (1.4 mmol) was added to a rapidly stirred solution of 3 equivalents of Cs\(_2\)CO\(_3\) (4.2 mmol) in THF (30 mL) at 30 °C open to the atmosphere for 15 min. followed by dropwise addition of 3 equivalents of MeI (4.2 mmol) and stirring continued for 6 h. The reaction was then cooled and quenched by addition of CH\(_2\)Cl\(_2\) (50 mL) and distilled water (50 mL) and the organic layer extracted with CH\(_2\)Cl\(_2\) (3 × 50 mL), the organic layers were combined and washed with saturated brine solution (50 mL). The organic fraction was dried (Na\(_2\)SO\(_4\)), filtered and solvent removed under reduced pressure to give a pale yellow oil. The oil was purified by column chromatography with silica using CH\(_2\)Cl\(_2\):MeOH 9:1 solvent system to afford the product C7 as a yellow solid (0.23 g, 54%).

\(\text{Mp} 100-102 \, °C \text{ (EtOAc/heptane);} \)

\(\text{IR } \nu_{\text{max}} \text{(film)/cm}^{-1} 1658, 1605 \text{ and } 1580;\)

\(\text{\textsuperscript{1}H NMR } \delta_{\text{H}}(400 \text{ MHz; CDCl}_3) 3.81 \text{ (3 H, s, Im CH}_3\text{), 3.93 \text{ (3 H, s, OCH}_3\text{), 3.94 \text{ (6 H, s, OCH}_3\text{), 7.03 \text{ (1 H, s, Im CH), 7.21 \text{ (1 H, s, Im CH), 7.36 \text{ (2 H, s, Ar CH), 7.68 \text{ (1 H, d, } J \text{ 15.0 Hz, COCH=CH) and 8.06 \text{ (1 H, d, } J \text{ 15.0 Hz, COCH=CH);}})\)

\(\text{\textsuperscript{13}C NMR } \delta_{\text{c}}(100\text{MHz; CDCl}_3) 33.0 \text{ (Im CH}_3\text{), 56.4 \text{ (OCH}_3\text{), 60.9 \text{ (OCH}_3\text{), 106.0 \text{ (Ar CH), 123.9 \text{ (HC=CH), 127.2 \text{ (Im CH), 130.3 \text{ (HC=CH), 131.4 \text{ (Im CH), 133.0 \text{ (Cq), 142.7 \text{ (Cq), 143.7 \text{ (Cq), 153.2 \text{ (Cq) and 188.1 \text{ (C=O);}})\)

\(\text{MS m/z (ES\(^+\)) Found 303.1360 (MH}\(^+\)\) and 325.1172 (MNa\(^+\)). C\(_{16}\)H\(_{19}\)N\(_2\)O\(_4\) (MH\(^+\)) requires 303.1345 and C\(_{16}\)H\(_{19}\)N\(_2\)O\(_4\)Na (MNa\(^+\)) requires 325.1164.\)
References


Compound B1

[Graph showing NMR spectral data for Compound B1 with peaks at various ppm values.]
Compound A3

[Chemical structure and spectrum images]
Compound C5

Compound C5 – diluted sample
HPLC traces at 214 nm

**Compound A1**

**Compound B1**

**Compound C1**
### Compound A2

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<th>Type</th>
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### Compound B2

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### Compound C2

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Compound C3-H$_2$

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Compound C4

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Compound C5

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Compound C7

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HPLC traces at 254 nm

**Compound A1**

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**Compound B1**

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<td>144.9597</td>
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### Compound C1

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<th>Amount</th>
<th>Type</th>
<th>Height</th>
<th>Rel.Area</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
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<td>9.987</td>
<td>120.746</td>
<td>n.a.</td>
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<td>140.517</td>
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<tr>
<td>2</td>
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<td>10.100</td>
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### Compound A2

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<th>Area</th>
<th>Amount</th>
<th>Type</th>
<th>Height</th>
<th>Rel.Area</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n.a.</td>
<td>5.867</td>
<td>0.426</td>
<td>n.a.</td>
<td>BMB</td>
<td>4.279</td>
<td>0.57</td>
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<td>3.911</td>
<td>0.45</td>
<td>19.73</td>
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<tr>
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</table>
### Compound B2

![Graph](image1)

<table>
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<th>Ret.Time</th>
<th>Area</th>
<th>Amount</th>
<th>Type</th>
<th>Height</th>
<th>Rel.Area</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>2.058</td>
<td>3.8519</td>
<td>n.a.</td>
<td>BMB</td>
<td>19.661</td>
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<td>BMB</td>
<td>1919.342</td>
<td>97.32</td>
<td>6.53</td>
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<td>5.446</td>
<td>0.49</td>
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Total: 171.3787 0.0000 1541.151 100.00

### Compound C2

![Graph](image2)

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<th>Ret.Time</th>
<th>Area</th>
<th>Amount</th>
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<th>Height</th>
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<th>Resolution</th>
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<td>0.92</td>
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Total: 48.1247 0.0000 448.763 100.00
### Compound A3

![Graph of Compound A3](image)

<table>
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<tr>
<th>No.</th>
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<th>Ret.Time (min)</th>
<th>Area (mAU.min)</th>
<th>Amount (mAU)</th>
<th>Type</th>
<th>Height (mAU)</th>
<th>Ret.Area %</th>
<th>Resolution</th>
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</thead>
<tbody>
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<td>94.3217</td>
<td>n.a.</td>
<td>BM</td>
<td>153.105</td>
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### Compound B3

![Graph of Compound B3](image)

<table>
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<th>Area (mAU.min)</th>
<th>Amount (mAU)</th>
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<th>Height (mAU)</th>
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<th>Resolution</th>
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### Compound C3

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### Compound C3-H₂

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**Compound C4**

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<th>Area mAU*min</th>
<th>Amount</th>
<th>Type</th>
<th>Height mAU</th>
<th>Rel Area %</th>
<th>Resolution</th>
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<tbody>
<tr>
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<td>84.573</td>
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**Compound C5**

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<th>Area mAU*min</th>
<th>Amount</th>
<th>Type</th>
<th>Height mAU</th>
<th>Rel Area %</th>
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<td>83.8075</td>
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### Compound C6

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<th>Area (mAU/min)</th>
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<th>Type</th>
<th>Height (mAU)</th>
<th>Rel. Area (%)</th>
<th>Resolution</th>
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### Compound C7

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<th>Area (mAU/min)</th>
<th>Amount</th>
<th>Type</th>
<th>Height (mAU)</th>
<th>Rel. Area (%)</th>
<th>Resolution</th>
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<tbody>
<tr>
<td>1</td>
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<td>BM6</td>
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</tbody>
</table>
Compound A1

HT29 Human Colon Carcinoma
Test Compound AC01:44
3 Day Exposure MTS

IC\text{50} = >500\mu M

HT29 Human Colon Carcinoma
Test Compound AC01:44
3 Day Exposure MTS

IC\text{50} = >500\mu M

1% DMSO only
Points are means ± s.d
n = 4
Compound A1

MDA231 Human Breast Carcinoma
Test Compound AC01:44
3 Day Exposure MTS

MDA231 Breast Carcinoma
Test Compound AC01:44
3 Day Exposure MTS

IC\textsubscript{50} = >500\mu M

1% DMSO only
Points are means ± s.d
n = 4
Compound A1

LNCAP Prostate Carcinoma
Test Compound AC01:44
3 Day Exposure MTS

![Graph 1](image1)

\[ \text{IC}_{50} = >500 \mu M \]

AC01:44 Concentration (nM)

LNCAP Prostate Carcinoma
Test Compound AC01:44
3 Day Exposure MTS

![Graph 2](image2)

\[ \text{IC}_{50} = >500 \mu M \]

AC01:44 Concentration (nM)

---

1% DMSO only
Points are means ± s.d
n = 4
Compound A1

FEK-4 Human Skin Fibroblast
Test Compound AC01:44
3 Day Exposure MTS

$IC_{50} = >500 \, \mu M$

FEK-4 Human Skin Cells
Test Compound AC01:44
3 Day Exposure MTS

$IC_{50} = >500 \, \mu M$

FEK-4 Human Skin Fibroblast
Test Compound AC01:44
3 Day Exposure MTS

$IC_{50} = >500 \, \mu M$

1% DMSO only
Points are means ± s.d
n = 4
**Compound B1**

HT29 Human Colon Carcinoma
Test Compound AC01:45
3 Day Exposure MTS

![Graph 1](Image)

$IC_{50} = 88.5 \mu M$

HT29 Human Colon Carcinoma
Test Compound AC01:45
3 Day Exposure MTS

![Graph 2](Image)

$IC_{50} = 87.2 \mu M$

HT29 Human Colon Carcinoma
Test Compound AC01:45
3 Day Exposure MTS

![Graph 3](Image)

$IC_{50} = 82.4 \mu M$

- 1% DMSO only
- Points are means ± s.d
- $n = 4$
Compound B1

**MDA231 Human Breast Carcinoma**
Test Compound AC01:45  
3 Day Exposure MTS

**MDA231 Breast Carcinoma**
Test Compound AC01:45  
3 Day Exposure MTS

![Graph 1](IC50 = 105.6 μM)

![Graph 2](IC50 = 98.3 μM)

**MDA231 Breast Carcinoma**
Test Compound AC01:45  
3 Day Exposure MTS

![Graph 3](IC50 = 103.3 μM)

1% DMSO only  
Points are means ± s.d  
n = 4
**Compound B1**

**LNCAP Prostate Carcinoma**
Test Compound AC01:45
3 Day Exposure MTS

**IC₅₀ = 114.0 µM**

**LNCAP Prostate Carcinoma**
Test Compound AC01:45
3 Day Exposure MTS

**IC₅₀ = 94.5 µM**

**LNCAP Prostate Carcinoma**
Test Compound AC01:45
3 Day Exposure MTS

---

1% DMSO only
Points are means ± s.d
n = 4

**IC₅₀ = 105.2µM**
Compound B1

FEK-4 Human Skin Fibroblast
Test Compound AC01:45
3 Day Exposure MTS

\[ \text{IC}_{50} = 176.0 \, \mu \text{M} \]

FEK-4 Human Skin Cells
Test Compound AC01:45
3 Day Exposure MTS

\[ \text{IC}_{50} = 126.1 \, \mu \text{M} \]

FEK-4 Human Skin Fibroblast
Test Compound AC01:45
3 Day Exposure MTS

\[ \text{IC}_{50} = 103.2 \, \mu \text{M} \]

- 1% DMSO only
- Points are means ± s.d
- n = 4
Compound C1

HT29 Human Colon Carcinoma
Test Compound SKL01:08
3 Day Exposure MTS

\[ \text{IC}_{50} = 49.2 \mu M \]

HT29 Human Colon Carcinoma
Test Compound SKL01:08
3 Day Exposure

\[ \text{IC}_{50} = 45.8 \mu M \]

HT29 Human Colon Carcinoma
Test Compound SKL01:08
3 Day Exposure

\[ \text{IC}_{50} = 34 \mu M \]

\[ 1\% \text{ DMSO only} \]
Points are means \( \pm \) s.d
\( n = 4 \)
Compound C1

**MDA231 Breast Carcinoma**

**Test Compound SKL01:08**

3 Day Exposure MTS

---

**IC$_{50}$ = 37.6 μM**

---

**MDA231 Breast Carcinoma**

**Test Compound SKL01:08**

3 Day Exposure MTS

---

**IC$_{50}$ = 51.2 μM**

---

**MDA231 Breast Carcinoma**

**Test Compound SKL01:08**

3 Day Exposure MTS

---

1% DMSO only

Points are means ± s.d

n = 4
Compound C1

IC₅₀ = 50.5 μM

IC₅₀ = 68.9 μM

IC₅₀ = 60.4 μM

1% DMSO only
Points are means ± s.d
n = 4

LNCAP Prostate Carcinoma
Test Compound SKL01:08
3 Day Exposure MTS
**Compound C1**

**FEK-4 Human Skin Cells**
Test Compound SKL01:08
3 Day Exposure MTS

![Graph 1](image1.png)

$IC_{50} = 144.6 \ \mu M$

**FEK-4 Human Skin Fibroblast**
Test Compound SKL01:08
3 Day Exposure MTS

![Graph 2](image2.png)

$IC_{50} = 125.4 \ \mu M$

**FEK-4**
Test Compound SKL01:08
3 Day Exposure MTS

![Graph 3](image3.png)

$IC_{50} = 198 \ \mu M$

- 1% DMSO only
- Points are means ± s.d
- n = 4
Compound A2

HT29 Human Colon Carcinoma
Test Compound AC01.08
3 Day Exposure MTS

\[ \text{IC}_{50} = 64.7 \ \mu M \]

HT29 Human Colon Carcinoma
MTS Cell Proliferation Assay
Test Compound: AC01.08
72h Exposure

\[ \text{IC}_{50} = 61.6 \ \mu M \]

\[ \text{IC}_{50} = 59 \mu M \]

1% DMSO only
Points are means ± s.d
n = 4
Compound A2

MDA231 Breast Carcinoma
Test Compound AC01:08
3 Day Exposure MTS

MDA231 Human Breast Carcinoma
Test Compound AC01:08
3 Day Exposure MTS

**IC₅₀** = 50.3 μM

**IC₅₀** = 50.1 μM

**IC₅₀** = 60.0 μM

1% DMSO only
Points are means ± s.d
n = 4
Compound A2

LNCAP Prostate Carcinoma
Test Compound AC01:08
3 Day Exposure MTS

IC_{50} = 68.2 \mu M

LNCAP Prostate Carcinoma
Test Compound AC01:08
3 Day Exposure MTS

IC_{50} = 78.0 \mu M

LNCAP Prostate Carcinoma
Test Compound AC01:08
3 Day Exposure MTS

1% DMSO only
Points are means ± s.d
n = 4

IC_{50} = 80.4 \mu M
Compound A2

FEK-4 Human Skin Fibroblast
Test Compound AC01.08
3 Day Exposure MTS

FEK-4 Human Skin Cells
Test Compound AC01.08
3 Day Exposure MTS

IC\textsubscript{50} = 293.2 \mu M

IC\textsubscript{50} = 122.9 \mu M

FEK-4 Human Skin Fibroblast
Test Compound AC01.08
3 Day Exposure MTS

IC\textsubscript{50} = 148.5 \mu M

1% DMSO only
Points are means \pm s.d
n = 4
Compound B2

**HT29 Human Colon Carcinoma**
- Test Compound AC01:13
- 3 Day Exposure MTS

![IC_{50} = 75.4 \mu M graph](image)

![IC_{50} = 54.9 \mu M graph](image)

**HT29 Human Colon Carcinoma**
- MTS Cell Proliferation Assay
- Test Compound: AC01:13
- 72h Exposure

![IC_{50} = 51 \mu M graph](image)

- 1% DMSO only
- Points are means ± s.d
- n = 4
 Compound B2

MDA231 Breast Carcinoma
Test Compound AC01:13
3 Day Exposure MTS

IC$_{50}$ = 40.1 µM

MDA231 Human Breast Carcinoma
Test Compound AC01:13
3 Day Exposure MTS

IC$_{50}$ = 33.4 µM

1% DMSO only
Points are means ± s.d
n = 4
Compound B2

LNCAP Prostate Carcinoma
Test Compound AC01:13
3 Day Exposure MTS

IC₅₀ = 68.1 μM

LNCAP Prostate Carcinoma
Test Compound AC01:13
3 Day Exposure MTS

IC₅₀ = 43.4 μM

LNCAP Prostate Carcinoma
Test Compound AC01:13
3 Day Exposure MTS

IC₅₀ = 57.3 μM

1% DMSO only
Points are means ± s.d
n = 4
Compound B2

FEK-4 Human Skin Fibroblast
Test Compound AC01:13
3 Day Exposure MTS

IC₅₀ = 202.11 µM

FEK-4 Human Skin Cells
Test Compound AC01:13
3 Day Exposure MTS

IC₅₀ = 108.4 µM

FEK-4 Human Skin Fibroblast
Test Compound AC01:13
3 Day Exposure MTS

IC₅₀ = 174.5 µM

1% DMSO only
Points are means ± s.d
n = 4
Compound C2

HT29 Human Colon Ca
Test Compound SKL01:05
3 Day Exposure MTS 2h

\[ IC_{50} = 12 \mu M \]

HT29 Human Colon Carcinoma
Test Compound SKL01:05
3 Day Exposure MTS

\[ IC_{50} = 17.6 \mu M \]

HT29 Human Colon Carcinoma
Test Compound AC01:04
3 Day Exposure MTS

- 1% DMSO only
- Points are means ± s.d
- n = 4

\[ IC_{50} = 8.0 \mu M \]
Compound C2

**MDA231 Breast Carcinoma**
Test Compound SKL01.05
3 Day Exposure MTS

**IC₅₀ = 9.5 µM**

**MDA231 Breast Carcinoma**
Test Compound SK01.06
3 Day Exposure MTS

**IC₅₀ = 20.0 µM**

**MDA231 Human Breast Carcinoma**
Test Compound ACD01.04
3 Day Exposure MTS

**IC₅₀ = 24.6 µM**

1% DMSO only
Points are means ± s.d
n = 4
Compound C2

**LNCAP Prostate Carcinoma**
Test Compound SKL01:05
3 Day Exposure MTS

**LNCAP Prostate Carcinoma**
Test Compound SKL01:05
3 Day Exposure MTS

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**IC**<sub>50</sub> = 59.6 μM

**IC**<sub>50</sub> = 81.3 μM

**IC**<sub>50</sub> = 67.5 μM

- 1% DMSO only
  Points are means ± s.d
  n = 4
Compound C2

FEK-4 Human Skin Cells
Test Compound SKL01:05
3 Day Exposure MTS

IC$_{50}$ = 145.7 μM

FEK-4 Human Skin Fibroblast
Test Compound SKL01:05
3 Day Exposure MTS

IC$_{50}$ = 100.7 μM

FEK-4
Test Compound SKL01:05
3 Day Exposure MTS

IC$_{50}$ = 106 μM

% DMSO only
Points are means ± s.d
n = 4
**Compound A3**

HT29 Human Colon Carcinoma
Test Compound AC01:21
3 Day Exposure MTS

**IC₅₀ = 24.5 µM**

AC01:21 Concentration (µM)

HT29 Human Colon Carcinoma
MTS Cell Proliferation Assay
Test Compound: AC01:21
72h Exposure

**IC₅₀ = 18.3 µM**

AC01:21 Concentration (µM)

1% DMSO only
Points are means ± s.d
n = 4

**IC₅₀ = 28 µM**

OD 450nm

Concentration (M)
Compound A3

MDA231 Breast Carcinoma
Test Compound AC01:21
3 Day Exposure MTS

IC$_{50}$ = 17.4 µM

MDA231 Human Breast Carcinoma
Test Compound AC01:21
3 Day Exposure MTS

IC$_{50}$ = 13.0 µM

MDA231 Human Breast Carcinoma
Test Compound AC01:21
3 Day Exposure MTS

IC$_{50}$ = 22.3 µM

1% DMSO only
Points are means ± s.d
n = 4
Compound A3

LNCAP Prostate Carcinoma
Test Compound AC01.21
3 Day Exposure MTS

IC$_{50}$ = 25.7 μM

LNCAP Prostate Carcinoma
Test Compound AC01.21
3 Day Exposure MTS

IC$_{50}$ = 30.7 μM

LNCAP Prostate Carcinoma
Test Compound AC01.21
3 Day Exposure MTS

IC$_{50}$ = 44.2 μM

1% DMSO only
Points are means ± s.d
n = 4
Compound A3

FEK-4 Human Skin Fibroblast
Test Compound AC01:21
3 Day Exposure MTS

![Graph 1](IC50 = 103.2 μM)

FEK-4 Human Skin Cells
Test Compound AC01:21
3 Day Exposure MTS

![Graph 2](IC50 = 80.8 μM)

- 1% DMSO only
- Points are means ± s.d
- n = 4
Compound B3

HT29 Human Colon Carcinoma
Test Compound AC01:20
3 Day Exposure MTS

IC₅₀ = 50.0 µM

AC01:20 Concentration (µM)

HT29 Human Colon Carcinoma
Test Compound AC01:20
3 Day Exposure MTS

IC₅₀ = 32.7 µM

AC01:20 Concentration (µM)

HT29 Human Colon Carcinoma
MTS Cell Proliferation Assay
Test Compound AC01:20
72h Exposure

IC₅₀ = 31 µM

Concentration (M)

1% DMSO only
Points are means ± s.d
n = 4
Compound B3

MDA231 Breast Carcinoma
Test Compound AC01:20
3 Day Exposure MTS

IC$_{50} = 17.1 \ \mu M$

MDA231 Breast Carcinoma
Test Compound AC01:20
3 Day Exposure MTS

IC$_{50} = 16.9 \ \mu M$

MDA231 Human Breast Carcinoma
Test Compound AC01:20
3 Day Exposure MTS

IC$_{50} = 20.6 \ \mu M$

1% DMSO only
Points are means ± s.d
n = 4
Compound B3

LNCAP Prostate Carcinoma
Test Compound AC01:20
3 Day Exposure MTS

IC₅₀ = 47.5 µM

LNCAP Prostate Carcinoma
Test Compound AC01:20
3 Day Exposure MTS

IC₅₀ = 52.2 µM

LNCAP Prostate Carcinoma
Test Compound AC01:20
3 Day Exposure MTS

IC₅₀ = 39.0 µM

1% DMSO only
Points are means ± s.d
n = 4
Compound B3

FEK-4 Human Skin Fibroblast
Test Compound AC01:20
3 Day Exposure MTS

![Graph 1](IC_50 = 94.3 \mu M)

AC01:20 Concentration (\mu M)

FEK-4 Human Skin Cells
Test Compound AC01:20
3 Day Exposure MTS

![Graph 2](IC_50 = 62.3 \mu M)

AC01:20 Concentration (\mu M)

FEK-4 Human Skin Fibroblast
Test Compound AC01:20
3 Day Exposure MTS

![Graph 3](IC_50 = 96.0 \mu M)

AC01:20 Concentration (\mu M)

- 1% DMSO only
- Points are means ± s.d
- n = 4
Compound C3

HT29 Human Colon Carcinoma
Test Compound AC01:18
3 Day Exposure MTS

IC$_{50}$ = 19.5 μM

HT29 Human Colon Carcinoma
Test Compound AC01:18
3 Day Exposure MTS

IC$_{50}$ = 20.1 μM

HT29 Human Colon Carcinoma
MTS Cell Proliferation Assay
Test Compound: AC01:18
72h Exposure

IC$_{50}$ = 19 μM

1% DMSO only
Points are means ± s.d
n = 4
Compound C3

MDA231 Breast Carcinoma
Test Compound AC01:18
3 Day Exposure MTS

IC₅₀ = 27.1 µM

MDA231 Breast Carcinoma
Test Compound AC01:18
3 Day Exposure MTS

IC₅₀ = 21.5 µM

MDA231 Breast Carcinoma
Test Compound AC01:18
3 Day Exposure MTS

1% DMSO only
Points are means ± s.d
n = 4

IC₅₀ = 20.1 µM
Compound C3

LNCAP Prostate Carcinoma
Test Compound AC01:18
3 Day Exposure MTS

LNCAP Prostate Carcinoma
Test Compound AC01:18
3 Day Exposure MTS

IC$_{50}$ = 53.7 μM

IC$_{50}$ = 51.1 μM

LNCAP Prostate Carcinoma
Test Compound AC01:18
3 Day Exposure MTS

IC$_{50}$ = 39.4 μM

1% DMSO only
Points are means ± s.d
n = 4
**Compound C3**

FEK-4 Human Skin Cells
Test Compound AC01:18
3 Day Exposure MTS

FEK-4 Human Skin Fibroblast
Test Compound AC01:18
3 Day Exposure MTS

---

**IC$_{50}$ = 45.2 µM**

1% DMSO only
Points are means ± s.d
n = 4
**Compound C3-H₂**

**HT29 Colon Carcinoma**
Test Compound AC01:39
3 Day Exposure MTS

**IC₅₀ = >500µM**

**HT29 Human Colon Carcinoma**
MTS Cell Proliferation Assay
Test Compound AC01:39
72h Exposure

1% DMSO only
Points are means ± s.d
n = 4

**IC₅₀ = 660µM**
Compound C3-H₂

MDA231 Breast Carcinoma
Test Compound AC01:39
3 Day Exposure MTS

MDA231 Human Breast Carcinoma
Test Compound AC01:39
3 Day Exposure MTS

IC₅₀ = 216.4 µM

MDA231 Human Breast Carcinoma
Test Compound AC01:39
3 Day Exposure MTS

IC₅₀ = 246.5 µM

IC₅₀ = 207.2 µM

1% DMSO only
Points are means ± s.d
n = 4
**Compound C3-H₂**

LNCAP Prostate Carcinoma
Test Compound AC01:39
3 Day Exposure MTS

IC₅₀ ≈ 500 μM

LNCAP Prostate Carcinoma
Test Compound AC01:39
3 Day Exposure MTS

IC₅₀ ≈ 385 μM

1% DMSO only
Points are means ± s.d
n = 4

IC₅₀ = 218.0 μM
Compound C3-H$_2$

FEK-4
Test Compound AC01:39
3 Day Exposure MTS

![Graph showing concentration vs. response]

IC$_{50}$ = >500 µM

1% DMSO only
Points are means ± s.d
n = 4
Compound C4

**HT29 Human Colon Carcinoma**
**Test Compound AC01:50**
**3 Day Exposure MTS**

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**HT29 Human Colon Carcinoma**
**Test Compound AC01:50**
**3 Day Exposure MTS**

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**IC\textsubscript{50} = 15.4 \mu M**

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**HT29 Human Colon Carcinoma**
**MTS Cell Proliferation Assay**
**Test Compound AC01:50**
**72h Exposure**

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1% DMSO only
Points are means ± s.d
n = 4

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**IC\textsubscript{50} = 18 \mu M**
Compound C4

MDA231 Breast Carcinoma
Test Compound AC01:50
3 Day Exposure MTS

\[ \text{IC}_{50} = 15.5 \mu M \]

MDA231 Breast Carcinoma
Test Compound AC01:50
3 Day Exposure MTS

\[ \text{IC}_{50} = 18.5 \mu M \]

MDA231 Breast Carcinoma
Test Compound AC01:50
3 Day Exposure MTS

\[ \text{IC}_{50} = 16.7 \mu M \]

1% DMSO only
Points are means ± s.d
n = 4
Compound C4

LNCAP Prostate Carcinoma
Test Compound AC01:50
3 Day Exposure MTS

IC₅₀ = 31.9 μM

AC01:50 Concentration (μM)

LNCAP Prostate Carcinoma
Test Compound AC01:50
3 Day Exposure MTS

IC₅₀ = 38.3 μM

AC01:50 Concentration (μM)

LNCAP Prostate Carcinoma
Test Compound AC01:50
3 Day Exposure MTS

IC₅₀ = 21.7 μM

AC01:50 Concentration (μM)

1% DMSO only
Points are means ± s.d
n = 4
Compound C4

FEK-4 Human Skin Fibroblast
Test Compound AC01:50
3 Day Exposure MTS

IC$_{50}$ = 50.0 µM

FEK-4 Human Skin Cells
Test Compound AC01:50
3 Day Exposure MTS

IC$_{50}$ = 51.7 µM

FEK-4 Human Skin Fibroblast
Test Compound AC01:50
3 Day Exposure MTS

IC$_{50}$ = 47.9 µM

Points are means ± s.d
n = 4

1% DMSO only
Compound C5

**HT29 Human Colon Carcinoma**
**Test Compound AC01:42T**
**3 Day Exposure MTS**

![Graph 1](image1)

**IC$_{50}$ = 4.28 µM**

![Graph 2](image2)

**IC$_{50}$ = 2.0 µM**

**HT29 Human Colon Carcinoma**
**Test Compound AC01:42T**
**3 Day Exposure MTS**

![Graph 3](image3)

**IC$_{50}$ = 2.5 µM**

- 1% DMSO only
- Points are means ± s.d
- $n = 4$
**Compound C5**

MDA231 Breast Carcinoma
Test Compound AC01:42T
3 Day Exposure MTS

IC$_{50} = 3.5 \mu M$

AC01:42T Concentration (uM)

MDA231 Breast Carcinoma
Test Compound AC01:42T
3 Day Exposure MTS

IC$_{50} = 7.4 \mu M$

AC01:42T Concentration (uM)

MDA231 Breast Carcinoma
Test Compound AC01:42T
3 Day Exposure MTS

1% DMSO only
Points are means ± s.d
n = 4

IC$_{50} = 3.5 \mu M$

AC01:42T Concentration (uM)
Compound C5

LNCAP Prostate Carcinoma
Test Compound AC01:42T
3 Day Exposure MTS

\[ \text{IC}_{50} = 63.9 \, \mu M \]

LNCAP Prostate Carcinoma
Test Compound AC01:42T
3 Day Exposure MTS

\[ \text{IC}_{50} = 41.2 \, \mu M \]

1% DMSO only
Points are means ± s.d
n = 4

\[ \text{IC}_{50} = 40.0 \, \mu M \]
Compound C5

FEK-4 Human Skin Fibroblast Cells
Test Compound AC01:42T
3 Day Exposure MTS

IC$_{50}$ = 75.9 µM

FEK4 Human Skin Fibroblast
Test Compound AC01:42T
3 Day Exposure MTS

IC$_{50}$ = 64.9 µM

FEK4 Human Skin Fibroblast
Test Compound AC01:42T
3 Day Exposure MTS

1% DMSO only
Points are means ± s.d
n = 4

IC$_{50}$ = 114.2 µM
Compound C6

HT29 Human Colon Carcinoma
Test Compound AC01:52
3 Day Exposure MTS

IC_{50} = 5.4 \mu M

HT29 Human Colon Carcinoma
Test Compound AC01:52
3 Day Exposure MTS

IC_{50} = 4.5 \mu M

MTS Cell Proliferation Assay
HT29 Human Colon Carcinoma
Test Compound AC01:52
72h Exposure

IC_{50} = 5.0 \mu M

1% DMSO only
Points are means ± s.d
n = 4
Compound C6

MDA231 Breast Carcinoma
Test Compound AC01:52
3 Day Exposure MTS

IC$_{50}$ = 3.8 µM

MDA231 Breast Carcinoma
Test Compound AC01:52
3 Day Exposure MTS

IC$_{50}$ = 5.6 µM

MDA231 Breast Carcinoma
Test Compound AC01:52
3 Day Exposure MTS

IC$_{50}$ = 5.2 µM

1% DMSO only
Points are means ± s.d
n = 4
Compound C6

LNCAP Prostate Carcinoma
Test Compound AC01:52
3 Day Exposure MTS

IC_{50} = 13.2 \, \mu M

LNCAP Prostate Carcinoma
Test Compound AC01:52
3 Day Exposure MTS

IC_{50} = 19.2 \, \mu M

LNCAP Prostate Carcinoma
Test Compound AC01:52
3 Day Exposure MTS

IC_{50} = 19.0 \, \mu M

1\% \text{DMSO only}
Points are means \pm s.d
n = 4
Compound C6

FEK-4 Human Skin Fibroblast Cells
Test Compound AC01:52
3 Day Exposure MTS

IC\textsubscript{50} = 23.1 μM

AC01:52 Concentration (μM)

FEK-4 Human Skin Fibroblast
Test Compound AC01:52
3 Day Exposure MTS

IC\textsubscript{50} = 40.9 μM

AC01:52 Concentration (μM)

FEK-4 Human Skin Fibroblast
Test Compound AC01:52
3 Day Exposure MTS

IC\textsubscript{50} = 21.8 μM

AC01:52 Concentration (μM)

1% DMSO only
Points are means ± s.d
n = 4
Compound C7

HT29 Human Colon Carcinoma
Test Compound AC01:54
3 Day Exposure MTS

IC$_{50}$ = 4.3 µM

AC01:54 Concentration (µM)

HT29 Human Colon Carcinoma
Test Compound AC01:54
3 Day Exposure MTS

IC$_{50}$ = 3.58 µM

AC01:54 Concentration (µM)

HT29 Human Colon Carcinoma
Test Compound AC01:54
3 Day Exposure MTS

IC$_{50}$ = 4.8 µM

AC01:54 Concentration (µM)
Compound C7

MDA231 Human Breast Carcinoma
Test Compound AC01:54
3 Day Exposure MTS

IC₅₀ = 5.0 μM

MDA231 Breast Carcinoma
Test Compound AC01:54
3 Day Exposure MTS

IC₅₀ = 4.6 μM

MDA231 Breast Carcinoma
Test Compound AC01:54
3 Day Exposure MTS

IC₅₀ = 5.0 μM

1% DMSO only
Points are means ± s.d
n = 4
Compound C7

LNCAP Prostate Carcinoma
Test Compound AC01.54
3 Day Exposure MTS

IC$_{50} = 7.2$ μM

1% DMSO only
Points are means ± s.d
n = 4

LNCAP Prostate Carcinoma
Test Compound AC01.54
3 Day Exposure MTS

IC$_{50} = 11.6$ μM

LNCAP Prostate Carcinoma
Test Compound AC01.54
3 Day Exposure MTS

IC$_{50} = 14.3$ μM
Compound C7

FEK4 Human Skin Fibroblast Test Compound AC01:54 3 Day Exposure MTS

IC<sub>50</sub> = 16.8 µM

FEK4 Human Skin Fibroblast Test Compound AC01:54 3 Day Exposure MTS

IC<sub>50</sub> = 15.6 µM

FEK4 Human Skin Fibroblast Test Compound AC01:54 3 Day Exposure MTS

IC<sub>50</sub> = 20.1 µM

1% DMSO only
Points are means ± s.d
n = 4
Doxorubicin

HT29 Human Colon Carcinoma

IC50 = 0.164 µM

MDA-MB-231 Human Breast Carcinoma

IC50 = 0.120 µM

LNCaP Prostate Carcinoma

IC50 = 0.154 µM

1% DMSO only
Points are means ± s.d
n = 4

MedChemComm, 2011, 2 (10), 1011-1015