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Full Paper

Synthesis of Migrastatin and its Macroketone Analogue and In Vivo FRAP Analysis of the Macroketone on E-Cadherin Dynamics

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Limiting migration: A 100 mg scale synthesis of a macroketone analogue of migrastatin is reported. Treatment of invasive pancreatic cancer cells with the macroketone led to inhibition of E-cadherin dynamics in vivo in a manner consistent with increased cell adhesion and reduced invasive potential.

Antiproliferation (not sure this is a relevant key word)

cell adhesion
cell-migration inhibitors
E-cadherin
photobleaching

An efficient and scalable synthesis of a key acyclic intermediate used for the preparation of migrastatin and its macroketone analogue is described; Brown alkoxyalloylation is the key step for this synthesis. The macroketone was prepared on 100 mg scale by this route. Treatment of invasive pancreatic cancer cells grown on a cell-derived matrix or as subcutaneous tumours
in nude mice with the macroketone inhibited E-cadherin dynamics in a manner consistent with increased cell adhesion and reduced invasive potential.

Introduction

The primary cause of cancer-related death is metastasis, which generally begins with misregulation of cell adhesion and migration of cancer cells away from the primary tumour. A key molecule in this process is the cell-adhesion molecule and tumour suppressor E-cadherin, which is often down- or misregulated in many cancer types. Inhibition of E-cadherin dynamics is associated with decreased cell migration,\(^1\) thus suggesting that the development of cell migration inhibitors and gaining an understanding of their mode of action could help in the identification of new anticancer drugs.\(^2\)

Migrastatin (1, Figure\(^1\)) is a natural product that contains a macrocyclic lactone ring, and has been reported to inhibit tumour cell migration. Simpler congeners of migrastatin, such as macrolactone\(^2\), have shown improved activity in\(^\text{vitro}\) compared to migrastatin; some of these data are summarised “derivatives and their IC\(_{50}\) values are shown”\(^{\text{[Y]es - OK}}\) in Figure\(^1\).\(^{[3]}\) Some of the simpler analogues (e.g. 4-6) have inhibited migration of highly metastatic tumour cells in mouse models.\(^4\)

Compound\(^8\) showed antibranching activity and interfered with the assembly of filopodia, thus preventing crosslinking of fascin1-dependent actin filaments in\(^\text{vitro}\).\(^5\)

It has been proposed that macroketone\(^4\) targets the actin bundling protein fascin1 and that this explains the observed effects on cell migration.\(^6\) However, there is debate about the mechanism. Danishefsky and co-workers have suggested that an intracellular protein such fascin1 might not be the primary target. \(^{<\text{please add this reference - N. Lecomte, J. T. Njardarson, P. Nagorny, G. Yang, R. Downey, O. Ouerfelli, M. A. Moore, S. J. Danishefsky, Proc. Natl. Acadl. Sci. USA 2011, 108, 15074-1507}}\)

Other changes suggested to figure 1

For compounds 2-5 the IC\(_{50}\) values & cell line was taken from Ref. 3

For compound 6 the IC\(_{50}\) values & cell line were taken from Ref. 4b


For compound 8 the IC\(_{50}\) & cell line was taken from Ref. 5
Compound\(^{7}\), which was a potent inhibitor of metastasis in an in\(^{\text{vivo}}\) lung cancer model, bears a carboxylic acid, which would be expected to reduce permeability of the small molecule through the cell membrane and lead to a reduction in activity.

A number of different preparations of migrastatin\(^{3,7}\) and its core \((2)\)^{8} have been reported, as well as the semisynthesis from isomigrastatin (a precursor generated by fermentation).\(^{9}\) The truncated migrastatin analogues 2--7 and other such compounds have been prepared by chemical synthesis.\(^{3}\) Published syntheses have shown that 1--7 can be obtained via alcohol\(^^{16}\) (Scheme\(^{\text{xscr1}}\)). However this was not the case and led these researchers to propose that the primary target could be a receptor at the cell surface rather than one inside the cell.

**Synthesis of macroketone and migrastatin**

In fragment\(^{16}\) there are three chiral centres, a terminal olefin and an allylic alcohol with an alkene that has Z-configuration (Scheme\(^{\text{xxscr1}}\)). The Z configuration at C2,3 can be prepared by olefination by using Ando’s phosphonate ester.\(^{7b-c,10}\) It was envisaged that the stereocentres at C5,6 could potentially be installed by using diastereoselective Brown alkoxyallylation\(^{11}\) from the allyl methyl ether\(^^{9}\) and the chiral aldehyde\(^{10}\) (the latter as prepared by Trost et al.).\(^{12}\) The synthesis began with a scale-up of the method of Trost et al.; hence, the highly diastereoselective addition of [(Z)-\(\gamma\)-methoxyallyl]diisopinocampheyborane to aldehyde\(^{10}\) gave \(11\) after work-up.

Subsequent cleavage of the benzoate protecting group\(^{13}\) afforded diol\(^{12}\) (\(-4^{\text{g}}\)) as a single diastereoisomer in 73\(^{\%}\) yield from \(10\). Protection of both alcohol groups of \(12\) as TBS ethers and subsequent regioselective cleavage of the TBS group were achieved by using a catalytic quantity of tosic acid in methanol to give the primary alcohol\(^{13}\). Dess--Martin
oxidation gave the desired aldehyde\(^{14}\), and its subsequent treatment with Ando phosphonate\(^{15}\) gave the trisubstituted alkene, with the reaction proceeding with high Z selectivity \((Z/E=97:3)\) and 79\(^\%\) yield on a 1\(^\text{g}\) scale. When the reaction was scaled up \((-4.0\text{ g})\) a lower yield (67\(^\%\)) and some decrease of the Z selectivity was observed \((Z/E=90:10)\). Primary alcohol\(^{16}\) was obtained efficiently after reaction of the intermediate ester with diisobutylaluminum hydride (DIBAL-H). The synthesis of 16 \(<?\rangle\) can be “was” \(OK?<\rangle\) \((\text{yes} ‘\text{was}’ \text{ is ok})\) carried out with an overall yield of 41\(^\%\) from 10. The preparation of 16 by this route is competitive with the other methods for the synthesis of 16\(^{[2,6a,7b]}\) or its precursor or analogues.\(^{[6b-c,7b]}\)

From 16 we completed the synthesis of 1, 4 and the other truncated analogues according to procedures of Danishefsky and co-workers.\(^{[3]}\) Given the interest in 4 (its reported activity in\(^\text{in vivo}\) and interest in its mechanism of action),\(^{[5]}\) it was prepared in 100\(^\text{mg}\) quantity. Macroketone\(^4\) can be purified by flash chromatography (petroleum ether/EtOAc, 10:1). However, the compound can be contaminated by hydrocarbon and silicone grease (NMR evidence).\(<?\rangle<\rangle\) \((\text{reference? (or is this in the Supp Info?)})<\rangle\) \(Please \text{ add reference} \)


Fortunately, 4 was distilled by using a Kugelrohr apparatus under reduced pressure \((0.05\text{ mbar}, \text{T}=90^\circ\text{C}, 18\text{h})\) to afford pure 4 as a white solid, with no trace of grease contaminants. Our data for 16, 1 and 4 were in excellent agreement with published analytical data: \(\alpha\)\(^\text{D}\) for 4 was +69.1 \((c=0.48, \text{CHCl}_3)\), which compares well with a reported value, +77.6 \((c=0.5, \text{CHCl}_3)\);\(^{[3]}\) \(\alpha\)\(^\text{D}\) for 1 was +112 \((c=0.4, \text{CHCl}_3)\) \((\text{there is an error in the } \alpha\text{D} \text{ for 1; it should be the same as shown in Scheme 1})\), which compares well with the reported value, +115.3 \((c=1.0, \text{CHCl}_3)\);\(^{[2]}\) thus indicating that enantioselectivity by this route is high and gives the enantiomer with the same configuration as natural migrastatin. (Details, analytical data and NMR spectra are in the Supporting Information.)

**Effect of macroketone treatment on E-cadherin dynamics in\(^\text{in vitro}\) and in\(^\text{in vivo}\)**
An important characteristic of cancer cells is the ability to detach from the primary tumour and to become invasive and metastatic. This feature has been associated with the misregulation of epithelial cadherin (E-cadherin).\[14\] E-Cadherin is a cell–cell adhesion protein responsible for maintaining cell polarity, epithelial architecture and cell differentiation “involved in cell differentiation, establishing cell polarity and maintaining epithelial architecture”?\[15\] Reduced levels of E-cadherin have been found in gastric, breast, colon, prostate, endometrial and ovarian cancer.\[16\] Cells with reduced levels of E-cadherin are more likely to become motile, and this characteristic is linked to the metastatic potential of epithelial cancers.\[1,15\]

It was previously demonstrated that E-cadherin dynamics assessed by fluorescence recovery after photobleaching (FRAP) can be used as a pharmacodynamic marker both in vitro and in vivo.\[1,17\] Photobleaching is a widely used technique for the study of molecular dynamics within cultured cells. In FRAP analysis, a small region of the sample is bleached with a laser, and recovered fluorescence in the bleached region is assessed over time.\[18\] Two variables are measured: 1) half-time recovery (HTR) and 2) immobile fraction (IF). Half-time recovery reflects the rate at which fluorescent molecules move into or out of the bleached region. The immobile fraction indicates how many of the fluorescent molecules remain trapped in the bleached region. This method is useful for evaluating the effects of small molecules on E-cadherin dynamics.\[1,19\] It was previously demonstrated that Fluorescence Recovery After Photobleaching (FRAP) of E-Cadherin–GFP can be used as a pharmacodynamic marker both in vitro and in vivo.\[1\] Serrels et al., Cancer Research 2009

No deleterious effects of E-cadherin–GFP in these cells were observed, and proliferation and cell junction formation appeared normal in the absence of small molecules. please add reference for this GFP experiment – cite to reference 1; also, “small molecules” have not been mentioned above (either absence or presence) -- please clarify (eg. which small molecules) \[^{ith reference to small molecules can be removed}^\]?

Therefore, FRAP was used to assess the effect of 4 on pancreatic ductal adenocarcinoma (PDAC) cells stably expressing E-cadherin–GFP. These cells are highly invasive because of expression of a mutant form of the tumour suppressor p53\[19\] and can
therefore serve as a potent model for the assessment of an antimetastatic therapy on cell adhesion. First, the effects of 4 were examined with cells cultured on a cell-derived matrix (CDM), a more biologically relevant substrate than glass for cell growth because it is compliant to forces exerted by cells and provides receptors for integrin engagement.\textsuperscript{[20]} PDAC cells were plated overnight, then control and macroketone-treated cells were subjected to FRAP analysis of E-cadherin dynamics. This analysis revealed that treatment with 4 had no effect on the immobile fraction of E-cadherin (Figure\textsuperscript{2A}, right), which is thought to be a measure of the amount of E-cadherin immobilised at cell--cell junctions. However, there was a significant increase in the half-time of recovery (Figure\textsuperscript{2A}, left), thus indicating an increase in transient binding of mobile E-cadherin with stationary E-cadherin clusters.

To assess the effects of macroketone treatment in\textsuperscript{vivo}, PDAC cells were injected subcutaneously into the flank of a nude mouse and a tumour was allowed to form. After seven days mice were treated with either vehicle or 20\textsuperscript{mg\textper kilogram} for three consecutive days, and intravital imaging was performed. Consistent with previous observations, we observed different effects on E-cadherin dynamics in\textsuperscript{vivo} compared to in\textsuperscript{vitro}.\textsuperscript{[1]} In\textsuperscript{vivo} treatment with 4 significantly increased the immobile fraction of E-cadherin in cell--cell junctions (Figure\textsuperscript{2B}, right), an effect expected to strengthen cell--cell adhesion and therefore impede metastasis. It also reduced the half-time of recovery (Figure\textsuperscript{2B}, left), however, this effect narrowly fell short of a test for significance.

Conclusions
We describe a new synthetic approach to key intermediate\textsuperscript{16}, which is used in the preparation of macroketone\textsuperscript{4}, migrastatin\textsuperscript{1} and other truncated migrastatin analogues. The synthetic route reported herein is competitive with other synthetic procedures. The effect of treatment with 4 on E-cadherin dynamics was studied by using FRAP. The immobile fraction of E-cadherin increased significantly in\textsuperscript{vivo} but not in\textsuperscript{vitro}. The differential effects in\textsuperscript{vitro} and in\textsuperscript{vivo} observed here are in general agreement with previous results using the Src inhibitor Dasatinib.\textsuperscript{[1]} Further experiments are warranted to confirm the effects of macroketone treatment in\textsuperscript{vivo}.\textsuperscript{[21]} The synthesis of new analogues from \textsuperscript{16} and their
Experimental Section

**General:** NMR spectra were recorded on a 500 MHz spectrometer at 30°C. Chemical shifts are reported relative to internal Me$_4$Si in CDCl$_3$ (δ=0.0 ppm) for $^1$H and CDCl$_3$ (δ=77.16 ppm) for $^{13}$C at 30°C, unless otherwise stated. $^{13}$C signals were assigned with the aid of HSQC. Coupling constants are reported in Hertz. High-resolution mass spectra were measured by using an LC-Orbitrap mass spectrometer and were measured in positive and/or negative mode as indicated. TLC was performed on aluminium sheets precoated with Silica Gel 60 (HF254, Merck Millipore) and spots were visualised by charring with vanillin solutions. Chromatography was carried out by using silica gel 60 (0.040–0.630 mm, E. Merck). Dichloromethane, tetrahydrofuran, MeOH and toluene were used as obtained from a Pure Solv solvent purification system. Petroleum ether is the fraction with b.p.=40–60°C.

$^{**}(2R,3S,4S)-4$-Methoxy-$2$-methylhex-$5$-ene-$1,3$-diol (12): sec-Butyllithium in cyclohexane (36.2 mmL, 1.3 M) was added to a stirred solution of allyl methyl ether$^9$ (5.4 mmL, 57.5 mmol) in THF (80 mmL) at $<$M$\text{->78}^\circ$C over 20 min. The mixture was stirred at $<$M$\text{->78}^\circ$C for an additional 40 min, then $\beta$-methoxydiisopinocampheylborane (17.09 mmg, 54.0 mmol) in THF (90 mmL) was added dropwise through a cannula. The mixture was stirred at $<$M$\text{->78}^\circ$C for 1.5 h, then boron trifluoride etherate (8.80 mmL, 69.7 mmol) was added dropwise at $<$M$\text{->78}^\circ$C. Immediately, a solution of aldehyde$^8$ (6.60 mmg, 34.9 mmol) in THF (80 mmL) was added, and the mixture was stirred at $<$M$\text{->78}^\circ$C for 28 h. Then aqueous NaOH (1 M, 24.9 mmL) was added followed by H$_2$O$_2$ (30%, 24.9 mmL), and the biphasic mixture was allowed to equilibrate to room temperature slowly. After stirring for another 16 h the mixture was diluted with ethyl ether, then the organic layer was separated, washed with saturated aqueous NH$_4$Cl and dried over Na$_2$SO$_4$, and the solvent was removed under reduced pressure. Chromatography of the residue (petroleum ether/EtOAc, 6:1) afforded a mixture of 11 and pinenol (total weight=23.5 mmg), which was then used directly in the next step. The mixture containing 11 (23.5 mmg) was dissolved in dry methanol (100 mmL), K$_2$CO$_3$...
(5.50 g) was added, the mixture was stirred at room temperature for 64 h, and the solvent was then removed under reduced pressure. The residue was taken up in a mixture of H₂O and EtOAc, and the organic layer was isolated. The aqueous layer was further extracted with EtOAc (10×100 mL), combined organic extracts were dried over Na₂SO₄, and the solvent was removed under reduced pressure. The residue was purified by chromatography (petroleum ether/EtOAc, 2:1) to give 12 (4.10 g, 73% from 10) as a colourless oil. ¹H NMR (500 MHz, CDCl₃): δ=5.59 (ddd, J₅,trans=17.0, J₅, cis=10.6, J₄,5=8.3 Hz, 1H; H-5), 5.38–5.29 (m, 2H; H-6), 3.76–3.69 (m, 2H; H-3, H-1), 3.68–3.62 (m, 1H; H-1'), 3.51 (appt t, J₂,₃=8.3 Hz, 1H; H-4), 3.32 (s, 3H; OCH₃), 2.84 (t, J₀,OH=1.6 Hz, 1H; OH at C-3), 2.36 (dd, J₀,OH=7.6, J₀,OH=3.8 Hz, 1H; OH at C-2), 1.81–1.73 (m, 1H; H-2), 0.97 ppm (d, J₁,₂=7.0 Hz, 3H; CH₃ at C-2); ¹³C NMR (125 MHz, CDCl₃): δ=134.4 (C-5), 120.6 (C-6), 85.0 (C-4), 75.8 (C-3), 67.5 (C-1), 56.4 (OCH₃), 35.8 (C-2), 9.7 ppm (CH₃ at C-2); HRMS-ESI: calc. for C₁₀H₁₆O₂Na: 183.0997, found: 183.0999.

<->(2R,3S,4S)-3-(tert-Butyldimethylsilyloxy)-4-methoxy-2-methylhex-5-en-1-ol (13): Triethylamine (10.7 mL, 76.9 mmol) and TBSOTf (14.1 mL, 61.6 mmol) were added to a solution of 12 (4.10 g, 25.6 mmol) in dichloromethane (270 mL) at 0°C. The reaction mixture was stirred at 0°C for 1.5 h, quenched with saturated aqueous NH₄Cl and extracted with dichloromethane (4×100 mL). The combined organic portion was dried over Na₂SO₄, and the solvent was removed under reduced pressure to give (2R,3S,4S)-1,3-di-(tert-butyldimethylsilyloxy)-4-methoxy-2-methylhex-5-en-1-ol. This was dissolved in methanol (270 mL), then p-TSA (487 mg, 2.57 mmol) was added at 0°C, and the mixture was stirred at room temperature for 2 h. Triethyl amine (0.35 mL, 2.55 mmol) was then added, and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether/EtOAc, 5:1) gave alcohol(^13) (5.89 g, 84% from 12) as a colourless oil. ¹H NMR (500 MHz, CDCl₃): δ=5.66 (ddd, J₅,trans=17.2, J₅, cis=10.5, J₄,5=7.9 Hz, 1H; H-5), 5.30–5.21 (m, 2H; H-6), 3.74 (dd, J=6.4, 3.6 Hz, 1H; H-3) 3.59–3.46 (m, 3H; H-4, H-1, H-1'), 3.25 (s, 3H; OCH₃), 2.19 (t, J₁,OH=4.8 Hz, 1H; OH), 1.85–1.74 (m, 1H; H-2), 0.89 (s, 9H; CH(CH₃)₃), 0.85 (d, J₂,₃=6.9 Hz, 3H; CH₃ at C-2), 0.08 (s, 3H; Si(CH₃)₃), 0.06 ppm (s, 3H; Si(CH₃)₃); ¹³C NMR (125 MHz, CDCl₃): δ=135.1 (C-5), 118.8 (C-6), 86.0 (C-4), 75.3 (C-3), 65.8 (C-1), 56.4 (OCH₃), 38.5 (C-2), 26.2 (CH(CH₃)₃), 18.6
(C(CH₃)₃), 11.3 (CH₃ at C-2), <M->3.8 (SiCH₃), <M->4.8±ppm (SiCH₃); HRMS-ESI: calcld for C₁₀H₄₀O₅SiNa: 297.1862, found: 297.1864.

1H NMR (500 MHz, CDCl₃): δ=5.56 (ddd, J₆,trans=17.1, J₃,trans=10.4, J₃=8.4 Hz, 1'H; H-5), 5.30-5.19 (m, 2'H; H-6); 3.81 (dd, J₃=8.0, 1.8 Hz, 1'H; H-3), 3.52 (dd, J₆=9.7, 8.3 Hz, 1'H; H-1), 3.42-3.35 (m, 2'H; H-1', H-4'), 3.22 (s, 3'H; OCH₃), 1.74-1.66 (m, 1'H; H-2), 0.89 (s, 9'H; C(CH₃)₃), 0.89 (s, 9'H; C(CH₃)₃), 0.76 (d, J=6.7 Hz, 3'H; CH₃ at C-2), 0.07 (s, 3'H; SiCH₃), 0.03±ppm (s, 6'H; SiCH₃); ¹³C NMR (125 MHz, CDCl₃): δ=135.3 (C-5), 118.6 (C-6), 87.0 (C-4), 73.6 (C-3), 65.9 (C-1), 56.1 (OCH₃), 38.2 (C-2), 26.3 (C(CH₃)₃), 26.1 (C(CH₃)₃), 18.8 (C(CH₃)₃), 18.4 (C(CH₃)₃), 9.4 (CH₃ at C-2), <M->3.6 (SiCH₃), <M->4.9 (SiCH₃), <M->5.1 (SiCH₃), <M->5.1±ppm (SiCH₃); HRMS-ESI: calcld for C₁₀H₄₀O₅SiNa: 411.2727, found: 411.2724.

The above paragraph could be removed from the paper if it is still confusing.

1H NMR (500 MHz, CDCl₃): δ=5.56 (ddd, J₆,trans=17.1, J₃,trans=10.4, J₃=8.4 Hz, 1'H; H-5), 5.30-5.19 (m, 2'H; H-6); 3.81 (dd, J₃=8.0, 1.8 Hz, 1'H; H-3), 3.52 (dd, J₆=9.7, 8.3 Hz, 1'H; H-1), 3.42-3.35 (m, 2'H; H-1', H-4'), 3.22 (s, 3'H; OCH₃), 1.74-1.66 (m, 1'H; H-2), 0.89 (s, 9'H; C(CH₃)₃), 0.89 (s, 9'H; C(CH₃)₃), 0.76 (d, J=6.7 Hz, 3'H; CH₃ at C-2), 0.07 (s, 3'H; SiCH₃), 0.03±ppm (s, 6'H; SiCH₃); ¹³C NMR (125 MHz, CDCl₃): δ=135.3 (C-5), 118.6 (C-6), 87.0 (C-4), 73.6 (C-3), 65.9 (C-1), 56.1 (OCH₃), 38.2 (C-2), 26.3 (C(CH₃)₃), 26.1 (C(CH₃)₃), 18.8 (C(CH₃)₃), 18.4 (C(CH₃)₃), 9.4 (CH₃ at C-2), <M->3.6 (SiCH₃), <M->4.9 (SiCH₃), <M->5.1 (SiCH₃), <M->5.1±ppm (SiCH₃); HRMS-ESI: calcld for C₁₀H₄₀O₅SiNa: 411.2727, found: 411.2724.
CDCl$_3$): $\delta$=9.70 (s, 1H; H-6), 5.68 (ddd, $J_{5,6$_{\text{trans}}}$=17.4, $J_{3,6$_{\text{cis}}}$=10.5, $J_{4,5}$=7.8 ppm, 1H; H-2), 5.32–5.25 (m, 2H; H-1), 4.11 (dd, $J_{3,4}$=6.0, $J_{2,3}$=3.8 ppm, 1H; H-4), 3.50 (dd, 1H; H-3), 3.21 (s, 3H; OCH$_3$), 2.41 (qd, $J_{3,4$_{\text{cis}}}$=7.0 ppm, 1H; H-5), 1.06 (d, 3H; CH$_3$ at C-5), 0.85 (s, 9H; C(CH$_3$)$_3$), 0.07 (s, 3H; Si(CH$_3$)$_3$), 0.05 ppm (s, 3H; Si(CH$_3$)$_3$); $^{13}$C NMR (125 ppm, CDCl$_3$): $\delta$=203.7 (C-6), 134.9 (C-2), 119.4 (C-1), 84.9 (C-3), 74.6 (C-4), 56.5 (OCH$_3$), 49.6 (C-5), 26.0 (C(CH$_3$)$_3$), 18.4 (C(CH$_3$)$_3$), 8.3 (CH$_3$ at C-2), <M->4.0 (Si(CH$_3$)$_3$), <M->4.9 ppm (Si(CH$_3$)$_3$); HRMS-ESI: calcd for C$_{14}$H$_{26}$O$_3$SiNa: 395.1705, found: 295.1699.

$(4R,5S,6S,Z)$-5-(tert-Butyldimethylsilyloxy)-6-methoxy-2,4-dimethylocta-2,7-dien-1-ol (16): Phosphonate$^{15}$ (1.39 g, 4.16 mmol) in THF (70 mL) was treated with NaH (60% dispersion in mineral oil, 233 mg, 5.82 mmol) at 0°C for 1 h. The mixture was cooled to <M->78°C, then 14 (1.133 g, 4.16 mmol) in THF (30 mL) was added, and stirring was continued for 30 min at <M->78°C. The resulting mixture was warmed to 0°C and left at this temperature for 15 h. The reaction was then quenched with saturated ammonium chloride (200 mL), and the aqueous layer was extracted with EtOAc (4×150 mL). The combined organic portion was dried over MgSO$_4$, and the solvent was then removed under reduced pressure. Chromatography of the residue (petroleum ether/EtOAc, 50:1) gave the intermediate ester (1.17 g, 79%), mixture of Z/E=97:3) as a colourless oil. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$=5.95 (dd, $J_{3,4}$=9.7, $J_{3,6$_{\text{cis}}}$=1.3, 1H; H-3), 5.64 (dd, $J_{3,8$_{\text{trans}}}$=17.2, $J_{3,8$_{\text{cis}}}$=10.3, $J_{6,7}$=8.5 ppm, 1H; H-7), 5.27 (dd, $J_{3,6$_{\text{cis}}}$=1.8, 1H; H-8$_{\text{cis}}$), 5.23 (dd, 1H; H-8$_{\text{trans}}$), 4.16 (q, $J$=7.1 ppm, 2H; OCH$_2$CH$_3$), 3.60 (dd, $J_{3,5}$=7.7, $J_{4,5}$=2.5 ppm, 1H; H-5), 3.36 (appt, 1H; H-6), 3.27–3.22 (m, 1H; H-4), 3.19 (s, 3H; OCH$_3$), 1.87 (d, 3H; CH$_3$ at C-2), 1.27 (t, 3H; OCH$_2$CH$_3$), 1.92 (d, $J_{3,6$_{\text{cis}}}$=6.7 ppm, 3H; CH$_3$; at C-4), 0.89 (s, 9H; C(CH$_3$)$_3$), 0.05 (s, 3H; SiCH$_3$), <M->0.00 ppm (s, 3H; SiCH$_3$); $^{13}$C NMR (125 ppm, CDCl$_3$): $\delta$=168.0 (C-1), 147.1 (C-3), 135.1 (C-7), 125.4 (C-2), 118.8 (C-8), 86.8 (C-6), 77.7 (C-5), 60.1 (OCH$_2$CH$_3$), 56.1 (OCH$_3$), 35.5 (C-4), 26.3 (C(CH$_3$)$_3$), 20.9 (CH$_3$ at C-2), 18.7 (C(CH$_3$)$_3$), 14.4 (OCH$_2$CH$_3$), 13.1 (CH$_3$ at C-4), <M->3.7 (SiCH$_3$), <M->4.9 ppm (SiCH$_3$); HRMS-ESI: calcd for C$_{14}$H$_{26}$O$_3$SiNa: 379.2281, found: 379.2269.

DIBAL-H (11.34 mL, 11.34 mmol, 1.0M in dichloromethane) was added to this intermediate (Z/E=97:3, 2.02 g, 5.67 mmol) in dichloromethane (300 mL) at <M->78°C. After stirring at <M->78°C for 10 min, the reaction was quenched with saturated aqueous potassium sodium tartrate (100 mL). The mixture was diluted with dichloromethane (120 mL) and vigorously stirred at room temperature for 2 h. The aqueous layer was extracted with dichloromethane (3×120 mL). The
combined organic portion was dried with MgSO_4_, then the solvent was removed under reduced pressure. Chromatography (cyclohexane/EtOAc, 10:1) of the residue gave Z-16 (1.599±g, 90±%) as a colourless oil. [α]_D^25±=+1.1° (c=0.71, CHCl_3); <2> correct reference? – change to reference

<3> Cell culture: Primary mouse PDAC cells were derived from tumours harvested from Pdx1-Cre, LSL-KRas^G12D^4, Trp53^LoxPl mice.<4>? What was the supplier for these mice?<4>?<5>.<10>


PDAC cells were subsequently stably transfected with E-cadherin-GFP and mutant p53^{175}. Cells were cultured in Dulbecco’s modified Eagle medium (DMEM) supplemented with FBS (10%) and L-glutamine (2±mM).

Cell-derived matrices (CDMs): Cell-derived matrices were grown on glass-bottom dishes as described previously.<2>–<3> Gelatin-coated glass-bottom dishes (MatTek) were crosslinked with glutaraldehyde, quenched, and equilibrated in DMEM containing 10% FCS. <2>NIH^3T3 fibroblasts or Tert-immortalised foetal fibroblasts telomerase-immortalised fibroblasts (TiFrFs) Which?

(and, where did they come from ?)<7> add the following citation "Human fibroblast replicative senescence can occur in the absence of extensive cell division and short telomeres, Munro J, Steeghs K, Morrison V, Ireland H, Parkinson EK, Oncogene, 2001 Jun 14;20(27):3541-52."<8> were seeded at near confluence (~2×10^4 cells/cm^2) and grown for eight days in DMEM containing 10%
FCS and 50 μg/mL ascorbic acid. Matrices were cleared of cells by incubation with PBS containing 20 mM NH₄OH and 0.5% Triton X-100, and DNA residues were “residual DNA was” (this change is ok) removed by incubation with DNaseI. Finally, matrices were blocked with 0.1% heat-denatured BSA prior to seeding of cells. 1×10⁶ cells were seeded unto the prepared dishes and allowed to adhere overnight. They were subsequently treated with 10 μM 4 and analysed by FRAP.

**Assessment of E-cadherin dynamics in vivo:**

Intravital imaging was preformed as described previously.[24] Following trypsinisation, cells (10⁶) were resuspended in 100 μL HBSS (Invitrogen) and subcutaneously injected into the rear flank of a CD1<sup>−/−</sup>/<sup>−/−</sup> nude mouse. Tumours were allowed to develop for seven days. Mice were subsequently treated for three days with daily intraperitoneal injections of either the PBS (vehicle) or 4 (20 mg/kg<sup>−1</sup>). Animals were kept in conventional animal facilities and all experiments were carried out in compliance with UK Home Office guidelines. To permit imaging, mice were terminally anaesthetised using an anaesthetic combination of hypnorm/hyponovel/H₂O (1:1:4). Following induction of anaesthesia the subcutaneous tumour was surgically exposed and the mouse restrained on a 37°C heated stage.

**Fluorescence recovery after photobleaching (FRAP):** FRAP was performed according to the method of Serrels et al.[1] Briefly, a focal plane was selected mid-way through a cell–cell junction, and a 30 pixel diameter circular region corresponding to 30 μm in the specimen plane was photobleached by using a 405 nm diode laser. Time-lapse recovery data were exported to SigmaPlot for analysis.

**Acknowledgements**

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<lit2><jnl>B. Hedley, E. Winquist, F. Chambers, Expert Opin. Ther. Targets 2004, 8, 527--536</jnl>.


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Scheme\textsuperscript{1} Synthesis of 16, migrastatin\textsuperscript{1} and macroketone\textsuperscript{4}. Reagents and conditions:
a)\textsuperscript{s}BuLi, THF, 15\textsuperscript{\textdegree}min, \textless M\textgreater 78\textsuperscript{\textdegree}C; b)\textsuperscript{(M+)}Ipc\textsubscript{2}BOMe, 1\textsuperscript{\textdegree}h, \textless M\textgreater 78\textsuperscript{\textdegree}C;
c)\textsuperscript{BF}_3\textsubscript{Et}_2O then \textless ?\textgreater 10 unit? \textless ?\textgreater; 20\textsuperscript{\textdegree}h, \textless M\textgreater 78\textsuperscript{\textdegree}C then 1\textsuperscript{\textdegree}m NaOH, 30\% H\textsubscript{2}O\textsubscript{2} 20\textsuperscript{\textdegree}h, RT; d)\textsuperscript{K}_2CO\textsubscript{3}, MeOH, RT, 18\textsuperscript{\textdegree}h, 73\% from 10; e)\textsuperscript{TBSOTf}, Et\textsubscript{3}N, CH\textsubscript{2}Cl\textsubscript{2}, 0\textsuperscript{\textdegree}C, 1.5\textsuperscript{\textdegree}h; f)\textsuperscript{p-TSA}, MeOH, 0\textsuperscript{\textdegree}C, 2\textsuperscript{\textdegree}h, 84\% over two steps; g)\textsuperscript{Dess--Martin reagent}, CH\textsubscript{2}Cl\textsubscript{2}, pyridine, 18\textsuperscript{\textdegree}h, 93\%; h)\textsuperscript{15}, THF, 1.5\textsuperscript{\textdegree}h at \textless M\textgreater 78\textsuperscript{\textdegree}C then, 0\textsuperscript{\textdegree}C for 15\textsuperscript{\textdegree}h, 79\%; i)\textsuperscript{2}. DIBAL, CH\textsubscript{2}Cl\textsubscript{2}, \textless M\textgreater 78\textsuperscript{\textdegree}C, 10\textsuperscript{\textdegree}min, 90\%. \textless ?\textgreater 2. \textless ?\textgreater DIBAL, CH\textsubscript{2}Cl\textsubscript{2}, \textless M\textgreater 78\textsuperscript{\textdegree}C, 10\textsuperscript{\textdegree}min, 90\%

\textbf{THF} = tetrahydrofuran

\textsuperscript{(+)}\textsuperscript{IPc}_2\textsubscript{BOMe} = (+)-8-Methoxydiisopinocampheylborane

\textsuperscript{TBSOTf} = tert-butyldimethylsilyl trifluoromethanesulfonate

\textsuperscript{p-TSA} = p-toluenesulfonic acid

\textsuperscript{DIBAL} = diisobutylaluminium hydride

Figure\textsuperscript{1} Migrastatin (1) and biologically active truncated analogues 2--8. IC\textsubscript{50} values (in parentheses) were determined in chamber cell migration assays against: \textsuperscript{4}4T1 mouse breast cancer cells; \textsuperscript{b}H1975 lung cancer cell lines; \textsuperscript{c}CMT-W2 canine mammary carcinoma cells.

\textless ?\textgreater There is no mention of “chamber cell” assay in the text, or of these cell lines. Please amend or add reference(s) if taken from published data\textless ?\textgreater

Figure\textsuperscript{2} Influence of macroketone\textsuperscript{4} on fluorescence recovery after photobleaching (FRAP) of E-cadherin--GFP in PDAC cells on a cell-derived matrix (CDM) and in\textsuperscript{\textdegree}vivo.

A)\textsuperscript{\textdegree}PDAC cells stably expressing E-cadherin--GFP were seeded onto matrices and treated with 10\textsuperscript{\textdegree}\textmu M\textsuperscript{4} 2\textsuperscript{\textdegree}h prior to FRAP imaging. B)\textsuperscript{\textdegree}PDAC cells stably expressing E-cadherin--GFP were injected subcutaneously into nude mice, and tumours were allowed to form for
seven days. One mouse was treated with 20 mg/kg macroketone for three days whereas the other mouse received vehicle treatment only. Mice were subsequently treated with vehicle or 20 mg/kg macroketone for three days and then subjected to live FRAP imaging. ns=not significant difference. Please specify p values for * and **. * p = 0.0285; ** p = 0.0026. Please specify number of animals used (n=1 implies one, but “mice” (plural) is used).