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Synthesis and evaluation of antimigratory and antiproliferative activities of lipid-linked [13]-macro-dilactones

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ABSTRACT

The biological activities of a family of novel, lipid-linked 13-membered-ring macro-dilactones are reported. These [13]-macro-dilactones were synthesized by diacylation of functionalized diols, followed by ring-closing metathesis under conditions we had previously reported. Antimigratory, cytostatic and cytotoxic activities of the compounds against cancer cells were evaluated. Compound **13** was the most potent in the series, while compound **10** had the broadest concentration range of subtoxic antiproliferative activity. These compounds share common structural components, namely the [13]-macro-dilactone templated by an octyl α-glucoside 4,6-diol.

A general objective of bioorganic chemistry and chemical biology is to generate synthetic small molecules that mimic the robust biological activities of complex natural products. Macrolactones are an enticing class of molecules in this regard because they possess a variety of molecular architectures and activities. Consideration of migrastatin (**1**)[1] and *iso*-migrastatin (*iso*-**1**)[2] (Fig. 1) illustrates some key points. These macrolactones are products of a polyketide synthase pathway.[3] The macrocycles thus contain an even number of atoms; **1** has a 14-membered ring and *iso*-**1** has a 12-membered ring. As their names imply, these and related molecules have been shown to inhibit the migration of a variety of human and mouse cancer cells[1a,4a–h] and tumor metastasis in mouse xenograft models.[4d,5] Cell migration is a fundamental biological phenomenon involved in a range of normal and disease states, including embryonic development, wound healing, blood vessel formation (angiogenesis), immune system function, and tumor metastasis. Cell migration has become recognized as a process that could be targeted for drug development, particularly for the treatment of cancer at the level of angiogenesis (which is involved in the early development of malignant solid tumors and depends on the growth and migration of vascular endothelial cells) and cancer cell invasion and metastasis.[6]

The antimigratory activity of *iso*-**1** exceeds that of **1** by several orders of magnitude,[4g] and **1** is thought to be a shunt metabolite of *iso*-**1**.[3b] A number of synthetic and semisynthetic analogs of migrastatin have also been shown to possess considerable antimigratory activity.[4b–h] Recently, the synthetic macroketone **2**, which potently inhibits cell migration,[4b] has been shown to bind and inhibit fascin, a crosslinker of actin filaments involved in cell migration, suggesting that fascin could be an antimetastatic drug target.[5] In addition, the natural product-like molecule **3**, which contains a macrocycle fused to a quinic acid scaffold, is also a potent inhibitor of cell migration.[4e] The ability of **2** and **3** to recapitulate the biological activity of the natural product itself suggests that other macrocycles of intermediate complexity may similarly inhibit the migration of cells.

We recently reported on the synthesis of some macro-dilactones that complement natural [12]- and [14]-macrocycles because they contain 13 atoms in their ring (e.g., compound **4** in Fig. 1).[7] A structural analysis of the new [13]-macro-dilactones demonstrated that they possessed helical chirality that arose from the size of the ring, the planar nature of their alkene and ester functional groups, and the stereochemistry of individual backbone stereocenters. As a consequence of the helical chirality, epoxidation of the double bond embedded in the macrocyclic backbone was highly selective. The unique molecular topology exhibited by the [13]-macro-dilactones and the fact that their ring size was similar to the migrastatin and *iso*-migrastatin isomers prompted us to consider their potential ability to inhibit cell migration. We were additionally inspired by the remarkable activity of the natural macrolactones despite their relatively sparse functionality and stereochemistry. Reported here are the synthesis and antimigratory and antiproliferative activities

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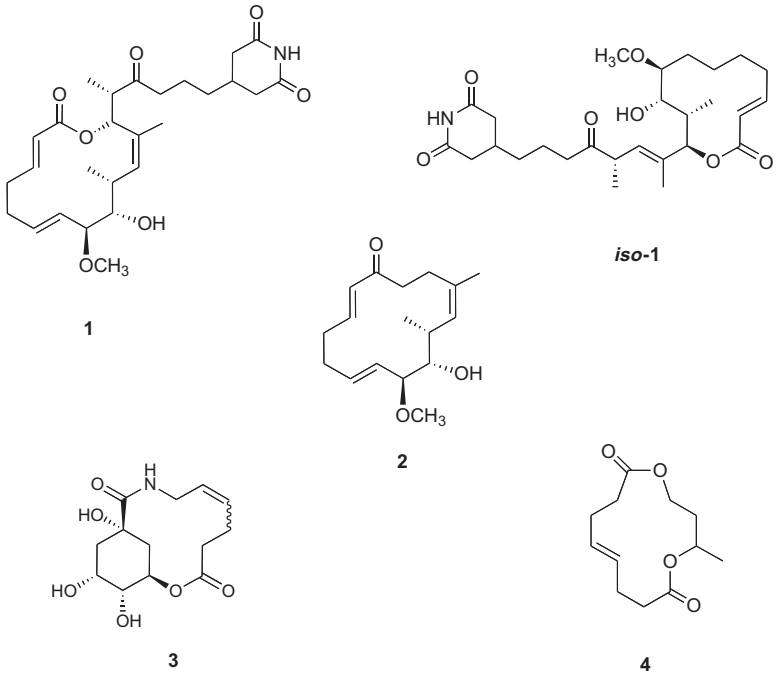


Figure 1. Migrastatin (**1**), *iso*-migrastatin (*iso*-**1**), 'macroketone' **2**, quinic acid-based macrolide **3**, and [13]-macro-dilactone **4**.

of a family of [13]-macro-dilactones (Fig. 2). A unifying feature among the active [13]-macro-dilactones was the presence of an octyl (C_8) glycoside. It is worth noting that two natural products containing a [13]-macrolactone, spongidepsin,[8] and amphidinolide P,[9] both have modest antiproliferative activity.

[13]-Macro-dilactones and related molecules evaluated in this investigation are shown in Figure 2. The molecules were designed to contain up to three components that might impart biological activity. The primary feature was the [13]-macro-dilactone itself, which is common among **4**–**13**. The nature of the 1,3-diol used to template the macro-dilactone was also varied. Macrocycles **4** and **7**, for example, utilized acyclic 1,3-diols from 1,3-butane diol and an acylated threoninol,[10] respectively. The 1*R*,2*S* and 1*S*,2*S* isomers of 2-hydroxycyclohexyl methanol[11] were used to template **5**

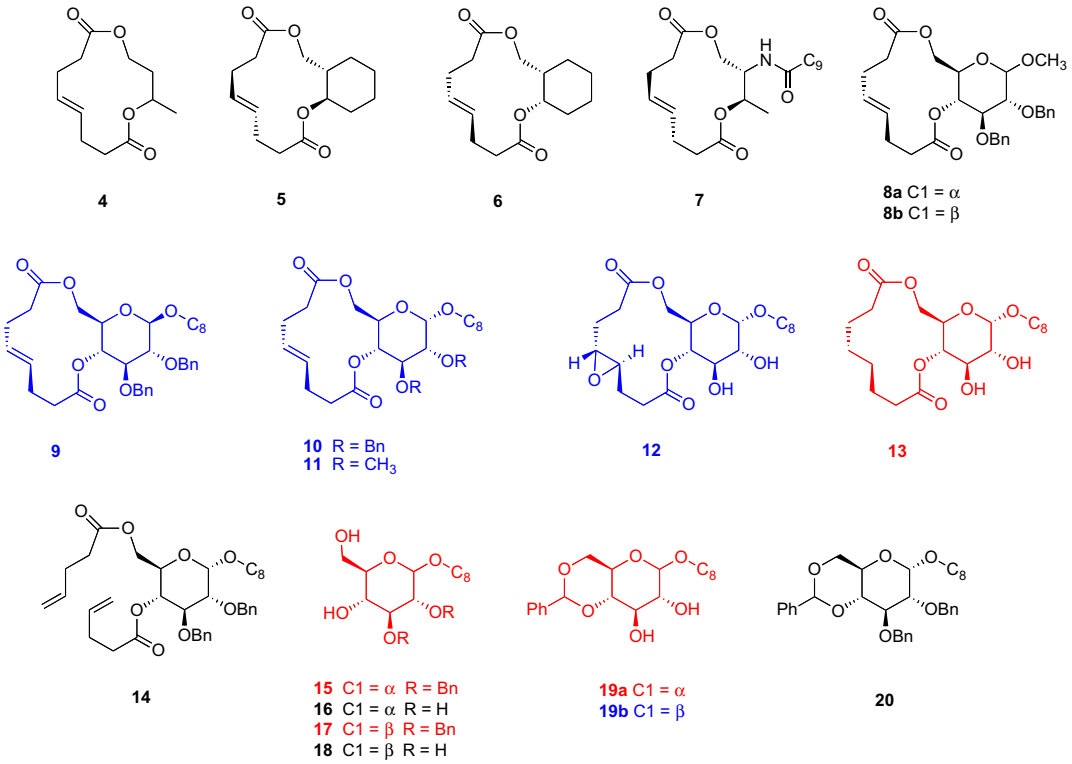
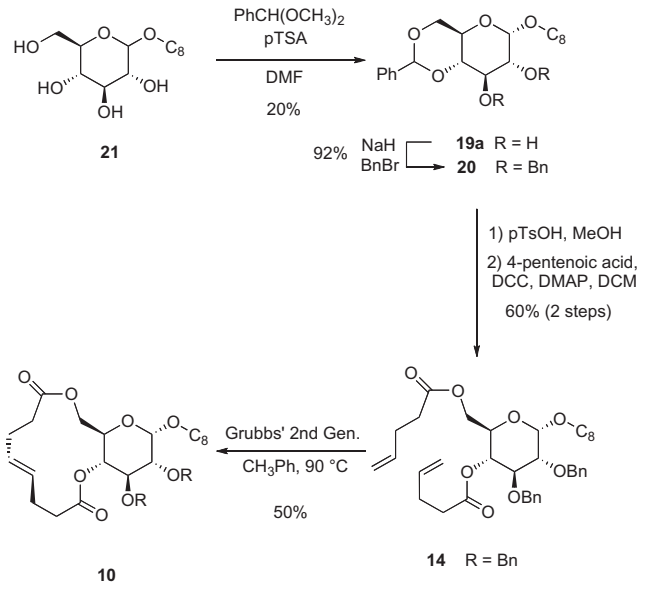


Figure 2. [13]-Macro-dilactones and related molecules synthesized and evaluated in this study.



Scheme 1. Synthesis of octyl glucoside-fused [13]-macro-dilactone **10**.

Table 1
Antimigratory activity of compounds **11**, **12**, and **13** in BT-20 breast cancer cells

Compound	MIC[a] (μM)	MLC[b] (μM)
11	75	125
12	50	125
13	50	75

[a] Minimum inhibitory concentration defined as the lowest concentration at which there was a statistically significant decrease by Student's *t*-test in the mean rate of migration in the wound closure assay compared to parallel controls from three independent experiments for each compound.

[b] Minimum lethal concentration defined as the lowest concentration at which there was evidence of cytotoxicity based on the trypan blue dye exclusion assay conducted at the end of each wound closure experiment.

and **6**. The remainder of the macrocycles were templated via the C4 and C6 hydroxyls of a protected glucoside, as in **8**–**13**. The attachment of a lipophilic alkyl chain to the molecule represented the final structural feature.

The [13]-macro-dilactones in Figure 2 were synthesized by a strategy we have previously described.[7] Scheme 1 depicts the synthesis of [13]-macro-dilactone **10**. It is illustrative of the approach taken for all the [13]-macro-dilactones. Preparation of **10** commenced with the protection of octyl glucoside **21**[12] as its 4,6-*O*-benzylidene. The α-anomer of the benzylidene, **19a**, was isolated in 20% yield, as was the β-anomer, from an initial ~1:1 mixture of C1-anomers. The C2 and C3 hydroxyl groups were then efficiently converted to their corresponding benzyl ethers to deliver **20** (92% yield). The 4,6-*O*-benzylidene of **20** was then removed, followed by acylation of the exposed hydroxyl groups with 4-pentenoic acid to give **14** in 60% yield over two steps. Ring-closing metathesis of the alkene moieties in **14** provided **10** (50% yield).

With the [13]-macro-dilactones in hand, the next objective was to assay their antimigratory activity. In addition, intermediates **14**–**20** from the synthesis of the macrocycles were also tested. We employed a scratch-wound assay, where a small wound—mechanically scratched in a cell monolayer—triggers cell migration and closure of the wound. The progress of wound closure was followed over time,[13] essentially as previously described.[14] The activity of these compounds was evaluated in BT-20 human breast carcinoma cells, T47D human breast carcinoma cells, MDA-MB-231 human breast carcinoma cells, MDA-MB-435 human melanoma cells, 4T1 mouse breast carcinoma cells, and Madin–Darby canine kidney (MDCK) epithelial cells. Compounds **10**, **11**, **12**, **13**, **19a**, and **19b** displayed weak antimigratory activity in BT-20, MDA-MB-435, and MDCK cells but not in T47D cells, MDA-MB-231, or 4T1 cells. Of these, **11**–**13** were the most bioactive, and they appeared to have the greatest activity in BT-20 cells of all the cell lines tested. The activity of these compounds was thus further examined in BT-20 cells. The rate of wound closure over a range of compound concentrations was determined from digital microscope images.[14,15] Potential cytotoxicity was determined at the end of each experiment. The concentration–response profiles revealed a very narrow range of subtoxic antimigratory activity between the minimum inhibitory concentration (MIC) and the minimum lethal concentration (MLC), as shown in Table 1. This precluded determination of meaningful half-maximal inhibitory

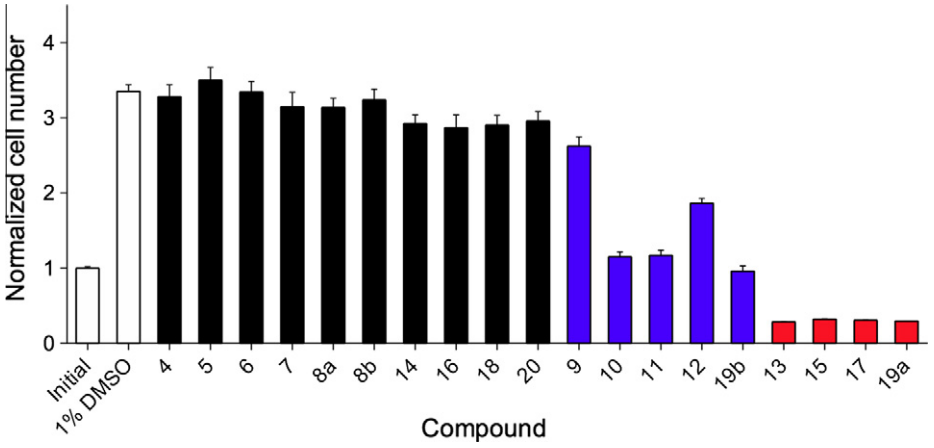


Figure 3. Antiproliferative activity at 100 μM in BT-20 breast cancer cells. Cells were plated at uniform density onto 96-well tissue culture plates and allowed to attach and grow for 24 h. At that point, the mean cell number ('initial') was determined in a tetrazolium salt-based assay. Compounds were added to experimental cultures, which were grown for another 48 h, along with parallel controls that had been treated with 1% DMSO alone. Values represent means with error bars signifying standard errors of the mean for three independent experiments for each treatment (with four replicate wells for each experiment for 12 total), normalized to the mean initial cell number. Black bars represent compounds that had no statistically significant effect on cell growth. Blue bars represent compounds that appeared cytostatic at 100 μM, defined as mean cell numbers that were significantly lower by Student's *t*-test than the mean control cell number at 48 h but not significantly reduced from the mean initial cell number. Red bars represent compounds that appeared cytotoxic at 100 μM, defined as cell numbers that were significantly lower by Student's *t*-test than the mean initial cell number.

Table 2
Activities of antiproliferative compounds in BT-20 breast cancer cells

Compound	MIC[a] (μM)	IC_{50}[b] (μM)	95% confidence interval[c] (μM)	MLC[d] (μM)	Therapeutic index (MLC/MIC)
10	10	85.1	82.8–87.4	300	30
11	50	91.4	89.2–93.6	300	6
12	50	107.0	102.6–111.5	>300	>6
13	10	38.1	37.3–38.8	50	5
15	25	52.9	51.2–53.8	75	3
17	25	61.1	60.1–62.1	100	4
19a	10	47.1	43.8–50.1	75	7.5
19b	50	72.1	69.4–74.8	300	6

[a] Minimum inhibitory concentration, defined as the lowest concentration at which there was a statistically significant reduction by Student's *t*-test in mean cell number from the mean control cell number at 48 h from three independent experiments (with four replicates for each treatment per experiment for 12 replicates total) in a tetrazolium-salt-based assay.
[b] Half-maximal concentration for inhibition of cell growth, calculated from mean cell numbers at 48 h for a range of concentrations of each compound.
[c] 95% confidence interval for the IC_{50}.
[d] Minimum lethal concentration, defined as the lowest concentration at which there was a statistically significant reduction by Student's *t*-test in mean cell number at 48 h from the mean initial cell number.

concentration (IC_{50}) values for the compounds' antimigratory activity. We concluded that the effects on wound closure observed at subtoxic concentrations were likely due to incipient toxicity.

We next evaluated the effects of the compounds on the viability and growth of BT-20 cells.[16] We first tested these compounds at 100 μM and found that compounds **9**, **10**, **11**, **12**, **13**, **15**, **17**, **19a**, and **19b** displayed either subtoxic antiproliferative (cytostatic) or cytotoxic activity at this concentration (Fig. 3). Compounds that were considered cytostatic at 100 μM (**9**, **10**, **11**, **12**, and **19b**) reduced the rate of cell proliferation over 48 h compared to controls treated with dimethyl sulfoxide (DMSO) alone but did not reduce cell numbers below the initial values. Compounds that were considered cytotoxic at this concentration (**13**, **15**, **17**, and **19a**) not only inhibited cell growth but also caused a reduction in cell numbers from the initial values due to cell death.

Compounds **10**, **11**, **12**, **13**, **15**, **17**, **19a**, and **19b** exhibited either pronounced cytostatic or cytotoxic activity in the initial assay at 100 μM and were tested over a range of concentration to establish concentration–response profiles for each compound. Compound **9** only very weakly inhibited cell proliferation, and, when tested over a range of concentrations, the concentration–response profile was virtually flat.[17] Compounds **10**, **11**, **12**, **13**, **15**, **17**, **19a**, and **19b**, on the other hand, displayed cytostatic activity that in most cases appeared separable from the cytotoxicity observed at higher concentrations (Table 2). The criterion for this conclusion was based on the MLC/MIC ratio ('therapeutic index,' often also defined as half-maximal lethal concentration divided by the IC_{50}). By this measure, therefore, these compounds have subtoxic cytostatic activity, particularly in the case of **10**, with a therapeutic index of 30.

The compounds possessing antiproliferative activity, **10**, **11**, **12**, **13**, **15**, **17**, **19a**, and **19b**, share common structural components that had been included in their design. To our surprise, the [13]-macro-dilactone unit was not essential for activity. All of the bioactive compounds contain a glucosyl unit and an octyl (C_8) chain. Furthermore, the majority of these molecules contain an α-linkage between the glucose unit and the alkyl chain. However, the most promising compounds, as measured by either therapeutic index (compound **10**) or IC_{50} (compound **13**), contained the [13]-macro-dilactone. In the case of **10** in particular, the cytostatic and cytotoxic activities were clearly separable, with a wide concentration range for its subtoxic antiproliferative activity. Based on this and its micromolar IC_{50}, it is likely that **10** does not act by merely having a non-specific effect on cells, such as disrupting the integrity of cellular membranes. Instead, this compound may target some factor(s) involved in cell cycle progression. In summary, we have demonstrated the application of our published synthesis to access a novel class of antiproliferative agents.

Acknowledgments

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Supplementary data

Supplementary data (synthetic schemes and spectral data) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.07.083.

References and notes

1. (a) Nakae, K.; Yoshimoto, Y.; Sawa, T.; Homma, Y.; Hamada, M.; Takeuchi, T.; Imoto, M. *J. Antibiot. (Tokyo)* **2000**, *53*, 1130; (b) Nakae, K.; Yoshimoto, Y.; Ueda, M.; Sawa, T.; Takahashi, Y.; Naganawa, H.; Takeuchi, T.; Imoto, M. *J. Antibiot. (Tokyo)* **2000**, *53*, 1228.
2. Woo, E. J.; Starks, C. M.; Carney, J. R.; Arslanian, R.; Cadapan, L.; Zavala, S.; Licari, P. *J. Antibiot. (Tokyo)* **2002**, *55*, 141.
3. (a) Ju, J.; Lim, S. K.; Jiang, H.; Seo, J. W.; Shen, B. *J. Am. Chem. Soc.* **2005**, *127*, 11930; (b) Ju, J.; Lim, S. K.; Jiang, H.; Shen, B. *J. Am. Chem. Soc.* **2005**, *127*, 1622; (c) Lim, S. K.; Ju, J.; Zazopoulos, E.; Jiang, H.; Seo, J. W.; Chen, Y.; Feng, Z.; Rajski, S. R.; Farnet, C. M.; Shen, B. *J. Biol. Chem.* **2009**, *284*, 29746.
4. (a) Gaul, C.; Njardarson, J. T.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 6042; (b) Gaul, C.; Njardarson, J. T.; Shan, D.; Dorn, D. C.; Wu, K.-D.; Tong, W. P.; Huang, X.-Y.; Moore, M. A. S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 11326; (c) Njardarson, J. T.; Gaul, C.; Shan, D.; Huang, X.-Y.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 1038; (d) Shan, D.; Chen, L.; Njardarson, J. T.; Gaul, C.; Ma, X.; Danishefsky, S. J.; Huang, X. Y. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 3772; (e) Metaferia, B. B.; Chen, L.; Baker, H. L.; Huang, X.-Y.; Bewley, C. A. *J. Am. Chem. Soc.* **2007**, *129*, 2434; (f) Ju, J.; Rajski, S. R.; Lim, S.-K.; Seo, J.-W.; Peters, N. R.; Hoffmann, F. M.; Shen, B. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5951; (g) Ju, J.; Rajski, S. R.; Lim, S. K.; Seo, J. W.; Peters, N. R.; Hoffmann, F. M.; Shen, B. *J. Am. Chem. Soc.* **2009**, *131*, 1370; (h) Oskarsson, T.; Nagorny, P.; Krauss, I. J.; Perez, L.; Mandal, M.; Yang, G.; Ouerfelli, O.; Xiao, D.; Moore, M. A. S.; Massagué, J.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2010**, *132*, 3224.
5. Chen, L.; Yang, S.; Jakoncic, J.; Zhang, J. J.; Huang, X. Y. *Nature* **2010**, *464*, 1062.
6. Fenteany, G.; Zhu, S. *Curr. Top. Med. Chem.* **2003**, *3*, 593.
7. (a) Fyvie, W. S.; Peczuh, M. W. *Chem. Commun.* **2008**, 4028; (b) Fyvie, W. S.; Peczuh, M. W. *J. Org. Chem.* **2008**, *73*, 3626.
8. Grassia, A.; Bruno, I.; Debitus, C.; Marzocco, S.; Pinto, A.; Gomez-Paloma, L.; Riccio, R. *Tetrahedron* **2001**, *57*, 6257.
9. Ishibashi, M.; Takahashi, M.; Kobayashi, J. *J. Org. Chem.* **1995**, *60*, 6062.
10. Gu, K.; Bi, L.; Zhao, M.; Wang, C.; Ju, J.; Peng, S. *Bioorg. Med. Chem.* **2007**, *15*, 6273.
11. Casas, J.; Sundén, H.; Córdova, A. *Tetrahedron Lett.* **2004**, *45*, 6117.
12. Porzelle, A.; Williams, C. M.; Schwartz, B. D.; Gentle, I. R. *Synlett* **2005**, 2923.
13. BT-20, T47D, MDA-MB-231, MDA-MB-435, 4T1, and MDCK cells were obtained from the American Tissue Culture Collection or Kam C. Yeung (University of Toledo). Cells were grown in a tissue culture incubator at 37 °C with 5% CO_2 in minimum essential medium with 10% fetal bovine serum (FBS) for BT-20 cells or 10% newborn calf serum for MDCK cells, RPMI 1640 with 10% FBS (T47D and

4T1 cells), Dulbecco's modified Eagle medium with 10% FBS (MDA-MB-231 and MDA-MB-435 cells). The medium was changed every 2 days. Cells were plated on 24-well tissue culture-treated plates. When the cells reached confluence, the medium was changed again one more time, and the experiment was started the next day. Cells were treated with 100 μM of each compound or 1% DMSO alone, corresponding to the concentration of DMSO carrier solvent in the experimental treatments. (We found in preparatory experiments that 1% DMSO had no detectable effect on the migration or growth of any of the cells compared to medium alone.) The compounds or DMSO alone, mixed with fresh medium before addition to the cell cultures, were delivered to the cells as the medium was changed. After 30 min, the cell monolayers were scraped with a micropipet tip to produce oval-shaped wounds of 0.5–1.0 mm^2 in area. Progress of wound closure was followed by observation on an inverted microscope every 12 h for 72 h. At each time point, wounds were scored as either closed or opened. Compounds that resulted in wounds that were still open after the wounds for the control DMSO treatments were closed were considered antimigratory. The trypan blue dye exclusion assay was performed at the end of each experiment to gauge potential cytotoxicity of the compounds.

14. Mc Henry, K. T.; Ankala, S. V.; Ghosh, A. K.; Fenteany, G. *ChemBioChem* **2002**, *3*, 1105.
15. Concentration–response profiles for compounds **11**, **12**, and **13**, which appeared most active in the initial wound closure assay, were obtained in a quantitative and kinetics wound closure assay. Confluent monolayers of BT-20 cells were wounded 30 min after treatment with different concentrations of these compounds or DMSO alone, and digital images of the wounds were captured at 3, 6, 9, 12, 24, 36, 48, 60, and 72 h post-wounding. The trypan blue dye exclusion assay was performed at the end of each experiment. The area of the wound at each time point was subsequently calculated from the digital images with NIH ImageJ software (http://rsbweb.nih.gov/ij/). Statistically significant differences were determined by unpaired, two-tailed Student's *t*-test ($p > 0.05$).
16. BT-20 cells were plated onto 96-well tissue culture-treated plates at 5000 cells per well in 100 μL of growth medium and incubated for 24 h in a tissue culture incubator at 37 °C with 5% CO_2. Initial cell numbers were determined for cells on a control plate by adding the tetrazolium salt WST-8 as per the manufacturer's instructions (Cell Counting Kit-8, Dojindo Molecular Technologies), incubating for 3 h at 37 °C and then measuring absorbance at 450 nm in a UV–visible absorbance plate reader (Molecular Devices Spectramax Plus 384). The experimental plates were treated with compounds or DMSO alone and incubated in the tissue culture incubator for another 48 h. Cell numbers were then measured as above. Statistically significant differences were determined by unpaired, two-tailed Student's *t*-test ($p > 0.05$). IC_{50} values were calculated with GraphPad Prism software from concentration–response data.
17. At concentrations above 500 μM, **9** by itself exhibited light scattering at the wavelength used in the spectrophotometric assay, preventing evaluation of this compound at high concentrations.