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

Annifer N. Magpusao, Richard T. Desmond, Katelyn J. Billings, Gabriel Fenteany *, Mark W. Peczuh *

Department of Chemistry, University of Connecticut, Storrs, CT 06269, USA

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 logical activities of complex natural products. Macrolactones are an is to generate synthetic small molecules that mimic the robust bio-

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* Corresponding authors. Tel.: +1 860 486 6645 (G.F.); tel.: +1 860 486 1605 (M.W.P.).
E-mail addresses: gabriel.fenteany@uconn.edu (G. Fenteany), mark.peczuh@uconn.edu (M.W. Peczuh).

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of a family of [13]-macro-dilactones (Fig. 2). A unifying feature among the active [13]-macro-dilactones was the presence of an octyl (C8) 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 1R,2S and 1S,2S isomers of 2-hydroxycyclohexyl methanol11 were used to template 5.

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

Figure 2. [13]-Macro-dilactones and related molecules synthesized and evaluated in this study.
conducted at the end of each wound closure experiment. There was evidence of cytotoxicity based on the trypan blue dye exclusion assay, which there was a statistically significant decrease 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 in BT-20 cells, M, defined as cell numbers that were significantly lower by Student’s t-test than the mean initial cell number. Black bars represent compounds that appeared cytostatic at 100 μM, defined as cell numbers that were significantly lower by Student’s t-test than the mean initial cell number.

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 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 microscopic 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 activity of these compounds.

Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>MICa (µM)</th>
<th>MLCa (µM)</th>
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<tbody>
<tr>
<td>11</td>
<td>75</td>
<td>125</td>
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<tr>
<td>12</td>
<td>50</td>
<td>125</td>
</tr>
<tr>
<td>13</td>
<td>50</td>
<td>75</td>
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Table 1: Antimigratory activity of compounds 11, 12, and 13 in BT-20 breast cancer cells

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.

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 allowed 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 microscopic 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 activity of these compounds.
concentration ([IC₅₀]) 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.⁶ 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 IC₅₀). 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 (C₈) 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 IC₅₀ (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 IC₅₀, 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.

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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


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% CO₂ 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 mm² 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.


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% CO₂. 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). IC₅₀ 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.