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Synthetic Macrolides that Inhibit Breast Cancer Cell Migration in Vitro

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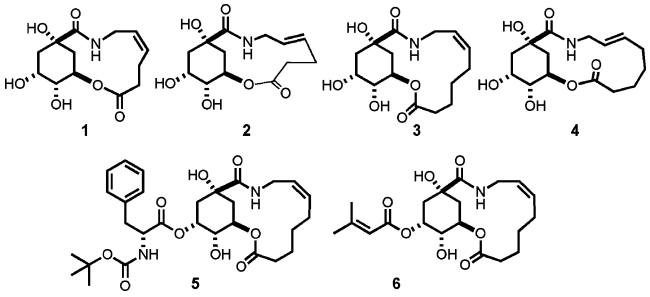
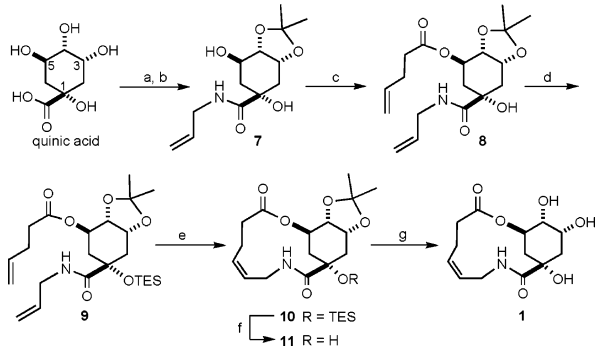
Historically, natural products have accounted for the majority of drugs and drug leads in various therapeutic areas including cancer, infectious diseases, and inflammation.[1−3] The three-dimensional structures of natural products are highly diverse and architecturally complex. Major classes of drugs or chemical probes that are derived from or inspired by natural products include non-ribosomal peptides, alkaloids, and polyketides or macrolides. In recent years, a number of elegant examples of synthetic libraries whose structural diversity and biological activities equals or even surpasses that of natural product libraries have been reported.[4−6] Inspired by such studies together with our studies of designing antimycobacterials, we report here the design and synthesis of six new macrolides (**1**−**6**) built upon a quinic acid-containing scaffold, some of which are potent inhibitors of tumor cell migration.



Our design of the quinic acid-derived scaffold present in macrolides **1**−**6** follows on our recent study in which we used quinic acid as starting material to construct a pseudo-disaccharide that mimics the small molecular weight thiol mycothiol.[7] In addition to being economical and commercially available, during that study, we were struck by the utility of quinic acid in that it is conformationally rigid and contains several stereochemically defined functional groups that can be readily derivatized. Moreover, because some polyketide macrolides are reminiscent of carbohydrates in that they are glycosylated, contain pyranose rings, are polyhydroxylated, or a combination of these features, we were further motivated to employ quinic acid as a scaffold upon which to build new macrolides. This design would further permit the use of ring-closing metathesis (RCM) chemistry to obtain macrocycles with varying ring sizes and stereochemistries.[8,9]

Mindful of the inherent arrangement of hydroxyl groups and the carboxylic acid in quinic acid, we sought to install the macrocycle between the C-1 carboxylic acid and the C-5 hydroxyl group. Preparation of the macrolides (Scheme 1) thus began by treating quinic acid with 2,2-dimethoxypropane and *p*-toluenesulfonic acid to give a protected lactone ketal in good yields. Treatment of this intermediate with allylamine in the presence of 2-hydroxypyridine opened the lactone to give amide **7**.[10] To generate the 12-membered

Scheme 1. Synthetic Scheme for Syntheses of **1**−**6** Exemplified by **1**[a]



[a] Reagents and conditions: (a) 2,2-dimethoxypropane, *p*-TsOH, acetone, reflux, 24 h (78%); (b) allylamine, 2-hydroxypyridine, THF (65%); (c) 4-pentenoic acid, EDCI/DMAP, CH_2Cl_2 (63%); (d) TESCl, DMF, imidazole (78%); (e) Grubb's-II catalyst, CH_2Cl_2, reflux 5 h; (f) TBAF/THF (92%); (g) TFA/CH_2Cl_2/H_2O (9:1:1) (82%).

macrocycles, the C-5 hydroxyl group was esterified chemoselectively with 4-pentenoic acid in the presence of EDCI/DMAP to give **8** in 63% yield.

We initially attempted to create the desired macrocycle from **8**, which contains a free tertiary hydroxyl group at C-1, but were unsuccessful despite trying a variety of RCM conditions. Compound **8** was thus converted to fully protected triethyl silyl ether **9**. Treatment of the protected diene with second generation Grubb's catalyst (23 mol %, 0.5 mM)[11] in refluxing dichloromethane afforded the desired 12-membered macrocycle **10** in 70% yield as a 5:1 ratio of *Z*/*E* isomers.

Finally, although global deprotection of **10** could be accomplished in a single step by treatment with TFA/CH_2Cl_2/H_2O (9:1:1), the observed yields were lower than desired. We therefore resorted to stepwise removal of the silyl group with TBAF/THF followed by treatment of acetonide **11** with TFA/CH_2Cl_2/H_2O to obtain macrolides **1** and **2** in high yields.

The same general strategy was used to synthesize compounds **3** and **4**, 14-membered macrocycles, with the exception that the C-5 hydroxyl group was esterified with 6-heptenoic acid. RCM afforded the protected macrocycles as an inseparable mixture of *Z*/*E* isomers (3:1 ratio) in 68% yield,[12] and the mixture could be purified by RP-HPLC following global deprotection. Last, two additional analogues of **3** were prepared by chemoselective esterification of the least hindered of the secondary alcohols, namely, the C-3 hydroxyl, with *N*-*tert*-Boc-phenylalanine or 3-methyl-2-butenoic acid to obtain **5** and **6**, respectively.

Once in hand, macrolides **1**−**6** were tested for antimicrobial and antifungal activity in a disk diffusion assay and for inhibition of tumor cell migration in two complementary assays. The inhibition observed in the antimicrobial screens was modest at best with compounds **1**, **3**, and **4** showing weak inhibition toward *Bacillus*

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Table 1. Results from Chamber Cell Migration Assay[a]

compound	IC_{50}	compound	IC_{60}
1	77 ± 17 nM	**4**	12 ± 2.9 μM
2	>250 μM	**5**	>500 μM
3	525 ± 163 nM	**6**	550 ± 189 nM

[a] 4T1 breast tumor cells used.

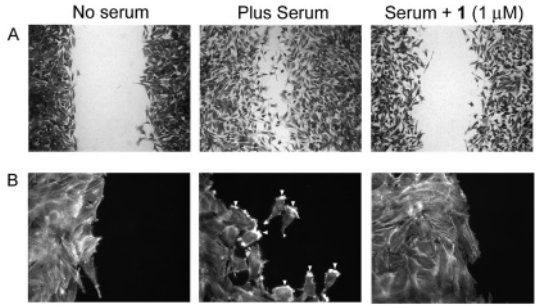
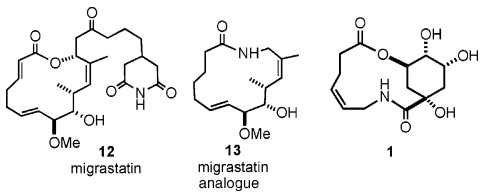


Figure 1. Micrographs of (A) in vitro wound healing and (B) lamellipodia formation using 4T1 breast cancer cells in the absence (left) or presence (center) of serum, and in the presence of serum plus compound **1** (right). See Supporting Information for experimental details.

subtilis. The results of the tumor cell migration assays, however, were much more gratifying and are presented below.

In recent years, migrastatin (**12**), a *Streptomycete*-derived macrolide natural product that inhibits tumor cell migration (IC_{50} = 29 μM),[13,14] has generated interest in developing synthetic analogues with increased potency and tumor cell selectivity. Danishefsky et al. have judiciously designed and synthesized migrastatin analogues,[15,16] such as **13**, that inhibit tumor cell migration at nanomolar concentrations in vitro and are active in a mouse model to suppress breast tumor metastasis.[17] Since the macrolides produced in this study bear some similarities to the core of migrastatin and **13**, we were hopeful that they might exhibit similar biological activities. Compounds **1**−**6** were tested for their effects on tumor cell migration using a quantitative chamber cell migration assay as well as a more qualitative wound healing assay using 4T1 breast cancer cells. Compound **1**, whose 12-membered macrocycle contains a cis double bond, inhibited tumor cell migration of 4T1 breast cancer cells with an IC_{50} of 77 nM, while macrolides **3** and **6**, 14-membered macrocycles also containing cis double bonds, inhibited tumor cell migration with sub-micromolar IC_{50} values. Compounds **2** and **5** were considered inactive, while macrolide **4** was approximately 20-fold less active than its *Z* isomer **3**. The activity of compound **1**



was further confirmed by testing in an in vitro wound healing assay. As shown in Figure 1A, addition of 1 μM of macrolide **1** completely inhibited serum-induced 4T1 tumor cell migration and prevented closure of the wound.

To test for cytotoxic effects, 4T1 cells were treated with increasing concentrations of **1** to concentrations as high as 100 μM. At this level, which would have completely inhibited 4T1 cell migration, macrolide **1** was found to have no effect on 4T1 cell proliferation. These observations strongly suggest that the inhibitory effect on cell migration by **1** is specific to this cellular event and not a global nonspecific effect on various cell functions.

To further demonstrate this specific effect on cell migration and to better understand the molecular mechanism by which these compounds inhibit cell migration, we investigated *rac*-mediated formation of lamellipodia.[18] Lamellipodia are actin-rich membrane structures present at the leading edge of migrating cells and are required for cell migration. Upon treatment of 4T1 cells with compound **1** (1 μM, Figure 1B), we found that lamellipodium formation at the leading edge of cells was completely inhibited, suggesting that the macrolides inhibit tumor cell migration by blocking *rac*-mediated formation of lamellipodia, similarly to migrastatin analogues.[17]

In summary, in an attempt to design and synthesize new macrocyclic antibiotics starting from quinic acid, we have discovered a new class of macrolides, some of which inhibit tumor cell migration with low nanomolar to sub-micromolar IC_{50} values via a mechanism that appears to be similar to that of migrastatin and its analogues. The synthetic protocol we have developed is straight forward and relatively high yielding. Although plasma stability tests have yet to be carried out, because the lactone is formed through a secondary alcohol and is linked to a cyclohexane ring, we are hopeful that these macrolides will be less susceptible to the action of esterases. Nonetheless, through the synthesis of additional analogues, it will be of great interest to determine requisite features of these and other macrolide scaffolds in the development of inhibitors of cancer metastasis.

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Supporting Information Available: Experimental details for syntheses, biological assays, and physical data for compounds **1**−**6**, **8**−**11**, and intermediates **S1** and **S2**, and ROESY spectra for **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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