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Evaluation of new migrastatin and dorrigocin congeners unveils cell migration inhibitors with dramatically improved potency

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ABSTRACT

Lactimidomycin (LTM, **1**), iso-migrastatin (iso-MGS, **2**) and migrastatin (MGS, **3**) are macrolide antitumor antibiotics differing in macrolide ring size but all bearing a glutarimide side chain. To further develop these natural products and related analogs as drug candidates we have produced and evaluated the biological activities of a small library of iso-MGS and LTM-derived agents; congeners evaluated bear either the MGS scaffold or related acyclic (dorrigocin) scaffolds. Scratch wound-healing (SWH) assays with 4T1 mouse and MDA-MB-231 human mammary tumor cell lines, respectively, reveal structural elements crucial to inhibition of cell migration by these compounds. Moreover, two substances, **14** and **17**, with activity far superior to that of MGS are unveiled by SWH assays.

Lactimidomycin (LTM, **1**) and iso-migrastatin (iso-MGS, **2**) are macrolide antibiotics characterized by a biosynthetically rare glutarimide side chain and unsaturated 12-membered lactone cores (Fig. 1).[1,2] The relationship shared by these two natural products is unique in that both compounds typify discreetly different, yet interrelated molecular scaffolds. LTM, first discovered in 1992 from fermentations of *Streptomyces amphibiosporus* ATCC53964, was found to display strong in vitro cytotoxicity against a number of human cell lines (IC_{50} = 3.0–65 nM), in vivo antitumor activity in mice, antifungal activity, and inhibited both DNA and protein syntheses.[1] Iso-MGS on the other hand has been only recently identified from fermentations of *S. platensis*, and investigations into its bioactivity have, to date, been limited.[2] However, enthusiasm for this compound is extremely high by virtue of its relationship to the very potent tumor cell migration inhibitor migrastatin (MGS, **3**).[3] Displaying molecular topology similar to **2**, **3** contains an expanded 14-membered macrolide in contrast to the 12-membered macrolide characteristic of both iso-MGS and LTM. In addition to **1**–**3**, a number of other glutarimide-containing polyketide natural products have been identified including the antifungal antibiotic cycloheximide,[4] streptimidone,[5] NK30424A,[6] dorrigocin (DGN) A (**4a**), 13-*epi*-DGN A (**4b**), and DGN B (**5**).[7]

MGS was first isolated from *Streptomyces* sp. MK929-43F1[3a] and later from *S. platensis*,[2] and represents a novel natural product lead for anticancer drug design, in part, because of its potent activity as an inhibitor of tumor cell migration. Association of this activity with that of antimetastatic activity has been validated by extensive chemical and biological studies.[8–10] Production and biological evaluation of truncated MGS analogs **6**–**8** has shown that macroketone **7** and lactam **8** show improved biological profiles (by up to three orders of magnitude for **6**) by inhibiting in vitro[8,9] and in vivo[10] tumor cell migration. Notably, macrolactone **6**, though significantly more active than **3** in cell migration assays, is extremely prone to degradation ($t_{1/2} \sim 5$ min) in mouse plasma whereas

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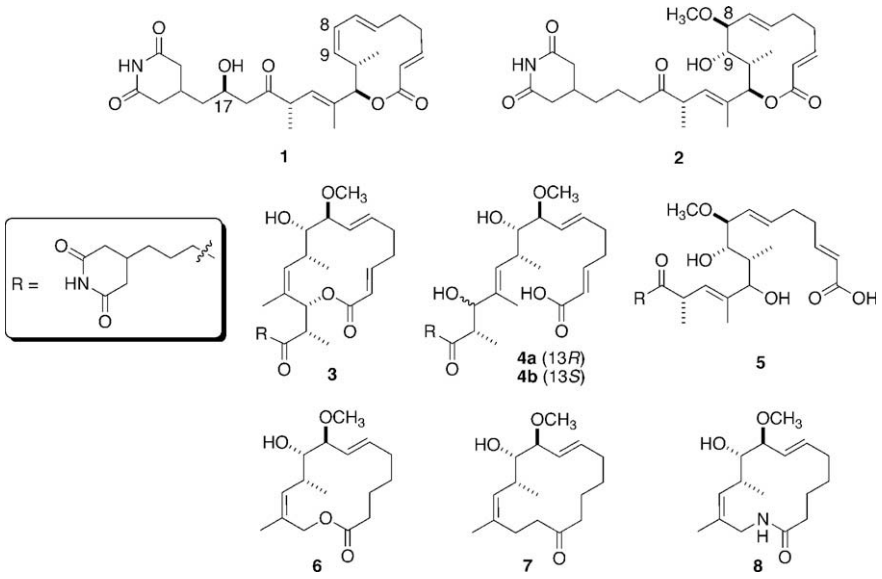

Figure 1. Structures of the natural products lactimidomicin (**1**), iso-migrastatin (**2**), migrastatin (**3**), dorrigocin A (**4a**), 13-*epi*-dorrigocin A (**4b**), and dorrigocin B (**5**) and fully synthetic macrolactone (**6**), macroketone (**7**), and macrolactam (**8**) analogs of **3**.

3 is not.[9] Total synthesis of **2** and **3** continues to hasten our understanding of this class of clinical candidates.[11,12]

Besides their fascinating and important bioactivities these natural products have provided extraordinarily fertile ground for biosynthetic study as reflected by our findings that (i) **3**–**5** are shunt metabolites of **2** despite the fact that **3** has been the predominantly studied product from *S. platensis*,[13] (ii) **2** and related congeners undergo H_2O-mediated rearrangement to afford the linear DGN and 14-membered macrolide MGS scaffolds,[14] (iii) **2** and related analogs undergo a thermally induced [3,3]-sigmatropic rearrangement to afford 14-membered macrolides; **1**, bearing the C-8, C-9 olefin, is incapable of such ring expansions,[15] and (iv) *S. amphibiosporus* produces, along with **1**, 12-membered macrolides capable of hydrolytic conversion to the DGN and MGS scaffolds.[16] We now present data validating a sequence of biosynthetic starting material production and subsequent semi-synthetic conversion of such natural products into linear DGN analogs and 14-membered macrolides of the MGS class as a way to improve upon **3**–**5**. These approaches complement strictly synthetic strategies[8–12] and shed insight into the structure and activity relationship for this family of natural products.

The thermolytic and hydrolytic lability of **2** and related congeners **28**–**36** is now widely appreciated, and has figured prominently in the production of compounds **9**–**27** (Figs. 2 and 3).[14,15] Similarly, recently identified compounds **37** and **38** have been found in optimized fermentations of *S. amphibiosporus* and are known to undergo hydrolysis to **16** and **17**, respectively (Fig. 3).[16]

The effects of **3**, **4a**, **4b**, **5**, and semi-synthetic derivatives **9**–**27** on the migration of 4T1 mouse mammary tumor cells were investigated. The rapid spread of 4T1 cells to lymph nodes, lungs, and other proximal organs mimics tumor cell metastasis in humans, and provides an excellent model.[8] In parallel MDA-MB-231 human breast tumor cells were also used to ascertain cell migration inhibition. Compound cytotoxicities were determined, and cell migration studies employed a standardized scratch wound-healing (SWH) assay at compound concentrations sufficient to avoid deleterious effects resulting from cell death. Standardized scratches (i.e., wounds) were made through confluent cell layers using a 96-well floating pin tool followed by addition of test compounds to each well. Incubation for 4 days at 37 °C followed by fixing, staining, and fluorescence measurement in the area of the wound allows for quantification of wound closure (Fig. 4).

Figure 4 depicts representative visual display of SWH results. Evident in the absence of test compound is that scratches in confluent cell layers are almost completely covered over ('healed') after 4 days. However, at concentrations of 50 μM **3** and 12.5 μM **14** the scratches originally incurred upon cell layers remain, the result of inhibited cell migration.[17,18] SWH assay data and the results of cytotoxicity assays (Table 1) provide clear insight into structure and activity relationships for these glutarimide-containing polyketides, and support earlier work with synthetic analogs **6**–**8**.[8–10]

As summarized in Table 1, of the compounds tested, **3**, **4a**, **4b**, **5**, and **9**–**27**, only **3**, **14**, and **17** displayed IC_{50}s below 100 μM for wound-healing inhibition. The poor activity displayed by the majority of MGS and DGN analogs tested reveals several key findings relating the activity of **3**, **4a**, **4b**, **5**, and **9**–**27** to their corresponding structures. First, and most generally, it is clear that inhibition of cell migration is critically dependent upon the macrolide structure; all DGNs and related acyclic analogs were completely inactive in SWH assays. The most vivid illustrations of macrolide importance come from comparison of activities of natural products **3** to **4a** and **4b** but also from comparison of MGS analog **14** to its hydrolysis product **23**. Cell migration inhibition by **14** is significantly greater than observed for **3** in both cell lines (by ~10-fold), yet **23** completely lacks inhibitory activity. Even more dramatic is the comparison of activities between macrolide **17** and its corresponding hydrolysis products **18** and **21**.

Secondly, comparison of cell migration inhibition by compound **14** to that of compounds **3** and **9** suggests a crucial role for the glutarimide side chain of these intact macrolides. Specifically, hydroxylation at C-17 profoundly improves activity of **14** relative to the fully saturated **3** and the 16,17-didehydro analog **9**. It is noteworthy also that compound **17**, which is almost two orders of magnitude more potent at cell migration inhibition than **3**, bears a C-17 OH moiety. These results contrast those of Danishefsky and coworkers in which **7** and **8**, both devoid of the glutarimide side chain, were found to inhibit cell migration far more potently than **3**. This suggests the possibility of multiple molecular targets or that different drug-target binding motifs avail themselves to different MGS congeners.

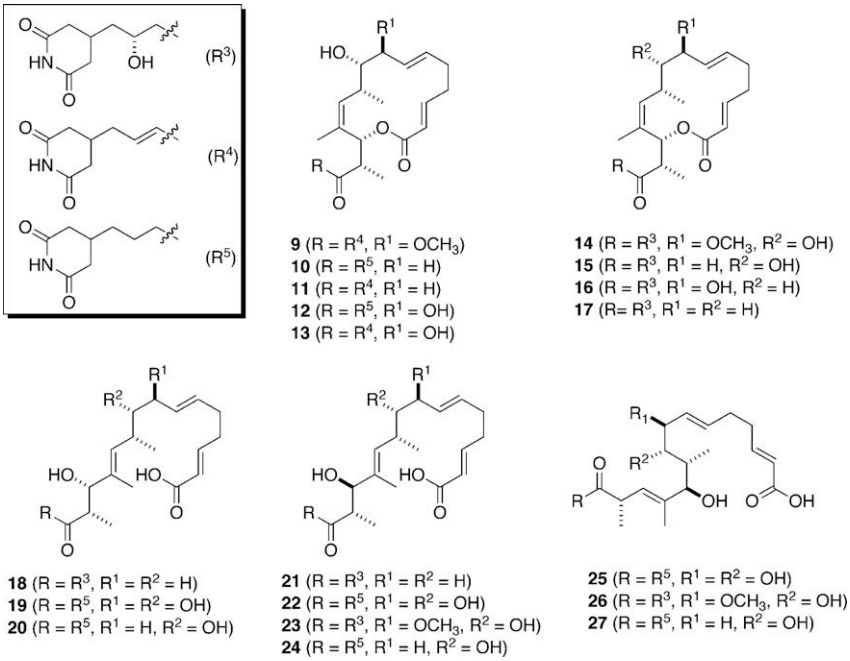

Figure 2. MGS and DGN analogs produced semi-synthetically from biosynthesized 12-membered macrolides. Compounds **9**–**13** are derived semi-synthetically via thermolytic [3,3]-sigmatropic rearrangement of **29**, **34**, **35**, **31**, and **32**, respectively.[15] Semi-synthetic hydrolysis of biosynthetic products (underlined) afforded the corresponding products (italics) as follows: **30** → ***14***, ***23***, ***26***;[14] **31** → ***19***, ***22***, ***25***;[14] **34** → ***20***, ***24***, ***27***;[14] **36** → ***15***;[14] **37** → ***16***;[16] **38** → ***17***, ***18***, ***21***.[16]

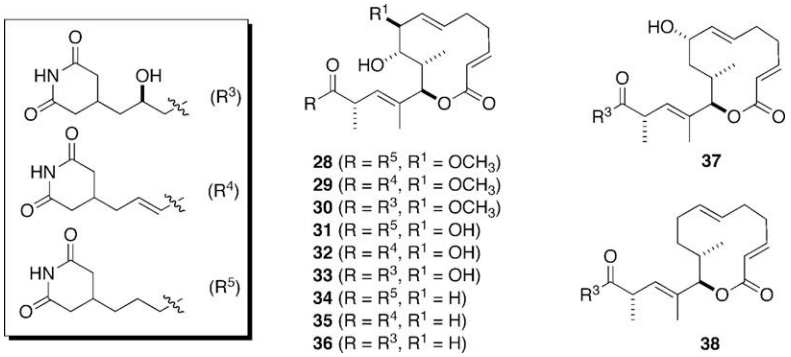

Figure 3. Biosynthetically derived starting materials for semi-synthetic production of **9**–**27**. Compounds **28**–**36** originate from fermentations of the iso-MGS producer *S. platensis*; **37** and **38** originate from *S. amphibiosporus*.

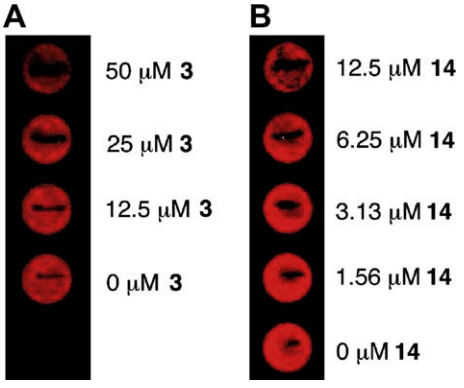

Figure 4. Representative SWH assays with 4T1 cells performed on (A) natural product **3** and (B) semi-synthetic MGS congener **14**.

Further evaluation of Table 1 reveals two additional key findings. It is clear by comparing the SWH data of compound **14** to those of **15** and **16** that, all other features remaining the same, subtle changes in macrolide substitution patterns impact activity. This too is noted in comparing cell migration inhibition by compound **17** to that of compounds **14**–**16**. These data support earlier assertions regarding the importance of the macrolide core. Finally, it is interesting to note that cytotoxicity data generated for all compounds were generally low indicating that results obtained for SWH assays are independent of effects induced by cell killing. Cytotoxic IC_{50}s for all new compounds were determined to be well above those found for cell migration inhibition, a property that is highly desirable for an antimetastatic agent.

The biosynthetic efforts detailed here, complementary to those of total synthesis-directed efforts, have afforded compounds otherwise difficult to access. The crucial nature of glutarimide side chain modifications and the integrity and substitution of the macrolide core of **3** and related congeners are apparent and will be crucial to the continued development of cell migration/metastasis inhibitors. However, the most striking feature of this work is the dramatic potency of **17** revealed by SWH assays. The activity of **17** rivals that of stable synthetic agents **7** and **8** previously reported

Table 1
Summary of cell migration inhibition[a] and cytotoxicity IC_{50} values for compounds **3**–**5** and **9**–**27**[b]

Compound	Migration inhibition IC_{50}[c] (μM)	Migration inhibition IC_{50}[d] (μM)	Cytotoxicity IC_{50}[c] (μM)	Cytotoxicity IC_{50}[d] (μM)
3	17 (1.1)	14 (1.2)	4.6 (1.0)	5.8 (0.61)
4a	>100	>100	>100	>100
4b	>100	>100	>100	>100
5	>100	>100	>100	>100
9[15]	>100	>100	>100	>100
10[15]	>100	>100	>100	>100
11[15]	>100	>100	>100	8.5
12[15]	>100	>100	>100	44
13[15]	>100	>100	29	32
14[14,15]	1.8 (0.21)	1.8 (0.11)	5.2 (0.72)	5.3 (1.4)
15[14]	>100	>100	>100	>100
16[16]	>100	>100	13	10
17[16]	70 nM (4.4 nM)	0.33 (0.010)	1.0 (0.16)	0.98 (0.16)
18[16]	>100	>100	>100	81
19[14]	>100	>100	>100	>100
20[14]	>100	>100	>100	>100
21[16]	>100	>100	>100	>100
22[14]	>100	>100	>100	>100
23[14]	>100	>100	>100	20
24[14]	>100	>100	>100	>100
25[14]	>100	>100	>100	>100
26[14]	>100	>100	>100	>100
27[14]	>100	>100	>100	>100

[a] Values derived from SWH assays.
[b] SE values in parentheses, references for **9**–**27** provided in left column.
[c] Using MDA-MB-231 human mammary adenocarcinoma cells.
[d] Using 4T1 mouse mammary adenocarcinoma cells.

to have IC_{50}s in 4T1 cell-based SWH assays of 100 and 255 nM, respectively.[9,10] Similar assays using MDA-MB-231 cells revealed **7** to have an IC_{50} of 350 nM and **8** to have an IC_{50} of 2.7 μM, clearly comparable to **17**.[10] The absence of C-8 or C-9 oxygenation of **17** suggests a possible correlation between compound hydrophobicity and activity since **17** is so much more potent than **14**, **15**, or **16**. However, because **14** with oxygen functionalities at both C-8 and C-9 is much more potent than either **15** or **16**, attempts to directly correlate hydrophobicity with activity would appear premature in the absence of a more detailed understanding of inhibitor-to-target contacts responsible for cell migration inhibition. Although not as potent a cell migration inhibitor as **17**, **14** also is significantly more active than its corresponding lead compound **3**.

In sum, these studies highlight structural features critical to the potential of MGS analogs as antimetastatic agents; no features investigated here are sufficient to compensate for the functionally deleterious impact of macrolide linearization observed for the DGNs. Macrolide integrity and elaboration play clear roles in activity attenuation as does glutarimide side chain modification. These findings and dramatically improved MGS analogs **14** and **17** advance efforts, both synthetic and biosynthetic, to develop antimetastatic agents for the control and eradication of many cancer types.

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