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Lactimidomycin, Iso-migrastatin and Related Glutarimide-containing 12-membered Macrolides are Extremely Potent Inhibitors of Cell Migration

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Abstract

Migrastatin (**1**), iso-migrastatin (**5**) and lactimidomycin (**7**) are all glutarimide-containing polyketides known for their unique structures and cytotoxic activities against human cancer cell lines. Migrastatin, a strong inhibitor of tumor cell migration, has been an important lead in the development of antimetastatic agents. Yet studies of the related 12-membered macrolides iso-migrastatin, lactimidomycin and related analogs have been hampered by their limited availability. We report here the production, isolation, structural characterization and biological activities of iso-migrastatin, lactimidomycin, and 23 related congeners. Our studies showed that, as a family, the glutarimide-containing 12-membered macrolides are extremely potent cell migration inhibitors with some members displaying activity on par or superior to that of migrastatin as exemplified by compounds **5**, **7**, and **9**–**12**. On the basis of these findings, the structures and activity of this family of compounds as cell migration inhibitors are discussed.

Migrastatin (MGS, **1**) is a glutarimide-containing 14-membered macrolide originally isolated from *Streptomyces* sp. MK929-43F1. It was later found in fermentation broths of *S. platensis*, and has been extensively studied for its ability to inhibit cell migration.[1–4] Potentially useful antimetastasis activities of this glutarimide-containing polyketide and numerous truncated synthetic derivatives including **2**–**4** (Fig. 1) have been experimentally correlated to in vitro and in vivo cell migration inhibition.[2,5,6] The MGS core **2** is the most potent cell migration inhibitor published to date with an IC_{50} of 22 nM.[2]

MGS is a shunt metabolite of the 12-membered macrolide-containing polyketide iso-migrastatin (iso-MGS, **5**)[4] that is known to undergo facile hydrolytic and thermolytic transformations yielding a wide array of ring expanded and ring-opened analogs.[7–9] Like **5**, lactimidomycin (LTM, **7**) is a 12-membered unsaturated macrolide antibiotic characterized by a biosynthetically rare glutarimide sidechain.[10] LTM, first discovered in 1992 from

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Supporting Information Available: Full experimental details, ^{1}H and ^{13}C NMR data and assignments for compounds **6**, **8**, and **17**–**27** are available free of charge via the Internet at http://pubs.acs.org.

fermentations of *Streptomyces amphibiosporus* ATCC53964, displays strong in vitro cytotoxicity against a number of human cell lines (IC_{50} = 3.0 ~ 65 nM), in vivo antitumor activity in mice, potent antifungal activity, and inhibits both DNA and protein synthesis.[10] Studies of **5** and **7** have been extremely limited, due principally to low fermentation titers. Although the total synthesis of **5** has recently been accomplished and will surely contribute to the development of new cell migration inhibitors, no total synthesis of LTM has yet appeared.[11]

We have optimized the fermentation of microorganisms leading to significant improvements in target natural product titers, the ability to recover previously undetected minor metabolites, and significantly more efficient, less costly, and greener secondary metabolite extractions. Exemplary in this regard has been our use of XAD-16 resin to extract **1**, **5**, and related metabolites **9**–**12**, and **14** from *S. platensis*[8] as well as **7** and **13** from *S. amphibiosporus* (Fig. 2).[12] We now report the isolation, structural characterization and biological activity of 12-membered structural relatives of 14-membered lead compound **1** and semi-synthetic derivatives thereof (**17**–**29**). Many of these 12-membered macrolides inhibited tumor cell migration with potencies far surpassing that of **1** and very much on par with the MGS core **2**.

The effects of **5**–**29** on the migration of 4T1 mouse mammary tumor and MDA-MB-231 cells were investigated using standardized scratch wound healing (SWH) assays and IC_{50}s for cell migration inhibition compared to the structurally related and extensively studied **1**.[2,14] Compound cytotoxicities were also determined to establish clear independence of cell migration inhibition from the possible effects of cell death.[14]

An impressive 14 compounds (**5**, **7**–**12**, **14**, **17**, **20**, **23**, and **25**–**27**) out of the collection tested inhibit tumor cell migration more potently than **1** suggesting the general superiority of the 12-membered glutarimide-capped macrolides relative to their 14-membered MGS cousins (Table 1).[14] Particularly striking are **5** and **7**; both inhibited cell migration with potencies in the low nM range, approximately three orders of magnitude better than **1** and superior in some cases to the synthetic MGS analog **2**. Of the compounds evaluated **13**, **15**, **16**, and **24** were found to be too toxic for SWH assays; these are therefore not included in Table 1.

The wide variance of activity among **5**–**29** gives insight into how structural features attenuate activity. Because **5**–**29** all retain the 12-membered macrolide, changes in activity can be correlated to one or a combination of alterations to three key functionalities including; (i) the glutarimide sidechain, (ii) oxidation state of the C2-C3 bond, and (iii) alteration of C8 and C9 substitution patterns.

We have found with MGS congeners that hydroxylation at C17 profoundly improves activity relative to the fully saturated and 16, 17-didehydro analogs.[14] Activity improvement by C17 hydroxylation is apparent also across **5**–**29**. Comparison of IC_{50} values for wound healing inhibition by **6** and **17** to inhibition by **20**, as well as wound healing inhibition by **18** and **19** to inhibition by **25** substantiates the influence of C17 hydroxylation. The latter case is particularly dramatic as both **18** and **19** are devoid of activity while **25** possesses an IC_{50} of ~260 nM. Iso-MGS, lacking the C17 hydroxyl moiety, displays cell migration inhibitory activity far greater than its hydroxylated counterpart **12** although multiple modes of target binding may account for this exception to the rule.[14]

Isolation of **5**, **7**, **9**–**12**, **14**–**16** permitted semi-synthetic production of 2, 3-dihydro analogs **6**, **8**, **17**–**27** (Supporting Information) and cysteine conjugates **28** and **29**.[8] In tandem with SWH assays, the importance of the C2, C3-olefin is made clear; reduction adducts **6** and **8** display IC_{50}s at least 100-fold higher than observed for their unsaturated precursors **5** and **7**, respectively. Comparison of IC_{50} values for the olefinic compounds **9**–**11**, and **14** to their reduced analogs **17**–**19**, and **22** respectively, also reveals a pronounced reduction in the ability

of reduced congeners to inhibit cell migration. Particularly striking is that reduction of compound **14** (IC_{50} = 3.9 nM) affords **22**, a compound completely devoid of activity. Among the glutarimide containing 12-membered macrolides, the C2, C3-olefin plays a vital role. Possibly important to understanding how best to optimize the pharmacokinetics of such compounds, this assertion is further supported by the inability of cysteine conjugates **28** and **29** derived from **5** and **14**, respectively, to inhibit tumor cell migration.

The results of SWH assays reveal that, all other factors being equal, the extent of macrolide oxygenation alters activity although not to the extent of C17 oxidation or enone reduction. As reflected by comparing the activity of compounds **9** to **11; 10** to **5** and **14**; and **20** to **23**, **25**, and **27**, it is evident that increased macrolide polarity via C8 and/or C9 oxygenation results in moderately improved activity. Comparison of the activities of **23** to **27** suggests that macrolide OH moieties may serve as vital H-bond donors. This is in contrast to previous studies in which increased polarity of 14-membered macrolide congeners of **1** led to reduced activity in SWH assays.[14]

Finally, cytotoxic IC_{50}s for the majority of new compounds were determined to be well above those found for cell migration inhibition, a property that is highly desirable for antimetastatic agents where cell killing is not the intended goal.[14]

These studies highlight structural features critical to the potential of glutarimide-containing polyketides as antimetastasis agents. However, the most striking feature of this work is the dramatic potency of **5** and **7** revealed by SWH assays. These, and related compounds such as **9**–**12** will serve as excellent leads to advance the development of this family of natural products and their analogs as antimetastasis agents for the control and eradication of human cancers.

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References & Notes

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13. Compounds 9–14 were isolated from *S. platensis* and *S. amphibiosporus* and have been previously reported[7,12] as have 28 and 29.[8] Compounds 15 and 16 were isolated from recombinant *S. amphibiosporus* and 17–27 were derived semi-synthetically (see Supporting Information).

14. For related studies of 1 and congeners see: Ju J, Rajski SR, Lim S-K, Seo J-W, Peters NR, Hoffmann FM, Shen B. Bioorg Med Chem Lett. 10.1016/j.bmcl.2008.07.072

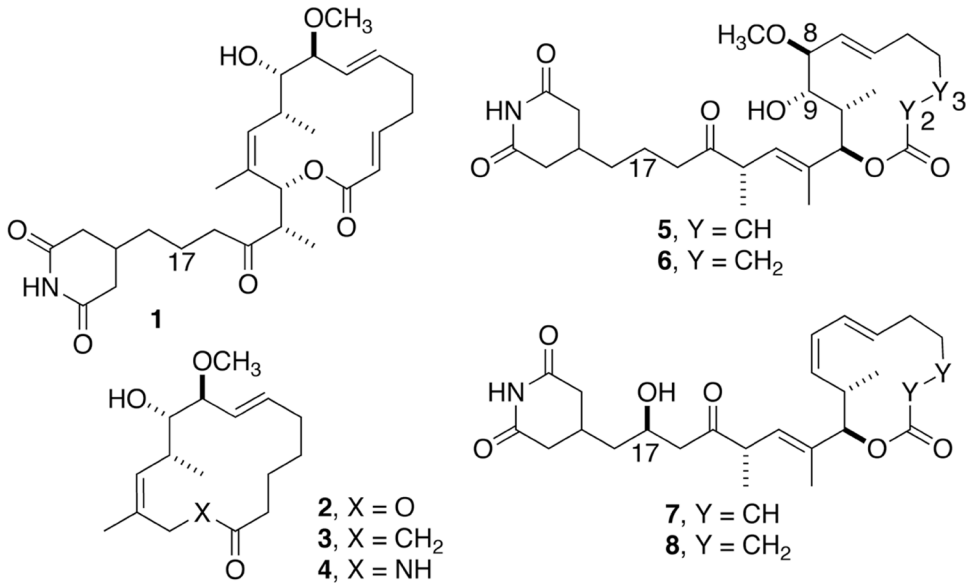

Figure 1.

Structures of the natural products migrastatin (**1**), iso-migrastatin (**5**) and lactimidomycin (**7**), fully synthetic macrolactone (**2**), macroketone (**3**), macrolactam (**4**),[2] and semi-synthetic 2, 3-dihydroiso-migrastatin (**6**), and 2, 3-dihydro-lactimidomycin (**8**).

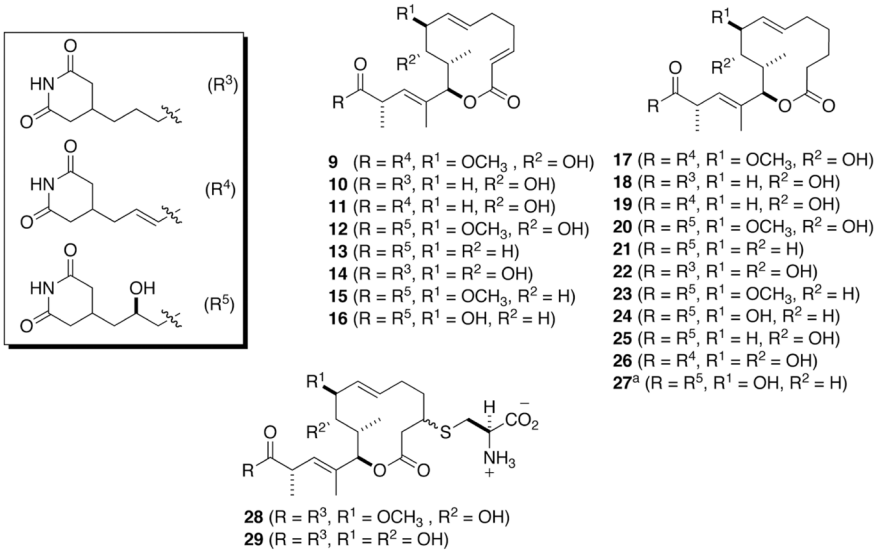

Figure 2.

Structures of LTM and iso-MGS congeners subjected to SWH assays *en route* to evaluation as cell migration inhibitors.[13a] Compound **27** is the 8-(*S*)-epimer of compound **24**.

Table 1

Summary IC_{50} values of cell migration inhibition[a] and cytotoxicity for 12-membered macrolides **5**–**29** in comparison with the 14-membered **1**.[b]

Compound	Migration inhibition IC_{50}, μM[d]	Migration inhibition IC_{50}, μM[e]	Cytotoxicity IC_{50}, μM[d]	Cytotoxicity IC_{50}, μM[e]
1[c]	14 (1.2)	17 (1.1)	5.8 (0.61)	4.6 (1.0)
5	23 nM (3.3)	32 nM (3.4)	0.17 (0.01)	0.33 (0.03)
6	19 (1.6)	13 (1.7)	> 100	> 100
7	5.03 nM (1.1)	0.60 nM (0.3)	0.11 (0.02)	4.3 nM (11)
8	2.1 (0.09)	1.6 (0.83)	44 (2.1)	71 (6.7)
9	60 nM (5.4)	0.87 (0.11)	1.44 (0.10)	0.13 (0.01)
10	57 nM (6.5)	0.5 nM (1.0)	1.95 (0.10)	0.18 (0.02)
11	73 nM (10)	7.1 nM (2.1)	0.80 (0.04)	0.10 (0.01)
12	0.31 (0.03)	62 nM (8.8)	0.80 (0.04)	76 nM (3.4)
14	> 50	3.9 nM (0.51)	1.0 (0.46)	0.11 (0.2)
17	2.0 (0.12)	0.59 (0.10)	20 (0.69)	3.5 (0.44)
18	> 50	> 50	> 100	> 100
19	> 50	> 50	44 (2.0)	17 (0.54)
20	0.12 (0.02)	80 nM (20)	4.8 (0.70)	0.75 (0.11)
21	> 50	> 50	20 (0.48)	13 (0.50)
22	> 50	> 50	> 100	98 (4.2)
23	5.5 (0.88)	1.70 (0.55)	41.7 (4.39)	3.7 (0.27)
25	0.69 (0.08)	0.26 (0.04)	12 (1.1)	1.7 (0.24)
26	> 50	1.24 (0.19)	30 (3.3)	8.0 (1.1)
27	1.7 (0.07)	0.43 (0.06)	24 (3.0)	4.2 (0.47)
28	> 50	> 50	> 100	43 (1.7)
29	> 50	> 50	> 100	69 (3.3)

[a] Values derived from SWH assays.

[b] SE values in parentheses in same units as value to which they refer.

[c] Values previously reported.[14]

[d] Using 4T1 mouse mammary adenocarcinoma cells.

[e] Using MDA-MB-231 human mammary adenocarcinoma cells