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Multifaceted Modes of Action for the Glutarimide-containing Polyketides Revealed

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The glutarimide-containing polyketides represent a fascinating class of natural products that exhibit a multitude of biological activities whose modes of action have remained unknown, until now. The 14-membered macrolide migrastatin (MGS, **1**), was first isolated from *Streptomyces* sp. MK929-43F1 by Imoto and co-workers in 2000 [1,2] and represents one of the three subclasses of the glutarimide-containing polyketides. Its structure was revealed to be a 14-membered macrolide with a glutarimide side chain on the basis of extensive spectroscopic analyses [2]. A second class of these natural products is represented by the linearized MGS congeners dorrigin A (DGN A, **2a**), 13-*epi*-dorrigin A (**2b**), and dorrigin B (DGN B, **3**). The acyclic DGNs were first isolated from *Streptomyces platensis* by McAlpine and co-workers in 1994 [3,4], with DGN A (**2a**) and DGN B (**3**) being regioisomers. Recognizing that **2a** is an acyclic isomer of **1**, Licari and co-workers reasoned that **1–3** might be biosynthesized by the same pathway. They re-cultured *S. platensis*, which was previously only known as a DGN producer, and carefully analyzed all metabolites produced. Indeed, they not only confirmed the production of **2a** and **3** but also isolated **1** as well as a new MGS analog, *iso*-migrastatin (*iso*-MGS, **4**) which, represents the third class of the glutarimide-containing polyketides, the 12-membered macrolides [5]. Consistent with the structural relationship between **2a** and **1**, **3** is an acyclic isomer of **4**. Finally, three additional representatives of the 12-membered macrolide class of glutarimide-containing polyketides are lactimidomycin (LTM, **5**), which was isolated from *Streptomyces amphibiosporus* by Sugawara and co-workers in 1992 [6], and NK30424 A/B (**6**), which were isolated from *Streptomyces* sp. NA30424 by Takayasu and co-workers in 2001 [7]. Interestingly, **6**, a mixture of stereoisomers at the thioether junction, displays structural elements characteristic of both **4** and **5**. In all, the glutarimide-containing polyketides are characterized by a bipartate structure composed of a macrolactone or hydrolyzed product thereof bearing a pendant glutarimide side chain.

A host of biological activities are displayed by the glutarimide-containing polyketides but by far the activities that have most captured the interest of biological, medicinal, and synthetic chemists have been the moderate cell migration inhibitory activity of **1** and the extremely potent cytotoxicity of **5**. It is now well established that tumor cell migration correlates directly

to tumor metastasis, arguably the single most important process dictating a tumor's ability to ultimately kill its host.^[8–11] Consequently, the discovery that **1** is a potent inhibitor of cell migration met with tremendous enthusiasm as it represents a potential antimetastasis agent.^[1,8–11] Indeed, only recently has it been recognized that **4** and **5** also inhibit cell migration with efficacies ~3 orders of magnitude superior to **1**, while the acyclic DGNs and the Cys-adducts of 12-membered macrolides as exemplified by **6** were found to be devoid of cell migration inhibitory activity.^[12]

Despite their very exciting potential the mode/s of action dictating and differentiating cell migration inhibition activities and cytotoxicity for the glutarimide-containing polyketides have not been clear. Two reports from 2010 now help to delineate the role that the glutarimide moiety of **1**, **4**, and **5** plays in the expression of biological activity by these novel polyketides. Central to these findings have been i) synthetic access to **7–10**, ii) the realization that truncated MGS analogs **7–9** display very potent activity as cell migration inhibitors and that **10** retains activity on par with **1**, and iii) inspiration by cycloheximide (CHX, **11**), another glutarimide-containing natural product, from *S. griseus*, to inhibit protein synthesis. Total syntheses of **1** and **5** were accomplished in 2003 and 2007, respectively.^[13,14] Of particular significance were subsequent diverted total synthesis (DTS) of **1**, which made possible the rapid production of an assortment of **1** congeners as well as assessment of their activities as cell migration inhibitors.^[15] Of the analogues produced and tested, a number of truncated variants (**7–9**) were found to possess cell migration inhibitory activities surpassing that of the natural product. Particularly notable was the MGS core **7**, which is the most potent cell migration inhibitor belonging to the 14-membered macrocyclic class (IC₅₀ = 22 nM) although the compound suffers from instability issues surrounding the lactone moiety.^[15] Truncated MGS analogs **8** and **9** also display 10³ improved cell migration inhibitory activity relative to **1** while also possessing enhanced stability relative to **7**. Advanced by the recognition that 14-membered macrocycles, lacking the glutarimide side chain of **1**, could still retain potent activity as cell migration inhibitors, the biotinylated macroketone **10** was synthesized and found to retain potent activity. More recent efforts have shown that it is possible, via DTS, to dispense with the carbonyl moiety altogether and to retain significant cell migration inhibition activity.^[16]

Huang and co-workers have now reported that treatment of 4T1 breast tumor cells with biotinylated **10** followed by affinity matrix chromatography and SDS-PAGE revealed the 58 kDa primary actin crosslinking protein fascin as the target of **10**, and by inference, **1** and synthetic macrocycles **7–9**.^[17] The well-established role of actin modeling in tumor cell migration and the role of fascin in coordinating this process makes clear that small molecules able to ablate or inhibit fascin function constitute excellent migration inhibitors.^[8–10] In depth study of the interaction of macroketone **8** with purified recombinant fascin in an F-actin pelleting assay and fluorescence microscopy revealed that i) **8** significantly diminished fascin-induced bundling of F-actin polymers, and ii) **8** decreased the thickness of actin bundles relative to bundles produced by fascin in the absence of macroketone. Furthermore, they solved the X-ray crystal structure of fascin in the absence and presence of **8**. Solution of the **8**:fascin cocrystal at 2.7 Å resolution revealed that **8**, and presumably **10** and **1** also, binds to fascin in one of the actin-binding sites thus interfering with the binding of actin to fascin. Follow up studies involving mutations at Glu391, His392, Lys471, His 474, and Ala488 revealed that several fascin residues involved in binding to **8** are, as one might predict, normally involved in binding to actin. These results suggest that **8** competitively binds to fascin although the structure reported for **8** in the **8**:fascin co-crystals has been found to differ from that shown in Figure 1 and thus requires further clarification.^[18]

Complementary to the synthetic means by which **7–10** have been produced we have applied biosynthetic methods to reveal that members of this family of natural products are related in so far as (i) **1–3** are shunt metabolites of **4** despite the fact that **1** has been the predominantly studied product from *S. platensis*,^[19] (ii) **4** and related congeners undergo H₂O-mediated rearrangement to afford the linear DGN and 14-membered macrolide MGS scaffolds,^[20] (iii) **4** and related congeners undergo a thermally induced [3,3] sigmatropic rearrangement to afford 14-membered macrolides; **5**, bearing the C-8, C-9 olefin, is incapable of such ring expansions,^[21] (iv) *S. amphibiosporus* produces, along with **5**, 12-membered macrolides capable of hydrolytic conversion to the DGN and MGS scaffolds.^[22] We have also unveiled parallels between the biosynthesis of **1–4** and the pathway to **5** via fermentation optimization and subsequent characterization of intermediates formed *en route* to **5**. Complementing these advances we have used combinatorial biosynthesis along with semisynthesis to produce structurally rich libraries of **1**, **4** and **5** congeners, as well as congeners featuring the DGN and **6**-scaffolds.^[12,23] Bioassays with these libraries revealed that, while the DGNs and **6** congeners completely lack cell migration inhibitory activity, the activities of the 12-membered macrolides to be far superior to those of **1** (by ~ 10³ fold). Of the 12-membered macrolides evaluated, **5** was the most potent thereby making it an ideal choice for further mode of action studies though **4** demonstrated highly analogous activity.

Efforts to elucidate the cytotoxic activities of **1 – 5** and related congeners have revealed the importance of translation elongation as a target for the 12-membered macrolides.^[17] Early metabolic labelling experiments in collaboration with Liu and co-workers revealed that, among natural products and engineered congeners tested, **5** and related analogues had a profound effect on protein synthesis, a property that inspired the parallel study of this family of compounds in comparison with CHX (**11**) based on their structural similarity. Concomitantly, the use of the glutarimide **11**, a well established protein synthesis inhibitor, afforded similar results albeit at concentrations roughly ten times greater than **5**. All representatives of the 14-membered macrolides, the acyclic DGNs, and the congeners featuring the **6**-scaffolds failed to impact protein synthesis. Only **4,5**, and related 12-membered macrolide congeners were found to inhibit translation. Subsequent work revealed that both **5** and **11** block translation elongation through binding to the E-site of the large ribosomal subunit. Differences do exist, such as the ability of **5** to stall the ribosome on the initiating AUG codon whereas **11** allows one round of translocation such that the ribosome stalls on the second codon of the template mRNA. Additionally, it is clear that **5** has markedly greater affinity for the ribosome than does **11**. Both the higher affinity of **5** and its ability to allow one round of translocation to proceed are presumably due to the presence of its 12-membered macrolactone moiety, which is absent in **11** and **2, 3, 6** as well as their congeners. In all, it is significant that **4** and **5** are excellent inhibitors of cell migration and potent translation inhibitors whereas the closely related **1** is capable of cell migration inhibition but was found to be completely devoid of translation inhibition activity. It is noteworthy that **4**, despite having a macrolactone substitution pattern more similar to **1** than to **5** also is a remarkable inhibitor of translation as well as tumor cell migration.

The results from Huang's group and our collaboration with Liu and co-workers provide a number of important lessons on how the glutarimide-containing polyketides work. First, it is now possible to assign to each hemisphere (glutarimide vs. macrolactone) a predominant function and to correlate this function to observed biological activities. Huang and co-workers provide very compelling evidence highlighting the macrolactone of **1**, as emulated by the hydrazone-tethered macroketone **11**, as a fascin-binding moiety responsible for compromised actin bundling and downstream cell migration inhibition. Whether the 12-membered macrolide **4** and **5** bind to fascin in a fashion similar to that of **1** and related congeners is not yet clear although one might readily envision how this might be possible,

begging the follow up experimental confirmation. Second, it has always been interesting that the truncated MGS analogs **7–9** possess $\sim 10^3$ greater activity as cell migration inhibitors than the parent natural product. This might now be rationalized, at least in part, by the realization that the glutarimide of **1**, by analogy to findings with **4** and **5**, may allow **1** to be titrated away from fascin, in favor of ribosome binding interactions. Titration of **7–9** away from fascin and to a glutarimide-binding target is simply not possible and may explain the enhanced ability of **7–9** to inhibit tumor cell migration compared to **1**. That the two hemispheres of the glutarimide-containing polyketides have different molecular targets provides a basis for understanding bioactivity differences and how best to attenuate that activity.

A third lesson invokes that the 12-membered macrolide along with the glutarimide moieties work together to define translation elongation inhibition activity. This conclusion is based on the fact that intact **4** and **5** were both found to inhibit translation whereas acyclic DGNs did not, nor did the 12-membered **6** and 14-membered **1**. The ability of **11** to inhibit translation, albeit at 10-fold greater concentrations than needed for **5**, the similarity of its glutarimide side chain to that of **5**, and its much simpler and smaller cyclic ketone relative to the macrolides of **4** and **5** support the essential nature of the glutarimide in ribosome binding. Translation elongation inhibition and inferred downstream cytotoxicity is clearly the result of molecular synergism between the glutarimide and macrolactone moieties of **4** and **5** and related compounds.

Clearly, there is still much fertile ground to be covered in developing a clearer understanding of how the glutarimide-containing polyketides express cell migration and/or protein synthesis inhibition activities. However, the advances seen in 2010 bode well for a very fruitful next decade when it comes to future mode of action studies. We anticipate that both organic synthesis as typified by the efforts resulting in **7–9**, **10**, as well as, combinatorial biosynthesis to produce a greater spectrum of structural diversity among analogues of **1**, **4**, and **5** will allow rapid refinement of our understanding of fascin binding and translation elongation inhibition by these natural products and how these processes can be translated to the clinic. The remainder of the decade promises to be exciting for the development of new antimetastasis agents by virtue of these highlighted advances.

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Scheme 1.

Glutarimide-containing polyketides. Migrastatin (**1**), dorrigocin A (**2a**), 13-*epi*-dorrigocin A (**2b**), dorrigocin B (**3**), *Iso*-migrastatin (**4**), dactimidomycin (**5**), and NK30424 A/B (**6**).

Scheme 2.

Compounds most crucial to determining the modes of action of **1**, **4**, and **5**. Truncated **1** analogues **7–9** achieved by DTS, biotinylated macroketone (**10**) used to identify fascin as the target of macrolactone moieties, and cycloheximide (**11**) inspired to elucidate the mechanism of cytotoxicity for **4** and **5** by inhibiting translation elongation