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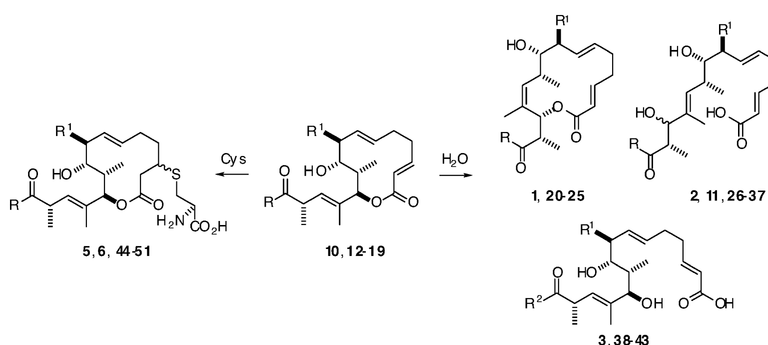

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Iso-Migrastatin Congeners from *Streptomyces platensis* and Generation of a Glutarimide Polyketide Library Featuring the Dorrigocin, Lactimidomycin, Migrastatin, and NK30424 Scaffolds

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Migrastatin (**1**) is a tumor cell migration specific inhibitor,[1,2] and synthetic analogues of **1** have been prepared as therapeutic candidates for treating tumor metastasis.[3] Dorrigocins A (**2**) and B (**3**) are novel natural product inhibitors of carboxyl methyltransferase involved in the processing of Ras-related proteins, serving as a valuable tool to study cellular signal transduction.[2,4] Structurally related to **1**, **2**, and **3** are lactimidomycin (**4**)[5] and NK30424 A (**5**) and B (**6**).[6] While **4** exhibits potent antitumor activity,[5] **5** and **6** inhibit LPS-induced TNF-α production by suppressing the NF-κB signaling pathway,[6] a property that could be exploited for the treatment of cancer and inflammation. These natural products belong to the glutarimide polyketide family, which also include antifungal antibiotic cycloheximide (**7**), streptimidone (**8**), and 9-methylstreptimidone (**9**).[7]

We have recently reported that iso-migrastatin (**10**)[2] is the main natural product of *Streptomyces platensis* NRRL18993, and **1**, **2**, and **3** as well as 13-*epi*-dorrigocin A (**11**) can be readily derived from **10** via a facile, H_2O-mediated rearrangement.[8] Here, we report the isolation and structural elucidation of eight new congeners (**12**−**19**) of **10** and generation of a 47-member library of glutarimide polyketides featuring the molecular scaffolds of **1**−**6**, **10**, and **11** (Figure 1), setting the stage to investigate the structure and activity relationship for this family of natural products. These results also established the absolute stereochemistry of **5** and **6** and shed new light into the post-polyketide synthase (PKS) steps for the biosynthesis of **10** (Figure 2).

In our early effort to characterize **10** from *S. platensis* fermentation, we noticed a plethora of minor metabolites whose identity as biosynthetic congeners of **10** is apparent upon HPLC−UV and LC-ESI-MS analyses.[8] Since the relative abundance of these congeners appeared to be fermentation condition dependent, we optimized their production by altering the age of the seed culture, level of dissolving oxygen, time of production fermentation, and nature of hydrophobic resin (XAD-16 versus HP-20) supplemented.[9] Under the optimized conditions, *S. platensis* was typically fermented in the presence of XAD-16 for its ability to sequester and, hence, stabilize the metabolites and increase their production titer. The resin was harvested by filtration and air-dried, and the metabolites were eluted with anhydrous ethanol and concentrated in vacuo to afford a crude mixture. Purification of these compounds proved to be challenging since they undergo a rapid, H_2O-mediated rearrangement, precluding any aqueous solvent-based preparative HPLC method. They were eventually separated by repeated flash silica gel column chromatography, eluted alternatively with two different solvent systems ($CHCl_3$/MeOH and hexane/EtOAc), and water was avoided throughout the purification. These procedures resulted in the identification of eight new compounds (**12**−**19**), together with **10** as the major metabolite (Figure 1). With the exception of **15** and **16**, the isolated yields of the new compounds range from 5 to 35 mg/L.[9]

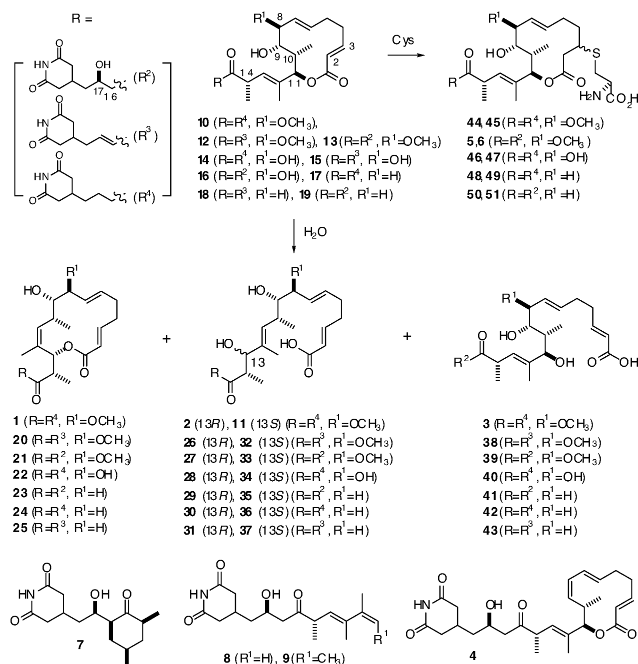

Figure 1. Structures of selected glutarimide-containing polyketide natural products and isolation of iso-migrastatin (**10**) and its congeners (**12**−**19**) and their conversion into a glutarimide polyketide library featuring the dorrigocin (**2**, **3**, **11**), lactimidomycin (**4**), migrastatin (**1**), and NK30442 A and B (**5**, **6**) scaffolds via H_2O-mediated rearrangement or cysteine 1,4-addition, respectively.

The ^{1}H and ^{13}C NMR spectra of the purified compounds were fully assigned on the basis of extensive 1D and 2D NMR (COSY, TOCSY, HMQC, and *g*HMBC) and ESI-MS and high-resolution MALDI-FT-MS analyses. These studies established the structures of **12**−**15** and **17**−**19**, as shown in Figure 1. Although we were not able to purify **16** due to its low abundance, its structure was supported by LC-ESI-MS analysis; its short HPLC retention time is also consistent with **16** being the most polar congener among the nine compounds identified.

The stereochemistry of these compounds was deduced from their ^{1}H and ^{13}C NMR spectra data in comparison with those of **10**.[9] The C-16/C-17 trans double bond (for **12**, **15**, and **18**) was based on its diagnostic coupling constant. The HO− group at C-17 (for **13** and **19**) was assigned *R* configuration on the basis of its near identical splitting patterns and chemical shifts at C-15, C-16, C-17, C-18, and C-19 to those of **8** (all measured in $CDCl_3$), the absolute *R* configuration of which had been determined previously.[7,9] Other asymmetric carbon centers (8*S*, 9*S*, 10*S*, 11*R*, and 14*S*) were

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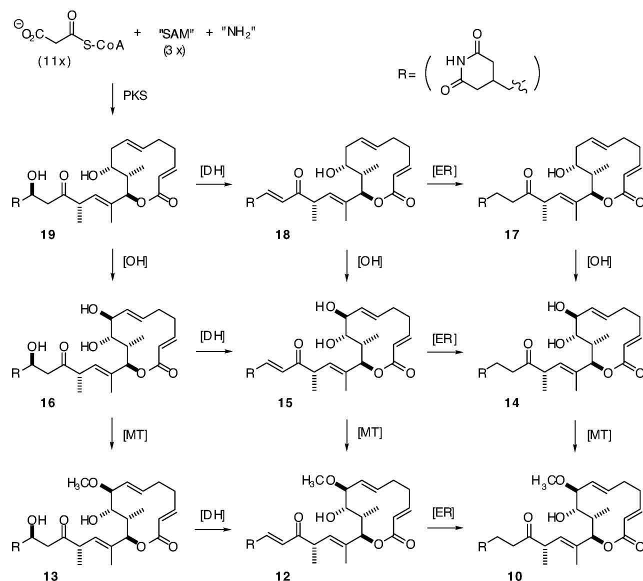

Figure 2. Post-PKS processing steps in the biosynthesis of iso-migrastatin (**10**) from the nascent polyketide intermediate (**19**) in *S. platensis* NRRL18993 (SAM, (*S*)-adenosylmethionine and NH_3, an unspecified amino donor). DH, dehydratase; ER, enoyl reductase; MT, methyltransferase; OH, hydroxylase.

postulated to be identical to those of **10** by comparative analyses of their ^{1}H and ^{13}C NMR splitting patterns and chemical shifts.[2,8,9]

The isolation of these congeners not only further supports the early conclusion of **10** as the main metabolite of *S. platensis*[8] but also provides new insight into the post-PKS steps for the biosynthesis of **10**. Four minimal steps, hydroxylation at C-8, O-methylation at HO−C-8, dehydration at HO−C-17/C-16, and enoyl reduction at C-16/C-17, could be conceptually envisaged to convert the nascent polyketide intermediate **19** to **10** (Figure 2). The fact that all possible intermediates have been identified strongly supports this proposal. While the current study falls short of determining the precise timing of individual steps, it certainly unveiled the substrate promiscuity of the enzymes for the post-PKS steps, a property that could be exploited to further expand the structural diversity for this family of natural products by processing additional analogues with modified polyketide scaffolds.

The availability of the new congeners inspired us to investigate if they can undergo the same H_2O-mediated rearrangement into the corresponding **1**, **2**, **3**, and **11** scaffolds as **10**. Incubation of **12**, **13**, **14**, **17**, **18**, or **19** in H_2O indeed afforded, in quantitative yield, the corresponding congeners of **1** (**20**−**25**), **2** (**26**−**31**), **11** (**32**−**37**), and **3** (**38**−**43**), respectively, whose structures were confirmed upon extensive 1D and 2D NMR as well as MS analyses[9] (Figure 1).

Isolation of **13** also prompted us to examine the biosynthetic relationship between **10** and **5** and **6**. Takayasu and co-workers first isolated **5** and **6** from *Streptomyces* sp. NA30424 and established their structures as two stereoisomers on the basis of NMR and MS data, with their stereochemistry undetermined.[6] Viewing **5** and **6** as a cysteine 1,4-adduct at C-2/C-3 of **13**, we incubated **13** with cysteine. The reaction was completed in 2 min at room temperature, yielding quantitatively two diastereomers at C-3 in an approximately 2:3 ratio whose identity as **5** and **6** was confirmed by their identical ^{1}H and ^{13}C NMR spectra compared to those reported previously.[6,9] Since all chiral centers in **13** remain intact during the 1,4-Michael addition, this result also established the absolute stereochemistry of **5** and **6** (i.e., the same configuration of 8*S*, 9*S*, 10*S*, 11*R*, 14*S*, and 17*R* as in **13**). The apparent intrinsic reactivity of the C-2/C-3 double bond as Michael acceptor further inspired us to test if **10** and its other congeners (**14**, **17**, and **19**) can undergo the same 1,4-addition. Incubation of these compounds with cysteine indeed afforded the corresponding adducts in quantitative yields, the structures of which as **44**−**51** were established by MS and 1D and 2D NMR analyses[9] (Figure 1). Rapid addition was also observed between cysteine and **12**, **15**, or **18**, but yielded a mixture of up to four adducts due to the presence of the second Michael acceptor at C-16/C-17. This facile reactivity also raises the question if **13** is the main metabolite of *S.* sp. NA30424 and **5** and **6**, in fact, are the result of adventitious addition between **13** and cysteine during fermentation or isolation.[6]

Synthetic analogues of **1**, **2**, **3**, **5**, and **6** with improved biological activities have been prepared,[3,6] validating the utility of this privileged molecular scaffold in lead optimization. None of the compounds produced in this study have been prepared previously, however, and nor can they be readily accessed via total synthesis. The current study, therefore, complements the synthetic effort and opens an alternative way to further diversify the glutarimide polyketide molecular scaffold. Combination of both biosynthetic and synthetic efforts holds the greatest promise to fully realize the potential of this family of natural products in drug discovery.

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Supporting Information Available: Full experimental details and MS and ^{1}H and ^{13}C NMR data and assignments for compounds **12**−**51**. This material is available free of charge via the Internet at http://pubs.acs.org.

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