Title

Studies on saccharothriolides, phenyl-substituted 10-membered macrolides from a rare actinomycete *Saccharothrix* sp. and precursor-directed *in situ* synthesis of saccharothriolide analogs

Keywords

saccharothriolides, *Sacharothrix* sp., 10-membered macrolides, precursor-directed *in situ* synthesis

Abstract

Expanding natural chemical diversity is important for drug leads discovery, since natural products can exhibit highly potent and/or selective biological activities. So far, over 10,000 bioactive secondary metabolites have been identified from a variety of actinomycetes. Rare actinomycetes (or non-streptomycete actinomycetes) are attractive resources of secondary metabolites that show biological activities with unprecedented chemical structures. Genus *Saccharothrix*, one of the rare actinomycetes, was first reported from a soil sample collected in Australia in 1984. A growing number of novel natural products with a structural and biological diversity have been reported from this genus over the past few years.

During the chemical screening, we found that the culture extract of *Saccharothrix* sp. A1506 contains novel metabolites and discovered a variety of phenyl-substituted 10-membered macrolides, designated as saccharothriolides (Chapter 1). Structure analyses implied the presence of common biosynthetic precursors, including precursor A (Chapter 2). Identification of precursor A allowed us to generate new saccharothriolide analogs by precursor-directed *in situ* synthesis (PDSS) method (Chapter 3). In this thesis, isolation and generation of unique metabolites by exploring rare resources and PDSS method are described.

Chapter 1

Isolation, structure elucidation, and biological activities of saccharothriolides A-F, produced by a rare actinomycete *Saccharothrix* sp. A1506.

We surveyed more than 30,000 microbe cultures by LC-MS analysis to find novel metabolites, and isolated six new 10-membered macrolides, saccharothriolides A-F (**1**-**6**) (Figure 1), from the rare actinomycete *Saccharothrix* sp. A1506. Their chemical structures were deduced by extensive spectroscopic analyses including advanced universal NMR database method and HR-ESI-MS data. The absolute configurations were determined by the modified Mosher's method and TDDFT-calculation of ECD spectra. Saccharothriolides A (**1**), B (**2**), D (**4**) and E (**5**) had an aryl amine substituent in the lactone ring through a C-N bond, while saccharothriolide C (**3**) possessed a hydroxy group at C-7. Saccharothriolides D (**4**) and E (**5**) were determined to be C-2 epimers of saccharothriolides A (**1**) and B (**2**), respectively. Saccharothriolide F (**6**) was identified to be a demethylated congener of saccharothriolides A (**1**) and D (**4**) at the C-2 position.

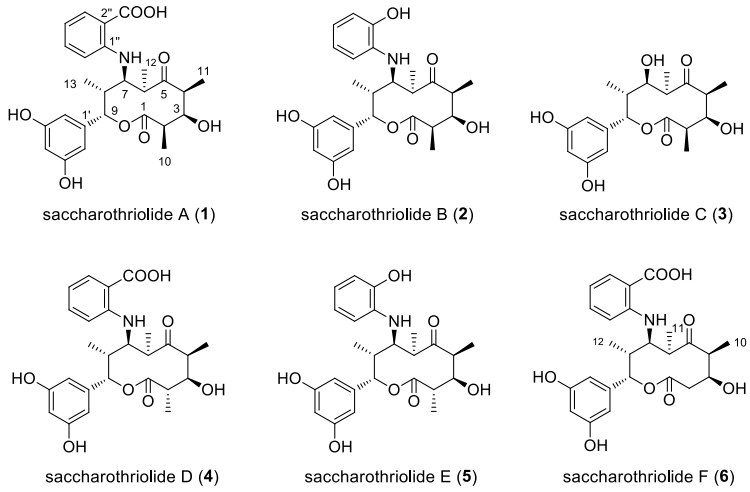

Figure 1. Chemical structures of saccharothriolides A-F (**1**-**6**).

Among saccharothriolides A-F (**1**-**6**), only saccharothriolide B (**2**) exhibited moderate cytotoxicity against human tumor cell lines HeLa and HT1080 with IC_{50} values of 17.9 and 13.9 μM, respectively, and showed weak antibacterial activity against *Staphylococcus aureus* in a paper disc assay.

The discovery of an array of new phenyl-substituted 10-membered macrolides demonstrates the potential of this genus *Saccharothrix* as drug discovery resources.

Chapter 2

Plausible biosynthetic pathway of saccharothriolides and isolation of a key precursor "precursor A"

Polyketide natural products are a structurally diverse group of highly oxygenated secondary metabolites biosynthesized by a complex cluster of enzymes known as a polyketide synthase (PKS). Macrolides are biosynthesized by type I modular polyketide synthase (PKS) pathways.

Saccharothriolides possess unique phenyl-substituted 10-membered macrolide structures that contain a variety of substituents at C-7. As in the case of other macrolides, saccharothriolides seem to be synthesized *via* polyketide biosynthetic pathway. An aryl starter unit and four units of methyl-malonyl-CoA seem to be conjugated followed by cyclization to yield precursor metabolites. The precursors with an α,β-unsaturated ketone that can function as a Michael acceptor, and are likely attacked by aryl amine groups or a water molecule to furnish metabolites saccharothriolides (Figure 2).

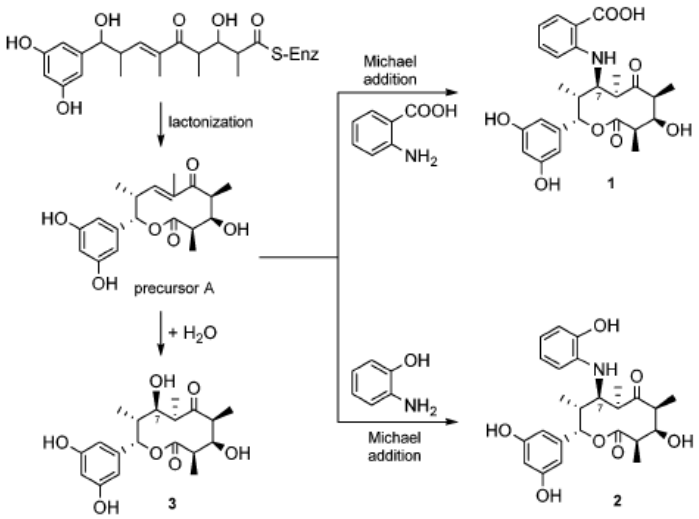

Figure 2. Plausible biosynthetic pathway of saccharothriolides A-C.

Saccharothriolides A-C (**1**-**3**) are likely generated from the precursor metabolite "precursor A". In fact, the culture broth included a significant amount of anthranilic acid and 2-aminophenol. Both of them could be generated from tryptophan, which was included in the culture media. As we expected, saccharothriolides A and B were not

detected when cultured in the absence of tryptophan. Instead, we could detect an ion peak corresponding to precursor A (**9**). We prepared an EtOAc extract from a large-scale tryptophan-free culture of *Saccharothrix* sp. A1506. MS-guided isolation, in which acidic or basic condition, and alcoholic solvents were not used, was carried out, and precursor A (**9**) was successfully isolated.

Chapter 3

Precursor-directed *in situ* synthesis of saccharothriolide analogs and their structure-activity relationship study

As described above, saccharothriolides were generated from precursors possessing α,β-unsaturated ketone via Michael-type addition. This reaction allowed us to explore precursor-directed *in situ* synthesis (PDSS) to obtain further saccharothriolide analogs simply by adding nucleophilic substituents to the culture.

The structure-activity relationship (SAR) study using saccharothriolides A-F (**1**-**6**) had revealed the importance of the substituent at C-2" on their cytotoxicity; metabolites possessing an alcohol group showed activity, while those possessing a carboxylic acid were less potent. To investigate the necessity of the free hydroxy group, the prepared 2- or 3-methoxyaniline-substituted saccharothriolide analogs G-J (**10**-**13**) will be examined their effect on the cytotoxicity against human fibrosarcoma HT1080 cells.

Conclusions

This thesis represents a systematic work on the isolation, structure elucidation, cytotoxicity evaluation of new metabolites saccharothriolides A-F and precursor A, precursor-directed *in situ* synthesis (PDSS) of saccharothriolide analogs G-J. The SAR study of saccharothriolides A-F revealed saccharothriolide B as a potential anticancer drug-lead from actinomycete source. The novel chemical scaffolds and bioactivities of saccharothriolides promote us to discover more potent analogs and investigate their mode of action. This effective PDSS method can provide functional modified analogs which can be obtained difficultly from total synthesis method.