Enantioselective Synthesis of 8-*epi*-Xanthatin and Biological Evaluation of Xanthanolides and Their Derivatives

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An enantioselective synthesis of 8-*epi*-xanthatin (9) has been accomplished starting from the bicyclic lactone 3, which has been used for the synthesis of other xanthanolides, sundiversifolide (4) and diversifolide (5), through a synthetic route without the use of a selenium species. Additionally we have evaluated antimicrobial activities of five natural xanthanolides and their derivatives. Although the synthetic xanthanolides did not show any activity against methicillin-resistant *Staphylococcus aureus* (MRSA), some of the synthetic intermediates did exhibit moderate antimicrobial activities.

Key words xanthanolide; enantioselective synthesis; antimicrobial activity; methicillin-resistant *Staphylococcus aureus*

Multidrug-resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) continue to pose clinical challenges.^{1,2)} Recently the emergence of community-acquired MRSA has compounded the problem^{3,4)} and focused increased attention on efforts to develop new *anti*-MRSA drugs.

In 1997, Sato *et al.*⁵⁾ reported that xanthatin (7), a member of the xanthanolides,⁶⁾ a sesquiterpene family possessing the characteristic oxabicyclo[5.3.0]decene core as its common basic carbon framework, exhibited potent *anti*-MRSA activity with an MIC value of 7.8 $\mu\text{g/mL}$. Xanthanolide sesquiterpenes were isolated from *Helianthus annuus* L. or *Xanthium* sp. and divided into two groups according to the configuration of their *cis*- and *trans*-ring junctions. Representative examples of the *cis*-fused xanthanolides are the sundiversifolide (4),^{7-10,11)} the diversifolide (5)^{10,12)} and the 8-*epi*-xanthatin (9)^{11,13,14)}; xanthatin (7)^{11,15)} and 11 α ,13-dihydroxanthatin (8) belong to the *trans* series.^{11,16)} They are known for their wide variety of

interesting allelopathic,¹⁷⁾ cytotoxic,¹⁸⁾ *anti*-leishmanial,¹⁹⁾ and *anti*-MRSA activity.⁵⁾ As part of our ongoing program directed towards the synthesis of xanthanolides, we have established an efficient access to both *cis*- and *trans*-xanthanolides starting from the bicyclic lactone 2 with a *cis* ring juncture, which can be prepared from the Evans aldol adduct 1 as the common key intermediate in an optically pure form, and have achieved the total synthesis of four xanthanolides, 4, 5, 6 and 7. In this report we describe an enantioselective synthesis of 8-*epi*-xanthatin (9) starting from 3, which is the intermediate for 4 and 5, and the biological evaluation of the compound library including the five xanthanolides and their derivatives (Chart 1).

8-*epi*-Xanthatin (9), commonly isolated from the extracts of the aerial parts of various species in the genus *Xanthium*, has also been obtained upon elimination of acetic acid from xanthumin (8-*epi*-2-acetoxy-3-hydroxanthatin). It has been shown

Chart 1. Synthetic Chart of the Xanthanolide

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Chart 2. Retrosynthetic Analysis of 8-*epi*-Xanthatin (**9**) Chart 3. Synthesis of 8-*epi*-Xanthatin (**9**)

to inhibit larval growth of *Drosophila melanogaster* (fruit fly) and the *in vivo* proliferation of several cultured human tumor cell lines. Not only does it display anti-malarial activity, but it was also found to inhibit the farnesylation process of human lamin-B by farnesyltransferase in a dose-dependent manner *in vitro*. A representative of the *cis*-fused γ -lactone series possessing a characteristic (*E*)-conjugated dienone functionality, this natural product was synthesized in 2005 by Martin *et al.*, in the first reported¹²⁾ total synthesis of **1**. In our previous synthesis of xanthatin (**7**),¹⁵⁾ which possesses a similar basic carbon framework and functionalities except for the stereochemistry on the ring junction, we employed two types of *syn*-elimination of selenoxides for the construction of the vinyl functionality at C1 (using the Nishizawa–Grieco protocol) and the *exo*-methylene at C11. In the present synthesis of 8-*epi*-xanthatin (**9**), out of ecological concerns, we decided not to use stoichiometric selenium reagents, as can be seen in our synthetic strategy in Chart 2. We envisaged the cross-metathesis for the installation of the enone part and an aldol reaction/ β -elimination sequence for the preparation of the *exo*-methylene moiety at C11 of **10** to give 8-*epi*-xanthatin (**9**). A 1,3-diene moiety in **10** would be assembled *via* a base-promoted β -elimination of hydrogen halide from the alkyl halide, which could be prepared from **3**.

The bicyclic lactone **3** was desilylated and the resulting alcohol **11** was converted to the iodide **12**. After several attempts to realize the base-promoted elimination of HI, it was found that treatment of a solution of **12** in toluene with DBU at 80°C produced the diene **10** in 87% yield over 2 steps. Hydroxymethylation of **10** with lithium 2,2,6,6-tetramethylpiperidide (LiTMP) and (1*H*-benzo[*d*][1,2,3]triazol-1-yl)methanol (**13**)²⁰⁾ provided the alcohol **14**, which was acetylated, and the resulting acetate **15** was exposed to DBU in CH₂Cl₂ at room temperature to give **16** in 62% yield over 3 steps. Finally **16** was treated with methyl vinyl ketone (MVK) in the presence

of the Grubbs' 2nd generation catalyst (**17**) (20 mol%) in refluxing CH₂Cl₂ to give 8-*epi*-xanthatin (**9**) in 81% yield. The spectral properties and optical rotation {[α]_D +42.0 (*c*=0.15, CHCl₃); lit.¹¹⁾ [α]_D +44.0 (*c*=0.25, CHCl₃)} of the synthetic compound were identical with those reported in the literature (Chart 3).

With the xanthanolide compound library in hand, we then explored active compounds against MRSA by the disc diffusion test. The screenings were carried out using two MRSA strains, *S. aureus* COL (a reference strain) and the clinical isolate 5. All strains of MRSA (19 strains) and the methicillin-sensitive *S. aureus* (MSSA; eight strains) used throughout this work were recovered from our laboratory stock culture collection.²¹⁾ The first screening was made by the disc diffusion test for five synthetic xanthanolides (**4**, **5**, **7**, **8**, **9**) and all synthetic intermediates (**2a**, **3**, **6**, **10–12**, **14–16**) described in this manuscript. The screening test indicated that only the bicyclic lactone **2a** exhibited zones of inhibition with both strains. Table 1 shows the MIC range, the MIC₅₀s, and the MIC₉₀s of the lactone **2a** and its desilylated analog **2b** against MRSA and MSSA. The MICs of the compounds were determined as described previously.²²⁾ As shown in Table 1, the *cis*-fused lactone **2a** showed activity with MIC₅₀ values of 31.3 μ g/mL against MRSA. In addition, the desilylated alcohol **2b** retained the *anti*-MRSA activity (MIC₅₀=62.6 μ g/mL). On the other hand, the *trans*-lactones did not show any activity at all. We also assessed the activity against Gram-negative *Pseudomonas aeruginosa*, but no active compounds could be found from our library.

Next we turned our attention to the antifungal activity of the synthetic xanthanolides. We found that the bicyclic lactone **2a** exhibited activity against *Candida* strains with an MIC₅₀ value of 31.3 μ g/mL, whereas the desilylated alcohol **2b** was completely inactive. However, the triene **16**, which has no activity against MRSA, showed moderate activity

Table 1. Evaluation of *anti*-MRSA Activities

Compound	MIC ($\mu\text{g/mL}$)					
	MRSA ($n=19$)			MSSA ($n=8$)		
	Range	MIC ₅₀	MIC ₉₀	Range	MIC ₅₀	MIC ₉₀
2a	0.98–31.3	31.3	31.3	15.6	15.6	15.6
2b	7.8–62.5	62.5	62.5	31.3–62.5	31.3	62.5

Table 2. Result of *anti*-Candida Assay

Compound	MIC ($\mu\text{g/mL}$) <i>Candida</i> strains ($n=21$)		
	Range	MIC ₅₀	MIC ₉₀
2a	<0.5–62.5	31.3	62.5
2b	>250	>250	>250
16	<0.5–62.5	62.5	62.5

Table 3. Biological Activities against Fungi

Fungi	MIC ($\mu\text{g/mL}$)	
	2a	16
<i>Aspergillus niger</i>	31.3	31.3
<i>Penicillium</i> sp.	15.6	31.3

(MIC₅₀=62.5 $\mu\text{g/mL}$) against *Candida* strains (Table 2).

Furthermore, as shown in Table 3, both **2a** and **16** showed a wide range of activities against two fungi (*Aspergillus niger* and *Penicillium* sp.).

In conclusion we have completed an enantioselective total synthesis of 8-*epi*-xanthatin (**9**) via a selenium-free synthetic route and evaluated the antimicrobial activities of the xanthanolides and their derivatives. It has been determined that the bicyclic *cis*-lactone **2a** showed a wide range of antimicrobial activities against both bacteria (MRSA and MSSA) and fungi (*Candida* species *A. niger* and *Penicillium* sp.). Although its MIC values are moderate, the lactone **2a** with a siloxy functionality interestingly showed higher activity compared with xanthatin (**7**) and 8-*epi*-xanthatin (**9**), which have been reported to have antimicrobial activities. We believe that these results will contribute to the further exploration of promising candidates as antimicrobial agents.

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