Bioorganic & Medicinal Chemistry Letters 27 (2017) 1274–1277

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis, and cytotoxicity of stabilized mycolactone analogs

Vaddela Sudheer Babu, Ya Zhou, Yoshito Kishi *

Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, MA 02138, USA

ARTICLE INFO

Article history:
Received 5 December 2016
Revised 11 January 2017
Accepted 12 January 2017
Available online 17 January 2017

Keywords:
Buruli ulcer
Mycolactones
Cytotoxicity
Dihydromycolactones
Photochemical [$_4\pi_s + {}_2\pi_a$] cycloaddition

ABSTRACT

On exposure to visible light, mycolactone A/B, the causative toxin of Buruli ulcer, rearranges to a mixture of four photo-mycolactones apparently via a rare photochemically-induced [$_4\pi_s + {}_2\pi_a$] cycloaddition. In order to prevent the rearrangement, two C6′-C7′ dihydromycolactone analogs 6′α-**15** and 6′β-**15** were designed and synthesized. 6′α-**15** and 6′β-**15** were shown to be stable under not only photochemical, but also acidic and basic conditions. Cytotoxicity was tested against arbitrarily chosen four cell lines (human Hek-293, human lung carcinoma A-549, human melanoma LOX-IMVI, and mouse L-929), thereby revealing that: (1) both analogs maintain potent cytotoxicity; (2) 6′β-**15** exhibits significantly higher potency against human cell lines than 6′α-**15**; (3) in comparison with parent mycolactone A/B, 6′β-**15** exhibits equal potency against human Hek-293, whereas significantly lower potency against human lung carcinoma A-549 and human melanoma LOX-IMVI.

Mycolactone A/B, the causative toxin of Buruli ulcer, was isolated from *Mycobacterium ulcerans* by Small and co-workers in 1999.[1] This devastating disease results in progressive necrotic lesions that, if untreated, can extend up to 15% of a patient's skin surface. Surgical intervention was the only practical curative therapy for Buruli ulcer. Encouragingly, combination treatments with rifampicin and either streptomycin or amikacin have recently been reported to prevent the growth of the bacteria, especially in early lesions.[2] Evidence from animal studies suggests that mycolactone A/B is directly responsible for the observed pathology, and recent studies have shed light on the mode of action of mycolactone A/B.[3–5] The gross structure of mycolactones A and B was elucidated with spectroscopic methods, whereas the stereochemistry was predicted via the universal NMR database approach and confirmed by total synthesis.[6–9] Under standard laboratory conditions, mycolactones A and B exist as a rapidly equilibrating 3:2 mixture of $\Delta^{4',5'}$-*Z* (major) and $\Delta^{4',5'}$-*E* (minor) isomers, and are referred to as mycolactone A/B in this paper (Scheme 1).

We have been interested in the chemical and biological properties of mycolactones and recently reported the photochemically-induced rearrangement of mycolactone A/B into four photo-mycolactones A1, A2, B1, and B2 (Scheme 1).[10] Interestingly, all four photo-mycolactones were found to exhibit significantly reduced cytotoxicity, compared with parent mycolactone A/B.

On exposure to light through a 365 nm filter at 30 °C in acetone, mycolactone A/B (**1**) rapidly yields an approximately 2:7:1:1 mixture of 4′*E*, 6′*E*, 8′*E*, 10′*E*, 4′*Z*, 6′*E*, 8′*E*, 10′*E*, 4′*E*, 6′*Z*, 8′*E*, 10′*E*, and 4′*Z*, 6′*Z*, 8′*E*, 10′*E* geometrical isomers. The facile *E*⇆*Z* isomerization is then followed by a slower photochemically induced [$_4\pi_s + {}_2\pi_a$] cyclization, to furnish the four photo-mycolactones (see: the structures depicted in the bracket in Scheme 1). According to the proposed mechanism, the C6′ double bond is required for the photochemical cyclization to proceed, thereby suggesting a possibility of synthesizing a photochemically-stabilized mycolactone analog with a replacement of the C6′-C7′ double bond for a C—C single bond. This operation of structure-modification results in two dihydromycolactones 6′α-**15** and 6′β-**15** (Scheme 2). In this letter, we report a synthesis of these mycolactone-A/B analogs and their photochemical and chemical stability, as well as cytotoxicity, in comparison with parent mycolactone A/B.

Scheme 2 outlines a retrosynthetic analysis of 6′α-**15** and 6′β-**15**. This analysis largely relies on our previous work, including: (1) final esterification step[11]; (2) choice of protecting groups for five hydroxyl groups[8]; (3) use of the two previous synthetic intermediates, i.e., the protected form of mycolactone core **14** and aldehyde **12**.[8] Thus, the major remaining question is concerned with the coupling of the C1′-C8′ building block with aldehyde **12**, to form the *E*-olefin **13**. For this case, Julia-Kocienski olefination[12] appears to be an obvious choice, i.e., 6′α-**10**/6′β-**10** + **12** → 6′α-**13**/6′β-**13**, respectively.

Scheme 3 summarizes the synthesis of sulfones 6′α-**10** and 6′β-**10** from commercially available (*S*)- and (*R*)-glycidols, respectively. For this synthesis, we planned to incorporate a chiral methyl group at C6′ into the fatty-acid backbone. Among several options, we chose to adopt the method developed for the synthesis of the

* Corresponding author.
E-mail address: kishi@chemistry.harvard.edu (Y. Kishi).

http://dx.doi.org/10.1016/j.bmcl.2017.01.036
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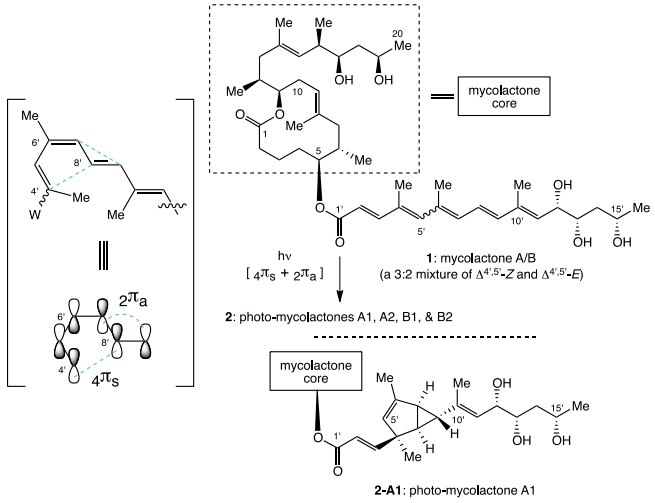
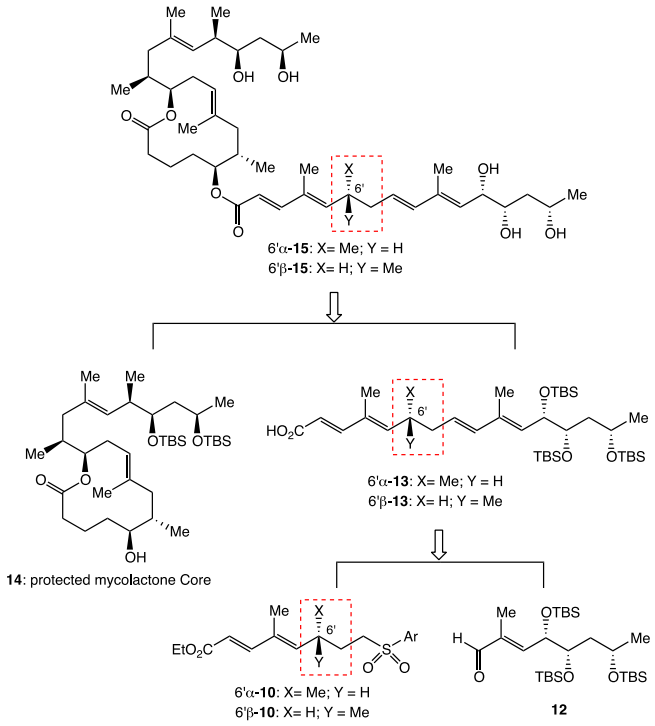


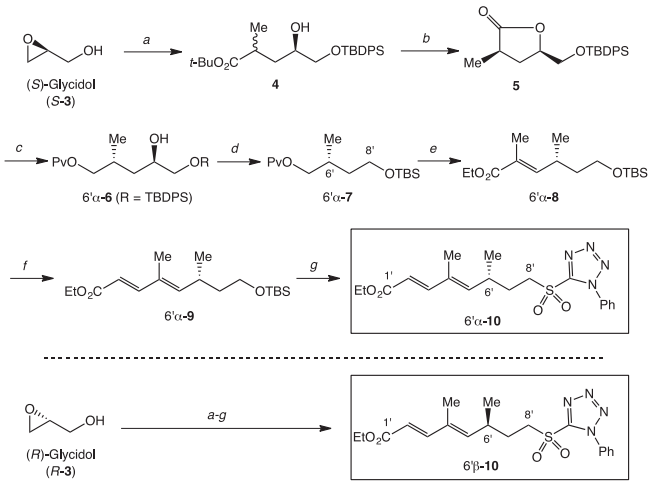
Scheme 1. Photochemical rearrangement of mycolactone A/B (**1**) into photo-mycolactones A1, A2, B1, & B2 (only one structure of the photo-mycolactones shown).



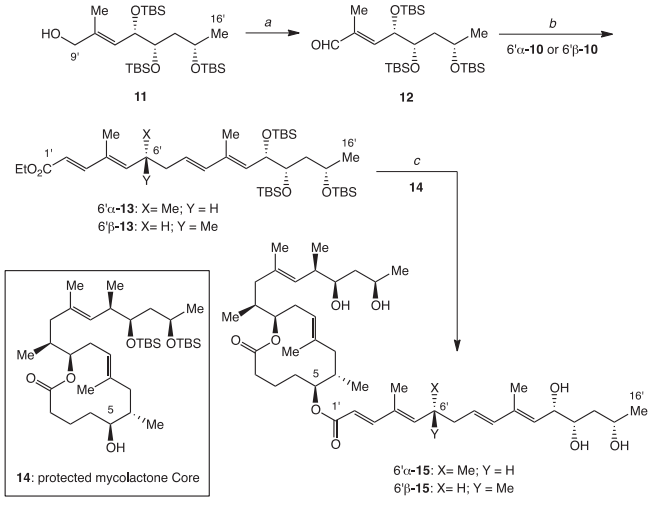
Scheme 2. Retrosynthetic analysis of C6′-C7′ dihydromycolactones 6′β-**15** and 6′α-**15**.

C23-C26 building block of halichondrins.[13] Thus, after protection of the primary alcohol with TBDPS ether, (*S*)-glycidol was treated with *tert*-butyl propionate under the condition reported by Taylor, to give *tert*-butyl ester **4** which, upon treatment with PTSA, resulted in the γ-lactone as a diastereomeric mixture.[14] Treatment with LDA, then with 2,6-di-*tert*-butylphenol, gave an 8–10:1 diastereomeric mixture of γ-lactones as a white solid. On single recrystallization from hexanes, the diastereomeric ratio was improved up to 50–100:1, to give γ-lactone **5**. Based on the consideration that the protonation took place preferentially from the direction opposite to the $CH_2OTBDPS$ group, we assigned, and proved, the stereochemistry of the major diastereomer as indicated.[15] $LiBH_4$-reduction of γ-lactone **5**, selective primary alcohol protection, TBDPS-deprotection, $NaIO_4$-oxidation, $NaBH_4$-reduction, and then TBS-protection treatment straightforwardly gave C5′-C8′ building block 6′α-**7**. We then followed the step-wise chain elongation route used in the previous work[16] to transform 6′α-**7** to 6′α-**9** via 6′α-**8**. TBS-deprotection of 6′α-**9**, followed by treatment with 1-phenyl-1H-tetrazole-5-thiol under the Mitsunobu condition,[17] gave the sulfide, which was oxidized with $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$/30% H_2O_2,[17] to furnish sulfone ester 6′α-**10**, required for the proposed Julia-Kocienski olefination.

Using the same sequence of reactions, 6′β-**10** was also prepared from (*R*)-glycidol.



Scheme 3. Stereoselective synthesis of sulfones 6′β-**10** and 6′α-**10**. Reagents and conditions: (a) 1. TBDPS-Cl, imidazole, CH_2Cl_2 (96%); 2. $MeCH_2CO_2Bu$-*t*, LiHMDS, $AlEt_3$, THF (95%). (*b*) 1. PTSA, $CHCl_3$, reflux (96%); 2. LDA, 2,6-di-*tert*-butylphenol, THF (*dr* = 8–10:1), then recrystallization (*dr* = 50–100:1; 65%). (*c*) 1. $LiBH_4$, THF, MeOH (97%); 2. Pv-Cl, Py, CH_2Cl_2 (90%). (*d*) 1. TBAF, THF (96%); 2. $NaIO_4$, aq·THF (93%); 3. $NaBH_4$, MeOH (96%); 4. TBS-Cl, imidazole, CH_2Cl_2. (94%) (e) 1. DIBAL, CH_2Cl_2 (92%); 2. SO_3·Py, $(i\text{-}Pr)_2(Et)N$, DMSO, CH_2Cl_2 (90%); 3. $Ph_3P=C(Me)CO_2Et$, CH_2Cl_2 (90%). (*f*) 1. DIBAL, CH_2Cl_2 (94%); 2. MnO_2, CH_2Cl_2 (92%); 3. $(EtO)_2P(O)CH_2CO_2Et$, *n*-BuLi, THF (93%); (*g*) 1. PPTS, EtOH (90%); 5. 1-phenyl-1H-tetrazole-5-thiol, DIAD, TPP, THF (94%); 2. H_2O_2, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, EtOH (90%). With use of the same sequence of reactions, sulfone ester 6′β-**10** was prepared from (*R*)-glycidol.



Scheme 4. Completion of the synthesis. Reagents and conditions: (a) MnO_2, CH_2Cl_2 (96%); (b) KHMDS, THF, −78 °C (90%); (c) 1. LiOH, THF, MeOH, Water (92%), 2. **14**, 2,4,6-Trichlorobenzoyl chloride, DMAP, DIPEA, Toluene (86%), 3. TBAF, THF (80%); Prepared 6′β-**15** using the same series of reaction sequence starting from 6′β-**10**.