Bioorganic & Medicinal Chemistry Letters 18 (2008) 5598–5600

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and biological activity of enantiomeric pairs of 5-[(*E*)-cycloalk-2-enylidenemethyl]thiolactomycin congeners

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ARTICLE INFO

Article history:
Received 29 July 2008
Revised 27 August 2008
Accepted 28 August 2008
Available online 31 August 2008

Keywords:
Antibiotic
Inhibitor of mammalian type I fatty acid synthase
5-[(*E*)-Cycloalk-2-enylidenemethyl]thiolactomycin

ABSTRACT

The title congeners were synthesized by employing our efficient synthetic route previously explored for preparing enantiomeric pairs of thiolactomycin and its 3-demethyl derivative. While all the synthesized congeners lacked in vitro antibacterial activity, some of the congeners bearing an (*E*)-cyclohept-2-enylidenemethyl or an (*E*)-cyclooct-2-enylidenemethyl group were found to exhibit more potent type I FAS inhibitory activity than (*S*)-3-demethylthiolactomycin having an unnatural configuration.

(*R*)-(+)-Thiolactomycin (TLM, **1**), a thiolactone antibiotic isolated from a soil bacterium, *Nocardia* sp.,[1] shows moderate in vitro activity against a number of pathogens, including Gram-positive and Gram-negative bacteria,[2] *Mycobacterium tuberculosis*[3] and malaria parasites (Fig. 1).[4] It has also been reported that **1** inhibits bacterial and plant type II fatty acid synthase (FAS).[5] These inhibitory activities are considered to explain the antibacterial and antiparasitical properties observed for **1**.[5] Interestingly, Townsend et al. recently disclosed that **1** and its derivatives also show inhibitory activity against mammalian type I FAS.[6] Therefore, **1** and its congeners attract much synthetic attention because they seem to constitute promising drug candidates with a hitherto unexplored mode of action for cancer and obesity treatments, as well as infective diseases.

Recently, we reported an efficient total synthesis of enantiomeric pairs of **1** and its 3-demethyl derivative (3-demethyl-TLM **2**) by employing novel deconjugative asymmetric α-sulfenylation of the chiral 3-(α,β,γ,δ-unsaturated-acyl)oxazolidin-2-one with a methanethiosulfonate as a key step.[7] The flexibility and efficiency of the explored synthetic route were next demonstrated by the successful synthesis of enantiomeric pairs of various structural types of 5-vinyl-TLM congeners (**3**).[8] From the biological activity assay carried out using **3** and *ent*-**3** along with **1**, *ent*-**1**, **2** and *ent*-**2**, it appeared evident that in vitro antibacterial and type I FAS inhibitory activity of TLM congeners can be cleanly separated by changing not only the structure but also the absolute configuration of the side chain at the C5-position.[8] In the course of our continuing studies on the synthesis and biological activity of **1** and its congeners, we next paid attention to exploring the conformational effects of the isoprenoid 1,3-diene moiety at the C5-position of **1** on in vitro antibacterial and type I FAS inhibitory activity. Accordingly, we designed enantiomeric pairs of 5-[(*E*)-cycloalk-2-enylidenemethyl]-TLM congeners and their 3-demethyl derivatives (**4**, *ent*-**4**, **5** and *ent*-**5**) bearing six- to eight-membered rings as the next targets.[9] In these compounds, the isoprenoid 1,3-diene moiety for **1** is clearly locked into an *s*-trans configuration as a consequence of their involvement in a ring system. It is also expected that successful synthesis of **4**, *ent*-**4**, **5** and *ent*-**5** might further demonstrate the flexibility and efficiency of our synthetic scheme developed for **1** and its congeners.[7,8] Here, we wish to report the synthesis of **4**, *ent*-**4**, **5** and *ent*-**5** and their in vitro antibacterial and type I FAS inhibitory activity. These studies disclosed novel aspects of the structural-activity relationships for TLM congeners especially concerning the isoprenoid 1,3-diene moieties at the C5-position.

According to the explored synthetic scheme,[7] the synthesis of (*R*)-5-[(*E*)-cycloalk-2-enylidenemethyl]-TLM and its 3-demethyl analogs (**4** and **5**) commenced from α,β-unsaturated carboxylic acids **7a–c**, which were prepared from α,β-unsaturated aldehydes **6a–c** by sequential Horner–Wadworth–Emmons reaction and alkaline hydrolysis (Scheme 1).[10] While **6a** was commercially available, **6b** and **6c** were prepared from 1-nitromethylcycloheptene[11] and *N*′-cyclooctylidenetosylhydrazide,[12] respectively, following the reported procedures.[11,12] Activation of **7a–c** with pivaloyl chloride followed by treating the formed mixed anhydrides with (*R*)-4-ben-

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doi:10.1016/j.bmcl.2008.08.103

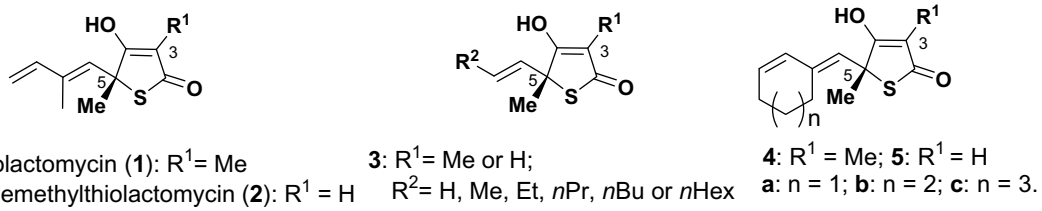


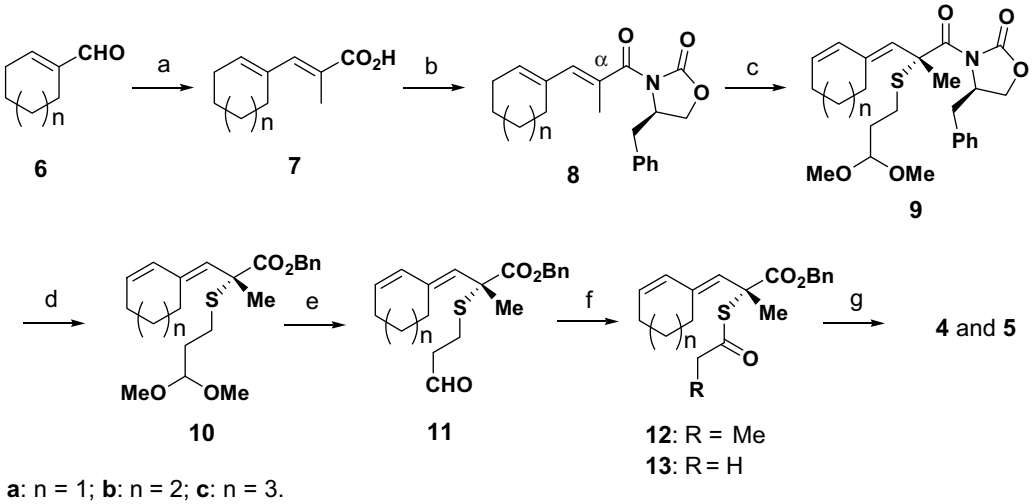
Figure 1. Structures of thiolactomycin (**1**), 3-demethylthiolactomycin (**2**), 5-vinylthiolactomycin congeners (**3**), 5-[(*E*)-cycloalk-2-enylidenemethyl]thiolactomycin and 5-[(*E*)-cycloalk-2-enylidenemethyl]-3-demethylthiolactomycin derivatives (**4** and **5**).

zyloxazolidin-2-one afforded (*R*)-*N*-acyloxazolidin-2-ones **8a**–**c**. With **8a**–**c** in hand, the key asymmetric deconjugative α-sulfenylation was attempted. Thus, treatments of **8a**,**b** with 3,3-dimethoxypropyl methanethiosulfonate using NaHMDS as a base in the presence of HMPA led to the asymmetric deconjugative α-sulfenylation, giving rise to the α-sulfenylated products **9a**,**b** highly diastereoselectively (>90% de) and in good yields.[13–16] However, the reaction of **8c** under the same conditions as for **8a**,**b** was found to give a lower yield of **9c**. After some experimentation, it was disclosed that the use of KHMDS as a base in the presence of 18-crown-6 was more effective, giving **9c** in a highly diastereoselective manner (>90% de) in a good yield.[13–16]

The α-sulfenylated products **9a**–**c** isolated in a pure state[14] were transformed into benzyl esters **10a**–**c** by imide-ester exchange using titanium benzyloxide.[17] Acidic hydrolysis of the dimethyl acetal moieties in **10a**–**c** produced aldehydes **11a**–**c**. Compounds **11a**–**c** were sequentially subjected to retro-Michael reaction using Cs_2CO_3 as a base and acylation with propionyl or acetyl chloride, furnishing α-acylthio esters **12a**–**c** or **13a**–**c**, respectively. Dieckmann condensation of **12a**–**c** and **13a**–**c** with LiHMDS gave rise to (*R*)-5-[(*E*)-cycloalk-2-enylidenemethyl]-TLM **4a**–**c** and its 3-demethyl derivatives **5a**–**c**.[18] By the same synthetic scheme, the enantiomers of **4a**–**c** and **5a**–**c** (*ent*-**4a**–**c** and *ent*-**5a**–**c**) were prepared from *ent*-**8a**–**c** obtained from **7a**–**c** and (*S*)-4-benzyloxazolidin-2-one.[18]

With **4a**–**c**, *ent*-**4a**–**c**, **5a**–**c** and *ent*-**5a**–**c** in hand, their in vitro antibacterial activity against various strains of bacteria[19] and inhibitory activity against mammalian type I FAS[20] were evaluated. The results are summarized in Table 1 along with those for **1**, *ent*-**1**, **2**, *ent*-**2**, ciprofloxacin (CPFX) and C75, the only potent inhibitor against type I FAS so far reported.[6,21] Being different from **1** and **2**, all the tested compounds were found to lack in vitro antibacterial activity against all the tested strains of bacteria even though **4a**–**c** and **5a**–**c** bear the same (*R*)-configurations as **1** and **2**. As for type I FAS inhibitory activity, fairly comparable activity was observed for *ent*-**5a**, **4b**, *ent*-**4b**, **5b**, *ent*-**5b**, *ent*-**4c**, **5c** and *ent*-**5c** whose isoprenoid 1,3-diene moieties are involved in a ring system. In particular, *ent*-**4b**, **5b** and *ent*-**4c** showed clearly more potent type I FAS inhibitory activity than that of the previously synthesized *ent*-**2**,[7] and the activity of the most potent *ent*-**4c** was almost twice as much as that of *ent*-**2**.

In conclusion, we have succeeded in the synthesis of six enantiomeric pairs of (*R*)-5-[(*E*)-cycloalk-2-enylidenemethyl]-TLM congeners (**4**, *ent*-**4**, **5** and *ent*-**5**) by employing our efficient synthetic route previously established for the total synthesis of TLM (**1**), 3-demethyl-TLM (**2**) and their enantiomers (*ent*-**1** and *ent*-**2**). From the results of in vitro antibacterial activity, it appeared evident that the free-rotational isoprenoid 1,3-diene moiety and the (*R*)-configuration at the C5-position are essential for **1** and its congeners to exhibit in vitro antibacterial activity. As for type I FAS inhibitory activity, some 5-[(*E*)-cycloalk-2-enylidenemethyl]-TLM congeners in which the isoprenoid 1,3-diene moieties are locked into an *s*-trans configuration as a consequence of their involvement in a ring system were found to exhibit inhibitory activity equal to (for *ent*-**5a**, **4b**, *ent*-**5b**, **5c** and *ent*-**5c**) or a little more potent (for *ent*-**4b**, **5b** and *ent*-**4c**) than that of the previously reported *ent*-**2**.[7] The results on biological activity collected in these studies should be useful for future attempts to design novel thiolactomycin congeners that might show more prominent and clinically useful activity than TLM.



Scheme 1. Reagents and conditions: (a) $(EtO)_2P(O)CHMeCO_2Et$, *t*BuOLi, hexane, rt, then 10% NaOH aq, EtOH, 50 °C, 84% for **7a**, 69% for **7b**, 81% for **7c**; (b) *t*BuCOCl, Et_3N, THF, −15 °C, then LiCl, (*R*)-4-benzyl-2-oxazolidinone, rt, 81% for **8a**, 90% for **8b**, 67% for **8c**; (c) NaHMDS, HMPA, −78 °C or KHMDS, 18-crown-6, −78 °C, then 3,3-dimethoxypropyl methanethiosulfonate, −78 to 0 °C, 56% for **9a**, 31% for **9b**, 50% for **9c**; (d) $Ti(OiPr)_4$, BnOH, 70 °C, 76% for **10a**, 69% for **10b**, 87% for **10c**; (e) 6% HCl aq, THF, rt, 87% for **11a**, 92% for **11b**, 96% for **11c**; (f) Cs_2CO_3, EtOH, 0 °C, then CH_3CH_2COCl or CH_3COCl, Et_3N, CH_2Cl_2, 0 °C, 66% for **12a**, 68% for **12b**, 73% for **12c**, 68% for **13a**, 63% for **13b**, 67% for **13c**; (g) LiHMDS, THF, −78 to 0 °C, 84% for **4a**, 89% for **4b**, 66% for **4c**, 99% for **5a**, 64% for **5b**, 46% for **5c**.

Table 1
In vitro antibacterial and mammalian type I FAS inhibitory activity of enantiomeric pairs of TLM and its congeners (**1**, *ent*-**1**, **2**, *ent*-**2**, **4a-c**, *ent*-**4a-c**, **5a-c** and *ent*-**5a-c**)

Compound	In vitro antibacterial activity, MIC (μg/mL)			Mammlian type I FAS inhibitory activity, IC_{50} (ng/mL) HepG2 ^{14}C
	S. aureus Smith	*M. catarrhalis* ATCC 25238	*H. influenzae* IID983	
TLM (**1**)	128	0.25	2	>80
ent-***1***	>128	>128	N.T.[b]	43.7
2	>128	16	32	>80
ent-1	>128	>128	N.T.[b]	19.0
4a	>64	>64	>64	>80
ent-***4a***	>64	>64	>64	>80
5a	>64	>64	>64	57.0
ent-***5a***	>64	>64	>64	18.9
4b	>64	>64	>64	25.4
ent-***4b***	>64	>64	>64	14.9
5b	>64	>64	>64	13.3
ent-***5b***	>64	>64	>64	22.6
4c	>128	64	>128	41.8
ent-***4c***	>128	64	>128	11.6
5c	>128	128	>128	19.5
ent-***5c***	>128	64	>128	24.6
CPFX[a]	0.063	0.031	0.008	N.T.[b]
C75[c]	N.T.[b]	N.T.[b]	N.T.[b]	7.4

[a] Ciprofloxacin.
[b] N.T., not tested.
[c] See the text.

Acknowledgments

We are grateful to Dr. T. Ishizaki of Kyorin Pharmaceutical Co., Ltd for his extensive support and Dr. Y. Fukuda of Kyorin Pharmaceutical Co., Ltd for his valuable suggestions and discussions. The in vitro antibacterial and mammalian type I FAS inhibitory activity assay were carried out by Drs. M. Takei and M. Tsunoda of Kyorin Pharmaceutical Co., Ltd, to whom the authors' thanks are due.

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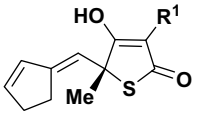
9. In addition to **4a–c**, **5a–c** and their enantiomers (*ent*-**4a–c** and *ent*-**5a–c**), 5-[(*E*)-cyclopent-2-enylidenemethyl]-TLM congeners (**4d**, *ent*-**4d**, **5d** and *ent*-**5d**) were designed and their synthesis was attempted following the same synthetic scheme shown in Scheme 1. Although the synthetic scheme smoothly proceeded to the stage of **10** ($n = 0$), aldehyde **11** ($n = 0$) derived from **10** ($n = 0$) was found to be very unstable, probably due to facile isomerization to the cyclopentadiene derivative and subsequent intramolecular Diels–Alder reaction (Ohata, K.; Terashima, S., to be published).



4d: R^1 = Me; **5d**: R^1 = H

10. The (*E*)-configuration of **7a** was determined by observing NOESY between two olefinic protons in its ^1H NMR spectrum. The structures of **7b**,**c** were assigned by comparing their ^1H NMR spectra with that of **7a**.
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13. In the asymmetric deconjugative α-sulfenylation of **8a–c**, formation of small amounts of the α-sulfenylated (*S*)-diastereomer and the α-sulfenylated (*Z*)-isomer [(*R*)- or (*S*)-diastereomer] was always observed. Their formation ratios estimated by the ^1H NMR spectra and/or HPLC analysis are as follows: **9**:(*S*)-isomer:(*Z*)-isomer; 12:1:1 for **9a**; 14:1:4 for **9b**; 12:1:1 for **9c**.
14. Pure samples of **9a–c** were obtained by sequential separation with column chromatography (SiO_2:solvent; hexane/AcOEt = 4/1 for **9a**; hexane/AcOEt = 5/1 for **9b**; hexane/AcOEt = 5/1 for **9c**) and HPLC. The HPLC conditions were as follows: **9a** [Daicel Chiralpak IA, φ 2.0 cm × 25 cm, hexane/EtOH = 90/10, followed by Daicel Chiralpak IA, φ 2.0 cm × 25 cm, hexane/*i*PrOH = 97/3]; **9b** [Daicel Chiralpak IA, φ 2.0 cm × 25 cm, hexane/EtOH = 91/9]; **9c** [Daicel Chiralpak IA, φ 2.0 cm × 25 cm, hexane/MTBE/*i*PrOH = 80:10:10, followed by Daicel Chiralpak IC, φ 2.0 cm × 25 cm, hexane/*i*PrOH = 75/25].
15. The absolute stereochemistries of newly created asymmetric centers for **9a–c** were assigned to have an (*R*)-configuration by comparing their ^1H NMR spectra with that of the corresponding α-sulfenylated intermediate for the synthesis of **1**.[7] Some representative data are as follows: ^1H NMR($CDCl_3$): 5.44 ppm [(Me)(SR)C–C*H*=C] for **9a**; 5.50 ppm [(Me)(SR)C–C*H*=C] for the (*S*)-diastereomer of **9a**; 5.72 ppm [(Me)(SR)C–C*H*=C] for the synthetic intermediate of **1**; 5.77 ppm [(Me)(SR)C–C*H*=C] for the (*S*)-diastereomer of the synthetic intermediate of **1**.
16. The α-sulfenylated products **9a**,**b** bearing (*R*)-configuration clearly showed NOESY between two olefinic protons in their ^1H NMR spectra. The structures of (*Z*)-isomers of **9a**,**b** were determined by observing the absence of NOESY between two olefinic protons in their ^1H NMR spectra. Assignment of the structures for (*E*)- and (*Z*)-isomer of **9c** was performed by comparing their ^1H NMR spectra with those of the corresponding isomers of **9a**,**b**.
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18. The physical data of **4a–c** and **5a–c** are as follows: **4a**: colorless crystals, mp 111–113 °C, $[\alpha]_D^{27}$ + 128° (*c* 0.30, MeOH); **4b**: light brown oil, $[\alpha]_D^{28}$ + 86.3° (*c* 0.24, MeOH); **4c**: colorless crystals, mp 105–111 °C, $[\alpha]_D^{27}$ + 77.3° (*c* 0.30, MeOH); **5a**: colorless solid, mp 105.5–110 °C, $[\alpha]_D^{25}$ + 138° (*c* 0.20, MeOH); **5b**: colorless crystals, mp 85–89 °C, $[\alpha]_D^{27}$ + 137° (*c* 0.21, MeOH); **5c**: colorless crystals, mp 131–137 °C, $[\alpha]_D^{27}$ + 88.9° (*c* 0.30, MeOH).
19. Determination of MICs by agar dilution methods was performed according to the guidelines M7–A6 of the Clinical and Laboratory Standards Institute, 2003.
20. Fatty acid synthesis was evaluated measuring incorporation of [1-^{14}C] acetate into cellular fatty acid as previously described with some modifications. See, Murakami, K.; Tobe, K.; Ide, T.; Mochizuki, T.; Ohashi, M.; Akanuma, Y.; Yazaki, Y.; Kadowaki, T. *Diabetes* **1998**, *47*, 1841.
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