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Synthesis and Structure–Activity Relationships of Thiotetronic Acid Analogues of Thiolactomycin

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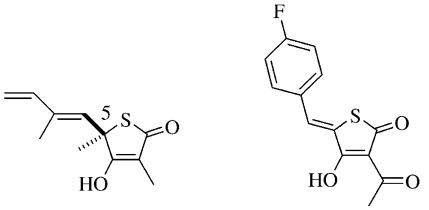
Abstract—3-Acetyl analogues of thiolactomycin, a thiotetronic acid natural product, were synthesized and profiled against livestock pathogens. Some analogues showed improved activity over thiolactomycin against *Staphylococcus aureus* and comparable activity against *Pasteurella multocida*. Several semisynthetically modified analogues of thiolactomycin showed no improvement in activity over thiolactomycin. © 2001 Elsevier Science Ltd. All rights reserved.

Thiolactomycin (**1**), a thiolactone antibiotic, was initially isolated from a soil bacterium, *Nocardia* sp.[1] It is effective in several in vivo murine infectious disease models, in spite of its moderate in vitro activity against a number of pathogenic bacteria.[2] Thiolactomycin inhibits beta-ketoacyl acyl carrier protein synthase (KAS) in type II fatty acid biosynthesis found in bacteria and plants.[3] This pathway is essential to the bacterial growth providing fatty acids as building blocks for the biosynthesis of membrane phospholipids and LPS. Therefore, this pathway is a valid antibacterial target. The details of the mechanism has emerged from recent reports on the crystal structures of the three bacterial beta-keto acyl carrier protein synthase I,[4] II,[5] and III.[6] The crystallographic information suggests that the thiolactomycin binds to the active site by mimicking the substrate malonyl-ACP in the active site. Thiolactomycin is an attractive lead due to its novel mechanism of action, which minimizes the cross-resistance to human therapeutics, and its proven in vivo mouse activity.

High-throughput screening of our compound library using *Escherichia coli* identified thiolactomycin (**1**) [minimum inhibitory concentration (MIC): 25 μg/mL] as a lead compound for our livestock antibacterial program. A sub-structural search based on the thiolactone ring in the compound library also identified compound **2** (MIC: 1.56–3.12 μg/mL) as another lead. They both had potent activity against a veterinary respiratory pathogen *Pasteurella multocida*. Thiolactomycin **1** demonstrated moderate in vivo activity in our murine *P. multocida* respiratory disease model ($ED_{50} = 90$ mg/kg) (Fig. 1).

The total synthesis of racemic thiolactomycin[7] and an asymmetric synthesis,[8] that proved the structure of the thiolactomycin structure as the (5*R*)-enantiomer (**1**), have been reported. Recently, two papers described the selective inhibition of the plant and mycobacterial synthases by thiolactomycin analogues.[9] In this paper we describe our synthetic efforts to improve the antibacterial activity of thiolactomycin via semisynthetic modification of thiolactomycin (**1**) and 3-acetyl thiotetronic acid analogues of **2**.

Acetyl thiotetronic acid analogues were prepared according to Scheme 1 and are shown in Tables 1 and 2.



1 Thiolactomycin **2**

Figure 1. Thiolactomycin (**1**) and 3-acetyl thiotetronic acid (**2**) lead.

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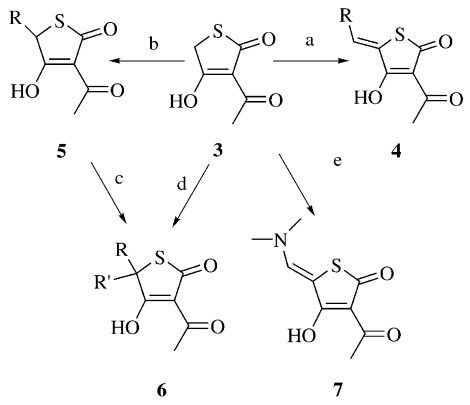
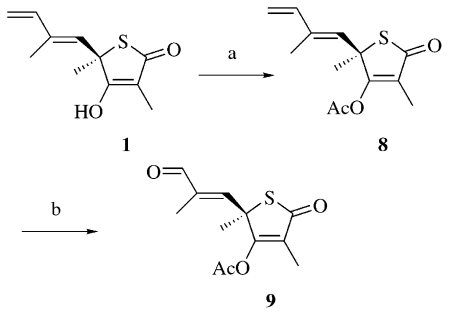
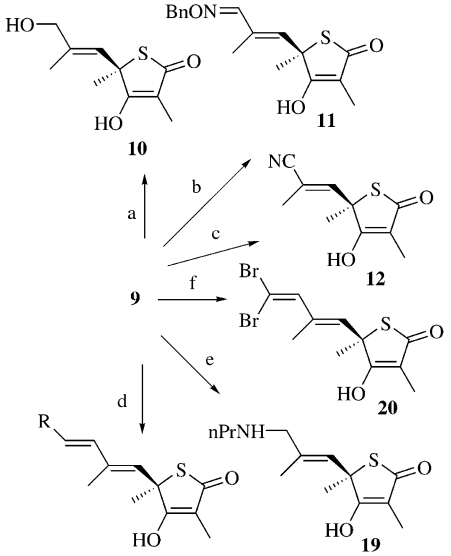


Scheme 1. (a) RCHO, piperidine, EtOH, 25 °C, 2–8 days; (b) NaHMDS, RBr, THF, −78 to 0 °C; (c) NaHMDS, R′Br, THF, −78 to 0 °C; (d) NaHMDS (2 equiv), RBr, then R′Br, THF, −78 to 0 °C; (e) $Me_2NCH(OEt)_2$, toluene, 100 °C.



Scheme 2. (a) Ac_2O, pyridine, 95%; (b) $NaIO_4$, cat OsO_4, THF/H_2O (5:2), 20–65%.



13 R = $CO(CH_2)_4CH_3$ (55%)
14 R = CO_2Et (24%)
15 R = COMe (65%)
16 R = CN (47%)
17 R = CH=CH-CO_2Et (26%)
18 R = Ph (27%)

Scheme 3. (a) $NaBH_4$, MeOH, 45%; (b) $PhONH_2.HCl$, $NaHCO_3$, MeOH, 55%; (c) $PhONH_2.HCl$, Na_2CO_3, MeOH, 45%; (d) $(RO)_2P(O)CH_2R$ or $PPh_3=CHR$, NaH, THF/DMF; (e) *n*$PrNH_2$, $NaCNBH_3$, CH_2Cl_2, 25%; (f) PPh_3, CBr_4, CH_2Cl_2; NaOMe, 35%.

The acetyl thiotetronic acid **3**[10] was condensed with aryl aldehydes in the presence of piperidine as base to provide the olefins **4**. These olefins were unstable to chromatographic purification and hence were purified by precipitation and trituration. Alkylation of **3** with various allylic or alkyl halides resulted in mono-alkylated products **5**. Di-alkylation could be achieved by repeating the alkylation of **5** under similar conditions to provide **6**. Alternatively, repeated alkylation of **3** in a single pot afforded the di-alkylated product **6**. One conjugated enamide derivative **7** was also prepared by condensing dimethyl formamide diethyl acetal in toluene at 100 °C.

We modified thiolactomycin to explore the importance of the diene moiety and the alcohol functionality. To that end, thiolactomycin (**1**) was first protected as an acetate **8** using acetic anhydride (95%) followed by

Table 1. Structure and activity of olefin analogues **4** and **7**

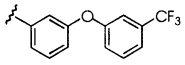
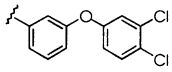
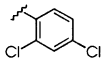
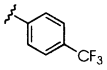
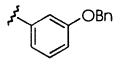
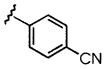
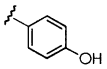
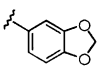
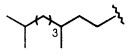
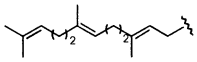
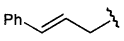
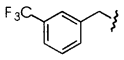
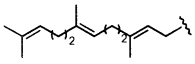
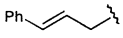
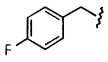
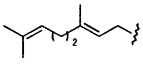
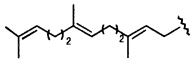
Compd	R	*P. multocida*	*S. aureus*
2		3.13	>200
4a		3.13	0.78
4b		0.2	1.56
4c		3.13	3.13
4d		3.13	3.13
4e		>200	3.13
4f		6.25	50
4g		1.56	>200
4h		>200	3.13
4i		>200	>200
4j		100	>200
4l		>200	>200
4k		>200	>200
7	Me_2N	>200	>200

Table 2. Structure and activity of mono-alkylated analogues **5** and dialkylated analogues **6**

		MIC (μg/mL)	
Compd	R	*P. multocida*	*S. aureus*
1	Thiolactomycin	0.2	>100
5a		3.13	0.78
5b		0.2	1.56
5c		3.13	3.13
5d		3.13	3.13
5e	$BnOCOCH_2$	>200	3.13
5f	Me	6.25	50

			MIC (μg/mL)	
Compd	R	R′	*P. multocida*	*S. aureus*
3	H	H	>200	>200
6a	Me		1.56	>200
6b	Me		>200	3.13
6c	Me		>200	>200
6d	Me		100	>200
6e	R′		>200	>200

selective cleavage of the terminal olefin using catalytic osmium tetraoxide and sodium periodate to provide the aldehyde **9** in modest yields (Scheme 2).

The aldehyde **9** was reduced to the alcohol **10** with sodium borohydride (Scheme 3). Reaction with *O*-phenyl hydroxylamine hydrochloride and sodium carbonate led to the vinyl nitrile **12** instead of the desired oxime. Use of milder base, sodium bicarbonate allowed for the formation of the *O*-phenyl oxime ether **11**. Several conjugated olefins (**13**–**18** and **20**) were prepared via Horner–Emmons or Wittig reactions. As expected, the Horner–Emmons reaction gave better yields than the Wittig reaction. In addition, use of excess reagent allowed for the in situ deprotection of the acetate. The exception in this case was **20,** where the acetate had to be deprotected with sodium methoxide. One allyl propyl amine analogue **19** was prepared via reductive amination under sodium cyanoborohydride conditions.[11]

All the compounds were profiled for in vitro activity using the micro-dilution method[12] against *P. multocida, Staphylococcus aureus*, and *E. coli*. None of the compounds except thiolactomycin and compound **2** showed any activity against *E. coli*. Among the aryl olefin analogues, biarylether containing analogues **4a** and **4b** had the best activity against both *P. multocida* and *S. aureus* (Table 1). Overall, the halogenated or trifluoromethyl substituted analogues had lower MICs than the non-halogenated analogues (**4a**, **4d**, **4c**, **4f**, **2**, and **4h** vs **4g**, **4l**, and **4p**) against both *P. multocida* and *S. aureus*. Some exceptions include the halogenated analogue **4h**, which had no activity against *S. aureus*, and a benzyloxy ether analogue **4e**, which had a moderate activity against *S. aureus*.

Among the mono-alkylated analogues shown in Table 2, **5b**, with the long lipophilic farnesyl side chain, had activity against both *P. multocida* and *S. aureus* (Table 2). In general, the lipophilic side chain seem to be

essential for activity. Di-alkylation does not appear to enhance the activity of the compounds over the mono alkylated case (**5b** vs **6a**; **5c** vs **6b**). In the case of di-alkylated analogues, a long lipophilic side-chain diminished the activity completely (**6e**). Overall, the aryl ether olefin analogues of **2** (**4a** and **4b**) possessed better activity than the alkylated analogues (**5b** and **6a**).

In comparison to the thiotetronic acid analogues, thiolactomycin (**1**) has an MIC of 0.2 μg/mL against *P. multocida* and is inactive against *S. aureus* (>100 μg/mL). The synthetic analogues **8**–**20**, however, all were inactive (MIC >100 μg/mL) against both *P. multocida* and *S. aureus*.

In summary, several of the thiotetronic acid analogues showed improved activity against both *P. multocida* and *S. aureus*. None of the analogues tested in our in vivo murine models[13] was active. Although the thiotetronic acid analogues possess a similar core structure to thiolactomycin, the target of action for these analogues has not yet been determined.

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References and Notes

1. Oishi, H.; Noto, T.; Sasaki, H.; Suzuki, K.; Hayashi, T.; Okazaki, H.; Ando, K.; Sawada, M. *J. Antibiot*. **1982**, *35*, 391.
2. Miyakawa, S.; Suzuki, K.; Noto, T.; Harada, Y.; Okazaki, H. *J. Antibiot*. **1982**, *35*, 411.
3. (a) Furukawa, H.; Tsay, J. T.; Jackowski, S.; Takamura, Y.; Rock, C. O. *J. Bacteriol*. **1993**, *175*, 3723. (b) Nishida, I.; Kawaguchi, A.; Yamada, M. *J. Biochem. (Tokyo)* **1986**, *99*, 1447. (c) Hayashi, T.; Yamamoto, O.; Sasaki, H.; Kawaguchi, A.; Okazaki, H. *Biochem. Biophys. Res. Commun*. **1983**, *115*, 1108.
4. Olsen, J. G.; Kadziola, A.; Von Wettstein-Knowles, P.; Siggaard-Andersen, M.; Larsen, S. *Structure (Cambridge, MA, U.S.)* **2001**, *9*, 233.
5. Moche, M.; Dehesh, K.; Edwards, P.; Lindqvist, Y. *J. Mol. Biol*. **2001**, *305*, 491.
6. (a) Davies, C.; Heath, R. J.; White, S. W.; Rock, C. O. *Structure (London)* **2000**, *8*, 185. (b) Qiu, X.; Janson, C. A.; Smith, W. W.; Head, M.; Lonsdale, J.; Konstantinidis, A. K. *J. Mol. Biol*. **2001**, *307*, 341.
7. Wang, C. L. J.; Salvino, J. M. *Tetrahedron Lett*. **1984**, *25*, 5243.
8. (a) Chambers, M. S.; Thomas, E. J. *J. Chem. Soc., Perkin Trans. 1* **1997**, 417. (b) Chambers, M. S.; Thomas, E. J. *J. Chem. Soc., Chem. Commun*. **1989**, 23.
9. (a) Kremer, L.; Douglas, J. D.; Baulard, A. R.; Morehouse, C.; Guy, M. R.; Alland, D.; Dover, L. G.; Lakey, J. H.; Jacobs, W. R., Jr.; Brennan, P. J.; Minnikin, D. E.; Besra, G. S. *J. Biol. Chem*. **2000**, *275*, 16857. (b) Jones, A. L.; Herbert, D.; Rutter, A. J.; Dancer, J. E.; Harwood, J. L. *Biochem. J*. **2000**, *347*, 205.
10. O'Mant, D. M. *J. Chem. Soc., C* **1968**, *1501*.
11. All the compounds were characterized by ^{1}H NMR and MS and are consistent with the structures.
12. Hayashi, S. F.; Norcia, L. J. L.; Seibel, S. B.; Silvia, A. M. *J. Antibiot*. **1997**, *50*, 514.
13. **Murine disease models**. Twenty-gram female CF-1 mice were infected intranasally with 50 μL of a suspension of 2×10^4 *P. multocida* serotype 5A or they were infected intraperitoneally with 1.6×10^5 *S. aureus* strain 6097 in 5% hog gastric mucin. Compounds were administered subcutaneously 0.5 h post-infection at doses of 10–90 mg/kg. The number of surviving mice was counted for 4 days.