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Bioorganic & Medicinal Chemistry Letters 15 (2005) 3857–3859
Bioorganic & Medicinal Chemistry Letters

New α-methylene-γ-butyrolactones with antimycobacterial properties

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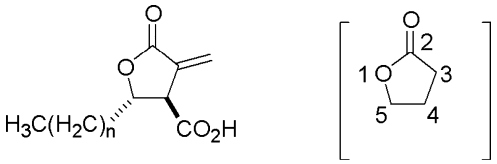
Received 11 March 2005; revised 26 May 2005; accepted 27 May 2005
Available online 5 July 2005

Abstract—The synthesis and antimycobacterial activity of a series of α-methylene-γ-butyrolactones based on the natural product protolichesterinic acid are described. Compounds **9**–**12** bearing an allylamide group at the C-4 position showed improved activity with MICs in the range of 6.25–12.5 μg/mL.

The α-methylene-γ-butyrolactone (AMBL) is a widely recognized component of natural products exhibiting a wide range of interesting biological activities including anticancer, fungicidal, and bactericidal properties.[1a,b,c] While the majority of these compounds appear in the sesquiterpene family, the efficacy of several naturally occurring AMBLs against mycobacteria suggests that these structures could serve as a new template for tuberculosis drug development.[2a] *Mycobacterium tuberculosis*, the causative agent of tuberculosis, infects one-third of the world's population with an estimated eight million new cases of active TB per year.[3] With a death occurring every 15 s, tuberculosis remains as a significant threat to human health. The problem today is exacerbated by the rise in multi-drug resistant (MDR) strains.[4]

Protolichesterinic acid (Fig. 1), isolated from *Cetraria islandica*, exhibits broad spectrum antibacterial properties with promising initial bactericidal activity against mycobacterium sp.[2a,b] The synthetic analog, C75 (Fig. 1), is an inactivator of mammalian type I fatty acid synthase at the β-ketoacyl synthase (KAS) domain.[5,6] This domain catalyzes the decarboxylative Claisen-type condensation of a carrier protein-linked malonyl unit onto an elongating fatty acid bound to the enzyme. C75 is thought to act, in part, as a malonyl CoA mimetic. It has been demonstrated to be selectively cytotoxic to cancer cells with the only side effect being reversible weight loss.[7]



n=12, Protolichesterinic acid
n= 7, C75

Figure 1. Structure of protolichesterinic acid and C75.

Fatty acid biosynthesis is an essential process for the survival of bacteria. The lipid products are important for the integrity and dynamic properties of cellular membranes, and play central roles in energy storage and metabolism. In mycobacteria, specialized biosynthetic pathways exist to extend normal fatty acids to mycolic acids, C_{60-90} fatty acids that are esterified to the arabinogalactan outer layer.[8] Unlike most bacteria, mycobacteria possess a eukaryotic-like type I FAS in addition to the expected type II FAS system. On the basis of the sequence similarity of the KAS domain in *M. tuberculosis* type I FAS to that of the human FAS KAS region (37%), we hypothesized that these compounds could serve as a new class of antimycobacterial agents with a similar mode of action. The bactericidal properties of FAS inhibitors, such as thiolactomycin[9a] and cerulenin,[9b] as well as the established first-line TB drugs, such as isoniazid and ethionamide, validate the usefulness of targeting enzymes involved in lipid biosynthesis.[9c] Furthermore, enzymatic studies using pyrazinamide, a first-line drug with remarkable in vivo potency,

Keywords: Mycobacteria; Butyrolactones.
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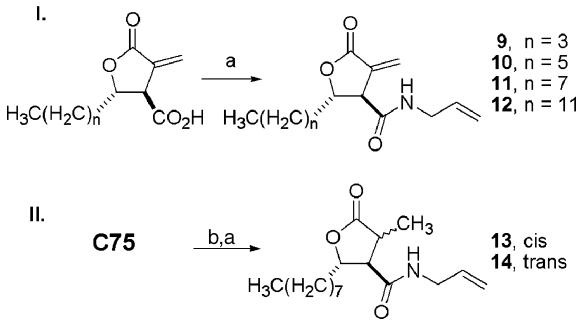
doi:10.1016/j.bmcl.2005.05.119

suggests that type I FAS inhibitors could be useful in addressing the issue of sterilizing activity as well.[9d]

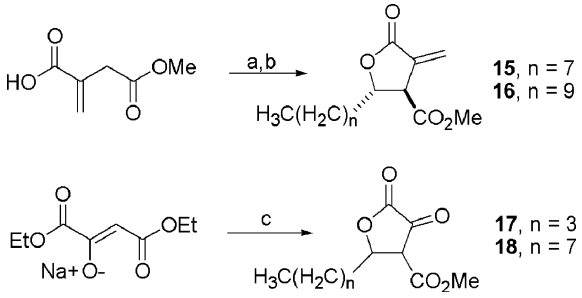
Most broad spectrum antibiotics are ineffective against mycobacteria as a consequence of limited intracellular uptake. Our synthetic strategy focused on substituted amide and ester modifications of the C-4 carboxylate of C75 to define more potent AMBLs. Preparations of the α-methylene-γ-butyrolactones are illustrated in Schemes 1–3. All compounds were prepared in racemic form and purified by silica gel chromatography to yield the desired diastereomers. Characterization was carried out by ^{1}H and ^{13}C NMR, IR and HRMS, or elemental analyses. Spectral data collected were consistent with structural assignments.[10]

Synthesis of the carboxylic acid starting material followed previously reported protocols.[5] Using tris(2-oxo-3-oxazolinyl)phosphine oxide[11] as a coupling agent, several amides were prepared with minimal isomerization of the double bond to the thermodynamically more stable endo-products (**1**–**12**) in 50–91% overall yield. The methyl ester analogs were obtained in one step by addition of the dianion of commercially available itaconic acid monomethyl ester to nonanal (**15**, 15% *trans*, 42% total yield) or undecanal (**16**, 15% *trans*, 35% total yield) followed by rapid lactonization upon acidic work-up. In a similar fashion, addition of sodium diethyl malonate to pentanal (**17**, 59% yield) and nonanal (**18**, 73% yield) generated 3-oxo analogs.

The antimycobacterial activities of the compounds were determined based on the inhibition of bacterial growth using the standard BACTEC 460 radiometric system.[12] Compounds were screened in vitro for activity against *M. bovis* BCG.[13]

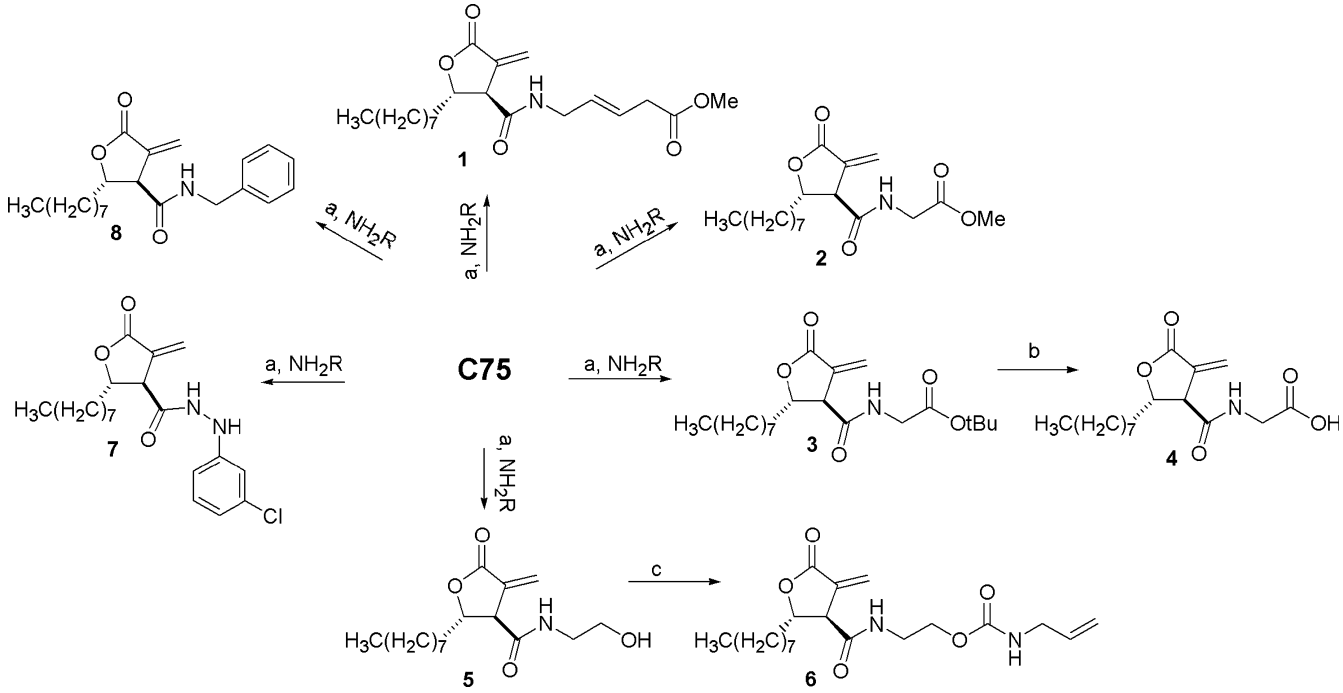


Scheme 2. Synthesis of allylamides. Reagents and conditions: (a) tris(2-oxo-3-oxazolinyl)phosphine oxide, CH_3CN, amine, rt, 30 min. (b) ethyl acetate, Pd (10% on carbon), H_2 (50 psi), 2 h.



Scheme 3. Synthesis of ester and 3-oxo analogs. Reagents and conditions: (a) LiHMDS/THF, −78 °C, 1 h. (b) aldehyde, 2 h at −78 °C then 6 M H_2SO_4/ether work-up. (c) ethanol, aldehyde, 50 °C, 24 h.

N-Decanesulfonyl acetamide (DSA) was used as a positive control for biological screens.[14] As evidenced in Table 1, modification of the C-4 carboxylate with amide substituents was more effective in improving the antimy-



Scheme 1. Synthesis of amide analogs. Reagents and conditions: (a) tris(2-oxo-3-oxazolinyl)phosphine oxide, CH_3CN, amine, rt, 30 min. (b) TFA, CH_2Cl_2, rt, 3 h. (c) DMAP, allyl isocyanate, CH_2Cl_2, rt, 1 h.

Table 1. In vitro efficacy of the synthesized butyrolactones in mycobacteria using the BACTEC radiometric system

Compound	MIC_{99} μg/mL[a]	Compound	MIC_{99} μg/mL[a]
Isoniazid[14]	0.1	9	12.5
DSA	0.75–1.5	10	12.5
C75	>25	11	12.5
1	25	12	6.25
2	25	13	>25
4	>25	14	>25
5	25	15	>25
6	>25	16	>25
7	>25	17	>25
8	25	18	>25

[a] Susceptibility in *M. bovis* BCG.

cobacterial activity of the compounds. This observation might suggest an important H-bonding network within the enzyme's active site that is essential for binding. Of the amides, the allylamide derivatives (**9**–**12**) gave the greatest activity, with compound **12** being the most active. The increase in activity of longer C-5 substituted alkyl groups is consistent with the role of FAS I in the synthesis of long chain fatty acids (C_{16}–C_{24}).[15a] Mycobacterial type I FAS readily incorporates long chain fatty acid CoAs as primers for chain elongation up to C_{18}.[15b]

Compounds **13** and **14** were prepared to examine the role of the exocyclic double bond on activity. Their synthesis was achieved in two steps as set out in Scheme 2. Briefly, reduction of C75 with H_2 over 10% Pd/C (1:1.8 for *cis* to *trans*, 92% overall yield) followed by tris(2-oxo-3-oxazolinyl)phosphine oxide mediated coupling afforded the desired products in 53% and 51% yields, respectively. AMBLs impart their activities through β-addition of biological nucleophiles.[1a] As expected, an appreciable loss of antibiotic activity was noted following reduction, in keeping with the proposed role of C75 in modification of the KAS active site cysteine.

These experiments provide an initial survey of the activity of C75 and related compounds against mycobacteria of the tuberculosis complex. While the SAR data are consistent with one or more FAS targets in BCG, they do not strictly prove it. In addition, additional analogs bearing modifications at the C-3 and C-5 positions are warranted to continue optimization of this structural series and to further examine the potential role of these compounds in TB chemotherapy.

Acknowledgments

We would like to thank Drs. James D. Dick and Nicole M. Parrish, and the Department of Pathology for the use of their facilities for susceptibility testing, and the National Institutes of Health (AI 054842) for support.

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10. *Representative spectral data*: (±)-α-methylene-γ-butyrolactone-5-octyl-4-allyl amide. (11) mp. 66–68 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, J = 6 Hz, 3 H), 1.23 (m, 11 H), 1.34–1.47 (m, 1 H), 1.60–1.71 (m, 2 H), 3.43–3.46 (m, 1 H), 3.87 (dt, J = 1.4, 5.7 Hz, 2 H), 4.74 (dt, J = 5, 7 Hz, 1 H), 5.12 (d, J = 10.6 Hz, 1 H), 5.16 (d, J = 17.3 Hz, 1 H), 5.72–5.85 (m, 1 H), 5.76 (d, J = 2.6 Hz, 1 H), 6.34 (d, J = 2.6 Hz, 1 H), 6.50 (br s, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 22.6, 24.9, 29.1, 29.2, 29.4, 31.8, 35.9, 42.3, 52.2, 80.5, 117.0, 124.3, 133.5, 135.4, 168.6, 168.6. IR (NaCl) 2922, 1771, 1756, 1642, 1557 cm^{-1}. Anal. Calcd. for $C_{17}H_{27}NO_3$: C, 69.5; H, 9.28. Found: C, 69.5; H, 9.09.
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