Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 373–376

Acetylene-based analogues of thiolactomycin, active against *Mycobacterium tuberculosis* mtFabH fatty acid condensing enzyme

Suzanne J. Senior,[a,b] Petr A. Illarionov,[a] Sudagar S. Gurcha,[a] Ian B. Campbell,[c] Merrill L. Schaeffer,[d] David E. Minnikin[a] and Gurdyal S. Besra[a,*]

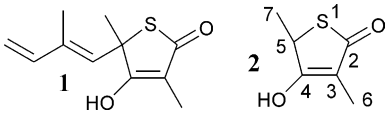
[a]*School of Biosciences, The University of Birmingham, Edgbaston, Birmingham B15 2TT, UK*
[b]*Department of Microbiology & Immunology, University of Newcastle, Newcastle upon Tyne NE2 4HH, UK*
[c]*GlaxoSmithKline Medicines Research Centre, Gunnels Wood Road, Stevenage SG1 2NY, UK*
[d]*GlaxoSmithKline, Collegeville, PA 19426, USA*

Received 3 October 2003; revised 9 October 2003; accepted 31 October 2003

Abstract—Analogues of the natural antibiotic thiolactomycin, with acetylene-based side chains, have the highest recorded in vitro inhibitory activity against the recombinant *Mycobacterium tuberculosis* β-ketoacyl-ACP synthase mtFabH condensing enzyme. In particular, 5-[3-(4-acetyl-phenyl)-prop-2-ynyl]-4-hydroxy-3,5-dimethyl-5H-thiophen-2-one exhibited more than an 18-fold increased potency, compared to thiolactomycin, against this key condensing enzyme, involved in *M. tuberculosis* mycolic acid biosynthesis. Analogues of the antibiotic thiolactomycin, with acetylene-based side chains, have the highest recorded activity against cloned mtFabH condensing enzyme.

1. Introduction

Mycobacterium tuberculosis continues to be a primary cause of morbidity and mortality worldwide; it is currently estimated that one-third of the world's population is infected with the bacillus.[1] The emergence of bacterial resistance to existing antitubercular agents has become a significant concern for the effective treatment of tuberculosis.[2] The determination of the whole-genome sequence of *M. tuberculosis* has pinpointed drug targets that potentially offer improved therapy.[3]



Thiolactomycin (TLM, **1**) is a thiolactone antibiotic isolated from a soil *Nocardia* spp.[4] TLM exhibits potent in vivo activity against many pathogenic bacteria, including Gram-negative and Gram-positive bacteria and *M. tuberculosis*.[5–7] It is relevant that TLM inhibits bacterial and plant type II fatty acid synthases (FAS-II) but not mammalian or yeast type I fatty acid synthases (FAS-I).[8] For instance, in *Escherichia coli*, TLM inhibits both β-ketoacyl-ACP synthase I to III and acetyl coenzyme A (CoA):ACP transacylase activities in vivo and in vitro.[9,10] In addition, TLM possesses encouraging anti-malarial activity, involving inhibition of the type II fatty acid biosynthetic pathway in apicoplasts.[11]

TLM **1** inhibits *M. tuberculosis* FAS-II through inhibition of both β-ketoacyl-ACP synthase condensing enzymes, mtFabH and KasA, in vitro and in vivo leading to inhibition of cell wall mycolic acid biosynthesis and to cell death.[12–15] Previous studies have shown that a number of TLM analogues, with aliphatic and other substituents linked to the 5-position of a thiolactone intermediate **2**, have significantly enhanced activity against mycolate synthesis.[13,16] The best results were obtained with 10-carbon isoprenoid-based side-chains. However, the use of more chemically stable and conformationally predictable substituents would be advantageous in a potential drug and assist in understanding modes of action. Recently, we have shown a number of biphenyl-based TLM analogues are highly active against

Keywords: Thiolactomycin; mycolic acids; *Mycobacterium tuberculosis*; inhibitors; in vitro assays.
* Corresponding author. Tel.: +44-121-415-8125; fax: +44-121-414-5925; e-mail: g.besra@bham.ac.uk

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.10.061

mtFabH, but the analogue with a benzyl 5-substituent had little activity.[17] These results demonstrated the importance of side-chain structure in TLM analogues, in accordance with previous predictions.[9,10] In this study, a propargyl substituted analogue **3** (Table 1) was used as an intermediate to produce a range of aromatic-acetylinic analogues, conveniently prepared under Sonogashira coupling[18] conditions (Table 1). These compounds possess π bonds with good potential to form hydrophobic and stacking interactions within the enzyme pocket.[9]

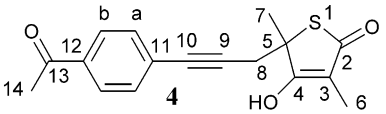
2. Chemical strategy

The key thiolactone intermediate **2** was synthesised essentially according to Wang and Salvino.[19] Lithium hexamethyldisilazane (LHMDS)[16,17] was used as a base to allow propargyl bromide to react at position 5 of the thiolactone ring **2** to produce the pivotal intermediate **3** (Table 1). Six aryl iodides containing electron-withdrawing substituents on the aryl ring were chosen (Table 1) and coupling reactions, using triethylamine, bis(triphenyl-phosphine)palladium(II) chloride (2 mol%) and copper(I) iodide (2 mol%) gave the desired products **4**–**9** (Table 1) in respectable yields (47–86%). ^{1}H NMR showed the absence of the alkynyl proton at δ 2.23 in each spectrum and a shift in the terminal carbon from δ 72.6 to the δ 90–95 region, indicating that the Sonogashira coupling had been successful. Details of the chemical synthesis of all these TLM analogues will be given elsewhere; the syntheses of intermediate **3** and compound **4** are described below, as examples.

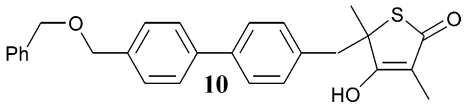
3. Biological activity

The propargyl substituted intermediate **3** showed better mtFabH inhibitory properties (IC_{50} 100±5.6 μM) than a previous[17] benzyl substituted compound (>250 μM) but it was still poorer than TLM **1** (74.9 μM) (Table 1).

Comparable activity to TLM was observed for compound **6**, with a *para*-methyl ester substituent (Table 1). Compound **7**, with the better hydrogen bonding *para*-nitro group, had improved activity against mtFabH (34±9.0 μM). Analogue **8**, containing a *para* nitrile group, was highly active, with an IC_{50} value of 7±0.5 μM. However, substitution at the *ortho* position, **9**, decreased activity to 48±4.1 μM. The best activity found so far, against mtFabH, was found for analogue **4**, the *para*-acetyl group increasing activity to 4±0.4 μM. Modification of compound **4**, by introduction of a hydroxyl group at the *meta* position of the aryl ring **5**, slightly decreases activity to 7±0.2 μM (Table 1).



4

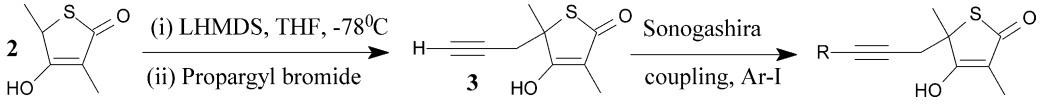


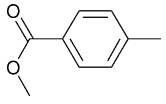
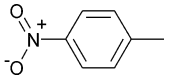
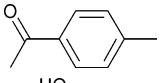
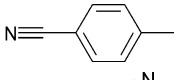
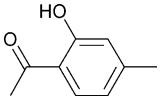
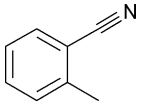
10

It can be concluded that the activity of a number of the acetylene-based compounds, **4**, **5** and **8** are significantly better than the best of those generated from Suzuki coupling reactions.[17] For instance, compare the best compound **4** (IC_{50} 4±0.4 μM) with the best biphenyl-based compound **10** (IC_{50} 17±1.3 μM).[17] The activity of analogues, such as **4** and **10**, is apparently not directly related to the length of the hydrophobic portion. The key feature of the highly enhanced activity of compounds, such as **4** and **10** is a linear π rich system containing hydrogen bond accepting substituents attached at the *para* position of the aromatic ring.

In conclusion, the new analogues **4**, **5** and **8** of TLM have the highest recorded activity against mtFabH. These types of condensing enzymes are of key importance in the synthesis of fatty acids in a number of microbial pathogens, as summarised above. It is now

Table 1. Synthetic route, structures and in vitro mtFabH activity



	R	IC_{50}, μM		R	IC_{50}, μM
1	TLM	74.9	**6**		74±3.4
3	H—	100±5.6	**7**		34±9.0
4		4±0.4	**8**		7±0.5
5		7±0.2	**9**		48±4.1

possible, therefore, to use such compounds to probe important biosynthetic mechanisms and pinpoint effective drug targets. The synthetic protocols, described, will readily allow systematic modifications to be made to effective lead compounds such as **4** (Table 1). These stable alkynyl compounds have an advantage over previous active, relatively labile, aliphatic-based analogues[13,17] in that their side-chains are conformationally defined. Other biological properties of these exciting TLM analogues are currently being investigated.

4. Experimental

4.1. 5-Prop-2-ynyl-4-hydroxy-3,5-dimethyl-5*H*-thiophen-2-one (3)

To a stirred solution of 4-hydroxy-3,5-dimethyl-5*H*-thiophen-2-one **2** (400 mg, 2.77 mmol) in anhydrous tetrahydrofuran (THF) (5 mL) at −78 °C was added lithium bis(trimethylsilyl)amide, 20% in THF (LHMDS) (15.65 mL, 16 mmol). The reaction mixture was stirred for 30 min at −78 °C before addition of propargyl bromide (0.74 mL, 8.33 mmol). The mixture was allowed to warm to room temperature and stirred for a further 3 h, acidified to pH 2 with 2 M aq acetic acid and extracted twice with dichloromethane. The organic layers were combined, washed with satd brine, dried and reduced in vacuo to yield the crude product. Purification on a 20 g SPE silica gel cartridge, eluting from 0 to 5% diethyl ether (1% increments) in dichloromethane, yielded **3** (420 mg, 83%) as a clear oil. δ_H (400 MHz; D_3COD,) 1.54 (6H, s, SCC*H*$_3$, CC*H*$_3$), 2.23 (1H, s, C*H*), 2.52–2.73 (2H, m, C*H*$_2$); δ_C (100.6 MHz; D_3COD) 8.0 (C-6), 25.6 (C-7), 31.0 (*C*H$_2$), 50.0 (C-5), 72.6 (H*C*≡), 82.0 (C*C*≡), 111.5 (C-3), 182.1 (C-2), 188.5 (C-4); *m*/*z* (ESP) 183.0482 (MH^+. $C_9H_{10}O_2S$ requires 183.0480).

4.1.1. 5-[3-(4-Acetyl-phenyl)-prop-2-ynyl]-4-hydroxy-3,5-dimethyl-5*H*-thiophen-2-one (4). To a stirred solution of intermediate **3** (100 mg, 0.549 mmol), dimethylformamide (DMF) (1.5 mL) and triethylamine (1.0 mL), under N_2, was added copper(I) iodide (2 mg, 0.109 mmol, 2 mol%), bis(triphenylphosphine)-palladium(II) chloride (6.5 mg, 0.274 mmol, 5 mol%) and 4-iodoacetophenone (162 mg, 0.659 mmol). The mixture was stirred overnight at room temperature, acidified to pH 4 with 10% aq citric acid and the product extracted into ethyl acetate. The organic layers were collected, washed with water, dried and reduced in vacuo to give the crude product. This was purified by Fisher Matrix silica gel 60 flash chromatography (0–10% ethyl acetate in cyclohexane) to give the title compound **4** (97 mg, 58%), as a pale yellow oil. δ_H (400 MHz; D_3COD) 1.71 (3H, s, SCC*H*$_3$), 1.74 (3H, s, CC*H*$_3$), 2.60 (3H, s, COC*H*$_3$), 2.93–3.13 (2H, C*H*$_2$), 7.46 (2H, d, C-b, J = 9 Hz), 7.95 (2H, d, C-a, J = 9 Hz); δ_C (100.6 MHz; D_3COD) 10.3 (C-6), 27.9 (C-7), 29.3 (C-8), 34.2 (C-14), 60.3 (C-5), 86.0 (C-9), 93.0 (C-10), 113.4 (C-11), 132.1 (C-3), 132.2 (C-b), 135.4 (C-a), 140.0 (C-12), 184.5 (C-4), 199.9 (C-2), 203.5 (C-13); *m*/*z* (ESP) (MH^+. 301.0912 (MH^+. $C_{17}H_{16}O_3S$ requires 301.0898).

4.2. MtFabH assay: determination of IC_{50} values

Direct, end-point scintillation proximity assays (SPA) were performed according to an established SPA assay.[17,20] The assay contained a mixture of recombinant *E. coli* produced mtFabH[12] (1.0 nM), biotinylated-malonyl-ecACP (2.5 μM), and myristoyl-CoA (0.4 μM) in 100 mM sodium phosphate buffer with 0.01% CHAPS and 1 mM DTT. Compounds in DMSO were added to the assay plate to provide concentrations ranging from 500 to 0.98 μM, followed by mtFabH and biotinylated-malonyl–ecACP. Following a 5 min pre-incubation at 30 °C, [^{3}H] myristoyl–CoA and unlabeled myristoyl–CoA were added to reach a final concentration of 0.4 μM containing approximately 30,000 cpm per well. Reactions were incubated at 30 °C and 100 μL of ethanol was added to quench the reaction after 20 min. Streptavidin-coated beads (40 μL) (10 mg/mL in DPBS) (Amersham) were added to each well, and the plates sealed with Topseal-A press-on, transparent adhesive sealing film (Packard) and shaken for 30 min. The plates were centrifuged for 1 min at 1700×*g* to pellet the beads in each well and the incorporated radioactivity was counted using a 1450 Microbeta Trilux liquid scintillation counter (Wallac).

Acknowledgements

This work was supported by GlaxoSmithKline, ActionTB; The Medical Research Council (G9901077, G9901078); National Institutes of Health, National Cooperative Drug Discovery Groups for the Treatment of Opportunistic Infections. G.S.B. is a Lister Institute Jenner Research Fellow.

References and notes

1. Enarson, D. A.; Chretien, J. *Curr. Opin. Pulm. Med.* **1999**, *5*, 128.
2. Kremer, L.; Besra, G. S. *Expert Opin. Investig. Drugs* **2002**, *11*, 1033.
3. Cole, S. T.; Brosch, R.; Parkhill, J.; Garnier, T.; Churcher, C.; Harris, D.; Gordon, S. V.; Eiglmeier, K.; Gas, S.; Barry, C. E.; Tekaia, F.; Badcock, K.; Basham, D.; Brown, D.; Chillingworth, T.; Connor, R.; Davies, R.; Devlin, K.; Feltwell, T.; Gentles, S.; Hamlin, N.; Holroyd, S.; Hornsby, T.; Jagels, K.; Krogh, A.; McLean, J.; Moule, S.; Murphy, L.; Oliver, K.; Osborne, J.; Quail, M. A.; Rajandream, M. A.; Rogers, J.; Rutter, S.; Seeger, K.; Skelton, J.; Squares, R.; Squares, S.; Sulston, J. E.; Taylor, K.; Whitehead, S.; Barrell, B. G. *Nature* **1998**, *393*, 537.
4. Oishi, H.; Noto, T.; Sasaki, H.; Suzuki, K.; Hayashi, T.; Okazaki, H.; Ando, K.; Sawada, M. *J. Antibiot. (Tokyo)* **1982**, *35*, 391.
5. Miyakawa, S.; Suzuki, K.; Noto, T.; Harada, Y.; Okazaki, H. *J. Antibiot. (Tokyo)* **1982**, *35*, 411.
6. Noto, T.; Miyakawa, S.; Oishi, H.; Endo, H.; Okazaki, H. *J. Antibiot. (Tokyo)* **1982**, *35*, 401.
7. Slayden, R. A.; Lee, R. E.; Armour, J. W.; Cooper, A. M.; Orme, I. M.; Brennan, P. J.; Besra, G. S. *Antimicrob. Agents Chemother.* **1996**, *40*, 2813.
8. Hayashi, T.; Yamamoto, O.; Sasaki, H.; Kawaguchi, A.;

Okazaki, H. *Biochem. Biophys. Res. Commun.* **1983**, *115*, 1108.
9. Heath, R. J.; White, S. W.; Rock, C. O. *Prog. Lipid. Res.* **2001**, *40*, 467.
10. Tsay, J. T.; Rock, C. O.; Jackowski, S. *J. Bacteriol.* **1992**, *174*, 508.
11. Waller, R. F.; Ralph, S. A.; Reed, M. B.; Su, V.; Douglas, J. D.; Minnikin, D. E.; Cowman, A. F.; Besra, G. S.; McFadden, G. I. *Antimicrob. Agents. Chemother.* **2003**, *47*, 297.
12. Choi, K. H.; Kremer, L.; Besra, G. S.; Rock, C. O. *J. Biol. Chem.* **2000**, *275*, 28201.
13. Kremer, L.; Douglas, J. D.; Baulard, A. R.; Morehouse, C.; Guy, M. R.; Alland, D.; Dover, L. G.; Lakey, J. H.; Jacobs, W. R.; Brennan, P. J.; Minnikin, D. E.; Besra, G. S. *J. Biol. Chem.* **2000**, *275*, 16857.
14. Schaeffer, M. L.; Agnihotri, G.; Volker, C.; Kallender, H.; Brennan, P. J.; Lonsdale, J. T. *J. Biol. Chem.* **2001**, *276*, 47029.
15. Kremer, L.; Dover, L. G.; Carrere, S.; Nampoothiri, K. M.; Lesjean, S.; Brown, A. K.; Brennan, P. J.; Minnikin, D. E.; Locht, C.; Besra, G. S. *Biochem. J.* **2002**, *364*, 423.
16. Douglas, J. D.; Senior, S. J.; Morehouse, C.; Phetsukiri, B.; Campbell, I. B.; Besra, G. S.; Minnikin, D. E. *Microbiology* **2002**, *148*, 3101.
17. Senior, S. J.; Illarionov, P. A.; Gurcha, S. S.; Campbell, I. B.; Schaeffer, M. L.; Minnikin, D. E.; Besra, G. S. *Biorg. Med. Chem. Lett.* **2003**, *13*, 3685.
18. Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, *16*, 4467.
19. Wang, C. L. J.; Salvino, J. M. *Tetrahedron Lett.* **1984**, *25*, 5243.
20. He, X.; Mueller, J. P.; Reynolds, K. A. *Anal. Biochem.* **2000**, *282*, 107.