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Synthesis and biological evaluation of a C5-biphenyl thiolactomycin library

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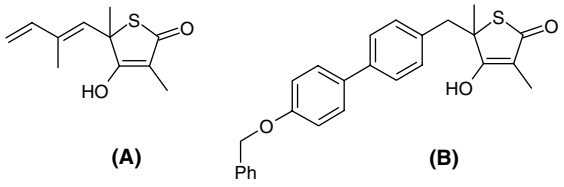
Abstract—Fifteen novel C5 analogues of thiolactomycin (13 biphenyl analogues and two biphenyl mimics) have been synthesised and assessed for their in vitro *mt*FabH and whole cell *Mycobacterium bovis* BCG activity, respectively. Analysis of the 15 compounds revealed that six possessed enhanced in vitro activity in a direct *mt*FabH assay. Encouragingly analogues **11**, **12** and **13** gave a significant enhancement in in vitro activity against *mt*FabH. Analogue **13** (5-(4-methoxycarbonyl-biphenyl-4-ylmethyl)-4-hydroxy-3,5-dimethyl-5H-thiophen-2-one) gave an IC_{50} value of 3 μM compared to the parent drug thiolactomycin (75 μM) against *mt*FabH. The biological analysis of this library reaffirms the requirement for a linear π-rich system containing hydrogen bond accepting substituents attached to the *para*-position of the C5 biphenyl analogue to generate compounds with enhanced activity.

Mycobacterium tuberculosis (*Mt*) still remains one of the leading causes of morbidity and mortality worldwide, contributing to an estimated 8.9 million new cases and 1.8 million deaths *per* annum.[1] Recently, the emergence of multi-drug-resistant tuberculosis (MDR-TB) and extensively drug-resistant TB (XDR-TB) strains, with resistance to at least three of the six classes of second-line drugs (aminoglycosides, polypeptides, fluoroquinolones, thioamides, cycloserine and *para*-aminosalicylic acid), has been reported.[2] In some regions approaching 20% of MDR-TB cases were classified as XDR-TB raising concerns over a future epidemic of virtually untreatable TB.[2] Given this backdrop, the need for rapid and continued progress in the development of new antitubercular agents and the identification and characterisation of novel drug targets to utilise medicinal chemistry is clearly evident.

Thiolactomycin (TLM) (**A**) possesses a thiolactone core and was originally isolated from a soil *Nocardia* spp.[3] TLM exhibits potent in vivo activity against many pathogenic bacteria, including *M. tuberculosis*.[4–6] TLM inhibits *M. tuberculosis* FAS-II through inhibition of β-ketoacyl-ACP synthase condensing enzymes, in vitro and in vivo, leading to the inhibition of cell wall mycolic acid biosynthesis and subsequent cell death.[7–10]

The β-ketoacyl-ACP synthase III condensing enzyme (*mt*FabH) is the pivotal link between the FAS-I and FAS-II systems involved in the biosynthesis of mycolic acids.[7,11] In a series of experiments, Senior et al.[12,13] determined that acetylene and biphenyl analogues of TLM possessed enhanced in vitro activity against *mt*FabH.



From this series of C5 biphenyl analogues of TLM, 5-(4′-benzyloxy-biphen-4-ylmethyl)-4-hydroxy-3,5-dimethyl-5H-thiophen-2-one (**B**) gave an approximate 4-fold increase in potency against *mt*FabH. It was apparent from this initial library that the key features required to obtain improved in vitro activity were a linear π-rich

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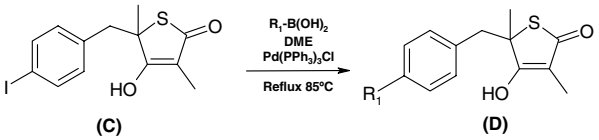
system containing hydrogen bond accepting substituents attached to the *para*-position of the aromatic ring. To further analyse the inhibition of *mt*FabH, we herein present synthetic and biological activity of fifteen novel C5 biphenyl analogues.

There has been much speculation into the validity of extending TLM analogues in the C-5 position. More recently Kim et al.[14] addressed this issue, determining that the only modification that can be tolerated in the C-5 position was an isoprene and that a slight modification, such as the reduction of the double bonds on the isoprene, resulted in markedly reduced activity. However, this does not correlate with the activity shown previously by C5 biphenyl and acetylene analogues[12,13] and the recent determination of the crystal structure of *M. tuberculosis* KasB and subsequent homology modelling of KasA, using the *mt*KasB structure as a template, supporting the potential for C5-derivatisation of the TLM scaffold.[15]

To generate the thiolactone core required for the synthesis of C5 TLM analogues, the Wang and Salvino method[16] was successfully employed with one modification. Instead of using triethylamine as the base in step two of the Wang and Salvino method to produce 2-acetylsulfanyl-2-methyl-3-oxopentanoic acid methyl ester,[17] cesium carbonate was used due to the phenomenon known as the "Cesium Effect"[18] This effect is widely used to describe the advantages concerning yield and reaction conditions of cesium assisted reactions compared to conventional non-cesium routes. This new method gave an improved product yield of 92% compared to the Wang and Salvino method that gave 60%. The reaction procedure is described in the notes.[17]

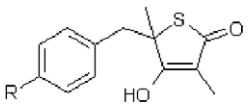
As previously published by Senior et al.[12] biphenyl analogues were synthesised by using three equivalents of lithium hexamethyldisilazide (LHMDS) to generate the dianion intermediate, onto which an aryl halide linker arm was introduced **(C)**. Suzuki coupling (Reaction Scheme 1) was then performed on the linker arm, resulting in the formation of the desired biphenyl analogue as a racemic mixture (**D**), where R^1 represents a substituted aromatic ring. Suzuki coupling reactions were achieved by heating the aryl halide intermediate, with a range of substituted boronic acids, bis(triphenylphosphine) palladium (II) chloride (5 mol %), dimethoxyethane (DME) and aqueous sodium carbonate under reflux for 6 h before quenching with acid. Fifteen novel analogues (Table 1) were developed of which thirteen were biphenyl analogues (**1**–**13**)[19] and two biphenyl mimics (**14** and **15**). Analogues **14** and **15** were synthesised in a similar procedure outlined by Senior et al. [12] by the direct allylation of the TLM core by 4-(bromomethyl) benzophenone to generate **14** and 3-phenoxybenzyl chloride to generate **15** under the standard conditions

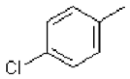
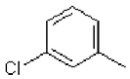
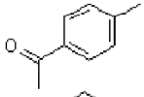
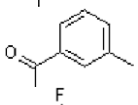
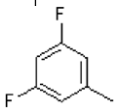
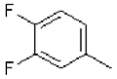
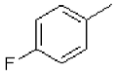
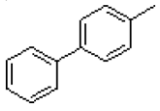
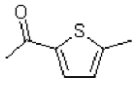
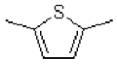
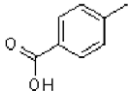
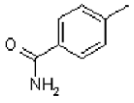
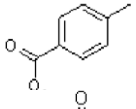
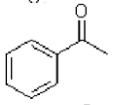
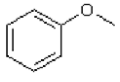


(C) (D)

Scheme 1.

Table 1. Structure and biological analysis of 13 novel biphenyl and two biphenyl mimic TLM analogues in a direct *mt*FabH assay[a]



	Structure	Yield (%)	IC_{50} (μM)
1		25	130
2		15	156
3		20	54
4		22	150
5		21	135
6		19	207
7		26	105
8		18	68
9		20	200
10		25	86
11		22	7
12		18	4
13		25	3
14		25	283
15		55	15
	TLM	—	75

[a] IC_{50} values in a direct *mt*FabH assay were measured in micromolar (μM).

of three equivalents of LHMDS. Analogues synthesised in this library were fully characterised by NMR (^1H and ^{13}C), HRMS and IR.

The *mt*FabH assay was performed using radiolabelled [2-^{14}C]malonyl-CoA. The assay mixture contained purified *mt*FabD, *mt*FabH and ACP/AcpM. The in vitro activity was determined by the incorporation of radiolabelled [2-^{14}C]malonyl-CoA into the acyl-1,3-diol formed upon reduction of the β-ketoacyl-AcpM generated by *mt*FabH.[20] From the 15 novel TLM analogues synthesised, six analogues (**3**, **8**, **11**, **12**, **13** and **15**) gave enhanced inhibitory in vitro *mt*FabH activity compared to the parent drug, TLM (Table 1). Analogues **11**, **12**, **13** and **15** gave a significant 4-fold increase in inhibitory activity, whereas analogues **3** and **8** gave only a slight improvement. The in vitro activity of compounds **11**, **12**, **13** and **15** is such that they are comparable to the activity of isoniazid against InhA (7.3 μM).[21] In terms of developing a structure–activity relationship (SAR) study, biphenyl analogues **2** and **4** containing *meta*-substituents gave markedly reduced in vitro inhibitory *mt*FabH activity compared to TLM. In contrast, analogue **3**, containing an acetyl function in the *para*-position, gave improved in vitro inhibitory *mt*FabH activity compared to analogue **4**, which contains the same modification, but in the *meta*-position. Furthermore, the requirement for *para*-modifications coupled with hydrogen bonding groups is evident in the analysis of analogues **11**, **12** and **13**. These results also reaffirm previously published data.[12] All the compounds in this series gave poor whole cell activity (above 250 μM) against *M. bovis* BCG in comparison to TLM (15 μM).[20] It is possible that the analogues may either, not permeate the cell wall of *M. bovis* BCG or are enzymatically modified, rendering them inactive.

Additional trends which are apparent in the library include: (i) halide-containing analogues **1**, **2**, **5**, **6** and **7** gave a marked decrease in inhibitory in vitro activity against *mt*FabH, (ii) disubstituted fluoride-containing analogues **5** and **6** also resulted in poor in vitro inhibition of *mt*FabH activity. It is plausible that such modifications may place structural constraints within the active site of *mt*FabH.

Interestingly, the triphenyl modified analogue **8** gave a comparable inhibitory in vitro *mt*FabH activity to TLM, however the simple introduction of an methylene oxy-group between the second and third aromatic ring (**B**) resulted in a 4-fold increase in potency against *mt*FabH activity.[12] There are two possible factors which may have governed the increase in the potency of **8**. Firstly, the presence of the oxy-group will provide hydrogen bonding interactions with neighbouring residues and secondly, the modification facilitates flexibility on the third aromatic ring. It is therefore clear that there is more scope to modify compound **8** and **B**. Finally, we explored the possibility of attaching a thiophene group to the C5 aryl linker arm of the thiolactone core in a Suzuki type coupling reaction. Although, analogue **10** gave a slight decrease in in vitro potency in the *mt*FabH assay as compared to TLM, it is difficult to suggest whether these modifications are viable without the generation and analysis of a more comprehensive library.

In conclusion, compounds **11**, **12** and **13** gave a significant increase in in vitro inhibitory activity against *mt*FabH compared to TLM. These analogues contained hydrogen bonding groups in the *para*-position of the biphenyl compound. As previously noted,[12] these contributing factors generate analogues with enhanced in vitro inhibitory activity. Although, the analysis of some C5 biphenyl analogues shows promising in vitro inhibitory *mt*FabH activity, to further develop these analogues as potential drugs several factors need to be considered. Importantly, the issue of poor in vivo inhibitory activity against *M. bovis* BCG must be addressed. The mycobacterial cell wall is unusually complex and provides a particularly formidable permeability barrier that protects the organism against various antibiotic and chemical insults. The disparity between improved in vitro performance and loss of antimycobacterial activity is likely related to their in ability to traverse this structure. Consequently, their utility against other TLM-sensitive organisms warrants investigation.[22]

Acknowledgments

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17. Synthesis of thiolactone core using cesium carbonate—cesium carbonate (13.22 g, m., 1 equiv) was dissolved in 30 ml of anhydrous acetonitrile and 25 ml of anhydrous methanol and left to stir. Thiolacetic acid (7.52 g, 8.02 ml, 0.099 mol, 1.1 equiv) was added dropwise and left to stir at rt for 1 h. The acetonitrile and methanol were removed in vacuo to yield the yellow solid product, $CsSCOCH_3$. The second step of the reaction requires dissolving (2RS, 4RS)-4-bromo-2-methyl-3-oxopentanoic acid methyl ester (20 g, 0.09 mol, 1 equiv) in 30 ml of DMF over molecular sieves (500 mg). The $CsSCOCH_3$ was dissolved in 10 ml of DMF and placed in an ice bath. (2RS, 4RS)-4-bromo-2-methyl-3-oxopentanoic acid methyl ester was added dropwise at rt for 3 h. The organic layer was extracted with chloroform and washed, dried and reduced in vacuo. The resulting red/brown oil was columned using ethyl acetate (0–20%) in petrol. This yielded 2-acetylsulfanyl-2-methyl-3-oxopentanoic acid methyl ester as a dark red oil (19.07 g, 97%). 1H NMR ($CDCl_3$, 300 MHz) δH: 1.24–1.30 (m, 3H, COCHCH_3), 1.34–1.50 (m, 3H, CHSCH_3), 2.33 (s, 3H, CH_3), 3.63 (s, 3H, OCH_3), 3.72–3.85 (m, 1H, COCHCH_3), 4.40–4.55 (m, 1H, SCHCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C: 12.47, 13.06 (C-6), 15.45, 15.88 (C-5), 29.57 (C-8), 45.19, 45.60 (C-2), 49.13, 49.46 (C-4), 51.95 (CH_3), 170.13 (C-7), 193.2 (C-3), 203.1 (C-1); *m/z* (EI) 218 (MH^+ 20%), 176 ($MH^+-SCOCH_3$, 100%); HRMS Calcd for $C_9H_{12}O_4S$ [MH^+] 218.4516 found 218.4523.
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19. Synthesis of biphenyl TLM analogue 3—compound 3 (4-acetyl (biphenyl-4-yl-methyl)-4-hydroxy-3,5-dimethyl-5H-thiophen-2-one) was synthesised as follows. A solution of 5-(4-iodobenzyl)-4-hydroxy-3,5-dimethyl-5H-thiophen-2-one (60 mg, 0.167 mmol, 1 equiv), DME (2 ml), aq sodium carbonate (0.5 ml, 1 M) and 4-acetylphenyl boronic acid (27 mg, 0.183 mmol, 1.2 equiv) was degassed for 10 min. Bis(triphenylphosphine) palladium (II) chloride (8 mg, 7×10^{-3}, 5 mol %) was added and the mixture was heated under reflux for 6 h. The mixture was portioned between water (10 ml) and ethyl acetate (10 ml) and separated. The aqueous layer was acidified to pH 2 with dilute HCl (2 M) and the product was extracted with ethyl acetate (2×10 ml). The organic layers were combined, washed with saturated brine (3×10 ml), dried and reduced to give the crude product. Purification was achieved via 2 separate columns to give a product yield of 36% (21 mg). ^{1}H NMR (CD_3OD, 300 MHz), δ_H; 1.50 (s, 3H, SCCH_3), 1.75 (s, 3H, CCH_3), 2.55 (s, 3H, COCH_3), 3.15–3.20 (q, 2H, CH_2), 7.20 (d, 2H, H-a), 7.35 (d, 2H, H-b), 7.55 (d, 2H, H-c), 8.05 (d, 2H, H-d); ^{13}C NMR (CD_3OD, 75 MHz) δ_C; 4.95 (C-6), 22.4 (C-7), 24.5 (C-14), 56.0 (C-5), 127.4, 127.8, 128.1, 128.3, 129.1, 129.4, 130.3, 130.6 (C-a, C-b, C-c, C-d), 137.8 (C-9); *m/z* (EI) 353.4 [MH^+] (100%), HRMS Calcd for $C_{21}H_{20}O_3HS$ [$M+H^+$] 353.4652 found 369.4659. All the analogues (**1**–**15**) were characterised by NMR, HRMS and IR.
20. Minimum inhibition concentration (MIC_{99}) and in vitro effect of TLM analogues on *mt*FabH activity—minimum inhibition concentration (MIC_{99}) of TLM analogues against *M. bovis* BCG were calculated by growth in liquid media using the Alamar blue 96-well plate standard protocol.[22] The full *mt*FabH assay was performed as published by Brown et al.[11] All the required enzymes were also generated as reported previously by Brown et al.[11]
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