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Bioorganic & Medicinal Chemistry
ELSEVIER
Bioorganic & Medicinal Chemistry 12 (2004) 683–692

Analogues of thiolactomycin as potential anti-malarial and anti-trypanosomal agents

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Received 10 July 2003; accepted 21 November 2003

Abstract—A series of analogues of the naturally occurring antibiotic thiolactomycin (TLM) have been synthesised and evaluated for their ability to inhibit the growth of the malaria parasite, *Plasmodium falciparum*. Thiolactomycin is an inhibitor of Type II fatty acid synthase which is found in plants and most prokaryotes, but not an inhibitor of Type I fatty acid synthase in mammals. A number of the analogues showed inhibition equal to or greater than TLM. The introduction of hydrophobic alkyl groups at the C3 and C5 positions of the thiolactone ring lead to increased inhibition, the best showing a fourteenfold increase in activity over TLM. In addition, some of the analogues showed activity when assayed against the parasitic protozoa, *Trypanosoma cruzi* and *Trypanosoma brucei*.

1. Introduction

Malaria is by far the world's most important tropical disease. In many developing countries and in Africa especially, malaria exacts an enormous toll in lives, in medical costs, and in days of labour lost. At present, at least 300 million people are affected by malaria globally, and there are between 1 and 1.5 million malaria deaths annually.[1,2] Over the last several decades, the rapid development of drug-resistant strains has compounded the already serious health problems. Of the four known human malaria parasites, *Plasmodium falciparum* is the predominant cause of mortality, with 120 million new cases and 1 million deaths per year globally. It is this particular species, which has given rise to formidable drug-resistant strains, resulting in the urgent need for new chemotherapeutic agents. The search for new agents has recently been aided with the completion of the *P. falciparum* genome.[3] Detailed studies[4] of this genome have identified new potential drug and vaccine targets. One such target appears to be fatty acid biosynthesis of *P. falciparum*.

Fatty acid synthesis is a crucial function of living cells. The main steps in this process in animals are carried out by a single, multifunctional polypeptide fatty acid synthase (Type I FAS). In contrast, plants and bacteria utilise a dissociable multienzyme system (Type II FAS).[5] The structural differences between these systems are sufficient for the development of selective antibiotics targeted against the β-ketoacyl-acyl carrier protein synthase (KAS) and other individual enzymes of the Type II FAS.[6,7]

In plants and bacteria, there appear to be three KAS enzymes, denoted KAS I, KAS II and KAS III. The initial C2-C4 step is catalysed by KAS III; thereafter KAS I and KAS II are involved in chain elongation.[8] In most organisms with a Type II fatty acid synthase, KAS I and KAS II show very high similarity at the protein level. Recent discoveries acknowledge that *Plasmodium* synthesises fatty acids in the apicoplast which is a vestigial organelle thought to be derived from a chloroplast.[9–12] Not surprisingly, *Plasmodium* fatty acid synthase (FAS) is a Type II enzyme complex and, thus, differs markedly from human Type I FAS. Analysis of the recently published *P. falciparum* genome[3] reveals just two different KAS enzymes, with the KAS I and KAS II of typical Type II systems replaced by a single enzyme, that we and others, have denoted KAS1/2, and a separate KAS III.[8]

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doi:10.1016/j.bmc.2003.11.023

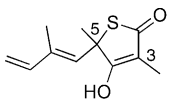

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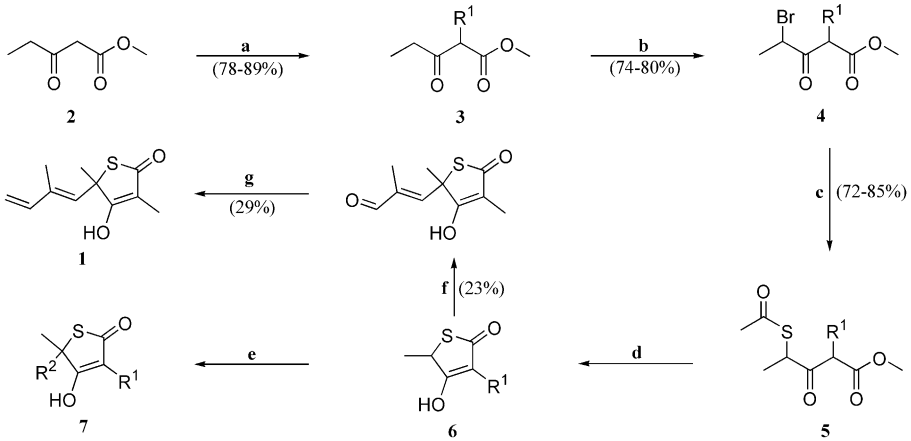
Figure 1. Structure of thiolactomycin.

A known inhibitor of the dissociable Type II FAS enzymes is the naturally occurring thiolactone antibiotic, thiolactomycin (TLM) **1**[13,14] (Fig. 1). Originally reported in 1982,[15] TLM has since become the most well-studied of the naturally occurring thiotetronic acids. The thiolactone has shown moderate in vitro activity against a broad spectrum of pathogens, including Gram-positive and Gram-negative[16] bacteria and *Mycobacterium tuberculosis*.[17] Furthermore, TLM analogues showed enhanced inhibition of pea (*Pisum sativum*) FAS,[18] and effective activity against *Staphylococcus aureus* and *Pasteurella multocida*.[19] Because of its negligible toxicity towards mammals,[15] TLM or its analogues are obvious candidates as new drugs for problem diseases such as malaria. In fact, recently Waller et al. have reported encouraging anti-malarial activity for TLM and various analogues.[20] Furthermore, because there are KAS isoforms in target organisms and each KAS isoform of Type II FAS complexes has been shown to be sensitive to TLM, then use of such inhibitors offers therapies with less chance of target-site resistance due to point mutations occurring readily. In this paper we present synthetic studies and findings in our attempts to improve the overall activity of TLM against *P. falciparum* cultured in red blood cells. Also studies against *Trypanosoma brucei rhodesiense* trypomastigotes and intracellular *Trypanosoma cruzi* amastigotes are reported.

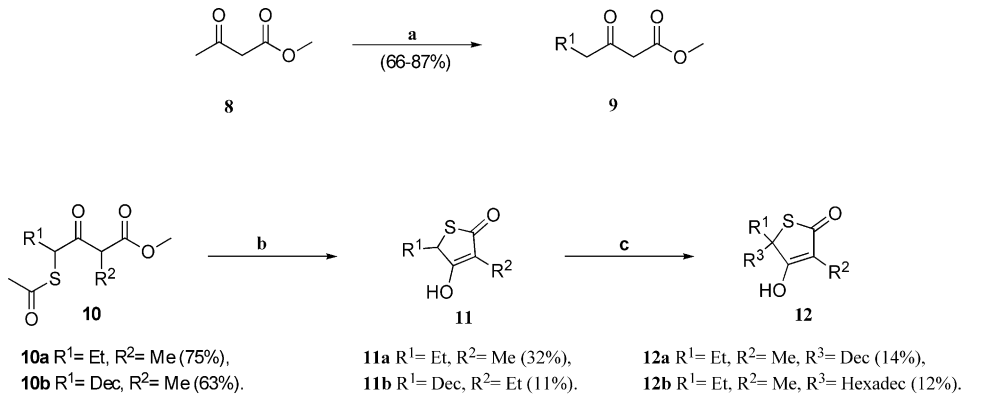
2. Chemistry

The first racemic total synthesis of TLM **1** was reported by Salvino et al. and involved the alkylation of a thiotetronic acid dianion with an isoprene cation equivalent.[21] A subsequent Wittig condensation with the resulting aldehyde afforded TLM **1**. The intermediate thiotetronic acid was prepared according to methodology developed by Benary.[22] Using a modification of this methodology we were able to synthesise TLM and a range of TLM analogues with varying chain length and saturation at the C5 and C3 positions of the ring (Scheme 1). Methyl propionylacetate **2** was treated with alkyl and benzyl halides in the presence of potassium carbonate as base to provide the alkylated products **3**. Treatment of the resulting β-ketoesters **3** with pyridinium tribromide in acetic acid afforded the bromides **4** as a mixture of diastereoisomers. Displacement of the bromides **4** proceeded smoothly using thiolacetic acid under basic conditions forming the thioacetates **5**. Cyclisation to the desired thiolactones **6** was accomplished upon treatment with aqueous potassium hydroxide solution. Isolated yields following flash chromatography were varied (30–93%). These derivatives were converted into the final compounds **7** after exposure to sodium hydride, butyllithium and the appropriate alkyl halides in dry THF at −78 °C. The alkylations tended to be generally low yielding; these findings are in agreement with previous publications in this area.[19] Attempts to protect the hydroxy functionality, in the hope that tying up of the free hydroxy group may improve the yields for the alkylation step, proved unsuccessful.

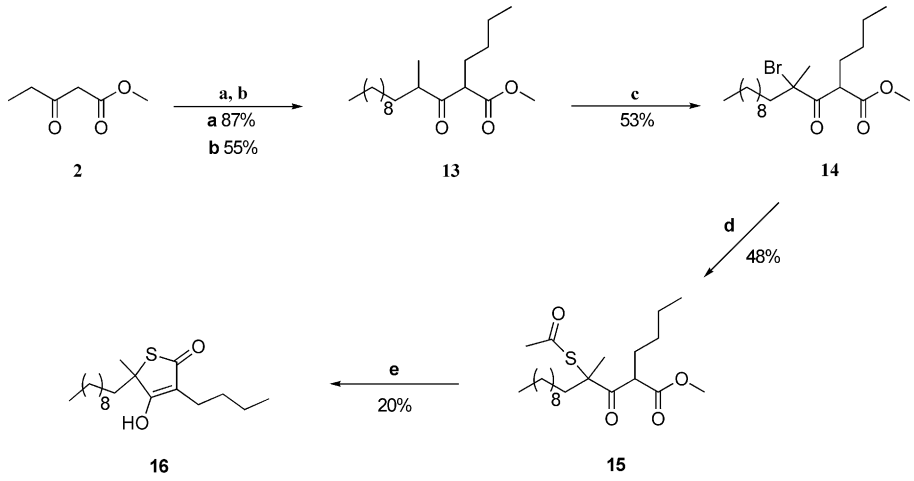
Derivatisation of the methyl substituent at the C5 position was accomplished as shown in Scheme 2. Following a literature procedure,[23] the β-ketoesters **9** were



Scheme 1. Reagents and conditions: (a) R^1X, K_2CO_3 (4 equiv), THF, reflux; (b) $PyBr_3$, AcOH, rt; (c) AcSH, Et_3N, EtOAc, rt; (d) KOH (2 equiv), H_2O/EtOH (1:1), 45 °C; (e) NaH (1.2 equiv), BuLi (1.1 equiv), R^2X, THF/DMPU (1:1), rt to −78 °C; (f) NaH, BuLi, $C_2H_5OCHC(CH_3)CHO$ (2 equiv), THF/Pyrimidone (1:1), rt to −78 °C; (g) BuLi (2 equiv), Ph_3P^+–$CH_3Br,^-$ THF, −78 °C to rt.



Scheme 2. Reagents and conditions: (a) NaH (1.2 equiv), BuLi (1.1 equiv), R^1I, 0 °C to rt; (b) KOH (2 equiv), H_2O/EtOH (1:1), 45 °C; (c) NaH (1.2 equiv), BuLi (1.1 equiv), R^3X, rt to −78 °C.



Scheme 3. Reagents and conditions: (a) NaH (1.2 equiv), BuLi (1.1 equiv), iododecane, 0 °C to rt; (b) iodobutane, K_2CO_3 (4 equiv), THF, reflux; (c) $PyBr_3$, AcOH, rt; (d) AcSH, Et_3N, EtOAc, rt; (e) KOH (2 equiv), H_2O/EtOH (1:1), 45 °C.

prepared in excellent yields by treatment of methyl acetoacetate **8** with sodium hydride, butyllithium and the relevant alkyl iodides. The corresponding β-keto-esters **10** were synthesised using the same methodology described in Scheme 1, and once formed were duly converted into the desired thiolactones **11** and **12**.

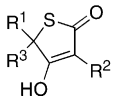
Using the same methodology described in Scheme 2, we were able to avoid the low yielding alkylation reaction at the C5 position with the construction of the desired carbon skeleton prior to cyclisation (Scheme 3). Subsequent alkylations of methyl propionylacetate **2** at the C4 and C2 positions respectively yielded the β-ketoester **13**. Bromination at the tertiary C4 position proceeded in reasonable yield (53%) forming the bromide **14**, which was duly converted to the thioacetate **15** in 48% yield. Treatment of the thioacetate **15** with potassium hydroxide delivered the derivitised thiolactone **16** (20%).

3. Results and discussion

All compounds were assayed in vitro for their activity against the malarial parasite, *P. falciparum* (Table 1). Furthermore, the compounds were also assayed against *T. brucei* and *T. cruzi* (Table 1). The screening procedures are briefly described in the Experimental (Section 5).

The basic structure of the thiolactomycin ring was retained with the analogues represented mainly by variations in the isoprenoid moiety and the methyl substituents at the C3 and C5 positions of the ring. Recently, thiolactomycin analogues have been tested in assays for fatty acid synthesis[18,20] and activities of the individual condensing enzymes.[17] The findings from these studies suggest that replacement of the isoprenoid side chain with longer tethers leads to an overall increase in inhibition of fatty acid synthesis. This notable trend is also evident in our own findings (Table 1). The analogues **7a** and **7b** showed inhibition comparable to that of thiolactomycin **1**. In comparison to **1**, the geranyl analogue **7c** showed almost a fourfold increase in activity, perhaps indicating that unsaturation in the hydrophobic chain enhances inhibition. Waller et al.[20] have reported a sixfold increase in efficacy for the geranyl analogue over TLM against the *P. falciparum* multidrug-resistant strain W2mef. The longer side-chain analogues **7d** (decyl) and **7e** (hexadecyl) also showed much improved activity over **1**, with **7e** registering a 6-fold (25 μM) increase in inhibition. The influence of the longer hydrophobic chain on activity is further

Table 1. Activities of compounds against *P. falciparum* cultured in red blood cells; *T. brucei* trypomastigotes; and *T. cruzi* amastigotes cultured in rat skeletal myoblasts



Compd	R^1	R^2	R^3	*P. falciparum* IC_{50} (μM)	*T. cruzi* IC_{50} (μM)	*T. brucei* IC_{50} (μM)
1	Me	Me	Isoprenoid	143	>427	256
6a	Me	Me	H	>347	>624	>624
6b	Me	Et	H	233	568	357
6c	Me	Pr	H	>290	>522	288
6d	Me	Bn	H	195	>408	408
7a	Me	Me	Hexyl	139	127	112
7b	Me	Me	Octyl	153	>350	194
7c	Me	Me	Geranyl	40	68	190
7d	Me	Me	Decyl	36	68	171
7e	Me	Me	Hexadecyl	25	28	62
7f	Me	Et	Hexyl	61	195	71
7g	Me	Et	Octyl	54	165	157
7h	Me	Et	Decyl	15	54	97
7i	Me	Et	Hexadecyl	19	43	47
7j	Me	Pr	Decyl	10	56	21
7k	Me	Bn	Decyl	50	64	132
11a	Et	Me	H	>316	>568	>568
11b	Decyl	Et	H	72	164	6
12a	Et	Me	Decyl	65	72	153
12b	Et	Me	Hexadecyl	35	49	130
16	Me	Bu	Decyl	71	77	8

Standards: For *P. falciparum*, chloroquine, $IC_{50}=0.126$ μM; artemisinin, $IC_{50}=0.005$ μM ; For *T. brucei*, melarsoprol, $IC_{50}=0.005$ μM ; For *T. cruzi*, benznidazole, $IC_{50}=1.68$ μM. The IC_{50} values are the means of four values of two independent assays done in duplicate. They were determined by linear interpolation between the two adjacent drug concentrations above and below the 50% incorporation line.

emphasised by considering the intermediate compounds **6a–d** and **11a**, all of which showed a marked decrease in inhibition (195 to >347) in comparison to **1**.

Recently, Price et al. have solved the crystal structure of TLM bound to *Escherichia coli* KAS I.[24,25] From this study a number of key interactions have been identified involving the thiolactone ring with the protein's active site. Specifically, the carbonyl oxygen at the C2 position binds with two histidines (His 298 and His 333). The isoprenoid moiety appears to occupy a hydrophobic crevice and is sandwiched between two pairs of amino acids (Gly 391 and Phe 392; Ala 271 and Pro 272). Interestingly, alignment of *P. falciparum* KAS1/2 with the *E. coli* KAS I indicates that these four amino acids are retained in the *P. falciparum* sequence (Gly 403 and Phe 404; Ala 274 and Pro 275).[25] Price et al.,[25] further observed that this crevice was not optimally filled by the isoprenoid side chain. Our findings lend support to this observation, and would suggest that alkyl chains of ten carbons and above better occupy this cleft, therefore increasing inhibition.

Price et al.[25] made a detailed study of the crystal structure of *E. coli* KAS I, and found that the two ring methyl substituents, at the C3 and C5 positions, are accommodated in hydrophobic pockets (the C3 methyl in a pocket bounded by Phe 229, Phe 392 and Thr 300; the C5 pocket by Pro 272 and Gly 305). The analogues **7f–i**, all possessing an ethyl substituent at the C3 position of the ring, all showed greater inhibition than TLM against *P. falciparum*. Once again, the longer chained analogues **7h** and **7i** gave the best inhibition, showing 10 and 8-fold increases in inhibition over TLM respectively. A further trend was also observed, analogues **7f–i** all displayed enhanced inhibition towards the parasite in comparison to analogues **7a–e** bearing a methyl substituent at C3 of the ring. This trend is highlighted when comparing the activities of analogues **7b** (153 μM) and **7g** (54 μM). Compound **7g** shows a 3-fold improvement in inhibition over **7b**. However with the more active compounds, there is a much smaller improvement in activity on addition of an ethyl group at the C3 position [compare **7h** (15 μM) with **7d** (36 μM); and **7i** (19 μM) with **7e** (25 μM)]. Clearly, the ethyl substituent is having a positive influence on the overall activity of the inhibitors. This led us to probe the C3 position further. Indeed, by extending the ethyl chain to a propyl group, and placing a decyl side chain at the C5 position (analogue **7j**) an IC_{50} of 10 μM was achieved, thus giving a 14-fold increase in activity over TLM. Increasing the propyl tether to a butyl derivative **16** resulted in a decrease in activity. A similar result was also obtained with the benzyl analogue **7k**, which showed an IC_{50} of 50 μM. Seemingly, it is plausible that we have reached an optimal chain length of three carbons at the C3 position, beyond which activity is diminished. To lend support to this assertion we were able to dock analogue **7j** into the *E. coli* KAS I using FlexX software (Figs 2 and 3). The propyl group appears to fit snugly into the hydrophobic pocket with the terminus of the propyl chain in close proximity to the Cys 163. This same amino acid is also present in the *P. falciparum* KAS1/2.[25]

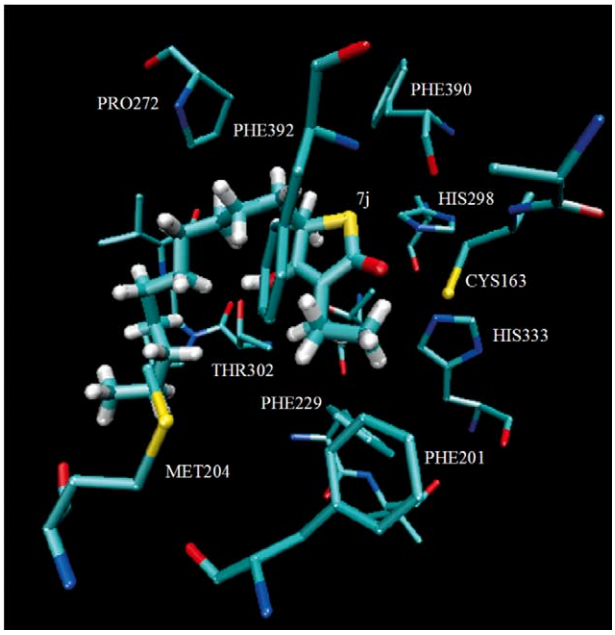


Figure 2. Compound **7j** docked into the *E. coli* KAS I. Part of the 3-propyl group is obscured.

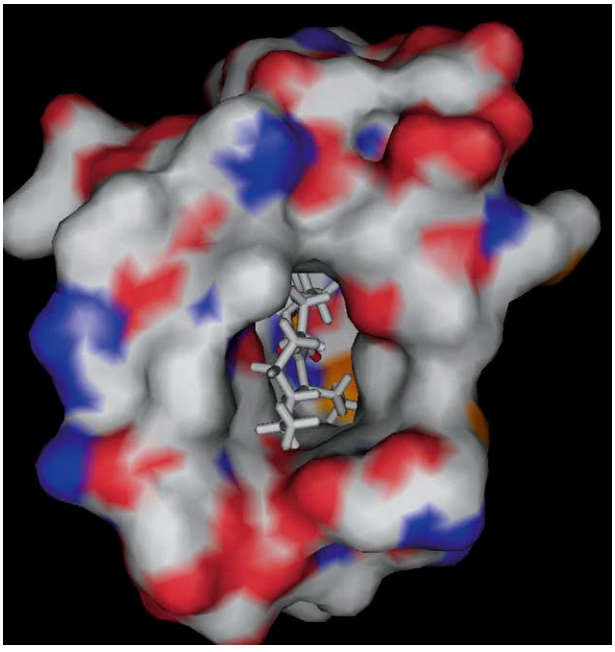


Figure 3. Inhibitor **7j** in relation to the surface of *E. coli* KAS I.

In an attempt to improve the hydrophobic interactions of the methyl substituent at the C5 position, an ethyl substituent was introduced in place of the methyl group. Both analogues **12a** (65 μM) and **12b** (35 μM) showed improved inhibition over TLM (Table 1). However, a direct comparison of these analogues with there methyl counterparts [compare **12a** (65 μM) with **7d** (36 μM); and **12b** (35 μM) with **7e** (25 μM)] suggests that the presence of the ethyl substituent at C5 serves only to reduce the activity of the inhibitors. Likewise, removal of the C5 methyl substituent (analogue **11b**) also appears to reduce the inhibitory effects of the analogue. This is highlighted with a direct comparison of the activities for analogues **11b** (72 μM) and **7h** (15 μM). Clearly, modification of the methyl substituent at the C5 position does not appear as influential to the overall activity of the analogues as modification at the C3 position.

All our compounds were also assayed in vitro against the *T. brucei* parasite, which is the cause of human sleeping sickness and livestock disease in Africa. Recently, Englund[26] identified thiolactomycin as a promising lead for antitrypanosomal drug development. *T. brucei* requires large amounts of myristate for synthesis of glycosyl phosphatidylinositol anchored variable surface glycoprotein. Thiolactomycin was shown to effectively inhibit trypanosomal myristate synthesis in vitro with a reported IC_{50} of ~150 μM.[26] A number of our compounds showed inhibition greater than thiolactomycin **1** (IC_{50} 256 μM in our assays), with the best two [analogues **11b** (6 μM) and **16** (8 μM)] showing a 42-fold improvement in activity (Table 1). The most active analogue (**7j**) against the malaria parasite also showed improved inhibition against *T. brucei*, registering an IC_{50} of 21 μM.

In vitro assays against *T. cruzi*, the causative organism of Chagas disease, showed that thiolactomycin **1** was inactive with an IC_{50} of >427 μM (Table 1). Several of the analogues tended to show enhanced activity towards the *T. cruzi* parasite over thiolactomycin. Analogue **7e** showed the greatest inhibition with an IC_{50} of 28 μM.

Finally, none of the compounds showed any appreciable activity against the *Leishmania donovani* parasite. The lack of activity against *Leishmania* may be due to the parasite being an intracellular parasite dwelling within a parasitophorous vacuole within the host cell, significantly reducing accessibility of compounds.

4. Conclusion

We have reported the design and synthesis of a number of thiolactomycin analogues as agents against *P. falciparum*, a causative organism of malaria. The compounds were designed with the intention of targeting the Type II FAS utilised for fatty acid biosynthesis. Several analogues showed inhibitory effects greater than thiolactomycin **1**, with the best compound (**7j**) showing a 14-fold increase in activity (10 μM). Furthermore, all compounds were assayed against three other parasitic protozoa, *T. cruzi*, *T. brucei* and *L. donovani*. Varied activities were found against the *T. brucei* and *T. cruzi* parasites. However, the analogues showed no appreciable activity against *L. donovani*. Although the inhibitory effects of these analogues are only in the micromolar range, these show significantly better activity than thiolactomycin. This study has demonstrated the scope that exists for the design of new fatty acid biosynthesis inhibitors with enhanced activity against the parasites which cause malaria, African trypanosomiasis and Chagas disease. Significantly improved inhibitors could offer a valuable new way of controlling these important diseases.

5. Experimental

Melting points were determined using a Gallenkamp melting point apparatus and are uncorrected. Infrared spectra were recorded as thin films for liquid samples, or as Nujol mulls for solid samples, on a Perkin–Elmer 1600 series FTIR spectrophotometer using sodium chloride plates. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advance DPX300 spectrometer operating at 300 and 75 M Hz, respectively, with tetramethylsilane as internal standard, using deuterated chloroform purchased from Goss unless stated otherwise. Low-resolution mass spectra, that is, electrospray [ES], were recorded using a fisons VG Platform II spectrometer. High-resolution spectra were obtained on a VG ZAB spectrometer from the EPSRC Mass Spectrometry Service at Swansea University, UK. Microanalyses were obtained from the analytical and chemical consultancy services MEDAC LTD. All reactions were performed in predried apparatus under an atmosphere of nitrogen unless otherwise stated. Solvents and reagents were purchased from chemical companies and used without further purification. Dry solvents were generally purchased in sure sealed bottles stored over molecular sieves. Thin-layer chromatography (tlc) was performed on Merck silica gel $60F_{254}$ plates. Column chromatography was carried out using Fisons matrix silica 60 (35–70 micron).

5.1. Cyclisation, general procedure A

Potassium hydroxide (2.0 equiv) in water (10 mL) was added dropwise to a stirred solution of the thioacetate (1.0 equiv) in ethanol (20 mL) at ambient temperature. The resulting solution was warmed to 40 °C and stirred for 3 h. The ethanol was then evaporated and water (~10 mL) added. The aqueous layer was washed with diethyl ether (2×10 mL) and acidified to ph 1 with the addition of 2 M HCl (~10 mL). The aqueous layer was extracted with diethyl ether. The organic layers were washed with brine, dried over anhydrous magnesium sulfate and the solvent removed in vacuo. The crude residue was purified by flash column chromatography (5–20% ethyl acetate in hexanes). The coupling constants (*J*) are in Hz.

5.2. 4-Hydroxy-3,5-dimethyl-2(5H)-thiophenone (6a)

As described in procedure A, starting from **5a** (1.0 g, 4.58 mmol) and potassium hydroxide (0.5 g, 9.16 mmol), **6a** was obtained as a white solid (0.60 g, 90%); mp 131–132 °C, ν_{MAX}/cm^{-1} 3197, 2923, 2878, 1555, 1291 and 1186; δ_H (acetone-d_6) 1.66 (3H, d, $J=7.0$, 5-CH_3), 1.74 (3H, s, 3-CH_3), 4.32 (1H, q, $J=7.0$, 5-H) and 10.22 (1H, br s, OH); δ_C (acetone-d_6) 12.6 (3-CH_3), 24.5 (5-CH_3), 47.9 (4-CH), 115.5 (3-C), 183.6 (4-C) and 199.3 (CO); MS (ES^-) *m/z* 142.6 ($M-H,^-$ 100%); HRMS (ES^+) $(M+H)^+$ $C_6H_9O_2S$ requires 145.0323, found 145.0321. Anal. calcd for $C_6H_8O_2S.0.1H_2O$: C, 49.4; H, 5.7. Found: C, 49.3; H, 5.4%.

5.3. 3-Ethyl-4-hydroxy-5-methyl-2(5H)-thiophenone (6b)

As described in procedure A, starting from **5b** (3.52 g, 7.58 mmol) and potassium hydroxide (1.70 g, 15.17 mmol), **6b** was obtained as a white solid (1.07 g, 89%); mp 75–76 °C, ν_{MAX}/cm^{-1} 3188, 2923, 2869, 1563, 1291 and 1184; δ_H (acetone-d_6) 1.94 (3H, t, $J=7.2$, 2′-CH_3), 1.53 (3H, d, $J=7.0$, CH_3), 2.18 (2H, q, $J=7.2$, 1′-CH_2), 4.21 (1H, q, $J=7.0$, 5-CH) and 10.11 (1H, br s, OH); δ_C (acetone-d_6) 13.4 (2′-CH_3), 17.1 (1′-CH_2), 20.2 (5-CH_3), 43.4 (5-C), 117.1 (3-C), 179.2 (4-C) and 194.8 (CO); MS (ES^-) *m/z* 156.7 ($M-H,^-$ 100%); HRMS (ES^-) $(M-H)^-$ $C_7H_9O_2S$ requires 157.0324, found 157.0323. Anal. calcd for $C_7H_{10}O_2S.0.1H_2O$: C, 52.5; H, 6.4. Found: C, 52.6; H, 6.4%.

5.4. 4-Hydroxy-5-methyl-3-propyl-2,5-dihydro-2-thiophene (6c)

As described in procedure A, starting from **5c** (3.50 g, 14.22 mmol) and potassium hydroxide (1.59 g, 28.44 mmol), **6c** was obtained as a colourless solid (1.04 g, 42%); mp 49–50 °C, δ_H (acetone-d_6) 0.91 (3H, t, $J=7.4$, 3′-CH_3), 1.41–1.54 (2H, m, 2′-CH_2), 1.65 (3H, d, $J=7.0$, CH_3), 2.22 (2H, t, $J=7.5$, 1′-CH_2) and 4.29 (1H, q, $J=7.0$, 5-CH); δ_C (acetone-d_6) 18.5 (3′-CH_3), 24.5 (CH_3), 26.6 (2′-CH_2), 29.8 (1′-CH_2), 47.6 (5-CH), 119.8 (3-C), 183.9 (4-C) and 199.1 (2-CO); MS ($ES^{''}$) *m/z* 170.9 ($M-H,^-$ 100%); HRMS (ES^+) $(M+NH_4)^+$ $C_8H_{12}O_2S$ requires 190.0896, found 190.0895. Anal. calcd for $C_8H_{12}O_2S$: C, 55.8; H, 7.0. Found: C, 55.4; H, 7.1%.

5.5. 3-Benzyl-4-hydroxy-5-methyl-2,5-dihydro-2-thiophene (6d)

As described in procedure A, starting from **5d** (6.00 g, 20.40 mmol) and potassium hydroxide (2.69 g, 40.80 mmol), **6d** was obtained as a white powder (1.32 g, 29%); mp 139–141 °C, δ_H (acetone-d_6) 1.59 (3H, d, $J=6.9$, CH_3), 3.50 (2H, s, CH_2Ph), 4.28 (1H, q, $J=6.9$, 5-CH), 7.08–7.21 (5H, m, ArH) and 10.33 (1H, br s, OH); δ_C (acetone-d_6) 20.2 (CH_3), 29.3 (CH_2Ph), 43.6 (5-CH), 115.0 (3-C), 127.1 (ArCH), 129.4 (ArCH), 129.5 (ArCH), 140.9 (ArC), 180.2 (4-C) and 194.6 (CO); MS (ES^-) *m/z* 219.5 ($M-H,^-$ 100%); HRMS (ES^-) $(M-H)^-$ $C_{12}H_{11}O_2S$ requires 219.0480, found 219.0484. Anal. calcd for $C_{12}H_{12}O_2S.0.1H_2O$: C, 64.9; H, 5.5. Found: C, 64.7; H, 5.5%.

5.6. 5-Ethyl-4-hydroxy-3-methyl-2,5-dihydro-2-thiophene (11a)

As described in procedure A, starting from **10a** (5.36 g, 23.10 mmol) and potassium hydroxide (2.59 g, 46.20 mmol), **11a** was obtained as a pale yellow solid (1.15 g, 32%); mp 95–96 °C, δ_H (acetone-d_6) 0.89 (3H, t, $J=7.4$, 2″-CH_3), 1.58 (3H, s, 1′-CH_3), 1.61–1.68 (1H, m, 1″-CH_AH_B), 2.07–2.17 (1H, m, 1″-CH_AH_B) and 4.14 (1H, dd, $J=6.2$ and 1.1, 5-CH); δ_C (acetone-d_6) 8.1 (7-CH_3), 11.4 (3-CH_3), 27.0 (6-CH_2), 50.8 (5-CH), 112.0 (3-C), 178.0 (4-C) and 195.2 (2-CO); MS (ES^-) *m/z* 156.8 ($M-H,^-$ 100%); HRMS (ES^+) $(M+NH_4)^+$ $C_7H_{14}NO_2S$ requires 176.0740; found 176.0740. Anal. calcd for $C_7H_{10}O_2S$: C, 53.1; H, 6.4. Found: C, 52.7; H, 6.4%.

5.7. 5-Decyl-3-ethyl-4-hydroxy-2(5H)-thiophenone (11b)

As described in procedure A, starting from **10b** (870 mg, 2.43 mmol) and potassium hydroxide (270 mg, 4.86 mmol), **11b** was obtained as a pale yellow solid (74 mg, 11%), mp 35–36 °C, δ_H 0.88 (3H, t, $J=6.8$, 15-CH_3), 1.04 (3H, t, $J=7.4$, 2′-CH_3), 1.26 (14H, m, 7×CH_2), 1.67–1.73 (2H, m, CH_2), 2.24–2.31 (4H, m, 6-CH_2 and 1′-CH_2) and 4.18 (1H, dd, $J=9.3$ and 2.6, 5-CH); δ_C 13.1 (15-CH_3), 14.5 (2′-CH_3), 16.5, 23.1, 27.9, 29.6, 29.7, 29.8, 30.0, 32.3, 33.3 (all CH_2), 50.1 (5-CH), 118.0 (3-C), 179.2 (4-C) and 199.9 (2-CO); MS (ES^-) m/z 283.1 ($M-H,^-$ 100%); HRMS $(ES)^+$ $(M+H)^+$ $C_{16}H_{29}O_2S$ requires 285.1883, found 285.1892. Anal. calcd for $C_{16}H_{28}O_2S.0.2H_2O$: C, 66.7; H, 9.9. Found: C, 66.8; H, 10.1%.

5.8. 3-Butyl-5-decyl-4-hydroxy-5-methyl-2(5*H*)-thiophenone (16)

As described in procedure A, starting from **15** (2.84 g, 7.39 mmol) and potassium hydroxide (0.82 g, 14.79 mmol), **16** was obtained as a pale yellow oil (0.47 g, 20%), δ_H 0.89–0.93 (6H, m, 15-CH_3 and 4′-CH_3), 1.23–1.37 (20H, m, 10×CH_2), 1.72 (3H, s, CH_3), 1.93–1.96 (2H, m, 6-CH_2) and 2.29 (2H, t, $J=7.2$, 1′-CH_2); δ_C 14.1, 14.3 (both CH_3), 22.8, 22.9, 23.1, 24.4, 25.4, 25.5, 26.4, 26.6, 29.7, 29.8, 29.9, 30.0, 32.0, 38.7 (all CH_2), 58.4 (5-C), 115.6 (3-C), 181.7 (4-C) and 198.6 (2-CO); MS (ES^-) m/z 325.4 ($M-H,^-$ 100%); HRMS (ES^-) $(M-H)^-$ $C_{19}H_{33}O_2S$ requires 325.2196, found 325.2199.

5.9. Alkylation, general procedure B

Thiophenone (1.0 equiv) was added portionwise to a stirred suspension of sodium hydride (1.2 equiv) in dry THF (4 mL) and DMPU (4 mL) at ambient temperature. The suspension was stirred for 0.5 h, then cooled to −78 °C and butyllithium (2.5 M solution in hexanes, 1.1 equiv) added dropwise. After stirring for 10 min, the mixture was allowed to warm to ambient temperature and stirred for 1 h. Once again the mixture was cooled to −78° and the halide (1.0 equiv) added dropwise. After stirring for 15 min, the mixture was allowed to warm to ambient temperature and stirred overnight. The reaction was quenched by the addition of 2 M HCl (10 mL) and the organic layer separated. The aqueous layer was then extracted with ethyl acetate (3×10 mL). The organic layers were washed with brine, dried over anhydrous magnesium sulfate and the solvent removed in vacuo. The crude residue was purified by flash column chromatography (0–20% ethyl acetate in hexanes).

5.10. Thiolactomycin 1

Butyllithium (1.6 mL of a 2.5 M solution in hexanes, 4.01 mmol) was added dropwise to a stirred suspension of the phosphonium salt (710 mg, 2.00 mmol) in dry THF (8 mL) at −78 °C. The resulting dark red solution was allowed to warm to ambient temperature and stirred for 1 h. The solution was then cooled to −78 °C and the aldehyde (430 mg, 2.00 mmol) added dropwise. A light brown suspension formed, which was then allowed to warm to ambient temperature and stirred overnight. 2 M HCl (10 mL) was added and the organic layer separated. The aqueous layer was extracted with ethyl acetate (3×10 mL). The combined organic solutions were washed with water (2×20 mL) and brine (2×20 mL), then dried and evaporated. Purification by column chromatography (20% ethyl acetate in hexanes) provided thiolactomycin **1** as a pale yellow oil (12 mg, 29% yield), ν_{MAX}/cm^{-1} 3600, 1700, 1630, 1450, 1380, 1325, 1280 and 1100; ^1H NMR δ 1.79 (3H, s, CH_3), 1.83 (3H, s, CH_3), 1.91 (3H, s, CH_3), 5.15 (1H, d, $J=11.1$, 4′-H_a), 5.33 (1H, d, $J=17.2$, 4′-H_b), 5.62 (1H, s, 1′-H) and 6.37 (1H, dd, $J=17.2$ and 11.1, 3′-H); δ_C 7.6 (3-CH_3), 12.1 (CH_3), 29.9 (5-CH_3), 58.6 (5-C), 110.8 (3-C), 114.0 (CH_2), 129.8, 140.6, 141.1, 181.3 (4-C) and 198.6 (CO). Spectroscopic data was identical to those recorded in the literature.[21]

5.11. 5-Hexyl-4-hydroxy-3,5-dimethyl-2(5H)-thiophenone (7a)

As described in procedure B, starting from **6a** (500 mg, 3.46 mmol), sodium hydride (160 mg, 4.16 mmol), butyllithium (1.52 mL, 3.80 mmol) and bromohexane (0.48 mL, 3.46 mmol), **7a** was obtained as a colourless solid (80 mg, 10%); mp 62–64 °C, ν_{MAX}/cm^{-1} 3145, 2929, 1600, 1454, 1247 and 1010; δ_H 0.87 (3H, t, $J=6.5$, 6′-CH_3), 1.26–1.34 (8H, m, 4×CH_2), 1.66 (3H, s, CH_3), 1.74 (3H, s, CH_3) and 1.85 (2H, t, $J=8.0$, 1′-CH_2); δ_C 7.92 (6′-CH_3), 14.4 (3-CH_3), 22.9 (CH_2), 25.5 (5-CH_3), 26.4, 29.6, 32.0, 38.9 (all CH_2), 58.0 (5-C), 110.9 (3-C), 183.6 (4-C) and 198.4 (CO); MS (ES^-) m/z 227.2 ($M-H,^-$ 100%); HRMS (ES^-) $(M-H)^-$ $C_{12}H_{19}O_2S$ requires 227.1106, found 227.1106. Anal. calcd for $C_{12}H_{20}O_2S.0.2H_2O$: C, 62.1; H, 8.9. Found: C, 61.9; H, 8.9%.

5.12. 4-Hydroxy-3,5-dimethyl-5-octyl-2(5H)-thiophenone (7b)

As described in procedure B, starting from **6a** (500 mg, 3.46 mmol), sodium hydride (160 mg, 4.16 mmol), butyllithium (1.52 mL, 3.80 mmol) and 1-iodooctane (1.05 mL, 3.46 mmol), **7b** was obtained as a pale yellow solid (108 mg, 12%); mp 81–83 °C, ν_{MAX}/cm^{-1} 1611 and 1076; δ_H 0.97 (3H, t, $J=6.9$, 8′-CH_3), 1.31 (10H, m, 5×CH_2), 1.75 (3H, s, CH_3), 1.83 (3H, s, CH_3) and 1.89–1.94 (2H, m, 1′-CH_2); δ_C 7.9 (8′-CH_3), 14.5 (3-CH_3), 23.0 (CH_2), 25.6 (5-CH_3), 26.3, 29.6, 29.8, 29.9, 32.2, 38.8 (all CH_2), 58.5 (5-C), 110.8 (3-C), 181.2 (4-C) and 198.3 (CO); MS (ES^-) m/z 255.2 ($M-H,^-$ 100%); HRMS (ES^+) $(M+H)^+$ $C_{14}H_{25}O_2S$ requires 257.1570, found 257.1568. Anal. calcd for $C_{14}H_{24}O_2S.0.1H_2O$: C, 65.1; H, 9.4. Found: C, 65.1; H, 9.6%. Spectroscopic data was identical to those recorded in the literature.[17]

5.13. 5-[(2*E*)-3,7-Dimethyl-2,6-octadienyl]-4-hydroxy-3,5-dimethyl-2(5H)-thiophenone (7c)

As described in procedure B, starting from **6a** (500 mg, 3.46 mmol), sodium hydride (160 mg, 4.16 mmol), butyllithium (1.52 mL, 3.80 mmol) and geranyl bromide (0.69 g, 3.46 mmol), **7c** was obtained as a thick yellow

oil (160 mg, 16%); ν_{MAX}/cm^{-1} 2972, 2927, 1600, 1450, 1383, 1280 and 969; δ_H 1.61 (3H, s, CH_3), 1.64 (3H, s, CH_3), 1.66 (3H, s, CH_3), 1.69 (3H, s, CH_3), 1.75 (3H, s, CH_3), 2.04–2.12 (4H, m, 2×CH_2), 2.52–2.69 (2H, m, CH_2), 5.08 (1H, m, CH) and 5.18 (1H, m, CH); δ_C 7.9 (CH_3), 16.9 (3-CH_3), 18.1 (CH_3), 25.2 (5-CH_3), 26.1 (CH_3), 26.8 (CH_2), 37.7, 40.2 (both CH_2), 58.2 (5-C), 110.7 (3-C), 119.2, 124.3 (both CH), 132.2, 140.5 (both C), 180.2 (4-C) and 203.2 (CO); MS (ES^-) *m/z* 279.1 (M−H,$^-$ 100%); HRMS (ES^-) $(M-H)^-$ $C_{16}H_{23}O_2S$ requires 279.1419, found 279.1416. Spectroscopic data was identical to those recorded in the literature.[17]

5.14. 5-Decyl-4-hydroxy-3,5-dimethyl-2(5H)-thiophenone (7d)

As described in procedure B, starting from **6a** (500 mg, 3.46 mmol), sodium hydride (160 mg, 4.16 mmol), butyllithium (1.52 mL, 3.80 mmol) and 1-iodododecane (0.73 mL, 3.46 mmol), **7d** was obtained as a white solid (110 mg, 10%); mp 74–75 °C, ν_{MAX}/cm^{-1} 3130, 2947, 1592, 1486, 1270, 1170 and 989; δ_H 0.82 (3H, t, $J=6.6$, 10′-CH_3), 1.20 (16H, m, 8×CH_2), 1.62 (3H, s, CH_3), 1.71 (3H, s, CH_3) and 1.80–1.85 (2H, m, 1′-CH_2); δ_C 7.9 (10′-CH_3), 14.5 (3-CH_3), 23.1 (CH_2), 25.6 (5-CH_3), 26.3, 29.7, 29.8, 29.9, 30.0, 32.3, 38.8 (all CH_2), 58.5 (5-C), 110.8 (3-C), 181.1 (4-C) and 198.3 (2-CO); MS (ES^-) *m/z* 283.0 (M−H,$^-$ 100%); HRMS (ES^+) $(M+NH_4)^+$ $C_{16}H_{32}NO_2S$ requires 302.2154, found 302.2149. Anal. calcd for $C_{16}H_{28}O_2S.0.2H_2O$: C, 66.7; H, 9.9. Found: C, 66.7; H, 9.9%.

5.15. 5-Hexadecyl-4-hydroxy-3,5-dimethyl-2(5H)-thiophenone (7e)

As described in procedure B, starting from **6a** (500 mg, 3.46 mmol), sodium hydride (160 mg, 4.16 mmol), butyllithium (1.52 mL, 3.80 mmol) and 1-bromohexadecane (1.05 mL, 3.46 mmol), **7e** was obtained as a white solid (87 mg, 7%), mp 56–57 °C, ν_{MAX}/cm^{-1} 2917, 2848, 1573, 1284, 1226 and 1145; δ_H 0.98 (3H, t, $J=6.9$, 16′-CH_3), 1.36 (28H, m, 14×CH_2), 1.77 (3H, s, CH_3), 1.85 (3H, s, CH_3) and 1.95 (2H, t, $J=7.9$, 1′-CH_2); δ_C 7.9 (16′-CH_3), 14.5 (3-CH_3), 23.1 (CH_2), 25.6 (5-CH_3), 26.3, 29.7, 29.8, 30.0, 30.1, 32.3, 38.9 (all CH_2), 58.2 (5-C), 110.9 (3-C), 180.1 (4-C) and 190.3 (2-CO); MS (ES^-) *m/z* 367.2 (M−H,$^-$ 100%); HRMS (ES^-) $(M-H)^-$ $C_{22}H_{39}O_2S$ requires 367.2671, found: 367.2666. Anal. calcd for $C_{22}H_{40}O_2S$: C, 71.7; H, 10.9. Found: C, 71.7; H, 11.2%.

5.16. 3-Ethyl-5-hexyl-4-hydroxy-5-methyl-2(5H)-thiophenone (7f)

As described in procedure B, starting from **6b** (400 mg, 2.53 mmol), sodium hydride (120 mg, 3.03 mmol), butyllithium (1.77 mL of a 1.57 M solution in hexanes, 2.78 mmol) and 1-iodohexaane (0.37 mL, 2.53 mmol), **7f** was obtained as a colourless oil (100 mg, 16%), δ_H 0.82 (3H, t, $J=6.8$, CH_3), 0.98 (3H, t, $J=7.5$, 2′-CH_3), 1.21 (8H, m, 4×CH_2), 1.62 (3H, s, CH_3), 1.80–1.83 (2H, m, CH_2) and 2.19 (2H, q, $J=7.5$, 1′-CH_2); δ_C 13.3 (CH_3), 14.4 (CH_3), 16.5 (CH_3), 23.0, 25.4, 26.3, 29.5, 31.9 and 38.8 (all CH_2), 58.1 (5-C), 116.9 (3-C), 180.4 (4-C) and 197.5 (2-CO); MS (ES^-) *m/z* 240.9 (M−H,$^-$ 100%). HRMS (ES^-) $(M-H)^-$ $C_{13}H_{21}O_2S$ requires 241.1262, found 241.1259. Anal. calcd for $C_{13}H_{22}O_2S.0.2H_2O$: C, 63.5; H, 9.2. Found: C, 63.6; H, 9.3%.

5.17. 3-Ethyl-4-hydroxy-5-methyl-5-octyl-2(5H)-thiophenone (7g)

As described in procedure B, starting from **6b** (300 mg, 1.89 mmol), sodium hydride (91 mg, 2.27 mmol), butyllithium (1.31 mL of a 1.57 M solution in hexanes, 2.07 mmol) and 1-iodooctane (0.31 mL, 1.89 mmol), **7g** was obtained as a pale yellow oil (40 mg, 18%); δ_H 0.92 (3H, t, $J=6.7$, 8″-CH_3), 1.09 (3H, t, $J=7.5$, 2″-CH_3), 1.30 (12H, m, 6×CH_2), 1.72 (3H, s, CH_3), 1.83–1.86 (2H, m, 1′-CH_2), 2.28 (2H, q, $J=7.5$, 1′-CH_2) and 7.68 (1H, br s, OH); δ_C 13.3, 14.5 and 16.4 (all (CH_3), 23.0, 25.4, 26.4, 29.5, 29.6, 29.9, 32.2 and 38.8 (all CH_2), 57.8 (5-C), 116.9 (3-C), 179.9 (4-C) and 196.7 (5-C); MS (ES^-) *m/z* 269.1 (M−H,$^-$ 100%); HRMS (ES^-) $(M-H)^-$ $C_{15}H_{25}O_2S$ requires 269.1576, found 269.1578.

5.18. 5-Decyl-3-ethyl-4-hydroxy-5-methyl-2(5H)-thiophene (7h)

As described in procedure B, starting from **6b** (300 mg, 1.89 mmol), sodium hydride (91 mg, 2.27 mmol), butyllithium (1.31 mL of a 1.57 M solution in hexanes, 2.07 mmol) and 1-iododecane (0.40 mL, 1.89 mmol), **7h** was obtained as a colourless solid (125 mg, 22%), mp 36–38 °C, δ_H 0.93 (3H, t, $J=6.8$, 10″-CH_3), 1.10 (3H, t, $J=7.5$, 2′-CH_3), 1.30 (14H, m. 7×CH_2), 1.71 (3H, s, OCH_3), 1.89 (2H, m, CH_2), 2.00 (2H, m, CH_2), 2.25 (2H, q, $J=7.5$, 1′-CH_2) and 6.85 (1H, br s, OH); δ_C 13.3, 14.5 and 16.5 (all CH_3), 23.1, 25.5, 26.4, 29.6, 29.7, 29.8, 29.9, 30.0, 32.3 and 38.8 (all CH_2), 57.9 (5-C), 117.0 (3-C), 179.7 (4-C) and 198.2 (2-C); MS (ES^-) *m/z* 297.3 (M−H,$^-$ 100%); HRMS (ES^-) $(M-H)^-$ $C_{17}H_{29}O_2S$ requires 297.1889, found 297.1888. Anal. calcd for $C_{17}H_{30}O_2S$: C, 68.4; H, 10.1. Found: C, 68.1; H, 10.3%.

5.19. 3-Ethyl-5-hexadecyl-4-hydroxy-5-methyl-2(5H)-thiophenone (7i)

As described in procedure B, starting from **6b** (300 mg, 1.89 mmol), sodium hydride (91 mg, 2.27 mmol), butyllithium (1.31 mL of a 1.57 M solution in hexanes, 2.07 mmol) and 1-bromohexadecane (0.31 mL, 1.89 mmol), **7i** was obtained as a colourless solid (160 mg, 22%), mp 86–87 °C, ν_{MAX}/cm^{-1} 3183, 2916, 2848, 1580, 1471, 1330, 1251 and 1145; δ_H 1.00 (3H, t, $J=6.6$, 16″-CH_3), 1.16 (3H, t, $J=7.5$, 2′-CH_3), 1.37 (28H, m, 14×CH_2), 1.80 (3H, s, 5-CH_3), 1.97–2.01 (2H, m, 1″-CH_2), 2.36 (1H, q, $J=7.5$, 1′-CH_2) and 8.04 (1H, br s, OH); δ_C 13.3, 14.5, 16.5, 20.7, 23.1, 25.5, 26.4, 29.8, 29.8, 29.9, 29.9, 30.0, 30.1, 32.3, 38.9, 57.9 (5-C), 117.1 (3-C), 179.5 (4-C) and 196.9 (2-CO); MS (ES^-) *m/z* 381.2 (M−H,$^-$ 100%); HRMS (ES^-) $(M-H)^-$ $C_{23}H_{41}O_2S$ requires 381.2828, found 381.2835. Anal. calcd for $C_{23}H_{42}O_2S$: C, 72.2; H, 11.1. Found: C, 72.3; H, 11.2%.

5.20. 5-Decyl-4-hydroxy-5-methyl-3-propyl-2,5-dihydro-2-thiophene (7j)

As described in procedure B, starting from **6c** (250 mg, 1.45 mmol), sodium hydride (69 mg, 1.74 mmol), butyllithium (0.63 mL of a 2.5 M solution in hexanes, 1.59 mmol) and 1-iododecane (0.31 mL, 0.38 g, 1.45 mmol), **7j** was obtained as a yellow oil (57 mg, 12%); δ_H 0.93 (6H, m, 2×CH_3), 1.29 (14H, m, 7×CH_2), 1.44–1.60 (2H, m, CH_2), 1.70 (3H, s, CH_3), 1.92–1.94 (4H, m, 2×CH_2) and 2.25 (2H, t, $J=7.3$, 1′-CH_2); δ_C 14.1, 14.5 (both CH_3), 21.8 (CH_2), 23.0, 25.5, 26.0 (all CH_2), 26.6 (CH_3), 29.7, 29.8, 29.9, 30.0, 30.5, 34.5 and 38.8 (all CH_2), 68.4 (5-C), 115.3 (3-C), 180.8 (4-C) and 196.8 (2-CO); MS (ES^-) *m*/*z* 311.2 ($M-H,^-$ 100%); HRMS (ES^-) $(M-H)^-$ $C_{18}H_{32}O_2S$ requires 313.2196, found 313.2195. Anal. calcd for $C_{18}H_{32}O_2S$: C, 69.2; H, 10.3. Found: C, 69.3; H, 10.7%.

5.21. 3-Benzyl-5-decyl-4-hydroxy-5-methyl-2,5-dihydro-2-thiophene (7k)

As described in procedure B, starting from **6d** (250 mg, 1.13 mmol), sodium hydride (54 mg, 1.36 mmol), butyllithium (0.49 mL of a 2.5 M solution in hexanes, 1.24 mmol) and 1-iododecane (0.24 mL, 0.30 g, 1.13 mmol), **7k** was obtained as a yellow oil (16 mg, 4%); δ_H 0.85 (3H, t, $J=6.8$, 10′-CH_3), 1.20–1.21 (16H, m, 8×CH_2), 1.58 (3H, s, CH_3), 1.79 (2H, t, $J=7.8$, 1′-CH_2), 3.52 (2H, s, CH_2Ph) and 7.10–7.21 (5H, m, ArH); δ_C 14.5 (CH_3), 23.1 (CH_2), 25.5 (CH_2), 26.4 (CH_3), 29.3, 29.7, 29.8, 29.9, 30.0, 32.3 and 38.9 (all CH_2), 57.9 (5-C), 114.0 (3-C), 127.3, 128.9 and 129.4 (all ArCH), 137.9 (ArC), 180.5 (4-C) and 196.1 (2-CO); MS (ES^-) *m*/*z* 359.3 ($M-H,^-$ 100%); HRMS (ES^-) $(M-H)^-$ $C_{22}H_{31}O_2S$ requires 359.2045, found 359.2048.

5.22. 5-Decyl-5-ethyl-4-hydroxy-3-methyl-2,5-dihydro-2-thiophene (12a)

As described in procedure B, starting from **11a** (250 mg, 1.58 mmol), sodium hydride (75 mg, 1.51 mmol), butyllithium (0.69 mL of a 2.5 M solution in hexanes, 1.89 mmol) and 1-iododecane (0.33 mL, 0.38 g, 1.58 mmol), **12a** was obtained as a yellow oil (34 mg, 14%); δ_H 0.90–0.98 (6H, m, 2×CH_3), 1.29 (14H, m, 7×CH_2), 1.78 (3H, s, CH_3) and 1.89–2.00 (4H, m, 2×CH_2); δ_C 6.3 (CH_3), 7.9 (CH_3), 13.1 (CH_3), 21.6, 23.6, 28.2, 28.3, 28.4, 28.5, 28.6, 29.7, 30.8, 36.6 (all CH_2), 61.9 (5-C), 110.9 (3-C), 177.1 (4-C) and 191.2 (2-CO).

5.22.1. 5-Ethyl-hexadecyl-4-hydroxy-3-methyl-2,5-dihydro-2-thiophene (12b)

As described in procedure B, starting from **11a** (200 mg, 1.26 mmol), sodium hydride (60 mg, 1.51 mmol), butyllithium (0.70 mL of a 2.0 M solution in hexanes, 1.38 mmol) and 1-bromohexadecane (0.38 mL, 0.38 g, 1.26 mmol), **12b** was obtained as a yellow oil (57 mg, 12%); δ_H 0.98–1.06 (6H, m, 2×CH_3), 1.37 (26H, m, 13×CH_2), 1.85 (3H, s, CH_3), 1,98–2.04 (4H, m, 2×CH_2) and 6.80 (1H, br s, OH). Anal. calcd for $C_{23}H_{42}O_2S$: C, 72.2; H, 11.1. Found: C, 72.0; H, 11.4%.

5.24. Biological assays

5.24.1. Plasmodium falciparum. In vitro activity against erythrocytic stages of *P. falciparum* was determined using a ^{3}H-hypoxanthine incorporation assay,[27,28] using the chloroquine and pyrimethamine resistant K1 strain and the standard drugs chloroquine (Sigma C6628) and artemisinin (Sigma 36,159-3). Compounds were dissolved in DMSO at 10 mg/mL and added to parasite cultures incubated in RPMI 1640 medium without hypoxanthine, supplemented with HEPES (5.94 g/L), $NaHCO_3$ (2.1 g/L), neomycin (100 U/mL), Albumax^R (5 g/L) and washed human red cells A^+ at 2.5% haematocrit (0.3% parasitaemia). Serial doubling dilutions of each drug were prepared in 96-well microtiter plates and incubated in a humidified atmosphere at 37 °C; 4% CO_2, 3% O_2, 93% N_2.

After 48 h 50 μL of ^{3}H-hypoxanthine (=0.5 μCi) was added to each well of the plate. The plates were incubated for a further 24 h under the same conditions. The plates were then harvested with a Betaplate^{TM} cell harvester (Wallac, Zurich, Switzerland), and the red blood cells transferred onto a glass fiber filter then washed with distilled water. The dried filters were inserted into a plastic foil with 10 mL of scintillation fluid, and counted in a Betaplate^{TM} liquid scintillation counter (Wallac, Zurich, Switzerland). IC_{50} values were calculated from sigmoidal inhibition curves using Microsoft Excel.

5.24.2. *Trypanosoma cruzi*. Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtiter plates at 2000 cells/well/100 μL in RPMI 1640 medium with 10% FBS and 2 mM L-glutamine. After 24 h, 5000 trypomastigotes of *T. cruzi* [Tulahuen strain C2C4 containing the β-galactosidase (Lac Z) gene] were added in 100 μL per well with 2× of a serial drug dilution. The plates were incubated at 37 °C in 5% CO_2 for 4 days. Then the substrate CPRG/Nonidet was added to the wells. The colour reaction, which developed during the following 2–4 h, was read photometrically at 540 nm. From the sigmoidal inhibition curve IC_{50} values were calculated.

5.24.3. *Trypanosoma brucei rhodesiense*. Minimum Essential Medium (50 μL) supplemented according to Baltz et al.[29] with 2-mercaptoethanol and 15% heat-inactivated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions were added to the wells. Then 50 μL of trypanosome suspension (*T.b. rhodesiense* STIB 900) was added to each well and the plate incubated at 37 °C under a 5% CO_2 atmosphere for 72 h. 10 μL Alamar Blue (Trinova, Giessen, Germany) was then added to each well and incubation continued for a further 2–4 h.[30] The plates are read in a microplate fluorescence scanner (Spectramax Gemini XS by Molecular Devices) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. From the sigmoidal inhibition curve IC_{50} values were calculated.

Acknowledgements

We would like to acknowledge the Cardiff Partnership Fund for financial support and the EPSRC National

Mass Spectrometry Service Centre Swansea for accurate mass spectrometry.

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