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Synthesis and biological evaluation of thiazolidine-2-one 1,1-dioxide as inhibitors of *Escherichia coli* β-ketoacyl-ACP-synthase III (FabH)

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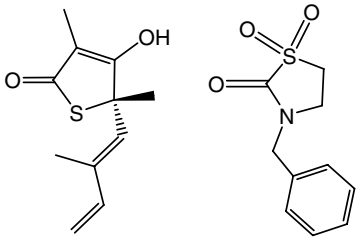
Abstract—A series of cyclic sulfones has been synthesized and their activity against β-ketoacyl-ACP-synthase III (FabH) has been investigated. The compounds are selectively active against *Escherichia coli* FabH (ecFabH), but not *Mycobacterium tuberculosis* FabH (mtFabH) or *Plasmodium falciparum* KASIII (PfKASIII). The activity against ecFabH ranges from 0.9 to >100 μM and follows a consistent general SAR trend. Many of the compounds were shown to have antimalarial activity against chloroquine (CQ)-sensitive (D6) *P. falciparum* (IC_{50} = 5.3 μM for the most potent inhibitor) and some were active against *E. coli* (MIC = 6.6 μg/ml for the most potent inhibitor).

β-Ketoacyl-ACP-synthase III (FabH) is a key condensing enzyme of the dissociated type II fatty acid synthase (FAS) system.[1,2] It catalyzes a cysteine-mediated Claisen condensation reaction between malonyl-ACP (MACP) and an enzyme-bound acyl unit formed by initial transacylation from a short-chain acyl-CoA primer to the active site Cys112.[2] The released β-ketoacyl-ACP product is subsequently reduced and elongated by other (FAS) condensing enzymes to generate a long-chain fatty acid.[3] This type of FabH is ubiquitous in both Gram-positive and -negative bacteria.[4,5] It is also present in *Plasmodium falciparum* and other parasitic organisms.[6] An unusual FabH is also present in *Mycobacterium tuberculosis* where it is required in the conversion of long-chain fatty acids into mycolic acid.[7,8] FabH bears no homology to the condensing enzymes in the mammalian type I FAS.[9,10] FabH inhibitors, therefore, have potential as general antibacterial, antiparasitic or even antimycobacterial agents.[11]

We have previously identified a series of potent FabH inhibitors[12] by searching the 3D National Cancer Institute (NCI) database for compounds bearing structural similarities to thiolactomycin (TLM) (Fig. 1).[13–15] One set of these compounds were 1,2-dithiole-3-ones.[12] Another set were cyclic sulfones such as compound **1a** (Fig. 1) which has been reported earlier as a herbicide and fungicide.[16] Here, we report our effort to further study and develop more potent analogues of **1a**.

The inhibitors were prepared following a general approach described by Aitken et al. and Le Core et al. as shown in Scheme 1.[17,18] The required N-substituted



TLM **1a**

Figure 1.

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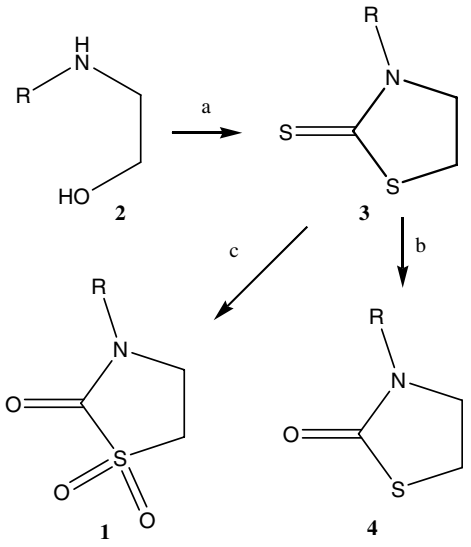
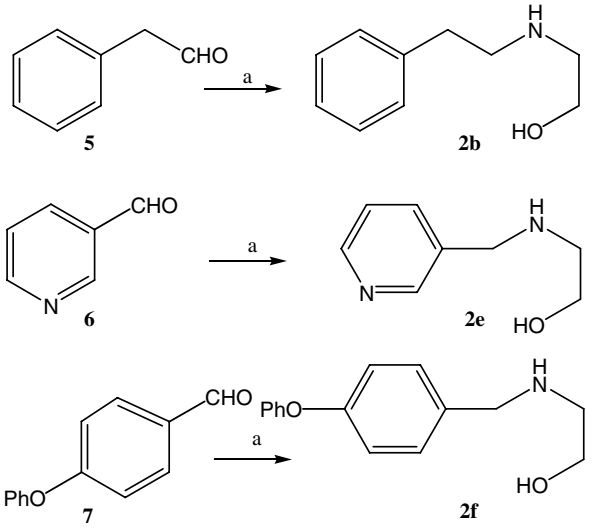
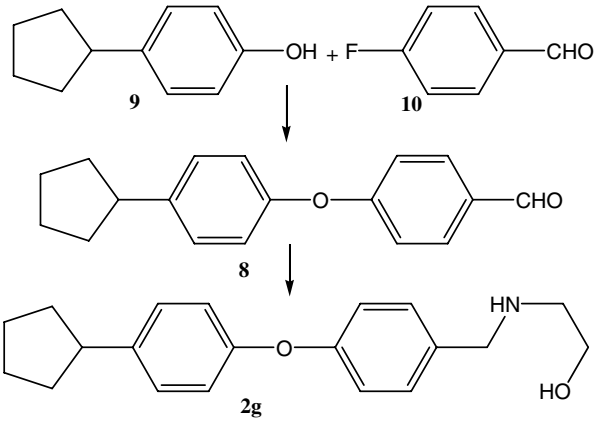


Scheme 1. Reagents and conditions: (a) CS_2, aq NaOH; (b) $KMnO_4$ (3 equiv), $PhCO_2H$, benzyltriethylammonium Cl^-, CH_2Cl_2–H_2O; (c) same as (b) except $KMnO_4$ (5 equiv).

aminoethanols **2b** and **2e–g** were readily obtained by condensation of aldehydes **5–8** with ethanolamine to give the corresponding imines which were subsequently reduced by sodium borohydride ($NaBH_4$) in methanol (Schemes 2 and 3). Aldehyde **8** was prepared by nucleophilic aromatic substitution of 4-fluorobenzaldehyde **10** by phenol **9** (Scheme 3). Aminoethanols **2** were subjected to reaction with carbon disulfide (CS_2) in aqueous sodium hydroxide to give N-substituted thiazolidine-2-thiones **3**.[17,18] Under phase-transfer conditions, the oxidation of **3** to the corresponding thiazolidin-2-ones **4** and thiazolidine-2-one 1,1-dioxides **1** was controlled using different amounts of $KMnO_4$ (3 and 5 equiv, respectively).[18] The desired products were purified and fully characterized by ^1H NMR, ^{13}C NMR, and high-resolution mass spectroscopy (HRMS).



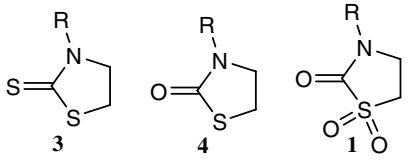
Scheme 2. Reagents: (a) i—benzene, $NH_2CH_2CH_2OH$; ii—$NaBH_4$, MeOH.



Scheme 3. Reagents and conditions: (a) K_2CO_3, DMF, 100 °C; (b) i—benzene, $NH_2CH_2CH_2OH$; ii—$NaBH_4$, MeOH.

The compounds synthesized (N-substituted analogues of **1**, **3**, and **4**) were tested against *E. coli* FabH (ecFabH). Preliminary SAR for these analogues against ecFabH are shown in Table 1. Investigations focused primarily on the N-substitution and the oxidation state of the sulfur moiety. Decreasing the oxidation state of parent compound **1a** to the thiazolidin-2-one **4a** resulted in a 13-fold decrease in the activity against ecFabH, while no activity was observed for the thiazolidine-2-thiones **3a** at 100 μM. Replacing the N-substituted benzyl of **1a** with a phenethyl group (**1b**) resulted in a ~2-fold decrease in activity. The lower oxidation state of **3b** gave similar activity pattern to the one observed for **3a**. Replacing the benzyl group with simple alkyl moieties (**3c**,**1c**, **3d**, and **1d**) resulted in a major loss of biological activity (IC_{50} > 100 μM). Introducing a 3-pyridyl ring

Table 1. IC_{50} against ecFabH and *P. falciparum* (D6)



R	Compound	EcFabH IC_{50} (μM)	*P. falciparum* D6 IC_{50} (μM)
Ph–CH_2	**3a**	>100	61.6
a	**4a**	13.7	90.9
	1a	1.1	67.6
Ph–CH_2–CH_2	**3b**	>100	55.5
b	**1b**	1.9	21.6
CH_3	**3c**	>100	>100
c	**1c**	>100	>100
$CH_3CH_2CH_2$	**3d**	>100	>100
d	**1d**	>100	>100
3-pyridyl–CH_2	**3e**	>100	73.4
e	**4e**	20.7	78.4
Ph–O–Ph–CH_2	**3f**	>100	55.6
f	**4f**	10.9	67.1
	1f	0.9	49.4
C_5H_9–Ph–O–Ph–CH_2	**3g**	17.7	5.3
g	**4g**	14.4	11.1
	1g	2.4	10.8

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MtFabH    42 WIYTRTGI 49    120 AGCA 123    160 DRGNCFIF 167    221 LEGPAVFRWAAFKM 234
EcFabH    32 WIVTRTGI 39    110 AACA 113    150 DRGTIIIF 157    207 MAGNEVFKVAVTEL 220
PfKASIII  80 WIRTRTGI 87    157 AACT 160    195 DRNTCVLF 202    261 MNGKEVFKYTISNI 274
             ** *****          *.*:            **.. .:*            : *  **: :  ::

MtFabH   258 HQANSRIN 265    284 IEHTGNTS 291    318 GYGAGLSY 325
EcFabH   244 HQANLRII 251    269 LDRHGNTS 276    303 AFGGGFTW 310
PfKASIII 298 HQANIRII 305    323 LDEYANTS 330    357 GFGAGMSY 364
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Figure 2. Alignment of mtFabH, ecFabH, and PfKASIII sequences (performed using ClustalW)[19] encompassing the enzyme active site and the walls and mouth of the CoA binding channel. Catalytic residues are indicated in red. (∗), (:), and (·) indicate identity, conserved substitution, and semi-conserved substitution, respectively.

(**4e**) in place of the phenyl (**4a**) resulted in decrease of activity. Based on this initial SAR data, we decided to retain the benzyl moiety and extend it at the *para*-position with another phenoxy group (**3f**, **4f**, and **1f**). This modification resulted in a slight improvement in bioactivity compared to our parent compound **1a**. Interestingly, the correlation between the oxidation state of these analogues and bioactivity was in full agreement with the one observed for the rest of the inhibitors (i.e., a higher oxidation gave higher bioactivity). Extending the size of the N-substituted aryl moiety of **1f** by an extra cyclopentyl group (**3g**, **4g**, and **1g**) resulted in ~2-fold decrease in activity (**1g** compared to **1a**). From these SAR studies we conclude that the most potent ecFabH inhibitors require both the sulfur in the oxidized sulfone form and an aromatic substitution at the 3-N position. While there is some flexibility in the N-substituent size, there appears to be an upper size limit.

A reversibility study was carried out to determine the mode of binding of **1a** with ecFabH. Compound **1a** and ecFabH were incubated under conditions that resulted in greater than 85% enzyme inhibition. Dialysis of the inhibited enzyme for 12 h in 50 mM phosphate buffer (pH 7.4 at 4 °C) resulted in a ~90% restoration of its activity (no restoration of activity was observed when these experiments were carried out with irreversible inhibitors). These data demonstrated the mode of binding of **1a** as a reversible inhibitor.

The activity of the 17 compounds against *M. tuberculosis* FabH (mtFabH), and *P. falciparum* KASIII (PfKASIII), was also evaluated. Surprisingly, no inhibition activity was observed for any of the compounds (at 100 μM). Thus for the most potent 1 μM inhibitors of the ecFabH, there is a decrease of more than two orders of magnitude for inhibition of the other two FabH enzymes. Attempts to determine the selective mode of binding of these inhibitors with the ecFabH through generation of a cocrystal structure were unsuccessful. Likewise docking studies failed to produce a compelling case for a specific mode of binding to the

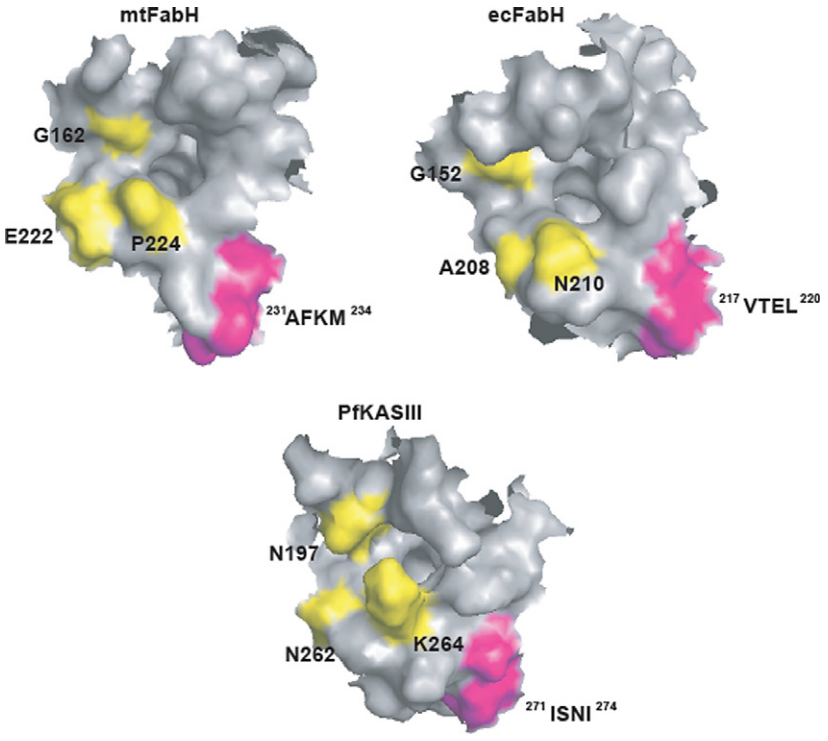

Figure 3. Surface representations of the conserved CoA binding channels of mtFabH (PDB ID 1U6S), ecFabH (PDB ID 1MZS), and PfKASIII (from homology model) created using Pymol.[20] All representations are shown in approximately the same orientation, looking at the opening of the channel.

ecFabH. Nonetheless, a comparison of the crystal structures of the ecFabH, mtFabH and a homology model of the PfKASIII revealed some structural differences which might account for the differing sensitivity to these and potentially other inhibitors. The three enzymes are 45% identical and 66% conserved in the region comprising the entire CoA binding channel (see alignment in Fig. 2). The channel itself is structurally quite similar; however, some differences do exist at the surfaces surrounding the mouth of the channel. These differences are highlighted in Figure 3. First, all three enzymes contain a patch of four variable residues distal to the opening of the channel (magenta in Fig. 3). The character of all three patches is principally hydrophobic, but the key difference among the patches is the nature of the polar/charged amino acid in each (K233, E219, and N273 for mtFabH, ecFabH, and PfKASIII, respectively). Closer to the mouth of the channel, three variable amino acids (indicated in yellow in Fig. 3) were also identified. The most striking of these (P224 in mtFabH, N210 in ecFabH, and K264 in PfKASIII) is located directly adjacent to the channel opening. Conformational restrictions imposed by proline in mtFabH may contribute to the poor inhibitor binding observed in this study. The long lysine side chain in PfKASIII provides a rather different surface in this region and may restrict access to the CoA binding channel. The steric bulk surrounding the opening of the PfKASIII channel is further increased by the presence of N197 and N262. In the mtFabH these residues are G162 and E222, with the latter also contributing to steric bulk. In ecFabH the equivalent positions are occupied by small aliphatic residues (G152 and A208). In summary, there are clear structural differences between the enzymes at the mouth of the CoA binding channel. These variable residues may limit access to the CoA binding channel for PfKASIII and mtFabH as compared to the ecFabH enzyme.

The activity of the 17 compounds against both *E. coli* and *P. falciparum* was determined. In the case of *E. coli*, only **1a** and **1b** showed ability to inhibit the growth of *E. coli* with a concentration below 50 μg/ml (MIC = 6.6 μg/ml for **1a** and 16.5 μg/ml for **1b**). An *E. coli* strain, carrying an ecFabH expression plasmid, grown under appropriate induction conditions also displayed an MIC value for **1a** of 6.6 μg/ml or less. The absence of increase in resistance to **1a** when the ecFabH was overexpressed in *E. coli* suggests that ecFabH is not the sole target for this class of inhibitors. The in vitro antimalarial activity of these analogues against chloroquine (CQ)-sensitive (D6) *P. falciparum* was also evaluated (Table 1).[21] In this case, 13 of the 17 inhibitors had shown activity with an IC_{50} value below 100 μM. Increasing the size of the aromatic N-substituent resulted in ~10-fold improvement of the antimalarial activity compared to the parent compound **1a** (IC_{50} for **3g** = 5.3 μM compared to IC_{50} for **1a** = 67.7 μM). There was no pattern regarding the oxidation state of the sulfur. As **1g**, **3g**, and **4g** do not inhibit the PfKASIII, the antimalarial activity of these compounds must arise through some other mechanism.

In conclusion, we have identified and synthesized a new series of ecFabH inhibitors. Preliminary SAR studies indicated that the most potent inhibitors require both the sulfur in the oxidized sulfone form and a limited-size aromatic substitution at the 3-N position. The selectivity of these inhibitors (against ecFabH and not mtFabH or PfKASIII) could provide structural insights and understanding of the different specificities of these three enzymes.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.11.067.

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