Bioorganic & Medicinal Chemistry Letters 18 (2008) 6402–6405

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological evaluation of novel sulfonyl-naphthalene-1,4-diols as FabH inhibitors

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ARTICLE INFO

Article history:
Received 25 August 2008
Revised 17 October 2008
Accepted 17 October 2008
Available online 25 October 2008

Keywords:
FabH
Fatty acid biosynthesis
SAR
Plasmodium falciparum
Inhibition

ABSTRACT

A series of analogs of 2-tosylnaphthalene-1,4-diol were prepared and were found to be potent 10–20 nM reversible inhibitors of the *Escherichia coli* FabH enzyme. The inhibitors were also effective but to a lesser degree (30 nM–5 μM), against the *Mycobacterium tuberculosis* and *Plasmodium falciparum* FabH enzymes. Preliminary SAR studies demonstrated that the sulfonyl group and naphthalene-1,4 diol were required for activity against all enzymes but the toluene portion could be significantly altered and leads to either modest increases or decreases in activity against the three enzymes. The in vitro activity of the analogs against *E. coli* FabH parallel the in vivo activity against *E. coli* TolC strain and many of the compounds were also shown to have antimalarial activity against *P. falciparum*.

β-Ketoacyl-ACP-synthase III (FabH) is a key condensing enzyme in bacterial fatty acid biosynthesis.[1] This enzyme is part of the dissociated fatty acid synthase (FAS). FabH is ubiquitous in both Gram-negative and -positive bacteria, while it bears little homology to the mammalian multifunctional type I FAS.[2,3] The enzyme is also present in *Plasmodium falciparum*[4] and *Mycobacterium tuberculosis*[5,6] and is considered a target for developing promising new antibacterial, antiparasitic, and antimycobacterial agents.[7,8]

2-Tosylnaphthalene-1,4-diol (**1**) (Fig. 1) was identified as potent inhibitor of *P. falciparum* FabH (PfFabH) through a virtual screen using a pharmacophore developed based on thiolactomycin.[9] Here, we report our effort to further study **1** and develop more potent sulfonyl-naphthalene-1,4-diols against various FabH enzymes. A series of 14 compounds were prepared (compounds **1**–**14**, Fig. 2). Compounds **1**–**11** were prepared following a general synthetic strategy as shown in Scheme 1. Nucleophilic addition of alkyl- or arylsulfinic acids to a variety of 1,4-quinones resulted in the formation of the corresponding alkyl- or arylsulfonylhydroquinones. The reaction was conducted using a two-phase dichloromethane-water system in the presence of trifluoroacetic acid.[10] The sulfinic acid salts **15**, **16**, and **17** were not commercially available and were prepared as shown in Scheme 2. Sodium 4-propylbenzenesulfinate **15** and sodium naphthalene-2-sulfinate **16** were prepared by reacting the corresponding sulfonyl chloride with sodium sulfite and sodium bicarbonate in water,[11] while sodium 2-carboxyethanesulfinate **17** was prepared by oxidation of 3-mercaptopropanoic acid using *meta*-chloroperoxybenzoic acid (mCPBA).[12]

The diaryl sulfone **12** was prepared by palladium-catalyzed coupling of 2-bromonaphthalene with toluene sulfinic acid as shown in Scheme 3.[13] Oxidation of compound **1** using manganese dioxide gave the 1,4-quinone **13** (Scheme 3). Nucleophilic addition of 4-methylbenzenethiol to 1,4-naphthoquinone gave compound **14**. Reduction of **14** using zinc dust/acetic acid gave the corresponding 1,4-hydroquinone **18** that converted back to **14** by air oxidation (Scheme 4). All analogs were purified and characterized by ^{1}H NMR, melting point, and high-resolution mass spectroscopy (HRMS).

As shown in Table 1, **1** is an effective inhibitor of all three enzymes and is most potent against the *Escherichia coli* FabH (ecFabH) (IC_{50} of 13 nM). It was least effective against the *M. tuberculosis* FabH (mtFabH). The analogs **2**–**14** were then synthesized and evaluated against all three enzymes to establish a preliminary SAR. These efforts focused primarily on modifying either the toluene

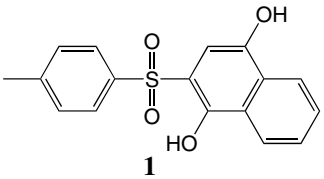

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Figure 1. FabH inhibitor **1**.

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0960-894X/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2008.10.097

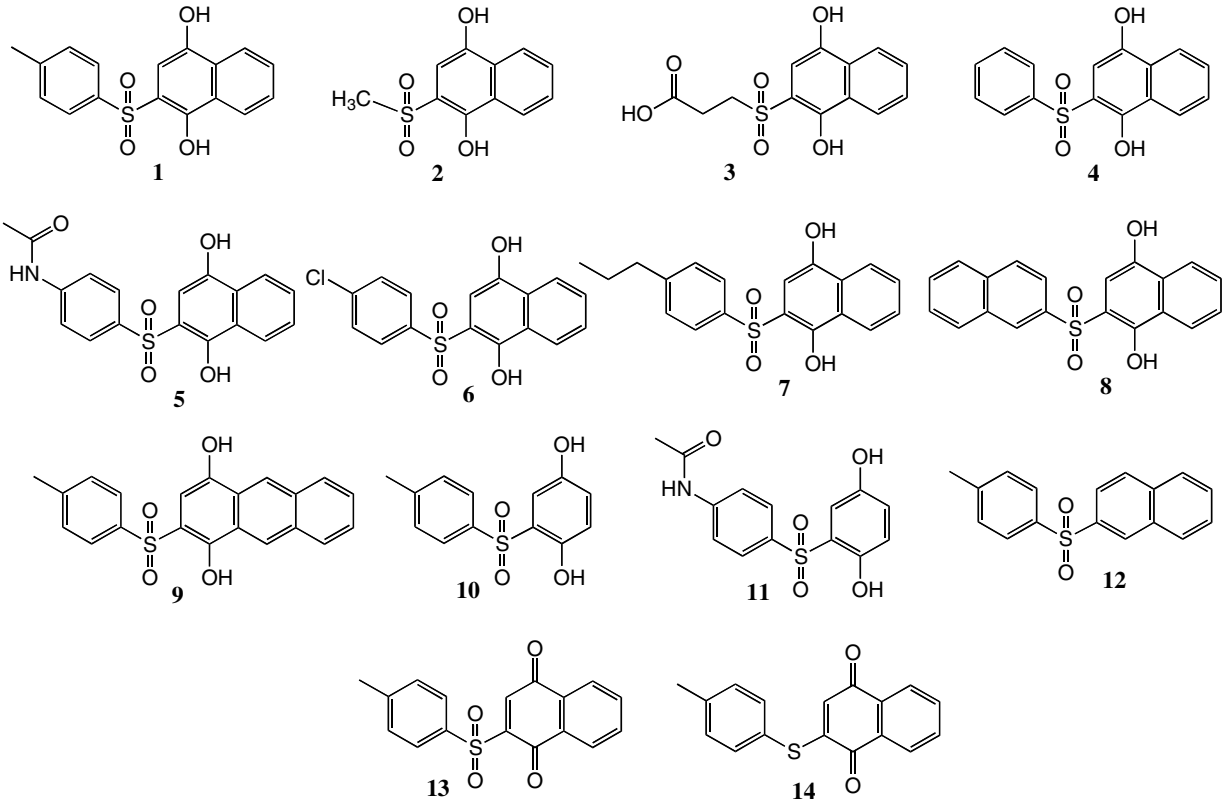
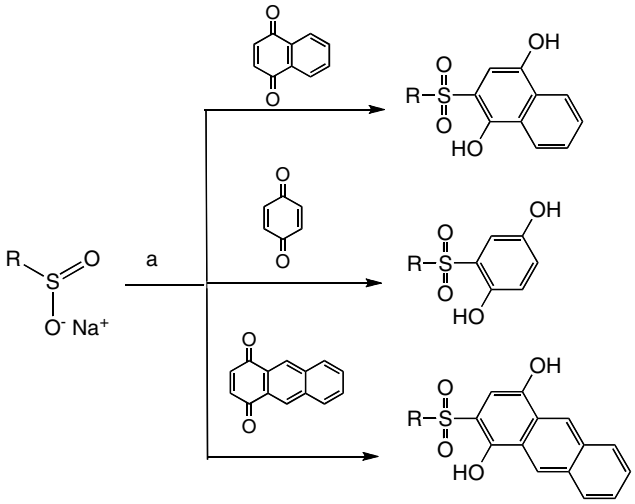


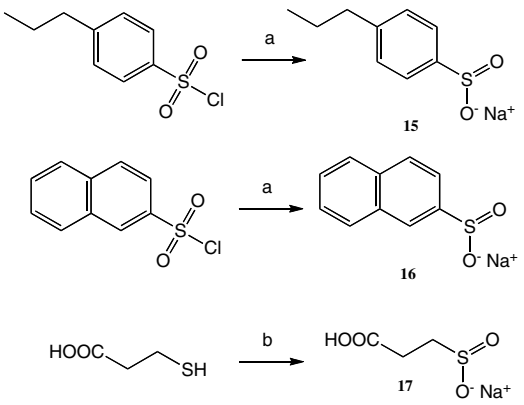
Figure 2. FabH inhibitors **1**–**14**.

or naphthalene portions of the molecule, or changing the oxidation state of the sulfur and 1,4-diol. The activity of each of these analogs with each enzyme is shown in Table 1. The toluene portion of **1** was very tolerant to modifications. Replacing the toluene ring with a methyl (**2**) or carboxy propyl (**3**) groups resulted in ~7-fold decrease in activity against ecFabH and ~5-fold decrease in activity against mtFabH. Interestingly, both compounds **2** and **3** were slightly more active against pfFabH than the parent compounds **1** (**2** was ~3 times more active against pfFabH than **1**). Replacing the toluene ring with a phenyl group (**4**) resulted in a decrease in activity against the three enzymes. Introducing a hydrophilic acetamide group (**5**) in place of the methyl group of **1** gave comparable activity against ecFabH and ~2.5 improvement in activity against pfFabH. A slight improvement in activity against ecFabH was observed when the methyl group of **1** was replaced by more hydrophobic groups such as chlolro and propyl (compounds **6** and **7**, respectively). However, these changes did not improve the activity against pfFabH or mtFabH. Increasing the size of the toluene portion of **1** by an extra phenyl group (**8**) resulted in no significant change in activity against ecFabH and a ~5 fold decrease in activity against pfFabH.

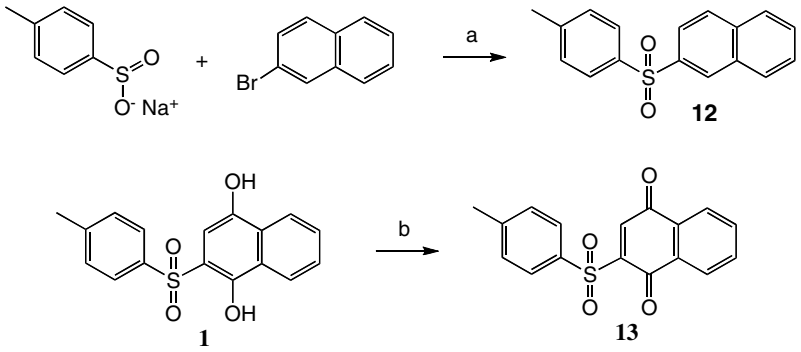
The naphthalene-1,4-diol portion of **1** was important for activity against all three FabH enzymes. Extending the size of the naphthalene-1,4-diol with an extra phenyl group (**9**) resulted in much lower activity against the three FabH enzymes. Decreasing the size of the naphthalene-1,4-diol by replacing it with a hydroquinone moiety (**10** and **11**) resulted in a major loss of activity against both



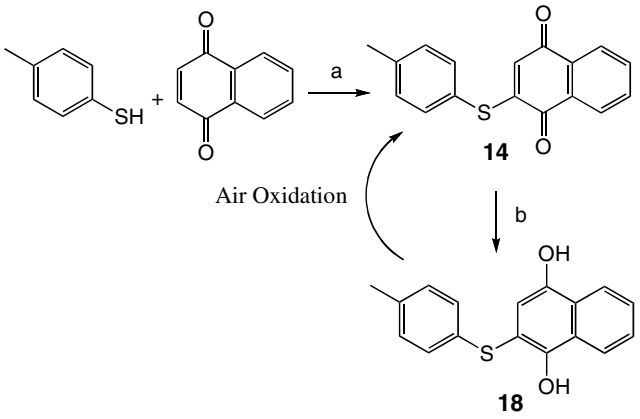
Scheme 1. Reagents: (a) TFA, CH_2Cl_2, H_2O.



Scheme 2. Reagents and condition: (a) Na_2SO_3, $NaHCO_3$, H_2O; (b) *m*CPBA, CH_2Cl_2, −30 °C.



Scheme 3. Reagents and condition: (a) $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, nBu$_4$NCl, Toluene, 80 °C; (b) MnO_2, acetonitrile.



Scheme 4. Reagents and conditions: (a) CH_2Cl_2; (b) Zinc dust, acetic acid.

ecFabH and pfFabH and ~50-fold decrease in activity against mtFabH. The 1,4-diol of **1** was crucial for activity. Replacing the naphthalene-1,4-diol with a simple naphthalene group (**12**) resulted in a drastic loss of activity against the three FabH enzymes. The quinone **13** (generated through oxidation of **1**) was also much less active against FabH, as compared to **1**. The sulfone moiety is also crucial for activity as compound **14** did not have significant activity against FabH.

These SAR data shows that toluene portion of **1** is tolerant to modifications. A hydrophobic group with a limited size is important for activity against ecFabH. On the other hand, pfFabH has is slightly more sensitive to analogs of **1** in which the toluene is replaced with a smaller (compound **2**) or more hydrophilic (compounds **3** and **5**) groups. There was no significant effect in changing the toluene part of **1** on the activity against mtFabH. The naphthalene-1,4-diol portion of **1** is important for activity and cannot be modified, as increasing its size reduced the activity, while decreasing its size resulted in major loss of activity. Changing the oxidation state of the naphthalene-1,4-diol moiety resulted also in major loss of activity. Interestingly, the major loss of activity observed by changes in the naphthalene-1,4-diol was universal among the three FabH enzymes. Finally, the loss of activity observed by changing the sulfone group (compound **14**) suggests a role of this group in critical interaction with the FabH enzymes.

Table 1
In vitro activity of **1**–**14** against three FabH enzymes.

Compound	ecFabH IC_{50} (nM)	pfFabH IC_{50} (nM)	mtFabH IC_{50} (nM)
1	13.6 ± 2.3	73.6	0.61 ± 0.16
2	87.3 ± 6.4	27.45	3.53 ± 0.12
3	96.5 ± 5.6	46.5	3.53 ± 0.11
4	21.2 ± 1.9	278.7	2.84 ± 0.17
5	11.8 ± 1.3	29.6	3.76 ± 0.68
6	8.8 ± 2.6	128.7	3.61 ± 0.94
7	9.7 ± 1.0	72.6	3.09 ± 0.66
8	15.4 ± 1.32	393	1.49 ± 0.12
9	112.4 ± 7.6	341.1	8.47 ± 1.63
10	NA	NA*	34.4 ± 2.85
11	NA	NA*	31.4 ± 2.7
12	NA	NA*	138.1 ± 6.4
13	187.2 ± 9.3	1425	1.99 ± 0.21
14	NA	NA*	112 ± 4.3

NA, not active below 10 μM; NA*, not active below 100 μM.

Table 2
In vitro activity of **1** and selected analogs against ecFabH and *E. coli tolC* mutant.

Compound	ecFabHIC_{50} (nM)	*E. coli tolC* mutant (MIC_{99}) (μg/ml)
1	13. 6 ± 2.3	14.1
2	87.3 ± 6.4	9.7
5	11.8 ± 1.3	12.3
10	>10 μM	>90
11	>10 μM	>90
12	>10 μM	>90

MIC_{99}, minimum concentration at which no visible growth was detected.

All FabH enzymes catalyze a Claisen-type condensation reaction between malonyl-ACP (MACP) and an acyl–enzyme intermediate formed by an initial transacylation from a short-chain acyl-CoA primer with a conserved active site cysteine.[1] We have recently shown that compounds that covalently bind to these active site cysteines are potent inhibitors of FabH enzymes.[14,15] A reversibility study, as previously reported,[16] was carried out to determine the mode of binding of **1** to FabH. Compound **1** and ecFabH were incubated under conditions that resulted in greater than 95% enzyme inhibition. Dialysis of the inhibited enzyme for 16 h in phosphate buffer (at 4 °C) resulted in a ~90% restoration of its activity. These data demonstrate that compound **1** is a reversible inhibitor of ecFabH and that there is likely no covalent interaction with the ecFabH active site cysteine (Cys112). We have shown previously that no restoration of activity was observed when these experiments were carried out with irreversible inhibitors that form covalent adducts with the active site cysteine.[14,15]

The activity of **1** and some of its analogs against *E. coli tolC* mutant was evaluated (Table 2). Interestingly, there is a clear correla-

Table 3
IC_{50} of **1** and selected analogs against *P. falciparum* D6.

Compound	*P. falciparum* D6 IC_{50} (μg/ml)
1	7.69
5	4.98
6	3.23
10	31.35
11	3.96
12	8.74

tion between the growth inhibition of this strain and the observed activity against ecFabH. Compounds that are ecFabH inhibitors (**1**, **2**, and **5**) inhibited the growth of *E. coli,* while compounds with no significant activity against ecFabH (**10**–**12**) did not show activity against *E. coli*. These data suggest that ecFabH might be the target of these compounds in *E. coli*. However, an *E. coli* strain, carrying an ecFabH expression plasmid, displayed a similar MIC value for **1** compared to the same *E. coli* strain lacking the ecFabH plasmid (data not shown). The absence of increase in resistance to **1** when the ecFabH was overexpressed in *E. coli* suggests that ecFabH might not be the sole target for this class of inhibitors.

Most compounds showed in vitro antimalarial activity against the chloroquine sensitive D6 strain of *P. falciparum* (IC_{50} between 3 and 32 μg/ml) (Table 3). However, there was no correlation between the inhibition of pfFabH and the antimalarial activity. Therefore, the antimalarial activity of these compounds must arise through some other mechanism.

Acknowledgments

Funding for this research was generously provided by the National Institutes of Health (AI52230). We thank Dr Martin Smilkstein (Portland Veterans Affairs Medical Center) for performing the in vitro antimalarial assay.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.10.097.

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