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Inhibitors of Bacterial Enoyl Acyl Carrier Protein Reductase (FabI): 2,9-Disubstituted 1,2,3,4-Tetrahydropyrido[3,4-*b*]indoles as Potential Antibacterial Agents

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Abstract—An SAR study of a screening lead has led to the identification of 2,9-disubstituted 1,2,3,4-tetrahydropyrido[3,4-*b*]indoles as inhibitors of *Staphylococcus aureus* enoyl acyl carrier protein reductase (FabI). © 2001 Elsevier Science Ltd. All rights reserved.

Introduction

The increasing resistance of clinically important pathogens to antibiotic treatment is of world-wide concern.[1] New antibacterial agents that operate with distinctly different mechanisms of action from current drug therapies offer hope towards combating these multidrug-resistant organisms.[2] In an effort to develop a novel class of antibacterial compounds, we targeted the inhibition of bacterial fatty acid synthesis (FAS-II). The FAS-II elongation cycle utilizes several discrete monofunctional enzymes with activity corresponding to individual polypeptides effecting fatty acid chain elongation and ultimately cell membrane production.[3] Enoyl acyl carrier protein reductase (FabI) is the component of FAS-II that catalyzes the final reaction in the enzymatic sequence.[4] In contrast, mammalian fatty acid synthesis (FAS-I) employs a multifunctional enzyme complex in which all enzymatic activities reside on a single polypeptide.[5] Thus, there exists a potential for selective inhibition of Gram-positive and Gram-negative bacterial cell growth by the inhibition of the FabI enzyme. Herein, we describe the SAR of a series of 2,9-disubstituted 1,2,3,4-tetrahydropyrido[3,4-*b*]indoles that exhibit antibacterial activity by inhibiting the FabI enzyme. We also report compounds tested for in vitro antibacterial activity against selected Gram-positive and Gram-negative FabI-containing organisms.[6] Triclosan, a commercial antibacterial agent, has recently been shown to inhibit FabI[7] and will be discussed in context with these results.

Chemistry

The FabI inhibitor **3** was synthesized as shown in Scheme 1. Carbobenzyloxy (Z) protection of commercially available 1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (**1**, Scheme 1) in quantitative yield followed by N-9 alkylation using sodium hydride and 4-benzyloxybenzyl chloride in DMF gave **2** (92% yield). Hydrogenolysis in methanol using $Pd(OH)_2$ at 45 psi and subsequent EDC-mediated acylation afforded compound **3** in 82% overall yield. Compounds **6**–**15** and **19**–**34** were likewise prepared with yields ranging from 72 to 90%. The regioisomeric derivative **16** was generated from 2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole and elaborated in the manner described in Scheme 1. Indole **18** was similarly constructed from Z-protected 2-(methylaminomethyl)indole. The racemic compound **17** was

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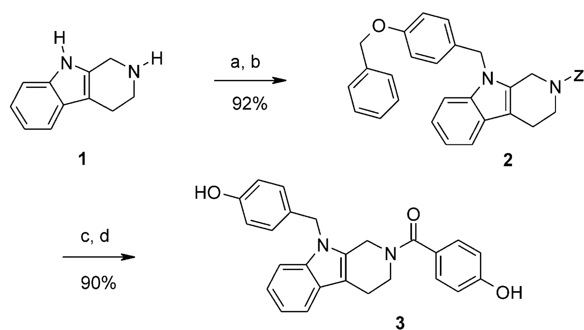


Scheme 1. Preparation of 2,9-disubstituted 1,2,3,4-tetrahydro-pyrido[3,4-*b*]indoles. Reagents: (a) Z-succinamide, Et_3N, DMF; (b) NaH, 4-benzyloxybenzyl chloride, rt; (c) $Pd(OH)_2$, 1 M HCl in dioxane, H_2 (3 atm), CH_3OH, rt; (d) 4-hydroxybenzoic acid, EDC, HOBt, DMF, rt.

synthesized in 89% isolated yield by the reaction of tyramine and 4-hydroxybenzaldehyde in a Pictet–Spengler condensation followed by acylation with 4-hydroxybenzoic acid.

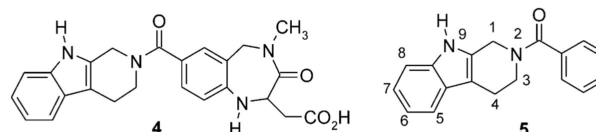
Biology

The *Escherichia coli* and *Staphylococcus aureus* FabI's were cloned, overexpressed and purified as described previously.[8a] FabI enzyme inhibition assays were carried out in half-area, 96-well microtitre plates. Compounds were evaluated in 150-µL assay mixtures containing 100 mM NaADA, pH 6.5 (ADA = *N*-[2-acetamido]-2-iminodiacetic acid), 4% glycerol, 0.25 mM crotonoyl CoA, 50 µM NADH, and an appropriate dilution of *E. coli* Fab I (usually 60 nM). Inhibitors were typically varied over the range of 0.01–10 µM. The consumption of NADH was monitored for 20 min at 30 °C by following the change in absorbance at 340 nm ($\varepsilon = 5.28\ mM^{-1}$). Initial velocities were estimated from an exponential fit of the nonlinear progress curves represented by the slope of the tangent at $t = 0$ min. IC_{50}'s were estimated from a fit of the initial velocities to a standard, four-parameter model and are typically reported as the mean ± SD of duplicate determinations. Triclosan, an inhibitor of Fab I, was included in all assays as a positive control. IC_{50}'s with *S. aureus* FabI were determined similarly except that NADPH was used as substrate, and the concentration of enzyme was typically 100 nM.

Whole-cell antimicrobial activity was determined by broth microdilution. Test compounds were dissolved in DMSO and diluted 1:10 in water to produce a 256 µg/mL stock solution. The stock solution was serially diluted using a Microlab AT Plus 2 (Hamilton Co., Reno, NV, USA) into cation adjusted Mueller–Hinton broth (Becton Dickenson, Cockeysville, MD, USA) on a 96-well microtitre plate. After dilution, a 50 µL aliquot of the test isolate ($\sim 1 \times 10^6$ cfu/mL) was added to each well of the microtitre plate. The final test concentrations ranged from 0.06 to 64 µg/mL. Inoculated plates were incubated at 35 °C in ambient air for 18–24 h. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of compound that inhibited visible growth.

Results and Discussion

Benzodiazepine **4** was identified as a weak lead compound ($IC_{50} = 7.8$ µM) from high throughput screening of our compound collection against *S. aureus* FabI. The key pharmacophore ($IC_{50} = 2.7$ µM) was determined to be 2-benzoyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (**5**).



Initial investigation focused on substitution at the N-9 position of the β-carboline subunit (Table 1). *para*-Hydroxybenzoyl was chosen as the N-2 acyl component because of its increased aqueous solubility relative to the benzoyl moiety of **5**. Increased inhibition of both *S. aureus* and *E. coli* FabI was observed for 4-substituted benzyl analogues, with the 4-hydroxybenzyl compound **3** exhibiting the greatest potency. Basic (**12**) and acidic (**15**) substituents on the benzyl ring were less well tolerated.

Further modifications to the most potent analogue **3** were subsequently investigated (Fig. 1). Regioisomer **16** and phenolic analogue **17** lost potency relative to **3**. Eliminating the conformational constraint afforded by the 4-methylene position of the 1,2,3,4-tetrahydro-pyrido[3,4-*b*]indole framework afforded compound **18**. Although this compound showed an improvement in activity over **16** and **17**, it was still significantly less active than the parent compound **3**. Additional alterations to the tricyclic structure were unsuccessful in defining a more potent template.

Table 1. Modifications to R^1

Compd	R^1	*S. aureus* FabI IC_{50} (µM)[a,b]	*E. coli* FabI IC_{50} (µM)[a,c]
6	H	1.4	18.0
7	CH_3	1.4	11.0
8	$CH_2CO_2CH_3$	9.8	34.9
9	$(CH_2)_4CO_2CH_3$	4.6	26.8
10	$C_6H_4CH_2$	1.3	27.0
11	$3\text{-}(OH)C_6H_4CH_2$	0.42	8.8
3	$4\text{-}(OH)C_6H_4CH_2$	0.11	4.2
12	$4\text{-}(NH_2)C_6H_4CH_2$	0.67	21.4
13	$4\text{-}(F)C_6H_4CH_2$	0.25	4.2
14	$4\text{-}(SO_2CH_3)C_6H_4CH_2$	0.33	4.5
15	$4\text{-}(CO_2H)C_6H_4CH_2$	8.7	> 50

[a]Values are the averages of at least two experiments, standard deviation is < 50% in all cases.
[b]*Staphylococcus aureus* Oxford.
[c]*Escherichia coli* WT.

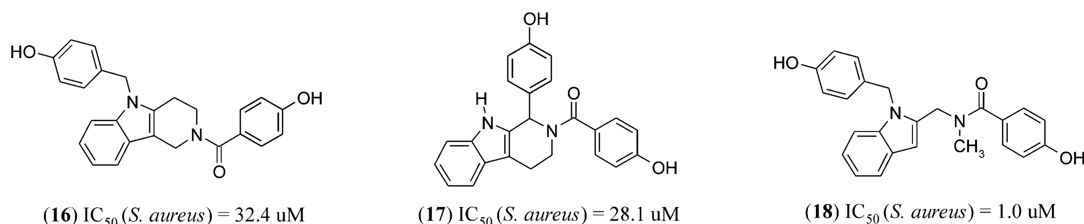


Figure 1. Effects of structural modifications to **3** on *S. aureus* FabI enzyme activity.

An investigation into substitution at N-2 of the β-carboline revealed that an aromatic acyl group at this position was required for FabI enzyme activity (data not shown). A simple one-dimensional, solution-phase array consisting of 9-(4-hydroxybenzyl)-1,2,3,4-tetrahydropyrido[3,4-*b*]indole and various substituted benzoic acids was prepared to rapidly determine the optimum substitution pattern on the benzoyl ring (Table 2).

Table 2. Modifications to R^2

Compd	R^2	*S. aureus* FabI IC_{50} (μM)[a]	*E. coli* FabI IC_{50} (μM)[a]
19	H	0.49	2.7
20	4-CH_3	0.24	5.2
21	4-C_4H_{10}	0.51	> 50
22	4-Cl	0.18	16.8
23	2,4-Cl	1.8	35.8
24	4-CO_2CH_3	2.0	> 50
25	4-NH_2	1.0	4.0
26	4-$NHCH_3$	2.1	38.6
27	2-OH	0.72	4.3
28	3-OH	3.4	> 50
3	4-OH	0.11	4.2
29	4-OCH_3	0.37	35.5
30	2-OH, 4-CH_3	0.12	8.2
31	2-OH, 4-Cl	0.16	6.1
32	3-CH_3, 4-OH	0.18	4.4
33	3-Cl, 4-OH	0.16	3.4
34	3,5-Cl, 2-OH	1.8	9.9
35	3,5-CH_3, 4-OH	2.9	23.0
36	3,6-CH_3, 2,4-OH	> 50	> 50
Triclosan	—	1.1	0.43

[a]See footnotes to Table 1.

The SAR revealed that maximum inhibition of *S. aureus* and *E. coli* FabI generally occurred when a small lipophilic or hydroxyl group was located at the 4-position of the benzamide ring. Compounds with two substituents in this ring maintained activity against both enzymes (**30**–**33**). Three or more substituents on the ring (**34**–**36**) led to a loss of FabI activity, possibly due to unfavorable steric interactions. It was also observed that small increases in substituent size decreased potency, especially in *E. coli* (**20** vs **21** and **25** vs **26**). A comparison of the *S. aureus* enzyme inhibition of **3** with that of triclosan reveals that compound **3** is 10 times more active against this specific enzyme. Conversely, triclosan is 10-fold more potent than **3** against the *E. coli* enzyme. These differences may be attributed to the time-dependent formation of a stable FabI-NAD^+-triclosan ternary complex in *E. coli*[8] as compared to the highly reversible binding activity characterized by the β-carboline inhibitors. Differences in the binding site conformations of *E. coli* and *S. aureus* could also contribute to these disparities in potency.

The X-ray co-crystallization study of **3** bound to *E. coli* FabI/NAD^+ assists in the interpretation of the β-carboline SAR (Fig. 2).[9] Although the full inhibitor has not been observed, the 4-hydroxybenzamide portion of the molecule is well ordered, and the data shows that the inhibitor is bound in the active site of the enzyme. The benzamide moiety of the inhibitor is surrounded by lipophilic residues in which Tyr 146 appears to participate in an orthogonal π-stacking interaction with the aromatic ring. The 4-hydroxy substituent on the ring is closely flanked by Met 206 and Phe 203 which may

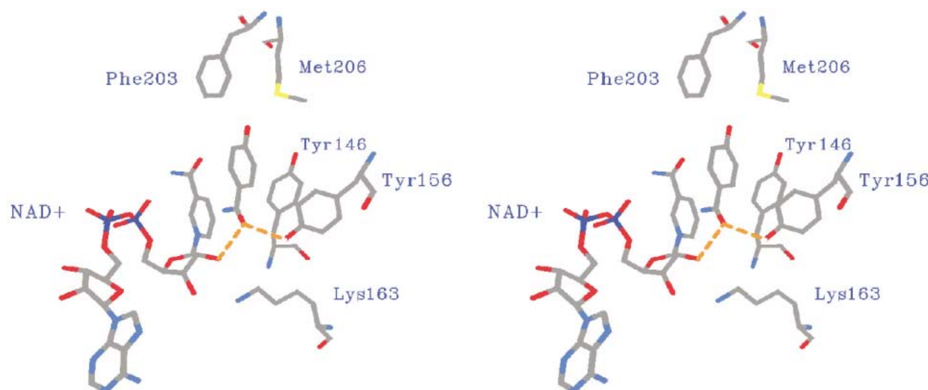


Figure 2. Stereoview of the benzamide portion of inhibitor **3** bound in the active site of *E. coli* FabI/NAD^+. For clarity, only the residues defining the hydrophobic pocket of FabI have been illustrated. The benzamide moiety was revealed by an electron-density map.

Table 3. In vitro antibacterial activity of selected inhibitors with <1 μM *S. aureus* FabI enzyme activity

Compd	MIC (μg/mL)[a]		
	S. aureus	*M. catarrhalis*	*E. coli*
20	0.5	>64	>64
22	4	>64	>64
27	4	8	>64
3	0.5	8	>64
30	1	4	>64
31	1	2	>64
32	1	8	>64
33	1	4	>64
Triclosan	0.03	0.06	4

[a]MICs were repeated at least twice ($n \geq 2$) with data variability of one dilution or less.

account for the steric effects observed in Table 2. The carbonyl oxygen of inhibitor **3** is apparently involved in a hydrogen bonding interaction to the 2′-ribose hydroxyl of NAD^+ and to the hydroxyl group of Tyr 156 (dashed orange lines). This particular active-site interaction is analogous to that reported for Triclosan in which a phenol hydroxyl functions in a similar capacity.[8]

Selected compounds with submicromolar *S. aureus* enzyme activity were tested for in vitro antibacterial activity against *S. aureus* Oxford, *Moraxella catarrhalis* 1502 and *E. coli* 7623 AcrABEFD+ organisms (Table 3). The MICs against *S. aureus* ranged from 0.5 to 4.0 (μg/mL) and corresponded well with FabI enzyme inhibition. Antibacterial activity against the Gram-negative *M. catarrhalis* strain was observed for the hydroxyl containing compounds (**3**, **27**, and **30–33**). While antibacterial activity was not observed against *E. coli*, this was expected due to the relatively poor *E. coli* FabI enzyme activity of these compounds. The MIC values of triclosan were lower than we would have predicted based on the corresponding IC_{50} levels. The high level of in vitro potency for triclosan may be the consequence of having multiple cellular targets,[10] and therefore MICs may not be exclusively attributable to FabI inhibition.

Each compound in Table 3 was tested against a FabI over-expressing strain of *S. aureus*. Thus, if FabI is an antibacterial target of a compound, an increase in MIC relative to the wild type strain would be expected. MIC values for the compounds in Table 3 showed increases of 4- to >16-fold, supporting that the mechanism of action does indeed involve FabI inhibition.

Conclusion

We have identified potent inhibitors of *S. aureus* enoyl acyl carrier protein reductase (FabI) that also demonstrate whole-cell antimicrobial activity in Gram-positive and Gram-negative organisms.

References and Notes

1. Neu, H. C. *Science* **1992**, *257*, 1073.
2. Chu, D. T. W.; Plattner, J. J.; Katz, L. *J. Med. Chem.* **1996**, *39*, 3853.
3. Fulco, A. J. *Prog. Lipid Res.* **1983**, *22*, 133.
4. (a) Bergler, H.; Fuchsbilder, S.; Hoegenauer, G.; Turnowsky, F. *Eur. J. Biochem.* **1996**, *242*, 689. (b) Heath, R. J.; Rock, C. O. *J. Biol. Chem.* **1995**, *270*, 26538.
5. Smith, S. *FASEB J.* **1994**, *8*, 1248.
6. (a) Heath, R. J.; Rock, C. O. *Nature* **2000**, *406*, 145. (b) Heath, R. J.; Su, N.; Murphy, C. K.; Rock, C. O. *J. Biol. Chem.* **2000**, *275*, 40128.
7. (a) Levy, C. W.; Roujeinikova, A.; Rice, D. W.; Rafferty, J. B. *Nature* **1999**, *398*, 383. (b) Heath, R. J.; Yu, Y.-T.; Shapiro, M. A.; Olson, E.; Rock, C. O. *J. Biol. Chem.* **1998**, *273*, 30316.
8. (a) Qiu, X.; Janson, C. A.; Court, R. I.; Smyth, M. G.; Payne, D. J.; Abdel-Meguid, S. S. *Protein Sci.* **1999**, *8*, 2529. (b) Heath, R. J.; Rubins, J. R.; Holland, D. R.; Zhang, E.; Snow, M. E.; Rock, C. O. *J. Biol. Chem.* **1999**, *274*, 11110.
9. Crystallization conditions and structure determination procedures are similar to previously published results.[8a] The flipping loop of the enzyme is disordered. The crystal structure has been solved and refined to 2.4 Å resolution ($R = 0.209$, R_f 0.277). The atomic coordinates have been deposited at the Protein Data Bank (PDB) (accession number 1I30).
10. (a) McDonnell, G.; Pretzer, D. Actions and Targets of Triclosan. *ASM News* **1998**, *64*, 670. (b) Russell, A. D. *J. Appl. Microbiol.* **1997**, *83*, 155.