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Inhibitors of FabI, an Enzyme Drug Target in the Bacterial Fatty Acid Biosynthesis Pathway

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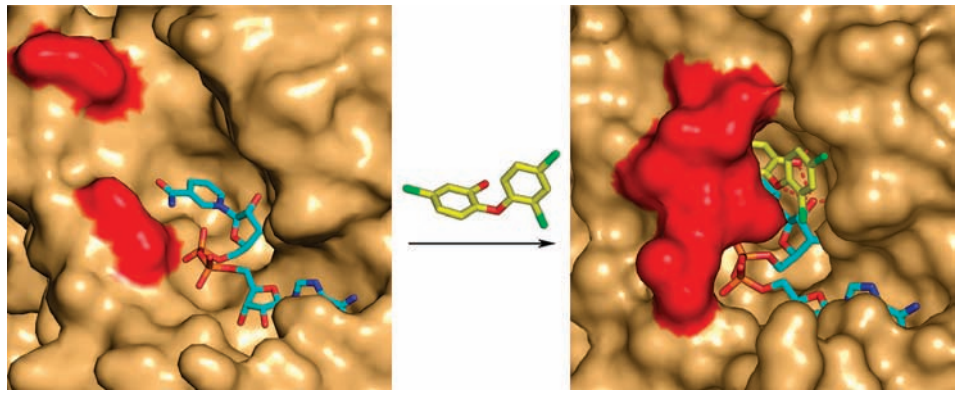

The modern age of drug discovery, which had been slowly gathering momentum during the early part of the twentieth century, exploded into life in the 1940s with the isolation of penicillin and streptomycin. The immense success of these early drug discovery efforts prompted the general view that many infectious diseases would now be effectively controlled and even eradicated. However this initial optimism was misplaced, and pathogens such as multidrug-resistant *Mycobacterium tuberculosis* and methicillin-resistant *Staphylococcus aureus* present a major current threat to human health. Drug resistance arises through the unrelenting pressure of natural selection, and there is thus a continuing need to identify novel drug targets and develop chemotherapeutics that circumvent existing drug resistance mechanisms. In this Account, we summarize current progress in developing inhibitors of FabI, the NADH-dependent enoyl reductase from the type II bacterial fatty acid biosynthesis pathway (FAS-II), a validated but currently underexploited target for drug discovery. The FabI inhibitors have been divided into two groups, based on whether they form a covalent adduct with the NAD^+ cofactor. Inhibitors that form a covalent adduct include the diazaborines, as well as the front-line tuberculosis drug isoniazid. The NAD adducts formed with these compounds are formally bisubstrate enzyme inhibitors, and we summarize progress in developing novel leads based on these pharmacophores. Inhibitors that do not form covalent adducts form a much larger group, although generally these compounds also require the cofactor to be bound to the enzyme. Using structure-based approaches, we have developed a series of alkyl diphenyl ethers that are nanomolar inhibitors of InhA, the FabI from *M. tuberculosis*, and that are active against INH-resistant strains of *M. tuberculosis*. This rational approach to inhibitor development is based on the proposal that high-affinity inhibition of the FabI enzymes is coupled to the ordering of a loop of amino acids close to the active site. Compounds that promote loop ordering are slow onset FabI inhibitors with increased residence time on the enzyme. The diphenyl ether skeleton has also been used as a framework by us and others to develop potent inhibitors of the FabI enzymes from other pathogens such as *Escherichia coli*, *S. aureus*, and *Plasmodium falciparum*. Meanwhile chemical optimization of compounds identified in high-throughput screening programs has resulted in the identification of several classes of heteroaromatic FabI inhibitors with potent activity both *in vitro* and *in vivo*. Finally, screening of natural product libraries may provide useful chemical entities for the development of novel agents with low toxicity. While the discovery that not all pathogens contain FabI homologues has led to reduced industrial interest in FabI as a broad spectrum target, there is substantial optimism that FabI inhibitors can be developed for disease-specific applications. In addition, the availability of genome sequencing data, improved methods for target identification and validation, and the development of novel approaches for determining the mode of action of current drugs will all play critical roles in the road ahead and in exploiting other components of the FAS-II pathway.

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Introduction

While there are documented uses of natural products to treat disease throughout human history, it was not until the latter part of the 19th and early 20th centuries that specific antibacterials started to be identified. Advances around this time included the synthesis of the arsenate-based compound Salvarsan by Ehrlich to treat syphilis,[1] the serendipitous discovery of lysozyme and penicillin by Fleming,[2,3] and the determination that sulfanilamide was the active metabolite in the dye-based drug Prontosil.[4] These discoveries laid the foundation for the modern age of drug development that started in the late 1930s with the isolation of penicillin from the mold *Penicillium notatum* by Chain and Florey.[5] This was almost immediately followed by the isolation of tyrothricine from *Bacillus brevis* by Dubos,[6] which prompted large scale efforts to identify antimicrobial products from soil bacteria and thence the isolation of streptomycin from *Streptomyces griseus* by Waksman and colleagues.[7]

Streptomycin was the first effective antibiotic for the treatment of tuberculosis (TB). The introduction of streptomycin together with additional TB drugs over the next 20 years caused a dramatic reduction in mortality from TB, which had been around 50%. Indeed, so profound was the impact of antibiotics that it was generally believed that diseases such as TB would soon be eradicated. Consequently the pace of antimicrobial drug discovery slowed,[8] and indeed no new classes of antibiotics were introduced between 1962 and 2000.[9] However, adaptation through natural selection has of course led to the emergence of drug-resistant pathogens,[10−12] and thus there is now a critical need to develop novel chemotherapeutics that can evade current resistance mechanisms. In the case of TB, the WHO estimates that a third of the world's population is infected with the latent form of *Mycobacterium tuberculosis* and that over 450 000 people worldwide are infected with multidrug-resistant TB (MDR-TB).[13,14] In addition, strains of extensively drug-resistant *M. tuberculosis* (XDR-TB) have now emerged that are resistant to both first and second line drugs. XDR-TB is almost impossible to treat, thus posing a serious threat to human health. Furthermore, while drugs for MDR-TB are needed, there must also be a major effort to shorten the course of the standard 6−9 month treatment regime and to develop drugs that are active against the latent, nonreplicating form of the disease.[15−17]

Although there are now many antibiotics, these compounds target only a small fraction of metabolic space, localizing primarily to targets in DNA replication, transcription, and translation, as well as in peptidoglycan synthesis.[18−21] The reason for this concentration on a relatively small number of targets is unclear and may be because only a small fraction of metabolic enzymes are actually essential[22] or because these are the preferred targets for natural products. Advances in DNA sequencing have provided additional impetus in the search for novel drug targets, and it is expected that in the next few years more than 100 bacterial DNA sequences will be completed.[23] While these efforts will assist in the discovery of genomics-derived targets, it will be important to ensure that the drug target is present in all strains.[24]

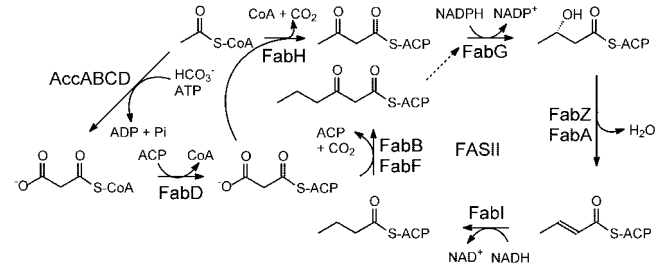

FIGURE 1. The fatty acid biosynthesis pathway in *E. coli*. Enzymes shown in the figure include the acetyl-CoA carboxylase (AccABCD), malonyl-CoA:ACP transacylase (FabD), β-ketoacyl reductase (FabG), β-hydroxyacyl dehydrase (FabA or FabZ in *E. coli*), and enoyl reductase (FabI). The initial condensation reaction is catalyzed by β-ketoacyl synthase III (FabH), while further rounds of elongation are initiated by FabB or FabF (β-ketoacyl synthase I and II).

A Novel Target for Drug Discovery: The Bacterial Fatty Acid Biosynthesis Pathway

The bacterial fatty acid biosynthesis pathway (FAS-II) (Figure 1) represents a validated and yet relatively unexploited target for drug discovery. Although fatty acids are essential for bacterial growth, they can not be scavenged from the host and must be synthesized *de novo*.[23,25] In addition, while components of the FAS-II pathway are highly conserved across many pathogens, the bacterial system, in which each reaction is performed by individual enzymes, is fundamentally distinct from the multienzyme FAS-I complex found in mammals.[26,27] Finally, genetic knockout and knockdown experiments coupled with the use of target-specific inhibitors have confirmed that the FAS-II pathway is essential for bacterial cell survival.[25,27] Access to complete bacterial genome sequences has led to the identification of many FAS-II components from different pathogens.[25] Moreover, structural data are available for many of the homologues,[28] which provides a good starting point for the rational design of novel FAS-II enzyme inhibitors. Below, we summarize the current status of efforts to inhibit the reaction in this pathway catalyzed by the enoyl reductase (FabI).

The Enoyl Reductase (FabI)

The last reaction in each round of elongation involves the reduction of an enoyl-ACP by the enoyl reductase enzyme. In most organisms, this reaction is catalyzed by FabI, an NADH-dependent enzyme encoded by the *fabI* gene. However, some organisms also contain the flavoprotein FabK in addition to FabI, while FabK is the sole enoyl reductase in *Streptococcus pneumoniae*.[29] In addition, *Bacillus subtilis* has been shown to contain both FabI and the enoyl reductase FabL.[30] Although FabI inhibitors have been reported that also have some activity against FabK, the discovery of FabK and the absence of FabI in some pathogens has reduced the likelihood that this enzyme class can be targeted by broad spectrum antibacterials.[24] As will be discussed below, however, the enoyl reductases remain an excellent target for narrow spectrum drug discovery.

FabI was initially shown to be essential for bacterial viability by Bergler et al.[31] The FabI enzymes are members of the short-chain alcohol dehydrogenase/reductase (SDR) superfamily characterized by a catalytic triad that includes a tyrosine and a lysine residue.[32] In the dehydrogenases, the third component of the triad is a serine, while in the reductases this residue is a tyrosine or phenylalanine.[28,33] While the overall structural homology between the FabIs is high, variability exists in a mobile loop of amino acids that covers the active site (the substrate binding loop).[34,35] Below we briefly summarize existing FabI inhibitors, separated into two general classes based on whether compounds form a covalent adduct with the NAD cofactor.

FabI Inhibitors That Covalently Modify the Cofactor. The diazaborines are a class of heterocyclic boron-containing compounds **1** (Figure 2) that inhibit FabI via the formation of a covalent bond between the boron atom and the 2′-hydroxyl of the NAD^+ ribose (Figure 3).[36] The diazaborine group binds in the active site where the enoyl substrate is normally located, and thus the diazaborine−NAD adduct is a bisubstrate FabI inhibitor.[36] SAR studies have shown that the diaza-moiety and the boron atom are essential for activity,[37,38] while chemical optimization has resulted in a series of derivatives **2** with antibacterial activity.[38] Benzodiazaborine **3** derivatives were also synthesized, with MIC values as low as 8 μg/mL against *M. tuberculosis* $H37_{RV}$.[39]

Although isoniazid (INH, **4**) was introduced in 1952, the cellular target for this drug remained obscure until 1994 when a missense mutation within the mycobacterial *inhA* gene was shown to confer resistance to INH.[40] Subsequently it was shown that the product of the *inhA* gene was the *M. tuberculosis* FabI protein (InhA).[41] Although mutations in InhA correlate with resistance to INH,[42] the majority of resistance arises from mutations in KatG, the mycobacterial catalase−peroxidase enzyme.[43] KatG is responsible for INH activation, resulting in the formation of an adduct with NAD^+ (the INH−NAD adduct) **5** (Figure 2).[44] We have shown that the adduct is a slow onset inhibitor of InhA, binding tightly to the enzyme with an overall K_i value of 0.75 nM.[45] Structural studies have revealed that, like the diazaborine−NAD adduct, the INH−NAD adduct is a bisubstrate inhibitor, and that enzyme-inhibition is coupled to ordering of the substrate binding loop (Figure 3).[36,46]

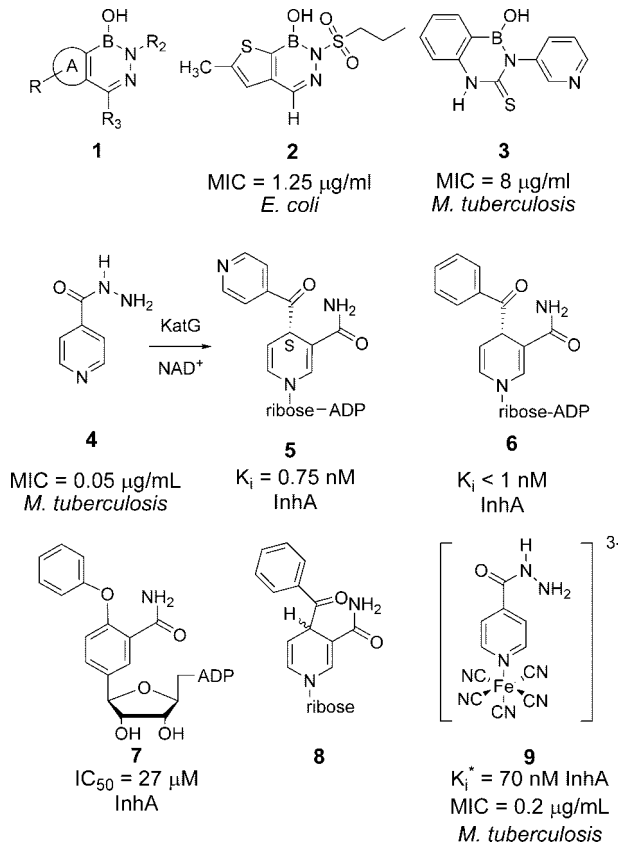

FIGURE 2. FabI inhibitors based on diazaborines and isoniazid. Structures of the diazaborine skeleton **1**, thiodiazaborine **2**, and benzodiazaborine **3** are shown. Ring A in structure **1** can be benzene, naphthalene, thiophene, furan, or pyrrole. Isoniazid **4** reacts with NAD^+ catalyzed by KatG to form the INH−NAD adduct **5**. Compounds **6**−**8** are derivatives of INH−NAD, while **9** is a derivative of INH. ADP is adenosine. Data were taken from refs 38 (**2**), 39 (**3**), 59 (**4**), 45 (**5**), 51 (**7**), 52 (**8**), and 54 (**9**) and unpublished work by Tonge (**6**).

Although InhA is clearly a target for INH, the mode of action of this drug is complex,[47] and it is likely that there are other targets in the cell. The observation that InhA mutations observed in INH-resistant clinical isolates have only a small impact on binding of the INH−NAD adduct to the enzyme

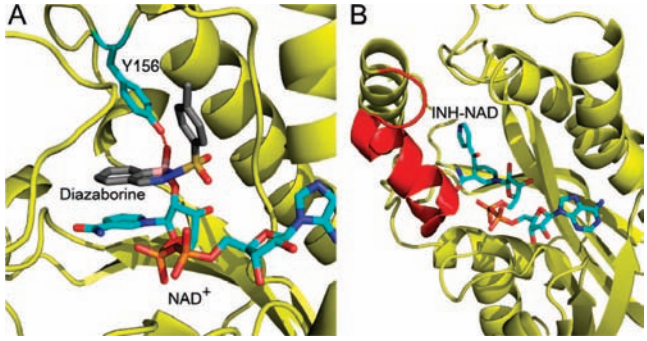

FIGURE 3. Structure of benzodiazaborine–NAD and isoniazid–NAD adducts bound to their target FabI enzymes: (A) structure of the 2-(toluene-4-sulfonyl)-benzodiazaborine–NAD adduct bound to ecFabI (yellow), in which a hydrogen bond is shown between the inhibitor and the conserved tyrosine, Y156, and the diazaborine is colored grey while the NAD and Y156 are cyan (1dfg.pdb);[36] (B) structure of the INH–NAD adduct (cyan) bound to InhA (yellow) (1zid.pdb).[46] The substrate binding loop is colored red. The figures were made using Pymol.[82]

prompted us to suggest that enzyme inhibition inside the cell is modulated by protein–protein interactions within a multienzyme noncovalent FAS-II complex.[45] Additionally, reaction of activated INH with NAD(P) results in several different chemically distinct structures that might inhibit other targets in the cell. In this regard, Blanchard and co-workers have demonstrated that the INH–NADP adduct is a high-affinity inhibitor of dihydrofolate reductase and have identified a number of proteins in mycobacteria that can bind adducts formed between INH and NAD(P).[48,49]

Efforts to build on the antimycobacterial potency of INH have proceeded in two directions. Several chemical classes of antimycobacterial compounds based on INH have been synthesized, although it is not known whether these compounds inhibit InhA directly.[50] In addition, synthetic analogues of the INH–NAD adduct have been explored (Figure 2) and while the benzoic hydrazide analogue **6** (BNH–NAD) is a potent InhA inhibitor (Tonge unpublished), other analogues such as **7** and **8** were less active but hold significant promise as lead compounds for InhA inhibitor discovery.[51,52] An inorganic complex **9** based on INH has also been synthesized and interestingly is a slow onset inhibitor of InhA.[53] *In vitro* kinetic assays and docking experiments suggest that inactivation of InhA buy this complex does not require the presence of NADH and that the inhibitor possibly interacts with the NADH binding site of the enzyme.[54] Hence it is reasonable to predict that inhibition of this compound does not require binding of NAD^+ to the enzyme. It is also important to point out that compounds such as **7–9** do not require activation by KatG and thus may represent useful leads for developing novel agents that are active against INH-resistant TB.

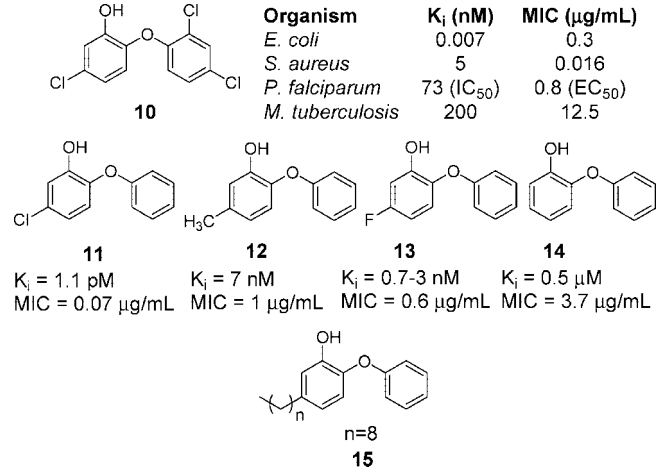

FIGURE 4. Diphenyl ether inhibitors of FabI. Structures of triclosan **10** and 5-substitued diphenyl ethers **11** Cl, **12** Me, **13** F, and **14** H are shown. Compound **15** is the generic structure of the alkyldiphenyl ether InhA inhibitors where the alkyl chain can be 1–14 carbons. Data for triclosan **10** were taken from ref 56 (*E. coli*), unpublished work by Tonge (*S. aureus*), ref 66 (*P. falciparum*), and refs 59 and 67 (*M. tuberculosis*). For compounds **11–15**, the data given are those for *E. coli* from ref 56.

FabI Inhibitors That Interact Noncovalently with the Enzyme–Cofactor Binary Complex. Diphenyl Ether Inhibitors of ecFabI, saFabI, and pfFabI. Triclosan **10** is a broad spectrum antimicrobial that is included in a wide range of consumer products (Figure 4). While triclosan was long thought to have no specific cellular target, genetic selection experiments[55] followed by kinetic and structural studies have revealed that triclosan is a potent inhibitor of the FabI enzymes from many organisms. Triclosan is a slow, tight-binding inhibitor of ecFabI, binding to the E/NAD^+ product complex with a K_i value of 7 pM.[56–58] The triclosan phenol ring forms a stacking interaction with the oxidized nicotinamide, while both the triclosan hydroxyl group and ether oxygen participate in hydrogen-bonding interactions involving the conserved active site tyrosine (Y156 in ecFabI) and the 2′ hydroxyl of NAD^+.[35] Thus, although triclosan is not covalently attached to NAD^+, the structural features of the triclosan–NAD^+ complex are strongly reminiscent of the complex formed between FabI, NAD^+, and the diazaborine inhibitors. Finally, binding of triclosan to ecFabI results in ordering of a loop of amino acids close to the active site (the substrate binding loop) (Figure 5),[35] and this has been proposed to account for the slow step in formation of the final enzyme–inhibitor complexes involving triclosan, as well as other slow onset inhibitors.[59]

We have performed extensive SAR studies and have shown that the A-ring chlorine (5-chloro) is critical for the high affinity inhibition of ecFabI.[56] Indeed, **11** binds 7-fold more tightly

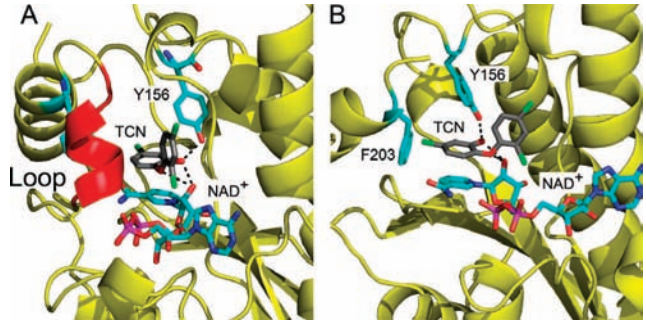

FIGURE 5. Structure of triclosan complexed with ecFabI and NAD^+: (A) structure of ecFabI (yellow) complexed with NAD^+ (cyan) and triclosan (grey) (1qsg.pdb)[35] with the substrate binding loop colored red and triclosan shown interacting with Y156 (cyan) and the NAD^+ ribose hydroxyl; (B) the same structure with the loop omitted for clarity in which F203 (cyan) can now be seen close to the triclosan A ring. The figures were made using Pymol.[82]

to ecFabI than triclosan (K_i = 1 pM), while **12** and **13** bind 1,000-fold less tightly. In addition, while **10** and **11** bind exclusively to the E:NAD^+ product complex, **12** binds to both the E/NAD^+ and E/NADH enzyme complexes, while **13** binds exclusively to the E/NADH complex. Thus, subtle steric and electronic changes to the triclosan A ring have a dramatic effect on interactions with ecFabI. In contrast to **10**–**13**, **14** is a classical rapid uncompetitive inhibitor of ecFabI with a K_i value of 0.5 μM, highlighting the importance of the 5-substituent for high-affinity slow onset inhibition. The alteration in affinity of **10**–**14** for ecFabI correlates well with their *in vitro* antibacterial activity toward *Escherichia coli*, with MIC_{99} values ranging from 0.07 μg/mL for **11** to 3.7 μg/mL for **14**.[56] The strong correlation between K_i and MIC suggests that the antibacterial activity of the diphenyl ether analogues results directly from inhibition of ecFabI. An important recent development shows that the experimental binding free energies for the diphenyl ether compound series can be reproduced using computational studies, paving the way for the rational design of additional high-affinity compounds.[60] The calculations utilized the molecular mechanics Poisson–Boltzmann surface area method, which showed that the van der Waals energies are the most correlated component of the total affinity, indicating that the shape of the inhibitor is very important in defining the binding energies for this system.

In addition to analoging studies, we have also explored the effect of mutagenesis on triclosan binding and have verified the importance of F203, M159, and G93 in high-affinity enzyme inhibition.[57] These residues, which were originally identified in the genetic selection experiments performed by Levy and co-workers,[55] surround the bound inhibitor, with F203 lying close to the 5-substituent (Figure 5). Resistance to triclosan in other organisms has also been correlated with mutations in FabI (*Staphylococcus aureus* and *M. smegmatis*),[61,62] as well as increased expression of FabI (*S. aureus* and *M. tuberculosis*),[62,63] supporting the hypothesis that FabI is the target for triclosan. Mutations in the FabI promoter or structural gene are not the only mechanisms of resistance, with efflux also playing an important role in organisms such as *Pseudomonas aeruginosa.*[64]

In addition to ecFabI, triclosan is also a potent inhibitor of the FabI enzymes from *S. aureus* and *Plasmodium falciparum* (Figure 4). Efforts to improve the affinity and antibacterial activity of triclosan toward *P. falciparum* have involved synthesizing a series of analogues with different substituents on both rings,[65] and recently several 5-substituted diphenyl ethers were reported that inhibit pfFabI with IC_{50} values in the low nanomolar range and that are active against the parasite with activity in the 1–2 μM range.[66]

Diphenyl Ether Inhibitors of InhA. While triclosan is a potent inhibitor of ecFabI, saFabI, and pfFabI, this compound only inhibits InhA with a K_i value of 0.2 μM.[67] In addition, triclosan has only modest activity toward *M. tuberculosis* (MIC_{99} = 12.5 μg/mL for $H37_{Rv}$).[59] Since resistance to INH results primarily from mutations in KatG, we and others have proposed that inhibitors of InhA that do not require activation should be effective against INH-resistant TB. X-ray structural data indicates that binding of triclosan to InhA in the presence of NAD^+ does not cause ordering of the substrate binding loop. Since triclosan is a rapid reversible inhibitor of InhA, these observations led to the hypothesis that high-affinity, slow binding inhibition of FabI enzymes was coupled to loop ordering. This hypothesis was supported by the knowledge that triclosan is a slow onset inhibitor of ecFabI while the INH–NAD adduct is a slow onset inhibitor of InhA and that in both cases inhibitor binding results in loop ordering. Slow onset inhibition of InhA by the INH-NAD adduct may be one reason why INH is such an effective drug, since compounds that bind slowly to form the final enzyme-inhibitor complex will also dissociate more slowly from the enzyme, thereby increasing their residence time on the target and thus improving their *in vivo* activity.[83] We thus set out to design compounds based on the diphenyl ether core that would increase contacts between the inhibitor and loop residues in InhA with the objective of promoting loop ordering and hence increasing the residence time of the inhibitors on the enzyme. We subsequently developed a series of alkyl diphenyl ethers, the most potent of which (8PP) inhibits InhA with a K_i value of 1 nM (**15** in Figure 4) .[59] Significantly, this compound is active against both sensitive and INH-resistant strains of *M. tuberculosis* with an MIC_{99} value of 1–2 μg/mL. For the alkyl diphenyl ethers, there is a good

TABLE 1. Diphenyl Ether InhA Inhibitors[a]

compound[b]	IC_{50} (nM)	K_i' (nM)	MIC_{99}, $\mu g\ mL^{-1}$ [c]			
			$H37_{Rv}$	$H37_{Rv}$ pMH29:*inhA*	TN587	NHN382
triclosan **(10)**	1000 ± 100	220 ± 20	12.5 ± 0	33.3 ± 12.9	12.5 ± 0	12.5 ± 0
2PP	2000 ± 700	*d*	3.8 ± 0	*d*	*d*	*d*
4PP	80 ± 15	*d*		*d*	*d*	*d*
5PP	17 ± 5	11.8 ± 4.5	1.0 ± 0	*d*	*d*	*d*
6PP	11 ± 1	9.4 ± 0.5	2.1 ± 0.9	18.8 ± 6.8	2.0 ± 1.0	3.1 ± 0
8PP	5.0 ± 0.3	1.1 ± 0.2	1.9 ± 0.5	22.9 ± 5.1	2.0 ± 1.0	2.6 ± 0.9
14PP	150 ± 24	30.3 ± 4.7	175	*d*	*d*	*d*
INH		0.75 ± 0.08	0.05 ± 0	*d*	2.4 ± 1.3	1.6 ± 0

[a] Data from ref 59. [b] 2PP, 4PP, 5PP, 6PP, 8PP, and 14PP are the diphenyl ethers with ethyl, butyl, pentyl, hexyl, octyl, and tetradecyl substituents at the 5 position (**15**). [c] MIC_{99} values are given for $H37_{Rv}$, a drug-sensitive strain of *M. tuberculosis*, and two INH-resistant strains, TN587 and NHN382. MIC_{99} values were also determined in a strain of $H37_{Rv}$ in which InhA was overexpressed using the pMH29:*inhA* vector. [d] Not determined.

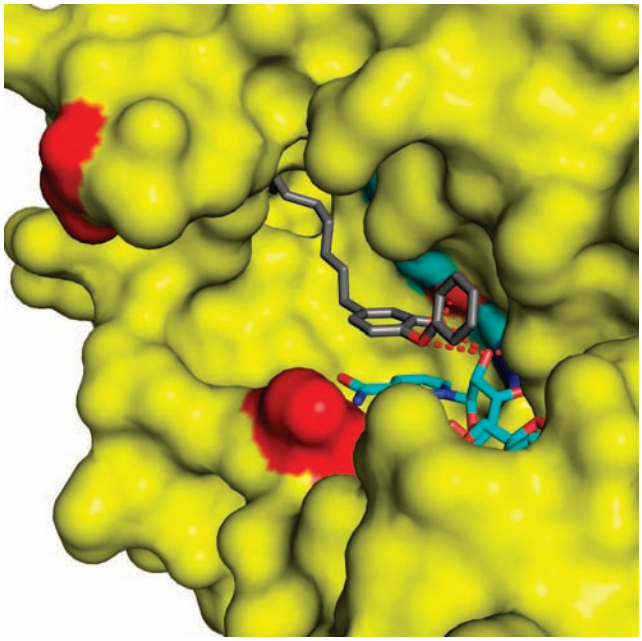

FIGURE 6. Structure of 5-octyl-2-phenoxyphenol (grey) (8PP; **15** $n = 7$) bound to InhA (yellow) in the presence of NAD^+ (cyan) (2b37.pdb)[59] The disordered loop termini are colored red. The figure was made using Pymol.[82]

correlation between the K_i and MIC_{99} values, while overexpression of InhA in *M. tuberculosis* results in a 10-fold increase in the MIC_{99} value for 8PP (Table 1). These data support the contention that the compounds target InhA within the mycobacterium and are consistent with the hypothesis that InhA inhibitors that circumvent the requirement for KatG activation should be active against INH-resistant strains of *M. tuberculosis*.[59,68]

Although the alkyl diphenyl ethers were designed with the intention of promoting loop ordering, structural studies reveal that the loop is disordered in the InhA/NAD^+/8PP complex (Figure 6).[59] This observation is in agreement with the knowledge that 8PP is a rapid reversible uncompetitive inhibitor of InhA and indicates that further structural elaboration is required to promote loop ordering.

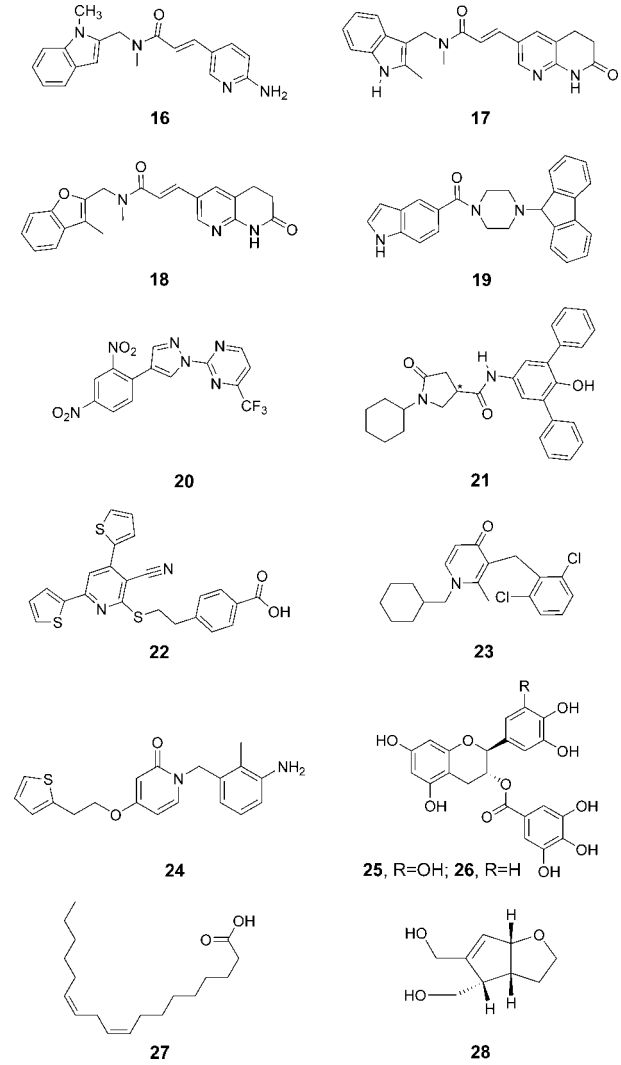

FIGURE 7. Structures of the FabI inhibitors identified through screening programs.

Current inhibitor development efforts are focused on improving the oral bioavailability of these compounds while retaining their antibacterial activity. The mode of action of the alkyl diphenyl ethers is also being explored using transcriptional profiling experiments, which have revealed that

TABLE 2. Other FabI Inhibitors[a]

		E. coli		*S. aureus*	
number	inhibitor/chemotype	IC_{50} (μM)	MIC (μg/mL)	IC_{50} (μM)	MIC (μg/mL)
16	indole aminopyridines	4.2[b]	>64[b]	2.4	0.5
17	indole naphthyridinones	<0.06	0.5	0.05	0.016
18	benzofuran derivative of **17**	*d*	0.03[c]	*d*	0.015
22	thiopyridine series	3	*d*	*d*	0.75
23	4-pyridone series	0.22	*d*	*d*	0.25
24	CG400549	*d*	*d*	*d*	0.5–8
25	(−)-gallocatechin gallate (GCG)	5	90	*d*	*d*
27	linoleic acid	*d*	*d*	35	56

		M. tuberculosis		*P. falciparum*	
number	inhibitor/chemotype	IC_{50} (μM)	MIC (μg/mL)	IC_{50} (μM)	MIC (μg/mL)
19	indole-piperazine (Genz-10850)	0.16	>12	18	6–13
20	pyrazole series (Genz-8575)	2.4	0.5–12	32	4–6
21	pyrrolidine carboxamide series	0.062	>56	*d*	*d*
26	(−)-catechin gallate (CG)	*d*	*d*	0.3	0.18
28	iridoid-related aglycone	*d*	*d*	600	40.6

[a] IC_{50} values are given for enzyme inhibition. MIC values are for *in vitro* antibacterial activity. Data were taken from references 69 (**16**), 70 (**17**), 71 (**18**), 68 (**19** and **20**), 72 (**21**), 73 (**22**), 74 (**23**), 75 (**24**), 76 (**25**), 77 (**26**), 79 (**27**), and 80 (**28**). [b] *H. influenzae*. [c] *S. epidermidis*. [d] Not reported.

the compounds fail to upregulate detoxification mechanisms triggered by triclosan.[59]

FabI Inhibitors Developed through Screening Programs. High-throughput screening programs at GlaxoSmithKline followed by chemical optimization have identified several classes of FabI inhibitors (Figure 7, Table 2). These efforts led to the development of several indole-based compounds such as **16** or **17**.[69,70] Both compound classes showed excellent *in vitro* and *in vivo* activity against *S. aureus*. Crystal structures of these compounds bound to FabI have been solved, demonstrating that they occupy the region of the active site where the enoyl substrate is expected to bind and that they cause ordering of the active site loop.[70] The observation that inhibition results in loop ordering suggests that these compounds are slow-onset enzyme inhibitors, and thus that their *in vivo* activity may result in part from increased residence time on the enzyme.[83] A benzofuran analogue of the napthopyridone series **18** is currently being developed by Affinium Pharmacueticals.[71] Interestingly, the napthopyridone **17** also showed limited activity against FabK. However, **17** was significantly less active against FabK than FabI, explaining the reduced antibacterial activity of this compound against pathogens such as *Str. pneumoniae*. This observation reinforces a point made earlier that the enoyl reductase enzyme class is likely a better target for the development of narrow rather than broad spectrum antibacterials.

Screening programs have also identified other chemical classes of InhA inhibitors including indole-piperazines **19**, pyrazole-based inhibitors **20**,[68] and pyrrolidine carboxamide inhibitors **21**.[72] In the case of **19** and **21**, structure-based chemical optimization produced compounds with sub-micromolar IC_{50} values. Structural data indicate that the inhibitors bind in the enoyl substrate binding site. Other classes of FabI inhibitors that have been discovered include compounds that incorporate thiopyridine **22** and 4-pyridone **23** (Figure 7, Table 2) moieties.[73,74] More recently, Crystal Genomics identified a 2-pyridone, CG400549 **24**, with potent antibacterial activity against several clinically drug-resistant strains of *S. aureus. In vivo* activity of this compound was also observed in an animal model of staphylococcal infection.[75] This compound bears interesting structural similarities to the diphenyl ether class of FabI inhibitors.

Natural Product FabI Inhibitors. Rock and co-workers screened a panel of green tea catechins and related plant polyphenols, and identified compounds such as (−)-gallocatechin gallate (GCG) **25** (Figure 7, Table 2) that inhibited bacterial fatty acid biosynthesis through an effect on both FabI and FabG.[76] In addition, screening of a flavonoid-containing series of natural products resulted in the identification of (−)-catechin gallate **26**, which had an IC_{50} value of 0.3 μM for pfFabI. The latter molecule had good antimicrobial activity with *in vitro* IC_{50} values of 3.2 and 0.4 μM for chloroquine-sensitive and -resistant strains of *P. falciparum*, respectively.[77] A recent study also indicated that these tea catechins and related polyphenols potentiate triclosan binding to pfFabI.[78] Finally, two other natural products, linoleic acid **27**[79] and an iridoid-related aglycone **28** extracted from *Scrophularia lepidota*,[80] were also shown to have moderate antimicrobial activity. The report that compound **25** inhibits FabG in addition to FabI indicates that some of these compounds might have multiple metabolic targets. In general, however, these natural compounds display very low toxicity to humans and thus rep-

resent potentially useful leads for the development of novel FabI inhibitors.

Summary

Aggressive efforts must be made to develop new antimicrobial drugs in order to keep pace with the emergence of organisms that are resistant to current chemotherapeutics, and there must be a concerted effort to identify and inhibit novel drug targets. As discussed above, while a significant fraction of metabolic space is not currently targeted by existing drugs, studies suggest that the range of new "druggable" targets may be limited. The inhibitor discovery efforts described in this Account firmly validate the microbial fatty acids biosynthesis pathway as a target for drug discovery. While the discovery of FabK in some organisms has reduced the likelihood that broad spectrum enoyl reductase inhibitors can be developed, there is clear evidence that FabI is an excellent target for the development of narrow spectrum antimicrobials that selectively target pathogens such as *M. tuberculosis*, *P. falciparum*, and drug-resistant *S. aureus.* Although outside the scope of the present Account, it is also important to note that other steps in the FAS-II pathway are candidates for drug development. In particular, the β-ketoacyl-ACP synthases are also validated targets for inhibitor discovery, although efforts have been hampered until recently by the lack of compound selectivity between the bacterial and mammalian FAS pathways.[81] In addition, the β-ketoacyl-ACP reductase is highly conserved and widely expressed with only a single isoform known in bacteria and thus provides a good drug target for board spectrum antimicrobial development. Conversely, the lack of obvious homologues for the β-hydroxyacyl dehydrase in some organisms suggests the presence of novel proteins that might be good targets for the development of selective antimicrobials. While the wealth of information from genome sequencing and analysis projects will be important for identifying FAS-II homologues in pathogens, the presence of multiple targets in this pathway presents the possibility of developing synergistic chemotherapeutic regimes that intervene simultaneously at multiple points in the biosynthesis of fatty acids.

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BIOGRAPHICAL INFORMATION

Hao Lu was born in Zhejiang, China, on September 13, 1983. He attended the Chinese University of Hong Kong and obtained his B.Sc. in Chemistry in 2005. He was admitted to the Chemistry Ph.D. program at Stony Brook University in 2005, where he is now a member of Professor Tonge's research group. His current research focuses on the enzymatic mechanism of FabI from *Francisella tularensis* and the synthesis of novel agents for treating tularemia.

Peter Tonge was born in Northampton, England, in 1961. He obtained his B.Sc. and Ph.D. degrees in biochemistry at the University of Birmingham and then moved to the National Research Council of Canada (NRC) in 1986 as a NATO-SERC postdoctoral fellow. After spells at NRC as a Research Associate and a Research Officer, followed by an appointment as a Staff Investigator at The Picower Institute for Medical Research, he joined the faculty at Stony Brook University in 1996 where he is currently a full Professor. His research focuses on using precise information on enzyme mechanisms to develop enzyme inhibitors with a specific focus on antibacterial drug discovery. He also uses steady-state and ultrafast vibrational spectroscopy to probe enzyme mechanisms and to understand the photochemistry of fluorescent as well as light-activated proteins.

FOOTNOTES

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