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1,4-Disubstituted Imidazoles are Potential Antibacterial Agents Functioning as Inhibitors of Enoyl Acyl Carrier Protein Reductase (FabI)

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Abstract—1,4-Disubstituted imidazole inhibitors of *Staphylococcus aureus* and *Escherichia coli* enoyl acyl carrier protein reductase (FabI) have been identified. Crystal structure data shows the inhibitor **1** bound in the enzyme active site of *E. coli* FabI.

Introduction

The emergence of pathogens resistant to known antibiotic therapy is becoming a serious global healthcare problem.[1] Enoyl acyl carrier protein reductase (FabI) was identified as a novel antibacterial target. This enzyme catalyzes the final reaction in bacterial fatty acid synthesis. It has recently been shown that FabI[2a] catalyzes this reaction in *Staphylococcus aureus* and *Escherichia coli* and that FabK catalyzes the reaction in other pathogens, such as *Streptococcus pneumoniae*.[2b] Eukaryotes produce fatty acids via a FAS I system, where the lipids are synthesized using a multifunctional enzyme complex in which all of the catalytic domains reside on one or two polypeptide chains.[3] The ACP is an integral part of this complex. In contrast, prokaryotes utilize the FAS II system where the enzymes which catalyze the individual steps are found on seperate polypeptide chains and the ACP is a discreet protein.[4] Therefore, there is considerable potential for selective inhibition of bacterial fatty acid biosynthesis. Here we describe a series of novel FabI inhibitors.

Compounds from our proprietary compound collection were screened against *S. aureus* FabI and imidazole **1** (Table 1) was identified as a lead ($IC_{50} = 1.24\,\mu M$). Herein we describe the results of our preliminary investigation into the structure–activity relationship (SAR) of a series of 1,4-disubstituted imidazoles related to **1** as FabI inhibitors. The in vitro anti-bacterial activity of selected compounds against a representative Gram-positive and Gram-negative organism is also reported.

Chemistry

The 1,4-disubstituted imidazoles were prepared using one of two methods illustrated in Scheme 1. *p*-Anisaldehyde was condensed with tosylmethyl isocyanide (TOSMIC) to give the corresponding oxazoline **3**. The crude oxazoline was heated with excess 4-methylbenzylamine to give **4** (method A).[5] Alternatively, 3-thiopheneboronic acid was coupled to 4-iodoimidazole[6] under Suzuki conditions[7] to give **7**. Treatment of **7** with 3-nitrobenzylbromide in the presence of K_2CO_3 gave **8** (method B).[8]

The regio-isomeric imidazole **11** was prepared according to the method shown in Scheme 2. The bromoketone **9** was condensed with the amidine **10** to give **11**.[9]

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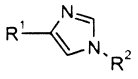
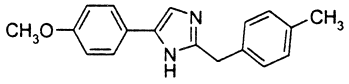
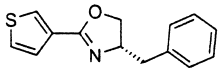
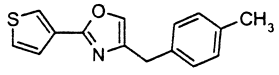
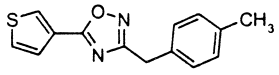
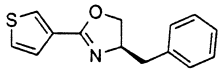
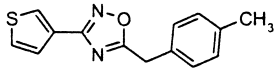
Table 1. *S. aureus* FabI and *E. coli* FabI inhibition data

No.	Structure		Method	*S. aureus* FabI IC_{50} (μM)	*E. coli* FabI IC_{50} (μM)
					
	R^1	R^2			
1	2-Thienyl	4-Me–C_6H_4–CH_2–	A	1.24±0.03	13.7±1.7
4	4-MeO–C_6H_4–	4-Me–C_6H_4–CH_2–	A,B	0.36±0.17	3.92±0.02
8	3-Thienyl	3-O_2N–C_6H_4–CH_2–	B	14.7±0.65	26.9±3.7
20	3-Thienyl	4-H_2N–C_6H_4–CH_2–	A	2.03±0.04	5.14±0.42
21	3-Thienyl	4-MeO–C_6H_4–CH_2–	A,B	1.90±0.08	9.40±0.35
22	3-Thienyl	3-(HO_2C)–C_6H_4–CH_2–	B	>100	>100
23	3-Thienyl	4-Me–C_6H_4–CH_2–	A	0.25±0.25	6.44±2.9
24	3-Thienyl	CH_3–	A	>100	—
25	3-Thienyl	3-Me–C_6H_4–CH_2–	A	4.24±0.86	12.2±0.63
26	3-Thienyl	2-Me–C_6H_4–CH_2–	A	36.5±7.9	65.4±0.3
27	3-Thienyl	4-(C_6H_5)–C_6H_4–CH_2–	B	7.66±0.23	1.18±0.21
28	4-MeO–C_6H_4–	C_6H_5–CH_2–	A	1.13±0.15	5.28±0.05
29	4-MeO–C_6H_4–	4-CF_3–C_6H_4–CH_2–	A	4.07±0.50	28.3±4.9
30	4-CF_3–C_6H_4–	C_6H_5–CH_2–	A	>100	—
31	2,3-Cl_2–C_6H_3–	4-Me–C_6H_4–CH_2–	A	>10	—
11				66.2±24.4	>100
15				>100	>100
16				>100	>100
19				51.0±12.2	>100
32				>100	>100
33				50.7±16.9	>100
	Triclosan			1.10±0.02	0.43±0.036

The oxazolines (**15** and **32**) and the oxazole **16** were prepared by condensing 3-thiophenecarboxylic acid with 2-amino-3-phenylpropan-1-ol using EDC to give the amide **14**. Cyclodehydration of **14** using Burgess' reagent[10] gave the corresponding oxazoline **15**. The oxazole **16** was prepared by oxidizing the oxazoline with DDQ (Scheme 3).

The oxadiazoles **19** and **33** were prepared according to the method of Bedford et al.[11] This is illustrated in Scheme 4 for the preparation of **19**. Condensation and cyclodehydration of the acyl chloride **17** with the amidoxime **18** gave the oxadiazole **19**.

Biology

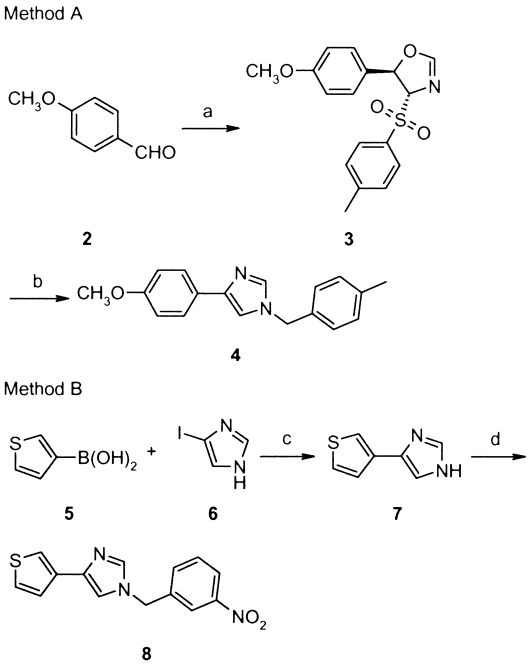
The FabI enzyme inhibition assays were carried out with varying concentrations of test compound and either *S. aureus* FabI or *E. coli* FabI in the presence of NADH using crotonoyl CoA as the substrate. The IC_{50} values were determined by monitoring the consumption of NADH.[12] Triclosan, a commercial antibacterial agent and inhibitor of FabI, was included in all assays as a positive control.[13a]

Whole-cell antimicrobial activity was determined by a broth microdilution procedure.[14] Compounds were tested in serial 2-fold dilutions ranging from 0.06 to 64 μg/mL. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of compound that inhibited visible growth.

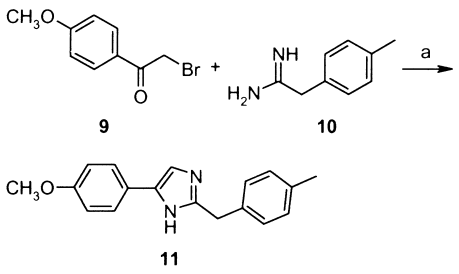
Results and Discussion

Solution-phase arrays were used to probe the scope of allowable substituents at the 1- and 4-positions of the imidazole lead **1**. The variable aromatic and aliphatic substituents at the 1- and 4-positions were chosen using

the Topliss analysis decision tree as a guide.[15] This semi-empirical method is designed to rapidly optimize biological activity by systematically probing electronic and steric effects of substituents. These studies revealed that electron-rich benzyl rings at the 1-position of the imidazole ring (R^2) are required for FabI inhibition (**4**, **20**, and **21** vs **8**, **22**, and **29**, Table 1). Additionally, small electron-donating groups, such as methyl and methoxy groups, are preferred at the 4-position of the aromatic ring (**21** and **23**). Larger groups, such as a phenyl group, were not tolerated at this position (**27**). Moving the methyl group from the 4-position to the 2- and 3-positions gave less active inhibitors (**25** and **26**). These studies also suggested that electron-rich aromatic groups are preferred at the 4-position (R^1) of the imidazole ring (**23** and **28** vs **30** and **31**).

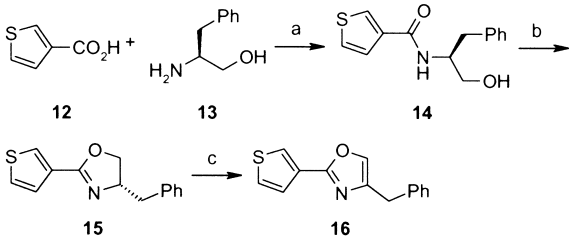


Scheme 1. Preparation of the 1,4-disubstituted imidazoles: (a) TOSMIC, NaCN, EtOH, rt, 30 min, quantitative; (b) 4-methylbenzylamine, toluene, reflux, 24 h, 38%; (c) $Pd(PPh_3)_4$, Na_2CO_3, DMF, 80 °C, 85%; (d) K_2CO_3, 3-nitrobenzyl bromide, DMF, 16%.

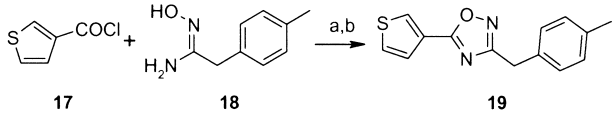


Scheme 2. Preparation of the 2,4-disubstituted imidazoles: (a) CH_2Cl_2, rt, 18 h, 75%.

These findings are consistent with results from X-ray co-crystallization studies with **1** bound to *E. coli* FabI/NAD^+. The crystal structure has been solved to 2.8 Å resolution (R 19.1%, R_f 27.3%) and shows that **1** is bound in the enzyme active site.[16] The thiophene ring of the inhibitor occupies a hydrophobic pocket and is engaged in a π-stacking interaction with the electron-poor nicotinamide ring of NAD^+. Electron-rich aromatic rings at the 4-position of the imidazole are expected to favorably influence the stacking interaction, while electron-poor rings should destabilize the complex. The unsubstituted imidazole nitrogen appears to be engaged in a hydrogen-bonding interaction with the phenolic hydroxyl of Tyr 156 from the enzyme (Fig. 1).



Scheme 3. Preparation of the oxazolines and oxazoles: (a) EDC, HOBt, Et_3N, DMF, 63%; (b) Burgess' reagent, 81%; (c) DDQ, 38%.



Scheme 4. Preparation of the oxadiazole: (a) THF, rt; (b) NaH, THF, reflux, 66% (two steps).

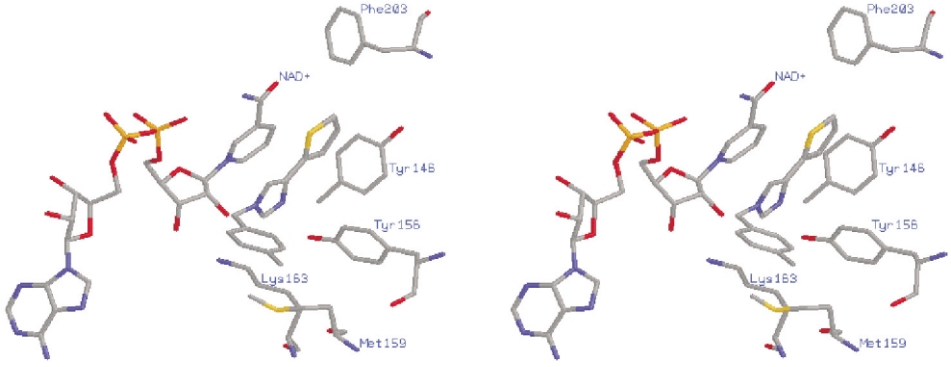


Figure 1. Stereoview of **1** and NAD^+ bound in the active site of *E. coli* FabI. For clarity, only the side chains of the residues which define the hydrophobic pocket of FabI have been shown. The thiophene ring and part of the imidazole ring of **1** were revealed by the electron-density map. The 4-methylbenzyl moiety represents a model since there was no electron density observed to define this part of the inhibitor.

Table 2. Antibacterial profile of **1**, **4**, **20**, **21**, **23**, **25**, and **28**

No.	MIC (ug/mL)	
	S. aureus Oxford	*M. catarrhalis* 1502
1	128	—
4	8	32
20	>64	>64
21	16	64
23	8	32
25	>64	32
28	64	64
Triclosan	0.03	0.06

The remainder of the inhibitor was disordered in the crystal structure. The interactions observed between **1** and *E. coli* FabI/NAD^+ are similar to the interactions observed between the known FabI inhibitor, triclosan, and *E. coli* FabI/NAD^+.[17] Namely, a π-stacking interaction is observed between the phenol of triclosan and the nicotinamide ring of NAD^+. A hydrogen bond is also observed between the phenolic hydroxyl group of triclosan and the hydroxyl group of Tyr 156.

Another array was prepared to assess the chain length requirements for the aryl groups attached to the central imidazole core. FabI inhibition was optimal when the aryl group at the 4-position (R^1) was directly attached to the imidazole core and when the aryl group at the 1-position (R^2) was separated from the imidazole nitrogen by a single methylene group (data not shown).

The 1,4-disubstituted imidazole ring appears to be critical for FabI activity. The regio-isomeric 2,4-disubstituted analogues are no longer effective inhibitors of FabI (**4** vs **11**). Oxazolines (**15** and **32**), oxazoles (**16**) and oxadiazoles (**19** and **33**) are also not tolerated as replacements for the 1,4-disubstituted imidazole ring. At this time we are unable to explain these results. There are no obvious unfavorable steric or electronic interactions when **11**, **15**, **16**, **19**, **32** and **33** are docked into the enzyme active site using the X-ray crystal structure solved for **1** bound to FabI.

Compounds with $IC_{50} < 5\,\mu M$ against *S. aureus* FabI were tested against a Gram-positive bacteria (*S. aureus* Oxford) and a Gram-negative bacteria (*Moraxella catarrhalis* 1502). The results show a correlation between the IC_{50} values in the enzyme assay and the MIC values against whole cell bacteria (Table 2). (Triclosan gives much lower MIC values than would be predicted from its enzyme IC_{50} level. This is presumably due to the multiple modes of action ascribed to Triclosan.[13b,c]) In addition, the antibacterial activity of **23** was evaluated in a *S. aureus* strain engineered to overexpress FabI. A greater than 8-fold increase in MIC values was observed in the overexpressing strain when compared to wild type. These results suggest that the antibacterial activity is primarily due to inhibition of FabI.

Conclusion

We have shown that appropriately functionalized 1,4-disubstituted imidazoles are effective inhibitors of FabI and that representative compounds have antibacterial activity against Gram-positive and Gram-negative bacteria. These results provide further validation of FabI as an antibacterial target.

References and Notes

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12. The *E. coli* and *S. aureus* FabI enzymes were cloned, overexpressed and purified as described previously.[17a] 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 non-linear progress curves represented by the slope of the tangent at $t = 0$ min. IC_{50} values were estimated from a fit of the initial velocities to a standard 4-parameter model and are typically reported as the mean ± SD of duplicate determinations. Triclosan was included in all assays as a positive control. IC_{50} determinations with *S. aureus* FabI were determined in a similar fashion.
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14. Compounds in DMSO were diluted 1:10 with water giving a 256 μg/mL solution. This solution (50 μL) was serially diluted into cation adjusted Mueller Hinton broth. A 50 μL aliquot of bacteria (ca. 1×10^6 cfu/mL) was added to each well. Inoculated plates were incubated at 35 °C for 24 h. Minimum inhibitory concentration (MIC) = the lowest concn of compound that inhibited visible growth.
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16. The atomic co-ordinates have been deposited at the Protein Data Bank (PDB) (accession number 1I2Z). The conditions used to crystalize the protein and solve the X-ray structure are similar to those described in ref 17a.

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