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Phenylimidazole derivatives as new inhibitors of bacterial enoyl-ACP reductase FabK

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Abstract—Novel FabK inhibitors with antibacterial activity against *Streptococcus pneumoniae* were synthesized and evaluated. Through SAR studies of our initial hit compound 2-(1*H*-benz[*d*]imidazol-2-ylthio)-*N*-(6-methoxycarbonylbenzo[*d*]thiazol-2-yl)acetamide, a series of novel phenylimidazole derivatives were discovered as potent FabK inhibitors.

Streptococcus pneumoniae is the main causative pathogen of community-acquired pneumonia. The infectious disease caused by *S. pneumoniae* remains a leading cause of morbidity and mortality. The increasing prevalence of penicillin-resistant *S. pneumoniae* (PRSP) is of concern, since most of the pathogens have acquired resistance to many antibacterial agents including macrolide and quinolone.[1–3] Since many conventional therapies are no longer effective against these drug-resistant pathogens, the discovery of new antibiotics is the most important. A key strategy to overcome drug-resistant pathogens is the discovery of antibacterial agents with novel mechanisms of action.

FabI is an enoyl-acyl carrier protein (ACP) reductase which catalyzes the final and rate-limiting step of bacterial fatty acid synthase (FAS).[4–7] Most bacteria possess FabI and bacterial FAS shows low overall sequence homology with mammalian enzymes. Therefore, FabI-targeting approach to antibacterial drug therapy seems feasible. Various compounds including isoniazid,[8] diazaborines,[9,10] triclosan,[11–16] indole naphthyridinones (**2**, **3**),[17–19] thiopyridine,[20] and 4-pyridones (**1**)[21,22] have been reported (Fig. 1).

However, recent studies have shown other bacterial enoyl-ACP reductases, in addition to FabI. A triclosan-resistant flavoprotein, termed FabK, has been shown to be the sole enoyl-ACP reductase in *S. pneumoniae*.[23,24] Consequently, a selective FabK inhibitor is expected to be a novel narrow-spectrum antibacterial agent against *S. pneumoniae* including PRSP, macrolide and quinolone resistant *S. pneumoniae*. There are very few reports of FabK inhibitors except for a small number of compounds. The reported FabK inhibitors **2** and **3** showed weak inhibitory activity and failed to show selective inhibition of bacterial FAS.[17,25] Atromentin and leucomelone were also reported as FabK inhibitor,[26] but these compounds did not show any antibacterial activity against *S. pneumoniae*. Unlike the reported FabK inhibitors, our developed phenylimidazole derivatives showed strong FabK-inhibitory activity and potent antibacterial activity against *S. pneumoniae* due to the inhibition of FabK. In this report, we present our investigation of a series of phenylimidazole derivatives as novel FabK inhibitors.

To discover novel small-molecule FabK inhibitors, we screened our compound library for inhibitory activity toward FabK of *S. pneumoniae*. Among them, amide compound **4** exhibited moderate FabK-inhibitory activity of *S. pneumoniae* (IC_{50} = 1.8 μM) and selective inhibition of bacterial FAS. Therefore, compound **4** was selected as the lead compound[27] and we studied for the structure–activity relationship (SAR).

Amide-type derivatives were prepared by means of standard procedures. For example, the synthesis of compound **9** is illustrated in Scheme 1. Commercially available 2-aminobenzothiazole **5** was treated with

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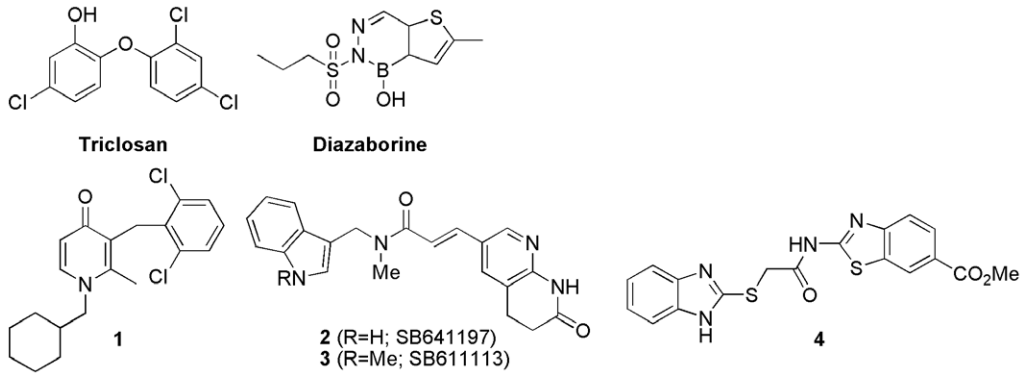
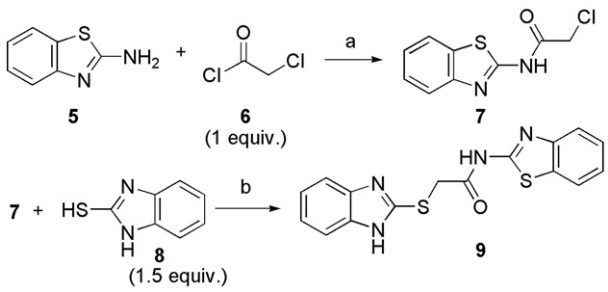


Figure 1. Inhibitors of FabI.



Scheme 1. Synthesis of compound **9**. Reagents and conditions: (a) toluene, 60 °C (70%); (b) NaOMe (1.5 equiv), DMF, rt (73%).

chloroacetyl chloride **6** in toluene at 60°C to afford compound **7** in 70% yield. The reaction of **7** with 2-mercaptobenzimidazole **8** was carried out in DMF at room temperature in the presence of sodium methoxide to afford the corresponding amide adduct **9** in 73% yield.

Although several of the amide derivatives showed good FabK-inhibitory activity, they showed no antibacterial activity against *S. pneumoniae* in the presence of blood. Examination of the stability of the compounds under the MIC measurement conditions by means of liquid chromatography–mass spectrometry indicated that **4** readily decomposed to compounds **10**–**12** (Fig. 2), which lacked FabK-inhibitory activity (Table 1). For improving the chemical stability of the amide bond, we prepared carbamate type **15** and ureido type **17**.

The synthesis of compound **15** is illustrated in Scheme 2. The activated amide **13** was prepared from 2-aminobenzothiazole **5** with 1,1′-carbonyldiimidazole (CDI), and used without purification. Reaction of commercially

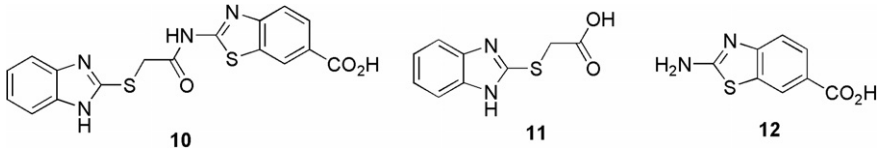
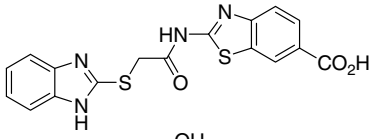
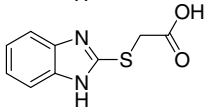
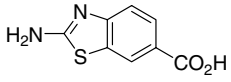


Figure 2. Expected compounds that were converted from compound **4**.

Table 1. Antibacterial activities for FabK inhibitors: Expected compounds that were converted from compound **4**

Compound	Structure	FabK IC_{50}[a](μM)*S. pneumoniae*	MIC(μg/mL)*S. pneumoniae*
10		>32	>32
11		>32	nt[b]
12		>32	nt[b]

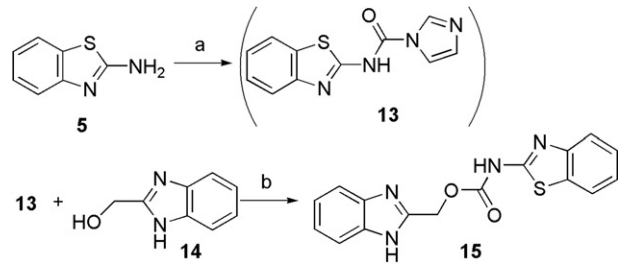
[a] *S. pneumoniae* KU197.
[b] nt, not tested.

available (1*H*-benz[*d*]imidazol-2-yl)methanol **14** with **13** in the presence of sodium methoxide gave compound **15**.

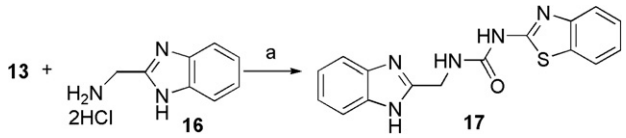
The ureido compound **17** was prepared as illustrated in Scheme 3. Reaction of commercially available (1*H*-benz[*d*]imidazol-2-yl)methylamine dihydrochloride salt **16** with **13** in THF at room temperature in the presence of *N*,*N*-diisopropylethylamine gave compound **17**.

FabK-inhibitory activity and antibacterial activity against *S. pneumoniae* of compounds **15** and **17** are shown in Table 2. Compound **15** having a carbamate group lacked FabK-inhibitory and antibacterial activities, while compound **17** having an ureido group showed weak FabK-inhibitory activity. Moreover, compound **17** showed antibacterial activity against *S. pneumoniae* due to the improved chemical stability in the standard MIC assay medium including 2% lysed horse blood. We therefore selected an ureido group as a basic structure.

SAR of (benzothiazol-2-yl)ureido derivatives is shown in Table 3. Compounds **26**–**28** having various functional



Scheme 2. Synthesis of compound **15**. Reagents and conditions: (a) CDI (2 equiv), THF, rt; (b) NaOMe (0.1 equiv), THF, 40 °C (26%).



Scheme 3. Synthesis of compound **17**. Reagents and condition: (a) *i*-Pr_2NEt (2 equiv), THF, rt (78%).

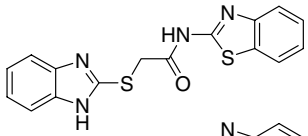
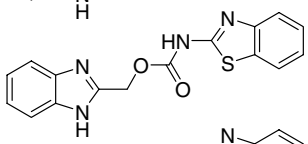
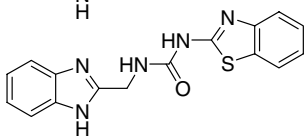
group at 6-position of benzothiazole showed moderate FabK-inhibitory activity. In particular, compound **27** having methylsulfonyl group exhibited a good FabK-inhibitory activity. Compounds **29** and **30** having methoxy group at 4- or 5-position of benzothiazole showed decreased FabK-inhibitory activities compared with **28**. These results indicate that substitutions at 6-position improve FabK-inhibitory activity.

Introduction of other heterocyclic rings (compounds **31** and **32**) instead of benzimidazole ring did not improve both activities, but the phenylimidazole derivative (compound **25**) showed strong FabK-inhibitory activity (0.14 μM) and antibacterial activity (0.5 μg/mL). Since compounds **17** and **25**–**30** did not show FabI-inhibitory activity (*Escherichia coli* FabI $IC_{50} > 32$ μM), these compounds appear to be selective FabK inhibitors. In addition, compound **25** did not show any antibacterial activity against *S. aureus* (>32 μg/mL) without FabK and elevated MIC value (>8-fold) was observed against *S. pneumoniae* mutant possessing an amino acid substitution in FabK.[27] These results indicate the antibacterial activity of compound **25** is due to the inhibition of FabK.

Compound **25** was prepared as illustrated in Scheme 4. Reaction of commercially available benzyl cyanomethylcarbamate **18** with sodium methoxide followed by ammonium chloride treatment gave compound **19** in 97% yield. Compound **19** was treated with 2-phenacylbromide **20** to give phenylimidazole derivative **21**, and cleavage of the benzyloxycarbonyl moiety afforded compound **22**. Compound **25** was prepared by similar procedures described for compounds **15** and **17**.

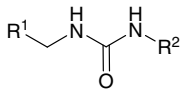
We discovered the phenylimidazole derivative **25** as a novel inhibitor of bacterial enoyl-ACP reductase (FabK). Compound **25** selectively exhibited strong FabK-inhibitory and potent antibacterial activities against *S. pneumoniae* in the standard MIC assay medium including blood. The present results support the idea that FabK is a valid antibacterial target, and that small-molecule FabK inhibitors are drugs for the treatment of bacterial infections, and further suggest that the

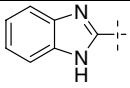
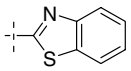
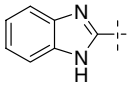
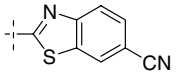
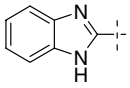
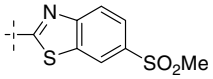
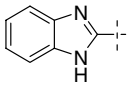
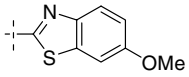
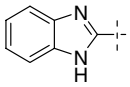
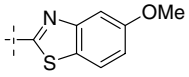
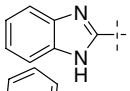
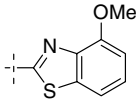
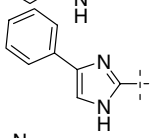
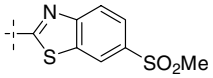
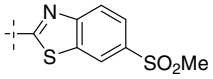
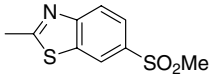
Table 2. Antibacterial activities for FabK inhibitors: Effect of combination units

Compound	Structure	FabK IC_{50} (μM) *S. pneumoniae*	MIC (μg/mL) *S. pneumoniae*[a]
9		>32	>32
15		>32	>32
17		28	32

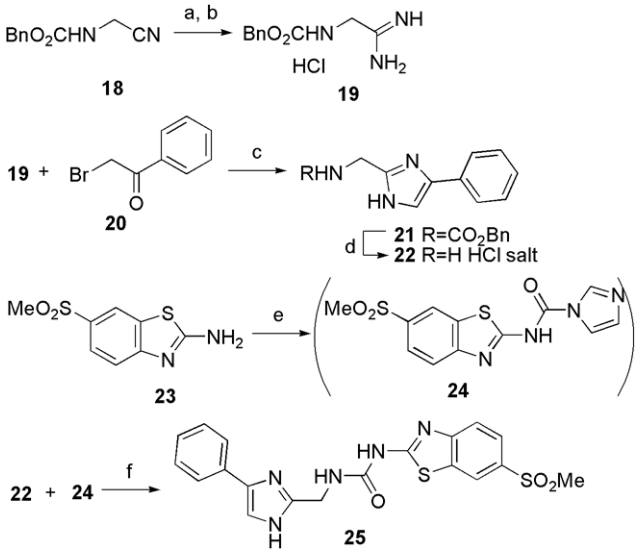
[a] *S. pneumoniae* KU197.

Table 3. SAR of (benzothiazol-2-yl)ureido derivatives



Compound	Structure		FabK IC_{50} [a] (μM) *S. pneumoniae*	MIC (μg/mL) *S. pneumoniae*[b]
	R^1	R^2		
17			28	32
26			4.7	8
27			0.76	4
28			7.6	16
29			>32	nt[c]
30			>32	nt[c]
25			0.14	0.5
31			24	>32
32			24	>32

[a] Average of two experiments.
[b] *S. pneumoniae* KU197.
[c] nt, not tested.



Scheme 4. Synthesis of compound **25**. Reagents and conditions: (a) NaOMe (0.1 equiv), MeOH, rt; (b) NH_4Cl (1 equiv), rt (97%); (c) K_2CO_3 (1 equiv), DMF, rt (29%); (d) 10% Pd/C (30 w/w%), H_2, MeOH, HClaq (76%); (e) CDI (2 equiv), THF, rt; (f) *i*-Pr_2NEt (2.2 equiv), THF, rt, (33%).

phenylimidazole derivatives represent a good lead structure. Further structural development studies are in progress.

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Supplementary data

Spectrum data of the new compounds and biological method. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.06.040.

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