Phenylimidazole Derivatives of 4-Pyridone as Dual Inhibitors of Bacterial Enoyl-Acyl Carrier Protein Reductases FabI and FabK

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Received May 7, 2007

FabI and FabK are bacterial enoyl-acyl carrier protein (ACP) reductases that catalyze the final and rate-limiting step of bacterial fatty acid biosynthesis (FAS) and are potential targets of novel antibacterial agents. We have reported 4-pyridone derivative **3** as a FabI inhibitor and phenylimidazole derivative **5** as a FabK inhibitor. Here, we will report phenylimidazole derivatives of 4-pyridone as FabI and FabK dual inhibitors based on an iterative medicinal chemistry and crystallographic study of FabK from *Streptococcus pneumoniae*/compound **26**. A representative compound **6** showed strong FabI inhibitory (IC_{50} = 0.38 μM) and FabK inhibitory (IC_{50} = 0.0045 μM) activities with potent antibacterial activity against *S. pneumoniae* (MIC = 0.5 μg/mL). Since elevated MIC value was observed against *S. pneumoniae* mutant possessing one amino acid substitution in FabK, the antibacterial activity of the compound was considered to be due to the inhibition of FabK. Moreover, this compound showed no significant cytotoxicity (IC_{50} > 69 μM). These results support compound **6** as a novel agent for the treatment of bacterial infections.

Introduction

The emergence of bacterial resistance to most of all antibiotics poses a threat to health care, and novel therapeutics are needed. In particular, the increase of methicillin-resistant *Staphylococcus aureus* (MRSA)[a,1] and penicillin-resistant *Streptococcus pneumoniae* (PRSP)[a] is of major concern, and two strains of vancomycin-resistant *S. aureus* have been isolated recently.[2−4] A key strategy to overcome antibiotic-resistant pathogens is the discovery of antibacterial agents with novel mechanisms of action and no cross-resistance. However, only three novel classes of antibiotics, oxazolidinones,[5] lipopeptides,[6] and glycylcyclines,[7] have reached the market.

Bacterial FAS[a] (type II)[8−10] provides essential fatty acids for use in the assembly of key cellular components such as cell envelope, phospholipids, lipoproteins, lipopolysaccharides, and mycolic acids. In bacteria, different monofunctional enzymes catalyze each of the reactions, and reaction intermediates are carried through the cytosol as a thioester of the small ACP[a]. On the other hand, mammalians use a single large multifunctional protein in which the growing chain is covalently attached to the protein to make fatty acids. This difference of fatty acid biosynthesis between bacteria and mammalian offers an attractive opportunity for selective inhibition of bacterial FAS[a]. Therefore, inhibitors of the bacterial enzymes are expected to be the candidates for novel antibacterial agents.

The first step in the bacterial biosynthetic cycle is the condensation of malonyl-ACP with acetyl-CoA by FabH.[11−13] In subsequent rounds, malonyl-ACP is condensed with the growing-chain acyl-ACP (FabB and FabF). In the elongation cycle of the second step, FabG mediates ketoester reduction by NADPH-dependent β-ketoacyl-ACP reductase.[14,15] Subsequent dehydration by FabA or FabZ,[16,17] which are β-hydroxyacyl-ACP dehydrases, leads to trans-2-enoyl-ACP. FabI is an enoyl-ACP reductase that catalyzes the conjugate reduction of an enoyl-ACP to the corresponding acyl-ACP using the cofactor NADPH or NADH as a hydride source.[18−20] Further rounds of this cycle, successively adding two carbon atoms per cycle, finally lead to palmitoyl-ACP. Then, the cycle is stopped due to feedback inhibition of FabH and FabI by palmitoyl-ACP. Therefore, FabI and FabH are rate-determining for the overall biosynthetic pathway[21](Figure 1).

In *Escherichia coli*, there is a single NADH-dependent isoform of this enzyme, FabI, which is essential for bacterial viability.[22,23] Various compounds including isoniazid,[24] diazaborines,[25,26] triclosan,[27−32] indole naphthyridinones,[33−35] and thiopyridine[36] have been reported as inhibitors of bacterial enoyl-ACP reductase, and a FabI-targeting approach to antibacterial drug therapy appears feasible (Figure 2).

However, recent studies have shown that other bacterial enoyl-ACP reductases exist in addition to FabI.[37] A triclosan-resistant flavoprotein, termed FabK, has been shown to be the sole enoyl-ACP reductase in *S. pneumoniae* and to exist together with FabI in key pathogens such as *Enterococcus faecalis* and *Pseudomonas aeruginosa*.[38] Inhibitors of both FabI and FabK are expected to have broad-spectrum antibacterial activity. The small-molecular FabI and FabK inhibitors **1** and **2** have been reported,[33] but they showed weaker inhibition of *S. pneumoniae* FabK than of *S. aureus* FabI.[39]

Therefore, we are interested in finding novel small-molecular FabI and FabK dual inhibitors. We have previously reported a potent FabI inhibitor (**3**)[40] with potent antibacterial activity against *S. aureus* and a FabK inhibitor (**5**) with potent antibacterial activity against *S. pneumoniae*[41, 42](Figure 3). The macromolecular synthesis assay demonstrated that a compound related to **3** and compound **4** selectively inhibited lipid biosynthesis.[42,45] We considered that it might be possible to combine the pharmacophores of these inhibitors to produce specific dual inhibitors.

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[a] Abbreviation: ACP, acyl carrier protein; FAS, fatty acid biosynthesis; MRSA, methicillin-resistant *Staphylococcus aureus*; PRSP, penicillin-resistant *Streptococcus pneumoniae*; SAR, structure−activity relationship; FMN, flavin mononucleotide; MOA, mode of action.

10.1021/jm0705354 CCC: $37.00 © 2007 American Chemical Society
Published on Web 08/22/2007

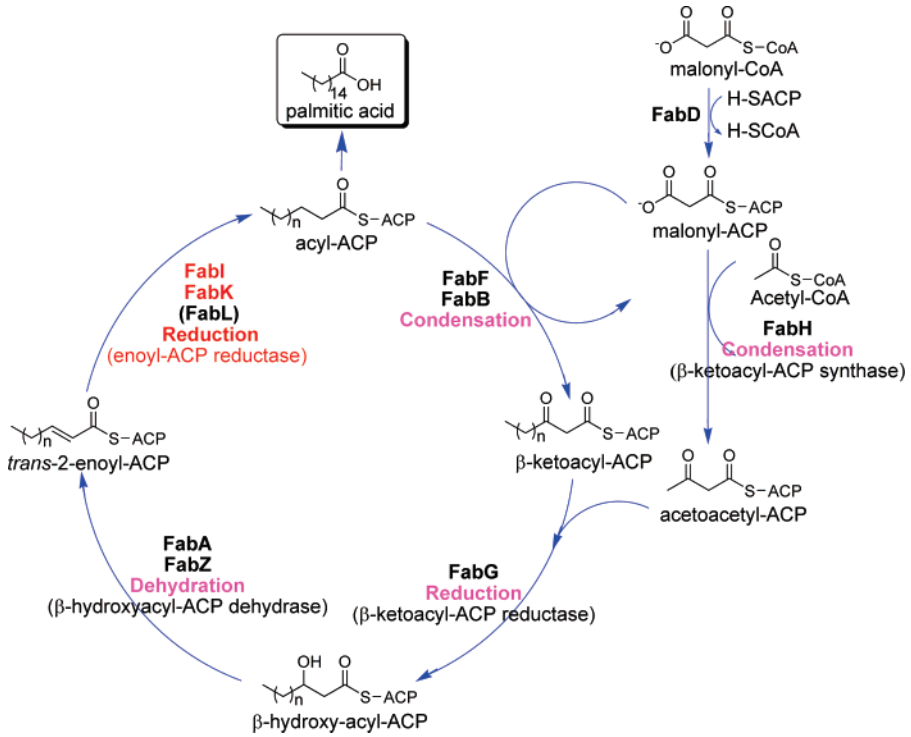


Figure 1. The bacterial biosynthetic cycle of fatty acids.

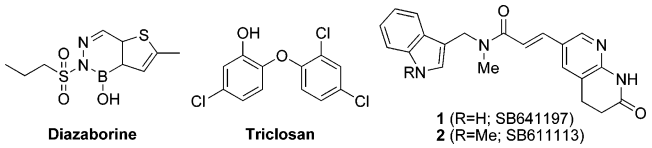


Figure 2. The reported FabI inhibitors.

Through iterative medicinal chemistry and X-ray crystal structure-based design,[43] we have developed novel phenylimidazole derivatives having a 4-pyridone moiety as FabI and FabK dual inhibitors. Among them, compound **6** exhibited strong FabI and FabK inhibitory activities, and it showed potent antibacterial activity against *S. pneumoniae*. Here, we would like to present details of the development of novel FabI and FabK dual inhibitors.

Chemistry

The phenylimidazole derivatives were prepared as follows. As an example, the synthesis of compound **11** is illustrated in Scheme 1. Commercially available benzyl cyanomethylcarbamate **7** was treated with sodium methoxide followed by ammonium bromide to give compound **8**. Compound **8** was treated with 4-bromophenacyl bromide **9** to give phenylimidazole **10** in 28% and then the amino group of the imidazole was protected with 2-(trimethylsilyl)ethoxymethyl to afford compound **11** in 60% yield.

The 4-pyridone derivative **17** was prepared as illustrated in Scheme 2.[40,44,45] Commercially available 4-methoxypyridine was treated with methyl magnesium bromide and carbobenzyloxy chloride in THF at −25 °C to afford compound **13** in 94% yield. The reaction of **13** with 2,6-dichlorobenzaldehyde was carried out in THF at −78 °C in the presence of lithium hexamethyldisilazide, and the corresponding aldol adduct **14** was obtained in 98% yield. The hydroxyl group of compound **14** was converted to the methanesulfonate **15** by treatment with methanesulfonyl chloride in 94% yield. Elimination of the methanesulfonyloxy group and isomerization of the *exo*-olefin by using potassium *tert*-butoxide gave compound **16** in 87% yield. Treatment of **16** with tetravinyltin in the presence of $Cu(OAc)_2$ in MeCN/DMF under an O_2 atmosphere at room temperature afforded compound **17**.

Compound **6**, a FabI and FabK dual inhibitor, was prepared as illustrated in Scheme 3. Compound **11** was coupled with **17** in the presence of $Pd_2(dba)_3$, $P(^tBu)_3$, and Cy_2NMe to afford compound **18** in 23% yield. Deprotection of the benzyloxycarbonyl and 2-(trimethylsilyl)ethoxymethyl groups under acidic condition afforded compound **19**. Hydrogenation of compound **19** gave the corresponding saturated amine (compound **20**). Coupling **20** with **22**, which is obtained from **21** as a crude intermediate, in the presence of *N*,*N*′-diisopropylethylamine

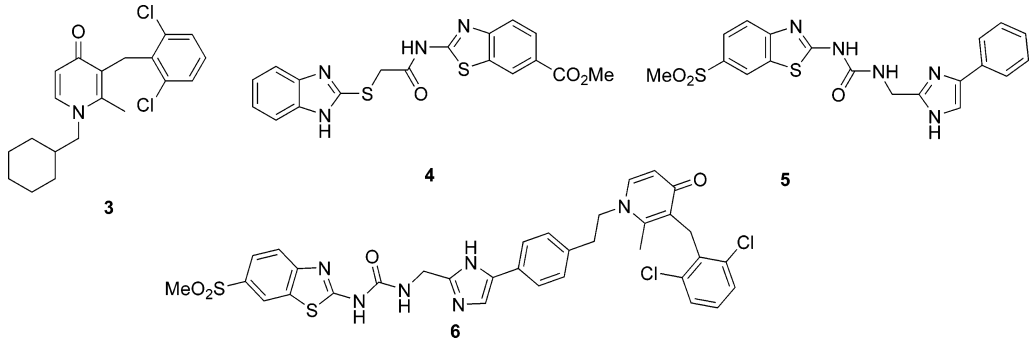
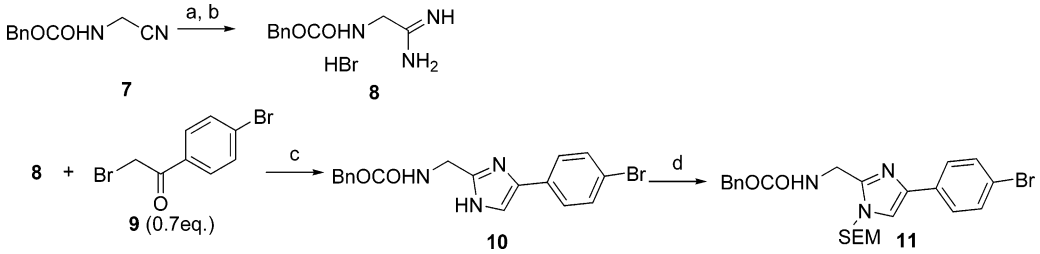


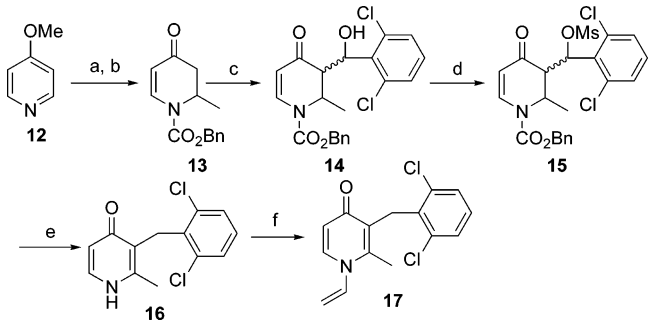
Figure 3. Our developed FabI and/or FabK inhibitors.

Scheme 1. Synthesis of Phenylimidazole Derivatives[a]



[a] Reagents and conditions: (a) NaOMe (0.1 equiv), MeOH, rt; (b) NH_4Br (1.05 equiv), rt (87%, two steps); (c) K_2CO_3 (1 equiv), THF/H_2O = 10/1, 80 °C (28%); (d) NaH (1 equiv), SEMCl (1 equiv), THF, rt to 40 °C (60%).

Scheme 2. Synthesis of 4-Pyridone Derivatives[a]



[a] Reagents and conditions: (a) BnOCOCl (1 equiv), CH_3MgBr (1.2 equiv), THF, −25 °C; (b) 3 N HCl, rt (94% for two steps); (c) 2,6-dichlorobenzaldehyde (1.3 equiv), LiHMDS (1.1 equiv), THF, −78 °C (98%); (d) MsCl (2 equiv), pyridine, 0 °C to rt (94%); (e) ^{t}BuOK (3 equiv), THF, rt (87%); (f) tetravinyltin (2 equiv), $Cu(OAc)_2$ (2 equiv), O_2, MeCN/DMF, rt (76%).

afforded compound **6**. The other related compounds were similarly prepared.

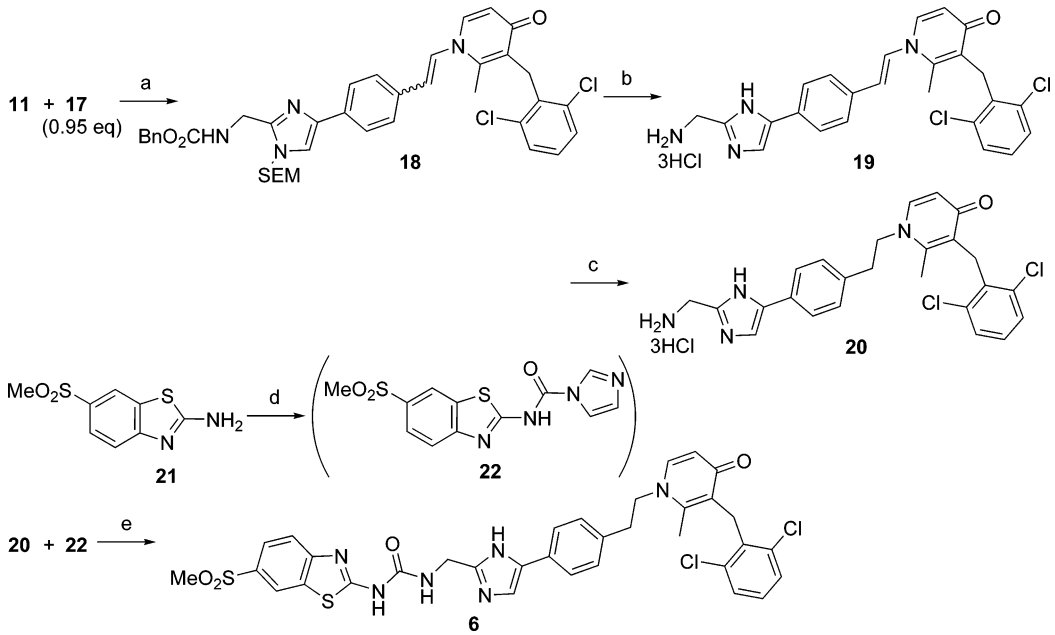
Results and Discussion

We have developed a series of small-molecule, phenylimidazole-based FabK inhibitors by optimizing a lead compound **4** discovered by screening our compound library for inhibitory activity toward FabK of *S. pneumoniae*. Among them, compound **5** showed strong FabK-inhibitory activity (IC_{50} = 0.14 μM) and potent antibacterial activity (MIC = 0.5 μg/mL) against *S. pneumoniae*.[41] However, it did not show either *E. coli* FabI-inhibitory activity (IC_{50} > 32 μM) or antibacterial activity against *S. aureus* (MIC > 64 μg/mL).

Evaluation of several derivatives structurally related to **5** revealed the importance of the phenylimidazole structure for FabK-inhibitory activity. Structure−activity relationship (SAR) studies concerning the benzothiazole ring in compound **5** revealed that compound **23**, having a pyridylthio group at the 5-position of thiazole, showed strong FabK inhibitory activity and potent antibacterial activity against *S. pneumoniae* (IC_{50} = 0.042 μM, MIC = 2 μg/mL). Therefore, we selected the thiazole moiety with a 5-pyridylthio group as a basic structure and prepared a range of phenylimidazole derivatives. SAR of these derivatives is shown in Table 1.

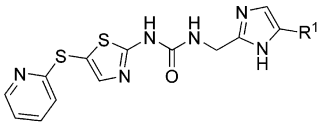
Compounds **24** and **25**, having a bromo substituent, and compound **26**, having a carboxylic acid group on the phenyl ring, showed strong FabK inhibitory activity. However, compound **25** showed decreased antibacterial activity, and compound **26** lacked antibacterial activity against *S. pneumoniae*. The reason for these results is not known, but several factors, such

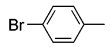
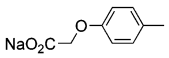
Scheme 3. Synthesis of Compound **6**[a]



[a] Reagents and conditions: (a) $Pd_2(dba)_3$ (0.2 equiv), $P(^tBu)_3$ (0.4 equiv), Cy_2NMe (2 equiv), DMF, 80 °C (23%); (b) 4 N HCl/1,4-dioxane, 100 °C (25%); (c) 10% Pd/C (10 w/w%), MeOH/HCl, H_2 atmosphere (44%); (d) CDI (2 equiv), THF, rt; (e) *N*,*N*′-diisopropylethylamine (4 equiv), DMF, rt (21%, two steps).

Table 1. Antibacterial Activities for Phenylimidazole Derivatives as FabK Inhibitors



Compound	R^1	IC_{50} (μM) FabI[a]	IC_{50} (μM) FabK[b]	MIC (μg/mL) *S. pneumoniae*[c]
Triclosan	—	0.9 ± 0.36	>32	32[d]
2	—	0.2 ± 0.02	>32	>32[e]
23	Ph	>32	0.042 ± 0.005	2
24		>32	0.0024 ± 0.0002	0.25
25		>32	0.0053 ± 0.0005	16
26		>32	0.034 ± 0.003	>32

[a] *E. coli* FabI. [b] *S. pneumoniae* FabK. [c] *S. pneumoniae* KU197. [d] *S. aureus* ATCC29213 MIC = 0.25 μg/mL. [e] *S. aureus* ATCC29213 MIC = 0.063 μg/mL.

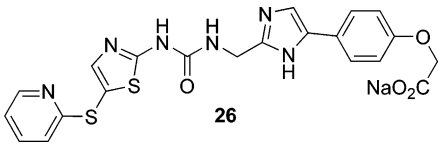


Figure 4. Structure of compound **26** used for the crystallographic study.

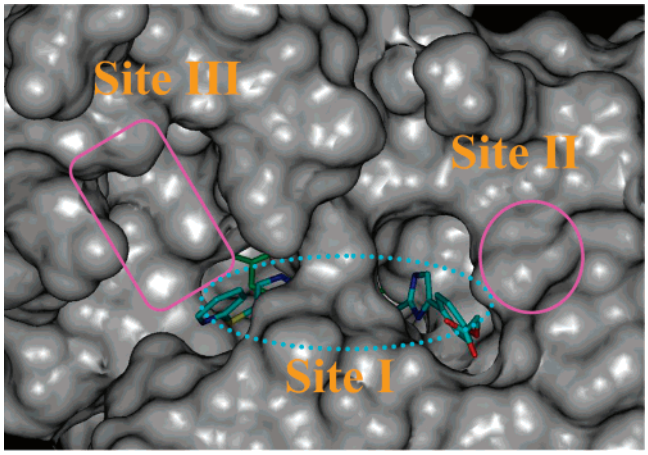


Figure 5. Crystal structure of compound **26** bound to the active site of FabK.

as poor penetration through the bacterial cell membrane or recognition by an efflux transporter of the bacteria, might be involved.

We have already crystallized FabK from *S. pneumoniae*.[43] Although compound **26** showed no antibacterial activity against *S. pneumoniae*, it was the only compound that had good solubility in aqueous 5% DMSO, and it was suitable for a crystallographic study. Therefore, to allow further optimization of inhibitors by structure-based drug design, we solved the crystal structure of the compound **26**/FabK complex (Figure 4).

The active pocket of FabK can be divided into three sites (Figures 5 and 6). The thiazole ring and a part of the ureido moiety of **26** are involved in a face-to-face $\pi-\pi$ stacking interaction with the isoalloxazine ring of flavin mononucleotide (FMN), with additional hydrophobic interactions between the thiazole ring and the side chains of hydrophobic amino acids.

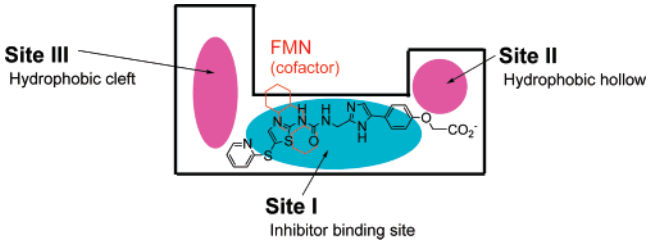


Figure 6. Schematic model of the active site of FabK.

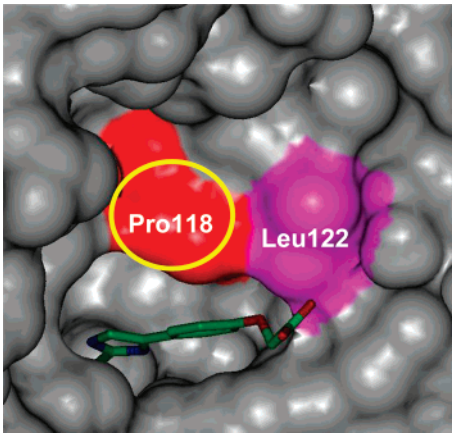


Figure 7. Expanded view of the complex of compound **26** and FabK around site II.

The phenylimidazole moiety is buried in a hydrophobic cleft. In addition, the hydrophobic amino acids Leu122 and Pro118 are located near the phenyl ring of compound **26** (Figure 7). The bromo substituent on the phenyl ring in compounds **24** and **25** may occupy the gap and reinforce the hydrophobic interaction with Leu122. Although Pro118 has a hydrophobic interaction with the phenyl ring of compounds, there is an additional shallow, hydrophobic hollow, called site II, extending to the plane of Pro118. Site III on the left side of site I is a hydrophobic cleft. The pyridine ring faces the empty hydrophobic pocket in site III. A detailed analysis of this crystal structure will be reported elsewhere.

We have reported that novel 4-pyridone derivatives having a hydrophobic group at the 1-position showed good *E. coli* FabI inhibitory activity and strong antibacterial activity against *S. aureus*.[40] In particular, compound **3**, having a cyclohexylmethyl group at the 1-position, exhibited strong activities.

We have also shown that it is possible to incorporate various bulky hydrophobic groups at the 1-position of 4-pyridones.[40] Therefore, we assumed that the substituent at the 1-position of 4-pyridones was recognized as an alkyl side chain of fatty acid. On the basis of this hypothesis, we speculated that introduction of a hydrophobic FabK inhibitory moiety at the 1-position of 4-pyridones might yield FabI and FabK dual inhibitors.

There are two possible strategies to hybridize FabI and FabK inhibitors (Figure 8). One is to connect the phenylimidazole side of the FabK inhibitory moiety to the FabI inhibitory moiety (type I), and the other is to connect the thiazole side of the FabK inhibitory moiety to the FabI inhibitory moiety (type II). From the perspective of a FabI inhibitor, the hydrophobic FabK inhibitory moiety expected to be recognized by the same site of the FabI active pocket in both types. On the other hand, from the perspective of a FabK inhibitor, the FabI inhibitory moiety or 4-pyridone added at either side of the FabK inhibitory moiety is expected to be recognized by different sites of the FabK active pocket. The 4-pyridone substructure in the case of types I and

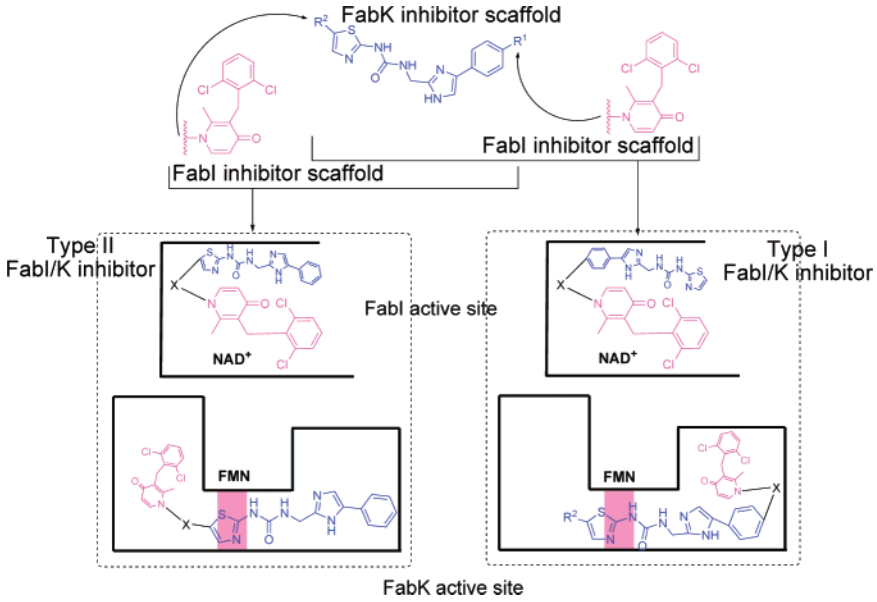
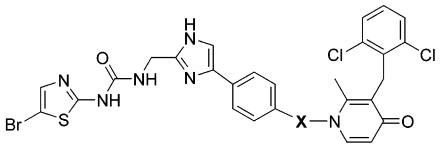
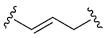
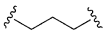


Figure 8. Synthetic strategy for FabI and FabK inhibitors.

Table 2. The Effect of Chain Length between 4-Pyridone and Phenylimidazole



Compound	X	IC$_{50}$(μM) FabI[a]	IC$_{50}$(μM) FabK[b]	MIC(μg/mL) *S. aureus*[c]	MIC(μg/mL) *S. pneumoniae*[d]
27		0.82 ± 0.21	11 ± 3	>32	>32
28		>32	>30	NT	NT
29		0.57 ± 0.07	0.029 ± 0.017	>32	8
30		0.29 ± 0.05	9.0 ± 2.9	>32	>32
31		0.34 ± 0.02	6.6 ± 1.8	>32	>32

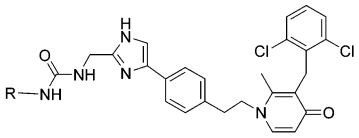
[a] *E. coli* FabI. [b] *S. pneumoniae* FabK. [c] *S. aureus* ATCC29213. [d] *S. pneumoniae* KU197.

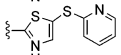
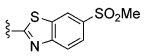
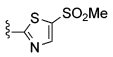
II is expected to be recognized by sites II and III of the FabK active pocket, respectively.

Since site II of the FabK active pocket is a hydrophobic hollow on Pro118, adding a plane structure such as 4-pyridone to the phenylimidazole moiety of a FabK inhibitor with an appropriate linker might improve the binding affinity to FabK. On the other hand, site III of FabK has larger space than site II, so it might be more difficult to introduce favorable interactions. Therefore, we first focused on type I inhibitors. The SAR of spacer connecting phenylimidazole and 4-pyridone is shown in Table 2.

Compound **27** having a one-carbon chain length showed FabI inhibitory activity, but weak FabK inhibitory activity. Although compound **28**, which has a *trans*-ethenyl fragment showed a decrease in both activities, the saturated compound **29** showed strong FabI and FabK inhibitory activities and moderate antibacterial activity against *S. pneumoniae*. Compounds **30** and **31**, having a three-carbon chain length, showed increased FabI inhibitory activity but decreased FabK inhibitory activity and lacked antibacterial activity against *S. pneumoniae*. In compounds **27** and **28**, the 4-pyridone may interfere sterically with binding to FabK. On the other hand, in compounds **30** and **31**, we considered that the 4-pyridone could not interact with the hydrophobic hollow on Pro118 of FabK because of the long linkers. These results indicated that our strategy for producing dual FabI/FabK inhibitors might be valid. Therefore, we chose a saturated two-carbon chain for the basic structure. Finally, we optimized the thiazole substitution. The SAR of substituted thiazole is shown in Table 3.

Table 3. Antibacterial Activities for Thiazole-Side Derivatives as FabI and FabK Inhibitors



Compound	R	IC$_{50}$(μM) FabI[a]	IC$_{50}$(μM) FabK[b]	MIC(μg/mL) *S. aureus*[c]	MIC(μg/mL) *S. pneumoniae*[d]
29		0.57 ± 0.07	0.029 ± 0.017	>32	8
32		0.56 ± 0.21	0.016 ± 0.004	>32	2
33		0.93 ± 0.37	>32	>32	>32
6		0.38 ± 0.07	0.0045 ± 0.0007	>32	0.5
34		0.31 ± 0.05	0.0094 ± 0.0010	>32	1
Triclosan		0.90 ± 0.36	>32	0.063	NT

[a] *E. coli* FabI. [b] *S. pneumoniae* FabK. [c] *S. aureus* ATCC29213. [d] *S. pneumoniae* KU197.

We previously found that 6-substituted benzo[*d*]thiazole[41] or 5-substituted thiazole derivatives showed potent FabK inhibitory activity and suggested that steric effects of those substituent groups were important for FabK inhibitory activity. More details of the SAR studies of our FabK inhibitors will be reported elsewhere. On the basis of these results, compound **32** showed strong FabI and FabK inhibitory activities. Compound **33**, having imidazole instead of thiazole, showed strong FabI inhibitory activity but lacked FabK inhibitory activity, indicating that the thiazole ring was essential for FabK inhibition, and **33** did not show antibacterial activity against *S. pneumoniae*. Compound **6** substituted at the 6-position on benzothiazole and compound **34** substituted at the 5-position of thiazole showed strong FabI and FabK inhibitory activities (FabI IC$_{50}$ = 0.38

μM, 0.31 μM, respectively; FabK IC_{50} = 0.0045 μM, 0.0094 μM, respectively) and potent antibacterial activity against *S. pneumoniae* (MIC = 0.5 μg/mL, 1 μg/mL, respectively). FabK inhibitory activities of these compounds tended to follow the SAR of our FabK inhibitors. Although the compounds in Table 3 showed the potent FabI inhibitory activity, they lacked antibacterial activity against *S. aureus*. We have reported that the 4-pyridone derivatives having longer alkyl chain at the 1-position did not show antibacterial activity against *S. aureus*, despite of strong FabI inhibitory activity.[40] Thus, we concluded that a 4-pyridone structure with a strongly hydrophobic side chain was unfavorable for antibacterial activity. The reason for these results is not clear now, but it might involve poor membrane permeability or exclusion of the compounds.

MICs of the representative compound **6** against other bacteria were evaluated. Compound **6** showed no antibacterial activities (MICs>64 μg/mL) against not only wild type strains (*E. coli*, *Haemophilus influenzae*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*) but also efflux pump deficient mutant (*E. coli* and *H. influenzae*). These results support the idea that compound **6** might have poor penetration.

On the other hand, compound **6** and other phenylimidazole derivatives in Tables 2 and 3 were evaluated against *S. pneumoniae* KU197 mutant with the alanine residue at position 141 in FabK replaced by serine,[42] and elevated MICs (>4-fold) versus a parent strain were observed. Furthermore, the phenylimidazole derivatives in Tables 2 and 3 showed no significant cytotoxicity (IC_{50} > 69 μM, in human K562-erythroleukemia cells). These studies support that the mode of action (MOA) of compound **6** is the inhibition of fatty acid synthesis.

Conclusion

We have discovered phenylimidazole derivatives of 4-pyridone as novel dual inhibitors of bacterial enoyl-ACP reductases FabI and FabK. On the basis of the iterative medicinal chemistry and crystallographic study of FabK from *S. pneumoniae*/compound **26**, we designed novel FabI and FabK dual inhibitors, where a FabK inhibitory moiety (phenylimidazoles) was combined with a FabI inhibitory moiety (4-pyridones). Since a saturated two-carbon chain was identified as a suitable spacer, related compounds were synthesized and evaluated. Among them, compound **6** showed strong FabI and FabK inhibitory activities with potent antibacterial activity against *S. pneumoniae*. This compound showed elevated MICs against FabK mutant of *S. pneumoniae* versus wild type strains. The macromolecular synthesis assay demonstrated that the 4-pyridone derivative and imidazole derivative selectively inhibited the lipid biosynthesis. Additionally, these compounds showed no significant cytotoxicity. These studies support a MOA for compound **6** as fatty acid synthesis inhibitor. Small-molecule FabI and FabK dual inhibitors could be candidates for systematically active agents for the treatment of clinically important bacterial infections. Further design and chemical modifications including the preparation of type II compounds to overcome clinically important bacterial infections are in progress.

Experimental Section

Chemistry. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz or 125 MHz) spectra were recorded on a JEOL Lambda 400 spectrometer or BRUKER Avance 500 spectrometer. Chemical shifts were reported in δ value (ppm) with tetramethylsilane (TMS) as the internal standard (NMR peak designations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad peak). Mass spectra were recorded with a JEOL JMS-700 spectorometer. High-resolution mass spectra (HRMS) were obtained on a JMS-FABmate spectrometer. LC−MS analyses were performed using the following methods. Method A: Capcellpak C_{18} MG (Shiseido) 3 mm × 150 mm column; linear gradient from 10% to 90% CH_3CN in H_2O over 10 min (0.01% trifluoroacetic acid) at a flow rate 0.4 mL/min. Method B: Capcellpak C_{18} MGII (Shiseido) 3 mm × 150 mm column; linear gradient from 10% to 90% CH_3CN in H_2O over 10 min (5 mM ammonium acetate) at a flow rate 0.4 mL/min. Elemental analyses were performed by Toray Research Center, Inc. Column chromatography was performed with silica gel (Kanto Chemical, 60N spherical, neutral). Preparative thin layer chromatography (preparative TLC) was performed with silica gel (Merck PLC plates, silica gel 60 F254). All the reagents and solvents were from commercial suppliers and were used without further purification.

Benzyl 2-Amino-2-iminoethylcarbamate Hydrobromide (8). To a solution of benzyl cyanomethylcarbamate (**7**) (Aldrich, 12.5 g, 66 mmol) in MeOH (190 mL) was added sodium methoxide (0.36 g, 6.6 mmol). The mixture was stirred at room temperature overnight and then to it was added ammonium bromide (6.8 g, 69 mmol). The mixture was stirred at room temperature for 1 h more, and then it was concentrated under reduced pressure. The resulting solid was dissolved in hexane/EtOAc = 1/1 (300 mL), stirred atroom temperature for 1.5 h, and filtered to obtain the title compound as an off-white powder (16.5 g, 87%). ^{1}H NMR (CD_3-OD): δ 7.39−7.31 (5H, m), 5.14 (2H, s), 4.10 (2H, s). MS (ESI+): 208 (M + H). HRMS: calcd for $C_{10}H_{13}N_3O_2$ (M + H) 208.1086, found 208.1085.

Benzyl (4-(4-Bromophenyl)-1*H*-imidazol-2-yl)methylcarbamate (10). To a solution of benzyl 2-amino-2-iminoethylcarbamate hydrobromide (**8**) (8.0 g, 28 mmol) in THF/H_2O = 10/1 (330 mL) were added potassium carbonate (3.8 g, 28 mmol) and 2, 4′-dibromoacetophenone (**9**) (Acros, 5.4 g, 19 mmol). The mixture was stirred at 80 °C for 30 min and then it was poured into brine, extracted with CH_2Cl_2, dried over Na_2SO_4, and concentrated under reduced pressure. To the resulting residue was added hexane/EtOAc to obtain the crude solid. Recrystallization from MeOH/$CHCl_3$ afforded the title compound as a white powder (1.13 g, 28%). ^{1}H NMR ($CDCl_3$): δ 7.60 (2H, d, J = 8.5 Hz), 7.47 (2H, d, J = 8.5 Hz), 7.37−7.32 (5H, m), 7.22 (1H, s), 5.63 (1H, br), 5.15 (2H, s), 4.41 (2H, d, J = 6.1 Hz). ^{13}C NMR (DMSO-d_6): δ 156.2, 145.8, 138.4, 136.9, 134.1, 131.1, 128.2, 127.70, 127.66, 126.0, 118.4, 113.3, 65.4, 38.2. MS (FAB+): 386 (M + H). HRMS: calcd for $C_{18}H_{16}BrN_3O_2$ (M + H) 386.0504, found 386.0496.

Benzyl 4-(4-Bromophenyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-2-yl)methylcarbamate (11). To a solution of benzyl (4-(4-bromophenyl)-1*H*-imidazol-2-yl)methylcarbamate (**10**) (386 mg, 1.0 mmol) in THF (8 mL) were added sodium hydride (24 mg, 1.0 mmol) and (2-(chloromethoxy)ethyl)trimethylsilane (175 μL, 1.0 mmol). The mixture was stirred at room temperature for 2 h and at 40 °C for another 1 h and then it was poured into brine, extracted with CH_2Cl_2, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by chromatography (hexane/EtOAc = 2/1) to obtain the title compound as a pale yellow powder (312 mg, 60%). ^{1}H NMR ($CDCl_3$): δ 7.62 (2H, d, J = 8.3 Hz), 7.49 (2H, d, J = 8.3 Hz), 7.38−7.33 (5H, m), 5.34 (2H, brs), 5.15 (2H, brs), 4.54 (2H, d, J = 5.6 Hz), 3.55 (2H, t, J = 8.2 Hz), 0.93 (2H, t, J = 8.2 Hz). ^{13}C NMR ($CDCl_3$): δ 156.2, 145.4, 139.5, 136.3, 132.6, 131.7, 128.5, 128.2, 126.4, 120.6, 116.2, 75.2, 67.0, 66.5, 37.3, 17.8, −1.4. MS (FAB+): 516 (M + H). HRMS: calcd for $C_{24}H_{30}BrN_3O_3Si$ (M + H) 516.1318, found 516.1331.

1-Benzyloxycarbonyl-2,3-dihydro-2-methylpyridin-4(1*H*)-one (13). To a solution of methylmagnesium bromide (178 mmol) and 4-methoxypyridine (**12**) (16.1 g, 148 mmol) in THF (300 mL) at −25 °C was added benzyl chloroformate over a period of 30 min. The resulting mixture was stirred at −25 °C for 4 h and then poured into 3 N HCl (300 mL). After stirring for 10 min at room temperature, the mixture was extracted with EtOAc twice and the combined organic extract was washed with 5% aqueous $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by chromatography

(hexane/EtOAc = 3/1) to obtain the title compound (34.1 g, 94%). ^{1}H NMR ($CDCl_3$) δ 7.74 (1H, d, J = 8.0 Hz), 7.45−7.28 (5H, m), 5.40−5.22 (3H, m), 4.73 (1H, br), 2.85 (1H, dd, J = 16.6 Hz, J = 6.8 Hz), 2.31 (1H, d, J = 16.6 Hz), 1.26 (3H, t, J = 6.8 Hz). MS (FAB+): 246 (M + H).

1-Benzyloxycarbonyl-3-((2,6-dichlorophenyl)hydroxymethyl)-2,3-dihydro-2-methylpyridin-4(1*H*)-one (14). To a solution of 1-benzyloxycarbonyl-2,3-dihydro-2-methylpyridin-4(1*H*)-one (**13**) (5.0 g, 20.4 mmol) in THF (60 mL) at −78 °C was added 1 M lithium bis(trimethylsilyl)amide in THF (22.4 mL), and the resulting mixture was stirred at 0 °C for 30 min and cooled to −78 °C. To this solution was added 2,6-dichlorobenzaldehyde (4.64 g, 26.5 mmol). The resulting solution was stirred at −78 °C for 1 h and then poured into saturated, aqueous NH_4Cl (200 mL). The mixture was extracted with EtOAc twice and the combined organic extract was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by chromatography (hexane/EtOAc = 3/1) to obtain the title compound (8.4 g, 98%). ^{1}H NMR ($CDCl_3$) δ 7.70−8.00 (1H, br), 7.50−7.05 (8H, br), 5.63−5.10 (4H, m), 4.15−3.90 (1H, br), 3.30−3.17 (1H, br), 3.12 (1H, d, J = 9.0 Hz), 1.20 (3H, d, J = 6.8 Hz). MS (FAB+): 420 (M + H).

1-Benzyloxycarbonyl-3-((2,6-dichlorophenyl)(methylsulfonyloxy)methyl)-2,3-dihydro-2-methylpyridin-4(1*H*)-one (15). To a solution of 1-benzyloxycarbonyl-3-((2,6-dichlorophenyl)hydroxymethyl)-2,3-dihydro-2-methylpyridin-4(1*H*)-one (**14**) (1.61 g, 3.83 mmol) in pyridine (10 mL) at 0 °C was added mesyl chloride (0.593 mL, 7.66 mmol). The resulting mixture was stirred at room temperature for 3 h and then poured into water (150 mL). The mixture was extracted with EtOAc (150 mL) and the organic extract was washed with water, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by chromatography (hexane/EtOAc = 3/1) to obtain the title compound (1.79 g, 94%). ^{1}H NMR ($CDCl_3$) δ 8.05−7.80 (1H, br), 7.52−7.05 (8H, br), 6.40 (1H, d, J = 10.7 Hz), 5.59−5.02 (3H, m), 4.15−3.91 (1H, br), 3.61−3.40 (1H, br), 2.89 (3H, s), 1.22 (3H, d, J = 6.8 Hz). MS (FAB): 498 (M + H).

3-(2,6-Dichlorobenzyl)-2-methylpyridin-4-(1*H*)-one (16). To a solution of 1-benzyloxycarbonyl-3-((2,6-dichlorophenyl)(methylsulfonyloxy)methyl)-2,3-dihydro-2-methylpyridin-4(1*H*)-one (**15**) (1.00 g, 2.01 mmol) in THF (35 mL) was added potassium *tert*-butoxide (675.5 mg 6.02 mmol). The resulting solution was stirred at room temperature for 10 min and then poured into 5% aqueous NH_4Cl (100 mL). The mixture was extracted with chloroform/2-propanol (5:1, 100 mL) twice and the combined organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by chromatography ($CHCl_3$/MeOH = 9/1) to obtain the title compound as a white powder (469 mg, 87%). IR (KBr) 3333, 1624, 1503, 1159, 842, 768, 543 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.26 (1H, br), 7.22 (2H, d, J = 8.1 Hz), 7.05 (1H, t, J = 8.1 Hz), 6.23 (1H, d, J = 7.1 Hz), 4.24 (2H, s), 2.10 (3H, s). ^{13}C NMR (CD_3OD): δ 180.4, 147.2, 137.63, 137.57, 137.2, 129.7, 129.3, 126.4, 115.3, 28.0, 17.5. HRMS: calcd for $C_{13}H_{12}Cl_2NO$ (M + H) 268.0296, found: 268.0294.

3-(2,6-Dichlorobenzyl)-2-methyl-1-vinylpyridin-4(1*H*)-one (17). To a solution of 3-(2,6-dichlorobenzyl)-2-methylpyridin-4(1*H*)-one (**16**) (804 mg, 3.0 mmol) in DMF/CH_3CN = 1/1 (30 mL) were added tetravinyltin (1.09 mL, 6.0 mmol) and copper(II) acetate (1.09 g, 6.0 mmol). The mixture was stirred at room temperature overnight under oxygen atmosphere, and then it was poured into aqueous $AcONH_4$, extracted with EtOAc, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by chromatography ($CHCl_3$/MeOH = 10/1) to obtain the title compound as a white powder (669 mg, 76%). ^{1}H NMR ($CDCl_3$): δ 7.48 (1H, d, J = 7.7 Hz), 7.30−7.25 (2H, m), 7.07 (1H, t, J = 8.0 Hz), 6.82 (1H, dd, J = 15 Hz, J = 7.3 Hz), 6.36 (1H, d, J = 7.7 Hz), 5.28 (1H, dd, J = 15 Hz, J = 1.7 Hz), 5.16 (1H, dd, J = 7.3 Hz, J = 1.7 Hz), 4.35 (2H, s), 2.10 (3H, s). ^{13}C NMR ($CDCl_3$): δ 178.2, 143.4, 136.98, 136.95, 135.9, 135.5, 128.4, 127.6, 126.7, 115.7, 109.2, 27.3, 16.8. MS (FAB+): 294 (M + H). HRMS: calcd for $C_{15}H_{13}Cl_2NO$ (M + H) 294.0452, found 294.0449.

Benzyl (4-(4-(2-(3-(2,6-Dichlorobenzyl)-2-methyl-4-oxopyridin-1(4*H*)-yl)vinyl)phenyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-2-yl)methylcarbamate (18). To a solution of benzyl (4-(4-bromophenyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-2-yl)methylcarbamate (**11**) (400 mg, 0.77 mmol) and 3-(2,6-dichlorobenzyl)-2-methyl-1-vinylpyridin-4(1*H*)-one (**17**) (216 mg, 0.74 mmol) in DMF (15 mL) were added tris(dibenzylideneacetone)dipalladium (142 mg, 0.16 mmol), tri-*tert*-butylphosphine (77 μL, 0.31 mmol), and dicyclohexylmethylamine (329 μL, 1.6 mmol). The mixture was stirred at 80 °C for 2 h, and then it was poured into brine, extracted with CH_2Cl_2, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by preparative TLC ($CHCl_3$/MeOH = 20/1). Further purification by LH-20 chromatography (Sephadex, CH_2Cl_2/MeOH = 1/1) provided the title compound as a yellow foam, which was a diastereomixture (121 mg, 23%, E/Z = ca. 7/1). ^{1}H NMR (*E* isomer, $CDCl_3$): δ 7.74 (2H, d, J = 8.4 Hz), 7.55 (1H, d, J = 7.8 Hz), 7.36−7.27 (10H, m), 7.17 (1H, d, J = 14 Hz), 7.07 (1H, t, J = 7.6 Hz), 6.63 (1H, d, J = 14 Hz), 6.38 (1H, d, J = 7.8 Hz), 5.35 (2H, s), 5.13 (2H, s), 4.54 (2H, d, J = 5.6 Hz), 4.38 (2H, s), 3.54 (2H, t, J = 8.0 Hz), 2.16 (3H, s), 0.92 (2H, t, J = 8.0 Hz), 0.01 (9H, m). ^{13}C NMR (*E* isomer, $CDCl_3$): δ 178.1, 156.2, 145.5, 143.9, 139.9, 137.7, 137.1, 136.3, 136.0, 134.3, 132.2, 128.6, 128.4, 128.2, 128.1, 128.0, 127.6, 126.9, 126.9, 126.4, 125.3, 116.6, 115.7, 75.3, 67.1, 66.6, 37.5, 27.4, 17.8, 17.1, −1.4. MS (FAB+): 729 (M + H). HRMS: calcd for $C_{39}H_{42}Cl_2N_4OSi$ (M + H) 729.2431, found 729.2427.

(*E*)-(4-(4-(2-(3-(2,6-Dichlorobenzyl)-2-methyl-4-oxopyridin-1(4*H*)-yl)vinyl)phenyl)-1*H*-imidazol-2-yl)methylamine Hydrochloride (19). Benzyl (4-(4-(2-(3-(2,6-dichlorobenzyl)-2-methyl-4-oxopyridin-1(4*H*)-yl)vinyl)phenyl)-1-((2-(trimethylsilyl)-ethoxy)methyl)-1*H*-imidazol-2-yl) methylcarbamate (**18**) (21 mg, 0.029 mmol) was dissolved to 4 N HCl/dioxane/H_2O (0.9 mL). The mixture was stirred at 100 °C for 1.5 h, and then it was washed with ether. The water layer was concentrated under reduced pressure. The resulting residue was recrystalyzed from $CHCl_3$/MeOH to obtain the title compound as a white powder (4.1 mg, 25%). ^{1}H NMR (CD_3OD): δ 8.63 (1H, d, J = 7.2 Hz), 8.01 (1H, s), 7.94−7.89 (3H, m), 7.80 (2H, d, J = 8.4 Hz), 7.41 (2H, d, J = 8.0 Hz), 7.29−7.19 (3H, m), 4.54 (4H, m), 2.68 (3H, m). ^{13}C NMR (CD_3OD): δ 172.1, 154.6, 144.6, 142.1, 137.1, 136.8, 136.7, 135.4, 133.9, 133.3, 130.2, 130.0, 129.8, 129.3, 126.5, 126.4, 118.0, 112.8, 36.7, 29.1, 18.3. MS (FAB+): 465 (M + H). HRMS: calcd for $C_{25}H_{22}Cl_2N_4O$ (M + H) 465.1249, found 465.1258.

(4-(4-(2-(3-(2,6-Dichlorobenzyl)-2-methyl-4-oxopyridin-1(4*H*)-yl)ethyl)phenyl)-1*H*-imidazole-2-yl)methylamine Hydrochloride (20). To a solution of (*E*)-(4-(4-(2-(3-(2,6-dichlorobenzyl)-2-methyl-4-oxopyridin-1(4*H*)-yl)vinyl)phenyl)-1*H*-imidazol-2-yl)methylamine hydrochloride (**19**) (25 mg, 0.44 mmol) in MeOH/HCl (1.1 mL) was added 10% Pd/C (2.5 mg, 10 w/w%). The reaction mixture was stirred at room temperature for 4.5 h under hydrogen atmosphere, and then it was filtered through Celite and concentrated under reduced pressure. The resulting residue was purified by preparative TLC ($CHCl_3$/MeOH/NH_3 aq = 5/1/0.1), and then it was acidified by HCl/EtOAc and concentrated under reduced pressure to obtain the title compound as pale yellow powder (11 mg, 44%). ^{1}H NMR (CD_3OD): δ 8.21 (2H, d, J = 7.2 Hz), 7.65 (2H, d, J = 8.2 Hz), 7.59 (1H, s), 7.35 (2H, d, J = 8.2 Hz), 7.18−7.12 (3H, m), 6.97 (2H, d, J = 7.2 Hz), 4.68 (2H, t, J = 6.8 Hz), 4.44 (2H, s), 4.32 (2H, s), 3.18 (2H, t, J = 6.8 Hz), 2.43 (3H, s). ^{13}C NMR (CD_3OD): δ 170.7, 155.1, 146.0, 141.6, 139.0, 137.0, 137.0, 135.4, 131.5, 130.8, 130.1, 130.0, 127.2, 126.6, 117.7, 112.4, 58.8, 36.9, 36.5, 29.0, 17.2. MS (FAB+): 467 (M + H). HRMS: calcd for $C_{25}H_{24}Cl_2N_4O$ (M + H) 467.1405, found 467.1410.

1-((4-(4-(2-(3-(2,6-Dichlorobenzyl)-2-methyl-4-oxopyridin-1(4*H*)-yl)ethyl)phenyl)-1*H*-imidazol-2-yl)methyl)-3-(6-(methylsulfonyl)benzo[*d*]thiazol-2-yl)urea (6). To a solution of 2-amino-6-(methylsulfonyl)benzo[*d*]thiazole (**21**) (2.28 g, 10 mmol) in THF was added 1,1-carbonyldiimidazole (3.24 g, 20 mmol). The mixture

was stirred at room temperature overnight, and then it was filtered. The filter cake was washed with THF to give *N*-(5-(6-(methylsulfonyl)benzo[*d*]thiazol-2-yl)-1*H*-imidazole-1-carboxamide (**22**) as a crude intermediate (3.01 g). To a solution of **22** (22.4 mg, 0.069 mmol) in DMF (1 mL) were added (4-(4-(2-(3-(2,6-dichlorobenzyl)-2-methyl-4-oxopyridin-1(4*H*)-yl)ethyl)phenyl)-1*H*-imidazol-2-yl)-methylamine hydrochloride (**20**) (40 mg, 0.069 mmol) and *N*,*N*′-diisopropylethylamine (47 μL, 0.28 mmol). The mixture was stirred at room temperature for 3 h, and then it was poured into water, extracted with $CHCl_3$, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by preparative TLC ($CHCl_3$/MeOH/NH_3 aq = 5/1/0.1) to obtain the title compound as a white powder (12 mg, 21%). ^{1}H NMR (CD_3OD): δ 8.43 (1H, d, J = 1.8 Hz), 7.92 (1H, dd, J = 8.6 Hz, J = 1.8 Hz), 7.80 (1H, d, J = 8.6 Hz), 7.57−7.51 (3H, m), 7.30 (1H, s), 7.22 (2H, d, J = 8.0 Hz), 7.01 (2H, d, J = 8.4 Hz), 6.94 (1H, t, J = 8.4 Hz), 6.29 (1H, d, J = 7.2 Hz), 4.58 (2H, s), 4.26−4.22 (4H, m), 3.14 (3H, m), 2.99 (2H, t, J = 6.4 Hz), 1.86 (3H, s). ^{13}C NMR (CD_3OD): δ 178.5, 165.4, 155.7, 153.9, 148.5, 146.8, 142.4, 138.1, 137.3, 136.7, 135.7, 133.5, 131.6, 130.3, 129.5, 128.8, 128.0, 126.1, 125.9, 122.4, 121.1, 116.8, 115.3, 56.0, 44.9, 37.9, 37.4, 27.9, 16.3. MS (FAB+): 721 (M + H). HRMS: calcd for $C_{34}H_{30}Cl_2N_6O_4S_2$ (M + H) 721.1225, found 721.1227. Anal. ($C_{34}H_{30}Cl_2N_6O_4S_2\cdot 3H_2O$) C, H, N.

1-((4-(4-Bromophenyl)-1*H*-imidazol-2-yl)methyl)-3-(5-(pyridin-2-ylthio)thiazol-2-yl)urea (24). To a solution of *N*-(5-(pyridin-2-ylthio)thiazol-2-yl)-1*H*-imidazole-1-carboxamide (30 mg, 0.10 mmol) in THF (1 mL) were added (4-(4-bromophenyl)-1*H*-imidazol-2-yl)methylamine hydrochloride (36 mg, 0.11 mmol) and *N*,*N*′-diisopropylethylamine (37 μL, 0.22 mmol). The mixture was stirred at room temperature overnight, and then it was poured into brine, extracted with $CHCl_3$, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting solid was filtered and the filter cake was washed with $CHCl_3$/MeOH to obtain the title compound as a white powder (17 mg, 34%). Recrystallization from $CHCl_3$/MeOH afforded completely pure compound. ^{1}H NMR ($CDCl_3/CD_3OD$): δ 8.38−8.36 (1H, m), 7.58−7.49 (6H, m), 7.23 (1H, s), 7.09 (1H, ddd, J = 8.2 Hz, J = 7.6 Hz, J = 0.6 Hz), 7.01 (1H, dd, J = 8.2 Hz, J = 0.6 Hz), 4.47 (2H, s). ^{13}C NMR (DMSO-d_6): δ 164.4, 160.0, 153.7, 149.3, 146.8, 145.5, 138.5, 137.6, 134.0, 131.2, 126.0, 120.6, 119.6, 118.5, 113.3, 112.8, 37.2. MS (FAB+): 487 (M + H). HRMS: calcd for $C_{19}H_{15}BrN_6OS_2$ (M + H) 487.0010, found 487.0017. Anal. ($C_{19}H_{15}BrN_6OS_2$) C, H, N.

1-((4-Phenyl-1*H*-imidazol-2-yl)methyl)-3-(5-(pyridin-2-ylthio)-thiazol-2-yl)urea (23). The title compound was obtained from *N*-(5-(pyridin-2-ylthio)thiazol-2-yl)-1*H*-imidazole-1-carboxamide and (4-phenyl-1*H*-imidazol-2-yl)methylamine hydrochloride according to the similar procedure used to prepare **24**. Recrystallization from MeOH/$CHCl_3$/diisopropyl ether afforded completely pure compound as a white powder (17 mg, 13%). ^{1}H NMR (DMSO-d_6): δ 8.41 (1H, m), 7.77−7.68 (3H, m), 7.64 (1H, s), 7.55 (1H, br), 7.36−7.32 (2H, m), 7.19−7.10 (3H, m), 7.03 (1H, dd, J = 8.0 Hz, J = 0.7 Hz), 4.41 (2H, d, J = 5.4 Hz). ^{13}C NMR (DMSO-d_6): δ 164.4, 160.0, 153.6, 149.6, 149.3, 146.8, 145.2, 137.6, 128.3, 125.9, 124.0, 120.6, 119.6, 115.5, 112.8, 112.6, 37.2. MS (FAB+): 409 (M + H). HRMS: calcd for $C_{19}H_{16}N_6OS_2$ (M + H) 409.0905, found 409.0905. Anal. ($C_{19}H_{16}N_6OS_2\cdot 0.25H_2O$) C, H, N.

1-((4-(3-Bromophenyl)-1*H*-imidazol-2-yl)methyl)-3-(5-(pyridin-2-ylthio)thiazol-2-yl)urea (25). The title compound was obtained from *N*-(5-(pyridin-2-ylthio)thiazol-2-yl)-1*H*-imidazole-1-carboxamide and (4-(3-bromophenyl)-1*H*-imidazol-2-yl)methylamine hydrochloride according to the similar procedure used to prepare **24**. Recrystallization from MeOH/$CHCl_3$/Et_2O afforded completely pure compound as a white powder (66 mg, 41%). ^{1}H NMR ($CDCl_3/CD_3OD$): δ 8.39−8.37 (1H, m), 7.82 (1H, brs), 7.58−7.52 (3H, m), 7.39−7.37 (1H, m), 7.27−7.23 (2H, m), 7.08−7.06 (1H, m), 7.00 (1H, d, J = 8.3 Hz), 4.46 (2H, s). ^{13}C NMR (DMSO-d_6): δ 164.6, 160.1, 153.9, 149.5, 146.9, 145.8, 138.2, 137.8, 137.3, 130.7, 128.6, 126.6, 123.0, 122.1, 120.8, 119.8, 114.0, 113.0, 37.4. MS (FAB+): 487 (M + H). HRMS: calcd for $C_{19}H_{15}BrN_6OS_2$ (M + H) 487.0010, found 487.019. Anal. ($C_{19}H_{15}BrN_6OS_2\cdot 0.1H_2O$) C, H, N.

Sodium 2-(4-(2-((3-(5-(Pyridin-2-ylthio)thiazol-2-yl)ureido)-methyl)-1*H*-imidazol-4-yl)phenoxy)acetate (26). To a solution of 1-((4-(4-((ethoxycarbonyl)methyoxy)phenyl)-1*H*-imidazol-2-yl)-methyl)-3-(5-(pyridin-2-ylthio)thiazol-2-yl)urea (99 mg, 0.19 mmol), which was obtained according to the similar procedure used to prepare **24**, in EtOH (3 mL) was added 5 N aqueous NaOH (0.39 mL, 1.9 mmol). The mixture was stirred at room temperature for 30 min, and then it was concentrated under reduced pressure. The resulting residue was purified by Dianion HP-20 (Mitsubishi Chemical) to obtain the title compound. Recrystallization from MeOH afforded completely pure compound as a white powder (28 mg, 28%). ^{1}H NMR (CD_3OD): δ 8.36−8.33 (1H, m), 7.67 (1H, ddd, J = 8.0 Hz, J = 7.8 Hz, J = 1.7 Hz), 7.57−7.54 (3H, m), 7.19−7.13 (2H, m), 7.07 (1H, d, J = 8.0 Hz), 6.94 (2H, d, J = 8.8 Hz) 4.52 (2H, s), 4.39 (2H, s). ^{13}C NMR (CD_3OD): δ 176.7, 167.4, 162.4, 159.3, 156.4, 150.2, 147.8, 146.9, 139.1, 127.0, 121.9, 116.0, 115.6, 68.5, 38.4. MS (ESI+): 483 (M + H). HRMS: calcd for $C_{21}H_{18}N_6O_4S_2$ (M + H) 483.0909, found 483.0914. Anal. ($C_{21}H_{17}N_6NaO_4S_2\cdot 3.4H_2O$) C, H, N.

1-((4-(4-(2-(3-(2,6-Dichlorobenzyl)-2-methyl-4-oxopyridin-1(4*H*)-yl)ethyl)phenyl)-1*H*-imidazol-2-yl)methyl)-3-(5-bromothiazol-2-yl)urea (29). To a solution of *N*-(5-bromothiazol-2-yl)-1*H*-imidazole-1-carboxamide (19 mg, 0.069 mmol) in DMF (1 mL) were added (4-(4-(2-(3-(2,6-dichlorobenzyl)-2-methyl-4-oxopyridin-1(4*H*)-yl)ethyl)phenyl)-1*H*-imidazol-2-yl)methylamine hydrochloride (**20**) (40 mg, 0.069 mmol) and *N*,*N*′-diisopropylethylamine (47 μL, 0.28 mmol). The mixture was stirred at room temperature for 3.5 h, and then it was poured into water, extracted with $CHCl_3$, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by preparative TLC ($CHCl_3$/MeOH/NH_3 aq = 7/1/0.1) to obtain the title compound. Recrystallization from MeOH/$CHCl_3$/DMSO afforded completely pure compound as an off-white powder (23 mg, 49%). ^{1}H NMR (CD_3OD): δ 7.56 (2H, d, J = 7.6 Hz), 7.51 (2H, d, J = 8.0 Hz), 7.30−7.21 (4H, m), 7.01 (2H, d, J = 8.2 Hz), 6.94 (1H, t, J = 8.2 Hz), 6.29 (1H, d, J = 7.6 Hz), 4.52 (2H, s), 4.27−4.22 (4H, m), 2.99 (2H, t, J = 6.2 Hz), 1.86 (3H, s). ^{13}C NMR (DMSO-d_6): δ 175.7, 160.3, 153.8, 145.1, 145.0, 140.9, 139.6, 138.4, 136.8, 134.9, 134.8, 133.2, 129.0, 128.5, 127.9, 125.6, 124.2, 113.7, 112.6, 99.6, 54.0, 37.3, 35.9, 27.4, 15.4. MS (FAB+): 671 (M + H). HRMS: calcd for $C_{29}H_{25}BrCl_2N_6O_2S$ (M + H) 671.0398, found 671.0404. LC−MS: method A, t_R = 8.41 min, 671 (M + H), purity 100%; method B, t_R = 10.37 min, 671 (M + H), purity 99.3%.

1-((4-(4-(2-(3-(2,6-Dichlorobenzyl)-2-methyl-4-oxopyridin-1(4*H*)-yl)methyl)phenyl)-1*H*-imidazol-2-yl)methyl)-3-(5-bromothiazol-2-yl)urea (27). The title compound was obtained from *N*-(5-bromothiazol-2-yl)-1*H*-imidazole-1-carboxamide and (4-(4-(2-(3-(2,6-dichlorobenzyl)-2-methyl-4-oxopyridin-1(4*H*)-yl)methyl)phenyl)-1*H*-imidazol-2-yl)methylamine hydrochloride according to the similar procedure used to prepare **29** as an off-white powder (5.6 mg, 21%). ^{1}H NMR (CD_3 OD): δ 7.85 (1H, d, J = 7.6 Hz), 7.67 (2H, d, J = 8.0 Hz), 7.37 (1H, s), 7.28 (2H, d, J = 7.8 Hz), 7.25 (1H, s), 7.12 (1H, t, J = 7.8 Hz), 7.03 (2H, d, J = 8.0 Hz), 6.43 (2H, d, J = 7.6 Hz), 5.26 (2H, s), 4.51 (2H, s), 4.33 (2H, s), 2.06 (3H, s). ^{13}C NMR (CD_3OD): δ 179.6, 162.5, 156.1, 149.1, 147.6, 143.9, 139.1, 137.7, 136.9, 135.8, 129.8, 129.3, 129.0, 127.4, 126.4, 115.7, 102.1, 58.5, 38.3, 28.5, 16.8. MS (FAB+): 657 (M + H). HRMS: calcd for $C_{28}H_{23}BrCl_2N_6O_2S$ (M + H) 657.0242, found 657.0229. LC−MS: method A, t_R = 8.47 min, 657 (M + H), purity 100%; method B, t_R = 10.33 min, 657 (M + H), purity 100%.

(*E*)-1-((4-(4-(2-(3-(2,6-Dichlorobenzyl)-2-methyl-4-oxopyridin-1(4*H*)-yl)vinyl)phenyl)-1*H*-imidazol-2-yl)methyl)-3-(5-bromothiazol-2-yl)urea (28). The title compound was obtained from *N*-(5-bromothiazol-2-yl)-1*H*-imidazole-1-carboxamide and (4-(4-(2-(3-(2,6-dichlorobenzyl)-2-methyl-4-oxopyridin-1(4*H*)-yl)vinyl)phenyl)-1*H*-imidazol-2-yl)methylamine hydrochloride (**19**) according to the similar procedure used to prepare **29** as an off-white powder (4.4 mg, 40%). ^{1}H NMR (DMSO-d_6): δ 7.90 (1H, d, J = 7.6 Hz), 7.76 (2H, d, J = 8.0 Hz), 7.64−7.52 (4H, m), 7.41−7.38 (3H, m), 7.23

(1H, t, J = 8.0 Hz), 7.10 (1H, br), 6.82 (1H, d, J = 13.6 Hz), 6.10 (1H, d, J = 7.6 Hz), 4.39 (1H, d, J = 4.8 Hz), 4.16 (2H, s), 2.23 (3H, s). ^{13}C NMR (DMSO-d_6): δ 176.4, 160.2, 153.8, 145.6, 144.5, 139.3, 138.39, 138.39, 136.6, 135.0, 134.7, 132.1, 128.6, 128.3, 128.2, 127.1, 125.2, 124.8, 124.3, 114.5, 113.4, 99.7, 37.3, 27.4, 16.7. MS (FAB+): 669 (M + H). HRMS: calcd for $C_{29}H_{23}BrCl_2N_6O_2S$ (M + H) 669.0242, found 669.0230. LC−MS: method A, t_R = 9.07 min, 669 (M + H), purity 98.2%; method B, t_R = 11.14 min, 669 (M + H), purity 97.9%.

(*E*)-1-((4-(4-(3-(3-(2,6-Dichlorobenzyl)-2-methyl-4-oxopyridin-1(4*H*)-yl)prop-1-enyl)phenyl)-1*H*-imidazol-2-yl)methyl)-3-(5-bromothiazol-2-yl)urea (30). The title compound was obtained from *N*-(5-bromothiazol-2-yl)-1*H*-imidazole-1-carboxamide and (4-(4-(2-(3-(2,6-dichlorobenzyl)-2-methyl-4-oxopyridin-1(4*H*)-yl)prop-1-enyl)phenyl)-1*H*-imidazol-2-yl)methylamine hydrochloride according to the similar procedure used to prepare **29** as a white powder (17 mg, 41%). ^{1}H NMR (CD_3OD): δ 7.76 (1H, d, J = 7.6 Hz), 7.63 (2H, d, J = 8.4 Hz), 7.36−7.31 (5H, m), 7.25 (1H, s), 7.14 (1H, t, J = 7.6 Hz), 6.41 (1H, d, J = 7.6 Hz), 6.34 (1H, dt, J = 16 Hz, J = 4.8 Hz), 6.18 (1H, d, J = 16 Hz), 4.80−4.77 (2H, m), 4.51 (2H, s), 4.04 (2H, s), 2.20 (3H, s). ^{13}C NMR (CD_3OD): δ 179.6, 162.5, 156.1, 149.0, 147.5, 143.2, 139.1, 137.9, 136.9, 135.7, 132.9, 129.8, 129.3, 128.7, 127.9, 126.0, 124.4, 115.9, 102.1, 56.9, 38.3, 28.5, 16.5. MS (FAB+): 683 (M + H). HRMS: calcd for $C_{30}H_{25}BrCl_2N_6O_2S$ (M + H) 683.0398, found 683.0388. Anal. ($C_{30}H_{25}BrCl_2N_6O_2S\cdot 2.46H_2O$) C, H, N.

1-((4-(4-(3-(3-(2,6-Dichlorobenzyl)-2-methyl-4-oxopyridin-1(4*H*)-yl)propyl)phenyl)-1*H*-imidazol-2-yl)methyl)-3-(5-bromothiazol-2-yl)urea (31). The title compound was obtained from *N*-(5-bromothiazol-2-yl)-1*H*-imidazole-1-carboxamide and (4-(4-(2-(3-(2,6-dichlorobenzyl)-2-methyl-4-oxopyridin-1(4*H*)-yl)propyl)phenyl)-1*H*-imidazol-2-yl)methylamine hydrochloride according to the similar procedure used to prepare **29** as a white powder (19 mg, 41%). ^{1}H NMR (CD_3OD): δ 7.90 (1H, s), 7.69 (1H, d, J = 7.6 Hz), 7.59 (2H, d, J = 8.4 Hz), 7.33−7.29 (3H, m), 7.25 (1H, s), 7.19−7.15 (3H, m), 6.35 (2H, d, J = 7.6 Hz), 4.51 (2H, s), 4.32 (2H, s), 3.99 (2H, t, J = 7.2 Hz), 2.62 (2H, t, J = 7.2 Hz), 2.08 (3H, s), 2.05−1.97 (2H, m). ^{13}C NMR (CD_3OD): δ 179.2, 162.5, 156.0, 148.5, 147.2, 142.8, 140.5, 139.1, 137.8, 136.9, 129.8, 129.7, 129.3, 128.5, 126.1, 115.7, 102.1, 54.7, 38.3, 33.1, 32.9, 28.5, 16.3. MS (FAB+): 685 (M + H). HRMS: calcd for $C_{30}H_{27}BrCl_2N_6O_2S$ (M + H) 685.0555, found 685.0556. LC−MS: method A, t_R = 8.72 min, 685 (M + H), purity 100%; method B, t_R = 10.81 min, 685 (M + H), purity 99.7%.

1-((4-(4-(2-(3-(2,6-Dichlorobenzyl)-2-methyl-4-oxopyridin-1(4*H*)-yl)ethyl)phenyl)-1*H*-imidazol-2-yl)methyl)-3-(5-(pyridin-2-ylthio)thiazol-2-yl)urea (32). The title compound was obtained from *N*-(5-(pyridin-2-ylthio)thiazol-2-yl)-1*H*-imidazole-1-carboxamide and (4-(4-(2-(3-(2,6-dichlorobenzyl)-2-methyl-4-oxopyridin-1(4*H*)-yl)ethyl)phenyl)-1*H*-imidazol-2-yl)methylamine hydrochloride (**20**) according to the similar procedure used to prepare **29** as an off-white powder (13.4 mg, 37%). ^{1}H NMR (CD_3OD): δ 8.34−8.32 (1H, m), 7.64 (1H, ddd, J = 8.0 Hz, J = 8.0 Hz, J = 2.0 Hz), 7.58 (1H, d, J = 7.4 Hz), 7.55 (1H, s), 7.28 (1H, s), 7.21 (2H, d, J = 8.0 Hz), 7.05−6.99 (3H, m), 6.91 (1H, t, J = 8.0 Hz), 6.31 (1H, d, J = 7.4 Hz), 4.53 (2H, s), 4.26−4.22 (4H, m), 2.99 (2H, d, J = 6.4 Hz), 1.83 (3H, s). ^{13}C NMR (DMSO-d_6): δ 175.6, 164.3, 160.0, 153.6, 149.3, 146.8, 145.0, 144.9, 140.9, 139.4, 137.6, 136.7, 134.81, 134.77, 133.0, 129.0, 128.4, 127.9, 125.5, 124.1, 120.6, 119.6, 113.7, 112.9, 112.5, 53.9, 37.2, 35.9, 27.3, 15.3. MS (FAB+): 702 (M + H). HRMS: calcd for $C_{34}H_{29}Cl_2N_7O_2S_2$ (M + H) 702.1279, found 702.1281. LC−MS: method A, t_R = 8.27 min, 702 (M + H), purity 99.6%; method B, t_R = 10.18 min, 702 (M + H), purity 99.7%.

1-((4-(4-(2-(3-(2,6-Dichlorobenzyl)-2-methyl-4-oxopyridin-1(4*H*)-yl)ethyl)phenyl)-1*H*-imidazol-2-yl)methyl)-3-(1*H*-imidazol-2-yl)urea (33). The title compound was obtained from *N*-(1*H*-imidazol-2-y)-1*H*-imidazole-1-carboxamide and (4-(4-(2-(3-(2,6-dichlorobenzyl)-2-methyl-4-oxopyridin-1(4*H*)-yl)ethyl)phenyl)-1*H*-imidazol-2-yl)methylamine hydrochloride (**20**) according to the similar procedure used to prepare **29** as a white powder (3.6 mg, 72%). ^{1}H NMR (CD_3OD): δ 7.56 (1H, d, J = 7.6 Hz), 7.52 (2H, d, J = 8.0 Hz), 7.28 (1H, brs), 7.22 (2H, d, J = 8.0 Hz), 7.01 (2H, d, J = 7.8 Hz), 6.94 (1H, t, J = 7.8 Hz), 6.72 (2H, s), 6.30 (1H, d, J = 7.6 Hz), 4.51 (2H, s), 4.27−4.23 (4H, m), 2.07 (2H, t, J = 5.2 Hz), 1.87 (3H, s). ^{13}C NMR ($CDCl_3$/CD_3OD): δ 177.9, 156.1, 146.5, 145.4, 142.8, 140.5, 137.3, 136.8, 135.9, 134.6, 131.9, 129.3, 128.6, 127.7, 127.6, 125.3, 117.3, 116.3, 115.0, 55.4, 37.3, 36.8, 27.4, 15.9. MS (FAB+): 576 (M + H). HRMS: calcd for $C_{29}H_{27}Cl_2N_7O_2$ (M + H) 576.1682, found 576.1688. LC−MS: method A, t_R = 7.21 min, 576 (M + H), purity 100%; method B, t_R = 8.97 min, 576 (M + H), purity 100%.

1-((4-(4-(2-(3-(2,6-Dichlorobenzyl)-2-methyl-4-oxopyridin-1(4*H*)-yl)ethyl)phenyl)-1*H*-imidazol-2-yl)methyl)-3-(5-(methylsulfonyl)thiazol-2-yl)urea (34). The title compound was obtained from *N*-(5-(methylsulfonyl)thiazol-2-yl)-1*H*-imidazole-1-carboxamide and (4-(4-(2-(3-(2,6-dichlorobenzyl)-2-methyl-4-oxopyridin-1(4*H*)-yl)ethyl)phenyl)-1*H*-imidazol-2-yl)methylamine hydrochloride (**20**) according to the similar procedure used to prepare **29** as a white powder (4.3 mg, 50%). ^{1}H NMR (CD_3OD): δ 7.88 (1H, s), 7.56 (1H, d, J = 7.6 Hz), 7.52 (1H, d, J = 8.0 Hz), 7.29 (1H, s), 7.23 (2H, d, J = 8.0 Hz), 7.01 (2H, d, J = 8.2 Hz), 6.94 (1H, t, J = 8.2 Hz), 6.29 (1H, d, J = 7.6 Hz), 4.55 (2H, s), 4.26−4.22 (4H, m), 3.21 (3H, s), 2.99 (2H, t, J = 6.4 Hz), 1.86 (3H, s). ^{13}C NMR (DMSO-d_6): δ 169.2, 164.8, 154.3, 153.0, 145.9, 144.6, 144.5, 137.3, 135.1, 134.3, 132.3, 129.9, 129.0, 128.8, 128.6, 125.9, 125.2, 124.6, 115.0, 111.2, 56.6, 45.8, 35.6, 35.1, 28.1, 16.4. MS (ESI+): 671 (M + H). HRMS: calcd for $C_{30}H_{28}Cl_2N_6O_4S_2$ (M + H) 671.1069, found 671.1072. Anal. ($C_{30}H_{28}Cl_2N_6O_4S_2$) C, H, N.

Biology. Preparation of His-Tagged FabI. The *fabI* gene from *E. coli* DH5α was amplified by PCR and cloned into pBAD/Myc-His B vector (Invitrogen). The resulting plasmid was transformed into *E. coli* TOP10. The expression of FabI protein fused with a His-tag was induced with 0.2% arabinose. The cell pellets were resuspended in lysis buffer (5 mM Tris-HCl, pH 8.0, 0.3 M NaCl, containing 1 mg/mL of lysozyme) and lysed by sonication. Cell lysates were applied to a Ni-NTA agarose column (QIAGEN) and eluted with 250 mM imidazole. The solvent was exchanged to 20 mM Tris-HCl, pH 7.5, 10 mM EDTA, pH 8.0, 1 mM DTT by dialysis, and the purified protein was stored at −80 °C until use.

Preparation of *S. pneumoniae* FabK. The *fabK* genes were amplified by PCR from *S. pneumoniae* R6 and cloned into pET-21b(+) expression vector (Novagen). The resulting plasmid was transformed into *E. coli* BL21(DE3). The expression of FabK protein fused with His-tag at C-terminal was induced by 1 mM isopropyl β-D-thiogalactoside, and the cells cultivated in LB broth were grown for a further 4 h before collecting by centrifugation. Purification of the His-tagged FabK protein was performed as above. Purified proteins were exchanged into 0.1 M sodium phosphate buffer, pH 7.0, by dialysis and stored at −80 °C until use.

Enzyme Inhibition Assay. Enzymatic activity of FabI and FabK was measured as the reduction of NADH and monitored by the change in absorbance at 340 nm. Assays were performed in 96-half-area plates in a final assay volume of 100 μL. For FabI inhibition assay, the reaction mixture consisted of 100 mM sodium phosphate (pH 7.4), 0.25 mM crotonoyl-CoA, 0.4 mM NADH, and 50 μg/mL of His-tagged *E. coli* FabI. For FabK inhibition assay, the reaction was performed in 100 mM 2-(*N*-morpholino)ethanesulfonic acid (pH 7.0), 100 mM NH_4Cl, 0.2 mM crotonoyl-CoA, 0.4 mM NADH, and 2 μg/mL of purified FabK. The reaction was initiated by addition of the enzyme and measured by absorption at 340 nm for 10 min at room temperature. Concentration giving 50% reduction in the enzymatic activity was determined as IC_{50}.

MIC Testing. Antibacterial activity was determined by the broth microdilution method according to the NCCLS document M7-A6. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of the test compound that prevented visible growth. *S. aureus* ATCC29213, *S. pneumoniae* KU197 (PRSP), *S. pneumoniae* KU197 (A141S) (PRSP, *fabk* mutant),[42] *H. influenzae* Rd, *H. influenzae* RdΔacrB (efflux mutant), *H. influenzae* PRC44,

E. coli W4680, *E. coli* WZM120 (efflux mutant), *E. faecalis* ATCC 19433, *P. aeruginosa* PAO1 were used.

Acknowledgment. The authors wish to thank Miss Shigeko Miki, Mrs. Takako Miyara, and Mrs. Kazue Sasaki of Meiji Seika Kaisha, Ltd. (http://www.meiji.co.jp/home.html) for their help with mass spectrometric analysis. The authors also wish to thank Mr. Takashi Watanabe and Mr. Seigo Sato for their help with computational or NMR study.

Supporting Information Available: Purity data including HPLC and elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0705354