Bioorganic & Medicinal Chemistry Letters xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# Discovery of a potent enoyl-acyl carrier protein reductase (FabI) inhibitor suitable for antistaphylococcal agent

Yun Gyeong Kim [a], Jae Hong Seo [a], Jin Hwan Kwak [b], Kye Jung Shin [a,*]

[a] Integrated Research Institute of Pharmaceutical Sciences, College of Pharmacy, The Catholic University of Korea, 43 Jibong-ro, Wonmi-gu, Bucheon, Gyeonggi-do 420-743, Republic of Korea
[b] School of Life and Food Sciences, Handong Global University, Pohang 791-7, Republic of Korea

ARTICLE INFO

*Article history:*
Received 22 June 2015
Revised 24 August 2015
Accepted 28 August 2015
Available online xxxx

*Keywords:*
Enoyl-acyl carrier protein reductase (FabI)
Antibiotic
MRSA
Pyridone

ABSTRACT

We report the discovery, synthesis, and biological activities of phenoxy-4-pyrone and phenoxy-4-pyridone derivatives as novel inhibitors of enoyl-acyl carrier protein reductase (FabI). Pyridone derivatives showed better activities than pyrone derivatives against FabI and *Staphylococcus aureus* strains, including methicillin-resistant *Staphylococcus aureus* (MRSA). Among the pyridone derivatives, compound **16l** especially exhibited promising activities against the MRSA strain and good pharmacokinetic profiles.



Since the discovery of antibiotics, the emergence of antimicrobial resistance is one of the most important threats to human health.[1] Among the resistant strains, methicillin-resistant *Staphylococcus aureus* (MRSA)[2] is the most common and serious strain of nosocomial infections. For the treatment of MRSA infection, glycopeptide antibiotics, such as vancomycin and teicoplanin, are now clinically used. However, the re-emergence of vancomycin-resistant *Staphylococcus aureus* (VRSA)[3] has prompted the development of novel antibiotics that are mechanistically different from present antibiotics.

During the last decade, as the genomic sequences of bacteria were reported, a number of novel antimicrobial targets were identified;[4] in particular, the pathway of bacterial fatty acid biosynthesis (FASII) has been recognized as a new antibacterial target.[5] In mammals, fatty acid biosynthesis is facilitated by a single multifunctional polypeptide complex. In contrast, fatty acids are synthesized by discrete proteins known as FabH, FabG, FabA, and FabI in bacteria. Based upon this difference, each enzyme involved in bacterial fatty acid biosynthesis may be a mechanistically distinct, antimicrobial target. Among these bacterial enzymes, the final reduction step catalyzed by FabI has been an attractive target for the development of novel antibiotics, because it is the rate-limiting step of chain elongation in bacterial fatty acid biosynthesis. Furthermore, FabI is a valid antibacterial target, because it is inhibited by antibacterial agents such as isoniazid (**1**)[6] and triclosan (**2**).[7]

As a part of our continuing effort to discover novel FabI inhibitors that could be developed as MRSA antibiotics, we have performed high throughput screening (HTS) of a library of compounds, to obtain hit compounds with new structural skeletons. We identified several hit compounds with new scaffolds. Among these compounds, compound **3**, consisting of a phenoxy-4-pyrone moiety, was an attractive hit, based on its inhibition of FabI and MRSA strains. Also, its structure was drug-like and distinct from known FabI inhibitors. In the following Letter, we describe the structural modification and optimization of phenoxy-4-pyrone in the development of an MRSA antibiotic candidate with enhanced potency, as well as the ability to be orally administered (see Fig. 1).

According to the in silico modeling study of hit compound **3** with FabI, the binding mode of this compound was similar to that of triclosan.[8] The oxygen of the ketone functional group in 4-pyrone occupies the same position as the phenolic hydroxyl group of triclosan that binds to the phenolic hydroxyl group of Tyr156 and the 2′-ribose hydroxyl group of the NADPH cofactor to form a hydrogen bond. The methoxymethyl group in the pyrone 6-position lies in a spacious hydrophobic pocket region where the *meta*-Cl group of the phenol in triclosan is located. On the basis of modeling results, we structurally modified hit compound **3** in the phenoxy and methoxymethyl parts to obtain lead compounds with better activities. In addition, we performed bioisosteric replacement of

* Corresponding author. Tel.: +82 2 2164 4060; fax: +82 2 2164 4059.
*E-mail address:* kyejung@catholic.ac.kr (K.J. Shin).

http://dx.doi.org/10.1016/j.bmcl.2015.08.077
0960-894X/

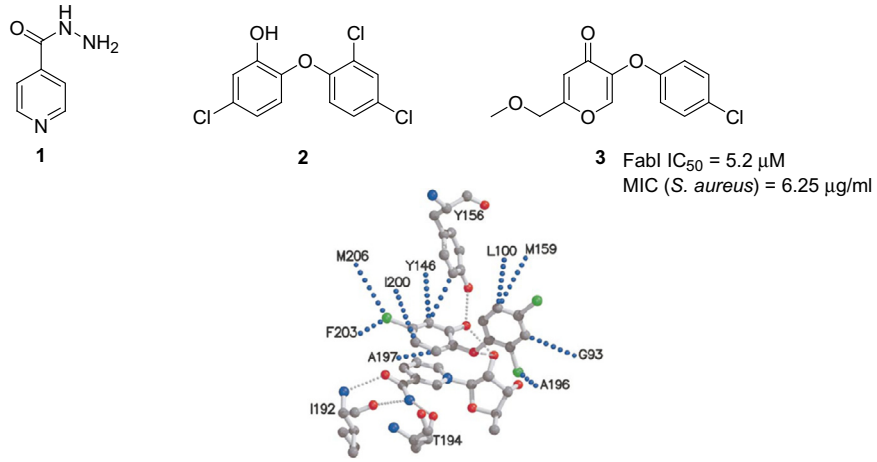


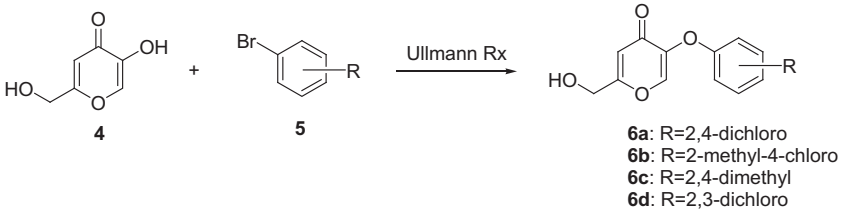

**Figure 1.** Structure of isoniazid (**1**), triclosan (**2**) and phenoxy-4-pyrone hit compound (**3**), and binding mode of triclosan (**2**) to *S. aureus* FabI enzyme.

pyrone into the pyridone moiety, to characterize biological activities resulting from a change of the main skeleton.

To investigate the antibacterial effect of the 3-phenoxy group on hit compound **3**, 3-phenoxy-6-hydroxymethyl-4-pyrones (**6**), as key intermediates of the pyrone and pyridone derivatives, were synthesized using two separate synthetic pathways, described in Schemes 1 and 2. To obtain 3-phenoxy-6-hydroxymethyl-4-pyrones (**6**) using a simple and expeditious synthetic method, Ullmann-type reactions with commercially available kojic acid (**4**) and various aryl halides (**5**) using various copper salts and ligands were initially used (Scheme 1).[9] However, the desired products (**6a–d**) were produced in very low yields (6–9%) in every Ullmann reaction. To overcome this problem, we used an alternative long-step approach to synthesize the pyrone skeleton (Scheme 2). Diverse phenols (**7**) were reacted with chloroacetone to produce phenoxyacetones (**8**), which were condensed with dimethylformamide dimethylacetal (DMF-DMA) to produce enaminones (**9**). Condensation of compounds **9** with diethyl oxalate and sodium ethoxide, followed by ring cyclization in acidic conditions, successfully produced pyrone fragments **11** in good yields. Pyrone carboxylic acid ethyl esters (**11**) were reduced to the desired alcohols (**6a–f**) using $NaBH_4$ in EtOH. Overall yields of 3-phenoxy-6-hydroxymethyl-4-pyrones (**6a–f**) using this alternative method ranged from 33% to 52%.

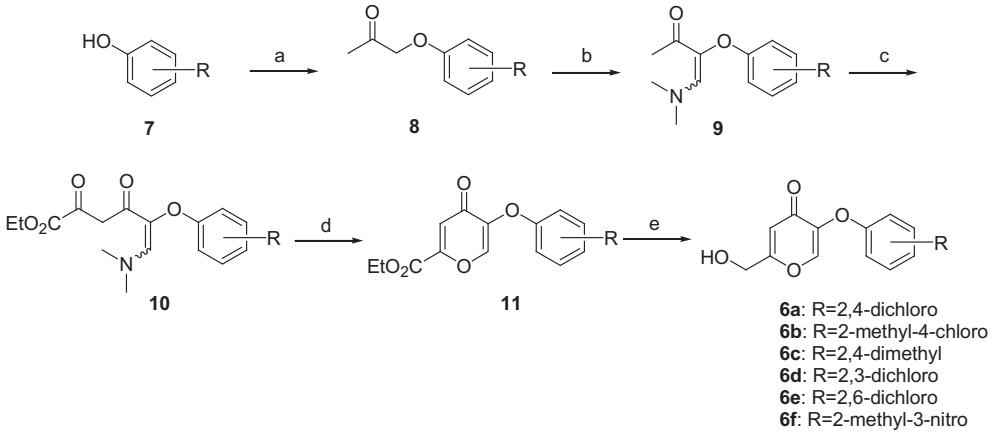
We modified the 6-position of pyrone, which is located in a hydrophobic region described in Scheme 3. In order to transform the 6-position of pyrone, we converted the hydroxyl group of **6** into the chloride (**12**) by treatment with $SOCl_2$. Reaction of the chloride with phenol and $K_2CO_3$ to obtain the bulkier lipophilic substituents produced the corresponding phenoxymethyl compounds **13** in good yields. Another reaction involved elongation of the carbon chain in the 6-position of pyrone. The Arbuzov reaction of **12** with triethylphosphite produced phosphonates **14**, which were subjected to the Horner–Wadsworth–Emmons reaction with a variety of aldehydes and *t*-BuOK, to produce **15**.

By using the structural modifications mentioned above, a series of 3-phenoxy-4-pyrone analogs (**13a–f**, **15a–u**) were synthesized. As the first step in hit optimization, they were used to investigate their inhibitory activities against FabI and against bacterial strains including MRSA (Table 1). Inhibitory activity against FabI was measured spectrophotometrically at 340 nm,[10] and the minimum inhibitory concentration (MIC) assay was performed using the conventional agar dilution method.[11] For comparison, triclosan and linezolid were also assayed. Phenoxymethyl compounds (**13a–f**) substituted in the 6-position of pyrone showed good to excellent inhibitory activities against FabI, compared to hit compound **3** and triclosan as reference compounds. In addition, MIC activities against *Staphylococcus aureus* and the MRSA strain increased in proportion to their enzymatic activities. We next investigated the effects of the phenoxy moiety at the 3-position of pyrone. Generally, 2,4-substituted phenoxy compounds (**13a–c**), whether the substituents were electron withdrawing or donating groups, produced desirable enzymatic and antibacterial activities compared with the 2,3- or 2,6-substituted phenoxy compounds (**13d–f**). A series of olefinic analogs (**15a–u**) substituted in the 6-position of pyrone possessed good to moderate inhibitory activities against FabI. However, they showed disappointing results for MIC activities, regardless of the phenoxy substituents in the 3-position. Although the reasons for these results are not definitively known, the formation of hydrogen bonding via the phenoxymethyl substituent or differences of lipophilicity are possible explanations. Using structure–activity relationship (SAR) analyses, there was a tendency for phenoxymethyl substituents in the 6-position of pyrone to show superior activities, as compared with alkenyl substituents, which are either aromatic or aliphatic.

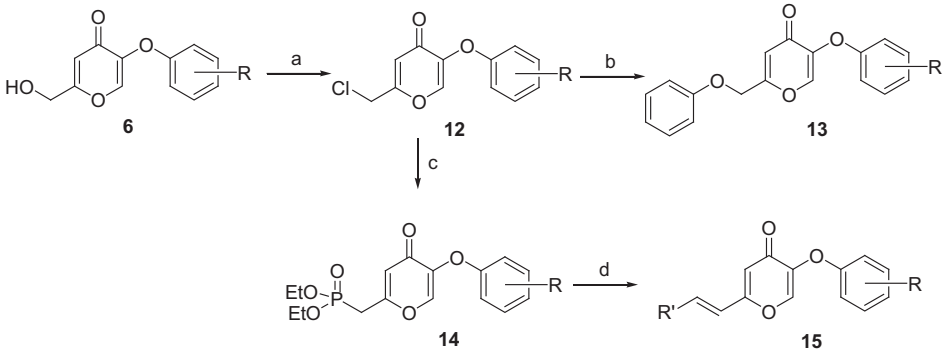



**Scheme 1.** Synthesis of 3-phenoxy-6-hydroxymethyl-4-pyrones (**6a–d**).

Please cite this article in press as: Kim, Y. G.; et al. *Bioorg. Med. Chem. Lett.* (2015), http://dx.doi.org/10.1016/j.bmcl.2015.08.077




**Scheme 2.** Reagents and condition: (a) chloroacetone, $K_2CO_3$, acetone, 60 °C, 16 h, 94–98%; (b) DMF-DMA, benzene, 80 °C, 10 h, 55–74%; (c) diethyl oxalate, NaOEt, EtOH, 80 °C, 8 h; (d) AcOH, 40 °C, 75–83% for 2 steps; (v) $NaBH_4$, MeOH, rt, 2 h, 88–93%.




**Scheme 3.** Reagents and condition: (a) $SOCl_2$, TEA, $CH_2Cl_2$, 40 °C, 5 h, 65–82%; (b) phenol, $K_2CO_3$, acetone, 60 °C, 3 h, 68–94%; (c) $P(OEt)_3$, 110 °C, 2 h, 88–100%; (d) R′CHO, *t*-BuOK, THF, 1–3 h, 0 °C, 43–89%.

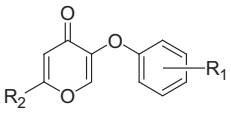
In order to further optimize compounds, we modified the main skeleton by replacing pyrone with the pyridone moiety. The rationale behind designing the pyridone moiety involves the observation that the physicochemical properties of the pyridone moiety, such as Log*P* and the solubility, are more drug-like than the pyrone moiety and the previous observations that some compounds with the pyridone moiety are known to inhibit FabI.[12] Using fixed 6-phenoxymethylpyrones (**13a–f**) that showed promising activities, we prepared a series of 1-substituted-4-pyridone compounds by reaction of **13a–f** with primary amines in refluxed MeOH, as depicted in Scheme 4.

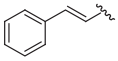
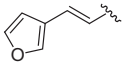
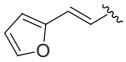
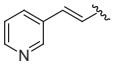
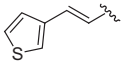
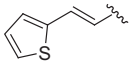
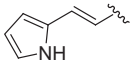
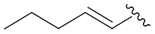
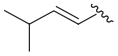
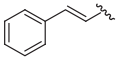
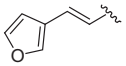
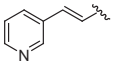
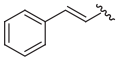
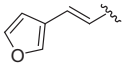
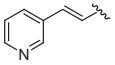
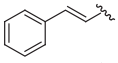
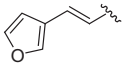
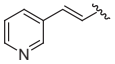
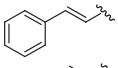
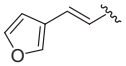
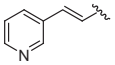
The inhibitory activities of various synthesized pyridone derivatives are summarized in Table 2. First, we explored the influence of pyridone substituents at the 1-position by introducing various alkyl groups, according to size. With increased bulk and length, there is a decrease in inhibitory activity for substituents at the 1-position of pyridone. The proton substituent, which was the smallest substituent, showed good enzymatic and strain growth inhibition; however, the optimal substituent in the 1-position was a methyl group in the pyridone skeleton. Next, we examined the effect on biological activities of phenoxy substituents at the 3-position of pyridone. Supporting the previous finding with pyrone compounds, that the 2,4-substituted phenoxy compounds gave more favorable results compared with the 2,3- and 2,6-substituted phenoxy compounds, similar inhibitory activities against FabI and the MRSA strain were observed for the pyridone core. Considering SAR studies with the pyridone skeleton, the best combination of substituents in the 3- and 1-positions involved 2,4-substituted phenoxy and methyl groups (**16b**, **16g**, **16l**).[13] In particular, compound **16l** which had 1-methyl and 3-(2,4-dimethylphenoxy) substituents showed excellent enzyme ($IC_{50}$ = 0.08 μM) and MRSA strain growth inhibition (MIC = 0.049 μg/mL). Based on these results, compound **16l** was selected for pharmacokinetic evaluation to assess the possibility of oral administration.

The pharmacokinetic properties of compound **16l**, after dissolving in a 1:2:7 mixed solution of *N*-methyl pyrrolidone, Tween 80, and water, were evaluated intravenously and orally in mice at a dose of 10 mg/kg (Table 3). Compound **16l** had an area under the concentration–time curve (AUC) of 355.2 μg min/mL, moderate clearance, a large volume distribution, and a terminal half-life of 47 min after intravenous administration. In administering compound **16l** orally, compound **16l** was rapidly absorbed into plasma with a $T_{max}$ time of 5 min and had an AUC of 149.4 μg min/mL. Taken together, compound **16l** possessed good pharmacokinetic profiles. The bioavailability of compound **16l** was 41.9%, so it should be possible to administer compound **16l** orally.

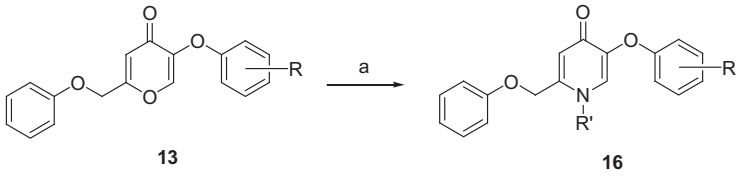
In summary, we have found the potent antistaphylococcal agents that have pyrone and pyridone structural moiety from a hit compound **3**. They strongly inhibit FabI, an important enzyme in fatty acid biosynthesis in bacteria. Importantly, pyridone compound **16l** showed excellent inhibitory activities against FabI and *Staphylococcus aureus* strains, including MRSA bacteria. Furthermore, compound **16l** had good pharmacokinetic properties in mice following intravenous and oral administration, and its

**Table 1**
Antibacterial activities of **13a–f** and **15a–u**



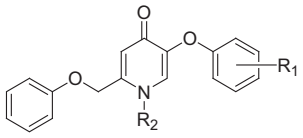

| Compound no. | $R_1$ | $R_2$ | $IC_{50}$ (FabI, μM) | MIC (μg/ml) | |
|---|---|---|---|---|---|
| | | | | *S. aureus* | MRSA |
| **13a** | 2,4-$Cl_2$ | $PhOCH_2$– | 0.68 | 3.125 | 6.25 |
| **13b** | 2-Me-4-Cl | $PhOCH_2$– | 0.45 | 1.563 | 6.25 |
| **13c** | 2,4-$Me_2$ | $PhOCH_2$– | 0.38 | 1.563 | 1.563 |
| **13d** | 2.3-$Cl_2$ | $PhOCH_2$– | 1.25 | 6.25 | 12.5 |
| **13e** | 2,6-$Cl_2$ | $PhOCH_2$– | 1.42 | 6.25 | 12.5 |
| **13f** | 2-Me-3-$NO_2$ | $PhOCH_2$– | 3.52 | 12.5 | 12.5 |
| **15a** | 2-Me-4-Cl |  | 4.88 | >20 | NT |
| **15b** | 2-Me-4-Cl |  | 2.45 | >20 | NT |
| **15c** | 2-Me-4-Cl |  | 3.89 | >20 | NT |
| **15d** | 2-Me-4-Cl |  | 2.94 | >20 | NT |
| **15e** | 2-Me-4-Cl |  | 3.12 | >20 | NT |
| **15f** | 2-Me-4-Cl |  | 4.57 | >20 | NT |
| **15g** | 2-Me-4-Cl |  | 5.82 | >20 | NT |
| **15h** | 2-Me-4-Cl |  | 6.44 | >20 | NT |
| **15i** | 2-Me-4-Cl |  | 5.78 | >20 | NT |
| **15j** | 2,4-$Cl_2$ |  | 4.53 | >20 | NT |
| **15k** | 2,4-$Cl_2$ |  | 1.89 | >20 | NT |
| **15l** | 2,4-$Cl_2$ |  | 2.04 | >20 | NT |
| **15m** | 2,4-$Me_2$ |  | 5.33 | >20 | NT |
| **15n** | 2,4-$Me_2$ |  | 2.29 | >20 | NT |
| **15o** | 2,4-$Me_2$ |  | 3.48 | >20 | NT |
| **15p** | 2.3-$Cl_2$ |  | 6.73 | >20 | NT |
| **15q** | 2.3-$Cl_2$ |  | 5.68 | >20 | NT |
| **15r** | 2.3-$Cl_2$ |  | 3.44 | >20 | NT |
| **15s** | 2,6-$Cl_2$ |  | 4.79 | >20 | NT |
| **15t** | 2,6-$Cl_2$ |  | 4.12 | >20 | NT |
| **15u** | 2,6-$Cl_2$ |  | 3.74 | >20 | NT |
| Triclosan | | | 0.44 | 0.391 | |
| Linezolid | | | | | 0.781 |

Please cite this article in press as: Kim, Y. G.; et al. *Bioorg. Med. Chem. Lett.* (2015), http://dx.doi.org/10.1016/j.bmcl.2015.08.077




**Scheme 4.** Reagents and condition: (a) R′-$NH_2$, MeOH, 60 °C, 12 h, 48–80%.

**Table 2**
Antibacterial activities of **16a–x**




| Compound no. | $R_1$ | $R_2$ | $IC_{50}$ (FabI, μM) | MIC (μg/ml) | |
|---|---|---|---|---|---|
| | | | | *S. aureus* | MRSA |
| **16a** | 2,4-$Cl_2$ | H | 1.82 | 0.781 | 1.563 |
| **16b** | 2,4-$Cl_2$ | $CH_3$ | 0.12 | 0.195 | 0.195 |
| **16c** | 2,4-$Cl_2$ | Cyclopropyl | 0.48 | 1.563 | 1.563 |
| **16d** | 2,4-$Cl_2$ | *n*-Butyl | 1.25 | 6.25 | 6.25 |
| **16e** | 2,4-$Cl_2$ | Benzyl | 1.42 | 6.25 | 12.5 |
| **16f** | 2-Me-4-Cl | H | 0.40 | 1.563 | 1.563 |
| **16g** | 2-Me-4-Cl | $CH_3$ | 0.11 | 0.098 | 0.098 |
| **16h** | 2-Me-4-Cl | Cyclopropyl | 0.47 | 0.391 | 0.781 |
| **16i** | 2-Me-4-Cl | *n*-Butyl | 0.63 | 3.125 | 3.125 |
| **16j** | 2-Me-4-Cl | Benzyl | 0.65 | 3.125 | 6.25 |
| **16k** | 2,4-$Me_2$ | H | 1.14 | 0.781 | 1.563 |
| **16l** | 2,4-$Me_2$ | $CH_3$ | 0.08 | 0.049 | 0.049 |
| **16m** | 2,4-$Me_2$ | Cyclopropyl | 3.17 | 0.391 | 1.563 |
| **16n** | 2,4-$Me_2$ | *n*-Butyl | 1.00 | 0.781 | 1.563 |
| **16o** | 2,4-$Me_2$ | Benzyl | 2.69 | 1.563 | 3.125 |
| **16p** | 2.3-$Cl_2$ | H | 0.84 | 1.563 | 1.563 |
| **16q** | 2.3-$Cl_2$ | $CH_3$ | 0.22 | 0.195 | 0.195 |
| **16r** | 2,3-$Cl_2$ | Cyclopropyl | 1.50 | 3.125 | 6.25 |
| **16s** | 2.6-$Cl_2$ | H | 4.98 | 3.125 | 3.125 |
| **16t** | 2,6-$Cl_2$ | $CH_3$ | 0.72 | 0.195 | 0.195 |
| **16u** | 2,6-$Cl_2$ | Cyclopropyl | 6.43 | 3.125 | 3.125 |
| **16v** | 2-Cl-3-$NO_2$ | H | 11.73 | >20 | >20 |
| **16w** | 2-Cl-3-$NO_2$ | $CH_3$ | 0.66 | 0.781 | 3.125 |
| **16x** | 2-Cl-3-$NO_2$ | Cyclopropyl | 17.64 | >20 | >20 |
| Triclosan | | | 0.44 | 0.391 | |
| Linezolid | | | | | 0.781 |

**Table 3**
Pharmacokinetic parameters after intravenous and oral administration (10 mg/kg) of **16l** to male ICR mice ($n$ = 3 per time point, time = 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h)

| Parameter | Intravenous | Oral |
|---|---|---|
| $AUC_{0-\infty}$ (μg min/ml) | 355.2 | 149.4 |
| $AUC_{last}$ (μg min/ml) | 354.7 | 148.7 |
| Terminal half-life (min) | 47.89 | 33.92 |
| $C_{max}$ (μg/ml) | | 4.043 |
| $T_{max}$ (min) | | 5 |
| CL (ml/mim/kg) | 28.15 | |
| MRT (min) | 36.5 | |
| $V_{ss}$ (ml/kg) | 1028 | |
| $F$ (%) | 41.92 | |

bioavailability ($F$ value) was over 40% in mice. Overall, these results strongly suggest that compound **16l** may be a potent and orally available FabI inhibitor for the treatment of MRSA infections.

## Acknowledgments

This work was financially supported by Research Fund 2012 of The Catholic University of Korea and the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea. (HI12C1087).

## References and notes

1. Levy, S. B.; Marshall, B. *Nat. Med.* **2004**, *10*, S122.
2. Voss, A.; Doebbeling, S. N. *Int. J. Antimicrob. Agents* **1995**, *5*, 101.
3. (a) Bax, R.; Mullan, N.; Verhoef, J. *Int. J. Antimicrob. Agents* **2000**, *16*, 51; (b) Pearson, H. *Nature* **2002**, *418*, 469.
4. Payne, D. J.; Gwynn, M. N.; Holmes, D. J.; Pompliano, D. L. *Nat. Rev. Drug Disc.* **2007**, *6*, 29.
5. (a) Wang, Y.; Ma, S. *ChemMedChem* **2013**, *8*, 1589; (b) Lu, H.; Tonge, P. J. *Acc. Chem. Res.* **2008**, *41*, 1; (c) Zhang, Y. M.; White, S. W.; Rock, C. O. *J. Biol. Chem.* **2006**, *281*, 17541; (d) Tasdemir, D. *Phytochem. Rev.* **2006**, *5*, 99.
6. Mdluli, K.; Slayden, R. A.; Zhu, Y.; Ramaswamy, S.; Pan, X.; Mead, D.; Crane, D. D.; Musser, J. M.; Barry, C. E., III *Science* **1998**, *280*, 1607.
7. Roujeinikova, A.; Levy, C. W.; Rowsell, S.; Sedelnikova, S.; Baker, P. J.; Minshull, C. A.; Mistry, A.; Colls, J. G.; Camble, R.; Stuitje, A. R.; Slabas, A. R.; Rafferty, J. B.; Pauptit, R. A.; Viner, R.; Rice, D. W. *J. Mol. Biol.* **1999**, *294*, 527.
8. (a) PDB Code; 1bvr: Rozwarski, D. A.; Vilchèze, C.; Sugantino, M.; Bittman, R.; Sacchettini, J. C. *J. Biol. Chem.* **1999**, *274*, 15582; (b) PDB Code; 1qsg: Stewart, M. J.; Parikh, S.; Xiao, G.; Tonge, P. J.; Kisker, C. *J. Mol. Biol.* **1999**, *290*, 859.
9. Hassan, J.; Sevignon, M.; Gozzi, C.; Schulz, E.; Lemaire, M. *Chem. Rev.* **2002**, *102*, 1359.
10. Assays were carried out in half-area, 96-well microtitre plates. Compounds were evaluated in 100 μl assay mixtures containing *S. aureus* FabI enzyme. Reduction of the *trans*-2-octenoyl *N*-acetylcysteamine substrate analog was measured spectrophotometrically by following the utilization of NADH or NADPH at 340 nm at 30 °C for the linear period of the assay. *S. aureus* FabI assays contained 50 mM sodium acetate, pH 6.5, 400 μM *trans*-2-octenoyl *N*-acetylcysteamine, 200 μM NADPH, and 150 nM *S. aureus* FabI. The rate of decrease in the amount of NADPH in each reaction well was measured by a microtiter ELISA reader using SOFTmax PRO software (Molecular Devices, California, USA). The inhibitory activity was calculated by the following formula: % of inhibition = 100 × [1 − (rate in the presence of compound/rate in the untreated control)]. $IC_{50}$ values were calculated by fitting the data to a sigmoid equation.
11. The antibacterial activities of the test compounds were evaluated using bacterial strains (Hoechst, Germany). The strains were inoculated into 3 ml of Fleisch extract broth (Beef extract 1%, peptone 1%, NaCl 0.3%, $Na_2HPO_4{\cdot}12H_2O$ 0.2%, pH 7.4–7.5) and cultured on a shaking incubator at 37 °C for 18 h. Test compounds were serially diluted in 2-fold dilutions from 2 mM to 15 μM. The 1.5 ml volume of each diluted solution was mixed with 13.5 ml of Muller Hinton agar (Difco, USA) and plated. The overnight-cultured strains were 100-fold diluted with broth on a 96-well plate. The diluted bacterial culture media were then inoculated (104 CFU/spot) on the prepared agar plates by an automatic inoculator (Dynatech, USA). The plates were incubated at 37 °C for 18 h. The lowest concentration that prevented the growth of each bacterium was determined to be MIC.
12. (a) Kitagawa, H.; Ozawa, T.; Takahata, S.; Iida, M. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4982; (b) Tipparaju, S. K.; Joyasawal, S.; Forrester, S.; Mulhearn, D. C.; Pegan, S.; Johnson, M. E.; Mesecar, A. D.; Kozikowski, A. P. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3565; (c) Kitagawa, H.; Ozawa, T.; Takahata, S.; Iida, M.; Saito, J.; Yamada, M. *J. Med. Chem.* **2007**, *50*, 4710.
13. **16b**: $^1H$ NMR ($CDCl_3$, 500 MHz): δ 7.40 (d, *J* = 2.4 Hz, 1H), 7.36–7.32 (m, 3H), 7.11 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.07 (m, 1H), 6.97 (d, *J* = 8.6 Hz, 2H), 6.82 (d, *J* = 8.7 Hz, 1H), 6.67 (s, 1H), 4.91 (s, 2H), 3.73 (s, 3H). $^{13}C$ NMR ($CDCl_3$, 125 MHz): δ 40.9, 66.6, 114.8, 117.8, 121.1, 122.3, 123.9, 127.7, 128.0, 29.9, 130.1, 134.4, 44.4, 144.6, 151.3, 157.3, 171.8. MS: calcd for $C_{19}H_{15}Cl_2NO_3 + H^+$, 376.04; found $[M+H]^+$, 376.12. **16g**: $^1H$ NMR ($CDCl_3$, 500 MHz): δ 7.36 (dd, *J* = 8.6, 2.2 Hz, 2H), 7.20 (d, *J* = 2.2 Hz, 1H), 7.08 (s, 1H), 7.08–7.06 (m, 2H), 6.89 (d, *J* = 8.6 Hz, 2H), 6.73 (d, *J* = 8.6 Hz, 1H), 6.68 (s, 1H), 4.91 (s, 2H), 3.69 (s, 3H), 2.30 (s, 3H). $^{13}C$ NMR ($CDCl_3$, 125 MHz): 16.1, 40.9, 66.8, 114.7, 117.8, 120.6, 122.3, 129.8, 128.3, 129.9, 130.1, 191.1, 131.8, 143.6, 146.6, 153.2, 157.3, 172.2. MS: calcd for $C_{20}H_{18}ClNO_3 + H^+$, 356.10; found $[M+H]^+$, 356.18. **16l**: $^1H$ NMR

(500 MHz, $CDCl_3$) $\delta$ 7.31–7.34 (m, 2H), 7.04 (d, $J$ = 8.2 Hz, 1H), 7.03 (s, 1H), 6.93–6.96 (m, 3H), 6.88 (s, 1H), 6.77 (d, $J$ = 8.2 Hz, 1H), 6.65 (s, 1H), 4.88 (s, 2H), 3.63 (s, 3H), 2.29 (s, 3H), 2.24 (s, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$ 172.2, 157.3, 151.7, 148.1, 143.1, 133.6, 132.1, 129.8, 129.7, 128.67, 127.6, 122.2, 119.7, 118.1, 114.7, 66.78, 40.8, 20.7, 16.0. MS: calcd for $C_{21}H_{21}NO_3 + H^+$, 336.15; found $[M+H]^+$, 336.28.

Please cite this article in press as: Kim, Y. G.; et al. *Bioorg. Med. Chem. Lett.* (2015), http://dx.doi.org/10.1016/j.bmcl.2015.08.077