Determination of Selectivity and Efficacy of Fatty Acid Synthesis Inhibitors

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Abbreviations: BABX: bischloroanthrabenzoxocinone; βME: β-mercaptoethanol; DTT: dithiothreitol; ACP: acyl carrier protein; CoA: coenzyme A; NAC: N-acetyl-cysteamine; cerulenin: (2S)(3R)2,3-epoxy-4-oxo-7,10-dodecadienoylamide; thiolactomycin: (4S)(2E,5E)-2,4,6 trimethyl-3-hydroxy-2,5,7-octatriene-4-thiolide; triclosan: 2,4,4'-trichloro-2'-hydroxydiphenylether; MIC: minimal inhibitory concentration.
Summary

Type II fatty acid synthesis (FASII) is essential to bacterial cell viability and is a promising target for the development of novel antibiotics. In the past decade, a few inhibitors have been identified for this pathway, but none of them lend themselves to drug development. In order to find better inhibitors that are potential drug candidates, we developed a high throughput assay that identifies inhibitors simultaneously against multiple targets within the FASII pathway of most bacterial pathogens. We demonstrated that the inverse t_{1/2} of the FASII enzymes-catalyzed reaction gives a measure of FASII activity. The Km values of octanoyl CoA and lauroyl CoA were determined to be 1.1±0.3 and 10±2.7 µM in *Staphylococcus aureus* and *Bacillus subtilis*, respectively. The effects of free metals and reducing agents on enzyme activity showed an inhibition hierarchy of Zn^{2+} > Ca^{2+} > Mn^{2+} > Mg^{2+}; no inhibition was found with β-mercaptoethanol or dithiothreitol. We used this assay to screen the natural product libraries and isolated an inhibitor, bischloroanthrabenzoxocinone (BABX) with a new structure. BABX showed IC_{50} values of 11.4 and 35.3 µg/ml in the *S. aureus* and *E. coli* FASII assays, respectively, and good antibacterial activities against *S. aureus* and permeable *E. coli* strains with MICs ranging from 0.2 to 0.4µg/ml. Furthermore, the effectiveness, selectivity and the *in vitro* and *in vivo* correlations of BABX as well as other fatty acid inhibitors were elucidated, which will aid in future drug discovery.
Introduction

Infectious disease is a global problem and the development of drug resistance is a major issue for all classes of antibiotics. Therefore, development of new high throughput assays for simultaneous screening of multiple targets in order to rapidly identify novel antibiotics is crucial. Fatty acids are essential for viability. The significant organizational and structural differences between the fatty acid synthesis of bacteria and humans make this system an attractive target for antibacterial drug discovery. The human fatty-acid synthase (FASI) is a multifunctional single polypeptide composed of distinct enzyme domains. In contrast, bacterial fatty acid synthesis (FASII) is carried out by a series of individual enzymes, which has been extensively reviewed (1,2) and is schematically described in Fig.1. Initially, acetyl CoA is carboxylated by AccABCD (3) to form malonyl CoA, which is, in turn, transferred to ACP (4) by FabD (5). Fatty acid synthesis is initiated by FabH (6) supplying substrates (acetoacetyl-ACP) to the fatty acid elongation cycle which includes FabG (7), FabA/Z (8,9), FabI (L/K) (10-12) and FabF/B (13,14) enzymes. In the cycle, the keto group of $\beta$-ketoacyl-ACP is reduced to a hydroxyl group by NADPH-dependent reductase FabG. $\beta$-Hydroxyacyl-ACP is dehydrated by dehydratase FabA or FabZ. The double bond of trans-2-enoyl-ACP is reduced by NADH-dependent reductase FabI(K/L) feeding the substrate back to FabF/B which adds an additional acetate unit (two carbons) and the cycle iterates.

Two marketed antibacterial agents, triclosan (antiseptic) and isoniazid (anti-TB), target the FabI enzyme of fatty acid synthesis (15,16). Two natural products, cerulenin (4) and
thiolactomycin (17) inhibit the condensation enzymes FabH and FabF/B, with cerulenin showing selectivity for FabF/B while thiolactomycin and its analogs (18,19) inhibit FabH and FabF/B. Thiolactomycin mimics malonyl-ACP in its binding site whereas cerulenin forms a covalent bond with the active site cysteine in FabF/B with its tail occupying a long hydrophobic cavity, which normally contains the growing acyl chain of the natural substrate (20,21). Lately, inhibition of FabH has drawn significant attention in drug discovery efforts (22,23).

In this study, we developed and validated a reliable high throughput fatty acid synthesis pathway assay that can simultaneously identify inhibitors of multiple targets including FabD, FabF/B, FabG, FabA/Z and FabI. We screened natural product extract libraries, identified and characterized bischloroanthrabenzoxocinone (BABX) as a new inhibitor of fatty acid synthesis. We also investigated the kinetics of pathway enzymes, the selectivity of fatty acid synthesis inhibitors as well as the relationship of biochemical activities and antibacterial activity.

**Experimental Procedures**

*Reagents*-All reagents were obtained from Sigma-Aldrich Chemical Co. unless otherwise indicated. DTT was from Fisher (BP172-5); perchloric acid (70%) was from FLUKA CHEMIKA (77230); β-mercaptoethanol (βME) was from Bio-Rad (161-0710). [14C]-malonyl CoA (60 mCi/mmol, NEC612), [3H]-acetyl CoA (NET290250UC), Phospholipid...
96-well Flashplates (SMP108) and other radio-labeled chemicals used in this study were from Perkin Elmer (NEN) Life Sciences. ACP (Sigma-Aldrich Chemical Co., A7303) was pretreated with 3 mM DTT on ice for 20 minutes, aliquoted and stored at -80 °C.

*Preparation of Type II Fatty Acid Synthesis (FASII) enzymes*- The procedure described previously (24) was used with some modification. Briefly, K12-derivative *E. coli* strains, *S. aureus* or *B. subtilis* were grown to stationary phase in 6 liters of LB medium. The cultures were centrifuged at 8000 rpm for 10 minutes using a Beckman JA-10 rotor. The pellets were washed twice with ice cold buffer A (0.1 M sodium phosphate, pH=7, 1 mM EDTA and 5 mM βME) and resuspended in 500 ml of the same buffer. The cells were lysed in a cold microfluidizer (Microfluidics Corp., M-110EH) at 18,000 lbs per square inch and centrifuged at 20,000 rpm for 15 min at 4 °C using a Beckman JA-20 rotor. The supernatant was collected and its volume was precisely measured. Ammonium sulfate (129 g) was added to each 500 ml of the supernatant in small quantities with low speed stirring at 4 °C to reach 45% ammonium sulfate saturation. The mixture was centrifuged at 10,000 rpm for 5 min and the supernatant was collected. Ammonium sulfate (113 g) was then added to each 500 ml of the supernatant to reach 80% ammonium sulfate saturation. The mixture was centrifuged again and the supernatant was then discarded. The 45-80% ammonium sulfate saturated protein fraction pellet, containing all necessary fatty acid synthesis (FASII) enzymes, was dissolved in 20 ml buffer A, dialyzed at 4 °C against four changes of buffer A using 10 kd molecular weight cutoff dialysis tubing (Invitrogen, 15961-022) and then concentrated. The protein concentration was
determined using the standard Bio-Rad protocol. The protein was aliquoted, flash frozen using liquid nitrogen and stored at -80 °C.

**FASII Assay** - The assay was performed in a Phospholipid 96-well Flashplate. Routinely, three micrograms of the partially purified protein containing fatty acid synthesis enzymes were pre-incubated with a serial dilution of natural products or synthetic compounds at room temperature for 20 minutes in 50 µl buffer containing 100 mM sodium phosphate (pH7.0), 5 mM EDTA, 1 mM NADPH, 1 mM NADH, 150 µM DTT, 5 mM βME, 20 µM n-octanoyl CoA (or lauroyl CoA), 4% DMSO and 5 µM of the pretreated ACP. The reaction was initiated by addition of 10 µl of water-diluted [14C]-malonyl CoA (the label is at C-2 of the malonyl group), which gave a final concentration of 4 µM malonyl CoA with total counts of about 20,000 (84 cpm/pmol) or 10,000 CPM (42 cpm/pmol) using Beckman Coulter LS6500 and Packard TopCount NXT Scintillation counters, respectively. The reaction was incubated at 37 °C for 30 minutes for *E. coli* and 60 minutes for *S. aureus* and *B. subtilis*. The reaction was terminated by adding 100 µl of 14% perchloric acid. The plates were sealed, incubated at room temperature overnight with mild shaking and counted for 5 minutes using the TopCount. Through hydrophobic interactions, long hydrophobic acyl chains of acyl-ACP bind to the phospholipids on the well’s surface which are coated with scintillant. This binding brings the incorporated [14C] into proximity of the scintillant resulting in the emission of a photon which is captured by a scintillation counter. All data were analyzed using Prism (GraphPad Software, Inc.)
Whole cell labeling assay- The assay was performed as previously described (25). Briefly, mid-log (A600 = 0.5 ~ 0.6) growth bacteria (E. coli and S. aureus) were incubated with 1 µCi/ml 2-[3H]glycerol, 1 µCi/ml 6-[3H]thymidine, 1 µCi/ml 5,6-[3H]uracil, 5 µCi/ml 4,5-[3H]leucine and 5 µCi/ml 2,3-[3H]alanine (or 2-[3H]glycine) for phospholipid, DNA, RNA, protein and cell wall, respectively, at an increasing concentration of each inhibitor at 37°C for 20 minutes. Cell wall labeling with 2,3-[3H]alanine (E. coli) or 2-[3H]glycine (S. aureus) was performed in the presence of 100 µg/ml chloramphenicol which blocks protein synthesis. The reaction was stopped by addition of 10% trichloroacetic acid and harvested using a glass fiber filter (Perkin Elmer Life Sciences, 1205-401). The filter was dried and counted with scintillation fluid.

Minimum inhibitory concentration (MIC)-The MIC against each of the strains was determined as previously described (26). Cells were inoculated at 10^5 CFU/ml followed by incubation at 37°C with a serial dilution of compounds in LB broth for 20 hr. MIC is defined as the lowest concentration of antibiotic inhibiting visible growth.

E. coli strain construction-All strains in this study are listed in Table I. MB4902 E. coli (lpxC), MB5747 E. coli (tolC) and MB5746 E. coli (lpxC, tolC) strains were constructed using standard P1 transduction methodology (27). CAG12184 was obtained from the Yale University E. coli Genetic Stock Center (28). In order to bring in a second Tn10-
linked mutation (tolC) via P1 transduction, the MB5008 strain was made by curing MB4902 of tetracycline resistance using a variation of the technique described (29). Concentrations of quinaldic acid and chlortetracycline were adjusted due to the increased sensitivity of the lpxC mutant.

**Results**

*Development of high throughput assay for type II fatty acid synthesis (FASII Assay)*-The conventional type II fatty acid synthesis pathway assay uses acetyl CoA and malonyl CoA as substrates (30). However, the FASII assay that we developed uses long chain acyl CoA (octanoyl CoA or lauroyl CoA) instead of acetyl CoA. Therefore, our FASII assay measures the cumulative activities of enzymes involved in the fatty acid elongation cycle only, avoiding the complexity of the chain initiation reaction steps catalyzed by acetyl CoA carboxylase (AccABCD), FabD and FabH. To determine enzyme activity and set up proper conditions for the assay, we titrated the FASII enzyme mixture from *E. coli* (*S. aureus* and *B. subtilis*, data not shown) in a time course study (Fig. 2A) at 37 °C. The t1/2 values are 3.2±0.3, 6.7±1.0, 12.4±1.0, 26.9±2.3 minutes respectively for 1.5, 3, 6 and 12 µg of the FASII enzyme mixture added per assay. The plot of t1/2 values against each concentration of the enzyme mixture results in a linear correlation (Fig. 2B), indicating that t1/2 (or apparent rate constant) gives a measure of the activity of the FASII enzymes (31).
Determination of enzyme kinetics - The assay was performed under standard conditions and the apparent Km (Km_{app}) and Vmax of the FASII enzymes were determined. The Km_{app} and Vmax of *S. aureus* FASII enzymes for octanoyl CoA were 1.1±0.3 µM and 88.9±4.5 pmol/min/mg, respectively (Fig. 3A). The Km_{app} and Vmax of *B. subtilis* FASII enzymes were 10.0±2.7 µM and 516±31 pmol/min/mg, respectively, for lauroyl CoA (Fig. 3B). Similar results were obtained by interchanging octanoyl CoA and lauroyl CoA (data not shown). The experiments were repeated at least twice with duplicates.

The effect of metals and reducing agents on FASII - In order to ascertain the robustness of the assay for use with natural product extracts which may contain metals and chelators, the effect of divalent cations and EDTA on FASII activity was examined (Fig. 4). Without the addition of EDTA, inhibition of FASII activity by Mg^{2+}, Ca^{2+}, Zn^{2+} and Mn^{2+} exhibited IC_{50} values of 81±17, 9.1±6.2, 5.6±3 and 19.4±7.6 mM, respectively. With the addition of 5 mM EDTA, the inhibition of FASII activity by these metals was reduced, resulting in IC_{50} values of 76±16, 4.0±0.9, 0.3±0.09 and 14±4.5 mM for Mg^{2+}, Ca^{2+}, Zn^{2+} and Mn^{2+}, respectively. Neither potassium nor sodium demonstrated any inhibitory effects on FASII activity until the concentration reached 300 mM (data not shown). These experiments were repeated at least three times in duplicate.

Efficacy and selectivity of FASII inhibitors across multiple bacterial species - Assays were performed with three FASII inhibitors in three different bacterial species. In the *E. coli*
FASII enzyme assay, cerulenin, thiolactomycin and triclosan showed IC$_{50}$ values of 1.0±0.3, 17.3±3.2, and 0.06±0.02 µg/ml, respectively (Fig 5A). Using *B. subtilis* FASII enzymes, IC$_{50}$ values of 0.10±0.03, 110±35 and 0.8±0.4 µg/ml were obtained for cerulenin, thiolactomycin and triclosan respectively (Fig. 5B). *S. aureus* enzymes revealed IC$_{50}$ values of 1.5±0.4 for cerulenin, 13.0±3.9 for thiolactomycin and 0.0079±0.002 µg/ml for triclosan (Fig. 5C). Experiments were performed in duplicate or triplicate at least three times.

**Screening, isolation and characterization of new inhibitors**—Using FASII assay, we screened both natural product extracts and a collection of synthetic compounds (not shown). The assay performed adequately to identify inhibitors of fatty acid synthesis for enzymes involved in chain elongation. The hit rate was 0.13 % and the Z’ factor (32) was 0.73. From Actinomycete active broth S18 (Fig. 6), we isolated a new compound, BABX (Fig. 7A), which accounted for the FASII inhibitory activity of the extract. The details of isolation and structure elucidation will be published elsewhere. BABX inhibited fatty acid synthesis, giving IC$_{50}$ values of 11.4 and 35.3 µg/ml in the *S. aureus* and *E. coli* FASII assays, respectively (Fig. 7B). BABX exhibited good antibacterial activities against *S. aureus* (MIC = 0.2 µg/ml) and poor antibacterial activities against wild type *E. coli* (MIC ≥250 µg/ml). To determine the reason for the failure of this compound to reach its intracellular target in *E. coli*, we generated permeable *E. coli* (lpxC) and/or efflux negative *E. coli* (tolC) strains (Table 1). This compound was tested against these strains and MIC’s were determined. It showed MIC values of 0.24, ≥250, and 0.39 µg/ml against
E. coli (lpxC), E. coli (tolC) and E. coli (lpxC, tolC), respectively (Table 2). Similar results were also observed in the Kirby-Bauer assay (Fig 7D). In the whole cell labeling assay, BABX inhibited S. aureus phospholipid synthesis with an IC₅₀ value of 0.21 µg/ml. However, it also inhibited DNA synthesis with a similar IC₅₀ value of 0.24 µg/ml. Due to the lack of penetration of BABX in E. coli, the whole cell labeling assay was carried out using E. coli (lpxC), showing 51%, 35%, 34%, 8% and no inhibition of phospholipids synthesis, DNA synthesis, protein synthesis, RNA synthesis and cell wall synthesis at 100 µg/ml, respectively (Table 2). To determine the possible target of BABX, the FASII elongation assay was performed. When acetyl CoA, a natural substrate, was used it produced longer chains of acyl-ACP but weaker signals in S. aureus compared to E. coli (Fig 7, lanes 2, 10). However, when we used n-octanoyl CoA, a substrate used in the FASII assay, as a substrate it produced better signals compared to acetyl CoA in S. aureus (Fig 7, lanes 2, 7). As a result it afforded a better signal to background window, which helped in the screening of natural product extracts against S. aureus enzymes. At 200 µg/ml, BABX completely blocked fatty acid elongation (Fig. 7, lanes 4, 8) similar to that of cerulenin unlike triclosan where an extra band can be seen (Fig. 7, Lane 9), BABX did not inhibit the FabD reaction which catalyze malonyl CoA and holo-ACP to malonyl-ACP (Fig. 7C, lanes 1, 4 and 8).

**Effects of cell permeability and efflux on fatty acid inhibitors**- In the process of discovery and optimization of new antibacterials, it is evident that whole-cell activity may vary independently of enzyme inhibition. Thus it is important to track their accumulation in
cells by measuring their ability to specifically inhibit the target pathway (phospholipid synthesis, in this case) and cell growth as well as their enzyme inhibitory activity. The effects of cerulenin, thiolactomycin and triclosan on macromolecular synthesis and MIC were examined as described in Experimental Procedures. All three compounds selectively inhibited phospholipid synthesis. The IC$_{50}$ values of whole cell labeling for RNA, protein and cell wall syntheses (data not shown) were similar to those of DNA synthesis. The results of MICs, whole cell labeling and IC$_{50}$s in the FASII assay against $E. coli$ (wild type), $E. coli$ ($lpxC$), $E. coli$ ($tolC$), $E. coli$ ($lpxC$, $tolC$) and $S. aureus$ are summarized in Table II. Rifampicin is a typical positive control against permeable gram-negative bacteria. Novobiocin is a positive control substrate of the AcrA/B TolC efflux pump of $E. coli$. Erythromycin is subject to both the outer membrane barrier and efflux. The MIC results against the $E. coli$ strains indicated that efflux played the primary role in modulating accumulation of the three compounds. In contrast, BABX had permeability characteristics similar to rifampicin, where TolC mediated efflux did not play a role.

**Discussion**

Acetyl CoA and malonyl CoA have been used as substrates for the *in vitro* fatty acid synthesis assay for decades. Acetyl CoA and malonyl-ACP are condensed by FabH to make acetoacetyl-ACP, which initiates the fatty acid elongation cycle. However, $E. coli$ and $S. aureus$ FabH can utilize short (up to six carbons) fatty acid chains as a substrate (22,33), which complicates the quantification of product formation. For example, cerulenin is a selective FabF/B inhibitor with an IC$_{50}$ range of 0.1-2 $\mu$g/ml. However, the
IC$_{50}$ of cerulenin for FabH is ~150 µg/ml (21) (data not shown). As such, a concentration of cerulenin which is sufficient to completely inhibit FabF activity may still allow FabH to continue the elongation of fatty acid chains to a maximum length of eight carbons. Unless mass spectrometry is used, the eight carbon fatty acids can not be separated from long chain fatty acids using ether extraction (data not shown). Using assay conditions described in Experimental Procedures, with the substitution of acetyl CoA for octanoyl CoA or lauroyl CoA, chain elongation inhibitors could be distinguished from fatty acid chain initiation inhibitors (Fig. 7C, data not shown).

As previously described (34), long chain fatty acids can be converted to acyl-ACP by acyl-acyl carrier protein synthase (Aas) in vitro. However, using long chain fatty acids as substrates for a high throughput assay is not easy due to their poor solubility. Conveniently, when water soluble octanoyl CoA or lauroyl CoA and $[^{14}C]$-malonyl CoA were used as substrates, the assay worked very well. To confirm that the assay is truly using either octanoyl CoA or lauroyl CoA as a substrate, we titrated these substrates using the extracts of FASII enzymes from *S. aureus* or *B. subtilis* (Fig. 3). As the concentration of octanoyl CoA or lauroyl CoA increased, the $[^{14}C]$-malonyl CoA incorporation into long chain fatty acid increased in a concentration dependent manner. The supporting data is visualized in Fig. 7C (lane 6). The affinity of *S. aureus* FASII enzymes for the substrates octanoyl CoA or lauroyl CoA was 10-fold higher than that of *B. subtilis*, while the Vmax of *B. subtilis* FASII enzymes for octanoyl CoA or lauroyl CoA was 5.8-fold higher than that of *S. aureus*. The *S. aureus* enzyme that catalyzes
octanoyl CoA or lauroyl CoA to their ACP counterpart, which can be utilized by the enzymes for the fatty acid chain elongation, is unknown. While it has been reported (35) that *E. coli* can carry out this reaction using condensing enzymes and that *B. subtilis* FabH1 can utilize octanoyl CoA as a substrate, albeit poorly (36), there is no reported evidence that identifies which *S. aureus* enzyme converts long chain (>6C) acyl CoA to acyl-ACP. Further investigation could lead to a better understanding of fatty acid metabolism. It was not obvious that the FASII system of the important gram-positive pathogen *S. aureus* would work under these conditions as we have been unable to carry out the reaction with enzymes from *Streptococcus pneumoniae* and *Streptococcus pyogenes*.

Enzyme activities are often regulated by divalent cations. This high throughput assay has been developed for use in screening natural product extracts, as shown in Fig 3. These extracts may contain metal ions, including divalent metals which modulate many enzyme activities, as well as chelators. To understand the effect of metals on FASII enzyme activity, we titrated Mg\(^{++}\), Ca\(^{++}\), Zn\(^{++}\), Mn\(^{++}\) (Fig 4). The FASII enzyme activity was inhibited by all divalent cations tested in the order Zn\(^{2+}\)>Ca\(^{2+}\)>Mn\(^{2+}\)>Mg\(^{2+}\). The addition of EDTA reduces free divalent metal concentrations, which can be calculated using the following formulas:

\[\text{[Total]} = [\text{Free}] + \text{fi}[\text{EDTA}]\]

\[\text{fi} = [\text{Free}]/([\text{Free}]+Kd)\]
[Total] is bound and unbound metal concentration; [Free] is unbound metal concentration. The pH dependent Kds of the metals for EDTA were theoretically calculated, providing values of 4 µM, 50 nM, 40 fM and 15.8 pM at pH 7 for Mg++, Ca++, Zn++ and Mn++, respectively (37,38). Although the mechanism of action of regulation by divalent cations remains a scientific interest, addition of a significant concentration of EDTA to the assay screen eliminates false positives due to metal effects and chelation.

Three known fatty acid synthesis inhibitors were evaluated in the high throughput screening format using extracts from *E. coli*, *B. subtilis* and *S. aureus* as enzyme sources. All compounds inhibited FASII activity (Fig. 5). Because cerulenin is a covalent inhibitor (13,39) and triclosan is a slow binding inhibitor, having a very slow koff (40), their IC50 values are completely dependent on assay conditions. The IC50s of thiolactomycin against FASII enzymes from *B. subtilis* and *S. aureus* are 17.3 and 110 µg/ml, which are similar to the IC50 values obtained in a single enzyme assay using *S. aureus* FabF and *B. subtilis* FabF (41,42). Since *E. coli* contains two beta-ketoacyl-acyl carrier protein synthases, FabF and FabB, comparison of the IC50 of thiolactomycin against FASII enzymes with a single enzyme (FabF or FabB) assay is required to ascertain each enzyme contribution. The IC50 of thiolactomycin against *E. coli* FASII enzymes is 17.3 µg/ml, which is two-fold higher than that obtained from the single enzyme assay with *E. coli* FabB (8.4 µg/ml) and 10-fold lower than that from *E. coli* FabF (170 µg/ml) (41). This is in agreement with the finding based on resistant mutants (40) that FabB is the most
sensitive thiolactomycin target in *E. coli*. Inhibition of FASII activity by triclosan reached a plateau, but failed to reach 100% inhibition. A possible explanation for this is that the FabI inhibitor does not block the first cycle of $[^{14}\text{C}]-\text{malonyl CoA}$ incorporation and this hypothesis was confirmed in the FASII elongation assay shown in Fig. 7C. Comparing lanes 6, 7, 8 and 9, lane 9 shows the inhibition by triclosan and contains an extra band, between C10:0-ACP and C12:0-ACP, which most likely is 10:1(Δ2t)-ACP that is observed in all replications.

Whole-cell activity of enzyme inhibitors may vary independently of enzyme inhibition. This is often due to variation in the ability of the compounds to reach their intracellular targets due to poor penetration (43) and/or active efflux (44,45). It is thus important in characterization and optimization of these compounds to ascertain their ability to accumulate in cells in parallel with their enzyme inhibitory activity. It is also important to show that the whole-cell activity is due to specific enzyme inhibition (as opposed to off-target activity). Thiolactomycin, cerulenin and triclosan resistance showed an association with efflux in *Pseudomonas aeruginosa* (46,47) as well as in *E. coli* (42). However, the role of outer membrane permeability and its relationship with the efflux of these fatty acid inhibitors have not been addressed. Therefore, we constructed the outer membrane permeable and/or efflux negative *E. coli* strains which were used in this study in whole cell labeling, MIC and cell free biochemical FASII assays. As described in Experimental Procedures, both FASII and whole cell labeling assays were performed for 20 minutes and MIC assays were done overnight. Therefore, the MIC presumably reflects the long term effects of accumulation of inhibitors inside the cell whereas the IC$_{50}$ of whole cell labeling reflects the combination of potency and the net accumulation
of inhibitors in a limited time. Direct comparison of FASII IC₅₀ values with those of PL labeling gives a measure of the effects of permeability and/or efflux. Comparison of IC₅₀ of PL labeling with MIC is more complex since multiple factors, such as rate of penetration versus efflux, mode of entry, nature of binding, feedback regulation, etc., play a role.

In this study, we provide both in vitro and in vivo evidence that the three fatty acid inhibitors are not only pumped out by efflux in a short period of time but are also subject to outer membrane permeability barriers. As expected, the IC₅₀ values of the cell free biochemical FASII assay for each of the fatty acid synthesis inhibitors showed similar results with FASII enzymes from the four *E. coli* strains. MICs of the three inhibitors decreased (antibacterial activity increased) two-fold against the outer membrane permeable *E. coli* (*lpxC*) strain compared to its wild type parent strain. In contrast, MICs decreased 32-, >64- and 125-fold against the efflux negative *E. coli* (*tolC*) strain for cerulenin, thiolactomycin and triclosan, respectively, indicating that efflux plays a critical role for their antibacterial activities. While it is possible that outer membrane permeable (*lpxC*) *E. coli* may lack efflux mechanisms in addition to those requiring TolC, and hence also reflect efflux rather than a simple barrier to entry, our findings indicate that the MICs of the compounds are not strongly affected by the *lpxC* mutation, as would be expected if the *lpxC* effect were exerted via efflux. In the whole cell labeling assay, however, IC₅₀s of phospholipid (PL) labeling showed a 4.4-fold decrease for cerulenin, a 50-fold decrease for thiolactomycin and a 267-fold decrease for triclosan with the
permeable *E. coli* (*lpxC*) strain compared to its wild type parent strain. Thus, the outer membrane appears to be a barrier to short term accumulation. With the efflux negative *E. coli* (*tolC*) strain, IC$_{50}$s of PL labeling for cerulenin, thiolactomycin and triclosan also showed 7-, 120- and 267-fold decreases, respectively. These findings demonstrate that both outer membrane permeability and efflux play roles in preventing the inhibitors from reaching their intracellular targets during short term exposure to the inhibitors. Consistent with this conclusion, the MICs of the permeable efflux negative *E. coli* (*lpxC, tolC*) strain were similar to that of *E. coli* (*tolC*) for all three inhibitors whereas the IC$_{50}$ values of PL labeling of the *E. coli* (*lpxC, tolC*) strain showed apparent additive effects caused by both the lack of efflux and increased permeability. The permeable efflux negative *E. coli* (*lpxC, tolC*) strain provides a tool to study the correlation of *in vitro* and *in vivo* activities of enzyme inhibitors in *E. coli* while the single mutants (*lpxC* or *tolC*) are useful in tracking chemical optimization of enzyme inhibitors with antibacterial activity.

In *E. coli* (*lpxC, tolC*), the IC$_{50}$ for cerulenin in PL labeling (3.55 µg/ml) is close to that of the cell-free FASII (0.75 µg/ml), which is consistent with the MIC (3.1 µg/ml). For thiolactomycin, the IC$_{50}$ of PL labeling (0.28 µg/ml) is closer to the MIC (3.1 µg/ml) than that of FASII (24.6 µg/ml) because the *E. coli* FASII assay does not involve FabH, one of the targets for thiolactomycin. On the other hand, while MICs for cerulenin and thiolactomycin are the same (3.1 µg/ml) in this strain, their effect on short term PL labeling is disparate (3.55 and 0.28 µg/ml respectively). The reason for this is unknown but might be due to feedback upregulation of FabH over time (affecting thiolactomycin),
differential effects of covalent (cerulenin) versus non-covalent (thiolactomycin) binding, or off-target activities of cerulenin. The IC$_{50}$ of triclosan for PL labeling (0.0002 µg/ml) is similar to its MIC (0.0004 µg/ml). However, it is more potent than that for FASII (0.05 µg/ml), suggesting the possible involvement of other unknown targets for triclosan activity in whole cells.

Interestingly, both IC$_{50}$ values of PL labeling (12.5 µg/ml) and MICs (64 µg/ml) are identical for cerulenin and thiolactomycin against \textit{S. aureus}. However, the IC$_{50}$ values of FASII are 9-fold apart (1.5 and 13 µg/ml, respectively), which likely reflects the fact that FabH does not play a role in the \textit{S. aureus} FASII assay, although differential effects of permeability/efflux cannot be ruled out. The IC$_{50}$ of triclosan for PL labeling and its MIC against \textit{S. aureus} are identical (0.002 µg/ml), which is 4-fold better than the IC$_{50}$ for FASII (0.008 µg/ml). Since little information about efflux and permeability on the fatty acid synthesis inhibitors for gram-positive bacteria has been documented to date, the correlation of potency between \textit{in vitro} and \textit{in vivo} activities against \textit{S. aureus} could not be determined. Further investigation of permeability and efflux would help to understand the mechanism of action of fatty acid synthesis inhibitors and resistance emergence on gram-positive bacteria.

When we screened the natural product extract libraries with the FASII assay, we used an assay for inhibition of FtsZ polymerization as a counter screen (26). The S18 broth (Fig.
Type II Fatty Acid Synthesis

6) showed selective inhibition in FASII assay, leading to the discovery of a new compound, BABX, as an inhibitor of fatty acid synthesis. This compound showed *in vitro* enzyme inhibitory activity with IC$_{50}$ values of 11.4 and 35.3 µg/ml in the *S. aureus* and *E. coli* FASII assays, respectively. BABX also showed potent antibacterial activities against *S. aureus* and permeable *E. coli* strains with MICs ranging from 0.2 to 0.4 µg/ml.

When evaluated in the whole cell labeling assay, BABX inhibited *E. coli* (*lpxC*) phospholipid synthesis (51%) with some selectivity compared to cell wall (no inhibition), RNA (8%), protein (34%) and DNA (35%) syntheses at 100µg/ml. BABX inhibited *S. aureus* phospholipid synthesis with an IC$_{50}$ of 0.2 µg/ml, which supported the MIC and Kirby-Bauer assay results. However, the compound also inhibited DNA synthesis at similar concentrations (IC$_{50}$ = 0.2 µg/ml). This indicates that inhibition of fatty acid synthesis is only one of the possible mechanisms for growth inhibition and illustrates the importance of evaluating the whole cell activity of inhibitors discovered in biochemical assays. In the *E. coli* case, the MIC in the *lpxC* strain was much lower than the IC$_{50}$s for whole cell labeling of all macromolecular synthesis (phospholipid, DNA, protein, RNA and cell wall) tested. Thus the primary target may be another system or growth inhibition may occur over a longer term than the labeling period.

Which fatty acid synthesis enzyme is a possible target for BABX? As we discussed earlier, any fatty acid synthesis inhibitor that inhibits elongation enzymes, other than condensation reactions, in the FASII assay initiated with octanoyl CoA cannot stop the addition of first two carbons to form 10 carbons of ß-ketoacyl-ACP, ß-hydroxyacyl-ACP...
or trans-2-enoyl-ACP (Fig. 1). From FASII assay (Fig. 7B) and FASII elongation assay (Fig. 7C), BABX did not inhibit FabD but fully inhibited acyl-ACP elongation, which is similar to that of cerulenin but different with that of triclosan, suggesting BABX is an inhibitor of the condensation enzyme in the elongation cycle. Further screening or potential chemical modification of BABX may lead to a better inhibitor of fatty acid synthesis and a more selective antibacterial agent.

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Table I. Properties of the *E. coli* strains

| Strain   | Relevant Markers | Other Markers                  | Reference |
|----------|------------------|-------------------------------|-----------|
| CAG12184 | *tolC::Tn10*     | rph-1                         | (28)      |
| MB4827   | wt<sup>1</sup>    | leu thr lac (thi) galK        | (43)      |
| MB4902<sup>2</sup> | *lpxC::Tn10* | leu thr lac (thi) galK        | (43)      |
| MB5008   | *lpxC*, cured of Tn10 | leu thr lac (thi) galK | this study |
| MB5747   | *tolC::Tn10*     | same as MB4827 but Tn10-linked *tolC* transductant (CAG12184 donor) | this study |
| MB5746   | *lpxC*, *tolC::Tn10* | same as MB5008 but Tn10-linked *tolC* transductant (CAG12184 donor) | this study |

<sup>1</sup> wild-type for outer membrane permeability and efflux, also known as C600galK.

<sup>2</sup> also known as LS583; the *envA* allele described in (41) has been renamed *lpxC*. 
Table II. Biological activity of fatty acid synthesis inhibitors (µg/ml)

| Organism | MB4827 (E. coli (wild type)) | MB4902 (E. coli (lpxC)) | MB5747 (E. coli (tolC)) | MB5746 (E. coli (lpxC, tolC)) | MB2985 (S. aureus (wild type)) |
|----------|-----------------------------|-------------------------|------------------------|-----------------------------|--------------------------------|
| Rifampicin | MIC | 5 | 0.005 | 2.5 | 0.005 | 0.0008 |
| Novobiocin | MIC | 200 | 50 | 0.8 | 0.4 | 0.05 |
| Erythromycin | MIC | 250 | 3.9 | 1.0 | 0.25 | 0.25 |
| Cerulenin | FASII (IC50) | 1.0 | 1.25 | 1.0 | 0.75 | 1.5 |
| | MIC | 100 | 50 | 3.1 | 3.1 | 64 |
| | PL labeling (IC50)a | 70.1 | 15.9 | 10.0 | 3.55 | 12.5 |
| | DNA labeling (IC50)b | >250 | 66.7 | 47.9 | 17.4 | >200 |
| Thiolactomycin | FASII (IC50) | 17.3 | 20.2 | 14.5 | 24.6 | 13.0 |
| | MIC | >200 | 200 | 3.1 | 3.1 | 64 |
| | PL labeling (IC50) | 79.4 | 1.58 | 0.66 | 0.28 | 12.5 |
| | DNA labeling (IC50) | >200 | >200 | >200 | >200 | >200 |
| Triclosan | FASII (IC50) | 0.06 | 0.05 | 0.05 | 0.05 | 0.008 |
| | MIC | 0.10 | 0.05 | 0.0008 | 0.0004 | 0.002 |
| | PL labeling (IC50) | 0.16 | 0.0006 | 0.0006 | 0.0002 | 0.002 |
| | DNA labeling (IC50) | >10 | 1.3 | 2.6 | 2.0 | >0.1 |
| BABX | FASII (IC50) | 37.5 | ND | ND | ND | 11.4 |
| | MIC | >250 | 0.24 | >250 | 0.39 | 0.2 |
| | PL labeling (IC50) | ND | 100c | ND | ND | 0.21 |
| | DNA labeling (IC50) | ND | >100 | ND | ND | 0.24 |

*a) PL labeling (IC50): IC50 of Whole cell phospholipid labeling with 1 µCi/ml 2-[3H]glycerol.
 b) DNA labeling (IC50): IC50 of Whole cell labeling with 1 µCi/ml 6-[3H]thymidine.

Under these conditions, the IC50s of Ciprofloxacin (a specific DNA synthesis inhibitor) are 0.011, 0.0079, <0.0078, <0.0078 and 0.5 µg/ml for E. coli (wild type), E. coli (lpxC), E. coli (tolC), E. coli (lpxC, tolC) and S. aureus (wild type), respectively. The data were averaged from at least three experiments. IC50s from FASII assay were obtained from at least 8 point titrations in duplicate. IC50s from the whole cell labeling assay were obtained from 16 points titrations in duplicate. Comparison of the IC50s of DNA labeling with those of PL labeling shows the selectivity of fatty acid synthesis inhibitors.

c) BABX was titrated starting at 100ug/ml and did not reach plateau or 100 % inhibition. At 100 µg/ml, 51% of phospholipids synthesis was inhibited showing some selectivity compared to cell wall (no inhibition), RNA (8%), protein (34%) and DNA (35%) syntheses.
Fig. 2
Fig. 3
Fig. 4
Fig. 5
Fig. 6
A

Bischloroanthrabenzoxocinone (BABX)

B

Log [BABX] (µg/ml) vs. % Control

-25 0 25 50 75 100 125

% Control

Log [BABX] (µg/ml)
Fig 7
Legends

Fig. 1. Type II Fatty acid synthesis pathway in bacteria.

Fig. 2. Determination of FASII activity. A, the assay was performed at 37 °C as described in Experimental Procedures with increasing amounts of the partially purified FASII enzymes from *E. coli* (□, 1.5 µg; ■, 3 µg; ◊, 6 µg; ●, 12 µg). The fatty acid was slowly captured by phospholipids on the flash plate surface and the elongated fatty acid was determined by measuring the incorporation of [14C]malonyl CoA at different times shown on the abscissa. Data are normalized to the background at zero time (202 cpm) determined by adding 100 µl of 14% perchloric acid before the assay was initiated. B, FASII activity, presented as an inverse of each t_{1/2} which was obtained from each data set of B, was plotted against the amount of FASII enzymes added. The goodness-of-fit of liner regression (r^2) is 0.9994. Data are means (±S.D.) of duplicate determinations and similar results were obtained from repeated experiments. The specific activity of [14C]malonyl CoA was 56.9 cpm/pmol using Packard TopCount NXT as described in Experimental Procedures. The maximum counts obtained from this experiment, however, were 6417 cpm, which implies the efficiency of flash plate vs. liquid scintillation is 67.7 %, giving 38.5 cpm/pmol.

Fig 3. Determination of steady state kinetic constants. A, 3 µg of the FASII enzymes from *S. aureus* were assayed as described in Experimental Procedures at increasing
concentrations of n-octanoyl CoA shown on the abscissa. Data (in duplicate) were fit to the Michaelis-Menten equation as well as to a Hanes-Wolff plot (inset). The $K_{m_{\text{app}}}$ is $1.1\pm0.3$ µM and $V_{\text{max}}$ is $88.9\pm4.5$ pmol/min/mg. B, an identical experiment was done as in A except using lauroyl CoA as substrate and the protein from $B.\ subtilis$ providing $K_{m_{\text{app}}} = 10.0\pm2.7$ µM and $V_{\text{max}}$ is $516\pm31$ pmol/min/mg.

Fig 4. The effect of divalent cat ions on FASII activity. The assay was performed as described in Experimental Procedures using increasing concentrations of metals with (●) or without (○) addition of 5mM EDTA. Without addition of EDTA the total metal concentrations equal free metal concentrations. The $IC_{50}$ of free Mg$^{++}$ (A) is 90 mM, free-Mn$^{++}$ (B) is 9mM, free Ca$^{++}$ (C) is 6mM and free Zn$^{++}$ (D) is 0.2 mM. Data was duplicated. Similar results were obtained from three other experiments with addition of varying concentrations of EDTA (0.8 - 5 mM, not shown). With addition of EDTA, the free metal calculation was described in the discussion.

Fig. 5. Efficacy and selectivity of FASII inhibitors on the enzymes from different species. The assay was performed as described in Experimental Procedures with addition of a serial dilution of inhibitors, cerulenin (●), thiolactomycin (○) or triclosan (□). A, the enzymes from $E.\ coli$ showed $IC_{50}$s of $1.0\pm0.3$ (cerulenin), $17.3\pm3.2$ (thiolactomycin) and $0.06\pm0.02$ µg/ml (triclosan). B, the enzyme from $B.\ subtilis$ displayed $IC_{50}$s of $0.10\pm0.03$ (cerulenin), $110\pm35$ (thiolactomycin) and $0.8\pm0.4$ µg/ml (triclosan). C, the enzyme from
S. aureus had IC$_{50}$s of 1.5±0.4 (cerulenin), 13.0±3.9 (thiolactomycin) and 0.0079±0.002 µg/ml (triclosan). Experiments were done at least three times in duplicates.

Fig 6. Screening of natural product extracts. An example of performance of FASII assay in 96 well plates. S18 shows inhibition greater than 50%. The background noise is below 30 % inhibition. Cerulenin was used as a positive control in each plate. Data are means (±S.D.) of duplicate determinations. The percent inhibition was calculated using the following equation: %INH=100-100*(data-BG)/(MAX-BG). Background (BG) is determined using 1 mM of cerulenin and Maximum Enzyme activity (MAX) is obtained by using 3.3 % DMSO.

Fig. 7. Identification and characterization of a new inhibitor, bischloroanthrabenzoxocinone (BABX). A, structure of BABX isolated from S18 (Fig. 6). B, FASII assay with a serial dilutions of BABX (333-0.01 µg/ml final concentration) using S. aureus (●) and E. coli (○) FASII enzymes, providing IC$_{50}$ values of 11.4 µg/ml (95% CI: 7.3-18.0) and 35.3 µg/ml (95% CI: 17.2-72.4), respectively. The graph shows the results of an average of two duplicate experiments. C, FASII elongation assay was done in identical conditions as the FASII assay with some exceptions. The reaction was performed with S. aurues FASII enzymes (lanes 2-9) and E. coli FASII enzymes (lane 10) using 4 µM of [14C]-malonyl CoA (60 mCi/mmol) as one substrate in polypropylene tubes. The second substrate in the assay was either 20 µM acetyl CoA (lanes 2-5, 10) or
20 µM of n-octanoyl CoA (lanes 6-9). After the reaction, 10 µl of each sample was directly applied to and resolved by a 16% polyacrylamide gel containing 4 M urea. The gel was blotted to a PVDF membrane and visualized by PhosphorImager. lane 1: a control of malonyl-ACP; lanes 2, 6, 10: without inhibitors; lanes 3, 7: 200 µg/ml cerulenin; lanes 4, 8: 200 µg/ml BABX; lanes 5, 9: 10µg/ml triclosan. The same samples were also resolved on 16% polyacrylamide gel containing between 0.5, 2 and 3.7 M urea (data not shown), which was used for confirming the results. Similar experiments were repeated 4 times with reproducible results. D, Kirby-Bauer assay with minor modifications. Briefly, 20 ml of melted LB agar was maintained at 44°C, seeded with 0.75 ml (A₆₀₀=0.3) of overnight culture and plated into an omni plate (NUNC-Nalgene). After agar solidified and dried for 15 minutes, 10 µg/ml of serial dilutions of BABX in LB containing 20% DMSO were placed on the seeded agar plate and incubated at 37°C for 20 hours. Zone sizes represent antibacterial activity. The results were confirmed by repeating three times.
References

1. Campbell, J. W., and Cronan, J. E. (2001) *Annual Review of Microbiology* **55**, 305-332
2. Heath, R., White, S., and Rock, C. (2001) *Prog Lipid Res.* **40**, 467-497
3. Fall, R. R., and Vagelos, P. R. (1972) *J. Biol. Chem.* **247**, 8005-8015
4. Matsumae, A., Nomura, S., and Hata, T. (1964) *J Antibiot (Tokyo).* **17**, 1-7
5. Joshi, V. C., and Wakil, S. J. (1971) *Archives Of Biochemistry And Biophysics* **143**, 493-505
6. Tsay, J., Oh, W., Larson, T., Jackowski, S., and Rock, C. (1992) *J. Biol. Chem.* **267**, 6807-6814
7. Rawlings, M., and Cronan, J., Jr. (1992) *J. Biol. Chem.* **267**, 5751-5754
8. Cronan, J., Jr Li, W., Coleman, R., Narasimhan, M., de Mendoza, D., and Schwab, J. (1988) *J. Biol. Chem.* **263**, 4641-4646
9. Mohan, S., Kelly, T., Eveland, S., Raetz, C., and Anderson, M. (1994) *J. Biol. Chem.* **269**, 32896-32903
10. Heath, R. J., and Rock, C. O. (1995) *J. Biol. Chem.* **270**, 26538-26542
11. Heath, R. J., and Rock, C. O. (2000) *Nature* **406**, 145-146
12. Heath, R. J., Su, N., Murphy, C. K., and Rock, C. O. (2000) *J. Biol. Chem.* **275**, 40128-40133
13. Kauppinen, S., Siggaard-Andersen, M., and von Wettstein-Knowles, P. (1988) *Carlsberg Research Communications* **53**, 357-370
14. Siggaard-Andersen, M., Wissenbach, M., Chuck, J.-A., Svendsen, I., Olsen, J. G., and Von Wettstein-Knowles, P. (1994) *Proceedings of the National Academy of Sciences of the United States of America* **91**, 11027-11031
15. Heath, R. J., Yu, Y.-T., Shapiro, M. A., Olson, E., and Rock, C. O. (1998) *J. Biol. Chem.* **273**, 30316-30320
16. Banerjee, A., Dubnau, E., Quemard, A., Balasubramanian, V., Um, K., Wilson, T., Collins, D., de Lisle, G., and Jacobs, W. J. (1994) *Science* **263**, 227-230
17. Noto, T., Miyakawa, S., Oishi, H., Endo, H., and Okazaki, H. (1982) *J Antibiot (Tokyo)* **35**, 401-410
18. Dolak, L., Castle, T., Truesdell, S., and Sebek, O. (1986) *J Antibiot (Tokyo)* **39**, 26-31
19. Omura, S., Y, I., Nakagawa, A., Iwata, R., Takahashi, Y., Shimizu, H., and Tanaka, H. (1983) *J Antibiot (Tokyo)* **36**, 109-114
20. Kauppinen, S., Siggaard-Anderson, M., and van Wettstein-Knowles, P. (1988) *Carlsburg. Res. Commun* **53**, 357-370
21. Price, A. C., Choi, K.-H., Heath, R. J., Li, Z., White, S. W., and Rock, C. O. (2001) *J. Biol. Chem.* **276**, 6551-6559
22. He, X., and Reynolds, K. A. (2002) *Antimicrob. Agents Chemother.* **46**, 1310-1318
23. Daines, R. A., Pendrak, I., Sham, K., Van Aller, G. S., Konstantinidis, A. K., Lonsdale, J. T., Janson, C. A., Qiu, X., Brandt, M., Khandekar, S. S., Silverman, C., and S., H. M. (2003) *J Med Chem.* **46**, 5-8
24. Tsay, J., Rock, C., and Jackowski, S. (1992) *J Bacteriol* **174**, 508-513
25. Onishi, H. R., Pelak, B. A., Gerckens, L. S., Silver, L. L., Kahan, F. M., Chen, M.-H., Patchett, A. A., Galloway, S. M., Hyland, S. A., Anderson, M. S., and Raetz, C. R. H. (1996) *Science* **274**, 980-982
26. Wang, J., Galgoci, A., Kodali, S., Herath, K. B., Jayasuriya, H., Dorso, K., Vicente, F., Gonzalez, A., Cully, D., Bramhill, D., and Singh, S. (2003) *J. Biol. Chem.* **278**, 44424-44428
27. Miller, J. H. (1972) in *Experiments in Molecular Genetics*, pp. 201-205, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
28. Singer, M., Baker, T. A., Schnitzler, G., Deische, S. M., Goel, M., Dove, W., Jaacks, K. J., Grossman, A. D. E., J. W., and Gross, C. A. (1989) *Microbiol Rev.* **53**, 1-24
29. Bochner, B. R., Huang, H. C., Schieven, G. L., and Ames, B. N. (1980) *J Bacteriol*. **143**, 926-933
30. Lynen, F. (1962) in *Methods in Enzymology* (Colowick, S., and Kaplan, N., eds) Vol. 5, pp. 443-451, Academic Press, New York and London
31. Wang, J., Tu, Y., Mukhopadhyay, S., Chidiac, P., Biddlecome, G. H., and Ross, E. M. (1999) in *G Proteins: Techniques of Analysis* (Manning, D. R., ed), pp. 123-151, CRC Press, Boca Raton
32. Zhang, J.-H., Chung, T. D. Y., and Oldenburg, K. R. (1999) *Journal of Biomolecular Screening* **4**, 67-73
33. Jackowski, S., and Rock, C. (1987) *J. Biol. Chem.* **262**, 7927-7931
34. Rock, C. O., and Cronan, J. E., Jr. (1979) *J. Biol. Chem.* **254**, 7116-7122
35. Alberts, A., Bell, R., and Vagelos, P. (1972) *J. Biol. Chem.* **247**, 3190-3198
36. Choi, K.-H., Heath, R. J., and Rock, C. O. (2000) *J. Bacteriol.* **182**, 365-370
37. Perrin, D. D., and Dempsey, B. (1974) (Hall, C. a., ed), New York
38. Wang, J., Tu, Y., Woodson, J., Song, X., and Ross, E. M. (1997) *J. Biol. Chem.* **272**, 5732-5740
39. Kawaguchi, A., Tomoda, H., Nozoe, S., Omura, S., and Okuda, S. (1982) *J Biochem (Tokyo)* **92**, 7-12
40. Ward, W., Holdgate, G., Rowsell, S., McLean, E., Pauptit, R., Clayton, E., Nichols, W., Colls, J., Minshull, C., Jude, D., Mistry, A., Timms, D., Camble, R., Hales, N., Britton, C., and Taylor, I. (1999) *Biochemistry* **38**, 12514-12525

41. Schujman, G. E., Choi, K.-H., Altabe, S., Rock, C. O., and de Mendoza, D. (2001) *J. Bacteriol.* **183**, 3032-3040

42. Jackowski, S., Zhang, Y.-M., Price, A. C., White, S. W., and Rock, C. O. (2002) *Antimicrob. Agents Chemother.* **46**, 1246-1252

43. Young, K., and Silver, L. (1991) *J Bacteriol* **173**, 3609-3614

44. Poole, K. (2001) *Current Opinion in Microbiology* **4**, 500-508

45. Markham, P. N., and Neyfakh, A. A. (2001) *Current Opinion in Microbiology* **4**, 509-514

46. Schweizer, H. P. (1998) *Antimicrob. Agents Chemother.* **42**, 394-398

47. Chuanchuen, R., Beinlich, K., Hoang, T. T., Becher, A., Karkhoff-Schweizer, R. R., and Schweizer, H. P. (2001) *Antimicrob. Agents Chemother.* **45**, 428-432
Sup. 1  Whole cell labeling of S. aureus, MB2985

FASII inhibitors
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Whole cell labeling of *S. aureus*, MB2985
Determination of selectivity and efficacy of fatty acid synthesis inhibitors
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