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Discovery of New Antibacterial Agents Inhibiting Bacterial Fatty Acid Biosynthesis

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The emergence of antibiotic-resistant bacteria, including methicillin-resistant *Staphylococcus aureus* and vancomycin resistant *Staphylococcus aureus,* has steadily increased to become a serious threat to the human. This is because most antibiotic drugs used today are derivatives of agents which have been in the clinic for more than 30 years. Thus, there is a need for new antibacterial agents with new mechanism of action.

The traditional method for discovering antibiotics involves screening for bacterial cell growth inhibitors and subsequent chemical improvements based on limited classes of antibiotics. Most antibiotics discovered by this method target a relatively small number of cellular processes including transcription, translation, DNA replication and cell wall biosynthesis. Consequently, any new class of antibiotic except for oxazolidinone has not been discovered in the last 35 years.

With the advent of microbial genomics, the complete genomes of nearly 80 microbes have been sequenced and about half of the genomes sequenced were from microbial pathogens. Bacterial genome sequence data has allowed the identification of novel classes of broad spectrum targets for therapeutic intervention. By *in silico* target identification and antisense RNA technology, 250 genes among 2600 genes in *S. aureus* have been selected as new possible targets which are over 10 times higher number than those used for the past 35 years. Among those selected targets, some including aminoacyl-tRNA synthetase, two-component signal transduction system, fatty acid biosynthesis etc, have already been validated as drug targets for new antibacterial agents development. The expansion of potential drug targets by genomic-based technology have facilitated a fundamental shift from direct antimicrobial screening program toward rational target-based strategies in the drug discovery paradigm.

Fatty acid synthesis (FAS) in bacteria is essential to the production of a number of lipid-containing components including the cell membranes. The bacterial fatty acid synthesis is carried out by a set of individual enzymes that are collectively known as the type II in conjunction with acyl carrier protein(ACP)-associated substrates. Mammalian fatty acid synthase called type I is different from type II in that lipid biosynthesis is mediated by a single multifunctional enzyme-ACP complex. The corresponding activities of the two FAS systems are related in structure and function, but generally lack overall sequence homolog. This differences in prokaryote and eukaryote fatty acid synthesis offer an attractive opportunity

for selective FAS II inhibition which is a potential strategy for the development of antibacterial agents. FabI is an enoyl-ACP reductase which catalyzes the final and rate-limiting step of the chain elongation process of the type II FAS. FabI catalyzes the stereospecific reduction of an enoyl-ACP using the cofactor NDAH and has been validated as an excellent target for antibacterial drug development.

A vast majority of the antibacterial agents in clinical use today are either microbial natural products or one of their analogs. Finding novel chemical entities with new mode of action and optimizing their activities by designing suitable derivatives has been the key to new drugs. Microbial natural products have been a proven, rich source of novel compound with diverse biological activities.

In this study, new enoyl reductase inhibitors have been screened from microbial natural resources for discovery of new antibacterial lead compound. The screening program led to the selection of a fungal strain producing a strong inhibitory metabolite. The active compound was purified as a single compound from solid state culture through bioassay-guided fractionation using ethyl acetate extraction, various chromatographic methods and HPLC. Chemical structure of the purified compound named compound A was elucidated by analyses of mass and various NMR spectral data. Compound A inhibited both *Staphylococcus aureus* FabI and *E. coli* FabI in a dose-dependent fashion with IC_{50} (ug/ml) values of 8.6 and 10.6, respectively. The inhibition of compound A was competitive with substrate. The K_i and K_m values for *E. coli* FabI were 6.42×10^{-4} M and 7.5×10^{-6} M, respectively. Compound A, however, exhibited weaker activity on FabK of *Streptococcus pneumoniae*, another isoform of enoyl-ACP reductase. In a whole cell assay, compound A showed antibacterial activity with MIC of 3 ug/ml against *Streptococcus pyogenes*, *Streptococcus faecium*, *Staphylococcus aureus*, and various MRSA strains. This study reports the first natural compound with FabI-inhibitory activity.

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