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Novel enoyl-ACP reductase (FabI) potential inhibitors of *Escherichia coli* from Chinese medicine monomers

Jingjing Yao †, Qingye Zhang †, Jun Min, Jin He *, Ziniu Yu *

State Key Laboratory of Agricultural Microbiology and National Engineering Research Center of Microbial Pesticides, Huazhong Agricultural University, Wuhan 430070, PR China

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ABSTRACT

By structure-based virtual screening and experimental verification, two Chinese medicine monomers, luteolin and curcumin, had been proved to be uncompetitive inhibitors of enoyl-ACP reductase from *Escherichia coli* (*Ec*FabI) with the inhibition constant (K_i) of 7.1 μM and 15.0 μM, respectively. In particular, curcumin had apparent antibacterial activity against *E. coli,* and the minimum inhibition concentration (MIC_{90}) was 73.7 μg/mL. Importantly, *fabI*-overexpressing *E. coli* showed reduced susceptibility to the inhibitor compared with the wild-type strains, demonstrating that its antibacterial action is mediated by the inhibition of *Ec*FabI.

The increasing resistances of clinically important pathogens to antibiotic treatments are of world-wide concern. One approach to combat antibiotic resistance is to identify new antibacterial agents that operate with distinctly different mechanisms from the actions of currently available drugs. Among various genomics driven metabolisms for antibacterial drug discovery, bacterial fatty acid synthesis (FAS) has been targeted since FAS is organized differently between bacteria (FASII) and mammals (FASI).[1] Enoyl-ACP reductase (ENR) catalyses the last and rate-limiting reaction in each round of the chain elongation cycle in FASII.[2] Thus, there exists a potential regarding selective inhibition of bacterial cellular growth by the inhibition of the ENR enzyme.[3,4] There are four isoforms, FabI, FabK, FabL and FabV, of ENR.[5] For most bacteria, FabI is unique ENR and shares high overall structural homology, and variability exists mainly in a mobile loop of amino acids that covers the active site (the substrate binding loop).[3]

Many FabI inhibitors have been reported in the past few years.[3,6–9] Triclosan, the widely employed antibacterial compound in many personal care products, that is, deodorants, soaps, hand washes and toothpastes, is a slow, tight-binding inhibitor of FabI, interacting specifically with the enzyme/NAD^+ product complex.[10] However, it has never been used for systemic therapeutic purposes because of its toxicity. Nowadays, more and more natural origin FabI inhibitors were reported.[6,8,11,12] Meanwhile, the structure-based virtual screening has been applied extensively in modern drug discovery. It is based on predicted binding interactions of small molecule with active site of the target.[13] Herein, the screen of novel potential FabI inhibitors from traditional Chinese medicine monomer library (mainly extracted and purified from plant and the purity of all compounds is HPLC ⩾ 98%. http://www.tcmtcm.com/standard) is reported. The body of the work includes structure-based virtual screening coupled with enzyme activity assay as well as bacteriostatic test. The discovered potential inhibitors are considered to be low toxicity since these parts of the plants had been used as drugs in China for several thousand years.

To identify a number of new hit compounds with potent inhibitory activity, FabI structure-based docking research was carried out by FlexX methods.[14] The active site was defined as follows, all atoms located within the range of 6.5 Å from any atom of the ligand of FabI were selected into the so-called active site and the amino acid residue was, therefore, involved into the active site if at least one of its atoms was selected. Other default parameters in the FlexX module were used in the calculations of molecular docking.[15,16] Five potential *Ec*FabI inhibitors, curcumin ((*E*,*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), imperatorin (9-[(3-methyl-2-buten-1-yl)oxy]-7*H*-furo[3,2-*g*][1]benzopyran-7-one), polydatin (3,4′,5-trihydroxystilbene-3-β-D-glucopyranoside), genistein (4′,5,7-trihydroxyisoflavone) and luteolin (3′,4′,5,7-tetrahydroxyflavone), were identified by the virtual screening from the traditional Chinese medicine monomer library. Their structures are shown in Figure 1. The representative hit compounds were selected by jointly using the docking score, binding mode, structural diversity, chemicophysical characteriza-

* Corresponding authors. Tel.: +86 27 87280802; fax: +86 27 87280670 (J.H.).
E-mail addresses: hejin@mail.hzau.edu.cn (J. He), yz41@mail.hzau.edu.cn (Z. Yu).
† Both the authors contributed equally and both are co-first authors.

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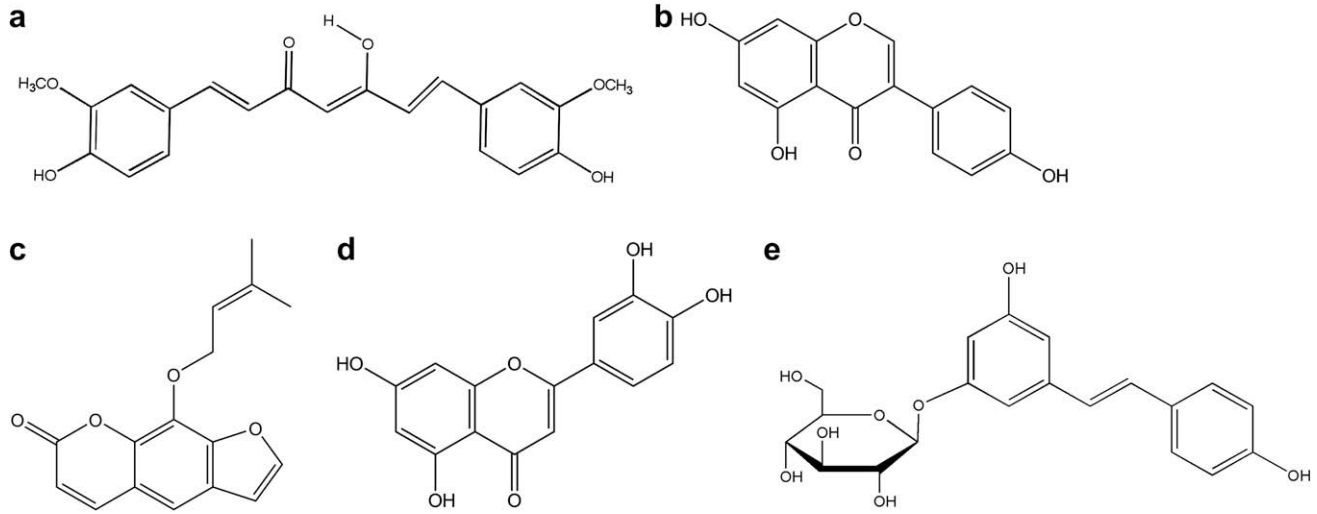

Figure 1. Chemical structures of (a) curcumin, (b) genistein, (c) imperatorin, (d) luteolin, and (e) polydatin.

tion for further bioactivity in vitro test. The predicted binding models of luteolin and curcumin within the enzyme active site superposed with triclosan are shown in Figures 2 and 3, respectively.

To establish the targeted enzymatic reaction system, at first, the *fabI* was amplified from the genome of *E. coli* MG1655 (GenBank Accession No. NP_415804) and was then inserted into pQE30 expression vector between the sites of *Bam*HI and *Hind*III. After that, the recombinant plasmid was transformed into *E. coli* M15

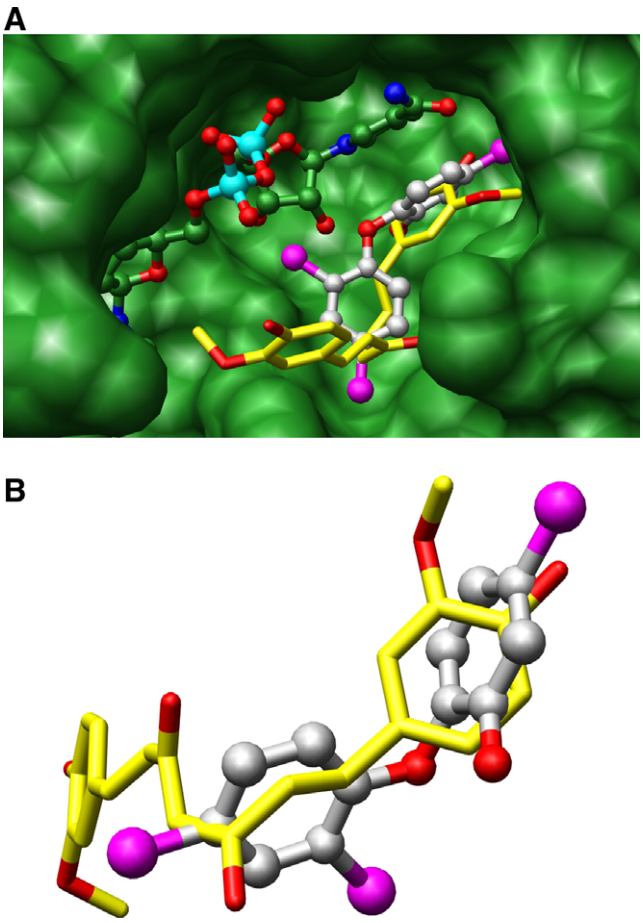

Figure 2. (A) the superposition of the binding model of curcumin (yellow stick) and tricloson (white ball-and-stick) in the active site of *Ec*FabI. The NAD^+ is shown in green ball-and-stick; (B) the superposition of docking model of curcumin (yellow stick) and triclosan binding model in the crystal structure (white ball-and-stick).

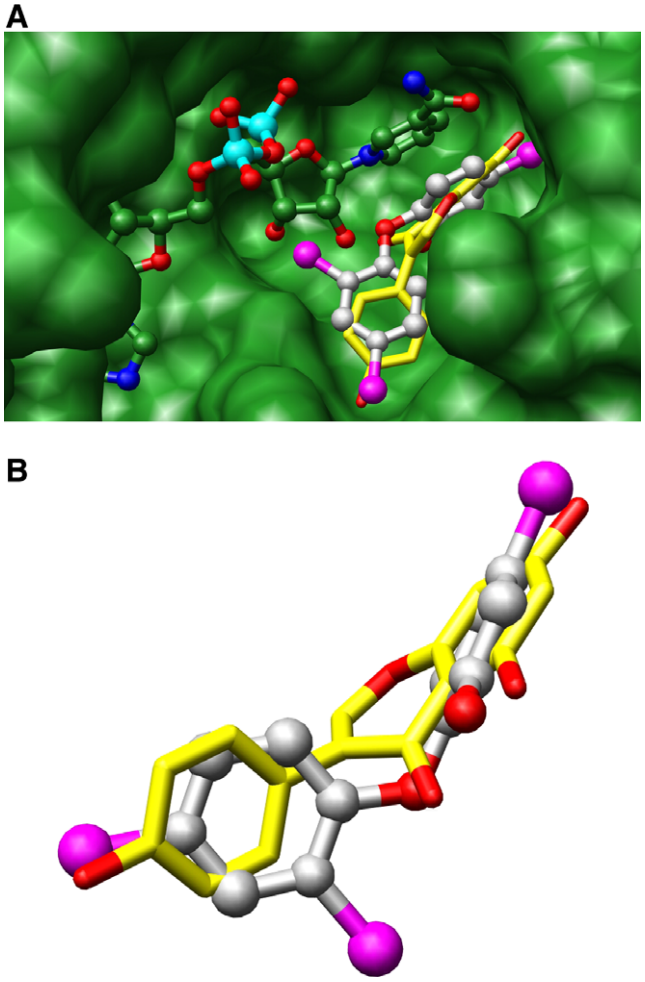

Figure 3. (A) the superposition of the binding model of luteolin (yellow stick) and triclosan (white ball-and-stick) in the active site of *Ec*FabI. The NAD^+ is shown in green ball-and-stick; (B) the superposition of docking model of luteolin (yellow stick) and triclosan binding model in the crystal structure (white ball-and-stick).

competent cells to obtain the *Ec*FabI over-expressed stain M15/pQE30-*fabI*. The six His-tagged *Ec*FabI was purified according to a reference.[17]

The standard reaction mixture contained 200 μM of crotonoyl-CoA, 100 μM of NADH, 50 nM of *Ec*FabI and 1% DMSO in 20 mM of Tris–HCl and 150 mM of NaCl buffer (pH 7.5) within a total volume of 100 μL. The individual compound was dissolved in DMSO and then preincubated with *Ec*FabI for 10 min before adding crotonoyl-CoA. *Ec*FabI inhibition assay as described previously.[11] In a inhibit system, the final concentration of the hit compound was 5 μM. The enzyme activity was determined by monitoring the oxidation of NADH to NAD^+ at 340 nm and room temperature for 5 min. The results of the enzymatic reaction catalyzed by *Ec*FabI showed that a commonly found flavones luteolin, which could dramatically reduce inflammation[18], was the most significant inhibitor followed by imperatorin, polydatin, curcumin and genistein (Fig. 4). The minimum inhibition concentration (MIC_{90}) was detected by K-B disc diffusion method and agar dilution technique.[19,20] The results indicated that only curcumin and genistein, possessed antibacterial activities against *E. coli* (Table 1). However, luteolin had no antibacterial activities to *E. coli* M15 and *E. coli* M15/pQE30-*fab*I. This may be caused by the decomposability in vivo or the low level of cellular entry.

Curcumin is an extract from spice turmeric, the powdered root of curcuma. It is widely used as food additive and appears to possess diverse pharmacologic effects including anti-inflammatory, anti-oxidant, anti-angiogenic and anti-proliferative activities.[21–23] The antibacterial activity of curcumin on the *fabI*-overexpressing strain was investigated to establish the effect of overexpression of FabI on the shift the MIC of curcumin for *E. coli*. As can be seen in Table 1, the MIC_{90} of curcumin for the *fabI*-overexpressing strain, M15/pQE30-*fabI*, increased at least sevenfold compared with those for the wild-type strain M15, and the vector-containing strain, M15/pQE30 (both were 73.7 μg/mL). The triclosan MIC_{90} for the *fabI*-overexpressing strain also increased greatly as a positive control. Antibiotics with different modes of action, spectinomycin, erythromycin, chloromycetin, gentamicin and rifampicin, as negative controls, did not change the MIC_{90} for the three strains, indicating that altered expression of *Ec*FabI does not change the susceptibility of the cells to antibiotics. Thus, these results suggest that curcumin inhibits the growth of the *E. coli* through inhibition of the FabI-encoded ENR.

To characterize the inhibition mechanism, further progress curves were obtained with various concentrations of crotonoly-CoA (0.1–0.5 mM). The potency of *Ec*FabI and inhibition patterns caused by luteolin and crucumin were examined via Lineweaver–Burk plot.[24] For each concentration of crotonoly-CoA, K_i was calculated from the progress curves. As can be seen in Figure 5, both luteolin and curcumin were uncompetitive inhibitors to *Ec*FabI, and their K_i values were 7.05 μM and 14.96 μM, respectively. As shown in Figures 2 and 3a, the two compounds form non-covalent bond with NAD^+ and surrounding residues separately, and the phenol rings of the two hits also makes the π-stacking interactions with the nicotinamide ring of NAD^+. Therefore, luteolin and curcumin had the same inhibit mechanisms with triclosan. The experiment results coincided well with the computer modeling.

Recently, a study claimed that it was not appropriate to develop antibiotic for Gram-positive pathogens based on FAS II pathway targets due to the fact that exogenous fatty acids fully bypass inhibition of this pathway both in vitro and in vivo conditions.[25,26]However, the pathogens were right in the environments full of fatty acids. The FASII-based antimicrobials still can be used for treating different infection pathways caused by Gram-positive pathogens, including respiratory and digestive tract infection and reproductive tract infection and mucosa infection, etc. In these cases, the content of exogenous fatty acids which can be used by the bacteria directly was limited. Even so, due to the differences in membrane structure and permeability, Gram-negative bacteria cannot suck exogenous fatty acids. So, FASII pathway is still an efficient target for omnipresent and formidable Gram-negative pathogens, such as *Escherichia coli*, *Salmonella*, *Shigella*, and *Pseudomonas*.

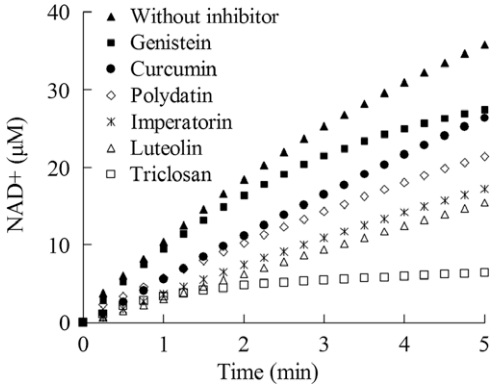

Figure 4. The progress curves for the inhibition of enoyl-ACP reductase from *E. coli* (*Ec*FabI). Five hit compounds of *Ec*FabI inhibitors, curcumin, imperatorin, polydatin, genistein and luteolin, were assayed at 5 μM of the final concentrations, respectively, with triclosan as positive control group. Each hit compound was preincubated with *Ec*FabI for 10 min and the progress was determined by monitoring the oxidation of NADH to NAD^+ at 340 nm for 5 min. All the experiments were carried out at room temperature.

Table 1
Antibacterial activities of potential inhibitors and relevant compounds against *E. coli* MG1655, M15, M15/pQE30 and M15/pQE30-*fab*I

Compound	MIC_{90} (μg/mL)			
	MG1655	M15	M15/pQE30	M15/pQE30-*fab*I
Triclosan	2.9	2.9	2.9	>500
Spectinomycin	20.0	20.0	20.0	20.0
Erythromycin	16.0	16.0	16.0	16.0
Chloromycetin	8.0	8.0	8.0	8.0
Gentamicin	8.0	8.0	8.0	8.0
Rifampicin	128	128	128	128
Curcumin	73.7	73.7	73.7	>500
Genistein	378	378	378	>500

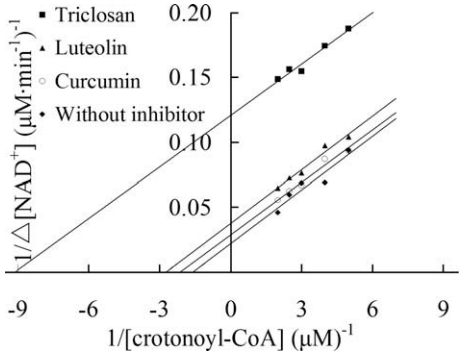

Figure 5. Inhibitory mechanisms of *E. coli* FabI (*Ec*FabI) by curcumin and luteolin. The reciprocals of the initial reaction crotonoyl-CoA concentrations were plotted. Curcumin and luteolin were assayed at 5 μM of the final concentrations, respectively, with triclosan as positive control group. They were preincubated with *Ec*FabI for 10 min and the progress curves were determined by monitoring the oxidation of NADH to NAD^+ at 340 nm for 5 min. All the experiments were carried out at room temperature.

In summary, curcumin and luteolin were verified as potential *Ec*FabI inhibitors by structure-based virtual screening and biological tests. Curcumin had apparent antibacterial activity against *E. coli* MG1655, and it is a promising FabI inhibitor for further study. Although it has no antibacterial activity, luteolin still has the potency to become antibacterial agents through structure reformation.

Acknowledgments

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