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Novel inhibitors of β-ketoacyl-ACP reductase from *Escherichia coli*

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ABSTRACT

Bacterial β-ketoacyl-[acyl carrier protein] (β-ketoacyl-ACP) reductase (FabG) is a highly conserved and ubiquitously expressed enzyme of the fatty-acid biosynthetic pathway of prokaryotic organisms that catalyzes NADPH-dependent reduction of β-ketoacyl-ACP intermediates. Therefore, FabG represents an appealing target for the development of new antimicrobial agents. A number of *trans*-cinnamic acid derivatives were designed and screened for inhibitory activities against FabG from *Escherichia coli*. These inhibited FabG enzymatic activity with IC_{50} values in the μM range, and were used as templates for the subsequent diversification of the chemotype. Introduction of an electron-withdrawing 4-cyano group to the phenol substituent showed improved inhibition over the non-substituted compound. The benzo-[1,3]-dioxol moiety also appeared to be essential for inhibitory activity of *trans*-cinnamic acid derivatives against FabG from *E. coli*. To explain the possible binding position, the best inhibitor from the present study was docked in the active site of FabG. The results for the best scoring conformers chosen by the docking programme revealed that cinnamic acid derivatives can be accommodated in the substrate-binding region of the active site, above the nicotinamide moiety of the NADPH cofactor. Additionally, a phage-displayed library of random linear 15-mer peptides was screened against FabG, to identify ligands with the common PPLTXY motif.

1. Introduction

Bacterial fatty-acid synthase (FAS II) consists of multiple individual enzymes, each encoded by a separate gene. This is in contrast to mammalian fatty-acid synthase, which is a homodimer of a single multifunctional polypeptide that is derived from a single gene [1]. The *fabG* gene product, β-ketoacyl-ACP reductase, is highly conserved and ubiquitously expressed in all bacteria, and is the only known isozyme that catalyzes the essential keto reduction step in the elongation cycle of FAS II [1]. Therefore, β-ketoacyl-ACP reductase (FabG) represents an appealing target that has yet to be exploited for the development of broad-spectrum antimicrobial agents.

The core reactions of FAS II occur in the elongation cycle that repeatedly extends the acyl chain by two-carbon units. The elongating substrate is attached as a thioester to the terminal sulfhydryl of the 4′-phospho-pantetheine moiety of the acyl carrier protein (ACP), and the growing acyl chain is carried by ACP through a series of four enzymes. First, a β-ketoacyl-ACP synthase (FabB or FabF) elongates the acyl-ACP C_n acyl chain to a C_{n+2} β-ketoacyl form. Next, the β-keto group is reduced by NADPH-dependent β-ketoacyl-ACP reductase (FabG), and the resulting β-hydroxy intermediate is then dehydrated by β-hydroxy acyl-ACP dehydratase (FabA or FabZ), to an enoyl-ACP. Finally, the reduction of the enoyl chain by a nucleotide-cofactor-dependent enoyl-ACP reductase (FabI, FabK, or FabL) produces an acyl-ACP with an elongated C_{n+2} acyl chain, which is ready to re-enter the cycle [2].

FabG is a member of the short-chain dehydrogenase/reductase (SDR) protein superfamily. The SDRs constitute one of the largest protein superfamilies known to date. The SDR proteins are non-metalloenzymes, are mostly of 25–35 kDa, and usually function as dimers or tetramers [3]. The sequence identities between different SDR proteins are low, being from 15% to 30%; however, the crystal structures of all of the SDR proteins revealed to date show a highly conserved α/β sandwich folding pattern, with a central β-sheet that is flanked by several helices, and which represents a typical Rossmann-fold motif [4]. The SDRs share a few distinct sequence motifs, like a co-enzyme binding site Gly-Xaa-Xaa-Xaa-Gly-Xaa-Gly in the N-terminal region, and a catalytic site Tyr-Xaa-Xaa-Xaa-Lys in the central region [5,6]. Site-directed mutagenesis of the SDRs has identified a Tyr, Lys and Ser as the catalytic triad residues. The Tyr acts as a basic catalyst, and the Lys binds NAD(P)H and lowers the pKa of the Tyr, while the Ser stabilizes the substrate, the reaction intermediate and the product during catalysis [7,8].

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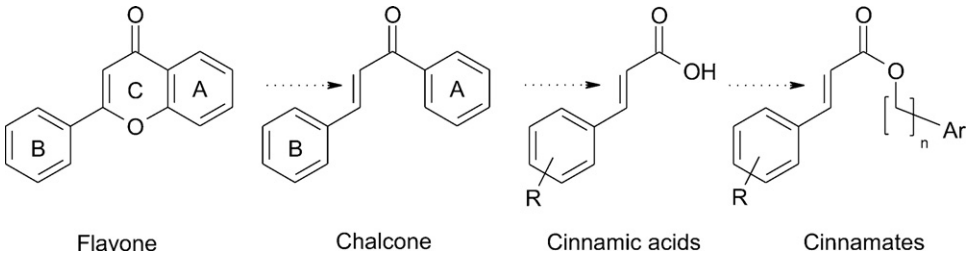

Fig. 1. Structural similarities of flavones and derivatives of cinnamic acid.

FabG is a tetrameric enzyme that undergoes significant conformational change upon NADPH binding, to bring residues Ser138, Tyr151 and Lys155 into their correct positions for active-site formation. An additional conformational change occurs following ACP binding [9]. The crystal structures of the apo-enzyme (1I01), FabG-$NADP^+$ binary complex (1Q7B), and non-active *Escherichia coli* FabG-Tyr151Phe mutant as an $NADP^+$ binary complex (1Q7C) from *E. coli* are available in the protein databank.

Natural products like (−)-epigallocatechin gallate (EGCG; green-tea catechin) and related flavonoids have been reported to be potent inhibitors of both FabG and FabI from *E. coli* (IC_{50} values of 5–100 μM). EGCG binds to both the free enzyme, to prevent the binding of the nucleotide cofactor, and the FabG/NADPH complex, to prevent binding of the substrate [1]. In another study, the C-3 gallic acid esters of the catechins were also identified as inhibitors of all three enzymes, FabG, FabI and FabZ, from *Plasmodium falciparum* (IC_{50} values of 0.2–1.1 μM) [10]. However, many polyphenols are known to be non-specific binders, and therefore their use as antimicrobial agents will presumably be limited [11].

Our initial idea was thus to evaluate *trans*-cinnamic acid esters, amides and related compounds as inhibitors of FabG from *E. coli*, as the biosynthesis of flavonoids proceeds via cinnamic acid or related phenolic acids (Fig. 1). In addition, we recently described cinnamic acid derivatives that inhibit 17β-hydroxysteroid dehydrogenase from the fungus *Cochliobolus lunatus*, a model enzyme of the SDR superfamily that is structurally related to FabG [12–14]. Therefore, a small focused library of cinnamic acid esters was synthesized and assayed for inhibition of FabG from *E. coli*. Additionally, a library of phage-displayed random linear peptides was screened against FabG to find novel hits with a peptide scaffold that would bind to the protein surface or bind in the active centre of FabG from *E. coli*.

2. Materials and methods

2.1. Synthesis and analyses of cinnamic acid derivatives

Target compounds **1**–**9** and **11** (Table 1) were synthesized from their corresponding carboxylic acids and alcohols or phenols. An ester bond was formed by standard methods (DCC [15] or BOP [16] -mediated esterification). Target compounds **10** and **12** (Table 1) were synthesized from their corresponding cinnamic or coumarin-3-carboxylic acids. For the formation of an amide bond, we used diphenylphosphoryl azide (DPPA) as the coupling agent [17].

All chemicals were obtained from commercial sources (Acros, Aldrich, Fluka, Merck, Jannsen and Sigma) and used without further purification. The solvents were used without purification or drying, unless otherwise stated. The reactions were monitored using analytical TLC plates (Merck, silica gel 60 F_{254}) with rhodamine G6 or sulphuric acid staining. Silica gel grade 60 (70–230 mesh, Merck) was used for column chromatography. The NMR spectra were obtained on a Bruker Avance DPX 300 instrument. ^{1}H NMR were recorded at 300.13 MHz with tetramethylsilane as the internal standard. Mass spectra were obtained with a VG-Analytical Autospec Q mass spectrometer with electro-spray (ES) or fast atom bombardment (FAB) ionization (MS Centre, Jožef Stefan Institute, Ljubljana). IR spectra were recorded on a PerkinElmer FTIR 1600 spectrometer. Elemental analyses were carried out by the Department of Organic Chemistry, Faculty of Chemistry and Chemical Technology, Ljubljana, on a 240 C PerkinElmer elemental analyzer. Melting points were determined using a Reichert hot-stage microscope, and are given without correction.

The analyses for compounds **1**, **2**, **4**, **7** and **11** were published previously [18].

2.1.1. (E)-phenyl 3-(benzo[d][1,3]dioxol-5-yl)acrylate (**3**)

Slightly yellow crystals; yield 70%; m.p. 100–102 °C (lit. [19] m.p. 104 °C); IR (KBr) ν_{max}: 1726, 1631, 1590, 1503, 1448, 1372, 1301, 1251, 1140, 1034, 968, 852, 802, 687 and 594 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.05 (s, 2H, CH_2), 6.47 (d, 1H, J = 15.8 Hz, CH=CH—CO), 6.87 (d, 1H, J = 7.9 Hz, aromatic), 7.06–7.14 (m, 2H, aromatic), 7.15–7.22 (m, 2H, aromatic), 7.23–7.31 (m, 1H, aromatic), 7.39–7.46 (m, 2H, aromatic), 7.80 (d, 1H, J = 15.8 Hz, CH=CH—CO); FAB MS: m/z 269 $[M+H]^+$; ESI HRMS: $[M+Na]^+$ $C_{16}H_{12}O_4Na$: 291.0634 (calcd 291.0633).

2.1.2. (E)-3-phenoxybenzyl 3-(benzo[d][1,3]dioxol-5-yl) acrylate (**5**)

White crystals; yield 66%; m.p. 75–78 °C; IR (KBr) ν_{max}: 1699, 1634, 1584, 1501, 1448, 1365, 1260, 1172, 1037, 982, 889, 846, 752 and 694 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 5.24 (s, 2H, CH_2), 6.03 (s, 2H, OCH_2O), 6.33 (d, 1H, J = 15.8 Hz, CH=CH—CO), 6.83 (d, 1H, J = 7.9 Hz, aromatic), 6.94–7.20 (m, 8H, aromatic), 7.30–7.42 (m, 3H, aromatic), 7.65 (d, 1H, J = 15.8 Hz, C=CH—CO); FAB MS: m/z 375 $[M+H]^+$; ESI HRMS: $[M+Na]^+$ $C_{23}H_{18}O_5Na$: 397.1064 (calcd 397.1052).

2.1.3. (E)-benzyl 3-(benzo[d][1,3]dioxol-5-yl)acrylate (**6**)

White crystals; yield 69%; m.p. 85–86 °C (lit. [20] m.p. 85–86 °C); IR (KBr) ν_{max}: 3028, 2919, 1702, 1633, 1500, 1450, 1364, 1267, 1169, 1038, 915, 848, 801, 757 and 701 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 5.26 (s, 2H, CH_2), 6.02 (s, 2H, OCH_2O), 6.33 (d, 1H, J = 15.8 Hz, CH=CH—CO), 6.83 (d, 1H, J = 7.9 Hz, aromatic), 7.00–7.06 (m, 2H, aromatic), 7.32–7.47 (m, 5H, aromatic), 7.66 (d, 1H, J = 15.8 Hz, CH=CH—CO); FAB MS: m/z 283 $[M+H]^+$; ESI HRMS: $[M+Na]^+$ $C_{17}H_{14}O_4Na$: 305.0795 (calcd 305.0790).

2.1.4. (E)-4-cyanophenyl 3-(benzo[d][1,3]dioxol-5-yl)acrylate (**8**)

White crystals: yield 65%; m.p. 164–167 °C; IR (KBr) ν_{max}: 2229, 1736, 1601, 1505, 1365, 1252, 1132, 1033, 925; 800 and 546 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.06 (s, 2H, OCH_2O), 6.45 (d, 1H, J = 15.8 Hz, CH=CH—CO), 6.87 (d, 1H, J = 7.5 Hz, aromatic), 7.05–7.14 (m, 2H, aromatic), 7.29–7.37 (m, 2H, aromatic), 7.67–7.77 (m, 2H, aromatic), 7.82 (d, 1H, J = 16.2 Hz, CH=CH—CO); FAB MS: m/z