Biochimica et Biophysica Acta 1861 (2017) 3180–3188

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

In silico investigation of lavandulyl flavonoids for the development of potent fatty acid synthase-inhibitory prototypes

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ARTICLE INFO

Article history:
Received 17 April 2016
Received in revised form 12 July 2016
Accepted 8 August 2016
Available online 13 August 2016

Keywords:
Sophora flavescens
Fatty acid synthase
Induced Fit docking
DP4 chemical-shift analysis
Per-residue analysis
Lavandulyl flavonoids

ABSTRACT

Background: Inhibition of fatty acid synthase (FAS) is regarded as a sensible therapeutic strategy for the development of optimal anti-cancer agents. Flavonoids exhibit potent anti-neoplastic properties.
Methods: The MeOH extract of *Sophora flavescens* was subjected to chromatographic analyses such as VLC and HPLC for the purification of active flavonoids. The DP4 chemical-shift analysis protocol was employed to investigate the elusive chirality of the lavandulyl moiety of the purified polyphenols. Induced Fit docking protocols and per-residue analyses were utilized to scrutinize structural prerequisites for hampering FAS activity. The FAS-inhibitory activity of the purified flavonoids was assessed via the incorporation of [^{3}H] acetyl-CoA into palmitate.
Results: Six flavonoids, including lavandulyl flavanones, were purified and evaluated for FAS inhibition. The lavandulyl flavanone sophoraflavanone G (**2**) exhibited the highest potency (IC_{50} of 6.7 ± 0.2 μM), which was more potent than the positive controls. Extensive molecular docking studies revealed the structural requirements for blocking FAS. Per-residue interaction analysis demonstrated that the lavandulyl functional group in the active flavonoids (**1**–**3** and **5**) significantly contributed to increasing their binding affinity towards the target enzyme.
Conclusion: This research suggests a basis for the *in silico* design of a lavandulyl flavonoid-based architecture showing anti-cancer effects via enhancement of the binding potential to FAS.
General significance: FAS inhibition by flavonoids and their derivatives may offer significant potential as an approach to lower the risk of various cancer diseases and related fatalities. *In silico* technologies with available FAS crystal structures may be of significant use in optimizing preliminary leads.

1. Introduction

Fatty acid synthase (FAS, EC 2.3.1.85) is a multifunctional enzyme participating in energy metabolism and it is responsible for the reductive *de novo* biosynthesis of long-chain fatty acids from acetyl-CoA and malonyl-CoA in the presence of NADPH [1]. This megasynthase is homodimeric and each subunit (270 kDa) is comprised of seven catalytic domains, i.e., the malonyl/acetyl transferase (MAT), β-ketoacyl synthase (KS), β-ketoreductase (KR), dehydratase (DH), enoyl reductase (ER), acyl carrier protein (ACP), and thioesterase (TE) domains. The main active sites of FAS are shown in Fig. 1 [2]. Long-chain fatty acids are covalently attached to ACP which carries them through the domains and fatty acids composed of 16–18 carbon atoms are released from the TE domain [3]. The expression and activity of FAS are strictly down-regulated under normal physiological conditions, whereas the enzyme is hyper-activated in early and advanced stages of many human malignancies including colon, ovary, and breast carcinomas [4]. The close correlation of FAS inhibition with anti-cancer activity was evidenced by a pioneering RNA interference (RNAi) study delineating that RNAi-mediated down-regulation of FAS expression inhibited lymph node carcinoma of prostate (LNCaP) cell growth, ultimately leading to the induction of apoptosis without influencing the viability of nonmalignant skin fibroblasts [5]. Owing to

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http://dx.doi.org/10.1016/j.bbagen.2016.08.001
0304-4165/

the overexpressed FAS in such neoplastic diseases and selective cytotoxicity on cancerous cells, inhibition of those domains has become a viable therapeutic strategy for the development of an ideal anti-cancer agent exclusively cytotoxic to carcinoma cells. For instance, cerulenin inhibits carcinoma cells via inducing apoptosis by binding to the KS domain irreversibly (Figs. 1, A and 2), and this was verified extensively by in vitro and in vivo studies [6–9]. Also, the proton pump inhibitor omeprazole is capable of impeding FAS activity [10] and exhibited remarkable cytotoxicity, especially against triple negative breast cancer, and the drug is currently in phase 2 clinical trials in the USA [11]. However, the toxicities of cerulenin and its synthetic analogue C75, originating from their irreversible inactivation mechanism, limit their clinical applications [12,13]. Moreover, orlistat (tetrahydrolipstatin) and GSK837149A, which interact with the TE (Figs. 1, D and 2) and KR domains, respectively, exhibited poor stability and bioavailability [14,15]. These studies collectively served as motivation to search for FAS antagonist prototypes that are structurally based on flavonoids, given that the polyphenolic architectures possess proven and potent FAS-inhibitory activity [16–18].

Flavonoids comprise the largest and a therapeutically vital group of polyphenols in plants. Numerous biological activities of flavonoids have been reported including anti-inflammatory activity, cancer chemoprevention, and alleviation of severe septic conditions [16,19–22]. In particular, several mechanisms of the antineoplastic effects have been investigated; epigallocatechin-3-*O*-gallate along with the flavones luteolin and apigenin, and the flavonols quercetin and kaempferol, and the dihydroflavonol taxifolin (Fig. 2) exhibited strong cytotoxic activity against prostate and breast cancer cells via inhibition of FAS [16]. Recently, the flavones apigenin and luteolin were shown to display powerful and selective inhibition of AKR1B10, a member of the aldo-keto reductase superfamily, that has been targeted for the control of breast and hepatocellular cancers. Their anti-neoplastic mechanism of action was addressed using computational molecular docking studies [23].

In silico technologies including structure–activity relationship modeling and virtual substrate docking are increasingly applied in drug discovery [24,25]. The computational approaches have been predominantly employed for predicting the affinity of a small molecule binding to a receptor domain, thereby offering detailed interpretations of the molecular mechanisms involved [24–26]. Prior to conducting accurate and reliable molecular docking predictions, elucidation of the 3D structures of the ligands, utilizing quantum mechanics-based calculations coupled with advanced statistics such as CP3 or DP4, is a prerequisite, since their spatial structures play a central role in determining binding affinity to a receptor, which consequently influences the elicited biological activity [26–30].

In continuing screenings aimed at the discovery of anti-FAS leads from medicinal plants [31,32], the FAS-inhibitory assay-guided fractionation of the methanol extract of *Sophora flavescens* roots exhibiting powerful FAS inhibition (95 and 65% inhibition at 100 and 50 μg/mL, respectively) was undertaken. The active extract yielded six active lavandulyl flavonoids that were evaluated for FAS-inhibitory potential and cytotoxic properties. Docking studies with the active flavonoids were carried out to investigate the computed molecular interactions with a FAS domain to gain insights into the structural requirements for inhibiting FAS activity. The enhanced inhibitory mechanism of the polyphenols was also probed utilizing detailed per-residue analysis between the ligands and active residues comprising the target domain.

2. Experimental procedures

2.1. General procedures

Optical rotations were measured using a JASCO DIP-1000 automatic digital polarimeter (Easton, MD). FT-IR spectra were recorded on a JASCO FT-IR 300E spectrophotometer and UV spectra were obtained using a JASCO V-550 spectrophotometer. ^{1}H and ^{13}C NMR experiments were performed using a Bruker 250 MHz (DMX 250, Billerica, MA) spectrometer. HRFAB mass spectra were acquired utilizing a JMS-700 mass spectrometer (JEOL, Tokyo, Japan). Column chromatography was carried out employing Merck silica gel (70–230 mesh, EMD Millipore, Darmstadt, Germany) and Merck LiChroprep RP-18 (40–63 μm).

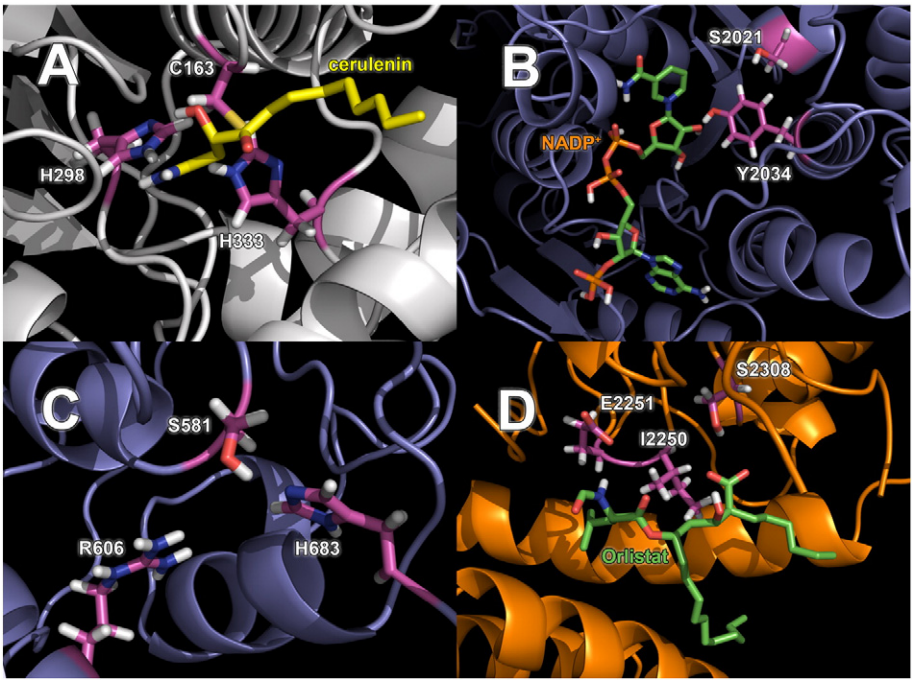

Fig. 1. The reported active sites of FAS. The labeled amino acids are key active site residues. (A) Cerulenin bound to the KS site (PDB ID: 1FJ8). The inhibitor forms a covalent bond with C163. (B) The KR site with bound $NADP^{+}$ (PDB ID: 2PX6) (C) Apoprotein of the MAT domain (PDB ID: 2PX6). (D) Orlistat bound to the TE site (PDB ID: 2PX6). The crystal structures were imported from the PDB data bank, optimized and minimized using Protein Preparation Wizard and redrawn using Pymol (ver. 3.0, Schrödinger LLC). Non-polar hydrogen atoms in the ligands are omitted for clear presentation.