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

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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 [³H] 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 (IC₅₀ 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

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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.

Keywords

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

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 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. ¹H and ¹³C 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).

2.2. Extraction and isolation of bioactive metabolites

Plant material. *S. flavescens* roots were purchased from a folk medicine market named “Yak-ryong-si” in Daegu, South Korea in October 2010. The plant material was identified by Dr. MinKyun Na and a voucher specimen (CNU0319) has been deposited in the Pharmacognosy laboratory at the College of Pharmacy, Chungnam National University.

The dried *S. flavescens* roots (70 g) were extracted with MeOH (2×2 L) at room temperature for one week and the organic extract was concentrated under reduced pressure to yield a crude extract (18 g). The MeOH extract was evaluated for its FAS inhibitory potential employing the in vitro assay protocols described below and the crude extract exhibited 95 and 65% inhibition at 100 $\mu\text{g/mL}$ and 50 $\mu\text{g/mL}$, respectively. The residue was suspended in H_2O (500 mL) and sequentially extracted with *n*-hexane (500 mL), EtOAc (500 mL), and *n*-BuOH (500 mL). Each organic layer was concentrated and evaluated for FAS inhibitory potency. The *n*-hexane and EtOAc fractions displayed FAS inhibition (95% at 100 $\mu\text{g/mL}$ for both fractions) and the major components of those active fractions comprised prenylated or lavandulyl flavonoids, based on the ^1H NMR spectra of the respective fractions. The active crude extracts were further fractionated by silica gel column chromatography [10 cm (i.d.) $\times$ 12 cm (length); 230–400 μm ; CHCl_3 –acetone 10:0, 8:2, 5:5, 3:7 and 0:10 v/v; 1 L for each step] to afford 35 fractions (Fr. 1–35). Fr. 3 to Fr. 7 were combined (1.2 g) and the mixture was purified by silica gel column chromatography [3.5 cm (i.d.) $\times$ 13 cm (length); 400–630 μm ; *n*-hexane–acetone 8:1 v/v; 1 L for isocratic elution] to yield compound **1** (107 mg). Fr. 9 to Fr. 14 were combined (1.4 g) based on a similar TLC profile and the mixture was subjected to silica gel column chromatography [5 cm (i.d.) $\times$ 10 cm (length); 230–400 μm ; CHCl_3 –MeOH 30:1 v/v; 1 L for isocratic elution] to afford compound **2** (270 mg). Fr. 26 to Fr. 29 were combined (Fr. A, 1.8 g) and the mixture was purified using silica gel column chromatography [3 cm (i.d.) $\times$ 15 cm (length); 400–630 μm ; *n*-hexane–EtOAc 3:1 v/v; 1 L under isocratic conditions] to yield compound **3** (210 mg) as well as six subfractions (Fr. A-1–Fr. A-6). Fr. A-4 exhibited FAS-inhibitory activity and the active fraction was chromatographed similar to the purification of compound **3** to afford compound **4** (50 mg). Fr. 31 to Fr. 35 were combined (Fr. B, 1.4 g) and the mixture was purified on a silica gel column [3 cm (i.d.) $\times$ 15 cm (length); 400–600 μm ; *n*-hexane/acetone 8:1, 6:3, 4:5, 3:6 v/v; 1 L for each step] to afford compound **5** (98 mg) along with eight subfractions (Fr. B-1–Fr. B-8). Compound **6** (80 mg) was obtained from Fr. B-7 under the chromatographic conditions used for purifying compound **5**.

2.3. FAS inhibition assay

Owing to the homology of the amino acid sequence and enzymatic characteristics of human FAS with those of chicken FAS [33], the latter was utilized for this study. The liver was prepared by utilizing stepwise ammonium sulfate precipitations, gel filtration, and anion exchange chromatography as described previously [34]. Briefly, FAS was 95% pure as evaluated using sodium dodecyl sulfate polyacrylamide gel electrophoresis with Coomassie blue staining. FAS activity was evaluated by analysis of the incorporation of [^3H] acetyl-

CoA into palmitate. To each microtube (final volume: 100 μ L) was added the prepared FAS (20–30 μ g) in a buffer containing 100 mM potassium phosphate (pH 7.0), 2.5 mM dithiothreitol, and 2.0 mM EDTA with or without the isolated flavonoids and positive controls. The reaction mixtures were pre-incubated at 37 $^{\circ}$ C for 60 min and the reaction was initiated by the addition of a substrate mixture containing 0.25 mM NADPH, 0.4 nmol of malonyl-CoA, and 0.02 μ Ci of [3 H] acetyl-CoA. After incubating at 37 $^{\circ}$ C for 10 min, the reaction was quenched with 60% HClO₄. Generated fatty acids were extracted with 1 mL of *n*-hexane and incorporation of radioactivity into the fatty acids was evaluated using scintillation counting.

2.4. Cytotoxicity assay

Cancer cell lines (COLO205, A549, and MCF-7) were maintained in RPMI 1640 media that contained L-glutamine with 10% FBS and 2% penicillin–streptomycin. Cancer cells were cultured at 37 $^{\circ}$ C in a 5% CO₂ incubator. The cytotoxicities of the purified flavonoids and positive controls were measured using a modified MTT assay [35]. Viable cells were seeded in the growth medium (180 μ L) into 96-well microtiter plates (1×10^4 cells per well) and incubated at 37 $^{\circ}$ C in a 5% CO₂ atmosphere. The compounds were dissolved in DMSO and adjusted to final sample concentrations ranging from 5.0 to 100 μ M by adding growth medium. After 2 h, the sample (20 μ L) was added to each well. An equal volume of DMSO was added to a well to serve as a negative control. After 48 h, the test sample and MTT (20 μ L) were added to each well and the final concentration was increased to 5 μ g/mL. After 2 h, the plate was centrifuged for 5 min at 1500 rpm, the media were removed, and the resulting formazan crystals were dissolved in DMSO (150 μ L). The optical density was measured at 570 nm using a Titertek microplate reader. The IC₅₀ value was calculated as the concentration of a test compound that reduced the absorbance by 50% relative to the DMSO-treated negative control.

2.5. Prediction of chirality of the C-2'' stereogenic center employing gauge invariant atomic orbital (GIAO) NMR chemical shift calculations

To assess the unknown chirality of the C-2'' stereogenic center of the lavandulyl flavonoids, GIAO NMR chemical shift calculations were performed [30]. The (2''-*R*) and (2''-*S*) diastereomers of kurarinol (**6**) were employed for computational prediction of 1 H and 13 C NMR chemical shifts because the hydroxy group in the geranyl chain restrains free rotation of the rotatable bonds by potentially generating hydrogen bonding with the 2'-hydroxy group (Supplementary Fig. 1). This led to a decrease in the number of possible conformers to consider, thus minimizing computational complexity and expense. The NMR deshielding properties were calculated as in previous studies [30,36]. Conformational searches were carried out using MacroModel [37] with the OPLS_2005 force field (gas phase), a 10 kcal/mol upper energy limit and 0.001 kJ (mol $\text{\AA}$)⁻¹ convergence threshold on the rms gradient. All the conformers of each diastereomer were optimized using the B3LYP hybrid density-functional theory (DFT) method with the 6-31+G(d,p) basis set in the gas phase, employing the Gaussian 09 package (Gaussian Inc) [38]. Chemical shift calculations were done at the B3LYP/6-311+G(2d,p) level in methanol for comparison with the experimental NMR data recorded in methanol-*d*₄. The predicted chemical shift data for each diastereomer of **6** were compared with the experimental data [39], utilizing the total absolute deviation

(TAD) ($\Sigma|\delta_{\text{calculated}} - \delta_{\text{experimental}}|$) and DP4 probability, employing an applet available at <http://www-jmg.ch.cam.ac.uk/tools/nmr/DP4/> [30]. In application of the DP4 statistical analyses, the comparison of calculated and experimental chemical shift values of diastereotopic carbons (C-6'' and C-7'') and protons (H-1'', H-3, H-3'', H-4'', and H-9'') was performed with their closest matches based on the original author's recommendation [30].

2.6. Docking and per-residue analysis

All the structures shown in Figs. 2 and 3 were prepared using LigPrep for docking simulations [40] under the OPLS_2005 force field with default settings. The obtained flavonoids and luteolin were docked to available mammalian FAS crystal structures from the Protein Data Bank and the docking results with the KS domain (2VZ9) [41] correlated cohesively with the FAS inhibitory activity of the examined polyphenols. Protein Preparation Wizard [42] was used for the preparation of the protein domain. Since this protein structure was not co-complexed with a ligand, the centroid of the active site residues of the KS domain (His331, Cys161, and His293) [41] was selected as a docking center for the Induced Fit Docking protocol [43]. Per-residue analysis was performed using Glide Cross (XGlide) Docking [44] to further specify interactions between a docked ligand and residues within 10 Å of the ligand.

3. Results and discussion

3.1. Identification of active flavonoids and evaluation of FAS inhibitory activity

FAS inhibitory assay-guided fractionation with the active methanol extract led to the isolation of six active flavonoids (Fig. 3). These polyphenols were identified as three lavandulyl flavanones (**1–3**), a lavandulyl chalcone (**5**), an oxygenated lavandulyl flavanone (**6**), and a prenylated flavanone (**4**) by comparison of experimental and reported spectroscopic and physicochemical data [39,45,46]. These polyphenols were evaluated for their FAS inhibitory potential by assessing the incorporation of [³H] acetyl-CoA into palmitate (Table 1). Compounds **1–3** and **5** displayed potent inhibitory activities ($IC_{50} = 6.7–9.6 \mu\text{M}$) while **4** and **6** were largely inactive ($IC_{50} > 100 \mu\text{M}$ and $IC_{50} = 98.2 \mu\text{M}$, respectively) (Table 1). This observation was in accord with previous findings validating that the presence of B-ring hydroxy group(s), a 7-hydroxy group, and the A-and B-rings was required for exerting FAS inhibitory activity [18]. In particular, the flavonoids possessing the lavandulyl group (**1–3** and **5**) exhibited more powerful inhibitory activities than cerulenin and luteolin, which were used as positive controls (Table 1). Among these active lavandulyl flavonoids, compound **2** showed the strongest inhibition ($IC_{50} = 6.7 \pm 0.2 \mu\text{M}$) followed by compound **1** ($8.5 \pm 0.2 \mu\text{M}$). This observation was reminiscent of the previous SAR study validating that the degree of hydroxylation in the B ring was proportional to the FAS inhibitory activity [47]. Moreover, it appeared that an intact lavandulyl functionality is vital for exerting potent FAS inhibitory activity as shown by inactive compounds **4** and **6** carrying a dimethylallyl motif and tertiary hydroxy group in the geranyl moiety, respectively.

3.2. Elucidation of stereochemical details using DP4

Molecular docking studies were utilized to elucidate how a lavandulyl group in the active flavonoids would contribute to reinforcement of their FAS inhibitory potential. Prior to conducting the molecular docking methodologies, ^1H and ^{13}C NMR chemical shift calculations were performed to assign the elusive C-2'' stereogenic center of lavandulyl flavonoids utilizing the hybrid density-functional theory (DFT) method B3LYP together with the 6-31+G(d,p) and 6-311+G(2d,p) basis sets for geometry optimization (Supplementary Tables 1–4) and chemical shift calculations (Supplementary Tables 5–8), respectively, for accurate computational outcomes [36]. Kurarinol (**6**) was chosen for the quantum mechanics calculation because a relatively low number of conformers of **6** was expected, due to the potential hydrogen bonding of the hydroxy functionality in the geranylated moiety to the 2'-hydroxy group (Fig. 3 and Supplementary Fig. 1). The computed chemical shifts of the (2'' *R*)- and (2'' *S*)-diastereomers of **6** were compared with the experimental values [45, 46] utilizing total absolute deviation (TAD) and DP4 probability analyses, both of which have been successfully applied in addressing stereochemical details of a wide array of compounds [30,36,48–51]. While the TAD analysis favored the (2'' *R*)-diastereomer, but with only a slight difference relative to the other diastereomer (Table 2), DP4 analysis predicted the structure of **6** as the (2'' *R*)-diastereomer of **6** with 100% probability (Fig. 4 and Supplementary Fig. 2) [30], leading to unequivocal assignment of the (2'' *R*) absolute configuration. A presumed similar biosynthesis pathway towards the formation of the lavandulyl group permitted tentative assignment of (2'' *R*) absolute configuration to the remaining lavandulyl flavonoids.

3.3. Induced Fit docking studies with a FAS domain

The fully elucidated structures of the purified compounds and luteolin were docked to the KS domain of FAS based on a kinetic study identifying that flavonoids bind to the KS domain [47]. A good correlation between the FAS inhibitory activity of examined flavonoids and their docking scores supported the importance of this domain for the bioactivity of the flavonoids. Induced Fit Docking was employed and the docking scores of the compounds showed good agreement with their inhibitory potency (Table 1). For instance, the lavandulyl flavonoids (**1–3** and **5**), which showed powerful inhibition of FAS activity, exhibited more negative (favorable affinity) docking scores to the domain, while the less-active flavonoids exhibited low docking scores (**4** and **6**). The most favorable docking pose for sophoraflavanone G (**2**) is shown in Fig. 5; the A-ring was positioned in a binding pocket comprising Tyr222, Thr295, and Met205 and the B ring was oriented in the other hydrophobic pocket comprising Phe393, Pro264, and Phe263. This positioning might explain the structural requirement of the presence of the two aromatic rings of the flavanone architecture for exhibiting FAS inhibitory potential. The binding pose was further stabilized by hydrogen bonding interactions between the C-4 carbonyl oxygen and the sulfanyl hydrogen of Cys161, between the 7-OH hydrogen and the carbonyl oxygen of Met205, and from the 4'-OH oxygen to the amino hydrogen of Ser265 and the carboxylate oxygen of Asp254 (Fig. 5). These hydrogen bonding interactions enhanced the binding affinity, which matches and helps to explain the aforementioned structural requirement of the presence of a 7-hydroxy group and B-ring hydroxy group(s) for inhibiting FAS. Among the factors

contributing to the overall calculated docking scores (Table 1), LipophilicEvdW, a score that represents a hydrophobic grid potential, displayed a distinctive difference between the active lavandulyl flavonoids and luteolin; the geranylated flavonoids exhibited calculated lipophilicity indices ranging from -7.4 to -9.3 , while luteolin showed -5.4 .

3.4. Per-residue analysis to reveal key moieties to enhance binding affinity

Per-residue van der Waals interactions of the active lavandulyl flavanones and luteolin were further computed using XGlide to specifically establish which functionalities of those active molecules (**1–3** and **5**) played a crucial role in enhancing the lipophilic interaction energy. The per-residue analysis of van der Waals interactions was calculated for the residues within $10 \text{ \AA}$ of these docked ligands (Fig. 6). The FAS active flavanones (**1–3** and **5**) showed more negative (more preferable) van der Waals interactions than luteolin with their proximal residues (Fig. 6). Particularly, preferable van der Waals interactions of the active flavanones (**1–3**, **5**), found with residues 204–206, 261–265, 296–298, and 394–396 compared to those of luteolin, at least partially validate the better docking properties of the lavandulyl flavanones. On the contrary, luteolin showed unfavorable (positive) van der Waals interactions with proximal residues and a remarkably repulsive interaction with Glu304, which substantially undermined its binding stability. To clarify which residues were mainly involved in generating the favorable lipophilic interaction energy, the van der Waals interaction scores of sophoraflavanone G (**2**) and luteolin were compared. The residues having significantly different interaction energies when comparing **2** and luteolin (Thr297, Met205, Leu203, Phe395, Thr262, Phe263, Gln269, Glu304, and Val299) were visualized with a space-filling model as shown in Fig. 7. Interestingly, these residues generating more favorable lipophilic interaction with **2** were located in close proximity to its lavandulyl group. This suggests the way in which the lavandulyl flavanones exhibited better binding affinities with enhanced van der Waals potentials towards the domain and why the lavandulyl flavanones exhibited more potent FAS inhibition than luteolin.

3.5. Assessment of cytotoxicity of anti-FAS flavonoids

All the purified flavonoids were evaluated for cytotoxic activity against MCF-7 (human breast adenocarcinoma), A549 (human lung adenocarcinoma), and COLO205 (human colon adenocarcinoma) cell lines. The lavandulyl flavanones showed moderate cytotoxicity against the tested cancer cell lines (Table 3). Among the assessed cell lines, the A549 cancer cell line exhibited a correlation between flavonoid-induced FAS inhibition and cancer cell proliferation, which suggests the dependence of human lung adenocarcinoma on the lipogenic enzymatic activity. Inhibition of COLO205 cancer cell growth by the cytotoxic polyphenols was not correlated to FAS inhibition. This implies that the mechanisms underlying the cytotoxic effect of the flavonoids on COLO205 involve more than their inhibition of FAS activity.

4. Conclusion

In silico docking studies and per-residue analyses evidenced that the KS domain of FAS could accommodate the flavonoids, as previously suggested [47]. The lavandulyl group of the flavanones addressed in this study substantially increased the hydrophobic binding

affinities to the KS domain, which could explain their enhanced inhibitory effects on FAS activity. Among the tested cancer cell lines, survival of the A549 (human lung adenocarcinoma) cells was most correlated to the FAS activity. The fatty acid synthesis pathway provides a wide range of channels for future application to diagnosis, treatment, and prevention of human cancer. In this sense, FAS inhibition may hold significant potential as a strategy to lower the risk of various cancer diseases and related fatalities. Given current pre-clinical or clinical trials of flavonoids such as the isoflavones daidzein and genistein for the treatment of oncological diseases [52], this research may facilitate the design of enhanced flavonoid-based cytotoxic prototypes capable of impeding oncogenic FAS activity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supplementary data

Detailed computational methods and results, including Cartesian coordinates of all optimized conformers. Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.bbagen.2016.08.001>.

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.

Fig. 2.
Basic flavonoid structure and previously reported FAS inhibitors.

Fig. 3.
Structures of FAS inhibitory flavonoids (1–6).

Fig. 4.

DP4 analysis by comparison of predicted ^1H and ^{13}C NMR data of the two plausible diastereomers of kurarinol (**6**) with the corresponding experimental NMR data. The DP4 probability was calculated utilizing an applet available at <http://www-jmg.ch.cam.ac.uk/tools/nmr/DP4/> (cf. Supplementary Fig. 2).

Fig. 5.

The most favorable docking pose of sophoraflavanone G (**2**). The labeled residues play a key role in enhancing the binding affinities. The dotted yellow lines denote hydrogen bonding interactions. The residues in pink represent the binding pockets for the A- and B-rings. Non-polar hydrogen atoms are omitted for clear presentation.

Fig. 6.

Per-residue analysis of van der Waals interaction energies of the lavandulyl flavanones and luteolin. Numbers on the X-axis refer to the FAS residues within 10 Å of these ligands bound to the KS site. More negative values indicate more preferable interactions between each ligand and residue. There are more preferable van der Waals interactions of the active flavonoids (**1–3, 5**) than those of luteolin with residues 204–206, 261–265, 296–298, and 394–396.

Fig. 7. The docking pose of sophoraflavanone (**2**) to the KS domain of FAS. The labeled residues represented in space-filling mode are those which demonstrated a substantial difference (>1 unit) in van der Waals interaction energy between **2** and luteolin. Non-polar hydrogen atoms are not shown for clarity.

Table 1

FAS inhibitory activities (IC₅₀), docking scores, and van der Waals energies to the KS domain.

Compound	XGlide docking score ^a	LipophilicEvdW ^{a,b}	IC ₅₀
Kushenol A (1)	-10.7	-8.4	8.5 ± 0.2 μM
Sophoraflavanone G (2)	-10.6	-7.4	6.7 ± 0.2 μM
(-)-Kurarinone (3)	-8.8	-8.6	10.3 ± 0.1 μM
Isoxanthohumol (4)	-6.1	-6.7	>100
Kurardin (5)	-9.4	-9.3	9.6 ± 0.4 μM
Kurarinol (6)	-6.8	-9.3	98.2 ± 0.4 μM
Luteolin ^c	-8.2	-5.4	62.5 μM

^a More negative values indicate more preferable binding affinities.

^b Lipophilic term that indicates the hydrophobic grid potential and fraction of the total protein-ligand van der Waals energy.

^c Luteolin was used as a positive control together with cerulenin (IC₅₀ of cerulenin is 13.0 μM).

Table 2

Calculated NMR chemical shift values^a of (2''*R*)- and (2''*S*)-kurarinol (**6**) at the B3LYP/6-311+G(2d,p) level in methanol and their experimental data (Exp.).^b

	¹³ C				¹ H						
	2''R	2''S	Exp.	2''R - Exp.	2''S - Exp.	2''R	2''S	Exp.	2''R - Exp.	2''S - Exp.	
C-2	73.8	80.9	76.3	2.5	4.6	H-2	5.7	5.2	5.5	0.2	0.3
C-3	43.9	43.1	46.4	2.5	3.3	H-3	3.2	3.8	2.9	0.4	0.9
C-4	192.0	191.7	194.7	2.7	3.0		2.3	2.3	2.7	0.4	0.4
C-4a	104.4	104.3	106.6	2.2	2.3						
C-5	160.2	159.8	162.7	2.5	2.9						
C-6	90.0	89.3	94.1	4.1	4.8	H-6	6.0	6.1	6.1	0.1	0.0
C-7	160.9	160.7	165.7	4.8	5.0						
C-8	107.0	107.7	110.3	3.3	2.6						
C-8a	163.2	161.4	165.5	2.3	4.1						
C-1'	118.6	118.0	119.2	0.6	1.2						
C-2'	155.8	157.0	157.4	1.6	0.4						
C-3'	102.8	102.2	104.2	1.4	2.0	H-3'	6.4	6.3	6.3	0.1	0.0
C-4'	157.7	157.7	160.3	2.6	2.6						
C-5'	105.1	103.5	108.4	3.3	4.9	H-5'	6.5	6.3	6.3	0.1	0.0
C-6'	127.7	130.2	129.3	1.6	0.9	H-6'	7.3	7.0	7.3	0.0	0.2
OCH ₃	52.7	53.0	56.7	4.0	3.7	OCH ₃	3.9	3.7	3.8	0.1	0.1
							3.7	3.9	3.8	0.0	0.1
C-1''	30.2	30.4	29.5	0.7	0.9	H-1''	3.7	3.7	3.8	0.1	0.1
C-2''	50.6	52.0	49.2	1.4	2.8	H-2''	2.8	2.3	2.6	0.2	0.4
C-3''	26.1	27.0	28.9	2.8	1.9	H-3''	2.3	2.7	2.6	0.4	0.1
C-4''	41.3	41.7	43.6	2.3	1.9	H-4''	2.2	2.1	2.4	0.2	0.3
							1.4	1.4	1.4	0.0	0.0
							1.3	1.7	1.4	0.1	0.4
							1.1	1.3	1.3	0.2	0.0
							1.4	1.1	1.3	0.2	0.2

c_{TAD} = total absolute deviation.

Table 3

Cytotoxic activity of compounds **1–6** (IC₅₀, μ M) against several carcinoma cell lines.

Compound	MCF-7	A549	COLO205
1	21.3	36.1	28.1
2	19.2	18.1	32.5
3	23.2	50.5	34.2
4	>100	NT	NT
5	23	57.1	29.4
6	>100	NT	NT
Adriamycin (positive control)	0.8	1.4	0.5

MCF-7: human breast adenocarcinoma, A549: human lung adenocarcinoma, COLO205 (human colon adenocarcinoma); NT stands for not tested.