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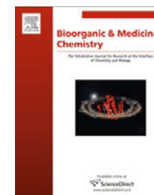

Synthesis of C(7) modified chrysin derivatives designing to inhibit β-ketoacyl-acyl carrier protein synthase III (FabH) as antibiotics

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ABSTRACT

As a naturally wide distributed flavone, chrysin exhibits numerous biological activities including anticancer, anti-inflammatory, and antimicrobials activities. β-Ketoacyl-acyl carrier protein synthase III (FabH) catalyzes the initial step of fatty acid biosynthesis via a type II fatty acid synthase in most bacteria. The important role of this essential enzyme combined with its unique structural features and ubiquitous occurrence in bacteria has made it an attractive new target for the development of antibacterial agents. We first used a structure-based approach to develop 18 novel chrysin analogues that target FabH for the development of new antibiotics. Structure-based design methods were used for the expansion of the chrysin derivatives including molecular docking and SAR research. Based on the results, 5-hydroxy-2-phenyl-7-(2-(piperazin-1-yl)ethoxy)-4H-chromen-4-one (**3g**) showed the most potent antibacterial activity with MIC of 1.56–6.25 μg/mL against the test bacterial stains, and docking simulation was performed to position compound **3g** into the *Escherichia coli* FabH active site to determine the probable binding conformation. The biological assays indicated that compound **3g** is a potent inhibitor of *E. coli* FabH as antibiotics.

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1. Introduction

Although several classes of antibacterial agents are presently available, resistance in most of the pathogenic bacteria to these drugs constantly emerges. In order to prevent this serious medical problem, the elaboration of new types of antibacterial agents or the expansion of bioactivity of the previous drugs is a very important task.[1] Therefore, in recent years the research has been focused toward development of new antibacterial agents, which may act through novel target, surpassing the problem of acquired resistance.

A promising target is the fatty acid synthase (FAS) pathway in bacteria. Fatty acid biosynthesis (FAB) is an essential metabolic process for prokaryotic organisms and is required for cell viability and growth.[2] Large multifunctional proteins termed type I fatty acid synthases (FAS I) catalyze these essential reactions in eukaryotes.[3–5] In contrast, bacteria use multiple enzymes to accomplish the same goal and are referred to as type II, or dissociated, fatty acid synthases (FAS II).[6,7] β-Ketoacyl-acyl carrier protein (ACP) synthase III, also known as FabH or KAS III, plays an essential and regulatory role in bacterial FAB.[8,9] The enzyme initiates the fatty acid elongation cycles,[10,11] and is involved in the feedback regulation of the biosynthetic pathway via product inhibition.[12] FabH catalyzes the condensation reaction between a CoA-attached acetyl group and an ACP-attached malonyl group, yielding acetoacetyl-ACP as its final product (Fig. 1). Two other condensing enzymes, FabB (KAS I) and FabF (KAS II), perform the chain elongation reactions in subsequent cycles leading to long-chain acyl ACP products.[6,8] While FabB and FabF are also condensing enzymes, FabH is structurally distinct.

FabH proteins from both Gram-positive and Gram-negative bacteria are highly conserved at the sequence and structural level while there are no significantly homologous proteins in humans. Importantly, the residues that comprise the active site are essentially invariant in various bacterial FabH molecules.[13–15] FabH represents a promising target for the design of novel antimicrobial drugs, since it regulates the fatty acid biosynthesis rate via an initiation pathway and its substrate specificity is a key factor in membrane fatty acid composition.[16–18] These attributes suggest that small molecule inhibitors of FabH enzymatic activity could be potential development candidates leading to selective, nontoxic, and broad-spectrum antibacterials.

Chrysin (1,5,7-dihydroxyflavone, shown in Scheme 1), a flavonoid widely distributed in nature, has been reported to have many different biological activities such as anti-oxidant,[19] anti-virus,[20] anti-microbial,[21] anti-anxiolytic activities[22] as well as antitumor effect.[23] In the reported experiments, the efforts were centered mostly on the substitutions on the aromatic rings (either A or C) of the chrysin, or chrysin was linked with heterocyclic moieties separated by different carbon spacers. These derivatives, compared with their parent compound, displayed significant biological activity against a panel of susceptible and resistant Gram-positive and Gram-

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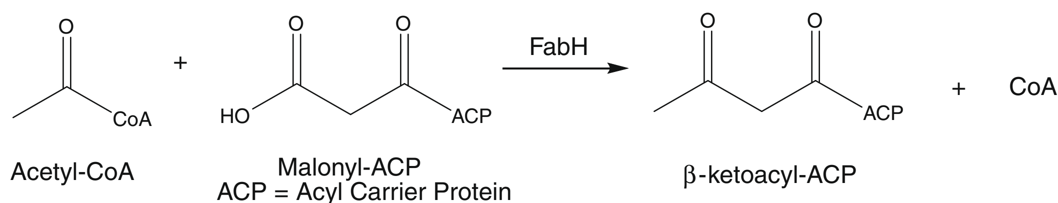
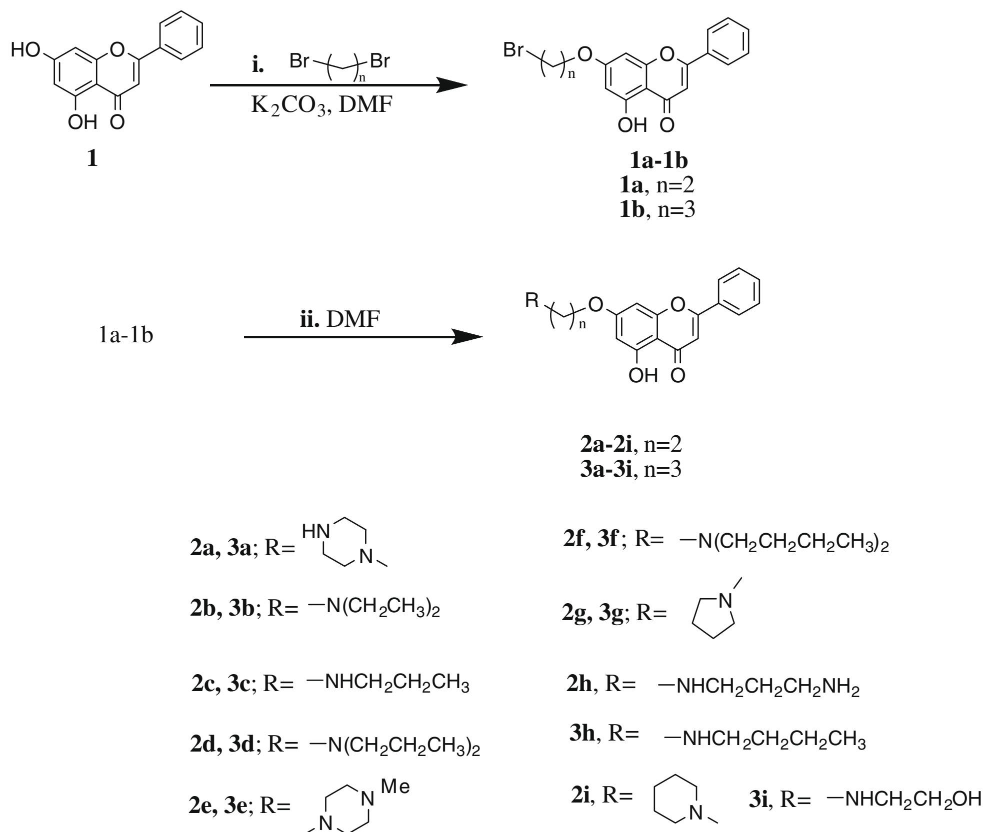


Figure 1. FabH-catalyzed initiation reaction of fatty acid biosynthesis.



Scheme 1. Synthesis of C(7) modified derivatives of chrysin. Reagents and conditions: (i) $BrCH_2CH_2Br$ or $BrCH_2CH_2CH_2Br$, K_2CO_3, DMF; (ii) R-amine, DMF, heating.

negative organisms.[23] Many flavonoids were reported to be good candidate KAS III inhibitors and may be utilized as effective antimicrobials.[24] Thus, it would be valuable to explore the inhibitory action of novel chrysin derivatives with heterocyclic and alkyl amines linked by different carbon spacers. In this paper, we described the synthesis of two series of analogues of chrysin and the evaluation of these compounds as FabH inhibitors for the first time. The antimicrobial activity against two Gram-negative bacterial strains, *Escherichia coli* and *Pseudomonas fluorescence*, and two Gram-positive bacterial strains, *Bacillus subtilis* and *Staphylococcus aureus*, of these analogues of chrysin were also been determined. Docking simulations were performed using the X-ray crystallographic structure of the FabH of *E. coli* in complex with an inhibitor to explore the binding modes of these compounds at the active site.

2. Results and discussion

2.1. Chemistry

The synthesis of compounds **2a–i** and **3a–i** was accomplished according to the general pathway illustrated in the Scheme 1. Compounds **1a** and **1b** were the key intermediates for synthesis of the compounds investigated. They were usually prepared from alkylation of 7-OH group by using 1,2- or 1,3-dihaloalkanes in the presence of bases such as NaOH or K_2CO_3 in anhydrous DMF.[25] To increase the antimicrobial properties of chrysin derivatives, chrysin derivatives in which the chrysin ring system is linked to the alkylamines by different spacers at C-7 position were investigated, with a view to modify their lipophilicity. The methods for alkylation of **1a** and **1b** were well documented.[26] The literature survey indicated that a method reported by Liu et al[27] is appropriate for N-alkylation of imidazole or benzimidazole, since the formation of quaternary imidazolium or benzimidazolium salts, as unwanted side product, was usually limited. Compounds in series 1 (**2a–2i**) contained a 2-carbon spacer in between chrysin and the substituent and compounds series 2 (**3a–3i**) contained a 3-carbon spacer in. All of the synthetic Compounds gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures.

2.2. Biological activity and molecular modeling

2.2.1. Antimicrobial activity

All the synthesized compounds (**2a–i** and **3a–i**) were screened for antibacterial activity against two Gram-negative bacterial

strains: *E. coli* and *P. fluorescence* and two Gram-positive bacterial strains: *B. subtilis* and *S. aureus* by MTT method. The MICs (minimum inhibitory concentrations) of the compounds against these bacteria were presented in Table 1. Standard antibacterial agent kanamycin B was also screened under identical conditions for comparison. The results revealed that most of the synthesized compounds exhibited significant antibacterial activity. It was observed that many compounds exhibited interesting antibacterial activity displaying MIC values between 1.56 and 50.0 μg/ml.

Among compounds in **3a–3i**, which contained a 3-carbon spacer, **3a**, **3e**, and **3g** with N-heterocyclic rings at C-7 position displayed potent zone of antibacterial activities ranging from 1.56–25.0 μg/ml, exhibiting higher potencies than chrysin which was inactive. Especially compound **3g** exhibited the most potent antibacterial activity with MIC of 1.56, 6.25, 6.25, and 3.13 μg/mL against *E. coli*, *P. fluorescence*, *B. subtilis*, and *S. aureus*, respectively, which was similar to the broad-spectrum antibiotic kanamycin B with corresponding MIC of 3.13, 3.13, 1.56, and 1.56 μg/mL. Compounds **3a** and **3e** also showed potent activity against the same strains. Other 3-carbon spacer derivatives with aliphatic chains substituent showed low inhibitory activity, this result disclosed that compounds with heterocyclic moieties at C-7 position of chrysin were more active than compounds with aliphatic chains. Compounds **2a–i** which contain a 2-carbon spacer, displayed moderate antibacterial activities. The N-heterocyclic rings displaced compounds **2a**, **2e**, **2g**, and **2i** showed better antibacterial activity than other compounds, and the same comparison was also exhibited in compounds **3a**–**i**. These results proved that the compounds with heterocyclic moieties at C-7 position of chrysin have a much higher activity than other species, although these compounds are not as good as kanamycin B which has an N-D-glucoside in the side chain in terms of broad-spectrum inhibition. A possible explanation for these results is that the lipophilicity of the chrysin derivatives and kanamycin B affected by their side chains played an important role for their antimicrobial activities.

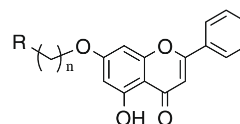
2.2.2. *E. coli* FabH inhibitory activity

The *E. coli* FabH inhibitory potency of the chrysin derivatives (**2a**, **2b**, **2c**, **3a**, **3e**, and **3g**) was examined and the results are summarized in Table 2. As shown in Table 2, most of the tested compounds displayed potent inhibitory activity. Among them, compounds **2b** and **2c**, with the alkyl amines at the C-7 position of chrysin had less inhibitory activity toward *E. coli* FabH. It suggested that a heterocyclic moiety at C-7 position of chrysin was essential for the FabH inhibitory activity. In addition to possessing antibacterial activity against both Gram-positive and Gram-negative bacteria, compound **3g** with pyrrolidine moiety at C-7 position was also shown to be a good inhibitor of *E. coli* FabH, indicating that **3g** may form a hydrophobic interaction of *E. coli* FabH. These biological assays indicated that compound **3g** is a potent inhibitor of *E. coli* FabH as antibiotics.

Table 1
Antimicrobial activity of synthetic compounds

Compounds	Minimum inhibitory concentrations (μg/mL)			
	Gram-negative		Gram-positive	
	E. coli	*P. fluorescence*	*B. subtilis*	*S. aureus*
2a	12.5	6.25	25	25
2b	>50	>50	>50	>50
2c	25	25	50	50
2d	>50	>50	>50	>50
2e	12.5	25	50	25
2f	>50	>50	>50	>50
2g	6.25	12.5	25	25
2h	>50	>50	>50	>50
2i	12.5	12.5	25	12.5
3a	3.13	12.5	12.5	6.25
3b	>50	>50	>50	>50
3c	12.5	12.5	25	12.5
3d	25	25	25	>50
3e	6.25	12.5	6.25	3.13
3f	>50	>50	>50	>50
3g	1.56	6.25	6.25	3.13
3h	12.5	12.5	25	12.5
3i	6.25	12.5	12.5	25
Kanamycin B	3.13	3.13	1.56	1.56
Chrysin	>50	>50	>50	>50

Table 2
FabH inhibitory activity of the target compounds (**2a**–**c**, *n* = 2; **3a**, **3e**, **3g**, *n* = 3)



Compound	R	*E.coli* FabH ‘ (μM)
2a	HN N	11.2
2b	$-N(CH_2CH_3)_2$	70
2c	$-NHCH_2CH_2CH_3$	54
3a	HN N	6.6
3e	N Me N	5.2
3g	N	3.1

Molecular docking of compound **3g** and *E. coli* FabH was performed on the binding model based on the *E. coli* FabH–CoA complex structure (1HNJ.pdb). The 3D structure of *E. coli* FabH is shown in Figure 2. The FabH active site generally contains a catalytic triad tunnel consisting of Cys-His-Asn, which is conserved in various bacteria. This catalytic triad plays an important role in the regulation of chain elongation and substrate binding. Since the alkyl chain of CoA is broken by Cys of the catalytic triad of FabH, interactions between Cys and substrate appear to play an important role in substrate binding. Qiu et al. have refined three-dimensional structure of *E. coli* FabH in the presence and absence of malonyl-CoA by X-ray spectroscopy. Since malonyl moiety is degraded by *E. coli* FabH, molecular docking studies for FabH and malonyl-CoA was carried out to identify a plausible malonyl-binding mode.[28] They found that in one of the binding modes appeared in the lower scored conformations, the malonyl carboxylate formed hydrogen bonds to the backbone nitrogen of Phe304. Enlightened by these facts, compound **3g** with the most potent inhibitory activity was hit by pharmacophore map I mentioned above. The binding model of compound **3g** and *E. coli* FabH is depicted in Figure 3. In the binding model, amino hydrogen of Asn247 forms hydrogen bond with 5-hydroxy of compound **3g**. pyrrolidine moiety at C-7 position may form a hydrophobic interaction with Asn274, Ile156, Phe157, and Met207 of *E. coli* FabH kinase.

Figure 2. 3D structure of *E. coli* FabH.

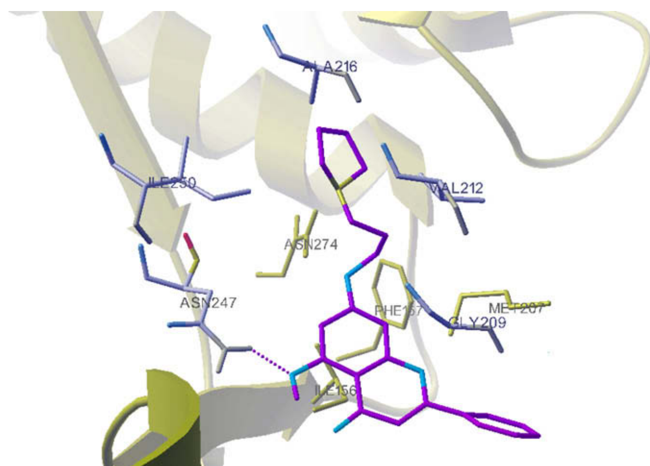


Figure 3. Binding model of compound **3g** and *E. coli* FabH.

3. Conclusion

In summary, two series of chrysin analogues (**2a–i**, **3a–i**) containing 2- and 3-carbon spacers were prepared and tested for their inhibitory activity against *E. coli* FabH and *E. coli*, *P. fluorescence*, *B. subtilis*, and *S. aureus*. Many synthesized compounds showed potent antibacterial and *E. coli* FabH inhibitory activities with compound **3g** being the most potent one. Preliminary structure–activity relationships and molecular modeling study provided further insight into interactions between the enzyme and its ligand. The results provided valuable information for the design of *E. coli* FabH inhibitors as antibiotics.

4. Experimental

4.1. Chemistry

All chemicals (reagent grade) used were commercially available. Chrysin (>98 %) was purchased from ShanXi Huike Co., Ltd, Jiangsu, China and was used without further purification. All the ^{1}H NMR spectra were recorded on a Bruker DRX 500 or DPX 300 model Spectrometer in DMSO-d_6. Chemical shifts (δ) for ^{1}H NMR spectra were reported in parts per million to residual solvent protons. ESI-MS spectra were recorded on a Mariner System 5304 Mass spectrometer. Elemental analyses were performed on a CHN-O-Rapid instrument and were within ±0.4% of the theoretical values. Melting points were measured on a Boetius micromelting point apparatus.

4.2. General method for synthesis of compounds 2a–3i

4.2.1. 5-Hydroxy-2-phenyl-7-(2-(piperazin-1-yl)ethoxy)-4H-chromen-4-one (2a)

To a solution of **1a** (0.36 g, 1 mmol) in 5 ml of anhydrous DMF was added piperazine (0.43 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared by TLC. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **2a** (0.30 g, 78%), mp 282–283 °C. ^{1}H NMR (DMSO-d_6): 2.01 (s, 1H), 2.29 (br s, 4H), 2.65–2.72 (m, 6H), 4.12 (t, *J* = 9.0 Hz, 2H), 6.40 (s, 1H), 6.48 (s, 1H), 6.70 (s, 1H), 7.56 (t, *J* = 6.5 Hz, 3H), 7.88 (t, *J* = 6.0 Hz, 1H), 12.60 (s, 1H). ESI-MS $C_{21}H_{22}N_2O_4$ $[M+H]^+$ 367.1. Anal. Calcd for $C_{21}H_{22}N_2O_4$: C, 68.84; H, 6.05; N, 7.65. Found: C, 68.87; H, 6.86; N, 7.48.

Compounds **2b–3i** were prepared in analogy.

4.2.2. 7-(2-(Diethylamino)ethoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (2b)

Mp 285–286 °C; ^{1}H NMR (DMSO-d_6): 1.16 (t, *J* = 7.0 Hz, 6H), 3.27–3.30 (m, 4H), 4.00 (t, *J* = 3.4 Hz, 2H), 4.12 (t, *J* = 3.4 Hz, 2H), 6.38 (s, 1H), 6.48 (s, 1H), 6.70 (s, 1H), 7.52 (t, *J* = 6.5 Hz, 3H), 7.84 (d, *J* = 6.0 Hz, 2H), 12.83 (s, 1H). ESI-MS $C_{21}H_{23}NO_4$ $[M+H]^+$ 354.1. Anal. Calcd for $C_{21}H_{23}NO_4$: C, 71.37; H, 6.56; N, 3.96. Found: C, 71.40; H, 6.49; N, 3.90.

4.2.3. 8-Hydroxy-3-phenyl-6-(2-(propylamino)ethoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (2c)

Mp 277–278 °C; ^{1}H NMR (DMSO-d_6): 0.91 (s, 3H), 1.46 (t, *J* = 7.1 Hz, 2H), 2.42 (t, *J* = 7.1 Hz, 2H), 2.72 (t, *J* = 5.6 Hz, 2H), 4.15 (t, *J* = 5.6 Hz, 2H), 6.39 (s, 1H), 6.51 (s, 1H), 6.94 (s, 1H), 7.52 (t, *J* = 6.5 Hz, 3H), 7.88 (d, *J* = 6.0 Hz, 2H), 12.44 (s, 1H). ESI-MS $C_{21}H_{23}NO_3$ $[M+H]^+$ 338.1. Anal. Calcd for $C_{21}H_{23}NO_3$: C, 74.75; H, 6.87; N, 4.15. Found: C, 74.72; H, 6.86; N, 4.17.

4.2.4. 7-(2-(Dipropylamino)ethoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (2d)

Mp 280–281 °C; ^{1}H NMR (DMSO-d_6): 0.86 (t, *J* = 7.2 Hz, 6H), 1.39–1.42 (m, 4H), 2.42 (t, *J* = 7.1 Hz, 4H), 2.78 (t, *J* = 5.6 Hz, 2H), 4.13 (t, *J* = 5.6 Hz, 2H), 6.39 (s, 1H), 6.54 (s, 1H), 6.98 (s, 1H), 7.52 (t, *J* = 6.5 Hz, 3H), 7.88 (d, *J* = 6.0 Hz, 2H), 12.60 (s, 1H). ESI-MS $C_{23}H_{27}NO_4$ $[M+H]^+$ 382.1. Anal. Calcd for $C_{23}H_{27}NO_4$: C, 72.42; H, 7.13; N, 3.67. Found: C, 72.62; H, 7.18; N, 3.59.

4.2.5. 5-Hydroxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)-2-phenyl-4H-chromen-4-one (2e)

Mp >300 °C; ^{1}H NMR (DMSO-d_6): 2.28 (s, 3H), 2.36 (br s, 8H), 3.17 (t, *J* = 5.5 Hz, 2H), 4.12 (t, *J* = 5.5 Hz, 2H), 6.18 (s, 1H), 6.52 (s, 1H), 6.66 (s, 1H), 7.60 (t, *J* = 7.5 Hz, 3H), 7.84 (d, *J* = 4.5 Hz, 2H), 12.83 (s, 1H). ESI-MS $C_{22}H_{24}N_2O_4$ $[M+H]^+$ 381.1. Anal. Calcd for $C_{22}H_{24}N_2O_4$: C, 69.46; H, 6.36; N, 7.36. Found: C, 69.69; H, 6.32; N, 7.48.

4.2.6. 7-(2-(Dibutylamino)ethoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (2f)

Mp 290–291 °C; ^{1}H NMR (DMSO-d_6): 0.92 (t, *J* = 7.1 Hz, 6H), 1.27–1.31 (m, 4H), 1.37–1.39 (m, 4H), 2.94 (m, 4H), 3.11 (br s,

2H), 4.18 (t, *J* = 5.5 Hz, 2H), 6.42 (d, *J* = 2.0 Hz, 1H), 6.62 (d, *J* = 2.0 Hz, 1H), 6.70 (s, 1H), 7.48 (t, *J* = 7.5 Hz, 3H), 7.82 (d, *J* = 6.0 Hz, 2H), 12.85 (s, 1H). ESI-MS $C_{25}H_{31}NO_4$ $[M+H]^+$ 410.5. Anal. Calcd for $C_{25}H_{31}NO_4$: C, 73.32; H, 7.63; N, 3.42. Found: C, 72.29; H, 7.66; N, 3.39.

4.2.7. 5-Hydroxy-2-phenyl-7-(2-(pyrrolidin-1-yl)ethoxy)-4H-chromen-4-one (2g)

Mp 280–281 °C; ^1H NMR (DMSO-d_6): 1.62 (brs, 4H), 2.61 (br s, 2H), 2.76 (m, 4H), 4.13 (t, *J* = 5.6 Hz, 2H), 6.39 (s, 1H), 6.67 (s, 1H), 6.74 (s, 2H), 7.52 (t, *J* = 7.5 Hz, 2H), 7.86 (d, *J* = 6.0 Hz, 2H), 12.87 (s, 1H). ESI-MS $C_{21}H_{21}NO_4$ $[M+H]^+$ 352.1. Anal. Calcd for $C_{21}H_{21}NO_4$: C, 71.78; H, 6.02; N, 3.99. Found: C, 71.63; H, 6.26; N, 3.90.

4.2.8. 5-Hydroxy-2-phenyl-7-(2-(piperidin-1-yl)ethoxy)-4H-chromen-4-one (2i)

Mp 283–285 °C; ^1H NMR (DMSO-d_6): 1.46–1.48 (m, 2H), 1.54–1.62 (m, 4H), 2.54 (br s, 4H), 2.67 (t, *J* = 5.6 Hz, 2H), 4.19 (t, *J* = 5.6 Hz, 2H), 6.42 (s, 1H), 6.62 (s, 1H), 6.70 (w, 1H), 7.48 (t, *J* = 7.5 Hz, 3H), 7.82 (d, *J* = 6.0 Hz, 2H), 12.70 (s, 1H). ESI-MS $C_{22}H_{23}NO_4$ $[M+H]^+$ 366.1. Anal. Calcd for $C_{21}H_{21}NO_4$: C, 72.31; H, 6.34; N, 3.83. Found: C, 72.53; H, 6.22; N, 3.71.

4.2.9. 5-Hydroxy-2-phenyl-7-(3-(piperazin-1-yl)propoxy)-4H-chromen-4-one (3a)

Mp 183–184 °C; ^1H NMR (DMSO-d_6): 1.82–1.84 (m, 2H), 2.10 (s, 1H), 2.30 (br s, 4H), 2.39 (t, *J* = 7.0 Hz, 2H), 2.72 (br s, 4H), 4.18 (t, *J* = 6.2 Hz, 2H), 6.38 (s, 1H), 6.46 (s, 1H), 6.64 (s, 1H), 7.50 (t, *J* = 7.5 Hz, 3H), 7.86 (d, *J* = 6.0 Hz, 2H), 12.60 (s, 1H). ESI-MS $C_{22}H_{24}N_2O_4$ $[M+H]^+$ 381.1. Anal. Calcd for $C_{22}H_{24}N_2O_4$: C, 69.46; H, 6.36; N, 7.36. Found: C, 69.61; H, 6.52; N, 7.33.

4.2.10. 7-(3-(Diethylamino)propoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (3b)

Mp 196–197 °C; ^1H NMR (DMSO-d_6): 1.01 (t, *J* = 7.1 Hz, 6H), 1.90–2.10 (m, 2H), 2.40–2.60 (m, 6H), 4.18 (t, *J* = 6.0 Hz), 6.39 (s, 1H), 6.46 (s, 1H), 6.64 (s, 1H), 7.46 (t, *J* = 7.5 Hz, 3H), 7.84 (d, *J* = 6.0 Hz, 2H), 12.70 (s, 1H). ESI-MS $C_{22}H_{25}NO_4$ $[M+H]^+$ 367.1. Anal. Calcd for $C_{22}H_{25}NO_4$: C, 71.91; H, 6.86; N, 3.81. Found: C, 71.95; H, 6.63; N, 3.68.

4.2.11. 8-Hydroxy-3-phenyl-6-(3-(propylamino)propoxy)naphthalen-1(4H)-one (3c)

Mp 214–216 °C; ^1H NMR (DMSO-d_6): 0.88 (s, 3H), 1.42 (t, *J* = 7.1 Hz, 2H), 2.36 (t, *J* = 7.1 Hz, 2H), 2.56–2.65 (m, 4H), 4.15 (t, *J* = 5.6 Hz, 2H), 6.39 (s, 1H), 6.54 (s, 1H), 6.94 (s, 1H), 7.52 (t, *J* = 6.5 Hz, 3H), 7.88 (d, *J* = 6.0 Hz, 2H), 12.44 (s, 1H). ESI-MS $C_{22}H_{25}NO_3$ $[M+H]^+$ 352.1. Anal. Calcd for $C_{22}H_{25}NO_3$: C, 75.19; H, 7.17; N, 3.99. Found: C, 75.22; H, 7.15; N, 4.02.

4.2.12. 7-(3-(Dipropylamino)propoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (3d)

Mp 153–155 °C; ^1H NMR (DMSO-d_6): 0.92 (t, *J* = 7.3 Hz, 6H), 1.55–1.57 (m, 4H), 1.90–2.10 (m, 2H), 2.40–2.60 (m, 2H), 3.06 (br s, 4H), 4.18 (t, *J* = 5.7 Hz, 2H), 6.42 (s, 1H), 6.62 (s, 1H), 6.70 (s, 1H), 7.56 (t, *J* = 7.5 Hz, 3H), 7.86 (d, *J* = 6.0 Hz, 2H), 12.70 (s, 1H). ESI-MS $C_{24}H_{29}NO_4$ $[M+H]^+$ 392.2. Anal. Calcd for $C_{24}H_{29}NO_4$: C, 72.89; H, 7.39; N, 3.54. Found: C, 72.64; H, 7.18; N, 3.37.

4.2.13. 5-Hydroxy-7-(3-(4-methylpiperazin-1-yl)propoxy)-2-phenyl-4H-chromen-4-one (3e)

Mp 135–137 °C; ^1H NMR (DMSO-d_6): 1.90–2.10 (m, 2H), 2.30 (s, 3H), 2.40–2.60 (m, 10H), 4.12 (br s, 2H), 6.18 (s, 1H), 6.52 (s, 1H), 6.64 (s, 1H), 7.58 (t, *J* = 7.5 Hz, 3H), 7.86 (d, *J* = 6.0 Hz, 2H), 12.70 (s, 1H). ESI-MS $C_{23}H_{26}N_2O_4$ $[M+H]^+$ 395.1. Anal. Calcd for $C_{23}H_{26}N_2O_4$: C, 70.03; H, 6.64; N, 7.10. Found: C, 70.25; H, 6.43; N, 7.31.

4.2.14. 7-(3-(Dibutylamino)propoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (3f)

Mp 188–189 °C; ^1H NMR (DMSO-d_6): 0.90 (t, *J* = 7.1 Hz, 6H), 1.30–1.34 (m, 4H), 1.52–1.57 (m, 4H), 2.08–2.09 (m, 2H), 2.87 (t, *J* = 7.8 Hz, 2H), 3.11 (br s, 2H), 4.20 (t, *J* = 5.6 Hz, 2H), 6.42 (s, 1H), 6.66 (s, 1H), 6.78 (s, 1H), 7.52 (t, *J* = 7.5 Hz, 3H), 7.88 (d, *J* = 6.0 Hz, 2H), 12.70 (s, 1H). ESI-MS $C_{26}H_{33}NO_4$ $[M+H]^+$ 423.2. Anal. Calcd for $C_{26}H_{33}NO_4$: C, 73.73; H, 7.85; N, 3.31. Found: C, 73.82; H, 7.60; N, 3.18.

4.2.15. 5-Hydroxy-2-phenyl-7-(3-(pyrrolidin-1-yl)propoxy)-4H-chromen-4-one (3g)

Mp 89–91 °C; ^1H NMR (DMSO-d_6): 1.68 (br s, 4H), 1.86–1.91 (m, 2H), 2.43 (br s, 4H), 2.52 (t, *J* = 7.2 Hz, 2H), 4.13 (t, *J* = 6.25 Hz, 2H), 6.38 (s, 1H), 6.48 (s, 1H), 6.64 (s, 1H), 7.52 (t, *J* = 7.5 Hz, 3H), 8.40 (d, *J* = 6.0 Hz, 2H), 12.60 (s, 1H). ESI-MS $C_{22}H_{23}NO_4$ $[M+H]^+$ 366.1. Anal. Calcd for $C_{22}H_{23}NO_4$: C, 72.31; H, 6.34; N, 3.83. Found: C, 72.52; H, 6.21; N, 3.57.

4.2.16. 8-Hydroxy-6-(3-(2-hydroxyethylamino)propoxy)-3-phenylnaphthalen-1(4H)-one (3i)

Mp 114–116 °C; ^1H NMR (DMSO-d_6): 1.42 (t, *J* = 7.1 Hz, 2H), 2.36 (t, *J* = 7.1 Hz, 2H), 2.56–2.65 (m, 4H), 3.61 (s, 1H), 4.15 (t, *J* = 5.6 Hz, 2H), 6.39 (s, 1H), 6.54 (s, 1H), 6.94 (s, 1H), 7.52 (t, *J* = 6.5 Hz, 3H), 7.88 (d, *J* = 6.0 Hz, 2H), 12.44 (s, 1H). ESI-MS $C_{21}H_{23}NO_4$ $[M+H]^+$ 354.1. Anal. Calcd for $C_{21}H_{23}NO_4$: C, 71.37; H, 6.56; N, 3.96. Found: C, 71.34; H, 6.51; N, 3.98.

4.3. Antibacterial activity

The antibacterial activity of the synthesized compounds was tested against *E. coli*, *P. fluorescence*, *B. subtilis*, and *S. aureus* using MH medium (Mueller-Hinton medium: casein hydrolysate 17.5 g, soluble starch 1.5 g, beef extract 1000 mL), The MICs (minimum inhibitory concentrations) of the test compounds were determined by a colorimetric method using the dye MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl trtrazolium bromide). A stock solution of the synthesized compound (100 μg/mL) in DMSO was prepared and graded quantities of the test compounds were incorporated in specified quantity of sterilized liquid MH medium. A specified quantity of the medium containing the compound was poured into microtitration plates. Suspension of the microorganism was prepared to contain approximately 10^5 cfu/mL and applied to microtitration plates with serially diluted compounds in DMSO to be tested and incubated at 37 °C for 24 h. After the MICs were visually determined on each of the microtitration plates, 50 μL of PBS (phosphate buffered saline 0.01 mol/L, pH 7.4: $Na_2HPO_4 \cdot 12H_2O$ 2.9 g, KH_2PO_4 0.2 g, NaCl 8.0 g, KCl 0.2 g, distilled water 1000 mL) containing 2 mg of MTT/mL was added to each well. Incubation was continued at room temperature for 4–5 h. The content of each well was removed, and 100 μL of isopropanol containing 5% 1 mol/L HCl was added to extract the dye. After 12 h of incubation at room temperature, the optical density (OD) was measured with a microplate reader at 550 nm.

4.4. *E. coli* FabH purification and activity assay

Native *E. coli* FabH protein was overexpressed in *E. coli* DH10B cells using the pET30 vector and purified to homogeneity in three chromatographic steps (Q-Sepharose, MonoQ, and hydroxyapatite) at 4 °C. The selenomethionine-substituted protein was expressed in *E. coli* BL21(DE3) cells and purified in a similar way. Harvested cells containing FabH were lysed by sonication in 20 mM Tris, pH

7.6, 5 mM imidazole, 0.5 M NaCl and centrifuged at 20,000 rpm for 30 min. The supernatant was applied to a Ni-NTA agarose column, washed, and eluted using a 5–500 mM imidazole gradient over 20 column volumes. Eluted protein was dialyzed against 20 mM Tris, pH 7.6, 1 mM DTT, and 100 mM NaCl. Purified FabD and FabHs were concentrated up to 2 mg/mL and stored at −80 °C in 20 mM Tris, pH 7.6, 100 mM NaCl, 1 mM DTT, and 20% glycerol for enzymatic assay.

In a final 20 μL reaction, 20 mM Na_2HPO_4, pH 7.0, 0.5 mM DTT, 0.25 mM $MgCl_2$, and 2.5 μM holo-ACP were mixed with 1 nM FabH, and H_2O was added to 15 μL. After 1 min incubation, a 2 μL mixture of 25 μM acetyl-CoA, 0.5 mM NADH, and 0.5 mM NADPH was added for FabH reaction for 25 min. The reaction was stopped by adding 20 μL of ice-cold 50% TCA, incubating for 5 min on ice, and centrifuging to pellet the protein. The pellet was washed with 10% ice-cold TCA and resuspended with 5 μL of 0.5 M NaOH. The incorporation of the 3H signal in the final product was read by liquid scintillation. When determining the inhibition constant (IC_{50}), inhibitors were added from a concentrated DMSO stock such that the final concentration of DMSO did not exceed 2%.

4.5. Docking simulations

Molecular docking of compound **3g** into the three-dimensional X-ray structure of *E. coli* FabH (PDB code: 1HNJ) was carried out using the AUTODOCK software package (version 4.0) as implemented through the graphical user interface AutoDockTools (ADT 1.4.6).[29]

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References and notes

1. Leeb, M. *Nature* **2004**, *431*, 892.
2. Lee, J.-Y.; Jeong, K.-W.; Lee, J.-U.; Kang, D.-I.; Kim, Y. *Bioorg. Med. Chem.* **2009**, *17*, 1506.
3. Lai, C. Y.; Cronan, J. E. *J. Biol. Chem.* **2003**, *19*, 51494.
4. Lu, Y. J.; Zhang, Y. M.; Rock, C. O. *Biochem. Cell Biol.* **2004**, *82*, 145.
5. Jayakumar, A.; Tai, M.-H.; Huang, W.-Y.; Al-Feel, W.; Hsu, M.; Abu-Elheiga, L.; Chirala, S.; Wakil, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 8695.
6. White, S. W.; Zheng, J.; Zhang, Y. M.; Rock, C. O. *Annu. Rev. Biochem.* **2005**, *74*, 791.
7. Veyron-Churlet, R.; Guerrini, O.; Mourey, L.; Daffé, M.; Zerbib, D. *Mol. Microbiol.* **2004**, *54*, 1161.
8. Khandekar, S. S.; Daines, R. A.; Lonsdale, J. T. *Curr. Protein Pept. Sci.* **2003**, *4*, 21.
9. Heath, R. J.; Rock, C. O. *Nat. Prod. Rep.* **2002**, *19*, 581.
10. Tsay, J. T.; Oh, W.; Larson, T. J.; Jakowski, S.; Rock, C. O. *J. Biol. Chem.* **1992**, *267*, 6807.
11. Clough, R. C.; Matthis, A. L.; Barnum, S. R.; Jaworski, J. G. *J. Biol. Chem.* **1992**, *267*, 20992.
12. Heath, R. J.; Rock, C. O. *J. Biol. Chem.* **1996**, *271*, 1833.
13. Christensen, C. E.; Kragelund, B. B.; Wettstein-Knowles, P.; Henriksen, A. *Protein Sci.* **2007**, *16*, 261.
14. Bylka, W.; Matlawska, I.; Pilewski, N. A. *JANA* **2004**, *7*, 24.
15. Huycke, M. M.; Sahm, D. F.; Gilmore, M. S. *Emerg. Infect. Dis.* **1998**, *4*, 239.
16. Puupponen-Pimi, R.; Nohynek, L.; Meier, C.; Kähkönen, M.; Heinonen, A. H. *J. Appl. Microbiol.* **2001**, *90*, 494.
17. Qiu, X.; Janson, C. A.; Konstantinidis, JA. K.; Nwagwu, S.; Silverman, C.; Smith, W. W.; Khandekar, S.; Lonsdale, J.; Abdel-Meguid, S. S. *J. Biol. Chem.* **1999**, *274*, 36465.
18. Vladimir, B.; Nikolai, P. *Exp. Rev. Clin. Immunol.* **2005**, *1*, 145.
19. Babu, K. S.; Babu, T. H.; Srinivas, P. V.; Kishore, K. H.; Murthy, U. S. N.; Rao, J. M. *Bioorg. Med. Chem.* **2006**, *16*, 221.
20. Chan, E. C. H.; Patchareewan, P. L.; Owen, W. *J. Cardiovasc. Pharmacol.* **2000**, *35*, 326.
21. Lee, J. H.; Kim, Y. S.; Lee, C. K.; Lee, H. K.; Han, S. S. *Saengyak Hakhoechi* **1999**, *30*, 34.
22. Shin, J. S.; Kim, K. S.; Kim, M. B.; Jeong, J. H.; Kim, B. K. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 869.
23. Cardenas, M.; Marder, M.; Blank, V. C.; Roguin, L. P. *Bioorg. Med. Chem.* **2006**, *14*, 2966.
24. Jeong, K.-W.; Lee, J.-Y.; Kang, D.-I.; Lee, J.-U.; Shin, S. Y.; Kim, Y. *J. Nat. Prod.* **2009**, *72*, 719.
25. Reinholz, E.; Becker, A.; Hagenbruxh, B.; Schafer, S.; Schmitt, A. *Synthesis* **1990**, *11*, 1069.
26. Chern, J. H.; Shia, K. S.; Hsu, T. A.; Tai, C. L.; Lee, C. C.; Lee, Y. C.; Chang, C. S.; Tseng, S. N.; Shih, S. R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2519.
27. Liu, Z. Z.; Chen, H. C.; Cao, S. L.; Li, R. T. *Synth. Commun.* **1993**, *23*, 2611.
28. Qiu, X.; Janson, C. A.; Smith, W. W.; Head, M.; Lonsdale, J.; Konstantinidis, A. *J. Mol. Biol.* **2001**, *307*, 341.
29. Huey, R.; Morris, G. M.; Olson, A. J.; Goodsell, D. S. *J. Comput. Chem.* **2007**, *28*, 1145.