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Design and synthesis of novel deoxybenzoin derivatives as FabH inhibitors and anti-inflammatory agents

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ABSTRACT

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 new FabH inhibitors. The synthesis and biological evaluation halide-deoxybenzoins derivatives are described in this Letter. Potent FabH inhibitory and selective anti-Gram-negative bacteria activities were observed in deoxybenzoin derivatives. Furthermore, compound **19** was able to reduce the ECE-induced IL-8 production in gastric mucosal cells significantly. Based on the biological data and molecular docking, compound **19** is a potential FabH inhibitor and anti-inflammatory agent deserving further research.

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 is a very important task.[1] Recent 10 years, different targets in key areas of the bacterial cell cycle have been studied that would be a new weapon against the problem of acquired resistance. One of the most attractive biochemical pathways to be used as the target for new antibacterial agents is the fatty acid biosynthesis (FAS). This pathway has been demonstrated to be essential for bacteria cell survival, and differs considerably from human FAS pathway.[2,3] While in humans fatty acid synthesis occurs in a homodimeric multifunctional enzyme,[4] in bacteria the pathway is composed of various discrete enzymes and each one can be considered a putative molecular target. Those features make the type II FAS pathway a potential target for new antimicrobial agents.

A key enzyme in this pathway is the β-ketoacyl-acyl carrier protein synthase III (FabH), which is the enzyme responsible for the first reaction in the pathway and plays an important regulatory role. FabH has also been demonstrated to be essential for organismal survival and is present in a large number of important human pathogens. Furthermore, some chemical compounds had shown to inhibit FabH from diverse microorganisms, including multi-drug resistant strains.[5,6] These facts support the idea that FabH can be used as an effective molecular target for the development of new antimicrobial agents. In our previous reports, isoflavone derivatives displayed potent antimicrobial, *Escherichia coli* FabH inhibitory[7] and anti-inflammatory activities.[8] Deoxybenzoins, intermediates in the synthesis of isoflavone, and their structural similarities with isoflavone led us to study the biological activity of the latter. The hypothesis is supported by the fact that deoxybenzoins exhibit a broad variety of biological activities including antibacterial[9] and *Helicobacter pylori* urease inhibitory activity[10] in our previous study. In this Letter, a novel series of haloid deoxybenzoin derivatives were designed for their *E. coli* FabH inhibitory activity. Docking simulations were performed using the X-ray crystallographic structure of the FabH of *E. coli* complexed with an inhibitor to explore the binding modes of these compounds at the active site. Moreover, the ECE (*E. coli* water extract) IL-8 expression inhibitory activity evaluation was also determined.

A series of new deoxybenzoin *Schiff* bases **7–36** were synthesized by the route outlined in Scheme 1. The synthesis started from phenols and phenylacetic acids. The dihydric phenols, resorcinols, and substituted resorcinols reacted with variously substituted phenylacetic acids,[11] giving excellent yields of deoxybenzoins catalyzed by $BF_3{\cdot}OEt_2$. Then compounds **7–36** were obtained in high yield using appropriately substituted anilines as the amine, and $TiCl_4$ as the carbonyl activator.[12,13] All new compounds (Table 1) were fully characterized by spectroscopic methods and elemental analysis.

All of the deoxybenzoins **1–6** and new deoxybenzoin Schiff bases (**7–36**) were evaluated for their antimicrobial activities against four bacteria strains (*Bacillus subtilis* ATCC 6633, *E. coli* ATCC35218, *Pseudomonas aeruginosa* ATCC 2785, and *Staphylococcus aureus* ATCC 6538), three fungi (*Aspergillus niger* ATCC 16404, *Candida albicans* ATCC 10231, and *Trichophyton rubrum* ATCC 10218). The Schiff bases showed potent selective inhibitory activities against Gram-negative

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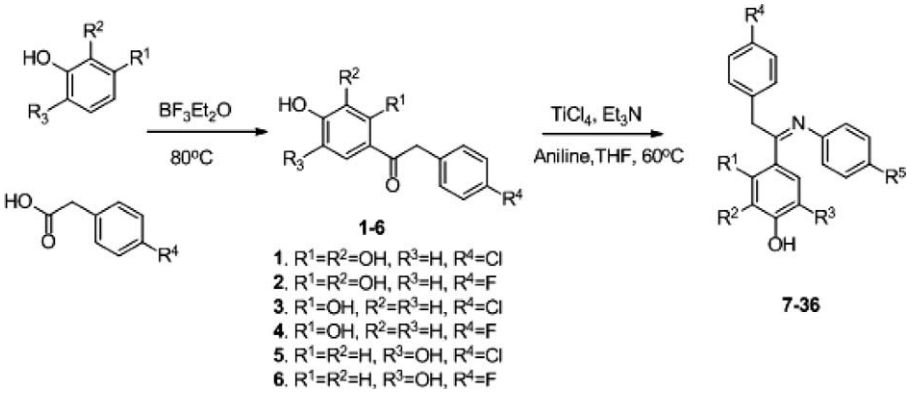

Scheme 1. The synthetic routes of compounds **1**–**36**.

Table 1
Chemical structures of Schiff bases **7**–**36**

Compd	R^1	R^2	R^3	R^4	R^5	Compd	R^1	R^2	R^3	R^4	R^5
7	OH	OH	H	Cl	Me	**22**	OH	H	H	F	Me
8	OH	OH	H	Cl	Br	**23**	OH	H	H	F	Br
9	OH	OH	H	Cl	Cl	**24**	OH	H	H	F	Cl
10	OH	OH	H	Cl	NO_2	**25**	OH	H	H	F	NO_2
11	OH	OH	H	Cl	OH	**26**	OH	H	H	F	OH
12	OH	OH	H	F	Me	**27**	H	H	OH	Cl	Me
13	OH	OH	H	F	Br	**28**	H	H	OH	Cl	Br
14	OH	OH	H	F	Cl	**29**	H	H	OH	Cl	Cl
15	OH	OH	H	F	NO_2	**30**	H	H	OH	Cl	NO_2
16	OH	OH	H	F	OH	**31**	H	H	OH	Cl	OH
17	OH	H	H	Cl	Me	**32**	H	H	OH	F	Me
18	OH	H	H	Cl	Br	**33**	H	H	OH	F	Br
19	OH	H	H	Cl	Cl	**34**	H	H	OH	F	Cl
20	OH	H	H	Cl	NO_2	**35**	H	H	OH	F	NO_2
21	OH	H	H	Cl	OH	**36**	H	H	OH	F	OH

E. coli and *P. aeruginosa*, but none of the compounds showed significant inhibitory activity against fungi, supported by the fact that their MICs were all high than 50 μg/mL (data not shown). Deoxybenzoins **1**–**6** showed no activity (MIC >50) while deoxybenzoin Schiff bases **7–36** displayed well antimicrobial activities. As shown in Table 2, the MIC values of the compounds differed greatly, ranging from 0.78 to 50 μg/mL, and compounds **18**, **19**, **23**, and **24** exhibited excellent activities against Gram-negative *E. coli* and *P. aeruginosa*. Especially, compounds **19** (MIC = 0.78 and 1.562 μg/mL) and **24** (MIC = 1.562 and 1.562 μg/mL) showed comparable activities to the positive control kanamycin. Compounds **17**–**26** with the resorcinol skeleton displayed higher activity than other compounds (catechol skeleton). Thus, the two meta hydroxyl groups on aromatic ring of deoxybenzoin may be responsible for inhibitory activity.

The *E. coli* FabH inhibitory potency of the selected compounds **16**–**25** was examined and the results were summarized in Table 3. As shown in Table 3, among the tested compounds, compounds **19** and **24** showed potent inhibitory activity with IC_{50} = 1.8 and 3.9 μM, respectively. Other tested compounds displayed moderate inhibitory activity with IC50 ranging from 8.3 to 63.4 μM. It also can be seen from Table 3 that the selected compounds displayed low hemolytic activity. These biological assays indicate that compounds **19** and **24** are potent inhibitors of *E. coli* FabH as antibacterial agents. In addition, molecular docking of compound **19** and *E. coli* FabH was performed on the binding model based on the *E. coli* FabH–CoA complex structure (1HNJ.pdb).[14] 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. (Fig. 1) 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

Table 2
Antimicrobial activity of the synthesized compounds

Compd	MIC (μg/mL)				Compd	MIC (μg/mL)			
	E[a]	*P*	*B*	*S*		*E*	*P*	*B*	*S*
7	50	50	25	25	**22**	25	25	25	25
8	12.5	25	50	50	**23**	3.125	6.25	50	50
9	12.5	25	50	50	**24**	1.562	1.562	50	50
10	50	50	50	50	**25**	6.25	12.5	50	50
11	25	50	50	25	**26**	12.5	25	50	25
12	50	50	50	50	**27**	25	50	50	50
13	25	25	25	25	**28**	12.5	12.5	25	25
14	12.5	12.5	50	50	**29**	6.25	6.25	50	50
15	25	25	50	25	**30**	25	25	25	12.5
16	25	50	50	50	**31**	25	50	50	50
17	12.5	12.5	25	12.5	**32**	25	50	25	12.5
18	3.125	3.125	12.5	25	**33**	12.5	25	25	50
19	0.78	1.562	25	25	**34**	6.25	6.25	25	25
20	6.25	6.25	25	12.5	**35**	25	25	50	50
21	6.25	12.5	25	>50	**36**	50	50	25	50
Kanamysin B	3.125	3.125	0.39	1.56	Penicillin G	6.25	6.25	1.562	1.562

[a] *B*, *Bacillus subtilis* ATCC 6633; *E*, *Escherichia coli* ATCC 35218; *P*, *Pseudomonas fluorescens* ATCC 13525; *S*, *Staphylococcus aureus* ATCC6538.

Table 3
E. coli FabH inhibitory activity of the selected compounds **16–25**

Compound	*E. coli* FabH IC_{50} (μM)	Hemolysis LC_{30}[a] (mg/mL)
16	63.4 ± 7.2	>10
17	15.1 ± 2.3	>10
18	9.1 ± 1.8	>10
19	1.8 ± 0.4	>10
20	8.7 ± 1.6	>10
21	20.5 ± 2.4	>10
22	35.3 ± 4.7	>10
23	8.3 ± 1.5	>10
24	3.9 ± 0.8	>10
25	28.7	>10

[a] Lytic concentration 30%.

out to identify a plausible malonyl-binding mode.[14] Enlightened by these facts, compound **19** with the most potent inhibitory activity was hit by pharmacophore map I mentioned above. The binding model of compound **19** and *E. coli* FabH is depicted in Figure 2. In the binding model, amino hydrogen of Cys112 and Gly306 forms hydrogen bonds with 3-hydroxy of compound **19**. The 3′-Cl aniline ring in compound **19** projects into a hydrophobic interaction region, which is comprised of the side chains of His244, Asn247, Asn274, and Phe157. The hydrophobic interaction between **19** and FabH was important for the potent inhibitory activity of **19**.

In general, gram-negative bacterial infections rapidly induce an inflammatory response in which the cytokine network plays a major role. Lipopolysaccharides (LPS) released from these bacteria may be a major immunogen contributing to the cytokine burst by the LPS ± LPS binding protein (BP) ± CD14 complex formation. IL-8 is a cytokine implicated in some cancers and a wide range of chronic inflammatory conditions, including rheumatoid arthritis heart and gastritis.[15,16] In addition, IL-8 has been shown previously to be derived after stimulation with *E. coli* in transformed epithelial cell-lines.[17] Here, Gram-negative bacteria *E. coli* was used to stimulate human gastric epithelial cancer cell line AGS to stimulate IL-8 expression, and the affect of compound **19** on IL-8 levels were assessed by ELISA assay. Fortunately, we found that compound **19** exhibited a strong attenuation of IL-8 production induced by *E. coli* water extract in AGS cells. Inactive compound **12** was also been evaluated as the negative control.

As shown in Figure 3, compound **19** showed well inhibitory activity against IL-8 levels which stimulated by *E. coli*. ECE (*E. coli* water extract) alone stimulated gastric mucosal cells to produce IL-8 as much as 1143.5 pg mL^{-1}. However, different dose-dependent attenuations of ECE-induced IL-8 production were seen with the addition of compound **19** and the reference compound genistein. Different dose-dependent attenuations of *E. coli*-induced IL-8 production were observed with the addition of aspirin and compound **19**. Compound **19** significantly reduced the IL-8 level in a dose-dependent way at concentrations of 15, 30, and 60 μmol L^{-1}, and the lowest IL-8 production was observed when the concentration of compound **19** was 60 μmol L^{-1}. Interestingly, compound **19** showed a more potent inhibitory activity against ECE-induced IL-8 production than genistein at the concentration of 30 μmol L^{-1} (P <0.05) and 60 μmol L^{-1} (P <0.01). However, the negative control compound **12** at the same concentration could only reduce the IL-8 level slightly. In addition, cell viability was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) assay to determine whether compound **19** and aspirin affect cell viability at the concentrations tested (15, 30, and 60 μmol L^{-1}). AGS cells were incubated with aspirin or compound **19** at serial concentrations of 15, 30, and 60 μmol L^{-1}. Then MTT assay was performed 48 h later. The result shown in Figure 4 demonstrated that compound **19** and aspirin did not affect cell viability at the concentrations tested (15, 30, and 60 μmol L^{-1}). Based on the data obtained in this study, it can be concluded that compound **19** would be a potential and promising anti-inflammatory agent.

In summary, a series of haloid deoxybenzoin derivatives were synthesized for the first time and evaluated for *E. coli* FabH inhib-

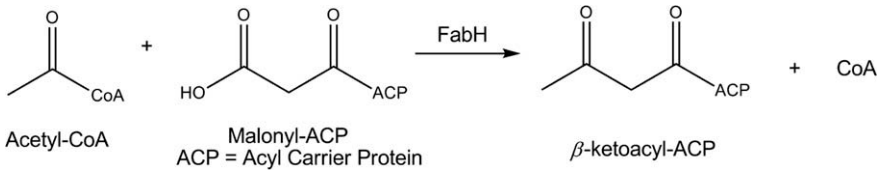

Figure 1. FabH-catalyzed initiation reaction of fatty acid biosynthesis.

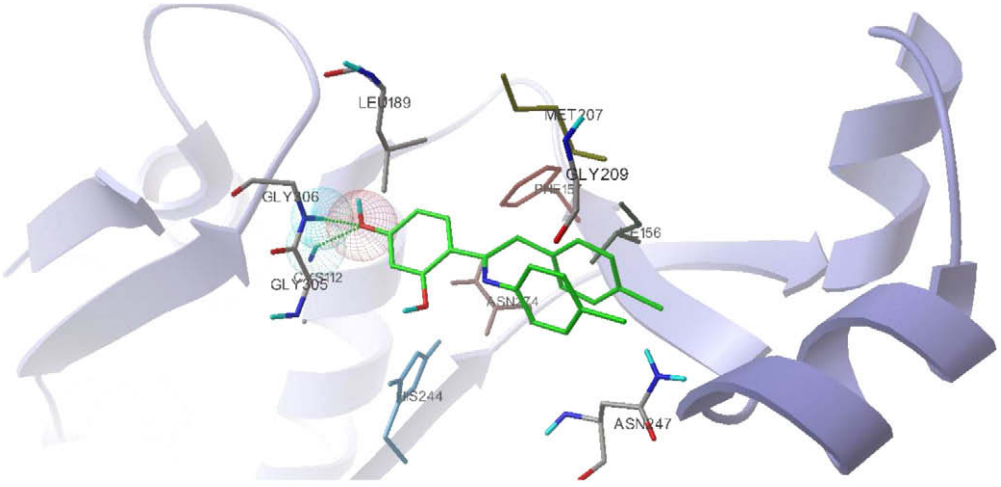

Figure 2. Binding model of compound **19** and *E. coli* FabH.

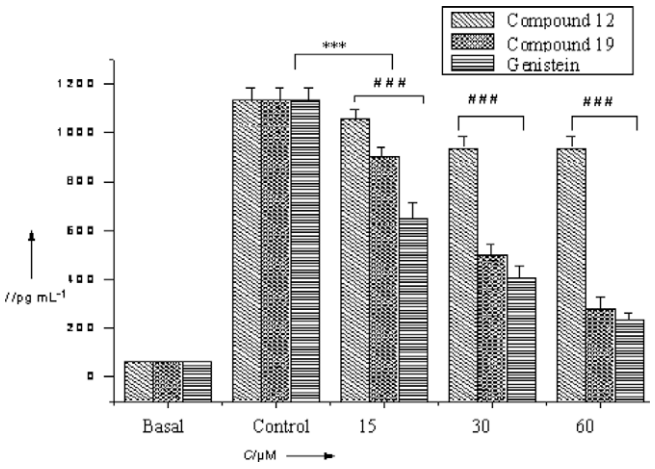

Figure 3. Inhibitory activities of compound **19** on IL-8 production induced by *E. coli* water extract in gastric mucosal cells. Results are means ± SEM of 3–5 experiments. Comparison 15, 30, and 60 μmol L^{-1} of all agents versus control: $^{*}P$ <0.05, $^{**}P$ <0.01, and $^{***}P$ <0.001; comparison compound **19** versus aspirin: $^{\#}P$ <0.05, $^{\#\#}P$ <0.01, and $^{\#\#\#}P$ <0.001.

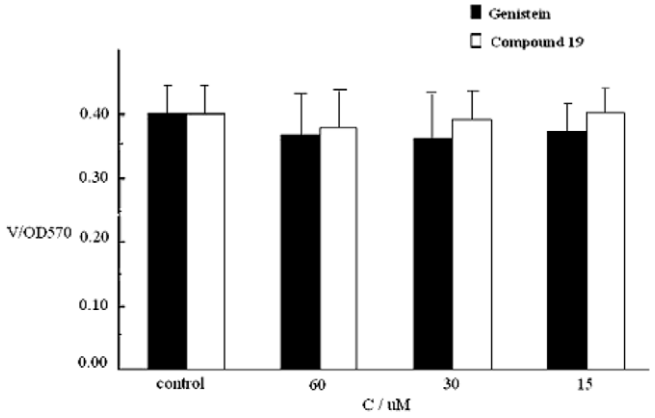

Figure 4. Effects of different agents on cell viability. V for cell viability and C for concentrations of the agents. Results are means ± SEM of 4–6 experiments.

itory activity as antibacterial agents. Among the compounds studied, potent and selective anti-Gram-negative bacteria activities were observed in Schiff bases from deoxybenzoins. Compounds **18**, **19**, **23**, and **24** exhibited excellent activities against Gram-negative *E. coli* and *P. aeruginosa*. Significantly, compound **19** showed the most potent *E. coli* FabH inhibitory activity with IC_{50} of 1.87 μM. Docking simulation was performed to position compound **19** into the *E. coli* FabH active site to determine the probable binding conformation. Furthermore, compound **19** was able to reduce the ECE-induced IL-8 production in gastric mucosal cells significantly. Based on the data obtained from this study, we conclude that compound **19** is a potential FabH inhibitor and anti-inflammatory agent worth of further study.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.01.032.23

References and notes

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12. *Selected data for compound* **3**: mp 132–133 °C. ^{1}H NMR (MeOD, 500 MHz): δ 8.17 (2H, d, J = 8.0 Hz, H-3, H-5), 7.85 (1H, d, J = 8 Hz, H-6), 7.51 (2H, d, J = 8.0 Hz, H-2, H-6), 6.38 (1H, dd, J = 8.0, 1.5 Hz, H-5), 6.27 (1H, d, J = 1.5 Hz, H-3), 4.42 (2H, s, CH_2). MS (ESI) $C_{14}H_{11}ClO_3$ $[M+H]^+$ 262.1. Anal. Calcd for $C_{14}H_{11}ClO_3$: C, 64.01; H, 4.22; Cl, 13.50. Found: C, 64.12; H, 4.26; Cl, 13.43.
13. *Selected data for compound* **30**: mp 154–156 °C. ^{1}H NMR (DMSO-d_6 500 MHz): δ 4.245 (s, 2H, CH_2), 6.57–6.62 (m, 4H, H-2′, H-3′, H-4′,H-5′), 7.344–7.393 (m, 3H, H-2, H-5, H-6), 7.919–7.970 (m, 4H, H-2″, H-3″, H-5″, H-6″), 9.356 (s, H, 2-OH), 9.889 (s, H, 3-OH). Anal. Calcd for $C_{20}H_{15}ClN_2O_4$: C, 62.75; H, 3.95; Cl, 9.26; N, 7.32. Found: C, 62.67; H, 3.89; Cl, 9.32; N, 7.41.
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