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Design, synthesis and biological evaluation of novel thiazole derivatives as potent FabH inhibitors

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ABSTRACT

Fatty acid biosynthesis is essential for bacterial survival. Components of this biosynthetic pathway have been identified as attractive targets for the development of new antibacterial agents. FabH, β-ketoacyl-acyl carrier protein (ACP) synthase III, is a particularly attractive target, since it is central to the initiation of fatty acid biosynthesis and is highly conserved among Gram positive and negative bacteria. Three series of Schiff bases containing thiazole template were synthesized and developed as potent inhibitors of FabH. This inhibitor class demonstrates strong antibacterial activity. *Escherichia coli* FabH inhibitory assay and docking simulation indicated that the compounds **11** and **18** were potent inhibitors of *E. coli* FabH.

The emergence of bacterial resistance to most of all antibiotics poses a threat to health care, and novel therapeutics are needed. Recently, the research has been focused toward development of new antibacterial agents with novel target. 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.[1] β-Ketoacyl-acyl carrier protein (ACP) synthase III, also known as FabH or KAS III, plays an essential and regulatory role in bacterial FAB.[2,3] The enzyme initiates the fatty acid elongation cycles[4,5] and is involved in the feedback regulation of the biosynthetic pathway via product inhibition.[6] Therefore, it represents a promising target for the design of novel antimicrobial drugs. Because of this, various kinds of compounds were screened by enzymatic assays to generate leads that were co-crystallized with various pathogenic FabH proteins and subsequently optimized using structure guided drug design methods.[7–11] For example, Kim and co-workers reported the YKAs3003, a Schiff base condensed by 4-hydroxy salicylaldehyde and cyclohexanamine as a potent inhibitor of *Escherichia coli* (*E. coli*) FabH with antimicrobial activity.[12] Further optimization of this compound is required to improve its antimicrobial activity.

Thiazoles and their derivatives have attracted continuing interest over the years because of their varied biological activities,[13,14] recently found application in drug development for the treatment of allergies,[15] hypertension,[16] inflammation,[17] schizophrenia,[18] bacterial,[19] HIV infections,[20] hypnotics,[21] and more recently for the treatment of pain.[22] Besides, Kitagawa et al. find that thiazole derivatives show strong FabI and FabK inhibitory activities with potent antibacterial activity.[23]

In view of the above mentioned findings, we report in the present work the synthesis of three series of Schiff bases containing thiazole template. This combination was suggested in an attempt to investigate the inhibitory activity against *E. coli* FabH. Biological evaluation indicated that compounds **11** and **18** displayed not only significant antibacterial activity against *E. coli* ATCC 35218, but also favorable activity against other five bacterial strains, indicating that they possessing broad-spectrum antibacterial activity. Besides, further *E. coli* FabH inhibitory assay was undertaken and the results suggested that compounds **11** and **18** were potent *E. coli* FabH inhibitors. In addition, docking simulation were performed to position compounds **11** and **18** into the *E. coli* FabH active site to determine the probable binding conformation and the results confirmed that the two compounds were potential inhibitors of *E. coli* FabH.

The synthesis of compounds **3–29** followed the general pathway outlined in Scheme 1. Nine aryl aldehydes reacted directly with thiosemicarbazide, and the obtained thiosemicarbazones subsequently reacted with suitable 2-halogenoketones to yield the 4-substituted thiazole ring derivatives. In the synthesis of all of the compounds, isopropyl alcohol proved to be the best solvent for our purpose. As a matter of fact, the reaction products precipitate upon cooling, and they can be filtered and purified by crystallization from ethanol or ethanol/isopropanol. All of the synthetic compounds gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures.

All the compounds prepared were evaluated for their antibacterial activities against three Gram positive bacterial strains (*Bacillus*

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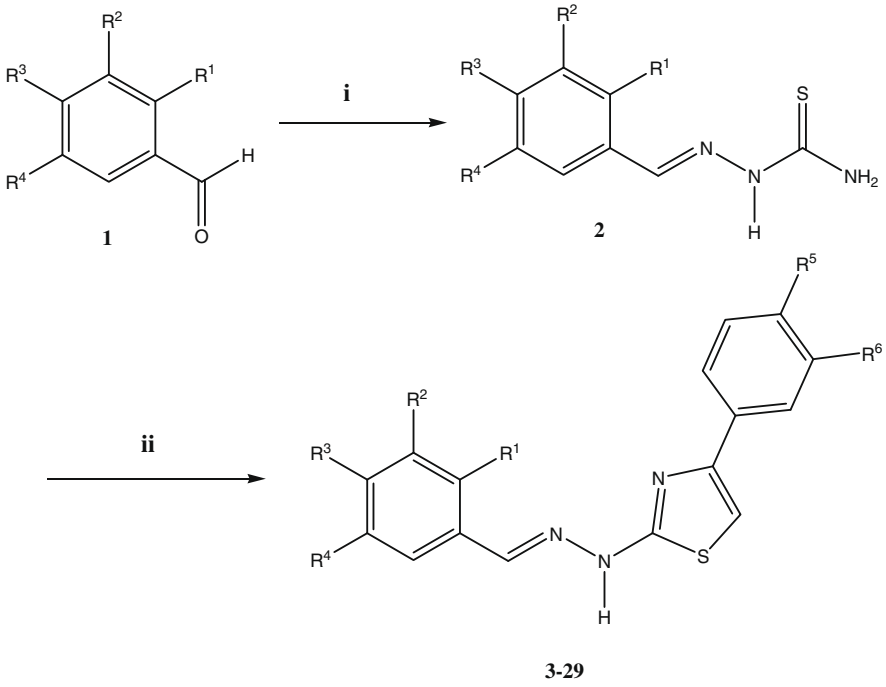


Scheme 1. General synthesis of compounds **3**–**29**. Reagents and conditions: (i) thiosemicarbazide, 2-propanol, acetic acid; (ii) 2-haloacetophenone, 2-propanol, rt.

subtilis ATCC 6633, *Staphylococcus aureus* ATCC 6538 and *Streptococcus faecalis* ATCC 9790) and three Gram negative bacterial strains (*E. coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 13525 and *Enterobacter cloacae* ATCC 13047) activities by MTT method, and the results are shown in Table 1. Also included was the activity of reference compounds Penicillin G and Kanamycin B. The results revealed that most of the synthesized compounds showed significant antibacterial activity.

As shown in Table 1, Compounds **10** (MIC = 1.56 μg/mL), **11** (MIC = 0.78 μg/mL) and **18** (MIC = 1.56 μg/mL) exhibited potent activity against *E. coli* ATCC 35218, which were superior to the positive control kanamycin B (MIC = 3.125 μg/mL). Compounds **17**

Table 1
MICs (minimum inhibitory concentrations) (μg/mL) of the synthetic compounds

Compounds	Microorganisms					
	Gram positive			Gram negative		
	B. subtilis ATCC 6633	*S. aureus* ATCC 6538	*S. faecalis* ATCC 9790	*P. aeruginosa* ATCC 13525	*E. coli* ATCC 35218	*E. cloacae* ATCC 13047
3	6.25	0.78	6.25	25	25	25
4	12.5	1.562	25	12.5	12.5	25
5	6.25	3.125	25	12.5	12.5	>50
6	>50	25	>50	25	25	12.5
7	25	25	>50	25	12.5	6.25
8	>50	12.5	12.5	12.5	6.25	>50
9	12.5	25	>50	12.5	6.25	12.5
10	12.5	6.25	12.5	6.25	1.56	12.5
11	3.125	6.25	6.25	3.125	0.78	3.125
12	12.5	6.25	12.5	25	50	25
13	>50	12.5	>50	6.25	25	>50
14	12.5	12.5	25	>50	25	12.5
15	6.25	25	12.5	25	12.5	6.25
16	12.5	25	>50	25	12.5	>50
17	6.25	12.5	25	12.5	3.125	12.5
18	25	6.25	12.5	6.25	1.56	6.25
19	>50	>50	12.5	6.25	6.25	>50
20	>50	25	>50	25	6.25	6.25
21	12.5	6.25	>50	12.5	25	>50
22	25	25	25	25	12.5	25
23	>50	12.5	1.562	12.5	>50	25
24	>50	25	>50	25	>50	>50
25	25	>50	>50	>50	25	>50
26	>50	>50	25	12.5	12.5	>50
27	12.5	25	12.5	25	12.5	25
28	25	>50	25	>50	25	12.5
29	12.5	>50	25	25	25	25
Penicillin G	1.562	1.562	1.562	6.25	6.25	3.125
Kanamycin B	0.39	1.562	3.125	3.125	3.125	1.562

showed significant activity with MIC values of 3.125 μg/mL against *E. coli* ATCC 35218, which was comparable to the positive control kanamycin B. Studies were performed by modification of the parent compounds to determine how the substituents of the subunits affected the antibacterial activities. The parent compounds can be divided into two rings: A ring and B ring (Scheme 1 and Table 3).

Compounds **3–11** have two chlorine atoms on the 3-position and 4-position of A ring. Among them**,** compounds **10** and **11** with two halogen atoms on 3-position and 5-position of A ring displayed better antibacterial activity against *E. coli* ATCC 35218 than that of compounds **3**–**9**. In addition, compounds **12–20** have a chlorine atom on the 4-position of B ring**,** among them, compounds **17** and **18** with a halogen atom on 5-position of A ring exhibited better antibacterial activity against *E. coli* ATCC 35218 than that of compounds **12–16, 19** and **20**.

Among compounds **3–11,** compound **3** displayed potent activity with MIC value of 0.78 μg/mL against *B. subtilis* ATCC 6633, which was superior to the positive control penicillin G. Compound **4** exhibited significant activity with MIC value of 1.562 μg/mL against *B. subtilis* ATCC 6633, which was comparable to the positive control penicillin G. A comparison of the substitution on A ring demonstrated that 4-position-halogen-substituted derivatives (compounds **3**–**5**) have more potent antibacterial activity against *B. subtilis* ATCC 6633 than others. It can be concluded that electron-withdrawing halogen groups on the 4-position of A ring were conductive to the antibacterial activity. Meanwhile, the derivatives (compounds **6** and **7**) which have electron-donating substituents (such as CH_3, OCH_3) on 4-position of A ring exhibited less potent activity against *B. subtilis* ATCC 6633, and their MICs values were 25 μg/mL. In general, compounds **11** and **18** displayed not only potent antibacterial activity against *E. coli* ATCC 35218, but also favorable activity against other five bacterial strains, indicating that they possessing broad-spectrum antibacterial activity.

The *E. coli* FabH inhibitory potency of the selected compounds **8**–**11**, **17**–**19** and **20** was examined and the results are summarized in Table 2. As shown in Table 2, among the tested compounds, compounds **11** and **18** showed potent inhibitory activity with IC_{50} of 3.6 and 6.8 μM, respectively. Other tested compounds displayed moderate inhibitory activity with IC_{50} ranging from 11.7 μM to 78.4 μM. It also can be seen from Table 2 that the selected compounds displayed low hemolytic activity. In addition, molecular docking of compound **11**, **18** and *E. coli* FabH were performed on the binding model based on the *E. coli* FabH-CoA complex structure (1HNJ·pdb).[24] 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.[24] 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 this report, Kim and coworkers designed pharmacophore maps considering the interaction with Phe304 and performed receptor-oriented pharmacophore based in silico screening of *E. coli* FabH.[12] YKAs3003, a Schiff base condensed by 4-hydroxy salicylaldehyde and cyclohexanamine, was hit by pharmacophore map I consisting of three features (two hydrogen bond donors that involving the backbone oxygen of Phe304 and Gly209, respectively, and one hydrophobic interaction with Ile156, Phe157 and Met207). The results of in silico screening indicated that YKAs3003 is a potent inhibitor of *E. coli* FabH with antibacterial activity.

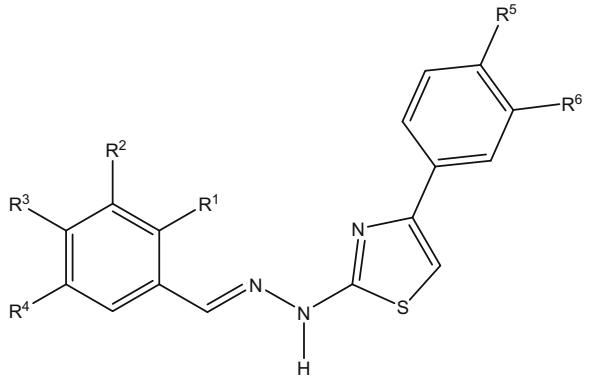
Based on these facts, in this present work, compound **11** and **18** with the most potent antibacterial activity were hit by pharmacophore map I mentioned above. The binding model of compounds **11**, **18** and *E. coli* FabH are depicted in Figures 1 and 2, respectively. In the binding model of compound **11** and *E. coli* FabH, amino hydrogen of Asn247 forms hydrogen bond with phenolic hydroxyl of compound **11**. However, in the binding model of compound **18** and *E. coli* FabH, amino hydrogen of GLY306 forms hydrogen bond with the sulfur atom of the thiazole ring of compound **18**. These results, along with the data of *E. coli* FabH inhibitory activity assay

Table 2
E. coli FabH inhibitory activity of the selected compounds

Compounds	*E. coli* FabH IC_{50} (μM)	Hemolysis LC_{30}[a] (mg/mL)
8	39.6	>10
9	43.4	>10
10	16.5	>10
11	3.6	>10
17	11.7	>10
18	6.8	>10
19	57.6	>10
20	78.4	>10

[a] Lytic concentration 30%.

Table 3
Chemical structures of compounds **3**–**29**



Compounds	R^1	R^2	R^3	R^4	R^5	R^6
3	H	H	F	H	Cl	Cl
4	H	H	Cl	H	Cl	Cl
5	H	H	Br	H	Cl	Cl
6	H	H	CH_3	H	Cl	Cl
7	H	H	OCH_3	H	Cl	Cl
8	OH	H	H	Cl	Cl	Cl
9	OH	H	H	Br	Cl	Cl
10	OH	Cl	H	Cl	Cl	Cl
11	OH	Br	H	Br	Cl	Cl
12	H	H	F	H	Cl	H
13	H	H	Cl	H	Cl	H
14	H	H	Br	H	Cl	H
15	H	H	CH_3	H	Cl	H
16	H	H	OCH_3	H	Cl	H
17	OH	H	H	Cl	Cl	H
18	OH	H	H	Br	Cl	H
19	OH	Cl	H	Cl	Cl	H
20	OH	Br	H	Br	Cl	H
21	H	H	F	H	H	H
22	H	H	Cl	H	H	H
23	H	H	Br	H	H	H
24	H	H	CH_3	H	H	H
25	H	H	OCH_3	H	H	H
26	OH	H	H	Cl	H	H
27	OH	H	H	Br	H	H
28	OH	Cl	H	Cl	H	H
29	OH	Br	H	Br	H	H

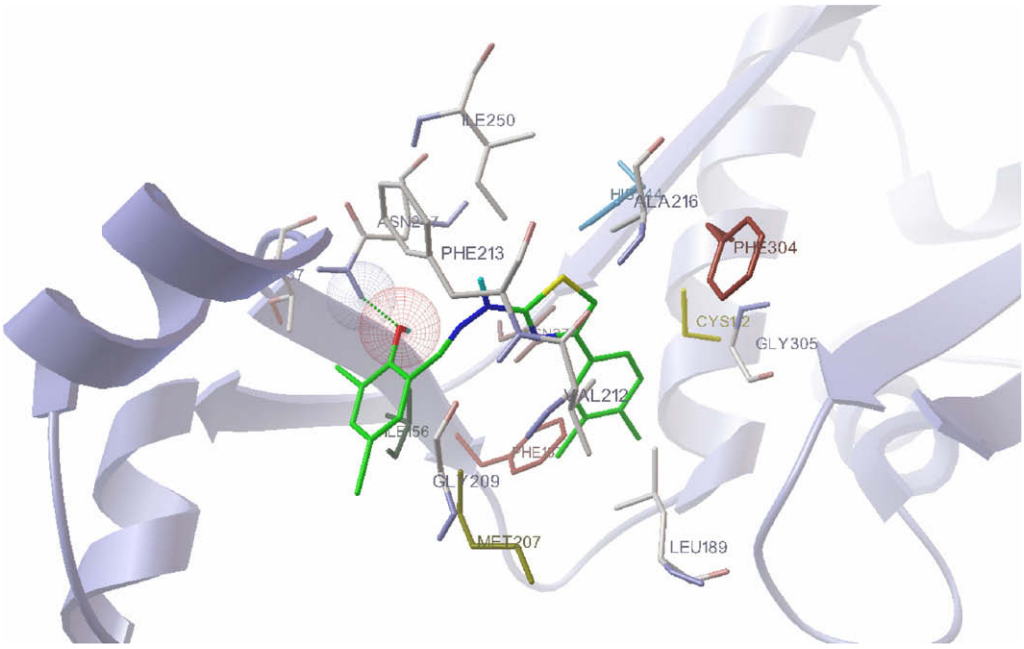


Figure 1. Binding model of compound **11** and *E. coli* FabH. H-bonds are displayed as dashed lines. Amino hydrogen of Asn247 forms hydrogen bond with phenolic hydroxyl of compound **11**.

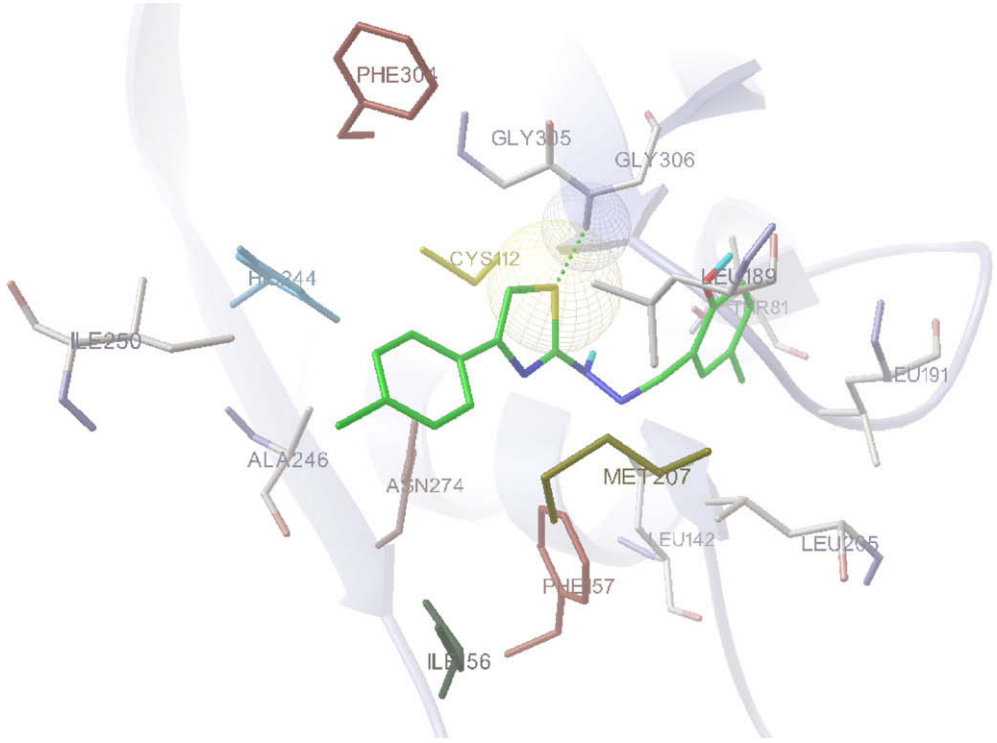


Figure 2. Binding model of compound **18** and *E. coli* FabH. H-bonds are displayed as dashed lines. Amino hydrogen of GLY306 forms hydrogen bond with the sulfur atom of the thiazole ring of compound **18**.

indicated that compounds **11** and **18** would be potential inhibitors of *E. coli* FabH with potent antibacterial activity.

In conclusion, three series of Schiff bases containing thiazole template were synthesized and evaluated for their antibacterial activity against three Gram positive bacterial strains (*B. subtilis* ATCC 6633, *S. aureus* ATCC 6538 and *S. faecalis* ATCC 9790) and three Gram negative bacterial strains (*E. coli* ATCC 35218, *P. aeruginosa* ATCC 13525, and *E. cloacae* ATCC 13047) activities by MTT method. Data obtained indicated that compounds **11** and **18** displayed potent antibacterial activity against *E. coli* ATCC 35218. Further *E. coli* FabH inhibitory activity assay suggested that they are potent *E. coli* FabH inhibitors. Besides, docking simulation were performed to position compound **11** and **18** into the *E. coli* FabH active site to determine the probable binding conformation and the

results confirmed that the two compounds were potential inhibitors of *E. coli* FabH.

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