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Design, synthesis and antibacterial activity studies of thiazole derivatives as potent ecKAS III inhibitors

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ABSTRACT

Two series of thiazole derivatives containing amide skeleton were synthesized and developed as potent *Escherichia coli* β-ketoacyl-(acyl-carrier-protein) synthase III (ecKAS III) inhibitors. All the 24 new synthesized compounds were assayed for antibacterial activity against the respective Gram-negative and Gram-positive bacterial strains, including *E. coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*. In which, 10 compounds with broad-spectrum antibacterial activities were further tested for their ecKAS III inhibitory activity. Last, we have successfully found that compound **4e** showed both the promising broad antibacterial activity with MIC of 1.56–6.25 μg/mL against the representative bacterial stains, and also processed the most potent ecKAS III inhibitory activity with IC_{50} of 5.3 μM. In addition, docking simulation also carried out in this study to give a potent prediction binding mode between the small molecule and ecKAS III (PDB code: 1hnj) protein.

The increasing appearance and prevalence of multidrug-resistant pathogenic bacteria have heightened the importance of the elaboration of new types of antibacterial agents or the expansion of bioactivity of the previous drugs.[1] Therefore, in recent years research has been focused toward development of new antibacterial agents, which may act through novel target, surpassing the problem of acquired resistance.

Saturated fatty acid biosynthesis has emerged as a prime candidate for development of such important and novel antibacterials.[2] The ubiquitous type II fatty acid synthesis system (FAS) in bacteria is not only essential to cell survival but also exhibits significant differences between bacterial and human fatty acid synthesis systems including the organization and structure of enzymes and the specific roles played by fatty acids, which make this system an attractive target for antibacterial drug discovery.[3,4] *Escherichia coli* β-ketoacyl-(acyl-carrier-protein) synthase III, also known as ecKAS III or FabH, plays an essential and regulatory role in bacterial FAS.[5] The enzyme initiates the fatty acid elongation cycles (Fig. 1), and is involved in the feedback regulation of the biosynthetic pathway via product inhibition.[6] In addition, ecKAS III proteins from both Gram-positive and -negative bacteria are highly conserved at the sequence and structural level, while there are no significantly homologous proteins in humans. Moreover, the residues that comprise the active site are essentially invariant among Gram-positive and -negative organisms.[7] These attributes suggest that ecKAS III could be a promising target for the design of novel antimicrobial drugs with selectivity, nontoxicity, and broad-spectrum antibacteria activity.

Thiazoles and their derivatives have attracted continuing interest over the years because of their varied biological activities,[8] recently found application in drug development for the treatment of allergies,[9] hypertension,[10] inflammation,[11] schizophrenia,[12] bacterial,[13] HIV infections,[14] hypnotics[15] and more recently for the treatment of pain.[16] Besides, Kitagawa et al. find that thiazole derivatives show strong FabI and FabK inhibitory activities with potent antibacterial activity.[17]

In view of the above mentioned findings, we described the synthesis of thiazole derivatives containing amide skeleton. This combination was suggested in an attempt to investigate the inhibitory activity against ecKAS III. The antimicrobial activity against two Gram-negative bacterial strains (*E. coli* and *Pseudomonas aeruginosa*), and two Gram-positive bacterial strains (*Bacillus subtilis* and *Staphylococcu saureus*) of these thiazole derivatives were also determined. Docking simulation was performed using the X-ray crystallographic structure of ecKAS III with the small molecule inhibitor **4e** to explore the binding mode and the activity relationship.

The synthesis of compounds **3a–l** and **4a–l** embarked on the preparation of two key intermediates **2a** and **2b** followed by the general synthetic procedure shown in Scheme 1. That is, treatment of bromides **1a** and **1b** with thiourea in refluxing EtOH afforded 4-phenylthiazol-2-amine (**2a**) and 4-(4-bromophenyl)thiazol-2-amine (**2b**) in 92% and 80% yields, respectively. The methods for acylation of **2a** and **2b** were well documented.[18] Here, acylation

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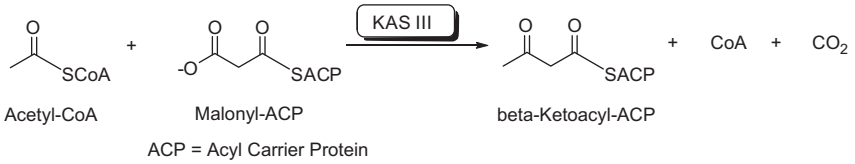


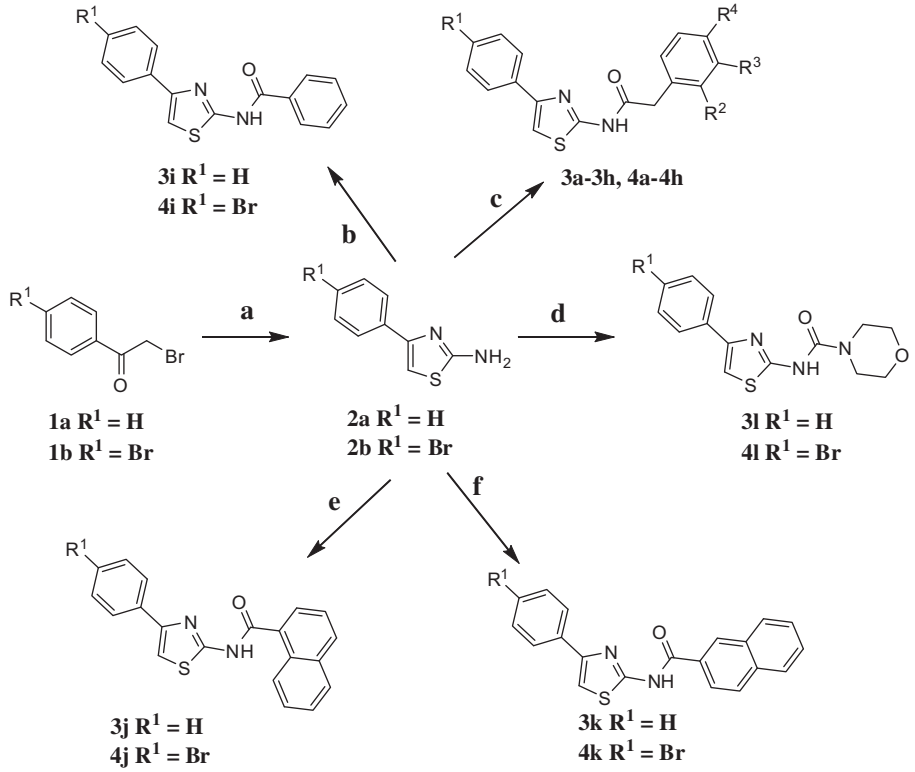
Figure 1. ecKAS III-catalyzed initiation reaction of fatty acid biosynthesis.

of amines **2a** and **2b** with a series of phenylacetic acid and naphthoic acid provided the corresponding final compounds **3a–h**, **3j–k**, **4a–h**, **4j–k** in the presence of EDC·HCl and HOBt in CH_2Cl_2. In addition, treatment of amines **2a** and **2b** with benzoyl chloride or 4-morpholinecarbonyl chloride in pyridine led to compounds **3i**, **3l**, **4i**, **4l**. All of the synthetic compounds (Table 1) gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures.

All the synthesized compounds (**3a–l** and **4a–l**) were screened for antibacterial activity against two Gram-negative bacterial strains: *E. coli* and *P. aeruginosa* 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 2. Standard antibacterial agent kanamycin B and Penicillin G were 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.

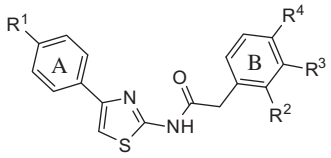
Out of the 24 compounds, compound **4e**, *N*-(4-(4-bromophenyl)thiazol-2-yl)-2-(3-chlorophenyl)acetamide, exhibited the most potent antibacterial activity with MIC of 1.56, 3.13, 6.25 and 3.13 μg/mL against *E. coli*, *P. aeruginos*a, *B. subtilis* and *S. aureus*, respectively, which was similar to the broad-spectrum antibiotic kanamycin B with corresponding MIC of 1.56, 3.13, 0.39, 3.13 μg/mL and penicillin G with corresponding MIC of 3.13, 6.25, 1.56, 6.25 μg/mL.

Modification of the parent compounds with various substituents such as halogen, methoxyl and ethoxy were performed to explore the structure–activity relationships (SAR) of theses thiazole derivatives containing amide skeleton. The parent compounds can be divided into two rings: A ring and B ring. As shown in Table 2, we found that compounds **4a–l** showed better antibacterial activity than compounds (**3a–l**). The slight structure difference between these two series was the substituent of A ring: for **3a–l**, there was no substituent on A ring while a chloro group on A ring for **4a–l**. These results demonstrated that chloro group on *para*-position of A ring was favorable to the antibacterial activity of synthetic compounds. Compared to **3a**, compounds **3b–f** showed more potent antibacterial activity. The MIC values of compound **3a** and **3b–f** were 25–100 μg/mL, 3.13–50 μg/mL, respectively. This result suggested that the introduction of halogen substituent on *meta*- or *para*-position of B ring increased the hydrophobicity of compounds and lead to the increase of antibacterial activity. In addition, the antibacterial activity of these compounds enhanced slightly in the order of substituent on *para*-position of B ring component: Br < Cl < F. The comparison

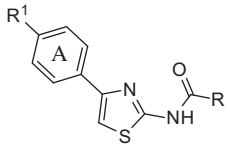


Scheme 1. General synthetic procedure for the preparation of compounds **3a–l** and **4a–l**. Reagents and conditions: (a) Thiourea, EtOH, rt; (b) benzoyl chloride, pyridine, 0 °C to rt; (c) substituted phenylacetic acid, EDC, HOBt, CH_2Cl_2, rt; (d) 4-morpholinecarbonyl chloride, pyridine, 0 °C to rt; (e) 1-naphthoic acid, EDC, HOBt, CH_2Cl_2, rt; (f) 2-naphthoic acid, EDC, HOBt, CH_2Cl_2, rt.

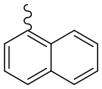
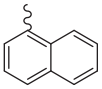
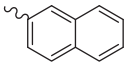
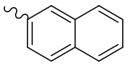
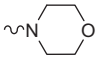
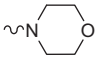
Table 1
Structure of compounds **3a–l** and **4a–l**



3a-3h, 4a-4h



3i-3l, 4i-4l

Compd	R^1	R^2	R^3	R^4	Compd	R^1	R^2	R^3	R^4
3a	H	H	H	H	**4a**	Br	H	H	H
3b	H	H	H	F	**4b**	Br	H	H	F
3c	H	H	H	Cl	**4c**	Br	H	H	Cl
3d	H	H	H	Br	**4d**	Br	H	H	Br
3e	H	H	Cl	H	**4e**	Br	H	Cl	H
3f	H	H	Br	H	**4f**	Br	H	Br	H
3g	H	H	OMe	H	**4g**	Br	H	OMe	H
3h	H	H	OEt	OEt	**4h**	Br	H	OEt	OEt
3i	H	*R* = 			**4i**	Br	*R* = 		
3j	H	*R* = 			**4j**	Br	*R* = 		
3k	H	*R* = 			**4k**	Br	*R* = 		
3l	H	*R* = 			**4l**	Br	*R* = 		

between **4a** and **4b–f** was similar to the example of **3a** and **3b–f**. Furthermore, compounds with substituent at *para*-position of B ring had less potent activity than those at *meta*-position, which can be seen from the comparison of **3b** and **3c**, **4b** and **4c**, respectively. This indicated that the location of substituent also had an influence on the activity.

Compounds **3g, 3h, 4g** and **4h** showed similar moderate antibacterial activity with MIC of 25–100 μg/mL against all the tested bacterial strains. This indicated that the introduction of electron-donating group could not enhance the inhibitory activity. Compounds **3i–l** and **4i–l** were found to be inactive against all the tested bacterial strains. This indicated that the introduction of morpholine or naphthalene lead to the decrease of antibacterial activity.

The ecKAS III inhibitory potency of the synthetic thiazole derivatives containing amide skeleton with potent antibacterial activities (**3b–c, 3e–f** and **4b–g**) was examined and the results were summarized in Table 3. Most of the tested compounds displayed potent ecKAS III inhibitory. The more detailed inhibitory assay procedure is described in the Supplementary data. Among them, compound **4e** showed the most potent inhibitory with IC_{50} of 5.3 μM. This result supported the potent antibacterial activities of **4e**. Compounds **4b–g** with substituent on A ring showed better ecKAS III inhibitory activity than compounds **3b–c** and **3e–f** with no substituent on A ring. Besides, a comparison of **4c** and **4e**, **4d** and **4f**, respectively, also indicated that compounds with *m*-substituted chloride and bromine group on B ring exhibited more potent inhibitory activity than those compounds with *p*-substituent on B ring. These results of ecKAS III inhibitory activity of the test compounds were corresponding to the structure–activity relationships (SAR) of their antibacterial activities. This demonstrated that the potent antibacterial activities of the synthetic compounds were probably correlated to their ecKAS III inhibitory activities.

Molecular docking of compound **4e** and ecKAS III was performed on the binding model based on the ecKAS III-CoA complex structure (1HNJ.pdb).[19] The ecKAS III 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 ecKAS III, interactions between Cys and substrate appear to play

Table 2
MICs (minimum inhibitory concentrations) (μg/mL) of the synthetic compounds

Compounds	Microorganisms			
	Gram-negative		Gram-positive	
	E. coli ATCC35218	*P. aeruginosa* ATCC13525	*B. subtilis* ATCC6633	*S. aureus* ATCC6538
3a	100	100	50	25
3b	12.5	25	12.5	12.5
3c	25	25	12.5	25
3d	50	50	25	50
3e	6.25	6.25	12.5	6.25
3f	12.5	12.5	6.25	3.13
3g	50	50	100	50
3h	50	50	>100	>100
3i	>100	>100	>100	>100
3j	>100	>100	>100	>100
3k	>100	>100	50	>100
3l	>100	>100	>100	>100
4a	50	50	25	25
4b	12.5	12.5	6.25	12.5
4c	12.5	25	12.5	12.5
4d	25	50	25	25
4e	1.56	3.13	6.25	3.13
4f	6.25	12.5	6.25	6.25
4g	25	50	25	50
4h	50	25	50	>100
4i	>100	>100	>100	>100
4j	50	100	>100	>100
4k	>100	>100	>100	>100
4l	50	>100	100	>100
Kanamycin B	1.56	3.13	0.39	3.13
Penicillin G	3.13	6.25	1.56	6.25

Table 3
E. coli ecKAS III inhibitory activity of synthetic compounds

Compounds	ecKAS III IC_{50} (μM)[a]	Hemolysis LC_{30} (mg/mL)
3b	38.4 ± 2.6	>10
3c	41.8 ± 5.3	>10
3e	12.3 ± 3.1	>10
3f	26.9 ± 2.7	>10
4b	29.3 ± 2.2	>10
4c	34.7 ± 1.9	>10
4d	48.1 ± 0.6	>10
4e	5.3 ± 1.1	>10
4f	9.8 ± 2.5	>10
4g	65.6 ± 3.7	>10

[a] IC_{50} average values and corresponding SD values are determined from the results of at least three independent repeats.

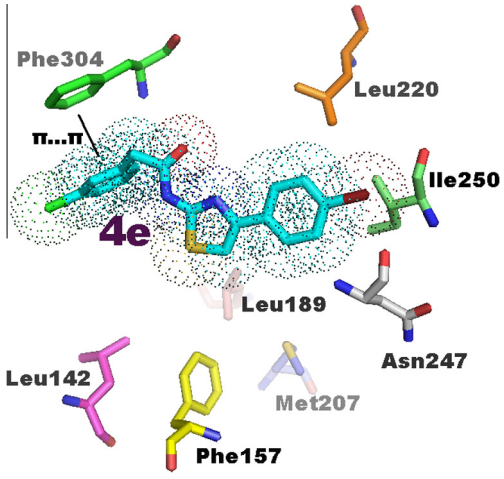


Figure 2. Molecular binding mode of compound **4e** with ecKAS III.

an important role in substrate binding. Qiu et al. have refined three-dimensional structure of ecKAS III in the presence and absence of malonyl-CoA by X-ray spectroscopy. Since malonyl moiety was degraded by ecKAS III, molecular docking studies for ecKAS III and malonyl-CoA were carried out to identify a plausible malonyl-binding mode.[19] 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.

The binding mode of compound **4e** and ecKAS III is depicted in Figure 2. In the binding mode, compound **4e** is projected into the active site with a quite smooth conformation. There is a π–π interaction between the benzyl ring of compound **4e** (C11, C12, C13, C14, C15, C16) and the benzyl ring of Phe304. The NH group of compound **4e** is also much close to the critical amino residue Phe304 in the active site. Besides, compound **4e** may form a hydrophobic interaction with Leu142, Phe157 and Leu189. Though there is no hydrogen bond found in the binding model, the dominant conformation and low binding energy (−8.56 kcal/mol) are still able to explain the potent ecKAS III inhibitory activity of compound **4e**.

In summary, a series of novel thiazole derivatives containing amide skeleton were synthesized and assayed for their antibacterial activities against *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus*. Most compounds showed potent antibacterial and ecKAS III inhibitory activities. In particular, compound **4e**, *N*-(4-(4-bromophenyl)thiazol-2-yl)-2-(3-chlorophenyl)acetamide showed both the promising broad antibacterial activity with MIC of 1.56–6.25 μg/mL against the representative bacterial stains, which was compared with the positive control kanamycin B and penicillin G, and also processed the most potent ecKAS III inhibitory activity with IC_{50} of 5.3 μM. Preliminary structure–activity relationships and molecular modeling study provided further insight into interactions between the enzyme and this small molecule ligand.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.05.006.

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