Design, synthesis and antimicrobial activities evaluation of Schiff base derived from secnidazole derivatives as potential FabH inhibitors

ARTICLE *in* BIOORGANIC & MEDICINAL CHEMISTRY · MARCH 2013

Impact Factor: 2.79 · DOI: 10.1016/j.bmc.2013.03.023 · Source: PubMed

7 AUTHORS, INCLUDING:

Xiao-Ming Wang
Nanjing University ,State Key Laboratory of…
27 PUBLICATIONS **155** CITATIONS

SEE PROFILE

Bioorganic & Medicinal Chemistry 21 (2013) 3120–3126

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis and antimicrobial activities evaluation of Schiff base derived from secnidazole derivatives as potential FabH inhibitors

Yao Li [†], Chang-Po Zhao [†], Hua-Ping Ma, Meng-Yue Zhao, Ya-Rong Xue *, Xiao-Ming Wang *, Hai-Liang Zhu *

State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, People's Republic of China

ARTICLE INFO

Article history:
Received 4 February 2013
Revised 12 March 2013
Accepted 18 March 2013
Available online 26 March 2013

Keywords:
Secnidazole derivatives
Schiff base
FabH inhibitors
Antibacterial activities

ABSTRACT

FabH, β-ketoacyl-acyl carrier protein (ACP) synthase III, is critically important to the initiation of fatty acid biosynthesis and is highly conserved among Gram-positive and Gram-negative bacteria. A series of novel secnidazole derivatives (**1**–**20**) were synthesized and fully characterized by spectroscopic methods and elemental analysis. Among these compounds, **6**, **8**, **11**, **13**, **14**, **16**–**20** were reported for the first time. These compounds were tested for antibacterial activities against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*. The compounds inhibitory assay and docking simulation indicated that compound **20** (*E*)-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-*N*′-(3,4,5-trimethylbenzylidene)acetohydrazide with MIC of 3.13–6.25 μg/mL against the tested bacterial strains was a potent inhibitor of *Escherichia coli* FabH.

1. Introduction

Microorganisms are almost everywhere in nature and infections caused by some small pathogenic microorganisms could lead to illnesses even a fatal one.[1] Although several class of antibacterial agents are still available and used for clinical treatment, their advances in medical care are threatened by a natural phenomenon known as 'antimicrobial resistance'. The incidences of drug resistance of microorganisms to antibacterial agents were constantly reported for the past few years.[2–5] In order to prevent the serious medical problem caused by microorganisms, the discovery of new types of antibacterial agents is a crucial task at present. Fortunately, much of the research effort is made to the design of new antibacterial agents with high efficiency.[6,7]

In recent 10 years, the research has been focused toward new antibacterial agents, which may act through different kinds of targets in key areas of the bacterial cell cycle, to surpass the problem of acquired resistance. The fatty acid synthesis (FAS) pathway in bacteria is a promising target in the recent research and the fatty acid biosynthesis (FAB) is a fundamental metabolic process for microorganisms and essential for cell viability and growth.[8,9] β-ketoacyl-acyl carrier protein synthase III (FabH) is the key enzyme responsible for the first reaction in the pathway and plays an important regulatory role.[10] FabH has also been demonstrated to be essential for initiating the fatty acid elongation cycles, and is involved in the feedback regulation of the biosynthetic pathway via product inhibition.[11,12] On the other hand, some novel compounds had been demonstrated to inhibit FabH from Gram-positive and Gram-negative bacteria, including multi-drug resistant strains. FabH proteins from 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.[13,14] FabH has been proved to be a promising target for the design of novel antimicrobial drugs because it adjust and control the fatty acid biosynthesis rate in an initiation pathway and its substrate specificity is a key factor in membrane fatty acid composition.[15–17] These facts indicate that small molecule inhibitors of FabH enzymatic activity could be potential candidates for selective, nontoxic, and broad-spectrum antibacterials.

Because of varied biological activities, nitroimidazoles derivatives have gained constant interests in drug research for antimicrobial chemotherapeutics and antiangiogenic hypoxic cell radiosensitizers. The metabolism and toxicology of nitroimidazoles derivatives, especially for secnidazole, have been characterized in recent reports.[18,19] Secnidazole (α, 2-dimethyl-5-nitro-1*H*-imidazole-1-ethanol) is extraordinary effective in the treatment of giardiasis, amebiasis and bacterial vaginosis. By oral administration, secnidazole can be rapidly and completely absorbed, and owns a longer terminal elimination half-life (17–29h) than popular medication.[20] On this occasion, the treatment outcome with secnidazole

* Corresponding author. Tel./fax: +86 25 83592672.
E-mail address: zhuhl@nju.edu.cn (H.-L. Zhu).
† These two authors equally contributed to this paper.

0968-0896/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2013.03.023

is more effective than the treatment using other imidazole drugs. Besides, the adverse effects of secnidazole are less drastic than others.[21] To sum up, secnidazole derivatives have been proved to be of great importance in pharmaceutical.

Schiff bases are the compounds with the structure of AC=NB, which are usually synthesized from the condensation of active carbonyl groups and primary amines. Lots of researchers studied the synthesis, characterization and structure-activity relationship (SAR) of Schiff bases and some of Schiff bases were reported to own antibacterial activities and.[22] 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.[23]

As a part of our research for new antibacterial drugs, a series of secnidazole derivatives containing Schiff base had been designed and synthesized. We chose Schiff base moiety as a part because it is a variety of biologically active substances, especially with antimicrobial activity. Compounds with the structure of C=N are known as Schiff bases, which are usually synthesized by the condensation of primary amines and active carbonyl groups. Some Schiff bases were reported to possess antibacterial, antifungal and antitumor activities.[22] Lots of researchers studied the synthesis, characterization and structure-activity relationship (SAR) of Schiff bases.[23–26] Kim et al. reported the YKAs3003, a Schiff base condensed by 4-hydroxy salicylaldehyde and cyclohexanamine as a potential inhibitor of *Escherichia coli* FabH with antimicrobial activity.[27]

In this study, we described the synthesis and structure-activity relationship of a new series of secnidazole derivatives containing Schiff base, and studied their antibacterial activities against *Escherichia coli*, *Pseudomonas aeruginosa* (*P. aeruginosa*), *Bacillus subtilis* (*B. subtilis*) and *Staphylococcus aureus* (*S. aureus*) *and E. coli* FabH inhibitory activities. Docking simulation was performed using the X-ray crystallographic structure of the FabH of *E. coli* in complex with the most potent inhibitor to explore the binding mode of the compound at the active site.

2. Results and discussion

2.1. Chemistry

As shown in Scheme 1, (2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide) was prepared by the reaction of 2-methyl-5-nitro-1*H*-imidazole in excess ethyl α-chloroacetate and then hydrazinolysis of ethyl ester group by hydrazine hydrate according to Mirzaei et al. method.[28]

Table 1
Structures of compounds **1–20**

Compounds	R	Compounds	R
1	4-F	11	3-Cl
2	4-Cl	12	3-NO_2
3	4-Br	13	3-OCH_3
4	4-OCH_3	14	2-Cl
5	4-NO_2	15	2-F
6	4-OH	16	2-NO_2
7	4-CH_3	17	2-OCH_3
8	4-N(CH_3)$_2$	18	2,4-2F
9	3-F	19	3,4-2OCH_3
10	3-Br	20	3,4,5-3CH_3

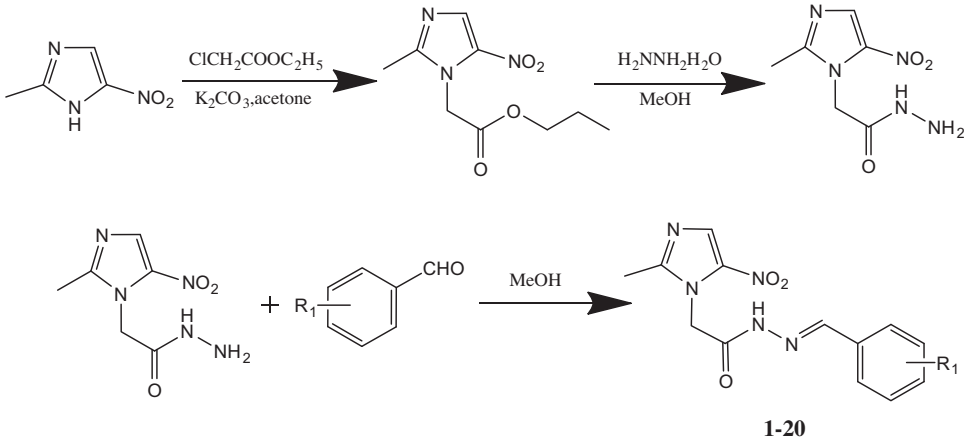
Then 2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide was reacted with substituted benzaldehyde to prepare the corresponding secnidazole derivatives **1**-**20**. The chemical structures of these secnidazole derivatives were summarized in Table 1. All these compounds were given satisfactory elementary analyses (±0.4%). ^{1}H NMR and ESI MS spectra data was consistent with the assigned structures. Among these compounds, **6**, **8**, **11**, **13**, **14**, **16**–**20** were reported for the first time.

2.2. Biological activity

2.2.1. Antimicrobial activity

All the synthesized compounds (**1–20**) were screened for their antibacterial activities against two Gram-negative bacterial strains: *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*), two Gram-positive bacterial strains: *Bacillus subtilis* ATCC 530 (*B. subtilis*) and *Staphylococcus aureus* ATCC 6538 (*S. aureus*) by serial dilution method. The MICs (minimum inhibitory concentrations) of the compounds against these bacteria were presented in Table 2. Also included was the activity of reference compound Kanamycin and DDCP (Compound **5** in Antimicrob. Agents Chemother. 2004, 48, 3093–3102)[29] under identical conditions for comparison. The results revealed that most of the synthesized compounds exhibited significant antibacterial activities.

Compared with the other synthetic secnidazole derivatives, compound **20**, (*E*)-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-*N*′-(3,4,5-trimethylbenzylidene)acetohydrazide, exhibited the most potent antibacterial activity with MIC of 3.13, 6.25, 3.13 and 3.13 μg/mL against *E. coli*, *P. aeruginosa, B. subtilis* and *S. aureus*, respectively, which was similar to the broad-spectrum antibiotic Kanamycin corresponding MIC of 3.13, 3.13, 1.56 and 1.56 μg/mL. From the data we got, the varieties of substitutes of benzaldehyde



Scheme 1. Synthesis of nitroimidazole derivatives **1**–**20**.

Table 2
Antibacterial activities of synthetic compounds

Compounds	Minimum inhibitory concentrations (μg/mL)			
	Gram-negative		Gram-positive	
	Escherichia coli	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Staphylococcus aureus*
1	>100	>100	>100	>100
2	50	50	>100	>100
3	25	12.5	25	25
4	6.25	6.25	6.25	12.5
5	25	25	50	25
6	50	>100	>100	>100
7	6.25	6.25	25	12.5
8	12.5	12.5	12.5	25
9	50	50	25	25
10	25	50	>100	>100
11	25	50	50	50
12	25	50	>100	50
13	25	50	50	25
14	>100	>100	50	50
15	>100	>100	>100	>100
16	25	25	12.5	12.5
17	50	50	>100	>100
18	50	50	50	25
19	12.5	12.5	25	12.5
20	3.13	6.25	3.13	3.13
Kanamycin	3.13	3.13	1.56	1.56
DDCP	1.56	3.13	12.5	25

such as halogen, nitro, methyl and methoxyl lead to the different antibacterial activities of these secnidazole derivatives. Structure-activity relationships in these secnidazole derivatives proved that compounds with electron-donating substituents (such as CH_3, OCH_3) (**4**, **7**, **8**, **19**, **20**), MICs value range from 3.13 to 12.5 μg/mL against *E. coil* ATCC 25922, exhibited more potential activities than those with electron-withdrawing substituents (such as F, Cl, Br). Based on the mention above, we presented ideas that electron-donating groups on benzaldehyde component were beneficial to the antibacterial activity and compounds with electron-withdrawing halogen groups on benzaldehyde component were not conducive for the activity, especially for compound **20** with three substituted methyl groups on benzaldehyde component.

According to Table 2, we found that compounds with an electron-donating group on the 4-position of benzaldehyde component (**4**, **7**, **19**, **20**), MICs value range from 3.13 to 12.5 μg/mL, displayed higher antibacterial activity against *E. coil* ATCC 25922 than compounds with an electron-donating group on the 3-position and 2-position of benzaldehyde component (**13**, **17**), MICs value range from 25 to 50 μg/mL. From this trend, we suggested the fact that electron-donating groups on the 4-position of benzaldehyde component were beneficial to the antibacterial activity.

2.2.2. *E. coli* FabH inhibitory activity

The *E. coli* FabH inhibitory potencies of the synthetic oximes with best antibacterial activities (**4**, **7**, **8**, **16**, **19**, **20**) and weakest antibacterial activities (**1**, **15**, **17**) were examined and the results were summarized in Table 3. Most of the tested compounds displayed prominent *E. coli* FabH inhibitory activities. Among them, compound **20** showed the best inhibitory activity with IC_{50} of 2.2 μM; compound **1** showed the weakest inhibitory activity with IC_{50} of 58.3 μM. This result supported the prominent antibacterial activity of **20**.

Compounds **7** with *para*-substituted methyl group showed better *E. coli* FabH inhibitory activity than compounds **1** with *para*-substituted F group on benzaldehyde component. Compounds **4** with *para*-substituted methoxy group showed better *E. coli* FabH inhibitory activity than compounds **17** with *ortho*-substituted methoxy group on benzaldehyde component. The results of *E. coli* FabH inhibitory activities of the test compounds were corresponding to the structure-activity relationships of their antibacterial activities. This demonstrated that antibacterial activities of the synthetic compounds were probably correlated to their FabH inhibitory activities.

Table 3
E. coli FabH inhibitory activities of selected compound **4–6**

E. coli FabH inhibitory activities of top compounds	
Compounds	IC_{50} (μM)
1	58.3 ± 3.9
4	4.5 ± 0.3
7	6.2 ± 0.4
8	15.9 ± 0.9
15	47.5 ± 4.2
16	19.8 ± 1.4
17	42.4 ± 3.5
19	8.8 ± 0.7
20	2.3 ± 0.2
DDCP	2.1 ± 0.1

Table 4
The docking calculation of the synthesized compounds (**1–20**)

Compounds	Cdocker interation energy ΔGb (kcal/mol)	Compounds	Cdocker interation energy ΔGb (kcal/mol)
1	−26.1226	**11**	−29.8734
2	−28.7742	**12**	−29.7453
3	−32.7251	**13**	−30.8633
4	−33.9155	**14**	−28.5753
5	−32.0342	**15**	−28.3193
6	−28.5169	**16**	−33.0668
7	−33.8697	**17**	−28.5587
8	−33.4241	**18**	−30.2775
9	−31.2451	**19**	−33.7007
10	−29.0816	**20**	−35.0512

2.2.3. Binding model of compound 11, 12 and *E. coli* FabH

Molecular docking of the compound **20** and *E. coli* FabH was performed on the binding model based on the *E. coli* FabH-CoA complex structure (1HNJ.pdb).[30]

All docking runs were applied Ligand Fit Dock protocol of Discovery Studio 3.1. The docking calculation of the synthesized compounds was showed in Table 4. The interaction energy of the compounds and their antibacterial activity showed the corresponding results. Among the docking calculation of the synthesized compounds, compound **20** showed the lowest interaction energy. The binding model of compound **20** and *E. coli* FabH was depicted in Figure 1 and Figure 2. In the binding model, compound **20** was nicely bound to the FabH kinase with 3 interaction bonds. The end amino of Trp32, Arg151 and Gly152 were respectively formed one hydrogen bonds interaction with oxygen atom and two pi cation monitor of secnidazole ring of compound **11**. This molecular docking result, along with the biological assay data, suggesting that compound **20** was a potential inhibitor of FabH.

3. Conclusions

To sum up, a series of novel nitroimidazole derivatives **1–20** were synthesized and tested for their inhibitory activities against *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus*. Among these compounds, compound **6**, **8**, **11**, **13**, **14**, **16**–**20** were reported for the

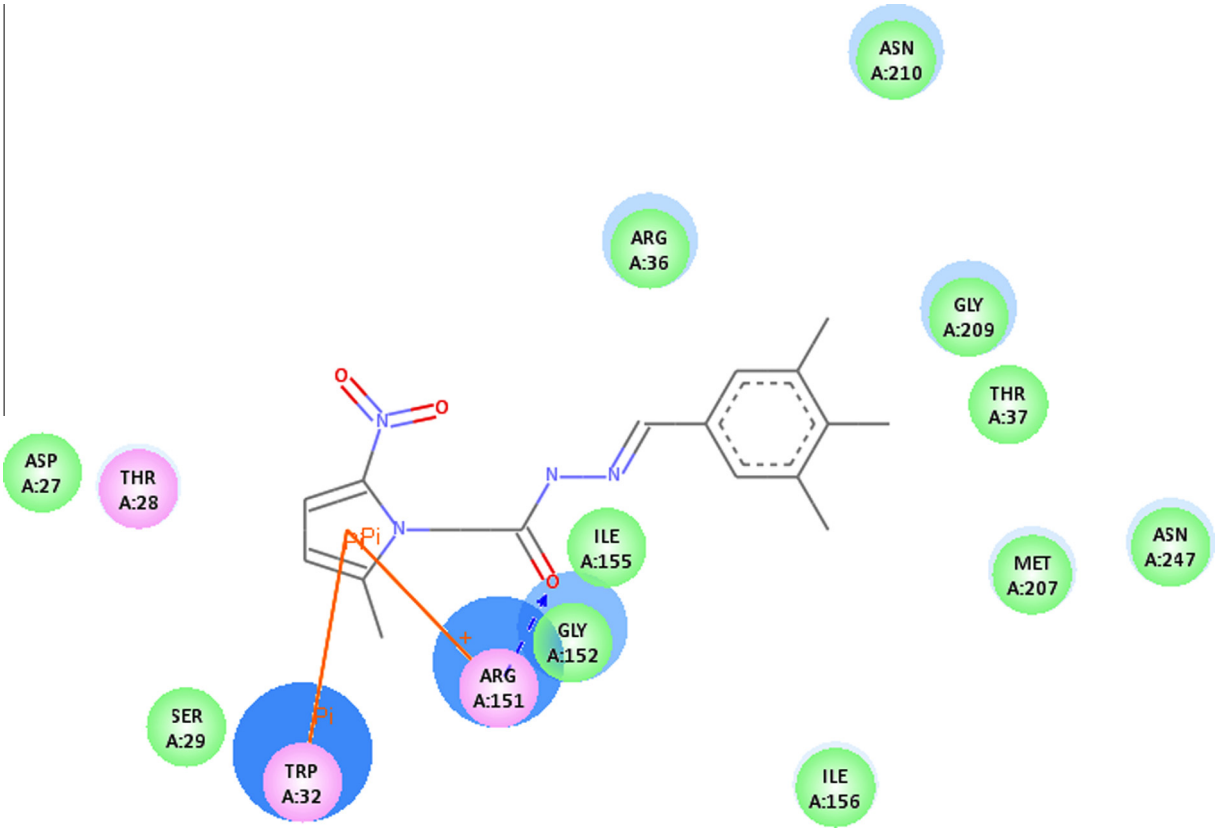


Figure 1. Binding model of compound **20** and *E. coli* FabH. H-bonds are displayed as dashed lines.

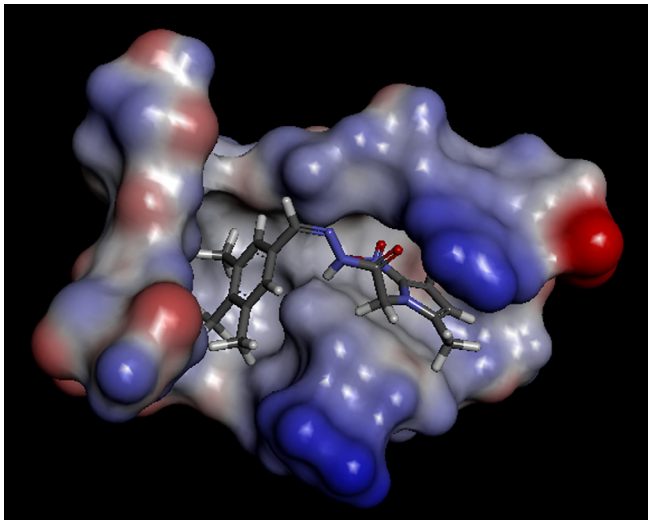


Figure 2. The surface model structure of compound **20** binding model with FabH complex.

first time. Many of them exhibited potent antibacterial and *E.coli* FabH inhibitory activities. Particularly, Compound **20** (*E*)-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-*N*′-(3,4,5-trimethylbenzylidene)acetohydrazide with MIC of 3.13–6.25 μg/mL against the tested bacterial strains were proved to be the most potent compounds. Preliminary SARs and molecular modeling study provided further insight into interactions between the enzyme and its ligand. The result provided valuable information for the design of *E. coli* FabH inhibitors as antibacterial agents.

4. Experiments

4.1. Materials and measurements

All chemicals (reagent grade) used were commercially available. Melting points (uncorrected) were determined on a X-4 MP apparatus (Taike Corp., Beijing, China). ESI mass spectra were obtained on a Mariner System 5304 mass spectrometer, and ^{1}H NMR spectra were recorded on a Bruker PX500 or DPX300 spectrometern at 25 °C with TMS and solvent signals allotted as internal standards. Chemical shifts were reported in ppm (δ). Elemental analyses were performed on a CHN–O–Rapid instrument and were within ±0.4% of the theoretical values.

4.2. General method of synthesis of secnidazole derivatives

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide was prepared according the previous procedure by Mirzaei et al.[29]

An equimolar mixture of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide (0.001 mol) and substituted benzaldehyde (0.001 mol) in methyl alcohol (15 mL) was reacted for 3–6 h with ice-bath. The resultant solid was filtered, washed with water and recrystallized from ethanol to give secnidazole derivatives **1–20**.

4.2.1. (*E*)-*N*′-(4-Fluorobenzylidene)-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide (1)

Mp: 259–260 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.31 (s, 3H), 5.39 (s, 2H), 7.27–7.32 (m, 2H), 7.78–7.83 (m, 2H), 8.06 (s, 1H), 8.27 (t, J = 17.55 Hz, 1H), 11.84 (s, 1H). MS (ESI):306.26 ($C_{13}H_{13}FN_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{12}FN_5O_3$: C, 51.15; H, 3.96; N, 22.94. Found: C, 51.17; H, 3.95; N, 22.93.

4.2.2. (*E*)-*N*′-(4-Chlorobenzylidene)-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide (2)

Mp: 254-255 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.31 (s, 3H), 5.39 (s, 2H), 7.52 (t, J = 10.45 Hz, 2H), 7.76 (t, J = 17.38 Hz, 2H), 8.05 (s, 1H), 8.27 (t, J = 18.65 Hz, 1H), 11.89 (s, 1H). MS (ESI):322.72 ($C_{13}H_{13}C_lN_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{12}C_lN_5O_3$: C, 48.52; H, 3.76; N, 21.77. Found: C, 48.54; H, 3.75; N, 21.78.

4.2.3. (*E*)-*N*′-(4-Bromobenzylidene)-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide (3)

Mp: 229–230 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.31 (s, 3H), 5.39 (s, 2H), 7.63–7.72 (m, 4H), 8.04 (s, 1H), 8.29 (d, J = 7.75 Hz, 1H), 11.89 (s, 1H). MS (ESI):367.17 ($C_{13}H_{13}BrN_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{12}BrN_5O_3$: C, 42.64; H, 3.30; N, 19.13. Found: C, 42.65; H, 3.31; N, 19.14.

4.2.4. (*E*)-*N*′-(4-Methoxybenzylidene)-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide (4)

Mp: 194–196 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.30 (d, J = 5.17 Hz, 3H), 3.81 (s, 3H), 5.37 (s, 2H), 7.02 (d, J=4.42 Hz, 2H), 7.65–7.70 (m, 2H), 8.00 (s, 1H), 8.30 (d, J = 5.80 Hz, 1H), 11.70 (d, J = 7.63 Hz, 1H). MS (ESI):318.30 ($C_{14}H_{16}N_5O_4$, $[M+H]^+$). Anal. Calcd for $C_{14}H_{15}N_5O_4$: C, 52.99; H, 4.76; N, 22.07. Found: C, 52.98; H, 4.75; N, 22.09.

4.2.5. (*E*)-2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)-*N*′-(4-nitrobenzylidene)acetohydrazide (5)

Mp: 247–248 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.32 (s, 3H), 5.45 (s, 2H), 8.00 (t, J = 9.87 Hz, 2H), 8.17 (s, 1H), 8.29–8.35 (m, 3H), 12.13 (s, 1H). MS (ESI):333.27 ($C_{13}H_{13}N_6O_5$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{12}N_6O_5$: C, 46.99; H, 3.64; N, 25.29. Found: C, 46.98; H, 3.65; N, 25.26.

4.2.6. (*E*)-*N*′-(4-Hydroxybenzylidene)-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide (6)

Mp: 294–296 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.29 (s, 3H), 5.34 (s, 2H), 6.83 (d, J = 4.31 Hz, 2H), 7.55 (t, J = 8.78 Hz, 2H), 7.95 (s, 1H), 8.30 (d, J = 3.12 Hz, 1H), 9.94 (s, 1H), 11.64 (d, J = 4.49 Hz, 1H). MS (ESI):304.27 ($C_{13}H_{14}N_5O_4$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{13}N_5O_4$: C, 51.48; H, 4.32; N, 23.09. Found: C, 51.49; H, 4.31; N, 23.07.

4.2.7. (*E*)-2-(2-Methyl-5-nitro-1H-imidazol-1-yl)-*N*′-(4-methylbenzylidene)acetohydrazide (7)

Mp: 208–210 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.29 (s, 3H), 2.34 (s, 3H), 5.33 (s, 2H), 6.75 (d, J = 4.38 Hz, 2H), 7.53 (t, J = 7.86 Hz, 2H), 7.91 (s, 1H), 8.31 (d, J = 2.30 Hz, 1H), 11.54 (d, J = 6.03 Hz, 1H). MS (ESI):302.30 ($C_{14}H_{16}N_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{14}H_{15}N_5O_3$: C, 55.81; H, 5.02; N, 23.24. Found: C, 56.81; H, 5.01; N, 23.25.

4.2.8. (*E*)-*N*′-(4-Hydroxybenzylidene)-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide (8)

Mp: 277–278 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.29 (s, 3H), 2.97 (s, 6H), 5.33 (s, 2H), 6.74 (d, J = 4.02 Hz, 2H), 7.53 (t, J = 7.68 Hz, 2H), 7.92 (s, 1H), 8.30 (d, J = 2.30 Hz, 1H), 11.54 (d, J = 5.85Hz, 1H). MS (ESI):331.34 ($C_{15}H_{19}N_6O_3$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{18}N_6O_3$: C, 54.54; H, 5.49; N, 25.44. Found: C, 54.55; H, 5.48; N, 25.42.

4.2.9. (*E*)-*N*′-(3-Fluorobenzylidene)-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide (9)

Mp: 222–223 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.29 (s, 3H), 5.41 (s, 2H), 7.28 (t, J = 8.40 Hz, 1H), 7.48–7.53 (m, 1H), 7.57 (d, J = 3.83 Hz, 1H), 7.62 (d, J = 5.05 Hz, 1H), 8.06 (s, 1H), 8.28 (t, J = 16.63 Hz, 1H), 11.93 (s, 1H). MS (ESI):306.26 ($C_{13}H_{13}FN_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{12}FN_5O_3$: C, 51.15; H, 3.96; N, 22.94. Found: C, 51.18; H, 3.97; N, 22.93.

4.2.10. (*E*)-*N*′-(3-Fluorobenzylidene)-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide (10)

Mp: 197–198 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.29 (s, 3H), 5.42 (s, 2H), 7.42 (t, J = 7.80 Hz, 1H), 7.63 (d, J = 3.95 Hz, 1H), 7.72 (d, J = 3.83 Hz, 1H), 7.99 (s, 1H), 8.06 (s, 1H), 8.03 (s, 1H), 8.27 (s, 1H), 11.94 (s, 1H). MS (ESI):367.17 ($C_{13}H_{13}BrN_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{12}BrN_5O_3$: C, 42.64; H, 3.30; N, 19.13. Found: C, 42.65; H, 3.31; N, 19.15.

4.2.11. (*E*)-*N*′-(3-Fluorobenzylidene)-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide (11)

Mp: 259–260 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.29 (s, 3H), 5.43 (s, 2H), 7.46–7.51 (m, 2H), 7.67–7.71 (m, 1H), 7.86 (s, 1H), 8.05 (s, 1H), 8.27 (t, J = 14.91 Hz, 1H), 11.96 (s, 1H). MS (ESI):322.72 ($C_{13}H_{13}C_lN_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{12}C_lN_5O_3$: C, 48.53; H, 3.76; N, 21.77. Found: C, 48.55; H, 3.75; N, 21.78.

4.2.12. (*E*)-2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)-*N*′-(3-nitrobenzylidene)acetohydrazide (12)

Mp: 256–257 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.30 (s, 3H), 5.46 (s, 2H), 7.46–7.51 (m, 1H), 7.67–7.71 (m, 2H), 8.28 (t, J = 5.20 Hz, 1H), 8.35 (d, J = 11.28 Hz, 1H), 8.56 (d, J = 6.40 Hz, 1H), 12.08 (s, 1H). MS (ESI):334.27 ($C_{13}H_{13}N_6O_5$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{12}N_6O_5$: C, 46.99; H, 3.64; N, 25.29. Found: C, 46.98; H, 3.65; N, 25.27.

4.2.13. (*E*)-*N*′-(3-Methoxybenzylidene)-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide (13)

Mp: 165–165 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.29 (s, 3H), 3.81 (s, 3H), 5.40(s, 2H), 7.02 (d, J = 4.58 Hz, 1H), 7.26–7.44 (m, 3H), 8.02 (s, 1H), 8.30 (d, J = 3.93 Hz, 1H), 11.85 (s, 1H). MS (ESI):318.30 ($C_{14}H_{16}N_5O_4$, $[M+H]^+$). Anal. Calcd for $C_{14}H_{16}N_5O_4$: C, 52.99; H, 4.76; N, 22.07. Found: C, 52.98; H, 4.75; N, 22.08.

4.2.14. (*E*)-*N*′-(2-Chlorobenzylidene)-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide (14)

Mp: 257–258 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.29 (s, 3H), 5.41 (s, 2H), 7.42–7.49 (m, 2H), 7.55 (t, J = 4.76 Hz, 1H), 8.05 (t, J = 4.76 Hz, 1H), 8.30 (d, J = 5.94 Hz, 1H), 8.45 (s, 1H), 12.03 (s, 1H). MS (ESI):322.72 ($C_{13}H_{13}C_lN_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{12}C_lN_5O_3$: C, 48.53; H, 3.76; N, 21.77. Found: C, 48.52; H, 3.75; N, 21.78.

4.2.15. (*E*)-*N*′-(2-Fluorobenzylidene)-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide (15)

Mp: 231–232 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.30 (s, 3H), 5.40 (s, 2H), 7.31 (t, J = 8.23 Hz, 2H), 7.50 (t, J = 6.25 Hz, 1H), 7.98 (t, J = 7.63 Hz, 1H), 8.28 (d, J = 3.38 Hz, 1H), 8.45 (s, 1H), 11.95 (s, 1H). MS (ESI):306.26 ($C_{13}H_{13}FN_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{12}FN_5O_3$: C, 51.15; H, 3.96; N, 22.94. Found: C, 51.14; H, 3.94; N, 22.96.

4.2.16. (*E*)-2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)-*N*′-(2-nitrobenzylidene)acetohydrazide (16)

Mp: 269–270 °C. ^{1}H NMR (300 MHz, DMSOd_6) δ: 2.30 (s, 3H), 5.40 (s, 2H), 7.70 (t, J = 7.77 Hz, 1H), 7.84 (t, J = 7.14 Hz, 1H), 8.08–8.17 (m, 2H), 8.28 (s, 1H), 8.46 (s, 1H), 12.15 (s, 1H). MS (ESI):333.27 ($C_{13}H_{13}N_6O_5$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{12}N_6O_5$: C, 46.99; H, 3.64; N, 25.29. Found: C, 46.97; H, 3.65; N, 25.31.

4.2.17. (*E*)-*N*′-(2-Methoxybenzylidene)-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide (17)

Mp: 279–280 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.29 (s, 3H), 3.86 (s, 3H), 5.38 (s, 2H), 7.01 (t, J = 6.87 Hz, 1H), 7.12 (d, J = 4.22 Hz, 1H), 7.41–7.46 (m, 1H), 7.89 (t, J = 3.86 Hz, 1H), 8.29 (s, 1H), 8.40 (s, 1H), 11.80 (s, 1H). MS (ESI):318.30 ($C_{14}H_{16}N_5O_4$,

$[M+H]^+$). Anal. Calcd for $C_{14}H_{15}N_5O_4$: C, 52.99; H, 4.76; N, 22.07. Found: C, 52.99; H, 4.76; N, 22.07.

4.2.18. (*E*)-*N*′-(2,4-Difluorobenzylidene)-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide (18)

Mp: 273–275 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.29 (s, 3H), 5.41 (s, 2H), 7.54–7.57 (m, 1H), 7.75 (d, J = 1.01 Hz, 1H), 8.06 (d, J = 4.31 Hz, 1H), 8.27 (s, 1H), 8.39 (s, 1H), 12.07 (s, 1H). MS (ESI):324.25 ($C_{13}H_{12}F_2N_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{13}F_2N_5O_3$: C, 48.30; H, 3.43; N, 21.67. Found: C, 48.31; H, 3.41; N, 21.66.

4.2.19. (*E*)-*N*′-(3,4-Dimethoxybenzylidene)-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide (19)

Mp: 273–275 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.30 (s, 3H), 3.82 (s, 6H), 5.39 (s, 2H), 7.54–7.57 (m, 1H), 7.75 (d, J = 1.01 Hz, 1H), 8.06 (d, J = 4.31 Hz, 1H), 8.27 (s, 1H), 8.39 (s, 1H), 12.07 (s, 1H). MS (ESI):324.25 ($C_{15}H_{18}N_5O_5$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{19}N_5O_5$: C, 51.87; H, 4.93; N, 20.16. Found: C, 51.88; H, 4.92; N, 20.17.

4.2.20. (*E*)-2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)-*N*′-(3,4,5-trimethylbenzylidene)acetohydrazide (20)

Mp: 245–246 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.31 (s, 3H), 3.70 (s, 3H), 3.83 (d, J = 2.21 Hz, 6H), 5.40 (s, 2H), 7.06 (s, 1H), 7.97 (s, 1H), 8.31 (s, 1H), 12.07 (s, 1H). MS (ESI):330.35 ($C_{16}H_{20}N_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{19}N_5O_5$: C, 58.35; H, 5.81; N, 21.26. Found: C, 58.34; H, 5.83; N, 21.28.

4.3. Antibacterial activity

The antibacterial activities of the synthetic compounds were tested against two Gram-negative bacterial strains: *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, two Gram-positive bacterial strains: *B. subtilis* ATCC 530 and *S. aureus* ATCC 25923, using method recommended by National Committee for Clinical Laboratory Standards (NCCLS).[31]

In vitro activities of the compounds were tested in Nutrient broth (NB) for bacteria by the twofold serial dilution method. Seeded broth (broth containing microbial spores) was prepared in NB from 24 h old bacterial cultures on nutrient agar (Hi-media) at 37 ± 1 °C. The bacterial suspension was adjusted with sterile saline to a concentration of 1×10^4–10^5 CFU. The tested compounds and reference drugs were prepared by twofold serial dilution to obtain the required concentrations of 100, 50, 25, 12.5, 6.25 and 3.13 μg/mL. The tubes were incubated in BOD incubators at 37 ± 1 °C for bacteria. The MICs were recorded by visual observations after 24 h (for bacteria) of incubation. Kanamycin and DDCP were used as standards for bacterial. The observed MICs are presented in Table 2

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

Full-length *E. coli* acyl carrier protein (ACP), acyl carrier protein synthase (ACPS), and β-ketoacyl-ACP synthase III (FabH) were individually cloned into pET expression vectors with an N-terminal His-tag (ACP, ACPS in pET19; FabH in pET28).

All proteins were expressed in *E. coli* strain BL21 (DE3). Transformed cells were grown on Luria–Bertani (LB) agar plates supplemented with kanamycin (30 μg/mL). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) analysis was used to screen colonies for overexpression of proteins. One such positive colony was used to inoculate 10 mL of LB medium with 30 μg/mL of kanamycin and grown overnight at 37 °C, 1 mL of which was used to inoculate 100 mL LB medium supplemented with 30 mg/mL of kanamycin. The culture was shaken for 4 h at 37 °C, and then induced with 0.5 mM isopropyl β-D-thiogalactopyranoside (IPTG). The culture was grown for 4 h, and harvested by centrifugation (30 min at 15,000 rpm).

Harvested cells containing His-tagged ACP, ACPS, and FabHs 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 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 assays.

Purified ACP contains the apo-form that needs to be converted into the holo-form. The conversion reaction is catalyzed by ACP synthase (ACPS). In the final volume of 50 mL, 50 mg ACP, 50 mM Tris, 2 mM DTT, 10 mM $MgCl_2$, 600 μM CoA, and 0.2 μM ACPS was incubated for 1 h at 37 °C. The pH of the reaction was then adjusted to approximately 7.0 using 1 M potassium phosphate. Holo-ACP was purified by fractionation of the reaction mixture by Source Q-15 ion exchange chromatography using a 0–500 mM NaCl gradient over 25 column volumes.

In a final 20 μL reaction, 20 mM Na_2HPO_4/NaH_2PO_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 mL. After 1 min incubation, a 2 μL mixture of 25 μM acetyl-CoA and 0.75 μCi [^{3}H] acetyl-CoA was added for FabH reaction for 25 min. The reaction was stopped by adding 20 mL 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 ^{3}H 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

The crystal structures of *E. coli* FabH (PDB code: 1HNJ)[31] was obtained from the Protein Data Bank (http://www.rcsb.org). Molecular docking of compound **20** into the three-dimensional X-ray structure of FabH was carried out using Ligand Fit Dock protocol of Discovery Studio 3.1.

Acknowledgements

This work was supported by Jiangsu National Science Foundation (SBC200910626).

References and notes

1. Gastmeier, P.; Sohr, D.; Geffers, C.; Behnke, M.; Daschner, F.; Rüden, H. *Infection* **2005**, *33*, 50.
2. Nikaido, H.; Pages, J. M. *FEMS Microbiol. Rev.* **2012**, *36*, 340–363.
3. Amabile-Cuevas, C. F.; Arredondo-Garcia, J. L.; Cruz, A.; Rosas, I. *J. Appl. Microbiol.* **2010**, *108*, 158–162.
4. Chopra, I.; Schofield, C.; Everett, M.; O'Neill, A.; Miller, K.; Wilcox, M.; Frere, J. M.; Dawson, M.; Czaplewski, L.; Urleb, U.; Courvalin, P. *Lancet Infect. Dis.* **2008**, *8*, 133–139.
5. Boyd, Becnel. L.; Maynard, M. J.; Morgan-Linnell, S. K.; Horton, L. B.; Sucgang, R.; Hamill, R. J.; Jimenez, J. R.; Versalovic, J.; Steffen, D.; Zechiedrich, L *Antimicrob. Agents Chemother.* **2009**, *53*, 229–234.
6. Zhang, H. J.; Qin, X.; Liu, K.; Zhu, D. D.; Zhu, H. L. *Bioorg. Med. Chem.* **2011**, *19*, 4413.
7. Li, Z. L.; Li, Q. S.; Zhang, H. J.; Hu, Y.; Zhu, D. D.; Zhu, H. L. *Bioorg. Med. Chem.* **2011**, *19*, 4413.
8. Lee, J. Y.; Jeong, K. W.; Lee, J. U.; Kang, D. I.; Kim, Y. *Bioorg. Med. Chem.* **2009**, *17*, 5408.
9. Zhang, H. J.; Li, Z. L.; Zhu, H. L. *Curr. Med. Chem.* **2012**, *19*, 1225.
10. Khandekar, S. S.; Daines, R. A.; Lonsdale, J. T. *Curr. Protein Pept. Sci.* **2003**, *4*, 21.
11. Tsay, J. T.; Oh, W.; Larson, T. J.; Jakowski, S. *J. Biol. Chem.* **1992**, *267*, 6807–6814.
12. Heath, R. J.; Rock, C. O. *J. Biol. Chem.* **1996**, *271*, 1833–1836.
13. Christensen, C. E.; Kragelund, B. B.; von Wettstein-Knowles, P.; Henriksen, A. *Protein Sci.* **2007**, *16*, 261.

14. Li, H. Q.; Shi, L.; Li, Q. S.; Lv, P. C.; Luo, Y.; Zhu, H. L. *Bioorg. Med. Chem.* **2009**, *20*, 6264.
15. Puupponen-Pimia, R.; Nohynek, L.; Meier, C.; Kahkonen, M.; Heinonen, M.; Hopia, A.; Oksman-Caldentey, K. M. *J. Appl. Microbiol.* **2001**, *90*, 494–507.
16. Luo, Y.; Zhang, L. R.; Hu, Y.; Zhang, S.; Fu, J.; Wang, X. M.; Zhu, H. L. *ChemMedChem* **2012**, *7*, 1587–1593.
17. Brusic, V.; Petrovsky, N. *Exp. Rev. Clin. Immunol.* **2005**, *1*, 145–157.
18. Uto, Y.; Nagasawa, H.; Jin, C. Z.; Nakayama, S.; Tanaka, A.; Kiyoi, S.; Nakashima, H.; Shimamura, M.; Inayama, S.; Fujiwara, T.; Takeuchi, Y.; Uehara, Y.; Kirk, K. L.; Nakata, E.; Hori, H. *Bioorg. Med. Chem.* **2008**, *16*, 6042.
19. Luo, Y.; Li, H. Q.; Zhou, Y.; Li, Z. L.; Yan, T.; Zhu, H. L. *ChemMedChem* **2010**, *5*, 1100.
20. Boza, A.; Gonzalez, R.; Novoa, H.; Cuéllar, D. M.; Valdés, M. *IL Farmaco* **2000**, *55*, 700.
21. Soedin, K.; Syukran, O.; Fadillah, A.; Sidabutar, P. *Pharmaceutica* **1985**, *4*, 251.
22. Zhang, H. J.; Qin, X.; Liu, K.; Zhu, D. D.; Wang, X. M.; Zhu, H. L. *Bioorg. Med. Chem.* **2011**, *19*, 5708–5715.
23. Lee, J. Y.; Jeong, K. W.; Lee, J. U.; Kang, D. I.; Kim, Y. *Bioorg. Med. Chem.* **2009**, *17*, 1506.
24. Shi, L.; Ge, H. M.; Tan, S. H.; Li, H. Q.; Song, Y. C.; Zhu, H. L.; Tan, R. X. *Eur. J. Med. Chem.* **2007**, *42*, 558.
25. Huang, G. S.; Liang, Y. M.; Wu, X. L.; Liu, W. M.; Ma, Y. X. *Appl. Organomet. Chem.* **2003**, *17*, 706.
26. Curini, M.; Epifano, F.; Maltese, F.; Marcotullio, M. C. *Tetrahedron Lett.* **2002**, *43*, 3821.
27. Yadav, L. D. S.; Yadav, B. S.; Rai, V. K. *Tetrahedron. Lett.* **2004**, *45*, 5351.
28. Zhang, L. X.; Liu, Y.; Cia, L. H.; Hu, Y. J.; Yin, J.; Hu, P. Z. *Thermochim. Acta* **2006**, *440*, 51.
29. Lee, J.-Y.; Jeong, K.-W.; Lee, J.-U.; Kang, D.-I.; Kim, Y. *Bioorg. Med. Chem.* **2009**, *17*, 1506.
30. Mirzaei, J.; Amini, M.; Pirelahi, H.; Shafiee, A. *J. Heterocycl. Chem.* **2008**, *45*, 921.
31. He, X.; Reeve, A. M.; Desai, U. R.; Kellogg, G. E.; Reynolds, K. A. *Antimicrob. Agents Chemother.* **2004**, *48*, 3093.