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Design, synthesis and antimicrobial activities evaluation of Schiff Base derived from secnidazole derivatives as Potential FabH Inhibitors

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Abstract

FabH, b-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 Gramnegative 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-1H-imidazol-1-yl)-N'-(3,4,5-trimethylbenzylidene)acetohydra zide with MIC of 3.13-6.25 µg/mL against the tested bacterial strains was a potent inhibitor of Escherichia coli FabH.

Key words:

Secnidazole derivatives

Schiff base

FabH inhibitors

Antibacterial activities

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 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 coworkers 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] Y. Kim *et. al* reported the YKAs3003, a Schiff base condensed by

4-hydroxy salicylaldehyde and cyclohexanamine as a potential inhibitor of Escherichia coli (E.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 (E. 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-1H-imidazol-1-yl)acetohydrazide was prepared by the reaction of 2-methyl-5-nitro-1H-imidazole in excess ethyl α -chloroacetate and then hydrazinolysis of ethyl ester group by hydrazine hydrate according to Javad Mirzaei *et al.*'s method.^[28]

Then 2-(2-Methyl-5-nitro-1H-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 ($\pm 0.4\%$). ¹H 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)^[39] 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-1H-imidazol-1-yl)-N'-(3,4,5-trimethylbenzylidene)acetohydra zide, 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 $1.56 \mu g/mL$. From the data we got, the varieties of substitutes of benzaldehyde 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 CH₃, OCH₃) (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 μ g/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 μ g/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 IC50 of 2.2 μ M; compound 1 showed the weakest inhibitory activity with IC50 of 58.3 μ M. 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.

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 PiCationMonitor 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. Conclusion

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 first time. Many of them exhibited potent antibacterial and *E.coli* FabH inhibitory activities. Particularly, Compound **20** (E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)-N'-(3,4,5-trimethylbenzylidene)acetohydrazide with MIC of $3.13-6.25 \mu g/mL$ 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 ¹H 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-1H-imidazol-1-yl)acetohydrazide was prepared according the previous procedure by JavadMirzaei *et al.*^[29]

An equimolar mixture of 2-(2-methyl-5-nitro-1H-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**.

(E)-N'-(4-fluorobenzylidene)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetohydrazi de (1)

mp: 259-260°C. 1H NMR (500 MHz, DMSO-d6) 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=17.55Hz, 1H), 11.84 (s,
1H). MS (ESI):306.26 (C13H13FN5O3, [M+H]+). Anal. Calcd for C13H12FN5O3:
C, 51.15; H, 3.96; N, 22.94. Found: C, 51.17; H, 3.95; N, 22.93.

(E)-N'-(4-chlorobenzylidene)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetohydrazi de (2)

mp: 254-255°C. 1H NMR (500 MHz, DMSO-d6) δ: 2.31 (s, 3H), 5.39 (s, 2H), 7.52 (t, J=10.45Hz, 2H), 7.76 (t, J=17.38Hz, 2H), 8.05 (s, 1H), 8.27 (t, J=18.65Hz, 1H), 11.89 (s, 1H). MS (ESI):322.72 (C13H13CIN5O3, [M+H]+). Anal. Calcd for C13H12CIN5O3: C, 48.52; H, 3.76; N, 21.77. Found: C, 48.54; H, 3.75; N, 21.78.

(E)-N'-(4-bromobenzylidene)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetohydrazi de (3)

mp: 229-230°C. 1H NMR (500 MHz, DMSO-d6) δ: 2.31 (s, 3H), 5.39 (s, 2H), 7.63-7.72 (m, 4H), 8.04 (s, 1H), 8.29 (d, J=7.75Hz, 1H), 11.89 (s, 1H). MS (ESI):367.17 (C13H13BrN5O3, [M+H]+). Anal. Calcd for C13H12BrN5O3: C, 42.64; H, 3.30; N, 19.13. Found: C, 42.65; H, 3.31; N, 19.14.

(E)-N'-(4-methoxybenzylidene)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetohydra zide (4)

mp: 194-196°C. 1H NMR (500 MHz, DMSO-d6) δ: 2.30 (d, J=5.17Hz, 3H), 3.81 (s, 3H), 5.37 (s, 2H), 7.02(d, J=4.42Hz, 2H), 7.65-7.70 (m, 2H), 8.00 (s, 1H), 8.30 (d, J=5.80Hz, 1H), 11.70 (d, J=7.63Hz, 1H). MS (ESI):318.30 (C14H16N5O4, [M+H]+). Anal. Calcd for C14H15N5O4: C, 52.99; H, 4.76; N, 22.07. Found: C, 52.98; H, 4.75; N, 22.09.

(E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)-N'-(4-nitrobenzylidene)acetohydrazide (5)

mp: 247-248°C. 1H NMR (300 MHz, DMSO-d6) δ: 2.32 (s, 3H), 5.45 (s, 2H), 8.00 (t, J=9.87Hz, 2H), 8.17 (s, 1H), 8.29-8.35 (m, 3H), 12.13 (s, 1H). MS (ESI):333.27

(C13H13N6O5, [M+H]+). Anal. Calcd for C13H12N6O5: C, 46.99; H, 3.64; N, 25.29. Found: C, 46.98; H, 3.65; N, 25.26.

(E)-N'-(4-hydroxybenzylidene)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetohydra zide (6)

mp: 294-296°C. 1H NMR (300 MHz, DMSO-d6) δ: 2.29 (s, 3H), 5.34 (s, 2H), 6.83 (d, J=4.31Hz, 2H), 7.55 (t, J=8.78Hz, 2H), 7.95 (s, 1H), 8.30 (d, J=3.12Hz, 1H), 9.94 (s, 1H), 11.64 (d, J=4.49Hz, 1H). MS (ESI):304.27 (C13H14N5O4, [M+H]+). Anal. Calcd for C13H13N5O4: C, 51.48; H, 4.32; N, 23.09. Found: C, 51.49; H, 4.31; N, 23.07.

(E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)-N'-(4-methylbenzylidene)acetohydrazi de (7)

mp: 208-210°C. 1H NMR (300 MHz, DMSO-d6) δ: 2.29 (s, 3H), 2.34 (s, 3H), 5.33 (s, 2H), 6.75 (d, J=4.38Hz, 2H), 7.53 (t, J=7.86Hz, 2H), 7.91 (s, 1H), 8.31 (d, J=2.30Hz, 1H), 11.54 (d, J=6.03Hz, 1H). MS (ESI):302.30 (C14H16N5O3, [M+H]+). Anal. Calcd for C14H15N5O3: C, 55.81; H, 5.02; N, 23.24. Found: C, 56.81; H, 5.01; N, 23.25.

(E)-N'-(4-hydroxybenzylidene)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetohydra zide (8)

mp: 277-278°C. 1H NMR (300 MHz, DMSO-d6) δ: 2.29 (s, 3H), 2.97 (s, 6H), 5.33 (s, 2H), 6.74 (d, J=4.02Hz, 2H), 7.53 (t, J=7.68Hz, 2H), 7.92 (s, 1H), 8.30 (d, J=2.30Hz, 1H), 11.54 (d, J=5.85Hz, 1H). MS (ESI):331.34 (C15H19N6O3, [M+H]+). Anal. Calcd for C15H18N6O3: C, 54.54; H, 5.49; N, 25.44. Found: C, 54.55; H, 5.48; N, 25.42.

(E)-N'-(3-fluorobenzylidene)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetohydrazi de (9)

mp: 222-223°C. 1H NMR (500 MHz, DMSO-d6) δ: 2.29 (s, 3H), 5.41 (s, 2H), 7.28 (t, J=8.40Hz, 1H), 7.48-7.53 (m, 1H), 7.57 (d, J=3.83Hz, 1H), 7.62 (d, J=5.05Hz, 1H), 8.06 (s, 1H), 8.28 (t, J=16.63Hz, 1H), 11.93 (s, 1H). MS (ESI):306.26

(C13H13FN5O3, [M+H]+). Anal. Calcd for C13H12FN5O3: C, 51.15; H, 3.96; N, 22.94. Found: C, 51.18; H, 3.97; N, 22.93.

(E)-N'-(3-fluorobenzylidene)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetohydrazi de (10)

mp: 197-198°C. 1H NMR (500 MHz, DMSO-d6) δ: 2.29 (s, 3H), 5.42 (s, 2H), 7.42 (t, J=7.80Hz, 1H), 7.63 (d, J=3.95Hz, 1H), 7.72 (d, J=3.83Hz, 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 (C13H13BrN5O3, [M+H]+). Anal. Calcd for C13H12BrN5O3: C, 42.64; H, 3.30; N, 19.13. Found: C, 42.65; H, 3.31; N, 19.15.

(E)-N'-(3-fluorobenzylidene)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetohydrazi de (11)

mp: 259-260°C. 1H NMR (300 MHz, DMSO-d6) 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=14.91Hz, 1H), 11.96 (s, 1H). MS (ESI):322.72 (C13H13CIN5O3, [M+H]+). Anal. Calcd for C13H12CIN5O3: C, 48.53; H, 3.76; N, 21.77. Found: C, 48.55; H, 3.75; N, 21.78.

(E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)-N'-(3-nitrobenzylidene)acetohydrazide (12)

mp: 256-257°C. 1H NMR (500 MHz, DMSO-d6) 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=5.20Hz, 1H), 8.35 (d, J=11.28Hz, 1H), 8.56 (d, J=6.40Hz, 1H), 12.08 (s, 1H). MS (ESI):334.27 (C13H13N6O5, [M+H]+). Anal. Calcd for C13H12N6O5: C, 46.99; H, 3.64; N, 25.29. Found: C, 46.98; H, 3.65; N, 25.27.

(E)-N'-(3-methoxybenzylidene)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetohydra zide (13)

mp: 165-165°C. 1H NMR (300 MHz, DMSO-d6) δ: 2.29 (s, 3H), 3.81 (s, 3H), 5.40(s, 2H), 7.02 (d, J=4.58Hz, 1H), 7.26-7.44 (m, 3H), 8.02 (s, 1H), 8.30 (d, J=3.93Hz, 1H), 11.85 (s, 1H). MS (ESI):318.30 (C14H16N5O4, [M+H]+). Anal. Calcd for C14H16N5O4: C, 52.99; H, 4.76; N, 22.07. Found: C, 52.98; H, 4.75; N,

22.08.

(E)-N'-(2-chlorobenzylidene)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetohydrazi de (14)

mp: 257-258°C. 1H NMR (300 MHz, DMSO-d6) δ: 2.29 (s, 3H), 5.41 (s, 2H), 7.42-7.49 (m, 2H), 7.55 (t, J=4.76Hz, 1H), 8.05 (t, J=4.76Hz, 1H), 8.30 (d, J=5.94Hz, 1H), 8.45 (s, 1H), 12.03 (s, 1H). MS (ESI):322.72 (C13H13ClN5O3, [M+H]+). Anal. Calcd for C13H12ClN5O3: C, 48.53; H, 3.76; N, 21.77. Found: C, 48.52; H, 3.75; N, 21.78.

(E)-N'-(2-fluorobenzylidene)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetohydrazi de (15)

mp: 231-232°C. 1H NMR (500 MHz, DMSO-d6) δ: 2.30 (s, 3H), 5.40 (s, 2H), 7.31 (t, J=8.23Hz, 2H), 7.50 (t, J=6.25Hz, 1H), 7.98 (t, J=7.63Hz, 1H), 8.28 (d, J=3.38Hz, 1H), 8.45 (s, 1H), 11.95 (s, 1H). MS (ESI):306.26 (C13H13FN5O3, [M+H]+). Anal. Calcd for C13H12FN5O3: C, 51.15; H, 3.96; N, 22.94. Found: C, 51.14; H, 3.94; N, 22.96.

(E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)-N'-(2-nitrobenzylidene)acetohydrazide (16)

mp: 269-270°C. 1H NMR (300 MHz, DMSO-d6) δ: 2.30 (s, 3H), 5.40 (s, 2H), 7.70 (t, J=7.77Hz, 1H), 7.84 (t, J=7.14Hz, 1H), 8.08-8.17 (m, 2H), 8.28 (s, 1H), 8.46 (s, 1H), 12.15 (s, 1H). MS (ESI):333.27 (C13H13N6O5, [M+H]+). Anal. Calcd for C13H12N6O5: C, 46.99; H, 3.64; N, 25.29. Found: C, 46.97; H, 3.65; N, 25.31.

(E)-N'-(2-methoxybenzylidene)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetohydra zide (17)

mp: 279-280°C. 1H NMR (300 MHz, DMSO-d6) δ: 2.29 (s, 3H), 3.86 (s, 3H), 5.38 (s, 2H), 7.01 (t, J=6.87Hz, 1H), 7.12 (d, J=4.22Hz, 1H), 7.41-7.46 (m, 1H), 7.89 (t, J=3.86Hz, 1H), 8.29 (s, 1H), 8.40 (s, 1H), 11.80 (s, 1H). MS (ESI):318.30 (C14H16N5O4, [M+H]+). Anal. Calcd for C14H15N5O4: C, 52.99; H, 4.76; N, 22.07. Found: C, 52.99; H, 4.76; N, 22.07.

(E)-N'-(2,4-difluorobenzylidene)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetohydr azide (18)

mp: 273-275°C. 1H NMR (300 MHz, DMSO-d6) δ: 2.29 (s, 3H), 5.41 (s, 2H), 7.54-7.57 (m, 1H), 7.75 (d, J=1.01Hz, 1H), 8.06 (d, J=4.31Hz, 1H), 8.27 (s, 1H), 8.39 (s, 1H), 12.07 (s, 1H). MS (ESI):324.25 (C13H12F2N5O3, [M+H]+). Anal. Calcd for C13H13F2N5O3: C, 48.30; H, 3.43; N, 21.67. Found: C, 48.31; H, 3.41; N, 21.66.

(E)-N'-(3,4-dimethoxybenzylidene)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetohy drazide (19)

mp: 273-275°C. 1H NMR (300 MHz, DMSO-d6) δ: 2.30 (s, 3H), 3.82 (s, 6H), 5.39 (s, 2H), 7.54-7.57 (m, 1H), 7.75 (d, J=1.01Hz, 1H), 8.06 (d, J=4.31Hz, 1H), 8.27 (s, 1H), 8.39 (s, 1H), 12.07 (s, 1H). MS (ESI):324.25 (C15H18N5O5, [M+H]+). Anal. Calcd for C15H19N5O5: C, 51.87; H, 4.93; N, 20.16. Found: C, 51.88; H, 4.92; N, 20.17.

(E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)-N'-(3,4,5-trimethylbenzylidene)acetoh ydrazide (20)

mp: 245-246°C. 1H NMR (300 MHz, DMSO-d6) δ: 2.31 (s, 3H), 3.70 (s, 3H), 3.83 (d, J=2.21Hz, 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 (C16H20N5O3, [M+H]+). Anal. Calcd for C15H19N5O5: 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 two-fold 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 ×104-105 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 mMTris, 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 mMTris, 2 mM DTT, 10 mM MgCl₂, 600 μ M CoA, and 0.2 μ M 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 μ L reaction, 20 mM Na₂HPO₄/NaH₂PO₄, pH 7.0, 0.5 mM DTT, 0.25 mM MgCl₂, and 2.5 μ Mholo-ACP were mixed with 1 nM FabH, and H₂O was added to 15 mL. After 1 min incubation, a 2 μ L mixture of 25 μ M acetyl-CoA and 0.75 μ Ci [³H] 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 pelletwaswashed with 10% ice-cold TCA and resuspended with 5 μ L of 0.5 MNaOH. The incorporation of the ³H signal in the final product was read by liquid scintillation. When determining the inhibition constant (IC₅₀), 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.

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Reference

[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] Becnel Boyd, 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. Hai-Liang Zhu. 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. Chem. Med. Chem. 2012, 7, 1587-1593.

[17] V. Brusic, N. Petrovsky, 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. Chem. Med. Chem.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. Organo-met. 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.

[32] Qiu, X.; Janson, C. A.; Smith, W. W.; Head, M.; Lonsdale, J.; Konstantinidis, A.
K. *J. Mol. Biol.* 2001, *307*, 341.

[33] National Committee for Clinical Laboratory Standards, in: Methods for Dilution

Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, fifth ed. National Committee for Clinical Laboratory Standards, Villanova, PA, 2000 Approved Standard. NCCLS Document M7-A5.

Legends for Figures and Schemes :

Scheme 1. Synthesis of nitroimidazole derivatives 1-20.

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

Scheme 1









Table 1

Structures of Compounds 1-20

Compounds	R	Compounds	R
1	4-F	11	3-Cl
2	4-C1	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	3,4-20CH ₃
10	3-Br	20	3,4,5-3CH ₃
Table 2			. 7

Table 2

Antibacterial activities of synthetic compounds.

	Compounds	Minimum inhibitory concentrations (µg/mL)				
		Gram-ne	egative	Gram-positive		
	-	Escherichia	Pseudomonas	Bacillus	Staphylococcus	
		coli	aeruginosa	subtilis	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	$\boldsymbol{\lambda}$
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	

Table 3

E. coli FabH inhibitory activities of selected compound 4-6.

E. coli FabH inhibitory activities of top compounds		
IC ₅₀ (μM)		
58.3±3.9		
4.5±0.3		
6.2±0.4		
15.9±0.9		
47.5±4.2		
19.8±1.4		
42.4±3.5		
8.8±0.7		
2.3±0.2		
2.1±0.1		

Table 4

The docking calculation of the synthesized compounds (1-20)

Compounds	CDOCKER INTERATION	Compounds CDOCKER INTERATION	
	ENERGY		ENERGY
	ΔGb (kcal/mol)		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

Design, synthesis and antimicrobial activities evaluation of Schiff Base

derived from secnidazole derivatives as Potential FabH Inhibitors

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FabH, b-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 Gramnegative 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-1H-imidazol-1-yl)-N'-(3,4,5-trimethylbenzylidene)acetohydraz ide with MIC of 3.13-6.25 μ g/mL against the tested bacterial strains was a potent inhibitor of Escherichia coli FabH.