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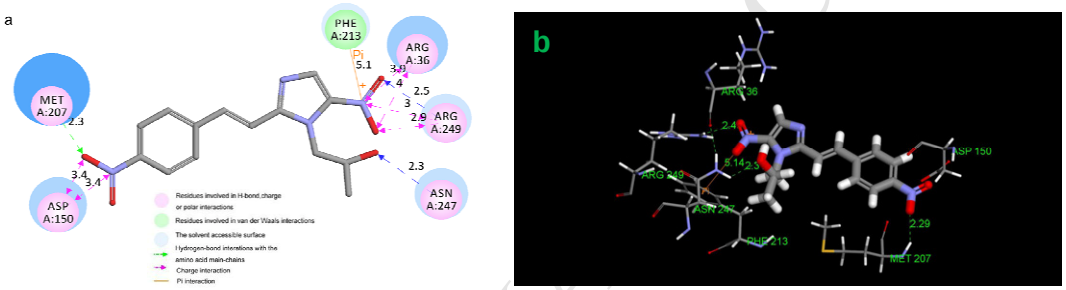

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Abstract

A series of novel 1-(2-hydroxypropyl)-2-styryl-5-nitroimidazole derivatives had been designed, synthesized, isolated and evaluated as potentiators of antibacterial agents. All these synthesized compounds were determined by elemental analysis, ^{1}H-NMR, and MS. Their biological activities were also evaluated against two Gram-negative bacterial strains: *Escherichia coli* and *Pseudomonas aeruginosa* and two Gram-positive bacterial strains: *Bacillus thuringiensis* and *Bacillus subtilis* by MTT method as potential FabH inhibitory. The results showed that compound **30** exhibited the most potent *E. coli* FabH inhibitory activity with IC_{50} of 4.6 μM. Molecular modeling simulation studies were performed in order to predict the biological activities of the proposed compounds. All compounds have been tested for toxicity by MTT assay on human macrophage.

Keywords:

Nitroimidazole derivatives

FabH inhibitors

Antibacterial activities

Cytotoxicity

MTT assay

1 Introduction：

Starting from the traditional antibiotics many antibacterials are known and commercially available [1]. However, the major obstacle in the antimicrobial drug therapy has come to be the drug resistance. Therefore, the spread of antibiotic resistance among pathogenic bacteria has become a serious puzzle for the clinical management of infectious diseases and has resulted in a clear need for novel antibacterial agents [2, 3]. To solve this severe medical problem, the urgent task of seeking out new types of antibacterial agents should be accomplished [4]. In recent years, diverse targets in key areas of the bacterial cell cycle have been studied and correlative researches showed the prospect of finding a new approach against the challenge of drug resistance. Among all the targets, the fatty acid synthase (FAS) pathway in bacteria is a promising one. Fatty acid biosynthesis (FAB) is an essential metabolic process for prokaryotic organisms and is required for cell viability and growth. This pathway has been demonstrated to be essential for bacteria cell survival, and differs considerably from human FAS pathway [5, 6]. A key enzyme responsible for initiation of bacterial fatty acid biosynthesis has so far escaped serious attention by the drug discovery industry. FabH, a β-keto-acyl-ACP synthase, plays an essential and regulatory role in bacterial FAB [7, 8]. As shown in Fig. 1, FabH catalyzes condensation reaction between a CoA-attached acetyl group and an ACP-attached malonyl group, which yields acetoacetyl-ACP as its final product [9].

Nitroimidazoles and their derivatives have been extensively used as antimicrobial chemotherapeutics and as antiangiogenic hypoxic cell radiosensitizers [10]. Therefore, nitroimidazole derivatives have attracted considerable attention as they show a tendency to penetrate and accumulate in regions of bacterium, especially in the organic synthesis [11, 12]. The 5-nitroimidazole core is the most commonly antimicrobial drug particularly secnidazole and metronidazole which is accepted as drug of choice for anti-infectious chemotherapy against bacteria [13]. Importantly, the toxicology and metabolism of nitroimidazoles have been characterized [14].

Secnidazole is rapidly and completely absorbed after oral administration and has a longer terminal elimination half-life (17-29 h) than commonly used drugs in this class [15]. In this case, the treatment with secnidazole is shorter and more effective than the treatment using other imidazole drugs and the adverse effects are not very drastic [16]. In addition, resistance has been induced in vitro against all commonly used antibacterial drugs, including different 5-nitroimidazole derivatives, furazolidone,

albendazole, and quinacrine, [17,18] further demonstrating the need for new compounds to preempt resistance development. Prior studies suggested that modifications in the 2-position of the imidazole ring (C2 position) can lead to effective 5-nitroimidazole derivatives drugs.[19,20] As a result, excellent works have been published on the activities and pharmacokinetics of 1-(2-hydroxypropyl)-2-styryl-5-nitroimidazole derivatives and their determination in pharmaceutical is of great importance [21, 22].

As a part of our search for basic information about the structural requirements for new antimicrobial activities of an existing drug, we designed and synthesized a series of novel 1-(2-hydroxypropyl)-2-styryl-5-nitroimidazole derivatives bearing a substituted styryl unit directly attached to the C2 position which present their *in vitro* antibacterial activity against *Escherichia coli* (*E.coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Bacillus thuringiensis* (*B. thuringiensis*), *Bacillus subtilis* (*B.subtilis*)

In this study, the synthesis, *in vitro* biological activity, and mode of binding of these compounds are described, and the structure-activity relationships (SAR) are discussed. Initial studies are performed by modification of the parent compound to determine if any of the subunits which comprise the secnidazole 2-substituent display antibacterial activity. Furthermore, docking simulations were performed using the X-ray crystallographic structure of the FabH in complex with an inhibitor to explore the binding modes of these compounds at the active site.

2. Chemistry:

A series of novel 1-(2-hydroxypropyl)-2-styryl-5-nitroimidazole derivatives FabH inhibitors (**22-42**) were synthesized following the synthetic pathway depicted in Scheme 1[a]. By reacting 1-(2-methyl-5-nitro-1H-imidazol-1-yl) propan-2-ol with the corresponding different substituted benzaldehydes in the presence of sodium methoxide in methanol, we achieved the corresponding target compounds in good yields. The reactions were monitored by thin layer chromatography (TLC) and the products were purified by recrystallization with ethanol. All of the target compounds gave satisfactory analytical and spectroscopic data, which in accordance with their depicted structures by ^{1}H NMR, ESI MS and single crystal X-ray structural analysis. In addition, these compounds were synthesized for the first time. Furthermore, the crystal structure of compounds **32, 33** and **34** were determined by single crystal X-ray diffraction analysis in Fig. 2a, Fig. 2b and Fig. 2c.

2.1 Molecular Modeling.

The protein structure of the *E. coli* FabH was downloaded from the PDB (1HNJ.pdb) [7]. And preprocessed using the Schrodinger Protein Preparation Guide [23], hydrogen atoms were added to the structure, H-bonds within the protein were optimized, and the protein was minimized to an rmsd of 0.3 Å. A 9.9 Å sphere of water molecules was added around the ligand and a short (3ps) dynamics run was carried out, followed by several cycles of minimization utilizing Quanta/CHARMm. The entire protein-ligand-water complex was free to move during these calculations [24].

The binding model of compounds **30** and *E. coli* FabH was depicted in Fig. 3. The amino acid residues which had interaction with FabH as well as bond length were labeled. In the binding mode with the **30** (Fig. 3), the hydroxy and nitro of the 1-(5-Nitro-2-(4-nitrostyryl)-1H-imidazol-1-yl) propan-2-ol forms a hydrogen bond with the backbone NH of Asn247 (angle O · H-D = 123.7.6^{o}, distance = 2.3 Å), Arg249 (angle O · H-H = 116.2^{o}, distance = 2. 5 Å), respectively. And the nitro of the benzene forms a hydrogen bond with the backbone NH of Met207 (angle O · H-N = 141.6^{o}, distance = 2.3 Å). In addition, compound **30** was also nicely bound to FabH via six charge interactions and one π-π interaction. The end group of Arg36, Arg249 and Asp150 respectively formed six charge interactions with two nitro group, which was accordant exactly with the previous work of FabH inhibitors [25]. Meanwhile, the nitrogen atom of nitro group formed a π-π interaction with Phe213. This molecular docking result, along with the biological assay data, suggested that compound **30** was a potential inhibitor of FabH.

3. Biological activity

3.1 Antimicrobial activity

Our interest in the secnidazole analogues as an alternative to antibacterial treatment was facilitated by the fact that not only secnidazole is effective but also the imidazole ring structure attached by side chain provides an opportunity to carry out various modifications. For these reasons, a series of novel 1-(2-hydroxypropyl)-2-styryl-5-nitroimidazole derivatives (**22**-**42**) had been designed, synthesized. And compounds **22**-**42** were assayed against two Gram-negative bacterial strains: *E. coli* and *P. aeruginosa* and two Gram-positive bacterial strains: *B. subtilis* and *B. thuringiensis* by MTT method. In this assay, the IC_{50} values of compounds possessing

sufficiently potent antibacterial activity were shown in Table 1. The results were compared with that provided by the known antibiotic: Kanamycin under identical conditions, which showed that most of the synthesized compounds exhibited significant antibacterial activities.

The results obtained from *in vitro* antibacterial evaluation of compounds **22**-**42** against susceptible and resistant strains were moderate. Most compounds exhibited moderate to good activity against all Gram-positive and Gram-negative strains tested. Best potent activities *in vitro* were got for compound **30** against *E. coil* with corresponding IC_{50} 36.8 μg/mL superior as compared to the positive control Kanamycin. In addition, these data suggested that compounds **30**, **36**, **36** and **33** exhibited distinguished activities with IC_{50} values of 36.8, 5.7, 3.4 and 6.1 μg/mL against *E.coli*, *P. aerμginosa*, *B. subtilis* and *B. thuringiensis*, respectively.

Based on the data obtained, we surveyed a variety of substituents at different positions on the phenyl ring of these 1-(2-hydroxypropyl)-2-styryl-5-nitroimidazole derivatives, and found that various substituents of such as halogen, methyl, methoxyl and nitro group led to distinct antibacterial activities of these target compounds. Compound **30** with a nitro group on the 4-position of phenyl ring manifested higher antibacterial activity with IC_{50} value of 36.8 μg/mL against *E. coil* than other compounds, while the simplest compound **22** of this styryl series with a bare phenyl ring displayed the worst antibacterial activity against *E. coil*. Substitutions at the phenyl with one methyl moieties did not improve the activity of compound **35** markedly, nor did halogen substitutions on the phenyl ring (**24**-**28**, **32**-**34**, **36**, **37**, **40**). Toxicity was only very slightly increased by most of these modifications, with the exceptions of the *m*-fluorine, and *o*-fluorine substitutions at the correspondence position of the phenyl ring, which increased toxicity moderately (**25** and **33** respectively) but not to the level attained by the compound **22**. Addition of larger side chains at the phenyl moiety, including *m*-OMe (**31**) and N $(CH_3)_2$ (**38**), or larger naphthalene ring (**42**), markedly reduced the activity against *E. coil*, while addition of *p*-OMe (**23**) did not affect antibacterial activity or toxicity. On the other hand, compound **30** with a nitro group on the 4-position of phenyl ring displayed higher antibacterial activity with IC_{50} value of 36.8 μg/mL against *E. coil* than compounds with a nitro group on the 2-position (40.3 μg/mL) and 3-position (38.8 μg/mL) of phenyl ring, while the activity gradient of substituent group on the phenyl ring in *m*-position was - NO_2 > - Cl >- F > - OCH_3. Together, these results illustrate that

derivatives of 5-nitroimidazole derivatives bearing an olefin directly attached to the C2 position of the imidazole ring have potent antibacterial activities.

In comparison, we found that these compounds, with bulky and electron-withdrawing group on the benzene ring (such as NO_2, F, Cl, Br), exhibited more potent antibacterial activities than those have electron-donating substituents (such as CH_3, OCH_3). From the above-mentioned analysis, it could be concluded that the compounds with a bare naphthoic ring (108.4 μg/mL) and nitro or halogen substituted benzene ring were found to be the most favorable for the antibacterial activity. More importantly, most of the analogues possess low cytoxicity.

3.2 *E. coli* FabH inhibitory activity

To generate data concerning the broad spectrum potential of these compounds in Table 1, the IC_{50} values of selected compounds against FabH enzymes were summarized in Table 1. Reference data for Kanamycin had also been included for comparison with the compounds reported in this study. It was possible that this result reflects a lack of selectivity and that these compounds inhibit other proteinases. In fact, we knew that the selectivity of these compounds for *E. coli* FabH can be modulated by changing the substitution pattern on the benzaldehyde. This would be the subject of future communications. While *E. coli* FabH activities would make a straightforward interpretation of these results difficult, some trends are evident. For the majority of the compounds, we found that compounds **30** and **39** with IC_{50} of 4.6 μM and 5.1 μM were better inhibitors than the positive control Kanamycin with IC_{50} of 6.3 μM, suggesting that, at least in part, inhibition of proliferation of the these lines be the result of *E. coli* FabH inhibition.

3.3 Cytotoxicity

All compounds were evaluated for their toxicity against human macrophage with the median cytotoxic concentration (CC_{50}) data of tested compounds by the MTT assay, as showed in Table 1. These compounds were tested at multiple doses to study the viability of macrophage.

Further more, after cells had been cultured for 24 h, morphological anomalies in macrophage exposed to different concentration compound **41** (2.5, 10, 40, 160 μg/mL) showed nothing different from morphological in control under phase contrast microscope (Fig. 4).

As shown in Table 1, 21 compounds demonstrated almost no cytotoxic activities *in*

vitro against macrophage.

4. Conclusion:

A series of novel 1-(2-hydroxypropyl)-2-styryl-5-nitroimidazole derivatives have been designed and synthesized by reaction of 1-(2-methyl-5-nitro-1H-imidazol-1-yl) propan-2-ol with the corresponding different substituted benzaldehydes. These compounds showed a very interesting profile for their inhibitory activities against *E. coli*, *P. aeruginosa*, *B. subtilis* and *B. thuringiensis* at low concentrations ranging from micrograms to milligrams per milliliter. Most of them exhibited *E. coli* FabH inhibitory activities and almost no toxicity towards morphological. The substitution pattern at the benzene ring is the main player in controlling the inhibitory power. Several beneficial substitution patterns were detected, among some 4-nitro, and 2-fluorine, and 2,4-disubstituted 1-(2-hydroxypropyl)-2-styryl-5-nitroimidazoles incorporating alkyl, alkoxy, aminomethyl, halogen, or nitro moieties. Compound **30** with a nitro group on the 4-position of phenyl ring displayed higher antibacterial activity with IC_{50} value of 36.8 μg/mL against *E. coil* than compounds with a nitro group on the 2-position (40.2 μg/mL) and 3-position (38.8 μg/mL) of phenyl ring. Docking simulation was performed to position compound **30** into the *E. coli* FabH active site to determine the probable binding conformation and the result indicated that compound **30** was a potent inhibitor of *E. coli* FabH. Given the unforeseen structural differences within the active site of some pathogenic enzymes, the key to discovering inhibitors with broad-spectrum antibacterial activity lies in a detailed understanding of the FabH active sites. Further studies on the FabH inhibition ability of this compound, new structural data were guiding further modifications of the current series with the hopes of improving both enzymatic inhibition and physical properties.

5. Experiments

5.1 Materials and measurements

All chemicals used were purchased from Aldrich (USA). All reagents used in current study were of analytical grade. Thinlayer chromatography (TLC) was performed on silica gel plates with fluorescent indicator. All analytical samples were homogeneous on TLC in at least two different solvent systems. Melting points (uncorrected) were determined on a X-4 MP apparatus (Taike Corp, Beijing, China). All the ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX 300 model Spectrometer in DMSO-d_6 and chemical shifts (δ) were reported as parts per million

(ppm). ESI-MS spectra were recorded on a Mariner System 5304 Mass spectrometer. Elemental analyses were performed on a CHN-O-Rapid instrument and were within ±0.4% of the theoretical values.

5.2 General Procedure for the Synthesis of Compounds 22-42.

To a solution of the 1-(2-methyl-5-nitro-1H-imidazol-1-yl) propan-2-ol (12 mmol) in DMSO (6 mL) at room temperature were added aromatic aldehydes **1-21** (16 mmol) and sodium methoxide in methanol (12.8 mmol). The reaction mixture was stirred at room temperature until TLC ($V_{EtOAc}/V_{hexanes}$ = 1:2) indicated reaction completion (generally 4-5 h) and poured into ice-water (250 mL). The precipitate formed was collected, washed three times with distilled water, and dried under vacuum. The crude products were purified by recrystallization with ethanol, ethyl acetate and acetone (1:1:0.05) washed by ice-water (25 mL) for three times to give a pure product.

5.2.1. 1-(5-Nitro-2-styryl-1H-imidazol-1-yl) propan-2-ol (**22**)

Light yellow crystals, yield 76.1%, m.p. 77~78 ˚C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.21 (s, 1H, CH), 7.80~7.75 (m, 3H, ArH, CH), 7.47~7.35 (m, 4H, ArH), 5.02 (d, J=5.4 Hz, 1H, OH), 4.58~4.39 (m, 2H, CH_2), 3.89~3.87 (m, 2H, CH), 1.16 (d, J=6.3 Hz, H, CH_3). ESI-MS: 273.20 $[M+H]^+$. Anal. Calcd for $C_{14}H_{15}N_3O_3$: C, 61.53; H, 5.53; N, 15.38; O, 17.56; Found: C, 61.52; H, 5.54; N, 15.37; O, 17.57%.

5.2.2. 1-(2-(4-Methoxystyryl)-5-nitro-1H-imidazol-1-yl) propan-2-ol (**23**)

Yellow crystals, yield 63.5%, m.p. 64~65 ˚C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.19 (s, 1H, CH), 7.76~7.70 (m, 2H, ArH), 7.24~7.19 (m, 2H, ArH), 6.99 (d, J=8.6 Hz, 2H, CH), 5.00 (d, J=5.4 Hz, 1H, OH), 4.56~4.37 (m, 2H, CH_2), 3.87~3.81 (m, 4H, CH, CH_3), 1.15 (d, J =6.2 Hz, 3H, CH_3). ESI-MS: 303.11 $[M+H]^+$. Anal. Calcd for $C_{15}H_{17}N_3O_4$: C, 59.40; H, 5.65; N, 13.85; O, 21.10; Found: C, 59.38; H, 5. 67; N, 13.79; O, 21.16%.

5.2.3. 1-(2-(4-Bromostyryl)-5-nitro-1H-imidazol-1-yl) propan-2-ol (**24**)

Orange crystals, yield 71.2%, m.p. 65~66 ˚C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.21 (s, 1H, CH), 7.77~7.72 (m, 3H, ArH), 7.63 (d, J=8.5 Hz, 2H, CH), 7.44~7.39 (m,

1H, ArH), 5.00 (d, *J*=5.4 Hz, 1H, OH), 4.58~4.40 (m, 2H, CH_2), 3.90~3.84 (m, 1H, CH), 1.16 (d, *J*=6.2 Hz, 3H, CH_3). ESI-MS: 352.01 $[M+H]^+$. Anal. Calcd for $C_{14}H_{14}BrN_3O_3$: C, 47.74; H, 4.01; Br, 22.69; N, 11.93; O, 13.63; Found: C, 47.69; H, 4.09; Br, 22.70; N, 11.91; O, 13.61%.

5.2.4. 1-(2-(3-Fluorostyryl)-5-nitro-1H-imidazol-1-yl) propan-2-ol (**25**)

Light yellow crystals, yield 73.4%, m.p. 82~83 ˚C; ^{1}H NMR (DMSO-*d*6, 300 MHz) *δ*: 8.21 (s, 1H, CH), 7.79~7.68 (m, 2H, CH), 7.58~7.40 (m, 3H, ArH), 7.23~7.17 (m, 1H, ArH), 5.01 (d, *J*=5.4 Hz, 1H, OH), 4.59~4.41 (m, 2H, CH_2), 3.88~3.86 (m, 1H, CH). 1.17 (d, *J*=6.6 Hz, 3H, CH_3). ESI-MS: 291.09 $[M+H]^+$. Anal. Calcd for $C_{14}H_{14}FN_3O_3$: C, 57.73; H, 4.84; F, 6.52; N, 14.43; O, 16.48; Found: C, 57.69; H, 4.85; F, 6.51; N, 14.42; O, 16.53%.

5.2.5. 1-(2-(4-Chlorostyryl)-5-nitro-1H-imidazol-1-yl) propan-2-ol (**26**)

Light yellow crystals, yield 62.5%, m.p. 78~79 ˚C; ^{1}H NMR (DMSO-d_6, 300 MHz) *δ*: 8.21 (s, 1H, CH), 7.82~7.73 (m, 3H, ArH), 7.51~7.37 (m, 3H, ArH, CH), 5.01 (d, *J*=5.4 Hz, 1H, OH), 4.57~4.45 (m, 2H, CH_2), 3.93~3.80 (m, 1H, CH), 1.17 (d, *J*=6.3 Hz, 3H, CH_3). ESI-MS: 307.56 $[M+H]^+$. Anal. Calcd for $C_{14}H_{14}ClN_3O_3$: C, 54.64; H, 4.59; Cl, 11.52; N, 13.65; O, 15.60; Found: C, 54.62; H, 4.60; Cl, 11.51; N, 13.63; O, 15.64%.

5.2.6. 1-(2-(4-Fluorostyryl)-5-nitro-1H-imidazol-1-yl) propan-2-ol (**27**)

Yellow crystals, yield 68.2%, m.p. 77~78 ˚C; ^{1}H NMR (DMSO-d_6, 300 MHz) *δ*: 8.20 (s, 1H, CH), 7.86~7.74 (m, 3H, ArH), 7.36~7.25 (m, 3H, ArH, CH), 5.00 (d, *J*=5.4 Hz, 1H, OH), 4.53~4.44 (m, 2H, CH_2), 3.93~3.8 (m, 1H, CH), 1.16 (d, *J*=2.4 Hz, 3H, CH_3). ESI-MS: 291.09 $[M+H]^+$. Anal. Calcd for $C_{14}H_{14}FN_3O_3$: C, 57.73; H, 4.84; F, 6.52; N, 14.43; O, 16.48; Found: C, 57.73; H, 4.79; F, 6.53; N, 14.41; O, 16.54%.

5.2.7. 1-(2-(3-Chlorostyryl)-5-nitro-1H-imidazol-1-yl) propan-2-ol (**28**)

Yellow crystals, yield 64.6%, m.p. 76~77 ˚C; ^{1}H NMR (DMSO-d_6, 300 MHz) *δ*:

8.22 (d, *J*=3.0 Hz, 1H, CH), 7.93 (s, 1H, ArH), 7.78~7.68 (m, 2H, CH), 7.48~7.41 (m, 3H, ArH), 5.00 (d, *J*=5.4 Hz, 1H, OH), 4.60~4.43 (m, 2H, CH_2), 3.86 (s, 1H, CH), 1.16 (d, *J*=6.3 Hz, 3H,CH_3). ESI-MS: 307.56 $[M+H]^+$. Anal. Calcd for $C_{14}H_{14}ClN_3O_3$: C, 54.64; H, 4.59; Cl, 11.52; N, 13.65; O, 15.60; Found: C, 54.61; H, 4.60; Cl, 11.51; N, 13.62; O, 15.66%.

5.2.8. 1-(2-(2-Methoxystyryl)-5-nitro-1H-imidazol-1-yl) propan-2-ol (**29**)

Yellow crystals, yield 69.4%, m.p. 69~70 ˚C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.19 (s, 1H, CH), 8.08 (d, *J*=15.8 Hz, 1H, CH), 7.85~7.82 (m, 1H, CH), 7.41~7.30 (m, 2H, ArH), 7.10~7.00 (m, 2H, ArH), 5.02 (d, *J*=5.4 Hz, 1H, OH), 4.55~4.36 (m, 2H, CH_2), 3.92~3.84 (m, 4H, CH, CH_3), 1.16 (d, *J*=6.3 Hz, 3H, CH_3). ESI-MS: 303.01 $[M+H]^+$. Anal. Calcd for $C_{15}H_{17}N_3O_4$: C, 59.40; H, 5.65; N, 13.85; O, 21.10; Found: C, 59.41; H, 5.66; N, 13.84; O, 21.09%.

5.2.9. 1-(5-Nitro-2-(4-nitrostyryl)-1H-imidazol-1-yl) propan-2-ol (**30**)

Light yellow crystals, yield 67.8%, m.p. 81~ 83 ˚C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.29~8.20 (m, 4H, ArH, CH), 8.05~8.00 (m, 2H, ArH), 7.87 (d, *J*=15.8 Hz, 1H, CH), 7.61 (d, *J*=15.8 Hz, 1H, CH), 5.01 (d, *J*=5.6 Hz, 1H, OH), 4.8 (t, *J*=5.0 Hz, 2H, CH_2), 3.75~3.70 (m, 2H, CH_2), 1.15 (d, *J*=6.2 Hz, 3H, CH_3). ESI-MS: 318.08 $[M+H]^+$. Anal. Calcd for $C_{14}H_{14}N_4O_5$: C, 52.83; H, 4.43; N, 17.60; O, 25.13; Found: C, 52.82; H, 4.44; N, 17.61; O, 25.12%.

5.2.10. 1-(2-(3-Methoxystyryl)-5-nitro-1H-imidazol-1-yl) propan-2-ol (**31**)

Yellow crystals, yield 66.1%, m.p. 72~73 ˚C; ^{1}H NMR (DMSO-d_6, 300MHz) δ: 8.21 (s, 1H, CH), 7.77~7.72 (m, 1H, CH), 7.39~7.32 (m, 4H, ArH), 6.99~6.95 (m, 1H, CH), 5.01 (d, *J*=5.6 Hz, 1H, OH), 4.59~4.41 (m, 2H, CH_2), 3.87~3.75 (m, 4H, CH, CH_3), 1.16 (d, *J*=6.3 Hz, 4H, CH_3, CH_3). ESI-MS: 303.11 $[M+H]^+$. Anal. Calcd for $C_{15}H_{17}N_3O_4$: C, 59.40; H, 5.65; N, 13.85; O, 21.10; Found: C, 59.38; H, 5.66; N, 13.86; O, 21.10%.

5.2.11. 1-(2-(3-Bromostyryl)-5-nitro-1H-imidazol-1-yl) propan-2-ol (**32**)

Light yellow crystals, yield 60.8%, m.p. 67~ 69 ˚C; ^{1}H NMR (DMSO-d_6, 300MHz) δ: 8.21 (s, 1H, CH), 8.06 (s, 1H, ArH), 7.76~7.71 (m, 2H, ArH), 7.57~7.36 (m, 3H, ArH, CH), 5.01 (d, J=5.5 Hz, 1H, OH), 4.60~4.43 (m, 2H, CH_2), 3.73~3.68 (m, 2H, CH_2). 3.90~3.83 (m, 1H, CH). 1.14 (d, J=9.1 Hz, 3H, CH_3). ESI-MS: 352.01 $[M+H]^+$. Anal. Calcd for $C_{14}H_{14}BrN_3O_3$: C, 47.74; H, 4.01; Br, 22.69; N, 11.93; O, 13.63; Found: C, 47.75; H, 4.02; Br, 22.68; N, 11.94; O, 13.61%.

5.2.12. 1-(2-(2-Fluorostyryl)-5-nitro-1H-imidazol-1-yl) propan-2-ol (**33**)

Light yellow crystals, yield 71.6%, m.p. 86~88 ˚C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.20 (s, 1H, CH), 7.77~7.72 (m, 1H, CH), 7.86 (d, J =16.0 Hz, 1H, CH), 7.50~7.22 (m, 4H, ArH), 5.01 (d, J =5.4 Hz, 1H, OH), 4.59~4.41 (m, 2H, CH_2), 3.94~3.81 (m, H, CH), 1.51 (d, J =6.3 Hz, 3H, CH_3). ESI-MS: 291.16 $[M+H]^+$. Anal. Calcd for $C_{14}H_{14}FN_3O_3$: C, 57.73; H, 4.84; F, 6.52; N, 14.43; O, 16.48; Found: C, 57.68; H, 4.86; F, 6.54; N, 14.42; O, 16.50%.

5.2.13. 1-(2-(2-Chlorostyryl)-5-nitro-1H-imidazol-1-yl) propan-2-ol (**34**)

Yellow crystals, yield 68.2%, m.p. 84~87 ˚C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.20 (s, 1H, CH), 7.77~7.72 (m, 1H, CH), 7.86 (d, J =16.0 Hz, 1H, CH), 7.50~7.22 (m, 4H, ArH), 5.01 (d, J =5.4 Hz, 1H, OH), 4.59~4.41 (m, 2H, CH_2), 3.94~3.81 (m, H, CH), 1.51 (d, J =6.3 Hz, 3H, CH_3). ESI-MS: 307.09 $[M+H]^+$. Anal. Calcd for $C_{14}H_{14}ClN_3O_3$: C, 54.64; H, 4.59; Cl, 11.52; N, 13.65; O, 15.60; Found: C, 54.61; H, 4.60; Cl, 11.54; N, 13.65; O, 15.60%.

5.2.14. 1-(2-(4-Methylstyryl)-5-nitro-1H-imidazol-1-yl) propan-2-ol (**35**)

Yellow crystals, yield 65.6%, m.p. 97~100 ˚C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.18 (s, 1H, CH), 7.85~7.72 (m, 3H, ArH, CH), 7.61~7.32 (m, 3H, ArH, CH), 5.00 (t, J= 5.4 Hz, 1H, OH), 4.59~4.41 (m, 2H, CH_2), 3.94~3.81 (m, H, CH), 2.33 (s, 3H, CH_3,), 1.51 (d, J=6.3 Hz, 3H, CH_3). ESI-MS: 287.06 $[M+H]^+$. Anal. Calcd for $C_{15}H_{17}N_3O_3$: C, 62.71; H, 5.96; N, 14.63; O, 16.71; Found: C, 62.73; H, 5.94; N, 14.62; O, 16.72%.

5.2.15. 1-(2-(2-Chloro-6-fluorostyryl)-5-nitro-1H-imidazol-1-yl) propan-2-ol (**36**)

Light yellow crystals, yield 62.4%, m.p. 70~72 ˚C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.22 (s, 1H, CH), 7.77~7.72 (m, 1H, CH), 7.50~7.22 (m, 4H, ArH, CH), 5.01 (d,

J=5.4 Hz,1H, OH), 4.59~4.41 (m, 2H, CH_2), 3.94~3.81 (m, H, CH), 1.51 (d, *J*=6.3 Hz, 3H, CH_3). ESI-MS: 325.11 $[M+H]^+$. Anal. Calcd for $C_{14}H_{13}ClFN_3O_3$: C, 51.62; H, 4.02; Cl, 10.88; F, 5.83; N, 12.90; O, 14.74; Found: C, 51.58; H, 4.04; Cl, 10.91; F, 5.84; N, 12.91; O, 14.75%.

5.2.16. 1-(2-(2,4-Dichlorostyryl)-5-nitro-1H-imidazol-1-yl) propan-2-ol (**37**)

Orange crystals, yield 66.8%, m.p. 90~92 ˚C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.22 (s, 1H, CH), 7.77~7.72 (m, 1H, CH), 7.50~7.22 (m, 4H, ArH, CH), 5.01 (d, *J*=5.4 Hz, 1H, OH), 4.59~4.41 (m, 2H, CH_2), 3.94~3.81 (m, H, CH), 1.51 (d, *J*=6.3 Hz, 3H, CH_3). ESI-MS: 342.06 $[M+H]^+$. Anal. Calcd for $C_{14}H_{13}Cl_2N_3O_3$: C, 49.14; H, 3.83; Cl, 20.72; N, 12.28; O, 14.03; Found: C, 49.10; H, 3.82; Cl, 20.75; N, 12.30; O, 14.03%.

5.2.17. 1-(2-(4-(Dimethylamino) styryl)-5-nitro-1H-imidazol-1-yl) propan-2-ol (**38**)

Orange crystals, yield 61.5%, m.p. 46~49 ˚C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.18 (s, 1H, CH), 7.85~7.72 (m, 3H, ArH, CH), 7.61~7.32 (m, 3H, ArH, CH), 5.00 (t, *J*= 5.4 Hz, 1H, OH), 4.59~4.41 (m, 2H, CH_2), 3.94~3.81 (m, H, CH), 3.02 (s, 6H, $CH_{3,}$), 1.51 (d, *J*=6.3 Hz, 3H, CH_3). ESI-MS: 316.03 $[M+H]^+$. Anal. Calcd for $C_{16}H_{20}N_4O_3$: C, 60.75; H, 6.37; N, 17.71; O, 15.17; Found: C, 60.72; H, 6.38; N, 17.72; O, 15.18%.

5.2.18. 1-(5-Nitro-2-(3-nitrostyryl)-1H-imidazol-1-yl) propan-2-ol (**39**)

Light yellow crystals, yield 63.9%, m.p. 175~177 ˚C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.64 (s, 1H, ArH), 8.18 (s, 1H, CH), 8.21~8.02 (m, 2H, ArH), 7.91~7.85 (m, 1H, CH), 7.83~7.70 (m, 1H, ArH, CH), 7.68~7.58 (m, 1H, CH), 5.01 (d, *J*=5.4 Hz, 1H, OH), 4.59~4.41 (m, 2H, CH_2), 3.88~3.86 (m, 1H, CH).,1.17 (d, *J*=6.6 Hz, 3H, CH_3). ESI-MS: 318.15 $[M+H]^+$. Anal. Calcd for $C_{14}H_{14}N_4O_5$: C, 52.83; H, 4.43; N, 17.60; O, 25.13; Found: C, 52.86; H, 4.43; N, 17.61; O, 25.15%.

5.2.19. 1-(2-(2-Bromostyryl)-5-nitro-1H-imidazol-1-yl) propan-2-ol (**40**)

Yellow crystals, yield 70.1%, m.p. 79~ 81 ˚C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.20 (s, 1H, CH), 7.77~7.72 (m, 1H, CH), 7.86 (d, *J* =16.0 Hz, 1H, CH), 7.50~7.22 (m, 4H, ArH), 5.01 (d, *J*=5.4 Hz, 1H, OH), 4.59~4.41 (m, 2H, CH_2), 3.94~3.81 (m, H,

CH), 1.51 (d, *J*=6.3 Hz, 3H, CH_3,). ESI-MS: 352.08 $[M+H]^+$. Anal. Calcd for $C_{14}H_{14}BrN_3O_3$: C, 47.74; H, 4.01; Br, 22.69; N, 11.93; O, 13.63; Found: C, 47.72; H, 4.00; Br, 22.70; N, 11.94; O, 13.64%.

5.2.20. 1-(5-Nitro-2-(2-nitrostyryl)-1H-imidazol-1-yl) propan-2-ol (**41**)

Light yellow crystals, yield 65.2%, m.p. 136~ 138 ˚C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.08 (s, 1H, CH), 7.77~7.72 (m, 1H, CH), 7.86 (d, *J*=16.0 Hz, 1H, CH), 7.50~7.22 (m, 4H, ArH), 5.01 (d, *J*=5.4 Hz,1H, OH), 4.59~4.41 (m, 2H, CH_2), 3.94~3.81 (m, H, CH), 1.51 (d, *J*=6.3 Hz, 3H, CH_3,). ESI-MS: 318.01 $[M+H]^+$. Anal. Calcd for $C_{14}H_{14}N_4O_5$: C, 52.83; H, 4.43; N, 17.60; O, 25.13; Found: C, 52.82; H, 4.44; N, 17.61; O, 25.12%.

5.2.21. 1-(2-(2-(Naphthalen-2-yl) vinyl)-5-nitro-1H-imidazol-1-yl) propan-2-ol (**42**)

Light yellow crystals, yield 65.8%, m.p. 97~ 99 ˚C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.26 (s, 1H, CH), 7.80~7.75 (m, 7H, ArH, CH), 7.47~7.35 (m, 4H, ArH), 5.02 (d, *J*=5.4 Hz, 1H, OH), 4.58~4.39 (m, 2H, CH_2), 3.89~3.87 (m, 2H, CH),1.16 (d, *J*=6.3 Hz, H, CH_3). ESI-MS: 323.08 $[M+H]^+$. Anal. Calcd for $C_{18}H_{17}N_3O_3$: C, 66.86; H, 5.30; N, 13.00; O, 14.84; Found: C, 66.84; H, 5.29; N, 13.03; O, 14.84%.

5.3 Crystal structure determination

X-ray diffraction studies of compounds **32**, **33** and **34** were performed on a Bruker-APEX diffractometer with a CCD area detector (Mo-Kα=0.71073 Å, monochromator: graphite) by the ω scan mode. Frames were collected at 293(2) via u/4-rotation at 10 sper frame (SMART) [26]. The measured intensities were reduced to F^2 and corrected for absorption with SADABS (SAINT-NT) [27]. Corrections were made for Lorentz and polarization effects. Structure solution, refinement and data output were carried out with the SHELXTL-NT program package [28].

All the non-hydrogen atoms were refined anisotropically. All the hydrogen atoms were placed in calculated positions and were assigned fixed isotropic thermal parameters at 1.2 times the equivalent isotropic U of the atoms to which they were attached and allowed to ride on their respective parent atoms. The contributions of these hydrogen atoms were included in the structure factors calculations. The crystal data, data collection and refinement parameters for the compound **32**, **33** and **34** were

listed in Table 2.

5.4 *E. coli* FabH inhibitory activity

All FabH proteins purified, tagged or tagless can use *E.coli* holo-ACP as their substrate. The protein was overexpressed in *E.coli* DH10B cells using the pET30 vector and purified to homogeneity in three hromatographic steps (Q-Sepharose, MonoQ, and hydroxyapatite) at 4 ℃. The selenomethionine-substituted protein was expressed cells containing FabH 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 mins. 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 FabD and FabHs were concentrated up to 2 mg/mL and stored at -80℃ in 20 mM tris, pH 7.6, 100 mM NaCl, 1 mM DTT, and 20% glycerol for enzymatic assay.

In a final 20 μL reaction, 20 mM Na_2HPO_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 (0.5 nM for *E. coli* FabH, 3 nM for *E. faecalis* and *H. influenzae* FabHs, 10 nM for *S. pyogenes* FabH, and 20 nM for *Staph aureus* FabH), and H_2O was added to 16 μL. After 1 min incubation, a 2 μL mixture of 25 μM acetyl-CoA, 0.5 mM NADH, and 0.5 mM NADPH was added for FabH reaction for 19 min. The reaction was stopped by adding 20 μL 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 4 μL of 2 M NaOH. The incorporation of the ^3H 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% [29].

5.5 Antibacterial activity

IC_{50} of the test compounds against *E. coli, B. thuringiensis, B. subtilis* and *P. aeruginosa* were determined by a broth microdilution method. They were grown to mid-log phase in Mueller-Hinton broth and then diluted 10000 fold in the same medium [30]. Afterwards the antibacterial activity of the synthesized compounds was tested against *E. coli, B. thuringiensis, B. subtilis* and *P. aeruginosa* using MH medium (Mueller-Hinton medium: casein hydrolysate 17.5 g, soluble starch 1.5 g, beef extract

1000 mL). A 10 μL aliquot of the diluted cell suspension (10^6 to 10^7 colony forming units) was used to inoculate each well of a 96-well plate containing 90 μL of MH broth with the indicated concentration of inhibitors. The plate was incubated at 37 ℃ for 24 h [31]. Then 100 μL a series concentration of drug-containing medium were added into wells to maintain the final concentration of drug as 40, 10, 2.5 and 0.25 μg/mL. After 12 h, bacterial survival was determined by the addition of an MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution (25 mL of 5 mg/mL MTT in PBS). 4 h later, the medium was discarded and 150 μL DMSO added. The plates were vibrated for 10 min to make completely dissolution. Optical absorbance was measured at 490 nm. The experiments were replicated at least three times to verify the methodology reproducibility when using the above-mentioned conditions.

5.6 Cytotoxicity

The cytotoxicity test of compounds was measured by the colorimetric MTT assay. Human macrophage suspension in DMEM medium supplemented with 10% FBS and 1 X antimycotic was added in 96-well microplates at 1.8×10^4 cells/well. Then, various concentrations of the test compounds (160, 40, 10, 2.5 and 0.25 μg/mL) dissolved in distilled 10% DMSO (10 μL) were added to each well. After incubation for 24 h at 37 ° C under 5% CO_2, 2.5 mg/mL of MTT solution (μL) was added to each well. The plate was incubated for a further 4 h. Then, the incubation medium was aspirated, and DMSO (100 μL) was added to solubilize the MTT formazan product. After mixing, the absorbances at 570 and 655 nm were measured. The difference between absorbance at 570 and 630 nm was used as an index of the cell viability. The morphology of the cells was observed using Giemsa stain under Phase contrast microscope [32, 33].

5.7 Docking simulations

Molecular docking of compounds into the three-dimensional X-ray structure of FabH was carried out using CDOCKER Dock protocol of Discovery Studio 3.1.

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Figure Captions

Table 1 Antibacterial activity of 1-(2-hydroxypropyl)-2- styryl -5-nitroimidazole derivatives (**22**-**42**) and their toxicity.

Table 2 Crystallographical and experimental data for compounds **32, 33** and **34**

Fig.1. FabH catalyzed initiation reaction of fatty acid biosynthesis.

Fig.2 (a) Crystal structure diagram of compound **32**; (b) Crystal structure diagram of compound **33**; (c) Crystal structure diagram of compound **34**, H atoms are shown as small spheres of arbitrary radii.

Fig.3. Molecular docking modeling of compound 30 with E. coli FabH: or clarity, only interacting residues are displayed. (a): 2D model of the interaction between compound 30 and the ATP binding site. P–sigma bond is displayed as orange lines. H-bonds are displayed as blue and green dashed lines. (b): 3D model of the interaction between compound 30 and the ATP binding site.

Fig.4. (a) Morphological changes in macrophage at 2.5 μg/mL of compound **41**. (b) Morphological changes in macrophage at 10 μg/mL of compound **41**. (c) Morphological changes in macrophage at 40 μg/mL of compound **41**. (d) Morphological changes in macrophage at 160 μg/mL of compound **41**.

Scheme 1[a]

Table 1 Antibacterial activity of 1-(2-hydroxypropyl)-2- styryl -5-nitroimidazole derivatives (**22-42**) and their toxicity.

Compounds	IC_{50} (μg/mL) [a]				*E. coli* FabH IC_{50} (μM)	CC_{50} (μM) [b]
	Gram-negative		Gram-positive			
	E. coli	*P. aeruginosa*	*B. thuringiensis*	*B. subtilis*		
22	>200	28.0	45.6	78.9	-	1.50
23	46.1	42.8	13.6	12.3	9.5	0.56
24	50.8	15.2	56.2	38.0	-	0.55
25	120.1	15.0	79.2	11.8	8.9	1.03
26	101.8	17.6	52.5	79.5	-	0.98
27	45.3	24.2	28.1	47.1	-	0.42
28	86.7	35.0	21.4	55.3	-	0.52
29	78.6	83.8	19.1	10.5	-	0.48
30	36.8	5.9	11.7	32.5	4.6	0.41
31	180.6	6.7	56.2	25.7	-	1.30
32	45.4	30.9	8.2	15.3	10.8	0.62
33	72.7	39.4	60.3	3.4	-	1.41
34	56.3	15.4	8.6	7.5	11.5	0.48
35	111.4	22.8	8.6	8.9	78.8	0.86
36	118.8	5.7	6.1	111.8	-	0.71
37	111.1	8.7	18.8	58.1	-	0.81
38	108.1	75.0	>200	56.5	-	0.68
39	38.8	32.7	20.6	31.1	5.1	0.43
40	63.6	11.1	11.4	6.0	-	0.49
41	40.3	7.4	72.9	12.3	11.0	1.09
42	108.4	42.1	52.2	23.5	43.2	0.79
Kanamycin	47.3	6.8	9.5	8.9	6.3	-

[a] Errors were in the range of (5-10% of the reported values, from three different assays.

[b] Minimum cytotoxic concentration required to cause a microscopically detectable alteration of normal cell morphology.

Table 2 Crystallographical and experimental data for compounds **32, 33** and **34**

Compound	**32**	**33**	**34**
Empirical formula	$C_{14}H_{14}BrN_3O_3$	$C_{14}H_{14}FN_3O_3$	$C_{14}H_{14}ClN_3O_3$
Formula weight	352.18	291.28	307.73
Temperature(K)	273(2)	273(2)	273(2)
Crystal system	Monoclinic	Monoclinic	Monoclinic
Space group	*P c*	*P c*	*P c*
a (Å)	10.2060(10)	11.9002(6)	11.4854(17)
b (Å)	13.4120(12)	14.5643(9)	15.508(2)
c (Å)	10.5874(10)	8.8348(5)	8.7486(13)
α (°)	90.00	90.00	90.00
β (°)	90.876(3)	101.589(2)	100.007(5)
γ (°)	90.00	90.00	90.00
V (Å)	1449.1(2)	1500.01(15)	1534.5(4)
Z	18	25	20
Dcalcd/g cm^{-3}	2.536	1.717	1.698
θ rang (deg)	2.45-25.73	2.24-25.22	2.23-25.73
F(000)	1026	775	780
Reflections collected	13419 (R_{int}= 0.0418)	14100 (R_{int}= 0.0295)	14920 (R_{int}=0.0492)
Data/restraints/parameters	2755/0/191	2705/0/207	2918/0/207
Absorption coefficient (mm^{-1})	12.502	0.193	0.967
R_1; wR_2 [I > 2σ(I)]	0.0644/0.1659	0.0721/0.2111	0.0504/0.1260
R_1; wR_2 (all data)	0.0883/0.1845	0.0922/0.2338	0.0819/0.1438
GOF	1.044	1.033	1.012
Larg.peak/hole(e. Å)	1.037/-0.884	1.114/-0.362	0.257/-0.328

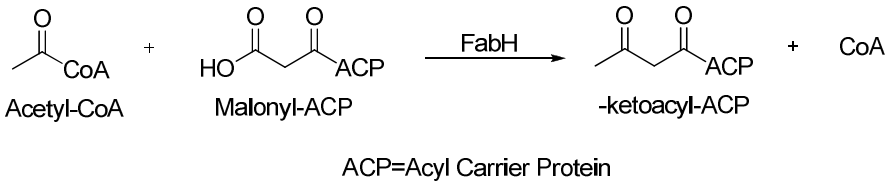


Fig.1. FabH catalyzed initiation reaction of fatty acid biosynthesis.

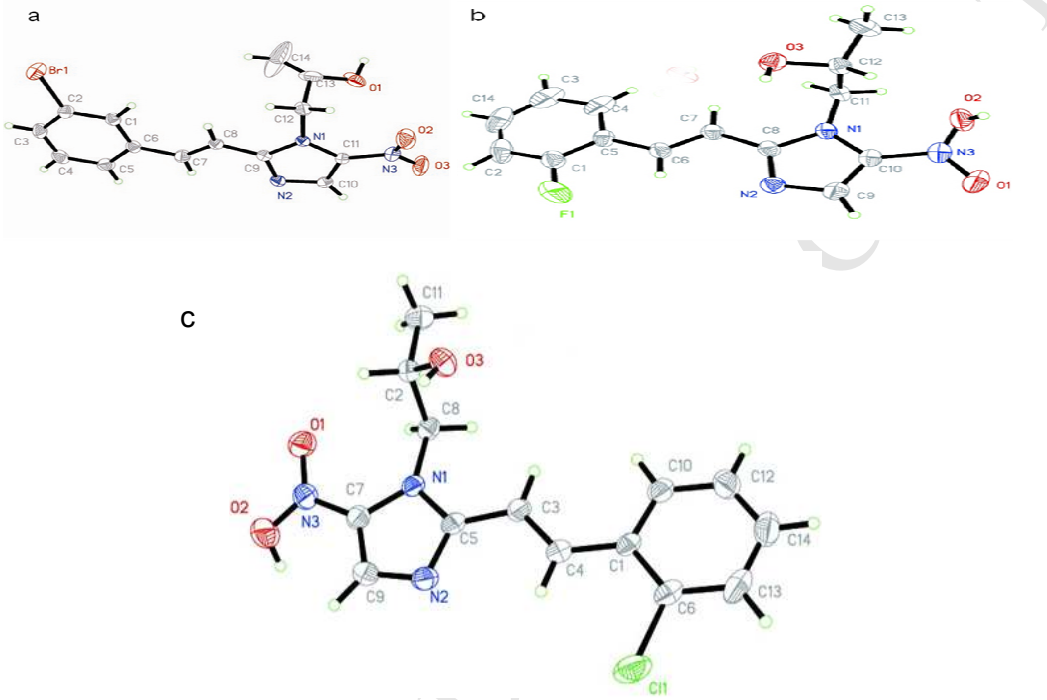


Fig.2 (a) Crystal structure diagram of compound **32**; (b) Crystal structure diagram of compound **33**; (c) Crystal structure diagram of compound **34**, H atoms are shown as small spheres of arbitrary radii.

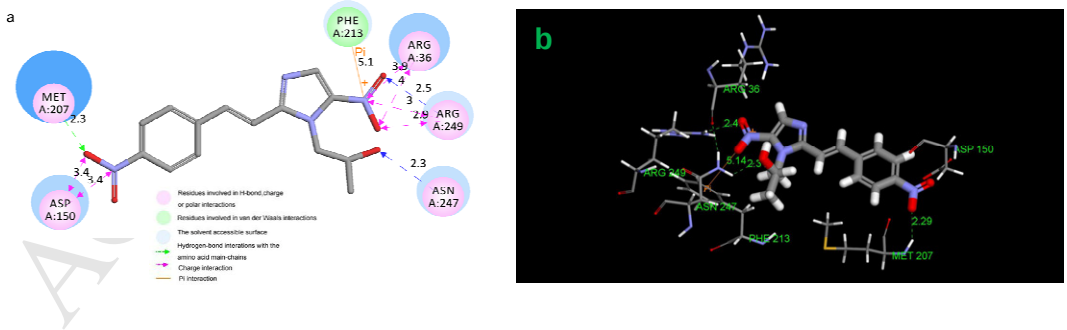


Fig.3. Molecular docking modeling of compound **30** with *E. coli* FabH: or clarity, only interacting residues are displayed. (a): 2D model of the interaction between compound **30** and the ATP binding site. *P*–sigma bond is displayed as orange lines. H-bonds are displayed as blue and green dashed lines. (b): 3D model of the interaction between compound **30** and the ATP binding site.

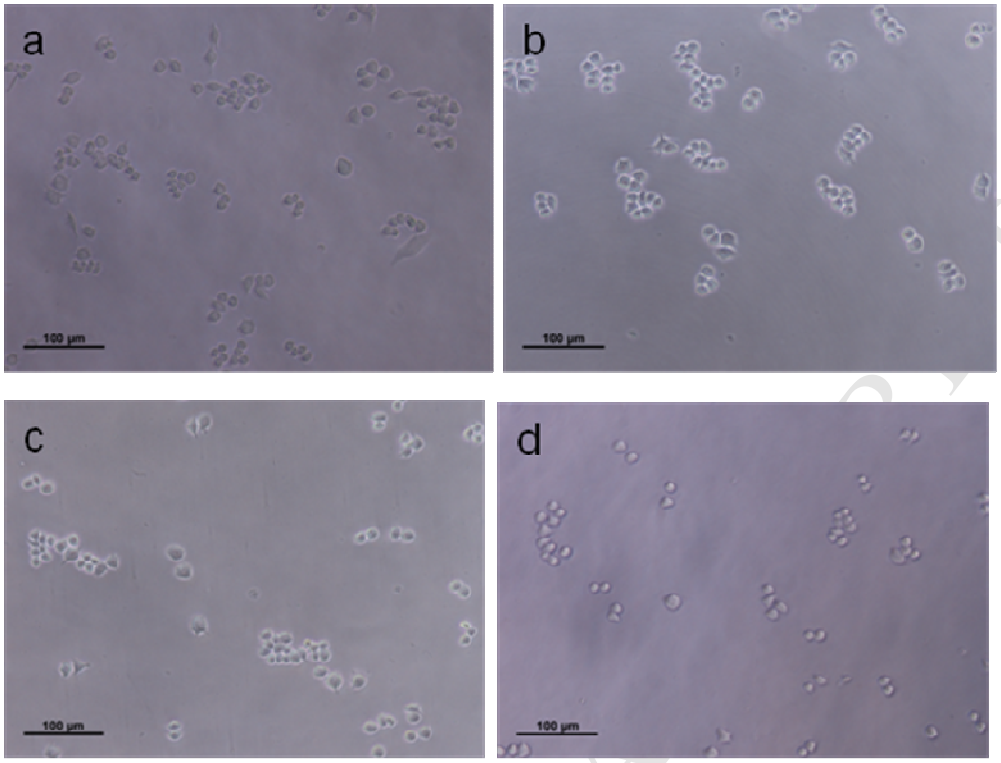
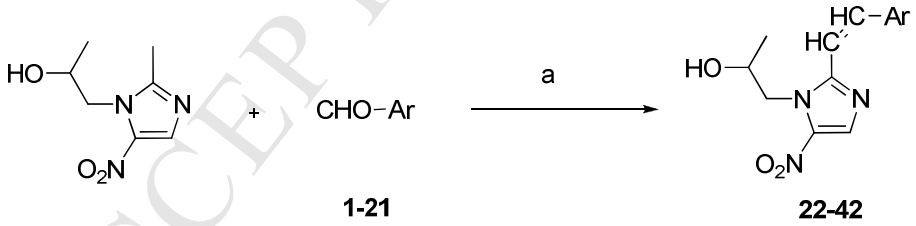


Fig.4. (a) Morphological changes in macrophage at 2.5 μg/mL of compound **41**. (b) Morphological changes in macrophage at 10 μg/mL of compound **41**. (c) Morphological changes in macrophage at 40 μg/mL of compound **41**. (d) Morphological changes in macrophage at 160 μg/mL of compound **41**.

Scheme 1[a]



1-21 **22-42**

Ar= 1, 22: phenyl; **2, 23:** 4-methoxyphenyl; **3, 24:** 4-bromophenyl;
4, 25: 3-fluorophenyl; **5, 26:** 4-chlorophenyl; **6, 27:** 4-fluorophenyl;
7, 28: 3-chlorophenyl; **8, 29:** 2-methoxyphenyl; **9, 30:** 4-nitrophenyl;
10, 31: 3-methoxyphenyl; **11, 32:** 3-bromophenyl; **12, 33:** 2-fluorophenyl;
13, 34: 2-chlorophenyl; **14, 35:** 4-methylphenyl; **15, 36:** 2-chloro-6-fluorophenyl;
16, 37: 2,4-dichlorophenyl; **17, 38:** 4-dimethylamiophenyl; **18,39:** 4-nitrophenyl;
19, 40: 3-bromophenyl; **20, 41:** 2-nitrophenyl; **21, 42:** 2-Naphthalphenyl;

[a] Reagents and conditions: (a) Sodium methoxide, DMSO, methanol, room temperature;

> 20 Novel 2-styryl-5-nitroimidazole derivatives have been synthesized.

> Their biological activities were evaluated against bacterial

> Most of the compounds showed low toxicity to human macrophage cells.

> Compound **30** showed the most potent and best selective *E. coli* FabH inhibition.

> Crystal structure of compounds **32, 33** and **34** were determined.