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Design, synthesis and antimicrobial activities of nitroimidazole derivatives containing 1,3,4-oxadiazole scaffold as FabH inhibitors

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ABSTRACT

Nitroimidazoles and their derivatives have drawn continuing interest over the years because of their varied biological activities, recently found application in drug development for antimicrobial chemotherapeutics and antiangiogenic hypoxic cell radiosensitizers. In order to search for novel antibacterial agents, we designed and synthesized a series of secnidazole analogs based on oxadiazole scaffold (**4**–**21**). Among these compounds, **4** and **7**–**21** were reported for the first time. These compounds were tested for antibacterial activities against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*. This new nitroimidazole derivatives class demonstrated strong antibacterial activities. *Escherichia coli* β-ketoacyl-acyl carrier protein synthase III (FabH) inhibitory assay and docking simulation indicated that the compounds 2-(2-methoxyphenyl)-5-((2-methyl-5-nitro-1*H*-imidazol-1-yl)methyl)-1,3,4-oxadiazole (**11**) with MIC of 1.56–3.13 μg/mL against the tested bacterial strains and 2-((2-methyl-5-nitro-1*H*-imidazol-1-yl)methyl)-5-(2-methylbenzyl)-1,3,4-oxadiazole (**12**) with MIC of 1.56–6.25 μg/mL were most potent inhibitors of *Escherichia coli* FabH.

1. Introduction

Infections triggered by some pathogenic microorganisms can bring illnesses even a fatal one.[1] Although many kinds of antibacterial agents were discovered and used for clinical treatment, the incidences of drug resistance of microorganisms to antibacterial agents were constantly reported.[2,3] Therefore, the development of new types of antibacterial agents is a very important task and much of the research effort is oriented to the design of new antibacterial agents with high efficiency.[4]

In recent years, different kinds of targets in key areas of the bacterial cell cycle have been studied that would be a new approach against the problem of drug resistance. One of the most attractive biochemical pathways to be used as the target for new antibacterial agents is the fatty acid biosynthesis (FAB). This pathway has been demonstrated to be essential for bacteria cell survival.[5,6] A key enzyme in this pathway is β-ketoacyl-acyl carrier protein synthase III (FabH), which is the enzyme responsible for the first reaction in the pathway and plays an important regulatory role.[7,8] The enzyme initiates the fatty acid elongation cycles,[9] and participates in the feedback regulation of the biosynthetic pathway via product inhibition.[10] As shown in Figure 1 FabH catalyzes the condensation reaction between a CoA-attached acetyl group and an ACP-attached malonyl group, yielding acetoacetyl-ACP as its final product.[11]

FabH proteins from both 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.[12–14] These facts suggest the idea that FabH can be used as an effective molecular target for the development of new antibacterial agents, since it regulates the fatty acid biosynthesis rate via an initiation pathway and its substrate specificity is a key factor in membrane fatty acid composition.[15–17]

Nitroimidazoles and their derivatives have drawn continuing interest over the years because of their varied biological activities, recently found application in drug development for antimicrobial chemotherapeutics and antiangiogenic hypoxic cell radiosensitizers.[18–20] Importantly, the toxicology and metabolism of nitroimidazoles, particularly secnidazole, have been characterized.[18] Secnidazole (α,2-dimethyl-5-nitro-1*H*-imidazole-1-ethanol) is particularly effective in the treatment of amebiasis, giardiasis, trichomoniasis, and bacterial vaginosis[21] as it 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.[21] In these cases, the treatment with secnidazole is shorter

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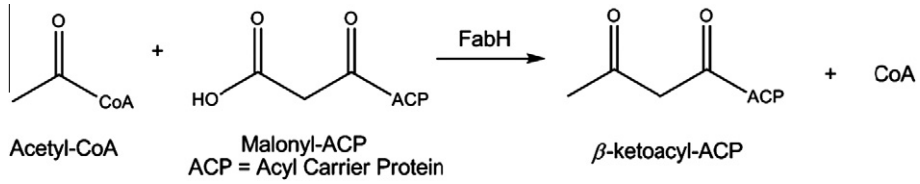


Figure 1. FabH-catalyzed initiation reaction of fatty acid biosynthesis.[11]

and significantly more effective than the treatment using other imidazole drugs and the adverse effects are not very drastic.[23] As a result, excellent works have been published on the activities and pharmacokinetics of secnidazole derivatives and their determination in pharmaceutical is of great importance.[18–23]

In order to search for novel antibacterial agents, we designed and synthesized a series of secnidazole analogs based on oxadiazole scaffold. We chose oxadiazole moiety because it was found in a variety of biologically substance and it showed widely biological activities such as anticancer activities, antidepressant properties and so on.[23–28] However, to our knowledge, few reports have been dedicated to the FabH inhibitory activities of nitroimidazole derivatives owing oxadiazole groups. Herein, to keep on research on antibacterial compounds with FabH inhibitory activity, we described the synthesis and structure–activity relationship (SAR) of a new series of nitroimidazole derivatives owing oxadiazole group, 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

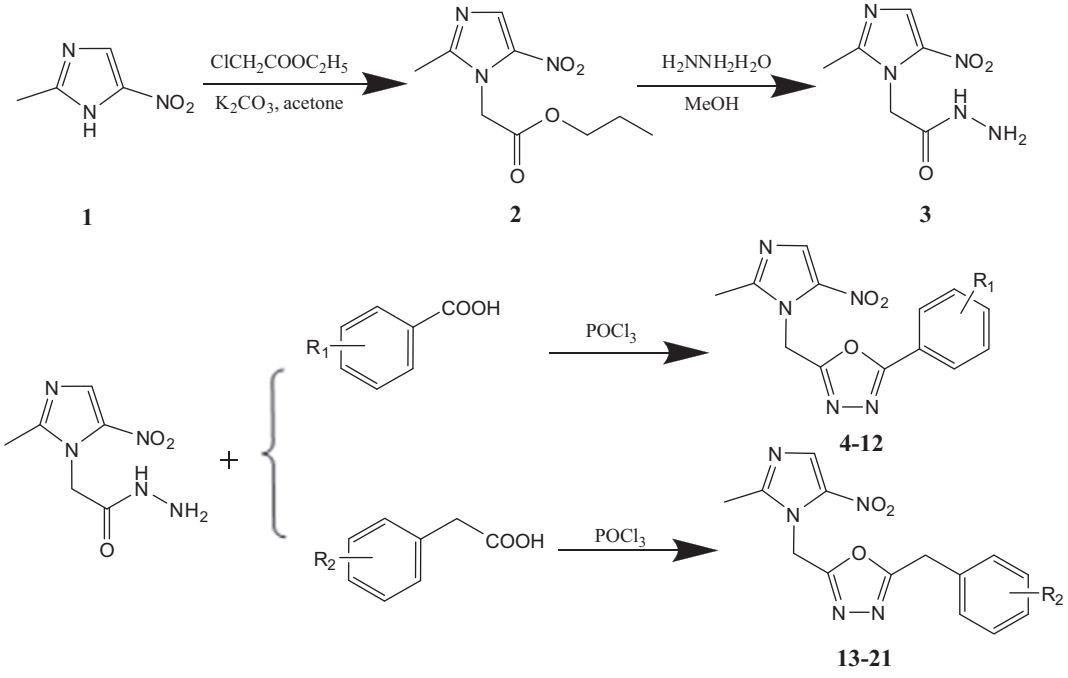
Compound 3 was synthesized by the routes outlined in Scheme 1 according to Mirzaei et al.'s method.[29] Compound 3 was prepared by the reaction of 2-methyl-5-nitro-1*H*-imidazole in excess ethyl α-chloroacetate and then hydrazinolysis of ethyl ester group by hydrazine hydrate.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide was then reacted with substituted benzoic acids and phenylacetic acids to prepare the corresponding nitroimidazole derivatives **4**–**21**. The chemical structures of these nitroimidazole derivatives were summarized in Table 1. These compounds gave satisfactory elementary analyses (±0.4%). ^{1}H NMR and ESI MS spectra data was consistent with the assigned structures. Among these compounds, **4** and **7**–**21** were reported for the first time. Compounds **4**–**12** were nitroimidazole derivatives prepared by substituted benzoic acids with 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide. Compounds **13**–**21** were nitroimidazole derivatives prepared by substituted phenylacetic acids with 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide.

2.2. Biological activity

2.2.1. Antimicrobial activity

All the synthesized compounds (**4**–**21**) were screened for their antibacterial activities against two Gram-negative bacterial strains: *E. coli* and *P. aeruginosa* and two Gram-positive bacterial strains: *B. subtilis* and *S. aureus* by MTT method. The MICs (minimum inhibitory concentrations) of the compounds against these bacteria were presented in Table 2. Also included was the activity of reference compound Kanamycin and DDCP (compound 5 in *Antimicrob. Agents Chemother.* **2004**, *48*, 3093)[30] under identical conditions for comparison. The results revealed that most of the synthesized compounds exhibited significant antibacterial



Scheme 1. Synthesis of nitroimidazole derivatives **4**–**21**.

Table 1
Structures of compounds **4**–**21**

Compounds	R_1	Compounds	R_2
4	4-F	**13**	4-F
5	4-Cl	**14**	4-Cl
6	4-Br	**15**	4-Br
7	3-F	**16**	3-F
8	3-Br	**17**	3-Br
9	4-NO_2	**18**	4-NO_2
10	3-OCH_3	**19**	3-OCH_3
11	2-OCH_3	**20**	2-OCH_3
12	2-CH_3	**21**	2-CH_3

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

Compounds	*E. coli* FabH IC_{50} (μM)	Hemolysis LC_{30}[a] (mg/mL)
4	18.6	>10
5	28.3	>10
6	43.8	>10
8	16.9	>10
9	40.5	>10
10	9.4	>10
11	4.3	>10
12	5.1	>10
DDCP	2.1	>10

[a] Lytic concentration 30%.

activities. Among the 18 synthetic new nitroimidazole derivatives, the benzoic acid derivatives **4**–**6** and **8**–**12**, whose MIC values ranging from 1.56 to 25 μg/mL, displayed higher antibacterial potencies than phenylacetic acid derivatives **13**–**21**, with MICs values ranging from 6.25 to 50 μg/mL, which showed the introduction of benzoic acid lead to the increase of the antibacterial activity.

Out of the eight nitroimidazole derivatives (compound **4**–**6** and **8**–**12**), compound **11** and **12** exhibited most potent activities with MIC values of both 1.56 μg/mL against *E. coil* ATCC35128, which are superior to the positive control kanamycin with corresponding MIC of 3.13 μg/mL and equivalent to the positive control DDCP with corresponding MIC of 1.56 μg/mL. Besides, the data showed compound **11** and **12** displayed significant activities with MIC values of 1.56, 1.56, 3.13 μg/mL and 3.13, 1.56, 6.25 μg/mL against *P. fluorescence*, *B. subtilis*, *S. aureus*, respectively, indicating that they possessed broad-spectrum antibacterial activities.

Based on the data obtained, we found that compounds with a methoxy group on the 2-position of benzoic acid displayed higher antibacterial activity with MIC values of 1.56 μg/mL against *E. coil* ATCC35128 than compounds with a methoxy group on the 3-position of benzoic acid with MIC values of 12.5 μg/mL and compounds with a methoxy group on the 2-position of phenylacetic acid with MIC values of 50 μg/mL. And the varieties of substitutes of benzoic acid and phenylacetic acid such as halogen, methyl and methoxyl also lead to the different antibacterial activities of these nitroimidazole derivatives. Among them, the derivatives which have electron-donating substituents (such as CH_3, OCH_3) exhibited more potent activities against *E. coil* ATCC35128 than those have electron-withdrawing substituents (such as F, Cl, Br), and their MICs value range from 6.25 to 25 μg/mL. We proposed that electron-donating groups on benzoic acid component were conducive to the antibacterial activity and compounds with electron-withdrawing halogen groups on benzoic acid component were not favorable for activity.

Moreover, according to the data in Table 2, among derivatives with electron-donating substituents, compounds **10**–**12** with substituted methoxy and methyl group on benzoic acid component exhibited significant antibacterial activities with MICs of 1.56–12.5 μg/mL against *E. coil* ATCC35128. Compound **19**–**21** with substituted methoxy and methyl group on phenylacetic acid component showed less potent activity with MIC of 12.5–50 μg/mL. These results demonstrated that the synthetic nitroimidazole derivatives with substituted electron-donating group on benzoic acid component showed more potent antibacterial activity than those of on phenylacetic acid component.

2.2.2. *E. coli* FabH inhibitory activity

The *E. coli* FabH inhibitory potency of the selected compounds **4**–**6** and **8**–**12** was examined and the results were summarized in Table 3. As shown in Table 3, among the tested compounds, compounds **11** and **12** showed potent inhibitory activities with IC_{50} of 4.3 and 5.1 μM, respectively, which were comparable to the positive control DDCP27 with IC_{50} of 2.1 μM. Other tested compounds displayed moderate inhibitory activities with IC_{50} ranging from 9.4 to 43.8 μM. It suggested that the introduction of benzoic acid and

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*
4	12.5	25	12.5	25
5	12.5	12.5	12.5	25
6	25	25	12.5	50
7	>100	>100	>100	>100
8	6.25	25	6.25	25
9	12.5	12.5	25	25
10	12.5	12.5	6.25	12.5
11	1.56	1.56	1.56	3.13
12	1.56	3.13	1.56	6.25
13	25	12.5	50	25
14	50	12.5	50	25
15	50	25	50	50
16	>100	>100	>100	>100
17	25	6.25	25	50
18	25	25	25	50
19	25	12.5	25	50
20	50	12.5	25	25
21	12.5	25	50	25
Kanamycin	3.13	3.13	1.56	1.56
DDCP	1.56	3.13	12.5	25

Table 4
The docking calculation of the synthesized compounds (**4**–**21**)

Compounds	CDOCKER INTERATION ENERGY ΔGb (kcal/mol)	Compounds	CDOCKER INTERATION ENERGY ΔGb (kcal/mol)
4	−31.5284	**13**	−29.7027
5	−31.3187	**14**	−27.9217
6	−30.6726	**15**	−27.0913
7	−26.9831	**16**	−27.0399
8	−32.2031	**17**	−30.2138
9	−30.9375	**18**	−29.0306
10	−32.7019	**19**	−29.171
11	−33.2629	**20**	−28.2003
12	−32.9295	**21**	−30.2406

the formation of nitroimidazole and oxadiazole structure were essential for the FabH inhibitory activity. It also can be seen from Table 3 that the selected compounds displayed low hemolytic activity.

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

Molecular docking of the synthesized compounds and *E. coli* FabH was performed on the binding model based on the *E. coli* FabH–CoA complex structure (1HNJ.pdb).[31] The coordinate of the input site sphere is 28.4494, 9.90349, 33.4428 and the radius of the sphere is 11 Å.

All docking runs were applied LigandFit 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, compounds 11 and **12** showed the lowest interaction energy. The binding model of compounds **11**, 12 and *E. coli* FabH was depicted in Figures 2 and 3. In the binding model, compound **11** was nicely bound to the FabH kinase with four interaction bonds. The end amino of Asn274, His244 and Asn247 were respectively formed four hydrogen bonds interaction with oxygen atom of oxadiazole ring of compound **11**. Besides, the hydrogen of Asn274 and His244 were respectively formed four hydrogen bonds interaction with oxygen atom of nitro group of compound **12**. This molecular docking result, along with the biological assay data, suggesting that compounds 11 and **12** was a potential inhibitor of FabH.

3. Conclusion

In summary, a series of novel nitroimidazole derivatives **4**–**21** were synthesized and tested for their inhibitory activities against *E. coli*, *P. fluorescence*, *B. subtilis* and *S. aureus*. Many of them exhibited potent antibacterial and *E. coli* FabH inhibitory activities. Particularly, Compounds 11 and **12** 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 nitroimidazole derivatives

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide (**1**–**3**) was prepared according the previous procedure by Mirzaei et al.[29] To a suspension of 2-methyl-5-nitroimidazole (0.01 mol) in ethyl α-chloroacetate (0.1 mol), propionic acid (6.66 mL) was added and refluxed for 16 h. The mixture was filtered and concentrated under reduced pressure to get compound **2**. To a stirring solution of hydrazine hydrate (6.5 mL) in an ice bath, a solution of **2** (0.03 mol) in methanol (40 mL) was added slowly (10 min).

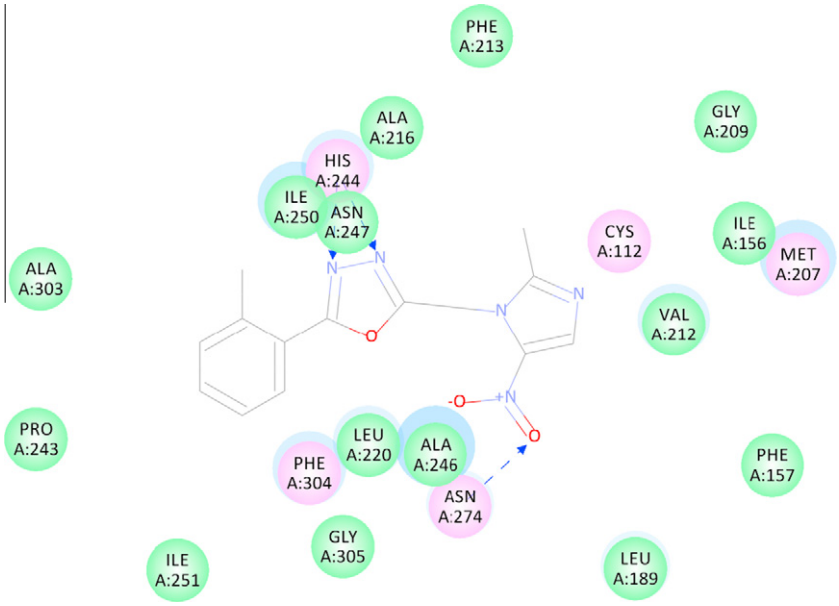


Figure 2. Binding model of compound **11** and *E. coli* FabH. H-bonds are displayed as dashed lines.

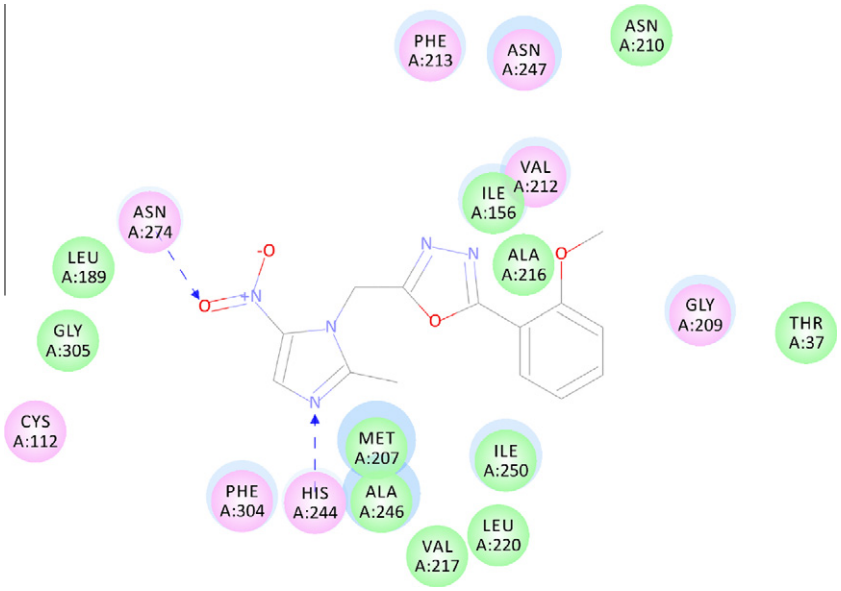


Figure 3. Binding model of compound **12** and *E. coli* FabH. H-bonds are displayed as dashed lines.

The stirring was continued for 4.5 h at 0 °C under argon atmosphere. The white precipitate which formed in −10 °C was filtered and recrystallized from chloroform to give compound **3**.

An equimolar mixture of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide (0.001 mol) and substituted carboxylic acids(0.001 mol) in phosphoryl chloride(15 mL) was refluxed for 10–16 h. Then reaction mixture was cooled, poured into ice-cold water and neutralized with 20% $NaHCO_3$ solution. The resultant solid was filtered, washed with water and recrystallized from ethanol to give nitroimidazoles derivatives **4**–**21**.

4.2.1. 2-(4-Fluorophenyl)-5-((2-methyl-5-nitro-1*H*-imidazol-1-yl)methyl)-1,3,4-oxadiazole (4)

Mp: 229–230 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.44 (s, 3H), 5.75 (s, 2H), 7.45 (t, J = 8.61 Hz, 2H), 8.04 (t, J = 8.40 Hz, 2H), 8.46 (s, 1H). MS (ESI): 304.25 ($C_{13}H_{11}FN_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{10}FN_5O_3$: C, 51.49; H, 3.32; N, 23.09. Found: C, 51.51; H, 3.33; N, 23.07.

4.2.2. 2-(4-Chlorophenyl)-5-((2-methyl-5-nitro-1*H*-imidazol-1-yl)methyl)-1,3,4-oxadiazole (5)

Mp: 225–226 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.49 (s, 3H), 5.75 (s, 2H), 7.56 (d, J = 8.43 Hz, 2H), 7.96 (d, J = 9.39 Hz, 2H), 8.46 (s, 1H). MS (ESI): 320.70 ($C_{13}H_{11}ClN_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{10}ClN_5O_3$: C, 48.84; H, 3.15; N, 21.91. Found: C, 48.85; H, 3.13; N, 21.90.

4.2.3. 2-(4-Bromophenyl)-5-((2-methyl-5-nitro-1*H*-imidazol-1-yl)methyl)-1,3,4-oxadiazole (6)

Mp: 220–223 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.45 (s, 3H), 5.68 (s, 2H), 7.69–7.80 (m, 2H), 7.84–7.93 (m, 2H), 8.46 (s, 1H). MS (ESI): 365.15 ($C_{13}H_{11}BrN_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{10}BrN_5O_3$: C, 42.88; H, 2.77; N, 21.94. Found: C, 42.87; H, 2.78; N, 21.92.

4.2.4. 2-(3-Fluorophenyl)-5-((2-methyl-5-nitro-1*H*-imidazol-1-yl)methyl)-1,3,4-oxadiazole (7)

Mp: 185–186 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.50 (s, 3H), 5.76 (s, 2H), 7.12 (d, J = 8.76 Hz, 1H), 7.92 (d, J = 8.79 Hz, 2H), 7.81 (d, J = 8.40 Hz, 1H), 8.46 (s, 1H). MS (ESI): 304.25 ($C_{13}H_{11}FN_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{10}FN_5O_3$: C, 51.49; H, 3.32; N, 23.09. Found: C, 51.51; H, 3.30; N, 23.09.

4.2.5. 2-(3-Bromophenyl)-5-((2-methyl-5-nitro-1*H*-imidazol-1-yl)methyl)-1,3,4-oxadiazole (8)

Mp: 214–216 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.43 (s, 3H), 5.72 (s, 2H), 7.14 (d, J = 3.42 Hz, 2H), 7.26 (d, J = 8.49 Hz, 2H), 7.81 (d, J = 3.42 Hz, 1H), 8.45 (s, 1H). MS (ESI): 365.15 ($C_{13}H_{11}BrN_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{10}BrN_5O_3$: C, 42.88; H, 2.77; N, 21.94. Found: C, 42.86; H, 2.79; N, 21.93.

4.2.6. 2-((2-Methyl-5-nitro-1*H*-imidazol-1-yl)methyl)-5-(4-nitrophenyl)-1,3,4-oxadiazol (9)

Mp: 214–216 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.34 (s, 3H), 4.97 (s, 2H), 8.09 (d, J = 8.76 Hz, 2H), 8.34 (d, J = 10.62 Hz, 3H). MS (ESI): 331.26 ($C_{13}H_{11}N_6O_5$, $[M+H]^+$). Anal. Calcd for $C_{14}H_{10}N_6O_5$: C, 47.28; H, 3.05; N, 25.45. Found: C, 47.30; H, 3.04; N, 25.42.

4.2.7. 2-(3-Methoxyphenyl)-5-((2-methyl-5-nitro-1*H*-imidazol-1-yl)methyl)-1,3,4-oxadiazole (10)

Mp: 216–217 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.49 (s, 3H), 3.84 (s, 3H), 5.76 (s, 2H), 7.49–7.67 (m, 3H), 7.79 (d, J = 9.72 Hz, 1H), 8.47 (s, 1H). MS (ESI): 316.28 ($C_{14}H_{14}N_5O_4$, $[M+H]^+$). Anal. Calcd for $C_{14}H_{13}N_5O_4$: C, 53.33; H, 4.16; N, 22.21. Found: C, 53.35; H, 4.14; N, 22.22.

4.2.8. 2-(2-Methoxyphenyl)-5-((2-methyl-5-nitro-1*H*-imidazol-1-yl)methyl)-1,3,4-oxadiazole (11)

Mp: 185–186 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.48 (s, 3H), 3.86 (s, 3H), 5.76 (s, 2H), 7.10–7.15 (m, 2H), 7.26 (t, J = 8.34 Hz, 1H), 7.68 (d, J = 8.40 Hz, 1H), 8.45 (s, 1H). MS (ESI): 317.28 ($C_{14}H_{14}N_5O_4$, $[M+H]^+$). Anal. Calcd for $C_{14}H_{13}N_5O_4$: C, 53.33; H, 4.16; N, 22.21. Found: C, 53.36; H, 4.15; N, 22.23.

4.2.9. 2-((2-Methyl-5-nitro-1*H*-imidazol-1-yl)methyl)-5-(2-methylbenzyl)-1,3,4-oxadiazole (12)

Mp: 233–236 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.49 (s, 3H), 2.69 (s, 3H), 5.75 (s, 2H), 7.21 (d, J = 7.53 Hz, 2H), 7.81–7.89 (m, 2H), 8.44 (s, 1H). MS (ESI): 300.28 ($C_{14}H_{14}N_5O_3$, $[M+H]^+$). Anal.

Calcd for $C_{14}H_{13}N_5O_3$: C, 56.18; H, 4.38; N, 23.40. Found: C, 56.16; H, 4.35; N, 23.43.

4.2.10. 2-(4-Fluorobenzyl)-5-((2-methyl-5-nitro-1*H*-imidazol-1-yl)methyl)-1,3,4-oxadiazole (13)

Mp: 264–265 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.33 (s, 3H), 3.57 (s, 2H), 4.91 (s, 2H), 7.20–7.34 (m, 4H), 8.29 (s, 1H). MS (ESI): 318.28 ($C_{14}H_{13}FN_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{14}H_{12}FN_5O_3$: C, 53.00; H, 3.81; N, 22.07. Found: C, 52.98; H, 3.82; N, 22.08.

4.2.11. 2-(4-Chlorobenzyl)-5-((2-methyl-5-nitro-1*H*-imidazol-1-yl)methyl)-1,3,4-oxadiazole (14)

Mp: 255–256 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.23 (s, 3H), 3.56 (s, 2H), 4.84 (s, 2H), 7.23–7.36 (m, 4H), 8.28 (s, 1H). MS (ESI): 334.73 ($C_{14}H_{13}ClN_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{14}H_{12}ClN_5O_3$: C, 50.39; H, 3.62; N, 20.99. Found: C, 50.36; H, 3.64; N, 20.96.

4.2.12. 2-(4-Bromobenzyl)-5-((2-methyl-5-nitro-1*H*-imidazol-1-yl)methyl)-1,3,4-oxadiazole (15)

Mp: 225–227 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.27 (s, 3H), 3.46 (s, 2H), 4.91 (s, 2H), 7.17–7.30 (m, 2H), 7.51 (t, J = 9.24 Hz, 2H) 8.33 (t, J = 9.60 Hz, 1H). MS (ESI): 379.18 ($C_{14}H_{13}BrN_5O_3$, $[M+H]^+$). Anal. Calcd Anal. Calcd for $C_{14}H_{12}BrN_5O_3$: C, 44.46; H, 3.20; N, 18.52. Found: C, 44.48; H, 3.21; N, 18.50.

4.2.13. 2-(3-Fluorobenzyl)-5-((2-methyl-5-nitro-1*H*-imidazol-1-yl)methyl)-1,3,4-oxadiazole (16)

Mp: 221–223 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.33 (s, 3H), 3.57 (s, 2H), 4.93 (s, 2H), 7.20 (d, J = 8.40 Hz, 2H), 7.35 (d, J =8.40 Hz, 2H) 8.33 (s, 1H). MS (ESI): 316.28 ($C_{14}H_{13}FN_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{14}H_{12}FN_5O_3$: C, 50.39; H, 3.62; N, 20.99. Found: C, 50.37; H, 3.64; N, 20.97.

4.2.14. 2-(3-Bromobenzyl)-5-((2-methyl-5-nitro-1*H*-imidazol-1-yl)methyl)-1,3,4-oxadiazole (17)

Mp: 215–218 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.23 (s, 3H), 3.46 (s, 2H), 4.84 (s, 2H), 7.23–7.35 (m, 4H), 8.29 (s, 1H). MS (ESI): 379.18 ($C_{14}H_{13}BrN_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{14}H_{12}BrN_5O_3$: C, 44.46; H, 3.20; N, 18.52. Found: C, 44.43; H, 3.22; N, 18.53.

4.2.15. 2-((2-Methyl-5-nitro-1*H*-imidazol-1-yl)methyl)-5-(4-nitrobenzyl)-1,3,4-oxadiazole (18)

Mp: 225–227 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.37 (s, 3H), 4.44 (s, 2H), 5.65 (s, 2H), 7.63 (d, J = 8.79 Hz, 2H), 8.22 (d, J = 8.76 Hz, 2H) 8.39 (s, 1H). MS (ESI): 345.28 ($C_{14}H_{13}N_6O_5$, $[M+H]^+$). Anal. Calcd for $C_{14}H_{12}N_6O_5$: C, 48.84; H, 3.51; N, 24.41. Found: C, 48.86; H, 3.50; N, 24.39.

4.2.16. 2-(3-Methoxybenzyl)-5-((2-methyl-5-nitro-1*H*-imidazol-1-yl)methyl)-1,3,4-oxadiazole (19)

Mp: 226–228 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.28 (s, 3H), 3.45 (s, 2H), 3.71 (s, 3H), 4.86 (s, 2H), 6.83–6.87 (m, 3H), 7.13 (d, J = 9.24 Hz, 1H), 8.28 (s, 1H). MS (ESI): 330.31 ($C_{15}H_{16}N_5O_4$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{15}N_5O_4$: C, 54.71; H, 4.59; N, 21.27. Found: C, 54.74; H, 4.57; N, 21.28.

4.2.17. 2-(2-Methoxybenzyl)-5-((2-methyl-5-nitro-1*H*-imidazol-1-yl)methyl)-1,3,4-oxadiazole (20)

Mp: 201–203 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.31 (s, 3H), 3.49 (s, 2H), 3.75 (s, 3H), 4.52 (s, 2H), 6.84–6.90 (m, 3H), 7.22 (d, J = 8.40 Hz, 1H), 8.28 (s, 1H). MS (ESI): 330.31 ($C_{15}H_{16}N_5O_4$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{15}N_5O_4$: C, 54.71; H, 4.59; N, 21.27. Found: C, 54.72; H, 4.58; N, 21.25.

4.2.18. 2-((2-Methyl-5-nitro-1*H*-imidazol-1-yl)methyl)-5-(2-methylbenzyl)-1,3,4-oxadiazole (21)

Mp: 218–220 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.36 (s, 3H), 2.54 (s, 3H), 3.52 (s, 2H), 4.52 (s, 2H), 7.29 (d, J = 8.04 Hz, 2H), 7.83 (d, J = 8.22 Hz, 2H), 8.29 (s, 1H). MS (ESI): 314.31 ($C_{15}H_{16}N_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{15}N_5O_3$: C, 57.50; H, 4.83; N, 22.35. Found: C, 76.53; H, 4.80; N, 22.32.

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).[32]

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 compounds 11 and **12** 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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