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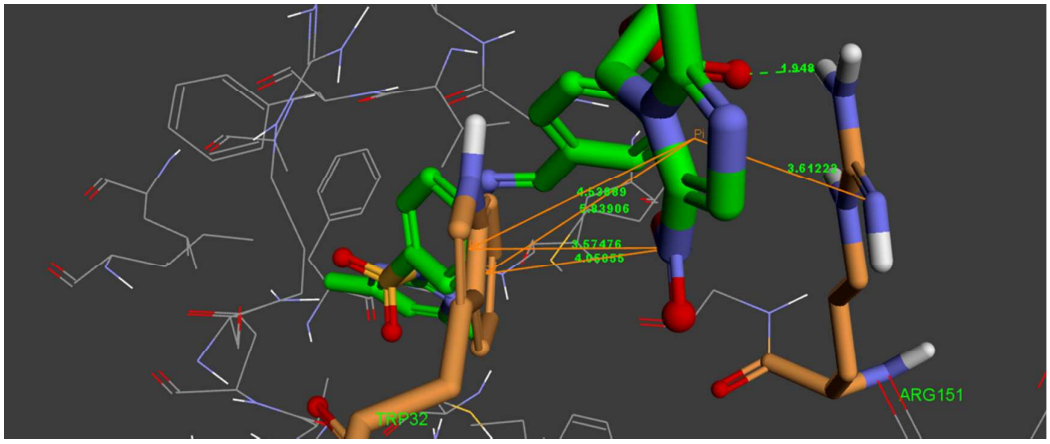

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Abstract

Series of novel Schiff's base derivatives have been synthesized by combing 2-(2-methyl-5-nitro-1*H*-imidazol-1-*yl*)ethyl 4-formylbenzoate **5, 6** with aromatic/heterocyclic amine **7a-r, 8**, **9a-r** in ethanol. All compounds were evaluated for antibacterial assay and inhibition against *E. coli* FabH. Among the compounds studied, most of the compounds showed effective antibacterial and potential inhibitory activity against *E. coli* FabH. Compound **10q** showed most potent inhibitory activity (IC_{50} = 2.6883 μM) by binding tightly into the active site of *E. coli* FabH receptor with minimum binding energy (ΔG_b = -55.3117 kcal/mol), in which molecular docking study indicated the binding mode was stabilized by one hydrogen bond and five π-π interactions.

Keywords:

Schiff's base

Nitroimidazole

Antibacterial activity

E. coli FabH inhibition

1 Introduction:

An alarming increment in pathogenic resistance to existing first line standard drugs is a serious problem in antimicrobial cure.[1] Moreover, the progression of drug-resistant strains has contributed to the inefficiency of the straight antimicrobial therapy. This crops up an enormous interest in antibacterial research and we strongly believe that there is an urgent call for the development of novel antibactieral drugs with divergent and unique structure. Consequently, this spot of research is accorded an enormous significance and keeps on attracting much attention from an increasing number of medicinal chemists. 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.[2]

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.[3,4]

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.[5] 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.[6,7] 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.[8,9] FabH has been proved to be a promising target for the design of novel antimicrobial drugs because it adjusts and control the fatty acid biosynthesis rate in an initiation pathway and its substrate

specificity is a key factor in membrane fatty acid composition.[10–12] 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, particularly for secnidazole, have been characterized in recent reports.[13,14] 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.[15] Also, the treatment achieve with secnidazole is more effective and displays less side effects.[16]

Moreover, 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. Schiff's bases constitute an important class of biologically active drug molecules which has attracted attention of medicinal chemists due to their wide range of pharmacological properties. These compounds are being synthesized as drugs by many researchers in order to combat diseases with minimal toxicity and maximal effects. These predictions has provided therapeutic pathway to develop new effective biologically active Schiff's base derivatives. Many researchers have studied the synthesis, characterization and structure-activity relationship (SAR) of Schiff bases and some of Schiff bases were reported to own antibacterial activities[17] as well as Kim and co-workers reported the YKAs3003, a Schiff base condensed by 4-hydroxy salicylaldehyde and cyclohexanamine as a potent inhibitor of *Escherichia coli* (*E. coli*) FabH with antimicrobial activity.[18]

Along the researches on FabH of our group based on nitroimidazole[19] and Schiff's base[20] respectively, we report here the synthesis and structure-activity relationship of a new series of Schiff base derivatives of nitroimidazole nuclei in a single scaffold and their antibacterial activities against *Escherichia coli*, *Pseudomonas*

aeruginosa (*P. aeruginosa*), *Bacillus subtilis* (*B. subtilis*) and *Staphylococcus aureus* (*S. aureus*) as well as *E. coli* FabH inhibitory activities.

2 Results and Discussion

2.1 Chemistry

Schiff's base derivatives **10a-r** have been synthesized by reaction between the intermediate **5** (2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl, which can be obtained by the simple two steps using compound **1** and metronidazole **3**) and various substituted aniline **7a-r** in ethanol, reflux overnight in good yield (45-87%) (Scheme 1). Besides, the addition of 2-phenylethanamine **8** into the intermediate **5** could produce target compound **11s** in ethanol, reflux overnight. Other compounds **12t-w** were derived from the reaction between compound **2** and secnidazole **4**, subsequently interacting with four substituted aniline **9t-w** in ethanol, reflux overnight.

[Scheme 1]

The structures of all the new synthesized compounds were established by ^{1}H NMR, elemental analysis, and molecular weight of compounds confirmed by mass spectrometry. Mass spectroscopy of compounds showed molecular ion peak (M^{+}) corresponding to the exact mass.

2.2 Biological activity

2.2.1 *In vitro* antibacterial and *E. Coli* FabH inhibitory activity

All the synthesized compounds 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 1. Kanamycin B and Penicillin G were taken as reference compound under identical conditions for comparison.

[Table 1]

Upon investigation of antibacterial activity (Table 1), it has been observed that majority of the compounds have shown effective activity against used strains. Against Gram negative bacteria *E. coli*, compound **10q** (MIC=1.56 µg/mL) showed most effective activity than other compounds of the series, Penicillin G B (MIC=3.13 µg/mL) and comparable activity to kanamycin B (MIC=1.56 µg/mL), while compounds **10f, 10l, 10o, 10r, 10v** and **10w** (MIC=3.13 µg/mL) showed comparable activity to Penicillin G (MIC=3.13 µg/mL), against *P. aeruginosa* compound **10q** (MIC=3.13 µg/mL) showed comparable activity against Kanamycin B (MIC=3.13 µg/mL). Against Gram positive bacterial *S. aureus*, compound **10q** (MIC=3.13 µg/mL) showed more effective activity as well as compounds **10b, 10f, 10k, 10o and 10p** (MIC=6.25 µg/mL) showed comparable activity as compared to Penicillin G (MIC=6.25 µg/mL) and less compare to Kanamycin B (MIC=1.56 µg/mL). Compounds **10q** (MIC=1.56 µg/mL) showed comparable activity and compound **10b, 10f, 10g, 10k, 10p, 10t and 10v** (MIC=3.13 µg/mL) showed less comparable activity as compared to Penicillin G (MIC=1.56 µg/mL) against *B. subtilis*. Of the compounds studied for *E. coli* FabH inhibitory activity (Table 2), compounds **10q** (IC_{50}=2.6883 µM), **12v** (IC_{50}=4.928 µM) and **10r** (IC_{50}=5.5923 µM) showed most potent activity as compared to other compounds of the series and Secnidazole (IC_{50}=28.5 µM), Metronidazole (IC_{50}=17.6 µM).

[Table 2]

Structure activity relationship (SAR) was carried out from *E. coli* FabH inhibitory and antibacterial activities. According to the activity data, it has been observed that the change in R substitution may lead to change in the activity against employed strains as well as *E. coli* FabH. Compounds **10o, 10p, 10q, 10r, 12v and 12w** having sulfonamide linkage (-SO_2NH) showed potent activity compares to other compounds. Electron releasing group R= 3-OCH_3 in compound **10f** give better activity then **10e** (R=3-CH_3), while there is no activity difference is observed in 2, 3 and 4-position of -CH_3 group. Secnidazole derivatives **12t-w** give good activity than derivatives. Introduction of alkyl chain in compound **11s** gives better activity than simple amine moiety in compound **10n**, also different halogen groups at 2, 3 and 4-position gives

different activity. The decreasing order of activity in halogen compounds is (4-Br > 4-F > 2-Cl > 2-Br > 4-Cl > 2, 4-Cl > 2-F). Moreover, reviewing and comparing the activity data, it is worthy to mention that the antibacterial activity against *E. coli* FabH of the target compounds depends not only on the heteroaromatic pharmacophore, but also on the nature of the substituents.

Besides, an acute oral toxicity test was conducted with mice to determine the toxicity from a single dose via the oral route. Based on the results (not listed), the single dose acute oral LD50 (half maximal concentration of lethal does) values of the compounds (10b, 10f, 10q, 10r, 12v) are all greater than 5000 mg/kg of bodyweight.

2.2.2 Molecular docking

Molecular docking of all compounds and *E. coli* FabH was performed on the binding model based on the *E. coli* FabH-CoA complex structure (1HNJ.pdb).[21] All docking runs were applied Ligand Fit Dock protocol of Discovery Studio 3.5. The binding energy calculation of the synthesized compounds is mentioned in Table 3. Among them, compound **10q** showed the lowest interaction energy (ΔG_b = -55.3117 kcal/mol). The binding model of compound 20 and *E. coli* FabH is depicted in Figure 1 and Figure 1A. In the binding model, compound 20 was nicely bound to the FabH kinase with one hydrogen bond and five π-π interactions. Among hydrogen bonds formed between O-atom of C=O (Carbonyl group) and ARG151 with distance: 1.948 Å; DHA angle: 133.0º and HAY angle: 149.3º, one π-π interaction formed between imidazole ring and ARG151 with distance 3.61222 Å, two π-π interactions formed between imidazole ring and TRP32 with distance 4.53889 Å and 5.83906 Å and two π-π interactions formed between -NO_2 group of imidazole ring and TRP32 with distance 3.57476 Å and 4.05855 Å. Also the binding model of compound 6 and *E. coli* FabH is depicted in Figure 2 and Figure 2A. In the binding model, compound **10f** was nicely bound to the FabH kinase with two hydrogen bonds and one π-π interaction. Among hydrogen bond formed between O-atom of -OCH_3 and ARG151 with distance: 2.4297 Å; DHA angle: 107.1º and HAY angle: 100.2º, while another one formed between O-atom of of C=O (Carbonyl group) and ASN247 with distance: 2.3913 Å; DHA angle: 125.3º and HAY angle: 117.0º. One π-π interaction formed

between imidazole ring and HIS244 with distance 6.62666 Å. This molecular docking result, along with the biological assay data, suggests that compound 20 and 6 proved as a potential inhibitor of *E. coli* FabH.

[Figure 1]

[Figure 1A]

[Figure 2]

[Figure 2A]

[Table 3]

3 Conclusion

New Schiff's base derivatives **10a-r, 11s and 12t-w** have been synthesized by reaction between 2-(2-methyl-5-nitro-1*H*-imidazol-1-*yl*)ethyl 4-formylbenzoate **5, 6** and aromatic/ heterocyclic amine in ethanol. This synthetic strategy allows the assimilation of two promising bioactive nuclei in a single scaffold through an easy way. Reviewing the biological activity data, it has been concluded that majority of the compounds have found to be most effective against applied bacterial strains. Compound **10q** showed most effective inhibition by binding in to the active site of *E. coli* FabH receptor with a minimum binding energy. According to this, it is worthy to mention that the Schiff's base derivatives having nitroimidazole nuclei have become a vital spot of antibacterial and *E. coli* FabH inhibition medicine research.

4 Experiments

4.1 Materials and measurements

All chemicals and reagents used in the current study were of analytical grade. Melting points were determined on an XT4 MP apparatus (Taike Corp., Beijing, China). All the ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX300 model Spectrometer in DMSO-d_6 and chemical shifts were reported in ppm (d). ESI-MS spectra were recorded on a Mariner System 5304 mass spectrometer. Elemental analyses were performed on a CHN-O-Rapid instrument. TLC was performed on the glass backed silica gel sheets (Silica Gel 60 GF254) and visualized

in UV light (254 nm).

4.2 General method for synthesis of Schiff's base derivatives.

2-(2-methyl-5-nitro-1*H*-imidazol-1-*yl*)ethyl 4-formylbenzoate 5, 6 (1 mol) and aromatic/heterocyclic amine **7a-r, 8**, **9t-w** (1.1 mol) were mixed together in ethanol as a solvent. The reaction mixture was stirred and reflux overnight. After the completion of reaction (checked by TLC), the solid separated was filtered, washed well with ethanol (10 mL) and water (10 mL), and finally dried and recrystallized from ethanol to get the pure solid sample **10a-r, 11s**, **12t-w**. Physical, analytic, and spectroscopic characterization data of the compounds are presented hereafter.

4.2.1. (E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-((p-tolylimino)methyl)benzoate (10a)

White powder, yield: 83%. m.p. 191-193 °C; 1H NMR (DMSO-d6) δ ppm: 2.33, 2.48 (s, 6H, 2×CH_3), 4.66-4.77 (t, 4H, 2×CH_2), 7.24-8.06 (m, 9H, ArHs), 8.73 (s, 1H, CH=N); Anal. Calcd. for $C_{21}H_{20}N_4O_4$ (392.15 g/mol): C, 64.28; H, 5.14; N, 14.28 (%); Found: C, 64.49; H, 5.22; N, 14.33 (%); MS (m/z): 392.1 (M+).

4.2.2. (E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-(((4-methoxyphenyl)imino)methyl) benzoate (10b)

White powder, yield: 87%. m.p. 197-199 °C; 1H NMR (DMSO-d6) δ ppm: 2.48 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 4.65-4.77 (t, 4H, 2×CH_2), 6.99-8.05 (m, 9H, ArHs), 8.74 (s, 1H, CH=N); Anal. Calcd. for $C_{21}H_{20}N_4O_5$ (408.41 g/mol): C, 61.76; H, 4.94; N, 13.72 (%); Found: C, 61.80; H, 5.12; N, 13.82 (%); MS (m/z): 408.1 (M+).

4.2.3. (E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-((o-tolylimino)methyl)benzoate (10c)

White powder, yield: 75%. m.p. 187-188 °C; 1H NMR (DMSO-d6) δ ppm: 2.35, 2.48 (s, 6H, 2×CH_3), 4.68-4.76 (t, 4H, 2×CH_2), 7.09-8.07 (m, 9H, ArHs), 8.70 (s, 1H, CH=N); Anal. Calcd. for $C_{21}H_{20}N_4O_4$ (392.15 g/mol): C, 64.28; H, 5.14; N, 14.28 (%); Found: C, 64.49; H, 5.22; N, 14.33 (%); MS (m/z): 392.1 (M+).

4.2.4. (E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-(((2-methoxyphenyl)imino)methyl)benzoate (10d)

White powder, yield: 80%. m.p. 196-197 °C; 1H NMR (DMSO-d6) δ ppm: 2.48 (s, 3H, CH_3), 3.80 (s, 3H, OCH_3), 4.68-4.76 (t, 4H, 2×CH_2), 6.95-8.05 (m, 9H, ArHs), 8.63 (s, 1H, CH=N); Anal. Calcd. for $C_{21}H_{20}N_4O_5$ (408.41 g/mol): C, 61.76; H, 4.94; N, 13.72 (%); Found: C, 61.80; H, 5.12; N, 13.82 (%); MS (m/z): 408.1 (M+).

4.2.5. (E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-((m-tolylimino)methyl)benzoate (10e)

White powder, yield: 75%. m.p. 185-187 °C; 1H NMR (DMSO-d6) δ ppm: 2.35, 2.48 (s, 6H, 2×CH_3), 4.68-4.76 (t, 4H, 2×CH_2), 7.09-8.07 (m, 9H, ArHs), 8.70 (s, 1H, CH=N); Anal. Calcd. for $C_{21}H_{20}N_4O_4$ (392.15 g/mol): C, 64.28; H, 5.14; N, 14.28 (%); Found: C, 64.49; H, 5.22; N, 14.33 (%); MS (m/z): 392.1 (M+).

4.2.6. (E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-(((3-methoxyphenyl)imino)methyl)benzoate (10f)

White powder, yield: 77%. m.p. 195-197 °C; 1H NMR (DMSO-d6) δ ppm: 2.49 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 4.68-4.78 (t, 4H, 2×CH_2), 6.86-8.08 (m, 9H, ArHs), 8.74 (s, 1H, CH=N); Anal. Calcd. for $C_{21}H_{20}N_4O_5$ (408.41 g/mol): C, 61.76; H, 4.94; N, 13.72 (%); Found: C, 61.80; H, 5.12; N, 13.82 (%); MS (m/z): 408.1 (M+).

4.2.7. (E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-(((2-chlorophenyl)imino)methyl)benzoate (10g)

White powder, yield: 76%. m.p. 199-200 °C; 1H NMR (DMSO-d6) δ ppm: 2.45 (s, 3H, CH_3), 4.64-4.75 (s, 4H, 2×CH_2), 7.21-8.02 (m, 9H, ArHs), 8.75 (t, 1H, CH=N); Anal. Calcd. for $C_{20}H_{17}ClN_4O_4$ (412.83 g/mol): C, 58.19; H, 4.15; N, 13.57 (%); Found: C, 58.25; H, 4.31; N, 13.62 (%); MS (m/z): 412.1 (M+).

4.2.8. (E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-(((2-bromophenyl)imino)methyl)benzoate (10h)

White powder, yield: 78%. m.p. 202-203 °C; 1H NMR (DMSO-d6) δ ppm: 2.45 (s, 3H, CH_3), 4.64-4.75 (s, 4H, 2×CH_2), 7.21-8.02 (m, 9H, ArHs), 8.76 (t, 1H, CH=N); Anal. Calcd. for $C_{20}H_{17}BrN_4O_4$ (457.28 g/mol): C, 52.53; H, 3.75; N, 12.25 (%); Found: C, 52.65; H, 3.81; N, 12.42 (%); MS (m/z): 456.1 (M+).

4.2.9. (E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-(((2-florophenyl)imino)methyl)benzoate (10i)

White powder, yield: 75%. m.p. 198-199 °C; 1H NMR (DMSO-d6) δ ppm: 2.45 (s, 3H, CH_3), 4.64-4.75 (s, 4H, 2×CH_2), 7.21-8.02 (m, 9H, ArHs), 8.74 (t, 1H, CH=N); Anal. Calcd. for $C_{20}H_{17}FN_4O_4$ (396.37 g/mol): C, 60.60; H, 4.32; N, 14.13 (%); Found: C, 60.65; H, 4.40; N, 14.20 (%); MS (m/z): 396.4 (M+).

4.2.10. (E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-(((4-chlorophenyl)imino)methyl)benzoate (10j)

White powder, yield: 86%. m.p. 201-202 °C; 1H NMR (DMSO-d6) δ ppm: 2.49 (s, 3H, CH_3), 4.68-4.78 (s, 4H, 2×CH_2), 7.35-8.08 (m, 9H, ArHs), 8.74 (t, 1H, CH=N); Anal. Calcd. for $C_{20}H_{17}ClN_4O_4$ (412.83 g/mol): C, 58.19; H, 4.15; N, 13.57 (%);

Found: C, 58.25; H, 4.31; N, 13.62 (%); MS (m/z): 412.1 (M+).

4.2.11. (E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-(((4-bromophenyl)imino)methyl)benzoate (10k)

White powder, yield: 87%. m.p. 203-204 °C; 1H NMR (DMSO-d6) δ ppm: 2.49 (s, 3H, CH_3), 4.68-4.78 (s, 4H, 2×CH_2), 7.35-8.08 (m, 9H, ArHs), 8.74 (t, 1H, CH=N); Anal. Calcd. for $C_{20}H_{17}BrN_4O_4$ (457.28 g/mol): C, 52.53; H, 3.75; N, 12.25 (%); Found: C, 52.65; H, 3.81; N, 12.42 (%); MS (m/z): 456.1 (M+).

4.2.12. (E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-(((4-florophenyl)imino)methyl)benzoate (10l)

White powder, yield: 85%. m.p. 199-200 °C; 1H NMR (DMSO-d6) δ ppm: 2.49 (s, 3H, CH_3), 4.68-4.78 (s, 4H, 2×CH_2), 7.35-8.08 (m, 9H, ArHs), 8.74 (t, 1H, CH=N); Anal. Calcd. for $C_{20}H_{17}FN_4O_4$ (396.37 g/mol): C, 60.60; H, 4.32; N, 14.13 (%); Found: C, 60.65; H, 4.40; N, 14.20 (%); MS (m/z): 396.4 (M+).

4.2.13. (E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-(((2,5-dichlorophenyl)imino)methyl)benzoate (10m)

White powder, yield: 65%. M.p. 205-206 °C; 1H NMR (DMSO-d6) δ ppm: 2.45 (s, 3H, CH_3), 4.64-4.75 (t, 4H, 2×CH_2), 7.31-8.07 (m, 8H, ArHs), 8.66(s, 1H, CH=N); Anal. Calcd. For $C_{20}H_{16}C_{l2}N_4O_4$ (447.27 g/mol): C, 53.71; H, 3.61; N, 12.53 (%); Found: C, 53.82; H, 3.49; N, 12.43 (%); MS (m/z): 446.1 (M+).

4.2.14. (E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-((phenylimino)methyl)benzoate (10n)

White powder, yield: 67%. m.p. 189-190 °C; 1H NMR (DMSO-d6) δ ppm: 2.49 (s, 3H, CH_3), 4.68-4.78 (t, 4H, 2×CH_2), 7.28-8.09 (m, 10H, ArHs), 8.73 (s, 1H, CH=N); Anal. Calcd. for $C_{20}H_{18}N_4O_4$ (378.38 g/mol): C, 63.48; H, 4.79; N, 14.81 (%); Found: C, 63.64; H, 4.63; N, 14.91 (%); MS (m/z): 378.1 (M+).

4.2.18. (E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-(((4-(N-(pyridin-2-yl)sulfamoyl)phenyl)imino) methyl) benzoate (10o)

White powder, yield: 45%. m.p. 234-235°C; 1H NMR (DMSO-d6) δ ppm: 2.48 (s, 3H, CH_3), 4.69-4.76 (t, 4H, 2×CH_2), 6.87-8.08 (m, 13H, ArHs), 8.72 (s, 1H, CH=N), 11.70 (s, 1H, SO_2NH); Anal. Calcd. for $C_{25}H_{22}N_6O_6S$ (534.54 g/mol): C, 56.17; H, 4.15; N, 15.72 (%); Found: C, 56.24; H, 3.93; N, 15.85 (%); MS (m/z): 534.1 (M+).

4.2.19. (E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-(((4-(N-(4-methylpyrimidin-2-yl)sulfamoyl) phenyl)imino)methyl)benzoate(10p)

White powder, yield: 50%. m.p. 245-246 °C; 1H NMR (DMSO-d6) δ ppm: 2.33,

2.48 (s, 6H, 2×CH_3), 4.68-4.76 (t, 4H, 2×CH_2), 6.91-8.34 (m, 11H, ArHs), 8.72 (s, 1H, CH=N), 11.72 (s, 1H, SO_2NH); Anal. Calcd. for $C_{25}H_{23}N_7O_6S$ (549.56 g/mol): C, 54.64; H, 4.22; N, 17.84 (%); Found: C, 56.79; H, 4.12; N, 17.58 (%); MS (m/z): 549.1 (M+).

4.2.20. (E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-(((4-(N-(4,6-dimethylpyrimidin-2-yl)sulfamoyl)phenyl)imino) methyl)benzoate (10q)

White powder, yield: 54%. m.p. 256-257 °C; 1H NMR (DMSO-d6) δ ppm: 2.24, 2.26, 2.47 (s, 9H, 3×CH_3), 4.67-4.75 (t, 4H, 2×CH_2), 5.94-8.04 (m, 10H, ArHs), 8.71 (s, 1H, CH=N), 11.75 (s, 1H, SO_2NH); Anal. Calcd. for $C_{26}H_{25}N_7O_6S$ (563.59 g/mol): C, 55.41; H, 4.47; N, 17.40 (%); Found: C, 55.31; H, 4.25; N, 17.63 (%); MS (m/z): 563.2 (M+).

4.2.21. (E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-(((4-(N-carbamimidoylsulfamoyl) phenyl)imino)methyl) benzoate (10r)

White powder, yield: 51%. m.p. 232-233 °C; 1H NMR (DMSO-d6) δ ppm: 2.48 (s, 3H, CH_3), 4.69-4.76 (t, 4H, 2×CH_2), 6.72-8.09 (m, 12H, ArHs+NH_2), 8.73 (s, 1H, CH=N), 11.73 (s, 1H, SO_2NH); Anal. Calcd. for $C_{21}H_{21}N_7O_6S$ (499.50 g/mol): C, 50.50; H, 4.24; N, 19.63 (%); Found: C, 50.37; H, 4.06; N, 19.48 (%); MS (m/z): 499.1 (M+).

4.2.15. (E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-((phenethylimino)methyl)benzoate (11s)

White powder, yield: 80%. m.p. 198-199 °C; 1H NMR (DMSO-d6) δ ppm: 2.47 (s, 3H, CH_3), 2.95 (s, 2H, CH_2), 3.86 (s, 2H, CH_2), 4.65-4.76 (t, 4H, 2×CH_2), 7.17-8.06 (m, 10H, ArHs), 8.35 (s, 1H, CH=N); Anal. Calcd. for $C_{22}H_{22}N_4O_4$ (406.43 g/mol): C, 65.01; H, 5.46; N, 13.78 (%); Found: C, 64.86; H, 5.64; N, 13.85 (%); MS (m/z): 406.2 (M+).

4.2.16. (E)-1-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-yl 4-(((4-methoxyphenyl)imino)methyl) benzoate (12t)

White powder, yield: 87%. m.p. 204-205 °C; 1H NMR (DMSO-d6) δ ppm: 1.57, 2.54 (s, 6H, 2×CH_3), 3.89 (s, 3H, OCH_3), 4.48-4.78 (d, 2H, CH_2), 5.59-5.64 (t, 1H, CH), 6.99-8.04 (m, 9H, ArHs), 8.58 (s, 1H, CH=N); Anal. Calcd. for $C_{22}H_{22}N_4O_5$ (422.43 g/mol): C, 62.55; H, 5.25; N, 13.26 (%); Found: C, 62.41; H, 5.04; N, 13.14 (%); MS (m/z): 422.2 (M+).

4.2.17. (E)-1-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-yl 4-((p-tolylimino)methyl)benzoate (12u)

White powder, yield: 86%. m.p. 203-205 °C; 1H NMR (DMSO-d6) δ ppm: 1.56, 2.43, 2.54 (s, 9H, 3×CH_3), 4.48-4.78 (d, 2H, CH_2), 5.60-5.64 (t, 1H, CH), 7.21-8.05 (m, 9H, ArHs), 8.56 (s, 1H, CH=N); Anal. Calcd. for $C_{22}H_{22}N_4O_4$ (406.43 g/mol): C, 65.01; H, 5.46; N, 13.78 (%); Found: C, 65.13; H, 5.30; N, 13.83 (%); MS (m/z): 406.2 (M+).

4.2.22. (E)-1-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-yl 4-(((4-(N-(4-methylpyrimidin-2-yl)sulfamoyl)phenyl)imino) methyl)benzoate (12v)

White powder, yield: 45%. m.p. 256-257 °C; 1H NMR (DMSO-d6) δ ppm: 2.24, 2.26, 2.47 (s, 9H, 3×CH_3), 4.67-4.75 (d, 2H, CH_2), 5.60-5.64 (t, 1H, CH), 5.94-8.04 (m, 11H, ArHs+NH_2), 8.71 (s, 1H, CH=N), 11.75 (s, 1H, SO_2NH); Anal. Calcd. for $C_{26}H_{25}N_7O_6S$ (563.59 g/mol): C, 55.41; H, 4.47; N, 17.40 (%); Found: C, 55.31; H, 4.25; N, 17.63 (%); MS (m/z): 563.2 (M+).

4.2.23. (E)-1-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-yl 4-(((4-(N-carbamimidoylsulfamoyl) phenyl)imino)methyl) benzoate (12w)

White powder, yield: 54%. m.p. 240-241 °C; 1H NMR (DMSO-d6) δ ppm: 1.46, 2.45 (s, 6H, 2×CH_3), 4.57-4.73 (d, 2H, CH_2), 5.71 (t, 1H, CH), 7.37-8.08 (m, 11H, ArHs+NH_2), 8.74(s, 1H, CH=N), 11.75 (s, 1H, SO_2NH); Anal. Calcd. for $C_{22}H_{23}N_7O_6S$ (513.53 g/mol): C, 51.46; H, 4.51; N, 19.09 (%); Found: C, 51.52; H, 4.40; N, 19.20 (%); MS (m/z): 513.1 (M+).

4.3 Biological Assays

4.3.1 Antibacterial activity assay

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).[22] *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 ºC. 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 ºC for bacteria. The MICs were recorded by visual observations after 24 h (for bacteria) of incubation. Kanamycin B and Penicilline G were used as standards for bacterial. The observed MICs are presented in Table 1.

4.3.2 *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 B (30 μg/mL). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) analysis was used to screen colonies for over expression of proteins. One such positive colony was used to inoculate 10 mL of LB medium with 30 μg/mL of kanamycin B and grown overnight at 37 ºC, 1 mL of which was used to inoculate 100 mL LB medium supplemented with 30 mg/mL of kanamycin B. The culture was shaken for 4 h at 37 ºC, 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 ºC 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 ºC. 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 [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 pellet was washed with 10% ice-cold TCA and resuspended with 5 μL of 0.5 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%.

4.3.3 Acute oral toxicity Assay

Five thousand milligrams of the compounds (10b, 10f, 10q, 10r, 12v) per kilogram of bodyweight were handled to 25 healthy rats by oral gavage, respectively. The animals were observed for mortality, signs of gross toxicity and behavioral changes at least once daily for 14 days. Bodyweights were recorded prior to administration and again on Day 7 and 14. All animals kept active and healthy during the whole study time. There were no signs of gross toxicity or abnormal behavior.

4.4 Docking simulations

The crystal structures of *E. coli* FabH (PDB code: 1HNJ) was obtained from the Protein Data Bank (http://www.rcsb.org). Molecular docking of compounds into the three-dimensional X-ray structure of FabH was carried out using Ligand Fit Dock protocol of Discovery Studio 3.5.

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Figure Captions

Table 1. The MICs (minimum inhibitory concentrations) of the compounds against these bacteria

Table 2. *E. coli* FabH inhibitory activities of compounds **10a-r, 11s, 12t-w**.

Table 3. Banding energy of synthesized compounds **10a-r, 11s, 12t-w**.

Figure 1. 3D binding model of compound **10q** into the active site of FabH.

Figure 1A. Surface model of compound **10q** into the active site of FabH (3D-model).

Figure 2. 3D binding model of compound **10f** into the active site of FabH.

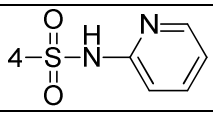
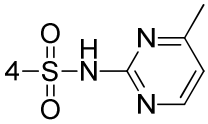
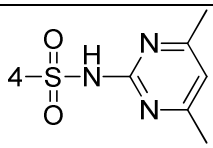
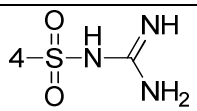
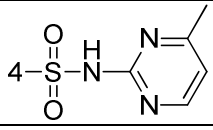
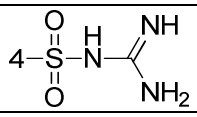
Figure 2A. Surface model of compound **10f** into the active site of FabH (3D-model).

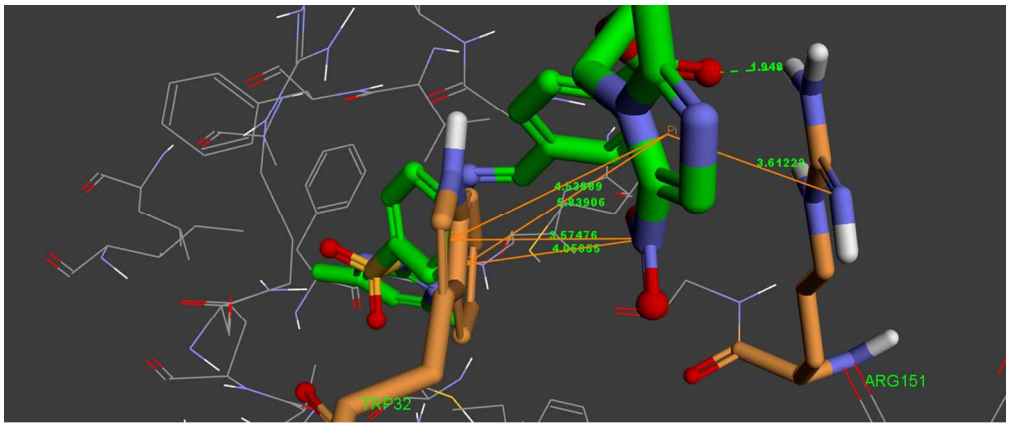
Scheme 1. Synthesis of the titled compounds.

Compound	Minimum inhibitory concentrations (μg/mL) of **10a-r, 11s, 12t-w**.			
	Gram-negative		Gram-positive	
	E.coli ATCC35218	*P.aeruginosa ATCC13525*	*B.subtilis ATCC6633*	*S.aureus ATCC6538*
10a	25	100	25	50
10b	6.25	12.5	3.13	6.25
10c	25	100	25	50
10d	12.5	100	25	25
10e	50	100	25	100
10f	3.13	12.5	3.13	6.25
10g	6.25	25	3.13	12.5
10h	6.25	100	12.5	12.5
10i	25	100	25	50
10j	12.5	100	12.5	25
10k	6.25	12.5	3.13	6.25
10l	3.13	25	12.5	25
10m	12.5	50	6.25	25
10n	12.5	50	12.5	25
10o	3.13	12.5	6.25	6.25
10p	6.25	25	3.13	6.25
10q	1.56	3.13	1.56	3.13
10r	3.13	12.5	6.25	12.5
11s	6.25	25	6.25	12.5
12t	12.5	25	3.13	12.5
12u	12.5	100	12.5	25
12v	3.13	25	3.13	12.5
12w	3.13	12.5	6.25	12.5
Penicillin G	3.13	6.25	1.56	6.25
Kanamycin B	1.56	3.13	0.78	1.56

Table 2. *E. coli* FabH inhibitory activities of compounds **10a-r, 11s, 12t-w**.

Compounds	IC_{50}(μM)	Hemolysis LC_{30} (mg/ml)
10a	31.4738	>10
10b	9.9087	>10
10c	39.0767	>10
10d	30.8408	>10
10e	31.0767	>10
10f	6.4994	>10
10g	12.2729	>10
10h	17.3407	>10
10i	43.4738	>10
10j	30.7389	>10
10k	11.1688	>10
10l	11.9535	>10
10m	14.8985	>10
10n	28.0284	>10
10o	6.1688	>10
10p	6.9193	>10
10q	2.6883	>10
10r	5.5923	>10
11s	10.0336	>10
12t	10.0857	>10
12u	28.4504	>10
12v	4.928	>10
12w	6.8125	>10
DCCP	3.1542	>10

Table 3. Banding energy of synthesized compounds **10a-r, 11s, 12t-w**.		
Compounds	R (Substitution)	CDOCKER interaction energy - ΔG_b (kcal/mol)
10a	4-CH_3	38.5262
10b	4-OCH_3	45.0913
10c	2-CH_3	37.9892
10d	2-OCH_3	39.1592
10e	3-CH_3	38.9233
10f	3-OCH_3	48.5006
10g	2-Cl	41.7271
10h	2-Br	40.6593
10i	2-F	36.9981
10j	4-Cl	39.2611
10k	4-Br	44.8312
10l	4-F	42.0465
10m	2,4-Cl	41.1015
10n	H	39.9716
10o		48.8312
10p		48.0807
10q		55.3117
10r		49.4077
11s	H	44.9664
12t	4-OCH_3	44.9143
12u	4-CH_3	39.5496
12v		51.0720
12w		48.1875



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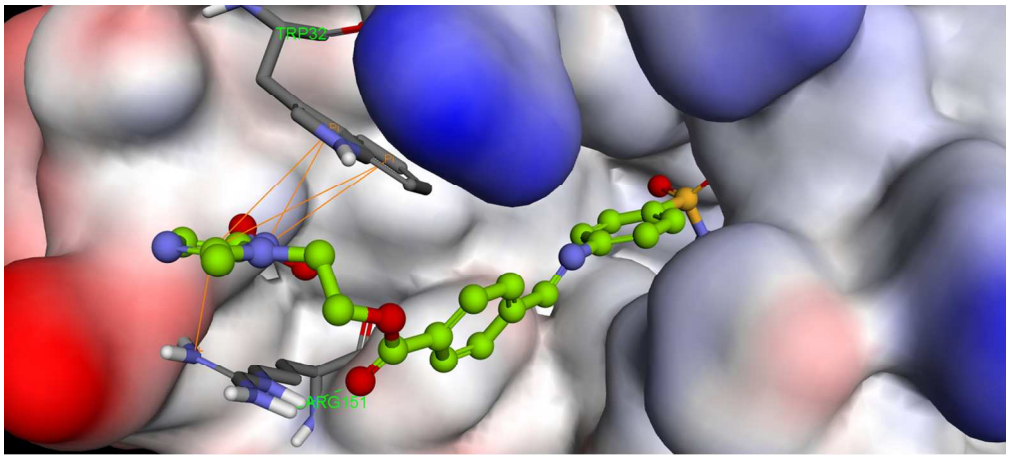

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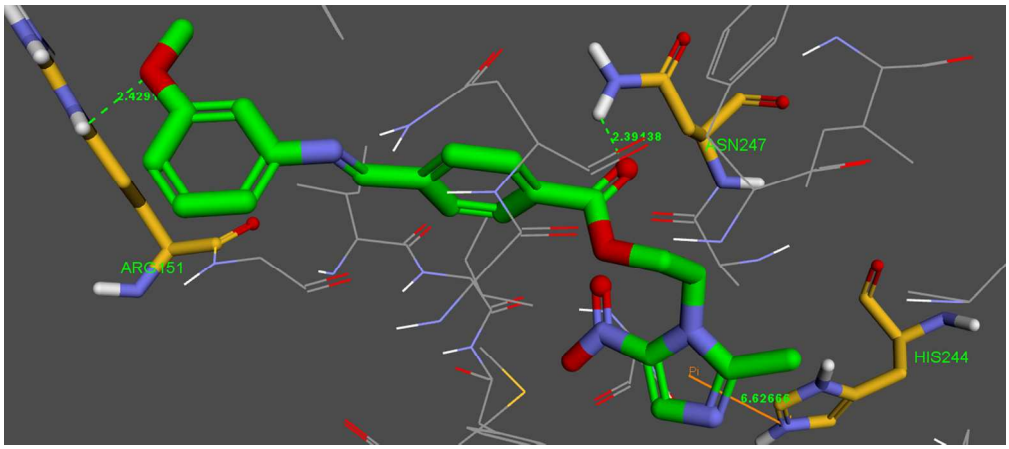

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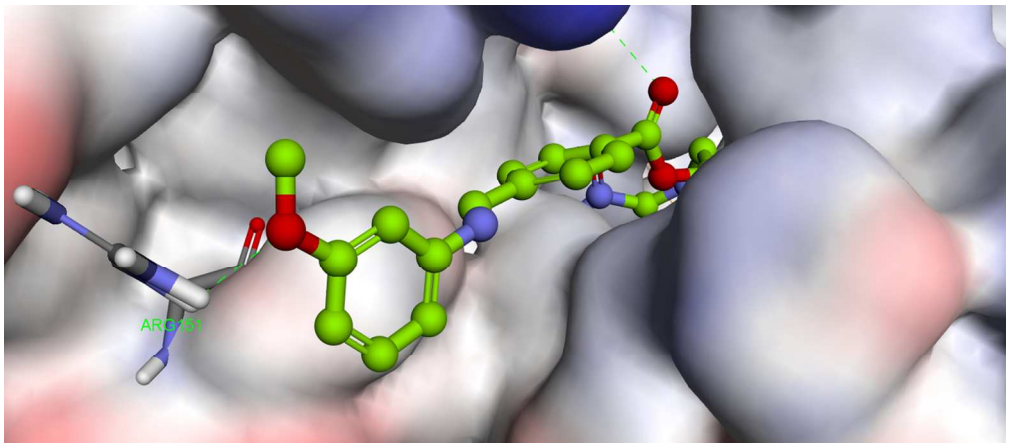

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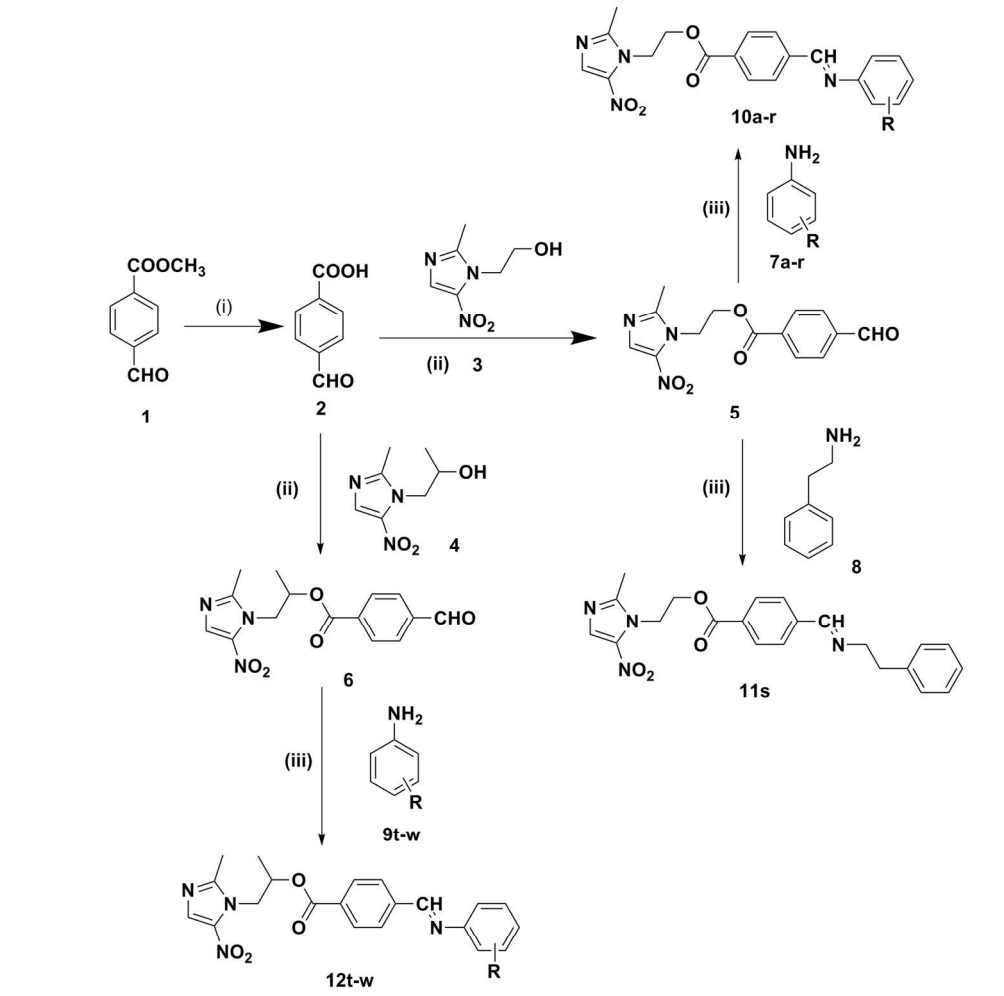

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