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Design, synthesis, and antibacterial evaluation of new Schiff's base derivatives bearing nitroimidazole and pyrazole nuclei as potent *E. coli* FabH inhibitors

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Abstract New Schiff's base derivatives **5a–j** have been synthesized by reaction between 5-aryloxypyrazole-4-carbaldehydes **3a–j** and 2-(2-methyl-5-nitro-1*H*-imidazol-1-*yl*)acetohydrazide **4** in the presence of nickel (II) nitrate as a catalyst in ethanol at room temperature with good yield (75–88 %). All compounds were tested for antibacterial properties and inhibition of *E. coli* FabH. Of the compounds studied, the majority of the compounds showed effective antibacterial properties and inhibition of *E. coli* FabH activity. Compound **5i** showed the most effective inhibition ($IC_{50} = 4.6 \pm 0.2$ μM) by binding into the active site of the *E. coli* FabH

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receptor with minimum binding energy ($\Delta G_b = -54.2961$ kcal/mol). The binding was stabilized by two hydrogen bonds, two π–π, and three π–cation interactions.

Keywords Pyrazole · Imidazol · Antibacterial activity · *E. coli* FabH

Introduction

An alarming increase in pathogenic resistance to existing first line standard drugs is a serious problem in antimicrobial cure and necessitates continuing research into new classes of antimicrobials [1]. Moreover, the progression of drug-resistant strains has contributed to the inefficiency of the straight antimicrobial therapy. Thus, there is enormous interest in antibacterial research, and we strongly believe that there is an urgent call for the development of new drugs with divergent and unique structure and probably with an unusual mechanism of action from that of existing first line drugs. Consequently, the enormous significance of this area of research continues to attract increasing numbers of medicinal chemists. In order to prevent serious illness caused by microorganisms, the discovery of new types of antibacterial agents is a crucial task at present. Fortunately, much of the research effort is put into the design of new antibacterial agents with high efficiency [2].

In the recent 10 years, research has been focused toward new antibacterial agents that may act through different targets in key areas of the bacterial cell cycle, in order to surpass the problem of acquired resistance. Fatty acid synthesis (FAS) pathway in bacteria is a promising target in the recent research, and fatty acid biosynthesis (FAB) is a fundamental metabolic process for microorganisms and is 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]. On the other hand, some novel compounds have 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 proved to be a promising target for the design of novel antimicrobial drugs because it adjusts and controls 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, there is gaining interest in nitroimidazole derivatives in drug research for antimicrobial chemotherapeutics and antiangiogenic hypoxic cell radiosensitizers. The metabolism and toxicology of nitroimidazole derivatives, particularly for secnidazole, have been characterized in recent reports [13, 14]. Secnidazole (α, 2-dimethyl-5-nitro-1*H*-imidazole-1-ethanol) is extraordinarily

effective in the treatment of giardiasis, amebiasis, and bacterial vaginosis. By oral administration, secnidazole can be rapidly and completely absorbed and has a longer terminal elimination half-life (17–29 h) than popular medication [15]. Also, the treatment achieved with secnidazole is more effective and has fewer side effects [16].

Moreover, Schiff's bases are compounds with the structure 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 that has attracted the attention of medicinal chemists due to their wide range of pharmacological properties. These compounds are being synthesized as drugs by many researchers in an effort to combat diseases with minimal toxicity and maximal effects. The results of these predictions have provided a 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's bases, and some were reported to have antibacterial activities [17]. Further, Kim et al. reported the YKAs3003, a Schiff's base condensed by 4-hydroxy salicylaldehyde and cyclohexanamine, as a potent inhibitor of *Escherichia coli* (*E. coli*) FabH with antimicrobial activity [18].

Encouraged by potential clinical applications of Schiff's base derivatives and our previous investigations on FabH inhibitory activity of nitroimidazole derivatives [19], and another literature survey on pyrazole having the same activity [20], we report here the synthesis and structure–activity relationship of a new series of Schiff's base derivatives having nitroimidazole and pyrazole 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 inhibitory activities against *E. coli* FabH.

Experiments

Materials and measurements

All chemicals and reagents used in the current study were of analytical grade. Melting points (uncorrected) 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*). FTIR spectra (KBr) were run on a Nexus 870 FT-IR spectrophotometer. 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). Compound **3a–j** was already reported by us [21].

General method for synthesis of Schiff's base derivatives 5a–j

5-aryloxypyrazole-4-carbaldehydes **3a–j** (1 mol) and 2-(2-methyl-5-nitro-1*H*-imidazol-1-*yl*) acetohydrazide **4** (1.1 mol) and $Ni(NO_3)_2{\cdot}6H_2O$ (10 mol %) were mixed together in ethanol as a solvent. The reaction mixture was stirred at room

temperature for 2.5–3 h. After the completion of reaction (checked by TLC ethyl acetate:hexane = 1:1), the solid compound was separated, filtered, washed well with ethanol (10 mL) and water (10 mL), and finally dried and recrystallized from ethanol to get the pure solid sample **5a**–**j**. Physical, analytic, and spectroscopic characterization data of the compounds **5a**–**j** are presented hereafter.

*(E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)-N′-((3-methyl-5-phenoxy-1-phenyl-1H-pyrazol-4-yl)methylene)acetohydrazide (**5a**)* Yield: 81 %; mp 238–240 °C; IR (KBr, ν_{max}, cm^{-1}): 3325 (str. of –N–H), 3035 (ArC–H), 1685 (C=O stretching), 1215 cm^{-1} (C–O–C ether stretching);^{1}H NMR (DMSO-d_6) δ ppm: 2.37, 2.48 (s, 6H, 2 × CH_3), 4.95 (s, 2H, CH_2), 7.02–7.93 (m, 11H, Ar–H), 8.78 (s, 1H, CH=N), 11.20 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) (δ): 14.32, 21.37 (Ar–CH_3), 37.81 (CH_2–CO), 109.35, 118.78, 121.93, 124.24, 128.47, 128.95, 130.38, 132.18, 136.73, 139.54, 143.26 (–CH=N), 150.76, 152.37, 153.48, 157.59, (Ar–C), 175.92 (C=O); Anal. Calcd. for $C_{23}H_{21}N_7O_4$ (459.46 g/mol): C, 60.12; H, 4.61; N, 21.34 (%); Found: C, 60.40; H, 4.39; N, 21.54 (%); MS (*m/z*): 460 $[M+H]^+$.

*(E)-N′-((3-methyl-1-phenyl-5-(p-tolyloxy)-1H-pyrazol-4-yl)methylene)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetohydrazide (**5b**)* Yield: 83 %; mp 249–251 °C; IR (KBr, ν_{max}, cm^{-1}): 3320 (str. of –N–H), 3025 (ArC–H), 1680 (C=O stretching), 1230 cm^{-1} (C–O–C ether stretching);^{1}H NMR (DMSO-d_6) δ ppm: 2.33, 2.35, 2.52 (s, 9H, 3 × CH_3), 5.04 (s, 2H, CH_2), 6.94–7.82 (m, 10H, Ar–H), 8.73 (s, 1H, CH=N), 11.23 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) (δ): 14.44, 20.90, 21.34 (Ar–CH_3), 37.84 (CH_2–CO), 108.52, 117.09, 123.68, 124.85, 127.93, 129.07, 130.48, 132.52, 137.13, 139.30, 143.72 (–CH=N), 150.37, 152.72, 153.25, 157.38 (Ar–C), 175.75 (C=O); Anal. Calcd. for $C_{24}H_{23}N_7O_4$ (473.48 g/mol): C, 60.88; H, 4.90; N, 20.71 (%); Found: C, 60.59; H, 5.12; N, 20.83 (%); MS (*m/z*): 474 $[M+H]^+$.

*(E)-N′-((5-(4-methoxyphenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetohydrazide (**5c**)* Yield: 84 %; mp 218-220 °C; IR (KBr, ν_{max}, cm^{-1}): 3325 (str. of –N–H), 3040 (ArC–H), 1700 (C=O stretching), 1210 cm^{-1} (C–O–C ether stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.45, 2.55 (s, 6H, 2 × CH_3), 3.42 (s, 3H, OCH_3), 4.90 (s, 2H, CH_2), 7.08–7.95 (m, 10H, Ar–H), 8.85 (s, 1H, CH=N), 11.18 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) (δ): 14.27, 21.29 (Ar–CH_3), 37.76 (CH_2–CO), 54.87 (Ar–OCH_3), 107.93, 119.24, 122.90, 124.18, 127.98, 129.26, 130.32, 132.42, 137.45, 139.78, 143.16 (–CH=N), 151.78, 152.24, 153.90, 157.52 (Ar–C), 176.05 (C=O); Anal. Calcd. for $C_{24}H_{23}N_7O_5$ (489.48 g/mol): C, 58.89; H, 4.74; N, 20.03 (%); Found: C, 59.65; H, 4.84; N, 20.40 (%); MS (*m/z*): 490 $[M+H]^+$.

*(E)-N′-((5-(4-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetohydrazide (**5d**)* Yield: 77 %; mp 267–269 °C; IR (KBr, ν_{max}, cm^{-1}): 3335 (str. of –N–H), 3025 (ArC–H), 1690 (C=O stretching), 1220 cm^{-1} (C–O–C ether stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.38, 2.50 (s, 6H, 2 × CH_3), 4.99 (s, 2H, CH_2), 6.90–7.87 (m, 10H, Ar–H), 8.80 (s, 1H, C<u>H</u>=N), 11.21 (s, 1H, N<u>H</u>); ^{13}C NMR (DMSO-d_6) (δ): 14.40, 21.38 (Ar–CH_3), 37.92 (<u>C</u>H_2–CO), 108.20, 118.62, 122.47, 127.68, 128.45, 129.15, 130.48, 132.93, 138.02, 139.96, 143.81 (–CH=N), 149.11, 153.15, 154.38, 158.38

(Ar–C), 175.68 (C=O); Anal. Calcd. for $C_{23}H_{20}FN_7O_4$ (477.45 g/mol): C, 57.86; H, 4.22; N, 20.54 (%); Found: C, 58.98; H, 4.53; N, 20.75 (%); MS (*m/z*): 478 $[M+H]^+$.

(E)-N′-((5-(4-chlorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetohydrazide ***(5e)*** Yield: 80 %; mp 210–212 °C; IR (KBr, ν_{max}, cm^{-1}): 3345 (str. of –N–H), 3035 (ArC–H), 1685 (C = O stretching), 1230 cm^{-1} (C–O–C ether stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.40, 2.49 (s, 6H, 2 × CH_3), 5.05 (s, 2H, CH_2), 6.95–7.86 (m, 10H, Ar–H), 8.74 (s, 1H, CH=N), 11.15 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) (δ): 14.35, 21.42 (Ar–CH_3), 37.89 (CH_2–CO), 106.94, 117.33, 122.47, 124.85, 128.09, 129.27, 130.28, 132.69, 137.24, 139.25, 143.19 (–CH=N), 150.64, 152.62, 153.37, 157.95 (Ar-C), 175.86 (C=O); Anal. Calcd. for $C_{23}H_{20}ClN_7O_4$ (493.90 g/mol): C, 55.93; H, 4.08; N, 19.85 (%); Found: C, 55.68; H, 4.43; N, 19.64 (%); MS (*m/z*): 494 $[M+H]^+$.

(E)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)-N′-((3-methyl-5-phenoxy-1-p-tolyl-1H-pyrazol-4-yl)methylene)acetohydrazide ***(5f)*** Yield: 79 %; mp 254–256 °C; IR (KBr, ν_{max}, cm^{-1}): 3330 (str. of -N–H), 3025 (ArC–H), 1690 (C=O stretching), 1225 cm^{-1} (C–O–C ether stretching);^{1}H NMR (DMSO-d_6) δ ppm: 227, 2.35, 2.45 (s, 9H, 3 × CH_3), 5.96 (s, 2H, CH_2), 6.96–7.81 (m, 10H, Ar–H), 8.83 (s, 1H, CH=N), 11.22 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) (δ): 14.28, 21.17, 21.49 (Ar–CH_3), 37.94 (CH_2–CO), 107.16, 117.19, 122.23, 124.50, 128.44, 129.83, 130.47, 132.38, 136.92, 139.47, 143.47 (–CH=N), 149.22, 152.69, 153.58, 157.73 (Ar–C), 176.21 (C=O); Anal. Calcd. for $C_{24}H_{23}N_7O_4$ (473.48 g/mol): C, 60.88; H, 4.90; N, 20.71 (%); Found: C, 61.12; H, 4.62; N, 20.93 (%); MS (*m/z*): 474 $[M+H]^+$.

(E)-N′-((3-methyl-1-p-tolyl-5-(p-tolyloxy)-1H-pyrazol-4-yl)methylene)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetohydrazide ***(5g)*** Yield: 76 %; mp 279–281 °C; IR (KBr, ν_{max}, cm^{-1}): 3335 (str. of –N–H), 3015 (ArC–H), 1695 (C=O stretching), 1230 cm^{-1} (C–O–C ether stretching);^{1}H NMR (DMSO-d_6) δ ppm: 2.20, 2.35, 2.43, 2.51 (s, 12H, 4 × CH_3), 5.10 (s, 2H, CH_2), 6.99–7.80 (m, 9H, Ar–H), 8.82 (s, 1H, CH=N), 11.19 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) (δ): 14.25, 20.93, 21.11, 21.46 (Ar–CH_3), 37.80 (CH_2–CO), 109.13, 116.26, 122.65, 124.35, 128.26, 129.96, 131.25, 132.50, 137.32, 139.62, 143.75 (–CH=N), 150.16, 152.28, 153.82, 157.30 (Ar–C), 175.93 (C=O); Anal. Calcd. for $C_{25}H_{25}N_7O_4$ (487.51 g/mol): C, 61.59; H, 5.17; N, 20.11 (%); Found: C, 61.85; H, 5.40; N, 20.37 (%); MS (*m/z*): 488 $[M+H]^+$.

(E)-N′-((5-(4-methoxyphenoxy)-3-methyl-1-p-tolyl-1H-pyrazol-4-yl)methylene)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetohydrazide ***(5h)*** Yield: 88 %; mp 262–264 °C; IR (KBr, ν_{max}, cm^{-1}): 3330 (str. of –N–H), 3025 (ArC–H), 1690 (C=O stretching), 1225 cm^{-1} (C–O–C ether stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 2.29, 2.37, 2.48 (s, 9H, 3 × CH_3), 3.41 (s, 3H, OCH_3), 5.02 (s, 2H, CH_2), 6.92–7.78 (m, 9H, Ar–H), 8.70 (s, 1H, CH=N), 11.25 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) (δ): 14.50, 21.22, 21.30 (Ar–CH_3), 37.85 (CH_2–CO), 54.85 (Ar–OCH_3), 108.82, 116.87, 123.11, 124.74, 128.20, 129.31, 130.53, 132.74, 137.78, 139.19, 143.87 (–CH=N), 150.45, 152.70, 153.74, 157.25 (Ar–C), 175.82 (C=O); Anal.

Calcd. for $C_{25}H_{25}N_7O_4$ (503.51 g/mol): C, 59.63; H, 5.00; N, 19.47 (%); Found: C, 59.29; H, 4.77; N, 19.64 (%); MS (*m/z*): 504 $[M+H]^+$.

(E)-N′-((5-(4-fluorophenoxy)-3-methyl-1-p-tolyl-1H-pyrazol-4-yl)methylene)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetohydrazide ***(5i)*** Yield: 77 %; mp 224–226 °C; IR (KBr, ν_{max}, cm^{-1}): 3340 (str. of –N–H), 3015 (ArC–H), 1680 (C=O stretching), 1215 cm^{-1} (C–O–C ether stretching);^{1}H NMR (DMSO-d_6) δ ppm: 2.32, 2.39, 2.47 (s, 9H, 3 × CH_3), 5.08 (s, 2H, CH_2), 7.05–7.97 (m, 9H, Ar–H), 8.84 (s, 1H, CH=N), 11.23 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) (δ): 14.37, 21.15, 21.43 (Ar–CH_3), 37.72 (CH_2–CO), 108.21, 115.37, 121.98, 125.67, 128.01, 129.23, 131.14, 132.46, 137.56, 139.73, 143.22 (–CH=N), 152.24, 153.46, 154.37, 157.35 (Ar–C), 175.77 (C=O); Anal. Calcd. for $C_{24}H_{22}FN_7O_4$ (491.47 g/mol): C, 58.65; H, 4.51; N, 19.95 (%); Found: C, 58.81; H, 4.73; N, 20.62 (%); MS (*m/z*): 492 $[M+H]^+$.

(E)-N′-((5-(4-chlorophenoxy)-3-methyl-1-p-tolyl-1H-pyrazol-4-yl)methylene)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetohydrazide ***(5j)*** Yield: 75 %; mp 242–244 °C;IR (KBr, ν_{max}, cm^{-1}): 3325 (str. of –N–H), 3020 (ArC–H), 1685 (C=O stretching), 1220 cm^{-1} (C–O–C ether stretching);^{1}H NMR (DMSO-d_6) δ ppm: 2.23, 2.42, 2.50 (s, 9H, 3 × CH_3), 5.05 (s, 2H, CH_2), 7.03–7.90 (m, 9H, Ar–H), 8.82 (s, 1H, CH=N), 11.18 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) (δ): 14.45, 21.18, 21.44 (Ar–CH_3), 37.90 (CH_2–CO), 107.90, 116.78, 122.44, 124.79, 128.84, 129.30, 130.47, 132.59, 137.22, 139.80, 143.51 (–CH=N), 151.47, 153.63, 154.25, 157.85 (Ar-C), 176.12 (C = O); Anal. Calcd. for $C_{24}H_{22}ClN_7O_4$ (507.93 g/mol): C, 56.75;

Table 1 In vitro antibacterial activity of compounds **5a–j**

Compounds	Minimum inhibitory concentrations (μg/mL)			
	Gram-positive		Gram-negative	
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
5a	50	25	>50	>50
5b	25	3.13	50	>50
5c	12.5	50	6.25	25
5d	6.25	50	25	3.13
5e	25	>50	6.25	25
5f	>50	25	12.5	3.13
5g	12.5	6.25	25	12.5
5h	25	25	3.13	>50
5i	25	12.5	3.13	25
5j	25	>50	50	50
Kanamycin B	1.56	1.56	3.13	3.13
DDCP	12.5	25	1.56	3.13

H, 4.37; N, 19.30 (%); Found: C, 57.05; H, 4.58; N, 19.17 (%); MS (*m/z*): 508 $[M+H]^+$.

Antibacterial activity assay

The antibacterial activities of the synthesized compounds **5a–j** were tested against Gram-negative bacterial strains (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853) and Gram-positive bacterial strains (*B. subtilis* ATCC 530 and *S. aureus* ATCC 25923) using the method recommended by National Committee for Clinical Laboratory Standards (NCCLS) [220]. In vitro activities of the compounds were tested in nutrient broth (NB) for bacteria using twofold serial dilution.

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 minimum inhibitory concentrations (μg/mL) MICs were recorded by visual observations after 24 h (for bacteria) of incubation. Kanamycin B and DDCP were used as standards for bacterial. The observed MICs are presented in Table 1.

E. coli FabH purification and activity assay

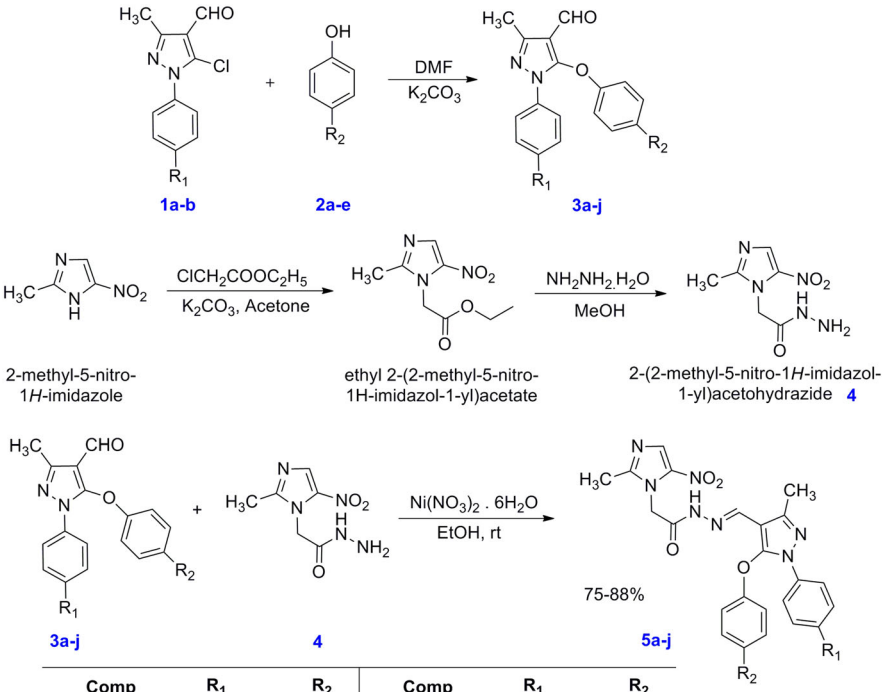
Full-length *E. coli* acyl carrier protein (ACP), acyl carrier protein synthase (ACPS), and β-ketoacyl-ACP synthase III (FabH) were individually cloned into pET expression vectors with an N-terminal His-tag (ACP, ACPS in pET19; FabH in pET28). All proteins were expressed in *E. coli* strain BL21 (DE3). 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 was grown overnight at 37 °C, 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 °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 FabH were lysed by sonication in 20 mM Tris, pH 7.6, 5 mM imidazole, and 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 FabH was 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 with 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 %.



Comp	R_1	R_2	Comp	R_1	R_2
5a	H	H	**5f**	CH_3	H
5b	H	CH_3	**5g**	CH_3	CH_3
5c	H	OCH_3	**5h**	CH_3	OCH_3
5d	H	F	**5i**	CH_3	F
5e	H	Cl	**5j**	CH_3	Cl

Scheme 1 Synthesis of compounds **5a**–**j**

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 3.1.

Results and discussion

Chemistry

Schiff's base derivatives **5a–j** have been synthesized by reaction between 5-arloxypyrazole-4-carbaldehydes [21] **3a–j** and 2-(2-methyl-5-nitro-1*H*-imidazol-1-*yl*)acetohydrazide **4** [23] in presence of nickel (II) nitrate as a catalyst in ethanol at room temperature in good yield (75–88 %) (Scheme 1). The reaction was also attempted using AcOH [24, 25], and conc. H_2SO_4 [26]; however, some shortcomings were observed such as incomplete reaction, long reaction time, loss of yield, and purification problems. Therefore, we applied $Ni(NO_3)_2{\cdot}6H_2O$ as a catalyst [27] for this reaction to avoid such drawbacks as well as to develop an easy and efficient method for the synthesis of new Schiff's base derivatives **5a–j**.

The structures of all the new synthesized compounds were established by ^1H NMR, ^{13}C NMR, FT-IR, elemental analysis, and molecular weight of compounds confirmed by mass spectrometry. Mass spectroscopy of compounds showed molecular ion peak $[M+H]^+$ corresponding to the exact mass.

Biological evaluation

In vitro antibacterial and E. Coli FabH inhibitory activity

All the synthesized compounds **5a–j** were screened for antibacterial activities against two Gram-negative bacterial strains, *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*); and two Gram-positive bacterial strains, *Bacillus subtilis* ATCC 530 (*B. subtilis*) and *Staphylococcus aureus* ATCC 6538 (*S. aureus*), by serial dilution. The MICs (minimum inhibitory concentrations) of the compounds against these bacteria are presented in Table 1. Kanamycin B and DDCP were taken as reference compound under identical conditions for comparison.

Upon investigation of antibacterial activity (Table 1), it has been observed that the majority of the compounds have shown effective activity against used strains. Against Gram-negative bacteria *E. coli*, compounds **5h** (MIC = 3.13 μg/mL) and **5i** (MIC = 3.13 μg/mL) showed more effective activity than other compounds of the series and comparable activity to kanamycin B (MIC = 3.13 μg/mL), while compounds **5d** (MIC = 3.13 μg/mL) and **5f** (MIC = 3.13 μg/mL) showed comparable activity to both reference compounds (MIC = 3.13 μg/mL) against Gram-negative bacteria *P. aeruginosa*. Against Gram-positive bacterial *S. aureus*,

compounds **5b** (MIC = 3.13 μg/mL), **5g** (MIC = 6.25 μg/mL), and **5i** (MIC = 12.5 μg/mL) showed more effective activity, and compounds **5a** (MIC = 25 μg/mL), **5f** (MIC = 25 μg/mL), and **5h** (MIC = 25 μg/mL) showed comparable activity as compared to DDCP (MIC = 25 μg/mL). Compounds **5d** (MIC = 6.25 μg/mL) showed more effective activity and compound **5g** (MIC = 12.5 μg/mL) showed comparable activity as compared to DDCP (MIC = 12.5 μg/mL) against *B. subtilis*. Of the compounds studied for *E. coli* FabH inhibitory activity (Table 2), compounds **5h** ($IC_{50} = 6.1 \pm 0.9$ μM) and **5i** ($IC_{50} = 4.6 \pm 0.2$ μM) showed the most potent activity as compared to other compounds of the series and less active than DDCP ($IC_{50} = 2.1 \pm 0.1$ μM).

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_1 and R_2 substitution may lead to change in the activity against employed strains as well as *E. coli* FabH. Compounds **5h** ($R_1 = CH_3$, $R_2 = OCH_3$), and **5i** ($R_1 = CH_3$, $R_2 = F$) showed potent activity against *E. coli* by introducing $-CH_3$ at R_1 position in compounds **5c** ($R_1 = H$, $R_2 = OCH_3$) and **5d** ($R_1 = H$, $R_2 = F$) respectively. Against *P. aeruginosa*, $R_2 = F$ and H may responsible for the good activity. The activity was completely lost as compared to the reference compounds by introducing other functional groups at the R_2 position. Compounds with $R_1 = H$ (**5d**) became more effective than $R_1 = CH_3$ (**5i**) against *B. subtilis*, while the reverse trend has been observed in the case of activity against *S. aureus*. Moreover, reviewing and comparing the activity data, it is worth mentioning that the antibacterial and activity against *E. coli* FabH of the target compounds depends not only on the bicyclic heteroaromatic pharmacophore, but also on the nature of the substituent.

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) [28]. All docking runs were applied Ligand Fit Dock protocol of Discovery Studio 3.1.

Table 2 *Escherichia coli* FabH inhibitory activities of compounds **5a–j**

Compounds	IC_{50} (μM)
5a	51.4 ± 1.4
5b	37.1 ± 0.3
5c	17.5 ± 0.7
5d	42.2 ± 2.1
5e	12.0 ± 0.1
5f	19.4 ± 0.6
5g	21.3 ± 1.2
5h	6.1 ± 0.9
5i	4.6 ± 0.2
5j	16.7 ± 0.3
DDCP	2.1 ± 0.1

Table 3 Banding energy of synthesized compounds **5a–j**

Compounds	CDOCKER interaction energy $-\Delta G_n$ (kcal/mol)
5a	42.2099
5b	45.2938
5c	47.745
5d	45.8859
5e	48.6371
5f	47.7319
5g	45.9751
5h	49.1384
5i	54.2961
5j	46.7888

The binding energy calculation of the synthesized compounds is mentioned in Table 3. Among them, compound **5i** showed the lowest interaction energy ($\Delta G_b = -54.2961$ kcal/mol). The binding model of compound **5i** and *E. coli* FabH is depicted in Figs. 1 and 2. In the binding model, compound **5i** was nicely bound to the FabH kinase with two hydrogen bonds, two π–π and three π–cation interactions. Among two hydrogen bonds, one formed between O-atom of $-NO_2$ and THR28 with distance: 2.12849 Å; DHA angle: 150.5° and HAY angle: 144.8°, while another one formed between F-atom of aryloxypyrazole nucleus and ARG36 with distance: 2.46349 Å; DHA angle: 126.5° and HAY angle: 95.9°. Two π–π interactions formed between imidazole ring and TRP32 with distance 3.66368 and 4.0845 Å. Among three π–cation interactions, one formed between imidazole ring and TRP32 with distance 3.65463 Å, the second between the imidazole ring and ARG151 with distance 4.07692 Å, the third with phenoxy ring of pyrazole nucleus

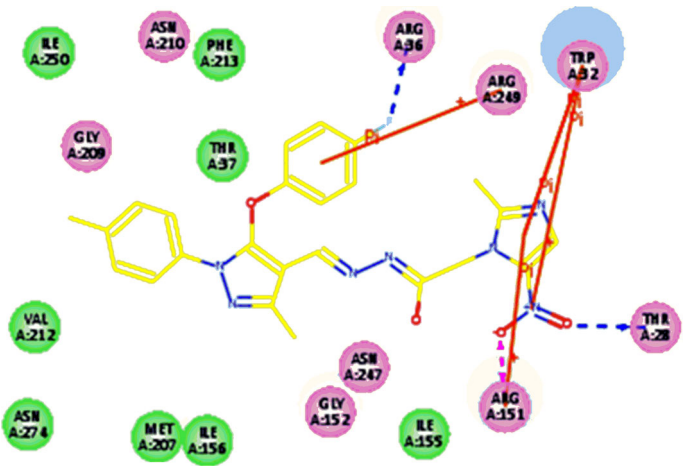


Fig. 1 2D binding model of compound **5i** into the active site of FabH

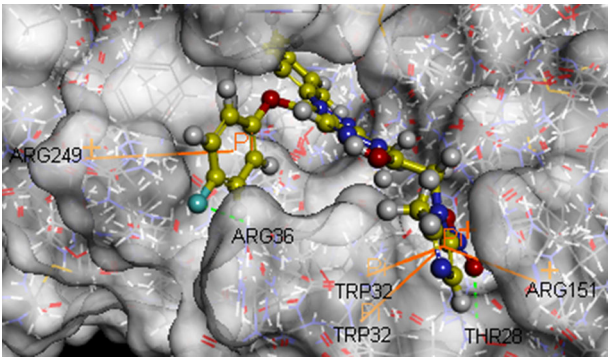


Fig. 2 Surface model of compound **5i** into the active site of FabH (3D-model)

and ARG249 with distance 5.83549 Å. This molecular docking result, along with the biological assay data, suggests that compound **5i** proved to be a potential inhibitor of *E. coli* FabH.

Conclusion

In conclusion, a new series of Schiff's base derivatives with nitroimidazole and pyrazole nuclei was synthesized by reaction between 5-aryloxypyrazole-4-carbaldehydes and 2-(2-methyl-5-nitro-1*H*-imidazol-1-*yl*)acetohydrazide in the presence of nickel (II) nitrate as a catalyst in ethanol at room temperature with a good yield. This synthetic strategy allows the assimilation of two promising bioactive nuclei in a single scaffold with an easy method. Reviewing the biological activity data, it has been concluded that the majority of the compounds are found to be the most effective against applied bacterial strains. Compound **5i** showed the most effective inhibition by binding into the active site of the *E. coli* FabH receptor with minimum binding energy. According to this, it is worth mentioning that the Schiff's base derivatives with nitroimidazole and pyrazole nuclei have become a vital area of antibacterial and *E. coli* FabH inhibition medicine research.

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