Synthesis of isoniazid-1,2,3-triazole conjugates: Antitubercular, antimicrobial evaluation and molecular docking study

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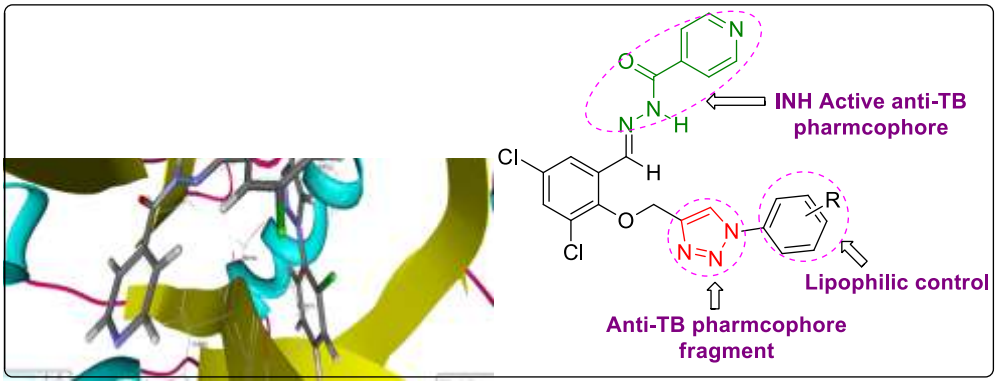
GRAPHICAL ABSTRACT



ABSTRACT

In the present study, a series of new isoniazid-1,2,3-triazole conjugates (**5a-k**) was synthesized *via* click chemistry approach. The newly synthesized compounds were assessed for their *in vitro* antimycobacterial and antimicrobial activities. The compound **5g** has displayed potent antitubercular activity against *M. tuberculosis* H37Rv (*Mtb*) with MIC value 1.56 µg/mL. The active compounds were screened for their cytotoxicity profile by MTT assay against RAW 264.7 cell line. The four compounds have shown good *in vitro* antimicrobial activities against both antibacterial and antifungal pathogens. A molecular

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docking study was accomplished to identify the probable mode of action of synthesized derivatives. These compounds have shown excellent binding affinity towards *Enoyl-acp reductase* (INHA) and *DNA gyrase*.

KEYWORDS: 1,2,3-Triazole, Isoniazid, Antitubercular Activity, Antimicrobial Activity, Cytotoxicity, Molecular Docking Study, Click Chemistry.

1. INTRODUCTION

Tuberculosis (TB) is a chronic disease that gets spread through air and caused by the bacillus *Mycobacterium tuberculosis*. It is one of the leading reason of death from a single infectious agent. It affects the lungs (pulmonary TB) and other sites (extra pulmonary TB).[1] As per Global Tuberculosis Report-**2019**, tuberculosis (TB) is among the top ten reasons of death worldwide.[2] It has more become difficult to combat the disease due to its poverty and co-infection with HIV. The development of resistance is a serious problem associated with the treatment of TB. The genetic mutation is one of the main reason of resistance. The current attempts in the drug discovery process are not sufficient to completely eliminate the tubercular epidemic.[3] It is observed that interest in tuberculosis research is rising, and the control of its spread has become one of the main health priorities in the world.[4] As limited treatment options for multi-drug resistant (MDR-TB) and extensively drug resistant (XDR-TB) are available, TB researchers have the challenge of inventing new anti-tubercular drugs with innovative modes of action.[5, 6]

A literature survey shown that 1,4-disubstituted 1,2,3-triazole derivatives have several therapeutic properties like antifungal,[7] antibacterial,[8] anticancer,[9] anti-HIV,[10] antidiabetic,[11] antitubercular[12] and antiviral.[13] They can exhibit hydrogen bonding, dipole-dipole and μ-staking interactions.[14] Recently, triazole has gained exceptional consideration in drug discovery.[15] It is an important scaffold of many drugs like tazobactum, cephalosporin, cetirizine, fluconazole, itraconazole, voriconazole and ketoconazole.[16] In addition, these are very stable to acidic and basic hydrolysis. It does not react with various oxidizing and reducing agents.[17] In view of these important features, numerous protocols for the synthesis of 1,2,3-triazole derivatives have been established.[18] The most useful method is the Huisgen's 1,3-dipolar Cycloaddition of azides and alkynes. Recently, Sharpless and Meldal group have reported new protocol for 1,3-dipolar cycloaddition reaction with dramatic rate enhancement and improved region-selectivity. "Click Chemistry" prescribed by Sharpless has turn out to be a premier module of synthetic organic chemistry.[19]

Isonicotinic acid hydrazide (Isoniazid) is one of the first-line drug used for the treatment of *Mycobacterium tuberculosis*. Its mode of action consist of several effects on lipids, glycolysis, biomembranes, proteins and nucleic acid synthesis.[20] Unfortunately, it has several adverse effects mainly, hepatitis, psychiatric, GI Intolerance, peripheral neuropathy, allergic reactions and drug interactions.[21] Hence, it is prime important to synthesis novel isoniazid derivatives.[22] To overcome the drug resistance and adverse effects, incorporation of isoniazid with other active moiety like 1,2,3-triazole is frequently applied (Figure 1).

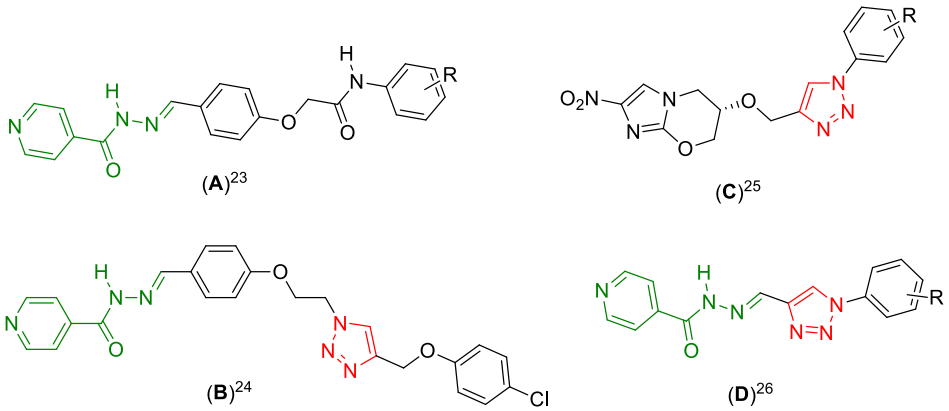


FIGURE 1 Representative isoniazid-1,2,3-triazole derivatives having antitubercular activity

It has been observed that the combination of lipophilic moieties with the skeleton of INH can enhance permeation of the drug into bacterial cells, hence increasing the anti-TB activity. Therefore, INH conjugates with better lipophilicity are evolving as one of the most potential anti-TB compounds. Rawat and coworkers have reported the synthesis of novel amido-ether (**A**) and 1,2,3-triazole (**B**) derivatives of isoniazid and evaluation of their *in vitro* and *in vivo* antitubercular activity (Figure 1).[23, 24] The compound PA-824 (**C**) has shown outstanding activities against the both replicating and non-replicating cultures of MTB including MDR-TB.[25] It is in phase II clinical trial for treatment of TB infection patients. Boechat and coworkers have reported isoniazid-*N*-phenyl-1,2,3-triazole derivatives and screened for *in vitro* for antimycobacterial activity against MTB H37Rv.[26] All of the hybrid molecules have shown substantial activity with MIC values ranging from 0.62 to 2.5 µg/mL. Thus, these the two entities on molecular hybridization to form one new single molecule may offer a new lead with potent anti-tubercular activity.[27] In extension of our efforts towards the identification of new anti-tuberculosis agents[28] herein, we have planned and synthesized a series of new isoniazid clubbed with 1,4-disubstituted 1,2,3-triazole conjugates. The newly

synthesized conjugates were screened for their *in vitro* antitubercular and antimicrobial activities. The cytotoxicity and molecular docking study of active compounds have also been performed.

2. MATERIALS AND METHODS

All the chemicals are obtained from commercial suppliers and used directly without further purification. Melting points were determined in open capillary tubes and are uncorrected. The IR spectra were obtained by using Brukar FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Brukar DRX-400 MHz NMR spectrometer. Tetramethylsilane (TMS) was used as an internal standard. High-resolution mass spectra (HRMS) was performed by using XEVO G2-XS QTOF (TOF MS ES^{+}) instrument.

2.1. Synthesis of 3,5-dichloro-2-(prop-2-yn-1-yloxy)benzaldehyde (2).

A mixture of commercially available 3, 5-dichloro salicylaldehyde (**1**) (1 mmol), propargyl bromide (1 mmol) and K_2CO_3 (2 mmol) was stirred in DMF at room temperature. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured on crushed ice. The solid obtained was filtered, washed with water and crystallized from ethanol.

Yield: 95%, M.P.: 81-83^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.55 (s, 1H, -CH), 4.89 (s, 2H, -OCH_2), 7.64 (d, J = 4 Hz, 1H, Ar-H), 7.76 (d, J = 4 Hz, 1H, Ar-H), 10.38 (s, 1H, -CHO); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 62.00, 77.08, 78.68, 126.57, 129.90, 131.48, 132.96, 135.73, 154.63, 188.29.

2.2. Synthesis of (*E*)-*N*'-(3,5-dichloro-2-(prop-2-yn-1-yloxy) benzylidene)isonicotino hydrazide (3)

3,5-Dichloro-2-(prop-2-yn-1-yloxy)benzaldehyde (**2**) (1 mmol) and isonicotinohydrazide (1 mmol) was stirred in diisopropylethylammonium aceate (DIPEAc) (10 ml) at room temperature for 1h. After completion of reaction, ice cold water was added in reaction mixture. Then, the solid compound obtained was filtered, washed with cold water and dried. The product obtained was crystallized by using ethanol.

(E)-N'-(3,5-Dichloro-2-(prop-2-yn-1-yloxy)benzylidene)isonicotinohydrazide (3)

Yield: 90%; M.P.: 160-162 ^{o}C; IR (Neat) ν cm^{-1}: 3320, 3024, 2930, 1640, 1598, 1530, 1489, 1278, 1208, 1050, 911, 830; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.55 (t, J = 4 Hz, 1H, -CH),

4.80 (d, J = 4 Hz, 2H, -OCH_2), 7.42-7.47 (m, 1H, Ar-H), 7.56-7.59 (m, 1H, Ar-H), 7.72-7.75 (m, 2H, Ar-H), 8.10-8.21 (m, 1H, Ar-H), 8.83 (s, 2H, Ar-H), 9.43 (s, 1H, -CON-H).

2.3. General procedure for the synthesis of (*E*)-*N*'-(3,5-dichloro-2-((1-phenyl-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)isonicotinohydrazide derivatives (5a-k)

(*E*)-*N*'-(3,5-Dichloro-2-(prop-2-yn-1-yloxy)benzylidene)isonicotino hydrazide (**3**) (1 mmol), substituted azidobenzenes (**4a-k**) (1 mmol), $CuSO_4.5H_2O$ (20 mol%) and sodium ascorbate (20 mol%) were stirred in DMF. After completion of reactions (6-8h), ice cold water was added in reaction mixture. The compounds obtained were filtered, washed with water, dried and crystallized by using ethanol.

(E)-N'-(3,5-Dichloro-2-((1-(p-tolyl)-1H-1,2,3-triazol-4-yl)methoxy)benzylidene)isonicotinohydrazide (5a)

Yield: 80%; M.P.: 213-215 ^oC; IR (Neat) ν cm^{-1}: 3222, 3020, 2941, 1675, 1607, 1570, 1540, 1455, 1301, 1270, 1178, 1052, 843, 812; ^1H NMR (400 MHz, $CDCl_3$) δ = 1.76 (s, 3H, -CH_3), 5.03 (s, 2H, -OCH_2), 6.90 (d, J = 8 Hz, 2H, Ar-H), 6.97-7.00 (m, 3H, Ar-H), 7.35 (d, J = 8 Hz, 2H, Ar-H), 7.81-7.84 (m, 2H, Ar-H), 8.27 (s, 1H, Ar-H), 8.37 (s, 1H, Ar-H), 8.41 (d, J = 2 Hz, 1H, Ar-H), 11.67 (s, 1H, -CON-H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6) δ = 17.52, 62.38, 112.68, 121.39, 121.92, 123.38, 125.50, 126.44, 126.60, 129.69, 131.20, 132.98, 135.78, 141.41, 141.56, 142.75, 149.85, 160.67, 161.97.

(E)-N'-(3,5-Dichloro-2-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)benzylidene) isonicotinohydrazide (5b)

Yield: 82%; M.P.: 130-132 ^oC; IR (Neat) ν cm^{-1}: 3218, 3031, 2917, 1666, 1599, 1556, 1495, 1310, 1261, 1144, 1050, 859, 838; ^1H NMR (400 MHz, $CDCl_3$) δ = 5.18 (s, 2H, -OCH_2), 7.47-7.51 (m, 4H, Ar-H), 7.61-7.68 (m, 2H, Ar-H), 7.72-7.83 (m, 2H, Ar-H), 8.01-8.06 (m, 2H, Ar-H), 8.67 (s, 1H, Ar-H), 7.9 (s, 1H, Ar-H), 10.76 (s, 1H, -CON-H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6) δ = 61.32, 112.83, 114.98, 117.02, 120.85, 121.47, 123.25, 126.89, 128.13, 129.73, 131.39, 137.25, 139.70, 143.20, 144.89, 150.31, 161.96, 163.62; HRMS $(ESI)^+$ calcd. for $C_{22}H_{15}Cl_3N_6O_2$ $[M+H]^+$: 501.0356 and found 501.0390.

(E)-N'-(3,5-Dichloro-2-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)benzylidene)isonicotinohydrazide (5c)

Yield: 86%; M.P.: 237-239 ^oC; IR (Neat) ν cm^{-1}: 3227, 3008, 2949, 1663, 1603, 1544, 1455, 1349, 1281, 1147, 1048, 912, 870, 819; ^1H NMR (400 MHz, $CDCl_3$) δ = 5.17 (s, 2H, -

OCH_2), 7.37-7.49 (m, 6H, Ar-H), 7.63-7.79 (m, 4H, Ar-H), 8.02 (s, 1H, Ar-H), 8.15 (s, 1H, Ar-H), 8.64 (s, 1H, Ar-H), 11.78 (s, 1H, -CON-H); HRMS $(ESI)^+$ calcd. for $C_{22}H_{16}Cl_2N_6O_2$ $[M+H]^+$: 467.0745 and found 467.0801.

(E)-N'-(2-((1-(4-Bromophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-3,5-dichlorobenzylidene) isonicotinohydrazide (5d)

Yield: 83%; M.P.: 240-242 ^oC; IR (Neat) ν cm^{-1}: 3237, 3053, 2912, 1644, 1617, 1560, 1513, 1389, 1250, 1107, 1050, 843, 822; ^1H NMR (400 MHz, $CDCl_3$) δ = 5.16 (s, 2H, -OCH_2), 7.45 (s, 1H, Ar-H), 7.55 (d, J = 8 Hz, 2H, Ar-H), 7.63 (d, J = 8 Hz, 2H, Ar-H), 7.66 (s, 1H, Ar-H), 7.79 (bs, 2H, Ar-H), 8.01 (d, J = 4 Hz, 1H, Ar-H), 8.07 (s, 1H, Ar-H), 8.67 (s, 1H, Ar-H), 8.77 (s, 1H, Ar-H), 10.88 (s, 1H, -CON-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 62.18, 111.56, 116.96, 118.49, 121.10, 122.03, 123.44, 124.59, 125.47, 127.34, 131.27, 132.77, 139.54, 140.27, 144.38, 150.60, 160.58, 161.34.

(E)-N'-(3,5-Dichloro-2-((1-(3-nitrophenyl)-1H-1,2,3-triazol-4-yl)methoxy)benzylidene) isonicotinohydrazide (5e)

Yield: 90%; M. P.: 138-140 ^oC; IR (Neat) ν cm^{-1}: 3220, 3008, 2939, 1655, 1598, 1560, 1507, 1447, 1274, 1139, 1056, 922, 858, 807; ^1H NMR (400 MHz, $CDCl_3$) δ = 5.23 (s, 2H, -OCH_2), 7.48 (s, 1H, Ar-H), 7.62 (d, J = 4 Hz, 1H, Ar-H), 7.76-7.80 (m, 3H, Ar-H), 8.01 (s, 1H, Ar-H), 8.20-8.25 (m, 1H, Ar-H), 8.30-8.34 (m, 2H, Ar-H), 8.60-8.68 (m, 2H, Ar-H), 8.80 (s, 1H, Ar-H), 10.64 (s, 1H, -CON-H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6) δ = 62.18, 112.65, 118.84, 120.10, 122.75, 123.51, 124.99, 126.02, 127.91, 128.61, 129.43, 131.02, 133.48, 136.79, 141.67, 142.40, 145.53, 150.48, 160.04, 161.04.

(E)-N'-(3,5-Dichloro-2-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy)benzylidene) isonicotinohydrazide (5f)

Yield: 87%; M.P.: 180-182 ^oC; IR (Neat) ν cm^{-1}: 3229, 3021, 2945, 1673, 1604, 1576, 1542, 1448, 1311, 1263, 1172, 1050, 930, 840, 802; ^1H NMR (400 MHz, $CDCl_3$) δ = 3.87 (s, 3H, -OCH_3), 5.22 (s, 2H, -OCH_2), 7.04-7.11 (m, 3H, Ar-H), 7.39-7.47 (m, 3H, Ar-H), 7.67 (d, J = 8 Hz, 1H, Ar-H), 7.73-7.75 (m, 1H, Ar-H), 8.09 (s, 2H, Ar-H), 8.27 (d, J = 4 Hz, 1H, Ar-H), 8.56 (s, 1H, Ar-H), 10.73 (s, 1H, -CON-H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6) δ = 55.89, 62.42, 112.40, 112.80, 120.93, 122.04, 123.49, 124.98, 125.42, 126.24, 127.85, 130.49, 132.69, 141.20, 141.55, 142.93, 150.87, 160.94, 162.94; HRMS $(ESI)^+$ calcd. for $C_{23}H_{18}Cl_2N_6O_3$ $[M+H]^+$: 497.0851 and found 497.0909.

(E)-N'-(3,5-Dichloro-2-((1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)benzylidene) isonicotinohydrazide (5g)

Yield: 85%; M.P.: 167-169 ^{o}C; IR (Neat) ν cm^{-1}: 3240, 3089, 2945, 1670, 1600, 1581, 1510, 1487, 1319, 1244, 1169, 1055, 861, 822; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.22 (s, 2H, -OCH_2), 7.42-7.49 (m, 4H, Ar-H), 7.49-7.59 (m, 2H, Ar-H), 7.78-7.87 (m, 2H, Ar-H), 7.98 (s, 1H, Ar-H), 8.05 (d, J = 4 Hz, 1H, Ar-H), 8.66 (s, 1H, Ar-H), 8.75 (s, 1H, Ar-H), 11.11 (s, 1H, -CON-H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6) δ = 62.41, 112.48, 120.97, 122.14, 123.52, 125.05, 126.42, 126.72, 127.78, 128.13, 130.61, 131.28, 134.29, 141.57, 141.78, 142.96, 145.88, 150.95, 160.91, 162.26; HRMS (ESI)$^{+}$ calcd. for $C_{22}H_{15}Cl_3N_6O_2$ $[M+H]^{+}$: 501.0356 and found 501.0406.

(E)-N'-(3,5-Dichloro-2-((1-(2-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy)benzylidene) isonicotinohydrazide (5h)

Yield: 82%; M.P.: 178-180 ^{o}C; IR (Neat) ν cm^{-1}: 3239, 3062, 2982, 1686, 1605, 1580, 1537, 1377, 1248, 1171, 1044, 887, 835; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.87 (s, 3H, -OCH_3), 5.22 (s, 2H, -OCH_2), 7.04-7.11 (m, 4H, Ar-H), 7.39-7.47 (m, 3H, Ar-H), 7.67 (d, J = 8 Hz, 1H, Ar-H), 7.74 (d, J = 8 Hz, 1H, Ar-H), 8.10 (s, 1H, Ar-H), 8.27 (d, J = 4 Hz, 1H, Ar-H), 8.59 (s, 1H, Ar-H), 10.88 (s, 1H, -CON-H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6) δ = 56.17, 61.17, 112.71, 117.96, 119.92, 121.20, 122.25, 123.76, 124.53, 125.27, 126.57, 127.73, 130.77, 135.31, 138.01, 140.52, 142.32, 143.13, 151.18, 161.23, 162.48.

(E)-N'-(3,5-Dichloro-2-((1-(3-chlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)benzylidene) isonicotinohydrazide (5i)

Yield: 86%; M.P.: 125-127 ^{o}C; IR (Neat) ν cm^{-1}: 3267, 3112, 3081, 2803, 1674, 1611, 1557, 1388, 1260, 1142, 1060, 934, 856, 809; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.20 (s, 2H, -OCH_2), 7.42-7.52 (m, 4H, Ar-H), 7.60-7.630 (m, 1H, Ar-H), 7.76-7.81 (m, 3H, Ar-H), 8.03-8.11 (m, 2H, Ar-H), 8.69 (s, 1H, Ar-H), 8.81 (s, 1H, Ar-H), 10.55 (s, 1H, -CON-H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6) δ = 62.52, 112.78, 115.69, 118.27, 120.10, 122.50, 123.42, 125.84, 126.68, 128.61, 130.97, 134.90, 137.39, 139.03, 141.57, 142.99, 143.35, 149.54, 160.77, 162.24.

(E)-N'-(3,5-Dichloro-2-((1-(3-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy)benzylidene) isonicotinohydrazide (5j)

Yield: 85%; M.P.: 190-192 ^{o}C; IR (Neat) ν cm^{-1}: 3210, 3123, 3017, 2939, 1681, 1618, 1550, 1490, 1322, 1269, 1143, 1052, 847, 819; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.86 (s, 3H, -OCH_3), 5.21 (s, 2H, -OCH_2), 6.98-7.01 (m, 1H, Ar-H), 7.20-7.23 (m, 2H, Ar-H), 7.29-7.33 (m, 1H, Ar-H), 7.40-7.44 (m, 1H, Ar-H), 7.47-7.50 (m, 1H, Ar-H), 7.88-7.92 (m, 1H, Ar-H), 8.05-8.09 (m, 2H, Ar-H), 8.66 (s, 1H, Ar-H), 8.79 (s, 1H, Ar-H), 10.50 (s, 1H, -CON-H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6) δ = 55.57, 62.56, 112.09, 112.63, 113.19, 114.31, 121.21, 121.94, 123.05, 123.42, 126.86, 130.74, 135.76, 137.54, 141.53, 142.68, 142.97, 144.43, 150.15, 160.32, 161.02; HRMS (ESI)$^{+}$ calcd. for $C_{23}H_{18}Cl_2N_6O_3$ $[M+H]^{+}$: 497.0851 and found 497.0883.

(E)-N'-(3,5-Dichloro-2-((1-(o-tolyl)-1H-1,2,3-triazol-4-yl)methoxy)benzylidene)isonicotinohydrazide (5k)

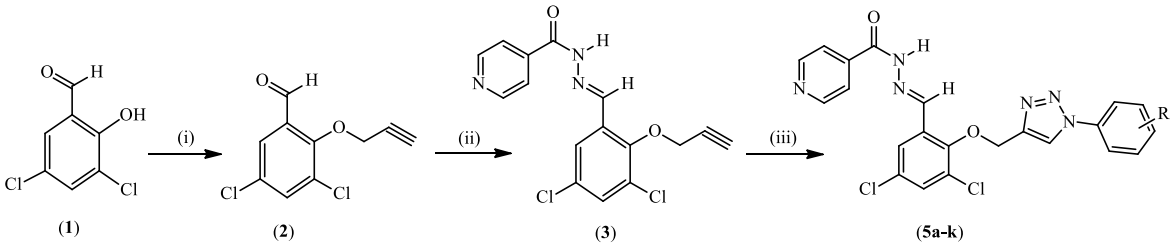
Yield: 83%; M.P.: 270-272 ^{o}C; IR (Neat) ν cm^{-1}: 3228, 3031, 2944, 1670, 1596, 1559, 1463, 1317, 1265, 1180, 1050, 850, 824; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.02 (s, 3H, -CH_3), 5.05 (s, 2H, -OCH_2), 6.85 (d, J = 8 Hz, 1H, Ar-H), 6.95-7.02 (m, 1H, Ar-H), 7.19 (d, J = 8 Hz, 1H, Ar-H), 7.27-7.29 (m, 4H, Ar-H), 7.41 (s, 1H, Ar-H), 7.85 (d, J = 8 Hz, 1H, Ar-H), 8.18 (d, J = 4 Hz, 1H, Ar-H), 8.39 (s, 1H, Ar-H), 8.45 (d, J = 4 Hz, 1H, Ar-H), 11.68 (s, 1H, -CON-H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6) δ = 17.52, 62.38, 112.68, 121.39, 121.92, 123.38, 125.00, 125.50, 126.44, 126.60, 129.69, 131.20, 132.98, 135.78, 140.05, 141.41, 141.56, 142.75, 149.85, 160.67, 161.97.

3. RESULT AND DISCUSSION

3.1. Chemistry

The target compounds (**5a-k**) were synthesized by following reaction sequence as shown in Scheme 1. In the first step, the propargylation of 3,5-dichloro-2-hydroxybenzaldehyde **(1)** was done by using propargyl bromide and K_2CO_3 in DMF at room temperature.[29] Then, the condensation of 3,5-dichloro-2-(prop-2-yn-1-yloxy)benzaldehyde (**2**) and isonicotinohydrazide was performed in diisopropylethylammonium acetate (DIPEAc) to furnish the (*E*)-*N*'-(3,5-dichloro-2-(prop-2-yn-1-yloxy)benzylidene)isonicotinohydrazide (**3**).[30] Cycloaddition reaction (Click) of (*E*)-*N*'-(3,5-dichloro-2-(prop-2-yn-1-yloxy)benzylidene)isonicotinohydrazide (**3**) and various substituted azidobenzenes (**4a-k**) by using $CuSO_4.5H_2O$ and sodium ascorbate was performed to obtain the corresponding (*E*)-*N*'-

(3,5-dichloro-2-((1-phenyl-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)isonicotinohydrazide derivatives (**5a-k**) in 80-90% yields.



SCHEME 1. *Reagents and conditions*: (i) Propargyl bromide, K_2CO_3, DMF, rt, 6h (92%); (ii) Isonicotinohydrazide, DIPEAc, rt, 1h (90%); (iii) Substituted azidobenzenes (**4a-k**), $CuSO_4$, Na ascorbate, DMF, rt (80-90%)

The structures of new compounds were assigned with the help of IR, ^{1}H NMR, ^{13}CNMR and HRMS spectral data. In IR spectrum of compound **5g**, the peaks at 3240 and 1670 cm^{-1} were due to N-H and C=O functional groups. The ^{1}H NMR of compound **5g** displayed a singlet at δ 5.22 due to –O-CH_2-triazolyl. The N-H proton of the amide group appeared at δ 11.11. All aromatic protons appeared in the region of δ 7.42 to 8.75. The ^{13}C NMR spectra showed peak at δ 62.41 was assigned to methylene carbon (–O-CH_2-triazolyl). The peak for C=O group is appeared at δ 162.26. The structure assigned to compound **5g** was further strengthen by its HRMS spectrum. It has exhibited $[M+H]^+$ ion peak at m/z 501.0406 for the molecular formula $C_{22}H_{15}Cl_3N_6O_2$.

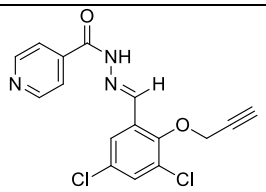
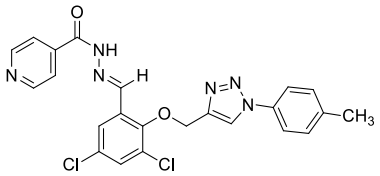
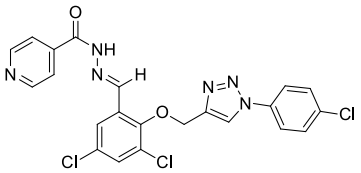
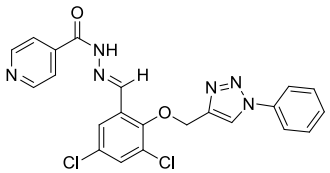
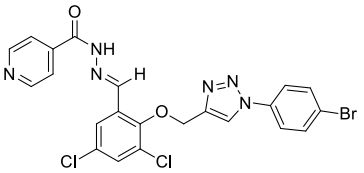
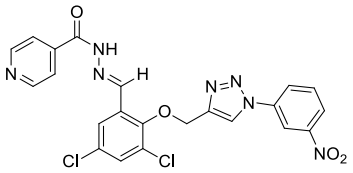
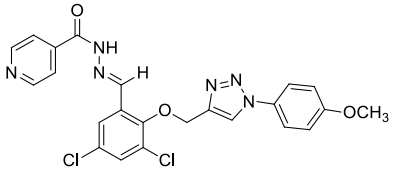
3.2. Antitubercular activity and cytotoxicity

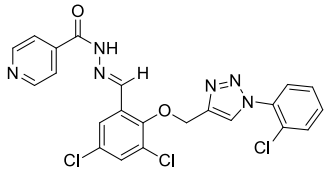
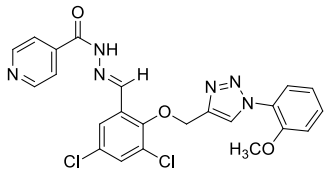
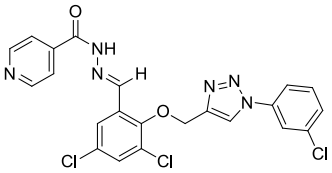
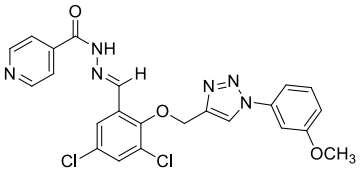
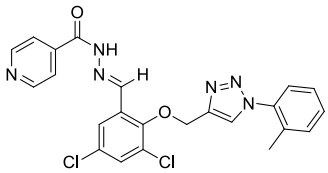
The antitubercular screening of isoniazid-1,2,3-triazole derivatives was performed by procedure reported in literature.[31] The four derivatives have exhibited good antitubercular activity. The compound **5g** is equally active to standard antitubercular drug, Ciprofloxacin with MIC value 1.56 µg/mL. It has shown better activity than the antitubercular drug, Ethambutol. The compound **5g** and **5h** have displayed antitubercular activity with 2-Cl and 2-OCH_3 substituents, respectively. Similarly, compound **5b** with 4-Cl substituent has shown good anti-TB activity. Thus, the chloro and methoxy substituents have played vital role in possessing the activity.

The *in vitro* cytotoxicity of the most active antitubercular derivatives with lower MIC value were evaluated against growth inhibition of RAW 264.7 cells at 25 µg/mL

concentration by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[32] The results obtained indicate that these compounds are not harmful to the normal cells (Table 1).

TABLE 1 Physical data and antitubercular activity of 1,2,3-triazole derivatives (**5a-k**)

Entry	Triazole derivatives	M.P. (°C)	Yield (%)	MIC against Mtb H37Rv strain (µg/mL)	% Inhibition Cytotoxicity at 25 µg/mL
3		160-162	90	>25	NA
5a		213-215	80	>25	NA
5b		130-132	82	12.5	NA
5c		237-239	86	6.25	24.54
5d		240-242	83	>25	NA
5e		138-140	90	>25	NA
5f		180-182	87	>25	NA

5g		167-169	85	1.56	22.04
5h		178-180	82	12.5	NA
5i		125-127	86	>25	NA
5j		190-192	85	>25	NA
5k		270-272	88	>25	NA
	Isoniazid			0.1	
	Ciprofloxacin			1.56	
	Ethambutol			3.125	

3.3. Antimicrobial activity

Newly synthesized isoniazid-1,2,3-triazole conjugates (**5a-k**) were screened for their *in vitro* antimicrobial activities by agar well diffusion assay.[33] Fluconazole and Tetracycline were used as reference standard for antifungal and antibacterial activities, respectively. The compounds were dissolved in DMSO and the concentration was adjusted to 1 mg/mL. Inoculums of each bacterial and fungal pathogen was developed by inoculating pathogens in nutrient broth and keeping them for 24 h at 37^0C. The turbidity was adjusted to the 0.5 McFarland standards by diluting bacterial suspension using sterile saline. The diluted suspension (200 µL) of each pathogen was inoculated on sterile Mueller Hinton agar plates. The wells were prepared in agar and filled with 100 µL of the samples. Incubation of all experimental Petri plates was allowed at 37^oC for 24h. After incubation, the plates were keenly observed and results were recorded.

The compounds **5d**, **5h**, **5i** and **5k** have exhibited good antimicrobial activities against both antibacterial and antifungal pathogens (Table 2). It has been observed that the

compounds with electron donating substituents like $–OCH_3$ and $–CH_3$ have shown better antimicrobial activities. We are expecting that on structural modifications in these active compounds will gain new antimicrobial agents.

TABLE 2 Antimicrobial activity of 1,2,3-triazole derivatives (**5a-k**)

Entry → **Pathogens ↓**	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**	**5h**	**5i**	**5j**	**5k**	**Standard**
S. aureus	07	--	--	17	16	14	--	18	13	--	18	29
B. cerus	--	10	--	12	14	16	--	16	14	12	16	33
B. subtilis	--	13	08	12	15	14	12	14	16	14	18	32
E. aerogenes	--	--	--	18	--	12	--	15	14	--	14	33
E. coli	09	12	--	19	14	12	10	17	08	--	18	29
S. typhi	--	10	--	16	--	--	--	16	15	10	16	33
P. aeruginosa	10	15	11	14	--	14	--	14	12	--	13	32
S. boydii	--	--	06	15	--	05	07	12	08	15	14	34
S. abony	--	08	--	13	--	--	--	10	06	12	15	27
A. niger	--	--	--	--	--	--	--	06		--	12	30
C. albicans	10	--	--	06	--	--	--	16	13	--	11	30
S. cerevisiae	--	07	--	--	--	--	--	09	09	--	11	30

(--): Not active

The MIC values were determined for the compounds having good antimicrobial activities. It was performed by using the method and guidelines of the Clinical and Laboratory Standard Institute (CLSI). The results are expressed in µg/mL (Table 3).

TABLE 3 MIC values of most potent isoniazid-1, 2, 3-triazole derivatives

Pathogens	**5d**	**5h**	**5j**	**5k**	**Tetracycline**
S. typhi	150	110	160	120	20
B. subtilis	180	130	90	80	25
E. coli	140	90	110	75	18
S. aureus	110	70	170	60	20

3.4. Molecular docking analysis for antitubercular activity

A molecular docking study was performed to find the possible mode of action of synthesized compounds for antitubercular and antibacterial activities. *Enoyl-ACP Reductase* (INHA)

important enzyme involved in the fatty acid biosynthesis in mycobacterium. It is responsible for the cell wall synthesis in mycobacteria. *DNA gyrase* is involved in bacterial cell growth. It is well-known antibacterial target for many established bactericidal agents like ciprofloxacin. Molecule structures were drawn using molecule builder module of V life MDS 4.6. They were further optimised *via* application of merck molecular force field (MMFF). The crystal structure of *Enoyl-Acp Reductase* (INHA) (PDB ID: 1ZID) and *S. aureus DNA gyrase* (PDB ID: 6QX1) were taken from the free protein database www.rcsb.org and utilised for the docking study.[34, 35] Grip based docking simulation was done keeping rotational angle to 10^{o} and number of placement to the 30. Molecular docking analysis was validated via redocking protocol. The RMSD for the co-crystallised ligand ZID in *Enoyl-Acp Reductase (INHA)* (PDB ID: 1ZID) was found to be 0.29 A^{o} and for co-crystallised ligand JK8 *aureus DNA gyrase* (PDB ID: 6QX1) was found to be 0.76 A^{o}, which indicated the applied docking protocol can efficiently predict key drug receptors interactions.

Derivative **5b** was interacting with *Enoyl-Acp Reductase (INHA)* with total docking score (PLP) of -53.95 kcal/mol. It was interacted *via* formation of hydrogen bond with LYS165, aromatic interaction with PHE97, hydrophobic interaction with ARG43, PHE97 and Vander wall interactions with ILE16, ILE21, PHE41, ASP42, ARG4, LEU63, SER94, ILE95, GLY96, PHE97, MET98, MET110, MET147, MET161, LYS165, and MET199 (Figure 2). Derivative **5c** was shown total docking score (PLP) of -63.87 kcal/mol and interacted *via* formation of hydrogen bond interactions with ILE95, GLY96, LYS165 aromatic interaction with PHE41, hydrophobic interaction with ILE16 and Vander wall interactions with ILE16, SER20, ILE21, PHE41, LEU63, ASP64, VAL65, SER94, ILE95, GLY96, MET147, LYS165, ILE194, THR196, LEU197, and ALA198 (Figure 3).

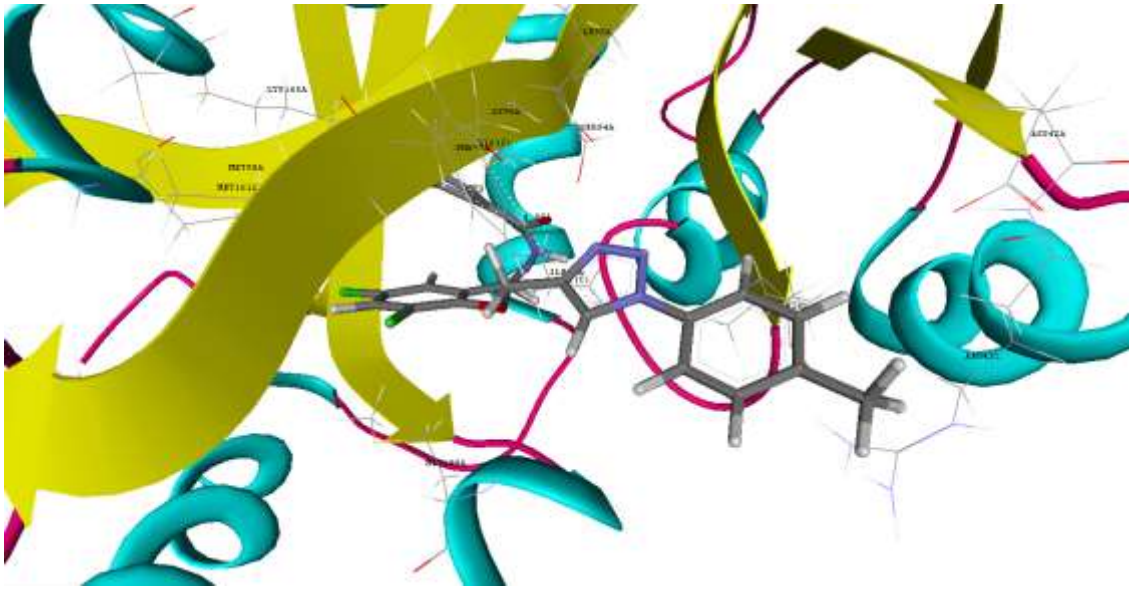


FIGURE 2 Docking interactions of **5b** with *Enoyl-Acp Reductase (INHA)*

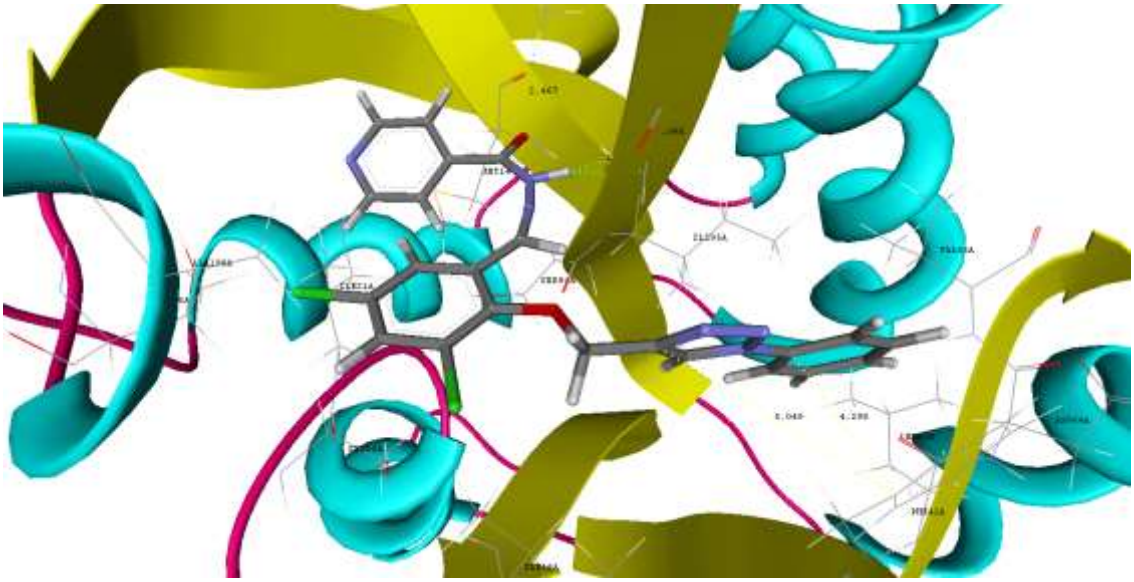


FIGURE 3 Docking interactions of **5c** with *Enoyl-Acp Reductase (INHA)*

Derivative **5g** was exhibited total docking score (PLP) of -77.30 kcal/mol and interacting *via* formation of hydrogen bond with GLY14, hydrophobic interactions with ILE16, SER20, ILE21 and Vander wall interactions with GLY14, ILE15, ILE16, SER20, ILE21, ALA22, VAL65, SER94, ILE95, GLY96, PHE97, ILE122, MET147, LYS165, ALA191, ILE194, THR196, LEU197, and ALA198 (Figure 4). Derivative **5h** was found to have total docking score (PLP) of -82.23 kcal/mol. It was interacting *via* formation of hydrogen bond with SER94, hydrophobic interactions with ILE16, GLY40, PHE41, ILE47, ILE95 and Vander wall interactions with SER13, GLY14, ILE16, SER20, ILE21, THR39, GLY40, PHE41,

ASP64, VAL65, SER94, ILE95, GLY96, ILE122, MET147, PHE149, LYS165, and ALA198 (Figure 5).

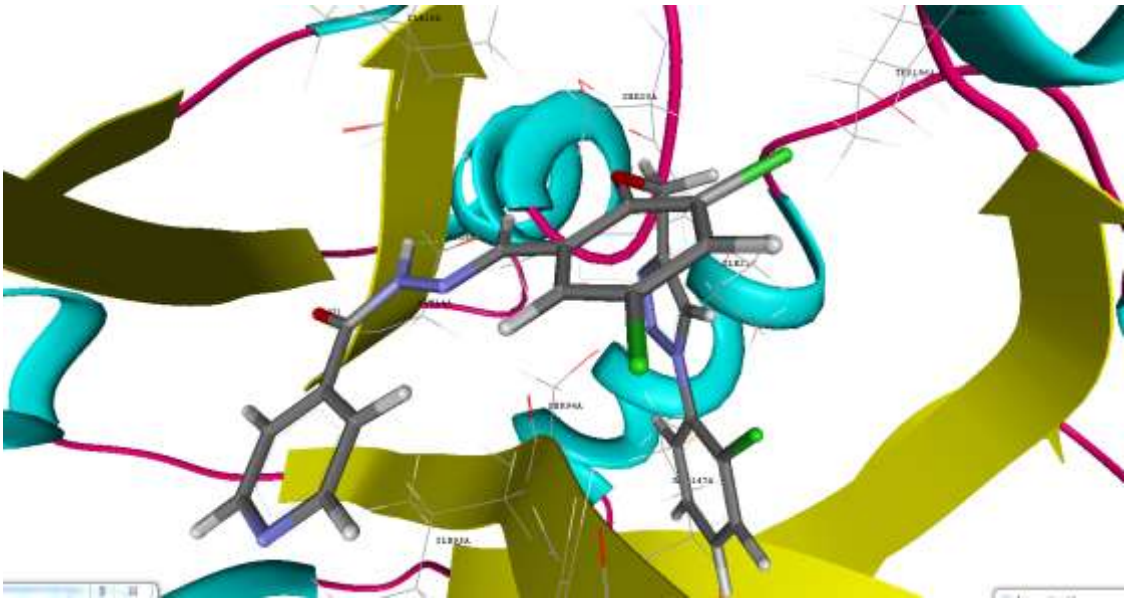


FIGURE 4 Docking interactions of **5g** with *Enoyl-Acp Reductase (INHA)*

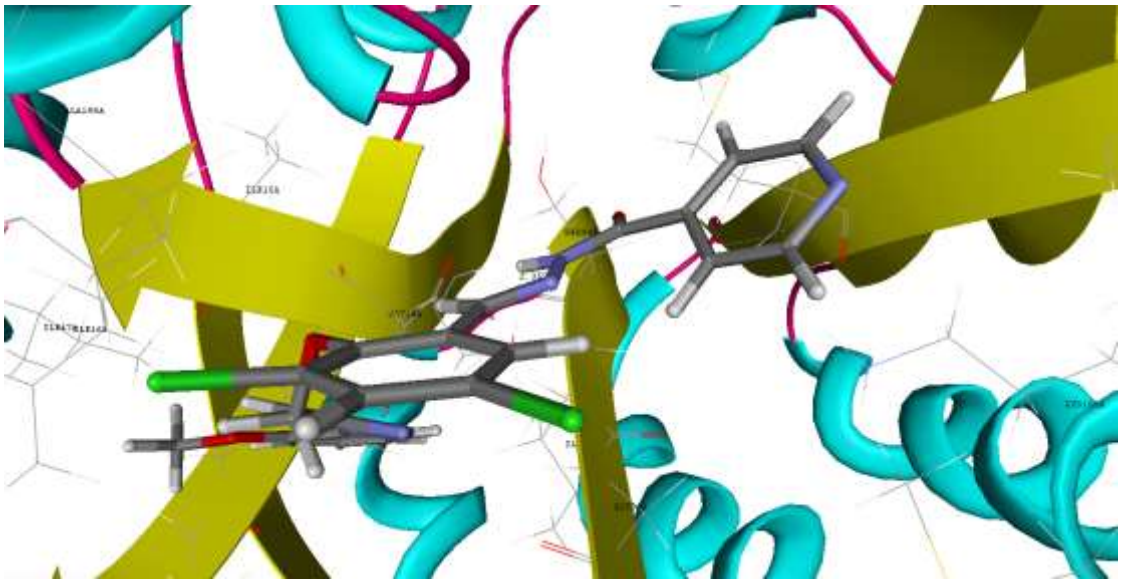


FIGURE 5 Docking interactions of **5h** with *Enoyl-Acp Reductase (INHA)*

3.5. Molecular docking analysis for antimicrobial activity

Derivative **5d** was found to have total docking score (PLP) of -62.62 kcal/mol. It was interacting with DNA gyrase *via* formation of hydrogen bond with ARG342, hydrophobic interactions with ARG342, GLU634 and Vander wall interactions with MET27, ILE30, VAL31, ARG342, PRO343, GLU634, and ALA637 (Figure 6). Derivative **5h** was exhibited

total docking score (PLP) of -58.71 kcal/mol. It was interacting *via* formation of hydrogen bond with LEU345 hydrophobic interactions with ARG342, GLU634 and Vander wall interactions with MET179, ILE336, ARG342, PRO343, LYS344, LEU345, ARG630, ILE633, GLU634, and ALA637 (Figure 7).

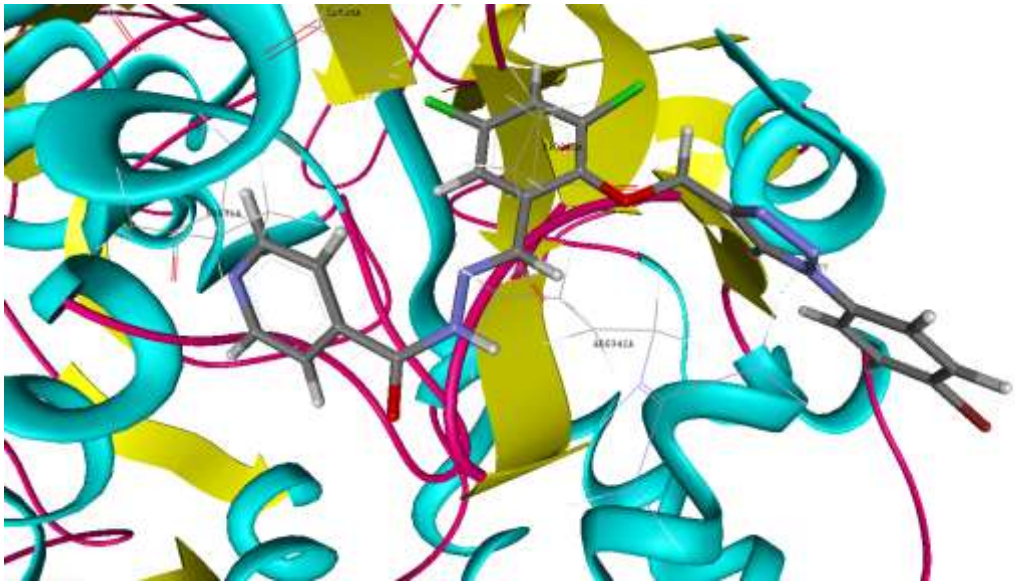


FIGURE 6 Docking interactions of **5d** with *DNA Gyrase*

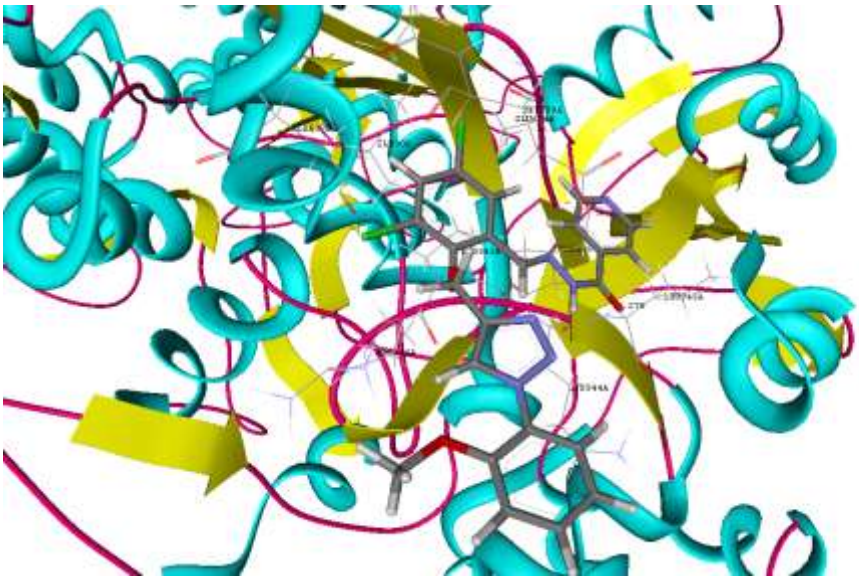


FIGURE 7 Docking interactions of **5h** with *DNA Gyrase*

Derivative **5j** have total docking score (PLP) of -63.00 kcal/mol. It was interacting *via* formation of hydrogen bond with ARG630, hydrophobic interactions with LYS344 and Vander wall interactions with ARG342, PRO343, LEU345, ARG630, GLU634, ALA637 (Figure 8). Derivative **5k** has total docking score (PLP) of -74.23 kcal/mol. It was found to be

interacting *via* the formation of hydrogen bond with LEU345, hydrophobic interactions with ARG342, GLU634 and Vander wall interactions with MET27, ARG342, PRO343, ARG630, ILE633, GLU634, and ALA637 (Figure 9).

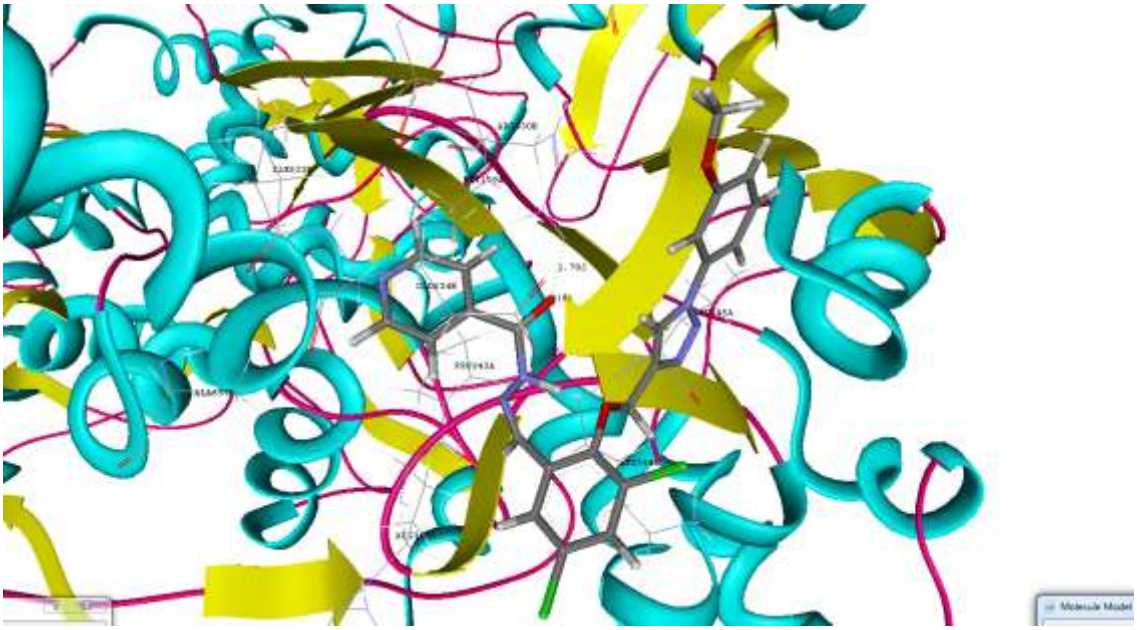


FIGURE 8 Docking interactions of **5j** with *DNA Gyrase*

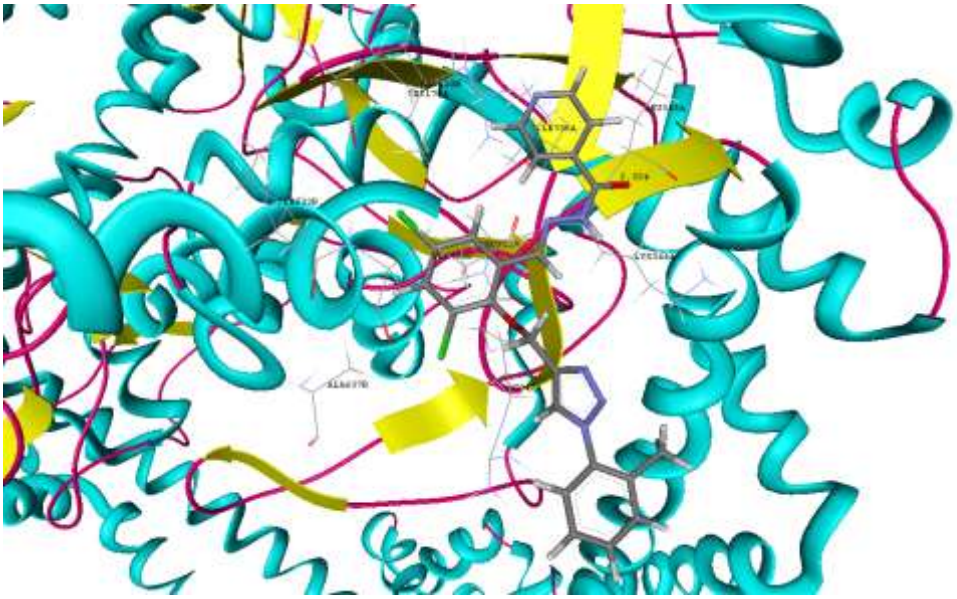


FIGURE 9 Docking interactions of **5k** with *DNA Gyrase*

4. CONCLUSION

In conclusion, a series of new isoniazid-1,2,3-triazole conjugates (**5a-k**) was synthesized *via* click reaction. These compounds were evaluated for their *in vitro* antimycobacterial and antimicrobial activities. The compound **5g** was identified as potent antitubercular agent against *M. tuberculosis* H37Rv (*Mtb*) with MIC value 1.56 μg/mL. However, the three compounds, **5b**, **5c** and **5h** have exhibited moderate antitubercular activity in the range of MIC value 6.25-12.5 μg/mL. These compounds have displayed low cytotoxicity profile by MTT assay against RAW 264.7 cell line. The four compounds, **5d**, **5h**, **5j** and **5k** have shown good *in vitro* antimicrobial activities against both antibacterial and antifungal pathogens. A molecular docking study was performed and these compounds have shown excellent binding affinity towards *Enoyl-acp reductase* (INHA) and *DNA gyrase*. We feel that these compounds with antitubercular and antimicrobial activities will be helpful for invention of potential lead molecules for further improvement.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPLEMENTARY MATERIAL

Additional supporting information may be found online in the Supporting Information Section at the end of this article.