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Ultrasound Assisted Synthesis of Triazole/Tetrazole Hybrids Based New Biquinoline Derivatives as a New Class of Antimicrobial and Antitubercular Agents

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ABSTRACT

A series of new biquinoline derivatives 8a-h and 9a-h, decorated with 1,2,4-triazole, tetrazole at C-4, and aromatic trifluoromethyl moiety at N-1 position were synthesized by multicomponent reactions of 2-amino triazole/amino tetrazole-3-formyl quinoline 5a-b/6a-b with malononitrile/isopropyl cyanoacetate 7a/b and synthesized β-enaminones 3a/b in the presence of ultrasound (soon-synthetic). The structures of synthesized compounds were characterized by infrared, ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, and mass spectroscopy. Furthermore, the synthesized compounds were evaluated for their in vitro antimicrobial activity against a representative panel of pathogenic strains, anti-tuberculosis (TB) activity against Mycobacterium TB $H_{37}Rv$.

Key words: *Ultrasound, Biquinoline, Triazole, Tetrazole, Antimicrobial, Anti-tubercular activity.*

1. INTRODUCTION

Tuberculosis (TB) is a disease which is caused by the bacterium *Mycobacterium TB* a huge menace to the mankind, it spread progressively in 2013, an estimated 9.0 million people developed TB, and 1.5 million died from the disease, 360,000 of whom were HIV-positive. TB is slowly declining each year, and it is estimated that 37 million lives were saved between 2000 and 2013 through effective diagnosis and treatment [1-3]. This has been increased by bacterial and fungal infections over the past years. To build up new antimicrobial and anti-tubercular drugs for efficient therapy is one of the most imperative challenges in the infectious disease research.

Multicomponent reactions (MCRs) with ultrasonic irradiation techniques play an important role that provides an efficient access to highly complex products in a single step, green, and an innocuous technique. They have been proven to be fast, atom-efficient reactions, convergent, and generally give higher yields of products with easy purifications [4-11]. In addition, triazole, tetrazole, quinoline, and its derivatives are more attracted due to their wide spectrum of the biological activities [12-21]. Furthermore, use trifluoromethyl moiety attached the molecule which enhances physical, biological and it has a higher electronegativity that also increase the metabolic stability biopotency, bioavailability, and lipophilicity [22].

In recent years, medicinal chemists have modified standard drugs based on the quinolone scaffold to develop the novel heterocycles with fascinating anti-tubercular and antimicrobial activities [23-25]. After an extensive literature search, we observed, there was no enough efforts have been made to synthesize the biquinoline containing triazole/tetrazole scaffold with ultrasound irradiation method [26-28]. Therefore, in our present study, we introduce triazole, tetrazole moieties, trifluoromethyl derivative to the targeted molecule and can observe the biological activity by changing the position of that group.

2. EXPERIMENTAL

2.1. Materials

Ultrasonication was performed in D-Compact ultrasonic cleaner with a frequency of 50 kHz and power of 250 W (EIE Inst. Pvt. Ltd, Ahmadabad, Gujarat). The reaction flask was suspended at the center of ultrasonic bath so as the surface of the reactants remained slightly lower than the level of water in the bath. All the reactions were performed with commercially available reagents and were used without further purification. All reactions were monitored by thin layer chromatography (TLC) (on aluminum plates coated with silica gel $^{60}F_{254}$, 0.25 mm thickness, Merck) carried on fluorescent-coated plates, and detection of the components was made by exposure to iodine vapors or ultraviolet light. The infrared (IR)

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spectra were recorded on a Perkin-Elmer Spectrum GX Fourier transform-IR (FT-IR) Spectrophotometer (Perkin-Elmer, USA) using potassium bromide pellets in the range 4000-400 cm^{-1} and frequencies of only characteristic peaks are expressed in cm^{-1}. ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra were recorded in dimethyl sulfoxide (DMSO)-d_6 on a Bruker Avance 400F (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using TMS as an internal standard at 400 and 100 MHz, respectively. Chemical shifts are reported in parts per million (ppm). Splitting patterns are designated as s, for singlet; d, for doublet, and m, for multiplet. The ESI mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan). Elemental analysis (% C, H, N) was carried out using a Perkin-Elmer 2400 series-II elemental analyzer (Perkin-Elmer, USA). All compounds are within ±0.4% of theory specified.

2.2. General Procedure for the Synthesis of Aldehydes 5a-b and 6a-b (Scheme 1)

Mix aldehydes 4a/b and ethylene glycol (45 mmol, 3 equiv) were dissolved in anhydrous toluene (25 ml). With this solution, a catalytic amount of *p*-toluenesulfonic acid (1.5 mmol, 0.1 equiv) was added. The reaction mixture was heated under reflux for 3-4 h by using a Dean–Stark condenser (TLC, ethyl acetate/petroleum ether 1:9). After removal of the solvent under reduced pressure, water was added to the reaction mixture, and then the aqueous layer was extracted with ethyl acetate, the combined organic layer was dried over sodium sulfate, and ethyl acetate was evaporated under vacuum. Then, replacement of chloro group by 4-amino triazole/5-amino tetrazole using dimethylformamide DMF as a solvent and K_2CO_3 as a base at reflux temperature. The final products were obtained by deprotection of aldehydes under reflux with acid in water for 1 h. The product was purified by silica gel column chromatography (mobile phase 1-3% ethyl acetate in petroleum ether).

2.3. General Procedure for the Synthesis of 8a-h and 9a-h by Conventional Method and Ultrasound Irradiation (Scheme 2)

2.3. (a) Synthesis of compounds 8a-h

A 50 mL round bottomed flask, fitted with a reflux condenser, was charged with a mixture of 2-(4H-1,2,4-triazol-4-ylamino)quinoline-3carbaldehyde 5a-b (1 mmol), β-enaminones 3a/b (1 mmol), malononitrile/ methyl cyanoacetate 7a/b (1 mmol) and pyridine (5 mol%) were add into ethanol (2 ml), reflux for 60 min at 80°C for conventional method. By ultrasound method, reflux for 20 min at 50°C. The progress of the reaction was monitored by TLC. After the completion of reaction (as evidenced by TLC), the reaction mixture was cooled to room temperature and stirred magnetically for further 20 min, the solid mass separated was collected by filtration, washed well with water and purified by leaching in equal volume ratio of chloroform and methanol (10 mL) to obtain pure solid sample.

2.3. (b) Synthesis of compounds 9a-h

In a 50 mL round-bottom flask, (1H-tetrazol-5-ylamino) quinoline-3-carbaldehyde 6a-b (1 mmol), β-enaminones 3a/b (1 mmol), malononitrile/methyl cyanoacetate 7a/b (1 mmol) and pyridine (5 mol%) mol%) were taken in ethanol. Reflux for 60 min at 80°C for conventional method and ultrasound method, reflux for 20 min at 50°C. After completion of the reaction, as indicated by TLC, the reaction mixture was cooled to room temperature and add into water. The precipitate separated was then filtered and washed well with water. The crude product was purified by leaching in equal volume ratio of chloroform and methanol (10 mL) to obtain pure solid sample.

2-(4*H*-1,2,4-triazol-4-ylamino)quinoline-3-carbaldehyde 5a-b/2-(1*H*-tetrazol-5-ylamino) quinoline-3-carbaldehyde 6a-b (1 mmol), 3 or 3,5-(trifluoromethyl)aniline 3a/b, malononitrile/ methyl cyanoacetate 7a/b (1 mmol), and pyridine (5 mol%) were add into ethanol (2 ml), reflux for 60 min at 80°C for conventional method. By ultrasound method, reflux for 20 min at 50°C. The reaction progress was monitored by TLC. After completion, the reaction mass was allowed to cool, filter, and wash with water and ethanol to afford the final product 8a-h and 9a-h.

2.3.1. 2-(4H-1,2,4-triazol-4-ylamino)quinoline-3-carbaldehyde (TA-1)

White solid; MP 120-125°C; IR (KBr, $\nu_{max,}$ cm^{-1}) = 2930 (Ar-C-H), 3330 (-NH), 1613 (C=O str.);^{1}H NMR (400 MHz, DMSO-d_6) δ=7.81-8.45 (m, 6H, Ar-H), 8.88 (s, 1H, Ar-H), 9.69 (s, 1H, Ar-NH), 10.45 (s, 1H, CHO) ppm; ^{13}C NMR (100 MHz DMSO-d_6) δ=121.54, 123.55, 125.36, 126.55, 128.66, 132.55, 140.36, 145.66, 146.87, 148.66, 158.57, 192.33 (C=O); MS Calc. for $C_{12}H_9N_5O[M]^+$ 239.15, found 239.08; Anal. Calc. C 60.25, H 3.79, N 29.27; Found: C 60.40, H 3.70, N 29.10%.

2.3.2. 2-(1H-tetrazol-5-ylamino) quinoline-3-carbaldehyde (TA-2)

White solid; mp 130-135°C; IR (KBr, $\nu_{max,}$ cm^{-1}) = 3025 (Ar-C-H), 3222 and 3350 (-NH), 1670 (C=O str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ=7.78-8.44 (m, 6H, Ar-H +tetrazole -NH), 9.07 (s, 1H, Ar-NH), 10.65 (s, 1H, CHO) ppm; ^{13}C NMR (100 MHz DMSO-d_6) δ=122.48, 123.64, 125.74, 127.12, 130.97, 132.74, 140.69, 147.55, 158.74, 162.52, 193.74 (C=O); MS Calc. for $C_{11}H_8N_6O[M]^+$ 240.20, found 240.22; Anal. Calc. C 55.00, H 3.36, N 34.98; Found: C 54.80, H 3.50, N 35.15%.

2.3.3. 2-(4H-1,2,4-triazol-4-ylamino)-2'-amino-5'-oxo-1'-(3-(trifluoromethyl)phenyl)-1',4',5',6',7',8'-hexahydro-3,4'-biquinoline-3'-carbonitrile (8a)

White solid; MP 180-185°C; IR (KBr, $\nu_{max,}$ cm^{-1}) = 3450 and 3345 (asym. & sym. str. of -NH_2), 2220 (C≡N str.), 1655 (C=O str.);^{1}H NMR (400 MHz, DMSO-d_6) δ=1.63-2.20 (m, 6H, 3xCH_2), 5.54 (s, 1H, CH), 7.23 (s, 2H, Ar-NH_2), 7.70-8.45 (m, 10H, Ar-H), 8.65 (s, 1H, Ar-H), 9.53 (s, 1H, -NH) ppm;^{13}C NMR (100 MHz DMSO-d_6) δ=26.30 (CH_2), 29.40 (CH_2), 34.50 (CH), 35.12 (CH_2CO), 59.26 (C-CN), 110.59, 112.72, 118.31, 122.36, 123.48, 125.84, 127.47, 127.98, 128.77, 129.80, 130.74, 132.63, 133.46, 135.89, 137.33, 140.35, 144.45, 145.23, 149.82, 151.69, 155.63, 163.73 (Ar-C), 194.46 (C=O) ppm; MS Calc. for $C_{28}H_{21}F_3N_8O[M]^+$ 542.20, found 542.51; Anal. Calc. C 61.99, H 3.90, N 20.65; Found: C 62.23, H 3.85, N 20.88%.

2.3.4. Isopropyl 2-(4H-1,2,4-triazol-4-ylamino)-2'-amino-5'-oxo-1'-(3-(trifluoromethyl)phenyl)-1',4',5',6',7',8'-hexahydro-3,4'-biquinoline-3'-carboxylate (8b)

White solid; MP 182-187°C; IR (KBr, $\nu_{max,}$ cm^{-1}) = 3425 and 3356 (asym. & sym. str. of -NH_2), 1682 and 1630 (C=O str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ=0.87 (d, 3H, J=6, CH_3), 1.09 (d, 3H, J=6, CH_3), 1.55-2.15 (m, 6H, 3xCH_2), 4.42 (m, 1H, $CH(CH_3)_2$), 5.63 (s, 1H, CH), 6.97 (s, 2H, Ar-NH_2), 7.18-7.68 (m, 8H, Ar-H), 8.34-8.47 (s, 3H,Ar-H), 9.43 (s,1H,-NH) ppm; ^{13}C NMR (100 MHz DMSO-d_6) δ=22.30 (CH_3), 24.65 (CH_3), 26.33 (CH_2), 30.58 (CH_2), 34.38 (CH), 36.74 (CH_2CO), 62.53 ($CH(CH_3)_2$), 78.12 (C-$COOCH_2CH_3$), 108.59, 114.39, 120.48, 122.74, 124.55, 125.81, 127.78, 127.38, 128.84, 129.13, 130.74, 132.51, 133.76, 134.89, 138.56, 141.36, 144.85, 143.33, 148.54, 151.89, 153.63 (Ar-C), 164.96 and 194.55 (C=O) ppm; MS calc. for $C_{31}H_{28}F_3N_7O_3[M]^+$ 603.60, found 603.59; Anal. Calc. C 61.69, H 4.68, N 16.24; Found: C 61.43, H 4.33, N 16.50%.

2.3.5. 2-(4H-1,2,4-triazol-4-ylamino)-2'-amino-6-methoxy-5'-oxo-1'-(3-(trifluoromethyl)phenyl)-1',4',5',6',7',8'-hexahydro-3,4'-biquinoline-3'-carbonitrile (8c)

White solid; MP 190-195°C; IR (KBr, $\nu_{max,}$ cm^{-1}) = 3470 and 3355 (asym. & sym. str. of -NH_2), 2212 (C≡N str.), 1675 (C=O str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ=1.67-2.10 (m, 6H, 3xCH_2), 3.59 (s, 3H, Ar-OCH_3), 5.57 (s, 1H, CH), 7.13 (s, 2H, Ar-HN_2), 7.53-8.30 (m, 9H, Ar-H), 8.49 (s, 1H, Ar-H), 9.24 (s, 1H, -NH) ppm; ^{13}C NMR (100 MHz DMSO-d_6) δ=25.45 (CH_2), 26.55 (CH_2), 33.86 (CH), 36.23 (CH_2CO), 58.26 (C-CN), 110.59, 115.66, 118.23, 120.68, 122.78, 123.69, 125.37, 126.56, 127.63, 128.87, 129.71, 130.36, 132.63, 132.99, 135.69, 138.78, 140.39, 144.56, 144.89, 150.69, 152.78, 156.46, 162.78 (Ar-C), 193.56 (C=O) ppm; MS calc. for $C_{29}H_{23}F_3N_8O_2[M]^+$ 572.21, found 572.54; Anal. Calc. C 60.84, H 4.05, N 19.57; Found: C 60.72, H 4.21, N 19.30%.

2.3.6. Isopropyl 2-(4H-1,2,4-triazol-4-ylamino)-2'-amino-6-methoxy-5'-oxo-1'-(3-(trifluoromethyl)phenyl)-1',4',5',6',7',8'-hexahydro-3,4'-biquinoline-3'-carboxylate (8d)

White solid; MP 194-198°C; IR (KBr, $\nu_{max,}$ cm^{-1}) = 3445 and 3370 (asym. & sym. str. of -NH_2), 1670 and 1652 (C=O str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ=0.75 (d, 3H, J=6, CH_3), 1.08 (d, 3H, J=6, CH_3), 1.54-2.12 (m, 6H, 3xCH_2), 3.49 (s, 3H, OCH_3), 4.98 (m, 1H, $CH(CH_3)_2$), 5.99 (s, 1H, CH), 7.19 (s, 2H, Ar-H), 7.48-8.21 (m, 9H, Ar-H), 8.39 (s, 1H, Ar-H), 9.33 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz DMSO-d_6) δ = 21.30 (CH_3), 23.74 (CH_3), 24.52 (CH_2) 27.33 (CH_2), 34.23 (CH), 36.52 (CH_2CO), 55.48 (OCH_3), 65.12 ($C(CH_3)_2$), 79.12 (C-$COOCH_2CH_3$), 107.31, 114.85, 120.51, 122.56, 124.87, 126.45, 127.28, 128.55, 128.96, 129.74, 131.56, 131.96, 133.74, 135.36, 136.34, 140.49, 144.74, 145.10, 150.36, 153.46, 165.33 (Ar-C), 172.93 and 194.66 (C=O) ppm; MS calc. for $C_{32}H_{30}F_3N_7O_4[M]^+$ 633.43, found 633.62; Anal. Calc. C 60.66, H 4.77, N 15.47; Found: C 60.33, H 4.51, N 15.25%.

2.3.7. 2-(4H-1,2,4-triazol-4-ylamino)-2'-amino-1'-(3,5-bis(trifluoromethyl)phenyl)-5'-oxo-1',4',5',6',7',8'-hexahydro-3,4'-biquinoline-3'-carbonitrile (8e)

White solid; MP 195-198°C; IR (KBr, $\nu_{max,}$ cm^{-1}) = 3447 and 3360 (asym. & sym. str. of -NH_2), 2231 (C≡N str.), 1681 (C=O str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 1.73-2.23 (m, 6H, 3xCH_2), 5.46 (s, 1H, CH), 7.19 (s, 2H, Ar-H), 7.78-8.47 (m, 9H, Ar-H), 8.69 (d, 1H, J=6, Ar-H), 9.69 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz DMSO-d_6) δ = 24.75 (CH_2), 29.40 (CH_2), 34.60 (CH), 37.10 (CH_2CO), 59.96 (C-CN), 107.11, 114.92, 119.16, 123.03, 125.12, 126.34, 127.86, 128.22, 129.15, 131.21, 133.41, 133.69, 138.50, 140.20, 141.82, 144.80, 145.17, 147.85, 148.26, 149.21, 153.69, 158.75, 163.40 (Ar-C), 196.02 (C=O) ppm; MS calc. for $C_{29}H_{20}F_6N_8O[M]^+$ 610.37, found 610.51; Anal. Calc. C 57.05, H 3.30, N 18.35; Found: C 56.95, H 3.61, N 18.60%.

2.3.8. Isopropyl 2-(4H-1,2,4-triazol-4-ylamino)-2'-amino-1'-(3,5-bis(trifluoromethyl)phenyl)-5'-oxo-1',4',5',6',7',8'-hexahydro-3,4'-biquinoline-3'-carboxylate (8f)

White solid; MP 210-214°C; IR (KBr, ν_{max}, cm^{-1}) = 3425 and 3356 (asym. & sym. str. of -NH_2), 1674 and 1633 (C=O str.); ^{1}H NMR (400MHz, DMSO-d_6) δ = 0.62 (d, 3H, J=6, CH_3), 1.07 (d, 3H, J=6, CH_3), 1.64-2.11 (m, 6H, 3xCH_2), 4.70 (m, 1H, $CH(CH_3)_2$), 5.66 (s, 1H, CH), 7.00 (s, 2H, Ar-NH_2), 7.67-8.23 (m, 9H, Ar-H), 8.65 (s, 1H, Ar-H), 9.17 (1H, s, -NH) ppm; ^{13}C NMR (100 MHz DMSO-d_6) δ=22.21 (CH_3), 23.48 (CH_3), 27.39 (CH_2), 28.91 (CH_2), 33.59 (CH), 35.37 (CH_2CO), 61.35 ($CH((CH)_3)_2$, 77.13 (C-$COOCH(CH_3)_2$), 105.17, 110.10, 114.94, 119.70, 121.90, 124.03, 124.71, 128.93,

129.46, 130.12, 132.75, 134.05, 138.04, 139.74, 141.69, 144.98, 145.83, 151.71, 155.00, 157.87, 162.56, 165.21 (Ar-C), 169.95 and 194.05 (C=O) ppm; MS calc. for $C_{32}H_{27}F_6N_7O_3[M]^+$ 671.31, found 671.59; Anal. calc. C 57.23, H 4.05, N 14.60; Found: C 57.55, H 3.85, N 14.30%.

2.3.9. 2-(4H-1,2,4-triazol-4-ylamino)-2'-amino-1'-(3,5-bis(trifluoromethyl)phenyl)-6-methoxy-5'-oxo-1',4',5',6',7',8'-hexahydro-3,4'-biquinoline-3'-carbonitrile (8g)

White solid; MP 205-208°C; IR (KBr, ν_{max}, cm^{-1}) = 3423 and 3346 (asym. & sym. str. of -NH_2), 2189 (C≡N str.), 1650 (C=O str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ=1.78-2.16 (m, 6H, 3xCH_2), 3.74 (s, 3H, Ar-OCH_3), 5.38 (s, 1H, CH), 7.16 (s, 2H, Ar-H), 7.43 (d, 1H, J=8.8, Ar-H), 7.59 (s, 1H, Ar-H), 7.88 (d, 1H, J=9.2, Ar-H), 8.27-8.56 (m, 6H, Ar-H), 9.70 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz DMSO-d_6) δ=20.94 (CH_2), 24.50 (CH_2), 34.15(CH), 37.80 (CH_2CO), 55.98 (C-CN), 57.30 (Ar-OCH_3), 109.66, 113.45, 117.06, 121.22, 123.02, 123.70, 124.32, 126.65, 127.40, 129.11, 129.29, 129.90, 130.90, 133.10, 133.95, 135.89, 139.79, 141.35, 145.18, 145.81, 150.80, 154.03, 165.91 (Ar-C), 193.11 (C=O) ppm; MS calc. for $C_{30}H_{22}F_6N_8O_2[M]^+$ 640.53, found 640.54; Anal. Calc. C 56.25, H 3.46, N 17.49; Found: C 55.95, H 3.78, N 17.28%.

2.3.10. Isopropyl 2-(4H-1,2,4-triazol-4-ylamino)-2'-amino-1'-(3,5-bis(trifluoromethyl)phenyl)-6-methoxy-5'-oxo-1',4',5',6',7',8'-hexahydro-3,4'-biquinoline-3'-carboxylate (8h)

White solid; MP 201-205°C; IR (KBr, ν_{max}, cm^{-1}) = 3441and 3373 (asym. & sym. str. of -NH_2), 1676 and 1640 (C=O str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ=0.74 (d, 3H, J=6, CH_3), 1.12 (d, 3H, J=6.4, CH_3), 1.79-2.19 (m, 6H, 3xCH_2), 3.65 (s, 3H, Ar-OCH_3), 4.34 (m, 1H, $CH((CH)_3)_2$, 5.64 (s, 1H, CH), 7.10 (s, 2H, Ar-H), 7.398-8.55 (m, 8H, Ar-H), 8.61 (s, 1H, Ar-H), 9.69 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz DMSO-d_6) δ=21.71 (CH_3), 23.55 (CH_3), 24.55 (CH_2), 26.55 (CH_2), 34.44 (CH), 56.11 (Ar-OCH_3), 63.55 ($C(CH_3)_2$), 77.63 (C-$COOCH_2CH_3$), 108.02, 110.27, 116.92, 120.04, 121.41, 122.54, 124.55, 126.33, 127.44, 128.98, 129.56, 130.65, 131.80, 133.41, 134.16, 137.21, 139.44, 145.58, 146.04, 151.42, 155.45, 158.33, 164.22 (Ar-C), 171.71 and 193.66 (C=O) ppm; MS calc. for $C_{33}H_{29}F_6N_7O_4[M]^+$ 701.34, found 701.62; Anal. calc. C 56.49,H 4.17, N 13.97; Found: C 56.12, H 3.95, N 14.15%.

2.3.11. 2-(1H-tetrazol-5-ylamino)-2'-amino-5'-oxo-1'-(3-(trifluoromethyl)phenyl)-1',4',5',6',7',8'-hexahydro-3,4'-biquinoline-3'-carbonitrile (9a)

White solid; MP 198-202°C; IR (KBr, ν_{max}, cm^{-1}) = 3425, 3352 and 3360 (asym. & sym. str. of -NH_2+ tetrazole-NH), 2189 (C≡N str.), 1662 (C=O str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ=1.69-2.40 (m, 6H, 3xCH_2), 5.68 (s, 1H, CH), 7.21 (s, 2H, Ar-NH_2), 7.56-8.32 (m, 9H, Ar-H+tetrazole-NH), 8.44 (s, 1H, Ar-H), 9.59 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz DMSO-d_6) δ=23.55 (CH_2), 25.58 (CH_2), 35.22 (CH), 37.58 (CH_2CO) 57.84 (C–CN), 108.23, 111.72, 116.41, 120.34, 121.98, 123.54, 124.21, 125.74, 126.48, 127.36, 128.64, 129.43,130.68, 132.43, 133.62, 135.83, 137.33, 145.63, 151.74, 155.43, 162.73 (Ar-C), 192.86 (C=O) ppm; MS Calc. for $C_{27}H_{20}F_3N_9O[M]^+$ 543.19, found 543.50; Anal. Calc. C 59.67, H 3.71, N 23.19; Found: C 59.90, H 3.95, N 22.90%.

2.3.12. Isopropyl 2-(1H-tetrazol-5-ylamino)-2'-amino-5'-oxo-1'-(3-(trifluoromethyl)phenyl)-1', 4', 5', 6', 7', 8'-hexahydro-3,4'-biquinoline-3'-carboxylate (9b)

White solid; MP 210-215°C; IR (KBr, ν_{max}, cm^{-1}) = 3410, 3379 and 3155 (asym. & sym. str. of -NH_2+ tetrazole-NH), 1674 and 1643 (C=O str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ=0.62 (d, 3H, J=6, CH_3), 1.05 (d, 3H, J=6.4, CH_3), 1.63-2.12 (m, 6H, 3xCH_2), 4.80 (m, 1H, $CH(CH_3)_2$), 5.69 (s, 1H, CH), 7.13 (s, 2H, Ar-NH_2), 7.68-8.41 (m, 9H, Ar-H+tetrazole-NH), 8.68 (s, 1H, Ar-H), 9.19 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz DMSO-d_6) δ=22.73 (CH_3), 24.56 (CH_3), 25.58 (CH_2), 29.55 (CH_2), 34.77 (CH), 37.55 (CH_2CO), 65.23 ($C(CH_3)_2$), 80.33 (C-$COOCH_2CH_3$), 108.13, 112.16, 118.35, 121.48, 122.58, 123.42, 124.39, 125.71, 128.53, 129.33, 130.69, 131.74, 132.65, 134.78, 135.35, 136.71, 138.22, 145.13, 150.35, 155.68 (Ar-C),172.46 and 195.15 (C=O) ppm; MS Calc. for $C_{30}H_{27}F_3N_8O_3[M]^+$ 604.61, found 604.58; Anal. Calc. C 59.60, H 4.50, N 18.53; Found: 59.42, H 4.31, N 18.70%.

2.3.13. 2-(1H-tetrazol-5-ylamino)-2'-amino-6-methoxy-5'-oxo-1'-(3-(trifluoromethyl)phenyl)-1',4',5',6',7',8'-hexahydro-3,4'-biquinoline-3'-carbonitrile (9c)

White solid; MP 220-224°C; IR (KBr, ν_{max}, cm^{-1}) = 3432, 3371 and 3145 (asym. & sym. str. of -NH_2+ tetrazole-NH), 2199 (C≡N str.), 1682 (C=O str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ=1.58-2.30 (m, 6H, 3xCH_2), 3.67 (s, 3H, Ar-OCH_3), 5.73 (1H, s, CH), 7.12 (s, 2H, Ar-NH_2), 7.59-8.49 (m, 8H, Ar-H+NH), 8.60 (s, 1H, Ar-H), 9.45 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz DMSO-d_6) δ=23.41 (CH_2), 25.66 (CH_2), 35.22 (CH), 36.98 (CH_2CO), 54.53 (OCH_3), 57.84 (C-CN), 109.23, 114.32, 117.45, 121.54, 121.89, 122.39, 124.78, 126.31, 127.85, 128.91, 130.43, 131.43, 133.48, 134.62, 135.11, 137.35, 142.66, 144.88, 154.73, 155.45, 162.45 (Ar-C), 194.86 (C=O) ppm; MS Calc. for$C_{28}H_{22}F_3N_9O_2[M]^+$ 573.65, found 573.53; Anal. Calc. C 58.64, H 3.87, N 21.98; Found: C 58.51, H 4.12, N 21.65%.

2.3.14. Isopropyl 2-(1H-tetrazol-5-ylamino)-2'-amino-6-methoxy-5'-oxo-1'-(3-(trifluoromethyl) phenyl)-1',4',5',6',7',8'-hexahydro-3,4'-biquinoline-3'-carboxylate (9d)

White solid; MP 210-214°C; IR (KBr, ν_{max}, cm^{-1}) = 3441, 3359 and 3165 (asym. & sym. str. of -NH_2+

tetrazole-NH),1625 and 1688 (C=O str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 0.79 (d, 3H, J=6, CH_3), 1.13 (d, 3H, J=6.4, CH_3), 1.49-2.20 (m, 6H, 3xCH_2), 3.54 (s, 3H, Ar-OCH_3), 4.58 (m, 1H, $CH(CH_3)_2$), 5.61 (s, 1H, CH), 7.22 (s, 2H, Ar-NH_2), 7.68-8.62 (m, 8H, Ar-H), 8.74 (s, 1H, Ar-H), 9.34 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz DMSO-d_6) δ=23.67 (CH_3), 25.74 (CH_3), 27.56 (CH_2), 29.98 (CH_2), 33.19 (CH), 36.45 (CH_2CO), 52.56 ($COOCH_3$), 56.75 (OCH_3) 111.23, 113.68, 118.65, 120.19, 121.55, 122.48, 124.67, 126.93, 127.80, 128.32, 129.35, 130.69, 134.29, 135.02, 137.89, 138.07, 140.67, 141.24, 144.57, 148.23, 154.08 (Ar-C), 170.67 and 195.59 (C=O) ppm; MS Calc. for $C_{31}H_{29}F_3N_8O_4[M]^+$ 634.60 found 634.61; Anal. Calc. C 58.67, H 4.61, N 17.66; Found: C 58.34, H 4.80, N 17.45%.

2.3.15. 2-(1H-tetrazol-5-ylamino)-2'-amino-1'-(3,5-bis(trifluoromethyl)phenyl)-5'-oxo-1',4',5',6',7',8'-hexahydro-3,4'-biquinoline-3'-carbonitrile (9e)

White solid; MP 220-222°C; IR (KBr, ν_{max}, cm^{-1}) = 3422, 3376 and 3120 (asym. & sym. str. of -NH_2+ tetrazole-NH), 2199 (C≡N str.), 1682 (C=O str.), 1203;[1] H NMR (400 MHz, DMSO-d_6) δ=1.82-2.26 (m, 6H, 3xCH_2), 5.66 (s, 1H, CH), 7.02 (s, 2H, Ar-NH_2), 7.59-8.12 (m, 7H, Ar-H+NH), 8.25 (s, 1H, Ar-H),8.47 (s, 1H, Ar-H), 9.36 (s, 1H, NH) ppm;^{13}C NMR (100 MHz DMSO-d_6) δ=24.33 (CH_2), 26.13 (CH_2) 33.41 (CH), 36.07 (CH_2CO), 58.40 (C-CN), 109.47, 113.70, 117.85, 120.35, 121.29, 122.05, 123.52, 124.46, 125.54, 126.72, 127.38, 128.23, 130.92, 131.39, 133.35, 135,17, 138.66, 140.55, 144.57, 152.64, 158.98, 164.03 (Ar-C), 195.98 (C=O) ppm; MS Calc. for$C_{28}H_{19}F_6N_9O[M]^+$ 611.31, found 611.50; Anal. Calc. C 55.00, H 3.13, N 20.61; Found: C 55.30, H 3.44, N 20.80%.

2.3.16. Isopropyl 2-(1H-tetrazol-5-ylamino)-2'-amino-1'-(3,5-bis(trifluoromethyl)phenyl)-5'-oxo-1',4',5',6',7',8'-hexahydro-3,4'-biquinoline-3'-carboxylate (9f)

White solid; MP 216-220°C; IR (KBr, ν_{max}, cm^{-1}) = 3439, 3369 and 3131 (asym. & sym. str. of -NH_2+ tetrazole-NH),1630 and 1682 (C=O str.);^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm)=0.69 (d, 3H, J=6, CH_3), 1.17 (d, 3H, J=6, CH_3), 1.54-2.21 (m, 6H, 3xCH_2), 4.51 (m, 1H, $CH(CH_3)_2$), 5.63 (1H, s, CH), 7.21 (s, 2H, Ar-NH_2), 7.65-8.49 (m, 9H, Ar-H+NH), 9.44 (s, 1H, NH) ppm;^{13}C NMR (100 MHz DMSO-d_6) δ (ppm)=23.56 (CH_3), 24.85 (CH_3), 27.45 (CH_2), 29.58 (CH_2), 33.19 (CH), 36.79 (CH_2CO), 63.63 ($CH((CH)_3)_2$, 81.63(C-$COOCH_2CH_3$), 109.63, 116.39, 121.39, 122.79, 123.81, 124.19, 127.76, 129.92, 130.11, 131.68, 132.79, 133.93, 135.86, 136.67,138.23, 140.39, 144.38, 145.79, 150.86, 158.68, 164.23 (Ar-C), 172.46 and 193.15 (C=O) ppm; MS Calc. for $C_{31}H_{26}F_6N_8O_3[M]^+$ 672.21, found 672. 58; Anal. Calc. C 55.36, H 3.90, N 16.66; Found: C 55.61, H 4.13, N 16.40%.

2.3.17. 2-(1H-tetrazol-5-ylamino)-2'-amino-1'-(3,5-bis(trifluoromethyl)phenyl)-6-methoxy-5'-oxo-1',4',5',6',7',8'-hexahydro-3,4'-biquinoline-3'-carbonitrile (9g)

White solid; MP 202-205°C; 220-225; IR (KBr, ν_{max}, cm^{-1}) = 3421, 3352 and 3122 (asym. & sym. str. of -NH_2+ tetrazole-NH), 2199 (C≡N str.), 1682 (C=O str.);^{1}H NMR (400 MHz, DMSO-d_6) δ=1.74-2.20 (m, 6H, 3xCH_2), 3.92 (s, 3H, Ar-OCH_3), 5.37 (s, 1H, H_4), 7.17 (s, 2H, Ar-NH_2), 7.42-8.54 (m, 8H, Ar-H+ tetrazole-NH), 9.56 (s, 1H, NH) ppm;^{13}C NMR (100 MHz DMSO-d_6) δ=23.73 (CH_2), 26.52 (CH_2), 33.16 (CH), 35.03 (CH_2), 54.25 (Ar-OCH_3), 58.42 (C-CN), 110.80, 115.91, 119.19, 121.10, 123.05, 125.12, 125.95, 126.46, 128.60, 128.65, 130.37, 132.45, 133.42, 134.37, 137.69, 139.05, 142.28, 146.53, 151.04, 154.20, 159.38, 164.02 (Ar-C), 192.02 (C=O) ppm; MS Calc. for $C_{29}H_{21}F_6N_9O_2[M]^+$ 641.52, found 641.53; Anal. Calc. C 54.29, H 3.30, N 19.65; Found: C 54.41, H 3.10, N 19.45%.

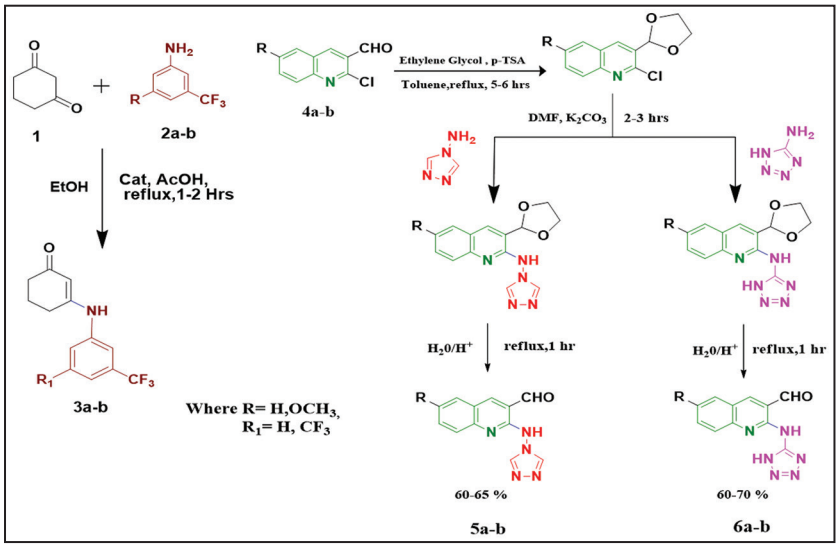
2.3.18. Isopropyl 2-(1H-tetrazol-5-ylamino)-2'-amino-1'-(3,5-bis(trifluoromethyl)phenyl)-6-methoxy-5'-oxo-1', 4', 5', 6', 7', 8'-hexahydro-3,4'-biquinoline-3'-carboxylate (9h)

White solid; MP 210-218°C; IR (KBr, ν_{max}, cm^{-1}) = 3394, 3310 and 3145 (asym. & sym. str. of -NH_2+ tetrazole-NH), 1666 and 1635 (C=O str.);^{1}H NMR (400 MHz, DMSO-d_6) δ=0.69 (d, 3H, J=6.4, CH_3), 1.07 (d, 3H, J=6.4, CH_3), 1.65-2.21 (m, 6H, 3xCH_2), 3.45 (s, 3H, OCH_3), 4.81 (m, 1H, $CH(CH_3)_2$), 5.74 (s, 1H, CH), 7.14 (s, 2H, Ar-NH_2), 7.74-8.52 (m, 7H, Ar-H+tetrazole-NH), 8.69 (s, 1H, Ar-H), 9.21 (s, 1H, NH) ppm;^{13}C NMR (100 MHz DMSO-d_6) δ=20.95 (CH_3), 23.56 (CH_3), 26.11 (CH_2), 30.89 (CH_2), 33.11 (CH), 36.78 (CH_2CO), 54.66 (OCH_3), 60.89 ($CH(CH_3)_2$), 79.56 ($COOCH_2CH_3$), 109.45, 116.86, 118.41, 120.61, 121.65, 122.15, 123.93, 124.66, 125.22, 126.78, 127.69, 130.58, 131.54, 133.74, 134.86, 136.79, 138.45, 141.69, 147.55, 152.89, 155.08 (Ar-C), 174.23 and 194.36 (C=O) ppm; MS Calc. for $C_{32}H_{28}F_6N_8O_4[M]^+$ 702.40, found 702.61; Anal. Calc. C 54.70, H 4.02, N 15.95; Found: C 54.45, H 4.22, N 15.70%.

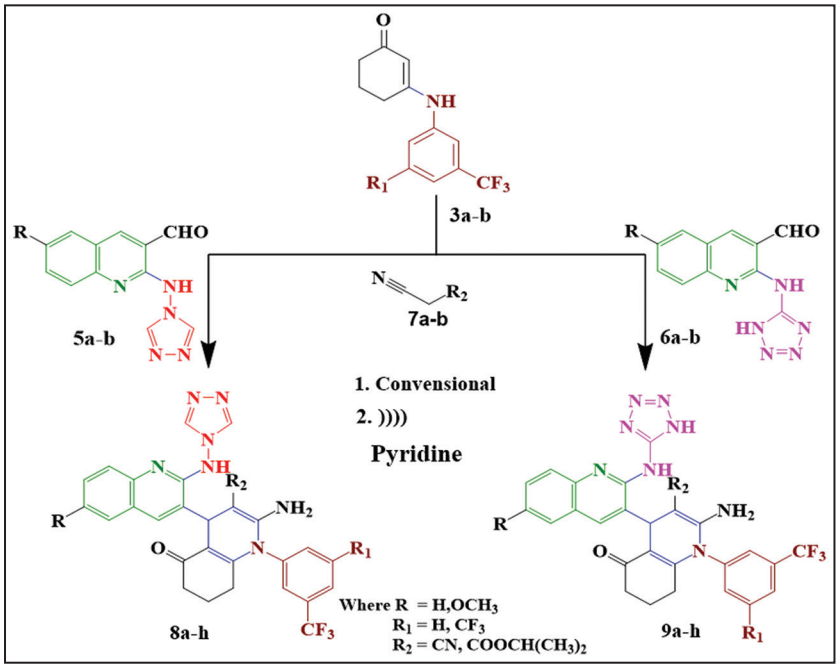
3. RESULT AND DISCUSSION

The synthesis of the target molecules is depicted in Scheme 1. From the literature procedure, the starting material 2-chloro-3-formyl quinoline 4a-b was prepared by Vilsmeier-Haack reaction of acetanilide and were conveniently converted into 5a-b/6a-b by nucleophilic displacement of chloro group at C-2 in 4a-b with 4-amino-1,2,4-triazole/5-amino tetrazole in the presence of anhydrous K_2CO_3 in DMF [29,30].

First, prepared the *β*-enaminones 3a-b by the reaction of cyclohexane-1, 3-dione 1 with 3 or 3, 5-(trifluoromethyl) aniline 2a-b in ethanol, add one



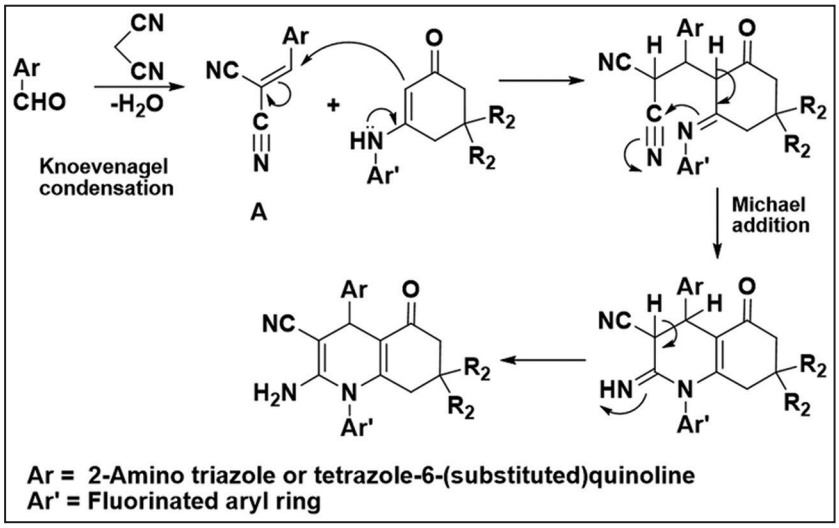
Scheme 1: General synthetic route for the synthesis of compounds 3(a-b), 5(a-b), and 6(a-b).



Scheme 2: General synthetic route for the compounds 8a-h and 9a-h.

drop of acetic acid as catalyst. Final, the *N*-aryl biquinoline 8a-h and 9a-h derivatives were synthesized by reaction of 2-substituted (amino-triazole/ tetrazole)-3-formyl quinoline 5a-b/6a-b, malononitrile 7a/isopropyl 2-cyanoacetate 7b with appropriate enaminones 3a-b in absolute ethanol in the presence of pyridine as a catalyst. The formation of compounds 8a-h and 9a-h may proceed via the initial formation of an intermediate.

The structures of all the synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR, and FT-IR spectrometry. Mass spectrometry and IR spectrometry were performed for few selected compounds. In the ^{1}H NMR (DMSO-d_6) spectrum, the peak of –CHO in TA-1 and TA-2 at δ=10.65, δ=10.45 which was disappearance in compound 8e and 9g singlet peak observed at δ=5.46 and δ=5.37 of –CH proton that conformed the cyclization of Knoevenagel intermediate, as well as –NH_2 protons, give singlet at δ=7.19 and δ=7.17 ppm that conformed the NH_2 group is present in our targeted molecule. In addition, ^{13}C NMR spectra of compounds 8e and 9g a peak observed at around δ=34.60 and δ=33.16 ppm for methane carbon(CH) which confirmed the cyclization. In FT-IR spectra, compounds 8a-h and 9a-h exhibited an absorption band at around 1620-1680 cm^{-1} for (C=O) stretching. The obtained elemental analysis



Scheme 3: A plausible mechanism Knoevenagel condensation followed by Michael addition to form the compounds 8 a-h and 9 a-h.

Table 1: Comparison between the yields obtained in the synthesis of compounds 8a-h and 9a-h using different reaction methodologies.

Entry	R	R_1	R_2	A[a]	B[b]	Atom economy (%)
8a	H	CN	H	75	91	96.79
8b	H	$COOCH((CH_3)_2)$	H	77	92	97.10
8c	OCH_3	CN	H	76	87	96.95
8d	OCH_3	$COOCH((CH_3)_2)$	H	78	90	97.23
8e	H	CN	CF_3	79	88	97.08
8f	H	$COOCH((CH_3)_2)$	CF_3	80	91	97.39
8g	OCH_3	CN	CF_3	75	90	97.26
8h	OCH_3	$COOCH((CH_3)_2)$	CF_3	74	89	97.49
9a	H	CN	H	77	92	96.79
9b	H	$COOCH((CH_3)_2)$	H	76	91	97.10
9c	OCH_3	CN	H	72	89	96.95
9d	OCH_3	$COOCH((CH_3)_2)$	H	76	87	97.24
9e	H	CN	CF_3	74	89	97.14
9f	H	$COOCH((CH_3)_2)$	CF_3	78	91	97.39
9g	OCH_3	CN	CF_3	75	88	97.27
9h	OCH_3	$COOCH((CH_3)_2)$	CF_3	77	90	97.50

[a]EtOH, 100°C, 60 min; [b]EtOH, 50°C, 20 min. [a,b]Isolated yield, A=Conventional, B=Ultrasound method

values are in consonance with theoretical data. Mass spectrometry of some selected compounds 8a, 8b, and 9d showed molecular ion peak $[M]^+$ corresponding to exact mass. Similarly, all these compounds were characterized on the basis of spectral studies as well as elemental analysis.

First, the initial formation of the heterylidenenitrile, *via* the reaction occurs an *in situ*, containing the electron-poor C=C double bond, from the Knoevenagel condensation (Scheme 3) between 5a-b/6a-b and malononitrile 7a or isopropyl cyanoacetate 7b by the loss of water molecules. In addition, Michael addition of *β*-enaminones 3a-b to the ylidenic bond in forming an acyclic intermediate which cyclized by nucleophilic attack of the -NH group on the cyano carbon, followed by tautomerization to the final products 8a-h and 9a-h.

Here, synthesized the compound 8a-h and 9a-h by using both methods such as conventional, ultrasound, and pyridine as a catalyst. From Table 1, we can say that using ultrasound irradiation increase the yield with atom economy compared with the conventional

method. Also required less time, no use of a toxic solvent which are the attractive feature for the medicinal chemist to synthesize the drugs. % Atom economy for ultrasound assisted synthesis compounds 8a-h and 9a-h were found in the range of 96-97%, as only one molecule of water were released in the process [31].

Table 2: Optimization of the reaction conditions of some selected target compound 8a, 8h, 9a, and 9h under ultrasound irradiation.

Entry	Temperature (°C)	Time (min)	Yield of percentage
1	30	20	0
2	40	20	Trace
3	50	5	5-10
4	50	10	20-35
5	50	15	35-50
6	50	20	86-92
7	50	25	80-85
8	50	60	70-80
9	50	70	50-60
10	50	100	30-40

Here, we increase the reaction temperature from 30 to 50°C led to an increase/decrease in the yield of the desired product (Table 2). Treatment of targeted product with 30°C at 20 min, under these conditions, failed to provide any of the desired products (Table 2, Entries 1), increase the temperature with time traces amount of the desired product was observed (Table 2, Entries 2).

Increasing the reaction temperature to 50°C and the reaction time increase by the difference of 5 min led to a significant yield increase/decrease of the desired (Table 2, Entries 3-10).We found that reacting 5a-b/6a-b, 3a-b, and 7a-b for 20 min at 50°C under ultrasound conditions afforded the product 8a-h and 9a-h with the highest yield.

Table 3: *In vitro* antimicrobial activity of *N*-aryl biquinoline 8a-h and 9a-h MICs, µg mL^{-1}.

	MIC, µg/ml							
Compound	**Gram-positive bacteria**			**Gram-negative bacteria**			**Fungi**	
	S.P.	***B.S.***	***C.T.***	***E.C.***	***S.T.***	***V.C.***	***C.A.***	***A.F.***
	MTCC 1936	**MTCC 441**	**MTCC 449**	**MTCC 443**	**MTCC 98**	**MTCC 3906**	**MTCC 227**	**MTCC 3008**
8a	250	250	250	500	200	500	500	1000
8b	250	250	250	200	200	200	500	500
8c	250	250	500	200	250	500	500	1000
8d	**200**	**200**	**62.5**	100	**62.5**	200	**250**	250
8e	**200**	**200**	250	250	200	200	500	1000
8f	**200**	250	250	500	500	200	1000	500
8g	500	**200**	**200**	500	250	200	1000	250
8h	**100**	**62.5**	**200**	**62.5**	500	**62.5**	**250**	500
9a	**100**	250	**200**	200	250	200	500	>1000
9b	**200**	250	250	100	200	500	**250**	250
9c	**200**	**200**	500	200	250	250	500	500
9d	**62.5**	**100**	**100**	100	**62.5**	500	**250**	500
9e	**200**	**200**	500	500	100	100	500	500
9f	500	500	**200**	250	200	200	250	1000
9g	250	**200**	250	200	250	250	500	500
9h	**62.5**	**100**	250	**62.5**	**62.5**	250	**250**	1000
Amp	250	250	250	100	100	100	-	-
Cipro	50	50	100	25	25	25	-	-
Nyst	-	-	-	-	-	-	100	100
Griseo	-	-	-	-	-	-	500	100

-=Not tested. Bold numbers indicate more potent compounds compared to standard drugs. MIC=Minimum inhibitory concentration, *B.S=Bacillus subtilis, C.T.=Clostridium tetani, S.P.=Streptococcus pneumonia, E.C.=Escherichia coli, S.T.=Salmonella typhi, V.C.=Vibrio cholera, A.F.=Aspergillus fumigatus, C.A.=Candida albicans, MTCC=Microbial type culture collection*, Ampi.=Ampicillin, Cipro.=Ciprofloxacin, Norflo.=Norfloxacin, Grise.=Griseofulvin, Nyst.=Nystatin

3.1. Antimicrobial Activity

Examination of the data (Table 3) revealed that many of the compounds showed good antibacterial and antifungal activity when compared with standard drugs. Compounds 8h and 9h (minimum inhibitory concentration [MIC]=62.5 μg mL^{-1}) were found to be exceedingly potent against most of the employed strains while 8d, 9b, and 9d (MIC=100 μg mL^{-1}) were found to have equivalent activity against Gram-negative bacteria *Escherichia coli*. On the other hand, *Streptococcus pneumoniae*, 9d, 9h (MIC=62.5 μg mL^{-1}) displayed excellent activity compound 8h, 9a (MIC=100 μg mL^{-1}), 8d, 8e, 8f, 9b, 9c, and 9e (MIC=200 μg mL^{-1}) displayed excellent activity as compared to the standard ampicillin, compounds 8a, 8b, 8c, and 9g were found comparable activity to the standard drug ampicillin. Against *Vibrio cholerae* 8h (MIC=62.5 μg mL^{-1}) exhibited excellent activity and 9e (MIC=100 μg mL^{-1}) exhibited comparable activity to ampicillin. Against *Bacillus subtilis* 8h (MIC=62.5 μg mL^{-1}), 9d, and 9h (MIC=100 μg mL^{-1}) and 8d, 8e, 8g, 9c, 9e, and 9g (MIC=200 μg mL^{-1}) displayed promising potency as compare to ampicillin (MIC=250 μg mL^{-1}).

Against *Salmonella typhi* compounds 8d, 9d, and 9h (MIC=62.5 μg mL^{-1}) displayed excellent activity, 9e (MIC=100 μg mL^{-1}) was found as a comparable activity to ampicillin (MIC=100 μg mL^{-1}). Against *Clostridium tetani* compounds 8d (MIC=62.5 μg mL^{-1}) and 9d (MIC=100 μg mL^{-1}), 8g, 8h, 9a, and 9f (MIC=200 μg mL^{-1}) were found to be more potent compare to ampicillin (MIC=250 μg mL^{-1}) while compounds 8a, 8b, 8e, 8f, 9b, 9g, and 9h were found to have equivalent activity against ampicillin (MIC=250 μg mL^{-1}). Moreover, against fungal pathogen *Candida albicans*, compounds 8d, 8h, 9b, 9d, 9f, and 9h (MIC=250 μg mL^{-1}) displayed excellent activity on comparison with griseofulvin (MIC=500 μg mL^{-1}). Unfortunately, none of the synthesized compounds was found sufficiently potent to inhibit fungal pathogen *Aspergillus fumigatus*.

The structural activity relationship analysis demonstrated that a change in the peripheral substituent might also affect the antimicrobial activity of title compounds 8a-h and 9a-h. Compounds with an electron donating group R=OCH_3, having a lipophilic group R_1=COOCH ($(CH_3)_2$) and R_2=CF_3 gave better results against a representative panel of employed species, e.g., 8h and 9h (MIC=62.5 μg mL^{-1}) compared to *N*-unsubstituted quinolone (R=H), e.g. 8f and 9f. In addition, 8d and 9d both having (R=OCH_3), lipophilic group R_1=COOCH($(CH_3)_2$), and R_2=H gave good result compare to 8b and 9b where R=H, R_1=COOCH($(CH_3)_2$), and R_2=H. Against *C. albicans*, compounds with electron donating group (R=OCH_3) 8d, 8h, 9d, and 9h increases the antifungal potency, but some exceptional compounds such as 9b and 9f have found a better antifungal activity where R=H. From these data, we can say that no. of tetrazole-containing compounds (9b, 9d, 9f, and 9h) have better antifungal activity compare with triazole containing compounds.

3.2. Anti-TB Activity

The screening results of the title compounds and the standard drugs are reported in Table 4. The compound 8h and 9h (R_1=COOCH ($(CH_3)_3$) and R_2=CF_3 of MIC=25 μg mL^{-1} displayed excellent activity against *M. TB* H_{37}Rv with 95% and 97% inhibition. This excellent activity could be credited to the combination effect of electron releasing OCH_3 and, electron withdrawing CF_3 groups, Also, compounds 8d and 9d with electron releasing OCH_3 at R position, R_1=COOCH (CH_3)$_3$ and R_2=H, substituent on *N*-aryl ring displayed moderate inhibition of 94% (MIC=50 μg mL^{-1}) and 96% with (MIC=50 μg mL^{-1}). Unfortunately, the majority of compounds showed poor inhibition of *M.* TB growth.

4. BIOLOGICAL EVALUATION

4.1. Antimicrobial Screening

The antimicrobial activity of synthesized compounds was carried out by broth microdilution method according to the "National Committee for Clinical

Table 4: *In vitro* anti-tuberculosis activity (% inhibition with MIC) of *N*-aryl biquinoline 8a-h and 9a-h *M*. TB H_{37}Rv (at concentration 6.25 μg ml^{-1}).

Compound	% Inhibition	MIC (μg/ml)	Compounds	% Inhibition	MIC (μg/ml)
8a	27	-	9a	20	-
8b	56	-	9b	65	-
8c	30	-	9c	52	-
8d	94	50	9d	96	50
8e	32	-	9e	63	-
8f	70	-	9f	74	-
8g	81	-	9g	68	-
8h	95	25	9h	97	25
Rifampicin	98	40	Isoniazid	99	0.20

MIC=Minimum inhibitory concentration, *M*. TB=*Mycobacterium tuberculosis*

Laboratory Standards" [32,33]. The antimicrobial screening data are shown in Table 3.

All the glass apparatus used were sterilized before use. Mueller-Hinton broth was used as a nutrient medium to grow and dilute the compound suspension for the test bacteria and sabouraud dextrose broth used for fungal nutrition. Inoculums size for test strain was adjusted to 10^8 colony forming unit per milliliter by comparing the turbidity. The strains used for the activity were procured from (microbial type culture collection [MTCC]) Institute of Microbial Technology, Chandigarh. Each synthesized compound was diluted obtaining 2000 µg mL^{-1} concentration, as a stock solution. The results are recorded in the form of primary and secondary screening. The compounds 8a-h and 9a-h were screened for their antibacterial activity against three Gram-negative (*V. cholerae* MTCC 3906, *E. coli* MTCC 443, and *S. typhi* MTCC 98) bacteria and three Gram-positive (*S. pneumoniae* MTCC 96, *C. tetani* MTCC 449, and *B. subtilis* MTCC 441) at concentrations of 1000, 500, and 250 µg mL^{-1} as primary screening. DMSO was used as a vehicle to get desired concentrations of compounds to test upon microbial strains. The compounds found to be active in the primary screening were further screened in a second set of dilution at concentrations of 200, 100, 62.5, and 50 µg mL^{-1}. 10 µl suspensions from each well were further inoculated, and growth was noted after 24 and 48 h. The lowest concentration, which showed no visible growth (turbidity) after spot subculture was considered as MIC for each compound. In this study, ampicillin and norfloxacin were used as standard antibacterial drugs, whereas griseofulvin was used as standard antifungal drugs. The protocols are summarized in Table 3.

4.2. Anti-TB Screening

Drug susceptibility and determination of anti-TB activity of the test compounds against *M.*TB $H_{37}Rv$ were performed by Lowenstein-Jensen slope method [33,34] with slight modification where 250 mg mL^{-1} dilution of each test compound were added liquid Lowenstein-Jensen medium, and then media were sterilized by inspissation method. A culture of *M.* TB $H_{37}Rv$ growing on Lowenstein-Jensen medium was harvested in 0.85% saline in bijou bottles. All test compound make solution of 250 mg mL^{-1} concentration of compounds was prepared in DMSO. These tubes were then incubated at 37°C for 24 h followed by streaking of *M.* TB $H_{37}Rv$ (5×10^4 bacilli per tube). These tubes were then incubated at 37°C. The growth of bacilli was seen after 12 days, 22 days, and finally 28 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with *M.* TB $H_{37}Rv$. The concentration at which no development of colonies occurred or <20 colonies was taken as MIC concentration of test compound. The screening results are summarized as % inhibition relative to standard drugs isoniazid and rifampicin.

5. CONCLUSION

Here, we have reported the synthesis of new quinoline based triazole-tetrazole-trifluoromethyl derivatives by conventional and ultrasound MCR system. But, using ultrasound protocol is more attractive compared to the conventional method in the concept of green techniques, save time, use less solvent, rapid, and automated synthesis with high atom economy of the target compounds. Also reviewing and comparing the activity data, with their spatial relationship and position changes. We identified the compound 8d, 8h, 9d, and 9h had found to be more efficient antimicrobial and anti-TB members.

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