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Synthesis of a novel class of some biquinoline pyridine hybrids via one-pot, three-component reaction and their antimicrobial activity

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Abstract. A small library of novel class of biquinoline containing pyridine moiety were synthesized by a one-pot cyclocondensation of 2-chloro-3-formyl quinoline, active methylene compounds and 3-(pyridine-3-ylamino)cyclohex-2-enone in the presence of catalytic amount of sodium hydroxide. The protocol offers rapid synthesis of structurally diverse novel class of some biquinoline pyridine hybrids for antimicrobial screening. These compounds were screened for their antibacterial activity against Gram-positive bacteria (*Bacillus subtilis*, *Clostridium tetani*, *Streptococcus pneumoniae*), Gram-negative bacteria (*Escherichia coli*, *Salmonella typhi*, *Vibrio cholerae*) and antifungal activity against *Aspergillus fumigatus*, *Candida albicans*. Some of the biquinoline compounds were found to be more potent or equipotent than the first line standard drugs. The compounds were evaluated for their *in vitro* antitubercular activity against *Mycobacterium tuberculosis* H37Rv strain using Lowenstein–Jensen medium. Compound **4g** showed a compelling activity at 6.25 μg/mL with a 96% inhibition and could be ideally suited for further modifications to obtain more efficacious compounds in the fight against tuberculosis.

Keywords. Quinoline; MIC; antimycobacterial; antimicrobial activity.

1. Introduction

Green chemistry emphasizes the development of environmentally benign chemical processes and technologies. Besides typical multi-step syntheses, an increasing number of organic chemical compounds are formed by multicomponent reactions (MCRs). MCRs often comply with the principles of green chemistry in terms of economy of steps as well as the many stringent criteria of an ideal organic synthesis. Multicomponent reactions offer greater possibilities for molecular diversity per step with a minimum of synthetic time, labour, cost, and waste production. The rapid assembly of molecular diversity utilizing multicomponent reactions has received a great deal of attention, most notably for the construction of heterocyclic 'drug-like' libraries.[1–3] These methods have significant utility, particularly, when they lead to the formation of privileged medicinal heterocyclic compounds.

Emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens still make the treatment of infectious diseases an important and pressing global problem. Therefore, a substantial research for the discovery and synthesis of new classes of antimicrobial agents is needed.[4,5]

Among the important pharmacophores responsible for antimicrobial activity, the quinoline scaffold is still considered a viable lead structure for the synthesis of more efficacious and broad spectrum antimicrobial agents. In the recent time, quinoline nucleus has gathered an immense attention among chemists as well as biologists as it is one of the key building elements for many naturally occurring compounds. The quinoline ring is endowed with various activities, such as antituberculosis,[6] antimalarial,[7] anti-inflammatory,[8] anticancer,[9] antimicrobial,[10] antihypertensive,[11] antioxidant,[12] tyrokinase PDGF-RTK inhibiting agents,[13] and antiHIV.[14] Amongst the various activities of their derivatives, antimicrobial activity is noteworthy.

The pyridine nucleus is prevalent in numerous natural products and is extremely important in chemistry of biological systems.[15] It plays a key role catalysing both biological and chemical systems. In many enzymes of living organisms it is the prosthetic pyridine nucleotide (NADP) that is involved in various oxidation–reduction processes. Other evidence of the potent activity of pyridine in biological systems is its presence in the important vitamins niacin and pyridoxine (vitamin B6) and also in highly toxic alkaloids such as nicotine.[16,17] The

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pyridine substructure is one of the most important heterocycles found in natural products, pharmaceuticals and functional materials.[18,19]

A thorough literature review reveals that more efficacious antibacterial compounds can be designed by joining two or more biologically active heterocyclic systems together in a single molecular framework.[20] In continuation of our efforts to develop new, green chemistry methods[21] as well as in continuation of our recent interest in the construction of heterocyclic scaffolds with antimicrobial activity[22–25] we report here the synthesis, antimicrobial evaluation of some novel structure hybrids incorporating both the quinoline moiety with pyridine. This combination was suggested in an attempt to investigate the influence of such hybridization and structure variation on the anticipated biological activities, hoping to add some synergistic biological significance to the target molecules.

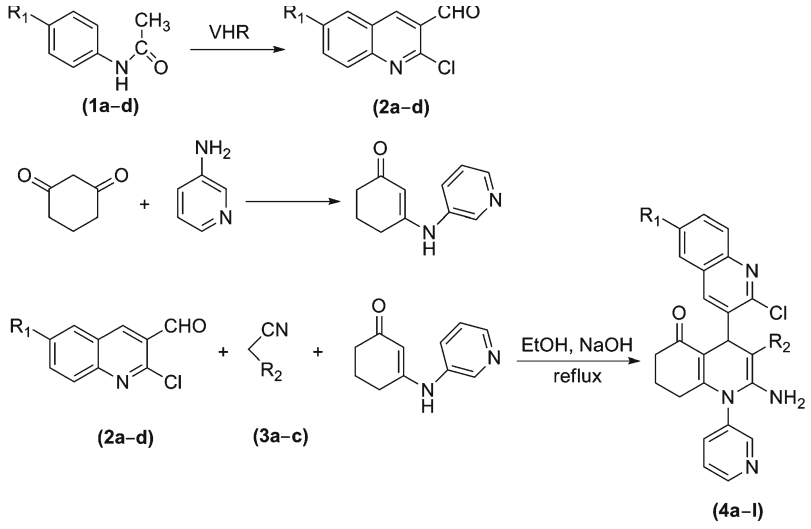
2. Experimental

2.1 *Materials and methods*

The reagents used in this work were obtained from Aldrich and were used without purification. All used solvents were of analytical grade. All melting points were taken in open capillaries and are uncorrected. Thin-layer chromatography (TLC, on aluminium plates precoated with silica gel, 60 F_{254}, 0.25 mm thickness) (Merck, Darmstadt, Germany) was used for monitoring the progress of all reactions. UV radiation and/or iodine were used as the visualizing agents. Elemental analysis (% C, H, N) was carried out by Perkin-Elmer 2400 series-II elemental analyzer (Perkin-Elmer, USA). The IR spectra were recorded in KBr on a Perkin-Elmer Spectrum GX FT-IR Spectrophotometer (Perkin-Elmer, USA) and only the characteristic peaks are reported in cm^{-1}. ^1H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Bruker Avance 400F (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using solvent peak as internal standard at 400 MHz and 100 MHz, respectively. Chemical shifts are reported in parts per million (ppm). Mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan).

2.2 *General procedure for the synthesis of 2-chloro-3-formyl quinolines (**2a–d**)*

The starting material, 2-chloro-3-formyl quinolines **2a–d** were prepared, according to literature procedure[26] by Vilsmeier–Haack reaction of acetanilide derivatives (**1a–d**) with phosphorus oxychloride in DMF (scheme 1).



Compd.	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	4k	4l
R_1	H	CH_3	OCH_3	Cl	H	CH_3	OCH_3	Cl	H	CH_3	OCH_3	Cl
R_2	CN	CN	CN	CN	COOEt	COOEt	COOEt	COOEt	COOMe	COOMe	COOMe	COOMe

Scheme 1. General synthetic route for the title compounds (**4a–l**). VHR: Vilsmeier-Haack Reaction.

2.3 *General procedure for the synthesis of 3-(pyridine-3-ylamino)cyclohex-2-enone*

1,3-Cyclohexanedione (1 mmol), 3-amino pyridine (1 mmol), methanol (15 mL) and 2 drops of acetic acid were charged in 100 mL round bottom flask equipped with refluxing condenser. The reaction mixture was slowly heated and refluxed for 1 h. On completion of reaction, monitored by TLC using 30% EtOAc in toluene as eluent, the reaction mixture was cooled to room temperature and the solid separated was filtered and washed with methanol to obtain the pure compound (scheme 1).

2.4 *General procedure for the synthesis of 2-amino-4-(2-chloro-6-(un)substituted (3-quinolyl))-5-oxo-1-pyridin-3-yl-1,4,6,7,8-pentahydro quinoline (**4a–l**)*

A mixture of 2-chloro-3-formyl quinoline (5 mmol) **(2a–d)**, malononitrile or ethyl cyanoacetate or methyl cyanoacetate (5 mmol) **(3a–c)** and 3-(pyridine-3-ylamino)cyclohex-2-enone (5 mmol) in ethanol (10 mL) containing NaOH (1 mmol) was heated under reflux for 2–3 h. On completion of reaction, monitored by TLC (ethyl acetate:hexane::3:7), the reaction mixture was cooled to room temperature and the solid separated was filtered, washed with ethanol, dried, and recrystallized to give the desired product. Analytical and spectroscopic characterization data of the synthesized compounds **(4a–l)** are given below.

2.4a *2-Amino-4-(2-chloro(3-quinolyl))-5-oxo-1-pyridin-3-yl-1,4,6,7,8-pentahydro quinoline-3-carbonitrile (**4a**)*: Yield, 84%; mp.: 242–244°C IR (KBr, ν, cm^{-1}): 3440 and 3160 (asym. and sym. stretching of NH_2), 2185 (C≡N stretching), 1665 (C=O stretching); ^{1}H NMR (400 MHz, DMSO-d_6): δ_H (ppm): 1.76–2.29 (m, 6H, 3×CH_2), 5.22 (s, 1H, quinoline H4), 5.63 (s, 2H, NH_2), 7.66–8.62 (m, 9H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm) 21.2, 28.7 (2C, CH_2), 34.6 (C4), 36.2 ($\underline{C}H_2$-CO), 59.5 ($\underline{C}$-CN), 112.3, 120.8, 125.6, 127.4, 128.5, 130.1, 131.2, 132.0, 136.2, 138.5, 140.1, 142.2, 144.6, 148.7, 150.1, 151.3, 153.5, 155.4 (18C, Ar-C), 195.4 (C=O); Anal. Calcd. for $C_{24}H_{18}ClN_5O$ (427.89 g/mol): C, 67.37; H, 4.24 ; N, 16.37. Found: C, 67.49; H, 4.15; N, 16.21.

2.4b *2-Amino-4-(2-chloro-6-methyl(3-quinolyl))-5-oxo-1-pyridin-3-yl-1,4,6,7,8-pentahydro quinoline-3-carbonitrile (**4b**)*: Yield 82%; mp.: 266–268°C IR (KBr, ν, cm^{-1}) 3435 and 3155 (asym. and sym. stretching of NH_2), 2185 (C≡N stretching), 1660 (C=O stretching); ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 1.74–2.27 (m, 6H, 3×CH_2), 2.45 (s, 3H, Ar-CH_3), 5.20 (s, 1H, quinoline H4), 5.67 (s, 2H, NH_2), 7.59–8.48 (m, 8H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 21.28 (CH_2), 21.54 (Ar-CH_3), 28.74 (CH_2), 34.67 (C4), 36.36 ($\underline{C}H_2$-CO), 59.62 ($\underline{C}$-CN), 112.5, 120.7, 123.5, 124.8, 127.8, 128.3, 130.2, 131.2, 133.3, 136.4, 138.4, 140.0, 142.6, 148.21, 150.3, 152.6, 153.2, 155.5 (18C, Ar-C), 195.5 (C=O); MS: m/z = 442.6 $[M+H]^+$; Anal. Calcd. for $C_{25}H_{20}ClN_5O$ (441.91 g/mol): C, 67.95; H, 4.56; N, 15.85. Found: C, 67.67; H, 4.63; N, 15.92.

2.4c *2-Amino-4-(2-chloro-6-methoxy(3-quinolyl))-5-oxo-1-pyridin-3-yl-1,4,6,7,8-pentahydro quinoline-3-carbonitrile (**4c**)*: Yield 78%; mp.: 218–220°C IR (KBr, ν, cm^{-1}) 3440 and 3150 (asym. and sym. stretching of NH_2), 2200 (C≡N stretching), 1665 (C=O stretching); ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 1.80–2.28 (m, 6H, 3×CH_2), 3.89 (s, 3H, Ar-OCH_3), 5.11 (s, 1H, quinoline H4), 5.59 (s, 2H, NH_2), 7.60–8.71 (m, 8H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 21.3, 28.6 (2C, CH_2), 34.6 (C4), 36.2 ($\underline{C}H_2$-CO), 55.8 (Ar-OCH_3), 59.4 ($\underline{C}$-CN), 105.3, 112.8, 120.8, 122.3, 124.6, 125.1, 128.6, 130.2, 132.4, 134.8, 136.1, 138.9, 140.2, 146.5, 148.3, 150.0, 153.3, 155.2 (18C, Ar-C), 195.5 (C=O); Anal. Calcd. for $C_{25}H_{20}ClN_5O_2$ (457.91 g/mol): C, 65.57; H, 4.40; N, 15.29. Found: C, 65.43; H, 4.52; N, 15.39.

2.4d *2-Amino-4-(2,6-dichloro(3-quinolyl))-5-oxo-1-pyridin-3-yl-1,4,6,7,8-pentahydro quinoline-3-carbonitrile (**4d**)*: Yield 86%; mp.: 272–274°C IR (KBr, ν, cm^{-1}) 3435 and 3150 (asym. and sym. stretching of NH_2), 2180 (C≡N stretching), 1665 (C=O stretching); ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 1.83–2.27 (m, 6H, 3×CH_2), 5.13 (s, 1H, quinoline H4), 5.69 (s, 2H, NH_2), 7.62–8.74 (m, 8H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 21.2, 28.6 (2C, CH_2), 34.6 (C4), 36.2 ($\underline{C}H_2$-CO), 59.5 ($\underline{C}$-CN), 112.1, 121.2, 125.1, 127.2, 129.0, 129.9, 131.2, 131.8, 133.5, 137.5, 138.8, 140.2, 144.6, 150.4, 150.9, 151.3, 151.8, 154.1 (18C, Ar-C), 195.4 (C=O); MS: m/z = 462.1 $[M+H]^+$; Anal. Calcd. for $C_{24}H_{17}Cl_2N_5O$ (462.33 g/mol): C, 62.35; H, 3.71; N, 15.15. Found: C, 62.47; H, 3.61; N, 15.08.

2.4e *Ethyl 2-amino-4-(2-chloro(3-quinolyl))-5-oxo-1-pyridin-3-yl-1,4,6,7,8-pentahydro quinoline-3-carboxylate (**4e**)*: Yield 81%; mp.: 227–229°C IR (KBr, ν, cm^{-1}) 3370 and 3180 (asym. and sym. stretching of NH_2), 1660 (C=O stretching), 1635 (C=O stretching); ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 0.98 (t, 3H, CH_3), 1.60–2.18 (m, 6H, 3×CH_2), 3.93 (q, 2H, OCH_2), 5.34 (s, 1H, quinoline H4), 7.11 (s, 2H, NH_2), 7.57–8.78 (m, 9H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 14.7 (CH_3), 21.1, 28.7 (2C, CH_2), 35.2 (C4), 36.4 ($\underline{C}H_2$-CO), 58.9 (OCH_2), 78.0 ($\underline{C}$-COOEt), 113.6, 125.3, 126.6, 127.1, 127.1, 128.2, 130.3, 133.5, 138.8, 139.7, 140.5, 145.8, 150.7, 151.0, 151.3, 153.0, 153.3 (17C, Ar-C), 169.3 ($\underline{C}$OO), 195.5 (C=O); Anal. Calcd. for $C_{26}H_{23}ClN_4O_3$ (474.94 g/mol): C, 65.75; H, 4.88; N, 11.80. Found: C, 65.84; H, 4.72; N, 12.05.

2.4f *Ethyl 2-amino-4-(2-chloro-6-methyl(3-quinolyl))-5-oxo-1-pyridin-3-yl-1,4,6,7,8-pentahydro quinoline-3-carboxylate (**4f**)*: Yield 77%; mp.: 254–256°C IR (KBr, ν, cm^{-1}) 3365 and 3185 (asym. and sym. stretching of NH_2), 1660 (C=O stretching), 1640 (C=O stretching); ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 0.97 (t, 3H, CH_3), 1.58–2.16 (m, 6H, 3×CH_2), 2.47 (s, 3H, Ar-CH_3), 3.95 (q, 2H, OCH_2), 5.35 (s, 1H, quinoline H4), 7.10 (s, 2H, NH_2), 7.52–8.72 (m, 8H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 14.7 (CH_3), 21.1 (CH_2), 21.5 (Ar-CH_3), 28.7 (CH_2), 35.2 (C4), 36.4 ($\underline{C}H_2$-CO), 58.9 (OCH_2), 78.0 ($\underline{C}$-COOEt), 113.5, 124.2, 125.8, 126.2, 127.3, 128.5, 130.1, 132.6, 138.5, 139.2, 142.3, 145.8, 149.4, 150.6, 151.2, 153.0, 153.8 (17C, Ar-C), 169.3 ($\underline{C}$OO), 195.5 (C=O); Anal. Calcd. for $C_{27}H_{25}ClN_4O_3$ (488.97 g/mol): C, 66.32; H, 5.15; N, 11.46. Found: C, 66.54; H, 5.27; N, 11.30.

2.4g *Ethyl 2-amino-4-(2-chloro-6-methoxy(3-quinolyl))-5-oxo-1-pyridin-3-yl-1,4,6,7,8-pentahydro quinoline-3-carboxylate (**4g**)*: Yield 78%; mp.: 246–248°C IR (KBr, ν, cm^{-1}) 3370 and 3190 (asym. and sym. stretching of NH_2), 1665 (C=O stretching), 1640 (C=O stretching); ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 0.97 (t, 3H, CH_3), 1.61–2.16 (m, 6H, 3×CH_2), 3.88 (s, 3H, Ar-OCH_3), 3.93 (q, 2H, OCH_2), 5.32 (s, 1H, quinoline H4), 7.12 (s, 2H, NH_2), 7.58–8.73 (m, 8H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 14.7 (CH_3), 21.2, 28.7 (2C, CH_2), 35.2 (C4), 36.5 ($\underline{C}H_2$-CO), 55.9 (Ar-OCH_3), 59.0 (OCH_2), 77.9 ($\underline{C}$-COOEt), 105.3, 113.1, 124.1, 125.3, 127.4, 128.8, 130.2, 132.5, 137.4, 138.1, 142.3, 144.5, 148.7, 150.6, 151.4, 153.0, 153.8 (17C, Ar-C), 169.4 ($\underline{C}$OO), 195.6 (C=O); Anal. Calcd. for $C_{27}H_{25}ClN_4O_4$ (504.96 g/mol): C, 64.22; H, 4.99; N, 11.10. Found: C, 64.34; H, 5.23; N, 11.18.

2.4h *Ethyl 2-amino-4-(2,6-dichloro(3-quinolyl))-5-oxo-1-pyridin-3-yl-1,4,6,7,8-pentahydro quinoline-3-carboxylate (**4h**)*: Yield 82%; mp.: 273–275°C IR (KBr, ν, cm^{-1}) 3360 and 3185 (asym. and sym. stretching of NH_2), 1665 (C=O stretching), 1640 (C=O stretching); ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 0.97 (t, 3H, CH_3), 1.62–2.20 (m, 6H, 3×CH_2), 3.91 (q, 2H, OCH_2), 5.38 (s, 1H, quinoline H4), 7.08 (s, 2H, NH_2), 7.60–8.75 (m, 8H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 14.7 (CH_3), 21.1, 28.7 (2C, CH_2), 35.1 (C4), 36.5 ($\underline{C}H_2$-CO), 58.9 (OCH_2), 78.0 ($\underline{C}$-COOEt), 113.5, 122.2, 125.5, 126.8, 129.1, 129.8, 131.4, 132.0, 133.1, 137.1, 138.9, 142.3, 144.6, 151.0, 152.5, 152.8, 153.2 (17C, Ar-C), 169.5 ($\underline{C}$OO), 195.5 (C=O); MS: m/z = 508.6 $[M+H]^+$; Anal. Calcd. for $C_{26}H_{22}Cl_2N_4O_4$ (509.38 g/mol): C, 61.31; H, 4.35; N, 11.00. Found: C, 61.11; H, 4.16; N, 11.21.

2.4i *Methyl 2-amino-4-(2-chloro(3-quinolyl))-5-oxo-1-pyridin-3-yl-1,4,6,7,8-pentahydro quinoline-3-carboxylate (**4i**)*: Yield 75%; mp.: 222–224°C IR (KBr, ν, cm^{-1}) 3350 and 3180 (asym. and sym. stretching of NH_2), 1660 (C=O stretching), 1640 (C=O stretching); ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 1.64–2.25 (m, 6H, 3×CH_2), 3.82 (s, 3H, OCH_3), 5.28 (s, 1H, quinoline H4), 7.14 (s, 2H, NH_2), 7.51–8.71 (m, 9H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 21.2, 28.7 (2C, CH_2), 35.3 (C4), 36.5 ($\underline{C}H_2$-CO), 52.8 (OCH_3), 78.4 ($\underline{C}$-$COOCH_3$), 113.7, 122.3, 125.6, 126.1, 127.0, 128.2, 128.9, 130.1, 132.4, 136.5, 138.3, 142.7, 146.3, 150.2, 151.5, 153.1, 153.6 (17C, Ar-C), 169.6 ($\underline{C}$OO), 195.5 (C=O); Anal. Calcd. for $C_{25}H_{21}ClN_4O_3$ (460.91 g/mol): C, 65.15; H, 4.59; N, 12.16. Found: C, 65.33; H, 4.40; N, 12.29.

2.4j *Methyl 2-amino-4-(2-chloro-6-methyl(3-quinolyl))-5-oxo-1-pyridin-3-yl-1,4,6,7,8-pentahydro quinoline-3-carboxylate (**4j**)*: Yield 74%; mp.: 262–264°C IR (KBr, ν, cm^{-1}) 3350 and 3190 (asym. and sym. stretching of NH_2), 1670 (C=O stretching), 1640 (C=O stretching); ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 1.65–2.22 (m, 6H, 3×CH_2), 2.47 (s, 3H, Ar-CH_3), 3.84 (s, 3H, OCH_3), 5.28 (s, 1H, quinoline H4), 7.11 (s, 2H, NH_2), 7.56–8.64 (m, 8H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 21.2 (CH_2), 21.5 (Ar-CH_3) 28.7 (CH_2), 35.2 (C4), 36.5 ($\underline{C}H_2$-CO), 52.8 (OCH_3), 78.5 ($\underline{C}$-$COOCH_3$), 113.6, 122.3,

124.3, 126.1, 127.0, 128.6, 130.3, 132.2, 136.5, 137.7, 142.0, 144.3, 147.4, 150.2, 151.2, 151.7, 153.8 (17C, Ar-C), 169.5 (COO), 195.5 (C=O); Anal. Calcd. for $C_{26}H_{23}ClN_4O_3$ (474.94 g/mol): C, 65.75; H, 4.88; N, 11.80. Found: C, 65.85; H, 4.98; N, 11.66.

2.4k *Methyl 2-amino-4-(2-chloro-6-methoxy(3-quinolyl))-5-oxo-1-pyridin-3-yl-1,4,6,7,8-pentahydro quinoline-3-carboxylate (**4k**)*: Yield 76%; mp.: 232–234°C IR (KBr, ν, cm^{-1}) 3365 and 3180 (asym. and sym. stretching of NH_2), 1660 (C=O stretching), 1635 (C=O stretching); ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 1.63–2.24 (m, 6H, 3×CH_2), 3.83 (s, 3H, OCH_3), 3.87 (s, 3H, Ar-OCH_3), 5.24 (s, 1H, quinoline H4), 7.04 (s, 2H, NH_2), 7.62–8.76 (m, 8H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 21.3, 28.7 (2C, CH_2), 35.4 (C4), 36.5 ($\underline{C}H_2$-CO), 52.8 (OCH_3), 55.9 (Ar-OCH_3), 78.2 ($\underline{C}$-$COOCH_3$), 105.2, 113.6, 124.5, 125.3, 126.6, 127.5, 130.2, 132.4, 136.5, 138.1, 142.2, 144.8, 150.1, 151.4, 152.6, 153.4, 154.4 (17C, Ar-C), 169.5 (COO), 195.6 (C=O); Anal. Calcd. for $C_{26}H_{23}ClN_4O_4$ (490.94 g/mol): C, 63.61; H, 4.72; N, 11.41. Found: C, 63.82; H, 4.81; N, 11.24.

2.4l *Methyl 2-amino-4-(2,6-dichloro(3-quinolyl))-5-oxo-1-pyridin-3-yl-1,4,6,7,8-pentahydro quinoline-3-carboxylate (**4l**)*: Yield 83%; mp.: 259–261°C IR (KBr, ν, cm^{-1}) 3360 and 3180 (asym. and sym. stretching of NH_2), 1665 (C=O stretching), 1645 (C=O stretching); ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 1.64–2.26 (m, 6H, 3×CH_2), 3.83 (s, 3H, OCH_3), 5.22 (s, 1H, quinoline H4), 7.05 (s, 2H, NH_2), 7.60–8.71 (m, 8H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 21.3, 28.7 (2C, CH_2), 35.2 (C4), 36.4 ($\underline{C}H_2$-CO), 52.8 (OCH_3), 78.1 ($\underline{C}$-$COOCH_3$), 112.8, 122.5, 124.2, 126.4, 127.9, 130.7, 132.1, 134.4, 136.3, 138.5, 140.0, 142.2, 145.6, 150.1, 151.6, 152.2, 154.6 (17C, Ar-C), 169.5 (COO), 195.6 (C=O); Anal. Calcd. for $C_{25}H_{20}Cl_2N_4O_3$ (495.36 g/mol): C, 60.62; H, 4.07; N, 11.31. Found: C, 60.39; H, 4.25; N, 11.15.

2.5 *Antimicrobial activity*

All the glass apparatus used were sterilized before use. The MICs of all the synthesized compounds was carried out by broth microdilution method.[27] Mueller Hinton broth was used as nutrient medium to grow and dilute the compound suspension for the test bacteria and Sabouraud Dextrose broth was used for fungal nutrition. Inoculum size for test strain was adjusted to 10^8 CFU [Colony Forming Unit] per milliliter by comparing the turbidity. The strains used for the activity were procured from [MTCC – Microbial Type Culture Collection] Institute of Microbial Technology, Chandigarh. Dimethyl sulfoxide (DMSO) was used as diluent to get desired concentration of drugs to test on standard bacterial strains. Serial dilutions were prepared in primary and secondary screening. The control tube containing no antibiotic was immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37°C overnight. The tubes were then incubated overnight. The MIC of the control organism was read to check the accuracy of the drug concentrations. The lowest concentration inhibiting growth of the organism was recorded as the MIC. All the tubes not showing visible growth (in the same manner as control tube described above) was subcultured and incubated overnight at 37°C. The amount of growth from the control tube before incubation (which represents the original inoculum) was compared.

Subcultures might show similar number of colonies indicating bacteriostatic; a reduced number of colonies indicating a partial or slow bactericidal activity and no growth if the whole inoculum has been killed. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity. Each synthesized drug was diluted to 2000 µg/mL concentration, as a stock solution. In primary screening 500, 250, 200 and 125 µg/mL concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. The drugs found active in primary screening were similarly diluted to obtain 200, 100, 50, 25, 12.5, 6.250, 3.125, and 1.5625 µg/mL concentrations. The highest dilution showing at least 99% inhibition is taken as MIC. The protocols were summarized in table 1.

2.6 *Antimycobacterial activity*

A primary screen was conducted at 6.25 µg/mL against *M. tuberculosis* H37Rv by Lowenstein–Jensen (LJ) MIC method,[28] where primary 6.25 µg/mL dilution of each test compound were added to liquid Lowenstein–Jensen medium and then media were sterilized by inspissation method. A culture of *M. tuberculosis* H37Rv growing on Lowenstein–Jensen medium was harvested in 0.85% saline in bijou bottles. DMSO was used as vehicle to get desired concentration. These

Table 1. Antimicrobial activity of the compounds (**4a–l**).

Compds	Minimum inhibitory concentration (MIC, μg/mL)							
	Gram-positive bacteria			Gram-negative bacteria			Fungi	
	S.P. MTCC 1936	C.T. MTCC 449	B.S. MTCC 441	S.T. MTCC 98	V.C. MTCC 3906	E.C. MTCC 443	A.F. MTCC 3008	C.A. MTCC 227
4a	500	500	**125**	200	250	250	>1000	**500**
4b	500	500	**250**	125	500	**62.5**	>1000	1000
4c	250	**250**	**250**	200	200	125	>1000	1000
4d	125	**200**	**200**	200	200	200	500	**250**
4e	200	**250**	**125**	250	125	**100**	500	**250**
4f	250	**250**	500	**100**	200	250	500	1000
4g	200	500	500	**62.5**	250	250	500	>1000
4h	**100**	**250**	**250**	250	200	250	500	>1000
4i	125	**200**	**250**	200	200	**62.5**	1000	**250**
4j	200	**100**	**200**	**100**	250	**100**	>1000	**500**
4k	200	**250**	**100**	**100**	500	125	>1000	**500**
4l	250	**200**	**200**	250	200	200	500	1000
Ampicillin	100	250	250	100	100	100	–	–
Chloramphencol	50	50	50	50	50	50	–	–
Ciprofloxacin	50	100	50	25	25	25	–	–
Norfloxacin	10	50	100	10	10	10	–	–
Griseofulvin	–	–	–	–	–	–	100	500
Nystatin	–	–	–	–	–	–	100	100

B.S., *Bacillus subtilis*; C.T., *Clostridium tetani*; S.P., *Streptococcus pneumoniae*; E.C., *Escherichia coli*; S.T., *Salmonella typhi*; V.C., *Vibrio cholerae*; A.F., *Aspergillus fumigatus*; C.A., *Candida albicans*
Bold entries = the compounds are found equipotent or more potent compared to the standard drugs used

tubes were then incubated at 37°C for 24 h followed by streaking of *M. tuberculosis* H37Rv (5 × 104 bacilli per tube). These tubes were then incubated at 37°C. Growth of bacilli was seen after 12, 22, and finally 28 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis* H37Rv. The concentration at which complete inhibition of colonies occurred was taken as active concentration of test compound. The standard strain *M. tuberculosis* H37Rv was tested with known drug Isoniazide and Rifampicin. The screening results are summarized as % inhibition relative to standard drug Isoniazide and Rifampicin. Compounds effecting <90% inhibition in the primary screen were not evaluated further. Compounds demonstrating at least 90% inhibition in the primary screen were re-tested at lower concentration (MIC) in a Lowenstein–Jensen medium. The protocols were summarized in tables 2 and 3.

Table 2. Antimycobacterial activity of the compounds (**4a–l**).

Compd.	Primary screen (6.25 μg/mL) % inhibition
4a	12
4b	20
4c	65
4d	14
4e	8
4f	74
4g	96
4h	52
4i	90
4j	25
4k	25
4l	84
Isoniazide	99
Rifampicin	98

Table 3. MIC of compounds against *M. tuberculosis* H37Rv.

Compound	MIC μg/mL
4g	12.5
4i	100
Isoniazide	0.2
Rifampicin	40

3. Results and discussion

3.1 *Chemistry*

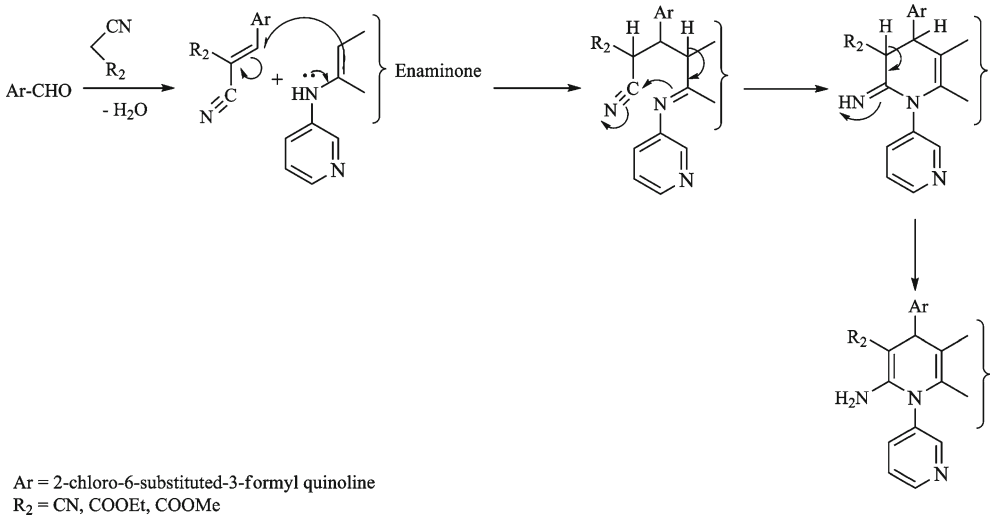
The synthesis of the target compounds is outlined in scheme 1. The core intermediate 2-chloro-3-formyl quinolines (**2a–d**) were prepared according to literature procedure[26] by Vilsmeier–Haack reaction. The required β-enaminone i.e., 3-(pyridine-3-ylamino)cyclohex-2-enone was prepared as described above by reaction of 1,3-cyclohexanedione and 3-amino pyridine. Subsequently, the one-pot three component cyclocondensation of a series of 2-chloro-3-formyl quinolines (**2a–d**), active methylene compounds (**3a–c**) and 3-(pyridine-3-ylamino)cyclohex-2-enone in ethanol containing NaOH afforded the target compounds (**4a–l**) in good to excellent yields.

To choose the most appropriate medium in this heterocyclization reaction, the reaction of 2-chloro-3-formyl quinolines (**2a–d**), malononitrile or ethyl cyanoacetate or methyl cyanoacetate (**3a–c**) and 3-(pyridine-3-ylamino)cyclohex-2-enone various reaction conditions were investigated. To search for the optimal reaction solvent, the reaction was examined in ethylene glycol, DMF, HOAc, THF, and ethanol as solvent under reflux, respectively. The reaction in ethanol resulted in higher yields with shorter reaction time compared to others. So ethanol was chosen as the appropriate solvent. Moreover, to further improve the reaction yields, different bases like NaOH, K_2CO_3, DMAP, Et_3N, and piperidine were examined in ethanol. The base NaOH afforded the target product **4a** with 84% yield. Therefore, NaOH was chosen as the most suitable base for all further reactions.

A possible mechanism for the reaction is outlined in scheme 2. The reaction occurs via an *in situ* initial formation of the heterylidenenitrile, containing the electron-poor C=C double bond, from the Knoevenagel condensation between 2-chloro-3-formyl quinolines (**2a–d**) and active methylene compounds (**3a–c**) by loss of water molecules. Michael addition of β-enaminone to the ylidenic bond in forming an acyclic intermediate which cyclizes by nucleophilic attack of the NH group on the cyano carbon, followed by tautomerisation to the final products (**4a–l**).

3.2 *Spectroscopic analysis*

The structures of newly synthesized compounds were elucidated by combined use of IR, ^{1}H and ^{13}C NMR, mass spectral data and elemental analysis. The absorption bands for compounds (**4a–d**) in IR-spectra were observed in the range of 2180–2200 cm^{-1} corresponding to C≡N. The NH_2 stretching and C=O stretching vibrations for all the compounds were observed in range of 3150–3440 cm^{-1} and 1635–1670 cm^{-1}, respectively. The ^{1}H NMR spectrum of compounds (**4a–l**) indicated the presence of one singlet in the range δ 5.11–5.38 ppm of C4H proton. Moreover, the ^{1}H NMR spectrum of all the compounds showed broad singlet in the range of δ 5.59–7.14 ppm due to the NH_2 protons. In the ^{13}C NMR spectra of (**4a–l**), the signals assigned to C4 δ 34.61–35.42 ppm and to the carbonyl group δ 195.45–195.65 were the most relevant features. The signal at around δ 59.48–59.62 ppm is assigned to carbon attached with carbonitrile in compounds (**4a–d**) while signals around δ 77.93–78.58 ppm



Ar = 2-chloro-6-substituted-3-formyl quinoline
R_2 = CN, COOEt, COOMe

Scheme 2. Plausible mechanistic pathway for the synthesis of biquinoline derivatives (**4a–l**).

is assigned to carbon attached with carboxylate in compounds (**4e–l**). The obtained elemental analysis values are in good agreement with theoretical data. Mass spectra of the title compounds gave $[M+H]^+$ peaks in agreement with their exact mass or molecular weight.

3.3 *Antimicrobial activity*

Reviewing of the antibacterial activities of biquinoline derivatives (table 1) indicate that compound **4b** (R_1 = CH_3, R_2 = CN), **4i** (R_1 = H, R_2 = COOMe) and **4g** (R_1 = OCH_3, R_2 = COOEt) showed highest activity (MIC = 62.5 μg/mL) against *E. coli* and *S. typhi*, respectively. So these compounds were found more potent than ampicillin (MIC = 100 μg/mL). Moreover, compounds **4e** (R_1 = H, R_2 = COOEt) and **4j** (R_1 = CH_3, R_2 = COOMe) (MIC = 100 μg/mL) were found equipotent to ampicillin (MIC = 100 μg/mL) towards *E. coli*. Towards the Gram-negative strain *S. typhi*, compounds **4f** (R_1 = CH_3, R_2 = COOEt), **4j** (R_1 = CH_3, R_2 = COOMe), and **4k** (R_1 = OCH_3, R_2 = COOMe) (MIC = 100 μg/mL) were equally active when compared to ampicillin (MIC = 100 μg/mL). Against *S. pneumoniae*, only one compound **4h** (R_1 = Cl, R_2 = COOEt) (MIC = 100 μg/mL) was found to have same activity as ampicillin (MIC = 100 μg/mL). Towards the Gram-positive strain *B. subtilis,* compounds **4a** (R_1 = H, R_2 = CN), **4d** (R_1 = Cl, R_2 = CN), **4e** (R_1 = H, R_2 = COOEt), **4j** (R_1 = CH_3, R_2 = COOMe), **4k** (R_1 = OCH_3, R_2 = COOMe) and **4l** (R_1 = Cl, R_2 = COOMe) (MIC < 250 μg/mL) possessed pronounced activity compared to ampicillin (MIC = 250 μg/mL) where as compounds **4b** (R_1 = CH_3, R_2 = CN), **4c** (R_1 = OCH_3, R_2 = CN), **4h** (R_1 = Cl, R_2 = COOEt) and **4i** (R_1 = H, R_2 = COOMe) were found to have equal activity as ampicillin (MIC = 250 μg/mL). Compound **4k** (R_1 = OCH_3, R_2 = COOMe) (MIC = 100 μg/mL) was also found equipotent to norfloxacin (MIC = 100 μg/mL) towards *B. subtilis*. Compounds **4d** (R_1 = Cl, R_2 = CN), **4i** (R_1 = H, R_2 = COOMe), **4j** (R_1 = CH_3, R_2 = COOMe) and **4l** (R_1 = Cl, R_2 = COOMe) (MIC < 250 μg/mL) displayed significant activity towards *C. tetani* compared to the standard ampicillin (MIC = 250 μg/mL). Compound **4j** (R_1 = CH_3, R_2 = COOMe) (MIC = 100 μg/mL) was also equally active as ciprofloxacin (MIC = 100 μg/mL) towards *C. tetani*. Against *C. tetani*, compounds **4c** (R_1 = OCH_3, R_2 = CN), **4e** (R_1 = H, R_2 = COOEt), **4f** (R_1 = CH_3, R_2 = COOEt), **4h** (R_1 = Cl, R_2 = COOEt) and **4k** (R_1 = OCH_3, R_2 = COOMe) (MIC = 250 μg/mL) exhibited comparable activity as the standard ampicillin (MIC = 250 μg/mL). None of the compounds was found sufficiently potent to inhibit *V. cholerae*. The remaining compounds showed moderate activity against other bacteria when compared with the remaining standard drugs. The data indicate that a change in the substituent might also affect the antibacterial activity of title compounds **4a–l**. Comparison of biological activities among **4a–l** shows functional groups as R_1 = CH_3/OCH_3 to be potentially more active against *S. typhi*. Also antibacterial potency of compounds among **4a–l** shows functional groups as R_1 = H/CH_3 found more active against *E. coli*.

Antifungal study revealed that all the synthesized biquinoline derivatives have poor activity against *A. fumigatus*. In comparison with standard fungicidal griseofulvin (MIC = 500 μg/mL), among biquinolines, compounds **4d** (R_1 = Cl, R_2 = CN), **4e** (R_1 = H, R_2 = COOEt) and **4i** (R_1 = H, R_2 = COOMe) (MIC = 250 μg/mL) exhibited excellent activity against *C. albicans* where as compounds **4a** (R_1 = H, R_2 = CN), **4j** (R_1 = CH_3, R_2 = COOMe) and **4k** (R_1 = OCH_3, R_2 = COOMe) showed comparable activity (MIC = 500 μg/mL). The data indicate that functional groups as R_1 = H interferes in the antifungal potency of title compounds (**4a–l**). Other compounds showed poor activity against the rest of the fungal species compared with the standard drugs nystatin and griseofulvin.

3.4 *Antimycobacterial activity*

The encouraging results from the antimicrobial studies prompted us to go for the preliminary screening of the title compounds for their *in vitro* antituberculosis activity against *M. tuberculosis* H37Rv.

Of the entire biquinoline derivatives compound, **4g** (R_1 = OCH_3, R_2 = COOEt) was the most active compound, with 96% inhibition. Compound **4i** (R_1 = H, R_2 = COOMe) also exhibited good inhibition of 90% with MIC = 100 μg/mL. Compound **4l** (R_1 = Cl, R_2 = COOMe) displayed moderate inhibition of 84%. Thus, the most potent compound of the series, compound **4g** (MIC = 12.5 μg/mL) opens up new door to optimize this series for new class of antituberculars.

4. Conclusion

For the first time, a series of novel biquinoline derivatives containing a pyridine moiety have been synthesized via one-pot, three-component reaction catalysed by non-hazardous NaOH and determined their antimicrobial and antimycobacterial activities. The one-pot

nature, the use of an ecocompatible catalytic reaction and the easy separation of products make it an interesting alternative to the reported approaches for the synthesis of such potent bio-active compounds. The use of an inorganic catalyst NaOH, not only gave good yields in shorter reaction time but also provided a procedure that does not use corrosive and hazardous organic bases like piperidine. The antimicrobial results revealed that numbers of compounds were found to be the most active against *C. tetani* and *B. subtilis* compared to rest of the employed species. In all the biquinolines synthesized and screened for antimicrobial activity compounds, **4b** and **4i** showed better inhibitory effects for *E. coli* and **4g** showed better results for *S. typhi*. Antifungal activity of the compounds shows that compounds **4d**, **4e** and **4i** are found to be potent against *C. albicans*. Moreover, compound **4g** have shown a great potential to serve as promising candidate for further development of antimycobacterial agents with improved potency. This suggests that hybrid compounds possessing biquinoline and pyridine moiety may have presented greater antimicrobial and antimycobacterial properties. These results suggested that further development of such compounds may be of interest.

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