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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and in vitro antimicrobial evaluation of penta-substituted pyridine derivatives bearing the quinoline nucleus

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Abstract A new series of penta-substituted pyridine derivatives bearing the quinoline nucleus have been synthesized by base-catalyzed cyclocondensation reaction through multi-component reaction (MCR) approach. All the synthesized compounds were subjected to in vitro antimicrobial screening against three Gram positive bacteria (*Bacillus subtilis*, *Clostridium tetani*, *Streptococcus pneumoniae*), three Gram negative bacteria (*Escherichia coli*, *Salmonella typhi*, *Vibrio cholerae*), and two fungi (*A. fumigatus*, *Candida albicans*). Reviewing the data, majority of the compounds were found to be active against *B. subtilis* and *C. tetani* while the most effective compounds **4c** and **4e** demonstrated MIC 62.5 μg/ml against *V. cholerae* and *E. coli* respectively.

Keywords Pyridine · Quinoline · One-pot reaction · Antimicrobial activity

Introduction

Many naturally occurring and synthetic compounds bearing pyridine scaffold possess interesting biological properties (Temple *et al.*, 1992). Among them, 2-amino-3-cyanopyridine derivatives are well known for their versatile biological activities like antimicrobial (Mahmoud *et al.*, 2007; Mungra *et al.*, 2009), antifungal (Costa *et al.*, 2008), isoniazid derivatives bearing the pyridine nucleus possess anti-tubercular (Ashrafali *et al.*, 2010; Lourenco *et al.*, 2007) and some of 3-cyano-2-pyridone derivatives having anti-inflammatory activity (Amr and Abdulla, 2006). Moreover, the polysubstituted pyridine represents molecular framework that serve as a platform for development of pharmaceutical agents. Of the pharmacologically active pyridine derivatives, pyridine-3,5-dicarbonitriles have attracted much interest in recent years because of their significant synthetic as well as medicinal utility. Thus, the synthesis of highly functionalized pyridine derivatives has become an active area of research (Anabha *et al.*, 2007; Fletcher *et al.*, 2006; Movassaghi and Hill, 2006; Shinde *et al.*, 2010; Sridhar *et al.*, 2009).

On the other hand, quinoline derivatives are possessing high activity profile due to their wide range of useful biological properties including antibacterial (Kalluraya *et al.*, 2008), antifungal (Rana *et al.*, 2008), antimycobacterial (Mital *et al.*, 2006), antimalarial (Charris *et al.*, 2005; Dave *et al.*, 2009), anti-inflammatory (Bava and Kumar 2009) and anticancer activity (Shi *et al.*, 2008). Encouraged by their potential clinical applications and in continuation of our previous investigations on biopotent heterocycles (Ladani *et al.*, 2009a, b; Ladani *et al.*, 2010; Mungra *et al.*, 2010; Nirmal *et al.*, 2009; Shah *et al.*, 2009; Thakor *et al.*, 2007; Thumar and Patel, 2009a, b), our efforts are focused to design and synthesize more biologically potent heterocyclic systems via combination of both therapeutically active moieties quinoline and pyridine together in a single scaffold.

Various methods for the synthesis of penta-substituted pyridines from aromatic and heterocyclic aldehydes involving different catalysts (Evdokimov *et al.*, 2006; Heravi *et al.*, 2010; Lakshmikantam *et al.*, 2010; Singh and Singh, 2009; Mamgain *et al.*, 2009; Sridhar *et al.*, 2009; Banerjee and Sereda, 2009; Ranu *et al.*, 2007) are available but not a single report has been found where 2-chloro-3-

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formyl quinoline is used for the synthesis of penta-substituted pyridines using piperidine as basic catalyst. Also, the most suitable protocol for the synthesis of functionalized organic compounds would be a one-pot reaction due to the fact that the synthesis can be performed without the isolation of the intermediates, without discharging any functional groups and within short reaction time (Boulard *et al.*, 2004). Thus, in a view to obtain more biologically potent heterocyclic system, containing both therapeutically active moieties quinoline and pyridine, we report herein the synthesis of some new penta-substituted pyridine derivatives bearing quinoline nucleus via MCR approach.

The constitutions of all the products were confirmed using ^{1}H NMR, ^{13}C NMR, FTIR, and elemental analysis. All synthesized compounds were screened for in vitro antimicrobial activity against eight human pathogens, of which three are Gram positive bacteria (*B. subtilis*, *C. tetani*, *S. pneumoniae*), three are Gram negative bacteria (*E. coli*, *S. typhi*, *V. cholerae*) and two fungi (*A. fumigatus*, *C. albicans*) using broth microdilution MIC (minimum inhibitory concentration) method.

Results and discussion

Chemistry

The required 2-chloro-3-formyl quinoline **1a–e** was prepared by Vilsmeier-Haack reaction according to literature procedure (Meth-Cohn and Bramha, 1978).

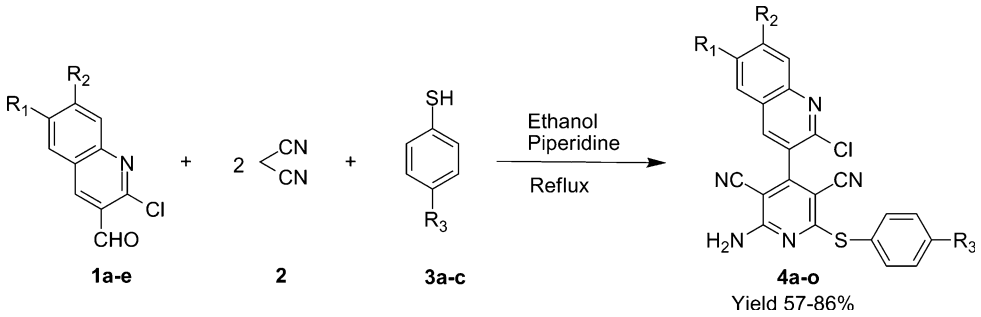
In this study, a series of penta-substituted pyridine derivatives **4a–o** has been synthesized by one-pot three-component cyclocondensation reaction of 2-chloro-3-formyl quinoline **1a–e**, malononitrile **2** and thiophenol **3a–c**, in presence of piperidine as catalyst. The above mixture in refluxing ethanol gives moderate to good yield, i.e., 57–86% (Scheme 1). In accordance with the mechanism suggested in literature (Evdokimov *et al.*, 2006), the first step of this process may involve the Knoevenagel condensation of an aldehyde with malononitrile to form the corresponding heterylidenenitrile derivative (A). The second molecule of malononitrile then undergoes Michael addition to (A) followed by simultaneous thiolate addition to –C≡N of the adduct and cyclization to dihydropyridine (B) which on aromatization and oxidation under the reaction conditions leads to pyridine derivatives **4a–o** (Scheme 2).

The structures of all the new synthesized compounds were established by ^{1}H NMR, ^{13}C NMR, FTIR, elemental analysis, and molecular weight of some selected compounds confirmed by mass spectrometry. In ^{1}H NMR (DMSO-d_6) spectra, aromatic protons as well as –NH_2 proton of pyridine derivatives **4a–o** resonate as multiplets at around δ 7.21–8.95 ppm. Aromatic carbons exhibited signals around δ 106.74–167.19 ppm while C-3 and C-5 carbons of the pyridine ring, attached to the –C≡N groups, exhibited distinctive signals around δ 94.04–94.87 ppm and δ 88.01–88.81 ppm, respectively, in ^{13}C NMR (DMSO-d_6). The IR spectrum of compounds **4a–o** exhibited characteristic absorption bands at 3480–3395 and 3390–3300 cm^{-1} for –NH_2 (asym. and sym. stretching) as well as 2215–2200 cm^{-1} for –C≡N stretching. Absorption band around 750–755 cm^{-1} is mainly attributed to thioether linkage at sixth position of pyridine. The obtained elemental analysis values are in good agreement with theoretical data. Further, the molecular weight of selected compounds, **4e** and **4i** were confirmed by its mass spectral studies. Mass spectroscopy of above mentioned compounds showed molecular ion peak (M + 1) corresponding to the exact mass. Mass spectra of compound **4e** gave molecular ion peak at *m/z* 462 (M + 1) corresponding to molecular formula $C_{23}H_{13}N_5SCl_2$ and **4i** gave molecular ion peak at *m/z* 458 (M + 1) corresponding to molecular formula $C_{24}H_{16}N_5OSCl$. All spectroscopic data have been given in experimental section.

Antimicrobial activity

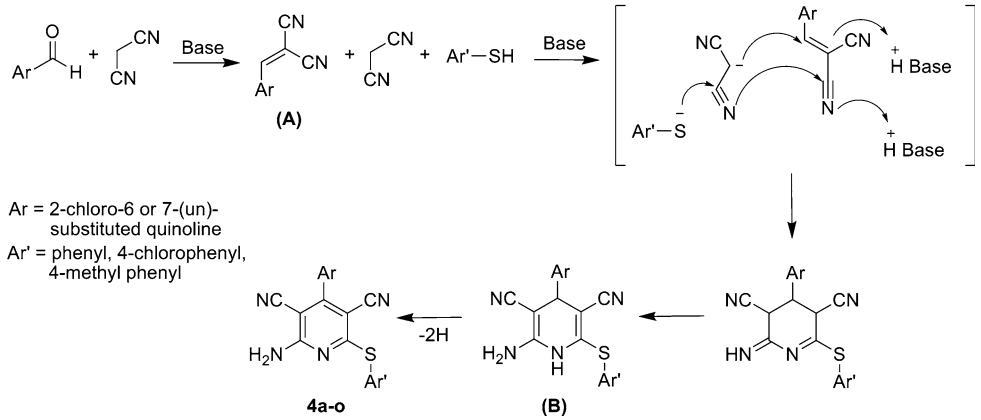
All the glass apparatus used were sterilized before use. Antimicrobial activity of all the synthesized compounds was carried out by broth microdilution method (National Committee for Clinical Laboratory Standards 2002). Mueller–Hinton broth was used as nutrient medium to grow and dilute the compound suspension for the test bacteria and Sabouraud Dextrose broth 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. Each synthesized compound was diluted obtaining 2000 μg/ml concentration, as a stock solution. The results are recorded in the form of primary and secondary screening. The compounds **4a–o** were screened for their antibacterial activity against *B. subtilis* (MTCC 441), *C. tetani* (MTCC 449), *S. pneumoniae* (MTCC 1936), *E. coli* (MTCC 443), *S. typhi* (MTCC 98) and *V. cholerae* (MTCC 3906) as well as for antifungal activity against *A. fumigatus* (MTCC 3008) and *C. albicans* (MTCC 227) at concentrations of 1000, 500, and 250 μg/ml as primary screening. DMSO was used as 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, 50 μg/ml. 10 μl suspensions from each well were further inoculated and growth was noted after 24 and 48 h. The lowest

Scheme 1 General synthetic route for the title compounds **4a–0**



Scheme 2 Plausible mechanistic pathway for the synthesis of title compounds **4a–0**

Compound	R_1	R_2	R_3	Compound	R_1	R_2	R_3
1a	H	H	-	**4e**	CH_3	H	Cl
1b	CH_3	H	-	**4f**	CH_3	H	CH_3
1c	OCH_3	H	-	**4g**	OCH_3	H	H
1d	Cl	H	-	**4h**	OCH_3	H	Cl
1e	H	CH_3	-	**4i**	OCH_3	H	CH_3
3a	-	-	H	**4j**	Cl	H	H
3b	-	-	Cl	**4k**	Cl	H	Cl
3c	-	-	CH_3	**4l**	Cl	H	CH_3
4a	H	H	H	**4m**	H	CH_3	H
4b	H	H	Cl	**4n**	H	CH_3	Cl
4c	H	H	CH_3	**4o**	H	CH_3	CH_3
4d	CH_3	H	H				



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 were summarized in Table 1.

The examination of the data (Table 1) revealed that majority of the compounds showed good antibacterial and antifungal activity when compared with ampicillin, norfloxacin, and griseofulvin.

Against Gram negative bacteria *E. coli*, compound **4e** (R_1=CH_3, R_2=H, R_3=Cl) and against *V. cholerae*, compound **4c** (R_1=H, R_2=H, R_3=CH_3) have shown excellent activity (MIC 62.5 μg/ml) as compared to ampicillin (MIC 100 μg/ml) as well as against Gram positive bacteria *B. subtilis*, compounds **4f** (R_1=CH_3, R_2=H, R_3=CH_3), **4j** (R_1=Cl, R_2=H, R_3=H) and **4 m** (R_1=H, R_2=CH_3, R_3=H) showed excellent activity (MIC 100 μg/ml) upon comparison with ampicillin (MIC 250 μg/ml). Compounds **4e** (R_1=CH_3, R_2=H, R_3=Cl), **4l** (R_1=H, R_2=CH_3, R_3=CH_3) and **4o** (R_1=Cl, R_2=H, R_3=CH_3) against *B. subtilis* as well as compounds **4g** (R_1=OCH_3, R_2=H, R_3=H), **4h** (R_1=OCH_3, R_2=H, R_3=Cl), **4l** (R_1=Cl, R_2=H, R_3=CH_3), **4m** (R_1=H, R_2=CH_3, R_3=H) and **4o** (R_1=H, R_2=CH_3, R_3=CH_3) against *C. tetani* were found to have better activity (MIC 200 μg/ml) than ampicillin (MIC 250 μg/ml).

Moreover, against fungal pathogen *C. albicans*, compound **4k** (R_1=Cl, R_2=H, R_3=Cl) showed excellent activity (MIC 200 μg/ml) while compounds **4f** (R_1=CH_3, R_2=H, R_3=CH_3), **4j** (R_1=Cl, R_2=H, R_3=H), and **4l** (R_1=Cl, R_2=H, R_3=CH_3) were found to possess better activity (MIC 250 μg/ml) as compared to griseofulvin (MIC 500 μg/ml).

Compounds **4a** (R_1=H, R_2=H, R_3=H), **4b** (R_1=H, R_2=H, R_3=Cl), **4c** (R_1=H, R_2=H, R_3=CH_3), **4g** (R_1=OCH_3, R_2=H,

Table 1 Antibacterial and antifungal activity of compounds **4a–o**

Minimum inhibitory concentration (MIC) expressed in μg/ml								
Compound	Gram-positive bacteria			Gram-negative bacteria			Fungal species	
	Bs. MTCC 441	Ct. MTCC 449	Sp. MTCC 1936	Ec. MTCC 443	St. MTCC 98	Vc. MTCC 3906	Af. MTCC 3008	Ca. MTCC 227
4a	250	500	250	200	200	200	500	500
4b	250	250	250	100	150	250	1000	1000
4c	250	250	200	100	100	62.5	>1000	>1000
4d	500	500	250	200	200	250	>1000	>1000
4e	200	250	200	62.5	100	200	500	500
4f	100	500	100	250	200	500	500	250
4g	250	200	250	500	500	500	1000	1000
4h	250	200	250	200	200	200	>1000	1000
4i	500	500	500	500	500	500	>1000	>1000
4j	100	250	200	200	250	200	250	250
4k	250	500	500	250	250	500	500	200
4l	200	200	250	200	200	200	500	250
4m	100	200	100	100	200	250	1000	1000
4n	250	250	250	500	500	500	>1000	>1000
4o	200	200	200	200	250	200	>1000	>1000
Ampicillin	250	250	100	100	100	100	–	–
Norfloxacin	100	50	10	10	10	10	–	–
Griseofulvin	–	–	–	–	–	–	100	500

Bs., *Bacillus subtilis*, Ct., *Clostridium tetani*, Sp., *Streptococcus pneumoniae,* Ec., *Escherichia coli*; St., *Salmonella typhi*; Vc., *Vibrio cholerae;* Af., *Aspergillus fumigatus*; Ca., *Candida albicans*

R_3=H), **4h** (R_1=OCH_3, R_2=H, R_3=Cl), **4k** (R_1=Cl, R_2=H, R_3=Cl), and **4n** (R_1=H, R_2=CH_3, R_3=Cl) as compared to ampicillin (MIC 250 μg/ml) as well as compounds **4f** (R_1=CH_3, R_2=H, R_3=CH_3), **4j** (R_1=Cl, R_2=H, R_3=H), and **4m** (R_1=H, R_2=CH_3, R_3=H) as compared to norfloxacin (MIC 100 μg/ml) have shown equipotent activity towards *B. subtilis*. Compounds **4b** (R_1=H, R_2=H, R_3=Cl), **4c** (R_1=H, R_2=H, R_3=CH_3), **4e** (R_1=CH_3, R_2=H, R_3=Cl), **4j** (R_1=Cl, R_2=H, R_3=H) and **4n** (R_1=H, R_2=CH_3, R_3=Cl) were found equipotent against *C. tetani* as compared to ampicillin (MIC 250 μg/ml), while compounds **4f** (R_1=CH_3, R_2=H, R_3=CH_3) and **4m** (R_1=H, R_2=CH_3, R_3=H) showed comparable activity to ampicillin (MIC 100 μg/ml) against *S. pneumoniae*.

Further, compounds **4b** (R_1=H, R_2=H, R_3=Cl), **4c** (R_1=H, R_2=H, R_3=CH_3) and **4m** (R_1=H, R_2=CH_3, R_3=H) against *Escherichia coli* as well as compounds **4c** (R_1=H, R_2=H, R_3=CH_3) and **4e** (R_1=CH_3, R_2=H, R_3=Cl) against *S. typhi* have been found to possess equipotent activity of ampicillin (MIC 100 μg/ml). Whereas, compounds **4a** (R_1=H, R_2==H, R_3=H) and **4e** (R_1=CH_3, R_2=H, R_3=Cl) were found to be equipotent with griseofulvin (MIC 500 μg/ml) against *C. albicans*. Unfortunately, none of the tested compounds were found to be active against *A. fumigatus*.

Experimental

All the reagents were obtained commercially and used with further purification. Solvents used were of analytical grade. All melting points were taken in open capillaries and are uncorrected. Thin-layer chromatography (TLC, on aluminum plates coated with silica gel 60 F_{254}, 0.25 mm thickness, Merck) was used for monitoring the progress of all reactions, purity and homogeneity of the synthesized compounds. Elemental analysis (% C, H, N) was carried out by Perkin-Elmer 2400 series-II elemental analyzer at Sophisticated Instrumentation Centre for Applied Research & Training (SICART), Vallabh Vidhyanagar and all compounds are within ±0.4% of theory specified. The FTIR spectra were recorded using potassium bromide disc on a Shimadzu FTIR 8401 spectrophotometer 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 using solvent peak as internal standard at 400 and 100 MHz, respectively. Chemical shifts are reported in parts per million (ppm). Mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer. Mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer.

General procedure for the synthesis of 2-amino-4-(2-chloro-6 or 7-(un)-substituted-quinolin-3-yl)-6-[(4-(un)-substituted phenyl)sulfanyl]pyridine-3,5-dicarbonitrile (**4a–o**)

A mixture of appropriate 2-chloro-3-formyl quinoline **1a–e** (5 mmol), malononitrile **2** (10 mmol), thiophenol **3a–c** (5 mmol) and 0.03 ml of piperidine in ethanol (10 ml) were charged in 100 ml round bottom flask equipped with condenser. The reaction mixture was stirred at reflux for 3 h. On completion of reaction, monitored by TLC, the solid separated was filtered, washed with methanol and recrystallized from chloroform to obtain the pure solid sample **4a–o**. The physicochemical and spectroscopic characterization data of the synthesized compounds **4a–o** are given below.

2-Amino-4-(2-chloroquinolin-3-yl)-6-(phenylsulfanyl)pyridine-3,5-dicarbonitrile (**4a**)

Yield 75%, m.p. 277–278°C, Anal. Calcd. for $C_{22}H_{12}N_5SCl$ (413.88 g/mol): C 63.84, H 2.92, N 16.92% Found: C 63.57, H 2.63, N 17.13%. IR (KBr, cm^{-1}): 3410 and 3315 (asym. and sym. stretching of $–NH_2$), 2212 ($–C\equiv N$ stretching), 753 (C–S–C thioether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 7.41-8.93 (m, 12H, Ar–H + NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 88.18 (C–CN), 94.82 (C–CN), 118.21, 120.53, 127.19, 127.62, 128.06, 128.28, 128.57, 129.00, 130.42, 130.16, 131.38, 134.61, 140.93, 142.91, 146.08, 149.94, 151.67, 166.17 (Ar–C).

2-Amino-4-(2-chloroquinolin-3-yl)-6-[(4-chlorophenyl)sulfanyl]pyridine-3,5-dicarbonitrile (**4b**)

Yield 68%, m.p. 230–232°C, Anal. Calcd. for $C_{22}H_{11}N_5SCl_2$ (448.33 g/mol): C 58.94, H 2.47, N 15.62% Found: C 58.72, H 2.71, N 15.51%. IR (KBr, cm^{-1}): 3415 and 3365 (asym. and sym. stretching of $–NH_2$), 2206 ($–C\equiv N$ stretching), 751 (C–S–C thioether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 7.51–8.68 (m, 11H, Ar–H + NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 88.21 (C–CN), 94.67 (C–CN), 114.66, 115.95, 126.13, 126.51, 127.74, 128.14, 130.37, 134.49, 137.13, 138.30, 139.45, 140.16, 143.44, 147.23, 152.71, 155.39, 159.16, 165.59 (Ar–C).

2-Amino-4-(2-chloroquinolin-3-yl)-6-[(4-methylphenyl)sulfanyl]pyridine-3,5-dicarbonitrile (**4c**)

Yield 73%, m.p. 258–260°C, Anal. Calcd. for $C_{23}H_{14}N_5SCl$ (427.91 g/mol): C 64.56, H 3.30, N 16.37% Found: C 64.43, H 2.97, N 16.08%. IR (KBr, cm^{-1}): 3395 and 3355 (asym. and sym. stretching of -NH_2), 2210 ($–C\equiv N$ stretching), 750 (C–S–C thioether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 2.35 (s, 3H, CH_3), 7.33-8.41 (m, 11H, Ar–H + NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.47 (CH_3), 88.71 (C–CN), 94.61 (C–CN), 113.46, 114.07, 125.17, 126.46, 127.73, 129.21, 131.17, 135.13, 137.54, 137.92, 140.28, 144.31, 146.59, 151.31, 155.17, 153.09, 159.46, 166.31 (Ar–C).

2-Amino-4-(2-chloro-6-methylquinolin-3-yl)-6-(phenylsulfanyl)pyridine-3,5-dicarbonitrile (**4d**)

Yield 78%, m.p. 268–270°C, Anal. Calcd. for $C_{23}H_{14}N_5SCl$ (427.91 g/mol): C 64.56, H 3.30, N 16.37% Found: C 64.73, H 3.03, N 16.56%. IR (KBr, cm^{-1}): 3400 and 3370 (asym. and sym. stretching of -NH_2), 2207 ($–C\equiv N$ stretching), 755 (C–S–C thioether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 2.37 (s, 3H, CH_3), 7.33–8.95 (m, 11H, Ar–H + NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.53 (CH_3), 88.33 (C–CN), 94.76 (C–CN), 118.14, 120.22, 127.04, 127.84, 128.27, 128.78, 129.17, 129.54, 130.22, 130.19, 131.23, 134.67, 140.80, 142.12, 146.74, 149.15, 151.26, 165.61 (Ar–C).

2-Amino-4-(2-chloro-6-methylquinolin-3-yl)-6-[(4-chlorophenyl)sulfanyl]pyridine-3,5- dicarbonitrile (**4e**)

Yield 86%, m.p. 283–284°C, Anal. Calcd. for $C_{23}H_{13}N_5SCl_2$ (462.35 g/mol): C 59.75, H 2.83, N 15.15% Found: C 59.56, H 3.12, N 14.87%. IR (KBr, cm^{-1}): 3480 and 3320 (asym. and sym. stretching of $–NH_2$), 2200 ($–C\equiv N$ stretching), 750 (C–S–C thioether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 2.54 (s, 3H, CH_3), 7.57–8.68 (m, 10H, Ar–H + NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.57 (CH_3), 88.59 (C–CN), 94.31 (C–CN), 114.70, 114.99, 126.01, 126.55, 127.40, 127.65, 128.12, 130.04, 135.22, 135.54, 137.32, 138.94, 140.19, 145.56, 146.44, 155.28, 159.76, 166.30 (Ar–C), MS: 462 (M + 1) (exact mass = 461.03 g/mol).

2-Amino-4-(2-chloro-6-methylquinolin-3-yl)-6-[(4-methylphenyl)sulfanyl]pyridine-3,5-dicarbonitrile (**4f**)

Yield 69%, m.p. 262–263°C, Anal. Calcd. for $C_{24}H_{16}N_5SCl$ (441.94 g/mol): C 65.23, H 3.65, N 15.85% Found: C 65.47, H 3.91, N 16.03%. IR (KBr, cm^{-1}): 3400 and 3323 (asym. and sym. stretching of $–NH_2$), 2215 ($–C\equiv N$ stretching), 752 (C–S–C thioether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 2.41 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 7.39–8.54 (m, 10H, Ar–H + NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.18 (CH_3), 21.42 (CH_3), 88.62 (C–CN), 94.51 (C–CN), 114.13, 125.23, 126.75, 127.33, 128.04, 128.94, 131.29, 134.20, 137.83, 138.22, 139.73, 145.17, 146.88, 150.61, 153.20, 155.77, 159.15, 166.43 (Ar–C).

2-Amino-4-(2-chloro-6-methoxyquinolin-3-yl)-6-(phenylsulfanyl)pyridine-3,5-dicarbonitrile (**4g**)

Yield 61%, m.p. 278-279°C, Anal. Calcd. for $C_{23}H_{14}N_5OSCl$ (443.91 g/mol): C 62.23, H 3.18, N 15.78% Found: C 62.34, H 2.94, N 15.49%. IR (KBr, cm^{-1}): 3415 and 3380 (asym. and sym. stretching of -NH_2), 2200 (–C≡N stretching), 751 (C–S–C thioether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.48 (s, 3H, OCH_3), 7.42-8.78 (m, 11H, Ar–H + NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 57.01 (OCH_3), 88.32 (C–CN), 94.04 (C–CN), 108.12, 113.93, 114.96, 123.51, 124.86, 127.54, 127.87, 129.26, 130.63, 135.84, 138.54, 139.02, 142.80, 143.87, 147.39, 153.03, 158.63, 166.25 (Ar–C).

2-Amino-4-(2-chloro-6-methoxyquinolin-3-yl)-6-[(4-chlorophenyl)sulfanyl]pyridine-3,5-dicarbonitrile (**4h**)

Yield 57%, m.p. 284–285°C, Anal. Calcd. for $C_{23}H_{13}N_5OSCl_2$ (478.35 g/mol): C 57.75, H 2.74, N 14.64% Found: C 57.51, H 2.41, N 14.88%. IR (KBr, cm^{-1}): 3430 and 3300 (asym. and sym. stretching of -NH_2), 2213 (–C≡N stretching), 753 (C–S–C thioether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.63 (s, 3H, OCH_3), 7.33–8.53 (m, 10H, Ar–H + NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 56.24 (OCH_3), 88.15 (C–CN), 94.25 (C–CN), 107.21, 114.03, 114.96, 121.23, 122.15, 124.20, 125.01, 126.34, 127.22, 129.76, 133.44, 136.45, 139.98, 142.08, 151.78, 156.21, 159.09, 166.73 (Ar–C).

2-Amino-4-(2-chloro-6-methoxyquinolin-3-yl)-6-[(4-methylphenyl)sulfanyl]pyridine-3,5-dicarbonitrile (**4i**)

Yield 82%, m.p. 291–292°C, Anal. Calcd. for $C_{24}H_{16}N_5OSCl$ (457.93 g/mol): C 62.95, H 3.52, N 15.29% Found: C 62.69, H 3.79, N 15.58%. IR (KBr, cm^{-1}): 3420 and 3310 (asym. and sym. stretching of -NH_2), 2205 (–C≡N stretching), 754 (C–S–C thioether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.38 (s, 3H, CH_3), 3.95 (s, 3H, OCH_3), 7.34–8.63 (m, 10H, Ar–H + NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.41 (CH_3), 56.32 (OCH_3), 88.28 (C–CN), 94.18 (C–CN), 106.74, 114.75, 115.06, 123.47, 125.46, 127.72, 127.95, 129.90, 130.72, 135.54, 139.34, 140.31, 143.78, 143.83, 155.28, 159.03, 159.79, 167.19 (Ar–C), MS: 458 (M + 1) (Exact mass = 457.08 g/mole).

2-Amino-4-(2,6-dichloroquinolin-3-yl)-6-(phenylsulfanyl)pyridine-3,5-dicarbonitrile (**4j**)

Yield 77%, m.p. 234–236°C, Anal. Calcd. for $C_{22}H_{11}N_5SCl_2$ (448.33 g/mol): C 658.94, H 2.47, N 15.62% Found: C 58.71, H 2.69, N 15.51%. IR (KBr, cm^{-1}): 3420 and 3380 (asym. and sym. stretching of -NH_2), 2210 (–C≡N stretching), 755 (C–S–C thioether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.24-8.67 (m, 11H, Ar–H + NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 88.01 (C–CN), 94.63 (C–CN), 115.21, 115.97, 125.46, 126.75, 127.11, 128.30, 130.82, 133.14, 137.23, 137.67, 139.24, 145.12, 146.22, 149.98, 155.64, 153.72, 160.81, 165.56 (Ar–C).

2-Amino-4-(2,6-dichloroquinolin-3-yl)-6-[(4-chlorophenyl)sulfanyl]pyridine-3,5-dicarbonitrile (**4k**)

Yield 64%, m.p. 254–256°C, Anal. Calcd. for $C_{22}H_{10}N_5SCl_3$ (482.77 g/mol): C 54.73, H 2.09, N 14.51% Found: C 54.88, H 2.31, N 14.41%. IR (KBr, cm^{-1}): 3455 and 3390 (asym. and sym. stretching of -NH_2), 2200 (–C≡N stretching), 750 (C–S–C thioether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.21–8.15 (m, 10H, Ar–H + NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 88.18 (C–CN), 94.87 (C–CN), 113.63, 115.63, 126.29, 127.10, 128.07, 128.22, 131.31, 134.73, 137.18, 137.93, 139.80, 141.20, 143.22, 149.19, 152.10, 155.33, 158.16, 166.83 (Ar–C).

2-Amino-4-(2,6-dichloroquinolin-3-yl)-6-[(4-methylphenyl)sulfanyl]pyridine-3,5-dicarbonitrile (**4l**)

Yield 69%, m.p. 286-288°C, Anal. Calcd. for $C_{23}H_{13}N_5SCl_2$ (462.35 g/mol): C 59.75, H 2.83, N 15.15% Found: C 59.58, H 2.52, N 14.90%. IR (KBr, cm^{-1}): 3435 and 3350 (asym. and sym. stretching of –NH_2), 2215 (–C≡N stretching), 755 (C–S–C thioether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.53 (s, 3H, CH_3), 7.40–8.53 (m, 10H, Ar–H + NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.47 (CH_3), 88.39 (C–CN), 94.20 (C–CN), 113.86, 114.13, 124.14, 126.54, 126.70, 128.93, 132.21, 135.75, 136.03, 137.13, 141.11, 144.15, 144.98, 150.51, 153.76, 155.44, 158.33, 165.87 (Ar–C).

2-Amino-4-(2-chloro-7-methylquinolin-3-yl)-6-(phenylsulfanyl)pyridine-3,5-dicarbonitrile (**4m**)

Yield 72%, mp 275–276°C, Anal. Calcd. for $C_{23}H_{14}N_5SCl$ (427.91 g/mol): C 64.56, H 3.30, N 16.37% Found: C 64.71, H 3.11, N 16.48%. IR (KBr, cm^{-1}): 3410 and 3365 (asym. and sym. stretching of –NH_2), 2213 (-C≡N stretching), 753 (C–S–C thioether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.52 (s, 3H, CH_3), 7.30–8.95 (m, 11H, Ar–H + NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.53 (CH_3), 88.31 (C–CN), 94.53 (C–CN), 118.31, 120.29, 126.85, 127.22, 128.32, 128.61, 129.08, 129.73, 130.24, 131.03, 131.35, 134.57, 139.88, 141.11, 146.79, 149.62, 151.30, 165.83 (Ar–C).

2-Amino-4-(2-chloro-7-methylquinolin-3-yl)-6-[(4-chlorophenyl)sulfanyl]pyridine-3,5-dicarbonitrile (**4n**)

Yield 79%, m.p. 250–252°C, Anal. Calcd. For $C_{23}H_{13}N_5SCl_2$ (462.35 g/mol): C 59.75, H 2.83, N 15.15% found: C 59.61, H 3.11, N 14.98%. IR (KBr, cm^{-1}): 3400 and 3385 (asym. and sym. stretching of -NH_2), 2209 (–C≡N stretching), 751 (C–S–C thioether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.54 (s, 3H, CH_3), 7.52–8.46 (m, 10H, Ar–H + NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.56 (CH_3), 88.61 (C–CN), 94.39 (C–CN), 114.53, 115.12, 125.94, 126.53, 127.45, 127.84, 128.20, 130.11, 135.28, 135.43, 137.56, 139.14, 140.27, 144.89, 146.41, 155.33, 159.64, 166.17 (Ar–C).

2-Amino-4-(2-chloro-7-methylquinolin-3-yl)-6-[(4-methylphenyl)sulfanyl]pyridine-3,5-dicarbonitrile (**4o**)

Yield 81%, m.p. 244–246°C, Anal. Calcd. for $C_{24}H_{16}N_5SCl$ (441.94 g/mol): C 65.23, H 3.65, N 15.85% Found: C 65.43, H 3.88, N 16.12%. IR (KBr, cm^{-1}): 3400 and 3340 (asym. and sym. stretching of -NH_2), 2212 (–C≡N stretching), 750 (C–S–C thioether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.55 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 7.41–8.69 (m, 10H, Ar–H + NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.25 (CH_3), 21.47 (CH_3), 88.56 (C–CN), 94.54 (C–CN), 114.18, 125.13, 126.56, 127.31, 128.13, 129.02, 131.32, 134.24, 137.65, 138.28, 139.20, 144.21, 146.73, 150.62, 153.36, 155.63, 158.93, 167.23 (Ar–C).

Conclusion

New penta-substituted pyridine derivatives bearing the quinoline nucleus have been synthesized through one-pot multicomponent reaction. This synthetic strategy allows the assimilation of two promising bioactive heterocycles through an easy way. Of the compounds studied, **4c** and **4e** have proved as the most efficient antimicrobial member. Majority of the compounds were found to be active against *C. tetani* and *B. subtilis*. Compounds **4j**, **4k**, and **4l** which are bearing chloro substituted quinoline ring are more potent against *C. albicans* upon comparison with griseofulvin. According to this conclusion, it is worth mentioning that quinolinylpyridines bearing thioether linkage in pyridine is emerged as a spot of antimicrobial medicine research.

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