Introduction

The emerging resistance of antibacterial agents is a worldwide problem, proving the need for new molecules [1,2]. However, in the last two decades only one new class of antibiotics has been commercialized, and there is a concerning dearth of antibacterial agents with a mechanism of action in development. Bacterial fatty acid biosynthesis is an essential process that supplies precursors for the assembly of important cellular components such as phospholipids, lipopolysaccharides, mycolic acids, and the cell envelope. In mammals, all enzymatic activities associated with acyl chain elongation are encoded by a single polypeptide. While in bacteria, the pathway is comprised of several discrete enzymes. This organizational difference makes the bacterial fatty acid biosynthetic enzymes potentially selective antibacterial targets [3,4]. Bacterial resistance to currently used antibiotics is a concern for public health (Monroe and Polk). The development of bacterial super resistant strain is resulting in currently used antibiotic agents failing to end many bacterial infections. For this reason the search is ongoing for new natural or synthetic antimicrobial agents [5]. Quinoline derivatives have demonstrated anti-leishmanial activity, antibacterial, antifungal, anti HIV and antitumor activities [15]. Quinolines are structurally diverse group of compounds present in numerous natural products and are also the object of extensive synthetic research. Quinoline derivatives have demonstrated anti-leishmanial activity, antibacterial, antifungal, anti HIV and antitumor activities [16-19]. Recently, 4-amino-7-chloro-quinoline derivatives have demonstrated mycobacterium tuberculosis activity [20-29]. Benzo[H][1,6]naphthyridine derivatives also showed antimalarial activity [5] [30].

Chemistry

One pot condensation of p-substituted aroylacetonitriles with 2-chloroquinolin-4-amine and triethylorthoester at 60-70°C in ethanol furnished amino butenenitrile quinoline derivatives 4(a-f).

Abstract

One pot reaction of 2-aminoquinoline 1 and ethyl orthoacetate with butynenitrile 2 yielded 4-amino butenenitrile quinoline 4 and with diethyl 2-(ethoxymethylene) malonate 5 yielded 4-amino methylene diethyl malonate 6 in good yield. The quinoline 6 was oxidized to quinolone 7 in acetic acid. The cyclization compound 6 was successfully attempted using Pb(OH)2 furnished 4-hydroxy benzo[h][1,6] naphthyride and on refluxing compound 6 in POCl3 yielded 4-chloro benzo[h][1,6] naphthyride derivatives 9. All new compounds showed good antimicrobial activity against standard ampicillin and streptomycin.

Keywords: Benzo[H][1,6]Naphthyridines; Methylene diethyl malonate; Antimicrobial activity

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as yellow color solid in 70-83% yield. The structure of compounds 4 (a-f) were illustrated by spectroscopic and analytical methods. For instance IR of 4b showed the presence of NH, CN and CO stretching frequency at 3443, 2206 and 1649 cm\(^{-1}\) respectively. The lowering of carbonyl frequency was due to conjugation and strong intramolecular H-bonding between carbonyl oxygen and NH group. The \(^1\)H NMR spectrum of 4b in CDCl\(_3\) showed singlet at \(\delta\) 2.54 assignable to CH\(_2\) group; the singlet at \(\delta\) 7.33 assignable to C\(_6\)H proton. The doublet at \(\delta\) 7.46 and triplet at \(\delta\) 7.61 (J=7.0 Hz) assignable to C\(_6\)H and C\(_5\)H protons respectively. The resonance singlet at \(\delta\) 9.95 assignable to NH proton present on secondary amino group. The remaining aromatic protons showed multiplet in between \(\delta\) 7.86-7.97. \(^{13}\)C NMR spectrum of 7b in CDCl\(_3\) showed the peaks at \(\delta\) 120.10 for CN group and at \(\delta\) 178.37 for the presence of ketone (CO). The EI-MS of 120.10 for CN group and at \(\delta\) 178.37 for the presence of ketone (CO). The EI-MS of 120.10 for CN group and at \(\delta\) 178.37 for the presence of ketone (CO).

Concentration of Compound: 1000 µg/ml (prepared in DMF)
Method used: Agar diffusion assay (disc method, disc size 5 mm)
Dilution of Drug: Stock prepared 1000 µg/ml prepared in DMF (100 µg per disc)

Results of antimicrobial activity: All the synthesized compounds 4(a-f), 6(a-b), 7(a-b), 8(a-b), 9(a-b) were tested against microorganism species at 1000 ppm concentration. The observed results of antibacterial screening reported in Table 1 indicate that 4-amino substituted quinoline and benzo[h][1,6]naphthyridine compounds 4c, 4d and 7a are active against S. aureus, compounds 4e, 4f and 6a are active against E. coli bacterial species. The Compounds 4b, 4c, 4d, 4e, 6b, and 7b showed activity against B. subsilis. The compounds 4d, 4f found active against B. ceriurus. The compounds 4c, 4d, 4f and 7b are active against B. megaterium species. However compounds 8a, 8b, 9a, 9b found totally inactive against bacterial species while the compounds 4c, 4d and 4f are most active against the bacterial species. The P. aeruginosa found stable against all compound. From the above observations it is clear that the 4-aminoquinoline derivatives 4c and 4f showed significant antibacterial activity against B. megaterium.

Experimental Section

General remarks
Melting points were determined on a Gallenkamp melting point apparatus in an open capillary tube and are uncorrected. The \(^1\)H (300 MHz) and \(^13\)C (75 MHz) NMR spectra were recorded on a Varian XL-300 spectrometer. Chemical shifts were reported in ppm relative to tetramethylsilane (TMS), and multiplicities are given as s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). Infrared spectra were recorded as KBr pellets on a Shimadzu FTIR-408 spectrophotometer. Mass spectra were recorded on a Shimadzu LC-MS: EI, 70 eV mass spectrometer with an ionization potential of 70 eV. Elemental analyses were performed on Thermo Quest Flash 1112 Series EA analyzer. Reactions were monitored by thin layer chromatography (TLC), carried out on 0.2 mm silica gel 60 F\(_{254}\) (Merck) plates using UV light (254 and 366 nm) for detection and compounds were purified by column chromatography by using silica gel of 5-20 µm (Merck, 60-120 mesh). Column dimension is 39 x 2 cm and elution volume used is about 200-400 mL for each

**Scheme 1**

Scheme 1 Synthesis of 2-(4-substituted benzoyl)-3-(2-chloroquinolin-4-ylamino)alkenenitrile 4(a-f) and 2-[(2-oxo-1,2-dihydro-quinolin-4-ylamino)methylene]malonic acid diethyl ester 7(a-b).

**Scheme 2**

Scheme 2 Synthesis of 5-chloro-4-hydroxy-benzo[h][1,6]naphthyridine-3-carboxylic acid ethyl ester 8(a-b) and 4,5-Dichloro-benzo[h][1,6]naphthyridine-3-carboxylic acid ethyl ester 9(a-b).
A mixture of 2-chloro-4-aminoquinoline 1 (0.005 mol), substituted benzoyl acetonitrile 2 and triethyl orthoformate or triethyl orthopropionate (0.006 mol) was refluxed in dry toluene at 3 h (TLC checked, toluene). After completion of the reaction, the solvent was removed under reduced pressure; the obtained solid was stirred in ethanol for 30 min. The solid separated was collected by suction filtration, dried, and recrystallized from ethanol to furnish title compound 4 in good yield.

2-(4-Bromobenzoyl)-3-(2-chloroquinolin-4-ylamino)but-2-enenitrile, 4a: Yellow prisms; Yield (1.545 g, 85%); M.P: 214°C; IR (KBr) ν\text{max}: 3353 (NH), 2817, 2223 (CN), 1649 (CO), 1570, 1270 cm\(^{-1}\); H NMR (CDCl\(_3\)): δ 2.51 (s, 3H, CH\(_3\)), 7.32 (s, 1H, C\(_{3}\)H), 7.45 (d, J=7.5 Hz, 1H, C\(_{7}\)), 7.61 (t, J=7.0 Hz, 1H, C\(_{7}\)), 7.86-7.97 (m, 5H, Ar-H), 9.95 (s, 1H, NH, D\(_2\)O exchangeable); MS: m/z (%): 381 (M+4, 20), 383 (M+2, 30), 385 (M+, 100), 246 (30), 192 (70), 190 (70), 139 (30); Anal. Calcd. For C\(_{20}\)H\(_{13}\)BrClN\(_2\)O (382.25); Calcd: C, 56.84; H, 3.28; N, 9.76; Found: C, 56.43; H, 2.99; N, 9.72.

Table 1 Antimicrobial activity of benzo[\(h\)]1,6]naphthyridine derivatives.

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Inhibition zone diameter (mm)</th>
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<tbody>
<tr>
<td></td>
<td>Gram negative (E. coli, P. aeruginosa, P. vulgaris)</td>
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<tr>
<td>4a</td>
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<td>4b</td>
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<td>9b</td>
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<tr>
<td>Ampicillin</td>
<td>35</td>
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<tr>
<td>Streptomycin</td>
<td>-</td>
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</table>

product where necessary. Common reagent-grade chemicals are either commercially available and were used without further purification or were prepared by standard literature procedures.

**Synthesis of 2-(4-chlorobenzoyl)-3-(2-chloroquinolin-4-ylamino)but-2-enenitrile, 4 (a-f)**

2-(4-Chlorobenzoyl)-3-(2-chloroquinolin-4-ylamino)but-2-enenitrile, 4c: Yellow prisms; Yield (1.50 g, 70%); M.P: 217°C; IR (KBr) ν\text{max}: 3337 (NH), 2817, 2223 (CN), 1649 (CO), 1570, 1270 cm\(^{-1}\); H NMR (CDCl\(_3\)): δ 1.31 (t, J=7 Hz, 3H, CH\(_3\)), 2.74 (q, J=7.0 Hz, 2H, CH\(_2\)), 7.37 (s, 1H, C\(_{3}\)H), 7.45 (d, J=7.5 Hz, 1H, C\(_{7}\)), 7.59 (J=7.5 Hz, C\(_{7}\)), 7.61-7.91 (m, 5H, Ar-H), 8.97 (s, 1H, NH, D\(_2\)O exchangeable); \(^{13}\)C NMR (CDCl\(_3\)): δ 15.28, 21.21, 79.52, 111.13, 116.23, 120.17, 122.70, 123.30, 123.47, 127.6, 130.09, 130.71, 132.20, 140.21, 156.26, 177.21; Anal. Calcd. For C\(_{30}\)H\(_{15}\)Cl\(_2\)N\(_2\)O (416.70); Calcd: C, 57.65; H, 2.90; N, 10.08; Found: C, 57.43; H, 2.99; N, 10.21.

2-(4-Chlorobenzoyl)-3-(2,8-dichloroquinolin-4-ylamino)-pent-2-enenitrile, 4d: Yellow prisms; Yield (1.42 g, 75%); M.P: 26°C; IR (KBr) ν\text{max}: 3475 (NH), 2908, 2215 (CN), 1645 (CO), 1570, 1170 cm\(^{-1}\); H NMR (CDCl\(_3\)): δ 2.51 (s, 3H, CH\(_3\)), 7.32 (s, 1H, C\(_{3}\)H), 7.45 (d, J=6.0 Hz, 1H, C\(_{7}\)), 7.60 (d, J=6.0 Hz, C\(_{7}\)), 7.65-7.96 (m, 5H, Ar-H), 8.21 (d, J=6.25 Hz, C\(_{7}\)), 10.08 (d, J=5.25 Hz, 1H, NH, D\(_2\)O exchangeable); MS: m/z (%): 385 (M+4, 20), 383 (M+2, 30), 381 (M+, 100), 246 (30), 192 (70), 190 (70), 139 (30); Anal. Calcd. For C\(_{30}\)H\(_{13}\)Cl\(_2\)N\(_2\)O (382.25); Calcd: C, 62.84; H, 3.43; N, 10.09; Found: C, 62.83; H, 3.59; N, 10.83.

2-(4-Chlorobenzoyl)-3-(2-chloro-7-methylquinolin-4-ylamino)acrylonitrile, 4e: Yellow prisms; Yield (1.28 g, 65%); M.P: 183°C; IR (KBr) ν\text{max}: 3237 (NH), 3008, 2205 (CN), 1647 (CO), 1545, 1169 cm\(^{-1}\); H NMR (CDCl\(_3\)): δ 2.51 (s, 3H, CH\(_3\)), 2.631 (s, 3H, CH\(_3\)).
1H, Ar-CH$_3$), 7.33 (s, 1H, C$_H$), 7.46 (d, J=7.5 Hz, 2H, ArH), 7.61 (d, J=7.5 Hz, C$_H$), 7.86-7.97 (m, 4H, ArH), 10.08 (s, 1H, NH, D$_2$O exchangeable); $\text{Anal. Calcld. For }$ C$_{12}$H$_{17}$Cl$_2$N$_2$O (396.28); $\text{Calcd. C}$, 63.65; H, 3.82; N, 10.60; $\text{Found: C}$, 63.69; H, 3.79; N, 10.55.

2-(4-Chlorobenzoyl)-3-(2-chloro-7-methyl-quinolin-4-ylamino)pent-2-enenitrile, 4f: Yellow prisms; Yield (1.22 g, 60%); M.P: 178°C; IR (KBr) $\nu_{max}$: 3337 (NH), 2917, 2215 (CN), 1645 (CO), 1570, 1170 cm$^{-1}$; $^{1}$H NMR (CDCl$_3$): $\delta$ 1.31 (t, J=8.0 Hz, 3H, CH$_3$), 2.61 (s, 3H, Ar-CH$_3$), 2.74 (q, J=8.0 Hz, 2H, CH$_2$), 7.35 (s, 1H, C$_H$), 7.45 (d, J=8.0 Hz, 1H, ArH), 7.45 (d, J=7.5 Hz, 1H, C$_H$), 7.59-7.91 (m, 2H, ArH), 10.17 (s, 1H, NH, D$_2$O exchangeable); $^{13}$C NMR (CDCl$_3$): $\delta$ 16.21, 23.17, 80.20, 111.31, 118.10, 120.14, 122.86, 123.09, 123.28, 123.84, 130.13, 130.94, 132.34, 139.25, 148.08, 163.30, 177.31; MS: m/z (%): 413 (M+4, 10), 411 (M+2, 30), 409 (M+, 100); 274 (30), 218 (70), 193 (80), 139 (40); $\text{Anal. Calcld. For }$ C$_{22}$H$_{17}$Cl$_2$N$_2$O (410.31); $\text{Calcd. C}$, 64.60; H, 4.18; N, 10.24; $\text{Found: C}$, 64.71; H, 4.10; N, 10.35.

**Synthesis of 2-[2-chloroquinolin-4-ylamino]methylene]malonic acid diethyl ester (6a-b)**

2-Chloro-4-amoquinoline 1a-b (0.005 mol) and diethoxymethylene malonate 5 (0.007 mol) was stirred at 120-130°C for 12 h. Progress of the reaction was monitored by TLC. After cooling the reaction mixture to room temperature, methanol (50 mol) was added to it. The crude product separated by suction filtration, dried, and recrystallized from the ethanol and DMF (8:2) to furnish yellow solid 6 in 83-90% yields.

$\text{[2-Chloroquinolin-4-ylamino]methylene]malonic acid diethyl ester (6a):}$ Yellow prisms; Yield (1.56 g, 90%); M.P: 205°C; IR (KBr) $\nu_{max}$: 3433 (NH), 2931, 1693 (CO), 1618, 1267, 806 cm$^{-1}$; $^{1}$H NMR (CDCl$_3$): $\delta$ 1.37 (t, J=7.0 Hz, 3H, CH$_3$), 1.42 (t, J=7.0 Hz, 3H, CH$_3$), 4.29 (q, J=7.0 Hz, 2H, CH$_2$), 4.32 (q, J=7.0 Hz, 2H, CH$_2$), 7.24 (s, 1H, C$_H$), 7.62 (t, J=6.5 Hz, 1H, C$_H$), 7.77 (t, J=6.5 Hz, 1H, C$_H$), 7.90 (d, J=6.5 Hz, 1H, C$_H$), 7.95 (d, J=12.6 Hz, 1H, =CH), 8.04 (d, J=6.5 Hz, 1H, C$_H$), 8.15 (d, J=12.6 Hz, 1H, =CH), 11.95 (d, J=12.6 Hz, 1H, NH, D$_2$O exchangeable); $^{13}$C NMR (CDCl$_3$): $\delta$ 14.20, 21.71, 169.30, 109.31, 122.23, 122.91, 123.20, 132.21, 132.02, 133.70, 140.20, 145.03, 159.01, 159.21; MS: m/z (%): 350 (M+2, 30), 348 (M+, 90), 278 (20), 178 (70), 170 (70); $\text{Anal. Calcld. For }$ C$_{13}$H$_{13}$Cl$_2$N$_2$O$_2$ (348.79); $\text{Calcd. C}$, 58.54; H, 4.91; N, 8.03; $\text{Found: C}$, 58.63; H, 4.87; N, 8.15.

$\text{[2-Chloroquinolin-4-ylamino]methylene]malonic acid diethyl ester (6b):}$ Yellow prisms; Yield (1.58 g, 83%); M.P: 183°C; IR (KBr) $\nu_{max}$: 3345 (NH), 2935, 1690 (CO), 1620, 1267, 870 cm$^{-1}$; $^{1}$H NMR (CDCl$_3$): $\delta$ 1.35 (t, J=7.2 Hz, 3H, CH$_3$), 1.43 (t, J=7.2 Hz, 3H, CH$_3$), 4.30 (q, J=7.2 Hz, 2H, CH$_2$), 4.32 (q, J=7.2 Hz, 2H, CH$_2$), 7.25 (s, 1H, C$_H$), 7.60 (t, J=7.0 Hz, 1H, C$_H$), 7.78 (d, J=7.0 Hz, 1H, C$_H$), 7.95 (d, J=7.0 Hz, 1H, C$_H$), 8.56 (d, J=13.5 Hz, 1H, =CH); $\text{Anal. Calcld. For }$ C$_{13}$H$_{13}$Cl$_2$N$_2$O$_2$ (383.23); $\text{Calcd. C}$, 53.28; H, 4.21; N, 7.31; $\text{Found: C}$, 53.20; H, 4.27; N, 7.41.
8.35 (d, J=7.5 Hz, 1H, C7H), 10.25 (s, 1H, OH, D2O exchangeable); 

**Conclusion**

The chemoselective synthesis of 4-aminoquinolines 6(a-b) were obtained from 2,4-dichloroquinoline derivatives at different reaction conditions. The N-alkylation on 4-aminoquinolines using benzoylacetonitrile, diethyl 2-(ethoxymethylene) malonate was carried under mild reaction conditions. The benzo[h][1,6]naphthyridine derivatives 8(a-b), 9(a-b) were obtained from open chain analog of 4-aminoquinolines 6(a-b). The open chain quinline derivatives showed antimicrobial activity while cyclic benzo[h][1,6]naphthyridine derivatives found totally against bacteria utilized.

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