Synthesis and Antimicrobial Activity of Benzo[H][1,6]Naphthyridine Derivatives

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 PbO furnished 4-hydroxy benzo[h][1,6] naphthyridine and on refluxing compound 6 in POCl₃ yielded 4-chloro benzo[h][1,6] naphthyridine 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

Introduction

The emerging resistance of antibacterial agents is worldwide problem proved the need of new molecules [1,2]. However, in last two decades only one new class of antibiotics has been commercialized, and there is a concerning dearth of antibacterial agents with mechanism of action in development. Bacterial fatty acid biosynthesis is an essential process that supplied precursors for the assembly of important cellular components such as phospholipids, lipoproteins, 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 becoming a concern to 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 activity [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 [30].

Chemistry

One pot condensation of p-substituted aroylacetonitriles with 2-chloroquinolin-4-amine 1 and triethylorthoester at 60-70°C in ethanol furnished amino butenenitrile quinoline derivatives 4(a-f).
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 1H NMR spectrum of 4b in CDCl₃ showed singlet at δ 2.54 assignable to CH₃ group; the singlet at δ 7.33 assignable to C₆H protons. The doublet at δ 7.46 and triplet at δ 7.61 (½=7.0 Hz) assignable to C₆H and C₆H protons respectively. The resonance singlet at δ 9.95 assignable to NH proton present on secondary amino group. The remaining aromatic protons showed multiplet in between δ 7.86-7.97. 13C NMR spectrum of 7b in CDCl₃ showed the peaks at δ 120.10 for CN group and at δ 178.37 for the presence of ketone (CO). The EI-MS of 7b showed M+, M+2 and M+4 at 426, 428 and 430 m/z respectively due to the presence of two chlorine atoms [31-42]. The neat reaction of compound M+4 at 426, 428 and 430 m/z respectively due to the presence of ketone (CO). The EI-MS of

The enamine 6 was cyclized by refluxing in diphenylether for 30 min yielded brown colored 4-amino methylene diethylmalonate 6 in 80-90% yield. The imine chloride group in compound 6 was oxidized to amide furnished derivative 7 on refluxing in acetic acid for 5 h. The structure of compound 7 was assigned using spectroscopic and analytical methods given in experimental section (Scheme 1).

Study of antimicrobial activity
Cultures (Bacteria) used:
Gram positive: S. aureus, B. subtulis, B. cerious, B. megaterium;
Gram negative: Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris

Media used: Nutrient agar (Hi-media)
Inoculum Size: X 10⁶ bacteria per ml

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 4d, 4c 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. subtulis. The compounds 4d, 4f found active against B. cerious. 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 are 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 1H (300 MHz) and 13C (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 QP 2010A mass spectrometer with an ionization potential of 70eV. 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 F₂₅₄ (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
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)**

A mixture of 2-chloro-4-aminoquinoline 1 (0.005 mol), substituted benzoyl acetonitrile 2 and triethyl orthoformate or triethyl orthoacetate 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 methanol for 30 min. The solid separated was collected by suction filtration, dried, and recrystallized from ethanol to furnish title compound 4 in good yield.

**Table 1 Antimicrobial activity of benzo[h][1,6]naphthyridine derivatives.**

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Gram negative</th>
<th>Inhibition zone diameter (mm)</th>
<th>Gram positive</th>
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<tbody>
<tr>
<td></td>
<td>E. coli</td>
<td>P. aeruginosa</td>
<td>P. vulgaris</td>
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<tr>
<td>4a</td>
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<td>-</td>
<td>11</td>
</tr>
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<td>4b</td>
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<td>14</td>
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<td>4c</td>
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<td>11</td>
</tr>
<tr>
<td>4d</td>
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<td>4e</td>
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<td>4f</td>
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<td>12</td>
</tr>
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<td>6a</td>
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<td>6b</td>
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<td>7a</td>
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<td>7b</td>
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<td>9b</td>
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<tr>
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<td>45</td>
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<tr>
<td>Streptomycin</td>
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</table>

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 max: 3353 (NH), 2924, 2223 (CN), 1649 (CO), 1570, 1270 cm⁻¹; H NMR (CDCl 3): δ 1.31 (t, J=7 Hz, 3H, CH₃), 7.37 (d, J=12.5 Hz, 1H, NH, D₂O exchangeable); MS: m/z (%): 430 (M+4, 40), 428 (M+2, 50), 426 (M⁺, 100), 345 (30), 250 (70), 176 (80), 185 (70); Anal. Calcld. For C₂₀H₁₃BrN₃O (426.70); Calcd: C, 56.30; H, 3.07; N, 9.85; Found: C, 56.43; H, 2.99; N, 9.72.

2-(4-Chlorobenzoyl)-3-(2-chloro-7-methylquinolin-4-ylamino)acrylonitrile, 4d: Yellow prisms; Yield (1.42 g, 75%); M.P: 26°C; IR (KBr) ν max: 3475 (NH), 2908, 2215 (CN), 1645 (CO), 1570, 1170 cm⁻¹; H NMR (CDCl 3): δ 2.51 (s, 3H, CH₃), 7.32 (s, 1H, CH₄), 7.45 (d, J=7.0 Hz, 1H, CH), 7.60 (d, J=7.0 Hz, 1H, CH) 7.65-7.96 (m, 5H, Ar-H), 8.21 (d, J=12.5 Hz, 1H=N=CH), 10.08 (d, J=12.5 Hz, 1H, NH, D₂O exchangeable); MS: m/z (%): 385 (M+4, 20), 383 (M+2, 30), 381 (M⁺, 100), 246 (30), 192 (70), 190 (70), 139 (30); Anal. Calcld. For C₂₀H₁₂BrN₃O (382.25); Calcd: C, 62.84; H, 3.43; N, 10.99; Found: C, 62.83; H, 3.59; N, 10.83.

2-(4-Chlorobenzoyl)-3-(2-chloro-7-methylquinolin-4-ylamino)but-2-enenitrile, 4e: Yellow prisms; Yield (1.28 g, 65%); M.P: 183°C; IR (KBr) ν max: v3237 (NH), 3008, 2205 (CN), 1647 (CO), 1545, 1169 cm⁻¹; H NMR (CDCl 3): δ 2.51 (s, 3H, CH₃), 2.631 (s,
1H, Ar-CH3), 7.33 (s, 1H, C-H), 7.46 (d, J=7.5 Hz, 2H, ArH), 7.61 (d, J=7.5 Hz, C6H), 7.86-7.97 (m, 4H, Ar-H), 10.08 (s, 1H, NH, D2O exchangeable); Anal. Calcd. For C17H17CL3N3O (396.28); Calcd.: C, 63.65; H, 3.82; N, 10.60; 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:

IR (KBr) νmax: 3433 (OH), 3050, 1720 (CO), 1624, 1566 cm-1; 1H NMR (CDCl3): δ 1.31 (t, J=7.2 Hz, 2H, CH2), 4.32 (q, J=7.2 Hz, 2H, CH2), 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, =CH2); Anal. Calcd. For C13H11CL2N2O2 (324.79); Calcd.: C, 53.20; H, 4.27; N, 7.41.

Synthesis of 2-[(2-oxo-1,2-dihydro-quinolin-4-ylamino)methylene]malonic acid diethyl ester, 7a-b

The open chain compound 6 was refluxed in acetic acid for 5 h (TLC check, toluene). Reaction mixture was cooled to room temperature, the solid separated was collected by suction filtration, dried, and recrystallized from ethanol/DMF (8:2) to afford 7 in 80-82% yield.

2-[(2-Oxo-1,2-dihydroquinolin-4-yl)amino]malonic acid diethyl ester, 7a:

IR (KBr) νmax: 3432 (OH), 3050, 1720 (CO), 1624, 1566 cm-1; 1H NMR (CDCl3): δ 1.30 (t, J=7.2 Hz, 2H, CH2), 4.26 (q, J=7.2 Hz, 2H, CH2), 7.57 (t, J=7.5 Hz, 1H, C-H), 7.78 (t, J=7.5 Hz, 1H, C-H), 8.05 (d, J=7.5 Hz, 1H, C-H),

Synthesis of 8-chloro-2-oxo-1,2-dihydroquinolin-4-yl)amino]malonic acid diethyl ester, 7b:

IR (KBr) νmax: 3433 (OH), 3072 (NH), 2982, 1725(CO), 1685 (CO) cm-1; 1H NMR (CDCl3): δ 1.28 (t, J=7.2 Hz, 2H, CH2), 1.30 (t, J=7.2 Hz, 3H, CH3), 4.15 (q, J=7.2 Hz, 2H, CH2), 4.26 (q, J=7.2 Hz, 2H, CH2), 6.55 (s, 1H, C-H), 7.38 (t, J=7.5 Hz, 1H, C-H), 7.56 (d, J=7.5 Hz, 1H, C-H), 7.75 (d, J=7.5 Hz, 1H, C-H), 8.47 (d, J=14 Hz, C-H), 11.18 (d, J=14 Hz, NH, 1H, NH, D2O exchangeable); MS: m/z (%): 360 (M+2, 30), 364 (M+, 90), 340 (24), 244 (60), 194 (70), 170 (20); Anal. Calcd. For C13H11CL2N2O2 (324.79); Calcd.: C, 55.97; H, 4.70; N, 7.68; Found: C, 55.89; H, 4.75; N, 7.69.

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)

Method A: The compound 6 (0.005 mol) in POCl3 was heated to reflux for 5 h (TLC checked, toluene). After completion of reaction, the reaction mixture was cooled to room temperature and then it was stirred in diethyl ether (50 mL). The solid obtained was filtered and washed with excess ether, dried, and recrystallized from ethanol/DMF (80:20) to afford 8 in 70-75% yield.

Method B: The compound 6 (0.005 mol) in POCI3 was heated to reflux for 5 h (TLC checked, toluene). After completion of reaction, excess POCI3 was evaporated. The red colored solid obtained was poured in ice water (1L) and solution was neutralized with solid sodium carbonate (10 g). The separated solid product was collected by suction filtration. The TLC analysis in toluene showed two products. The mixture of crude product was separated by column chromatography on silica gel eluting with toluene, yields title compound 8 (20%) and 9 (40%).
8.35 (d, J=7.5 Hz, 1H, C7H), 10.25 (s, 1H, OH, D2O exchangeable); 


5,7-Dichloro-4-hydroxy-benzo[h][1,6]naphthyridine-3-carboxylic acid ethyl ester, 8b: Yellow prisms; Yield; (Method A=1.17 g, 75%; B=0.337 g, 20%); M.P: 243°C; IR (KBr) νmax: 3425 (OH), 3055, 1714 (CO), 1624, 1516, 2280, 769 cm⁻¹; 1H NMR (CDCl3): δ 1.31 (t, J=7.0 Hz, 3H, CH3), 4.25 (q, J=7.0 Hz, 2H, CH2), 7.77 (t, J=8.1 Hz, 1H, C9H), 8.10 (d, J=8.1 Hz, 1H, C8H), 8.42 (s, 1H, C2H), 8.54 (d, J=8.1 Hz, 1H, C10H), 10.80 (s, 1H, OH, D2O exchangeable);MS: m/z (%): 341 (M+4, 20), 339 (M+2, 30), 337 (M+, 90), 300 (70), 255 (70), 185 (80); Anal. Calcd. For C15H10Cl2N2O3 (337.17); Calcd: C, 53.44; H, 2.99; N, 8.31; Found: C, 53.50; H, 3.09; N, 8.40.

4,5-Dichlorobenzo[h][1,6]naphthyridine-3-carboxylic acid ethyl ester, 9a: Yellow prisms; Yield; (Method A=0.642 g, 40%); M.P: 188°C; IR (KBr) νmax: 2955, 1728 (CO), 156, 1174, 770 cm⁻¹; 1H NMR (CDCl3): δ 1.48 (t, J=6.9 Hz, 3H, CH3), 4.51 (q, J=6.9 Hz, 2H, CH2), 7.76 (t, J=7.8 Hz, 1H, C9H), 7.88 (t, J=7.8 Hz, 1H, C8H), 8.05 (d, J=7.8 Hz, 1H, C10H), 9.05 (d, J=7.8 Hz, 1H, C10H), 9.12 (s, 1H, C2H); MS: m/z (%): 325 (M+4, 10), 323 (M+2, 30), 321 (M+, 100), 284 (30), 187 (40); Anal. Calcd. For C15H10Cl2N2O2 (321.17); Calcd: C, 56.10; H, 2.99; N, 8.32; Found: C, 56.22; H, 3.09; N, 8.69.

Conclusion

The chemoselective synthesis of 4-aminquinolines 6(a-b) were obtained from 2,4-dichloroquinoline derivatives at different reaction conditions. The N-alkylation on 4-aminquinolines 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-aminquinolines 6(a-b). The open chain quinline derivatives showed antimicrobial activity while cyclic benzo[h][1,6]naphthyridine derivatives found totally against bacteria utilized.

Acknowledgements

Authors thank UGC, New Delhi and BCUD, Savitribai Phule Pune University for financial support, CIF, Department of Chemistry, Savitribai Phule Pune University for spectral analysis and Principal, KTHM College, Nashik-422 002, Maharashtra for providing facilities.
References


