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

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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_2O furnished 4-hydroxy benzo[h][1,6] naphthyride and on refluxing compound 6 in $POCl_3$ 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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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 been used for the treatment of malaria [6]. Systematic modification of quinine led to the potent antimalarial chloroquine **2** drug [7]. After worldwide development, chloroquine drug was found resistant against malaria. Therefore chemist focused to synthesize new compounds. The screening test of mefloquine **3**, quinacrine **4** and other new potent quinolines showed good activity against malaria [8-11]. The quinoline ring system and aliphatic side chain is crucial for the mode of action of chloroquine [12-14]. 4-Aminoquinoline bioisoster and their platinum (II) complexes showed anti-leishmanial and antitubercular 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 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 **5** [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 $CDCl_3$ showed singlet at δ 2.54 assignable to CH_3 group; the singlet at δ 7.33 assignable to C_3H proton. The doublet at δ 7.46 and triplet at δ 7.61 (J=7.0 Hz) assignable to C_7H and C_6H 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. ^{13}C NMR spectrum of **7b** in $CDCl_3$ 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 **1** and diethyl 2-(ethoxymethylene) malonate **5** at 120°C for 12 h furnished a yellow 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**).

The enamine **6** was cyclized by refluxing in diphenylether for 30 min yielded brown colored to desired benzo[*h*][1,6]naphthyridine derivatives **8** in 70-75% yield. The compound **6a** refluxed in $POCl_3$ furnished mixture of compounds **8** and **9** as yellow coloured solids in 20 % and 40% yield respectively. The mixture of compounds **8** and **9** was separated by using column chromatography eluting with toluene. The structures of synthesized compounds were based on analytical and spectroscopic data (**Scheme 2**).

Study of antimicrobial activity

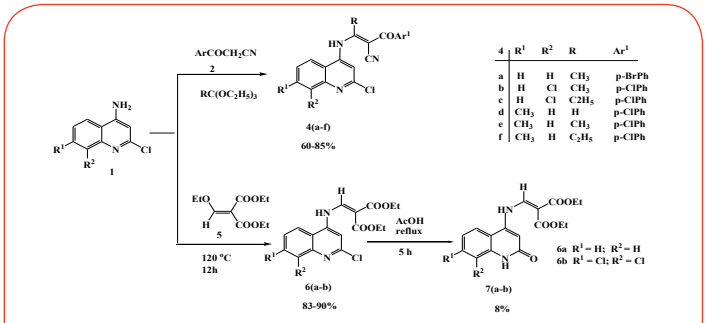
Cultures (Bacteria) used:

Gram positive: S. aureus, *B. substilis, B. cerious, B. megaterium;*

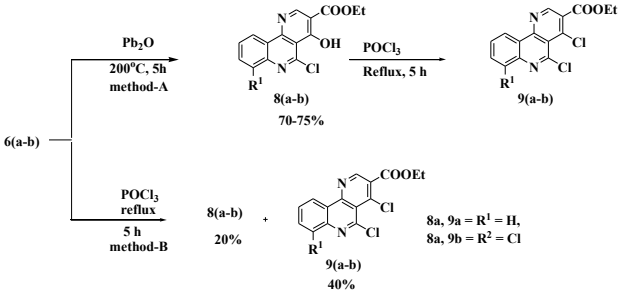
Gram negative: Escherichia coli, *Pseudomonas aeruginosa, Proteus valgaris*

Media used: Nutrient agar (Hi-media)

Inoculum Size: X 10^6 bacteria per ml



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 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).

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. substilis.* 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 ^{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 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 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

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

Compound No.	*Inhibition zone diameter* (mm)						
	Gram negative			Gram positive			
	E. coli	*P. aeruginosa*	*P. vulgaris*	*S. aureus*	*B. subtilis*	*B. cerious*	*B. megaterium*
4a	-	-	11	-	-	-	-
4b	-	-	**14**	-	8	-	-
4c	-	-	11	**14**	**13**	-	**18**
4d	-	-	12	**11**	**10**	11	**13**
4e	10	-	-	-	**-**	-	**-**
4f	10	-	12	-	**11**	14	**19**
6a	8	-	-	-	-	-	-
6b	-	-	10	-	8	-	-
7a	-	-	-	8	-	-	-
7b	-	-	-	-	8	-	10
8a	-	-	-	-	-	-	-
8b	-	-	-	-	-	-	-
9a	-	-	-	-	-	-	-
9b	-	-	-	-	-	-	-
Ampicillin	35	45	34	45	40	33	15
Streptomycin	-	-	34	13	15	40	-

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.

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, 2230 (CN), 1640 (CO), 1539, 126 cm^{-1};^{1}H NMR ($CDCl_3$): δ2.54 (s, 3H, CH_3), 7.23 (s, 1H, C_3H), 7.55 (t, *J*=7.5 Hz, 1H, C_6H), 7.2 (t, *J*=7.5 Hz, 1H, C_7H), 7.77 (d, *J*=7.5 Hz, 1H, C_5H), 7.82-7.93(m, 5H, Ar-H), 10.15 (d, *J*=12.6 Hz, 1H, NH, D_2O exchangeable); MS: *m/z* (%): 430 (M+4, 40), 428 (M+2, 50), 426 (M+, 100), 345 (30), 250 (70), 176 (80), 185 (70); *Anal. Calcd. For* $C_{20}H_{13}BrClN_3O$ (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-(2,8-dichloroquinolin-4-ylamino)but-2-enenitrile, 4b: Yellow prisms; Yield (1.46 g, 71%); M.P: 230°C; IR (KBr) ν_{max}: *v* 3443 (NH), 2924, 2206 (CN), 1649 (CO), 1575, 1280 cm^{-1};^{1}H NMR ($CDCl_3$): δ 2.54 (s, 3H, CH_3), 7.33 (s, 1H, C_3H), 7.46 (d, *J*=7.0 Hz, 1H, C_7H), 7.61 (t, *J*=7.0 Hz, 1H, C_6H), 7.86-7.97(m, 5H, Ar-H), 9.95 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR ($CDCl_3$): δ 22.32, 83.23, 110.48, 117.10, 120.07, 122.86, 123.06, 123.17, 123.26, 128.84, 130.05, 130.16, 130.87, 139.51, 148.06, 161.37, 178.37; MS: *m/z* (%): 422 (M+6, 20), 420 (M+, 20), 418 (M+2, 30), 416 (M+, 100), 212 (30), 204 (70), 141 (40); *Anal. Calcd. For* $C_{20}H_{12}Cl_3N_3O$ (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, 4c: Yellow prisms; Yield (1.50 g, 70%); M.P: 217°C; IR (KBr) ν_{max}: *v*3337 (NH), 2817, 2223 (CN), 1649 (CO), 1570, 1270 cm^{-1};^{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_3H), 7.45 (d, *J*=7.5 Hz, 1H, C_7H), 7.59 (t, *J*=7.5 Hz, C_6H) 7.61-7.91(m, 5H, Ar-H), 9.87 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR ($CDCl_3$): δ 15.28, 21.21, 79.52, 111.13, 116.23, 120.17, 122.70, 123.03, 123.30, 124.07, 127.6, 130.09, 130.71, 132.20, 140.21, 146.30, 160.20, 177.21; *Anal. Calcd. For* $C_{21}H_{14}Cl_3N_3O$ (430.72); *Calcd*: C, 58.56; H, 3.28; N, 9.76; *Found*: C, 58.43; H, 3.19; N, 9.6.

2-(4-Chlorobenzoyl)-3-(2-chloro-7-methyl-quinolin-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};^{1}H NMR ($CDCl_3$): δ 2.51 (s, 3H, CH3), 7.32 (s, 1H, C_3H), 7.45 (d, *J*=7.0 Hz, 1H, C_6H), 7.60 (d, *J*=7.0 Hz, C_5H) 7.65-7.96(m, 5H, Ar-H), 8.21(d, *J*=12.5 Hz, 1H,=CH), 10.08 (d, *J*=12.5 Hz, 1H, NH, D_2O 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* $C_{20}H_{13}Cl_2N_3O$ (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-methyl-quinolin-4-ylamino) but-2-enenitrile, 4e: Yellow prisms; Yield (1.28 g, 65%); M.P: 183°C; IR (KBr) ν_{max}: *v*3237 (NH), 3008, 2205 (CN), 1647 (CO), 1545, 1169 cm^{-1};^{1}H NMR ($CDCl_3$): δ 2.51 (s, 3H, CH_3), 2.631 (s,

1H, Ar-CH_3), 7.33 (s, 1H, C_3H), 7.46 (d, *J*=7.5 Hz, 2H, ArH), 7.61 (d, *J*=7.5 Hz, C_6H) 7.86-7.97 (m, 4H, Ar-H), 10.08 (s, 1H, NH, D_2O exchangeable); *Anal. Calcd. For* $C_{21}H_{15}Cl_2N_3O$ (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: Yellow prisms; Yield (1.22 g, 60%); M.P: 178°C; IR (KBr) ν_{max}: 3337 (NH), 2917, 2215 (CN), 1645 (CO), 1570, 1170 cm^{-1};^{1}H NMR ($CDCl_3$): δ 1.31 (t, *J*=8.0 Hz, 3H, CH_3), 2.61 (s, 3h, $ArCH_3$), 2.74 (q, *J*=8.0 Hz, 2H, CH_2), 7.35 (s, 1H, C_3H), 7.45 (d, *J*=8.0 Hz, 1H, ArH), 7.45 (d, *J*=7.5 Hz, 1H, C_6H), 7.59-7.91(m, 5H, Ar-H), 10.17 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR ($CDCl_3$): δ 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); *Anal. Calcd. For* $C_{22}H_{17}Cl_2N_3O$ (410.31); *Calcd*: C, 64.40; H, 4.18; N, 10.24; *Found*: C, 64.71; H, 4.10; N, 10.35.

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

2-Chloro-4-aminoquinoline **1(a-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 mL) was added to it. The crude product separated was collected by suction filtration, dried, and recrystallized from the ethanol and DMF (8:2) to furnish yellow solid **6** in 83-90% yields.

2-[(2-Chloro-quinolinin-4-ylamino)methylene]malonic acid diethyl ester (6a): Yellow prisms; Yield (1.56 g, 90%); M.P: 205°C; IR (KBr) ν_{max}: 3433 (NH), 2931, 1693 (CO), 1618, 1267, 806 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 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_3H), 7.62 (t, *J*=6.5 Hz, 1H, C_7H), 7.77 (t, *J*=6.5 Hz, 1H, C_6H), 7.79 (d, *J*=6.5 Hz, 1H, C_8H), 8.04 (d, *J*=6.5 Hz, 1H, C_5H), 8.57 (d, *J*=12.6 Hz, 1H,=CH), 11.95 (d, *J*=12.6 Hz, 1H, NH, D_2O exchangeable); ^{13}C NMR ($CDCl_3$): δ 14.20, 21.71, 169.30, 109.31, 122.23, 122.91, 123.20, 132.21, 133.02, 133.70, 140.20, 145.03, 159.01, 169.21; MS: *m/z* (%): 350 (M+2, 30), 348 (M+, 90), 278 (20), 178 (70), 170 (70); *Anal. Calcd. For* $C_{17}H_{17}ClN_2O_4$ (348.79); *Calcd*: C, 58.54; H, 4.91; N, 8.03; *Found*: C, 58.63; H, 4.87; N, 8.15.

2-[(2,8-Dichloro-quinolinin-4-ylamino)methylene]malonicacid diethyl ester (6b): Yellow prisms; Yield (1.58 g, 83%); M.P: 183°C; IR (KBr) ν_{max}: 3345 (NH), 2935, 1690 (CO), 1620, 1267, 870 cm^{-1};^{1}H NMR ($CDCl_3$): δ 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_3H), 7.60 (t, *J*=7.0 Hz, 1H, C_6H), 7.78 (d, *J*=7.0 Hz, 1H, C_7H), 7.95 (d, *J*=7.0 Hz, 1H, C_5H), 8.56 (d, *J*=13.5 Hz, 1H,=CH), 11.95 (d, *J*=13.5 Hz, 1H, NH, D_2O exchangeable); *Anal. Calcd. For* $C_{17}H_{16}Cl_2N_2O_4$ (383.23); *Calcd*: C, 53.28; H, 4.21; N, 7.31; *Found*: 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-ylamino)methylene]malonic acid diethyl ester,7a: Yellow prisms; Yield (1.32 g, 80%); M.P: 230°C; IR (KBr) ν_{max}: 3439 (NH), 3281 (NH), 2980, 1724(CO), 1699 (CO)cm^{-1};^{1}H NMR ($CDCl_3$): δ 1.27 (t, *J*=7.0 Hz, 3H, CH_3), 1.29 (t, *J*=7.0 Hz, 3H, CH_3), 4.14 (q, *J*=7.0 Hz, 2H, CH_2), 4.26 (q, *J*=7.0 Hz, 2H, CH_2), 6.52 (s, 1H, C_3H), 7.31 (t, *J*=7.2 Hz, 1H, C_6H), 7.37 (t, *J*=7.7 Hz, 1H, C_7H), 7.56-7.60 (m, 2H, C_5H& C_8H), 8.49 (d, *J*=12.3 Hz,=CH), 11.21 (d, *J*=12.3 Hz, NH, 1H, NH, D_2O exchangeable); *Anal. Calcd. For* $C_{17}H_{18}N_2O_5$ (330.34); *Calcd*: C, 61.81; H, 5.49; N, 8.48; *Found*: C, 61.78; H, 5.30; N, 8.51.

2-[(8-Chloro-2-oxo-1,2-dihydroquinolin-4-ylamino)methylene] malonic acid diethyl ester, 7b: Yellow prisms; Yield (1.49 g, 82%); M.P: 197°C; IR (KBr) ν_{max}: 3433 (NH), 3270 (NH), 2982, 1725(CO), 1685 (CO) cm^{-1};^{1}H NMR ($CDCl_3$): δ 1.28 (t, *J*=7.2 Hz, 3H, CH_3), 1.30 (t, *J*=7.2 Hz, 3H, CH_3), 4.15 (q, *J*=7.2 Hz, 2H, CH_2), 4.26 (q, *J*=7.2 Hz, 2H, CH_2), 6.55 (s, 1H, C_3H), 7.38 (t, *J*=7.5 Hz, 1H, C_6H), 7.56 (d, *J*=7.5 Hz, 1H, C_7H), 7.75 (d, *J*=7.5 Hz, 1H, C_5H), 8.47 (d, *J*=14 Hz,=CH), 11.18 (d, *J*=14 Hz, NH, 1H, NH, D_2O exchangeable);MS: *m/z* (%): 366 (M+2, 30), 364 (M+, 90), 304 (40), 244 (60), 194 (70), 170 (80); *Anal. Calcd. For* $C_{17}H_{17}ClN_2O_5$ (364.79); *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 diphenyl ether was refluxed (200°C) for 30 min (TLC checked, toluene). After completion of reaction, the reaction mixture was cool 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** in70-75% yield.

Method B: The compound **6** (0.005 mol) in $POCl_3$ was heated to reflux for 5 h (TLC checked, toluene). After completion of reaction, excess $POCl_3$ vacuum evaporated. The red colored residue 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%).

5-Chloro-4-hydroxybenzo[h][1,6]naphthyridine-3-carboxylic acid ethyl ester, 8a: Yellow prisms; Yield; (Method A=1.05 g, 70%, B=0.302 g, 20%); M.P: 250°C; IR (KBr) ν_{max}: 3423 (OH), 3050, 1720 (NH), 2982, 1720 (CO), 1624, 1566 cm^{-1};^{1}H NMR ($CDCl_3$): δ 1.30 (t, *J*=7.2 Hz, 3H, CH_3), 4.26 (q, *J*=7.2 Hz, 2H, CH_2), 7.57 (t, *J*=7.5 Hz, 1H, C_9H), 7.78 (t, *J*=7.5 Hz, 1H, C_8H), 8.05 (d, *J*=7.5 Hz, 1H, $C_{10}H$),

8.35 (d, *J*=7.5 Hz, 1H, C_7H), 10.25 (s, 1H, OH, D_2O exchangeable); *Anal. Calcd. For* $C_{15}H_{11}ClN_2O_3$ (302.72); *Calcd*: C, 59.52; H, 3.66; N, 9.25; *Found*: C, 59.60; H, 3.61; N, 9.40.

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) ν_{max}: 3425 (OH), 3055, 1714 (CO), 1624, 1516, 2280, 769 cm^{-1};^{1}H NMR ($CDCl_3$): δ 1.31 (t, *J*=7.0 Hz, 3H, CH_3), 4.25 (q, *J*=7.0 Hz, 2H, CH_2), 7.77 (t, *J*=8.1 Hz, 1H, C_9H), 8.10 (d, *J*=8.1 Hz, 1H, C_8H), 8.42 (s, 1H, C_2H), 8.54 (d, *J*=8.1 Hz, 1H, $C_{10}H$), 10.80 (s, 1H, OH, D_2O exchangeable);MS: *m/z* (%): 341 (M+4, 20), 339 (M+2, 30), 337 (M+, 90), 300 (70), 255 (70), 185 (80); *Anal. Calcd. For* $C_{15}H_{10}Cl_2N_2O_3$ (337.16); *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) ν_{max}: 2955, 1728 (CO), 156, 1174, 770 cm^{-1};^{1}H NMR ($CDCl_3$): δ 1.48 (t, *J*=6.9 Hz, 3H, CH_3), 4.51 (q, *J*=6.9 Hz, 2H, CH_2), 7.76 (t, *J*=7.8 Hz, 1H, C_9H), 7.88 (t, *J*=7.8 Hz, 1H, C_8H), 8.05 (d, *J*=7.8 Hz, 1H, $C_{10}H$), 9.05 (d, *J*=7.8 Hz, 1H, C_7H), 9.12 (s, 1H, C_2H); MS: *m/z* (%): 325 (M+4, 10), 323 (M+2, 30), 321 (M+, 100), 284 (30), 187 (40); *Anal. Calcd. For* $C_{15}H_{10}Cl_2N_2O_2$ (321.17); *Calcd*: C, 56.10; H, 3.14; N, 8.72; *Found*: C, 56.22; H, 3.09; N, 8.69.

4,5,7-Trichlorobenzo[h][1,6]naphthyridine-3-carboxylic acid ethyl ester, 9b: Yellow prisms; Yield; (Method A=0.71 g, 40%); M.P: 149°C; IR (KBr) ν_{max}: 2923, 160 (CO), 1537, 1179, 770 cm^{-1};^{1}H NMR ($CDCl_3$): δ 1.48 (t, *J*=7.0 Hz, 3H, CH_3), 4.52 (q, *J*=7.0 Hz, 2H, CH_2), 7.70 (t, *J*=7.2 Hz, 1H, C_9H), 7.80 (d, *J*=7.2 Hz, 1H, C_8H), 8.05 (d, *J*=7.2 Hz, 1H, $C_{10}H$), 9.10(s, 1H, C_2H); *Anal. Calcd. For* $C_{15}H_9Cl_3N_2O_2$ (355.61); *Calcd*: C, 50.66; H, 2.55; N, 7.88; *Found*: C, 50.79; H, 2.61; N, 7.79.

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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