One-pot synthesis and antimicrobial activity of novel naphthyridine derivatives

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A one-pot synthetic route was developed to synthesise novel naphthyridine derivatives from amino acids and a series of new naphthyridines was prepared and characterised by IR, ^{1}H NMR, MS and elemental analysis. The preliminary bioassay results reveal that they have medium antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Aspergillus*, *Escherichia coli* and *Monilia albican*. The compounds containing aliphatic amino acids have greater bioactivity than that of those containing aromatic amino acids against *Bacillus subtilis* and *Staphylococcus aureus*.

Keywords: naphthyridines, amino acids, antimicrobial activity, antifungal activity

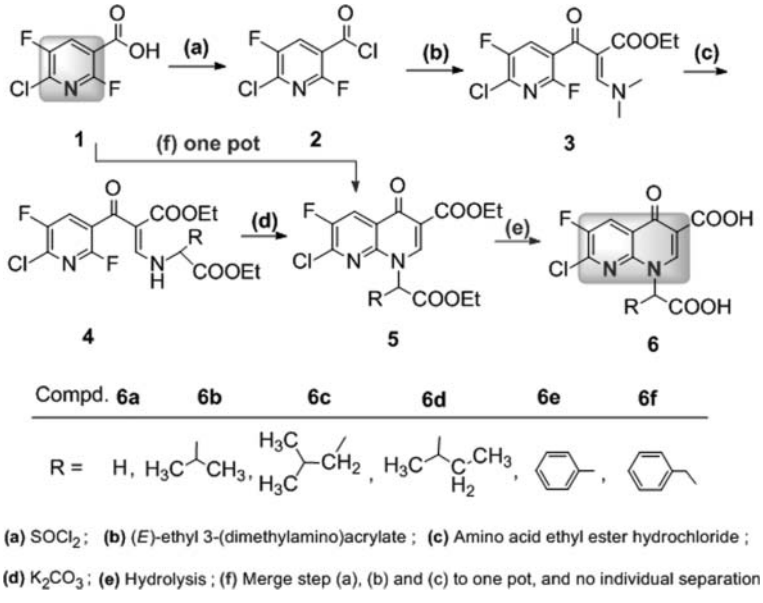
Interest in heterocyclic compounds,[1–3] especially the naphthyridines, has continued for many years, largely based on their widespread use as anti-asthmatic, anti-bacterial, and anti-malarial agents. [4–7] As part of our ongoing interests to find new naphthyridine derivatives, we have focused our attention on introducing natural amino acids into naphthyridines at the N1-position.

Often, several steps and much complicated post-treatment and purification processes are necessary to synthesise the naphthyridine derivatives. For example, Okada and Kimura *et al.* reported a muti-step reaction to synthesise some of these heterocyclic derivatives.[8,9] We prepared a series of naphthyridine heterocyclic derivatives by a multistep reaction from an analogous compound **1** to give, via the intermediates **2–4**, the compound **5**. We wish to develop a concise route with simple work-up procedures to synthesise a series of these novel heterocyclic compounds. We now report that this procedure goes well and via steps a–d shown in Scheme 1 we can merge the different steps into a one-pot procedure. The title compounds were characterised by ^{1}H NMR, IR spectra, mass spectra and elemental analyses. In order to investigate their preliminary antimicrobial bioactivities, all the compounds were screened against *Bacillus subtilis*, *Staphylococcus aureus*, *Aspergillus*, *Escherichia coli* and *Monilia albican*. The preliminary bioassay results reveal that they have medium antimicrobial activity against the above bacteria. The synthetic route is shown in Scheme 1.

Experimental

All the reagents were purchased commercially and used without further purification. The melting points were determined using a XT-4 melting-point apparatus and uncorrected. IR spectra were recorded on a Bruker Equinox-55 spectrophotometer using KBr discs in the 4000–400 cm^{-1} region. The ^{1}H NMR data were obtained on a Bruker AC-400 (400 MHz) instrument in DMSO-d_6 using TMS as internal standard. Chemical shifts (δ) are expressed in ppm and coupling constants *J* are given in Hz. Mass spectra were obtained on an Agilent 5973N mass spectrometer operating at 70 eV by electron ionisation technique (EI/MS). Elemental analyses were performed on an EA-1110 instrument.

1-(Carboxymethyl)-7-chloro-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (**6a**): 2,6-Di- chloro-5-fluoronicotinic acid **1** (4.18 g, 0.02 mol) was dissolved in thionyl chloride (23 mL) in a 50 mL round-bottomed flask, stirred and refluxed for 3 h, The excess thionyl chloride was removed on a rotary evaporator. (E)-Ethyl-3-(dimethylamino) acrylate (2.85 g, 0.02 mol) was added dropwise to the toluene solution, and it was stirred at 50–60 °C, monitoring with TLC (PE/EA=1/1 (v/v)) to show at the end of reaction the absence of reactants. Then the amino acid ethyl ester hydrochloride (0.02 mol) was added directly and stirring was continued for about 5–8 h at room temperature, checking the reaction via TLC. After completion, potassium carbonate (3.08 g, 0.025 mol) was added and the reaction



Scheme 1 The synthetic route to novel naphthyridine derivatives.

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was stirred for 8–10 h at 110 °C. When the reaction was over (determined by TLC), the cold mixture was acidified with dilute hydrochloric acid (5%) to pH=1–2. The reaction mixture was poured into a separating funnel and separated. The organic layer was concentrated under reduced pressure and the residue was chromatographed on silica gel (PE/EA=2/1 (v/v)) to give compound **5a**. Compound **5a** was added to 20% sulfuric acid solution, refluxed for about 6–8 h, then cooled to room temperature and the mixture was filtered. The filter cake was recrystallised using 15% alcohol aqueous solution to afford compounds **6a** in 50% yield; m.p. 259–261 °C. IR (KBr) ν_{max}: 3487 (–OH), 2929 (CH_2), 1746 (C=O), 1634 (Ar), 1489 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 5.33 (s, 2H, CH_2), 8.74 (d, *J* = 7.60 Hz, 1H, ArH), 9.29 (s, 1H, C=CH), 14.12 (br s, 2H, COOH); MS (70 eV): *m/z* (%) = 300 (M^+). Anal. Calcd for $C_{11}H_6ClFN_2O_5$: C, 43.95; H, 2.01; N, 9.32. Found: C, 44.02; H, 2.05; N, 9.44%.

1-(1-Carboxy-2-methylpropyl)-7-chloro-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (**6b**): This compound was obtained in similar manner to **6a** with 52% yield; m.p. 254–256 °C. IR (KBr) ν_{max}: 3469 (-OH), 2972 (CH_3), 1754 (C=O), 1611 (Ar), 1474 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 0.91 (d, *J* = 7.20 Hz, 6H, CH_3), 2.02–2.04 (m, 1H, CH), 3.65–3.67 (m, 1H, CH), 8.77 (d, *J* = 7.60 Hz, 1H, ArH), 9.22 (s, 1H, C=CH), 13.53 (br s, 1H, COOH), 14.22 (br s, 1H, COOH); MS (70 eV): *m/z* (%) = 342 (M^+). Anal. Calcd for $C_{14}H_{12}ClFN_2O_5$: C, 49.07; H, 3.53; N, 8.17. Found: C, 49.21; H, 3.55; N, 8.24%.

1-(1-Carboxy-3-methylbutyl)-7-chloro-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (**6c**): This compound was obtained in similar manner to **6a** with 49% yield; m.p. 271–273 °C. IR (KBr) ν_{max}: 3313 (–OH), 2972 (CH_3), 1753 (C=O), 1611 (Ar), 1473 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 0.93 (d, *J* = 7.20 Hz, 6H, CH_3), 1.41–1.43 (m, 1H, CH), 2.31–2.33 (m, 2H, CH_2), 3.71–3.74 (m, 1H, CH), 8.68 (d, *J* = 8.00 Hz, 1H, ArH), 9.27 (s, 1H, C=CH), 13.65 (br s, 1H, COOH), 14.13 (br s, 1H, COOH); MS (70 eV): *m/z* (%) = 356 (M^+). Anal. Calcd for $C_{15}H_{14}ClFN_2O_5$: C, 50.50; H, 3.96; N, 7.85. Found: C, 50.56; H, 4.00; N, 7.94%.

1-(1-Carboxy-2-methylbutyl)-7-chloro-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (**6d**): This compound was obtained in similar manner **6a** with 48% yield; m.p. 282–284 °C. IR (KBr) ν_{max}: 3387 (-OH), 2963 (CH_3), 1751 (C=O), 1630 (Ar), 1472 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 0.85-0.89 (m, 6H, CH_3), 0.93–0.96 (m, 2H, CH_2), 2.21–2.24 (m, 1H, CH), 3.79–3.82 (m, 1H, CH), 8.42 (d, *J* = 8.00 Hz, 1H, ArH), 9.29 (s, 1H, C=CH), 13.62 (br s, 1H, COOH), 14.18 (br s, 1H, COOH); MS (70 eV): *m/z* (%) = 356 (M^+). Anal. Calcd for $C_{15}H_{14}ClFN_2O_5$: C, 50.50; H, 3.96; N, 7.85. Found: C, 50.55; H, 4.02; N, 7.96%.

1-(Carboxy(phenyl)methyl)-7-chloro-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (**6e**): This compound was obtained in similar manner with 50% yield; m.p. 172–174 °C. IR (KBr) ν_{max}: 3435 (–OH), 1747 (C=O), 1688 (Ar), 1470 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 4.19 (s, 1H, CH), 7.52–7.61 (m, 5H, ArH), 8.36 (d, *J* = 7.60 Hz, 1H, ArH), 9.26 (s, 1H, C=CH), 13.58 (br s, 1H, COOH), 14.23 (br s, 1H, COOH); MS (70 eV): *m/z* (%) = 376 (M^+). Anal. Calcd for $C_{17}H_{10}ClFN_2O_5$: C, 54.20; H, 2.68; N, 7.44. Found: C, 54.29; H, 2.55; N, 7.47%.

1-(1-Carboxy-2-phenylethyl)-7-chloro-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (**6f**): This compound was obtained in similar manner with 51% yield; m.p. 247–249 °C. IR (KBr) ν_{max}: 3494 (-OH), 2970 (CH_2), 1746 (C=O), 1650 (Ar), 1470 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 3.43–3.49 (m, 2H, CH_2), 3.92–3.99 (m, 1H, CH), 7.29–7.41 (m, 5H, ArH), 8.27 (d, *J* = 7.60 Hz, 1H, ArH), 9.31 (s, 1H, C=CH), 13.56 (br s, 1H, COOH), 14.25 (br s, 1H, COOH); MS (70 eV): *m/z* (%) = 390 (M^+). Anal. Calcd for $C_{18}H_{12}ClFN_2O_5$: C, 55.33; H, 3.10; N, 7.17. Found: C, 55.39; H, 2.99; N, 7.24%.

All the target compounds were tested for their antibacterial and antifungal activity by a broth dilution method with *Bacillus subtilis*, *Staphylococcus aureus*, *Aspergillus*, *Escherichia coli* and *Monilia albican* according to the literature.[10,11] The antimicrobial discs (diameter, 0.55 cm) were prepared at concentrations of 1 mg mL^{-1}, 0.2 mg mL^{-1} and 0.1 mg mL^{-1}, and applied to each of the culture plates previously seeded with the test bacteria. These culture plates were then incubated at 37 °C for 24 h. The preliminary antimicrobial activity was determined by the diameter of the inhibition zone. For each compound, three replicate trials were conducted against each organism.

Results and discussion

The structures of these novel naphthyridine heterocyclic compounds **6a**–**f** were confirmed by IR, ^1H NMR, MS and elemental analysis. The IR spectra of all compounds showed easily distinguishable stretching vibration peaks, such as carboxylic group (1746–1754 cm^{-1}), aromatic ring (1611–1688 cm^{-1}) and carbon-carbon double bond (1470–1489 cm^{-1}). In ^1H NMR spectrum, two kind of carboxylic group signals appear at 13.53–13.65 ppm and 14.12–14.25 ppm, which demonstrate the existence of amino acids. All the MS spectral data and elemental analyses agree well with the target molecule structures.

It should be noted that, after confirmation by IR, ^1H NMR, MS and elemental analysis, the structures of compounds **6a**–**f** prepared by one-pot method were exactly the same as those prepared by a four-step method.

All the title compounds were tested for their antibacterial and antifungal activities. From the screening results, it was discovered that these compounds have medium bioactivity against *Bacillus subtilis* and *Staphylococcus aureus*, and compounds **6b** and **6c** have some bioactivity against *Aspergillus*, *Escherichia coli* and *Monilia albican*. The detailed results are listed in Table 1.

From the preliminary biological results, all the new compounds have medium antibacterial and antifungal bioactivity. Generally speaking, the compounds containing aliphatic amino acids have greater bioactivities than that of those containing aromatic amino acids against *Bacillus subtilis* and *Staphylococcus aureus*. Although the series of compounds have less bioactivity with regard to the control, it is a preliminary research to develop new bioactive compounds and it gives valuable information. These results can evoke our keen interests to design better compounds. Especially, the compound **6b** has a wider antimicrobial spectrum than other compounds, and it has the anti-*Aspergillus* and anti-*Monilia albican* activities

Table 1 The preliminary antimicrobial activity of title compounds

	Conc. (mg mL^{-1})	6a	6b	6c	6d	6e	6f	Control[c]
Bacillus subtilis	1	1.60[a]	2.00	1.90	1.10	0.75	0.65	4.60
	0.2	1.00	1.15	1.50	0.65	0.65	–	3.80
	0.1	–[b]	1.00	0.70	–	0.60	–	3.60
Staphylococcus aureus	1	1.15	1.75	2.10	0.90	1.10	0.75	3.30
	0.2	1.00	0.95	1.50	0.60	0.65	0.60	2.90
	0.1	–	0.65	1.15	–	0.60	–	2.60
Aspergillus	1	–	0.75	0.75	0.65	–	–	–
Escherichia coli	1	–	0.60	0.65	–	–	–	2.70
Monilia albican	1	–	0.65	–	–	–	–	–

[a] Diameter of inhibition zone (cm).
[b] No inhibition.
[c] Levofloxacin hydrochloride.

which the commercial control drug Levofloxacin Hydrochloride does not possess. The results intrigued us to study its QSAR further and synthesise higher activity compounds in our following study and in-depth research work is ongoing.

Conclusions

In conclusion, we have developed a one-pot method of preparing naphthyridine heterocyclic compounds and synthesised a series of novel heterocyclic derivatives which contain natural amino acids. The preliminary bioassays results reveal that they have medium antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Aspergillus*, *Escherichia coli* and *Monilia albican*. Generally speaking, the compounds containing aliphatic amino acids have greater bioactivities than that of those containing aromatic amino acids against *Bacillus subtilis* and *Staphylococcus aureus* in naphthyridines heterocycle.

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