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ORIGINAL ARTICLE

Synthesis and antibacterial properties of new N_4-acetylated hexahydro-2,7-dioxopyrido[2,3-*f*]quinoxaline-8-carboxylic acids

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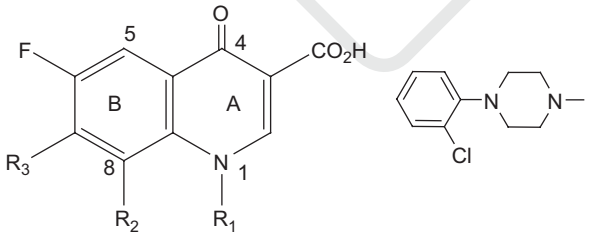
Abstract

Direct interaction between 7-chloro-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid and primary α-amino acids (exemplified by glycine, alanine, and L-valine) in aqueous ethanolic $NaHCO_3$ at 70–80°C for 24–72 h produced the respective *N*-(4-oxoquinolin-7-yl)-α-amino acids (**6a**–**c**). The latter derivatives underwent reductive lactamization upon treatment with $Na_2S_2O_4$ in aqueous ethanol to afford moderate yields of the corresponding pyrido[2,3-*f*]quinoxaline-8-carboxylic acids (**8a**–**c**). Acetylation of **8a**–**c** using acetyl chloride afforded N_4-acetylated hexahydro-2,7-dioxopyrido[2,3-*f*]quinoxaline-8-carboxylic acids (**9a**–**c**). The structures, assigned to these new heterocyclic products, are supported by analytical and spectral data. The synthesized compounds (**6a**–**c**/**9a**–**c**) showed appreciable antibacterial activity as compared with ciprofloxacin.

Keywords: Synthesis, hexahydro-2,7-dioxopyrido[2,3-f]quinoxaline-8-carboxylic acids, antibacterial activity, reductive lactamization

Introduction

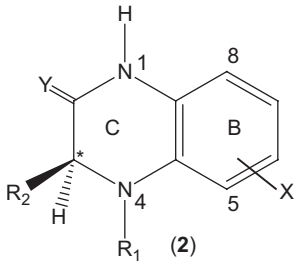
Synthetic fluoroquinolones (e.g. ciprofloxacin **1a**) represent a successful achievement towards the design and development of potent anti-infectious drugs.[1–5] Some derivatives such as 7-(4-arylpiperazin-1-yl)-6-fluoro-8-trifluoromethylquinolones, for example **1b**, were reported to act as non-nucleoside reverse transcriptase inhibitors (NNRTIs) of HIV-1 reverse transcriptase.[6–9]



1a (R_1 = *c*-C_3H_5; R_2 = H; R_3 = *N*-piperazinyl)

Ib (R_1 =Me; R_2 = CF_3; R_3 =)

On the other hand, simple or heterocyclic-fused quinoxalinones have become interesting compounds for study as antiviral agents[10–27] exemplified by the 3,4-dihydroquinoxalin-2-one core (**2**, Y=O), a second generation of NNRTIs, that is *opaviraline* (**2a**)[22–27] and the related 2-thione *talveraline* (**2b**),[14–19] are in clinical development.



X = F; Cl; OH; OMe; OEt; OPr
Y = O; S
R_1 = C_1-C_6 alkoxycarbonyl or C_2-C_6 alkenyloxycarbonyl
R_2 = C_1-C_4 havingOH;alkoxy or alkylthiosubstituents

(*S*)-**2a** (R_1 = CO_2CHMe_2; R_2 = Et; X=6-F; Y = O)

(*S*)-**2b** (R_1 = CO_2CHMe_2; R_2 = CH_2SMe; X = 6-OMe; Y = S)

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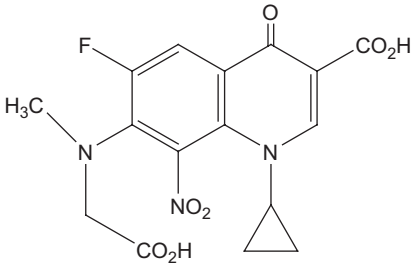
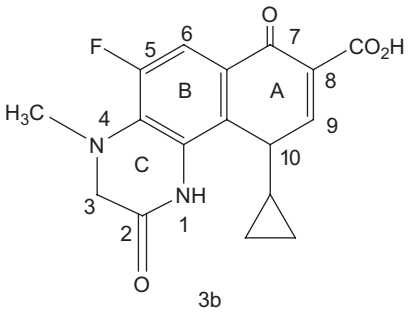
The antibacterial potency of new trihybrid system of both systems (**1** and **2**), 4-methyl-2,7-dioxo-1,2,3,4,7,10-hexahydropyrido[2,3-*f*]-quinoxaline-8-carboxylic acid, selected [*a*]-fused heterocycles, and acyclic precursors (**3a** and **b**) has been reported by our group earlier.[28]



3a



3b

Owing to the potential biological interest in these heterocyclic compounds, the present work aims at the synthesis and characterization of new 3-(substituted)-N_4-acetylated-10-cyclopropyl-5-fluoro-2,7-dioxo-hexahydropyrido[2,3-*f*]quinoxaline-3-carboxylic acids (**8a**–**c**/**9a**–**c**) (Scheme 1), a tricyclic system encompassing the structural features of both 4-quinolone and 3,4-dihydroquinoxalin-2-one chemotypes. Such hybrid heterocyclic derivatives might have interesting potential bioactive properties such as antimicrobial and/or antiviral activity, which prompted us to prepare an additional set of title compound and to examine the effect of alkylation at C-3 and/or N_4-acetylation on antibacterial activity.

Materials and methods

Chemistry

The primary α-amino acids, employed in this study, are biochemical grades (Aldrich, UK) and were used as received. 2,4-Dichloro-5-fluoro-3-nitrobenzoic acid was purchased from Acros-Belgium through local agents. Melting points (uncorrected) were determined on a Gallenkamp electrothermal melting temperature apparatus.

^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker DPX-300 instrument with TMS as internal reference. High-resolution MS-ESI (HRMS) data of selected compounds were obtained in positive ion mode using electrospray ion trap technique by collision-induced dissociation on Bruker Apex-IV (7 Tesla) or on Bruker Bio TOF III instruments. IR spectra were recorded as KBr discs on a Nicolet Impact-400 FT-IR spectrophotometer. Microanalyses were preformed at the Microanalytical Laboratory–Medicinal Chemistry Division, Faculty of Pharmacy, University of Jordan, Amman, Jordan.

7-Chloro-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (4)

It was prepared using reported methods with minor modifications.[30,31]

7-[(Carboxymethyl)amino]-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6a)

A well-stirred mixture of glycine (**5a**/0.68 g, 9 mmol), **4** (1.0 g, 3 mmol), and sodium hydrogen carbonate (1.5 g, 18 mmol) in aqueous ethanol (140 mL, 1:1 v/v) was heated at 70–80°C. The reaction mixture slowly developed a light yellow colour that changed into bright yellow, then into clear orange solution. The progress of the reaction was monitored by thin-layer chromatography (TLC), and was completed within 40–48 h. The resultant orange solution was first extracted with dichloromethane (50 mL), then the aqueous layer was separated, acidified with 3N HCl to pH 6.5, and re-extracted with dichloromethane (50 mL). The aqueous layer was separated and acidified again with 3N HCl to pH 2–3, whereby the title compound was precipitated as fluorescent yellowish solid, which was filtered, washed with cold water (3 × 20 mL), and recrystallized from ethanol.

Yield 0.90 g (82 %), mp 236–238°C (decomposition)

Anal. Calcd for $C_{15}H_{12}FN_3O_7$ (365.27): C, 49.32; H, 3.31; N, 11.50; found C, 49.02; H, 3.22; N, 11.41

IR (KBr): ν = 3090, 2915, 1740 (br), 1610, 1455, 1319, 1260, 1210 cm^{-1}

^{1}H NMR (300 MHz, DMSO-d_6): δ 0.95 (m, 4H, H_2-2′/H_2-3′), 3.67 (br m, 1H, H-1′), 4.25 (br, dd, J = 5.4 Hz, 6.2 Hz, 2H, a-CH_2–NH), 7.57 (br s, 1H, N-H), 7.95 (d, $^3J_{H\text{-}F}$ = 13.9 Hz, 1H, H-5), 8.72 (s, 1H, H-2), 13.43 (br s, 1H, CH_2–CO_2H), 14.63 (br s, 1H, C(3)–CO_2H)

^{13}C NMR (75 MHz, DMSO-d_6): δ 10.2 (C-2′/C-3′), 40.6 (C-1′), 47.1 (d, $J_{C\text{-}F}$ = 12.5 Hz, a-*C*H$_2$NH), 109.5 (C-3), 114.4 (d, $^2J_{C\text{-}F}$ = 23.0 Hz, C-5), 116.6 (d, $^3J_{C\text{-}F}$ = 7.1 Hz, C-4a), 128.0 (d, $^3J_{C\text{-}F}$ = 5.6 Hz, C-8), 135.5 (C-8a), 138.8 (d, $^2J_{C\text{-}F}$ = 14.3 Hz, C-7), 150.4 (d, $^1J_{C\text{-}F}$ = 247 Hz, C-6), 152.0 (C-2), 165.4 (C(3)–*C*O$_2$H), 171.7 (d, $J_{C\text{-}F}$ = 2.5 Hz, CH_2–*C*O$_2$H), 175.5 (d, $^4J_{C\text{-}F}$ = 2.6 Hz, C-4).

(S)-7-[(1-Carboxyethyl)amino]-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid [(S)-6b]

A stirred mixture of (*S*)-α-alanine [(*S*)-**5b**/0.8 g, 9 mmol], **4** (1.0 g, 3 mmol), and sodium hydrogen carbonate (1.5 g, 18 mmol) in 50% aqueous ethanol (140 mL) was heated at 75–80°C for 24–30 h under reflux conditions. Workup of the reaction mixture as described for **6a** above, produced the title compound as fluorescent yellow solid, which was recrystallized from ethanol.

Yield 0.86 g (76%), mp 219–221°C (decomposition)

Anal. Calcd for $C_{16}H_{14}FN_3O_7$ (379.30): C, 50.67; H, 3.72; N, 11.08; found C, 50.44; H, 3.61; N, 10.95

IR (KBr): ν = 3091, 2915, 2880, 1730, 1610, 1450, 1310, 1250, 1215 cm^{-1}

MS (EI): *m*/*z* (% rel. int.): 379 (6, M^+), 359 (2), 335 (20), 301 (9), 289 (24), 257 (50), 244 (58), 217 (30), 188 (21), 174 (13), 147 (11), 107 (9), 85 (61), 83 (100)

HRMS Calcd for $C_{16}H_{14}FN_3O_7$ 379.08153, Found 379.08219

^{1}H NMR (300 MHz, DMSO-d_6): δ 0.94 (m, 4H, H_2-2′/H_2-3′), 1.46 (d, *J* = 6.8 Hz, 3H, CH_3), 3.67 (m, 1H, H-1′), 4.62 (dq, *J* = 6.8 Hz, 7.1 Hz, 1H, α-C*H*Me), 7.36 (d, *J* = 7.1 Hz, 1H, N-H), 8.03 (d, $^3J_{H\text{-}F}$ = 13.7 Hz, 1H, H-5), 8.75 (s, 1H, H-2), 13.30 (br s, 1H, CH–CO_2H), 14.59 (br s, 1H, C(3)–CO_2H)

^{13}C NMR (75 MHz, DMSO-d_6): δ 10.1 (C-2′/C-3′), 19.8 (d, $J_{C\text{-}F}$ = 2.9 Hz, CH_3), 40.7 (C-1′), 53.8 (d, $J_{C\text{-}F}$ = 12 Hz, α-*C*HMe), 109.7 (C-3), 114.9 (d, $^2J_{C\text{-}F}$ = 22.9 Hz, C-5), 117.3 (d, $^3J_{C\text{-}F}$ = 7.1 Hz, C-4a), 128.7 (d, $^3J_{C\text{-}F}$ = 5 Hz, C-8), 135.7 (C-8a), 137.9 (d, $^2J_{C\text{-}F}$ = 14.7 Hz, C-7), 150.2 (d, $^1J_{C\text{-}F}$ = 248 Hz, C-6), 152.1 (C-2), 165.3 (C(3)–*C*O_2H), 174.2 (CH–*C*O_2H), 175.5 (d, $^4J_{C\text{-}F}$ = 2.2 Hz, C-4).

(S)-7-(1-Carboxy-2-methylpropylamino)-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid [(S)-6c]

A stirred mixture of (*S*)-L-valine [1.0 g, 9 mmol], **4** (1.0 g, 3 mmol), and sodium hydrogen carbonate (1.5 g, 18 mmol) in 50% aqueous ethanol (140 mL) was heated at 75–80°C for 100–120 h under reflux conditions. Workup of the reaction mixture as described for **6a** above produced the title compound as fluorescent yellow solid, which was recrystallized from ethanol.

Yield 1.1 g (90%), mp 217–222°C (decomposition)

Anal. Calcd for $C_{18}H_{18}FN_3O_7$ (407.35): C, 53.07; H, 4.45; N, 10.32; found: C, 52.83; H, 4.69; N, 10.68

IR (KBr): ν = 3308, 3070, 2971, 1733, 1700, 1629, 1549, 1518, 1469, 1321, 1257, 1216, 1144, 1033 cm^{-1}

MS (FAB): *m*/*z* (% rel. int.): 408 [100, $(M+H)^+$/Calcd. for $C_{18}H_{18}FN_3O_7$ 407 (M)]

^{1}H NMR (300 MHz, DMSO-d_6): δ 0.89, 0.95 (2d, *J* = 6.8 Hz, 6.0 H, (CH_3)$_2$–CH), 0.92 (m, 4H, H_2-2′/H_2-3′), 2.21 (m, 1H, C*H*Me_2), 3.68 (m, 1H, H-1′), 4.50 (br d, *J* = 7.2 Hz, C*H*–CO_2H), 7.21 (d, *J* = 8.0 Hz, 1H, *N*-H), 8.07 (d, $^3J_{H\text{-}F}$ = 13.5 Hz, 1H, H-5), 8.77 (s, 1H, H-2), 13.39 (br s, 1H, CH–CO_2H), 14.55 (br s, 1H, C_3–CO_2H)

^{13}C NMR (75 MHz, DMSO-d_6): δ 10.1 (C-2′/C-3′), 18.0, 18.5 ((CH_3)$_2$), 31.6 (*C*HMe_2), 40.9 (C-1′), 63.2 (d, $J_{C\text{-}F}$ = 11.3 Hz, *C*H-NH), 109.8 (C-3), 115.2 (d, $^2J_{C\text{-}F}$ = 22.7 Hz, C-5), 117.8 (d, $^3J_{C\text{-}F}$ = 7.1 Hz, C-4a), 129.3 (d, $^3J_{C\text{-}F}$ = 5.4 Hz, C-8), 135.8 (C-8a), 138.1 (d, $^2J_{C\text{-}F}$ = 14.9 Hz, C-7), 150.4 (d, $^1J_{C\text{-}F}$ = 248 Hz, C-6), 152.3 (C-2), 165.3 (C_3–*C*O_2H), 172.7 (CH–*C*O_2H), 175.6 (d, $^4J_{C\text{-}F}$ = 2.5 Hz, C-4).

10-Cyclopropyl-5-fluoro-2,7-dioxo-1,2,3,4,7,10-hexahydropyrido[2,3-f]quinoxaline-8-carboxylic acid [8a]

To a vigorously stirred solution of 7-[(carboxymethyl) amino]-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6a**/0.37 g, 1.0 mmol) and potassium carbonate (0.96 g, 7.0 mmol) in water (20 mL), was added portion wise a solution of sodium dithionite (1.22 g, 7.0 mmol) in water (5 mL). Following each addition, the solution acquired a dark brown colour, which shortly faded away (1–2 min). The reaction mixture was further stirred at room temperature for 25 min. Thereafter, the pH of the resultant solution was adjusted to about 4 by slow addition of 3N HCl, the precipitated product was filtered, washed successively with water and methanol, air-dried, and recrystallized from ethanol.

Yield 0.17 g (54%), mp 290-292°C (decomposition/the crystals darken at 270°C)

Anal. Calcd for $C_{15}H_{12}FN_3O_4$ (317.27): C, 56.78; H, 3.81; N, 13.24; found C, 56.54; H, 3.77; N, 13.08

IR (KBr): ν = 3270, 2980, 1730, 1680, 1615, 1583, 1525, 1444, 1380, 1315, 1185, 1082, 1010 cm^{-1}

MS (TOF, ES$^+$): *m*/*z* 318 $(M + H)^+$, HRMS Calcd for $C_{15}H_{13}FN_3O_4$ 318.0890, found 318.0885; *m*/*z* 340 $(M + Na)^+$, HRMS Calcd for $C_{15}H_{12}FN_3O_4Na$ 340.0709, found 340.0704

^{1}H NMR (300 MHz, 3% NaOD in D_2O): δ 0.21, 0.69 (2m, 4H, H_2-2′/H_2-3′), 3.27 (s, 2H, H_2-3), 3.98 (m, 1H, H-1′), 7.00 (d, $^3J_{H\text{-}F}$ = 10.8 Hz, 1H, H-6), 8.17 (s, 1H, H-9)

^{13}C NMR (75 MHz, 3% NaOD in D_2O): δ 9.4 (C-2′/C-3′), 40.1 (C-1′), 42.9 (C-3), 102.3 (d, $^2J_{C\text{-}F}$ = 20.2 Hz, C-6), 114.8 (C-8), 119.9 (d, $^3J_{C\text{-}F}$ = 7 Hz, C-6a), 128.0 (d, $^3J_{C\text{-}F}$ = 3.7 Hz, C-10b), 130.0 (d, $^2J_{C\text{-}F}$ = 15.5 Hz, C-4a), 131.9 (C-10a), 148.9 (C-9),149.2 (d, $^1J_{C\text{-}F}$ = 240 Hz, C-5), 168.1 (C-2), 173.1 (C(8)–CO_2^-), 175.6 (d, $^4J_{C\text{-}F}$ = 2.9 Hz, C-7).

(S)-10-Cyclopropyl-5-fluoro-3-methyl-2,7-dioxo-1,2,3,4,7,10-hexahydropyrido-[2,3-f]-quinoxaline-8-carboxylic acid [(S)-8b]

To a stirred solution of **6b** (0.38 g, 1.0 mmol) and potassium carbonate (0.96 g, 7.0 mmol) in water (20 mL), was added portion wise a solution of sodium dithionite (1.22 g, 7.0 mmol) in water (5 mL). The reaction mixture was further stirred at room temperature for 10 min, and worked up as described for **8a** above. The title product was recrystallized from chloroform/methanol (3:1, v/v) to furnish pale yellow fine crystals.

Yield 0.21 g (64%), mp 315–317°C (decomposition/the crystals darken at 290°C)

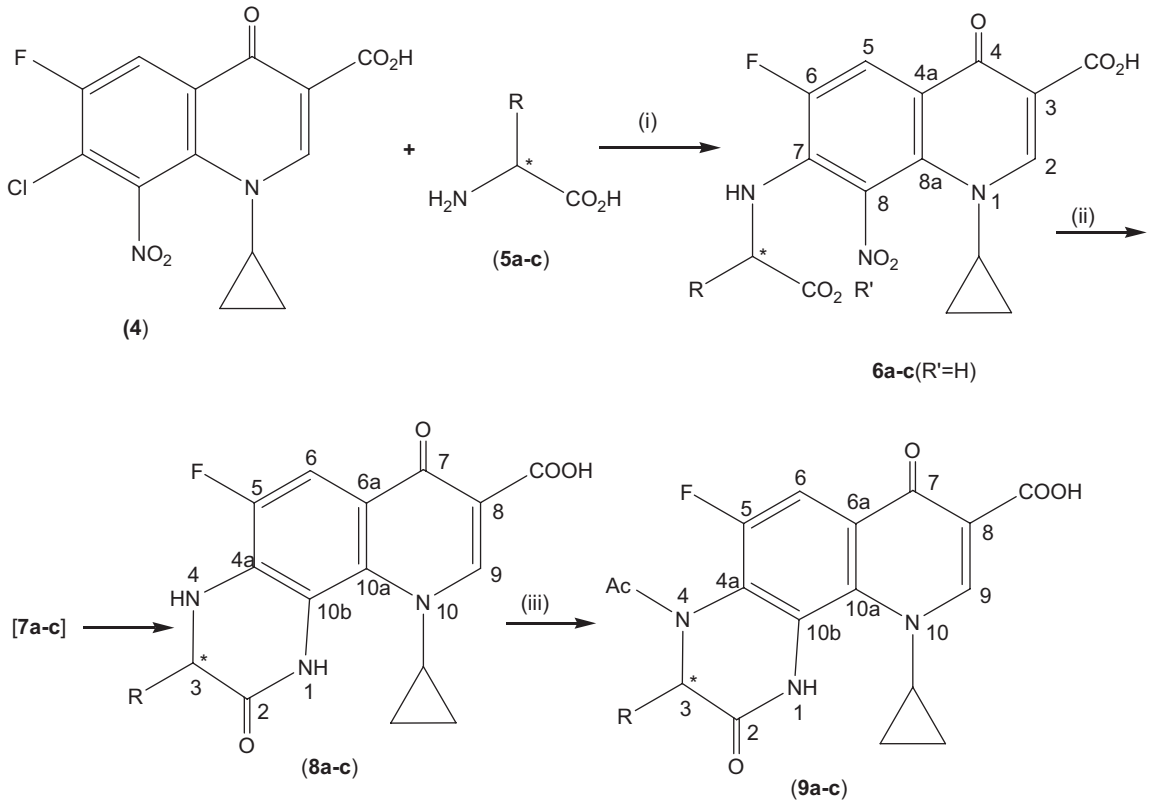
Anal. Calcd for $C_{16}H_{14}FN_3O_4$ (331.30): C, 58.01; H, 4.26; N, 12.68; found C, 57.86; H, 4.18; N, 12.55

IR (KBr): ν = 3270, 2996, 1724, 1675, 1611, 1585, 1520, 1435, 1380, 1311, 1180, 1080, 1017 cm^{-1}

MS (TOF ES$^+$): *m*/*z* 354 $(M+Na)^+$, HRMS Calcd for $C_{16}H_{14}FN_3O_4Na$ 354.0866, found 354.0861

^{1}H NMR (300 MHz, DMSO-d_6): δ 0.97, 1.05 (2m, 4H, H_2-2′/H_2-3′), 1.32 (d, *J* = 6.6 Hz, 3H, CH_3), 3.93 (br q, *J* = 6.6 Hz, 1H, H-3), 4.48 (m, 1H, H-1′), 7.48 (br s, 1H, N(4)-H), 7.62 (d, $^3J_{H\text{-}F}$ = 10.6 Hz, 1H, H-6), 8.65 (s, 1H, H-9), 10.34 (s, 1H, lactam N(1)-H), 15.13 (s, 1H, CO_2H)

^{13}C NMR (75 MHz, DMSO-d_6): δ 10.2 (C-2′/C-3′), 16.2 (CH_3), 39.0 (C-1′), 50.4 (C-3), 105.4 (d, $^2J_{C\text{-}F}$ = 19.6 Hz, C-6), 107.2 (C-8), 116.1 (d, $^3J_{C\text{-}F}$ = 6.3 Hz, C-10b), 117.2 (d, $^3J_{C\text{-}F}$ = 7.1 Hz, C-6a), 129.3 (C-10a), 131.9 (d, $^2J_{C\text{-}F}$ = 18.2 Hz, C-4a), 149.4 (d, $^1J_{C\text{-}F}$ = 242 Hz, C-5), 151.0 (C-9), 166.2 (CO_2H), 166.5 (C-2), 176.6 (d, $^4J_{C\text{-}F}$ = 3.1 Hz, C-7).



compounds **5-9**

entry	a	b	c
R	H	Me	i-*pr*

reagents and conditions:
(i) $NaHCO_3$, 50% aq.EtOH/Δ
(ii) $Na_2S_2O_4$, aq.K_2CO_3 /20°C
(iii) CH_3COCl, TEA, 60–65°C, 48hr

Scheme 1. Synthesis of N_4-acetylated hexahydro-2,7-dioxopyrido[2,3-*f*]quinoxaline-8-carboxylic acids (**9a-c**).

(S)-10-Cyclopropyl-5-fluoro-3-isopropyl-2,7-dioxo-1,2,3,4,7,10-hexahydropyrido-[2,3-f]quinoxaline-8-carboxylic acid [(S)-8c]

To a stirred solution of (±)-7-(1-carboxy-2-methylpropylamino)-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6c**, 0.36 g, 1 mmol) and (0.96 g, 7 mmol) of potassium carbonate in 20 mL water, an aqueous solution of sodium dithionite (0.87 g, 5 mmol) in 5 mL water was added drop wise. The reaction mixture was further stirred at room temperature for 15–25 min. Thereafter, the pH of the solution was adjusted to about 4.0. The precipitated product was filtered, washed with water, and air-dried. Then further re-crystallization was carried out from acetone and ethanol (1:1), yielding faint yellow crystals.

Yield 0.33 g (92%), mp 285–291°C (decomposition)

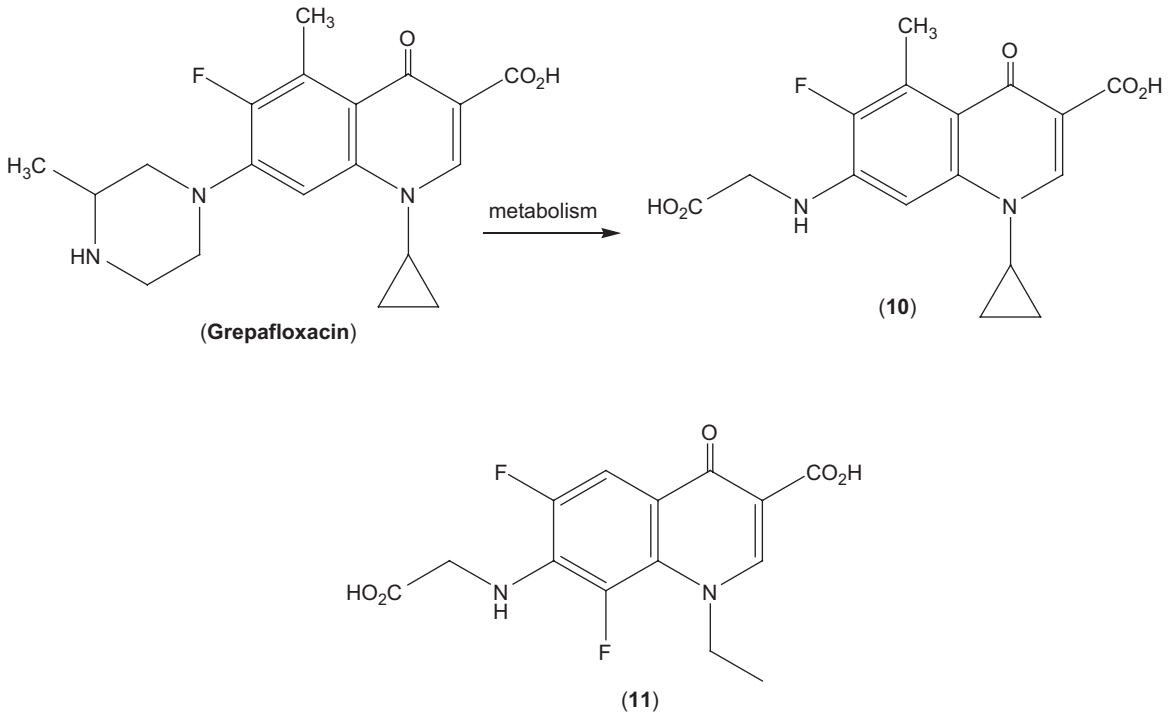
Anal. Calcd for $C_{18}H_{18}FN_3O_4$ (359.35): C, 60.16; H, 5.05; N, 11.69; found: C, 60.22; H, 5.04, N, 11.76

IR (KBr): ν = 3282, 2996, 1724, 1683, 1621, 1581, 1530, 1436, 1385, 1322, 1184, 1083, 1027 cm^{-1}

MS (EI): *m*/*z* (% rel. int.): 359 (38), 315 (100), 288 (7), 272 (39), 257 (55), 244 (40), 230 (39), 216 (33), 203 (44), 188 (52), 175 (74), 163 (20), 147 (21), 135 (11), 121 (9), 108 (7), 95 (3) HRMS Calcd for $C_{18}H_{18}FN_3O_4$ 359.12810, found 359.12940

^{1}H NMR (300 MHz, DMSO-d_6): δ 0.79, 0.89 (2m, 2H, overlapped with a CH_3 doublet), and 1.06, 1.26 (2m, 2H) (H_2-2′/H_2-3′), 0.90 (d, *J* = 6.5 Hz, 3H) and 0.94 (d, *J* = 6.7 Hz, 3H) [CH(CH_3)$_2$], 1.88 (m, 1H, C*H*Me$_2$), 3.53 (dd, *J* = 8.1, 2.9 Hz, 1H, H-3), 4.50 (br m, 1H, H-1′), 7.60 (d, $^3J_{H\text{-}F}$ = 10.5 Hz, 1H, H-6), 7.77 (br s, 1H, N(4)-*H*), 8.62 (s, 1H, H-9), 10.36 (s, 1H, lactam N(1)-*H*), 15.11 (s, 1H, CO_2H)

^{13}C NMR (75 MHz, DMSO-d_6): δ 9.0, 11.3 (C-2′/C-3′), 18.9, 19.2 [CH(*C*H_3)$_2$], 29.5 (*C*HMe$_2$), 39.0 (C-1′), 61.2 (C-3), 105.4 (d, $^2J_{C\text{-}F}$ = 19.4 Hz, C-6), 107.1 (C-8), 115.8 (d, $^3J_{C\text{-}F}$ = 6.4 Hz, C-10b), 116.9 (d, $^3J_{C\text{-}F}$ = 7.1 Hz, C-6a), 129.3 (C-10a), 130.7 (d, $^2J_{C\text{-}F}$ = 18.3 Hz, C-4a), 149.3 (d, $^1J_{C\text{-}F}$ = 242



Scheme 2. Fluoroquinolones (**10, 11**) with glycine substituent.

Hz, C-5), 150.9 (C-9), 164.5 (C-2), 166.2 (CO_2H), 176.6 (d, $^4J_{C\text{-}F}$ = 3.2 Hz, C-7).

4-Acetyl-10-cyclopropyl-5-fluoro-2,7-dioxo-1,2,3,4,7,10-hexahydropyrido[2,3-f]quinoxaline-8-carboxylic acid (9a)

To a stirred solution of 10-cyclopropyl-5-fluoro-2,7-dioxo-1,2,3,4,7,10-hexahydropyrido[2,3-*f*]quinoxaline-8-carboxylic acid (**8a**, 0.32 g, 1 mmol) and (0.3 mL) of triethylamine in 20 mL chloroform, acetyl chloride (0.08 mL, 1 mmol) in 5 mL chloroform was added drop wise. The reaction mixture was refluxed for 48 h at 60–65°C. The chloroform was removed under pressure. The product was collected, washed with ice-cold water, and air-dried. Then further re-crystallization was carried out from acetone and ethanol (1:1), yielding white crystals.

Yield 0.19 g (53%), mp 300–306°C (decomposition)

Anal. Calcd for $C_{17}H_{14}FN_3O_5$ (359.31): C, 56.83; H, 3.93; N, 11.69; found C, 56.84; H, 3.97; N, 11.58

IR (KBr): ν = 3270, 2996, 1730, 1680, 1620, 1580, 1523, 1440, 1376, 1313, 1183, 1070, 1000 cm^{-1}

HRMS Calcd for $C_{17}H_{13}FN_3O_5Na$ 381.29039, found 381.29077

^{1}H NMR (300 MHz, 3% NaOD in D_2O): δ 0.25, 0.71 (2m, 4H, H_2-2′/H_2-3′), 1.80 (m, 1H, H-1′), 2.25 (s, 3H,–CO–CH_3), 4.07 (s, 2H, H_2-3), 7.50 (d, $^3J_{H\text{-}F}$ = 10.8 Hz, 1H, H-6), 8.17 (s, 1H, H-9)

^{13}C NMR (75 MHz, 3% NaOD in D_2O): δ 9.3 (C-2′/C-3′), 21.2 (–CO*C*H_3), 40.2 (C-1′), 42.0 (C-3), 102.6 (d, $^2J_{C\text{-}F}$ = 20.2 Hz, C-6), 115.0 (C-8), 120.0 (d, $^3J_{C\text{-}F}$ = 7 Hz, C-6a), 128.1 (d, $^3J_{C\text{-}F}$ = 3.7 Hz, C-10b), 130.3 (d, $^2J_{C\text{-}F}$ = 15.5 Hz, C-4a), 132.1 (C-10a), 148.7 (C-9), 149.1 (d, $^1J_{C\text{-}F}$ = 240 Hz, C-5), 168.5 (C-2), 170.2 (–*C*OCH_3), 173.2 (C(8)–CO_2^-), 175.6 (d, $^4J_{C\text{-}F}$ = 2.9 Hz, C-7).

Table 1. *In vitro* antibacterial activity (minimum inhibitory concentration (MIC) values, μg/mL) of model compounds of the formulae **6a–c**/**9a–c** as compared with the reference ciprofloxacin.

Compound number	Log*P* (cLog*P*)*	*S. aureus* ATCC 6538a MIC (μg/mL)	*E. coli* ATCC 8739 MIC (μg/mL)
6a	0.46 (1.57)	10.1	20.1
6b	0.95 (1.88)	9.5	19.5
6c	1.83 (2.81)	0.4	22.5
8a	–0.36 (0.79)	20.0	22.0
8b	0.13 (1.32)	5.8	11.5
8c	1.01 (2.24)	80.0	19
9a	–0.72 (0.65)	>100	>100
9b	–0.22 (1.17)	>100	>100
9c	0.66 (2.09)	>100	>100
3a	1.24 (1.76)	*12.0*	*12.0*
3b	0.42 (1.48)	*10.5*	*0.7*
Ciprofloxacin	—	*1.23*	*0.31*

*Log*P* (cLog*P*) were calculated using CambridgeSoft Chem Draw Ultra Version 8.0.

(S)-4-Acetyl-10-cyclopropyl-5-fluoro-3-methyl-2,7-dioxo-1,2,3,4,7,10-hexahydropyrido[2,3-f]quinoxaline-8-carboxylic acid [(S)-9b]

Yield 0.24 g (65%), mp 302–305°C (decomposition)

Anal. Calcd for $C_{18}H_{16}FN_3O_5$ (373.34): C, 57.91; H, 4.32; N, 11.26; found C, 57.86; H, 4.35; N, 11.21

IR (KBr): ν = 3270, 2996, 1724, 1675, 1611, 1585, 1520, 1435, 1380, 1311, 1180, 1080, 1017 cm^{-1}

MS (TOF, ES+): *m/z* 374 (M + H)+, HRMS Calcd for $C_{18}H_{16}FN_3O_5$ 373.10740, found 373.1108

^{1}H NMR (300 MHz, DMSO-d_6): δ 0.90, 1.11 (2m, 4H, H_2-2′/H_2-3′), 1.35 (d, *J* = 6.6 Hz, 3H, CH_3), 1.89 (m, 1H, H-1′), 2.30 (s, 3H,–CO–CH_3), 3.95 (br q, *J* = 6.6 Hz, 1H, H-3), 7.62 (d, $^3J_{H\text{-}F}$ = 10.6 Hz, 1H, H-6), 8.60 (s, 1H, H-9), 9.90 (s, 1H, lactam N(1)-H), 15.13 (s, 1H, CO_2H)

^{13}C NMR (75 MHz, DMSO-d_6): δ 10.1 (C-2′/C-3′), 16.5 (CH_3), 20.9 (–COCH_3), 39.0 (C-1′), 51.0 (C-3), 105.0 (d, $^2J_{C\text{-}F}$ = 19.6 Hz, C-6), 107.8 (C-8), 116.4 (d, $^3J_{C\text{-}F}$ = 6.3 Hz, C-10b), 118.1 (d, $^3J_{C\text{-}F}$ = 7.1 Hz, C-6a), 130.1 (C-10a), 132.1 (d, $^2J_{C\text{-}F}$ = 18.2 Hz, C-4a), 148.4 (d, $^1J_{C\text{-}F}$ = 242 Hz, C-5), 151.2 (C-9), 166.3 (CO_2H), 166.8 (C-2), 171.1 (–COCH_3), 176.6 (d, $^4J_{C\text{-}F}$ = 3.1 Hz, C-7).

(S)-4-Acetyl-10-cyclopropyl-5-fluoro-1,2,3,4,7,10-hexahydro-3-isopropyl-2,7-dioxopyrido[2,3-f] quinoxaline-8-carboxylic acid [(S)-9c]

Yield 0.32 g (80%), mp 305–309°C (decomposition)

Anal. Calcd for $C_{20}H_{20}FN_3O_5$ (401.39): C, 59.85; H, 5.02; N, 10.47; found: C, 59.91; H, 5.03, N, 10.50

IR (KBr): *ν* = 3182, 2996, 1724, 1683, 1621, 1581, 1530, 1436, 1385, 1322, 1184, 1083, 1027 cm^{-1}

MS (TOF, ES+): *m/z* 402 (M + H)+, HRMS Calcd for $C_{20}H_{20}FN_3O_5$ 401.13870, found 301.14010

^{1}H NMR (300 MHz, DMSO-d_6): δ 0.78 , 0.82 (2m, 2H, overlapped with a CH_3 doublet), and 0.90, 0.92 (2m, 2H) (H_2-2′/H_2-3′), 0.96 (d, *J* = 6.5 Hz, 3H) and 1.14 (d, *J* = 6.7 Hz, 3H) [CH(CH_3)$_2$], 1.52 (m, 1H, C*H*Me$_2$), 2.05 (br m, 1H, H-1′), 2.65 (s, 3H,–CO–CH_3); 3.51 (dd, *J* = 8.1, 2.9 Hz, 1H, H-3), 5.25 (s, 1H, lactam N(1)-*H*), 7.55 (d, $^3J_{H\text{-}F}$ = 10.2 Hz, 1H, H-6), 8.85 (s, 1H, H-9), 14.01 (s, 1H, CO_2H)

^{13}C NMR (75 MHz, DMSO-d_6): δ 6.2, 9.5 (C-2′/C-3′), 18.0, 19.1 (CH(*C*H$_3$)$_2$), 21.1 (–CO*C*H$_3$), 28.5 (*C*HMe$_2$), 37.0 (C-1′), 64.1 (C-3), 108.1 (d, $^2J_{C\text{-}F}$ = 17.4 Hz, C-6), 107.5 (C-8), 115.1 (d, $^3J_{C\text{-}F}$ = 6.3 Hz, C-10b), 117.8 (d, $^3J_{C\text{-}F}$ = 7.1 Hz, C-6a), 127.7 (d, $^2J_{C\text{-}F}$ = 18.3 Hz, C-4a), 130.3 (C-10a), 150.1 (d, $^1J_{C\text{-}F}$ = 241 Hz, C-5), 153.1 (C-9), 166.2 (*C*O$_2$H), 170.5 (C-2), 171.1 (–*C*O–CH$_3$), 176.5 (d, $^4J_{C\text{-}F}$ = 3.2 Hz, C-7).

Antibacterial activity

The minimum inhibitory concentrations (MICs) were determined by the conventional broth dilution method using two serial dilution techniques. The standardization of bacterial test suspension was carried out according to *Mcfarland standard method* as described by the National Committee for Clinical Laboratories Standard (NCCLS) (1993). Stock solutions of the test compounds were prepared using dimethyl sulphoxide (DMSO) to obtain concentration of 200 μg/cm^3. Serial dilutions were prepared to obtain test concentrations ranging from 100 to 0.098 μg/cm^3. Each tube was then inoculated with 0.1 cm^3 of the cultured bacteria (containing approximately 1 to 2×10^8 CFU/cm^3), mixed, and incubated at 37°C for 24 h. Growth inhibitions with concentrations at 10 μg/cm^3 or lower were carried out in duplicates.

All test tubes showing positive/negative growth were confirmed by the agar plate method. The results were recorded according to presence and absence of growth. The MICs were calculated as the average concentration of the test agent in the broth tubes showing consecutive positive and negative growth. *In vitro* antibacterial activity of the model compounds are given in Table 1.

Control tests for each experiment were performed. Positive growth control was performed by inoculating 0.1 cm^3 of the cultured bacteria to a test tube of the culture medium without the test compound. Negative growth control was also performed using uninoculated tube of medium without the test compound. Both were incubated for 24 h at 37°C for both types of bacteria.

Positive and negative controls were performed with DMSO at the same dilution as in experiment to assure that it is incapable of inhibiting the growth of bacteria. Test tubes were incubated at 37°C 24 h and found to have no influence on microbial growth at tested concentration.[31]

Results and discussion

Chemistry

The desired 10-cyclopropyl-5-fluoro-2,7-dioxo-hexahydropyrido[2,3-*f*]quinoxaline-3-carboxylic acids (e.g. **8a**–**c**) and N_4-acetylated compounds (**9a**–**c**), incorporating masked α-amino acid residues are hitherto undescribed and for which a synthetic route is outlined herein (Scheme 1). This preparative approach entails the construction of 3,4-dihydropyrazinone moiety onto an 1-cyclopropyl-8-nitro-4-quinolone skeleton bearing α-amino acid at C-7 (compounds **6a**–**c**; Scheme 1), and is analogous to the methodology reported for the preparation of 3,4-dihydroquinoxalinones starting from *N*-(2-nitrophenyl)- or *N*-(2,4-dinitrophenyl)amino acids.[29] The new compounds **6a**–**c**, required in this study, are readily prepared by direct interaction of 7-chloro-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4**) with the corresponding α-amino acid (**5a**–**c**) in aqueous ethanolic sodium hydrogen carbonate solution at 60–90°C for 1–5 days as detailed in the "Materials and methods" section. The required synthon (**4**), substituted 4(1*H*)-oxoquinoline-3-carboxylic acid, is prepared from 2,4-dichloro-5-fluoro-3-nitrobenzoic acid and ethyl 3-(dimethylamino)-acrylate according to a reported procedure.[30,31]

Over the last two decades, a plethora of secondary amines[1–5] and amino alcohols[32] were introduced at C-7 of 6-fluoro-4-oxoquinoline-3-carboxylic acid for enhancement of the antibacterial activity, attainment of good safety profile and optimal blend of favourable bioproperties. However, incorporation of α-amino acids thereat is limited to two reports on the glycine derivatives (**10** and **11**; Scheme 2). The former compound (**10**) was detected as a metabolite (beside others) of *grepafloxacin* following its oral administration in mammalians (Scheme 2), and was authenticated by a preparative sample obtainable

from the reaction of glycine with 7-chloro-1-cyclopropyl-6-fluoro-5-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.[33] The other related glycine derivative (**11**) has likewise been prepared via the reaction of glycine with 1-ethyl-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.[34]

Reduction of **6a–c** with sodium dithionite in aqueous potassium carbonate medium converts the nitro group to an amino group, and is followed by spontaneous lactamization of the resultant intermediates (**7a–c**) to afford good yields of the corresponding target product **8a–c** in fairly pure form. Experimentally, this reductive cyclization procedure has added advantages of being quite simple and fast (5–30 min).[28,31] Acetylation of **8a–c** with acetyl chloride in presence of triethylamine produces N_4-acetylated compounds (**9a–c**).

The constitutions of the new hexahydropyrido[2,3-*f*] quinoxalines (**8a–c**/**9a–c**) and their fluoroquinolone precursors (**6a–c**) were based on IR, MS, ^{1}H NMR and ^{13}C NMR spectral data, which were fully consistent with the proposed structures. Signal assignments to the various protons and carbons followed from DEPT and 2D (COSY, HMQC, and HMBC) experiments. For **8a–c**/**9a–c**, long-range correlations are observed between H-9 and each of C-10a, C-7, C-1′, and C(3)–*C*O_2H, as well as between H-6 and each of C-10a, C-7, and C-4a. Corresponding long-range correlations are also observed in **6a–c** between H-2, H-5, and their neighbour carbons. Those skeletal carbons (**4a,5,6,7,8,8a**) of the fused benzenoid ring of compounds **6a–c** are recognizable by their signal splitting (doublet) arising from coupling with the fluorine atom, while through space ^{13}C…^{19}F coupling is noticeable for the stereogenic α-*C**H carbon (d, $J_{C\text{-}F}$ δ 12 Hz) in **6a–c**.

It turned out that the H-5 in **6a–c**, which resonates at ca. 8 ppm (d, $^3J_{H\text{-}F}$ δ 13.5 Hz), shows sizable upfield shift in the corresponding annulated products **8a–c** and **9a–c** [H-6: d δ 7.6 ppm (d, $^3J_{H\text{-}F}$ δ10.3 Hz)]. Similarly, the α-*C***H* proton in **8a–c** is shielded as compared with its resonance position in the ^{1}H NMR spectra of the respective uncyclized precursors (**6a–c**). Yet, the cyclopropyl methine proton (H-1′), which resonates at ca. 3.6 ppm in **6a–c**, experiences downfield shift (to about 4.4 ppm) in the annulated compounds (**8a–c**/**9a–c**), probably due to the deshielding effect caused by the proximal electron pair of the pyrazinoid ring nitrogen (N1).

Antibacterial activity

In vitro antibacterial screening of the compounds **9a–c** along with **3a** and **b** indicates that acetylation of the–N_4– of the quinoxaline ring (**9**) decreases the over all antibacterial activity, whereas methylation of–N_4– produces more active compound (**3b**), in particular against Gram-negative bacteria.[28] The quinoxaline derivative **8b** is most active derivative of the non-acetylated series (**8a–c**) with MIC 5.8 µg/mL against *Staphylococcus aureus* ATCC 6538a and 11.5 µg/mL against *Escherichia coli* ATCC 8739. Methylation of the C-3 of quinoxaline increases the activity against *S. aureus* ATCC 6538a as well as *E. coli* ATCC 8739 (compound **8b** vs. **8a**), although introduction of isopropyl group reduces the antibacterial activity (compound **8c** vs. **8b**). The compound **6a** is more active against Gram-positive bacteria (*S. aureus* ATCC 6538a) than Gram-negative (*E. coli* ATCC 8739). The fluoroquinolone precursors **6a–c** have excellent antibacterial activity than corresponding quinoxaline **8a–c** derivatives. It might be due to change in the lipophilicity of the compound. Finally, it has been observed that the preference of quinolone antibacterial for Gram-positive or Gram-negative bacteria is related to the lipophilicity of the side chain at C-7 of the quinolone antibacterials (**6a–c**). It is generally assumed that the more lipophilic quinolone can penetrate better the lipophilic cell membrane of Gram-positive bacteria, whereas less lipophilic compounds are more liable to penetrate the cell wall of Gram-negative bacteria. However, the overall activity of the compound depends on the hydrophilic/lipophilic balance of the compound.

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