Synthesis and Antibacterial Potency of 4-Methyl-2,7-dioxo-1,2,3,4,7,10-hexahydropyrido[2,3-f]quinoxaline-8-carboxylic acid, Selected [$\alpha$]-Fused Heterocycles and Acyclic Precursors

Yusuf M. Al-Hiaria, Ali M. Qaisi a, Mohammad Y. Abu Shuheil b, Mustafa M. El-Abadelah b, and Wolfgang Voelterc

a Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan, Amman, Jordan
b Chemistry Department, Faculty of Science, University of Jordan, Amman, Jordan
c Interfakultäres Institut für Biochemie, Universität Tübingen, Hoppe-Seyler Straße 4, D-72076 Tübingen, Germany

Reprint requests to Prof. W. Voelter. E-mail: wolfgang.voelter@uni-tuebingen.de or to Prof. M. M. El-Abadelah. E-mail: mustelab@ju.edu.jo

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The reaction of 7-chloro-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (7) with each of sarcosine and (±)-pipercolinic acid afforded the corresponding N-(4-oxoquinolin-7-yl)-$\alpha$-amino acids 8 and 9. Reductive lactamization of the latter with sodium dithionite gave hexahydropyrido[2,3-f]quinoxaline (10) and octahydrodipyrido[1,2-a:2,3-f]quinoxaline (11) derivatives, respectively. Compounds 8 – 11 and their homologs 1 – 6, accessible from (S)-proline, (2S,4R)-4-hydroxyproline and (S)-tetrahydroisoquinoline-3-carboxylic acid exhibit good to excellent antibacterial activities against E. coli and S. aureus.

Key words: Pipecolinic Acid, Sarcosine, 7-Chloro-8-nitro-4-oxoquinoline-3-carboxylic Acid, SNAr Reactions, Reductive Lactamization, Antibacterial Activity

Introduction

New drugs are desperately searched for in order to counteract the alarming increase in evolution of antimicrobial resistance to existing antibiotics. There is a continuing need for the discovery and development of new agents against resistant strains with broad therapeutic index [1]. Synthetic fluoroquinolones [2, 3], e.g. ciprofloxacin [2], represent a recent successful achievement towards the design and development of potent antiinfectious drugs. On the other hand, substituted quinoxalinones have become interesting compounds for study of their bioproperties such as antibacterial [4] and antitumor [5] activity. Quite recently, we have described the synthesis of heterocycles [$\alpha$]-fused onto pyrido[2,3-f]quinoxaline-3-carboxylic acids, exemplified by compounds 4 – 6 via reductive lactamization of their respective acyclic fluoroquinolone precursors 1 – 3 [6]. The heterocyclic derivatives 4 – 6 exhibit excellent potential as antitumor therapeutic agents [6]

In this study, we have investigated the antibacterial activity of compounds 1 – 6, which were shown to display good to excellent potency against Gram negative and Gram positive bacterial strains. These findings have prompted us to prepare an additional set of related heterocycles 8 – 11 (Schemes 1 and 2) for further antibacterial testing.

Results and Discussion

Direct interaction between 7-chloro-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (7) with each of sarcosine and (±)-pipercolinic acid afforded the respective N-(4-oxoquinolin-7-yl)-$\alpha$-amino acids 8a, 9a which were converted into their methyl esters 8b, 9b upon reaction with diazomethane in ether (Scheme 1).

Reductive lactamization of 8a, 9a with sodium dithionite delivered the respective tri- and tetra cyclic derivatives, namely hexahydro[2,3-f]quinoxaline 10 and octahydrodipyrido[1,2-a:2,3-f]quinoxaline 11 (Scheme 2). This synthetic approach is similar to that described for compounds 1 – 6 [6] and is analogous to the methodology recently reported [7] for the preparation of heterocyclic [$c$]-fused 3,4-di-
hydroquinoxalin-2-ones starting from \( N-(2\text{-nitrophenyl}) \) cyclic imino acids.

The spectral (IR, MS, NMR) and microanalytical data for the new compounds 8–11 are in accordance with the assigned structures, and are given in the Experimental Section. Thus, the mass spectra of 8–11 display the correct molecular ions for which the measured high-resolution (HRMS) data are in good agreement with the calculated values. The \(^1\)H and \(^{13}\)C signal assignments are based on DEPT and 2D (COSY, HMQC, HMBC) experiments wherein the associated spectra showed correlations that helped in assigning the various signals to the different carbon and their attached / neighboring hydrogen atoms.

**Antimicrobial Activity**

*In vitro* antibacterial screening results of compounds 1–6 and 8–11 have shown that all tested compounds exhibit good to excellent antimicrobial po-
Compound No. | 1 | 2 | 3 | 4 | 5 | 6 | 8a | 9a | 10 | 11 | Ref |
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<td><em>Staphylococcus aureus</em> ATCC 6538p</td>
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Table 1. *In vitro* antibacterial activity (MIC values, µg/mL) of model compounds 1–6 and 8–11 as compared with ciprofloxacin as the reference agent (Ref*).

Scheme 2.

(8a) 

(9a) 

(i) Na₂S₂O₄, aq. K₂CO₃ / 20 °C;  
(ii) Na₂S₂O₄, aq. K₂CO₃ / 0–3 °C

Scheme 2.

Experimental Section

The secondary α-amino acids sarcosine and (±)-pipercolic acid, employed in this study, were of biochemical grades (Acros) and used as received. 2,4-Dichloro-5-fluoro-3-nitrobenzoic acid was purchased from Acros. Melting points (uncorrected) were determined on a Gallenkamp electrothermal melting temperature apparatus. ¹H and ¹³C NMR spectra were recorded on a Bruker DPX-300 instrument with Me₄Si as internal reference. EIMS spectra were obtained using a Finnigan MAT TSQ-70 spectrometer at 70 eV, ion source temperature = 200 °C. High-resolution MS-ESI data were obtained with a Bruker Bio TOF III instrument. IR spectra were recorded as KBr discs on a Nicolet Impact-400 FT-IR spectrophotometer. Microanalyses were performed at the Microanalytical Laboratory – Medicinal Chemistry Division, Faculty of Pharmacy, University of Jordan, Amman.

Pharmacological tests

The minimal inhibitory concentrations (MICs) were determined by the conventional broth dilution method using the two serial dilution technique. The standardization of bacterial test suspension was carried out according to the McFarland standard method as described by the National Committee for Clinical Laboratories Standard (NCCLS) (1993). Stock solutions of the test compounds were prepared using DMSO. Serial dilutions were prepared to obtain test concentrations ranging from 156 µg/mL to 0.3 µg/mL. Each tube was then inoculated with 0.1 mL of the cultured bacteria (containing approximately 1 to 2 × 10⁶ CFU/mL), mixed and incubated at 37 °C for 24 h. Growth inhibition with concentrations of 156 µg/mL 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.

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

This compound was prepared from 2,4-dichloro-5-fluoro-3-nitrobenzoic acid, ethyl 3-(N,N-dimethylamino)acrylate and cyclopropylamine by following literature procedures [8].

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

A stirred mixture of sarcosine (0.80 g, 9 mmol), 7-chloro-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 7 (1.00 g, 3.1 mmol) and sodium hydrogen carbonate (1.50 g, 18 mmol) in 50% aqueous ethanol (140 mL) was heated at 75–80 °C under reflux conditions. The mixture slowly developed a light yellow color that changed into a bright yellow, then into a clear...
orange solution. The progress of the reaction was monitored by TLC and was completed within 20–24 h. The orange solution was extracted with dichloromethane (50 mL) and the aqueous layer was separated, acidified with 3 N HCl to pH 6.5 and re-extracted with dichloromethane (50 mL). The aqueous layer was again separated, cooled and re-acidified with 3 N HCl to pH 3–4, whereby the title compound was precipitated as a yellowish solid which was collected by suction filtration, washed with cold water (2 × 10 mL), dried and recrystallized from ethanol. Yield of 8a: 0.93 g (82%); m.p. 214–216 °C (dec). – IR (KBr): ν = 3098, 2911, 1745 (br), 1609, 1455, 1319, 1260, 1217 cm⁻¹. – MS (FAB): m/z (%): 380 (100) [M+H]+ (calcld. 379 for C16H14FN3O7 [M]+). – 1H NMR (300 MHz, D2O): δ = 1.00, 1.09 (2 m, CH3). 3.89 (t, 2H, OMe). 7.51 (t, 1H, 1H-CH3), 8.18 (d, J=1.9 Hz, H-5). 8.24 (d, J=3.2 Hz, C-8a), 151.7 (C-2), 153.0 (CH=O), 157.7 (d, 1H, C-2). – C18H18FN3O7 (407.35): calcd. C 53.07, H 4.45, N 10.32; found C 53.17, H 4.44, N 10.24. 

Methyl 1-cyclopropyl-6-fluoro-7-[(methoxycarbonylmethyl)(methyl)amino]-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (8b)

To a fine powder of 8a (0.38 g, 1.0 mmol), suspended in cold diethyl ether (20 mL), was added portionwise a cold fresh ethereal diazomethane solution until evolution of nitrogen ceased. The reaction mixture was kept at 5–10 °C for 20 min, and the solvent was then evaporated at r.t., whereby the title compound was obtained as a yellow solid. Yield of 8b: 0.34 g (83%); m.p. 145–146 °C (dec). – IR (KBr): ν = 3086, 3002, 2953, 2851, 2808, 1745, 1702, 1651, 1617, 1459, 1408, 1328, 1240, 1208, 1044 cm⁻¹. – MS (TOF (+)-ES): m/z = 430 [M+Na]+. – HRMS: m/z = 430.1021 (calcld. 430.1026 for C16H14FN3O7Na). – 1H NMR (300 MHz, D2O): δ = 1.03, 1.12 (2 m, 4H, 2′H-3′H). 2.83 (d, J=1.9 Hz, 3H, N-CH3). 3.66 (m, 1H, 1′-H). 3.88 (s, 3H, CH2CONH2). 3.87 (s, 2H, N-CH2). 3.88 (s, 3H, CH2CONH2). 8.25 (d, J=1.9 Hz, 1H, 2′′-H). 8.60 (s, 1H, 2′′-H). – 13C NMR (75 MHz, CDCl3): δ = 10.9 (C-2′-CH3). 37.7 (C-5′-CH3). 52.4 (C(3)-CH2). 57.2 (CH2CONH2). 111.1 (C-3). 116.1 (d, 2JCF=22.6 Hz, C-7). 128.4 (d, 2JCF=6.9 Hz, C-4a). 130.7 (d, 2JCF=2.2 Hz, C-8a). 138.4 (d, 2JCF=17.1 Hz, C-7). 144.1 (d, 2JCF=3.2 Hz, C-8). 151.7 (C-2). 157.4 (d, 2JCF=253 Hz, C-6). 165.1 (C(3)-CO2Me). 169.8 (d, CH2=CO2Me). 171.3 (d, 2JCF=21 Hz, C-4). – C18H18FN3O7 (407.35): calcd. C 53.07, H 4.44, N 10.32; found C 53.17, H 4.44, N 10.24.

A stirred mixture of (±)-pipecolinic acid (1.20 g, 9.3 mmol), 7 (1.0 g, 3.1 mmol) and sodium hydroxide carbonate (1.50 g, 18 mmol) in 50% aqueous ethanol (140 mL) was heated at 85–90 °C for 4–5 days under reflux conditions. Work up of the resulting reaction mixture was followed as described for 8a above and produced the title compound as a light yellow solid which was recrystallized from ethanol.

Yield of (±)-9a: 0.83 g (64%); m.p. 229–230 °C (dec). – IR (KBr): ν = 2351 (br), 2945, 2851, 1770, 1702 (br), 1617, 1532, 1438, 1328, 1268 cm⁻¹. – MS (FAB): m/z (%): 380 (100) [M+H]+ (calcld. 419 for C16H16F2N3O7 [M]+). – MS (EI): m/z (%): 399 (1, [M+H]+, 374 (3), 371 (2), 344 (4). 339 (5), 297 (12), 295 (17), 278 (9), 244 (11), 219 (100). 190 (14), 164 (8), 123 (5), 106 (5). – 1H NMR (300 MHz, D6DMSO): δ = 0.91 (m, 1H, 1H, 1H, 1H, 1H, 1H, 1H). 1.13 (m, 2H, 2H-3′-H). 1.27 (m, 2H, 2H-3′-H). 1.57 (m, 2H, 2H-3′-H). 2.88 (m, 1H, 6′H). 3.40 (m, 1H, 6′H). 3.69 (m, 1H, 1′H). 4.03 (m, 1H, 2′′-H). 8.20 (d, J=11.7 Hz, 1H, H-5). 8.77 (s, 1H, 2H, 2H). 12.67 (br s, 1H, C(3)-CO2H). 13.94 (br s, 1H, C(3)-CO2H). – 13C NMR (75 MHz, D6DMSO): δ = 10.5, 11.5 (C-2′-CH3). 22.9 (C-5′-CH3), 25.7 (C-5′-CH3), 30.1 (C-3′-CH3), 39.5 (C-1′-CH3). 54.0 (C-6′-CH3), 63.3 (d, J=6.2 Hz, C-2′-CO2H). 109.0 (C-3′). 114.4 (d, J=22.8 Hz, C-5). 125.3 (C-4a). 130.4 (C-4a). 130.5 (d, J=17.7 Hz, C-7). 141.1 (C-8). 153.0 (C-2). 157.7 (d, J=253 Hz, C-6). 165.1 (C(3)-CO2H). 172.8 (C(2′)-CO2H). 175.9 (d, J=2.3 Hz, C-4). – C19H18F2N3O7 (419.36): calcd. C 54.42, H 4.33, N 10.02; found C 54.30, H 4.39, N 10.22.

(±)-1-Methyl-1-cyclopropyl-6-fluoro-7-[2-(methoxycarbonyl) Piperidin-1-yl]-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (±)-9b

This compound was prepared via the reaction of (±)-9a (0.42 g, 1.0 mmol) with diazomethane in ether following the procedure described above for 8b. Yield of (±)-9b: 0.38 g (85%); m.p. 181–182 °C (dec). – IR (KBr): ν = 3100, 2939, 2843, 1737, 1702, 1635, 1604, 1544, 1468, 1326, 1289, 1178, 1092 cm⁻¹. – MS (TOF (+)-ES): m/z = 470 [M+Na]+, 917 [2M+Na]+. – HRMS: m/z = 470.1334 (calcld. 470.1339 for C22H22F2N3O7Na). 917.2776 (calcld. 917.2778 for C22H22F2N3O7Na). – 1H NMR (300 MHz,
[D$_6$]DMSO): $\delta = 1.03$ (m, 3H) and 1.18 (m, 1H) (2'-H$_2$ + 3'-H$_2$), 1.54 (m, 1H, 4'-H$_2$), 1.65 (m, 2H, 5'-H$_2$), 1.81 (m, 1H, 4'-H$_3$), 1.95 (m, 2H, 3'-H$_2$), 2.98 (m, 1H, 6'-H$_3$), 3.38 (br m, 1H, 6'-H$_3$), 3.52 (s, 3H, C$_2$-$^2$CO$_2$CH$_3$), 3.62 (m, 1H, 1'-H$_3$), 3.88 (s, 3H, C$_3$-CO$_2$CH$_3$), 4.13 (m, 1H, 2'-H$_3$). 8.20 (d, $^3$J$_{1-f}$ = 11.7 Hz, 1H, 5'-H), 8.59 (s, 1H, 2'-H). $^{13}$C NMR (75 MHz, [D$_6$]DMSO): $\delta = 10.2$, 11.7 (C-2' + C-3'), 23.4 (C-4'), 25.5 (C-5'), 30.3 (C-3'), 37.6 (C-1'), 51.9 (C$_2$-$^2$CO$_2$CH$_3$), 52.4 (C$_3$-CO$_2$CH$_3$), 54.4 (C-6'), 63.0 (d, $^2$J$_{1-C}$ = 5.4 Hz, C-2), 111.1 (C-3), 115.9 (d, $^2$J$_{1-C}$ = 22.7 Hz, C-5), 128.6 (d, $^3$J$_{1-C}$ = 7.0 Hz, C-4a), 130.9 (C-8a), 138.6 (d, $^2$J$_{1-C}$ = 17.6 Hz, C-7), 141.4 (d, $^3$J$_{1-C}$ = 4.8 Hz, C-8), 151.7 (C-2), 157.5 (d, $^3$J$_{1-C}$ = 252 Hz, C-6), 165.2 (C$_3$-CO$_2$Me), 171.3 (d, $^3$J$_{1-C}$ = 1.8 Hz, C-3'), 172.0 (C$_2$-$^2$CO$_2$Me). – C$_{12}$H$_{12}$F$_{2}$O$_7$ (447.41): calcld. C 56.37, H 4.96, N 9.39; found C 56.24, H 5.04, N 9.16.

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


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