

Detailed Information on the Synthesis and Characterization

General: Melting points were determined on a Electrothermal IA9100 melting point apparatus and are uncorrected. Nuclear magnetic resonance (^1H and ^{13}C) spectra were recorded on a Varian Gemini 200 spectrometer or Varian-Unity-400 spectrometer. Chemical shifts were expressed in parts per million (δ) with tetramethylsilane (TMS) as an internal standard. Thin-layer chromatography was performed on silica gel 60 F-254 plates purchased from E. Merck and Co.. The elemental analyses were performed in the Instrument Center of National Science Council at National Cheng-Kung University and National Chung-Hsing University using Heraeus CHN-O Rapid EA, and all values are within $\pm 0.4\%$ of the theoretical compositions.

General Procedure for the Preparation of 1-Ethyl-6,8-difluoro-7-[4-[2-(4-substituted-phenyl)-2-oxoethyl]-1-piperazinyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic Acids 4a, 4d, 4f, and 4h. A mixture of the 8-fluoronorfloxacin (**2**, 0.34 g, 1 mmol), sodium bicarbonate (0.11 g, 1.28 mmol), and 2-bromoacetophenone (0.25 g, 1.28 mmol) in DMF (10 mL) was stirred at room temperature for 24 h, then poured into ice-water (20mL), and extracted with CH_2Cl_2 (20 mL x 3). The extract was washed with water, dried over MgSO_4 , and evaporated. Recrystallization from CH_2Cl_2 -MeOH (10:1) yielded 1-ethyl-6,8-difluoro-4-oxo-7-[4-(2-phenyl-2-oxoethyl)-1-piperazinyl]-1,4-dihydroquinoline-3-carboxylic acid (**4a**) (73 % yield): mp: 167 °C dec; ^1H NMR (400MHz, DMSO-d_6): δ 1.44 (t, 3H, $J = 6.6$ Hz), 2.70 and 3.32 (two m, 8H), 3.97 (s, 2H), 4.59 (m, 2H), 7.54 and 8.02 (m, 5H), 7.85 (d, 1H, $J = 13.6$ Hz), 8.92 (s, 1H), 14.95 (br s, 1H). Anal. ($\text{C}_{24}\text{H}_{23}\text{F}_2\text{N}_3\text{O}_4 \cdot 0.5 \text{H}_2\text{O}$) C, H, N.

1-Ethyl-6,8-difluoro-7-[4-[2-(4-fluorophenyl)-2-oxoethyl]-1-piperazinyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (4d). Treatment of 8-fluoronorfloxacin under the same conditions described for the synthesis of **4a**, but using 2-bromo-4'-fluoroacetophenone instead of 2-bromoacetophenone, gave **4d** (77 % yield): mp: 228 °C dec; ^1H NMR (400MHz, DMSO-d_6) δ

1.44 (t, 3H, $J = 6.4$ Hz), 2.69 and 3.32 (two m, 8H), 3.94 (s, 2H), 4.58 (m, 2H), 7.36 and 8.11 (m, 4H), 7.85 (d, 1H, $J = 11.2$ Hz), 8.92 (s, 1H), 14.90 (br s, 1H). Anal. ($C_{24}H_{22}F_3N_3O_4 \cdot 0.5 H_2O$) C, H, N.

1-Ethyl-7-[4-[2-(4-chlorophenyl)-2-oxoethyl]-1-piperazinyl]-6,8-difluoro-4-oxo-1,4-

dihydroquinoline-3-carboxylic acid (4f). Treatment of 8-fluoronorfloxacin under the same conditions described for the synthesis of **4a**, but using 2-bromo-4'-chloroacetophenone instead of 2-bromoacetophenone, gave **4f** (69 % yield): mp: 235 °C; 1H NMR (400MHz, DMSO- d_6) δ 1.43 (t, 3H, $J = 6.6$ Hz), 2.69 and 3.29 (two m, 8H), 3.94 (s, 2H), 4.58 (m, 2H), 7.61 and 8.03 (m, 4H), 7.87 (d, 1H, $J = 12.0$ Hz), 8.93 (s, 1H), 14.94 (br s, 1H). Anal. ($C_{24}H_{22}ClF_2N_3O_4 \cdot H_2O$) C, H, N.

1-Ethyl-6,8-difluoro-7-[4-[2-(4-methoxyphenyl)-2-oxoethyl]-1-piperazinyl]-4-oxo-1,4-

dihydroquinoline-3-carboxylic acid (4h). Treatment of 8-fluoronorfloxacin under the same conditions described for the synthesis of **4a**, but using 2-bromo-4'-methoxyacetophenone instead of 2-bromoacetophenone, gave **4h** (81 % yield): mp: 189 °C dec; 1H NMR (400MHz, DMSO- d_6) δ 1.44 (t, 3H, $J = 6.8$ Hz), 2.69 and 3.24 (two m, 8H), 3.84 (s, 3H), 3.87 (s, 2H), 4.58 (m, 2H), 7.05 (m, 2H), 7.85 (d, 1H, $J = 12.0$ Hz), 8.01 (m, 2H), 8.92 (s, 1H), 14.92 (br s, 1H). Anal. ($C_{25}H_{25}F_2N_3O_5 \cdot 0.25 H_2O$) C, H, N.

(Z)-1-Ethyl-6-fluoro-4-oxo-7-[4-(2-phenyl-2-semicarbazonoethyl)piperazinyl]-1,4-

dihydroquinoline-3-carboxylic acid (5b). To a suspension of the 1-ethyl-6-fluoro-7-[4-(2-phenyl-2-oxoethyl)-1-piperazinyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3a**, 0.44 g, 1 mmole) in absolute methanol (10mL) and CH_2Cl_2 (10 mL) was added semicarbamide hydrogen chloride (0.22 g, 2 mmol) and sodium bicarbonate (0.17 g, 2 mmol). The reaction mixture was heated at reflux for 48 h, then concentrated in vacuo to give a solid which was washed with EtOH (10mL) and purified by flash column chromatography (silica gel, with CH_2Cl_2 -MeOH (10:1) as the eluent) and crystallization from CH_2Cl_2 -EtOH (5:1) to give an off-yellow solid (0.34 g, 68 %): mp: >300 °C dec; 1H NMR (400 MHz, DMSO- d_6) δ 1.41 (t, 3H, $J = 7.2$ Hz), 2.71 and 3.35 (two m, 8H), 3.83 (s, 2H), 4.61 (q, 2H, $J = 7.3$ Hz), 6.62 (br s, 2H), 7.23 (d, 1H, $J = 7.2$ Hz), 7.38 (m, 3H), 7.90 (m, 3H),

8.96 (s, 1H), 10.56 (br s, 1H), 15.37 (br s, 1H). ^{13}C NMR (200 MHz, DMSO- d_6) δ 14.39, 49.07, 49.51, 49.58, 51.60, 54.69, 106.18 ($J = 2.9$ Hz), 107.10, 111.13 ($J = 23.0$ Hz), 119.46 ($J = 7.4$ Hz), 126.26, 128.28, 128.58, 137.18, 137.53, 142.28, 145.32 ($J = 10.3$ Hz), 148.49, 152.94 ($J = 247.9$ Hz), 156.56, 166.10, 176.14. Anal. ($\text{C}_{25}\text{H}_{27}\text{FN}_6\text{O}_4 \cdot \text{H}_2\text{O}$) C, H, N.

(Z)-1-Ethyl-6-fluoro-4-oxo-7-[4-(2-phenyl-2-thiosemicarbazonoethyl)piperazinyl]-1,4-

dihydroquinoline-3-carboxylic acid (5c) was prepared according to the synthesis of **5b**, using thiosemicarbamide in 42 % yield: mp: 243 °C dec; ^1H NMR (400 MHz, DMSO- d_6) δ 1.42 (t, 3H, $J = 7.0$ Hz), 2.75 and 3.42 (two m, 8H), 3.96 (s, 2H), 4.60 (q, 2H, $J = 7.2$ Hz), 7.22 (d, 1H, $J = 7.2$ Hz), 7.41 (m, 3H), 7.96 (m, 3H), 8.03 (br s, 1H), 8.41 (br s, 1H), 8.96 (s, 1H), 12.38 (br s, 1H), 15.36 (br s, 1H). ^{13}C NMR (200 MHz, DMSO- d_6) δ 14.38, 49.08, 49.60, 51.46, 55.86, 105.89 ($J = 2.4$ Hz), 107.09, 111.22 ($J = 22.7$ Hz), 119.39 ($J = 7.8$ Hz), 126.71, 128.35, 129.28, 136.82, 137.20, 144.33, 145.13 ($J = 10.0$ Hz), 148.47, 152.82 ($J = 248.2$ Hz), 166.10, 176.13 ($J = 2.6$ Hz), 178.08. Anal. ($\text{C}_{25}\text{H}_{27}\text{FN}_6\text{O}_3\text{S} \cdot \text{H}_2\text{O}$) C, H, N.

(Z)-1-Ethyl-6-fluoro-7-[4-[2-(4-fluorophenyl)-2-hydrazinoethyl]-1-piperazinyl]-4-oxo-1,4-

dihydro-3-quinolinecarboxylic acid (5e). To a suspension of the 1-ethyl-6-fluoro-7-[4-[2-(4-fluorophenyl)-2-oxoethyl]-1-piperazinyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3d**, 0.23 g, 0.5 mmole) in absolute methanol (20 mL) was added hydrazine monohydrate (0.1 g, 2 mmol). The reaction mixture was heated at reflux for 24 h and allowed to cool to room temperature. CH_2Cl_2 (50 mL) was added, and the layers were separated. The organic phase was washed successively with water and brine, dried over Na_2SO_4 , filtered and concentrated in vacuo to give a solid which was purified by flash column chromatography (silica gel, with CH_2Cl_2 -MeOH (10:1) as the eluent) and crystallization from CH_2Cl_2 -MeOH (5:1) to gave **5e** as a white solid (0.17 g, 72 %): mp: 182 °C; ^1H NMR (DMSO- d_6) δ 1.41 (t, 3H, $J = 7.2$ Hz), 2.65 and 3.33 (two m, 8H), 3.62 (s, 2H), 4.58 (q, 2H, $J = 7.2$ Hz), 7.14 (m, 3H), 7.26 (s, 2H), 7.71 (m, 2H), 7.91 (d, 1H, $J = 13.3$ Hz), 8.95 (s, 1H), 15.33 (br s, 1H). ^{13}C NMR (200 MHz, DMSO- d_6) δ 14.30, 49.02, 49.49, 49.58, 51.83, 53.14, 106.13 ($J = 3.4$), 107.04, 111.10 ($J = 22.8$), 114.81 ($J = 21.1$), 119.28 ($J = 7.6$),

126.91 ($J = 7.7$), 135.80 ($J = 2.8$), 137.13, 139.79, 145.36 ($J = 10.5$), 148.45, 152.86 ($J = 247.8$), 161.43 ($J = 242.6$), 166.05, 176.11 ($J = 2.6$). Anal. ($C_{24}H_{25}F_2N_5O_3 \cdot 0.25 H_2O$) C, H, N.

1-Ethyl-6-fluoro-7-{4-[2-(4-chlorophenyl)-2-methoxyiminoethyl]-1-piperazinyl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5g, $E/Z = 1:2.9$) was prepared according to the synthesis of **5e**, using O-methylhydroxylamine hydrochloride instead of hydrazine monohydrate (68 % yield): mp: 168-202 °C dec; 1H NMR (200MHz, DMSO- d_6) δ 1.42 (t, 3H, $J = 6.6$ Hz), 2.55 and 3.25 (two m, 8H), 3.36 and 3.70 (two s, 2H, NCH_2 for E/Z stereoisomers), 3.79 and 3.93 (two s, 3H, $NOCH_3$ for E/Z stereoisomers), 4.56 (m, 2H), 7.48 and 7.85 (two m, 5H), 8.90 (s, 1H), 14.88 (br s, 1 H). Anal. ($C_{25}H_{25}ClF_2N_4O_4$) C, H, N.

1-Ethyl-6-fluoro-7-{4-[2-methoxyimino-2-(4-methoxyphenyl)ethyl]-1-piperazinyl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5i, $E/Z = 1:2.3$) was prepared according to the synthesis of **5e**, using O-methylhydroxylamine hydrochloride instead of hydrazine monohydrate (71 % yield): mp: 168-169 °C dec; 1H NMR (DMSO- d_6) δ 1.39 (t, 3H, $J = 7.0$ Hz), 2.61 and 3.24 (two m, 8H), 3.69-3.80 (m, 5H), 3.90 (m, 3H), 4.55 (q, 2 H, $J = 7.0$ Hz), 6.95 (m, 2H), 7.14 (d, 1H, $J = 7.2$ Hz), 7.64 and 7.72 (m, 2H for E/Z stereoisomers), 7.88 (d, 1H, $J = 13.6$ Hz), 8.93 (s, 1H), 15.33 (br s, 1H). Anal. ($C_{26}H_{29}FN_4O_5 \cdot H_2O$) C, H, N.

(Z)-1-Ethyl-6,8-difluoro-4-oxo-7-[4-(2-phenyl-2-semicarbazonoethyl)piperazinyl]-1,4-dihydroquinoline-3-carboxylic acid (6b) was prepared according to the synthesis of **5b**, using **4a** instead of **3a** in 71 % yield: mp: 242 °C dec; 1H NMR (200 MHz, DMSO- d_6) δ 1.44 (t, 3H, $J = 7.0$ Hz), 2.65 and 3.28 (two m, 8H), 3.81 (s, 2H), 4.58 (q, 2H, $J = 7.2$ Hz), 6.61 (br s, 2H), 7.39 (m, 3H), 7.88 (m, 3H), 8.91 (s, 1H), 10.66 (br s, 1H), 14.91 (br s, 1H). Anal. ($C_{25}H_{26}F_2N_6O_4 \cdot H_2O$) C, H, N.

(Z)-1-Ethyl-6,8-difluoro-4-oxo-7-[4-(2-phenyl-2-thiosemicarbazonoethyl)piperazinyl]-1,4-dihydroquinoline-3-carboxylic acid (6c) was prepared according to the synthesis of **5b**, using thiosemicarbamide in 58 % yield: mp: 245 °C dec; 1H NMR (400 MHz, DMSO- d_6) δ 1.45 (t, 3H, $J = 7.0$ Hz), 2.68 and 3.45 (m, 8H), 3.95 (s, 2H), 4.60 (q, 2H, $J = 7.0$ Hz), 7.41 (m, 3H), 7.88 (dd,

1H, $J = 12.0, 1.6$), 7.96 (m, 2H), 8.02 (br s, 1H), 8.41 (br s, 1H), 8.93 (s, 1H), 12.50 (br s, 1H), 14.91 (br s, 1H). FABMS found $[M + H]^+$ 529.1833; $C_{25}H_{27}F_2N_6O_3S$ require 529.1833.

1-Ethyl-6,8-difluoro-7-{4-[2-(4-fluorophenyl)-2-hydroxyiminoethyl]-1-piperazinyl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6d, $E/Z = 1:2.9$) was prepared according to the synthesis of **5e**, using **4d** in 79 % yield: mp: 201 °C dec; 1H NMR (400 MHz, DMSO- d_6) δ 1.41 (t, 3H, $J = 6.6$ Hz), 2.57 and 3.27 (two m, 8H), 3.47 and 3.70 (two s, 2H, NCH₂ for E/Z stereoisomers), 4.56 (q, 2H, $J = 6.6$ Hz), 7.19-7.23 (m, 2H), 7.81-7.84 (m, 3H), 8.90 (s, 1H), 11.09 and 11.49 (two s, 1H for E/Z stereoisomers), 14.92 (br s, 1H). Anal. ($C_{24}H_{23}F_3N_4O_4 \cdot 0.5 H_2O$) C, H, N.

1-Ethyl-6,8-difluoro-7-{4-[2-(4-chlorophenyl)-2-hydroxyiminoethyl]-1-piperazinyl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6f, $E/Z = 1:3.8$) was prepared according to the synthesis of **5e**, using **4f** in 76 % yield: mp: 199 °C dec; 1H NMR (400 MHz, DMSO- d_6) δ 1.42 (t, 3H, $J = 6.8$ Hz), 2.56 and 3.27 (m, 8H), 3.41 and 3.70 (two s, 2H, NCH₂ for E/Z stereoisomers), 4.56 (q, 2H, $J = 6.8$ Hz), 7.43-7.48 (m, 2H), 7.78-7.85 (m, 3H), 8.91 (s, 1H), 11.15 and 11.59 (two s, 1H for E/Z stereoisomers), 14.95 (br s, 1H). Anal. ($C_{24}H_{23}ClF_2N_4O_4$) C, H, N.

1-Ethyl-6,8-difluoro-7-{4-[2-(4-chlorophenyl)-2-methoxyiminoethyl]-1-piperazinyl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6g, $E/Z = 1:2.9$) was prepared according to the synthesis of **5e**, using **4g** in 68 % yield: mp: 202 °C dec; 1H NMR (200 MHz, DMSO- d_6) δ 1.42 (t, 3H, $J = 6.6$ Hz), 2.55 and 3.25 (two m, 8H), 3.36 and 3.70 (two s, 2H, NCH₂ for E/Z stereoisomers), 3.79 and 3.93 (two s, 3H, NOCH₃ for E/Z stereoisomers), 4.56 (q, 2H, $J = 6.6$ Hz), 7.48-7.58 (m, 5H), 8.90 (s, 1H), 14.88 (br s, 1H). Anal. ($C_{25}H_{25}ClF_2N_4O_4$) C, H, N.

1-Ethyl-6,8-difluoro-7-{4-[2-hydroxyimino-2-(4-methoxyphenyl)ethyl]-1-piperazinyl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6h, $E/Z = 1:3.2$) was prepared according to the synthesis of **5e**, using **4h** in 87 % yield: mp: 182 °C dec; 1H NMR (400 MHz, DMSO- d_6) δ 1.42 (t, 3H, $J = 6.8$ Hz), 2.58 and 3.29 (two m, 8H), 3.40 and 3.69 (two s, 2H, NCH₂ for E/Z stereoisomers), 3.78 (s, 3H), 4.57 (m, 2H), 6.94 (m, 2H), 7.73 (m, 2H), 7.83 (d, 1H, $J = 12.0$ Hz), 8.91 (s, 1H), 10.94 and 11.25 (two s, 1H for E/Z stereoisomers), 14.90 (br s, 1H). Anal.

(C₂₅H₂₆F₂N₄O₅ · 0.5 H₂O) C, H, N.

6,7-Difluoro-1-(2-fluoro-4-nitrophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (8c). A mixture of 6,7-Difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**7**), 1.27 g, 5 mmol), anhydrous potassium carbonate (1.73 g, 12.5 mmol) and 3,4-difluoronitrobenzene (1.19 g, 7.5 mmol) in dry DMF (20 mL) was stirred at 90-100°C for 12 h. After removing the solvent, the residue was taken up by CH₂Cl₂, and the mixture was washed with H₂O, dried with MgSO₄, and evaporated. Recrystallization from ethyl acetate yielded **8c** (67 % yield): mp: 192 °C; ¹H NMR (400 MHz, CDCl₃) δ 1.38 (t, 3H, *J* = 7.2 Hz), 4.36 (q, 2H, *J* = 7.2 Hz), 6.68 (ddd, 1H, *J* = 10.4, 6.4, 1.6 Hz), 7.93 (dd, 1H, *J* = 8.8, 7.2 Hz), 8.24, (dd, 1H, *J* = 10.0, 8.4 Hz), 8.32 (dd, 1H, *J* = 8.8, 2.4 Hz), 8.36 (s, 1H), 8.39 (m, 1H). Anal. (C₁₈H₁₁F₃N₂O₅) C, H, N.

6,7-Difluoro-1-(2-fluoro-4-nitrophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (8d). To a suspension of **8c** (1.57 g, 4 mmol) in AcOH (20 mL) was added a solution of 2.0 N HCl (4.5 mL). The mixture was heated at reflux for 2 h and then the solvent was evaporated under reduced pressure. The residue thus obtained was dissolved in H₂O (30 mL) and the precipitate was filtered, washed with H₂O and dried to afford **8d** (95 % yield) : mp: 265 °C dec; ¹H NMR (200 MHz, DMSO-*d*₆) δ 7.58 (ddd, 1H, *J* = 11.6, 6.6, 1.3 Hz), 8.16 (dd, 1H, *J* = 8.8, 7.4 Hz), 8.37 (m, 2H), 8.58 (dd, 1H, *J* = 9.8, 2.4 Hz), 9.07 (s, 1H), 14.31 (br s, 1H). Anal. (C₁₆H₇F₃N₂O₅) C, H, N.

6-Fluoro-1-(2-fluoro-4-nitrophenyl)-7-(1-piperazinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (9b). A mixture of **8d** (1.46 g, 4 mmol), piperazine (1.13 g, 13.2 mmol) and acetonitrile (30 mL) was stirred at 70-80 °C under nitrogen atmosphere. After 16 h, the mixture was evaporated to dryness and H₂O was added to the residue. The resulting solid was filtered off, washed with H₂O, dried, and crystallized from ethyl acetate-EtOH to give **9b** (78 % yield): mp: 188 °C; ¹H NMR (400 MHz, DMSO-*d*₆ + TFA) δ 3.27 and 3.33 (two m, 8H), 6.49 (d, 1H, *J* = 6.8 Hz), 8.07 (d, 1H, *J* = 13.2 Hz), 8.21 (m, 1H), 8.41 (m, 1H), 8.55 (dd, 1H, *J* = 10.0, 2.8 Hz), 8.95 (s, 1H). Anal. (C₂₀H₁₆F₂N₄O₅ · 2.0 H₂O) C, H, N.

1-(4-Amino-2-fluorophenyl)-6-fluoro-7-(1-piperazinyl)-4-oxo-1,4-dihydroquinoline-3-

carboxylic acid (9c). A mixture of **9b** (0.43 g, 1 mmol) and 10 % Pd/C (0.2 g) in CH₂Cl₂ (50 mL) was stirred under H₂ for 24 h. The resulting mixture was filtered and the solvent was evaporated under reduced pressure. The residue was crystallized from EtOH to give a solid **9c** (85 % yield): mp: 193 °C dec; ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.81 and 2.99 (two m, 8H), 6.05 (br s, 2H), 6.44 (d, 1H, *J* = 7.2 Hz), 6.58 (m, 2H), 7.36 (m, 1H), 7.94 (d, 1H, *J* = 13.6 Hz), 8.61 (s, 1H). Anal. (C₂₀H₁₈F₂N₄O₂ · 1.8 H₂O) C, H, N.

General Procedure for the Preparation of 6-Fluoro-7-{4-[2-(4-substituted-phenyl)-2-oxoethyl]-1-piperazinyl}-1-(2-fluoro-4-nitrophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic

Acids 10a-c. A mixture of the 1-(4-nitrophenyl)-6-fluoro-7-(1-piperazinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**9a**, 0.41 g, 1 mmol), sodium bicarbonate (0.11 g, 1.28 mmol), and 2-bromo-4'-fluoroacetophenone (0.28 g, 1.28 mmol) in DMF (10 mL) was stirred at room temperature for 24 h, then poured into ice-water (20 mL), and extracted with CH₂Cl₂ (20 mL x 3). The extract was washed with water, dried over MgSO₄, and evaporated. Recrystallization from CH₂Cl₂-MeOH (10:1) yielded 6-fluoro-7-{4-[2-(4-fluorophenyl)-2-oxoethyl]-1-piperazinyl}-1-(4-nitrophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**10a**) (86 % yield): mp: 201 °C dec; ¹H NMR (200 MHz, DMSO-*d*₆) δ 2.64 and 3.08 (two m, 8H), 3.86 (s, 2H), 6.43 (d, 1H, *J* = 7.2 Hz), 7.32 (m, 2H), 7.96-8.09 (m, 5H), 8.51 (m, 2H), 8.72 (s, 1H), 15.01 (br s, 1H). Anal. (C₂₈H₂₂F₂N₄O₆ · 0.5 H₂O) C, H, N.

6-Fluoro-7-{4-[2-(4-methoxyphenyl)-2-oxoethyl]-1-piperazinyl}-1-(4-nitrophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (10b). It was obtained using the same conditions employed to obtain **10a** using 2-bromo-4'-methoxyacetophenone instead of 2-bromo-4'-fluoroacetophenone (95 % yield): mp: 206 °C dec; ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.63 and 3.08 (two m, 8H), 3.81 (s, 2H), 3.83 (s, 3H), 6.43 (d, 1H, *J* = 6.8 Hz), 7.01 (d, 2H, *J* = 9.2 Hz), 7.95-8.04 (m, 5H), 8.51 (d, 2H, *J* = 9.2 Hz), 8.73 (s, 1H), 15.04 (br s, 1H). Anal. (C₂₉H₂₅FN₄O₇ · 0.5 H₂O) C, H, N.

6-Fluoro-1-(2-fluoro-4-nitrophenyl)-7-{4-[2-(4-methoxyphenyl)-2-oxoethyl]-1-piperazinyl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (10c). It was obtained using the same procedure

employed to obtain **10a** using 6-fluoro-1-(2-fluoro-4-nitrophenyl)-7-(1-piperazinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid instead of **9b** (86 % yield): mp: 136 °C dec; ¹H NMR (200 MHz, DMSO-*d*₆) δ 2.63 and 3.02 (two m, 8H), 3.81-3.83 (m, 5H), 6.40 (d, 1H, *J* = 6.8 Hz), 7.01 (d, 2H, *J* = 9.0 Hz), 7.94-8.59 (m, 6H), 8.92 (s, 1H), 14.88 (br s, 1H). Anal. (C₂₉H₂₄F₂N₄O₇) C, H, N.

1-(4-Amino-2-fluorophenyl)-6-fluoro-7-{4-[2-(4-methoxyphenyl)-2-oxoethyl]-1-piperazinyl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (10d). A mixture of **10c** (0.58 g, 1 mmol) and 10 % Pd/C (0.2 g) in CH₂Cl₂ (50 mL) was stirred under H₂ for 24 h. The resulting mixture was filtered and the solvent was evaporated under reduced pressure. The residue was crystallized from EtOH to give a solid **10d** (51 % yield): mp: 143 °C dec; ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.66 and 3.09 (two m, 8H), 3.82-3.84 (m, 5H), 6.05 (s, 2H), 6.47 (d, 1H, *J* = 7.2 Hz), 6.56-7.36 (m, 3H), 7.02 (d, 2H, *J* = 8.4 Hz), 7.94-8.00 (m, 3H), 8.62 (s, 1H), 15.06 (br s, 1H). Anal. (C₂₉H₂₆F₂N₄O₅ · H₂O) C, H, N.

General Procedure for the Hydroxyimination of 6-Fluoro-1-(4-nitrophenyl)-7-{4-[2-(4-substituted-phenyl)-2-oxoethyl]-1-piperazinyl}-4-oxo-1,4-dihydro-3-quinolinecarboxylic

Acids 11a-d. To a suspension of the 6-fluoro-7-{4-[2-(4-fluorophenyl)-2-oxoethyl]-1-piperazinyl}-1-(4-nitrophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**10a**, 0.55 g, 1 mmol) in absolute ethanol (20 mL) was added a solution of hydroxylamine hydrochloride (0.14 g, 2 mmol) and sodium bicarbonate (0.17 g, 2 mmol) in water (2 mL). The mixture was heated at reflux for 20 h and allowed to cool to room temperature. CH₂Cl₂ (50 mL) was added, and the layers were separated. The organic phase was washed successively with water and brine, dried over MgSO₄, filtered and concentrated in vacuo to give a solid which was purified by flash column chromatography (silica gel, with CH₂Cl₂-MeOH (10:1) as the eluent) and crystallization from CH₂Cl₂-MeOH (5:1) to give 6-fluoro-7-{4-[2-(4-fluorophenyl)-2-hydroxyiminoethyl]-1-piperazinyl}-1-(4-nitrophenyl)-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid (**11a**, *E* / *Z* = 1:4.6) (85 % yield): mp: 204 °C dec; ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.51 and 2.99 (two m, 8H), 3.65

(s, 2H), 6.40 (d, 1H, $J = 7.2$ Hz), 7.16 (m, 2H), 7.76 and 7.99 (two m, 5H), 8.49 (m, 2H), 8.71 (s, 1H), 11.09 and 11.47 (two s, 1H for *E/Z* stereoisomers), 15.00 (br s, 1H). Anal. ($C_{28}H_{23}F_2N_5O_6 \cdot 0.7 H_2O$) C, H, N.

6-Fluoro-7-{4-[2-(4-methoxyphenyl)-2-hydroxyiminoethyl]-1-piperazinyl}-1-(4-nitrophenyl)-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid (11b, *E / Z* = 1:4.4) was prepared according to the synthesis of **11a**, using **10b** in 85 % yield: mp: 216 °C dec; 1H NMR (400 MHz, DMSO- d_6) δ 2.54 and 2.99 (two m, 8H), 3.62 (s, 2H), 3.76 (s, 3H), 6.40 (d, 1H, $J = 7.6$ Hz), 6.88 and 7.67 (two m, 4H), 7.99 (m, 3H), 8.49 (d, 2H), 8.71 (s, 1H), 10.90 and 11.21 (two s, 1H for *E/Z* stereoisomers), 15.00 (br s, 1H). Anal. ($C_{29}H_{26}FN_5O_7 \cdot H_2O$) C, H, N.

(*E*)-6-Fluoro-1-(2-fluoro-4-nitrophenyl)-7-{4-[2-(4-methoxyphenyl)-2-hydroxyiminoethyl]-1-piperazinyl}-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid (11c) was prepared according to the synthesis of **11a**, using **10c** in 80 % yield: mp: 216 °C dec; 1H NMR (400 MHz, DMSO- d_6) δ 2.54 and 3.02 (two m, 8H), 3.63 (s, 2H), 3.75 (s, 3H), 6.36 (d, 1H, $J = 6.8$ Hz), 6.88 and 7.67 (two m, 4H), 7.98 (d, 1H, $J = 13.6$ Hz), 8.14 (m, 1H), 8.36 (m, 1H), 8.53 (dd, 1H, $J = 9.6, 2.4$ Hz), 8.91 (s, 1H), 11.22 (br s, 1H), 14.85 (br s, 1H). Anal. ($C_{29}H_{25}F_2N_5O_7 \cdot 2.5 H_2O$) C, H, N.

1-(4-Amino-2-fluorophenyl)-6-fluoro-7-{4-[2-(4-methoxyphenyl)-2-hydroxyiminoethyl]-1-piperazinyl}-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid (11d, *E / Z* = 1:3.8) was prepared according to the synthesis of **11a**, using **10d** in 81 % yield: mp: 207 °C dec; 1H NMR (400 MHz, DMSO- d_6) δ 2.56 and 3.01 (two m, 8H), 3.64 (s, 2H), 3.77 (s, 3H), 6.03 (m, 2H), 6.43 (d, 1H, $J = 7.2$ Hz), 6.56 (m, 2H), 6.92 (m, 2H), 7.36 (m, 1H), 7.67 (m, 2H), 7.94 (d, 1H, $J = 13.2$ Hz), 8.61 (s, 1H), 10.93 and 11.23 (two s, 1H for *E/Z* stereoisomers), 15.05 (br s, 1H). Anal. ($C_{29}H_{27}F_2N_5O_5 \cdot 2.1 H_2O$) C, H, N.

Table 1. In Vitro Antibacterial Activity of 4-Oxoquinoline-3-carboxylic Acids [MIC, μM ($\mu\text{g}/\text{mL}$)]^a

Compd.	E. co.	M. ra.	P. ae.	K. pn.	P. vu.	MRSA	SpEAR	EfVRE
2	0.30	9.28	0.59	0.074	0.039	2.31	18.5	18.5
	(0.10)	(3.13)	(0.20)	(0.025)	(0.013)	(0.78)	(6.25)	(6.25)
3a	0.46	7.15	3.57	0.03	0.23	7.15	>200	>200
	(0.20)	(3.13)	(1.56)	(0.013)	(0.10)	(3.13)	(>100)	(>100)
3d	0.44	13.7	3.42	0.055	0.44	6.87	>200	>200
	(0.20)	(6.25)	(1.56)	(0.025)	(0.20)	(3.13)	(>100)	(>100)
3f	1.56	>200	53.0	0.053	1.65	>200	>200	>200
	(0.74)	(>100)	(25.2)	(0.025)	(0.78)	(>100)	(>100)	(>100)
3h	0.83	>200	13.4	0.053	0.83	6.70	>200	>200
	(0.39)	(>100)	(6.25)	(0.025)	(0.39)	(3.13)	(>100)	(>100)
4a	0.22	3.42	0.86	0.055	0.22	1.71	13.7	13.7
	(0.10)	(1.56)	(0.39)	(0.025)	(0.10)	(0.78)	(6.25)	(6.25)
4h	0.62	20.6	>200	0.06	0.21	0.62	>200	>200
	(0.30)	(10.0)	(>100)	(0.03)	(0.10)	(0.30)	(>100)	(>100)
5a	0.86	55.3	13.8	0.22	3.45	0.44	>200	>200
	(0.39)	(25)	(6.25)	(0.10)	(1.56)	(0.20)	(>100)	(>100)
5b	3.15	101	25.3	0.79	3.15	3.15	25.3	25.3
	(1.56)	(50)	(12.5)	(0.39)	(1.56)	(1.56)	(12.5)	(12.5)
5c	3.06	>200	>200	0.76	6.13	1.53	24.5	12.2
	(1.56)	(>100)	(>100)	(0.39)	(3.13)	(0.78)	(12.5)	(6.25)
5d	6.38	>200	>200	0.21	6.38	2.13	>200	>200
	(3.0)	(>100)	(>100)	(0.10)	(3.0)	(1.0)	(>100)	(>100)
5e	0.83	107	6.67	0.11	1.66	1.66	26.6	26.6
	(0.39)	(50)	(3.13)	(0.050)	(0.78)	(0.78)	(12.5)	(12.5)
5f	6.16	>200	>200	0.21	6.16	>200	>200	>200
	(3.0)	(>100)	(>100)	(0.10)	(3.0)	(>100)	(>100)	(>100)
5g	nd ^b	>200	>200	2.00	59.9	nd ^b	>200	>200
		(>100)	(>100)	(1.0)	(30)		(>100)	(>100)
5h	1.62	104	25.9	0.027	3.23	0.41	13.0	3.23
	(0.78)	(50)	(12.5)	(0.013)	(1.56)	(0.20)	(6.25)	(1.56)

5i	6.30 (3.13)	>200 (>100)	>200 (>100)	1.57 (0.78)	6.30 (3.13)	1.57 (0.78)	12.6 (6.25)	12.6 (6.25)
6b	3.04 (1.56)	>200 (>100)	12.2 (6.25)	0.76 (0.39)	3.04 (1.56)	0.39 (0.20)	6.11 (3.13)	6.11 (3.13)
6c	5.92 (3.13)	>200 (>100)	>200 (>100)	0.38 (0.20)	5.92 (3.13)	0.74 (0.39)	>200 (>100)	>200 (>100)
6d	0.61 (0.30)	>200 (>100)	6.14 (3.0)	0.06 (0.030)	0.61 (0.30)	0.61 (0.30)	6.14 (3.0)	2.05 (1.0)
6f	1.98 (1.0)	59.4 (30)	19.8 (10)	0.59 (0.30)	1.98 (1.0)	0.59 (0.30)	19.8 (10)	19.8 (10)
6g	>200 (>100)	>200 (>100)	>200 (>100)	193 (100)	>200 (>100)	>200 (>100)	>200 (>100)	>200 (>100)
6h	0.40 (0.20)	6.25 (3.13)	3.12 (1.56)	0.10 (0.050)	0.40 (0.20)	0.20 (0.10)	6.25 (3.13)	3.12 (1.56)
9b	0.12 (0.050)	7.27 (3.13)	0.91 (0.39)	0.058 (0.025)	7.27 (3.13)	7.27 (3.13)	0.46 (0.20)	29.0 (12.5)
9c	0.97 (0.39)	7.82 (3.13)	1.95 (0.78)	0.12 (0.050)	7.82 (3.13)	0.97 (0.39)	3.90 (1.56)	15.6 (6.25)
10a	1.82 (1.0)	>200 (>100)	>200 (>100)	0.18 (0.10)	>200 (>100)	>200 (>100)	>200 (>100)	>200 (>100)
10b	0.70 (0.39)	>200 (>100)	11.15 (6.25)	0.18 (0.10)	>200 (>100)	5.58 (3.13)	>200 (>100)	>200 (>100)
10c	0.35 (0.20)	>200 (>100)	10.8 (6.25)	0.17 (0.10)	>200 (>100)	2.70 (1.56)	5.41 (3.13)	43.2 (25)
10d	0.55 (0.30)	18.2 (10)	5.47 (3.0)	0.055 (0.030)	18.2 (10)	0.55 (0.30)	1.82 (1.0)	5.47 (3.0)
11a	5.32 (3.0)	>200 (>100)	>200 (>100)	nd ^b	>200 (>100)	>200 (>100)	>200 (>100)	>200 (>100)
11b	0.17 (0.10)	10.9 (6.25)	2.71 (1.56)	0.087 (0.050)	10.9 (6.25)	1.36 (0.78)	10.9 (6.25)	21.7 (12.5)
11c	1.31 (0.78)	21.1 (12.5)	42.1 (25)	0.084 (0.050)	>200 (>100)	0.34 (0.20)	1.31 (0.78)	5.27 (3.13)
11d	1.77	53.2	17.7	0.18	17.7	0.53	1.77	5.32

	(1.0)	(30)	(10)	(0.10)	(10)	(0.30)	(1.0)	(3.0)
Nf ^c	0.63	9.80	1.22	0.041	0.16	4.89	19.6	9.80
	(0.20)	(3.13)	(0.39)	(0.013)	(0.050)	(1.56)	(6.25)	(3.13)

^aOrganisms selected are as follows: E. co., *Escherichia coli*; M. ra., *Mycobacterium ranae*; P. ae., *Pseudomonas aeruginosa*; K. pn., *Klebsilla pneumoniae*; P. vu., *Proteus vulgaris*; MRSA, *Staphylococcus aureus*, Methicillin Resistant; SpEAR, *Staphylococcus pneumoniae*, Erythromycin and Ampicillin resistant, clinical isolates; EfVRE, *Enterococcus faecalis*, Vancomycin resistant, clinical isolates. ^bnd, not determined. ^cNf, norfloxacin.

Table 2. Inhibitory Activity of Quinolones in a Panel of Human Renal Cancer Cell Lines [log

GI₅₀ (M)]^a

Compd.	786-0	A498	ACHN	CAKI-1	RXF 393	SN12C	TK-10	UO-31	Mean ^b
3a	-4.39	> -4.0	-4.76	nd ^b	nd ^b	> -4.0	-4.52	-5.73	-4.16
3f	-4.33	> -4.0	-4.28	nd ^b	nd ^b	> -4.0	-4.11	-4.89	-4.14
3h	-4.80	-4.41	-4.86	nd ^b	nd ^b	> -4.0	-4.77	-4.87	-4.29
4a	> -4.0	> -4.0	> -4.0	nd ^b	nd ^b	> -4.0	> -4.0	-4.84	-4.02
4h	-4.53	> -4.0	-4.08	nd ^b	nd ^b	> -4.0	-4.47	-4.32	-4.09
10a	-4.62	-4.18	-4.34	-4.55	-4.70	-4.41	-4.54	-4.43	-4.47
10b	-4.53	> -4.0	-4.04	> -4.0	> -4.0	-4.35	> -4.0	-4.34	-4.30
10c	-4.30	-4.45	-4.40	-4.35	-4.35	-4.31	> -4.0	-4.25	-4.33
10d	-4.70	-4.74	-4.61	-4.77	-4.88	-4.69	-4.46	-4.96	-4.67
5a	-4.47	nd ^b	-4.73	-4.49	-4.44	-4.54	-4.12	-6.51	-4.53
5c	-5.63	-5.95	-5.87	-5.97	-5.50	-5.57	-5.41	-6.98	-5.56
5d	> -4.0	-4.68	-4.58	-5.72	-6.77	-4.23	-4.28	-6.88	-4.67
5e	-4.90	-4.89	-4.67	nd ^b	nd ^b	-4.72	-4.53	nd ^b	-4.74
5f	-4.48	-5.27	-4.87	-6.40	-6.14	-4.42	-4.65	-7.54	-5.03
5g	-4.83	-5.33	-5.57	-6.20	-4.84	-4.79	-4.78	-7.35	-5.17
5h	-4.03	-4.41	-4.50	-5.16	nd ^b	> -4.0	-4.19	-6.26	-4.50
5i	-4.63	-4.70	-5.23	nd ^b	nd ^b	-4.61	-4.55	-5.88	-4.72
6c	-4.85	-5.41	-5.68	-5.53	-4.81	-4.80	-4.43	-6.65	-4.87
6d	-4.43	-4.69	-5.68	nd ^b	nd ^b	-4.52	-4.29	-6.29	-4.62
11a	-4.62	-4.44	-4.47	-4.67	-4.70	-4.63	-4.63	-4.75	-4.59
11b	-5.34	-5.54	-5.51	-5.68	-5.68	-5.49	-5.23	-5.87	-5.50

^aData obtained from NCI's in vitro disease-oriented tumor cells screen. GI₅₀: Drug molar concentration causing 50% cell growth inhibition.

^bNot determined.

^cMean values over all cell lines tested. The cell lines used in these experiments were leukemia (CCRF-CEM, HL-60 (TB), K-562, MOLT-4, PRMI-8226, and SR); non-small cell lung cancer (A549/ATCC, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, and NCI-H522); colon cancer (COLC 205, HCC-2998, HCT-116, HCT-15, HT29, KM12, and SW-620); CNS

cancer (SF-268, SF-295, SF-539, SNB-19, SNB-75, and U251); melanoma (LOX IMVI, MALME-3M, M14, SK-MEL-2, SK-MEL-28, SK-MEL-5, and UACC-257); ovarian cancer (IGROV1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, and SK-OV-3); renal cancer (786-0, A498, ACHN, CAKI-1, RXF 393, SN12C, TK-10, and UO-31); prostate cancer (PC-3 and DU-145); and breast cancer (MCF7, MCF7/ADR-RES, MDA-MB-231/ ATCC, HS 578T, MDA-MB-435, MDA-N and T-47D).