MICROWAVE-ASSISTED SYNTHESIS OF FLUOROQUINOLONES AND THEIR NUCLEOSIDES AS INHIBITORS OF HIV INTEGRASE

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Dedicated to Professor Antonín Holý on the occasion of his 70th birthday in recognition of his outstanding contributions to bioactive nucleic acid components.

Six fluoroquinolone ribonucleosides were synthesized by using microwave irradiation starting from fluoroanilines. In most cases the microwave application proved superior in time and yield, especially the one step decarboxylation of the carboxyquinolone esters **3a**–**3c** and the Vorbrüggen glycosylation. The former led to the new type of fluoroquinolone ribosides **8a**–**8c**. Compound **8c** in the crystal structure showed C3′-endo and anti conformation. The nucleosides were examined, but found inactive against the replication of HIV-1(IIIB) in cell culture, while they were toxic for the cells at a 50% cytotoxic concentration ranging from 31 to >125 μg/ml. But measurements of the inhibitory effects against HIV-1 integrase enzymatic activity showed an interesting activity for compound **8c**.

Keywords: Nucleosides; Microwaves; Fluoroquinolones; Glycosidation; Decarboxylation; Anti-HIV activity; HIV integrase; Antivirals.

Human immunodeficiency virus (HIV), a retrovirus which causes the acquired immune deficiency syndrome (AIDS), has a replication cycle characterized by the reverse transcription of its genomic RNA into double-stranded DNA and by the integration of this viral DNA copy into the host chromosome. Integrase is an enzyme of particular interest, since integration is an essential step in the replication cycle of HIV and no human counterpart of integrase has been found so far[1–4]. The only integrase inhibitors with antiviral effect reported to date are the diketo acids (DKA) and the pyranopyrimidines (PDP)[5,6].

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Since several aminoquinolone derivatives were previously shown to exhibit potent activity against replication of HIV-1 in de novo-infected human lymphoblastoid cells, we decided to work towards new synthetic quinolones[7,8]. Their easy and reliable synthesis was introduced by Gould and Jacobs[9] in 1939. But it was not until 1963 that nalidixic acid, a systemic Gram-negative antibacterial agent, was introduced as a drug[10]. The outstanding antibacterial and antiviral activity of nalidixic acid and its numerous analogues led to research activities towards new synthetic quinolones[11–13]. Natural nucleoside analogues have been investigated for antiviral and anticancer therapy[14]. Considering the pharmacological effects of both substance classes we decided to synthesize monofluorinated quinolone nucleosides since only one (the free acid and ethyl ester of compound **6**) were previously described in literature[15]. A few groups synthesized quinolone nucleosides, all bearing a carboxy group in 3 position during the last decade for biological evaluation but none of them showed considerable biological effects[10,15,16]. In this paper we report the first full synthesis of fluoroquinolone nucleosides by microwave-induced chemistry with good to excellent yields, the antiviral evaluation of all compounds and their integrase inhibition.

EXPERIMENTAL

General

All ^{1}H and ^{13}C NMR spectra were measured on a Bruker AM 250 (250 MHz) spectrometer or on a Bruker AMX 400 (400 MHz) spectrometer (δ in ppm, *J* in Hz). MALDI mass spectra were recorded on a Fisons VG Tofspec spectrometer and ESI mass spectra on a Fisons VG Plattform II spectrometer. Column chromatography was carried out on silica gel 60 (Merck 9385, 34–62 μm). Melting points were measured on a Büchi apparatus after Totolli and are uncorrected. The microwave-supported syntheses were accomplished in equipment of the company CEM, model Discover. All reactions were carried out in dry flasks under argon.

Diethyl [(Fluoroanilino)methylidene]malonates (**2a**–**2c**). General Procedure

Stoichiometric amounts of a fluoroaniline and diethyl (ethoxymethylidene)malonate (DEMM) were mixed in a round-bottom flask and microwave-irradiated (150 W) at 250 °C for 2 min. After cooling the obtained solid was dried under reduced pressure.

Diethyl [(2-fluoroanilino)methylidene]malonate (**2a**). 2-Fluoroaniline (**1a**; 1.11 g, 10 mmol) and diethyl (ethoxymethylidene)malonate (DEMM; 2.16 g, 10 mmol) were reacted according to the general procedure. Yield 2.81 g (100%), m.p. 80 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 10.89 (d, *J* = 13.5, NH); 8.47 (d, *J* = 13.5, H8); 7.63 (t, *J* = 8.25, 1 H, H5); 7.26 (m, 3 H, H3, H4, H6); 4.17 (m, 4 H, CH_2); 1.27 (m, 6 H, CH_3). ^{13}C NMR (63 MHz, DMSO-d_6): 167.63 (C=O), 164.32 (C=O), 154.15 (C7), 150.34 (C-F), 127.35 (C1), 125.45 (C5), 117.45 (C4), 116.13 (C3), 115.83 (C6), 94.41 (C8), 59.92 (CH_2), 14.16 (CH_3). ESI (+), *m/z*: 282.0 $(M + H)^+$.

Diethyl [(3-fluoroanilino)methylidene]malonate (**2b**). 3-Fluoroaniline (**1b**; 14.92 g, 0.134 mol) and DEMM (29.03 g, 0.134 mol) were reacted according to the general procedure. Yield 37.32 g (99%), m.p. 48 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 10.68 (d, J = 13.5, 1 H, NH); 8.39 (d, J = 13.75, 1 H, H8); 7.40 (m, 2 H, H5, H2); 7.22 (m, 1 H, H6); 6.98 (m, 1 H, H4); 4.18 (m, 4 H, CH_2); 1.27 (m, 6 H, CH_3). ^{13}C NMR (63 MHz, DMSO-d_6): 167.06 (C=O), 164.80 (C=O), 160.65 (C-F), 150.64 (C8), 141.44 (C1), 131.34 (C5), 113.48 (C6), 115.13 (C4), 94.25 (C9), 59.73 (CH_2), 14.19 (CH_3). ESI (+), *m/z*: 282.0 $(M + H)^+$.

Diethyl [(4-fluoroanilino)methylidene]malonate (**2c**). 4-Fluoroaniline (**1c**; 11.70 g, 0.105 mol) and DEMM (22.80 g, 0.105 mol) were reacted according to the general procedure. Yield 28.66 g (97%), m.p. 66 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 10.71 (d, J = 14, 1 H, NH); 8.34 (d, J = 14, 1 H, H8); 7.43 (m, 2 H, H2, H6); 7.24 (m, 2 H, H3, H5); 4.17 (m, 4 H, CH_2); 1.25 (m, 6 H, CH_3). ^{13}C NMR (63 MHz, DMSO-d_6): 167.25 (C=O), 164.92 (C=O), 161.13 (C-F), 151.66 (C7), 136.12 (C1), 119.78–114.46 (C2, C3, C5, C6), 93.04 (C8), 59.58 (CH_2), 14.21 (CH_3). ESI (+), *m/z*: 282.1 $(M + H)^+$.

Ethyl Fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylates (**3a**–**3c**). General Procedure

A diethyl [(fluoroanilino)methylidene]malonate was suspended in diphenyl ether and refluxed for 1 h. After cooling, a white precipitate was filtered off and dried in vacuum.

Ethyl 8-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**3a**). Diethyl [(2-fluoroanilino)-methylidene]malonate (**2a**; 25 g, 89 mmol) was suspended in diphenyl ether (270 ml) and it was proceeded according to the general procedure. Yield 20.91 g (100%).

Microwave synthesis. Diethyl [(2-fluoroanilino)methylidene]malonate (**2a**; 5.62 g, 20 mmol) was suspended in diphenyl ether (56 ml) and microwave-irradiated (300 W) at 280 °C for 30 min. After cooling the precipitate was filtered off, washed with toluene and dried under reduced pressure. Yield of **3a** 1.42 g (30%), m.p. 178 °C. ^{1}H NMR (400 MHz, TFA-*d*): 9.75 (s, H2); 8.86 (t, J = 3.04, H5); 8.34 (m, 2 H, H6, H7); 5.08 (m, 2 H, CH_2); 1.917 (t, J = 7.12, 3 H, CH_3). ESI (+), *m/z*: 236.0 $(M + H)^+$. For $C_{12}H_{10}NO_3F$ (235.2) calculated: 61.28% C, 4.29% H, 5.96% N; found: 61.20% C, 4.05% H, 6.10% N.

Ethyl 7-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**3b**). Diethyl [(3-fluoroanilino)-methylidene]malonate (**2b**; 11.24 g, 40 mmol) was suspended in diphenyl ether (110 ml) and proceeded after the general procedure. Yield 6.23 g (66%).

Microwave synthesis. Diethyl [(3-fluoroanilino)methylidene]malonate (**2b**; 5.62 g, 20 mmol) was suspended in diphenyl ether (56 ml) and microwave-irradiated (300 W) at 280 °C for 30 min. After cooling the precipitate was filtered off, washed with toluene and dried under vacuum. Yield of **3b** 4.21 g (90%), m.p. 296 °C. ^{1}H NMR (400 MHz, TFA-*d*): 9.69 (s, 1 H, H2); 9.09 (m, 1 H, H8); 8.18 (m, 1 H, H5); 8.07 (m, 1 H, H6); 5.05 (q, J = 7.09, 2 H, CH_2) 1.90 (t, J = 7.09, 3 H, CH_3). ESI (+), *m/z*: 235.8 $(M + H)^+$. For $C_{12}H_{10}NO_3F$ (235.2) calculated: 61.28% C, 4.29% H, 5.96% N; found: 61.05% C, 4.29% H, 6.14% N.

Ethyl 6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**3c**). Diethyl [(4-fluoroanilino)-methylidene]malonate (**2c**; 3.03 g, 10.8 mmol) was suspended in diphenyl ether (56 ml) and proceeded after the general procedure. Yield 2.22 g (88%).

Microwave synthesis. Diethyl [(4-fluoroanilino)methylidene]malonate (**2c**; 4 g, 14.2 mmol) was suspended in diphenyl ether (35 g) and microwave-irradiated (300 W) at 280 °C for 15 min. After cooling the precipitate was filtered off, washed with toluene and dried under reduced pressure. Yield of **3c** 2.57 g (77%), m.p. 299 °C. ^{1}H NMR (250 MHz, TFA-*d*): 9.17 (s, 1 H, CH); 8.12 (m, 2 H, H5, H7); 7.79 (m, 1 H, H8); 4.59 (q, J = 6.5, 2 H, CH_2); 1.35 (t, J =

6.5, 3 H, CH_3). ^{13}C NMR (100.62 MHz, TFA-*d*): 175.35 (C4), 169.00 (C11), 158.62 (C-F), 147.04 (C9), 138.64 (C2), 131.92 (C7), 126.4 (C10), 120.95 (C5), 118.10 (C8), 107.48 (C3), 67.24 (C12), 14.50 (C13). ESI (+), *m/z*: 235.8 $(M + H)^+$.

Fluoroquinolin-4(1*H*)-ones (**4a**–**4c**). General Procedure

An ethyl fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate was mixed with diphenyl ether and a catalytic amount of water in a pressure vial with magnetic stirrer. The suspension was microwave-irradiated (300 W) at 20 bar and 260 °C for 20 min. After cooling the precipitate was filtered off, washed with toluene and dried in vacuo.

8-*Fluoroquinolin-4(1H)-one* (**4a**). Ethyl 8-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**3a**; 0.35 g, 1.5 mmol) was suspended in diphenyl ether (6.2 ml) and water (6.5 µl, 0.36 mmol) was added. Yield 0.235 g (96%), m.p. 262 °C. ^1H NMR (250 MHz, DMSO-d_6): 7.88 (m, 2 H, H2, H5); 7.53 (m, 1 H, H6); 7.26 (m, 1 H, H7); 6.09 (d, *J* = 7.25, 1 H, H3). ^{13}C NMR (63 MHz, DMSO-d_6): 175.67 (C4), 154.06 (C-F), 150.12 (C10), 140.26 (C2), 130.20 (C9), 127.30 (C6), 120.61 (C7), 116.01 (C5), 109.37 (C3). ESI (+), *m/z*: 163.8 $(M + H)^+$.

7-Fluoroquinolin-4(1H)-one (**4b**). Ethyl 7-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**3b**; 0.35 g, 1.5 mmol) was suspended in diphenyl ether (6.2 ml) and water (6.5 µl, 0.36 mmol) was added. Yield 0.240 g (98%), m.p. 207 °C. ^1H NMR (250 MHz, DMSO-d_6): 12.80 (bs, 1 H, NH); 8.14 (m, 1 H, H8); 7.92 (d, *J* = 7.5, 1 H, H2); 7.36 (m, 2 H, H5, H6); 6.04 (d, *J* = 7.25, 1 H, H3). ^{13}C NMR (63 MHz, DMSO-d_6): 176.24 (C4), 165.67 (C-F), 141.46 (C2), 139.91 (C9), 128.33 (C8), 122.83 (C10), 112.04 (C5), 111.66 (C6), 108.98 (C3). ESI (+), *m/z*: 163.7 $(M + H)^+$.

6-Fluoroquinolin-4(1H)-one (**4c**). Ethyl 6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**3c**; 0.235 g, 1 mmol) was suspended in diphenyl ether (4.1 ml) and water (4.3 µl, 0.24 mmol) was added. Yield 0.163 g (100%), m.p. 197 °C. ^1H NMR (250 MHz, DMSO-d_6): 11.91 (s, 1 H, NH); 7.93 (d, *J* = 7.25, 1 H, H2); 7.62 (m, 3 H, H5, H7, H8); 6.03 (d, *J* = 7.25, 1 H, H3). ^{13}C NMR (63 MHz, DMSO-d_6): 175.89 (C4), 160.14 (C-F), 139.61 (C2), 136.77 (C9), 126.86 (C10), 121.13 (C7), 120.66 (C5), 109.01 (C8), 107.76 (C3). ESI (+), *m/z*: 163.7 $(M + H)^+$.

Synthesis of Methyl Fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate Nucleosides (**7a**–**7c**). General Procedure

An ethyl fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**3a**–**3c**; 1.18 g, 5 mmol) and 1,2,3,5-*O*-tetraacetyl-β-D-ribofuranose (2.07 g, 1.3 equiv., 6.5 mmol) were suspended in dry acetonitrile (100 ml) and *N*,*O*-bis(trimethylsilyl)acetamid (1.32 g, 1.3 equiv., 6.5 mmol) was added. The colorless solution was refluxed for 30 min. After cooling to room temperature trimethylsilyl triflate (1.44 g, 1.3 equiv., 6.5 mmol) was added and the solution refluxed for another 3.5 h. After cooling the solution was quenched with saturated $NaHCO_3$ solution, the precipitate filtered off and identified as unreacted nucleobase. The solution was extracted with methylene chloride three times, dried over anhydrous $MgSO_4$ and the solvent was evaporated in vacuo.

*8-Fluoro-4-oxo-1-(2,3,5-tri-O-acetyl-*β*-D-ribofuranosyl)-1,4-dihydroquinoline-3-carboxylate* (**5a**). The brown oil was not purified. Yield 1.03 g (42%). ESI (+), *m/z*: 494.1 $(M)^+$.

*7-Fluoro-4-oxo-1-(2,3,5-tri-O-acetyl-*β*-D-ribofuranosyl)-1,4-dihydroquinoline-3-carboxylate* (**5b**). The brown oil was purified by column chromatography (CH_2Cl_2–MeOH 95:5). Yield 0.93 g (38%). R_F 0.32 (CH_2Cl_2–MeOH 95:5). ESI (+), *m/z*: 494.2 $(M)^+$.

6-Fluoro-4-oxo-1-(2,3,5-tri-O-acetyl-β-D-*ribofuranosyl)-1,4-dihydroquinoline-3-carboxylate* (**5c**). The orange foam was not purified. Yield 1.58 g (64%). R_F 0.63 (CH_2Cl_2–MeOH 9:1). ESI (+), *m/z*: 494.0 $(M)^+$; 516.0 $(M + Na)^+$.

Deprotection of Acetylated Nucleosides. General Procedure

An ethyl fluoro-4-oxo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,4-dihydroquinoline-3-carboxylate (**5a**–**5b**) was dissolved in dry methanol and a 5.4 M sodium methoxide solution was added. The solution was stirred for 1 h, neutralized with Dowex 50WX8, filtered, washed with methanol and the solvent evaporated in vacuo. The product was reprecipitated from methanol.

Methyl 8-fluoro-4-oxo-1-(2,3,5-tri-O-acetyl-β-D-*ribofuranosyl)-1,4-dihydroquinoline-3-carboxylate* (**7a**). 8-Fluoro-4-oxo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,4-dihydroquinoline-3-carboxylate (**5a**; 0.5 g, 1 mmol), dry methanol (9 ml) and the sodium methoxide solution (0.06 ml) were reacted according to the general procedure. Yield 0.26 g (76%). R_F 0.75 (CH_2Cl_2–MeOH 4:1). ^{1}H NMR (250 MHz, DMSO-d_6): 9.21 (m, 1 H, H2); 8.40 (m, 1 H, H5); 8.31 (m, 1 H, H6); 8.27 (m, 1 H, H7); 5.69 (m, 1 H, H1′); 4.69 (m, 1 H, H2′); 4.21 (m, 2 H, H3′, H4′); 3.87 (m, 2 H, H5′a, H5′b); 3.59 (s, 3 H, CH_3). MALDI (+), *m/z*: 353.93 $(M + H)^+$.

Methyl 7-fluoro-4-oxo-1-(2,3,5-tri-O-acetyl-β-D-*ribofuranosyl)-1,4-dihydroquinoline-3-carboxylate* (**7b**). 7-Fluoro-4-oxo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,4-dihydroquinoline-3-carboxylate (**5b**; 0.59 g, 1.2 mmol), dry methanol (9 ml) and the sodium methoxide solution (0.06 ml) were reacted according to the general procedure. Yield 0.24 g (60%). R_F 0.25 (CH_2Cl_2–MeOH 9:1). ^{1}H NMR (250 MHz, DMSO-d_6): 9.35 (m, 1 H, H2); 8.31 (m, 1 H, H5); 7.61 (d, J = 11, 1 H, H6); 7.23 (t, J = 7.75, 1 H, H8); 5.80 (d, J = 3.5, 1 H, H1′); 4.62 (m, 1 H, H2′); 4.26 (m, 2 H, H3′, H4′); 3.89 (m, 2 H, H5′a, H5′b); 3.68 (s, 3 H, CH_3). ESI (–), *m/z*: 352.0 $(M - H)^-$.

Methyl 6-fluoro-4-oxo-1-(2,3,5-tri-O-acetyl-β-D-*ribofuranosyl)-1,4-dihydroquinoline-3-carboxylate* (**7c**). 6-Fluoro-4-oxo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,4-dihydroquinoline-3-carboxylate (**5c**; 1.58 g, 3.2 mmol), dry methanol (24 ml) and the sodium methoxide solution (0.18 ml) were reacted according to the general procedure. Yield 0.68 g (67%). R_F 0.2 (CH_2Cl_2–MeOH 9:1). ^{1}H NMR (250 MHz, DMSO-d_6): 8.95 (s, 1 H, H2); 7.91 (m, 3 H, H5, H7, H8); 6.07 (m, 1 H, H1′); 4.19 (m, 1 H, H2′); 4.06 (m, 2 H, H4′, H3′); 3.68 (m, 2 H, H5′a, H5′b); 3.58 (s, 3 H, CH_3). ^{13}C NMR (63 MHz, DMSO-d_6): 169.32 (C4), 169.18 (C-F), 123.2 (C7), 118.08 (C5), 109.99 (C8), 108.04 (C3), 103.76 (C5), 83.56 (C4′), 74.19 (C2′), 63.15 (C3′), 60.06 (C5′). MALDI (+), *m/z*: 354.95 $(M + H)^+$.

Synthesis of Fluoroquinolin-4(1*H*)-one Nucleosides (**6a**–**6c**). General Procedure

The fluoroquinolin-4(1*H*)-ones (**4a**–**4c**) and 1,2,3,5-*O*-tetraacetyl-β-D-ribofuranose were suspended in dry acetonitrile and *N*,*O*-bis(trimethylsilyl)acetamide was added. The solution was refluxed for 30 min. After cooling to room temperature, trimethylsilyl triflate was added and the solution refluxed for 3 h. After cooling the solutions were quenched with saturated $NaHCO_3$ solution, extracted with methylenechloride three times, dried over anhydrous $MgSO_4$ and the solvent evaporated in vacuo. Purification was done as stated for the different substances.

8-Fluoro-1-(2,3,5-tri-O-acetyl-β-D-*ribofuranosyl)quinolin-4(1H)-one* (**6a**). 8-Fluoroquinolin-4(1*H*)-one (**4a**; 0.82 g, 5 mmol), 1,2,3,5-*O*-tetraacetyl-β-D-ribofuranose (2.07 g, 1.3 equiv., 6.5 mmol), acetonitrile (100 ml), *N*,*O*-bis(trimethylsilyl)acetamide (1.32 g, 1.3 equiv., 6.5 mmol)

and trimethylsilyl triflate (1.44 g, 1.3 equiv., 6.5 mmol) were reacted according to the general procedure. The product was deprotected without further purification. Yield 0.81 g (39%).

Microwave synthesis. The reaction was carried out in a dry flask under argon. 8-Fluoroquinolin-4(1*H*)-one (**4a**; 0.816 g, 5.0 mmol) and 1,2,3,5-*O*-tetraacetyl-β-D-ribofuranose (1.91 g, 6 mmol) were suspended in dry acetonitrile (50 ml) and *N*,*O*-bis(trimethylsilyl)-acetamide (1.22 g, 1.1 equiv., 6 mmol) was added. The solution was microwave-irradiated (150 W) at 80 °C for 15 min. After cooling to room temperature, trimethylsilyl triflate (1.33 g, 1.1 equiv., 6 mmol) was added and the solution microwave-irradiated (150 W) at 80 °C for 50 min. After cooling the solution was quenched with saturated $NaHCO_3$ solution, extracted with methylene chloride three times, dried over anhydrous $MgSO_4$ and the solvent evaporated in vacuo. The oil was purified by column chromatography (CH_2Cl_2–MeOH 95:5). Yield of **6a** 1.69 g (80%). ESI (+), *m/z*: 422.0 $(M + H)^+$.

*7-Fluoro-1-(2,3,5-tri-O-acetyl-*β*-D-ribofuranosyl)quinolin-4(1H)-one* (**6b**). 7-Fluoroquinolin-4(1*H*)-one (**4b**; 1.63 g, 10 mmol), 1,2,3,5-*O*-tetraacetyl-β-D-ribofuranose (4.14 g, 1.3 equiv., 13 mmol), acetonitrile (100 ml), *N*,*O*-bis(trimethylsilyl)acetamide (2.64 g, 1.3 equiv., 13 mmol) and trimethylsilyl triflate (2.88 g, 1.3 equiv., 13 mmol) were reacted according to the general procedure. The foam was deprotected without further purification. ESI (+), *m/z*: 422.0 $(M - H)^+$.

*6-Fluoro-1-(2,3,5-tri-O-acetyl-*β*-D-ribofuranosyl)quinolin-4(1H)-one* (**6c**). 6-Fluoroquinolin-4(1*H*)-one (**4c**; 0.82 g, 5 mmol), 1,2,3,5-*O*-tetraacetyl-β-D-ribofuranose (2.07 g, 1.3 equiv., 6.5 mmol), acetonitrile (100 ml), *N*,*O*-bis(trimethylsilyl)acetamide (1.32 g, 1.3 equiv., 6.5 mmol) and trimethylsilyl triflate (1.44 g, 1.3 equiv., 6.5 mmol) were reacted according to the general procedure. The product was purified by column chromatography (CH_2Cl_2–MeOH 95:5). Yield 1.34 g (64%).

Microwave synthesis. The reaction was carried out in a dry flask under argon. 6-Fluoroquinolin-4(1*H*)-one (**4c**; 0.90 g, 5.5 mmol) and 1,2,3,5-*O*-tetraacetyl-β-D-ribofuranose (1.91 g, 6 mmol) were suspended in dry acetonitrile (50 ml) and *N*,*O*-bis(trimethylsilyl)acetamide (1.22 g, 1.1 equiv., 6 mmol) was added. The solution was microwave-irradiated (150 W) at 80 °C for 15 min. After cooling to room temperature, trimethylsilyl triflate (1.33 g, 1.1 equiv., 6 mmol) was added and the solution microwave-irradiated (150 W) at 80 °C for 50 min. After cooling the solution was quenched with saturated $NaHCO_3$ solution, extracted with methylenechloride three times, dried over anhydrous $MgSO_4$ and the solvent evaporated in vacuo. The oil was purified by colomn chromatography (CH_2Cl_2–MeOH 95:5). Yield of **6c** 2.23 g (96%). R_F 0.66 (CH_2Cl_2–MeOH 9:1). ^{1}H NMR (250 MHz, DMSO-d_6): 8.15 (d, J = 8, 1 H, H2); 7.88 (m, 1 H, H5); 7.76 (m, 1 H, H7); 7.60 (m, 1 H, H8); 6.41 (d, J = 5.5, 1 H, H1′); 6.15 (d, J = 8, 1 H, H3); 5.45 (m, 1 H, H2′); 5.29 (m, 1 H, H3′); 4.36 (m, 3 H, H4′, H5′a, H5′b); 2.06 (s, 3 H, C2′-Ac); 2.02 (s, 3 H, C3′-Ac); 1.99 (s, 3 H, C5′-Ac). ^{13}C NMR (100.61 MHz, DMSO-d_6): 175.75 (C4), 170.00 (C=O, acetyl), 169.41 (C=O, acetyl), 169.16 (C=O, acetyl), 159.17 (C-F), 139.28 (C9), 136.14 (C2), 127.79 (C7), 120.80 (C10), 120.50 (C5), 119.10 (C8), 109.20 (C3), 89.39 (C1′), 79.06 (C4′), 72.20 (C2′), 69.01 (C3′), 62.65 (C5′), 20.51 (C-Ac). ESI (+), *m/z*: 422.1 $(M + H)^+$.

Deprotection of Nucleosides **6a**–**6c**. General Procedure

A fluoro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)quinolin-4(1*H*)-one (**6a**–**6c**) was dissolved in dry methanol and a 5.4 M sodium methoxide solution was added. The solution was stirred

for 1 h, neutralized with Dowex 50WX8, filtered, washed with methanol and the solvent was evaporated in vacuo. The products were reprecipitated from methanol.

8-Fluoro-1-(β-D-ribofuranosyl)quinolin-4(1H)-one (**8a**). 8-Fluoro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)quinolin-4(1*H*)-one (**6a**; 0.5 g, 1.19 mmol), dry methanol (9 ml) and the sodium methoxide solution (0.06 ml) were reacted according to the general procedure. Yield 0.24 g (68%). R_F 0.48 (CH_2Cl_2–MeOH 4:1). ^{1}H NMR (250 MHz, DMSO-d_6): 8.32 (d, J = 8.25, 1 H, H2); 8.08 (m, 1 H, H7); 7.59 (m, 1 H, H5); 7.36 (m, 1 H, H6); 6.10 (d, J = 9.25, 1 H, H3); 5.70 (m, 2 H, H1′, 2′-OH); 5.22 (d, J = 5.25, 1 H, 3′-OH); 5.18 (d, J = 5, 1 H 5′-OH); 4.11 (m, 1 H, H2′); 3.90 (m, 1 H, H3′); 3.84 (m, 1 H, H4′); 3.70 (m, 2 H, H5′a, H5′b). ^{13}C NMR (63 MHz, DMSO-d_6): 175.61 (C4), 169.71 (C-F), 169.18 (C9), 140.71 (C2), 128.91 (C7), 123.21 (C10), 110.55 (C6), 107.96 (C3), 103.43 (C5), 91.33 (C1′), 85.29 (C4′), 73.66 (C2′), 62.07 (C3′), 60.06 (C5′). ESI (–), *m/z*: 292.9 $(M - 2H)^{2-}$.

7-Fluoro-1-(β-D-ribofuranosyl)quinolin-4(1H)-one (**8b**). 7-Fluoro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)quinolin-4(1*H*)-one (**6b**; 5.9 g, impure), dry methanol (100 ml) and the sodium methoxide solution (0.79 ml) were reacted according to the general procedure. The product was purified by column chromatography and reprecipitated from methanol. Yield 1.2 g (43%). R_F 0.47 (CH_2Cl_2–MeOH 4:1). ^{1}H NMR (250 MHz, DMSO-d_6): 8.45 (d, J = 8.25, 1 H, H2); 8.22 (m, 1 H, H8); 7.66 (m, 1 H, H5); 7.27 (m, 1 H, H6); 6.10 (d, J = 8.25, 1 H, H3); 5.94 (d, J = 4.25, 1 H, H1′); 5,72 (d, J = 6.0, 1 H, 2′-OH); 5.24 (m, 2 H, 3′-OH, 5′-OH); 4.13 (m, 1 H, H2′); 4.02 (m, 2 H, H3′, H4′); 3.66 (m, 2 H, H5′a, H5′b). ^{13}C NMR (63 MHz, DMSO-d_6): 175.95 (C4), 162.61 (C-F), 141.05 (C9), 138.96 (C2), 129.03 (C8), 123.18 (C10), 112.20 (C6), 109.25 (C3), 102.26 (C5), 91.54 (C1′), 85.06 (C4′), 74.59 (C2′), 62.28 (C3′), 60.13 (C5′). MALDI (+), *m/z*: 296.5 $(M + H)^+$.

6-Fluoro-1-(β-D-ribofuranosyl)quinolin-4(1H)-one (**8c**). 6-Fluoro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)quinolin-4(1*H*)-one (**6c**; 1.899 g, 4.506 mmol), dry methanol (35 ml) and the sodium methoxide solution (0.30 ml) were reacted according to the general procedure. The product was recrystallized from methanol. Yield 1.293 g (97%). R_F 0.125 (CH_2Cl_2–MeOH 9:1). ^{1}H NMR (250 MHz, DMSO-d_6): 8.50 (d, J = 8.75, 1 H, H2); 7.84 (m, 2 H, H5, H7); 7.65 (m, 1 H, H8); 6.11 (d, J = 7.75, 1 H, H3); 6.01 (d, J = 4, 1 H, H1′); 4.19 (m, 1 H, H2′); 4.02 (m, 2 H, H4′, H3′); 3.67 (m, 2 H, H5′a, H5′b). ^{13}C NMR (63 MHz, DMSO-d_6): 174.87 (C4), 168.89 (C-F), 138.54 (C9), 128.93 (C7), 122.32 (C10), 117.47 (C5), 109.29 (C8), 77.91 (C4′), 71.10 (C2′), 67.82 (C3′), 61.05 (C5′). ESI (+), *m/z*: 296.0 $(M + H)^+$.

X-ray analysis of **8c**: $C_{14}H_{14}FNO_5 \cdot 0.5CH_3OH \cdot 0.25H_2O$. Crystal system: monoclinic, space group: $P2_1\uparrow$, crystal color: colorless, unit cell parameters: a = 8.0476(12) Å, b = 43.884(7) Å, c = 8.1096(12) Å, β = 90.432(6)°, V = 2863.9(8) Å^3, Z = 8, R = 0.102, GOF = 1.03. A single crystal was measured on a Siemens Smart CCD diffractometer, radiation MoKα at temperature of ca. –114 °C. The structure was determined by direct methods using program SHELXS. CCDC 600092 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge, CB2 1EZ, UK; fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk).

In vitro Anti-HIV Assay and Drug Susceptibility Assay

The antiviral activity of these compounds on the HIV-induced CPE in human lymphocyte MT-4 cell culture was determined by the MT-4/MTT-assay[17]. All compounds proved to be inactive against HIV-1 replication at subtoxic concentrations.

The overall integration assay. The enzymatic overall integration reaction was carried out as described previously with minor modifications[18,19]. In the overall integration assay, binding of integrase to DNA substrate, 3′-processing and DNA strand transfer can be detected. The final reaction mixture for this assay was 20 mmol in HEPES, pH 7.5, 5 mmol in dithiothreitol (DTT), 10 mmol in $MgCl_2$, 75 mmol in NaCl, 15% (v/v) in poly(ethylene glycol) 8000, 30 nmol in the oligonucleotide substrate, and 760 nmol in the His-tag IN (10 μl was the final volume). Reactions were started by the addition of the enzyme. Inhibitors were incubated shortly with the reaction components before the addition of IN. Reactions were allowed to proceed at 37 °C, stopped by the addition of formamide loading buffer (95% formamide, 30 mM EDTA, 0.1% Xylene Cyanole, 0.1% Bromophenol Blue, 0.1% SDS). The products were separated in a 15% denaturing polyacrylamide/urea gel. Quantification of the results was performed using the PhosphorImager (Molecular Dynamics, Sunnyvale (CA), U.S.A.). Specific activities of different enzyme preparations (1 μM) were determined in this assay. The extent of 3′-processing or DNA strand transfer were based on the amounts of –2 bands or strand transfer products relative to the intensity of the total radioactivity present in the lane as determined using the software OptiQuant Acquisition and Analysis (Perkin Elmer Corporate, Fremont (CA), U.S.A.).

In vitro anti-HIV and drug susceptibility assays. The inhibitory effect of antiviral drugs on the HIV-induced CPE in human lymphocyte MT-4 cell culture was determined by the MT-4/MTT-assay[17]. This assay is based on the reduction of the yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) by mitochondrial dehydrogenase of metabolically active cells to a blue formazane derivative, which can be measured spectrophotometrically. The 50% cell culture infective dose ($CCID_{50}$) of different HIV strains was determined by titration of the virus stock using MT-4 cells. For the drug-susceptibility assays, MT-4 cells were infected with 100–300 $CCID_{50}$ of the virus stock in the presence of five-fold serial dilutions of the antiviral drugs. The concentration of various compounds providing 50% protection against the CPE of different HIV strains, (EC_{50}) was determined.

RESULTS

Synthesis

Conventional methods for synthesizing fluoroquinolones were slightly varied after Koga et al. (Scheme 1)[20]. Since high temperatures are needed in the synthesis of the heterocycle, we decided to make use of a microwave applicator. Therefore we modified the procedure of Kidwai et al.[21] for the first step. 2-, 3- and 4-Fluoroaniline were *N*-substituted with diethyl (ethoxymethylidene)malonate to give diethyl [(fluoroanilino)methylidene]malonates (**2a**–**2c**). The electrophilic ring closure was performed in diphenyl ether following Koga's procedure. The synthesis of quinolones (**4a**–**4c**) was reduced from a two-step synthesis by hydrolysis and decarboxylation to a single-step, one-pot reaction, modified after Curran et al.[22] Using this method we were able to shorten the synthesis by one step and to obtain very good yields (96–100%).

SCHEME 1

Reagents and conditions: (i) DEMM, MW, 2 min, 250 °C, 97–100%; (ii) diphenyl ether, Δ, 1 h, 66–100% or diphenyl ether, MW, 30 min, 280 °C, 30–90%; (iii) diphenyl ether, water, MW, 20 min, 20 bar, 260 °C, 96–100%; (iv) 1,2,3,5-*O*-tetraacetyl-D-ribofuranose, MeCN, bis(trimethylsilyl)acetamide, trimethylsilyl triflate, Δ, 3 h, 38–64% or 1,2,3,5-*O*-tetraacetyl-D-ribofuranose, MeCN, bis(trimethylsilyl)acetamide, trimethylsilyl triflate, MW, 50 min, 80–96%; (v) NaOMe, MeOH, r.t., 1 h, 43–97%

Several attempts to synthesize the nucleoside failed due to the lack of solubility of the quinolones in the Vorbrüggen reaction. A slightly modified Vorbrüggen method of Moore et al.[23] worked best for the general procedure, but we obtained excellent results (96% yields) by using microwave irradiation in the synthesis of **6c** instead of 64% and 80% for **6a** instead of 39% for the standard procedure. We do explain these results by a combination of optimal solubility and heat distribution due to the microwave irradiation. Deprotection was carried out using standard procedures with sodium methoxide in methanol. Nucleosides (**7a**–**7c** and **8a**–**8c**) were obtained in acceptable yields (20–69% in 4 steps or 5 steps for decarboxylated nucleobases).

We were able to crystallize compound (**8c**) from methanol. The crystal structure (Fig. 1) shows that in the solid state the nucleobase is anti to the sugar and the sugar conformation is C3′-endo-envelope. The planar quinolone rings of the nucleosides are stacked but flipped by 180° so that the sugar moieties avoid steric interference.

The fluorine molecules do not show hydrogen bonding in the crystal cluster (Fig. 2). Hydrogen bonding can be observed only between the carbonyl group and the hydroxy groups of the sugar. The crystal cluster contains hydrophilic and hydrophobic layers. Hydrophilic layers are formed by sugar moieties whereas hydrophobic layers by relatively nonpolar quinolone rings.

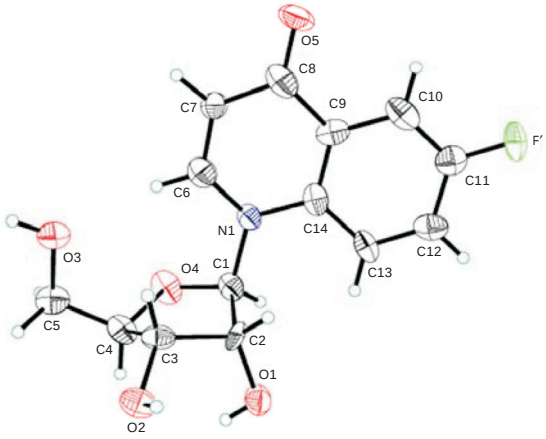

FIG. 1
Crystal structure of 6-fluoro-1-(β-D-ribofuranosyl)quinolin-4(1*H*)-one (**8c**)

Inhibition of HIV-1 IN Activity

Compounds (**7a**–**7c** and **8a**–**8c**) were tested in IN inhibition assays which have been recently reviewed[18,24]. Activity below 100 μg/ml was displayed by compound (**8c**) with IC_{50} = 63.4 ± 24.0 μg/ml. This biological activity is striking since compound (**8c**) lacks the carboxyl group at C-3. Non-nucleosidic quinolones tested for HIV-1-IN activity possessed the C-3 carboxyl moiety[8,25]. Therefore it is even more surprising that the carboxylated compounds did not show any considerable biological activity. These data are very promising in comparison with other unmodified dinucleosides, while mononucleosides have no activity at this concentration[26]. The integrase of HIV is an attractive target for antiviral therapy since there is no known functional homolog in human cells[27].

DISCUSSION

Compound **8c** is the first quinolone nucleoside with an interesting and promising biological activity. Considering this activity, it should be a good substance for structure-activity relationship tests and an interesting precursor of other modified nucleosides aiming at a higher HIV-integrase activity.

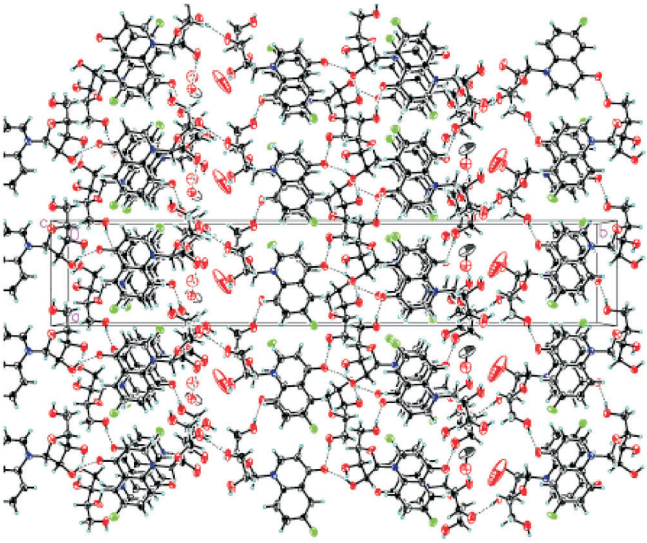

FIG. 2
Crystal cluster of 6-fluoro-1-(β-D-ribofuranosyl)quinolin-4(1*H*)-one (**8c**)

This is surprising considering that carboxylated nucleosides did not show any activity although this functional group is crucial for the antibacterial activity of fluoroquinolones. Compound **8c** is one of the most active anti-integrase nucleosides known sofar and could be a promising precursor of a new range of antiretroviral structures.

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