

RESEARCH ARTICLE

Synthesis and biological evaluation of 5-fluoroquinolone-3-carboxylic acids as potential HIV-1 integrase inhibitors

Qiu-Qin He¹, Xuan Zhang^{2,3}, Liu-Meng Yang², Yong-Tang Zheng², and Fener Chen¹

¹Department of Chemistry, Fudan University, Shanghai, P R China, ²Key Laboratory of Animal Models and Human Disease Mechanisms of Chinese Academy of Sciences & Yunnan Province, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, P R China, and ³Faculty of Pharmacy, Kunming Medical University, Kunming, P R China

Abstract

A series of new quinolone-3-carboxylic acids as HIV-1 integrase inhibitors featuring a fluorine atom at C-5 position were synthesized and evaluated for their antiviral activity in C8166 cell culture. These newly synthesized compounds showed anti-HIV activity against wild-type virus with an EC₅₀ value ranging from 29.85 to 0.032 μM. The most active compound **4e** exhibited activity against wild-type virus and the mutant virus A17 with an EC₅₀ value of 0.032 and 0.082 μM, respectively. Preliminary structure–activity relationship of these 5-fluoroquinolone-3-carboxylic acids was also investigated.

Keywords: HIV-1 integrase inhibitor, 5-fluoroquinolone-3-carboxylic acids, anti-HIV activity, metal chelating, structure–activity relationship

Introduction

Insertion of proviral DNA into the host chromosome is a crucial step in the HIV replication cycle. This process is catalyzed by virally encoded and packaged enzyme, namely HIV integrase (IN)^{1,2}. The recent approval of Raltegravir (Figure 1)^{3–5} by FDA and the encouraging late-stage clinical trials with Elvitegravir (Figure 1)^{6,7} have validated IN as a novel HIV therapeutic target.

Elvitegravir and its derivatives, firstly reported by Sato et al. in 2006, have emerged as promising therapeutic IN inhibitors, due to their high activity and favorable pharmacokinetic property⁸. Subsequent chemical modifications of their quinolone core focused on N-1, C-6, 7 and 8 position have been introduced in order to obtain more potent and selective IN inhibitors^{9–11}. However, very few modifications have been carried out at the C-5 position.

Considering the above facts, we have focused our attention on the introduction of the substituent at the C-5 position. Very recently, we identified a series of 5-hydroxyquinolone-3-carboxylic acids (Figure 1) with

low micromolar to submicromolar EC₅₀ values against HIV-1 virus^{12,13}. Among these 5-hydroxyquinolone analogues, the most active compound **3e** characterized by a hydroxymethyl moiety at the 1S-position of the isobutyl group exhibited 0.13 μM EC₅₀ value¹². As part of our ongoing effort to find potent anti-HIV 5-substituted-quinolone analogues, as well as the incorporation of fluorine into the target molecules could affect a variety of properties (e.g., enhanced binding interactions, metabolic stability, changes in physical properties, and selective reactivities) due to its special nature such as the high electronegativity, small size, and its van der Waals radius closer to that of oxygen^{14–16}, a series of new 5-fluoroquinolone-3-carboxylic acids (Figure 1) were synthesized and evaluated as potential IN inhibitors.

Chemistry

Two series of 5-fluoroquinolone-3-carboxylic acids (N-alkyl-substituted compounds **4a–e** and

Address for Correspondence: Professor Fener Chen, Department of Chemistry, Fudan University, Shanghai 200433, P R China. Tel: +86 21 65643809. Fax: +86 21 65643811. E-mail: rfchen@fudan.edu.cn

(Received 20 January 2012; revised 13 February 2012; accepted 13 February 2012)

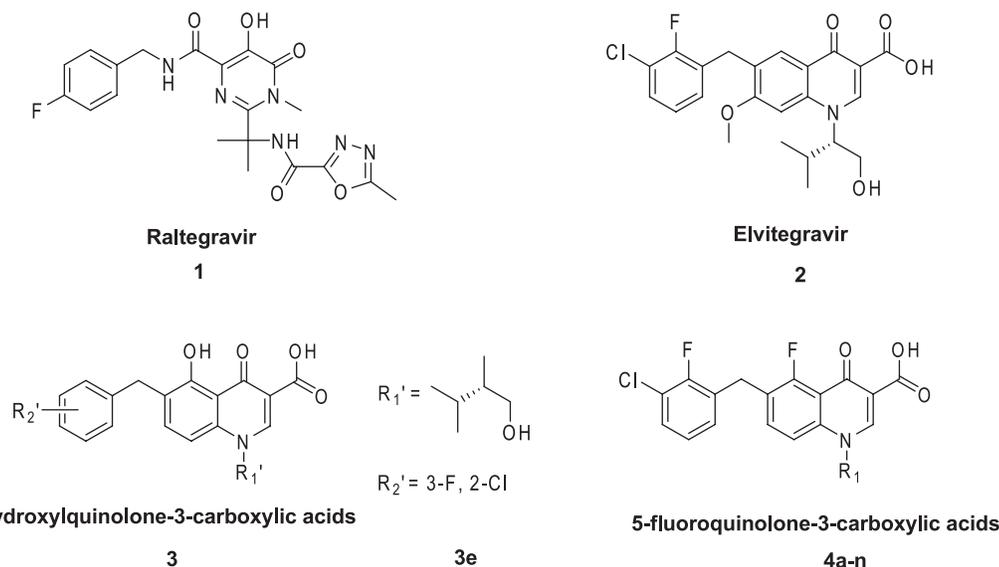
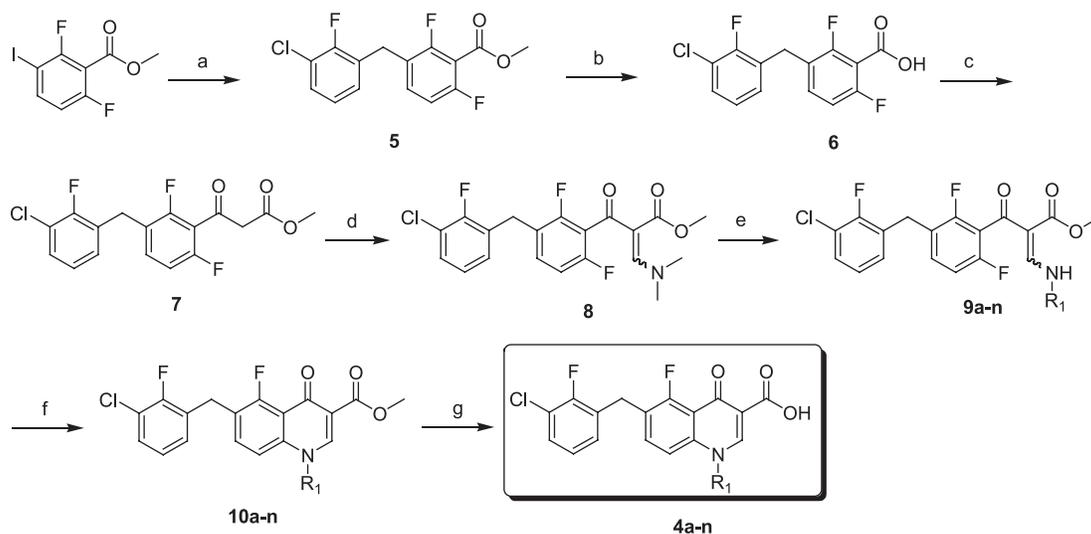


Figure 1. Chemical structures of HIV-1 IN inhibitors.



Scheme 1. Reagents and conditions.

N-aryl-substituted compounds **4f-n**) were synthesized similar to our previously reported protocol^{12,13} as depicted in Scheme 1. Compound **8** were prepared starting from **2**, 6-difluoro-3-iodobenzoate according to reported procedures¹². Condensation of **8** with *N,N*-dimethylformamide dimethyl acetal and followed by substitution with appropriate amines led to acrylates **9a-n**, which were then cyclized using 1,8-Diazabicycloundec-7-ene as a base to furnish quinolone esters **10a-n**. Ester hydrolysis of **10a-n** with satd aq LiOH afforded the target compounds.

Results and discussion

Biological evaluation

All the target compounds were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay^{17,18} for cytotoxicity and antiviral activity in

C8166 cells infected with the wild-type HIV-1 (LAI strain IIIB) and HIV-1 mutant virus (A17). Elvitegravir and **3e**¹² were included as the reference compounds. The results, expressed as EC₅₀, CC₅₀ and SI, are illustrated in Table 1.

The newly synthesized quinolone-3-carboxylic acids **4a-n** exhibited EC₅₀ values against HIV-1 IIIB in the range of 0.032–29.85 μM. Compound **4a**, bearing a propyl group at *N*-1 position of quinolone core, showed antiviral activity with 13.44 μM EC₅₀ value. The replacement of straight chain alkyl group of **4a** with branched (**4b**) or cyclic one (**4c**) led to an improvement of antiviral activity against wild-type HIV-1 IIIB. Compound **4d** with an *i*-butyl group at *N*-1 position showed more potent than *i*-propyl counterparts (**4b**). The introduction of a hydroxymethyl group at the 1*S*-position of butyl moiety significantly further enhanced its potency. Compound **4e** was the most active of this new series and was more potent than the reference compound **3e**. These results

Table 1. Anti-HIV-1 activity and cytotoxicity of compounds 4a–n in C8166 cells^a.

Compound	R ₁	EC ₅₀ ^b (μM)		CC ₅₀ ^c (μM)	SI ^d
		IIIB	A17		
4a	<i>n</i> -Propyl	13.44	ND	23.27	1.73
4b	<i>i</i> -Propyl	4.18	ND	92.19	22.06
4c	Cyclopropyl	2.90	2.69	>512.82	>176.83
4d	<i>i</i> -Butyl	2.49	20.20	48.25	19.38
4e	2 <i>S</i> -1-hydroxy-3-methylbutan-2-yl	0.032	0.082	41.08	1295.90
4f	<i>o</i> -Chlorophenyl	29.85	79.09	75.20	2.52
4g	<i>m</i> -Chlorophenyl	9.98	24.23	22.93	2.30
4h	<i>p</i> -Chlorophenyl	3.26	6.85	26.50	8.13
4i	<i>p</i> -Fluorophenyl	9.59	15.09	45.65	4.76
4j	<i>p</i> -Bromophenyl	3.82	6.57	28.97	7.58
4k	<i>p</i> -Trifluoromethylphenyl	2.47	15.22	23.08	9.34
4l	<i>m</i> -Methylphenyl	21.91	31.02	57.07	2.60
4m	<i>p</i> -Methylphenyl	11.89	52.09	65.70	5.53
4n	2,6-Dimethylphenyl	3.52	22.16	46.54	13.22
3e		0.13	0.078	125.03	961.77
Elvitegravir		0.00021	0.00096	17.20	81904.76

^aAll data represent mean values from at least two separate experiments.

^bEC₅₀: compound concentration required to protect the cell against viral cytopathogenicity by 50% in C8166 cells.

^cCC₅₀: compound concentration that decreases the normal uninfected C8166 cell viability by 50%.

^dSI: selectivity index; ratio CC₅₀/EC₅₀ (wild-type).

ND, not determined.

are consistent with the SAR for the 5-hydroxylquinolone analogues that the replacement of straight chain alkyl group with branched ones caused an increase in antiviral activity and the introduction of a hydroxymethyl group at the 1*S*-position of butyl moiety resulted in significant improvement of activity¹².

For the *N*-aryl-substituted compounds **4f–n**, *p*-chloro compound **4h** showed higher activity against both wild-type HIV-1 IIIB and HIV-1 mutant virus A17 than *o*-chloro (**4f**) and *m*-chloro (**4g**) compounds. Since the effect of the introduction of a withdrawing group at the para position was marked, a fluoro (**4i**), a bromo (**4j**), or a trifluoromethyl (**4k**) was introduced. Among these compounds, only **4k** exhibited slightly increased activity against HIV-1 IIIB compared to **4h**. The introduction of a methyl group at the para position resulted in **4m**, which showed less potent than **4h**; however, the introduction of dual methyl group at ortho position (**4n**) led to comparable activity to **4h**.

Molecular modeling calculations

In an attempt to investigate the binding model of our newly synthesized compounds with IN, molecular docking study was performed.

Compound **4e**, which displayed the most activity against both wild-type HIV-1 and the mutant virus A17, was docked into our previously constructed model of the HIV-1 IN catalytic core domain (CCD)/viral DNA complex¹² using SURFLEX-DOCK SYBYLX 1.1. For comparison, the binding modes of Elvitegravir and **3e** were also investigated. All the molecules were energy minimized by the conjugated gradient method with Gasteiger-Hückel charge until a convergence value of 0.01 kcal/(Å mol), using the Tripos force field. After the hydrogen atoms were added to the HIV-1

IN CCD/DNA complex, atomic charges were recalculated by Kollman all-atom for the protein and Gasteiger-Hückel for ligand. The protocol generated using a threshold of 0.50 and bloat of zero (default values). Other parameters were set as defaults for Surflex-Dock. The docking results showed that 4-ketone and 3-carboxylate in compound **4e** could form Mg²⁺ chelation with HIV-1 IN (Figure 2a). The quinolone ring exhibit π–π stacking interaction with A17, like **3e** and Elvitegravir do (Figure 2b and 2c). No interaction could be detected between the substituent on N-1 or C-5 fluoro atom and IN. Although the anti-HIV activity of **3e** (Figure 2b) might involve a two-metal chelating mechanism¹², **4e** showed more potent against HIV-1 IIIB than **3e**, this might due to the incorporation of fluorine into **4e** favorite its physicochemical properties.

Conclusions

In conclusion, we designed and synthesized a series of new 5-fluoroquinolone-3-carboxylic acids. All the compounds showed moderate to good activity against wild-type virus with an EC₅₀ value ranging from 29.85 to 0.032 μM. Compound **4e** was identified as the most active compound of this new series (EC₅₀ = 0.032 μM, SI = 1295.90) associated with high activity against HIV-1 mutant strain A17 (EC₅₀ = 0.082 μM). Preliminary structure–activity relationship of the newly synthesized quinolone analogues was also investigated.

Experimental

General procedures

Melting points were measured on a WRS-1 digital melting point apparatus and are uncorrected. ¹H NMR and

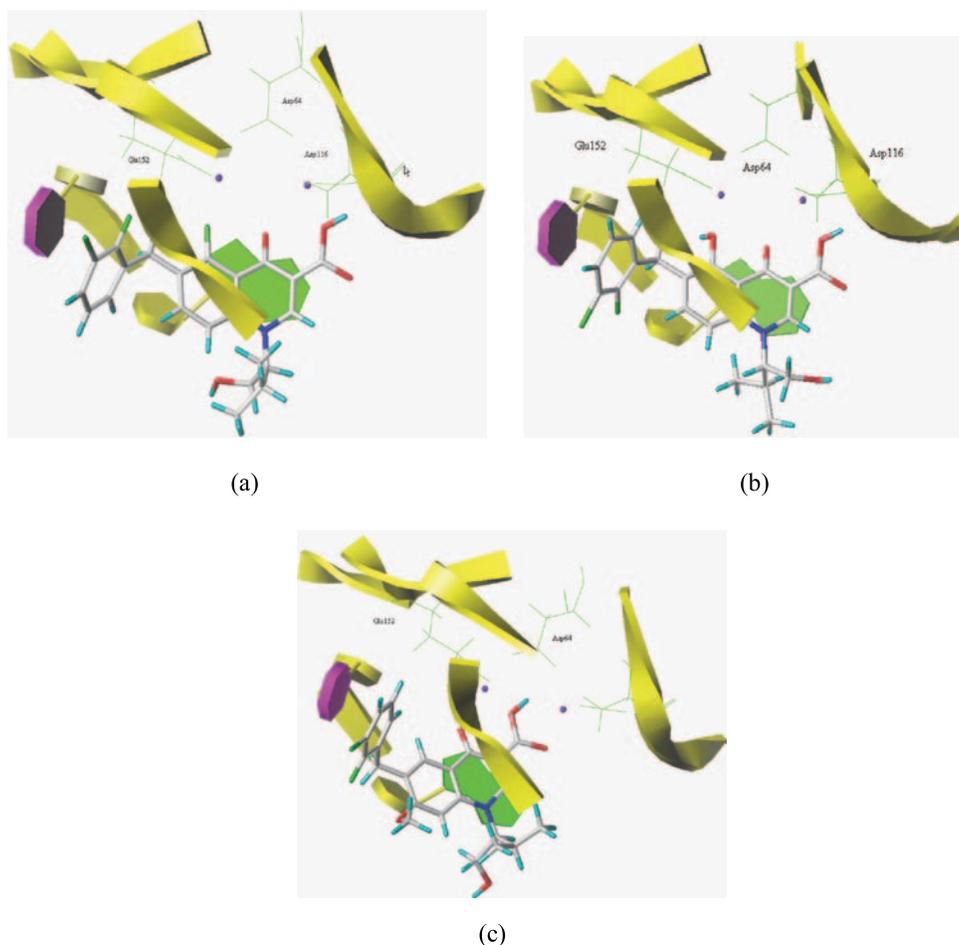


Figure 2. Binding models of compounds (a) **4e**, (b) **3e** and (c) Elvitegravirin the active site of new homology model of HIV-1 IN.

^{13}C NMR spectra on a Bruker AV 400 MHz spectrometer were recorded in CDCl_3 . Chemical shifts are reported in δ (ppm) units relative to the internal standard tetramethylsilane. Mass spectra were obtained on an Agilent MS/5975 mass spectrometer. All chemicals and solvents used were of reagent grade and were purified and dried by standard methods before use. All air-sensitive reactions were run under a nitrogen atmosphere. All the reactions were monitored by thin layer chromatography on pre-coated silica gel G plates at 254 nm under a UV lamp using ethyl acetate/hexane as eluents. Flash chromatography separations were obtained on silica gel (300–400 mesh).

General procedure for the preparation of **9a–n**

A mixture of 2-(3-(3-Chloro-2-fluorobenzyl)-2,6-difluoro benzoyl)-3-(dimethylamino) acrylate **8** (618 mg, 1.5 mmol) and appropriate amines (1.8 mmol) in tetrahydrofuran (15 mL) was stirred at 50°C for 5–10 min and then concentrated under reduced pressure. The resulting residue **9a–n** was used directly for the next step without further purification.

General procedure for the preparation of **10a–n**

A mixture of **9a–n** (1.0 mmol) and K_2CO_3 (2.5 mmol) in DMF (15 mL) was stirred at 60°C overnight, filtered

and poured into ice-water. The mixture was extracted by dichloromethane (5 mL \times 3). The combined organic solution was washed with brine (5 mL \times 2), dried over MgSO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, petroleum ether/ethyl acetate 5/1 to 3/1, v/v) to give the desired compound **10a–n**.

General procedure for the preparation of **4a–n**

To a solution of satd aq LiOH (5 mL) in dioxane (8 mL) was added **10a–n** (1.2 mmol). After being stirred at 50°C for 3 h, the mixture was cooled, poured into ice-water and acidified with 4 M HCl to pH \sim 2. The resulting precipitate was collected by filtration, washed by water and ethanol, dried to give the desired compound **4a–n**.

6-(3-chloro-2-fluorobenzyl)-5-fluoro-4-oxo-1-propyl-1,4-dihydroquinoline-3-carboxylic acid (4a) Yield 75%. Mp $192\text{--}193^\circ\text{C}$. ^1H NMR (CDCl_3) δ 1.01–1.05 (t, 3 H, $J = 7.2$ Hz, CH_3), 1.92–1.97 (m, 2 H, CH_2), 4.14 (s, 2 H, CH_2), 4.22–4.26 (t, 2 H, $J = 7.2$ Hz, CH_2), 7.01–7.04 (t, 1 H, $J = 8.0$ Hz, ArH), 7.17–7.21 (m, 1 H, ArH), 7.26–7.30 (m, 1 H, ArH), 7.32–7.35 (d, $J = 8.8$ Hz, 1 H, ArH), 7.65–7.66 (m, 1 H, ArH), 8.65 (s, 1 H, CH), 14.84 (s, 1 H, COOH). MS (ESI)

m/z 392 $[M+H]^+$. Anal. Calcd for $C_{20}H_{16}ClF_2NO_3$: C 61.31, H 4.12, N 3.58, found: C 61.59, H 4.36, N 3.32.

6-(3-chloro-2-fluorobenzyl)-5-fluoro-1-isopropyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4b**) Yield 82%. Mp 227–229°C. 1H NMR ($CDCl_3$) δ 1.63–1.65 (d, 6 H, $J = 6.0$ Hz, 2CH₃), 4.15 (s, 2 H, CH₂), 4.94–4.96 (m, 1 H, CH), 7.01–7.05 (t, 1 H, $J = 8.0$ Hz, ArH), 7.18–7.22 (m, 1 H, ArH), 7.26–7.30 (m, 1 H, ArH), 7.47–7.49 (d, $J = 8.8$ Hz, 1 H, ArH), 7.67–7.71 (m, 1 H, ArH), 8.85 (s, 1 H, CH), 14.93 (s, 1 H, COOH). MS (ESI) m/z 392 $[M+H]^+$. Anal. Calcd for $C_{20}H_{16}ClF_2NO_3$: C 61.31, H 4.12, N 3.58, found: C 61.52, H 3.86, N 3.35.

6-(3-chloro-2-fluorobenzyl)-1-cyclopropyl-5-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4c**) Yield 80%. Mp 239–240°C. 1H NMR ($CDCl_3$) δ 1.18–1.19 (d, 2 H, $J = 3.2$ Hz, CH₂), 1.39–1.40 (d, 2 H, $J = 3.2$ Hz, CH₂), 3.54–3.57 (m, 1 H, CH), 4.15 (s, 2 H, CH₂), 7.00–7.04 (t, 1 H, $J = 8.0$ Hz, ArH), 7.17–7.21 (m, 1 H, ArH), 7.26–7.30 (m, 1 H, ArH), 7.67–7.71 (m, 1 H, ArH), 7.83–7.85 (d, $J = 8.8$ Hz, 1 H, ArH), 8.80 (s, 1 H, CH), 14.72 (s, 1 H, COOH). MS (ESI) m/z 390 $[M+H]^+$. Anal. Calcd for $C_{20}H_{14}ClF_2NO_3$: C 61.63, H 3.62, N 3.59, found: C 61.85, H 3.38, N 3.32.

6-(3-chloro-2-fluorobenzyl)-5-fluoro-1-isobutyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4d**) Yield 82%. Mp 152–154°C. 1H NMR ($CDCl_3$) δ 0.99–1.01 (d, 6 H, $J = 6.4$ Hz, 2 CH₃), 2.24–2.27 (m, 1 H, CH), 4.05–4.07 (d, 2 H, $J = 6.4$ Hz, CH₂), 4.14 (s, 2 H, CH₂), 7.00–7.04 (t, 1 H, $J = 8.0$ Hz, ArH), 7.18–7.21 (m, 1 H, ArH), 7.26–7.28 (d, 1 H, $J = 6.8$ Hz, ArH), 7.29–7.32 (m, 1 H, ArH), 7.65–7.66 (m, 1 H, ArH), 8.65 (s, 1 H, CH), 14.84 (s, 1 H, COOH). MS (ESI) m/z 406 $[M+H]^+$. Anal. Calcd for $C_{21}H_{18}ClF_2NO_3$: C 62.15, H 4.47, N 3.45, found: C 62.41, H 4.68, N 3.22.

(S)-6-(3-chloro-2-fluorobenzyl)-5-fluoro-1-(1-hydroxy-3-methylbutan-2-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4e**) Yield 70%. Mp 95–97°C. 1H NMR ($CDCl_3$) δ 0.71–0.73 (d, 3 H, $J = 6.4$ Hz, CH₃), 1.11–1.12 (d, 3 H, $J = 6.0$ Hz, CH₃), 2.34–2.35 (m, 1 H, CH), 3.74–3.77 (brs, 1 H, OH), 4.04–4.11 (m, 4 H, 2 CH₂), 4.43–4.44 (m, 1 H, CH), 6.93–6.97 (t, 1 H, $J = 8.0$ Hz, ArH), 7.09–7.12 (m, 1 H, ArH), 7.19–7.22 (m, 1 H, ArH), 7.46–7.48 (d, 1 H, $J = 8.8$ Hz, ArH), 7.55–7.57 (m, 1 H, ArH), 8.78 (s, 1 H, CH), 15.10 (s, 1 H, COOH). MS (ESI) m/z 436 $[M+H]^+$. Anal. Calcd for $C_{22}H_{20}ClF_2NO_4$: C 60.63, H 4.63, N 3.21, found: C 60.40, H 4.36, N 3.45.

6-(3-chloro-2-fluorobenzyl)-1-(2-chlorophenyl)-5-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4f**) Yield 87%. Mp 236–238°C. 1H NMR ($CDCl_3$) δ 4.11–4.20 (dd, $J = 15.2, 21.6$ Hz, 2 H, CH₂), 6.65–6.67 (d, $J = 8.8$ Hz, 1 H, ArH), 7.02–7.06 (t, $J = 8.0$ Hz, 1 H, ArH), 7.19–7.23 (m, 1 H, ArH), 7.27–7.31 (m, 1 H, ArH), 7.49–7.54 (m, 2 H, ArH), 7.57–7.61 (m, 1 H, ArH), 7.63–7.67 (m, 1 H, ArH),

7.70–7.73 (m, 1 H, ArH), 8.65 (s, 1 H, CH), 14.66 (s, 1 H, COOH); MS (ESI) m/z 460 $[M+H]^+$. Anal. Calcd for $C_{23}H_{13}Cl_2F_2NO_3$: C 60.02, H 2.85, N 3.04, Found: C 59.81, H 3.11, N 3.28.

6-(3-chloro-2-fluorobenzyl)-1-(3-chlorophenyl)-5-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4g**) Yield 89%. Mp 215–217°C. 1H NMR ($CDCl_3$) δ 4.06 (s, 2 H, CH₂), 6.79–6.81 (d, $J = 8.8$ Hz, 1 H, ArH), 6.83–7.05 (m, 4 H, ArH), 7.20–7.35 (m, 3 H, ArH), 7.45–7.47 (d, $J = 8.8$ Hz, 1 H, ArH), 8.81 (s, 1 H, CH), 13.24 (s, 1 H, COOH); MS (ESI) m/z 460 $[M+H]^+$. Anal. Calcd for $C_{23}H_{13}Cl_2F_2NO_3$: C 60.02, H 2.85, N 3.04, Found: C 60.31, H 2.66, N 3.25.

6-(3-chloro-2-fluorobenzyl)-1-(4-chlorophenyl)-5-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4h**) Yield 90%. Mp >250°C. 1H NMR ($CDCl_3$) δ 4.16 (s, 2 H, CH₂), 6.84–6.86 (d, $J = 8.8$ Hz, 1 H, ArH), 7.03–7.07 (t, $J = 7.6$ Hz, 1 H, ArH), 7.20–7.24 (m, 1 H, ArH), 7.31–7.33 (m, 1 H, ArH), 7.36–7.39 (d, $J = 8.8$ Hz, 2 H, ArH), 7.50–7.56 (m, 1 H, ArH), 7.64–7.66 (d, $J = 8.8$ Hz, 2 H, ArH), 8.73 (s, 1 H, CH), 14.64 (s, 1 H, COOH); MS (ESI) m/z 460 $[M+H]^+$. Anal. Calcd for $C_{23}H_{13}Cl_2F_2NO_3$: C 60.02, H 2.85, N 3.04, Found: C 60.27, H 3.09, N 2.78.

6-(3-chloro-2-fluorobenzyl)-5-fluoro-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4i**) Yield 93%. Mp 225–227°C. 1H NMR ($CDCl_3$) δ 4.14 (s, 2 H, CH₂), 6.83–6.85 (d, $J = 8.8$ Hz, 1 H, ArH), 7.01–7.03 (t, $J = 8.0$ Hz, 1 H, ArH), 7.18–7.22 (m, 1 H, ArH), 7.26–7.30 (m, 1 H, ArH), 7.33–7.37 (m, 2 H, ArH), 7.44–7.47 (m, 2 H, ArH), 7.51–7.55 (m, 1 H, ArH), 8.70 (s, 1 H, CH), 14.62 (s, 1 H, COOH); MS (ESI) m/z 444 $[M+H]^+$. Anal. Calcd for $C_{23}H_{13}ClF_3NO_3$: C 62.25, H 2.95, N 3.16, Found: C 62.47, H 3.19, N 2.89.

6-(3-chloro-2-fluorobenzyl)-1-(4-bromophenyl)-5-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4j**) Yield 88%. Mp >250°C. 1H NMR ($CDCl_3$) δ 4.15 (s, 2 H, CH₂), 6.85–6.87 (d, $J = 8.8$ Hz, 1 H, ArH), 7.02–7.06 (t, $J = 8.0$ Hz, 1 H, ArH), 7.19–7.23 (t, $J = 7.2$ Hz, 1 H, ArH), 7.28–7.30 (m, 1 H, ArH), 7.32–7.34 (d, $J = 8.4$ Hz, 2 H, ArH), 7.52–7.56 (t, $J = 8.0$ Hz, 1 H, ArH), 7.80–7.82 (d, $J = 8.4$ Hz, 2 H, ArH), 8.71 (s, 1 H, CH), 14.62 (s, 1 H, COOH). MS (ESI) m/z 504 $[M+H]^+$. Anal. Calcd for $C_{23}H_{13}BrClF_2NO_3$: C 54.73, H 2.60, N 2.78, Found: C 54.47, H 2.36, N 2.49.

6-(3-chloro-2-fluorobenzyl)-5-fluoro-4-oxo-1-(4-(trifluoromethyl)phenyl)-1,4-dihydroquinoline-3-carboxylic acid (**4k**) Yield 85%. Mp >250°C. 1H NMR ($CDCl_3$) δ 4.17 (s, 2 H, CH₂), 6.81–6.84 (d, $J = 8.8$ Hz, 1 H, ArH), 7.03–7.07 (t, $J = 8.0$ Hz, 1 H, ArH), 7.20–7.24 (m, 1 H, ArH), 7.31–7.33 (m, 1 H, ArH), 7.53–7.57 (m, 1 H, ArH), 7.60–7.62 (d, $J = 8.4$ Hz, 2 H, ArH), 7.95–7.97 (d, $J = 8.4$ Hz, 2 H, ArH), 8.73 (s, 1 H, CH), 14.56 (s, 1 H, COOH). MS (ESI) m/z 494 $[M+H]^+$. Anal. Calcd for $C_{24}H_{13}ClF_5NO_3$: C 58.37, H 2.65, N 2.84, Found: C 58.59, H 2.38, N 2.59.

6-(3-chloro-2-fluorobenzyl)-5-fluoro-4-oxo-1-m-tolyl-1,4-dihydroquinoline-3-carboxylic acid (**4l**) Yield 89%. Mp 218–220°C. ¹H NMR (CDCl₃) δ 2.48 (s, 3 H, CH₃), 4.15 (s, 2 H, CH₂), 6.90–6.92 (d, 1 H, *J* = 8.8 Hz, ArH), 7.02–7.05 (t, 1 H, *J* = 8.0 Hz, ArH), 7.18–7.22 (m, 3 H, ArH), 7.27–7.30 (m, 1 H, ArH), 7.44–7.46 (d, 1 H, *J* = 8.0 Hz, ArH), 7.49–7.52 (m, 2 H, ArH), 8.74 (s, 1 H, CH), 14.78 (s, 1 H, COOH); MS (ESI) *m/z* 440 [M+H]⁺. Anal. Calcd for C₂₄H₁₆ClF₂NO₃: C 65.54, H 3.67, N 3.18, Found: C 65.82, H 3.43, N 3.45.

6-(3-chloro-2-fluorobenzyl)-5-fluoro-4-oxo-1-p-tolyl-1,4-dihydroquinoline-3-carboxylic acid (**4m**) Yield 90%. Mp 230–232°C. ¹H NMR (CDCl₃) δ 2.51 (s, 3 H, CH₃), 4.14 (s, 2 H, CH₂), 6.89–6.91 (d, 1 H, *J* = 8.8 Hz, ArH), 7.01–7.05 (t, 1 H, *J* = 8.0 Hz, ArH), 7.18–7.22 (m, 1 H, ArH), 7.26–7.28 (m, 1 H, ArH), 7.28–7.30 (d, 1 H, *J* = 7.6 Hz, ArH), 7.43–7.44 (d, 1 H, *J* = 7.6 Hz, ArH), 7.48–7.52 (m, 1 H, ArH), 8.73 (s, 1 H, CH), 14.78 (s, 1 H, COOH); MS (ESI) *m/z* 440 [M+H]⁺. Anal. Calcd for C₂₄H₁₆ClF₂NO₃: C 65.54, H 3.67, N 3.18, Found: C 65.28, H 3.39, N 3.42.

6-(3-chloro-2-fluorobenzyl)-1-(2,6-dimethylphenyl)-5-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4n**) Yield 85%. Mp 200–202°C. ¹H NMR (CDCl₃) δ 2.01 (s, 6 H, 2CH₃), 4.16 (s, 2 H, CH₂), 6.64–6.67 (d, *J* = 8.8 Hz, 1 H, ArH), 7.04–7.07 (t, *J* = 8.0 Hz, 1 H, ArH), 7.22–7.32 (m, 4 H, ArH), 7.42–7.46 (m, 1 H, ArH), 7.49–7.53 (m, 1 H, ArH), 8.62 (s, 1 H, CH), 14.81 (s, 1 H, COOH); MS (ESI) *m/z* 454 [M+H]⁺. Anal. Calcd for C₂₅H₁₈ClF₂NO₃: C 66.16, H 4.00, N, 3.09, Found: C 66.42, H 3.73, N 3.31.

Declaration of interest

This project was funded by China Postdoctoral Science Foundation (20100470625). The work was also supported in part by grants from the National Natural Science Foundation of China (20872018), from Chinese Academy of Sciences (KSCX2-YW-R-185), from the Eleventh Five-Year Key Scientific and Technological Program of China (2009ZX09501-029), and from National Basic Research Program of China (2009CB5223006).

References

- Craigie R. HIV integrase, a brief overview from chemistry to therapeutics. *J Biol Chem* 2001;276:23213–23216.
- Hare S, Vos AM, Clayton RF, Thuring JW, Cummings MD, Cherepanov P. Molecular mechanisms of retroviral integrase inhibition and the evolution of viral resistance. *Proc Natl Acad Sci USA* 2010;107:20057–20062.
- Cabrera C. Raltegravir, an HIV-1 integrase inhibitor for HIV infection. *Curr Opin Investig Drugs* 2008;9:885–898.
- Anker M, Corales RB. Raltegravir (MK-0518): a novel integrase inhibitor for the treatment of HIV infection. *Expert Opin Investig Drugs* 2008;17:97–103.
- Hatano H, Hayes TL, Dahl V, Sinclair E, Lee TH, Hoh R et al. A randomized, controlled trial of raltegravir intensification in antiretroviral-treated, HIV-infected patients with a suboptimal CD4+ T cell response. *J Infect Dis* 2011;203:960–968.
- Klibanov OM. Elvitegravir, an oral HIV integrase inhibitor, for the potential treatment of HIV infection. *Curr Opin Investig Drugs* 2009;10:190–200.
- Mathieu M, Nick V, Kasthuraiah M, Alena N, Zhang XM, David R, Christophe M, Yves P. Elvitegravir overcomes resistance to raltegravir induced by integrase mutation Y143. *AIDS* 2011;25:1175–1178.
- Sato M, Motomura T, Aramaki H, Matsuda T, Yamashita M, Ito Y et al. Novel HIV-1 integrase inhibitors derived from quinolone antibiotics. *J Med Chem* 2006;49:1506–1508.
- Pasquini S, Mugnaini C, Tintori C, Botta M, Trejos A, Arvela RK et al. Investigations on the 4-quinolone-3-carboxylic acid motif. 1. Synthesis and structure-activity relationship of a class of human immunodeficiency virus type 1 integrase inhibitors. *J Med Chem* 2008;51:5125–5129.
- Sato M, Kawakami H, Motomura T, Aramaki H, Matsuda T, Yamashita M et al. Quinolone carboxylic acids as a novel monoketo acid class of human immunodeficiency virus type 1 integrase inhibitors. *J Med Chem* 2009;52:4869–4882.
- Nagasawa JY, Song J, Chen H, Kim HW, Blazel J, Ouk S et al. 6-Benzylamino 4-oxo-1,4-dihydro-1,8-naphthyridines and 4-oxo-1,4-dihydroquinolines as HIV integrase inhibitors. *Bioorg Med Chem Lett* 2011;21:760–763.
- He QQ, Gu SX, Liu J, Wu HQ, Zhang X, Yang LM et al. Structural modifications of quinolone-3-carboxylic acids with anti-HIV activity. *Bioorg Med Chem* 2011;19:5039–5045.
- He QQ, Zhang X, Wu HQ, Gu SX, Ma XD, Yang LM et al. Synthesis and biological evaluation of HQCAs with aryl or benzyl substituents on N-1 position as potential HIV-1 integrase inhibitors. *Bioorg Med Chem* 2011;19:5553–5558.
- Purser S, Moore PR, Swallow S, Gouverneur V. Fluorine in medicinal chemistry. *Chem Soc Rev* 2008;37:320–330.
- Kirk KL. Fluorination in medicinal chemistry: methods, strategies, and recent developments. *Org Process Res Dev* 2008;12:305–321.
- Hagmann WK. The many roles for fluorine in medicinal chemistry. *J Med Chem* 2008;51:4359–4369.
- Zheng YT, Zhang WF, Ben KL, Wang JH. *In vitro* immunotoxicity and cytotoxicity of trichosanthin against human normal immunocytes and leukemia-lymphoma cells. *Immunopharmacol Immunotoxicol* 1995;17:69–79.
- Pannecouque C, Daelemans D, De Clercq E. Tetrazolium-based colorimetric assay for the detection of HIV replication inhibitors: revisited 20 years later. *Nat Protoc* 2008;3:427–434.