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Quinolone Carboxylic Acids as a Novel Monoketo Acid Class of Human Immunodeficiency Virus Type 1 Integrase Inhibitors

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Human immunodeficiency virus type 1 (HIV-1) integrase is a crucial target for antiretroviral drugs, and several keto−enol acid class (often referred to as diketo acid class) inhibitors have clinically exhibited marked antiretroviral activity. Here, we show the synthesis and the detailed structure−activity relationship of the quinolone carboxylic acids as a novel monoketo acid class of integrase inhibitors. 6-(3-Chloro-2-fluorobenzyl)-1-((2*S*)-1-hydroxy-3,3-dimethylbutan-2-yl)-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **51**, which showed an IC_{50} of 5.8 nM in the strand transfer assay and an ED_{50} of 0.6 nM in the antiviral assay, and 6-(3-chloro-2-fluorobenzyl)-1-((2*S*)-1-hydroxy-3-methylbutan-2-yl)-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **49**, which had an IC_{50} of 7.2 nM and an ED_{50} of 0.9 nM, were the most potent compounds in this class. The monoketo acid **49** was much more potent at inhibiting integrase-catalyzed strand transfer processes than 3′-processing reactions, as is the case with the keto−enol acids. Elvitegravir **49** was chosen as a candidate for further studies and is currently in phase 3 clinical trials.

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Introduction

Human immunodeficiency virus type 1 (HIV-1[a]) integrase plays a central role in the insertion of viral DNA into the genome of host cells, which first catalyzes removal of the terminal dinucleotide from each 3′-end of the viral DNA (3′-processing) and subsequently mediates joining of the 3′-end of the viral DNA to the host DNA (strand transfer).[1] This virally encoded enzyme is thus essential for viral replication and represents a crucial target for antiretroviral drugs.[2]

Among numerous attempts to develop integrase inhibitors, the keto−enol acid class (often referred to as the diketo acid class) of compounds has been most aggressively developed because of the marked antiretroviral activities exhibited.[2,3] Since the keto−enol acid compounds often exhibit adverse hepatic effects,[4] only raltegravir from this class of integrase inhibitors has been approved for therapeutic use by the FDA.[5] γ-Ketone, α-enol, and carboxylic acid in the keto−enol acid moiety are believed to be essential for the inhibitory activity of this series of integrase inhibitors.[6] Keto−enol triazole (S1360),[7] keto−enol tetrazole (5CITEP),[8] 7-carbamoyl-8-hydroxy-1,6-naphthyridine (L-870810),[9−11] and 4-carbamoyl-5-hydroxy-6-pyrimidinone (raltegravir)[5,12,13] were identified as bioisosters of the keto−enol acid pharmacophore (Figure 1). The carboxylic acid in the keto−enol acid motif can be replaced with not only well-known bioisosters of a carboxylic acid group, such as triazole and tetrazole,[14] but also by a basic aromatic heterocycle bearing a lone pair donor atom, such as a pyridine ring.[9] The heteroaromatic nitrogen in the pyridine ring is considered to mimic the corresponding carboxyl oxygen in the keto−enol acid motif as a Lewis base equivalent.[9] Moreover, the carboxylic acid moiety of the keto−enol acid motif can be replaced with a neutral carbonyl group (Figure 1).[15] Phenolic hydroxyl groups as alternatives to α-enol confirmed that the biologically active conformation was coplanar.[5,9−13] All bioisosters of the keto−enol acid motif have the three functional groups that mimic a ketone, enol, and carboxyl oxygen and can assume a coplanar conformation. On the other hand, we previously disclosed several structures of novel quinolone HIV-1 integrase inhibitors possessing a monoketo acid motif as an alternative to the keto−enol acid motif.[16,17]

In this paper, the more detailed information on the SAR of the novel class of quinolone integrase inhibitors and their effects on strand transfer reaction, 3′-processing, and viral replication are presented.

Chemistry

Preparation of the quinolone analogues is shown in Schemes 1 and 2. Palladium-catalyzed coupling of benzylzinc halides **53**, which were derived from the corresponding benzyl halides, with 1-iodo-4-nitrobenzenes **54** (Negishi coupling) and subsequent reduction of the nitro group, gave the aniline **57**. Palladium-catalyzed coupling of halobenzenes **55** with 4-methylnitrobenzene **56** and subsequent reduction of the nitro group also gave the aniline **57**. Condensation of **57** with diethyl ethoxymethylenemalonate and subsequent thermal cyclization of the aminoacrylate products in phenyl ether led to the quinolone ester **58**.[18] Benzylation of **58** and subsequent hydrolysis gave the 3-quinolinecarboxylic acid **59**. Amidation of **59** and subsequent reductive deprotection of benzyl group gave the quinolone carboxamide **6**. Esterification of **59** and subsequent reduction and oxidation led to the aldehyde **60**.

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[a] Abbreviations: 5CITEP, 1-(5-chloroindol-3-yl)-3-hydorxy-3-(tetrazol-5-yl)-propenone; HIV-1, human immunodeficiency virus type 1; SAR, structure−activity relationship.

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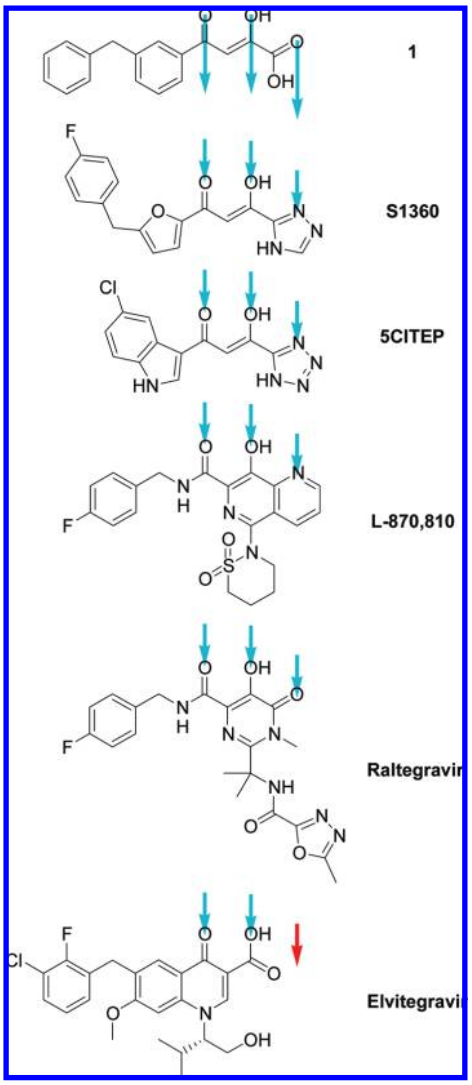

Figure 1. Structures of the keto−enol acid family and the novel monoketo acid.

Coupling of **60** with trimethylsilyl (TMS) cyanide gave the trimethylsilyloxyacetonitrile **61**. Ethanolysis of **61** and subsequent hydrolysis of the imidate, oxidation of hydroxyl group, N-methylation, and hydrolysis of the ethyl ester gave the diketo acid **2**. Hydrolysis of **58** gave **62**. N-Alkylation of **58** with substituted alkyl halides and subsequent hydrolysis of protecting groups resulted in **63**. In all cases, N-alkylation proceeded predominantly over O-alkylation. The acid chlorides of 5-iodinated 2-fluorobenzoic acids **64** were coupled with ethyl 3-(dimethylamino)acrylate to produce the acrylate **65**. Substitution with amino alcohols and subsequent cyclization with potassium carbonate and protection of the alcohol with *tert*-butyldimethylsilyl (TBS) chloride gave the quinolone **66**.[19] Negishi coupling of **66** with **53** led to the quinolone ester **67**. Hydrolysis of **67** gave **68**. Methoxylation of **67** with sodium methoxide produced **69**.

Results and Discussion

In order to obtain a new scaffold as an alternative to keto−enol acids, we designed quinolone-3-glyoxylic acid **2** as a nonenolizable diketo acid. However, compound **2** did not exhibit integrase inhibitory activity (Table 1). This indicates the importance of the central ionizable enol in the keto−enol acid motif. On the other hand, we found that 4-quinolone-3-carboxylic acid **3**, which had only two functional coplanar groups, did exhibit integrase inhibitory activity (Table 1). Since compound **3** potentially tautomerizes, nontautomerizable compound **4** was confirmed to be as potent as **3** (Table 1). Therefore, the planar monoketo acid motif was considered to be a new alternative scaffold to the keto−enol acid motif, although the metal-chelating functions of the keto−enol acid group, in simultaneous coordination with two divalent metal ions, are considered to be relevant to the mechanism of their inhibitory activity.[6,20] The carboxylic acid in the monoketo acid motif would be an alternative to the central ionizable enol in the keto−enol acid moiety. Neither ester **5** nor amide **6** displayed activity, indicating the importance of ionizable carboxylic acid (Table 2). Monoketo acids (**3** and **4**) having only two functional groups that did not fully coordinate with the two divalent metal ions were far less potent than keto−enol acid **1**, so the metal-chelating functions are still considered important for their inhibitory effect.

For the purpose of lead optimization, we first examined the effect of introducing substituents into the terminal benzene ring of compound **3**, which had an IC_{50} of 1.63 μM in the strand transfer assay (Table 2). Although introduction of a chloro group at the para position of the terminal benzene ring (**7**) caused a loss of integrase inhibitory activity, introduction of a chloro group at the meta (**8**) or ortho (**9**) position of the benzene ring caused an increase. Since the effect of the introduction at the ortho position was marked, a fluoro (**10**), a methyl (**11**), a methoxy (**12**), or a trifluoromethyl (**13**) group was introduced at the ortho position of the benzene ring. Among the compounds, only the fluoro compound **10** was as potent as **9**. We next introduced dichloro groups into the terminal benzene ring (**14**−**16**) and found that 2,3-dichloro compound **16** showed a significant improvement in the inhibition of strand transfer (IC_{50} = 0.07 μM) and in the appearance of antiviral activity (EC_{50} = 3.4 μM).

Next, we examined the effect of introducing substituents at the 1-position of the quinolone ring of **16** (Table 3). 1-Methyl compound **17** did not enhance its integrase inhibitory activity but improved its antiviral activity (EC_{50} = 0.45 μM) compared with **16** (EC_{50} = 3.4 μM). This would be due to improvement in its physicochemical properties. Introduction of an ethyl group (**18**) or an isopropyl group (**20**) at the 1-position of quinolone ring caused an increase in both integrase inhibitory activity and antiviral activity. However, 1-*n*-propyl compound **19** and 1-*n*-butyl compound **21** were less potent in antiviral activity than **17**. Introduction of a carboxymethyl acid (**22**) or a carboxyethyl acid (**23**) at the 1-position of the quinolone ring caused an increase in integrase inhibitory activity but a decrease or a loss their antiviral activities. These would be too polar to permeate cell membrane in antiviral assay. 1-Carbamoylmethyl (**24**) was approximately 2-fold more potent at inhibiting strand transfer and as potent at inhibiting HIV-1 replication as **17**. Introduction of a carbamoylethyl (**25**) or an aminoethyl group (**26**) at the 1-position of the quinolone ring did not enhance inhibitory activity. Compound **27**, bearing a hydroxyethyl group at the 1-position of the quinolone ring, was 1.5-fold more potent at inhibiting strand transfer (IC_{50} = 0.021 μM) and displayed 3-fold stronger antiviral activity (EC_{50} = 0.10 μM) than **18**. On the other hand, 1-hydroxypropyl compound **28** was less potent in integrase inhibitory activity than **19** or **27**. Therefore, the hydroxyl group of **27** evidently plays an important role in the binding to integrase.

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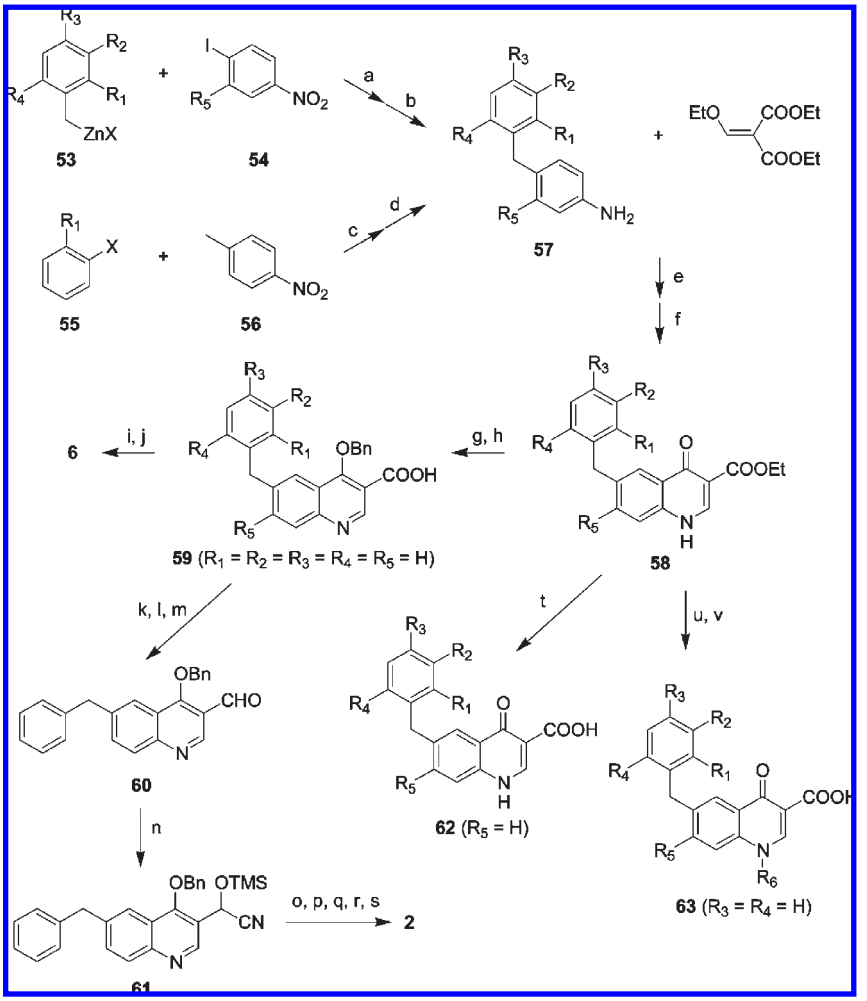
Scheme 1[a]



[a] Reagents and conditions: (a) $Pd(dba)_2$, tri-2-furylphosphine, THF, reflux; (b) Zn, AcOH; (c) $Pd(OAc)_2$, PPh_3, Cs_2CO_3, DMF, 140 °C; (d) H_2, Pd−C, EtOH; (e) toluene, reflux; (f) Ph_2O, 250 °C; (g) BnOH, DIAD, Ph_3P, THF; (h) LiOH, THF, H_2O; (i) NH_4Cl, EDC, HOBt, Et_3N, DMF; (j) H_2, Pd−C, THF, MeOH; (k) MeI, K_2CO_3, DMF; (l) DIBAH, THF, −20 °C; (m) MnO_2, EtOAc; (n) TMSCN, NMO, $CHCl_3$; (o) AcCl, EtOH, $CHCl_3$, 0 °C; (p) H_2O, reflux; (q) MnO_2, THF; (r) MeI, K_2CO_3, DMF; (s) NaOH, EtOH; (t) NaOH, EtOH/H_2O, reflux; (u) alkyl halides, K_2CO_3, DMF, 80 °C; (v) deprotection.

To confirm the effect of the fluoro substituent on the terminal benzene ring, we changed the chloro group of **27** to a fluoro group. 2-Fluoro-3-chlorobenzyl compound **30** was as potent as **27**, but 2-chloro-3-fluorobenyl compound **29** was less potent than **27**.

Next, we examined the effect of introducing substituents into the central benzene ring of **27** (Table 4). Although introduction of a fluoro group at the 5- or 8-position of the quinolone ring of **27** (**31** and **33**) led to a decrease in the inhibition of strand transfer, 7-fluoroquinolone **32** was as potent at inhibiting both strand transfer and HIV-1 replication as **27**. Since the substitution at the 7-position of the quinolone ring was only tolerable, we attempted to introduce a methoxy group (**34**), a chloro group (**35**), a methyl group (**36**), a trifluoromethyl group (**37**), or a cyano group (**38**) at the 7-position of the quinolone ring of **27**. Among these, compound **34** was approximately 2-fold as potent as **27**.

We replaced the chloro substituent at the 2-position of the terminal benzene ring of **34** with a fluoro group (**39**) and confirmed that **39** was as potent at inhibiting HIV-1 integrase as **34**. To a greater extent than anticipated, **39** was approximately 3-fold more potent at inhibiting HIV-1 replication (EC_{50} = 0.02 μM) than **34**.

The independent introduction of a small alkyl group or a hydroxyethyl group at the 1-position of the quinolone ring led to a significant improvement of activity, so we examined the effect of the combination of the small alky groups and the hydroxyethyl group (Table 5). First, we introduced a methyl group at the 1*S*-position of the hydroxyethyl moiety of **30** (**40**), which was 1.6-fold more potent at inhibiting strand transfer (IC_{50} = 14.8 nM) and displayed an approximately 2.7-fold stronger antiviral activity (EC_{50} = 27.7 nM) than **30**. On the other hand, compound **41**, the enantiomer of **40**, was less potent at inhibiting both strand transfer (IC_{50} = 38.3 nM) and HIV-1 replication (EC_{50} = 115.5 nM) than **30**. Thus, we introduced several alkyl groups, such as an ethyl (**42**), a *n*-propyl (**43**), an isopropyl (**44**), a *tert*-butyl (**45**), or a cyclohexyl (**46**) group, at the 1*S*-position of the hydroxyethyl moiety of **30**. Among these, compound **45** displayed the strongest antiviral activity (EC_{50} = 1.3 nM). Compounds **43**, **44**, and **46** also displayed strong antiviral activity, with EC_{50} values of 9.8, 7.5, and 7.3 nM, respectively. On the other hand, an introduction of a phenyl group at the 1*S*-position of the hydroxyethyl

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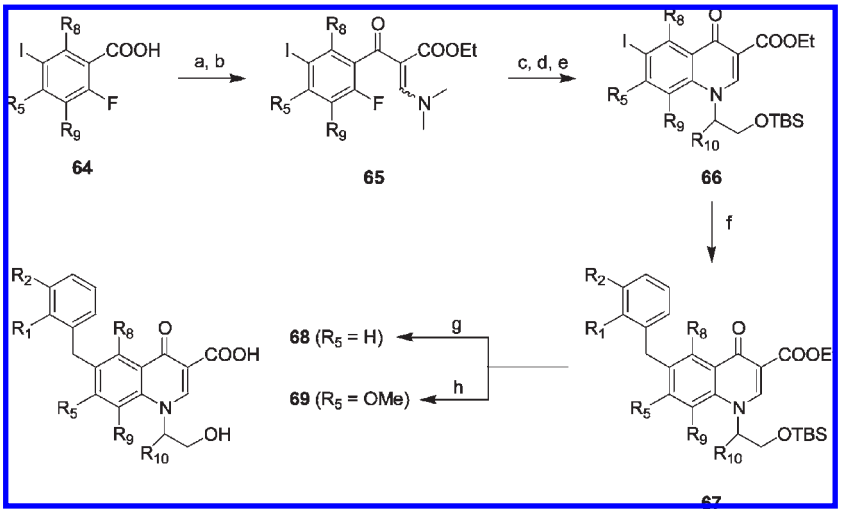
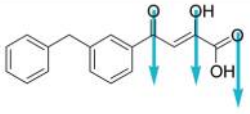
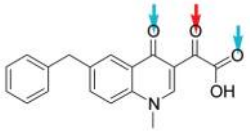
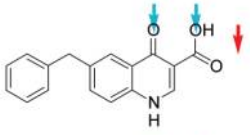
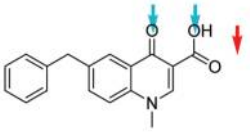
Scheme 2[a]



[a] Reagents and conditions: (a) $SOCl_2$, DMF, toluene, reflux; (b) ethyl 3-(dimethylamino)acrylate, Et_3N, THF, 50 °C; (c) amino alcohol, THF; (d) K_2CO_3, DMF, 70 °C; (e) TBSCl, imidazole, DMF; (f) **53**, $Pd(dba)_2$, trifurylphosphine, THF, reflux; (g) NaOH, EtOH/H_2O, reflux; (h)NaOMe, MeOH, reflux.

Table 1. Inhibition of HIV-1 Integrase Catalytic Activities and HIV-1 Replication in Cells by Keto−Enol Acid, Diketo Acid, and Monoketo Acids[b]

	compd	Inhibition of Strand transfer IC_{50} (μM)	Antiviral Activity EC_{50} (μM)	Cytotoxicity CC_{50} (μM)
1[a]		0.05 ± 0.02	0.44 ±0.07	>30
2		> 100	> 30	> 30
3		1.63 ± 0.30	> 30	> 30
4		2.30 ± 0.39	>30	>30

[a] Prepared according to the reported method.[28] [b] Data are given as the mean ± SD (n = 3).

moiety of **30** (**47**) led to a slight improvement of both integrase inhibitory activity and antiviral activity. Introduction of both a methoxy group at the 7-position of the quinolone ring and an alkyl group, such as a *n*-propyl (**48**), an isopropyl (**49**), a *tert*-butyl (**51**), and a cyclohexyl (**52**), at the 1*S*-position of the hydroxyethyl moiety of **30** led to synergistic improvement of antiviral activity, with EC_{50} values of 1.3, 0.9, 0.6, and 2.8 nM, respectively, but there was no additive or synergistic improvement in the inhibition of HIV-1 integrase. This may be due to the condition of the strand transfer assay using 5 nM target DNA, which influences the potency of inhibitors. The enantiomer of **49** (**50**) was confirmed to be far less potent at inhibiting HIV-1 replication (EC_{50} = 108.8 nM) than **49** or **39**.

The monoketo acid integrase inhibitor **49** was more than 100-fold as potent at inhibiting integrase-catalyzed strand transfer processes as 3′-processing reactions (Figure 2), as previously reported for compounds of the keto−enol acid class.[21,22] This indicates that the monoketo acids probably inhibit HIV-1 integrase via a mechanism similar to that of keto−enol acid, although there is no direct evidence, such as cocrystal data.

Conclusions

The initial lead monoketo acid **3** was far less potent than the keto−enol acid **1**, probably because the structurally modified

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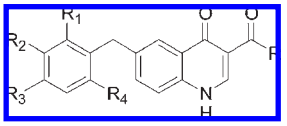
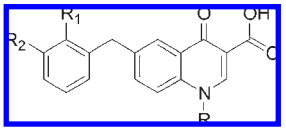
Table 2. Inhibition of HIV-1 Integrase Catalytic Activities and HIV-1 Replication in Cells by Monoketo Acids **5**−**16**[a]



compd	R_1	R_2	R_3	R_4	R	inhibition of strand transfer, IC_{50} (μM)	antiviral activity, EC_{50} (μM)	cytotoxicity, CC_{50} (μM)
5	H	H	H	H	OEt	> 100	> 30	> 30
6	H	H	H	H	NH_2	> 100	> 30	> 30
7	H	H	Cl	H	OH	> 100	> 6	> 6
8	H	Cl	H	H	OH	0.80 ± 0.03	> 6	> 6
9	Cl	H	H	H	OH	0.41 ± 0.07	> 6	> 6
10	F	H	H	H	OH	0.50 ± 0.11	15.9 ± 20.6	> 30
11	Me	H	H	H	OH	1.08 ± 0.38	> 6	18.5 ± 4.9
12	OMe	H	H	H	OH	1.17 ± 0.15	> 6	16.6 ± 1.5
13	CF_3	H	H	H	OH	0.72 ± 0.11	> 6	14.8 ± 2.2
14	Cl	H	H	Cl	OH	0.37 ± 0.14	> 6	14.9 ± 0.4
15	H	Cl	H	Cl	OH	0.25 ± 0.07	> 6	13.2 ± 0.6
16	Cl	Cl	H	H	OH	0.07 ± 0.02	3.4 ± 0.8	12.6 ± 1.2

[a] Data are given as the mean ± SD (n = 3).

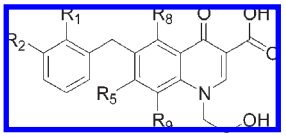
Table 3. Inhibition of HIV-1 Integrase Catalytic Activities and HIV-1 Replication in Cells by Monoketo Acids **17**−**30**[a]



compd	R_1	R_2	R	inhibition of strand transfer, IC_{50} (μM)	antiviral activity, EC_{50} (μM)	cytotoxicity, CC_{50} (μM)
17	Cl	Cl	Me	0.083 ± 0.047	0.45 ± 0.07	14.4 ± 5.6
18	Cl	Cl	Et	0.031 ± 0.012	0.29 ± 0.06	16.2 ± 0.2
19	Cl	Cl	Pr	0.055 ± 0.031	0.64 ± 0.14	15.6 ± 0.2
20	Cl	Cl	iPr	0.026 ± 0.008	0.33 ± 0.07	> 6
21	Cl	Cl	Bu	0.065 ± 0.027	2.34 ± 0.25	15.0 ± 0.5
22	Cl	Cl	CH_2CO_2H	0.032 ± 0.003	13.59 ± 1.38	> 30
23	Cl	Cl	$CH_2CH_2CO_2H$	0.038 ± 0.014	> 30	> 30
24	Cl	Cl	CH_2CONH_2	0.035 ± 0.010	0.54 ± 0.05	22.6 ± 2.0
25	Cl	Cl	$CH_2CH_2CONH_2$	0.116 ± 0.039	2.04 ± 0.99	15.1 ± 3.7
26	Cl	Cl	$CH_2CH_2NH_2$	0.215 ± 0.103	0.57 ± 0.06	5.3 ± 3.3
27	Cl	Cl	CH_2CH_2OH	0.021 ± 0.005	0.10 ± 0.02	> 6
28	Cl	Cl	$CH_2CH_2CH_2OH$	0.077 ± 0.025	0.47 ± 0.04	15.1 ± 0.3
29	Cl	F	CH_2CH_2OH	0.044 ± 0.014	0.34 ± 0.08	> 6
30	F	Cl	CH_2CH_2OH	0.024 ± 0.012	0.08 ± 0.00	> 6

[a] Data are given as the mean ± SD (n = 3).

Table 4. Inhibition of HIV-1 Integrase Catalytic Activities and HIV-1 Replication in Cells by Monoketo Acids **31**−**39**[a]



compd	R_1	R_2	R_5	R_8	R_9	inhibition of strand transfer, IC_{50} (μM)	antiviral activity, EC_{50} (μM)	cytotoxicity, CC_{50} (μM)
31	Cl	Cl	H	F	H	0.084 ± 0.022	1.68 ± 0.60	14.3 ± 0.4
32	Cl	Cl	F	H	H	0.025 ± 0.010	0.13 ± 0.01	9.8 ± 3.6
33	Cl	Cl	H	H	F	0.034 ± 0.007	0.53 ± 0.12	> 30
34	Cl	Cl	OMe	H	H	0.012 ± 0.003	0.06 ± 0.01	4.2 ± 0.5
35	Cl	Cl	Cl	H	H	0.043 ± 0.030	0.51 ± 0.09	> 6
36	Cl	Cl	Me	H	H	0.041 ± 0.005	0.18 ± 0.02	> 30
37	Cl	Cl	CF_3	H	H	0.674 ± 0.225	> 6	10.0 ± 2.8
38	Cl	Cl	CN	H	H	0.050 ± 0.013	2.00 ± 0.16	13.9 ± 1.8
39	F	Cl	OMe	H	H	0.009 ± 0.002	0.02 ± 0.00	5.3 ± 1.1

[a] Data are given as the mean ± SD (n = 3).

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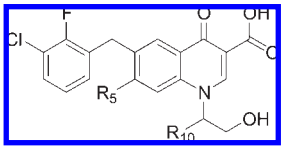
Table 5. Inhibition of HIV-1 Integrase Catalytic Activities and HIV-1 Replication in Cells by Monoketo Acids **40**−**52**[a]



compd	R_5	R_{10}	inhibition of strand transfer, IC_{50} (nM)	antiviral activity, EC_{50} (nM)	cytotoxicity, CC_{50} (μM)
40	H	(*S*)-Me	14.8 ± 6.4	27.7 ± 3.0	13.7 ± 0.8
41	H	(*R*)-Me	38.3 ± 14.0	115.5 ± 12.6	14.4 ± 1.3
42	H	(*S*)-Et	9.0 ± 1.3	17.1 ± 2.5	13.0 ± 1.4
43	H	(*S*)-Pr	8.2 ± 2.5	9.8 ± 3.3	12.5 ± 1.8
44	H	(*S*)-^{i}Pr	8.2 ± 1.7	7.5 ± 0.8	14.0 ± 2.0
45	H	(*S*)-^{t}Bu	6.0 ± 2.6	1.3 ± 0.5	14.4 ± 1.8
46	H	(*S*)-cyclohexyl	5.6 ± 1.6	7.3 ± 0.8	7.7 ± 3.2
47	H	(*S*)-Ph	9.8 ± 3.5	51.6 ± 6.7	8.7 ± 4.0
48	OMe	(*S*)-Pr	5.8 ± 1.6	1.3 ± 0.2	3.1 ± 0.5
49	OMe	(*S*)-^{i}Pr	7.2 ± 2.2	0.9 ± 0.4	4.0 ± 0.8
50	OMe	(*R*)-^{i}Pr	14.4 ± 3.1	108.8 ± 14.0	4.2 ± 0.0
51	OMe	(*S*)-^{t}Bu	5.8 ± 2.0	0.6 ± 0.2	3.4 ± 0.4
52	OMe	(*S*)-cyclohexyl	6.7 ± 1.9	2.8 ± 1.2	3.0 ± 0.3

[a] Data are given as the mean ± SD (n = 3).

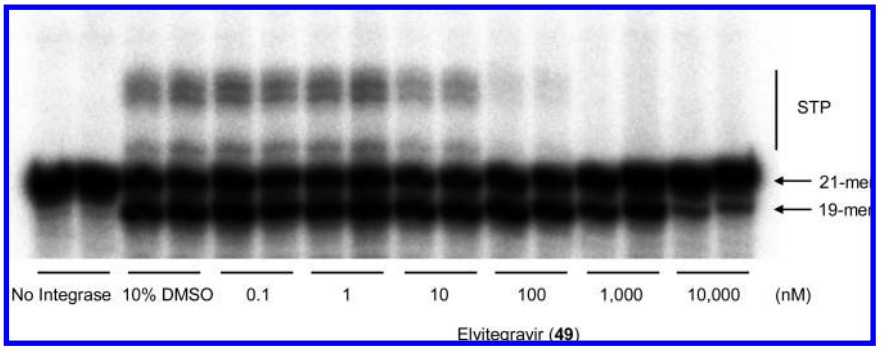


Figure 2. Inhibition of 3′-processing and strand transfer by compound **49**. Gel electrophoresis shows the viral DNA substrate (21-mer oligonucleotide), 3′-processing product (19-mer oligonucleotide), and strand transfer products (>21-mer oligonucleotides).

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monoketo acids cannot fully mimic the chelating function of the diketo acids, which can simultaneously coordinate with two divalent metal ions. However, structure−activity relationship studies on a novel series of monoketo acid HIV-1 integrase strand transfer inhibitors disclosed the structural requirements for high potency and led to the optimum compounds **49** and **51**. Furthermore, the selectivity indexes (CC_{50}/EC_{50}) of these compounds are more than 4000. Elvitegravir **49**, which inhibits HIV-1 replication in human peripheral blood mononuclear cells with an EC_{90} value of 9.8 nM in the presence of 50% human serum and has good oral bioavailability,[23−25] was chosen as a candidate for further studies[26,27] and is currently undergoing phase 3 clinical trials.

Experimental Section

Chemistry. Melting points were obtained with a Yanagimoto micro melting point apparatus or a Stanford Research Systems MPA100 and were uncorrected. Combustion analyses were performed with a Perkin-Elmer 2400 series II CHNS/O analyzer, and all values were within ±0.4% of the calculated values. Mass spectra were recorded on an Agilent Technologies 1100 series LC/MS (ESI) spectrometer. ^{1}H NMR spectra were recorded on a JEOL JNM-A300W, Bruker DPX300, Bruker ARX400, or Varian MERCURYplus-AS400 spectrometer in a solution of either $CDCl_3$ or DMSO-d_6, using tetramethylsilane as the internal standard. Chemical shifts are expressed as δ (ppm) values for protons relative to the internal standard. All compounds gave spectra consistent with their assigned structures. Optical rotation was measured with a Rudolph Research Analytical AUTOPOL V spectrometer. The purity of all the tested compounds was determined by combustion analyses and was ≥95%.

6-Benzyl-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid Ethyl Ester (5). To a stirred mixture of 4-benzylaniline (2.01 g, 11.0 mmol) in toluene (20 mL) was added 2-ethoxymethylenemalonic acid diethyl ester (2.4 mL, 12 mmol). The mixture was heated to reflux overnight, cooled, and concentrated in vacuo. Under an argon atmosphere this residue was dissolved in phenyl ether (20 mL), and the mixture was heated to 250 °C for 30 min with removal of the low boiling point material. The mixture was cooled and a precipitate was collected by filtration and washed with ethyl acetate to give **5** (2.09 g, 62%). Mp 270 °C. ^{1}H NMR (DMSO-d_6) δ 12.28 (1H, br s), 8.50 (1H, s), 7.97 (1H, s), 7.61−7.52 (2H, m), 7.34−7.17 (5H, m), 4.20 (2H, q, J = 7.1 Hz), 4.08 (2H, s), 1.27 (3H, t, J = 7.2 Hz). MS (ESI) m/z 308 $(M + H)^+$. Anal. ($C_{19}H_{17}NO_3$) C, H, N.

6-Benzyl-4-benzyloxyquinoline-3-carboxylic Acid (59). Step 1. To a mixture of **5** (3.82 g, 12.4 mmol), benzyl alcohol (1.56 mL, 15.1 mmol), and triphenylphosphine (6.52 g, 24.9 mmol) in tetrahydrofuran (100 mL) was slowly added diisopropyl azodicarboxylate (3.6 mL, 18 mmol) over a period of 10 min. After being stirred for 2.5 h at room temperature, the mixture was concentrated in vacuo. The residue was purified by column chromatography (silica gel, *n*-hexane/ethyl acetate 4/1 to 3/1 v/v as eluent) to give

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6-benzyl-4-benzyloxyquinoline-3-carboxylic acid ethyl ester (4.72 g, 96%). ^{1}H NMR (DMSO-d_6) δ 9.05 (1H, s), 7.98 (1H, d, J = 8.6 Hz), 7.93 (1H, d, J = 1.4 Hz), 7.75 (1H, dd, J = 8.6, 2.1 Hz), 7.42−7.37 (5H, m), 7.35−7.21 (5H, m), 5.23 (2H, s), 4.41 (2H, q, J = 7.1 Hz), 4.14 (2H, s), 1.35 (3H, t, J = 7.1 Hz).

Step 2. A mixture of the compound from the previous step (4.72 g, 11.9 mmol), tetrahydrofuran (375 mL), and 1 M aqueous lithium hydroxide (75 mL) was stirred for 24 h at room temperature. After being acidified with 5% aqueous potassium hydrogen sulfate at 0 °C, this mixture was diluted with brine and extracted with ethyl acetate. The organic solution was washed with brine, dried over sodium sulfate, and concentrated in vacuo. The residue was treated with isopropyl ether, and the resulting solid was collected by filtration to give **59** (3.05 g, 70%). ^{1}H NMR (DMSO-d_6) δ 13.56 (1H, br s), 9.06 (1H, s), 7.96 (1H, d, J = 8.6 Hz), 7.92 (1H, d, J = 1.4 Hz), 7.73 (1H, dd, J = 8.6, 2.1 Hz), 7.44−7.37 (5H, m), 7.35−7.29 (2H, m), 7.27−7.21 (3H, m), 5.25 (2H, s), 4.14 (2H, s).

6-Benzyl-4-benzyloxyquinoline-3-carbaldehyde (60). Step 1. A mixture of **59** (2.68 g, 7.25 mmol) and potassium carbonate (1.50 g, 10.9 mmol) in *N*,*N*-dimethylformamide (25 mL) was stirred for 0.5 h at 60 °C and then cooled at 0 °C. To this suspension was added methyl iodide (0.54 mL, 8.7 mmol), and the mixture was stirred for 1 h at room temperature. Water was added to this mixture at 0 °C, and the precipitate was collected by filtration to give 6-benzyl-4-benzyloxyquinoline-3-carboxylic acid methyl ester (2.61 g, 94%). ^{1}H NMR (DMSO-d_6) δ 9.06 (1H, s), 7.98 (1H, d, J = 8.6 Hz), 7.94 (1H, d, J = 1.4 Hz), 7.76 (1H, dd, J = 8.7, 2.0 Hz), 7.42−7.38 (5H, m), 7.35−7.29 (2H, m), 7.27−7.20 (3H, m), 5.22 (2H, s), 4.15 (2H, s), 3.94 (3H, s).

Step 2. To a stirred solution of the compound from the previous step (1.94 g, 5.07 mmol) in tetrahydrofuran (40 mL), 0.99 M solution of diisobutylaluminum hydride in toluene (15 mL, 15 mmol) was added slowly over 10 min under an argon atmosphere in dry ice/ethanol bath. The reaction mixture was allowed to rise to −20 °C over 2 h and then stirred for 4 h at this temperature. After the addition of 10% aqueous Rochelle salt solution, the mixture was stirred for 1 h. The mixture was separated, and the aqueous phase was extracted with ethyl acetate. The combined organic solution was washed with brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, *n*-hexane/ethyl acetate, 1/3 v/v, to ethyl acetate only as eluent) to give (6-benzyl-4-benzyloxyquinolin-3-yl)methanol (1.17 g, 62%). ^{1}H NMR (DMSO-d_6) δ 8.85 (1H, s), 7.98 (1H, d, J = 8.6 Hz), 7.94 (1H, d, J = 1.4 Hz), 7.76 (1H, dd, J = 8.6, 1.9 Hz), 7.42−7.38 (5H, m), 7.36−7.29 (2H, m), 7.28−7.20 (3H, m), 5.22 (2H, s), 4.15 (2H, s), 3.94 (3H, s).

Step 3. A mixture of the compound from the previous step (1.82 g, 4.92 mmol) with active manganese dioxide (5.51 g) in ethyl acetate (70 mL) was stirred for 24 h at room temperature, filtered though Celite, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, *n*-hexane/ethyl acetate, 3/1 v/v, as eluent) to give **60** (1.53 g, 85%). ^{1}H NMR (DMSO-d_6) δ10.35 (1H, br s), 9.05 (1H, s), 8.02−8.00 (2H, m), 7.81 (1H, dd, J = 8.6, 2.1 Hz), 7.42 (5H, s), 7.36−7.22 (5H, m), 5.45 (2H, s), 4.18 (2H, s).

(6-Benzyl-4-benzyloxyquinolin-3-yl)trimethylsiloxyacetonitrile (61). To a stirred solution of **60** (854 mg, 2.32 mmol) and *N*-methylmorphorin *N*-oxide (42 mg, 0.47 mmol) in chloroform (10 mL) was added cyanotrimethylsilane (500 μL, 3.8 mmol) slowly. The reaction mixture was stirred for 14 h at room temperature, diluted with ethyl acetate, and washed successively with water twice and with brine. The organic solution was dried over magnesium sulfate and concentrated in vacuo to give **61** (1131 mg, quantitative yield). ^{1}H NMR ($CDCl_3$) δ 9.04 (1H, s), 8.11 (1H, d, J = 8.8 Hz), 7.86−7.86 (1H, m), 7.66 (1H, dd, J = 8.7, 2.0 Hz), 7.49−7.45 (3H, m), 7.42−7.37 (2H, m), 7.37−7.33 (2H, m), 7.31−7.24 (3H, m), 5.73 (1H, s), 5.21 (2H, dd, J = 17.6, 11.1 Hz), 4.21 (2H, s), 0.20 (9H, s).

(6-Benzyl-1-methyl-4-oxo-1,4-dihydroquinolin-3-yl)oxoacetic Acid (2). Step 1. Under an argon atmosphere, acetyl chloride (9.5 mL) was added slowly over 30 min to a mixture of ethanol (8.6 mL) and chloroform (9.5 mL) at 0 °C. To this mixture was slowly added **61** (1128 mg, 2.32 mmol) in chloroform (9.5 mL), and the solution was stirred for 1 h at 0 °C. Water (1.45 mL) was added followed by ethanol (9.5 mL). The reaction mixture was heated to reflux for 1 h and then concentrated in vacuo. To the residue was added saturated aqueous sodium hydrogen carbonate, and the mixture was extracted with a mixture of ethyl acetate and ethanol. The organic solution was washed with brine twice, dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, chloroform/methanol, 50/1 to 25/1 v/v, as eluent) to give (6-benzyl-4-oxo-1,4-dihydroquinolin-3-yl)hydroxyacetic acid ethyl ester (580 mg, 74%). ^{1}H NMR (DMSO-d_6) δ 11.84 (1H, d, J = 6.0 Hz), 7.92−7.88 (2H, m), 7.53 (1H, dd, J = 8.5, 2.0 Hz), 7.48 (1H, d, J = 8.6 Hz), 7.31−7.16 (5H, m), 5.77 (1H, d, J = 6.5 Hz), 5.14 (1H, d, J = 6.3 Hz), 4.11−3.98 (4H, m), 1.12 (3H, t, J = 7.1 Hz).

Step 2. A mixture of the compound from the previous step (184 mg, 0.55 mmol) with active manganese dioxide (558 mg) in tetrahydrofuran (30 mL) was stirred for 9 h at room temperature, filtered through Celite, and concentrated in vacuo. The residue was dissolved in *N*,*N*-dimethylformamide (2 mL), and methyl iodide (50 μL, 0.80 mmol) was added followed by potassium carbonate (223 mg, 1.6 mmol). The mixture was stirred for 1.5 h at room temperature under an argon atmosphere, diluted with ethyl acetate, and poured into ice. The mixture was separated, and the aqueous phase was further extracted with ethyl acetate. The combined organic solution was washed successively with water three times, saturated aqueous sodium thiosulfate, and brine. This solution was dried over magnesium sulfate and concentrated in vacuo. The residue was treated with ethyl acetate, and the resulting solid was collected by filtration to give (6-benzyl-1-methyl-4-oxo-1,4-dihydroquinolin-3-yl)oxoacetic acid ethyl ester (159 mg, 84%). ^{1}H NMR (DMSO-d_6) δ 8.77 (1H, s), 8.07 (1H, s), 7.77 (2H, d, J = 1.2 Hz), 7.33−7.25 (4H, m), 7.23−7.18 (1H, m), 4.29 (2H, q, J = 7.1 Hz), 4.12 (2H, s), 3.98 (3H, s), 1.29 (3H, t, J = 7.1 Hz).

Step 3. To a mixture of the compound from the previous step (158 mg, 0.45 mmol) in tetrahydrofuran/ethanol/water (2 mL/8 mL/8 mL) was added 1 M aqueous sodium hydroxide (1.2 mL), and the mixture was stirred for 1.5 h at 0 °C. The solution was poured into 0.5 M hydrochloric acid, and the resulting precipitate was collected by filtration to give **2** (134 mg, 92%). Mp 222 °C. ^{1}H NMR (DMSO-d_6) δ 13.55 (1H, br s), 8.72 (1H, s), 8.06 (1H, s), 7.75 (2H, d, J = 1.1 Hz), 7.33−7.24 (4H, m), 7.23−7.18 (1H, m), 4.13 (2H, s), 3.97 (3H, s). MS (ESI) m/z 322 $(M + H)^+$. Anal. ($C_{19}H_{15}NO_4$) C, H, N.

6-Benzyl-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (3). To a stirred mixture of **5** (1.80 g, 5.86 mmol) in tetrahydrofuran (10 mL) and ethanol (10 mL) was added 4 M aqueous sodium hydroxide (4.4 mL). The mixture was heated to reflux for 3.5 h, cooled, and concentrated in vacuo. The residue was diluted with water, and the mixture was acidified with 6 M hydrochloric acid. A precipitate was collected by filtration to give **3** (1.33 g, 81%). Mp 240−241 °C. ^{1}H NMR (DMSO-d_6) δ 15.42 (1H, s), 13.40 (1H, br s), 8.86 (1H, s), 8.13 (1H, s), 7.77 (1H, s), 7.34−7.21 (5H, m), 4.15 (2H, s). MS (ESI) m/z 279 $(M+H)^+$. Anal. ($C_{17}H_{13}NO_3$) C, H, N.

6-Benzyl-4-oxo-1,4-dihydroquinoline-3-carboxamide (6). Step 1. A mixture of **59** (257 mg, 0.70 mmol), ammonium chloride (152 mg, 2.8 mmol), *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide (419 mg, 2.2 mmol), 1-hydorxybenzotriazole (125 mg, 0.93 mmol), and triethylamine (395 μL, 2.8 mmol) in *N*,*N*-dimethylformamide (2.5 mL) was stirred for 19 h at room temperature and poured into water. The mixture was extracted with ethyl acetate, and the organic solution was washed successively with 5% aqueous potassium hydrogen sulfate twice,

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water, saturated aqueous sodium hydrogen carbonate, and brine. This solution was dried over magnesium sulfate and concentrated in vacuo. The residue was purified by preparative thin layer chromatography (silica gel, chloroform/methanol, 10/1 v/v, as eluent) to give 6-benzyl-4-benzyloxyquinoline-3-carboxamide (168 mg, 65%). ^{1}H NMR (DMSO-d_6) δ 8.82 (1H, s), 8.07 (1H, br s), 7.93 (1H, d, J = 8.8 Hz), 7.91 (1H, d, J = 1.4 Hz), 7.85 (1H, br s), 7.68 (1H, dd, J = 8.6, 2.1 Hz), 7.43−7.37 (5H, m), 7.35−7.30 (2H, m), 7.27−7.21 (3H, m), 5.26 (2H, s), 4.15 (2H, s).

Step 2. A mixture of the compound from the previous step (50 mg, 0.14 mmol) and 5% palladium on carbon (18 mg) in methanol/tetrahydrofuran (4 mL, 1/1 v/v) was stirred under a hydrogen atmosphere for 0.5 h at room temperature. The mixture was filtered through Celite, and the solvent was removed in vacuo. The residue was treated with *n*-hexane/ethyl acetate (1/1 v/v), and the resulting solid was collected by filtration to give **6** (34 mg, 89%). Mp 279−282 °C. ^{1}H NMR (DMSO-d_6) δ 12.60 (1H, br s), 9.35 (1H, d, J = 4.6 Hz), 8.68 (1H, s), 8.07 (1H, d, J = 0.9 Hz), 7.63 (1H, dd, J = 8.5, 1.7 Hz), 7.60 (1H, d, J = 7.9 Hz), 7.38 (1H, d, J = 4.4 Hz), 7.33−7.24 (4H, m), 7.23−7.18 (1H, m), 4.10 (2H, s). MS (ESI) m/z 279 $(M + H)^+$. Anal. ($C_{17}H_{14}N_2O_2 \cdot 0.5H_2O$) C, H, N.

6-(2,3-Dichlorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (16). Step 1. A vigorously stirred mixture of zinc powder (55.1 g, 843 mmol) in tetrahydrofuran (56 mL) was heated to reflux under an argon atmosphere. After 1,2-dibromoethane (2.91 mL, 33.8 mmol) was added, the mixture was stirred for 5 min at the same temperature. The suspension was cooled to 0 °C, and to it was added chlorotrimethylsilane (8.56 mL, 67.5 mmol) followed by 2,3-dichlorobenzyl chloride (82.4 g, 421 mmol) in tetrahydrofuran (330 mL) dropwise. The resulting gray suspension was used as 2,3-dichlorobenzylzinc chloride in the next step.

Step 2. A round-bottomed flask was charged with 4-iodonitrobenzene (70.0 g, 281 mmol), $Pd_2(dba)_3$ (3.23 g, 5.62 mmol), and tri-2-furylphosphine (2.61 g, 11.24 mmol), evacuated, and backfilled with argon. To the mixture was added tetrahydrofuran (1000 mL) followed by 2,3-dichlorobenzylzinc chloride (421 mmol) under an argon atmosphere. The reaction mixture was stirred for 2 h at room temperature and diluted with saturated aqueous ammonium chloride. A precipitate was filtered by Celite and washed with ethyl acetate. The combined filtrate was concentrated in vacuo. To the residue were added ethyl acetate and water. The organic phase was separated, washed successively with water and brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was treated with *n*-hexane, and the resulting solid was collected by filtration to give 4-(2,3-dichlorobenzyl)nitrobenzene (60.2 g, 76%). ^{1}H NMR ($CDCl_3$) δ 8.15 (2H, d, J = 8.7 Hz), 7.44−7.40 (1H, m), 7.33−7.31 (2H, d, J = 8.7 Hz), 7.20−7.16 (1H, m), 4.24 (2H, s).

Step 3. To a stirred solution of the compound from the previous step (25.0 g, 88.6 mmol) in acetic acid (400 mL) was added zinc powder (70.0 g) in small portions at room temperature. The reaction mixture was stirred for 0.5 h, filtered through Celite, and concentrated in vacuo. The residue was diluted with saturated aqueous sodium hydrogen carbonate, and the mixture was extracted with ethyl acetate. The organic solution was washed with water, dried over sodium sulfate, and concentrated in vacuo. The residue was treated with *n*-hexane, and the resulting solid was collected by filtration to give 4-(2,3-dichlorobenzyl)aniline (36.3 g, 81%). ^{1}H NMR ($CDCl_3$) δ 7.34−7.31 (1H, m), 7.13−6.96 (4H, m), 6.67−6.62 (2H, m), 4.03 (2H, s), 3.61 (2H, br s).

Step 4. The compound from the previous step (10.0 g, 39.7 mmol) was treated as described for the synthesis of **5** to afford 6-(2,3-dichlorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (10.14 g, 68%). ^{1}H NMR (DMSO-d_6) δ 8.51 (1H, s), 7.90 (1H, s), 7.58−7.55 (3H, m), 7.42−7.34 (2H, m), 4.27 (2H, s), 4.20 (2H, q, J = 7.1 Hz), 1.27 (3H, t, J = 7.1 Hz).

Step 5. The compound of the previous step (120.0 mg, 0.32 mmol) was treated as described for the synthesis of **3** to give **16** (76.6 mg, 69%). Mp 261 °C. ^{1}H NMR (DMSO-d_6) δ 15.13 (1H, br s), 13.19 (1H, br s), 8.85 (1H, d, J = 5.7 Hz), 8.05 (1H, s), 7.80−7.77 (2H, m), 7.58−7.56 (1H, m), 7.44−7.35 (2H, m), 4.34 (2H, s). MS (ESI) m/z 348 $(M + H)^+$. Anal. ($C_{17}H_{11}Cl_2NO_3$) C, H, N.

The following compounds (**7**−**11**, **14**, and **15**) were prepared using the above procedures.

6-(4-Chlorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (7): 45% yield. Mp 260 °C. ^{1}H NMR (DMSO-d_6) δ 15.33 (1H, br s), 13.36 (1H, br s), 8.84 (1H, d, J = 4.5 Hz), 8.13 (1H, s), 7.76 (2H, s), 7.37−7.36 (2H, m), 7.31−7.29 (2H, m), 4.15 (2H, s). MS (ESI) m/z 314 $(M+H)^+$. Anal. ($C_{17}H_{12}ClNO_3 \cdot 0.5H_2O$) C, H, N.

6-(3-Chlorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (8): 76% yield. Mp 238 °C. ^{1}H NMR (DMSO-d_6) δ 15.33 (1H, br s), 13.52 (1H, br s), 8.82 (1H, s), 8.15 (1H, s), 7.79 (2H, s), 7.37−7.32 (2H, s), 7.28−7.25 (2H, m), 4.17 (2H, s). MS (ESI) m/z 314 $(M+H)^+$. Anal. ($C_{17}H_{12}ClNO_3$) C, H, N.

6-(2-Chlorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (9): 48% yield. Mp 254 °C. ^{1}H NMR (DMSO-d_6) δ 15.29 (1H, br s), 13.35 (1H, br s), 8.84 (1H, d, J = 4.1 Hz), 8.05 (1H, s), 7.77 (2H, s), 7.48−7.42 (2H, m), 7.34−7.30 (2H, m), 4.28 (2H, s). MS (ESI) m/z 314 $(M+H)^+$. Anal. ($C_{17}H_{12}ClNO_3$) C, H, N.

6-(2-Fluorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (10): 35% yield. Mp 256 °C. ^{1}H NMR (DMSO-d_6) δ 15.33 (1H, br s), 13.41 (1H, br s), 8.86 (1H, d, J = 6.4 Hz), 7.78 (1H, s), 7.41−7.36 (1H, m), 7.32−7.28 (1H, m), 7.22−7.15 (2H, m), 4.18 (2H, s). MS (ESI) m/z 298 $(M + H)^+$. Anal. ($C_{17}H_{12}FNO_3$) C, H, N.

6-(2-Methylbenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (11): 94% yield. Mp 252 °C. ^{1}H NMR (DMSO-d_6) δ 15.32 (1H, br s), 13.37 (1H, br s), 8.84 (1H, d, J = 6.5 Hz), 7.99 (1H, s), 7.81−7.72 (2H, m), 7.18 (4H, s), 4.16 (2H, s), 2.20 (3H, s). MS (ESI) m/z 294 $(M + H)^+$. Anal. ($C_{18}H_{15}NO_3$) C, H, N.

6-(2,6-Dichlorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (14): 47% yield. Mp 282 °C. ^{1}H NMR (DMSO-d_6) δ 15.20 (1H, br s), 13.38 (1H, br s), 8.84 (1H, d, J = 4.3 Hz), 7.92 (1H, s), 7.81−7.76 (2H, m), 7.58−7.56 (2H, m), 7.42−7.38 (1H, m), 4.46 (2H, s). MS (ESI) m/z 348 $(M + H)^+$. Anal. ($C_{17}H_{11}Cl_2NO_3$) C, H, N.

6-(2,5-Dichlorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (15): 60% yield. Mp 252 °C. ^{1}H NMR (DMSO-d_6) δ 13.44 (1H, br s), 8.87 (1H, s), 8.07 (1H, s), 7.80 (2H, s), 7.59 (1H, d, J = 2.0 Hz), 7.52 (1H, d, J = 8.6 Hz), 7.40 (1H, dd, J = 8.3, 2.3 Hz), 4.28 (2H, s). MS (ESI) m/z 348 $(M + H)^+$. Anal. ($C_{17}H_{11}Cl_2NO_3$) C, H, N.

6-(2-Methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (12). Step 1. A round-bottomed flask was charged with 2-bromoanisole (3.26 g, 12.00 mmol), 4-methylnitrobenzene (1.77 g, 10.00 mmol), palladium acetate (112 mg, 0.50 mmol), triphenylphosphine (524 mg, 2.00 mmol), and cesium carbonate (3.92 g, 12.00 mmol), evacuated, and backfilled with argon. *N*,*N*-Dimethylformamide (40 mL) was added under an argon atmosphere. The reaction mixture was heated to 140 °C for 2 h, cooled, and diluted with water. The mixture was extracted with ethyl acetate, and the organic solution was washed successively with water twice and brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, *n*-hexane/ethyl acetate = 20/1 v/v as eluent) to give 4-(2-methoxybenzyl)nitrobenzene (992 mg, 41%). ^{1}H NMR (DMSO-d_6) δ 8.09 (2H, d, J = 8.8 Hz), 7.33 (2H, d, J = 8.4 Hz), 7.28−7.20 (1H, m), 7.11−7.08 (1H, m), 6.90−6.80 (2H, m), 4.04 (2H, s), 3.78 (3H, s).

Step 2. To a mixture of 4-(2-methoxybenzyl)nitrobenzene (992 mg, 4.08 mmol) in ethanol (20 mL) was added 10%

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palladium on carbon (500 mg). The mixture was hydrogenated for 2 h at room temperature under atmospheric pressure. The catalyst was filtered and washed with ethanol. The filtrate combined was concentrated in vacuo to give 4-(2-methoxybenzyl)aniline (822 mg). This material was treated as described for the synthesis of **5** to give 6-(2-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester. The title compound was prepared from this ester as described for the synthesis of **3** (238 mg, 19%). Mp 243 °C. ^{1}H NMR (DMSO-d_6) δ 15.39 (1H, br s), 13.37 (1H, br s), 8.84 (1H, d, J = 6.0 Hz), 8.06 (1H, s), 7.75 (2H, s), 7.27−7.18 (2H, m), 7.00 (1H, d, J = 7.7 Hz), 6.93−6.87 (1H, m), 4.08 (2H, s), 3.77 (3H, s). MS (ESI) m/z 310 $(M + H)^+$. Anal. ($C_{18}H_{15}NO_4$) C, H, N.

4-Oxo-6-(2-trifluoromethylbenzyl)-1,4-dihydroquinoline-3-carboxylic Acid (13). The title compound was prepared from 2-bromotrifluoromethylbenzene and 4-methylnitrobenzene as described for the synthesis of **12** (22% yield). Mp 245 °C. ^{1}H NMR (DMSO-d_6) δ 15.26 (1H, br s), 13.38 (1H, br s), 8.85 (1H, s), 7.98 (1H, s), 7.80−7.76 (2H, m), 7.72−7.70 (1H, m), 7.67−7.63 (1H, m), 7.52−7.48 (1H, m), 7.43−7.41 (1H, m), 4.36 (2H, s). MS (ESI) m/z 348 $(M + H)^+$. Anal. ($C_{18}H_{12}F_3NO_3$) C, H, N.

6-(2,3-Dichlorobenzyl)-1-(2-hydroxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (27). Step 1. To a stirred mixture of 6-(2,3-dichlorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (6.0 g, 16.0 mmol) and potassium carbonate (14.3 g, 103.5 mmol) in *N*,*N*-dimethylformamide (240 mL) was added 2-bromoethyl acetate (6.0 mL, 24.2 mmol). The reaction mixture was heated to 80 °C for 3.5 h, cooled, and diluted with water. A precipitate was collected by filtration and then treated with *n*-hexane/ethyl acetate (3/2 v/v). The resulting solid was collected by filtration to give 1-(2-acetoxyethyl)-6-(2,3-dichlorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (6.0 g, 82%). ^{1}H NMR ($CDCl_3$) δ 8.59 (1H, s), 7.98 (1H, d, J = 2.1 Hz), 7.83 (1H, d, J = 8.8 Hz), 7.68−7.65 (1H, m), 7.57 (1H, dd, J = 7.6, 1.9 Hz), 7.43 (1H, dd, J = 7.7, 2.0 Hz), 7.39−7.34 (1H, m), 4.63 (2H, t, J = 5.0 Hz), 4.37 (2H, t, J = 5.0 Hz), 4.29 (2H, s), 4.21 (2H, q, J = 7.1 Hz), 1.90 (3H, s), 1.27 (3H, t, J = 7.1 Hz).

Step 2. The title compound was prepared from the compound of the previous step (6.0 g, 16.0 mmol) as described for the synthesis of **3** (4.5 g, 72%). Mp 245−247 °C. ^{1}H NMR (DMSO-d_6) δ 15.21 (1H, br s), 8.87 (1H, s), 8.15 (1H, d, J = 2.3 Hz), 8.03 (1H, d, J = 8.7 Hz), 7.82 (1H, dd, J = 9.0, 2.3 Hz), 7.58 (1H, dd, J = 7.7, 1.7 Hz), 7.46 (1H, dd, J = 7.7, 1.7 Hz), 7.41−7.36 (1H, m), 5.01 (1H, br s), 4.61 (2H, t, J = 4.9 Hz), 4.37 (2H, s), 3.80−3.71 (2H, m). MS (ESI) m/z 392 $(M+H)^+$. Anal. ($C_{19}H_{15}Cl_2NO_4$) C, H, N.

The following compounds (**4**, **17**−**22**, **24**, **28**−**30**, and **34**−**39**) were prepared using the above procedures.

6-Benzyl-1-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (4): 91% yield. Mp 233−235 °C. ^{1}H NMR ($CDCl_3$) δ 14.97 (1H, br s), 8.72 (1H, s), 8.41 (1H, d, J = 1.9 Hz), 7.68 (1H, dd, J = 8.9, 2.1 Hz), 7.53 (1H, d, J = 8.7 Hz), 7.35−7.18 (5H, m), 4.17 (2H, s), 4.00 (3H, s). MS (ESI) m/z 294 $(M + H)^+$. Anal. ($C_{18}H_{15}NO_3$) C, H, N.

6-(2,3-Dichlorobenzyl)-1-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (17): 84% yield. Mp 257 °C. ^{1}H NMR (DMSO-d_6) δ 9.01 (1H, s), 8.12 (1H, s), 7.93 (1H, d, J = 8.9 Hz), 7.85 (1H, dd, J = 8.9, 2.0 Hz), 7.58 (1H, dd, J = 7.8, 1.7 Hz), 7.46 (1H, dd, J = 7.6, 1.6 Hz), 7.40−7.35 (1H, m), 4.37 (2H, s), 4.09 (3H, s). MS (ESI) m/z 362 $(M + H)^+$. Anal. ($C_{18}H_{13}Cl_2NO_3$) C, H, N.

6-(2,3-Dichlorobenzyl)-1-ethyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (18): 98% yield. Mp 222−223 °C. ^{1}H NMR (DMSO-d_6) δ 15.20 (1H, br s), 9.04 (1H, s), 8.15 (1H, d, J = 1.9 Hz), 8.02 (1H, d, J = 8.7 Hz), 7.84 (1H, dd, J = 9.0, 2.3 Hz), 7.58 (1H, dd, J = 7.9, 1.9 Hz), 7.46 (1H, dd, J = 7.5, 1.9 Hz), 7.41−7.36 (1H, m), 4.59 (2H, q, J = 7.2 Hz), 4.37 (2H, s), 1.41 (3H, t, J = 7.2 Hz). MS (ESI) m/z 376 $(M + H)^+$. Anal. ($C_{19}H_{15}Cl_2NO_3 \cdot 0.25H_2O$) C, H, N.

6-(2,3-Dichlorobenzyl)-4-oxo-1-propyl-1,4-dihydroquinoline-3-carboxylic acid (19): 92% yield. Mp 220−221 °C. ^{1}H NMR (DMSO-d_6) δ 15.19 (1H, br s), 9.02 (1H, s), 8.14 (1H, d, J = 1.9 Hz), 8.02 (1H, d, J = 9.0 Hz), 7.83 (1H, dd, J = 8.9, 2.1 Hz), 7.58 (1H, dd, J = 7.9, 1.9 Hz), 7.46 (1H, dd, J = 7.7, 1.7 Hz), 7.41−7.36 (1H, m), 4.51 (2H, t, J = 7.3 Hz), 4.36 (2H, s), 1.88−1.76 (2H, m), 0.91 (3H, t, J = 7.3 Hz). MS (ESI) m/z 390 $(M + H)^+$. Anal. ($C_{20}H_{17}Cl_2NO_3$) C, H, N.

6-(2,3-Dichlorobenzyl)-1-(1-methylethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (20): 55% yield. Mp 212−214 °C. ^{1}H NMR (DMSO-d_6) δ 8.86 (1H, s), 8.18−8.14 (2H, m), 7.85 (1H, dd, J = 8.9, 2.1 Hz), 7.58 (1H, dd, J = 7.8, 1.7 Hz), 7.47−7.45 (1H, m), 7.41−7.36 (1H, m), 5.29−5.20 (1H, m), 4.37 (2H, s), 1.57 (6H, d, J = 6.5 Hz). MS (ESI) m/z 390 $(M + H)^+$. Anal. ($C_{20}H_{17}Cl_2NO_3$) C, H, N.

1-Butyl-6-(2,3-dichlorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (21): 94% yield. Mp 213−214 °C. ^{1}H NMR (DMSO-d_6) δ 15.19 (1H, br s), 9.02 (1H, s), 8.15 (1H, d, J = 1.9 Hz), 8.01 (1H, d, J = 9.0 Hz), 7.84 (1H, dd, J = 8.9, 2.1 Hz), 7.58 (1H, dd, J = 7.9, 1.9 Hz), 7.46 (1H, dd, J = 7.5, 1.9 Hz), 7.41−7.36 (1H, m), 4.55 (2H, t, J = 7.3 Hz), 4.37 (2H, s), 1.83−1.71 (2H, m), 1.40−1.28 (2H, m), 0.90 (3H, t, J = 7.3 Hz). MS (ESI) m/z 404 $(M + H)^+$. Anal. ($C_{21}H_{19}Cl_2NO_3$) C, H, N.

1-Carboxymethyl-6-(2,3-dichlorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (22): 67% yield. Mp 269 °C. ^{1}H NMR (DMSO-d_6) δ 15.38 (1H, s), 8.85 (1H, s), 8.09 (1H, d, J = 1.9 Hz), 7.77 (1H, dd, J = 8.9, 2.1 Hz), 7.70 (1H, d, J = 9.0 Hz), 7.57 (1H, dd, J = 7.5, 1.9 Hz), 7.43 (1H, dd, J = 7.5, 1.9 Hz), 7.40−7.35 (1H, m), 4.79 (2H, s), 4.34 (2H, s). MS (ESI) m/z 406 $(M + H)^+$. Anal. ($C_{19}H_{13}Cl_2NO_5$) C, H, N.

1-Carbamoylmethyl-6-(2,3-dichlorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (24): 94% yield. Mp 276 °C. ^{1}H NMR (DMSO-d_6) δ 15.09 (1H, br s), 9.02 (1H, s), 8.13 (1H, d, J = 1.9 Hz), 7.90 (1H, br s), 7.85 (1H, dd, J = 8.8, 2.1 Hz), 7.64 (1H, d, J = 9.0 Hz), 7.58 (1H, dd, J = 7.9, 1.6 Hz), 7.54 (1H, br s), 7.45 (1H, dd, J = 7.8, 1.5 Hz), 7.40−7.36 (1H, m), 5.24 (2H, s), 4.35 (2H, s). MS (ESI) m/z 405 $(M + H)^+$. Anal. ($C_{19}H_{14}Cl_2N_2O_4 \cdot 0.67H_2O$) C, H, N.

6-(2,3-Dichlorobenzyl)-1-(3-hydroxypropyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (28): 21% yield. Mp 205−208 °C. ^{1}H NMR (DMSO-d_6) δ 15.16 (1H, br s), 8.96 (1H, s), 8.14 (1H, s), 8.00 (1H, d, J = 6.7 Hz), 7.86−7.83 (1H, m), 7.57 (1H, d, J = 5.9 Hz), 7.46−7.44 (1H, m), 7.40−7.36 (1H, m), 4.68 (1H, br s), 4.61−4.58 (2H, m), 4.36 (2H, s), 3.47−3.44 (2H, m), 1.97−1.94 (2H, m). MS (ESI) m/z 406 $(M + H)^+$. Anal. ($C_{20}H_{17}Cl_2NO_4$) C, H, N.

6-(2-Chloro-3-fluorobenzyl)-1-(2-hydroxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (29): 87% yield. Mp 227−228 °C. ^{1}H NMR (DMSO-d_6) δ 15.25 (1H, br s), 8.90 (1H, s), 8.19 (1H, s), 8.08−8.05 (1H, m), 7.88−7.80 (1H, m), 7.45−7.29 (3H, m), 5.03 (1H, br s), 4.68−4.57 (2H, m), 4.39 (2H, s), 3.82−3.73 (2H, m). MS (ESI) m/z 376 $(M + H)^+$. Anal. ($C_{19}H_{15}ClFNO_4$) C, H, N.

6-(3-Chloro-2-fluorobenzyl)-1-(2-hydroxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (30): 80% yield. Mp 225−226 °C. ^{1}H NMR (DMSO-d_6) δ 8.87 (1H, s), 8.22 (1H, s), 8.04−8.01 (1H, m), 7.85−7.82 (1H, m), 7.51−7.46 (1H, m), 7.43−7.38 (1H, m), 7.24−7.19 (1H, m), 5.00 (1H, br s), 4.61 (2H, t, J = 5.1 Hz), 4.26 (2H, s), 3.80−3.71 (2H, m). MS (ESI) m/z 376 $(M + H)^+$. Anal. ($C_{19}H_{15}ClFNO_4$) C, H, N.

6-(2,3-Dichlorobenzyl)-1-(2-hydroxyethyl)-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (34): 85% yield. Mp 288 °C. ^{1}H NMR (DMSO-d_6) δ 15.40 (1H, s), 8.80 (1H, s), 7.83 (1H, s), 7.59 (1H, dd, J = 8.0, 1.5 Hz), 7.37−7.33 (2H, m), 7.25 (1H, dd, J = 7.7, 1.4 Hz), 5.04 (1H, t, J = 5.7 Hz), 4.65 (2H, t, J = 4.8 Hz), 4.20 (2H, s), 4.03 (3H, s), 3.81 (2H, dt, J = 5.0, 2.5 Hz). MS (ESI) m/z 422 $(M + H)^+$. Anal. ($C_{20}H_{17}Cl_2NO_5$) C, H, N.

7-Chloro-6-(2,3-dichlorobenzyl)-1-(2-hydroxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (35): 83% yield. Mp 282 °C.

^{1}H NMR (DMSO-d_6) δ 14.87 (1H, br s), 8.89 (1H, s), 8.32 (1H, s), 8.00 (1H, s), 7.62 (1H, dd, J = 8.0, 1.5 Hz), 7.39−7.35 (1H, m), 7.20 (1H, dd, J = 7.7, 1.4 Hz), 5.02 (1H, t, J = 5.6 Hz), 4.64 (2H, t, J = 4.9 Hz), 4.40 (2H, s), 3.76 (2H, dd, J = 10.1, 5.5 Hz). MS (ESI) m/z 426 $(M + H)^+$. Anal. ($C_{19}H_{14}Cl_3NO_4$) C, H, N.

6-(2,3-Dichlorobenzyl)-1-(2-hydroxyethyl)-7-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (36): 49% yield. Mp 286 °C. ^{1}H NMR (DMSO-d_6) δ 15.23 (1H, br s), 8.82 (1H, s), 7.96 (1H, s), 7.79 (1H, s), 7.62 (1H, dd, J = 8.0, 1.5 Hz), 7.39−7.35 (1H, m), 7.17 (1H, dd, J = 7.7, 1.4 Hz), 5.02 (1H, t, J = 5.6 Hz), 4.64−4.59 (2H, m), 4.29 (2H, s), 3.80−3.75 (2H, m), 2.50 (3H, s). MS (ESI) m/z 406 $(M + H)^+$. Anal. ($C_{20}H_{17}Cl_2NO_4$) C, H, N.

6-(2,3-Dichlorobenzyl)-7-trifluoromethyl-1-(2-hydroxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (37): 83% yield. Mp 246−248 °C. ^{1}H NMR (DMSO-d_6) δ 14.62 (1H, br s), 9.00 (1H, s), 8.40 (1H, s), 7.95 (1H, s), 7.65 (1H, dd, J = 8.1, 1.4 Hz), 7.42−7.39 (1H, m), 7.25 (1H, dd, J = 7.7, 1.4 Hz), 5.06 (1H, t, J = 5.6 Hz), 4.75 (2H, t, J = 4.9 Hz), 4.48 (2H, s), 3.82−3.77 (2H, m). MS (ESI) m/z 460 $(M + H)^+$. Anal. ($C_{20}H_{14}Cl_2F_3NO_4$) C, H, N.

6-(2,3-Dichlorobenzyl)-7-cyano-1-(2-hydroxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (38): 75% yield. Mp 265 °C. ^{1}H NMR (DMSO-d_6) δ 8.96 (1H, s), 8.79 (1H, s), 8.07 (1H, s), 7.64 (1H, dd, J = 8.0, 1.5 Hz), 7.43−7.39 (1H, m), 7.32 (1H, dd, J = 7.7, 1.6 Hz), 5.01 (1H, t, J = 5.6 Hz), 4.68 (2H, t, J = 4.9 Hz), 4.52 (2H, s), 3.79−3.74 (2H, m). MS (ESI) m/z 417 $(M + H)^+$. Anal. ($C_{20}H_{14}Cl_2N_2O_4$) C, H, N.

6-(3-Chloro-2-fluorobenzyl)-1-(2-hydroxyethyl)-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (39): 76% yield. Mp 253−255 °C. ^{1}H NMR (DMSO-d_6) δ 15.45 (1H, br s), 8.80 (1H, s), 8.03 (1H, s), 7.50−7.46 (1H, m), 7.31 (1H, s), 7.26−7.16 (2H, m), 5.03 (1H, t, J = 5.6 Hz), 4.64 (2H, t, J = 4.8 Hz), 4.12 (2H, s), 4.01 (3H, s), 3.84−3.77 (2H, m). MS (ESI) m/z 406 $(M + H)^+$. Anal. ($C_{20}H_{17}ClFNO_5$) C, H, N.

1-(2-Carboxyethyl)-6-(2,3-dichlorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (23). Step 1. To a stirred mixture of 6-(2,3-dichlorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (700 mg, 1.86 mmol) and Triton B (0.15 mL, 40 wt % in methanol) in *N*,*N*-dimethylformamide (150 mL) was added acrylic acid *tert*-butyl ester (2.67 mL, 18.62 mmol). The mixture was heated to 90 °C for 2 h, cooled, and diluted with water, and the mixture was extracted with ethyl acetate. The organic solution was washed successively with water twice and with brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, chloroform/methanol = 50/1 v/v as eluent) to give 1-(2-*tert*-butoxycarbonylethyl)-6-(2,3-dichlorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (468 mg, 50%). ^{1}H NMR ($CDCl_3$) δ 8.54 (1H, s), 8.38 (1H, d, J = 2.1 Hz), 7.52 (1H, dd, J = 8.7, 2.2 Hz), 7.37−7.34 (2H, m), 7.14 (1H, d, J = 3.9 Hz), 7.13 (1H, s), 4.44 (2H, t, J = 6.8 Hz), 4.39 (2H, q, J = 7.1 Hz), 4.26 (2H, s), 2.80 (2H, t, J = 6.8 Hz), 1.43 (9H, s), 1.40 (3H, t, J = 7.1 Hz).

Step 2. A mixture of the compound of the previous step (428 mg, 0.85 mmol) in chloroform (3.4 mL) and trifluoroacetic acid (3.3 mL) was stirred for 1.5 h at room temperature and concentrated in vacuo. The residue was purified by column chromatography (silica gel, chloroform/methanol = 20/1 v/v as eluent) to give 1-(2-carboxyethyl)-6-(2,3-dichlorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (363 mg, 96%). ^{1}H NMR ($CDCl_3$) δ 9.27 (1H, s), 8.42 (1H, s), 7.89 (1H, dd, J = 8.9, 2.0 Hz), 7.85 (1H, d, J = 9.0 Hz), 7.45 (1H, dd, J = 7.7, 1.9 Hz), 7.26−7.22 (1H, m), 7.20 (1H, dd, J = 7.7, 2.1 Hz), 4.90 (2H, t, J = 6.3 Hz), 4.49 (2H, q, J = 7.2 Hz), 4.39 (2H, s), 3.10 (2H, t, J = 6.3 Hz), 1.45 (3H, t, J = 7.2 Hz).

Step 3. The title compound was prepared from the compound of the previous step (130 mg, 0.29 mmol) as described for the synthesis of **3** (88 mg, 94%). Mp 238 °C. ^{1}H NMR (DMSO-d_6) δ 15.13 (1H, br s), 9.01 (1H, s), 8.14 (1H, d, J = 2.1 Hz), 8.00 (1H, d, J = 9.0 Hz), 7.84 (1H, dd, J = 8.8, 2.1 Hz), 7.58 (1H, dd, J = 7.9, 1.6 Hz), 7.46 (1H, dd, J = 7.7, 1.6 Hz), 7.40−7.36 (1H, m), 4.73 (2H, t, J = 6.7 Hz), 4.36 (2H, s), 2.83 (2H, t, J = 6.7 Hz). MS (ESI) m/z 419 $(M + H)^+$. Anal. ($C_{20}H_{15}Cl_2NO_5$) C, H, N.

1-(2-Carbamoylethyl)-6-(2,3-dichlorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (25). Step 1. To a stirred mixture of **28** (203 mg, 0.50 mmol) and acetic anhydride (0.094 mL, 1.00 mmol) in acetic acid (2.0 mL) was added sodium acetate (12.3 mg, 0.15 mmol). The mixture was heated to 130 °C for 4.5 h, cooled, and diluted with water. The mixture was extracted with ethyl acetate. The organic solution was washed with water, dried over sodium sulfate, and concentrated in vacuo to give 1-(3-acetoxypropyl)-6-(2,3-dichlorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (160 mg, 74%). ^{1}H NMR ($CDCl_3$) δ 8.79 (1H, s), 8.36 (1H, s), 7.72−7.69 (1H, m), 7.62−7.59 (1H, m), 7.42−7.38 (1H, m), 7.27−7.16 (2H, m), 4.82 (2H, t, J = 7.0 Hz), 4.32 (2H, s), 4.18 (2H, t, J = 5.5 Hz), 2.30−2.25 (2H, m), 2.08 (3H, s).

Step 2. To a stirred mixture of the compound from the previous step (160 mg, 0.37 mmol) in toluene (2.0 mL) was added *N*,*N*-dimethylformamide di-*tert*-butyl acetal (0.50 mL, 2.00 mmol). The mixture was heated to reflux for 2 h, cooled, and diluted with water. The mixture was extracted with ethyl acetate. The organic solution was washed successively with 1 M hydrochloric acid and water three times, dried over sodium sulfate, and concentrated in vacuo. The residue was dissolved in tetrahydrofuran/methanol (2.0 mL, 1/1 v/v). To the stirred solution was added potassium carbonate (276 mg, 2.00 mmol). The mixture was stirred for 1.5 h at room temperature, diluted with water, and extracted with ethyl acetate. The organic solution was washed successively with 1 M hydrochloric acid and water, dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, chloroform/ethyl acetate = 4/1 v/v as eluent) to give 6-(2,3-dichlorobenzyl)-1-(3-hydroxypropyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid *tert*-butyl ester (90 mg, 54%). ^{1}H NMR ($CDCl_3$) δ 8.45 (1H, s), 8.36 (1H, br s), 7.50−7.44 (2H, m), 7.37−7.32 (1H, m), 7.12−7.08 (2H, m), 4.34 (2H, t, J = 7.0 Hz), 4.23 (2H, s), 3.72 (2H, t, J = 5.5 Hz), 2.26 (1H, br s), 2.13−2.05 (2H, m), 1.59 (9H, s).

Step 3. To a stirred mixture of the compound from the previous step (43 mg, 0.10 mmol) in acetone/1,4-dioxane (4.0 mL, 1/1 v/v) was added Jones reagent (0.68 mL) at 0 °C. The mixture was stirred for 0.5 h at 0 °C, diluted with water, and extracted with ethyl acetate twice. The organic solution combined was washed with water twice, dried over sodium sulfate, and concentrated in vacuo. The residue was dissolved in *N*,*N*-dimethylformamide (1.0 mL). To the stirred solution were added triethylamine (0.012 mL, 0.09 mmol), 4-dimethylaminopyridine (10 mg, 0.08 mmol), ammonium chloride (5.0 mg, 0.09 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (16 mg, 0.08 mmol). The mixture was stirred for 16 h at room temperature, diluted with water, and extracted with ethyl acetate. The organic solution was washed successively with 1 M hydrochloric acid and water thee times, dried over sodium sulfate, and concentrated in vacuo. The residue was dissolved in formic acid (1.0 mL). The mixture was heated to 70 °C for 2 h and concentrated in vacuo. The residue was treated with ethyl ether, and the resulting solid was collected by filtration to give **25** (6.0 mg, 15%). Mp 240 °C. ^{1}H NMR (DMSO-d_6) δ 8.93 (1H, s), 8.15 (1H, s), 8.02−7.99 (1H, m), 7.88−7.83 (1H, m), 7.59−7.56 (1H, m), 7.48−7.45 (1H, m), 7.42−7.36 (2H, m), 6.97 (1H, br s), 4.73 (2H, t, J = 3.9 Hz), 4.37 (2H, s), 2.67 (2H, t, J = 3.9 Hz). MS (ESI) m/z 419 $(M + H)^+$. Anal. ($C_{20}H_{16}Cl_2N_2O_4 \cdot 0.67H_2O$) C, H, N.

1-(2-Aminoethyl)-6-(2,3-dichlorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (26). Step 1. To a stirred mixture of *N*-(*tert*-butoxycarbonyl)ethanolamine (1.61 g, 10.0 mmol) in ethyl ether (8.0 mL) was added triethylamine (3.1 mL, 22.0 mmol) followed by the slow addition of methanesulfonyl chloride (0.85 mL, 11.0 mmol) at 0 °C. The mixture was stirred for 0.5 h at 0 °C, diluted with water, and extracted with ethyl ether. The organic

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solution was washed successively with water and brine, dried over sodium sulfate, and concentrated in vacuo to give methanesulfonic acid 2-*tert*-butoxycarbonylaminoethyl ester (2.44 g, quantitative yield). ^{1}H NMR ($CDCl_3$) δ 4.91 (1H, br s), 4.31 (2H, t, J = 5.2 Hz), 3.52−3.50 (2H, m), 3.05 (3H, s), 1.47 (9H, s).

Step 2. 1-(2-*tert*-Butoxycarbonylaminoethyl)-6-(2,3-dichlorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester was prepared from 6-(2,3-dichlorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (730 mg, 1.94 mmol) and the compound of the previous step (2.09 g, 8.75 mmol) as described in step 1 for the synthesis of **27** (685 mg, 66%). ^{1}H NMR ($CDCl_3$) δ 8.41−8.40 (2H, m), 7.54 (2H, s), 7.39−7.37 (1H, m), 7.16−7.15 (1H, m), 4.74 (1H, br s), 4.40 (2H, q, J = 7.1 Hz), 4.35 (2H, t, J = 4.3 Hz), 4.28 (2H, s), 3.54 (2H, dt, J = 6.1, 4.3 Hz), 1.45 (9H, s), 1.42 (3H, t, J = 7.1 Hz).

Step 3. To a stirred solution of the compound of the previous step (683 mg, 1.28 mmol) in chloroform (40 mL) was added trifluoroacetic acid (20 mL). The mixture was stirred for 2.5 h at room temperature and concentrated in vacuo. To the residue was added aqueous sodium hydrogen carbonate, and the mixture was extracted with ethyl acetate/tetrahydrofuran (1/1 v/v). The organic solution was washed with water and brine, dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, chloroform/methanol = 50/1 to 20/1 to 10/1 v/v as eluent) to give 1-(2-aminoethyl)-6-(2,3-dichlorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (271 mg, 51%). ^{1}H NMR (DMSO-d_6) δ 8.59 (1H, s), 7.98 (1H, d, J = 2.1 Hz), 7.78 (1H, d, J = 8.8 Hz), 7.63 (1H, dd, J = 8.8, 2.1 Hz), 7.56 (1H, dd, J = 7.8, 1.7 Hz), 7.42 (1H, dd, J = 7.8, 1.7 Hz), 7.36 (1H, t, J = 7.8 Hz), 4.30−4.25 (4H, m), 4.20 (2H, q, J = 7.1 Hz), 2.88 (2H, t, J = 5.9 Hz), 1.85 (1H, br s), 1.26 (3H, t, J = 7.1 Hz).

Step 4. To a suspension of the compound of the previous step (269 mg, 0.64 mmol) in ethanol (10 mL) was added 1 M aqueous sodium hydroxide (10 mL). This mixture was heated to reflux for 0.5 h, diluted with water, and cooled to room temperature. Acetic acid (1.75 mL) was added, and the suspension was stirred at room temperature overnight. The precipitate was collected by filtration to give **26** (237 mg, 87%). Mp 210 °C. ^{1}H NMR (DMSO-d_6) δ 8.92 (1H, s), 8.14 (1H, s), 8.02 (1H, d, J = 9.2 Hz), 7.83−7.77 (1H, m), 7.59−7.56 (1H, m), 7.47−7.46 (1H, m), 7.41−7.35 (1H, m), 4.48 (2H, t, J = 5.4 Hz), 4.36 (2H, s), 2.93 (2H, t, J = 5.4 Hz). MS (ESI) m/z 391 $(M + H)^+$. Anal. ($C_{19}H_{16}Cl_2N_2O_3 \cdot 2H_2O$) C, H, N.

6-(3-Chloro-2-fluorobenzyl)-1-((2*S*)-1-hydroxy-3-methylbutan-2-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (44). Step 1. To a stirred mixture of 2-fluoro-5-iodobenzoic acid (3.00 g, 11.28 mmol) in toluene (30 mL) was added thionyl chloride (4.1 mL, 56.21 mmol) followed by *N*,*N*-dimethylformamide (0.02 mL). The mixture was heated to reflux for 1.5 h, cooled, and concentrated in vacuo. A solution of this residue in tetrahydrofuran (20 mL) was added to a stirred solution of ethyl 3-(dimethylamino)acrylate (1.78 g, 12.43 mmol) and triethylamine (1.89 mL, 13.56 mmol) in tetrahydrofuran (20 mL). The mixture was heated to 50 °C for 2.5 h, cooled, and diluted with water. The mixture was extracted with ethyl acetate. The organic solution was washed successively with water and brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (silica, *n*-hexane/ethyl acetate = 2/1 to 1/1 v/v as eluent) to give 3-dimethylamino-2-(2-fluoro-5-iodobenzoyl)acrylic acid ethyl ester (2.30 g, 52%).

Step 2. To a stirred solution of the compound from the previous step (300 mg, 0.77 mmol) in tetrahydrofuran (1.5 mL) was added (*S*)-(+)-2-amino-3-methyl-1-butanol (0.10 mL, 0.920 mmol). The mixture was heated to 60 °C for 1.5 h, then cooled and concentrated in vacuo. The residue was dissolved in *N*,*N*-dimethylformamide (1.2 mL), and to it was added potassium carbonate (318 mg, 2.30 mmol). The mixture was stirred for 4 h at 70 °C, cooled, and acidified with 1 M hydrochloric acid. The slurry was filtered, and the solid was washed with ethanol/water (6.0 mL, 1/2 v/v) and *n*-hexane/ethyl ether (5.0 mL, 2/1 v/v) to give 1-((2*S*)-1-hydroxy-3-methylbutan-2-yl)-6-iodo-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (280 mg, 85%).

Step 3. A mixture of the compound from the previous step (280 mg, 0.65 mmol), imidazole (53 mg, 0.78 mmol), and *tert*-buthyldimethylchlorosilane (128 mg, 0.85 mmol) in *N*,*N*-dimethylformamide (1.0 mL) was stirred for 0.5 h at room temperature. The mixture was diluted with water and extracted with ethyl acetate. The organic solution was washed successively with water twice and with brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (silica, *n*-hexane/ethyl acetate = 2/1 to 1/1 v/v as eluent) to give 1-((2*S*)-1-*tert*-buthyldimethylsilyloxy-3-methylbutan-2-yl)-6-iodo-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (327 mg, 92%). ^{1}H NMR ($CDCl_3$) δ 8.89 (1H, d, J = 2.2 Hz), 8.68 (1H, s), 7.90 (1H, dd, J = 8.8, 2.2 Hz), 7.30 (1H, d, J = 9.2 Hz), 4.43−4.35 (2H, m), 4.28−4.20 (1H, m), 4.08−4.00 (1H, m), 3.95−3.87 (1H, m), 2.53−2.36 (1H, m), 1.40 (3H, t, J = 7.2 Hz), 1.18 (3H, d, J = 6.6 Hz), 0.83 (3H, d, J = 6.6 Hz), 0.77 (9H, s), −0.05 (3H, s), −0.08 (3H, s).

Step 4. 1-((2*S*)-1-*tert*-Buthyldimethylsilyloxy-3-methylbutan-2-yl)-6-(3-chloro-2-fluorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester was prepared from the compound of previous step (327 mg, 0.60 mmol) and 3-chloro-2-fluorobenzylzinc bromide (1 M solution of tetrahydrofurn, 1.2 mL, 1.2 mmol) as described in step 2 for the synthesis of **16** (245 mg, 73%). ^{1}H NMR ($CDCl_3$) δ 8.68 (1H, s), 8.44 (1H, s), 7.48 (2H, s), 7.30−7.24 (1H, m), 7.10−7.06 (1H, m), 7.02−6.96 (1H, m), 4.42−4.35 (2H, m), 4.33−4.24 (1H, m), 4.13 (2H, s), 4.07−3.99 (1H, m), 3.91−3.89 (1H, m), 2.53−2.41 (1H, m), 1.40 (3H, t, J = 7.2 Hz), 1.17 (3H, d, J = 6.3 Hz), 0.84 (3H, d, J = 6.3 Hz), 0.75 (9H, s), −0.07 (3H, s), −0.09 (3H, s).

Step 5. To a stirred solution of the compound of the previous step (245 mg, 0.44 mmol) in ethanol (1.0 mL) was added 1 M aqueous sodium hydroxide (2.0 mL). The mixture was heated to reflux for 1 h, then cooled and diluted with water. The mixture was acidified with acetic acid (0.35 mL) and extracted with chloroform. The organic solution was washed successively with water and brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was treated with chloroform/ethyl acetate/*n*-hexane (1/1/4 v/v/v), and a precipitate was collected by filtration to give **44** (135 mg, 74%). Mp 152−153 °C. ^{1}H NMR (DMSO-d_6) δ 15.21 (1H, br s), 8.92 (1H, s), 8.25 (1H, d, J = 9.0 Hz), 8.22 (1H, d, J = 1.9 Hz), 7.83 (1H, dd, J = 8.8, 1.6 Hz), 7.51−7.46 (1H, m), 7.43−7.38 (1H, m), 7.24−7.19 (1H, m), 5.18 (1H, t, J = 4.9 Hz), 4.88−4.81 (1H, m), 4.25 (2H, s), 4.00−3.93 (1H, m), 3.79−3.72 (1H, m), 2.41−2.31 (1H, m), 1.12 (3H, d, J = 6.5 Hz), 0.70 (3H, d, J = 6.7 Hz). MS (ESI) m/z 418 $(M + H)^+$. Anal.($C_{22}H_{21}ClFNO_4$) C, H, N.

The following compounds (**31**−**33**, **40**−**43**, and **45**−**47**) were prepared using the above procedures.

6-(2,3-Dichlorobenzyl)-5-fluoro-1-(2-hydroxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (31): 19% yield. Mp 239 °C. ^{1}H NMR (DMSO-d_6) δ 8.86 (1H, s), 7.84−7.81 (1H, m), 7.73−7.68 (1H, m), 7.58 (1H, dd, J = 7.7, 1.8 Hz), 7.38−7.29 (2H, m), 5.05−4.96 (1H, m), 4.58 (2H, t, J = 4.8 Hz), 4.29 (2H, s), 3.77−3.69 (2H, m). MS (ESI) m/z 410 $(M + H)^+$. Anal. ($C_{19}H_{14}Cl_2FNO_4$) C, H, N.

6-(2,3-Dichlorobenzyl)-7-fluoro-1-(2-hydroxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (32): 91% yield. Mp 271 °C. ^{1}H NMR (DMSO-d_6) δ 14.99 (1H, br s), 8.88 (1H, s), 8.11−8.03 (2H, m), 7.63−7.60 (1H, m), 7.41−7.34 (2H, m), 5.04−4.97 (1H, m), 4.62−4.54 (2H, m), 4.34 (2H, s), 3.80−3.70 (2H, m). MS (ESI) m/z 410 $(M + H)^+$. Anal. ($C_{19}H_{14}Cl_2FNO_4$) C, H, N.

6-(2,3-Dichlorobenzyl)-8-fluoro-1-(2-hydroxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (33): 93% yield. Mp 235 °C. ^{1}H NMR (DMSO-d_6) δ 14.83 (1H, br s), 8.81 (1H, s), 8.01 (1H, s), 7.79 (1H, dd, J = 15.8, 1.9 Hz), 7.60 (1H, dd, J = 7.9, 1.9 Hz), 7.48

(1H, dd, J = 7.5, 1.5 Hz), 7.42−7.37 (1H, m), 5.00 (1H, t, J = 5.7 Hz), 4.69−4.62 (2H, m), 4.35 (2H, s), 3.81−3.74 (2H, m). MS (ESI) m/z 410 $(M + H)^+$. Anal. ($C_{19}H_{14}Cl_2FNO_4$) C, H, N.

6-(3-Chloro-2-fluorobenzyl)-1-((2*S*)-1-hydroxypropan-2-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (40): 90% yield. Mp 246−248 °C. ^{1}H NMR (DMSO-d_6) δ 8.88 (1H, s), 8.24 (1H, s), 8.17 (1H, d, J = 8.8 Hz), 7.89−7.82 (1H, m), 7.53−7.48 (1H, m), 7.45−7.40 (1H, m), 7.26−7.20 (1H, m), 5.29−5.18 (2H, m), 4.26 (2H, s), 3.87−3.76 (2H, m), 1.53 (3H, d, J = 6.5 Hz). MS (ESI) m/z 390 $(M + H)^+$. Anal. ($C_{20}H_{17}ClFNO_4$) C, H, N.

6-(3-Chloro-2-fluorobenzyl)-1-((2*R*)-1-hydroxypropan-2-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (41): 89% yield. Mp 247−249 °C. ^{1}H NMR (DMSO-d_6) δ 8.88 (1H, s), 8.24 (1H, s), 8.16 (1H, d, J = 9.2 Hz), 7.89−7.81 (1H, m), 7.52−7.47 (1H, m), 7.44−7.38 (1H, m), 7.25−7.19 (1H, m), 5.25−5.17 (2H, m), 4.26 (2H, s), 3.83−3.73 (2H, m), 1.53 (3H, d, J = 6.8 Hz). MS (ESI) m/z 390 $(M + H)^+$. Anal. ($C_{20}H_{17}ClFNO_4$) C, H, N.

6-(3-Chloro-2-fluorobenzyl)-1-((2*S*)-1-hydroxybutan-2-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (42): 56% yield. Mp 224 °C. ^{1}H NMR (DMSO-d_6) δ 15.24 (1H, s), 8.86 (1H, s), 8.23−8.19 (2H, m), 7.86−7.83 (1H, m), 7.51−7.46 (1H, m), 7.44−7.39 (1H, m), 7.24−7.19 (1H, m), 5.17 (1H, t, J = 5.4 Hz), 5.03 (1H, br s), 4.26 (2H, s), 3.92−3.70 (2H, m), 2.10−1.83 (2H, m), 0.86 (3H, t, J = 7.3 Hz). MS (ESI) m/z 404 $(M + H)^+$. Anal. ($C_{21}H_{19}ClFNO_4$) C, H, N.

6-(3-Chloro-2-fluorobenzyl)-1-((2*S*)-1-hydroxypentan-2-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (43): 65% yield. Mp 160−161 °C. ^{1}H NMR (DMSO-d_6) δ 15.22 (1H, br s), 8.85 (1H, s), 8.24−8.18 (2H, m), 7.83 (1H, d, J = 8.1 Hz), 7.51−7.46 (1H, m), 7.43−7.38 (1H, m), 7.23−7.19 (1H, m), 5.19−5.10 (2H, m), 4.25 (2H, s), 3.86−3.71 (2H, m), 2.02−1.83 (2H, m), 1.35−1.16 (2H, m), 0.87 (3H, t, J = 7.4 Hz). MS (ESI) m/z 418 $(M + H)^+$. Anal. ($C_{22}H_{21}ClFNO_4$) C, H, N.

6-(3-Chloro-2-fluorobenzyl)-1-((2*S*)-1-hydroxy-3,3-dimethylbutan-2-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (45): 78% yield. Mp 216 °C. ^{1}H NMR (DMSO-d_6) δ 15.17 (1H, s), 8.81 (1H, s), 8.37 (1H, d, J = 9.3 Hz), 8.21 (1H, d, J = 1.9 Hz), 7.82 (1H, dd, J = 9.0, 2.1 Hz), 7.51−7.46 (1H, m), 7.43−7.38 (1H, m), 7.24−7.19 (1H, m), 5.13−5.07 (2H, m), 4.26 (2H, s), 4.12−4.00 (2H, m), 0.96 (9H, s). MS (ESI) m/z 432 $(M + H)^+$. Anal. ($C_{23}H_{23}ClFNO_4$) C, H, N.

6-(3-Chloro-2-fluorobenzyl)-1-((1*S*)-1-cyclohexyl-2-hydroxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (46): 62% yield. Mp 217 °C. ^{1}H NMR (DMSO-d_6) δ 15.05 (1H, br s), 8.95 (1H, s), 8.26 (1H, d, J = 9.1 Hz), 8.22 (1H, s), 7.85−7.80 (1H, m), 7.53−7.38 (2H, m), 7.26−7.19 (1H, m), 5.22−5.17 (1H, m), 4.94−4.87 (1H, m), 4.26 (2H, s), 4.04−3.94 (1H, m), 3.81−3.71 (1H, m), 2.11−1.88 (2H, m), 1.82−1.72 (1H, m), 1.64−1.49 (2H, m), 1.34−1.05 (5H, m), 0.96−0.82 (1H, m). MS (ESI) m/z 458 $(M + H)^+$. Anal. ($C_{25}H_{25}ClFNO_4$) C, H, N.

6-(3-Chloro-2-fluorobenzyl)-1-((1*S*)-1-phenyl-2-hydroxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (47): 35% yield. Mp 191−193 °C. ^{1}H NMR (DMSO-d_6) δ 15.11 (1H, br s), 9.03 (1H, s), 8.21 (1H, s), 8.03−7.95 (1H, m), 7.77−7.71 (1H, m), 7.50−7.43 (1H, m), 7.40−7.28 (6H, m), 7.23−7.17 (1H, m), 6.31−6.24 (1H, m), 5.59−5.53 (1H, m), 4.36−4.26 (1H, m), 4.23−4.16 (1H, m). MS (ESI) m/z 452 $(M + H)^+$. Anal. ($C_{25}H_{19}ClFNO_4$) C, H, N.

6-(3-Chloro-2-fluorobenzyl)-1-((2*S*)-1-hydroxypentan-2-yl)-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (48). A mixture of 1-((2*S*)-1-(*tert*-butyldimethylsilyloxy)pentan-2-yl)-6-(3-chloro-2-fluorobenzyl)-7-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (194 mg, 0.33 mmol), which was prepared from 2,4-difluoro-5-iodobenzoic acid and (*S*)-2-aminopentan-1-ol as described in steps 1−4 for the synthesis of **44**, and 28% sodium methoxide in methanol (3.0 mL) was heated to reflux for 3 h, cooled, and filtered through Celite. The filtrate was acidified with acetic acid, and the suspension was filtered. The solid was washed successively with ethanol/water (3/7 v/v) and *n*-hexane to give **48** (136 mg, 90%). Mp 201−202 °C. ^{1}H NMR (DMSO-d_6) δ 15.43 (1H, br s), 8.80 (1H, s), 8.03 (1H, s), 7.50−7.45 (1H, m), 7.44 (1H, s), 7.26−7.21 (1H, m), 7.20−7.15 (1H, m), 5.19 (2H, br s), 4.11 (2H, s), 4.02 (3H, s), 3.87−3.73 (2H, m), 1.99−1.86 (2H, m), 1.37−1.17 (2H, m), 0.89 (3H, t, J = 7.3 Hz). MS (ESI) m/z 448 $(M + H)^+$. Anal. ($C_{23}H_{23}ClFNO_5$) C, H, N.

The following compounds (**49**−**52**) were prepared using the above procedures.

6-(3-Chloro-2-fluorobenzyl)-1-((2*S*)-1-hydroxy-3-methylbutan-2-yl)-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (49): 88% yield. $[\alpha]_D^{25}$ −29.7° (*c* 1.03, MeOH). Mp 151−152 °C. ^{1}H NMR (DMSO-d_6) δ 15.43 (1H, br s), 8.87 (1H, s), 8.03 (1H, s), 7.50−7.44 (2H, m), 7.26−7.22 (1H, m), 7.21−7.16 (1H, m), 5.21−5.18 (1H, m), 4.88 (1H, br s), 4.11 (2H, s), 4.04 (4H, s), 4.01−3.95 (4H, m), 3.82−3.75 (1H, m), 2.45−2.32 (1H, m), 1.16 (3H, d, J = 6.5 Hz), 0.73 (3H, d, J = 6.5 Hz). MS (ESI) m/z 448 $(M + H)^+$. Anal. ($C_{23}H_{23}ClFNO_5$) C, H, N.

6-(3-Chloro-2-fluorobenzyl)-1-((2*R*)-1-hydroxy-3-methylbutan-2-yl)-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (50): 66% yield. Mp 150−151 °C. ^{1}H NMR (DMSO-d_6) δ 15.44 (1H, br s), 8.88 (1H, s), 8.04 (1H, s), 7.53−7.44 (2H, m), 7.29−7.16 (2H, m), 5.20 (1H, t, J = 5.3 Hz), 4.92−4.83 (1H, m), 4.12 (2H, s), 4.04 (3H, s), 4.00−3.94 (1H, m), 3.85−3.74 (1H, m), 2.44−2.32 (1H, m), 1.16 (3H, d, J = 6.4 Hz), 0.73 (3H, d, J = 6.4 Hz). MS (ESI) m/z 448 $(M + H)^+$. Anal. ($C_{23}H_{23}ClFNO_5$) C, H, N.

6-(3-Chloro-2-fluorobenzyl)-1-((2*S*)-1-hydroxy-3,3-dimethylbutan-2-yl)-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (51): 66% yield. Mp 149−152 °C. ^{1}H NMR (DMSO-d_6) δ 15.37 (1H, br s), 8.78 (1H, s), 8.02 (1H, s), 7.52 (1H, s), 7.50−7.46 (1H, m), 7.26−7.21 (1H, m), 7.21−7.16 (1H, m), 5.19−5.14 (1H, m), 5.11 (1H, t, J = 4.9 Hz), 4.11 (2H, s), 4.09−4.05 (2H, m), 4.03 (3H, s), 0.99 (9H, s). MS (ESI) m/z 462 $(M + H)^+$. Anal. ($C_{24}H_{25}ClFNO_5$) C, H, N.

6-(3-Chloro-2-fluorobenzyl)-1-((1*S*)-1-cyclohexyl-2-hydroxyethyl)-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (52): 73% yield. Mp 131−134 °C. ^{1}H NMR (DMSO-d_6) δ 15.45 (1H, br s), 8.87 (1H, s), 8.02 (1H, s), 7.52−7.38 (2H, m), 7.29−7.13 (2H, m), 5.22−5.14 (1H, m), 4.96−4.84 (1H, m), 4.10 (2H, s), 4.06−3.94 (1H, m), 4.03 (3H, s), 3.83−3.72 (1H, m), 2.10−1.92 (2H, m), 1.83−1.73 (1H, m), 1.65−1.51 (2H, m), 1.33−0.87 (6H, m). MS (ESI) m/z 488 $(M + H)^+$. Anal. ($C_{26}H_{27}ClFNO_5 \cdot 0.5H_2O$) C, H, N.

HIV-1 Strand Transfer Assay. Donor DNA (processed at the 3′ end of the strand and biotinylated at the 5′ end) was immobilized on streptavidin-coated microtiter plates. Recombinant integrase (300 nM) was assembled on the immobilized donor DNA (0.5 pmol per well) in 100 μL of reaction buffer (30 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS), 5 mM $MgCl_2$, 3 mM dithiothreitol (DTT), 0.1 mg/mL bovine serum albumin (BSA), 5% glycerol, 10% DMSO, 0.01% Tween-20) by incubation for 60 min at 37 °C. Then excess enzyme was removed and the test compound was added. The strand transfer reaction was initiated by the addition of target DNA (5 nM), which was labeled at the 3′ end with digoxigenin. After incubation at 37 °C for 10 min, the plates were washed with phosphate-buffered saline (PBS) containing 0.1% Tween-20. The digoxigenin-labeled products were detected using anti-digoxigenin-peroxidase (POD) Fab fragments (Roche Diagnostics) and a POD substrate, tetramethylbenzidine (TMB). Then 100 μL of anti-digoxigenin-POD Fab fragment solution was added to each well, and the plates were incubated at 37 °C for 60 min. After the samples were washed with PBS containing 0.1% Tween-20, an amount of 100 μL of the POD substrate (TMB) was added to each well, and the plates were incubated at room temperature. The colorimetric reaction was stopped by addition of 100 μL of 0.5 M H_2SO_4, and the absorbance was measured at 450 nm by a microplate reader (SPECTRA max 340, Molecular Devices).

Antiviral Assay. MT-4 human T lymphoid cells (1×10^5 cells/mL) in RPMI 1640 medium containing 10% fetal bovine serum, 100 U/mL of penicillin, and 100 μg/mL of streptomycin were infected with HIV-1 strain IIIb at a multiplicity of 0.01 and were

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distributed into 96-well microtiter plates. Test compounds were added to the wells, and cultures were incubated at 37 °C for 5 days. Cell viability was determined by the MTT assay, which measures living cells on the basis of mitochondrial dehydrogenase activity. The cells were incubated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (7.5 mg/mL). The MTT formazan crystals were dissolved in acidic isopropanol containing 4% Triton-X, and the absorbance was measured at 595 nm.

Cytotoxicity. MT-4 human T lymphoid cells (1×10^5 cells/mL) in RPMI 1640 medium containing 10% fetal bovine serum, 100 U/mL of penicillin, and 100 μg/mL of streptomycin were distributed into 96-well microtiter plates. Test compounds were added to the wells, and cultures were incubated at 37 °C for 5 days. Cell viability was determined by the MTT assay on the basis of mitochondrial dehydrogenase activity. The cells were incubated with MTT solution (7.5 mg/mL). The MTT formazan crystals were dissolved in acidic isopropanol containing 4% Triton-X and the absorbance was measured at 595 nm.

HIV-1 Integrase Assay. 21-Mer oliligonucleotide H-U5V1 (5′-ATG TGG AAA ATC TCT AGC AGT-3′, FASMAC Co., Ltd.) was 5′-end labeled using T4 polynucleotide kinase (Invitrogen Corporation) and [γ-^{32}P]ATP (GE Healthcare Bio-Sciences Corporation). The mixture (60 μL) of H-U5V1 (300 pM), T4 polynucleotide kinase (30 U), and [γ-^{32}P]ATP (5.55 MBq) in forward reaction buffer (Invitrogen Corporation) was incubated at 37 °C for 1 h and stopped by heating at 70 °C for 10 min. Unincorporated radioactive nucleotide was removed by gel filtration using a spin column (Micro Bio-Spin 6 column, Bio-Rad Laboratories, Inc.). After pre-equilibration of the column with TE buffer (pH 8.0), an amount of 60 μL of the reaction mixture was applied to the column, then the filtrate containing labeled H-U5V1 was collected by centrifugation. Labeled H-U5V1 was annealed with unlabeled H-U5V2 (5′-ACT GCT AGA GAT TTT CCA CAT-3′, FASMAC Co., Ltd.) at a ratio of 1:1.2. The mixture of the two oligonucleotides was heated at 95 °C for 5 min, then allowed to slowly cool to room temperature. The annealed DNA was used as the ^{32}P-labeled DNA substrate (21-mer). The mixture (20 μL) of compound **49**, ^{32}P-labeled 21-mer oligonucleotide (50 nM), and HIV-1 NL4-3 integrase (1.5 μM) in reaction buffer (20 mM MOPS (pH 7.2), 30 mM $MgCl_2$, 10 mM DTT, 0.1 mg/mL BSA, 5% glycerol, 0.02% Tween-20, and 10% DMSO) was incubated at 37 °C for 60 min. After incubation, the reaction mixtures were quickly chilled on ice and then mixed with 25 μL of 2× TBE urea sample buffer (Invitrogen Corporation). Reaction products were electrophoresed on 25% polyacrylamide gel with 5 M urea in TBE buffer at 450 V for approximately 200 min.

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Supporting Information Available: Combustion analysis data for **2**−**52** and NMR data of intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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