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Structural modifications of quinolone-3-carboxylic acids with anti-HIV activity

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ABSTRACT

A series of new quinolone-3-carboxylic acids featuring a hydroxyl group at C-5 position were synthesized and evaluated for their in vitro activity against HIV in C8166 cell culture. All the compounds showed anti-HIV-1 activity with low micromolar to submicromolar EC_{50} values. The most active compound **2k** exhibited activity against wild-type HIV-1 with an EC_{50} value of 0.13 μM. Preliminary structure–activity relationship of the newly synthesized quinolone analogues was also investigated. Further docking study revealed that the anti-HIV activity of these compounds might involve a two-metal chelating mechanism.

1. Introduction

Since its discovery in 2006, quinolone-3-carboxylic acids believed as one-metal chelating inhibitors of HIV-1 integrase (IN), have attracted a great deal of attention due to their high activity and favorable pharmacokinetic property.[1] Considerable structural modifications of quinolones focused on N-1, C-6, 7 and 8 position, have been introduced in order to obtain more potent and selective IN inhibitors. These efforts have led to the identification of many new quinolone or quinolone-like 3-carboxylic acids such as 4-oxoquinoline 3-carboxylic acids and 4-oxonaphthyridine 3-carboxylic acids with high anti-HIV activity (**1a**,**b**, Fig. 1).[1,2] However, chemical modifications at C-5 position are scarce.[2] This paper described a series of new 5-hydroxylquinolone-3-carboxylic acids (**2a–k**, Fig. 1) in an attempt to investigate whether the introduction of OH at C-5 position would provide an additional metal binding site and result in an improved activity against HIV-1.

2. Chemistry

The synthesis of the target compounds **2a–k** is outlined in Scheme 1. The iodination of 2,6-difluorobenzoic acid with *N*-iodobutanimide (NIS) in concd H_2SO_4 gave the corresponding aryl iodide **3**, which was subjected to acid-catalyzed esterification with methanol to afford benzoic ester **4**. The Negishi cross-coupling of **4** with 3-chloro-2-flurobenzylzinc bromide in the presence of $Pd(PPh_3)_4$ in THF/toluene and subsequent hydrolysis of the resulting coupling product **5** with satd aq LiOH yielded benzoic acid **6**. Treatment of the carbonyl diimidazole (CDI) actived **6** with potassium methylmalonate and anhydrous $MgCl_2$ produced β-ketoester **7**. Condensation of **7** with *N*,*N*-dimethylformamide dimethyl acetal (DMFDMA) and followed by substitution with

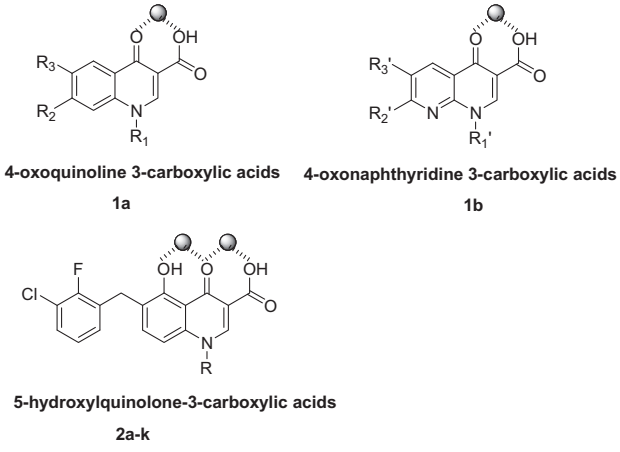

Figure 1. The structures of quinolone-3-carboxylic acids.

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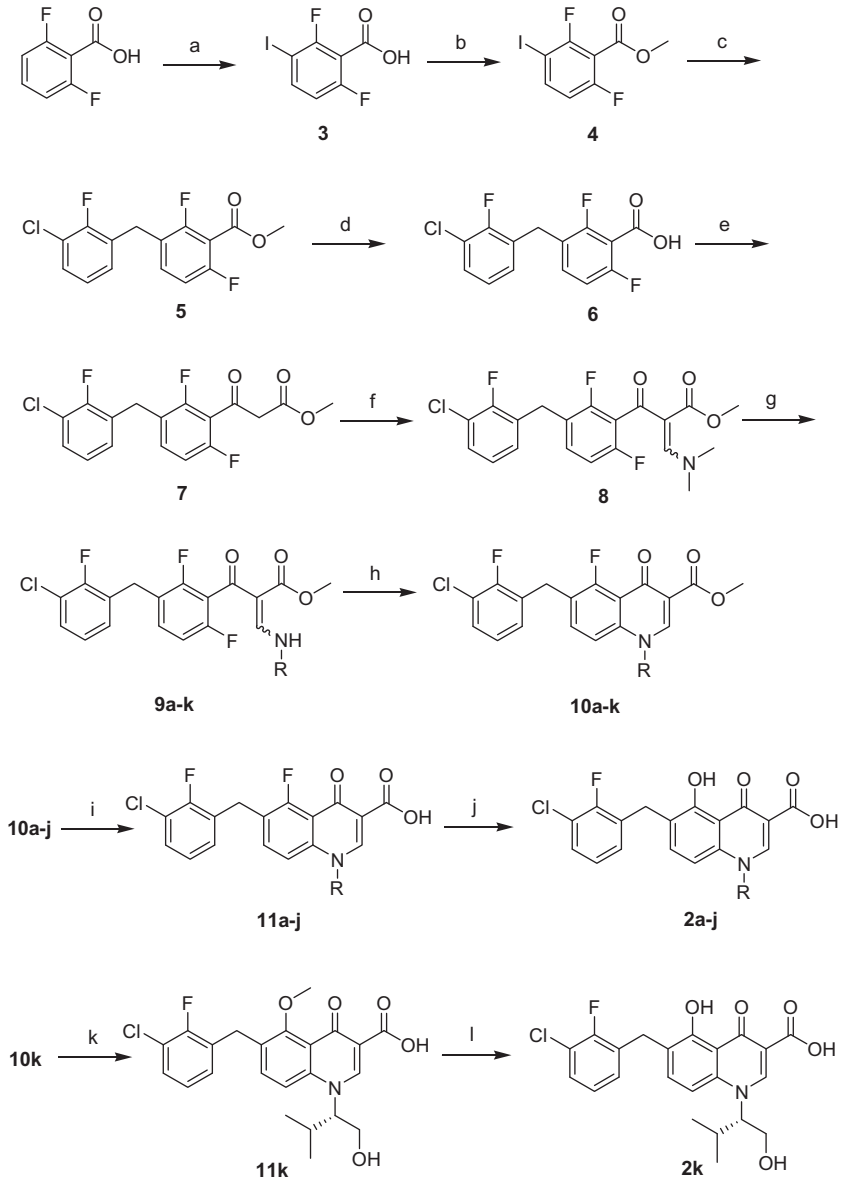

Scheme 1. Reagents and conditions: (a) NIS, concd H_2SO_4, 0 °C, 3.5 h; (b) MeOH, $SOCl_2$, reflux, 3 h; (c) (1) 3-chloro-2-flurobenzyl bromide, Zn, 1,2-dibromoethane, chlorotrimethylsilane, THF, 60 °C, 1 h, (2) $Pd(PPh_3)_4$, toluene, reflux, overnight; (d) satd aq LiOH, THF, 50 °C, 3 h; (e) (1) CDI, THF, rt, 2 h; (2) potassium methylmalonate, $MgCl_2$, THF, 60 °C, overnight; (f) DMFDMA, THF, 50 °C, 3 h; (g) aliphatic amines, THF, 50 °C, 5–10 min; (h) DBU, DMF, 60–90 °C, overnight; (i) satd aq LiOH, dioxane, 50 °C, 3 h; (j) 12.5 M NaOH, dioxane, 80 °C, 1–2 d. (k) MeONa, MeOH, reflux, 20 h. (l) 2 M BBr_3, CH_2Cl_2, −40 °C to rt, overnight.

appropriate aliphatic amines led to acrylates **9a–k**, which were then cyclized using DBU as a base to furnish quinolone esters **10a–k**. The target compounds **2a–j** were obtained from **10a–j** by ester hydrolysis and subsequent 5-OH incorporation under basic conditions. The target compound **2k** was synthesized from **10k** by methoxylation with sodium methoxide and subsequent demethylation with BBr_3.

3. Results and discussion

3.1. Biological evaluation

The synthesized compounds **2a–k** were evaluated by 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) assay[3] for cytotoxicity and antiviral activity in C8166 cells infected with the wild-type HIV-1 (LAI strain IIIB). Elviltegravir, currently being in phase III trials,[4] was included as reference compound. The cytotoxicity and antiviral activities of these compounds are listed in Table 1.

All of the target compounds **2a–k** exhibited anti-HIV-1 activity with low micromolar to submicromolar EC_{50} values in infected C8166 cells. Compounds **2a–c** bearing a straight alkyl chain of variable length showed significant decrease in activity with the length of the chain. The replacement of straight chain alkyl group of **2b** with branched ones (**2e–g**) led to an improvement of antiviral activity. The compound **2k**, characterized by a hydroxymethyl moiety at the 1*S*-position of the isobutyl group, appeared to be the most active one against HIV-1. The compounds with N-substituted cyclic groups **2h**, **2i** were more active than the corresponding ones with linear or branched moieties **2a**, **2c**, and **2d**. The compound **2g** with *t*-butyl group displayed no significant cytotoxicity with a CC_{50} of 400.17 μM.

Table 1
Anti-HIV-1 activity and cytotoxicity of compounds **2a**–**k** in C8166 cells[a]

Compd	R	EC_{50}[b] (μM)	CC_{50}[c] (μM)	SI[d]
2a	*n*-Propyl	2.79	24.17	8.66
2b	*n*-Butyl	8.09	19.95	2.47
2c	*n*-Pentyl	23.54	39.93	1.70
2d	*i*-Propyl	4.44	26.49	5.97
2e	*i*-Butyl	3.47	19.53	5.63
2f	*s*-Butyl	1.83	30.72	16.79
2g	*t*-Butyl	4.53	400.17	88.34
2h	Cyclopropyl	2.01	39.12	19.46
2i	Cyclopentyl	3.87	12.41	3.21
2j	Cyclohexyl	2.81	17.37	6.18
2k		0.13	125.03	961.77
Elvitegravir		0.00021	17.20	81904.76

[a] All data represent mean values from at least two separate experiments.
[b] EC_{50}: compound concentration required to protect the cell against viral cytopathogenicity by 50% in C8166 cells.
[c] CC_{50}: compound concentration that decreases the normal uninfected C8166 cell viability by 50%.
[d] SI: selectivity index; ratio CC_{50}/EC_{50}.

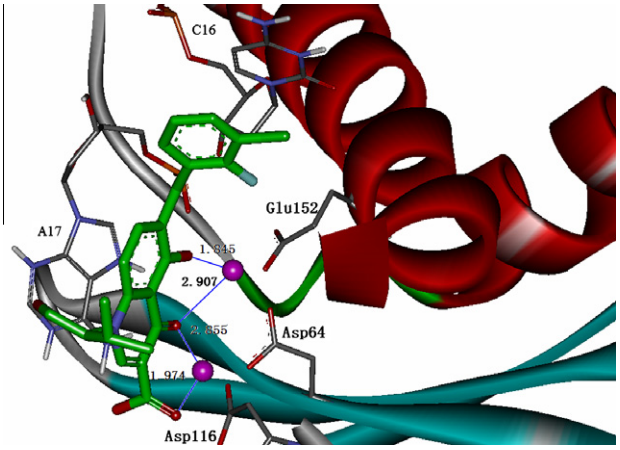

Figure 2. Binding model of compound **2k** in the active site of new homoly model of HIV-1 IN.

3.2. Molecular modeling calculations

In an attempt to determine the interaction between the hydroxyl group at C-5 position and HIV-1 IN, molecular docking study was performed.

Crystallographic analysis of inhibitors bound to the HIV IN/viral DNA complex continues to be a daunting challenge. Nevertheless, the recently reported crystal structures of DNA-bound retroviral IN[5] made it possible to model the HIV-1 intasome. The model of the HIV-1 catalytic core domain (CCD)/viral DNA complex was homology constructed using Discovery Studio 2.5 based on the original X-ray crystal structures of IN from prototype foamy virus (PFV) (pdb: 3OYA). Compound **2k**, as a representative of the target molecules, was docked into the HIV IN core domain using AUTODOCK 4.0 program. The docking result suggested that the C-5 hydroxyl, together with 4-ketone and 3-carboxylate in compound **2k** could form a two-Mg^{2+} chelation with HIV-1 IN as shown in Figure 2.

The binding mode of **2k** in the active site of IN showed that C-6 substituted benzyl group and quinolone ring exhibit π–π stacking interaction with C16 and A17, respectively. No interaction could be detected between the hydroxyalkyl group on N-1 of **2k** and IN, which indicates that this part of the molecule might contribute to favorable physicochemical properties of **2K** and lead to an improvement in antiviral activity.

4. Conclusion

In conclusion, we designed and synthesized a series of new 5-hydroxylquinolone-3-carboxylic acids. All the target compounds exhibited anti-HIV-1 activity with low micromolar to submicromolar EC_{50} values in infected C8166 cells. The most active compound **2k** exhibited activity against wild-type HIV-1 with an EC_{50} value of 0.13 μM. Further docking study revealed that the anti-HIV activity of these compounds might involve a two-metal chelating mechanism. However, in all cases, the measured activities were lower than that of Elvitegravir. This might be due to the introduction of C-5 hydroxyl group resulting in unfavorable physicochemical property or the lack of the influence of C-7 methoxyl group which together with N-1 hydroxyalkyl moiety could lead to a synergistic improvement of antiviral activity.[1] Further studies are ongoing to clarify the structure–activity relationship for this quinolone series.

5. Experimental

5.1. General procedures

Melting points were measured on a WRS-1 digital melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra on a Brucker AV 400 MHz spectrometer were recorded in $CDCl_3$. Chemical shifts are reported in δ (ppm) units relative to the internal standard tetramethylsilane (TMS). 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 TLC 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).

5.2. 2,6-Difluoro-3-iodobenzoic acid (3)

To a solution of 2,6-difluorobenzoic acid (1.58 g, 10.0 mmol) in concd H_2SO_4 (25 mL) was added NIS (2.25 g, 10.0 mmol) portionwise at 0 °C. After being stirred at 0 °C for 3.5 h, the mixture was poured onto crushed ice (10 g), and 3% Na_2SO_3 (10 mL) was added. The resulting slurry was stirred at 0 °C for 1 h, filtered, washed by water, dried to give the compound **3** as a white solid (2.05 g, 72%): ^{1}H NMR ($CDCl_3$) δ 6.78–6.82 (t, 1H, J = 8.4 Hz, ArH), 7.78–7.83 (m, 1H, ArH); GC–MS: 240 $[M-44]^+$.

5.3. Methyl 2,6-difluoro-3-iodobenzoate (4)

To a mixture of 2,6-difluoro-3-iodobenzoic acid **3** (2.84 g, 10.0 mmol) in methanol (30 mL) was added $SOCl_2$ (0.1 mL) dropwise at 0 °C. After being stirred at reflux for 3 h, the mixture was concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, petroleum ether/ethyl acetate 15:1 to 10:1, v/v) to give the desired compound **4** as a white solid (2.75 g, 92%): mp 54–55 °C (lit.[6] mp 53–55 °C); ^{1}H NMR ($CDCl_3$) δ 3.95 (s, 3H, CH_3), 6.78–6.82 (t, 1H, J = 8.4 Hz, ArH), 7.78–7.83 (m, 1H, ArH); ^{13}C NMR ($CDCl_3$) δ 53.10, 75.42, 75.46, 75.69, 75.73 (dd, J_{C-F} = 4.2, J'_{C-F} = 26.8 Hz), 111.49, 111.69, 111.89 (t, J_{C-F} = 19.8 Hz), 113.81, 113.84, 114.03, 114.07 (dd, J_{C-F} = 3.9, J'_{C-F} = 22.4 Hz), 141.42, 141.45, 141.51, 141.54 (dd, J_{C-F} = 3.1, J'_{C-F} = 9.4 Hz), 157.99, 158.05, 160.52, 160.58 (dd, J_{C-F} = 6.2, J'_{C-F} = 252.6 Hz), 159.48, 159.53, 162.04, 162.09 (dd, J_{C-F} = 5.1, J'_{C-F} = 256.2 Hz), 161.16; GC–MS: 298 M^+.

5.4. Methyl 3-(3-chloro-2-fluorobenzyl)-2,6-difluorobenzoate (5)

Under N_2, zinc powder (156 mg, 2.4 mmol) was suspended in THF (5 mL), then 1,2-dibromoethane (catalytic amount) and trimethylsilyl chloride (catalytic amount) were added at 60 °C. After being stirred for 30 min, a solution of 3-chloro-2-fluorobenzyl bromide (488 mg, 2.2 mmol) in THF (3 mL) was added dropwise at 60 °C. The mixture was stirred for further 1 h to give the solution of 3-chloro-2-fluorobenzylzinc bromide. To a mixture of methyl 2,6-difluoro-3-iodobenzoate **4** (596 mg, 2.0 mmol) and $Pd(PPh_3)_4$ (13 mg, 0.011 mmmol) in toluene (10 mL) was added the above solution of 3-chloro-2-fluorobenzylzinc bromide dropwise at 60 °C under N_2. After completion of the addition, the mixture was heated under reflux overnight. After allowing the mixture to cool, toluene (15 mL) and 20% aqueous NH_4Cl solution (10 mL) were added to the reaction solution, and the mixture was stirred and partitioned. The organic layer was washed twice with 20% aqueous NH_4Cl solution (5 mL) and twice with satd $NaHCO_3$ (5 mL), then dried over anhydrous $MgSO_4$. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, petroleum ether/ethyl acetate 40:1 to 25:1, v/v) to give the desired compound ***5*** as a colorless oil (290 mg, 46%): ^{1}H NMR ($CDCl_3$): δ 3.95 (s, 3H, CH_3), 4.00 (s, 2H, CH_2), 6.87–6.92 (td, 1H, J = 8.8, J'' = 1.6 Hz, ArH), 6.99–7.03 (m, 1H, ArH), 7.05–7.09 (m, 1H, ArH), 7.24–7.30 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$): δ 27.82, 27.85, 27.88 (t, J_{C-F} = 3.2 Hz), 52.79, 110.74, 110.93, 111.11 (t, J_{C-F} = 18.6 Hz), 111.77, 111.81, 111.99, 112.03 (dd, J_{C-F} = 4.1, J'_{C-F} = 21.7 Hz), 121.12, 121.30 (d, J_{C-F} = 17.9 Hz), 122.71, 122.75, 122.87, 122.91 (dd, J_{C-F} = 3.9, J'_{C-F} = 16.1 Hz), 124.65, 124.70 (d, J_{C-F} = 4.6 Hz), 127.48, 127.63 (d, J_{C-F} = 15.5 Hz), 129.16, 129.20 (d, J_{C-F} = 3.9 Hz), 129.23, 133.51, 133.57, 133.59, 133.66 (dd, J_{C-F} = 6.5, J'_{C-F} = 8.7 Hz), 155.15, 157.62 (d, J_{C-F} = 246.7 Hz), 157.04, 157.10, 159.58, 159.64 (dd, J_{C-F} = 6.2, J'_{C-F} = 254.6 Hz), 158.04, 158.10, 160.58, 160.64 (dd, J_{C-F} = 6.1, J'_{C-F} = 253.9 Hz), 162.07; MS (ESI) *m/z* 337 $[M+Na]^+$.

5.5. 3-(3-Chloro-2-fluorobenzyl)-2,6-difluorobenzoic acid (6)

To a solution of methyl 3-(3-chloro-2-fluorobenzyl)-2,6-difluorobenzoate **5** (315 mg, 1.0 mmol) in THF (8 mL) was added satd aq LiOH (5 mL). 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 ~2. The precipitate was filtered off, washed by water, and dried to afford **6** as a white solid. This crude was used directly for the next step without further purification.

5.6. Methyl 3-(3-(3-chloro-2-fluorobenzyl)-2,6-difluorophenyl)-3-oxo-propanoate (7)

To a suspension of CDI (389 mg, 2.4 mmol) in THF (5 mL) was added a solution of 3-(3-chloro-2-fluorobenzyl)-2,6-difluorobenzoic acid **6** (602 mg, 2.0 mmol) in THF (5 mL) dropwise, the mixture was stirred at rt for 2 h. After a mixture of potassium 3-methoxy-3-oxopropanoate (374 mg, 2.4 mmol) and $MgCl_2$ (228 mg, 2.4 mmol) in THF (10 mL) stirring at 50 °C for 2 h was added the above solution. The resulting mixture was stirred at 50 °C overnight, filtrated. The filtrate was diluted with water (5 mL) and acidified with 4 M HCl to pH 5~6. The mixture was extracted with ethyl acetate (15 mL) and washed with water (5 mL), satd aq $NaHCO_3$ (5 mL), and brine (5 mL), then dried over $MgSO_4$. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, petroleum ether/ethyl acetate 20:1 to 15:1, v/v) to give the desired compound **7** as a colorless oil (320 mg, 45%): keto form/enol form = 2.3:1; keto form ^{1}H NMR ($CDCl_3$) δ 3.75 (s, 3H, CH_3), 3.95 (s, 2H, CH_2), 4.03 (s, 2H, CH_2), 6.91–6.96 (m, 1H, ArH), 7.02–7.12 (m, 2H, ArH), 728–7.34 (m, 2H, ArH); Enol form ^{1}H NMR ($CDCl_3$) δ 3.83 (s, 1.3H, CH_3), 4.03 (s, 0.9H, CH_2), 5.44 (s, 0.4H, OH), 6.89–6.96 (m, 0.4H, ArH), 7.02–7.12 (m, 0.9H, ArH), 7.21–7.34 (m, 0.9H, ArH), 12.31 (s, 0.4H, CH); MS (ESI) *m/z* 357 $[M+H]^+$.

5.7. Methyl 2-(3-(3-chloro-2-fluorobenzyl)-2,6-difluorobenzoyl)-3-(dimethylamino)acrylate (8)

A mixture of methyl 3-(3-(3-chloro-2-fluorobenzyl)-2,6-difluorophenyl)-3-oxopropanoate **7** (320 mg, 0.9 mmol) and DMFMDA (129 mg, 1.1 mmol) in THF (10 mL) was heated at 50 °C for 3 h. The reaction mixture was cooled to room temperature and then concentrated under reduced pressure. The residue was used directly for the next step without further purification.

5.8. General procedure for the preparation of 9a–j

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

5.9. General procedure for the preparation of 10a–j

A mixture of **9a–j** (1.5 mmol) and DBU (2.3 mmol) in DMF (10 mL) was stirred at 60–90 °C overnight, then cooled down and poured into ice-water. The mixture was extracted by dichloromethane (5 mL × 3). The combined organic solution was washed with brine (5 mL × 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–j**.

5.9.1. Methyl 6-(3-chloro-2-fluorobenzyl)-5-fluoro-4-oxo-1-propyl-1,4-dihydroquinoline-3-carboxylate (10a)

Yield 54%. ^{1}H NMR ($CDCl_3$) δ 1.02–1.06 (t, 3H, J = 7.2 Hz, CH_3), 1.89–1.95 (m, 2H, CH_2), 3.94 (s, 3H, OCH_3), 4.08–4.12 (t, 2H, J = 7.2 Hz, CH_2), 4.13 (s, 2H, CH_2), 7.01–7.05 (t, 1H, J = 8.0 Hz, ArH), 7.14–7.16 (d, 1H, J = 9.2 Hz, ArH), 7.18–7.21 (m, 1H, ArH), 7.28–7.31 (m, 1H, ArH), 7.49–7.53 (m, 1H, ArH), 8.41 (s, 1H, CH).

5.9.2. Methyl 6-(3-chloro-2-fluorobenzyl)-1-butyl-5-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (10b)

Yield 61%. ^{1}H NMR ($CDCl_3$) δ 0.97–1.00 (t, 3H, J = 7.2 Hz, CH_3), 1.37–1.45 (m, 2H, CH_2), 1.80–1.85 (m, 2H, CH_2), 3.91 (s, 3H, OCH_3), 4.08–4.12 (t, 2H, J = 7.4 Hz, CH_2), 4.10 (s, 2H, CH_2), 6.98–7.02 (t, 1H, J = 8.0 Hz, ArH), 7.12–7.14 (d, 1H, J = 9.2 Hz, ArH), 7.16–7.19 (m, 1H, ArH), 7.24–7.28 (m, 1H, ArH), 7.46–7.50 (m, 1H, ArH), 8.37 (s, 1H, CH).

5.9.3. Methyl 6-(3-chloro-2-fluorobenzyl)-1-butyl-5-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (10c)

Yield 63%. ^{1}H NMR ($CDCl_3$) δ 0.91–0.94 (t, 3H, J = 6.8 Hz, CH_3), 1.37–1.39 (m, 4H, $2CH_2$), 1.84–1.86 (m, 2H, CH_2), 3.92 (s, 3H, OCH_3), 4.09–4.13 (t, 2H, J = 7.4 Hz, CH_2), 4.11 (s, 2H, CH_2), 7.00–7.04 (t, 1H, J = 8.0 Hz, ArH), 7.13–7.15 (d, 1H, J = 9.2 Hz, ArH), 7.16–7.20 (m, 1H, ArH), 7.26–7.29 (m, 1H, ArH), 7.47–7.50 (m, 1H, ArH), 8.38 (s, 1H, CH).

5.9.4. Methyl 6-(3-chloro-2-fluorobenzyl)-5-fluoro-1-*iso*-propyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (10d)

Yield 62%. ^{1}H NMR ($CDCl_3$) δ 1.56–1.57 (d, 6H, J = 6.4 Hz, $2CH_3$), 3.90 (s, 3H, OCH_3), 4.09 (s, 2H, CH_2), 4.77–4.80 (m, 1H, CH),

6.96–7.00 (t, 1H, J = 8.0 Hz, ArH), 7.13–7.17 (m, 1H, ArH), 7.22–7.29 (m, 2H, ArH), 7.45–7.47 (m, 1H, ArH), 8.53 (s, 1H, CH).

5.9.5. Methyl 6-(3-chloro-2-fluorobenzyl)-5-fluoro-1-*iso*-butyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (10e)

Yield 71%. ^{1}H NMR ($CDCl_3$) δ 0.95–0.96 (d, 6H, J = 6.4 Hz, $2CH_3$), 2.16–2.23 (m, 1H, CH), 3.86 (s, 3H, OCH_3), 3.86–3.88 (d, 2H, J = 8.4 Hz, CH_2), 4.05 (s, 2H, CH_2), 6.95–6.99 (t, 1H, J = 8.0 Hz, ArH), 7.07–7.09 (d, 1H, J = 8.8 Hz, ArH), 7.11–7.15 (m, 1H, ArH), 7.21–7.25 (m, 1H, ArH), 7.40–7.44 (m, 1H, ArH), 8.29 (s, 1H, CH).

5.9.6. Methyl 6-(3-chloro-2-fluorobenzyl)-5-fluoro-1-*sec*-butyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (10f)

Yield 68%. ^{1}H NMR ($CDCl_3$) δ 1.82–1.92 (m, 6H, $2CH_3$), 2.23–2.26 (m, 2H, CH_2), 3.87 (s, 3H, OCH_3), 4.05 (s, 2H, CH_2), 4.78–4.81 (m, 1H, CH), 6.94–6.98 (t, 1H, J = 8.0 Hz, ArH), 7.10–7.14 (m, 1H, ArH), 7.20–7.24 (m, 1H, ArH), 7.29–7.31 (d, 1H, J = 8.8 Hz, ArH), 7.41–7.45 (m, 1H, ArH), 8.48 (s, 1H, CH).

5.9.7. Methyl 6-(3-chloro-2-fluorobenzyl)-5-fluoro-1-*tert*-butyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (10g)

Yield 57%. ^{1}H NMR ($CDCl_3$) δ 1.84 (s, 9H, $3CH_3$), 3.91 (s, 3H, OCH_3), 4.10 (s, 2H, CH_2), 6.99–7.03 (t, 1H, J = 8.0 Hz, ArH), 7.17–7.20 (m, 1H, ArH), 7.24–7.29 (m, 1H, ArH), 7.41–7.45 (m, 1H, ArH), 7.59–7.61 (d, 1H, J = 8.8 Hz, ArH), 8.80 (s, 1H, CH).

5.9.8. Methyl 6-(3-chloro-2-fluorobenzyl)-5-fluoro-1-cyclopropyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (10h)

Yield 45%. ^{1}H NMR ($CDCl_3$) δ 1.10–1.14 (m, 2H, CH_2), 1.28–1.35 (m, 2H, CH_2), 3.38–3.43 (m, 1H, CH), 3.92 (s, 3H, OCH_3), 4.12 (s, 2H, CH_2), 7.00–7.04 (t, 1H, J = 8.0 Hz, ArH), 7.17–7.20 (m, 1H, ArH), 7.26–7.30 (m, 1H, ArH), 7.50–7.54 (m, 1H, ArH), 7.65–7.67 (d, 1H, J = 8.8 Hz, ArH), 8.52 (s, 1H, CH).

5.9.9. Methyl 6-(3-chloro-2-fluorobenzyl)-5-fluoro-1-cyclopentyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (10i)

Yield 55%. ^{1}H NMR ($CDCl_3$) δ 1.87–1.99 (m, 6H, $3CH_2$), 2.28–2.33 (m, 2H, CH_2), 3.94 (s, 3H, OCH_3), 4.13 (s, 2H, CH_2), 4.82–4.85 (m, 1H, CH), 7.00–7.04 (t, 1H, J = 8.0 Hz, ArH), 7.17–7.21 (m, 1H, ArH), 7.26–7.30 (m, 1H, ArH), 7.33–7.36 (d, 1H, J = 8.8 Hz, ArH), 7.49–7.53 (m, 1H, ArH), 8.55 (s, 1H, CH).

5.9.10. Methyl 6-(3-chloro-2-fluorobenzyl)-5-fluoro-1-cyclohexyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (10j)

Yield 50%. ^{1}H NMR ($CDCl_3$) δ 1.25–1.85 (m, 4H, $2CH_2$), 2.00–2.14 (m, 4H, $2CH_2$), 3.90 (s, 3H, OCH_3), 4.09 (s, 2H, CH_2), 4.24–4.31 (m, 1H, CH), 6.97–7.01 (t, 1H, J = 8.0 Hz, ArH), 7.14–7.17 (m, 1H, ArH), 7.24–7.26 (m, 2H, ArH), 7.45–7.49 (m, 1H, ArH), 8.54 (s, 1H, CH).

5.9.11. (*S*)-Methyl 6-(3-chloro-2-fluorobenzyl)-5-fluoro-1-(1-hydroxy-3-methylbutan-2-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (10k)

Yield 62%. ^{1}H NMR ($CDCl_3$) δ 0.74–0.76 (d, 3H, J = 6.8 Hz, CH_3), 1.22–1.23 (d, 3H, J = 6.0 Hz, CH_3), 2.52–2.57 (m, 1H, CH), 3.72–3.90 (m, 2H, CH_2), 3.82 (s, 3H, OCH_3), 4.13–4.18 (m, 2H, CH_2), 4.28–4.32 (m, 1H, CH), 5.38–5.41 (t, 1H, J = 6.8 Hz, OH), 6.93–6.97 (dd, 1H, $J = J'$ = 8.0 Hz, ArH), 6.99–7.02 (m, 1H, ArH), 7.22–7.25 (m, 1H, ArH), 7.34–7.37 (d, 1H, J = 8.8 Hz, ArH), 7.39–7.43 (m, 1H, ArH), 8.59 (s, 1H, CH).

5.10. General procedure for the preparation of 11a–j

To a solution of satd aq LiOH (5 mL) in dioxane (8 mL) was added **10a–j** (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 ~2. The resulting precipitate was collected by filtration, washed by water, and then dried to give the crude desired compound **11a–j**. This crude was used directly for the next step without further purification.

5.11. (*S*)-6-(3-Chloro-2-fluorobenzyl)-1-(1-hydroxy-3-methylbutan-2-yl)-5-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (11k)

To a solution of MeONa (2.16 g, 40 mmol) in methanol (20 mL) was added **10k** (450 mg, 1.0 mmol) and one drop of water. After being stirred at reflux for 20 h, the mixture was cooled, poured into ice-water and acidified with 4 M HCl to pH ~2, extracted by dichloromethane (10 mL × 3) and concentrated. The residue was purified by column chromatography (silica gel, dichloromethane/methanol 60:1 to 40:1, v/v) to give the desired compound **11k** as a white solid (390 mg, 87%): ^{1}H NMR ($CDCl_3$) δ 0.82–0.84 (d, 3H, J = 6.4 Hz, CH_3), 1.20–1.22 (d, 3H, J = 6.8 Hz, CH_3), 2.44–2.46 (m, 1H, CH), 3.48–3.53 (m, 1H, OH), 3.94 (s, 3H, OCH_3), 4.13–4.22 (m, 4H, $2CH_2$), 4.52–4.53 (m, 1H, CH), 7.01–7.05 (dd, 1H, $J = J'$ = 8.0 Hz, ArH), 7.13–7.16 (m, 1H, ArH), 7.28–7.31 (m, 1H, ArH), 7.51–7.53 (d, 1H, J = 9.2 Hz, ArH), 7.60–7.62 (d, 1H, J = 9.2 Hz, ArH), 8.86 (s, 1H, CH), 15.80 (s, 1H, COOH).

5.12. General procedure for the preparation of 2a–j

To a solution of 12.5 N NaOH (5 mL) in dioxane (8 mL) was added **11a–j** (1.2 mmol). After being stirred at 80 °C for 1 to 2 days, the mixture was cooled, poured into water and acidified with 4 M HCl to pH ~2, then extracted by dichloromethane (5 mL × 3). The combined organic solution was washed with water (5 mL × 2), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, dichloromethane) to afford the desired compound **2a–j**.

5.12.1. 6-(3-Chloro-2-fluorobenzyl)-5-hydroxy-4-oxo-1-propyl-1,4-dihydroquinoline-3-carboxylic acid (2a)

Yield 45%. Mp 207–209 °C; ^{1}H NMR ($CDCl_3$) δ 1.01–1.04 (t, 3H, J = 7.4 Hz, CH_3), 1.93–1.98 (m, 2H, CH_2), 4.10 (s, 2H, CH_2), 4.19–4.22 (t, 2H, J = 7.4 Hz, CH_2), 6.92–6.94 (d, 1H, J = 8.4 Hz, ArH), 6.99–7.02 (t, 1H, J = 8.0 Hz, ArH), 7.21–7.28 (m, 2H, ArH), 7.57–7.59 (d, 1H, J = 8.4 Hz, ArH), 8.68 (s, 1H, CH), 13.35 (s, 1H, OH), 13.70 (s, 1H, COOH); ^{13}C NMR ($CDCl_3$): δ = 10.98, 22.02, 28.36, 28.38 (d, J_{C-F} = 2.5 Hz), 56.93, 105.43, 107.13, 113.42, 120.93, 121.11 (d, J_{C-F} = 18.0 Hz), 123.17, 124.46, 124.51 (d, J_{C-F} = 4.6 Hz), 128.32, 128.48 (d, J_{C-F} = 15.5 Hz), 128.85, 129.78, 129.82 (d, J_{C-F} = 4.0 Hz), 136.78, 138.88, 148.83, 155.34, 157.80 (d, J_{C-F} = 246.0 Hz), 160.00, 165.92, 181.62. MS (ESI) m/z 390 $[M+H]^+$. Anal. Calcd for $C_{20}H_{17}ClFNO_4$: C, 61.62; H, 4.40; N, 3.59. Found: C, 61.37; H, 4.64; N, 3.81.

5.12.2. 6-(3-Chloro-2-fluorobenzyl)-1-butyl-5-hydroxy-4-oxo-1,4-dihydro quinoline-3-carboxylic acid (2b)

Yield 41%. Mp 190–192 °C; ^{1}H NMR ($CDCl_3$) δ 0.97–1.01 (t, 3H, J = 7.4 Hz, CH_3), 1.38–1.48 (m, 2H, CH_2), 1.85–1.92 (m, 2H, CH_2), 4.10 (s, 2H, CH_2), 4.22–4.25 (t, 2H, J = 7.4 Hz, CH_2), 6.93–6.95 (d, 1H, J = 8.8 Hz, ArH), 6.99–7.03 (t, 1H, J = 8.0 Hz, ArH), 7.21–7.28 (m, 2H, ArH), 7.56–7.59 (d, 1H, J = 8.8 Hz, ArH), 8.67 (s, 1H, CH), 13.34 (s, 1H, OH), 13.71 (s, 1H, COOH); ^{13}C NMR (100 MHz, $CDCl_3$-d_3): δ 13.53, 19.91, 28.38, 28.41 (d, J_{C-F} = 2.4 Hz), 30.66, 55.32, 105.42, 107.18, 113.44, 120.92, 121.11 (d, J_{C-F} = 18.3 Hz), 123.17, 124.47, 124.52 (d, J_{C-F} = 4.6 Hz), 128.32, 128.47 (d, J_{C-F} = 15.4 Hz), 128.87, 129.80, 129.84 (d, J_{C-F} = 3.8 Hz), 136.78, 138.88, 148.76, 155.34, 157.80 (d, J_{C-F} = 246.0 Hz), 160.01, 165.97, 181.61; MS (ESI) m/z 404 $[M+H]^+$. Anal. Calcd for $C_{21}H_{19}ClFNO_4$: C, 62.46; H, 4.74; N, 3.47. Found: C, 62.24; H, 5.02; N, 3.21.

5.12.3. 6-(3-Chloro-2-fluorobenzyl)-5-hydroxy-4-oxo-1-pentyl-1,4-dihydro quinoline-3-carboxylic acid (2c)

Yield 32%. Mp: 185–187 °C; ^{1}H NMR ($CDCl_3$) δ 0.90–0.93 (t, 3H, J = 6.8 Hz, CH_3), 1.37–1.39 (m, 4H, $2CH_2$), 1.88–1.92 (m, 2H, CH_2), 4.09 (s, 2H, CH_2), 4.21–4.25 (t, 2H, J = 7.6 Hz, CH_2), 6.92–6.94 (d, 1H, J = 8.8 Hz, ArH), 6.98–7.02 (t, 1H, J = 8.0 Hz, ArH), 7.21–7.27 (m, 2H, ArH), 7.56–7.58 (d, 1H, J = 8.8 Hz, ArH), 8.67 (s, 1H, CH), 13.31 (s, 1H, OH), 13.68 (s, 1H, COOH); ^{13}C NMR ($CDCl_3$) δ 13.84, 22.18, 28.35, 28.38 (d, J_{C-F} = 2.8 Hz), 28.43, 28.67, 55.55, 105.45, 107.15, 113.40, 120.91, 121.09 (d, J_{C-F} = 18.0 Hz), 123.13, 124.47, 124.51 (d, J_{C-F} = 4.7 Hz), 128.32, 128.47 (d, J_{C-F} = 15.5 Hz), 128.85, 129.79, 129.83 (d, J_{C-F} = 4.0 Hz), 136.77, 138.87, 148.76, 155.33, 157.79 (d, J_{C-F} = 246.0 Hz), 159.97, 165.94, 181.57; MS (ESI) m/z 418 $[M+H]^+$. Anal. Calcd for $C_{22}H_{21}ClFNO_4$: C, 63.24; H, 5.07; N, 3.35. Found: C, 63.01; H, 5.29; N, 3.08.

5.12.4. 6-(3-Chloro-2-fluorobenzyl)-5-hydroxy-1-isopropyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (2d)

Yield 43%. Mp: 190–192 °C; ^{1}H NMR ($CDCl_3$) δ 1.62–1.64 (d, 6H, J = 6.8 Hz, $2CH_3$), 4.10 (s, 2H, CH_2), 4.90–4.97 (m, 1H, CH), 6.98–7.02 (t, 1H, J = 7.6 Hz, ArH), 7.06–7.08 (d, 1H, J = 8.8 Hz, ArH), 7.21–7.28 (m, 2H, ArH), 7.57–7.59 (d, 1H, J = 8.8 Hz,ArH), 8.83 (s, 1H, CH), 13.49 (s, 1H, OH), 13.75 (s, 1H, COOH); ^{13}C NMR ($CDCl_3$) δ 22.16, 28.35, 28.38 (d, J_{C-F} = 2.9 Hz), 53.01, 104.74, 107.42, 113.49, 120.91, 121.10 (d, J_{C-F} = 18.2 Hz), 123.05, 124.46, 124.50 (d, J_{C-F} = 4.6 Hz), 128.36, 128.51 (d, J_{C-F} = 15.4 Hz), 128.84, 129.77, 129.81 (d, J_{C-F} = 4.0 Hz), 136.72, 139.37, 144.14, 155.34, 157.80 (d, J_{C-F} = 245.9 Hz), 160.23, 166.10, 181.33; MS (ESI) m/z 390 $[M+H]^+$. Anal. Calcd for $C_{20}H_{17}ClFNO_4$: C, 61.62; H, 4.40; N, 3.59. Found: C, 61.87; H, 4.12; N, 3.86.

5.12.5. 6-(3-Chloro-2-fluorobenzyl)-5-hydroxy-1-isobutyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (2e)

Yield 41%. Mp: 195–196 °C; ^{1}H NMR ($CDCl_3$) δ 0.99–1.01 (d, 6H, J = 6.8 Hz, $2CH_3$), 2.26–2.32 (m, 1H, CH), 4.02–4.04 (d, 2H, J = 7.6 Hz, CH_2), 4.09 (s, 2H, CH_2), 6.89–6.92 (d, 1H, J = 8.8 Hz, ArH), 6.98–7.02 (t, 1H, J = 8.0 Hz, ArH), 7.21–7.27 (m, 2H, ArH), 7.55–7.57 (d, 1H, J = 8.8 Hz, ArH), 8.62 (s, 1H, CH), 13.33 (s, 1H, OH), 13.68 (s, 1H, COOH); ^{13}C NMR ($CDCl_3$): δ = 19.80, 27.39, 28.36, 28.39 (d, J_{C-F}=2.8 Hz), 28.39, 62.65, 105.66, 106.89, 113.39, 120.92, 121.10 (d, J_{C-F} = 17.9 Hz), 123.17, 124.47, 124.51 (d, J_{C-F} = 4.6 Hz), 128.30, 128.46 (d, J_{C-F} = 15.4 Hz), 128.86, 129.80, 129.84 (d, J_{C-F} = 3.9 Hz), 136.69, 139.04, 149.15, 155.34, 157.80 (d, J_{C-F} = 246.1 Hz), 159.95, 165.88, 181.63; MS (ESI) m/z 404 $[M+H]^+$. Anal. Calcd for $C_{21}H_{19}ClFNO_4$: C, 62.46; H, 4.74; N, 3.47. Found: C, 62.25; H, 4.51; N, 3.66.

5.12.6. 6-(3-Chloro-2-fluorobenzyl)-1-*sec*-butyl-5-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (2f)

Yield 40%. Mp: 161–163 °C; ^{1}H NMR ($CDCl_3$) δ 0.94–0.97 (t, 3H, J = 7.2 Hz, CH_3), 1.59–1.60 (d, 3H, J = 6.8 Hz, CH_3), 1.91–2.02 (m, 2H, CH_2), 4.10 (s, 2H, CH_2), 4.67–4.72 (m, 1H, CH), 6.98–7.02 (t, 1H, J = 8.0 Hz, ArH), 7.05–7.07 (d, 1H, J = 8.8 Hz, ArH), 7.21–7.27 (m, 2H, ArH), 7.56–7.58 (d, 1H, J = 8.4 Hz, ArH), 8.75 (s, 1H, CH), 13.50 (s, 1H, OH), 13.75 (s, 1H, COOH); ^{13}C NMR ($CDCl_3$) δ 10.48, 20.13, 28.35, 28.38 (d, J_{C-F} = 2.8 Hz), 29.06, 67.11, 104.67, 107.41, 113.50, 120.91, 121.09 (d, J_{C-F} = 18.0 Hz), 122.99, 124.45, 124.50 (d, J_{C-F} = 4.7 Hz), 128.36, 128.51 (d, J_{C-F} = 15.4 Hz), 128.84, 129.79, 129.83 (d, J_{C-F} = 4.0 Hz), 136.69, 136.71 (d, J_{C-F} = 2.0 Hz), 139.82, 145.67, 155.34, 157.80 (d, J_{C-F} = 246.0 Hz), 160.21, 166.06, 181.27; MS (ESI) m/z 404 $[M+H]^+$. Anal. Calcd for $C_{21}H_{19}ClFNO_4$: C, 62.46; H, 4.74; N, 3.47. Found: C, 62.71; H, 4.98; N, 3.25.

5.12.7. 6-(3-Chloro-2-fluorobenzyl)-1-*tert*-butyl-5-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (2g)

Yield 31%. Mp: 234–235 °C; ^{1}H NMR ($CDCl_3$) δ 1.90 (s, 9H, $3CH_3$), 4.10 (s, 2H, CH_2), 6.99–7.03 (t, 1H, J = 7.6 Hz, ArH), 7.23–7.28 (m, 2H, ArH), 7.38–7.40 (d, 1H, J = 8.8 Hz, ArH), 7.51–7.53 (d, 1H, J = 9.2 Hz, ArH), 9.06 (s, 1H, CH), 13.74 (s, 2H, OH, COOH); ^{13}C NMR ($CDCl_3$) δ 28.24, 28.27 (d, J_{C-F} = 2.8 Hz), 30.68, 65.49, 106.42, 109.84, 114.50, 120.92, 121.10 (d, J_{C-F} = 18.0 Hz), 122.79, 124.47, 124.51 (d, J_{C-F} = 4.5 Hz), 128.35, 128.51 (d, J_{C-F} = 15.5 Hz), 128.84, 129.86, 129.90 (d, J_{C-F} = 3.8 Hz), 135.03, 138.97, 146.07, 155.37, 157.83 (d, J_{C-F} = 246.3 Hz), 160.46, 166.40, 181.21; MS (ESI) m/z 404 $[M+H]^+$. Anal. Calcd for $C_{21}H_{19}ClFNO_4$: C, 62.46; H, 4.74; N, 3.47. Found: C, 62.67; H, 4.52; N, 3.28.

5.12.8. 6-(3-Chloro-2-fluorobenzyl)-1-cyclopropyl-5-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (2h)

Yield 32%. Mp: 208–209 °C; ^{1}H NMR ($CDCl_3$) δ 1.20–1.21 (m, 2H, CH_2), 1.40–1.41 (m, 2H, CH_2), 3.54–3.56 (m, 1H, CH), 4.13 (s, 2H, CH_2), 7.01–7.05 (t, 1H, J = 8.0 Hz, ArH), 7.23–7.28 (m, 2H, ArH), 7.42–7.44 (d, 1H, J = 8.4 Hz, ArH), 7.62–7.64 (d, 1H, J = 8.4 Hz, ArH), 8.84 (s, 1H, CH), 13.20 (s, 1H, OH), 13.70 (s, 1H, COOH); ^{13}C NMR ($CDCl_3$) δ 8.23, 28.41, 28.43 (d, J_{C-F} = 2.4 Hz), 36.01, 106.27, 107.23, 112.80, 120.92, 121.11 (d, J_{C-F} = 18.2 Hz), 123.32, 124.46, 124.51 (d, J_{C-F} = 4.4 Hz), 128.37, 128.53 (d, J_{C-F} = 15.5 Hz), 128.85, 129.76, 129.80 (d, J_{C-F} = 4.0 Hz), 136.78, 140.61, 148.64, 155.34, 157.80 (d, J_{C-F} = 246.0 Hz), 159.66, 165.82, 181.14; MS (ESI) m/z 388 $[M+H]^+$. Anal. Calcd for $C_{20}H_{15}ClFNO_4$: C, 61.94; H, 3.90; N, 3.61. Found: C, 61.74; H, 4.19; N, 3.38.

5.12.9. 6-(3-Chloro-2-fluorobenzyl)-1-cyclopentyl-5-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (2i)

Yield 30%. Mp: 170–171 °C; ^{1}H NMR ($CDCl_3$): δ 1.86–2.03 (m, 6H, $3CH_2$), 2.31–2.38 (m, 2H, CH_2), 4.10 (s, 2H, CH_2), 4.92–4.98 (m, 1H, CH), 6.98–7.02 (t, 1H, J = 8.0 Hz, ArH), 7.10–7.12 (d, 1H, J = 8.8 Hz, ArH), 7.20–7.28 (m, 2H, ArH), 7.56–7.58 (d, 1H, J = 8.8 Hz, ArH), 8.79 (s, 1H, CH), 13.47 (s, 1H, OH), 13.74 (s, 1H, COOH); ^{13}C NMR ($CDCl_3$) δ 23.69, 28.33, 28.36 (d, J_{C-F} = 2.8 Hz), 32.41, 63.21, 105.67, 107.03, 113.45, 120.90, 121.08 (d, J_{C-F} = 18.0 Hz), 123.05, 124.45, 124.50 (d, J_{C-F} = 4.7 Hz), 128.37, 128.53 (d, J_{C-F} = 15.4 Hz), 128.82, 129.76, 129.80 (d, J_{C-F} = 4.0 Hz), 136.55, 136.57 (d, J_{C-F} = 1.6 Hz), 139.91, 144.47, 155.32, 157.79 (d, J_{C-F} = 246.2 Hz), 160.01, 166.09, 181.28; MS (ESI) m/z 416 $[M+H]^+$. Anal. Calcd for $C_{22}H_{19}ClFNO_4$: C, 63.54; H, 4.61; N, 3.37. Found: C, 63.31; H, 4.85; N, 3.58.

5.12.10. 6-(3-Chloro-2-fluorobenzyl)-1-cyclohexyl-5-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (2j)

Yield 31%. Mp: 178–179 °C; ^{1}H NMR ($CDCl_3$) δ 1.25–2.19 (m, 10H, $5CH_2$), 4.08 (s, 2H, CH_2), 4.41–4.47 (m, 1H, CH), 6.97–7.01 (t, 1H, J = 8.0 Hz, ArH), 7.04–7.06 (d, 1H, J = 8.8 Hz, ArH), 7.19–7.26 (m, 2H, ArH), 7.55–7.57 (d, 1H, J = 8.8 Hz, ArH), 8.81 (s, 1H, CH), 13.46 (s, 1H, OH), 13.74 (s, 1H, COOH); ^{13}C NMR ($CDCl_3$) δ 25.14, 25.84, 28.36, 28.37 (d, J_{C-F} = 1.7 Hz), 32.82, 61.08, 104.74, 107.24, 113.52, 120.91, 121.09 (d, J_{C-F} = 17.9 Hz), 122.97, 124.45, 124.49 (d, J_{C-F} = 4.6 Hz), 128.39, 128.54 (d, J_{C-F} = 15.4 Hz), 128.82, 129.76, 129.80 (d, J_{C-F} = 3.9 Hz), 136.61, 139.39, 144.65, 155.33, 157.79 (d, J_{C-F} = 246.0 Hz),160.19, 166.15, 181.24; MS (ESI) m/z 430 $[M+H]^+$. Anal. Calcd for $C_{23}H_{21}ClFNO_4$: C, 64.26; H, 4.92; N, 3.26. Found: C, 64.03; H, 4.69; N, 3.52.

5.12.11. (*S*)-6-(3-Chloro-2-fluorobenzyl)-5-hydroxy-1-(1-hydroxy-3-methylbutan-2-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (2k)

To a solution of **11k** (224 mg, 0.5 mmol) in dichloromethane (10 mL) was added BBr_3 (2 M, 5.0 mmol) dropwise at −40 °C. After being stirred for 1 h, the mixture was allowed to warm to rt and stirred overnight. Ice-water (5 mL) was added dropwise to the reaction solution. The resulting mixture was extracted by dichloromethane (10 mL × 3), washed with cold 5% aqueous $NaHCO_3$ solution (5 mL), dried and concentrated. The residue was purified by

column chromatography (silica gel, dichloromethane/methanol 60:1 to 50:1, v/v) to give the desired compound **2k** as a colorless oil (53 mg, 24%): $[\alpha]_D^{20} = -24.8$ (*c* 0.5, $CHCl_3$), ^1H NMR ($CDCl_3$) δ 0.80–0.82 (d, 3H, J = 6.8 Hz, CH_3), 1.18–1.19 (d, 3H, J = 6.4 Hz, CH_3), 2.44–2.49 (m, 1H, CH), 3.37–3.38 (m, 1H, OH), 4.06–4.20 (m, 4H, $2CH_2$), 4.43–4.45 (m, 1H, CH), 7.01–7.07 (dd, 1H, $J = J'$ = 8.0 Hz, ArH), 7.07–7.09 (d, 1H, J = 8.8 Hz, ArH), 7.24–7.26 (m, 2H, ArH), 7.55–7.57 (d, 1H, J = 8.8 Hz, ArH), 8.85 (s, 1H, CH), 13.37 (s, 1H, OH), 13.78 (s, 1H, COOH); ^{13}C NMR ($CDCl_3$) δ 19.51, 19.69, 28.31, 28.33 (d, J_{C-F} = 2.6 Hz), 29.42, 61.10, 68.03, 105.01, 106.49, 113.32, 120.90, 121.08 (d, J_{C-F} = 18.1 Hz), 123.00, 124.45, 124.49 (d, J_{C-F} = 4.5 Hz), 128.36, 128.51 (d, J_{C-F} = 15.5 Hz), 128.81, 129.78, 129.82 (d, J_{C-F} = 4.0 Hz), 136.79, 140.95, 146.27, 155.35, 157.81 (d, J_{C-F} = 245.9 Hz),160.05, 166.93, 181.12; MS (ESI) *m/z* 434 $[M+H]^+$. Anal. Calcd for $C_{22}H_{21}ClFNO_5$: C, 60.90; H, 4.88; N, 3.23. Found: C, 60.72; H, 5.16; N, 3.55.

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