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Synthesis and biological evaluation of HQCAs with aryl or benzyl substituents on N-1 position as potential HIV-1 integrase inhibitors

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ABSTRACT

A series of new 5-hydroxylquinolone-3-carboxylic acids (HQCAs) with various aryl or benzyl substituents on N-1 position were synthesized and evaluated for their anti-HIV activity in C8166 cell culture. Most of the target compounds displayed activity against wide-type HIV-1 in the low micromolar range in infected C8166 cells. The most active compound **5g** exhibited activity against wild-type HIV-1 and HIV-1 mutant virus A17 with an EC_{50} value of 3.17 and 17.88 μM, respectively. The biological results and the docking study revealed that the substitution pattern on N-1 position of the quinolone core might contribute to physicochemical properties of HQCAs and resulted in great influence on their antiviral potency.

1. Introduction

As a promising therapeutic HIV-1 integrase (IN) inhibitor, Elvitegravir[1a,b] (**1**, Fig. 1) has drawn a lot of attention due to its favorable pharmacokinetic property and clinical profiles. A variety of **1** analogs such as 4-oxoquinoline 3-carboxylic acids (OQCAs),[2a,b] 4-oxonaphthyridine 3-carboxylic acids (ONCAs)[3] and 5-hydroxylquinolone-3-carboxylic acids (HQCAs)[4] (**2**–**4**, Fig. 1) have been developed in an effort to obtain more potent and selective IN inhibitors. Although considerable linear, branched and cyclic alkyl groups have been introduced on N-1 of these analogs, the effect of aryl or benzyl group at N-1 on biological activity have not yet been studied.

HQCAs,[4] recently reported by us, have shown anti-HIV-1 activity with low micromolar to submicromolar EC_{50} values. Further docking study revealed that the anti-HIV activity of these compounds might involve a two-metal chelating mechanism. With the aim of contributing to a better understanding of the structure–activity relationships (SAR) of HQCAs, a series of new HQCAs with various aryl or benzyl substituents on N-1 (**5a**–**m**, Fig. 1) were synthesized and evaluated as potential IN inhibitors.

2. Chemistry

Two series of HQCAs (*N*-aryl-substituted compounds **5a**–**e** and *N*-benzyl-substituted compounds **5f**–**m**) were synthesized as depicted in Scheme 1. The key intermediate acrylates **9a**–**b** were prepared from methyl 2,6-difluoro-3-iodobenzoate similar to our previously reported protocol.[4] Substitution of **9a**–**b** with appropriate aryl amines or benzyl amines led to acrylates **10a**–**m**, which were then cyclized using DBU as a base to furnish quinolone esters **11a**–**m**. The target compounds **5a**–**e** were obtained from **11a**–**e** by ester hydrolysis and subsequent 5-OH incorporation under basic conditions. The target compounds **5f**–**m** was synthesized from **11f**–**m** by methoxyl substitution with sodium methoxide and then demethylation with BBr_3.

3. Results and discussion

3.1. Biological evaluation

The synthesized compounds **5a**–**m** were evaluated by 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) assay[5] for cytotoxicity and antiviral activity in C8166 cells infected with the wild-type HIV-1 (LAI strain IIIB), and the compounds **5b**–**m** were also assayed for their biological activity against HIV-1 mutant virus (A17). Compound **4k**[4] ($R_1'' = 2S$-1-hydroxy-3-*methylbutan*-2-yl) and Elvitegravir were included as reference

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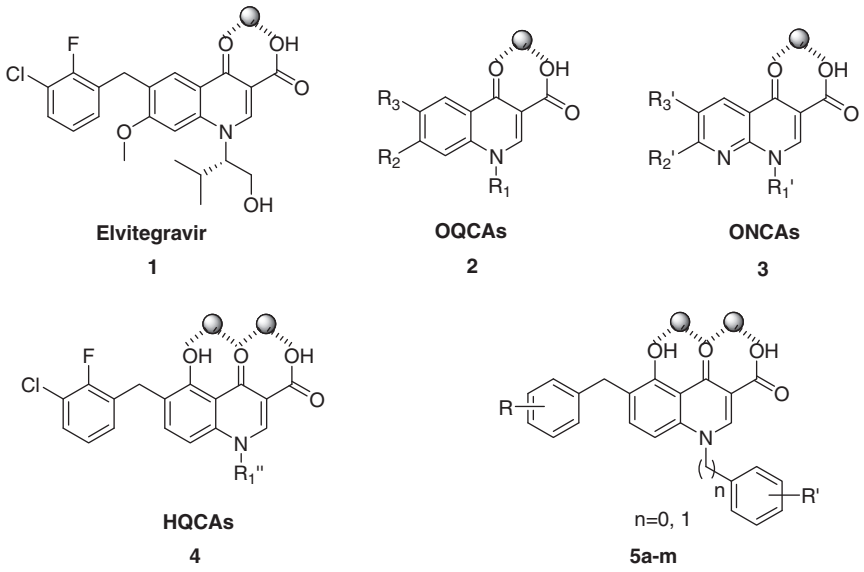


Figure 1. Chemical structures of HIV-1 IN inhibitors.

compounds. The cytotoxicity and antiviral activities of these compounds are listed in Table 1.

First we compared the antiviral activity of the compounds with *N*-substituted benzene ring **5a** and *N*-substituted benzyl group **5g** against wild-type HIV-1, the biological results showed that **5a** displayed much weak active than that of **5g**.

Next we examined the effect of introducing substituents into the benzene ring and benzyl group of **5a** and **5g**, respectively. Interestingly, the introduction of methyl group or bromide atom at the meta or para position of the benzene ring (**5b**–**e**) led to significantly increased activity against wild-type HIV-1, however, the introduction of methyl group or fluoro atom at the ortho, meta or para position of the benzene ring (**5h**–**m**) caused decreased antiviral activity. And compound **5c** incorporated a methyl group at the para position of **5a** was approximately 80 times more potent than that of **5a** and showed EC_{50} value similar to that of **5g**. The introduction of a second methyl group (**5d**) at the meta position of **5c** caused a decrease of antiviral activity against wild-type HIV-1 but enhanced activity against HIV-1 mutant virus A17.

To confirm the effect of 2-fluoro and 3-chloro substituents on the C-6 benzyl group, we replaced the chloro substituent at 3-position of **5g** and **5j** with a hydrogen atom (**5f** and **5i**). 2-Fluoro-3-chlorobenzyl compounds **5g** and **5j** appeared to be more active against wild-type HIV-1 than the corresponding 2-fluorobenzyl compounds **5f** and **5i**, respectively. And the compound **5j** also displayed slightly stronger activity against A17 than that of **5i**.

3.2. Molecular modeling calculations

With the aim to investigate the binding model of our newly synthesized compounds with IN, molecular docking study was performed.

Compounds **5c** and **5g**, which displayed the most activity against wild-type HIV-1 of *N*-aryl-substituted HQCAs and *N*-benzyl-substituted HQCAs, were docked into our previously constructed model of the HIV-1 IN catalytic core domain (CCD)/viral DNA complex[4] using SURFLEX-DOCK SYBYLX 1.1. The docking results showed that both **5c** and **5g** positioned a similar orientation in the active site of the homology model of HIV-1 IN (Fig. 2). As shown in the previous theoretical binding model of compound **4k** with IN,[4] the C-5 hydroxyl, 4-ketone and 3-carboxylate in compounds **5c** and **5g** also could form a two-Mg^{2+} chelation with HIV-1 IN. The substituted benzyl group and quinolone ring exhibit π–π stacking interaction with C16 and A17, respectively. No interaction could be detected between the substituent on N-1 and IN, suggesting that this part of the molecule might just contribute to favorable physicochemical properties of **5c** and **5g**.

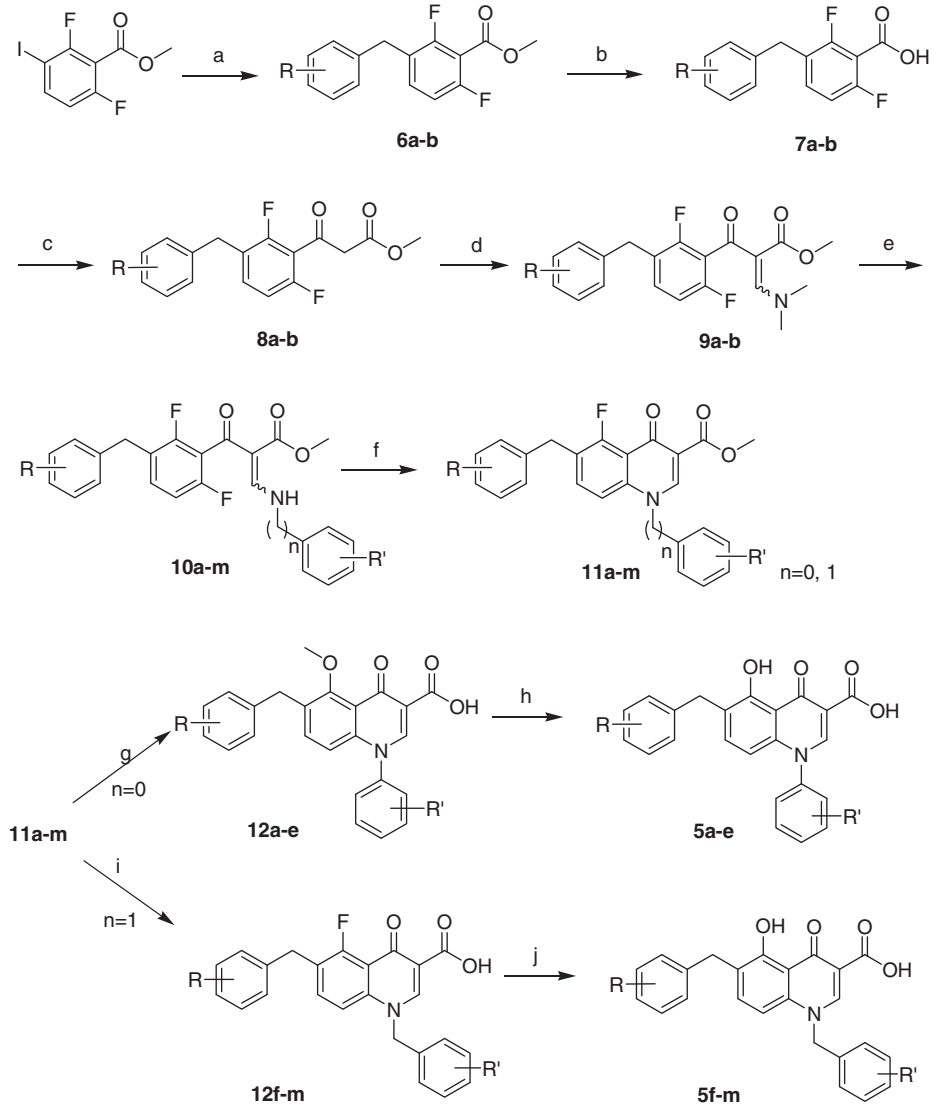
4. Conclusion

In conclusion, we designed and synthesized a series of new HQCAs with various aryl or benzyl substituents on N-1 position of a quinolone. All the target compounds exhibited activity against wide-type HIV-1 in the low micromolar range in infected C8166 cells. The most active compound **5g** exhibited activity against wild-type HIV-1 and HIV-1 mutant virus A17 with an EC_{50} value of 3.17 and 17.88 μM, respectively. Although in all cases, the measured activities against wild-type HIV-1 were lower than that of **4k** and Elvitegravir. The biological results showed that variation in substitution at N-1 position of the quinolone core had great influence on antiviral potency of HQCAs. Interestingly, no interaction between the substituent on N-1 and IN could be detected from the docking study. These findings suggested that the substitution pattern on N-1 position of the quinolone core might contribute to physicochemical properties of HQCAs and strongly affect their antiviral activity, which is consistent with the observations previously reported by us[4] and Dayam et al.[6]

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).



Scheme 1. *Reagents and conditions:* (a) (1) 3-chloro-2-flurobenzyl bromide, Zn, 1,2-dibromoethane, chlorotrimethylsilane, THF, 60 °C, 1 h, (2) $Pd(PPh_3)_4$, toluene, reflux, overnight; (b) satd aq LiOH, THF, 50 °C, 3 h; (c) (1) CDI, THF, rt, 2 h; (2) potassium methylmalonate, $MgCl_2$, 60 °C, overnight; (d) DMFDMA, THF, 50 °C, 3 h; (e) aryl amines or benzyl amines, THF, 60 °C, 3–8 h; (f) DBU, DMF, 90 °C, overnight; (g) MeONa, toluene, DMF, reflux, 5–10 h; (h) BBr_3, CH_2Cl_2, −40 °C to rt, overnight; (i) satd aq LiOH, dioxane, 50 °C, 3 h; (j) 12.5 M NaOH, dioxane, 80 °C, 1–2 days.

5.2. General procedure for the synthesis of 6a–b

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) or 2-fluorobenzyl bromide (414 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 benzylzinc bromide. To a mixture of methyl 2,6-difluoro-3-iodobenzoate (596 mg, 2.0 mmol) and $Pd(PPh_3)_4$ (13 mg, 0.011 mmol) in toluene (10 mL) was added the above solution of benzylzinc 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) and 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 40/1 to 25/1, v/v) to give the desired compound.

Compound **6a**[4] (R = 2-F, 3-Cl, 290 mg, 46%): colorless oil; ^1H 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).

Compound **6b** (R = 2-F, 280 mg, 50%): ^1H NMR ($CDCl_3$): colorless oil; ^1H NMR ($CDCl_3$): δ 3.97 (s, 3H, CH_3), 4.01 (s, 2H, CH_2), 6.87–6.92 (td, 1H, J = 8.8, J' = 1.2 Hz, ArH), 7.04–7.11 (m, 2H, ArH), 7.17–7.29 (m, 3H, ArH).

5.3. General procedure for the synthesis of 7a–b

To a solution of **6a**–**b** (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 **7** as a white solid. This crude was used directly for the next step without further purification.

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

Compd	R	R′	EC_{50}[b] (μM)		CC_{50}[c] (μM)	SI[d]
			WT (IIIB)	A17		
5a	2-F, 3-Cl	H	307.57	ND[e]	388.14	1.26
5b	2-F, 3-Cl	3-CH_3	20.48	43.68	71.55	3.49
5c	2-F, 3-Cl	4-CH_3	3.58	67.88	193.88	54.16
5d	2-F, 3-Cl	3,4-2CH_3	24.93	32.81	315.73	12.66
5e	2-F, 3-Cl	4-Br	7.12	33.24	84.97	11.93
5f	2-F	H	20.87	51.19	156.28	7.49
5g	2-F, 3-Cl	H	3.17	17.88	20.34	6.42
5h	2-F, 3-Cl	4-CH_3	5.55	29.25	255.51	46.04
5i	2-F	2-F	6.46	27.22	67.14	10.39
5j	2-F, 3-Cl	2-F	3.27	24.23	44.91	13.73
5k	2-F, 3-Cl	3-F	4.93	19.08	69.04	14.00
5l	2-F, 3-Cl	4-F	9.34	22.52	65.79	7.04
5m	2-F, 3-Cl	3,4-2F	5.74	24.25	71.35	12.43
4k			0.13	0.085	125.03	961.77
1			0.00021	0.0010	17.20	81,904.76

[a] All data represent mean values for at least two separate experiments.
[b] Compound concentration required to protect the cell against viral cytopathogenicity by 50% in C8166 cells.
[c] Compound concentration that decreases the normal uninfected C8166 cell viability by 50%.
[d] Selectivity index; ratio CC_{50}/EC_{50}.
[e] Not detected.

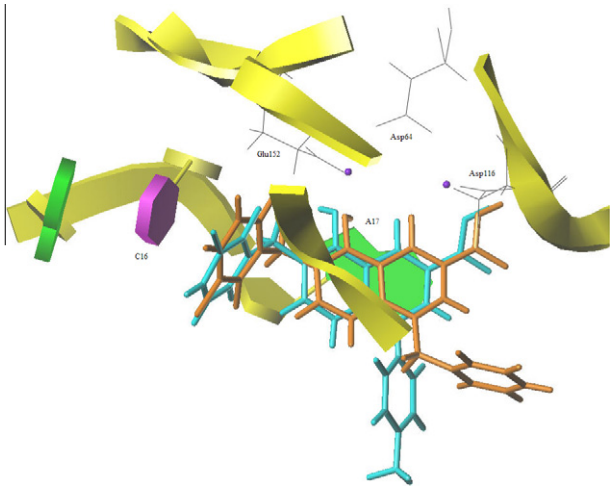


Figure 2. Binding model of compounds **5c** (cyan) and **5g** (orange) in the active site of new homoly model of HIV-1 IN.

5.4. General procedure for the synthesis of 8a–b

To a suspension of CDI (389 mg, 2.4 mmol) in THF (5 mL) was added a solution of **7a**–**b** (2.0 mmol) in THF (5 mL) dropwise. The resulting mixture was stirred at room temperature for 2 h, and then potassium 3-methoxy-3-oxopropanoate (374 mg, 2.4 mmol) and $MgCl_2$ (228 mg, 2.4 mmol) were added portion-wise. The reaction mixture was stirred at 60 °C overnight. The resulting precipitate was removed by filtration, and the filtrate was diluted with water (5 mL) and acidified with 2 M HCl to pH 6–7. 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.

Compound **8a**[4] (R = 2-F, 3-Cl, 320 mg, 45%): colorless oil; 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, CH), 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, OH).

Compound **8b** (R = 2-F, 335 mg, 52%): colorless oil; Keto form/Enol form = 2/1; Keto form ^{1}H NMR ($CDCl_3$) δ 3.74 (s, 3H, CH_3), 3.95 (s, 2H, CH_2), 4.02 (s, 2H, CH_2), 6.89–6.93 (m, 1H, ArH), 7.00–7.12 (m, 2H, ArH), 7.18–7.29 (m, 3H, ArH); Enol form ^{1}H NMR ($CDCl_3$) δ 3.83 (s, 1.5H, CH_3), 4.02 (s, 1H, CH_2), 5.44 (s, 0.5H, CH), 6.87–6.91 (m, 0.5H, ArH), 7.00–7.12 (m, 1H, ArH), 7.18–7.29 (m, 1.5H, ArH), 12.31 (s, 0.5H, OH).

5.5. General procedure for the synthesis of 9a–b

A mixture of **8a**–**b** (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.

5.6. General procedure for the preparation of 10a–m

A mixture of **9a**–**b** (1.5 mmol) and aryl amines or benzyl amines (1.8 mmol) in THF (15 mL) was stirred at 60 °C for 3–8 h and then concentrated under reduced pressure. The resulting residue **10a**–**m** was used directly for the next step without further purification.

5.7. General procedure for the preparation of 11a–m

A mixture of **10a**–**m** (1.5 mmol) and DBU (2.3 mmol) in DMF (10 mL) was stirred at 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 recrystallized from ethyl acetate to give the desired compound **11a**–**m**.

5.8. General procedure for the preparation of 12a–e

To a solution of MeONa (270 mg, 5 mmol) in toluene (15 mL) was added **11a**–**e** (1.0 mmol) and one drop of DMF. After being stirred at reflux for 5–10 h, the mixture was cooled, poured into ice-water and acidified with 4 M HCl to pH ~ 2, extracted by ethyl acetate (10 mL × 3) and concentrated to give the desired compound **12a**–**e**.

5.9. General procedure for the preparation of 12f–m

To a solution of satd aq LiOH (5 mL) in dioxane (8 mL) was added **11f**–**m** (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 **12f**–**m**. This crude was used directly for the next step without further purification.

5.10. General procedure for the preparation of 5a–e

To a solution of **12a**–**e** (1 mmol) in dichloromethane (10 mL) was added BBr_3 (2 M, 10 mmol) dropwise at −40 °C. After being stirred for 1 h, the mixture was allowed to warm to rt and stirred overnight. Ice-water (8 mL) was added dropwise to the reaction solution. The resulting mixture was extracted by dichloromethane (10 mL × 3), washed with 5% aqueous $NaHCO_3$ solution (5 mL), dried and concentrated to give the desired compound **5a**–**e**.

5.10.1. 6-(3-Chloro-2-fluorobenzyl)-5-hydroxy-4-oxo-1-phenyl-1,4-dihydroquinoline-3-carboxylic acid (5a)

Yield 82%. mp 218–220 °C; ^{1}H NMR ($CDCl_3$): δ 4.09 (s, 2H, CH_2), 6.44–6.46 (d, J = 8.8 Hz, 1H, ArH), 6.98–7.01 (t, J = 8.0 Hz, 1H, ArH), 7.18–7.25 (m, 2H, ArH), 7.37–7.39 (m, 2H, ArH), 7.40–7.42 (d, J = 8.8 Hz, 1H, ArH), 7.62–7.64 (m, 3H, ArH), 8.72 (s, 1H, CH), 13.15 (s, 1H, OH), 13.62 ppm (s, 1H, COOH). MS (ESI) m/z 446 $[M+Na]^+$; Anal. Calcd for $C_{23}H_{15}ClFNO_4$: C, 65.18; H, 3.57; N, 3.30. Found: C, 64.92; H, 3.89; N, 3.59.

5.10.2. 6-(3-Chloro-2-fluorobenzyl)-5-hydroxy-4-oxo-1-m-tolyl-1,4-dihydroquinoline-3-carboxylic acid (5b)

Yield 80%. mp 206–208 °C; ^{1}H NMR ($CDCl_3$): δ 2.48 (s, 3H, CH_3), 4.10 (s, 2H, CH_2), 6.50–6.52 (d, J = 8.8 Hz, 1H, ArH), 6.99–7.03 (t, J = 8.0 Hz, 1H, ArH), 7.19–7.20 (m, 2H, ArH), 7.22–7.26 (m, 2H, ArH), 7.42–7.46 (m, 2H, ArH), 7.49–7.53 (m, 1H, ArH), 8.72 (s, 1H, CH), 13.16 (s, 1H, OH), 13.72 ppm (s, 1H, COOH). MS (ESI) m/z 460 $[M+Na]^+$; Anal. Calcd for $C_{24}H_{17}ClFNO_4$: C, 65.83; H, 3.91; N, 3.20. Found: C, 66.14; H, 3.59; N, 3.44.

5.10.3. 6-(3-Chloro-2-fluorobenzyl)-5-hydroxy-4-oxo-1-*p*-tolyl-1,4-dihydroquinoline-3-carboxylic acid (5c)

Yield 78%. mp 234–236 °C; ^{1}H NMR ($CDCl_3$): δ 2.51 (s, 3H, CH_3), 4.11 (s, 2H, CH_2), 6.48–6.50 (d, J = 8.8 Hz, 1H, ArH), 6.99–7.04 (t, J = 8.0 Hz, 1H, ArH), 7.22–7.28 (m, 5H, ArH), 7.41–7.43 (d, J = 8.0 Hz, 2H, ArH), 8.73 (s, 1H, CH), 13.18 (s, 1H, OH), 13.76 ppm (s, 1H, COOH). MS (ESI) m/z 460 $[M+Na]^+$; Anal. Calcd for $C_{24}H_{17}ClFNO_4$: C, 65.83; H, 3.91; N, 3.20. Found: C, 65.63; H, 3.53; N, 3.40.

5.10.4. 6-(3-Chloro-2-fluorobenzyl)-1-(3,4-dimethylphenyl)-5-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5d)

Yield 81%. mp 210–212 °C; ^{1}H NMR ($CDCl_3$): δ 2.36 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 4.10 (s, 2H, CH_2), 6.51–6.54 (d, J = 8.8 Hz, 1H, ArH), 6.99–7.03 (t, J = 8.0 Hz, 1H, ArH), 7.10–7.14 (m, 2H, ArH), 7.22–7.26 (m, 2H, ArH), 7.35–7.37 (d, J = 8.0 Hz, 1H, ArH), 7.41–7.43 (d, J = 8.8 Hz, 1H, ArH), 8.72 (s, 1H, CH), 13.18 (s, 1H, OH), 13.76 ppm (s, 1H, COOH). MS (ESI) m/z 474 $[M+Na]^+$; Anal. Calcd for $C_{25}H_{19}ClFNO_4$: C, 66.45; H, 4.24; N, 3.10. Found: C, 66.17; H, 3.92; N, 3.36.

5.10.5. 6-(3-Chloro-2-fluorobenzyl)-1-(4-bromophenyl)-5-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5e)

Yield 76%. mp 207–209 °C; ^{1}H NMR ($CDCl_3$): δ 4.10 (s, 2H, CH_2), 6.44–6.46 (d, J = 8.8 Hz, 1H, ArH), 7.00–7.03 (t, J = 8.0 Hz, 1H, ArH), 7.21–7.25 (m, 2H, ArH), 7.29–7.31 (d, J = 8.8 Hz, 2H, ArH), 7.44–7.46 (d, J = 8.8 Hz, 1H, ArH), 7.78–7.80 (d, J = 8.8 Hz, 2H, ArH), 8.69 (s, 1H, CH), 13.11 (s, 1H, OH), 13.59 ppm (s, 1H, COOH). MS (ESI) m/z 524 $[M+Na]^+$; Anal. Calcd for $C_{23}H_{14}BrClFNO_4$: C, 54.95; H, 2.81; N, 2.79. Found: C, 54.74; H, 2.64; N, 3.09.

5.11. General procedure for the preparation of 5f–m

To a solution of 12.5 N NaOH (5 mL) in dioxane (8 mL) was added **12f**–**m** (1.5 mmol). After being stirred at 80 °C for 1–2 days, the mixture was cooled, poured into water and acidified with 2 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 **5f**–**m**.

5.11.1. 6-(2-Fluorobenzyl)-1-benzyl-5-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5f)

Yield 58%. mp 208–210 °C; ^{1}H NMR ($CDCl_3$): δ 4.05 (s, 2H, CH_2), 5.41 (s, 2H, CH_2), 6.83–6.85 (d, J = 8.8 Hz, 1H, ArH), 6.99–7.07 (m, 2H, ArH), 7.13–7.22 (m, 3H, ArH), 7.27–7.29 (m, 1H, ArH), 7.36–7.37 (m, 3H, ArH), 7.40–7.42 (d, J = 8.8 Hz, 1H, ArH), 8.81 (s, 1H, CH), 13.26 (s, 1H, OH), 13.74 ppm (s, 1H, COOH). MS (ESI) m/z 426 $[M+Na]^+$; Anal. Calcd for $C_{24}H_{18}FNO_4$: C, 71.46; H, 4.50; N, 3.47. Found: C, 71.14; H, 4.71; N, 3.77.

5.11.2. 6-(3-Chloro-2-fluorobenzyl)-1-benzyl-5-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5g)

Yield 60%. mp 216–218 °C; ^{1}H NMR ($CDCl_3$): δ 4.05 (s, 2H, CH_2), 5.43 (s, 2H, CH_2), 6.86–6.88 (d, J = 7.6 Hz, 1H, ArH), 6.97–6.98 (m, 1H), 7.15–7.26 (m, 5H), 7.36–7.37 (m, 2H), 7.43–7.45 (d, J = 7.2 Hz, 1H, ArH), 8.82 (s, 1H, CH), 13.26 (s, 1H, OH), 13.68 ppm (s, 1H, COOH). MS (ESI) m/z 460 $[M+Na]^+$; Anal. Calcd for $C_{24}H_{17}ClFNO_4$: C, 65.83; H, 3.91; N, 3.20. Found: C, 65.61; H, 4.16; N, 3.44.

5.11.3. 6-(3-Chloro-2-fluorobenzyl)-1-(4-methylbenzyl)-5-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5h)

Yield 62%. mp 238–240 °C; ^{1}H NMR ($CDCl_3$): δ 2.34 (s, 3H, CH_3), 4.06 (s, 2H, CH_2), 5.37 (s, 2H, CH_2), 6.87–6.90 (d, J = 8.8 Hz, 1H, ArH), 6.97–7.01 (t, J = 8.0 Hz, 1H, ArH), 7.03–7.05 (d, J = 8.0 Hz, 2H, ArH), 7.16–7.18 (d, J = 8.0 Hz, 2H, ArH), 7.20–7.24 (m, 2H, ArH), 7.44–7.46 (d, J = 8.8 Hz, 1H, ArH), 8.80 (s, 1H, CH), 13.28 (s, 1H, OH), 13.71 ppm (s, 1H, COOH). MS (ESI) m/z 474 $[M+Na]^+$; Anal. Calcd for $C_{25}H_{19}ClFNO_4$: C, 66.45; H, 4.24; N, 3.10. Found: C, 66.73; H, 4.02; N, 3.42.

5.11.4. 6-(2-Fluorobenzyl)-1-(2-fluorobenzyl)-5-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5i)

Yield 48%. mp 225–226 °C; ^{1}H NMR ($CDCl_3$): δ 4.08 (s, 2H, CH_2), 5.47 (s, 2H, CH_2), 6.89–6.91 (d, J = 8.8 Hz, 1H, ArH), 7.02–7.23 (m, 6H, ArH), 7.30–7.32 (m, 1H, ArH), 7.36–7.41 (m, 1H, ArH), 7.47–7.49 (d, J = 8.8 Hz, 1H, ArH), 8.85 (s, 1H, CH), 13.28 (s, 1H, OH), 13.71 ppm (s, 1H, COOH). MS (ESI) m/z 444 $[M+Na]^+$; Anal. Calcd for $C_{24}H_{17}F_2NO_4$: C, 68.41; H, 4.07; N, 3.32. Found: C, 68.71; H, 4.32; N, 3.07.

5.11.5. 6-(3-Chloro-2-fluorobenzyl)-1-(2-fluorobenzyl)-5-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5j)

Yield 52%. mp 215–217 °C; ^{1}H NMR ($CDCl_3$): δ 4.07 (s, 2H, CH_2), 5.45 (s, 2H, CH_2), 6.89–6.91 (d, J = 8.8 Hz, 1H, ArH), 7.00–7.01 (m, 2H, ArH), 7.11–7.23 (m, 4H, ArH), 7.35–7.40 (m, 1H, ArH), 7.48–7.50 (d, J = 8.8 Hz, 1H, ArH), 8.83 (s, 1H, CH), 13.27 (s, 1H, OH), 13.65 ppm (s, 1H, COOH). MS (ESI) m/z 478 $[M+Na]^+$; Anal. Calcd for $C_{24}H_{16}ClF_2NO_4$: C, 63.24; H, 3.54; N, 3.07. Found: C, 63.02; H, 3.84; N, 2.85.

5.11.6. 6-(3-Chloro-2-fluorobenzyl)-1-(3-fluorobenzyl)-5-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5k)

Yield 53%. mp 214–215 °C; ^{1}H NMR ($CDCl_3$): δ 4.06 (s, 2H, CH_2), 5.42 (s, 2H, CH_2), 6.79–6.81 (d, J = 8.8 Hz, 1H, ArH), 6.83–6.85 (d, J = 8.8 Hz, 1H, ArH), 6.91–6.93 (d, J = 8.0 Hz, 1H, ArH), 6.97–7.01 (t, J = 8.0 Hz, 1H, ArH), 7.03–7.07 (m, 1H, ArH), 7.19–7.25 (m, 2H, ArH), 7.33–7.38 (m, 1H, ArH), 7.45–7.47 (d, J = 8.8 Hz, 1H, ArH), 8.12 (s, 1H, CH), 13.24 (s, 1H, OH), 13.64 ppm (s, 1H, COOH). MS (ESI) m/z 478 $[M+Na]^+$; Anal. Calcd for $C_{24}H_{16}ClF_2NO_4$: C, 63.24; H, 3.54; N, 3.07. Found: C, 62.93; H, 3.38; N, 3.27.

5.11.7. 6-(3-Chloro-2-fluorobenzyl)-1-(4-fluorobenzyl)-5-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5l)

Yield 48%. mp 236–238 °C; ^{1}H NMR ($CDCl_3$): δ 4.06 (s, 2H, CH_2), 5.39 (s, 2H, CH_2), 6.82–6.84 (d, J = 8.8 Hz, 1H, ArH), 6.97–7.01 (t, J = 8.0 Hz, 1H, ArH), 7.05–7.10 (m, 2H, ArH), 7.13–7.16 (m, 2H, ArH), 7.19–7.26 (m, 2H, ArH), 7.45–7.47 (d, J = 8.8 Hz, 1H, ArH), 8.80 (s, 1H, CH), 13.26 (s, 1H, OH), 13.65 ppm (s, 1H, COOH). MS (ESI) m/z 478 $[M+Na]^+$; Anal. Calcd for $C_{24}H_{16}ClF_2NO_4$: C, 63.24; H, 3.54; N, 3.07. Found: C, 63.55; H, 3.36; N, 3.25.

5.11.8. 1-(3,4-Difluorobenzyl)-6-(3-chloro-2-fluorobenzyl)-5-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5m)

Yield 30%. mp 222–224 °C; ^{1}H NMR ($CDCl_3$): δ 4.07 (s, 2H, CH_2), 5.38 (s, 2H, CH_2), 6.75–6.78 (d, J = 8.8 Hz, 1H, ArH), 6.87–6.90 (m, 1H, ArH), 6.96–7.02 (m, 2H, ArH), 7.16–7.23 (m, 3H, ArH), 7.46–7.48 (d, J = 8.8 Hz, 1H, ArH), 8.79 (s, 1H, CH), 13.24 (s, 1H, OH), 13.61 ppm (s, 1H, COOH). MS (ESI) m/z 496 $[M+Na]^+$; Anal. Calcd for $C_{24}H_{15}ClF_3NO_4$: C, 60.84; H, 3.19; N, 2.96. Found: C, 61.10; H, 3.37; N, 2.68.

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