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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

The simple synthesis and antimicrobial activity of novel fluoroquinolone derivatives from natural amino acid salts

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Abstract A simple synthetic route was developed to prepare novel fluoroquinolone derivatives from amino acid salts. A facile route preparing the quinolone intermediates in one pot was explored which can facilitate the industrial operation. A series of new compounds were prepared conveniently and characterized by IR, ^{1}H NMR, MS, and elemental analysis. The preliminary bioassays results revealed that they have certain antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, and *Aspergillus fumigatus*.

Keywords Fluoroquinolone · Amino acid salts · Antimicrobial

Introduction

Quinoline and fluoroquinolone derivatives are receiving much attention for their broad-spectrum bioactivities, such as antiasthmatic, antibacterial, antimalarial, and tyrosine-kinase PDGF-RTK inhibitor agent, etc. (Silva *et al.*, 2003; Chide and Orisakwe 2007; Chen *et al.*, 2001; Miyauchi *et al.*, 2009). Due to such a wide range of applicability in medicine, bioorganic chemistry, as well as synthetic organic chemistry, there has been increasing interests in the development of new quinolone and fluoroquinolone derivatives to enrich this domain (Sakineh *et al.*, 2009; Murugesan *et al.*, 2008; Chai *et al.*, 2009). Structure–activity relationship studies discovered that N1, C2-H, C3-carboxylic acid, C4-carbonyl, C6-F, and C7-piperazine are essential or beneficial for antibacterial activity (Najma *et al.*, 2009; Jayashree *et al.*, 2009; Patel and Patel 2009). As part of our ongoing interests to find new fluoroquinolone derivatives, we have focused our attention on introducing natural amino acid piece to the fluoroquinolone ring at N1-position. Herein a series of novel fluoroquinolone derivatives from natural amino acid salts were designed and prepared. A simple synthetic route was developed and gives good yields. The preliminary bioassays results revealed that they have certain bactericidal activity against *Bacillus subtilis*, *Staphylococcus aureus*, and *Aspergillus fumigatus*. Our primary objective was to find the new bioactive compounds and then optimize it further in our subsequent research. The synthetic routes are shown in Fig. 1.

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Experimental

Materials and methods

All the reagents were purchased commercially and used without further purification. The melting point was determined using a XT-4 melting-point apparatus and uncorrected. IR spectra were recorded on a Bruker Equinox-55 spectrophotometer using KBr discs in the 4000–400 cm^{-1} region. The ^{1}H NMR was obtained on a Bruker AC-400 (400 MHz) instrument in DMSO-d_6 using TMS as internal standard. Chemical shifts (δ) are expressed in ppm and coupling constants J are given in Hz. Mass spectra were obtained on an Agilent 5973N mass spectrometer operating at 70 eV by electron ionization technique (EI MS). Elemental analyses were performed on an EA-1110 instrument.

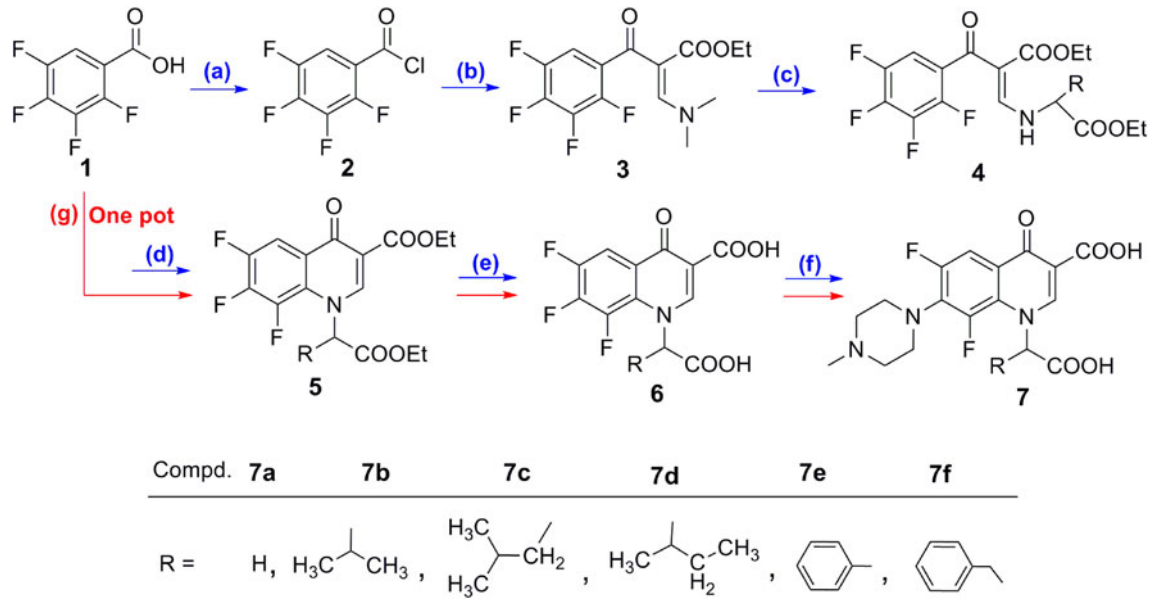

Fig. 1 The synthesis of novel fluoroquinolone derivatives. (*a*) $SOCl_2$; (*b*) (*E*)-ethyl-3-(dimethylamino)acrylate; (*c*) amino acid ethyl ester hydrochloride; (*d*) K_2CO_3; (*e*) hydrolysis; (*f*) substitute reaction; (*g*) merge step (*a*), (*b*), and (*c*) to one pot, and no individual separation

General procedure

2,3,4,5-Tetrafluorobenzoic acid **1** 1.94 g (0.01 mol) was dissolved in thionyl chloride 14.5 ml in a 50-ml round-bottomed flask, stirring, and reflux for 3 h, removing the superfluous $SOCl_2$ on a rotary evaporator. (*E*)-ethyl-3-(dimethylamino)acrylate 1.43 g (0.01 mol) was added dropwise to the toluene solution of above leavings with stirring at 50°C, monitoring with TLC (PE/EA = 1/1 (v/v)) to the end of reactants, adding amino acid ethyl ester hydrochloride (0.01 mol) directly and stirring for about 5 h at room temperature, checking the reaction via TLC, then potassium carbonate 1.66 g (0.012 mol) was added and stirring for 8 h at 100°C. When the reaction was complete (determined by TLC), the mixture was acidified with diluted hydrochloride acid (5%) to pH = 2–3. the reaction mixture was poured into separatory funnel and separated. The organic layer was concentrated under reduced pressure and the residual was chromatographed on silica gel (PE/EA = 2/1 (v/v)) to give compound **5**, which was added to the 20% sulfuric acid solution, refluxing for about 6 h, cooled to room temperature and filter. The filter cake **6** was dissolved in 9 ml sulfoxide and added to 1-methylpiperazine 0.04 mol, raising the temperature slowly to 100–110°C and maintaining this temperature for about 5 h. After concentration, the mixture was poured into 20 ml water and separated. The crude product was recrystallized from ethanol solvent, giving compound **7**.

Antimicrobial activity

All of the synthesized compounds were tested for their antibacterial and antifungal activity in vitro by broth dilution method with some bacteria *Bacillus subtilis*, *Staphylococcus aureus*, *Aspergillus fumigatus*, and *Candida albicans* according to the literature (Kong *et al.*, 2009; Andre and Patrick 1984). The antimicrobial discs (diameter, 0.55 cm) were prepared at concentrations of 1, 0.2, and 0.1 mg/ml and applied to each of the culture plates previously seeded with the test bacteria. These culture plates were then incubated at 37°C for 24 h. The preliminary antimicrobial activity was determined by the diameter of inhibition zone. For each compound, three replicate trials were conducted against each organism.

Results and discussion

Chemistry

The common synthetic routes of fluoroquinolone derivatives are carried out from compounds **1** to **5** step by step with separation and purification, then to the final products. To simplify the synthetic routes, we explored a facile and simple route from compounds **1** to **5** in one pot without separation, which is very beneficial for the industrial operation to save manufacturing costs.

The structures of synthesized compounds of **7a**–**7f** were confirmed by IR, ^{1}H NMR, MS, and elemental analysis. The Infrared spectra of all synthesized compounds showed easily distinguishable carbonyl stretching of the carboxylic acid group at 1728–1752 cm^{-1}, and 1628–1557 cm^{-1} is assigned to ring carbonyl stretching. Furthermore, the absence of free NH stretching between 3300 and 3200 cm^{-1} confirmed that the reaction had taken place at NH of the piperazine ring as well as amino acids derivatives. In ^{1}H NMR spectrum, common signals of carboxylic group appear between 12.99 and 14.74 ppm and the two kind of carboxylic group signals demonstrate that the piece of amino acids combined to the title compounds. The characteristic data are almost similar with the literature (Murugesan *et al.*, 2008; Zhu *et al.*, 2009).

Biological activities

All the title compounds were tested for their antibacterial and antifungal activity. From the screening results, all of the title compounds have activity against *Bacillus subtilis* and no activity against C*andida albicans*. Some compounds have certain activity against *Staphylococcus aureus* and *Aspergillus fumigatus*. The detail results are listed in Table 1. Compared to the activity of control compound, the biological value is not so good, while some new compounds exhibit certain activities against *Aspergillus fumigatus* which the control agent has not owned. The results show that those new compounds can expand the application of fluoroquinolone compounds on the *Aspergillus fumigatus*.

It was found that all the new synthesized compounds have certain antibacterial and antifungal bioactivity, although the biological results are not as good as our wishes. It maybe attribute to the lack of crucial functionality in these compounds to block bacterial replication which intrigued us to study its QSAR further and synthesize higher activity compound, the in-depth research work is undergoing.

Chemical data and analyses

*Synthesis of ethyl 1-(2-ethoxy-2-oxoethyl)-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**5a**)*

Yield: 75%. Mp: 198–200°C. IR (KBr) v: 3426, 3070, 2986, 1760, 1678, 1488, 1381, 1329, 1269, 1108, 1078 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.31 (t, $J = 7.2$ Hz, 3H, CH_3), 1.38 (t, $J = 7.2$ Hz, 3H, CH_3), 4.30 (q, $J = 6.8$ Hz, $J = 14.8$ Hz, 2H, CH_2), 4.36 (q, $J = 6.4$ Hz, $J = 14.6$ Hz, 2H, CH_2), 5.03 (d, $J = 6.0$ Hz, 2H, CH_2), 8.12 (t, $J = 9.2$ Hz, 1H, ArH), 8.33 (s, 1H, CH). ^{13}C NMR ($CDCl_3$, 100 MHz): 13.21, 14.17, 59.83, 61.23, 62.26, 109.82, 110.15 (d, $^2J_{C–F} = 13.8$ Hz), 125.97 (d, $^3J_{C–F} = 7.1$ Hz), 128.13 (d, $^2J_{C–F} = 14.2$ Hz), 142.44 (dd, $^1J_{C–F} = 105.7$ Hz, $^2J_{C–F} = 14.1$ Hz), 145.37, 146.92 (dd, $^1J_{C–F} = 106.1$ Hz, $^2J_{C–F} = 14.2$ Hz), 150.12 (dd, $^1J_{C–F} = 103.3$ Hz, $^2J_{C–F} = 14.2$ Hz), 166.34, 168.11, 179.15. MS (70 eV): *m/z* (%) = 357 (M^+).

*Synthesis of ethyl 1-(1-ethoxy-3-methyl-1-oxobutan-2-yl)-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**5b**)*

Yield: 77%. Mp: 72–74°C. IR (KBr) v: 3459, 3068, 2982, 2937, 1739, 1696, 1523, 1486, 1415, 1370, 1241, 1188, 1056 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): 0.92 (d, $J = 6.8$ Hz, 3H, CH_3), 1.16 (d, $J = 6.8$ Hz, 3H, CH_3), 1.28 (t, $J = 7.2$ Hz, 3H, CH_3), 1.39 (t, $J = 7.2$ Hz, 3H, CH_3), 2.55-2.59 (m, 1H, CH), 4.21–4.30 (m, 2H, CH_2), 4.35–4.41

Table 1 The preliminary antimicrobial activity of title compounds

	Conc. (mg/ml)	**7a**	**7b**	**7c**	**7d**	**7e**	**7f**	Control[c]
Bacillus subtilis	1	1.1[a]	1.0	1.15	1.2	1.05	1.2	4.60
	0.2	0.9	0.7	0.95	1.15	0.9	0.95	3.80
	0.1	0.65	–	–	0.6	0.6	–	3.60
Staphylococcus aureus	1	0.7	–	0.6	0.6	0.6	0.8	3.30
	0.2	–[b]	–	–	–	–	–	2.90
	0.1	–	–	–	–	–	–	2.60
Aspergillus fumigatus	1	1.0	–	–	–	0.6	0.75	–
	0.2	0.6	–	–	–	–	–	–
	0.1	–	–	–	–	–	–	–

[a] Diameter of inhibition zone (cm)

[b] No inhibition

[c] Levofloxacin Hydrochloride

(m, 2H, CH_2), 5.14 (s, 1H), 8.13–8.19 (m, 1H, ArH), 8.73 (s, 1H, CH). ^{13}C NMR ($CDCl_3$, 100 MHz): 13.34, 14.25, 21.88, 22.12, 29.54, 60.13, 61.12, 61.63, 109.11, 111.42 (d, $^2J_{C–F} = 14.3$ Hz), 125.92 (d, $^3J_{C–F} = 7.2$ Hz), 127.34 (d, $^2J_{C–F} = 14.1$ Hz), 141.45 (dd, $^1J_{C–F} = 104.6$ Hz, $^2J_{C–F} = 14.8$ Hz), 144.11, 146.39 (dd, $^1J_{C–F} = 105.3$ Hz, $^2J_{C–F} = 13.8$ Hz), 150.09 (dd, $^1J_{C–F} = 103.6$ Hz, $^2J_{C–F} = 13.6$ Hz), 168.42, 169.79, 177.25. MS (70 eV): *m/z* (%) = 399 (M^+).

Synthesis of ethyl 1-(1-ethoxy-4-methyl-1-oxopentan-2-yl)-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate ***(5c)***

Yield: 73%. Mp: 98-100°C. IR (KBr) v: 3452, 3061, 2988, 2943, 1742, 1686, 1533, 1476, 1410, 1373, 1239, 1183, 1088 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): 0.95 (d, $J = 6.0$ Hz, 6H, CH_3), 1.24 (t, $J = 7.2$ Hz, 3H, CH_3), 1.39 (t, $J = 7.2$ Hz, 3H, CH_3), 1.58–1.59 (m, 1H, CH), 2.05–2.19 (m, 2H, CH_2), 4.25 (q, $J = 7.2$ Hz, $J = 14.2$ Hz, 2H, CH_2), 4.38 (q, $J = 7.2$ Hz, $J = 14.2$ Hz, 2H, CH_2), 5.54 (s, 1H), 8.14–8.19 (m, 1H, ArH), 8.50 (s, 1H, CH). ^{13}C NMR ($CDCl_3$, 100 MHz): 13.75, 14.23, 22.12, 22.89, 28.34, 35.87, 60.14, 61.42, 63.33, 108.92, 112.22 (d, $^2J_{C–F} = 14.2$ Hz), 125.97 (d, $^3J_{C–F} = 7.2$ Hz), 127.39 (d, $^2J_{C–F} = 14.1$ Hz), 142.15 (dd, $^1J_{C–F} = 104.3$ Hz, $^2J_{C–F} = 14.2$ Hz), 145.51, 146.37 (dd, $^1J_{C–F} = 105.1$ Hz, $^2J_{C–F} = 13.9$ Hz), 149.89 (dd, $^1J_{C–F} = 104.1$ Hz, $^2J_{C–F} = 13.9$ Hz), 166.32, 167.69, 178.17. MS (70 eV): *m/z* (%) = 413 (M^+).

Synthesis of ethyl 1-(1-ethoxy-3-methyl-1-oxopentan-2-yl)-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate ***(5d)***

Yield: 72%. Mp: 58–60°C. IR (KBr) v: 3456, 3069, 2990, 2965, 1744, 1609, 1511, 1427, 1405, 1356, 1206, 1108, 1072 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): 0.71 (t, $J = 7.6$ Hz, 3H, CH_3), 0.84 (t, $J = 7.6$ Hz, 1H, CH), 0.97 (d, $J = 6.4$ Hz, 3H, CH_3), 1.11 (t, $J = 7.2$ Hz, 3H, CH_3), 1.20 (t, $J = 7.2$ Hz, 3H, CH_3), 4.07–4.21 (m, 4H, CH_2), 5.11–5.13(m, 1H, CH), 7.90–7.95 (m, 1H, ArH), 8.60 (s, 1H, CH). ^{13}C NMR ($CDCl_3$, 100 MHz): 12.69, 13.29, 14.35, 15.54, 23.42, 32.67, 61.24, 62.48, 63.33, 109.62, 112.34 (d, $^2J_{C–F} = 14.2$ Hz), 126.77 (d, $^3J_{C–F} = 7.1$ Hz), 128.21 (d, $^2J_{C–F} = 14.4$ Hz), 142.35 (dd, $^1J_{C–F} = 104.6$ Hz, $^2J_{C–F} = 14.2$ Hz), 146.01, 147.29 (dd, $^1J_{C–F} = 106.1$ Hz, $^2J_{C–F} = 14.2$ Hz), 149.78 (dd, $^1J_{C–F} = 105.2$ Hz, $^2J_{C–F} = 14.2$ Hz), 165.13, 166.89, 177.37. MS (70 eV): *m/z* (%) = 413 (M^+).

Synthesis of ethyl 1-(2-ethoxy-2-oxo-1-phenylethyl)-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate ***(5e)***

Yield: 69%. Mp: 121–123°C. IR (KBr) v: 3434, 2958, 1748, 1690, 1631, 1510, 1483, 1341, 1317, 1242, 1188, 1059 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.22 (t, $J = 7.2$ Hz, 3H, CH_3), 1.28 (t, $J = 7.2$ Hz, 3H, CH_3), 4.17–4.22 (m, 2H, CH_2), 4.29–4.39 (m, 2H, CH_2), 6.65 (s, 1H, CH), 7.37–7.39 (m, 2H, ArH), 7.52–7.54 (m, 3H, ArH), 8.11–8.16 (m, 1H, ArH), 8.12 (s, 1H, CH). ^{13}C NMR ($CDCl_3$, 100 MHz): 14.11, 15.12, 61.24, 62.13, 68.93, 110.82, 112.44 (d, $^2J_{C–F} = 14.1$ Hz), 126.67 (d, $^3J_{C–F} = 7.3$ Hz), 128.34, 128.55 (d, $^2J_{C–F} = 14.6$ Hz), 129.65, 130.22, 131.59, 142.76 (dd, $^1J_{C–F} = 104.2$ Hz, $^2J_{C–F} = 14.3$ Hz), 146.79, 147.70 (dd, $^1J_{C–F} = 106.4$ Hz, $^2J_{C–F} = 14.4$ Hz), 150.28 (dd, $^1J_{C–F} = 106.1$ Hz, $^2J_{C–F} = 14.3$ Hz), 166.23, 167.49, 178.11. MS (70 eV): *m/z* (%) = 435 (M^+).

Synthesis of ethyl 1-(1-ethoxy-1-oxo-3-phenylpropan-2-yl)-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate ***(5f)***

Yield: 72%. Mp: 133–135°C. IR (KBr) v: 3425, 2968, 1724, 1668, 1621, 1532, 1443, 1321, 1221, 1125, 1064 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.21 (t, $J = 7.2$ Hz, 3H, CH_3), 1.31 (t, $J = 7.2$ Hz, 3H, CH_3), 3.18–3.21 (m, 2H, CH_2), 3.57–3.59 (m, 1H, CH), 4.19–4.24 (m, 4H, CH_2), 7.55–7.61 (m, 5H, ArH), 8.13–8.17 (m, 1H, ArH), 8.21 (s, 1H, CH). ^{13}C NMR ($CDCl_3$, 100 MHz): 14.16, 14.92, 37.97, 60.34, 61.77, 71.62, 111.12, 113.41 (d, $^2J_{C–F} = 14.2$ Hz), 127.68 (d, $^3J_{C–F} = 7.1$ Hz), 128.95 (d, $^2J_{C–F} = 13.6$ Hz), 127.14, 128.62, 129.32, 131.29, 142.71 (dd, $^1J_{C–F} = 103.4$ Hz, $^2J_{C–F} = 14.1$ Hz), 147.75, 148.12 (dd, $^1J_{C–F} = 105.8$ Hz, $^2J_{C–F} = 14.1$ Hz), 150.45 (dd, $^1J_{C–F} = 105.9$ Hz, $^2J_{C–F} = 14.1$ Hz), 166.43, 168.29, 177.39. MS (70 eV): *m/z* (%) = 447 (M^+).

Synthesis of 1-(carboxymethyl)-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(6a)***

Yield: 68%. Mp: 249–251°C. 248-250IR (KBr) v: 3422, 3058, 2982, 1725, 1619, 1523, 1492, 1448, 1351, 1226, 1078 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): 5.42 (d, $J = 6.8$ Hz, 2H, CH_2), 8.19 (m, 1H, ArH), 9.07 (s, 1H), 13.67 (s, br, 1H, COOH), 14.24 (s, br, 1H, COOH). ^{13}C NMR (DMSO-d_6, 100 MHz): 59.83, 108.41, 112.35 (d, $^2J_{C–F} = 14.1$ Hz), 126.42 (d, $^3J_{C–F} = 7.3$ Hz), 128.64 (d, $^2J_{C–F} = 14.6$ Hz), 141.39 (dd, $^1J_{C–F} = 106.4$ Hz, $^2J_{C–F} = 15.2$ Hz), 143.62, 147.38 (dd, $^1J_{C–F} = 104.3$ Hz, $^2J_{C–F} = 13.6$ Hz), 149.92 (dd, $^1J_{C–F} = 102.1$ Hz, $^2J_{C–F} = 14.2$ Hz), 169.41, 171.32, 179.15. MS (70 eV): *m/z* (%) = 301 (M^+).

Synthesis of 1-(1-carboxy-2-methylpropyl)-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(6b)***

Yield: 70%. Mp: 221–223°C. IR (KBr) v: 3425, 2986, 1735, 1617, 1465, 1349, 1232, 1122, 1065 cm^{-1}. ^{1}H NMR

(DMSO-d_6, 400 MHz): 0.54–1.43 (m, 6H, CH_3), 2.57–2.65 (m, 1H, CH), 5.29–5.49 (m, 1H, CH), 8.18–8.23 (m, 1H), 9.17 (s, 1H, CH), 14.02 (s, br, 2H, COOH). ^{13}C NMR (DMSO-d_6, 100 MHz): 21.88, 22.12, 29.54, 61.63, 109.11, 111.42 (d, $^2J_{C–F} = 14.3$ Hz), 125.92 (d, $^3J_{C–F} = 7.2$ Hz), 127.34 (d, $^2J_{C–F} = 14.1$ Hz), 141.45 (dd, $^1J_{C–F} = 104.6$ Hz, $^2J_{C–F} = 14.8$ Hz), 144.11, 146.39 (dd, $^1J_{C–F} = 105.3$ Hz, $^2J_{C–F} = 13.8$ Hz), 150.09 (dd, $^1J_{C–F} = 103.6$ Hz, $^2J_{C–F} = 13.6$ Hz), 167.31, 172.12, 178.91. MS (70 eV): *m/z* (%) = 343 (M^+).

*Synthesis of 1-(1-carboxy-3-methylbutyl)-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6c**)*

Yield: 72%. Mp: 230–232°C. IR (KBr) v: 3427, 2988, 1727, 1607, 1484, 1349, 1239, 1112, 1045 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): 0.81–0.96 (m, 6H, CH_3), 1.49–1.50 (m, 1H, CH), 2.17–2.22 (m, 2H, CH_2), 5.72–5.76 (m, 1H, CH), 8.18–8.23 (m, 1H), 9.21 (s, 1H, CH), 13.99 (s, br, 2H, COOH). ^{13}C NMR (DMSO-d_6, 100 MHz): 22.13, 22.45, 26.34, 31.16, 63.89, 108.34, 112.12 (d, $^2J_{C–F} = 14.1$ Hz), 126.02 (d, $^3J_{C–F} = 6.8$ Hz), 128.51 (d, $^2J_{C–F} = 14.3$ Hz), 142.14 (dd, $^1J_{C–F} = 105.8$ Hz, $^2J_{C–F} = 14.4$ Hz), 146.45, 147.21 (dd, $^1J_{C–F} = 106.2$ Hz, $^2J_{C–F} = 13.9$ Hz), 149.69 (dd, $^1J_{C–F} = 104.8$ Hz, $^2J_{C–F} = 14.2$ Hz), 167.65, 169.69, 177.85. MS (70 eV): *m/z* (%) = 357 (M^+).

*Synthesis of 1-(1-carboxy-2-methylbutyl)-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6d**)*

Yield: 69%. Mp: 197–199°C. IR (KBr) v: 3432, 2989, 1731, 1612, 1482, 1329, 1242, 1121, 1062 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): 0.59–0.62 (m, 6H, CH_3), 1.19–1.21 (m, 1H, CH), 2.07–2.12(m, 2H, CH_2), 5.13–5.16 (m, 1H, CH), 8.21–8.26 (m, 1H), 9.08 (s, 1H, CH), 13.56 (s, br, 2H, COOH). ^{13}C NMR (DMSO-d_6, 100 MHz): 11.87, 15.89, 25.64, 35.89, 68.97, 107.14, 112.19 (d, $^2J_{C–F} = 14.8$ Hz), 125.92 (d, $^3J_{C–F} = 7.1$ Hz), 128.62 (d, $^2J_{C–F} = 14.1$ Hz), 142.65 (dd, $^1J_{C–F} = 106.2$ Hz, $^2J_{C–F} = 14.1$ Hz), 146.99, 147.67 (dd, $^1J_{C–F} = 106.1$ Hz, $^2J_{C–F} = 14.2$ Hz), 149.82 (dd, $^1J_{C–F} = 104.2$ Hz, $^2J_{C–F} = 13.8$ Hz), 166.88, 169.89, 178.22. MS (70 eV): *m/z* (%) = 357 (M^+).

*Synthesis of 1-(carboxy(phenyl)methyl)-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6e**)*

Yield: 74%. Mp: 221–223°C. IR (KBr) v: 3433, 1729, 1631, 1484, 1349, 1243, 1115, 1052 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): 7.11 (s, 1H, CH), 7.43–7.45 (m, 5H, ArH), 8.20–8.22 (m, 1H, ArH), 8.39 (s, 1H, CH), 14.04 (s, br, 2H, COOH). ^{13}C NMR (DMSO-d_6, 100 MHz): 69.79, 108.98, 112.69 (d, $^2J_{C–F} = 14.2$ Hz), 126.45 (d, $^3J_{C–F} = 7.2$ Hz), 127.62, 128.57 (d, $^2J_{C–F} = 14.6$ Hz), 129.68, 130.02, 131.56, 142.74 (dd, $^1J_{C–F} = 106.8$ Hz, $^2J_{C–F} = 14.4$ Hz), 146.57, 147.39 (dd, $^1J_{C–F} = 106.5$ Hz, $^2J_{C–F} = 13.8$ Hz), 150.21 (dd, $^1J_{C–F} = 104.8$ Hz, $^2J_{C–F} = 14.2$ Hz), 165.69, 168.98, 178.68. MS (70 eV): *m/z* (%) = 377 (M^+).

*Synthesis of 1-(1-carboxy-2-phenylethyl)-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline 3-carboxylic acid (**6f**)*

Yield: 72%. Mp: 218–220°C. IR (KBr) v: 3442, 1735, 1645, 1442, 1336, 1213, 1125, 1047 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): 3.71–3.76 (m, 2H, CH_2), 6.06–6.08 (m, 1H, CH), 7.00–7.16 (m, 5H, ArH), 8.18–8.20 (s, 1H, ArH), 8.54 (s, 1H, CH), 13.90 (s, br, 2H, COOH). ^{13}C NMR (DMSO-d_6, 100 MHz): 36.98, 74.88, 109.38, 111.54 (d, $^2J_{C–F} = 13.6$ Hz), 125.54 (d, $^3J_{C–F} = 7.1$ Hz), 126.36, 127.98, 128.69 (d, $^2J_{C–F} = 14.2$ Hz), 129.35, 135.78, 142.88 (dd, $^1J_{C–F} = 107.1$ Hz, $^2J_{C–F} = 14.2$ Hz), 146.68 (dd, $^1J_{C–F} = 106.2$ Hz, $^2J_{C–F} = 14.3$ Hz), 148.27, 149.91 (dd, $^1J_{C–F} = 104.5$ Hz, $^2J_{C–F} = 13.8$ Hz), 166.19, 169.34, 179.11. MS (70 eV): *m/z* (%) = 391 (M^+).

*Synthesis of 1-(carboxymethyl)-6,8-difluoro-7-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7a**)*

Total yield: 56%. Mp: 277–279°C. IR (KBr) v: 3542, 3128, 2988, 2872, 1728, 1628, 1513, 1488, 1462, 1321, 1236, 1088 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): 2.71 (s, 3H, CH_3), 2.82-2.85 (m, 4H, CH_2), 3.14-3.17 (m, 4H, CH_2), 5.78 (d, $J = 6.8$ Hz, 2H, CH_2), 8.19 (m, 1H, ArH), 9.03 (s, 1H), 13.97 (s, br, 1H, COOH), 14.74 (s, br, 1H, COOH). MS (70 eV): *m/z* (%) = 381 (M^+). ^{13}C NMR (DMSO-d_6, 100 MHz): 45.89, 56.89, 56.93, 57.99, 58.03, 59.97, 109.61, 110.38 (d, $^2J_{C–F} = 13.7$ Hz), 123.64 (d, $^3J_{C–F} = 7.2$ Hz), 126.56 (d, $^2J_{C–F} = 14.2$ Hz), 139.22 (dd, $^1J_{C–F} = 103.6$ Hz, $^2J_{C–F} = 12.9$ Hz), 146.62, 147.22 (d, $^1J_{C–F} = 105.1$ Hz), 151.52 (d, $^1J_{C–F} = 104.6$ Hz), 166.67, 170.62, 178.35. Anal. Calcd. for $C_{17}H_{17}F_2N_3O_5$: C, 53.54; H, 4.49; N, 11.02. Found: C, 53.37; H, 4.54; N, 10.98.

*Synthesis of 1-(1-carboxy-2-methylpropyl)-6,8-difluoro-7-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7b**)*

Total yield: 55%. Mp: 274–276°C. IR (KBr) v: 3468, 3028, 2982, 2932, 1742, 1672, 1533, 1488, 1414, 1361, 1246, 1098 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): 0.95 (d, $J = 6.8$ Hz, 6H, CH_3), 2.51–2.53 (m, 1H, CH), 2.64 (s, 3H, CH_3), 2.67–2.69 (m, 4H, CH_2), 3.26–3.28 (m, 1H, CH), 3.66–3.68 (m, 4H, CH_2), 8.16–8.19 (m, 1H, ArH), 9.22 (s,

1H), 12.99 (s, br, 1H, COOH), 13.64 (s, br, 1H, COOH). ^{13}C NMR (DMSO-d_6, 100 MHz): 21.62, 22.23, 46.11, 56.35, 56.41, 57.78, 57.91, 61.07, 109.43, 111.28 (d, $^{2}J_{C-F}$ = 14.2 Hz), 122.85 (d, $^{3}J_{C-F}$ = 6.8 Hz), 126.77 (d, $^{2}J_{C-F}$ = 14.1 Hz), 140.10 (dd, $^{1}J_{C-F}$ = 104.1 Hz, $^{2}J_{C-F}$ = 13.4 Hz), 145.32, 148.11 (d, $^{1}J_{C-F}$ = 104.8 Hz), 150.33 (d, $^{1}J_{C-F}$ = 105.1 Hz), 165.47, 171.92, 179.23. MS (70 eV): *m/z* (%) = 410 (M^+). Anal. Calcd. for $C_{20}H_{23}F_2N_3O_5$: C, 56.73; H, 5.48; N, 9.92. Found: C, 56.82; H, 5.32; N, 10.02.

*Synthesis of 1-(1-carboxy-3-methylbutyl)-6,8-difluoro-7-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7c**)*

Total yield: 51%. Mp: 238–240°C. IR (KBr) v: 3439, 3033, 2968, 2936, 1752, 1645, 1557, 1448, 1434, 1323, 1236, 1078 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): 0.93 (d, J = 6.8 Hz, 6H, CH_3), 1.49–1.51 (m, 1H, CH), 1.62–1.64 (m, 2H, CH_2), 2.03–2.05 (m, 2H, CH_2), 2.74 (s, 3H, CH_3), 3.51–3.54 (m, 1H, CH), 3.78–3.79 (m, 4H, CH_2), 8.12–8.14 (m, 1H, ArH), 9.02 (s, 1H), 13.22 (s, br, 1H, COOH), 13.94 (s, br, 1H, COOH). ^{13}C NMR (DMSO-d_6, 100 MHz): 22.12, 22.34, 25.98, 30.99, 46.45, 56.23, 56.33, 57.45, 57.52, 62.27, 108.86, 112.18 (d, $^{2}J_{C-F}$ = 13.8 Hz), 125.55 (d, $^{3}J_{C-F}$ = 6.9 Hz), 127.13 (d, $^{2}J_{C-F}$ = 14.2 Hz), 141.31 (dd, $^{1}J_{C-F}$ = 104.8 Hz, $^{2}J_{C-F}$ = 14.5 Hz), 146.78, 147.39 (d, $^{1}J_{C-F}$ = 104.8 Hz), 149.89 (d, $^{1}J_{C-F}$ = 104.6 Hz), 166.89, 170.42, 178.14. MS (70 eV): *m/z* (%) = 408 (M^+). Anal. Calcd. for $C_{21}H_{25}F_2N_3O_5$: C, 57.66; H, 5.76; N, 9.61. Found: C, 57.81; H, 5.72; N, 9.72.

*Synthesis of 1-(1-carboxy-2-methylbutyl)-6,8-difluoro-7-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7d**)*

Total yield: 55%. Mp: 239–241°C. IR (KBr) v: 3433, 3023, 2988, 2843, 1734, 1642, 1555, 1447, 1432, 1321, 1232, 1078 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): 0.91 (t, J = 6.8 Hz, 3H, CH_3), 1.09 (d, J = 6.8 Hz, 3H, CH_3), 1.31–1.34 (m, 2H, CH_2), 1.62–1.64 (m, 1H, CH), 2.23 (s, 3H, CH_3), 2.51–2.54 (m, 4H, CH_2), 3.28–3.29 (m, 4H, CH_2), 3.67–3.69 (m, 1H, CH), 8.12–8.14 (m, 1H, ArH), 9.13 (s, 1H), 13.14 (s, br, 1H, COOH), 13.59 (s, br, 1H, COOH). ^{13}C NMR (DMSO-d_6, 100 MHz): 11.56, 15.43, 23.63, 29.38, 46.67, 56.31, 56.65, 57.67, 57.86, 66.49, 108.89, 111.98 (d, $^{2}J_{C-F}$ = 14.1 Hz), 124.89 (d, $^{3}J_{C-F}$ = 7.1 Hz), 126.63 (d, $^{2}J_{C-F}$ = 14.6 Hz), 141.39 (dd, $^{1}J_{C-F}$ = 105.2 Hz, $^{2}J_{C-F}$ = 13.9 Hz), 145.89, 147.54 (d, $^{1}J_{C-F}$ = 105.1 Hz), 150.06 (d, $^{1}J_{C-F}$ = 104.9 Hz), 165.99, 169.69, 179.32. MS (70 eV): *m/z* (%) = 437 (M^+). Anal. Calcd. for $C_{21}H_{25}F_2N_3O_5$: C, 57.66; H, 5.76; N, 9.61. Found: C, 57.75; H, 5.78; N, 9.73.

*Synthesis of 1-(carboxy(phenyl)methyl)-6,8-difluoro-7-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7e**)*

Total yield: 54%. Mp: 205–207°C. IR (KBr) v: 3343, 3091, 3072, 1736, 1631, 1484, 1349, 1243, 1114, 1052 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): 2.42–2.46 (m, 4H, CH_2), 2.67 (s, 3H, CH_3), 3.31–3.33 (m, 4H, CH_2), 3.75–3.77 (m, 1H, CH), 7.44–7.46 (m, 5H, ArH), 8.20–8.24 (m, 1H, ArH), 9.16 (s, 1H), 13.96 (s, br, 1H, COOH), 14.11 (s, br, 1H, COOH). ^{13}C NMR (DMSO-d_6, 100 MHz): 45.78, 55.98, 56.11, 57.45, 57.65, 70.12, 109.31, 112.12 (d, $^{2}J_{C-F}$ = 13.8 Hz), 124.59 (d, $^{3}J_{C-F}$ = 6.9 Hz), 126.72 (d, $^{2}J_{C-F}$ = 14.1 Hz), 127.66, 128.76, 128.98, 129.34, 130.21, 134.54, 142.22 (dd, $^{1}J_{C-F}$ = 104.6 Hz, $^{2}J_{C-F}$ = 13.8 Hz), 146.27, 147.66 (d, $^{1}J_{C-F}$ = 104.8 Hz), 149.25 (d, $^{1}J_{C-F}$ = 104.2 Hz), 165.49, 169.22, 178.82. MS (70 eV): *m/z* (%) = 457 (M^+). Anal. Calcd. for $C_{23}H_{21}F_2N_3O_5$: C, 60.39; H, 4.63; N, 9.19. Found: C, 59.99; H, 4.70; N, 9.23.

*Synthesis of 1-(1-carboxy-2-phenylethyl)-6,8-difluoro-7-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7f**)*

Total yield: 56%. Mp: 225-227°C. IR (KBr) v: 3342, 3106, 3087, 1735, 1642, 1493, 1380, 1257, 1112, 1065 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): 2.33–2.35 (m, 4H, CH_2), 2.71 (s, 3H, CH_3), 3.42–3.44 (m, 4H, CH_2), 3.53–3.54 (m, 1H, CH), 3.71–3.75 (m, 2H), 7.11–7.16 (m, 5H, ArH), 8.11–8.20 (m, 1H, ArH), 8.55 (s, 1H), 13.21 (s, br, 1H, COOH), 13.98 (s, br, 1H, COOH). ^{13}C NMR (DMSO-d_6, 100 MHz): 39.67, 46.18, 55.11, 55.89, 57.15, 57.79, 75.17, 109.76, 112.92 (d, $^{2}J_{C-F}$ = 14.5 Hz), 125.12 (d, $^{3}J_{C-F}$ = 7.1 Hz), 126.12 (d, $^{2}J_{C-F}$ = 14.6 Hz), 127.69, 128.21, 128.69, 129.54, 129.77, 130.15, 142.46 (dd, $^{1}J_{C-F}$ = 104.2 Hz, $^{2}J_{C-F}$ = 14.1 Hz), 146.95 (d, $^{1}J_{C-F}$ = 104.6 Hz), 148.17, 150.18 (d, $^{1}J_{C-F}$ = 103.9 Hz), 165.57, 168.82, 179.96. MS (70 eV): *m/z* (%) = 471 (M^+). Anal. Calcd. for $C_{24}H_{23}F_2N_3O_5$: C, 61.14; H, 4.92; N, 8.91. Found: C, 61.31; H, 5.01; N, 9.01.

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