Statistical analysis was performed with SPSS Version 11.0 statistic software package. Data were expressed as means ± standard deviation (SD). Comparisons between groups were performed with analysis of non-parametric test. A value of $P < 0.05$ was considered statistically significant.

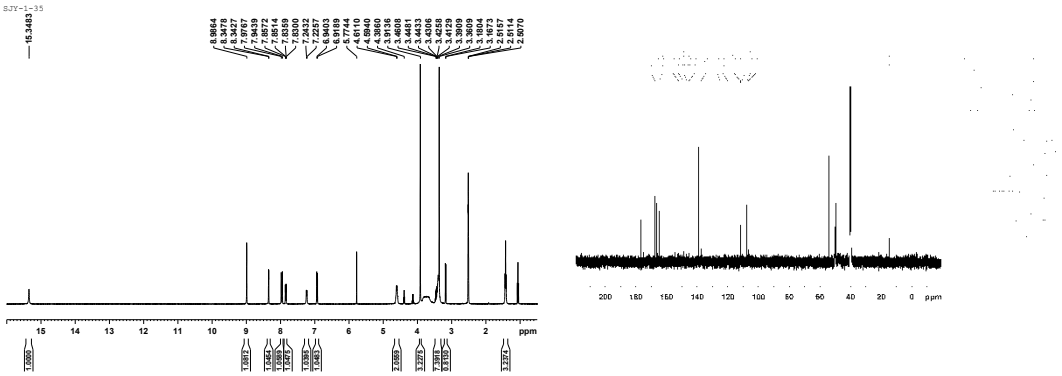
All of the synthesized compounds were chemically characterized by thin layer chromatography (TLC), proton nuclear magnetic resonance (^{1}H NMR) and elemental microanalyses (CHN). ^{1}H NMR spectra were measured on a Bruker AV-400 spectrometer at 25°C and referenced to Me_4Si. Chemical shifts were reported in ppm (δ) using the residual solvent line as internal standard. Splitting patterns are designed as s, singlet; d, doublet; t, triplet; m, multiplet. ESI-MS spectra were recorded on a Mariner System 5304 Mass spectrometer. Elemental analyses were performed on a CHN-O-Rapid instrument and were within ± 0.4 % of the theoretical values. Melting points were determined on a WRS-1B apparatus (Jingke Corp., Shanghai, China) and are as read. Analytic thin-layer chromatography (TLC) was performed on the glass-backed silica gel sheets (silica gel 60 Å GF254). All compounds were detected using UV light (254 nm or 365 nm).

The synthesis of series fluoroquinolone derivatives was started from different substituted nicotinic or salicylic acid by dissolved in $SOCl_2$ to prepare the corresponding chlorides. Then, these corresponding chlorides gave fluoroquinolone derivatives by using carbodiimide hydrochloride and N-hydroxybenzotriazole in anhydrous CH_2Cl_2 with ciprofloxacin or norfloxacin.

1-Ethyl-6-fluoro-7-(4-(6-methoxynicotinoyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (D1)

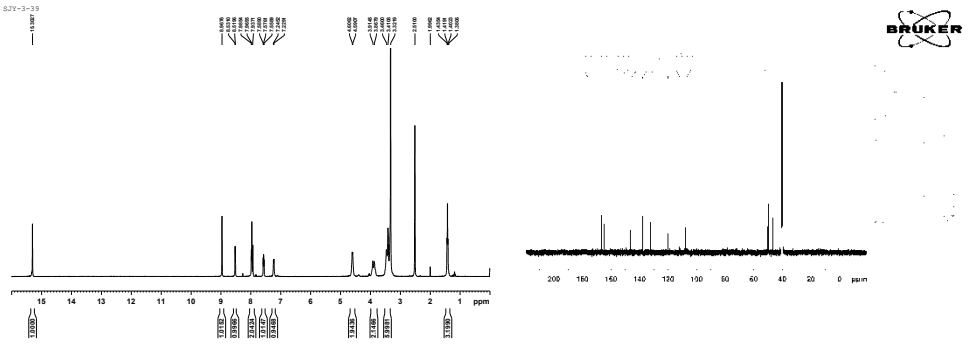
Mp: 258.7-259.7°C. ^{1}H NMR (400 MHz, DMSO): δ 1.40-1.44 (m, 3H), 3.17-3.18 (d, J = 5.2 Hz, 1H), 3.36-3.46 (m, 7H), 3.91 (s, 3H), 4.59-4.61 (d, J = 6.8 Hz, 2H), 6.92-6.94 (d, J = 8.4 Hz, 1H), 7.23-7.24 (d, J = 6.8 Hz, 1H), 7.83-7.86 (m, 1H), 7.94-7.98 (m, 1H), 8.34-8.35 (m, 1H), 8.99 (s, 1H), 15.35 (s, 1H). ^{13}C NMR (100 MHz, DMSO-

d_6):176.57, 167.53, 166.51, 164.63, 152.02, 148.97, 146.69, 145.60(d, J=10.36 Hz), 139.07, 137.56, 125.16, 120.02(d, J=7.8 Hz), 111.66(d, J=2.00 Hz), 110.80, 107.59, 106.67, 53.98(2), 49.97, 49.53, 21.21, 14.86(2). IR (KBr, ν, cm^{-1}): 3460, 3051, 2980, 2947, 2907, 2841, 1727, 1625, 1516, 1468, 1372, 1287, 1252, 1215, 1159, 1111, 1007. MS (ESI): 455 ($C_{23}H_{24}FN_4O_5$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{23}FN_4O_5$: C, 60.79; H, 5.10; N, 12.33%; Found: 60.73; H, 5.14; N, 12.30%.



7-(4-(2-Chloronicotinoyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (D2)

Mp: 216.7-217.9°C. ^{1}H NMR (400 MHz, DMSO): δ 1.38-1.44 (m, 3H), 3.41-3.46 (m, 6H), 3.87-3.92 (m, 2H), 4.59-4.61 (m, 2H), 7.23-7.25 (d, J = 6.4 Hz, 1H), 7.56-7.59 (m, 1H), 7.94-7.99 (m, 2H), 8.52-8.53 (d, J = 4.4 Hz, 1H), 8.97 (s, 1H), 15.30 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6):176.62(d, J=2.65 Hz), 166.51, 164.68, 150.84, 149.06, 146.31, 145.47(d, J=10.17 Hz), 138.12, 137.59, 132.23, 124.02, 120.12(d, J=7.79 Hz), 111.85, 111.62, 107.62, 106.83, 50.08, 49.54, 46.49, 41.42, 14.89(2). IR (KBr, ν, cm^{-1}):3483, 3049, 2988, 2863, 1721, 1627, 1556, 1475, 1395, 1295, 1260, 1214, 1169, 1114, 1007. MS (ESI): 459 ($C_{22}H_{21}ClFN_4O_4$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{20}ClFN_4O_4$: C, 57.58; H, 4.39; N, 12.21%; Found: C, 57.62; H, 4.33, N, 12.18%.



1-Cyclopropyl-6-fluoro-7-(4-(6-methoxynicotinoyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (C1)

Mp: 234.4-235.6°C. ^{1}H NMR (400 MHz, DMSO): δ 1.19 (s, 2H), 1.33-1.34 (d, J = 6.4 Hz, 2H), 3.35 (s, 8H), 3.81-3.83 (m, 1H), 3.89-3.91 (d, J = 11.6 Hz, 3H), 6.92-6.94 (d, J = 8.4 Hz, 1H), 7.60-7.62 (d, J = 7.2 Hz, 1H), 7.83-7.86 (m, 1H), 7.94-7.98 (d, J = 13.2 Hz, 1H), 8.35-8.35 (m, 1H), 8.69 (s, 1H), 15.21 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6):176.81, 167.54, 166.34, 164.63, 152.16, 148.49, 146.71, 145.30(d, J=10.19 Hz), 139.58, 139.11, 125.17, 119.37, 111.59, 111.36, 110.81, 107.24, 107.16, 53.99(2), 49.88, 36.35(2), 8.06(2). IR (KBr, ν, cm^{-1}):3440, 3060, 2961, 2854, 1722, 1628, 1541, 1455, 1337, 1284, 1252, 1216, 1160, 1109, 1004. MS (ESI): 467 ($C_{24}H_{24}FN_4O_5$, [M+H]$^+$). Anal. Calcd for $C_{24}H_{23}FN_4O_5$: C, 61.80; H, 4.97; N, 12.01%; Found: C, 61.77; H, 5.02; N, 11.99%.

7-(4-(2-Chloronicotinoyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (C2)

Mp: 273.9-274.7°C. ^{1}H NMR (400 MHz, DMSO): δ 1.19 (s, 2H), 1.32-1.34 (m, 2H), 3.35-3.43 (m, 8H), 3.83 (s, 1H), 7.57-7.62 (m, 2H), 7.93-8.00 (m, 2H), 8.53-8.57 (m, 1H), 8.64 (s, 1H), 15.19 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6):176.72, 166.33, 164.71, 160.41, 150.85, 148.55, 146.32, 139.60, 138.15, 132.23, 124.04, 120.46, 119.03, 115.38, 111.65, 107.28, 49.94, 49.47, 46.45, 41.37, 36.37, 8.06(2). IR (KBr, ν, cm^{-1}):3477, 3276, 3096, 2988, 2887, 2845, 1711, 1629, 1548, 1470, 1399, 1264, 1251, 1213, 1167, 1096, 1007. MS (ESI): 471 ($C_{23}H_{21}ClFN_4O_4$, [M+H]$^+$). Anal. Calcd for $C_{23}H_{20}ClFN_4O_4$: C, 58.67; H, 4.28%; N, 11.90; Found: C, 58.71; H, 4.31; N, 11.85%.

7-(4-(5-Chloro-2-hydroxybenzoyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (A1)

Mp: 256.3-259.6°C. ^{1}H NMR (400 MHz, DMSO): δ 1.17-1.18 (m, 2H), 1.32-1.34 (m, 2H), 2.00 (s, 1H), 3.36 (s, 8H), 4.03-4.05 (m, 1H), 6.91-6.93 (d, J = 8.4 Hz, 1H), 7.24-7.24 (m, 1H), 7.30-7.32 (m, 1H), 7.59-7.60 (m, 1H), 7.93-7.96 (d, J = 13.2 Hz, 1H), 8.68 (s, 1H), 10.24 (s, 1H). 13C NMR (100 MHz, DMSO-d6):176.86, 166.35, 164.23, 163.55, 159.04, 148.58, 146.07, 138.44, 133.54(d, J=10.19 Hz), 131.93, 130.75, 126.16, 120.59, 119.22, 116.47, 110.28, 107.29, 60.21, 46.71, 41.43, 36.35, 14.55, 8.07(2). IR (KBr, ν, cm^{-1}):3402, 3266, 3072, 2920, 2827, 2711, 1711, 1627, 1551, 1446, 1390,137, 1285, 1251, 1205, 1150, 1044, 1012. MS (ESI): 486 ($C_{24}H_{22}ClFN_3O_5$, [M+H] $^{+}$). Anal. Calcd for $C_{24}H_{21}ClFN_3O_5$: C, 59.33; H, 4.36; N, 8.65%; Found: C, 59.38; H, 4.31; N, 8.70%.

1-Cyclopropyl-6-fluoro-7-(4-(2-hydroxy-4-methoxybenzoyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (A2)

Mp: 261.3-261.9°C. ^{1}H NMR (400 MHz, DMSO): δ 1.16-1.19 (m, 2H), 1.33-1.34 (m, 2H), 2.00 (m, 1H), 3.35 (s, 8H), 3.36 (s, 3H), 4.18-4.19 (m, 1H), 6.98-7.00 (m, 2H), 7.26-7.28 (m, 2H), 7.96-7.97 (m, 1H), 8.70 (s, 1H), 10.55 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6):176.83, 166.32(t, J=35.09 Hz), 160.78, 148.52, 147.80, 146.76, 141.13, 139.56, 130.93, 128.13, 127.48, 126.80, 124.09, 121.23, 118.19, 110.47, 107.27, 107.04, 60.21, 46.73, 36.33, 21.79, 21.18, 14.55, 8.03(2). IR (KBr, ν, cm^{-1}): 3429, 3182, 3089, 3028, 2938, 2921, 2860, 2707, 1721, 1626, 1542, 1469, 1335, 1299, 1252, 1209, 1147, 1011. MS (ESI): 482 ($C_{25}H_{25}FN_3O_6$, [M+H] $^{+}$). Anal. Calcd for $C_{25}H_{24}FN_3O_6$: C, 62.36; H, 5.02; N, 8.73%; Found: C, 62.40; H, 4.96; N, 8.70%.

1-Cyclopropyl-6-fluoro-7-(4-(2-hydroxy-3,5-dinitrobenzoyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (A3)

Mp: 261.3-261.9°C. ^{1}H NMR (400 MHz, DMSO): δ 1.16-1.18 (m, 2H), 1.33-1.36 (m, 2H), 2.00 (s, 1H), 3.34 (s, 8H), 4.15-4.18 (m, 1H), 6.97-6.98 (m, 2H), 7.33-7.36 (m,

1H), 7.95-7.97 (m, 1H), 8.73 (s, 1H), 10.50 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6):176.57, 166.79, 161.32, 163.13, 158.21, 153.38, 149.20, 146.13, 141.28, 136.37, 132.54(d, J=8.12 Hz), 129.48, 128.01, 126.60, 113.27, 111.52, 107.29, 58.72, 43.76, 41.62, 36.36, 14.20, 8.07(2). IR (KBr, ν, cm^{-1}):3441, 3216, 3035, 2932, 2887, 2734, 2553, 1712, 1657, 1597, 1526, 1484, 1423, 1350, 1270, 1159, 1083, 1059. MS (ESI): 542 ($C_{24}H_{21}FN_5O_9$, [M+H]$^+$). Anal. Calcd for $C_{24}H_{20}FN_5O_9$: C, 53.24; H, 3.72; N, 12.93%; Found: C, 53.35; H, 3.69; N, 12.98%.

7-(4-(5-Bromo-2-hydroxybenzoyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (A4)

Mp: 270.8-271.3°C. ^{1}H NMR (400 MHz, DMSO): δ 1.16-1.20 (m, 2H), 1.32-1.35 (m, 2H), 2.00 (s, 1H), 3.36-3.42 (m, 8H), 6.87-6.89 (d, J = 8.8 Hz, 1H), 7.35-7.36 (m, 1H), 7.41-7.44 (m, 1H), 7.59-7.60 (d, J = 6.4 Hz, 1H), 7.91-7.95 (m, 1H), 8.67 (s, 1H), 10.27 (s, 1H), 15.20 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6):176.79, 166.32, 165.92, 164.23, 163.55, 159.05, 153.16, 148.49(d, J=7.45 Hz), 146.07, 139.55, 133.36, 131.92, 130.86(d, J=21.02 Hz), 126.25(d, J=25.85 Hz), 120.58, 119.22, 116.44, 107.25(d, J=3.22 Hz), 60.21, 36.35(2), 21.22, 14.55, 8.06(2). IR (KBr, ν, cm^{-1}): 3455, 3261, 3069, 3000, 2883, 2829, 2711, 1711, 1627, 1549, 1469, 1388, 1283, 1253, 1205, 1108, 1013. MS (ESI): 530 ($C_{24}H_{22}BrFN_3O_5$, [M+H]$^+$). Anal. Calcd for $C_{24}H_{21}BrFN_3O_5$: C, 54.35; H, 3.99; N, 7.92%; Found: C, 54.41; H, 4.05; N, 7.86%.

1-Cyclopropyl-6-fluoro-7-(4-(2-hydroxy-5-nitrobenzoyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (A5)

Mp: 270.8-271.3°C. ^{1}H NMR (400 MHz, DMSO): δ 1.17-1.19 (m, 2H), 1.33-1.36 (m, 2H), 2.00 (s, 1H), 3.36-3.40 (m, 8H), 6.89-6.90 (m, 2H), 7.33-7.34 (m, 1H), 7.41-7.43 (m, 1H), 7.58-7.79 (m, 1H), 8.67 (s, 1H), 10.27 (s, 1H), 15.20 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6):176.77, 166.32, 165.25, 160.09, 154.53, 151.91, 148.45, 145.26(d, J=10.15 Hz), 140.07, 139.56, 132.47, 130.56, 126.95, 124.88, 116.78, 111.74, 107.23, 51.34, 49.35, 42.56, 36.33, 18.20, 8.05(2). IR (KBr, ν, cm^{-1}): 3455, 3261, 3069, 3000, 2883, 2829, 2711, 1711, 1627, 1549, 1469, 1388, 1283, 1253, 1205, 1108, 1013. MS

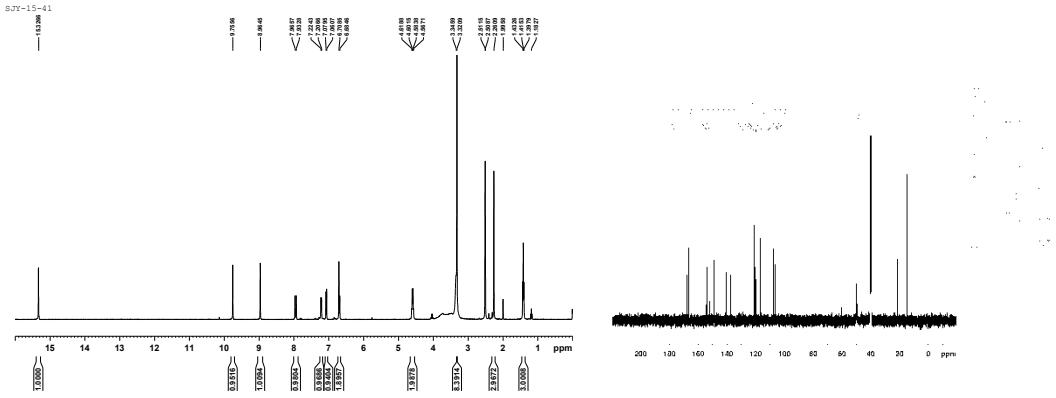
(ESI): 497 ($C_{24}H_{22}FN_4O_7$, $[M+H]^+$). Anal. Calcd for $C_{24}H_{21}FN_4O_7$: C, 58.06; H, 4.26; N, 11.29%; Found: C, 58.12; H, 4.21; N, 11.33%.

7-(4-(5-Chloro-2-hydroxybenzoyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (B1)

Mp: 311.2-311.3°C. ^{1}H NMR (400 MHz, DMSO): δ 1.40-1.43 (m, 3H), 3.42 (s, 6H), 3.83 (s, 2H), 4.57-4.62 (m, 2H), 6.91-6.93 (d, J = 8.4 Hz, 1H), 7.21-7.23 (d, J = 7.6 Hz, 2H), 7.29-7.31 (d, J = 8.8 Hz, 1H), 7.94-7.97 (m, 1H), 8.97 (s, 1H), 10.21 (s, 1H), 15.32 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6):176.61, 166.53, 166.01, 152.71, 148.98, 145.62(d, J=10.08 Hz), 137.59, 130.44, 128.11, 125.87, 123.15, 119.96(d, J=7.66 Hz), 117.95, 111.79, 111.56, 107.58, 106.63, 50.24, 49.52, 46.45, 41.35, 14.88(2). IR (KBr, ν, cm^{-1}):3440, 3049, 2987, 2912, 2696, 2577, 1727, 1627, 1554, 1474, 1444, 1368, 1285, 1255, 1214, 1113, 1016. MS (ESI): 474 ($C_{23}H_{22}ClFN_3O_5$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{21}ClFN_3O_5$: C, 58.30; H, 4.47; N, 8.87%; Found: C, 58.34; H, 4.45; N, 8.11%.

1-Ethyl-6-fluoro-7-(4-(2-hydroxy-6-methylbenzoyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (B2)

Mp: 260.5-261.7°C. ^{1}H NMR (400 MHz, DMSO): δ 1.18-1.33 (m, 3H), 2.26 (s, 3H), 3.32 (s, 8H), 4.57-4.62 (m, 2H), 6.69-6.71 (m, 1H), 6.69-6.71 (m, 1H), 7.06-7.08 (d, J = 7.6 Hz, 1H), 7.21-7.22 (d, J = 6.8 Hz, 1H), 7.93-7.97 (m, 1H), 8.96 (s, 1H), 9.76 (s, 1H), 15.33 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6):176.56(d, J=2.56 Hz), 167.82, 166.52, 153.81, 152.02, 148.93, 145.66(d, J=10.18 Hz), 140.58, 137.57, 128.71, 121.13, 120.41, 119.94, 119.87, 116.59, 111.62(d, J=22.71 Hz), 107.56, 106.61, 60.21, 50.15, 49.51, 21.41, 14.86(2). IR (KBr, ν, cm^{-1}):3421, 3052, 2965, 2891, 2734, 2603, 1722, 1624, 1555, 1481, 1384, 1307, 1254, 1204, 1110, 1014. MS (ESI): 454 ($C_{24}H_{25}FN_3O_5$, $[M+H]^+$). Anal. Calcd for $C_{24}H_{24}FN_3O_5$: C, 63.57; H, 5.33; N, 9.27%; Found: C, 63.59; H, 5.29; N, 9.26%.



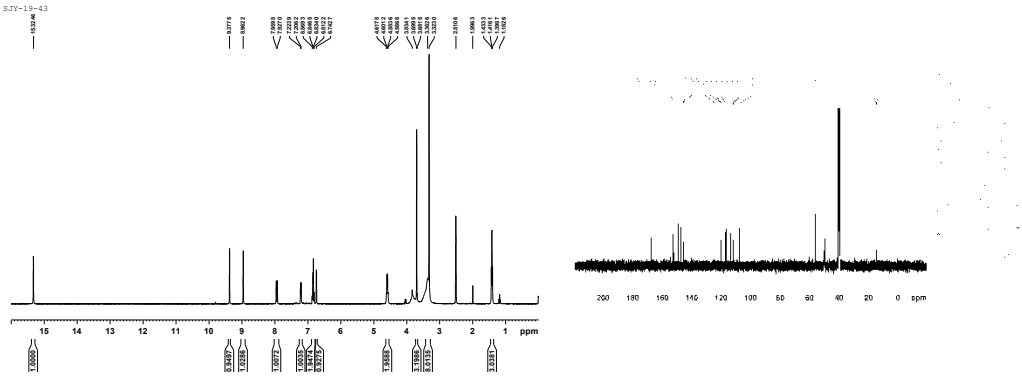
7-(4-(5-Bromo-2-hydroxybenzoyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (B3)

Mp: 249.1-251.8°C. ^{1}H NMR (400 MHz, DMSO): δ 1.40-1.44 (m, 3H), 3.41 (s, 6H), 4.03-4.05 (m, 2H), 4.59-4.61 (m, 2H), 6.87-6.89 (m, 1H), 7.21-7.26 (m, 1H), 7.35 (s, 1H), 7.41-7.45 (m, 1H), 7.95-7.98 (m, 1H), 8.97 (s, 1H), 10.24 (s, 1H), 15.33 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6):176.58, 166.52, 165.90, 153.14, 148.98, 145.62(d, J=12.00 Hz), 137.59, 133.32, 130.93, 126.42, 119.96(d, J=7.73 Hz), 118.45, 111.79, 111.56, 110.60, 107.58, 106.62, 50.23, 49.52, 46.46, 41.40, 14.88(2). IR (KBr, ν, cm^{-1}):3467, 3051, 2984, 2909, 2837, 2691, 1724, 1626, 1581, 1474, 14445, 1368, 1285, 1255, 1214, 1086, 1016. MS (ESI): 518 ($C_{23}H_{22}BrFN_3O_5$, [M+H]$^{+}$). Anal. Calcd for $C_{23}H_{21}BrFN_3O_5$: C, 53.30; H, 4.08; N, 8.11%; Found: C, 53.31; H, 4.09; N, 8.15%.

1-Ethyl-6-fluoro-7-(4-(2-hydroxy-5-methoxybenzoyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (B4)

Mp: 322.8-324.8°C. ^{1}H NMR (400 MHz, DMSO): δ 1.40-1.43 (m, 3H), 3.32-3.36 (m, 8H), 3.68-3.70 (m, 3H), 4.57-4.62 (m, 2H), 6.74 (s, 1H), 6.81-6.87 (m, 2H), 7.21-7.22 (d, J = 7.2 Hz, 1H), 7.93-7.96 (m, 1H), 8.96 (s, 1H), 9.38 (s, 1H), 15.33 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6):176.61, 167.24, 166.53, 152.55, 152.04, 148.98, 147.43, 145.67(d, J=10.08 Hz), 137.61, 124.40, 119.93(d, J=7.51 Hz), 117.09, 116.51, 113.33, 111.67(d, J=23.13 Hz), 107.58, 106.63, 55.93(2), 50.11, 49.52, 14.87(2), 14.54. IR

(KBr, ν, cm^{-1}):3472, 3229, 3045, 2981, 2832, 2713, 1706, 1630, 1506, 1475, 1383, 1252, 1203, 1112, 1015. MS (ESI): 470 ($C_{24}H_{25}FN_3O_6$, $[M+H]^+$). Anal. Calcd for $C_{24}H_{24}FN_3O_6$: C, 61.40; H, 5.15; N, 8.95%; Found: C, 61.44; H, 5.13; N, 8.92%.



Single crystal *X*-ray diffraction data was collected on a Bruker *D*-8 venture diffractometer at room temperature (293 K). The *X*-ray generator was operated at 50 KV and 35 mA using Mo Kα radiation (λ = 0.71073 Å). The data was collected using SMART software package. The data were reduced by SAINT-PLUS, an empirical absorption correction was applied using the package SADABS and XPREP were used to determine the space group. The crystal structure was solved by direct methods using SIR92 and refined by full-matrix least-squares method using SHELXL97 [29, 30]. All non-hydrogen atoms were refined anisotropic ally and hydrogen atoms have been refined in the riding mode on their carrier atoms wherever applicable.

The activity of the gamma isoforms was evaluated using the purified catalytic p110*γ* subunit (PIK3CG) (ref PV4786 from Invitrogen).The activity of the alpha isoforms of PI3K was evaluated using a purified heterodimer (ref PV4788 from Invitrogen) composed of the catalytic p110*α* subunit (PIK3CA) and the regulatory p85*α* subunit (PIK3R1). The activity of the beta isoforms of PI3*β* was evaluated using

a purified heterodimer composed of the catalytic p110β subunit (PIK3CB) and the regulatory p85β subunit. The ADAPTA^{TM} kinase assay kit (Invitrogen) is a fluorescence-based immunoassay for the detection of ADP. The experiments were performed according to the manufacturer's instructions.

The in vitro anticancer activities of the prepared compounds against tumor cell lines were evaluated as described in the literature (Chen et al., 2005) with some modifications. Target tumor cells were grown to log phase in DMEM medium supplemented with 10% fetal bovine serum. After reaching a dilution of 5×10^4 cells mL^{-1} with the medium, 100 μL of the obtained cell suspension was added to each well of 96-well culture plates. Subsequently, incubation was performed at 37 ˚C in 5% CO_2 atmosphere for 48 h before the cytotoxicity assessment. Tested samples at preset concentrations were added to 6 wells with CAL-101 being employed as a positive reference. After 72 h exposure period, 25 μL of PBS containing 2.5 mg mL^{-1} of MTT was added to each well. After 4 h, the medium was replaced by 150 μL DMSO to dissolve the purple formazan crystals produced. The absorbance at 570 nm of each well was measured with an ELISA plate reader. The data represented the mean of three independent experiments in triplicate and were expressed as means ± SD. The IC_{50} value was defined as the concentration at which 50% of the cells could survive.

Molecular docking of compound **A3** into the three dimensional X-ray structure of human PI3K (PDB code: 1E8Z) was carried out using the Discovery Studio (version 3.5) as implemented through the graphical user interface DS-CDOCKER protocol. The three-dimensional structures of the aforementioned compounds were constructed using Chem. 3D ultra 12.0 software [Chemical Structure Drawing Standard; Cambridge Soft corporation, USA (2010)], then they were energetically minimized by using MMFF94 with 5000 iterations and minimum RMS gradient of 0.10. The crystal structures of protein complex were retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). All bound waters and ligands were eliminated from the protein and the polar hydrogen was added to the proteins.

Molecular docking of all thirteen compounds was then carried out using the Discovery Stutio (version 3.5) as implemented through the graphical user interface CDOCKER protocol. CDOCKER is an implementation of a CHARMm based molecular docking tool using a rigid receptor. It includes the following steps:

(1) A series of ligand conformations are generated using high temperature molecular dynamics with different random seeds.

(2) Random orientations of the conformations are generated by translating the center of the ligand to a specified position within the receptor active site, and making a series of random rotations. A softened energy is calculated and the orientation is kept when it is less than a specified limit. This process repeats until either the desired number of low-energy orientations is obtained, or the test times of bad orientations reached the maximum number.

(3) Each orientation is subjected to simulated annealing molecular dynamics. The temperature is heated up to a high temperature then cooled to the target temperature. A final energy minimization of the ligand in the rigid receptor using non-softened potential is performed.

(4) For each of the final pose, the CHARMm energy (interaction energy plus ligand strain) and the interaction energy alone are figured out. The poses are sorted according to CHARMm energy and the top scoring (most negative, thus favorable to binding) poses are retained. CHARMm was selected as the force field. The molecular docking was performed with a simulated annealing method. The heating steps were 2000 with 700 of heating target temperature. The cooling steps were 5000 with 300 cooling target temperature. Ten molecular docking poses saved for each ligand were ranked according to their dock score function. The pose with the highest -CDOCKER energy was chosen as the most suitable pose.