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Conformationally Restricted Quinazolone Derivatives as PI3Kδ-selective Inhibitors: the Design, Synthesis and Biological Evaluation

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1. Experimental

1.1. Chemistry

The reagents and solvents for reaction were purchased from common commercial suppliers. If necessary, purification was carried out prior to use. ^{1}H NMR and ^{13}C NMR spectra were recorded on 500 MHz (or 400 MHz) and 100 MHz instruments (Bruker Bioscience, Billerica, MA, USA), respectively, with tetramethylsilane (TMS) as internal standard. ESI-MS spectral data were obtained by Esquire-LC-00075 spectrometer (Bruker Bioscience) and HRMS spectral data were obtained by Waters Q-TOF Micromass. Flash column chromatography was performed using silica gel (200–300 mesh). HPLC of target compounds was performed using a Shimadzu Essentia LC-16 system with UV detection at 254 nm, eluting with a binary solvent system containing A and B [A: H_2O with 0.05% phosphoric acid (W/V); B: CH_3CN]. Analytical purity of all tested compounds was over 95%.

1.1.1. General procedure for the preparation of amide intermediates 11-16

To a solution of 2-nitrobenzoic acid or 2-fluoro-6-nitrobenzoic acid (1.0 equiv) in thionyl chloride (1 mL/1 mmol substrate) was added an catalytic amount of DMF and the mixture was heated to reflux for 2 h. After being cooled to the room temperature, it was concentrated in vacuo and the residue was dissolved in DCM (2 mL/1 mmol substrate). The resultant solution was then added dropwise to a solution of corresponding amine (1.0 equiv), such as benzylamine, cyclobutylamine, cyclopentylamine, cyclohexylamine or n-butylamine, and TEA (1.2 equiv) in DCM (2 mL/1 mmol substrate) at 0 °C. After being stirred at room temperature for 0.5 h, the reaction mixture was quenched with saturated $NaHCO_3$ solution. The organic layer was then washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to provide the crude product. It was purified by flash column chromatography using EA/PE (1:6) as eluent to give corresponding amide intermediate as light yellow solid (Yield: 83-94%).

1.1.2. General procedure for the preparation of imide intermediates 17-22

To a solution of the amide **11** (or **12-16**, 1.0 equiv) in $SOCl_2$ (3 mL/1 mmol substrate) was added an catalytic amount of DMF and the mixture was heated to reflux for 4 h. After removal of $SOCl_2$, the residue was disolved in anhydrous DCM (2.5 mL/1 mmol substrate). The resultant solution was then added dropwise to a solution of (*S*)-1-*N*-Boc-proline (1.2 equiv) and TEA (1.2 equiv) in anhydrous DCM (2.5 mL/1 mmol substrate) at 0 °C under N_2 atmosphere. The reaction mixture was then stirred at room temperature for 2 h. Afterwards, it was quenched with saturated $NaHCO_3$ solution, and the

organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was finally purified by flash column chromatography using EA/PE (1:3) as eluent to give the imide **17** (or **18-22**) as the intermediate. The ^{1}H NMR spectra of **18-22** indicated a mixture of rotamers.

1.1.2.1. *tert*-butyl (*S*)-2-(benzyl (2-nitrobenzoyl) carbamoyl) pyrrolidine-1-carboxylate (**17**)

White foam; yield: 65%; ESI-MS: m/z = 454 $[M+H]^+$.

1.1.2.2. *tert*-butyl (*S*)-2-(benzyl (2-fluoro-6-nitrobenzoyl) carbamoyl) pyrrolidine-1-carboxylate (**18**)

White foam; yield: 71%; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 8.24–8.12 (m, 1H), 7.95–7.68 (m, 2H), 7.61–7.01 (m, 5H), 5.33–4.61 (m, 3H), 3.30–3.17 (m, 2H), 2.28–2.02 (m, 1H), 1.86–1.47 (m, 3H), 1.45–1.09 (1.35, 1.34, 1.23, three singlets, 9H); ESI-MS: m/z = 472 $[M+H]^+$.

1.1.2.3. *tert*-butyl (*S*)-2-(cyclobutyl (2-fluoro-6-nitrobenzoyl) carbamoyl) pyrrolidine-1-carboxylate (**19**)

White foam; yield: 69%; ^{1}H NMR (500 MHz, DMSO-*d6*): δ 8.21–8.11 (m, 1H), 7.90–7.74 (m, 2H), 4.99–4.93 (m, 0.6H), 4.88–4.81 (m, 0.4H), 4.61–4.48 (m, 1H), 3.31–3.25 (m, 2H, partially overlaps with H_2O signal), 2.74–2.58 (m, 2H), 2.39–2.29 (m, 1H), 2.26–2.12 (m, 2H), 1.88–1.75 (m, 3H), 1.71–1.61 (m, 2H), 1.43–1.23 (1.36, 1.33, two singlets, 9H); ESI-MS: m/z = 436 $[M+H]^+$.

1.1.2.4. *tert*-butyl (*S*)-2-(cyclopentyl (2-fluoro-6-nitrobenzoyl) carbamoyl) pyrrolidine-1-carboxylate (**20**)

White foam; yield: 58%; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 8.22–8.09 (m, 1H), 7.91–7.81 (m, 1H), 7.79–7.69 (m, 1H), 5.13–4.92 (m, 1H), 4.56–4.39 (m, 1H), 3.30–3.22 (m, 2H, partially overlaps with H_2O signal), 2.43–2.29 (m, 1H), 2.14–1.97 (m, 2H), 1.95–1.74 (m, 6H), 1.72–1.60 (m, 1H), 1.58–1.45 (m, 2H), 1.38–1.28 (1.36, 1.34, 1.32, three singlets, 9H); ESI-MS: m/z = 450 $[M+H]^+$.

1.1.2.5. *tert*-butyl (*S*)-2-(cyclohexyl (2-fluoro-6-nitrobenzoyl) carbamoyl) pyrrolidine-1-carboxylate (**21**)

White foam; yield: 57%; ^{1}H NMR (500 MHz, DMSO-*d6*): δ 8.21–8.06 (m, 1H), 7.88–7.66 (m, 2H), 5.04–4.82 (m, 1H), 3.96–3.72 (m, 1H), 3.31–3.19 (m, 2H, partially overlaps with H_2O signal), 2.44–2.22 (m, 3H), 1.89–1.71 (m, 6H), 1.66–1.51 (m, 2H), 1.43–1.20 (m, 11H), 1.17–1.08 (m, 1H); ESI-MS: m/z = 464 $[M+H]^+$.

1.1.2.6. *tert*-butyl (*S*)-2-(butyl (2-fluoro-6-nitrobenzoyl) carbamoyl) pyrrolidine-1-carboxylate (**22**)

White foam; yield: 63%; ^{1}H NMR (500 MHz, DMSO-*d6*): δ 8.22–8.09 (m, 1H), 7.89–7.81 (m, 1H), 7.79–7.70 (m, 1H), 4.93–4.70 (m, 1H), 4.17–3.89 (m, 1H), 3.79–3.55 (m, 1H), 3.28 (t, *J* = 6.5 Hz, 2H), 2.48–2.38 (m, 0.8H), 2.36–2.26 (m, 0.2H), 1.89–1.52 (m, 5H), 1.46–1.22 (m, 11H), 0.99–0.85 (m, 3H);

ESI-MS: m/z = 438 $[M+H]^+$.

1.1.3. General procedure for the preparation of quinazolone intermediates 23-28

To a solution of the imide **17** (or **18-22**, 1.0 equiv) in HAc (4 mL/1 mmol substrate) was added activated zinc powder (10.0 equiv) slowly at room temperature. The resultant mixture was stirred at 40 °C under N_2 atmosphere for 8 h. The mixture was then filtered and the filtrate was concentrated in vacuo. After dissolving the residue in DCM, the resultant solution was washed successively with saturated $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was finally purified by flash column chromatography using EA/PE (1:9) as eluent to give the quinazolones **23** (or **24-28**) as the intermediate. The ^1H NMR spectra of **24-28** indicated a mixture of rotamers.

1.1.3.1. *tert*-butyl (*S*)-2-(3-benzyl-4-oxo-3,4-dihydroquinazolin-2-yl) pyrrolidine-1-carboxylate (**23**)

White foam; yield: 56%; ESI-MS: m/z = 406 $[M+H]^+$.

1.1.3.2. *tert*-butyl (*S*)-2-(3-benzyl-5-fluoro-4-oxo-3,4-dihydroquinazolin-2-yl) pyrrolidine-1-carboxylate (**24**)

White foam; yield: 63%; ^1H NMR (400 MHz, DMSO-*d6*): δ 7.85–7.74 (m, 1H), 7.40 (d, *J* = 8.0 Hz, 1H), 7.37–7.24 (m, 6H), 5.45–5.27 (m, 2H), 4.91 (dt, *J* = 2.8, 8.8 Hz, 1H), 3.64–3.52 (m, 1H), 3.44–3.37 (m, 1H), 2.15–2.03 (m, 1H), 1.97–1.70 (m, 3H), 1.37 (s, 3H), 1.04 (s, 6H); ESI-MS: m/z = 424 $[M+H]^+$.

1.1.3.3. *tert*-butyl (*S*)-2-(3-cyclobutyl-5-fluoro-4-oxo-3,4-dihydroquinazolin-2-yl) pyrrolidine-1-carboxylate (**25**)

White foam; yield: 61%; ^1H NMR (500 MHz, DMSO-*d6*): δ 7.78–7.68 (m, 1H), 7.34–7.28 (m, 1H), 7.25–7.18 (m, 1H), 5.11–5.06 (m, 0.3H), 4.99–4.88 (m, 1H), 4.87–4.79 (m, 0.7H), 3.62–3.54 (m, 1H), 3.47–3.39 (m, 1H), 3.06–2.93 (m, 1.3H), 2.88–2.77 (m, 0.7H), 2.47–2.25 (m, 3H), 2.08–1.74 (m, 5H), 1.38 (s, 3H), 1.08 (s, 6H); ESI-MS: m/z = 388 $[M+H]^+$.

1.1.3.4. *tert*-butyl (*S*)-2-(3-cyclopentyl-5-fluoro-4-oxo-3,4-dihydroquinazolin-2-yl) pyrrolidine-1-carboxylate (**26**)

White solid; yield: 59 %; ^1H NMR (400 MHz, DMSO-*d6*): δ 7.79–7.68 (m, 1H), 7.37–7.28 (m, 1H), 7.26–7.16 (m, 1H), 5.21–5.08 (m, 1H), 4.86–4.67 (m, 1H), 3.65–3.56 (m, 1H), 3.48–3.41 (m, 1H), 2.48–2.36 (m, 1H), 2.30–2.19 (m, 1H), 2.18–2.06 (m, 1H), 2.05–1.71 (m, 7H), 1.70–1.52 (m, 2H), 1.39 (s, 3H), 1.08 (s, 6H); ESI-MS: m/z = 402 $[M+H]^+$.

1.1.3.5. *tert*-butyl (*S*)-2-(3-cyclohexyl-5-fluoro-4-oxo-3,4-dihydroquinazolin-2-yl) pyrrolidine-1-carboxylate (**27**)

White foam; yield: 61%; ^{1}H NMR (500 MHz, DMSO-*d6*): δ 7.77–7.70 (m, 1H), 7.31 (d, *J* = 8.5 Hz, 1H), 7.24–7.17 (m, 1H), 5.10–5.05 (m, 0.3H), 5.03–4.98 (m, 0.7H), 4.12–3.93 (m, 1H), 3.65–3.57 (m, 1H), 3.51–3.43 (m, 1H), 2.73–2.62 (m, 1H), 1.98–1.87 (m, 3H), 1.85–1.72 (m, 3H), 1.70–1.62 (m, 2H), 1.57–1.47 (m, 1H), 1.42–1.30 (m, 4H), 1.26–1.15 (m, 2H), 1.13–1.02 (m, 7H); ESI-MS: m/z = 416 $[M+H]^+$.

1.1.3.6. *tert*-butyl (*S*)-2-(3-butyl-5-fluoro-4-oxo-3,4-dihydroquinazolin-2-yl) pyrrolidine-1-carboxylate (**28**)

White foam; yield: 70%; ^{1}H NMR (500 MHz, DMSO-*d6*): δ 7.82–7.70 (m, 1H), 7.40–7.32 (m, 1H), 7.28–7.19 (m, 1H), 5.01–4.89 (m, 1H), 4.34–4.21 (m, 1H), 3.79–3.68 (m, 1H), 3.65–3.61 (m, 1H), 3.50–3.40 (m, 1H), 2.48–2.34 (m, 1H), 2.06–1.84 (m, 3H), 1.72–1.55 (m, 2H), 1.50–1.33 (m, 5H), 1.12 (s, 6H), 1.00–0.90 (m, 3H); ESI-MS: m/z = 390 $[M+H]^+$.

1.1.4. General procedure for the preparation of target compounds (29-38)

To a solution of the quinazolone intermediate in DCM (5 mL/1 mmol substrate) was added TFA (1.25 mL/1 mmol substrate) at 0 °C and the resultant solution was stirred at room temperature for 4 h. The reaction was then quenched with saturated $NaHCO_3$ solution and the organic layer was washed with brine. After being dried over anhydrous Na_2SO_4, the organic layer was concentrated in vacuo to give the Boc-deprotected quinazolone-based secondary amine intermediates, which were used directly for the next step without further purification.

To a solution of the quinazolone-based secondary amine intermediate (1.0 equiv) in *t*-BuOH (5 mL/1 mmol substrate) was added 6-chloro purine (or 4-amino-5-carbonitrile-6-chloro pyrimidine, 6-chloro-2-fluoro-9*H*-purine, 1.5 equiv) and DIPEA (4.0 equiv). The resultant mixture was then stirred at 80 °C under N_2 atmosphere for 8 h. After removal of the solvent in vacuo, the residue was dissolved in DCM, and the mixture was washed succesively with saturated $NaHCO_3$ solution and brine. When preparing 4-amino-5-carbonitrile pyrimidine derivatives, the mixture was washed successively with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo to afford the crude product, which was subjected to flash column chromatography to provide compound **29** (or **30-38**). As for the purine and 2-fluoro purine derivatives, EA was utilized as the eluent for flash column chromatography. As for the 4-amino-5-carbonitrile pyrimidine derivatives, EA/PE (1:4) was

used as the eluent. The yield of target compound was calculated with corresponding crude Boc-deprotected secondary amine intermediate. The ^{1}H NMR and ^{13}C NMR spectra of **29**-**36** indicated a mixture of rotamers.

1.1.4.1. (*S*)-2-(1-(9*H*-purin-6-yl)pyrrolidin-2-yl)-3-benzylquinazolin-4(3*H*)-one (**29**)

White solid; yield: 72%; ^{1}H NMR (500 MHz, DMSO-*d6*): δ 12.97 (s, 0.6H), 12.91 (s, 0.4H), 8.26 (s, 0.4H), 8.17–8.08 (m, 1.6H), 7.98 (s, 0.6H), 7.93 (s, 0.4H), 7.75–7.67 (m, 1H), 7.54–7.36 (m, 6H), 7.34–7.27 (m, 1H), 6.17–6.10 (m, 0.4H), 6.00–5.91 (m, 0.6H), 5.69–5.58 (m, 1.5H), 5.46–5.40 (m, 0.5H), 4.45–4.30 (m, 1H), 4.06–3.96 (m, 0.5H), 3.84–3.74 (m, 0.5H), 2.32–2.17 (m, 1H), 2.15–2.00 (m, 1H), 1.98–1.78 (m, 2H) ; ^{13}C NMR (100 MHz, DMSO-*d6*): δ 161.50, 159.03, 158.80, 152.22, 151.57, 151.51, 150.99, 146.88, 146.55, 138.73, 138.49, 136.99, 136.60, 134.37, 128.56, 128.45, 127.42, 127.30, 127.17, 127.10, 126.92, 126.46, 126.32, 119.97, 119.10, 59.96, 59.09, 49.48, 48.02, 46.19, 46.07, 31.97, 30.22, 23.97, 20.90. HRMS: calcd for $C_{24}H_{22}N_7O$ $[M+H]^+$, 424.1886; found 424.1878. HPLC: t_R = 8.91 min, flow rate 0.8 mL/min, Agilent TC-C18(2) 250 × 4.6mm 5μm, 35 °C, eluent A-60%, eluent B-40%.

1.1.4.2. (*S*)-2-(1-(9*H*-purin-6-yl)pyrrolidin-2-yl)-3-benzyl-5-fluoroquinazolin-4(3*H*)-one (**30**)

White solid; yield: 58%; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 8.20–7.83 (m, 2H), 7.73–7.63 (m, 1H), 7.61–7.45 (m, 2H), 7.44–7.35 (m, 2H), 7.34–7.28 (m, 1H), 7.27–7.15 (m, 2H), 6.25–6.05 (m, 0.5H), 5.98–5.82 (m, 0.5H), 5.80–5.74 (m, 0.4H), 5.72–5.49 (m, 1.6H), 5.47–5.28 (m, 0.6H), 4.48–4.24 (m, 1.4H), 2.26–1.76 (m, 4H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 160.81 (d, $J_{C\text{-}F}$ = 263 Hz), 160.79, 159.05, 152.66, 151.98, 151.58, 149.39, 140.74, 136.99, 135.50 (d, $J_{C\text{-}F}$ = 11.0 Hz), 128.98, 127.72, 127.49, 123.74 (d, $J_{C\text{-}F}$ = 3.0 Hz), 119.73, 113.21 (d, $J_{C\text{-}F}$ = 21.0 Hz), 110.02 (d, $J_{C\text{-}F}$ = 5.0 Hz), 65.49, 59.63, 55.39, 49.86, 46.36, 30.65, 24.52. HRMS: calcd for $C_{24}H_{21}FN_7O$ $[M+H]^+$, 442.1792; found 442.1795. HPLC: t_R = 7.19 min, flow rate 0.6 mL/min, Agilent TC-C18(2) 250 × 4.6mm 5μm, 35 °C, eluent A-55%, eluent B-45%.

1.1.4.3. (*S*)-2-(1-(9*H*-purin-6-yl)pyrrolidin-2-yl)-3-cyclobutyl-5-fluoroquinazolin-4(3*H*)-one (**31**)

White solid; yield: 65%; ^{1}H NMR (500 MHz, DMSO-*d6*): δ 12.96 (s, 0.4H), 12.84 (s, 0.6H), 8.24 (s, 0.6H), 8.13 (s, 0.4H), 7.99 (s, 0.4H), 7.90 (s, 0.6H), 7.65–7.54 (m, 1H), 7.19–7.04 (m, 2H), 6.33–6.24 (m, 0.6H), 5.61–5.55 (m, 0.4H), 5.28–5.10 (m, 1H), 4.45–4.29 (m, 0.7H), 4.02–3.94 (m, 0.6H), 3.89–3.80 (m, 0.7H), 3.29–3.17 (m, 1.3H), 3.05–2.94 (m, 0.7H), 2.66–2.55 (m, 1H), 2.48–2.37 (m, 1H), 2.35–2.23 (m, 1H), 2.15–1.81 (m, 5H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 160.15 (d, $J_{C\text{-}F}$ = 261 Hz),

159.89, 159.61 (d, $J_{C\text{-}F}$ = 3.0 Hz), 159.31, 152.35, 152.20, 151.71, 150.93, 150.33, 148.54, 148.27, 138.66, 138.31, 134.45 (d, $J_{C\text{-}F}$ = 10.0 Hz), 122.85, 122.72, 119.21, 112.49 (d, $J_{C\text{-}F}$ = 20.0 Hz), 110.43 (d, $J_{C\text{-}F}$ = 5.0 Hz), 60.20, 58.83, 51.45, 49.39, 47.76, 31.47, 30.13, 28.09, 26.98, 26.60, 24.00, 21.21, 14.64, 14.40; HRMS: calcd for $C_{21}H_{21}FN_7O$ $[M+H]^+$, 406.1762; found 406.1780. HPLC: t_R = 10.08 min, flow rate 0.8 mL/min, Agilent TC-C18(2) 250 × 4.6mm 5μm, 35 °C, eluent A-65%, eluent B-35%.

1.1.4.4. (*S*)-2-(1-(9*H*-purin-6-yl)pyrrolidin-2-yl)-3-cyclopentyl-5-fluoroquinazolin-4(3*H*)-one (**32**)

White solid; yield: 63%; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 12.87 (brs, 1H), 8.25 (s, 0.7H), 8.14 (s, 0.3H), 7.99 (s, 0.3H), 7.91 (s, 0.7H), 7.66–7.51 (m, 1H), 7.14 (d, *J* = 8.4 Hz, 1H), 7.10 (t, *J* = 8.0 Hz, 1H), 6.37 (d, *J* = 8.4 Hz, 0.8H), 5.79–5.72 (m, 0.2H), 5.08–5.00 (m, 1H), 4.48–4.32 (m, 0.5H), 4.05–3.94 (m, 0.7H), 3.93–3.82 (m, 0.8H), 2.69–2.55 (m, 0.8H), 2.40–2.22 (m, 3.2H), 2.18–2.09 (m, 1H), 2.08–1.97 (m, 4H), 1.95–1.84 (m, 1H), 1.74–1.58 (m, 2H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 160.72 (d, $J_{C\text{-}F}$ = 262 Hz), 160.08, 158.86, 152.90, 152.71, 150.82, 148.87, 138.78, 134.84 (d, $J_{C\text{-}F}$ = 10.0 Hz), 123.39, 119.75, 112.89 (d, $J_{C\text{-}F}$ = 21.0 Hz), 110.93 (d, $J_{C\text{-}F}$ = 5.0 Hz), 60.99, 59.50, 58.57, 49.86, 48.24, 31.95, 30.59, 29.15, 28.90, 28.33, 26.20, 26.13, 24.47, 21.74; HRMS: calcd for $C_{22}H_{23}FN_7O$ $[M+H]^+$, 420.1948; found 420.1944. HPLC: t_R = 9.94 min, flow rate 0.8 mL/min, Agilent TC-C18(2) 250 × 4.6mm 5μm, 35 °C, eluent A-60%, eluent B-40%.

1.1.4.5. (*S*)-2-(1-(9*H*-purin-6-yl)pyrrolidin-2-yl)-3-cyclohexyl-5-fluoroquinazolin-4(3*H*)-one (**33**)

White solid; yield: 81%; ^{1}H NMR (500 MHz, DMSO-*d6*): δ 12.97 (s, 0.4H), 12.85 (s, 0.6H), 8.26 (s, 0.6H), 8.14 (s, 0.4H), 7.99 (s, 0.4H), 7.92 (s, 0.6H), 7.62–7.54 (m, 1H), 7.19–7.04 (m, 2H), 6.29–6.24 (m, 0.6H), 5.65–5.59 (m, 0.4H), 4.46–4.29 (m, 2H), 4.01–3.85 (m, 2H), 2.79–2.57 (m, 3H), 2.33–2.19 (m, 1H), 2.16–2.08 (m, 1H), 2.07–2.01 (m, 1H), 1.91–1.82 (m, 2H), 1.81–1.73 (m, 1H), 1.72–1.66 (m, 1H), 1.58–1.47 (m, 1H), 1.47–1.37 (m, 1H), 1.29–1.22 (m, 1H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 160.24 (d, $J_{C\text{-}F}$ = 262 Hz), 159.07, 152.46, 152.21, 151.68, 150.93, 150.35, 148.62, 148.34, 138.69, 138.30, 134.38 (d, $J_{C\text{-}F}$ = 10.0 Hz), 122.89, 122.77, 119.20, 112.43 (d, $J_{C\text{-}F}$ = 21.0 Hz), 110.57 (d, $J_{C\text{-}F}$ = 5.0 Hz), 60.43, 59.26, 58.97, 49.49, 47.86, 31.32, 30.11, 27.85, 27.40, 25.89, 25.74, 25.60, 25.15, 25.01, 24.01, 21.26; HRMS: calcd for $C_{23}H_{25}FN_7O$ $[M+H]^+$, 434.2105; found 434.2092. HPLC: t_R = 7.47 min, flow rate 0.8 mL/min, Agilent TC-C18(2) 250 × 4.6mm 5μm, 35 °C, eluent A-50%, eluent B-50%.

1.1.4.6. (*S*)-2-(1-(9*H*-purin-6-yl)pyrrolidin-2-yl)-3-butyl-5-fluoroquinazolin-4(3*H*)-one (**34**)

White solid; yield: 78%; ^{1}H NMR (500 MHz, DMSO-*d6*): δ 13.17–12.69 (m, 1H), 8.27 (s, 0.4H), 8.15 (s, 0.6H), 7.99 (s, 0.5H), 7.90 (s, 0.5H), 7.62 (s, 1H), 7.29–7.01 (m, 2H), 6.16–6.01 (m, 0.4H),

5.59–5.42 (m, 0.6H), 4.55–4.31 (m, 2H), 4.10–3.80 (m, 2H), 2.69–2.58 (m, 0.4H), 2.38–2.17 (m, 1.6H), 2.16–1.96 (m, 3H), 1.92–1.78 (m, 1H), 1.56–1.39 (m, 2H), 1.08–0.91 (m, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 160.76 (d, $J_{C\text{-}F}$ = 262 Hz), 160.68 (d, $J_{C\text{-}F}$ = 261 Hz), 160.51, 159.52, 159.01 (d, $J_{C\text{-}F}$ = 4.0 Hz), 158.66 (d, $J_{C\text{-}F}$ = 4.0 Hz), 152.89, 152.70, 152.11, 152.05, 151.45, 150.88, 149.42, 149.09, 139.30, 138.80, 135.15 (d, $J_{C\text{-}F}$ = 10.0 Hz), 135.05 (d, $J_{C\text{-}F}$ = 10.0 Hz), 123.62 (d, $J_{C\text{-}F}$ = 2.0 Hz), 123.55 (d, $J_{C\text{-}F}$ = 3.0 Hz), 119.63, 119.52, 113.96 (d, $J_{C\text{-}F}$ = 20.0 Hz), 113.92 (d, $J_{C\text{-}F}$ = 21.0 Hz), 110.99 (d, $J_{C\text{-}F}$ = 5.0 Hz), 60.49, 59.19, 50.03, 49.06, 48.59, 43.72, 42.94, 32.62, 30.73, 30.49, 29.51, 24.49, 21.57, 20.38, 20.11, 14.24; HRMS: calcd for $C_{21}H_{23}FN_7O$ $[M+H]^+$, 408.1948; found 408.1942. HPLC: t_R = 12.01 min, flow rate 0.8 mL/min, Agilent TC-C18(2) 250 × 4.6mm 5μm, 35 °C, eluent A-65%, eluent B-35%.

1.1.4.7. **(***S***)-3-benzyl-5-fluoro-2-(1-(2-fluoro-9*H*-purin-6-yl)pyrrolidin-2-yl)quinazolin-4(3*H*)-one (35)**

White solid; yield: 59%; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 13.44–12.78 (m, 1H), 8.17 (s, 0.5H), 7.95 (s, 0.5H), 7.78–7.54 (m, 2H), 7.53–7.11 (m, 6H), 6.23–6.05 (m, 0.4H), 5.97–5.80 (m, 0.4H), 5.68–5.31 (m, 2.2 H), 4.63–4.14 (m, 1.2H), 4.03–3.87 (m, 0.4H), 3.85–3.69 (m, 0.4H), 2.41–1.69 (m, 3.7H), 1.58–1.46 (m, 0.3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 160.29 (d, $J_{C\text{-}F}$ = 263 Hz), 159.20, 158.92, 158.77, 158.25 (d, $J_{C\text{-}F}$ = 3.0 Hz), 158.02 (d, $J_{C\text{-}F}$ = 253 Hz), 157.27, 153.47 (d, $J_{C\text{-}F}$ = 20.0 Hz), 152.82 (d, $J_{C\text{-}F}$ = 22.0 Hz), 152.36 (d, $J_{C\text{-}F}$ = 21.0 Hz), 151.68 (d, $J_{C\text{-}F}$ = 20.0 Hz), 148.77, 148.51, 139.31, 139.07, 136.65, 136.35, 135.05 (d, $J_{C\text{-}F}$ = 10.0 Hz), 128.53, 128.39, 127.40, 127.30, 127.21, 126.91, 123.25, 117.56, 112.92 (d, $J_{C\text{-}F}$ = 20.0 Hz), 112.87 (d, $J_{C\text{-}F}$ = 20.0 Hz), 109.56 (d, $J_{C\text{-}F}$ = 5.0 Hz), 60.21, 59.63, 49.73, 48.37, 46.03, 31.83, 30.04, 23.74, 20.92; HRMS: calcd for $C_{24}H_{20}F_2N_7O$ $[M+H]^+$, 460.1697; found 460.1700. HPLC: t_R = 11.19 min, flow rate 0.8 mL/min, Agilent TC-C18(2) 250 × 4.6mm 5μm, 35 °C, eluent A-40%, eluent B-60%.

1.1.4.8. (*S*)-3-cyclopentyl-5-fluoro-2-(1-(2-fluoro-9*H*-purin-6-yl)pyrrolidin-2-yl)quinazolin-4(3*H*)-one (**36**)

White solid; yield: 52%; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 13.00 (brs, 1H), 8.14 (s, 0.2H), 7.91 (s, 0.8H), 7.66–7.54 (m, 1H), 7.24–7.06 (m, 2H), 6.44–6.31 (m, 0.8H), 5.76–5.66 (m, 0.2H), 5.08–4.94 (m, 1H), 4.47–4.31 (m, 0.5H), 4.00–3.92 (m, 0.7H), 3.90–3.76 (m, 0.8H), 2.68–2.55 (m, 1H), 2.38–2.22 (m, 3H), 2.19–2.13 (m, 1H), 2.08–1.97 (m, 4H), 1.94–1.83 (m, 1H), 1.72–1.59 (m, 2H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 160.72 (d, $J_{C\text{-}F}$ = 262 Hz), 159.84, 159.79, 159.44, 158.82 (d, $J_{C\text{-}F}$ = 4.0 Hz), 158.50 (d, $J_{C\text{-}F}$ = 255 Hz), 157.83, 154.13 (d, $J_{C\text{-}F}$ = 21.0 Hz), 152.07 (d, $J_{C\text{-}F}$ = 20.0 Hz), 148.76, 139.33, 134.95, 134.84, 123.45 (d, $J_{C\text{-}F}$ = 4.0 Hz), 123.33 (d, $J_{C\text{-}F}$ = 3.0 Hz), 118.22 (d, $J_{C\text{-}F}$ = 3.0 Hz), 113.01 (d,

$J_{C\text{-}F}$ = 21.0 Hz), 110.95 (d, $J_{C\text{-}F}$ = 5.0 Hz), 61.16, 59.83, 58.64, 50.03, 48.59, 31.89, 28.91, 28.29, 26.19, 26.12, 21.70; HRMS: calcd for $C_{22}H_{22}F_2N_7O$ $[M+H]^+$, 438.1854; found 438.1853. HPLC: t_R = 10.57 min, flow rate 0.8 mL/min, Agilent TC-C18(2) 250 × 4.6mm 5μm, 35 °C, eluent A-40%, eluent B-60%.

1.1.4.9. (*S*)-4-amino-6-(2-(3-benzyl-5-fluoro-4-oxo-3,4-dihydroquinazolin-2-yl)pyrrolidin-1-yl)pyrimidine-5-carbonitrile (**37**)

White solid; yield: 74%; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.89–7.60 (m, 2H), 7.55–7.04 (m, 9H), 5.57–5.24 (m, 3H), 4.15–3.84 (m, 2H), 2.21–1.94 (m, 3H), 1.90–1.77 (m, 1H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 166.20, 160.84 (d, $J_{C\text{-}F}$ = 262 Hz), 159.87, 159.39, 158.91 (d, $J_{C\text{-}F}$ = 4.0 Hz), 158.74, 149.34, 136.76, 135.59 (d, $J_{C\text{-}F}$ = 10.0 Hz), 128.90, 127.66, 127.35, 123.79 (d, $J_{C\text{-}F}$ = 3.0 Hz), 117.85, 113.31 (d, $J_{C\text{-}F}$ = 21.0 Hz), 110.02 (d, $J_{C\text{-}F}$ = 6.0 Hz), 68.30, 60.99, 49.25, 46.25, 30.07, 24.18. HRMS: calcd for $C_{24}H_{21}FN_7O$ $[M+H]^+$, 442.1792; found 442.1794. HPLC: t_R = 8.70 min, flow rate 0.8 mL/min, Agilent TC-C18(2) 250 × 4.6mm 5μm, 35 °C, eluent A-40%, eluent B-60%.

1.1.4.10. (*S*)-4-amino-6-(2-(3-cyclopentyl-5-fluoro-4-oxo-3,4-dihydroquinazolin-2-yl)pyrrolidin-1-yl)pyrimidine-5-carbonitrile (**38**)

White solid; yield: 81%; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 8.17–7.74 (m, 1H), 7.72–7.61 (m, 1H), 7.44–6.90 (m, 3H), 7.24 (d, *J* = 8.0 Hz, 1H), 5.99–5.63 (m, 1H), 5.01–4.75 (m, 1H), 4.19–3.76 (m, 2H), 2.31–2.13 (m, 3H), 2.12–1.93 (m, 5H), 1.92–1.76 (m, 2H), 1.71–1.53 (m, 2H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 165.63, 160.25 (d, $J_{C\text{-}F}$ = 262 Hz), 159.16, 158.63, 158.33, 148.35, 134.64, 134.53, 122.99, 117.49, 112.69, 110.47, 67.83, 60.22, 58.05, 48.69, 28.51, 27.89, 25.72, 25.60. HRMS: calcd for $C_{22}H_{23}FN_7O$ $[M+H]^+$, 420.1948; found 420.1947. HPLC: t_R = 8.10 min, flow rate 0.8 mL/min, Agilent TC-C18(2) 250 × 4.6mm 5μm, 35 °C, eluent A-40%, eluent B-60%.

1.2. ADP-Glo assay for class I PI3Ks (PI3Kα, β, γ and δ)

Class I PI3Ks (PI3Kα, β, γ and δ) inhibitory activities were evaluated by ADP-Glo assay. PI3Kδ and ADP-Glo kit were purchased from Promega, PI3Kα, β and γ were purchased from Carna, and DMSO were purchased from Sigma. The assays were performed according to the standard protocols of Promega. The kinase buffer contained HEPES (pH 7.5), $MgCl_2$, EGTA, NaCl, and CHAPS. The kinase solution was prepared by dissolving the kinase (PI3Kα, β, γ or δ) in the kinase buffer, while the substrate solution was prepared by dissolving PIP_2 and ATP in the kinase buffer. Compound solution, kinase solution and substrate solution were added successively to the well of the assay plate. The reaction mixture was incubated at room temperature for 2 h, and then stopped by ADP-Glo reagent.

Subsequently, the mixture need be mixed briefly with centrifuge and incubated at room temperature for 3 h. Before reading on a plate reader for luminescence, the kinase detection reagent was added to the mixture, and the resultant mixture was incubated at room temperature for 1 h. After calculating the percent inhibition, the curves were fitted by log(inhibitor) vs. response-Variable slope in Graphpad Prism 5 to give IC_{50} values of tested compounds.

1.3. Anti-proliferative assay

The anti-proliferative acitivity against SU-DHL-6 cell line was evaluated by CellTiter-Glo luminescent cell viability assay purchased from Promega. SU-DHL-6 cells purchased from ATCC were seeded in 96-well plates (Corning) at the density of 1.5×10^4 cells per well, and incubated with medium alone or with the tested compounds or the reference drugs at the indicated concentrations for 72 h. 100 μL CellTiter-Glo reagent was added to each well for inducing cell lysis, and the plate was shaken for 10 min while being protected from light and incubated at room temperature for 10 min to stabilize luminescent signal. The luminescence was read on Envision and the inhibition rates calculated according to the luminescence. GI_{50} values were calculated using four-parameter logistic model with XLFit software.

1.4. Western blot assay

The capability of **38** to down-regulate phos-Akt (S473) and phos-S6K1 (T389) in SU-DHL-6 cells was determined by Western blot analysis. SU-DHL-6 cells were seeded into six-well plate at 1×10^6 cells per well and then incubated at 37 °C (5% CO_2) overnight. Cells were subsequently treated with compound **38 (**100, 300 and 1000 nM) or **1**, and incubated at 37 °C for 2 h. Afterwards, culture medium was discarded and cells were washed twice with ice-cold PBS, and cell lysis buffer was added. Then the cellular debris was pelleted by centrifugation at 13,000 rpm for 30 min at 4 °C and the supernatant was collected. For western blot analysis, the proteins were separated on SDS-PAGE and then transferred onto PVDF membrane. The membranes were incubated with antibodies against phos-Akt (S473) (Abcam, Cambridge, UK), Akt (Abcam, Cambridge, UK), phos-S6K1 (T389) (Abcam, Cambridge, UK), S6K1 (Abcam, Cambridge, UK) and GAPDH (Abcam, Cambridge, UK), washed by TBST, and then incubated with secondary antibodies. Membrane was imaged using Tanon 6600 system and the optical density was measured using Image pro plus 6.0.

1.5. *In vitro* metabolic stability in liver microsomes

After preparing the compound and control working solutions, an appropriate amount of NADPH

powder was weighed and diluted into $MgCl_2$ solution (10 mM). The microsome (HLM, Corning; SD rat RLM, Xenotech) working solutions were prepared with potassium phosphate buffer (100 mM). Cold CH_3CN containing 100 ng/mL tolbutamide and 100 ng/mL labetalol as internal standards (IS) was used as the stop solution. Compound or control working solution (10 μL/well) was added to all plates (T0, T5, T10, T20, T30, T60, NCF60) except matrix blank. Microsome solution was then dispensed to every plate (80 μL/well) and the mixture was incubated at 37 °C for about 10 min. After potassium phosphate buffer (100 mM) was added to the plate NCF60 (10 μL/well), the mixture was incubated at 37 °C. The pre-warmed NADPH solution was added to start the reaction of other plates (10 μL/well). At each time point, the stop solution (cold in 4 °C) was added to terminate the reaction (300 μL/well). After shaking and centrifuging, 100 μL supernatant was mixed with an appropriate amount of water for LC/MS/MS analysis.

1.6. PK study

SD rats were utilized for the PK study of **38** following oral gavage at the dosage of 10 mg/kg. The oral dose was formulated in a homogenous opaque suspension of 0.5% methylcellulose at 2 mg/mL. The animal was restrained manually at the designated time points, and blood sample was collected *via* tail vein for terminal bleeding into EDTA-2K tubes. A solution of 100 ng/mL dexamethasone (IS) in CH_3CN was used as the internal control. An aliquot of 8 μL sample was treated with 160 μL IS solution for protein precipitation, and the mixture was vortex-mixed well and centrifuged at 3220 g for 15 min at 4 °C. Finally, 1 μL supernatant was injected for LC-MS/MS analysis. The study was carried out in accordance with institutional guidelines of the Animal Research Committee at Jiaxing University (log number JXU2015120812). The protocol was approved by the institution.

1.7. Molecular docking

The co-crystal structure of PI3Kδ complexed with **1** (PDB code 4XE0) was used for the docking calculation in C-DOCKER module of Discovery Studio (version 2.5; Accelrys, San Diego, CA, USA, 2008). For the preparation of ligands, their 3D structures were generated and the energy minimization was performed. After removal of **1** and solvent molecules, the CHARMm-force field was applied to the protein. The active site of the receptor was determined according to the location of **1** in PI3Kδ enzyme and the ligand was docked into the defined site. The final binding conformation was determined based on the calculated C-DOCKING ENERGE. Afterwards, the location of compound **38** was overlapped with that of **1** in the PI3Kδ catalytic site

2. Copies of the NMR and HRMS spectra, as well as HPLC of target compounds

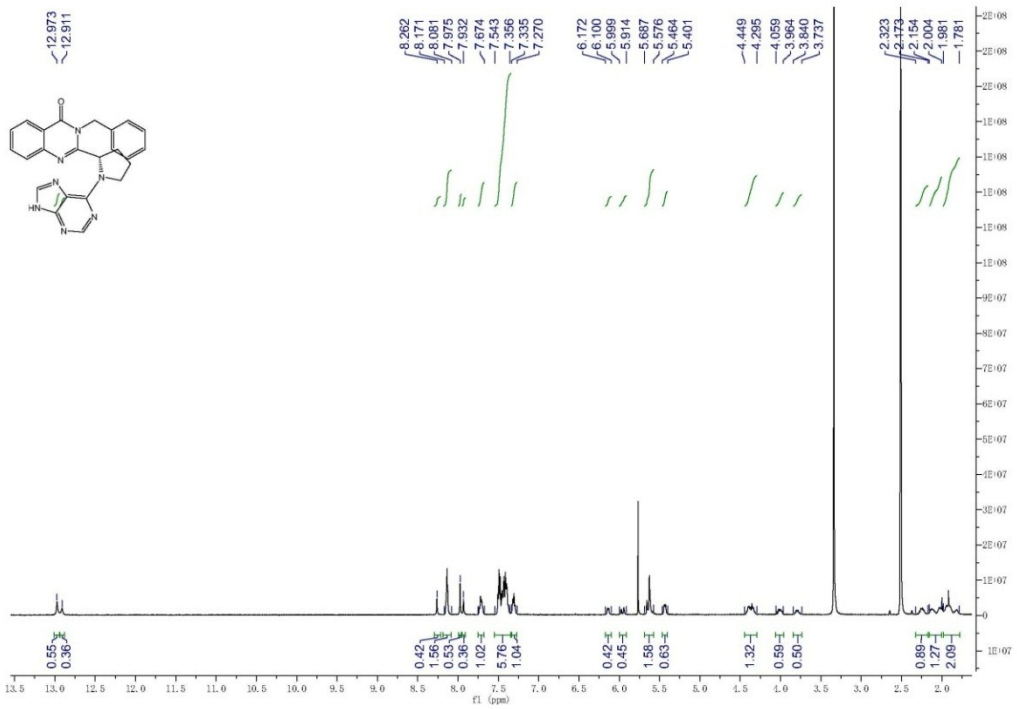

Figure S1 (a) ^{1}H NMR spectrum of compound 29

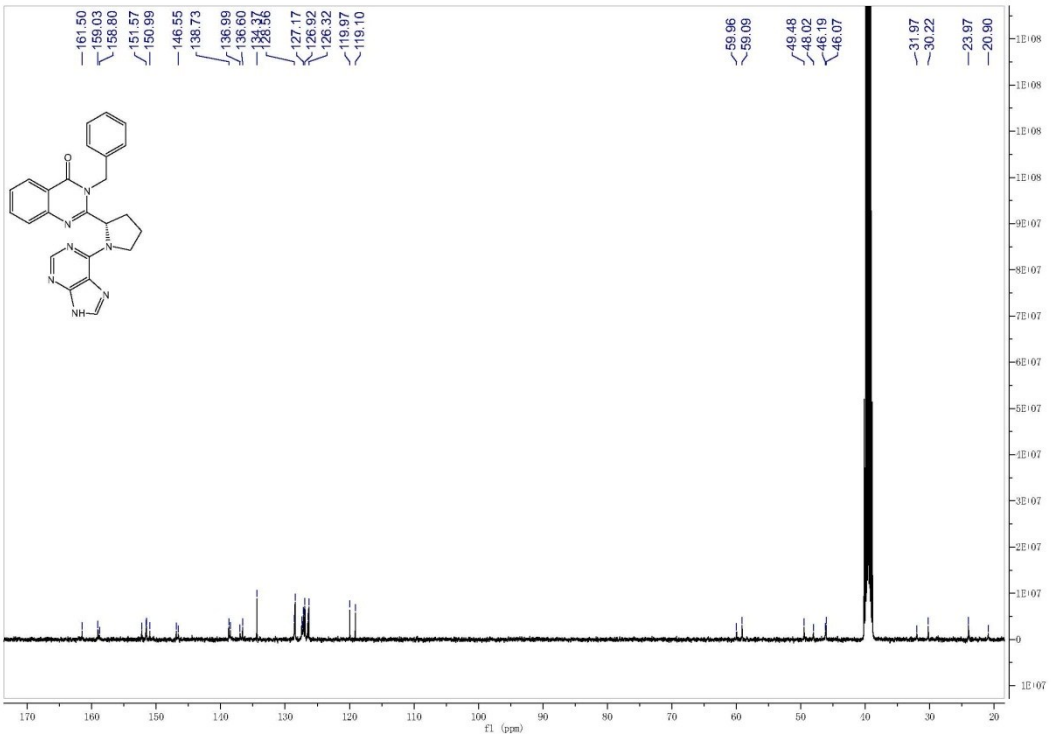

Figure S1 (b) ^{13}C NMR spectrum of compound 29

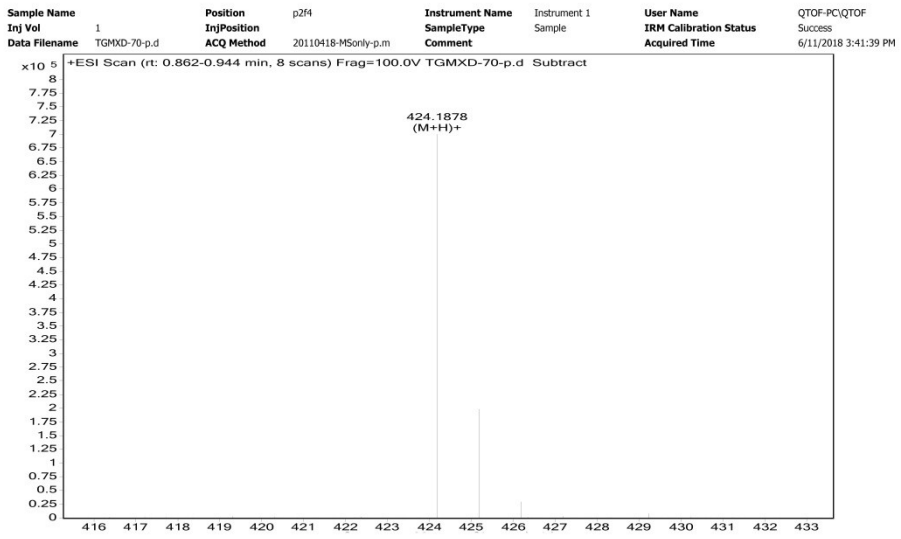

Figure S1 (c) HRMS spectrum of compound 29

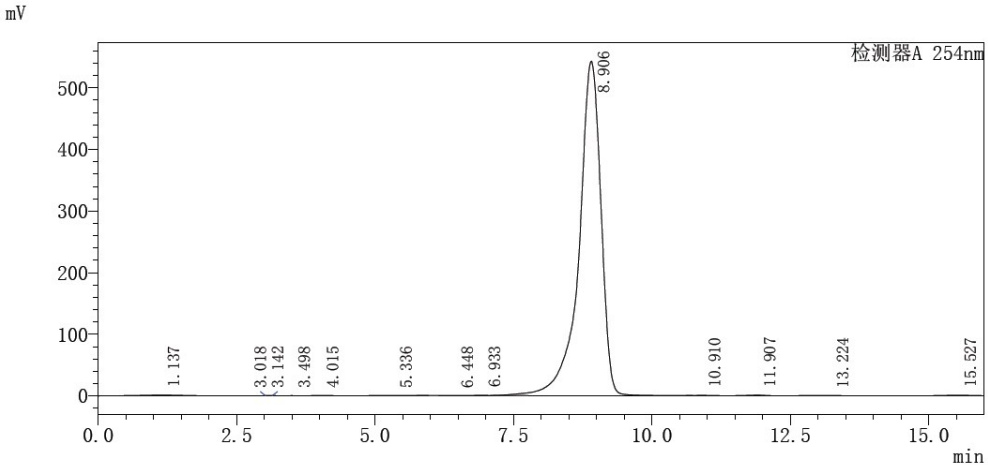

Figure S1 (d) HPLC of compound 29

Cpd.	Area of cpd.	Total area	Area %
29	15029891	15177125	99.0

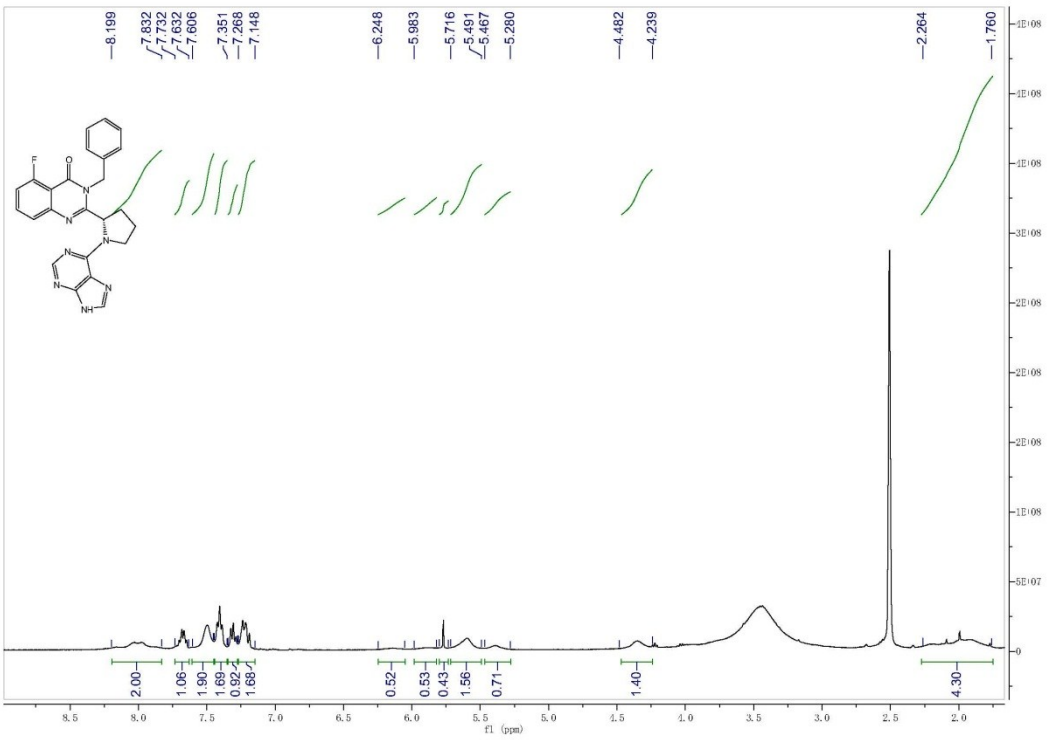

Figure S2 (a) ^{1}H NMR spectrum of compound 30

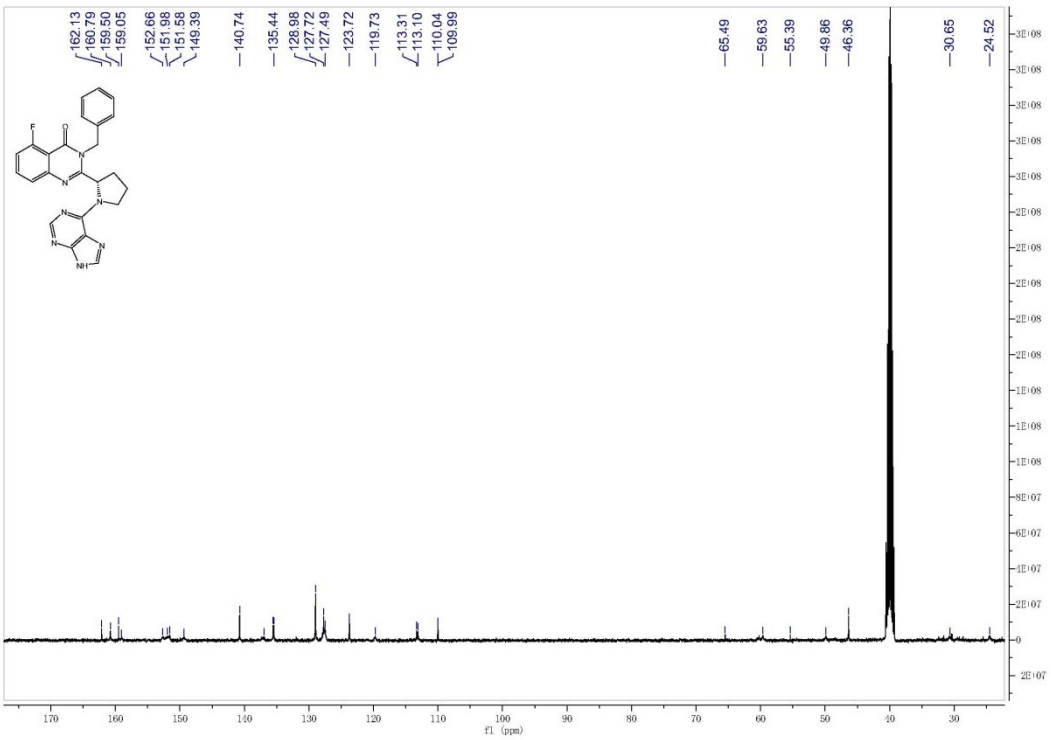

Figure S2 (b) ^{13}C NMR spectrum of compound 30

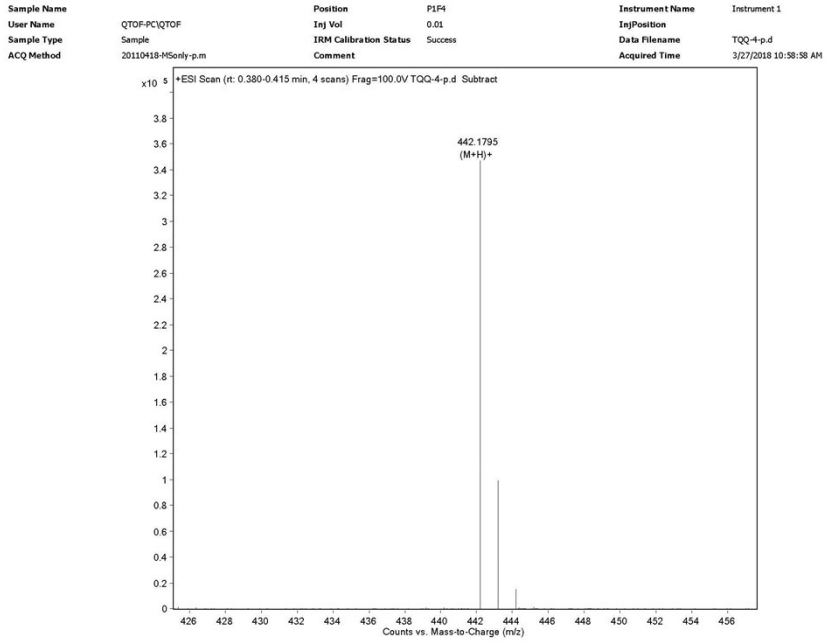

Figure S2 (c) HRMS spectrum of compound 30

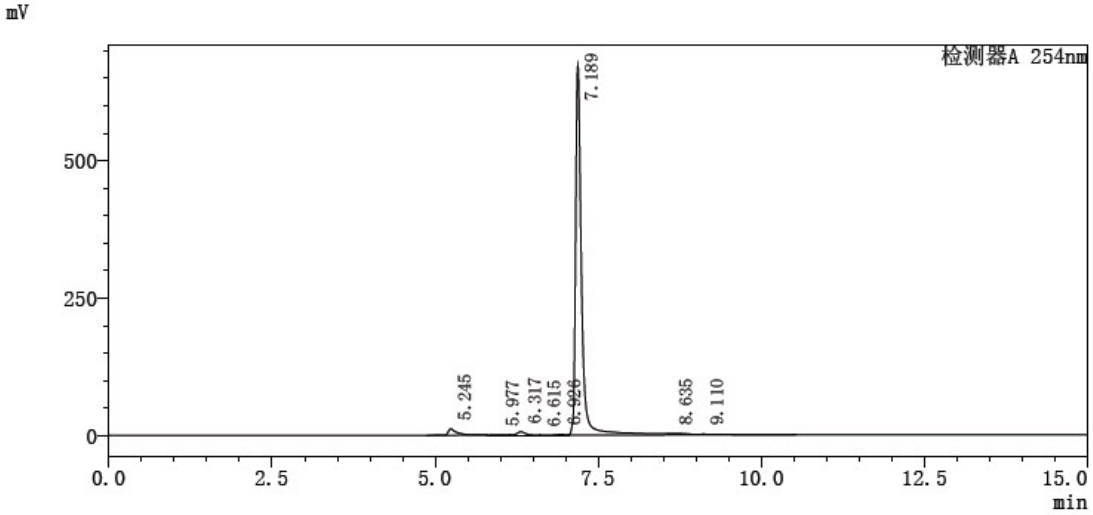

Figure S2 (d) HPLC of compound 30

Cpd.	Area of cpd.	Total area	Area %
30	4345106	4557024	95.3

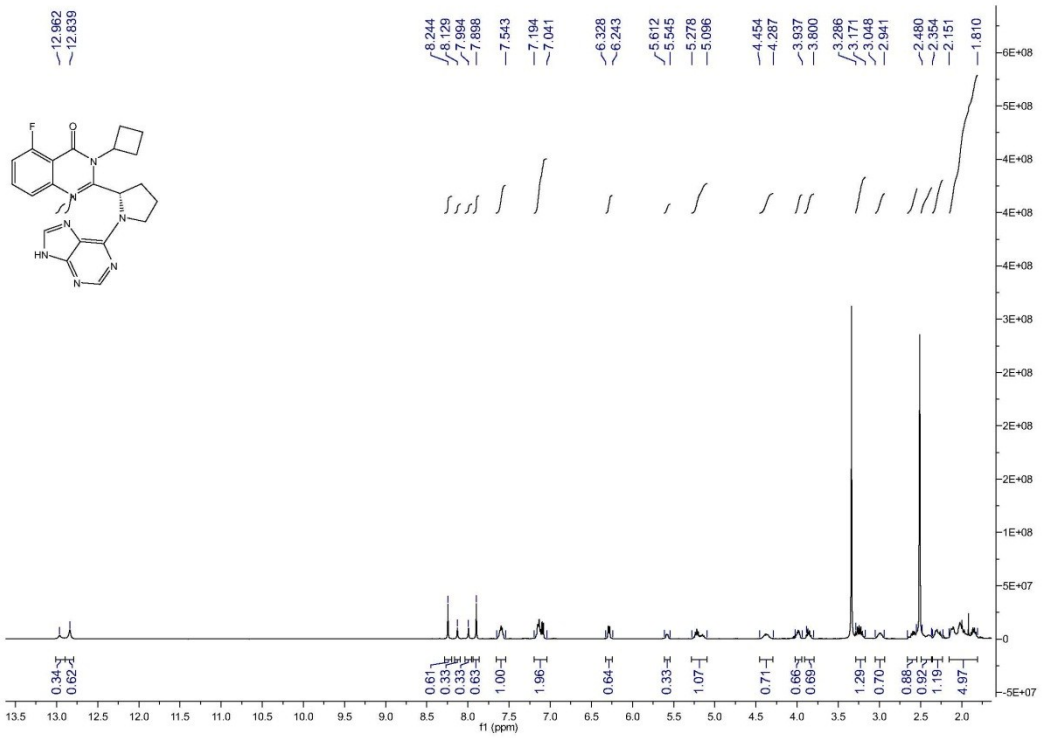

Figure S3 (a) ^{1}H NMR spectrum of compound 31

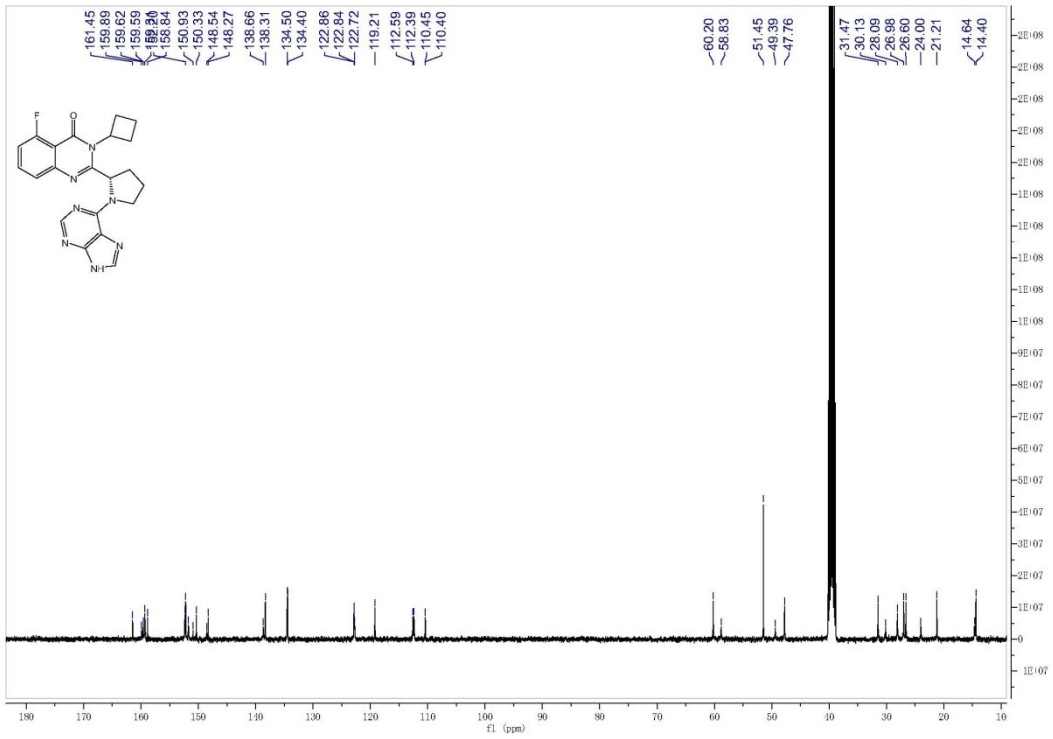

Figure S3 (b) ^{13}C NMR spectrum of compound 31

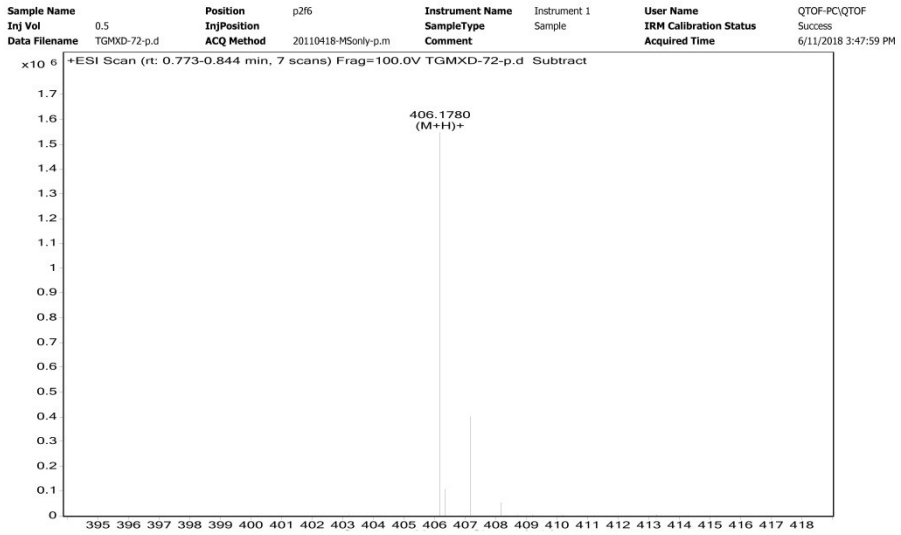

Figure S3 (c) HRMS spectrum of compound 31

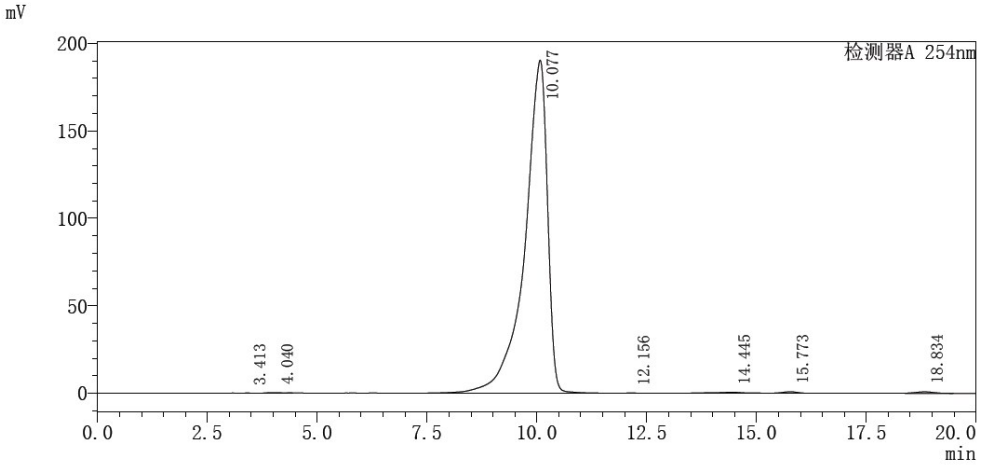

Figure S3 (d) HPLC of compound 31

Cpd.	Area of cpd.	Total area	Area %
31	6805855	6886806	98.8

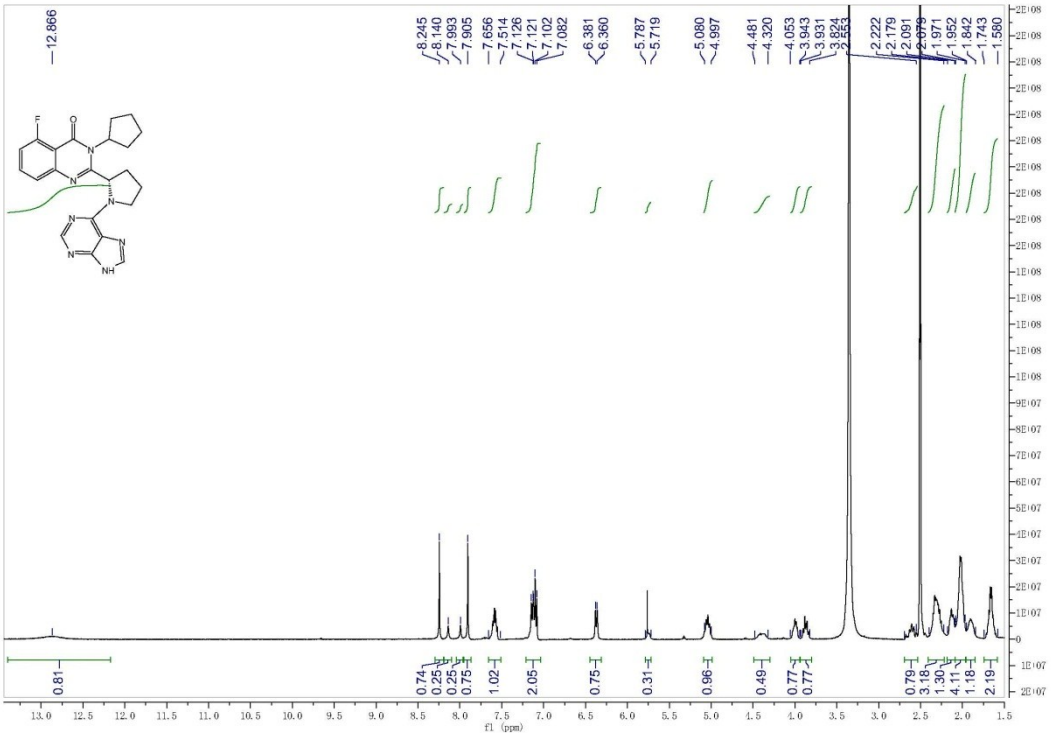

Figure S4 (a) ^{1}H NMR spectrum of compound 32

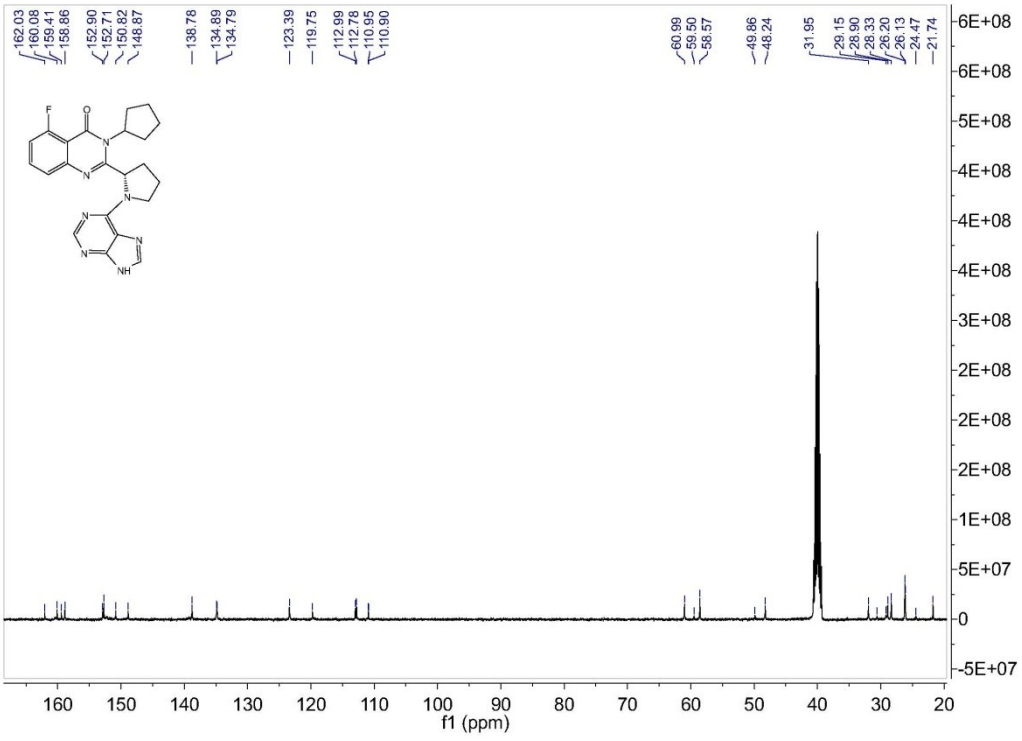

Figure S4 (b) ^{13}C NMR spectrum of compound 32

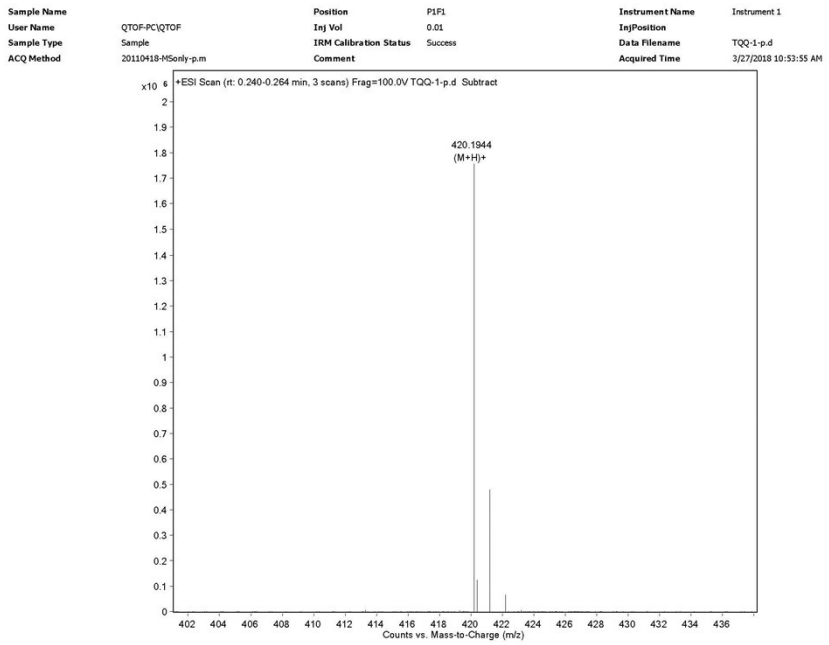

Figure S4 (c) HRMS spectrum of compound 32

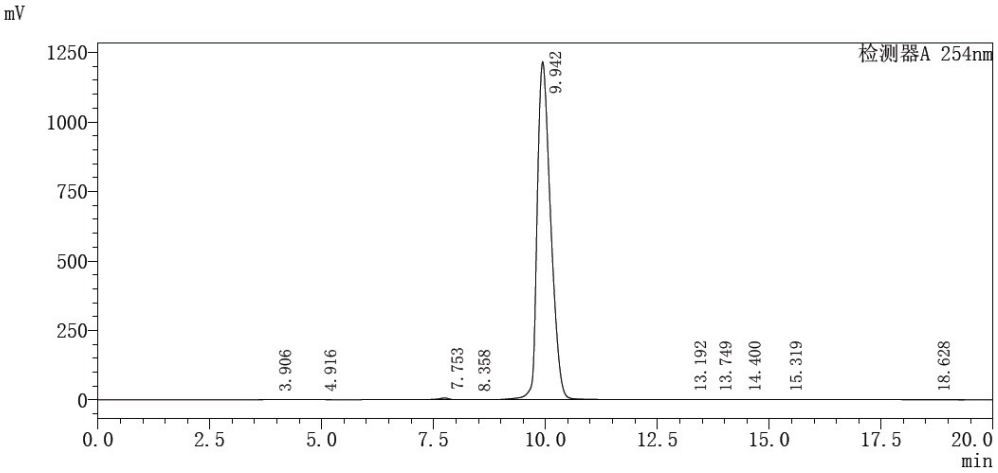

Figure S4 (d) HPLC of compound 32

Cpd.	Area of cpd.	Total area	Area %
32	25280509	25469551	99.3

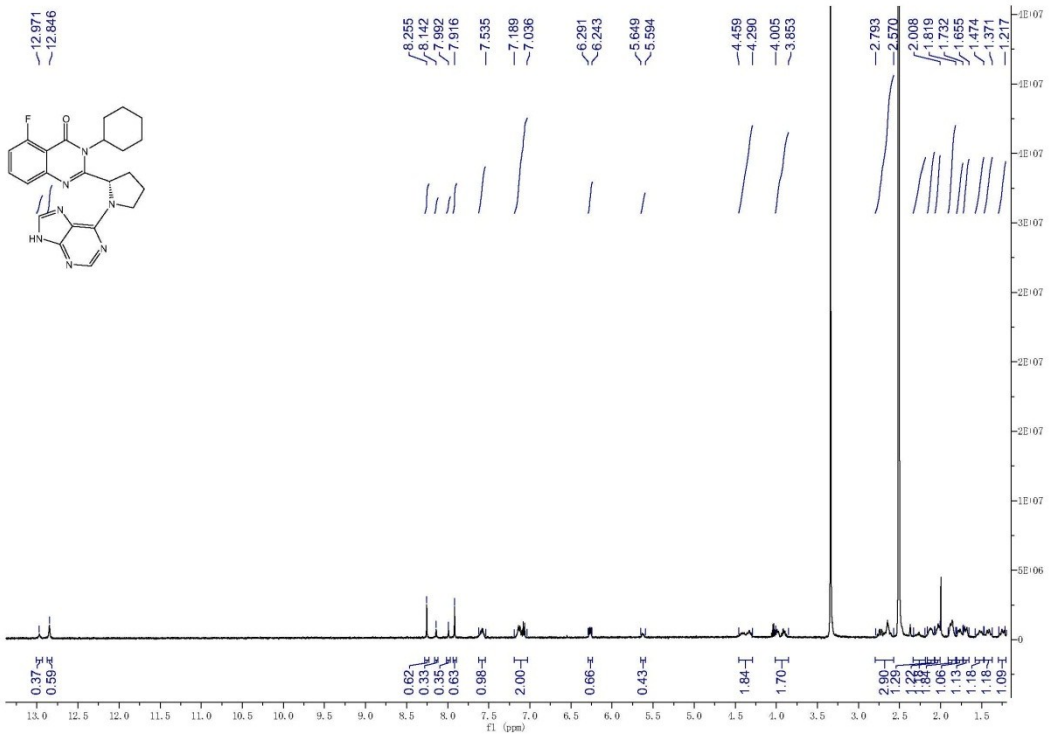

Figure S5 (a) ^{1}H NMR spectrum of compound 33

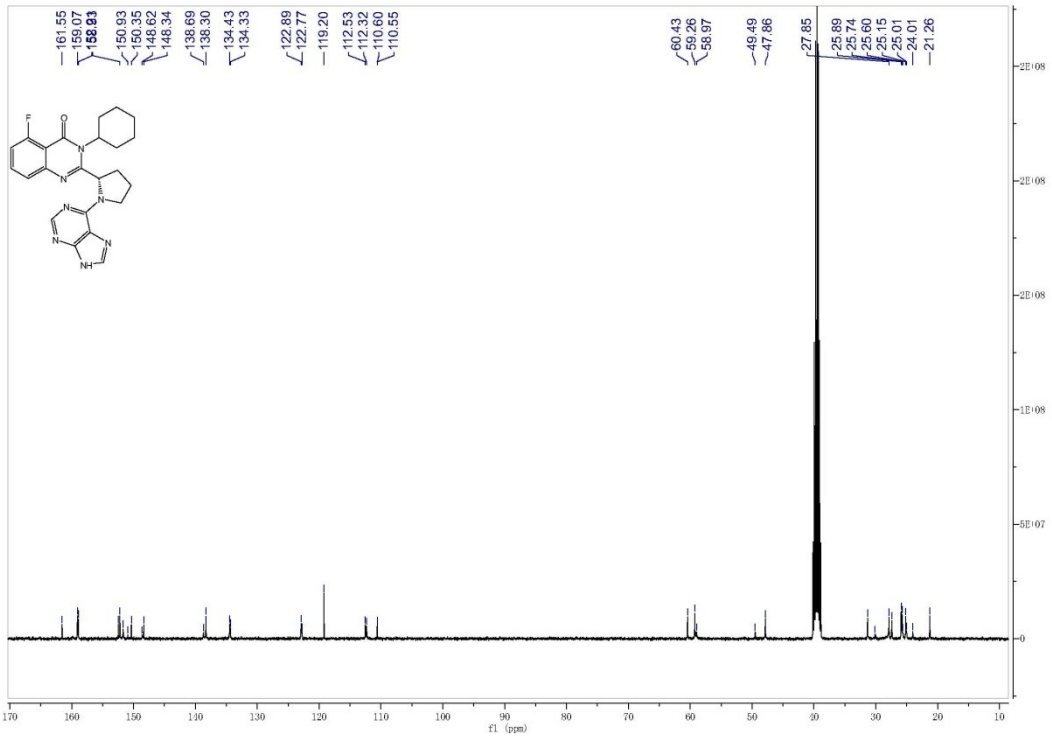

Figure S5 (b) ^{13}C NMR spectrum of compound 33

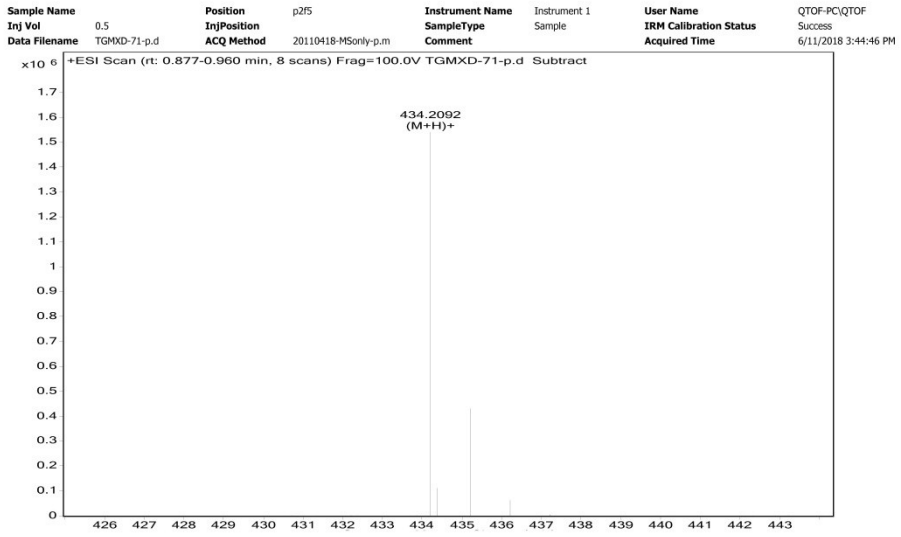

Figure S5 (c) HRMS spectrum of compound 33

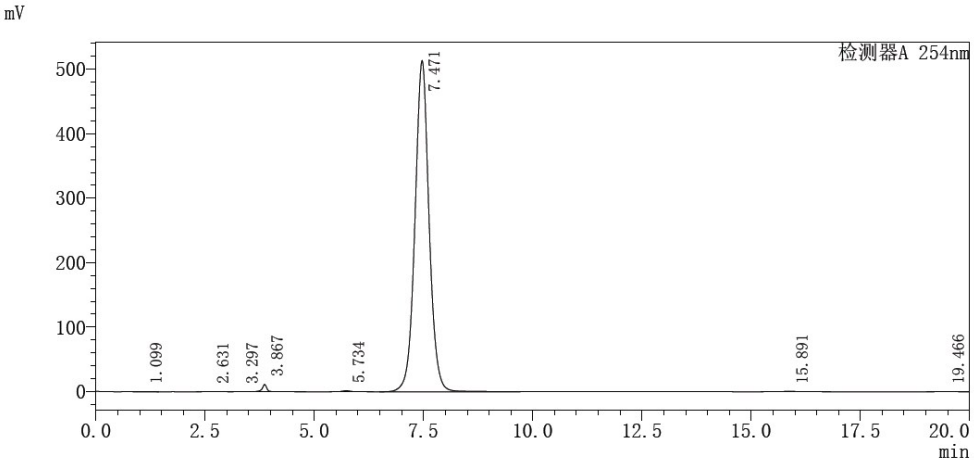

Figure S5 (d) HPLC of compound 33

Cpd.	Area of cpd.	Total area	Area %
33	11576660	11727435	98.7

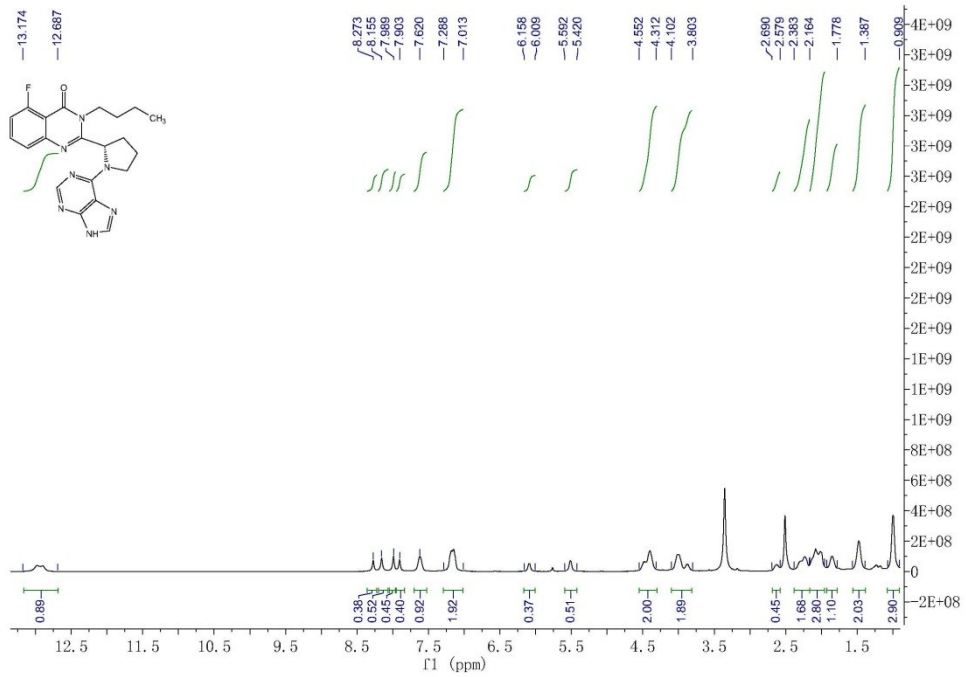

Figure S6 (a) ^{1}H NMR spectrum of compound 34

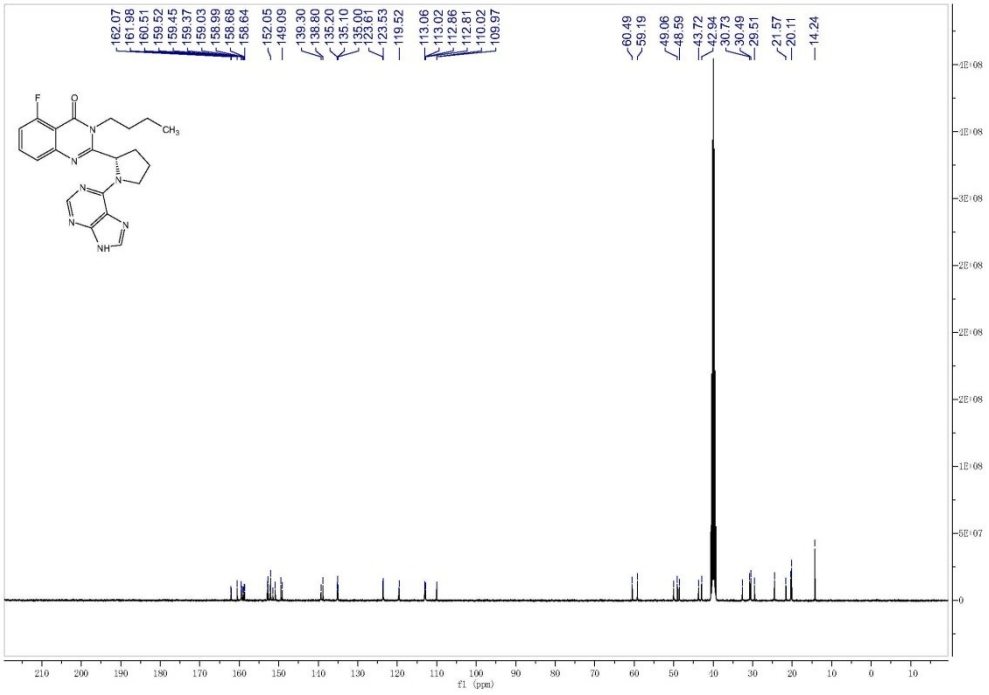

Figure S6 (b) ^{13}C NMR spectrum of compound 34

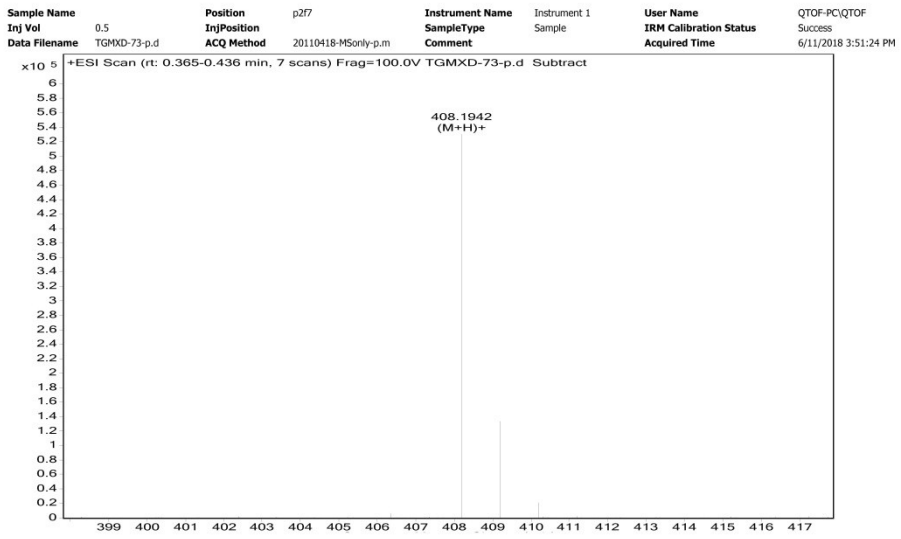

Figure S6 (c) HRMS spectrum of compound 34

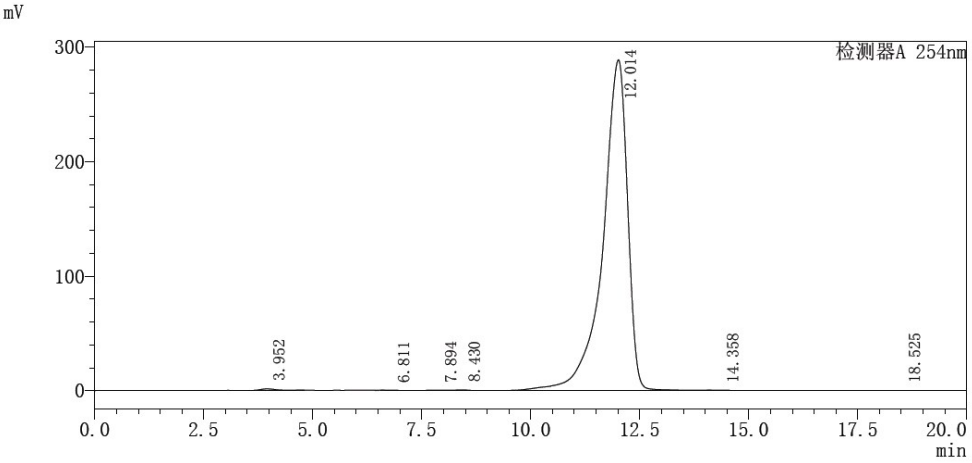

Figure S6 (d) HPLC of compound 34

Cpd.	Area of cpd.	Total area	Area %
34	11343933	11393471	99.6

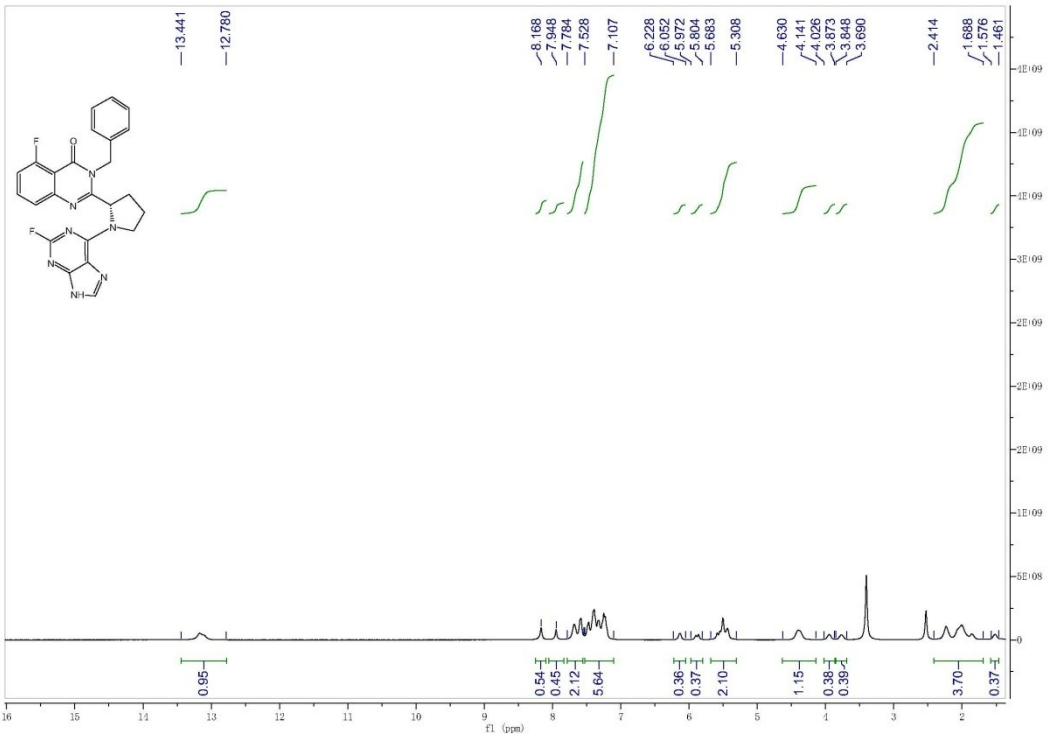

Figure S7 (a) ^{1}H NMR spectrum of compound 35

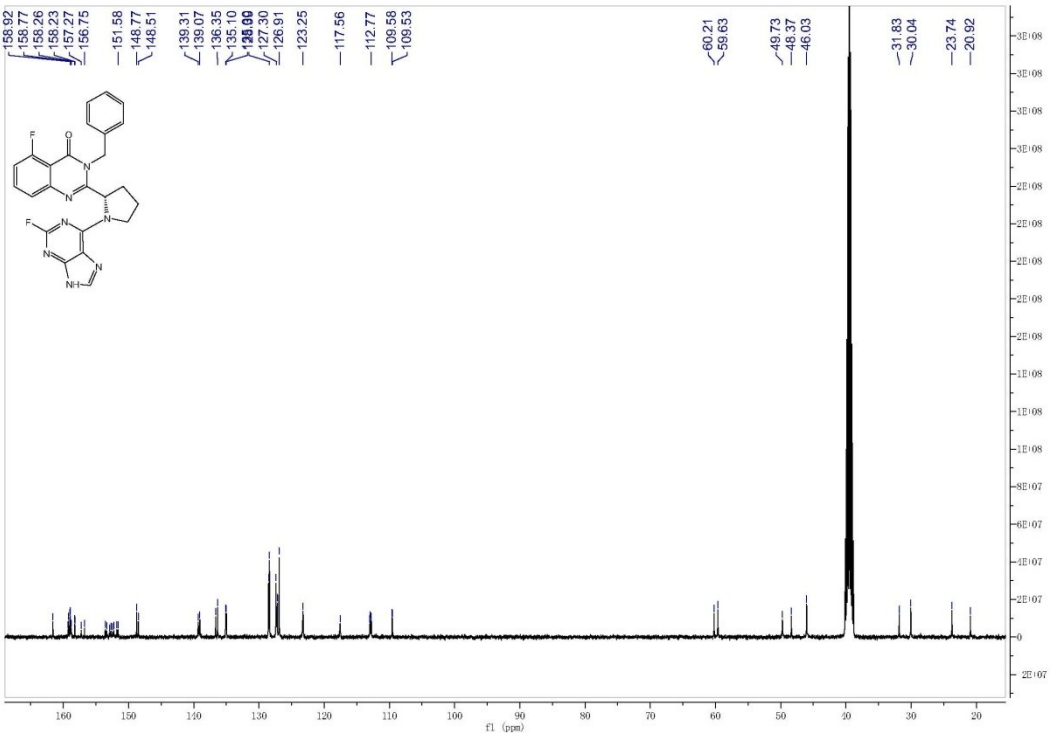

Figure S7 (b) ^{13}C NMR spectrum of compound 35

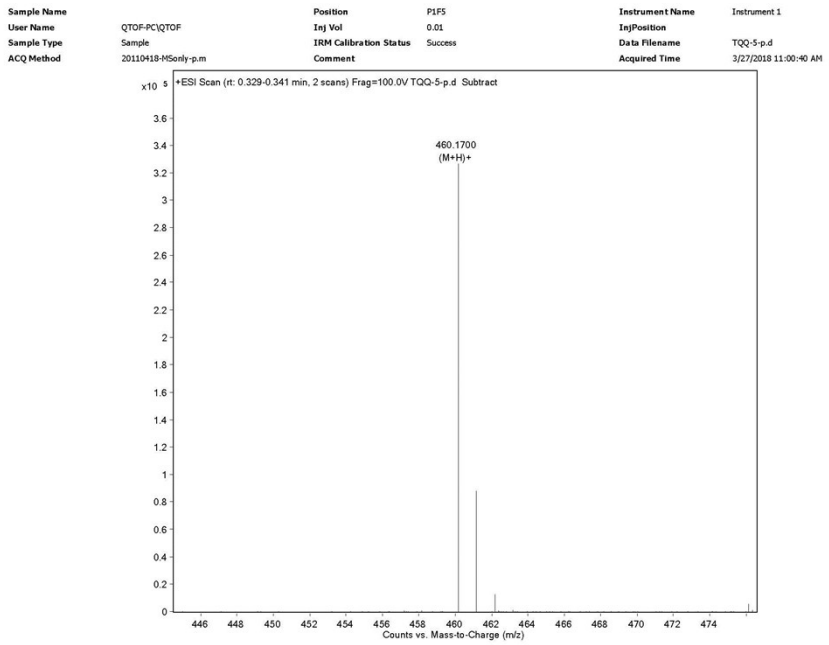

Figure S7 (c) HRMS spectrum of compound 35

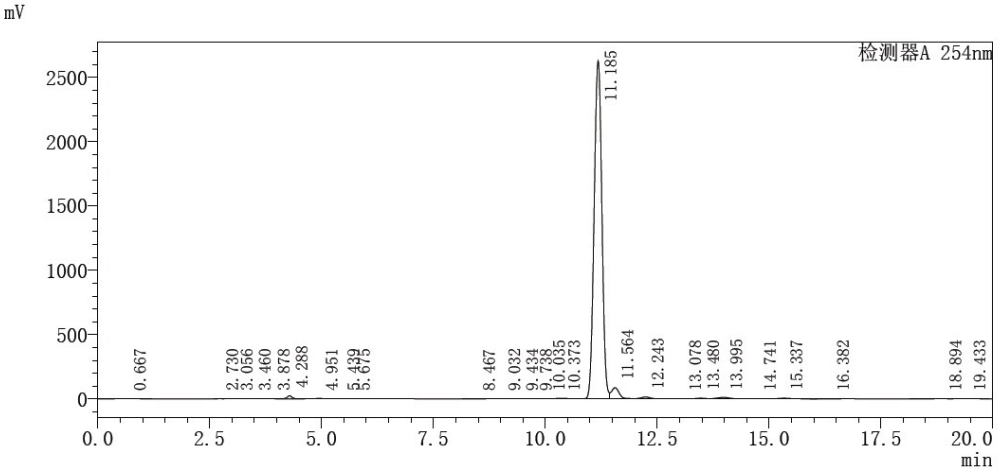

Figure S7 (d) HPLC of compound 35

Cpd.	Area of cpd.	Total area	Area %
35	31579647	33057949	95.5

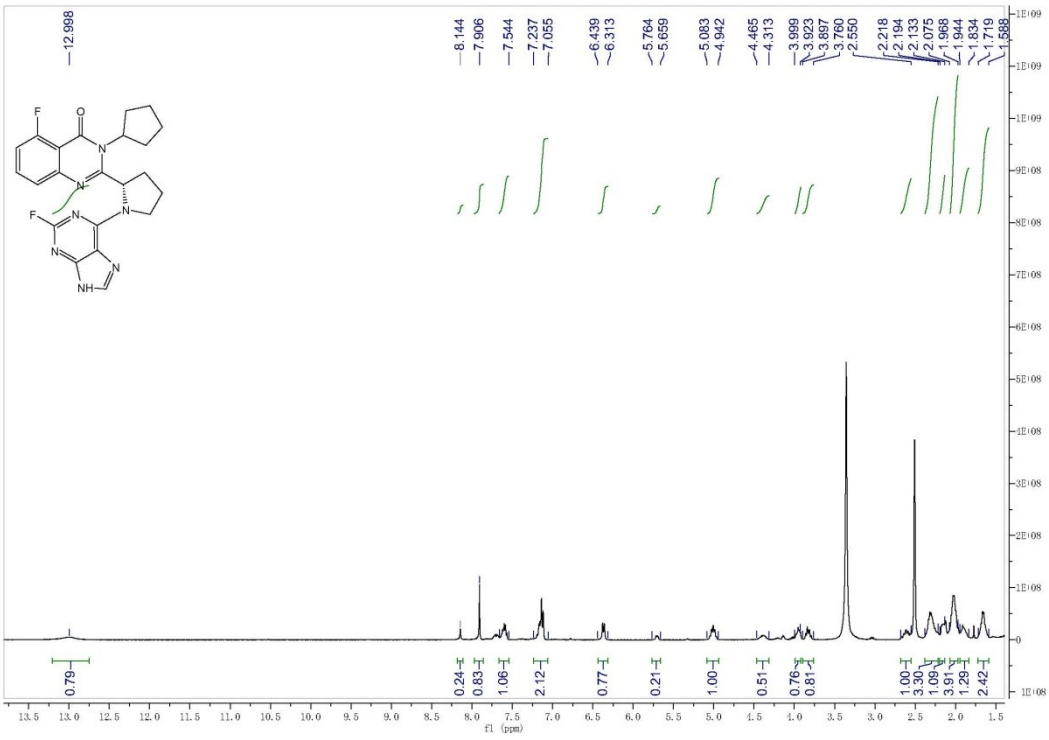

Figure S8 (a) ^{1}H NMR spectrum of compound 36

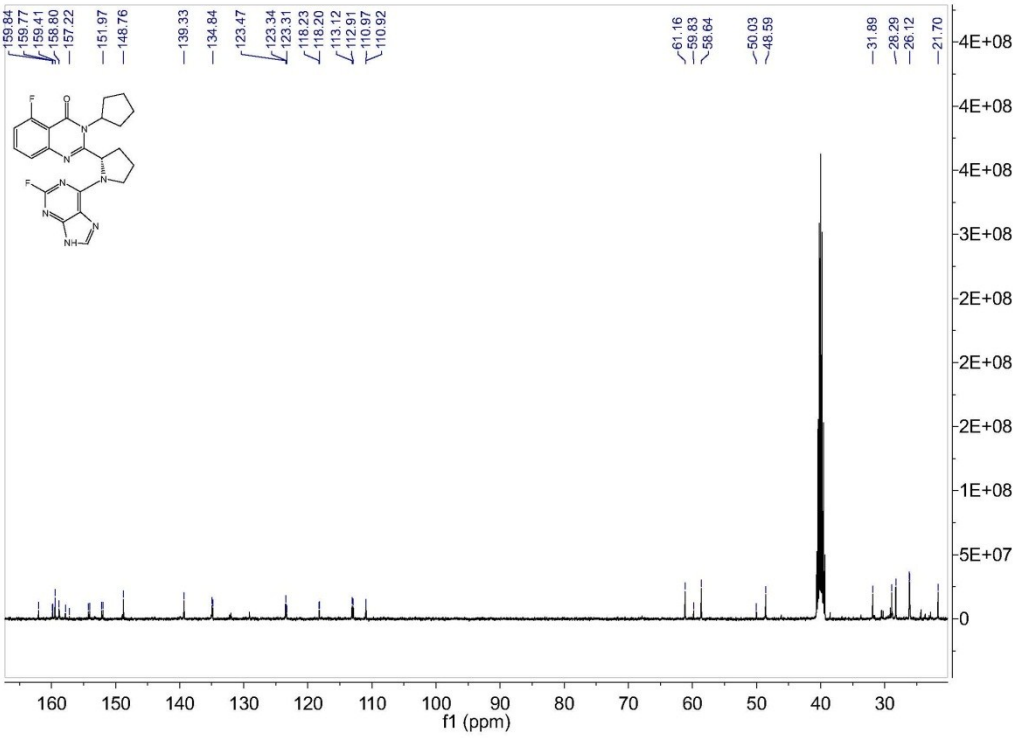

Figure S8 (b) ^{13}C NMR spectrum of compound 36

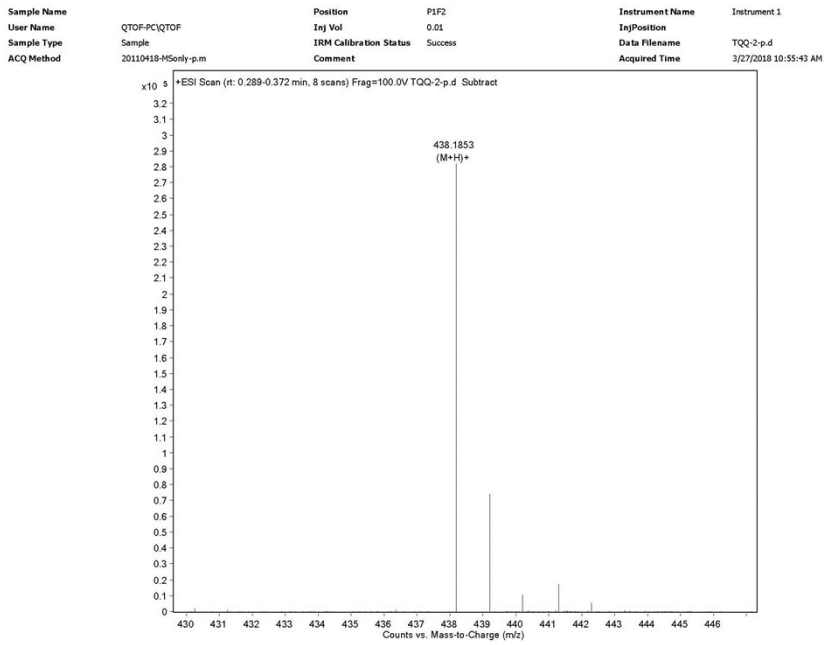

Figure S8 (c) HRMS spectrum of compound 36

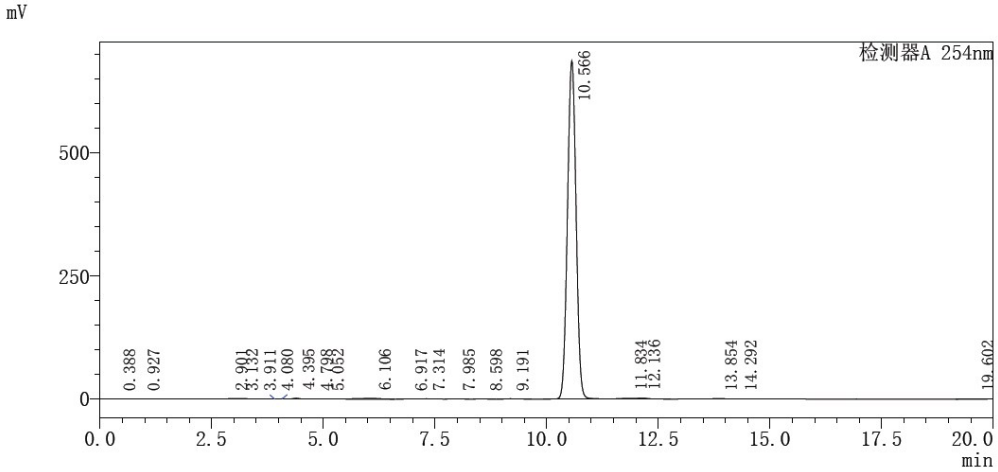

Figure S8 (d) HPLC of compound 36

Cpd.	Area of cpd.	Total area	Area %
36	9214290	9414717	97.9

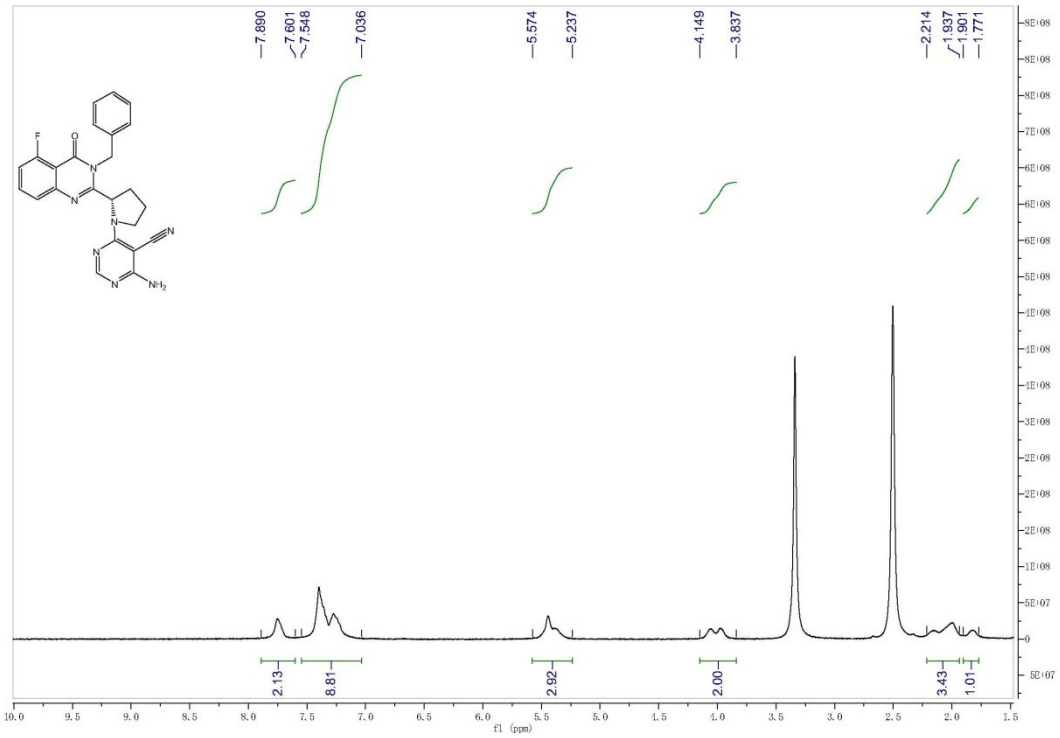

Figure S9 (a) ^{1}H NMR spectrum of compound 37

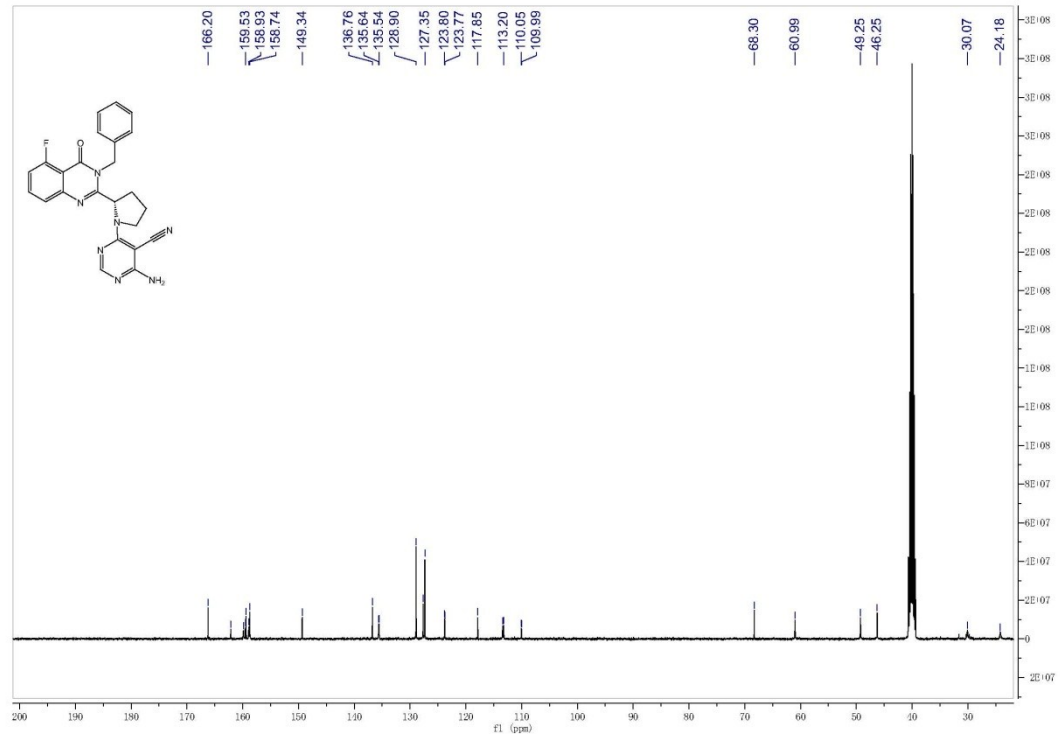

Figure S9 (b) ^{13}C NMR spectrum of compound 37

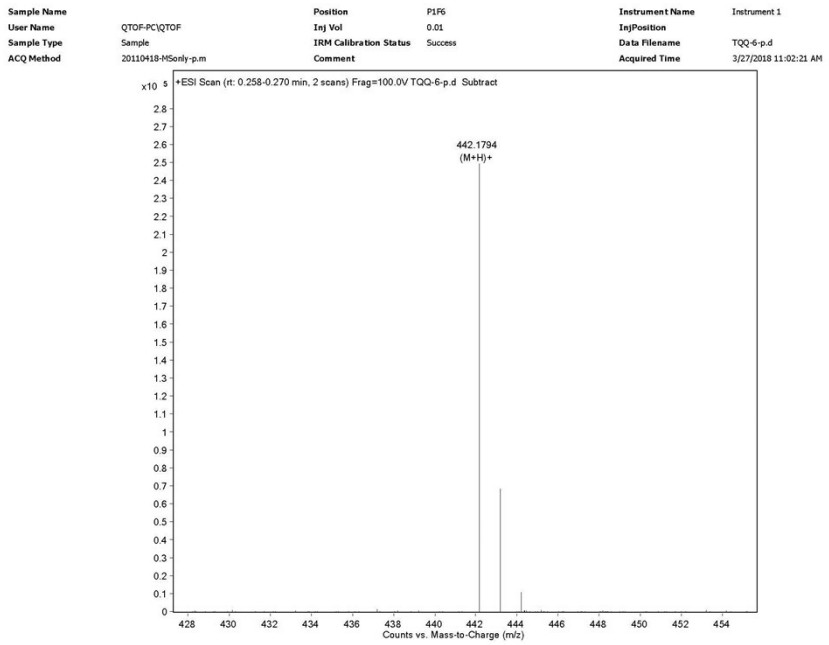

Figure S9 (c) HRMS spectrum of compound 37

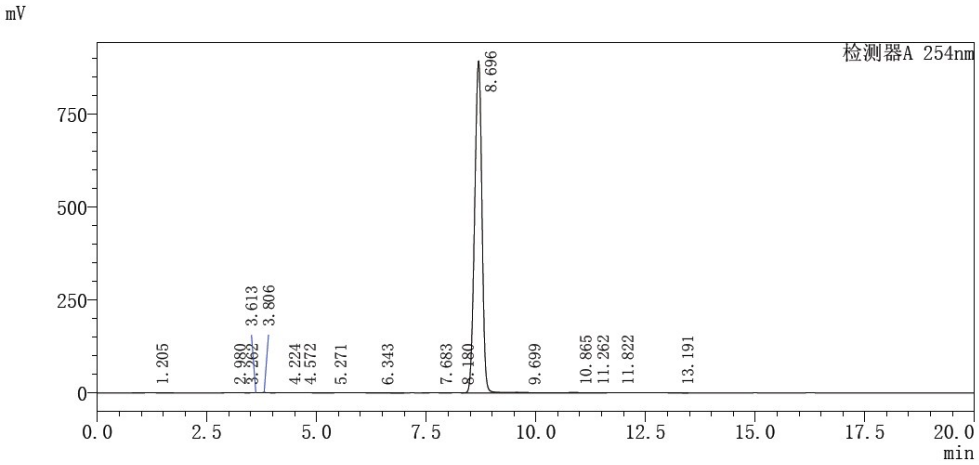

Figure S9 (d) HPLC of compound 37

Cpd.	Area of cpd.	Total area	Area %
37	10360572	10426629	99.4

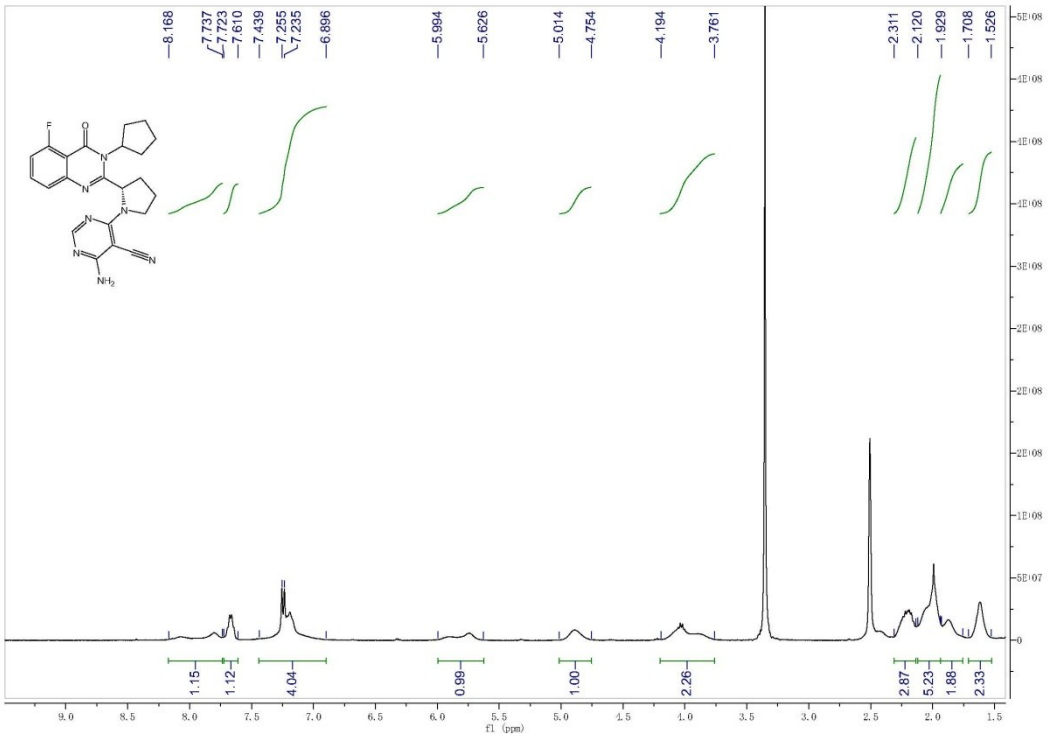

Figure S10 (a) ^{1}H NMR spectrum of compound 38

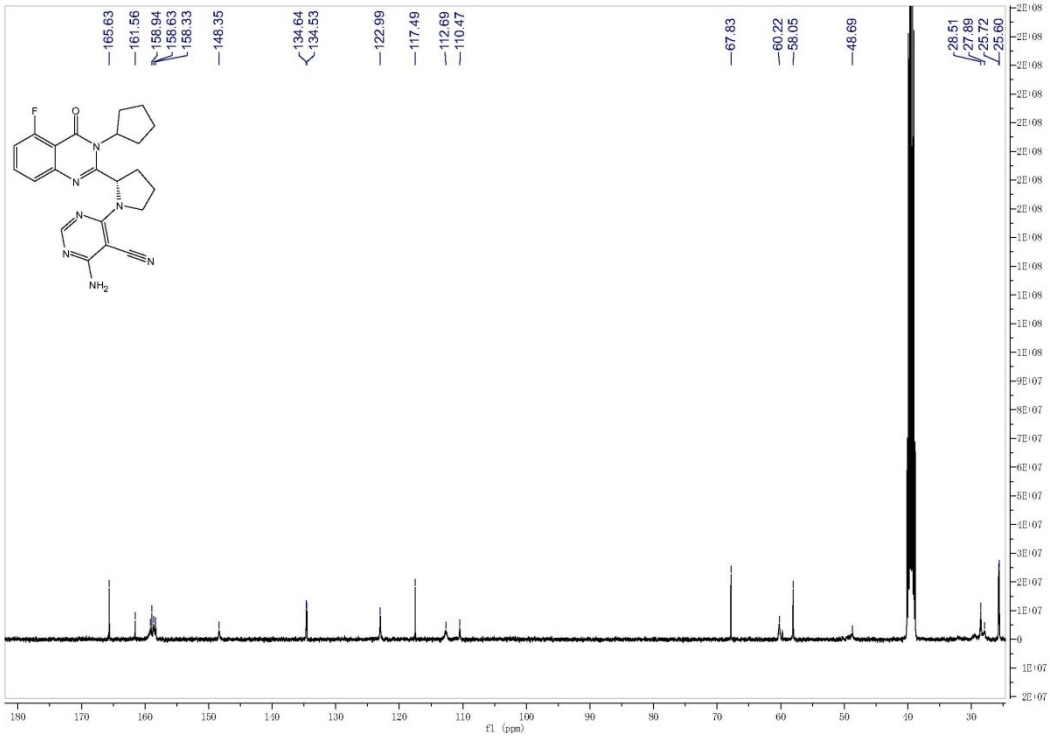

Figure S10 (b) ^{13}C NMR spectrum of compound 38

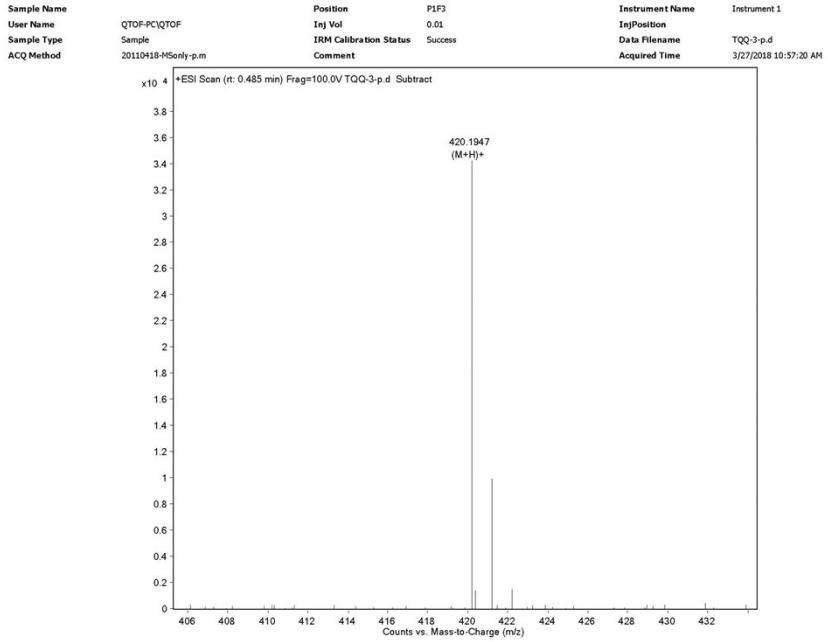

Figure S10 (c) HRMS spectrum of compound 38

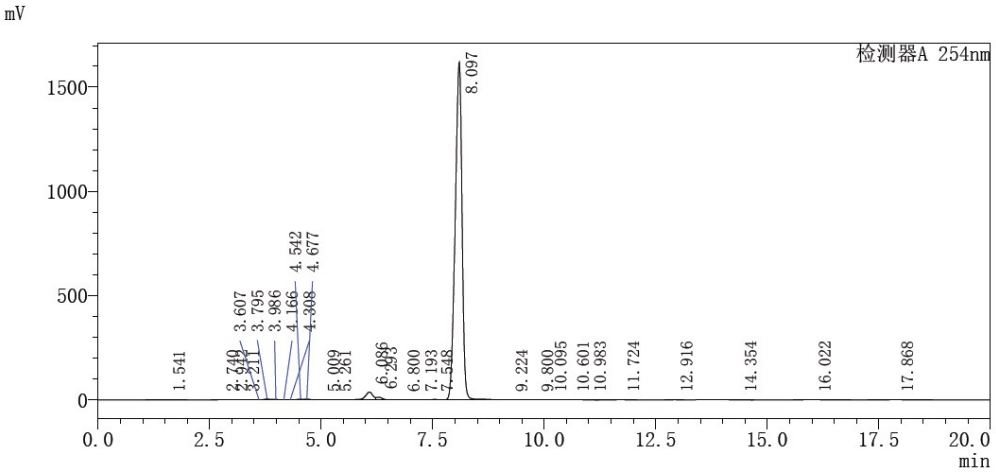

Figure S10 (d) HPLC of compound 38

Cpd.	Area of cpd.	Total area	Area %
38	17146183	17978359	95.4

3. Uncropped western blot images of compound 38

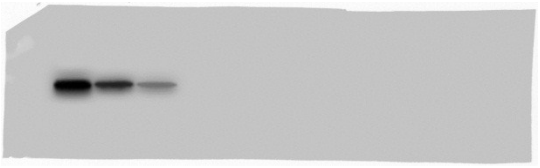

Figure S11 (a) Phos-S6K1 (T389)-1

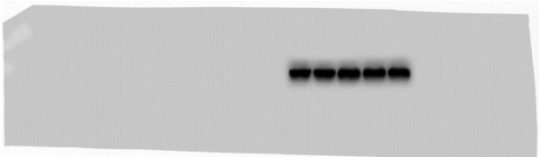

Figure S11 (b) Total S6K1-1

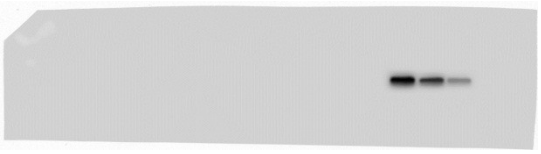

Figure S11 (c) Phos-Akt (S473)-1

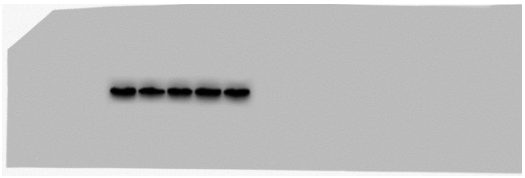

Figure S11 (d) Total Akt-1

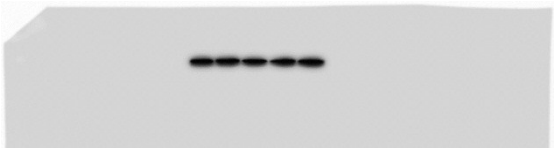

Figure S11 (e) GAPDH-1

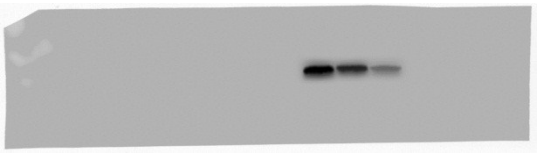

Figure S12 (a) Phos-S6K1 (T389)-2

Figure S12 (b) Total S6K1-2

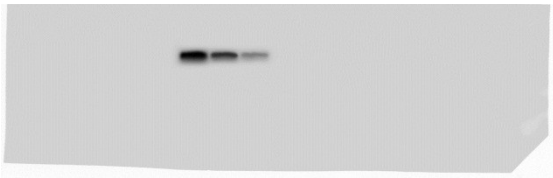

Figure S12 (c) Phos-Akt (S473)-2

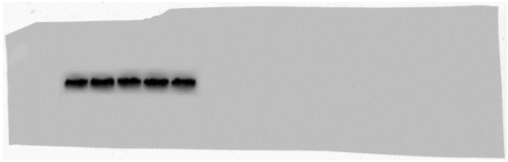

Figure S12 (d) Total Akt-2

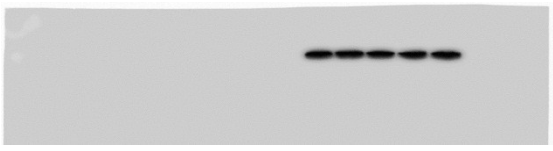

Figure S12 (e) GAPDH-2

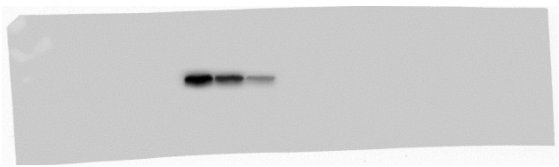

Figure S13 (a) Phos-S6K1 (T389)-3

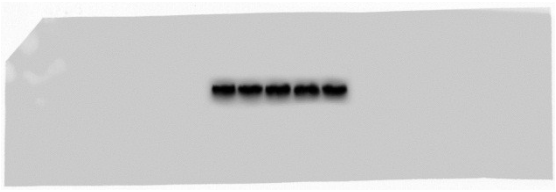

Figure S13 (b) Total S6K1-3

Figure S13 (c) Phos-Akt (S473)-3

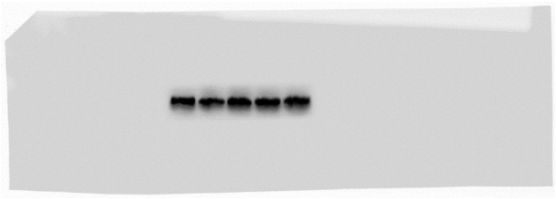

Figure S13 (d) Total Akt-3

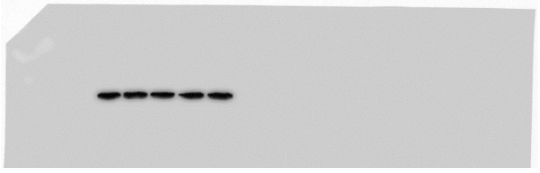

Figure S13 (e) GAPDH-3