Electronic Supplementary Material (ESI) for Chemical Communications.


# Supplementary Information

## Discovery of Novel Photocaged ERK1/2 Inhibitors as Light-controlled Anticancer Agents

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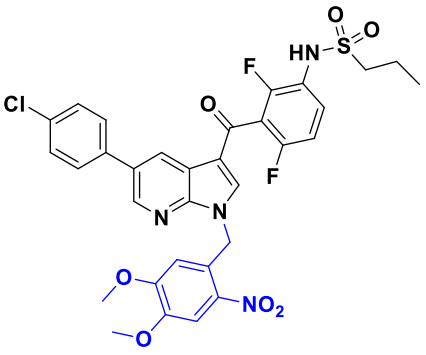
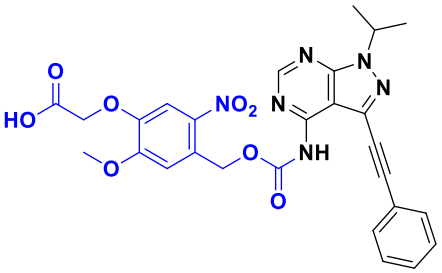
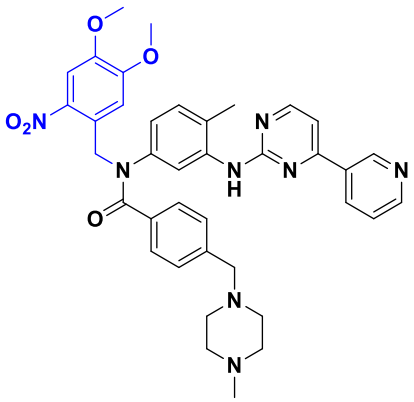


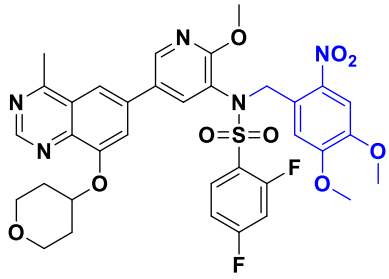
Caged vemurafenib



Caged RET kinase inhibitor

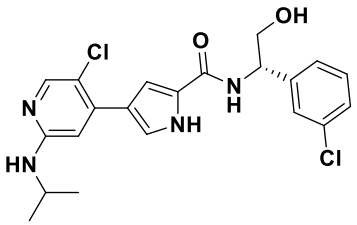


Caged Imatinib

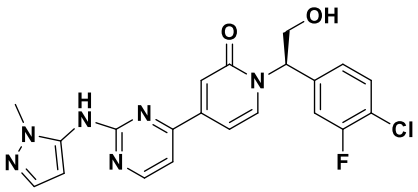


Caged PI3K inhibitor

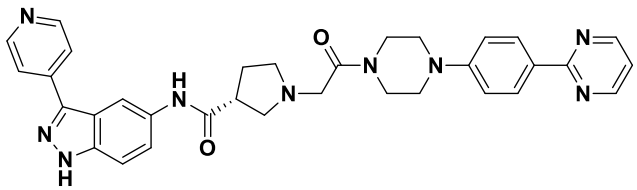
**Figure S1.** Reported photoactivatable caged kinase inhibitors.



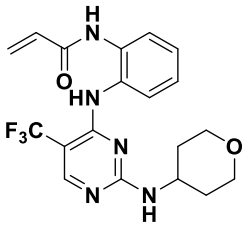
BVD-523 (Ulixertinib)



GDC-0994 (Ravoxertinib)



SCH772984



AZ13767370

**Figure S2.** Representative ERK1/2 inhibitors.

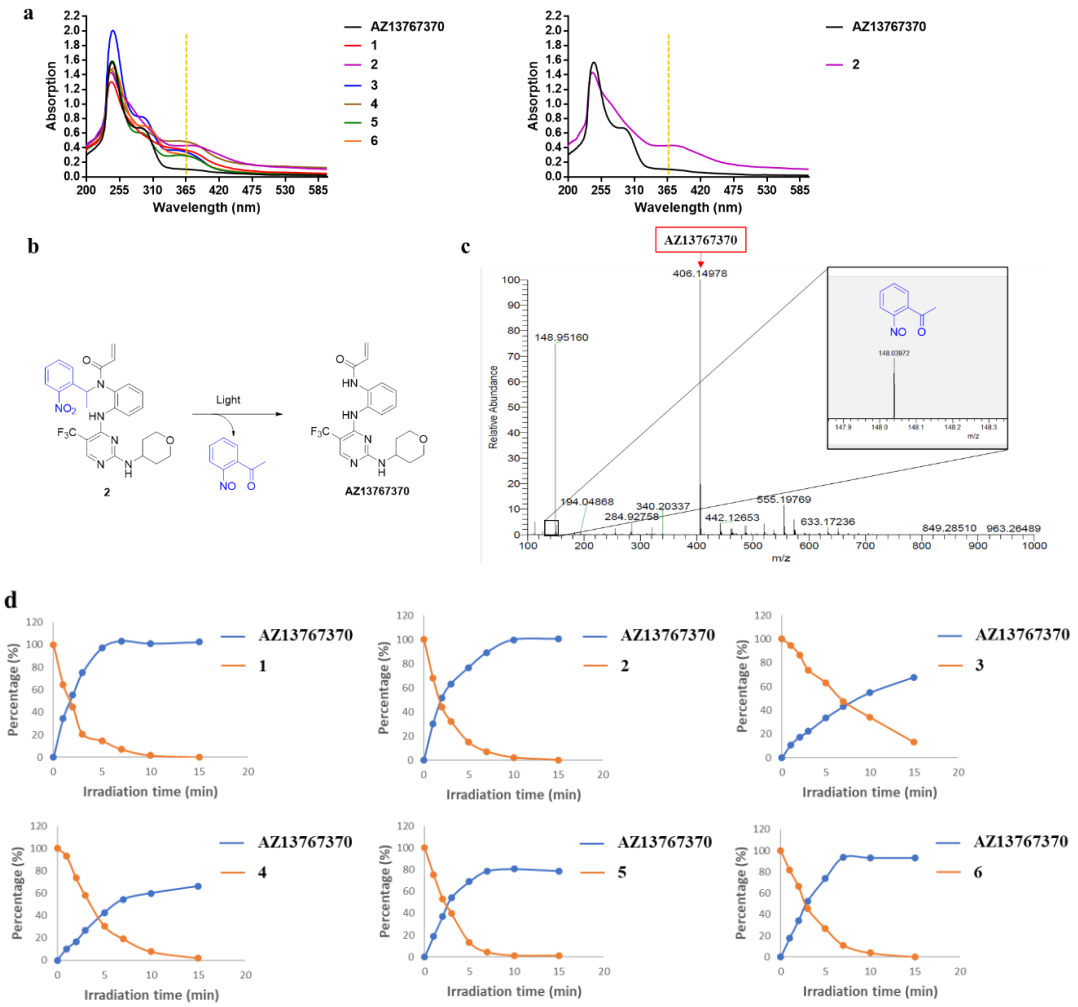
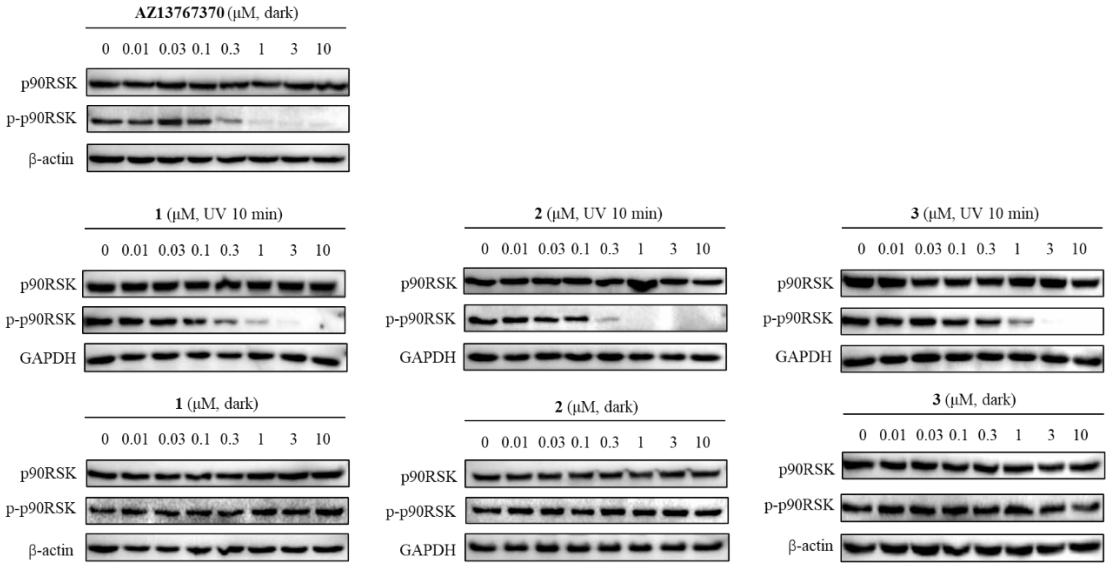



**Figure S3. a)** The absorption spectra of **AZ13767370** and photocaged ERK1/2 inhibitors **1-6** (150 μM in PBS buffer), which were measured on a UV-vis spectrometer (UV-1800, Shimadzu). **b)** Photolysis reaction of photocaged ERK1/2 inhibitor **2**. **c)** Mass spectra of the reaction mixture of photocaged ERK1/2 inhibitor **2** in MeCN irradiated by 365 nm light for 10 minutes. **d)** Photo induced release of **AZ13767370** by photocaged ERK1/2 inhibitors **1-6** upon irradiation.



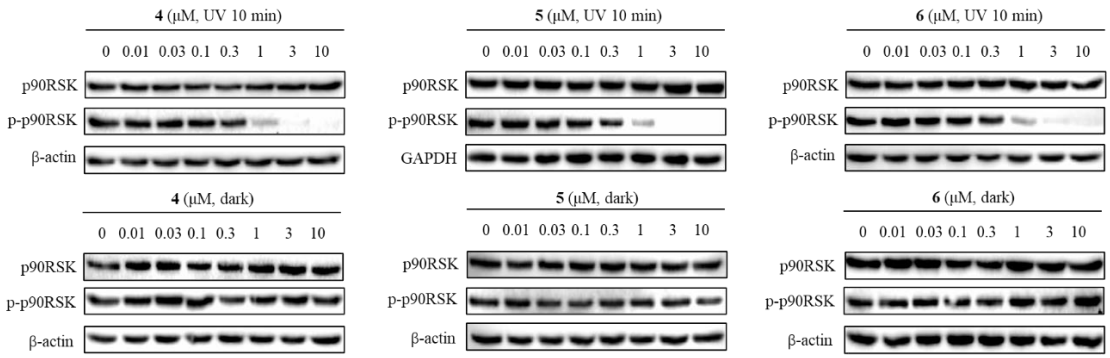



**Figure S4.** Activation of inhibitory effects of different photocaged ERK1/2 inhibitors on ERK signaling *in vitro*. A375 cells were incubated with DMSO and various concentrations of **AZ13767370** and different photocaged ERK1/2 inhibitors **1-6** for 1 h. After irradiation for 10 min or no irradiation at 365 nm (3 mW/cm$^2$), the cells were cultured in the dark for 2 h, and lysates were analyzed with immunoblotting.

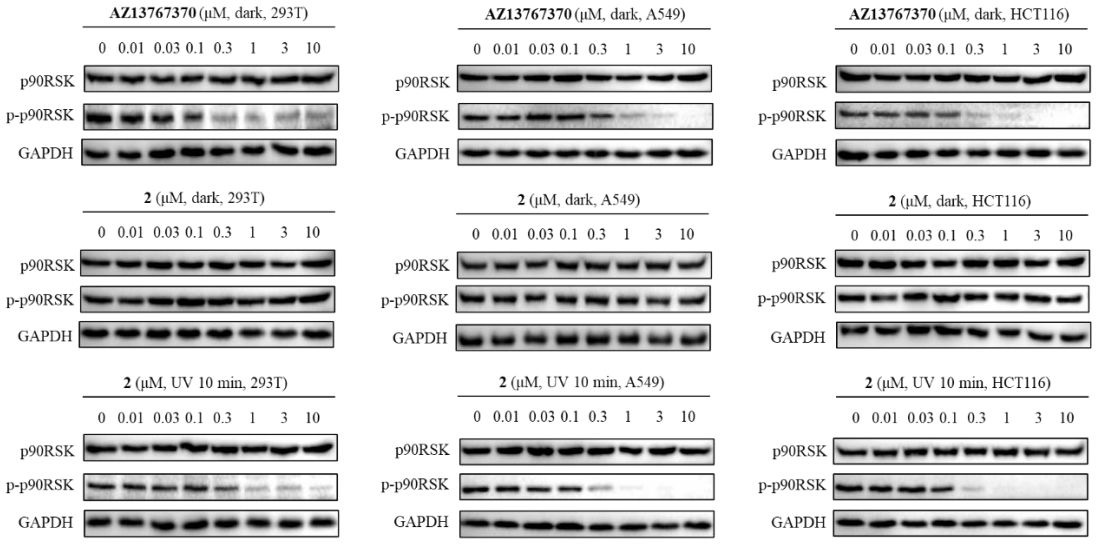



**Figure S5.** Activation of inhibitory effects of photocaged ERK1/2 inhibitor **2** on ERK signaling *in vitro* in different cells including 293T, A549 and HCT116. Different cells were incubated with DMSO and various concentrations of **AZ13767370** and compound **2** for 1 h. After irradiation for 10 min or no irradiation at 365 nm (3 mW/cm$^2$), the cells were cultured in the dark for 2 h, and lysates were analyzed with immunoblotting.

# EXPERIMENTAL SECTION

## Chemistry and chemical methods.

### General

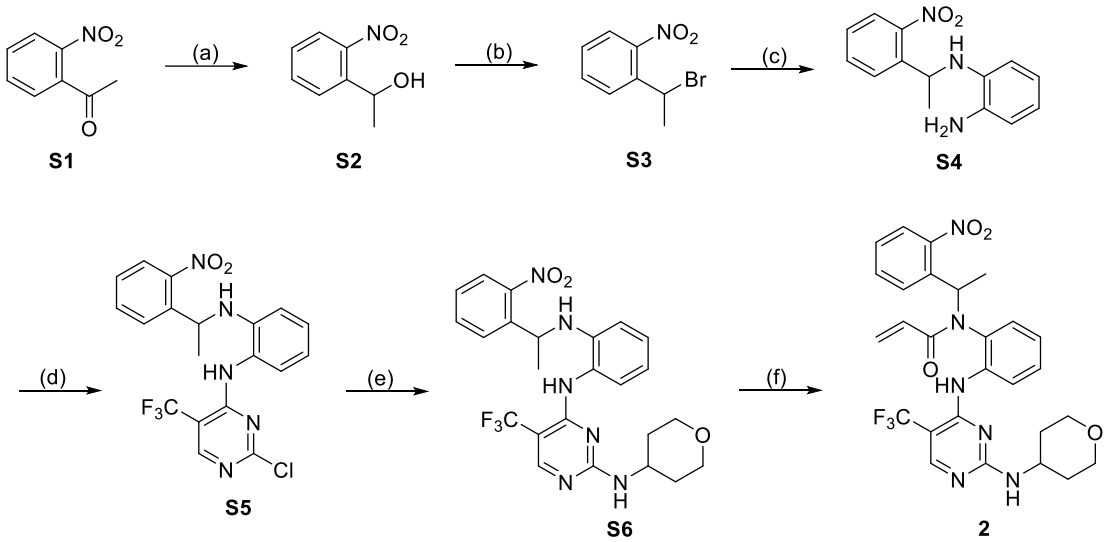
Unless specified otherwise, all the starting materials, reagents and solvents were commercially available. Reaction using air- or moisture-sensitive reagents were performed under an atmosphere of nitrogen. All the reactions were monitored by TLC and/or LC-MS (LCMS2020, shimadzu). Flash column chromatography was conducted using silica gel. The purities of all final compounds

were >95% and were analyzed by HPLC on an Agilent PN880975-902 ZORBAX SB-C18 4.6 × 250 mm column monitored at 254 nm. NMR spectra were measured using Bruker 400 or 500 MHz spectrometers, and chemical shifts are reported in units of ppm downfield from TMS using residual nondeuterated solvent as internal standards (DMSO, 2.50 ppm; $CHCl_3$, 7.26 ppm; $(CD_3)_2CO$, 2.05 ppm). In the NMR tabulation, these following abbreviations were used to describe peak splitting patterns: s indicates singlet, d indicates doublet, t indicates triplet, q indicates quartet, m indicates multiplet, dd indicates doublet of doublets and br indicates broad peak. High resolution mass spectra (HRMS) were performed by Thermo Q Exactive Focus instrument.

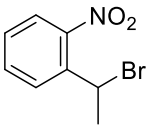
## Photolysis of Photocaged ERK1/2 Inhibitors.

Compounds (2 mM) were dissolved in PBS/MeCN (1:1) in 96-well plate and exposed to UV light (365 nm light source, CL-1000 L, UV Closslinkers, P/N number 95-0228-02, Ultraviolet Products Ltd., Cambridge, UK) at indicated times. Then the samples were analyzed by HPLC (Agilent PN880975-902 ZORBAX SB-C18 4.6 × 150 mm column, 5 μM), using water and MeCN as eluents.

## Synthesis of Photocaged ERK1/2 Inhibitors.



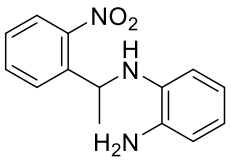

**Supporting Scheme S1: (a)** $NaBH_4$, MeOH, 0 ℃-R.T., 2 h; **(b)** $PBr_3$, DCM, 0 ℃-reflux, 2 h, 87%; **(c)** benzene-1,2-diamine, $K_2CO_3$, KI, MeCN, 60 ℃, 6 h, 83%; **(d)** 2,4-dichloro-5-(trifluoromethyl) pyrimidine, DIPEA, Ethanol, 0.5 h, 43%; **(e)** tetrahydro-*2H*-pyran-4-amine, HCl (conc.), 1,4-dioxane, M.W. 105 ℃, 3 h, 88%; **(f)** acryloyl chloride, DIPEA, DCM, 0 ℃, 12 h, 67%.




**S3**

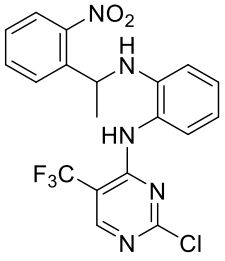
$NaBH_4$ (0.57 g, 15 mmol) were added slowly to 1-(1-bromoethyl)-2-nitrobenzene **S1** (1.65 g, 10 mmol) in dry MeOH (50 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 2 hours. The reaction mixture was quenched with ice water (20 mL). The organic layer was evaporated and then the residue was extracted with EtOAc (3 x 20 mL), and washed sequentially with saturated brine (50 mL). The organic layer was dried over $Na_2SO_4$, filtered and evaporated to afford crude product 1-(2-nitrophenyl) ethan-1-ol **S2**. Phosphorus tribromide $PBr_3$ (1.2 mL, 13 mmol) was added

dropwise to **S2** in DCM (50 mL) at 0 °C over a period of 20 minutes. The resulting mixture was stirred at 0 °C for 1 hours and then refluxed for 1 hours. The reaction mixture was quenched with ice water (30 mL). The organic layer was collected and then evaporated to dryness to afford crude product. The crude product was purified by flash silica chromatography to get 1-(1-bromoethyl)-2-nitrobenzene **S3** (2 g, 87%) as yellow soild.$^{1}$H NMR (400 MHz, Chloroform-*d*) δ 7.86 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.79 (dd, *J* = 8.2, 1.4 Hz, 1H), 7.62 (td, *J* = 7.7, 1.4 Hz, 1H), 7.41 (ddd, *J* = 8.6, 7.4, 1.4 Hz, 1H), 5.79 (q, *J* = 6.8 Hz, 1H), 2.05 (d, *J* = 6.9 Hz, 3H). $^{13}$C NMR (101 MHz, Chloroform-*d*) δ 147.42, 137.52, 133.42, 129.75, 128.94, 124.27, 41.84, 27.01. HRMS: ESI$^{+}$ *m/z* calcd. for $C_8H_7BrNO_2$ [M-H]$^{-}$ 227.9666, found 227.9666.



**S4**

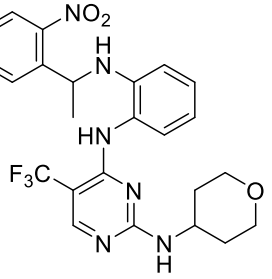
1-(1-bromoethyl)-2-nitrobenzene **S3** (2.30 g, 10 mmol) was added to 1,2-diaminobenzene (1.30 g, 12 mmol), potassium carbonate ($K_2CO_3$, 2.07 g, 15 mmol) and potassium iodide (KI, 166 mg, 1 mmol) in MeCN (50 mL). The resulting mixture was stirred at 60 °C for 6 hours. The resulting mixture was filtered and evaporated to dryness to afford crude product. The crude product was purified by flash silica chromatography to get N$^{1}$-(1-(2-nitrophenyl) ethyl) benzene-1,2-diamine **S4** (2.14 g, 83%) as yellow solid. $^{1}$H NMR (400 MHz, DMSO-*d*$_6$) δ 7.90 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.73 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.60 (td, *J* = 7.6, 1.2 Hz, 1H), 7.45 – 7.37 (m, 1H), 6.58 (dd, *J* = 7.5, 1.6 Hz, 1H), 6.38 (td, *J* = 7.5, 1.4 Hz, 1H), 6.31 (td, *J* = 7.6, 1.6 Hz, 1H), 6.03 (dd, *J* = 7.9, 1.4 Hz, 1H), 5.19 (d, *J* = 6.6 Hz, 1H), 5.00 (q, *J* = 6.4 Hz, 1H), 4.77 (s, 2H), 1.56 (d, *J* = 6.6 Hz, 3H). $^{13}$C NMR (101 MHz, DMSO-*d*$_6$) δ 148.99, 140.60, 135.31, 133.83, 133.53, 127.94, 127.35, 123.98, 117.51, 117.43, 114.55, 110.51, 48.00, 23.52. HRMS: ESI$^{+}$ *m/z* calcd. for $C_{14}H_{16}N_3O_2$ [M+H]$^{+}$ 258.1237, found 258.1237.



**S5**

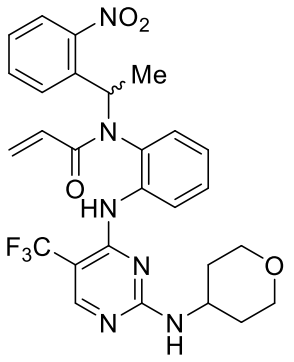
2,4-dichloro-5-(trifluoromethyl) pyrimidine (1.69 g, 7.8 mmol) was added to N$^{1}$-(1-(2-nitrophenyl) ethyl) benzene-1,2-diamine **S4** (1.54 g, 6 mmol) and N-ethyl-N-isopropylpropan-2-amine DIPEA (1.55 mL, 9 mmol) in ethanol (20 mL) at room temperature under nitrogen. The resulting mixture was stirred at room temperature for 0.5 hour. The reaction was evaporated to dryness, dissolved in EtOAc and washed with saturated brine. The aqueous layer was extracted with further EtOAc. Combined organic layers were dried over $Na_2SO_4$, filtered and evaporated to dryness to afford crude product. The crude product was purified by flash silica chromatography to get N$^{1}$-(2-chloro-5-(trifluoromethyl) pyrimidin-4-yl)-N$^{2}$-(1-(2-nitrophenyl) ethyl) benzene-1,2-diamine **S5** (1.13g, 43%) as yellow solid. $^{1}$H NMR (400 MHz, DMSO-*d*$_6$) δ 9.84 (s, 1H), 8.71 (s, 1H), 7.94 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.82 (d, *J* = 7.9 Hz, 1H), 7.65 (td, *J* = 7.6, 1.3 Hz, 1H), 7.53 – 7.42 (m, 1H), 7.13 (dd,

*J* = 7.8, 1.5 Hz, 1H), 6.91 (td, *J* = 7.8, 1.6 Hz, 1H), 6.55 (td, *J* = 7.5, 1.3 Hz, 1H), 6.22 (dd, *J* = 8.4, 1.2 Hz, 1H), 5.77 (s, 1H), 5.12 – 4.95 (m, 1H), 1.49 (d, *J* = 6.6 Hz, 3H). $^{13}$C NMR (101 MHz, DMSO-*d*$_6$) δ 162.62, 158.07, 158.03, 148.76, 142.07, 139.97, 133.72, 128.14, 127.75, 127.44, 127.41, 124.57, 124.14, 122.96, 121.89, 116.11, 111.03, 110.70, 110.37, 110.05, 47.87, 23.26. HRMS: ESI$^+$ *m/z* calcd. for $C_{19}H_{16}ClF_3N_5O_2$ $[M+H]^+$ 438.0939, found 438.0941.



**S6**

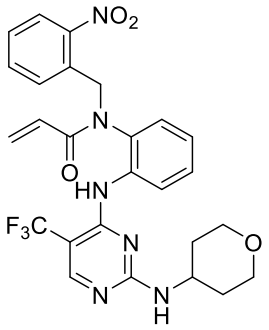
$N^1$-(2-chloro-5-(trifluoromethyl) pyrimidin-4-yl)-$N^2$-(1-(2-nitrophenyl) ethyl) benzene-1,2-diamine **S5** (438 mg, 1 mmol), tetrahydro-2H-pyran-4-amine (131 mg, 1.3 mmol) and concentrated hydrochloric acid (20 μL) were dissolved in 1,4-dioxane (5 mL) and sealed into a microwave tube. The reaction was heated to 105 °C for 3 hours in the microwave reactor and cooled to room temperature. The reaction mixture was extracted with EtOAc and saturated brine. The organic layer was dried over $Na_2SO_4$, filtered and evaporated to dryness to afford crude product. The crude product was purified by flash silica chromatography to get $N^4$-(2-((1-(2-nitrophenyl) ethyl) amino) phenyl)-$N^2$-(tetrahydro-2H-pyran-4-yl)-5-(trifluoromethyl) pyrimidine-2,4-diamine **S6** (440 mg, 88%) as yellow solid. $^1$H NMR (400 MHz, DMSO-*d*$_6$) δ 8.88 (s, 1H), 8.18 (s, 1H), 7.93 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.81 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.67 – 7.53 (m, 1H), 7.44 (td, *J* = 7.8, 1.4 Hz, 1H), 7.32 (d, *J* = 7.8 Hz, 1H), 6.81 (td, *J* = 7.7, 1.6 Hz, 1H), 6.63 – 6.47 (m, 2H), 6.19 (dd, *J* = 8.3, 1.3 Hz, 1H), 5.69 (s, 1H), 4.98 (q, *J* = 6.6 Hz, 1H), 4.12 (s, 1H), 3.82 (dd, *J* = 11.5, 3.6 Hz, 2H), 3.18 (d, *J* = 11.7 Hz, 2H), 1.66 (dt, *J* = 15.4, 7.3 Hz, 4H), 1.51 (d, *J* = 6.6 Hz, 3H). $^{13}$C NMR (101 MHz, DMSO-*d6*) δ 162.07, 157.32, 154.78, 148.81, 140.70, 140.09, 133.63, 128.11, 127.65, 126.41, 125.56, 124.90, 124.10, 123.73, 116.07, 111.07, 97.50, 97.18, 96.86, 96.54, 66.50, 66.47, 48.08, 47.06, 31.95, 31.86, 23.49. HRMS: ESI$^+$ *m/z* calcd. for $C_{24}H_{26}F_3N_6O_3$ $[M+H]^+$ 503.2013, found 503.2015.



**2**

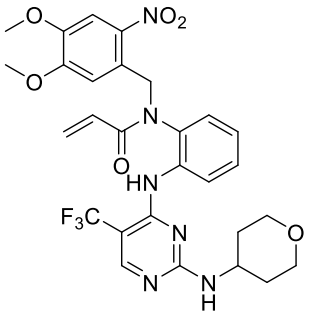
Acryloyl chloride (33 μL, 0.4 mmol) was added to $N^4$-(2-((1-(2-nitrophenyl) ethyl) amino) phenyl)-$N^2$-(tetrahydro-*2H*-pyran-4-yl)-5-(trifluoromethyl) pyrimidine-2,4-diamine **S6** (200 mg, 0.4 mmol) in DCM (20 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 12 hours. The reaction mixture was extracted with EtOAc and saturated brine. The organic layer was dried over $Na_2SO_4$,

filtered and evaporated to dryness to afford crude product. The crude product was purified by flash silica chromatography to get N-(1-(2-nitrophenyl) ethyl)-N-(2-((2-((tetrahydro-*2H*-pyran-4-yl) amino)-5-(trifluoromethyl) pyrimidin-4-yl) amino) phenyl) acrylamide **2** (Rotamers, 149 mg, 67%) as yellow solid. $^{1}$H NMR (400 MHz, Chloroform-*d*) δ 8.39 – 7.89 (m, 2H), 7.79 (d, *J* = 8.0 Hz, 0.56H), 7.66 (d, *J* = 8.0 Hz, 0.43H), 7.59 – 7.29 (m, 3H), 7.27 – 6.74 (m, 3.4H), 6.57 (br, 0.47H), 6.48 – 6.33 (m, 1H), 6.22 (q, *J* = 7.2 Hz, 1H), 5.85 (br, 1H), 5.59 – 4.89 (m, 2H), 4.01 (m, 3H), 3.49 (s, 2H), 2.16 – 1.84 (m, 2H), 1.69 (m, 2H), 1.65 – 1.47 (m, 3H). $^{13}$C NMR (101 MHz, Chloroform-*d*) δ 166.20, 165.82, 162.02, 161.70, 156.97, 156.56, 156.11, 156.06, 150.02, 149.53, 136.95, 136.32, 134.62, 132.21, 130.71, 130.47, 129.85, 129.25, 128.69, 128.32, 128.12, 127.92, 127.61, 126.02, 125.72, 125.21, 124.80, 124.63, 124.04, 123.34, 123.04, 99.01, 98.53, 98.22, 97.88, 66.79, 66.75, 51.31, 51.00, 50.45, 48.01, 47.79, 47.40, 33.19, 32.88, 19.83, 18.69. HRMS: ESI$^{+}$ *m/z* calcd. for $C_{27}H_{28}F_3N_6O_4[M+H]^+$ 557.2119, found 557.2115.



**1**

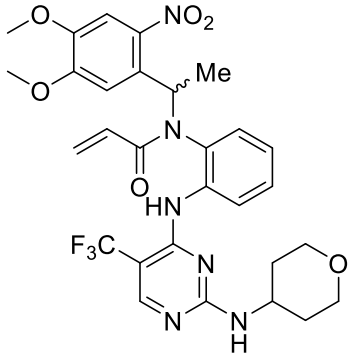
N-(2-nitrobenzyl)-N-(2-((2-((tetrahydro-2H-pyran-4-yl) amino)-5-(trifluoromethyl) pyrimidin-4-yl) amino) phenyl) acrylamide **1** (Rotamers). $^{1}$H NMR (500 MHz, Chloroform-*d*) δ 10.28 (d, *J* = 6.7 Hz, 0.64H), 10.04 (br, 0.37H), 8.46 (s, 0.65H), 8.04 (s, 1H), 7.89 (br, 0.37H), 7.87 – 7.68 (m, 2H), 7.51 (m, 1H), 7.47 – 7.31 (m, 4H), 7.15 (d, *J* = 5 Hz, 0.67H), 7.11 (br, 0.61H), 6.75 – 6.53 (m, 1.32H), 6.06 – 5.90 (m, 1H), 5.69 (d, *J* = 10.3 Hz, 0.65H), 5.53 (br, 0.35H), 5.43 (br, 0.36H), 5.40 – 5.30 (m, 1H), 4.04 – 3.15 (m, 5H), 1.91 – 1.50 (m, 4H). $^{13}$C NMR (126 MHz, Chloroform-*d*) δ 167.20, 165.83, 163.81, 163.51, 157.30, 154.32, 153.96, 149.35, 148.15, 134.55, 133.53, 132.95, 132.78, 132.63, 131.57, 131.26, 130.18, 129.48, 129.22, 128.98, 128.88, 128.81, 128.50, 127.46, 127.00, 126.76, 126.46, 126.04, 125.49, 124.46, 123.51, 121.36, 117.36, 115.06, 99.56, 99.29, 99.02, 98.73, 66.50, 66.27, 52.13, 49.19, 48.89, 47.58, 32.04, 31.58. HRMS: ESI$^{+}$ *m/z* calcd. for $C_{26}H_{26}F_3N_6O_4[M+H]^+$ 543.1962, found 543.1968.



**3**

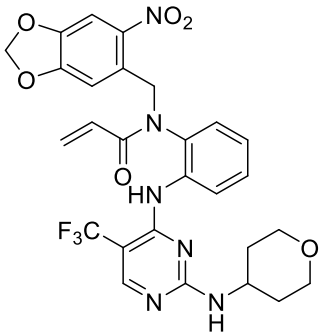
N-(4,5-dimethoxy-2-nitrobenzyl)-N-(2-((2-((tetrahydro-2H-pyran-4-yl) amino)-5-(trifluoromethyl) pyrimidin-4-yl) amino) phenyl) acrylamide **3** (Rotamers). $^{1}$H NMR (400 MHz, Chloroform-*d*) δ

10.39 (d, *J* = 6.7 Hz, 0.76H), 10.18 (br, 0.30H), 8.37 (br, 0.30H), 7.97 (s, 1H), 7.65 (m, 0.77H), 7.46 – 7.38 (m, 2H), 7.34 (m, 1H), 7.28 (s, 1H), 7.21 (s, 1H), 6.99 (s, 0.78H), 6.86 (br, 0.31H), 6.68 (m, 0.28H), 6.57 (m, 1H), 6.01 (m, 1H), 5.73 (m, 0.77H), 5.62 (m, 0.30H), 5.55 (m, 0.77H), 5.31 (m, 1H), 4.06 – 3.58 (m, 9H), 3.50 – 3.13 (m, 2H), 2.00 – 1.52 (m, 4H). $^{13}$C NMR (101 MHz, Chloroform-*d*) δ 167.03, 165.64, 163.50, 163.13, 162.76, 156.98, 153.97, 153.63, 153.21, 148.74, 148.26, 144.79, 141.56, 140.43, 135.84, 134.65, 132.90, 132.51, 132.24, 131.49, 129.45, 128.98, 128.68, 128.37, 127.23, 126.82, 126.27, 125.58, 125.11, 123.64, 120.95, 117.42, 114.43, 112.91, 107.99, 106.88, 99.02, 98.68, 98.33, 97.98, 66.46, 66.13, 56.43, 56.23, 52.27, 48.99, 47.30, 32.10, 31.41, 31.11. HRMS: ESI$^+$ *m/z* calcd. for $C_{28}H_{30}F_3N_6O_6$ $[M+H]^+$ 603.2173, found 603.2174.



**4**

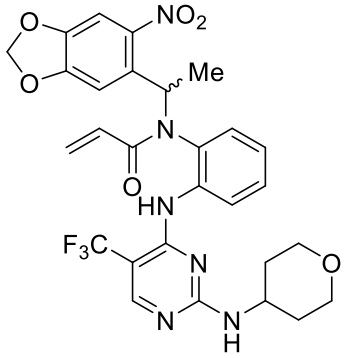
N-(1-(4,5-dimethoxy-2-nitrophenyl)ethyl)-N-(2-((2-((tetrahydro-2H-pyran-4-yl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)phenyl)acrylamide **4** (Rotamers). $^1$H NMR (500 MHz, Acetone-$d_6$) δ 8.48 – 7.80 (m, 2H), 7.67 – 6.50 (m, 7H), 6.45 – 6.16 (m, 2H), 5.91 (m, 1H), 5.54 (m, 1H), 4.16 – 3.51 (m, 9H), 3.41 (m, 2H), 1.93 (m, 2H), 1.64 (m, 3H), 1.60 – 1.39 (m, 2H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) δ 164.67, 162.38, 162.04, 161.80, 156.61, 155.78, 152.51, 152.42, 152.31, 151.77, 148.33, 148.11, 148.02, 147.83, 142.40, 142.27, 141.65, 136.86, 131.98, 131.45, 131.33, 131.04, 129.68, 129.28, 129.02, 128.77, 128.42, 126.48, 125.89, 125.53, 125.38, 124.90, 124.72, 124.31, 111.64, 111.15, 110.88, 107.82, 107.73, 106.69, 96.19, 95.94, 66.89, 66.57, 56.46, 56.38, 56.22, 56.05, 55.93, 55.86, 50.86, 48.52, 48.07, 47.55, 47.32, 33.45, 32.84, 32.68, 32.43, 31.85, 19.41. HRMS: ESI$^+$ *m/z* calcd. for $C_{29}H_{32}F_3N_6O_6$ $[M+H]^+$ 617.2330, found 617.2330.



**5**

N-((6-nitrobenzo[d][1,3]dioxol-5-yl)methyl)-N-(2-((2-((tetrahydro-2H-pyran-4-yl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)phenyl)acrylamide **5** (Rotamers).$^1$H NMR (500 MHz, Chloroform-*d*) δ 10.26 (d, *J* = 6.3 Hz, 0.63H), 10.07 (d, *J* = 6.1 Hz, 0.52H), 8.50 (s, 0.52H), 8.06 (s, 1H), 7.72 (d, *J* = 7.2 Hz, 0.63H), 7.50 (d, *J* = 4 Hz, 0.62H), 7.42 (m, 2H), 7.35 (d, *J* = 4 Hz, 0.50H), 7.26 – 7.10 (m, 2H), 6.68 (m, 0.64H), 6.60 (m, 1H), 6.21 – 5.95 (m, 3H), 5.74 (m, 0.52H), 5.50 (s,

1H), 5.41 (m, 0.64H), 5.30 (m, 0.52H), 4.05 – 3.10 (m, 5H), 1.94 – 1.42 (m, 4H). $^{13}$C NMR (101 MHz, Chloroform-*d*) δ 166.21, 162.01, 157.22, 155.95, 151.96, 147.34, 143.42, 134.86, 133.31, 130.87, 130.10, 128.90, 128.46, 127.58, 127.12, 126.58, 126.34, 126.24, 123.56, 110.69, 110.22, 105.28, 104.99, 103.18, 98.13, 98.07, 97.87, 97.72, 66.93, 66.75, 47.96, 47.82, 47.47, 33.23, 32.88. HRMS: ESI$^+$ *m/z* calcd. for $C_{27}H_{26}F_3N_6O_6$ $[M+H]^+$ 587.1860, found 587.1858.



**6**

N-(1-(6-nitrobenzo[d][1,3]dioxol-5-yl)ethyl)-N-(2-((2-((tetrahydro-2H-pyran-4-yl)amino)-5-(trifluoromethyl) pyrimidin-4-yl)amino)phenyl)acrylamide **6** (Rotamers). $^1$H NMR (500 MHz, Chloroform-*d*) δ 8.16 (m, 2H), 7.35 (m, 1.48H), 7.20 (s, 1H), 7.17 – 7.02 (m, 1.47H), 7.02 – 6.50 (m, 2H), 6.44 (m, 1H), 6.34 – 6.02 (m, 2H), 6.02 – 5.69 (m, 2H), 5.65 – 4.92 (m, 2H), 4.20 – 3.70 (m, 3H), 3.51 (br, 2H), 2.12 – 1.53 (m, 7H). $^{13}$C NMR (126 MHz, Chloroform-*d*) δ 166.35, 165.95, 162.06, 157.24, 156.71, 156.35, 156.15, 151.33, 151.07, 146.97, 143.89, 136.61, 136.31, 131.93, 131.45, 131.07, 130.43, 129.92, 129.30, 128.60, 128.07, 125.88, 125.63, 125.36, 124.89, 123.74, 123.48, 116.48, 107.84, 105.10, 102.97, 102.91, 98.62, 98.43, 98.30, 98.14, 66.91, 66.87, 53.18, 52.83, 51.54, 50.54, 48.04, 47.53, 33.33, 33.12, 32.91, 19.95, 19.51. HRMS: ESI$^+$ *m/z* calcd. for $C_{28}H_{28}F_3N_6O_6$ $[M+H]^+$ 601.2017, found 601.2017.

## Biology

### Enzymatic assays

The enzymatic activities against ERK2 (PV3313, Thermo) were tested with the Thermo fisher Z'-LYTE Kit (PV3176, Z'-LYTE$^{TM}$ kinase assay kit-Ser/Thr3 Peptide). All protocols are available from the supplier.

### Cell culture

A375, A549, HCT116, 293T cell lines were obtained from Type Culture Collection of the Chinese Academy of Science, Shanghai, China. A375 and 293T cell lines were cultured in DMEM (11995065, Gibco) containing 10% fetal bovine serum. A549 cell line was cultured in F-12K Nutrient Mixture (21127-022, Gibco) containing 10% fetal bovine serum. HCT116 cell line was cultured in McCoy's 5A (16600-082, Gibco) containing 10% fetal bovine serum. All cell lines were incubated in a humidified atmosphere containing 5% $CO_2$ at 37 ℃.

### Western-Blot

A375, A549, HCT116, 293T were seeded in 12-well plates at 2 × $10^6$ cells per well, which were treated with various concentrations of compounds for 1 h. After irradiation or not, the cells were incubated in the dark for 2 h and then collected and lysed in lysis buffer (Beyotime P0013) with

protease inhibitors and phosphatase inhibitors I/II on the ice for 30 min and centrifuged at 12000 rpm for 10 min at 4 ℃, later removed the insoluble material. The lysates were analyzed by SDS PAGE and transferred to PVDF membrane. After blocked in milk, the membranes were incubated with primary antibodies at 4 °C overnight, followed by rabbit secondary antibodies incubated for 1 h at room temperature. The results were visualized by using Millpore reagent (Luminata TM, Cat. No. WBLUR0500) on Chemidoc MP imaging system and analyzed with Image Lab software. The primary antibodies against p90RSK (#610225, BD bioscience), p-p90RSK (#9344S, CST), GAPDH (Prod#2118S, CST), and β-actin (#4970S, CST) and secondary antibodies (Mouse #7076S, CST; Rabbit #7074S, CST) were diluted at 1:1000 with milk.

**Cell Viability Assays**

$6\times 10^3$ cells were seeded in 96-well plates and then incubated in a humidified atmosphere containing 5% $CO_2$ at 37 ℃ for 8 h or overnight. And then compounds of serially diluted concentrations were added to treat cells for 1 h before irradiation (UV 365 nm) for 10 min or no irradiation. After 72 h of incubation in the dark, cell viability was measured by using the CellTiter-Glo® luminescent cell viability assay (Promega, G7573). Data was analyzed by GraphPad Prism 6.0 software.

**Colony-Formation Assay**

A375 Cells were seeded to 12-well plates at a density of $10^4$ cells per well with various concentration of compound **2** or **AZ13767370** and then incubated in a humidified atmosphere containing 5% $CO_2$ at 37 ℃ for 1 h. After irradiation (UV 365 nm) for 10 min or not, the cells were cultured for 10 days. After culture medium was removed, the cells were wash with PBS for three times and then were fixed with 4% formalin (Biosharp, P0099) for 1 h. Finally, the cells were stained with crystal violet staining solution (Beyotime, C0121) for 15 min. Crystal violet was washout with PBS and the pictures of the cell colonies were recorded with a camera.

***In Vivo* Studies.**

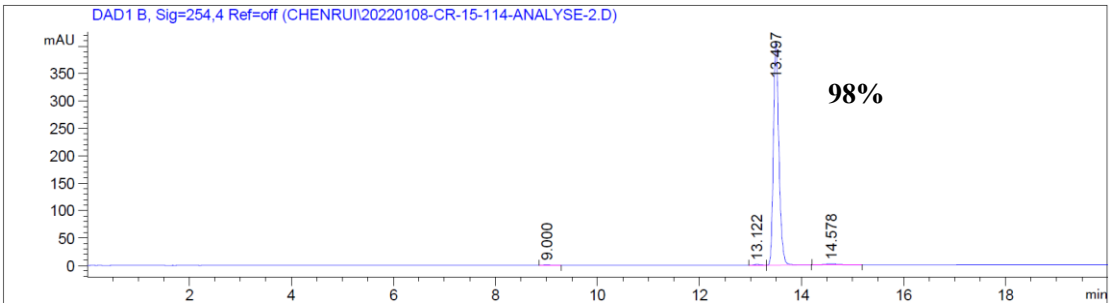
zebrafish was used as a model organism to evaluate the *in vivo* activity of compound **2**. Human melanoma cells (A375) labeled by CM-DiI were transplanted into the 2 dpf wild-type AB strain zebrafish yolk sac by microinjection with about 200 cells per tail to establish a zebrafish human tumor transplantation model. After cultured at 35 °C to 3 dpf, the zebrafish with good tumor cell growth consistency were selected under the microscope to assign randomly to a 6-well plate with 30 zebrafish per well. And then every zebrafish was injected by the yolk sac with different concentrations of compound **2** and **AZ13767370** or DMSO. After irradiation (UV 365 nm,15 min) or not, all groups were placed into a dark incubator for another 48 h.12 zebrafishes were randomly selected from each experimental group and photographed under a fluorescence microscope. The data was collected with NIS-Elements D 3.20 software to analyze the fluorescence intensity of zebrafish tumor cells.

# Purities of all final compounds by HPLC

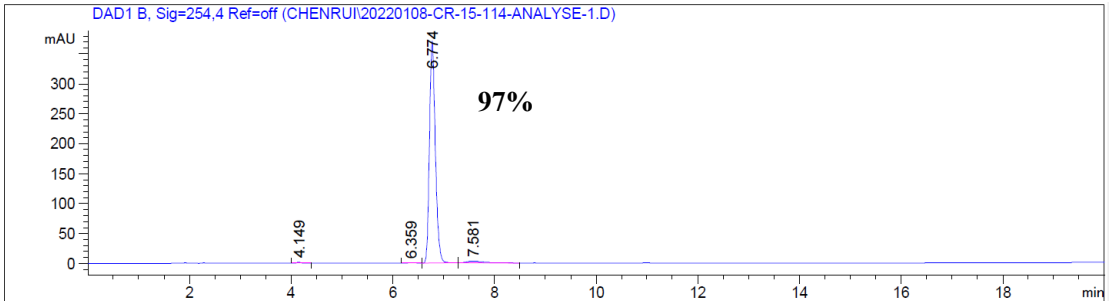
The samples were analyzed by HPLC (Agilent PN880975-902 ZORBAX SBC18 4.6 × 250 mm column), using water and MeCN as eluents.

## Pc-ERK inhibitor 1

A: Gradient elution 40-90% MeCN, 20 min.

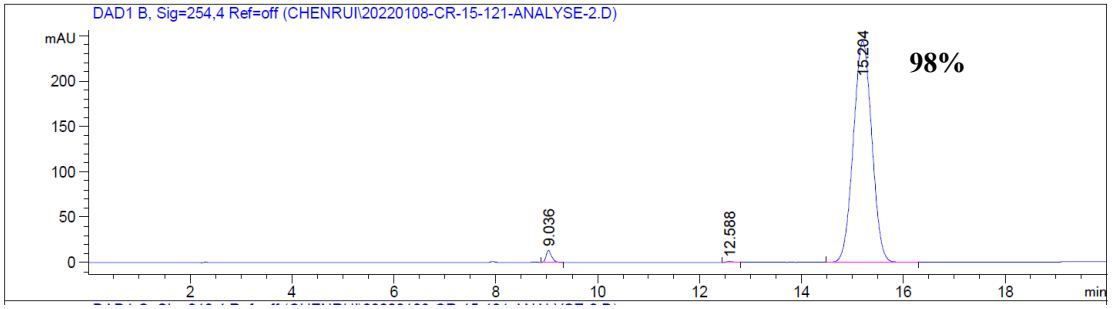



B: Gradient elution 60-90% MeCN, 20 min.

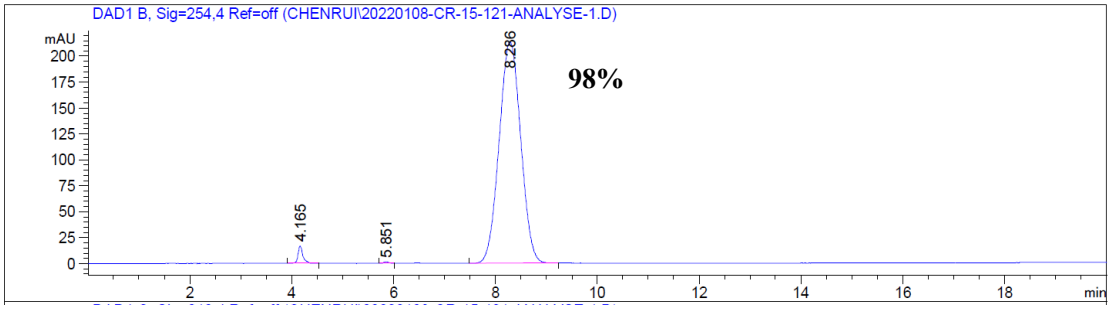



## Pc-ERK inhibitor 2

A: Gradient elution 40-90% MeCN, 20 min.

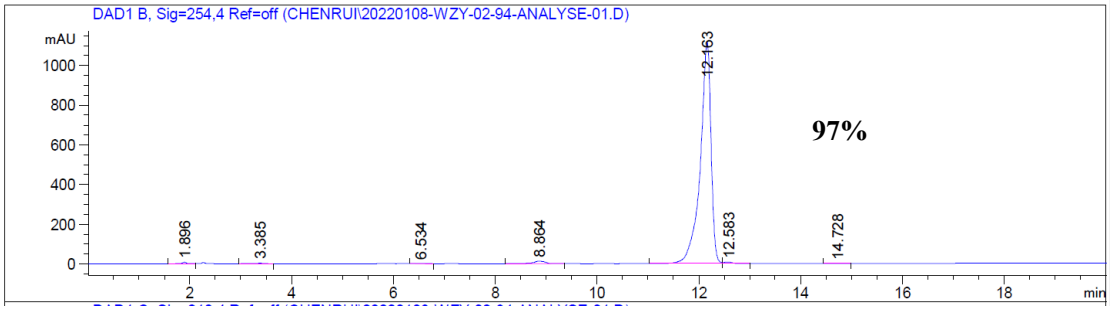



B: Gradient elution 60-90% MeCN, 20 min.

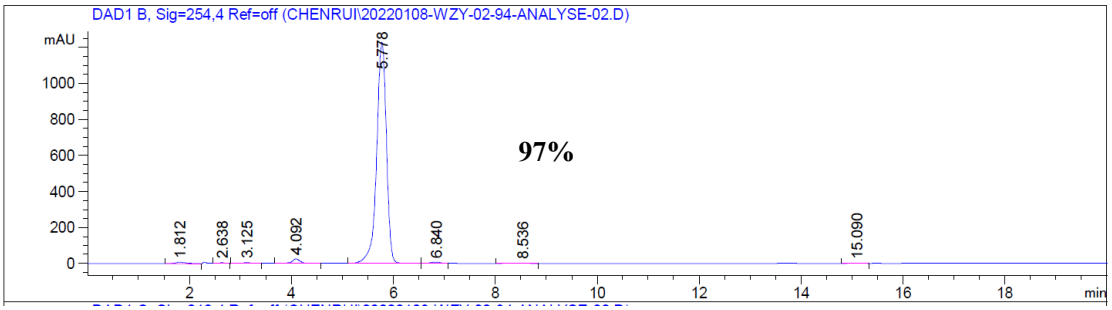


## Pc-ERK inhibitor 3

A: Gradient elution 40-90% MeCN, 20 min.

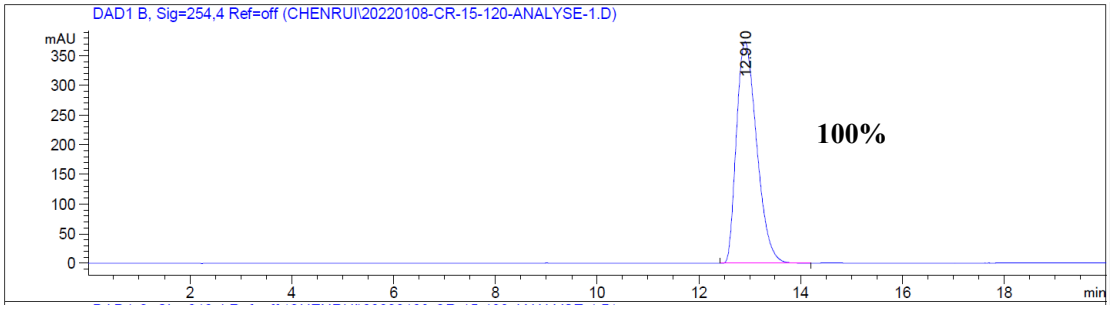



B: Gradient elution 60-90% MeCN, 20 min.

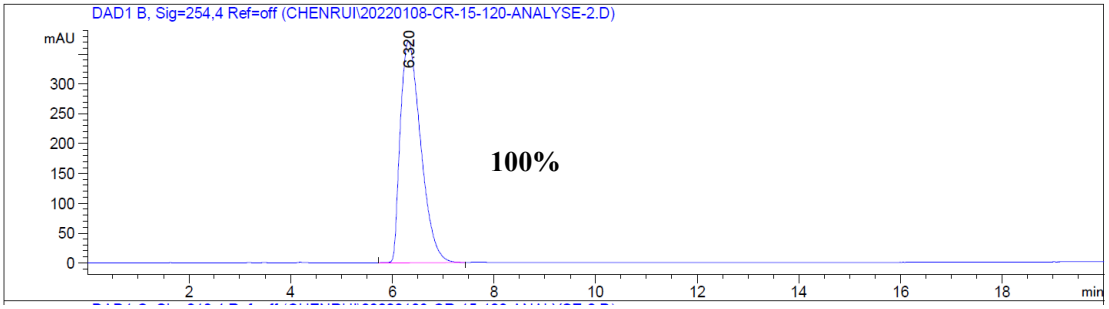



## Pc-ERK inhibitor 4

A: Gradient elution 40-90% MeCN, 20 min.

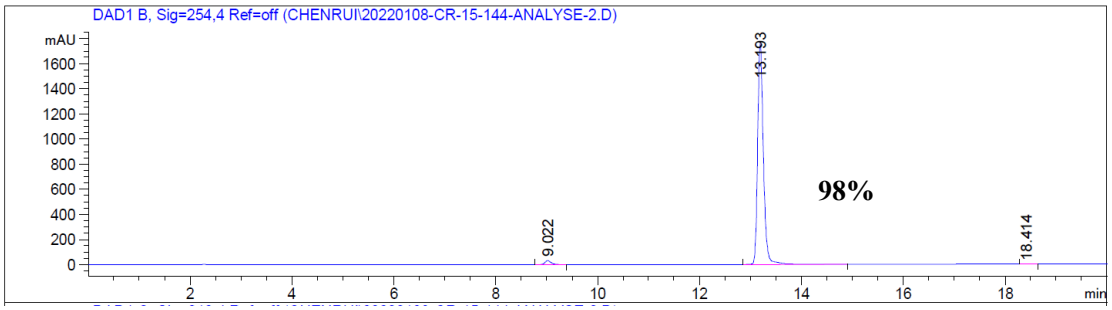



B: Gradient elution 60-90% MeCN, 20 min.

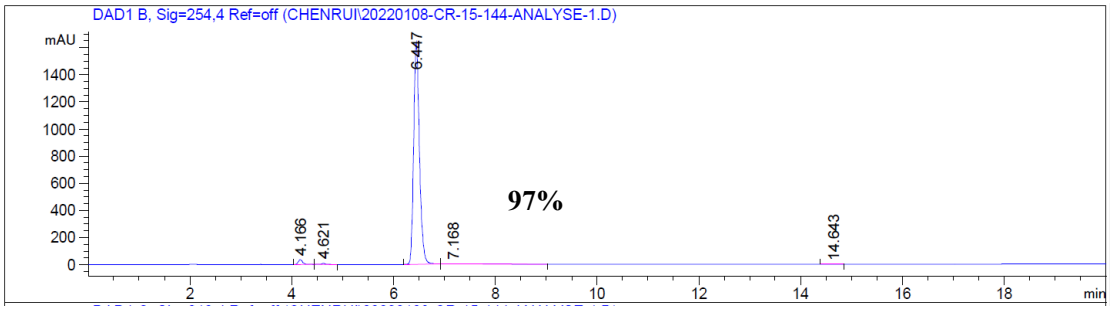


## Pc-ERK inhibitor 5

A: Gradient elution 40-90% MeCN, 20 min.

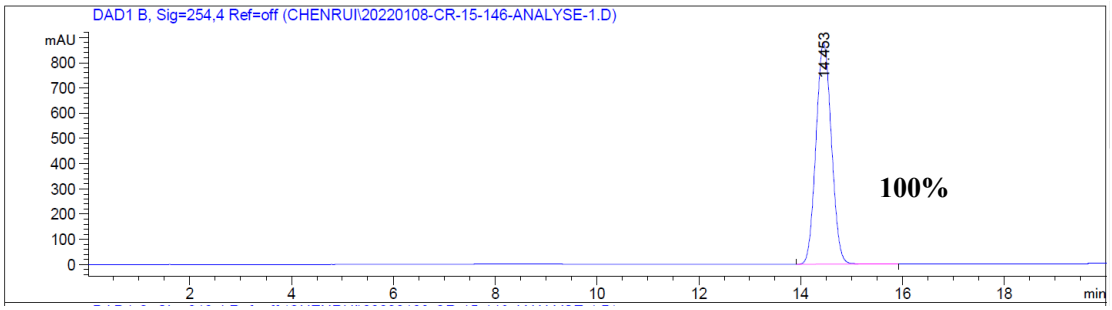



B: Gradient elution 60-90% MeCN, 20 min.

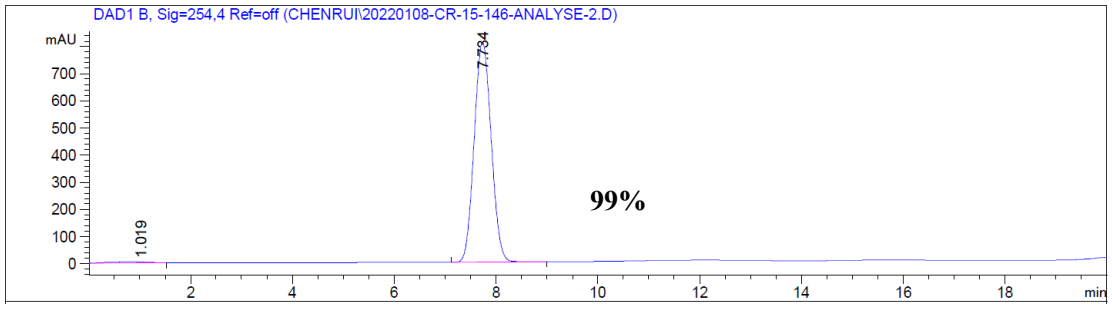



## Pc-ERK inhibitor 6

A: Gradient elution 40-90% MeCN, 20 min.



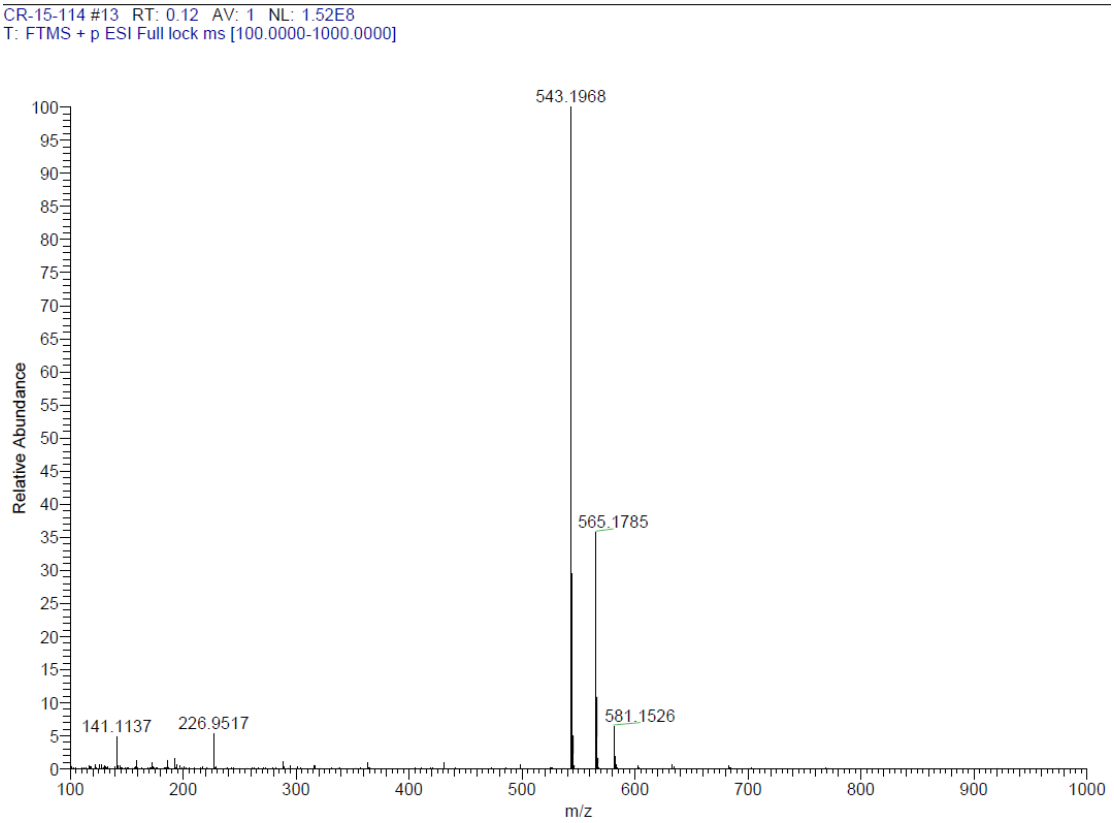

B: Gradient elution 60-90% MeCN, 20 min.



# HRMS Data

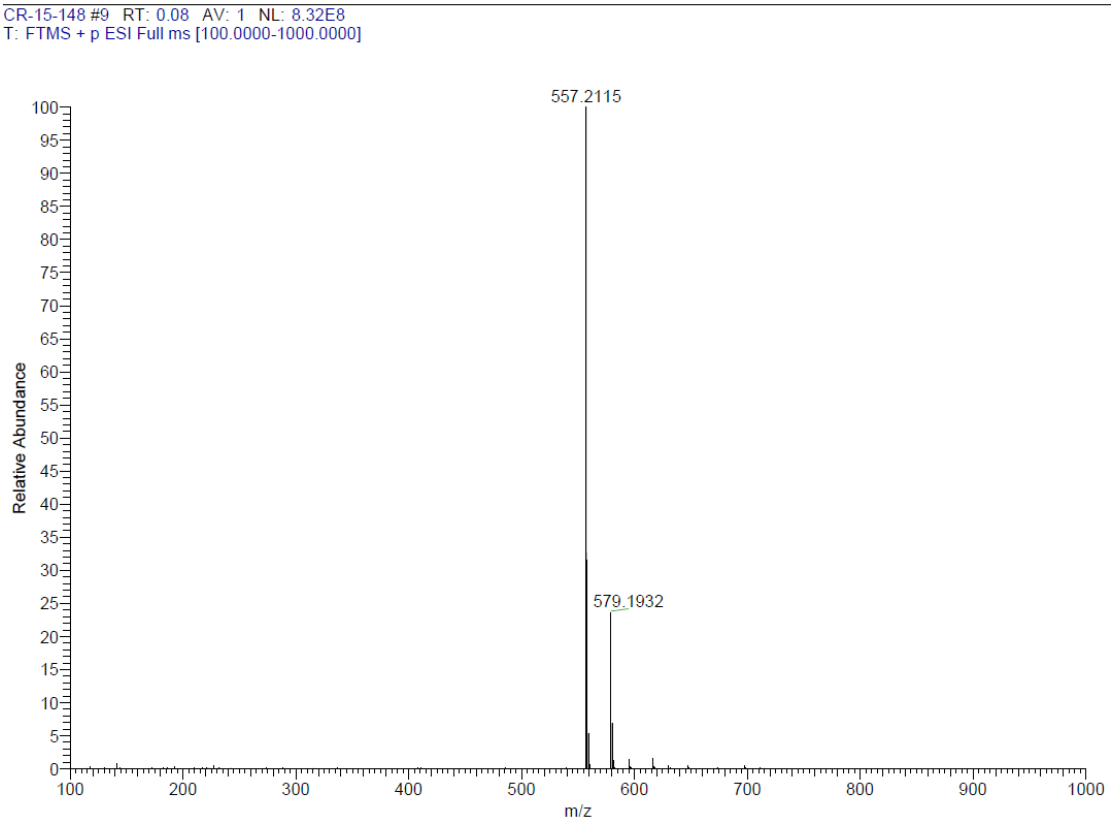
N-(2-nitrobenzyl)-N-(2-((2-((tetrahydro-2H-pyran-4-yl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)phenyl)acrylamide **1** (Rotamers).

HRMS: ESI$^+$ *m/z* calcd. for $C_{26}H_{26}F_3N_6O_4[M+H]^+$ 543.1962, found 543.1968.



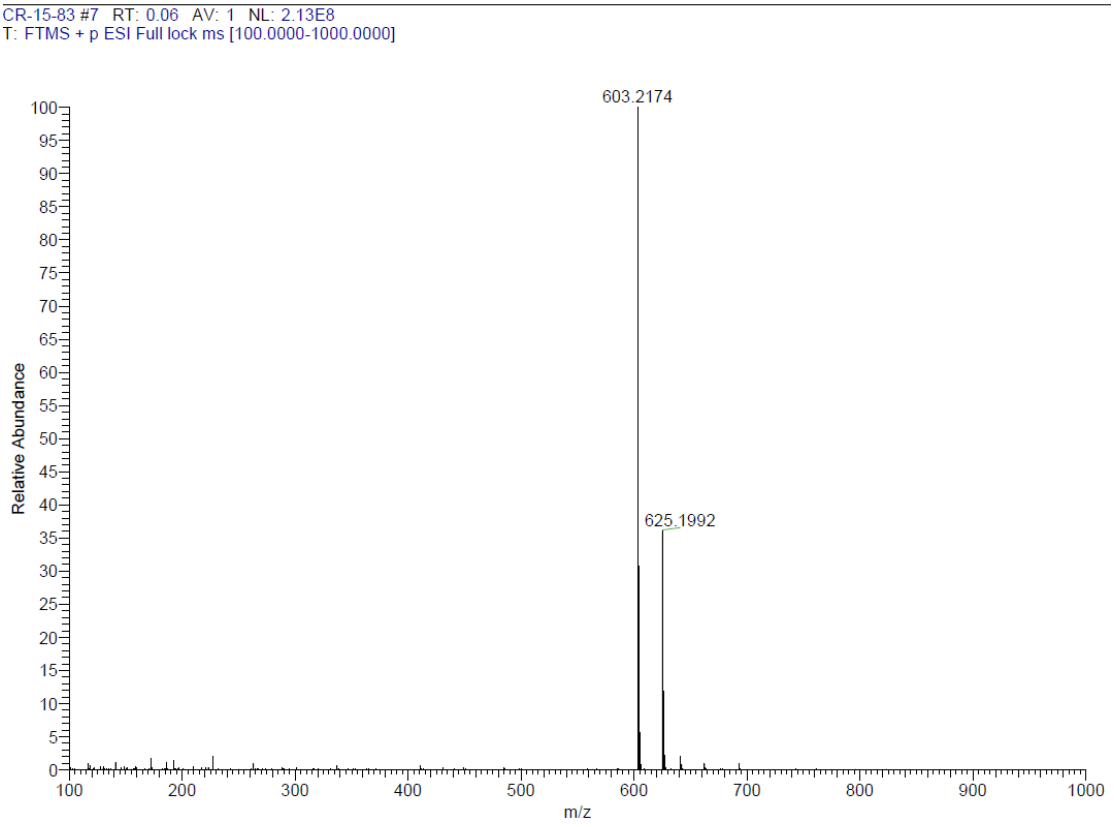
N-(1-(2-nitrophenyl)ethyl)-N-(2-((2-((tetrahydro-2H-pyran-4-yl)amino)-5-(trifluoromethyl) pyrimidin-4-yl)amino)phenyl)acrylamide **2** (Rotamers)

HRMS: ESI$^+$ *m/z* calcd. for $C_{27}H_{28}F_3N_6O_4[M+H]^+$ 557.2119, found 557.2115.



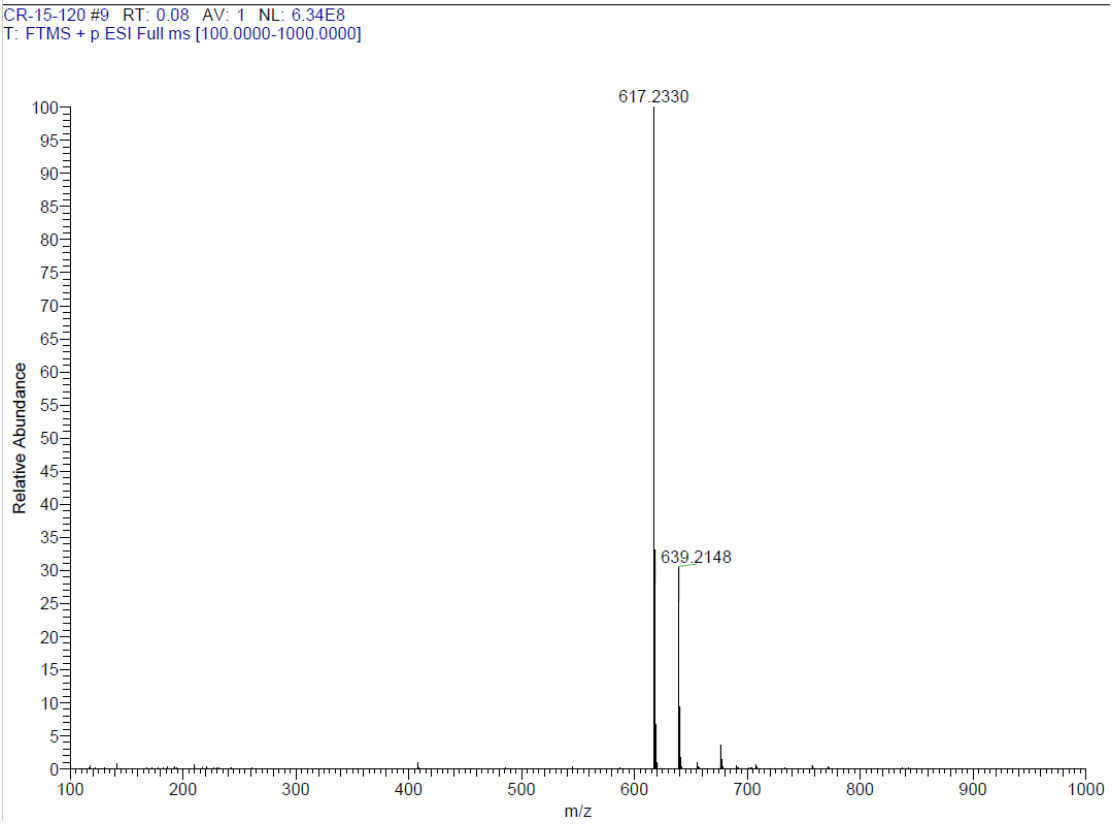
N-(4,5-dimethoxy-2-nitrobenzyl)-N-(2-((2-((tetrahydro-2H-pyran-4-yl)amino)-5-(trifluoromethyl) pyrimidin-4-yl)amino)phenyl)acrylamide **3** (Rotamers).

HRMS: ESI$^+$ *m/z* calcd. for $C_{28}H_{30}F_3N_6O_6$ $[M+H]^+$ 603.2173, found 603.2174.



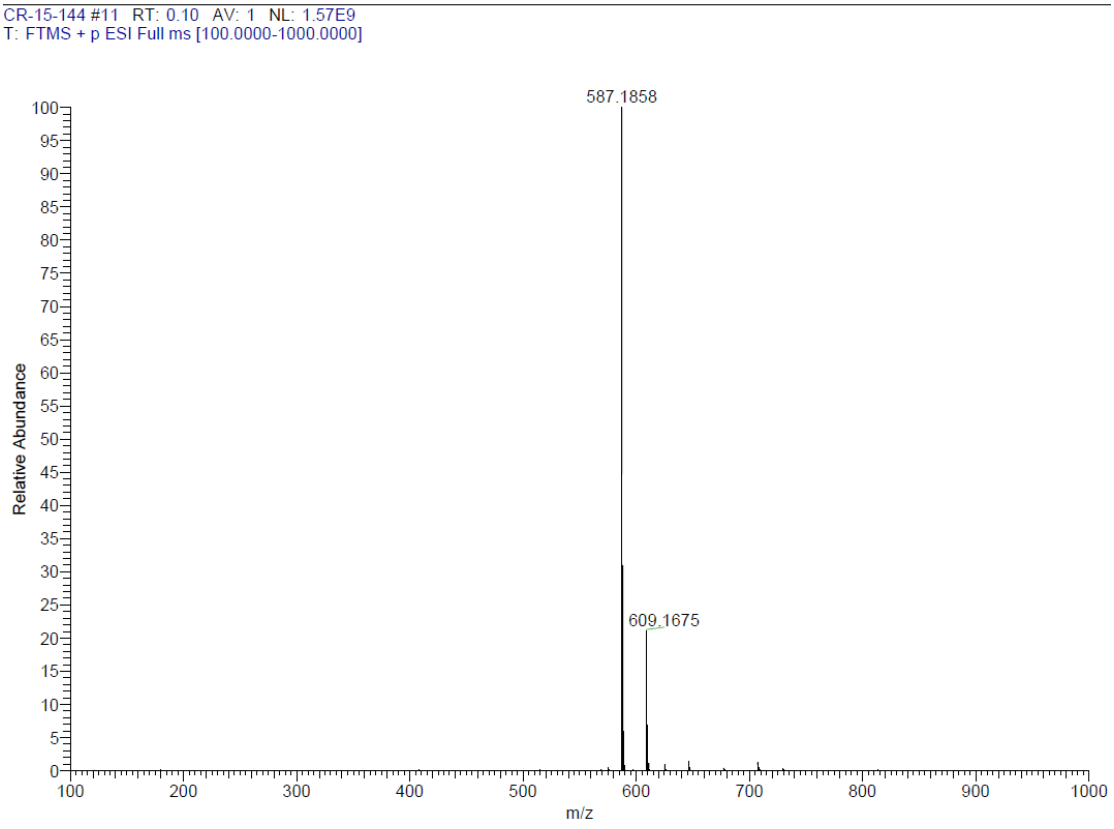
N-(1-(4,5-dimethoxy-2-nitrophenyl)ethyl)-N-(2-((2-((tetrahydro-2H-pyran-4-yl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)phenyl)acrylamide **4** (Rotamers).

HRMS: ESI$^+$ *m/z* calcd. for $C_{29}H_{32}F_3N_6O_6$ $[M+H]^+$ 617.2330, found 617.2330.



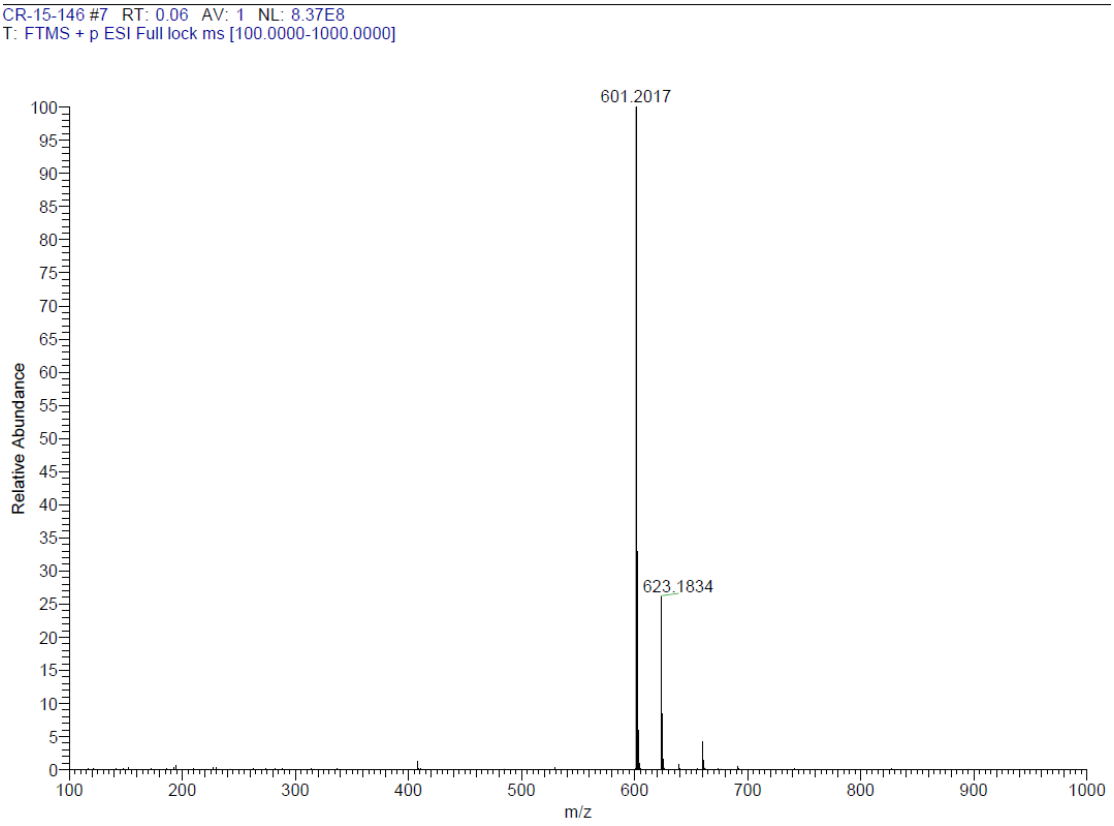
N-((6-nitrobenzo[d][1,3]dioxol-5-yl)methyl)-N-(2-((2-((tetrahydro-2H-pyran-4-yl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)phenyl)acrylamide **5** (Rotamers)

HRMS: ESI$^+$ *m/z* calcd. for $C_{27}H_{26}F_3N_6O_6$ $[M+H]^+$ 587.1860, found 587.1858.



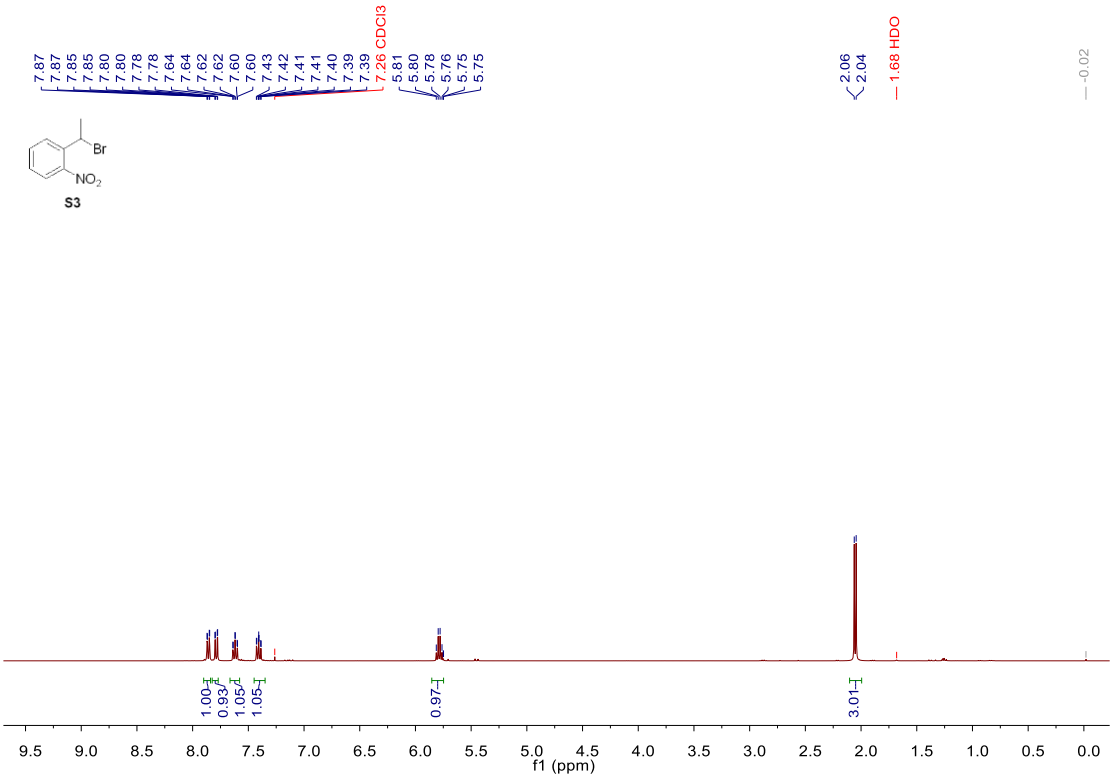
N-(1-(6-nitrobenzo[d][1,3]dioxol-5-yl)ethyl)-N-(2-((2-((tetrahydro-2H-pyran-4-yl)amino)-5-(trifluoromethyl) pyrimidin-4-yl)amino)phenyl)acrylamide **6** (Rotamers).

HRMS: ESI$^+$ *m/z* calcd. for $C_{28}H_{28}F_3N_6O_6$ $[M+H]^+$ 601.2017, found 601.2017.

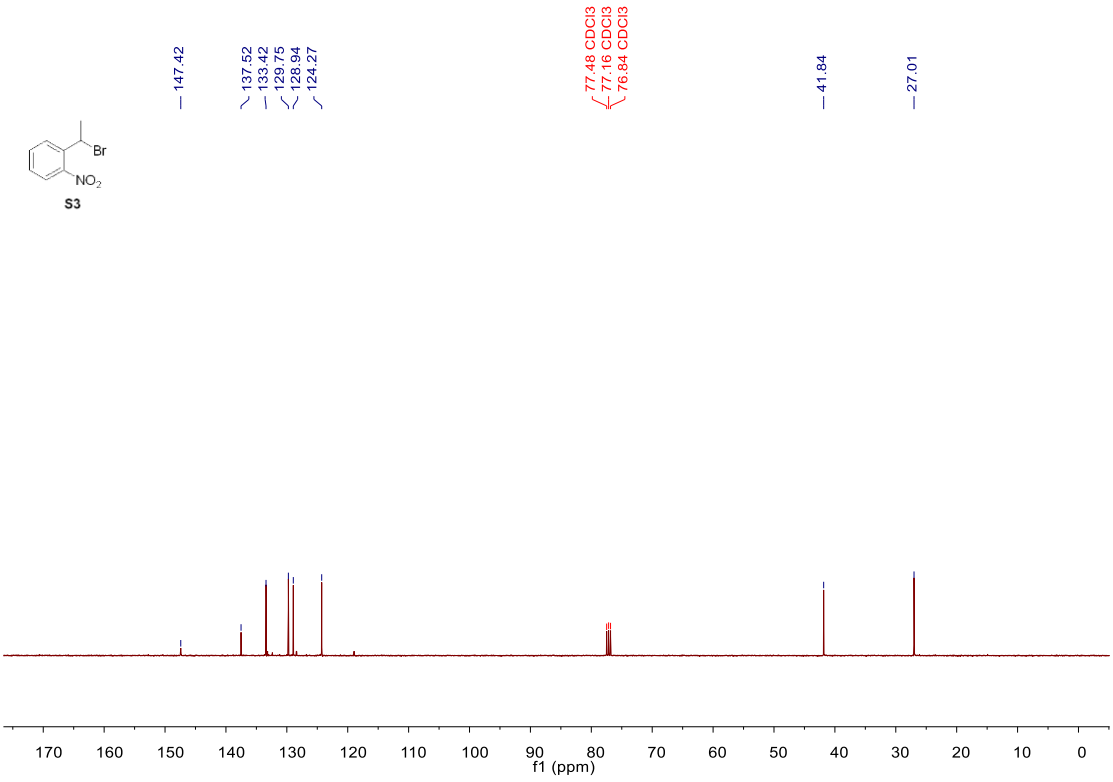


# NMR spectra

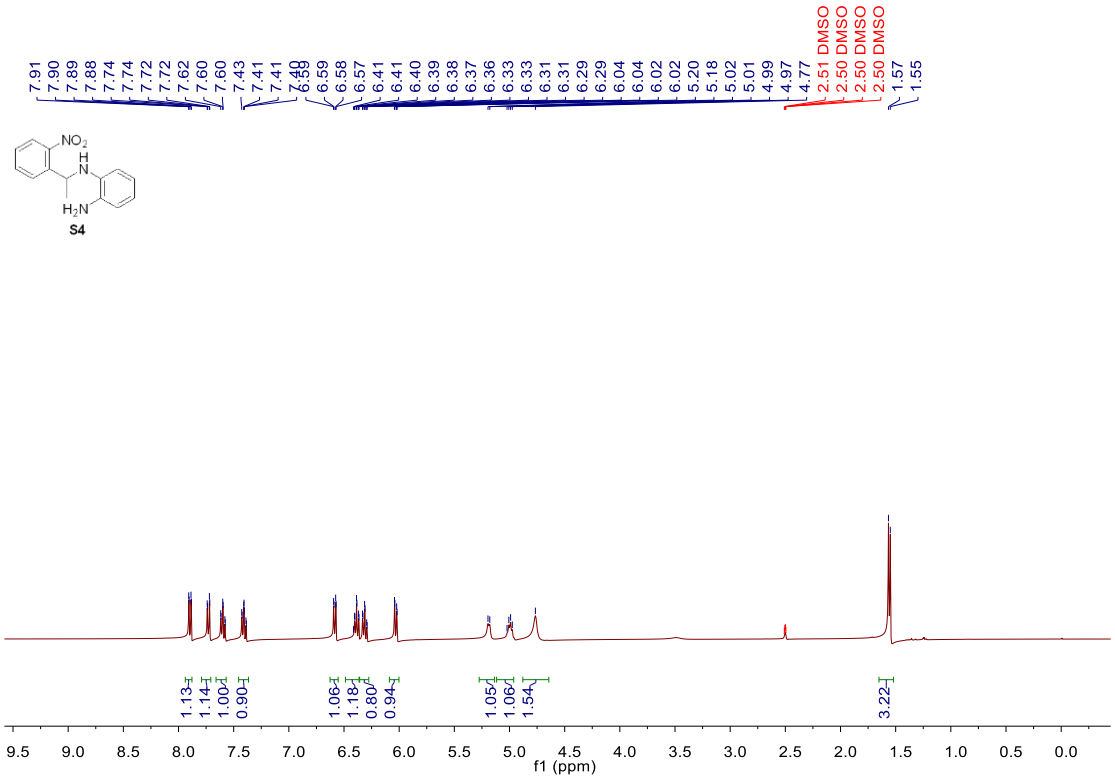
$^{1}$H NMR (400 MHz, Chloroform-*d*)



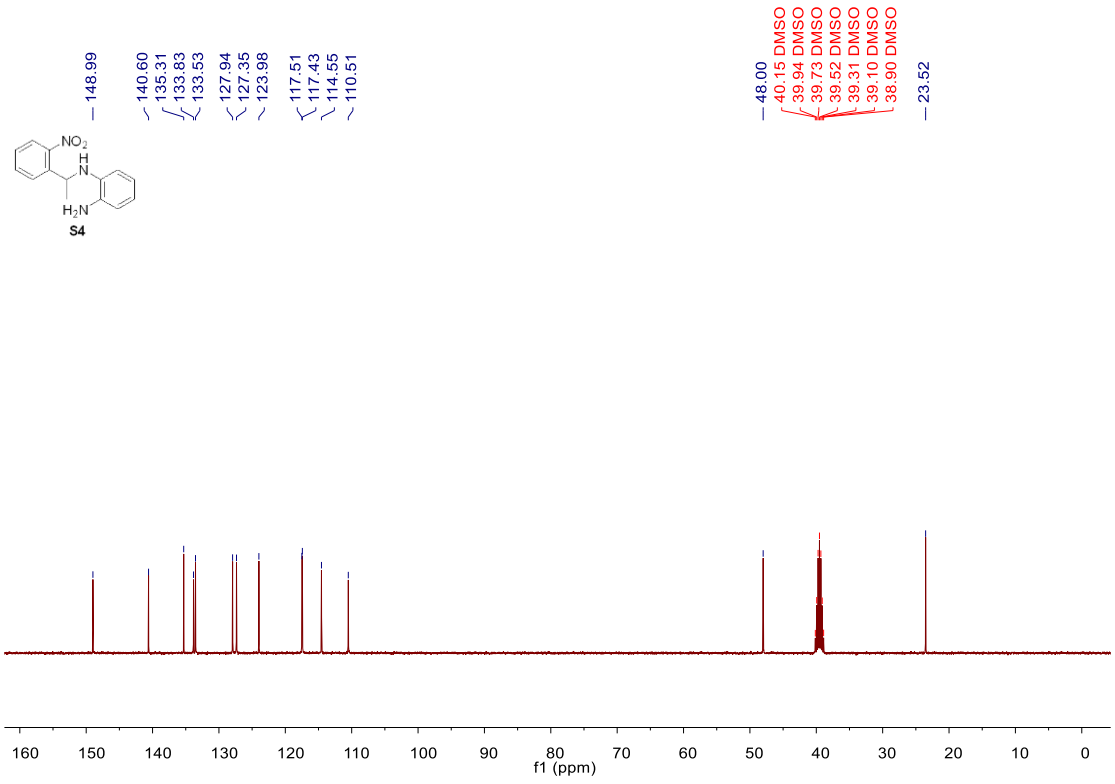

$^{13}$C NMR (101 MHz, Chloroform-*d*)



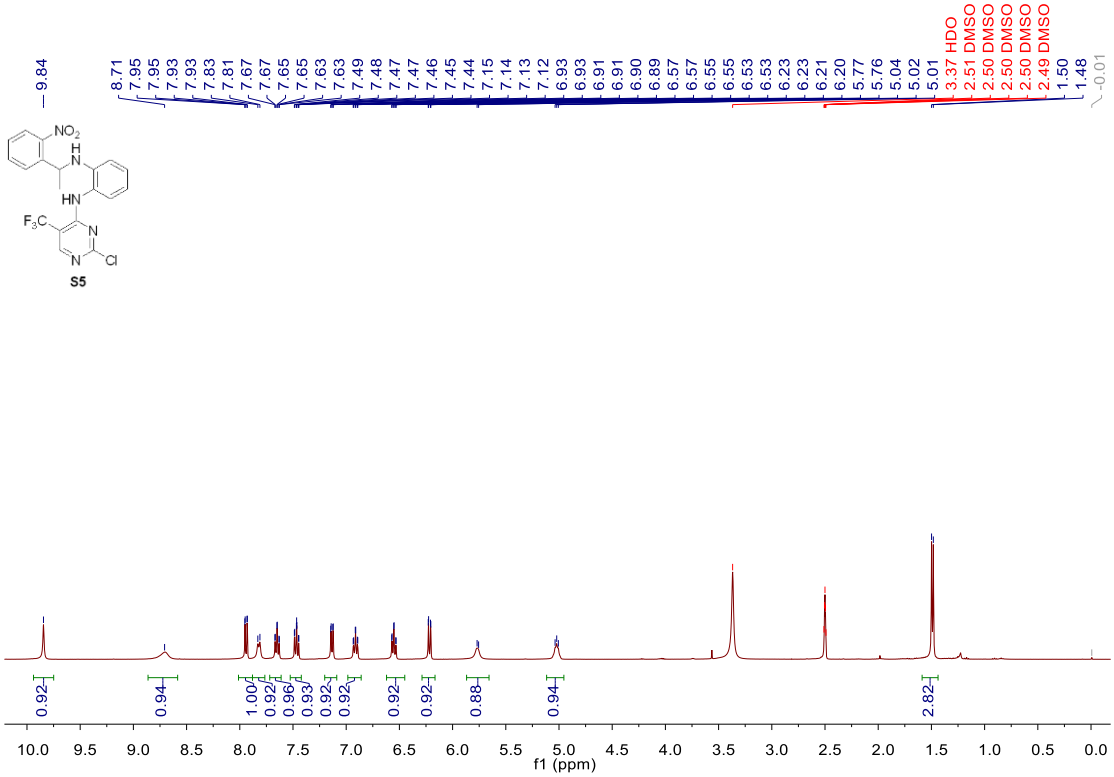
$^{1}$H NMR (400 MHz, DMSO-$d_6$)



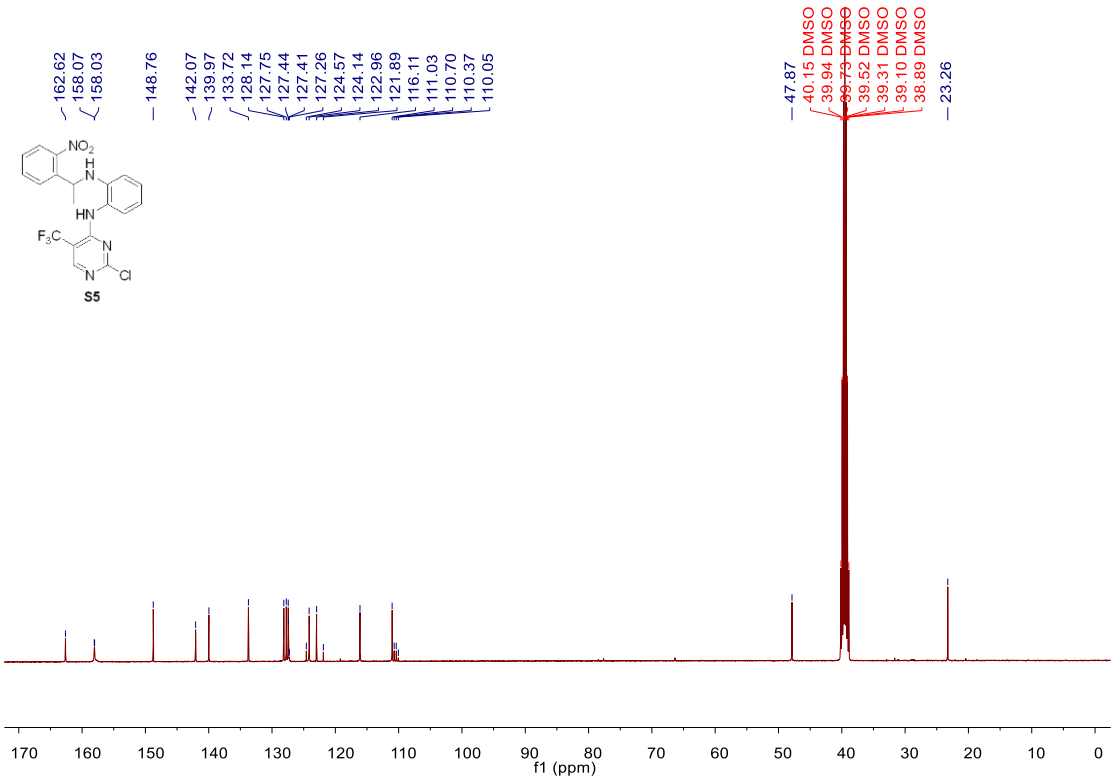

$^{13}$C NMR (101 MHz, DMSO-$d_6$)



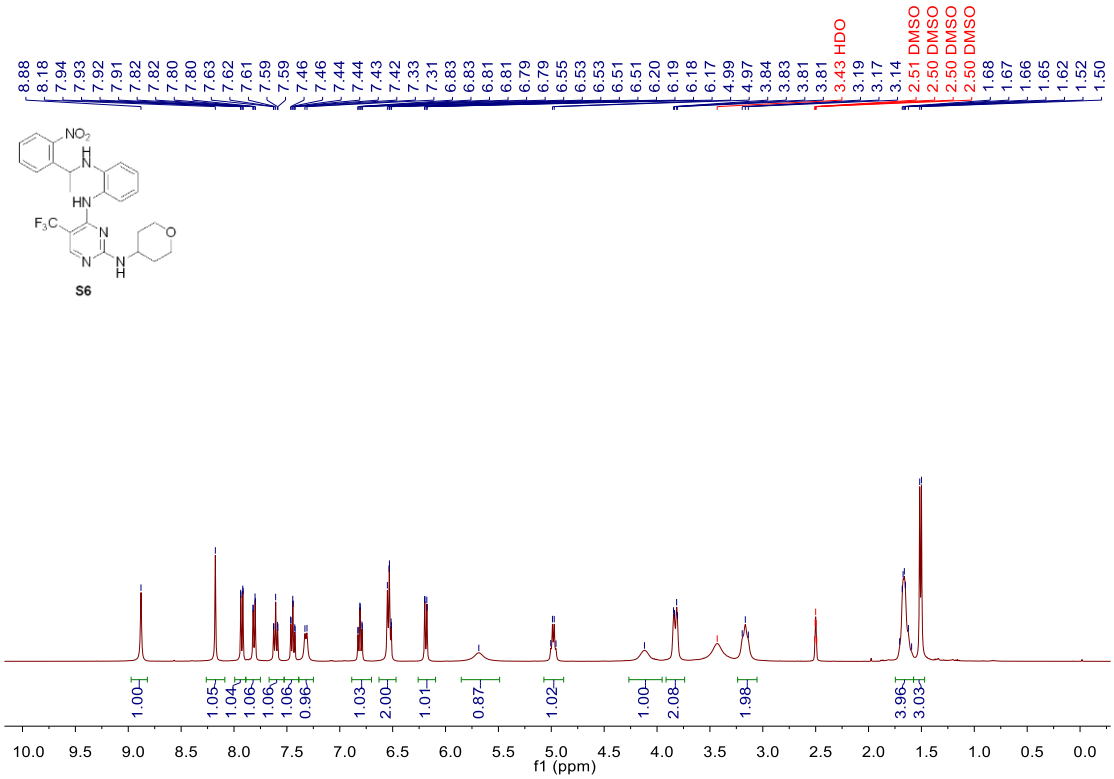
$^{1}$H NMR (400 MHz, DMSO-$d_6$)



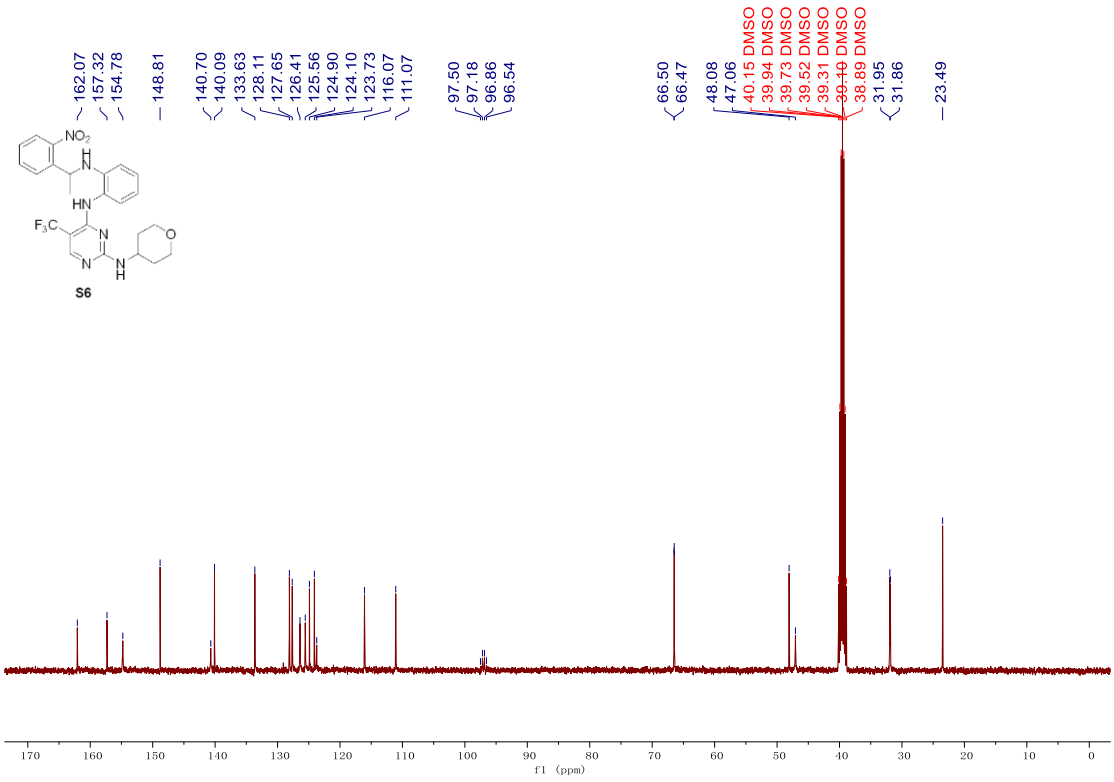

$^{13}$C NMR (101 MHz, DMSO-$d_6$)



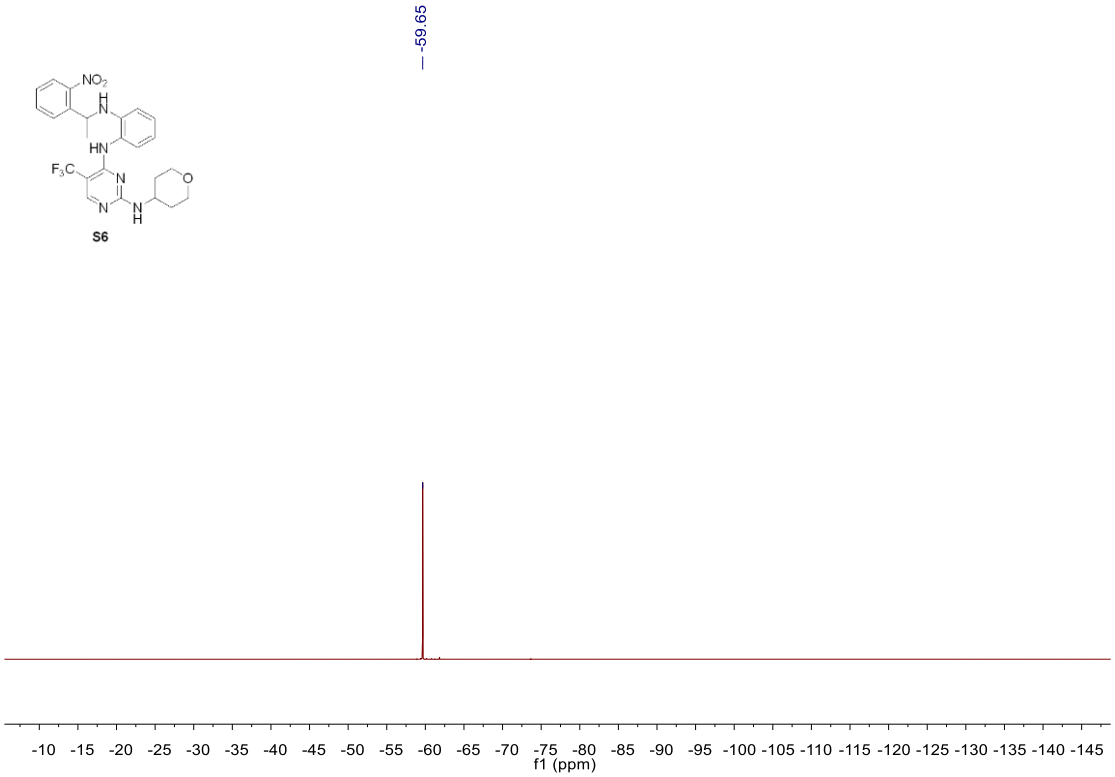
$^{1}$H NMR (400 MHz, DMSO-$d_6$)



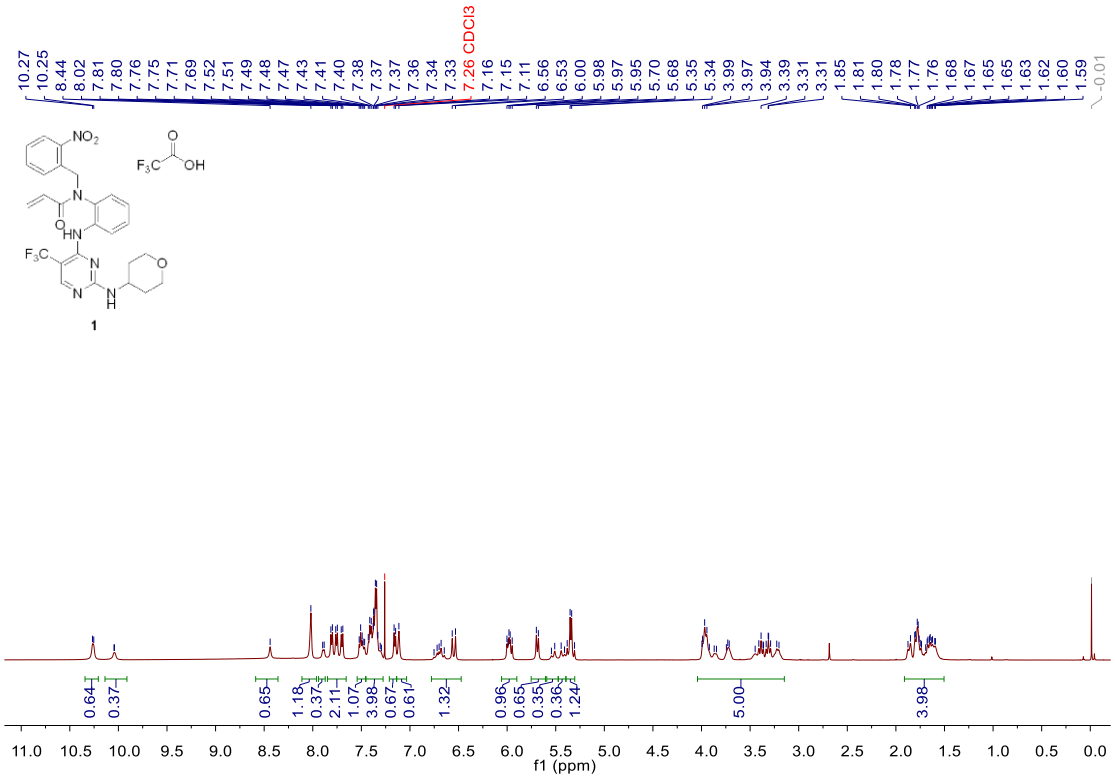

$^{13}$C NMR (101 MHz, DMSO-$d_6$)



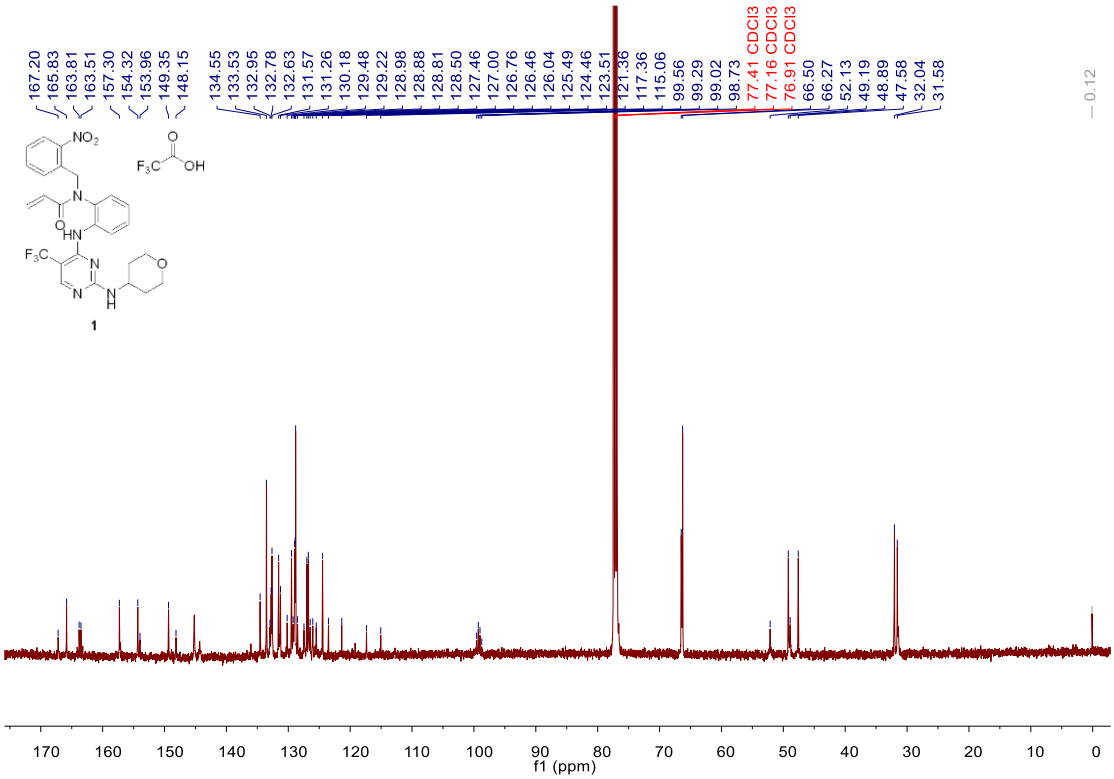
$^{19}$F NMR (376 MHz, DMSO-$d_6$)



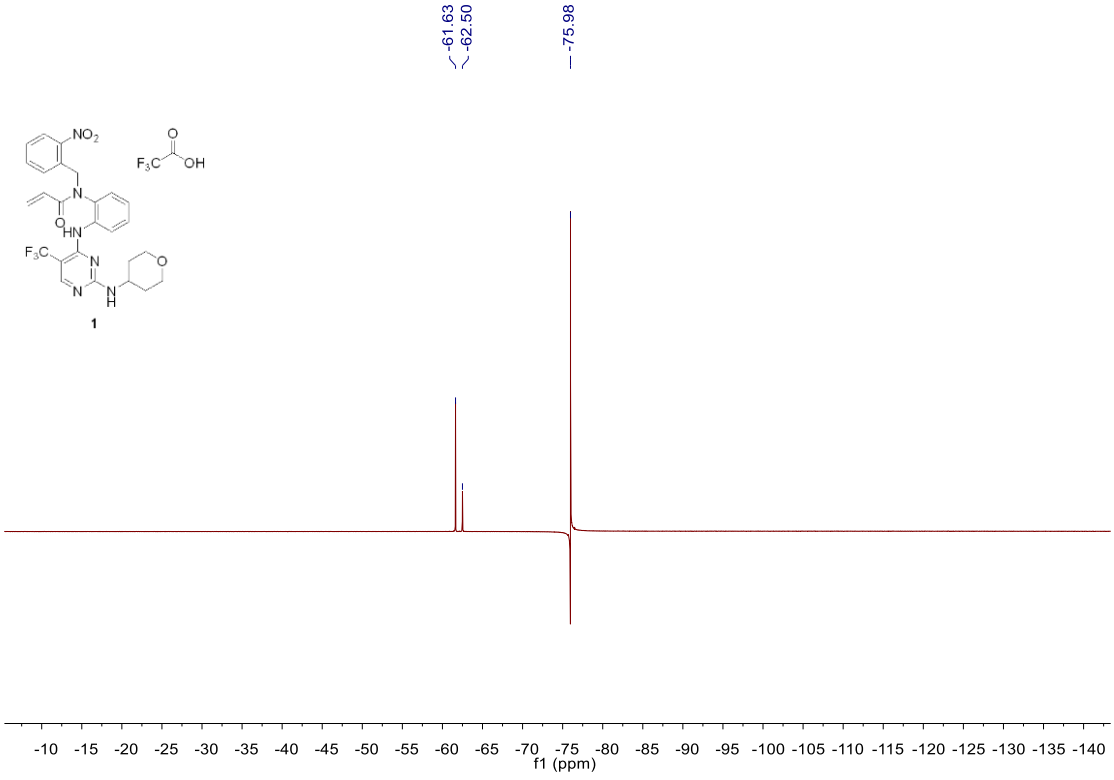

$^{1}$H NMR (500 MHz, Chloroform-$d$)



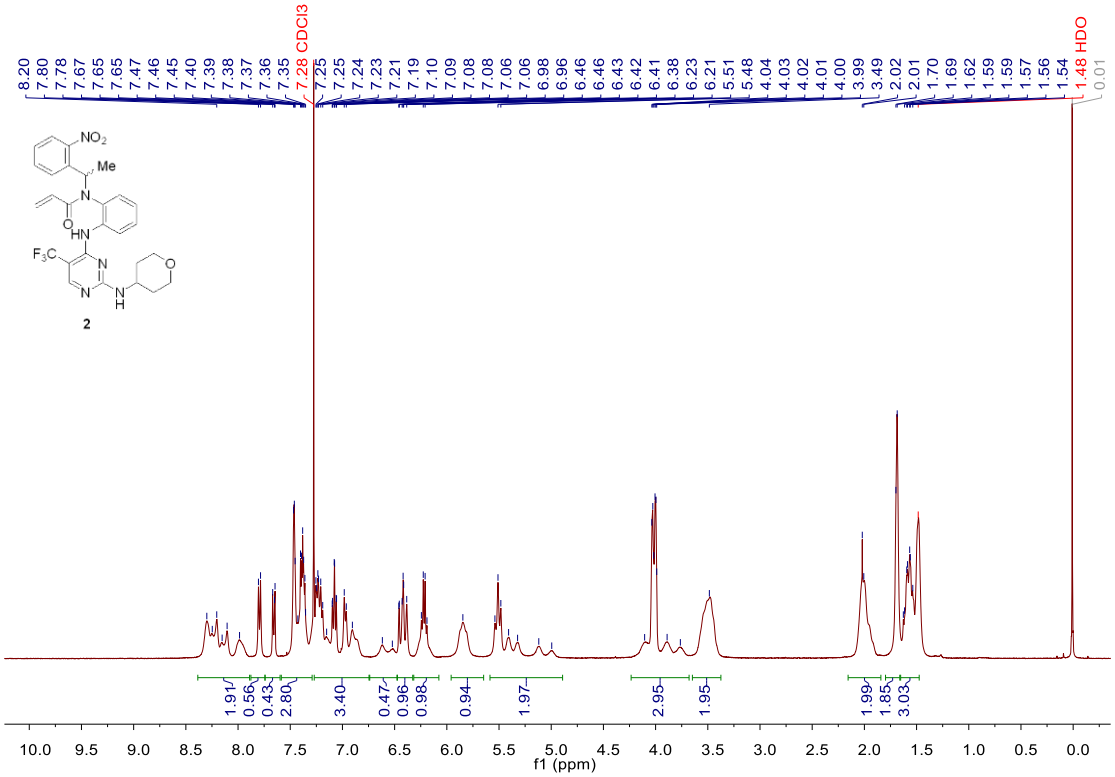
$^{13}$C NMR (126 MHz, Chloroform-*d*)



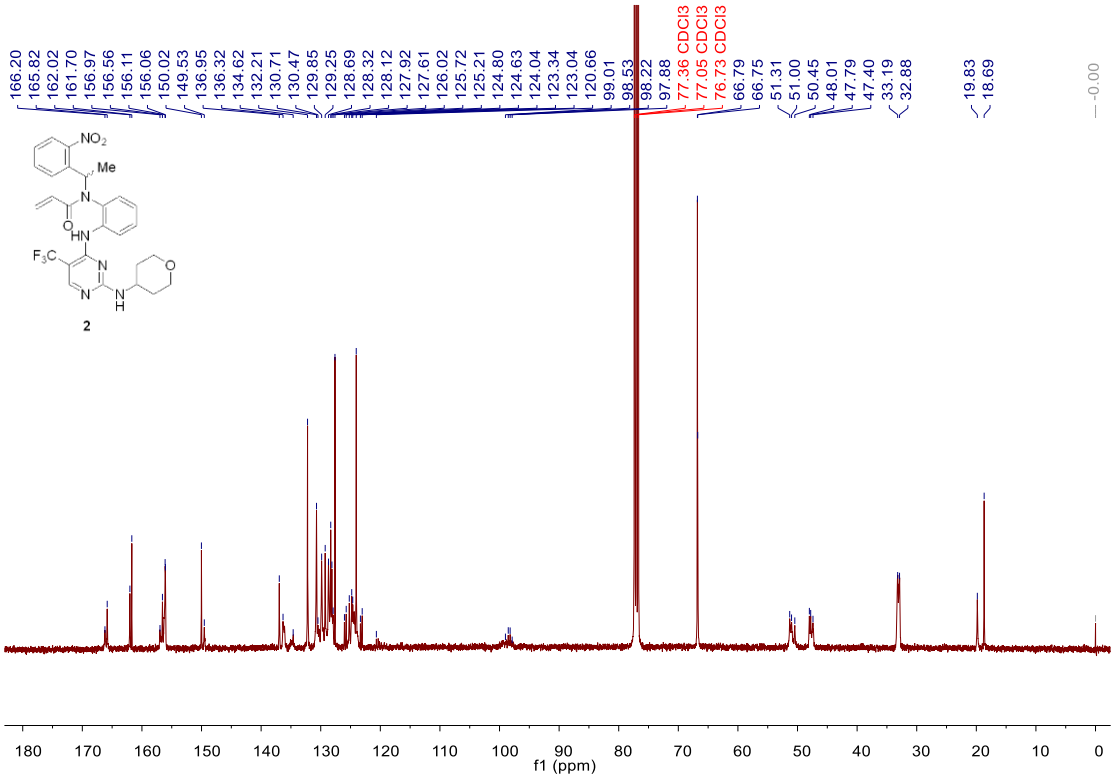

$^{19}$F NMR (376 MHz, Chloroform-*d*)



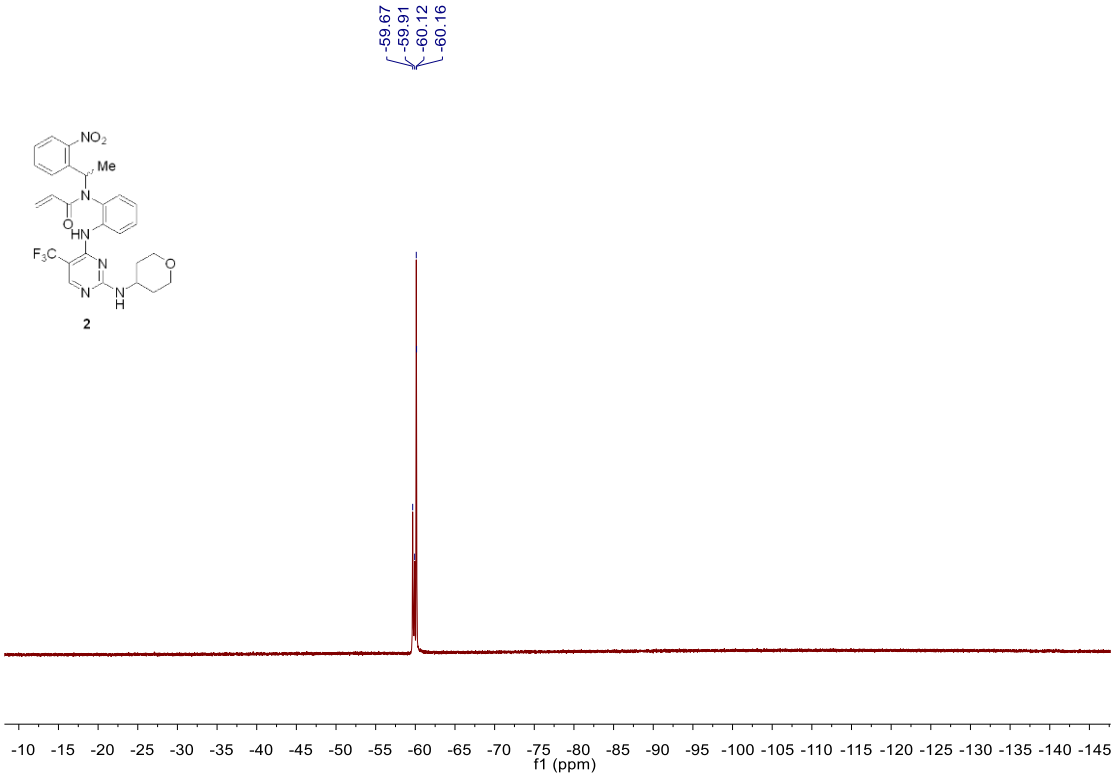
$^{1}$H NMR (400 MHz, Chloroform-*d*)



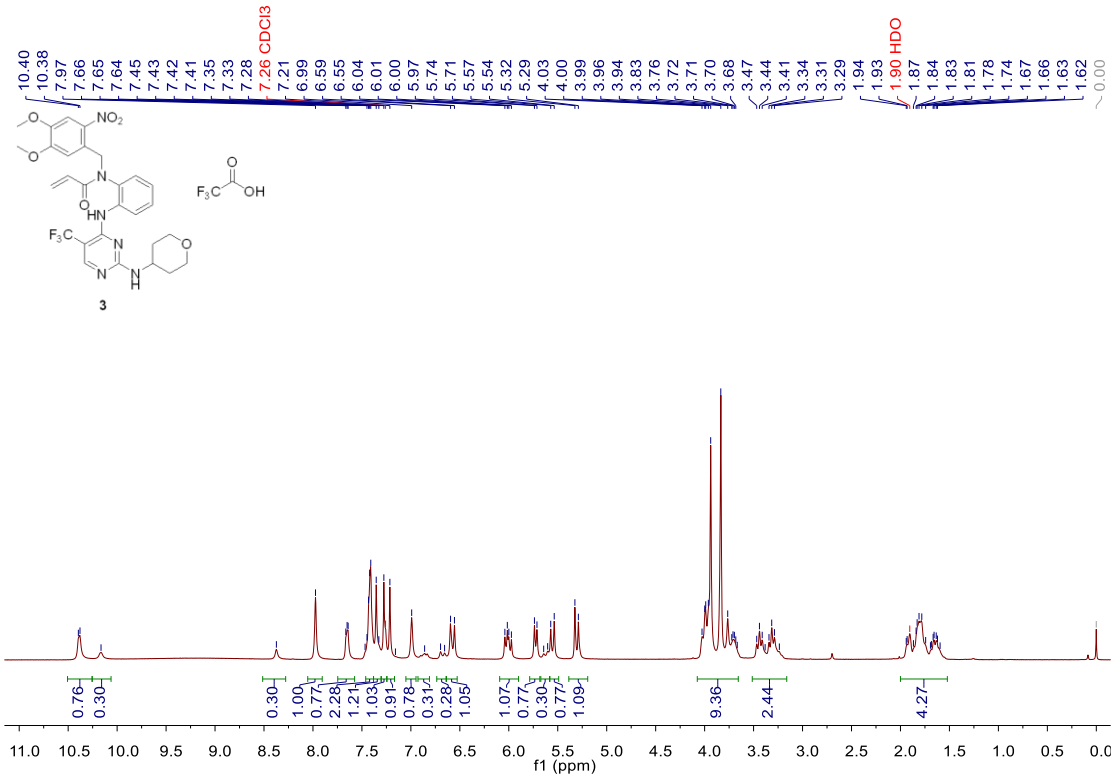

$^{13}$C NMR (101 MHz, Chloroform-*d*)



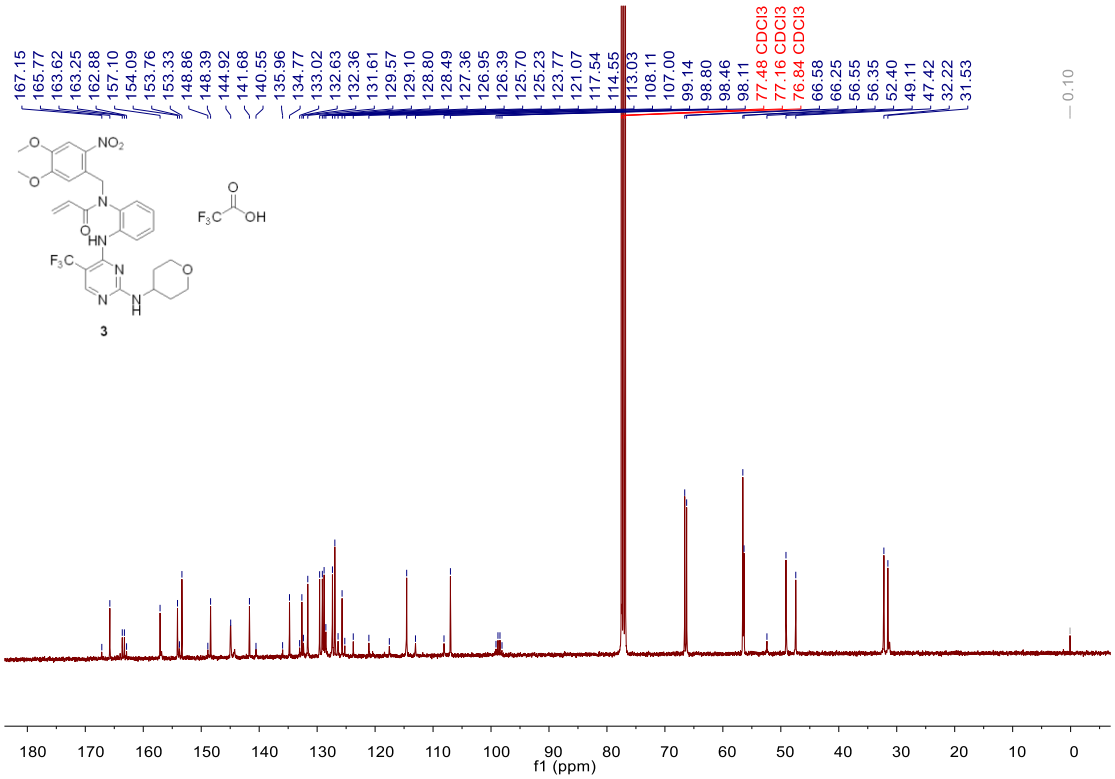
$^{19}$F NMR (376 MHz, Chloroform-*d*)



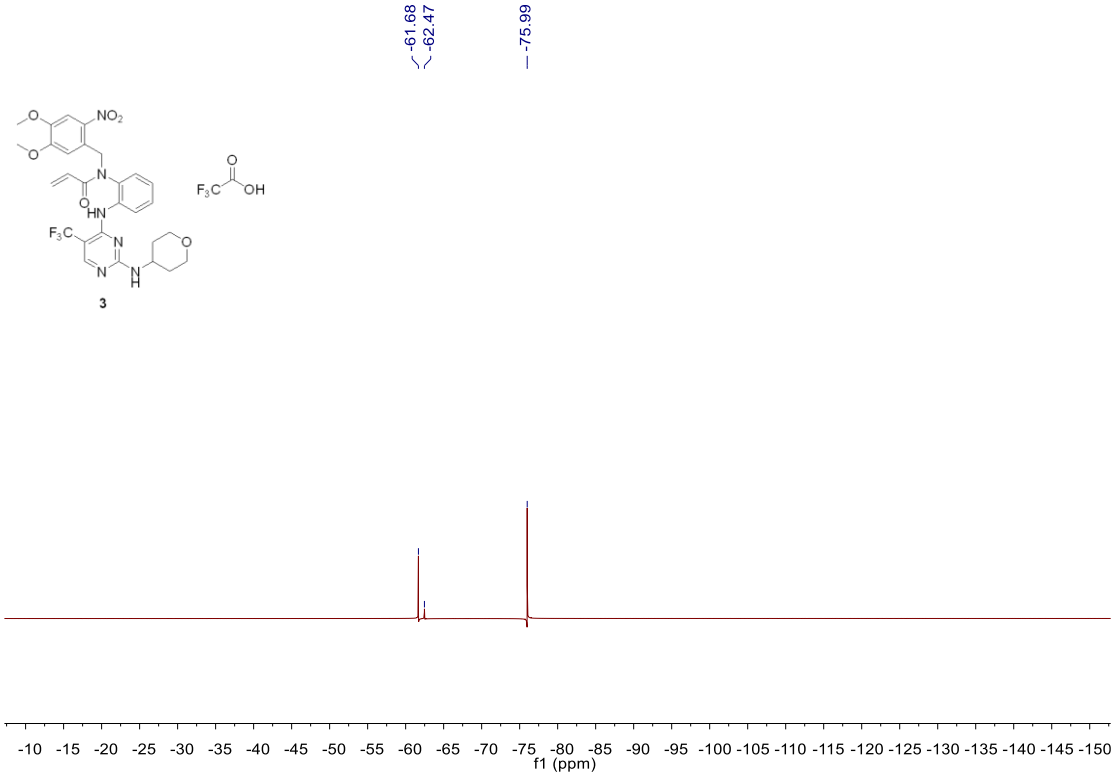

$^{1}$H NMR (400 MHz, Chloroform-*d*)



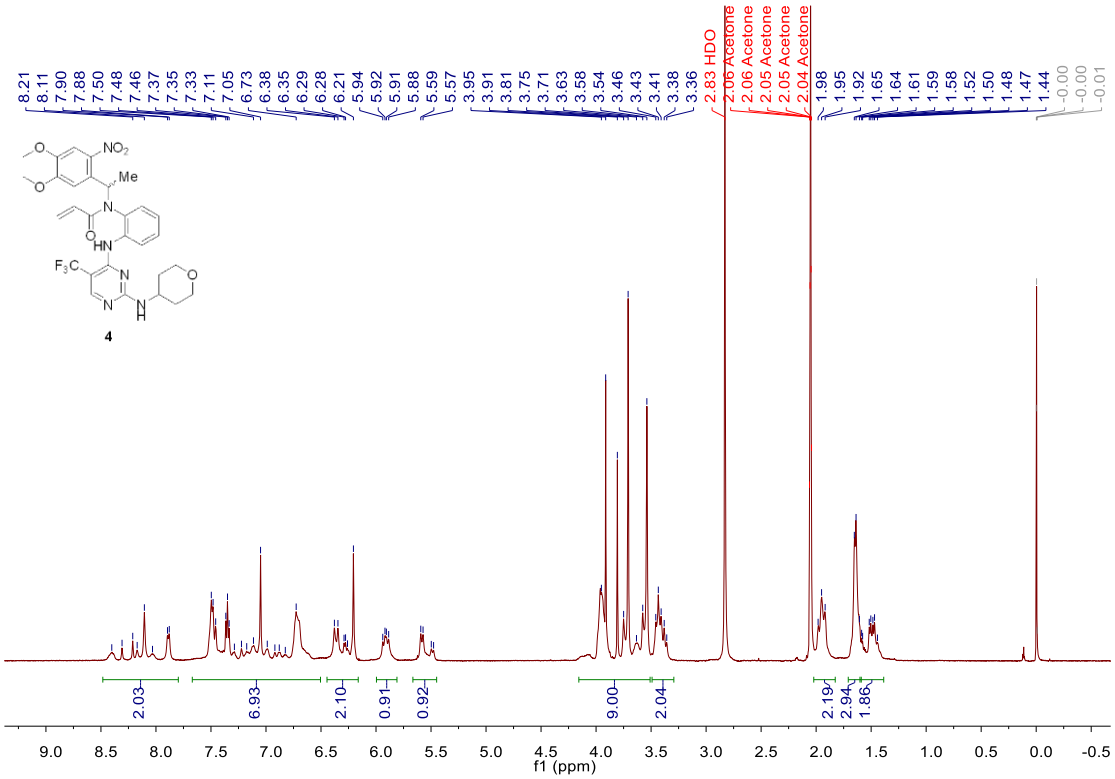
$^{13}$C NMR (101 MHz, Chloroform-*d*)



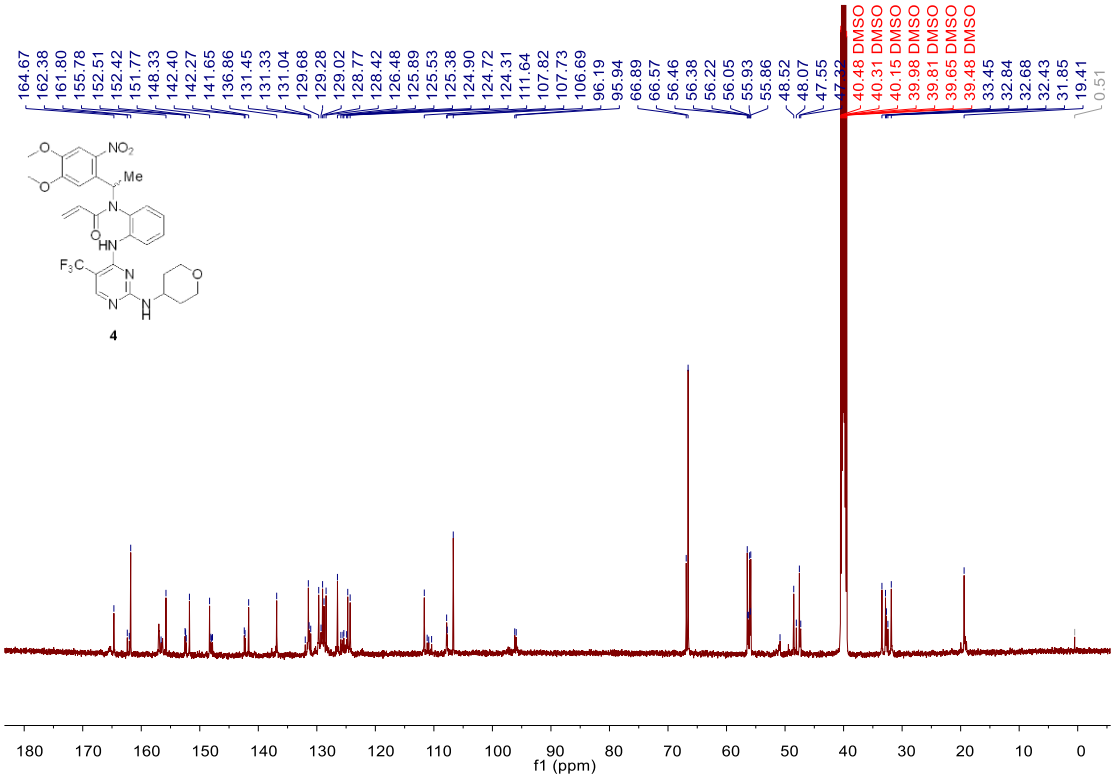

$^{19}$F NMR (376 MHz, Chloroform-*d*)



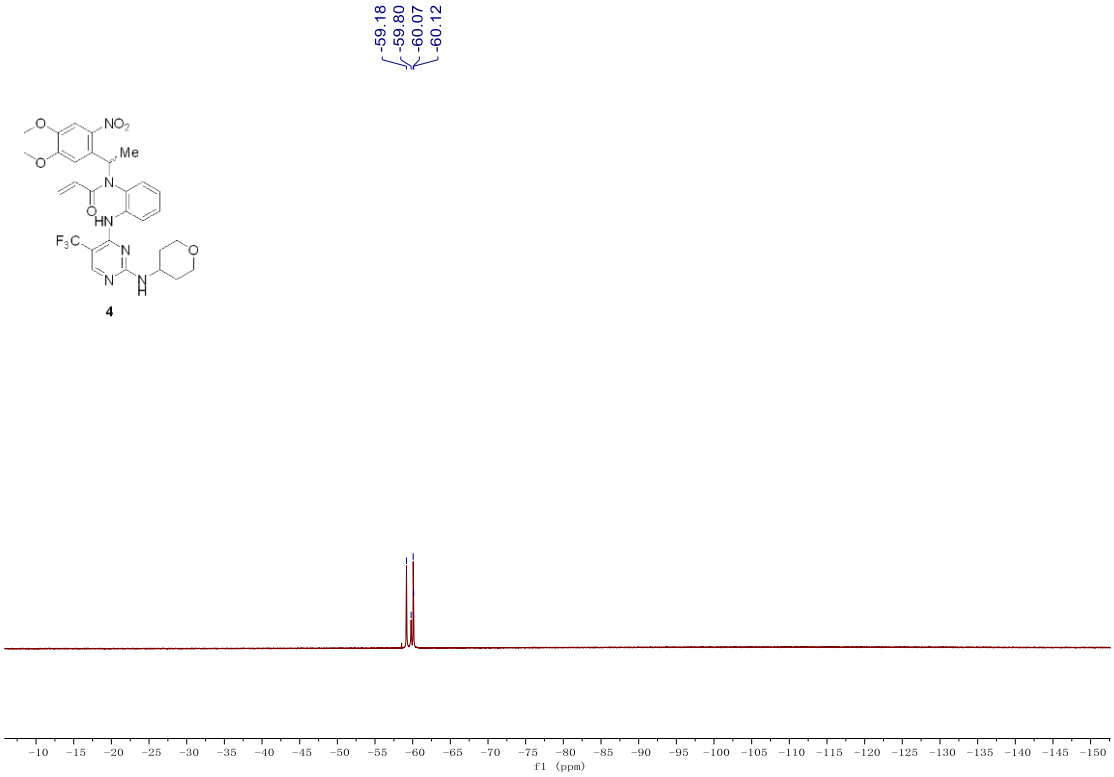
$^{1}$H NMR (500 MHz, Acetone-$d_6$)



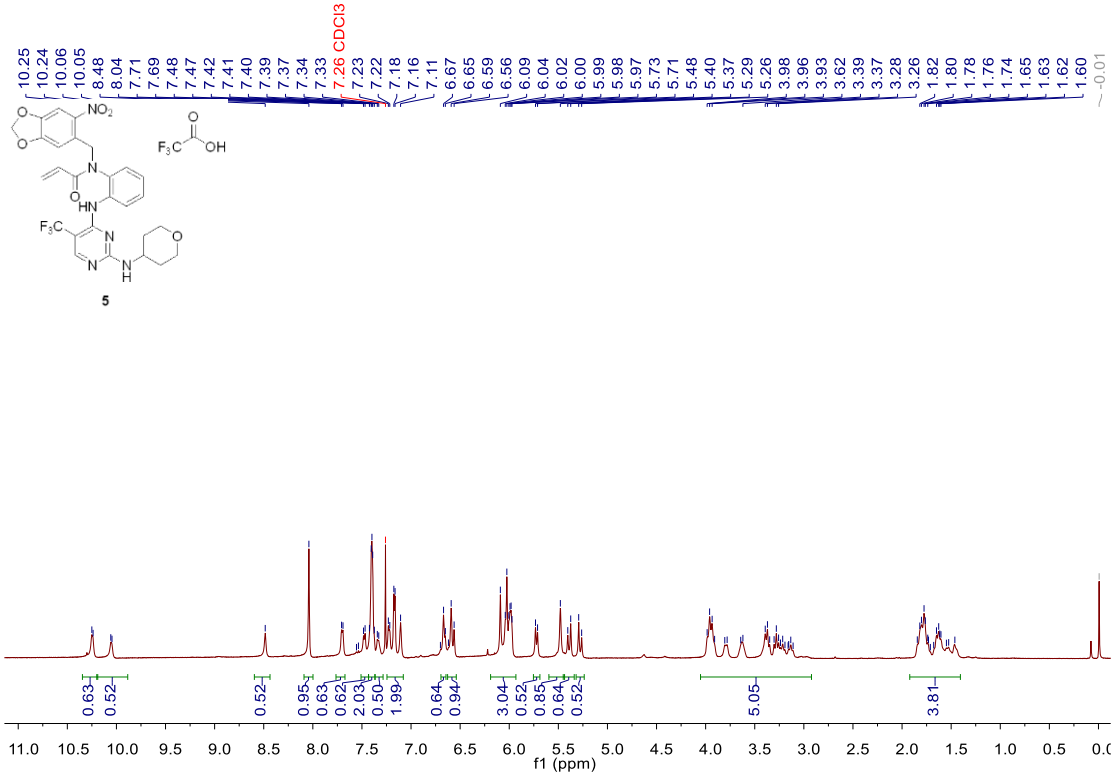

$^{13}$C NMR (126 MHz, DMSO-$d_6$)



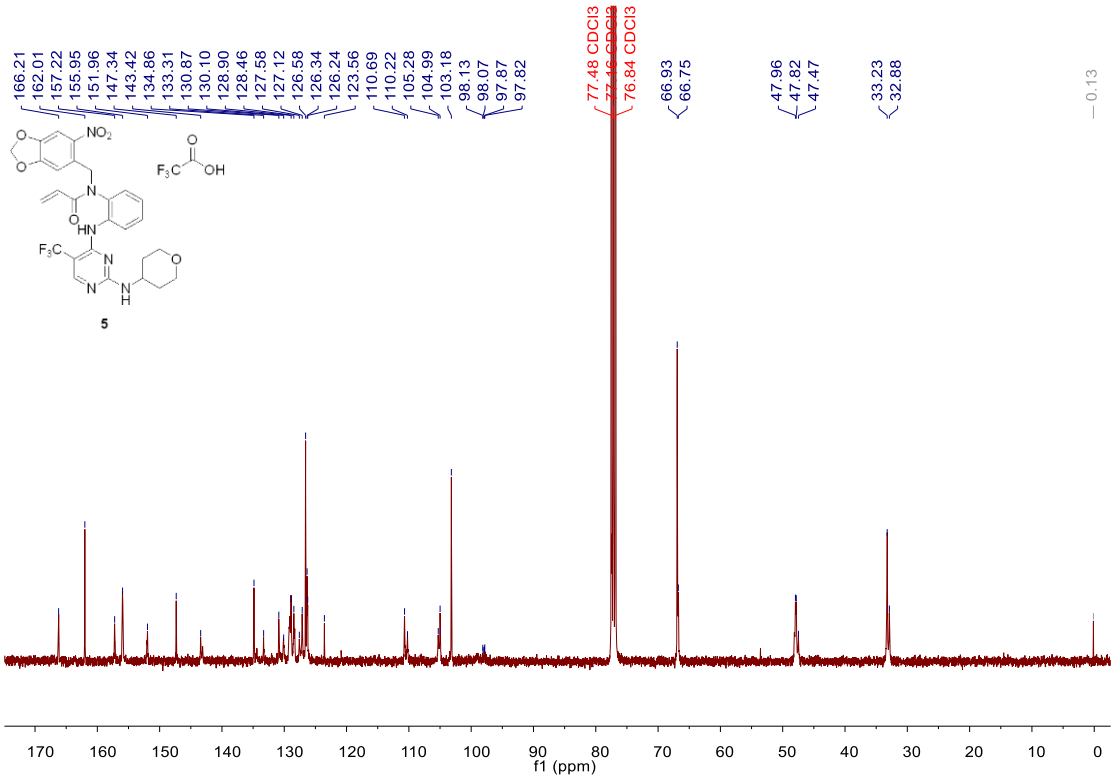
$^{19}$F NMR (376 MHz, Acetone-$d_6$)



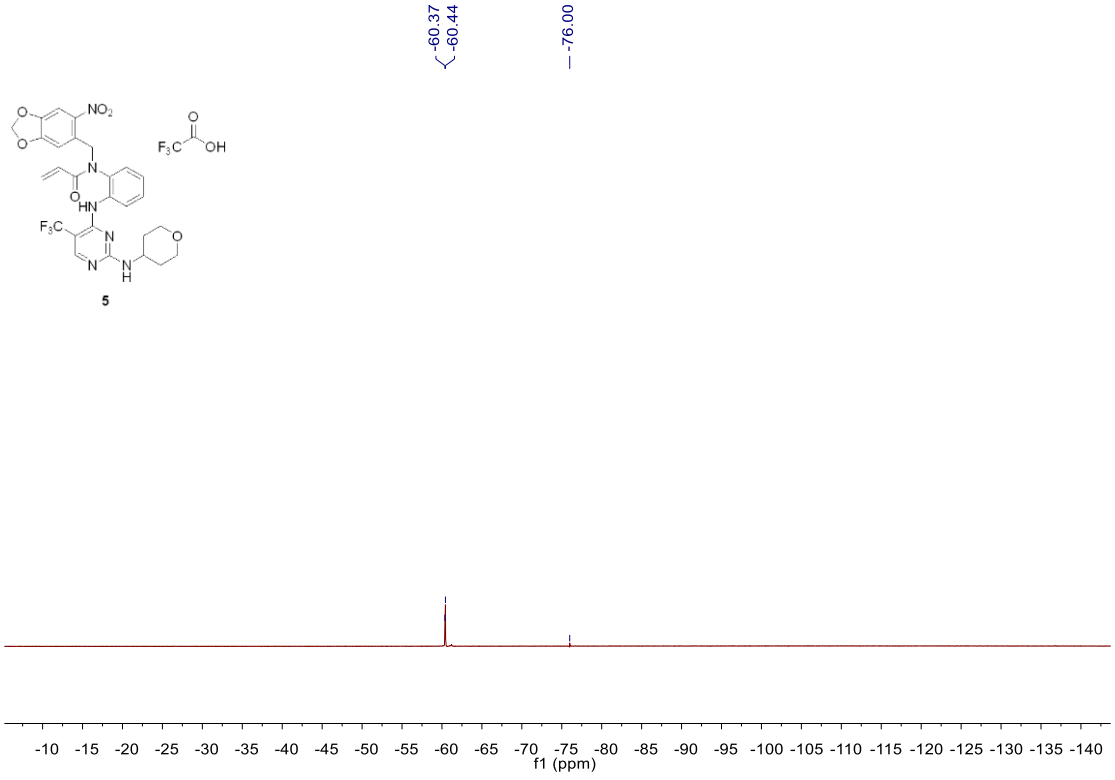

$^{1}$H NMR (500 MHz, Chloroform-$d$)



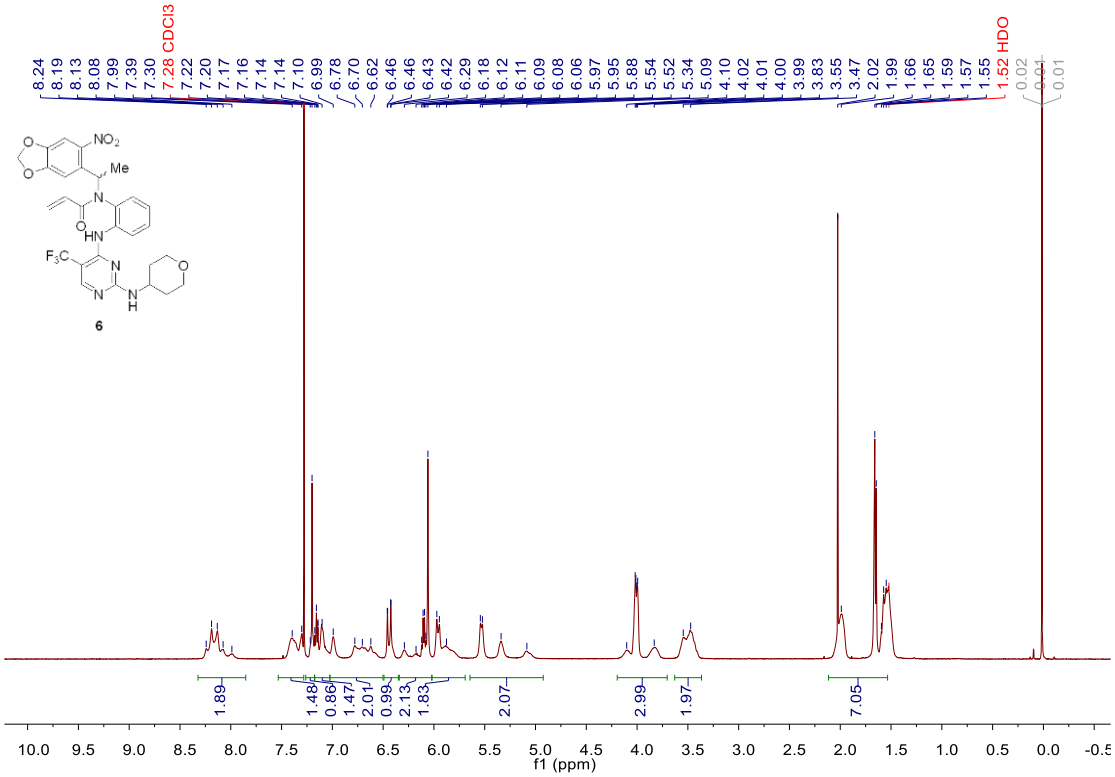
$^{13}$C NMR (101 MHz, Chloroform-*d*)



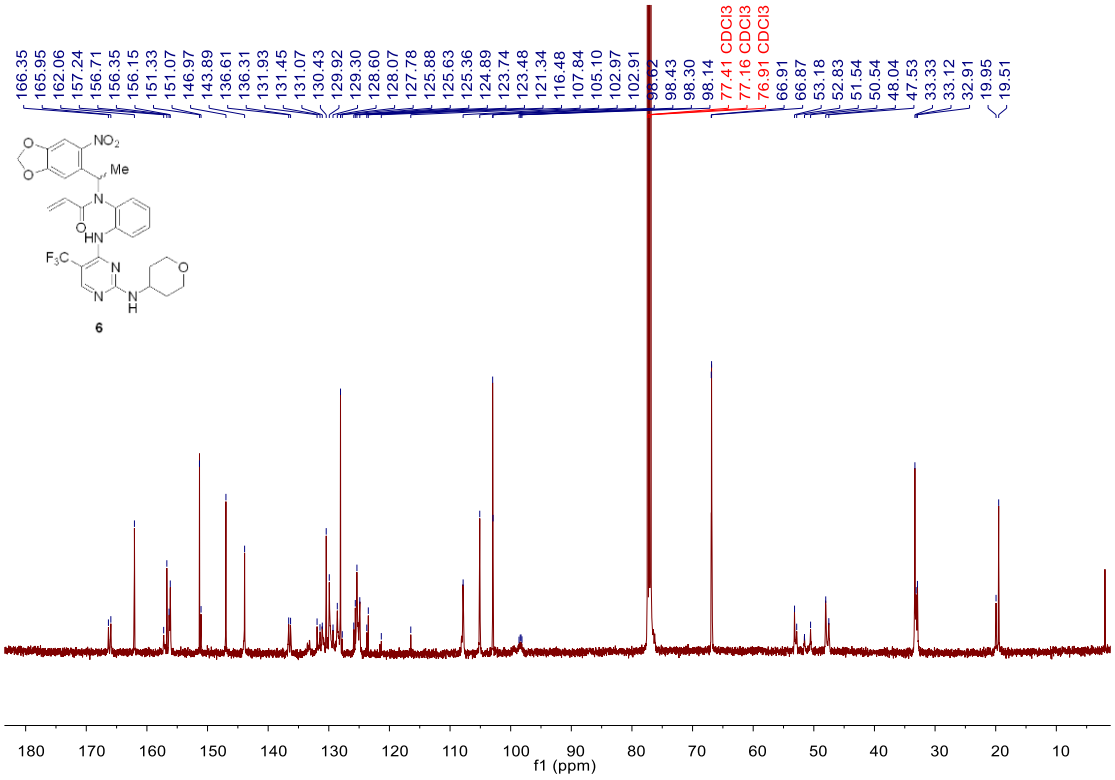

$^{19}$F NMR (376 MHz, Chloroform-*d*)



$^{1}$H NMR (500 MHz, Chloroform-*d*)




$^{13}$C NMR (126 MHz, Chloroform-*d*)



$^{19}$F NMR (376 MHz, Chloroform-*d*)