

Supporting Information

Tetrahydroisoquinoline-7-carboxamide Derivatives as New Selective Discoidin Domain Receptor 1 (DDR1) Inhibitors

Zhen Wang,^{†, #, ‡} Yali Zhang,^{§, ‡} Sergio G. Bartual,^{||} Jinfeng Luo,[#] Tingting Xu,^Ψ
Wenting Du,^ϕ Qiuju Xun,[#] Zhengchao Tu,[#] Rolf A. Brekken,^ϕ Xiaomei Ren,[#] Alex N.
Bullock,^{||, *} Guang Liang,^{§, *} Xiaoyun Lu^{†, *} and Ke Ding^{†, *}

[†]School of Pharmacy, Jinan University, 601 Huangpu Avenue West, Guangzhou 510632, China.

[#]State Key Laboratory of Respiratory Diseases, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, 190 Kaiyuan Avenue, Guangzhou 510530, China.

[§]Chemical Biology Research Center, School of Pharmaceutical Sciences, Wenzhou Medical University, Wenzhou, People's Republic of China

^ΨDepartment of Pulmonary Medicine, the 2nd affiliated hospital, Wenzhou Medical University, Wenzhou, People's Republic of China

^{||}Structural Genomics Consortium, University of Oxford, Old Road Campus, Roosevelt Drive, Oxford OX3 7DQ, U.K.

^ϕDivision of Surgical Oncology, Department of Surgery and the Hamon Center for Therapeutic Oncology Research, UT Southwestern Medical Center, Dallas Texas, 75390-8593, United States

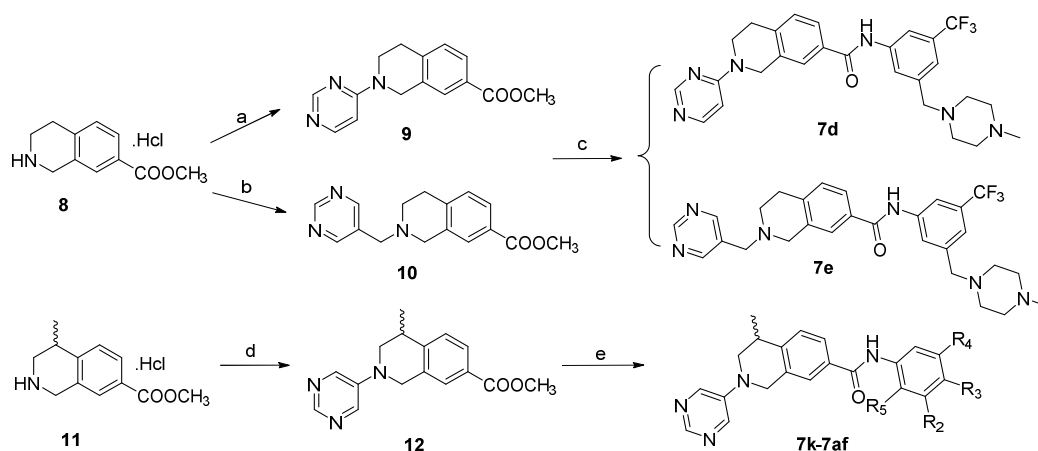
Contents of SI

Synthetic procedures for compounds 7d-7af	S2
The results of the selectivity profiling study of compound 7ae	S16
Active-site dependent competition binding assay–Kinome^{scan} Screening	S21
<i>In vitro</i> kinase assay	S23
Western blot analysis	S23
<i>In vitro</i> anti-inflammatory activity	S24
Determination of pharmacokinetic parameters in rats	S24
<i>In vivo</i> anti-inflammatory experiments	S24
Protein expression and purification	S25
Crystallization and structure determination	S25
Computational study	S27
The ¹H and ¹³C NMR spectra of compounds 7d-7af	S28
References	S56

Synthetic procedures for compounds 7d-7af

Synthesis of the designed compounds **7d**, **7e**, **7k-7af** was outlined in Scheme S1. Briefly, the previously prepared intermediate methyl 1, 2, 3, 4-tetrahydroisoquinoline-7-carboxylate hydrochloride (**8**) was reacted with 4-chloropyrimidine hydrochloride or 5-chloromethylpyrimidine to yield compounds **9** or **10**, respectively, which were further treated with 3-((4-methylpiperazin-1-yl)methyl)-5-(trifluoromethyl)aniline to produce final products **7d** and **7e** under basic conditions. The key intermediate **12** was synthesized by coupling methyl (*R, S*)-4-methyl-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxylate hydrochloride (**11**) with 5-bromopyrimidine under the Buchwald-Hartwig amination conditions. Intermediate **12** was reacted with different anilines to produce the corresponding products **7k-7af** in good or moderate yields.

Scheme 1. Synthesis of Compounds **7d**, **7e**, **7k-7af**.^a

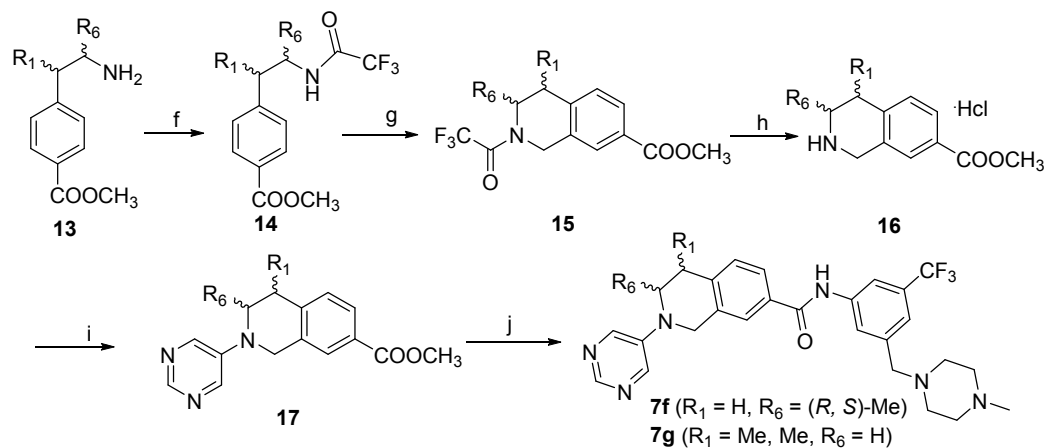


^aReagents and conditions: (a) 4-chloropyrimidine hydrochloride, triethylamine (Et₃N), tert-butyl alcohol (t-BuOH), room temperature (rt)~82 °C, 66%; (b) 5-chloromethylpyrimidine, potassium carbonate (K₂CO₃), *N, N*-dimethyl formamide (DMF), rt, 87%; (c) 3-((4-methylpiperazin-1-yl)methyl)-5-(trifluoromethyl)aniline, potassium tert-butanolate (t-BuOK), tetrahydrofuran (THF), 67-76%; (d) 5-bromopyrimidine, bis(dibenzylideneacetone)palladium (Pd(dba)₂), 2-dicyclohexyl phosphino-2',6'-diisopropoxy-1,1'-biphenyl (Ruphos), cesium carbonate (Cs₂CO₃), toluene, 80 °C, 86%; (e) substituted anilines, t-BuOK, THF, -20 °C to rt, 40-86%.

Compounds **7f** and **7g** were synthesized by utilizing a similar protocol to that for compound **7a** (Scheme S2), whereas the preparation of a racemic mixture of **7h** and **7i** as well as **7j** was outlined in Scheme S3. Briefly, (*R, S*)-2, 2, 2-trifluoro-*N*-(2-phenylpropyl)acetamide (**19**) was prepared by the trifluoroacetylation of (*R, S*)-2-phenylpropan-1-amine (**18**). A straightforward nitration of compound **19** yielded intermediate **20** which was converted to a tetrahydroisoquinoline derivative **21** through a classical Pictet-Spengler procedure. Compound **21** was deprotected and reacted with a hydrochloric acid solution in methanol to form the key intermediate **22**, which was coupled with 5-bromopyrimidine through Buchwald-Hartwig amination and followed by hydrogenation to generate compound **24**. The final product (a racemic mixture of **7h** and **7i**) was obtained by coupling intermediate **24** with 3-((4-methylpiperazin-1-yl)methyl)-5-(trifluoromethyl)benzoic acid.

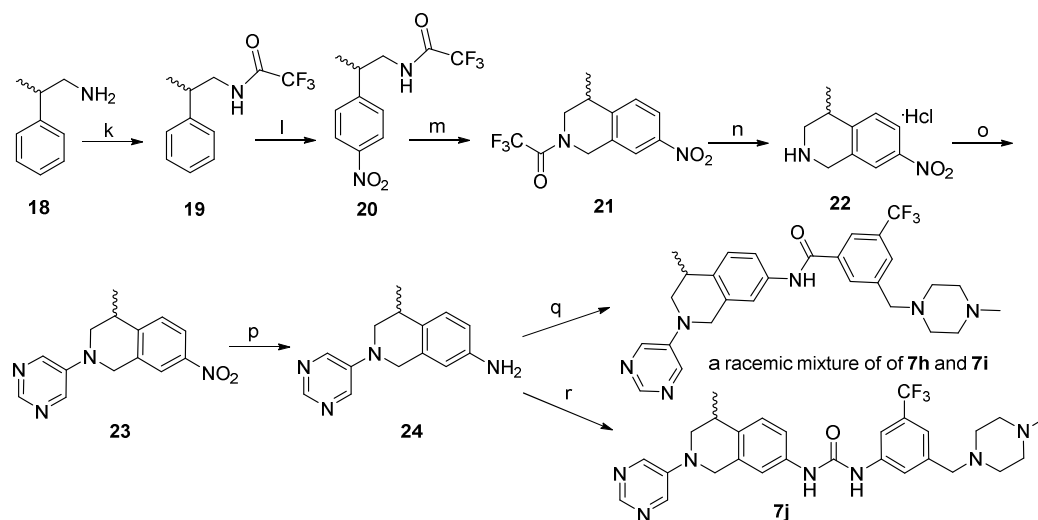
The enantiomers of **7h** and **7i** were obtained by chiral separation from the racemic mixture using HPLC. Compound **7j** was produced by treating **24** with 1-(3-isocyanato-5-(trifluoromethyl)benzyl)-4-methylpiperazine.

Scheme S2. Synthesis of Compounds **7f** and **7g**.^a



^aReagents and conditions: (f) Trifluoroacetic anhydride, 0 °C to rt, 63-68%; (g) (HCHO)_n, conc. H₂SO₄, 0 °C to rt, 58-76%; (h) i) K₂CO₃, methanol (MeOH)/H₂O (2: 1), rt; ii) HCl.MeOH, MeOH, rt, 90-92% (two steps); (i) 5-bromopyrimidine, Pd(dba)₂, Ruphos, Cs₂CO₃, toluene, 80 °C, 37-73%; (j) 3-((4-methylpiperazin-1-yl)methyl)-5-(trifluoromethyl)aniline, t-BuOK, THF, 71-78%.

Scheme S3. Synthesis of Compounds **7j** and a Mixture Rotamers of **7h** and **7i**.^a



^aReagents and conditions: (k) Trifluoroacetic anhydride, 0 °C to rt, 71%; (l) 65% HNO₃, acetic anhydride (Ac₂O), 0 °C to rt, 63%; (m) (HCHO)_n, conc. H₂SO₄, 0 °C to rt, 44%; (n) i) K₂CO₃, MeOH/H₂O (2: 1), rt; ii) HCl.MeOH, MeOH, rt, 70% (two steps); (o) 5-bromopyrimidine, Pd(dba)₂, Ruphos, Cs₂CO₃, toluene, 80 °C, 57%; (p) Pd/C, H₂, MeOH, rt, 98%; (q) 3-((4-methylpiperazin-1-yl)methyl)-5-(trifluoromethyl)benzoic acid,

2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyl uronium hexafluoro phosphate (HATU), *N,N*-diisopropylethylamine (DIPEA), DCM, 64%; (r)
3-((4-methylpiperazin-1-yl)methyl)-5-(trifluoromethyl)aniline, triphosgene, Et₃N, 0 °C to rt to 45 °C, 60%.

General Methods for Chemistry. All reagents and solvents were purchased from commercial sources without further purification. Flash chromatography was performed using 300 mesh silica gel. All reactions were monitored by thin-layer chromatography (TLC) using silica gel plates with fluorescence F254 and UV light visualization. ¹H NMR spectra were recorded on a Bruker AV-400 spectrometer at 400 MHz or a Bruker AV-500 spectrometer at 500 MHz. ¹³C NMR spectra were recorded on a Bruker AV-500 spectrometer at 125 MHz. Coupling constants (J) are expressed in hertz (Hz). Chemical shifts (δ) of NMR are reported in parts per million (ppm) units relative to an internal standard (TMS). Low resolution ESI-MS were recorded on an Agilent 1200 HPLC-MSD mass spectrometer and high resolution ESI-MS on an Applied Biosystems Q-STAR Elite ESI-LC-MS/MS mass spectrometer. Purity of compounds was determined by reverse-phase high performance liquid chromatography [HPLC, Dionex Summit HPLC (Column: Diamonsil C18, 5.0 μm, 4.6 × 250 mm (Dikma Technologies); detector: PDA-100 photodiode array; injector: ASI-100 autoinjector; pump: p-680A)] to be >95%. A flow rate of 1.0 mL/min was used with mobile phase of 85%MeOH in H₂O with 0.1% modifier (ammonia, v/v).

Methyl 2-(pyrimidin-4-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxylate (9).

To a solution of 4-chloropyrimidine hydrochloride (911 mg, 4.0 mmol) and methyl 1, 2, 3, 4-tetrahydroisoquinoline-7-carboxylate hydrochloride (910 mg, 4.0 mmol) in 20 ml of t-BuOH was added Et₃N (2.2 ml, 16.0 mmol) at rt. The reaction mixture was stirred at 82 °C overnight and quenched with water. The mixture was extracted with ethyl acetate twice. The combined organic layer was washed with brine, dried over Na₂SO₄, concentrated in vacuo and further purified by flash chromatography on silica gel to give the title compound **9** (708 mg, 66% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.65 (s, 1 H), 8.25 (d, J = 5.2 Hz, 1 H), 7.91-7.88 (m, 2 H), 7.27 (d, J = 8.0 Hz, 1 H), 6.57 (d, J = 6.0 Hz, 1 H), 4.79 (s, 2 H), 3.92 (s, 3 H), 3.92-3.88 (m, 2 H), 3.02 (t, J = 9.2 Hz, 2 H).

***N*-(3-((4-methylpiperazin-1-yl)methyl)-5-(trifluoromethyl)phenyl)-2-(pyrimidin-4-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7d).**

To a solution of methyl 2-(pyrimidin-4-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxylate (1.0 g, 3.7 mmol) and 3-((4-methylpiperazin-1-yl)methyl)-5-(trifluoromethyl)aniline (1.0 g, 3.7 mmol) in anhydrous THF (20 mL) was added potassium tert-butoxide (1.2 g, 11.1 mmol) portionwise at -20 °C. The reaction mixture was slowly warmed to room temperature and stirred at rt for 1 hour. After the reaction was finished by TLC, the mixture was poured into ice water with stirring and extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous Na₂SO₄. The filtrate was concentrated under vacuum. The resulting residue was purified by silica gel column to give the desired product **7d** (1.4 g, 76% yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.48 (s, 1 H), 8.54 (s, 1 H), 8.22 (d, J = 6.0 Hz, 1 H), 8.19 (s, 1 H), 8.00 (s, 1 H), 7.90 (s, 1 H), 7.82 (d, J = 8.0 Hz, 1 H), 7.37 (d, J = 8.0 Hz, 1 H), 7.34 (s, 1 H), 6.89 (d, J = 6.0 Hz, 1 H), 4.85 (s, 1 H), 3.86 (t, J = 5.2 Hz, 2 H), 3.52 (s, 1 H), 2.97 (t, J = 5.6 Hz, 2 H), 2.39 (br s, 4 H), 2.35 (br s, 4 H), 2.15 (s, 3 H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ 165.5, 160.6, 157.8, 155.4, 140.7, 140.0,

139.2, 133.8, 132.2, 129.2 (q, J = 31.2 Hz), 128.4, 125.9, 125.9, 124.1 (q, J = 270.8 Hz), 123.7, 119.8, 114.9, 103.5, 61.4, 54.6, 52.5, 45.6, 45.2, 40.9, 27.8. HRMS (ESI) for C₂₇H₂₉F₃N₆O [M + H]⁺, calcd: 511.2428, Found: 511.2421. Purity 98.7% (t_R = 7.03 min).

Methyl 2-(pyrimidin-5-ylmethyl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxylate (10).

To a solution of methyl 1, 2, 3, 4-tetrahydroisoquinoline-7-carboxylate hydrochloride (911 mg, 4.0 mmol) in 10 ml of DMF was added K₂CO₃ (829 mg, 6.0 mmol) and 5-chloromethylpyrimidine (566 mg, 4.4 mmol). The mixture was stirred at rt overnight and quenched with ice-water. The mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over Na₂SO₄, concentrated in vacuo and purified by flash chromatography on silica gel to give the title compound 10 (986 mg, 87% yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.11 (s, 1 H), 8.78 (s, 2 H), 7.70 (d, J = 7.6 Hz, 1 H), 7.65 (s, 1 H), 7.24 (d, J = 8.0 Hz, 1 H), 3.84 (s, 3 H), 3.71 (s, 2 H), 3.65 (s, 2 H), 2.88-2.86 (m, 2 H), 2.73-2.69 (m, 2 H).

N-(3-((4-methylpiperazin-1-yl)methyl)-5-(trifluoromethyl)phenyl)-2-(pyrimidin-5-ylmethyl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7e).

Compound 7e was prepared by following a similar procedure as that of 7d. Yield, 67%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.37 (s, 1 H), 9.12 (s, 1 H), 8.80 (s, 2 H), 8.15 (s, 1 H), 7.97 (s, 1 H), 7.74 (d, J = 6.0 Hz, 1 H), 7.68 (s, 1 H), 7.32 (s, 1 H), 7.26 (d, J = 6.4 Hz, 1 H), 3.74 (s, 2 H), 3.65 (s, 2 H), 3.51 (s, 2 H), 2.89 (s, 2 H), 2.74 (s, 2 H), 2.37 (br s, 8 H), 2.15 (s, 3 H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ 165.5, 157.4, 157.2, 140.6, 140.0, 138.4, 134.5, 131.6, 129.1 (q, J = 31.3 Hz), 128.6, 125.8, 125.4, 124.1 (q, J = 271.1 Hz), 123.6, 119.7, 114.9, 61.3, 56.1, 54.9, 54.6, 52.4, 49.7, 45.6, 28.5. HRMS (ESI) for C₂₈H₃₁F₃N₆O [M + H]⁺, calcd: 525.2584, Found: 525.2577. Purity 96.7% (t_R = 5.89 min).

Methyl (R, S)-4-methyl-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxylate (12). An oven-dried test tube, which was equipped with a magnetic stir bar and fitted with a teflon septum, was charged with the Pd(dba)₂ (253 mg, 10 mmol%), Ruphos (411 mg, 20 mmol%), CsCO₃ (4.3 g, 13.2 mmol), methyl (R, S)-4-methyl-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxylate hydrochloride (1.1 g, 4.4 mmol), and 5-bromopyrimidine (769 mg, 4.8 mmol). The vessel was evacuated and backfilled with argon and then toluene (20 mL) was added via syringe. The solution was heated to 80°C overnight, and then cooled to room temperature. The reaction mixture was filtered through a pad of Celite and concentrated under vacuum, and then purified by flash column to yield the title compound 12 (1.1 g, 86% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.70 (s, 1 H), 8.48 (s, 2 H), 7.93 (d, J = 8.0 Hz, 1 H), 7.89 (s, 1 H), 7.35 (d, J = 8.0 Hz, 1 H), 4.59 (d, J = 15.2 Hz, 1 H), 4.48 (d, J = 15.2 Hz, 1 H), 3.92 (s, 3 H), 3.63-3.60 (m, 1 H), 3.45-3.41 (m, 1 H), 3.23-3.22 (m, 1 H), 1.42 (d, J = 7.2 Hz, 3 H).

(R, S)-4-methyl-N-(3-((4-methylpiperazin-1-yl)methyl)phenyl)-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7k).

Compound 7k was prepared by following a similar procedure as that of 7d. Yield, 40%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.18 (s, 1 H), 8.59-8.55 (m, 3 H), 7.84-7.82 (m, 2 H), 7.72-7.70 (m, 2 H), 7.44 (d, J = 7.6 Hz, 1 H), 7.28 (t, J = 8.0 Hz, 1 H), 7.01 (d, J = 8.0 Hz, 1 H), 4.64 (d, J = 16.0 Hz, 1 H), 4.52 (d, J = 16.0 Hz, 1 H), 3.66 (dd, J = 12.4, 4.4 Hz, 1 H), 3.47-3.43 (m, 3 H), 3.20-3.17 (m, 1 H), 2.37 (br s, 4 H), 2.32 (br s, 4 H), 2.14 (s, 3 H), 1.34 (d, J = 6.8 Hz, 3 H). ¹³C NMR (125 MHz,

DMSO-d₆) δ 165.3, 147.7, 143.4, 143.2, 141.8, 139.1, 138.8, 133.0, 132.8, 128.3, 127.2, 126.0, 125.8, 124.2, 120.6, 118.9, 62.2, 54.7, 52.6, 50.2, 48.0, 45.7, 32.1, 19.2. HRMS (ESI) for C₂₇H₃₂N₆O [M + H]⁺, calcd: 457.2710, Found: 457.2704. Purity 95.5% (tR = 12.08 min, flow rate: 1.0 mL/min, mobile phase: 70%MeOH in H₂O with 0.1% modifier (ammonia, v/v)).

(R, S)-N-(3-ethyl-5-((4-methylpiperazin-1-yl)methyl)phenyl)-4-methyl-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7l).

Compound 7l was prepared by following a similar procedure as that of 7d. Yield, 45%. ¹H NMR (400 MHz, DMSO-d₆) δ 10.12 (s, 1 H), 8.65-8.50 (m, 3 H), 7.84-7.81 (m, 2 H), 7.57 (s, 1 H), 7.54 (s, 1 H), 7.44(d, J = 7.2 Hz, 1 H), 6.86 (s, 1 H), 4.64 (d, J = 15.6 Hz, 1 H), 4.52 (d, J = 15.6 Hz, 1 H), 3.67-3.64 (m, 1 H), 3.47-3.40 (m, 3 H), 3.23-3.11 (m, 1 H), 2.60-2.58 (m, 2 H), 2.46-2.22 (m, 8 H), 2.14 (s, 3 H), 1.34 (d, J = 6.0 Hz, 3 H), 1.19 (t, J = 6.4 Hz, 3 H). ¹³C NMR (125 MHz, DMSO-d₆) δ 165.2, 147.7, 143.4, 143.2, 141.8, 139.1, 138.7, 133.0, 132.8, 127.2, 126.0, 125.8, 123.7, 118.3, 118.2, 62.3, 54.7, 52.6, 50.2, 48.0, 45.7, 32.1, 28.3, 19.2, 15.5. HRMS (ESI) for C₂₉H₃₆N₆O [M + H]⁺, calcd: 485.3023, Found: 485.3019. Purity 95.3% (tR = 8.80 min).

(R, S)-N-(3-isopropyl-5-((4-methylpiperazin-1-yl)methyl)phenyl)-4-methyl-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7m).

Compound 7m was prepared by following a similar procedure as that of 7d. Yield, 58%. ¹H NMR (400 MHz, DMSO-d₆) δ 10.11 (s, 1 H), 8.61-8.53 (m, 3 H), 7.84-7.81 (m, 2 H), 7.58 (s, 1 H), 7.57 (s, 1 H), 7.44 (d, J = 7.6 Hz, 1 H), 6.88 (s, 1 H), 4.64 (d, J = 16.0 Hz, 1 H), 4.52 (d, J = 16.0 Hz, 1 H), 3.67-3.64 (m, 1 H), 3.48-3.41 (m, 3 H), 3.18-3.17 (m, 1 H), 2.87-2.84 (m, 1 H), 2.36-2.33 (m, 8 H), 2.14 (s, 3 H), 1.34 (d, J = 6.4 Hz, 3 H), 1.21 (d, J = 6.4 Hz, 6 H). ¹³C NMR (125 MHz, DMSO-d₆) δ 165.2, 148.6, 147.7, 143.4, 143.2, 141.8, 139.1, 138.6, 133.0, 132.8, 127.2, 126.0, 125.9, 122.4, 118.4, 116.9, 62.4, 54.7, 52.6, 50.2, 48.1, 45.7, 33.5, 32.2, 23.9, 19.2. HRMS (ESI) for C₃₀H₃₈N₆O [M + H]⁺, calcd: 499.3180, Found: 499.3173. Purity 95.7% (tR = 7.64 min).

(R, S)-N-(3-(tert-butyl)-5-((4-methylpiperazin-1-yl)methyl)phenyl)-4-methyl-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7n).

Compound 7n was prepared by following a similar procedure as that of 7d. Yield, 60%. ¹H NMR (500 MHz, DMSO-d₆) δ 10.12 (s, 1 H), 8.58 (s, 2 H), 8.57 (s, 1 H), 7.84-7.82 (m, 2 H), 7.70 (s, 1 H), 7.63 (s, 1 H), 7.44(d, J = 8.0 Hz, 1 H), 7.04 (s, 1 H), 4.65 (d, J = 16.0 Hz, 1 H), 4.52 (d, J = 16.0 Hz, 1 H), 3.68-3.64 (m, 1 H), 3.49-3.45 (m, 1 H), 3.44 (s, 1 H), 3.20-3.16 (m, 1 H), 2.48-2.18 (m, 8 H), 2.15 (s, 3 H), 1.34 (d, J = 7.0 Hz, 3 H), 1.28 (s, 9 H). ¹³C NMR (125 MHz, DMSO-d₆) δ 165.2, 150.9, 147.7, 143.4, 143.3, 141.8, 138.9, 138.2, 133.0, 132.9, 127.2, 126.0, 125.9, 121.1, 118.0, 116.1, 62.5, 54.7, 52.5, 50.2, 48.1, 45.7, 34.4, 32.1, 31.2, 19.2. HRMS (ESI) for C₃₁H₄₀N₆O [M + H]⁺, calcd: 513.3336, Found: 513.3330. Purity 98.1% (tR = 8.61 min).

(R, S)-N-(3-cyclopropyl-5-((4-methylpiperazin-1-yl)methyl)phenyl)-4-methyl-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7o).

Compound 7o was prepared by following a similar procedure as that of 7d. Yield, 41%. ¹H NMR

(500 MHz, DMSO-d₆) δ 10.09 (s, 1 H), 8.65-8.54 (m, 3 H), 7.83-7.81 (m, 2 H), 7.51 (s, 1 H), 7.44 (d, J = 7.5 Hz, 1 H), 7.39 (s, 1 H), 6.76 (s, 1 H), 4.64 (d, J = 16.0 Hz, 1 H), 4.52 (d, J = 16.0 Hz, 1 H), 3.67-3.64 (m, 1 H), 3.48-3.44 (m, 1 H), 3.38 (s, 2 H), 3.22-3.13 (m, 1 H), 2.47-2.21 (m, 8 H), 2.14 (s, 3 H), 1.90-1.89 (m, 1 H), 1.34 (d, J = 6.5 Hz, 3 H), 0.96-0.94 (m, 2 H), 0.63-0.62 (m, 2 H). ¹³C NMR (125 MHz, DMSO-d₆) δ 165.6, 147.8, 144.2, 143.6, 143.4, 142.0, 139.2, 138.8, 133.2, 132.9, 127.4, 126.2, 126.1, 122.0, 118.3, 116.0, 62.4, 54.8, 52.7, 50.4, 48.2, 45.8, 32.3, 19.4, 15.3, 9.5. HRMS (ESI) for C₃₀H₃₆N₆O [M + H]⁺, calcd: 497.3023, Found: 497.3015. Purity 96.6% (tR = 6.81 min).

(R,

S)-N-(3-cyclohexyl-5-((4-methylpiperazin-1-yl)methyl)phenyl)-4-methyl-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7p).

Compound 7p was prepared by following a similar procedure as that of 7d. Yield, 48%. ¹H NMR (400 MHz, DMSO-d₆) δ 10.09 (s, 1 H), 8.63-8.53 (m, 3 H), 7.84-7.81 (m, 2 H), 7.58 (s, 1 H), 7.55 (s, 1 H), 7.44 (d, J = 7.6 Hz, 1 H), 6.86 (s, 1 H), 4.64 (d, J = 16.0 Hz, 1 H), 4.52 (d, J = 16.0 Hz, 1 H), 3.67-3.65 (m, 1 H), 3.49-3.44 (m, 2 H), 3.41 (s, 2 H), 3.22-3.12 (m, 1 H), 2.34-2.21 (m, 8 H), 2.15 (s, 3 H), 1.88-1.75 (m, 4 H), 1.73-1.70 (m, 1 H), 1.40-1.33 (m, 7 H), 1.29-1.17 (m, 1 H). ¹³C NMR (125 MHz, DMSO-d₆) δ 165.4, 148.0, 147.7, 143.5, 143.3, 141.9, 139.1, 138.6, 133.1, 132.9, 127.4, 126.1, 126.0, 123.0, 118.6, 117.5, 62.5, 54.8, 52.6, 50.3, 48.2, 45.8, 44.0, 34.2, 32.3, 26.5, 25.8, 19.3. HRMS (ESI) for C₃₃H₄₂N₆O [M + H]⁺, calcd: 539.3493, Found: 539.3488. Purity 95.7% (tR = 13.57 min).

(R,

S)-4-methyl-N-(5-((4-methylpiperazin-1-yl)methyl)-[1, 1'-biphenyl]-3-yl)-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7q).

Compound 7q was prepared by following a similar procedure as that of 7d. Yield, 45%. ¹H NMR (500 MHz, DMSO-d₆) δ 10.29 (s, 1 H), 8.58-8.57 (m, 3 H), 8.04 (s, 1 H), 7.87-7.85 (m, 2 H), 7.76 (s, 1 H), 7.64-7.63 (m, 2 H), 7.50-7.46 (m, 3 H), 7.38 (t, J = 7.0 Hz, 1 H), 7.30 (s, 1 H), 4.65 (d, J = 16.0 Hz, 1 H), 4.53 (d, J = 16.0 Hz, 1 H), 3.68-3.65 (m, 1 H), 3.52 (s, 2 H), 3.49-3.46 (m, 1 H), 3.19-3.18 (m, 1 H), 2.42 (br s, 4 H), 2.36 (br s, 4 H), 2.15 (s, 3 H), 1.34 (d, J = 6.7 Hz, 3 H). ¹³C NMR (125 MHz, DMSO-d₆) δ 166.3, 148.6, 144.3, 144.2, 142.8, 141.3, 140.7, 140.5, 134.0, 133.6, 129.9, 128.5, 128.2, 127.6, 127.0, 126.8, 123.4, 120.6, 118.2, 63.2, 55.7, 53.6, 51.1, 49.0, 46.7, 33.1, 20.1. HRMS (ESI) for C₃₃H₃₆N₆O [M + H]⁺, calcd: 533.3023, Found: 533.3027. Purity 96.9% (tR = 10.98 min).

(R, S)-4-methyl-2-(pyrimidin-5-yl)-N-(3-(trifluoromethyl)phenyl)-1, 2, 3, 4 -tetrahydroisoquinoline-7-carboxamide (7r).

Compound 7r was prepared by following a similar procedure as that of 7d. Yield, 65%. ¹H NMR (400 MHz, DMSO-d₆) δ 10.52 (s, 1 H), 8.58 (s, 2 H), 8.57 (s, 1 H), 8.24 (s, 1 H), 8.06 (d, J = 8.0 Hz, 1 H), 7.86-7.84 (m, 2 H), 7.60 (t, J = 8.0 Hz, 1 H), 7.49-7.44 (m, 2 H), 4.65 (d, J = 16.0 Hz, 1 H), 4.53 (d, J = 16.0 Hz, 1 H), 3.66 (dd, J = 12.4, 4.4 Hz, 1 H), 3.47 (dd, J = 12.4, 6.4 Hz, 1 H), 3.21-3.17 (m, 1 H), 1.34 (d, J = 6.8 Hz, 3 H). ¹³C NMR (125 MHz, DMSO-d₆) δ 165.8, 147.7, 143.7, 143.4, 141.8, 140.0, 133.2, 132.3, 129.9, 129.4 (q, J = 31.4 Hz), 127.4, 126.1, 125.9, 124.2 (q, J = 270.5 Hz), 123.7, 119.9, 116.3, 50.2, 48.0, 32.2, 19.2. HRMS (ESI) for C₂₂H₁₉F₃N₄O [M + H]⁺, calcd: 413.1584, Found: 413.1576. Purity 95.2% (tR = 7.40 min).

(R, S)-4-methyl-N-(2-((4-methylpiperazin-1-yl)methyl)-5-(trifluoromethyl)phenyl)-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7s).

Compound 7s was prepared by following a similar procedure as that of 7d. Yield, 79%. ¹H NMR (500 MHz, DMSO-d₆) δ 10.70 (s, 1 H), 8.72 (s, 1 H), 8.58-8.57 (m, 3 H), 7.83-7.82 (m, 2 H), 7.52-7.50 (m, 2 H), 7.43 (d, J = 8.0 Hz, 1 H), 4.65 (d, J = 16.0 Hz, 1 H), 4.55 (d, J = 16.0 Hz, 1 H), 3.82 (s, 2 H), 3.66 (dd, J = 12.5, 4.0 Hz, 1 H), 3.49 (dd, J = 12.5, 6.0 Hz, 1 H), 3.23-3.20 (m, 1 H), 2.50-2.18 (m, 8 H), 2.16 (s, 3 H), 1.35 (d, J = 7.0 Hz, 3 H). ¹³C NMR (125 MHz, DMSO-d₆) δ 165.2, 147.8, 144.3, 143.5, 141.9, 139.4, 133.7, 132.5, 131.2, 130.4, 128.9 (q, J = 31.5 Hz), 127.9, 125.7, 124.4 (q, J = 270.6 Hz), 120.0, 116.3, 60.7, 54.6, 52.2, 50.3, 48.3, 45.7, 32.4, 19.3. HRMS (ESI) for C₂₈H₃₁F₃N₆O [M + H]⁺, calcd: 525.2584, Found: 525.2582. Purity 95.1% (t_R = 8.23 min).

(R, S)-4-methyl-N-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7t).

Compound 7t was prepared by following a similar procedure as that of 7d. Yield, 80%. ¹H NMR (400 MHz, DMSO-d₆) δ 10.48 (s, 1 H), 8.59-8.55 (m, 3 H), 8.20 (s, 1 H), 8.05 (d, J = 8.4 Hz, 1 H), 7.85-7.83 (m, 2 H), 7.70 (d, J = 8.4 Hz, 1 H), 7.47 (d, J = 8.4 Hz, 1 H), 4.65 (d, J = 16.0 Hz, 1 H), 4.53 (d, J = 16.0 Hz, 1 H), 3.66 (dd, J = 12.8, 4.4 Hz, 1 H), 3.56 (s, 2 H), 3.47 (dd, J = 12.4, 6.0 Hz, 1 H), 3.20-3.16 (m, 1 H), 2.38 (br s, 4 H), 2.33 (br s, 4 H), 2.15 (s, 3 H), 1.34 (d, J = 6.8 Hz, 3 H). ¹³C NMR (125 MHz, DMSO-d₆) δ 165.5, 147.7, 143.5, 143.3, 141.8, 138.3, 133.1, 132.3, 131.9, 131.2, 127.4 (q, J = 29.5 Hz), 127.2, 126.1, 125.9, 124.3 (q, J = 272.5 Hz), 123.4, 117.1, 57.5, 54.7, 52.7, 50.2, 48.0, 45.7, 32.2, 19.1. HRMS (ESI) for C₂₈H₃₁F₃N₆O [M + H]⁺, calcd: 525.2584, Found: 525.2582. Purity 97.7% (t_R = 7.72 min).

(R, S)-4-methyl-N-(3-(4-methylpiperazin-1-yl)-5-(trifluoromethyl)phenyl)-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7u).

Compound 7u was prepared by following a similar procedure as that of 7d. Yield, 78%. ¹H NMR (400 MHz, DMSO-d₆) δ 10.30 (s, 1 H), 8.59-8.55 (m, 3 H), 7.84-7.82 (m, 2 H), 7.68 (s, 1 H), 7.63 (s, 1 H), 7.47 (d, J = 8.0 Hz, 1 H), 6.94 (s, 1 H), 4.65 (d, J = 16.0 Hz, 1 H), 4.53 (d, J = 16.0 Hz, 1 H), 3.66 (dd, J = 12.8, 4.4 Hz, 1 H), 3.47 (dd, J = 12.4, 6.4 Hz, 1 H), 3.23-3.16 (m, 5 H), 2.47 (t, J = 4.8 Hz, 4 H), 2.23 (s, 3 H), 1.34 (d, J = 6.8 Hz, 3 H). ¹³C NMR (125 MHz, DMSO-d₆) δ 165.6, 151.6, 147.7, 143.6, 143.4, 141.8, 140.8, 133.1, 132.4, 130.1 (q, J = 30.8 Hz), 127.3, 126.0, 125.8, 124.4 (q, J = 271.0 Hz), 109.5, 106.6, 106.4, 54.3, 50.2, 48.0, 47.6, 45.7, 32.2, 19.2. HRMS (ESI) for C₂₇H₂₉F₃N₆O [M + H]⁺, calcd: 511.2428, Found: 511.2423. Purity 99.8% (t_R = 6.80 min).

(R, S)-4-methyl-N-(3-(2-(4-methylpiperazin-1-yl)ethyl)-5-(trifluoromethyl)phenyl)-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7v).

Compound 7v was prepared by following a similar procedure as that of 7d. Yield, 82%. ¹H NMR (400 MHz, DMSO-d₆) δ 10.42 (s, 1 H), 8.59-8.56 (s, 3 H), 8.07 (s, 1 H), 7.91 (s, 1 H), 7.85-7.83 (m, 2 H), 7.47 (d, J = 8.0 Hz, 1 H), 7.34 (s, 1 H), 4.65 (d, J = 16.0 Hz, 1 H), 4.53 (d, J = 16.0 Hz, 1 H), 3.66 (dd, J = 12.4, 4.4 Hz, 1 H), 3.47 (dd, J = 12.4, 6.4 Hz, 1 H), 3.21-3.17 (m, 1 H), 2.82 (t, J = 7.2 Hz, 2 H), 2.54 (t, J = 7.2 Hz, 2 H), 2.45 (br s, 4 H), 2.31 (br s, 4 H), 2.14 (s, 3 H), 1.34 (d, J = 6.8 Hz, 3 H). ¹³C NMR (125 MHz, DMSO-d₆) δ 165.7, 147.7, 143.6, 143.4, 142.7, 141.8, 139.9, 133.2, 132.3, 129.3 (q, J = 31.2 Hz), 127.3, 126.1, 125.9, 124.3 (q, J = 270.8 Hz), 123.8,

120.3, 114.2, 59.1, 54.7, 52.6, 50.2, 48.0, 45.7, 32.6, 32.2, 19.2. HRMS (ESI) for C₂₉H₃₃F₃N₆O [M + H]⁺, calcd: 539.2741, Found: 539.2733. Purity 98.7% (tR = 6.91 min).

(R, S)-N-(3-((4-ethylpiperazin-1-yl)methyl)-5-(trifluoromethyl)phenyl)-4-methyl-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7w).

Compound 7w was prepared by following a similar procedure as that of 7d. Yield, 79%. ¹H NMR (400 MHz, DMSO-d₆) δ 10.50 (s, 1 H), 8.60-8.55 (m, 3 H), 8.19 (s, 1 H), 8.00 (s, 1 H), 7.86-7.84 (m, 2 H), 7.47 (d, J = 8.0 Hz, 1 H), 7.34 (s, 1 H), 4.65 (d, J = 16.0 Hz, 1 H), 4.53 (d, J = 16.0 Hz, 1 H), 3.66 (dd, J = 12.4, 4.4 Hz, 1 H), 3.54 (s, 2 H), 3.47 (dd, J = 12.8, 6.4 Hz, 1 H), 3.21-3.16 (m, 1 H), 2.48-2.33 (m, 8 H), 2.30 (q, J = 7.2 Hz, 3 H), 1.34 (d, J = 6.8 Hz, 3 H), 0.97 (t, J = 7.2 Hz, 3 H). ¹³C NMR (125 MHz, DMSO-d₆) δ 165.7, 147.7, 143.6, 143.3, 141.8, 140.7, 133.1, 132.2, 129.2 (q, J = 31.3 Hz), 127.3, 126.1, 125.9, 124.2 (q, J = 270.8 Hz), 123.7, 119.9, 115.0, 61.4, 52.6, 52.3, 51.6, 50.2, 48.0, 32.2, 19.2, 11.9. HRMS (ESI) for C₂₉H₃₃F₃N₆O [M + H]⁺, calcd: 539.2741, Found: 539.2732. Purity 98.8% (tR = 7.76min).

(R, S)-N-(3-((4-cyclohexylpiperazin-1-yl)methyl)-5-(trifluoromethyl)phenyl)-4-methyl-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7x).

Compound 7x was prepared by following a similar procedure as that of 7d. Yield, 75%. ¹H NMR (400 MHz, DMSO-d₆) δ 10.49 (s, 1 H), 8.63-8.51 (m, 3 H), 8.19 (s, 1 H), 7.99 (s, 1 H), 7.86-7.84 (m, 2 H), 7.46 (d, J = 8.0 Hz, 1 H), 7.33 (s, 1 H), 4.64 (d, J = 16.4 Hz, 1 H), 4.52 (d, J = 16.0 Hz, 1 H), 3.67-3.63 (m, 1 H), 3.51 (s, 2 H), 3.49-3.44 (m, 1 H), 3.19-3.17 (m, 1 H), 2.50-2.39 (m, 8 H), 2.24-2.10 (m, 1 H), 1.73-1.69 (m, 4 H), 1.55-1.53 (m, 1 H), 1.34 (d, J = 6.8 Hz, 3 H), 1.24-1.11 (m, 4 H), 1.10-0.97 (m, 1 H), ¹³C NMR (125 MHz, DMSO-d₆) δ 165.9, 147.8, 143.8, 143.5, 141.9, 140.8, 140.1, 133.2, 132.3, 129.4 (q, J = 31.0 Hz), 127.5, 126.2, 126.0, 124.3 (q, J = 270.9 Hz), 124.0, 120.1, 115.1, 62.7, 61.7, 53.2, 50.3, 48.4, 48.1, 32.3, 28.5, 26.0, 25.4, 19.3. HRMS (ESI) for C₃₃H₃₉F₃N₆O [M + H]⁺, calcd: 593.3210, Found: 593.3203. Purity 99.2% (tR = 15.81 min).

(R, S)-4-methyl-N-(3-(morpholinomethyl)-5-(trifluoromethyl)phenyl)-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7y).

Compound 7y was prepared by following a similar procedure as that of 7d. Yield, 73%. ¹H NMR (400 MHz, DMSO-d₆) δ 10.49 (s, 1 H), 8.61-8.54 (m, 3 H), 8.19 (s, 1 H), 8.03 (s, 1 H), 7.87-7.85 (m, 2 H), 7.47 (d, J = 8.0 Hz, 1 H), 7.37 (s, 1 H), 4.65 (d, J = 16.0 Hz, 1 H), 4.53 (d, J = 16.0 Hz, 1 H), 3.66 (dd, J = 12.4, 4.0 Hz, 1 H), 3.60-3.59 (m, 4 H), 3.55 (s, 2 H), 3.47 (dd, J = 12.4, 6.0 Hz, 1 H), 3.21-3.17 (m, 1 H), 2.43-2.35 (m, 4 H), 1.34 (d, J = 6.8 Hz, 3 H). ¹³C NMR (125 MHz, DMSO-d₆) δ 165.6, 147.7, 143.6, 143.3, 141.8, 140.2, 140.0, 133.1, 132.2, 129.3 (q, J = 31.3 Hz), 127.3, 126.1, 125.9, 124.2 (q, J = 270.9 Hz), 123.8, 120.0, 115.1, 66.2, 61.8, 53.1, 50.1, 48.0, 32.2, 19.1. HRMS (ESI) for C₂₇H₂₈F₃N₅O₂ [M + H]⁺, calcd: 512.2268, Found: 512.2261. Purity 96.4% (tR = 6.20 min).

(R, S)-4-methyl-2-(pyrimidin-5-yl)-N-(3-(thiomorpholinomethyl)-5-(trifluoromethyl)phenyl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7z).

Compound 7z was prepared by following a similar procedure as that of 7d. Yield, 79%. ¹H NMR (400 MHz, DMSO-d₆) δ 10.51 (s, 1 H), 8.62-8.53 (m, 3 H), 8.19 (s, 1 H), 8.01 (s, 1 H), 7.86-7.84 (m, 2 H), 7.47 (d, J = 8.0 Hz, 1 H), 7.35 (s, 1 H), 4.65 (d, J = 16.4 Hz, 1 H), 4.53 (d, J = 16.0 Hz, 1

H), 3.66 (dd, J = 12.4, 4.4 Hz, 1 H), 3.60-3.59 (m, 2 H), 3.47 (dd, J = 12.4, 6.0 Hz, 1 H), 3.19-3.18 (m, 1 H), 2.65-2.64 (m, 8 H), 1.34 (d, J = 6.4 Hz, 3 H). ¹³C NMR (125 MHz, DMSO-d₆) δ 165.6, 147.7, 143.5, 143.3, 141.8, 140.5, 140.0, 133.1, 132.2, 129.2 (q, J = 31.3 Hz), 127.2, 126.0, 125.8, 124.1 (q, J = 270.9 Hz), 123.6, 119.8, 114.9, 61.8, 54.3, 50.1, 48.0, 32.1, 27.1, 19.1. HRMS (ESI) for C₂₈H₃₀F₃N₅O [M + H]⁺, calcd: 528.2039, Found: 528.2037. Purity 95.9% (tR = 9.27 min).

(R, S)-4-methyl-N-(3-(piperidin-1-ylmethyl)-5-(trifluoromethyl)phenyl)-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7aa).

Compound 7aa was prepared by following a similar procedure as that of 7d. Yield, 82%. ¹H NMR (400 MHz, DMSO-d₆) δ 10.50 (s, 1 H), 8.61-8.54 (m, 3 H), 8.18 (s, 1 H), 7.99 (s, 1 H), 7.86-7.84 (m, 2 H), 7.47 (d, J = 7.6 Hz, 1 H), 7.34 (s, 1 H), 4.65 (d, J = 16.0 Hz, 1 H), 4.53 (d, J = 16.0 Hz, 1 H), 3.66 (dd, J = 12.0, 4.0 Hz, 1 H), 3.50 (s, 2 H), 3.48-3.45 (m, 1 H), 3.19-3.18 (m, 1 H), 2.40-2.30 (m, 4 H), 1.51-1.50 (m, 4 H), 1.45-1.37 (m, 2 H), 1.34 (d, J = 6.8 Hz, 3 H). ¹³C NMR (125 MHz, DMSO-d₆) δ 165.6, 147.7, 143.6, 143.3, 141.8, 141.1, 140.0, 133.1, 132.2, 129.2 (q, J = 31.1 Hz), 127.3, 126.1, 125.9, 124.2 (q, J = 270.9 Hz), 123.7, 119.8, 114.9, 62.2, 53.9, 50.1, 48.0, 32.2, 25.5, 23.9, 19.2. HRMS (ESI) for C₂₈H₃₀F₃N₅O [M + H]⁺, calcd: 510.2475, Found: 510.2470. Purity 96.5% (tR = 11.56 min).

(R, S)-4-methyl-2-(pyrimidin-5-yl)-N-(3-(pyrrolidin-1-ylmethyl)-5-(trifluoromethyl)phenyl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7ab).

Compound 7ab was prepared by following a similar procedure as that of 7d. Yield, 82%. ¹H NMR (400 MHz, DMSO-d₆) δ 10.48 (s, 1 H), 8.60-8.55 (m, 3 H), 8.18 (s, 1 H), 8.02 (s, 1 H), 7.87-7.85 (m, 2 H), 7.47 (d, J = 8.0 Hz, 1 H), 7.35 (s, 1 H), 4.65 (d, J = 16.4 Hz, 1 H), 4.53 (d, J = 16.4 Hz, 1 H), 3.68-3.64 (m, 3 H), 3.49-3.45 (m, 1 H), 3.21-3.16 (m, 1 H), 2.50-2.47 (m, 4 H), 1.76-1.70 (m, 4 H), 1.34 (d, J = 6.8 Hz, 3 H). ¹³C NMR (125 MHz, DMSO-d₆) δ 166.5, 148.6, 144.5, 144.2, 142.7 (d, J = 5.0 Hz), 140.9, 134.0, 133.1, 130.1 (q, J = 31.1 Hz), 128.1, 127.0, 126.8, 125.1 (q, J = 270.7 Hz), 124.3, 120.4 (q, J = 3.5 Hz), 115.7 (d, J = 3.8 Hz), 60.0, 54.4, 51.1, 48.9, 33.1, 24.1, 20.0. HRMS (ESI) for C₂₇H₂₈F₃N₅O [M + H]⁺, calcd: 496.2318, Found: 496.2310. Purity 96.6% (tR = 8.77 min).

(R, S)-N-(3-((dimethylamino)methyl)-5-(trifluoromethyl)phenyl)-4-methyl-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7ac).

Compound 7ac was prepared by following a similar procedure as that of 7d. Yield, 86%. ¹H NMR (400 MHz, DMSO-d₆) δ 10.48 (s, 1 H), 8.58-8.57 (m, 3 H), 8.18 (s, 1 H), 8.02 (s, 1 H), 7.87-7.85 (m, 2 H), 7.47 (d, J = 8.0 Hz, 1 H), 7.35 (s, 1 H), 4.65 (d, J = 16.4 Hz, 1 H), 4.53 (d, J = 16.4 Hz, 1 H), 3.66 (dd, J = 12.4, 4.4 Hz, 1 H), 3.50-3.45 (m, 3 H), 3.21-3.17 (m, 1 H), 2.19 (s, 6 H), 1.34 (d, J = 7.2 Hz, 3 H). ¹³C NMR (125 MHz, DMSO-d₆) δ 165.7, 147.7, 143.7, 143.4, 141.8, 141.4, 140.0, 133.2, 132.2, 129.3 (q, J = 31.5 Hz), 127.4, 126.1, 125.9, 124.2 (q, J = 271.0 Hz), 123.7, 119.9, 115.0, 62.8, 50.2, 48.0, 45.0, 32.2, 19.2. HRMS (ESI) for C₂₅H₂₆F₃N₅O [M + H]⁺, calcd: 470.2162, Found: 470.2155. Purity 97.8% (tR = 6.66 min).

(R, S)-4-methyl-N-(3-((4-methyl-1,4-diazepan-1-yl)methyl)-5-(trifluoromethyl)phenyl)-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7ad).

Compound 7ad was prepared by following a similar procedure as that of 7d. Yield, 76%.

Preparation HPLC: CHIRALPAK AS-H (0.46 cm I.D. × 15 cm L), MeOH/DEA=100/0.1 (V/V), 35 °C, 1.0 ml/min. The absolute chirality of 7ae and 7af was analogous to 7b based on their optical rotation values.

(R)-4-methyl-N-(3-((4-methyl-1,4-diazepan-1-yl)methyl)-5-(trifluoromethyl)phenyl)-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7ae).

$[\alpha]_D^{25}$ -20.0 (c 0.100, MeOH). ¹H NMR (500 MHz, DMSO-d₆) δ 10.50 (s, 1 H), 8.58 (s, 3 H), 8.16 (s, 1 H), 8.03 (s, 1 H), 7.86-7.84 (m, 2 H), 7.47 (d, J = 7.5 Hz, 1 H), 7.37 (s, 1 H), 4.66 (d, J = 16.0 Hz, 1 H), 4.53 (d, J = 16.0 Hz, 1 H), 3.69-3.65 (m, 3 H), 3.49-3.45 (m, 1 H), 3.19-3.18 (m, 1 H), 2.66-2.65 (m, 4 H), 2.57-2.52 (m, 4 H), 2.25 (s, 3 H), 1.75-1.70 (m, 2 H), 1.34 (d, J = 6.0 Hz, 3 H). ¹³C NMR (125 MHz, DMSO-d₆) δ 166.6, 148.7, 144.6, 144.3, 143.0, 142.8, 140.9, 134.1, 133.2, 130.2 (q, J = 30.9 Hz), 128.3, 127.1, 126.9, 125.2 (q, J = 270.7 Hz), 124.4, 120.6, 115.8, 62.2, 58.2, 57.2, 55.3, 54.7, 51.1, 49.0, 47.5, 33.1, 27.9, 20.1. HRMS (ESI) for C₂₉H₃₃F₃N₆O [M + H]⁺, calcd: 539.2741, Found: 539.2734. Purity 98.9% (t_R = 19.42 min). Analytical chiral HPLC (CHIRALPAK AS-H, MeOH/DEA=100/0.1(V/V), 1.0 ml/min), >99%, t_R = 6.35 min.

(S)-4-methyl-N-(3-((4-methyl-1,4-diazepan-1-yl)methyl)-5-(trifluoromethyl)phenyl)-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7af).

$[\alpha]_D^{25}$ +22.4 (c 0.098, MeOH). ¹H NMR (500 MHz, DMSO-d₆) δ 10.51 (s, 1 H), 8.59-8.58 (s, 3 H), 8.17 (s, 1 H), 8.03 (s, 1 H), 7.87-7.85 (m, 2 H), 7.48 (d, J = 8.0 Hz, 1 H), 7.38 (s, 1 H), 4.66 (d, J = 16.0 Hz, 1 H), 4.53 (d, J = 16.0 Hz, 1 H), 3.70-3.65 (m, 3 H), 3.50-3.46 (m, 1 H), 3.20-3.19 (m, 1 H), 2.68-2.65 (m, 4 H), 2.58-2.51 (m, 4 H), 2.25 (s, 3 H), 1.75-1.72 (m, 2 H), 1.35 (d, J = 7.0 Hz, 3 H). ¹³C NMR (125 MHz, DMSO-d₆) δ 166.6, 148.7, 144.5, 144.3, 143.0, 142.8, 140.9, 134.3, 133.2, 130.1 (q, J = 30.8 Hz), 128.2, 127.1, 126.8, 125.5 (q, J = 270.4 Hz), 124.4, 120.5, 115.8, 62.2, 58.2, 57.2, 55.2, 54.7, 51.1, 48.9, 47.5, 33.1, 27.9, 20.1. HRMS (ESI) for C₂₉H₃₃F₃N₆O [M + H]⁺, calcd: 539.2741, Found: 539.2734. Purity 98.7% (t_R = 19.33 min). Analytical chiral HPLC (CHIRALPAK AS-H, MeOH/DEA=100/0.1(V/V), 1.0 ml/min), >97%, t_R = 3.47 min.

Methyl (R, S)-4-(2-(2, 2, 2-trifluoroacetamido)propyl)benzoate (14f).

Methyl (R, S)-4-(2-aminopropyl)benzoate (10 g, 52 mmol) was added portionwise to the well stirred trifluoroacetic anhydride (50 ml). The reaction mixture was stirred at room temperature for 3 hours. After the reaction was finished as determined by TLC, the reaction mixture was poured into 100 ml of ice water, and stirred for 30 mins. The resulting solid was filtered, washed with water, and dried under vacuum to give the pure compound 14f (10 g, 63% yield). ¹H NMR (400 MHz, DMSO-d₆) δ 9.31 (d, J = 8.4 Hz, 1 H), 7.87 (d, J = 8.0 Hz, 2 H), 7.34 (d, J = 8.0 Hz, 2 H), 4.14-4.07 (m, 1 H), 3.83 (s, 3 H), 2.85 (d, J = 7.2 Hz, 2 H), 1.17 (d, J = 6.8 Hz, 3 H).

Methyl (R, S)-3-methyl-2-(2, 2, 2-trifluoroacetyl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxylate (15f).

Methyl (R, S)-4-(2-(2, 2, 2-trifluoroacetamido)propyl)benzoate (9.7 g, 33.5 mmol) was stirred at room temperature with (HCHO)_n (4.5 g) and conc. H₂SO₄ for 5 hours. The clear solution was added to cold water and extracted with ethyl acetate. The organic layer was washed with saturated

Na₂CO₃, water, and dried over anhydrous Na₂SO₄. The filtrate was concentrated under vacuum to give the title compound 15f (5.8 g, 58% yield). ¹H NMR (400 MHz, DMSO-d₆) δ 7.93-7.80 (m, 2 H), 7.40-7.34 (m, 1 H), 5.13-4.43 (m, 3 H), 3.85 (s, 3 H), 3.19-3.15 (m, 1 H), 2.89-2.79 (m, 1 H), 1.15-1.11 (m, 3 H).

Methyl (R, S)-3-methyl-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxylate hydrochloride (16f).

Methyl (R, S)-3-methyl-2-(2, 2, 2-trifluoroacetyl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxylate (9.0 g, 29.8 mmol) was added to K₂CO₃ (6.2 g, 44.7 mmol) in methanol/water (2:1), and stirred at room temperature for 3 hours. Methanol was removed from the reaction mixture and water was added, extracted with ethyl acetate, followed by washing with water. The organic layer was dried over anhydrous Na₂SO₄, and evaporated under reduced pressure to give the compound as colorless oil. Then the oil was diluted with methanol, and HCl solution in methanol was added dropwise. The mixture was stirred for 1 hour, and the white solid was collected, and dried under reduced pressure to give the title compound (6.5 g, 90% yield). ¹H NMR (400 MHz, DMSO-d₆) δ 9.78 (s, 1 H), 9.52 (s, 1 H), 7.87 (s, 1 H), 7.83 (d, J = 8.0 Hz, 1 H), 7.35 (d, J = 8.0 Hz, 1 H), 4.43-4.31 (m, 2 H), 3.85 (s, 3 H), 3.61-3.50 (m, 1 H), 3.13 (dd, J = 15.6, 4.4 Hz, 1 H), 2.94-2.87 (m, 1 H), 1.39 (d, J = 6.4 Hz, 3 H).

Methyl (R, S)-3-methyl-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxylate (17f). Compound 17f was prepared by following a similar procedure as that of 12. Yield, 37%. ¹H NMR (400 MHz, CDCl₃) δ 8.66 (s, 1 H), 8.39 (s, 2 H), 7.91-7.89 (m, 2 H), 7.28-7.26 (m, 1 H), 4.59 (d, J = 15.6 Hz, 1 H), 4.40-4.33 (m, 2 H), 3.31-3.26 (m, 1 H), 2.80 (d, J = 8.0 Hz, 1 H), 1.25 (d, J = 7.2 Hz, 3 H).

(R, S)-3-methyl-N-(3-((4-methylpiperazin-1-yl)methyl)-5-(trifluoromethyl)phenyl)-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7f).

Compound 7f was prepared by following a similar procedure as that of 7d. Yield, 71%. ¹H NMR (500 MHz, DMSO-d₆) δ 10.52 (s, 1 H), 8.56-8.54 (m, 3 H), 8.19 (s, 1 H), 8.00 (s, 1 H), 7.90 (s, 1 H), 7.85 (d, J = 7.5 Hz, 1 H), 7.41 (d, J = 7.5 Hz, 1 H), 7.35 (s, 1 H), 4.72 (d, J = 16.5 Hz, 1 H), 4.60-4.50 (m, 1 H), 4.33 (d, J = 16.5 Hz, 1 H), 3.54 (s, 2 H), 3.25-3.22 (m, 1 H), 2.86-2.83 (m, 1 H), 2.46-2.21 (m, 8 H), 2.15 (s, 3 H), 0.99 (d, J = 6.0 Hz, 3 H). ¹³C NMR (125 MHz, DMSO-d₆) δ 165.9, 147.5, 142.2, 141.6, 140.8, 140.1, 137.1, 132.9, 132.3, 129.5, 129.2, 126.1, 126.0, 124.3 (q, J = 270.8 Hz), 123.9, 120.1, 115.1, 61.5, 54.7, 52.5, 46.4, 45.7, 46.4, 44.2, 34.5, 15.5. HRMS (ESI) for C₂₈H₃₁F₃N₆O [M + H]⁺, calcd: 525.2584, Found: 525.2576. Purity 97.4% (t_R = 6.78 min).

Methyl 4-(2-methyl-1-(2, 2, 2-trifluoroacetamido)propan-2-yl)benzoate (14g).

Compound 14g was prepared by following a similar procedure as that of 14f. Yield, 68%. ¹H NMR (400 MHz, DMSO-d₆) δ 9.31-9.28 (m, 1 H), 7.89 (d, J = 7.6 Hz, 2 H), 7.53 (d, J = 7.6 Hz, 2 H), 3.84 (s, 3 H), 3.41-3.39 (m, 2 H), 1.29 (s, 6 H).

Methyl 4, 4-dimethyl-2-(2, 2, 2-trifluoroacetyl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxylate (15g).

Compound 15g was prepared by following a similar procedure as that of 15f. Yield, 76%. ¹H NMR (400 MHz, DMSO-d₆) δ 7.88-7.81 (m, 2 H), 7.60 (dd, J = 8.4, 3.2 Hz, 1 H), 4.91 (s, 1 H),

4.84 (s, 1 H), 3.84 (s, 3 H), 3.69 (s, 1 H), 3.60 (s, 1 H), 1.27 (s, 3 H), 1.25 (s, 3 H).

Methyl 4, 4-dimethyl-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxylate hydrochloride (16g).

Compound 16g was prepared by following a similar procedure as that of 16f. Yield, 92%. ¹H NMR (400 MHz, DMSO-d₆) δ 9.73 (s, 2 H), 7.87-7.85 (m, 2 H), 7.64 (d, J = 8.0 Hz, 1 H), 4.32 (s, 2 H), 3.85 (s, 3 H), 3.22 (s, 2 H), 1.37 (s, 6 H).

Methyl 4, 4-dimethyl-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxylate (17g).

Compound 17g was prepared by following a similar procedure as that of 17f. Yield, 73%. ¹H NMR (400 MHz, DMSO-d₆) δ 8.59 (s, 2 H), 8.57 (s, 1 H), 7.84-7.78 (m, 2 H), 7.60 (s, 1 H), 4.57 (s, 2 H), 3.85 (s, 3 H), 3.43 (s, 2 H), 1.33 (s, 6 H).

4, 4-dimethyl-N-(3-((4-methylpiperazin-1-yl)methyl)-5-(trifluoromethyl)phenyl)-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline-7-carboxamide (7g).

Compound 7g was prepared by following a similar procedure as that of 7d. Yield, 78%. ¹H NMR (400 MHz, DMSO-d₆) δ 10.37 (s, 1 H), 8.65-8.53 (s, 3 H), 8.18 (s, 1 H), 8.00 (s, 1 H), 7.90-7.77 (m, 2 H), 7.60 (d, J = 6.8 Hz, 1 H), 7.34 (s, 1 H), 4.58 (s, 2 H), 3.53 (s, 2 H), 3.45 (s, 2 H), 2.39 (br s, 8 H), 2.15 (s, 3 H), 1.35 (s, 6 H). ¹³C NMR (125 MHz, DMSO-d₆) δ 165.6, 147.8, 147.3, 143.4, 141.7, 140.7, 140.0, 132.2, 132.1, 129.2 (q, J = 31.4 Hz), 126.1, 125.9, 125.5, 124.2 (q, J = 270.7 Hz), 123.6, 119.8, 114.9, 61.4, 55.9, 54.6, 52.5, 48.4, 45.7, 35.3, 27.2. HRMS (ESI) for C₂₉H₃₃F₃N₆O [M + H]⁺, calcd: 539.2741, Found: 539.2735. Purity 99.7% (t_R = 8.09 min).

(R, S)-2, 2, 2-trifluoro-N-(2-phenylpropyl)acetamide (19).

Compound 19 was prepared by following a similar procedure as that of 14. Yield, 71%. ¹H NMR (400 MHz, DMSO-d₆) δ 9.43 (s, 1 H), 7.32-7.28 (m, 2 H), 7.23-7.18 (m, 3 H), 3.39-3.27 (m, 2 H), 3.04-2.96 (m, 1 H), 1.19 (d, J = 6.8 Hz, 3 H).

(R, S)-2, 2, 2-trifluoro-N-(2-(4-nitrophenyl)propyl)acetamide (20).

To a solution of (R, S)-2,2,2-trifluoro-N-(2-phenylpropyl)acetamide (12 g, 52 mmol) in acetic anhydride (50 ml) was added a solution of HNO₃ (10.8 ml, 156 mmol) in acetic anhydride (50 ml) dropwise at 0 °C. The mixture was warmed to rt slowly and stirred for another 4 hours. After completion of the reaction, the mixture was poured into ice-water. The precipitate was collected to give the title compound 20 (9 g, 63%). ¹H NMR (400 MHz, DMSO-d₆) δ 9.47 (s, 1 H), 8.17 (d, J = 6.8 Hz, 2 H), 7.53 (d, J = 6.8 Hz, 2 H), 3.46-3.38 (m, 2 H), 3.21-3.15 (m, 1 H), 1.24 (d, J = 5.6 Hz, 3 H).

(R, S)-2, 2, 2-trifluoro-1-(4-methyl-7-nitro-3,4-dihydroisoquinolin-2(1H)-yl)ethan-1-one (21).

Compound 21 was prepared by following a similar procedure as that of 15. Yield, 44%. ¹H NMR (400 MHz, DMSO-d₆) δ 8.28-8.22 (m, 1 H), 8.10-8.06 (m, 1 H), 7.57 (d, J = 8.4 Hz, 1 H), 5.03-4.97 (m, 1 H), 4.88-4.72 (m, 1 H), 3.86-3.76 (m, 1 H), 3.70-3.66 (m, 1 H), 3.23-3.22 (m, 1 H), 1.24-1.20 (m, 3 H).

(R, S)-4-methyl-7-nitro-1, 2, 3, 4-tetrahydroisoquinoline hydrochloride (22).

Compound 22 was prepared by following a similar procedure as that of 16. Yield, 70%. ¹H NMR (400 MHz, DMSO-d₆) δ 9.91 (br s, 1 H), 9.71 (br s, 1 H), 8.21 (s, 1 H), 8.13 (d, J = 8.4 Hz, 1 H), 7.67 (d, J = 8.4 Hz, 1 H), 4.38 (s, 2 H), 3.51-3.48 (m, 1 H), 3.37-3.32 (m, 1 H), 3.10-3.02 (m, 1 H),

1.36 (d, J = 6.8 Hz, 3 H).

(R, S)-4-methyl-7-nitro-2-(pyrimidin-5-yl)-1,2,3,4-tetrahydroisoquinoline (23).

Compound 23 was prepared by following a similar procedure as that of 17. Yield, 57%. ¹H NMR (400 MHz, DMSO-d₆), δ 8.57 (s, 3 H), 8.15 (s, 1 H), 8.08 (d, J = 8.4 Hz, 1 H), 7.59 (d, J = 8.4 Hz, 1 H), 4.71 (d, J = 16.4 Hz, 1 H), 4.57 (d, J = 16.8 Hz, 1 H), 3.67-3.63 (m, 1 H), 3.50-3.46 (m, 1 H), 3.25-3.21 (m, 1 H), 1.34 (d, J = 6.8 Hz, 3 H).

(R, S)-4-methyl-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinolin-7-amine (24).

To a solution of (R, S)-4-methyl-7-nitro-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinoline (678 mg, 2.5 mmol) in methanol was added Pd/C. The mixture was filled with H₂ and stirred at rt for 3 hours. After completion of the reaction, the reaction mixture was filtered through a pad of Celite and concentrated under vacuum to yield the title compound 24 (589 mg, 98% yield). ¹H NMR (400 MHz, DMSO-d₆), δ 8.52-8.50 (m, 3 H), 6.92 (d, J = 8.0 Hz, 1 H), 6.44 (d, J = 8.0 Hz, 1 H), 6.0 (s, 1 H), 4.92 (s, 2 H), 4.36 (d, J = 16.0 Hz, 1 H), 4.28 (d, J = 16.0 Hz, 1 H), 3.58-3.54 (m, 1 H), 3.30-3.25 (m, 1 H), 2.93-2.90 (m, 1 H), 1.20 (d, J = 6.8 Hz, 3 H).

(R, S)-N-(4-methyl-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinolin-7-yl)-3-((4-methylpiperazin-1-yl)methyl)-5-(trifluoromethyl)benzamide (a racemic mixture of 7h and 7i).

To a solution of 3-((4-methylpiperazin-1-yl)methyl)-5-(trifluoromethyl)benzoic acid (831 mg, 2.8 mmol) in 20 mL of dichloromethane, (R, S)-4-methyl-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinolin-7-amine (589 mg, 2.5 mmol), (1-[bis(dimethylamino)methylene]-1H-1, 2, 3-triazolo[4, 5-b]pyridinium 3-oxid hexafluorophosphate) (HATU) (1.4 g, 3.8 mmol), and N, N-diisopropylethylamine (DIPEA) (0.9 mL, 5 mmol) were added. The resulting mixture was stirred at rt overnight. The reaction was quenched with water and extracted with dichloromethane. The combined organic layer was dried over anhydrous sodium sulfate, concentrated under vacuum, and then purified by column chromatography over silica gel to afford a racemic mixture of 7h and 7i (839 mg, 64% yield). Preparation HPLC: CHIRALCEL OJ-H (0.46 cm I.D. × 15 cm L), Hexane/iPrOH/TEA=90/10/0.1 (V:V:V), 35 °C, 1.0 ml/min. The absolute chirality of 7h and 7i was determined based on DDR1-7h co-crystal structure.

(R)-N-(4-methyl-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinolin-7-yl)-3-((4-methylpiperazin-1-yl)methyl)-5-(trifluoromethyl)benzamide (7h).

$[\alpha]_D^{25}$ -20.2 (c 0.106, MeOH). ¹H NMR (400 MHz, DMSO-d₆) δ 10.44 (s, 1 H), 8.57 (s, 2 H), 8.55 (s, 1 H), 8.19 (s, 1 H), 8.17 (s, 1 H), 7.84 (s, 1 H), 7.69 (s, 1 H), 7.58 (d, J = 8.0 Hz, 1 H), 7.29 (d, J = 8.0 Hz, 1 H), 4.55 (d, J = 16.0 Hz, 1 H), 4.45 (d, J = 16.0 Hz, 1 H), 3.68-3.59 (m, 3 H), 3.42-3.38 (m, 1 H), 3.08-3.07 (m, 1 H), 2.41 (br s, 4 H), 2.33 (br s, 4 H), 2.15 (s, 3 H), 1.30 (d, J = 6.8 Hz, 3 H). ¹³C NMR (125 MHz, DMSO-d₆) δ 163.9, 147.6, 143.4, 141.8, 140.7, 136.7, 135.8, 135.2, 133.1, 131.9, 129.2 (q, J = 31.8 Hz), 127.9, 127.3, 124.0 (q, J = 271.4 Hz), 122.8, 119.3, 118.4, 60.9, 54.6, 52.4, 50.5, 48.2, 45.6, 31.6, 19.2. HRMS (ESI) for C₂₈H₃₁F₃N₆O [M + H]⁺, calcd: 525.2584, Found: 525.2578. Purity 98.3% (t_R = 7.91 min). Analytical chiral HPLC (OJ-H, Hexane/iPrOH/TEA=90/10/0.1 (V:V:V), 1.0 ml/min), >99%, t_R = 41.91 min.

(S)-N-(4-methyl-2-(pyrimidin-5-yl)-1,2,3,4-tetrahydroisoquinolin-7-yl)-3-((4-methylpiperaz

in-1-yl)methyl)-5-(trifluoromethyl)benzamide (7i).

$[\alpha]_D^{25} +23.0$ (c 0.114, MeOH). $^1\text{H NMR}$ (400 MHz, DMSO- d_6) δ 10.45 (s, 1 H), 8.57 (s, 2 H), 8.55 (s, 1 H), 8.19 (s, 1 H), 8.17 (s, 1 H), 7.85 (s, 1 H), 7.69 (s, 1 H), 7.58 (d, $J = 8.4$ Hz, 1 H), 7.30 (d, $J = 8.4$ Hz, 1 H), 4.55 (d, $J = 16.0$ Hz, 1 H), 4.45 (d, $J = 16.0$ Hz, 1 H), 3.68-3.60 (m, 3 H), 3.43-3.38 (m, 1 H), 3.08 (m, 1 H), 2.41 (br s, 4 H), 2.33 (br s, 4 H), 2.15 (s, 3 H), 1.30 (d, $J = 6.8$ Hz, 3 H). $^{13}\text{C NMR}$ (125 MHz, DMSO- d_6) δ 163.9, 147.6, 143.4, 141.8, 140.7, 136.7, 135.8, 135.2, 133.1, 131.9, 129.2 (q, $J = 31.9$ Hz), 127.9, 127.3, 124.0 (q, $J = 271.1$ Hz), 122.9, 119.3, 118.4, 60.9, 54.6, 52.5, 50.5, 48.2, 45.7, 31.6, 19.2. HRMS (ESI) for $\text{C}_{28}\text{H}_{31}\text{F}_3\text{N}_6\text{O}$ $[\text{M} + \text{H}]^+$, calcd: 525.2584, Found: 525.2578. Purity 98.6% ($t_R = 7.90$ min). Analytical chiral HPLC (OJ-H, Hexane /iPrOH /TEA=90/10/0.1 (V:V:V), 1.0 ml/min), >99%, $t_R = 31.09$ min.

(R, S)-1-(4-methyl-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinolin-7-yl)-3-(3-((4-methylpiperazin-1-yl)methyl)-5-(trifluoromethyl)phenyl)urea (7j).

To solution of 3-((4-methylpiperazin-1-yl)methyl)-5-(trifluoromethyl)aniline (300 mg, 1.1 mmol) in THF at 0 °C was added dropwise a solution of triphosgene (326 mg, 1.1 mmol) in THF and followed by added Et₃N (0.4 ml, 2.3 mmol) dropwise. The mixture was stirred at rt for 2 hours. Then the solvent was removed under reduced pressure. The mixture was redissolved in THF and (R, S)-4-methyl-2-(pyrimidin-5-yl)-1, 2, 3, 4-tetrahydroisoquinolin-7-amine (264 mg, 1.1 mmol) was added. The reaction mixture was stirred at 45 °C for 2 hours. After completion of the reaction, the solvent was removed under reduced pressure. Ethyl acetate and water were added to the mixture. The organic layer was separated and dried over anhydrous sodium sulfate, concentrated under vacuum, and purified by column chromatography over silica gel to afford 7j (356 mg, 60% yield). $^1\text{H NMR}$ (500 MHz, DMSO- d_6) δ 9.06 (s, 1 H), 8.72 (s, 1 H), 8.59-8.51 (m, 3 H), 7.93 (s, 1 H), 7.51 (s, 1 H), 7.45 (s, 1 H), 7.25-7.20 (m, 3 H), 4.52 (d, $J = 16.0$ Hz, 1 H), 4.42 (d, $J = 16.0$ Hz, 1 H), 3.62 (dd, $J = 12.0, 3.5$ Hz, 1 H), 3.50 (s, 2 H), 3.37 (dd, $J = 12.0, 6.0$ Hz, 1 H), 3.04-3.03 (m, 1 H), 2.47-2.18 (m, 8 H), 2.15 (s, 3 H), 1.27 (d, $J = 6.5$ Hz, 3 H). $^{13}\text{C NMR}$ (125 MHz, DMSO- d_6) δ 152.8, 147.6, 143.7, 141.9, 140.9, 140.8, 137.4, 133.7, 133.4, 129.7 (q, $J = 31.3$ Hz), 127.6, 124.4 (q, $J = 270.7$ Hz), 122.0, 118.4, 117.7, 116.6, 113.1, 61.5, 54.8, 52.6, 50.8, 48.4, 45.8, 31.6, 19.5. HRMS (ESI) for $\text{C}_{28}\text{H}_{32}\text{F}_3\text{N}_7\text{O}$ $[\text{M} + \text{H}]^+$, calcd: 540.2693, Found: 540.2690. Purity 99.4% ($t_R = 7.46$ min).

The results of the selectivity profiling study of compound 7ae

Gene Symbol	%Ctrl @ 1000nM	Gene Symbol	%Ctrl @ 1000nM
AAK1	90	BRK	92
ABL1(E255K)-phosphorylated	83	BRSK1	98
ABL1(F317I)-nonphosphorylated	73	BRSK2	85
ABL1(F317I)-phosphorylated	58	BTK	98
ABL1(F317L)-nonphosphorylated	17	BUB1	84
ABL1(F317L)-phosphorylated	58	CAMK1	82
ABL1(H396P)-nonphosphorylated	70	CAMK1B	100
ABL1(H396P)-phosphorylated	83	CAMK1D	91
ABL1(M351T)-phosphorylated	59	CAMK1G	95
ABL1(Q252H)-nonphosphorylated	12	CAMK2A	97
ABL1(Q252H)-phosphorylated	75	CAMK2B	94
ABL1(T315I)-nonphosphorylated	93	CAMK2D	85
ABL1(T315I)-phosphorylated	85	CAMK2G	88
ABL1(Y253F)-phosphorylated	66	CAMK4	77
ABL1-nonphosphorylated	3.3	CAMKK1	93
ABL1-phosphorylated	64	CAMKK2	88
ABL2	32	CASK	86
ACVR1	94	CDC2L1	94
ACVR1B	84	CDC2L2	99
ACVR2A	100	CDC2L5	70
ACVR2B	100	CDK11	9.4
ACVRL1	81	CDK2	97
ADCK3	88	CDK3	100
ADCK4	95	CDK4	100
AKT1	100	CDK4-cyclinD1	94
AKT2	93	CDK4-cyclinD3	99
AKT3	87	CDK5	98
ALK	100	CDK7	70
ALK(C1156Y)	89	CDK8	16
ALK(L1196M)	83	CDK9	82
AMPK-alpha1	97	CDKL1	84
AMPK-alpha2	96	CDKL2	100
ANKK1	84	CDKL3	75
ARK5	97	CDKL5	93
ASK1	100	CHEK1	96
ASK2	87	CHEK2	96
AURKA	100	CIT	94
AURKB	90	CLK1	91
AURKC	77	CLK2	83
AXL	95	CLK3	87
BIKE	81	CLK4	79
BLK	96	CSF1R	80
BMPR1A	93	CSF1R-autoinhibited	98
BMPR1B	95	CSK	85
BMPR2	100	CSNK1A1	93
BMX	83	CSNK1A1L	92
BRAF	86	CSNK1D	94
BRAF(V600E)	53	CSNK1E	98
		CSNK1G1	99

Gene Symbol	%Ctrl @ 1000nM
CSNK1G2	89
CSNK1G3	91
CSNK2A1	89
CSNK2A2	100
CTK	95
DAPK1	97
DAPK2	85
DAPK3	100
DCAMKL1	73
DCAMKL2	93
DCAMKL3	84
DDR1	0.1
DDR2	0
DLK	100
DMPK	98
DMPK2	98
DRAK1	88
DRAK2	100
DYRK1A	96
DYRK1B	62
DYRK2	83
EGFR	92
EGFR(E746-A750del)	94
EGFR(G719C)	85
EGFR(G719S)	94
EGFR(L747-E749del, A750P)	88
EGFR(L747-S752del, P753S)	92
EGFR(L747-T751del,Sins)	77
EGFR(L858R)	99
EGFR(L858R,T790M)	96
EGFR(L861Q)	77
EGFR(S752-I759del)	88
EGFR(T790M)	79
EIF2AK1	90
EPHA1	84
EPHA2	14
EPHA3	57
EPHA4	35
EPHA5	55
EPHA6	33
EPHA7	11
EPHA8	0.8
EPHB1	57
EPHB2	20
EPHB3	80
EPHB4	67
EPHB6	99
ERBB2	98
ERBB3	86

Gene Symbol	%Ctrl @ 1000nM
ERBB4	86
ERK1	99
ERK2	87
ERK3	96
ERK4	78
ERK5	98
ERK8	98
ERN1	75
FAK	100
FER	98
FES	100
FGFR1	81
FGFR2	81
FGFR3	95
FGFR3(G697C)	81
FGFR4	100
FGR	98
FLT1	95
FLT3	64
FLT3(D835H)	79
FLT3(D835V)	28
FLT3(D835Y)	51
FLT3(ITD)	51
FLT3(ITD,D835V)	16
FLT3(ITD,F691L)	81
FLT3(K663Q)	52
FLT3(N841I)	60
FLT3(R834Q)	88
FLT3-autoinhibited	73
FLT4	96
FRK	21
FYN	90
GAK	95
GCN2(Kin.Dom.2,S808G)	96
GRK1	72
GRK2	91
GRK3	96
GRK4	83
GRK7	93
GSK3A	91
GSK3B	90
HASPIN	100
HCK	72
HIPK1	83
HIPK2	91
HIPK3	100
HIPK4	92
HPK1	84
HUNK	0

Gene Symbol	%Ctrl @ 1000nM
ICK	96
IGF1R	100
IKK-alpha	95
IKK-beta	100
IKK-epsilon	93
INSR	94
INSRR	100
IRAK1	100
IRAK3	95
IRAK4	100
ITK	94
JAK1(JH1domain-catalytic)	98
JAK1(JH2domain-pseudokinase)	97
JAK2(JH1domain-catalytic)	99
JAK3(JH1domain-catalytic)	96
JNK1	76
JNK2	77
JNK3	92
KIT	89
KIT(A829P)	88
KIT(D816H)	86
KIT(D816V)	77
KIT(L576P)	90
KIT(V559D)	86
KIT(V559D,T670I)	92
KIT(V559D,V654A)	94
KIT-autoinhibited	98
LATS1	89
LATS2	100
LCK	36
LIMK1	96
LIMK2	100
LKB1	88
LOK	33
LRRK2	100
LRRK2(G2019S)	100
LTK	91
LYN	73
LZK	86
MAK	90
MAP3K1	98
MAP3K15	81
MAP3K2	79
MAP3K3	90
MAP3K4	96
MAP4K2	90
MAP4K3	97
MAP4K4	100
MAP4K5	98

Gene Symbol	%Ctrl @ 1000nM
MAPKAPK2	100
MAPKAPK5	93
MARK1	92
MARK2	100
MARK3	98
MARK4	94
MAST1	98
MEK1	100
MEK2	100
MEK3	76
MEK4	100
MEK5	87
MEK6	87
MELK	99
MERTK	97
MET	96
MET(M1250T)	94
MET(Y1235D)	84
MINK	86
MKK7	100
MKMK1	88
MKMK2	73
MLCK	89
MLK1	98
MLK2	64
MLK3	100
MRCKA	100
MRCKB	92
MST1	97
MST1R	93
MST2	69
MST3	100
MST4	96
MTOR	98
MUSK	20
MYLK	88
MYLK2	73
MYLK4	96
MYO3A	76
MYO3B	73
NDR1	86
NDR2	100
NEK1	99
NEK10	72
NEK11	100
NEK2	100
NEK3	84
NEK4	91
NEK5	98

Gene Symbol	%Ctrl @ 1000nM
NEK6	96
NEK7	100
NEK9	100
NIK	84
NIM1	98
NLK	92
OSR1	99
p38-alpha	82
p38-beta	88
p38-delta	92
p38-gamma	84
PAK1	100
PAK2	94
PAK3	94
PAK4	93
PAK6	88
PAK7	87
PCK1	96
PCK2	91
PCK3	97
PDGFRA	78
PDGFRB	83
PDPK1	83
PFCDPK1(P.falciparum)	94
PFPK5(P.falciparum)	93
PFTAIRE2	100
PFTK1	99
PHKG1	100
PHKG2	87
PIK3C2B	100
PIK3C2G	68
PIK3CA	93
PIK3CA(C420R)	78
PIK3CA(E542K)	88
PIK3CA(E545A)	89
PIK3CA(E545K)	89
PIK3CA(H1047L)	79
PIK3CA(H1047Y)	100
PIK3CA(I800L)	92
PIK3CA(M1043I)	99
PIK3CA(Q546K)	84
PIK3CB	90
PIK3CD	97
PIK3CG	100
PIK4CB	87
PIKFYVE	94
PIM1	97
PIM2	99
PIM3	95

Gene Symbol	%Ctrl @ 1000nM
PIP5K1A	87
PIP5K1C	92
PIP5K2B	67
PIP5K2C	93
PKAC-alpha	99
PKAC-beta	87
PKMYT1	83
PKN1	97
PKN2	90
PKNB(M.tuberculosis)	88
PLK1	100
PLK2	90
PLK3	95
PLK4	100
PRKCD	74
PRKCE	74
PRKCH	80
PRKCI	84
PRKCQ	62
PRKD1	87
PRKD2	96
PRKD3	99
PRKG1	100
PRKG2	79
PRKR	77
PRKX	91
PRP4	93
PYK2	98
QSK	90
RAF1	71
RET	97
RET(M918T)	83
RET(V804L)	92
RET(V804M)	85
RIOK1	78
RIOK2	36
RIOK3	70
RIPK1	100
RIPK2	100
RIPK4	86
RIPK5	96
ROCK1	100
ROCK2	100
ROS1	94
RPS6KA4(Kin.Dom.1-N-terminal)	93
RPS6KA4(Kin.Dom.2-C-terminal)	95
RPS6KA5(Kin.Dom.1-N-terminal)	90
RPS6KA5(Kin.Dom.2-C-terminal)	100
RSK1(Kin.Dom.1-N-terminal)	92

Gene Symbol	%Ctrl @ 1000nM
RSK1(Kin.Dom.2-C-terminal)	100
RSK2(Kin.Dom.1-N-terminal)	85
RSK2(Kin.Dom.2-C-terminal)	92
RSK3(Kin.Dom.1-N-terminal)	84
RSK3(Kin.Dom.2-C-terminal)	85
RSK4(Kin.Dom.1-N-terminal)	85
RSK4(Kin.Dom.2-C-terminal)	97
S6K1	96
SBK1	86
SGK	96
SgK110	100
SGK2	96
SGK3	78
SIK	85
SIK2	100
SLK	84
SNARK	88
SNRK	99
SRC	71
SRMS	84
SRPK1	93
SRPK2	84
SRPK3	98
STK16	93
STK33	100
STK35	97
STK36	100
STK39	87
SYK	89
TAK1	69
TAOK1	91
TAOK2	61
TAOK3	87
TBK1	92
TEC	95
TESK1	92
TGFBR1	82
TGFBR2	89
TIE1	68
TIE2	46
TLK1	100
TLK2	94
TNIK	99
TNK1	100
TNK2	97
TNNI3K	52
TRKA	1.5
TRKB	0.55
TRKC	0.6

Gene Symbol	%Ctrl @ 1000nM
TRPM6	96
TSSK1B	82
TSSK3	95
TTK	79
TXK	100
TYK2(JH1domain-catalytic)	95
TYK2(JH2domain-pseudokinase)	83
TYRO3	78
ULK1	76
ULK2	98
ULK3	90
VEGFR2	78
VPS34	81
VRK2	80
WEE1	100
WEE2	92
WNK1	100
WNK2	99
WNK3	99
WNK4	96
YANK1	90
YANK2	83
YANK3	85
YES	87
YSK1	98
YSK4	80
ZAK	32
ZAP70	83

Table S1. S-score table for **7ae**

Compound Name	Selectivity Score Type	Number of Hits	Number of Non-Mutant Kinases	Screening Concentration (nM)	Selectivity Score
7ae	S(35)	19	403	1000	0.047
7ae	S(10)	9	403	1000	0.022
7ae	S(1)	6	403	1000	0.015

Active-site dependent competition binding assay–Kinome^{scan} Screening

The binding affinity of **7ae** with DDR1 was analyzed by KINOME scan system conducted by Ambit Bioscience (San Diego, USA). Briefly, kinases were tagged with DNA. The ligands were biotinylated and immobilized to streptavidin-coated beads. The binding reactions were assembled by incubating DNA-tagged kinases, immobilized ligands, and test compounds in binding reactions (20% SeaBlock, 0.17 × PBS, 0.05% tween-20, 6 mM DTT) for 1.0 h at room temperature. The affinity beads were washed with washing buffer (1× PBS, 0.05% Tween-20) first and then elution buffer (1× PBS, 0.05% Tween 20, 0.5 μM nonbiotinylated affinity ligands). The kinase concentration in the eluate was determined by quantitative PCR of the DNA tagged to the kinase. The ability of the test compound to bind to the kinase was evaluated with percent control (%) as (test compound signal – positive control signal)/negative control signal – positive control signal) × 100%. Negative control is DMSO control (100% ctrl) and positive control is control compound (0% ctrl).

Table S2. Binding affinities or kinase inhibition of compound **7ae** against a panel of “off target” kinases.

Kinase	K _d value or IC ₅₀ (nM)	Kinase	K _d value or IC ₅₀ (nM)
Abl	8100 ^b	CDK11	400 ^a
DDR2	255 ^b	EPHA8	35 ^a
HUNK	>1000 ^a	TRKA	38 ^a (160) ^b
TRKB	2 ^a (53) ^b	TRKC	2.4 ^a (18) ^b

^aThe binding affinities (K_d) were determined by using DiscoverX's platform. ^b The kinase inhibitory activities (IC₅₀) were evaluated by using “in-house” kinase assays. Reported data are mean values from two independent experiments in which the variation is less than 20%.

***In vitro* kinase assay**

The functional assays of compounds on the kinase activities of c-Kit and Abl were determined using the FRET-based Z'-Lyte assay system according to the manufacturer's instructions (Invitrogen, USA). Tyrosine 2 peptide was used as Abl substrate, and Ser/Thr 6 peptide was used as the substrate for c-Kit. The reactions were carried out in 384-well plates in a 10 μ L of reaction volume with appropriate amount of kinases in 50 mM HEPES (pH 7.5), 10 mM MgCl₂, 1 mM EGTA, and 0.01% Brij-35. The reactions were incubated 1 h at room temperature in the presence of 2 μ M of substrate with 10 mM of ATP (for Abl1 assays) or 300 μ M of ATP (kit assay) and in the presence of various concentrations of the compounds. The development reagent was then added for a further 2 h room temperature incubation followed by the addition of stop solution. Fluorescence signal ratio of 445 nm (Coumarin)/520 nm (fluorescein) was examined on a EnVision Multilabel Reader (Perkin-Elmer, Inc.).

The effects of compounds on the kinases DDR1 and DDR2 were assessed by using a LanthaScreen Eu kinase activity assay technology (Invitrogen, USA). Kinase reactions were performed in a 10 μ L solution in low-volume 384-well plates. The kinase reaction buffer consisted of 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, and 1 mM EGTA; the concentration of Fluorescein-Poly GAT substrate (Invitrogen, USA) in the assay was 100 nM. Kinase reactions were initiated by the addition of 100 nM ATP in the presence of serially diluted compounds. The reactions were allowed to proceed for 1 h at room temperature before a 10 μ L preparation of EDTA (20 mM) and Eu-labeled antibody (4 nM) in TR-FRET dilution buffer were added. The final concentration of antibody in the assay well was 2 nM, and the final concentration of EDTA was 10 mM. The plate was allowed to incubate at room temperature for one more hour before the TR-FRET emission ratios of 665 nm/340 nm were acquired on a PerkinElmer EnVision multilabel reader (Perkin-Elmer, Inc.). Data analysis and curve fitting were performed using GraphPad Prism4 software.

Western blot analysis

Primary human lung fibroblast was cultured in Medium199 (Sigma Aldrich) containing 10% fetal bovine serum (FBS) and maintained at 37 °C in a humidified incubator with 5% CO₂ and 95% air. Cells were cultured in 100 mm tissue culture dishes in complete media (M199 with 10% FBS) until they reached a high density (~80% confluence). Then cells were starved for 4 hours in M199 with 1% FBS. After that cells were cultured in 5mL of complete media with 10 μ g/mL collagen and 4-fold dilution of **7ae** for 24 hours. Collagen, collagen with DMSO and collagen with 1 μ M **7rh** were added as controls. Cells were lysed, supernatants were recovered by centrifugation at 13,000 rpm, protein concentration was measured and equal amounts of total protein were separated by SDS-PAGE. Proteins were transferred to PVDF membranes (Bio-Rad; Hercules, CA) followed by blockade for 1 hour in 5% donkey serum in TBS-T. Membranes were incubated overnight at 4 °C with primary antibody phospho-DDR1 (Tyr792, Cell Signaling #11994) and phospho-p38 MAP kinase(Thr180/Tyr182, Cell Signaling #9211). Membranes were incubated with corresponding HRP-conjugated secondary antibody (Pierce Biotechnologies; Rockford, IL) for 1 hour. Specific bands were detected using the enhanced chemiluminescence reagent (ECL, Perkin Elmer Life Sciences; Boston, MA) on autoradiographic film.

***In vitro* anti-inflammatory activity**

Cells and treatment. MPMs were prepared and cultured from C57BL/6 mice using the method described in our previous paper.¹ MPMs were incubated in DMEM media (Gibco) supplemented with 10% FBS, 100 U/ml penicillin, and 100 mg/mL streptomycin at 37 °C with 5% CO₂. Compounds were added into cell cultural medium in DMSO solution with the final concentration of DMSO is 0.1%.

Reagents. LPS was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Mouse TNF- α and IL-6 ELISA kits were purchased from eBioscience (San Diego, CA, USA). Anti-CD68 was from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Trizol-reagent and primers were purchased from Invitrogen (Invitrogen, Carlsbad, CA, USA).

Determination of Pharmacokinetic Parameters in Rats

Compound **7ae** was dissolved in mixed solvents (EtOH: H₂O =5: 95 and a bit of 0.1 mol/L HCl) as clear solution. The final concentrations were 4 mg/mL and 2 mg/mL. Sprague–Dawley (SD) rats (male, three animals per group) weighing 180–220 g were injected intravenously or administrated orally at doses of 2 mg/mL (iv) or 4 mg/mL (po), respectively. After dose administration, 0.5 mL of the orbital blood was taken at 0.03 h, 0.17 h, 0.5 h, 1.0 h, 2.0 h, 3.0 h, 4.0 h, 6.0 h, 8.0 h, 12.0 h, 24.0 h. Samples were stored at –80 °C until shipment to the analytical laboratory and tested by HPLC/MS using propranolol as internal standard to measure the compound concentration in the blood. The pharmacokinetic parameters were calculated using DAS (Drug and Statistics) 2.0 software (Mathematical Pharmacology Professional Committee of China, Shanghai, China).

Table S3. Pharmacokinetic profile of compound **7ae** in rats^a

	7ae	
	oral (20 mg/kg)	iv (4 mg/kg)
AUC _(0-∞) ug/L·h	1916.5 ± 547.0	1071.2 ± 56.2
T _{1/2} (h)	3.55 ± 0.46	3.09 ± 0.92
T _{max} (h)	3.67 ± 0.58	--
C _{max} (ug/L)	273.33 ± 99.16	1230±125.3
CLz (L/h/kg)	--	3.7 ± 0.2
BA (%)	35.8	

^aSD rats (male, 3 animals per group) weighted 180–220g were used for the study.

In vivo anti-inflammatory experiments

Animals. Male C57BL/6 mice (6–8 weeks age) were obtained from the Animal Center of Wenzhou Medical University (Wenzhou, China). Animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals. All animal experimental procedures were approved by the Wenzhou Medical University Animal Policy and Welfare Committee.

LPS-induced ALI. Male C57BL/6 mice were randomly divided into four groups, designated “CON” (8 mice, only received the vehicle of 0.9% saline), “LPS” (8 mice, received 5 mg/kg LPS alone), “LPS+20 mg/kg BID **7ae**” (8 mice, received both compound **7ae** at 20 mg/kg and 5 mg/kg LPS), and “LPS+40 mg/kg BID **7ae**” (8 mice, received both compound **7ae** at 40 mg/kg and 5

mg/kg LPS). Prior to intratracheal injection of LPS, the mice were treated orally with **7a** at the dosages of 20 mg/kg and 40 mg/kg continuously for one week. Mice were then euthanized with ketamine 6 h after LPS induction. The chest cavity of each animal was carefully opened, and the collection of BALF was performed.

BALF Analysis. The collected BALF was centrifuged at 1000 rpm for 10 min at 4 °C, the supernatant was used for protein concentration detection and subsequent cytokine determinations. The precipitation was resuspended using 50 µL physiological saline. The total number of cells on BALF was detected by cell counting instrument. The number of neutrophils on BALF was examined using Wright-Gimesa stain.

Lung Wet/dry Weight Ratio. The right upper lobe of lung was excised. After removal of the excessive water on the tissue surface, the wet weight was recorded. The sample was then dried at 60 °C for 48 h until no weight change to record the dry weight. The wet weight/dry weight ratio (W/D) was calculated and used as an index of lung edema.

Pulmonary Histopathology and Immunohistochemistry Analysis. The right lower lobe of lung was excised and fixed with 4% formalin. The lung tissues were embedded with paraffin, sliced to 5 µm sections, and stained with hematoxylin and eosin (HE). Mice lung histopathology images were acquired using a microscope (Nikon Model Eclipse 80i, Nikon, Tokyo, Japan).

Statistical analysis. All *in vitro* experiments were assayed in triplicate. Data are expressed as means ± SD. All statistical analyses were performed using GraphPad Pro. Prism 6.0 (GraphPad, SanDiego, CA). Student's t-test and ANOVA multiple comparisons were employed to analyze the differences between sets of data. A p value < 0.05 was considered statistically significant.

Protein expression and purification

Human DDR1 kinase domain (Uniprot Q08345, residues 601-913) fused to a N-terminal hexahistidine tag was recombinantly expressed in Sf9 insect cells and purified as previously described (Kim et al. 2013). The tag was removed by overnight TEV cleavage followed by size exclusion chromatography. The purified DDR1 protein was concentrated to 10 mg/mL and kept frozen at -80 until needed.

Crystallization and structure determination

DDR1 was co-crystallized with the inhibitor **7h** by the vapour-diffusion method at 20°C. Crystals grew when a solution of the protein-inhibitor complex at 9 mg/mL (buffered in 25 mM HEPES pH 7.4, 200 mM NaCl, 5% glycerol, 5 mM L-arginine, 5 mM L-glutamate, 2mM DTT), was mixed in 1:1 ratio with precipitant solution consisting of 20% polyethylene glycol 3350, 10% ethylene glycol, 0.1 M Bis-Tris propane pH 6.5, 0.2 M sodium acetate in 150 nL (end volume) sitting drops. Crystals were harvested in precipitant solution supplemented with 15% ethylene glycol, mounted in nylon loops and vitrified in liquid nitrogen. Data collection was carried out at the Diamond Light Source I04 and I04-1 beamlines (Harwell, UK). Crystals of the DDR1-**7h** complex belonged to the Monoclinic P21 Space group (a=45.55, b=120.34, c=63.02) with two molecules in the asymmetric unit.

The best dataset was processed with imosflm², scaled up and truncated with Aimless³. Initial phases were obtained by molecular replacement using PDB ID: 4CKR as an initial model in Phaser⁴. The DDR1 structure was subject to several cycles of refinement with Refmac⁵ coupled with manual building with coot⁶. Ligand restraints were generated with Phenix elbow⁷ and final

refinement cycles were performed with autoBuster⁸. Geometry of the final model was checked with MOLPROBITY⁹. The excellent electron density maps allowed the modelling of ligands, solvent molecules and most of the polypeptide chain, except for two flexible loops. Data statistics are given in **Table S4**. Figures were generated using PyMol¹⁰.

Table S4. Diffraction data collection and refinement statistics

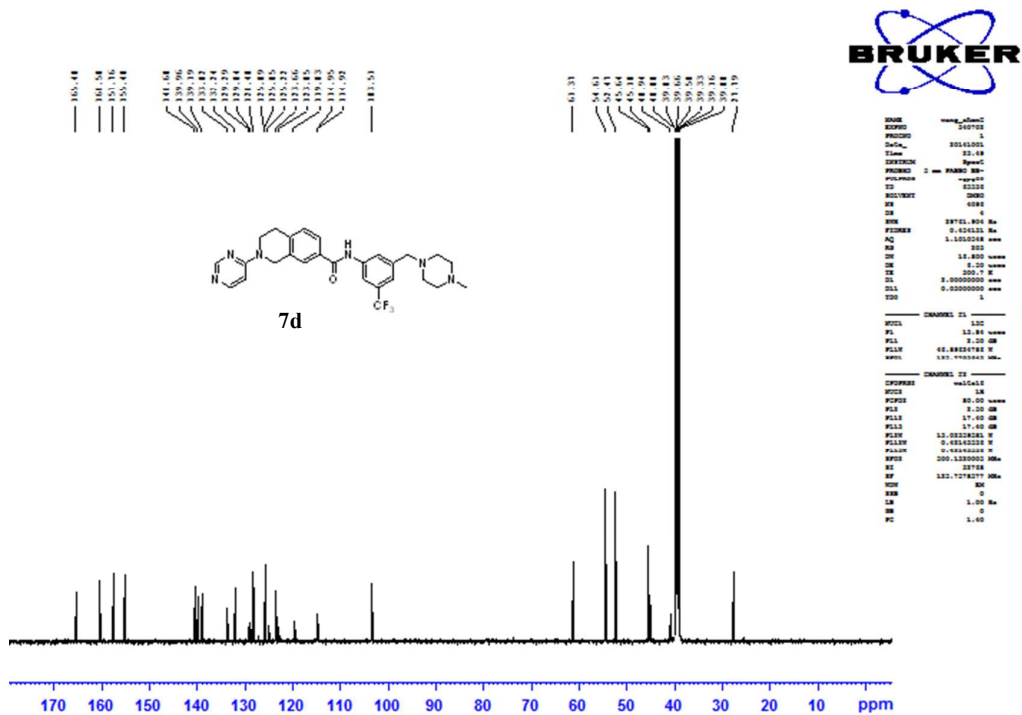
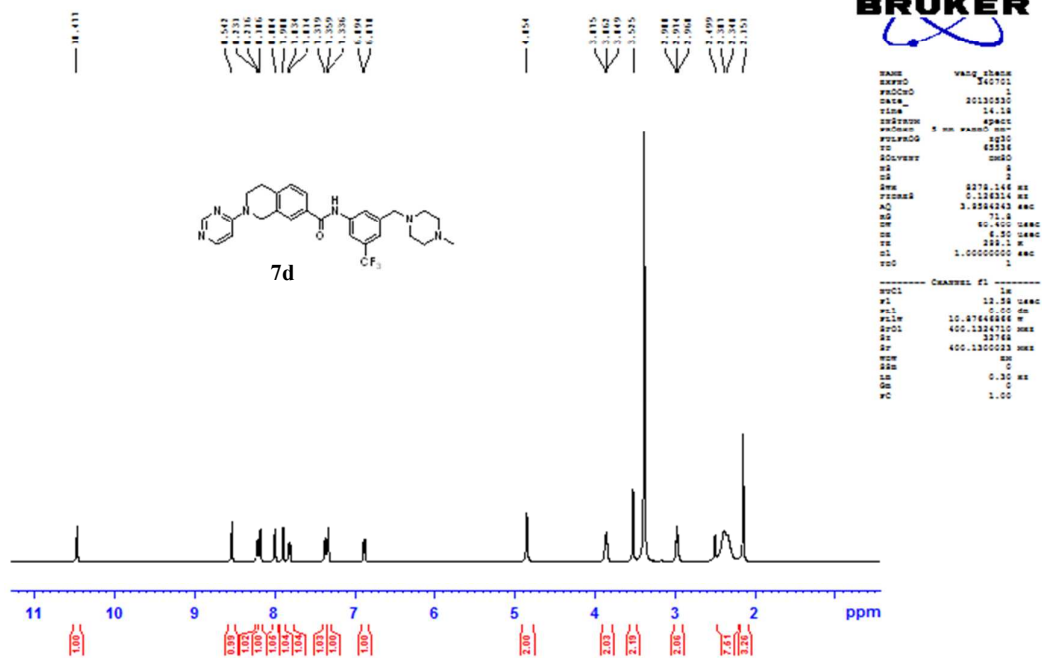
	DDR1-7ae complex
Data collection	
Space group	P2 ₁
a, b, c (Å)	45.55 120.34 63.02
α, β, γ (°)	90.00 94.87 90.00
T (K)	100
Wavelength (Å)	0.928
X-Ray Source	Synchrotron
Resolution (Å) ¹	43.44 (2.78-2.65)
Total no. of reflections ¹	65242 (8686)
No. unique reflections ¹	19393 (2583)
R_{sym} ¹	0.093 (0.057)
$I/\sigma(I)$ ¹	5.5 (0.8)
CC (1/2) ¹	0.993 (0.752)
Completeness (%) ¹	98.6 (99.2)
Redundancy ¹	3.4 (3.4)
Refinement	
Resolution (Å)	
R_{work}/R_{free} ¹	0.2056/0.2756
No. Atoms	
Protein	4372
Ligand	76
Polyethylene glycol	14
Ethylene glycol	24
Chloride	-
Solvent	148
B-factor (Å ²)	
Protein	56.4505
Ligand	47.171
Polyethylene glycol	65.964
Ethylene glycol	56.729
Chloride	-
Solvent	44.559
<i>R.m.s. deviations</i>	
Bond length (Å)	0.010
Bond angles (°)	1.07
PDB CODE	5FDX

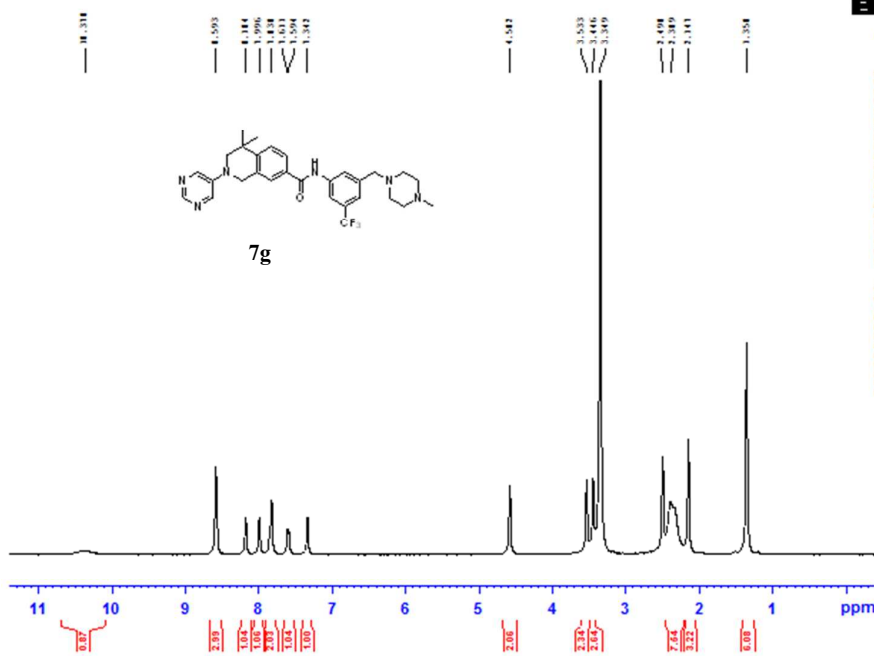
¹Values in parentheses show the statistics for the highest resolution shells.

Computational Study

All procedures were performed in Maestro 9.9 (Schrodinger LLC). The crystal structures of DDR1 protein with their corresponding inhibitors were taken from the PDB (5FDP). The protein was processed using the “Protein Preparation Wizard” workflow in Maestro 9.9 (Schrodinger LLC) to add bond orders and to add hydrogens. All heteroatom residues and crystal water molecules beyond 5 Å from het group were removed. Inhibitors were built in the LigPrep module using the OPLS-2005 force field. Glide module was used as the docking program. The grid-enclosing box was placed on the centroid of the binding ligand in the optimized crystal structure as described above, and a scaling factor of 1.0 was set to van der Waals (VDW) radius of those receptor atoms with partial atomic charges of less than 0.25. Standard precision (SP) approach of Glide was adopted to dock **7b** into DDR1 with the default parameters, and the top-ranking pose was selected for energy minimization using Prime MM-GBSA, under the solvation model of VSGB.

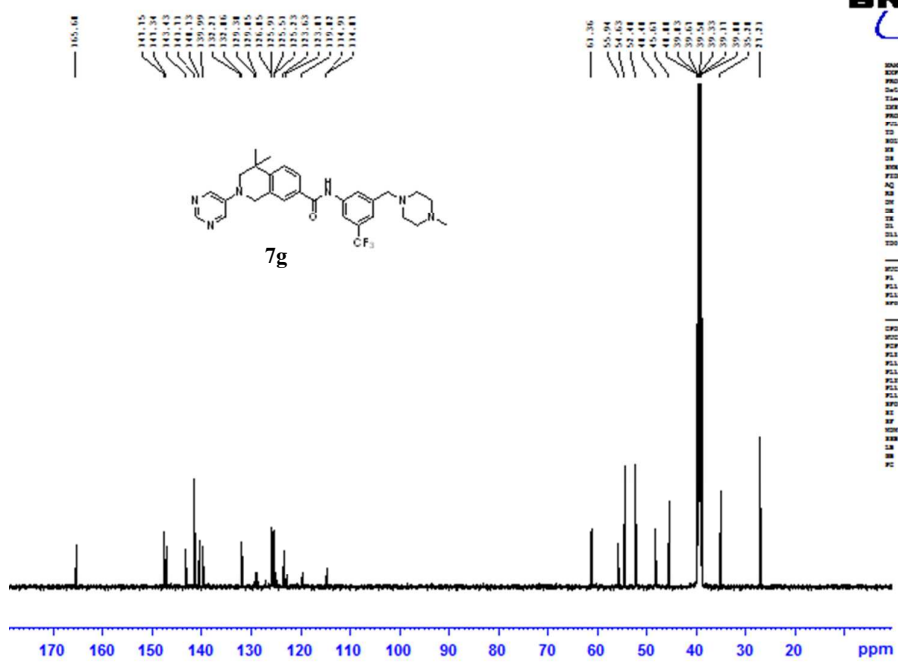
The ¹H and ¹³C NMR spectra of compounds 7d-7af





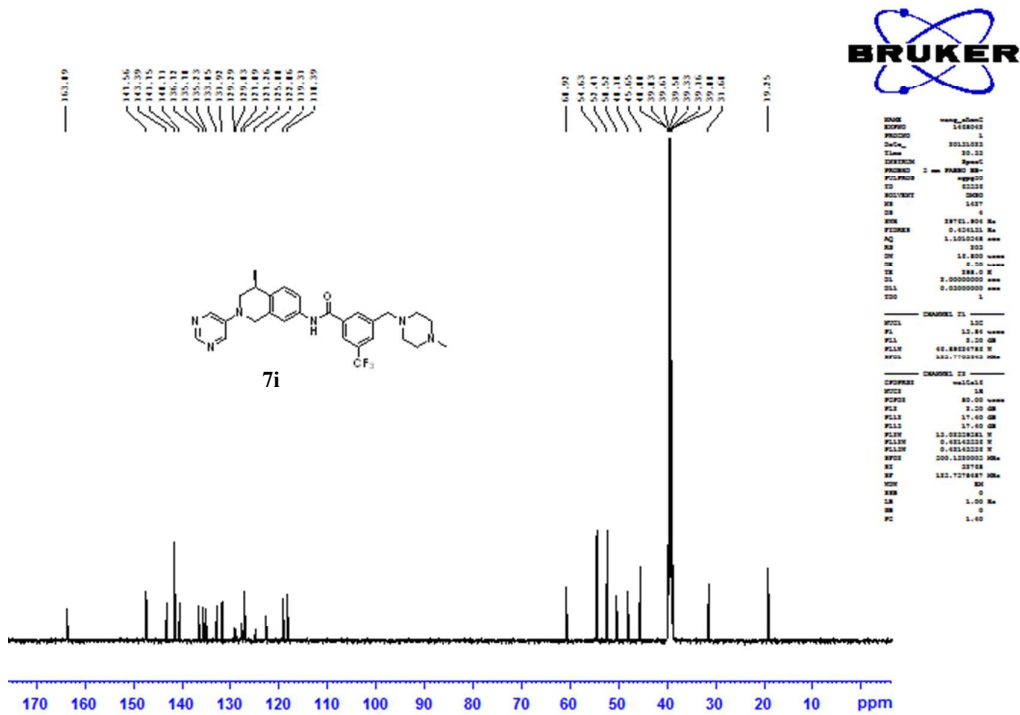
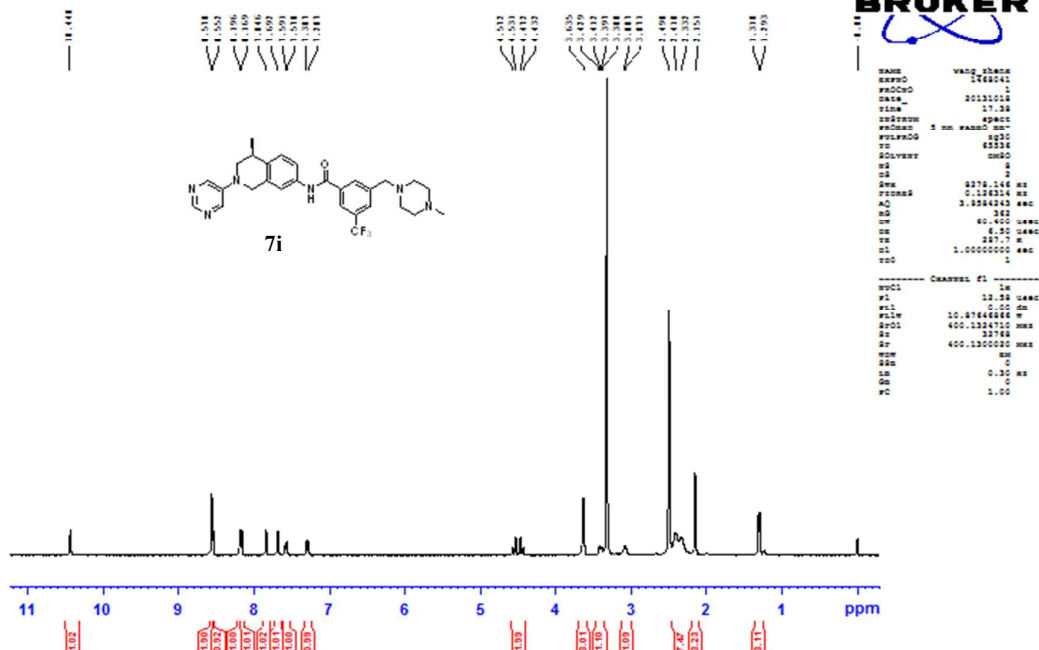
```

NAME       VADG_040601
EXPNO     1
PROCNO    1
DATA_     20131029
TIME      8.24
INSTRUM   spect
PROBHD    5 mm WBBO 50
PULPROG   zgpg30
TD         65536
SOLVENT   DMSO
NS         4
DS         4
SWH        8279.146 Hz
FIDRES    0.124314 Hz
AQ         3.1504943 sec
RG         341.3
SF         400.146013 MHz
DE         6.50 uM
TE         300.2 K
D1         1.00000000 sec
TDO        1
----- CHANNEL f1 -----
NUC1       13
P1         12.50 uM
PC1        0.00 dB
PL1W      10.8744644 W
SFO1       400.1324712 MHz
SI         32
SF         400.1300004 MHz
WDW        EM
SSB        0
GB         0.30 Hz
PC         1.00
  
```

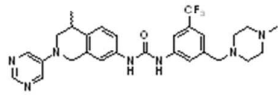


```

NAME       VADG_040601
EXPNO     1
PROCNO    1
DATA_     20131029
TIME      11.39
INSTRUM   spect
PROBHD    5 mm WBBO 50
PULPROG   zgpg30
TD         65536
SOLVENT   DMSO
NS         4
DS         4
SWH        8279.146 Hz
FIDRES    0.124314 Hz
AQ         3.1504943 sec
RG         341.3
SF         400.146013 MHz
DE         6.50 uM
TE         300.2 K
D1         1.00000000 sec
TDO        1
----- CHANNEL f1 -----
NUC1       13
P1         12.50 uM
PC1        0.00 dB
PL1W      10.8744644 W
SFO1       400.1324712 MHz
SI         32
SF         400.1300004 MHz
WDW        EM
SSB        0
GB         0.30 Hz
PC         1.00
----- CHANNEL f2 -----
CPDPRG2   waltz16
NUC2       15
P2         80.00 uM
PC2        0.00 dB
PL2W      17.40 W
SFO2       101.6234418 MHz
SI2        12
SF2        101.6234418 MHz
PULPROG2  zgpg30
TD2        65536
SOLVENT2   DMSO
NS2         4
DS2         4
SWH2       131.7278442 MHz
FIDRES2    0.124314 Hz
AQ2        3.1504943 sec
RG2        341.3
SF2        400.1300004 MHz
DE2        6.50 uM
TE2        300.2 K
D12        1.00000000 sec
TDO2       1
  
```

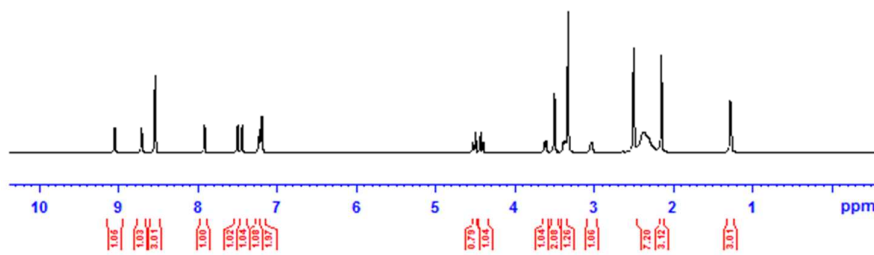
5.159
4.331
4.251
1.995
1.546
1.449
1.319
1.242
4.537
4.344
4.183
3.633
3.626
3.626
3.647
3.547
3.481
3.389
3.356
3.141
3.131
3.144
3.151
1.312
1.314



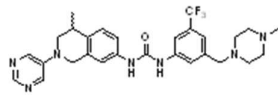
7j



```
NAME      VANG_18426
EXPNO    1
PROCNO   1
DATE_    20150429
TIME     22.44
INSTRUM  spect
PROBHD   5 mm WALTZ
PULPROG  zgpg30
TD        65536
SOLVENT  DMSO
NS        14
DS        4
SWH       10330.574 Hz
FREQ0    500.136300 MHz
AQ        3.1718203 sec
RG         203
SF        49.999 MHz
OR        0.30 uarc
DE        0.30 uarc
TE        300.2 K
SI        1.00000000 sec
RG        1
----- CHANNEL f1 -----
NUC1      13C
P1        14.00 uarc
PL1       0.00 dB
PL12      13.0232450 W
SFO1      100.6283540 MHz
SI        32768
SF        100.6283540 MHz
WDW       EM
SSB       0
LB        0.30 Hz
GB        0
PC        1.00
```



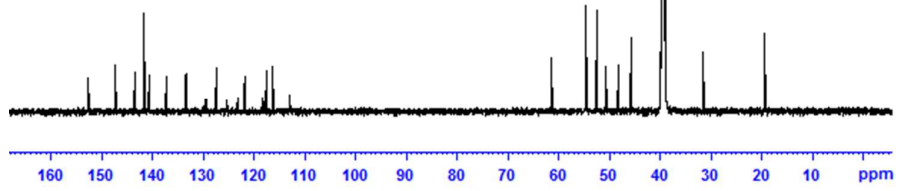
152.41
141.56
141.46
141.46
134.41
133.42
133.41
129.54
121.63
121.53
121.44
121.43
121.43
121.34
121.33
61.53
54.19
52.51
52.51
44.39
44.39
44.44
44.44
39.51
39.51
39.54
39.33
39.34
39.44
31.63
19.54

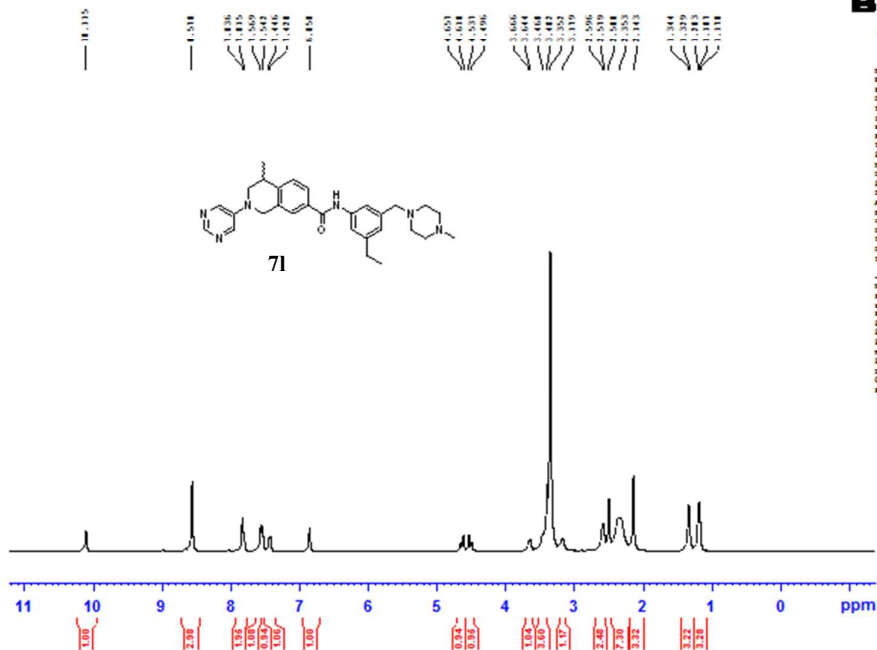


7j

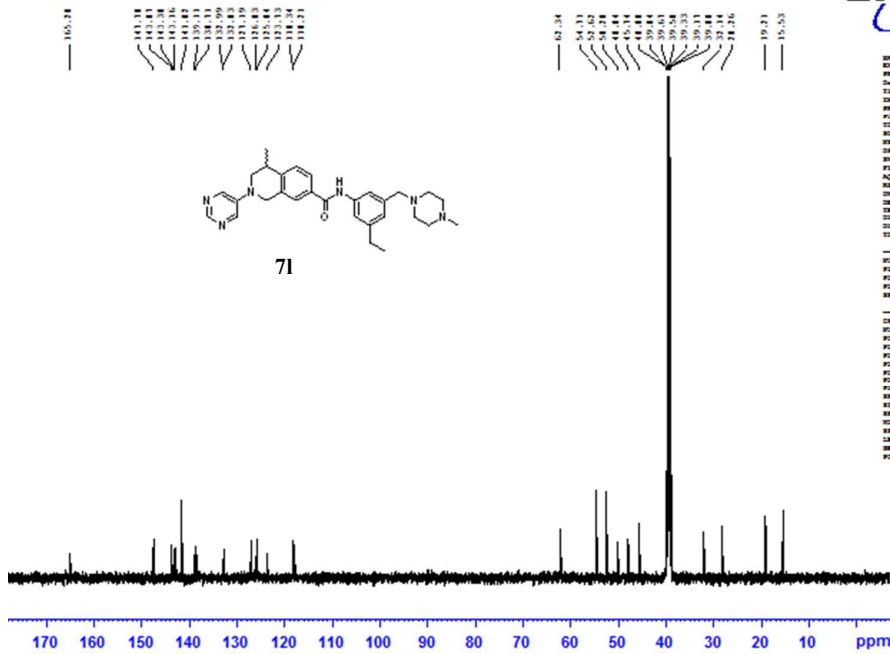


```
NAME      VANG_18426
EXPNO    1
PROCNO   1
DATE_    20150429
TIME     22.44
INSTRUM  spect
PROBHD   5 mm WALTZ
PULPROG  zgpg30
TD        65536
SOLVENT  DMSO
NS        14
DS        4
SWH       10330.574 Hz
FREQ0    500.136300 MHz
AQ        3.1718203 sec
RG         203
SF        49.999 MHz
OR        0.30 uarc
DE        0.30 uarc
TE        300.2 K
SI        1.00000000 sec
RG        1
----- CHANNEL f1 -----
NUC1      13C
P1        14.00 uarc
PL1       0.00 dB
PL12      13.0232450 W
SFO1      100.6283540 MHz
SI        32768
SF        100.6283540 MHz
WDW       EM
SSB       0
LB        0.30 Hz
GB        0
PC        1.00
```

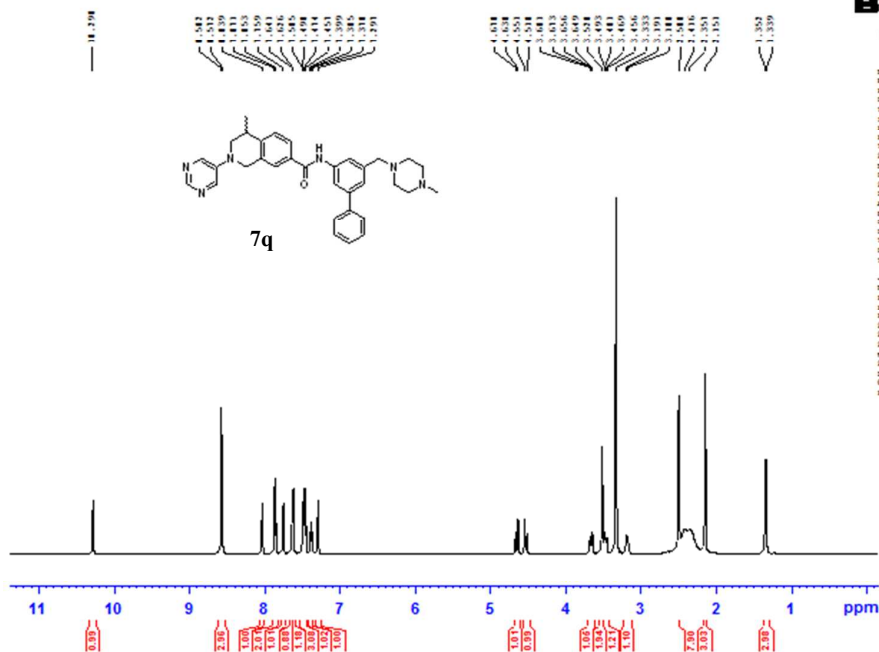




```
NAME      VAND_21624
EXPNO    1
PROCNO   1
DATA_    20140417
TIME     22.48
INSTRUM  spect
PROBHD   5 mm cryo 150-1
PULPROG  zgpg30
TD        65536
SOLVENT  DMSO
NS        4
DS         2
SWH       8278.146 Hz
F2       51.62814 MHz
AQ        3.3284343 sec
RG         134
SR        40.400 MHz
OR         6.50 uARC
TE        300.2 K
SI         1.00000000 sec
TO        1
----- CHANNEL f1 -----
NUC1      13C
P1        12.50 uARC
PL1       0.00 dB
PL12      10.8764666 Hz
SFO1      400.1324710 MHz
SI         32768
SF         400.1320024 MHz
WDW        EM
SSB         0
LB         0.30 Hz
GB         0
PC         1.00
```



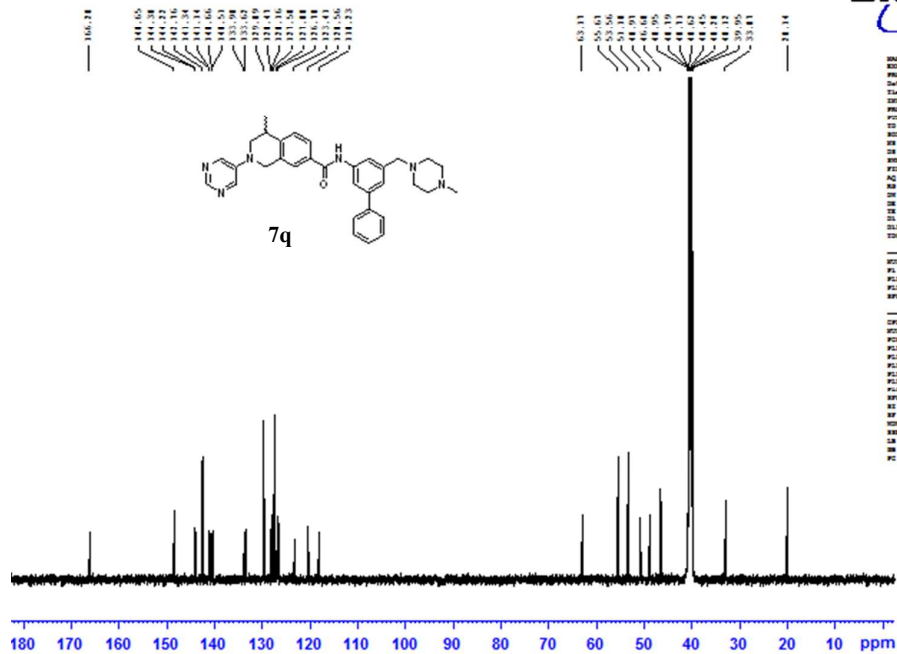
```
NAME      VAND_21624
EXPNO    1
PROCNO   1
DATA_    20140418
TIME     14.50
INSTRUM  spect
PROBHD   5 mm WALTZ 160-
PULPROG  zgpg30
TD        65536
SOLVENT  DMSO
NS        4
DS         2
SWH       18761.804 MHz
F2       101.62814 MHz
AQ        1.1550248 sec
RG         250
SR        10.000 MHz
OR         6.50 uARC
TE        300.2 K
SI         3.00000000 sec
DIL        0.02000000 sec
TO        1
----- CHANNEL f1 -----
NUC1      13C
P1        12.50 uARC
PL1       0.00 dB
PL12      66.8828782 Hz
SFO1      101.6281400 MHz
----- CHANNEL f2 -----
NAME      VAND_21624
EXPNO    1
PROCNO   1
DATA_    20140418
TIME     14.50
INSTRUM  spect
PROBHD   5 mm WALTZ 160-
PULPROG  zgpg30
TD        65536
SOLVENT  DMSO
NS        4
DS         2
SWH       18761.804 MHz
F2       101.62814 MHz
AQ        1.1550248 sec
RG         250
SR        10.000 MHz
OR         6.50 uARC
TE        300.2 K
SI         3.00000000 sec
DIL        0.02000000 sec
TO        1
----- CHANNEL f3 -----
NAME      VAND_21624
EXPNO    1
PROCNO   1
DATA_    20140418
TIME     14.50
INSTRUM  spect
PROBHD   5 mm WALTZ 160-
PULPROG  zgpg30
TD        65536
SOLVENT  DMSO
NS        4
DS         2
SWH       18761.804 MHz
F2       101.62814 MHz
AQ        1.1550248 sec
RG         250
SR        10.000 MHz
OR         6.50 uARC
TE        300.2 K
SI         3.00000000 sec
DIL        0.02000000 sec
TO        1
```

```

NAME          VADG 13092
EXPNO         33500341
PROCNO        1
DATA_         20150423
TIME          22.41
VIEW          SPAC1
INSTRUM       spect
PROBHD        5 mm PABBO 1H
PULPROG       zgpg30
TD            65536
SOLVENT       DMSO
NS            16
DS            2
SWH           10330.574 Hz
FREQ0        0.1576930 MHz
AQ           3.1176223 sec
RG            203
SF            400.1460000 MHz
DE           6.50
TE            293.15 K
DIL           1.000000000
FID           1
----- CHANNEL f1 -----
NUC1          1H
P1            14.00 usec
PC1           2.00 dB
PL1          13.023523380 W
PR1          500.13302881 MHz
RG           32768
SF           500.13302881 MHz
WDW           EM
SSB           0
LB           0.30 kHz
GB           1.00
PC           1.00

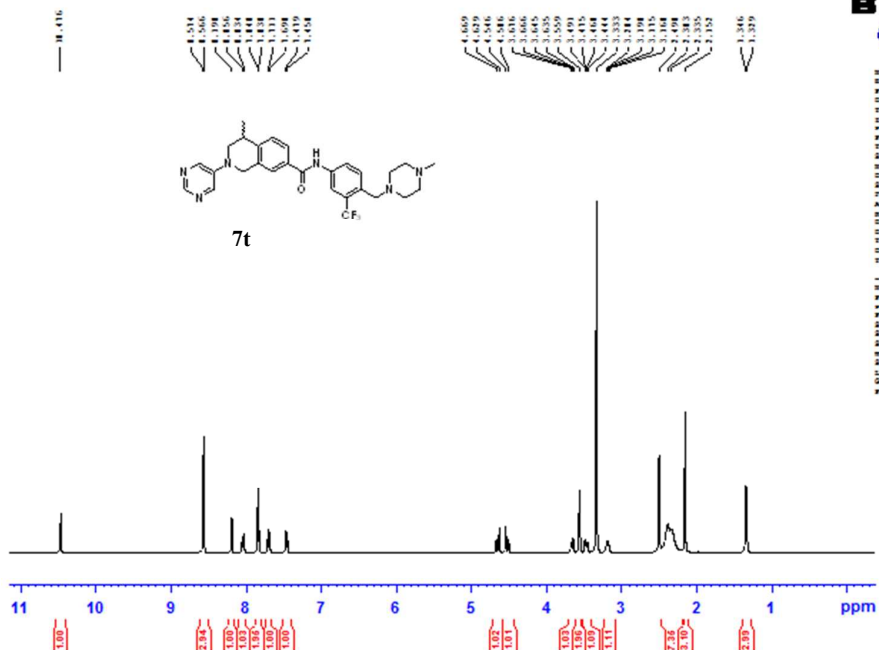
```



```

NAME          VADG 13092
EXPNO         33500341
PROCNO        1
DATA_         20150423
TIME          22.41
VIEW          SPAC1
INSTRUM       spect
PROBHD        5 mm PABBO 1H
PULPROG       zgpg30
TD            65536
SOLVENT       DMSO
NS            16
DS            2
SWH           10330.574 Hz
FREQ0        0.1576930 MHz
AQ           3.1176223 sec
RG            203
SF            400.1460000 MHz
DE           6.50
TE            293.15 K
DIL           1.000000000
FID           1
----- CHANNEL f1 -----
NUC1          13C
P1            12.00 usec
PC1           2.00 dB
PL1          16.88224762 W
PR1          125.7702643 MHz
RG           32768
SF           125.7702643 MHz
WDW           EM
SSB           0
LB           0.30 kHz
GB           1.00
PC           1.00

```

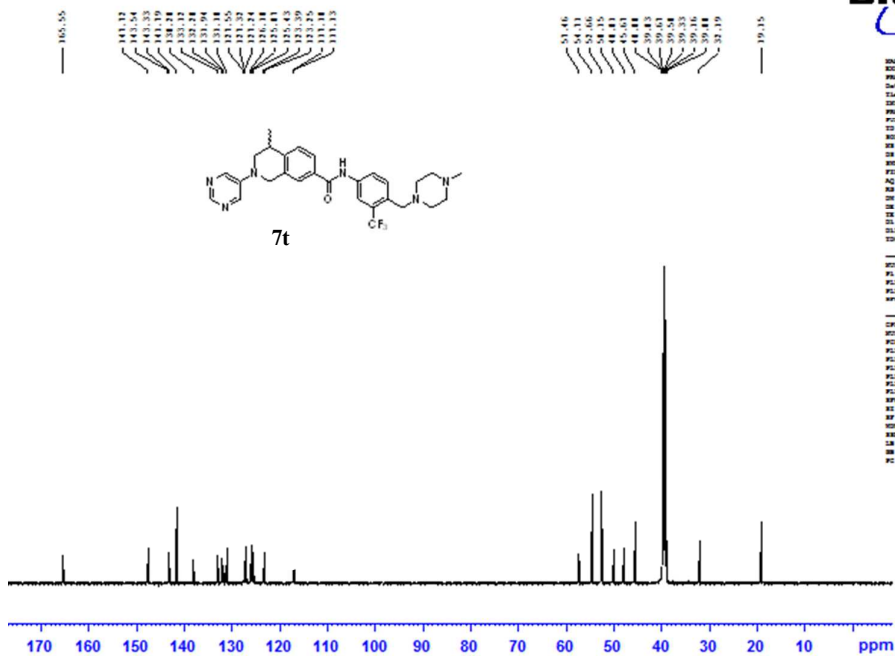



```

NAME      VANG_20001
EXPNO    1
PROCNO   1
SOLVENT  DMSO
INSTRUM  spect
PROBHD   5 mm WALTZ
PULPROG  zgpg30
TD        65536
SOLVENT  DMSO
NS        16
DS        4
SWH       8270.144 Hz
FIDRES    0.124314 Hz
AQ        3.1254243 sec
RG        328
DE        60.000 uS
TE        300.2 K
PC        1.00000000 sec
TDC       1
  
```

```

CHANNEL F1
NUC1      1H
P1        12.00 uS
PC1       0.00 dB
PL1       0.00 dB
PL12      10.00 dB
PL13      10.00 dB
PL14      10.00 dB
PL15      10.00 dB
PL16      10.00 dB
PL17      10.00 dB
PL18      10.00 dB
PL19      10.00 dB
PL20      10.00 dB
PL21      10.00 dB
PL22      10.00 dB
PL23      10.00 dB
PL24      10.00 dB
PL25      10.00 dB
PL26      10.00 dB
PL27      10.00 dB
PL28      10.00 dB
PL29      10.00 dB
PL30      10.00 dB
PL31      10.00 dB
PL32      10.00 dB
PL33      10.00 dB
PL34      10.00 dB
PL35      10.00 dB
PL36      10.00 dB
PL37      10.00 dB
PL38      10.00 dB
PL39      10.00 dB
PL40      10.00 dB
PL41      10.00 dB
PL42      10.00 dB
PL43      10.00 dB
PL44      10.00 dB
PL45      10.00 dB
PL46      10.00 dB
PL47      10.00 dB
PL48      10.00 dB
PL49      10.00 dB
PL50      10.00 dB
  
```

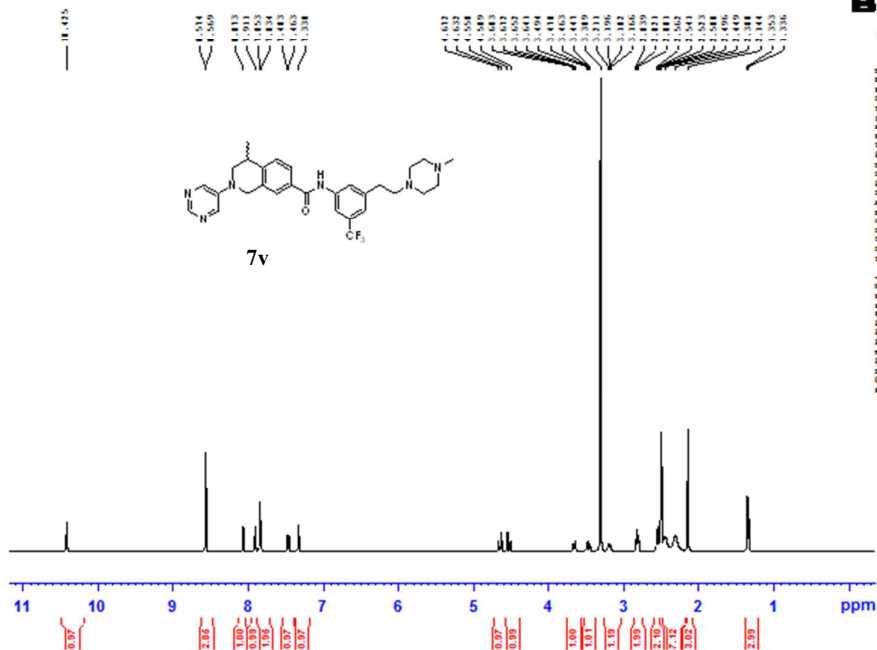


```

NAME      VANG_20001
EXPNO    1
PROCNO   1
SOLVENT  DMSO
INSTRUM  spect
PROBHD   5 mm WALTZ
PULPROG  zgpg30
TD        65536
SOLVENT  DMSO
NS        16
DS        4
SWH       8270.144 Hz
FIDRES    0.124314 Hz
AQ        3.1254243 sec
RG        328
DE        60.000 uS
TE        300.2 K
PC        1.00000000 sec
TDC       1
  
```

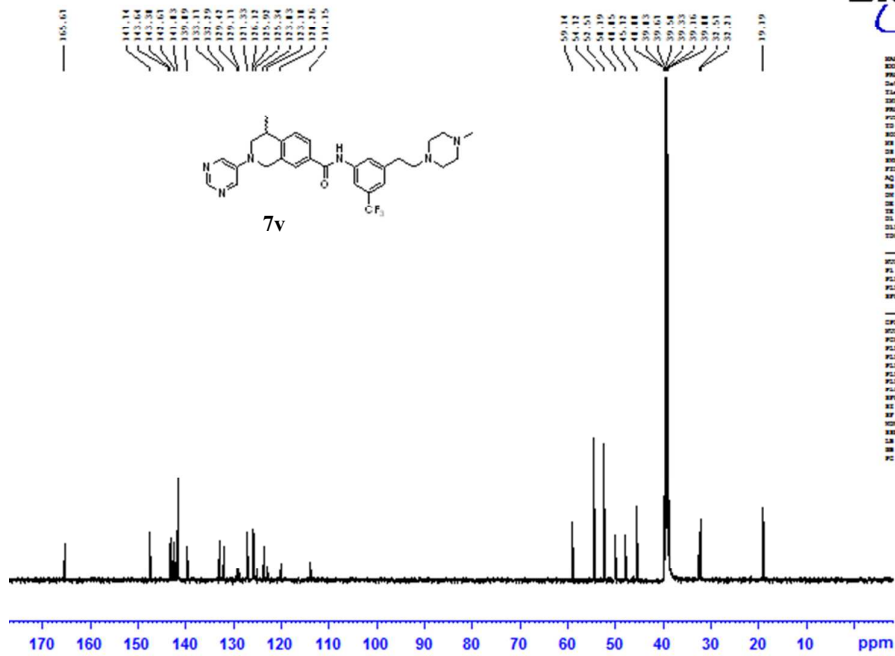
```

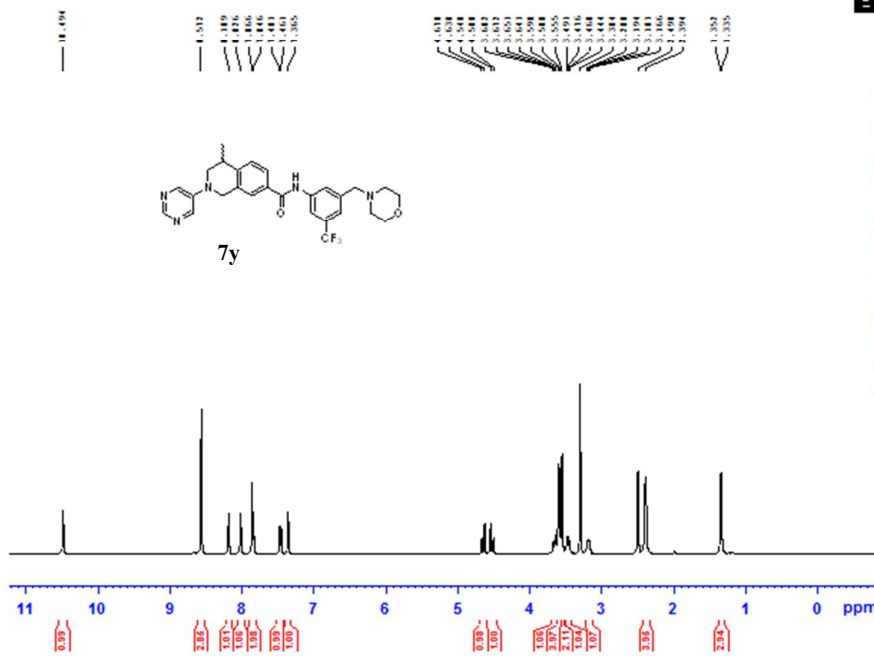
CHANNEL F1
NUC1      13C
P1        12.00 uS
PC1       0.00 dB
PL1       0.00 dB
PL12      10.00 dB
PL13      10.00 dB
PL14      10.00 dB
PL15      10.00 dB
PL16      10.00 dB
PL17      10.00 dB
PL18      10.00 dB
PL19      10.00 dB
PL20      10.00 dB
PL21      10.00 dB
PL22      10.00 dB
PL23      10.00 dB
PL24      10.00 dB
PL25      10.00 dB
PL26      10.00 dB
PL27      10.00 dB
PL28      10.00 dB
PL29      10.00 dB
PL30      10.00 dB
PL31      10.00 dB
PL32      10.00 dB
PL33      10.00 dB
PL34      10.00 dB
PL35      10.00 dB
PL36      10.00 dB
PL37      10.00 dB
PL38      10.00 dB
PL39      10.00 dB
PL40      10.00 dB
PL41      10.00 dB
PL42      10.00 dB
PL43      10.00 dB
PL44      10.00 dB
PL45      10.00 dB
PL46      10.00 dB
PL47      10.00 dB
PL48      10.00 dB
PL49      10.00 dB
PL50      10.00 dB
  
```

```

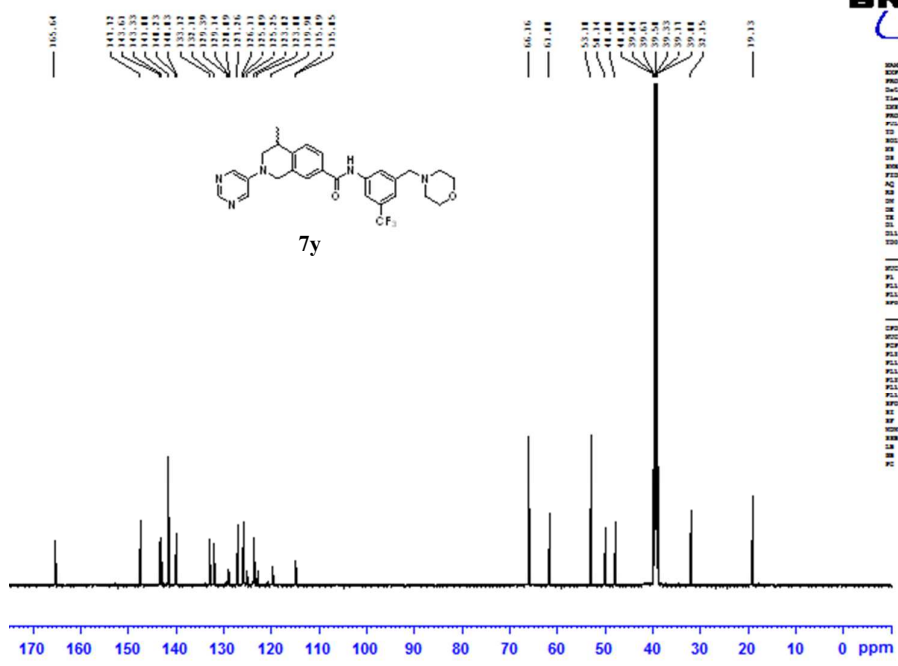
NAME      VANG_010001
EXPNO    2
PROCNO   1
DATA_    20140707
TIME     12.44
INSTRUM  spect
PROBHD   5 mm mmx 150-
PULPROG  zgpg30
TD        65536
SOLVENT  DMSO
NS        4
DS        2
SWH       8278.144 Hz
FREQ0    512.8214 MHz
AQ        0.1048143 sec
RG         256
SH        40.000 usec
OR         0.50 usec
TE        300.2 K
SI        1.0000000 sec
F2        1
----- CHANNEL f1 -----
NUC1      13
P1        12.50 usec
PC1       0.00 dB
PL1       10.87644646 W
SFO1      400.1324712 MHz
SI        32768
SF        400.1320058 MHz
WDW       EM
SSB       0
LB         0.30 Hz
GB         0
PC        1.00
  
```





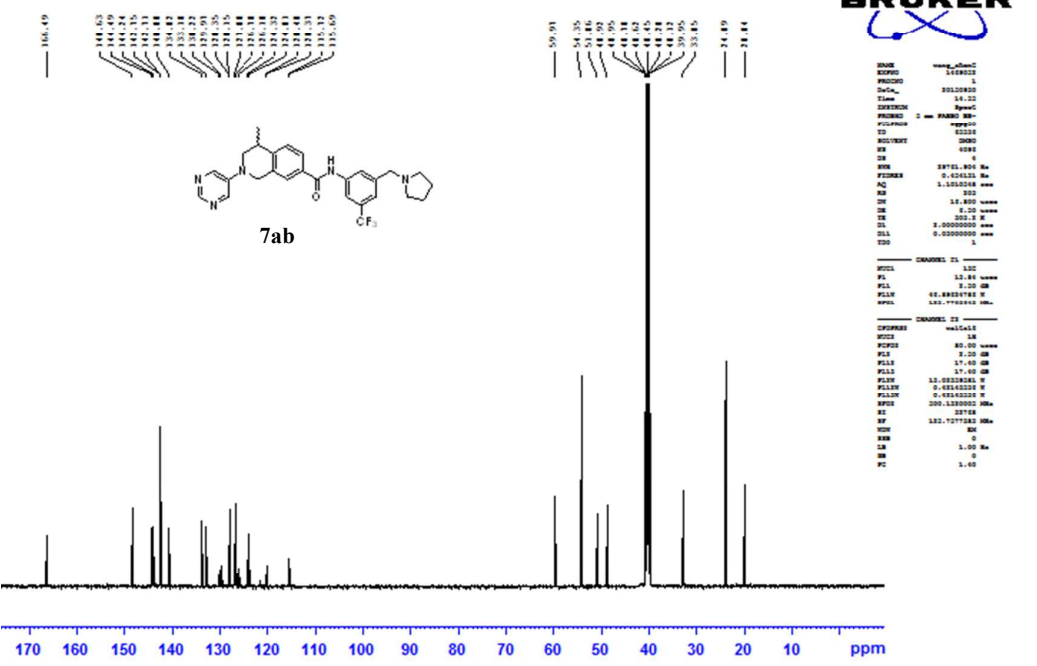
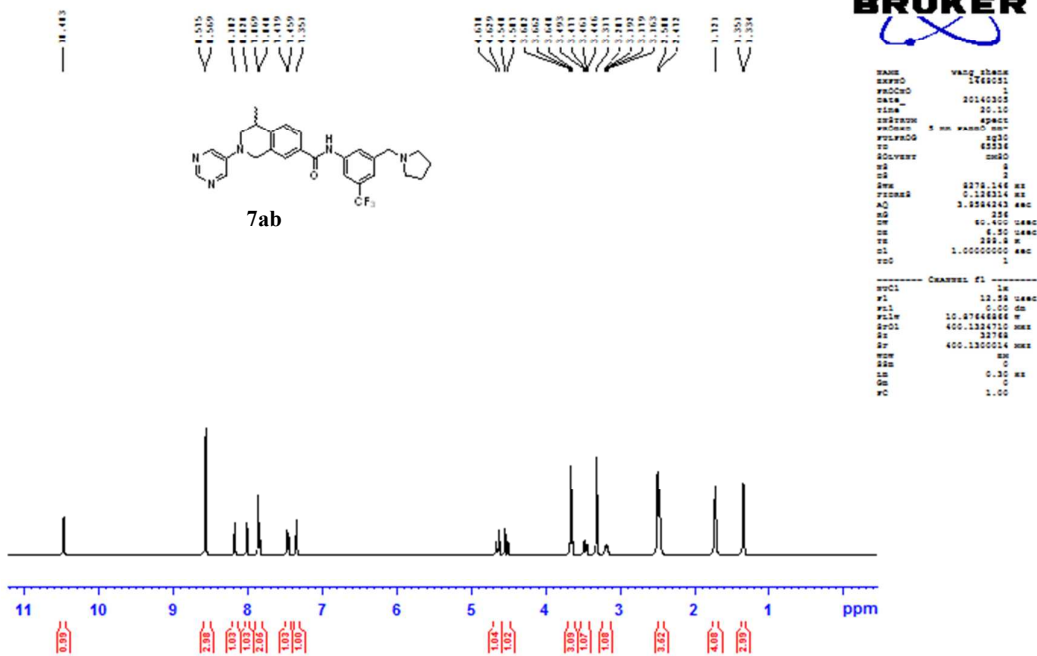
```

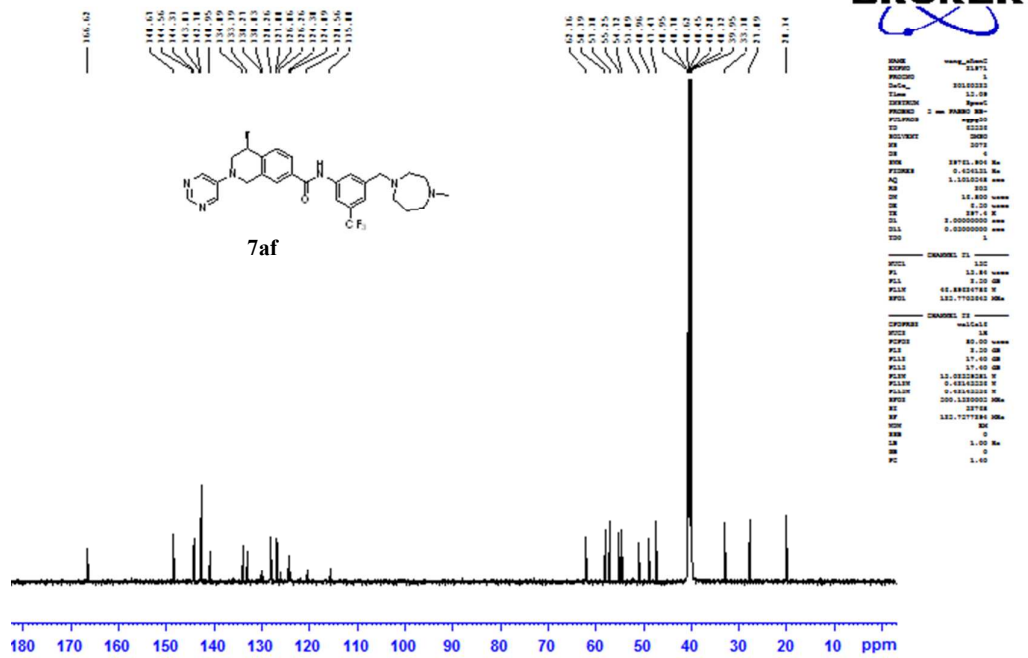
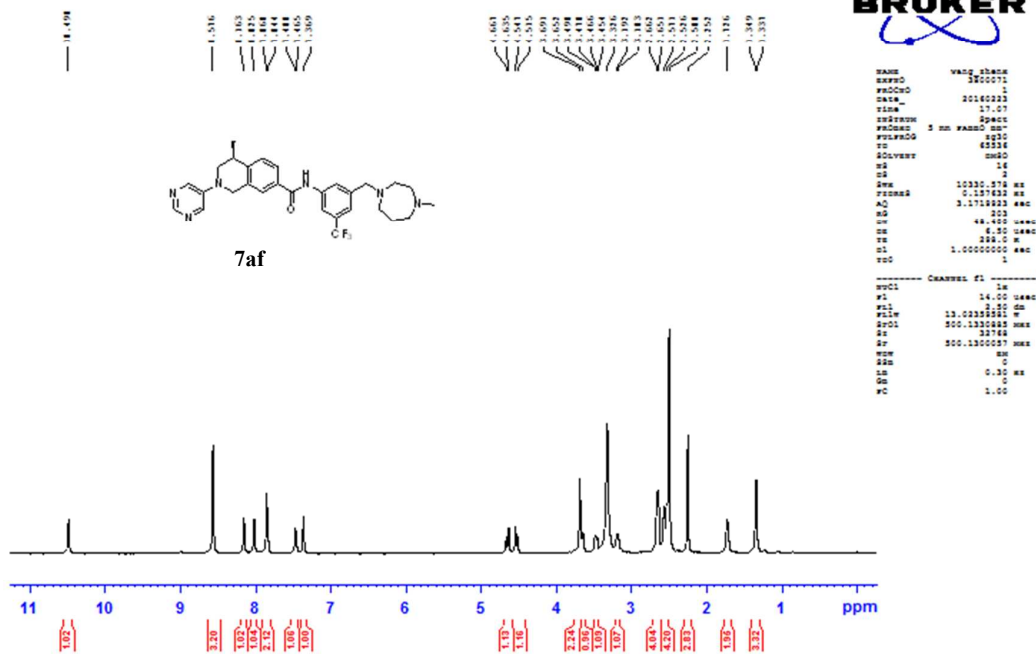
NAME      W400_13000
EXPNO    1
PROCNO   1
DATA_    20140303
TIME     22.05
INSTRUM  spect
PROBHD   5 mm PABBO MM
PULPROG  zgpg30
TD        65536
SOLVENT  DMSO
NS        4
DS        2
SWH       2078.146 Hz
F2       0.128334 Hz
AQ        3.1284243 sec
RG        322.5
SF        400.130000 MHz
DS        4.50 SARC
DE        258.2 Hz
DI        1.0000000 sec
TD0       1
----- CHANNEL f1 -----
NUC1      13C
P1        12.00 SARC
PC1       0.00 dB
PL1       10.87644846 Hz
PR1       400.1324710 MHz
RG        322.5
SF        400.1300018 MHz
SFO       100
WDW       EM
SSB       0
LB        0.30 Hz
GB        0
PC        1.00
  
```



```

NAME      W400_13000
EXPNO    1
PROCNO   1
DATA_    20140303
TIME     22.05
INSTRUM  spect
PROBHD   5 mm PABBO MM
PULPROG  zgpg30
TD        65536
SOLVENT  DMSO
NS        4
DS        2
SWH       2078.146 Hz
F2       0.128334 Hz
AQ        3.1284243 sec
RG        322.5
SF        400.130000 MHz
DS        4.50 SARC
DE        258.2 Hz
DI        1.0000000 sec
TD0       1
----- CHANNEL f1 -----
NUC1      13C
P1        12.00 SARC
PC1       0.00 dB
PL1       10.87644846 Hz
PR1       400.1324710 MHz
RG        322.5
SF        400.1300018 MHz
SFO       100
WDW       EM
SSB       0
LB        0.30 Hz
GB        0
PC        1.00
  
```



References

- 1 Chen, G. *et al.* Discovery of a New Inhibitor of Myeloid Differentiation 2 from Cinnamide Derivatives with Anti-Inflammatory Activity in Sepsis and Acute Lung Injury. *Journal of medicinal chemistry* **59**, 2436-2451, doi:10.1021/acs.jmedchem.5b01574 (2016).
- 2 Batty, T. G., Kontogiannis, L., Johnson, O., Powell, H. R. & Leslie, A. G. iMOSFLM: a new graphical interface for diffraction-image processing with MOSFLM. *Acta Crystallogr D Biol Crystallogr* **67**, 271-281, doi:10.1107/S0907444910048675 (2011).
- 3 Evans, P. R. & Murshudov, G. N. How good are my data and what is the resolution? *Acta Crystallogr D Biol Crystallogr* **D69** (2013).
- 4 Read, R. J. Pushing the boundaries of molecular replacement with maximum likelihood. *Acta Crystallogr D Biol Crystallogr* **57**, 1373-1382 (2001).
- 5 Murshudov, G. N., Vagin, A. A. & Dodson, E. J. Refinement of macromolecular structures by the maximum-likelihood method. *Acta Crystallogr D Biol Crystallogr* **53**, 240-255, doi:10.1107/S0907444996012255 (1997).
- 6 Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. Features and development of Coot. *Acta Crystallogr D Biol Crystallogr* **66**, 486-501, doi:10.1107/S0907444910007493 (2010).
- 7 Adams, P. D. *et al.* PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr D Biol Crystallogr* **66**, 213-221, doi:10.1107/S0907444909052925 (2010).
- 8 Bricogne, G. *et al.* BUSTER version 2.10.2. *Global Phasing Ltd.* (2011).
- 9 Chen, V. B. *et al.* MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr D Biol Crystallogr* **66**, 12-21, doi:10.1107/S0907444909042073 (2010).
- 10 Schrodinger, LLC. *The PyMOL Molecular Graphics System, Version 1.3r1* (2010).