Supporting Information

N-(3-ethynyl-2, 4-difluorophenyl)sulfonamide Derivatives as Selective Raf Inhibitors

Yingjun Li,[†,‡] Huimin Cheng,[†] Zhang Zhang,[†,§] Xiaoxi Zhuang,[†] Jinfeng Luo,[†] Huoyou Long,[†] Yang Zhou[†,‡], Yong Xu[†], Rana Taghipouran,[⊥] Dan Li,[⊥] Adam Patterson,[⊥,ϕ] Jeff Smaill,[⊥,ϕ] Zhengchao Tu,[†] Donghai Wu,[†] Xiaomei Ren,[†,*] and Ke Ding[†,*]

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

‡University of Chinese Academy of Sciences, #19 Yuquan Road, Beijing 100049, China

§Biotechnological Institute of Chinese Materia Medica and Department of pharmacology, Jinan University, #601 Huangpu Avenue West, Guangzhou 510632, China.

⊥Auckland Cancer Society Research Centre, University of Auckland, #92019 Private Bag , Auckland 1142, New Zealand

ϕMaurice Wilkins Centre for Molecular Biodiscovery, University of Auckland, #92019 Private Bag, Auckland 1142, New Zealand

* Tel: +86-20-32015276. Fax: +86-20-32015299. E-Mail: ding_ke@gibh.ac.cn； ren_xiaomei@gibh.ac.cn

Contents:

Figure S1. Chemical structures of FDA approved B-Raf^{V600E} inhibitor **2**……..……..……..s3

Abbreviations……...……. s3

General methods for chemistry……...…….s3

Synthetic procedures and compound characterization……... s2-14

Scheme S1. Synthesis of compounds **14** ……s11

Scheme S2. Synthesis of compounds **19** ……s12

Computational study ……s14

Materials and methods for biological assays…… s15-19

Table S1. Pharmacokinetic profile of selected compounds in Rats……s19

Figure S2. Dose-response behaviour of $BRAF_{Kd}$ upon treatment with inhibitor **3s**……s19

Table S2. Matrix of compound screen for inhibitor **3s** ……..……s19-30

Table S3. TREEspot™ Interaction Maps for **3s**…… s31

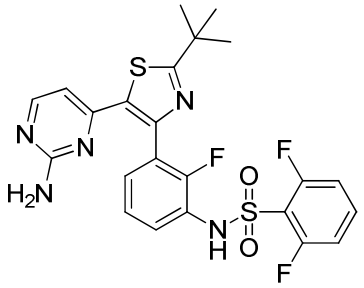
Table S4. S-score Table for **3s**……...…… s31

Figure S3. **3o** and **3s** dose-dependently inhibit the activation of MAPK pathway in colon cancer cells……..…… s32

Table S5. Enzymatic and Cellular Activities of Dabrafenib (IC50, μM/L) ……s32

Figure S6. **3s** do not affect the body weight of mice in xenograft model of Colo205….. s33

^{1}H NMR and ^{13}C NMR spectra of compounds **3a-s** ……s34-51



2 (dabrafenib)

Figure S1. Chemical structure of FDA approved B-Raf^{V600E} inhibitor 2.

Abbreviations

MAPK, mitogen-activated protein kinase signaling pathway; ERK, extracellular signal-regulated kinase; Val (V), valine; Glu (E), glutamic acid; FDA, Food and Drug Administration; DGF: Asp-Phe-Gly; rt, room temperature; IC_{50}, the half maximal (50%) inhibitory concentration (IC) of a substance; DCM, dichloromethane; THF, tetrahydrofuran; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromi; SD, standard deviation; PK, pharmacokinetic ; TGI, tumor growth inhibition.

General Methods for Chemistry. All reagents and solvents were used directly as purchased from commercial sources. Flash chromatography was performed using silica gel (200-300 mesh). All reactions were monitored by TLC, using silica gel plates with fluorescence F_{254} and UV light visualization. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV-400 spectrometer at 400 MHz and 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 control (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. The purity of compounds was determined by reverse-phase high performance liquid chromatography (HPLC) analysis to be >95%. HPLC instrument, 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). A flow rate of 1.0 ml/min was used with a mobile phase of MeOH in H_2O with a 0.1% modifier (ammonia or trifluoroacetate, v/v).

2-bromo-1,3-difluoro-4-nitrobenzene 5. To a 500 ml three-neck flask containing **4** (50g, 259 mmol) was added 250 ml concentrated sulfuric acid under iced base. Potassium nitrate (75g, 518 mmol, 2 eq) was added in small potions during 30min. After that the reaction mixture was warmed to rt and stirred overnight. The mixture was poured into 1L cooled water with stirring for 2h. The precipitated light yellow solid was filtered, washed with 50 ml water three times and dried under reduced pressure. The desired product compound (55g, 231 mmol） was dissolved in 50 ml EtOH in a 500 ml three-neck flask with reflux condenser. 200 ml concentrated hydrochloric acid was added then $SnCl_2$ (131g, 693 mmol, 3 eq) was added in small potions and the mixture was heated to reflux for 3h. Another 1 eq $SnCl_2$ (43g) was added and the reaction was refluxed for 2h. After the completion of the reaction, the mixture was taken up in 1L ice-water mixture and adjusted the pH value to 8 with NaOH aqueous solution. The resulting solid was filtered and washed well with EtOAc. The aqueous layer was extracted with EtOAc 3 times. The combined EtOAc layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford a brown crystal solid. Yield 43.5g (93%). 1H NMR (400 MHz, $CDCl_3$) δ 6.77(m, 1H), 6.67(m, 1H), 3.61-3.82(brs, 1H)

2,4-difluoro-3-methylaniline 6. A mixture of **5** (5g, 24 mmol), Cu(Ⅰ) iodide (0.456g, 2.4 mmol, 0.1 eq), $Pd(dba)_2$ (690mg, 1.2 mmol. 0.05 eq), K_2CO_3 (6.6g, 48 mmol, 2 eq), and THF (50 ml) were added to a 150 ml sealed tube. The mixture was bubbled with argon for 20 min. Trimethylsilylacetylene (10 ml, 72 mmol, 3 eq) and 10% Tri-tert-butylphosphine solution in toluene (4.8 ml, 2.4 mmol, 0.1 eq) was added and the sealed reaction mixture was stirred at 120℃ for 48h. After the reaction was finished, the mixture was filtered through celite and concentrated. The residue was dissolved in THF (30 ml) with addiction of tetrabutylammonium fluoride (1g). The reaction mixture was stirred as rt for 1h and then extracted with EtOAc, washed with brine and dried on Na_2SO_4. The crude product was purified through flash column chromatography, eluting with petroleum ether/ EtOAc (30:1), petroleum ether/ EtOAc (10:1) to give the desired product(1.7g, yield: 46%). 1H NMR (400 MHz, $CDCl_3$) δ 6.74(m, 2H), 3.49(s, 1H), 3.49-3.91(brs, 2H).

3-((1H-pyrazolo[3,4-b]pyridin-5-yl)ethynyl)-2,4-difluoroaniline 7. A mixture of **6** (1.7g, 11 mmol), 6-bromo-3H-pyrazolo[3,4-b]pyridine (2.18g, 11 mmol, 1 eq), Cu(Ⅰ) iodide (0.209g, 1.1 mmol, 0.1 eq), $Pd(dba)_2$ (322mg, 0.56 mmol. 0.05 eq), K_2CO_3 (3g, 22 mmol, 2

eq), and dry THF (30 ml) were added to a 100 ml sealed tube. The mixture was bubbled with argon for 20 min. 10% Tri-tert-butylphosphine solution in toluene (2.2 ml, 1.1 mmol, 0.1 eq) was added and the sealed reaction mixture was stirred at 120℃ for 24h. After the reaction was finished, the mixture was filtered through celite and concentrated. The residue was purified through flash column chromatography, eluting with petroleum ether/ EtOAc (2:1) to give the desired product as a brown solid (2.1g, yield: 63%). 1H NMR (400 MHz, DMSO-d6) δ 13.96(s, 1H), 8.67(s, 1H), 8.51(d, *J* = 1.7 Hz, 1H), 8.21(s, 1H), 6.94(t, *J* = 9 Hz, 1H), 6.81-6.87(m, 1H), 5.10-5.35(brs. 2H).

N-(3-((1H-pyrazolo[3,4-b]pyridin-5-yl)ethynyl)-2,4-difluorophenyl)benzenesulfo-nami de 3a. benzenesulfonyl chloride(194mg, 1.1 mmol, 1.1 eq) was added dropwise to a solution of **7** (270mg, 1 mmol) in dry DCM (10 ml), following by addition of pyridine (0.12 ml, 1.5m ml, 1.5 eq). The reaction mixture was stirred at rt overnight. After the reaction was finished, 1 ml MeOH was added to the resulting suspension and the mixture was stirred for 10min and then concentrated. The residue was purified through flash column chromatography, eluting with DCM/ MeOH (100:1), DCM/ MeOH (40:1). The obtained solid was recrystallized with DCM to give the desired product (0.369mg, yield: 90%). ^{1}H NMR(400 MHz, DMSO-d_6): δ 13.97 (s, 1H), 10.35 (s, 1H), 8.65 (d,J = 2Hz, 1H), 8.51 (d,J = 2Hz, 1H), 8.21 (s, 1H), 7.71-7.74 (m, 2H), 7.66-7.69(m, 1H), 7.57-7.61 (m, 2H), 7.25-7.31 (m, 1H), 7.19-7.24 (m, 1H),

N-(3-ethynyl-2,4-difluborophenyl)pyridine-3-sulfonamide 8. 3-pyridine sulphonyl chloride (2.15g, 12.1 mmol, 1.1 eq) was added dropwise to a solution of **6** (1.7g, 11 mmol) in dry DCM (55 ml), following by addition of pyridine (1.35 ml, 16.5m ml, 1.5 eq). The reaction mixture was stirred at rt overnight. After the reaction was finished, 20 ml hexane was added and the precipitation was filtered and collected. The filtrate was concentrated and purified through flash column chromatography, eluting with DCM/ MeOH (100:1), DCM/ MeOH (40:1). Combined the solid to give the desired product (3g, yield: 93%).1H NMR (400 MHz, $CDCl_3$) δ10.51-10.72(brs, 1H) 8.83(m, 2H), 8.08(d, *J* = 8.4 Hz, 1H), 8.63(m, 1H), 7.30(m, 1H), 7.21(m, 1H), 4.82(s, 1H),

N-(3-((3H-pyrrolo[2,3-b]pyridin-6-yl)ethynyl)-2,4-difluorophenyl)pyridine-3-sulfonamid e 3f. A mixture of **8** (294, 1 mmol), 5-bromo-1H-pyrrolo[2,3-b]pyridine (197mg, 1 mmol, 1

eq), Cu(Ⅰ) iodide (19mg, 0.1 mmol, 0.1 eq), $Pd(dba)_2$ (29mg, 0.05 mmol. 0.05 eq), K_2CO_3 (276mg, 2 mmol, 2 eq), and dry THF (5 ml) were added to a 10 ml sealed tube. The tube was evacuated and backfilled with argon three times. 10% Tri-tert-butylphosphine solution in toluene (2.2 ml, 1.1 mmol, 0.1 eq) was added and the sealed reaction mixture was stirred at 120℃ for 24h. After the reaction was finished, the mixture was filtered through celite and washed with THF repeatedly. Water was added to the filtrate and the pH was adjusted to 6 with 1N HCl. The resulting suspension was extracted with EtOAc three times. The combined organic layers were dried, filtered and concentrated. The residue was purified through flash column chromatography, eluting with DCM/ MeOH (100:1), DCM/ MeOH (40:1). The obtained solid was recrystallized with DCM to give the desired product (260mg, yield: 64%). 1H NMR (400 MHz, $CDCl_3$) δ12.00 (s, 1H), 10.60 (s, 1H), 8.86-8.88 (m, 2H), 8.37 (s, 1H), 8.19 (d,J = 1.6Hz, 1H), 8.12 (d,J = 8.1Hz, 1H), 7.66 (dd, J = 4.8, 8.0Hz, 1H), 7.57-7.59 (m, 1H), 7.27-7.33 (m, 1H), 7.20-7.25 (m, 1H), 6.52 (dd, J = 1.8, 3.3Hz, 1H).

N-(3-((1H-pyrazolo[3,4-b]pyridin-5-yl)ethynyl)-2,4-difluorophenyl)benzenesulfonamide 3a

^{1}H NMR(400 MHz, DMSO-d_6): δ(ppm) 13.97 (s, 1H), 10.35 (s, 1H), 8.65 (d,J = 2Hz, 1H), 8.51 (d,J = 2Hz, 1H), 8.21 (s, 1H), 7.71-7.74 (m, 2H), 7.66-7.69(m, 1H), 7.57-7.61 (m, 2H), 7.25-7.31 (m, 1H), 7.19-7.24 (m, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ(ppm) 160.99(dd, J = 250,4 Hz, 1C), 157.48(dd, J = 253,5 Hz, 1C), 151.29, 151.08, 140.02, 134.41, 134.06, 133.56, 129.74(2C), 128.88(d,J = 10 Hz, 1C), 126.98(2C), 121.75(dd, J = 12,4 Hz, 1C), 114.36, 112.36(dd, J = 21,4 Hz, 1C), 111.06, 101.89(t,J = 20 Hz, 1C), 97.87, 76.71. HRMS (ESI) calcd for C20H12F2N4O2S $[M+H]^+$: 411.072; found 411.0722. HPLC purity = 98.19%, Rt 2.40 min.

N-(3-((1H-pyrazolo[3,4-b]pyridin-5-yl)ethynyl)-2,4-difluorophenyl)-2-fluorobenzenesulfonamide 3b

^{1}H NMR(400 MHz, DMSO-d_6): δ(ppm) 13.98(s, 1H), 10.64(s, 1H), 8.66(s, 1H), 8.52(s, 1H), 8.22(s, 1H), 7.74(t, J = 7.1 Hz, 2H), 7.49(t, J = 9.5 Hz, 1H), 7.33-7.38(m, 2H), 7.23(t, J = 8.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ(ppm) 161.66(dd, J = 250,4 Hz, 1C), 160.08(d, J = 253 Hz, 1C), 158.32(dd, J = 253,6 Hz, 1C), 151.31, 136.40(d,J = 8 Hz, 1C), 134.39, 133.95, 130.28, 129.58(d,J = 10 Hz, 1C), 128.38(d,J = 14 Hz, 1C), 125.33(d,J = 36 Hz, 1C),

121.42(dd, J = 13,3 Hz, 1C), 117.87, 117.66, 114.46, 112.31(dd, J = 21,4 Hz, 1C), 111.25, 102.09(t,J = 19 Hz, 1C), 98.05, 76.77. HRMS (ESI) calcd for C20H11F3N4O2S $[M+H]^+$: 429.0628; found 429.0629. HPLC purity = 99.33%, Rt 2.01 min.

N-(3-((1H-pyrazolo[3,4-b]pyridin-5-yl)ethynyl)-2,4-difluorophenyl)-3-fluorobenzenesulfonamide 3c

^{1}H NMR(400 MHz, DMSO-d_6): δ(ppm) 13.99(1s), 10.51(1s), 8.66(1s), 8.52(1s), 8.21(1s), 7.66(dd, J = 14.1,8.0 Hz, 1H), 7.54-7.58(m, 3H), 7.28(dd, J = 15.0,8.7 Hz, 1H), 7.23(t, J = 8.8 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ(ppm): 163.05(d,J = 247 Hz, 1C), 161.19(dd, J = 250,4 Hz, 1C), 157.67(dd, J = 253,5 Hz, 1C), 151.30, 151.08, 142.10(d,J = 6 Hz, 1C), 134.43, 134.10, 132.26(d,J = 8 Hz, 1C), 129.18(d,J = 10 Hz, 1C), 123.34(d,J = 3 Hz, 1C), 121.39(dd, J = 12,4 Hz, 1C), 120.88(d,J = 41 Hz, 1C), 114.36, 114.09(d,J = 24 Hz, 1C), 112.49(dd, J = 42,4 Hz, 1C), 111.04, 101.98(t,J = 20 Hz, 1C), 97.95, 76.65. HRMS (ESI) calcd for C20H11F3N4O2S $[M+H]^+$: 429.0628; found 429.0627. HPLC purity = 98.10%, Rt 2.09 min.

N-(3-((1H-pyrazolo[3,4-b]pyridin-5-yl)ethynyl)-2,4-difluorophenyl)-4-fluorobenzenesulfonamide 3d

^{1}H NMR(400 MHz, DMSO-d_6): δ(ppm) 13.98(1s), 10.38(1s), 8.66(1s), 8.52(1s), 8.22(1s), 7.79(t, J = 6.4 Hz, 2H), 7.44(t, J = 8.6 Hz, 2H), 7.29(dd, J = 15.2,8.5 Hz, 1H), 7.22(t, J = 8.8 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ(ppm): 165.88(d, J = 250 Hz, 1C), 161.14(dd, J = 250,4 Hz, 1C), 157.65(dd, J = 253,5 Hz, 1C), 151.29, 151.07, 136.37(d,J = 3 Hz, 1C), 134.41, 134.04, 130.18(d,J = 10 Hz, 2C), 129.16(d,J = 10 Hz, 1C), 121.52(dd, J = 12,3 Hz, 1C), 117.05(d,J = 22 Hz, 2C), 114.36, 112.43(dd, J = 22,3 Hz, 1C), 111.05, 101.98(t,J = 20 Hz, 1C), 97.92, 76.67. HRMS (ESI) calcd for C20H11F3N4O2S $[M+H]^+$: 429.0628; found 429.0630. HPLC purity = 99.48%, Rt 2.26 min.

N-(3-((1H-pyrazolo[3,4-b]pyridin-5-yl)ethynyl)-2,4-difluorophenyl)pyridine-2-sulfonamide 3e

^{1}H NMR(400 MHz, DMSO-d_6): δ(ppm) 13.99(1s), 10.56(1s), 8.77(d,J = 4.3 Hz, 1H), 8.67(1s), 8.53(1s), 8.22(1s), 8.09(t, J = 7.8 Hz, 1H), 7.92(d,J = 7.8 Hz, 1H), 7.70(t, J = 6.1 Hz, 1H), 7.38(dd, J = 15.0,8.7 Hz, 1H), 7.21(t, J = 8.8 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ(ppm) 165.78(dd, J = 250,4 Hz, 1C),162.41(dd, J = 253,5 Hz, 1C),162.00, 156.09, 155.85, 155.38, 144.05, 139.26(d,J = 16 Hz, 1C), 138.90(d,J = 15 Hz, 1C),133.93, 132.73, 127.17,

126.67(dd, J = 12,3 Hz, 1C),119.14, 16.97(d,J = 10 Hz, 1C),115.87, 106.58(t,J = 20 Hz, 1C),102.57, 81.55. HRMS (ESI) calcd for C19H11F2N5O2S $[M+H]^+$: 412.0674; found 412.0672. HPLC purity = 99.18%, Rt 1.91 min.

N-(3-((3H-pyrazolo[3,4-b]pyridin-6-yl)ethynyl)-2,4-difluorophenyl)pyridine-3-sulfonamide 3f

^{1}H NMR(400 MHz, DMSO-d_6): δ(ppm) 13.97(s, 1H), 10.62(brs, 1H), 8.88(d,J = 2.1 Hz, 1H), 8.85(dd, J = 4.8,1.4 Hz, 1H), 8.65(d,J = 2.0 Hz, 1H), 8.51(d,J = 1.9 Hz, 1H), 8.21(s, 1H), 8.11(td, J = 8.3,1.9 Hz, 1H), 7.65(dd, J = 8.0,4.8 Hz, 1H), 7.29-7.35(m, 1H), 7.24(t, J = 8.9 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ(ppm) 161.31, (dd, J = 250,4 Hz, 1C), 157.73 (dd, J = 253,5 Hz, 1C), 154.09, 151.29, 151.08, 147.39, 136.58, 135.07, 134.41, 134.08, 129.52(d,J = 10 Hz, 1C), 124.79, 121.22(dd, J = 13,3 Hz, 1C), 114.35, 112.57(dd, J = 22,4 Hz, 1C), 111.03, 102.05(t,J = 20 Hz, 1C), 97.99, 76.61. HRMS (ESI) calcd for C19H11F2N5O2S $[M+H]^+$: 412.0674; found 412.0674. HPLC purity = 98.39%, Rt 10.61 min.

N-(3-((3H-pyrrolo[2,3-b]pyridin-6-yl)ethynyl)-2,4-difluorophenyl)pyridine-3-sulfonamide 3g

^{1}H NMR(400 MHz, DMSO-d_6): δ(ppm) 12.00 (s, 1H), 10.60 (s, 1H), 8.86-8.88 (m, 2H), 8.37 (s, 1H), 8.19 (d,J = 1.6Hz, 1H), 8.12 (d,J = 8.1Hz, 1H), 7.66 (dd, J = 4.8, 8.0Hz, 1H), 7.57-7.59 (m, 1H), 7.27-7.33 (m, 1H), 7.20-7.25 (m, 1H), 6.52 (dd, J = 1.8, 3.3Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ(ppm) 161.30(dd, J = 250,4 Hz, 1C), 157.69(dd, J = 253,6 Hz, 1C), 154.12, 148.29, 147.41, 145.50, 136.64, 135.08, 131.74, 129.18(d,J = 10 Hz, 1C), 128.32, 124.91, 121.06(dd, J = 12,3 Hz, 1C), 119.63, 112.53(dd, J = 22,4 Hz, 1C), 109.65, 102.63, 102.31(t,J = 20 Hz, 1C), 99.40, 75.75. HRMS (ESI) calcd for C20H12F2N4O2S $[M+H]^+$: 411.0722; found 411.0722. HPLC purity = 97.10%, Rt 1.86 min.

N-(3-((1H-indazol-5-yl)ethynyl)-2,4-difluorophenyl)pyridine-3-sulfonamide 3h

^{1}H NMR(400 MHz, DMSO-d_6): δ(ppm) 11.38(s, 1H), 8.86(d,J = 1.5 Hz, 1H), 8.76(d,J = 3.8 Hz, 1H), 8.08(td, J = 8.1,1.8 Hz, 1H), 7.78(s, 1H), 7.58(dd, J = 7.9,4.9 Hz, 1H), 7.43-7.46(m, 2H), 7.20-7.26(m, 2H), 7.08(t, J = 8.9 Hz, 1H), 6.49(s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ(ppm) 161.30(dd, J = 250,4 Hz, 1C), 157.72 (dd, J = 252,6 Hz, 1C),154.07, 147.41, 140.06, 136.74, 135.05, 134.67, 129.23, 129.06(d,J = 10 Hz, 1C), 125.57, 124.91, 123.28, 121.15 (dd, J = 13,4 Hz, 1C),113.05, 112.48(dd, J = 21, 3 Hz, 1C), 111.39, 102.50(t,J = 20 Hz, 1C),

101.15, 74.00. HRMS (ESI) calcd for C20H12F2N4O2S $[M+H]^+$: 411.0722; found 411.0721. HPLC purity = 95.37%, Rt 6.60 min.

N-(2,4-difluoro-3-(pyrazolo[1,5-a]pyrimidin-6-ylethynyl)phenyl)pyridine-3-sulfonamide 3i ^{1}H NMR(400 MHz, DMSO-d_6): δ(ppm) 10.64 (s, 1H), 9.58 (d,J = 1.2Hz, 1H), 8.88 (m, 2H), 8.62 (d,J = 2.0Hz, 1H), 8.34 (d,J = 2.3Hz, 1H), 8.12 (d,J = 8.0Hz, 1H), 7.66 (dd, J = 4.6, 7.9Hz, 1H), 7.33-7.39 (m, 1H), 7.24-7.28 (m, 1H), 6.84 (d,J = 1.6Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ(ppm) 161.42(dd, J = 252,4 Hz, 1C), 157.74(dd, J = 542,5 Hz, 1C), 154.06, 150.90, 147.28, 147.11, 146.98, 139.11, 136.74, 135.09, 130.10(d,J = 10 Hz, 1C), 125.12, 121.00(dd, J = 12,3 Hz, 1C), 112.65(dd, J = 22,4 Hz, 1C), 104.00, 101.50(t,J = 20 Hz, 1C), 97.97, 93.50, 79.01. HRMS (ESI) calcd for C19H11F2N5O2S $[M+H]^+$: 412.0674; found 412.0674. HPLC purity = 96.22%, Rt 6.69 min.

N-(2,4-difluoro-3-((3-methoxy-1H-pyrazolo[3,4-b]pyridin-5-yl)ethynyl)phenyl)pyridine-3-sulfonamide 3j

^{1}H NMR(400 MHz, DMSO-d_6): δ(ppm) 12.96 (s, 1H), 10.62 (s, 1H), 8.85-8.86 (m, 2H), 8.60 (d,J = 1.8Hz, 1H), 8.32(d,J = 1.6Hz, 1H), 8.09(d,J = 8.1Hz, 1H), 7.65 (dd, J = 4.8, 8.0 Hz, 1H), 7.30-7.32 (m, 1H), 7.22-7.26 (m, 1H), 4.02 (s,3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ(ppm) 161.31 (d,J = 251 Hz, 1C), 157.70 (dd, J = 253,5 Hz, 1C), 155.38, 154.11, 152.40, 151.38, 136.53, 135.07, 132.74, 129.48 (d,J = 4 Hz, 1C), 124.81, 121.07(d,J = 13 Hz, 1C), 112.54 (d,J = 21 Hz, 1C), 109.75, 103.49, 102.12 (t,J = 20 Hz, 1C), 97.97, 76.51, 56.22. HRMS (ESI) calcd for C20H13F2N5O3S $[M+H]^+$: 442.0780; found442.0779. HPLC purity = 97.56%, Rt 3.90 min.

N-(3-((1-ethoxy-3H-pyrazolo[3,4-b]pyridin-6-yl)ethynyl)-2,4-difluorophenyl)pyridine-3-sulfonamide 3k

^{1}H NMR(400 MHz, DMSO-d_6): δ(ppm) 12.91(s, 1H), 10.61(s, 1H), 8.92(m, 2H), 8.60(s, 1H), 8.30(m, 1H), 8.12(d,J = 7.6 Hz, 1H), 7.67(s, 1H), 7.30(m, 1H), 7.23(m, 1H), 4.41(q,J = 7.4 Hz, 2H), 1.40(t,J = 7.4 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ(ppm) 161.30(d,J = 250 Hz, 1C), 157.69(d,J = 255 Hz, 1C), 154.79, 154.10, 152.32, 151.23, 147.41, 135.01, 132.76, 129.47(d,J = 11 Hz, 1C), 125.18, 121.14(d,J = 11 Hz, 1C), 112.56(dd, J = 21,3 Hz, 1C), 109.72, 103.72, 102.13(t,J = 20 Hz, 1C), 97.98, 76.51, 64.79, 14.97. HRMS (ESI) calcd for C21H15F2N5O3S $[M+H]^+$: 456.0936; found 456.0940. HPLC purity = 96.02%, Rt 4.99 min.

N-(2,4-difluoro-3-((1-methyl-3H-pyrazolo[3,4-b]pyridin-6-yl)ethynyl)phenyl)pyridine-3-sulfonamide 3l

^{1}H NMR(400 MHz, DMSO-d_6): δ(ppm) 13.53 (s, 1H), 10.61 (s, 1H), 8.87 (d,J = 1.9Hz, 1H), 8.85 (m, 1H), 8.60 (d,J = 1.8Hz, 1H), 8.51 (d,J = 1.2Hz, 1H), 8.11 (d,J = 8.1Hz, 1H), 7.65 (dd, J = 4.8, 8.0Hz, 1H), 7.29-7.35 (m, 1H), 7.22-7.26 (m, 1H), 2.51 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ(ppm) 161.34(dd, J = 251,4 Hz, 1C), 157.72(dd, J = 254,6 Hz, 1C), 154.13, 151.75, 151.17, 147.39, 142.50, 136.55, 135.08, 133.67, 129.50(d,J = 9 Hz, 1C), 124.82, 121.13(dd, J = 12,4 Hz, 1C), 113.97, 112.60(dd, J = 21,4 Hz, 1C), 110.08, 102.14(t,J = 20 Hz, 1C), 98.25, 76.43, 12.51. HRMS (ESI) calcd for C20H13F2N5O2S $[M+H]^+$: 426.0831; found 426.0833. HPLC purity = 99.15%, Rt 1.87 min.

N-(3-((3-cyclopropyl-1H-pyrazolo[3,4-b]pyridin-5-yl)ethynyl)-2,4-difluorophenyl)pyridine-3-sulfonamide 3m ^{1}H NMR(400 MHz, DMSO-d_6): δ(ppm) 13.48(s, 1H), 10.63(brs, 1H), 8.88(s, 1H), 8.85(d,J = 4.5 Hz, 1H), 8.60(s, 1H), 8.55(s, 1H), 8.12(d,J = 7.9 Hz, 1H), 7.65(t, J = 6.5 Hz, 1H), 7.32(dd, J = 14.6,8.5 Hz, 1H), 7.24(t, J = 8.8 Hz, 1H), 2.35-2.40(m, 1H), 0.99-1.02(m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6): δ(ppm) 161.25(d,J = 252 Hz, 1C), 157.69(dd, J = 252,6 Hz, 1C), 154.08, 151.75, 151.31, 147.70, 147.38, 136.62, 135.07, 133.39, 129.40(d,J = 10 Hz, 1C), 124.81, 121.30(d,J = 12 Hz, 1C), 113.26, 112.56(dd, J = 22,3 Hz, 1C), 110.09, 102.11(t,J = 20 Hz, 1C), 98.21, 76.50, 8.48, 8.31(2 C). HRMS (ESI) calcd for C22H15F2N5O2S $[M+H]^+$: 452.0987; found 452.0990. HPLC purity = 98.46%, Rt 7.68 min.

N-(2,4-difluoro-3-((1-phenyl-3H-pyrazolo[3,4-b]pyridin-6-yl)ethynyl)phenyl)pyridine-3-sulfonamide 3n

^{1}H NMR(400 MHz, DMSO-d_6): δ(ppm) 14.14(s, 1H), 10.63(s, 1H), 8.90(m, 2H), 8.80(s, 1H), 8.71(s, 1H), 7.13(d,J = 7.9 Hz, 1H), 8.08(d,J = 7.5 Hz, 1H), 7.67(m, 1H), 7.54(t, J = 7.4 Hz, 2H), 7.45(s, 1H), 7.33(dd, J = 14.5,8.4 Hz, 1H), 7.26(t, J = 8.7 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ(ppm) 161.40(dd, J = 251,14 Hz, 1C), 157.78(dd, J = 253,5 Hz, 1C), 154.09, 152.23, 151.56, 147.39, 143.84, 136.82, 135.04, 134.23, 132.86, 129.61(d,J = 10 Hz, 1C), 129.53(2C), 128.91, 127.20(2C), 125.09, 121.18(dd, J = 12,3 Hz, 1C), 112.61(dd, J = 22,3 Hz, 1C), 111.93, 111.66, 102.09(t,J = 20 Hz, 1C), 98.02, 76.95. HRMS (ESI) calcd for C25H15F2N5O2S $[M+H]^+$: 488.0987; found 488.0987. HPLC purity = 94.74%, Rt 2.22 min.

N-(2,4-difluoro-3-((1-(pyridin-4-yl)-3H-pyrazolo[3,4-b]pyridin-6-yl)ethynyl)phenyl)pyri

dine-3-sulfonamide 3o

^{1}H NMR(400 MHz, DMSO-d_6): δ(ppm): 14.48(s, 1H), 10.54-10.79(brs, 1H) 8.97(s, 1H) 8 90(m, 1H), 8.87(d, J = 3.95 Hz, 1H), 8.76(m, 2H), 8.12(m, 3H), 7.68(dd, J = 4.85，7.95 Hz, 1H), 7.35(m, 1H), 7.28(t, J = 8.7 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ(ppm) 161.42(d,J = 250 Hz, 1C), 157.80(dd, J = 254,5 Hz, 1C), 154.15, 152.28, 151.90, 150.76(2C), 147.40, 141.31, 139.85, 136.58, 135.09, 134.13, 129.74(d,J = 9 Hz, 1C), 124.84, 121.29(2C), 121.20, 112.65(dd, J = 22,3 Hz, 1C), 112.33, 112.09, 101.99(t,J = 20 Hz, 1C), 97.76, 77.26. HRMS (ESI) calcd for C24H14F2N6O2S $[M+H]^+$: 489.0940; found 489.0939. HPLC purity = 95.81%, Rt 8.49 min.

N-(2,4-difluoro-3-((1-(4-fluorophenyl)-3H-pyrazolo[3,4-b]pyridin-6-yl)ethynyl)phenyl)py ridine-3-sulfonamide 3s

^{1}H NMR(400 MHz, DMSO-d_6): δ(ppm): 14.14(s, 1H), 10.62(s, 1H), 8.90(m, 2H), 8.80(s, 1H), 8.70(s, 1H), 8.13(m, 3H), 7.67(m, 1H), 7.33(m, 3H), 7.35(m, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ(ppm) 163.65 (d,J = 244 Hz, 1C), 161.31(d,J = 250 Hz, 1C), 157.78(d,J = 252 Hz, 1C), 154.08, 152.18, 151.61, 147.42, 142.94, 134.95, 134.19, 129.47-129.60(m, 1C), 129.36(2C), 129.29, 125.56, 121.34-121.24(m, 1C), 116.42(d,J = 21 Hz, 2C), 112.56(d,J = 21 Hz, 1C), 111.76(d,J = 7 Hz, 1C), 101.84(t,J = 19 Hz, 1C), 77.01. HRMS (ESI) calcd for C25H14F3N5O2S $[M+H]^+$: 506.0893; found 506.0893. HPLC purity = 95.06%, Rt 7.65 min.

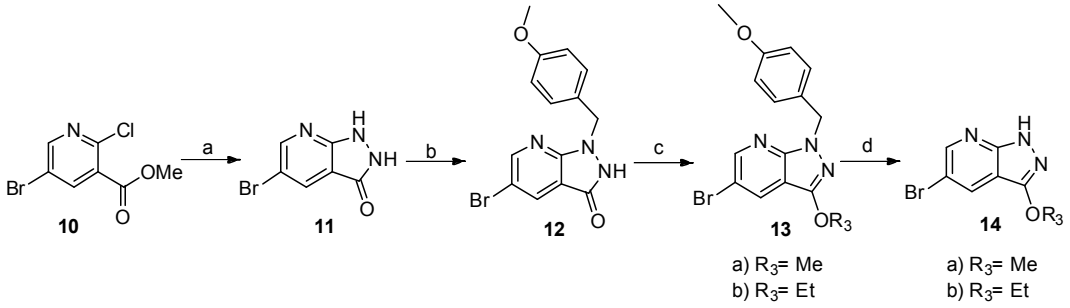
N-(3-((3-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridin-5-yl)ethynyl)-2,4-difluorophenyl)py ridine-3-sulfonamide 3r ^{1}H NMR(400 MHz, DMSO-d_6): δ(ppm) 12.36(s, 1H), 10.40-10.80(brs. 1H), 8.80-8.25(brs. 1H), 8.45(m, 2H), 8.12(d, J = 8.4 Hz, 1H), 8.05(s, 1H), 7.79(d, J = 8.2 Hz, 2H), 7.68(m, 1H), 7.49(d, J = 8.2 Hz, 2H), 7.29(m, 1H), 7.23(m, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ(ppm) 161.39(dd, J = 251,3Hz, 1C), 157.78(dd, J = 254,50 Hz, 1C), 154.12, 152.20, 151.66, 147.40, 142.66, 136.84, 135.04, 134.14, 133.53, 131.68, 129.65(d,J = 11 Hz, 1C), 129.43(2C), 128.84(2C), 125.02, 121.18(dd, J = 12,4 Hz, 1C), 112.61(dd, J = 9,3 Hz, 1C), 111.80, 102.03(t,J = 20 Hz, 1C), 97.91, 77.05. HRMS (ESI) calcd for C25H14ClF2N5O2S $[M+H]^+$: 522.0598; found 522.0597. HPLC purity = 96.09%, Rt 9.79 min.

N-(3-((3-(3-chlorophenyl)-1H-pyrrolo[2,3-b]pyridin-5-yl)ethynyl)-2,4-difluorophenyl)py ridine-3-sulfonamide 3q ^{1}H NMR(400 MHz, DMSO-d_6): δ(ppm) 14.26(s, 1H), 10.61(m, 1H),

8.88(d, J = 2.0 Hz, 1H), 8.85(m, 2H), 8.72(d, J = 1.8 Hz, 1H), 8.13(m, 1H), 8.06(m, 2H), 7.66(dd, J = 4.8 , 8.1 Hz, 1H), 7.56(t, J = 7.6 Hz, 8.1H), 7.52(m, 1H), 7.35(m, 1H), 7.26(t, J = 8.6 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ(ppm) 161.34(d,J = 251 Hz, 1C), 157.72(d,J = 254 Hz, 1C), 154.10, 151.55, 147.38, 142.80, 136.51, 135.05, 134.12, 132.69, 132.56, 131.41, 130.91, 130.50, 129.61(d,J = 9 Hz, 1C), 127.90, 124.79, 121.09(dd, J = 12,3 Hz, 1C), 113.13, 112.58(dd, J = 21,3 Hz, 1C), 111.50, 102.00(t,J = 20 Hz, 1C), 97.75, 76.96. HRMS (ESI) calcd for C25H14ClF2N5O2S $[M+H]^+$: 522.0598; found 522.0598. HPLC purity = 98.5%, Rt 7.68 min.

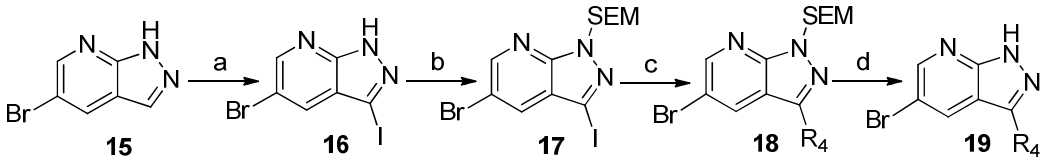
N-(3-((3-(2-chlorophenyl)-1H-pyrrolo[2,3-b]pyridin-5-yl)ethynyl)-2,4-difluorophenyl)pyridine-3-sulfonamide 3p ^{1}H NMR(400 MHz, DMSO-d_6): δ(ppm) 10.60(s, 1H), 8.86(d, J = 1.9 Hz, 1H), 8.84, 8.72(d, J = 2.0 Hz, 1H), 8.34(d, J = 1.9 Hz, 1H), 8.10(m, 1H), 7.67(m, 3H), 7.53(m, 12H), 7.31(m, 1H), 7.23(t, J = 8.7 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ(ppm) 161.33(dd, J = 250,4 Hz, 1C), 157.71(dd, J = 254,6 Hz, 1C), 154.11, 148.81, 147.40, 146.13, 136.84, 136.63, 135.06, 134.11, 131.16, 130.93, 129.26 (d,J = 10 Hz, 1C),126.83, 126.26, 125.48, 124.88, 121.09(d,J = 9 Hz, 1C), 116.98, 113.87, 112.54(dd, J = 22,4 Hz, 1C), 110.56, 102.37(t,J = 20 Hz, 1C), 99.10, 76.21. HRMS (ESI) calcd for C25H14ClF2N5O2S $[M+H]^+$: 522.0598; found 522.0599. HPLC purity = 98.4%, Rt 10.61 min.

Scheme S1. Synthesis of Compounds 14a and 14b[a]



[a]Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$, EtOH, 80 °C, overnight, 93%; (b) NaOH, DMSO, p-methoxybenzyl chloride, rt, 1 h, 62%; (c) NaH, R_3I, DMF, rt, overnight, 47%-53%; (d) TFA, reflux, overnight, 87%.

Scheme S2. Synthesis of Compounds 19[a]



[a]Reagents and conditions: (a) NIS, 1,2-dichloroethane, reflux, overnight, 68%; (b) SEM-Cl, NaH, DMF, 0℃-rt, 2h, 63%; (c) $R_4B(OH)_2$, $Pd(dppf)Cl_2$, K_3PO_4, 1,4-dioxane, 80℃, 5h, 37−73%; (d) DCM:TFA=1:1, reflux, overnight, 70-84%.

5-Bromo-1H-pyrazolo[3,4-b]pyridin-3-ol 11. A solution of **10** (4.0 g, 16.0 mmol) in ethanol (40.0 ml) was added hydrazine hydrate (12.9 ml, 48 mmol, 3 eq) and then stirred overnight at 80℃. After completion, the precipitated white solid was filtered, washed with ethanol and water, and dried to afford the desired product. (3.16 g, yield: 93%). 1H NMR (400 MHz, *d*-DMSO), δ 8.46 (d, *J* = 2.0 Hz, 1 H), 8.34 (d, *J* = 2.0 Hz, 1 H).

5-Bromo-1-(4-methoxybenzyl)-1H-pyrazolo[3,4-b]pyridin-3-ol 12. To a solution of **11** (1.5 g, 7.0 mmol) and NaOH (0.42 g, 10.5 mmol, 1.5 eq) in DMSO (20.0 ml) was added 4-methoxybenzyl-chloride (1.7g, 10.5 mmol, 1.5 eq) slowly under Ar atmosphere. After stirring for 1h at room temperature, the reaction mixture was diluted with ethyl acetate, washed with water, $NaHCO_3$ (aq.) and brine. The organic layer was dried over anhydrous Na2SO4 and concentrated to afford the product which was further purification through recrystallization in EA-PE solvent. (1.85 g, yield: 61.8 %) 1H NMR (400 MHz, *d*-DMSO), δ 10.82-11.18(brs, 1H), 8.56(d, *J* = 2.2 Hz, 1H), 8.33(d, *J* = 2.2 Hz, 1H), 7.18(d, *J* = 8.6 Hz, 1H), 6.86(d, *J* = 8.6 Hz, 1H), 6.33(s, 2H), 3.70(s, 3H).

5-Bromo-3-methoxy-1-(4-methoxybenzyl)-1H-pyrazolo[3,4-b]pyridine13a. Compound **12** (1.85 g, 5.5 mmol) was dissolved in DMF (20.0 ml) under ice base and then sodium hydride (60% in oil) (0.27g, 6.7 mmol, 1,2 eq) was added and stirred for 15 minutes. After addition of methyl iodide (0.42 ml, 6.7 mmol, 1.2 eq), the reaction mixture was stirred overnight. The residue was then diluted with ethyl acetate, washed with water (3 times) and brine, dried over anhydrous Na_2SO_4 and concentrated, which was purified by column chromatography, eluting with petroleum ether/ EtOAc (30:1) to give the desired product. (0.9 g, yield: 46.6%) 1H NMR (400 MHz, *d*-DMSO), δ 8.61 (d, *J* = 2.4 Hz, 1H), 8.40 (d, *J* = 2.0Hz, 1H), 7.17 (d, *J* = 8.8 Hz, 2H), 6.85 (d, *J* = 8.8 Hz, 2H), 5.41 (s, 2H), 3.99 (s, 3H), 3.70

(s, 3H).

5-bromo-3-methoxy-1H-pyrazolo[3,4-b]pyridine 14a. A solution of **13a**(900mg, 2.5 mmol) in trifluoroacetic acid (10 ml) was heated to reflux overnight. After the reaction was finished, the solvent was evaporated under reduced pressure. The residue was added 10 ml water, adjusted the pH value to 7 and then filtered. The solid was washed with water and ether and dried to give the desired product. (496mg, yield: 87%) 1H NMR (400 MHz, *d*-DMSO), δ 12.80(s, 1H), 8.54(d, *J* = 2.0 Hz, 1H), 8.36(d, *J* = 1.6 Hz, 1H), 4.00(s, 3H).

5-bromo-3-iodo-1H-pyrazolo[3,4-b]pyridine 16. A mixture of **15** (19.7 g, 100 mmol) and NIS (24.8 g, 110 mmol, 1.1 eq) in 1,2-dichloroethane (150 ml) was heated reflux overnight. After cooling to room temperature, the reaction mixture was diluted with THF, washed with $Na_2S_2SO_3$ (aq.) and brine, dried over anhydrous Na_2SO_4 and concentrated. The crude product was further purified by washing with ether to afford a brown solid. (21.9g, yield: 68%) 1H NMR (400 MHz, *d*-DMSO), δ 14.30 (s, 1 H), 8.63 (d, J = 2.0 Hz, 1 H), 8.18 (d, J = 2.0 Hz, 1 H).

5-Bromo-3-iodo-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazolo[3,4-b]pyridine 17. To a solution of **16** (20 g, 61.7 mmol) in DMF (154 ml) was added NaH (60% in oil) (6.17 g, 154 mmol, 2.5 eq) potionwisely at 0℃. The reaction mixture was stirred for 0.5 hr and then 2-(trimethylsilyl)ethoxymethyl chloride (17.5 g, 74 mmol, 1.2 eq) was added. The reaction was stirred for another 2.0 h at the same temperature. The ice-water was carefully added to quench the excess sodium hydride. The resulting slurry was extracted with EtOAc three times, washed with brine, dried and concentrated. The residue was purified through flash column chromatography, eluting with petroleum ether/ EtOAc (30:1) to give the desired product as a white solid. (17.7 g, yield: 63.2%) 1HNMR (400 MHz, *d*-DMSO), δ 8.72 (d, *J*= 2.0 Hz, 1 H), 8.25 (d, *J* = 2.0 Hz, 1 H), 5.73 (s, 2 H), 3.58 (t, *J* = 8.0 Hz, 2 H), 0.82 (t, *J* = 8.4Hz, 2 H), -0.12 (s, 9 H).

5-bromo-3-(4-fluorophenyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrazolo[3,4-b]pyridine 18a. A mixture of **21a** (1.0 g, 2.2 mmol), 4-fluorobenzeneboronic acid (0.308 g, 2.2 mmol, 1 eq), K_3PO_4 (1.17 g, 4.4 mmol, 2 eq) and Pd(dppf)Cl_2-DCM (0.18 g, 0.22 mmol, 0.1 eq) in 1,4-dioxane (5.0 ml) was added to a sealed tube. The tube was evacuated and backfilled with argon (3 cycles). After stirring at 80℃ for 5h, the reaction mixture was filtered and

concentrated. The residue was purified by flash column chromatography on silica gel, eluting with petroleum ether/ EtOAc (20:1) to afford the desired product as white solid. (0.687 g, yield: 74%). 1H NMR (400 MHz, $CDCl_3$), δ 8.57(d, *J* = 2.0 Hz, 1H), 8.36(d, *J* = 2.4 Hz, 1H), 7.87(m, 1H), 7.16(t, *J* = 8.8 Hz, 1H), 5.85 3.69(t, *J* = 8.4 Hz, 1H), 0.94(t, *J* = 8.4 Hz, 1H), -0.06(s, 9H).

5-bromo-3-(4-fluorophenyl)-1H-pyrazolo[3,4-b]pyridine 19b. A solution of **22**(0.687g, 1.62 mmol) in 20 ml DCM and trifluoroacetic acid (3:1) was heated to reflux overnight. After the reaction was finished, the solvent was evaporated under reduced pressure. The residue was added 10 ml water, adjusted the pH value to 7 and then filtered. The solid was washed with water and ether and dried to give the desired product. (396mg, yield: 84%) 1H NMR (400 MHz, *d*-DMSO), δ 8.71(d, *J* = 1.6 Hz, 1H), 8.38(d, *J* = 2.0 Hz, 1H), 7.82-7.85 (m, 2H), 7.14-7.19(m, 3H).

Computational Study. All the procedure was performed in Maestro 9.9 (Schrodinger LLC). The crystal structures of B-Raf protein and vemurafenib were taken from PDB ID 3OG7. The protein was processed using the "Protein Preparation Wizard" workflow in Maestro 9.9 (Schrodinger LLC) to adding bond orders and add hydrogens. All hetatm residues and crystal water molecules beyond 5 A from het group were removed. **3a** and **3o** were built by in LigPrep module using OPLS-2005 force field. Glide module was used as 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. Extra precision (XP) approach of Glide was adopted to dock **3a** and **3s** to B-Raf with the default parameters, and the top-ranking pose was selected to minimized the energy using Prime MM-GBSA, under the solvation model of VSGB.

***In Vitro* Enzymatic Activity Assay.** $B\text{-}Raf^{V600E}$ (as $B\text{-}Raf^{V599E}$ in supplier's catalogue) and the Z′- Lyte Kinase Assay Kit were purchased from Invitrogen. The experiments were performed according to the instructions of the manufacturer. The concentrations of kinases were determined by optimization experiments and the respective concentration was: $BRAF^{V600E}$ (PV4173, Invitrogen) 0.22 μg/ μL. First, the compounds were diluted three-fold

from 5.1×10^{-9} M to $1x10^{-4}$ M in DMSO and a 400 μM compound solution was prepared (4 μL compound dissolved in 96 μL water). Second, a 100 μM ATP solution in1.33×Kinase Buffer was prepared. Third, a kinase/peptide mixture containing 2×kinase and 4 μM Tyr 4 peptide (Invitrogen, PV3193) was prepared right before use.

Kinase/peptide mixture was prepared by diluting Z'-LYTE Ser/Thr3 peptide (Invitrogen, PV3176) and three kinases (B-Raf, MAP2K1/MEK1 (Invitrogen, P3093), MAPK1/ERK2 (Invitrogen, PV3314)) in 1×Kinase Buffer, and 0.2 μM Ser/Thr3 phospho-peptide solution was made by adding Z'-LYTE Ser/Thr3 phospho-peptide to 1×Kinase Buffer. The final 10 μL reaction consists of 0.002 ng of B-Raf,10 ng inactive MAP2K1 (MEK1),100 ng inactive MAPK1 (ERK2), 2 μM Ser/Thr3 peptide in 1×kinase buffer.

For each assay, 10 μL kinase reactions were made at first (including 2.5 μL compound solution, 5 μL Kinase/Peptide Mixture, and 2.5 μL ATP solution). Mixed the plate thoroughly and incubated for one hour at room temperature. Then 5 μL development solution was added to each well and the plate was incubated for 1h at room temperature; the nonphosphopeptides were cleaved at this time. In the end, 5 μL stop reagent was loaded to stop the reaction. For the control setting, 5 μL phospho-peptide solution instead of kinase/peptide mixture was used as 100% phosphorylation control. 2.5 μL 1.33×Kinase Buffer instead of ATP solution was used as 100% inhibition control, and 2.5 μL 4% DMSO instead of compound solution was used as the 0% inhibitor control. The plate was measured on an *EnVision* Multilabel Reader (Perkin-Elmer). Curve fitting and data presentations were performed using Graph Pad Prism, version 5.0. Every experiment was repeated at least 2 times.

Cell Proliferation and Growth Inhibition Assay. a. The human colorectal adenocarcinoma cell lines HT-29, HCT-116, Colo205, LOVO and malignant melanoma cell lines A375, SK-MEL-28, SK-MEL-2, SK-MEL-1 were purchased from ATCC. HT-29 and HCT116 were maintained in McCoy's 5a with 10% FBS, Colo205, LOVO and A375 were maintained in RPMI-1640, F12K and DMEM with 10% FBS respectively, while SK-MEL-2, SK-MEL-1 and SK-MEL-28 were grown in Eagle's Minimum Essential Medium with 10% FBS. Cells of log phase were used. 1000-3000 cells/well were seeded in 96-well plates with a 100 μL volume, and 6 parallels and 8 rows were designed. Compounds were dissolved to 10 μM with DMSO, and a 5-fold serial dilution of the compounds from 1×10^{-5} M to 0.64×10^{-9} M

was performed. 2 μL of compound solution was added to 998 μL of growth medium, the mixture was vortexed sufficiently. 100 μL of the mixture was correspondingly added to the 96-well plate. 2 μL DMSO instead of compound solution was used as the 0% inhibitor control. After coincubation for 68 h, 20 μL MTT (5mg/ ml) was added. 4h later, the supernatant was discarded completely and 150 μL DMSO was added. After shaking for 10 min, the plates were read in the Synerg HT (Bio Tek) at 570 nm. The data was calculated using Graph Pad Prism version 4.0. The IC_{50} were fitted using a nonlinear regression model with a sigmoidal dose response. **b.** Monolayer cultures of primary melanoma cells were harvested, counted and seeded into 96-well plates at appropriate densities (2500 cells/well for NZM07, 6000 cells/well for NZM09, 4000 cells/well for NZM20 and 500-1000 cells/well for NZM40). Cells were allowed to settle for 24 hours in 5% O_2 incubators. Cells were then treated with each test compound for a continuous exposure of 5 days under 5% O_2 conditions. Cells were fixed in 1% trichloroacetic acid (TCA) and stained with Sulforhodamine B (SRB) to measure total cells. Cell density was determined using Biotek ELx808 Absorbance Microplate Reader. IC50 values for each compound were determined by interpolation as the drug concentration reducing staining to 50% of untreated control wells on the same plate.

Western Blot. **a.** log-phase primary melanoma NZM20 and NZM40 cells were seeded into 6-well plates at a density of 1 million cells per well and were incubated for 24 hours under 5% O_2 conditions. Drug stock solutions were diluted in media to achieve final concentrations in each well. Following 2 hours of drug exposure, cells were washed in ice-cold PBS and lysed on ice for 30 minutes with radioimmunoprecipitation assay (RIPA) buffer containing 100× protease inhibitor, and sodium orthovanadate and sodium fluoride at final concentrations of 1 mM each. Cell debris was pelleted out by centrifugation (13,000 rpm for 2 minutes). Protein concentrations in each sample were determined by a bicinchoninic acid (BCA) assay. Equivalent amounts of protein (20 μg) were denatured (98 °C for 5 minutes) and loaded onto 4-20% pre-cast Bis-Tris protein gels for protein separation. Following transfer to a nitrocellulose membrane, each membrane was blocked with 5% bovine serum albumin (in Tris-buffered saline) for 1 hour. Primary antibody against ERK/MAPK (total or phospho-specific) or α-tubulin was added overnight at 4 ˚C in 5% bovine serum albumin (with

Tris-buffered saline). Excess primary antibody in each membrane was washed off with Tris-buffered saline. Secondary antibody was added to each membrane for 2 hours. For protein detection, the membrane was washed in Tris-buffered saline to remove excess secondary antibody and chemiluminescent substrate was added (Pierce Supersignal West Pico Chemiluminescent Substrate) for 5 minutes. The membrane was viewed using a Fujifilm LAS 4000 imager. **b.** 1×10^6 Cells of Colo205, HTC116 cells were seeded into 6-cm dishes overnight. The medium was changed, and 1, 0.3, 0.1, 0.03, 0.01 μM/L of **3s** was added the next day; medium with 1‰ DMSO was used as the control. Cells were exposed to treatment after indicated hours. The dishes was washed twice using precold PBS and 400 μL of RIPA then. After incubating plates on ice for 15 min, cells were scraped carefully and centrifuged for 10 minutes at 14,000g at 4 ˚C immediately. The remaining supernatant and lysates were maintained at -70˚C. A BCA protein assay kit (23227, Thermo) was used to quantitate the cell lysates. Proper 5× loading buffer was loaded before use, and the samples were denatured by boiling. The same amount of quantitated sample was loaded, and proteins were transferred to the PVDF membrane (Milipore) then. After blocking for 1.0 h at room temperature, diluted primary antibody ERK (CST, 9102), phospho-ERK (t202/y204) (CST, 9101), and GAPDH (KC-5G5, KangChen) were added. A second antibody with horseradish peroxidase (HRP, sigma) conjugated was used then. Blots were developed by enhanced chemiluminescence (Thermo).

Mice Xenograft Using Colo205. Male SCID mice were purchased from Vital River Laboratory Animal Technology Inc. (Beijing, China). All animal studies were approved by the Institutional Animal Use and Care Committee of Guangzhou Institute of Biomedicine and Health, Chinese Academy of Science. COLO205 cells were resuspended in normal saline (NS) solution (2.5 ×107 cell/mL). A 0.2 mL amount of cell suspension was injected subcutaneously into the right flank of each mouse. Mice were randomly grouped based on the tumor volume when the mean tumor volume reached 100−200 mm^3. Compound **3s** (formulated as sodium salts) and drug **1** (used as original power) were dissolved in sodium carboxymethyl cellulose. Mice were treated for the 14 consecutive days by oral gavage with **3a** (50 mg/kg once daily, 100mg/kg once daily, 100mg/kg twice daily), drug **1** (30 mg/kg once daily), and vehicle,

respectively. Tumor volume and body weight were monitored once every 2 days. Tumor volume was calculated as the L × W (L and W are the length and width of the tumor, respectively). After the last measurement, mice were sacrificed and the tumor were separated and photographed.

KINOME*scan* ™: kinase-tagged T7 phage strains were prepared in an *E. coli* host derived from the BL21 strain. *E. coli* were grown to log-phase and infected with T7 phage and incubated with shaking at 32°C until lysis. The lysates were centrifuged and filtered to remove cell debris. The remaining kinases were produced in HEK-293 cells and subsequently tagged with DNA for qPCR detection. Streptavidin-coated magnetic beads were treated with biotinylated small molecule ligands for 30 minutes at room temperature to generate affinity resins for kinase assays. The liganded beads were blocked with excess biotin and washed with blocking buffer (SeaBlock (Pierce), 1% BSA, 0.05% Tween 20, 1 mM DTT) to remove unbound ligand and to reduce non-specific binding. Binding reactions were assembled by combining kinases, liganded affinity beads, and test compounds in 1x binding buffer (20% SeaBlock, 0.17x PBS, 0.05% Tween 20, 6 mM DTT). All reactions were performed in polystyrene 96-well plates in a final volume of 0.135 ml. The assay plates were incubated at room temperature with shaking for 1 hour and the affinity beads were washed with wash buffer (1x PBS, 0.05% Tween 20). The beads were then re-suspended in elution buffer (1x PBS, 0.05% Tween 20, 0.5 μM non-biotinylated affinity ligand) and incubated at room temperature with shaking for 30 minutes. The kinase concentration in the eluates was measured by qPCR.

For Kd determination, an 11-point 3-fold serial dilution of compound **3s** was prepared in 100% DMSO at 100x final test concentration and subsequently diluted to 1x in the assay (final DMSO concentration = 1%). Binding constants (Kds) were calculated with a standard dose-response curve using the Hill equation.

For primary screening, compound **3s** were screened at the concentration of 2μM/L, and the results are reported as `% Ctrl`.

Table S1. Pharmacokinetic Profile of Selected Compounds in Rats [a]

compd	iv (5 mg/kg)			p.o. (25 mg/kg)			F (%)
	AUC(0-∞) μg/L*h	Cmax μg/L	T1/2 (h)	AUC(0-∞) μg/L*h	Cmax μg/L	T1/2 (h)	
3n	17,033	22,125	2.2	28,790	11,833	1.1	33.8%
3o	10,643	27,250	2.3	3,829	1,628	1.4	7.2%
3s	33,060	36,250	2.2	96,342	45,850	0.9	54%

[a]SD rats (male, 3-4 animals per group) weighted 180−220g were used for the study.

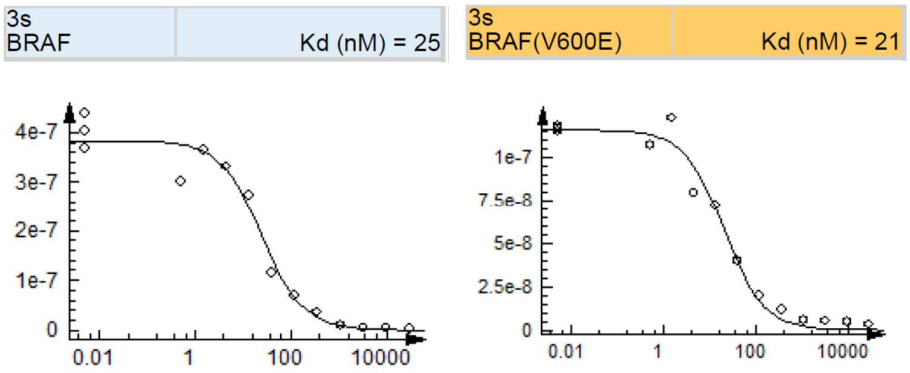


Figure S2. Dose-response behaviour of BRAF(wild type)$_{Kd}$ and BRAF(V600E)$_{Kd}$ upon treatment with inhibitor **3s.** The amount of kinase measured by qPCR (Signal; y-axis) is plotted against the corresponding compound concentration in nM in log10 scale (x-axis).

Table S2. Matrix of Compound Screen for Inhibitor 3s.

Target	**3s**
Gene Symbol	%Ctrl @ 1000nM
AAK1	80
ABL1(E255K)-phosphorylated	73
ABL1(F317I)-nonphosphorylated	84
ABL1(F317I)-phosphorylated	100
ABL1(F317L)-nonphosphorylated	91
ABL1(F317L)-phosphorylated	75
ABL1(H396P)-nonphosphorylated	92
ABL1(H396P)-phosphorylated	88
ABL1(M351T)-phosphorylated	70
ABL1(Q252H)-nonphosphorylated	78
ABL1(Q252H)-phosphorylated	93
ABL1(T315I)-nonphosphorylated	86

ABL1(T315I)-phosphorylated	80
ABL1(Y253F)-phosphorylated	85
ABL1-nonphosphorylated	76
ABL1-phosphorylated	71
ABL2	95
ACVR1	95
ACVR1B	97
ACVR2A	80
ACVR2B	89
ACVRL1	90
ADCK3	81
ADCK4	100
AKT1	90
AKT2	79
AKT3	100
ALK	99
ALK(C1156Y)	92
ALK(L1196M)	97
AMPK-alpha1	99
AMPK-alpha2	100
ANKK1	99
ARK5	87
ASK1	96
ASK2	94
AURKA	87
AURKB	79
AURKC	86
AXL	96
BIKE	88
BLK	80
BMPR1A	82
BMPR1B	75
BMPR2	77
BMX	67
BRAF	4.1
BRAF(V600E)	3.3
BRK	49
BRSK1	100
BRSK2	100
BTK	100
BUB1	45
CAMK1	89

CAMK1B	84
CAMK1D	97
CAMK1G	98
CAMK2A	64
CAMK2B	83
CAMK2D	98
CAMK2G	92
CAMK4	94
CAMKK1	59
CAMKK2	70
CASK	86
CDC2L1	90
CDC2L2	91
CDC2L5	97
CDK11	17
CDK2	97
CDK3	98
CDK4	100
CDK4-cyclinD1	87
CDK4-cyclinD3	86
CDK5	91
CDK7	71
CDK8	41
CDK9	100
CDKL1	82
CDKL2	100
CDKL3	100
CDKL5	92
CHEK1	73
CHEK2	70
CIT	77
CLK1	95
CLK2	81
CLK3	98
CLK4	85
CSF1R	97
CSF1R-autoinhibited	94
CSK	91
CSNK1A1	66
CSNK1A1L	90
CSNK1D	95
CSNK1E	90

CSNK1G1	77
CSNK1G2	70
CSNK1G3	92
CSNK2A1	84
CSNK2A2	85
CTK	84
DAPK1	100
DAPK2	86
DAPK3	79
DCAMKL1	86
DCAMKL2	100
DCAMKL3	75
DDR1	65
DDR2	97
DLK	83
DMPK	92
DMPK2	100
DRAK1	87
DRAK2	88
DYRK1A	62
DYRK1B	61
DYRK2	90
EGFR	79
EGFR(E746-A750del)	91
EGFR(G719C)	87
EGFR(G719S)	92
EGFR(L747-E749del, A750P)	100
EGFR(L747-S752del, P753S)	82
EGFR(L747-T751del,Sins)	93
EGFR(L858R)	89
EGFR(L858R,T790M)	96
EGFR(L861Q)	77
EGFR(S752-I759del)	95
EGFR(T790M)	100
EIF2AK1	86
EPHA1	97
EPHA2	91
EPHA3	100
EPHA4	100
EPHA5	95
EPHA6	100
EPHA7	88

EPHA8	88
EPHB1	88
EPHB2	90
EPHB3	100
EPHB4	94
EPHB6	3.2
ERBB2	100
ERBB3	89
ERBB4	100
ERK1	83
ERK2	95
ERK3	100
ERK4	100
ERK5	96
ERK8	95
ERN1	84
FAK	95
FER	99
FES	91
FGFR1	100
FGFR2	100
FGFR3	81
FGFR3(G697C)	100
FGFR4	100
FGR	88
FLT1	89
FLT3	33
FLT3(D835H)	47
FLT3(D835V)	85
FLT3(D835Y)	56
FLT3(ITD)	52
FLT3(ITD,D835V)	69
FLT3(ITD,F691L)	92
FLT3(K663Q)	39
FLT3(N841I)	66
FLT3(R834Q)	91
FLT3-autoinhibited	76
FLT4	93
FRK	47
FYN	97
GAK	90
GCN2(Kin.Dom.2,S808G)	2.2

GRK1	90
GRK2	100
GRK3	98
GRK4	92
GRK7	95
GSK3A	80
GSK3B	83
HASPIN	90
HCK	56
HIPK1	68
HIPK2	87
HIPK3	67
HIPK4	64
HPK1	83
HUNK	47
ICK	73
IGF1R	76
IKK-alpha	100
IKK-beta	93
IKK-epsilon	87
INSR	100
INSRR	100
IRAK1	89
IRAK3	100
IRAK4	98
ITK	100
JAK1(JH1domain-catalytic)	83
JAK1(JH2domain-pseudokinase)	70
JAK2(JH1domain-catalytic)	100
JAK3(JH1domain-catalytic)	94
JNK1	73
JNK2	100
JNK3	99
KIT	55
KIT(A829P)	77
KIT(D816H)	100
KIT(D816V)	88
KIT(L576P)	42
KIT(V559D)	47
KIT(V559D,T670I)	95
KIT(V559D,V654A)	90
KIT-autoinhibited	91

LATS1	100
LATS2	60
LCK	96
LIMK1	52
LIMK2	75
LKB1	88
LOK	74
LRRK2	96
LRRK2(G2019S)	75
LTK	84
LYN	89
LZK	100
MAK	100
MAP3K1	72
MAP3K15	87
MAP3K2	93
MAP3K3	92
MAP3K4	100
MAP4K2	100
MAP4K3	100
MAP4K4	75
MAP4K5	76
MAPKAPK2	88
MAPKAPK5	92
MARK1	100
MARK2	82
MARK3	74
MARK4	100
MAST1	97
MEK1	89
MEK2	81
MEK3	74
MEK4	66
MEK5	21
MEK6	92
MELK	86
MERTK	98
MET	77
MET(M1250T)	88
MET(Y1235D)	95
MINK	79
MKK7	84

MKNK1	80
MKNK2	94
MLCK	30
MLK1	80
MLK2	74
MLK3	95
MRCKA	95
MRCKB	93
MST1	80
MST1R	89
MST2	98
MST3	98
MST4	92
MTOR	100
MUSK	94
MYLK	85
MYLK2	100
MYLK4	63
MYO3A	85
MYO3B	100
NDR1	81
NDR2	97
NEK1	94
NEK10	99
NEK11	100
NEK2	89
NEK3	63
NEK4	90
NEK5	100
NEK6	76
NEK7	89
NEK9	89
NIK	82
NIM1	98
NLK	100
OSR1	100
p38-alpha	94
p38-beta	94
p38-delta	82
p38-gamma	99
PAK1	97
PAK2	87

PAK3	90
PAK4	93
PAK6	96
PAK7	100
PCTK1	100
PCTK2	91
PCTK3	95
PDGFRA	73
PDGFRB	62
PDPK1	87
PFCDPK1(P.falciparum)	10
PFPK5(P.falciparum)	100
PFTAIRE2	98
PFTK1	99
PHKG1	100
PHKG2	92
PIK3C2B	91
PIK3C2G	96
PIK3CA	94
PIK3CA(C420R)	93
PIK3CA(E542K)	65
PIK3CA(E545A)	81
PIK3CA(E545K)	67
PIK3CA(H1047L)	56
PIK3CA(H1047Y)	96
PIK3CA(I800L)	77
PIK3CA(M1043I)	95
PIK3CA(Q546K)	76
PIK3CB	79
PIK3CD	93
PIK3CG	82
PIK4CB	74
PIKFYVE	96
PIM1	100
PIM2	67
PIM3	90
PIP5K1A	83
PIP5K1C	69
PIP5K2B	100
PIP5K2C	97
PKAC-alpha	94
PKAC-beta	93

PKMYT1	97
PKN1	86
PKN2	100
PKNB(M.tuberculosis)	94
PLK1	97
PLK2	85
PLK3	88
PLK4	71
PRKCD	84
PRKCE	100
PRKCH	94
PRKCI	78
PRKCQ	87
PRKD1	100
PRKD2	95
PRKD3	68
PRKG1	96
PRKG2	87
PRKR	83
PRKX	100
PRP4	86
PYK2	100
QSK	97
RAF1	0.55
RET	98
RET(M918T)	89
RET(V804L)	90
RET(V804M)	100
RIOK1	90
RIOK2	90
RIOK3	92
RIPK1	100
RIPK2	90
RIPK4	83
RIPK5	91
ROCK1	88
ROCK2	83
ROS1	100
RPS6KA4(Kin.Dom.1-N-terminal)	82
RPS6KA4(Kin.Dom.2-C-terminal)	66
RPS6KA5(Kin.Dom.1-N-terminal)	95
RPS6KA5(Kin.Dom.2-C-terminal)	90

RSK1(Kin.Dom.1-N-terminal)	56
RSK1(Kin.Dom.2-C-terminal)	100
RSK2(Kin.Dom.1-N-terminal)	63
RSK2(Kin.Dom.2-C-terminal)	100
RSK3(Kin.Dom.1-N-terminal)	90
RSK3(Kin.Dom.2-C-terminal)	96
RSK4(Kin.Dom.1-N-terminal)	75
RSK4(Kin.Dom.2-C-terminal)	99
S6K1	90
SBK1	100
SGK	76
SgK110	100
SGK2	75
SGK3	98
SIK	53
SIK2	100
SLK	92
SNARK	93
SNRK	89
SRC	95
SRMS	54
SRPK1	91
SRPK2	87
SRPK3	55
STK16	61
STK33	83
STK35	99
STK36	89
STK39	80
SYK	77
TAK1	79
TAOK1	100
TAOK2	91
TAOK3	100
TBK1	90
TEC	92
TESK1	86
TGFBR1	90
TGFBR2	93
TIE1	100
TIE2	95
TLK1	95

TLK2	99
TNIK	85
TNK1	61
TNK2	97
TNNI3K	68
TRKA	60
TRKB	81
TRKC	74
TRPM6	68
TSSK1B	90
TSSK3	98
TTK	92
TXK	99
TYK2(JH1domain-catalytic)	100
TYK2(JH2domain-pseudokinase)	93
TYRO3	100
ULK1	100
ULK2	92
ULK3	79
VEGFR2	96
VPS34	68
VRK2	86
WEE1	91
WEE2	91
WNK1	97
WNK2	100
WNK3	100
WNK4	100
YANK1	91
YANK2	99
YANK3	78
YES	88
YSK1	91
YSK4	75
ZAK	1.6
ZAP70	82

Table S3. TREEspot™ Interaction Maps for 3s.

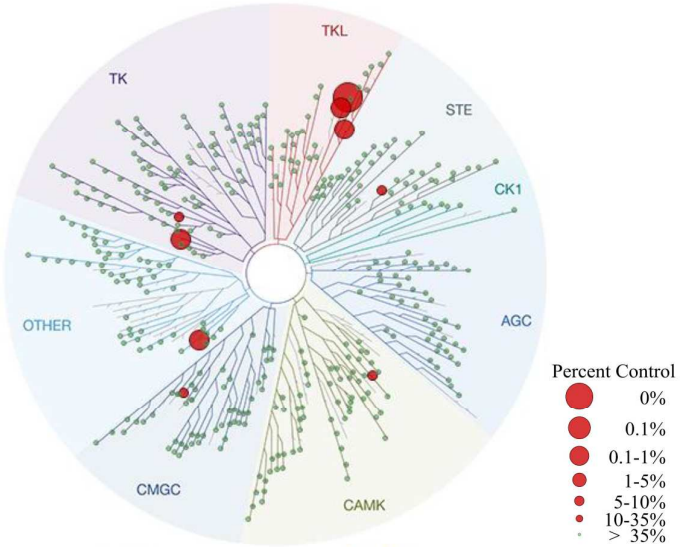


Table S4. S-score Table for 3s.

Compound	Selectivity Score type	Number of Hits	Number of Non-Mutant Kinases	Screening Concentration (nM)	Selectivity Score
3s	S(35)	10	403	1000	0.025
3s	S(10)	5	403	1000	0.012
3s	S(1)	1	403	1000	0.002

Note: S(35) = (number of non-mutant kinases with %Ctrl <35)/(number of non-mutant kinases tested). S(10) = (number of non-mutant kinases with %Ctrl <10)/(number of non-mutant kinases tested). S(1) = (number of non-mutant kinases with %Ctrl <1)/(number of non-mutant kinases tested)

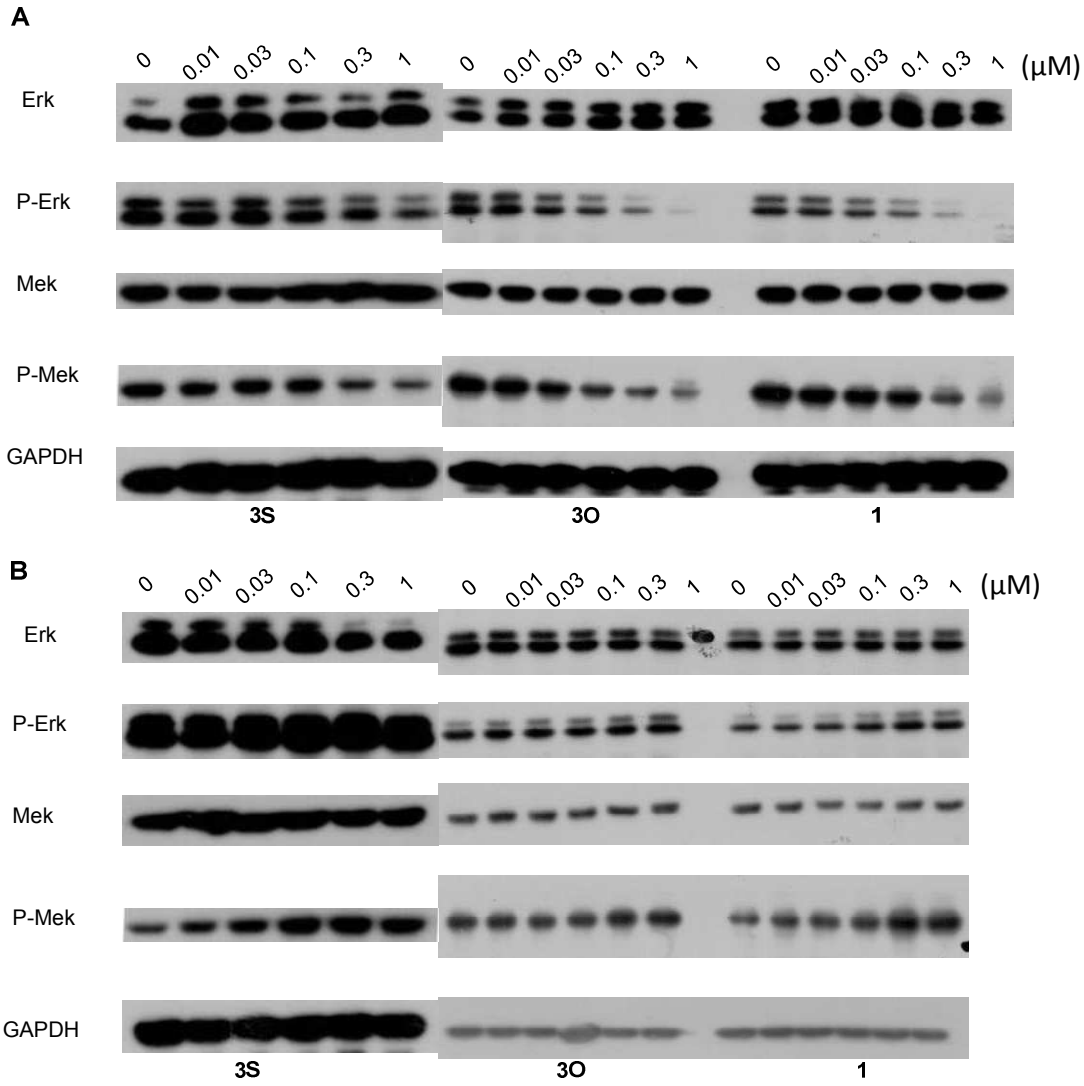


Figure S3. 3o and **3s** dose-dependently inhibit the activation of MAPK pathway in COLO205 colon cancer cells with B-Raf^{V600E}, in a similar way of vemurafenib (**1**) (**A**), but elevate the phosphorylation of Mek and Erk in HTC116 cells harboring B-Raf^{WT} (**B**).

Table S5. Enzymatic and Cellular Activities of Dabrafenib (IC_{50}, μM/L)

	B-Raf^{V600E} kinase	B-Raf^{V600E}					B-Raf^{WT}	
		colo205	435s	HT29	WiDR	sk-mel-28	HCT-116	H460
dabrafenib	0.001	0.006	>10	0.012	0.865	0.008	6.346	>10

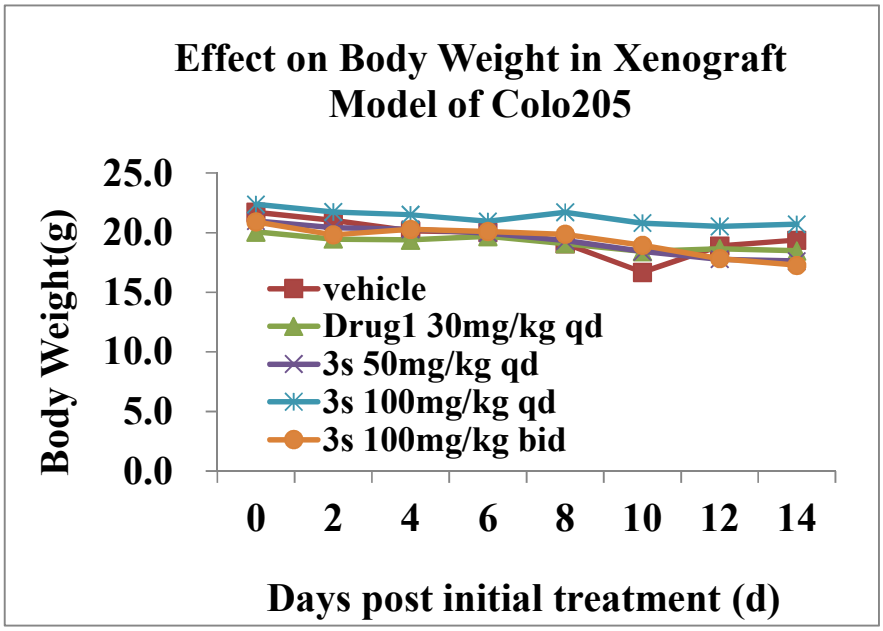
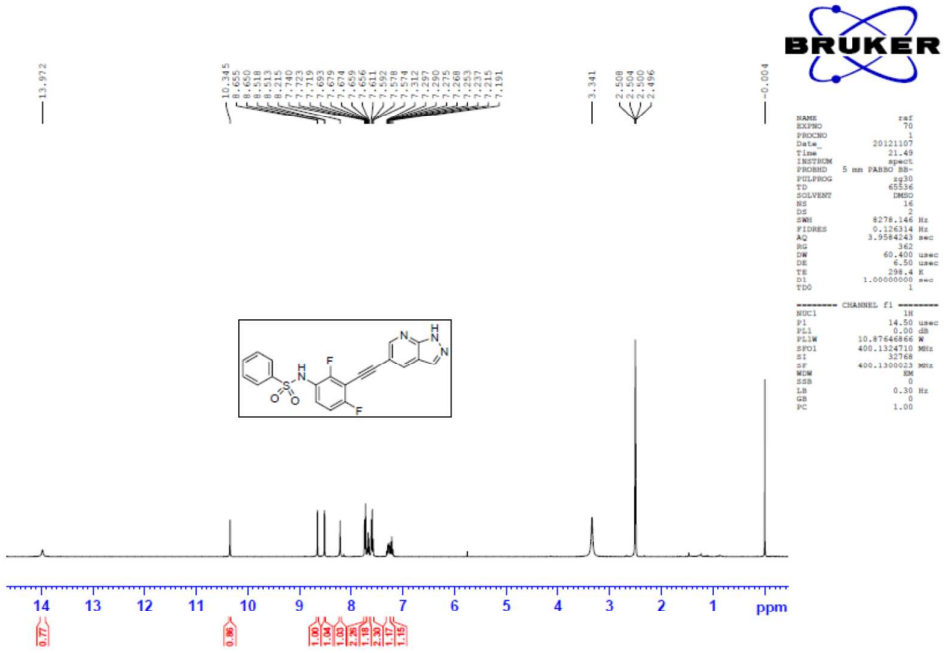
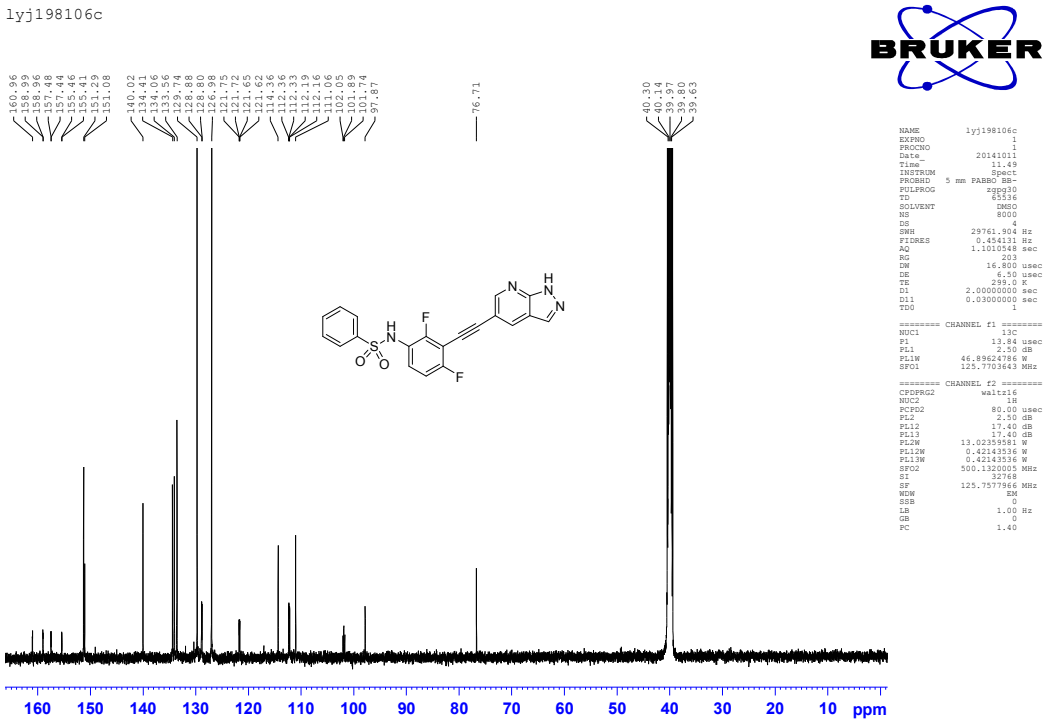


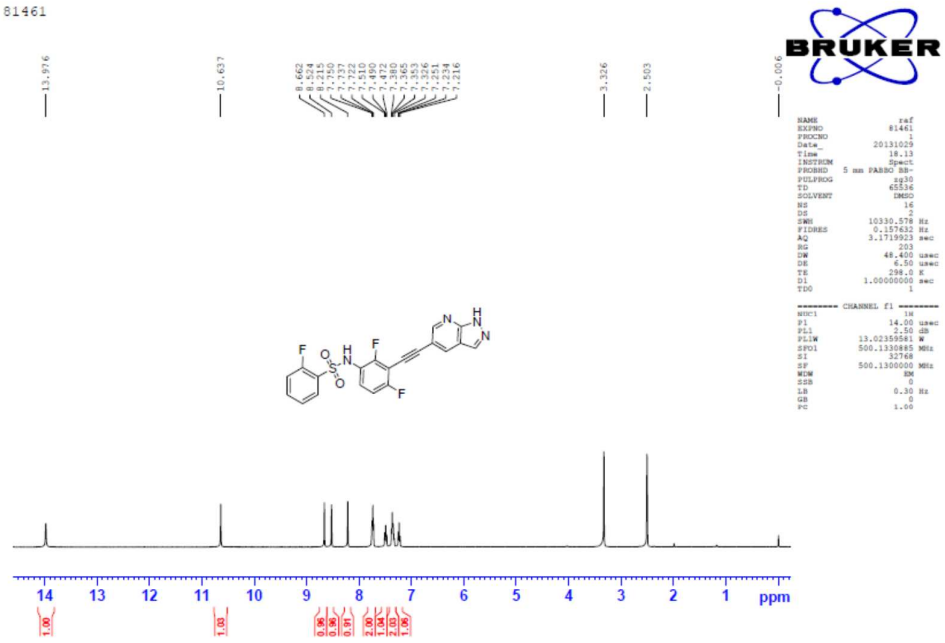
Figure S4. 3s do not affect the body weight of mice in xenograft model of Colo205. Days post initial treatment (d; y-axis) is plotted against the corresponding body weight (g; x-axis).

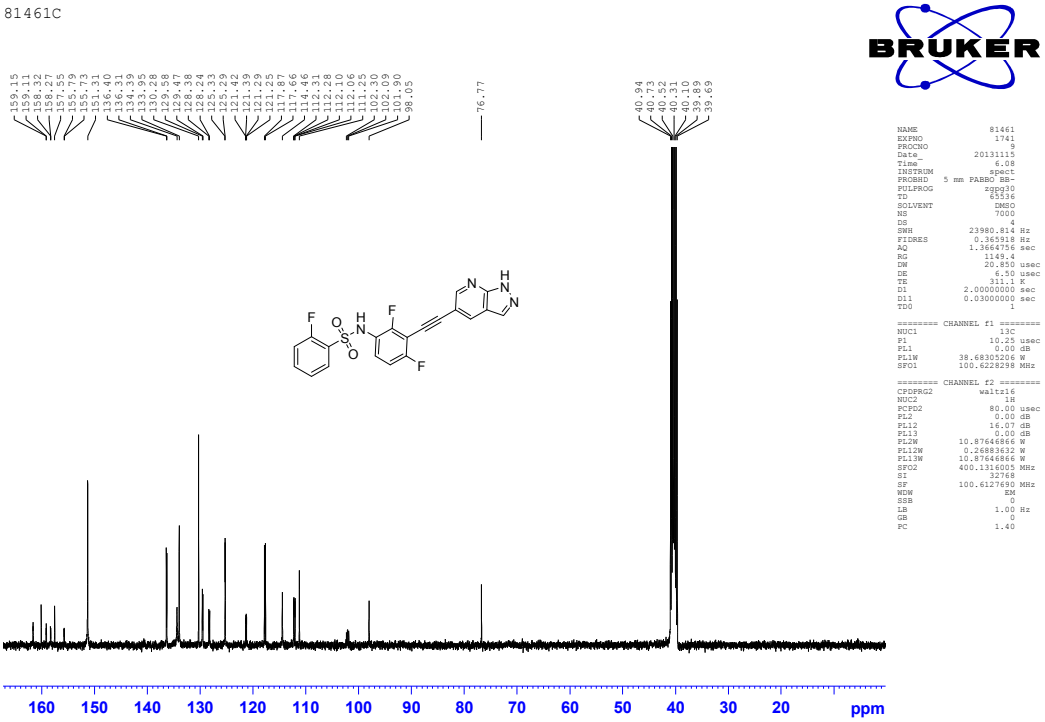
198014

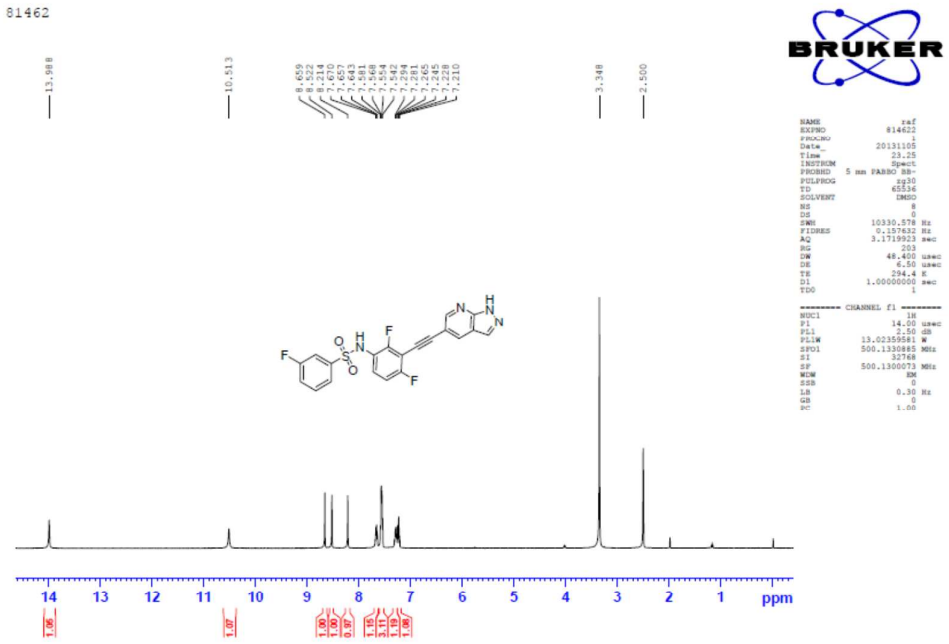


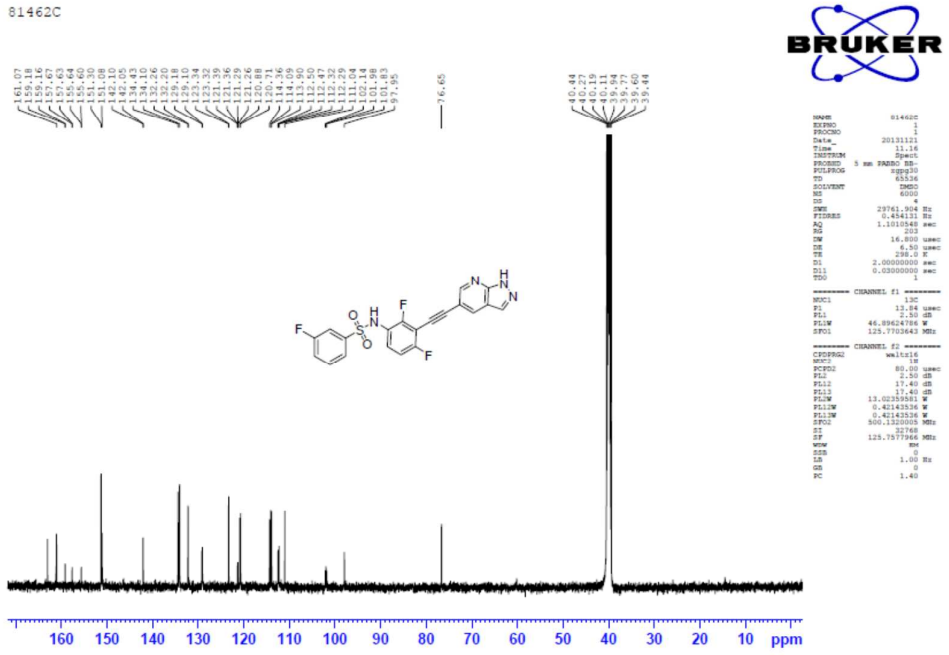
lyj198106c

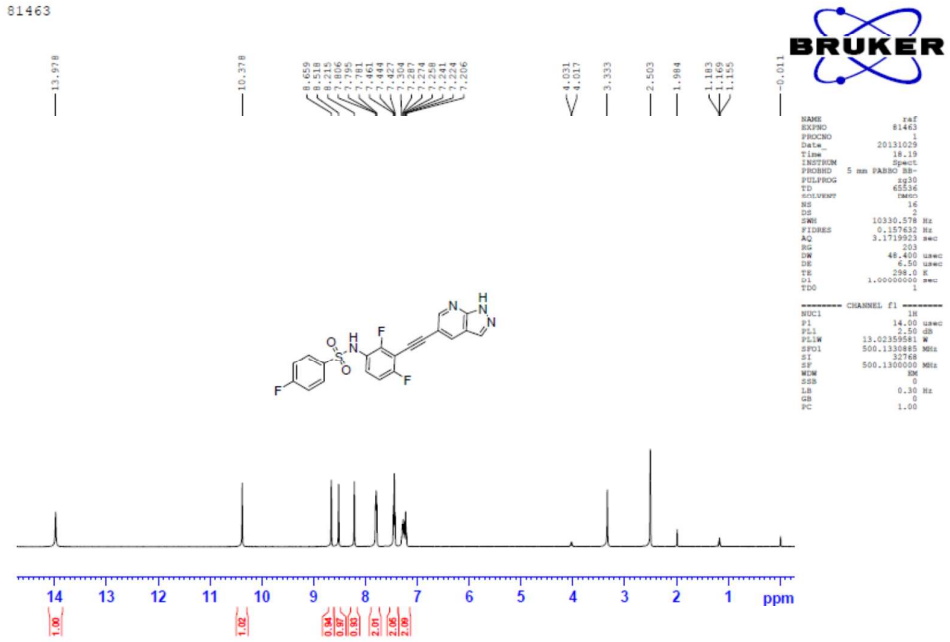


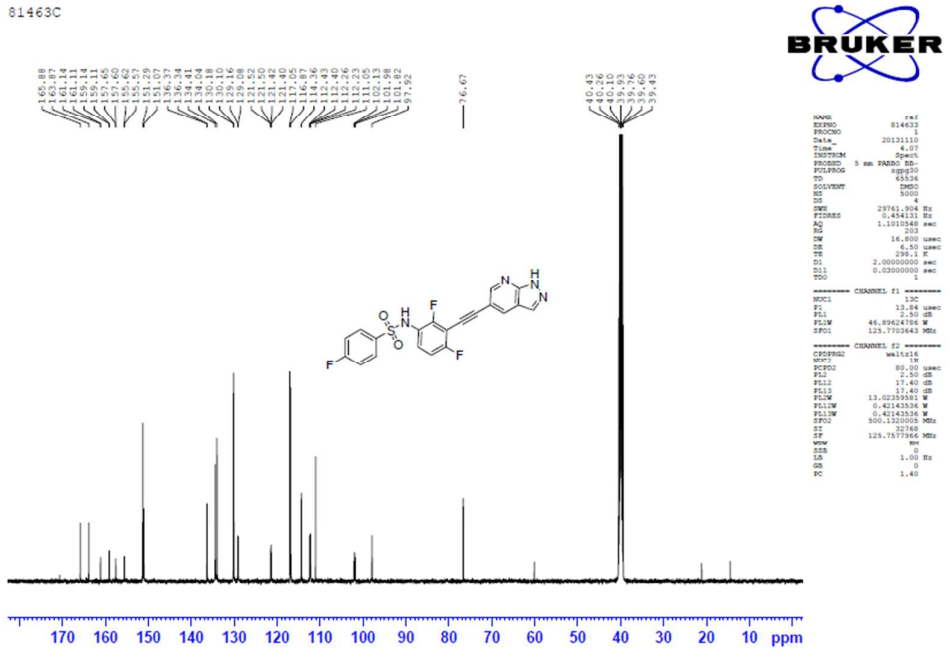

81461
13.976
10.637
8.662
8.524
8.215
7.750
7.727
7.722
7.510
7.490
7.472
7.340
7.365
7.353
7.336
7.251
7.234
7.216
3.326
2.503
-0.006
BRUKER
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm

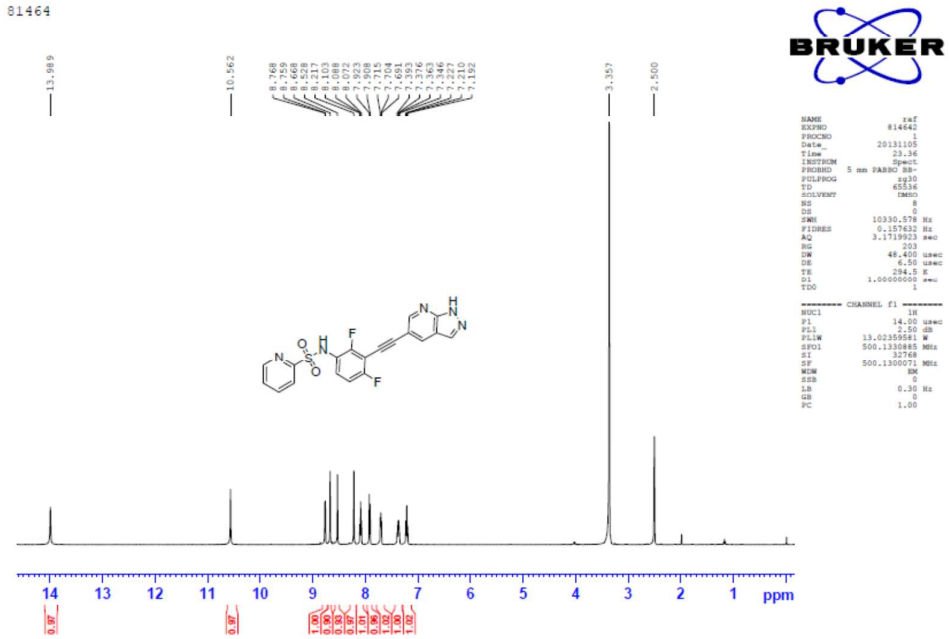

81461C
159.15
159.11
158.32
158.27
157.55
157.49
155.73
151.31
136.40
136.31
134.39
134.35
130.28
129.58
129.47
128.38
128.24
128.13
125.29
121.42
121.39
121.29
121.25
121.07
117.66
114.46
112.31
112.28
112.10
112.06
111.25
102.30
102.09
101.90
98.05
76.77
40.94
40.73
40.52
40.31
40.10
39.89
39.69
BRUKER
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

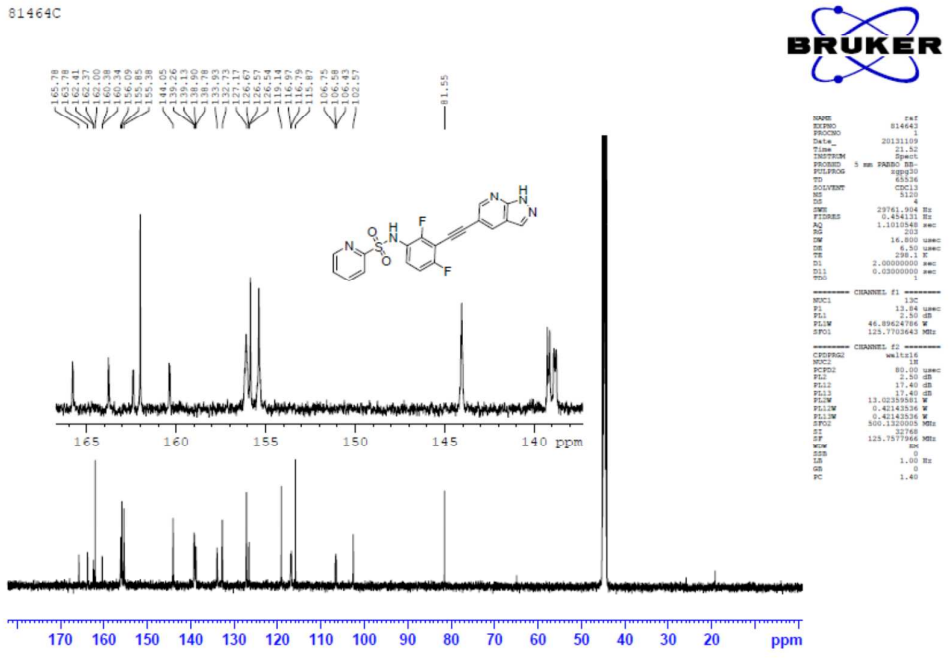

81462
13.988
10.513
8.659
8.522
8.214
8.030
7.657
7.643
7.581
7.568
7.554
7.542
7.284
7.281
7.265
7.245
7.218
7.210
3.348
2.500
BRUKER
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm

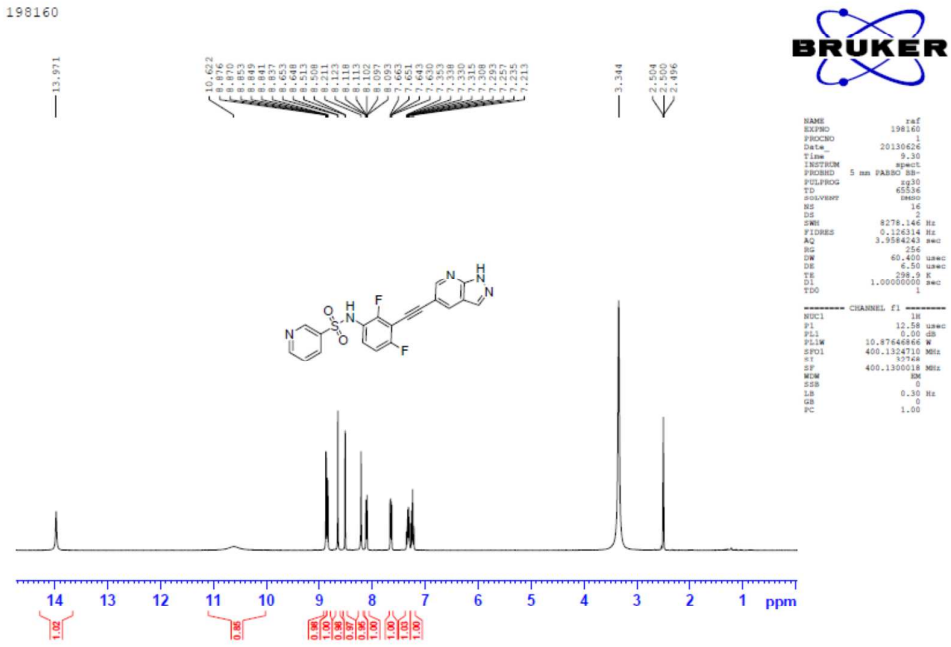

81462C
161.07
159.18
159.16
157.67
157.63
155.64
155.60
151.30
151.08
142.10
142.05
134.43
134.10
132.26
132.20
129.18
129.10
123.34
123.32
121.39
121.36
121.29
121.26
120.88
120.71
114.36
114.29
113.90
112.50
112.47
112.32
112.29
111.04
102.14
101.88
101.83
97.95
76.65
40.44
40.27
40.19
40.11
39.94
39.77
39.60
39.44
BRUKER
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

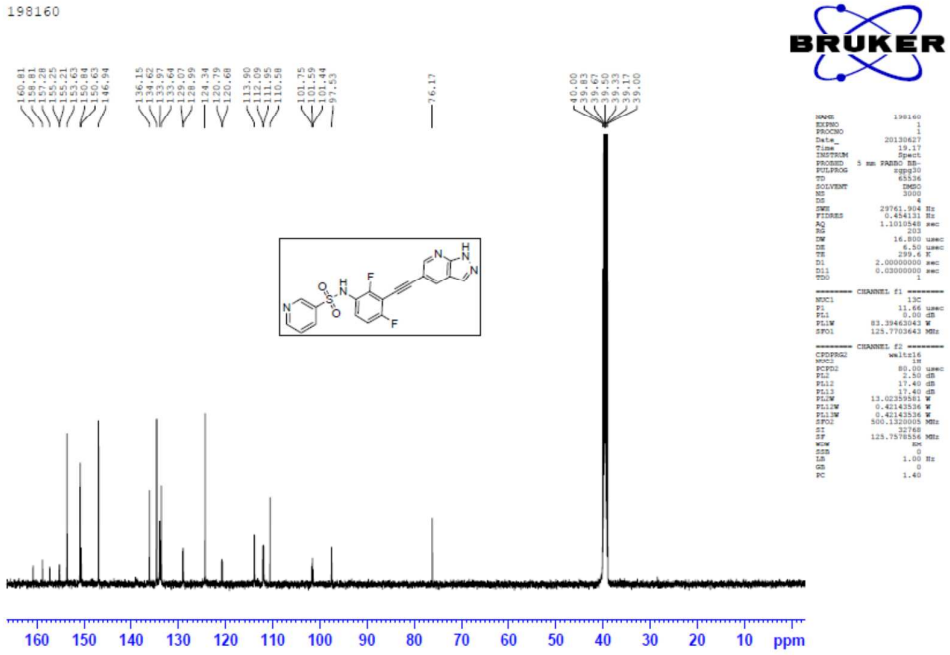

81463
BRUKER
13.978
10.378
8.659
8.638
8.215
7.806
7.795
7.781
7.461
7.444
7.427
7.409
7.287
7.274
7.258
7.244
7.224
7.206
4.031
4.017
3.333
2.503
1.984
1.183
1.169
1.155
-0.011
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm


81463C
BRUKER
165.89
163.87
161.14
161.11
159.14
159.11
157.65
157.60
155.62
155.57
151.29
151.07
136.37
136.34
134.41
134.04
130.18
130.10
129.16
129.08
121.52
121.50
121.42
121.40
117.65
117.62
114.36
112.43
112.40
112.26
112.23
111.05
102.13
102.08
101.82
97.92
76.67
40.43
40.26
40.09
39.93
39.76
39.60
39.43
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

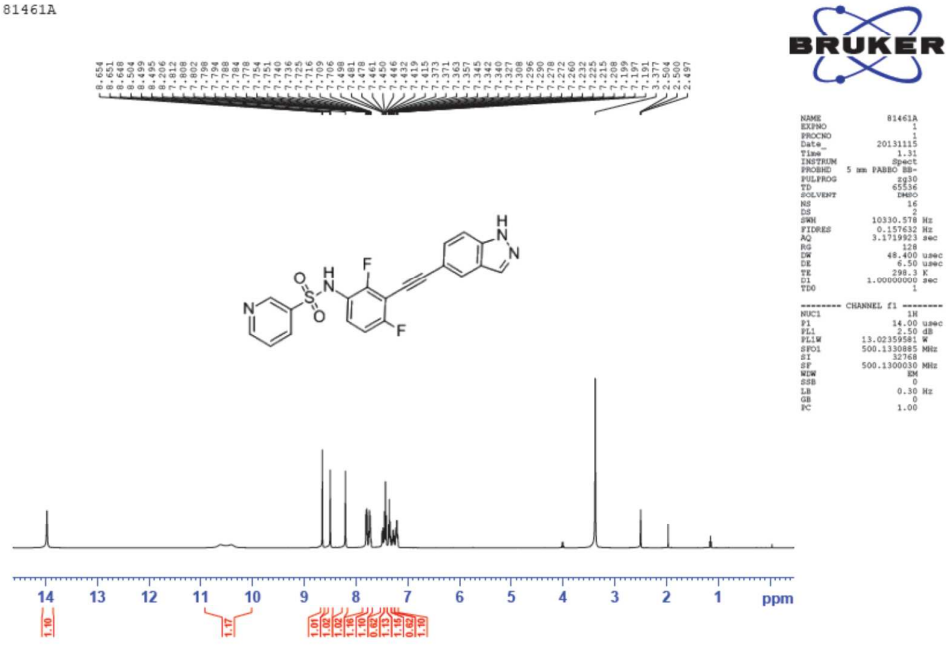

81464
13.989
10.562
8.768
8.759
8.668
8.638
8.217
8.103
8.088
8.072
7.923
7.908
7.715
7.704
7.691
7.393
7.376
7.365
7.346
7.227
7.210
7.192
3.357
2.500
BRUKER
14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm


81464C
165.78
163.38
162.41
162.37
162.00
161.38
160.34
156.09
155.85
155.38
144.05
139.26
139.13
138.90
138.78
133.93
132.73
127.67
126.67
126.57
126.54
119.54
118.85
116.79
115.87
106.75
106.58
106.43
102.57
81.55
BRUKER
165 160 155 150 145 140 ppm
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

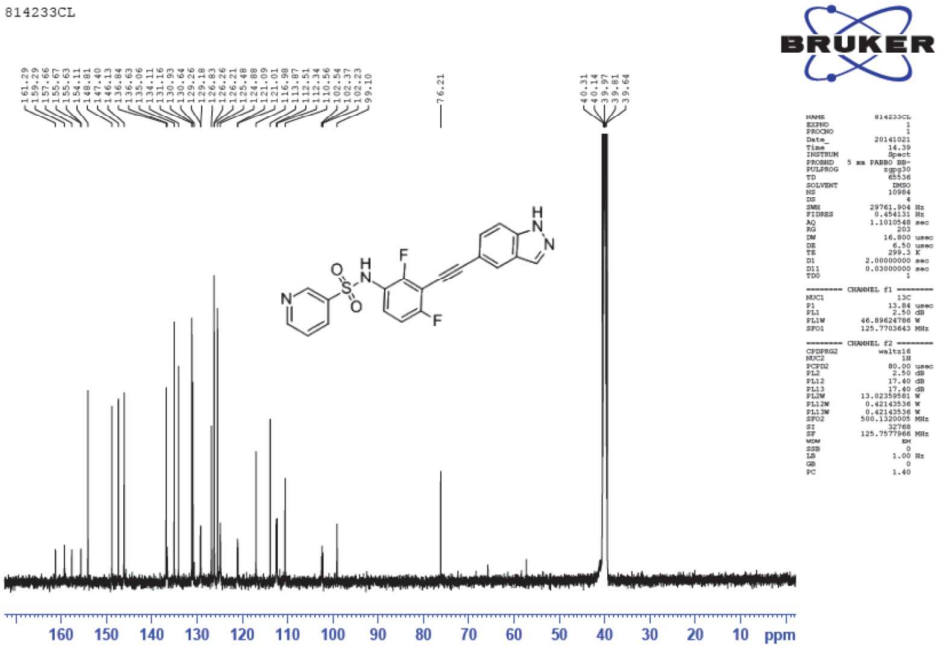

198160
13.971
10.622
8.876
8.870
8.853
8.849
8.841
8.837
8.653
8.648
8.513
8.508
8.211
8.123
8.118
8.113
8.102
8.097
8.093
7.663
7.651
7.643
7.640
7.353
7.330
7.315
7.308
7.293
7.257
7.225
7.213
3.344
2.504
2.500
2.496
BRUKER
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm

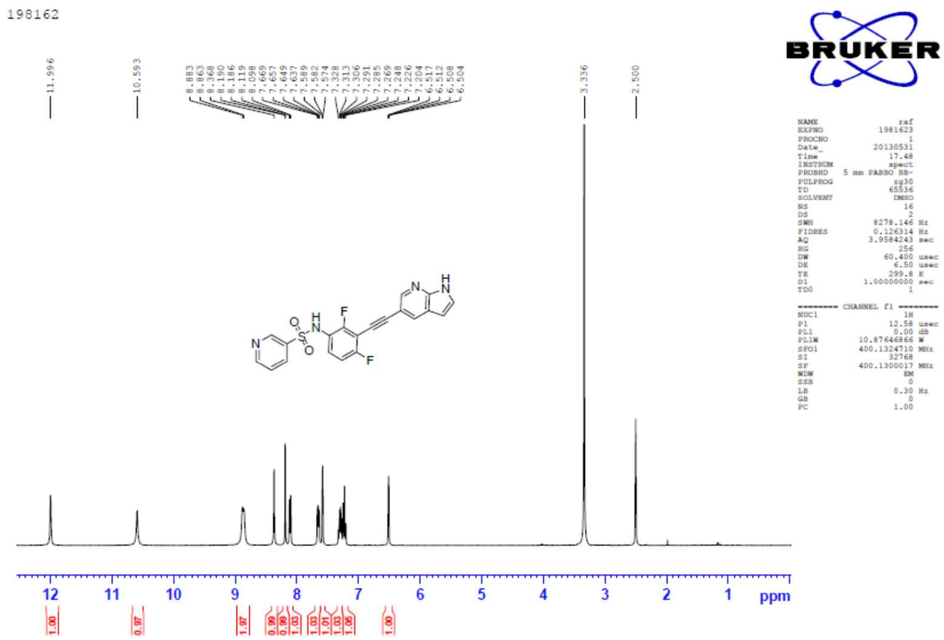

198160
160.81
158.81
157.28
155.35
155.21
153.63
150.84
150.63
146.94
136.15
134.62
133.97
133.64
129.07
128.98
124.34
120.79
120.68
113.90
112.09
111.95
110.58
101.75
101.59
101.44
91.53
76.17
40.00
39.83
39.67
39.50
39.33
39.17
39.00
BRUKER
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

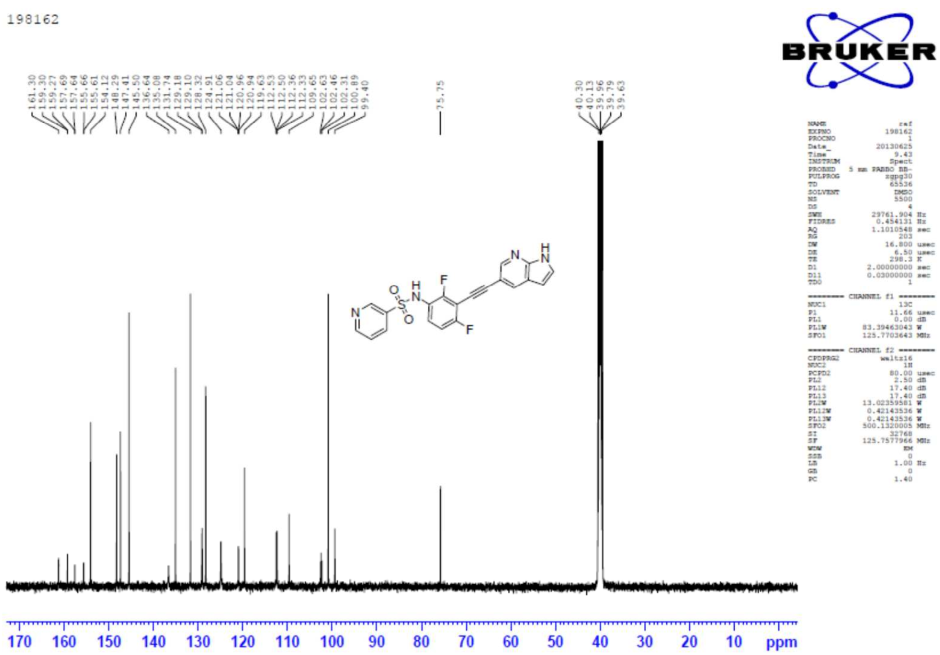
81461A



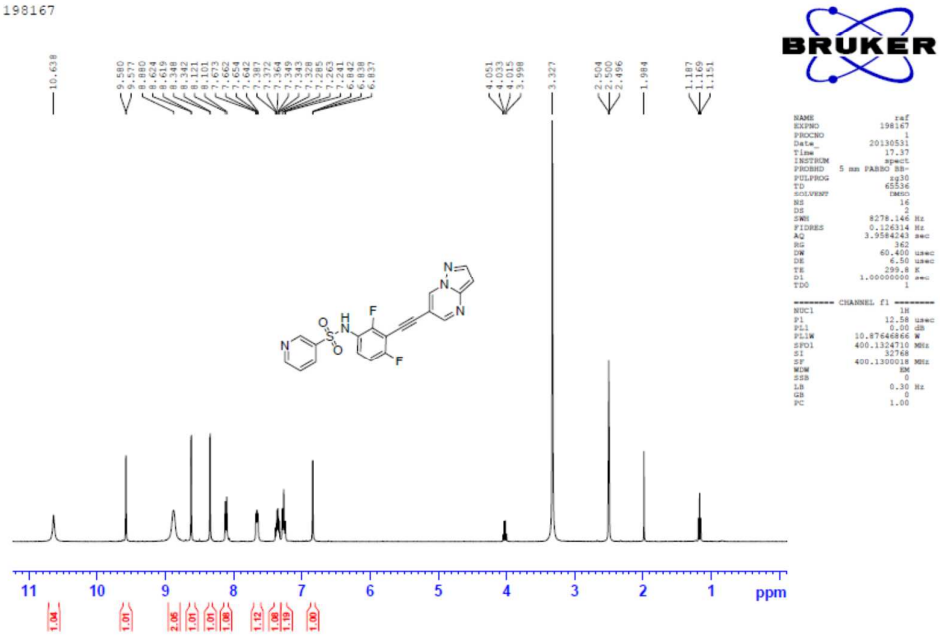
814233CL



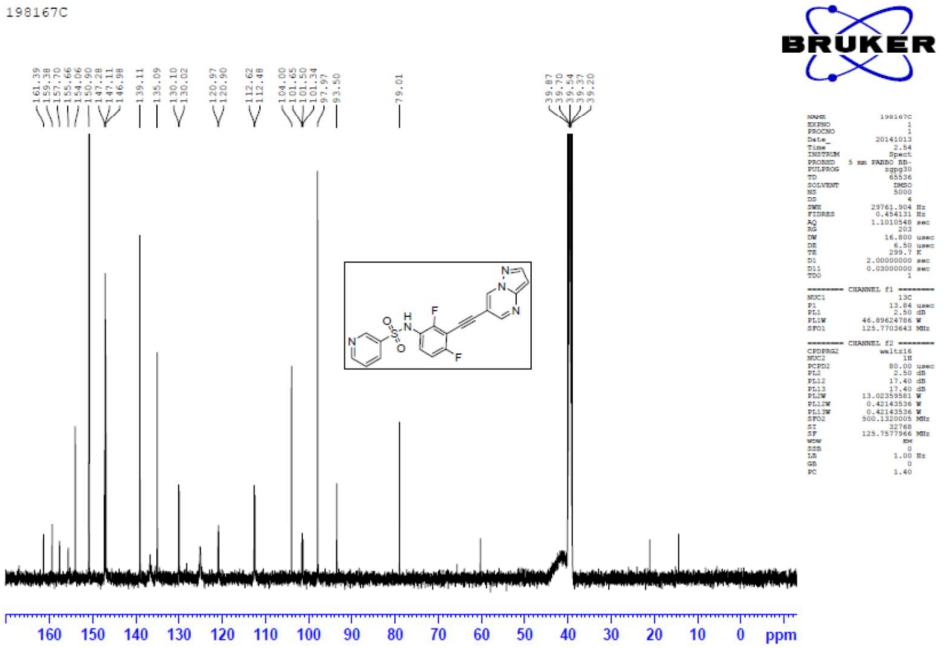

198162
11.996
10.593
8.883
8.863
8.368
8.190
8.186
8.119
8.098
7.669
7.657
7.649
7.637
7.589
7.582
7.574
7.328
7.313
7.306
7.291
7.285
7.269
7.248
7.226
7.204
6.517
6.512
6.508
6.504
3.336
2.500
BRUKER
12 11 10 9 8 7 6 5 4 3 2 1 ppm

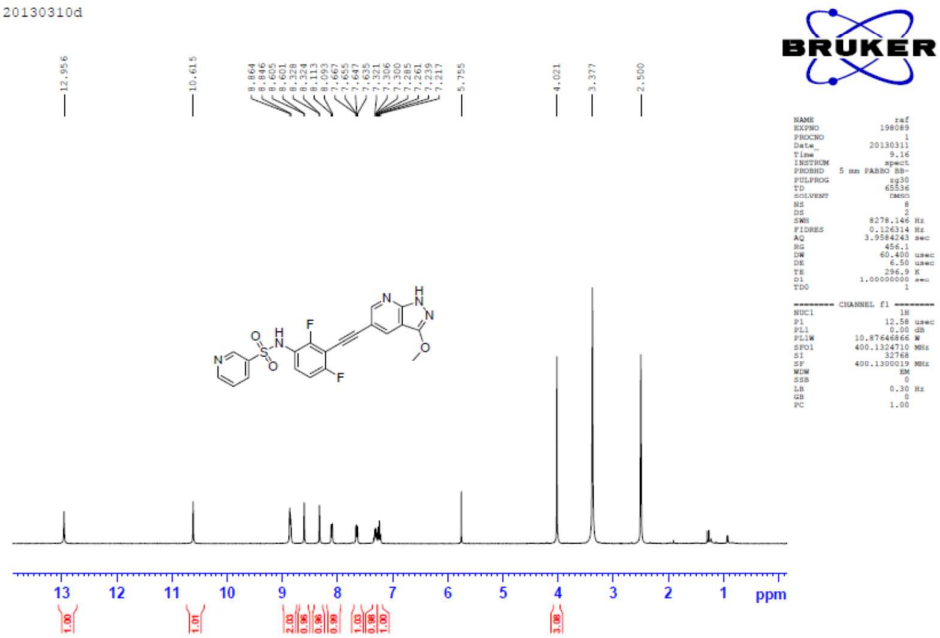

198162
161.30
159.30
157.27
157.69
157.64
155.66
155.61
154.12
148.29
147.41
145.50
136.64
135.08
131.74
129.18
129.10
128.32
124.91
121.06
121.04
120.96
120.94
119.63
112.53
112.50
112.36
112.33
109.65
102.63
102.46
102.31
100.89
99.40
75.75
40.30
40.13
39.96
39.79
39.63
BRUKER
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

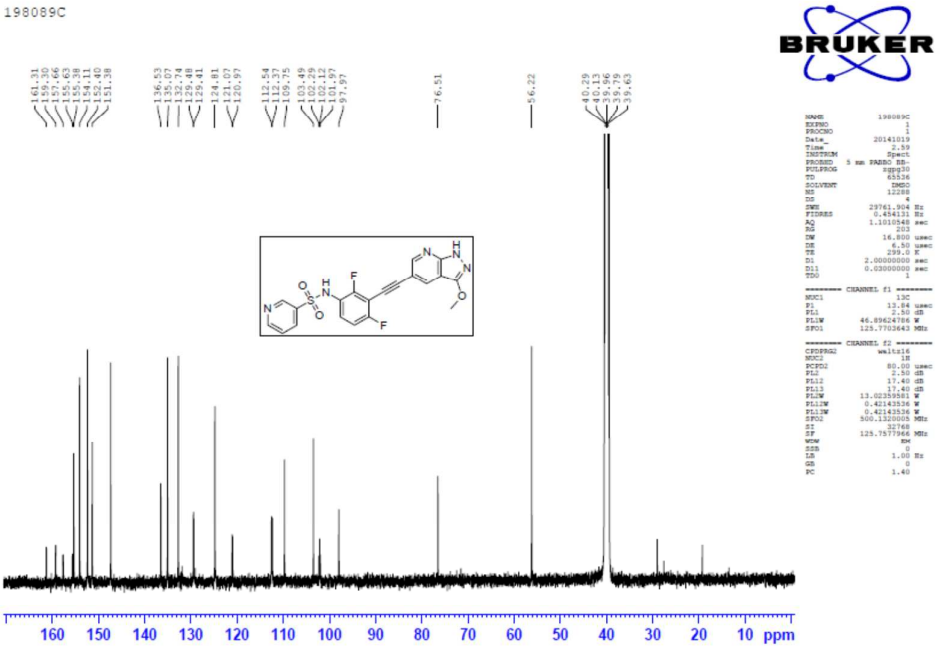
198167

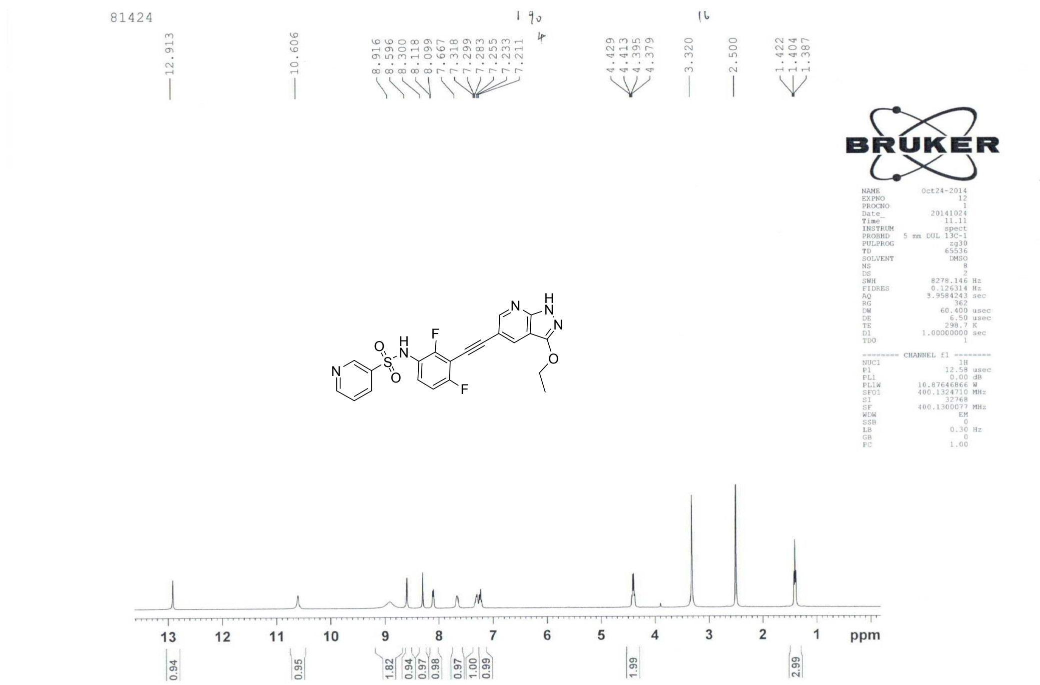


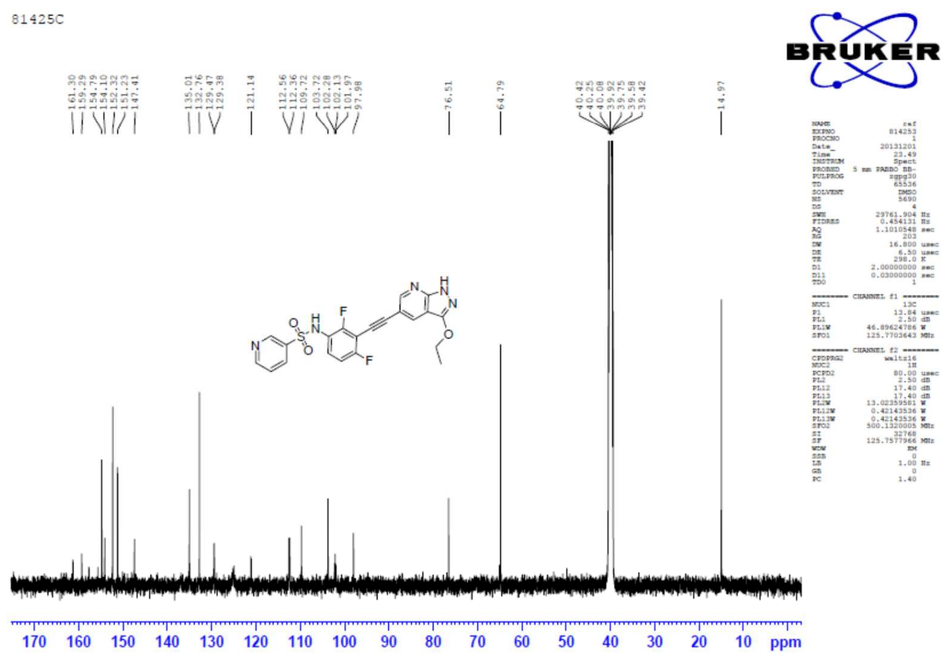
198167C

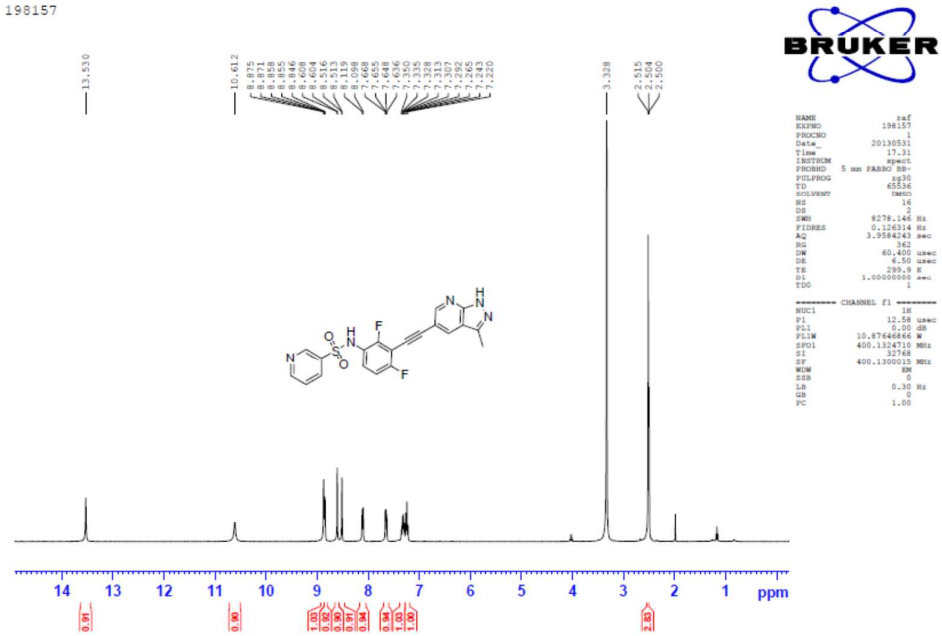


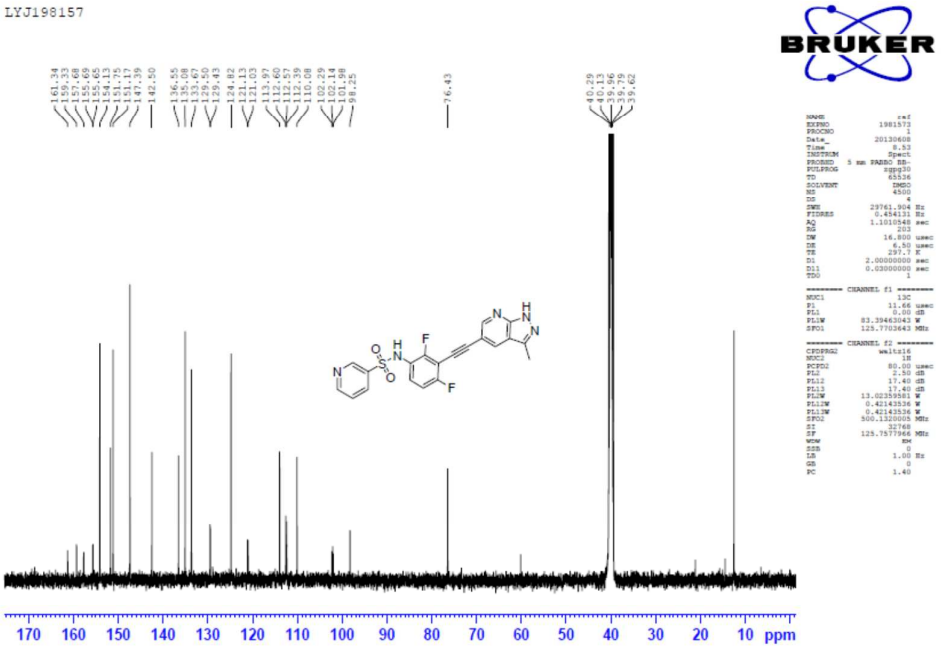

20130310d
BRUKER
12.956
10.615
8.864
8.846
8.685
8.601
8.328
8.324
8.113
8.093
7.667
7.655
7.647
7.635
7.321
7.306
7.300
7.285
7.261
7.239
7.217
5.755
4.021
3.377
2.500
ppm


198089C
BRUKER
161.31
159.30
157.66
155.63
154.38
154.11
152.40
151.38
136.53
135.07
132.74
130.48
129.41
124.81
121.07
120.97
112.54
112.37
107.75
103.49
102.29
102.12
101.97
97.97
76.51
56.22
40.29
40.13
39.96
39.79
39.63
ppm

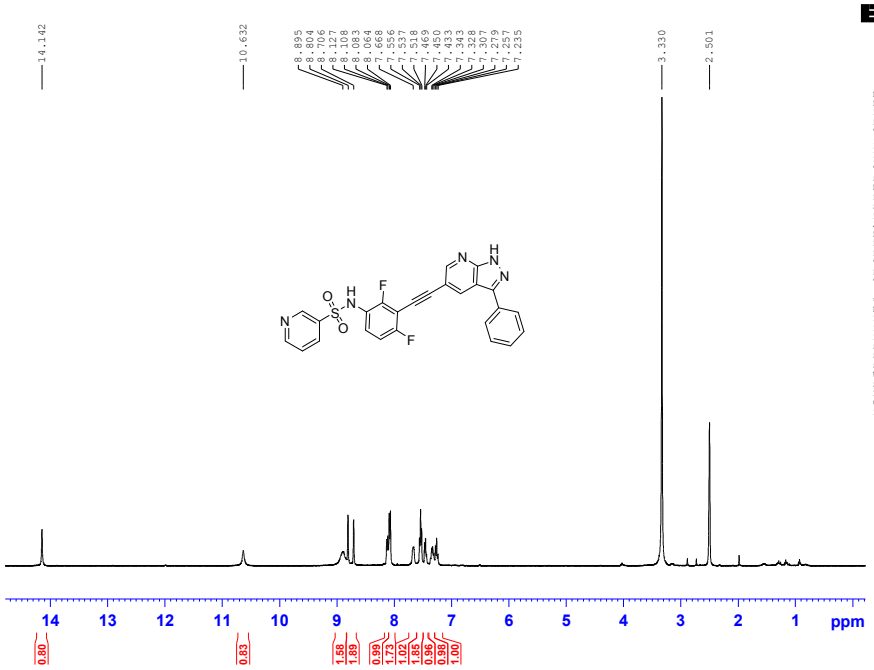




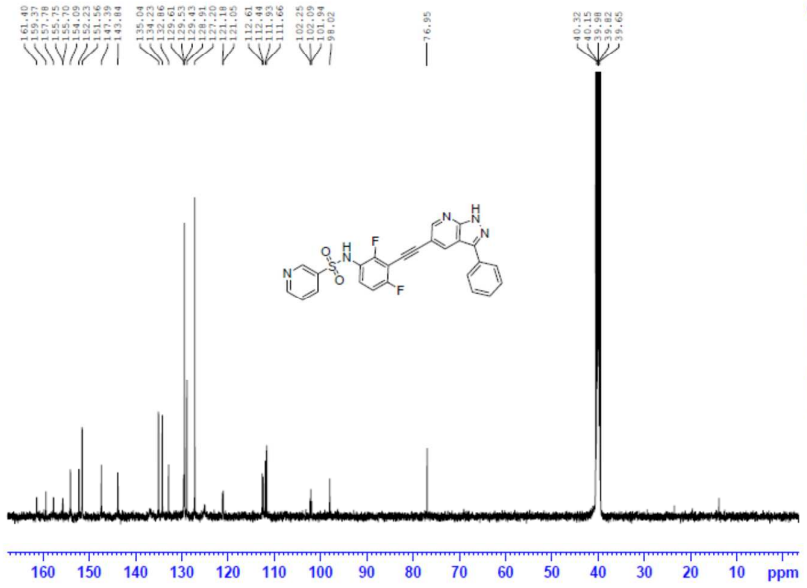

198157
BRUKER
13.530
10.612
8.875
8.871
8.859
8.855
8.846
8.608
8.604
8.516
8.513
8.119
8.099
7.668
7.655
7.648
7.636
7.350
7.335
7.328
7.313
7.307
7.292
7.265
7.243
7.220
3.328
2.515
2.504
2.500
14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm

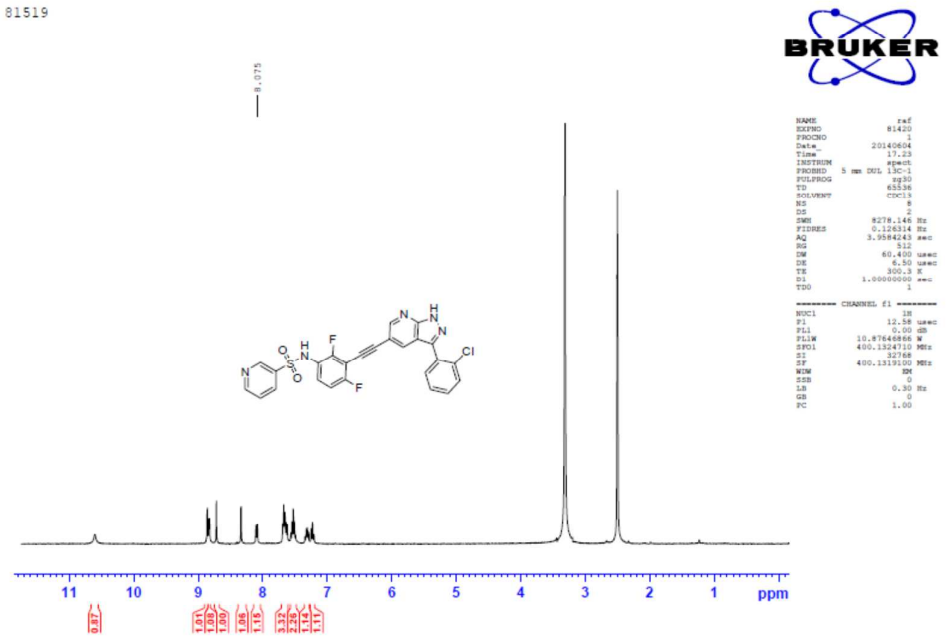

LYJ198157
BRUKER
161.34
157.23
157.08
155.69
155.65
154.13
151.75
151.17
147.39
142.50
136.55
135.08
133.67
129.30
129.15
124.82
121.13
121.03
113.97
112.60
112.57
112.39
110.08
102.29
102.14
101.98
98.25
76.43
40.29
40.13
39.96
39.79
39.62
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

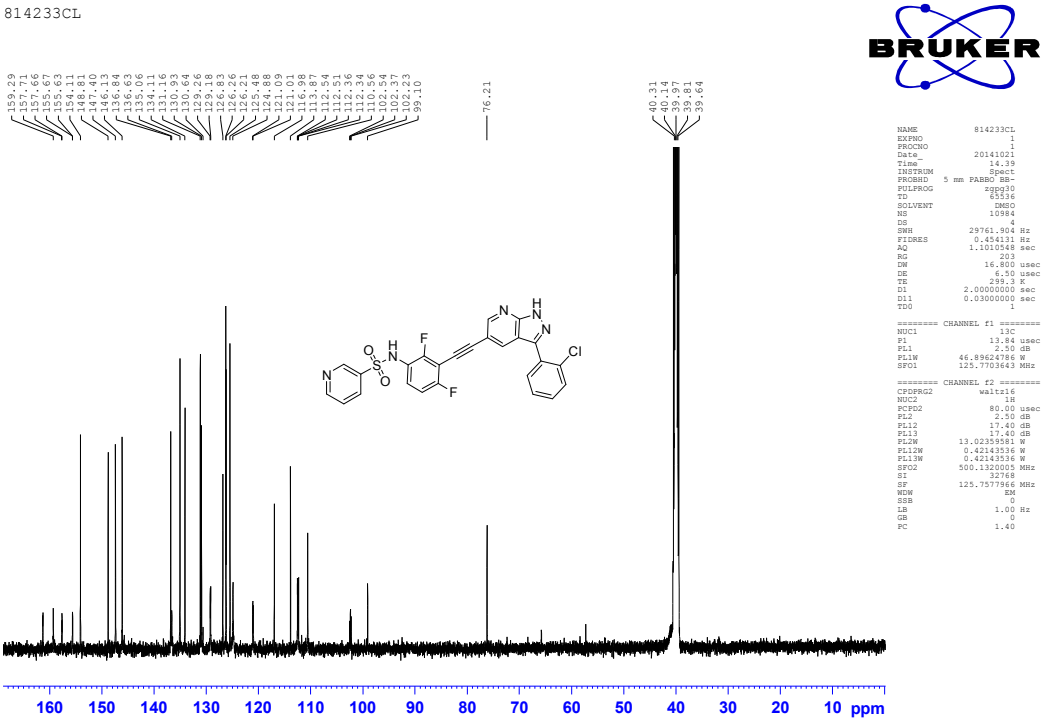
198184

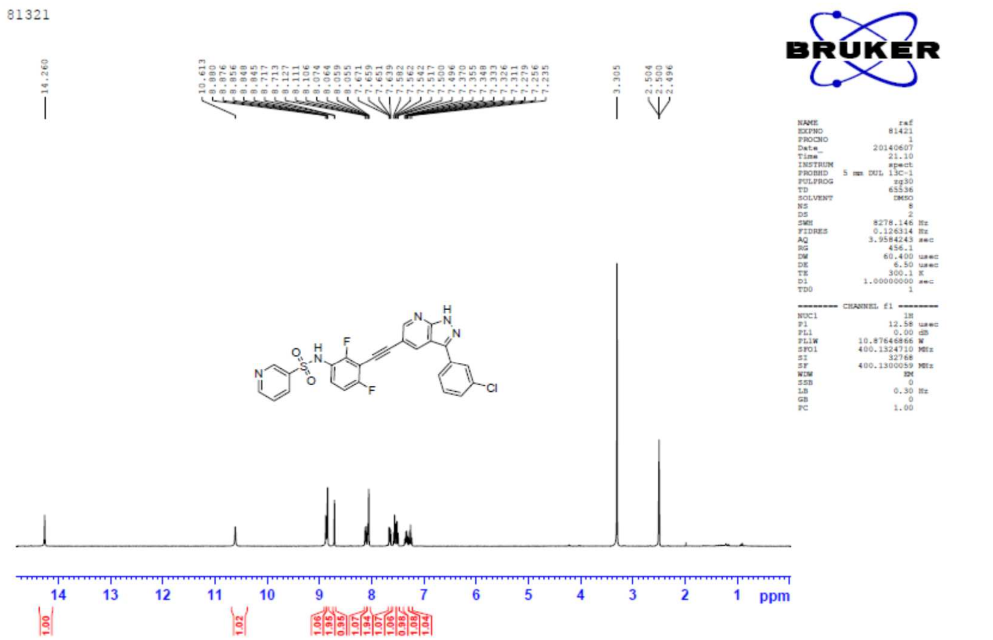


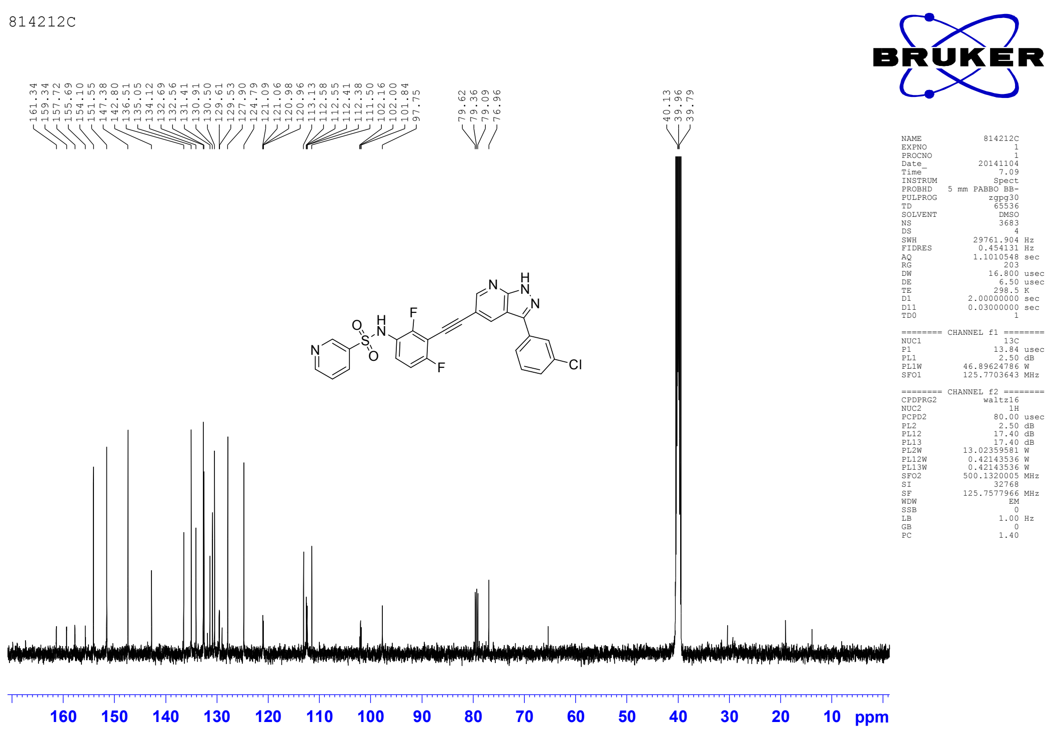
198175C

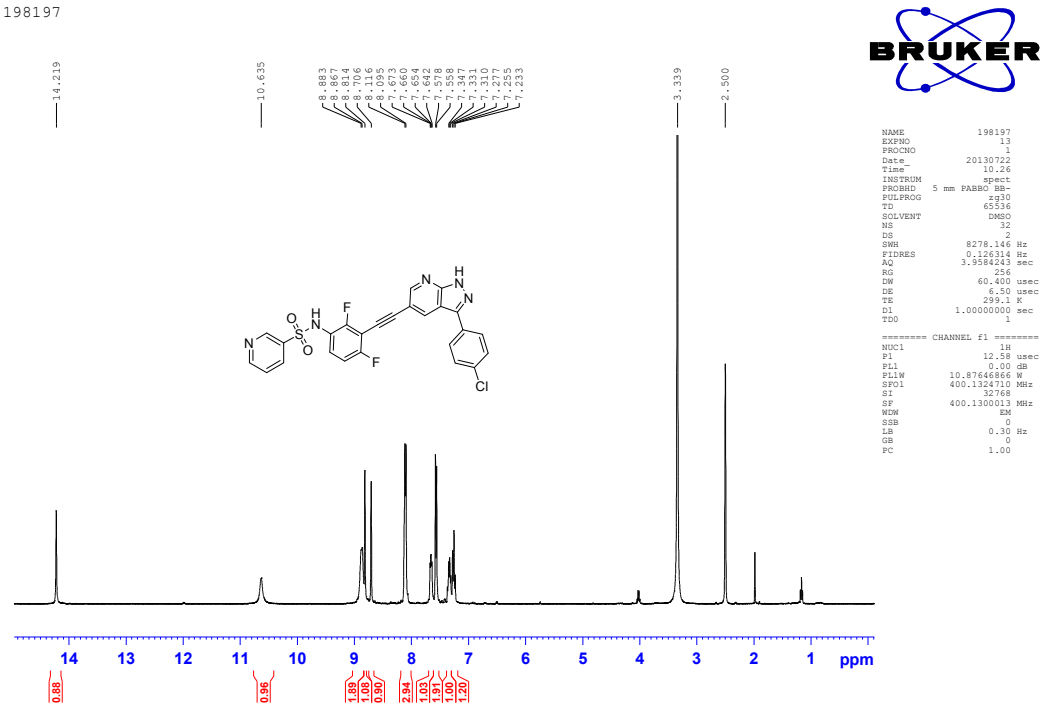


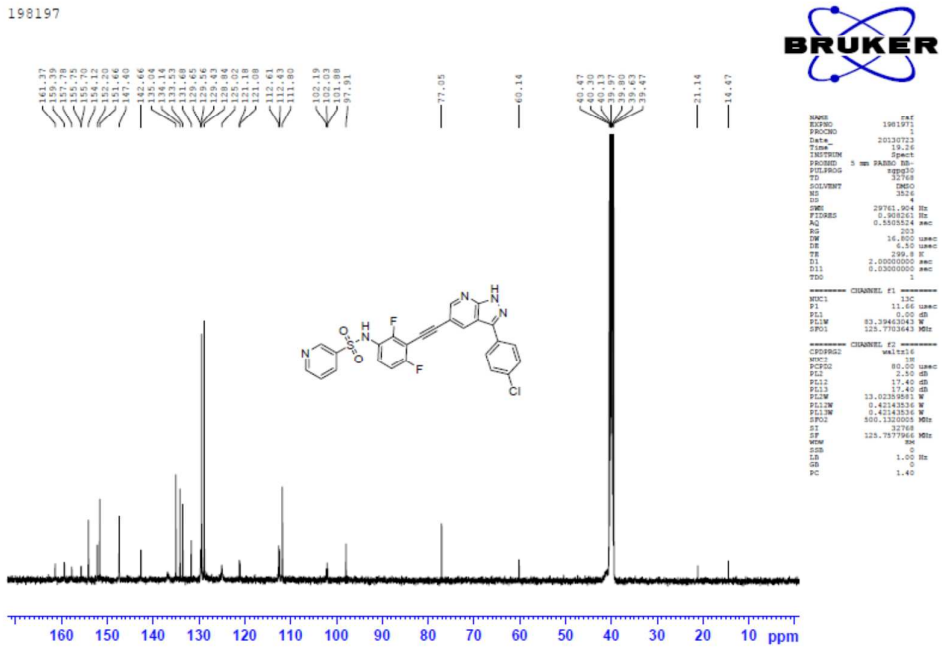

81519
8.075
11
10
9
8
7
6
5
4
3
2
1
ppm

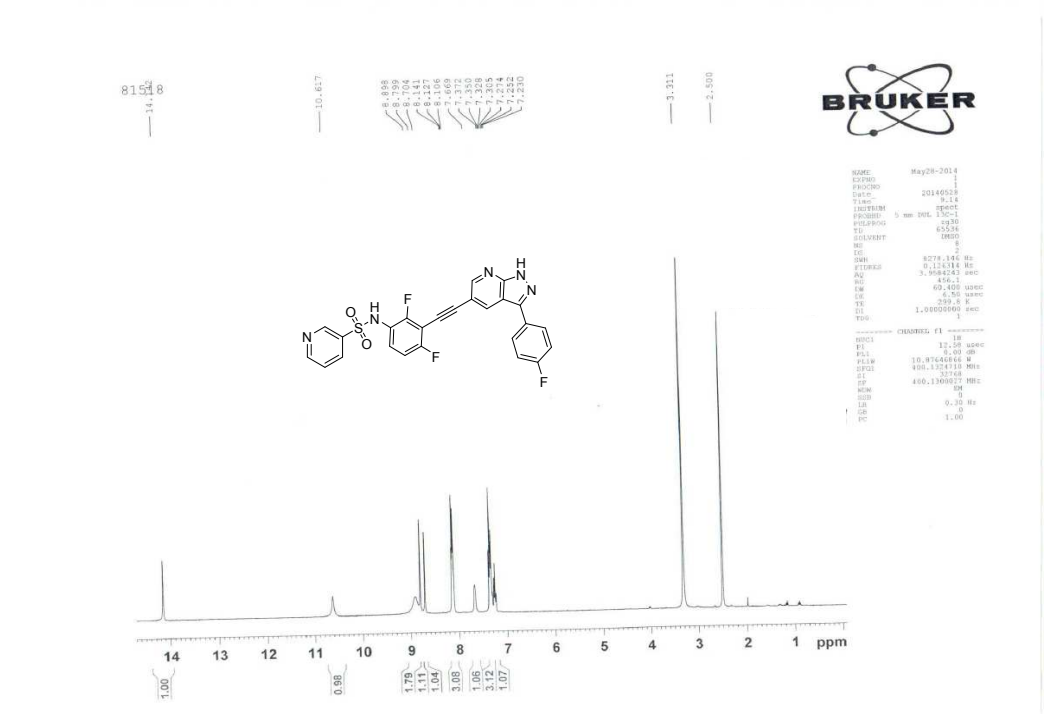

814233CL
159.29
157.71
157.66
155.67
155.63
154.11
148.81
147.40
146.13
136.84
136.63
135.06
134.11
131.16
130.93
130.64
130.46
129.18
126.83
126.26
126.21
125.48
124.88
121.09
121.01
116.98
113.87
112.54
112.31
112.36
112.34
110.56
102.54
102.37
102.33
99.10
76.21
40.31
40.14
39.97
39.81
39.64
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm



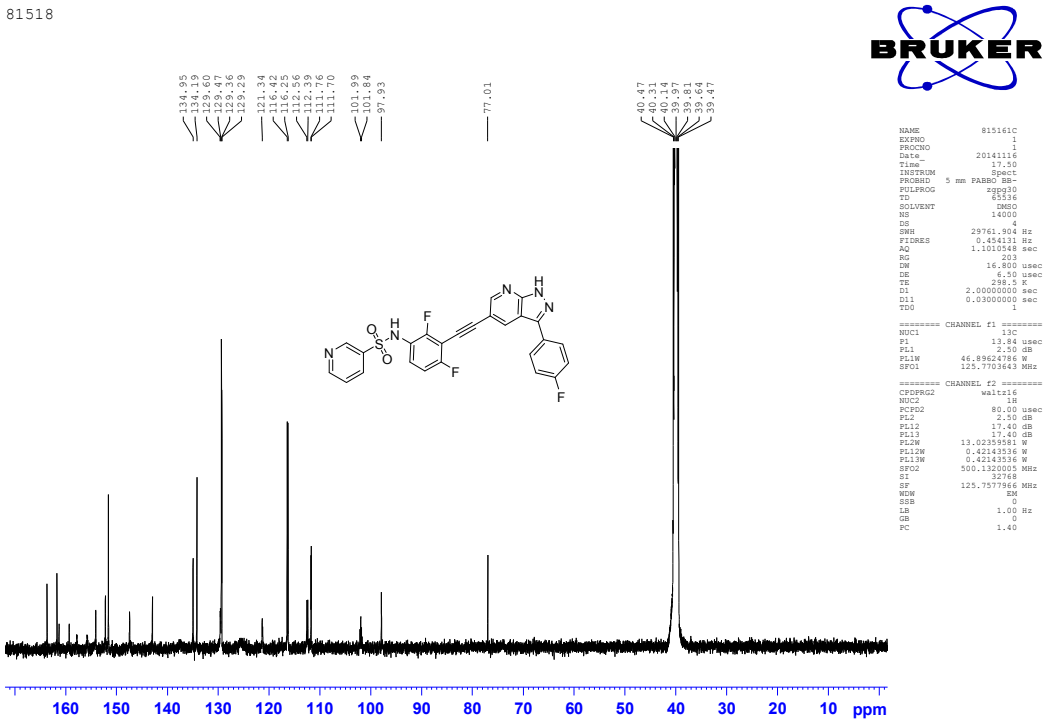



198197
14.219
10.635
8.883
8.867
8.814
8.706
8.116
8.095
8.073
7.660
7.654
7.642
7.578
7.558
7.347
7.331
7.310
7.277
7.255
7.233
3.339
2.500
BRUKER
NAME 198197
EXPNO 13
PROCNO 1
Date_ 20130722
Time 10.26
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 2
SWH 8278.146 Hz
FIDRES 0.126314 Hz
AQ 3.9584243 sec
RG 256
DW 60.400 usec
DE 6.50 usec
TE 299.1 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 12.58 usec
PL1 0.00 dB
PL1W 10.87646866 W
SFO1 400.1324710 MHz
SI 32768
SF 400.1300013 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.88 0.96 1.88 1.08 0.90 2.94 1.63 1.91 1.08 1.20


198197
161.37
159.79
157.79
155.75
155.70
154.12
152.26
151.66
147.40
142.66
136.14
134.14
133.53
131.68
129.65
129.56
129.43
128.84
125.02
121.18
121.08
112.61
112.43
111.80
102.19
102.03
101.98
97.91
77.05
60.14
60.47
60.30
60.13
59.97
59.80
59.63
59.47
21.14
14.47
BRUKER
NAME raf
EXPNO 19819711
PROCNO 1
Date_ 20130723
Time 19.26
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 32768
SOLVENT DMSO
NS 3526
DS 4
SWH 29761.904 Hz
FIDRES 0.908261 Hz
AQ 0.5505524 sec
RG 203
DW 16.800 usec
DE 6.50 usec
TE 299.8 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 11.66 usec
PL1 0.00 dB
PL1W 83.39463043 W
SFO1 125.7703643 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 2.50 dB
PL12 17.40 dB
PL13 17.40 dB
PL2W 13.02359581 W
PL12W 0.42143536 W
PL13W 0.42143536 W
SFO2 500.1320005 MHz
SI 32768
SF 125.7577966 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


BRUKER
14.02
10.617
8.888
8.799
8.784
8.141
8.127
8.106
7.660
7.372
7.350
7.308
7.295
7.274
7.252
7.230
3.311
2.500
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
0.98
1.78
1.11
1.04
5.08
1.06
3.12
1.07

81518


BRUKER
134.95
134.19
129.60
129.47
129.36
129.29
121.34
116.42
116.25
112.56
112.39
111.76
111.70
101.99
101.84
97.93
77.01
40.47
40.31
40.14
39.97
39.81
39.64
39.47
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm