Supplementary Information

Structure-based design of potent and selective inhibitors of the metabolic kinase PFKFB3

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1. Experimental methods

Assessment of enzyme activity:

Compound preparation: Serial dilutions were prepared as previously described (Turmel *et al.* JALA (2010) 15:297-305) and 40 nL volumes at each concentration dispensed into a 384 well test plate. All test wells were back filled with dimethyl sulphoxide (DMSO) such that the final concentration in each well was 1% v/v.

Assay Reagents: All fine chemicals, with the exception of ATP, were supplied by Sigma Aldrich, UK. Ultrapure ATP and ADP Glo kits were supplied by Promega, USA (Cat. No: V9102). Purified kinase enzymes were supplied by AstraZeneca's internal protein supply capability from insect cell lysates infected with virus carrying one of the recombinant human PFKFB genes.

Biochemical Assay Principle: PFKFB enzyme activity was estimated by calculating the amount of ADP generated in a kinase reaction. ADP generation was measured using an ADP Glo kit with no deviation from the recommended protocol. DMSO was added to test wells to estimate the maximum uninhibited activity of the enzyme, a PFKFB inhibitor at a final concentration of 0.1 mM was added to wells to fully inhibit enzyme activity.

Kinase Protocol: Base buffer was prepared as standard for all kinase reactions. This buffer contained 100 mM HEPES (pH7.0); 400 mM KCl; 10mM potassium phosphate (pH7.0); 10 mM $MgCl_2$; 1 mM dithiothreitol; 0.2 mM Triton X100. Immediately prior to starting the assay kinase enzyme was added to the base buffer. For PFKFB3 testing the enzyme was diluted to a concentration estimated to be 17.5 nM, for PFKFB1 the dilution was to 70 nM and PFKFB2 it was 20 nM. Each well received 2 µL of enzyme diluted in base buffer. A substrate buffer addition of 2 µL containing fructose 6 phosphate and ATP was made to each well to start the reaction. Each kinase was tested at the respective K_m for both substrate and ATP. For PFKFB3 the substrate solution contained 30 µM Fructose 6 phosphate and 68 µM ATP; for PFKFB1, 4 µM substrate and 28 µM ATP; for PFKFB2, 50 µM substrate and 60 µM ATP. The reactions

were left to incubate at room temperature for 1 hour before the addition of 4 µL of ADP Glo reagent 1. A second incubation for 50 minutes followed, again at room temperature, before the addition of 8 µL ADP Glo reagent 2. After an additional hour, a PerkinElmer Envision® with enhanced luminescence module was used to measure the luminescence signal generated in each well.

IC_{50} estimates were calculated using a commercially available software package from GeneData Screener®. Curve fitting was carried out using the Condeseo® module which applied the appropriate multivariate fit for each compound. The enzyme activity in the Max and Min wells was used to establish the window and performance of each assay.

Assessment of cellular activity:

F16BP: A549 cell samples were treated with test compound for 4 hours then extracted in acetronitrile/methanol/water (40%/40%/20% v/v) before carrying out the LC-MS/MS analysis. Once extracted the samples were run using a CTC Analytics PAL autosampler connected via an Agilent 1100 liquid chromatography unit running a 5.5 min gradient (100%A reducing to 50%A in 4 mins, 100%A by 4.1 mins until 5.5 mins. Buffer A was 10 mM tributylamine, 15 mM acetic acid, buffer B was 80% methanol, 20% isopropanol) on a Waters Xbridge C18 3.5 uM, 2.1x50 mm column at 600C, with a flow rate of 0.6 ml/min to inject into an AB Sciex 4000 mass spec in negative mode via an ESI spray (IS -4000V, TEM 4000C, CAD 4, CUR 30, GS1 40, GS2 80, unit resolution quad 1 and 3, 50 msec dwell). The MSMS analysis was carried out on fructose 1, 6 bisphosphate (339 m/z Q1, 97 m/z Q3) and 13C labelled fructose 1,6 bisphosphate (345m/z Q1, 97m/z Q3) as an internal standard using Analyst version 1.4.2.

Lactate: This test is a cell based assay developed to identify inhibitors of PFKFB3 by measuring a decrease in lactate secretions in A549 cells following treatment with compound. Cells are plated directly onto compound and incubated for 4 hours. Following the incubation, a media sample is taken to which the lactate kit reagent is added. After a 10 minute incubation, the absorbance readings are determined using the Envision. Cell viability is then determined using CyQuant. The assay is a 12 pt singlicate dose response. Cells are cultured to maximum of 80 %

confluency. Reconstitute the required number of Trinity Biotech reagent vials with 10 ml water per vial. Invert gently to mix. Do not shake. For Plasticware use Cell plate Costar 384 well black, clear bottom # 3712. Remove the growth media out of the flasks. Wash once with PBS and add 5ml Accutase to the cells (5ml per T175). Once detached re-suspend cells in 10 ml culture media. Centrifuge at 1000 rpm for 5 mins in a bench top centrifuge. Re-suspend cells in plating media and make up the final cell dilution to 1 x 105 cells / ml in plating media. Use a Wellmate to add 40 µl of cell suspension to all wells of the dosed 384 well plate required, equivalent to 4000 cells/well. Incubate for 4hrs at 37^{o}C / 5% CO2. Before transferring the media samples, prepare the lactate reagents (see below). Transfer 5 µl media from the wells to empty Costar 384 well plates using the Biomek. Reconstitute the required number of Trinity Biotech reagent vials with 10 ml water per vial, using a Wellmate/Multidrop add 30µl reagent per well. Leave in the dark for a minimum of 10 mins (maximum of 3 hours) then read on the Envision using the Lactate protocol. For viability assay reagent is added to the cells after transfer of media for the lactate assay, make up the required volume of CyQUANT, Dilute in PBS (1/250 dilution of Cyquant direct nucleic acid stain, 1/50 dilution of Cyquant direct background suppressor). Add 10µl CyQUANT/well using a wellmate, cover and leave at room temperature for one hour then read on the Envision using the Viability protocol.

2. Synthesis details

Chemistry Unless otherwise stated, commercially available reagents were used as supplied. All reactions requiring anhydrous conditions were conducted in dried apparatus under an atmosphere of nitrogen. ^{1}H NMR spectra were recorded using a Bruker AV400, AV500 or AV700 NMR. Chemical shifts δ are reported in ppm and multiplicity of signals are denoted s = singlet, d = doublet, t = triplet and m = multiplet respectively, with coupling constants (*J*) reported in hertz (Hz). HRMS were recorded using a Thermo Accela CTC - LTQ FT instrument (ESI+). Reactions and intermediates were also characterised by mass spectroscopy following liquid chromatography (LCMS or UPLC); UPLC was carried out using a Waters UPLC fitted with Waters SQ mass spectrometer (Column temp 40, UV = 220-300nm, MS = ESI with pos/neg switching) at a flow rate of 1ml/min using a solvent system of 97% A + 3% B to 3% A to 97% B over 1.50mins (total runtime with equilibration back to starting conditions etc 1.70min), where A = 0.1% Formic acid in water (for acid work) or 0.1% Ammonia in water (for base work) B = Acetonitrile. For acid analysis the column used was Waters Acquity HSS T3 1.8um 2.1 x50 mm, for base analysis the column used was Waters Acquity BEH 1.7um 2.1x50mm.; LCMS was carried out using a Waters Alliance HT (2795) fitted with a Waters ZQ ESCi mass spectrometer and a Phenomenex Gemini –NX (50x2.1 5um) column at a flow rate of 1.1mL/min 95%A to 95%B over 4 min with a 0.5 min hold. The modifier is kept at a constant 5% C (50:50 acetonitrile:water 0.1% Formic acid) or D (50:50 acetonitrile:water 0.1% ammonium hydroxide (0.88 SG) depending on whether it is an acidic or basic method. Ion exchange purification was generally peformed using a SCX-2 (Biotage, Propylsulfonic acid functionalized silica. Manufactured using a trifunctional silane. Non end-capped) cartridge. Individual purification methods referred to here are detailed in the Supplementary section.

Purification methods

Flash Chromatography apparatus

Standard flash chromatography procedures were performed using Kieselgel 60 (40-63 μm) or with a TeleDyne Isco CombiFlash Rf automated purification system.

Preparative LCMS methods

Method A

A SunFire, 5 micron pore size, C18 column of dimensions 50x19 mm was used. The flow rate was 25 mL/min and the mobile phases of water and acetonitrile contained 0.1% formic acid. The elution was started at 95% water:5% acetonitrile which is held for 1.5 minutes ramping up to 5% water:95% acetonitrile over 10 minutes. This is then held for 30 seconds, the complete length of the run was 12 minutes.

Method B

A SunFire, 5 micron pore size, C18 column of dimensions 50x19 mm was used. The flow rate was 25 mL/min and the mobile phases of water and acetonitrile contained 0.1% formic acid. The elution was started at 95% water:5% acetonitrile which is held for 1.5 minutes ramping up to 50% water:50% acetonitrile over 7.5 minutes. This is ramped up to 5% water:95% acetonitrile over 30 seconds then held for 0.9 minutes. This ramps up 95% water:5% acetonitrile over 6 seconds then held until 12 minutes.

Method C

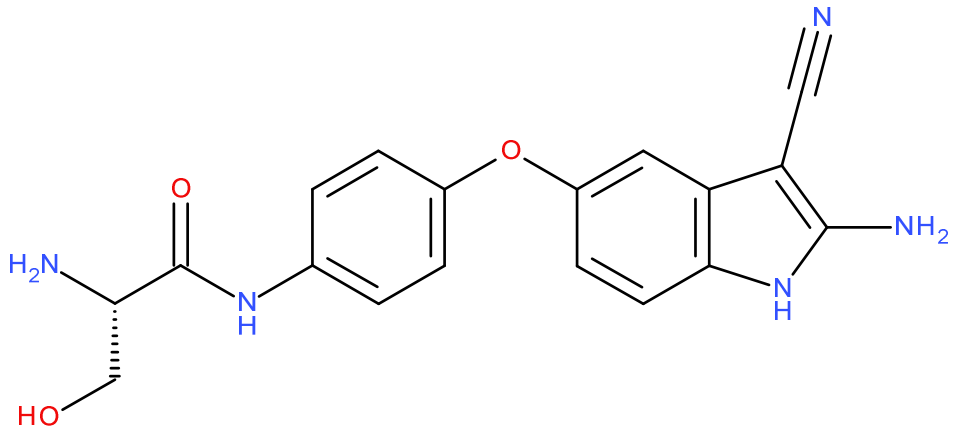
A SunFire, 5 micron pore size, C18 column of dimensions 50x19 mm was used. The flow rate was 25 mL/min and the mobile phases of water and acetonitrile contained 0.1% formic acid. The elution was started at 95% water:5% acetonitrile and held at this for 0.3 minutes ramping up to 5% water:95% acetonitrile over 5 minutes. The eluent is held at 95% acetonitrile until 5.8 minutes.

Method D (basic method)

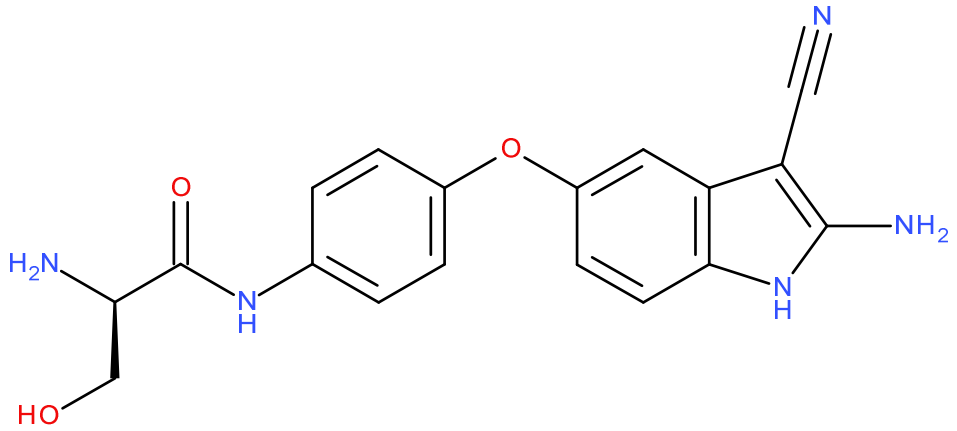
A SunFire, 5 micron pore size, C18 column of dimensions 50x19 mm was used. The flow rate was 25 mL/min and the mobile phases were water containing 0.1% ammonium bicarbonate and acetonitrile . The elution was started at 95% water:5% acetonitrile and held at this for 1.5 minutes ramping up to 5% water:95% acetonitrile over 10 minutes. The eluent is held at 95% acetonitrile until 12 minutes.

Method E

A XSelect, 5 micron pore size, C18 column of dimensions 150x19 mm was used. The flow rate was 25 mL/min and the mobile phases of water and acetonitrile contained 0.1% formic acid. The elution was started at 75% water:25% acetonitrile ramping up to 69% water:31% acetonitrile over 10 minutes then upto 100% acetonitrile over 1 minute and held for 1 minute. The eluentwas then ramped down to 75% water:25% acetonitrile over 2 minutes.

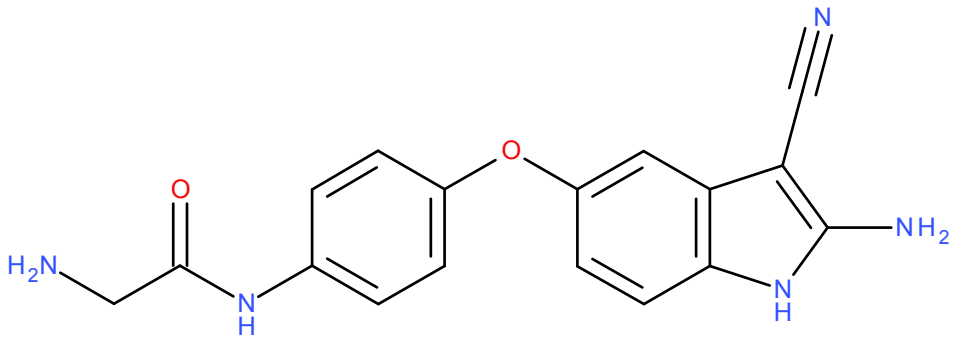


(2*S*)-2-Amino-*N*-[4-[(2-amino-3-cyano-1*H*-indol-5-yl)oxy]phenyl]-3-hydroxy-propanamide (44). Using the same procedure as for 2-amino-*N*-[4-(2-amino-3-cyano-1-methyl-1*H*-indol-5-yloxy)-phenyl]-acetamide **11** using *N*-BOC-*L*-serine instead of *N*-(*tert*-butoxycarbonyl)glycine (omitting the indole alkylation step). LCMS (ES^+) 352.20 $(M+H)^+$. ^1H NMR δ (d^6-DMSO) 10.71 (br s, 1H), 7.62 (d, *J* = 9.1Hz, 2H), 7.10 (d, *J* = 8.4Hz, 1H), 6.91 (d, 9.1Hz, 2H), 6.82 (br s, 2H), 6.65 (d, 2.3Hz, 1H), 6.59 (dd, *J* = 8.4Hz, 2.3Hz, 1H), 4.85 (t, *J* =.4Hz, 1H), 3.55 (m, 2H), 3.37 (t, *J* = 5.6Hz, 1H), 3 exchangeable protons not observed.

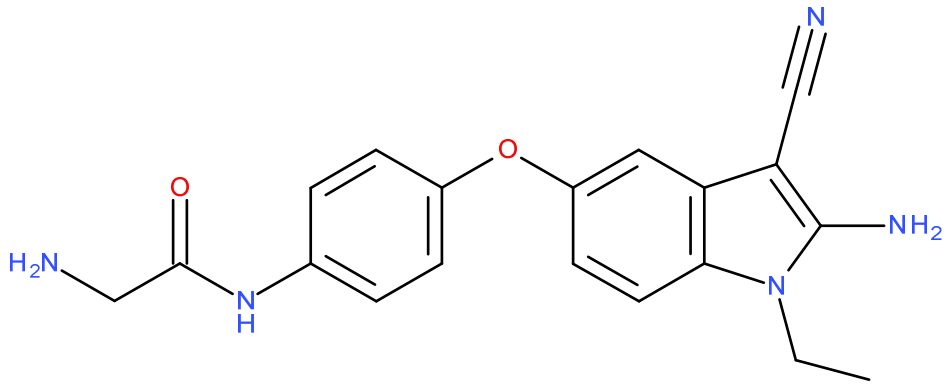


(2*R*)-2-Amino-*N*-[4-[(2-amino-3-cyano-1*H*-indol-5-yl)oxy]phenyl]-3-hydroxy-propanamide (45). Using the same procedure as for 2-amino-*N*-[4-(2-amino-3-cyano-1-methyl-1*H*-indol-5-yloxy)-phenyl]-acetamide **11** using *N*-BOC-*R*-serine instead of *N*-(*tert*-butoxycarbonyl)glycine

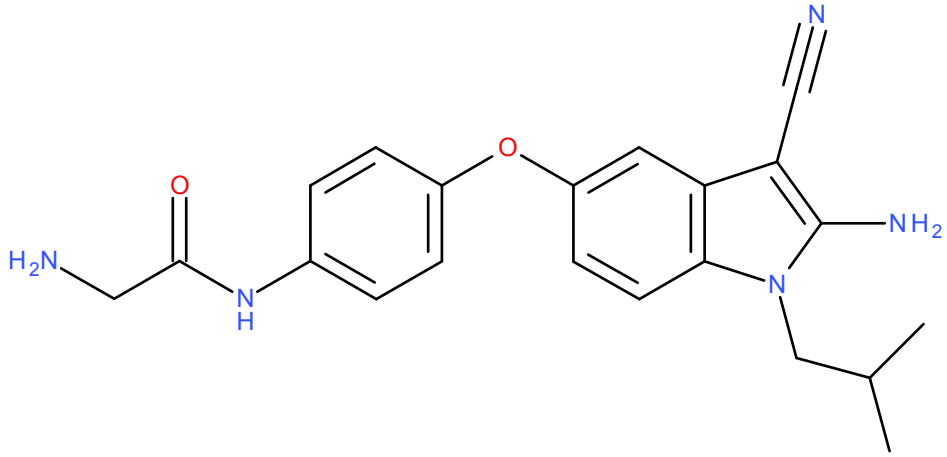
(omitting the indole alkylation step). HRMS ESI+ *m/z* observed 352.1405, $C_{18}H_{18}N_5O_3$ requires 352.1410. ^{1}H NMR δ (d^6-DMSO) 10.71 (br s, 1H), 7.61 (d, *J* = 9.1Hz, 2H), 7.10 (d, *J* = 8.4Hz, 1H), 6.91 (d, *J* = 9.1Hz, 2H), 6.82 (br s, 2H), 6.66 (d, *J* = 2.3Hz, 1H), 6.59 (dd, *J* = 8.4Hz, 2.3Hz, 1H), 4.85 (t, *J* = 5.5Hz, 1H), 3.55 (m, 2H), 3.37 (t, *J* = 5.6Hz, 1H), 3 exchangeable protons not observed.



2-Amino-*N*-[4-[(2-amino-3-cyano-1*H*-indol-5-yl)oxy]phenyl]acetamide (46). Using the same procedure as for 2-amino-*N*-[4-(2-amino-3-cyano-1-methyl-1*H*-indol-5-yloxy)-phenyl]-acetamide **11** (but omitting the indole alkylation step). HRMS ESI+ *m/z* observed 322.1305, $C_{17}H_{16}N_5O_2$ requires 322.1304. ^{1}H NMR δ (d^6-DMSO) 10.70 (br s, 1H), 7.60 (d, *J* = 9.0Hz, 2H), 7.10 (d, *J* = 8.4Hz, 1H), 6.91 (d, *J* = 9.0Hz, 2H), 6.82 (br s, 2H), 6.66 (d, *J* = 2.3Hz, 1H), 6.59 (dd, *J* = 8.4Hz, 2.4Hz, 1H), 3.26 (s, 2H), 3 exchangeable protons not observed.

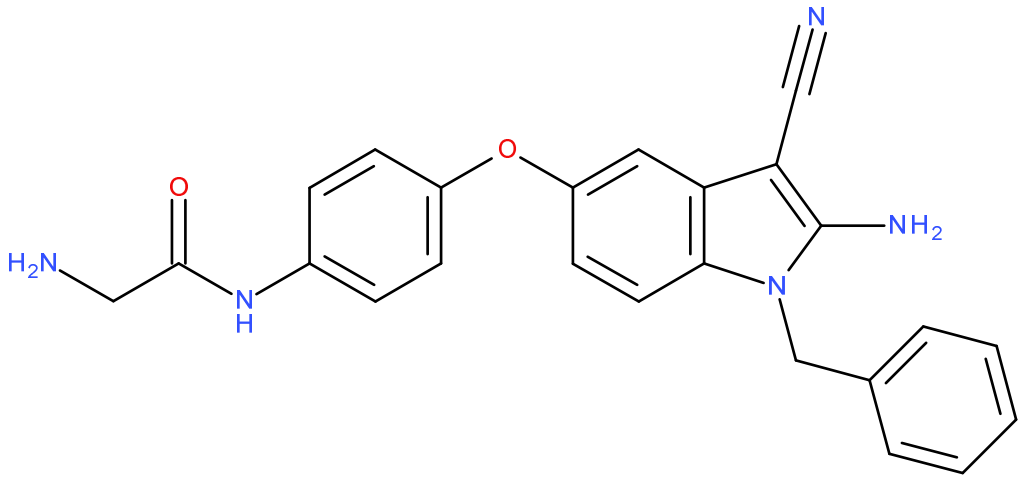


2-Amino-*N*-[4-(2-amino-3-cyano-1-ethyl-1*H*-indol-5-yloxy)-phenyl]-acetamide (47). Using the same procedure as for 2-amino-*N*-[4-(2-amino-3-cyano-1-methyl-1*H*-indol-5-yloxy)-phenyl]-acetamide **11** using ethyl iodide instead of methyl iodide. HRMS ESI+ *m/z* observed 350.1611, $C_{19}H_{20}N_5O_2$ requires 350.1617. ^{1}H NMR δ (d^6-DMSO) 7.60 (d, *J* = 9.0Hz, 2H), 7.23 (d, *J* = 8.5Hz, 1H), 7.06 (br s, 2H), 6.92 (d, *J* = 9.0Hz, 2H), 6.70 (d, *J* = 2.3Hz, 1H), 6.65 (dd, *J* = 8.5Hz, 2.3Hz, 1H), 4.06 (q, *J* = 7.0Hz, 2H), 3.26 (s, 2H), 1.17 (t, *J* = 7.0Hz, 3H), 3 exchangeable protons not observed.

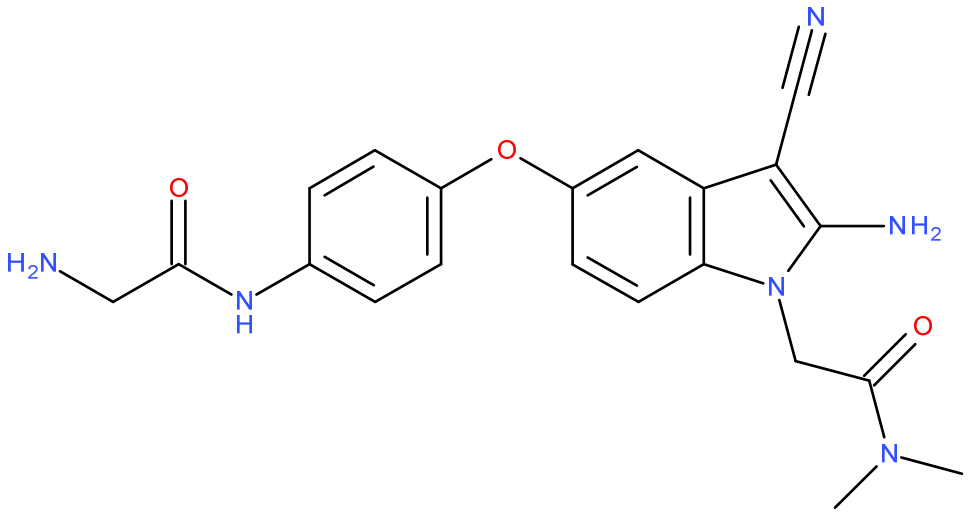


2-Amino-*N*-[4-(2-amino-3-cyano-1-isobutyl-indol-5-yl)oxyphenyl]acetamide (48). Using the same procedure as for 2-amino-*N*-[4-(2-amino-3-cyano-1-methyl-1*H*-indol-5-yloxy)-phenyl]-

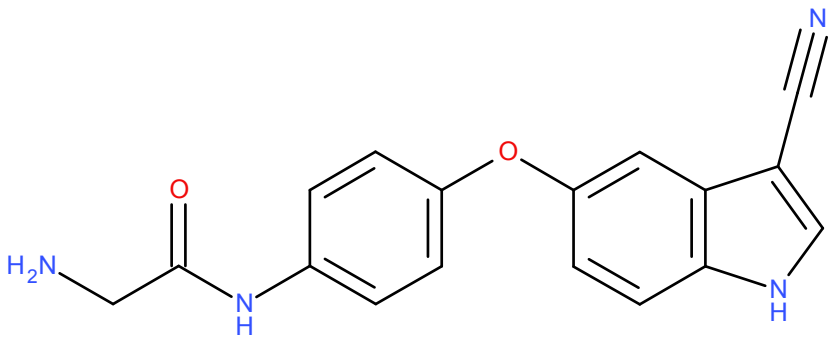
acetamide **11** using 1-bromo-2-methylpropane instead of methyl iodide. HRMS ESI+ *m/z* observed 378.1935, $C_{21}H_{24}N_5O_2$ requires 378.1930. ^{1}H NMR δ (d^6-DMSO) 7.61 (d, *J* = 9.0Hz, 2H), 7.22 (d, *J* = 8.6Hz, 1H), 7.03 (s, 2H), 6.93 (d, *J* = 9.0Hz, 2H), 6.68 (d, *J* = 2.2Hz, 1H), 6.63 (dd, *J* = 8.6Hz, 2.2Hz, 1H), 3.83 (d, *J* = 7.7Hz, 2H), 3.25 (s, 2H), 2.09 (m, 1H), 0.86 (d, *J* = 6.6Hz, 6H), 3 exchangeable protons not observed.



2-Amino-*N*-[4-(2-amino-1-benzyl-3-cyano-1*H*-indol-5-yloxy)-phenyl]-acetamide (49). Using the same procedure as for 2-amino-*N*-[4-(2-amino-3-cyano-1-methyl-1*H*-indol-5-yloxy)-phenyl]-acetamide **11** using benzyl bromide instead of methyl iodide. HRMS ESI+ *m/z* observed 412.1784, $C_{24}H_{22}N_5O_2$ requires 412.1773. ^{1}H NMR δ (d^6-DMSO) 7.61 (d, *J* = 9Hz, 2H), 7.36 – 7.30 (m, 2H), 7.26 (m, 1H), 7.21 (br s, 2H), 7.16 – 7.12 (m, 3H), 6.92 (d, *J* = 9Hz, 2H), 6.71 (d, *J* = 2.3Hz, 1H), 6.60 (dd, *J* = 8.6Hz, 2.3Hz, 1H), 5.32 (s, 2H), 3.25 (s, 2H), 3 exchangeable protons not observed.

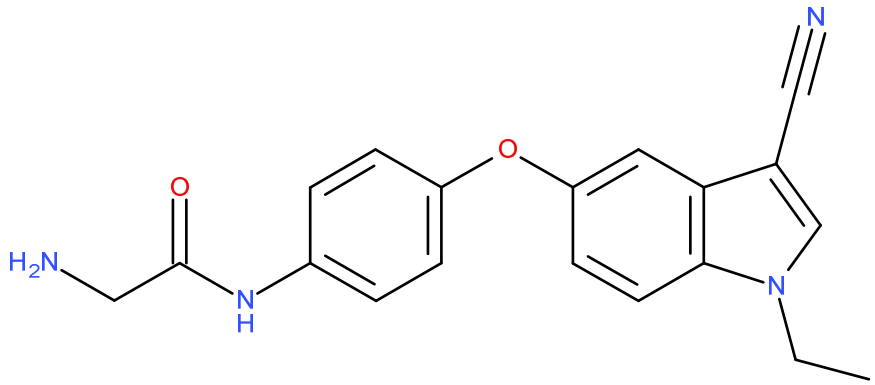


2-Amino-*N*-[4-(2-amino-3-cyano-1-dimethylcarbamoylmethyl-1*H*-indol-5-yloxy)-phenyl]-acetamide (50). Using the same procedure as for 2-amino-*N*-[4-(2-amino-3-cyano-1-methyl-1H-indol-5-yloxy)-phenyl]-acetamide **11** using 2-chloro-*N,N*-dimethyl-acetamide instead of methyl iodide. HRMS ESI+ *m/z* observed 407.1814, $C_{21}H_{23}N_6O_3$ requires 407.1832. ^{1}H NMR δ (d^6-DMSO) 7.43 (d, *J* = 9.0Hz, 2H), 6.94 (d, *J* = 8.6Hz, 1H), 6.82 (br s, 2H), 6.75 (d, *J* = 9.0Hz, 2H), 6.53 (d, *J* = 2.3Hz, 1H), 6.44 (dd, *J* = 8.6Hz, 2.3Hz, 1H), 4.78 (s, 2H), 3.21 (s, 2H), 2.92 (s, 3H), 2.68 (s, 3H), 3 exchangeable protons not observed.

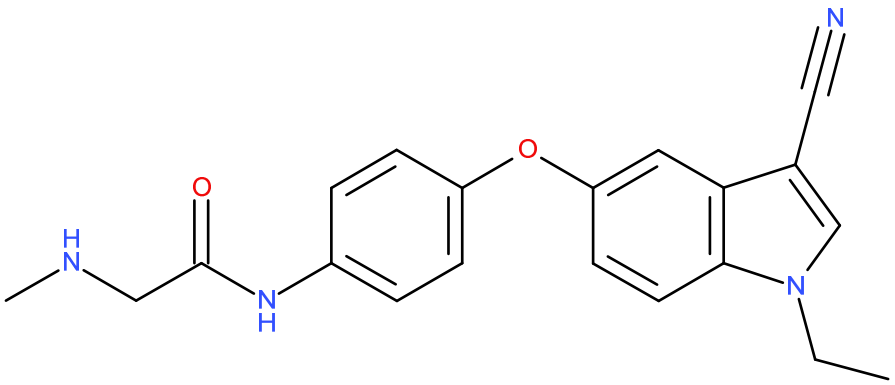


2-Amino-*N*-[4-(3-cyano-1*H*-indol-5-yloxy)-phenyl]-acetamide (51). Using the same procedure as for (2*S*)-*N*-[4-(3-cyano-1-isobutyl-indol-5-yl)oxyphenyl]pyrrolidine-2-carboxamide

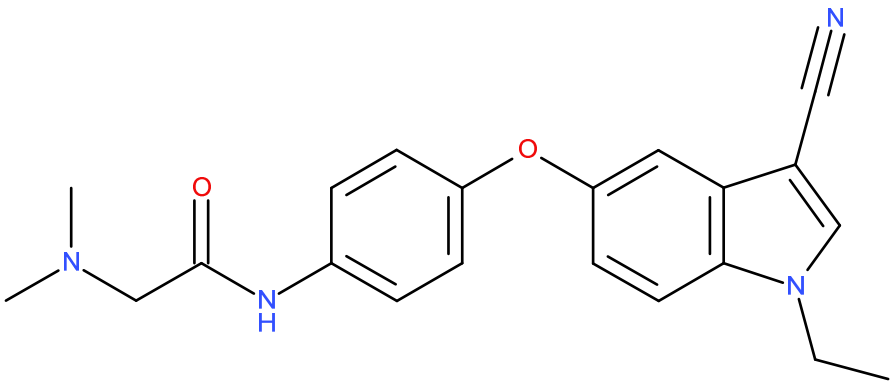
26 using *N*-BOC glycine instead of (2*S*)-1-*tert*-butoxycarbonylpyrrolidine-2-carboxylic acid (but omitting the indole alkylation step). HRMS ESI+ *m/z* observed 307.1205, $C_{17}H_{15}N_4O_2$ requires 307.1195. ^{1}H NMR δ (d^6-DMSO) 8.27 (s, 1H), 7.64 (d, *J*= 9Hz, 2H), 7.56 (d, *J* = 8.8Hz, 1H), 7.09 (d, *J* =1.9Hz, 1H), 7.02 (dd, *J* = 8.8, 2.3, 1H), 6.98 (d, *J* = 9Hz, 2H), 3.26 (s, 2H), exchangeable protons not observed.



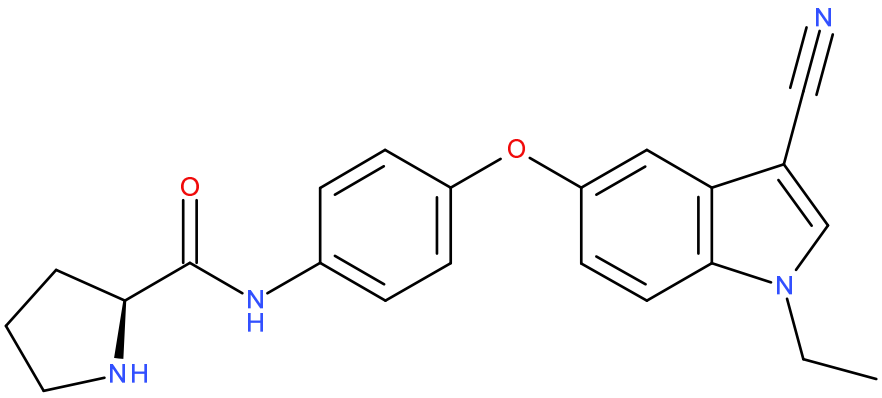
2-Amino-*N*-[4-(3-cyano-1-ethyl-indol-5-yl)oxyphenyl]acetamide (52). Using the same procedure as for (2*S*)-*N*-[4-(3-cyano-1-isobutyl-indol-5-yl)oxyphenyl]pyrrolidine-2-carboxamide **26** using *N*-BOC glycine instead of (2*S*)-1-*tert*-butoxycarbonylpyrrolidine-2-carboxylic acid and ethyl iodide instead of 1-bromo-2-methyl-propane. 1-Hydroxybenzotriazole/ 3-(Ethyliminomethyleneamino)-*N,N*-dimethylpropan-1-amine were used instead of *N,N,N′,N′*-tetramethyl-*O*-(1H-benzotriazol-1-yl)uronium hexafluorophosphate and 4M HCl in dioxane instead of TFA. HRMS ESI^+ *m/z* observed 335.1483, $C_{19}H_{19}N_4O_2$ requires 335.1508. ^{1}H NMR δ (500 MHz, DMSO) 8.30 (s, 1H), 7.66 – 7.72 (m, 1H), 7.58 – 7.65 (m, 2H), 7.07 (dd, *J* = 0.46, 2.21 Hz, 1H), 7.04 (dd, *J* = 2.32, 8.87 Hz, 1H), 6.91 – 7 (m, 2H), 4.26 (q, *J* = 7.24 Hz, 2H), 3.24 (s, 2H), 1.38 (t, *J* = 7.23 Hz, 3H), exchangeable protons not observed.



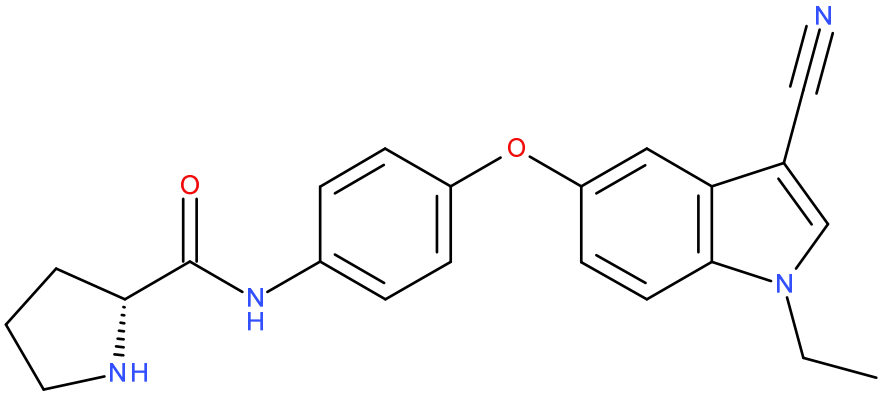
***N*-[4-(3-Cyano-1-ethyl-indol-5-yl)oxyphenyl]-2-(methylamino)acetamide (53).** Using the same procedure as for (2*S*)-*N*-[4-(3-cyano-1-isobutyl-indol-5-yl)oxyphenyl]pyrrolidine-2-carboxamide **26** using 2-[*tert*-butoxycarbonyl(methyl)amino]acetic acid instead of (2*S*)-1-*tert*-butoxycarbonylpyrrolidine-2-carboxylic acid and ethyl iodide instead of 1-bromo-2-methyl-propane. 1-Hydroxybenzotriazole/ 3-(Ethyliminomethyleneamino)-*N,N*-dimethylpropan-1-amine were used instead of *N,N,N′,N′*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate and 4M HCl in dioxane instead of TFA. HRMS ESI$^+$ *m/z* observed 349.1658, $C_{20}H_{21}N_4O_2$ requires 349.1665. ^{1}H NMR δ (500 MHz, DMSO) 8.30 (s, 1H), 7.69 (dd, *J* = 0.44, 8.85 Hz, 1H), 7.57 – 7.66 (m, 2H), 7.07 – 7.1 (m, 1H), 7.04 (dd, *J* = 2.36, 8.87 Hz, 1H), 6.93 – 7 (m, 2H), 4.26 (q, *J* = 7.24, 7.24, 7.24 Hz, 2H), 3.21 (s, 2H), 2.30 (s, 3H), 1.38 (t, *J* = 7.24, 7.24 Hz, 3H), exchangeable protons not observed.



***N*-[4-(3-Cyano-1-ethyl-indol-5-yl)oxyphenyl]-2-(dimethylamino)acetamide (54).** Using the same procedure as for (2*S*)-*N*-[4-(3-cyano-1-isobutyl-indol-5-yl)oxyphenyl]pyrrolidine-2-carboxamide **26** using 2-(dimethylamino)acetic acid instead of (2*S*)-1-*tert*-butoxycarbonylpyrrolidine-2-carboxylic acid and ethyl iodide instead of 1-bromo-2-methyl-propane. 1-Hydroxybenzotriazole/ 3-(Ethyliminomethyleneamino)-*N,N*-dimethylpropan-1-amine were used instead of *N,N,N′,N′*-tetramethyl-*O*-(1H-benzotriazol-1-yl)uronium hexafluorophosphate and omitting the deprotection step (isolated as the formate salt). HRMS ESI^+ *m/z* observed 363.1817, $C_{21}H_{23}N_4O_2$ requires 363.1821. ^1H NMR δ (500 MHz, DMSO) 10.74 (s, 1H), 9.90 (s, 1H), 8.32 (s, 1H), 7.72 (d, *J* = 8.76 Hz, 1H), 7.57 – 7.64 (m, 2H), 7.08 – 7.1 (m, 1H), 7.07 (dd, *J* = 2.36, 8.80 Hz, 1H), 7.01 – 7.04 (m, 2H), 4.28 (q, *J* = 7.23, 7.23, 7.24 Hz, 2H), 4.12 (s, 2H), 2.86 (s, 6H), 1.39 (t, *J* = 7.24, 7.24 Hz, 3H).

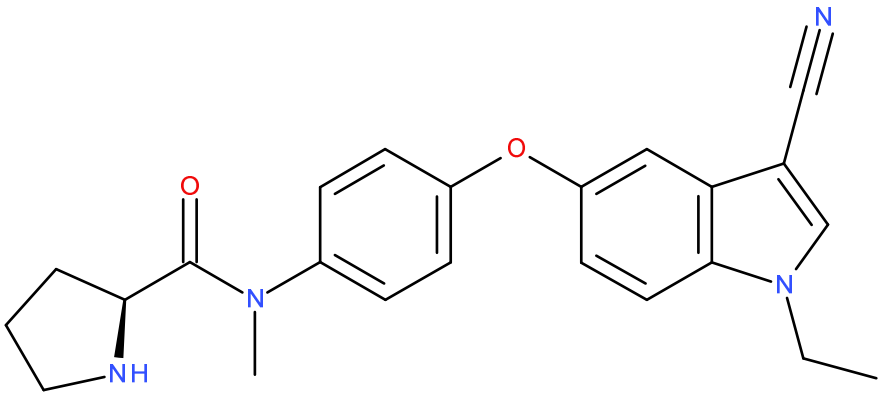


(2*S*)-*N*-[4-(3-Cyano-1-ethyl-indol-5-yl)oxyphenyl]pyrrolidine-2-carboxamide (55). Using the same procedure as for (2*S*)-*N*-[4-(3-cyano-1-isobutyl-indol-5-yl)oxyphenyl]pyrrolidine-2-carboxamide **26** using ethyl iodide instead of 1-bromo-2-methyl-propane. HRMS ESI^+ *m/z* observed 375.18158, $C_{22}H_{23}N_4O_2$ requires 375.18155. ^1H NMR δ (Methanol-d4) 8.01 (s, 1H), 7.60 – 7.53 (m, 3H), 7.15 (d, *J* = 2.0Hz, 1H), 7.07 (dd, *J* = 9.0Hz, 2.0Hz, 1H), 6.97 (d, *J* = 9.0Hz, 2H), 4.30 (q, *J* = 7.2Hz, 2H), 3.98 (m, 1H), 3.24 – 3.05 (m, 2H), 2.37 – 2.27 (m, 1H), 2.04 – 1.85 (m, 3H), 1.49 (t, *J* = 7.2Hz, 3H), exchangeable protons not observed.



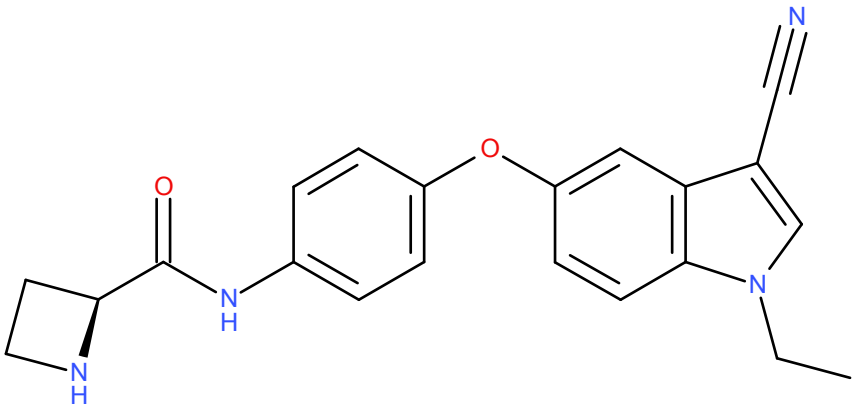
(2*R*)-*N*-[4-(3-Cyano-1-ethyl-indol-5-yl)oxyphenyl]pyrrolidine-2-carboxamide (56). Using the same procedure as for (2*S*)-*N*-[4-(3-cyano-1-isobutyl-indol-5-yl)oxyphenyl]pyrrolidine-2-carboxamide **26** using (2*R*)-1-*tert*-butoxycarbonylpyrrolidine-2-carboxylic acid instead of (2*S*)-

1-*tert*-butoxycarbonylpyrrolidine-2-carboxylic acid and ethyl iodide instead of 1-bromo-2-methyl-propane. HRMS ESI$^+$ *m/z* observed 375.18167, $C_{22}H_{23}N_4O_2$ requires 375.18155. ^{1}H NMR δ (Methanol-d4) 8.01 (s, 1H), 7.60 – 7.53 (m, 3H), 7.14 (d, *J* = 2.3Hz, 1H), 7.07 (dd, *J* = 9.0Hz, 2.3Hz, 1H), 6.97 (d, *J* = 9.0Hz, 2H), 4.30 (q, *J* = 7.3Hz, 2H), 4.10 (m, 1H), 3.24 – 3.10 (m, 2H), 2.42 – 2.33 (m, 1H), 2.07 – 1.91 (m, 3H), 1.48 (t, *J* = 7.3Hz, 3H), exchangeable protons not observed.

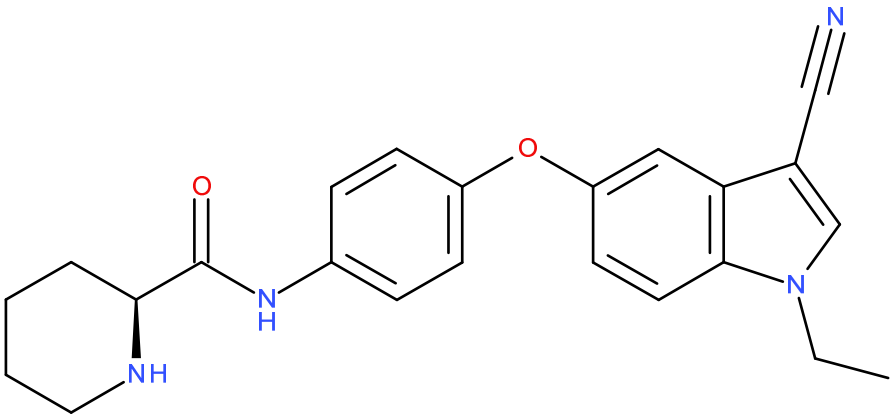


(2*S*)-*N*-[4-(3-Cyano-1-ethyl-indol-5-yl)oxyphenyl]-N-methyl-pyrrolidine-2-carboxamide (57). (*S*)-2-[4-(3-Cyano-1-ethyl-1*H*-indol-5-yloxy)-phenylcarbamoyl]-pyrrolidine-1-carboxylic acid tert-butyl ester (intermediate from synthesis of **55**) (50 mg, 0.1 mmol) was dissolved in THF (2 mL) and treated with sodium hydride 60% in mineral oil (5 mg, 0.12 mmol) and stirred for 10 minutes before the addition of methyl iodide (0.006 mL, 0.1 mmol). The reaction mixture was stirred at room temperature for 30 minutes then treated with water (2 mL). The mixture was extracted into DCM (5 mL) which was evaporated to dryness. The residue was dissolved in DCM (1 mL) and TFA (1 mL) was added before stirring for 16 hours. The DCM and TFA were evaporated and the residue was neutralised by 1 mL of 2M ammonia in methanol which was then evaporated. The residue was then purified by prep Method C. Fractions containing product were

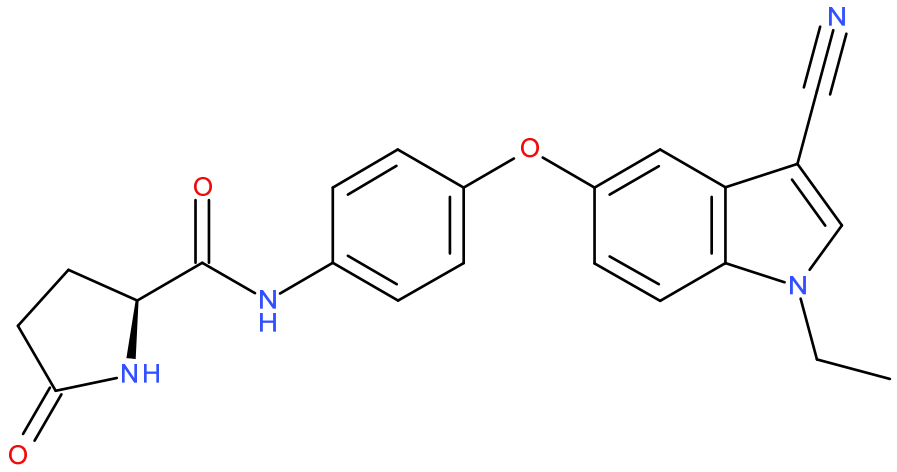
passed down an SCX column washing with methanol and eluting with 2M ammonia in methanol. This was then evaporated dried in the vacuum oven to yield **57** (10 mg, 25%). HRMS ESI$^+$ *m/z* observed 389.19727, $C_{23}H_{25}N_4O_2$ requires 389.19720. ^{1}H NMR δ (d6-DMSO) 8.38 (s, 1H), 7.78 (d, *J*= 8.9Hz, 1H), 7.34(d, *J* = 8.6Hz, 2H), 7.27 (s, 1H), 7.14 (dd, *J* = 8.9, 2.1Hz, 1H), 7.04 (d, *J* = 8.6Hz, 2H), 4.31 (q, *J* = 7.2Hz, 2H), 3.35-3.41 (m, 1H), 3.16 (s, 3H), 2.95-3(m, 2H), 1.45-1.6 (m, 4H), 1.41 (t, *J* = 7.2Hz, 3H).



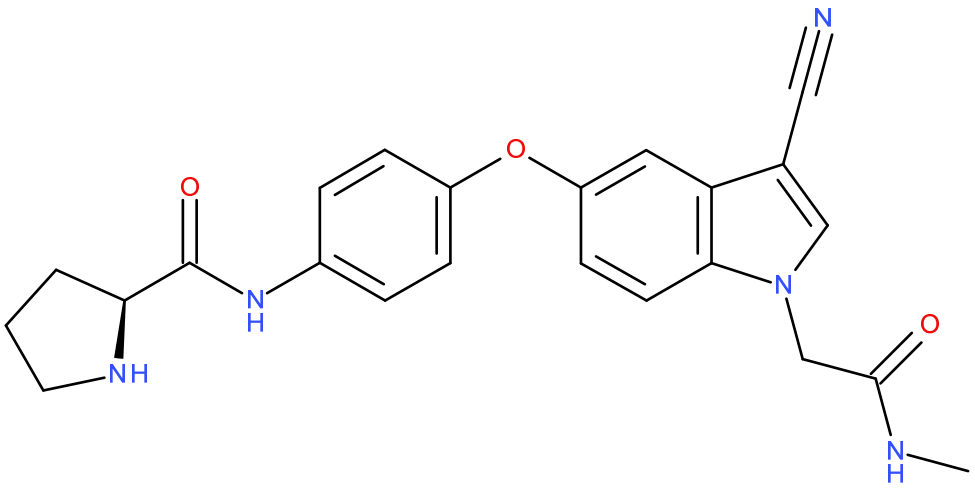
(2*S*)-*N*-[4-(3-cyano-1-ethyl-indol-5-yl)oxyphenyl]azetidine-2-carboxamide (58). Using the same procedure as for (2*S*)-*N*-[4-(3-cyano-1-isobutyl-indol-5-yl)oxyphenyl]pyrrolidine-2-carboxamide **26** using (2*S*)-1-*tert*-butoxycarbonylazetidine-2-carboxylic acid instead of (2*S*)-1-*tert*-butoxycarbonylpyrrolidine-2-carboxylic acid and ethyl iodide instead of 1-bromo-2-methyl-propane. 1-Hydroxybenzotriazole/ 3-(Ethyliminomethyleneamino)-*N,N*-dimethylpropan-1-amine were used instead of *N,N,N′,N′*-tetramethyl-*O*-(1H-benzotriazol-1-yl)uronium hexafluorophosphate and 4M HCl in dioxane instead of TFA. HRMS ESI$^+$ *m/z* observed 361.1675, $C_{21}H_{21}N_4O_2$ requires 361.1665.



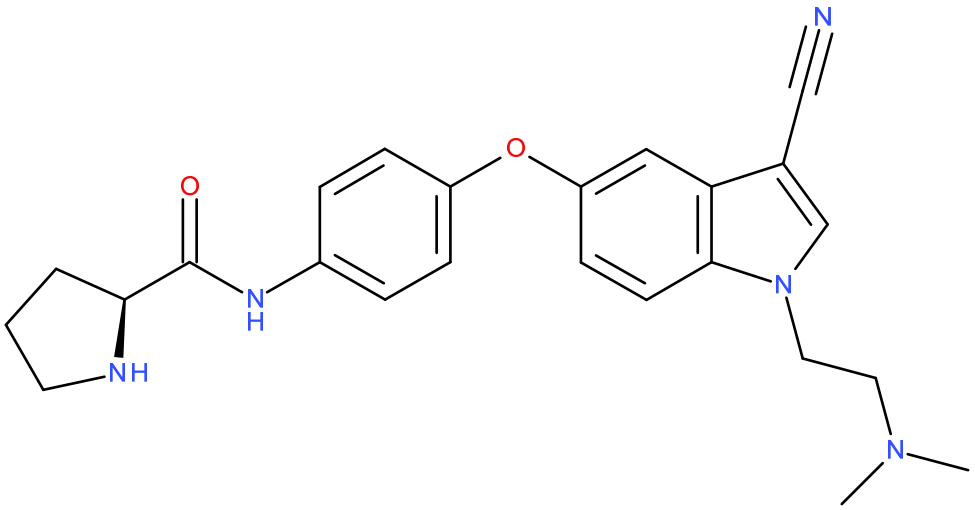
(2*S*)-*N*-[4-(3-Cyano-1-ethyl-indol-5-yl)oxyphenyl]piperidine-2-carboxamide (59). Using the same procedure as for (2*S*)-*N*-[4-(3-cyano-1-isobutyl-indol-5-yl)oxyphenyl]pyrrolidine-2-carboxamide **26** using (2*S*)-1-tert-butoxycarbonylpiperidine-2-carboxylic acid instead of (2*S*)-1-*tert*-butoxycarbonylpyrrolidine-2-carboxylic acid and ethyl iodide instead of 1-bromo-2-methyl-propane. 1-Hydroxybenzotriazole/ 3-(Ethyliminomethyleneamino)-*N,N*-dimethylpropan-1-amine were used instead of *N,N,N′,N′*-tetramethyl-*O*-(1H-benzotriazol-1-yl)uronium hexafluorophosphate and 4M HCl in dioxane instead of TFA. HRMS ESI^+ *m/z* observed 389.1965, $C_{23}H_{25}N_4O_2$ requires 389.1978. ^{1}H NMR δ (500 MHz, DMSO) 9.68 (s, 1H), 8.31 (s, 1H), 7.70 (dd, *J* = 0.50, 8.84 Hz, 1H), 7.62 – 7.66 (m, 2H), 7.08 (dd, *J* = 0.46, 2.20 Hz, 1H), 7.05 (dd, *J* = 2.40, 8.85 Hz, 1H), 6.95 – 6.98 (m, 2H), 4.27 (q, *J* = 7.21 Hz, 2H), 3.22 – 3.34 (m, 4H), 2.98 (d, *J* = 13.13 Hz, 1H), 2.54 – 2.66 (m, 1H), 1.72 – 1.87 (m, 2H), 1.50 (d, *J* = 11.15 Hz, 1H), 1.39 (t, *J* = 7.23 Hz, 3H), one exchangeable proton not observed.



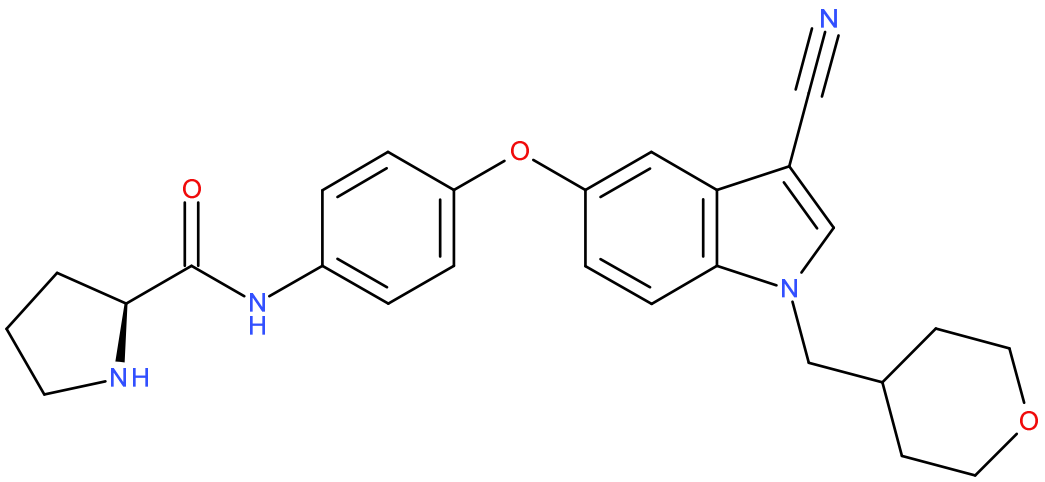
(2*S*)-*N*-[4-(3-cyano-1-ethyl-indol-5-yl)oxyphenyl]-5-oxo-pyrrolidine-2-carboxamide (60). Using the same procedure as for (2*S*)-*N*-[4-(3-cyano-1-isobutyl-indol-5-yl)oxyphenyl]pyrrolidine-2-carboxamide **26** using (2*S*)-5-oxopyrrolidine-2-carboxylic acid instead of (2*S*)-1-*tert*-butoxycarbonylpyrrolidine-2-carboxylic acid and ethyl iodide instead of 1-bromo-2-methyl-propane. 1-Hydroxybenzotriazole/ 3-(Ethyliminomethyleneamino)-*N,N*-dimethylpropan-1-amine were used instead of *N,N,N′,N′*-tetramethyl-*O*-(1H-benzotriazol-1-yl)uronium hexafluorophosphate and omitting the deprotection step. HRMS ESI^+ *m/z* observed 389.1602, $C_{22}H_{21}N_4O_3$ requires 389.1614. ^{1}H NMR δ (500 MHz, DMSO) 9.98 (s, 1H), 8.27 (s, 1H), 7.80 (s, 1H), 7.66 (dd, *J* = 0.47, 8.85 Hz, 1H), 7.53 – 7.63 (m, 2H), 7.05 (dd, *J* = 0.44, 2.22 Hz, 1H), 7.01 (dd, *J* = 2.40, 8.88 Hz, 1H), 6.92 – 6.97 (m, 2H), 4.23 (q, *J* = 7.24, 7.24, 7.24 Hz, 2H), 4.07 – 4.18 (m, 1H), 2.23 – 2.33 (m, 1H), 2.04 – 2.21 (m, 2H), 1.9 – 1.99 (m, 1H), 1.35 (t, *J* = 7.24, 7.24 Hz, 3H).



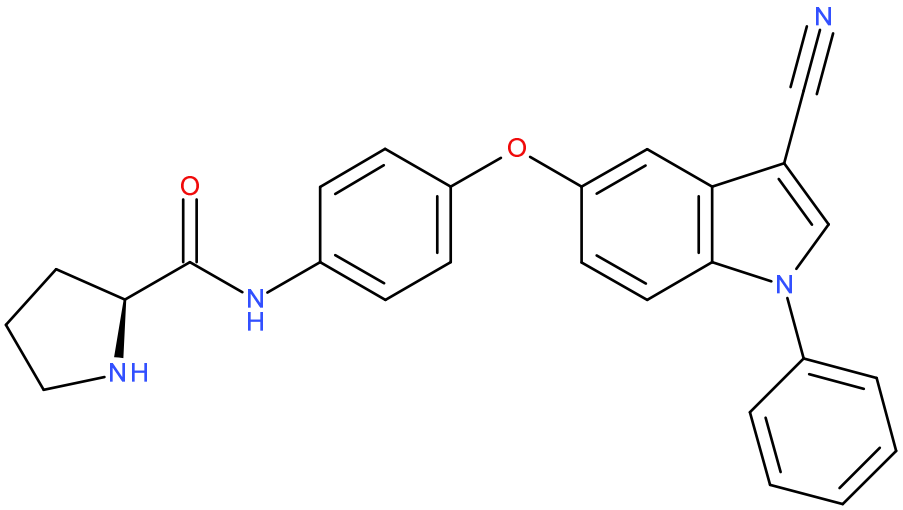
(2*S*)-*N*-[4-[3-cyano-1-[2-(methylamino)-2-oxo-ethyl]indol-5-yl]oxyphenyl]pyrrolidine-2-carboxamide (61). Using the same procedure as for (2*S*)-*N*-[4-(3-cyano-1-isobutyl-indol-5-yl)oxyphenyl]pyrrolidine-2-carboxamide **26** using 2-chloro-*N*-methyl-acetamide instead of 1-bromo-2-methyl-propane. HRMS ESI$^+$ *m/z* observed 418.1849, $C_{23}H_{24}N_5O_3$ requires 418.1879. ^{1}H NMR δ (d6-DMSO) 9.98 (s, 1H), 8.24 (s, 1H), 8.21 (m, 1H), 7.66 (d, *J* = 9.0Hz, 2H), 7.55 (d, *J* = 8.9Hz, 1H), 7.11 (d, *J* = 2.2Hz, 1H), 7.06 (dd, *J* = 8.9Hz, 2.2Hz, 1H), 6.98 (d, *J* = 9.0Hz, 2H), 4.94 (s, 2H), 3.69 (m, 1H), 2.90 (t, *J* = 6.6Hz, 2H), 2.64 (d, *J* = 4.6Hz, 3H), 2.07 – 1.98 (m, 1H), 1.83 – 1.74 (m, 1H), 1.70 – 1.62 (m, 2H), one exchangeable proton not observed.



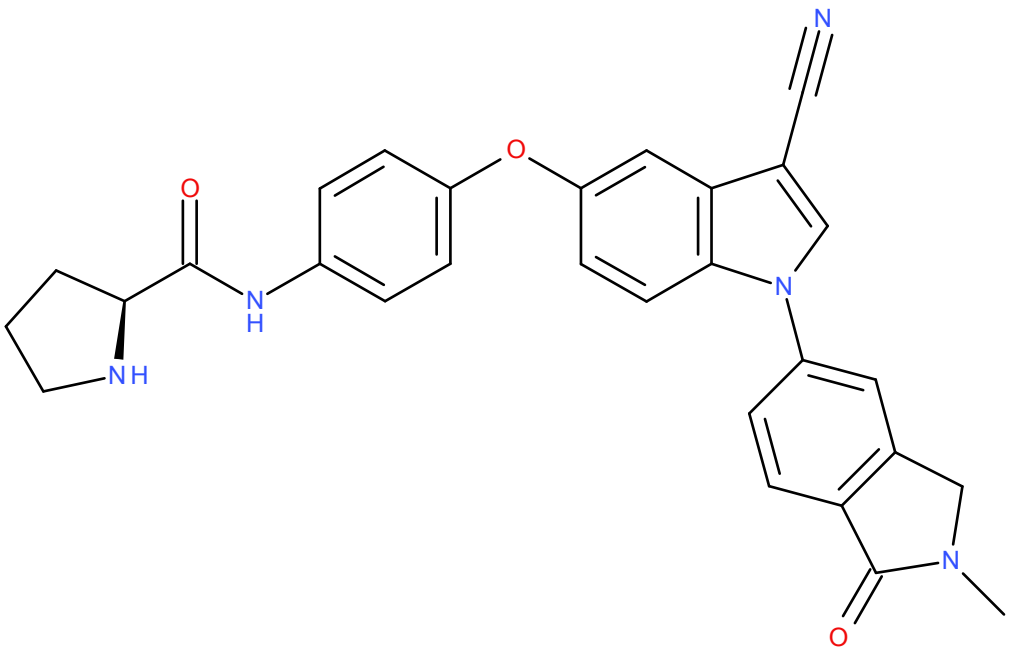
(2*S*)-*N*-[4-[3-Cyano-1-[2-(dimethylamino)ethyl]indol-5-yl]oxyphenyl]pyrrolidine-2-carboxamide (62). To a solution of (*S*)-2-[4-(3-cyano-1-ethyl-1*H*-indol-5-yloxy)-phenylcarbamoyl]-pyrrolidine-1-carboxylic acid *tert*-butyl ester (intermediate from synthesis of **55**) (0.2 mmol, 1.0 eq) and 2-chloro-*N,N*-dimethyl-ethanamine (0.3 mmol, 1.5 eq) in dioxane (1.5 mL) was added potassium *tert*-butoxide (0.4 mmol, 2.0 eq), and the reaction was shaken at 105°C overnight. The crude product was purified by preparative prep-TLC to afford alkylated product. The intermediate was stirred in 2*N* HCl/dioxane (2 mL) overnight at room temperature before the solvent was removed in vacuo to afford **62**. HRMS ESI^+ *m/z* observed 418.2226, $C_{24}H_{28}N_5O_2$ requires 418.2243.



(2*S*)-*N*-[4-[3-Cyano-1-(tetrahydropyran-4-ylmethyl)indol-5-yl]oxyphenyl]pyrrolidine-2-carboxamide (63). Using the same procedure as for (2*S*)-*N*-[4-(3-cyano-1-isobutyl-indol-5-yl)oxyphenyl]pyrrolidine-2-carboxamide **26** using 4-(bromomethyl)tetrahydro-2*H*-pyran instead of 1-bromo-2-methyl-propane. HRMS ESI^+ *m/z* observed 445.2247, $C_{26}H_{29}N_4O_3$ requires 445.2240. ^{1}H NMR δ (400 MHz, DMSO) 9.92 (s, 1H), 8.29 (s, 1H), 7.77 (d, *J* = 8.69 Hz, 1H), 7.61 – 7.71 (m, 2H), 7.03 – 7.13 (m, 2H), 6.94 – 7.03 (m, 2H), 4.17 (d, *J* = 7.30 Hz, 2H), 3.77 – 3.89 (m, 2H), 3.68 (dd, *J* = 5.60, 8.78 Hz, 1H), 3.22 (td, *J* = 2.24, 11.54, 11.55 Hz, 2H), 2.90 (t, *J* = 6.58, 6.58 Hz, 2H), 1.97 – 2.17 (m, 2H), 1.73 – 1.85 (m, 1H), 1.66 (p, *J* = 6.63, 6.63, 6.65, 6.65 Hz, 2H), 1.21 – 1.43 (m, 4H), one exchangeable proton not observed.

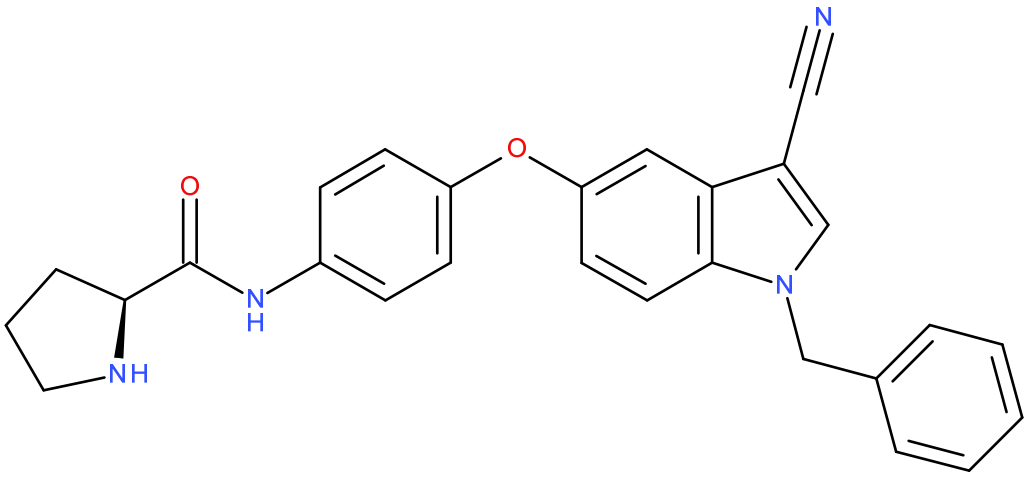


(2*S*)-*N*-[4-(3-Cyano-1-phenyl-indol-5-yl)oxyphenyl]pyrrolidine-2-carboxamide (64). Phenyl boronic acid (48 mg, 0.4 mmol), *tert*-butyl (2*S*)-2-[[4-[(3-cyano-1*H*-indol-5-yl)oxy]phenyl]carbamoyl]pyrrolidine-1-carboxylate **24** (90 mg, 0.2 mmol), copper acetate (18 mg, 0.1 mmol), 4Å mol sieves (50 mg) and pyridine (80 mg, 1 mmol) were added to DCM (2 mL). Compressed air was bubbled through the reaction mixture and then left to stir for 3 days at room temperature. The reaction mixture was washed with water (2 mL) then treated with TFA (1 mL) and left to stir overnight. The mixture was then evaporated and purified by prep LCMS (Method C). This was then passed down an SCX washing with methanol and eluting with 2M ammonia in methanol. Fractions were evaporated and dried to yield **64** (30 mg, 38%). HRMS ESI+ *m/z* observed 423.18158, $C_{26}H_{23}N_4O_2$ requires 423.18155. ^{1}H NMR δ (DMSO) 9.97 (s, 1H), 8.62 (s, 1H), 7.5-7.7 (m, 8H), 7.19 (d, *J* = 2.4Hz, 1H), 7.09 (dd, *J* = 9, 2.4Hz, 1H), 7.01 (d, *J* = 9Hz, 2H), 3.68 (dd, *J* = 8.7, 5.6Hz, 1H), 2.89 (t, *J*=6.6Hz, 2H), 1.99-2.08 (m, 1H), 1.73-1.82(m, 1H), 1.61-1.68 (m, 2H), NH not observed.

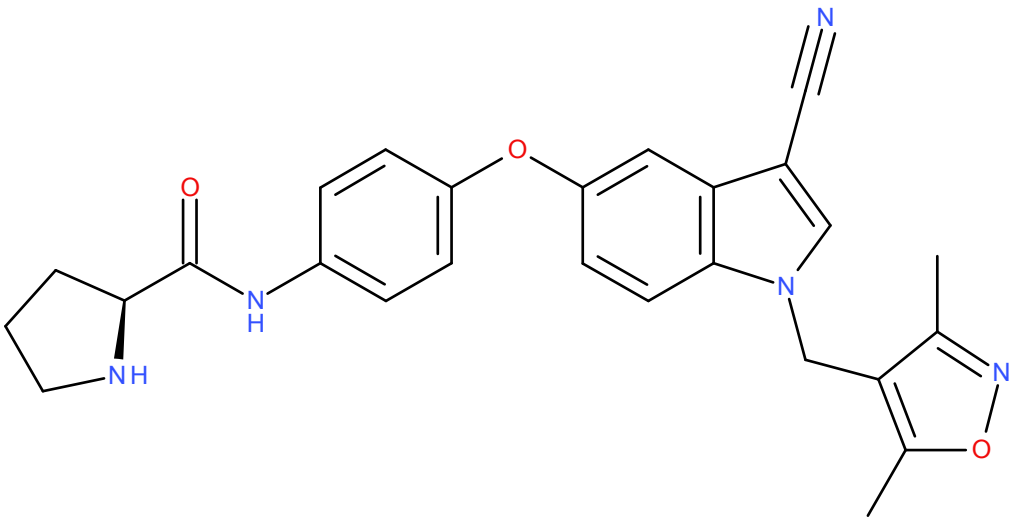


(2*S*)-*N*-[4-[3-Cyano-1-(2-methyl-1-oxo-isoindolin-5-yl)indol-5-yl]oxyphenyl]pyrrolidine-2-carboxamide (65). Into a 40-mL sealed tube (1 atm) purged and maintained with an inert atmosphere of nitrogen, was placed *tert*-butyl (2*S*)-2-[[4-[(3-cyano-1*H*-indol-5-yl)oxy]phenyl]carbamoyl]pyrrolidine-1-carboxylate **24** (156 mg, 0.35 mmol), 5-bromo-2-methyl-isoindolin-1-one (158 mg, 0.70 mmol), K_3PO_4 (223 mg, 1.05 mmol), dioxane (10 mL), (1*S*, 2*S*)-1-*N*,2-*N*-dimethylcyclohexane-1,2-diamine (25 mg, 0.18 mmol) and CuI (7 mg, 0.04 mmol). The resulting solution was stirred for 6 hours at 100°C. The residue was dissolved in 30 mL of H_2O. The resulting solution was extracted with 3 x 30 mL of DCM and the organic layers combined and dried over anhydrous sodium sulfate. The solids were filtered and the resulting mixture was concentrated under vacuum. The product was dissolved in dichloromethane (10 mL) and to the solution was added trifluoroacetic acid (1.5 mL). The resulting solution was stirred for 2 hours at 25°C. The resulting mixture was concentrated under vacuum and dissolved in 30 mL of ethyl acetate. The mixture was washed with 2 x 10 mL of aq. sodium bicarbonate before the organic layer was concentrated *in vacuo*. residue was then purified by prep Method E. This gave

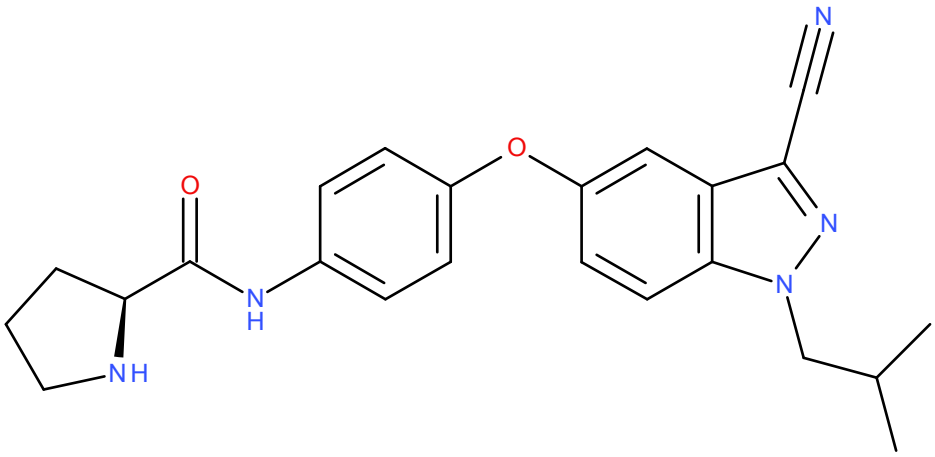
65 (88 mg, 77%) as a white solid. HRMS ESI$^+$ *m/z* observed 492.2021, $C_{29}H_{26}N_5O_3$ requires 492.2036. 1H NMR (700 MHz, DMSO) δ 10.08 (s, 1H), 8.66 (s, 1H), 7.89 – 7.93 (m, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.74 (dd, *J* = 8.0, 1.8 Hz, 1H), 7.63 – 7.7 (m, 3H), 7.20 (d, *J* = 2.3 Hz, 1H), 7.12 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.02 (dq, *J* = 8.4, 3.0 Hz, 2H), 4.56 (s, 2H), 3.76 – 3.9 (m, 1H), 3.11 (s, 3H), 2.98 (t, *J* = 6.0 Hz, 2H), 2.11 (dq, *J* = 15.6, 7.5 Hz, 1H), 1.83 (dq, *J* = 13.1, 7.1 Hz, 1H), 1.72 (p, *J* = 6.7 Hz, 2H), one exchangable proton not observed.



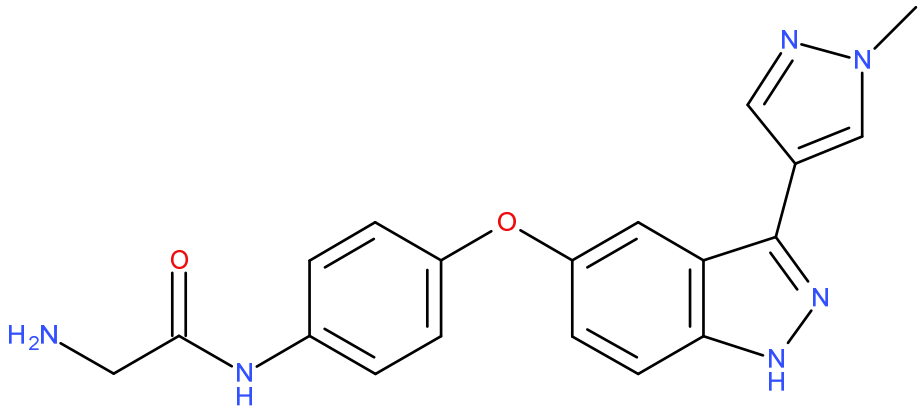
(2*S*)-*N*-[4-(1-Benzyl-3-cyano-indol-5-yl)oxyphenyl]pyrrolidine-2-carboxamide (66). Using the same procedure as for (2*S*)-*N*-[4-(3-cyano-1-isobutyl-indol-5-yl)oxyphenyl]pyrrolidine-2-carboxamide **26** using benzyl bromide instead of 1-bromo-2-methyl-propane. HRMS ESI$^+$ *m/z* observed 437.19714, $C_{27}H_{25}N_4O_2$ requires 437.19720. ^{1}H NMR δ (d6-DMSO) 9.95 (s, 1H), 8.49 (s, 1H), 7.70 – 7.63 (m, 3H), 7.38 – 7.27 (m, 5H), 7.09 (d, *J* = 2.2Hz, 1H), 7.03 (dd, *J* = 8.9Hz, 2.2Hz, 1H), 6.97 (d, *J* = 9.0Hz, 2H), 5.51 (s, 2H), 3.67 (m, 1H), 2.89 (t, *J* = 6.6Hz, 2H), 2.08 – 1.97 (m, 1H), 1.83 – 1.72 (m, 1H), 1.68 – 1.60 (m, 2H), one exchangeable proton not observed.



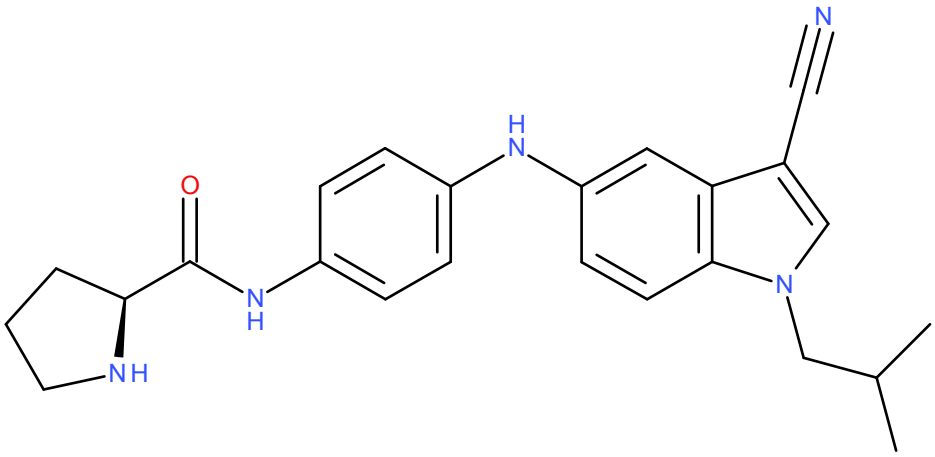
(2*S*)-*N*-[4-[3-Cyano-1-[(3,5-dimethylisoxazol-4-yl)methyl]indol-5-yl]oxyphenyl]pyrrolidine-2-carboxamide (67). Using the same procedure as for (2*S*)-*N*-[4-[3-cyano-1-[2-(dimethylamino)ethyl]indol-5-yl]oxyphenyl]pyrrolidine-2-carboxamide **63** using 4-(chloromethyl)-3,5-dimethyl-isoxazole instead of 2-chloro-*N,N*-dimethyl-ethanamine. HRMS ESI^+ *m/z* observed 456.2026, $C_{26}H_{26}N_5O_3$ requires 456.2036. ^1H NMR δ (500 MHz, DMSO) 10.77 (s, 1H), 8.26 (s, 1H), 7.57 – 7.65 (m, 3H), 7.05 – 7.11 (m, 2H), 6.98 – 7.05 (m, 2H), 5.32 (s, 2H), 4.26 – 4.41 (m, 1H), 3.19 – 3.27 (m, 2H), 2.40 (s, 3H), 1.99 (s, 3H), 1.88 – 1.97 (m, 4H), one exchangeable proton not observed.



(2*S*)-*N*-[4-(3-Cyano-1-isobutyl-indazol-5-yl)oxyphenyl]pyrrolidine-2-carboxamide (68). Using the same procedure as for (2S)-N-[4-[3-cyano-1-[(3,5-dimethylisoxazol-4-yl)methyl]indazol-5-yl]oxyphenyl]pyrrolidine-2-carboxamide 33 using 1-bromo-2-methyl-propane instead of 4-(chloromethyl)-3,5-dimethyl-isoxazole. HRMS ESI+ m/z observed 404.2085, $C_{23}H_{26}N_5O_2$ requires 404.2087. 1H NMR (700 MHz, DMSO) δ 10.07 (s, 1H), 7.99 (dd, *J* = 9.1, 4.3 Hz, 1H), 7.65 – 7.73 (m, 2H), 7.33 (dd, *J* = 9.1, 2.3 Hz, 1H), 7.19 (d, *J* = 2.0 Hz, 1H), 7.05 (dq, *J* = 8.2, 3.0 Hz, 2H), 4.36 (d, *J* = 7.3 Hz, 2H), 3.78 (dd, *J* = 8.7, 6.0 Hz, 1H), 2.95 (t, *J* = 6.7 Hz, 2H), 2.24 (dp, *J* = 13.7, 6.8 Hz, 1H), 2.09 (td, *J* = 8.1, 5.1 Hz, 1H), 1.81 (dq, *J* = 13.0, 7.2 Hz, 1H), 1.63 – 1.74 (m, 2H), 0.86 (d, *J* = 6.7 Hz, 6H), one exchangable proton not observed.



2-Amino-*N*-[4-[[3-(1-methylpyrazol-4-yl)-1*H*-indol-5-yl]oxy]phenyl]acetamide (69). Using the same procedure as for (2*S*)-*N*-[4-[[3-(1-methylpyrazol-4-yl)-1*H*-indol-5-yl]oxy]phenyl]pyrrolidine-2-carboxamide **40** using 2-(benzyloxycarbonylamino)acetic acid instead of (2*S*)-1-*tert*-butoxycarbonylpyrrolidine-2-carboxylic acid. Instead of TFA deprotection the intermediate was dissolved in methanol (2 mL) and added to 10% palladium on charcoal (5 mg) under nitrogen. The reaction mixture was evacuated and flushed with nitrogen (x 3) then hydrogen (x 3) then stirred under an atmosphere of hydrogen at atmospheric pressure for 2 hours. The mixture was evacuated and flushed with nitrogen (x 3) then filtered through Celite and evaporated. The crude material was purified by prep LCMS (Method A) then free based by absorbing onto 1g SCX cartridge, washing with methanol then eluting with 2M ammonia in methanol. Methanol fractions containing desired product were evaporated under a stream of nitrogen then vacuum oven at 40°C overnight to afford **69** (11 mg, 54%) as a white solid. HRMS ESI+ m/z observed 362.1602, $C_{20}H_{19}N_5O_2$ requires 362.1617. ^{1}H NMR δ (d6-DMSO) 11.21 (br s, 1H), 8.05 (s, 1H), 7.71 (s, 1H), 7.60 (d, *J* = 2.5Hz, 1H), 7.56 (d, *J* = 9.0Hz, 2H), 7.44 – 7.39 (m, 2H), 6.88 (d, *J* = 9.0Hz, 2H), 6.83 (dd, *J* = 8.7Hz, 2.3Hz, 1H), 3.85 (s, 3H), 3.12 – 2.88 (br s, 2H). CH_2 protons under DMSO peak, amide NH not observed.



(*S*)-*N*-(4-((3-Cyano-1-isobutyl-1*H*-indol-5-yl)amino)phenyl)pyrrolidine-2-carboxamide (70). 1-Bromo-2-methylpropane (1.86 mL, 17.10 mmol) was added to 5-nitro-1*H*-indole-3-carbonitrile (2 g, 10.69 mmol), and cesium carbonate (5.57 g, 17.10 mmol) in DMF (25 mL) at 20°C under nitrogen. The resulting mixture was stirred at 80°C for 3.5 hours. The reaction mixture was diluted with ethyl acetate (500 mL), and water (100 mL). The organic phase was washed sequentially with water (100 mL), and saturated brine (100 mL) then dried over $MgSO_4$, before being filtered and evaporated to afford a solid. The crude product was purified by flash silica chromatography, elution gradient 10 to 50% ethyl acetate in heptane. Pure fractions were evaporated to dryness to afford 1-isobutyl-5-nitro-1*H*-indole-3-carbonitrile (2.02 g, 78%) as a beige solid. *m/z*: ES- $[M-H]^-$ 242. ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.68 – 8.77 (m, 1H), 8.24 (dd, *J* = 2.19, 9.13 Hz, 1H), 7.75 (s, 1H), 7.49 (d, *J* = 9.10 Hz, 1H), 4.03 (d, *J* = 7.40 Hz, 2H), 2.09 – 2.36 (m, 1H), 0.98 (d, *J* = 6.67 Hz, 6H).

10% Palladium on carbon (0.20 g, 0.19 mmol) was added to a solution of 1-isobutyl-5-nitro-1*H*-indole-3-carbonitrile (2.02 g, 8.30 mmol) in THF (50 mL) and ethanol (50 mL). This solution was allowed to stir under a hydrogen atmosphere supplied by a balloon for 4 hours. The reaction mixture was filtered through Celite and washed with THF and evaporated. The crude product

was purified by flash silica chromatography, elution gradient 10 to 60% ethyl acetate in heptane. Pure fractions were evaporated to dryness to afford 5-amino-1-isobutyl-1*H*-indole-3-carbonitrile (1.61 g, 91%) as a brown oil which solidified on standing. *m/z*: ES+ $[M+MeCN]^+$ 255. ^{1}H NMR δ (400 MHz, CDCl3) 7.43 (s, 1H), 7.13 – 7.2 (m, 1H), 6.97 – 7.02 (m, 1H), 6.74 (dd, *J* = 2.21, 8.70 Hz, 1H), 3.86 (d, *J* = 7.37 Hz, 2H), 3.66 (s, 2H), 2.17 (dt, *J* = 7.01, 7.01, 13.75 Hz, 1H), 0.92 (d, *J* = 6.65 Hz, 6H).

Brettphos pre-catalyst (0.060 g, 0.08 mmol) was added to a degassed mixture of 5-amino-1-isobutyl-1*H*-indole-3-carbonitrile (0.8 g, 3.75 mmol), 1-bromo-4-nitrobenzene (0.76 g, 3.75 mmol) and sodium *tert*-butoxide (0.433 g, 4.50 mmol) in dioxane (16 mL). The mixture was stirred in the microwave at 85 °C for 4 hours. The reaction mixture was diluted with water (10 mL), and extracted with ethyl acetate (2 x 20 mL). The organic layers were combined, washed with saturated brine, dried with $MgSO_4$ and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 10 to 50% ethyl acetate in heptane. Pure fractions were evaporated to dryness to afford 1-isobutyl-5-((4-nitrophenyl)amino)-1*H*-indole-3-carbonitrile (1.14 g, 91%) as a brown solid. *m/z*: ES+ $[M+H]^+$ 335. ^{1}H NMR δ (500 MHz, DMSO) 9.31 (s, 1H), 8.31 (s, 1H), 8.03 – 8.13 (m, 2H), 7.75 (d, *J* = 8.80 Hz, 1H), 7.45 (d, *J* = 2.03 Hz, 1H), 7.24 (dd, *J* = 2.09, 8.81 Hz, 1H), 6.96 – 7.06 (m, 2H), 4.09 (d, *J* = 7.39 Hz, 2H), 2.09 – 2.24 (m, 1H), 0.88 (d, *J* = 6.65 Hz, 6H).

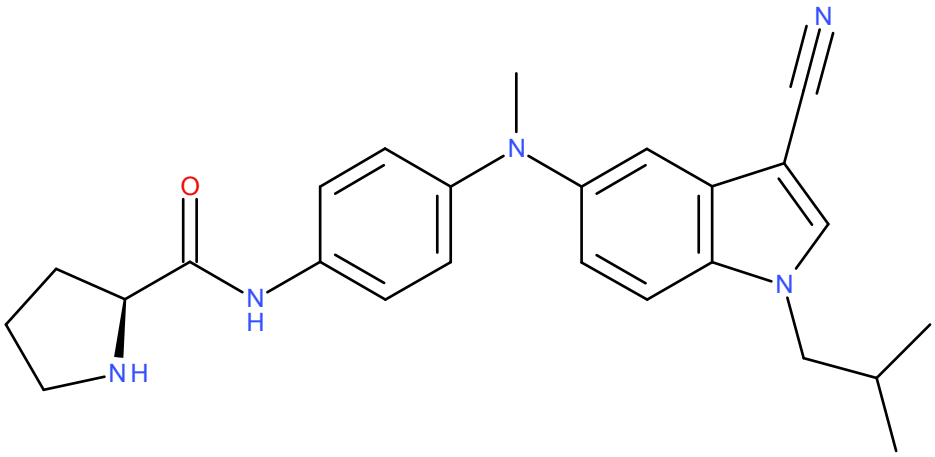
10% Palladium on carbon (0.03 g, 0.03 mmol) was added to a solution of 1-isobutyl-5-((4-nitrophenyl)amino)-1*H*-indole-3-carbonitrile (0.3 g, 0.90 mmol) in THF (15 mL) and ethanol (15 mL). This solution was allowed to stir under a hydrogen atmosphere supplied by a balloon for 4 hours. The reaction mixture was filtered through Celite and washed with THF and evaporated. The crude product was purified by flash silica chromatography, elution gradient 20 to 80% ethyl

acetate in heptane. Pure fractions were evaporated to dryness to afford 5-((4-aminophenyl)amino)-1-isobutyl-1*H*-indole-3-carbonitrile (0.16 g, 59%) as a purple oil. *m/z*: ES+ $[M+H]^+$ 305. ^{1}H NMR δ (400 MHz, $CDCl_3$) 7.45 (s, 1H), 7.16 – 7.23 (m, 2H), 6.94 – 6.99 (m, 2H), 6.92 (dt, *J* = 2.62, 2.62, 8.82 Hz, 1H), 6.64 – 6.71 (m, 2H), 5.40 (s, 1H), 3.87 (d, *J* = 7.36 Hz, 2H), 3.53 (s, 2H), 2.18 (dp, *J* = 6.83, 6.83, 6.86, 6.86, 13.84 Hz, 1H), 0.93 (d, *J* = 6.65 Hz, 6H).

O-(7-Azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (0.24 g, 0.63 mmol) was added portionwise to (*S*)-1-(tert-butoxycarbonyl)pyrrolidine-2-carboxylic acid (0.1 g, 0.47 mmol), 5-((4-aminophenyl)amino)-1-isobutyl-1*H*-indole-3-carbonitrile (0.16 g, 0.53 mmol) and DIPEA (0.37 mL, 2.10 mmol) in DMF (5 mL) at room temperature under nitrogen. The resulting solution was stirred at room temperature for 2 hours. The reaction mixture was partitioned between ethyl acetate (25 mL) and water (20 mL). The aqueous layer was extracted into ethyl acetate (20 mL) and the combined organics washed with water (40 mL) and saturated brine (20 mL), dried over $MgSO_4$, filtered and evaporated to give crude product. The crude product was purified by flash silica chromatography, elution gradient 20 to 70% ethyl acetate in heptane. Pure fractions were evaporated to dryness to afford (*S*)-*tert*-butyl 2-((4-((3-cyano-1-isobutyl-1H-indol-5-yl)amino)phenyl)carbamoyl)pyrrolidine-1-carboxylate as a purple solid. *m/z*: ES- $[M-H]^-$ 500. ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.25 (s, 1H), 7.49 (s, 1H), 7.41 (d, *J* = 8.67 Hz, 2H), 7.36 (s, 1H), 7.28 (s, 1H), 7.06 (dd, *J* = 2.11, 8.81 Hz, 1H), 6.97 – 7.03 (m, 2H), 5.64 (s, 1H), 4.42 (s, 1H), 3.90 (d, *J* = 7.35 Hz, 2H), 3.46 (s, 2H), 2.53 (s, 1H), 2.20 (dp, *J* = 6.93, 6.93, 6.95, 6.95, 13.89 Hz, 1H), 1.93 (s, 3H), 1.50 (s, 9H), 0.95 (d, *J* = 6.65 Hz, 6H).

Trifluoroacetic acid (0.25 mL, 3.39 mmol) was added to (*S*)-*tert*-butyl 2-((4-((3-cyano-1-isobutyl-1*H*-indol-5-yl)amino)phenyl)carbamoyl)pyrrolidine-1-carboxylate (0.17 g, 0.34 mmol)

in DCM (5 mL). The reaction mixture was stirred at room temperature for 20 hours and then purified directly by ion exchange chromatography, using an SCX column. The desired product was eluted from the column using 7M NH_3/methanol and pure fractions were evaporated to dryness to afford a purple oil. The crude product was purified by flash silica chromatography, elution gradient 0 to 20% methanol in DCM. Pure fractions were evaporated to dryness to afford (*S*)-*N*-(4-((3-cyano-1-isobutyl-1*H*-indol-5-yl)amino)phenyl)pyrrolidine-2-carboxamide **70** (0.05 g, 38%) as a beige solid. HRMS ESI$^+$ *m/z* observed 402.2313, $C_{24}H_{28}N_5O$ requires 402.2294. ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.62 (s, 1H), 7.48 – 7.54 (m, 3H), 7.36 (d, *J* = 2.04 Hz, 1H), 7.28 (s, 1H), 7.06 (dd, *J* = 2.15, 8.83 Hz, 1H), 7 – 7.04 (m, 2H), 5.65 (s, 1H), 3.84 – 3.94 (m, 3H), 3.05 (ddt, *J* = 6.55, 6.55, 10.23, 36.79 Hz, 2H), 2.13 – 2.29 (m, 2H), 2.06 (dd, *J* = 6.01, 12.35 Hz, 1H), 1.76 (ddt, *J* = 6.09, 6.09, 12.60, 19.18 Hz, 3H), 0.95 (d, *J* = 6.66 Hz, 7H).



(*S*)-*N*-(4-((3-Cyano-1-isobutyl-1*H*-indol-5-yl)(methyl)amino)phenyl)pyrrolidine-2-carboxamide (71).

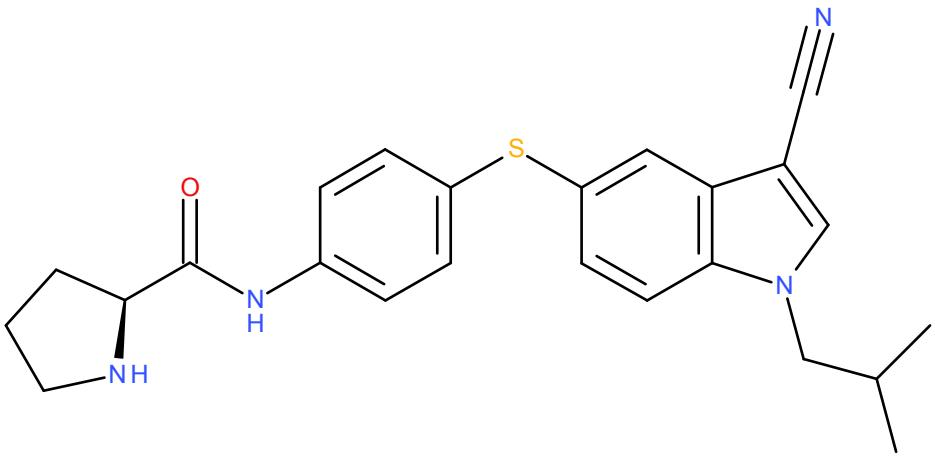
Sodium hydride (0.073 g, 1.83 mmol) was added to 1-isobutyl-5-((4-nitrophenyl)amino)-1*H*-indole-3-carbonitrile (0.51 g, 1.53 mmol) in DMF (30 mL) at 0°C under nitrogen. The resulting solution was stirred at 20°C for 30 minutes and re-cooled to 0°C. Methyl iodide (0.11 mL, 1.83 mmol) was added and the reaction stirred at 20°C for 2 hours. The mixture was poured onto ice/ water and extracted into ethyl acetate (2 x 80 mL) and the combined organic layer was washed with saturated brine (100 mL), dried over Na_2SO4, filtered and evaporated to give crude product. The crude product was purified by flash silica chromatography, elution gradient 10 to 30% Ethyl acetate in heptane. Pure fractions were evaporated to dryness to afford 1-isobutyl-5-(methyl(4-nitrophenyl)amino)-1*H*-indole-3-carbonitrile (0.42 g, 80%) as a yellow solid. *m/z*: ES+ $[M+H]^+$ 349. ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.01 – 8.12 (m, 2H), 7.57 – 7.66 (m, 2H), 7.46 (d, *J* = 8.81 Hz, 1H), 7.17 (dd, *J* = 2.04, 8.74 Hz, 1H), 6.5 – 6.68 (m, 2H), 3.99 (d, *J* = 7.36 Hz, 2H), 3.45 (s, 3H), 2.13 – 2.35 (m, 1H), 0.99 (d, *J* = 6.68 Hz, 6H).

10% Palladium on carbon (0.043 g, 0.04 mmol) was added to a solution of 1-isobutyl-5-(methyl(4-nitrophenyl)amino)-1*H*-indole-3-carbonitrile (0.42 g, 1.21 mmol) in THF (15 mL) and ethanol (15 mL). This solution was allowed to stir under a hydrogen atmosphere supplied by a balloon for 4 hours. The reaction mixture was filtered through Celite and washed with THF and evaporated. The crude product was purified by flash silica chromatography, elution gradient 20 to 80% ethyl acetate in heptane. Pure fractions were evaporated to dryness to afford 5-((4-aminophenyl)(methyl)amino)-1-isobutyl-1*H*-indole-3-carbonitrile (0.29 g, 75%) as a yellow oil. *m/z*: ES+ $[M+MeCN]^+$ 360. ^{1}H NMR δ (400 MHz, $CDCl_3$) 7.45 (s, 1H), 7.11 – 7.16 (m, 2H), 6.9 – 6.97 (m, 2H), 6.84 (dd, *J* = 2.19, 9.13 Hz, 1H), 6.64 – 6.71 (m, 2H), 3.86 (d, *J* = 7.35 Hz, 2H), 3.51 (d, *J* = 55.09 Hz, 2H), 3.27 (s, 3H), 2.17 (dt, *J* = 6.92, 6.92, 13.62 Hz, 1H), 0.92 (d, *J* = 6.66 Hz, 6H).

O-(7-Azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (0.41 g, 1.09 mmol) was added portionwise to (*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxylic acid (0.195 g, 0.90 mmol), 5-((4-aminophenyl)(methyl)amino)-1-isobutyl-1*H*-indole-3-carbonitrile (0.29 g, 0.90 mmol) and DIPEA (0.630 mL, 3.62 mmol) in DMF (8 mL) at room temperature under nitrogen. The resulting solution was stirred at room temperature for 2 hours before the reaction mixture was partitioned between ethyl acetate (25 mL) and water (20 mL). The aqueous was re-extracted into ethyl acetate (20 mL) and the combined organics washed with water (40 mL) and saturated brine (20 mL), dried over $MgSO_4$, filtered and evaporated to give crude product. The crude product was purified by flash silica chromatography, elution gradient 20 to 70% ethyl acetate in heptane. Pure fractions were evaporated to dryness to afford (S)-*tert*-butyl 2-((4-((3-cyano-1-isobutyl-1*H*-indol-5-yl)(methyl)amino)phenyl)carbamoyl)pyrrolidine-1-carboxylate (0.34 g, 73%) as a white solid. *m/z*: ES- $[M-H]^-$ 514. ^1H NMR δ (400 MHz, $CDCl_3$) 9.27 (s, 1H), 7.52 (s, 1H), 7.39 (d, *J* = 8.66 Hz, 3H), 7.23 (s, 1H), 6.97 – 7.09 (m, 1H), 6.91 (d, *J* = 8.87 Hz, 2H), 4.44 (s, 1H), 3.90 (d, *J* = 7.36 Hz, 2H), 3.36 – 3.67 (m, 2H), 3.33 (s, 3H), 2.55 (s, 1H), 2.20 (dp, *J* = 6.90, 6.90, 6.91, 6.91, 13.79 Hz, 1H), 1.83 – 2.03 (m, 3H), 1.49 (s, 9H), 0.95 (d, *J* = 6.66 Hz, 6H).

Trifluoroacetic acid (0.49 mL, 6.59 mmol) was added to (*S*)-*tert*-butyl 2-((4-((3-cyano-1-isobutyl-1*H*-indol-5-yl)(methyl)amino)phenyl)carbamoyl)pyrrolidine-1-carboxylate (0.34 g, 0.66 mmol) in DCM (10 mL). The reaction mixture was stirred at room temperature for 20 hours and then purified by ion exchange chromatography, using an SCX column. The desired product was eluted from the column using 7M NH_3/methanol and pure fractions were evaporated to dryness to afford a brown oil. The crude product was purified by flash silica chromatography, elution gradient 0 to 20% methanol in DCM. Pure fractions were evaporated to dryness to afford (*S*)-*N*-

(4-((3-cyano-1-isobutyl-1*H*-indol-5-yl)(methyl)amino)phenyl)pyrrolidine-2-carboxamide **71** (0.17 g, 61%) as a white solid. HRMS ESI$^+$ *m/z* observed 416.2447, $C_{25}H_{30}N_5O$ requires 416.2450. ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.62 (s, 1H), 7.44 – 7.54 (m, 3H), 7.35 (d, *J* = 2.02 Hz, 1H), 7.21 – 7.26 (m, 1H), 7.02 (dd, *J* = 2.19, 8.92 Hz, 1H), 6.89 – 6.96 (m, 2H), 3.76 – 3.97 (m, 3H), 3.33 (s, 3H), 3.04 (ddt, *J* = 6.57, 6.57, 10.23, 37.24 Hz, 2H), 2.11 – 2.31 (m, 2H), 2.01 – 2.1 (m, 1H), 1.64 – 1.87 (m, 2H), 0.94 (d, *J* = 6.67 Hz, 6H), NH not observed.



(*S*)-*N*-(4-((3-Cyano-1-isobutyl-1*H*-indol-5-yl)thio)phenyl)pyrrolidine-2-carboxamide (72). 1-Bromo-2-methylpropane (7.1 mL, 65.29 mmol) was added to 5-bromo-1*H*-indole (8 g, 40.81 mmol), and cesium carbonate (21.27 g, 65.29 mmol) in DMF (100 mL) at 20°C under nitrogen. The resulting mixture was stirred at 80°C for 20 hours. The reaction mixture was diluted with ethyl acetate (500 mL), and water (100 mL). The organic phase was washed sequentially with water (100 mL), saturated brine (100 mL) and then was dried over $MgSO_4$ before being filtered and evaporated to afford a solid. The crude product was purified by flash silica chromatography, elution gradient 5 to 25% ethyl acetate in heptane. Pure fractions were evaporated to dryness to afford 5-bromo-1-isobutyl-1*H*-indole (7.51 g, 73%) as a yellow oil. HRMS ESI+ *m/z* observed

251.0298, $C_{12}H_{14}NBr$ requires 251.0310. ^{1}H NMR δ (400 MHz, $CDCl_3$) 7.72 – 7.77 (m, 1H), 7.23 – 7.3 (m, 1H), 7.19 (d, *J* = 8.70 Hz, 1H), 7.06 (d, *J* = 3.12 Hz, 1H), 6.41 (dd, *J* = 0.74, 3.10 Hz, 1H), 3.88 (d, *J* = 7.35 Hz, 2H), 2.03 – 2.28 (m, 1H), 0.91 (d, *J* = 6.65 Hz, 6H).

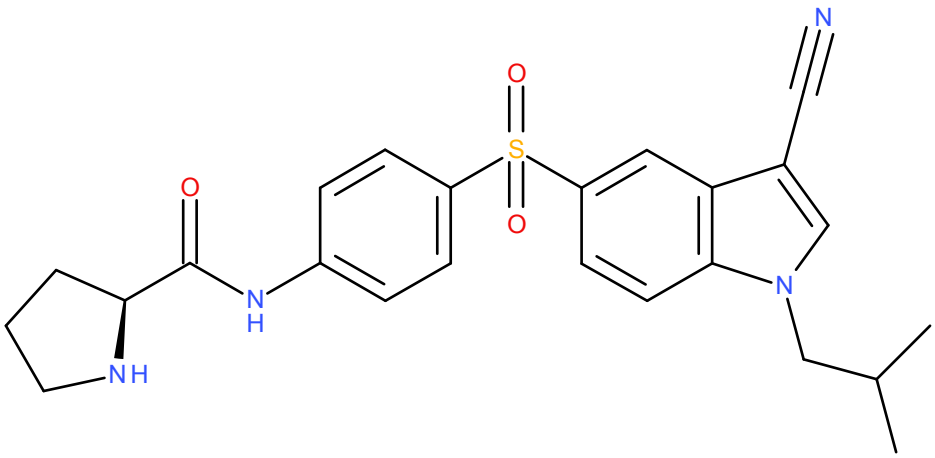
Chlorosulfonyl isocyanate (1.38 mL, 15.86 mmol) was added dropwise as a solution in acetonitrile (10 mL) to 5-bromo-1-isobutyl-1*H*-indole (2 g, 7.93 mmol) in acetonitrile (80 mL)/DMF (8 mL) at 0°C over a period of 5 minutes under nitrogen. The resulting solution was stirred at 0°C for 10 minutes, warmed to room temperature and stirred for 2 hours. The mixture was then poured into ice-water and basified (carefully) to pH 7/8 with sat. aq. $NaHCO_3$ solution. The mixture was then extracted with ethyl acetate (2 x 100 mL), the organic layer was washed with brine (100 mL), dried (Na_2SO_4), filtered and evaporated to yield a brown gum. The crude product was purified by flash silica chromatography, elution gradient 10 to 30% ethyl acetate in heptane. Pure fractions were evaporated to dryness to afford 5-bromo-1-isobutyl-1*H*-indole-3-carbonitrile (1.88 g, 85%) as a yellow oil which solidified on standing. HRMS ESI+ *m/z* observed 277.0271, $C_{13}H_{13}N_2Br$ requires 277.0262. ^{1}H NMR δ (400 MHz, $CDCl_3$) 7.89 – 7.92 (m, 1H), 7.56 (s, 1H), 7.42 (dd, *J* = 1.85, 8.78 Hz, 1H), 7.28 (s, 1H), 3.93 (d, *J* = 7.40 Hz, 2H), 2.1 – 2.28 (m, 1H), 0.94 (d, *J* = 6.69 Hz, 6H).

A solution of *N,N′*-Dimethylethylenediamine (0.078 mL, 0.72 mmol) in dioxane (18 mL) was added to a stirred mixture of *tert*-butyl (4-mercaptophenyl)carbamate (2.44 g, 10.82 mmol), Neocuproine Reagent (0.15 g, 0.72 mmol), 5-bromo-1-isobutyl-1*H*-indole-3-carbonitrile (1 g, 3.61 mmol), sodium iodide (1.190 g, 7.94 mmol), sodium *tert*-butoxide (0.693 g, 7.22 mmol) and copper(I)iodide (0.172 g, 0.90 mmol) in a microwave vial under nitrogen. The resulting suspension was stirred in the microwave at 130°C for 8 hours. The reaction mixture was evaporated to dryness and redissolved in ethyl acetate (100 mL), and washed sequentially with

water (100 mL) and saturated brine (100 mL) and filtered to remove copper salts. The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 10 to 50% ethyl acetate in heptane. Pure fractions were evaporated to dryness to afford 5-((4-aminophenyl)thio)-1-isobutyl-1*H*-indole-3-carbonitrile (0.033 g, 3%) as a beige gum. *m/z*: ES- $[M-H]^-$ 320. ^{1}H NMR δ (400 MHz, $CDCl_3$) 7.59 (dd, *J* = 0.73, 1.55 Hz, 1H), 7.51 (s, 1H), 7.27 – 7.32 (m, 2H), 7.2 – 7.25 (m, 2H), 6.62 – 6.7 (m, 2H), 3.89 (d, *J* = 7.38 Hz, 2H), 3.78 (s, 2H), 2.16 (dt, *J* = 7.03, 7.03, 13.74 Hz, 1H), 0.92 (d, *J* = 6.65 Hz, 6H).

O-(7-Azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (0.047 g, 0.12 mmol) was added portionwise to (*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxylic acid (0.020 g, 0.09 mmol), 5-((4-aminophenyl)thio)-1-isobutyl-1*H*-indole-3-carbonitrile (0.033 g, 0.10 mmol) and DIPEA (0.072 mL, 0.41 mmol) in DMF (2 mL) at room temperature under nitrogen. The resulting solution was stirred at room temperature for 24 hours. The reaction mixture was partitioned between ethyl acetate (25 mL) and water (20 mL) and the aqueous was re-extracted into ethyl acetate (20 mL). The combined organic layer was washed with water (40 mL) and saturated brine (20 mL), dried over $MgSO_4$, filtered and evaporated to yield crude product. The crude product was purified by flash silica chromatography, elution gradient 20 to 70% ethyl acetate in heptane. Pure fractions were evaporated to dryness to afford (*S*)-*tert*-butyl 2-((4-((3-cyano-1-isobutyl-1*H*-indol-5-yl)thio)phenyl)carbamoyl)pyrrolidine-1-carboxylate (0.04 g, 77%) as a white solid. *m/z*: ES- $[M-H]^-$ 517. ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.55 (s, 1H), 7.78 (s, 1H), 7.55 (s, 1H), 7.47 (d, *J* = 8.63 Hz, 2H), 7.30 (d, *J* = 8.68 Hz, 4H), 4.44 (s, 1H), 3.92 (d, *J* = 7.37 Hz, 2H), 3.42 (s, 2H), 2.55 (s, 1H), 2.19 (dt, *J* = 6.96, 6.96, 13.71 Hz, 1H), 1.93 (s, 3H), 1.49 (s, 9H), 0.94 (d, *J* = 6.68 Hz, 6H).

Trifluoroacetic acid (0.059 mL, 0.79 mmol) was added to (*S*)-*tert*-butyl 2-((4-((3-cyano-1-isobutyl-1*H*-indol-5-yl)thio)phenyl)carbamoyl)pyrrolidine-1-carboxylate (0.04 g, 0.08 mmol) in DCM (2 mL). The reaction mixture was stirred at room temperature for 20 hours before being diluted with DCM (50 mL), basified with saturated aq. $NaHCO_3$ solution, and washed with water (25 mL) then saturated brine (25 mL). The organic layer was dried over $MgSO_4$ and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 20% methanol in DCM. Pure fractions were evaporated to dryness to afford (*S*)-*N*-(4-((3-cyano-1-isobutyl-1H-indol-5-yl)thio)phenyl)pyrrolidine-2-carboxamide **72** (0.017 g, 51%) as a white solid. HRMS ESI^+ *m/z* observed 419.19006, $C_{24}H_{27}N_4OS$ requires 419.19001. ^1H NMR δ (400 MHz, $CDCl_3$) 9.80 (s, 1H), 7.75 (t, *J* = 1.16, 1.16 Hz, 1H), 7.53 – 7.6 (m, 3H), 7.28 – 7.36 (m, 4H), 3.92 (d, *J* = 7.38 Hz, 2H), 3.86 – 3.91 (m, 1H), 3.05 (ddt, *J* = 6.57, 6.57, 10.27, 39.65 Hz, 2H), 2.11 – 2.3 (m, 3H), 1.98 – 2.11 (m, 1H), 1.68 – 1.87 (m, 2H), 0.94 (d, *J* = 6.65 Hz, 6H).

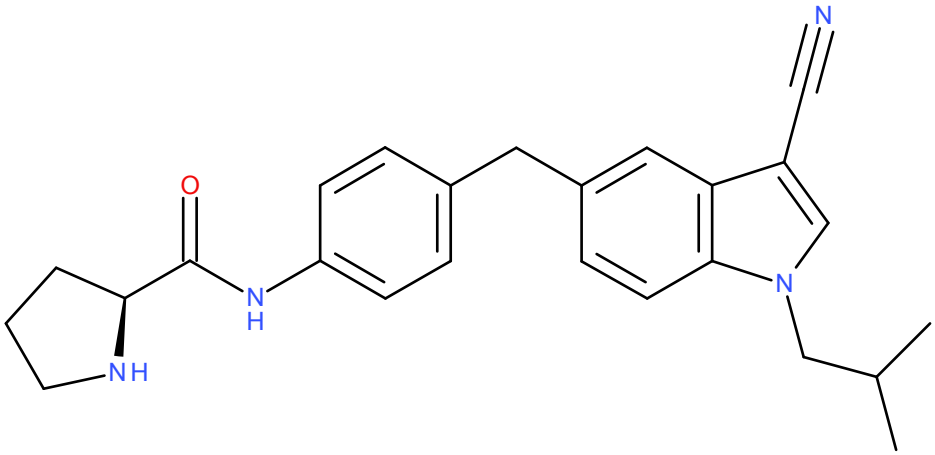


(*S*)-*N*-(4-((3-Cyano-1-isobutyl-1*H*-indol-5-yl)sulfonyl)phenyl)pyrrolidine-2-carboxamide (73).

3-Chloroperoxybenzoic acid (70%) (0.68 g, 2.78 mmol) was added in one portion to (*S*)-tert-butyl 2-((4-((3-cyano-1-isobutyl-1*H*-indol-5-yl)thio)phenyl)carbamoyl)pyrrolidine-1-carboxylate (0.72 g, 1.39 mmol) in DCM (40 mL) at 0ºC. The resulting solution was stirred at 20°C for 3 hours. The reaction mixture was diluted with DCM (50 mL) and washed sequentially with saturated aq. $NaHCO_3$ (75 mL) and saturated brine (75 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 10 to 50% ethyl acetate in heptane. Pure fractions were evaporated to dryness to afford (*S*)-*tert*-butyl 2-((4-((3-cyano-1-isobutyl-1*H*-indol-5-yl)sulfonyl)phenyl)carbamoyl)pyrrolidine-1-carboxylate (0.64 g, 84%) as a white solid. *m/z*: ES-$[M-H]^-$ 549. ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.99 (s, 1H), 8.39 (d, *J* = 1.34 Hz, 1H), 7.91 (d, *J* = 8.61 Hz, 2H), 7.85 (dd, *J* = 1.63, 8.78 Hz, 1H), 7.68 (s, 1H), 7.64 (d, *J* = 8.82 Hz, 2H), 7.46 (d, *J* = 8.71 Hz, 1H), 4.44 (s, 1H), 3.97 (d, *J* = 7.39 Hz, 2H), 3.38 (s, 2H), 2.52 (s, 1H), 2.17 (hept, *J* = 6.73, 6.73, 6.86, 6.86, 13.81 Hz, 1H), 1.82 – 2 (m, 3H), 1.48 (s, 9H), 0.93 (d, *J* = 6.65 Hz, 6H).

Trifluoroacetic acid (0.87 mL, 11.62 mmol) was added to (*S*)-*tert*-butyl 2-((4-((3-cyano-1-isobutyl-1*H*-indol-5-yl)sulfonyl)phenyl)carbamoyl)pyrrolidine-1-carboxylate (0.64 g, 1.16 mmol) in DCM (40 mL). The reaction mixture was stirred at room temperature for 20 hours then purified by ion exchange chromatography, using an SCX column. The desired product was eluted from the column using 7M NH_3/methanol and pure fractions were evaporated to dryness to afford a colourless gum. The crude product was purified by flash silica chromatography, elution gradient 0 to 20% methanol in DCM . Pure fractions were evaporated to dryness to afford (*S*)-*N*-(4-((3-cyano-1-isobutyl-1*H*-indol-5-yl)sulfonyl)phenyl)pyrrolidine-2-carboxamide **73**

(0.29 g, 55%) as a white solid. HRMS ESI$^+$ *m/z* observed 451.1794, $C_{24}H_{27}N_4O_3S$ requires 451.1804. ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.02 (s, 1H), 8.38 (d, *J* = 1.41 Hz, 1H), 7.9 – 7.98 (m, 2H), 7.86 (dd, *J* = 1.76, 8.80 Hz, 1H), 7.7 – 7.78 (m, 2H), 7.67 (s, 1H), 7.46 (d, *J* = 8.82 Hz, 1H), 3.97 (d, *J* = 7.39 Hz, 2H), 3.86 (dd, *J* = 5.16, 9.31 Hz, 1H), 3.03 (ddt, *J* = 6.55, 6.55, 10.28, 49.58 Hz, 2H), 2.08 – 2.31 (m, 2H), 2.00 (dq, *J* = 6.70, 6.72, 6.72, 12.92 Hz, 1H), 1.74 (p, *J* = 6.74, 6.74, 6.78, 6.78 Hz, 3H), 0.93 (d, *J* = 6.65 Hz, 6H).



(*S*)-*N*-(4-((3-Cyano-1-isobutyl-1*H*-indol-5-yl)methyl)phenyl)pyrrolidine-2-carboxamide (74)

1-Bromo-2-methylpropane (3.58 mL, 32.92 mmol) was added to 5-iodo-1*H*-indole (5 g, 20.57 mmol), and cesium carbonate (10.72 g, 32.92 mmol) in DMF (70 mL) at 20°C under nitrogen. The resulting mixture was stirred at 80°C for 20 hours. The reaction mixture was diluted with ethyl acetate (500 mL) and water (100 mL). The organic phase was washed sequentially with water (100 mL) and saturated brine (100 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford a solid. The crude product was purified by flash silica chromatography, elution gradient 5 to 25% ethyl acetate in heptane. Pure fractions were evaporated to dryness to afford 5-iodo-1-isobutyl-1H-indole (5.04 g, 82%) as a yellow oil. HRMS ESI+ *m/z* observed

299.0216, $C_{12}H_{14}NI$ requires 299.0171. ^{1}H NMR δ (400 MHz, $CDCl_3$) 7.95 (d, *J* = 1.68 Hz, 1H), 7.42 (dd, *J* = 1.70, 8.62 Hz, 1H), 7.10 (d, *J* = 8.63 Hz, 1H), 7.02 (d, *J* = 3.13 Hz, 1H), 6.40 (d, *J* = 3.08 Hz, 1H), 3.87 (d, *J* = 7.35 Hz, 2H), 2.16 (dt, *J* = 6.88, 6.88, 13.64 Hz, 1H), 0.90 (d, *J* = 6.69 Hz, 6H).

Chlorosulfonyl isocyanate (1.75 mL, 20.06 mmol) was added dropwise as a solution in acetonitrile (10 mL) to 5-iodo-1-isobutyl-1*H*-indole (3 g, 10.03 mmol) in acetonitrile (80 mL)/DMF (8 mL) at 0°C over a period of 5 minutes under nitrogen. The resulting solution was stirred at 0°C for 10 minutes, warmed to room temperature and stirred for 2 hours. The mixture was then poured into ice-water and basified (carefully) to pH 7/8 with saturated aq. $NaHCO_3$ solution. The mixture was then extracted with ethyl acetate (2 x 100 mL), combined organics washed with brine (100 mL), dried (Na_2SO_4), filtered and evaporated to give a brown gum. The crude product was purified by flash silica chromatography, elution gradient 10 to 30% ethyl acetate in heptane. Pure fractions were evaporated to dryness to afford 5-iodo-1-isobutyl-1*H*-indole-3-carbonitrile (2.82 g, 87%) as a yellow oil. HRMS ESI+ *m/z* observed 324.0173, C13H13N2I requires 324.0124. ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.09 – 8.14 (m, 1H), 7.59 (dd, *J* = 1.63, 8.69 Hz, 1H), 7.51 (s, 1H), 7.17 (d, *J* = 8.65 Hz, 1H), 3.93 (d, *J* = 7.39 Hz, 2H), 2.09 – 2.28 (m, 1H), 0.93 (d, *J* = 6.69 Hz, 6H).

Tetrakis(Triphenylphosphine)Palladium(0) (0.18 g, 0.15 mmol) was added to a degassed solution of 5-iodo-1-isobutyl-1*H*-indole-3-carbonitrile (1 g, 3.08 mmol) in THF (15 mL) at 20°C under nitrogen. (4-Bromobenzyl)zinc(II) bromide (0.5M in THF) (6.79 mL, 3.39 mmol) was added to the resulting suspension and stirred at 65°C for 2 hours. The reaction mixture was quenched with saturated aq. NH_4Cl (5 mL) solution, extracted with ethyl acetate (2 x 25 mL) and the organic layer was dried over $MgSO_4$, filtered and evaporated to afford a yellow gum. The crude product

was purified by flash silica chromatography, elution gradient 5 to 25% ethyl acetate in heptane. Pure fractions were evaporated to dryness to afford 5-(4-bromobenzyl)-1-isobutyl-1*H*-indole-3-carbonitrile (0.68 g, 60%) as a yellow gum. HRMS ESI+ *m/z* observed 366.0724, $C_{20}H_{19}N_2Br$ requires 366.0732. ^{1}H NMR δ (400 MHz, $CDCl_3$) 7.57 (dd, *J* = 0.68, 1.50 Hz, 1H), 7.54 (s, 1H), 7.38 – 7.43 (m, 2H), 7.29 (d, *J* = 8.50 Hz, 1H), 7.06 – 7.14 (m, 3H), 4.04 (s, 2H), 3.92 (d, *J* = 7.37 Hz, 2H), 2.19 (s, 1H), 0.93 (d, *J* = 6.64 Hz, 6H).

5-(4-Bromobenzyl)-1-isobutyl-1*H*-indole-3-carbonitrile (0.682 g, 1.86 mmol), cesium carbonate (0.91 g, 2.79 mmol), *tert*-butyl carbamate (0.33 g, 2.79 mmol), dicyclohexyl(2',4',6'-triisopropylbiphenyl-2-yl)phosphine (0.19 g, 0.39 mmol) and palladium(II) acetate (0.029 g, 0.13 mmol) were placed under a nitrogen atmosphere. Dry, degassed dioxane (16 mL) was added and the reaction was warmed to 110°C in a microwave. After reaction completion (3 hours) the reaction was diluted with ethyl acetate (20 mL) and water (10 mL) and the layers were separated. The aqueous layer was washed with a further portion of ethyl acetate (10 mL) and the combined organic layers were dried ($MgSO_4$), filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 30 to 70% ethyl acetate in isohexane. Pure fractions were evaporated to dryness to afford *tert*-butyl (4-((3-cyano-1-isobutyl-1*H*-indol-5-yl)methyl)phenyl)carbamate (0.54 g, 72%) as a colourless gum. *m/z*: ES-$[M-H]^-$ 402. ^{1}H NMR δ (400 MHz, $CDCl_3$) 7.55 – 7.59 (m, 1H), 7.52 (s, 1H), 7.28 (s, 3H), 7.1 – 7.17 (m, 3H), 6.39 (s, 1H), 4.04 (s, 2H), 3.91 (d, *J* = 7.37 Hz, 2H), 2.18 (dt, *J* = 6.88, 13.66 Hz, 1H), 1.51 (s, 9H), 0.93 (d, *J* = 6.66 Hz, 6H).

Trifluoroacetic acid (1 mL, 13.38 mmol) was added to *tert*-butyl (4-((3-cyano-1-isobutyl-1*H*-indol-5-yl)methyl)phenyl)carbamate (0.54 g, 1.34 mmol) in DCM (20 mL). The reaction mixture was stirred at room temperature for 20 hours. The crude product was purified by ion exchange

chromatography, using an SCX column and the desired product was eluted from the column using 7M NH_3/methanol. Pure fractions were evaporated to dryness to afford 5-(4-aminobenzyl)-1-isobutyl-1*H*-indole-3-carbonitrile (0.3 g, 74%) as a beige solid. *m/z*: ES+ $[M+MeCN]^+$ 345. ^{1}H NMR δ (400 MHz, $CDCl_3$) 7.56 (d, *J* = 0.86 Hz, 1H), 7.51 (s, 1H), 7.28 (s, 1H), 7.14 (dd, *J* = 1.58, 8.53 Hz, 1H), 6.96 – 7.04 (m, 2H), 6.49 – 6.72 (m, 2H), 3.98 (s, 2H), 3.90 (d, *J* = 7.36 Hz, 2H), 3.52 (s, 2H), 2.18 (dt, *J* = 6.96, 13.68 Hz, 1H), 0.92 (d, *J* = 6.65 Hz, 6H).

O-(7-Azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (0.454 g, 1.19 mmol) was added portionwise to (*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxylic acid (0.193 g, 0.90 mmol), 5-(4-aminobenzyl)-1-isobutyl-1*H*-indole-3-carbonitrile (0.302 g, 1.00 mmol) and DIPEA (0.694 ml, 3.98 mmol) in DMF (8 ml) at room temperature under nitrogen. The resulting solution was stirred at room temperature for 3 days. The reaction mixture was partitioned between ethyl acetate (25 mL) and water (20 mL). The aqueous was re-extracted into ethyl acetate (20 mL) and the combined organics washed with water (40 mL) and saturated brine (20 mL), dried over $MgSO_4$, filtered and evaporated to yield crude product. The crude product was purified by flash silica chromatography, elution gradient 20 to 70% ethyl acetate in heptane. Pure fractions were evaporated to dryness to afford (*S*)-*tert*-butyl 2-((4-((3-cyano-1-isobutyl-1*H*-indol-5-yl)methyl)phenyl)carbamoyl)pyrrolidine-1-carboxylate (0.36 g, 72%) as a white solid. *m/z*: ES- $[M-H]^-$ 499. ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.38 (s, 1H), 7.57 (s, 1H), 7.52 (s, 1H), 7.44 (d, *J* = 8.41 Hz, 2H), 7.26 (s, 1H), 7.09 – 7.21 (m, 3H), 4.44 (s, 1H), 4.05 (s, 2H), 3.91 (d, *J* = 7.36 Hz, 2H), 3.42 (s, 2H), 2.54 (s, 1H), 2.18 (dt, *J* = 6.91, 13.70 Hz, 1H), 1.92 (s, 3H), 1.48 (s, 9H), 0.93 (d, *J* = 6.66 Hz, 6H).

Trifluoroacetic acid (0.52 mL, 6.99 mmol) was added to (*S*)-*tert*-butyl 2-((4-((3-cyano-1-isobutyl-1*H*-indol-5-yl)methyl)phenyl)carbamoyl)pyrrolidine-1-carboxylate (0.35 g, 0.70 mmol) in DCM (20 mL). The reaction mixture was stirred at room temperature for 20 hours. The crude product was purified by ion exchange chromatography, using an SCX column. The desired product was eluted from the column using 7M NH_3/methanol and pure fractions were evaporated to dryness to afford a colourless gum. The crude product was purified by flash silica chromatography, elution gradient 0 to 20% methanol in DCM . Pure fractions were evaporated to dryness to afford (*S*)-*N*-(4-((3-cyano-1-isobutyl-1*H*-indol-5-yl)methyl)phenyl)pyrrolidine-2-carboxamide **74** (0.14 g, 51%) as a white solid. HRMS ESI+ *m/z* observed 401.2342, $C_{25}H_{29}N_4O$ requires 401.2341. ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.67 (s, 1H), 7.56 (s, 1H), 7.49 – 7.54 (m, 3H), 7.24 – 7.3 (m, 1H), 7.17 (d, *J* = 8.40 Hz, 2H), 7.13 (dd, *J* = 1.40, 8.53 Hz, 1H), 4.06 (s, 2H), 3.91 (d, *J* = 7.36 Hz, 2H), 3.85 (dd, *J* = 5.19, 9.13 Hz, 1H), 2.88 – 3.15 (m, 2H), 2.19 (dq, *J* = 7.00, 13.69 Hz, 2H), 2.03 (dq, *J* = 6.65, 12.58 Hz, 1H), 1.76 (tt, *J* = 6.35, 12.67 Hz, 3H), 0.92 (d, *J* = 6.65 Hz, 6H).

3. Kinase Selectivity

Compound **26** was screened in a suite of kinase assays at a single concentration of 1 μM at Millipore (http://www.emdmillipore.com):

Kinase	Percent Inhibition at 1 μM
MELK(h)	25
DCAMKL2(h)	23
DRAK1(h)	21
TrkA(h)	20
EphB4(h)	19
Mer(h)	18
Aurora-B(h)	17

MST1(h)	16
Lck(h)	15
Lyn(h)	14
B-Raf(h)	13
IGF-1R(h)	13
TAO2(h)	13
LOK(h)	12
MLK1(h)	11
TLK1(h)	11
WNK3(h)	11
CLK3(h)	10
EphA2(h)	10
LRRK2(h)	10
PAK4(h)	10
GRK1(h)	9
PKCζ(h)	9
TrkB(h)	9
PI3KC2γ(h)	9
EphA1(h)	8
MKK4(m)	8
Pyk2(h)	8
Tec(h) activated	8
CK1δ(h)	7
IKKε(h)	7
*J*AK1(h)	7
*J*NK3(h)	7
PDGFRα(h)	7
TSSK2(h)	7
CDK2/cyclinE(h)	6
SAPK3(h)	6
SAPK4(h)	6
Snk(h)	6
TAO3(h)	6
ACK1(h)	5
CK2α2(h)	5
c-RAF(h)	5
DAPK1(h)	5
Fer(h)	5
FGFR2(h)	5

mTOR(h)	5
mTOR/FKBP12(h)	5
PKCδ(h)	5
STK25(h)	5
Wee1(h)	5
AMPKα2(h)	4
eEF-2K(h)	4
Haspin(h)	4
MAPKAP-K3(h)	4
MINK(h)	4
MST3(h)	4
Pim-1(h)	4
Tie2 (h)	4
Bmx(h)	3
CHK2(h)	3
EphB1(h)	3
Fyn(h)	3
GCN2(h)	3
GRK5(h)	3
IKKα(h)	3
KDR(h)	3
Mnk2(h)	3
PKCθ(h)	3
PKD2(h)	3
Plk3(h)	3
TGFBR1(h)	3
TYK2(h)	3
PI3 Kinase (p110δ/p85α)(h)	3
PI3 Kinase (p110α/p85α)(h)	3
CaMKIδ(h)	2
CaMKIIδ(h)	2
CSK(h)	2
ErbB2(h)	2
FAK(h)	2
FGFR4(h)	2
HIPK3(h)	2
IR(h)	2
MARK1(h)	2
NEK9(h)	2

PhKγ2(h)	2
Ron(h)	2
CaMKI(h)	1
CaMKK2(h)	1
CK1(y)	1
CK2(h)	1
Fms(h)	1
GCK(h)	1
MST2(h)	1
PDK1(h)	1
PKBβ(h)	1
PKG1β(h)	1
Rse(h)	1
Rsk3(h)	1
SAPK2b(h)	1
SRPK2(h)	1
ULK2(h)	1
PIP4K2α(h)	1
CDK5/p35(h)	0
DMPK(h)	0
DYRK2(h)	0
EGFR(h)	0
EphA3(h)	0
FGFR1(h)	0
*J*NK2α2(h)	0
LKB1(h)	0
Met(h)	0
MLCK(h)	0
NLK(h)	0
PAR-1Bα(h)	0
PKCγ(h)	0
PTK5(h)	0
RIPK2(h)	0
STK33(h)	0
Txk(h)	0
VRK2(h)	0
PI3 Kinase (p120γ)(h)	0
PI3KC2α(h)	0
PIP5K1γ(h)	0

ALK4(h)	-1
Arg(h)	-1
Axl(h)	-1
CDK5/p25(h)	-1
cSRC(h)	-1
Fgr(h)	-1
Hck(h)	-1
Hck(h) activated	-1
IRAK1(h)	-1
Itk(h)	-1
Lck(h) activated	-1
MST4(h)	-1
PKCα(h)	-1
PKCε(h)	-1
PKCμ(h)	-1
SGK3(h)	-1
PI3 Kinase (p110β/p85α)(h)	-1
PI3 Kinase (p110β/p85β)(m)	-1
PIP5K1α(h)	-1
CDK3/cyclinE(h)	-2
CK1γ3(h)	-2
HIPK2(h)	-2
IGF-1R(h), activated	-2
MRCKβ(h)	-2
MSK1(h)	-2
NEK2(h)	-2
NEK11(h)	-2
PAK1(h)	-2
PAK5(h)	-2
PEK(h)	-2
TAK1(h)	-2
TBK1(h)	-2
TSSK1(h)	-2
DDR2(h)	-3
LIMK1(h)	-3
MRCKα(h)	-3
NEK3(h)	-3
PKCβI(h)	-3
PKCη(h)	-3

Ros(h)	-3
TAO1(h)	-3
ULK3(h)	-3
WNK2(h)	-3
ZIPK(h)	-3
ALK2(h)	-4
BrSK1(h)	-4
BrSK2(h)	-4
BTK(h)	-4
CDK6/cyclinD3(h)	-4
CK1γ1(h)	-4
CLK2(h)	-4
EphA8(h)	-4
IR(h), activated	-4
*J*AK3(h)	-4
MSSK1(h)	-4
Yes(h)	-4
CaMKIIβ(h)	-5
CaMKIV(h)	-5
CHK1(h)	-5
IKKβ(h)	-5
IRAK4(h)	-5
*J*NK1α1(h)	-5
MSK2(h)	-5
PAK2(h)	-5
PKBγ(h)	-5
Plk1(h)	-5
ROCK-II(h)	-5
TLK2(h)	-5
TrkC(h)	-5
ZAP-70(h)	-5
ARK5(h)	-6
MAPK1(h)	-6
MAPK2(h)	-6
PKG1α(h)	-6
Ret(h)	-6
SGK(h)	-6
CDK2/cyclinA(h)	-7
DAPK2(h)	-7

EphB2(h)	-7
GSK3α(h)	-7
IRR(h)	-7
MuSK(h)	-7
SRPK1(h)	-7
AMPKα1(h)	-8
BRK(h)	-8
EphA5(h)	-8
EphB3(h)	-8
PAK6(h)	-8
PKCι(h)	-8
Rsk4(h)	-8
SNRK(h)	-8
Syk(h)	-8
ASK1(h)	-9
CLK4(h)	-9
Fes(h)	-9
MAPKAP-K2(h)	-9
MEK1(h)	-9
PASK(h)	-9
PDGFRβ(h)	-9
Blk(h)	-10
EphA4(h)	-10
PKCβII(h)	-10
ROCK-I(h)	-10
Abl(h)	-11
Aurora-C(h)	-11
CDK1/cyclinB(h)	-11
Flt3(h)	-11
GRK6(h)	-11
p70S6K(h)	-11
PKBα(h)	-11
PRAK(h)	-11
Rsk1(h)	-11
SAPK2a(h)	-11
CaMKIIγ(h)	-12
FGFR3(h)	-12
Flt4(h)	-12
CDK7/cyclinH/MAT1(h)	-13

CDK9/cyclin T1(h)	-14
cKit(h)	-14
EphA7(h)	-14
GRK7(h)	-14
GSK3β(h)	-14
NEK6(h)	-14
PKA(h)	-14
PrKX(h)	-14
NEK7(h)	-15
Pim-2(h)	-15
Pim-3(h)	-15
SGK2(h)	-15
HIPK1(h)	-16
IRE1(h)	-16
ALK(h)	-18
Aurora-A(h)	-18
ErbB4(h)	-19
*J*AK2(h)	-19
Rsk2(h)	-19
SIK(h)	-19
CK1γ2(h)	-20
MKK7β(h)	-20
Flt1(h)	-24
CLK1(h)	-25
PRK2(h)	-34
MKK6(h)	-38

4. Crystallography

The full length PFKFB3 protein was expressed as described earlier, purified and stored in the presence of 20 mM Tris pH 7.5, 10 mM NaPi, 0.5 mM EDTA, 0.5 mM TCEP, 1.2mM ADP,1.2 mM fructose-6-phosphate to increase protein stability. Diffraction quality crystals were obtained using the sitting drop vapour diffusion method with droplets containing a 2:1 ratio of protein and precipitant. A solution containing 0.2M sodium malonate, 18w/v% PEG3350 and 0.1 m PCTP buffer pH 7.0 was used as precipitant. Compound complex crystals were prepared by soaking the ADP bound crystals in 5-25% dilutions of 100mM DMSO stock solutions of compounds. Data collections were carried out at the Diamond Light Source synchrotron beamlines at cryogenic

temperatures, using ethylene glycol as cryoprotectant. The pipedream and autoBUSTER software packages were used to solve and refine the structures, the program Coot was used for manual building of the models. Data collection and refinement statistics together with PDB accession codes of the final models are listed below:

Compound	**44**	**49**	**67**	**69**	**51**	**43**
PDB accession code	**5AJV**	**5AJW**	**5AJX**	**5AJY**	**5AJZ**	**5AK0**
Data collection statistics						
Space group and cell par:	P6522	P6522	P6522	P6522	P6522	P6522
a = b [Å]	102.63	102.53	102.28	102.817	102.48	103.42
c [Å]	258.21	260.31	259.71	261.79	258.96	260.71
Resolution [Å]	3.01	2.50	2.58	2.37	2.45	2.14
Unique reflections	14554 (2379)	28866 (4104)	26253 (2482)	34160 (3296)	34476 (4906)	53660 (7514)
Multiplicity	17.8 (7.6)	7.1 (7.3)	12.3 (13.1)	7.0 (6.9)	6.5 (6.8)	6.6 (6.7)
Completeness [%]	92.0 (94.2)	99.8 (100)	99.9 (100)	99.8 (100)	99.8 (100)	99.5 (98.0)
R_{sym} [%]	11.0 (62.0)	7.1 (48.9)	8.8 (92)	7.1 (99)	7.3 (51.9)	5.5 (47.5)
Mean(I)/sd	16.5 (4.6)	16.9 (3.7)	16.9 (2.2)	14.5 (1.9)	14.8 (2.9)	18.5 (3.4)
Refinement statistics						
Number of reflections (working /test)	14554 (766)	28821 (1496)	26171 (2893)	34066 (2748)	34367 (1733)	53614 (3617)
R/R_{free} [%]	18.4/22.5	21.36/25.49	23.95/25.42	24.42 (25.28)	20.14/26.22	18.08/20.37
Deviation from ideal geometry						
bond lengths [Å]	0.014	0.009	0.009	0.009	0.009	0.010
bond angles [◦]	1.932	1.060	1.040	1.020	1.030	0.980
Ramachandran plot [%]						

preferred region	91.6	97.4	97.4	97.7	97.7	97.2
generously allowed region	5.3	1.6	2.0	1.6	1.4	1.8
disallowed region	3.3	0.9	0.6	0.7	0.9	0.9

[‡] Data in parentheses refer to the highest resolution shell.