Supporting Information

Type II kinase inhibitors targeting Cys-gatekeeper kinases display orthogonality with wild type and Ala/Gly-gatekeeper kinases

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Supplementary Figures

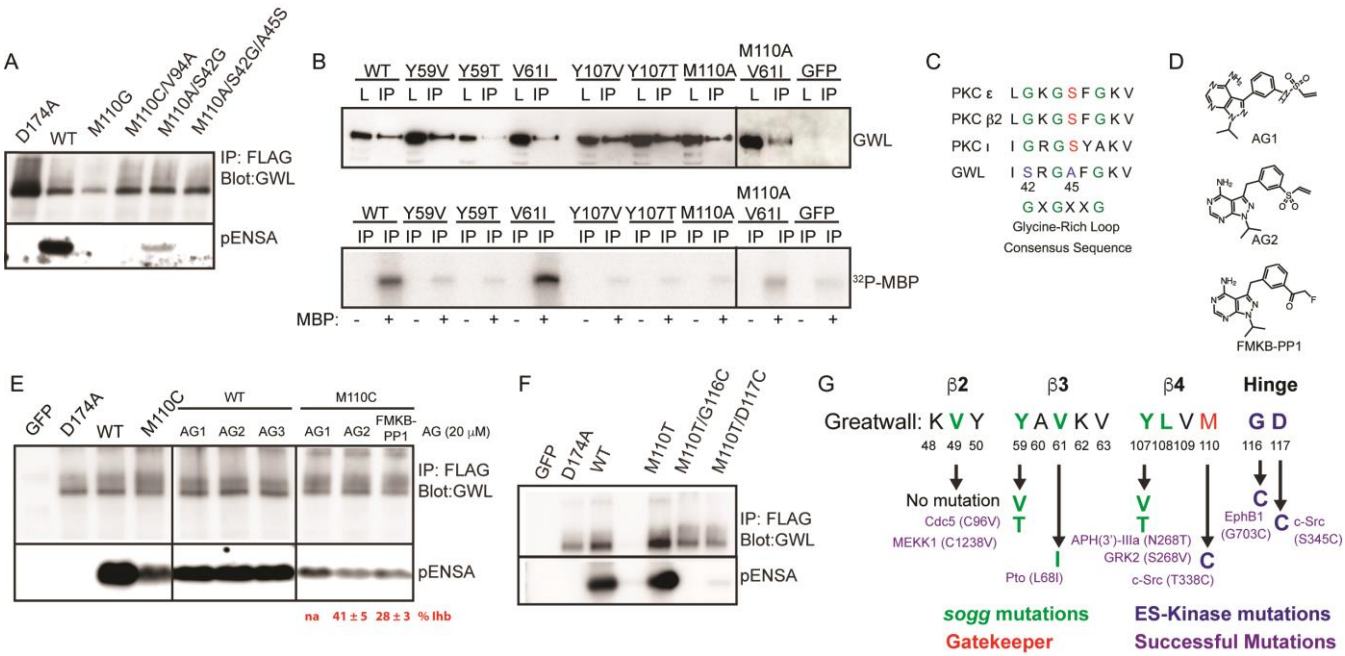


Figure S1. Attempts to identify rescuing *sogg* mutations and establish an ES-kinase system for Greatwall kinase. A) FLAG-tagged GWL or mutants D174A, M110G and the double and triple mutants M110C/V94A, M110A/S42G and M110A/S42G/A45S were expressed in human HEK 293T cells, immunoprecipitated and taken into kinase assays using ENSA as substrate. **B**) FLAG-tagged GWL or mutants Y59V, Y59T, V61I, Y106V, Y107T, M110A and double mutant M110A/V61I were expressed in human HEK 293T cells, immunoprecipitated and taken into kinase assays using myelin basic protein as substrate. **C**) Alignment of the glycine-rich loop region of GWL with those of other AGC kinases such as PKC isoforms ε, β2 and ι reveals incongruous amino acids at positions S42 and A45 of GWL. **D,E**) Analogue-sensitive, electrophilic kinase inhibitors **AG1-3** (**D**) were tested against immunoprecipitated WT and M110C GWL (**E**) in kinase assays using ESNA as substrate. **F**) FLAG-tagged GWL or mutants D174A, M110T and double mutants M110T/G116C and M110T/D117C were expressed in human HEK 293T cells, immunoprecipitated and taken into kinase assays using ENSA as substrate. **G**) Diagram illustrating *sogg* mutations made within β-sheets 2 – 4 and mutations of the gatekeeper position and select hinge region amino acids to cysteine. Mutations Y59V and Y59T were rationally incorporated particularly due to the stabilising effect of β-branched amino acids on β-sheets.

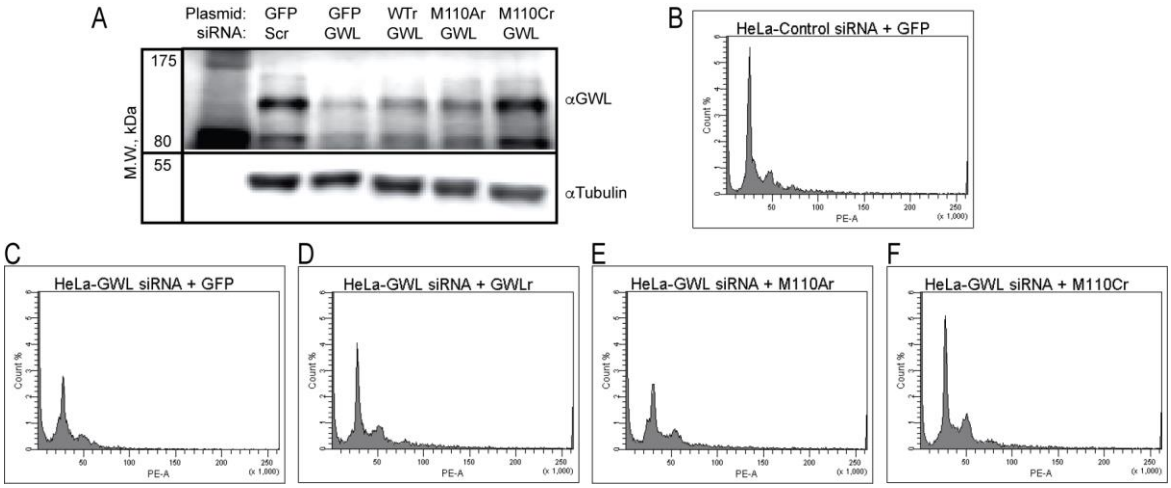


Figure S2. Cell cycle analysis of HeLa cells expressing siRNA-resistant GWL constructs. A) Rescue of RNAi-mediated GWL depletion by co-expression with siRNA-resistant WT (GWL)-, M110A-

and MC-GWL constructs (GWLr = WT, M110Ar and M110Cr). **B-F**) FACS-cell cycle analysis of HeLa cells.

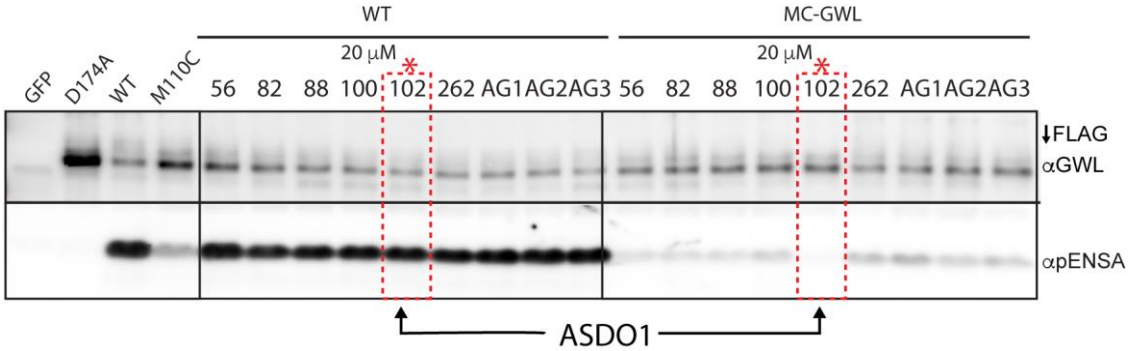


Figure S3. Screen of AD57-analogues against WT- and MC-GWL. FLAG-tagged GWL and M110C mutant were expressed in human HEK293T cells, immunoprecipitated and taken into kinase assays in the presence of 20 μM concentrations of AD57-analogues 56, 82, 88, 100, 102 (ASDO1), 262 and AG1-2 and FMKB-PP1 (AG3) using ENSA as substrate.

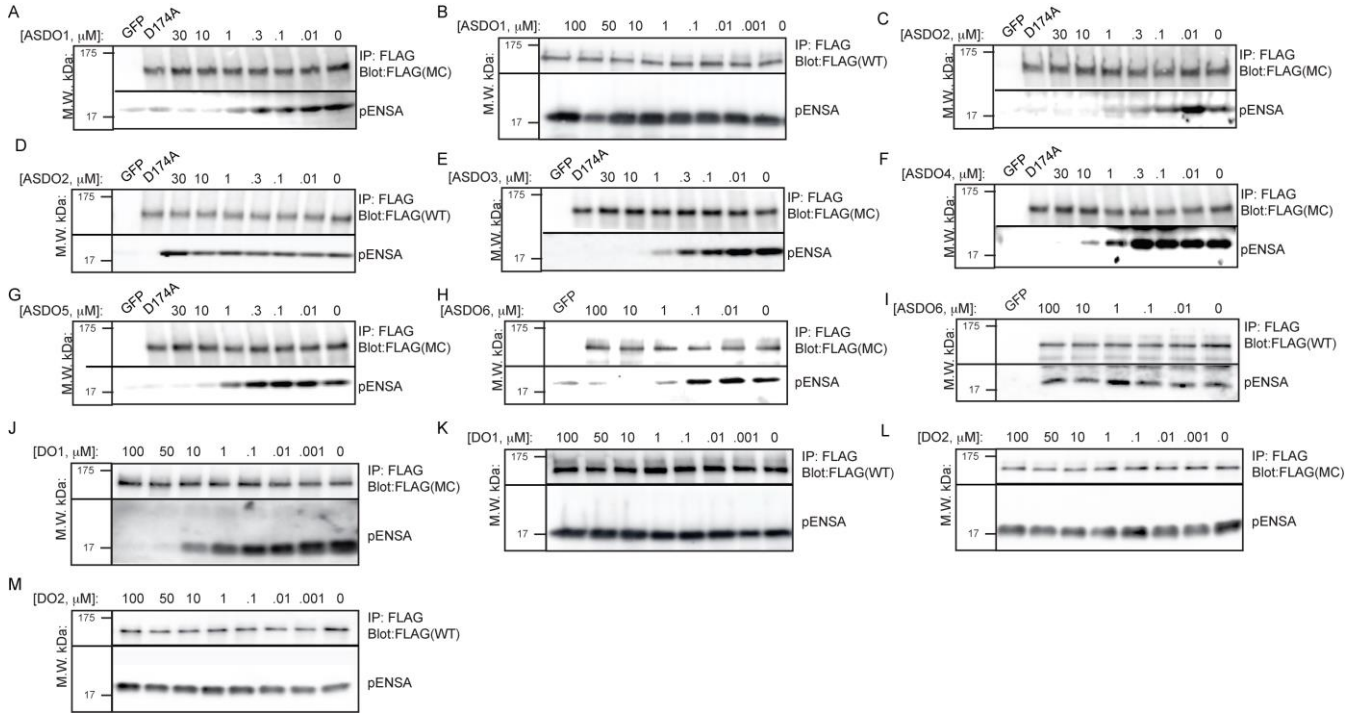
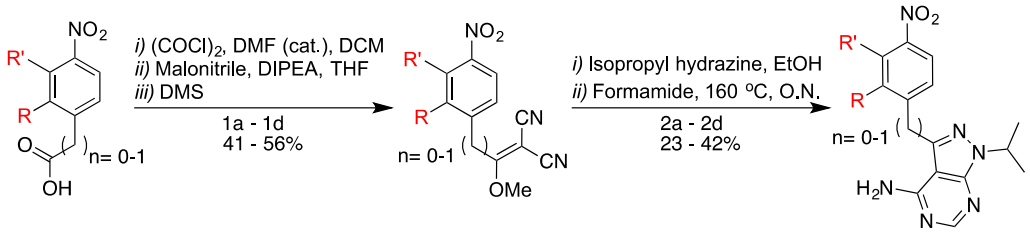
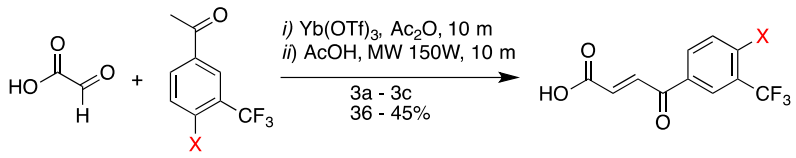


Figure S4. *In vitro* analysis of ASDO analogues by IP kinase assay and western analysis. A-M) FLAG-tagged GWL and M110C mutant were expressed in human HEK 293T cells, immunoprecipitated and taken into kinase assays in the presence of increasing concentrations of ASDO analogues and **DO1** and DO2 using ENSA as substrate.

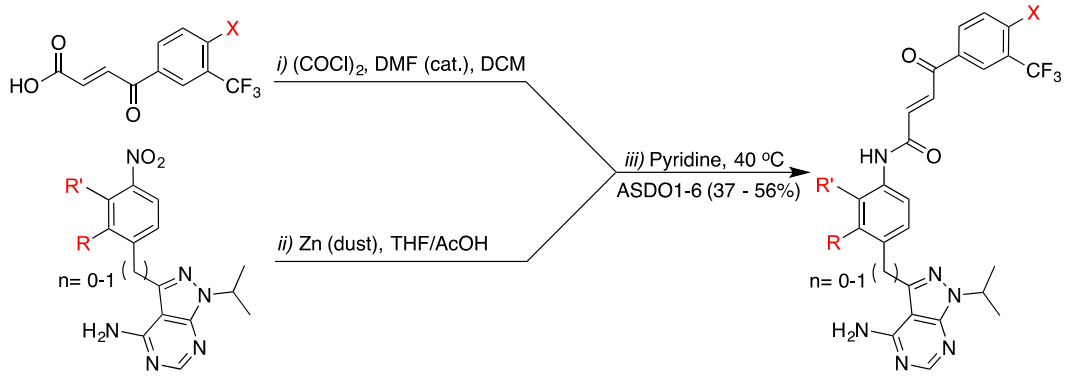
Supplementary Scheme 1. Synthetic route for nitrophenyl (or nitrophenylmethyl) pyrazolopyrimidine intermediates.



Supplementary Scheme 2. Synthetic route for 3-benzoylacrylic acids.



Supplementary Scheme 3. Synthesis of **ASDO1-6** by coupling of pyrazolopyrimidines with 3-benzoylacrylic acids.



A

Plate Barcode D6745PM	ASDO2 (1 μM) % Activity Remaining	% Inhibition	SD
Src	5	95	0
RIPK2	6	94	2
Lck	8	92	1
TrkA	9	91	2
Aurora B	11	89	0
HIPK2	19	81	2
MLK3	24	76	2
HER4	26	74	3
MKK1	27	73	2
VEG-FR	27	73	2
TAK1	30	70	9
IRAK4	30	70	1
CHK2	32	68	4
S6K1	36	64	6
CAMK1	40	60	21
CK1δ	51	49	1
ROCK 2	55	45	7
MST2	55	45	4
RSK1	62	38	6
BTK	63	37	15
GSK3b	67	33	14
MSK1	70	30	0
PAK4	70	30	6
PLK1	71	29	0
PKD1	73	27	1
PDK1	74	26	26
PKCa	74	26	14
JAK2	75	25	3
AMPK (hum)	75	25	2
EPH-A2	76	24	13
LKB1	76	24	6
TTK	79	21	3
DYRK1A	80	20	5
MARK3	80	20	1
SGK1	81	19	3
IGF-1R	82	18	9
CAMKKb	87	13	3
PRK2	87	13	4
SRPK1	88	12	2
PKA	91	9	1
PKBa	92	8	9
SYK	94	6	4
p38a MAPK	94	6	8
CK2	98	2	9
TBK1	99	1	14
JNK1	101	-1	4
PIM1	102	-2	3
EF2K	108	-8	8
NEK6	111	-11	2
SmMLCK	120	-20	3

B

Plate Barcode D6769PM	ASDO6 (1uM) % activity	% Inhibition	SD
SRPK1	16	84	1
RIPK2	27	73	1
MLK3	32	68	5
Src	38	62	1
VEG-FR	51	49	10
Lck	60	40	13
HER4	63	37	1
TrkA	65	35	6
ROCK 2	69	31	4
AMPK (hum)	70	30	4
TTK	70	30	3
PKD1	71	29	0
BTK	73	27	4
MKK1	73	27	4
MST2	77	23	4
PKCa	78	22	4
TAK1	78	22	7
DYRK1A	80	20	7
CHK2	82	18	0
PDK1	85	15	8
p38a MAPK	87	13	2
CAMK1	88	12	1
RSK1	91	9	3
SGK1	91	9	4
PIM1	92	8	9
CAMKKb	94	6	3
JAK3	94	6	10
GSK3b	95	5	7
MSK1	95	5	3
S6K1	95	5	3
TBK1	96	4	8
CK1δ	96	4	6
JNK1	96	4	0
EPH-A2	97	3	31
PKA	97	3	4
PKBa	97	3	2
EF2K	98	2	11
PAK4	98	2	3
PRK2	99	1	0
SYK	99	1	5
IRAK4	100	0	9
SmMLCK	101	-1	3
HIPK2	102	-2	13
PLK1	103	-3	5
CK2	104	-4	3
LKB1	104	-4	15
MARK3	106	-6	16
NEK6	123	-23	2
IGF-1R	142	-42	13

Figure S5. Kinase Profiling Express Screen. ASDO2 (**A**) and **ASDO6** (**B**) were tested against 50 select kinases at a concentration of 1 μM. The Kinase Profiling Express Screen was conducted at the International Centre for Kinase Profiling, University of Dundee.

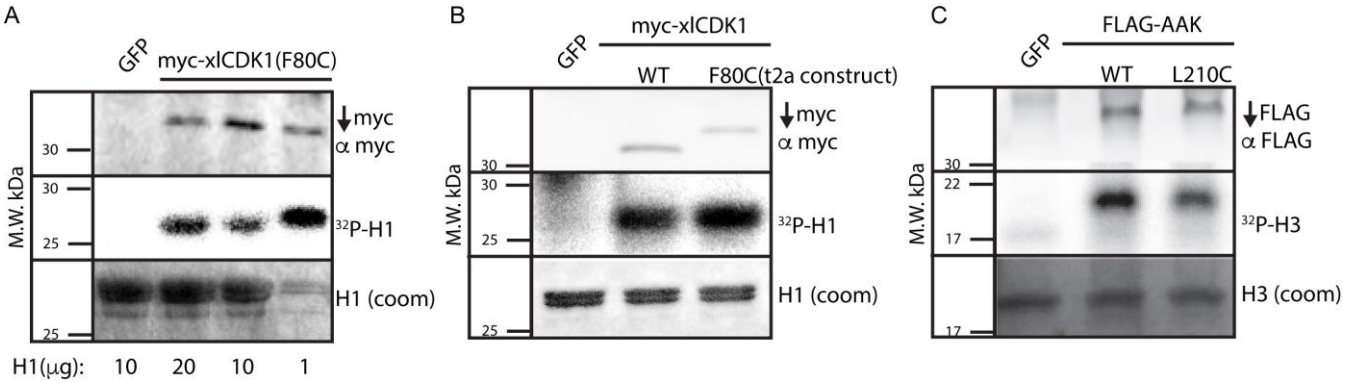


Figure S6. xlCDK1 and Aurora A kinase assays. A) In order to establish an xlCDK1 kinase assay, MYC-tagged xlCDK1 and F80C mutant were expressed in HEK293T cells, immunoprecipitated with 4 μg of anti-MYC antibody and 5 μL Protein G magnetic Dynabeads and taken into radioactive ^{32}P-ATP kinase assays with increasing amounts of the substrate histone H1. **B**) After optimisation of the xlCDK1 kinase assay, WT and F80C mutant xlCDK1 were assayed again to establish the relative activity of the mutant vs. WT kinase, revealing the mutant was significantly more active than WT. **C**) FLAG-tagged WT and L210C Aurora A kinase (AAK) were expressed in HEK293T cells, immunoprecipitated and taken into radioactive ^{32}P-ATP kinase assays using histone H3 as substrate. L210C-AAK demonstrated significant activity compared to GFP expressing cells, but slightly less activity than WT AAK.

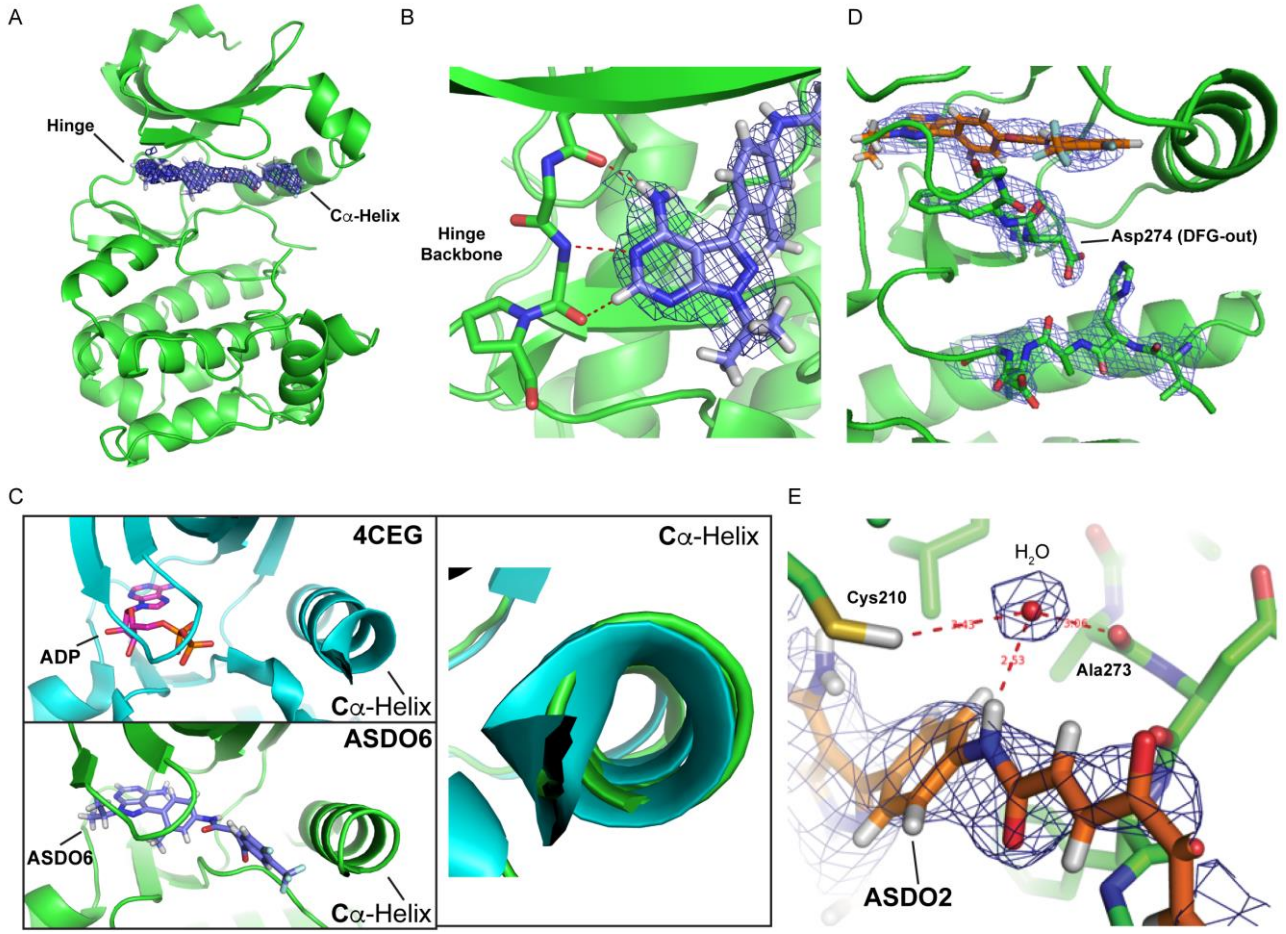


Figure S7. X-ray co-crystal structures of ASDO6 and ASDO2 help reveal the molecular level basis for cysteine-gatekeeper specificity. A) The final 2mFo-DFc electron density map is shown as a mesh contoured at 1 σ with **ASDO6** (PDB: 6HJJ) bound to the ATP-binding pocket and stretching from the hinge to the Cα-helix. **B**) **ASDO6** makes the expected interactions with the hinge region of Aurora-A kinase, typical of an ATP-competitive inhibitor. P-loop has been removed for clarity. **C**) Front view (left panels) of compound **ASDO6** (purple) and ADP (pink) showing the Cα-helix and end view (right panel) of the Cα-helix in **ASDO6** structure (green) and ADP structure (cyan, PDB: 4CEG). **D**) **ASDO2**-bound Aurora A kinase (PDB: 6HJK) is in the DFG-out conformation as expected with a type II inhibitor.

Compound is bound adjacent to Cα-helix, distorting it. **E**) Water-mediated hydrogen bonds between **ASDO2**, the carbonyl of Ala273 and the side-chain of Cys210.

Table 1: Crystal data and structure refinement (**ASDO2; PDB code 6HJK**)

Resolution range (Å…)	76.88 - 2.4 (2.486 - 2.4)
Space group	P 32 2 1
Unit cell	88.769 88.769 77.28 90 90 120
Total reflections	28217 (2745)
Unique reflections	14112 (1373)
Multiplicity	2.0 (2.0)
Completeness (%)	100.00 (100.00)
Mean I/sigma(I)	23.07 (4.63)
Wilson B-factor	57.84
R-merge	0.01263 (0.1532)
R-meas	0.01786
CC1/2	1 (0.93)
CC*	1 (0.982)
R-work	0.2022 (0.2668)
R-free	0.2354 (0.3553)
Number of non-hydrogen atoms	2054
macromolecules	1993
ligands	43
water	18
Protein residues	255
RMS(bonds)	0.013
RMS(angles)	1.33
Ramachandran favored (%)	90
Ramachandran outliers (%)	2.8
Clashscore	13.13
Average B-factor	74.4
macromolecules	74
ligands	97
solvent	69.2

Table 2: Crystal data and structure refinement **(ASDO6; PDB code 6HJJ**)

Resolution range (Å…)	71.04 - 2.13 (2.206 - 2.13)
Space group	P 61 2 2
Unit cell	82.027 82.027 172.609 90 90 120
Total reflections	39980 (3856)
Unique reflections	19990 (1928)
Multiplicity	2.0 (2.0)
Completeness (%)	99.94 (99.33)
Mean I/sigma(I)	17.84 (1.51)
Wilson B-factor	44.14
R-merge	0.03359 (0.7864)
R-meas	0.0475
CC1/2	0.999 (0.427)
CC*	1 (0.774)
R-work	0.2086 (0.3889)
R-free	0.2682 (0.3975)
Number of non-hydrogen atoms	2193
macromolecules	2037
ligands	80
water	76
Protein residues	256
RMS(bonds)	0.011
RMS(angles)	1.11
Ramachandran favored (%)	96
Ramachandran outliers (%)	0.4
Clashscore	6.46
Average B-factor	60.8
macromolecules	59.7
ligands	87.9
solvent	61.6

Supplementary Methods

Antibodies and Western Analysis

Mouse monoclonal anti-FLAG® M2 and rabbit anti-MASTL (GWL, Prestige Antibodies) antibodies were purchased from Sigma-Aldrich and polyclonal rabbit anti-phospho(Ser67)-ENSA/ARPP19 and anti-ENSA antibodies were purchased from Cell Signaling Technology. Mouse monoclonal anti-c-Myc (9E10) and mouse and rabbit derived antibodies for loading controls α-tubulin (DM1A) and GAPDH respectively were purchased from Abcam. Secondary antibodies were HRP-conjugated, polyclonal goat-derived antibodies against mouse and rabbit (Dako, Agilent Technologies). Western blot analysis of GWL, ENSA, phospho-ENSA, and the loading controls α-tubulin and GAPDH was accomplished through SDS-PAGE of cell lysates in 1X SDS-loading buffer containing β-mercaptoethanol (5X buffer: 0.25 % w/v Bromophenol blue, 0.5 M DTT, 50% v/v glycerol, 10% w/v SDS (sodium dodecyl sulphate)

and 0.25M Tris-HCl pH 6.8) and transfer of protein onto PVDF using the Trans-Blot Turbo System (Bio-Rad). Quantitation of western blot protein intensity was performed by densitometry using ImageJ and data were plotted using Prism 6.0.

Expression Constructs

The mammalian expression construct for N-terminally FLAG-tagged full-length human GWL and Aurora A kinase have previously been described[1, 2]. The plasmid for expression of *Xenopus laevis* MYC-Cdk1 was a kind gift from Helfrid Hochegger. Recombinant His-tagged ENSA was purified using Ni-NTA agarose (QIAGEN) from BL21 *E. coli* following the manufacturer's recommended protocol. FLAG-GWL (WT or M110C) was cloned into the pLVX-IRES-Puro (Clontech) lentiviral vector through restriction digestion of the FLAG-GWL construct with NotI and ligation of this product with NotI digested and phosphatase (CIP) treated pLVX-IRES-Puro plasmid. Ligation with T4 DNA Ligase (NEB) proceeded at 15 ^{o}C overnight after which 2 μl was transformed into supercompetent DH5α. After plating and growing transformants overnight, colonies were selected, amplified in the presence of ampicillin and then harvested to prepare DNA minipreps. Miniprepped DNA was digested with BamHI and analysed by agarose gel electrophoresis to confirm that GWL expressing pLVX clones contained properly oriented inserts. Clones were then amplified and used to prepare lentiviral particles according to the instructions in the Lenti-X^{TM} Lentiviral Expression System Manual (Clontech).

Mutagenesis

All mutagenesis reactions were carried out using the QuikChange II Site-Directed or Lightning Multi Site-Directed Mutagenesis Kits (Agilent) according to the manufacturer's instructions. All plasmids were sent to GATC Biotech for Sanger sequencing to confirm the mutations. Mutagenic primers were purchased from Eurofins and are listed below.

GWL

Y59V: F – GAA AGG CGG CAA ATT GGT TGC AGT AAA GGT TG,
R – CAA CCT TTA CTG CAA CCA ATT TGC CGC CTT TC

Y59T: F – GAA AGG CGG CAA ATT GAC TGC AGT AAA GGT TG,
R – CAA CCT TTA CTG CAG TCA ATT TGC CGC CTT TC

V61I: F – GGC AGA AAG GCG GCA AAT TGT ATG CAA TAA AGG TTG,
R – CAA CCT TTA TTG CAT ACA ATT TGC CGC CTT TCT GCC

Y107V: F – GCA GTC TGC AAA CAA TGT CGT CTT GGT AAT GG,
R – CCA TTA CCA AGA CGA CAT TGT TTG CAG ACT GC

Y107T: F – GCA GTC TGC AAA CAA TGT CAC CTT GGT AAT GG,
R – CCA TTA CCA AGG TGA CAT TGT TTG CAG ACT GC

D174A: F – GGGTCATATTAAACTGACGGCTTTTGGCCTTTCAAAAGTTAC,
R – GTAACTTTTGAAAGGCCAAAAGCCGTCAGTTTAATATGACCC

M110V: F – CAATGTCTACTTGGTAGTGGAATATCTTATTGGGG,

R – CCCCAATAAGATATTCCACTACCAAGTAGACATTG

M110T: F – GTCTACTTGGTAACGGAATATCTTATTGGGGG,

R – CCCCCAATAAGATATTCCGTTACCAAGTAGAC

M110C: F – GTCTACTTGGTATGCGAATATCTTATTGGG,

R – CCCAATAAGATATTCGCATACCAAGTAGAC

M110S: F – CAAACAATGTCTACTTGGTATCGGAATATCTTATTG,

R – CAATAAGATATTCCGATACCAAGTAGACATTGTTTG

M110A: F – CAATGTCTACTTGGTAGCGGAATATCTTATTGGGG,

R – CCCCAATAAGATATTCCGCTACCAAGTAGACATTG

M110G: F – CAATGTCTACTTGGTAGGGGAATATC,

R – CAATAAGATATTCCCCTACCAAGTAGACATTGTTTG

V94A: F – CATTCATTGCCCATTTGTATTATTCACTG,

R – CAGTGAATAATACAAATGGGCAATGAATG

S42G: F – CATAGTGAAGCCCATTGGCCGGGGCGCCTTCG,

R – CGAAGGCGCCCCGGCCAATGGGCTTCACTATG

A45S: F – CATTGGCCGGGGCTCCTTCGGGAAAG,

(with S42G) R – CTTTCCCGAAGGAGCCCCGGCCAATG

G116C: F – CTTATTGGGTGTGATGTCAAG,

R – CTTGACATCACACCCAATAAG

G117C: F – CTTATTGGGGGATGTGTCAAGTCTC,

R – GAGACTTGACACATCCCCCAATAAG

siRNA_ resistant GWL mutations (Figure S1):

F – CAAAAGGACACTACTCCTTACTCTTCAAAACTCCTAAAATCATG

R – CATGATTTTAGGAGTTTTGAAGAGTAAGGAGTAGTGTCCTTTTG

Aurora-A Kinase

L210C: F – GTCTACCTAATTTGCGAATATGCACC,

R – GGTGCATATTCGCAAATTAGGTAGAC

xlCDK1

F80C: F – CAAGGTTGTATCTTATCTGTGAGTTTCTCTCCATGG,

R – CCATGGAGAGAAACTCACAGATAAGATACAACCTTG

Cell Culture, Compound Preparation and FACS analysis

MEFs and HEK 293T cells were cultured in Dulbecco's modified Eagle Medium (DMEM) supplemented with 10% v/v FCS, 2 mM L-glutamine, 100 U ml^{-1} penicillin and 0.1 mg ml^{-1} streptomycin in a 37 °C, 5% CO_2 incubator. For GWL depletion, 10 μl of AdCre (Vector Biolabs) was added to a 10 cm dish of MEFs in culture. After 3 d, cells were then treated with 500 μl of GWL expressing lentivirus (0.45 micron filtered).

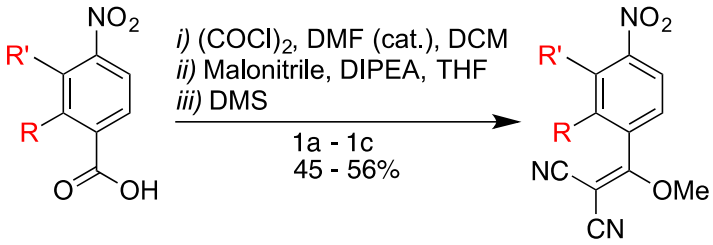
Synthetically derived ASDO compounds, 4-(4-ethoxy-phenyl)-4-oxo-but-2-enoic acid (4-ethyl-phenyl)-amide (DO2, Sigma-Aldrich/Merck), Staurosporine (Cell Signalling Technology) and AD57 were dissolved in DMSO and stored at –20 ^{o}C in glass vials. Cells transfected with or without GWL targeting siRNA (HS_MASTL_6)[1] and Qiagen AllStars negative control were harvested, washed with PBS and fixed in 70% EtOH overnight at 4 ^{o}C. Next, cells were pelleted and washed with 1% BSA in PBS. After pelleting, cells were re-suspended and incubated with propidium iodide (PI) solution for at least 6 h or overnight (PI solution: 20 μg ml^{-1} PI, 0.1% Triton X-100, 200 μg ml^{-1} RNAse (all from Sigma) diluted in 0.5% BSA in PBS). Cell cycle analysis was performed on the BD Accuri™ C6 Plus personal flow cytometer and data analysed using FCS Express 6 flow cytometry software.

Proliferation Assays

MEF cells were seeded at a density of 1000 and 2000 cells/well, respectively, in clear 96-well plates and allowed to grow for 5–6 d in the presence of inhibitors or DMSO. After the indicated time-point, all cells were treated with CellTiter-Blue Reagent (Promega) and allowed to incubate at 37 ^{o}C for an additional 3 h. After 3 h, fluorescence was measured using the GloMax-Multi Detection System. All concentration-dependent cellular proliferation assays were performed 5 times with replicates ranging from 4 to 10 per point and quantitation of average normalized proliferation was reported as % of the DMSO control ± S.D. and plotted using Prism 6.0. The t-test statistical module of Prism 6.0 was used to determine p-values (ns (not statistically significant): P ≤ 0.01; ***: P ≤ 0.001; ****).

Chemical Synthesis

All commercial reagents were purchased from Sigma-Aldrich, Alfa Aesar, Combi-Blocks, TCI (UK) and Princeton Biomolecular and were of the highest available purity. Anhydrous solvents were purchased from Acros (AcroSeal) or Sigma-Aldrich (SureSeal) and were stored under nitrogen. Proton nuclear magnetic resonance spectra were recorded at 500 or 400 MHz on a Varian VNMRS 500 MHz spectrometer, at 25 °C. Carbon Nuclear Magnetic Resonance spectra were recorded at 125 MHz on a Varian 500 MHz spectrometer. It should be noted that we did not detect C-F heteronuclear coupling due to CF_3 in the ^{13}C NMR mainly due to overlapping peaks. LCMS data were recorded on a Waters 2695 HPLC using a Waters 2487 UV detector and a Thermo LCQ ESI-MS. Samples were eluted through a Phenomenex Lunar 3μ C18 50 mm × 4.6 mm column, using water and acetonitrile acidified with 0.1% formic acid at 1 ml min^{-1} and detected at 254 nm. The gradient employed was a 4 or 7 min. method of 5-95% MeCN. High-resolution Mass Spectra (HRMS) were recorded at the University of Sussex Mass Spectrometry Centre on a high-resolution Orbitrap-XL instrument (Thermofisher). All experiments were carried out under an inert atmosphere of N_2 unless otherwise stated.

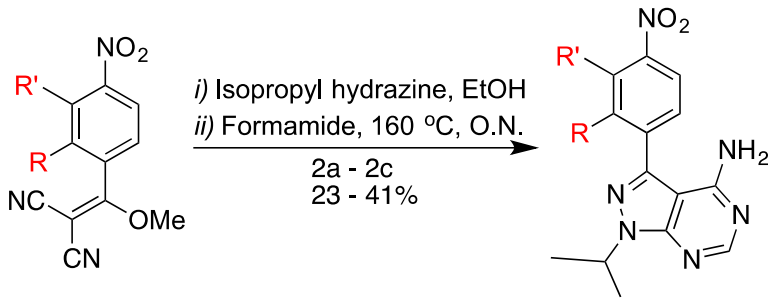


Intermediate	R	R'	%
1a	H	H	46
1b			45
1c	Me	H	56

General procedure for the synthesis of 2-[methoxy(4-nitrophenyl)methylidene]propanedinitriles (**1a** – **1c**)[3]. 2-[Methoxy(4-nitrophenyl)methylidene]propanedinitrile (**1a**) was prepared by slowly adding a 2.0 M solution of oxalyl chloride in DCM (0.30 mL, 3.52 mmol, 1.2 eq.) to a solution of benzoic acid (0.49 g, 2.93 mmol, 1.0 eq.) in 15 mL DCM containing 1 drop of DMF at RT for 30 min. This solution was then concentrated *in vacuo* and then re-dissolved in 5 ml of THF containing malonitrile (0.23 g, 3.52 mmol, 1.2 eq.) This mixture was cooled to 0 °C before drop-wise addition of DIPEA (1.28 mL, 7.33 mmol, 2.5 eq.). This reaction was allowed to warm to room temperature before being mixed for an additional 2 h. Next, dimethyl sulfate (DMS, 0.33 mL, 3.52 mmol, 1.2 eq.) was added, after which the temperature was increased to 70 °C. This was allowed to react for 4 h before bringing the temperature down to RT. At this stage, the reaction proceeded overnight before being worked-up with 1 N HCl (aq.) and extracted with ethyl acetate. Extracts were washed with water and brine and then dried with $MgSO_4$. The crude mixture was purified by silica chromatography using a hexanes/ethyl acetate solvent system (ethyl acetate gradient increased over time from 0 – 60%). Concentration of the extract yielded 0.31 g (1.37 mmol, 46%) of a yellow, oily material. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.99 (d, *J* = 6.5 Hz, 2H), 7.24 (d, *J* = 6.5 Hz, 2H), 4.01 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 183.8, 161.7, 132.6, 126.9, 125.8, 110.0, 49.8.

2-[Methoxy(4-nitronaphthalen-1-yl)methylidene]propanedinitrile (**1b**) was made according to the general procedure reported for **1a**. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.54 (d, *J* = 8.2 Hz, 1H), 8.25 (d, *J* = 7.8 Hz, 1H), 7.94 – 7.82 (m, 3H), 7.72 (d, *J* = 7.8 Hz, 1H), 3.76 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 182.3, 149.6, 130.8, 130.7, 130.7, 130.2, 126.8, 125.0, 124.2, 123.8, 122.2, 111.7, 110.4, 60.3.

2-[Methoxy(2-methyl-4-nitrophenyl)methylidene]propanedinitrile (**1c**) was made according to the general procedure reported for **1a**. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.11 (d, *J* = 6.7 Hz, 1H), 7.52 (m, 2H), 4.02 (s, 3H), 2.68 (s, 3H).

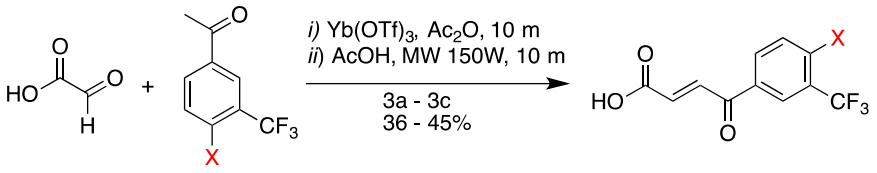


Intermediate	R	R'	%
2a	H	H	41
2b			36
2c	Me	H	23

General procedure for the synthesis of 3-(4-nitrophenyl)-1-(propan-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amines (**2a** – **2c**)[3]. 3-(4-Nitrophenyl)-1-(propan-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**2a**) was prepared by mixing **1a** (0.31 g, 1.37 mmol, 1 eq.) with isopropyl hydrazine (0.12 g, 1.65 mmol, 1.2 eq.) and TEA (3.71 mL, 27.5 mmol, 20 eq.) in 5 mL of EtOH. After 1h, the reaction was concentrated and generation of the pyrazole was confirmed by LCMS-LCQ: 7 mins, 5-95% MeCN, Rt = 3.75 mins; $[M+H]^+$ calculated for $C_{13}H_{13}N_5O_2$ 272.11; found 272.17. The pyrazole was then diluted with 5 mL of formamide (0.3M **1a**) and left to reflux ON at 160 ^oC. After cooling, the reaction was diluted with 50 mL of ice cold water, which was then filtered over a pad of celite. Extracting the filtrate with ethyl acetate and concentrating the extracts gave 190 mg (0.64 mmol, 41%) of a yellow powder that was used in the next reaction without further purification. ^1H NMR (500 MHz, DMSO-d_6) δ 8.30 (d, J = 8.7 Hz, 1H), 8.21 (s, 1H), 7.89 (d, J = 8.5 Hz, 1H), 6.59 (s, 1H), 5.09 (hept, J = 6.7 Hz, 1H), 1.51 (d, J = 6.6 Hz, 3H). ^{13}C NMR (126 MHz, dmso) δ 158.3, 155.7, 147.6, 141.4, 140.2, 129.5, 124.3, 123.9, 110.0, 30.9, 22.0. LCMS-LCQ: 4 mins, 5-95% MeCN, Rt = 1.16 mins; $[M+H]^+$ calculated for $C_{14}H_{14}N_6O_2$ 299.30; found 299.17.

3-(4-Nitronaphthalen-1-yl)-1-(propan-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**2b**) was made according to the general procedure reported for **2a**. After 1h of mixing **1d**, isopropyl hydrazine and TEA in ethanol, the reaction was concentrated and generation of the pyrazole was confirmed by LCMS-LCQ: 4 mins, 5-95% MeCN, Rt = 0.71 mins; $[M+H]^+$ calculated for $C_{17}H_{15}N_5O_2$ 322.12; found 321.98. ^1H NMR (500 MHz, DMSO-d_6) δ 8.44 (d, J = 8.7 Hz, 1H), 8.37 (d, J = 7.8 Hz, 1H), 8.28 (s, 1H), 8.04 (d, J = 8.4 Hz, 1H), 7.84 (t, 1H), 7.77 – 7.68 (m, 2H), 7.37 – 5.83 (s br, 2H), 5.14 (hept, J = 6.7 Hz, 1H), 1.53 (d, J = 6.7 Hz, 6H). ^{13}C NMR (126 MHz, dmso) δ 158.2, 156.2, 153.6, 147.2, 140.6, 137.1, 133.0, 129.9, 128.5, 127.8, 127.1, 125.4, 124.0, 122.9, 110.0, 49.0, 22.2. LCMS-LCQ: 4 mins, 5-95% MeCN, Rt = 0.74 mins; $[M+H]^+$ calculated for $C_{18}H_{16}N_6O_2$ 349.35; found 349.20.

3-(2-Methyl-4-nitrophenyl)-1-(propan-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**2c**) was made according to the general procedure reported for **2a**. After 1h of mixing **1b**, isopropyl hydrazine and TEA in ethanol, the reaction was concentrated and generation of the pyrazole was confirmed by LCMS-LCQ: 4 mins, 5-95% MeCN, Rt = 3.07 mins; $[M+H]^+$ calculated for $C_{14}H_{15}N_5O_2$ 286.12; found 286.02. ^1H NMR (500 MHz, DMSO-d_6) δ 8.18 (s, 1H), 7.22 (s, 2H), 7.17 (d, J = 8.1 Hz, 1H), 6.73 (d, J = 8.2 Hz, 2H), 5.16 (s, 2H), 5.01 (hept, J = 6.7 Hz, 1H), 2.12 (s, 3H), 1.46 (d, J = 6.7 Hz, 6H). LCMS-LCQ: 4 mins, 5-95% MeCN, Rt = 2.13 mins; $[M+H]^+$ calculated for $C_{15}H_{16}N_6O_2$ 313.33; found 313.17.

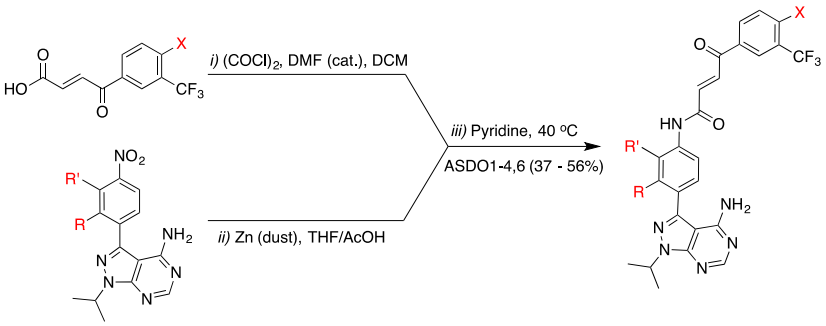


Intermediate	X	%
3a	H	39
3b	F	36
3c	Cl	45

General procedure for the synthesis of 3-benzoyl acrylic acids (**3a** – **3c**)[4]. To prepare (2E)-4-oxo-4-[3-(trifluoromethyl)phenyl]but-2-enoic acid (**3a**), glyoxylic acid monohydrate (2.2 g, 23.9 mmol), acetic anhydride (2.4 mL, 25.2 mmol) and ytterbium triflate (0.37 g, 0.59 mmol) were combined in a microwave reaction vial and stirred at room temperature for 10 min. Then, *m*-(trifluoromethyl)benzoylacetophenone (5 g, 24 mmol) and glacial acetic acid (4 mL) were added. The reaction vial was sealed, and the mixture was subjected to microwave irradiation for 10 min. at 150 W (< 6 bar internal pressure). After completion of the reaction, the dark brown/black mixture was diluted with water, basified with K_2CO_3 (25% aq.) and washed with DCM (5X 60 mL). Then, the aqueous phase was cooled in an ice-bath and acidified with HCl (concentrated aq.). The solid product was filtered off, washed with water and dried in air at 50 °C for 24 h to give 2.3 g (39%) of a light brown solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.06 (s, 1H), 8.33 (d, *J* = 7.8 Hz, 1H), 8.27 (s, 1H), 8.06 (d, *J* = 7.9 Hz, 1H), 7.92 (d, *J* = 15.7 Hz, 1H), 7.80 (t, *J* = 7.9 Hz, 1H), 6.71 (d, *J* = 15.5 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.0, 171.4, 142.2, 141.0, 138.9, 138.0, 135.7, 135.6, 135.3, 133.2, 109.9.

(2E)-4-[4-Fluoro-3-(trifluoromethyl)phenyl]-4-oxobut-2-enoic acid (**3b**) was made according to the general procedure reported for **3a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.17 (s, 1H), 8.43 (ddd, *J* = 8.1, 6.8 (H-F), 2.2 (H-F) Hz, 1H), 8.33 (dd, *J* = 6.8 (H-F), 2.0 (H-F) Hz, 1H), 7.92 (d, *J* = 15.6 Hz, 1H), 7.71 (t, *J* = 8.5, 6.8 (H-F) Hz, 1H), 6.71 (d, *J* = 15.6 Hz, 1H). ^{13}C NMR (126 MHz, dmso) δ 188.1, 166.6, 136.8, 136.7, 136.3, 136.1, 134.2, 128.6, 118.7, 118.5, 110.0.

(2E)-4-[4-Chloro-3-(trifluoromethyl)phenyl]-4-oxobut-2-enoic acid (**3c**) was made according to the general procedure reported for **3a.** ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.06 (s, 1H), 8.33 – 8.31 (m, 2H), 7.93 (d, *J* = 8.8 Hz, 1H), 7.89 (d, *J* = 15.6 Hz, 1H), 6.72 (d, *J* = 15.5 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 193.2, 171.3, 140.8, 140.5, 139.5, 139.0, 139.0, 137.7, 134.5, 133.1, 110.0.



Intermediate	R	R'	X	%
ASDO1	H	H	H	41
ASDO2	H	H	F	43
ASDO3	H	H	Cl	56
ASDO4			H	37
ASDO6	Me	H	F	37

General procedure for the coupling of the 3-phenyl acrylic acids with 3-phenyl pyrazolopyrimidines (ASDO1-4 & 6). To a dry round bottom flask was added **2a** (20 mg, 0.067 mmol, 1.2 eq.) in 1 mL of AcOH/THF (1:1). Zn dust (13 mg, 0.20 mmol, 3.5 eq.) was added slowly and in

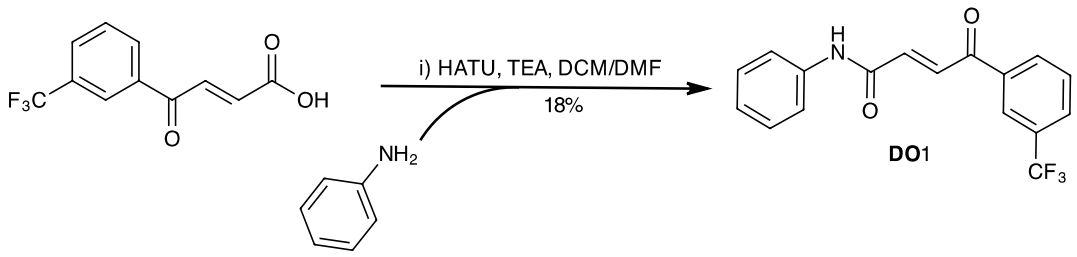
portions over 5 mins. The reaction was mixed vigorously at 40 °C over the next 3 h and monitored closely by TLC (DCM/ethyl acetate (1:1)). Next, the reaction was diluted in ethyl acetate, filtered over a pad of celite and washed with water thrice. This was then washed with brine, dried with magnesium sulfate and concentrated *in vacuo* to yield a brownish solid amine (deep purple colour with ninhydrin stain). This amine was diluted with 500 ul of pyridine and chilled on ice before being used as a coupling partner in the next step. In parallel to this reaction, the conversion of the acrylic acid to an acid chloride was effected by combining **3a** (13.7 mg, 0.057 mmol, 1 eq.) with oxalyl chloride $(COCl)_2$ (2M in DCM, 28.5 ul, 1 eq.) and 1 drop of DMF (catalytic). This solution was allowed to stir for 30 min. before removal of DCM by roto-evaporation; the resulting acid chloride was diluted with 200 ul of DMF. This acid chloride solution was then slowly added to a solution of the chilled amine solution. After 10 min., the reaction was allowed to warm to room temperature for 30 min. and then 40 °C for 1 h. Finally, the reaction was diluted with ethyl acetate, washed with water and brine thrice, concentrated and purified by silica chromatography using a DCM/ethyl acetate solvent system (ethyl acetate gradient increased over time from 0 – 90%). To ensure that the remaining traces of the amine were removed, the resulting compound was purified further by preparatory TLC (Analtech Uniplates, Silica GF, 250 μM, 20 cm X 20 cm) under isocratic conditions using DCM/ethyl acetate (1:1). After etching off the correct band from the TLC plate, the silica was pulverised, mixed with ethyl acetate, filtered through a frit and the resulting filtrate was concentrated to yield 11.6 mg (0.023 mmol, 41%) of (2E)-N-{4-[4-amino-1-(propan-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl]phenyl}-4-oxo-4-[3-(trifluoromethyl)phenyl]but-2-enamide (**ASDO1**). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.80 (s, 1H), 8.37 (d, *J* = 4.5 Hz, 1H), 8.29 (s, 1H), 8.21 (s, 1H), 7.97 (d, J = 4.5 Hz, 1H), 7.88 (d, J = 16 Hz, 1H), 7.83 (d, J = 12 Hz, 1H), 7.81 (t, J = 4.5 Hz, 1H), 7.64 (d, J = 12 Hz, 1H), 7.25 (d, J = 16 Hz, 1H), 6.74 (s, 2H), 5.04 (hept, J = 6.5 Hz, 1H), 1.45 (d, J = 6.5 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 188.2, 162.0, 157.2, 147.7, 144.3, 137.2, 136.5, 131.9, 131.9, 131.6, 131.6, 130.1, 130.1, 129.5, 129.7, 129.1, 125.6, 125.5, 120.9, 100.3, 49.7, 21.8. LCMS-LCQ: 7 mins, 5-95% MeCN, Rt = 3.64 mins, > 99% purity; $[M+H]^+$ calculated for $C_{25}H_{21}F_3N_6O_2$ 495.17; found 495.27. HRMS (ESI): $[M+H]^+$ calculated for $C_{25}H_{21}F_3N_6O_2$ 495.1678, found 495.1836.

(2E)-N-{4-[4-Amino-1-(propan-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl]phenyl}-4-[4-fluoro-3-(trifluoromethyl)phenyl]-4-oxobut-2-enamide (**ASDO2**) was made according to the general procedure reported for **ASDO1**. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 10.29 (s, 1H), 8.58 (d, *J* = 4.6 Hz, 1H), 8.25 – 8.18 (m, 2H), 7.89 (d, J = 15 Hz, 1H), 7.82 (d, J = 10 Hz, 2H), 7.57 (d, J = 10 Hz, 2H), 7.33 (d, J = 15 Hz, 1H), 7.30 – 7.25 (m, 2H), 6.03 (s, 2H), 5.05 (hept, *J* = 6.5 Hz, 1H), 1.48 (d, *J* = 6.5 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 190.8, 156.9, 153.7, 150.4, 146.9, 143.7, 143.5, 139.3, 138.0, 134.7, 131.6, 129.0, 128.9, 128.5, 120.6, 120.6, 120.0, 117.7, 117.5, 100.2, 39.4, 20.8. LCMS-LCQ: 4 mins, 5-95% MeCN, Rt = 0.62 mins, > 99% purity; $[M+H]^+$ calculated for $C_{25}H_{20}F_4N_6O_2$ 513.16; found 513.19. HRMS (ESI): $[M+H]^+$ calculated for $C_{25}H_{20}F_4N_6O_2$ 513.1583, found 513.1659.

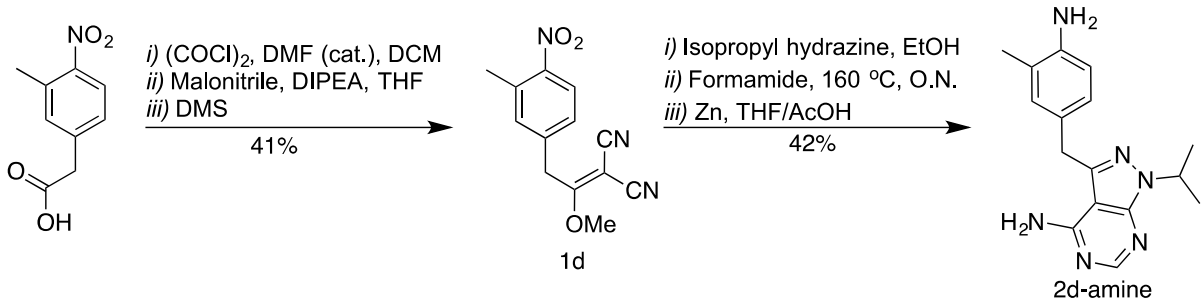
(2E)-N-{4-[4-Amino-1-(propan-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl]phenyl}-4-[4-chloro-3-(trifluoromethyl)phenyl]-4-oxobut-2-enamide (**ASDO3**) was made according to the general procedure reported for **ASDO1**. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 10.40 (s, 1H), 9.66 (s, 1H), 8.28 (s, 1H), 8.21 (s, 1H), 8.09 (d, *J* = 8.3 Hz, 1H), 7.93 (s, 1H), 7.90 – 7.87 (m, 3H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.55 – 7.52 (m, 2H), 7.34 (d, *J* = 14.9 Hz, 1H), 6.86 (s, 2H), 5.10 (hept, *J* = 6.8 Hz, 1H), 1.52 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 188.2, 152.2, 150.8, 146.9, 143.9, 143.8, 137.3, 136.4, 135.5, 131.9, 130.6, 130.1, 130.1, 129.5, 129.5, 126.8, 125.6, 125.6, 124.5, 97.6, 34.5, 21.8. LCMS-LCQ: 4 mins, 5-95% MeCN, Rt = 0.65 mins, > 99% purity; $[M+H]^+$ calculated for $C_{25}H_{20}ClF_3N_6O_2$ 529.12; found 529.15. HRMS (ESI): $[M+H]^+$ calculated for $C_{25}H_{20}ClF_3N_6O_2$ 529.1288, found 529.1359.

(2E)-N-{4-[4-amino-1-(propan-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl]naphthalen-1-yl}-4-oxo-4-[3-(trifluoromethyl)phenyl]but-2-enamide (**ASDO4**) was made according to the general procedure reported for **ASDO1**. ^{1}H NMR (500 MHz, Chloroform-*d*) ^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.55 (s, 1H), 8.39 (s, 1H), 8.34 (s, 2H), 8.27 (s, 1H), 8.18 (d, *J* = 14.7 Hz, 1H), 8.01 (d, *J* = 8.7 Hz, 1H), 7.91 (d, *J* = 7.9 Hz, 1H), 7.72 (d, *J* = 7.6 Hz, 1H), 7.64 (t, *J* = 8.0 Hz, 1H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.45 (d, *J* = 14.7 Hz, 1H), 5.49 (s, 2H), 5.28 (hept, *J* = 6.5 Hz, 1H), 1.67 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 188.09, 176.93, 164.36, 157.07, 154.49, 137.26, 136.07, 133.89, 133.13, 131.95, 131.91, 130.27, 129.98, 129.74, 129.62, 128.36, 127.67, 127.61, 127.55, 127.32, 126.59, 125.68, 125.65, 125.62, 120.25, 100.23, 49.31, 22.05. LCMS-LCQ: 4 mins, 5-95% MeCN, Rt = 0.61 mins, > 99% purity; $[M+H]^+$ calculated for $C_{29}H_{23}F_3N_6O_2$ 545.18; found 545.25. HRMS (ESI): $[M+H]^+$ calculated for $C_{29}H_{23}F_3N_6O_2$ 545.1835, found 545.2167.

(2E)-N-{4-[4-Amino-1-(propan-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl]-3-methylphenyl}-4-[4-fluoro-3-(trifluoromethyl)phenyl]-4-oxobut-2-enamide (**ASDO6**) was made according to the general procedure reported for **ASDO1**. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.97 (s, 1H), 8.37 (s, 1H), 8.35 (d, 1H), 8.32 – 8.26 (m, 1H), 8.24 (d, *J* = 8.2 Hz, 1H), 8.09 (d, *J* = 14.8 Hz, 1H), 7.62 – 7.57 (m, 2H), 7.38 (t, *J* = 9.1 Hz, 1H), 7.29 (s, 1H), 5.75 (s, 2H), 5.19 (hept, *J* = 6.7 Hz, 1H), 2.43 (s, 3H), 1.61 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 186.6, 161.6, 157.2, 154.5, 153.3, 143.4, 136.3, 135.9, 134.8, 134.7, 133.2, 133.1, 130.8, 130.7, 129.7, 128.6, 128.5, 126.9, 123.2, 118.0, 117.8, 98.4, 77.3, 49.1, 22.0, 17.9. LCMS-LCQ: 4 mins, 5-95% MeCN, Rt = 2.89 mins, > 95% purity; $[M+H]^+$ calculated for $C_{26}H_{22}F_4N_6O_2$ 527.17; found 527.21. HRMS (ESI): $[M+H]^+$ calculated for $C_{26}H_{22}F_4N_6O_2$ 527.1740, found 527.2500.



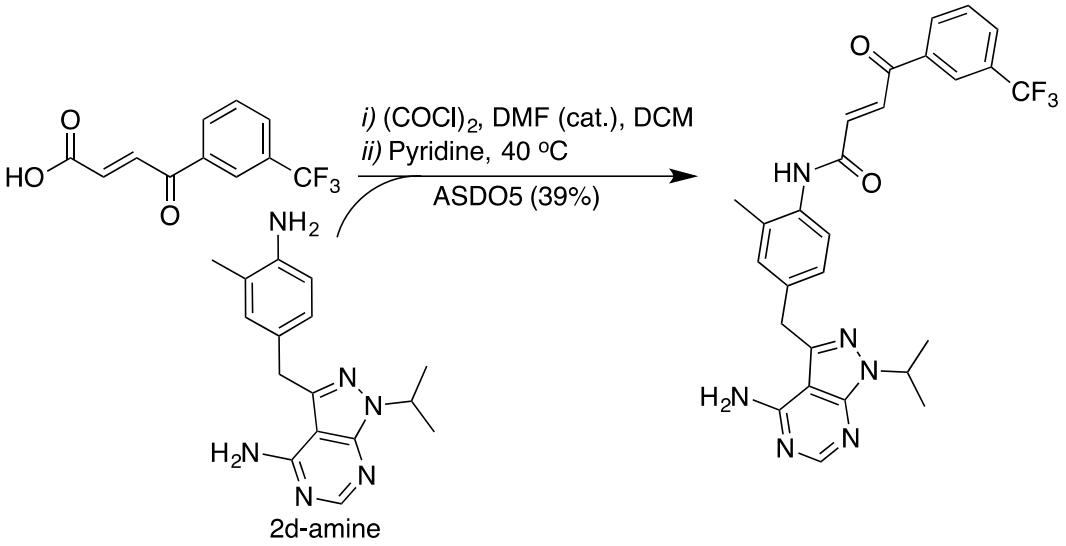
(*E*)-4-Anilino-1-[*m*-(trifluoromethyl)phenyl]-2-butene-1,4-dione (**DO1**) was made by dissolving (E)-4-oxo-4-[3-(trifluoromethyl)phenyl]but-2-enoic acid (80 mg, 0.33 mmol, 1.5 eq.) in DCM (2mL) and DMF (200uL) in an oven dried, Ar (g) cooled 20 mL scintillation vial with septum top. TEA (61 uL, 0.44 mmol, 2 eq.) and HATU (133 mg, 0.35 mmol, 1.6 eq.) were then added and the mixture was allowed to stir at RT for 15 min. Next, the solution was chilled to 0 ^{o}C before the drop-wise addition of aniline (20 uL, 0.22 mmol, 1 eq.). After 10 min., the reaction was warmed to RT, stirred for an hour and then warmed to 40 ^{o}C. After mixing for 1 h at 40 ^{o}C, the reaction was cooled and then quenched with 25 mL $NAHCO_3$. This mixture was extracted 2X with DCM, dried with $MgSO_4$ and concentrated *in vacuo*. Purification was achieved by silica chromatography using a hexanes/ethyl acetate system (ethyl acetate gradient increased over time from 0 – 100%). Concentration of the fractions yielded 12.9 mg (18%) of **DO1**.^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (s, 1H), 8.38 (d, *J* = 7.9 Hz, 1H), 8.31 (s, 1H), 8.12 – 8.06 (m, 1H), 7.95 (d, *J* = 15.2 Hz, 1H), 7.85 (t, *J* = 7.8 Hz, 1H), 7.73 (dd, *J* = 8.6, 1.0 Hz, 2H), 7.41 – 7.34 (m, 2H), 7.26 (d, *J* = 15.2 Hz, 1H), 7.16 – 7.09 (m, 1H). ^{13}C NMR (100 MHz, DMSO) δ 189.2, 162.1, 139.1, 137.8, 137.7, 133.2, 133.0, 130.8, 130.5, 130.4, 130.3, 130.0, 129.4, 125.4, 125.4, 124.6, 119.9.



2-[1-methoxy-2-(3-methyl-4-nitrophenyl)ethylidene]propanedinitrile (**1d**) was made according to the general procedure reported for **1a**. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.96 (d, 1H), 7.26 (m, 2H), 3.73 (s, 3H), 3.68 (s, 2H), 2.61 (s, 3H).

3-[(4-amino-3-methylphenyl)methyl]-1-(propan-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**2d-amine**) was made according to the general procedure reported for **2a** followed by reduction with Zn dust as reported in the first Zn-reduction step of the general procedure for the coupling of 3-benzoylacrylic acids with 3-phenyl pyrazolopyrimidines. The amine was purified by silica chromatography using a DCM/ethyl acetate solvent system (ethyl acetate gradient increased over time from 0 – 50%). This coupling partner is hindered, and thus we needed to reduce the nitro substituted precursor and obtain a very pure sample of the amine to carry on through to the next coupling step. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.11 (s,

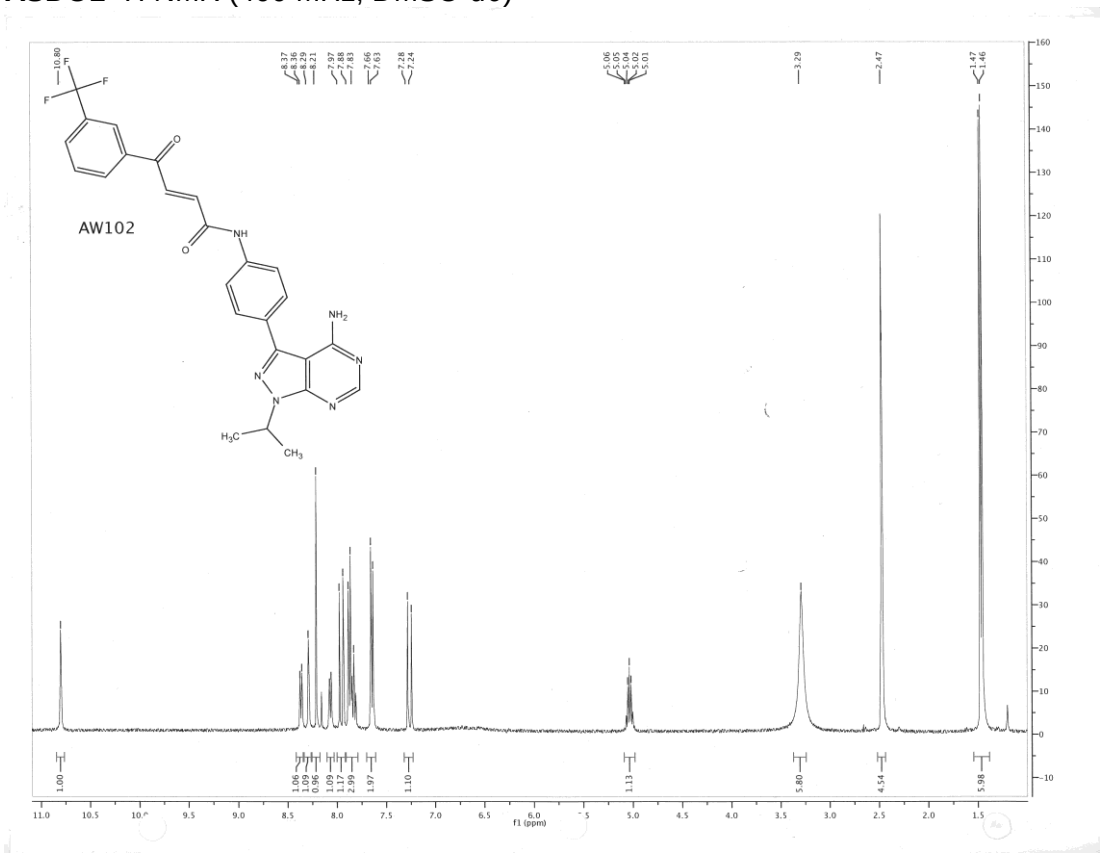
1H), 6.77 (s, 1H), 6.75 (d, *J* = 7.9 Hz, 1H), 6.52 (d, *J* = 8.1 Hz, 1H), 5.47 (s, 2H), 4.97 (hept, *J* = 6.7 Hz, 1H), 4.06 (s, 2H), 3.55 (s, 2H), 2.00 (s, 3H), 1.46 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 157.0, 153.8, 152.7, 144.2, 144.0, 132.0, 130.3, 126.7, 123.1, 115.4, 99.9, 48.4, 34.2, 21.8, 17.3.



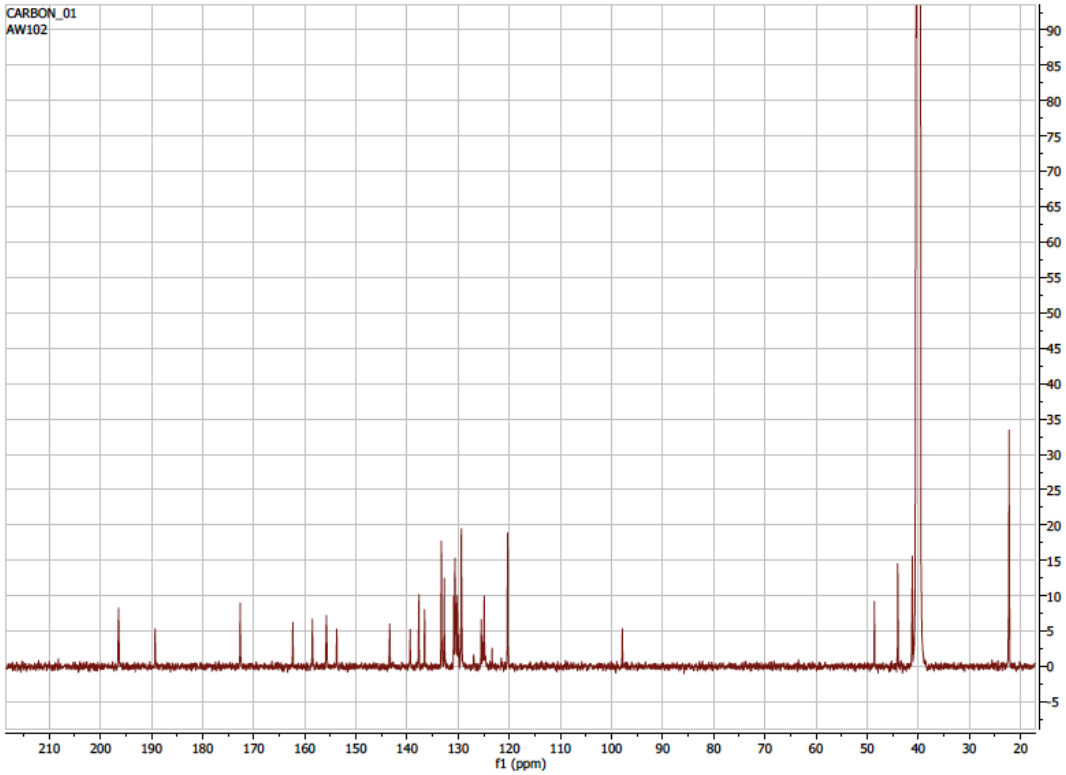
(2E)-N-(4-{[4-amino-1-(propan-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl]methyl}-2-methylphenyl)-4-oxo-4-[3-(trifluoromethyl)phenyl]but-2-enamide (**ASDO5)** was made according to the general procedure reported for **ASDO1** starting from the amine. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.33 (s, 1H), 8.32 (s, 1H), 8.25 (d, *J* = 8.0 Hz, 1H), 8.19 (s, 1H), 8.12 – 8.06 (m, 2H), 7.90 (d, *J* = 7.8 Hz, 1H), 7.75 (s, 1H), 7.69 (t, *J* = 7.9 Hz, 1H), 7.32 (d, *J* = 14.9 Hz, 1H), 7.14 (d, *J* = 8.4 Hz, 1H), 5.58 (s, 2H), 5.16 (hept, *J* = 6.8 Hz, 1H), 4.33 (s, 2H), 2.34 (s, 3H), 1.63 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 188.2, 176.4, 173.3, 167.0, 165.8, 152.3, 150.0, 146.9, 143.9, 137.3, 136.5, 135.5, 131.9, 130.7, 130.1, 129.5, 126.8, 125.6, 124.5, 97.6, 50.2, 34.5, 21.8, 18.2. LCMS-LCQ: 7 mins, 5-95% MeCN, Rt = 3.09 mins, > 99% purity; $[M+H]^+$ calculated for $C_{27}H_{25}F_3N_6O_2$ 523.1990; found 523.21. HRMS (ESI): $[M+H]^+$ calculated for $C_{27}H_{25}F_3N_6O_2$ 523.1990, found 523.4167.

^{1}H and ^{13}C NMR Spectra and LCMS Analysis

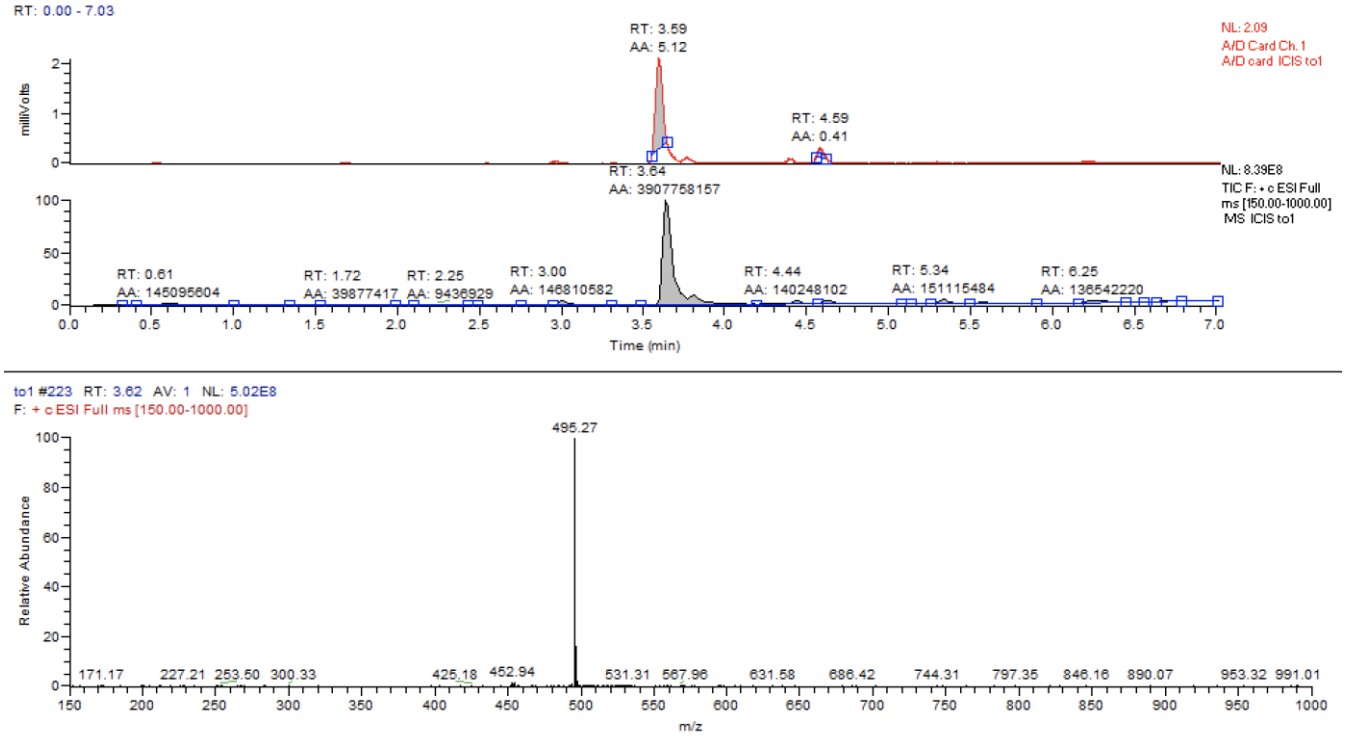
ASDO1 ^{1}H NMR (400 MHz, DMSO-*d6*)



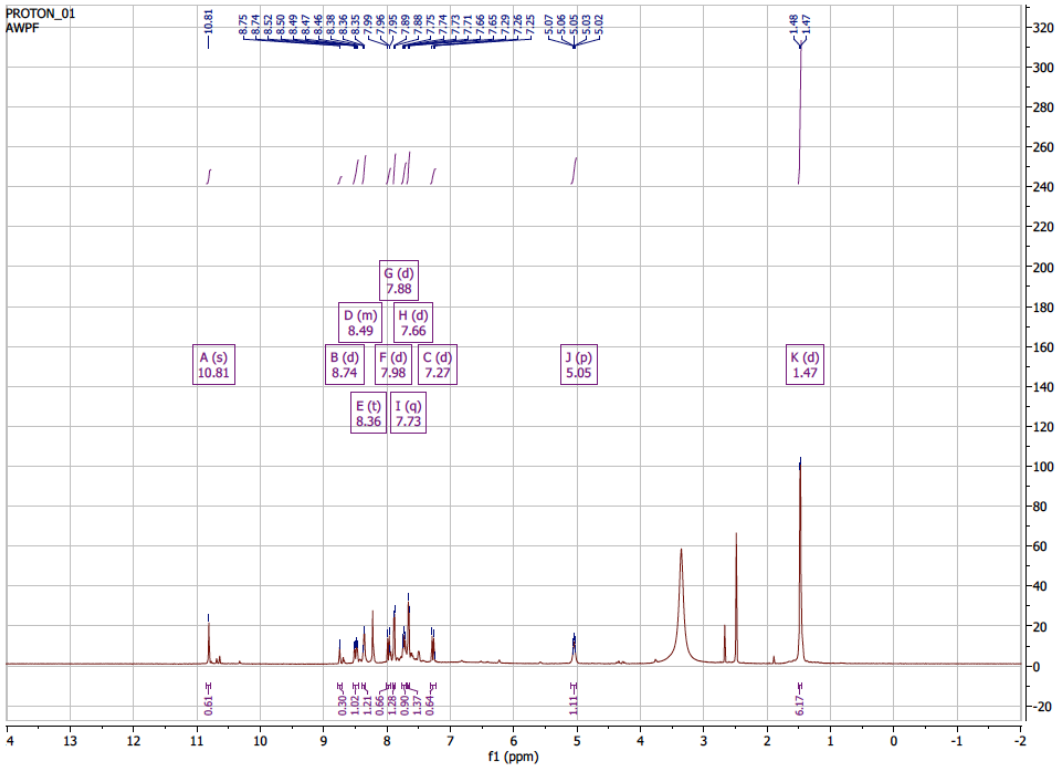
ASDO1 ^{13}C NMR (126 MHz, $CDCl_3$): It should be noted that we did not detect C-F heteronuclear coupling due to CF_3 in the ^{13}C NMR mainly due to overlapping peaks.



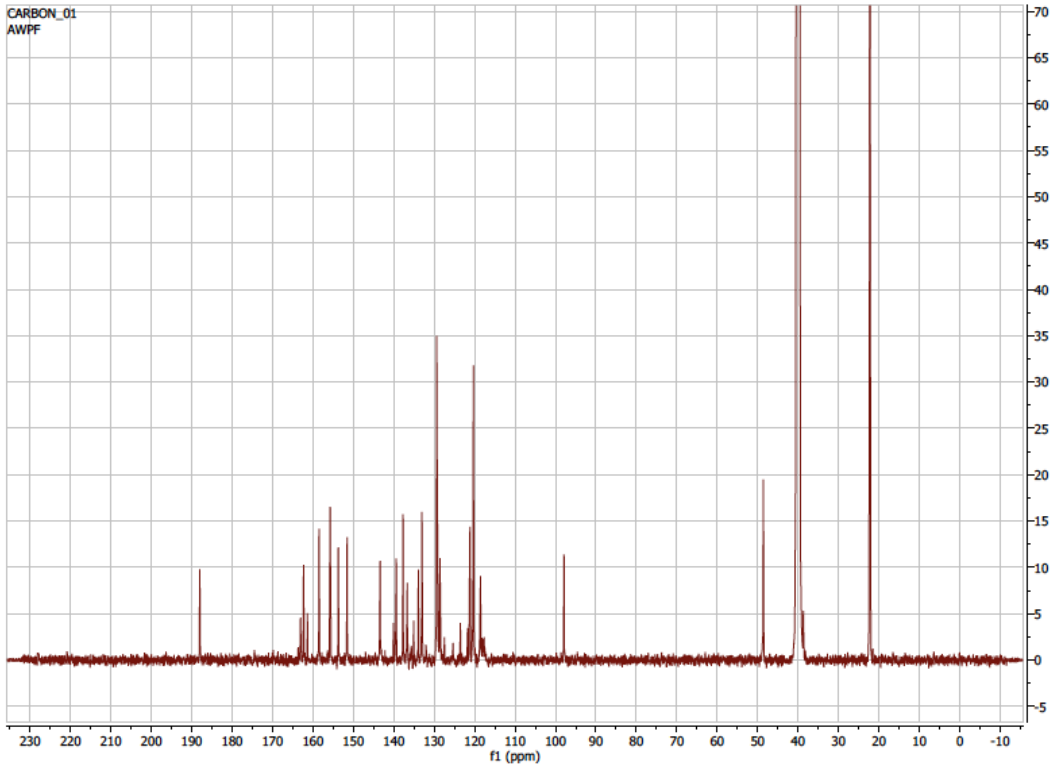
ASDO1 LCMS



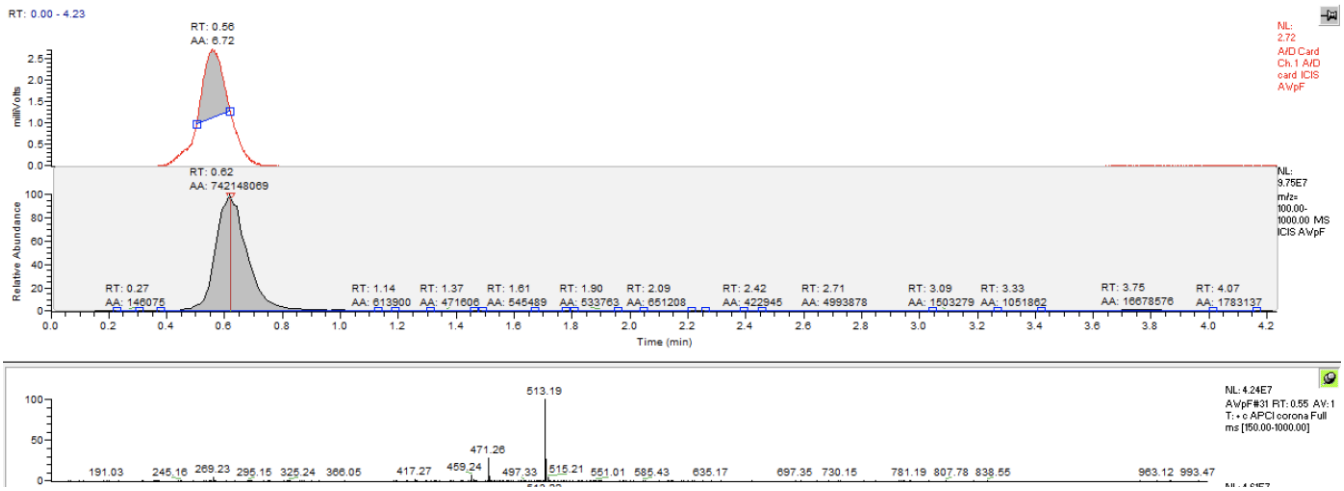
ASDO2 ^{1}H NMR (500 MHz, 1:1 Chloroform-*d*:DMSO-*d6*)



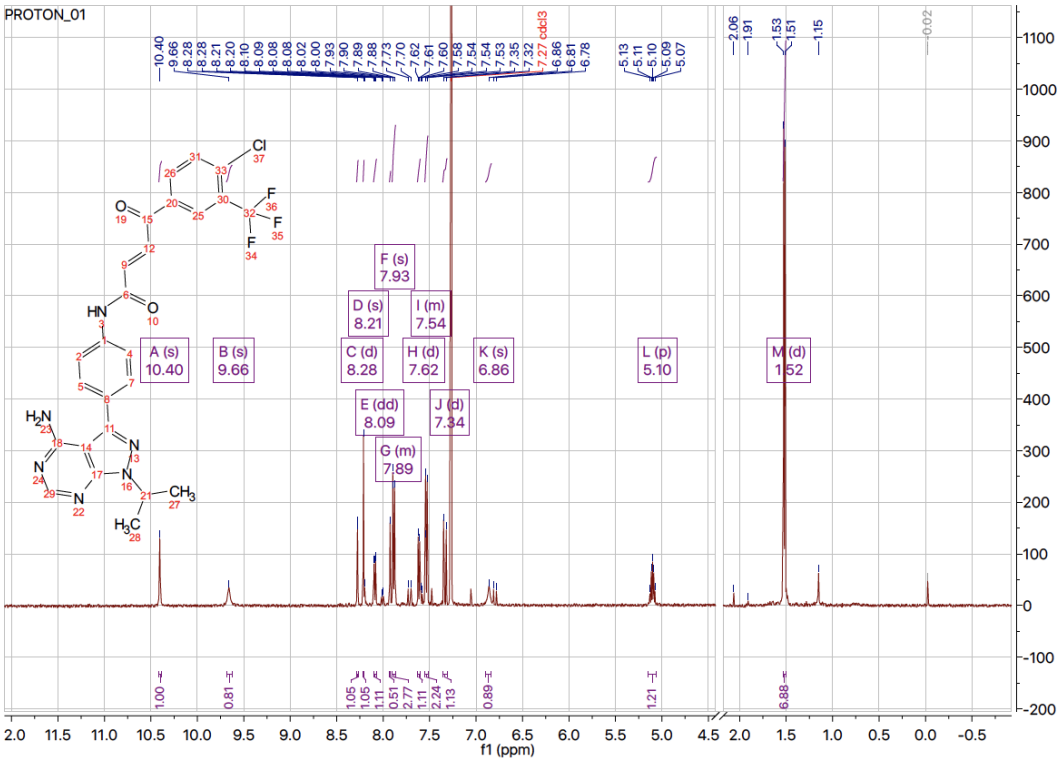
ASDO2 ^{13}C NMR (126 MHz, 1:1 $CDCl_3$:DMSO-*d6*): It should be noted that we did not detect C-F heteronuclear coupling due to CF_3 in the ^{13}C NMR mainly due to overlapping peaks.



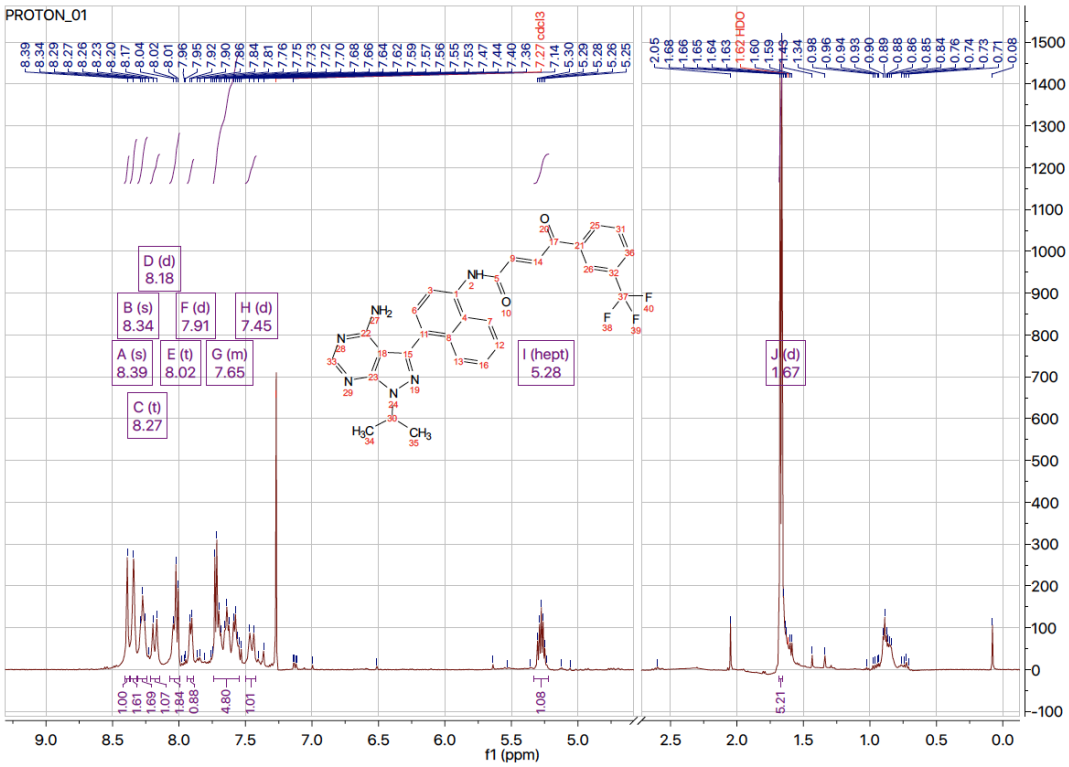
ASDO2 LCMS



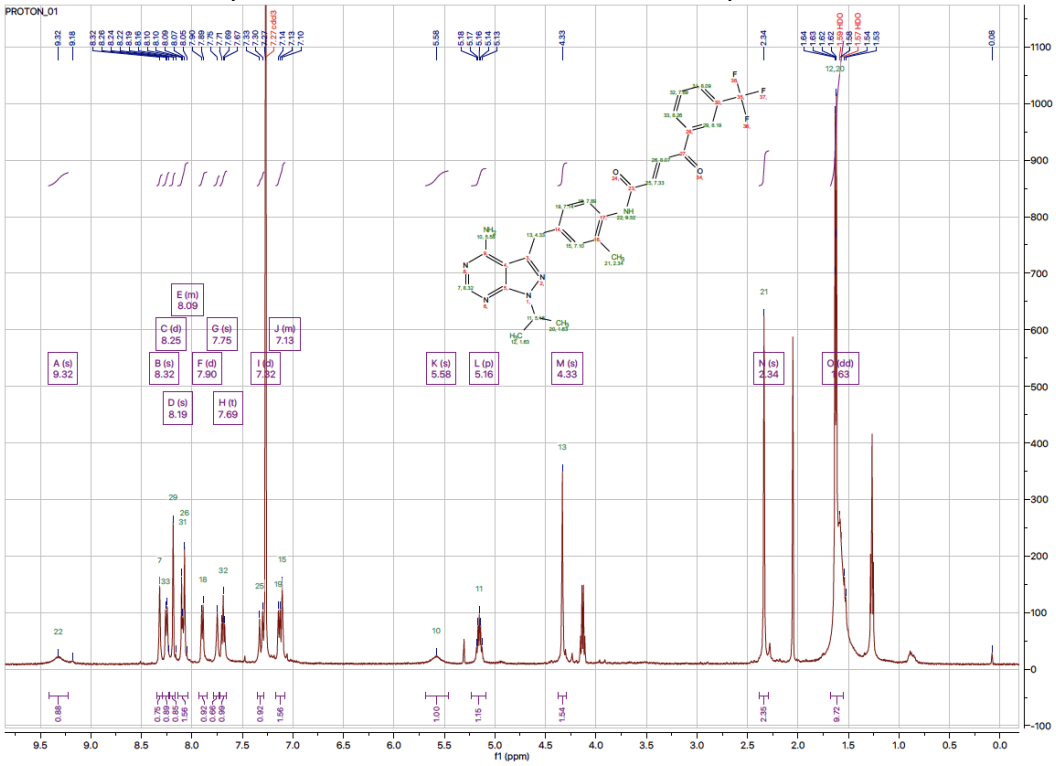
ASDO3 ^{1}H NMR (500 MHz, 1:1 Chloroform-*d*:DMSO-*d6*)



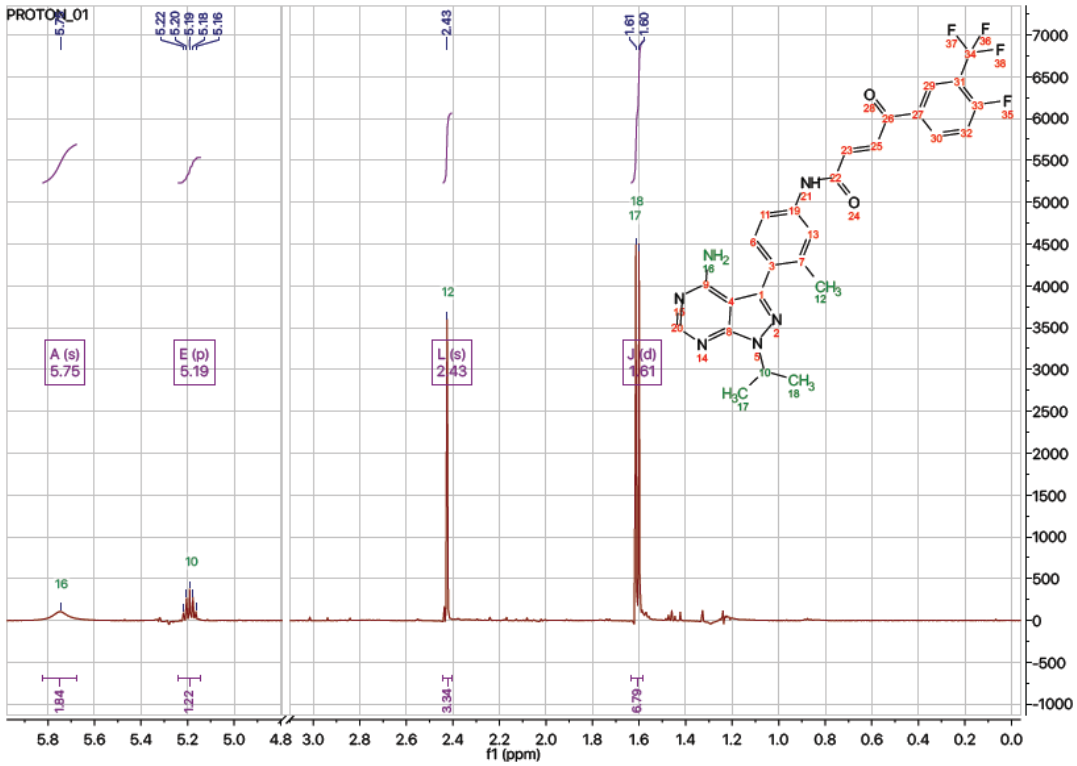
ASDO4 ^{1}H NMR (500 MHz, 1:1 Chloroform-*d*:DMSO-*d6*)

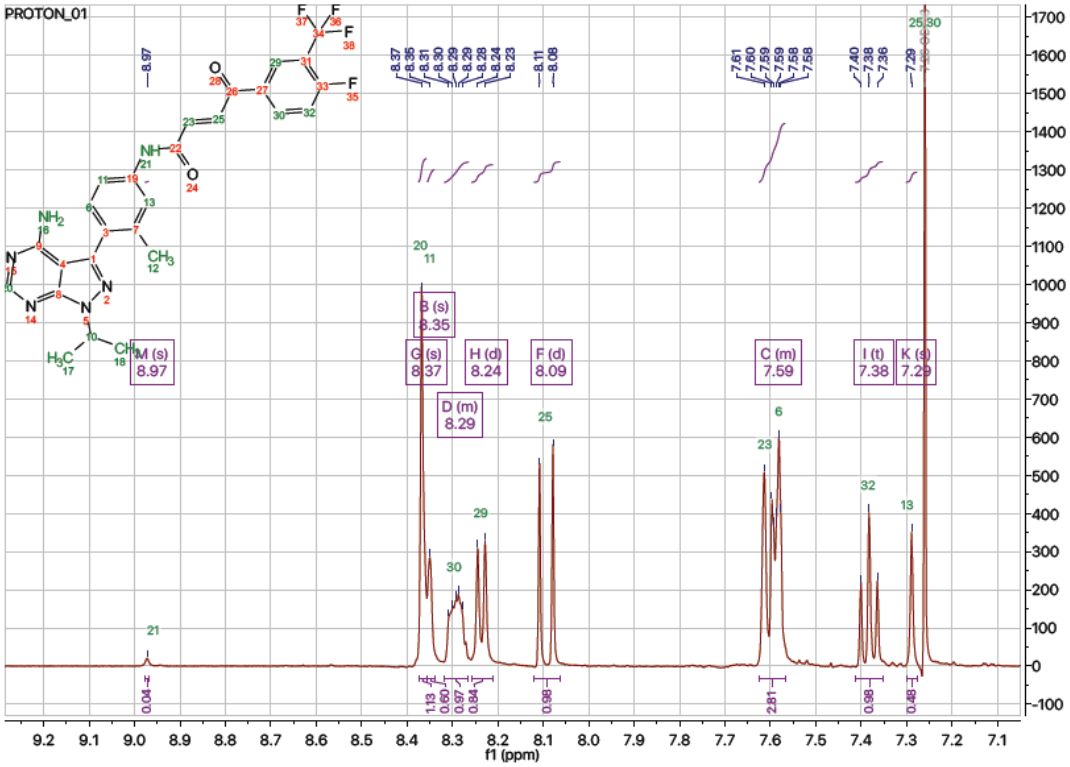


ASDO5 ^{1}H NMR (500 MHz, 1:1 Chloroform-*d*:DMSO-*d6*)

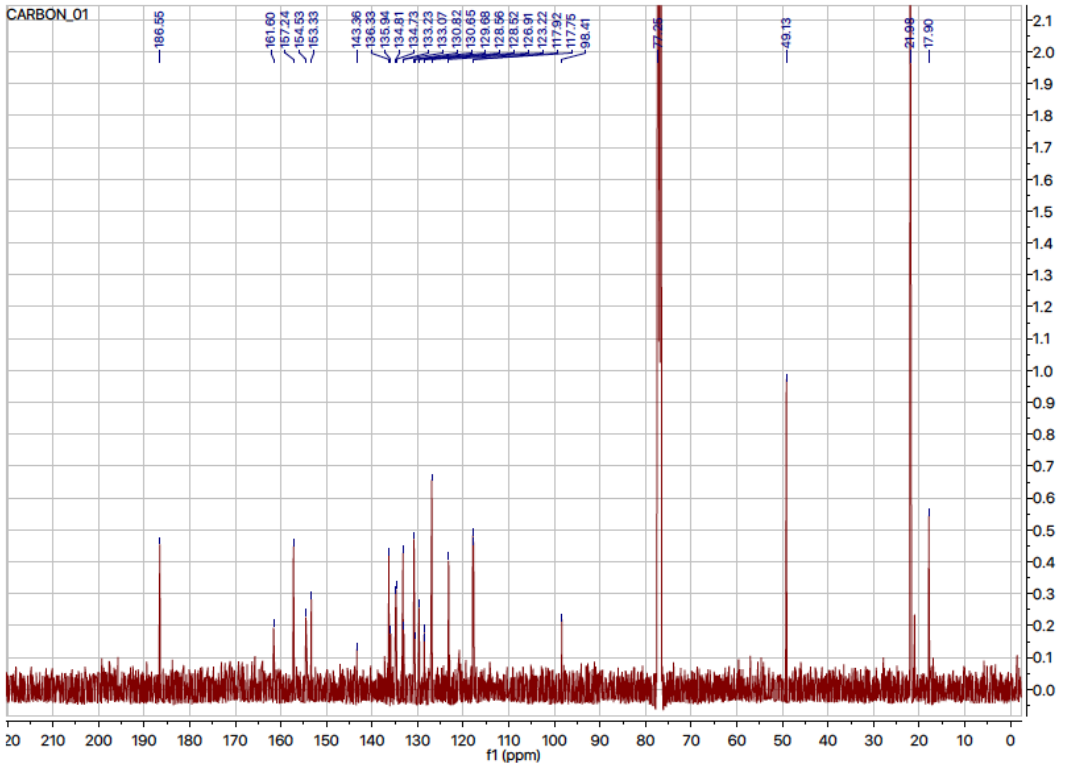


ASDO6 ^{1}H NMR (500 MHz, Chloroform-*d*)

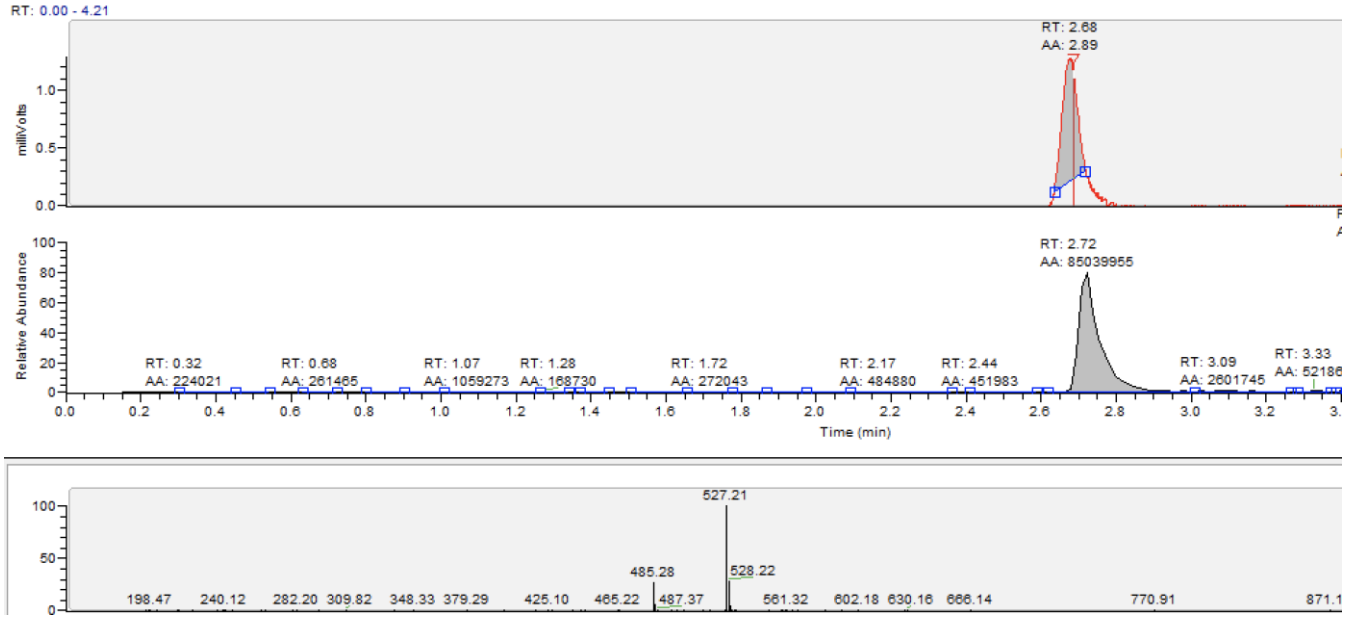




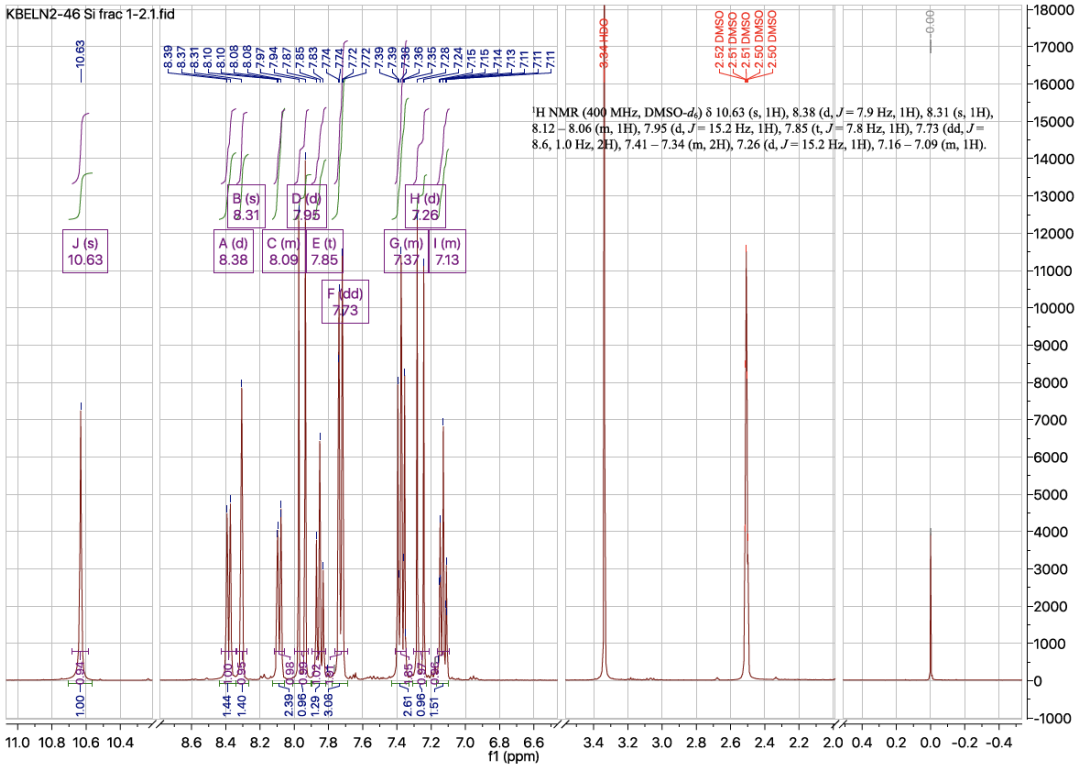
ASDO6 ^{13}C NMR (126 MHz, $CDCl_3$): It should be noted that we did not detect C-F heteronuclear coupling due to CF_3 in the ^{13}C NMR mainly due to overlapping peaks.



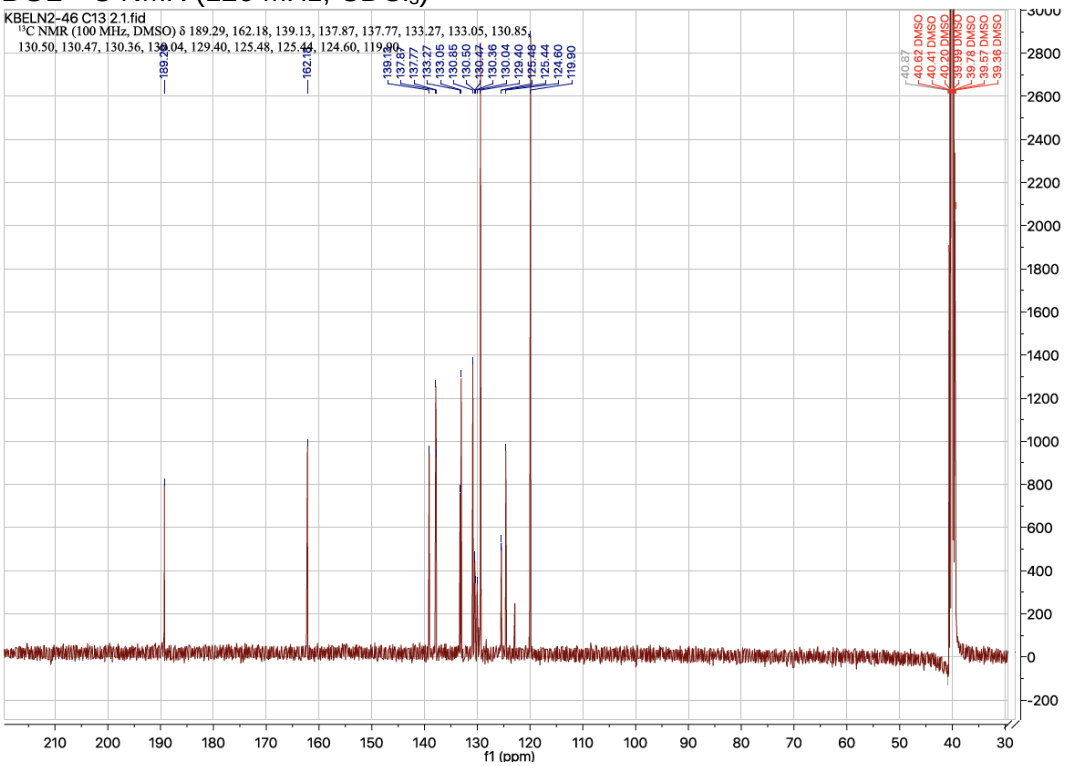
ASDO6 LCMS



DO1 ^{1}H NMR (500 MHz, Chloroform-*d*)



DO1 ^{13}C NMR (126 MHz, $CDCl_3$)



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