

Supplementary Information

Discovery of KRAS(G12D) selective degrader ASP3082

Tomohiro Yoshinari*, Takeyuki Nagashima, Hiroki Ishioka, Kohei Inamura, Yoshihiro Nishizono, Mamoru Tasaki, Kanako Iguchi, Atsushi Suzuki, Chikako Sato, Ayako Nakayama, Yasushi Amano, Yukihiro Tateishi, Yosuke Yamanaka, Fumio Osaki, Masayasu Yoshino, Kazuyuki Kuramoto, Tomoyoshi Imaizumi & Masahiko Hayakawa

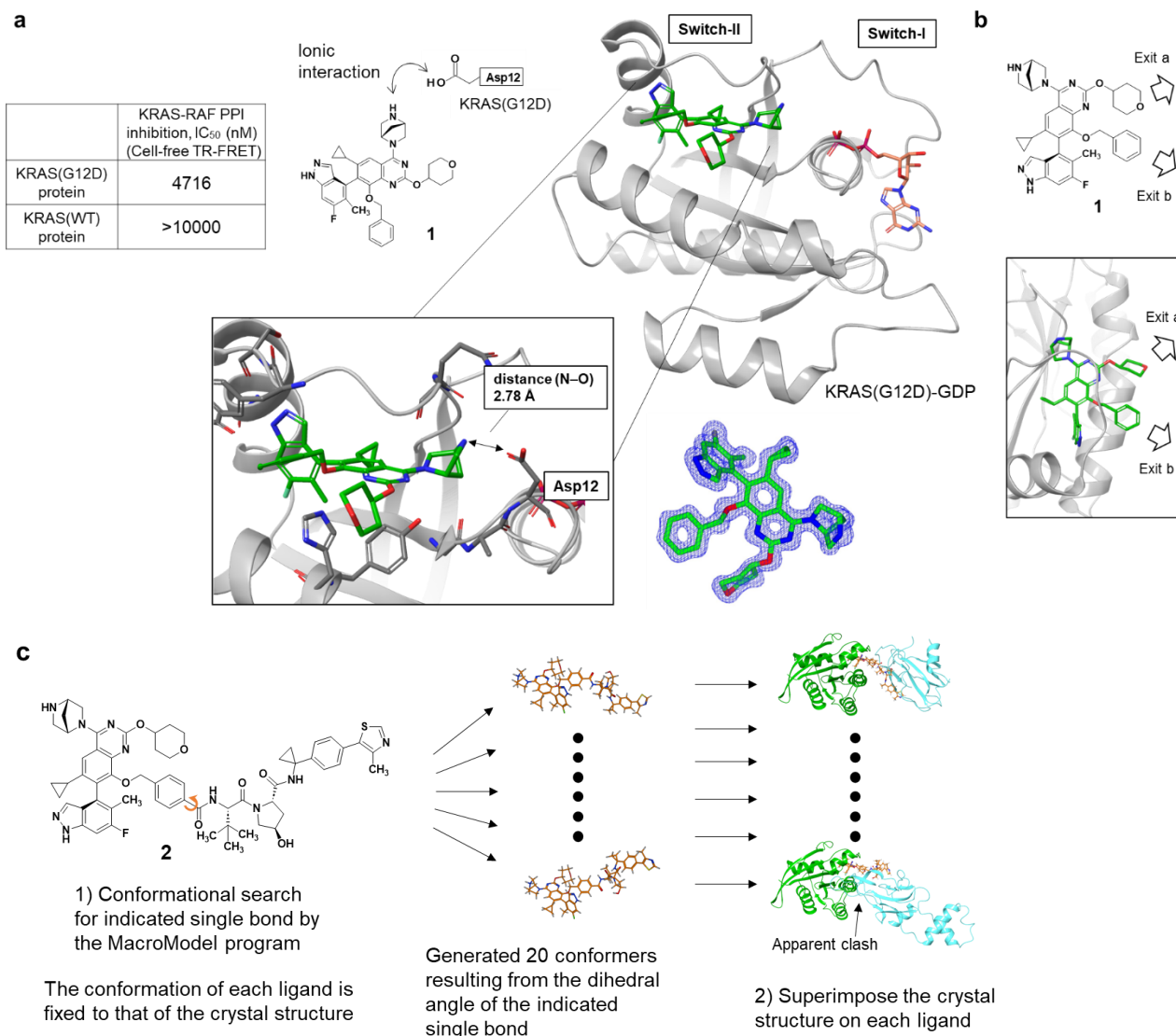
Astellas Pharma Inc.

Supplementary Figures 1 – 7

Supplementary Tables 1 – 7

Supplementary Note (Synthesis of compounds)

Single crystal X-ray crystallography (Supplementary Figures 8, 9)



Supplementary Figure 1. | Structural information of KRAS(G12D) binder.

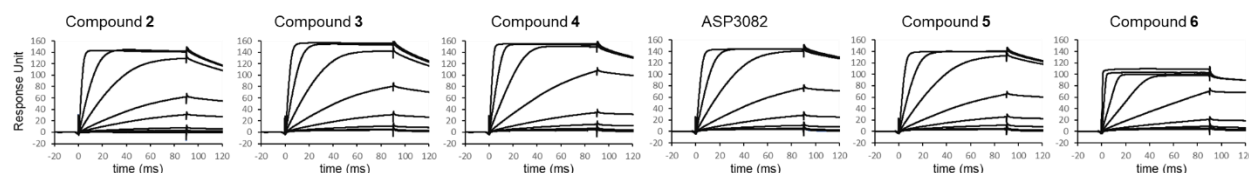
a, Chemical structure of KRAS(G12D) binder (compound **1**), and X-Ray crystal structure of compound **1** in complex with GDP-bound KRAS(G12D) (PDB: 9L6A). Inset depicts ligand binding site and the $2F_o - F_c$ map for the ligand **1** (1.5σ) in mesh. For X-ray crystallography data collection and refinement statistics, see Supplementary Table 7. IC₅₀ values of compound **1** on SOS-mediated KRAS-RAF interaction inhibition were determined in table. Data are presented as the geometric mean ($n = 1$, duplicate).

b, Information of linker attachment sites of compound **1**.

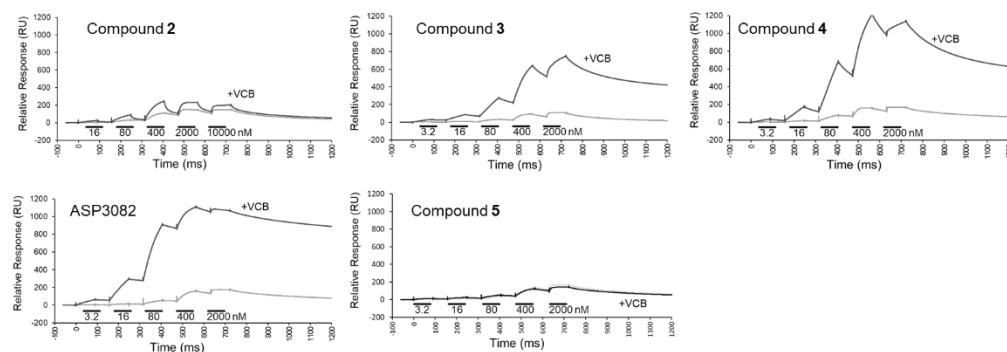
c, Illustration of our modeling method for construction of the ternary complex model based on conformation of degrader.

GDP-bound KRAS(G12D) immobilized SPR Binary affinity (multicycle mode, kinetic analysis)					Binary affinity (single-cycle mode, kinetic analysis)					Ternary affinity with VCB (single-cycle mode, kinetic analysis)				
	Geometric Mean					Geometric Mean					Geometric Mean			
	K_D (nM)	R_{max} (RU)	k_a ($M^{-1} s^{-1}$)	k_d (s^{-1})		K_D (nM)	R_{max} (RU)	k_a ($M^{-1} s^{-1}$)	k_d (s^{-1})		K_D (nM)	R_{max} (RU)	k_a ($M^{-1} s^{-1}$)	k_d (s^{-1})
Cmpd. 2	183	82	3.8×10^4	6.9×10^{-3}	Cmpd. 2	71	148	2.2×10^5	1.6×10^{-2}	Cmpd. 2	40	214	3.3×10^8	13
Cmpd. 3	158	87	5.2×10^4	8.2×10^{-3}	Cmpd. 3	56	134	1.7×10^5	9.4×10^{-3}	Cmpd. 3	14	880	3.8×10^6	5.4×10^{-2}
Cmpd. 4	42	86	1.5×10^5	6.3×10^{-3}	Cmpd. 4	17	115	4.7×10^5	7.9×10^{-3}	Cmpd. 4	5.6	763	2.1×10^6	1.2×10^{-2}
ASP3082	56	88	6.0×10^4	3.4×10^{-3}	ASP3082	20	150	1.8×10^5	3.4×10^{-3}	ASP3082	0.95	952	4.6×10^5	4.4×10^{-4}
Cmpd. 5	85	89	5.5×10^4	4.7×10^{-3}	Cmpd. 5	36	148	1.2×10^5	4.2×10^{-3}	Cmpd. 5	26	127	1.3×10^5	3.1×10^{-3}
Cmpd. 6	1.1	59	3.6×10^6	3.9×10^{-3}										

Sensorgrams of multicycle mode



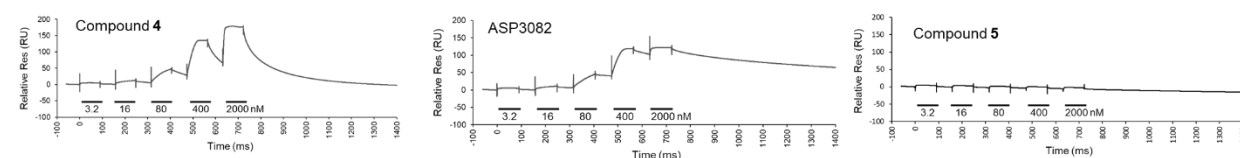
Sensorgrams of single-cycle mode



b

VCB-immobilized SPR
Binary affinity (single-cycle mode, kinetic analysis)

	Geometric Mean			
	K_D (nM)	R_{max} (RU)	k_a ($M^{-1} s^{-1}$)	k_d (s^{-1})
Cmpd. 2	1446	170	3.1×10^4	4.5×10^{-2}
Cmpd. 3	714	169	7.8×10^4	5.5×10^{-2}
Cmpd. 4	113	169	1.4×10^5	1.6×10^{-2}
ASP3082	7.5	127	2.7×10^5	2.1×10^{-3}
Cmpd. 5	ND	ND	ND	ND

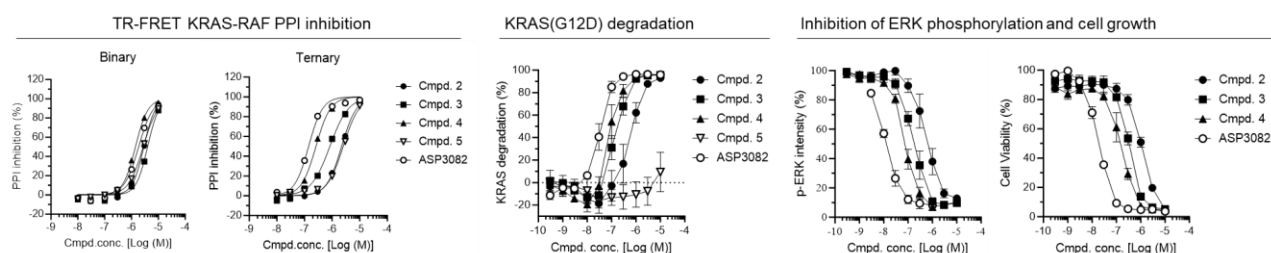
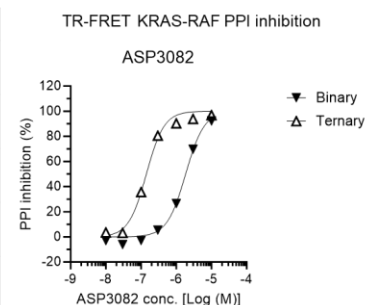


Supplementary Figure 2. | SPR assay data of KRAS(G12D) degraders.

a, Binary (without VCB) or ternary (with VCB) affinities to KRAS(G12D) for indicated compounds were determined using GDP-bound KRAS(G12D)-immobilized SPR assay in multicycle or single-cycle kinetic format. K_D values were calculated from fitted kinetic data ($K_D = k_d/k_a$). In the multicycle mode, it was conducted at 9 concentrations ranging from 1 nM to 10 μ M. Data are presented as the geometric mean (multicycle, $n = 5$; single-cycle, $n = 3$). Sensorgrams are representatives of three or five independent experiments.

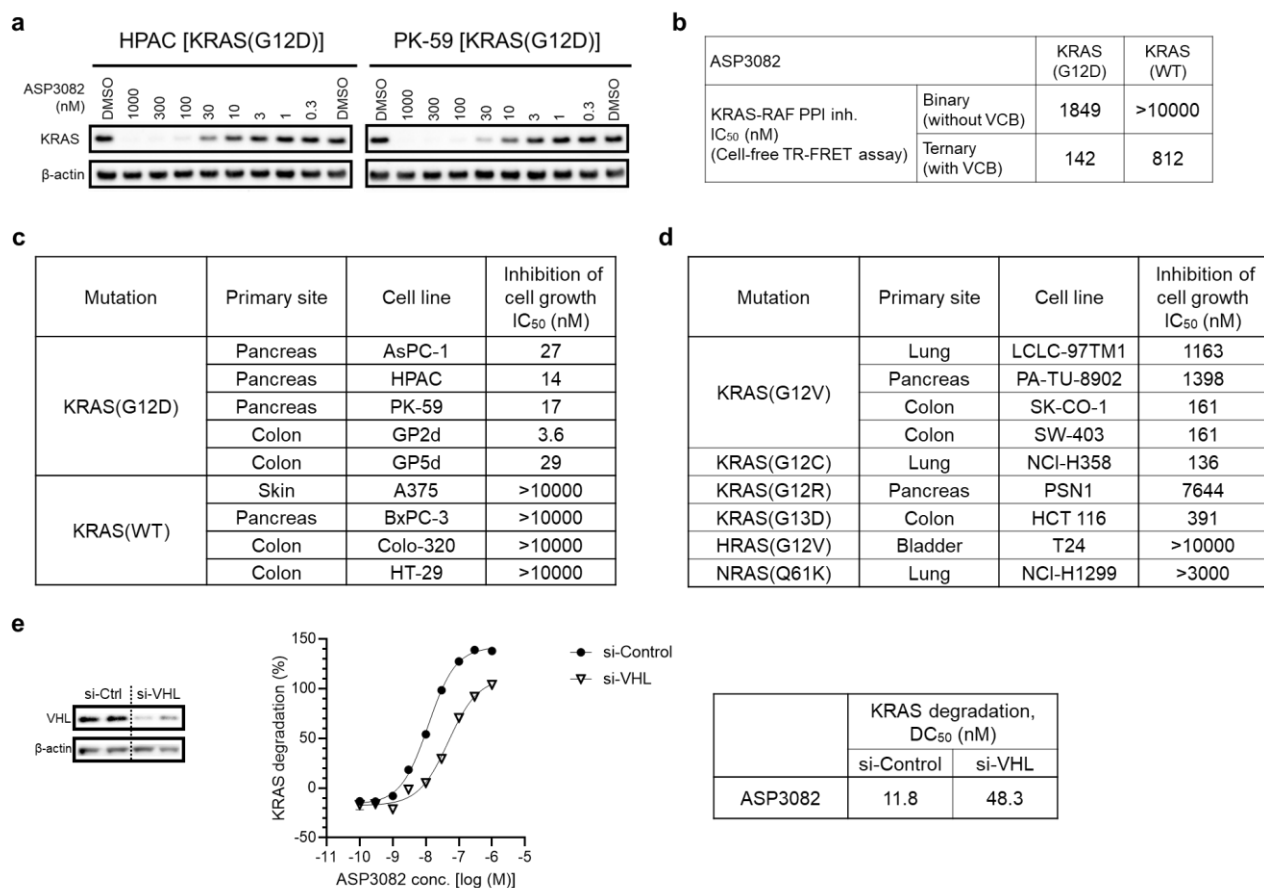
b, Binary affinities to VHL for indicated compounds were determined using VCB-immobilized SPR assay in single-cycle kinetic format. K_D values were calculated from fitted kinetic data ($K_D = k_d/k_a$). Data are presented as the geometric mean ($n = 3$). Sensorgrams are representatives of three independent experiments.

		Cmpd. 2	Cmpd. 3	Cmpd. 4	ASP3082	Cmpd. 5
KRAS-RAF PPI inhibition IC ₅₀ (nM) (Cell-free TR-FRET assay)	Binary	2880	3973	1401	1849	2726
	Ternary (with VCB)	2221	833	261	142	2530
KRAS(G12D) degradation, DC ₅₀ (nM) (In-Cell ELISA assay)		682	181	106	38	>10000
Inhibition of ERK phosphorylation, IC ₅₀ (nM) (In-Cell ELISA assay)		665	185	80	14	N.T.
Inhibition of cell growth, IC ₅₀ (nM) (Luminescent cell viability assay)		853	307	128	19	N.T.



Supplementary Figure 3. | Profiles of KRAS(G12D) degraders.

IC₅₀ values of KRAS(G12D)-RAF PPI inhibition for indicated compounds were determined using cell-free TR-FRET assay and presented in the table as the geometric mean (n = 1, duplicate). Each point in the graph represents the mean (n = 1, duplicate). DC₅₀ and IC₅₀ values of KRAS degradation and p-ERK inhibition were determined using In-Cell ELISA assays (24 h treatment), and IC₅₀ values of cell viability were determined using CellTiter Glo assay (6 days treatment) in AsPC-1 pancreatic cancer cells harboring KRAS(G12D) mutation. The values of cellular assays in the table are presented as the geometric mean (n = 3). Each point in the graph represents the mean ± SEM (n = 3).



Supplementary Figure 4. | Pharmacological profiles and mechanistic study of selective KRAS(G12D) degrader, ASP3082.

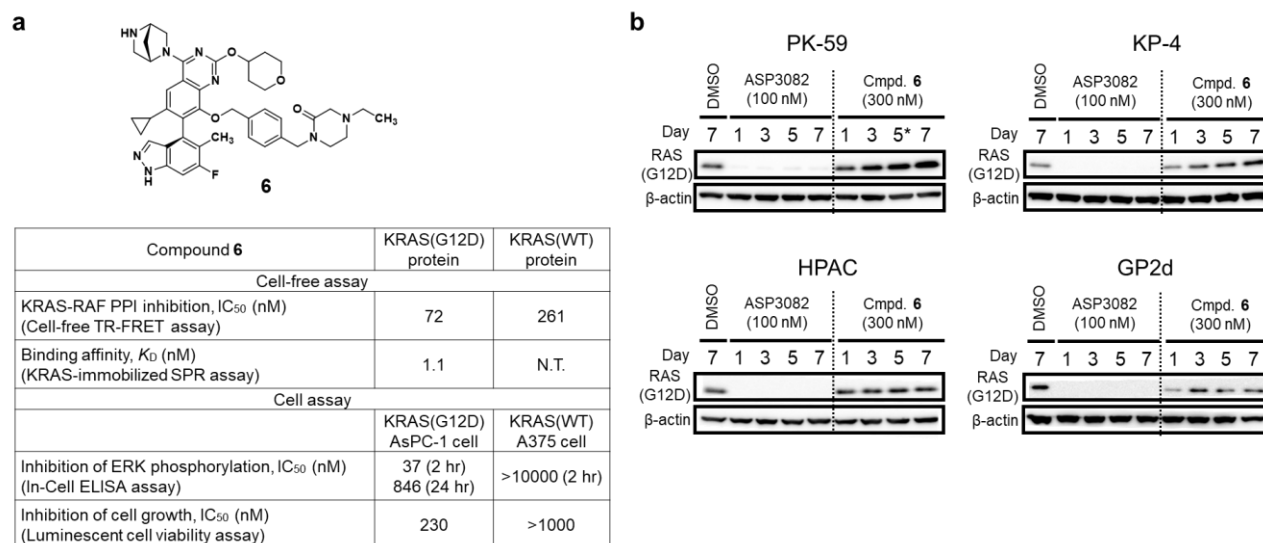
a, Immunoblot analysis of cancer cell lines treated with the indicated concentrations of ASP3082 or DMSO as a control for 24 h was performed.

b, IC₅₀ values of ASP3082 on SOS-mediated KRAS-RAF binary interactions or KRAS-RAF-VHL ternary complex inhibition were determined (n = 1, duplicate).

c, IC₅₀ values on cell viability of human cancer cells with KRAS(G12D) mutation and wild-type (WT) KRAS treated with ASP3082 for 6 days were determined (n = 1, quadruplicate).

d, IC₅₀ values on cell viability of human cancer cells with various RAS mutations treated with ASP3082 for 6 days were determined (n = 1, quadruplicate).

e, VHL-mediated degradation by ASP3082. (Left) Immunoblot analysis of PK-59 cells treated with control or VHL siRNA for 2 days. (Middle, Right) In-Cell ELISA for KRAS degradation of PK-59 cells treated with control or VHL siRNA for 3 days and then treated with ASP3082 for 24 h (PK-59 cells were treated with KRAS siRNA as positive control (100% degradation)).

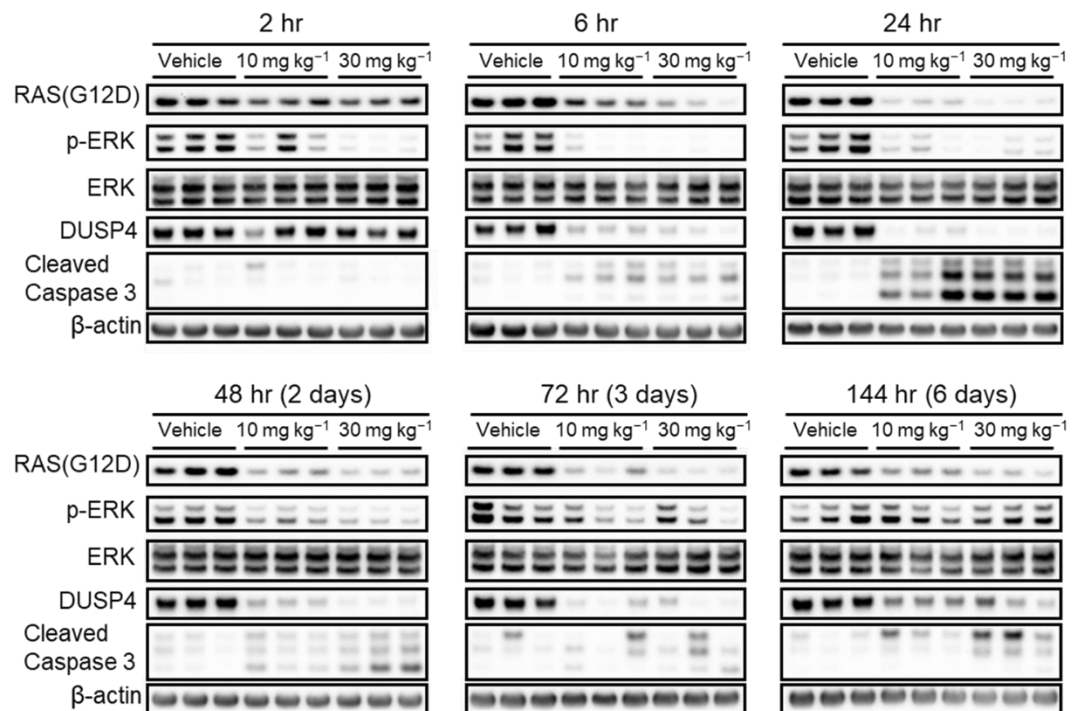


Supplementary Figure 5. | Profiles of KRAS(G12D) inhibitor 6 and mechanistic differences between KRAS degrader ASP3082 and inhibitor 6.

a, Selectivity profile of compound **6**. IC₅₀ values for compound **6** were determined using cell-free TR-FRET assay for inhibitory activity of KRAS-RAF interaction, SPR assay for binding affinity to KRAS(G12D), In-Cell ELISA assay for inhibitory activity of ERK phosphorylation (2 or 24 h treatment) or cell viability assay (6 days treatment) in AsPC-1 pancreatic cancer cells harboring the KRAS(G12D) mutation or A375 skin cancer cells harboring KRAS(WT). TR-FRET (n = 1), ERK phosphorylation (n = 2 for AsPC-1 (24 h) and n = 1 for AsPC-1 (2 h) and A375) and cell viability (n = 2 for AsPC-1 (24 h) and n = 1 for AsPC-1 (2 h) and A375) assay data are presented. Binding experiments were performed by KRAS(G12D)-immobilized SPR assay in multicycle kinetic format. K_D values were calculated from fitted kinetic data ($K_D = k_d/k_a$). Data are presented as the geometric mean (n = 5). Full details are shown in Supplementary Fig. 2a.

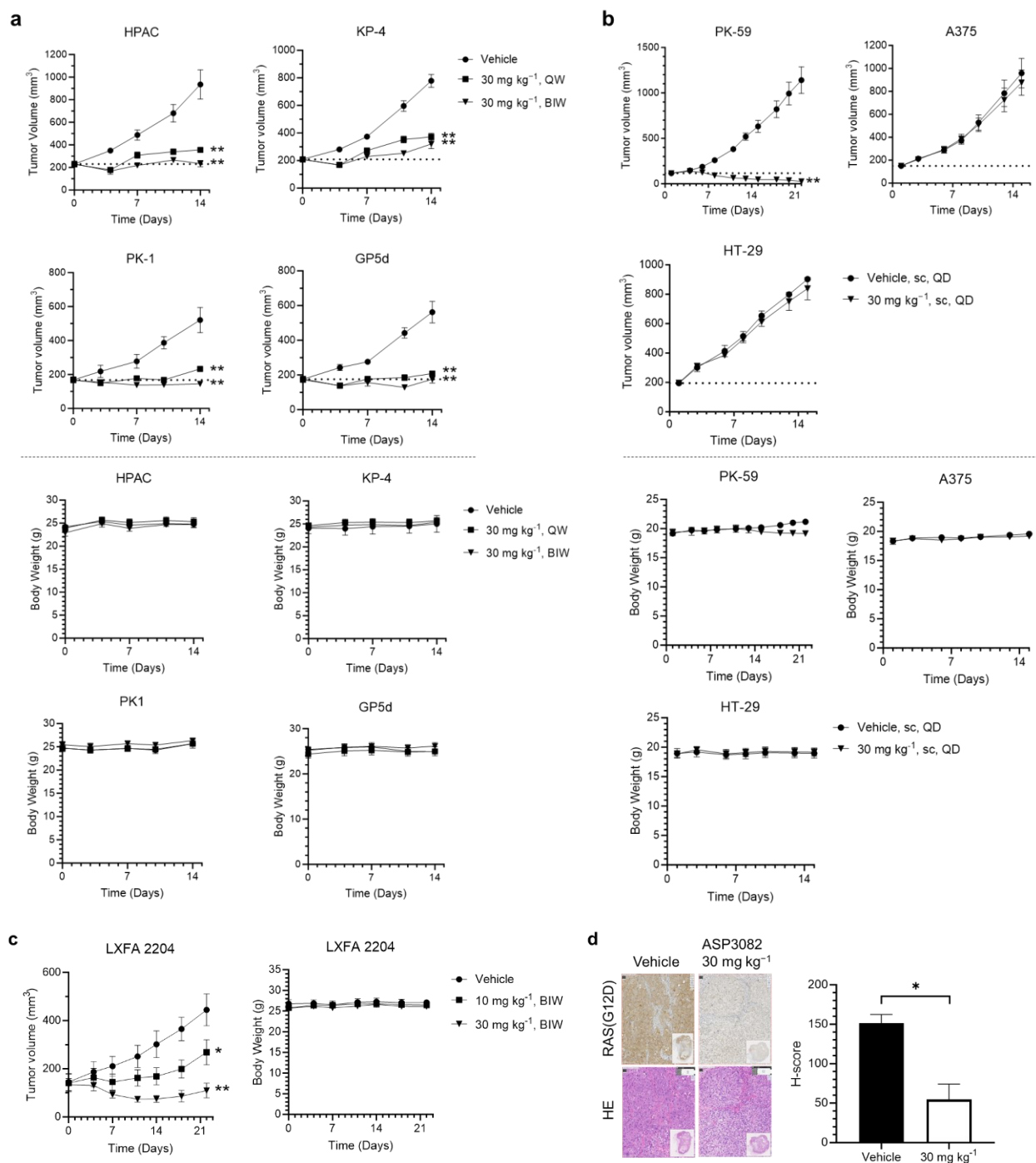
b, Effects of KRAS(G12D) degrader or inhibitor on total KRAS protein expression. Immunoblot analysis of cancer cell lines treated with ASP3082 or compound **6** for the indicated days was performed. Compounds were added only on day 0 and maintained in the cell cultures. *Due to a technical error, the sample treated with compound **6** in PK-59 cells for 5 days had approximately 1.2 times more sample volume than the other samples.

N.T., not tested.



Supplementary Figure 6. | In vivo pharmacodynamics of selective KRAS(G12D) degrader ASP3082.

Immunoblot analysis of PK-59 xenograft tumors treated with ASP3082 or the vehicle for the indicated hours was performed (n = 3 per each time point).



Supplementary Figure 7. | Anti-tumor activity of selective KRAS(G12D) degrader ASP3082.

a, Anti-tumor activity of ASP3082 in CDX (HPAC, KP-4, PK-1 and GP5d) models. Mice were treated intravenously with the vehicle or ASP3082 (30 mg kg^{-1}) on the following schedule. In the ASP3082 once-weekly group, ASP3082 was administered on days 1 and 8, and the vehicle was administered on days 5 (or 4 for PK-1) and 12 (or 11 for PK-1). In the ASP3082 twice-weekly or vehicle group, ASP3082 or the vehicle was administered on days 1, 5 (or 4 for PK-1), 8 and 12 (or 11 for PK-1),

respectively. Tumor sizes and body weights were measured twice weekly until day 14. Dotted line represents the mean tumor volume of all groups at day 0. Statistical analysis of tumor sizes was performed for the values on day 14. $**P < 0.01$ compared with the value of the vehicle group on day 14 (Dunnett's multiple comparisons test). Each point represents the mean $\pm$ SEM (n = 5).

b, Anti-tumor activity of ASP3082 in CDX (PK-59 (G12D), A375 and HT-29(WT)) models. Mice were treated subcutaneously with the vehicle or ASP3082 once daily, and tumor sizes and body weights were measured three times a week. Dotted line represents the mean tumor volume of all groups at day 1. Statistical analysis of tumor sizes was performed for the values on day 22 (PK-59). $**P < 0.01$ compared with the value of the vehicle group on day 22 (Unpaired *t*-test). Each point represents the mean $\pm$ SEM (n = 5).

c, Anti-tumor activity of ASP3082 in a NSCLC PDX (LXFA2204) model. Mice were treated intravenously with the vehicle or ASP3082 on days 1, 4, 8, 11, 15 and 18. Tumor sizes and body weights were measured twice weekly until day 22. Statistical analysis of tumor sizes was performed for the values on day 22. $*P < 0.05$, $**P < 0.01$ compared with the value of the vehicle treated group on day 22 (Dunnett's multiple comparisons test). Each point represents the mean $\pm$ SEM (n = 8).

d, Immunohistochemical staining with anti-RAS(G12D) antibody or HE in the tumors harvested from the vehicle or ASP3082-treated mice (LXFA 2204 NSCLC PDX model) 24 h after the last dose of ASP3082. (Left) Representative immunohistochemistry images of tumor sections from each treatment group. (Right) H-score of each treatment group. Statistical analysis was performed for the values. $*P < 0.05$ compared with the value of the vehicle treated group (Mann–Whitney test). Each bar represents the mean value and SEM (vehicle group, n = 7; ASP3082-treated group, n = 8). The H-score is calculated as follows: (1 $\times$ percentage of weak staining) + (2 $\times$ percentage of moderate staining) + (3 $\times$ percentage of strong staining) within the target region, ranging from 0 to 300.

QW, Once-weekly; BIW, Twice-weekly; QD, Once daily.

Supplementary Tables.

Supplementary Table 1. Cell lines and culture media.

Cell Line Table							
Name	Tumor Type	KRAS Status	Oncogenic Alterations (excluding KRAS)	RRID	Vendor	Catalogue Number	Medium
AsPC-1	Pancreas	G12D	SMAD4 (R100T)	CVCL_0152	ATCC	CRL-1682	RPMI-1640
HPAC	Pancreas	G12D	TP53 (G187R)	CVCL_3517	ATCC	CRL-2119	RPMI-1640
KP-4	Pancreas	G12D	-	CVCL_1338	JCRB	JCRB0182	IMDM
PK-1	Pancreas	G12D	TP53 (M237I)	CVCL_4717	RIKEN BRC	RCB1972	RPMI-1640
PK-59	Pancreas	G12D	RAC1 (P29S)	CVCL_4897	RIKEN BRC	RCB1901	RPMI-1640
PSN1	Pancreas	G12R	TP53 (K132Q)	CVCL_1644	ECACC	94060601	RPMI-1640 (with L-glutamine)
PA-TU-8902	Pancreas	G12V	TP53 (C176S), SPOP (M117I)	CVCL_1845	DSMZ	ACC 179	DMEM
BxPC-3	Pancreas	WT	TP53 (Y220C)	CVCL_0186	ATCC	CRL-1687	RPMI-1640
GP2d	Colon	G12D	RHOA (Y34C), PIK3CA(H1047L)	CVCL_2450	ECACC	95090714	DMEM (with sodium pyruvate)
GP5d	Colon	G12D	RHOA (Y34C), PIK3CA(H1047L)	CVCL_1235	ECACC	95090715	DMEM (with sodium pyruvate)
SK-CO-1	Colon	G12V	-	CVCL_0626	ATCC	HTB-39	EMEM
SW-403	Colon	G12V	PIK3CA (Q546K)	CVCL_0545	DSMZ	ACC 294	Leibovitz's L-15
HCT 116	Colon	G13D	CTNNB1 (S45del), PIK3CA (H1047R)	CVCL_0291	ATCC	CCL-247	McCoy's 5A
HT-29	Colon	WT	TP53 (R273H), PIK3CA (P449T), BRAF (V600E)	CVCL_0320	ATCC	HTB-38	McCoy's 5A
COLO-320	Colon	WT	TP53 (R248W)	CVCL_1989	RIKEN BRC	RCB1193	RPMI-1640
NCI-H358	Lung	G12C	-	CVCL_1559	ATCC	CRL-5807	RPMI-1640
LCLC-97TM1	Lung	G12V	-	CVCL_1376	DSMZ	ACC 388	RPMI-1640
NCI-H1299	Lung	WT	NRAS (Q61K)	CVCL_0060	ATCC	CRL-5803	RPMI-1640
A375	Skin	WT	BRAF (V600E)	CVCL_0132	ATCC	CRL-1619	DMEM (with sodium pyruvate)
T24	Bladder	WT	HRAS (G12V)	CVCL_0554	JCRB	JCRB0711	McCoy's 5A

Supplementary Table 2. Results of proteomic analysis.

See Excel file (Supplementary Data 1).

Supplementary Table 3. Antibodies.

Antibody Table						
Type	Antibodies and Conjugations	Clone	RRID	Host	Manufacturer	Catalogue Number
Primary	anti-Akt		AB_329827	Rabbit	Cell Signaling Technology	9272
Primary	anti-cleaved Caspase-3 (Asp175)	5A1E	AB_2070042	Rabbit	Cell Signaling Technology	9664
Primary	anti-DUSP4	D9A5	AB_2750867	Rabbit	Cell Signaling Technology	5149
Primary	anti-p44/42 MAPK (Erk1/2)		AB_330744	Rabbit	Cell Signaling Technology	9102
Primary	anti-GST-Tag	91G1	AB_490796	Rabbit	Cell Signaling Technology	2625
Primary	anti-KRAS	F234	AB_627865	Mouse	Santa Cruz	sc-30
Primary	anti-phospho-Akt (Ser473)	D9E	AB_2315049	Rabbit	Cell Signaling Technology	4060
Primary	anti-phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204)	D13.14.4E	AB_2315112	Rabbit	Cell Signaling Technology	4370
Primary	anti-phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204)	197G2	AB_331775	Rabbit	Cell Signaling Technology	4377
Primary	anti-phospho-S6 Ribosomal Protein (Ser235/236)	D57.2.2E	AB_916156	Rabbit	Cell Signaling Technology	4858
Primary	anti-RAS (G12D Mutant)	D8H7	AB_2728748	Rabbit	Cell Signaling Technology	14429
Primary	anti-S6 Ribosomal Protein	54D2	AB_2238583	Mouse	Cell Signaling Technology	2317
Primary	anti-VHL		AB_2716279	Rabbit	Cell Signaling Technology	68547
Primary	anti- β actin		AB_2305186	Rabbit	Abcam	ab8227
Primary	anti- β actin	mAbcam 8226	AB_306371	Mouse	Abcam	ab8226
Primary	anti- β actin	AC-15	AB_476692	Mouse	SIGMA-ALDRICH	A1978
Primary	anti- β actin	8H10D10	AB_2242334	Mouse	Cell Signaling Technology	3700
Secondary	anti-mouse IgG, horseradish peroxidase (HRP)-linked		AB_772210	Sheep	Cytiva	NA931-1ML
Secondary	anti-mouse IgG, horseradish peroxidase (HRP)-linked		AB_330924	Horse	Cell Signaling Technology	7076
Secondary	anti-rabbit IgG, horseradish peroxidase (HRP)-linked		AB_2099233	Goat	Cell Signaling Technology	7074
Secondary	anti-rabbit IgG, horseradish peroxidase (HRP)-linked		AB_772191	Donkey	Cytiva	NA9340-1ML
Secondary	anti-mouse IgG, IRDye [®] 680RD-conjugated		AB_10953628	Donkey	LI-COR	926-68072
Secondary	anti-rabbit IgG, IRDye [®] 800CW-conjugated		AB_621843	Goat	LI-COR	926-32211

Supplementary Table 4. siRNAs for VHL-knockdown experiment.

siRNA Table			
siRNA	Product Name	Manufacturer	Catalogue Number
Anti-human VHL	ON-TARGETplus Human VHL	Dharmacon	LQ-003936-00-0005
Anti-human KRAS	ON-TARGETplus Human KRAS	Dharmacon	J-005069-08
Control	ON-TARGETplus Non-targeting Pool	Dharmacon	D-001810-10-05

Supplementary Table 5. LC-MS/MS conditions for pharmacokinetic assay.

LC-MS/MS conditions							
Reagents	Test Sample	Calibration Standard					
Plasma sample	5 µL	-					
Blank plasma	-	5 µL					
50% Acetonitrile	5 µL	-					
Test substance solution for calibration curve	-	5 µL					
IS solution	50 µL	50 µL					
Reagents	Test Sample	Calibration Standard					
Tumor or pancreas homogenate sample	20 µL	-					
Blank homogenate of tumor or pancreas	-	20 µL					
50% Acetonitrile	20 µL	-					
Test substance solution for calibration curve	-	20 µL					
IS solution	200 µL	200 µL					
Analytical column:	TSKgel ODS-120H, 2.0 mm ID × 30 mm, 1.9 µm						
Mobile phase (A):	20 mmol/L HCOONH ₄						
Mobile phase (B):	Acetonitrile						
Gradient program:	Time	0	0.5	0.85	0.86	1.2 [min]	
	(A)	80	15	15	80	80 [%]	
	(B)	20	85	85	20	20 [%]	
Flow rate:	0.85 mL/min						
Autosampler temperature:	10 °C						
Column temperature:	50 °C						
Injection volume:	0.5 µL						
Mass spectrometer:	QTRAP5500 (AB SCIEX)						
Ionization method:	ESI						
Polarity:	Positive						
Monitoring ion:		Precursor ion	Product ion				
	Test substance (ASP3082)	559.39	218.06 [m/z]				
	IS (methyltestosterone)	303.20	97.20 [m/z]				
Calibration range:	3-10000 ng/mL (plasma)						
	1-1000 ng/mL (plasma, re-analysis)						
	1.5-5000 ng/g (tumor)						
	1.5-5000 ng/g (pancreas)						

Supplementary Table 6. Information of primers and probes for qPCR.

TaqMan [®] Assays (primers and probes) for qPCR		
Gene Name	Gene Symbol	Assay ID
beta-2-microglobulin	B2M	Hs99999907_ml
ETS variant 4	ETV4	Hs00385910_ml
dual specificity phosphatase 4	DUSP4	Hs00154826_ml
dual specificity phosphatase 6	DUSP6	Hs01044001_ml
sprouty RTK signaling antagonist 4	SPRY4	Hs00540086_ml
v-myc avian myelocytomatosis viral oncogene homolog	MYC	Hs00153408_ml
FOS like 1, AP-1 transcription factor subunit	FOSL1	Hs04187685_ml

Supplementary Table 7. Data collection and refinement statistics

	KRasG12D(GDP)/compound 1	KRasG12D(GDP)/VCB/ASP3082
Data collection		
Space group	$H3_2$	$P2_1$
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (Å)	67.94, 67.94, 200.10	144.35, 58.91, 165.98
α , β , γ (°)	90, 90, 120	90, 104.3, 90
Resolution (Å)	38.11-1.36 (1.38-1.36)	58.9-3.18 (3.29-3.18)
<i>R</i> _{sym} or <i>R</i> _{merge}	0.073 (0.967)	0.045 (0.272)
<i>I</i> / σI	20.4 (3.0)	2.4 (1.9)
Completeness (%)	99.9 (99.9)	100.0 (100.0)
Redundancy	9.3 (9.2)	1.9 (1.9)
Refinement		
Resolution (Å)	38.11-1.36	48.76-3.18
No. reflections	36710	43994
<i>R</i> _{work} / <i>R</i> _{free}	0.223 / 0.245	0.241 / 0.305
No. atoms		
Protein	1339	16093
Ligand/ion	75	440
Water	128	
<i>B</i> -factors		
Protein	17.9	142.7
Ligand/ion	13.0	128.0
Water	24.3	
R.m.s. deviations		
Bond lengths (Å)	0.011	0.003
Bond angles (°)	2.038	1.103

*Values in parentheses are for highest-resolution shell.

Supplementary Note

SYNTHESIS OF COMPOUNDS

General Experiment

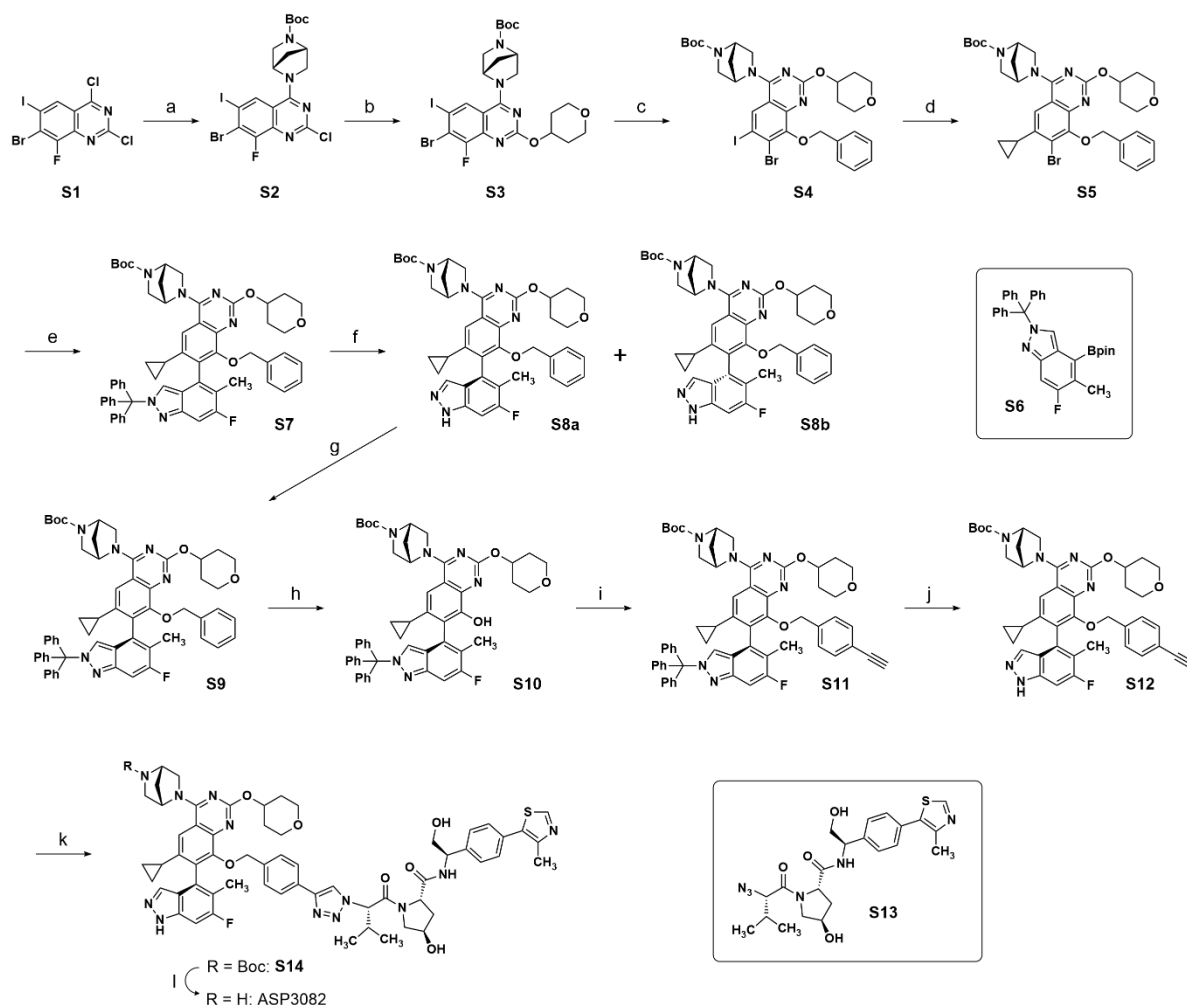
^1H NMR and ^{13}C NMR spectra were recorded on an Agilent (Varian) VNS 400 or a Bruker AVANCE III-HD500 spectrometer. Chemical shifts were expressed in δ values (ppm) and referenced to tetramethylsilane or the residual solvent signals. Signal splitting patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br), or a combination thereof. Coupling constants (J) were measured in hertz (Hz). Low-resolution mass spectra (MS) were recorded on a Waters ACQUITY UPLC/SQD system or a Thermo Scientific Exactive Plus Orbitrap coupled with a Waters ACQUITY UPLC system. High-resolution mass spectra (HRMS) were recorded on a Thermo Scientific Exactive Plus Orbitrap using electrospray ionization (ESI) coupled with a Waters ACQUITY UPLC system. X-ray Diffraction data were collected at 93 K on a Rigaku XtaLAB P200 using multi-layer mirror monochromated $\text{CuK}\alpha$ radiation.

Unless otherwise noted, all reactions were performed using commercially available reagents and solvents without further purification. Preparative column chromatography was performed using a Silica gel 60N (Kanto Chemical), Presep (Fujifilm Wako Pure Chemical), Purif-Pack-EX (Shoko Science), Hi-Flash column (Yamazen) or Chromatorex (Fuji Silysia Chemical).

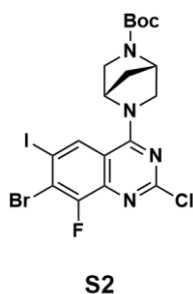
The following abbreviations are used: DABCO, 1,4-diazabicyclo[2.2.2]octane; DIPEA, *N,N*-diisopropylethylamine; DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide; DOX, 1,4-dioxane; Et_3N , triethylamine; LDA, lithium diisopropylamide; HATU, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate; MeCN, acetonitrile; MeOH, methanol; ODS, octadecylsilyl; $\text{PdCl}_2(\text{dppf})\cdot\text{CH}_2\text{Cl}_2$, [1,1'-bis(diphenylphosphino)ferrocene]palladium(II) dichloride dichloromethane adduct; Pd/C, palladium on carbon; *t*-BuOH, *tert*-butanol; *t*-BuOK, potassium *tert*-butoxide; $\text{Pd}(\text{OAc})_2$, palladium(II) acetate;

Ph₃P, triphenylphosphine; KOAc, potassium acetate; RuPhos, dicyclohexyl(2',6'-diisopropoxy-[1,1'-biphenyl]-2-yl)phosphine; RuPhos Pd G3, (2-dicyclohexylphosphino-2',6'-diisopropoxy-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II) methanesulfonate; MeI, methyl iodide; 2-MeTHF, 2-methyltetrahydrofuran; TBAF, tetrabutylammonium fluoride; and THF, tetrahydrofuran.

Synthesis of ASP3082



Reagents and conditions: a) *tert*-butyl (1*S*,4*S*)-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate, DIPEA, DOX, THF, 0 °C to rt; b) tetrahydro-2*H*-pyran-4-ol, Cs₂CO₃, DABCO, DMF, THF, rt; c) benzyl alcohol, *t*-BuOK, THF, 0 °C; d) cyclopropylboronic acid, PdCl₂(dppf)·CH₂Cl₂, K₃PO₄, MeCN, DOX, water, 100 °C; e) **S6**, RuPhos Pd G3, RuPhos, K₃PO₄, DOX, water, 80 °C; f) *p*-TsOH·H₂O, MeOH, 0 °C to rt; g) trityl chloride, Et₃N, CH₂Cl₂, 0 °C to rt; h) H₂, Pd/C, NaHCO₃, THF, MeOH, rt; i) 1-(chloromethyl)-4-ethynylbenzene, Cs₂CO₃, DMF, rt; j) *p*-TsOH·H₂O, MeOH, rt; k) **S13**, CuI, sodium ascorbate, *t*-BuOH, THF, water, rt; l) MsOH, MeOH, 50 °C.

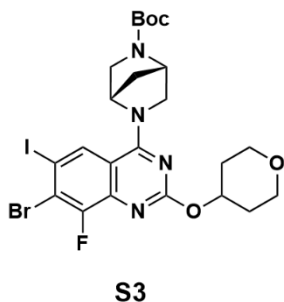


tert-Butyl (1*S*,4*S*)-5-(7-bromo-2-chloro-8-fluoro-6-iodoquinazolin-4-yl)-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate (**S2**):

A mixture of 7-bromo-2,4-dichloro-8-fluoro-6-iodoquinazoline (**S1**, 100 g, 237 mmol), DOX (1000 mL), DIPEA (240 mL, 1.40 mol) and THF (500 mL) was cooled with ice bath, and then *tert*-butyl (1*S*,4*S*)-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate (48 g, 242 mmol) was added. The mixture was stirred at room temperature overnight. Water was added to the reaction mixture, and the mixture was extracted with ethyl acetate. The organic layer was washed with an aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure until the total amount of the solution became about 400 mL. A mixed solvent (hexane/ethyl acetate = 4/1, 1000 mL) was added to the resulting solution, and the mixture was stirred at room temperature for 2 h. The precipitated solid was collected by filtration, washed with hexane/ethyl acetate (4/1, 600 mL), and dried overnight to give the title compound (123 g, 89% yield) as a solid.

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.41–8.32 (m, 1H), 5.26–5.14 (m, 1H), 4.62–4.51 (m, 1H), 4.40–4.17 (m, 1H), 3.92–3.72 (m, 1H), 3.54–3.35 (m, 2H), 2.04–1.90 (m, 2H), 1.44–1.31 (m, 9H);

MS (ESI) *m/z* = 583.0, 585.0, 587.1 [M+H]⁺.

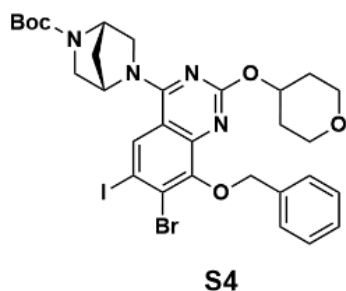


tert-Butyl (1*S*,4*S*)-5-{7-bromo-8-fluoro-6-iodo-2-[(oxan-4-yl)oxy]quinazolin-4-yl}-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate (**S3**):

To a mixture of **S2** (30 g, 51.4 mmol), tetrahydro-2*H*-pyran-4-ol (15 mL, 158 mmol), DABCO (1.15 g, 10.3 mmol), DMF (150 mL) and THF (100 mL) was added cesium carbonate (50.3 g, 154 mmol) at room temperature, and the mixture was stirred at room temperature overnight under argon atmosphere. About 1 kg of ice water was added to the reaction mixture, and the mixture was stirred at room temperature for 6 h. The resultant precipitate was collected by filtration, washed with water, and dried at 50 °C overnight under reduced pressure to give the title compound (32.8 g, 98% yield) as a solid.

¹H NMR (500 MHz, DMSO-*d*₆) δ 8.29–8.23 (m, 1H), 5.23–5.10 (m, 2H), 4.60–4.50 (m, 1H), 4.30–4.20 (m, 1H), 3.92–3.84 (m, 2H), 3.80–3.68 (m, 1H), 3.55–3.37 (m, 4H), 2.10–1.89 (m, 4H), 1.74–1.62 (m, 2H), 1.43–1.32 (m, 9H);

MS (ESI) *m/z* = 649.1, 651.0 [M+H]⁺.

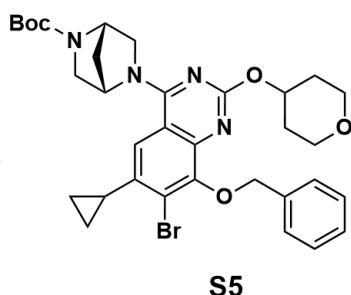


tert-Butyl (1*S*,4*S*)-5-{8-(benzyloxy)-7-bromo-6-iodo-2-[(oxan-4-yl)oxy]quinazolin-4-yl}-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate (**S4**):

To a mixture of **S3** (11.9 g, 18.3 mmol), benzyl alcohol (2.37 g, 21.9 mmol) and THF (40 mL) was added *t*-BuOK (2.54 g, 22.0 mmol) under ice-bath cooling and argon atmosphere, and the mixture was stirred at the same temperature for 1.5 h. Ice water and saturated aqueous ammonium chloride solution were added to the reaction mixture, and the mixture was extracted twice with ethyl acetate. The organic layer was dried over anhydrous magnesium sulfate and concentrated under reduced pressure. To the residue was added a mixed solvent (hexane/ethyl acetate = 6/1), and the mixture was stirred for a while. The resultant precipitate was collected by filtration, washed with a mixed solvent (hexane/ethyl acetate = 6/1), and dried to give the title compound (11.8 g, 87% yield) as a solid.

¹H NMR (500 MHz, DMSO-*d*₆) δ 8.29–8.22 (m, 1H), 7.60–7.54 (m, 2H), 7.46–7.41 (m, 2H), 7.40–7.35 (m, 1H), 5.36–5.27 (m, 1H), 5.22–5.06 (m, 3H), 4.59–4.48 (m, 1H), 4.30–4.20 (m, 1H), 3.89–3.79 (m, 2H), 3.76–3.62 (m, 1H), 3.51–3.37 (m, 2H), 3.35–3.27 (m, 2H), 2.08–1.87 (m, 4H), 1.71–1.61 (m, 2H), 1.43–1.32 (m, 9H);

MS (ESI) *m/z* = 737.1, 739.1 [M+H]⁺.

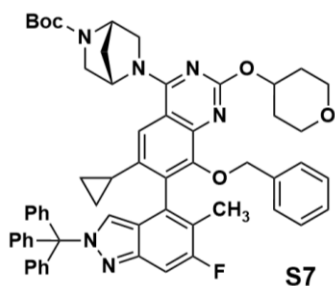


tert-Butyl (1*S*,4*S*)-5-{8-(benzyloxy)-7-bromo-6-cyclopropyl-2-[(oxan-4-yl)oxy]quinazolin-4-yl}-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate (**S5**):

A mixture of **S4** (5.47 g, 7.42 mmol), cyclopropylboronic acid (1.27 g, 14.8 mmol), tripotassium phosphate (5.67 g, 26.7 mmol), PdCl₂(dppf)·CH₂Cl₂ (600 mg, 0.735 mmol), MeCN (88 mL), DOX (10 mL) and water (22 mL) was stirred at 100 °C for 3 h under argon atmosphere. After the reaction mixture was allowed to cool to room temperature, the mixture was concentrated under reduced pressure. To the residue was added a saturated aqueous sodium chloride solution, and the mixture was extracted with CHCl₃. The organic layer was dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 70/30 to 30/70) to give the title compound (3.8 g, 79% yield) as a solid.

¹H NMR (500 MHz, DMSO-*d*₆) δ 7.62–7.55 (m, 2H), 7.47–7.41 (m, 2H), 7.40–7.35 (m, 2H), 5.36–5.28 (m, 1H), 5.22–5.04 (m, 3H), 4.59–4.48 (m, 1H), 4.29 (d, *J* = 8.7 Hz, 1H), 3.87–3.81 (m, 2H), 3.72–3.61 (m, 1H), 3.51–3.40 (m, 2H), 3.35–3.27 (m, 2H), 2.19–2.11 (m, 1H), 2.09–1.87 (m, 4H), 1.71–1.59 (m, 2H), 1.42–1.32 (m, 9H), 1.07–0.97 (m, 2H), 0.78–0.65 (m, 2H);

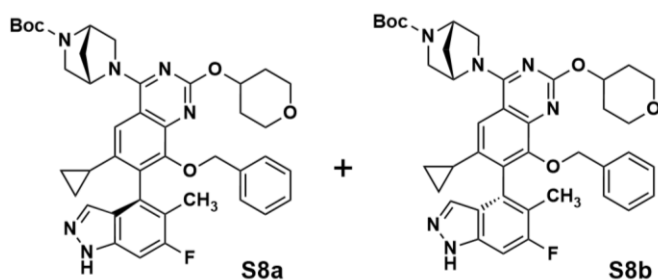
MS (ESI) *m/z* = 651.4, 653.4 [M+H]⁺.



tert-Butyl (1*S*,4*S*)-5-{8-(benzyloxy)-6-cyclopropyl-7-[6-fluoro-5-methyl-2-(triphenylmethyl)-2*H*-indazol-4-yl]-2-[(oxan-4-yl)oxy]quinazolin-4-yl}-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate (**S7**):

A mixture of **S5** (1 g, 1.53 mmol), 6-fluoro-5-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2-(triphenylmethyl)-2*H*-indazole (1.2 g, 2.31 mmol), tripotassium phosphate (1.3 g, 6.12 mmol), RuPhos (63 mg, 0.154 mmol), RuPhos Pd G3 (120 mg, 0.154 mmol), DOX (20 mL) and water (4 mL) was stirred at 80 °C for 2 h under an argon atmosphere. After the reaction mixture was allowed to cool to room temperature, water was added and the mixture was extracted with ethyl acetate. The organic layer was washed with an aqueous sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (amino silica gel, hexane/ethyl acetate = 95/5 to 75/25) to give the title compound (ca. 1:1 mixture of axial isomers, 1.46 g, 99%) as a solid.

MS (ESI) m/z = 963.5 $[M+H]^+$.



tert-Butyl (1*S*,4*S*)-5-{(7*M*)-8-(benzyloxy)-6-cyclopropyl-7-(6-fluoro-5-methyl-1*H*-indazol-4-yl)-2-[(oxan-4-yl)oxy]quinazolin-4-yl}-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate (**S8a**) and *tert*-butyl (1*S*,4*S*)-5-{(7*P*)-8-(benzyloxy)-6-cyclopropyl-7-(6-fluoro-5-methyl-1*H*-indazol-4-yl)-2-[(oxan-4-yl)oxy]quinazolin-4-yl}-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate (**S8b**):

To a solution of **S7** (1.19 g, 1.24 mmol) in MeOH (18 mL) was added *p*-TsOH·H₂O (240 mg, 1.26 mmol) under ice-bath cooling, and the mixture was stirred at room temperature for 2 h. Saturated aqueous sodium hydrogen carbonate solution was added and the mixture was extracted with CHCl₃. The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (amino silica gel, hexane/ethyl acetate = 90/10 to 10/90) to give **S8a** (441 mg, 50%) (low polar) as a solid, and **S8b** (348 mg, 39%) (high polar) as a solid.

S8a

¹H NMR (500 MHz, DMSO-*d*₆) δ 13.07 (s, 1H), 7.51 (s, 1H), 7.42–7.34 (m, 2H), 7.19–7.11 (m, 3H), 6.79–6.73 (m, 2H), 5.29–5.21 (m, 1H), 5.20–5.13 (m, 2H), 4.69 (d, *J* = 11.3 Hz, 1H), 4.64–4.52 (m, 1H), 4.35 (d, *J* = 8.4 Hz, 1H), 3.89–3.81 (m, 2H), 3.81–3.68 (m, 1H), 3.54–3.45 (m, 2H), 3.35–3.23 (m, 2H), 2.10–1.89 (m, 7H), 1.71–1.61 (m, 2H), 1.46–1.31 (m, 10H), 0.70–0.49 (m, 4H);

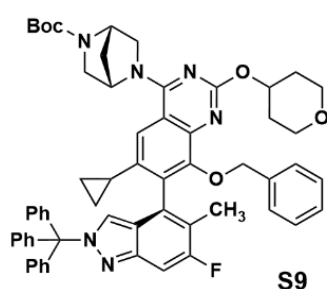
MS (ESI) *m/z* = 721.5 [M+H]⁺.

S8b

¹H NMR (500 MHz, DMSO-*d*₆) δ 13.06 (s, 1H), 7.46 (s, 1H), 7.43–7.34 (m, 2H), 7.20–7.12 (m, 3H),

6.80–6.75 (m, 2H), 5.21–5.13 (m, 2H), 5.10 (d, $J = 11.6$ Hz, 1H), 4.88 (t, $J = 12.5$ Hz, 1H), 4.63–4.52 (m, 1H), 4.34 (d, $J = 8.9$ Hz, 1H), 3.87–3.81 (m, 2H), 3.81–3.69 (m, 1H), 3.55–3.45 (m, 2H), 3.34–3.24 (m, 2H), 2.10–1.89 (m, 7H), 1.72–1.60 (m, 2H), 1.45–1.30 (m, 10H), 0.71–0.59 (m, 3H), 0.55–0.47 (m, 1H);

MS (ESI) $m/z = 721.5$ $[M+H]^+$.



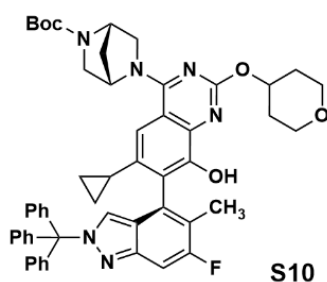
tert-Butyl (1*S*,4*S*)-5-[(7*M*)-8-(benzyloxy)-6-cyclopropyl-7-[6-fluoro-5-methyl-2-(triphenylmethyl)-2*H*-indazol-4-yl]-2-[(oxan-4-yl)oxy]quinazolin-4-yl]-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate (**S9**):

To a solution of **S8a** (338 mg, 0.469 mmol) in CH_2Cl_2 (4 mL) were added Et_3N (100 μL , 0.718 mmol) and trityl chloride (170 mg, 0.610 mmol) under ice-bath cooling, and the mixture was stirred at room temperature overnight. Additional Et_3N (100 μL , 0.718 mmol) and trityl chloride (170 mg, 0.610 mmol) were added under ice-bath cooling, and the mixture was stirred at room temperature for 2 h. Saturated aqueous ammonium chloride solution was added and the mixture was extracted with CHCl_3 . The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (amino silica gel, hexane/ethyl acetate = 90/10 to 70/30) to give the title compound (371 mg, 82%) as an oil.

^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 7.48 (d, $J = 10.9$ Hz, 1H), 7.39–7.33 (m, 1H), 7.32–7.14 (m, 13H), 7.02–6.95 (m, 6H), 6.77 (d, $J = 7.2$ Hz, 2H), 5.37–5.28 (m, 1H), 5.19–5.09 (m, 2H), 4.60–4.49 (m,

2H), 4.28 (d, $J = 8.7$ Hz, 1H), 3.89–3.82 (m, 2H), 3.74–3.62 (m, 1H), 3.50–3.41 (m, 2H), 3.39–3.29 (m, 2H), 2.09–1.87 (m, 7H), 1.71–1.61 (m, 2H), 1.45–1.34 (m, 10H), 0.81–0.67 (m, 2H), 0.58–0.48 (m, 1H), 0.37–0.28 (m, 1H);

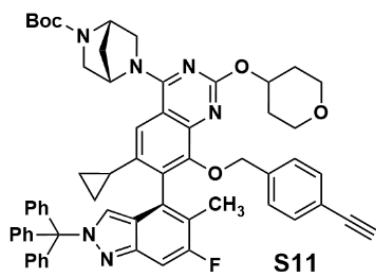
MS (ESI) $m/z = 963.7$ $[M+H]^+$.



tert-Butyl (1*S*,4*S*)-5-{(7*M*)-6-cyclopropyl-7-[6-fluoro-5-methyl-2-(triphenylmethyl)-2*H*-indazol-4-yl]-8-hydroxy-2-[(oxan-4-yl)oxy]quinazolin-4-yl}-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate (**S10**) :

A mixture of **S9** (287 mg, 0.298 mmol), 10% Pd/C (50% water content, 87 mg), sodium hydrogen carbonate (130 mg, 1.55 mmol), THF (3 mL) and MeOH (3 mL) was stirred under H₂ atmosphere at room temperature for 19 h. The mixture was filtered through a pad of Celite[®] and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (amino silica gel, hexane/ethyl acetate = 80/20 to 20/80) to give the title compound (204 mg, 78%) as a solid.

¹H NMR (500 MHz, DMSO-*d*₆) δ 8.88–8.66 (m, 1H), 7.43 (d, $J = 10.9$ Hz, 1H), 7.38–7.26 (m, 10H), 7.07–7.01 (m, 6H), 6.93 (s, 1H), 5.39–5.32 (m, 1H), 5.16–5.10 (m, 1H), 4.59–4.48 (m, 1H), 4.25 (d, $J = 8.9$ Hz, 1H), 3.90–3.82 (m, 2H), 3.66 (d, $J = 7.5$ Hz, 1H), 3.55–3.28 (m, 4H), 2.05–1.88 (m, 7H), 1.67–1.57 (m, 2H), 1.46–1.28 (m, 10H), 0.76–0.61 (m, 2H), 0.54–0.45 (m, 1H), 0.29–0.21 (m, 1H); MS (ESI) $m/z = 873.7$ $[M+H]^+$.

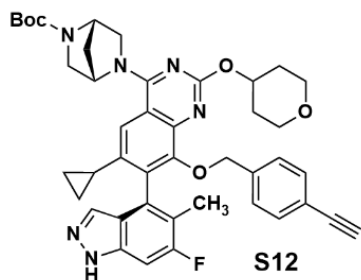


tert-Butyl (1*S*,4*S*)-5-{(7*M*)-6-cyclopropyl-8-[(4-ethynylphenyl)methoxy]-7-[6-fluoro-5-methyl-2-(triphenylmethyl)-2*H*-indazol-4-yl]-2-[(oxan-4-yl)oxy]quinazolin-4-yl}-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate (**S11**) :

To a mixture of **S10** (3.15 g, 3.60 mmol), cesium carbonate (3.52 g, 10.8 mmol) and DMF (30 mL) was added 1-(chloromethyl)-4-ethynylbenzene (540 mg, 3.59 mmol) at room temperature, and the mixture was stirred at room temperature under N₂ flow for 2 h. Water was added and the mixture was stirred at room temperature for 1 h. The resultant precipitate was collected by filtration and dried under reduced pressure. The obtained solid was purified by silica gel column chromatography (amino silica gel, hexane/ethyl acetate = 90/10 to 40/60) to give the title compound (2.69 g, 76%) as a solid.

¹H NMR (500 MHz, DMSO-*d*₆) δ 7.48 (d, *J* = 10.9 Hz, 1H), 7.39–7.33 (m, 1H), 7.32–7.22 (m, 12H), 7.02–6.95 (m, 6H), 6.78 (d, *J* = 7.6 Hz, 2H), 5.42–5.32 (m, 1H), 5.17–5.08 (m, 2H), 4.61–4.48 (m, 2H), 4.29 (d, *J* = 9.0 Hz, 1H), 4.16 (s, 1H), 3.88–3.80 (m, 2H), 3.74–3.61 (m, 1H), 3.51–3.40 (m, 2H), 3.39–3.25 (m, 2H), 2.07–1.87 (m, 7H), 1.70–1.59 (m, 2H), 1.44–1.33 (m, 10H), 0.82–0.67 (m, 2H), 0.58–0.46 (m, 1H), 0.37–0.27 (m, 1H);

MS (ESI) *m/z* = 987.7 [M+H]⁺.

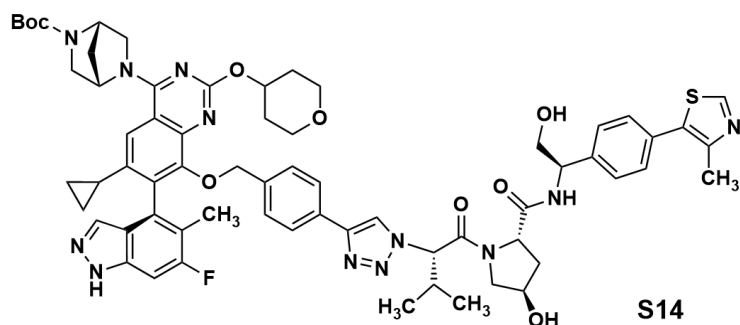


tert-Butyl (1*S*,4*S*)-5-{(7*M*)-6-cyclopropyl-8-[(4-ethynylphenyl)methoxy]-7-(6-fluoro-5-methyl-1*H*-indazol-4-yl)-2-[(oxan-4-yl)oxy]quinazolin-4-yl}-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate (**S12**) :

To a solution of **S11** (745 mg, 0.755 mmol) in MeOH (8 mL) was added *p*-TsOH·H₂O (145 mg, 0.762 mmol) at room temperature, and the mixture was stirred at room temperature for 1 h. Saturated aqueous sodium hydrogen carbonate solution was added and the mixture was extracted twice with ethyl acetate. The combined organic layer was washed with saturated aqueous sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was dissolved in ethyl acetate (3 mL) and hexane (3 mL). The solution was diluted with hexane (3 mL), stirred at 90 °C for 30 min, and then stirred at room temperature for 1 h. The resultant precipitate was collected by filtration and dried under reduced pressure to give the title compound (408 mg, 73%) as a solid.

¹H NMR (500 MHz, DMSO-*d*₆) δ 13.06 (s, 1H), 7.49 (s, 1H), 7.41–7.33 (m, 2H), 7.26–7.21 (m, 2H), 6.80–6.75 (m, 2H), 5.31–5.23 (m, 1H), 5.20–5.08 (m, 2H), 4.74 (d, *J* = 11.6 Hz, 1H), 4.63–4.52 (m, 1H), 4.35 (d, *J* = 8.4 Hz, 1H), 4.12 (s, 1H), 3.87–3.68 (m, 3H), 3.55–3.44 (m, 2H), 3.33–3.20 (m, 2H), 2.07–1.89 (m, 7H), 1.70–1.59 (m, 2H), 1.45–1.30 (m, 10H), 0.70–0.49 (m, 4H);

MS (ESI) *m/z* = 745.5 [M+H]⁺.



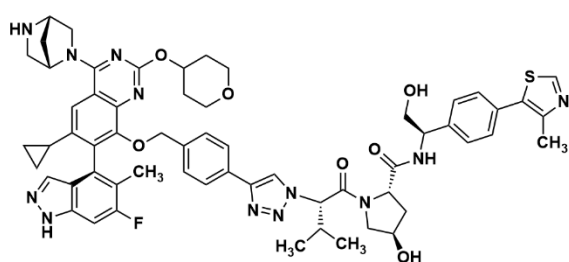
tert-Butyl (1*S*,4*S*)-5-((7*M*)-6-cyclopropyl-7-(6-fluoro-5-methyl-1*H*-indazol-4-yl)-8-([4-(1-((2*S*)-1-[(2*S*,4*R*)-4-hydroxy-2-((1*R*)-2-hydroxy-1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]ethyl)carbamoyl)pyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl)-1*H*-1,2,3-triazol-4-yl)phenyl]methoxy)-2-[(oxan-4-yl)oxy]quinazolin-4-yl)-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate (**S14**) :

To a mixture of **S12** (310 mg, 0.416 mmol), **S13** (200 mg, 0.423 mmol), *t*-BuOH (0.4 mL), THF (0.5 mL) and water (0.4 mL) were added sodium ascorbate (80 mg, 0.404 mmol) and copper(I) iodide (20 mg, 0.105 mmol) at room temperature. The mixture was stirred at room temperature for 4 h. Additional copper(I) iodide (20 mg, 0.105 mmol) was added and the mixture was stirred at room temperature for 15 h. To the mixture was added a solution of ethylenediaminetetraacetic acid disodium salt (750 mg, 2.23 mmol) in water (20 mL), and the mixture was diluted with ethyl acetate (20 mL) and stirred at room temperature for 1 h. The mixture was extracted twice with ethyl acetate. The combined organic layer was washed with water and saturated aqueous sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (amino silica gel, CHCl₃/MeOH = 100/0 to 92/8) to give the title compound (452 mg, 88% yield) as a solid.

¹H NMR (500 MHz, DMSO-*d*₆) δ 13.09 (s, 1H), 8.99 (s, 1H), 8.65 (s, 1H), 8.47 (d, *J* = 7.8 Hz, 1H), 7.67 (d, *J* = 7.4 Hz, 2H), 7.53 (s, 1H), 7.47–7.36 (m, 6H), 6.79 (d, *J* = 8.3 Hz, 2H), 5.36–5.25 (m, 2H), 5.23–5.12 (m, 3H), 4.89–4.82 (m, 2H), 4.72 (d, *J* = 11.0 Hz, 1H), 4.65–4.53 (m, 1H), 4.46 (t, *J*

= 8.0 Hz, 1H), 4.39–4.29 (m, 2H), 3.90–3.83 (m, 2H), 3.83–3.67 (m, 3H), 3.67–3.56 (m, 2H), 3.56–3.46 (m, 2H), 3.41–3.32 (m, 2H), 2.56–2.50 (m, 1H), 2.46 (s, 3H), 2.15–1.89 (m, 8H), 1.80 (ddd, J = 12.9, 8.3, 4.8 Hz, 1H), 1.73–1.64 (m, 2H), 1.46–1.33 (m, 10H), 1.08 (d, J = 6.6 Hz, 3H), 0.72 (d, J = 6.6 Hz, 3H), 0.71–0.50 (m, 4H); Minor peaks derived from rotamers are omitted.

MS (ESI) m/z = 1215.6 $[M-H]^-$.



ASP3082

(4*R*)-1-[(2*S*)-2-(4-{4-[(*(7M)*-6-Cyclopropyl-4-[(1*S*,4*S*)-2,5-diazabicyclo[2.2.1]heptan-2-yl]-7-(6-fluoro-5-methyl-1*H*-indazol-4-yl)-2-[(oxan-4-yl)oxy]quinazolin-8-yl}oxy)methyl]phenyl}-1*H*-1,2,3-triazol-1-yl)-3-methylbutanoyl]-4-hydroxy-*N*-{(1*R*)-2-hydroxy-1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]ethyl}-L-prolinamide (ASP3082) :

To a solution of **S14** (200 mg, 0.164 mmol) in MeOH (1 mL) was added methanesulfonic acid (100 μ L, 1.54 mmol) at room temperature, and the mixture was stirred at 50 °C for 4 h. The resulting reaction mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (ODS, 0.1% formic acid in H₂O/0.1% formic acid in MeCN = 90/10 to 70/30). 5% aqueous sodium hydrogen carbonate solution was added to fractions containing the target compound, and the mixture was extracted twice with CHCl₃/MeOH (9/1). The combined organic layer was dried over anhydrous sodium sulfate, and the solution was concentrated under reduced pressure. The resulting product was dissolved in ethanol and then concentrated under reduced pressure to give the title compound (139 mg, 76%) as a solid.

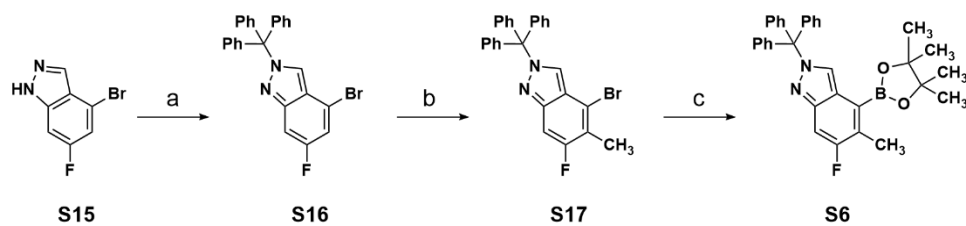
^1H NMR (500 MHz, DMSO- d_6 , 60 °C) δ 12.96 (s, 1H), 8.94 (s, 1H), 8.55 (s, 1H), 8.27 (d, J = 8.0 Hz, 1H), 7.65 (d, J = 8.3 Hz, 2H), 7.47 (s, 1H), 7.46 (s, 1H), 7.45–7.37 (m, 4H), 7.35 (d, J = 9.9 Hz, 1H), 6.80 (d, J = 8.1 Hz, 2H), 5.30 (d, J = 9.9 Hz, 1H), 5.27 (d, J = 11.3 Hz, 1H), 5.19 (tt, J = 9.1, 4.3 Hz, 1H), 5.09 (s, 1H), 5.01 (d, J = 4.0 Hz, 1H), 4.91–4.83 (m, 1H), 4.76 (d, J = 11.3 Hz, 1H), 4.68 (t, J = 5.7 Hz, 1H), 4.49 (t, J = 7.9 Hz, 1H), 4.37–4.31 (m, 1H), 4.20 (dd, J = 9.0, 1.6 Hz, 1H), 3.89–3.83 (m, 2H), 3.82 (dd, J = 10.6, 4.4 Hz, 1H), 3.77–3.71 (m, 2H), 3.70–3.59 (m, 3H), 3.43–3.33 (m, 2H), 3.11 (d, J = 10.1 Hz, 1H), 3.02 (dd, J = 9.6, 1.5 Hz, 1H), 2.57–2.50 (m, 1H), 2.46 (s, 3H), 2.11–2.01 (m, 3H), 2.00 (d, J = 2.3 Hz, 3H), 1.90–1.82 (m, 2H), 1.76–1.64 (m, 3H), 1.41–1.34 (m, 1H), 1.08 (d, J = 6.6 Hz, 3H), 0.74 (d, J = 6.6 Hz, 3H), 0.69–0.50 (m, 4H); Minor peaks derived from rotamers are omitted.

^{13}C NMR (126 MHz, DMSO- d_6 , 60 °C) δ 170.55, 166.21, 161.70, 160.40 (d, $^1J_{\text{C-F}}$ = 241.8 Hz), 159.96, 151.18, 149.67, 147.78, 146.17, 145.69, 141.08, 138.19 (d, $^3J_{\text{C-F}}$ = 14.5 Hz), 137.39, 135.43, 134.21 (d, $^4J_{\text{C-F}}$ = 2.5 Hz), 132.52, 131.10, 130.12 (d, $^3J_{\text{C-F}}$ = 6.5 Hz), 129.95, 129.87, 128.55 (2C), 127.41 (2C), 127.27 (2C), 124.76 (2C), 120.30, 120.12, 117.06 (d, $^2J_{\text{C-F}}$ = 20.9 Hz), 115.41, 114.52, 94.69 (d, $^2J_{\text{C-F}}$ = 27.4 Hz), 74.13, 70.53, 68.68, 66.45, 65.03, 64.99, 64.51, 61.67, 59.98, 58.99, 56.16, 56.02, 54.76, 50.54, 37.81, 36.02, 32.17, 32.13, 31.64, 18.31, 18.28, 15.87, 13.21, 11.86 (d, $^3J_{\text{C-F}}$ = 5.0 Hz), 8.23, 7.99;

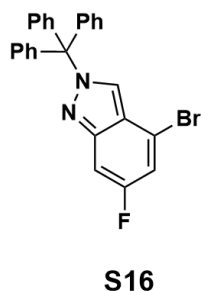
^{19}F NMR (470 MHz, DMSO- d_6 , 60 °C) δ -119.4 ($\text{C}_6\text{H}_5\text{CF}_3$ was used as internal standard.);

HRMS (ESI) m/z $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{60}\text{H}_{66}\text{FN}_{12}\text{O}_7\text{S}$, 1117.4877; found, 1117.4878.

Synthesis of indazole boronic ester (S6)



Reagents and conditions: a) trityl chloride, Et₃N, CH₂Cl₂, rt; b) LDA (2 M in THF), 2-MeTHF, -78 °C, 2.5 h; then MeI, -78 to 25 °C; c) bis(pinacolato)diboron, Pd(OAc)₂, Ph₃P, KOAc, DOX, 100 °C.

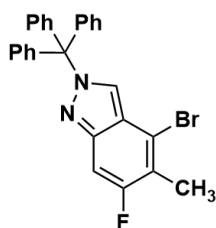


4-Bromo-6-fluoro-2-(triphenylmethyl)-2H-indazole (**S16**):

To a mixture of 4-bromo-6-fluoro-1H-indazole (**S15**) (235 g, 1.09 mol), Et₃N (183 mL, 1.31 mol) and CH₂Cl₂ (1880 mL) was added trityl chloride (335 g, 1.20 mol) at room temperature, and the mixture was stirred at 25 °C for 16 h. The reaction mixture was poured into ice water (1.5 L), and the layers were separated. The aqueous layer was extracted three times with CH₂Cl₂ (400 mL). The combined organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. Petroleum ether (550 mL) was added to the residue for trituration (0 °C, 2 h), and the resultant precipitate was collected by filtration and dried under reduced pressure to give the title compound (509 g, 85%) as a solid.

^1H NMR (500 MHz, DMSO- d_6) δ 7.89 (d, J = 0.9 Hz, 1H), 7.54–7.50 (m, 1H), 7.43–7.37 (m, 10H), 7.14–7.08 (m, 6H);

MS (ESI) m/z = 479.0, 481.0 $[\text{M}+\text{Na}]^+$.



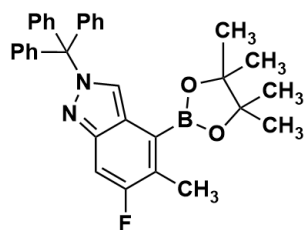
S17

4-Bromo-6-fluoro-5-methyl-2-(triphenylmethyl)-2H-indazole (**S17**) :

To a mixture of **S16** (100 g, 214 mmol) and 2-MeTHF (1000 mL) was added LDA (2 M THF solution, 214 mL, 428 mmol) at $-78\text{ }^\circ\text{C}$ under nitrogen atmosphere, and the mixture was stirred at $-78\text{ }^\circ\text{C}$ for 2.5 h. MeI (26.7 mL, 429 mmol) was added at $-78\text{ }^\circ\text{C}$, and the mixture was stirred at $25\text{ }^\circ\text{C}$ for 2.5 h. Water (2000 mL) was added to quench the reaction, and the mixture was extracted twice with ethyl acetate (800 mL). The combined organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. Ethyl acetate (50 mL)/petroleum ether (50 mL) were added to the residue for trituration, and the resultant precipitate was collected by filtration and dried under reduced pressure to give the title compound (81 g, 72%) as a solid.

^1H NMR (500 MHz, DMSO- d_6) δ 7.79 (d, J = 0.9 Hz, 1H), 7.51 (d, J = 10.4 Hz, 1H), 7.43–7.37 (m, 9H), 7.14–7.07 (m, 6H), 2.34 (d, J = 2.4 Hz, 3H);

MS (ESI) m/z = 493.1, 495.0 $[\text{M}+\text{Na}]^+$.



S6

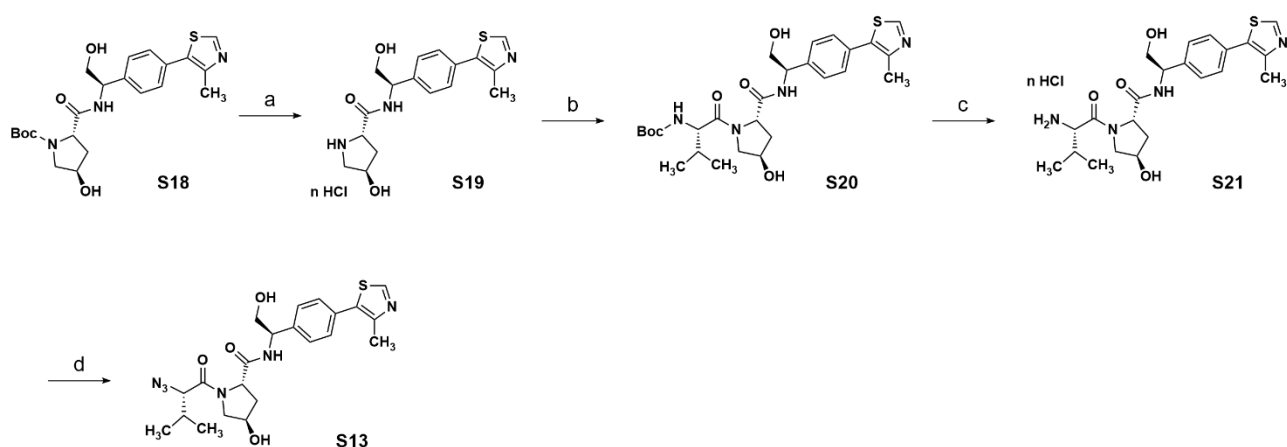
6-Fluoro-5-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2-(triphenylmethyl)-2*H*-indazole (**S6**):

To a mixture of **S17** (100 g, 202 mmol), bis(pinacolato)diboron (61.4 g, 242 mmol), Ph_3P (10.6 g, 40.3 mmol), KOAc (59.3 g, 605 mmol) and DOX (1000 mL) was added $\text{Pd}(\text{OAc})_2$ (4.52 g, 20.2 mmol) under nitrogen atmosphere at room temperature. After the reaction mixture was degassed and filled with nitrogen gas for three times each, the mixture was stirred at 100 °C for 12 h under nitrogen atmosphere. After cooling, water (1500 mL) was added, and the mixture was extracted three times with ethyl acetate (900 mL). The combined organic layer was dried over anhydrous sodium sulfate, and then the insoluble materials were removed by filtration. Activated carbon (50 g) was added to the filtrate, and the mixture was stirred at 20 °C for 1 h and filtered while washing with ethyl acetate (50 mL) three times. The filtrate was concentrated under reduced pressure. MeOH (200 mL) was added to the residue for trituration, and the resultant precipitate was collected by filtration and dried under reduced pressure to give the title compound (110 g, 84%) as a solid.

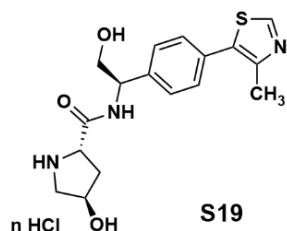
^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 8.09 (d, $J = 0.6$ Hz, 1H), 7.49 (d, $J = 11.3$ Hz, 1H), 7.41–7.37 (m, 9H), 7.10–7.05 (m, 6H), 2.44 (d, $J = 3.1$ Hz, 3H), 1.21 (s, 12H);

MS (ESI) $m/z = 541.2$ $[\text{M}+\text{Na}]^+$.

Synthesis of VHL ligand for ASP3082



Reagents and conditions: a) 4 M HCl in DOX, CH₂Cl₂, MeOH, 0 °C to rt; b) *N*-Boc-L-valine, HATU, DIPEA, DMF, 0 °C to rt; c) 4 M HCl in DOX, CH₂Cl₂, MeOH, -20 to -15 °C to rt; d) 2-azido-1,3-dimethylimidazolium hexafluorophosphate (ADMP), Et₃N, MeCN, THF, 0 °C.



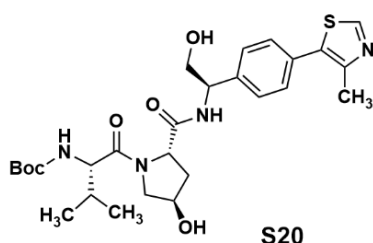
(4*R*)-4-Hydroxy-*N*-((1*R*)-2-hydroxy-1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]ethyl)-L-prolinamide hydrochloride (**S19**):

To a solution of *tert*-butyl (2*S*,4*R*)-4-hydroxy-2-((1*R*)-2-hydroxy-1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]ethyl)carbamoylpyrrolidine-1-carboxylate (**S18**) (3.09 g, 6.90 mmol) in CH₂Cl₂ (18 mL) and MeOH (18 mL) was added hydrogen chloride (4 M DOX solution, 17 mL, 68 mmol) under ice-cooling, and the mixture was stirred under ice-bath cooling for 1 h and then at room

temperature for 4 h. The reaction mixture was concentrated under reduced pressure to give the title compound (2.92 g, quant.) as a solid.

^1H NMR (500 MHz, DMSO- d_6) δ 9.90–9.81 (m, 1H), 9.20 (d, J = 7.6 Hz, 1H), 9.02 (s, 1H), 8.66–8.56 (m, 1H), 7.48–7.44 (m, 2H), 7.42–7.37 (m, 2H), 4.95–4.89 (m, 1H), 4.47–4.39 (m, 2H), 3.70–3.58 (m, 2H), 3.33–3.25 (m, 1H), 3.12–3.06 (m, 1H), 2.46 (s, 3H), 2.44–2.38 (m, 1H), 1.81 (ddd, J = 13.2, 10.9, 4.1 Hz, 1H);

MS (ESI) m/z = 348.2 $[\text{M}+\text{H}]^+$.

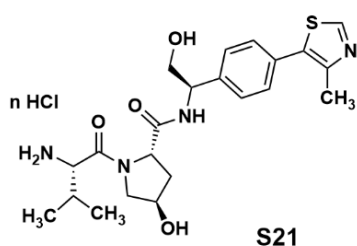


N-(*tert*-Butoxycarbonyl)-L-valyl-(4*R*)-4-hydroxy-*N*-{(1*R*)-2-hydroxy-1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]ethyl}-L-prolinamide (**S20**):

To a mixture of **S19** (3.81 g, 9.06 mmol), *N*-(*tert*-butoxycarbonyl)-L-valine (2.16 g, 9.94 mmol) and DMF (45 mL) was added DIPEA (6.2 mL, 36.2 mmol), and then HATU (3.61 g, 9.49 mmol) was added portionwise under ice-bath cooling. The mixture was stirred for 1 h under ice-bath cooling and for 1 h at room temperature. Water, saturated aqueous sodium chloride solution and ethyl acetate were added under ice-bath cooling, and the layers were separated. The aqueous layer was extracted twice with ethyl acetate and then extracted with ethyl acetate/isopropyl alcohol (9/1). The combined organic layer was washed with saturated aqueous sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($\text{CHCl}_3/\text{MeOH}$ = 100/0 to 90/10) to give the title compound (4.43 g, 89%) as a solid.

^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 8.98 (s, 1H), 8.35 (d, $J = 8.1$ Hz, 1H), 7.45–7.41 (m, 2H), 7.41–7.36 (m, 2H), 6.65 (d, $J = 8.7$ Hz, 1H), 5.15 (d, $J = 3.5$ Hz, 1H), 4.88–4.80 (m, 2H), 4.48 (t, $J = 7.9$ Hz, 1H), 4.33–4.28 (m, 1H), 4.03 (t, $J = 8.0$ Hz, 1H), 3.65–3.55 (m, 4H), 2.46 (s, 3H), 2.08–2.00 (m, 1H), 1.97–1.87 (m, 1H), 1.86–1.76 (m, 1H), 1.38 (s, 9H), 0.89 (d, $J = 6.6$ Hz, 3H), 0.83 (d, $J = 6.7$ Hz, 3H); Minor peaks derived from rotamers are omitted.

MS (ESI) $m/z = 547.4$ $[\text{M}+\text{H}]^+$.

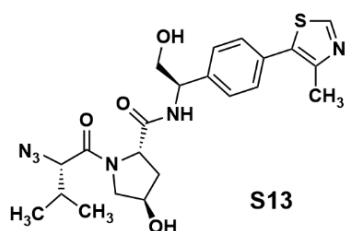


L-Valyl-(4R)-4-hydroxy-*N*-{(1R)-2-hydroxy-1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]ethyl}-L-prolinamide hydrochloride (**S21**) :

To a solution of **S20** (4.43 g, 8.10 mmol) in CH_2Cl_2 (35 mL) and MeOH (35 mL) was added hydrogen chloride (4 M DOX solution, 20 mL, 80 mmol) under cooling at -20 to -15 $^\circ\text{C}$, and the mixture was stirred at room temperature for 6 h. The reaction mixture was concentrated under reduced pressure to give the title compound (4.21 g, quant.) as a solid.

^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 9.03 (s, 1H), 8.57 (d, $J = 8.0$ Hz, 1H), 8.11 (d, $J = 4.3$ Hz, 3H), 7.49–7.36 (m, 4H), 4.86–4.80 (m, 1H), 4.61–4.55 (m, 1H), 4.35–4.30 (m, 1H), 4.03–3.96 (m, 1H), 3.68–3.50 (m, 4H), 2.47 (s, 3H), 2.18–2.07 (m, 2H), 1.78 (ddd, $J = 13.0, 8.7, 4.6$ Hz, 1H), 1.02 (d, $J = 7.0$ Hz, 3H), 0.94 (d, $J = 7.0$ Hz, 3H); Minor peaks derived from rotamers are omitted.

MS (ESI) $m/z = 447.3$ $[\text{M}+\text{H}]^+$.



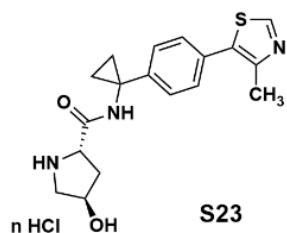
(4*R*)-1-[(2*S*)-2-Azido-3-methylbutanoyl]-4-hydroxy-*N*-{(1*R*)-2-hydroxy-1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]ethyl}-*L*-prolinamide (**S13**) :

To a mixture of **S21** (1.71 g, 3.29 mmol), Et₃N (3.2 mL, 23.0 mmol), THF (20 mL) and MeCN (20 mL) was added a solution of 2-azido-1,3-dimethylimidazolinium hexafluorophosphate (1.06 g, 3.72 mmol) in MeCN (5 mL) dropwise slowly over 10 minutes under ice-bath cooling and argon atmosphere, and the mixture was stirred under ice-bath cooling for 5 h. Water, saturated aqueous sodium chloride solution and ethyl acetate were added, and the layers were separated. The aqueous layer was extracted with ethyl acetate. The combined organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CHCl₃/MeOH = 95/5) to give the title compound (1.07 g, 69%) as a solid.

¹H NMR (500 MHz, DMSO-*d*₆) δ 8.98 (s, 1H), 8.45 (d, *J* = 8.0 Hz, 1H), 7.45–7.36 (m, 4H), 5.14 (d, *J* = 3.4 Hz, 1H), 4.86–4.81 (m, 2H), 4.54 (t, *J* = 8.3 Hz, 1H), 4.31–4.26 (m, 1H), 3.73 (d, *J* = 8.3 Hz, 1H), 3.66–3.47 (m, 4H), 2.47–2.45 (m, 3H), 2.13–2.02 (m, 2H), 1.77 (ddd, *J* = 12.9, 8.5, 4.6 Hz, 1H), 0.98 (d, *J* = 6.7 Hz, 3H), 0.96 (d, *J* = 6.7 Hz, 3H); Minor peaks derived from rotamers are omitted.

MS (ESI) *m/z* = 473.3 [M+H]⁺.

Reagents and conditions: a) 4 M HCl in DOX, CH₂Cl₂, MeOH, 0 °C to rt; b) For **S24**: *N*-Boc-L-valine, HATU, DIPEA, DMF, 0 °C to rt; c) 4 M HCl in DOX, CH₂Cl₂, MeOH, 0 °C to rt; d) methyl 4-(bromomethyl)benzoate, Cs₂CO₃, DMF, rt; e) 1 M NaOH aq., MeOH, THF, rt; f) For **S30**: **S27**, HATU, DIPEA, DMF, 0 °C to rt; For **S31**: **S26**, HATU, DIPEA, DMF, 0 °C to rt; For **S32**: **S21**, HATU, DIPEA, DMF, 0 °C to rt; g) 4 M HCl in DOX, CH₂Cl₂, MeOH, 0 °C to rt.

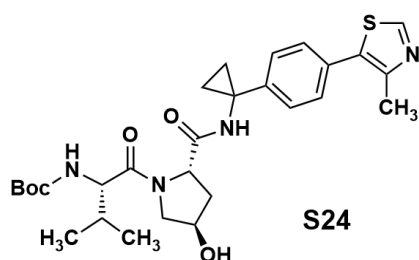


(4*R*)-4-Hydroxy-*N*-{1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]cyclopropyl}-*L*-prolinamide hydrochloride (**S23**):

To a mixture of *tert*-butyl (2*S*,4*R*)-4-hydroxy-2-({1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]cyclopropyl} carbamoyl)pyrrolidine-1-carboxylate (**S22**) (19 g, 42.8 mmol), CH₂Cl₂ (95 mL) and MeOH (95 mL) was added hydrogen chloride (4 M DOX solution, 107 mL, 428 mmol) under ice-bath cooling, and the mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated under reduced pressure. Diethyl ether (100 mL), ethyl acetate (100 mL) and ethanol (50 mL) were added to the residue, and the mixture was stirred at room temperature for 30 min. The resultant precipitate was collected by filtration and dried overnight to give the title compound (17.8 g, quant.) as a solid.

¹H NMR (500 MHz, DMSO-*d*₆) δ 10.03–9.90 (m, 1H), 9.44 (s, 1H), 9.04 (s, 1H), 8.70–8.55 (m, 1H), 7.44–7.37 (m, 2H), 7.27–7.22 (m, 2H), 4.47–4.42 (m, 1H), 4.40–4.31 (m, 1H), 3.36–3.28 (m, 1H), 3.13–3.06 (m, 1H), 2.45 (s, 3H), 2.40–2.33 (m, 1H), 1.93 (ddd, *J* = 13.2, 10.8, 4.4 Hz, 1H), 1.35–1.18 (m, 4H);

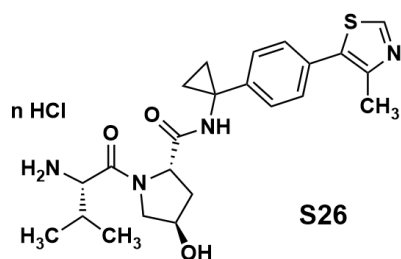
MS (ESI) *m/z* = 344.3 [M+H]⁺.



N-(*tert*-Butoxycarbonyl)-L-valyl-(4*R*)-4-hydroxy-*N*-{1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]cyclopropyl}-L-prolinamide (**S24**):

To a mixture of **S23** (2.00 g, 4.80 mmol) and DMF (20 mL) were added *N*-(*tert*-butoxycarbonyl)-L-valine (1.25 g, 5.75 mmol) and DIPEA (3.3 mL, 19 mmol) under ice-bath cooling, and then HATU (2.10 g, 5.52 mmol) was added portionwise under ice-bath cooling (internal temperature: below 4 °C). The mixture was stirred at room temperature for 3 h. The mixture was cooled with ice-bath, and then saturated aqueous sodium hydrogen carbonate solution and water were added. The mixture was extracted twice with ethyl acetate. The combined organic layer was washed with water and saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CHCl₃/MeOH = 100/0 to 90/10) to give the title compound (2.57 g, 99%) as a solid. ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.96 (s, 1H), 8.70 (s, 1H), 7.34–7.31 (m, 2H), 7.27–7.24 (m, 2H), 6.69 (d, *J* = 8.7 Hz, 1H), 5.11 (d, *J* = 2.6 Hz, 1H), 4.43–4.34 (m, 2H), 4.08–4.02 (m, 1H), 3.66–3.61 (m, 1H), 3.61–3.55 (m, 1H), 2.44 (s, 3H), 2.05–1.84 (m, 3H), 1.39 (s, 9H), 1.27–1.11 (m, 4H), 0.87 (d, *J* = 6.7 Hz, 3H), 0.82 (d, *J* = 6.6 Hz, 3H); Minor peaks derived from rotamers are omitted.

MS (ESI) *m/z* = 565.4 [M+Na]⁺.

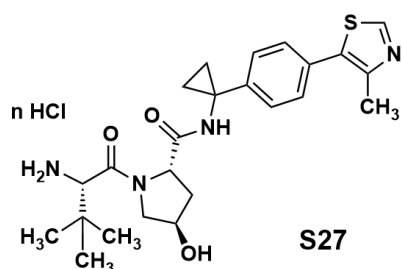


L-Valyl-(4*R*)-4-hydroxy-*N*-{1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]cyclopropyl}-L-prolinamide hydrochloride (**S26**):

To a solution of **S24** (2.57 g, 4.74 mmol) in CH₂Cl₂ (13 mL) and MeOH (13 mL) was added hydrogen chloride (4 M DOX solution, 12 mL, 48 mmol) under ice-bath cooling, and the mixture was stirred at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure. Diethyl ether was added to the residue and the resultant precipitate was collected by filtration, washed with diethyl ether, and dried overnight to give the title compound (2.58 g, quant.) as a solid.

¹H NMR (500 MHz, DMSO-*d*₆) δ 9.02 (s, 1H), 8.90 (s, 1H), 8.17 (d, *J* = 4.3 Hz, 3H), 7.36–7.32 (m, 2H), 7.28–7.23 (m, 2H), 4.52–4.46 (m, 1H), 4.41–4.37 (m, 1H), 4.08–4.00 (m, 1H), 3.71–3.66 (m, 1H), 3.58–3.53 (m, 1H), 2.45 (s, 3H), 2.17–2.07 (m, 2H), 1.88 (ddd, *J* = 13.0, 9.0, 4.3 Hz, 1H), 1.27–1.20 (m, 2H), 1.20–1.12 (m, 2H), 0.99 (d, *J* = 6.9 Hz, 3H), 0.93 (d, *J* = 6.9 Hz, 3H); Minor peaks derived from rotamers are omitted.

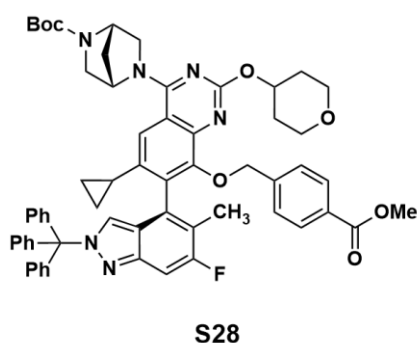
MS (ESI) *m/z* = 443.4 [M+H]⁺.



3-Methyl-L-valyl-(4*R*)-4-hydroxy-*N*-{1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]cyclopropyl}-L-prolinamide hydrochloride (**S27**):

To a mixture of **S25** (11.5 g, 20.7 mmol) in CH₂Cl₂ (58 mL) and MeOH (58 mL) was added hydrogen chloride (4 M DOX solution, 58 mL, 232 mmol) under ice-bath cooling, and the mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated under reduced pressure. Diethyl ether (200 mL) was added to the residue, and the mixture was stirred at room temperature for 30 min. The resultant precipitate was collected by filtration, washed with diethyl ether, and dried overnight to give the title compound (10.9 g, quant.) as a solid.

¹H NMR (500 MHz, DMSO-*d*₆) δ 9.01 (s, 1H), 8.97 (s, 1H), 8.14 (br d, *J* = 4.3 Hz, 3H), 7.33–7.27 (m, 4H), 4.52 (dd, *J* = 9.0, 7.8 Hz, 1H), 4.41–4.37 (m, 1H), 3.94–3.89 (m, 1H), 3.78–3.71 (m, 1H), 3.54 (dd, *J* = 11.0, 4.0 Hz, 1H), 2.44 (s, 3H), 2.12–2.05 (m, 1H), 1.87 (ddd, *J* = 13.1, 9.3, 4.3 Hz, 1H), 1.29–1.17 (m, 3H), 1.16–1.10 (m, 1H), 1.02 (s, 9H); Minor peaks derived from rotamers are omitted. MS (ESI) *m/z* = 457.2 [M+H]⁺.

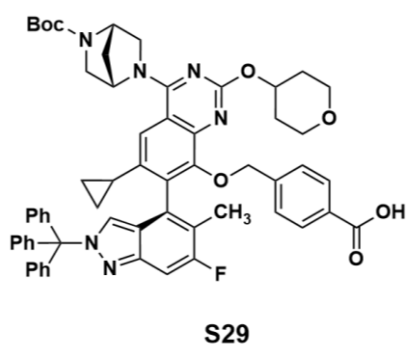


tert-Butyl (1*S*,4*S*)-5-{(7*M*)-6-cyclopropyl-7-[6-fluoro-5-methyl-2-(triphenylmethyl)-2*H*-indazol-4-yl]-8-{[4-(methoxycarbonyl)phenyl]methoxy}-2-[(oxan-4-yl)oxy]quinazolin-4-yl}-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate (**S28**):

To a mixture of **S10** (900 mg, 1.03 mmol), methyl 4-(bromomethyl)benzoate (290 mg, 1.27 mmol) and DMF (9 mL) was added cesium carbonate (680 mg, 2.09 mmol) at room temperature, and the mixture was stirred at room temperature for 2 h. Water was added and the mixture was extracted twice with ethyl acetate. The combined organic layer was washed with water and saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (amino silica gel, hexane/ethyl acetate = 90/10 to 40/60) to give the title compound (903 mg, 86%) as a solid.

^1H NMR (500 MHz, DMSO- d_6) δ 7.74 (d, J = 7.8 Hz, 2H), 7.49 (d, J = 10.9 Hz, 1H), 7.40–7.35 (m, 1H), 7.34–7.21 (m, 10H), 7.00–6.95 (m, 6H), 6.90 (d, J = 7.3 Hz, 2H), 5.51–5.42 (m, 1H), 5.16–5.08 (m, 2H), 4.66 (d, J = 12.7 Hz, 1H), 4.61–4.48 (m, 1H), 4.29 (d, J = 8.9 Hz, 1H), 3.88–3.80 (m, 5H), 3.75–3.62 (m, 1H), 3.51–3.41 (m, 2H), 3.31–3.24 (m, 2H), 2.07–1.87 (m, 7H), 1.70–1.59 (m, 2H), 1.44–1.34 (m, 10H), 0.82–0.67 (m, 2H), 0.58–0.47 (m, 1H), 0.38–0.29 (m, 1H);

MS (ESI) m/z = 1021.5 $[\text{M}+\text{H}]^+$.

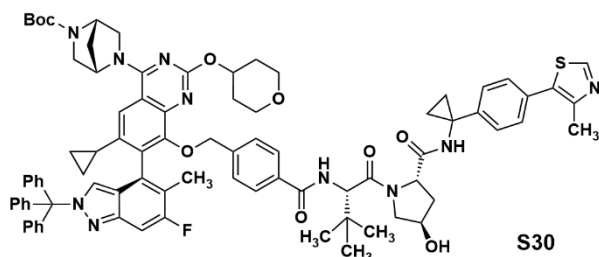


4-[(*(7M)*-4-[(*(1S,4S)*-5-(*tert*-Butoxycarbonyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl]-6-cyclopropyl-7-[6-fluoro-5-methyl-2-(triphenylmethyl)-2*H*-indazol-4-yl]-2-[(oxan-4-yl)oxy]quinazolin-8-yl}oxy)methyl]benzoic acid (**S29**):

To a mixture of **S28** (400 mg, 0.392 mmol), MeOH (2 mL) and THF (2 mL) was added 1 M

NaOH aq. (1 mL, 1.00 mmol) at room temperature, and the mixture was stirred at room temperature for 18 h. The mixture was cooled with ice-bath and neutralized by addition of 1 M hydrochloric acid, and then extracted twice with CHCl₃/MeOH (5/1). The combined organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure to give the title compound (410 mg, quant.) as a solid.

¹H NMR (500 MHz, DMSO-*d*₆) δ 12.89 (br s, 1H), 7.73 (d, *J* = 8.0 Hz, 2H), 7.49 (d, *J* = 10.9 Hz, 1H), 7.41–7.35 (m, 1H), 7.34–7.20 (m, 10H), 7.00–6.94 (m, 6H), 6.88 (d, *J* = 7.6 Hz, 2H), 5.50–5.41 (m, 1H), 5.17–5.08 (m, 2H), 4.64 (d, *J* = 12.9 Hz, 1H), 4.61–4.48 (m, 1H), 4.29 (d, *J* = 8.4 Hz, 1H), 3.88–3.80 (m, 2H), 3.75–3.63 (m, 1H), 3.51–3.40 (m, 2H), 3.31–3.24 (m, 2H), 2.07–1.87 (m, 7H), 1.70–1.59 (m, 2H), 1.46–1.33 (m, 10H), 0.82–0.67 (m, 2H), 0.57–0.46 (m, 1H), 0.37–0.28 (m, 1H); MS (ESI) *m/z* = 1007.7 [M+H]⁺.



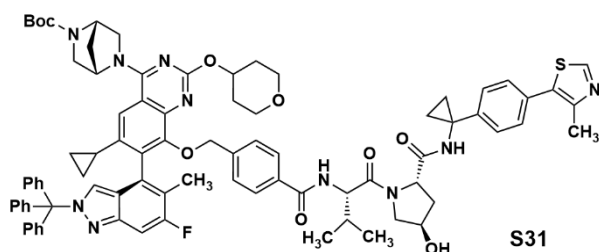
N-{4-[(*(7M)*-4-[(*(1S,4S)*-5-(*tert*-Butoxycarbonyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl]-6-cyclopropyl-7-[6-fluoro-5-methyl-2-(triphenylmethyl)-2*H*-indazol-4-yl]-2-[(oxan-4-yl)oxy]quinazolin-8-yl]oxy)methyl]benzoyl}-3-methyl-L-valyl-(4*R*)-4-hydroxy-*N*-{1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]cyclopropyl}-L-prolinamide (**S30**):

To a mixture of **S29** (200 mg, 0.199 mmol), **S27** (116 mg, 0.219 mmol) and DMF (2 mL) were added DIPEA (140 μ L, 0.818 mmol) and HATU (91 mg, 0.239 mmol) under ice-bath cooling and argon atmosphere. The mixture was stirred at room temperature for 1 h. Saturated aqueous sodium hydrogen carbonate solution was added and the mixture was extracted with ethyl acetate. The

organic layer was washed with water and saturated aqueous sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CHCl₃/MeOH = 100/0 to 95/5) to give the title compound (275 mg, 96%) as a solid.

¹H NMR (500 MHz, DMSO-d₆) δ 8.96 (s, 1H), 8.82 (s, 1H), 7.99 (d, *J* = 9.2 Hz, 1H), 7.74 (d, *J* = 8.0 Hz, 2H), 7.50 (d, *J* = 10.9 Hz, 1H), 7.44–7.38 (m, 1H), 7.35–7.21 (m, 14H), 7.01–6.95 (m, 6H), 6.83 (d, *J* = 5.2 Hz, 2H), 5.47–5.36 (m, 1H), 5.18–5.10 (m, 3H), 4.80 (d, *J* = 9.2 Hz, 1H), 4.61–4.49 (m, 2H), 4.45 (t, *J* = 8.2 Hz, 1H), 4.41–4.36 (m, 1H), 4.29 (d, *J* = 8.9 Hz, 1H), 3.90–3.82 (m, 2H), 3.78–3.63 (m, 3H), 3.52–3.41 (m, 2H), 3.39–3.32 (m, 2H), 2.44 (s, 3H), 2.11–1.86 (m, 9H), 1.71–1.61 (m, 2H), 1.46–1.33 (m, 10H), 1.30–1.18 (m, 3H), 1.17–1.11 (m, 1H), 1.04 (s, 9H), 0.82–0.69 (m, 2H), 0.60–0.50 (m, 1H), 0.40–0.31 (m, 1H);

MS (ESI) *m/z* = 1445.7 [M+H]⁺.



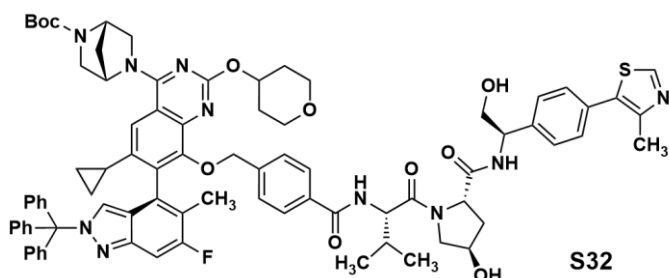
N-{4-[(*(7M)*-4-[(*(1S,4S)*-5-(*tert*-Butoxycarbonyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl]-6-cyclopropyl-7-[6-fluoro-5-methyl-2-(triphenylmethyl)-2*H*-indazol-4-yl]-2-[(oxan-4-yl)oxy]quinazolin-8-yl}oxy)methyl]benzoyl}-L-valyl-(4*R*)-4-hydroxy-*N*-{1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]cyclopropyl}-L-prolinamide (**S31**):

To a mixture of **S29** (140 mg, 0.139 mmol), **S26** (80 mg, 0.155 mmol) and DMF (2 mL) were added DIPEA (100 μL, 0.584 mmol) and HATU (64 mg, 0.168 mmol) under ice-bath cooling. The mixture was stirred at room temperature for 1 h. Saturated aqueous sodium hydrogen carbonate

solution was added and the mixture was extracted with ethyl acetate. The organic layer was washed with water and saturated aqueous sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($\text{CHCl}_3/\text{MeOH} = 100/0$ to $95/5$) to give the title compound (195 mg, 98%) as a solid.

^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 8.96 (s, 1H), 8.72 (s, 1H), 8.43 (d, $J = 8.3$ Hz, 1H), 7.77 (d, $J = 7.8$ Hz, 2H), 7.50 (d, $J = 10.9$ Hz, 1H), 7.44–7.38 (m, 1H), 7.36–7.30 (m, 3H), 7.30–7.21 (m, 11H), 7.01–6.95 (m, 6H), 6.82 (d, $J = 7.3$ Hz, 2H), 5.47–5.48 (m, 1H), 5.18–5.09 (m, 3H), 4.61–4.49 (m, 3H), 4.45–4.37 (m, 2H), 4.29 (d, $J = 8.6$ Hz, 1H), 3.90–3.82 (m, 2H), 3.82–3.77 (m, 1H), 3.76–3.63 (m, 2H), 3.51–3.41 (m, 2H), 3.40–3.30 (m, 2H), 2.44 (s, 3H), 2.23–2.14 (m, 1H), 2.11–1.87 (m, 9H), 1.71–1.61 (m, 2H), 1.45–1.34 (m, 10H), 1.26–1.13 (m, 4H), 0.96 (d, $J = 6.7$ Hz, 3H), 0.93 (d, $J = 6.7$ Hz, 3H), 0.82–0.69 (m, 2H), 0.60–0.50 (m, 1H), 0.39–0.31 (m, 1H);

MS (ESI) $m/z = 1431.6$ $[\text{M}+\text{H}]^+$.



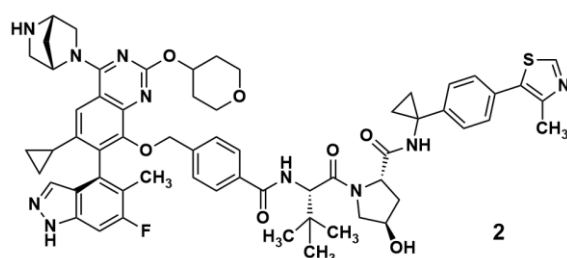
N-{4-[(*(7M)*-4-[(*(1S,4S)*-5-(*tert*-Butoxycarbonyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl]-6-cyclopropyl-7-[6-fluoro-5-methyl-2-(triphenylmethyl)-2*H*-indazol-4-yl]-2-[(oxan-4-yl)oxy]quinazolin-8-yl}oxy)methyl]benzoyl}-L-valyl-(4*R*)-4-hydroxy-*N*-{(1*R*)-2-hydroxy-1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]ethyl}-L-prolinamide (**S32**):

To a mixture of **S29** (180 mg, 0.179 mmol), **S21** (105 mg, 0.202 mmol) and DMF (2 mL) were added DIPEA (120 μL , 0.701 mmol) and HATU (82 mg, 0.216 mmol) under ice-bath cooling.

The mixture was stirred at room temperature for 1 h. Saturated aqueous sodium hydrogen carbonate solution was added and the mixture was extracted with ethyl acetate. The organic layer was washed with water and saturated aqueous sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($\text{CHCl}_3/\text{MeOH} = 100/0$ to $95/5$) to give the title compound (227 mg, 89%) as a solid.

^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 8.98 (s, 1H), 8.41 (d, $J = 8.1$ Hz, 1H), 8.36 (d, $J = 8.1$ Hz, 1H), 7.76 (d, $J = 8.0$ Hz, 2H), 7.50 (d, $J = 10.9$ Hz, 1H), 7.46–7.38 (m, 5H), 7.35–7.30 (m, 1H), 7.30–7.21 (m, 9H), 7.01–6.95 (m, 6H), 6.82 (d, $J = 6.9$ Hz, 2H), 5.47–5.38 (m, 1H), 5.17–5.08 (m, 3H), 4.91–4.84 (m, 1H), 4.79 (t, $J = 5.8$ Hz, 1H), 4.61–4.47 (m, 4H), 4.35–4.26 (m, 2H), 3.90–3.82 (m, 2H), 3.81–3.75 (m, 1H), 3.75–3.56 (m, 4H), 3.51–3.41 (m, 2H), 3.41–3.30 (m, 2H), 2.46 (s, 3H), 2.20–2.11 (m, 1H), 2.10–1.88 (m, 8H), 1.85 (ddd, $J = 12.7, 7.8, 4.9$ Hz, 1H), 1.71–1.61 (m, 2H), 1.45–1.35 (m, 10H), 0.98 (d, $J = 6.7$ Hz, 3H), 0.94 (d, $J = 6.6$ Hz, 3H), 0.82–0.69 (m, 2H), 0.60–0.49 (m, 1H), 0.39–0.31 (m, 1H);

MS (ESI) $m/z = 1435.6$ $[\text{M}+\text{H}]^+$.



N-{4-[(*(7M)*-6-Cyclopropyl-4-[(*(1S,4S)*)-2,5-diazabicyclo[2.2.1]heptan-2-yl]-7-(6-fluoro-5-methyl-1*H*-indazol-4-yl)-2-[(oxan-4-yl)oxy]quinazolin-8-yl}oxy)methyl]benzoyl}-3-methyl-L-valyl-(*(4R)*)-4-hydroxy-*N*-{1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]cyclopropyl}-L-prolinamide (**2**):

To a solution of **S30** (213 mg, 0.147 mmol) in CH_2Cl_2 (1 mL) and MeOH (1 mL) was added hydrogen chloride (4 M DOX solution, 1 mL, 4 mmol) under ice-bath cooling, and the mixture was

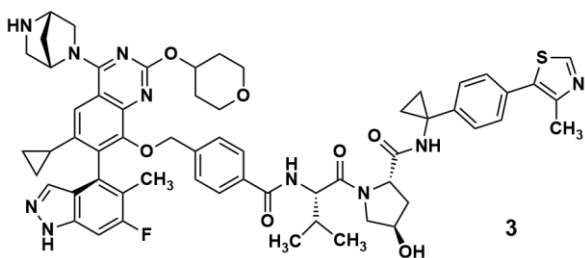
stirred at room temperature for 3 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (ODS, 0.1% formic acid in H₂O/0.1% formic acid in MeCN = 90/10 to 65/35). Saturated aqueous sodium hydrogen carbonate solution was added to fractions containing the target compound, and the mixture was extracted twice with CHCl₃/MeOH (9/1). The combined organic layer was dried over anhydrous sodium sulfate, and the solution was concentrated under reduced pressure. Diethyl ether was added to the residue and the resultant precipitate was collected by filtration, washed with diethyl ether, and dried to give the title compound (103 mg, 63%) as a solid.

¹H NMR (500 MHz, DMSO-*d*₆, 60 °C) δ 12.95 (s, 1H), 8.92 (s, 1H), 8.62 (s, 1H), 7.69 (d, *J* = 9.0 Hz, 1H), 7.62 (d, *J* = 8.3 Hz, 2H), 7.46 (s, 2H), 7.36–7.29 (m, 5H), 6.82 (d, *J* = 8.3 Hz, 2H), 5.30 (d, *J* = 11.9 Hz, 1H), 5.19–5.12 (m, 1H), 5.10–5.08 (m, 1H), 4.99 (d, *J* = 3.8 Hz, 1H), 4.81 (d, *J* = 11.9 Hz, 1H), 4.73 (d, *J* = 9.2 Hz, 1H), 4.45 (t, *J* = 8.0 Hz, 1H), 4.41–4.36 (m, 1H), 4.19 (dd, *J* = 9.1, 1.9 Hz, 1H), 3.85 (dt, *J* = 11.7, 4.1 Hz, 2H), 3.78–3.67 (m, 4H), 3.38–3.30 (m, 2H), 3.11 (br d, *J* = 10.1 Hz, 1H), 3.02 (dd, *J* = 9.8, 1.8 Hz, 1H), 2.43 (s, 3H), 2.08–1.98 (m, 6H), 1.95–1.85 (m, 2H), 1.76–1.63 (m, 3H), 1.40–1.33 (m, 1H), 1.26–1.13 (m, 4H), 1.06–0.98 (m, 9H), 0.68–0.49 (m, 4H);

¹³C NMR (126 MHz, DMSO-*d*₆, 60 °C) δ 172.34, 169.58, 166.03, 161.65, 160.35 (d, ¹*J*_{C-F} = 242.3 Hz), 159.95, 150.96, 149.51, 147.53, 145.61, 143.62, 141.08, 138.15 (d, ³*J*_{C-F} = 14.0 Hz), 135.41, 134.15 (d, ⁴*J*_{C-F} = 2.0 Hz), 133.13, 132.52, 131.20, 130.02 (d, ³*J*_{C-F} = 7.0 Hz), 128.36, 128.30 (2C), 127.09 (2C), 126.33 (2C), 124.86 (2C), 120.24, 117.03 (d, ²*J*_{C-F} = 20.9 Hz), 115.48, 114.51, 94.60 (d, ²*J*_{C-F} = 27.9 Hz), 73.81, 70.54, 68.99, 65.00, 64.97, 61.66, 59.98, 58.90, 57.30, 56.38, 56.15, 50.54, 37.58, 36.01, 35.54, 33.72, 32.15, 32.10, 26.45 (3C), 19.33, 18.58, 15.81, 13.20, 11.83 (d, ³*J*_{C-F} = 4.5 Hz), 8.22, 7.98;

¹⁹F NMR (470 MHz, DMSO-*d*₆, 60 °C) δ –119.3 (C₆H₅CF₃ was used as internal standard.);

HRMS (ESI) *m/z* [M+H]⁺ calculated for C₆₁H₆₈FN₁₀O₇S, 1103.4972; found, 1103.4970.



N-{4-[(*(7M)*-6-Cyclopropyl-4-[(*(1S,4S)*-2,5-diazabicyclo[2.2.1]heptan-2-yl]-7-(6-fluoro-5-methyl-1*H*-indazol-4-yl)-2-[(oxan-4-yl)oxy]quinazolin-8-yl}oxy)methyl]benzoyl}-*L*-valyl-(4*R*)-4-hydroxy-*N*-{1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]cyclopropyl}-*L*-prolinamide (**3**):

To a solution of **S31** (120 mg, 0.0838 mmol) in CH₂Cl₂ (1 mL) and MeOH (1 mL) was added hydrogen chloride (4 M DOX solution, 1 mL, 4 mmol) under ice-bath cooling, and the mixture was stirred at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (ODS, 0.1% formic acid in H₂O/0.1% formic acid in MeCN = 90/10 to 65/35). Saturated aqueous sodium hydrogen carbonate solution was added to fractions containing the target compound, and the mixture was extracted twice with CHCl₃/MeOH (9/1). The combined organic layer was dried over anhydrous sodium sulfate, and the solution was concentrated under reduced pressure. Diethyl ether was added to the residue and the resultant precipitate was collected by filtration, washed with diethyl ether, and dried to give the title compound (64 mg, 70%) as a solid.

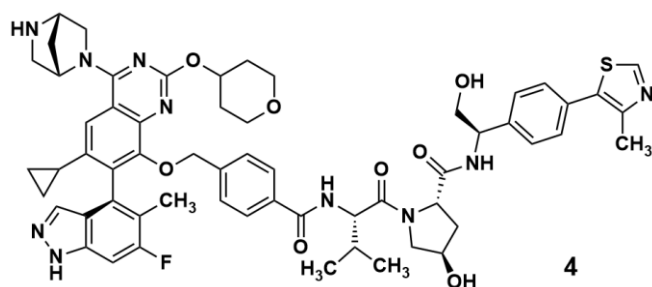
¹H NMR (500 MHz, DMSO-*d*₆, 60 °C) δ 12.95 (s, 1H), 8.92 (s, 1H), 8.54 (s, 1H), 8.09 (d, *J* = 8.6 Hz, 1H), 7.66 (d, *J* = 8.3 Hz, 2H), 7.46 (s, 2H), 7.37–7.31 (m, 3H), 7.30–7.25 (m, 2H), 6.81 (d, *J* = 8.3 Hz, 2H), 5.30 (d, *J* = 11.9 Hz, 1H), 5.19–5.12 (m, 1H), 5.10–5.07 (m, 1H), 4.96 (d, *J* = 3.8 Hz, 1H), 4.80 (d, *J* = 11.9 Hz, 1H), 4.53 (t, *J* = 8.3 Hz, 1H), 4.42 (t, *J* = 7.8 Hz, 1H), 4.41–4.36 (m, 1H), 4.19 (dd, *J* = 9.2, 1.8 Hz, 1H), 3.88–3.82 (m, 2H), 3.77–3.68 (m, 4H), 3.39–3.30 (m, 2H), 3.11 (d, *J* = 9.8 Hz, 1H), 3.02 (dd, *J* = 9.6, 1.8 Hz, 1H), 2.44 (s, 3H), 2.20–2.11 (m, 1H), 2.09–1.97 (m, 6H), 1.96–1.89 (m, 1H), 1.89–1.84 (m, 1H), 1.75–1.63 (m, 3H), 1.40–1.33 (m, 1H), 1.25–1.13 (m, 4H), 0.94 (d,

$J = 6.7$ Hz, 3H), 0.91 (d, $J = 6.7$ Hz, 3H), 0.68–0.50 (m, 4H);

^{13}C NMR (126 MHz, DMSO- d_6 , 60 °C) δ 172.09, 170.25, 165.95, 161.65, 160.35 (d, $^1J_{\text{C-F}} = 241.8$ Hz), 159.95, 150.97, 149.52, 147.58, 145.62, 143.63, 141.03, 138.14 (d, $^3J_{\text{C-F}} = 13.5$ Hz), 135.41, 134.15 (d, $^4J_{\text{C-F}} = 2.0$ Hz), 133.09, 132.50, 131.17, 130.02 (d, $^3J_{\text{C-F}} = 6.5$ Hz), 128.43, 128.36 (2C), 127.09 (2C), 126.28 (2C), 124.87 (2C), 120.25, 117.04 (d, $^2J_{\text{C-F}} = 20.9$ Hz), 115.48, 114.51, 94.59 (d, $^2J_{\text{C-F}} = 27.9$ Hz), 73.82, 70.54, 68.89, 65.00, 64.97, 61.66, 59.98, 58.83, 56.50, 56.15, 55.43, 50.53, 37.59, 36.01, 33.71, 32.14, 32.10, 30.14, 18.99, 18.57 (2C), 18.50, 15.82, 13.19, 11.83 (d, $^3J_{\text{C-F}} = 5.0$ Hz), 8.23, 7.98;

^{19}F NMR (470 MHz, DMSO- d_6 , 60 °C) δ -119.3 ($\text{C}_6\text{H}_5\text{CF}_3$ was used as internal standard.);

HRMS (ESI) m/z $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{60}\text{H}_{66}\text{FN}_{10}\text{O}_7\text{S}$, 1089.4815; found, 1089.4811.



N-{4-[(*(7M)*-6-Cyclopropyl-4-[(*(1S,4S)*)-2,5-diazabicyclo[2.2.1]heptan-2-yl]-7-(6-fluoro-5-methyl-1*H*-indazol-4-yl)-2-[(oxan-4-yl)oxy]quinazolin-8-yl}oxy)methyl]benzoyl}-*L*-valyl-(*(4R)*)-4-hydroxy-*N*-(*(1R)*)-2-hydroxy-1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]ethyl}-*L*-prolinamide (**4**):

To a solution of **S32** (172 mg, 0.120 mmol) in CH_2Cl_2 (1 mL) and MeOH (1 mL) was added hydrogen chloride (4 M DOX solution, 1 mL, 4 mmol) under ice-bath cooling, and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (ODS, 0.1% formic acid in H_2O /0.1% formic acid in MeCN = 90/10 to 65/35). Saturated aqueous sodium hydrogen carbonate solution was

added to fractions containing the target compound, and the mixture was extracted twice with $\text{CHCl}_3/\text{MeOH}$ (9/1). The combined organic layer was dried over anhydrous sodium sulfate, and the solution was concentrated under reduced pressure. Diethyl ether was added to the residue and the resultant precipitate was collected by filtration, washed with diethyl ether, and dried to give the title compound (55 mg, 42%) as a solid.

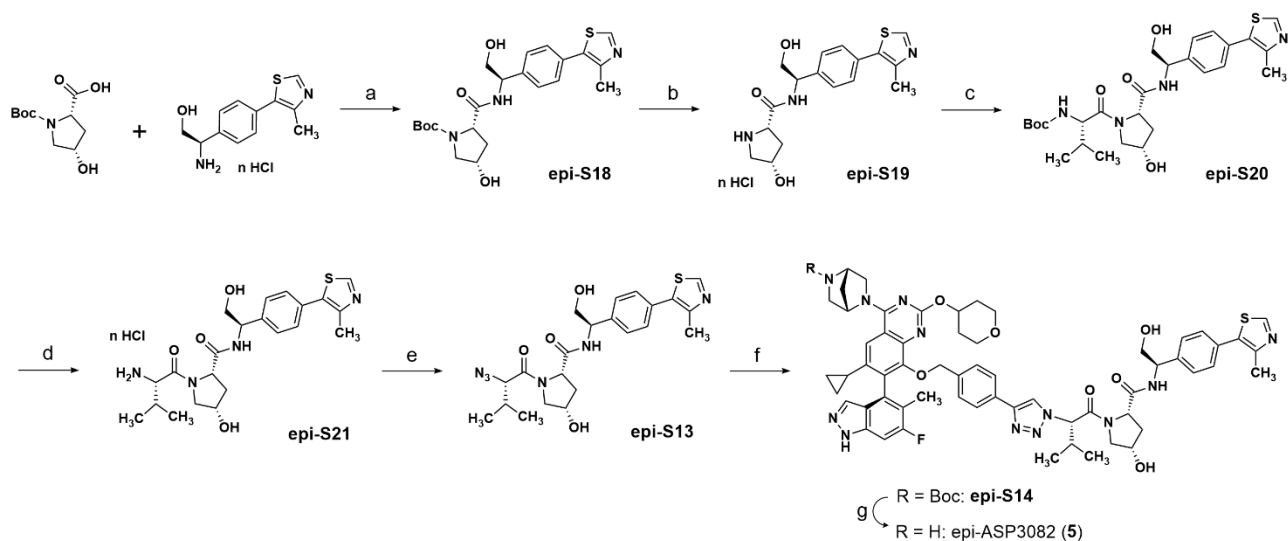
^1H NMR (500 MHz, $\text{DMSO}-d_6$, 60 °C) δ 12.95 (s, 1H), 8.94 (s, 1H), 8.14 (d, $J = 8.0$ Hz, 1H), 8.09 (d, $J = 8.3$ Hz, 1H), 7.65 (d, $J = 8.4$ Hz, 2H), 7.47–7.45 (m, 2H), 7.44–7.38 (m, 4H), 7.34 (d, $J = 9.9$ Hz, 1H), 6.81 (d, $J = 8.4$ Hz, 2H), 5.30 (d, $J = 11.9$ Hz, 1H), 5.19–5.12 (m, 1H), 5.10–5.07 (m, 1H), 4.95 (d, $J = 4.0$ Hz, 1H), 4.90–4.84 (m, 1H), 4.80 (d, $J = 11.9$ Hz, 1H), 4.63 (t, $J = 5.7$ Hz, 1H), 4.55–4.48 (m, 2H), 4.36–4.30 (m, 1H), 4.19 (dd, $J = 9.2, 1.8$ Hz, 1H), 3.89–3.82 (m, 2H), 3.78–3.59 (m, 6H), 3.38–3.30 (m, 2H), 3.10 (d, $J = 9.2$ Hz, 1H), 3.02 (dd, $J = 9.7, 1.8$ Hz, 1H), 2.46 (s, 3H), 2.19–2.10 (m, 1H), 2.08–1.97 (m, 6H), 1.92–1.85 (m, 2H), 1.76–1.63 (m, 3H), 1.40–1.33 (m, 1H), 0.97 (d, $J = 6.7$ Hz, 3H), 0.92 (d, $J = 6.7$ Hz, 3H), 0.68–0.50 (m, 4H);

^{13}C NMR (126 MHz, $\text{DMSO}-d_6$, 60 °C) δ 170.97, 170.38, 165.95, 161.65, 160.36 (d, $^1J_{\text{C-F}} = 242.3$ Hz), 159.95, 151.17, 149.51, 147.77, 145.62, 141.05, 141.00, 138.16 (d, $^3J_{\text{C-F}} = 14.5$ Hz), 135.42, 134.15 (d, $^4J_{\text{C-F}} = 2.0$ Hz), 133.07, 132.48, 131.10, 130.03 (d, $^3J_{\text{C-F}} = 7.0$ Hz), 129.93, 128.53 (2C), 127.43 (2C), 127.09 (2C), 126.28 (2C), 120.25, 117.03 (d, $^2J_{\text{C-F}} = 20.9$ Hz), 115.48, 114.50, 94.60 (d, $^2J_{\text{C-F}} = 27.9$ Hz), 73.80, 70.54, 68.74, 65.00, 64.97, 64.57, 61.65, 59.97, 58.80, 56.64, 56.15, 55.37, 54.68, 50.53, 37.64, 36.01, 32.14, 32.10, 30.06, 19.03, 18.60, 15.86, 13.19, 11.83 (d, $^3J_{\text{C-F}} = 5.0$ Hz), 8.22, 7.98;

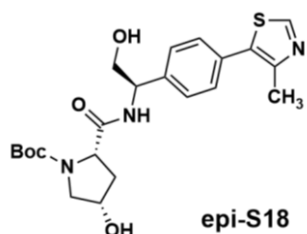
^{19}F NMR (470 MHz, $\text{DMSO}-d_6$, 60 °C) δ -119.3 ($\text{C}_6\text{H}_5\text{CF}_3$ was used as internal standard.);

HRMS (ESI) m/z $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{59}\text{H}_{66}\text{FN}_{10}\text{O}_8\text{S}$, 1093.4764; found, 1093.4756.

Synthesis of epi-ASP3082 (**5**)



Reagents and conditions: a) HATU, DIPEA, DMF, 0 °C to rt; b) 4 M HCl in DOX, CH₂Cl₂, MeOH, 0 °C to rt; c) *N*-Boc-L-valine, HATU, DIPEA, DMF, 0 °C to rt; d) 4 M HCl in DOX, CH₂Cl₂, MeOH, 0 °C to rt; e) 2-azido-1,3-dimethylimidazolinium hexafluorophosphate (ADMP), Et₃N, DMAc, MeCN, −6 to 0 °C; f) **S12**, CuI, sodium ascorbate, *t*-BuOH, THF, water, rt; g) 4 M HCl in DOX, CH₂Cl₂, MeOH, 0 °C to rt.



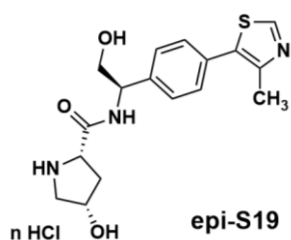
tert-Butyl (2*S*,4*S*)-4-hydroxy-2-((1*R*)-2-hydroxy-1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]ethyl)carbamoylpyrrolidine-1-carboxylate (**epi-S18**):

To a mixture of (2*S*,4*S*)-1-(*tert*-butoxycarbonyl)-4-hydroxypyrrolidine-2-carboxylic acid

(160 mg, 0.692 mmol), (2*R*)-2-amino-2-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]ethanol hydrochloride (200 mg, 0.651 mmol) and DMF (2 mL) were added DIPEA (450 μ L, 2.63 mmol) and HATU (265 mg, 0.697 mmol) under ice-bath cooling. The mixture was stirred at room temperature for 1 h. Water and saturated aqueous sodium hydrogen carbonate solution were added and the mixture was extracted three times with ethyl acetate/isopropyl alcohol (9/1). The combined organic layer was washed with saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($\text{CHCl}_3/\text{MeOH} = 100/0$ to 90/10) to give the title compound (279 mg, 96%) as a solid.

^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 8.98 (s, 1H), 8.33–8.19 (m, 1H), 7.47–7.38 (m, 4H), 5.23–5.12 (m, 1H), 4.93–4.85 (m, 1H), 4.85–4.75 (m, 1H), 4.22–4.11 (m, 2H), 3.71–3.58 (m, 2H), 3.49 (dd, $J = 10.8$, 5.4 Hz, 1H), 3.26–3.15 (m, 1H), 2.45 (s, 3H), 2.40–2.27 (m, 1H), 1.76–1.65 (m, 1H), 1.47–1.26 (m, 9H);

MS (ESI) $m/z = 448.3$ $[\text{M}+\text{H}]^+$.



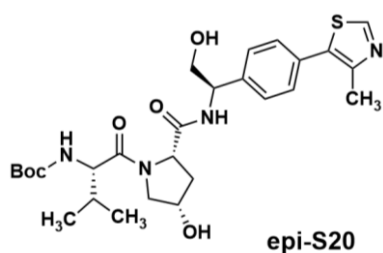
(4*S*)-4-Hydroxy-*N*-{(1*R*)-2-hydroxy-1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]ethyl}-L-prolinamide hydrochloride (**epi-S19**):

The title compound was prepared from **epi-S18** in a similar manner as described for compound **S19**, with a yield of 84% as a solid.

^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 10.03–9.93 (m, 1H), 9.11 (d, $J = 7.8$ Hz, 1H), 9.04 (s, 1H), 8.57–

8.46 (m, 1H), 7.48–7.40 (m, 4H), 4.96–4.90 (m, 1H), 4.39–4.27 (m, 2H), 3.69–3.59 (m, 2H), 3.27–3.18 (m, 1H), 3.14–3.06 (m, 1H), 2.57 (ddd, $J = 13.5, 9.7, 5.5$ Hz, 1H), 2.47 (s, 3H), 1.93–1.86 (m, 1H);

MS (ESI) $m/z = 348.3$ $[M+H]^+$.

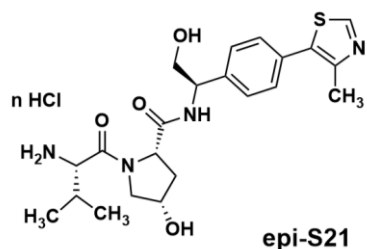


N-(*tert*-Butoxycarbonyl)-L-valyl-(4*S*)-4-hydroxy-*N*-{(1*R*)-2-hydroxy-1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]ethyl}-L-prolinamide (**epi-S20**) :

The title compound was prepared from **epi-S19** and *N*-(*tert*-butoxycarbonyl)-L-valine in a similar manner as described for compound **S20**, with a quantitative yield as a form-like solid.

^1H NMR (500 MHz, DMSO- d_6) δ 8.98 (s, 1H), 8.34 (d, $J = 7.8$ Hz, 1H), 7.45–7.36 (m, 4H), 6.83 (d, $J = 8.3$ Hz, 1H), 5.30 (d, $J = 6.7$ Hz, 1H), 4.89–4.82 (m, 1H), 4.78 (t, $J = 5.7$ Hz, 1H), 4.40 (dd, $J = 8.6, 5.9$ Hz, 1H), 4.26–4.16 (m, 1H), 3.96 (t, $J = 8.1$ Hz, 1H), 3.90 (dd, $J = 10.2, 5.6$ Hz, 1H), 3.65–3.56 (m, 2H), 3.38–3.33 (m, 1H), 2.46 (s, 3H), 2.37–2.28 (m, 1H), 1.97–1.86 (m, 1H), 1.71–1.62 (m, 1H), 1.37 (s, 9H), 0.90 (d, $J = 6.7$ Hz, 3H), 0.86 (d, $J = 6.6$ Hz, 3H); Minor peaks derived from rotamers are omitted.

MS (ESI) $m/z = 547.5$ $[M+H]^+$.

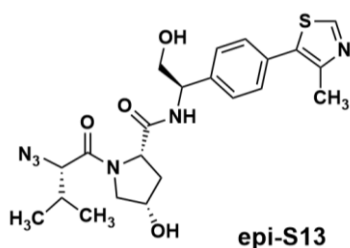


L-Valyl-(4*S*)-4-hydroxy-*N*-{(1*R*)-2-hydroxy-1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]ethyl}-L-prolinamide hydrochloride (**epi-S21**) :

The title compound was prepared from **epi-S20** in a similar manner as described for compound **S21**, with a yield of 94% as a solid.

¹H NMR (500 MHz, DMSO-*d*₆) δ 9.01 (s, 1H), 8.50 (d, *J* = 7.8 Hz, 1H), 8.11 (d, *J* = 4.3 Hz, 3H), 7.46–7.41 (m, 2H), 7.41–7.36 (m, 2H), 4.87–4.80 (m, 1H), 4.47 (dd, *J* = 8.5, 7.3 Hz, 1H), 4.25–4.17 (m, 1H), 4.01–3.97 (m, 1H), 3.95–3.91 (m, 1H), 3.66–3.58 (m, 2H), 3.24 (dd, *J* = 10.1, 6.4 Hz, 1H), 2.46 (s, 3H), 2.45–2.37 (m, 1H), 2.16–2.06 (m, 1H), 1.67–1.60 (m, 1H), 1.01 (d, *J* = 7.0 Hz, 3H), 0.95 (d, *J* = 6.9 Hz, 3H); Minor peaks derived from rotamers are omitted.

MS (ESI) *m/z* = 447.2 [M+H]⁺.



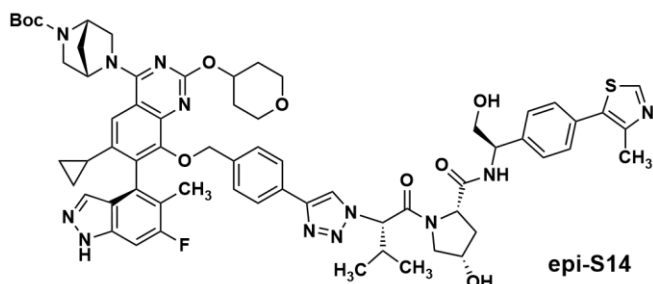
(4*S*)-1-[(2*S*)-2-Azido-3-methylbutanoyl]-4-hydroxy-*N*-{(1*R*)-2-hydroxy-1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]ethyl}-L-prolinamide (**epi-S13**) :

The title compound was prepared from **epi-S21** in a similar manner as described for

compound **S13**, with a yield of 80% (impure) as an oil.

^1H NMR (500 MHz, DMSO- d_6) δ 8.98 (s, 1H), 8.45 (d, J = 7.8 Hz, 1H), 7.46–7.42 (m, 2H), 7.41–7.37 (m, 2H), 5.27 (d, J = 6.9 Hz, 1H), 4.88–4.82 (m, 2H), 4.45 (dd, J = 8.7, 6.3 Hz, 1H), 4.26–4.18 (m, 1H), 3.86–3.79 (m, 2H), 3.68–3.58 (m, 2H), 3.30–3.24 (m, 1H), 2.46 (s, 3H), 2.42–2.35 (m, 1H), 2.11–2.01 (m, 1H), 1.62 (dt, J = 12.7, 6.3 Hz, 1H), 1.01–0.93 (m, 6H); Minor peaks derived from rotamers are omitted.

MS (ESI) m/z = 473.2 $[\text{M}+\text{H}]^+$.



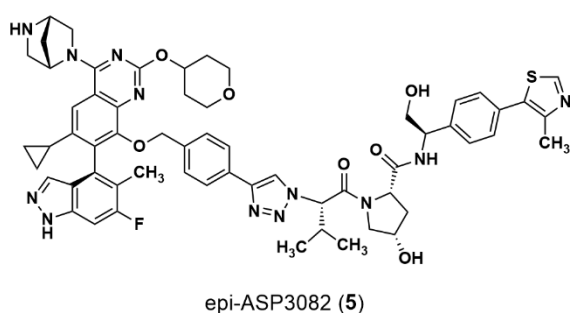
tert-Butyl (1*S*,4*S*)-5-((7*M*)-6-cyclopropyl-7-(6-fluoro-5-methyl-1*H*-indazol-4-yl)-8-{[4-(1-((2*S*)-1-[(2*S*,4*S*)-4-hydroxy-2-((1*R*)-2-hydroxy-1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]ethyl)carbamoyl)pyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl)-1*H*-1,2,3-triazol-4-yl)phenyl]methoxy}-2-[(oxan-4-yl)oxy]quinazolin-4-yl)-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate (**epi-S14**) :

The title compound was prepared from **S12** and **epi-S13** in a similar manner as described for compound **S14**, with a yield of 86% as a solid.

^1H NMR (500 MHz, DMSO- d_6) δ 13.09 (s, 1H), 8.99 (s, 1H), 8.66 (s, 1H), 8.48 (d, J = 7.8 Hz, 1H), 7.68 (d, J = 8.1 Hz, 2H), 7.53 (s, 1H), 7.47–7.37 (m, 6H), 6.80 (d, J = 8.1 Hz, 2H), 5.39 (d, J = 10.1 Hz, 1H), 5.33–5.25 (m, 2H), 5.23–5.14 (m, 2H), 4.90–4.84 (m, 2H), 4.74–4.69 (m, 1H), 4.64–4.53 (m, 1H), 4.41–4.33 (m, 2H), 4.27–4.21 (m, 1H), 3.93 (dd, J = 10.4, 5.8 Hz, 1H), 3.89–3.82 (m, 2H),

3.82–3.70 (m, 1H), 3.69–3.55 (m, 3H), 3.55–3.47 (m, 2H), 3.39–3.32 (m, 2H), 2.57–2.52 (m, 1H), 2.47 (s, 3H), 2.40–2.33 (m, 1H), 2.13–2.07 (m, 1H), 2.07–1.90 (m, 6H), 1.73–1.61 (m, 3H), 1.46–1.33 (m, 10H), 1.08 (d, $J = 6.7$ Hz, 3H), 0.74 (d, $J = 6.6$ Hz, 3H), 0.71–0.50 (m, 4H); Minor peaks derived from rotamers are omitted.

MS (ESI) $m/z = 1217.5$ $[M+H]^+$.



(4*S*)-1-[(2*S*)-2-(4-{4-[(*(7M)*)-6-Cyclopropyl-4-[(1*S*,4*S*)-2,5-diazabicyclo[2.2.1]heptan-2-yl]-7-(6-fluoro-5-methyl-1*H*-indazol-4-yl)-2-[(oxan-4-yl)oxy]quinazolin-8-yl}oxy)methyl]phenyl]-1*H*-1,2,3-triazol-1-yl)-3-methylbutanoyl]-4-hydroxy-*N*-{(1*R*)-2-hydroxy-1-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]ethyl}-L-prolinamide (epi-ASP3082, **5**):

The title compound was prepared from **epi-S14** in a similar manner as described for compound **4**, with a yield of 40% as a solid.

^1H NMR (500 MHz, DMSO- d_6 , 60 °C) δ 12.97 (s, 1H), 8.94 (s, 1H), 8.56 (s, 1H), 8.29 (d, $J = 7.8$ Hz, 1H), 7.67–7.62 (m, 2H), 7.48 (d, $J = 0.9$ Hz, 1H), 7.46 (s, 1H), 7.46–7.39 (m, 4H), 7.36 (d, $J = 9.9$ Hz, 1H), 6.81 (d, $J = 8.4$ Hz, 2H), 5.36 (d, $J = 9.8$ Hz, 1H), 5.27 (d, $J = 11.3$ Hz, 1H), 5.24–5.16 (m, 2H), 5.10 (s, 1H), 4.93–4.86 (m, 1H), 4.76 (d, $J = 11.5$ Hz, 1H), 4.70 (br s, 1H), 4.41 (dd, $J = 9.0, 5.4$ Hz, 1H), 4.29–4.17 (m, 2H), 3.92–3.83 (m, 3H), 3.78–3.72 (m, 2H), 3.72–3.59 (m, 3H), 3.41–3.32 (m, 2H), 3.12 (d, $J = 9.8$ Hz, 1H), 3.03 (dd, $J = 9.6, 1.3$ Hz, 1H), 2.60–2.51 (m, 1H), 2.47 (s, 3H), 2.39–2.31 (m, 1H), 2.11–2.01 (m, 2H), 2.01 (d, $J = 2.4$ Hz, 3H), 1.88 (d, $J = 9.2$ Hz, 1H), 1.77–1.64

(m, 4H), 1.41–1.34 (m, 1H), 1.08 (d, $J = 6.7$ Hz, 3H), 0.76 (d, $J = 6.6$ Hz, 3H), 0.69–0.50 (m, 4H);

Minor peaks derived from rotamers are omitted.;

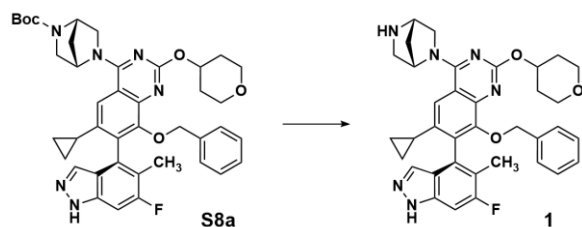
^{13}C NMR (126 MHz, DMSO- d_6 , 60 °C) δ 171.15, 166.36, 161.69, 160.38 (d, $^1J_{\text{C-F}} = 241.8$ Hz), 159.94, 151.20, 149.64, 147.79, 146.14, 145.67, 140.80, 138.17 (d, $^3J_{\text{C-F}} = 14.0$ Hz), 137.41, 135.43, 134.20 (d, $^4J_{\text{C-F}} = 2.5$ Hz), 132.52, 131.08, 130.10 (d, $^3J_{\text{C-F}} = 7.0$ Hz), 129.99, 129.83, 128.55 (2C), 127.41 (2C), 127.26 (2C), 124.75 (2C), 120.29, 120.23, 116.97 (d, $^2J_{\text{C-F}} = 20.4$ Hz), 115.41, 114.51, 94.57 (d, $^2J_{\text{C-F}} = 27.9$ Hz), 74.10, 70.52, 68.95, 66.29, 65.02, 64.98, 64.35, 61.63, 59.96, 59.10, 56.16, 55.39, 54.77, 50.53, 36.98, 36.00, 32.15, 32.11, 31.38, 18.31, 18.25, 15.86, 13.20, 11.85 (d, $^3J_{\text{C-F}} = 5.0$ Hz), 8.22, 7.99;

^{19}F NMR (470 MHz, DMSO- d_6 , 60 °C) δ –119.4 ($\text{C}_6\text{H}_5\text{CF}_3$ was used as internal standard.);

HRMS (ESI) m/z $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{60}\text{H}_{66}\text{FN}_{12}\text{O}_7\text{S}$, 1117.4877; found, 1117.4861.

Synthesis of Inhibitors

Synthesis of compound 1



Reagents and conditions: 4 M HCl in DOX, CH₂Cl₂, MeOH, 0 °C to rt.

(7*M*)-8-(Benzyloxy)-6-cyclopropyl-4-[(1*S*,4*S*)-2,5-diazabicyclo[2.2.1]heptan-2-yl]-7-(6-fluoro-5-methyl-1*H*-indazol-4-yl)-2-[(oxan-4-yl)oxy]quinazoline (**1**):

To a solution of **S8a** (75 mg, 0.104 mmol) in CH₂Cl₂ (1 mL) and MeOH (1 mL) was added hydrogen chloride (4 M DOX solution, 1 mL, 4 mmol) under ice-bath cooling, and the mixture was stirred at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure. Saturated aqueous sodium hydrogen carbonate solution was added to the residue, and the mixture was extracted twice with CHCl₃/MeOH (9/1). The combined organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (amino silica gel, CHCl₃/MeOH = 100/0 to 97/3). To the obtained material was added diethyl ether and the resultant precipitate was collected by filtration, washed with diethyl ether, and dried to give the title compound (38 mg, 59%) as a solid.

¹H NMR (500 MHz, DMSO-*d*₆) δ 13.11 (s, 1H), 7.48 (d, *J* = 0.8 Hz, 1H), 7.45 (s, 1H), 7.37 (d, *J* = 9.9 Hz, 1H), 7.20–7.11 (m, 3H), 6.79–6.73 (m, 2H), 5.25 (d, *J* = 11.2 Hz, 1H), 5.20–5.12 (m, 1H), 5.05 (s, 1H), 4.70 (d, *J* = 11.2 Hz, 1H), 4.23 (d, *J* = 8.3 Hz, 1H), 3.88–3.80 (m, 2H), 3.77–3.71 (m, 2H), 3.33–3.24 (m, 2H), 3.09 (d, *J* = 9.6 Hz, 1H), 2.99 (d, *J* = 8.6 Hz, 1H), 2.10–1.99 (m, 2H), 1.98 (d, *J* = 2.4 Hz, 3H), 1.88 (d, *J* = 9.3 Hz, 1H), 1.72 (d, *J* = 9.3 Hz, 1H), 1.71–1.61 (m, 2H), 1.38–1.31 (m, 1H), 0.69–0.50 (m, 4H);

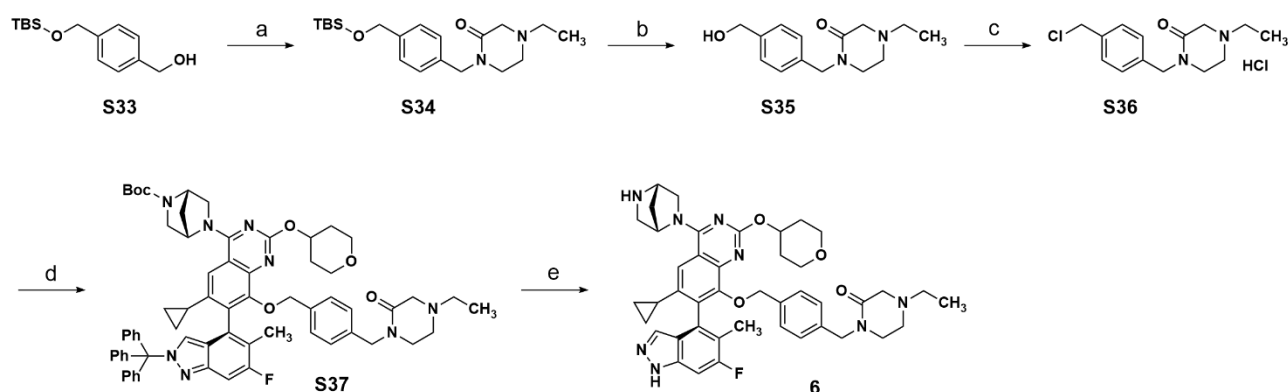
¹³C NMR (126 MHz, DMSO-*d*₆) δ 161.57, 159.78, 159.29 (d, ¹*J*_{C-F} = 242.3 Hz), 149.52, 145.45,

137.96 (d, $^3J_{C-F}$ = 13.5 Hz), 137.64, 135.36, 134.16 (d, $^4J_{C-F}$ = 2.5 Hz), 132.45, 129.93 (d, $^3J_{C-F}$ = 7.0 Hz), 127.83 (2C), 127.30, 126.72 (2C), 120.14, 116.95 (d, $^2J_{C-F}$ = 20.9 Hz), 115.30, 114.47, 94.53 (d, $^2J_{C-F}$ = 27.9 Hz), 74.26, 70.47, 65.04, 64.96, 61.74, 59.85, 56.08, 50.36, 35.80, 32.06, 32.02, 13.14, 11.88 (d, $^3J_{C-F}$ = 4.5 Hz), 8.30, 8.10;

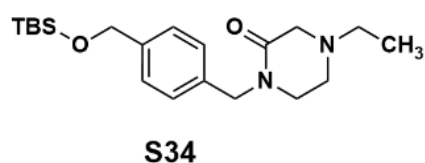
^{19}F NMR (470 MHz, DMSO- d_6 , 60 °C) δ -119.4 ($\text{C}_6\text{H}_5\text{CF}_3$ was used as internal standard.);

HRMS (ESI) m/z $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{36}\text{H}_{38}\text{FN}_6\text{O}_3$, 621.2984; found, 621.2975.

Synthesis of compound **6**



Reagents and conditions: a) methanesulfonic anhydride (Ms_2O), DIPEA, CH_2Cl_2 , 0 °C, then 4-ethylpiperazin-2-one, $t\text{-BuOK}$, DMF, 0 °C to rt; b) 1 M TBAF in THF, THF, rt; c) SOCl_2 , CH_2Cl_2 , 0 °C to rt; d) **S10**, Cs_2CO_3 , DMF, 0 °C to 60 °C; e) 4 M HCl in DOX, CH_2Cl_2 , MeOH, 0 °C to rt.

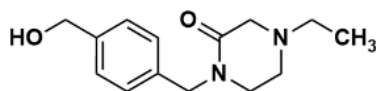


1-([4-($\{tert\text{-Butyl}(\text{dimethyl})\text{silyl}\}$ oxy)methyl]phenyl)methyl-4-ethylpiperazin-2-one (**S34**):

To a solution of [4-({*tert*-butyl(dimethyl)silyl}oxy)methyl]phenyl]methanol (**S33**) (1.5 g, 5.9 mmol) in CH₂Cl₂ (30 mL) were added methanesulfonic anhydride (1.9 g, 11 mmol) and DIPEA (2.2 mL, 13 mmol) under ice-bath cooling. The mixture was stirred under ice-bath cooling for 1 h under argon atmosphere. Water was added under ice-bath cooling, and the layers were separated. The aqueous layer was extracted twice with CH₂Cl₂. The combined organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. To the residue were added 4-ethylpiperazin-2-one (840 mg, 6.55 mmol) and DMF (30 mL), and the mixture was cooled with ice bath. *t*-BuOK (870 mg, 7.75 mmol) was added, and the mixture was stirred under ice-bath cooling for 1 h and then at room temperature for 1 h. Saturated aqueous ammonium chloride solution was added under ice-bath cooling, and the mixture was extracted with ethyl acetate. The organic layer was washed with an aqueous sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give the title compound (impure, 2.6 g, quant.) as an oil.

¹H NMR (500 MHz, DMSO-*d*₆) δ 7.27 (d, *J* = 8.0 Hz, 2H), 7.19 (d, *J* = 8.1 Hz, 2H), 4.68 (s, 2H), 4.49 (s, 2H), 3.18–3.14 (m, 2H), 3.04 (s, 2H), 2.61–2.56 (m, 2H), 2.37 (q, *J* = 7.1 Hz, 2H), 0.99 (t, *J* = 7.2 Hz, 3H), 0.90 (s, 9H), 0.07 (s, 6H);

MS (ESI) *m/z* = 363.5 [M+H]⁺.



S35

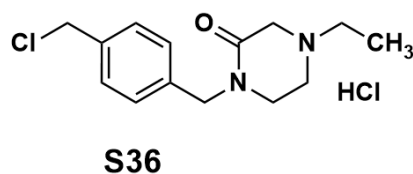
4-Ethyl-1-([4-(hydroxymethyl)phenyl]methyl)piperazin-2-one (**S35**):

To a solution of **S34** (impure, 2.2 g, 6.1 mmol) in THF (22 mL) was added TBAF (1 M THF solution, 8.0 mL, 8.0 mmol) at room temperature, and the mixture was stirred at room temperature

overnight. The reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (amino silica gel, CHCl₃/MeOH = 100/0 to 99/1) to give the title compound (impure, 1.58 g, quant.) as an oil.

¹H NMR (500 MHz, DMSO-*d*₆) δ 7.32 (d, *J* = 8.1 Hz, 2H), 7.22 (d, *J* = 8.0 Hz, 2H), 5.17 (t, *J* = 5.7 Hz, 1H), 4.52 (s, 2H), 4.51 (d, *J* = 5.7 Hz, 2H), 3.22–3.18 (m, 2H), 3.08 (s, 2H), 2.65–2.60 (m, 2H), 2.41 (q, *J* = 7.2 Hz, 2H), 1.03 (t, *J* = 7.2 Hz, 3H);

MS (ESI) *m/z* = 249.3 [M+H]⁺.

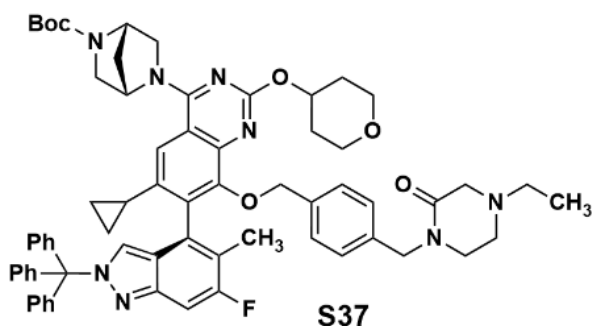


1-{[4-(Chloromethyl)phenyl]methyl}-4-ethylpiperazin-2-one hydrochloride (**S36**):

To a solution of **S35** (impure, 400 mg, 1.61 mmol) in CH₂Cl₂ (4 mL) was added thionyl chloride (981 mg, 8.25 mmol) under ice-bath cooling. The mixture was stirred at room temperature for 4 h under argon atmosphere. The mixture was concentrated under reduced pressure. To the residue was added toluene and the mixture was concentrated under reduced pressure to give the title compound (487 mg, quant.) as a foam-like solid.

¹H NMR (500 MHz, DMSO-*d*₆) δ 11.21 (br s, 1H), 7.44–7.40 (m, 2H), 7.34–7.30 (m, 2H), 4.76 (s, 2H), 4.70–4.63 (m, 1H), 4.56–4.50 (m, 1H), 4.03–3.95 (m, 1H), 3.91–3.83 (m, 1H), 3.71–3.62 (m, 2H), 3.36–3.28 (m, 2H), 3.22–3.15 (m, 2H), 1.26 (t, *J* = 7.3 Hz, 3H);

MS (ESI) *m/z* = 267.1, 269.2 [M+H]⁺.

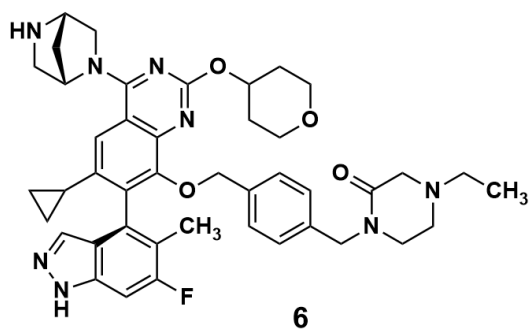


tert-Butyl (1*S*,4*S*)-5-((7*M*)-6-cyclopropyl-8-(4-[(4-ethyl-2-oxopiperazin-1-yl)methyl]phenyl}methoxy)-7-[6-fluoro-5-methyl-2-(triphenylmethyl)-2*H*-indazol-4-yl]-2-[(oxan-4-yl)oxy]quinazolin-4-yl}-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate (**S37**):

To a mixture of **S10** (500 mg, 0.573 mmol) and DMF (10 mL) was added cesium carbonate (900 mg, 2.76 mmol) followed by a solution of **S36** (310 mg, 1.02 mmol) in DMF (2 mL) under ice-bath cooling. The mixture was stirred at room temperature under for 1 h and then at 60 °C for 2 h. After cooling to room temperature, water was added and the mixture was extracted with ethyl acetate. The organic layer was washed with water and an aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (amino silica gel, hexane/ethyl acetate = 70/30 to 30/70) to give the title compound (557 mg, 88%) as an oil.

¹H NMR (500 MHz, DMSO-*d*₆) δ 7.48 (d, *J* = 11.0 Hz, 1H), 7.40–7.34 (m, 1H), 7.32–7.20 (m, 10H), 7.08–7.03 (m, 2H), 7.02–6.95 (m, 6H), 6.75–6.70 (m, 2H), 5.35–5.27 (m, 1H), 5.18–5.09 (m, 2H), 4.60–4.40 (m, 4H), 4.28 (d, *J* = 8.3 Hz, 1H), 3.88–3.82 (m, 2H), 3.74–3.62 (m, 1H), 3.51–3.41 (m, 2H), 3.35–3.27 (m, 2H), 3.11 (t, *J* = 5.5 Hz, 2H), 3.04 (s, 2H), 2.58–2.53 (m, 2H), 2.39–2.34 (m, 2H), 2.09–1.87 (m, 7H), 1.69–1.60 (m, 2H), 1.44–1.34 (m, 10H), 0.99 (t, *J* = 7.2 Hz, 3H), 0.81–0.68 (m, 2H), 0.59–0.49 (m, 1H), 0.37–0.29 (m, 1H);

MS (ESI) *m/z* = 1125.7 [M+Na]⁺.



1-({4-[({(7*M*)-6-Cyclopropyl-4-[(1*S*,4*S*)-2,5-diazabicyclo[2.2.1]heptan-2-yl]-7-(6-fluoro-5-methyl-1*H*-indazol-4-yl)-2-[(oxan-4-yl)oxy]quinazolin-8-yl}oxy)methyl]phenyl}methyl)-4-ethylpiperazin-2-one (**6**):

To a solution of **S37** (465 mg, 0.422 mmol) in CH₂Cl₂ (5 mL) and MeOH (5 mL) was added hydrogen chloride (4 M DOX solution, 5 mL, 20 mmol) under ice-bath cooling, and the mixture was stirred at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure. Saturated aqueous sodium hydrogen carbonate solution was added to the residue, and the mixture was extracted twice with CHCl₃/MeOH (9/1). The combined organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (amino silica gel, CHCl₃/MeOH = 100/0 to 97/3) to give the title compound (235 mg, 73%) as a solid.

¹H NMR (500 MHz, DMSO-*d*₆) δ 13.10 (s, 1H), 7.46 (s, 1H), 7.44 (s, 1H), 7.37 (d, *J* = 9.8 Hz, 1H), 7.00 (d, *J* = 8.3 Hz, 2H), 6.71 (d, *J* = 8.0 Hz, 2H), 5.23 (d, *J* = 11.3 Hz, 1H), 5.18–5.12 (m, 1H), 5.05 (s, 1H), 4.68 (d, *J* = 11.3 Hz, 1H), 4.41 (s, 2H), 4.23 (d, *J* = 8.3 Hz, 1H), 3.87–3.80 (m, 2H), 3.77–3.71 (m, 2H), 3.27–3.23 (m, 2H), 3.12–3.07 (m, 3H), 3.03 (s, 2H), 3.00 (d, *J* = 8.6 Hz, 1H), 2.56 (t, *J* = 5.5 Hz, 2H), 2.36 (q, *J* = 7.0 Hz, 2H), 2.08–1.98 (m, 2H), 1.97 (d, *J* = 2.1 Hz, 3H), 1.88 (d, *J* = 9.2 Hz, 1H), 1.73 (d, *J* = 9.5 Hz, 1H), 1.70–1.60 (m, 2H), 1.37–1.30 (m, 1H), 0.99 (t, *J* = 7.2 Hz, 3H), 0.68–0.51 (m, 4H);

^{13}C NMR (126 MHz, DMSO- d_6) δ 166.06, 161.58, 160.23 (d, $^1J_{\text{C-F}} = 241.4$ Hz), 159.76, 149.52, 145.41, 137.93 (d, $^3J_{\text{C-F}} = 13.0$ Hz), 136.66, 136.25, 135.37, 134.15 (d, $^4J_{\text{C-F}} = 3.0$ Hz), 132.44, 129.92 (d, $^3J_{\text{C-F}} = 7.0$ Hz), 127.35 (2C), 126.93 (2C), 120.13, 116.95 (d, $^2J_{\text{C-F}} = 20.9$ Hz), 115.26, 114.45, 94.53 (d, $^2J_{\text{C-F}} = 27.9$ Hz), 74.05, 70.47, 65.03, 64.95, 61.55, 59.79, 56.58, 56.12, 50.41, 50.35, 48.64, 47.91, 45.43, 35.77, 32.05, 32.01, 13.14, 11.88 (d, $^3J_{\text{C-F}} = 5.0$ Hz), 11.63, 8.30, 8.10;

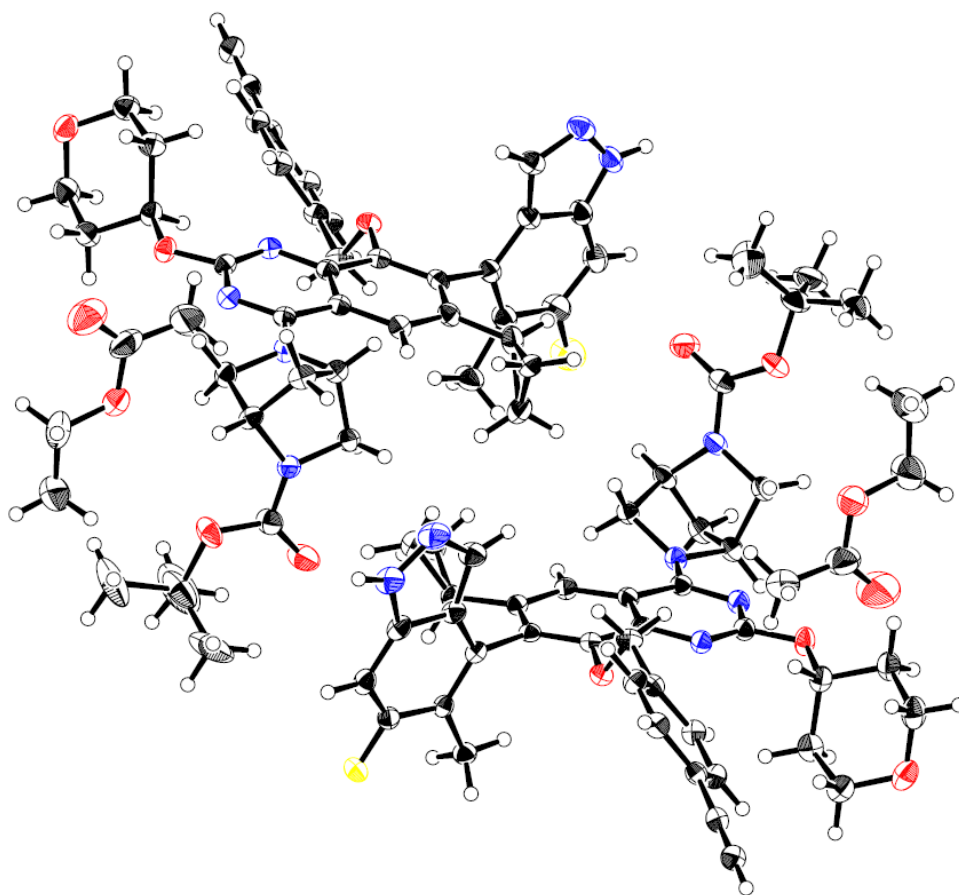
^{19}F NMR (470 MHz, DMSO- d_6 , 60 °C) δ -119.4 ($\text{C}_6\text{H}_5\text{CF}_3$ was used as internal standard.);

HRMS (ESI) m/z $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{43}\text{H}_{50}\text{FN}_8\text{O}_4$, 761.3934; found, 761.3931.

Single crystal X-ray crystallography

Supplementary Figure 8. ORTEP-style illustration of compound **S12** with ellipsoids at 50% probability.

Single crystals for X-ray studies were grown by dissolving **S12** in ethyl acetate and then slowly adding heptane and leaving it to stand. The X-ray data of **S12** is deposited in the Cambridge Crystallographic Data Centre with a number of CCDC 2412699.



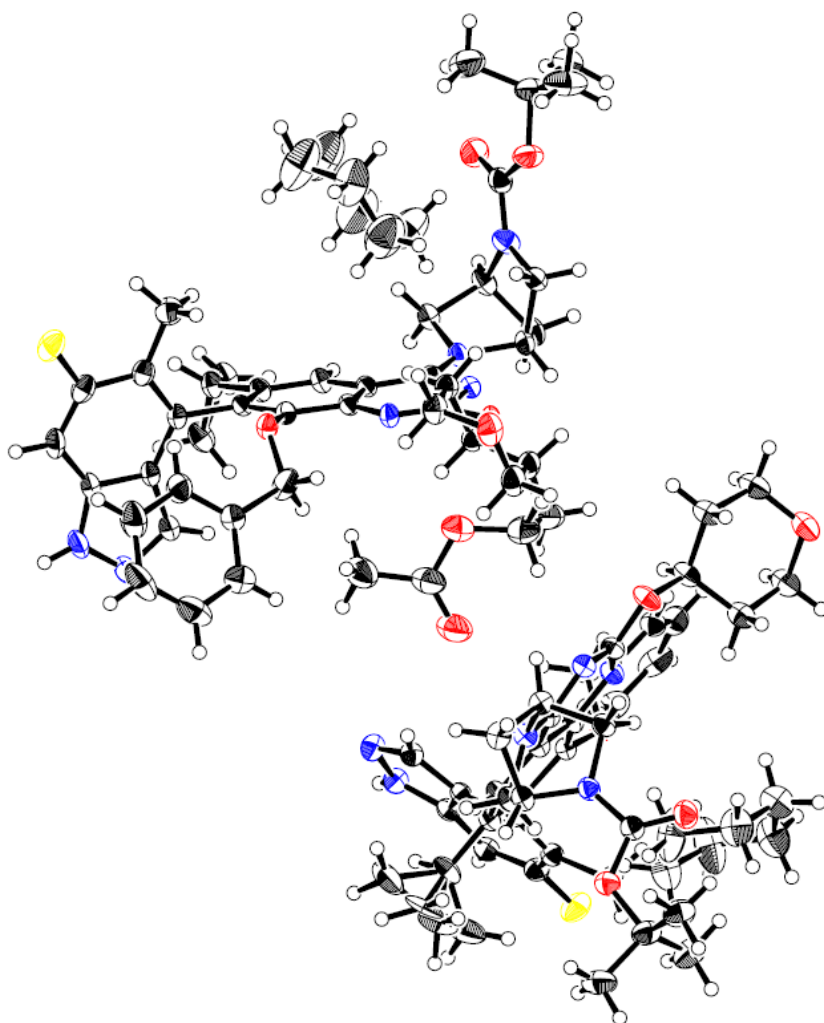
Crystal Data for C₄₇H₅₃FN₆O₇ (M.W. = 832.97):

triclinic, space group P1, $a = 13.02574(5)$ Å, $b = 13.03315(6)$ Å, $c = 13.31113(5)$ Å, $\alpha = 74.0847(4)^\circ$,
 $\beta = 81.8181(3)^\circ$, $\gamma = 86.2528(3)^\circ$, $V = 2150.218(16)$ Å³, $Z = 2$, $T = 93$ K, $\mu(\text{CuK}\alpha) = 7.383$ mm⁻¹,
 $D_{\text{calc}} = 1.286$ g/cm³. The final R_1 was 0.0321 ($I > 2\sigma(I)$) and wR_2 was 0.0889 (all data)

Identification code	
Empirical formula	C ₄₇ H ₅₃ FN ₆ O ₇
Formula weight	832.97
Temperature/K	93
Crystal system	Triclinic
Space group	P1
a/Å	13.02574(5)
b/Å	13.03315(6)
c/Å	13.31113(5)
α/°	74.0847(4)
β/°	81.8181(3)
γ/°	86.2528(3)
Volume/Å ³	2150.218(16)
Z	2
ρ _{calc} /cm ³	1.286
μ/mm ⁻¹	7.383
F(000)	884
Crystal size/mm ³	1.58 × 10 ⁻³
Radiation	CuKα (λ = 1.54184)
2θ range for data collection/°	6.858 to 148.306
Index ranges	-16 < h < 15, -16 < k < 16, -16 < l < 16
Reflections collected	206301
Independent reflections	16471
Data/restraints/parameters	16471/3/1111
Goodness-of-fit on F ²	1.011
Final R indexes [I ≥ 2σ (I)]	R ₁ = 0.0321 wR ₂ = 0.0887
Final R indexes [all data]	R ₁ = 0.0324 wR ₂ = 0.0889
Largest diff. peak/hole / e Å ⁻³	0.57/-0.28
Flack parameter ¹	-0.00(2)

Supplementary Figure 9. ORTEP-style illustration of **S8b** with ellipsoids at 50% probability

Single crystals for X-ray studies were grown by dissolving **S8b** in ethyl acetate and then slowly adding cyclohexane and leaving it to stand. The X-ray data of **S8b** is deposited in the Cambridge Crystallographic Data Centre with a number of CCDC 2412700.



Crystal Data for $C_{49}H_{61}FN_6O_6$ (M.W. = 849.06):

monoclinic, space group $P2_1$, $a = 10.25239(11)$ Å, $b = 40.5410(3)$ Å, $c = 12.03868(13)$ Å, $\alpha = 90^\circ$, $\beta = 114.0263(13)^\circ$, $\gamma = 90^\circ$, $V = 4570.25(9)$ Å³, $Z = 4$, $T = 93$ K, $\mu(\text{CuK}\alpha) = 0.684$ mm⁻¹, $D_{\text{calc}} = 1.234$ g/cm³. The final R_1 was 0.0443 ($I > 2\sigma(I)$) and wR_2 was 0.1221 (all data)

Identification code	
Empirical formula	C ₄₉ H ₆₁ FN ₆ O ₆
Formula weight	849.06
Temperature/K	93
Crystal system	monoclinic
Space group	P2 ₁
a/Å	10.25239(11)
b/Å	40.5410(3)
c/Å	12.03868(13)
α/°	90
β/°	114.0263(13)
γ/°	90
Volume/Å ³	4570.25(9)
Z	4
ρ _{calc} /g/cm ³	1.234
μ/mm ⁻¹	0.684
F(000)	1816
Crystal size/mm ³	0.112 × 10 ⁻³
Radiation	CuKα (λ = 1.54184)
2θ range for data collection/°	8.04 to 149.446
Index ranges	-11 < h < 12, -50 < k < 50, -15 < l < 14
Reflections collected	54518
Independent reflections	18129
Data/restraints/parameters	18129/4/1146
Goodness-of-fit on F ²	1.043
Final R indexes [I ≥ 2σ (I)]	R ₁ = 0.0443 wR ₂ = 0.1197
Final R indexes [all data]	R ₁ = 0.0458 wR ₂ = 0.1221
Largest diff. peak/hole / e Å ⁻³	0.56/-0.34
Flack parameter ¹	0.04(4)

Supplementary References

1. Parsons, S. & Flack, H. *Acta Cryst. A* **60**, s61 (2004).