Supporting Information

Quinoxaline-Based Linear HCV NS3/4A Protease Inhibitors Exhibit Potent Activity Against Drug Resistant Variants

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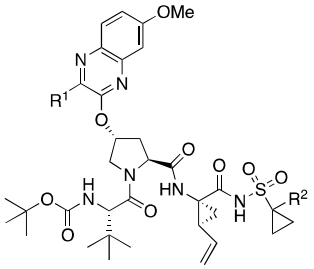
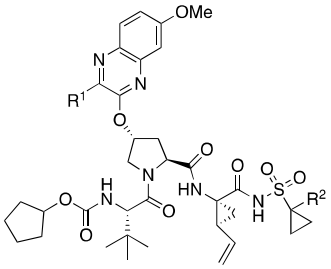
Table S1. Inhibitory activity against wild-type HCV NS3/4A protease and drug resistant variants



3, 9b-e; 10a-e



11a-e; 12a-e

Compound	R^1	R^2	*K*i (nM) (fold change)		
			WT	R155K	D168A
3	Et	H	19 ± 2.7	17 ± 2.3 (0.9)	642 ± 101 (34)
10a	Et	Me	16 ± 1.3	14 ± 1.1 (0.9)	385 ± 31 (24)
11a	Et	H	9.8 ± 2.0	15 ± 2.2 (1.5)	350 ± 30 (36)
12a	Et	Me	6.9 ± 0.5	13 ± 2.7 (1.9)	145 ± 14 (21)
9b	Me	H	18 ± 1.6	8.5 ± 2.1 (0.5)	290 ± 24 (16)
10b	Me	Me	14 ± 2.1	14 ± 1.7 (1.0)	265 ± 26 (19)
11b	Me	H	9.2 ± 0.9	9.6 ± 0.9 (1.0)	144 ± 23 (16)
12b	Me	Me	7.1 ± 1.1	10 ± 1.3 (1.4)	140 ± 13 (20)
9c	i-Pr	H	32 ± 5.1	49 ± 11 (1.5)	1086 ± 137 (34)
10c	i-Pr	Me	29 ± 9.4	27 ± 5.6 (0.9)	1179 ± 170 (41)
11c	i-Pr	H	17 ± 3.2	55 ± 11 (3)	985 ± 106 (58)
12c	i-Pr	Me	21 ± 2.6	43 ± 11 (2.0)	1000 ± 80 (48)
9d	Cl	H	7.8 ± 1.1	2.2 ± 0.4 (0.3)	128 ± 16 (16)
10d	Cl	Me	6.1 ± 1.1	3.8 ± 0.6 (0.7)	119 ± 16 (31)
11d	Cl	H	3.8 ± 0.6	4.1 ± 0.5 (1.1)	99 ± 10 (26)
12d	Cl	Me	3.9 ± 0.7	5.2 ± 0.8 (1.3)	51 ± 6.0 (13)
9e	CF3	H	87 ± 18	24 ± 3.3 (0.3)	723 ± 80 (8)
10e	CF3	Me	46 ± 9.6	12 ± 1.7 (0.3)	513 ± 50 (11)
11e	CF3	H	34 ± 8.3	26 ± 7.7 (0.8)	703 ± 63 (21)
12e	CF3	Me	22 ± 3.4	22 ± 6.9 (1.0)	516 ± 61 (24)
2			2.0 ± 0.1	3.1 ± 0.34 (1.6)	91 ± 10 (46)
1			0.20 ± 0.1	0.80 ± 0.3 (4)	40 ± 5.0 (200)

Table S2. X-ray data collection and crystallographic refinement statistics.

	WT1a-**12b**	WT1a-**12c**	WT1a-**12d**
PDB code:	6CVW	6CVX	6CVY
Resolution	1.78 Å	1.78 Å	1.80 Å
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Molecules in AU[a]	1	1	1
Cell dimensions			
a (Å)	55.2	55.3	55.5
b (Å)	58.5	58.5	58.5
c (Å)	59.9	59.8	59.7
β (°)	90	90	90
Completeness (%)	98.3	99.1	96.4
Total reflections	70790	119454	116526
Unique reflections	18870	19054	17991
Average I/σ	18.5	15.1	15.2
Redundancy	3.8	6.3	6.5
R_{sym} (%)[b]	7.8 (25.6)	7.1 (27.3)	8.5 (30.7)
RMSD[c] in			
Bond lengths (Å)	0.014	0.019	0.009
Bond angles (°)	1.4	1.5	1.1
R_{factor} (%)[d]	14.9	15.6	14.7
R_{free} (%)[e]	18.5	19.4	18.4

[a]AU, asymmetric unit.
[b]$R_{sym} = \Sigma \mid I - \langle I \rangle \mid / \Sigma I$, where I = observed intensity, $\langle I \rangle$ = average intensity over symmetry equivalent; values in parentheses are for the highest resolution shell.
[c]RMSD, root mean square deviation.
[d]$R_{factor} = \Sigma \mid\mid F_o \mid - \mid F_c \mid\mid / \Sigma \mid F_o \mid$.
[e]R_{free} was calculated from 5% of reflections, chosen randomly, which were omitted from the refinement process.

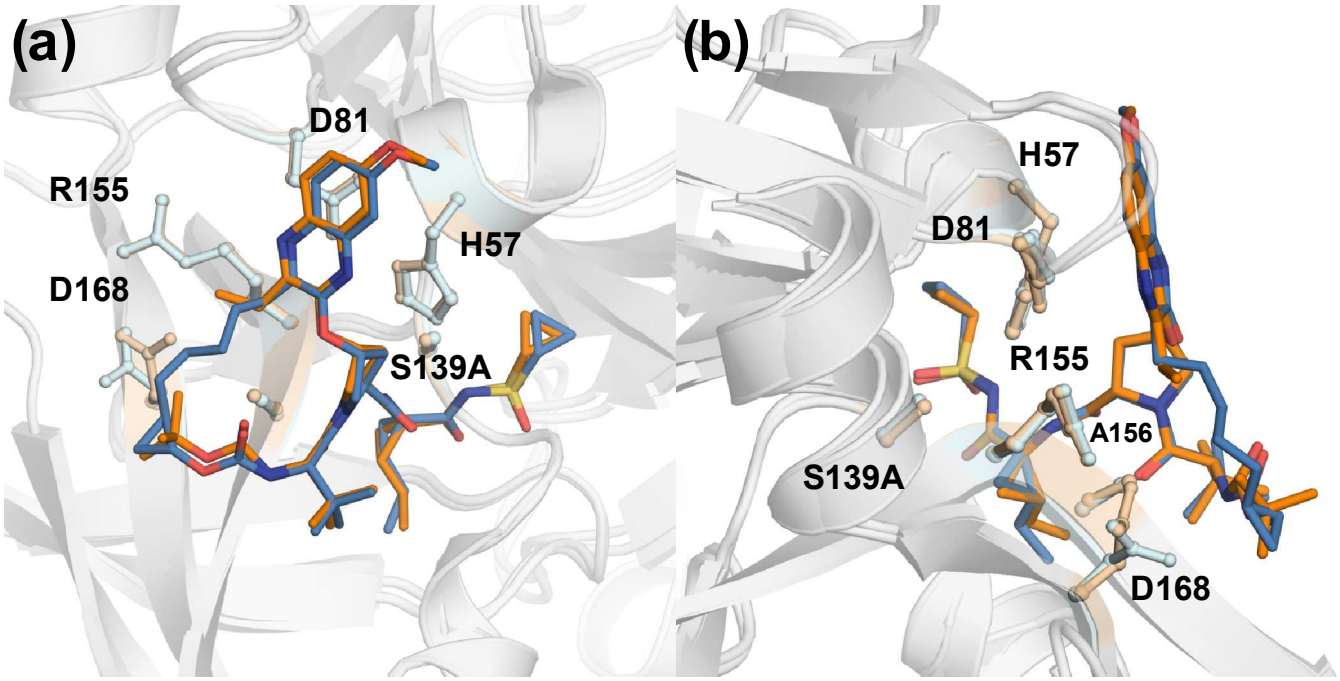


Figure S1. Superposition of WT-**1** and WT-**3** complexes, focusing on the differences at the P2 quinoxaline. The protease is in ribbon representation (light grey), with bound inhibitors **1** (blue) and **3** (orange) depicted as sticks. The side chains of catalytic triad and drug resistance residues Arg155, Ala156, and Asp168 are shown as ball and sticks.

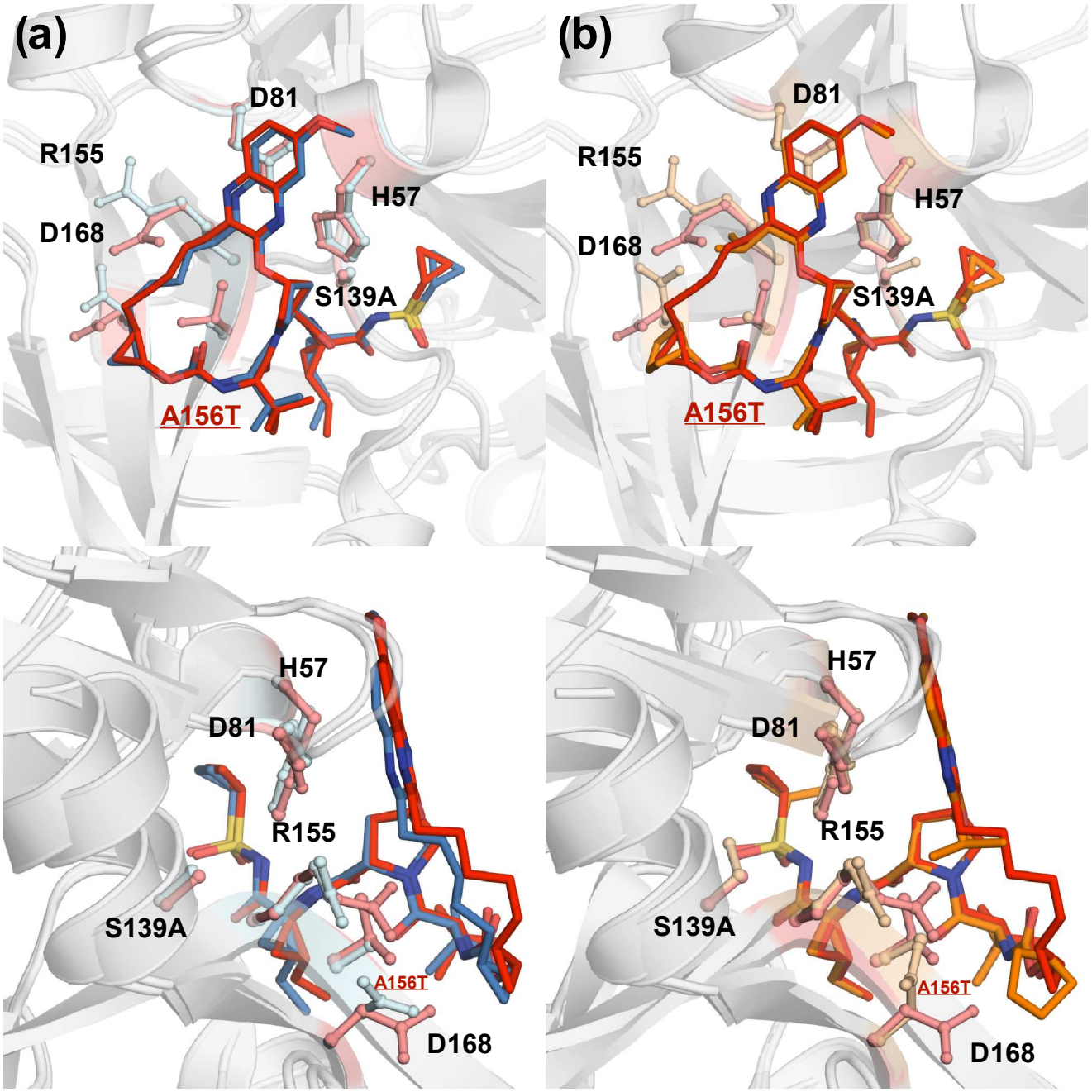


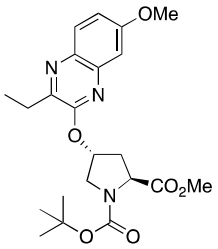
Figure S2. (a) Superposition of WT-**1** and A156T-**1** and (b) A156T-**1** and WT-**12d** complexes, focusing on the differences at the P2 quinoxaline. The protease is in ribbon representation (light grey) with bound inhibitor **1** depicted as sticks in blue (WT) and red (A156T), and inhibitor **12d** (WT) in orange. The side chains of catalytic triad and drug resistance residues Arg155, Ala156, and Asp168 are shown as ball and sticks.

CHEMISTRY

General. All reactions were performed in oven-dried round bottomed or modified Schlenk flasks fitted with rubber septa under argon atmosphere, unless otherwise noted. All reagents and solvents, including anhydrous solvents, were purchased from commercial sources and used as received. Flash column chromatography was performed using silica gel (230–400 mesh, EMD Millipore). Thin-layer chromatography (TLC) was performed using silica gel (60 F-254) coated aluminum plates (EMD Millipore), and spots were visualized by exposure to ultraviolet light (UV), exposure to iodine adsorbed on silica gel, and/ or exposure to an acidic solution of p-anisaldehyde (anisaldehyde) followed by brief heating. 1H NMR and 13C NMR spectra were acquired on Varian Mercury 400 MHz and Bruker Avance III HD 500 MHz NMR instruments. Chemical shifts are reported in ppm (δ scale) with the residual solvent signal used as reference and coupling constant (J) values are reported in hertz (Hz). Data are presented as follows: chemical shift, multiplicity (s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, m = multiplet, br s = broad singlet), coupling constant in Hz, and integration. High-resolution mass spectra (HRMS) were recorded on a Thermo Scientific Orbitrap Velos Pro mass spectrometer coupled with a Thermo Scientific Accela 1250 UPLC and an autosampler using electrospray ionization (ESI) in the positive mode. The purity of final compounds was determined by analytical HPLC and was found to be ≥95% pure. HPLC was performed on an Agilent system equipped with a photodiode array detector under the following conditions: column, Agilent Zorbax Eclipse XDB RP-C8 (5 μm, 4.6 × 150 mm, 80 Å); solvent A, H2O containing 0.1% trifluoroacetic acid (TFA), solvent B, CH3CN containing 0.1% TFA; gradient, 0% B to 100% B over 10 min followed by 100% B over 3 min; injection volume, 20 μL; flow rate, 1.4 mL/ min. Retention times and purity data for each target compound are provided in the experimental section.

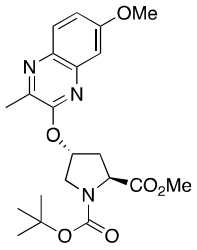
Synthesis of Intermediates and Final Compounds:

1-(*tert*-Butyl) 2-methyl (2*S*,4*R*)-4-((3-ethyl-7-methoxyquinoxalin-2-yl)oxy)pyrrolidine-1,2-dicarboxylate (4a).



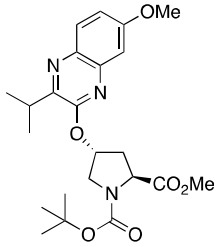
A solution of 3-ethyl-7-methoxyquinoxalin-2(1*H*)-one[1] (3.0 g, 14.7 mmol) in anhydrous NMP (45 mL) was treated with Cs_2CO_3 (7.40 g, 22.7 mmol). After stirring the reaction mixture at room temperature for 15 min, brosylated *cis*-hydroxyproline derivative 1-(*tert*-butyl) 2-methyl (2*S*,4*S*)-4-(((4-bromophenyl)sulfonyl)oxy)pyrrolidine-1,2-dicarboxylate (6.20 g, 13.3 mmol) was added in one portion. The reaction mixture was heated to 55 °C, stirred for 4 h, and then another portion of brosylated *cis*-hydroxyproline derivative (0.48 g, 1.0 mmol) was added. The resulting reaction mixture was stirred at 55 °C for additional 2 h, cooled to room temperature, quenched with aqueous 1 N HCl solution (150 mL), and extracted with EtOAc (300 mL). The organic fraction was washed successively with saturated aqueous $NaHCO_3$ and NaCl (150 mL each), dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by flash column chromatography using 15–30% EtOAc/hexanes as the eluent to provide **4a** (5.50 g, 87%) as a white foamy solid. ^{1}H NMR (400 MHz, $CDCl_3$) (mixture of rotamers, major rotamer) δ 7.85 (d, J = 9.0 Hz, 1 H), 7.18 (m, 1 H), 7.11 (d, J = 2.8 Hz, 1 H), 5.73 (br s, 1 H), 4.47 (t, J = 8.0 Hz, 1 H), 3.98–3.86 (m, 5 H), 3.78 (s, 3 H), 2.92 (q, J = 7.2 Hz, 2 H), 2.68–2.60 (m, 1 H), 2.43–2.36 (m, 1 H), 1.43 (s, 9 H), 1.31 (t, J = 7.2 Hz, 3 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.56, 160.59, 155.38, 154.02, 148.95, 141.26, 134.12, 129.07, 119.02, 106.11, 80.76, 73.81, 58.43, 55.93, 52.73, 52.40, 36.88, 28.47, 26.68, 11.97 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{22}H_{30}N_3O_6$, 432.2129; found 432.2135.

1-(*tert*-Butyl) 2-methyl (2*S*,4*R*)-4-((7-methoxy-3-methylquinoxalin-2-yl)oxy)pyrrolidine-1,2-dicarboxylate (4b).



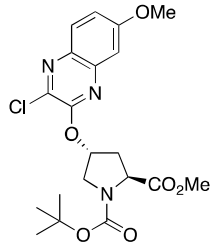
The same procedure was used as described above for compound **4a**. 7-Methoxy-3-methylquinoxalin-2(1*H*)-one[1] (6.2 g, 32.6 mmol) in NMP (100 mL) was treated with Cs_2CO_3 (16.0 g, 49.0 mmol) and activated *cis*-hydroxyproline derivative (15.0 g, 32.3 mmol) to provide **4b** (10.0 g, 74%) as a white foamy solid. ^{1}H NMR (500 MHz, $CDCl_3$) (mixture of rotamers, major rotamer) δ 7.80 (d, J = 9.0 Hz, 1 H), 7.17 (dd, J = 9.0, 3.0 Hz, 1 H), 7.11 (d, J = 2.5 Hz, 1 H), 5.71 (br s, 1 H), 4.48 (t, J = 8.0 Hz, 1 H), 3.99–3.91 (m, 4 H), 3.87 (d, J = 12.5 Hz, 1H), 3.78 (s, 3 H), 2.67–2.58 (m, 1 H), 2.56 (s, 3 H), 2.43–2.37 (m, 1 H), 1.43 (s, 9 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.36, 160.24, 155.51, 153.81, 144.60, 141.04, 134.22, 128.95, 118.63, 105.95, 80.54, 73.59, 58.20, 55.68, 52.48, 52.20, 36.70, 28.26, 19.93 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{21}H_{28}N_3O_6$, 418.1973; found 418.1976.

1-(*tert*-Butyl) 2-methyl (2*S*,4*R*)-4-((3-isopropyl-7-methoxyquinoxalin-2-yl)oxy)pyrrolidine-1,2-dicarboxylate (4c).



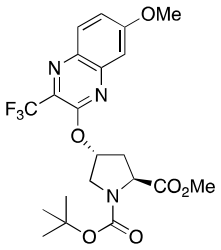
The same procedure was used as described above for compound **4a**. 3-Isopropyl-7-methoxyquinoxalin-2(1*H*)-one[1] (4.0 g, 18.3 mmol) in NMP (65 mL) was treated with Cs_2CO_3 (9.0 g, 27.6 mmol) and activated *cis*-hydroxyproline derivative (8.30 g, 17.9 mmol) to provide **4c** (7.30 g, 90%) as a colorless gummy solid. ^{1}H NMR (500 MHz, $CDCl_3$) (mixture of rotamers, major rotamer) δ 7.83 (d, J = 8.0 Hz, 1 H), 7.16 (d, J = 8.4 Hz, 1 H), 7.10 (s, 1 H) 5.74 (br s, 1 H), 4.48 (t, J = 7.5 Hz, 1 H), 3.92–3.87 (m, 5 H), 3.78 (s, 3 H), 3.41–3.36 (m, 1 H), 2.68–2.59 (m, 1 H), 2.42–2.35 (m, 1 H), 1.43 (s, 9 H), 1.31 (t, J = 7.0 Hz, 6 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.37, 160.19, 154.62, 153.82, 152.00, 140.68, 134.31, 129.39, 118.41, 105.80, 80.49, 73.36, 58.28, 55.67, 52.58, 52.19, 36.68, 30.81, 28.25, 20.43, 20.38 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{23}H_{32}N_3O_6$, 446.2286; found 446.2287.

1-(*tert*-butyl) 2-methyl (2*S*,4*R*)-4-((3-chloro-7-methoxyquinoxalin-2-yl)oxy)pyrrolidine-1,2-dicarboxylate (4d).



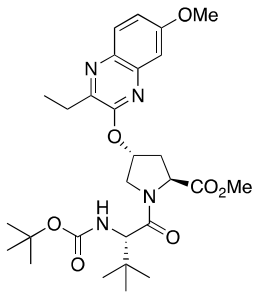
The same procedure was used as described above for compound **4a**. 3-Chloro-7-methoxyquinoxalin-2(1*H*)-one[2] (4.0 g, 19.0 mmol) in NMP (60 mL) was treated with Cs_2CO_3 (9.30 g, 28.6 mmol) and activated *cis*-hydroxyproline derivative (8.40 g, 18.1 mmol) to provide **4d** (6.30 g, 76%) as an off-white foamy solid. ^{1}H NMR (400 MHz, $CDCl_3$) (mixture of rotamers, major rotamer) δ 7.80 (d, J = 8.8 Hz, 1 H), 7.21 (dd, J = 8.8, 2.8 Hz, 1 H), 7.12 (d, J = 2.8 Hz, 1 H), 5.69 (br s, 1 H), 4.52 (t, J = 7.6 Hz, 1 H), 4.0–3.94 (s, 4 H), 3.88 (d, J = 12.8 Hz, 1 H), 3.78 (s, 3 H), 2.72–2.62 (m, 1 H), 2.45–2.37 (m, 1 H), 1.43 (s, 9 H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$) δ 173.32, 162.35, 153.84, 152.48, 141.03, 136.11, 134.06, 129.97, 119.95, 105.83, 80.60, 75.02, 58.10, 55.81, 52.36 , 52.10, 36.64, 28.27 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{20}H_{25}ClN_3O_6$, 438.1426; found 438.1438.

1-(*tert*-Butyl) 2-methyl (2*S*,4*R*)-4-((7-methoxy-3-(trifluoromethyl)quinoxalin-2-yl)oxy)pyrrolidine-1,2-dicarboxylate (4e).



The same procedure was used as described above for compound **4a**. 7-Methoxy-3-(trifluoromethyl)quinoxalin-2(1*H*)-one[1] (4.76 g, 19.5 mmol) in anhydrous NMP (65 mL) was treated with Cs_2CO_3 (9.80 g, 30.0 mmol) and activated *cis*-hydroxyproline derivative (9.0 g, 19.4 mmol) to provide **4e** (6.50 g, 71%) as a pale yellow foamy solid. ^{1}H NMR (500 MHz, $CDCl_3$) (mixture of rotamers, major rotamer) δ 7.77 (d, J = 9.0 Hz, 1 H), 7.48–7.43 (m, 2 H), 5.76 (br s, 1 H), 4.50 (t, J = 8.0 Hz, 1 H), 3.97–3.91 (m, 5 H), 3.78 (s, 3 H), 2.69–2.64 (m, 1 H), 2.41–2.34 (m, 1 H), 1.42 (s, 9 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.43, 159.58, 153.98, 152.11, 138.39, 137.22, 127.99, 125.73, 120.70 (q, J = 273.4 Hz), 107.64, 80.69, 74.62, 58.27, 56.02, 52.32, 52.11, 36.70, 28.34 ppm; ^{19}F NMR (470 MHz, $CDCl_3$); −67.73 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{21}H_{25}F_3N_3O_6$, 472.1690; found 472.1689.

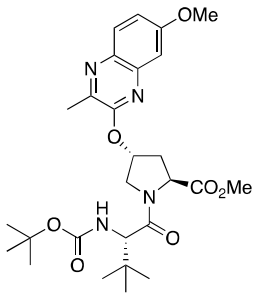
Methyl (2*S*,4*R*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3,3-dimethylbutanoyl)-4-((3-ethyl-7-methoxyquinoxalin-2-yl)oxy)pyrrolidine-2-carboxylate (5a).



A solution of P2 intermediate **4a** (2.75 g, 6.4 mmol) in anhydrous CH_2Cl_2 (20 mL) was treated with a solution of 4 N HCl in 1,4-dioxane (20 mL). After stirring the reaction mixture at room temperature for 3 h, solvents were evaporated under reduced pressure, and the residue was dried under high vacuum. The pale yellow solid was triturated with diethyl ether (20 mL), filtered and washed with diethyl ether diethyl ether (3 × 5 mL) to yield the proline amine salt (2.30 g, 98%) as an off-white powder.

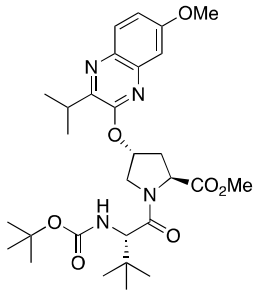
A mixture of above amine salt (1.15 g, 3.1 mmol) and Boc-Tle-OH (0.88 g, 3.8 mmol) in anhydrous DMF (20 mL) was treated with DIEA (2.52 mL, 15.2 mmol) and HATU (2.17 g, 5.7 mmol). The resulting reaction mixture was stirred at room temperature for 4 h, then diluted with EtOAc (150 mL), and washed successively with aqueous 0.5 N HCl, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl (75 mL each). The organic portion was dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by flash chromatography using 25–30% EtOAc/hexanes as the eluent to provide **5a** (1.45 g, 85%) as a white foamy solid. ^{1}H NMR (400 MHz, $CDCl_3$) (mixture of rotamers, major rotamer) δ 7.87 (d, J = 9.2 Hz, 1 H), 7.19 (dd, J = 8.8, 2.8 Hz, 1 H), 7.13 (d, J = 2.8 Hz, 1 H), 5.86 (br s, 1 H), 5.18 (d, J = 9.2 Hz, 1 H), 4.73 (t, J = 8.4 Hz, 1 H), 4.27–4.22 (m, 2 H), 4.11–4.04 (m, 1 H), 3.94 (s, 3 H), 3.78 (s, 3 H), 2.87 (q, J = 7.2 Hz, 2 H), 2.71–2.65 (m, 1 H), 2.39–2.31 (m, 1 H), 1.33 (s, 9 H), 1.27 (t, J = 7.2 Hz, 3 H), 1.05 (s, 9 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.50, 171.60, 160.57, 155.92, 155.11, 149.02, 141.11, 134.13, 129.13, 118.99, 106.20, 79.79, 74.37, 58.76, 58.19, 55.92, 53.92, 52.57, 35.85, 35.23, 28.43, 26.50, 11.85 ppm; HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{28}H_{41}N_4O_7$, 545.2970; found 545.2973.

Methyl (2*S*,4*R*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3,3-dimethylbutanoyl)-4-((7-methoxy-3-methylquinoxalin-2-yl)oxy)pyrrolidine-2-carboxylate (5b).



The same procedure was used as described above for compound **5a**. Compound **4b** (3.60 g, 8.6 mmol) was treated with 4 N HCl (25 mL) to afford the proline amine salt (3.0 g, 8.5 mmol), which was coupled with Boc-Tle-OH (2.40 g, 10.4 mmol) using DIEA (7.0 mL, 42.4 mmol) and HATU (5.65 g, 14.8 mmol) to provide **5b** (3.50 g, 78%) as a white foamy solid. ^{1}H NMR (500 MHz, $CDCl_3$) (mixture of rotamers, major rotamer) δ 7.80 (d, J = 9.0 Hz, 1 H), 7.18 (dd, J = 9.0, 2.5 Hz, 1 H), 7.12 (d, J = 3.0 Hz, 1 H), 5.84 (br s, 1 H), 5.18 (d, J = 9.5 Hz, 1 H), 4.75 (t, J = 8.5 Hz, 1 H), 4.27–4.22 (m, 2 H), 4.07 (dd, J = 11.5, 4.5 Hz, 1 H), 3.94 (s, 3 H), 3.77 (s, 3 H), 2.70–2.65 (m, 1 H), 2.52 (s, 3 H), 2.38–2.32 (m, 1 H), 1.34 (s, 9 H), 1.05 (s, 9 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.42, 171.57, 160.36, 155.87, 155.39, 144.86, 141.05, 134.51, 129.15, 118.76, 106.19, 79.71, 74.30, 58.70, 58.03, 55.79, 53.81, 52.48, 35.70, 35.15, 28.36, 26.42, 19.97 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{27}H_{39}N_4O_7$, 531.2813; found 531.2807.

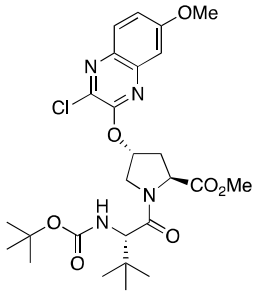
Methyl (2*S*,4*R*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3,3-dimethylbutanoyl)-4-((3-isopropyl-7-methoxyquinoxalin-2-yl)oxy)pyrrolidine-2-carboxylate (5c).



The same procedure was used as described above for compound **5a**. Compound **4c** (1.30 g, 2.92 mmol) was treated with 4 N HCl (12 mL) to afford the proline amine salt (1.05 g, 2.75 mmol), which was coupled with Boc-Tle-OH (0.83 g, 3.60 mmol) using DIEA (2.38 mL, 14.4 mmol) and HATU (1.85 g, 4.86 mmol) to provide **5c** (1.30 g, 85%) as a white foamy solid. ^{1}H NMR (500 MHz, $CDCl_3$) (mixture of rotamers, major rotamer) δ 7.83 (d, J = 9.0 Hz, 1 H), 7.17 (dd, J = 9.0, 3.0 Hz, 1 H), 7.11 (d, J = 3.0 Hz, 1 H), 5.88 (br s, 1 H), 5.20 (d, J = 9.5 Hz, 1 H), 4.72 (t, J = 8.5 Hz, 1 H), 4.26–4.21 (m, 2 H), 4.07 (dd, J = 11.5, 4.0 Hz, 1 H), 3.94 (s, 3 H), 3.77 (s, 3 H), 3.39–3.33 (m, 1 H), 2.71–2.65 (m, 1 H), 2.38–2.32 (m, 1 H), 1.33 (s, 9 H), 1.28 (t, J = 7.0 Hz, 6 H), 1.05 (s, 9 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.41,

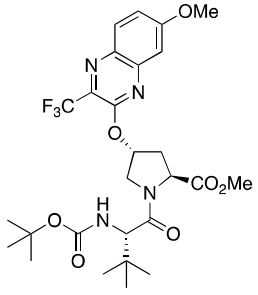
171.45, 160.33, 155.77, 154.47, 152.19, 140.67, 134.59, 129.56, 118.57, 106.01, 79.64, 74.04, 58.66, 58.14, 55.78, 53.91, 52.46, 35.88, 35.16, 30.80, 28.36, 26.40, 20.61, 20.52 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{29}H_{43}N_4O_7$, 559.3126; found 559.3112.

Methyl (2*S*,4*R*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3,3-dimethylbutanoyl)-4-((3-chloro-7-methoxyquinoxalin-2-yl)oxy)pyrrolidine-2-carboxylate (5d).



The same procedure was used as described above for compound **5a**. Compound **4d** (1.05 g, 2.40 mmol) was treated with 4 N HCl (10 mL) to afford the proline amine salt (0.89 g, 2.40 mmol), which was coupled with Boc-Tle-OH (0.66 g, 2.86 mmol) using DIEA (1.90 mL, 11.5 mmol) and HATU (1.41 g, 3.72 mmol) to provide **5d** (1.0 g, 76%) as an off-white foamy solid. ^{1}H NMR (500 MHz, $CDCl_3$) (mixture of rotamers, major rotamer) δ 7.81 (d, J = 9.0 Hz, 1 H), 7.22 (dd, J = 9.0, 2.5 Hz, 1 H), 7.15 (d, J = 3.0 Hz, 1 H), 5.81 (br s, 1 H), 5.21 (d, J = 10.0 Hz, 1 H), 4.79 (t, J = 8.5 Hz, 1 H), 4.31 (d, J = 12.0 Hz, 1 H), 4.21 (d, J = 10.0 Hz, 1 H), 4.09 (dd, J = 11.5, 4.0 Hz, 1 H), 3.96 (s, 3 H), 3.78 (s, 3 H), 2.74–2.68 (m, 1 H), 2.40–2.34 (m, 1 H), 1.32 (s, 9 H), 1.04 (s, 9 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.36, 171.54, 161.40, 155.87, 152.36, 140.99, 136.27, 134.26, 129.05, 120.05, 106.05, 79.77, 75.69, 58.63, 58.02, 55.92, 53.43, 52.50, 35.74, 34.98, 28.36, 26.40 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{26}H_{36}ClN_4O_7$, 551.2267; found 551.2257.

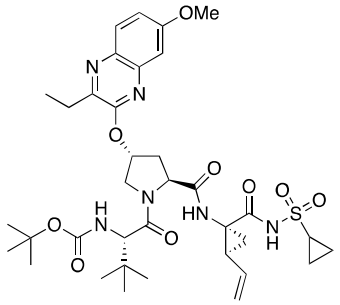
Methyl (2*S*,4*R*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3,3-dimethylbutanoyl)-4-((7-methoxy-3-(trifluoromethyl)quinoxalin-2-yl)oxy)pyrrolidine-2-carboxylate (5e).



The same procedure was used as described above for compound **5a**. Compound **4e** (1.30 g, 2.76 mmol) was treated with 4 N HCl (10 mL) to afford the proline amine salt (1.10 g, 2.70 mmol), which was

coupled with Boc-Tle-OH (0.81 g, 3.50 mmol) using DIEA (2.30 mL, 14.0 mmol) and HATU (2.0 g, 5.25 mmol) to provide **5e** (1.50 g, 95%) as a pale yellow foamy solid. ^{1}H NMR (500 MHz, $CDCl_3$) (mixture of rotamers, major rotamer) δ 7.78 (d, J = 9.0 Hz, 1 H), 7.47 (dd, J = 9.5, 3.0 Hz, 1 H), 7.43 (d, J = 2.5 Hz, 1 H), 5.87 (br s, 1 H), 5.22 (d, J = 9.5 Hz, 1 H), 4.74 (t, J = 8.5 Hz, 1 H), 4.27 (d, J = 12.0 Hz, 1 H), 4.20 (d, J = 9.5 Hz, 1 H), 4.11–4.07 (m, 1 H), 3.94 (s, 3 H), 3.77 (s, 3 H), 2.71–2.66 (m, 1 H), 2.38–2.32 (m, 1 H), 1.30 (s, 9 H), 1.03 (s, 9 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.36, 171.47, 159.64, 155.84, 151.89, 138.51, 137.09, 134.59 (q, J = 35.9 Hz), 128.04, 125.73, 120.69 (d, J = 273.8 Hz), 107.65, 79.69, 75.05, 58.58, 57.92, 56.03, 53.47, 52.49, 35.75, 34.97, 28.26, 26.37 ppm; ^{19}F NMR (470 MHz, $CDCl_3$); −67.84 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{27}H_{36}F_3N_4O_7$, 585.2531; found 585.2516.

***tert*-Butyl ((*S*)-1-((2*S*,4*R*)-2-(((1*R*,2*S*)-1-((cyclopropylsulfonyl)carbamoyl)-2-vinylcyclopropyl)carbamoyl)-4-((3-ethyl-7-methoxyquinoxalin-2-yl)oxy)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (3).**

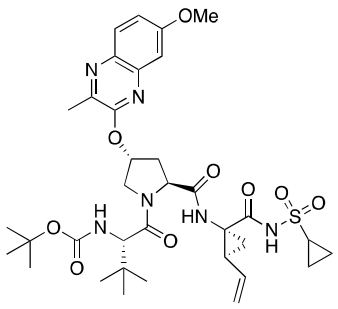


A solution of P2–P3 intermediate **5a** (1.80 g, 3.31 mmol) in THF-H_2O mixture (1:1, 50 mL) was treated with LiOH.H_2O (0.46 g, 11.0 mmol). The resulting reaction mixture was stirred at room temperature for 24 h. The reaction mixture was cooled to ~5 °C, acidified to a pH of 2.0 by slow addition of aqueous 0.50 N HCl (~ 75 mL), and extracted with EtOAc (2 × 150 mL). The organic portions were washed separately with saturated aqueous NaCl (75 ml), dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The gummy residue was dissolved in $CHCl_3$ (20 mL), concentrated under reduced pressure, and the residue was dried under high vacuum overnight to yield the acid **6a** (1.75 g, 100%) as a white foamy solid.

A mixture of acid **6a** (0.88 g, 1.64 mmol) and P1–P1' amine salt **7**[3] (0.48 g, 1.80 mmol) in anhydrous DMF (15 mL) was treated with DIEA (1.10 mL, 6.60 mmol) and HATU (0.94 g, 2.46 mmol). The resulting reaction mixture was stirred at room temperature for 2 h, then diluted with EtOAc (100 mL) and washed successively with aqueous 0.5 N HCl, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl (50 mL each). The organic portion was dried (Na_2SO_4), filtered, and evaporated under reduced

pressure. The residue was purified by flash chromatography using 50–80% EtOAc/hexanes as the eluent to provide compound **3** (0.95 g, 78%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.08 (s, 1 H), 7.84 (d, J = 9.2 Hz, 1 H), 7.20–7.14 (m, 2 H), 7.07 (s, 1 H), 5.90 (br s, 1 H), 5.79–5.72 (m, 1 H), 5.28–5.22 (m, 2 H), 5.15 (d, J = 10.4 Hz, 1 H), 4.48 (t, J = 8.4 Hz, 1 H), 4.30 (d, J = 12.0 Hz, 1 H), 4.24 (d, J = 9.6 Hz, 1 H), 4.03 (dd, J = 11.6, 3.2 Hz, 1 H), 3.94 (s, 3 H), 2.94–2.83 (m, 3 H), 2.56–2.52 (m, 2 H), 2.12 (q, J = 8.4 Hz, 1 H), 1.97 (dd, J = 8.0, 6.0 Hz, 1 H), 1.48 (dd, J = 9.2, 5.6 Hz, 1 H), 1.40–1.22 (m, 13 H), 1.10–0.96 (m, 12 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.05, 172.77, 168.64, 160.56, 155.87, 154.99, 148.86, 141.05, 134.51, 132.76, 129.32, 119.01, 118.85, 106.19, 80.06, 74.37, 60.09, 58.96, 55.92, 54.51, 42.05, 38.85, 35.81, 35.75, 34.51, 31.49, 28.43, 26.71, 22.63, 11.84, 6.50, 6.45 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{36}H_{51}N_6O_9S$, 743.3433; found 743.3431. Anal. RP-HPLC: t_R 9.57 min, purity 97%.

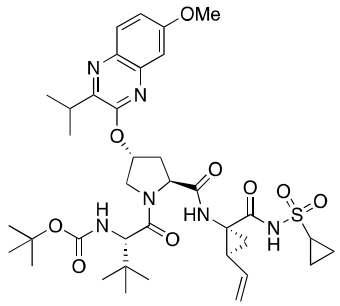
***tert*-Butyl ((*S*)-1-((2*S*,4*R*)-2-(((1*R*,2*S*)-1-((cyclopropylsulfonyl)carbamoyl)-2-vinylcyclopropyl)carbamoyl)-4-((7-methoxy-3-methylquinoxalin-2-yl)oxy)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (9b).**



The same procedure was used as described above for compound **3**. Ester **5b** (1.30 g, 2.45 mmol) was treated with $LiOH.H_2O$ (0.36 g, 8.60 mmol) to afford acid **6b** (1.25 g, 2.42 mmol). A portion of acid **6b** (0.62 g, 1.20 mmol) was reacted with amine salt **7** (0.40 g, 1.50 mmol) using DIEA (0.80 mL, 4.84 mmol) and HATU (0.70 g, 1.84 mmol) to provide compound **9b** (0.64 g, 74%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.08 (s, 1 H), 7.79 (d, J = 9.2 Hz, 1 H), 7.18 (dd, J = 8.8, 2.4 Hz, 1 H), 7.13 (d, J = 2.8 Hz, 2 H), 5.86 (br s, 1 H), 5.80–5.71 (m, 1 H), 5.28–5.23 (m, 2 H), 5.13 (d, J = 10.8 Hz, 1 H), 4.51 (t, J = 8.4 Hz, 1 H), 4.30 (d, J = 11.6 Hz, 1 H), 4.24 (d, J = 9.2 Hz, 1 H), 4.03 (dd, J = 12.0, 4.0 Hz, 1 H), 3.94 (s, 3 H), 2.93–2.86 (m, 1 H), 2.56–2.50 (m, 5 H), 2.11 (q, J = 9.2 Hz, 1 H), 1.95 (dd, J = 8.0, 6.0 Hz, 1 H), 1.47 (dd, J = 9.2, 5.6 Hz, 1 H), 1.38–1.30 (m, 10 H), 1.07–0.97 (m, 12 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.89, 172.49, 168.48, 160.25, 155.67, 155.12, 144.48, 140.89, 134.31, 132.51, 128.94, 118.78, 118.64, 106.0, 79.84, 74.25, 59.79, 58.72, 55.68, 54.23, 41.83, 35.55, 35.42, 34.21,

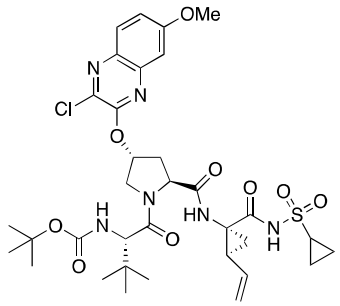
31.23, 28.22, 26.48, 22.32, 19.81, 6.27, 6.22 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{35}H_{49}N_6O_9S$, 729.3276; found 729.3283. Anal. RP-HPLC: t_R 9.13 min, purity 99%.

***tert*-Butyl ((*S*)-1-((2*S*,4*R*)-2-(((1*R*,2*S*)-1-((cyclopropylsulfonyl)carbamoyl)-2-vinylcyclopropyl)carbamoyl)-4-((3-isopropyl-7-methoxyquinoxalin-2-yl)oxy)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (9c).**



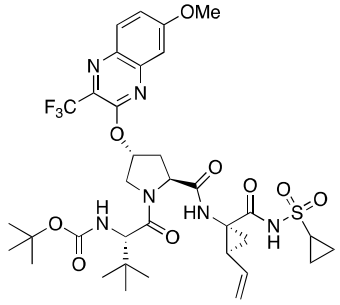
The same procedure was used as described above for compound **3**. Ester **5c** (2.25 g, 4.03 mmol) was treated with $LiOH.H_2O$ (0.68 g, 16.1 mmol) to afford acid **6c** (2.15 g, 3.95 mmol). A portion of acid **6c** (1.0 g, 1.84 mmol) was coupled with amine salt **7** (0.60 g, 2.25 mmol) using DIEA (1.25 mL, 7.40 mmol) and HATU (1.0 g, 2.63 mmol) to provide compound **9c** (1.25 g, 90%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.11 (s, 1 H), 7.83 (d, J = 9.2 Hz, 1 H), 7.18 (dd, J = 8.8, 2.4 Hz, 1 H), 7.12 (d, J = 2.8 Hz, 1 H), 7.02 (s, 1 H), 5.91 (br s, 1 H), 5.82–5.73 (m, 1 H), 5.28–5.23 (m, 2 H), 5.14 (d, J = 10.4 Hz, 1 H), 4.46 (t, J = 8.4 Hz, 1 H), 4.29–4.22 (m, 2 H), 4.06 (dd, J = 11.6, 4.0 Hz, 1 H), 3.94 (s, 3 H), 3.37–3.30 (m, 1 H), 2.94–2.87 (m, 1 H), 2.57–2.49 (m, 2 H), 2.12 (q, J = 8.4 Hz, 1 H), 1.96 (dd, J = 8.4, 6.0 Hz, 1 H), 1.48 (dd, J = 9.6, 6.0 Hz, 1 H), 1.40–1.20 (m, 16 H), 1.05–0.95 (m, 12 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.79, 172.42, 168.52, 160.23, 155.60, 154.19, 151.78, 140.52, 134.42, 132.55, 129.37, 118.61, 105.81, 79.78, 74.02, 59.97, 58.68, 55.68, 54.34, 41.75, 35.70, 35.57, 34.37, 31.25, 30.67, 28.21, 26.47, 22.57, 20.51, 20.44, 6.29, 6.21 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{37}H_{53}N_6O_9S$, 757.3589; found 757.3588. Anal. RP-HPLC: t_R 10.13 min, purity 98%.

***tert*-Butyl ((*S*)-1-((2*S*,4*R*)-4-((3-chloro-7-methoxyquinoxalin-2-yl)oxy)-2-(((1*R*,2*S*)-1-((cyclopropylsulfonyl)carbamoyl)-2-vinylcyclopropyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (9d).**



The same procedure was used as described above for compound **3**. Ester **5d** (2.0 g, 3.63 mmol) was treated with LiOH.H_2O (0.60 g, 14.3 mmol) to afford acid **6d** (1.90 g, 3.54 mmol). A portion of acid **6d** (0.92 g, 1.71 mmol) was coupled with amine salt **7** (0.50 g, 1.88 mmol) using DIEA (1.15 mL, 6.96 mmol) and HATU (0.95 g, 2.50 mmol) to provide compound **9d** (1.0 g, 78%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.07 (s, 1 H), 7.81 (d, J = 8.8 Hz, 1 H), 7.26–7.22 (1 H), 7.18 (d, J = 2.4 Hz, 1 H), 7.04 (s, 1 H), 5.87 (br s, 1 H), 5.81–5.74 (m, 1 H), 5.29–5.20 (m, 2 H), 5.14 (d, J = 10.4 Hz, 1 H), 4.55 (t, J = 8.4 Hz, 1 H), 4.37 (d, J = 11.6 Hz, 1 H), 4.21 (d, J = 9.6 Hz, 1 H), 4.05 (dd, J = 12.0, 3.6 Hz, 1 H), 3.96 (s, 3 H), 2.93–2.88 (m, 1 H), 2.57 (dd, J = 7.6, 2.8 Hz, 2 H), 2.12 (q, J = 8.8 Hz, 1 H), 1.98 (dd, J = 7.6, 6.0 Hz, 1 H), 1.48 (dd, J = 9.6, 6.0, 1 H), 1.38–1.29 (m, 10 H), 1.09–0.98 (m, 12 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.94, 172.63, 168.57, 161.46, 155.82, 152.21, 141.01, 136.09, 134.25, 132.65, 129.00, 120.22, 118.77, 106.05, 80.04, 75.77, 60.55, 59.97, 58.85, 54.01, 42.01, 35.69, 35.53, 34.36, 31.39, 28.34, 26.60, 22.46, 6.40, 6.35 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{34}H_{46}ClN_6O_9S$, 749.2730; found 749.2736. Anal. RP-HPLC: t_R 9.82 min, purity 97%.

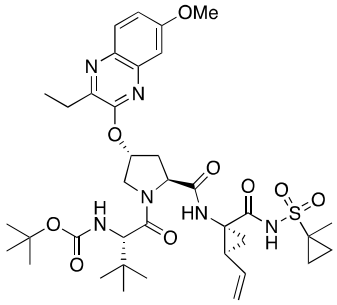
***tert*-Butyl ((*S*)-1-((2*S*,4*R*)-2-(((1*R*,2*S*)-1-((cyclopropylsulfonyl)carbamoyl)-2-vinylcyclopropyl)carbamoyl)-4-((7-methoxy-3-(trifluoromethyl)quinoxalin-2-yl)oxy)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (9e).**



The same procedure was used as described above for compound **3**. Ester **5e** (1.60 g, 2.74 mmol) was treated with LiOH.H_2O (0.40 g, 9.53 mmol) to afford acid **6e** (1.56 g, 2.74 mmol). A portion of acid **6e** (0.78 g, 1.37 mmol) was coupled with amine salt **7** (0.45 g, 1.69 mmol) using DIEA (0.95 mL, 5.75 mmol) and HATU (0.85 g, 2.24 mmol) to provide compound **9e** (0.80 g, 75%) as an off-white solid. ^{1}H

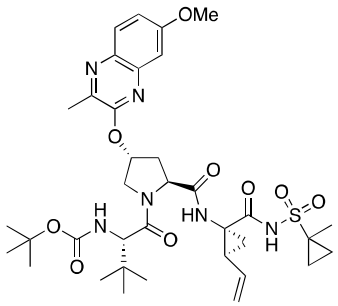
NMR (400 MHz, $CDCl_3$) δ 10.09 (s, 1 H), 7.80 (d, J = 8.8 Hz, 1 H), 7.48 (dd, J = 8.8, 2.8 Hz, 1 H), 7.42 (d, J = 2.8 Hz, 1 H), 7.15 (s, 1 H), 5.92 (br s, 1 H), 5.81–5.72 (m, 1 H), 5.29–5.23 (m, 2 H), 5.14 (d, J = 11.2 Hz, 1 H), 4.50 (t, J = 8.8 Hz, 1 H), 4.32 (d, J = 12.0 Hz, 1 H), 4.19 (d, J = 9.6 Hz, 1 H), 4.04 (dd, J = 12.4, 4.0 Hz, 1 H), 3.94 (s, 3 H), 2.93–2.87 (m, 1 H), 2.53 (dd, J = 8.8, 2.8 Hz, 2 H), 2.13 (q, J = 8.4 Hz, 1 H), 1.96 (dd, J = 8.4, 6.0 Hz, 1 H), 1.48 (dd, J = 9.6, 6.0 Hz, 1 H), 1.36–1.32 (m, 1 H), 1.28 (s, 9 H), 1.05–0.96 (m, 12 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.74, 172.37, 168.52, 159.38, 155.61, 151.59, 138.37, 136.95, 134.24 (q, J = 35.8 Hz), 132.54, 127.97, 125.70, 120.57 (d, J = 273.6 Hz), 118.60, 106.42, 79.76, 75.0, 59.79, 58.60, 55.90, 53.93, 41.79, 35.57, 35.45, 34.27, 31.24, 28.11, 26.43, 22.45, 6.26, 6.19 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{35}H_{46}F_3N_6O_9S$, 783.2994; found 783.3000. Anal. RP-HPLC: t_R 9.89 min, purity 98%.

***tert*-Butyl ((*S*)-1-((2*S*,4*R*)-4-((3-ethyl-7-methoxyquinoxalin-2-yl)oxy)-2-(((1*R*,2*S*)-1-(((1-methylcyclopropyl)sulfonyl)carbamoyl)-2-vinylcyclopropyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (10a).**



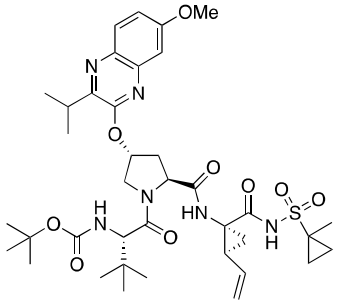
The same procedure was used as described above for compound **3**. Acid **6a** (0.73 g, 1.38 mmol) was coupled with amine salt **8**[4] (0.45 g, 1.60 mmol) using DIEA (0.95 mL, 5.75 mmol) and HATU (0.85 g, 2.24 mmol) to provide compound **10a** (0.80 g, 77%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.80 (s, 1 H), 7.83 (d, J = 9.0 Hz, 1 H), 7.20–7.15 (m, 3 H), 5.89 (br s, 1 H), 5.72–5.64 (m, 1 H), 5.28 (d, J = 17.0 Hz, 1 H), 5.21 (d, J = 9.5 Hz, 1 H), 5.15 (d, J = 10.0 Hz, 1 H), 4.57 (t, J = 8.0 Hz, 1 H), 4.31 (d, J = 12.0 Hz, 1 H), 4.24 (d, J = 9.5 Hz, 1 H), 4.02 (dd, J = 12.0, 4.0 Hz, 1 H), 3.95 (s, 3 H), 2.86 (q, J = 7.5 Hz, 2 H), 2.67–2.61 (m, 1 H), 2.57–2.52 (m, 1 H), 2.11 (q, J = 8.5 Hz, 1 H), 1.94 (dd, J = 8.0, 6.0 Hz, 1 H), 1.73–1.68 (m, 1 H), 1.64–1.60 (m, 1 H), 1.50 (s, 3 H), 1.39 (dd, J = 9.0, 6.5 Hz, 1 H), 1.33 (s, 9 H), 1.27 (t, J = 7.5 Hz, 3 H), 1.03 (s, 9 H), 0.90–0.81 (m, 2 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.56, 172.86, 167.24, 160.42, 155.77, 154.92, 148.85, 140.95, 134.54, 132.74, 129.28, 118.89, 118.84, 106.14, 79.98, 74.18, 59.64, 58.95, 55.83, 54.36, 42.55, 36.69, 35.58, 35.06, 34.16, 28.34, 26.60, 21.42, 18.54, 14.17, 13.66, 11.70 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{37}H_{53}N_6O_9S$, 757.3589; found 757.3587. Anal. RP-HPLC: t_R 9.78 min, purity 99%.

***tert*-Butyl ((*S*)-1-((2*S*,4*R*)-4-((7-methoxy-3-methylquinoxalin-2-yl)oxy)-2-(((1*R*,2*S*)-1-(((1-methylcyclopropyl)sulfonyl)carbamoyl)-2-vinylcyclopropyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (10b).**



The same procedure was used as described above for compound **3**. Acid **6b** (0.62 g, 1.20 mmol) was coupled with amine salt **8** (0.40 g, 1.43 mmol) using DIEA (0.80 mL, 4.84 mmol) and HATU (0.70 g, 1.84 mmol) to provide compound **10b** (0.70 g, 79%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.80 (s, 1 H), 7.80 (d, J = 9.2 Hz, 1 H), 7.23 (s, 1 H), 7.20–7.14 (m, 2 H), 5.86 (br s, 1 H), 5.72–5.63 (m, 1 H), 5.29–5.21 (m, 2 H), 5.15 (d, J = 10.0 Hz, 1 H), 4.60 (t, J = 8.4 Hz, 1 H), 4.32 (d, J = 11.2 Hz, 1 H), 4.23 (d, J = 9.2 Hz, 1 H), 4.01 (dd, J = 11.6, 3.6 Hz, 1 H), 3.94 (s, 3 H), 2.69–2.61 (m, 1 H), 2.57–2.50 (m, 4 H), 2.10 (q, J = 8.4 Hz, 1 H), 1.93 (dd, J = 8.0, 5.6 Hz, 1 H), 1.73–1.67 (m, 1 H), 1.63–1.59 (m, 1 H), 1.50 (s, 3 H), 1.37 (dd, J = 9.6, 6.0 Hz, 1 H), 1.33 (s, 9 H), 1.02 (s, 9 H), 0.88–0.81 (m, 2 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.67, 172.99, 167.35, 160.48, 155.89, 155.39, 144.74, 141.14, 134.57, 132.82, 129.19, 119.0, 106.25, 80.10, 74.40, 59.69, 59.04, 55.92, 54.41, 42.64, 36.74, 35.61, 35.12, 34.14, 28.45, 26.71, 21.44, 20.08, 18.62, 14.26, 13.72 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{36}H_{51}N_6O_9S$, 743.3433; found 743.3428. Anal. RP-HPLC: t_R 9.34 min, purity 99%.

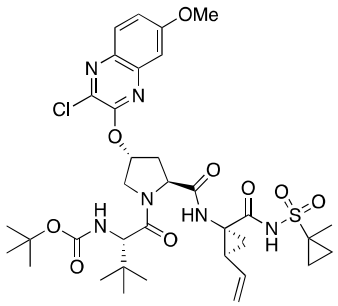
***tert*-Butyl ((*S*)-1-((2*S*,4*R*)-4-((3-isopropyl-7-methoxyquinoxalin-2-yl)oxy)-2-(((1*R*,2*S*)-1-(((1-methylcyclopropyl)sulfonyl)carbamoyl)-2-vinylcyclopropyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (10c).**



The same procedure was used as described above for compound **3**. Acid **6c** (1.0 g, 1.84 mmol) was coupled with amine salt **8** (0.67 g, 2.39 mmol) using DIEA (1.25 mL, 7.56 mmol) and HATU (1.0 g,

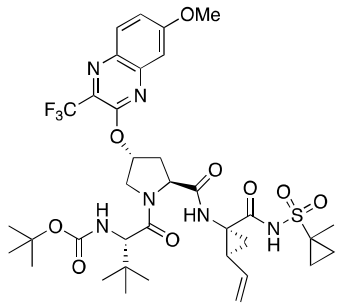
2.63 mmol) to provide compound **10c** (1.20 g, 85%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.85 (s, 1 H), 7.83 (d, J = 9.2 Hz, 1 H), 7.18–7.12 (m, 3 H), 5.89 (br s, 1 H), 5.73–5.64 (m, 1 H), 5.30–5.24 (m, 2 H), 5.15 (d, J = 10.4 Hz, 1 H), 4.53 (t, J = 8.4 Hz, 1 H), 4.30–4.21 (m, 2 H), 4.04 (dd, J = 12.0, 4.0 Hz, 1 H), 3.94 (s, 3 H), 3.38–3.29 (m, 1 H), 2.60–2.50 (m, 2 H), 2.13 (q, J = 8.4 Hz, 1 H), 1.93 (dd, J = 8.0, 6.0 Hz, 1 H), 1.73–1.53 (m, 2 H), 1.50 (s, 3 H), 1.44–1.23 (m, 16 H), 1.02 (s, 9 H), 0.90–0.80 (m, 2 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.45, 172.52, 167.27, 160.22, 155.58, 154.23, 151.78, 140.54, 134.40, 132.63, 129.36, 118.73, 118.59, 105.83, 79.78, 73.98, 59.60, 58.75, 55.68, 54.28, 42.32, 36.52, 35.59, 34.96, 34.20, 30.65, 28.21, 26.45, 21.47, 20.49, 20.45, 18.39, 13.97, 13.56 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{38}H_{55}N_6O_9S$, 771.3746; found 771.3735. Anal. RP-HPLC: t_R 10.33 min, purity 98%.

***tert*-Butyl ((*S*)-1-((2*S*,4*R*)-4-((3-chloro-7-methoxyquinoxalin-2-yl)oxy)-2-(((1*R*,2*S*)-1-(((1-methylcyclopropyl)sulfonyl)carbamoyl)-2-vinylcyclopropyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (10d).**



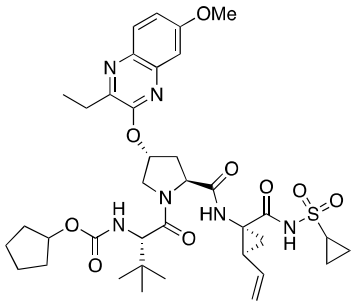
The same procedure was used as described above for compound **3**. Acid **6d** (0.92 g, 1.71 mmol) was coupled with amine salt **8** (0.53 g, 1.88 mmol) using DIEA (1.15 mL, 6.96 mmol) and HATU (0.95 g, 2.50 mmol) to provide compound **10d** (1.0 g, 77%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.83 (s, 1 H), 7.80 (d, J = 8.8 Hz, 1 H), 7.26–7.22 (m, 2 H), 7.18 (d, J = 2.4 Hz, 1 H), 5.84 (br s, 1 H), 5.72–5.63 (m, 1 H), 5.27 (d, J = 16.4 Hz, 1 H), 5.23 (d, J = 9.2 Hz, 1 H), 5.15 (d, J = 10.4 Hz, 1 H), 4.62 (t, J = 8.0 Hz, 1 H), 4.38 (d, J = 12.0 Hz, 1 H), 4.21 (d, J = 9.6 Hz, 1 H), 4.03 (dd, J = 11.6, 4.4 Hz, 1 H), 3.96 (s, 3 H), 2.64–2.54 (m, 2 H), 2.13 (q, J = 8.8 Hz, 1 H), 1.94 (dd, J = 8.0, 6.0 Hz, 1 H), 1.75–1.58 (m, 2 H), 1.50 (s, 3 H), 1.44–1.38 (m, 1 H), 1.31 (s, 9 H), 1.02 (s, 9 H), 0.88–0.80 (m, 2 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.35, 172.64, 167.17, 161.29, 155.66, 152.08, 140.87, 135.95, 134.08, 132.60, 128.85, 120.08, 118.75, 105.89, 79.91, 75.56, 59.53, 58.73, 55.82, 53.80, 42.38, 36.50, 35.43, 34.87, 34.00, 28.21, 26.44, 21.32, 18.40, 13.99, 13.52 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{35}H_{48}ClN_6O_9S$, 763.2887; found 763.2878. Anal. RP-HPLC: t_R 9.82 min, purity 99%.

***tert*-Butyl ((*S*)-1-((2*S*,4*R*)-4-((7-methoxy-3-(trifluoromethyl)quinoxalin-2-yl)oxy)-2-(((1*R*,2*S*)-1-(((1-methylcyclopropyl)sulfonyl)carbamoyl)-2-vinylcyclopropyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (10e).**



The same procedure was used as described above for compound **3**. Acid **6e** (0.78 g, 1.37 mmol) was coupled with amine salt **8** (0.48 g, 1.71 mmol) using DIEA (0.95 mL, 5.75 mmol) and HATU (0.85 g, 2.24 mmol) to provide compound **10e** (0.82 g, 75%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.80 (s, 1 H), 7.81 (d, J = 8.8 Hz, 1 H), 7.48 (dd, J = 8.8, 2.8 Hz, 1 H), 7.43 (d, J = 2.8 Hz, 1 H), 7.27–7.25 (m, 1 H), 5.92 (br s, 1 H), 5.73–5.64 (m, 1 H), 5.28 (d, J = 17.2 Hz, 1 H), 5.20 (d, J = 9.6 Hz, 1 H), 5.15 (d, J = 10.8 Hz, 1 H), 4.56 (t, J = 8.4 Hz, 1 H), 4.34 (d, J = 12.0 Hz, 1 H), 4.18 (d, J = 9.6 Hz, 1 H), 4.0 (dd, J = 11.6, 3.6 Hz, 1 H), 3.94 (s, 3 H), 2.64–2.51 (m, 2 H), 2.13 (q, J = 8.4 Hz, 1 H), 1.95 (dd, J = 8.0, 6.0 Hz, 1 H), 1.73–1.60 (m, 2 H), 1.59 (s, 3 H), 1.43–1.38 (m, 1 H), 1.28 (s, 9 H), 1.00 (s, 9 H), 0.89–0.81 (m, 2 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.53, 172.82, 167.41, 159.76, 155.80, 151.85, 138.61, 137.20, 134.48 (q, J = 35.9 Hz), 132.86, 128.21, 125.90, 120.81 (d, J = 274.0 Hz), 118.96, 107.68, 80.0, 75.16, 59.69, 58.90, 56.13, 54.12, 42.60, 36.75, 35.69, 35.17, 34.28, 28.34, 26.65, 21.66, 18.65, 13.79, 13.44 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{36}H_{48}F_3N_6O_9S$, 797.3150; found 797.3146. Anal. RP-HPLC: t_R 10.10 min, purity 98%.

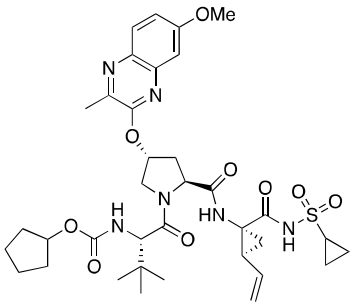
Cyclopentyl ((*S*)-1-((2*S*,4*R*)-2-(((1*R*,2*S*)-1-((cyclopropylsulfonyl)carbamoyl)-2-vinylcyclopropyl)carbamoyl)-4-((3-ethyl-7-methoxyquinoxalin-2-yl)oxy)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (11a).



Compound **3** (0.40 g, 0.54 mmol) was treated with a solution of 4 N HCl in 1,4-dioxane (10 mL). After stirring the reaction mixture at room temperature for 3 h, solvents were evaporated under reduced pressure. The residue was triturated with diethyl ether (3 × 10 mL) and dried to yield the amine salt product (0.37 g, 100%) as a white powder.

A solution of the above amine salt (0.37 g, 0.54 mmol) in anhydrous CH_3CN (15 mL) was treated with DIEA (0.37 mL, 2.24 mmol) and *N*-(cyclopentyloxycarbonyloxy)-succinimide (0.15 g, 0.66 mmol). The reaction mixture was stirred at room temperature for 36 h, then concentrated under reduced pressure and dried under high vacuum. The residue was purified by flash chromatography using 50–90% EtOAc/hexanes as the eluent to provide the target compound **11a** (0.36 g, 88%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.03 (s, 1 H), 7.83 (d, J = 9.0 Hz, 1 H), 7.19 (dd, J = 9.0, 2.8 Hz, 1 H), 7.14 (d, J = 2.5 Hz, 2 H), 5.90 (br s, 1 H), 5.80–5.73 (m, 1 H), 5.32 (d, J = 9.5 Hz, 1 H), 5.26 (d, J = 17.0 Hz, 1 H), 5.15 (d, J = 10.6 Hz, 1 H), 4.90–4.86 (m, 1 H), 4.51 (t, J = 8.6 Hz, 1 H), 4.32–4.26 (m, 2 H), 4.05 (dd, J = 11.6, 3.6 Hz, 1 H), 3.94 (s, 3 H), 2.93–2.84 (m, 3 H), 2.57–2.53 (m, 2 H), 2.12 (q, J = 8.4 Hz, 1 H), 1.96 (dd, J = 8.0, 6.0 Hz, 1 H), 1.76–1.45 (m, 9 H), 1.36–1.33 (m, 1 H), 1.28 (t, J = 7.5 Hz, 3 H), 1.08–0.98 (m, 12 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.93, 172.61, 168.54, 160.41, 156.49, 154.92, 148.91, 140.92, 134.60, 132.66, 129.31, 118.81, 106.13, 78.06, 74.17, 60.02, 59.22, 55.82, 54.40, 41.96, 35.68, 35.61, 34.38, 32.92, 32.71, 32.53, 31.41, 26.61, 23.80, 22.53, 11.72, 6.40, 6.17 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{37}H_{51}N_6O_9S$, 755.3433; found 755.3429. Anal. RP-HPLC: t_R 9.59 min, purity 95%.

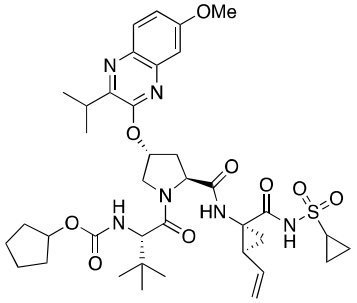
Cyclopentyl ((*S*)-1-((2*S*,4*R*)-2-(((1*R*,2*S*)-1-((cyclopropylsulfonyl)carbamoyl)-2-vinylcyclopropyl)carbamoyl)-4-((7-methoxy-3-methylquinoxalin-2-yl)oxy)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (11b).



The same procedure was used as described above for compound **11a**. Compound **9b** (0.31 g, 0.42 mmol) was treated with 4 N HCl in 1,4-dioxane (10 mL) to yield the amine salt product, which was treated with DIEA (0.30 mL, 1.82 mmol) and *N*-(cyclopentyloxycarbonyloxy)-succinimide (0.12 g, 0.53 mmol) to provide the target compound **11b** (0.26 g, 84%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.05 (s,

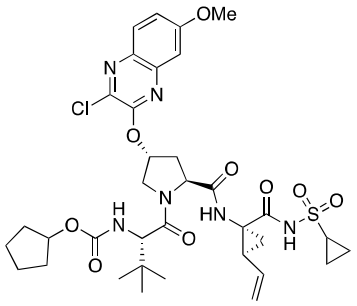
1 H), 7.80 (d, J = 8.8 Hz, 1 H), 7.19 (dd, J = 9.2, 2.8 Hz, 1 H), 7.14 (d, J = 2.8 Hz, 1 H), 7.08 (s, 1 H), 5.88 (br s, 1 H), 5.81–5.72 (m, 1 H), 5.34 (d, J = 9.6 Hz, 1 H), 5.26 (d, J = 17.2 Hz, 1 H), 5.15 (d, J = 10.0 Hz, 1 H), 4.92–4.87 (m, 1 H), 4.52 (t, J = 8.0 Hz, 1 H), 4.32–4.25 (m, 2 H), 4.05 (dd, J = 11.2, 4.0 Hz, 1 H), 3.94 (s, 3 H), 2.94–2.87 (m, 1 H), 2.57–2.51 (m, 5 H), 2.11 (q, J = 8.4 Hz, 1 H), 1.97 (dd, J = 7.6, 5.6 Hz, 1 H), 1.78–1.45 (m, 9 H), 1.36–1.32 (m, 1 H), 1.08–0.98 (s, 12 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.73, 172.46, 168.40, 160.26, 156.40, 155.16, 144.55, 140.89, 134.35, 132.48, 128.95, 118.80, 118.69, 106.0, 77.94, 74.16, 59.87, 59.05, 55.69, 54.20, 41.79, 35.57, 35.50, 34.17, 32.78, 32.60, 31.24, 26.47, 23.68, 22.41, 19.84, 6.28, 6.22 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{36}H_{49}N_6O_9S$, 741.3276; found 741.3275. Anal. RP-HPLC: t_R 9.17 min, purity 99%.

Cyclopentyl ((*S*)-1-((2*S*,4*R*)-2-(((1*R*,2*S*)-1-((cyclopropylsulfonyl)carbamoyl)-2-vinylcyclopropyl)carbamoyl)-4-((3-isopropyl-7-methoxyquinoxalin-2-yl)oxy)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (11c).



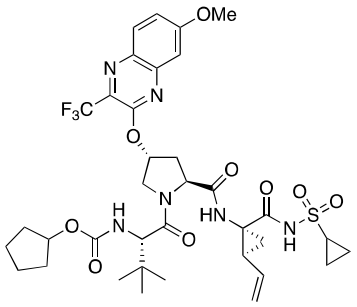
The same procedure was used as described above for compound **11a**. Compound **9c** (0.50 g, 0.66 mmol) was treated with 4 N HCl in 1,4-dioxane (10 mL) to yield the amine salt product, which was treated with DIEA (0.44 mL, 2.66 mmol) and *N*-(cyclopentyloxycarbonyloxy)-succinimide (0.16 g, 0.70 mmol) to provide the target compound **11c** (0.48 g, 95%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.07 (s, 1 H), 7.83 (d, J = 9.2 Hz, 1 H), 7.19–7.12 (m, 3 H), 5.91 (br s, 1 H), 5.80–5.72 (m, 1 H), 5.38 (d, J = 9.6 Hz, 1 H), 5.26 (d, J = 17.2 Hz, 1 H), 5.13 (d, J = 10.4 Hz, 1 H), 4.90–4.86 (m, 1 H), 4.48 (t, J = 8.8 Hz, 1 H), 4.32–4.27 (m, 2 H), 4.06 (dd, J = 12.0, 4.0 Hz, 1 H), 3.94 (s, 3 H), 3.38–3.32 (m, 1 H), 2.93–2.87 (m, 1 H), 2.55–2.51 (m, 2 H), 2.12 (q, J = 8.8 Hz, 1 H), 1.96 (dd, J = 8.4, 6.0 Hz, 1 H), 1.78–1.47 (m, 9 H), 1.38–1.23 (m, 7 H), 1.07–0.97 (m, 12 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.81, 172.34, 168.52, 160.22, 156.33, 154.23, 151.80, 140.53, 134.43, 132.54, 129.36, 118.59, 105.82, 77.86, 73.99, 59.92, 59.02, 55.67, 54.31, 41.77, 35.64, 35.51, 34.33, 32.78, 32.56, 31.24, 30.65, 26.48, 26.18, 23.66, 22.50, 20.50, 20.46, 6.29, 6.20 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{38}H_{53}N_6O_9S$, 769.3589; found 769.3587. Anal. RP-HPLC: t_R 10.15 min, purity 96%.

Cyclopentyl ((*S*)-1-((2*S*,4*R*)-4-((3-chloro-7-methoxyquinoxalin-2-yl)oxy)-2-(((1*R*,2*S*)-1-((cyclopropylsulfonyl)carbamoyl)-2-vinylcyclopropyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (11d).



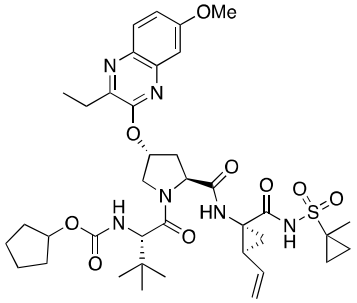
The same procedure was used as described above for compound **11a**. Compound **9d** (0.50 g, 0.67 mmol) was treated with 4 N HCl in 1,4-dioxane (10 mL) to yield the amine salt product, which was treated with DIEA (0.45 mL, 2.72 mmol) and *N*-(cyclopentyloxycarbonyloxy)-succinimide (0.16 g, 0.70 mmol) to provide the target compound **11d** (0.46 g, 90%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.03 (s, 1 H), 7.81 (d, J = 9.0 Hz, 1 H), 7.23 (dd, J = 9.0, 3.0 Hz, 1 H), 7.18 (d, J = 3.0 Hz, 1 H), 7.14 (br s, 1 H), 5.86 (br s, 1 H), 5.78–5.72 (m, 1 H), 5.33 (d, J = 8.4 Hz, 1 H), 5.27 (d, J = 17.0 Hz, 1 H), 5.15 (d, J = 10.6 Hz, 1 H), 4.88–4.85 (m, 1 H), 4.58 (t, J = 8.4 Hz, 1 H), 4.38 (d, J = 12.0 Hz, 1 H), 4.23 (d, J = 10.0 Hz, 1 H), 4.04 (dd, J = 11.6, 3.6 Hz, 1 H), 3.96 (s, 3 H), 2.92–2.88 (m, 1 H), 2.57 (dd, J = 8.0, 2.4 Hz, 2 H), 2.12 (q, J = 8.6 Hz, 1 H), 1.97 (dd, J = 7.6, 6.0 Hz, 1 H), 1.76–1.43 (m, 9 H), 1.36–1.32 (m, 1 H), 1.08–0.96 (m, 12 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.86, 172.61, 168.53, 161.47, 156.58, 152.27, 141.03, 136.25, 134.30, 132.63, 129.01, 120.21, 118.81, 106.07, 78.16, 75.68, 59.99, 59.17, 55.95, 53.93, 42.04, 35.64, 35.50, 34.26, 32.92, 32.72, 31.40, 26.60, 23.82, 22.40, 6.40, 6.35 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{35}H_{46}ClN_6O_9S$, 761.2730; found 761.2730. Anal. RP-HPLC: t_R 9.63 min, purity 98%.

Cyclopentyl ((*S*)-1-((2*S*,4*R*)-2-(((1*R*,2*S*)-1-((cyclopropylsulfonyl)carbamoyl)-2-vinylcyclopropyl)carbamoyl)-4-((7-methoxy-3-(trifluoromethyl)quinoxalin-2-yl)oxy)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (11e).



The same procedure was used as described above for compound **11a**. Compound **9e** (0.40 g, 0.51 mmol) was treated with 4 N HCl in 1,4-dioxane (10 mL) to yield the amine salt product, which was treated with DIEA (0.35 mL, 2.10 mmol) and *N*-(cyclopentyloxycarbonyloxy)-succinimide (0.13 g, 0.57 mmol) to provide the target compound **11e** (0.35 g, 86%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.05 (s, 1 H), 7.81 (d, J = 9.2 Hz, 1 H), 7.48 (dd, J = 9.2, 2.8 Hz, 1 H), 7.43 (d, J = 2.8 Hz, 1 H), 7.18 (s, 1 H), 5.93 (br s, 1 H), 5.81–5.72 (m, 1 H), 5.32–5.25 (m, 2 H), 5.15 (d, J = 11.2 Hz, 1 H), 4.81–4.77 (m, 1 H), 4.53 (t, J = 8.4 Hz, 1 H), 4.35 (d, J = 12.0 Hz, 1 H), 4.19 (d, J = 9.6 Hz, 1 H), 4.02 (dd, J = 12.0, 4.0 Hz, 1 H), 3.94 (s, 3 H), 2.94–2.88 (m, 1 H), 2.56–2.52 (m, 2 H), 2.13 (q, J = 8.4 Hz, 1 H), 1.97 (dd, J = 7.6, 5.6 Hz, 1 H), 1.74–1.42 (m, 9 H), 1.37–1.33 (m, 1 H), 1.25–1.0 (m, 12 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.69, 172.39, 168.42, 159.54, 156.34, 151.65, 138.40, 136.96, 134.34 (q, J = 35.8 Hz), 132.50, 127.96, 125.67, 120.60 (d, J = 273.6 Hz), 118.66, 107.42, 77.82, 74.94, 59.77, 58.92, 55.90, 53.87, 41.82, 35.47, 35.43, 34.16, 32.75, 32.42, 31.24, 26.41, 23.67, 23.62, 22.37, 6.26, 6.20 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{36}H_{46}F_3N_6O_9S$, 795.2994; found 795.2996. Anal. RP-HPLC: t_R 9.93 min, purity 97%.

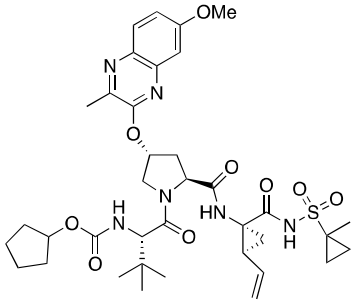
Cyclopentyl ((*S*)-1-((2*S*,4*R*)-4-((3-ethyl-7-methoxyquinoxalin-2-yl)oxy)-2-(((1*R*,2*S*)-1-(((1-methylcyclopropyl)sulfonyl)carbamoyl)-2-vinylcyclopropyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (12a).



The same procedure was used as described above for compound **11a**. Compound **10a** (0.35 g, 0.46 mmol) was treated with 4 N HCl in 1,4-dioxane (10 mL) to yield the amine salt product, which was treated with DIEA (0.31 mL, 1.94 mmol) and *N*-(cyclopentyloxycarbonyloxy)-succinimide (0.13 g, 0.57 mmol) to provide the target compound **12a** (0.34 g, 96%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.77 (s, 1 H), 7.83 (d, J = 9.0 Hz, 1 H), 7.25 (s, 1H, overlapping), 7.19 (dd, J = 9.0, 2.5 Hz, 1 H), 7.15 (d, J = 2.5 Hz, 1 H), 5.89 (br s, 1 H), 5.72–5.64 (m, 1 H), 5.33–5.25 (m, 2 H), 5.15 (d, J = 10.5 Hz, 1 H), 4.87 (m, 1 H), 4.58 (t, J = 8.0 Hz, 1 H), 4.33 (d, J = 12.0 Hz, 1 H), 4.27 (d, J = 10.0 Hz, 1 H), 4.02 (dd, J = 12.0, 4.0 Hz, 1 H), 3.95 (s, 3 H), 2.86 (q, J = 7.5 Hz, 2 H), 2.67–2.62 (m, 1 H), 2.56–2.51 (m, 1 H), 2.11 (q, J = 8.5 Hz, 1 H), 1.93 (dd, J = 8.0, 6.5 Hz, 1 H), 1.77–1.05 (m, 13 H), 1.38 (dd, J = 9.0, 6.0 Hz,

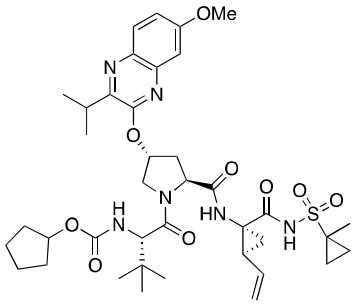
1 H), 1.27 (t, J = 7.5 Hz, 3 H), 1.03 (s, 9 H), 0.90–0.80 (m, 2 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.55, 172.78, 167.25, 160.40, 156.48, 154.95, 148.92, 140.95, 134.60, 132.73, 129.30, 118.90, 118.79, 106.15, 78.07, 74.10, 59.64, 59.25, 55.82, 54.31, 42.55, 36.68, 35.54, 35.01, 34.10, 32.93, 32.71, 26.59, 23.80, 21.38, 18.54, 14.15, 13.67, 11.70 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{38}H_{53}N_6O_9S$, 769.3589; found 769.3584. Anal. RP-HPLC: t_R 9.79 min, purity 98%.

Cyclopentyl ((*S*)-1-((2*S*,4*R*)-4-((7-methoxy-3-methylquinoxalin-2-yl)oxy)-2-(((1*R*,2*S*)-1-(((1-methylcyclopropyl)sulfonyl)carbamoyl)-2-vinylcyclopropyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (12b).



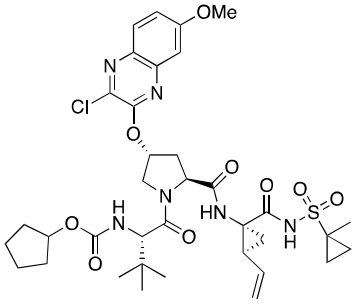
The same procedure was used as described above for compound **11a**. Compound **10b** (0.40 g, 0.54 mmol) was treated with 4 N HCl in 1,4-dioxane (10 mL) to yield the amine salt product, which was treated with DIEA (0.36 mL, 2.18 mmol) and *N*-(cyclopentyloxycarbonyloxy)-succinimide (0.14 g, 0.62 mmol) to provide the target compound **12b** (0.34 g, 96%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.77 (s, 1 H), 7.80 (d, J = 9.0 Hz, 1 H), 7.32 (s, 1 H), 7.19–7.14 (m, 2 H), 5.87 (br s, 1 H), 5.71–5.64 (m, 1 H), 5.38 (d, J = 9.6 Hz, 1 H), 5.27 (d, J = 17.0 Hz, 1 H), 5.15 (d, J = 10.6 Hz, 1 H), 4.94–4.87 (m, 1 H), 4.61 (t, J = 8.0 Hz, 1 H), 4.32 (d, J = 11.6 Hz, 1 H), 4.27 (d, J = 9.6 Hz, 1 H), 4.03 (dd, J = 11.6, 4.0 Hz, 1 H), 3.94 (s, 3 H), 2.66–2.60 (m, 1 H), 2.56–2.50 (m, 4 H), 2.11 (q, J = 8.4 Hz, 1 H), 1.92 (dd, J = 8.0, 6.0 Hz, 1 H), 1.80–1.49 (m, 13 H), 1.37 (dd, J = 9.0, 6.0 Hz, 1 H), 1.02 (s, 9 H), 0.88–0.79 (m, 2 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.57, 172.73, 167.33, 160.40, 156.55, 155.34, 144.74, 141.06, 134.50, 132.74, 129.08, 118.86, 106.20, 78.07, 74.25, 59.61, 59.26, 55.81, 54.28, 42.50, 36.68, 35.50, 34.97, 34.11, 32.92, 32.75, 26.60, 23.81, 21.41, 19.96, 18.52, 14.05, 13.71 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{37}H_{51}N_6O_9S$, 755.3433; found 755.3433. Anal. RP-HPLC: t_R 9.38 min, purity 98%.

Cyclopentyl ((*S*)-1-((2*S*,4*R*)-4-((3-isopropyl-7-methoxyquinoxalin-2-yl)oxy)-2-(((1*R*,2*S*)-1-(((1-methylcyclopropyl)sulfonyl)carbamoyl)-2-vinylcyclopropyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (12c).



The same procedure was used as described above for compound **11a**. Compound **10c** (0.62 g, 0.80 mmol) was treated with 4 N HCl in 1,4-dioxane (12 mL) to yield the amine salt product, which was treated with DIEA (0.53 mL, 3.20 mmol) and *N*-(cyclopentyloxycarbonyloxy)-succinimide (0.20 g, 0.88 mmol) to provide the target compound **12c** (0.58 g, 93%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.83 (s, 1 H), 7.84 (d, J = 9.2 Hz, 1 H), 7.23 (s, 1 H), 7.20–7.13 (m, 2 H), 5.89 (br s, 1 H), 5.73–5.64 (m, 1 H), 5.35 (d, J = 9.2 Hz, 1 H), 5.27 (d, J = 16.4 Hz, 1 H), 5.14 (d, J = 10.4 Hz, 1 H), 4.90–4.84 (m, 1 H), 4.56 (t, J = 8.0 Hz, 1 H), 4.33–4.24 (m, 2 H), 4.04 (dd, J = 12.0, 4.4 Hz, 1 H), 3.94 (s, 3 H), 3.38–3.31 (m, 1 H), 2.67–2.52 (m, 2 H), 2.12 (q, J = 8.0 Hz, 1 H), 1.93 (dd, J = 8.0, 6.0 Hz, 1 H), 1.77–1.50 (m, 13 H), 1.41–1.25 (m, 7 H), 1.02 (s, 9 H), 0.89–0.80 (m, 2 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.37, 172.52, 167.22, 160.22, 156.29, 154.26, 151.81, 140.55, 134.42, 132.59, 129.35, 118.76, 118.57, 105.84, 77.86, 73.89, 59.55, 59.06, 55.67, 54.22, 42.32, 36.51, 35.51, 34.96, 34.10, 32.80, 32.56, 30.65, 26.44, 23.66, 21.43, 20.50, 20.42, 18.40, 13.95, 13.57 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{39}H_{55}N_6O_9S$, 783.3746; found 783.3745. Anal. RP-HPLC: t_R 10.37 min, purity 98%.

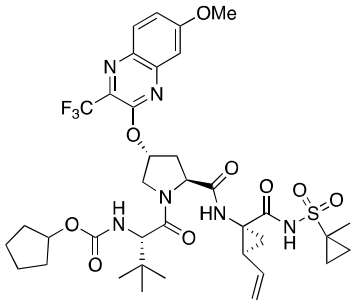
Cyclopentyl ((*S*)-1-((2*S*,4*R*)-4-((3-chloro-7-methoxyquinoxalin-2-yl)oxy)-2-(((1*R*,2*S*)-1-(((1-methylcyclopropyl)sulfonyl)carbamoyl)-2-vinylcyclopropyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (12d).



The same procedure was used as described above for compound **11a**. Compound **10d** (0.50 g, 0.65 mmol) was treated with 4 N HCl in 1,4-dioxane (10 mL) to yield the amine salt product, which was treated with DIEA (0.43 mL, 2.60 mmol) and *N*-(cyclopentyloxycarbonyloxy)-succinimide (0.16 g, 0.70 mmol) to provide the target compound **12d** (0.45 g, 89%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$)

δ 9.79 (s, 1 H), 7.80 (d, J = 8.8 Hz, 1 H), 7.30 (s, 1 H), 7.26–7.21 (m, 1 H), 7.18 (d, J = 2.0 Hz, 1 H), 5.85 (br s, 1 H), 5.73–5.64 (m, 1 H), 5.35 (d, J = 9.2 Hz, 1 H), 5.27 (d, J = 16.8 Hz, 1 H), 5.15 (d, J = 10.8 Hz, 1 H), 4.87–4.84 (m, 1 H), 4.64 (t, J = 7.6 Hz, 1 H), 4.40 (d, J = 11.6 Hz, 1 H), 4.23 (d, J = 10.0 Hz, 1 H), 4.02 (dd, J = 11.6, 4.0 Hz, 1 H), 3.96 (s, 3 H), 2.64–2.54 (m, 2 H), 2.12 (q, J = 8.4 Hz, 1 H), 1.94 (dd, J = 7.6, 6.0 Hz, 1 H), 1.78–1.49 (m, 13 H), 1.39 (dd, J = 9.2, 5.6 Hz, 1 H), 1.01 (s, 9 H), 0.88–0.80 (m, 2 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.30, 172.60, 167.17, 161.27, 156.42, 152.14, 140.88, 136.12, 134.10, 132.57, 128.82, 120.05, 118.80, 105.89, 78.01, 75.48, 59.53, 59.02, 55.82, 53.74, 42.36, 36.50, 35.34, 34.86, 33.90, 32.78, 32.58, 26.43, 23.70, 21.32, 18.40, 13.95, 13.56 ppm; HRMS (ESI) m/z: $[M + H]^+$ calcd for $C_{36}H_{48}ClN_6O_9S$, 775.2887; found 775.2890. Anal. RP-HPLC: t_R 9.87 min, purity 98%.

Cyclopentyl ((*S*)-1-((2*S*,4*R*)-4-((7-methoxy-3-(trifluoromethyl)quinoxalin-2-yl)oxy)-2-(((1*R*,2*S*)-1-(((1-methylcyclopropyl)sulfonyl)carbamoyl)-2-vinylcyclopropyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (12e).



The same procedure was used as described above for compound **11a**. Compound **10e** (0.40 g, 0.50 mmol) was treated with 4 N HCl in 1,4-dioxane (10 mL) to yield the amine salt product, which was treated with DIEA (0.35 mL, 2.10 mmol) and *N*-(cyclopentyloxycarbonyloxy)-succinimide (0.13 g, 0.57 mmol) to provide the target compound **12e** (0.38 g, 94%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.79 (s, 1 H), 7.81 (d, J = 9.2 Hz, 1 H), 7.48 (dd, J = 8.8, 2.8 Hz, 1 H), 7.43 (d, J = 2.8 Hz, 1 H), 7.32 (s, 1 H), 5.91 (br s, 1 H), 5.73–5.63 (m, 1 H), 5.30–5.25 (m, 2 H), 5.16 (d, J = 10.8 Hz, 1 H), 4.80–4.75 (m, 1 H), 4.60 (t, J = 7.6 Hz, 1 H), 4.37 (d, J = 12.0 Hz, 1 H), 4.19 (d, J = 9.6 Hz, 1 H), 3.98 (dd, J = 11.6, 3.6 Hz, 1 H), 3.94 (s, 3 H), 2.67–2.51 (m, 2 H), 2.12 (q, J = 8.0 Hz, 1 H), 1.94 (dd, J = 8.0, 6.0 Hz, 1 H), 1.73–1.48 (m, 13 H), 1.38 (dd, J = 9.2, 5.6 Hz, 1 H), 0.99 (s, 9 H), 0.89–0.81 (m, 2 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.26, 172.60, 167.14, 159.51, 156.31, 151.68, 138.39, 136.96, 134.38 (q, J = 36.0 Hz), 132.58, 127.98, 125.62, 120.60 (d, J = 274.4 Hz), 118.76, 107.42, 77.82, 74.86, 59.41, 58.95, 55.89, 53.79, 42.36, 36.50, 35.30, 34.91, 33.89, 32.76, 32.40, 26.38, 23.66, 23.62, 21.34,

18.40, 13.95, 13.56 ppm; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{37}H_{48}F_3N_6O_9S$, 809.3150; found 809.3157. Anal. RP-HPLC: t_R 10.16 min, purity 96%.

Biology:

Expression and Purification of NS3/4A Protease Constructs

The HCV GT1a NS3/4A protease gene described in the Bristol Myers Squibb patent[5] was synthesized by GenScript and cloned into a PET28a expression vector. The D168A and R155K genes were engineered using the site-directed mutagenesis protocol from Stratagene. Protein expression and purification were carried out as previously described. Briefly, transformed *Escherichia coli* BL21(DE3) cells were grown in LB media containing 30 μg/mL of kanamycin antibiotic at 37 °C. After reaching an OD_{600} of 0.8, cultures were induced with 1 mM IPTG and harvested after 4 h of expression. Cells were pelleted by centrifugation, resuspended in Resuspension buffer [50 mM phosphate buffer, 500 mM NaCl, 10% glycerol, 2 mM β-ME, pH 7.5] and frozen at −80 °C for storage.

Cell pellets were thawed and lysed via cell disruptor (Microfluidics Inc.) two times to ensure sufficient DNA shearing. Lysate was centrifuged at 19,000 rpm, for 25 min at 4 °C. The soluble fraction was applied to a nickel column (Qiagen) pre-equilibrated with Resuspension buffer. The beads and soluble fraction were incubated at 4 °C for 1.5 h and the lysate was allowed to flow through. Beads were washed with Resuspension buffer supplemented with 20 mM imidazole and eluted with Resuspension buffer supplemented with 200 mM imidazole. The eluent was dialyzed overnight (MWCO 10 kD) to remove the imidazole, and the His-tag was simultaneously removed with thrombin treatment. The eluate was judged >90% pure by polyacrylamide gel electrophoresis, concentrated, flash and stored at −80 °C.

Enzyme Inhibition Assays

For each assay, 2 nM of NS3/4A protease (GT1a, R155K and D168A) was pre-incubated at room temperature for 1 h with increasing concentration of inhibitors in assay buffer (50 mM Tris, 5% glycerol, 10 mM DTT, 0.6 mM LDAO, and 4% dimethyl sulfoxide, pH 7.5). Inhibition assays were performed in non-binding surface 96-well black half-area plates (Corning) in a reaction volume of 60 μL. The proteolytic reaction was initiated by the injection of 5 μL of HCV NS3/4A protease substrate (AnaSpec), to a final concentration of 200 nM and kinetically monitored using a Perkin Elmer EnVision plate reader (excitation at 485 nm, emission at 530 nm). Three independent data sets were collected for each inhibitor with each protease construct. Each inhibitor titration included at least 12 inhibitor

concentration points, which were globally fit to the Morrison equation to obtain the K_i value. Gibbs free energy of binding was calculated using the following equation: $\Delta G = RT\ln K_i$

Cell-Based Drug Susceptibility Assays

Mutations (R155K, D168A and A156T) were constructed by site-directed mutagenesis using a Con1 (genotype 1b) luciferase reporter replicon containing the H77 (genotype 1a) NS3 sequence.[6] Replicon RNA of each protease variant was introduced into Huh7 cells by electroporation. Replication was then assessed in the presence of increasing concentrations of protease inhibitors by measuring luciferase activity (relative light units) 96 h after electroporation. The drug concentrations required to inhibit replicon replication by 50% (EC_{50}) were calculated directly from the drug inhibition curves.

Crystallization and Structure Determination

Protein expression and purification were carried out as previously described (see Supporting Information for details). The Ni-NTA purified WT1a protein was thawed, concentrated to 3 mg/mL, and loaded on a HiLoad Superdex75 16/60 column equilibrated with gel filtration buffer (25 mM MES, 500 mM NaCl, 10% glycerol, and 2 mM DTT, pH 6.5). The protease fractions were pooled and concentrated to 25 mg/mL with an Amicon Ultra-15 10 kDa filter unit (Millipore). The concentrated samples were incubated for 1 h with 3:1 molar excess of inhibitor. Diffraction-quality crystals were obtained overnight by mixing equal volumes of concentrated protein solution with precipitant solution (20–26% PEG-3350, 0.1 M sodium MES buffer, 4% ammonium sulfate, pH 6.5) at RT in 24-well VDX hanging drop trays. Crystals were harvested and data was collected at 100 K. Cryogenic conditions contained the precipitant solution supplemented with 15% glycerol or ethylene glycol.

X-ray diffraction data were collected in-house using our Rigaku X-ray system with a Saturn 944 detector. All datasets were processed using HKL-3000.[7] Structures were solved by molecular replacement using PHASER.[8] Model building and refinement were performed using Coot[9] and PHENIX,[10] respectively. The final structures were evaluated with MolProbity[11] prior to deposition in the PDB. To limit the possibility of model bias throughout the refinement process, 5% of the data were reserved for the free R-value calculation.[12] Structure analysis, superposition and figure generation were done using PyMOL.[13] X-ray data collection and crystallographic refinement statistics are presented in Table S1.

References:

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