Supplementary Material

The Discovery and Characterization of a Novel Scaffold as Potent Hepatitis C Virus Inhibitors

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1. Chemical synthesis and Structural Characterization of the intermediates and target compounds

Reagent and chemicals. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker 300 MHz and 600 MHz spectrometers at room temperature, with tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) are reported in parts per million (ppm), and signals are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br s (broad singlet). High-resolution mass spectrometry data were collected on a Kratos Concept mass spectrometer. TLC analysis was carried out on silica gel plates GF254 (Qingdao Haiyang Chemical, China). Silica gel column chromatography wasperformed with Silica gel 60 G (Qingdao Haiyang Chemical, China). Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification.

Synthesis of (S)-Benzyl-[2-[(6-nitrobenzo[d]thiazol-2-yl)carbamoyl] pyrrolidine-1-yl]carboxylate (4). A mixture of *N*-Cbz-*L*-proline (**2**, 9.58 g, 38.4 mmol) and EDC·HCl (7.37 g, 38.4 mmol) in DCM (100 mL) was stirred at room temperature for 30 min. Then 2-amino-6-nitrobenzo[*d*]thiazol (**3**, 5.00 g, 25.6 mmol) and DMAP (0.31 g, 2.54 mmol) were added. The reaction mixture was stirred at room temperature overnight, concentrated under reduced pressure, and purified by silica gel chromatography (hexane: EtOAc = 3: 1) to yield the intermediate **4** (10.05 g, 92.03%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.33 (brs, 1H), 8.71 (s, 1H), 8.29 (d, J = 8.73 Hz, 1H), 7.82 (d, J = 8.94 Hz, 1H), 7.30-7.48 (m, 4H), 7.00-7.16 (m, 1H), 5.17-5.32 (m, 2H), 4.69 (m, 1H), 3.52-3.59 (m, 2H), 2.52 (m, 1H), 2.03-2.05 (m, 3H). MS (ESI) m/z: 427.28 (M+1). HRMS-ESI$^+$: $[M + H]^+$ calcd for $C_{20}H_{19}N_4O_5S$, 427.1011; found, 427.1011.

Synthesis of (S)-Benzyl-[2-[(6-aminobenzo[d]thiazol-2-yl)carbamoyl]pyrrolidine -1-yl]carboxylate (5). Iron powder (1.15 g, 18.8 mmol) was added to a solution of compound **4** (2.00 g, 4.69 mmol) in EtOH (50 mL). The mixture was heated at 78 °C. Diluted hydrochloric acid (7.50 mL, 1 mol/L) was added dropwise. The resulting mixture was refluxed for 2 h. The cooled mixture was filtered through Celite and concentrated in vacuo. The crude product was purified by silica gel chromatography

(DCM: MeOH = 100: 1) to yield the intermediate **5** (1.52 g, 81.72%) as a light yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.21(brs, 1H), 7.37-7.42 (m, 3H), 7.1 -7.19 (m, 1H), 6.99-7.12 (m, 3H), 6.69-6.73 (m, 1H), 5.14-5.26 (m, 2H), 4.91-5.10 (m, 2H), 4.44-4.53 (m, 1H), 3.42-3.55 (m, 2H), 2.16-2.37 (m, 1H), 1.82-1.97 (m, 3H). MS (ESI) m/z: 397.27 (M+1). HRMS-ESI$^+$: $[M + H]^+$ calcd for $C_{20}H_{21}N_4O_3S$, 397.1321; found, 397.1321.

Synthesis of (S)-Benzyl-[2-[[6-(cyclopropanecarboxamido)benzo[d]thiazol-2-yl] carbamoyl]pyrrolidine-1-yl]carboxylate (1). A mixture of cyclopropanecarboxylic acid (28.23 mg, 0.328 mmol) and EDC·HCl (62.86 mg, 0.328 mmol) in DCM (20mL) was stirred at room temperature for 30 min. Then compound **5** (100 mg, 0.252 mmol) and DMAP (3.08 mg, 0.0252 mmol) were added. The reaction mixture was stirred at room temperature overnight, concentrated, and purified by silica gel chromatography (DCM: MeOH = 100: 1) to yield the compound **1** (90.1 mg, 76.97%) as a light yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.45-12.48 (s, 1H), 10.36 (s, 1H), 8.33 (dd, J_1 = 1.62 Hz, J_2 = 6.00 Hz, 1H), 7.67 (dd, J_1 = 4.50 Hz, J_2 = 8.70 Hz, 1H), 7.50-7.53 (m, 1H), 7.37 (d, J = 4.38 Hz, 2H), 7.16 (d, J = 7.01 Hz, 1H), 7.03-7.10 (m, 2H), 4.91-5.11 (m, 2H), 4.49-4.55 (m, 1H), 3.42-3.56 (m, 2H), 2.21-2.32 (m, 1H), 1.84-1.99 (m, 3H), 1.78-1.83 (m, 1H), 0.78-0.82 (m, 4H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.93, 171.51， 156.56, 154.03， 153.39, 144.22, 136.57, 135.47, 131.94, 128.35, 127.46, 126.90, 120.46, 118.44, 111.25, 65.98, 59.17, 47.13, 31.03, 23.20, 14.50, 7.11. MS (ESI) m/z: 465.26 (M+1). HRMS-ESI$^+$: $[M + H]^+$ calcd for $C_{24}H_{25}N_4O_4S$, 465.1518; found, 465.1518.

Synthesis of (S)-Benzyl-2-[[6-[N-(phenylsulfonyl)phenylsulfonamido]benzo[d] thiazol-2-yl]carbamoyl]pyrrolidine-1-carboxylate (D01). Compound **5** (100 mg, 0.252 mmol) and DMAP (3.08 mg, 0.0252 mmol) were dissolved in DCM (20mL) and cooled to 0 °C. Benzene sulfonyl chloride (98.01 mg, 0.555 mmol) was added slowly. The reaction was stirred at room temperature overnight, concentrated, and purified by silica gel chromatography (DCM: MeOH = 100: 5) to yield the compound **D01** (120.0 mg, 88.63%) as a grey solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.58-12.96 (brs, 1H), 7.79-7.85 (m, 6H), 7.73-7.77 (m, 1H), 7.66-7.70 (m, 5H), 7.37 (d, J =

4.38 Hz, 2H), 7.16 (d, J = 7.08 Hz, 1H), 7.03-7.10 (m, 2H), 6.94-6.96 (m, 1H), 4.91-5.11 (m, 2H), 4.49-4.54 (m, 1H), 3.42-3.57 (m, 2H), 2.22-2.32 (m, 1H), 1.82-2.00 (m, 3H). MS (ESI) m/z: 677.32 (M+1). HRMS-ESI$^+$: [M + H]$^+$calcd for $C_{32}H_{29}N_4O_7S_3$, 677.1137; found, 677.1137. Chemical synthesis of compounds **9, D02 ~ D14** was similar to that of compound **D01.**

(*S*)-*Tert*-butyl[2-[(6-aminobenzo[*d*]thiazol-2-yl)carbamoyl]pyrrolidine-1-yl]carboxylate (8). Compound **8** was prepared by the method for compound **5**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.12-12.22 (brs, 1H), 7.39-7.42 (m, 1H), 7.00-7.05 (m, 1H), 6.72 (dd, J_1 = 1.89 Hz, J_2 = 8.52 Hz, 1H), 5.36 (br s, 1H), 4.30-4.40 (m, 1H), 3.41-3.47 (m, 2H), 2.18-2.22 (m, 1H), 1.84-1.91 (m, 3H), 1.20-1.28 (m, 9H). HRMS-ESI$^+$: [M + H]$^+$ calcd for $C_{17}H_{23}N_4O_3S$, 363.1407; found, 363.1407. **(*S*)-*Tert*-butyl-2-[[6-[2-fluoro-*N*-[(2-fluorophenyl)sulfonyl]phenylsulfonamido]benzo[*d*]thiazol-2-yl]carbamoyl]pyrrolidine-1-carboxylate (9).** ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.70-12.78 (brs, 1H), 8.00 (d, J = 1.59 Hz, 1H), 7.74-7.92 (m, 5H), 7.42-7.61 (m, 4H), 7.14-7.17 (m, 1H), 4.37-4.47 (m, 1H), 3.43-3.54 (m, 2H), 2.21-2.27 (m, 1H), 1.82-1.96 (m, 3H), 1.23-1.24 (m, 9H). HRMS-ESI$^+$: [M + H]$^+$ calcd for $C_{29}H_{29}F_2N_4O_7S_3$, 679.1172; found, 679.1172.

(*S*)-Benzyl-2-[[6-[2-fluoro-*N*-[(2-fluorophenyl)sulfonyl]phenylsulfonamido]benzo[*d*]thiazol-2-yl]carbamoyl]pyrrolidine-1-carboxylate (D02). Light yellow solid (149.6 mg, yield 83.20%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.73-12.76 (brs, 1H), 7.93 (dd, J_1 = 2.22 Hz, J_2 = 21.54 Hz, 1H), 7.82-7.90 (m, 4H), 7.74-7.77 (m, 1H), 7.51-7.54 (m, 2H), 7.45-7.47 (m, 1H), 7.43-7.45 (m, 1H), 7.38 (d, J = 4.44 Hz, 2H), 7.18-7.21 (m, 1H), 7.14-7.15 (m, 1H), 6.98-7.07 (m, 2H), 4.89-5.12 (m, 2H), 4.51-4.56 (m, 1H), 3.45-3.56 (m, 2H), 2.23-2.34 (m, 1H), 1.84-2.00 (m, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.48, 172.15, 160.63, 159.15, 157.44, 154.05, 153.34, 149.89, 138.09, 136.77, 136.43, 132.14, 131.55, 129.30, 128.37, 127.92, 127.48, 127.08, 125.57, 125.24, 120.77, 117.71, 66.09, 59.23, 47.13, 30.95, 23.24.MS (ESI) m/z: 713.20 (M+1). HRMS-ESI$^+$: [M + H]$^+$ calcd for $C_{32}H_{27}F_2N_4O_7S_3$, 713.0988; found, 713.0988.

(*S*)-Benzyl-2-[[6-[3-fluoro-*N*-[(3-fluorophenyl)sulfonyl]phenylsulfonamido]benzo[*d*]thiazol-2-yl]carbamoyl]pyrrolidine-1-carboxylate (D03). White solid (141.5 mg, yield 78.70%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.78 (brs, 1H), 7.93 (dd, J_1 = 1.95 Hz, J_2 = 7.95 Hz, 1H), 7.62-7.75 (m, 9H), 7.36-7.37 (m, 2H), 7.15 (d, J = 6.03 Hz, 1H), 7.02-7.09 (m, 3H), 4.89-5.13 (m, 2H), 4.52-4.58 (m, 1H), 3.43-3.54 (m, 2H), 2.18-2.34 (m, 1H), 1.87-2.00 (m, 3H). MS (ESI) m/z: 713.44 (M+1). HRMS-ESI$^+$: [M + H]$^+$ calcd for $C_{32}H_{27}F_2N_4O_7S_3$, 713.0988; found, 713.0988.

(*S*)-Benzyl-2-[[6-[4-fluoro-*N*-[(4-fluorophenyl)sulfonyl]phenylsulfonamido]benzo[*d*]thiazol-2-yl]carbamoyl]pyrrolidine-1-carboxylate (D04). White solid (143.1 mg, yield 79.59%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.75 (brs, 1H), 7.89-7.94 (m, 4H), 7.85 (dd, J_1= 2.04 Hz, J_2 = 19.56 Hz, 1H), 7.74-7.77 (m, 1H), 7.54 (t, J = 8.46 Hz, 4H), 7.38 (d, J = 4.26 Hz, 2H), 7.17 (d, J = 7.26 Hz, 1H), 7.02-7.10 (m, 3H), 4.93-5.12 (m, 2H), 4.53-4.58 (m, 1H), 3.44-3.58 (m, 2H), 2.25-2.35 (m, 1H), 1.84-2.03 (m, 3H). MS (ESI) m/z: 713.01 (M+1). HRMS-ESI$^+$: [M + H]$^+$ calcd for $C_{32}H_{27}F_2N_4O_7S_3$, 713.0988; found, 713.0988.

(*S*)-Benzyl-2-[[6-[2-(trifluoromethyl)-*N*-[[2-(trifluoromethyl)phenyl]sulfonyl]phenylsulfonamido]benzo[*d*]thiazol-2-yl]carbamoyl]pyrrolidine-1-carboxylate (D05). Light yellow solid (60.7 mg, yield 29.61%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.77 (brs, 1H), 8.33-8.39 (m, 1H), 7.93-8.03 (m, 7H), 7.65-7.70 (m, 1H), 7.36-7.37 (m, 2H), 6.92-7.13 (m, 4H), 4.85-5.11 (m, 2H), 4.49-4.52 (m, 1H), 3.48-3.61(m, 2H), 2.25-2.32 (m, 1H), 1.86-2.00 (m, 3H). MS (ESI) m/z: 813.45 (M+1). HRMS-ESI$^+$: [M + H]$^+$ calcd for $C_{34}H_{27}F_6N_4O_7S_3$, 813.0945; found, 813.0945.

(*S*)-Benzyl-2-[[6-[3-(trifluoromethyl)-*N*-[[3-(trifluoromethyl)phenyl]sulfonyl]phenylsulfonamido]benzo[*d*]thiazol-2-yl]carbamoyl]pyrrolidine-1-carboxylate (D06). Yellow solid (88.4 mg, yield 43.12%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.80 (brs, 1H), 8.28 (d, J = 7.47 Hz, 2H), 8.11-8.13 (m, 2H), 7.75-8.03 (m, 6H), 7.37-7.38 (m, 2H), 7.14-7.16 (m, 1H), 7.03-

7.05 (m, 3H), 4.88-5.08 (m, 2H), 4.48-4.56 (m, 1H), 3.47-3.49 (m, 2H), 2.26-2.34 (m, 1H), 1.85-1.91 (m, 3H). MS (ESI) m/z: 813.44 (M+1). HRMS-ESI$^+$: $[M + H]^+$ calcd for $C_{34}H_{27}F_6N_4O_7S_3$, 813.0945; found, 813.0945.

(*S*)-Benzyl-2-[[6-[4-(trifluoromethyl)-*N*-[[4-(trifluoromethyl)phenyl]sulfonyl]phenylsulfonamido]benzo[*d*]thiazol-2-yl]carbamoyl]pyrrolidine-1-carboxylate (D07). Light yellow solid (96.1 mg, yield 46.88%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.79 (brs, 1H), 8.09-8.12 (m, 8H), 7.96 (dd, J_1 = 2.10 Hz, J_2 = 19.38 Hz, 1H), 7.78-7.81 (m, 1H), 7.38 (d, J = 4.20 Hz, 2H), 7.18 (d, J = 6.84 Hz, 1H), 7.10-7.13 (m, 1H), 7.05-7.09 (m, 2H), 4.94-5.13 (m, 2H), 4.53-4.59 (m, 1H), 3.45-3.59 (m, 2H), 2.25-2.36 (m, 1H), 1.84-2.03 (m, 3H). MS (ESI) m/z: 813.01 (M+1). HRMS-ESI$^+$: $[M + H]^+$ calcd for $C_{34}H_{27}F_6N_4O_7S_3$, 813.0945; found, 813.0945.

(*S*)-Benzyl-2-[[6-[4-chloro-*N*-[(4-chlorophenyl)sulfonyl]phenylsulfonamido]benzo[*d*]thiazol-2-yl]carbamoyl]pyrrolidine-1-carboxylate (D08). Light yellow solid (158.2 mg, yield 84.11%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.77 (brs, 1H), 7.94-7.91 (m, 5H), 7.76-7.81 (m, 5H), 7.39 (d, J = 4.20 Hz, 2H), 7.18 (d, J = 7.02 Hz, 1H), 7.04-7.11 (m, 3H), 4.93-5.13 (m, 2H), 4.53-4.59 (m, 1H), 3.45-3.59 (m, 2H), 2.25-2.39 (m, 1H), 1.85-2.03 (m, 3H). MS (ESI) m/z: 746.97 (M+1). HRMS-ESI$^+$: $[M + H]^+$ calcd for $C_{32}H_{27}Cl_2N_4O_7S_3$, 745.0366; found, 745.0366.

(*S*)-Benzyl-2-[[6-[4-bromo-*N*-[(4-bromophenyl)sulfonyl]phenylsulfonamido]benzo[*d*]thiazol-2-yl]carbamoyl]pyrrolidine-1-carboxylate (D09). Light yellow solid (143.6 mg, yield 68.22%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.76 (brs, 1H), 7.93 (d, J = 8.34 Hz, 4H), 7.89 (dd, J_1 = 2.16 Hz, J_2 = 15.60 Hz, 1H), 7.76-7.79 (m, 5H), 7.39 (d, J = 4.32 Hz, 2H), 7.17 (d, J = 6.96 Hz, 1H), 7.04-7.11 (m, 3H), 4.93-5.13 (m, 2H), 4.53-4.58 (m, 1H), 3.45-3.59 (m, 2H), 2.25-2.36 (m, 1H), 1.84-2.03 (m, 3H). MS (ESI) m/z: 834.80 (M+1). HRMS-ESI$^+$: $[M + H]^+$ calcd for $C_{32}H_{27}Br_2N_4O_7S_3$, 832.9324; found, 832.9324.

(*S*)-Benzyl-2-[[6-[4-iodo-*N*-[(4-iodophenyl)sulfonyl]phenylsulfonamido]benzo[*d*]thiazol-2-yl]carbamoyl]pyrrolidine-1-carboxylate (D10). White solid (202.9 mg, yield 86.64%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.78 (brs, 1H), 8.08 (d, J = 7.92 Hz, 4H), 7.88 (d, J = 6.09 Hz, 1H), 7.73-7.77 (m, 1H), 7.56-7.58 (m, 4H), 7.36 (m, 2H), 7.16 (d, J = 6.57 Hz, 1H), 7.04-7.06 (m, 3H), 4.90-5.08 (m, 2H), 4.46-4.63 (m, 1H), 3.46-3.54 (m, 2H), 2.23-2.30 (m, 1H), 1.89-1.91 (m, 3H). HRMS-ESI$^+$: $[M + H]^+$ calcd for $C_{32}H_{27}I_2N_4O_7S_3$ (M+1) 927.9053, found 928.9112.

(*S*)-Benzyl-2-[[6-[2-methyl-*N*-(o-tolylsulfonyl)phenylsulfonamido]benzo[*d*]thiazol-2-yl]carbamoyl]pyrrolidine-1-carboxylate (D11). Light yellow solid (163.6 mg, yield 92.03%). ^{1}H NMR δ (300 MHz, DMSO-d6): 12.76 (brs, 1H), 7.88-7.90 (m, 2H), 7.61-7.73 (m, 4H), 7.40-7.45 (m, 4H), 7.36-7.37(m,2H), 6.95-7.15 (m,4H), 4.86-5.13 (m, 2H), 4.46-4.61 (m, 1H), 3.45-3.53(m, 2H), 2.28-2.43 (m, 7H), 1.84-2.00 (m, 3H). MS (ESI) m/z: 705.54(M+1). HRMS-ESI$^+$: $[M + H]^+$ calcd for $C_{34}H_{33}N_4O_7S_3$, 705.1439; found, 705.1439.

(*S*)-Benzyl-2-[[6-[4-ethyl-*N*-[(4-ethylphenyl)sulfonyl]phenylsulfonamido]benzo[*d*]thiazol-2-yl]carbamoyl]pyrrolidine-1-carboxylate (D12). White solid (160.0 mg, yield 87.17%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.77 (brs, 1H), 7.71-7.79 (m, 6H), 7.49 (d, J = 7.92 Hz, 4H), 7.36-7.37 (m, 2H), 7.16 (d, J = 6.51 Hz, 1H), 7.00-7.05 (m, 3H), 4.90-5.09 (m, 2H), 4.45-4.64 (m, 1H), 3.46-3.54 (m, 2H), 2.69-2.76 (m, 4H), 2.17-2.40 (m, 1H), 1.88-1.91 (m, 3H), 1.19-1.23 (m, 6H). MS (ESI) m/z: 733.59(M+1). HRMS-ESI$^+$: $[M + H]^+$ calcd for $C_{36}H_{37}N_4O_7S_3$, 733.1727; found, 733.1727.

(*S*)-Benzyl-2-[[6-[2-cyano-*N*-[(2-cyanophenyl)sulfonyl]phenylsulfonamido]benzo[*d*]thiazol-2-yl]carbamoyl]pyrrolidine-1-carboxylate (D13). Yellow solid (25.8 mg, yield 14.08%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.79(brs, 1H), 8.17-8.26 (m, 4H), 8.04-8.09 (m, 4H), 7.92-7.98 (m, 1H), 7.79-7.82 (m, 1H), 7.39 (d, J = 4.44 Hz, 2H), 7.21-7.24 (m, 1H), 7.16 (d, J = 6.96 Hz, 1H), 7.04-7.11 (m, 2H), 4.91-5.13 (m, 2H), 4.53-4.59 (m, 1H), 3.44-3.61 (m, 2H), 2.25-2.35 (m, 1H), 1.86-2.03 (m, 3H). MS (ESI) m/z:

727.01(M+1). MS (ESI) m/z: 733.59(M+1). HRMS-ESI$^+$: [M + H]$^+$ calcd for $C_{34}H_{27}N_6O_7S_3$, 727.1075; found, 727.1075.

(*S*)-Benzyl-2-[[6-[*N*-([1,1'-biphenyl]-4-ylsulfonyl)-[1,1'-biphenyl]-4-ylsulfonamido]benzo[*d*]thiazol-2-yl]carbamoyl]pyrrolidine-1-carboxylate (D14). Yellow solid (108.3 mg, yield 51.79%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.76 (brs, 1H), 7.99 (d, J = 8.16 Hz, 4H), 7.91-7.95 (m, 5H), 7.77-7.81 (m, 5H), 7.55 (t, J = 7.68 Hz, 4H), 7.48 (t, J = 7.38 Hz, 2H), 7.37-7.38 (m, 2H), 7.16-7.17 (m, 1H), 7.09-7.12 (m, 1H), 7.03-7.08 (m, 2H), 4.92-5.12 (m, 2H), 4.53-4.58 (m, 1H), 3.44-3.58 (m, 2H), 2.24-2.34 (m, 1H), 1.83-2.03 (m, 3H). MS (ESI) m/z: 829.11(M+1). HRMS-ESI$^+$: [M + H]$^+$ calcd for $C_{44}H_{37}N_4O_7S_3$, 829.1742; found, 829.1742.

(*S*)-N-(6-(2-fluoro-N-((2-fluorophenyl)sulfonyl)phenylsulfonamido)benzo[d]thiazol-2-yl)pyrrolidine-2-carboxamide (D15). 9 (500 mg, 73.7 mmol) was dissolved in DCM (10mL). CF_3COOH (5 mL) was added. The reaction was stirred at room temperature for 2 h, concentrated, and purified by silica gel chromatography (DCM: MeOH = 100: 1) to yield the product (412.0 mg, 96.71%) as a light yellow solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.08 – 7.95 (m, 3H), 7.77 (s, 1H), 7.70 (dd, J_1 = 18.9 Hz, J_2 = 7.5 Hz, 4H), 7.23 (m, 3H), 3.47 (d, J = 24.2 Hz, 2H), 2.47 (s, 1H), 2.19 (m, 1H), 2.12 – 1.95 (m, 3H). HRMS-ESI$^+$: [M + H]$^+$calcd for $C_{24}H_{20}F_2N_4O_5S_3$,579.0643; found,579.0643.

(*S*)-*N*-[6-[2-Fluoro-*N*-[(2-fluorophenyl)sulfonyl]phenylsulfonamido]benzo[*d*]thiazol-2-yl]-1-(quinolin-2-ylmethyl)pyrrolidine-2-carboxamide (D16). Light yellow solid (67.3 mg, yield 50.26%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.87 (brs, 1H), 8.37 (dd, J_1= 8.28 Hz, J_2= 19.60 Hz, 1H), 7.80-7.93 (m, 6H), 7.73-7.78 (m, 1H), 7.62 (d, J = 8.37 Hz, 1H), 7.52-7.57 (m,2H), 7.41-7.49 (m, 2H), 7.37 (d, J = 7.50 Hz, 1H), 7.24-7.28 (m, 1H), 7.19 (d, J = 8.91 Hz, 1H), 6.89 (d, J = 5.22 Hz, 1H), 4.29 (d, J = 14.92 Hz, 1H), 4.06 (d, J = 14.65 Hz, 1H), 3.76-3.81 (m, 1H), 3.38-3.51(m, 2H), 2.15-2.20 (m, 1H), 1.82-2.00 (m, 3H). HRMS-ESI$^+$: [M + H]$^+$ calcd for $C_{34}H_{28}F_2N_5O_5S_3$, 719.1146; found, 719.1146.

(*S*)-*N*-[6-[2-Fluoro-*N*-[(2-

fluorophenyl)sulfonyl]phenylsulfonamido]benzo[*d*]thiazol-2-yl]-1-(naphthalen-1-ylmethyl)pyrrolidine-2-carboxamide (D17). Light yellow solid (77.4 mg, yield 72.42%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.01 (brs, 1H), 8.41 (d, J = 8.40 Hz, 1H), 7.80-7.91 (m, 6H), 7.76 (d, J = 8.25 Hz, 1H), 7.70 (d, J = 8.67 Hz, 1H), 7.34-7.56 (m, 8H), 7.15 (dd, J_1 = 2.16 Hz, J_2 = 8.46 Hz, 1H), 4.29 (d, J = 12.70 Hz, 1H), 3.99 (d, J = 12.61 Hz, 1H), 3.53-3.58 (m, 1H), 3.37-3.41 (m, 2H), 2.14-2.18 (m, 1H), 1.75-1.91 (m, 3H). HRMS-ESI$^+$: $[M + H]^+$ calcd for $C_{35}H_{29}F_2N_4O_5S_3$, 719.1278; found, 719.1278.

(*S*)-*N*-[6-[2-fluoro-*N*-[(2-fluorophenyl)sulfonyl]phenylsulfonamido]benzo[*d*]thiazol-2-yl]-1-(phenylpropanoyl)pyrrolidine-2-carboxamide (D18). Light yellow solid (94.0 mg, yield 72.42%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.63 (brs, 1H), 7.79-7.90 (m, 5H), 7.75 (d, J = 8.67 Hz, 1H), 7.41-7.55 (m, 4H), 7.21-7.28 (m, 4H), 7.13-7.18 (m, 2H), 4.53-4.56 (m, 1H), 3.47-3.68 (m, 2H), 2.76-2.81 (m, 2H), 2.59-2.64 (m, 2H), 2.13-2.20 (m, 1H), 1.88-1.98 (m, 3H). HRMS-ESI$^+$: $[M + H]^+$ calcd for $C_{33}H_{29}F_2N_4O_6S_3$, 711.1153; found, 711.1153.

2. Potocols for biological assays

Cells and viruses. Huh-7.5 cells were maintained in DMEM medium supplemented with 10% fetal calf serum, and non-essential amino acids). Jc1FLAG2(p7-nsGluc2A), Jc1-Gluc for short, is a genotype 2a HCVcc system expression secreted Gaussia luciferase (Gluc). JFH1 is a full length genotype 2a infectious HCV clone. BB7 and BB7-Gluc systems are Con-1 (genotype 1b) derived subgenomic replicons which are maintained by blasticidin.

Determination of antiviral activity and cell toxicity. The initial antiviral activity screening was performed on Jc1-Gluc system. Antiviral activity in genotype 1b virus was tested in BB7-Gluc system. For genotype 2a, confluent cells in 96-well plates were infected with Jc1-Gluc for 8 h and then treated with serially diluted compounds (four wells per dilution) and cultured for 72 h. For genotype 1b, similar procedures were performed except no HCVcc was innoculated since a subgenomic replicon was

used. The replication status of this virus can be quantified by measuring Gluc acitivity in the supernatant using Renilla luciferase assay systemin a Glomax 96 well luminometer (Promega). These experiments were repeated at least twice. CC_{50} determination was performed using the CCK-8 assay (Dojindo Laboratories, Japan). The IC_{50} and CC_{50} value and 95% CI (confidence interval) range of each drug was estimated using non-linear regression functionality in GraphPad 5.0 with a variable slope and least-square fit method. The error bars stand for SEM (standard eror of the mean).

RNA extraction andqRT-PCR. Total RNA from cells was isolated using TRIzol reagent (Invitrogen, city, CA) and standard phenol chloroform extraction procedure. The amount and quality of RNA were measured by Nanodrop. For quantification of HCV RNA, one microgram of total RNA was first reverse transcribed (ReverTra, Toyobo, Japan) using random hexamer as primer followed by quantitative PCR using Thunderbird qPCR mastermix (both from Toyobo, Japan). The copy number of HCV RNA was normalized with GAPDH mRNA. Ther primers used are as follows: HCV forward, CCCTGTGAGGAACTWCTGTCTTCACGC, HCV reverse, GCTCATGRTGCACGGTCTACGAGACCT. GAPDH forward, GGTATCGTGGAAGGACTCATGAC, GAPDH reverse, ATGCCAGTGAGCTTCCCGTTCAGC.

Selection of drug resistance mutants and sequencing of NS5A region. The BB7 subgenomic replicon was maintained with Blasticidin (2 μM) and treated with compound **1** initially at a concentration of 10 μM followed by 20 μM and 50 μM in a mixed population. Each step took around 2-4 passages depending on the viability and growth of the cells under selection pressure. The resultant cell population was obtained after around one month after selection. For the sanger sequencing of NS5A region in resistant replicons, cells were lysed and RNA was extracted and reverse transcbied as described above and the NS5A domain I region was PCR amplified using primers as follows: forward, CCATGCTCCGGMTCSTGG, reverse, gtctccgccgtRatgtggg and cloned into pZeroback using Zeroback fast ligation kit (Tiangen, China). The resultant plasmid clones were individually sanger sequenced

and aligned with the BB7 NS5A sequence. The amplified products were purified and subjected to sanger sequencing.

Immunofluorescence staining and confocal microscopy. Cells were plated on 8-well Labtek chamber slides (Nunc) and treated and/or infected as indicated. After fixation with 4% paraformaldehyde in PBS, cells were permeablized with 0.1% Triton X-100 in PBS and blocked with 10% fetal calf serum (FCS) for 30min. Primary antibody (mouse monoclonal anti-NS5A,9E10) was diluted 500 fold in 10% FCS and detected by anti-mouse IgG Alexa Fluor 594 (1:500, Molecular Probes) in combination with BODIPY 495/505 (Molecular Probes). Cells were finally counterstained with DAPI and imaged with the Zeiss Axiovert 200 or Leica SP5 II confocal microscope.

Expression and purification of NS5A-Chis and DARTS analysis. Full length NS5A was expressed from *Escherichia coli* essentially as described[43]. Briefly, pET-Ub-NS5A-CHis and pCG1 (generous gifts from Prof. Craig. E Cameron) were co-transformed into BL21(DE3) cells. Cells in logarithmic growth phase were treated with IPTG (0.5 mM) and grown for an additional 4hr at 20 °C. NS5A-Chis was purified by Ni–NTA–agarose as described and reached >90% purity. DARTS analysis was performed essentially as reported[40]. Briefly, purified NS5A-Chis (final concentration 20-40μg/ml) was co-incubated with DMSO, compound **1** or GL100953 in binding buffer (50 mM HEPES pH 7.4, 100 mM KCl, 2 mM $MgCl_2$ and 0.5% NP-40). Thermolysin (Sigma) was added to a final concentration of 0.5 μg/mL and incubated in 25 °C. Digestion reaction was stopped by EDTA and protein lysed before and 5, 10 min after protease addition and subject to SDS-PAGE and silver staining.

3. DARTS analysis

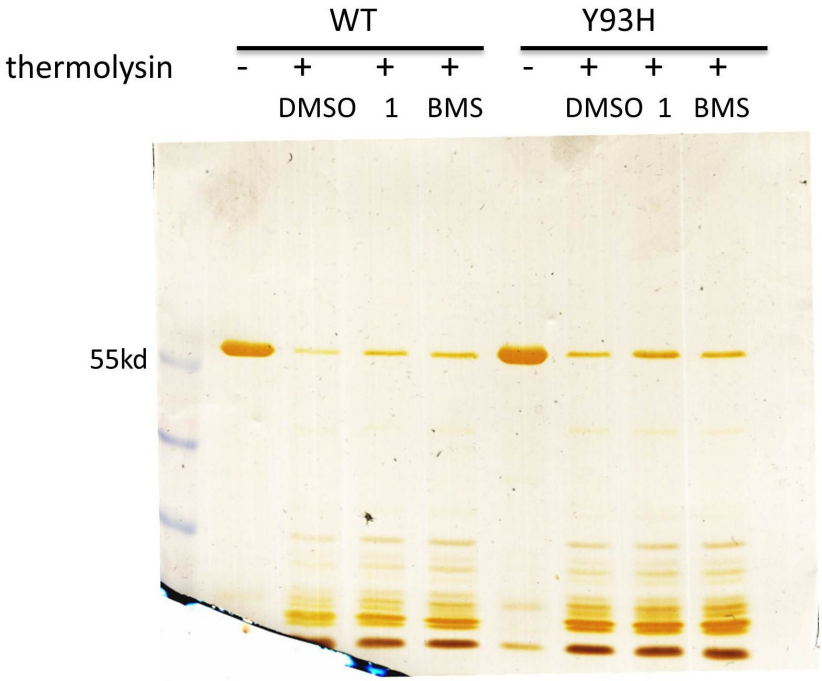
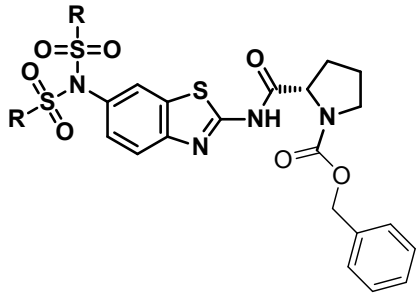
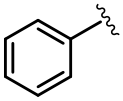
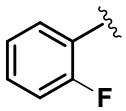
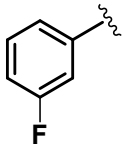
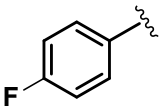
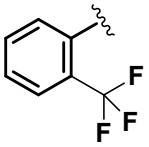
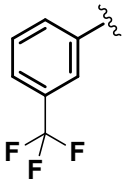
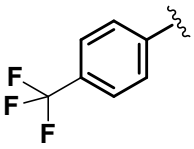
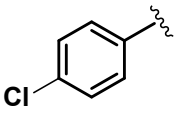
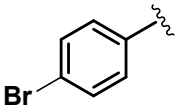
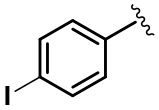
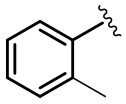
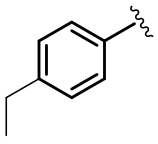
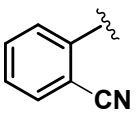


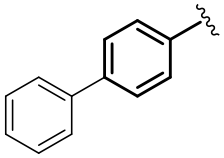
Figure S1 In vitro interaction between BMS-790052, compound 1 and NS5A identified by DARTS analysis. Purified NS5A-CHis was co-incubated with compound 1 (10μM) or BMS-790052 (0.1μM) in the presence of thermolysin and digest for 5 min. The mixture was lysed and subject to SDS-PAGE and silver staining.

Supplementary Table S1. *In vitro* activityof the benzothiazole-disulfoamide derivatives against HCV GT2a in the replicon assay.

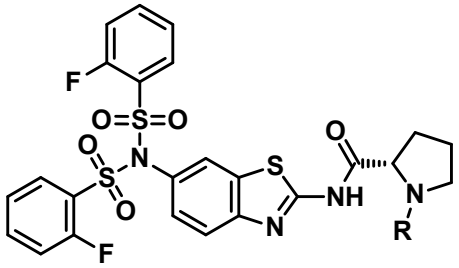


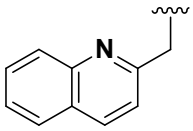
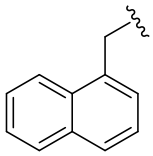
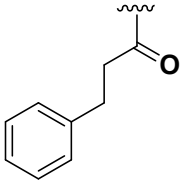
Compound	R	IC_{50} (μM)	CC_{50} (μM)	SI (CC_{50}/IC_{50})
1		26.81	>50	>1.9
D01		2.99	>50	>16.7

D02		0.49	>50	>102.8
D03		>10	>50	ND
D04		>10	>50	ND
D05		3.18	>50	>15.7
D06		>10	>50	ND
D07		>10	>50	ND
D08		2.17	>50	>23.0
D09		>10	>50	ND
D10		>10	>50	ND
D11		1.96	>50	>25.5
D12		>10	>50	ND
D13		2.10	>50	>23.8

D14		>10	>50	ND

Supplementary Table S2. *In vitro* activity of the benzothiazole-disulfoamide derivatives against HCV GT2a in the replicon assay.



Compound	R	IC_{50} (μM)	CC_{50} (μM)	SI (CC_{50}/IC_{50})
D15	H	0.40	>50	>125
D16		1.00	>50	>50
D17		8.90	>50	>5.62
D18		0.19	>50	>263.2