Supporting Information

Discovery of *N*-[4-[6-*tert*-Butyl-5-methoxy-8-(6-methoxy-2-oxo-1*H*-pyridin-3-yl)-3-quinolyl]phenyl]methanesulfonamide (RG7109), a Potent Inhibitor of the Hepatitis C Virus NS5B Polymerase

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***N*-[4-[6-*tert*-Butyl-8-(5-fluoro-2-oxo-1*H*-pyridin-3-yl)-3-quinolyl]phenyl] methanesulfonamide (34).** A microwave vial was charged with 2-bromo-4-*tert*-butylaniline (587 mg, 2.57 mmol), (5-fluoro-2-methoxy-3-pyridyl)boronic acid (660 mg, 3.86 mmol), $Pd(PPh_3)_4$ (148 mg, 0.13 mmol), Na_2CO_3 (818 mg, 7.7 mmol), MeOH (0.7 mL), and CH_2Cl_2 (3.5 mL). The vial was sealed and irradiated in a microwave reactor at 115 °C for 2 h. The reaction mixture was cooled to rt and diluted with EtOAc. The organic layer was washed with water, dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified over SiO_2 (1/4, EtOAc/hexane) to afford 4-*tert*-butyl-2-(5-fluoro-2-methoxy-3-pyridyl)aniline (460 mg, 65%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 3.0 Hz, 1 H), 7.39 (dd, *J* = 8.2, 3.0 Hz, 1 H), 7.24 (dd, *J* = 8.2, 2.2 Hz, 1 H), 7.08 (d, *J* = 2.1 Hz, 1 H), 6.74 (d, *J* = 8.2 Hz, 1 H), 3.95 (s, 3 H), 3.66 (br s, 2 H), 1.30 (s, 9 H).

A solution of Br_2 (191 mg, 1.2 mmol) in AcOH (5 mL) was added to a solution of 2-bromoprop-2-enal (230 mg, 1.71 mmol) in AcOH (5 mL) at rt until the appearance of a faint reddish color of excess Br_2. After stirring at rt for 15 min, a solution of 4-*tert*-butyl-2-(5-fluoro-2-methoxy-3-pyridyl)aniline (437 mg, 1.59 mmol) in AcOH (5 mL) was added. The reaction mixture was heated at 100 °C for 2 h. The reaction mixture was carefully poured into a cold saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified over SiO_2 (gradient: 20 to 50% EtOAc in hexane) to afford 3-(3-bromo-6-*tert*-butyl-8-quinolyl)-5-fluoro-1*H*-pyridin-2-one (260 mg, 43%) as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.83 (d, *J* = 2.4 Hz, 1 H), 8.33 (d, *J* = 2.4 Hz, 1 H), 7.96 (d, *J* = 2.2 Hz, 1 H), 7.71 (d, *J* = 2.2 Hz, 1 H), 7.64 (dd, *J* = 7.9, 3.3 Hz, 1 H), 7.32 (t, *J* = 3.3 Hz, 1 H), 1.45 (s, 9 H).

A microwave vial was charged with 3-(3-bromo-6-*tert*-butyl-8-quinolyl)-5-fluoro-1*H*-pyridin-2-one (60 mg, 0.16 mmol), 4-(methanesulfonamido)phenylboronic acid (52 mg, 0.241 mmol), $Pd(PPh_3)_4$ (10 mg, 0.008 mmol), Na_2CO_3 (51 mg, 0.48 mmol), MeOH (0.1 mL), and CH_2Cl_2 (0.5 mL). The vial was sealed and irradiated in a microwave reactor at 115 °C for 2 h. The reaction mixture was cooled to rt and diluted with EtOAc. The organic layer was washed with water, dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified over SiO_2 (1/1, EtOAc/hexane) to afford desmethoxyquinoline **34** (32 mg, 42%) as an off-white solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.93 (br s, 1 H), 9.14 (d, J = 2.0 Hz, 1 H), 8.61 (d, J = 2.3 Hz, 1 H), 7.98 (d, J = 2.3 Hz, 1 H), 7.91–7.83 (m, 3 H), 7.76–7.65 (m, 2 H), 7.38 (d, J = 8.3 Hz, 2 H), 3.06 (s, 3 H), 1.42 (s, 9 H). LC/MS (ES/APCI): 466.0 $(M + H)^+$.

***N*-[4-[6-*tert*-Butyl-5-methoxy-8-(6-methyl-2-oxo-1*H*-pyridin-3-yl)-3-quinolyl]phenyl] methanesulfonamide (40) hydrobromide.** Using the two steps procedure for the preparation of quinoline **33** from 8-bromoquinoline **14** but using (2-methoxy-6-methyl-3-pyridyl)boronic acid, methylpyridone **40** (55 mg, 38% two steps) was obtained as yellow crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.03 (br s, 1 H), 9.21 (d, J = 1.7 Hz, 1 H), 8.84 (br s, 1 H), 7.96 (d, J = 8.5 Hz, 2 H), 7.80 (s, 1 H), 7.59 (d, J = 7.0 Hz, 1 H), 7.41 (d, J = 8.5 Hz, 2 H), 6.21 (d, J = 6.6 Hz, 1 H), 4.04 (s, 3 H), 3.08 (s, 3 H), 2.3 (s, 3 H), 1.5 (s, 9 H). LC/MS (ESI): 492.1 $(M + H)^+$.

***N*-[4-[6-*tert*-Butyl-5-methoxy-8-(2-oxohexahydropyrimidin-1-yl)-3-quinolyl]phenyl] methanesulfonamide (43).** A vial charged with the corresponding 8-bromoquinoline **14** (80 mg, 0.172 mmol), 1,3-propanediamine (0.3 mL), D,L-proline (8 mg, 0.069 mmol), CuI (8 mg, 0.036 mmol), K_2CO_3 (96 mg, 0.695 mmol) and degassed DMSO (0.5 mL) was sealed and heated at 150 °C for 48 h. The reaction mixture was cooled to rt and diluted with EtOAc. The organic layer was washed with water, dried over $MgSO_4$, filtered, and concentrated. The crude residue

was dissolved in THF (5 mL) and treated with carbonyl diimidazole (350 mg, 2.1 mmol). After stirring at rt for 4 h, the reaction was diluted with EtOAc. The organic layer was washed with water, dried over $MgSO_4$, filtered, and concentrated. The amine was not completely cyclized, thus the material was dissolved in dioxane (15 mL) and irradiated in a microwave reactor at 150 °C for 30 min. The reaction mixture was cooled to rt and concentrated. The crude residue was purified on a SiO_2 preparative TLC (5/95, MeOH/CH_2Cl_2) to afford cyclic urea **43** (15 mg, 18%) as a light yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.61 (br s, 1 H), 8.70 (d, *J* = 2.0 Hz, 1 H), 8.24 (d, *J* = 2.0 Hz, 1 H), 7.70 (s, 1 H), 7.15 (d, *J* = 8.4 Hz, 2 H), 6.80 (d, *J* = 8.5 Hz, 2 H), 5.69 (br s, 1 H), 3.94 (s, 3 H), 3.88–3.72 (m, 2 H), 3.69–3.53 (m, 2 H), 2.91 (s, 3 H), 2.27 (quintuplet, *J* = 5.1 Hz, 2 H), 1.54 (s, 9 H). LC/MS (ES): 483.1 $(M + H)^+$.

***N*-[4-[6-*tert*-Butyl-5-methoxy-8-(1-methyl-2,4-dioxo-pyrimidin-5-yl)-3-quinolyl]phenyl] methanesulfonamide (44) hydrobromide.** A mixture of 8-bromoquinoline **14** (263 mg, 0.57 mmol), 2,4-dimethoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidine (259 mg, 0.68 mmol), Pd(dppf)Cl_2·CH_2Cl_2 adduct (23 mg, 0.028 mmol), Cs_2CO_3 (555 mg, 1.7 mmol), dioxane (4.5 mL) and water (1.1 mL) in a sealed vial was heated at 100 °C for 30 min. The reaction mixture was poured into water and extracted with EtOAc. The organic layers were combined, washed with water, brine, dried over Na_2SO_4, and concentrated. The crude material was purified over SiO_2 (gradient: 10 to 100% EtOAc in hexane) to obtain *N*-[4-[6-*tert*-butyl-8-(2,4-dimethoxypyrimidin-5-yl)-5-methoxy-3-quinolyl]phenyl]methanesulfonamide (140 mg, 47%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.98 (br s, 1 H), 9.14 (d, *J* = 2.0 Hz, 1 H), 8.57 (d, *J* = 2.1 Hz, 1 H), 8.33 (s, 1 H), 7.90 (d, *J* = 8.4 Hz, 2 H), 7.71 (s, 1 H), 7.39 (d, *J* = 8.5 Hz, 2 H), 4.02 (s, 3 H), 3.98 (s, 3 H), 3.83 (s, 3 H), 3.07 (s, 3 H), 1.50 (s, 9 H).

In a screw-capped vial, *N*-[4-[6-*tert*-butyl-8-(2,4-dimethoxypyrimidin-5-yl)-5-methoxy-3-quinolyl]phenyl]methanesulfonamide (80 mg, 0.15 mmol), MeI (0.15 mL, 2.4 mmol), and CH_2Cl_2 (1 mL) were stirred at rt for 18 h. The solvent was evaporated and the crude material was purified over SiO_2 (gradient: 0 to 5% MeOH in CH_2Cl_2) to obtain *N*-[4-[6-*tert*-butyl-5-methoxy-8-(4-methoxy-1-methyl-2-oxo-pyrimidin-5-yl)-3-quinolyl]phenyl] methanesulfonamide (53 mg, 62%) as an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.06 (d, *J* = 2.2 Hz, 1 H), 8.53 (d, *J* = 2.1 Hz, 1 H), 7.75–7.67 (m, 4 H), 7.42 (d, *J* = 8.5 Hz, 2 H), 6.93 (br s, 1 H), 4.04 (s, 3 H), 3.94 (s, 3 H), 3.60 (s, 3 H), 3.11 (s, 3 H), 1.57 (s, 9 H).

A solution of *N*-[4-[6-*tert*-butyl-5-methoxy-8-(4-methoxy-1-methyl-2-oxo-pyrimidin-5-yl)-3-quinolyl]phenyl] methanesulfonamide (49 mg, 0.094 mmol) and 48% HBr (38 mg, 0.025 mL, 0.047 mmol) in AcOH (1 mL) was heated at 60 °C for 1 h. The reaction mixture was diluted with water and filtered through sintered glass. The isolated solid was dried under vacuum (50 °C) to afford C-linked uracil **44** (45 mg, 81%) as a light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.39 (s, 1 H), 9.97 (s, 1 H), 9.16 (d, *J* = 2.3 Hz, 1 H), 8.55 (d, *J* = 2.3 Hz, 1 H), 7.90 (d, *J* = 8.4 Hz, 2 H), 7.86 (s, 1 H), 7.70 (s, 1 H), 7.39 (d, *J* = 8.9 Hz, 2 H), 4.0 (s, 3 H), 3.30 (s, 3 H), 3.07 (s, 3 H), 1.49 (s, 9 H).

***N*-[4-[6-*tert*-Butyl-5-methoxy-8-(6-methyl-2-oxo-1*H*-pyridin-3-yl)-3-quinolyl]butyl] methanesulfonamide (45).** In a Parr vessel, **46** (60 mg, 0.13 mmol) and 10% Pd/C (14 mg) were combined with EtOAc and MeOH then subjected to hydrogenation at 50 psi for 20 h. Additional 10% Pd/C (13 mg) was added and resubmitted to hydrogenation at 50 psi for 72 h. The reaction mixture was filtered through Celite®, washed with CH_2Cl_2, and concentrated. The crude material was purified over SiO_2 (gradient: 0 to 10% MeOH in EtOAc) to obtain *N*-alkyl metahnesulfonamide **45** (30 mg, 49%) as a light yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ

8.67 (d, J = 1.5 Hz, 1 H), 8.13 (d, J = 1.3 Hz, 1 H), 7.76 (s, 1 H), 7.56 (d, J = 7.1 Hz, 1 H), 6.18 (d, J = 7.1 Hz, 1 H), 5.42 (t, J = 5.9 Hz, 1 H), 3.96 (s, 3 H), 3.10 (q, J = 6.6 Hz, 2 H), 2.91 (s, 3 H), 2.80 (t, J = 7.6 Hz, 2 H), 2.31 (s, 3 H), 1.79–1.69 (m, 2 H), 1.68–1.56 (m, 2 H), 1.50 (s, 9 H). LC/MS (ES): 472.2 $(M + H)^+$.

***N*-[4-[6-*tert*-Butyl-5-methoxy-8-(6-methyl-2-oxo-1*H*-pyridin-3-yl)-3-quinolyl]but-3-ynyl]methanesulfonamide (46).** A screw-capped vial was charged with 3-(3-bromo-6-*tert*-butyl-5-methoxy-8-quinolyl)-6-methyl-1*H*-pyridin-2-one (prepared as described for **17**) (250 mg, 0.62 mmol), *tert*-butyl *N*-but-3-ynyl-*N*-methylsulfonyl carbamate (300 mg, 1.29 mmol), $Pd(PPh_3)_4$ (83 mg, 0.072 mmol), CuI (12 mg), Et_3N (0.5 mL), and DMF (2 mL). The vial was sealed and heated at 90 °C overnight. The reaction mixture was cooled, diluted with EtOAc, washed sequentially with water, brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified over SiO_2 (gradient: 50 to 100% EtOAc in hexane) to afford *tert*-butyl *N*-[4-[6-*tert*-butyl-5-methoxy-8-(6-methyl-2-oxo-1*H*-pyridin-3-yl)-3-quinolyl]but-3-ynyl]-*N*-methylsulfonyl-carbamate. The pure product was dissolved in CH_2Cl_2 (3 mL) and TFA (1 mL) and the resulting solution was stirred at rt for 18 h. The solvents were evaporated and the residue purified by flash chromatography over SiO_2 (gradient: 50 to 100% EtOAc in hexane) and purified again (gradient 0 to 80% MeOH in EtOAc) to afford alkyne **46** (80 mg, 27% two steps) as an oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.67 (s, 1 H), 8.74 (d, J = 1.9 Hz, 1 H), 8.44 (d, J = 2.1 Hz, 1 H), 7.72 (s, 1 H), 7.42 (d, J = 6.9 Hz, 1 H), 7.36 (br t, J = 5.1 Hz, 1 H), 6.09 (d, J = 6.7 Hz, 1 H), 3.94 (s, 3 H), 3.25 (q, J = 6.0 Hz, 2 H), 2.97 (s, 3 H), 2.72 (t, J = 7.0 Hz, 2 H), 2.24 (s, 3 H), 1.46 (s, 9 H). LC/MS (ES): 468.2 $(M + H)^+$.

***N*-[[1-[6-*tert*-Butyl-5-methoxy-8-(2-oxo-1*H*-pyridin-3-yl)-3-quinolyl]azetidin-3-yl]methyl] methanesulfonamide (47).** A screw-capped vial was charged with **17** (301 mg, 0.77 mmol), *N*-

(azetidin-3-ylmethyl)methanesulfonamide hydrochloride (200 mg, 1 mmol), $Pd(OAc)_2$ (18 mg, 0.08 mmol), P(*tert*-Bu)$_3$ (24 μL, 0.079 mmol), *tert*-BuONa (298 mg, 3.1 mmol), and toluene (3 mL). The mixture was heated at 100 °C for 20 h. The reaction mixture was cooled to rt and diluted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified over SiO_2 (gradient: 0 to 10% MeOH in CH_2Cl_2) to afford azetidine **47** (95 mg, 26%) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.40 (br s, 1 H), 8.11 (d, *J* = 2.2 Hz, 1 H), 7.57 (d, *J* = 6.5 Hz, 1 H), 7.42 (s, 1 H), 7.40–7.34 (br s, 2 H), 6.92 (d, *J* = 2.1 Hz, 1 H), 6.41 (t, *J* = 6.6 Hz, 1 H), 3.85 (s, 3 H), 3.78 (t, *J* = 7.5 Hz, 2 H), 3.54 (t, *J* = 5.6 Hz, 2 H), 3.22 (br t, *J* = 5.3 Hz, 2 H), 2.89 (s, 3 H), 2.80 (br s, 1 H), 1.49 (s, 9 H). LC/MS (ES): 471.1 $(M + H)^+$.

***N*-[[1-[6-*tert*-Butyl-8-(6-methyl-2-oxo-1*H*-pyridin-3-yl)-3-quinolyl]-3-piperidyl]methyl] methanesulfonamide (49).** This compound was prepared using the same procedure as azetidine **47** but using 3-(3-bromo-6-*tert*-butyl-8-quinolyl)-6-methyl-1*H*-pyridin-2-one and *N*-(3-piperidylmethyl)methanesulfonamide to obtain piperidine **49** (29 mg, 22%) as a solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68 (d, *J* = 2.3 Hz, 1 H), 7.66 (d, *J* = 1.8 Hz, 1 H), 7.61–7.55 (m, 2 H), 7.32 (d, *J* = 2.6 Hz, 1 H), 6.17 (d, *J* = 6.9 Hz, 1 H), 5.39 (br s, 1 H), 3.60 (t, *J* = 13.4 Hz, 2 H), 3.09 (t, *J* = 6.2 Hz, 2 H), 2.96 (s, 3 H), 2.81 (br t, *J* = 10.8 Hz, 1 H), 2.60 (br t, *J* = 10.7 Hz, 1 H), 2.31 (s, 3 H), 1.95–1.50 (m, 3 H), 1.40 (s, 9 H).

Table S1. Data collection and refinement statistics

	41 (4MIA)	**48** (4MIB)
Data collection		
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions		
a, *b*, *c* (Å)	84.981, 104.816, 125.284	86.413, 105.549, 126.006
α, β, γ (°)	90, 90, 90	90, 90, 90
Resolution (Å)	2.8 (2.90-2.80)	2.3 (2.38-2.30)
R_{merge}	0.186 (0.944)	0.143 (0.572)
$I / \sigma I$	9.8 (1.6)	11.5 (3.2)
Completeness (%)	99.7 (99.0)	97.4 (99.7)
Redundancy	6.7 (5.6)	6.3 (6.2)
Refinement		
Resolution (Å)	2.80	2.30
No. reflections	28,155	51,902
R_{work} / R_{free}	20.21/27.88	20.00/24.85
No. atoms		
Protein	8592	8784
Ligand	72	68
Water	230	493
B-factors		
Protein	48.6	31.9
Ligand	71.3	21.7
Water	36.1	37.9
R.m.s. deviations		
Bond lengths (Å)	0.009	0.009
Bond angles (°)	1.05	1.02
Ramachandran		
Preferred	997 (95.6%)	1026 (96.7%)
Allowed	45 (4.3%)	35 (3.3%)
Outliers	1 (0.1%)	0 (0%)