viruses

MDPI

Supplementary Materials

Structure-Based Discovery of *N*-Sulfonylpiperidine-3-carboxamides as Novel Capsid Assembly Modulators for Potent Inhibition of HBV Replication

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Table S1. Primer sequences for RT-qPCR.

HBV 3.5 kb RNA (nt) 2150 to 2300	Former primer : 5′-CCTAGTAGTCAGTTATGTCAAC-3′
	Reverse primer: 5′-TCTATAAGCTGGAGGAGTGCGA-3′
Total HBV RNAs (nt) 3058 to 3158	Former primer : 5′- ACCGACCTTGAGGCATACTT-3′
	Reverse primer : 5′- GCCTACAGCCTCCTAGTACA -3′
HBV core DNA (nt) 1770 to 2105	Former primer : 5′-CCTCTTCATCCTGCTGCT-3′
	Reverse primer : 5′-AACTGAAAGCCAAACAGTG-3′
HBV cccDNA (nt) 1545 to 1899	Former primer : 5′-CTCCCCGTCTGTGCCTTCT-3′
	Reverse primer : 5′-CCCCAAAGCCACCCAAG-3′
	cccDNA probe: FAM-ACGTCGCATGGAGACCAC-CGTGAACGCC-TAMRA

Table S2. Chemical structures and anti-HBV DNA activities of C-1 – C-24.

Compound	Vendor ID	Structure	Residual HBV DNA Level (%) at 20 μM
C-1	STL173465		62
C-2	STL390663		44
C-3	STL102917		22

C-4	STK654654		21
C-5	STL344232		51
C-6	STK607709		36
C-7	5236-0418		13
C-8	D359-0746		28
C-9	L949-2795		11
C-10	C453-0014		29
C-11	G214-4780		59
C-12	8018-8334		36
C-13	K889-0488		44
C-14	K838-0091		68

C-15	G808-0068		60
C-16	L860-0145		4
C-17	F550-3276		21
C-18	F184-1704		2
C-19	K781-8075		3
C-20	L810-0114		32
C-21	C326-0005		51
C-22	D473-1628		29
C-23	8017-8758		27
C-24	F589-0013		53

Figure S1. C-18 inhibits HBV replication and capsid formation in HepG2-HBV1.3 cells. (**A**) Effects of C-18 on intracellular HBV capsid formation and HBc. HepG2-HBV1.3 cells were incubated with indicated concentrations of C-18 for 6 days. Capsid and HBV core protein were probed with anti-HBV core antibody, and GAPDH was used as a loading control. (**B-E**) Effect of C-18 on HBV replication. HepG2-HBV1.3 cells were incubated with indicated concentrations of C-18 for 6 days. Secreted HBeAg (**B**) and HBsAg (**C**) were determined by ELISA, intracellular HBV 3.5 kb RNA (**D**) and HBV DNA (**E**) were measured by qPCR. ETV (25 nM), and BAY 41-4109 (2 μM) were used as controls. The data in (B-E) are representative of three independent experiments and are expressed as mean ± SD (Student's *t*-test was used to assess the statistical significance between DMSO group against any other treatment, $^{*}p < 0.05$; $^{**}p < 0.01$).

Figure S2. C-39 significantly inhibits HBV replication in HepG2-HBV1.3 cells. HepG2-HBV1.3 cells were incubated with the indicated concentrations of C-39 for 6 days. (**A**) Effects of C-39 on intracellular HBV capsid formation and HBc. HBV capsid and core protein were assessed by anti-HBV core antibody. (**B-F**) Effects of C-39 on HBV replication. Secreted HBeAg (**B**) and HBsAg (**C**) were determined by ELISA, intracellular HBV 3.5 kb RNA (**D**) and core DNA (**E**) were measured by qPCR, and HBV replication intermediates (RIs) were assessed by Southern blotting assay (**F**). ETV (25 nM) and BAY 41-4109 (2 μM) were used as controls. The data in (**B-E**) are representative of three independent experiments and are expressed as mean ± SD (Student's *t*-test was used to assess the statistical significance between DMSO group against any other treatment, $^{*}p < 0.05$; $^{**}p < 0.01$).

Figure S3. C-39 significantly inhibits HBV replication in de novo infection model. HepG2-NTCP cells were incubated with the indicated concentrations of C-39 for 6 days. (**A**) Effects of C-39 on intracellular HBV capsid assembly. HBV capsids and core protein were assessed by anti-HBV core antibody. (**B-F**) Effects of C-39 on HBV replication. Secreted HBeAg (**B**) and HBsAg (**C**) were determined by ELISA, intracellular HBV 3.5 kb RNA (D) and core DNA (**E**) were measured by qPCR. ETV (25 nM) and BAY 41-4109 (2 μM) were used as controls. (**F-G**) HBV cccDNA levels in HepG2-NTCP cells when HepG2-NTCP cells were infected with HBV and treated with different compounds either together with the viral inoculum (day 0, F) or at 4 days after infection (day 4, **G**). C-18, C-19, and C-39 were used at 5 μM. ETV (25 nM) and BAY 41-4109 (2 μM) were used as controls. The data in (**B-G**) are representative of three independent experiments and are expressed as mean ± SD (Student's *t*-test was used to assess the statistical significance between DMSO group against any other treatment, $*p < 0.05$; $**p < 0.01$).

Figure S4. C-39 inhibits ETV-resistant HBV replication and the combination of C-39 and ETV enhances the suppression of HBV replication. Antiviral activity of ETV (**A**) and C-39 (**B**) by presence or absence of nucleos(t)ide-resistant mutations. Secreated HBV DNA (**C**) and intracellular HBV core DNA(**D**) levels in response to the treatment of ETV in combination with increasing doses of C-39. The data are representative of three independent experiments and are expressed as mean ± SD (Student's *t*-test was used to assess the statistical significance between combination against any other treatment, $*p < 0.05$; $**p < 0.01$).

Figure S5. Electron microscopy analysis of the effects on capsid assembly**.** Recombinant Cp149 was treated with DMSO, 5 μM BAY 41-4109 for 3 days or 20 μM C-39 for 3 and 7 days in 300 mM NaCl and 150 mM HEPES (pH 7.5). Electron microscopic images of capsids are shown. Scale bars = 200 nm (up) and 50 nm (down).

^{1}H NMR Spectra of SPCs

C-18

C-29

C-30

C648-0183
C648-0183 DM03 C648_0084_Ac21153
C20H19ClFN3O5S 467.90
File name: C648-0183
Expert: BLJ
SF: 399.9525 MHz
NSC: 12
PW: 12.00 usec, RG: 28, SI: 16384
Grade: OK(00)
Date: 11-Jun-2003
Solvent: DMSO-d6+CCL4
SW: 7502 Hz
TE: 300 K
AQ: 1.09 sec, RD: 1,5 sec
* C648- 0183. 11- Jun- 2003. 00*

C-31

e570-1769
E570-1769 DM03 C23H25ClFN3O4S
LOW solubility
File name: e570-1769
Operator: VVN
SF: 399.9525 MHz
NSC: 12
PW: 12.00 usec, RG: 28
Grade: OK(00)
Date: 01-May-2004
Solvent: DMSO
SW: 7502 Hz
TE: 300 K
AQ: 1.09 sec, RD: 1.50 sec
* e570- 1769. 01- May- 2004. 00*

C-32

E589-5256
E589-5256 DM03 C21H19ClFN3O4S
463.91
File name: E589-5256
Expert: BLJ
SF: 399.9525 MHz
NSC: 12
PW: 12.00 usec, RG: 28, SI: 16384
Grade: OK(00)
Date: 02-Jun-2004
Solvent: DMSO-d6+CCl4
SW: 7502Hz
TE: 300K
AQ: 1.09 sec, RD: 1,5 sec
* E589- 5256. 02- Jun- 2004. 00*

C-33

F061-0168
F061-0168 DM03 1-1_Ac21153
C25H25ClFN3O4S 518.00
File name: F061-0168
Operator: POK
SF: 300.1300 MHz
NSC: 4
PW: 0.00 usec, RG: 362, SI: 32768
Grade: OK(00)
Date: 05-Feb-2006
Solvent: DMSO
SW: 6173 Hz
TE: 300 K
AQ: 2.00 sec, RD: 0.00 sec
* F061- 0168. 05- Feb- 2006. 00*

C-34

F061-1016
F061-1016 DM03 1-3_Ac21153
C24H22ClF2N3O4S 521.96
File name: F061-1016 Operator: ChSZR, Expert: PEB SF: 399.9525 MHz NSC: 8 PW: 8.00 usec, RG: 14, SI: 16384 Grade: OK(00)
Date: 07-Mar-2006 Solvent: DMSO-d6 + CCl4 SW: 7502 Hz TE: 300 K AQ: 1.09 sec, RD: 1.50 sec * F061- 1016. 07- Mar - 2006. 00*

C-35

F821-0553
F821-0553 DM03 1-2_Ac21153
C21H21ClFN3O5S 481.92
File name: F821-0553 Operator: NVV, Expert: NVV SF: 399.9525 MHz NSC: 4 PW: 7.00 usec, RG: 22. SI: 16384 Grade: OK(00)
Date: 09-Aug-2006 Solvent: DMSO SW: 7502 Hz TE: 300 K AQ: 1.09 sec, RD: 1.50 sec * F821- 0553. 09- Aug- 2006. 00*

C-36

F894-0160
F894-0160 C20H20ClFN4O4S2 498.97
File name: F894-0160
Operator: KLP
SF: 300.1300 MHz
NSC: 8
PW: 0.00 usec, RG: 4
Grade: OK(10), Expert: SAI
MSL-300. 19-Oct-2007
Solvent: DMSO-d6 + CCl4
SW: 6024 Hz
TE: 298 K
AQ: 1.20 sec, RD: 0.00 sec

C-37

G070-0348
G070-0348 1-2_Ac21153 C21H25ClFN3O5S
485.96
CH+CH2+DMSO
File name: G070-0348
Operator: ChSZR, Expert: PEB
SF: 399.9525 MHz
NSC: 8
PW: 8.00 usec, RG: 13, SI: 16384
Grade: OK(00)
Date: 14-Dec-2005
Solvent: DMSO-d6 + CCl4
SW: 7502 Hz
TE: 300 K
AQ: 1.09 sec, RD: 1.50 sec
G070-0348. 14-Dec-2005. 00

C-38

G935-2712
G935-2712 DM03 1-7_Ac21153
C23H29ClFN5O4S 526.02
File name: G935-2712 Expert: BLJ SF: 399.9525 MHz NS: 8 PW: 12.00usec, RG: 14, SI: 16384 Grade: OK(00)
Date: 12-Jul-2006 Solvent: DMSO-d6+CCL4 SW: 7502Hz TE: 300K AQ: 1.09 sec, RD: 1,5sec *G935- 2712. 12- Jul - 2006. 00*

C-39

G937-1732
G937-1732 DM03 1-5_Ac21153
C22H27ClFN3O5S 499.98
File name: G937-1732 Operator: , Expert: NVV SF: 399.9525 MHz NSC: 3 PW: 8.00 usec, RG: 14, SI: 16384 Grade: OK(00)
Date: 15-Dec-2005 Solvent: DMSO + CCl4 SW: 7502 Hz TE: 300 K AQ: 1.09 sec, RD: 1.50 sec *G937- 1732. 15- Dec- 2005. 00*

C-40

F899-0160
F899-0160 DM03 1-1_Ac21153
C21H24ClFN4O4S 482.96
File name: F899-0160 Operator: NVV Expert: NVV SF: 399.9525 MHz NSC: 4 PW: 7.00 usec, RG: 20, SI: 16384 Grade: OK(00)
Date: 17-Aug-2006 Solvent: DMSO + CCl4 SW: 7502 Hz TE: 300 K AQ: 1.09 sec, RD: 1.50 sec *F899- 0160. 17- Aug- 2006. 00*

C-41

L501-0160
L501-0160 C19H18ClFN4O4S2 484.95
File name: L501-0160 Operator: KLP SF: 300.1300 MHz NSC: 8 PW: 0.00 usec, RG: 4 Grade: OK(10), Expert: SAI
MSL-300. 19-Oct-2007 Solvent: DMSO-d6 + CCl4 SW: 6024 Hz TE: 298 K AQ: 1.20 sec, RD: 0.00 sec