Discovery of RG7834: The First-in-Class Selective and Orally Available Small Molecule Hepatitis B Virus Expression Inhibitor with Novel Mechanism of Action

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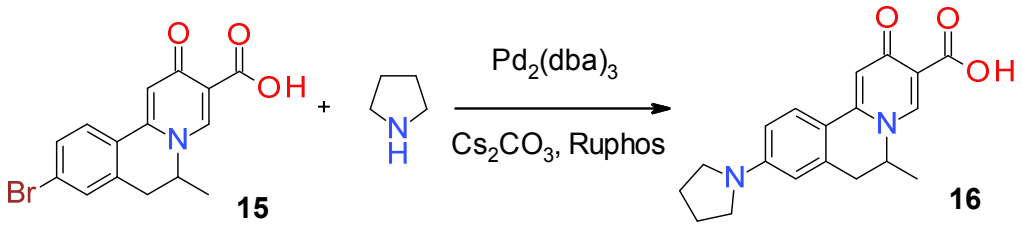
Support information

Content

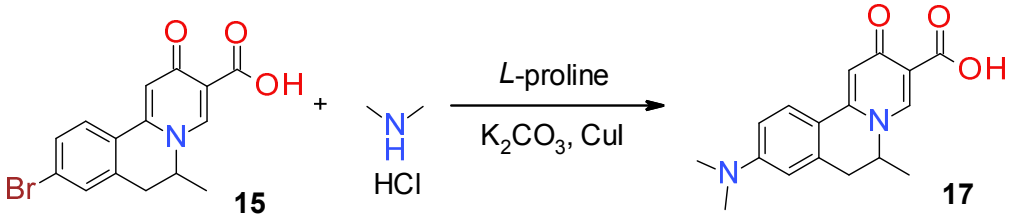
1. Synthesis of compounds 16, 17, 18 and 35

9-(dimethylamino)-6-methyl-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (16)



To a solution of 9-bromo-6-methyl-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (100 mg, 0.30 mmol) in Dioxane (3 mL) was added pyrrolidine (32 mg), Cs_2CO_3 (195 mg, 0.60 mmol), $Pd_2(dba)_3$ (27.5 mg, 0.03 mmol) and Ruphos (28 mg, 0.06 mmol), the reaction was stirred for one hour at 120 ^oC under atmosphere of argon and microwave irradiation. After LCMS indicated the starting material was consumed completely, the reaction mixture was cooled to room temperature and filtered. The filtrate was washed with brine and extracted with ethyl acetate (50 mL). The organic layer was concentrated in vacuum and the residue was purified by preparative HPLC to 6-methyl-2-oxo-9-pyrrolidin-1-yl-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (4 mg). ^1H NMR (400 MHz, METHANOL-d_4) δ ppm 8.76 (br s, 1 H), 7.80 (d, 1H), 7.23 (br s, 1H), 6.81 (dd, 1H) 6.69 (d, 1H), 3.42 - 3.55 (m, 1H), 3.12 (s, 6H), 2.95 (br m, 1H), 2.72 (s, 1H), 1.34 (d, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 299.

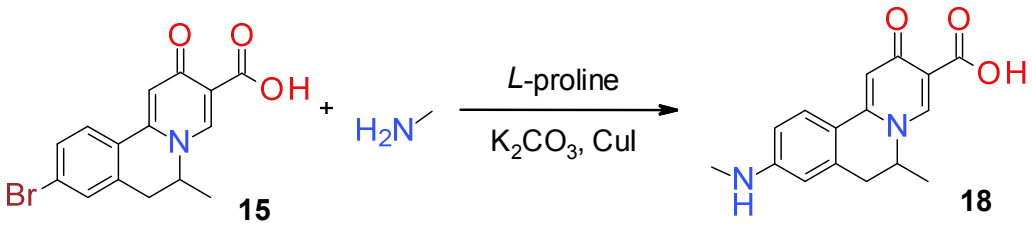
6-methyl-2-oxo-9-pyrrolidin-1-yl-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (17)



To a solution of 9-bromo-6-methyl-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (100 mg, 0.30 mmol) in DMSO (3 mL) was added Dimethylamine hydrochloride (7.2 mg, 0.9 mmol), K_2CO_3 (208 mg, 0.15 mmol), CuI (5.7 mg, 0.03 mmol) and *L*-proline (6.9 mg, 0.06 mmol), the reaction was stirred for 18 hours at 100 ^oC under atmosphere of argon. After LCMS indicated the starting material was consumed completely, the reaction mixture was cooled to room temperature and filtered. The filtrate was washed with brine and extracted with ethyl acetate (50 mL). The organic layer was concentrated in vacuum and the residue was purified by Preparative HPLC to give 9-(dimethylamino)-6-methyl-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (5 mg). ^1H NMR (400 MHz, METHANOL-d_4) δ ppm 8.69

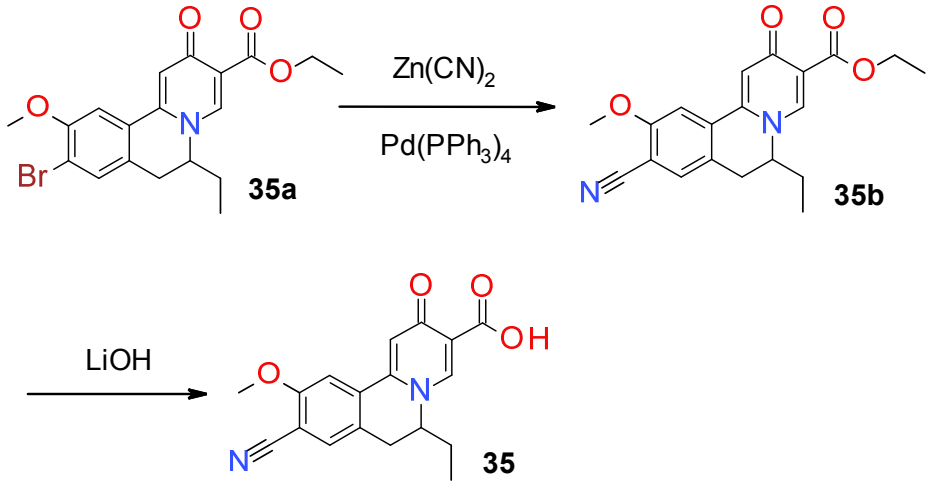
(s, 1H), 7.74 (d, 1H), 7.44 (br s, 1H), 7.16 (s, 1H), 6.64 (dd, 1H), 6.50 (d, 1H), 3.37 - 3.53 (m, 4H), 3.21 (m, 1H), 2.92 (m, 1H), 2.04 - 2.18 (m, 3H), 1.29 - 1.41 (m, 4H). MS obsd. (ESI^+) [$(M+H)^+$]: 325.

6-methyl-9-(methylamino)-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (18)



To a solution of 9-bromo-6-methyl-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (100 mg, 0.30 mmol) in DMSO (3 mL) was added the solution of methylamine in MeOH (7N, 2 mL), K_2CO_3 (82.8 mg, 0.60 mmol), CuI (5.7 mg, 0.03 mmol) and *L*-proline (6.9 mg, 0.06 mmol), the reaction was stirred for 18 hours at 100 °C under atmosphere of argon. After LCMS indicated the starting material was consumed completely, the reaction mixture was cooled to room temperature and filtered. The filtrate was washed with brine and extracted with ethyl acetate (50 mL). The organic layer was concentrated in vacuum and the residue was purified by Preparative HPLC to give6-methyl-9-(methylamino)-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (48 mg). ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 8.89 (br s, 1H), 7.77 (br d, 1H), 7.35 (br s, 1H), 6.67 (br d, 1H), 6.55 (s, 1H), 4.00 (s, 1H), 3.44 (br d, 1H), 2.87 - 2.93 (m, 1H), 2.89 (s, 3H), 2.68 (s, 1H), 1.36 (br d, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 285.

9-cyano-6-ethyl-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (35)



Intermediate **35a** can be obtained through general procedure for the preparation of **G11** mentioned in article.

Step 1: Preparation of ethyl 9-cyano-6-ethyl-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylate (35b)

To a solution of ethyl 9-bromo-6-ethyl-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylate (**35a**, 0.12 g, 0.3 mmol) in DMF (10 mL) was added zinc cyanide (53 mg, 0.45 mmol) and tetrakis(triphenylphosphine) palladium(0) (70 mg, 0.06 mmol). The resultant mixture was stirred at 100 ^{o}C for 10 hours. After being cooled to room temperature, the mixture was concentrated under reduced pressure, and the residue was dissolved in ethyl acetate (50 mL). The resultant solution was washed with water (25 mL x 2) and brine, and then dried over anhydrous Na_2SO_4 and then concentrated to give ethyl 9-cyano-6-ethyl-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylate (100 mg) which was used in the next step without purification.

Step 2: Preparation of 9-cyano-6-ethyl-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (35)

To a solution of ethyl 9-cyano-6-ethyl-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylate (0.1 g, 0.3 mmol) in THF (2 mL) and methanol (6 mL) was added 1.0 M LiOH (0.9 mL) aqueous solution at room temperature. The resultant mixture was stirred for 4 hours, and then acidified to pH 1-2 with 2 M hydrochloric acid. The mixture was extracted with DCM (50 mL x 2), and the combined organic layers were washed with brine, and then dried over anhydrous Na_2SO_4 and then concentrated to give a yellow solid, which was purified by prep-HPLC to give 9-cyano-6-ethyl-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (6 mg). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 16.35 (s, 1H), 8.91 (s, 1H), 7.86 (s, 1H), 7.82 (s, 1H), 7.79 (s, 1H), 4.80 (m, 1H), 4.10 (s, 3H), 3.35 (m, 1H) 3.11 (m, 1H), 1.41 (m, 2H), 0.80 (t, 3H). MS obsd. (ESI$^+$) [(M+H)$^+$]: 325.

2. X-ray Crystal Structure of (*S*)-22.

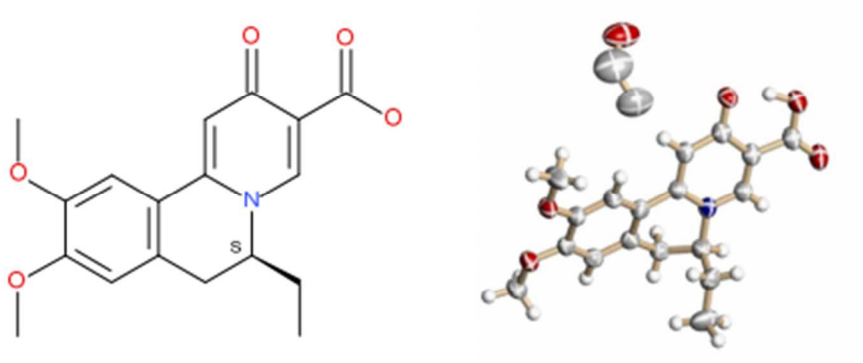


Figure S1. X-ray crystal structure of (*S*)-22

Empirical formula	C19 H19 N O5.50
Formula weight	349.35
Temperature	160(2) K
Wavelength	1.54178 A
Crystal system, space group	Orthorhombic, P2(1)2(1)2(1)
Unit cell dimensions	a = 11.93780(10)A alpha = 90 deg. b = 12.27040(10)A beta = 90 deg. c = 24.9356(2) A gamma = 90 deg.
Volume	3652.61(5) A^3
Z, Calculated density	8, 1.271 Mg/m^3
Absorption coefficient	0.781 mm^-1
F(000)	1472
Crystal size	0.40 x 0.15 x 0.10 mm
Theta range for data collection	3.54 to 66.50 deg.
Limiting indices	-14<=h<=14, -14<=k<=14, -29<=l<=29
Reflections collected / unique	115261 / 6418 [R(int) = 0.0369]
Completeness to theta = 66.71	99.8 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	1.00000 and 0.87885
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	6418 / 0 / 612
Goodness-of-fit on F^2	1.081
Final R indices [I>2sigma(I)]	R1 = 0.0309, wR2 = 0.0911

R indices (all data)	R1 = 0.0314, wR2 = 0.0916
Absolute structure parameter	-0.02(12)
Largest diff. peak and hole	0.504 and -0.171 e.A^-3

3. X-ray Crystal Structure of RG7834

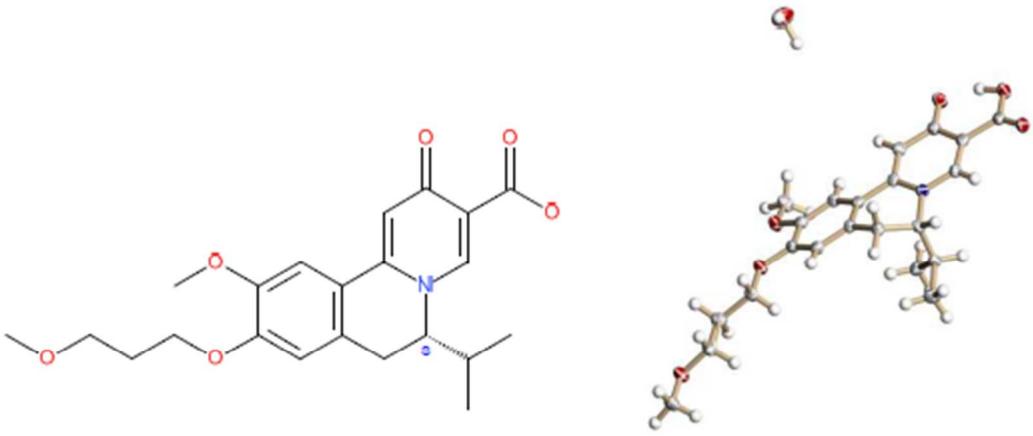
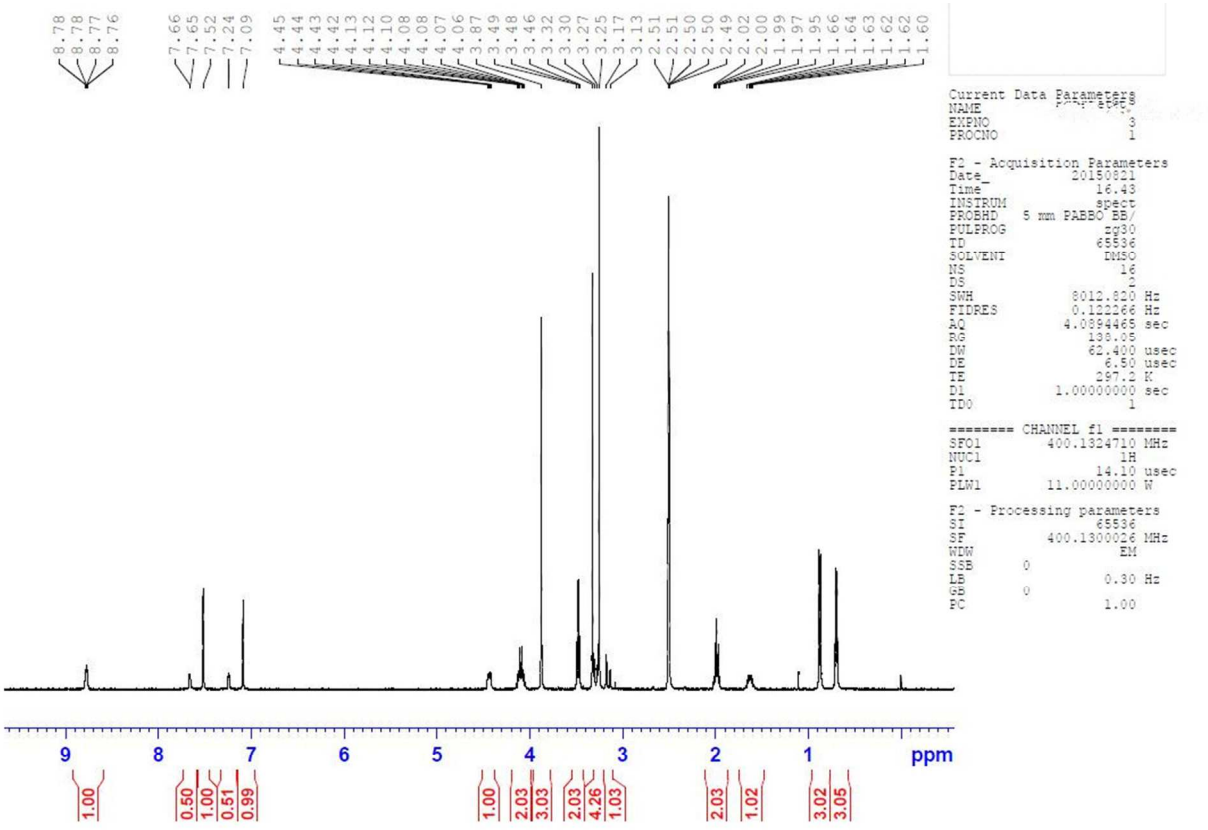


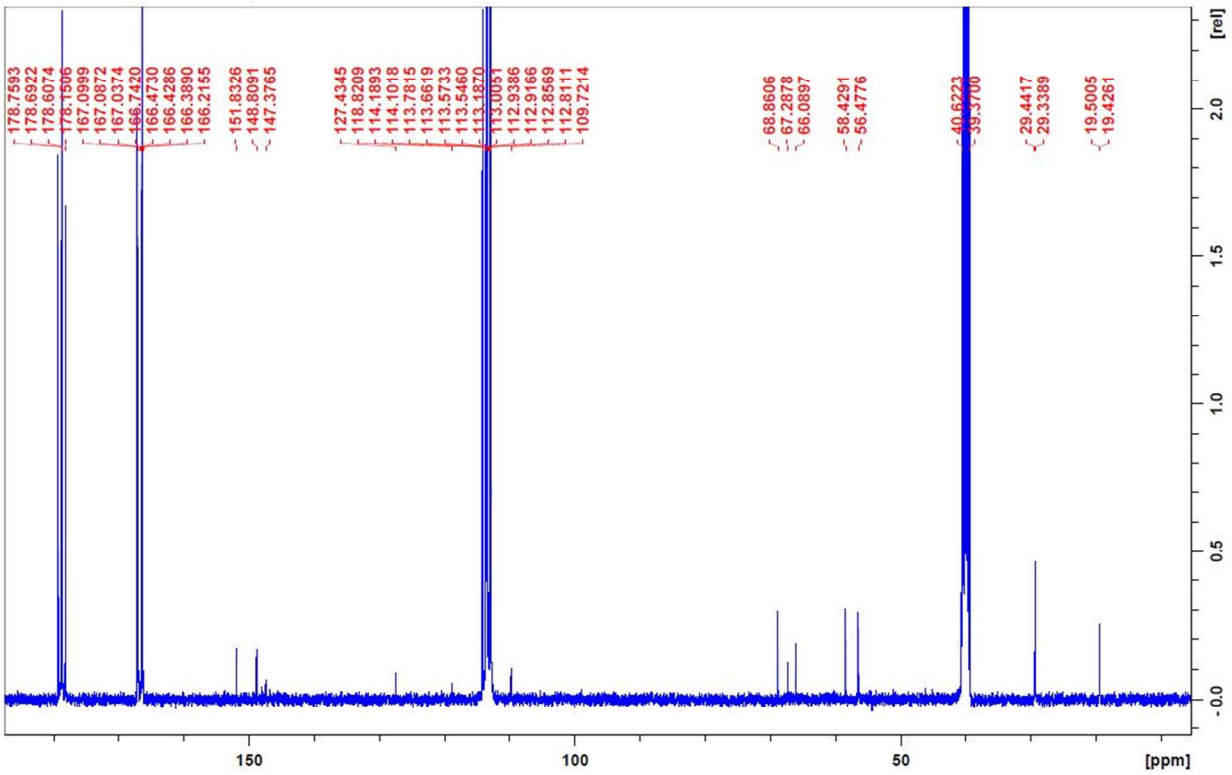
Figure S2. X-ray crystal structure of 64*S*.

Empirical formula	C22 H29 N O7
Formula weight	419.46
Temperature	100(2) K
Wavelength	1.54184 A
Crystal system, space group	Monoclinic, P2(1)
Unit cell dimensions	a = 9.30570(10) A alpha= 90 deg. b = 11.73100(10)A beta = 95.1650(10)deg. c = 9.39180(10) A gamma= 90 deg.
Volume	1021.095(18) A^3
Z, Calculated density	2, 1.364 Mg/m^3
Absorption coefficient	0.842 mm^-1
F(000)	448
Crystal size	0.50 x 0.50 x 0.40 mm
Theta range for data collection	4.73 to 66.71 deg.
Limiting indices	-10<=h<=11, -13<=k<=13, -11<=l<=11

Reflections collected / unique	33729 / 3562 [R(int) = 0.0276]
Completeness to theta = 66.71	99.1 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	1.00000 and 0.74149
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3562 / 1 / 287
Goodness-of-fit on F^2	1.048
Final R indices [I>2sigma(I)]	R1 = 0.0223, wR2 = 0.0617
R indices (all data)	R1 = 0.0224, wR2 = 0.0618
Absolute structure parameter	-0.01(10)
Largest diff. peak and hole	0.160 and -0.162 e.A^-3

4. ^{1}H NMR and ^{13}C NMR Spectra of RG7834

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