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Synthesis and SAR Study of Carbamoyl Pyridone Bicycle Derivatives as Potent Inhibitors of Influenza Cap-dependent Endonuclease

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KEYWORDS

Anti-influenza drug, Cap-dependent endonuclease, Carbamoyl Pyridone, Chelator, Virtual modeling

Abstract

The medicinal chemistry and structure activity relationships (SAR) for a novel series of CArbamoyl pyridone Bicycle (CAB) compounds as influenza Cap-dependent EndoNuclease (CEN) inhibitors are disclosed. Substituent effects were evaluated at the C (N)-1, N-3, and C-7 positions of the CAB ring system using docking study. Submicromolar EC50 values were achieved in the cellular assay with C-7-unsubstituted CAB which possessed a benzhydryl group on either the C-1 or N-1 position. An N-3 substituent was found to be critical for the plasma protein binding effect in vitro, and the CAB-N analogue (**2v**) exhibited reasonable total clearance (CLtot). More importantly, the compound **2v** displayed significant efficacy in a mouse model infected with influenza viruses.

Text

1. Introduction

Influenza is an acute respiratory infectious disease that affects 5-10% of the world population every winter, resulting in 3-5 million severe cases with 250 000-50 000 deaths.[1] In the past century, four influenza pandemics have occurred, each causing the death of millions.[2] While vaccination is a reasonable preventive, its efficacy is heavily dependent on accurate prediction of the predominant infectious strains for each season.[3]

Major antiviral drugs, such as zanamivir, oseltamivir and peramivir, target viral neuraminidase and, can be useful for treatment but must be administered within 48 hours of infection.[4, 5] These therapeutics also can cause undesirable side effects, including unusual neurologic or psychiatric events such as delirium, hallucinations, confusion, and abnormal behavior, primarily in children.[5, 6] Moreover, problems such as the appearance of resistant strains, as well as worldwide epidemics caused by new strains of influenza viruses possessing high pathogenicity and mortality,[7-9] have raised the need for the development of novel anti-influenza drugs that function via different mechanisms.[10]

The influenza virus is a lipid-enveloped virus with a negative-sense single-strand RNA with the viral genome having eight genomic segments; PB2, PB1, PA, HA, NP, NA, M and NS. PB2, PB1 and PA compose the polymerase complex and are responsible for both transcription and replication of the viral genome.[11] The viral replication starts with "cap-snatching" and occurs in three steps . First, the PB2 subunit binds the 5'-mRNA cap of a host cell, and in the second step the PA subunit cleavages 10-13 nucleotides downstream to yield 5'-capped RNA fragments. These fragment serve as primers for viral mRNA elongation catalyzed by the PB1 subunit in the third step.[12] Those three subunits are essential for proliferation of the influenza virus, in particular, Cap-dependent EndoNuclease (CEN) in the N-terminal domain of the PA subunit is highly conserved

across various influenza types and subtypes.[13, 14] Therefore CEN inhibitors are expected to have broad efficacy against multiple influenza viruses. However, there are no currently approved drugs.

Biochemical and crystallographic studies have revealed CEN to contain a dinuclear metal in the active site, employing two Mn^{2+} or Mg^{2+} ions.[14]

In previous researches, several potent inhibitors which bind the CEN active site, have been reported (Figure 1). Almost all of them(**1a**~**1g**) belonged to 1 metal, 2 metal or water-mediated 2 metal chelator which has a hydrophobic region behind the chelate side to enhance CEN inhibitory potency.[15-22] However their antiviral activities are very weak compared to their enzyme inhibitory potencies. This may be due to low cell membrane permeability caused by the high polarity of their chelate motifs. Recently, the non-chelate compound **1h** was reported,[23] to have low dissociation of enzyme and virus inhibition activity, but its in vitro activity was not sufficient for clinical applications.

Our research effort focused on a new type of scaffold, a 2-metals chelator, namely, CArbamoyl pyridone Bicycle (CAB) shown in Figure 2. First, we identified the hit compound **2a**, which binds two metals, as a CEN inhibitor from our compound library. As described below, the evolution from **2a** to the promising lead CAB compounds was

accompanied by a marked increase in enzyme inhibitory and antiviralactivity. In addition, one CAB congener which had acceptable in vivo clearance in rat, showed significant efficacy in a mouse model infected with influenza viruses.

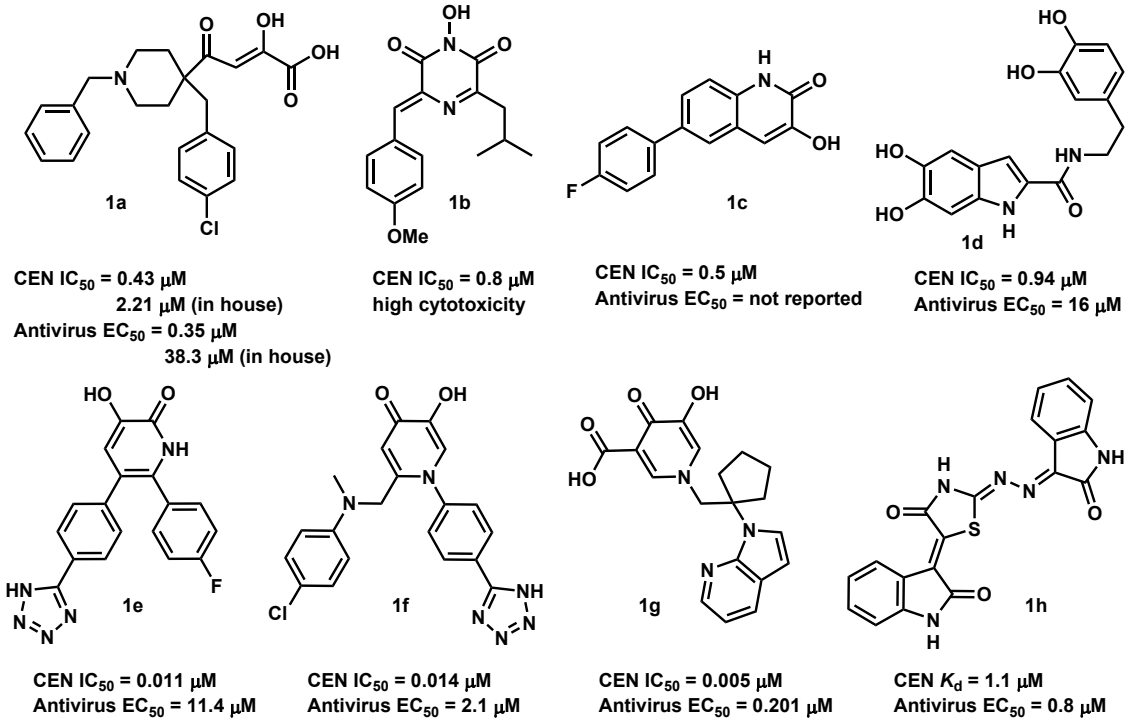


Figure 1. Reported potent CEN inhibitors. The results of compound 1a tested in our conditions were shown.

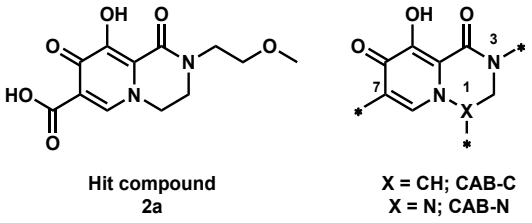
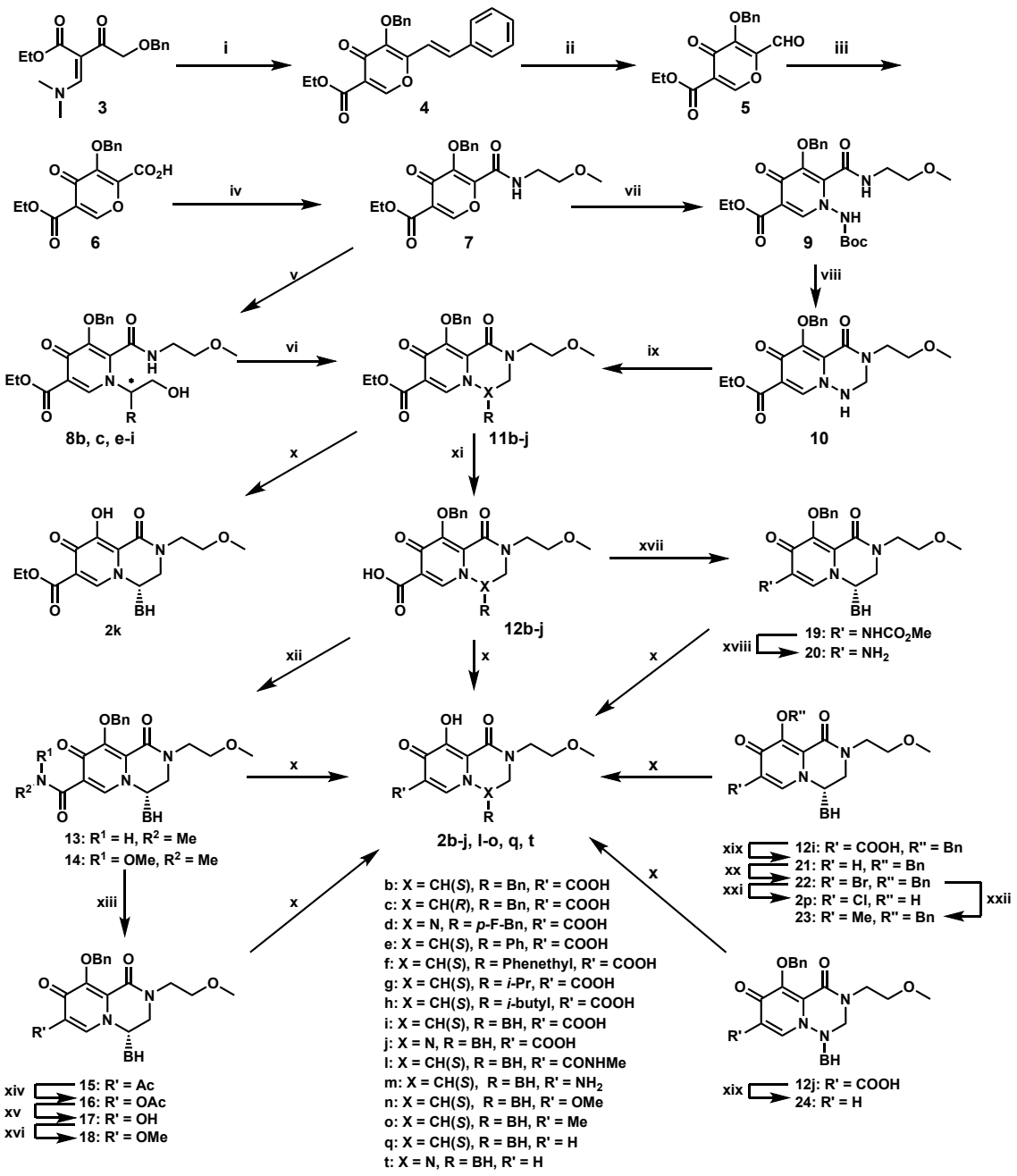


Figure 2. Screening hit compound **2a**, and the chemical structure of the CAB scaffold, and each substituent positions.

2. Chemistry

Scheme 1 shows the synthetic routes to the Carbamoyl Pyridone Bicycle (CAB) moiety. Cyclization of **3**[24] with cinnamoyl chloride and LHMDS yielded pyrone **4**. Ruthenium-catalyzed oxidative cleavage of olefin provided aldehyde **5**. This compound **5** was oxidized with sodium sulfite to produce the corresponding carboxylic acid **6**. Condensation of **6** with 2-methoxyethylamine afforded compound **7**. Insertion of various amino-alcohol yielded pyridone derivatives **8**. Cyclization of **8** under Mitsunobu conditions gave CAB-C moiety **11**. Insertion of N-Boc hydrazine to compound **7** yielded N-amino pyridone **9**. Compound **9** was deprotected with TFA followed by cyclization to provide CAB-N moiety **10**. Alkylation of **10** with various alkyl bromides gave **11**(**d**, **j**). Compound **11k** was deprotected with TFA to produce **2k**. Hydrolysis of ethyl ester and deprotection of benzyl ether furnished compound **2**(**b-j**). 1-Benzhydryl-7-carboxylic acid **12i** was converted to its N-methyl amide **13** and Weinreb amide **14** derivatives by condensation with the corresponding amines. Compound **13** was deprotected with TFA to produce **2l**. Treatment of **14** with MeMgBr in THF afforded 7-acetyl compound **15**. Baeyer-Villiger oxidation of **15** with m-CPBA and hydrolysis of acetyl group gave 7-

hydroxy pyridone **17**. Compound **17** was alkylated with MeI in the presence of NaH to afford 7-methoxy-pyridone **18**. Compound **18** was treated with TFA to yield **2n**. On the other hand, Curtius rearrangement of **12i** by stepwise method provided 7-methoxycarbonylaminopyridone **19**. Hydrolysis of **19** and deprotection of benzyl group gave **2m**. Decarboxylation of **12i** at high temperature (245 °C) in sealed tube under microwave condition produced 7-nonsubstituted pyridone **21**. Deprotection of the benzyl group gave compound **2q**. In the same way, compound **2t** was synthesized from compound **12j** via compound **24**. Compound **21** was treated with NBS to convert 7-bromo-pyridone **22**. Treatment of **22** with CuCl in DMSO afforded 7-chloro derivative 2p in which benzyl ether was deprotected under the reaction conditions. Palladium-catalyzed methylation of compound **22** at the C-7 position was performed to give compound **23**. Deprotection of benzyl ether furnished compound **2o**.

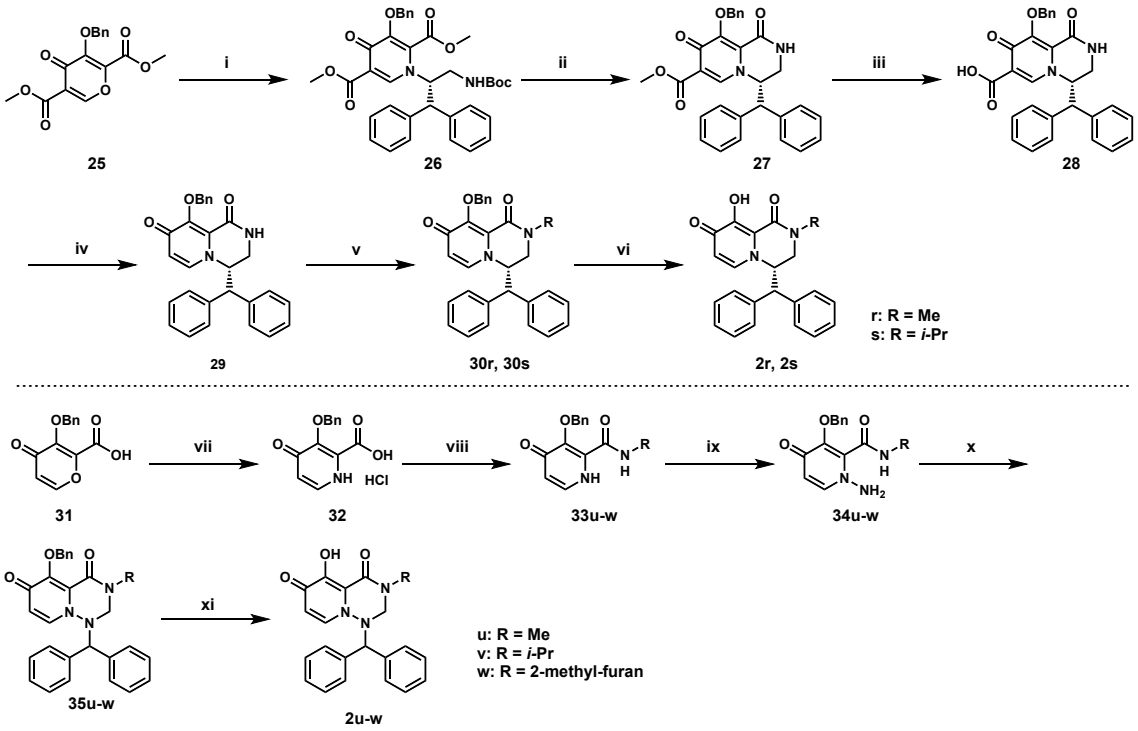


Scheme 1. Synthesis of Compounds **2b-q** and **2t**

Reagents and conditions: (i) cinnamoyl chloride, LHMDS, THF, -78 °C; (ii) $RuCl_3$, $NaIO_4$, conc.H_2SO_4, MeCN, r.t.; (iii) conc.H_2SO_4, amidosulfuric acid, sodium sulfite, MeCN, r.t.; (iv) (1) WSC-HCl, HOBt, DMF, rt; (2) 2-methoxyethanamine, r.t.; (v) amino-alcohol, xylene, 120 °C; (vi) PPh_3, DEAD, THF, r.t.; (vii) N-Boc hydrazine, AcOH, toluene, reflux; (viii) (1) TFA, DCM, r.t.; (2) formaldehyde, EtOH, microwave, 100 °C; (ix) R-Br, Cs_2CO_3, DMF, r.t.; (x) TFA, r.t.; (xi) 2N NaOH aq., EtOH, r.t.; (xii) (1) ethyl

chloroformate, TEA, DMF, r.t.; (2) amine, DMAP, r.t.; (xiii) MeMgBr, THF, -50 °C; (xiv) m-CPBA, DCM, r.t.; (xv) EtOH, reflux; (xvi) MeI, NaH, DMF, r.t.; (xvii) (1) ethyl chloroformate, TEA, DMF, r.t.; (2) NaN_3, r.t.; (3) MeOH, 50 °C; (xviii) 2N NaOH aq., EtOH, 60 °C; (xix) microwave, Ph_2O, 245 °C; (xx) NBS, DCM, reflux; (xxi) CuCl, DMSO, 120 °C; (xxii) hexamethyldistannane, $Pd(PPh_3)_4$, toluene, reflux; BH means benzhydryl

The synthetic routes to 7-decarboxylated CAB-C and CAB-N analogues are shown in Scheme 2. The commercially available pyrone **25** was converted to pyridone **26** via amination with N-Boc-ethylene diamine derivatives. Deprotection of Boc group and neutralization led **26** to CAB-C ring **27**. Hydrolysis of methyl ester followed by decarboxylation yielded compound **29**. Alkylation of **29** with the corresponding alkyl bromides gave **30r** and **30s**. Deprotection of the benzyl ether furnished compound **2r** and **2s**. On the other hand, amination of the commercially available pyrone **31** with ammonia gave pyridone **32**. After condensation of carboxylic acid with the corresponding amine, N-amination was performed to give intermediates **34u-w**. Cyclization of **34** and followed by alkylation provided CAB-N ring **35u-w**. Deprotection of the benzyl group gave compounds **2u-w**.



Scheme 2. Synthesis of Compounds **2r**, **2s**, and **2u-w**

Reagents and conditions: (i) amine, toluene, reflux; (ii) (1) 4N HCl EtOAc, r.t.; (2) $NaHCO_3$ aq., r.t.; (iii) 2N NaOH aq., THF-MeOH, r.t.; (iv) microwave, Ph_2O, 240 °C; (v) R-Br, Cs_2CO_3, r.t.; (vi) TFA, r.t.; (vii) 28% NH_3 aq., r.t.; (viii) (1) WSC-HCl, HOBt, DMF, r.t., (2) amine, r.t.; (ix) O-(2,4-dinitrophenyl)hydroxylamine, K_2CO_3, DMF, r.t.; (x) (1) paraformaldehyde, AcOH, toluene, 100 °C, then concentration, (2) bromodiphenylmethane, Cs_2CO_3, DMF, r.t., (xi) TFA, r.t.

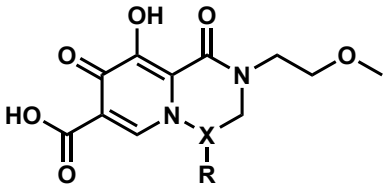
3. Results and Discussion

3.1. In Vitro SAR.

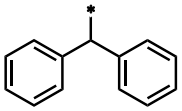
We identified several hit compounds in our library. One of them was compound **2a** which was a CEN inhibitor without a hydrophobic region. From our previous research[22],

introduction of the hydrophobic domain to the chelator moiety led to enhanced inhibitory activity of CEN. A compound substituted by a benzyl group at the C-1 position, **2b** showed remarkable activity compared to **2a**, whereas its enantiomer **2c** had very low activity. In addition, replacement of the nitrogen atom at the 1-position (CAB-N) maintained activity against CEN. On the other hand, either a shorter or longer linker, **2e** and **2f**, was clearly associated with a dramatic loss of potency compared to **2b**. In the same way, conversion to an alkyl group, **2g** and **2h**, reduced in the potency. A branched structure closer to the CAB ring was found to be favorable for the enzyme inhibition. From these findings, we hypothesized that substitution at the benzylic position of compound **2b** and **2d** would be effective. Benzhydryl-substituted compounds **2i** and **2j**, were found to show, 4-5 fold higher inhibition potency than **2b** and **2d,** respectively (corresponding benzyl analogues).

Table 1. SAR of 1-Substitited CAB Inhibitors



Compound	X (Chirality)	R	CEN IC_{50}[a] (μM)	CPE EC_{50}[b] (μM)
2a	CH	H	68.6	N.D.
2b	CH (S)	Bn	0.241	> 50

2c	CH (R)	Bn	27.4	> 50
2d	N	*p*-F-Bn	0.419	> 25
2e	CH (S)	Ph	27.8	> 50
2f	CH (S)	Phenethyl	1.35	N.D.
2g	CH (S)		2.99	N.D.
2h	CH (S)		9.31	N.D.
2i	CH (S)		0.0478	0.293
2j	N		0.116	1.47

[a]Reported values are the means of three or more experiments. [b]Enzyme inhibitory activity of CEN was measured by a fluorescence recovery assay of the enzyme reaction. [c]MDBK cells were incubated with test compounds and influenza A virus (A/WSN/33) for 72 hr, and the concentration of test compound resulting in 50% cell protection was reported as the EC_{50}. N.D., not determined.

We attempted to validate the chelating mode of our inhibitors to the active site in CEN. We hypothesized that chelation by the CAB scaffold could done by the four modes shown in Figure 3. In order to estimate the chelate binding mode, constrained docking and minimization studies were performed for a reported CEN crystal structure (PDB code 2W69)[14] with Glide and MacroModel (Schrödinger Inc. U.S.A.). Metal binding oxygen atoms of **2i** were fixed in each optimal geometry to bind the two metals in the active site. The ligand molecule **2i** and the receptor protein were minimized in the flexible mode.

The coordinating phenolic oxygen atom of inhibitor **2i** was deprotonated and the charge on each metal center was assigned as 2+ in the simulation.

As a result, docking analysis showed that the CAB derivative **2i** could chelate to the active site metals in the most stabilized mode (c). C-7 carboxylic acid on **2i** did not chelate to metals, but interacted with the side chains of Lys134 and Tyr130 mediated by the hydrogen bond. More interestingly, the two benzenes on **2i** nicely filled two hydrophobic pockets (Pocket 1 and 2 in Figure 4). In addition, the methoxyethyl moiety binding pocket neighboring N-3 position (Pocket 3) could tolerate a variety of substitutions, which suggests that this region of the binding site is not spatially constrained.

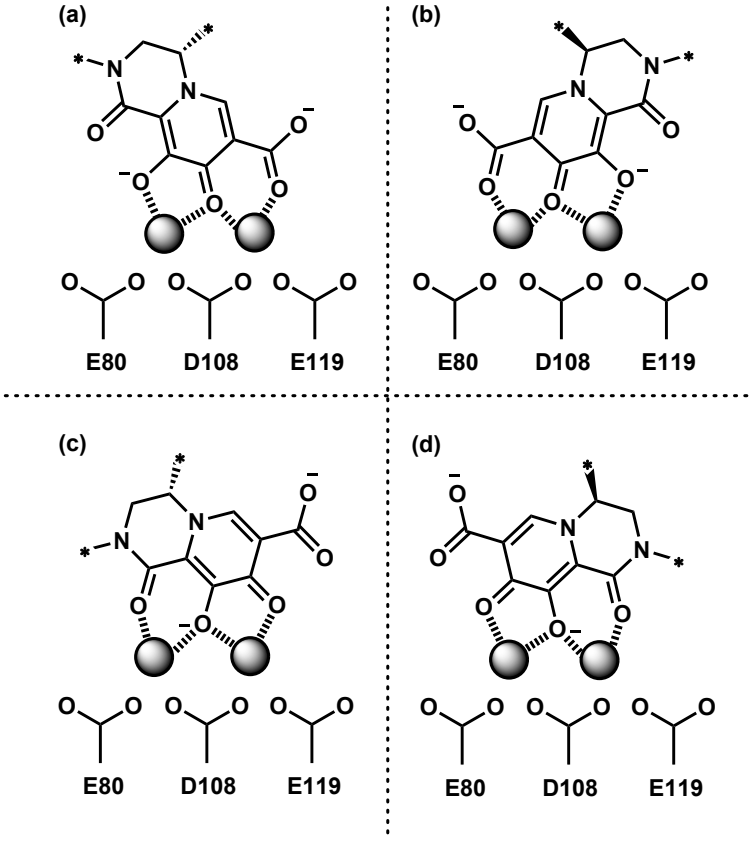


Figure 3. Conceivable chelate modes of the CEN active site with CAB-C analogue.

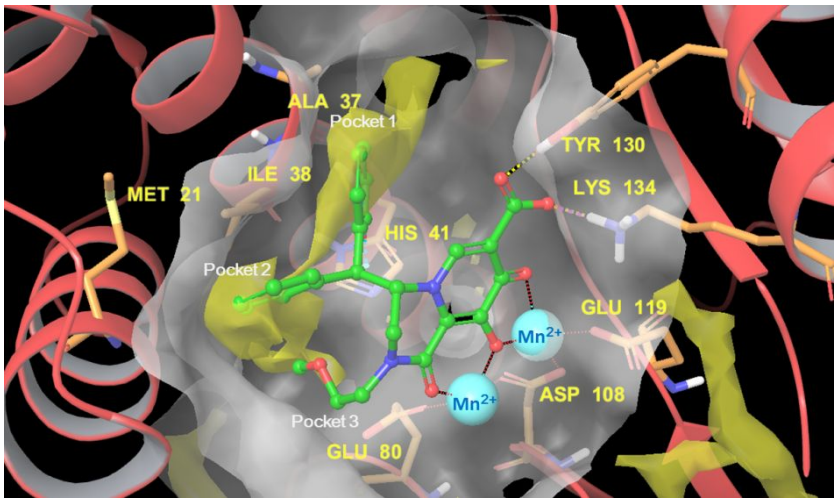
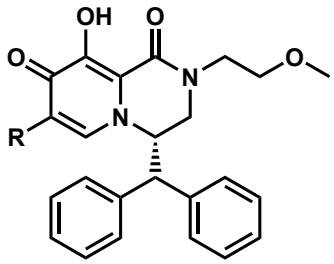


Figure 4. Docking simulation of **2i** (green) binding to the active site of CEN. Two metals and lipophilic sites are shown in light blue and yellow respectively.

In the light of these findings, we considered the conversion of carboxylic acid at C-7 to be acceptable for the CEN active pocket. Ethyl ester **2k** and N-methyl amide **2l** were less potent in both the CEN and CPE assays but the activities did not disappear. When sterically restricted and non-chelatable substituents, such as an amino, methoxy, methyl or chloro group were evaluated, the potency decreased by 2~8 fold. By removing the carboxylic acid at the C-7 position, we obtained a new type of inhibitor **2q** that was 2-fold less potent than **2i** in the CEN assay. This may have been due to the absence of

the hydrogen bond. However, compound **2q** showed the same range of activity in the CPE assay. Consequently, it is reasonable to assume that non-substitution at the C-7 position increases membrane permeability to result in enhancing cell activity, because decarboxylation reduces down the molecular weight and increases the lipophilicity. (Calculated LogP of **2i** and **2q** were 1.29 and 2.06, respectively)[25]

Table 2. SAR of 7-Substitited CAB Inhibitors[a]



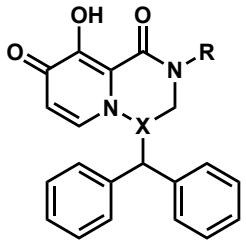
Compound	R	CEN IC_{50}[b] (μM)	CPE EC_{50}[c] (μM)
2i	COOH	0.0478	0.293
2k	CO_2Et	0.298	2.53
2l	CONHMe	1.59	7.11
2m	NH_2	0.358	3.86
2n	OMe	0.110	1.68
2o	Me	0.281	2.47
2p	Cl	0.114	0.541
2q	H	0.115	0.134

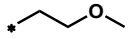
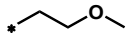
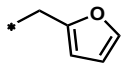
[a]Reported values are the mean of three or more experiments. [b]Enzyme inhibitory activity of CEN was measured by a fluorescence recovery assay of the enzyme reaction. [c]MDBK cells were incubated with test compounds and influenza A virus (A/WSN/33)

for 72 hr, and the concentration of test compound resulting in 50% cell protection was reported as the EC_{50}.

Next, we explored the N-3 position of the CAB scaffold. We thought conversion of the N-3 position could adjust the physicochemical property of molecules without loss of potency, because the docking study indicated that this region was not spatially constrained in the CEN active site. The presence of an N-3 methyl or isopropyl substituent in CAB-C series (**2r**, **2s**) appeared to lead to the retaining of antiviral potency. The CAB-N series (**2t**, **2u**, **2v**) were similarly found to be equipotent to CAB-C analogues in the CPE assay except for the 2-methyl-furan-substituted **2w** which showed reduced potency in both assays. In silico docking study using **2v** demonstrated the isopropyl group at N-3 position did not interact any residues in CEN, because it was located in the solvent exposure resion (Figure S3). Interestingly, PK study with rat showed that more hydrophobic substituent groups improved rat iv clearance in each series. This was caused by thet decreasing fu value in plasma in a group with high metabolic stability (**2q-2v**). On the other hand, **2w** was lower than the clearance value in spite of having the lowest fu value. It may be due to low stability in the rat microsome. As a result, we obtained the promising inhibitor **2v** which exhibited good antiviral potency with 81.6 nM and reasonable in vivo clearance with 10.9 mL/min/kg.

Table 3. SAR of 3-Substitited CAB Inhibitors[a] and Rat PK Parameters[b]



					Rat		
Compound	X (*Chirality*)	R	CEN IC_{50}[c] (nM)	CPE EC_{50}[d] (nM)	Met. Stab. Microsome[e] (%)	iv Cl[f] (mL/min/kg)	fu[g] (%)
2q			115	134	79.7	42.2	29.7
2r	CH (*S*)	Me	80.4	144	79.9	31.6	28.5
2s		*i*-Pr	239	57.5	78.7	25.3	18.2
2t			103	75.2	83.4	47.4	12.9
2u		Me	164	81.6	92.1	18.8	7.00
2v	N	*i*-Pr	286	81.6	86.0	10.9	3.77
2w			588	478	50.6	42.6	2.17

[a]Reported values are the means of three or more experiments. [b]Values are the means of duplicate experiments. [c]Enzyme inhibitory activity of CEN was measured by a fluorescence recovery assay of the enzyme reaction. [d]MDBK cells were incubated with test compounds and influenza A virus (A/WSN/33) for 72 hr, and the concentration of test compound resulting in 50% cell protection was reported as the EC_{50}. [e]Metabolic stability in the presence of rat liver microsomes was represented as the %compound remaining at a concentration of 2 μM after 30 min, incubated at 37 °C. [f]Rat clearance was measured by LC/MS/MS after a single intravenous administration. [g]Free fraction ratio in the presence of rat serum.

As the active site of CEN is highly conserved among all influenza virus families, we confirmed the broad antiviral spectrum using compound **2s** and **2v** . They showed similar antiviral behavior in the CPE assay. The atom at the 1-position (C or N) may not have affected the antiviral activity as described previously. They displayed equivalent antiviral activities against the H1N1 type, including the reverse genetic strain (rgA/WSN/33), oseltamivir-resistant strain (rgA/WSN/33–NA/H274Y), and the clinically isolated strain (A/PR/8/34). Although their antiviral activities against H3N2 (A/Victoria/3/75, A/HongKong/8/68) were slightly reduced, those of influenza B exhibited the same range as that against H1N1.

Table 4. Antiviral potency of compound **2s** and **2v** against various influenza viruses

Virus		Mean CPE EC_{50}[a] (nM)	
		2s	2v
A / H1N1	A/PR/8/34	194.4±6.0	183.0±11.3
	rgA/WSN/33 [b]	154.8±45.9	165.1±15.8
	rgA/WSN/33 –NA/H274Y [b]	89.2±67.7	80.4±50.0
A / H3N2	A/Victoria/3/75	487.6±185.5	828.8±94.4
	A/HongKong/8/68	232.4±17.4	301.5±77.9
B	B/Hong Kong/5/72	100.1±19.0	124.3±39.8

	B/Maryland/1/59	177.0±11.6	176.0±6.4

[a]MDCK cells were incubated with test compounds and influenza viruses for 72 hr, and the concentration of test compound resulting in 50% cell protection was reported as the EC_{50}. [b]rg; means reverse genetics.

3.2. In vivo efficacy

Finally, compound **2v** was advanced to a mouse influenza B (B/Maryland/1/59) model (Figure 5) where it showed dose-dependent efficacy in an immediate treatment model after infection at 0.08-10 mg/kg(q.d., 1 day) by intravenous administration (ED_{50}=0.89 mg/kg/day). Moreover, we confirmed 100% survival rate at 10 mg/kg. This demonstrated that CEN is an attractive target for influenza treatment and its efficacy against influenza B is superior to other target of influenza virus (PB2)[26]. However, we confirmed that **2v** was deficient with respect to antiviralpotency because a low dose (0.08, 0.4, and 2 mg/kg) of **2v** was not able to protect all mice infected with influenza virus. Furthermore, thet oral bioavailability of **2v** was not sufficient to confirm its in vivo activity with the same model.

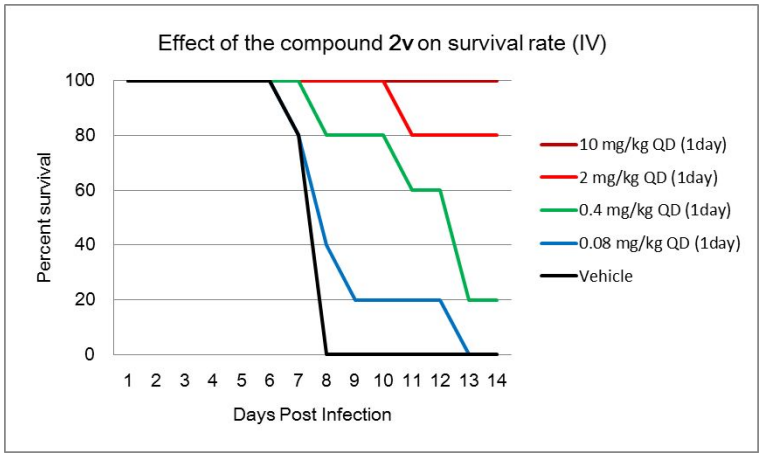


Figure 5. In vivo activity of **2v** in mouse influenza B model when administered immediately after infection: survival curve of female BALB/C mice (5 mice/group) inoculated with influenza viruses B/Maryland/1/59 (100 $TCID_{50}$/mouse) by intranasal instillation.

4. Conclusion

In summary, we established the synthesis of two carbamoyl pyridone bicycle analogues (CAB-C, N) focused on the 1-, 3-, and 7-positions, which were identified asCEN inhibitors of the influenza virus starting from our library compounds. Our research on the substituent effects of 1- and 7-positions of the core templates, led to improved enzyme inhibition and antiviral activity with corroboration by docking analysis. Additionally, more hydrophobic substituents at the 3-position, in particular of thtet CAB-N moiety, presenteda reasonable pharmacokinetic profile in rat coupled with increased protein

binding. Further testing showed that compound **2s** and **2v** were potent against all influenza A and B strains tested, including pandemic H1N1 flu strains. Moreover, **2v** was effective in the mouse model infected with influenza B virus in a dose-dependent manner. Additional SAR studies in the title series leading to the discovery and development of clinical candidate S-033188 will be reported in future publications.

EXPERIMENTAL SECTION

Compound Purity and Identity. 1H NMR(400 MHz) spectra were measured on a Varian MERCURY or Bruker in a solution of either $CDCl_3$ or DMSO-d_6, using tetramethylsilane as the internal standard. Chemical shifts are expressed as δ (ppm) values for protons relative to the internal standard. The purity of the test compounds was confirmed by UPLC-MS with UV diode array detection to determine purity and by MS to confirm molecular weight. A Waters Acquity UPLC system comprising Binary Solvent manager, Sample Manager, PDA Detector, Waters ZQ or SQD mass spectrometer, and Waters Acquity evaporative light scattering detector were employed. The Column: ACQUITY UPLC® BEH C18 (1.7 μm、i.d.2.1x 50 mm) (Waters) Flow rate: 0.8 mL/min, UV detection wavelength: 254 nm. Mobile phase: [A] is 0.1 % formic acid-containing aqueous solution, and [B] is 0.1 % formic acid-containing acetonitrile solution. Gradient: Linear gradient of 5 % to 100 % solvent [B] for 3.5 minutes was performed, and 100 %

solvent [B] was maintained for 0.5 minute. Purity of all tested compounds were ≥95%. Analytical HPLC was also used in some cases to monitor reactions and establish final compound purity using a C-18 column (5.0 μm, 0→100% CH_3CN /water with 0.1% TFA and UV detection with mass spectrometer detection. Preparative HPLC conditions were as follows: C-18 column, 5 μm, 21.2 mm × 150 mm; flow rate = 4 mL/min;mobile phase: 10→100% CH_3CN/H_2O/0.1%HCOOH (10 min run).

Ethyl-(E)-5-(benzyloxy)-4-oxo-6-styryl-4H-pyran-3-carboxylate (4). A 1N lithiumhexamethyldisilazane THF solution (4.29 ml, 4.29 mmol) was cooled to -78° C., and a THF solution (4 ml) of compound 3 (500 mg, 1.72 mmol) and cinnamoyl chloride (343.2 mg, 2.06 mmol) were added dropwise thereto over 3 minutes while the same temperature was retained. After the reaction solution was stirred at the same temperature for 25 minutes, 2N hydrochloric acid (10 ml) was added, and the mixture was further stirred at room temperature for 10 minutes. To the reaction solution was added ethyl acetate, the organic layer was separated, and the aqueous layer was extracted with ethyl acetate three times. The combined extracts were dried with sodium sulfate. The solvent was distilled off and the resulting oil was purified by silica gel column chromatography. From fraction eluted with n-hexane-ethyl acetate (1:1, v/v), 364.3 mg (yield 56%) of

compound **4** was obtained as a white solid. ^{1}H NMR ($CDC1_3$) δ 1.40 (3H, t, J = 7.2 Hz), 4.39 (2H, q, J = 7.2 Hz), 5.27 (2H, s), 6.99 (1H, d, J = 16.2 Hz), 7.23 (1H, d, J = 16.2), 7.26-7.48 (10H, m), 8.45 (1H, s).

Ethyl-5-(benzyloxy)-6-formyl-4-oxo-4H-pyran-3-carboxylate (5). To a MeCN (5 ml) solution of compound **4** and ruthenium chloride (2.76 mg, 0.0133 mmol) was added dropwise an aqueous solution (8 ml) of sodium periodate (625.8 mg, 2.93 mmol) and 96% sulfuric acid (287.4 mg, 2.93 mmol) over 10 minutes at room temperature under nitrogen stream. After the reaction solution was stirred at the same temperature for 5 minutes, ethyl acetate was added, the organic layer was separated, and the aqueous layer was extracted with ethyl acetate two times. The combined extracts were dried with sodium sulfate. The solvent was distilled off, and the resulting oil was purified by silica gel column chromatography. From fraction eluted with n-hexane-ethyl acetate (1:1, v/v), 303.2 mg (yield 75%) of compound **5** was obtained as a colorless oil. ^{1}H NMR ($CDC1_3$) δ 1.39 (3H, t, J = 6.9 Hz), 4.40 (2H, q, J = 6.9 Hz), 5.54 (2H, s), 7.37 (5H, s), 8.48 (1H, s), 9.85 (1H, s).

3-(Benzyloxy)-5-(ethoxycarbonyl)-4-oxo-4H-pyran-2-carboxylic acid (6). To a MeCN (15 ml) solution of compound **5** (1.00 g, 3.31 mmol) was added an aqueous solution (10 ml) of 96% sulfuric acid (421.7 mg, 4.30 mmol) and amidosulfuric acid

(642.7 mg, 6.62 mmol) at room temperature, the mixture was stirred, and an aqueous solution (10 ml) of sodium chlorite (388.9 mg, 4.30 mmol) was added dropwise over 5 minutes while the same temperature was retained. After the reaction solution was stirred at the same temperature for 5 minutes, an aqueous saturated sodium chloride solution was added, and the mixture was extracted with ethyl acetate three times. The combined extracts were dried with sodium sulfate. The solvent was distilled off, and the resulting oil was purified by silica gel column chromatography. The materials were eluted firstly with chloroform and then with chloroform-MeOH (7:3, v/v). Concentration of the objective fraction afforded 748.8 mg (yield 71%) of compound **6** as a colorless oil. ^{1}H NMR ($CDC1_3$) δ 1.40 (3H, t, J = 7.2 Hz), 3.93 (1H, br s), 4.40 (2H, q, J = 7.2 Hz), 5.61 (2H, s), 7.38-7.44 (10H, m), 8.52 (1H, s).

Ethyl-5-(benzyloxy)-6-((2-methoxyethyl)carbamoyl)-4-oxo-4H-pyran-3-carboxylate (7). To a DMF (10 ml) solution of compound **6** (1.00 g, 3.14 mmol) were added WSC.HC1 (1.20 g, 6.28 mmol) and HOBt (551.6 mg, 4.08 mmol) at room temperature, and the mixture was stirred at the same temperature for 90 minutes. The reaction solution was cooled to 0° C, and a DMF (2 ml) solution of 2-methoxyethanamine (236.0 mg, 3.14 mmol) was added dropwise over 3 minutes. The reaction solution was stirred at the same temperature for 1 hour, water was added, and the mixture was extracted

with ethyl acetate three times. The extract was washed with water three times, and dried with sodium sulfate. The solvent was distilled off and the resulting oil was purified by silica gel chromatography. The materials were eluted firstly with n-hexane-ethyl acetate (1:1, v/v) and, then, with n-hexane-ethyl acetate (1:9, v/v). Concentration of the objective fraction afforded 928.5 mg (yield 79%) of compound **7** as a brown oil. ^{1}H NMR ($CDC1_3$) δ 1.39 (3H, t, J = 7.2 Hz), 3.29 (3H, s), 3.41 (2H, t, J = 5.4 Hz), 3.47-3.53 (2H, m), 4.39 (2H, q, J = 7.2 Hz), 5.44 (2H, s), 7.36 (3H, m), 7.44-7.47 (2H, m), 8.07 (1H, br s), 8.54 (1H, s).

Ethyl-(S)-5-(benzyloxy)-1-(1-hydroxy-3-phenylpropan-2-yl)-6-((2-methoxyethyl) carbamoyl)-4-oxo-1,4-dihydropyridine-3-carboxylate (8b). A xylene (2 ml) solution of compound **7** (500 mg, 1.33 mmol) and (S)-2-amino-3-phenylpropan-l-ol (604.2 mg, 4.0 mmol) was heated to 120° C, and stirred for 30 minutes. After the reaction solution was cooled to room temperature, the solvent was distilled off, and the resulting oil was purified by silica gel chromatography. The materials were eluted first with chloroform and then with chloroform-MeOH (9:1, v/v). Concentration of the objective fraction afforded 487 mg (yield 72%) of compound **8b** as a colorless oil. ^{1}H NMR ($CDC1_3$) 8:1.41 (3H, t, J=6.9 Hz), 2.24-2.34 (1H, m), 2.24-3.00 (1H, m), 3.03-3.16 (1H, m), 3.05 (3H, m), 3.25-3.32 (2H, m), 4.13-4.19 (1H, m), 4.17-4.30 (1H, m), 4.36-4.47 (1H, m), 4.51-4.54

(1H, m), 4.55 (1H, d, J = 10.5 Hz), 5.78 (1 H, t, J = 6.9 Hz), 7.17-7.26 (4H, m), 7.28-7.35 (5H, m), 7.49 (1H, t, J 5.4 Hz), 6.32 (1H, s).

Compound **8i** was prepared by the procedure used for compound **11b**.

Ethyl-(S)-5-(benzyloxy)-1-(3-hydroxy-1,1-diphenylpropan-2-yl)-6-((2-methoxyethyl)carbamoyl)-4-oxo-1,4-dihydropyridine-3-carboxylate (8i). Brown oil, 52 % yield. ^{1}H NMR ($CDCl_3$) δ 8.15 (1H, s), 7.58-7.27 (12H, m), 7.20 (1H, d, J = 7.1 Hz), 7.09 (2H, t, J = 7.3 Hz), 7.00 (1H, d, J = 7.2 Hz), 5.02-4.92 (2H, m), 4.74 (1H, d, J = 11.4 Hz), 4.58 (1H, d, J = 10.4 Hz), 4.46-4.32 (1H, m), 4.12 (2H, q, J = 7.2 Hz), 3.65 (1H, t, J = 9.5 Hz), 3.45-3.34 (2H, m), 3.17-2.97 (5H, m), 1.35 (3H, t, J = 7.1 Hz).

Compound **8c, 8e, 8f, 8g,** and **8h** were not identified because of the parallel synthesis. Used reagents were same as the synthesis of **8b.**

Ethyl-5-(benzyloxy)-1-((tert-butoxycarbonyl)amino)-6-((2-methoxyethyl)carbamoyl)-4-oxo-1,4-dihydropyridine-3-carboxylate (9). To a solution of compound **7** (3.5 g, 9.32 mmol) in toluene (30 ml) was added tert-butyl hydrazinecarboxylate (2.465 g, 18.65 mmol) and acetic acid (0.213 ml, 3.73 mmol) at r.t. under N_2 atm.. The mixture was stirred at reflux for 3 hours. After evaporation, the crude product was purified by silica gel chromatography. The material was eluted with chloroform-MeOH (20:1, v/v). Collected fractions were evaporated to afford compound

9 (3.8 g, 7.76 mmol, 83 %) as a white solid. ^{1}H NMR ($CDCl_3$) δ 8.12 (1H, br s), 7.41-7.29 (5H, m), 5.20 (2H, s), 4.35 (2H, q, J = 7.2 Hz), 3.39-3.24 (7H, m), 1.46 (9H, s), 1.38 (3H, t, J = 7.2 Hz).

Ethyl-5-(benzyloxy)-3-(2-methoxyethyl)-4,6-dioxo-2,3,4,6-tetrahydro-1H-pyrido[2,1-f][1,2,4]triazine-7-carboxylate (10). To a solution of compound **9** (500 mg, 1.021 mmol) in DCM(10 mL) was added TFA (2 mL) at room temperature. The mixture was stirred at room temperature for 3 hours. After toluene (10 mL) was added, the mixture was concentrated under reduced pressure. The resulting residue was diluted with sat $NaHCO_3$, and then extracted with CH_2Cl_2 to afford the deprotected crude product. The crude compound was taken to the next step without purification. To a dried sealed tube were added crude product in EtOH (10 mL) and paraformaldehyde (92 mg, 3.06 mmol) at room temperature. After the mixture was stirred at 140 °C for 10 minutes, the reaction mixture was concentrated under reduced pressure. The crude product was subjected to a silica gel chromatography and eluted with $CHCl_3$/MeOH. Collected fractions were evaporated to afford compound **10** (352 mg, 85.9 % yield) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 8.16 (1H, s), 7.37-7.28 (5H, m), 6.18 (1H, t, J = 7.8 Hz), 5.27 (2H, s), 4.46 (2H, d, J = 7.8 Hz), 4.33 (2H, q, J = 7.2 Hz), 3.57-3.51 (2H, m), 3.51-3.46 (2H, m), 3.31 (3H, s), 1.35 (3H, t, J = 7.2 Hz).

Ethyl-(S)-4-benzyl-9-(benzyloxy)-2-(2-methoxyethyl)-1,8-dioxo-1,3,4,8-tetrahydro-2H-pyrido[1,2-a]pyrazine-7-carboxylate (11b). To a THF (6 ml) solution of compound **8b** (2.86 g, 5.63 mmol) and triphenylphosphine (2.21 g, 8.45 mmol) was added dropwise a DEAD 40 wt % toluene solution (3.68 g, 8.45 mmol) at room temperature over 3 minutes. The reaction solution was stirred at the same temperature for 30 minutes, the solvent was distilled off, and the resulting oil was purified by silica gel chromatography. From a fraction eluted with ethyl acetate-MeOH (9:1, v/v), 1.37 g (yield 50%) of compound **11b** was obtained as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.31 (3H, t, J = 7.2 Hz), 3.07 (2H, d, J = 6.9 Hz), 3.33 (3H, s), 3.57-3.80 (4H, m), 3.95 (1H, dd, J = 3.0 Hz, 6.6 Hz), 4.01-4.14 (1H, m), 4.16-4.34 (2H, m), 5.24 (1H, d, J = 9.9 Hz), 5.51 (1H, d, J = 9.9 Hz), 7.01-7.03 (2H, m), 7.21-7.37 (5H, m), 7.41-7.58 (1H, m), 7.64-7.69 (2H, m).

Ethyl-5-(benzyloxy)-1-(4-fluorobenzyl)-3-(2-methoxyethyl)-4,6-dioxo-2,3,4,6-tetrahydro-1H-pyrido[2,1-f][1,2,4]triazine-7-carboxylate (11d). To a solution of compound **10** (50 mg, 0.125 mmol) in DMF (0.5 ml) was added 1-(bromomethyl)-4-fluorobenzene (0.023 ml, 0.187 mmol) and Cs_2CO_3 (101 mg, 0.311 mmol) at room temperature. After the mixture had been stirred at r.t. for 18 hours, the reaction mixture was diluted with H_2O, then extracted with $CHCl_3$ (2 x 20 mL). The organic layers were

combined and washed with H_2O, and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by silica gel chromatography. From a fraction eluted with $CHCl_3$/MeOH, collected fractions were evaporated to afford compound **11d** (45.8 mg, 72 % yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.70 (1H, s), 7.64 (2H, d, J = 7.3 Hz), 7.38-7.28 (3H, m), 7.18 (2H, t, J = 6.5 Hz), 7.05 (2H, t, J = 8.2 Hz), 5.44 (2H, br s), 4.29 (2H, q, J = 6.9 Hz), 4.10 (2H, s), 3.68-3.50 (4H, m), 3.31 (3H, s), 1.31 (3H, t, J = 7.2 Hz).

Compound **11i** was prepared by the procedure used for compound **11b**.

Ethyl-(S)-4-benzhydryl-9-(benzyloxy)-2-(2-methoxyethyl)-1,8-dioxo-1,3,4,8-tetrahydro-2H-pyrido[1,2-a]pyrazine-7-carboxylate (11i). Yellow solid, 33% yield. ^{1}H NMR (DMSO-d_6) δ 1.18 (3H, m), 3.11 (3H, s), 3.16 (1H, m), 3.28 (1H, m), 3.76 (1H, m), 3.97-4.13 (3H, m), 4.31(1H, d, J = 11.3 Hz),5.08(2H, s), 5.52 (1H, d, J = 12.0Hz), 7.18-7.25 (6H, m), 7.25-7.45 (6H, m), 7.55-7.66 (6H, m). MS: m/z 567.7 $[M+H]^+$.

Compound **11j** was prepared by the procedure used for compound **11d**.

Ethyl-1-benzhydryl-5-(benzyloxy)-3-(2-methoxyethyl)-4,6-dioxo-2,3,4,6-tetrahydro-1H-pyrido[2,1-f][1,2,4]triazine-7-carboxylate (11j). White solid, 71 % yield. ^{1}H NMR ($CDCl_3$) δ 7.66 (2H, d, J = 7.3 Hz), 7.59 (1H, s), 7.53 (2H, d, J = 7.2 Hz), 7.44 (2H, t, J = 7.1 Hz), 7.40-7.27 (5H, m), 7.15 (4H, d, J = 13.1 Hz), 5.44 (2H, q, J =

10.2 Hz), 5.23 (1H, s), 4.88 (1H, d, J = 13.7 Hz), 4.46 (1H, d, J = 13.6 Hz), 4.28-4.15 (2H, m), 4.08 (1H, d, J = 14.1 Hz), 3.49-3.36 (2H, m), 3.17 (3H, s), 3.03-2.90 (1H, m), 1.28 (3H, t, J = 7.1 Hz).

Compound **11c, 11e, 11f, 11g,** and **11h** were not identified because of the parallel synthesis. Used reagents were same as the synthesis of **11b.**

(S)-4-Benzyl-9-(benzyloxy)-2-(2-methoxyethyl)-1,8-dioxo-1,3,4,8-tetrahydro-2H-pyrido[1,2-a]pyrazine-7-carboxylic acid (12b). To an EtOH (6 ml) solution of compound **11b** (1.0 g, 2.04 mmol) was added a 2N aqueous sodium hydroxide solution (6 ml), and the mixture was stirred at room temperature for 30 minutes. The reaction solution was neutralized with 2N hydrochloric acid, and the precipitated solid was filtered, and dried to obtain 754 mg (yield 80%) of compound **12b** as a white solid. ^{1}H NMR ($CDCI_3$) δ 3.10 (2H, d, J = 7.8 Hz), 3.33 (3H, s), 3.57-3.69 (4H, m), 3.82-3.90 (1H, m), 3.95 (1H, dd, J = 3.3Hz, 13.8Hz),4.36(1H, dd, J = 6.3Hz, 7.5Hz),5.36(1H, d, J = 10.2 Hz), 5.45 (1H, d, J = 10.2 Hz), 6.98-7.01 (2H, m), 7.28-7.39 (6H, m), 7.59 (2H, dd, J 1.8 Hz, 8.1 Hz), 7.87 (1H, s).

Compound **12d, 12i,** and **12j** were prepared by the procedure used for compound **12b**.

5-(Benzyloxy)-1-(4-fluorobenzyl)-3-(2-methoxyethyl)-4,6-dioxo-2,3,4,6-tetrahydro-1H-pyrido[2,1-f][1,2,4]triazine-7-carboxylic acid (12d). White solid, 80 %

yield. ^{1}H NMR ($CDCl_3$) δ 8.11 (1H, s), 7.58 (2H, dd, J = 7.5, 1.8 Hz), 7.41-7.31 (3H, m), 7.21-7.14 (2H, m), 7.07 (2H, t, J = 8.5 Hz), 5.47 (2H, br s), 4.14 (3H, br s), 3.68-3.50 (4H, m), 3.33-3.25 (4H, m).

(S)-4-Benzhydryl-9-(benzyloxy)-2-(2-methoxyethyl)-1,8-dioxo-1,3,4,8-tetrahydro-2H-pyrido[1,2-a]pyrazine-7-carboxylic acid (12i). White solid, 94% yield. ^{1}H NMR (DMSO-d_6) δ 3.11 (3H, s), 3.16 (1H, m), 3.25(1H, m), 3.75 (1H, m),4.11 (1H, m), 4.36 (1H, d, J = 11.6 Hz), 5.18 (2H, dd, J = 15.7 Hz, 10.4 Hz), 5.71 (1H, d, J = 11.6 Hz), 7.08-7.20 (5H, m), 7.29-7.45 (6H, m), 7.55 (2H, d, J = 6.7 Hz), 7.61 (2H, d, J = 7.5 Hz), 7.98 (1H, s). MS: m/z 539.4 $[M+H]^+$.

1-Benzhydryl-5-(benzyloxy)-3-(2-methoxyethyl)-4,6-dioxo-2,3,4,6-tetrahydro-1H-pyrido[2,1-f][1,2,4]triazine-7-carboxylic acid (12j). White solid, 83 % yield. ^{1}H NMR (DMSO-d_6) δ: 3.13 (3H, s), 3.25-3.34 (3H, m), 3.79 (1H, d, J = 13.73 Hz), 4.42 (1H, d, J = 14.03 Hz), 5.11-5.27 (3H, m), 5.48 (1H, s), 7.18-7.21 (5H, m), 7.33-7.49 (6H, m), 7.56-7.58 (2H, m), 7.74 (2H, d, J = 7.32 Hz), 8.01 (1H, s).

Compound **12c, 12e, 12f, 12g,** and **12h** were not identified because of the parallel synthesis. Used reagents were same as the synthesis of **12b.**

Compound **13** was prepared by the procedure used for compound **14**.

(S)-4-Benzhydryl-9-(benzyloxy)-2-(2-methoxyethyl)-N-methyl-1,8-dioxo-1,3,4,8-tetrahydro-2H-pyrido[1,2-a]pyrazine-7-carboxamide (13). 99% yield, white solid ^{1}H NMR ($CDCl_3$) δ 9.72 (1H, d, J = 4.1 Hz), 7.63 (2H, d, J = 7.3 Hz), 7.59 (1H, s), 7.46-7.28 (8H, m), 7.23-7.14 (3H, m), 7.02 (2H, d, J = 6.8 Hz), 5.36 (2H, s), 4.67 (1H, d, J = 11.2 Hz), 4.30 (1H, d, J = 11.3 Hz), 4.04 (1H, d, J = 14.3 Hz), 3.95 (1H, d, J = 12.0 Hz), 3.56-3.35 (3H, m), 3.17 (3H, s), 3.12-3.01 (1H, m), 2.87 (3H, d, J = 4.8 Hz).

(S)-4-Benzhydryl-9-(benzyloxy)-N-methoxy-2-(2-methoxyethyl)-N-methyl-1,8-dioxo-1,3,4,8-tetrahydro-2H-pyrido[1,2-a]pyrazine-7-carboxamide (14). Compound **12i** (112 mg, 0.208 mmol) was dissolved in DMF (2 mL), triethylamine (0.144 ml, 1.04 mmol) and, subsequently, ethyl chloroformate (0.040 mL, 0.42 mmol) was added under ice-cooling, the mixture was stirred at room temperature for 10 minutes. Next, N,O-dimethylhydroxyamine hydrochloride (41 mg, 0.42 mmol) and then DMAP (3 mg, 0.02 mmol) were added, and the mixture was stirred at room temperature for 1 hour. To the reaction solution was added water and ethyl acetate, the ethyl acetate layer was separated, and the aqueous layer was extracted with ethyl acetate. To the combined extracts was added sodium sulfate, the mixture was filtered, and the solvent was distilled off. The resulting residue was purified by silica gel column chromatography. From a fraction eluted with EtOAc/MeOH, collected fractions were evaporated to afford 127 mg (quant

yield) of compound **14** as a yellow oil including DMF. ^{1}H NMR ($CDCl_3$) δ 7.61 (2H, d, J = 6.9 Hz), 7.47-7.27 (8H, m), 7.16-7.10 (3H, m), 7.01-6.94 (2H, m), 6.71 (1H, s), 5.45 (2H, d, J = 9.9 Hz), 4.50 (1H, d, J = 11.5 Hz), 4.28 (1H, d, J = 11.5 Hz), 3.90 (1H, dd, J = 13.8, 2.9 Hz), 3.44 (2H, t, J = 13.2 Hz), 3.17 (3H, s), 3.07-2.99 (2H, m), 2.96 (3H, s), 2.88 (3H, s). MS: m/z 582.20 $[M+H]^+$.

(S)-7-Acetyl-4-benzhydryl-9-(benzyloxy)-2-(2-methoxyethyl)-3,4-dihydro-2H-pyrido[1,2-a]pyrazine-1,8-dione (15). Compound **14** (137 mg, 0.236 mmol) was dissolved in THF (8 mL), a 2M THF solution of methyl magnesium bromide (0.444 ml, 0.471 mmol) was added at -78° C. under nitrogen stream, and the mixture was stirred for 30 minutes while temperature was raised to -50° C. To the reaction solution was added 1M hydrochloric acid (4 ml), the mixture was stirred at 0° C for 20 minutes, ethyl acetate was added, the ethyl acetate layer was separated, and the aqueous layer was extracted with ethyl acetate. The combined extracts were neutralized with an aqueous saturated sodium bicarbonate solution, sodium sulfate was added to the organic layer, the mixture was filtered, and the solvent was distilled off. The resulting residue was purified by silica gel column chromatography. From a fraction eluted with EtOAc/MeOH, collected fractions were evaporated to afford 67 mg (53% yield) of compound **15** as a yellow oil. ^{1}H NMR ($CDC1_3$) δ 2.55 (3H, s), 3.01-3.14 (1H, m), 3.16 (3H, s), 3.37-3.54 (3H, m),

3.91-4.07 (2H, m), 4.28 (1H, d, J = 11.3 Hz), 4.50-4.60 (1H, m), 5.42 (2H, d, J = 1.2 Hz), 6.97-6.99 (2H, m), 7.14-7.17 (4H, m), 7.31-7.45 (8H, m), 7.65 (2H, d, J 6.5 Hz). MS: m/z 537.20 $[M+H]^+$.

(S)-4-Benzhydryl-9-(benzyloxy)-7-hydroxy-2-(2-methoxyethyl)-3,4-dihydro-2H-pyrido[1,2-a]pyrazine-1,8-dione (17). Compound **15** (67 mg, 0.13 mmol) was dissolved in dichloromethane (4 mL), mCPBA (32 mg, 0.19 mmol) was added at 0° C under nitrogen stream, and the mixture was stirred at room temperature for 3 hours. The reaction solution was ice-cooled, then an aqueous sodium thiosulfate solution, and ethyl acetate were added, the ethyl acetate layer was separated, and the aqueous layer was extracted with ethyl acetate. The combined extracts were neutralized with an aqueous saturated sodium bicarbonate solution, sodium sulfate was added to the organic layer, the mixture was filtered, and the solvent was distilled off to obtain 64 mg (89% yield) of compound **16** as a white solid. The crude product was directly used for the next step. Compound **16** (64 mg, 0.12 mmol) was dissolved in ethanol (8 mL), and the solution was heated to reflux for 4 hours. The reaction solution was concentrated, and the resulting residue was purified by silica gel column chromatography. From a fraction eluted with EtOAc/MeOH, collected fractions were evaporated to afford 42 mg (69 % yield) of compound **17** as a white solid. ^{1}H NMR ($CDCl_3$) δ 2.93-3.09 (1H, m), 3.16 (3H, s), 3.33-3.53 (4H, m), 3.90-

4.07 (2H, m), 4.29-4.47 (2H, m), 5.41 (2H, q, J = 10.4 Hz), 6.34 (1H, s), 6.95-6.99 (2H, m), 7.12-7.21 (4H, m), 7.33-7.42 (8H, m), 7.64 (2H, d, J = 6.9 Hz). MS: m/z 511.21 $[M+H]^+$.

(S)-4-Benzhydryl-9-(benzyloxy)-7-methoxy-2-(2-methoxyethyl)-3,4-dihydro-2H-pyrido[1,2-a]pyrazine-1,8-dione (18). Compound **17** (41 mg, 0.080mmol) was dissolved in DMF (1 mL), sodium hydride (6.4 mg, 0.16 mmol) was added under ice-cooling, the mixture was stirred for 10 minutes, methyl iodide (0.010 ml, 0.16 mmol) was added, and the mixture was stirred at room temperature for 1.5 hours. To the reaction solution were added ice water and ethyl acetate, the ethyl acetate layer was separated, and the aqueous layer was extracted with ethyl acetate. To the combined extracts was added sodium sulfate, the mixture was filtered, and the solvent was distilled off. The resulting residue was purified by silica gel column chromatography. From a fraction eluted with EtOAc/MeOH, collected fractions were evaporated to afford 41 mg (98 % yield) of compound **18** as a white solid. ^{1}H NMR ($CDCl_3$) δ 2.99-3.09 (1H, m), 3.16 (3H, s), 3.25 (3H, s), 3.32-3.38 (1H, m), 3.42-3.50 (2H, m), 3.94-4.03 (2H, m), 4.28 (1H, d, J = 11.3 Hz), 4.43 (1H, br s), 5.40 (2H, dd, J = 28.3 Hz, 10.2 Hz), 6.01 (1H, s), 6.90-7.19 (5H, m), 7.28-7.44 (8H, m), 7.66 (2H, d, J = 6.4 Hz). MS: m/z 525.21 $[M+H]^+$.

Methyl-(S)-(4-benzhydryl-9-(benzyloxy)-2-(2-methoxyethyl)-1,8-dioxo-1,3,4,8-tetrahydro-2H-pyrido[1,2-a]pyrazin-7-yl)carbamate (19). A DMF (5 ml) solution of compound **12i** (424 mg, 0.787 mmol) was ice-cooled,then triethylamine (327 ul, 2.36 mmol) and, subsequently, ethyl chloroformate (150 ul, 1.57 mmol) were added. After the reaction solution was stirred at room temperature for 10 minutes, it was ice-cooled again, sodium azido (154 mg, 2.36 retool) was added, and the mixture was stirred for 1 hour. To the reaction solution were added dichloromethane, water and a small amount of methanol, the dichloromethane layer was separated, and the aqueous layer was extracted once with dichloromethane. The combined extracts were concentrated, methanol (8 ml) was added to the resulting residue, the mixture was stirred at 50° C for 3 hours, and the solvent was distilled off. The resulting oil was purified by silica gel column chromatography. The materials were eluted firstly with n-hexane-ethyl acetate (1 : 1, v/v) and, then, with only ethyl acetate. Concentration of objective fraction afforded 160 mg (36 % yield) of compound **19** as a white solid. ^{1}H NMR ($CDC1_3$) δ 3.08-3.18 (4H, m), 3.35-3.49 (3H, m), 3.68 (3H, s), 3.98 (2H, dt, J = 23.1, 5.6 Hz), 4.32 (1H, d, J = 11.3Hz), 4.59 (1H, d, J= 11.3 Hz), 5.37 (2H, dd, J = 12.0, 10.4 Hz), 6.98-7.70 (15H, m). MS: m/z 568.25 $[M+H]^+$.

(S)-7-Amino-4-benzhydryl-9-(benzyloxy)-2-(2-methoxyethyl)-3,4-dihydro-2H-pyrido[1,2-a]pyrazine-1,8-dione (20). Compound **19** (160 mg, 0.102 mmol) was

dissolved in EtOH (10 mL), a 2N aqueous sodium hydroxide solution (14 ml) was added, and the mixture was stirred at 60°C for 2 hours. After the reaction solution was concentrated under reduced pressure, the residue was distributed between dichloromethane and water. The dichloromethane layer was separated, and the aqueous layer was extracted with dichloromethane three times. The solvent was distilled off to obtain 143 mg (quant yield) of compound **20** as a pale yellow solid. ^{1}H NMR ($CDC1_3$) δ 2.97-3.06 (1H, m), 3.15 (3H, s), 3.38-3.44 (3H, m), 3.71 (2H, s), 3.93-3.99 (2H, m), 4.35 (2H, dd, J = 19.3, 11.1 Hz), 5.37 (2H, dd, J = 31.6, 10.1 Hz), 6.04 (1H, s), 6.98 (2H, dd, J = 6.4, 2.9 Hz), 7.17 (4H, t, J = 3.3 Hz), 7.28-7.69 (12H, m). MS: m/z 509.23 $[M+H]^+$.

(S)-4-Benzhydryl-9-(benzyloxy)-2-(2-methoxyethyl)-3,4-dihydro-2H-pyrido[1,2-a]pyrazine-1,8-dione (21). Compound **12i** (164 mg, 0.304 mmol) was dissolved in diphenyl ether (1 mL), the mixture was stirred at 245 °C for 1 hour using a microwave apparatus and, thereafter, the reaction solution was purified by silica gel column chromatography. Concentration of the objective fraction afforded 72 mg (48 % yield) of compound **21** as a brown solid. ^{1}H NMR ($CDC1_3$) δ 2.92-3.01 (1H, m), 3.16 (3H, s), 3.32-3.50 (3H, m), 3.90-4.46 (4H, m), 5.42 (2H, dd, J = 26.1, 10.3 Hz), 5.94 (1H, d, J = 7.4 Hz), 6.28 (1H, d, J = 7.5 Hz), 6.96-6.99 (2H, m), 7.15-7.19 (3H, m), 7.28-7.44 (8H, m), 7.62-7.65 (2H, m). MS: m/z 495.21 $[M+H]^+$.

(S)-4-Benzhydryl-9-(benzyloxy)-7-bromo-2-(2-methoxyethyl)-3,4-dihydro-2H-pyrido[1,2-a]pyrazine-1,8-dione (22). To a dichloromethane (4 mL) solution of compound **21** (21 mg, 0.042 mmol) was added NBS (11 mg, 0.062 mmol), and the mixture was heated to reflux for 1 hour. The reaction solution was allowed to cool and then purified by silica gel column chromatography. Concentration of the objective fraction afforded 26 mg (quant yield) of compound **22** as a white solid. ^{1}H NMR ($CDC1_3$) δ 3.01-3.09 (1H, m), 3.16 (3H, s), 3.35-3.53 (3H, m), 3.92-4.47 (4H, m), 5.41 (2H, dd, J = 32.6 Hz, 10.0 Hz), 6.72 (1H, s), 6.97-7.00 (2H, brm), 7.20-7.22 (3H, m), 7.30-7.46 (8H, m), 7.66-7.70 (2H, m). MS: m/z 573.20 $[M+H]^+$.

Compound **24** was prepared by the procedure used for compound **21**.

1-Benzhydryl-5-(benzyloxy)-3-(2-methoxyethyl)-2,3-dihydro-1H-pyrido[2,1-f][1,2,4]triazine-4,6-dione (24). White solid, 48 % yield ^{1}H NMR ($CDC1_3$) δ 2.92-3.01 (1H, m), 3.16 (3H, s), 3.32-3.50 (3H, m), 3.90-4.46 (4H, m), 5.42 (2H, dd, J = 26.1, 10.3 Hz), 5.94 (1H, d, J = 7.4 Hz), 6.28 (1H, d, J = 7.5 Hz), 6.96-6.99 (2H, m), 7.15-7.19 (3H, m), 7.28-7.44 (8H, m), 7.62-7.65 (2H, m). MS: m/z 495.21 $[M+H]^+$.

Dimethyl-(S)-3-(benzyloxy)-1-(3-((tert-butoxycarbonyl)amino)-1,1-diphenylpropan-2-yl)-4-oxo-1,4-dihydropyridine-2,5-dicarboxylate (26). Dimethyl 3-(benzyloxy)-4-oxo-4H-pyran-2,5-dicarboxylate (974 mg, 3.06 mmol), and tert-butyl

(S)-(2-amino-3,3-diphenylpropyl)carbamate (999 mg, 3.06 mmol) were added to toluene (10 ml), and the mixture was stirred at 110° C for 5 hours. After the solvent was distilled off under reduced pressure, the resulting crude product was purified by silica gel column chromatography (chloroform-methanol, 98:2, v/v) to obtain 1.51 g of compound **26** as a pale yellow solid (79% yield). ^{1}H NMR ($CDC1_3$) δ 1.36 (9H, s), 3.40 (1H, m), 3.53 (1H, m), 3.82 (3H, s), 3.91 (3H, s), 4.29 (1H, d, J = 11.3 Hz), 4.78 (1H, m), 4.82 (1H, m), 5.11 (1.9H, d, J = 7.5 Hz), 7.10-7.38 (10H, m), 8.27 (1H, s).

Methyl-(S)-4-benzhydryl-9-(benzyloxy)-1,8-dioxo-1,3,4,8-tetrahydro-2H-pyrido[1,2-a]pyrazine-7-carboxylate (27). To compound **26** (1.45 g, 2.31 mmol) was added 4N HCl (ethyl acetate solution, 20 ml), and the mixture was stirred at room temperature for 1.5 hours. After the solvent was distilled off under reduced pressure, sodium bicarbonate water was added, and the mixture was stirred at room temperature for 1.5 hours. This was extracted with chloroform, and dried with sodium sulfate. After the solvent was distilled off under reduced pressure, the resulting crude product was purified by silica gel column chromatography (chloroform-methanol, 95:5, v/v) to obtain 1.01 g of compound **27** as a colorless solid (89% yield). ^{1}H NMR ($CDC1_3$) δ 3.40 (1H, dd, J = 13.6, 6.6 Hz), 3.78 (3H, s), 3.80 (1H, m), 4.37 (1H, d, J = 11.6 Hz), 4.59 (1H, d, J

= 11.0 Hz), 5.43 (2H, d, J = 10.2 Hz), 5.93 (1H, d, J = 5.8 Hz), 7.03-7.21 (5H, m), 7.37 (9H, m), 7.63 (2H, m).

(S)-4-Benzhydryl-9-(benzyloxy)-1,8-dioxo-1,3,4,8-tetrahydro-2H-pyrido[1,2-a]pyrazine-7-carboxylic acid (28). Compound **27** (460 mg, 0.930 mmol) was dissolved in THF (2.5 ml) and methanol (2.5 ml), a 2N aqueous sodium hydroxide solution (2.33 ml, 4.65 mmol) was added at room temperature, and the mixture was stirred for 1.5 hours. After 1N hydrochloric acid was added, the mixture was extracted with ethyl acetate, and the extract was dried with sodium sulfate. After the solvent was distilled off under reduced pressure, 405 mg (91% yield) of compound **28** was obtained as a colorless solid. ^{1}H NMR ($CDC1_3$) δ 3.45 (1H, ddd, J = 13.8, 6.9, 1.3 Hz), 3.80 (1H, dd, J = 13.5, 2.1 Hz), 4.35 (1H, d, J = 11.6 Hz), 4.77 (1H, d, J = 11.3 Hz),5.46 (1H, d, J = 10.5Hz),5.52 (1H, d, J = 10.5 Hz), 6.11 (1H, d, J = 5.8 Hz), 6.94-6.98 (2H, m), 7.17 (3H, m), 7.31-7.46 (8H, m), 7.58 (3H, m).

(S)-4-Benzhydryl-9-(benzyloxy)-3,4-dihydro-2H-pyrido[1,2-a]pyrazine-1,8-dione (29). Compound **27** (402 mg, 0.837 mmol) was added to diphenyl ether (5 ml), and the mixture was stirred at 245° C for 1 hour under microwave irradiation. The reaction solution was poured into n-hexane, and the precipitated solid was filtered. The resulting crude product was purified by amino column chromatography (chloroform-methanol,

99:1, v/v) to obtain 164 mg of compound **28** as a colorless solid (45% yield). ^{1}H NMR ($CDC1_3$) δ 3.36 (1H, dd, J = 13.0, 7.0 Hz), 3.72 (1H, d, J = 11.1 Hz), 4.35 (1H, d, J = 11.4Hz), 4.49 (1H, d, J = 10.2 Hz), 5.38 (1H, d, J = 10.5 Hz), 5.43 (1H, d, J = 10.4 Hz), 5.94 (1H, d, J = 7.2 Hz), 6.29 (1H, d, J = 6.6 Hz), 6.38 (1H, d, J = 7.5 Hz), 6.99 (2H, m), 7.17 (3H, m), 7.36 (8H, m), 7.60 (2H, m).

(S)-4-Benzhydryl-9-(benzyloxy)-2-methyl-3,4-dihydro-2H-pyrido[1,2-a]pyrazine-1,8-dione (30r). Compound **29** (33 mg, 0.076 mmol) was dissolved in DMF (0.7 ml), and cesium carbonate (123 mg, 0.38 mmol) was added. After stirring at room temperature for 30 minutes, iodomethane (0.024 ml, 0.38 mmol) was added, and the mixture was stirred at room temperature for 3 hours. After the reaction solution was poured into water, and the mixture was extracted with ethyl acetate, the extract was dried with sodium sulfate. After the solvent was distilled off under reduced pressure, the resulting crude product was purified by silica gel column chromatography (chloroform-methanol, 95:5, v/v) to obtain 18 mg of compound **30r** as a white solid (53% yield). ^{1}H-NMR ($CDCl_3$) δ 7.65 (2H, d, J = 7.3 Hz), 7.43 (2H, t, J = 7.5 Hz), 7.37-7.29 (6H, m), 7.19 (3H, t, J = 3.0 Hz), 7.06-7.00 (2H, m), 6.31 (1H, d, J = 7.6 Hz), 5.96 (1H, d, J = 7.6 Hz), 5.41 (2H, dd, J = 36.4, 10.1 Hz), 4.43 (1H, d, J = 11.1 Hz), 4.22 (1H, d, J = 11.1 Hz), 3.86 (1H, dd, J = 13.6, 3.0 Hz), 3.25 (1H, d, J = 14.4 Hz), 2.91 (3H, s).

Compound **30s** was prepared by the procedure used for compound **29r**.

(S)-4-Benzhydryl-9-(benzyloxy)-2-isopropyl-3,4-dihydro-2H-pyrido[1,2-a]pyrazine-1,8-dione (30s). White solid, 93 % yield. ^{1}H NMR ($CDC1_3$) δ 0.76 (3H, d, J = 6.7 Hz), 0.98 (3H, d, J = 6.9 Hz), 3.43-3.52 (2H, m), 3.62 (1H, dd, J = 13.6, 3.5 Hz), 4.22 (1H, d, J = 11.6 Hz), 4.52 (1H, d, J = 11.6 Hz), 4.86-4.95 (1H, m), 5.37 (1H, d, J = 10.2 Hz), 5.45 (1H, d, J = 10.2 Hz), 5.90 (1H, d, J = 7.5 Hz), 6.22 (1H, d, J = 7.5 Hz), 6.89 (2H, m), 7.15 (3H, m), 7.36 (8H, m), 7.67 (2H, m).

3-(Benzyloxy)-4-oxo-1,4-dihydropyridine-2-carboxylic acid hydrochloride (32). Compound **31** (1.0 g, 4.06 mmol) was dissolved in 28% aqueous ammonia, and the solution was stirred at room temperature for 12 hours. After concentration of the reaction solution, the resulting residue was neutralized with 2N hydrochloric acid, and the precipitated solid was suspended in ethyl acetate, filtered, and dried to obtain 1.14 g (yield 100%) of compound **32** as a white solid. ^{1}H NMR (DMSO-d_6) δ 5.14 (2H, s), 7.31 (1H, d, J = 6.6 Hz), 7.34-7.41 (3H, m), 7.45-7.51 (2H, m), 8.17 (1H, d, J = 6.6 Hz).

3-(Benzyloxy)-N-methyl-4-oxo-1,4-dihydropyridine-2-carboxamide (33u). To a DMF (10 ml) solution of compound **32** (3.00 g, 10.65 mmol) were added WSC HC1 (3.06 g, 15.98 mmol) and HOBt (1.58 g, 11.7 mmol) at room temperature, the mixture was stirred for 10 minutes, and a methylamine 33 wt % ethanol solution (1.50 g, 15.98 mmol)

was added dropwise. After the reaction solution was stirred at the same temperature for 2 hours, water was added, and the mixture was extracted with chloroform five times. The extract was dried with sodium sulfate, the solvent was distilled off, and the resulting oil was purified by silica gel chromatography. From a fraction eluted with ethyl acetate-MeOH (6:4, v/v), 2.62 g (yield 95%) of compound **33u** was obtained as a pale brown solid. ^{1}H NMR ($CDC1_3$) δ 2.77 (3H, d, J = 4.8 Hz), 5.49 (2H, s), 6.57 (1H, d, J = 6.9 Hz), 7.25-7.43 (5H, m), 7.48 (1H, t, J = 6.0 Hz), 8.23 (1H, brs), 9.77 (1H, brs).

Compound **33v** and **33w** were prepared by the procedure used for compound **33u**.

3-(Benzyloxy)-N-isopropyl-4-oxo-1,4-dihydropyridine-2-carboxamide (33v). Yellow solid. 80% yield ^{1}H NMR ($CDCl_3$) δ 9.71 (1H, s), 8.22 (1H, s), 7.53-7.41 (6H, m), 6.59 (1H, dd, J = 7.2, 2.2 Hz), 5.57 (2H, s), 4.14-3.99 (1H, m), 0.99 (6H, d, J = 6.5 Hz).

3-(Benzyloxy)-N-(furan-2-ylmethyl)-4-oxo-1,4-dihydropyridine-2-carboxamide (33w). Yellow solid. 70 % yield ^{1}H NMR ($CDCl_3$) δ 9.65 (1H, br s), 8.66 (1H, br s), 7.46 (1H, t, J = 6.6 Hz), 7.38-7.28 (6H, m), 6.54 (1H, dd, J = 7.2, 2.0 Hz), 6.33 (1H, t, J = 2.5 Hz), 6.16 (1H, d, J = 3.2 Hz), 5.50 (2H, s), 4.45 (2H, d, J = 5.6 Hz).

1-Amino-3-(benzyloxy)-N-methyl-4-oxo-1,4-dihydropyridine-2-carboxamide (34u). Into a DMF (10 ml) solution of compound **33u** (2.62 g, 10.14 mmol) was

suspended potassium carbonate (4.20 g, 30.42 mmol) at room temperature, the suspension was stirred for 5 minutes, O-(2,4-dinitrophenyl)hydroxylamine (3.03 g, 15.21 mmol) was added, and the mixture was stirred at the same temperature for 3 hours. To the reaction solution was added water, then the mixture was extracted with chloroform five times, and the extract was dried with sodium sulfate. After the solvent was distilled off, the resulting oil was purified by silica gel chromatography. From a fraction eluted with ethyl acetate-MeOH (6:4, v/v), 1.41 g (yield 51%) of compound **34u** was obtained as a brown solid. ^{1}H NMR ($CDC1_3$) δ 2.62 (3H, d, J = 5.1 Hz), 5.06 (2H, s), 5.22 (2H, s), 6.18 (1H, d, J = 7.8 Hz), 7.25-7.36 (5H, m), 5.89 (1H, d, J = 7.8 Hz), 7.57 (1H, q, J = 5.1 Hz).

Compound **34v** and **34w** were prepared by the procedure used for compound **34u**.

1-Amino-3-(benzyloxy)-N-isopropyl-4-oxo-1,4-dihydropyridine-2-carboxamide (34v). Brown solid. 25 % yield. ^{1}H NMR ($CDCl_3$) δ 7.44-7.30 (6H, m), 6.26 (1H, d, J = 7.8 Hz), 5.59 (2H, s), 5.21 (2H, s), 4.06 (1H, dq, J = 14.3, 6.6 Hz), 1.07 (6H, d, J = 6.6 Hz).

1-Amino-3-(benzyloxy)-N-(furan-2-ylmethyl)-4-oxo-1,4-dihydropyridine-2-carboxamide (34w). Brown solid. 50% yield. ^{1}H NMR ($CDCl_3$) δ 7.73 (1H, br s), 7.42 (1H, d, J = 7.8 Hz), 7.34-7.23 (6H, m), 6.32 (1H, t, J = 2.4 Hz), 6.27-6.22 (2H, m), 5.55 (2H, s), 5.14 (2H, s), 4.33 (2H, d, J = 5.8 Hz).

1-Benzhydryl-5-(benzyloxy)-3-methyl-2,3-dihydro-1H-pyrido[2,1-f][1,2,4]triazine-4,6-dione (35u). To a toluene (10 ml) solution of compound **34u** (1.0 g, 3.66 mmol) were added paraformaldehyde (109.9 mg, 3.66 mmol) and acetic acid (22 mg, 0.37 mmol), and the mixture was heated with stirring at 100° C for 40 minutes. After cooling, the solvent was distilled off, the residue was dissolved in DMF (10 ml) without purification, cesium carbonate (3.58 g, 10.98 mmol) was added under ice-cooling, and the mixture was stirred for 10 minutes. To the reaction solution was added bromodiphenylmethane (1.36 g, 5.49 mmol), then the mixture was stirred at room temperature for 3 hours, water was added, and the mixture was extracted with ethyl acetate three times. The extract was washed with water three times and dried with sodium sulfate. The solvent was distilled off, and the resulting oil was purified by silica gel chromatography. From a fraction eluted with ethyl acetate-MeOH (9:1, v/v), 1.26 g (yield 71%) of compound **35u** was obtained as a white solid. ^{1}H NMR ($CDC1_3$) δ 2.91 (3H, s), 4.26 (1H, d, J = 13.2 Hz), 4.77 (1H, d, J = 13.2 Hz), 5.12 (1H, s), 5.42 (1H, J = 13.2 Hz), 5.45 (1H, d, J = 13.2 Hz), 5.82 (1H, J = 7.5 Hz), 6.71 (1H, d, J = 7.5 Hz), 7.10-7.23 (5H, m), 7.27-7.46 (6H, m), 7.52 (2H, d, J = 6.9 Hz), 7.60-7.64 (2H, m).

Compound **35v** and **35w** were prepared by the procedure used for compound **35u**.

1-Benzhydryl-5-(benzyloxy)-3-isopropyl-2,3-dihydro-1H-pyrido[2,1-f][1,2,4]triazine-4,6-dione (35v). White solid. 46% yiled. ^{1}H NMR ($CDCl_3$) δ 7.70-7.60 (2H, m), 7.55-7.28 (8H, m), 7.20-7.07 (3H, m), 6.99 (2H, d, J = 6.7 Hz), 6.65 (1H, d, J = 7.8 Hz), 5.76 (1H, d, J = 7.8 Hz), 5.43 (2H, dd, J = 14.0, 10.4 Hz), 5.18 (1H, s), 4.90-4.80 (1H, m), 4.64 (1H, d, J = 13.3 Hz), 4.44 (1H, d, J = 13.3 Hz), 1.00 (3H, d, J = 6.7 Hz), 0.93 (3H, d, J = 6.7 Hz).

1-Benzhydryl-5-(benzyloxy)-3-(furan-2-ylmethyl)-2,3-dihydro-1H-pyrido[2,1-f][1,2,4]triazine-4,6-dione (35w). White solid. 63% yield. ^{1}H NMR ($CDCl_3$) δ 7.60 (2H, dd, J = 7.7, 1.8 Hz), 7.40-7.26 (8H, m), 7.22 (1H, dd, J = 1.8, 0.8 Hz), 7.16-7.09 (3H, m), 7.02 (2H, dd, J = 7.8, 1.5 Hz), 6.69 (1H, d, J = 7.8 Hz), 6.25 (1H, dd, J = 3.2, 1.8 Hz), 6.14 (1H, d, J = 3.2 Hz), 5.77 (1H, d, J = 7.8 Hz), 5.43 (2H, dd, J = 13.3, 10.6 Hz), 5.05 (1H, s), 4.79-4.60 (2H, m), 4.45-4.34 (2H, m).

General Procedure for O-Benzyl Deprotection

The indicated starting material (**12b-j, 13, 18, 20, 21, 24, 30r, 30s,** and **35u-w**) was dissolved in trifluoroacetic acid (2 ml), and the mixture was stirred at room temperature for 1 hour. The solvent was distilled off, the residue was dissolved in dichloromethane, and the solution was neutralized with a saturated aqueous sodium bicarbonate solution. The resulting solution was made acidic with an aqueous citric acid solution, and the

organic layer was separated. The aqueous layer was extracted with dichloromethane, and the combined organic layers were washed with brine, and dried with anhydrous sodium sulfate. After the solvent was distilled off, the resulting solid was recrystallized from diisopropyl ether/dichloromethane or purified by preparative HPLC to obtain the desired compounds.

(S)-4-Benzyl-9-hydroxy-2-(2-methoxyethyl)-1,8-dioxo-1,3,4,8-tetrahydro-2H-pyrido[1,2-a]pyrazine-7-carboxylic acid (2b). White solid, 64% yield. ^{1}H NMR ($CDCl_3$) δ 3.14 (2H, d, J = 6.3 Hz), 3.36 (3H, s), 3.60-3.86 (5H, m), 4.14 (1H, d, J = 12.9 Hz), 4.47 (1H, s), 7.03-7.05 (2H, m), 7.30-7.35 (3H, m), 7.88 (1H, s), 12.68 (1H, s), 14.83 (1H, s).

(R)-4-Benzyl-9-hydroxy-2-(2-methoxyethyl)-1,8-dioxo-1,3,4,8-tetrahydro-2H-pyrido[1,2-a]pyrazine-7-carboxylic acid (2c). White solid, 58 % yield, ^{1}H NMR ($CDCl_3$) δ 14.74 (1H, br s), 12.64 (1H, br s), 7.81 (1H, s), 7.38-7.29 (3H, m), 7.02 (2H, d, J = 5.9 Hz), 4.37 (1H, s), 4.13 (1H, d, J = 13.3 Hz), 3.89-3.64 (5H, m), 3.36 (3H, s), 3.18-3.06 (2H, m).

1-(4-Fluorobenzyl)-5-hydroxy-3-(2-methoxyethyl)-4,6-dioxo-2,3,4,6-tetrahydro-1H-pyrido[2,1-f][1,2,4]triazine-7-carboxylic acid (2d). White solid, 75 % yield. ^{1}H NMR ($CDC1_3$) δ 3.34 (3H, s), 3.57-3.68 (2H, m), 3.73 (2H, br s), 4.18 (2H, s), 4.75 (2H,

br s), 7.06-7.12 (2H, m), 7.21-7.24 (2H, m), 8.10 (1H, s), 11.96 (1H, br s), 14.52 (1H, brs).

(S)-9-Hydroxy-2-(2-methoxyethyl)-1,8-dioxo-4-phenyl-1,3,4,8-tetrahydro-2H-pyrido[1,2-a]pyrazine-7-carboxylic acid (2e). white solid, 72% yield, ^{1}H NMR ($CDCl_3$) δ 14.82 (1H, s), 12.76 (1H, s), 8.19 (1H, s), 7.48-7.42 (3H, m), 7.12-7.05 (2H, m), 5.40 (1H, t, J = 4.2 Hz), 4.24 (1H, dd, J = 13.8, 3.9 Hz), 4.11 (1H, dd, J = 13.6, 4.8 Hz), 3.79-3.70 (1H, m), 3.66-3.57 (1H, m), 3.55-3.50 (1H, m), 3.40-3.35 (1H, m).

(S)-9-Hydroxy-2-(2-methoxyethyl)-1,8-dioxo-4-phenethyl-1,3,4,8-tetrahydro-2H-pyrido[1,2-a]pyrazine-7-carboxylic acid (2f). White solid, 46 % yield. ^{1}H NMR (DMSO-d_6) δ 2.07 (2H, m), 2.55 (1H, m), 2.74 (1H, m), 3.17 (1H, s), 3.23 (3H, s), 3.48-3.65 (4H, m), 3.79 (1H, d, J = 13.6 Hz), 3.87 (1H, m), 4.09 (1H, d, J = 13.6 Hz), 4.80 (1H, s), 7.10-7.29 (5H, m), 8.59 (1H, s), 12.77 (1H, s), 15.49 (1H, s). MS: m/z=387.3 $[M+H]^+$.

(S)-9-Hydroxy-4-isopropyl-2-(2-methoxyethyl)-1,8-dioxo-1,3,4,8-tetrahydro-2H-pyrido[1,2-a]pyrazine-7-carboxylic acid (2g). White solid, 50 % yield. ^{1}H NMR ($CDCl_3$) δ 14.88 (1H, br s), 12.56 (1H, br s), 8.24 (1H, s), 4.07 (1H, d, J = 13.8 Hz), 3.97-3.84 (2H, m), 3.78 (1H, d, J = 9.3 Hz), 3.69-3.58 (3H, m), 3.37 (3H, s), 2.27-2.14 (1H, m), 1.12 (3H, d, J = 6.4 Hz), 0.83 (3H, d, J = 6.8 Hz).

(S)-9-Hydroxy-4-isobutyl-2-(2-methoxyethyl)-1,8-dioxo-1,3,4,8-tetrahydro-2H-pyrido[1,2-a]pyrazine-7-carboxylic acid (2h). White solid, 55 % yield. ^{1}H NMR ($CDCl_3$) δ 14.91 (1H, brs), 12.59 (1H, brs), 8.25 (1H, s), 4.27 (1H, s), 4.11 (1H, d, J = 13.2 Hz), 3.96-3.85 (1H, m), 3.74-3.60 (4H, m), 3.37 (3H, s), 1.90-1.78 (1H, m), 1.03 (3H, d, J = 5.6 Hz), 0.97 (3H, d, J = 5.5 Hz).

(S)-4-Benzhydryl-9-hydroxy-2-(2-methoxyethyl)-1,8-dioxo-1,3,4,8-tetrahydro-2H-pyrido[1,2-a]pyrazine-7-carboxylic acid (2i). White solid, 92% yield. ^{1}H NMR (DMSO-d_6) δ 3.15 (3H, s), 3.50-3.70 (5H, m),4.19(1H, dd, J 13.8Hz, 3.1Hz),4.49(1H, d,J 11.6 Hz), 5.78 (1H, d, J 9.6 Hz), 7.10-7.27 (6H, m), 7.34 (1H, m), 7.46 (2H, t, J 7.5 Hz), 7.63 (2H, t, J 7.7 Hz), 7.94 (1H, s), 12.94 (1H, s), 15.08 (1H, s). MS: m/z 449.4 $[M+H]^+$.

1-Benzhydryl-5-hydroxy-3-(2-methoxyethyl)-4,6-dioxo-2,3,4,6-tetrahydro-1H-pyrido[2,1-f][1,2,4]triazine-7-carboxylic acid (2j). White solid, 56 % yield. ^{1}H NMR (DMSO-d_6) δ 3.13 (3H, s), 3.41-3.56 (4H, m), 4.50 (1H, d, J = 13.57 Hz), 5.21 (1H, d, J = 13.42 Hz), 5.58 (1H, s), 7.16-7.50 (8H, m), 7.72 (2H, d, J = 7.32 Hz), 7.93 (1H, s), 12.12 (1H, s).

Ethyl-(S)-4-benzhydryl-9-hydroxy-2-(2-methoxyethyl)-1,8-dioxo-1,3,4,8-tetrahydro-2H-pyrido[1,2-a]pyrazine-7-carboxylate (2k). White solid, 82 % yield. ^{1}H NMR(DMSO-d_6) δ 1.17 (3H, t, J = 6.9Hz), 3.11 (3H, s), 3.48-3.58 (2H, m), 3.95-4.12

(3H, m), 4.40 (1H, d, J = 11.4 Hz), 5.59 (1H, d, J = 11.4Hz),7.11 (1H, d, J = 7.3 Hz), 7.17 (2H, t, J = 7.2 Hz), 7.26 (2H, d, J = 7.1 Hz), 7.30 (1H, t, J = 7.3 Hz), 7.42 (2H, t, J = 7.2 Hz), 7.60 (3H, m), 12.55 (1H, brs). MS: m/z 477.2 $[M+H]^+$.

(S)-4-Benzhydryl-9-hydroxy-2-(2-methoxyethyl)-N-methyl-1,8-dioxo-1,3,4,8-tetrahydro-2H-pyrido[1,2-a]pyrazine-7-carboxamide (2l). White solid, 4.5 % yield. ^{1}H NMR (DMSO-d_6) δ 12.56 (1H, br s), 9.56 (1H, d, J = 5.0 Hz), 7.87 (1H, s), 7.61 (3H, d, J = 7.9 Hz), 7.31-7.07 (7H, m), 5.72 (1H, d, J = 12.2 Hz), 4.41 (1H, d, J = 11.4 Hz), 4.10 (1H, dd, J = 13.7, 3.8 Hz), 3.53 (2H, t, J = 5.2 Hz), 3.40-3.30 (5H, m), 2.70 (3H, d, J = 4.7 Hz).

(S)-7-Amino-4-benzhydryl-9-hydroxy-2-(2-methoxyethyl)-3,4-dihydro-2H-pyrido[1,2-a]pyrazine-1,8-dione (2m). White solid, 15 % yield. ^{1}H NMR (DMSO-d_6) δ 7.57 (2H, d, J = 8.3 Hz), 7.40 (2H, t, J = 8.5 Hz), 7.33-7.25 (1H, m), 7.23-7.08 (5H, m), 6.65 (1H, s), 5.31 (1H, d, J = 12.5 Hz), 4.45 (1H, br s), 4.36 (1H, d, J = 11.4 Hz), 3.99 (2H, d, J = 13.0 Hz), 3.40-3.30 (m, 2H), 3.12 (3H, s).

(S)-4-Benzhydryl-9-hydroxy-7-methoxy-2-(2-methoxyethyl)-3,4-dihydro-2H-pyrido[1,2-a]pyrazine-1,8-dione (2n). Pink solid, 21 % yield. ^{1}H NMR ($CDC1_3$) δ 3.17 (3H, s), 3.22 (3H, s), 3.40-3.53 (4H, m), 3.63-3.71 (1H, m), 4.24 (1H, d, J = 11.5 Hz),

4.45 (1H, d, J = 13.3 Hz), 4.60 (1H, d, J = 11.2 Hz), 6.08 (1H, d, J = 11.7 Hz), 6.96-6.99 (2H, br m), 7.13-7.17 (3H, m), 7.30-7.43 (5H, m). MS: m/z 435.15 $[M+H]^+$.

(S)-4-Benzhydryl-9-hydroxy-2-(2-methoxyethyl)-7-methyl-3,4-dihydro-2H-pyrido[1,2-a]pyrazine-1,8-dione (2o). To a toluene (2 ml) solution of compound **22** (58 mg, 0.101 mmol) were added tetrakistriphenylphosphinepalladium (10 mg, 8.65 μmol) and hexamethylditin (80.4 mg, 0.242 mmol) at room temperature, and the mixture was heated with stirring at reflux for 10 hours. After the solvent was distilled off, the resulting oil was purified by silica gel column chromatography (ethyl acetate/methanol, 70:30, v/v) to obtain 18.4 mg of crude compound 23 as a colorless oil (included compound 21). This crude compound (18.4 mg) was dissolved in DCM (2 mL), TFA (2 ml) was added, and the mixture was stirred at room temperature for 2 hours. After the solvent was distilled off, the resulting oil was purified by prep HPLC to obtain 3.6 mg (yield 8.5 % for 2 steps) of compound **2o** as a pale red solid. ^{1}H NMR ($CDCl_3$) δ 7.47-7.29 (5H, m), 7.20-7.13 (3H, m), 7.03-6.97 (2H, m), 6.27 (1H, s), 4.51 (1H, d, J = 11.2 Hz), 4.30 (1H, d, J = 11.3 Hz), 4.09 (1H, d, J = 11.7 Hz), 3.78 (1H, d, J = 14.2 Hz), 3.61-3.31 (4H, m), 3.19 (3H, s), 1.65 (3H, s).

(S)-4-Benzhydryl-7-chloro-9-hydroxy-2-(2-methoxyethyl)-3,4-dihydro-2H-pyrido[1,2-a]pyrazine-1,8-dione (2p). To a DMSO (2 ml) solution of compound **22** (69

mg, 0.120 mmol) was added copper(I) chloride (39 mg, 0.396 mmol) at room temperature, and the mixture was heated to stir at 120° C for 2 hours. To that mixture was added copper(I) chloride (50 mg, 0.505 mmol) with stirring at reflux temperature for 1 hour. After the reaction solution was cooled to room temperature, the solvent was distilled off, and the resulting oil was purified by preparative HPLC to obtain 20.9 mg (yield 40%) of compound **2p** as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.43 (2H, t, J = 7.2 Hz), 7.37-7.30 (3H, m), 7.23-7.19 (3H, m), 7.00 (2H, dd, J = 6.7, 2.7 Hz), 6.59 (1H, s), 4.56 (1H, d, J = 11.6 Hz), 4.29 (1H, d, J = 11.4 Hz), 4.17 (1H, dd, J = 13.7, 3.5 Hz), 3.82-3.72 (1H, m), 3.58 (1H, d, J = 13.4 Hz), 3.50-3.34 (3H, m), 3.20 (3H, s).

(S)-4-Benzhydryl-9-hydroxy-2-(2-methoxyethyl)-3,4-dihydro-2H-pyrido[1,2-a]pyrazine-1,8-dione (2q). White solid, 35 % yield. ^{1}H NMR (DMSO-d_6) δ 3.12 (3H, s), 3.51 (5H, m), 4.05 (1H, dd, J = 13.9, 3.5 Hz), 4.37 (1H, d, J = 11.4 Hz), 5.38 (1H, d,J = 11.6 Hz), 5.60 (1H, d, J = 7.3 Hz), 6.90 (1H, d, J = 7.5 Hz), 7.22 (6H, m), 7.40 (2H, t, J = 7.5 Hz), 7.56 (2H, d, J = 7.2 Hz). MS: m/z 405 $[M+H]^+$.

(S)-4-Benzhydryl-9-hydroxy-2-methyl-3,4-dihydro-2H-pyrido[1,2-a]pyrazine-1,8-dione (2r). White solid, 73 % yield. ^{1}H NMR (DMSO-d_6) δ 2.93 (3H, s), 3.17 (1H, d, J = 13.0Hz),4.13(1H, dd, J = 13.6, 3.4 Hz), 4.47 (1H, d, J = 11.4 Hz),5.52(1H, dd, J =

9.3, 3.4 Hz), 5.99 (1H, d, J = 7.3Hz),7.18 (4H, m), 7.30 (3H, m), 7.41 (2H, t, J =7.5 Hz), 7.60 (2H, d, J = 7.2 Hz). MS: m/z 361 $[M+H]^+$.

(S)-4-Benzhydryl-9-hydroxy-2-isopropyl-3,4-dihydro-2H-pyrido[1,2-a]pyrazine-1,8-dione (2s). White solid, 65 % yield. ^{1}H NMR (DMSO-d_6) δ 0.82 (3H, d, J = 6.7 Hz), 1.05 (3H, d, J = 6.7 Hz), 3.90 (1H, dd, J = 13.6, 3.4 Hz), 4.39 (1H, d, J = 11.9 Hz), 4.77-4.86 (1H, m), 5.50 (1H, d, J = 8.6 Hz), 5.69 (1H, d, J = 7.4Hz), 6.92 (1H, d, J = 7.4Hz), 7.15-7.48 (8H, m), 7.63 (2H, d, J = 7.7 Hz) 12.51 (1H, brs). MS: m/z 389 $[M+H]^+$.

1-Benzhydryl-5-hydroxy-3-(2-methoxyethyl)-2,3-dihydro-1H-pyrido[2,1-f][1,2,4]triazine-4,6-dione (2t). White solid, 44 % yield. ^{1}H NMR ($CDCl_3$) δ 7.62-7.34 (6H, m), 7.28-7.16 (4H, m), 6.79 (1H, d, J = 7.7 Hz), 5.75 (1H, d, J = 7.7 Hz), 5.32 (1H, s), 5.04 (1H, d, J = 13.3 Hz), 4.56 (1H, d, J = 13.4 Hz), 4.00-3.89 (1H, m), 3.59-3.44 (2H, m), 3.26-3.15 (2H, m), 3.25 (3H, s).

1-Benzhydryl-5-hydroxy-3-methyl-2,3-dihydro-1H-pyrido[2,1-f][1,2,4]triazine-4,6-dione (2u). White solid, 64 % yield. ^{1}H NMR ($CDC1_3$) δ 2.95 (3H, s), 4.36 (1H, d, J = 13.2 Hz), 4.95 (1H, d, J = 13.2 Hz), 5.22 (1H, s), 5.71 (1H, d, J = 7.8 Hz), 6.75 (1H, d, J = 7.8 Hz), 7.21 (5H, br s), 7.33-7.47 (4H, m), 7.55 (2H, d, J = 6.6 Hz).

1-Benzhydryl-5-hydroxy-3-isopropyl-2,3-dihydro-1H-pyrido[2,1-f][1,2,4]triazine-4,6-dione (2v). White solid, 68 % yield. ^{1}H NMR ($CDC1_3$) δ 0.93 (3H,

d, J = 6.9 Hz), 1.09 (3H, d, J = 6.9 Hz), 4.58 (1H, d, J = 12.6 Hz), 4.79 (1H, d, J = 12.6 Hz), 4.83-4.90 (1H, m), 5.20 (1H, s), 5.67 (1H, d, J = 7.5 Hz), 6.66 (1H, d, J = 7.5 Hz), 7.07-7.09 (2H, m), 7.13-7.19 (3H, m), 7.34-7.46 (3H, m), 7.52 (1H, d, J = 7.5 Hz).

1-Benzhydryl-3-(furan-2-ylmethyl)-5-hydroxy-2,3-dihydro-1H-pyrido[2,1-f][1,2,4]triazine-4,6-dione (2w). White solid, 61 % yield. ^{1}H NMR ($CDC1_3$) δ 4.54 (1H, d, J = 12.9 Hz), 4.56 (2H, s), 4.94 (1H, d, J = 12.9 Hz), 5.14 (1H, s), 5.68 (1H, d, J = 7.8 Hz), 6.20 (1H, d, J = 3.0 Hz), 6.25-6.27 (1H, m), 6.72 (1H, d, J = 7.8 Hz), 7.10-7.37 (11H, m).

ASSOCIATED CONTENT

Molecular formula strings (CSV)

Molecular modeling with CEN (PDF)

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Notes

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Abbreviations used

CEN, cap-dependent endonuclease; BH, benzhydryl; LHMDS, lithium bis(trimethylsilyl)amide; THF, tetrahydrofuran; $RuCl_3$, ruthenium(III) chloride; $NaIO_4$, sodium periodate; H_2SO_4, sulfuric acid; MeCN, acetonitrile; WSC-HCl, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole; DMF, N,N-dimethylformamide; PPh_3, triphenylphosphine; DEAD, diethyl azodicarboxylate; N-Boc hydrazine, tert-butoxycarbonylhydrazine; AcOH, acetic acid; TFA, trifluoroacetic acid; DCM, dichloromethane; EtOH, ethanol; Cs_2CO_3, caesium carbonate; NaOH, sodium hydroxide; TEA, triethylamine; DMAP, N,N-dimethyl-4-aminopyridine; MeMgBr, methylmagnesium bromide; m-CPBA, m-chloroperoxybenzoic acid; MeI, iodomethane; NaH, sodium hydride; NaN_3, sodium azide; MeOH, methanol; Ph_2O, Diphenyl ether; NBS, N-bromosuccinimide; CuCl, copper(I) chloride; DMSO, dimethyl sulfoxide; $Pd(PPh_3)_4$, tetrakis(triphenylphosphine)palladium(0); HCl, hydrochloric acid; EtOAc, ethyl acetate; $NaHCO_3$, sodium hydrogen carbonate; aq., aqueous solution; NH_3, ammonia; K_2CO_3, potassium carbonate; CPE, cytopathogenic effect; PK, pharmacokinetics; $CDCl_3$,

deuterated chloroform; DMSO-d_6, deuterated dimethyl sulfoxide; UPLC-MS, ultra performance liquid chromatography - mass spectrometer; UV, ultraviolet; PDA, photodiode array; N_2, nitrogen

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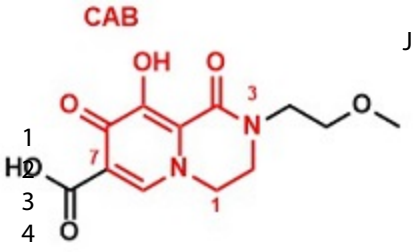
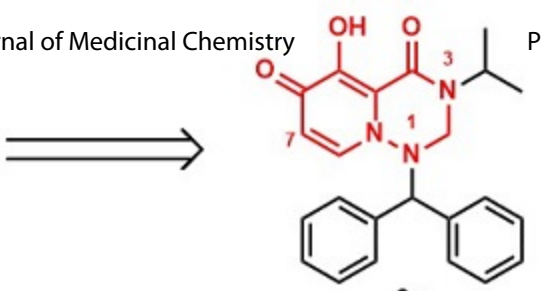
Table of Contents graphic.



hit compound **2a**

CEN (A/WSN/33) IC_{50} = 68.6 μM



2v

CEN (A/WSN/33) IC_{50} = 0.286 μM

CPE (A/WSN/33) EC_{50} = 0.0816 μM

MDBK cell CC_{50} > 25.0 μM

ED_{50} (B/Maryland/1/59) = 0.89 mg/kg/day (mouse, iv)

Rat CL_{tot} = 10.9 mL/min/kg