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Semisynthesis of (−)-Rutamarin Derivatives and Their Inhibitory Activity on Epstein−Barr Virus Lytic Replication

Yongsheng Lin,[†,#] Qian Wang,[‡,#] Qiong Gu,[*,†] Hongao Zhang,[†] Cheng Jiang,[†] Jiayuan Hu,[‡] Yan Wang,[§] Yuan Yan,[*,‡,⊥] and Jun Xu[*,†]

[†]Research Center for Drug Discovery, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, People's Republic of China

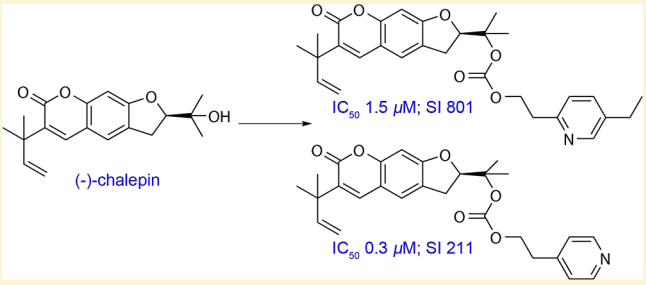
[‡]The Institute of Human Virology, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, Guangdong 510080, People's Republic of China

[§]Guanghua School of Stomatology, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Stomatology, Guangzhou 510080, People's Republic of China

[⊥]Department of Microbiology, School of Dental Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, United States

Supporting Information

ABSTRACT: (+)-Rutamarin inhibits EBV lytic DNA replication with an IC_{50} of 7.0 μM. (−)-Chalepin, a (−)-rutamarin derivative, was isolated from the whole plant of *Ruta graveolens* and used as a precursor of (−)-rutamarin. Altogether, 28 (−)-rutamarin derivatives were synthesized starting from (−)-chalepin. Of these, 16 compounds (**2a−e**, **3b−e**, **3g**, **4f**, **4k**, **4m−p**) were found to be more potent against EBV lytic DNA replication than (−)-chalepin. Compounds **4m**, **4n**, and **4p** exhibited IC_{50} values of 1.5, 0.32, and 0.83 μM and showed selectivity index values (SI) of 801, 211, and >120, respectively. Thus, compounds **4m**, **4n**, and **4p** are considered promising leads for further laboratory investigation.



Epstein−Barr virus (EBV) is a human gamma-herpes virus, which widely infects more than 90% of the human population.[1] EBV preferentially infects B lymphocytes and epithelial cells and causes various diseases, such as Hodgkin's disease, Burkitt's lymphoma, nasopharyngeal carcinoma, and gastric carcinoma.[2,3] EBV exhibits two distinct life cycles, latency and lytic viral replication. EBV lytic replication is directly linked to infectious mononucleosis,[4] chronic active EBV infection (CAEBV),[5] and other EBV-associated diseases.[6] Therefore, the inhibition of EBV lytic replication may be a strategy for the treatment of some EBV-associated diseases. Presently, there are no effective drugs available to treat EBV infections except for some broad-spectrum antiviral agents. These drugs often exhibit only a weak therapeutic effect[7] and develop drug resistance.[8] Therefore, there is a demand for new efficient antiviral agents against EBV for the treatment of EBV-associated human diseases.

The coumarin (+)-rutamarin, isolated from the plant *Ruta graveolens* L. (Rutaceae), exhibited cytotoxic activity[9] and an inhibitory spasmogenic effect on isolated smooth muscle organs.[10] It was also reported as a dual inducer of both GLUT4 translocation and expression[11] and as an agonist of TRPM5 and TRPV1.[12] In a previous study, (+)-rutamarin showed potential inhibition of EBV lytic replication.[13] However, its potency (IC_{50} 7.0 μM) was not considered good enough for further laboratory development. Therefore, the discovery of new (+)-rutamarin analogues with improved activity is desirable.

Since the initial isolation of (+)-rutamarin, Massanet and co-workers synthesized it as a racemic compound.[14] In 2008, Zhang and associates first reported the total synthesis of (+)-rutamarin via 10 steps.[15] However, this procedure is not efficient for synthesizing analogues, due to the production of the chiral benzodihydrofuran, the low overall yield (12%), and the expensive and toxic reagents used. As a result of these problems, (−)-chalepin, an abundant constituent of *R. graveolens*, was isolated as the starting material as a substitute for (+)-rutamarin. However, in an anti-EBV assay, (−)-chalepin exhibited 10 times less potent activity of EBV lytic replication inhibition (IC_{50} 69.9 μM) than (+)-rutamarin. (−)-Rutamarin was produced by acetylating (−)-chalepin.[16] (−)-Rutamarin exhibited similar anti-EBV activity (IC_{50} 13.8 μM) to its enantiomer (+)-rutamarin (IC_{50} 7.0 μM). Hence, this result indicated that the chiral center at C-2′ is not crucial for the anti-EBV activity, and it is possible to discover new analogues with improved anti-EBV activity by modifying (−)-rutamarin. Until now, there has been no such report on (−)-rutamarin and its derivatives. Herein, the synthesis and biological testing of these compounds for their anti-EBV activity are reported.

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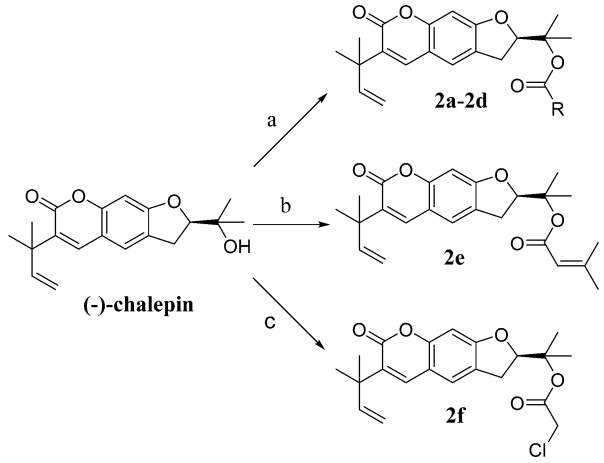
RESULTS AND DISCUSSION

Twenty-eight (−)-rutamarin derivatives were designed and synthesized by introducing acyl, glycinate, and carbonic ester/carbamate units at C-4′. The potential of each derivative in the inhibition of EBV lytic DNA replication and its cytotoxicity were determined as described previously.[17,18]

Due to the steric hindrance of a tertiary alcohol, the esterification reactions were not able to take place under conventional conditions (Table S1, Supporting Information). It was necessary, therefore, to establish reaction conditions under which the tertiary alcohol could be activated in an esterification reaction. According to a literature method,[19] the Lewis acid $Bi(OTf)_3$ catalyzes the esterification reactions of alcohols, acids, and anhydrides (Scheme 1). Compounds **2a**−**d** were synthesized

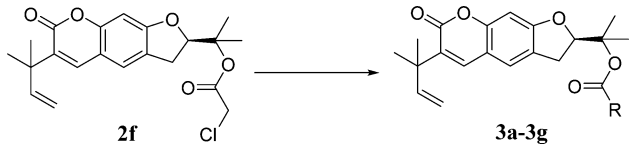
Scheme 1. Synthesis of (−)-Chalepin Derivatives 2a−f[a]



[a]Reagents and conditions: (a) $Bi(OTf)_3$, anhydride, rt, 4−6 h, 62%−83%; (b) CuCN, 3-methylcrotonoyl chloride, toluene, 50 °C, 14 h, 71%; (c) chloroacetyl chloride, DMAP, anhydrous CH_2Cl_2, N_2, 0 °C → rt, 66%.

by reaction of (−)-chalepin with anhydrides, in high yields in the 62−83% range (Scheme 1). Compound **2e** was obtained from (−)-chalepin and 3-methylcrotonoyl chloride, which was catalyzed by cuprous cyanide.[20] Compound **2f** was synthesized from (−)-chalepin by acylation with chloraoacetyl chloride in 66% yield. Seven aliphatic amine analogues (**3a**−**g**) were produced by the reaction of **2f** with the corresponding amines (Scheme 2).[21] The carbonic ester and carbamate derivatives of

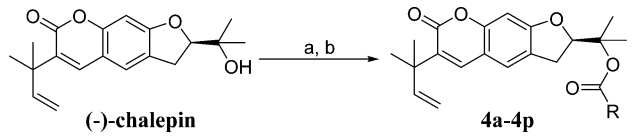
Scheme 2. Synthesis of Compounds 3a−g[a]



[a]Reagents and conditions: amines, CH_3CN/THF (2:1), rt, 44−61%.

(−)-rutamarin[22−24] (**4a**−**q**) were prepared using triphosgene green reagents (Scheme 3). A mixture of (−)-chalepin and triphosgene was stirred in anhydrous dichloromethane at room temperature for 15 min in the presence of 4-dimethylaminopyridine (DMAP) under nitrogen protection, and then the corresponding amine or alcohol was added directly. The yields ranged from 30% to 65%. After reaction, the remaining (−)-chalepin was recycled.

Scheme 3. Synthesis of Compounds 4a−q[a]



[a]Reagents and conditions: (a) triphosgene, DMAP, anhydrous CH_2Cl_2, rt; (b) amine or alcohol, 30−65%.

Compounds **2a**−**d**, with the larger lipophilic groups including an ethyl, isopropyl, tertiary butyl, or phenyl group, showed better activity than (−)-chalepin and (−)-rutamarin (Table 1; Table S2, Supporting Information). These results suggest that increasing the size of lipophilic groups improved the anti-EBV activity. Moreover, **2e**, with a 3-methylcrotonoyl group at C-4′, showed the most potent anti-EBV activity, with an IC_{50} value of 2.1 μM in series 1. Due to the alkyl chloride substituted at C-4′, compound **2f** exhibited high cytotoxicity levels for the P3HR-1 cell line at a concentration of 10 μM. Thus, the anti-EBV activity of **2f** was not given.

The results with compounds **3a**, **3b**, and **3c** indicated that the substituents with a longer alkyl chain connected to nitrogen led to increased anti-EBV activity (Table S2, Supporting Information). However, compounds **3a**, **3b**, and **3c** also showed high cytotoxicity, leading to low selectivity (selectivity index, SI < 3). In contrast, compound **3d**, possessing a cyclopentyl substituent fragment, exhibited antiviral activity (IC_{50} 2.0 μM) and low cytotoxicity (SI > 20). The anti-EBV activity of compounds **3e** and **3f** decreased when a cyclopentyl group was replaced by an oxygen-containing heterocyclic ring. More specifically, the 4-fluorophenethyl-containing compound **3g** showed potent anti-EBV activity (Table 1) with an IC_{50} value of 0.76 μM and a SI value of 56. Therefore, the amine-containing compounds **3a**−**f** exhibited improved anti-EBV activity in comparison to (+)- or (−)-rutamarin.

When compared to (−)-rutamarin, the carbamate derivatives **4a**−**e** showed low anti-EBV lytic replication activity (Table S2, Supporting Information). However, the 4-fluorophenethyl carbamate derivative **4f** exhibited greater potency than (−)-rutamarin, with an IC_{50} value of 1.4 μM. While the carbonic ester derivatives **4g**−**p** showed weak to potent anti-EBV activity, compounds with an aliphatic chain (**4g**, **4i**) or heterocyclic (**4h**) or phenyl (**4j**) groups exhibited reduced anti-EBV activity (Table S2, Supporting Information). The aromatic heterocyclic-containing compounds **4k**−**p** showed increased anti-EBV activity. When the number of carbons between oxygen and the aromatic heterocycle was reduced to one, the activity, as for example in compound **4l**, was lowered. This suggested that the length of the side chain might affect anti-EBV activity. The phenyl ethyl carbonic acid ester-containing compound **4j** showed no activity and high cytotoxicity. When the phenyl group was changed to 2-pyridine, **4k** showed significant activity and weak cytotoxicity (IC_{50} 6.2 μM; CC_{50} 42.7 μM; SI 7; Table S2, Supporting Information). Compound **4m**, with a 5-ethyl group substituted at the 2-pyridine ring, exhibited weak anti-EBV activity but superior selectivity (IC_{50} 1.5 μM; CC_{50} 1202 μM; SI 801; Figure 2). The above results indicated that the presence of a substituted 2-pyridine favored the resultant anti-EBV activity. Compound **4n**, with a 4-pyridine-ethyl carbonic acid ester substituted at C-4′, exhibited better activity and fairly low cytotoxicity (IC_{50} 0.32 μM; CC_{50} 67.6 μM; SI 211; Figure 2) compared with the 2-pyridine-ethyl-substituted **4k** (Table S2, Supporting Information). This suggests that a 4-pyridine-ethyl

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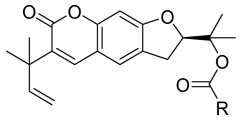
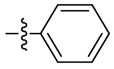
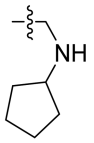
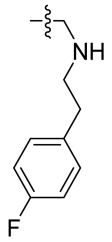
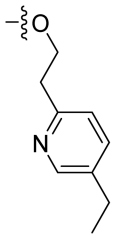
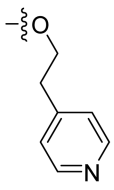
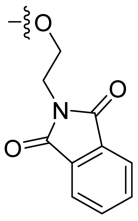
Table 1. Anti-EBV Lytic Replication Activities of (−)-Rutamarin Derivatives (SI ≥ 20)



compound	R	IC_{50}[a]	R^2[d]	CC_{50}[b]	R^2[d]	SI[c]
(−)-chalepin	-	69.9	0.95	-	-	-
(−)-rutamarin	-	13.8	0.91	>140	-	>10
(+)-rutamarin[e]	-	7.0	0.88	>150	-	>21
2d		4.5	0.95	370.4	0.96	83
2e		2.1	0.93	>100	-	>47
3d		2.0	0.99	40.8	1.00	20
3g		0.76	0.94	42.6	0.74	56
4m		1.5	0.98	1202	0.90	801
4n		0.32	0.99	67.6	0.89	211
4p		0.83	0.90	>100	0.83	>120

[a]The inhibitory effects of compounds against EBV lytic replication were tested and expressed as IC_{50} values (μM). [b]Cytotoxicities were measured after 2 days of compound treatment and expressed as CC_{50} values (μM). [c]Selective index (SI) = CC_{50}/IC_{50}. [d]Regression coefficients of the dose−response curves. [e]Positive control.

group is more favorable than a 2-pyridine-ethyl group for anti-EBV activity. On the basis of the above results, it is speculated that a substituted 4-pyridine-ethyl moiety may contribute to higher activity and lower cytotoxicity.

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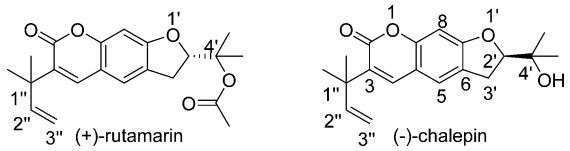

Figure 1. Structures of (+)-rutamarin and (−)-chalepin.

EXPERIMENTAL SECTION

General Experimental Procedures. Reagents were used without further purification unless otherwise specified. Solvents were dried and redistilled prior to use with the usual methods. All reactions were monitored by thin-layer chromatography using silica gel (Marine Chemical Ltd., Qingdao, People's Republic of China) and visualized by UV light. Flash column chromatography was performed using silica gel (200−300 mesh) purchased from Qingdao Marine Chemical Ltd. Optical rotations were measured on a PerkinElmer 341 automatic polarimeter. The ^{1}H NMR and ^{13}C NMR spectra were recorded using tetramethylsilane as the internal standard on a BrukerBioSpin GmbH NMR spectrometer (AvanceIII, Switzerland) at 400 and 100 MHz. Coupling constants are given in Hz. High-resolution mass spectra were obtained using a Shimadzu LCMS-IT-TOF mass spectrometer.

Plant Material. The aerial parts of *R. graveolens* (10 kg) were collected in Foshan, Guangdong Province, in July 2013. They were identified by Dr. Chunyan Han of the Kunming Institute of Botany, Chinese Academy of Sciences. A voucher sample (SYSU-20130901) has been deposited at the School of Pharmaceutical Sciences, Sun Yat-sen University.

Isolation of (−)-Chalepin. The aerial parts of *R. graveolens* were dried at room temperature, powdered, and extracted with 95% EtOH to afford an extract (200 g), which was partitioned with EtOAc (4 × 4 L), *n*-BuOH (3 × 3 L), and H_2O. The EtOAc fraction (80 g) was chromatographed on a silica gel column eluting with CH_2Cl_2−EtOAc (100:0 to 0:100) to obtain 10 fractions (A−G). (−)-Chalepin (500 mg) was recrystallized from fraction D and exhibited $[\alpha]^{25}_{D}$ −24 (*c* 0.4, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 7.47 (1H, s, H-4), 7.18 (1H, s, H-5), 6.69 (1H, s, H-8), 6.15 (1H, dd, *J* = 17.2, 10.9 Hz, H-2″), 5.05 (2H, m, H-3″), 4.70 (1H, t, *J* = 9.3 Hz, H-2′), 3.18 (2H, m, H-3′), 1.46 (6H, s, CH_3-1″), 1.35 (3H, s, CH_3-4′), 1.22 (3H, s, CH_3-4′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 162.4 (C, C-2), 160.3 (C, C-7), 154.8 (C, C-9), 145.7 (CH, C-4), 138.2 (CH, C-2″), 131.0 (C, C-3), 124.7 (C, C-6), 123.4 (CH, C-5), 113.3 (C, C-10), 112.2 (CH_2, C-3″), 97.2 (CH, C-8), 91.0 (CH, C-2′), 71.8 (C, C-4′), 40.4 (C, C-1″), 29.7 (CH_2, C-3′), 26.2 (CH_3 × 2, CH_3-4′), 24.4 (CH_3 × 2, CH_3-1″); EIMS *m*/*z* 315.2 $[M + H]^+$.

Synthesis of (−)-Rutamarin. A mixture of (−)-chalepin (31.4 mg, 0.1 mmol) and *p*-toluensulfonic acid (1.9 mg, 0.011 mmol) in acetic anhydride (2 mL) was stirred for 1 h at room temperature. Then, ice water was added, and the mixing continued for an additional 10 min. The suspension was filtered, and the residue was washed with water to give (−)-rutamarin (32.7 mg, 92%): $[\alpha]^{25}_{D}$ −8 (*c* 0.4, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) 7.48 (1H, s, H-4), 7.18 (1H, d, *J* = 1.5 Hz, H-5), 6.71 (1H, s, H-8), 6.16 (1H, dd, *J* = 17.3, 10.8 Hz, H-2″), 5.07 (3H, m, H-2′, H-3″), 3.20 (2H, m, H-3′), 1.98 (3H, s, $COCH_3$), 1.55 (3H, s, CH_3-4′), 1.50 (3H, s, CH_3-4′), δ_H 1.46 (6H, s, CH_3-1″); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 170.4 (C, $COCH_3$), 162.5 (C, C-2), 160.3 (C, C-7), 154.9 (C, C-9), 145.7 (CH, C-4), 138.2 (CH, C-2″), 131.0 (C, C-3), 124.0 (C, C-6), 123.2 (CH, C-5), 113.2 (C, C-10), 112.2 (CH_2, C-3″), 97.3 (CH, C-8), 88.4 (CH, C-2′), 82.3 (C, C-4′), 40.4 (C, C-1″), 29.8 (CH_2, C-3′), 26.2 (CH_3 × 2, CH_3-1″), 22.5 (CH_3, CH_3-4′), 22.1 (CH_3, CH_3-4′), 21.2 (CH_3, $COCH_3$); EIMS *m*/*z* 357.2 $[M + H]^+$.

General Procedure for Synthesizing Compounds 2a−d. A solution of (−)-chalepin (31.4 mg, 0.1 mmol) and the corresponding anhydride (0.15 mmol) in dichloromethane was stirred at room temperature in the presence of $Bi(OTf)_3$ (2.18 mg, 3.0%/mol, calculated as the tetrahydrate) for 4−6 h. Methanol (1 mL, unpurified) was added, and the mixture was stirred at 50 °C for 7 h. The mixture was passed through a Celite pad with hexane, and the filtrate was concentrated under reduced pressure. The crude product was dissolved in EtOAc (5 mL) and portioned with aqueous $NaHCO_3$ three times. The organic phase was separated and dried over $MgSO_4$. Removal of solvent in vacuo afforded an oily residue. The residue was purified by silica gel column chromatography (petroleum ether−EtOAc, 5:1) to give the corresponding ester.

Synthesis of Compound ***2a.*** Compound **2a** (32.6 mg, 80%) was synthesized from (−)-chalepin and propionic anhydride by the general procedure: ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.03 (3H, t, *J* = 7.5 Hz), 1.45 (6H, s), 1.50 (3H, s), 1.55 (3H, s), 2.22 (2H, q, *J* = 7.6 Hz), 3.19 (2H, qd, *J* = 16.0, 8.5 Hz), 5.04 (2H, m), 5.08 (1H, s), 6.15 (1H, dd, *J* = 17.3, 10.8 Hz), 6.68 (1H, s), 7.18 (1H, s), 7.47 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 9.2, 21.2, 22.2, 26.2, 28.8, 29.8, 40.4, 82.0, 88.5, 97.2, 112.2, 113.1, 123.2, 124.1, 130.9, 138.1, 145.7, 154.8, 160.3, 162.6, 173.7; EIMS *m*/*z* 371.2 $[M + H]^+$; HREIMS *m*/*z* 371.1845 $[M + H]^+$ (calcd for $C_{22}H_{26}O_5$, 371.1853).

Synthesis of Compound ***2b.*** Compound **2b** (29.6 mg, 77%) was synthesized from (−)-chalepin and isobutyric anhydride by the general procedure: ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.05 (6H, dd, *J* = 7.0, 2.2 Hz), 1.46 (6H, s), 1.51 (3H, s), 1.55 (3H, s), 2.41 (1H, hept, *J* = 7.0 Hz), 3.19 (2H, m), 5.05 (3H, m), 6.17 (1H, dd, *J* = 17.1, 11.1 Hz), 6.69 (1H, s), 7.18 (1H, s), 7.47 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 19.0, 21.2, 22.3, 26.2, 29.7, 35.0, 40.4, 81.8, 88.7, 97.2, 112.2, 113.1, 123.1, 124.1, 131.0, 138.1, 145.8, 154.9, 160.3, 162.6, 176.3; EIMS *m*/*z* 385.2 $[M + H]^+$; HREIMS *m*/*z* 385.2006 $[M + H]^+$ (calcd for $C_{23}H_{28}O_5$, 385.2010).

Synthesis of Compound ***2c.*** Compound **2c** (33.1 mg, 83%) was synthesized from (−)-chalepin and trimethylacetic anhydride by the

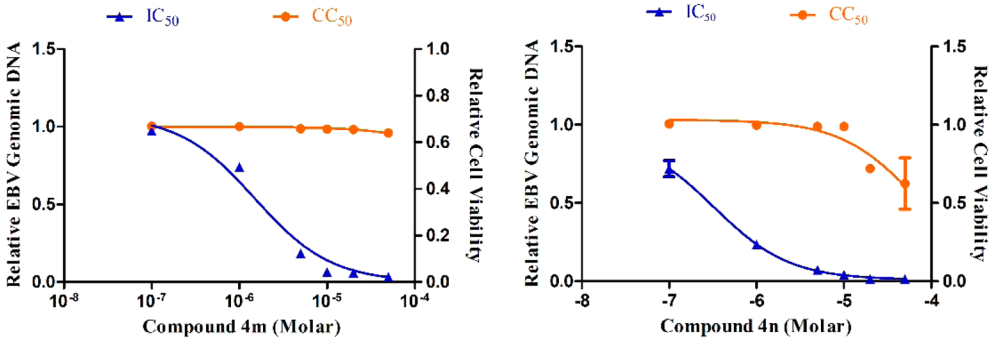

Figure 2. Effects of compounds **4m** and **4n** on EBV lytic replication and their associated cytotoxicity in P3HR-1 cells. EBV lytic replication in P3HR-1 was induced with TPA and sodium butyrate. Compounds **4m** and **4n** were added to the cell culture for 3 h after induction. Intracellular EBV DNA (blue) and cell viability (orange) were determined for each concentration. These values were compared to those from the control cells (nondrug treatment). Mean values of results from at least three independent experiments and standard deviations are presented on the *y*-axes of dose−response curves.

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J. Nat. Prod. 2017, 80, 53−60

general procedure: ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.03 (9H, s), 1.46 (6H, s), 1.53 (6H, d, J = 9.6 Hz), 3.22 (2H, m), 4.94 (1H, dd, J = 9.5, 7.1 Hz), 5.05 (1H, m), 5.08 (1H, s), 6.16 (1H, dd, J = 10.2, 10.2 Hz), 6.67 (1H, s), 7.18 (1H, s), 7.47 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 21.2, 22.2, 26.2, 27.1, 29.7, 39.5, 40.4, 81.7, 89.0, 97.0, 112.1, 113.1, 123.1, 124.1, 130.9, 138.2, 145.7, 154.8, 160.3, 162.7, 177.6; EIMS m/z 399.2 $[M + H]^+$; HREIMS m/z 399.2172 $[M + H]^+$ (calcd for $C_{24}H_{30}O_5$, 399.2142).

Synthesis of Compound ***2d***. Compound **2d** (25.9 mg, 62%) was synthesized from (−)-chalepin and benzoic anhydride by the general procedure: ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.48 (6H, s), 1.68 (3H, s), 1.70 (3H, s), 3.33 (2H, m), 5.11 (3H, m), 6.18 (1H, dd, J = 17.2, 10.9 Hz), 6.75 (1H, s), 7.23 (1H, s), 7.31 (2H, t, J = 7.7 Hz), 7.48 (1H, d, J = 7.5 Hz), 7.51 (1H, s), 7.75 (2H, d, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 21.4, 22.3, 26.3, 29.9, 40.5, 83.1, 89.0, 97.3, 112.2, 113.2, 123.2, 124.1, 128.4, 129.5, 131.1, 131.2, 133.0, 138.1, 145.7, 154.9, 160.3, 162.7, 165.5; EIMS m/z 419.2 $[M + H]^+$; HREIMS m/z 419.1857 $[M + H]^+$ (calcd for $C_{26}H_{26}O_5$, 419.1853).

Synthesis of Compound ***2e***. To a stirred suspension of (−)-chalepin (31.4 mg, 0.1 mmol) and CuCN (26.7 mg, 0.3 mmol) in toluene (1 mL) was added a solution of 3-methylcrotonoyl chloride (0.2 mmol) in toluene (1 mL) at room temperature. The mixture was stirred for 14 h at 50 °C and was filtered through a Celite pad. The residue was washed with EtOAc. The combined filtrates were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether−EtOAc, 5:1) to give **2e** (28.2 mg, 71%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.44 (6H, s), 1.50 (3H, s), 1.56 (3H, s), 1.83 (3H, s), 2.08 (3H, s), 3.18 (2H, dd, J = 17.1, 8.9 Hz), 5.07 (3H, m), 5.54 (1H, s), 6.14 (1H, dd, J = 17.3, 10.8 Hz), 6.68 (1H, s), 7.16 (1H, s), 7.46 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 20.3, 21.4, 22.5, 26.3, 27.6, 29.9, 40.5, 81.6, 88.8, 97.3, 112.3, 113.2, 117.2, 123.3, 124.3, 131.0, 138.3, 145.8, 154.9, 156.8, 160.4, 162.7, 166.1; EIMS m/z 397.2 $[M + H]^+$; HREIMS m/z 397.2010 $[M + H]^+$ (calcd for $C_{24}H_{28}O_5$, 397.2003).

Synthesis of Compound ***2f***. A mixture of chloroacetyl chloride (510 mg, 3 mmol) in anhydrous cyclohexane was added dropwise into a mixture of (−)-chalepin (314 mg, 1 mmol) and 4-dimethylaminopyridine (488 mg, 4 mmol) in anhydrous cyclohexane (10 mL) under nitrogen at 0 °C. The reaction mixture was stirred at room temperature for 24 h, washed with HCl (5%, 10 mL × 3) and then water (10 mL × 3) successively, and then dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (petroleum ether−EtOAc, 5:1) to obtain **2f** (261 mg, 66%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.44 (6H, s), 1.57 (6H, d, J = 5.8 Hz), 3.20 (2H, m), 3.94 (2H, s), 5.03 (3H, m), 6.14 (1H, dd, J = 17.3, 10.8 Hz), 6.68 (1H, s), 7.17 (1H, s), 7.45 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 21.2, 21.9, 26.3, 29.9, 40.5, 41.9, 84.9, 88.2, 97.4, 112.3, 113.4, 123.3, 123.8, 131.2, 138.2, 145.8, 154.9, 160.3, 162.4, 166.3; EIMS m/z 391.1 $[M + H]^+$; HREIMS m/z 391.1300 $[M + H]^+$ (calcd for $C_{21}H_{23}ClO_5$, 391.1307).

General Procedure for Preparation of 3a−g. To a solution of **2f** (20 mg, 0.05 mmol) in CH_3CN (1 mL) was added the corresponding amine (0.05 mmol) in THF (0.5 mL) at 0 °C. The reaction mixture was stirred at room temperature for 24−36 h and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (CH_2Cl_2−MeOH, 30:1) to give **3a−g**.

Synthesis of Compound ***3a***. Compound **3a** (11.7 mg, 61%) was synthesized from compound **2f** and methylamine by the general procedure: ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.45 (6H, s), 1.51 (3H, s), 1.54 (3H, s), 2.46 (2H, s), 3.18 (2H, m), 3.35 (2H, s), 5.05 (3H, m), 6.15 (1H, dd, J = 17.2, 10.9 Hz), 6.67 (1H, s), 7.18 (1H, s), 7.47 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 21.3, 22.0, 26.2, 29.9, 40.4, 42.0, 57.8, 83.2, 88.2, 97.2, 112.2, 113.2, 123.3, 123.8, 131.1, 138.1, 145.7, 154.8, 160.2, 162.4, 170.0; EIMS m/z 384.9 $[M - H]^-$; HREIMS m/z 386.1960 $[M + H]^+$ (calcd for $C_{22}H_{27}NO_5$, 386.1962).

Synthesis of Compound ***3b***. Compound **3b** (10.0 mg, 50%) was synthesized from compound **2f** and ethylamine by the general procedure: ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.09 (3H, t, J = 7.1 Hz), 1.46 (6H, s), 1.55 (3H, s), 1.57 (3H, s), 2.65 (2H, q, J = 7.1 Hz), 3.21 (2H, dd, J = 24.5, 8.6 Hz), 3.33 (2H, s), 5.06 (3H, dd, J = 18.1, 9.5 Hz), 6.16 (1H, dd, J = 17.4, 10.7 Hz), 6.70 (1H, s), 7.19 (1H, s), 7.47 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 14.9, 21.4, 22.1, 26.3, 29.9, 40.5, 43.8, 51.2, 83.4, 88.4, 97.3, 112.2, 113.3, 123.2, 123.9, 131.2, 138.1, 145.7, 154.9, 160.2, 162.4, 171.2; EIMS m/z 400.2 $[M + H]^+$; HREIMS m/z 400.2118 $[M + H]^+$ (calcd for $C_{23}H_{29}NO_5$, 400.2117).

Synthesis of Compound ***3c***. Compound **3c** (11.1 mg, 52%) was synthesized from **2f** and butylamine by the general procedure: ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 0.82 (3H, t, J = 7.2 Hz), 1.23 (2H, m), 1.34 (2H, m), 1.39 (6H, s), 1.48 (3H, s), 1.50 (3H, s), 2.48 (2H, t, J = 7.1 Hz), 3.13 (2H, m), 3.22 (2H, s), 4.99 (3H, m), 6.09 (1H, dd, J = 17.3, 10.8 Hz), 6.62 (1H, s), 7.12 (1H, s), 7.41 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 14.1, 20.4, 21.3, 22.1, 26.2, 29.8, 32.2, 40.4, 49.3, 51.8, 83.1, 88.4, 97.2, 112.2, 113.2, 123.2, 123.9, 131.0, 138.1, 145.7, 154.8, 160.2, 162.4, 171.7; EIMS m/z 428.3 $[M + H]^+$; HREIMS m/z 428.2436 $[M + H]^+$ (calcd for $C_{23}H_{33}NO_5$, 428.2431).

Synthesis of Compound ***3d***. Compound **3d** (12.1 mg, 55%) was synthesized from **2f** and cyclopentylamine by the general procedure: ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.28 (2H, dt, J = 11.7, 3.9 Hz), 1.45 (6H, s), 1.50 (2H, dt, J = 8.6, 4.7 Hz), 1.54 (3H, s), 1.56 (3H, s), 1.63 (2H, m), 1.74 (2H, m), 2.99 (1H, m), 3.18 (2H, m), 3.28 (2H, s), 5.04 (3H, m), 6.15 (1H, dd, J = 17.3, 10.8 Hz), 6.69 (1H, s), 7.18 (1H, s), 7.47 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 21.3, 22.1, 24.1, 26.2, 29.8, 33.0, 40.4, 50.6, 59.4, 83.1, 88.4, 97.2, 112.2, 113.2, 123.2, 123.9, 131.0, 138.1, 145.7, 154.8, 160.2, 162.4, 171.8; EIMS m/z 440.3 $[M + H]^+$; HREIMS m/z 440.2429 $[M + H]^+$ (calcd for $C_{25}H_{33}NO_5$, 440.2431).

Synthesis of Compound ***3e***. Compound **3e** (12.3 mg, 54%) was synthesized from **2f** and 2-tetrahydrofurfurylamine by the general procedure: ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.45 (6H, s), 1.52 (3H, d, J = 2.7 Hz), 1.56 (3H, s), 1.91 (4H, m), 2.63 (2H, m), 3.20 (2H, m), 3.33 (2H, s), 3.71 (1H, m), 3.87 (2H, m), 5.06 (3H, m), 6.15 (1H, dd, J = 17.3, 10.8 Hz), 6.68 (1H, s), 7.18 (1H, s), 7.46 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 21.3, 22.2, 25.8, 26.2, 29.3, 29.8, 40.4, 51.8, 53.8, 68.1, 78.4, 83.1, 88.4, 97.2, 112.2, 113.2, 123.2, 123.9, 131.0, 138.1, 145.7, 154.8, 160.2, 162.5, 171.5; EIMS m/z 456.2 $[M + H]^+$; HREIMS m/z 456.2375 $[M + H]^+$ (calcd for $C_{26}H_{33}NO_6$, 456.2381).

Synthesis of Compound ***3f***. Compound **3f** (12.2 mg, 54%) was synthesized from **2f** and furfurylamine following the same procedure: ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.46 (6H, s), 1.53 (3H, s), 1.56 (3H, s), 3.19 (2H, m), 3.30 (2H, s), 3.75 (2H, s), 5.05 (3H, m), 6.15 (2H, m), 6.29 (1H, dd, J = 3.2, 1.9 Hz), 6.69 (1H, s), 7.17 (1H, s), 7.34 (1H, m), 7.46 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 21.3, 22.1, 26.2, 29.8, 40.4, 45.6, 50.5, 83.3, 88.3, 97.2, 107.5, 110.3, 112.2, 113.2, 123.2, 123.8, 131.1, 138.1, 142.2, 145.7, 153.1, 154.9, 160.2, 162.4, 171.3; EIMS m/z 452.2 $[M + H]^+$; HREIMS m/z 452.2063 $[M + H]^+$ (calcd for $C_{26}H_{29}NO_6$, 452.2068).

Synthesis of Compound ***3g***. Compound **3g** (10.8 mg, 44%) was synthesized from **2f** and 4-fluorophenethylamine by the general procedure: ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.46 (6H, s), 1.52 (3H, s), 1.55 (3H, s), 2.77 (4H, m), 3.17 (2H, m), 3.30 (2H, s), 5.06 (3H, m), 6.15 (1H, dd, J = 17.3, 10.8 Hz), 6.69 (1H, s), 6.97 (2H, t, J = 8.6 Hz), 7.14 (2H, dd, J = 8.4, 5.5 Hz), 7.17 (1H, s), 7.47 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 21.3, 22.0, 26.2, 29.8, 35.8, 40.4, 50.8, 51.6, 83.2, 88.3, 97.3, 112.2, 113.2, 115.4 (d, J_{CF} = 21.2 Hz), 123.2, 123.8, 130.1 (d, J_{CF} = 7.9 Hz), 130.2, 131.1, 135.4 (d, J_{CF} = 3.3 Hz), 138.1, 145.7, 160.2, 161.6 (d, J_{CF} = 244.2 Hz), 162.4, 171.5; EIMS m/z 494.2 $[M + H]^+$; HREIMS m/z 494.2324 $[M + H]^+$ (calcd for $C_{29}H_{32}FNO_5$, 494.2337).

General Procedure for Preparation of 4a−p. (−)-Chalepin (20.7 mg, 0.066 mmol) and triphosgene (6.5 mg, 0.022 mmol) were dispersed in anhydrous dichloromethane (2 mL), to which DMAP (24.2 mg, 0.198 mmol) was added under nitrogen. The mixture was stirred at room temperature for 15 min, and then the corresponding amine or alcohol (0.066 mmol) was added. The solution was stirred at room temperature overnight, diluted with dichloromethane (10 mL), and washed with HCl (pH 1, 3 × 5 mL) and then with water (3 × 5 mL). The organic phase was dried with Na_2SO_4. After the solvent was removed under reduced pressure, the crude product was purified by silica gel chromatography (CH_2Cl_2−MeOH, 30:1) to give **4a−p**.

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J. Nat. Prod. 2017, 80, 53−60

Synthesis of Compound ***4a***. Compound **4a** (8.7 mg, 33%) was synthesized from (−)-chalepin and 1,4-diaminobutane by the general procedure: ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.43 (6H, s), 1.47 (3H, s), 1.52 (3H, s), 2.78 (2H, s), 3.18 (4H, m), 5.04 (4H, m), 6.13 (1H, dd, J = 17.2, 10.9 Hz), 6.66 (1H, s), 7.15 (1H, s), 7.44 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 21.7, 22.5, 26.3, 29.9, 40.5, 41.9, 43.6, 81.4, 88.8, 97.3, 112.2, 113.2, 123.3, 124.2, 131.0, 138.2, 145.8, 154.9, 155.7, 160.4, 162.7; EIMS m/z 401.2 $[M + H]^+$; HREIMS m/z 401.2071 $[M + H]^+$ (calcd for $C_{22}H_{28}N_2O_5$, 401.1998).

Synthesis of Compound ***4b***. Compound **4b** (8.5 mg, 30%) was synthesized from (−)-chalepin (0.100 g, 0.23 mmol) and 1,4-diaminobutane by the general procedure: ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.47 (6H, s), 1.51 (2H, m), 1.52 (3H, s), 1.55 (3H, s), 1.57 (2H, m)2.73 (2H, t, J = 6.6 Hz), 3.18 (4H, dq, J = 24.5, 8.1, 6.6 Hz), 4.90 (1H, t, J = 6.1 Hz), 5.11 (3H, m), 6.17 (1H, dd, J = 17.3, 10.9 Hz), 6.71 (1H, s), 7.19 (1H, s), 7.48 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 21.7, 22.4, 26.2, 27.5, 29.8, 30.5, 40.4, 40.7, 41.7, 81.2, 88.8, 97.2, 112.2, 113.1, 123.2, 124.2, 130.9, 138.1, 145.7, 154.8, 155.5, 160.3, 162.6; EIMS m/z 429.2 $[M + H]^+$; HREIMS m/z 429.2384 $[M + H]^+$ (calcd for $C_{24}H_{32}N_2O_5$, 429.2390).

Synthesis of Compound ***4c***. Compound **4c** (10.6 mg, 40%) was synthesized from (−)-chalepin and 3-aminopropionitrile by the general procedure, and the residue was purified by silica gel chromatography (petroleum ether−EtOAc, 5:1): ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.43 (6H, s), 1.49 (3H, s), 1.53 (3H, s), 2.57 (2H, dd, J = 11.9, 6.0 Hz), 3.18 (2H, dd, J = 15.7, 8.6 Hz), 3.37 (2H, dd, J = 9.4, 6.4 Hz), 5.04 (3H, m), 5.13 (1H, t, J = 5.8 Hz) 6.13 (1H, dd, J = 17.3, 10.9 Hz), 6.65 (1H, s), 7.15 (1H, s), 7.44 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 19.0, 21.6, 22.2, 26.2, 29.8, 37.0, 40.4, 82.2, 88.6, 97.2, 112.2, 113.2, 118.1, 123.3, 124.0, 131.0, 138.1, 145.7, 154.8, 155.1, 160.3, 162.5; EIMS m/z 411.2 $[M + H]^+$; HREIMS m/z 411.1924 $[M + H]^+$ (calcd for $C_{23}H_{26}N_2O_5$, 411.1914).

Synthesis of Compound ***4d***. Compound **4d** (19.2 mg, 32%) was synthesized from (−)-chalepin (0.15 mmol) and ethanolamine by the general procedure: ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.44 (6H, s), 1.49 (3H, s), 1.53 (3H, s), 3.19 (2H, dd, J = 12.0, 8.7 Hz), 3.26 (2H, m), 3.68 (2H, t, J = 5.1 Hz), 4.04 (3H, m), 5.20 (1H, t, J = 5.5 Hz), 6.13 (1H, dd, J = 17.3, 10.8 Hz), 6.65 (1H, s), 7.16 (1H, s), 7.45 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 21.7, 22.2, 26.2, 29.8, 40.4, 43.3, 62.3, 81.6, 88.8, 97.1, 112.2, 113.1, 123.2, 124.2, 130.8, 138.2, 145.6, 154.7, 156.2, 160.4, 162.5; EIMS m/z 402.2 $[M + H]^+$; HREIMS m/z 402.1916 $[M + H]^+$ (calcd for $C_{22}H_{26}O_5$, 402.1911).

Synthesis of Compound ***4e***. To a stirred solution of **4d** (0.025 mmol) in dichloromethane (2 mL) were added EDC·HCl (0.03 mmol), DMAP (0.1 equiv), and acetic acid (0.075 mmol), and the resulting mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure, and the residue was purified by silica gel chromatography (CH_2Cl_2−EtOAc, 3:1) to give **4e** (7.8 mg, 70%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.45 (6H, s), 1.49 (3H, s), 1.54 (3H, s), 2.05 (3H, s), 3.18 (2H, m), 3.38 (2H, q, J = 5.6 Hz), 4.11 (2H, m), 4.91 (1H, t, J = 5.4 Hz), 5.09 (3H, m), 6.15 (1H, dd, J = 17.3, 10.9 Hz), 6.69 (1H, s), 7.17 (1H, s), 7.46 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 21.0, 21.7, 22.3, 26.3, 29.9, 40.0, 40.5, 63.7, 81.8, 88.7, 97.3, 112.3, 113.2, 123.3, 124.2, 131.1, 138.2, 145.8, 154.9, 155.3, 160.3, 162.6, 171.1; EIMS m/z 444.2 $[M + H]^+$; HREIMS m/z 444.2017 $[M + H]^+$ (calcd for $C_{24}H_{29}NO_7$, 444.2015).

Synthesis of Compound ***4f***. Compound **4f** (11.4 mg, 36%) was synthesized from (−)-chalepin and 4-fluorophenethylamine by the general procedure: ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.46 (6H, s), 1.47 (3H, s), 1.53 (3H, s), 2.76 (2H, t, J = 7.1 Hz), 3.16 (2H, m), 3.35 (2H, q, J = 6.8 Hz), 4.66 (1H, t, J = 6.2 Hz), 5.09 (3H, m), 6.16 (1H, dd, J = 17.3, 10.8 Hz), 6.68 (1H, s), 6.97 (2H, t, J = 8.5 Hz), 7.13 (2H, t, J = 6.9 Hz), 7.17 (1H, s), 7.46 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 21.7, 22.3, 26.2, 29.8, 35.5, 40.4, 42.1, 81.4, 88.6, 97.2, 112.2, 113.1, 115.5 (d, J_{CF} = 20.8 Hz), 115.6, 123.2, 124.1, 130.2(d, J_{CF} = 7.9 Hz), 130.3, 130.9, 134.5 (d, J_{CF} = 3.6 Hz), 138.1, 145.7, 154.8, 155.3, 155.3, 160.4 (d, J_{CF} = 23.8 Hz), 161.2 (d, J_{CF} = 244.5 Hz), 162.8 (d, J_{CF} = 37.9 Hz); EIMS m/z 480.2 $[M + H]^+$; HREIMS m/z 480.2181 $[M + H]^+$ (calcd for $C_{28}H_{30}FNO_5$, 480.2181).

Synthesis of Compound ***4g***. Compound **4g** (14.4 mg, 53%) was synthesized from (−)-chalepin and 3-hydroxypropionitrile by the general procedure, and the residue was purified by silica gel chromatography (petroleum ether−EtOAc, 10:1): ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.45 (6H, s), 1.54 (3H, s), 1.58 (3H, s), 2.72 (2H, t, J = 6.4 Hz), 3.22 (2H, m), 4.25 (2H, m), 4.54 (3H, m), 6.15 (1H, dd, J = 17.2, 10.9 Hz), 6.70 (1H, s), 7.19 (1H, s), 7.47 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 18.2, 20.7, 21.7, 26.3, 30.0, 40.5, 61.6, 84.8, 87.9, 97.4, 112.3, 113.4, 116.6, 123.4, 123.7, 131.2, 138.2, 145.8, 152.7, 154.9, 160.3, 162.3; EIMS m/z 412.2 $[M + H]^+$; HREIMS m/z 412.1755 $[M + H]^+$ (calcd for $C_{23}H_{25}NO_6$, 412.1756).

Synthesis of Compound ***4h***. Compound **4h** (19.3 mg, 62%) was synthesized from (−)-chalepin and 4-(2-hydroxyethyl)morpholine by the general procedure, and the residue was purified by silica gel chromatography (CH_2Cl_2−EtOAc, 4:1): ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.45 (6H, s), 1.50 (3H, s), 1.57 (3H, s), 2.51 (4H, t, J = 4.7 Hz), 2.64 (2H, t, J = 5.8 Hz), 3.21 (2H, m), 3.70 (4H, t, J = 4.7 Hz), 4.20 (2H, t, J = 5.8 Hz), 5.07 (3H, m), 6.15 (1H, dd, J = 17.3, 10.9 Hz), 6.70 (1H, s), 7.18 (1H, s), 7.46 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 20.7, 21.9, 26.2, 29.9, 40.4, 53.9, 57.1, 64.3, 66.9, 83.8, 87.8, 97.4, 112.2, 113.3, 123.3, 123.8, 131.1, 138.1, 145.7, 153.3, 154.9, 160.2, 162.4; EIMS m/z 472.2 $[M + H]^+$; HREIMS m/z 472.2321 $[M + H]^+$ (calcd for $C_{26}H_{33}NO_7$, 472.2330).

Synthesis of Compound ***4i***. Compound **4i** (25.0 mg, 62%) was synthesized from (−)-chalepin (0.1 mmol) and ethylene glycol by the general procedure, and the residue was purified by silica gel chromatography (CH_2Cl_2−EtOAc, 3:1): ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.43 (6H, s), 1.50 (3H, s), 1.55 (3H, s), 3.19 (2H, m), 3.80 (2H, m), 4.17 (2H, m), 5.04 (3H, m), 6.12 (1H, dd, J = 17.2, 10.9 Hz), 6.68 (1H, s), 7.16 (1H, d, J = 1.3 Hz), 7.44 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 20.7, 21.7, 26.2, 29.9, 40.4, 61.0, 69.0, 84.0, 88.0, 97.4, 112.2, 113.3, 123.3, 123.8, 131.1, 138.1, 145.7, 153.5, 154.8, 160.2, 162.3; EIMS m/z 403.2 $[M + H]^+$; HREIMS m/z 403.1749 $[M + H]^+$ (calcd for $C_{22}H_{26}O_7$, 403.1751).

Synthesis of Compound ***4j***. Compound **4j** (19.9 mg, 65%) was synthesized from (−)-chalepin and phenethyl alcohol following the same procedure, and the residue was purified by silica gel chromatography (petroleum ether−EtOAc, 5:1): ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.39 (6H, s), 1.41 (3H, s), 1.49 (3H, s), 2.89 (2H, t, J = 7.3 Hz), 3.13 (2H, m), 4.20 (2H, t, J = 7.3 Hz), 5.01 (3H, m), 6.09 (1H, dd, J = 17.3, 10.9 Hz), 6.64 (1H, s), 7.17 (6H, m), 7.40 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 20.7, 22.0, 26.3, 29.9, 35.4, 40.5, 68.0, 83.7, 88.0, 97.4, 112.3, 113.4, 123.3, 123.9, 126.9, 128.7, 129.1, 131.2, 137.4, 138.2, 145.8, 153.4, 154.9, 160.3, 162.5; EIMS m/z 463.2 $[M + H]^+$; HREIMS m/z 463.2114 $[M + H]^+$ (calcd for $C_{28}H_{30}O_6$, 463.2115).

Synthesis of Compound ***4k***. Compound **4k** (15.9 mg, 52%) was synthesized from (−)-chalepin and 2-pyridine-ethanol by the general procedure, and the residue was purified by silica gel chromatography (CH_2Cl_2−EtOAc, 10:1): ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.42 (6H, s), 1.45 (3H, s), 1.52 (3H, s), 3.11 (3H, m), 3.21 (1H, dd, J = 16.7, 9.6 Hz), 4.44 (2H, t, J = 6.9 Hz), 5.04 (3H, m), 6.12 (1H, dd, J = 17.2, 10.9 Hz), 6.66 (1H, s), 7.13 (3H, m), 7.43 (1H, s), 7.58 (1H, td, J = 7.7, 1.8 Hz), 8.50 (1H, d, J = 4.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 20.7, 21.9, 26.3, 29.9, 37.4, 40.5, 66.7, 83.7, 87.9, 97.4, 112.2, 113.3, 122.0, 123.3, 123.8, 123.9, 131.1, 136.8, 138.2, 145.8, 149.5, 153.3, 154.9, 157.6, 160.3, 162.4; EIMS m/z 464.2 $[M + H]^+$; HREIMS m/z 464.2060 $[M + H]^+$ (calcd for $C_{27}H_{29}NO_6$, 464.2068).

Synthesis of Compound ***4l***. Compound **4l** (17.8 mg, 60%) was synthesized from (−)-chalepin and 2-(hydroxymethyl)pyridine by the general procedure, and the residue was purified by silica gel chromatography (CH_2Cl_2−EtOAc,10:1): ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.43 (6H, s), 1.51 (3H, s), 1.58 (3H, s), 3.19 (2H, m), 5.06 (3H, m), 5.19 (2H, s), 6.13 (1H, dd, J = 17.3, 10.8 Hz), 6.68 (1H, s), 7.16 (1H, s), 7.22 (1H, dd, J = 12.0, 5.5 Hz), 7.33 (1H, d, J = 7.8 Hz), 7.44 (1H, s), 7.68 (1H, t, J = 7.7 Hz), 8.56 (1H, d, J = 4.7 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 20.8, 22.0, 26.3, 30.0, 40.5, 69.6, 84.2, 88.0, 97.5, 112.3, 113.4, 121.8, 123.2, 123.4, 123.9, 131.2, 137.0, 138.2, 145.8, 149.7, 153.2, 154.9, 155.4, 160.3, 162.4; EIMS m/z 450.2 $[M + H]^+$; HREIMS m/z 450.1915 $[M + H]^+$ (calcd for $C_{26}H_{27}NO_6$, 450.1911).

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J. Nat. Prod. 2017, 80, 53−60

*Synthesis of Compound **4m**.* Compound **4m** (16.2 mg, 50%) was synthesized from (−)-chalepin and 5-ethyl-2-pyridine-ethanol by the general procedure, and the residue was purified by silica gel chromatography (CH_2Cl_2−EtOAc, 10:1): ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.23 (3H, t, J = 7.6 Hz), 1.45 (6H, s), 1.47 (3H, s), 1.55 (3H, s), 2.61 (2H, q, J = 7.6 Hz), 3.09 (2H, t, J = 6.9 Hz), 3.20 (2H, m), 4.44 (2H, t, J = 7.0 Hz), 5.06 (3H, m), 6.15 (1H, dd, J = 17.2, 10.9 Hz), 6.69 (1H, s), 7.08 (1H, m), 7.17 (1H, s), 7.42 (1H, dd, J = 7.9, 2.4 Hz), 7.46 (1H, s), 8.36 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 15.5, 20.6, 21.9, 25.8, 26.2, 29.9, 37.1, 40.4, 66.8, 83.6, 87.8, 97.4, 112.2, 113.3, 123.2, 123.3, 123.8, 131.1, 135.9, 137.3, 138.1, 145.7, 149.3, 153.3, 154.8, 154.9, 160.2, 162.4; EIMS m/z 492.2 $[M + H]^+$; HREIMS m/z 492.2380 $[M + H]^+$ (calcd for $C_{29}H_{33}NO_6$, 492.2381).

*Synthesis of Compound **4n**.* Compound **4n** (14.7 mg, 48%) was synthesized from (−)-chalepin and 4-pyridine-ethanol by the general procedure, and the residue was purified by silica gel chromatography (CH_2Cl_2−EtOAc, 10:1): ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.45 (6H, s), 1.48 (3H, s), 1.54 (3H, s), 2.96 (2H, t, J = 6.8 Hz), 3.14 (2H, m), 4.31 (2H, t, J = 6.8 Hz), 5.06 (3H, m), 6.15 (1H, dd, J = 17.4, 10.7 Hz), 6.71 (1H, s), 7.14 (2H, d, J = 6.0 Hz), 7.18 (1H, s), 7.47 (1H, s), 8.52 (2H, d, J = 5.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 20.7, 21.7, 26.2, 29.9, 34.5, 40.4, 66.5, 84.0, 87.8, 97.4, 112.2, 113.3, 123.3, 123.7, 124.4, 131.1, 138.1, 145.7, 146.5, 150.0, 153.1, 154.8, 160.2, 162.3; EIMS m/z 464.2 $[M + H]^+$; HREIMS m/z 464.2068 $[M + H]^+$ (calcd for $C_{27}H_{29}NO_6$, 464.2068).

*Synthesis of Compound **4o**.* Compound **4o** (17.2 mg, 54%) was synthesized from (−)-chalepin and 5-(2-hydroxyethyl)-4-methylthiazole by the general procedure, and the residue was purified by silica gel chromatography (CH_2Cl_2−EtOAc, 10:1): ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.46 (6H, s), 1.51 (3H, s), 1.56 (3H, s), 2.40 (3H, s), 3.11 (2H, t, J = 6.8 Hz), 3.22 (2H, m), 4.23 (2H, t, J = 6.8 Hz), 5.06 (3H, m), 6.16 (1H, dd, J = 17.3, 10.8 Hz), 6.71 (1H, s), 7.19 (1H, s), 7.47 (1H, s), 8.62 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 14.9, 20.7, 21.8, 26.0, 26.3, 29.8, 29.9, 40.5, 66.8, 84.0, 87.9, 97.4, 112.2, 113.3, 123.3, 123.8, 126.6, 131.2, 138.1, 145.7, 150.3, 153.1, 154.9, 160.2, 162.4; EIMS m/z 484.2 $[M + H]^+$; HREIMS m/z 484.1786 $[M + H]^+$ (calcd for $C_{26}H_{29}NO_6S$, 484.1716).

*Synthesis of Compound **4p**.* Compound **4p** (21.1 mg, 60%) was synthesized from (−)-chalepin and *N*-(2-Hydroxyethyl)phthalimide by the general procedure, and the residue was purified by silica gel chromatography (CH_2Cl_2−EtOAc, 20:1): ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.45 (6H, s), 1.47 (3H, s), 1.56 (3H, s), 3.21 (2H, qdd, J = 16.1, 8.6, 1.3 Hz), 3.98 (2H, m), 4.30 (2H, m), 4.54 (3H, m), 6.15 (1H, dd, J = 17.4, 10.8 Hz), 6.68 (1H, s), 7.17 (1H, s), 7.46 (1H, s), 7.72 (2H, dd, J = 5.5, 3.0 Hz), 7.84 (2H, dd, J = 5.5, 3.1 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 20.5, 21.8, 26.2, 29.8, 37.0, 40.4, 64.4, 84.1, 87.9, 97.3, 112.2, 113.2, 123.3, 123.5, 123.9, 131.0, 132.1, 134.2, 138.1, 145.7, 153.1, 154.8, 160.2, 162.4, 168.2; EIMS m/z 532.2 $[M + H]^+$; HREIMS m/z 532.1958 $[M + H]^+$ (calcd for $C_{30}H_{29}NO_8$, 532.1966).

Analysis of Intracellular EBV Genomic DNA Content and Inhibition of EBV DNA Replication. P3HR-1 (clone 16) is an EBV-positive Burkitt's lymphoma cell line, a clonally derived subline of Jiyoye, and obtained from Dr. George Miller Laboratory (Yale University). P3HR-1 cells were cultured in RPMI 1640 medium (Gibco-BRL, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (Gibco-BRL), streptomycin (100 μg/mL), and penicillin (100 units/mL). For viral lytic replication, P3HR-1 cells were induced with TPA (20 ng/mL) and sodium butyrate (0.3 mM). After 3 h, the cells were treated with varied concentrations of the test compounds. Forty-eight hours postinduction, the total DNA in the cells was purified using the DNeasy tissue kit, according to the manufacturer's protocol (Takara Bio. Inc., Shiga, Japan). The EBV genomic copy number was quantified by real-time PCR on a Roche 480 Light Cycler instrument using the Light Cycler Fast Start DNA Master^{plus} SYBR green kit with primers for the detection of EBNA1 (sense: 5′-CATTGAGTCGTCTCCCCTTTGGAAT-3′; antisense: 5′-TCATAACAAGGTCCTTAATCGCATC-3′). The half-maximal antiviral effective concentration IC_{50} value of each compound was determined from a dose−response curve of EBV DNA content values from TPA/butyrate-induced and compound-treated cells. The viral DNA content was reduced by the content of noninduced cells, divided by that from the control cells without drug treatment, and then represented on the y-axes of dose−response curves; y-axis value = $(TPA_x - \text{no } TPA_x)/(TPA_0 - \text{no } TPA_0)$, where x is any concentration of the test compound and 0 represents no test compound treatment. The IC_{50} value for viral DNA synthesis for each compound was calculated with the aid of GraphPad Prism software (San Diego, CA, USA).

Cytotoxicity Assays. The viabilities of P3HR-1 cells after being treated or untreated with compounds were assessed by counting Trypan blue-stained cells 2 days post-treatment using a light microscope. Cell viabilities were defined relative to the control cells (non-drug-treated). Each half-maximal cytotoxic concentration (CC_{50}) was calculated from the dose−response curves with GraphPad Prism software.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b00415.

NMR and MS spectra of the synthesized derivatives and reaction process (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*Tel/Fax: +86-20-39943077. E-mail: guqiong@mail.sysu.edu.cn (Q. Gu).

*E-mail: yuan2@pobox.upenn.edu (Y. Yuan).

*Tel/Fax: +86-20-39943023. E-mail: junxu@biochemomes.com (J. Xu).

ORCID

Qiong Gu: 0000-0001-6011-3697

Jun Xu: 0000-0002-1075-0337

Author Contributions

#Y. Lin and Q. Wang contributed equally.

Notes

The authors declare no competing financial interest.

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