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Research Paper

Effects of piperine, a component of pepper (*Piper nigrum*), on herpes simplex virus type-1 in Vero cells

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ABSTRACT

The fruit of pepper (*Piper nigrum*, Piperaceae) is used as a spice for its pungency and aroma all over the world. Piperine is the major secondary metabolite responsible for the strong pungency. We found that piperine activates herpes simplex virus type-1 (HSV-1) in Vero cells. Specifically, piperine increased both the number and size of plaques formed by HSV-1 replication (175% activation at 100 μM compared with control cells) in a plaque assay. To confirm the effect of piperine on the activation of HSV-1, we synthesized 15 piperine derivatives. The synthesis of the piperine derivatives with an amide moeity [four 5-(3.4-methylenedioxyphenyl)penta-2,4-dienamides, a 5-(3.4-methylenedioxyphenyl)pentanamide, and eight 5-phenylpenta-2,4-dienamides] was accomplished by the condensation reactions of carboxylic acids with various amines in the presence of condensing agents. Next, we conducted plaque assays to examine their effects on HSV-1. Piperine derivatives having an amide moiety increased the number of plaques, suggesting that they activate HSV-1. To the best of our knowledge, this is the first example of a plant component with the ability to activate HSV-1. The mechanism of HSV-1 activation by piperine remains unclear. Since piperine is the major secondary metabolite of pepper (*P. nigrum*), an excessive intake of pepper may have an effect on HSV-1 infection. The detailed mechanistic studies are needed to determine whether piperine affects the virus or the host (i.e., Vero cells).

Key words: pepper, *Piper nigrum*, Piperaceae, piperine, herpes simplex virus type-1, plaque assay, aggravation of HSV-1 infection

1. Introduction

Pepper (*Piper nigrum*, Piperaceae) is widely distributed in tropical and subtropical regions. Its fruit is used as a spice for its pungency and aroma worldwide. The substance responsible for the strong pungency is piperine, an alkaloid known to be present in a few percent of dried fruits. Piperine is also a pungent component of other Piper plants including *P. longum* and *P. retrofractum*.

Herpes simplex virus type-1 (HSV-1) is a well-known human pathogen. The WHO estimates that approximately 3.7 billion people aged < 50 years are infected with HSV-1. HSV-1 infection mainly causes herpes labialis and, in serious cases, herpes simplex encephalitis. Against this backdrop, searching for compounds with anti-HSV-1 activity from among natural medicines and foods has become a focus of our efforts. We examined the activity of piperine isolated from pepper and found that, contrary to our expectations, piperine increases both the number and size of plaques formed by HSV-1 replication in Vero cells, suggesting that it activates HSV-1 (Figure 1). In addition, we synthesized piperine derivatives and conducted plaque assays to examine their effects on the activation of HSV-1. In this report, we describe the effects of piperine and its derivatives on the activation of HSV-1.

2. Material & Methods

2.1. General

IR spectra, Shimadzu FTIR-8100 spectrometer; EIMS and HREIMS, JEOL JMS-GCMATE mass spectrometer; ^{1}H-NMR spectra, JEOL JNM-ECS 400 (400 MHz), JNM-LA 500 (500 MHz), and JNM-ECA 600 (600 MHz,) spectrometer; ^{13}C-NMR spectra, JEOL JNM-ECS 400 (100 MHz), JNM-LA 500 (125 MHz,), and JNM-ECA 600 (150 MHz,) spectrometer; The following experimental materials were used for chromatography: normal phase silica gel column chromatography, silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150-350 mesh); TLC, precoated TLC plates

with Silica gel $60F_{254}$ (Merck, 0.25mm) (ordinary phase). Detection was achieved by UV irradiation.

2.2. Chemicals and Reagents

Reagents: Chemicals were purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan).

2.3. Synthesis of compounds 1 and 7

Compounds **1** (92% from piperine) and **7** (90% from **6**) were obtained by basic hydrolysis under basic condition using KOH to remove the piperidine ring as previously described (Mishra et al., 2005). The structures of **1** and **7** were identified by comparison of ^{1}H-NMR and MS spectra with reported values (Mishra et al., 2005; Reen et al., 1993).

2.4. Synthesis of compound 6

Compound **6** (85% from piperine) was prepared by the same method as described in reference (Venkatasamy et al., 2004).

2.5. Synthesis of compound 9

Malonic acid (416 mg, 4 mmol) was added to a solution of *trans*-cinnamaldehyde (264 mg, 2.0 mmol) in piperidine (3 mL) and pyridine (30 mL). The reaction mixture was heated under reflux for 1 h. After cooling, the reaction mixture was evaporated and neutralized with 5% aqueous HCl. Ethyl acetate (EtOAc) was added to the reaction mixture. The EtOAc layer was separated and evaporated to dryness, and the residue was subjected to normal-phase silica gel column chromatography [*n*-hexane–EtOAc (1:1, v/v)] to give (4E)-5-phenylpenta-2,4-dienoic acid (**9**, 314 mg, 90%) as a mixture of *trans* and *cis* isomers. Compound **9** was used in the subsequent reaction without further purification.

2.6. Synthesis of compounds 10 to 13

1 (100 mg, 0.46 mmol), **7** (100 mg, 0.45 mmol), and **9** (100 mg, 0.57 mmol) in CH_2Cl_2 (5 mL) were treated with amines (1.0 eq.) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl, 1.0 eq.) and 1-hydroxybenzotriazole (HOBt, 1.0 eq.). In each case, the reaction mixture was stirred at room temperature for 12 h, and after evaporating the reaction solvent, EtOAc was added. The EtOAc extract was washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4 powder, filtered, and evaporated to dryness. The obtained residues were subjected to normal-phase silica gel column chromatography [*n*-hexane → *n*-hexane–EtOAc (2:1 → 1:1, v/v)] to give **2** (107 mg, 90% from **1**), **3** (112 mg, 94% from **1**), **4** (116 mg, 92% from **1**), **5** (122 mg, 92% from **1**), **8** (115 mg, 92% from **7**), **10a** (76 mg, 62% from **9**), **10b** (27 mg, 22% from **9**), **11a** (82 mg, 63% from **9**), **11b** (31 mg, 24% from **9**), **12a** (81 mg, 66% from **9**), **12b** (29 mg, 24% from **9**), **13a** (84 mg, 64% from **9**), and **13b** (27 mg, 21% from **9**). The structures of known compounds **2** (Venkatasamy et al., 2004), **3** (Sangwan et al., 2008), **4** (Venkatasamy et al., 2004), **5** (de Paula et al., 2000), **8** (Sangwan et al., 2008), **10a** (Werbel et al., 1697), **11a** (Marques et al., 2010), **12a** (Riedel, 1908), and **13a** (Riedel, 1908) were identified by comparison of ^{1}H-NMR and MS spectra with reported values. The structures of new compounds **10b**, **11b**, **12b**, and **13b** and three known compounds **10a**, **12a**, and **13a** (no NMR data have been reported) were identified by analysis of IR, ^{1}H-NMR, ^{13}C-NMR, and HR-MS spectral data.

2 [5-(3.4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid isopropyl amide): a colorless powder; ^{1}H-NMR ($CDCl_3$) δ 1.20 (6H, d, J = 6.6 Hz, (CH_3)$_2$CH-), 4.20 (1H, m, NHC*H*), 5.31 (1H, br d, J = 8.1, NH), 5.86 (1H, d, J = 14.8 Hz, H-2), 5.97 (2H, s, OCH_2O), 6.67 (1H, dd, J = 10.7, 15.5, H-4), 6.76 (1H, d, J = 7.9, H-5'), 6.77 (1H, d, J = 15.8, H-5), 6.89 (1H, dd, J = 1.7, 7.9, H-6'), 6.98 (1H, d, J = 1.7, H-2'), 7.36 (1H, dd, J = 10.7, 14.8, H-3).

3 [5-(3.4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid propyl amide): a colorless powder; ^{1}H-NMR ($CDCl_3$) δ 0.93 (3H, t, J = 7.4 Hz, CH_3CH$_2$-), 1.58 (2H, m, CH$_3$CH_2-), 3.32 (2H, m, NHCH_2-), 5.49 (1H, m, NHC*H*), 5.90 (1H, d, J = 14.8 Hz, H-2), 5.98 (2H, s, OCH_2O), 6.67 (1H, dd, J = 10.6, 15.4, H-4), 6.76 (1H, d, J = 8.0, H-5'), 6.77 (1H, d, J = 15.4, H-5), 6.89 (1H, dd, J = 1.7, 8.0, H-6'), 6.98 (1H, d, J = 1.7, H-2'), 7.36 (1H, dd, J = 10.6, 14.8, H-3).

4 [5-(3.4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid butyl amide): a colorless powder; ^{1}H-NMR ($CDCl_3$) δ 0.93 (3H, t, J = 7.4 Hz, CH_3CH$_2$-), 1.35 (2H, m, CH$_3$CH_2-), 1.54 (2H, m, CH$_3$CH$_2$CH_2-), 3.36 (2H, q like, J = 7.2 Hz, NHCH_2-), 5.47 (1H, m, NHC*H*), 5.91 (1H, d, J = 14.8 Hz, H-2), 5.98 (2H, s, OCH_2O), 6.67 (1H, dd, J = 10.6, 15.4, H-4), 6.76 (1H, d, J = 8.0, H-5'), 6.77 (1H, d, J = 15.4, H-5), 6.89 (1H, dd, J = 1.7, 8.0, H-6'), 6.98 (1H, d, J = 1.7, H-2'), 7.36 (1H, dd, J = 10.6, 14.8, H-3).

5 [5-(3.4-methylenedioxyphenyl)-2*E*,4*E*-pentadienoic acid pentyl amide): a colorless powder; ^{1}H-NMR ($CDCl_3$) δ 0.90 (3H, t, J = 7.7 Hz, CH_3CH$_2$-), 1.32 (4H, m, CH$_3$CH_2CH_2-), 1.55 (2H, m, CH$_3$CH$_2$CH$_2$CH_2-), 3.36 (2H, m, NHCH_2-), 5.48 (1H, m, NHC*H*), 5.91 (1H, d, J = 14.8 Hz, H-2), 5.98 (2H, s, OCH_2O), 6.67 (1H, dd, J = 10.6, 14.7, H-4), 6.78 (1H, d, J = 8.0, H-5'), 6.79 (1H, d, J = 14.8, H-5), 6.89 (1H, dd, J = 1.7, 8.0, H-6'), 6.98 (1H, d, J = 1.7, H-2'), 7.36 (1H, dd, J = 10.6, 14.8, H-3).

8 [5-(3,4-methylenedioxyphenylpentanoic acid isobutyl amide): a colorless powder; ^{1}H-NMR ($CDCl_3$) δ 0.90 (6H, d, J = 6.7 Hz, (CH_3)$_2$CH-), 2.32 (1H, m, (CH_3)$_2$C*H*-), 3.07 (2H, dd like, J = 6.6, 6.6 Hz, NHCH_2), 2.18 (1H, t, J = 7.4 Hz, H-2), 1.65 (4H, m, H-3, 4), 2.56 (1H, t like, J = 6.9 Hz, H-5), 5.91 (2H, s, OCH_2O), 6.61 (1H, dd like, J = 1.6, 7.9, H-6'), 6.66 (1H, d like, J = 1.6, H-2'), 6.71 (1H, d, J = 7.9, H-5').

10a (5-phenyl-2*E*,4*E*-pentadienoic acid isopropyl amide): a colorless powder; IR(film): ν_{max} 3289, 2975, 1646, 1611, and 1262 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.23 (6H, d, J = 6.5 Hz, (CH_3)$_2$CH-), 4.20 (1H, m, NHC*H*), 5.92 (1H, d, J = 14.9 Hz, H-2), 6.84 (2H, m, H-4,5), 7.34 (4H, m, H-3, 3',4',5'), 7.45 (2H, dd like, J = 1.9, 8.4 Hz, H-2',6'); ^{13}C-NMR ($CDCl_3$) δ 22.9, 41.5, 124.2, 126.4, 127.0, 128.7 x 2, 136.3, 139.0, 140.7, 165.1; HREI-MS: *m/z* 215.1319 (calcd for $C_{14}H_{17}NO$ $[M]^+$, 215.1310).

10b (5-phenyl-2*Z*,4*E*-pentadienoic acid isopropyl amide): a colorless powder; IR(film): ν_{max} 3285, 2973, 1646, 1600, and 1254 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.21 (6H, d, J = 6.5 Hz, (CH_3)$_2$CH-), 4.18 (1H, m, NHC*H*), 5.58 (1H, d, J = 11.1 Hz, H-2), 6.56 (1H, dd, J = 11.1, 11.1 Hz, H-3), 6.72 (1H, d, J = 15.9 Hz, H-5), 7.28 (3H, m, H-3',4',5'), 7.52 (2H, br d like, J = 8.4, H-2',6'), 8.30 (1H, dd like, J = 11.1, 15.9 Hz, H-4); ^{13}C-NMR ($CDCl_3$) δ 22.8, 41.2, 120.5, 125.2, 127.3, 128.5, 128.6, 136.6, 139.5, 141.1, 165.5; HREI-MS: *m/z* 215.1303 (calcd for $C_{14}H_{17}NO$ $[M]^+$, 215.1310).

11a (5-phenyl-2*E*,4*E*-pentadienoic acid isobutyl amide): a colorless powder; ^{1}H-NMR ($CDCl_3$) δ 0.96 (6H, d, J = 6.6 Hz, (CH_3)$_2$CH-), 1.83 (1H, m, (CH_3)$_2$C*H*-), 3.20 (2H, dd, J = 6.2, 6.2 Hz, NHCH_2), 5.96 (1H, d, J = 14.9 Hz, H-2), 6.85 (2H, m, H-4,5), 7.34 (4H, m, H-3,3',4',5'), 7.44 (2H, dd like, J = 1.6, 8.6 Hz, H-2',6').

11b (5-phenyl-2*Z*,4*E*-pentadienoic acid isobutyl amide): a colorless powder; IR(film): ν_{max} 3285, 2961, 1645, 1601, and 1255 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.94 (6H, d, J = 6.8 Hz, (CH_3)$_2$CH-), 1.83 (1H, m, (CH_3)$_2$C*H*-), 3.16 (2H, dd, J = 6.2, 6.2 Hz, NHCH_2), 5.64 (1H, d, J = 11.0 Hz, H-2), 6.57 (1H, dd, J = 11.0, 11.0 Hz, H-3), 6.72 (1H, d, J = 16.0 Hz, H-5), 7.28 (3H, m, H-3',4',5'), 7.52 (2H, br d like, J = 7.3, H-2',6'), 8.30 (1H, dd, J = 11.0, 16.0 Hz, H-4); ^{13}C-NMR ($CDCl_3$) δ 20.1, 28.6, 46.7, 120.3, 125.3, 127.3, 128.5, 128.6, 136.6, 139.5, 141.2, 166.4; HREI-MS: *m/z* 229.1473 (calcd for $C_{15}H_{19}NO$ $[M]^+$, 229.1467).

12a (5-phenyl-2*E*,4*E*-pentadienoic acid propyl amide): a colorless powder; IR(film): ν_{max} 3289, 2961, 1646, 1614, 1262 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.96 (3H, t, J = 7.3 Hz, CH_3CH$_2$-), 1.58 (2H, td, J = 7.3, 7.3 Hz, CH$_3$CH_2-), 3.33 (2H, td, J = 7.3, 7.3 Hz, NHCH_2), 5.96 (1H, d, J = 14.9 Hz, H-2), 6.85 (2H, m, H-4,5), 7.34 (4H, m, H-3,3',4',5'), 7.44 (2H, dd like, J = 1.9, 7.8 Hz, H-2',6'); ^{13}C-NMR ($CDCl_3$) δ 11.4, 23.0, 41.4, 124.0, 126.4, 127.0, 128.7, 128.8, 136.3, 139.1, 140.8, 166.0; HREI-MS: *m/z* 215.1310 (calcd for $C_{14}H_{17}NO$ $[M]^+$, 215.1310).

12b (5-phenyl-2*Z*,4*E*-pentadienoic acid propyl amide): a colorless powder; IR(film): ν_{max} 3291, 2965, 1644, 1601, 1256 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.96 (3H, t, J = 7.3 Hz, CH_3CH$_2$-), 1.58 (2H, td, J = 7.3, 7.3 Hz, CH$_3$CH_2-), 3.31 (2H, td, J = 7.3, 7.3 Hz, NHCH_2), 5.62 (1H, d, J = 11.1 Hz, H-2), 6.57 (1H, dd, J = 11.1, 11.1 Hz, H-3), 6.73 (1H, d, J = 15.9 Hz, H-5), 7.28 (3H, m, H-3',4',5'), 7.52 (2H, d like, J = 7.8, H-2',6'), 8.30 (1H, dd, J = 11.1, 15.9 Hz, H-4); ^{13}C-NMR ($CDCl_3$) δ 11.5, 23.0, 41.2, 120.3, 125.3, 127.4, 128.6 x 2, 136.6, 139.6, 141.2, 166.4; HREI-MS: *m/z* 215.1310 (calcd for $C_{14}H_{17}NO$ $[M]^+$, 215.1310).

13a (5-phenyl-2*E*,4*E*-pentadienoic acid butyl amide): a colorless powder; IR(film): ν_{max} 3289, 2957, 1646, 1617, 1265 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.94 (3H, t, J = 7.0 Hz, CH_3CH$_2$-), 1.37, 1.54 (2H each, both m, CH$_3$CH_2CH_2-), 3.33 (2H, td, J = 7.0, 5.9 Hz, NHCH_2), 5.95 (1H, d, J = 14.9 Hz, H-2), 6.84 (2H, m, H-4,5), 7.34 (4H, m, H-3,3',4',5'), 7.45 (2H, dd like, J = 1.6, 8.4 Hz, H-2',6'); ^{13}C-NMR ($CDCl_3$) δ 13.8, 20.1, 31.8, 39.4, 124.0, 126.3, 127.0, 128.7, 128.8, 136.3, 139.1, 140.8, 166.0; HREI-MS: *m/z* 229.1459 (calcd for $C_{15}H_{19}NO$ $[M]^+$, 229.1467).

13b (5-phenyl-2*Z*,4*E*-pentadienoic acid butyl amide): a colorless powder; IR(film): ν_{max} 3293, 2959, 1645, 1601, 1252 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.94 (3H, t, J = 7.0 Hz, CH_3CH$_2$-), 1.37, 1.55 (2H each, both m, CH$_3$CH_2CH_2-), 3.33 (2H, td, J = 6.8, 5.9 Hz, NHCH_2), 5.61 (1H, d, J = 11.0 Hz, H-2), 6.57 (1H, dd, J = 11.0, 11.0 Hz, H-3), 6.73 (1H, d, J = 15.9 Hz, H-5), 7.28 (3H, m, H-3',4',5'), 7.52 (2H, br d like, J = 7.8, H-2',6'), 8.30 (1H, dd, J = 11.0, 15.9 Hz, H-4); ^{13}C-NMR ($CDCl_3$) δ 13.8, 20.2, 31.8, 39.2, 120.3, 125.3, 127.4, 128.6 x 2, 136.6, 139.6, 141.2, 166.4; HREI-MS: *m/z* 229.1460 (calcd for $C_{15}H_{19}NO$ $[M]^+$, 229.1467).

2.7. Protocol for plaque assay

The effects against HSV-1 (HF strain) were evaluated with the plaque assay with some modifications as previous reports (Ogawa et al., 2018). Vero cells were maintained in DMEM medium supplemented with 10% fetal calf serum. Vero cells (1.7–2.8 × 10^5 cells/well) were seeded onto a 12-well tissue culture plate and precultured for 1-2 days. The cells were inoculated with 100 plaque forming units (PFU) of HSV-1 in FBS-free DMEM (0.1 mL). The inoculum was removed after 30 minutes. HSV-infected cells were maintained in DMEM which contains 10% FBS, methylcellulose, and serially diluted piperine and synthetic compounds. The medium was removed after 48 h incubation at 37 °C. The cell sheets were stained with 1% crystal violet dissolved in 50% methanol, then the number of plaques with larger than 0.4 mm was counted. HSV-1 replication causes cytopathic effects resulting in plaque formation. The number and size of plaques reflecting the virus yields. To examine the effect of virus yield, the total plaque number in compound-untreated cells was defined as 100% and set as control group. Standard deviations were determined by analyzing the data from three experiments and are indicated by error bars.

Significant difference from the control group was calculated by the Dunnett test. Probability (p) values less than 0.05 were considered to be significant ($^{*}p < 0.05$, $^{**}p < 0.01$).

3. Results

Our group has conducted studies to identify natural compounds with anti-HSV-1 activity. We previously reported that several triterpenes and dolabellane diterpenes have anti-HSV-1 activity (Ogawa et al., 2018). During the course of characterization of anti-HSV-1 constituents from natural medicines and foods, we evaluated the effects of piperine, the major secondary metabolite of pepper (*P. nigrum*, fruit), on HSV-1 (HF strain) in Vero cells using a plaque assay, a method for quantifying virus titer. When cultured cells (i.e., Vero cells) are infected with a virus (i.e., HSV-1), cytopathic effects due to viral replication cause changes in cell shape, leading to plaque formation. The number and size of plaques, which can be detected visually, reflect the virus titer. To examine the effect of virus titer, the total number of plaques in untreated cells (control group) was defined as 100%. Surprisingly, piperine had no anti-HSV-1 effect; rather, it increased the number of plaques formed by HSV-1 replication in Vero cells (175% activation at 100 μM compared with the control group) and also increased the plaque size, suggesting that it activated HSV-1 (Figure 1).

To confirm the effect of piperine on the activation of HSV-1, we synthesized 15 piperine derivatives (Scheme 1). First, carboxylic acid **1** was derived from piperine by

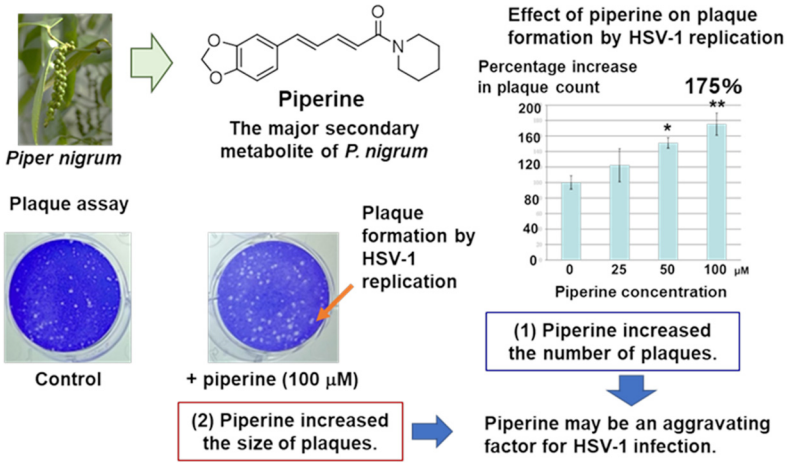
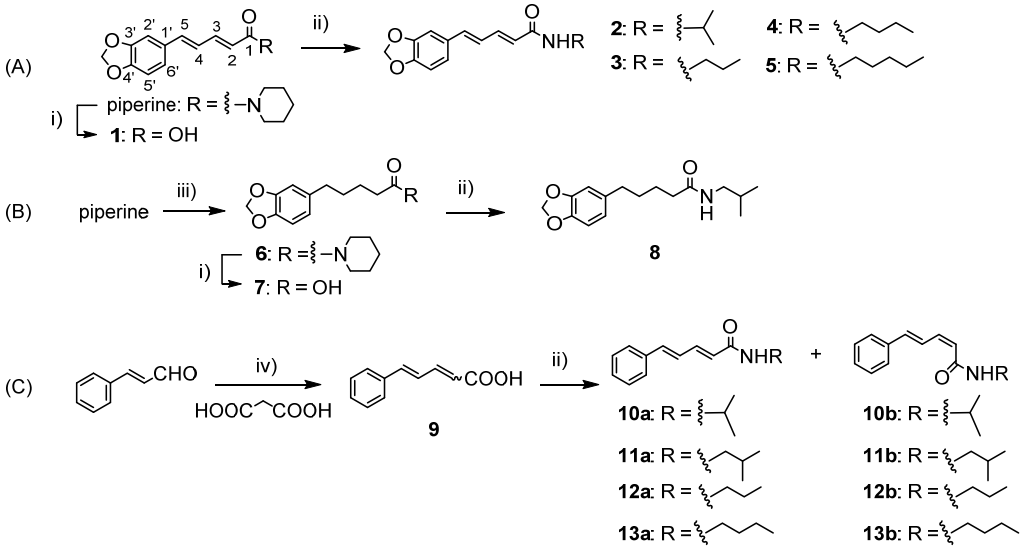


Figure 1. Effects of piperine on the number and size of plaques formed by HSV-1 replication in Vero cells.



Scheme 1: i) KOH, EtOH (**1**: 92%., **7**: 90%); ii) amine, EDC, HOBt (**2**: 90%, **3**: 94%, **4**: 92%, **5**: 92%, **8**: 92%, **10a**: 62%, **10b**: 22%, **11a**: 63%, **11b**: 24%, **12a**: 66%, **12b**: 24%, **13a**: 64%, **13b**: 21%); iii) H_2, Pd/C, CH_2Cl_2 (**6**: 85%); (iv) piperidine, pyridine (**9**: 90%).

Scheme 1. Synthesis of piperine derivatives.

Table 1. Effects of piperine and its derivatives on plaque formation by HSV-1 replication in Vero cells (plaque assay).

Compound	Percentage increase in plaque count (%) [a]			
	0 μM	25 μM	50 μM	100 μM
Piperine	100.0 ± 8.8	122.4 ± 21.4	151.3 ± 6.8*	175.4 ± 14.3**
2	100.0 ± 21.6	152.1 ± 16.0*	143.7 ± 18.8	213.4 ± 3.0**
3	100.0 ± 5.0	143.4 ± 6.9**	154.5 ± 10.8**	178.3 ± 5.4**
4	100.0 ± 28.6	159.7 ±19.7	198.5 ± 7.06*	205.4 ± 19.9**
5	100.0 ± 11.3	190.5 ± 4.4**	207.9 ± 13.0**	202.8 ± 5.9**
6	100.0 ± 11.9	134.4 ± 13.1*	144.2 ± 11.8*	174.9 ± 3.2**
7	100.0 ± 19.2	99.6 ± 8.6	111.5 ± 9.6	116.7 ± 4.0
8	100.0 ± 14.3	147.8 ± 6.7	161.6 ± 21.9*	187.0 ± 8.1**
10a	100.0 ± 3.7	114.5 ± 11.8	121.1 ± 13.8	119.4 ± 11.4
10b	100.0 ± 12.4	99.6 ± 9.1	107.1 ± 7.2	110.6 ± 7.9
11a	100.0 ± 12.5	146.4 ± 15.4*	137.1 ± 7.9	137.8 ± 16.5
11b	100.0 ± 20.6	146.1 ± 26.8	139.9 ± 26.0	116.6 ± 19.4
12a	100.0 ± 5.6	86.7 ± 12.2	95.6 ± 4.0	124.1 ± 10.3*
12b	100.0 ± 6.6	113.0 ± 12.9	131.6 ± 10.4*	130.5 ± 8.1*
13a	100.0 ± 8.5	116.6 ± 11.9	168.3 ± 14.0**	200.5 ± 4.4**
13b	100.0 ± 23.4	170.1 ± 13.4*	217.9 ± 14.5**	226.6 ± 11.9**

[a]: Significantly different from the control group, *$p < 0.05$, **$p < 0.01$.

hydrolysis under basic condition. Second, **6** was obtained by the catalytic reduction of piperine and then hydrolyzed to give **7**. Third, 5-phenylpenta-2,4-dienoic acid **9** as a mixture of *trans* and *cis* isomers was obtained by Knoevenagel condensation of cinnamaldehyde and malonic acid. Finally, the condensation reactions of carboxylic acids **1**, **7**, and **9** with various amines using EDC·HCl afforded 5-(3.4-methylenedioxyphenyl)penta-2,4-dienamides **2**–**5**, 5-(3.4-methylenedioxyphenyl)pentanamide **8**, and 5-phenylpenta-2,4-dienamides **10**–**13** with *cis*/*trans* isomers at 2-position, respectively. The synthesized compounds were evaluated by the plaque assay to examine their effects on HSV-1 (HF strain) in Vero cells (Table 1). Compounds **2**, **4**, **5**, **13a**, and **13b** significantly increased the number of plaques formed by HSV-1 replication by > 200% at 100 μM compared with the control group [percentage increase in plaque count (%): 213.4 ± 3.0 (**2**), 205.4 ± 19.9 (**4**), 202.8 ± 5.9 (**5**), 200.5 ± 4.4 (**13a**), and 226.6 ± 11.9 (**13b**)]. These results show that piperine derivatives, which have an amide moiety, can activate HSV-1 in Vero cells.

4. Conclusion

The results of the plaque assay suggested that piperine activates HSV-1 in Vero cells. Moreover, the piperine derivatives having an amide moiety were also shown to activate HSV-1. To the best of our knowledge, this is the first example of a plant component that has the ability to activate HSV-1. The mechanism of HSV-1 activation by piperine remains unclear. Since piperine is the major secondary metabolite of pepper (*P. nigrum*), an excessive intake of pepper may have an effect on HSV-1 infection. The detailed mechanistic studies are needed to determine whether piperine affects the virus or the host (i.e., Vero cells).

Conflict of Interest

The authors declare no conflict of interest.

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