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Antiviral activities against herpes simplex virus type 1 by HPH derivatives and their structure–activity relationships

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Abstract—The compound named Histidine–pyridine–histidine (HPH) is an oxygen-activating ligand derived from the structure of bleomycin. We synthesized HPH derivatives, namely HPH-1 to -8, and investigated their antiviral activities against herpes simplex virus type 1. HPH-8 showed potent antiviral activity with an EC_{50} of 15 μM, and relatively high cytotoxicity with a CC_{50} of 37 μM. In contrast, HPH-4 indicated a weaker antiviral activity with an EC_{50} of 79 μM, but exhibited a far more less cytotoxicity (CC_{50} 500 μM). Other HPH derivatives showed no effects against antiviral activities and cytotoxicities.

A high percentage of the world's population are infected with herpes simplex virus type 1 (HSV-1), and infection can cause a wide range of diseases and symptoms such as ulcerative mucocutaneous diseases, severe complications in HIV infected persons, and herpetic encephalitis.[1–4] In addition to acute infections, HSV establish life-long latent infections and resistance to anti-virus drugs, and these two characters have been major clinical problem.[5] Development of a new class of compounds with potent antiviral activity is important not only in the development of therapeutic agents against various viral diseases, but also in the study of the mechanisms of antiviral effects because it could lead to the identification of novel molecular targets which affect viral replication cycles. The anti-virus agent with a different mechanism of action could offer an additional strategy against drug resistance of viruses.

Histidine–pyridine–histidine (HPH) derivatives are oxygen-activating ligands derived from the structure of bleomycin, an antibiotic used as a chemotherapeutical drug against many kinds of cancer.[6] HPH derivatives have been shown to inhibit the activity of farnesyltransferase, which catalyzes the transfer of a farnesyl group onto a conserved cysteine residue of a number of proteins, and to induce apoptosis in human pancreatic adenocarcinoma cells and mouse leukemia cells.[7–10] These biological activities of HPH derivatives have been thought to be dependent on their metal-chelating action. Bergeron et al. reported that metal-chelating agents inhibit the replication of HSV-1 in monkey kidney cells.[11]

In this short communication, we show anti-HSV-1 activities of HPH derivatives (HPH-4 and -8) and discuss their structure–activity relationships.

The compounds were synthesized from 2,6-pyridine dicarboxylic acid (**1**) according to the procedures outlined in Scheme 1. Transformation of the amide to the thioamide was effected by treatment with Lawesson's reagent. 2-*tert*-Butyl sulfenylethylamine hydrochloride was prepared according to Tombeux's method.[12] The structures of HPH derivatives[13] used in this study are shown in Figure 1. The compounds **3**[14] and **4**[15] correspond with HPH-4 and HPH-8, respectively.

Keywords: Histidine–pyridine–histidine; HPH; Antiviral activity; Herpes simplex virus type 1; Structure–activity relationship.
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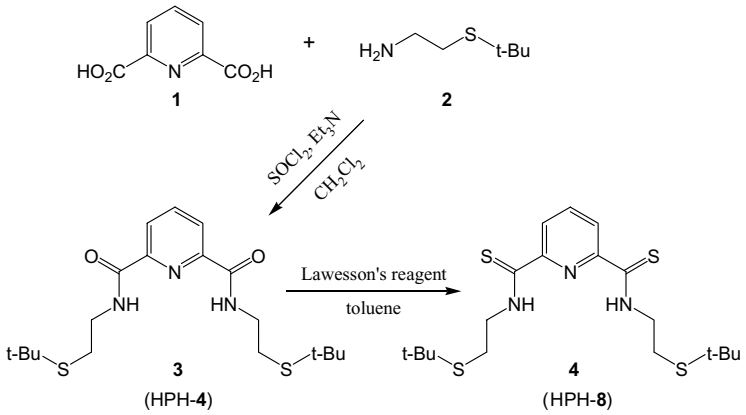

Scheme 1.

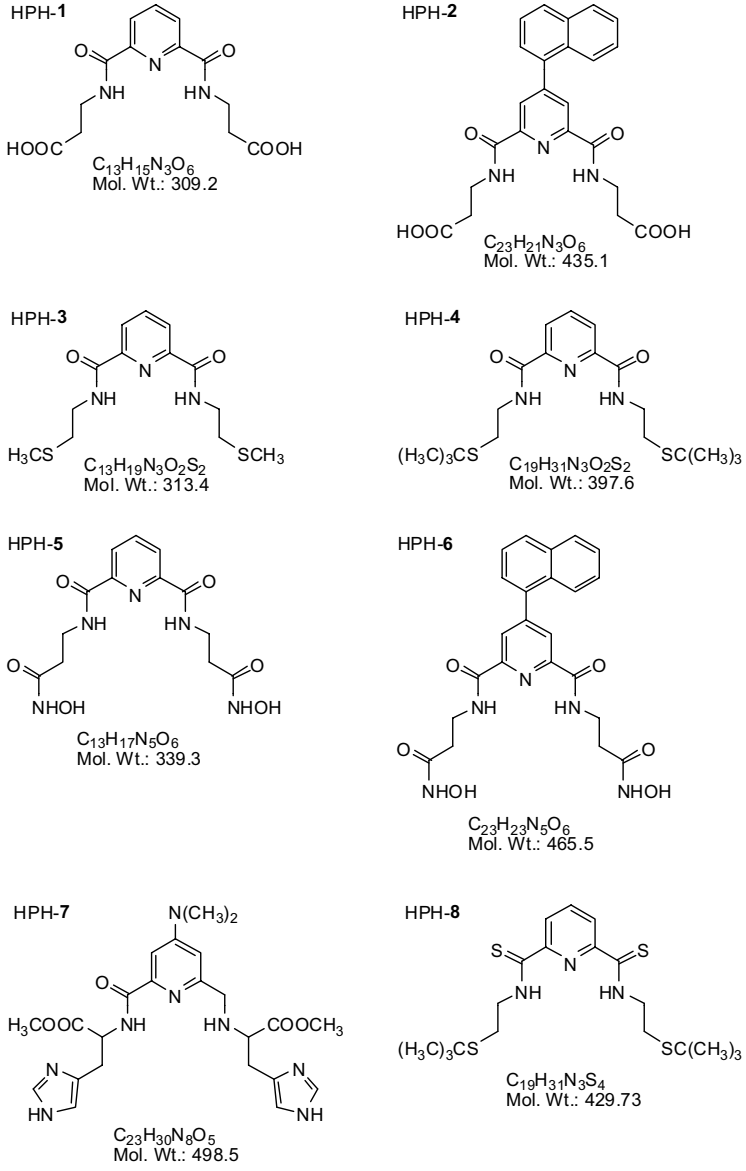

Figure 1. The structure of HPH derivatives.

The antiviral activities of the compounds, shown in Figure 1, were measured by the plaque reduction assay.[16] Calculated EC_{50} values for HPH derivatives in the tested compounds are summarized in Table 1. As shown in Ta-

Table 1. Antiviral activities and cytotoxicities of HPH derivatives

HPH	Antiviral activity Inhibition (EC_{50}, μM)	Cytotoxicity Inhibition (CC_{50}, μM)
HPH-1	>100	>100
HPH-2	>100	>100
HPH-3	>100	>100
HPH-4	79	>100
HPH-5	>100	>100
HPH-6	>100	>100
HPH-7	>100	>100
HPH-8	15	37
Acyclovir[a]	6	>100

[a] Standard as antiviral reagent.

ble 1, HPH-8 showed potent antiviral activity against HSV-1 with an EC_{50} of 15 μM, the value is almost of the same order as in the standard antiviral drug, acyclovir. HPH-4 showed weaker inhibition, and other HPH derivatives showed no activities. Within HPH derivatives tested, only two compounds, HPH-4 and HPH-8, with antiviral activity, have $SC(CH_3)_3$ residues. These results suggest that $SC(CH_3)_3$ residue is important for antiviral activity.

The cytotoxicities of the compounds, shown in Figure 1, were examined by Alamar blue assay.[17] Calculated cytotoxicity (CC_{50}) values for the tested compounds are summarized in Table 1. As shown in Table 1, HPH-8 indicated the obvious cytotoxicity with a CC_{50} of 37 μM. Other HPH derivatives showed no cytotoxicities against Vero cells. HPH-4 showed cytotoxicity with a CC_{50} of 500 μM (data not shown). These results indicated that antiviral coefficient (CC_{50}/EC_{50}) of HPH-4 and HPH-8 is 6.3 and 2.5, respectively. The structures of these HPH derivatives are almost same except for the peptide bonds, an oxygen for HPH-4 and a sulfur for HPH-8. The difference in cytotoxicity between HPH-4 and HPH-8 could be caused by structural difference in peptide bonds.

We have shown a potential of HPH derivatives as a novel anti-HSV-1 agent. It has been reported that HPH derivatives inhibit the farnesyltransferase activity and induce apoptosis in human pancreatic adenocarcinoma AsPC-1 cells and mouse leukemia cells by metal-chelating action.[7–10] The exact mechanism of HPH-4 and HPH-8 to inhibit HSV-1 replication is not evident at present, however, their effect as a chelating agent might be one explanation. Bergeron et al. have reported that several metal-chelators inhibited HSV-1 replication, and these effects were fully prevented by exogenous Fe ion.[11] In addition, the Log *P* value of HPH-4 and HPH-8 is 2.7 and 4.4, respectively, and is higher than those of other HPH derivatives (data not shown). It is not clear whether the anti-HSV-1 activity and high Log *P* value are related. To clarify the anti-HSV-1 mechanism of HPH-4 and HPH-8, further investigation is needed.

In HPH derivatives tested, we could detect anti-HSV-1 activity only in HPH-4 and HPH-8. These two compounds commonly have $SC(CH_3)_3$ residues and have different peptide bonds. To exert anti-HSV activity, $SC(CH_3)_3$ residues seem to be critical, and a pair of their peptide bonds might have enhancement effect. To screen the compounds with higher anti-HSV-1 activity and lower cytotoxicity, HPH-4 and HPH-8 could be candidates as lead compounds.

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13. Synthesis of HPHs will be published elsewhere. Analytical or spectral data for HPHs are as follows. HPH-1, MS (EI) *m/z* 309 (M^+); Anal. Calcd for $C_{13}H_{15}N_3O_6{\cdot}H_2O$: C 47.71, H 5.24, N 12.84; found: C 47.64, H 5.00, N 12.96. HPH-2, MS (EI) *m/z* 435 (M^+). HPH-3, MS (FAB) *m/z* 314 (MH^+); Anal. Calcd for $C_{13}H_{19}N_3O_2S_2$: C 49.81, H 6.11, N 13.41; found: C 49.88, H 6.19, N 13.30. HPH-5, MS (FAB) *m/z* 340 (MH^+). HPH-6, MS (FAB) *m/z* 466 (MH^+); Anal. Calcd for $C_{23}H_{23}N_5O_6$: C 59.35, H 4.98, N 15.05; found: C 59.33, H 4.75, N 15.04. HPH-7, see Matsumoto, M.; Okuno, Y.; Ogata, Y.; Kurosaki, H.; Okamoto, Y.; Otsuka, M. *Heterocycles* **2006**, *69*, 311.
14. *Experimental procedure for the preparation of 2,6-bis(N-(2-tert-butylsulfenylethyl)carbamoyl)pyridine* (**3**, *HPH-4*): To a stirred solution of 2,6-pyridine dicarboxylic acid (167 mg, 1 mmol) (**1**), triethylamine (0.70 mL, 5 mmol), and thionyl chloride (0.18 mL, 2.5 mmol) in dist dichloromethane (5 mL) was added dropwise a solution of 2-*tert*-butylsulfenylethylamine hydrochloride (339 mg, 2.5 mmol) (**2**) and triethylamine (0.30 mL, 2 mmol) in dist dichloromethane (10 mL). The reaction mixture was stirred at room temperature for 2 h water was added, and then extracted with dichloromethane 3 times. The organic layer was dried over magnesium sulfate. The solvent was evaporated and the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 1:1). Yield: 219 mg (55%); ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 18H), 2.80 (t, J = 6.8, 4H), 3.65 (q, J = 6.8, 4H), 8.04 (t, J = 7.9, 1H), 8.36 (d, J = 7.9, 2H), 8.55 (t, J = 6.8, 2H); ^{13}C NMR ($CDCl_3$) δ 28.21 (CH_2), 30.95 (CH_3), 39.73 (CH_2), 42.40 (C), 124.77 (CH), 138.90 (CH), 148.55 (C), 163.47 (C); MS (FAB) *m/z* 398 (MH^+); IR (KBr): 3300, 1640; Anal. Calcd for $C_{19}H_{31}N_3O_2S_2$: C 57.40, H 7.86, N 10.57; found: C 57.17, H 7.96, N 10.44.

15. *Experimental procedure for the preparation of 2,6-bis(N-(2-tert-butylsulfenylethyl)thiocarbamoyl)pyridine* (**4**, *HPH-8*): 2,6-Bis(*N*-(2-*tert*-butylsulfenylethyl)carbamoyl)pyridine (199 mg, 0.5 mmol) (**3**) and Lawesson's reagent (404 mg, 1 mmol) were suspended in dry toluene (10 mL). The reaction mixture was stirred at 80 °C for 2 h. After removal of toluene, the residue was dissolved in chloroform and washed with brine once. The organic layer was dried over sodium sulfate. Chloroform was evaporated and the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 5:1 to 2:1). Yield: 210 mg (98%); ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 18H), 3.00 (t, J = 6.8, 4H), 4.09 (q, J = 6.8, 4H), 7.95 (t, J = 7.9, 1H), 8.36 (d, J = 7.9, 2H), 10.12 (t, J = 6.8, 2H); ^{13}C NMR ($CDCl_3$) δ 26.84 (CH_2), 31.14 (CH_3), 43.03 (C), 45.35 (CH_2), 127.21 (CH), 138.33 (CH), 148.78 (C), 190.08 (C); MS (FAB) *m*/*z* 430 (MH^+); Anal. Calcd for $C_{19}H_{31}N_3S_4$: C 53.10, H 7.27, N 9.78; found: C 52.82, H 7.24, N 9.56.
16. *Plaque reduction assay.* Confluent monolayers of Vero cells (*ca.* 1×10^6 cells/well) in 6-well plastic plates were infected with 100 PFU of HSV-1 (KOS strain). After a 1 h adsorption period at 37 °C, the cultures were overlaid with 2 mL of Dulbecco's modified Eagle's minimum essential medium (DMEM) containing 2% heat-inactivated fetal calf serum (FCS), 2% γ-globulin, and various concentrations of the target compounds. The cultures infected with HSV-1 were incubated in a CO_2 incubator, fixed with formalin, and stained with crystal violet in methanol at 3 days after infection. After washes with water and drying, the plaques were counted.
17. *Alamar blue assay.* Confluent monolayers of Vero cells were seeded in 96-well plastic plates at 5×10^4 cells per well. After 1 day, the cells were re-fed with 100 μL of DMEM containing 5% FCS and various concentrations of the target compounds. After 69-h incubation, 10 μL of Alamar blue reagent (Cosmo Bio Co., Ltd, Carlsbad, CA) was added to each culture, then the plates were reincubated for 4 h. The optical density of each culture at 570 nm was determined by spectrophotometer using a reference wavelength of 630 nm.