In Vitro Anti-influenza Viral Activities of Stilbenoids from the Lianas of *Gnetum pendulum*

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Abstract

The anti-influenza viral activities of six stilbenoids from the lianas of *Gnetum pendulum* C.Y. Cheng were evaluated with two different assays, neuraminidase (NA) activity assay and cytopathic effect (CPE) reduction assay. The NA assay results showed that all six stilbenoids exerted an NA inhibitory effect, while the CPE assay indicated that among them, isorhapontigenin (**2**), gnetupendin B (**3**), shegansu B (**4**), and gnetin D (**6**) exhibit significant *in vitro* anti-influenza viral activity in MDCK cells, with IC_{50} values from 0.67 to 11.99 µg/mL in comparison to the positive controls oseltamivir acid and ribavirin whose IC_{50} values were 0.040 and 5.54 µg/mL, respectively.

Key words

Gnetum pendulum · Gnetaceae · influenza virus · neuraminidase · stilbenoid

Abbreviations

NA:	neuraminidase
CPE:	cytopathic effect
MDCK:	Madin-Darby canine kidney

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

Influenza is an infectious disease, which is responsible for 500 million cases and 0.25–0.5 million deaths worldwide annually, while in pandemic influenza years, still more people will be killed [1]. However, there are only two classes of drugs with elucidated action mechanisms which are used for influenza treatment. One of them is the M2 blockers, such as amantadine and rimantadine, which are only effective on influenza A virus. This kind of drug is of limited use because of its side effects and especially rapid emergence of resistant virus strains during treatment [2]. The other class of drugs are neuraminidase (NA) inhibitors, which are effective on both influenza A and B viruses. NA inhibitors, such as zanamivir and oseltamivir, have less adverse effects, and their usefulness in the clinical treatment of influenza has been reported [3]. Oseltamivir is the suggested drug for influenza treatment and prevention in many countries [4]. However, the use of oseltamivir is also limited because of the shortage of the raw material anise for manufacturing the drug [5]. In addition, more and more drug-resistance events related to oseltamivir were reported although much less often than in the case of M2 inhibitors [6]. Therefore, new drug research and development for influenza treatment and prevention have become very urgent and important.

NA is one of the two glycoproteins on the surface of the influenza virus, which takes charge of catalyzing the cleavage of neuraminic acid residues to facilitate the movement of the virus to and from sites of infection in the respiratory tract. Because of the importance of this enzyme in the pathogenesis of influenza virus infection and the close correspondence of the conserved residues of the active sites from NAs of all influenza A and B viruses, the enzyme is still regarded as an attractive drug target for new drug development [2, 7].

Stilbenoids, especially oligostilbenoids, have attracted much attention for their various biological properties, including antifungal and antimicrobial [8, 9], antiproliferative [10], anticancer [11], anti-inflammatory [12, 13], protein kinase C inhibitory [14], hepatoprotective and hepatotoxic [15], as well as anti-HIV activities [16]. Nevertheless, anti-influenza viral activities have not been reported.

In this study, six stilbenoids (**1**–**6**), resveratrol, isorhapontigenin, shegansu B, gnetupendin B, gnetulin, and gnetin D (**Fig. 1**), from the lianas of *Gnetum pendulum* (Gnetaceae) [17, 18], were evaluated by NA activity assay. They all exerted inhibitory effects on three influenza virus NAs with IC_{50} values ranging from 5.0 to 26.3 µg/mL (**Table 1**).

Their *in vitro* anti-influenza virus activities were also evaluated with the influenza virus A/Guangdong/243/72 (H3N2)-induced CPE reduction assay in MDCK cells (**Table 2**). The CPE assay results showed that the antiviral activities of resveratrol (**1**) and gnetulin (**5**) were less than 50% at their maximal noncytotoxic concentrations (MNCC), while isorhapontigenin (**2**), gnetupendin B (**3**), shegansu B (**4**), and gnetin D (**6**) obviously exhibited anti-

Table 1 Inhibitory effects of the stilbenoids from the lianas of *Gnetum pendulum* on three influenza virus NAs.

Compound	IC_{50} (µg/mL)[a,b,c]		
	A/PR/8/34 (H1N1)	A/Guangdong/243/72 (H3N2)	B/Jiangsu/10/2003
Resveratrol (**1**)	18.2 ± 2.2	14.8 ± 0.6	20.2 ± 1.0
Isorhapontigenin (**2**)	9.2 ± 1.2	16.4 ± 0.4	15.4 ± 0.5
Gnetupendin B (**3**)	5.0 ± 0.4	9.4 ± 2.0	17.1 ± 0.5
Shegansu B (**4**)	16.5 ± 0.5	20.8 ± 0.9	25.6 ± 1.1
Gnetulin (**5**)	18.6 ± 0.1	21.2 ± 0.6	18.0 ± 0.5
Gnetin D (**6**)	14.2 ± 2.3	18.0 ± 1.5	26.3 ± 0.8
Oseltamivir acid	0.0010 ± 0.0002	0.00047 ± 0.00042	0.0039 ± 0.0022

[a] Average of four determinations; [b] five concentrations 40, 8, 1.6, 0.32, 0.064 µg/mL of compounds from the plant were tested, and six concentrations 10, 1, 0.1, 0.01, 0.001, 0.0001 µg/mL of oseltamivir acid were tested; [c] IC_{50} = average ± SD

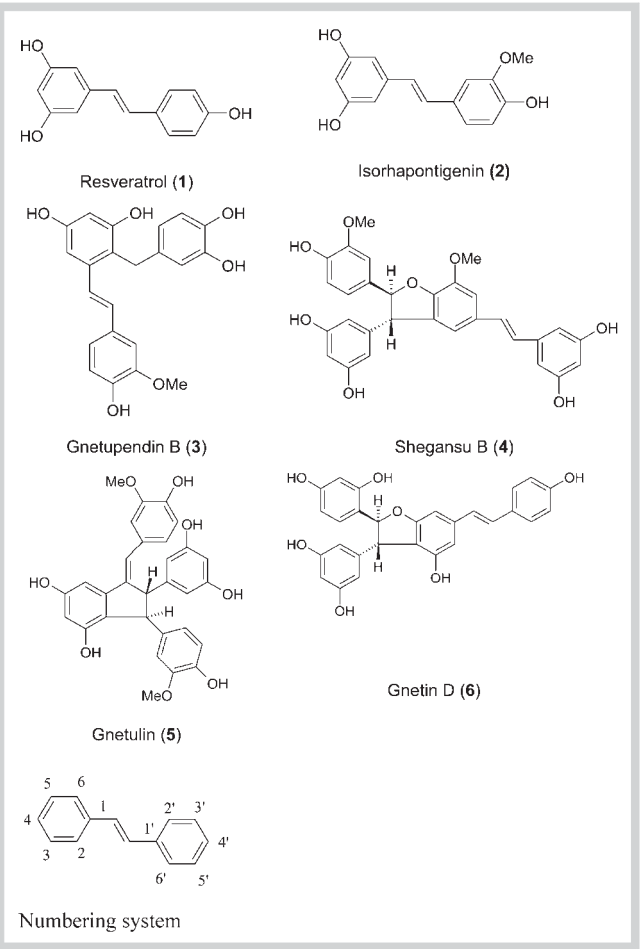

Fig. 1 Chemical structures of the six stilbenoids from the lianas of *Gnetum pendulum* [17, 18].

influenza virus activities. However, the activities of gnetupendin B (**3**) and shegansu B (**4**) were weaker than those of ribavirin and oseltamivir acid, while gnetin D (**6**) exhibited the highest activity, with an IC_{50} value approximately eight times lower than that of ribavirin and approximately seventeen times higher than that of oseltamivir acid.

Obviously, different scaffolds and substituents result in different activities. Isorhapontigenin (**2**) displayed much higher activity than resveratrol (**1**) because of the presence of 3′-OMe which suggested that a hydrophobic group at the C3′ position may significantly increase the antiviral activity. Interestingly, although shegansu B (**4**), gnetulin (**5**), and gnetin D (**6**) are all stilbene dimers, their *in vitro* anti-influenza viral activities were very different. The comparison of the structures and activities of these stilbene dimers suggested that gnetin D (**6**) may have the most favorable scaffold for anti-influenza viral activity. Moreover, the scaffolds of these stilbenoids are novel compared with NA inhibitors found in the drug market. These findings will provide important information for new drug design with higher NA inhibitory activities and anti-influenza viral effects.

In addition, our findings showed for the first time that the six stilbenoids from the lianas of *G. pendulum* possess different anti-influenza virus activities *in vitro*, and NA inhibition is obviously one of the major mechanisms of action against the influenza virus. Isorhapontigenin (**2**), gnetupendin B (**3**), shegansu B (**4**), and gnetin D (**6**) possess remarkable *in vitro* anti-influenza virus activity (**Table 2**), so we can presume that the traditional and clinical therapeutic effects of the lianas of *G. pendulum* against influenza viral infection of the respiratory tract are probably attributable to these constituents and/or other structurally related unknown compounds present in the plant.

Materials and Methods

Influenza viruses A/PR/8/34 (H1N1) and B/Jiangsu/10/2003 were kindly donated by the China Center for Disease Control. Influenza virus A/Guangdong/243/72 (H3N2) was provided by the Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences. MDCK cells were used for viral replication.

The stilbenoids in this paper were isolated by Professor M. Lin, Institute of Materia Medica, Chinese Academy of Medical Sciences. Their isolation and identification have been reported in the literature. The purity of the compounds ranged from 95% to 98% as determined by analytical HPLC and ^{1}H NMR spectral data [17, 18]. The properties of these stilbenoids from the lianas of *G. pendulum* were tested and verified by NA activity and CPE reduction assays, described in Supporting Information.

Supporting information

Details on the NA activity assay and CPE reduction assay are available as Supporting Information.

Table 2 *In vitro* anti-influenza virus activities of the six NA inhibitors against influenza virus A/Guangdong/243/72 (H3N2) in MDCK cells using the CPE reduction assay.

Compound [a]	CC_{50} (μg/mL)[b]	MNCC (μg/mL)[c]	IC_{50} (μg/mL)[d]	SI[e]
Resveratrol (**1**)	66.67	22.22	> 22.22	ND[f]
Isorhapontigenin (**2**)	38.49	22.22	4.28	8.99
Gnetupendin B (**3**)	200.00	66.67	6.17	32.40
Shegansu B (**4**)	115.47	66.67	11.99	9.60
Gnetulin (**5**)	115.47	66.67	> 66.67	ND
Gnetin D (**6**)	38.49	22.22	0.67	57.44
Oseltamivir acid	3.21	0.74	0.040	80.25
Ribavirin	> 200.00	ND	5.54	> 36.10

[a] The compounds were tested in triplicate; [b] CC_{50}: median (50%) cytotoxic concentration; [c] MNCC: maximal noncytotoxic concentration; [d] IC_{50}: median (50%) inhibitory concentration; [e] SI: selective index, CC_{50}/IC_{50}; [f] ND: not determined

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