In vitro Anti-Influenza Viral Activities of Constituents from *Caesalpinia sappan*

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Abstract

Six constituents with neuraminidase (NA) inhibitory activity, namely brazilein, brazilin, protosappanin A, 3-deoxysappanchalcone, sappanchalcone and rhamnetin, were isolated from the hearthwood of *Caesalpinia sappan* (Leguminosae). Their *in vitro* anti-influenza virus activities were evaluated with the cytopathic effect (CPE) reduction method. The results showed that 3-deoxysappanchalcone and sappanchalcone exhibited the highest activity against influenza virus (H3N2) with IC_{50} values of 1.06 and 2.06 μg/mL, respectively, in comparison to the positive control oseltamivir acid and ribavirin with IC_{50} values of 0.065 and 9.17 μg/mL, respectively.

Key words

Caesalpinia sappan · Leguminosae · influenza virus · neuraminidase · sappanchalcone

Abbreviations

CPE:	cytopathic effect
NA:	neuraminidase

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

Influenza is a globally important contagious disease, which leads to significant morbidity and mortality each year. Because of the importance of influenza neuraminidase (NA) in viral replication, release, and pathogenesis, NA is regarded as an attractive drug target for the development of new drugs for influenza treatment [1], and thus, the NA activity assay has been used to screen for new NA inhibitors.

In this study, the isolation of the constituents from *Caesalpinia sappan* (Leguminosae) was guided by their effects on NA activity. The structures of the active compounds (**1 – 6**) were identified as brazilein, brazilin, protosappanin A, 3-deoxysappanchalcone, sappanchalcone and rhamnetin (**Fig. 1**), respectively, by comparison of their spectral data with literature values [2], [3], [4], [5]. They exerted inhibitory effects on NAs with IC_{50} values ranging from 13.9 to 35.6 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 anti-viral activities of brazilein (**1**), brazilin (**2**) and protosappanin A (**3**) were less than 50% at their max-

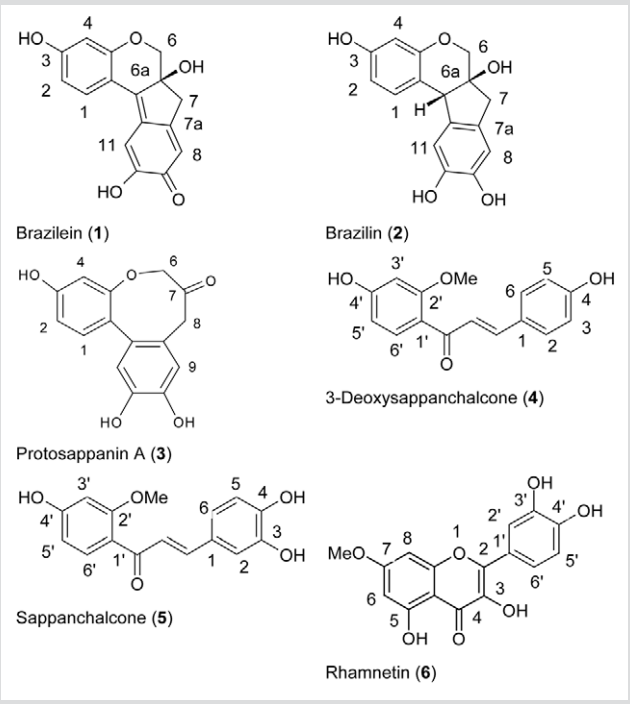

Fig. 1 Chemical structures of the six constituents isolated from *Caesalpinia sappan*.

imal non-cytotoxic concentrations (MNCC), while rhamnetin (**6**) obviously exhibited anti-influenza virus activity, which was weaker than those of ribavirin and oseltamivir acid.

Interestingly, 3-deoxysappanchalcone (**4**) exhibited significant *in vitro* anti-influenza virus activity. Its IC_{50} value was approximately eight times lower than that of ribavirin and approximately 15 times higher than that of oseltamivir acid. Another constituent, sappanchalcone (**5**), which has the same scaffold as 3-deoxysappanchalcone (**4**), displayed a potency similar to that of 3-deoxysappanchalcone (**4**).

Although the six constituents from *C. sappan* have been reported previously, our findings show, for the first time, that they possess different anti-influenza virus activities *in vitro*, and NA inhibition is obviously the major mechanism of action against influenza virus. Among the six NA inhibitors identified, brazilein (**1**) is an oxidation product of brazilin (**2**). The NA inhibitory activity of brazilein (**1**) was weaker than that of brazilin (**2**), indicating that the scaffold of brazilin (**2**) is more favorable to this inhibition than that of brazilein (**1**), although both compounds did not show good inhibitory activity in the CPE assay. 3-Deoxysappanchalcone (**4**), sappanchalcone (**5**) and rhamnetin (**6**) exhibited higher anti-influenza virus activity *in vitro* (▶ **Table 2**), so we can presume that the traditional and clinical therapeutic effects of *C. sappan* against colds and flu are probably attributable to these constituents and/or other structurally related unknown compounds present in the plant.

Table 1 Inhibitory effects of the constituents isolated from *Caesalpinia sappan* on three influenza virus NAs

Compound	IC_{50} (μg/mL)[a,b]		
	A/PR/8/34 (H1N1)	A/Guangdong/243/72 (H3N2) (H3N2)	B/Jiangsu/10/2003
Brazilein (**1**)	26.5 ± 0.3	24.6 ± 0.1	28.3 ± 2.3
Brazilin (**2**)	19.9 ± 0.7	14.5 ± 1.7	22.0 ± 0.5
Protosappanin A (**3**)	25.7 ± 1.8	21.5 ± 0.1	35.6 ± 3.4
3-Deoxysappanchalcone (**4**)	14.6 ± 0.2	17.4 ± 0.9	18.6 ± 1.1
Sappanchalcone (**5**)	14.3 ± 0.1	21.2 ± 0.6	18.0 ± 0.5
Rhamnetin (**6**)	15.4 ± 1.0	24.1 ± 2.3	13.9 ± 0.6
Oseltamivir acid	0.0010 ± 0.0002	0.00047 ± 0.00042	0.0039 ± 0.0022

[a] Average of four determinations.

[b] IC_{50} = average ± S.D.

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

Compound	CC_{50} (μg/mL)[a]	MNCC (μg/mL)[b]	IC_{50} (μg/mL)[c]	SI[d]
Brazilein (**1**)	2.38	1.37	> 1.37	ND[e]
Brazilin (**2**)	2.38	1.37	> 1.37	ND
Protosappanin A (**3**)	333.33	111.11	> 111.11	ND
3-Deoxysappanchalcone (**4**)	17.25	2.47	1.06	16.27
Sappanchalcone (**5**)	12.83	7.41	2.06	6.23
Rhamnetin (**6**)	115.47	66.67	15.40	7.50
Oseltamivir acid	8.01	0.74	0.065	123.23
Ribavirin	> 333.33	ND	9.17	> 36.34

[a] CC_{50}: mean (50 %) cytotoxic concentration.

[b] MNCC: maximal non-cytotoxic concentration.

[c] IC_{50}: mean (50 %) inhibitory concentration.

[d] SI: selective index, CC_{50}/IC_{50}.

[e] ND: not determined.

3-Deoxysappanchalcone (**4**) and sappanchalcone (**5**) both belong to the chalcones. However, sappanchalcone (**5**) showed less inhibitory activity than 3-deoxysappanchalcone (**4**). This suggests that the presence of the 3-OH group in sappanchalcone (**5**) slightly reduces its activity. These findings provide insight into the rational design of chemical compounds with higher NA inhibitory activities and anti-influenza viral effects.

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 plants of *Caesalpinia sappan* L. (Leguminosae) were collected in Hebei Province, China, in June 2005, and identified by Prof. L. Ma, Chinese Academy of Medical Sciences. A voucher specimen (ID-S-2239) was deposited at the Herbarium of the Department of Botany, Institute of Materia Medica, Chinese Academy of Medical Sciences, China.

The isolation and identification of the active constituents from *C. sappan* were guided by the NA inhibitory activity. The active compounds **1** (2 g) and **2** (1.5 g) were isolated from the BuOH extract as previously described [2]. While the active compounds **3** (2 g), **4** (30 mg), **5** (125 mg) and **6** (4 mg) were isolated from the EtOAc extract by a silica gel column (150 cm × 12 cm, 200 – 300 mesh), eluted with $CHCl_3$-MeOH of increasing polarity ($CHCl_3$ 50 : 1 – 10 : 1 MeOH). The purity of the compounds ranged from 94.5 % (**3**) to 98.0 % (**6**) as determined by analytical HPLC with ^{1}H-NMR.

The activities of the extracts, fractions and constituents from *C. sappan* were tested and verified in the NA activity assay and CPE reduction assay, which are described in the Supporting Information.

Supporting Information

Detailed experimental protocols are available as Supporting Information.

Acknowledgements

We would like to thank the Platform Program of the National Scientific and Technological Foundation (no. 2005DKA32400), the National Natural Science Foundation (no. 30171148) and the Basic Scientific Research Program of the Institute of Materia Medica, CAMS (no. 2007ZD01).

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received July 1, 2008
revised October 28, 2008
accepted November 4, 2008

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DOI 10.1055/s-0028-1112208
Planta Med 2009; 75: 337–339

Published online January 15, 2009
ISSN 0032-0943

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