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Bacterial neuraminidase inhibitory effects of prenylated isoflavones from roots of *Flemingia philippinensis*

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ABSTRACT

Bacterial neuraminidase (NA) is one of the key enzymes involved in pathogenesis of inflammation during infection. The organic extract of the roots of *Flemingia philippinensis* showed high bacterial NA inhibitory activity with an IC_{50} of around 5 μg/mL. Activity-guided separation of the methanol extract yielded nine prenylated isoflavones together with the novel species isoflavone (**2**) which was given the name flemingsin. Isolated prenylated isoflavones (**1**–**9**) were evaluated for NA inhibition and their IC_{50} values were determined to range between 0.30 and 56.8 μM. The most potent inhibitor **4** (IC_{50} = 300 nM, K_i = 130 nM) features a catechol motif in the B-ring and a furan in the A-ring. Structure–activity analysis also showed a 4-hydroxyl group within the B-ring was essential for NA inhibitory activity, because isoflavone (**9**) having protected 4-hydroxyl group was much less potent than its hydroxylated counterpart. All neuraminidase compounds screened were found to be reversible noncompetitive inhibitors. Furthermore, the most active NA inhibitors (**1**–**9**) were proven to be present in the native roots in high quantities by HPLC and LC-DAD-ESI/MS.

1. Introduction

Sialic acid, a nine carbon α-keto aldonic acid, plays essential roles in biological recognition since it is situated on the outer periphery of the cell.[1] It is uniquely able to interact with its microenvironment. The neuraminidase family (EC 3.2.1.18) is a group of exo-acting enzymes that hydrolyzes terminal sialic acid from a variety of glycoproteins.[2] This enzyme specifically cleaves *N*-acetyl neuraminic acid (NeuSAc) from cell surface glycoproteins when sialic acid is joined to galactose via a α-2 → 3 or α-2 → 6 linkage.[3] Glycoconjugates of membrane proteins on the surface of epithelial cells are implicated in pathogenesis, bacterial nutrition and cellular interactions.[4] In particular, microbial sialic acid metabolism has been linked with numerous important processes required for infection to occur including cell attachment.[5]

Various sialylated structures are involved in the innate and adaptive immune responses. These include sialyl lewis X (LeX) antigen and selectin binding during leukocyte migration.[6] Inflammation and infection also typically involve desialylation of glycoconjugates on the cell surface.[7] For instance, in bacterial infections of the lung, pathogenic neuraminidase is released into the infected region. Thus neuraminidase activity can be detected in bronchoalveolar lavage fluids from patients with inflammation.[8] Recently, the action of neuraminidase in mucosal infection has been linked to biofilm formation.[9] In addition, neuraminidase activity is increased in the serum of patients and animal models of sepsis. This indicates that neuraminidase is associated with the pathogenesis of inflammation during infection.[10,11] However in spite of the prominent role NA plays in infectivity, it remains under investigated as an antibacterial drug target. Nonetheless NA is a key validated drug target in commercially available antiviral drugs.

Flemingia philippinesis is renowned as a polyphenol-rich plant which is mainly distributed in tropical areas of China.[12] This species belongs to the family of legumes, the roots of which have been used throughout history to cure rheumatism, arthropathy, leucorrhea, meralgia and for improving bone mineral density.[13] It is also an important component of commercial nutraceutics for anti-inflammation. Previous phytochemical investigations have reported that *F. philippinensis* contains various isoflavones, benzofurans, flavanones and coumaronochromones.[13–16] In recent years, the phytochemical composition of *F. philippinesis* has drawn increasing attention due to the structural complexity and diverse bioactivities.[14,17,18] However, to the best of our knowledge, there is no report that the roots of *F. philippinesis* elicit neuraminidase inhibition. In this study, we isolated nine prenylated isoflavones from the roots of *F. philippinesis* and their structures were identified using spectroscopic methods. Their inhibitory behaviors were determined by Lineweaver–Burk and Dixon plots. We also assessed the relative abundance of these extracts within the native root using an HPLC chromatogram profile.

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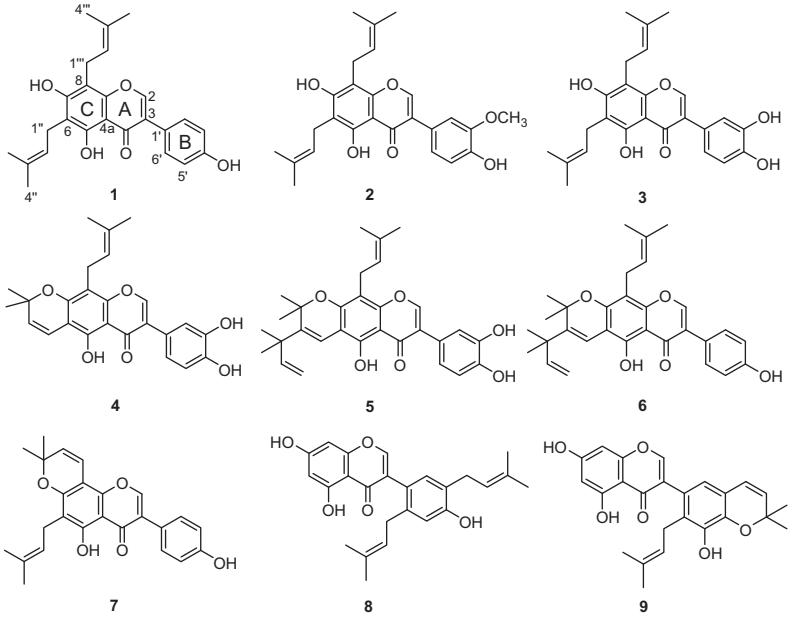

Fig. 1. Chemical structures of isolated isoflavone **1**–**9** from the root bark of *F. philippinensis*.

Table 1
^{1}H and ^{13}C NMR spectroscopic data of new compound **2** in CD_3OD

Position	2		
	$\delta^{13}C$	$\delta^{1}H$ (*J* in Hz)	HMBC
2	155.3	8.14 (s)	C-3, 1′, 8a, 4
3	124.5		
4	183.1		
4a	106.9		
5	158.8		
6	113.6		
7	161.1		
8	108.2		
8a	155.2		
1′	124.8		
2′	114.6	7.18 (d,1.8)	C-3, 1′, 3′, 4′, 6′
3′	149.2		
4′	148.3		
5′	116.6	6.87 (d,8.1)	C-1′, 3′, 4′
6′	123.4	6.98 (dd,1.8, 8.1)	C-3, 1′, 2′, 4′
1″	23.0	3.40 (d,7.0)	C-5, 6, 7, 2″, 3″
2″	123.7	5.21 (t,7.1)	C-1″, 4″, 5″
3″	133.2		
4″	18.4	1.81 (s)	C-2″, 3″, 5″
5″	26.3	1.67 (s)	C-2″, 3″, 4″
1‴	23.1	3.49 (d,7.0)	C-7, 8, 2‴, 3‴, 8a
2‴	123.6	5.21 (t,7.1)	C-1‴, 4‴, 5‴
3‴	133.4		
4‴	18.4	1.84 (s)	C-2‴, 3‴, 5‴,
5‴	26.4	1.70 (s)	C-2‴, 3‴, 4‴,
OCH_3	56.9	3.91 (s)	C-3′

Table 2
Inhibitory effects of compounds (**1**–**9**) on neuraminidase activities

Compound	Neuraminidase	
	IC_{50}[a]	Kinetic mode (K_i[b], μM)
1	4.04 ± 1.2 μM	Noncompetitive (4.29)
2	3.75 ± 0.4 μM	Noncompetitive (3.68)
3	1.07 ± 0.8 μM	Noncompetitive (1.02)
4	0.30 ± 0.6 μM	Noncompetitive (0.13)
5	1.63 ± 0.9 μM	Noncompetitive (1.36)
6	7.90 ± 0.9 μM	Noncompetitive (7.27)
7	3.06 ± 1.1 μM	Noncompetitive (3.21)
8	7.85 ± 0.6 μM	Noncompetitive (7.86)
9	56.80 ± 1.5 μM	Noncompetitive (53.02)
Genistein	12.05 ± 1.3 μM	NT[c]

[a] All compounds were examined in a set of experiments repeated three times; IC_{50} values of compounds represent the concentration that caused 50% enzyme activity loss.
[b] Values of inhibition constant.
[c] NT is not tested.

2. Results and discussion

2.1. Bacterial neuraminidase inhibitory potential of extracts

F. philippinensis is a popular cultivated plant in southern China because it is an important ingredient of neutraceutics as well as traditional Chinese medicine. Several papers have reported that *F. philippinensis* contains many phenolic compounds including isoflavones, flavonones and benzofurans. But biological studies have been limited to cytotoxic, estrogenic and antioxidant activities. *F. philippinensis* root extracts made using five different polar solvents ($CHCl_3$, EtOH, MeOH, 50% EtOH in water and 100% water) were tested for their enzymatic inhibitory activity against bacterial neuraminidase (NA). All extracts apart from the water extract exhibited high NA inhibitory activity with IC_{50} around 5 μg/mL (Supplementary data). Methanol was found to be the best solvent in terms of extraction yield as well as inhibitory potency and this solvent was used for large scale extraction.

2.2. Structure identification of the isolated prenylated isoflavones

Activity guided fractionation of the methanol extract gave nine prenylated isoflavones **1**–**9** (Fig. 1), which were purified over silica gel, Sephadex LH-20 and octadecyl-functionalized silica gel. Isolated compounds (**1**–**9**) were identified as the known species 8-γ,γ-dimethylallylwighteone **1**, 6,8-diprenylorobol **3**, auriculasin **4**, flemiphilippinin A **5**, flemiphilippinin E **6**, osajin **7**, 5,7,3′,4′-tetrahydroxy-2′,5′-di(3-mehylbut-2-enyl)isoflavone **8**, 5,7,3′-trihydroxy-2′-(3-methylbut-2-enyl)-4′,5′-(3,3-dimethylpyrano) isoflavone **9**, through analysis of spectroscopic data and comparison with previous studies.[15,19–22]