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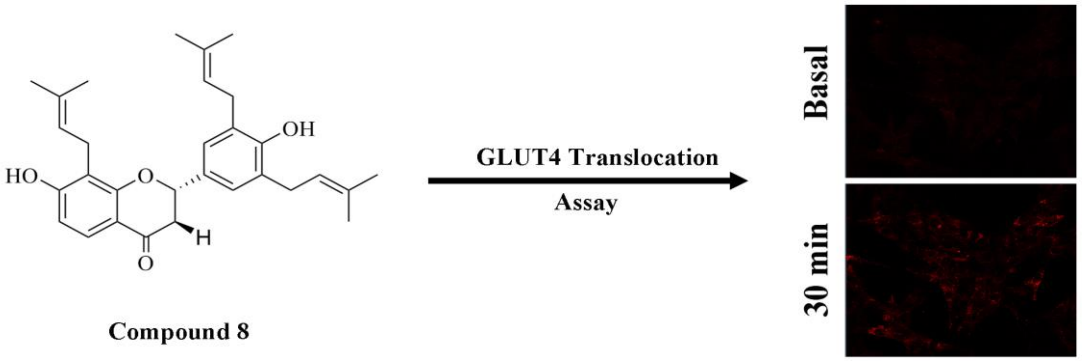

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Chemical constituents from *Sophora tonkinensis* and their glucose transporter 4 translocation activities

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ABSTRACT

Bioassay-guided phytochemical investigation of the EtOAc fraction (ST-EtOAc) from the roots of *Sophora tonkinensis* resulted in the isolation of a new compound 1-hydroxy-4-isoprenyl-maackiain (**1**), along with 12 known compounds (**2-13**). The structure of the new compound was established by 1D and 2D NMR, MS data and circular dichroism analysis. Polyprenylated flavonoids **6**-**9** and **11**-**13** increased GLUT-4 translocation by the range of 1.35-2.75 folds. Sophoranone **(8)** exerted the strongest activity with 2.75 folds GLUT-4 translocation enhancement at the concentration of 10 μM. This is the first report of the GLUT-4 translocation activity of the plant *Sophora tonkinensis*.

[1] These authors contributed to this article equally.

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Diabetes Mellitus (DM) is a metabolic disorder with symptoms of high blood sugar and many other complications.[1] 90% patients worldwide have type 2 diabetes (T2DM) which is resulted from a loss of glucose homeostasis or insulin resistance.[2] The costs to prevent and treat T2DM and its associated complications have become global health and economic burdens.[3,4] This problem is particularly prevalent in China due to its rapid economic development, improved survival rate from communicable diseases, and the genetic susceptibility.[5]

GLUT-4, one of the 13 sugar transporter proteins, is highly expressed in adipose tissue and skeletal muscle and catalyzes hexose transport across cell membranes.[6] The protein also mediates glucose removal from the circulation and regulates glucose homeostasis.[7,8] Given its role in sugar regulation, GLUT-4 has been used as an important target for anti-diabetic drug discovery.

In this study, a cell-based GLUT-4 translocation system was established using L6 recombinant GLUT-4. Confocal imaging technique was used to quantify the translocation activity. The extracts and fractions from 800 traditional Chinese medicines (TCMs) were screened against GLUT4 translocation.[9-12] One EtOAc fraction (ST-EtOAc) from the roots of *Sophora tonkinensis* exerted a promising stimulatory effect on GLUT-4 translocation. Bioassay-guided phytochemical investigation on the active ST-EtOAc led to the isolation of a new compound, 1-hydroxy-4-isoprenyl-maackiain (**1**), along with 12 known flavonoids.

S. tonkinensis, a traditional medicine used for the treatment of asthma, allergic dermatitis, gastrointestinal hemorrhage, chronic bronchitis, acute pharyngolaryngeal infections and throat inflammation, was collected from Jingxi County, Guangxi Zhuang Autonomous Region, China, in June, 2012. A specimen (No. SC0060) of this plant has been deposited in the Herbarium of South-Central University for Nationalities, Wuhan, China. The roots of the plant were triturated and then extracted sequentially by maceration with *n*-hexane, EtOAc, methanol successively. The EtOAc fraction was subjected to repeated flash chromatography on silica gel, MCI gel CHP20P and Sephadex LH-20, followed by C_{18} reverse phase HPLC to afford a new compound, 1-hydroxy-4-isoprenyl-maackiain (**1**, 8 mg), with an isoflavanone conjugate skeleton, as well as 12 known compounds (**2**-**13**) (**Fig 1**.). The known compounds were identified as trifolirhizin (**2**),[13] trifolirhizin-6"-monoacetate (**3**),[13] maackiain (**4**),[14] medicarpin (**5**),[15] 7,4'-dihydroxy-6,8-diprenylflavanone (**6**),[16] glabrol (**7**),[17] sophoranone (**8**),[18] sophoranochromene (**9**),[18] quercetin (**10**),[19] 6,8-diprenylkaempferol (**11**),[20] 2-(2',4'-dihydroxyphenyl}-8,8-dimethyl-10-(3-methyl-2-butenyl)-8H-pyrano[2,3-d]chroman-4-one (**12**)[21] and dehydrolupinifolinol (**13**),[22] by the comparison of their spectroscopic data with those previously reported in literature. Herein, we describe the structure elucidation of the new compound and the GLUT-4 translocation activities of compounds **1**-**13**.

Compound **1** was obtained as an optically active light yellow powder. The molecular formula of $C_{21}H_{20}O_6$ was established according to the quasi-molecular ion $[M - H]^-$ at *m/z* 367.1186 in the HRESIMS, which was in agreement with its ^{13}C-NMR data. The UV spectrum showed absorption maximum at 235, 285, 305 nm, typical absorptions of pterocarpanes.[14]

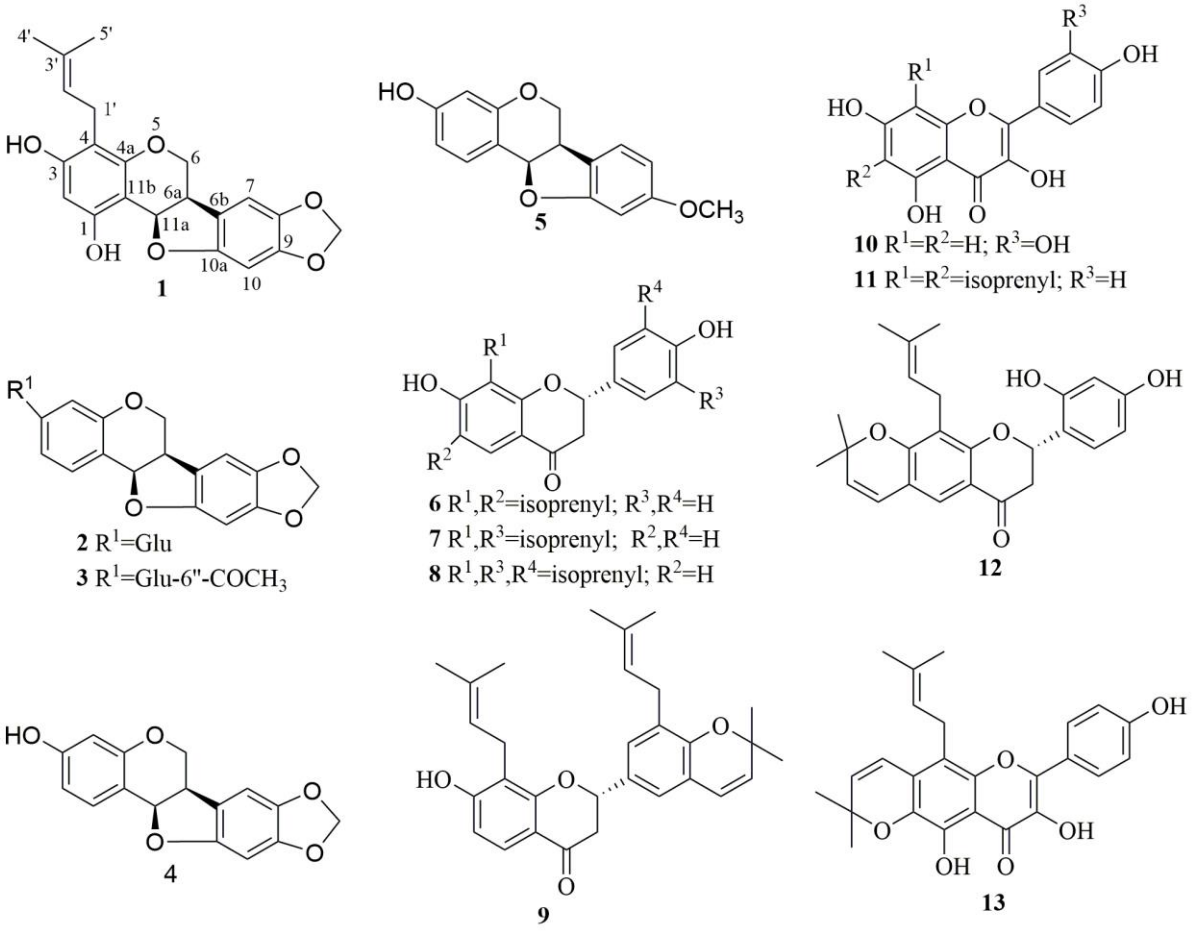

Figure 1. Chemical structures of compounds **1**-**13**.

Table 1. ^{1}H and ^{13}CNMR data for compound **1** in CD_3OD.

Position	δ_H (*J* in Hz)[a]	δ_C	HMBC
1		148.6	
2	6.24, s	100.0	C-1, 3, 4, 4a
3		147.3	
4		108.8	
4a		137.2	
6α	3.43, d, 10.9 Hz	65.1	C-1, 6a, 6b, 11a
6β	4.10, dd, 10.9, 4.9 Hz		C-1, 6a, 6b, 11a
6a	3.33, obs	39.6	C-6, 6b, 10a
6b		118.2	
7	6.39, s	92.2	C-6b, 8, 9, 10, 10a
8		141.2	
9		147.1	
10	6.79, s	103.9	C-7, 8, 9, 6a, 6b,
10a		153.8	
11a	5.47, d, 7.0 Hz	77.1	C-4, 6, 6b, 11b
11b		128.2	
OCH_2O	5.87, s	100.5	C-8, 9
	5.85, s		
1'	3.65, m	24.5	C-4, 2', 4a, 3
	3.47, m		
2'	5.18, m	122.2	C-4', 5'
3'		130.0	
4'	1.76, s	16.1	C-3', 4', 5'
5'	1.67, s	24.0	C-2', 3', 4'

[a] Spectra were recorded for ^{1}H and ^{13}C NMR data at 30 °C.

The ^{1}H-NMR spectrum disclosed signals (**Table 1**) for two isolated aromatic protons at δ_H 6.79 (1H, s) and 6.39 (1H, s), signals for a -OCHCHCH$_2$O- moiety [5.47 (1H, d, *J* = 7.0 Hz), 4.10 (1H, dd, *J* = 10.9, 4.9 Hz), 3.43 (1H, d, *J* = 10.9 Hz), 3.33 (1H, obs)], and a dioxygenated methylene at δ_H 5.87 (1H , s) and 5.85 (1H, s), suggesting a chemical structure similar to the known compound maackiain.[14] Extra set of signals in **1**, including two methyls at δ_H 1.76 (3H, s) and 1.67 (3H, s), an olefin proton at δ_H 5.18 (1H, m), and a methylene moiety at δ_H 3.65 (1H, m) and δ_H 3.47 (1H, m) were assigned to an isoprenyl functionality based on the COSY correlation data from H-1′ to H-2′, and from H-2′ to H-4′ and H-5.

On the basis of above partial structures, the molecular framework was constructed by the HMBC analysis (**Fig. 2**). The characteristic HMBC correlations of the methyl groups at δ_H 1.76 and 1.67 to two olefin carbons at δ_c 122.2 (C-2') and 130.0 (C-3'), and the olefin proton at δ_H 5.18 to the methylene carbon at δ_c 24.5 (C-1') confirmed the presence of an isoprenyl group. The HMBC correlations of the olefin proton at δ_H 5.18 (H-2') to the aromatic carbon at δ_c 108.8 (C-4), and the methylene at δ_H 3.65 and 3.47 (H_2-1') to two aromatic carbons at δc 137.2 (C-4a) and C-4, confirmed that the isoprenyl group was attached at C-4. Moreover, the key HMBC correlations of the isolated aromatic protons at δ_H 6.24 to C-4 and C-4a, and two oxygenated aromatic carbons at δc 148.6 and 147.3 confirmed that the proton at δ_H 6.24 located at C-2, and two hydroxyl groups were attached to C-1 and C-3, respectively. Thus, **1** was elucidated as 1-hydroxy-4-isoprenyl-maackiain.[23]

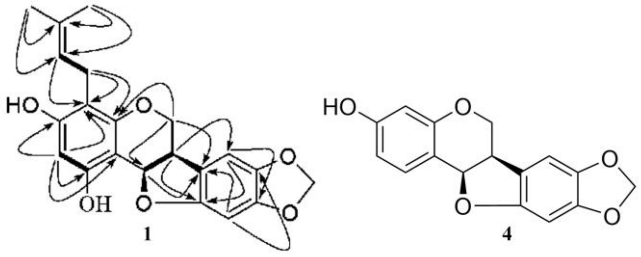

Figure 2. ^{1}H-^{1}H COSY (—) and key HMBC (⟶) correlations for **1**.

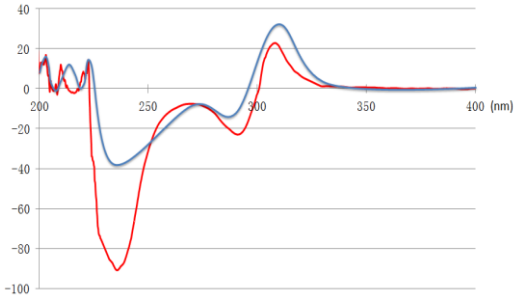

Figure 3. The CD spectra of compound **1** (red) and maackiain (blue).

As shown in **Figure 3**, the circular dichroism (CD) spectrum of compound **1** (red line) showed a positive absorption at 308 nm (Δε = + 6.7) and negative absorptions at 235 nm (Δε = - 26.9) and 291 nm (Δε = - 6.9), similar to those of (-)-maackiain (blue line). Based the combined analysis of CD spectra of compound **1** and maackiain. The absolute configuration of **1** was, therefore, determined to be 6a*R*,11a*R*-, the same as that of maackiain.[14]

The potential GLUT-4 translocation activity of compounds **1-13** was tested against pIRAP-mOrange cDNAs transfected L6 cells .[9-12] Insulin (100 nM) was used as the positive control.

Compound **1** showed weak GLUT-4 translocation effect with 0.79-fold enhancement within 30 minutes. Compounds **2-5** and **10** displayed no activity on GLUT-4 translocation (**Fig. 4**). Polyprenylated flavonoids **6-9** and **11-13** exerted moderate to strong activity, increasing GLUT-4 translocation by 1.35-2.75 folds, respectively. Sophoranone (**8**) exerted the strongest activity with 2.75 folds GLUT-4 translocation enhancement (**Fig. 4**, **5A**). Dose-response analysis showed that compound **8** increased GLUT4 translocation of L6 cells in a dose-dependent manner at the lower concentrations (≤ 10 μM). At higher concentrations (> 10 μM), the compound maintained its GLUT-4 translocation activity at around 2.7 folds (**Fig. 5B**). Initial examination of the structure–activity relationship revealed that the active compounds **6-9** and **11-13** all possessed a flavonoid ring system with an aromatic substituent at the C-6 position and an isoprenyl functionality at the C-3 position, suggesting that these structural features may contribute to their GLUT-4 translocation activity.

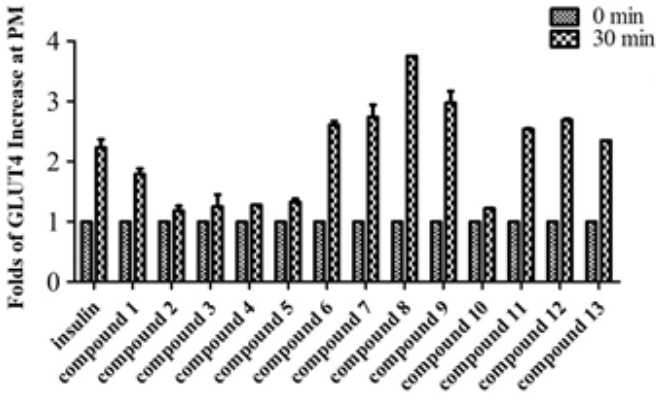

Figure 4. GLUT-4 translocation effects of compounds **1-13**.

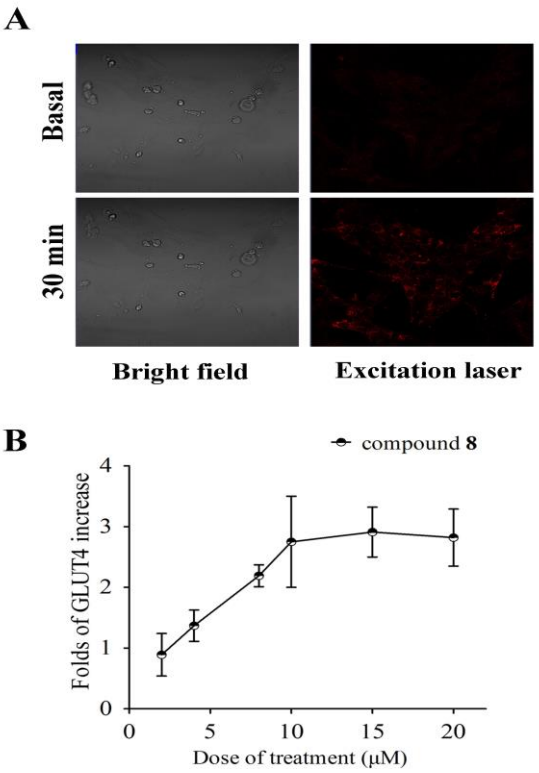

Figure 5. (**A**) Confocal images in L6 cells incubated in the absence (basal) or presence of compound **8** for 30 minutes (L6 cells were infected with pIRAP-mOrange in order to detect externalized IRAP by confocal microscopy). (**B**) Dose response curve of compounds **8**.

In conclusion, 6a*R*,11a*R*-1-hydroxy-4-prenyl-maackiain (**1**), a new isoprenyl substituted derivative of maackiain was isolated from the roots of *S. tonkinensis*, along with 12 known compounds (**2-13**). Detailed CD spectroscopic data analysis confirmed the the absolute stereochemistry of compound **1**. Polyprenylated flavonoids **6-9** and **11-13** increased GLUT-4 translocation by 1.35-2.75 folds, respectively. Polyprenylated flavonoids may be the active principles responsible for GLUT-4 translocation activity of the ST-EtOAc. This is the first report of GLUT-4 translocation activity of the herbal medicine *Sophora tonkinensis.*

Acknowledgments

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Supplementary material

UV, IR, ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC and CD spectra for 6a*R*,11a*R*-1-hydroxy-4-isoprenyl-maackiain (**1**), detailed experimental procedures including general experimental procedure, plant material, extraction, isolation procedure and ^{1}H NMR for compounds **2-13**, and GLUT-4 translocation assay.

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23. 6a*R*,11a*R*-1-hydroxy-4-isoprenyl-maackiain (**1**): yellow powder; UV (CH_3OH) max (log ε) 235 (1.96), 285 (1.02) and 305 (2.08) nm; IR (film) 3432, 2913, 1702, 1616, 1469, 1326, 1126, 1037, 844 cm^{-1}; ^{1}H and ^{13}C NMR data (CD_3OD) see **Table 1**; (-)-HRESIMS m/z 367.1186 $[M - H]^-$ (calcd. for $C_{21}H_{19}O_6$, 367.1186).