NOTE

A New Chromone from the Flower Buds of *Magnolia fargesii* and its Anti HIV-1 Activity

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A new chromone, fargesiichromone A (**1**) was isolated from the flower buds of *Magnolia fargesii*. The structure of newly isolated fargesiichromone (**1**) was elucidated by spectroscopic methods including extensive 1D and 2D NMR techniques. Compound **1** was also evaluated for the anti HIV-1 activity and it showed anti HIV-1 activity with EC₅₀ 4.18 µg/mL, CC₅₀ > 200 µg/mL and therapeutic index value above 47.8.

Key Words: *Magnolia fargesii*, Chromone, Anti HIV-1 activity.

The genus of *Magnolia* (Magnoliaceae) has been used as herb medicine in China for treatment of inflammatory-related diseases, such as nasal congestion, empyema, sinusitis and allergic rhinitis for a long time^{1,2}. Previous phytochemical investigations had revealed that this species contains several secondary metabolites such as lignans³⁻⁵, neolignans^{6,7}, sesquiterpenes^{2,8} and essential oils⁹, which show various biological activities. To search for more new bioactive compound from this plant, we reexamined the flower buds of *M. fargesii*, which led to the isolation of a new chromone, named fargesiichromone A (**1**). In addition, the anti HIV-1 activity of **1** was evaluated. The structure elucidation and biological activity of the new compound are described in this paper.

General procedures: Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on DRX-500 spectrometers with TMS as internal standard. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. HRESIMS was performed on an API QSTAR time-of-flight spectrometer and a VG Autospec-3000 spectrometer, respectively. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a ZORBAX PrepHT GF (21.2 mm × 25 cm, 7 µm) column or a Venusil MP C₁₈ (20 mm × 25 cm, 5 µm) column. Column chromatography was performed with Si gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China), Lichroprep RP-18 gel (40-

63 µm, Merck, Darmstadt, Germany) and MCI gel (75-150 µm, Mitsubishi Chemical Corporation, Tokyo, Japan). The fractions were monitored by TLC and spots were visualized by heating Si gel plates sprayed with 5 % H₂SO₄ in EtOH.

The flower buds of *M. fargesii*, indigenous to Nanzhao country, Henang province, were purchased from Kunming Herb Medicine Market in September 2010. A voucher specimen (YNNI-10-9-28) has been deposited in our laboratory.

Extraction and isolation: The air-dried and powdered flower buds of *M. fargesii* (2.0 kg) were extracted three times with 70 % aqueous MeOH (3 × 3.5 L) at room temperature and filtered to yield a filtrate, which was successively evaporated under reduced pressure to obtained a crude extract (152 g). This crude extract was applied to Si gel (200-300 mesh) column chromatography eluting with a CHCl₃-MeOH gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5) to give six fractions A-F. The separation of fraction B (22.6 g) by Si gel column chromatography eluted with CHCl₃-acetone (20:1-1:2) yielded mixtures B1-B6. Fraction B2 (3.58 g) was subjected to Si gel column chromatography using petroleum ether-acetone and preparative HPLC (55 % MeOH-H₂O, flow rate 12 mL/min) to give compound **1** (15.6 mg).

Anti HIV-1 assay: The cytotoxicity assay against C8166 cells (CC₅₀) was assessed using the MTT method and anti HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC₅₀)¹⁵.

Fargesiichromone A (1): Yellow gum; UV (MeOH) λ_{max} (log ε) 218 (4.48), 245 (3.97), 270 (4.32), 362 (3.95) nm; IR (KBr, ν_{max}, cm⁻¹): 3434, 2925, 2878, 1726, 1665, 1613, 1562,

1435, 1357, 1146, 955, 795; ^1H and ^{13}C NMR (CDCl_3 , 500 and 125 MHz), see Table-1; positive ESIMS m/z 295 $[\text{M} + \text{Na}]^+$; HRESIMS m/z 295.0953 $[\text{M} + \text{Na}]^+$ (calcd. (%) for $\text{C}_{16}\text{H}_{16}\text{O}_4\text{Na}$, 295.0946).

TABLE-1

 ^{13}C and ^1H NMR DATA OF COMPOUND **1** (δ IN ppm, IN CDCl_3)

Position	δ_{C} (m)	δ_{H} (m, J, Hz)
2	162.4 s	—
3	108.5 d	6.34 s
4	181.8 s	—
5	138.6 s	—
6	115.8 d	6.72, d, $J = 2.2$
7	168.2 s	—
8	102.2 d	6.86, d, $J = 2.2$
9	159.7 s	—
10	116.0 s	—
11	131.3 d	6.48, d, $J = 15.8$
12	124.8 d	6.40 m
13	19.9 q	1.69, d, $J = 6.5$
14	50.0 t	4.15, s
15	208.1 s	—
16	30.4 q	2.26 s
OMe-7	55.8 q	3.85 s

The air-dried and powdered flower Buds of *M. fargesii* (2.0 kg) was extracted with 70 % aqueous methanol (3 L $\times$ 3.5 L) at room temperature and filtered to yield a filtrate, which was successively evaporated under reduced pressure to obtained a crude extract (152 g). This crude extract was subjected repeatedly to column chromatography on Si gel, Sephadex LH-20, RP-18 and preparative HPLC to afford compound **1**. The structure of the fargesiiichromone (**1**) was shown in Fig. 1 and its NMR data were listed in Table-1.

Fig. 1. Structure of new chromone

Fargesiiichromone (**1**) was obtained as a yellow gum and assigned the molecular formula, $\text{C}_{16}\text{H}_{16}\text{O}_4$, from its HRESIMS at m/z 295.0953 $[\text{M} + \text{Na}]^+$ (calcd. m/z 295.0946). IR absorptions implied the presence of hydroxy (3434 cm^{-1}), ketone ($1726, 1665\text{ cm}^{-1}$) and aromatic ring ($1613, 1562, 1435\text{ cm}^{-1}$) functions. UV absorptions at 218, 245, 270 and 362 nm also indicated a conjugated aromatic ring system. Its ^1H , ^{13}C and DEPT NMR spectra showed signals for 15 carbons and 16 hydroxy atoms, corresponding to one chromone ring system^{10,11} (δ_{C} 162.4 s, 108.5 d, 181.8 s, 138.6 s, 115.8 d, 168.2 s, 102.2 d, 159.7 s, 116.0 s) with three aromatic protons (δ_{H} 6.34 s, 6.72 d $J = 2.2$ and 6.86 d $J = 2.2$), a (E)-propenyl group ($-\text{CH}=\text{CH}-\text{CH}_3$; δ_{C} 131.3 d, 124.8 d, 19.9 q; δ_{H} 6.48 d $J = 15.8$, 6.40 m, 1.69 $J = 6.5$), an acetyl group ($-\text{CH}_2\text{C}(\text{O})\text{CH}_3$; δ_{C} 50.0 t, 208.1 s, 30.4 q; δ_{H} 4.15 s, 2.26 s) and a methoxyl group (δ_{C} 55.8 q, δ_{H} 3.85 s). The HMBC correlations of H-12 with C-2, of H-3 with C-11 and C-12 indicated that the propenyl group located at C-2. The HMBC correlations of H-14 with

C-5, C-6 and C-10, of H-6 with C-14 indicated that the acetyl group located at C-5. Whereas, the methoxyl group located C-7 was supported by the HMBC correlations of the methoxyl proton signal (δ_{H} 3.85) with, C-7 (δ_{C} 168.2). Thus, the structure of **1** was established as shown and given the name as fargesiiichromone A (Fig. 2).

Fig. 2. Selected HMBC ($\curvearrowright$) correlations of compound **1**

Since certain of chromone derivatives exhibit potential antivirus activities¹²⁻¹⁴, the compound **1** was tested for their the anti HIV-1 activity according to the literature¹⁵.

In anti HIV-1 activity test, the cytotoxicity assay against C8166 cells (CC_{50}) and anti HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}), using azidothymidine (AZT) as a positive control ($\text{EC}_{50} = 0.034\text{ mg/mL}$ and $\text{CC}_{50} > 200\text{ mg/mL}$). Compound **1** showed anti HIV-1 activity with EC_{50} 4.18 $\mu\text{g/mL}$, $\text{CC}_{50} > 200\text{ }\mu\text{g/mL}$ and TI (Therapeutic Index) value above 47.8. This compound shows modest anti HIV-1 activity.

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REFERENCES

- M. Miyazawa, H. Kasahara and H. Kameoka, *Phytochemistry*, **31**, 3666 (1992).
- Y.J. Lee, Y.M. Lee, C.K. Lee, J.K. Jung, S.B. Han and J.T. Hong, *Pharm. Therap.*, **130**, 157 (2011).
- Y.L. Huang, C.C. Chen, Y.P. Chen, H.Y. Hsu and Y.H. Kuo, *Planta Med.*, **56**, 237 (1990).
- M. Miyazawa, Y. Ishikawa, H. Kasahara, J. Yamanaka and H. Kameoka, *Phytochemistry*, **35**, 611 (1994).
- J. Lee, D. Lee, D.S. Jang, J.W. Nam, J.P. Kim, K.H. Park, M.S. Yang and E.K. Seo, *Chem. Pharm. Bull.*, **55**, 137 (2007).
- J. Lee, E.K. Seo, D.S. Jang, T.J. Ha, J.P. Kim, J.W. Nam, G. Bae and J.S. Kim, *Chem. Pharm. Bull.*, **57**, 298 (2009).
- M.A. Kelm and M.G. Nair, *Stud. Nat. Prod. Chem.*, **24**, 845 (2000).
- K.Y. Jung, D.S. Kim, S.H. Park, I.S. Lee, S.R. Oh, J.J. Lee, E.H. Kim and H.K. Lee, *Phytochemistry*, **48**, 1383 (1998).
- J.Y. Fang, T.H. Tsai, C.F. Hung and W.W. Wong, *J. Pharm. Pharmacol.*, **56**, 1493 (2004).
- S. Oshimi, Y. Tomizawa, Y. Hirasawa, T. Honda, A. Widyawaruyanti, W. Ekasari, M. Rudyanto and H. Morita, *Bioorg. Med. Chem. Lett.*, **18**, 3761 (2008).
- K.M. Biswas and H. Mallik, *Phytochemistry*, **25**, 1727 (1986).
- L.Y. Ma, S.C. Ma, F. Wei, R.C. Lin, P.P. But, S.H. Lee and S. F. Li, *Chem. Pharm. Bull.*, **51**, 1264 (2003).
- J. Ungwitayatorn, C. Wiwat, W. Samee, P. Nunthanavanit and N. Phosrihong, *J. Mol. Struct.*, **1001**, 152 (2011).
- Y.Y. Sun, G.M. Liu, H. Huang and P.Z. Yu, *Phytochemistry*, **75**, 169 (2012).
- P.L. Darke and J.R. Huff, *Adv. Pharmacol.*, **25**, 399 (1994).