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Inhibition of tyrosinase activity by polyphenol compounds from *Flemingia philippinensis* roots

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ABSTRACT

Flemingia philippinensis is used as a foodstuff or medicinal plant in the tropical regions of China. The methanol (95%) extract of the roots of this plant showed potent tyrosinase inhibition (80% inhibition at 30 μg/ml). Activity-guided isolation yielded six polyphenols that inhibited both the monophenolase (IC_{50} = 1.01–18.4 μM) and diphenolase (IC_{50} = 5.22–84.1 μM) actions of tyrosinase. Compounds **1**–**6** emerged to be three new polyphenols and three known flavanones, flemichin D, lupinifolin and khonklonginol H. The new compounds (**1**–**3**) were identified as dihydrochalcones which we named fleminchalcones (A–C), respectively. The most potent inhibitor, dihydrochalcone (**3**) showed significant inhibitions against both the monophenolase (IC_{50} = 1.28 μM) and diphenolase (IC_{50} = 5.22 μM) activities of tyrosinase. Flavanone (**4**) possessing a resorcinol group also inhibited monophenolase (IC_{50} = 1.79 μM) and diphenolase (IC_{50} = 7.48 μM) significantly. In kinetic studies, all isolated compounds behaved as competitive inhibitors. Fleminchalcone A was found to have simple reversible slow-binding inhibition against monophenolase.

1. Introduction

Tyrosinase is the rate limiting enzyme for production of melanin and other pigments via the oxidation of L-tyrosine. It uses a redox active copper as cofactor within its active site to oxidize arene rings and is involved in two distinct reactions which occur in a processive fashion: the hydroxylation of a monophenol followed by the conversion of the product *o*-diphenol to the corresponding *o*-quinone.[1] The *o*-quinone product then spontaneously converts to melanin.[2] The production of melanin by tyrosinase is essential for the protection of skin from solar radiation.[3] Tyrosinase also serves a protective function in plants and is responsible for browning of damaged fruits during post-harvest handling and processing. In certain cases, the browning of some fruits, beverage and vegetables catalyzed by tyrosinase causes a significant decrease in their nutritional and aesthetic value.[4] In addition, tyrosinase appears to play much more complex roles in higher order organisms than previously thought.[5] For instance tyrosinase may contribute to neurodegeneration associated with Parkinson's disease.[6] These new findings underscore the importance of tyrosinase inhibitor discovery and development. Tyrosinase inhibitors typically fall into two classes either chelating to the copper within the active site, or obstructing the substrate–enzyme interaction.[7]

Flemingia philippinensis belongs to the family of legumes and has been cultivated as food ingredient in tropical parts of China. It contains various isoflavones, benzofurans, flavanones and coumarono chromanes.[8–10] Polyphenols were reported to have many human health benefits, including antioxidant and anti-inflammatory effects.[11,12] Some studies have reported that *F. phillippinesis* displays antioxidant, cytotoxicity, antiestrogenic and immunosuppressive activities,[13–16] but these studies have been relatively restricted. Herein we disclose that this important plant is a rich source of tyrosinase inhibitors.

As part of our continued search for new tyrosinase inhibitors from natural resources,[17,18] in a preliminary screen, we evaluated the MeOH (95%) extract of *F. philippinensis* roots for tyrosinase inhibitory activity. This extract exhibited good activity with 80% inhibition occurring at 30 μg/ml. The aim of the present work is to investigate the tyrosinase inhibitory activities of *F. philippinensis* extracts. We isolated six tyrosinase inhibitory polyphenols together with three new dihydrochalcones. All isolated compounds were evaluated for their inhibitory activities towards both monophenolase and diphenolase activities of tyrosinase. Their inhibition mechanisms were assessed using Lineweaver–Burk and Dixon plots.

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2. Results and discussion

2.1. Tyrosinase inhibitory potential of extract and isolation of inhibitors

In preliminary screening, we observed that the methanol extract of *F. philippinensis* roots showed significant inhibition (80% inhibition at 30 μg/ml) of mushroom tyrosinase catalyzed oxidation of both L-tyrosine and L-DOPA. The high potency of methanol extract encouraged us to identify the compounds responsible for tyrosinase inhibition. Six polyphenols **1**–**6** (Fig. 1) were purified over silicagel, Sephadex LH-20 and octadecyl-functionalized silica gel as described in Section 2.4. Isolated compounds were identified as known flavanones **4**–**6** and three new dihydrochalcones **1**–**3**. The flavanones **4**–**6** were identified as flemichin D, lupinifolin and khonklonginol H by comparing their spectroscopic data with the previous report.[19–21]

2.2. Structural elucidation of new compounds

Compounds **1**–**3** emerged to be new dihydrochalcone derivatives, which were named fleminchalcones (A–C). The structural characterization is delineated below.

Compound **1** was isolated as a yellow powder with molecular formula $C_{26}H_{32}O_6$ established by the $[M^+]$ ion at 440.2199 (calcd 440.2199) in HREIMS. ^1H and ^{13}C NMR data in conjunction with DEPT experiments indicated the presence of 26 carbons consisting of the following functional groups: 5 methines (sp^2), 1 methine (sp^3), 4 methylene (sp^3), 5 methyls and 11 quaternary carbons (Table 1). The analysis of degrees of unsaturation indicated a tricyclic skeleton with two aromatic rings. HMBC correlation (Fig. 2) of C–αH (δ_H = 3.21) to the ketone C=O (δ_C = 204.7) and also correlation of C–βH (δ_H = 2.79) with C-6 (δ_C = 130.6) confirmed the dihydrocalcone skeleton. One set of AA′BB′ resonances at δ_H = 7.08 (2H, d, J = 8.5 Hz) and δ_H = 6.7 (2H, d, J = 8.5 Hz) was found on the A-ring, due to a *para*-disubstituted aromatic ring. A strong HMBC

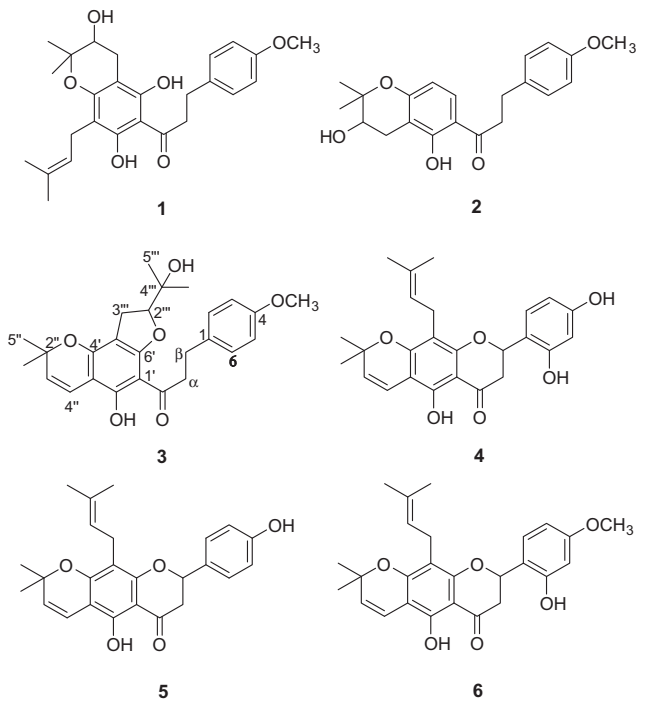

Figure 1. Chemical structures of compounds **1**–**6** isolated from the root bark of *F. philippinensis*.

Table 1
^{13}C NMR data of new compounds **1**–**3**[a]

Position	**1** δ, mult.	**2** δ, mult.	**3** δ, mult.
1	134.9 s	133.5 s	133.7 s
2	130.6 d	129.6 d	129.6 d
3	114.9 d	114.4 d	114.3 d
4	159.4 s	158.4 s	158.4 s
5	114.9 d	114.4 d	114.3 d
6	130.6 d	129.6 d	129.6 d
α	45.4 t	45.4 t	44.6 t
β	30.6 t	30.1 t	30.1 t
1′	102.4 s	107.2 s	101.8 s
2′	163.3 s	160.9 s	156.3 s
3′	108.7 s	117.9 s	103.0 s
4′	159.0 s	163.1 s	160.2 s
5′	105.3 s	109.9 d	104.8 s
6′	162.4 s	131.9 d	162.7 s
2″	79.6 s	72.0 s	78.6 s
3″	92.6 d	92.0 d	125.3 d
4″	28.1 t	29.8 t	116.7 d
5″	26.4 q	26.5 q	29.1 q
6″	26.3 q	25.0 q	29.1 q
1‴	22.5 t		
2‴	124.6 d		92.2 d
3‴	131.3 s		27.0 t
4‴	18.3 q		72.0 s
5‴	26.6 q		26.5 q
6‴			24.8 q
C=O	204.7	205.2	203.6

[a] NMR solvents: acetone-d_6 for **1**; $CDCl_3$ for **2** and **3**.

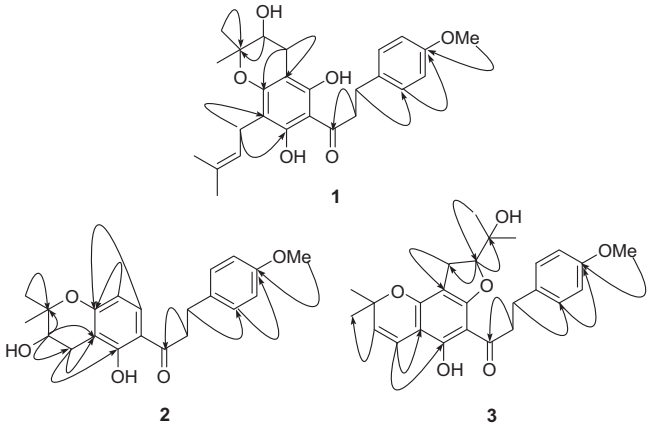

Figure 2. Key HMBC correlations of new compounds **1**–**3**.

correlation between OCH_3 (δ_H = 3.62) and C-4 (δ_C = 159.4) confirmed the location of the methoxy group. The proton within the hydroxyl group at C-2′ (δ_C = 163.3) was observed at δ_H = 13.41. This is consistent with the C-2′OH forming an H-bond to the carbonyl group at δ_C = 204.7. The presence of a 1,1-dimethylallyl group on C-3′ was deduced from successive connectivities between H-1‴ (δ_H = 3.13), H-2‴ (δ_H = 5.08) and H-4‴/5‴ (δ_H = 1.61/1.50) in the COSY spectrum. The location of this 1,1-dimethylallyl group was confirmed by HMBC correlation of H-1‴ with C-3′ (δ_C = 108.7). The prenyl-derived side chain was unveiled to be a 2,2-dimethyl-3-hydroxy-3,4-dihydro-2*H*-pyran moiety by HMBC correlation of C-2″ with H-5″, H-6″ and H-4″, and strong COSY correlations between H-3″ and H-4″. Coupling of diastereotopic protons of H-4″α (δ_H = 2.96, dd) and H-4″β (δ_H = 3.04, dd) with oxygenated methine H-3″ (δ_H = 4.68), also proved the presence of a 3-hydroxy-3,4-dihydro-2*H*-pyran moiety. The absolute stereochemistry of C-3″ asymmetric center of **1** was not ascertained. HMBC correlation of H-4″ with C-4′ (δ_C = 159.0), C-5′ (δ_C = 105.3) and C-6′ (δ_C = 162.4) affirmed the cyclization of this side chain with C-4′