Highly potent tyrosinase inhibitor, neorauflavane from Campylotropis hirtella and inhibitory mechanism with molecular docking

Xuefei Tan a, Yeong Hun Song a, Chanin Park b, Ki-Won Lee b, Jeong Yoon Kim a, Dae Wook Kim a, Kwang Dong Kim a, Keun Woo Lee b, Marcus J. Curtis-Long c, Ki Hun Park a,*

a Division of Applied Life Science (BK21 plus), IALS, Gyeongsang National University, Jinju 660-701, Republic of Korea
b Division of Applied Life Science (BK21 plus), PMBBRC, RINS, Gyeongsang National University, Jinju 660-701, Republic of Korea
c Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853, United States

A R T I C L E   I N F O

Article history:
Received 8 November 2015
Revised 28 November 2015
Accepted 28 November 2015
Available online 30 November 2015

Keywords:
Tyrosinase
Campylotropis hirtella
Neorauflavane
Competitive inhibitor
Molecular docking

A B S T R A C T

Tyrosinase inhibition may be a means to alleviate not only skin hyperpigmentation but also neurodegeneration associated with Parkinson’s disease. In the course of metabolite analysis from tyrosinase inhibitory methanol extract (80% inhibition at 20 μg/ml) of Campylotropis hirtella, we isolated fourteen phenolic compounds, among which neorauflavane C3 emerged as a lead structure for tyrosinase inhibition. Neorauflavane C3 inhibited tyrosinase monophenolase activity with an IC50 of 30 nM. Thus this compound is 400-fold more active than kojic acid. It also inhibited diphenolase (IC50 = 500 nM), significantly. Another potent inhibitor C1 (IC50 = 2.9 μM) was found to be the most abundant metabolite in C. hirtella. In kinetic studies, compounds C3 showed competitive inhibitory behavior against both monophenolase and diphenolase. It manifested simple reversible slow-binding inhibition against monophenolase with the following kinetic parameters: Kpp = 1.48 nM, k1 = 0.0033 nM⁻¹ min⁻¹ and k4 = 0.0049 min⁻¹. Neorauflavane C3 efficiently reduced melanin content in B16 melanoma cells with 12.95 μM IC50. To develop a pharmacophore model, we explored the binding mode of neorauflavane C3 in the active site of tyrosinase. Docking results show that resorcinol motif of B-ring and methoxy group in A-ring play crucial roles in the binding the enzyme.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Tyrosinase (EC 1.14.18.1) catalyzes two distinct reactions both of which are essential for biosynthesis of melanin. This process proceeds via conversion of tyrosine to 3,4-dihydroxy phenyllalnine (DOPA), a process termed tyrosinase monophenolase activity. The next step is the oxidation of DOPA into DOPA quinone, a process called diphenolase activity. The reactive ortho quinone, DOPA quinone, spontaneously polymerizes to high molecular weight melanin nonenzymatically.¹,² Tyrosinase contains two copper ions that coordinate histidine residues in the active site. The two copper ions are critical for both catalytic activities of tyrosinase. Tyrosinase inhibitors usually chelate the copper ion within the tyrosinase active site, obstruct the substrate–enzyme interaction or prevent oxidation via an electrochemical process.³,⁴ Tyrosinase is involved in numerous cellular processes in a vast number of different species including insect molting and the browning of damaged fruit.⁵ In humans melanin is mainly used to protect skin from UV radiation, but overproduction of melanin results in skin hyperpigmentation, characterized by age spots, melasma and chloasma. Tyrosinase is also known to oxidize dopamine to form melanin in the brain. Thus tyrosinase is also implicated in the pathogenesis of Parkinson’s disease and related neurodegenerative disorders.⁶ Accordingly, control of melanin formation is directly linked to human disease prevention and fruit preservation.

It has been observed that hydroxylated flavonoids are a class of compounds that display potent tyrosinase inhibition because they share structural similarities with L-tyrosine and they are very good donor ligands. In the course of searching for new tyrosinase inhibitors, we found out that the extracts of Campylotropis hirtella showed significant tyrosinase inhibition. C. hirtella has been cultivated as food ingredient in subtropical parts of China such as the...
Yunnan, Sichuan and Guizhou province. Its roots are used as a traditional Chinese medicine to treat diseases such as irregular menstruation, dysmenorrhea, metrorrhagia and gastric ulcers.\(^7\),\(^8\) Its main bioactive constituents are tannin, triterpene, flavonoids, coumarins, lignan, and C-glycosylflavones, many of which have been proven to have immunosuppressive activities and inhibit prostate specific antigen.\(^9\),\(^10\) Although it is an edible, polyphenol rich plant, its biological functions have not been investigated intensively. There is no report about tyrosinase inhibitors being derived from \(C.\ hirtella\). The aim of this study was to investigate phenolic metabolites of \(C.\ hirtella\) to identify a new lead structure that can elicit tyrosinase inhibition and thus suppress melanogenesis in B16 melanoma cells. In the course of the work we successfully identified several potent inhibitors and the inhibition kinetics of the most active compounds were fully characterized. Furthermore, we explored the binding mode of most active compounds (1 and 3) at active site of enzyme. These compounds also showed antimelanogenesis efficacy in a cell culture model.

2. Results and discussion

Polyphenol compounds have been attractive targets for tyrosinase inhibitors because of their structural similarity with tyrosine and antioxidant properties.\(^11\),\(^12\) Many of these efforts have focused on finding a lead structure from abundant polyphenols in plants. In the course of a screening program geared at finding a lead structure for tyrosinase inhibition, the methanolic extract of root barks of \(C.\ hirtella\) (80% inhibition at 20 μg/ml) showed a potent inhibition against mushroom tyrosinase. Fourteen phenolic compounds were purified from the most potent extract (methanol) and their structural elucidation was performed through 2D-NMR results in conjunction with previously reported data (Supplementary material).\(^13\),\(^14\) Among of them, four isoflavonoids (1–4) showed potent inhibition of tyrosinase. These compounds were elucidated to be 3-geranyl-5,7,2’4’-tetrahydroxyisoflavone (1), 3-geranyl-5,7,2’,5’-tetrahydroxy-diosyflavone (2), neorauflavane (3), (6)-(3-(3,7-dimethylocta-2,6-dienyl)-2,4-dihydroxyphenyl)-3,5,7-trihydroxy-chroman-4-one (4), as shown in Figure 1. The most active compound was found to be neorauflavane (3). This compound is 400-fold more active as compared to kojic acid, a commonly used positive control for tyrosinase inhibition, against the monophenolase activity of tyrosinase (see Fig. 2).

Compound 3 was first reported in 1974, but no detailed spectroscopic data have yet been reported and there have been no reports about its biological function so far.\(^14\) Thus we briefly describe structural elucidation of 3. Compound 3 had the molecular formula \(C_{14}H_{15}O_8\) and 4 degrees of unsaturation, as deduced from HREIMS (m/z 354.1467 [M^+]).\(^14\)CNMR data including DEPT experiments showed the presence of twenty one carbon atoms: two methylenes (sp²), seven methines (sp), three methyls (sp³), and nine quaternary carbons. The extra 4 degrees of unsaturation after counting C–C double bonds were ascribed to tetracyclic skeleton. The presence of a resorcinol group was confirmed by ABX coupling between H-3’, H-5’, and H-6’. Isoflavan motif was deduced from proton coupling networks across H-2 (δH 3.98, 4.26), H-3 (δH 3.40) and H-4 (δH 2.78, 2.96) in the COSY spectrum. The location of C5-OCH3 was proved by HMBC correlation of OCH3 (δH 3.72) with C-5 (δc 154.11), and C-5 with H-4” (δH 6.51). The CD spectrum showed a positive cotton effect at 281 nm and a negative cotton effect at 241 nm, suggesting a R-configuration at C-3.\(^16\)

Compounds 1–4 showed a dose dependent inhibitory effect on tyrosinase. Figure 2 shows that neorauflavane 3 inhibited enzyme much more significantly than the other inhibitors (1, 2 and 4) and kojic acid. In fact, compound 3 was 400-fold more active than kojic acid (IC50 = 13.2 μM) against monophenolase. Polyphenols constitute the largest group of tyrosinase inhibitors found so far. It is known that inhibitory activities depend on the chemical skeleton, position of hydroxyl groups, and additional substitutions.\(^17\) Remarkably, despite the rich literature surrounding polyphenol tyrosinase inhibitors, compound 3 is actually the most potent member of this class.\(^17\) The two most potent compounds (1 and 3) could perform a dual role, inhibiting both monophenolase and diphenolase. For example, neorauflavane 3 inhibited monophenolase (IC50 = 30 nM) and diphenolase (IC50 = 500 nM), respectively. The geranylated isoflavane 1 also inhibited monophenolase and diphenolase with IC50 values 2.9 and 128.2 μM, respectively. However, isoflavane 2 was found to be much less effective on monophenolase (IC50 = 92 μM) and diphenolase (IC50 > 200 μM) than its corresponding isoflavane 3. Another isoflavane 4 bearing hydroxyl group on C-3 position inhibited tyrosinase efficiently with 18.4 μM of IC50.

All inhibitors manifested a similar relationship between enzyme activity and enzyme concentration. The inhibition of monophenolase by compound 3, the most potent inhibitor (Kᵢ = 19 nM), is illustrated in Figure 3, representatively. This was done by using both Lineweaver–Burk and Dixon plots. As shown in Figure 3A, the kinetic plot shows that compound 3 is competitive inhibitor. This is because increasing concentration of 3 resulted in a family of lines with a common intercept on the 1/V axis but with different gradients (Fig. 3A). The Kᵢ value of 3 was determined to be 19 nM by using a Dixon plot (Table 1). The inhibitory behaviors of the isolated compounds on tyrosinase-catalyzed oxidation of L-DOPA were subsequently investigated. Compounds 1, 3 and 4 were found to also be competitive inhibitors of diphenolase. As shown in Figure 4, the Lineweaver–Burk plots of 1/V versus 1/[S] resulted in a family of straight lines with the same y-axis intercept, as illustrated, respectively, for the most potent inhibitor 3. The Kᵢ value of 3 was estimated by Dixon plots to be 130 nM.

To further investigate the inhibition mechanism, the time dependence of the inhibition by inhibitor 3 was subsequently
Inhibitory effects of compounds 1–4 on tyrosinase activities

Table 1

Inhibitory effects of compounds 1–4 on tyrosinase activities

<table>
<thead>
<tr>
<th>Compds</th>
<th>l-Tyrosine</th>
<th>l-DOPA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (µM)</td>
<td>Inhibition type (K&lt;sub&gt;i&lt;/sub&gt;, µM)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>2.9 ± 0.3</td>
<td>Competitive (1.8)</td>
</tr>
<tr>
<td>2</td>
<td>92.0 ± 0.2</td>
<td>Competitive (49.7)</td>
</tr>
<tr>
<td>3</td>
<td>0.03 ± 0.006</td>
<td>Competitive (0.019)</td>
</tr>
<tr>
<td>4</td>
<td>18.4 ± 0.5</td>
<td>Competitive (7.5)</td>
</tr>
<tr>
<td>Kojic acid&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.2 ± 0.8</td>
<td>Not tested</td>
</tr>
</tbody>
</table>

<sup>a</sup> All compounds were examined in a set of experiments repeated three times; IC<sub>50</sub> values of compounds represent the concentration that caused 50% enzyme activity loss.

<sup>b</sup> Values of inhibition constant.

<sup>c</sup> Kojic acid is positive control.

Figure 3. (A) Lineweaver–Burk plots for the effect of compound 3 on the monophenolase activity of tyrosinase. (B) Inhibition as a function of preincubation time (○: 0, ▼: 5, △: 15, ■: 30, □: 45, ○: 60 min) for compound 3 at 35.0 nM. Inset: ○: 0, ▼: 35.0 nM. (C) Time course of the inactivation of tyrosinase by 3 (○: 0, ▼: 17.5, △: 35.0, ■: 70.0, □: 140.0 nM). Inset: plot of K<sub>obs</sub> as a function of inhibitor 3 concentration.

Figure 4. (A) Dixon plots for the inhibition of the diphenolase activity of tyrosinase by compound 3. (B) Dixon plots for the inhibition of the diphenolase activity of tyrosinase by compound 3.

by measuring residual activity of the enzyme treated with different concentrations of 3 (0, 17.5, 35, 70 and 140 nM) over different time points. The resulting data were fit to (1) and (2) to determine K<sub>obs</sub>:<sup>18</sup>

\[
\frac{[P]_t}{[P]_0} = \left( \frac{V_i}{V_s} \right) + \frac{(V_i - V_s)}{k_{obs}} \left( 1 - e^{-k_{obs}t} \right) \tag{1}
\]

\[
\frac{V}{V_i} = e^{-k_{obs}t} \tag{2}
\]

where [P]<sub>t</sub> is the concentration of product formed, [E] is the total enzyme concentration, V<sub>i</sub> is the initial velocity, V<sub>s</sub> is the steady state velocity, t is time, k<sub>obs</sub> is the exponential rate constant for equilibration. Then, we identified which of four possible inhibition modes (uninhibited, simple reversible show binding, enzyme isomerization, and mechanism-based inhibition) that can lead to time dependent inhibition kinetics was at play. A plot of k<sub>obs</sub> versus inhibitor showed no deviation from linearity, the mechanism can be modeled by a simple reversible slow binding model. Given these parameters, k<sub>app</sub> is a function of both the on and off rates of the inhibitor and thus the parameters k<sub>1</sub> (second order on rate), k<sub>4</sub> (off rate) and K<sub>app</sub> can be fitted to Eqs. 3 and 4. We thus established the following parameters: k<sub>1</sub> = 0.0033 nM<sup>−1</sup> min<sup>−1</sup>, k<sub>4</sub> = 0.0049 min<sup>−1</sup> and K<sub>app</sub> = 1.48 nM for compound 3.

Enz + I \[\xrightarrow{k_3}\] Enz − I

\[
k_{obs} = k_3 + \frac{k_4[I]}{K_1^{app}} \tag{3}
\]

\[
K_1 = \frac{k_4}{k_3} \tag{4}
\]
pigment formation in melanoma cells. Both compounds elicited a significant reduction in melanin content with IC50 = 13.0 \mu M and IC50 = 1.48 \mu M for compounds 1 and 3, respectively. They were also not toxic to B16 cells at these concentrations.

To further understand the binding modes of these inhibitors, we explored the binding mode of compounds 1 and 3 at the active site of the enzyme. The geranylated isoflavanone 1 also showed a competitive characteristic feature and a typical progress curve of simple reversible slow-binding inhibition against tyrosinase. The second candidate, isoflavonanone 3 was reasonably potent, but has the added advantage of being the most abundant secondary metabolite in C. hirtella. Both inhibitors manifested competitive, simple reversible slow-binding inhibition against tyrosinase.

The geranylated isoflavanone 1 also showed a competitive characteristic feature and a typical progress curve of simple reversible slow-binding inhibition against tyrosinase. The second candidate, isoflavonanone 3 was reasonably potent, but has the added advantage of being the most abundant secondary metabolite in C. hirtella. Both inhibitors manifested competitive, simple reversible slow-binding inhibition against tyrosinase.

### 3. Conclusion

This study validated C. hirtella as a significant source of tyrosinase inhibitors. We isolated two potent inhibitors from this important plant. The first candidate, neorauflavane 3 showed not only very low nanomolar inhibition against tyrosinase but also had strong depigmentation activity in B16 melanoma cells. The second candidate, isoflavonanone 1 was reasonably potent, but has the added advantage of being the most abundant secondary metabolite in C. hirtella. Both inhibitors manifested competitive, simple reversible slow-binding inhibition against tyrosinase.

### 4. Materials and methods

#### 4.1. Chemical and instruments

1D and 2D NMR spectra were recorded on a Bruker (AM 500 MHz) spectrometer (Billerica, MA), using acetone-D6, as solvent and tetramethylsilane (TMS) as an internal standard. Electron ionization (EI) and El high resolution (HR) mass spectra were obtained on a JEOL JMS-700 instrument (JEOL, Tokyo, Japan). Optical rotations were measured on a Perkin-Elmer 343 polarimeter (Perkin-Elmer, Bridgeport, USA). Column chromatography was performed using silica gel (230–400 mesh; Merck Co., Darmstadt, Germany), YMC-gel ODS-A (5–7.5 \mu m; YMC), and Sephadex LH-20 (GE GE healthcare Bio-Science AB, Uppsala, Sweden). Thin-layer chromatography (TLC) was performed on precoated silica gel 60 F254 (0.25 mm; Merck). Spots were detected by UV light (245 nm) and spraying with 10% H2SO4 followed by heating. Enzymatic assays were carried out on a SpectraMaxM3 Multi-Mode Microplate Reader (Molecular Devise, USA). All other chemicals were of analytical grade and used as received.

#### 4.2. Extraction and isolation

Tyrosinase inhibitors (1–4) were isolated from methanol extract of C. hirtella root barks by MPLC over silica gel and reversed phase silica gel. The air dried root bark (0.5 kg) was extracted with MeOH (5 L × 3) at room temperature for one week. The combined filtrate was concentrated to yield a dark red gum (58 g). The MeOH extract (30 g) was subjected to column chromatography on silica gel (10 × 40 cm, 230–400 mesh, 750 g) using n-hexane to ethylacetic acid gradient (50:1 → 1:1) to give 5 fractions (A–E). Fraction C (3.2 g) was fractionated via MPLC using a C18 column (130 g) with elution using a gradient of increasing MeOH (0–100%) in H2O and 20 ml/ min flow rate to afford 30 subfractions (B1–B30). Subfractions B11–B15 (520 mg) enriched with compounds 3 and 4 were further chromatographed over MPLC using a gradient of MeOH (0–100%) in H2O to afford compounds 3 (28 mg) and 4 (17 mg). Subfractions B17–B21 (920 mg), enriched with compounds 1 and 2 were further chromatographed over a Sephadex LH-20 column using MeOH–H2O (90:10) to afford compounds 1 (96 mg) and 2 (12 mg). The remaining twelve compounds were also isolated and evaluated on tyrosinase inhibition.
Compound 1: pale yellow oil; HREIMS [M]+ 424.1885 (calcd for C25H28O6 424.1886); 1H NMR (500 MHz, CDCl3) δ 1.51 (3H, s, H-9), 1.58 (3H, s, H-10), 1.73 (3H, s, H-4), 1.99 (2H, m, H-5), 2.11 (2H, m, H-6), 3.39 (2H, d, J = 6.59 Hz, H-1), 3.92 (1H, t, J = 8.32, 4.16 Hz, H-3), 4.60 (2H, dd, J = 11.67, 4.39 Hz, H-2), 4.73 (2H, dd, J = 11.72, 4.47 Hz, H-2), 4.97 (1H, br s, H-7), 5.86 (1H, br s, H-8), 6.33 (1H, d, J = 8.08 Hz, H-5), 7.08 (1H, d, J = 8.27 Hz, H-6). 13C NMR (125 MHz, CDCl3) δ 197.56 (C-4), 165.86 (C-7), 165.51 (C-5), 163.59 (C-8a), 155.76 (C-2), 139.61 (C-3), 132.41 (C-8), 125.95 (C-6), 124.15 (C-2), 121.79 (C-1), 115.99 (C-3), 115.43 (C-1'), 109.07 (C-5'), 102.32 (C-4'), 97.32 (C-6'), 95.80 (C-8), 70.17 (C-2), 45.67 (C-3), 40.09 (C-5'), 26.78 (C-1'), 26.03 (C-9'), 23.18 (C-6'), 18.07 (C-10'), 16.60 (C-4').

Compound 2: pale yellow oil; HREIMS [M]+ 422.1729 (calcd for C25H26O6 422.1729); 1H NMR (500 MHz, acetone-D6) δ 1.56 (3H, s, H-9), 1.62 (3H, s, H-10), 1.79 (3H, s, H-4), 2.06 (2H, m, H-5), 2.09 (2H, m, H-6'), 3.46 (2H, d, J = 7.07 Hz, H-1'), 5.09 (1H, t, J = 14.00, 6.98 Hz, H-7'), 5.34 (1H, t, J = 14.16, 7.16 Hz, H-2'), 6.27 (1H, br s, H-6), 6.34 (1H, br s, H-8), 6.51 (1H, d, J = 8.31 Hz, H-5'), 6.94 (1H, d, J = 8.31 Hz, H-6'), 8.18 (1H, s, H-2). 13C NMR (125 MHz, acetone-D6) δ 183.31 (C-4), 165.95 (C-7), 163.99 (C-5), 163.72 (C-8a), 159.57 (C-2), 158.24 (C-4), 157.26 (C-2), 135.16 (C-3'), 131.97 (C-8'), 129.47 (C-6'), 125.64 (C-7'), 124.50 (C-3), 123.86 (C-2'), 111.76 (C-3), 108.91 (C-1'), 106.20 (C-5'), 100.67 (C-4a), 96.98 (C-6), 95.05 (C-8), 41.05 (C-5'), 27.90 (C-1'), 26.21 (C-6'), 23.78 (C-9'), 18.11 (C-10'), 16.71 (C-4').

Compound 3: colorless powder; HREIMS [M]+ 354.1467 (calcd for C21H22O5 354.1467); [α]D = +3.8 (c 0.55, in CHCl3); 1H NMR (500 MHz, CDCl3) δ 1.39 (3H, s, H-9), 1.40 (3H, s, H-8), 2.78 (2H, dd, J = 15.95, 10.60 Hz, H-4), 2.96 (2H, dd, J = 16.00, 3.90 Hz, H-4), 3.40 (1H, br s, H-3'), 3.72 (3H, s, OCH3), 3.98 (2H, t, J = 10.05 Hz, H-2), 4.26 (1H, d, J = 9.85 Hz, H-2), 5.51 (1H, d, J = 9.90, H-5'), 6.18 (1H, s, H-8), 6.26 (1H, br s, H-3'), 6.31 (1H, d, J = 8.0 Hz, H-6), 7.08 (1H, d, J = 8.27 Hz, H-6).

Figure 6. Molecular docking of compounds 1 and 3 to mushroom tyrosinase. Binding conformations of (A) compound 1 and (B) compound 3 to the active site. Compounds 1 and 3, and copper ions are colored in green, cyan, and brown, respectively. Key residues interacting with the inhibitor are shown as white and pink (copper ion binding residues); Hydrogen bond is denoted by green dashed line. π–π and π–σ interactions are represented as yellow and orange dashed lines, respectively. Geometry of the active site as surface representation with (C) compound 1 and (D) compound 3. 2D diagrams of molecular interactions for (E) compound 1 and (F) compound 3. Gray, green, dark pink, and light pink dashed lines represent metal, hydrogen bond, π–π, and π–σ interactions, respectively.
Activity \(= \frac{J}{C_{138}} \) ÷ \( C_{104} \) ppm, for each curve; all other parameters were set as default. The interaction energies between the enzyme and two inhibitors were then calculated using Calciate Interaction Energy protocol implemented in DS.4.6. Cell culture

The B16 mouse melanoma cell line was purchased from American Type of Culture Collection (Manassas, VA, USA). The cells were cultured in DMEM supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin (Sigma–Aldrich, St. Louis, USA) at 37°C in a 5% CO₂ humidified incubator.

4.7. Measurement of melanin content

B16 cells were seeded in 6-well plate at a density of 5 × 10⁴ cells per well and incubated overnight. The cells were treated with α-MSH (1 μM) in the presence or absence of various concentrations of compounds for 72 h. The cells were harvested and dissolved in 1 N NaOH containing 10% DMSO at 65°C for 24 h. The melanin content was measured at 415 nm using a microplate reader (Bio-Rad, Hercules, CA, USA).

4.8. Cell viability assay

B16 cells were seeded in 96-well plate and incubated overnight. The cells were then incubated with various concentrations of compounds for 72 h. After incubation, 5 μg/ml of MTT (Amresco, Solon, OH, USA) was then added and the cells were incubated for 3 h. The culture medium was removed and replaced with DMSO for 20 min at room temperature. Absorbance was determined at 595 nm.

Acknowledgements

This research was supported by Agriculture Bio-industry Technology Development Program (No. 315032-04-1-S010) for Ministry of Agriculture, Food and Rural Affairs and Next-Generation BioGreen 21 Program (SSAC, No. PJ01107001), Rural Development
Administration, Republic of Korea. All students were supported by a scholarship from the BK21 plus program.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.11.040.

References and notes

10. Li, X. P.; Xuan, B. X.; Shou, Q. Y.; Shen, Z. W. Fitoterapia 2014, 95, 220.