Supporting Information

Dodoviscin A Inhibits Melanogenesis in Mouse B16-F10 Melanoma Cells

Guirui Yan¹, Jingjie Zhu¹, Laibin Zhang², Zhijian Xu¹, Gaihong Wang¹, Weiliang Zhu¹, Aijun Hou², Heyao Wang¹

Affiliations

¹Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China
²Department of Pharmacognosy, School of Pharmacy, Fudan University, Shanghai, China

Correspondence

Prof. Dr. Heyao Wang
Shanghai Institute of Materia Medica
Chinese Academy of Sciences
555 Zu Chong Zhi Road
Shanghai 201203
China
Phone: +86 21 508 057 85
Fax: +86 21 508 070 88
hywang@mail.shcnc.ac.cn
Materials and Methods

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity was assessed by the method described in [1] with a minor modification. 50 μL of freshly prepared DPPH (Sigma-Aldrich) solution (0.8 mM in methanol) and 50 μL of various concentrations of sample in methanol were mixed and allowed to react for 30 min in the dark. The scavenged DPPH was monitored by measuring the absorbance at 517 nm.

Metal chelating assay

Metal ion chelating effect of dodoviscin A for ferrous ions (Fe$^{2+}$) was measured according to the method described previously [2]. Briefly, 50 μL of various concentrations of dodoviscin A in methanol was added to 50 μL of a solution of 0.75 mM FeSO$_4$. These components were then mixed thoroughly and incubated at room temperature for 5 min. Then, 50 μL of 0.3 mM ferrozine (Sigma-Aldrich) dissolved in methanol was added, mixed by vigorously shaking, and the mixture was left to incubate in the dark at room temperature for another 5 min. The absorbance was measured at 562 nm.
Fig. 1S Antioxidant activities of dodoviscin A.

DPHP radical scavenging activity (A) and ferrozine ion chelating (FIC) ability (B) of dodoviscin A were measured as described. Quercetin (IC$_{50}$ value of 26.87 ± 11.42 μM) and EDTA (IC$_{50}$ value of 23.21 ± 8.91 μM) were used as positive controls. Dodoviscin A did not show significant antioxidant activity up to 400 μM (n=3).
Fig. 2S Effect of dodoviscin A on the phosphorylation of MEK, ERK1/2, AKT, and GSK3α/β in B16F10 cells. Cells were depleted of serum for 10 h and then exposed to 3 μM dodoviscin A for 0-240 min. The expression levels of phospho-MEK, phospho-ERK1/2, phospho-AKT, and phospho-GSK3α/β were examined by Western blot. Equal protein loading was confirmed by actin expression. No visible time dependent changes in the phosphorylation levels of GSK3α/β, MEK, ERK, and AKT were observed (n=3).
References
