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BRIEF COMMUNICATION

A concise total synthesis and PPAR activation activity of hericerin from *Hericium erinaceum*

Zhongyong Zheng[1,2] · Baosong Chen[1,2] · Kai Wang[3] · Li Bao[1,2] · Zhengdi Wang[4] · Liping Xie[4] · Changbin Guo[4] · Hongwei Liu[1,2]

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Abstract

Hericerin is an isoindolinone meroterpenoid alkaloid isolated from medicinal mushroom *Hericium erinaceum* with some bioactivities. Herein, a concise total synthesis of hericerin was described, with four steps and 30% overall yield starting from commercially available methyl 3-hydroxy-5-methoxybenzoate and geranyl bromide. A comprehensive effect of hericerin on HepG2 cell line was observed and confirmed by transcriptomic analysis. Furthermore, hericerin was found to be a new PPARγ agonist.

Isoindolinone, featured as benzo-fused five-membered *N*-heterocyclic skeleton, are widely present in an array of biologically active natural products, such as stachyflin with antiviral activity [1], stachybotrylactam I with HIV-1 protease inhibition activity [2], stachybotrin with neuritogenic properties [3], and staurosporine with inhibition activities against PKC, GSK-3, PKA, PKG, and TPK kinases [4]. Secondary natural products derived from fungi provide a wealth of lead compounds for drug development [5, 6]. Hericerin, an isoindolinone isolated from the well-known edible and medicinal mushroom *Hericium erinaceum* [6], shows various bioactivities such as anti-inflammation, cytotoxicity, and inhibitory activity against pine pollen germination and tea pollen growth [6–9]. Recently, we and other groups found that hericerin was a strong α-glucosidase inhibitor, which may play a beneficial role in the treatment of diabetes [10, 11]. However, the lower yields of hericerin in the fruiting body of *H. erinaceum* along with the multi-step chemical synthesis routes [12–14] limit the further research and development of hericerin as an important leading compound.

Hericerin was first synthesized by Kobayashi et al. employing the geranylated lactone as the starting material that was synthesized by $CuBr_2$-mediated multistep reactions, with a relatively low yield (~3%) [12], and the structure of hericerin was revised to be identical with that of isohericerin based on this total synthesis. In a following report, hericerin was synthesized with a 34% overall yield from commercially available 2-hydroxy-4-methoxybenzaldehyde by employing a ether–phenol rearrangement and a $Pd(OAc)_2$-catalyzed carbonylative ring closure reactions [13]. However, the harsh reaction conditions and the use of environmentally unfriendly CO gas limits the application of this synthetic route [13]. In 2017, Mun et al. used a Mannich reaction and one-pot lactamization to establish the common isoindolinone core structure from the commercially available hydroxybenzoate. Hericerin was further obtained by a Suzuki–Miyaura coupling reaction to link isoindolinone unit with geranyl boronate [14]. In our study, we developed a new route to total synthesis of hericerin.

Scheme 1 describes the total synthesis of hericerin (**1**). According to a previous report by the Lee group [14], the

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✉ Hongwei Liu
liuhw@im.ac.cn

1 State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, No. 1 Beichenxi Road, Chaoyang District, 100101 Beijing, PR China

2 Savaid Medical School, University of Chinese Academy of Sciences, 100049 Beijing, PR China

3 Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Peking University, and the Key Laboratory of Molecular Cardiovascular Science, Ministry of Education, 100191 Beijing, PR China

4 Department of chemistry, Capital Normal University, 100048 Beijing, PR China

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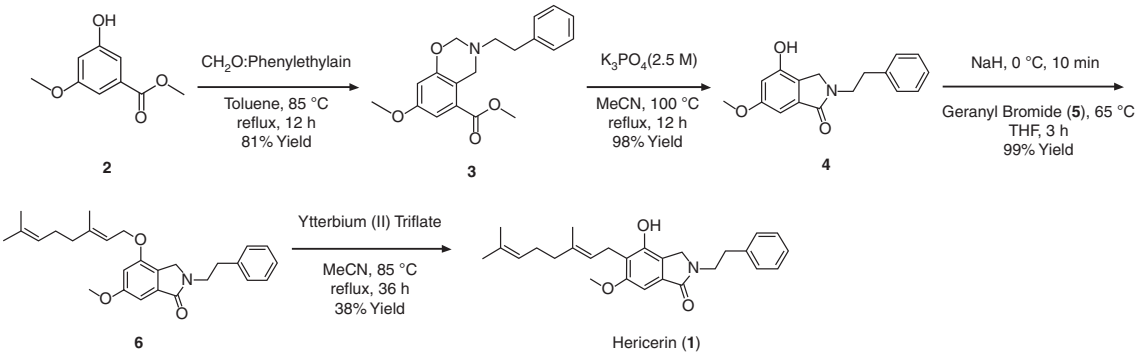

Scheme 1 Concise total synthesis of hericerin

Table 1 Optimization of reaction conditions for the ether–phenol rearrangement

Entry	Catalyst	Time(h)	Reagent	Temperature (°C)	Yield (%)
1	Montmorillonite	24	Toluene	Reflux	–[a]
2	Montmorillonite	24	DMF	130	–
3	Montmorillonite	24	CH_2Cl_2	Reflux	–
4	Montmorillonite K10	8	Toluene	Reflux	–
5	Montmorillonite K10	24	DMF	130	–
6	Montmorillonite K10	24	CH_2Cl_2	Reflux	–
7	Montmorillonite K10	12	CH_2Cl_2	0	–
8	Montmorillonite KSF	24	Toluene	Reflux	–
9	Montmorillonite KSF	24	DMF	130	–
10	Montmorillonite KSF	24	CH_2Cl_2	Reflux	–
11	Montmorillonite KSF	12	CH_2Cl_2	0	–
12	DIBAL-H	12	CH_2Cl_2	0	–
13	DMA	24	benzene	Reflux	–
14	$AlMe_3 + H_3O^+$	8	CH_2Cl_2	−20	–
15	$AlMe_3$	8–12	CH_2Cl_2	From −20 to reflux	–
16	$AlCl_3$	8–12	CH_2Cl_2	From −20 to reflux	–
17	$Bi(OTf)_3$	24	Toluene	Reflux or R.T.	–
18	$Bi(OTf)_3$	24	MeCN	Reflux	–
19	$Yb(OTf)_3$	24	MECN	From –20 to R.T.	–
20	$Yb(OTf)_3$	24	MeCN	Reflux	23%
21	$Yb(OTf)_3$	72	MeCN	Reflux	38%
22	$Yb(OTf)_3$	72	DMSO	130	15%
23	$Yb(OTf)_3$	72	DMF	130	–

[a]Reaction product not obtained

aromatic precursor isoindolinone **4** was synthesized. Due to the strict conditions for the $Pd(dppf)Cl_2$-catalyzed C–C bond coupling reaction, we performed an alkylation reaction with the geranyl bromide **5** and **4** under KOH-basic phase transfer conditions to provide the phenol ether **6**. Next, we attempted a [1,3]-sigmatropic rearrangement of geranyl ethers to *ortho*- and *para*-phenols, unfortunately, the target product was not obtained by frequently used rearrangement agents including montmorillonite and its derivatives, DIBAL-H, and DMA [13, 15–17]. The eletrophilicity of the amide group in **6** might not favor the [1,3]-sigmatropic rearrangement reaction. After exploring a range of metal salts $AlCl_3$, $Bi(OTf)_3$, or $Yb(OTf)_3$ as stronger Lewis acids [18], $Yb(OTf)_3$ was found to transform compound **6** into **1** with a satisfactory yield (38%, Table 1). Herein, $Yb(OTf)_3$ was reported as a [1,3]-sigmatropic rearrangement reagent for geranyl ether while it has been used as a [3,3]-sigmatropic rearrangement reagent in the synthesis of β-amino-α,β,ε,ζ-unsaturated-γ,δ-disubstituted esters [19]. Thus, a concise four-step total synthesis of hericerin was performed, with 30% overall yield starting from commercially available methyl 3-hydroxy-5-methoxybenzoate (**2**) and geranyl bromide (**5**).

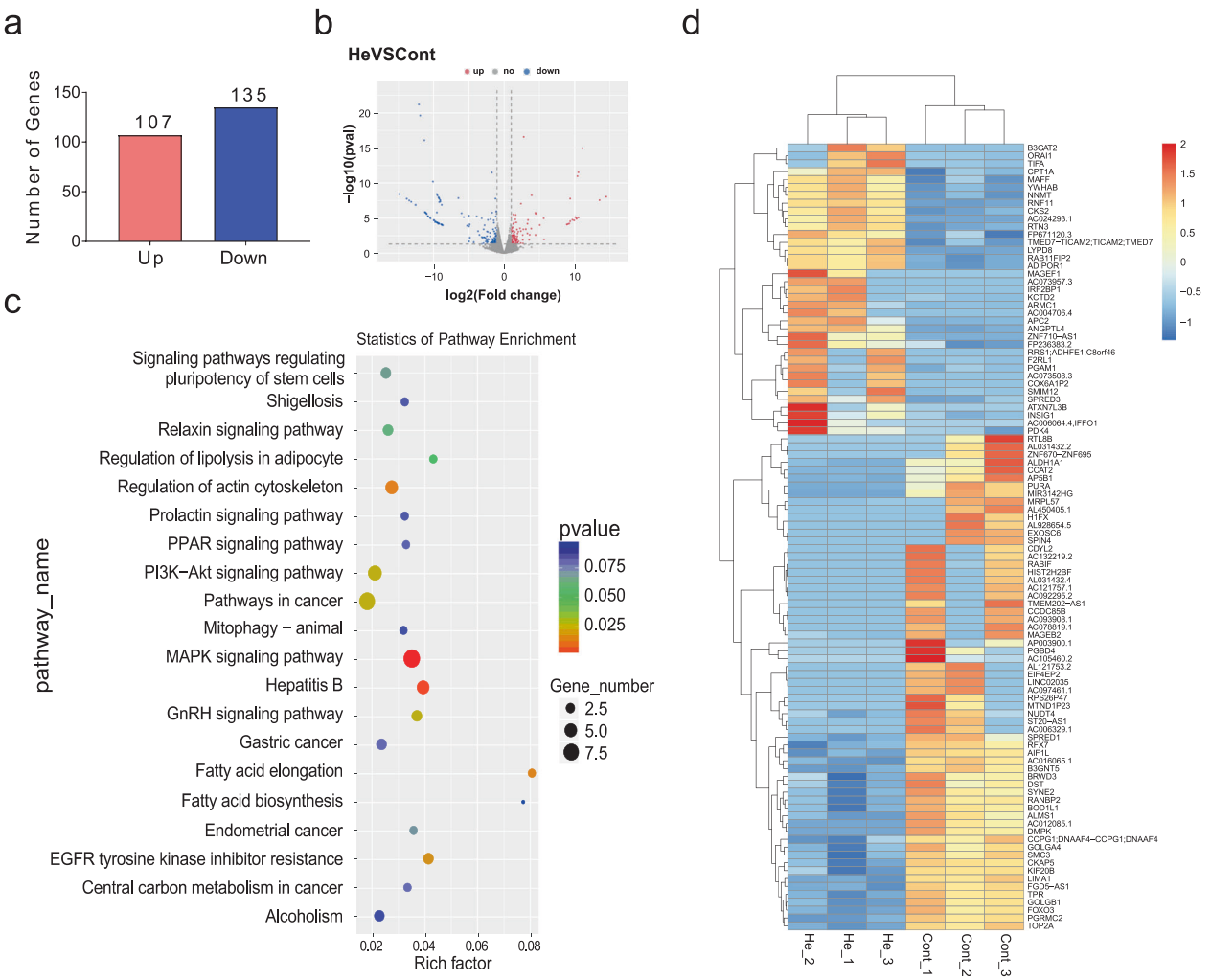

Fig. 1 Transcriptome analysis of the gene expression profile in hericerin-treated HepG2 cell line. **a** Gene expression changes. **b** Volcano Plots. **c** KEGG pathway enrichment analyses of regulated genes. **d** Hierarchical clustering of the differentially expressed genes by heatmap. $N = 3$ per group

Transcriptomic analysis of hericerin on HepG2 cell

To explore new bioactivities of hericerin (**1**), we analyzed transcriptome alterations using high-depth next generation sequencing with an Illumina HiSeq 4000 on HepG2 cell lines. Among the 26,432 genes detected by RNA-seq, hericerin significantly altered the levels of 242 genes ($p < 0.05$; Fig. 1a). Of these, 107 upregulated genes and 135 downregulated genes were differentially expressed by twofold or more (Fig. 1b). A Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of these genes showed biological pathways involved in the lipid and glucose metabolism (e.g., regulation of lipolysis in adipocyte, peroxisome proliferator-activated receptor (PPAR) signaling pathway, PI3K-Akt signaling pathway, MAPK signaling pathway, fatty acid biosynthesis, and fatty acid biosynthesis; Fig. 1c). Further gene analysis also revealed that hericerin treatment significantly decreased the expression of genes participating in lipogenesis (*Was*, *Acpp*, *Cd74*, *Ear2*, *Wisp1*, *Trp*, *Lima1*, *Top2a*, and *Aif1l*). These data confirmed the beneficial effect of hericerin on lipid metabolism.

Notably, mRNA expression of genes involved in PPARγ signaling pathway including *Tca1*, *Cd79b*, *Camk1g*, *Cdh1*, *Ptprn*, and *Hs3st1* was upregulated (Fig. 1d and Supplementary information). PPARγ, one of the members of PPAR family, functions as a regulator of fat gene programs and plays an important role in the control of lipid and glucose homeostasis [20]. Above results indicated that hericerin be a potential PPARγ agonist. To verify the effect on PPARγ, hericerin was assayed in vitro for PPARγ transactivation activity. As shown in Fig. S9, hericerin showed dose-dependent activation of GAL4-PPARc by ~7.47-fold with an EC_{50} of 2.17 μM. Thus, hericerin is defined as a strong PPARγ agonist with potential hypoglycemic and hypolipidemic effects.

In summary, a revised concise total synthesis route for hericerin was reported with four-step and 30% overall yield. The transcriptomic analysis on HepG2 cell line together with the PPARγ activation assay confirmed hericerin to be a new PPARγ agonist with a new scaffold.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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