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Selesconol, a Fungal Polyketide That Induces Stem Cell Differentiation

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Supporting Information

ABSTRACT: Owing to the negligible or acceptable immunogenicity, small molecules capable of inducing the differentiation of undifferentiated stem cells into organ-specific cell types are particularly promising in developing replacement therapy, but such compounds with undescribed architectures are extremely rare. Selesconol (**1**) is discovered from the culture of *Daldinia eschscholzii* IFB-TL01 as a skeletally unprecedented inducer for the differentiation of rat bone marrow mesenchymal stem cells into neural cells, with its unique framework clarified to derive from the intermediate tautomerization of the dalesconol A biosynthetic pathway.

Stem cells are unspecialized and able to exist in the undifferentiated state before differentiating in an organ-specific manner into more specialized cell types upon exposure to instructive signals including lineage-specific transcription factors.[1] Because of this, stem-cell-based replacement therapy is widely expected to be effective in treating currently life-threatening diseases such as Alzheimer's and Parkinson's diseases, diabete, cancer, and heart falure.[2] Distinct from most genetic approaches, many small molecules including some prescribed small-molecule drugs may reversibly regulate specific functions of proteins (or protein complexes) with desired precision. Thus, there is an urgent need to identify promising small molecules that can modulate the stem cell fate and developmental potential.[2]

Many kinds of natural products and some of their substructures have been demonstrated to benefit nerve regeneration.[3−5] Some stem cell differentiations can be regulated by diverse nature-derived polyphenols such as resveratrol,[6] ellagic acid,[7] epigallocatechin-3-gallate,[8] and curcumin.[9] But to our knowledge, few reports deal with fungal polyphenol(s) capable of modulating stem cell differentiations, although many fungi are efficient producers of phenolic secondary metabolites.[10] *Daldinia eschscholzii* IFB-TL01, derived from the mantis (*Tenodera aridifolia*) gut, has been investigated to afford arrays of bioactive polyphenols including the immunosuppressive dalesconols A and B.[10] Based on the fungal genomic information, the dalesconol architecture has been addressed to be constructed through stepwise couplings of naphthol radicals.[12] The dalesconol biosynthetic pathway was therefore selected as the topic of the present investigation in view of the shunt product formation from the intermediate tautomerism.[11] Presented herein is the discovery of selesconol (**1**), a skeletally unprecedented polyphenol capable of inducing the differentiation of rat bone marrow mesenchymal stem cells (rMSCs) into neuronal cells, thereby being valuable for the development of cell replacement-based therapies.

The work began with a 500 L scaled-up growth of *D. eschscholzii*, followed by extraction with ethyl acetate. Evaporation of the solvent from the extract gave a residue which was fractionated through repeated chromatographies to give selesconol (**1**, 65 mg) as a polyketide with an unprecedented skeletal class; see the Supporting Information (SI).

Selesconol (**1**) was evidenced to have a molecular formula of $C_{29}H_{18}O_6$ from the protonated molecular ion at m/z 463.1175 (calcd 463.1176) displayed using high resolution electrospray ionization mass spectrometry (HR-ESI-MS). The ultraviolet (UV) spectrum of **1** strikingly differed from that of dalesconol A[11a] owing to its prolonged conjugation system (Figure 3). Subsequent structure determination was accommodated by our correlative interpretation of its ^{1}H and ^{13}C NMR, ^{1}H−^{1}H COSY, ROESY, HSQC, and HMBC spectra (Table S1 and Figures S5−S11). The spectrally elucidated structure of **1** was substantiated by its single crystal X-ray diffraction analysis with Cu Kα radiation (Figure 1). Surprisingly, the space group (Cc)

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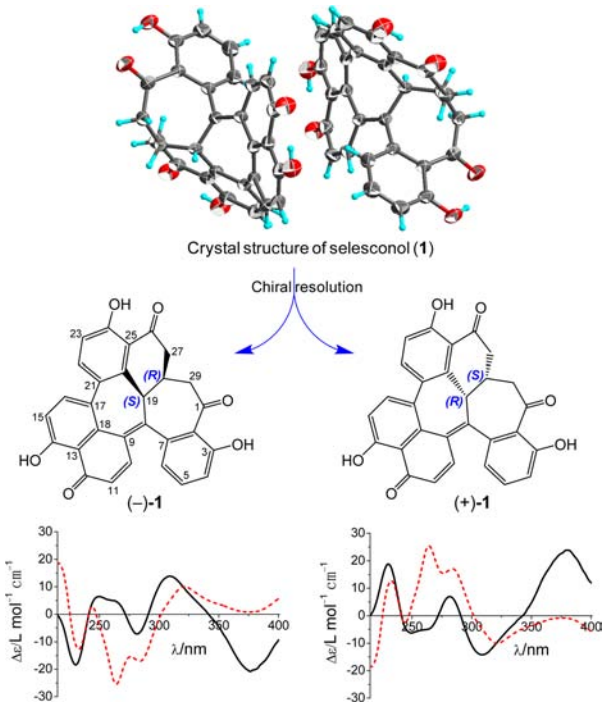

Figure 1. Absolute configuration of (−)-**1** and (+)-**1** derived from the crystal selesconol racemate was assigned by comparison between the recorded (solid line) and computed ECD spectra (dotted line).

of crystal **1** indicated its racemate nature (see the SI). Chiral HPLC separation of **1** afforded the corresponding enantiomers (+)-**1** and (−)-**1** (Figure 1), which were addressed to have (19*R*,28*S*)- and (19*S*,28*R*)-configurations, respectively, by comparing their electronic circular dichroism (ECD) spectral data with those calculated for all of its optional stereoisomers (Figure 1 and Tables S2). The recorded positive Cotton Effects (CEs) at 230, 282, and 375 nm in the ECD spectra were reproduced by the calculated counterparts around 233, 287, and 376 nm, respectively. These positive CEs arise from the electronic transitions from filled C═C molecular orbital and lone pair orbital of oxygen ($\pi_{C=C}$ and n_o) to the antibonding C═C and C═O orbitals ($\pi^*_{C=C}$ and $\pi^*_{C=O}$) (MO 120 → 129, MO 115 → 123, and MO 120 → 126). The negative CEs around 249 and 309 nm in the acquired CD spectra matched well the computed counterparts centered at 246 and 320 nm, respectively. The electronic transitions from σ_{C-C} and $\sigma_{C=C}$ to $\pi^*_{C=C}$ and $\pi^*_{C=O}$ orbitals were predicted to contribute to these absorption bands (MO 109 → 121 and MO 120 → 124).

With the absolute configuration assigned, we were curious about whether **1** could be an artifact formed from dalesconol A during the fractionation procedure. To address whether the assumed transformation was triggered by acid or base,[13] dalesconol A and **1** were separately treated with acetic acid (1 mol), trifluoroacetic acid (1 mol), potassium carbonate (50%), and sodium hydroxide (50%). No transformation between the two compounds could be detected in any of those treatments. To clarify whether the presumed conversion could be facilitated by fungal enzyme or photocatalysts,[14] dalesconol A and **1** were exposed separately to intra- and extracellular fungal proteins, and to 24 h irradiations with fluorescent light (26 W) in the presence of one of the photocatalysts ($Ir(ppy)_3$, $Ir(p\text{-}MeO\text{-}ppy)_3$, and $Ir(ppy)_2(dtbbpy)PF_6$). However, no interconversion between **1** and dalesconol A could be found in any of those experimentations (Figure 2).

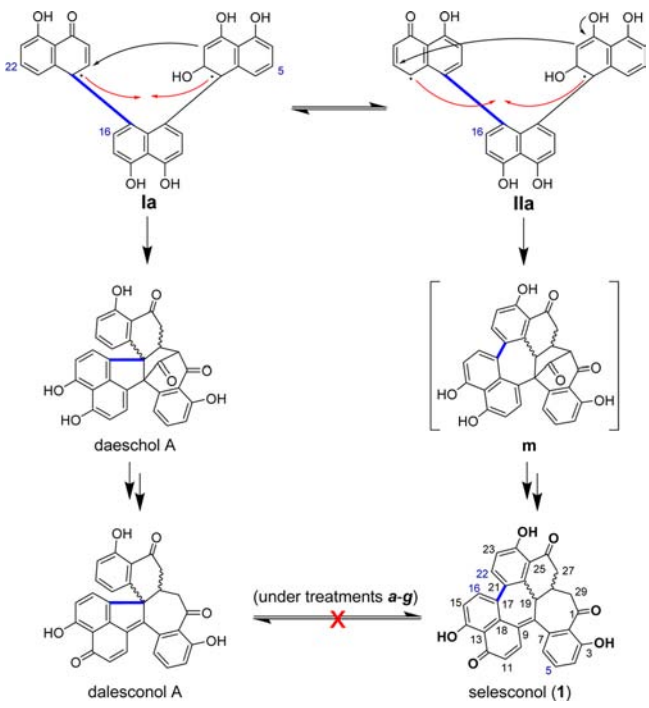

Figure 2. Tautomerism of isomeric precursors **Ia** and **IIa**. Dalesconol A and selesconol (**1**), derived from **Ia** and **IIa**, respectively, are irreversible under any treatments with (a) 1 mol of CH_3CO_2H, (b) 1 mol of CF_3CO_2H, (c) 50% K_2CO_3, (d) 50% NaOH, (e) intracellular and extracellular (f) fungal proteins, and (g) fluorescent light (26 W) irradiations with separate exposure to $Ir(ppy)_3$, $Ir(p\text{-}MeO\text{-}ppy)_3$, and $Ir(ppy)_2(dtbbpy)PF_6$ at 1 mol %.

Accordingly, selesconol (**1**) could only be the shunt product originated from the dalesconol A biosynthetic pathway, which was undetected during our previous investigations.[10] By analogy with the dalesconol A biosynthesis,[12] we hypothesized that **1** might have been derived from the promiscuous couplings of naphthol radicals as well (Figure 3A). To confirm this assumption, we deleted the *pksTL* gene encoding polyketide synthase catalyzing the fungal generation of 1,3,6,8-tetrahydroxynaphthalene, the common precursor for constructing dalesconols A−C.[12] The mutant strain (*pksTL*KO) was unable to form **1** (Figure 3B). Next, the *lacTL* gene encoding fungal laccase catalyzing naphthol radical couplings[5] was deleted from *D. eschscholzii*, and the obtained mutant strain was incapable of producing **1** (Figure 3). Such experimental observations pinpointed that **1** was a shunt metabolite from the dalesconol A biosynthetic pathway.[12]

The *α,β*-unsaturated carbonyl moiety of **1** may be susceptible to some nucleophilic amino and mercapto groups in protein peptide chains.[15] Interestingly, the *α,β*-unsaturated ketone(-like) substructures happen to be motifs of some stem cell differentiation inducers such as retinoic acid (RA),[2] vitamin C,[16] NanoScript,[17] and others.[18] Selesconol (**1**) was therefore assayed for the induction of the stem cell differentiation since the small molecule inducers are indispensable to the development of cell replacement-based therapies under the rising public expectation.[2,16−18] We tried to determine the cytotoxicity of (+)- and (−)-selesconols toward both stem and differentiated cells, but we failed to calculate the corresponding

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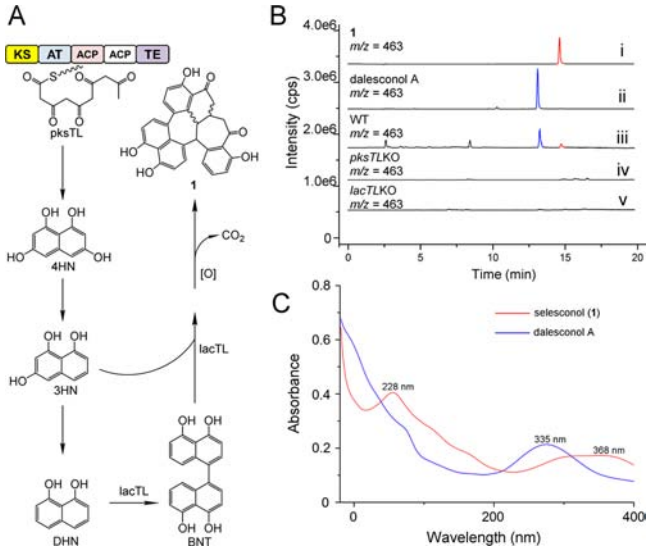

Figure 3. Proposed biosynthetic pathway of selesconol (**1**). (A) Schematic illustration of the selesconol biosynthesis governed by *pksTL* and *lacTL*. (B) Comparisons of LC-UV-MS profiles (EIC mode) of wild-type (WT), *pksTL* gene knockout (*pksTL*KO), and *lacTL* gene knockout (*lacTL*KO) strains of *D. eschscholzii*. The authentic materials of **1** and dalesconol A were shown in trace i and ii, respectively. (C) UV spectra of **1** and dalesconol A.

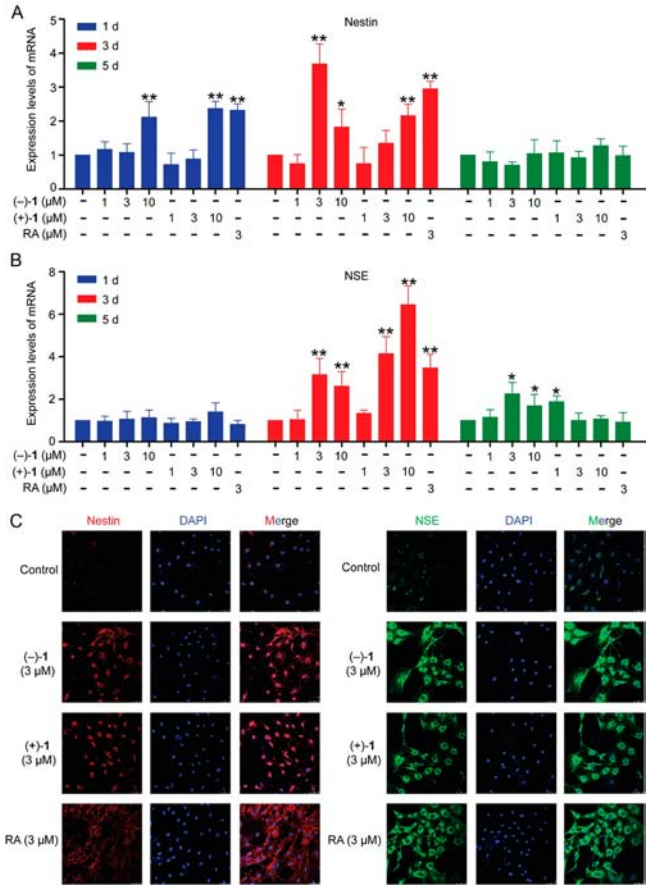

Figure 4. Effects of (+)-**1**, (−)-**1**, and RA on the *in vitro* differentiation of rat bone marrow mesenchymal stem cells into neuronal cells. After the 3 day treatment with (+)-**1**, (−)-**1** and RA, respectively, the mRNA expression level in differentiated rMSCs are measured by Q-PCR method. (A) The effect of (+)-**1**, (−)-**1** and RA on the mRNA expression of Nestin (a marker for neural stem cells). (B) The effect of (+)-**1**, (−)-**1** and RA on the mRNA expression of NSE (a marker for neurons); After the 3 day exposure to (+)-**1**, (−)-**1** and RA, respectively, the proteins expression level in differentiated rMSCs are measured by immunofluorescence cytochemistry method. (C) The effect of (+)-**1**, (−)-**1** and RA on the protein expression of Nestin and NSE in the differentiated rMSCs.

IC_{50} value since selesconols displayed almost no inhibition on the proliferation of stem and differentiated cells even after a 48 h treatment at the highest dose of 30 μmol/L (μM). In particular, mesenchymal stem cells (MSCs) can be differentiated into neuron-like cells under specific conditions, thereby holding great promise for treating neuron loss related disorders such as Alzheimer's and Parkinson's diseases.[19] Therefore, selesconol enantiomers (+)-**1** and (−)-**1** were tested *in vitro* as described[20] for the inducing effect on the differentiation of rat bone marrow mesenchymal stem cells (rMSCs) into neuronal cells. The rMSCs were cultured with separate exposures to (+)-**1** or (−)-**1** with RA coassayed as a positive control. A dose range of 1−10 μM was taken for (+)-**1**, (−)-**1**, and RA (Figure 4) since they were tested to be safe to rMSCs up to 30 μM. Using real-time quantitative PCR (Q-PCR), we evaluated the mRNA expression level of markers for neural stem cells and neurons in rMSCs. As a result, (+)-**1**, (−)-**1** could induce a high mRNA expression of Nestin and neuron specific enolase (NSE) in rMSCs on the third day after the treatment, with the potency comparable to those of RA (Figure 4A and 4B).

To reinforce the finding, we measured the effect of (+)-**1** and (−)-**1** on the protein expression of Nestin and NSE in differentiated rMSCs using the immunofluorescence method. The obtained results showed that Nestin and NSE are more highly expressed in the differentiated rMSCs under the stimulation of (+)-**1** and (−)-**1** with the magnitude similar to those of RA (Figure 4C).

In summary, we report the discovery of selesconol (**1**) as a new inducer for the differentiation of rat bone marrow mesenchymal stem cells into neural cells. Following the structure elucidation, enantiomeric resolution, and absolute configuration clarification, selesconol (**1**) is biosynthetically deduced, and its unique skeleton is formed from the tautomerization of the triple naphthol composed intermediate leading dominantly to the biosynthesis of dalesconol A. The endeavor visualizes as well another challenging issue concerning how to dictate or at least discernibly regulate the tautomerization direction of natural product bioassembly lines to create more unprecedented skeletal classes with remarkably expanded chemical space and of important biological properties.

The privileged structure of natural products enshrines usually biological functions if bioassay models are diverse enough, including those for testing for impact on the survival of their producing organisms. Though in its infancy, the search for stem cell differentiation inducers from nature has been kindled by such effects of phenolic (e.g., resveratrol, epigallocatechin-3-gallate, and curcumin; see above) and nonphenolic natural products (e.g., forskolin).[21] The present discovery of selesconol (**1**) as a skeletally undescribed natural modulator of stem cell differentiations may activate to some extent this frontier of natural product sciences.

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■ ASSOCIATED CONTENT

Supporting Information

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The general methods and fractionation procedure, 1D and 2D NMR spectra, crystallographic details (PDF)

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Author Contributions

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Notes

The authors declare no competing financial interest.

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