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Total syntheses and endoplasmic reticulum stress suppressive activities of hericenes A−C and their derivatives

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ABSTRACT

We report the syntheses and neuroprotective activities of hericenes and their derivatives against endoplasmic reticulum (ER) stress-dependent cell death. Four natural products, including hericenes A−C and hericenol A, and five synthetic derivatives were synthesized and their protective activities were evaluated. In designing the synthetic derivatives, we focused on the binding position of the fatty chain. Hericenes B and C showed moderate protective activity against thapsigargin-induced ER stress-dependent cell death. In contrast, their regioisomers (with respect to the position of the fatty chain) exhibited higher protective activity against tunicamycin-induced ER stress. This study clearly shows that the number and the binding position of the fatty chain are critical for protective activity against ER stress-dependent cell

death.

Keywords:

Geranyl resorcylate; Total synthesis; Structure-activity relationship; Endoplasmic reticulum stress; Neuroprotective agent

Hericium erinaceum, an edible mushroom named yamabushitake in Japan, houtouku in China, and lion's mane mushroom in Europe, is known as a medicinal food that is effective for the prevention of various diseases, including cancer, inflammation, diabetes, immune disorders, and dementia.[1] Its unique and broader medicinal effects have stimulated many scientists to elucidate the structures and compositions of its bioactive components and their modes of actions.[2] Among its reported medicinal virtues, the observed anti-dementia effect is specific to this mushroom.[1d–f] Kawagishi et al. showed in a pioneering study that hericenones C−E isolated from the fruiting bodies of *H. erinaceum* stimulate mouse astrocytes to produce nerve growth factor (NGF), a neurotrophic factor responsible for the growth, maintenance, and survival of neurons.[3] In 2014, the Sabaratnam group independently demonstrated that hericenones C−E increased NGF levels in culture medium and potentiated neurite outgrowth in PC12 cells when induced with a low concentration of NGF.[4]

Kawagishi and one of the authors of this paper reported the neuroprotective effect of 3-hydroxyhericenone F (**1a**) against endoplasmic reticulum (ER) stress-dependent cell death (Fig. 1).[5] The ER is the major organelle responsible for Ca^{2+} storage and release, as well as protein synthesis, folding, and export. Accumulation of unfolded or misfolded proteins and alterations in calcium homeostasis lead to a disturbance in ER function, which is collectively called ER stress.[6] While various signal transduction pathways, including the unfolded protein response, are activated in response to ER stress, intense and prolonged ER stress ultimately leads to apoptosis.[7] A number of pathological analyses suggest that ER stress is involved in neurodegenerative

disorders.[8] Therefore, ER stress suppressive compounds that enhance neuronal cell viability have attracted attention for the prevention and treatment of neurodegenerative disorders such as Alzheimer's, Parkinson's, and prion diseases.[9]

Structurally, 3-hydroxyhericenone F (**1a**) consists of a poly-substituted chromane core and a palmitate chain (Fig. 1). It can be assumed that hericenone C (i.e., 5′-oxohericene A, **1b**) is a biosynthetic precursor of **1a** and the tetrahydropyran ring is constructed by epoxidation of the C2′-C3′ double bond, followed by 6-*endo* cyclization. Another characteristic of **1a** is the presence of a hydroxyl group at C3, which is not found in other naturally-occurring geranyl resorcylates.[1b,2] Of the fungal-derived geranyl resorcylates isolated to date, only **1a** has been shown to possess cytoprotective activity against ER stress-dependent cell death.[5] It remains unknown if other geranyl resorcylates possess similar protective activities.[10] Hence, we undertook a synthesis and structure-activity relationship (SAR) study to clarify the fundamental structure critical for cytoprotective activity, and to discover novel drug lead compounds that can suppress ER stress-dependent cell death. Herein, we report the synthesis and neuroprotective activity of hericenes A−C (5′-deoxohericenones) (**2a–c**) and their derivatives. The results show that the number and the binding position of the fatty chain are important for protective activity.

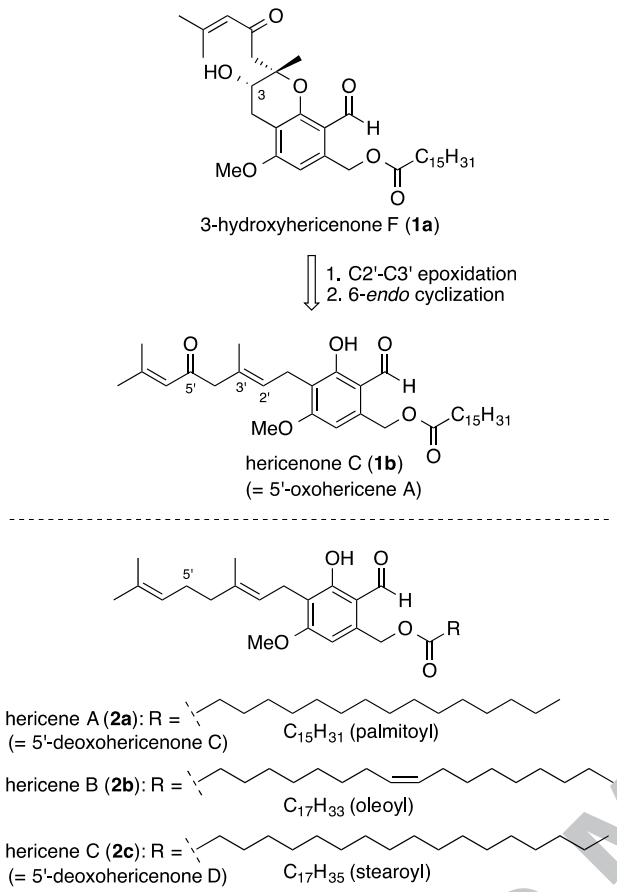
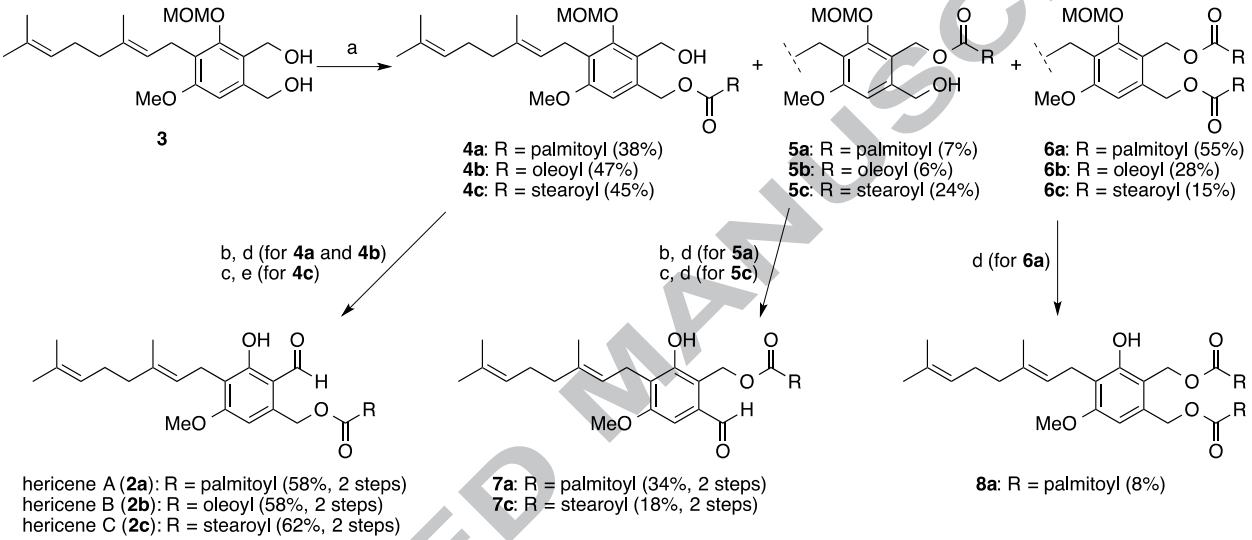


Fig. 1. Structures of 3-hydroxyhericenone F (**1a**), hericenone C (**1b**) and hericenes A−C (**2a–c**)

In 2011, we reported the first total syntheses of hericenone J and hericene A (5′-deoxohericenone C, **2a**), using $CuBr_2$-mediated one-pot phthalide formation and Stille coupling as key reactions.[11,12] In the present study, the common intermediate **3**[11] was prepared using our established method (Scheme 1). Esterification of **3** with various acid chlorides afforded monoesters **4a–c** (38−47%), along with their regioisomers **5a–c** (6−24%) and diesters **6a–c** (15−55%). Importantly, these reactions were initiated at low temperature to suppress diesterification. In addition, LiCl was added with the expectation that one of the hydroxyl groups would be deactivated by lithium-mediated chelation with a MOM-oxygen. Although the effect was subtle, the natural-type compounds **4a–c** prevailed over their regioisomers **5a–c**.[13] Oxidation[14,15] of **4a–c** followed by deprotection[11,16] afforded hericenes A–C (**2a–c**) in 58–62% yield over two steps. Similarly, two selected regioisomers (**7a** and **7c**) and diester **8a** were

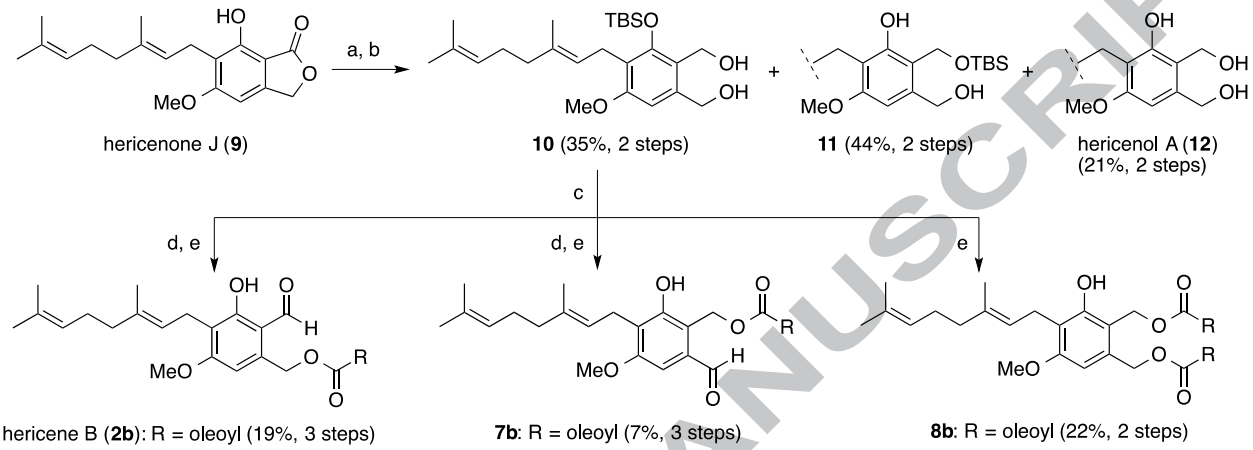
synthesized from **5a**, **5c**, and **6a**, respectively. It is noted that in the synthesis of **2a–c**, deprotection was facilitated by the presence of the ortho formyl group, which could coordinate with the Lewis acid, whereas this interaction was absent in the case of the regioisomers (**7a** and **c**) and diester **8a**. The low solubility of **6a** in CH_2Cl_2 at low temperature may also account for the unsatisfactory formation of **8a**.



Scheme 1. Syntheses of hericenes A–C (**2a–c**) and their derivatives (**7a**, **7c** and **8a**). Reagents and conditions: (a) RCOCl, pyridine, LiCl, THF, −78 or −30 °C to −30 ~ 0 °C; (b) 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO), $PhI(OAc)_2$, CH_2Cl_2, rt; (c) 2-azaadamantane *N*-oxyl (AZADO), $PhI(OAc)_2$, CH_2Cl_2, rt; (d) Me_2BBr, amylene, CH_2Cl_2, −78 °C; (e) $TiCl_4$, 2,6-di-*tert*-butyl-4-methylpyridine, CH_2Cl_2, −78 to −30 °C.

To circumvent this deprotection issue, the regioisomer of hericene B (**7b**) and its diester derivative (**8b**) were prepared by changing the phenolic protective group (Scheme 2). Thus, the phenolic hydroxyl group of hericenone J (**9**), synthesized previously,[11] was protected as its TBS ether and the lactone moiety was reduced with $LiAlH_4$. Unexpectedly, the phenolic TBS group migrated to the primary alcohol and the regioisomeric alcohol **11** predominated over the expected product **10**. Furthermore, the fully deprotected triol **12** (i.e., hericenol A[17]) was isolated in 21%

yield, which represents the second total synthesis of this molecule.[18] The alcohol **10** was then esterified with oleoyl chloride and converted into hericene B (**2b**), the regioisomer **7b**, and diester **8b**.[19]



Scheme 2. Syntheses of hericene B (**2b**), the derivatives (**7b** and **8b**) and hericenol A (**12**). Reagents and conditions: (a) TBSOTf, 2,6-lutidine, rt; (b) $LiAlH_4$, THF, 0 °C to rt; (c) oleoyl chloride, pyridine, DMAP, CH_2Cl_2, −60 °C to rt; (d) AZADO, $PhI(OAc)_2$, CH_2Cl_2, rt; (e) TBAF, THF, −78 °C.

With these natural and synthetic hericenes in hand, a cell protection assay was carried out using murine neuroblastoma cell line (Neuro2a).[9e] Tunicamycin (TM), an inhibitor of *N*-linked glycosylation in the ER, and thapsigargin (TG), an inhibitor of sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase, were used as inducers of ER stress.[6,20] In a typical experiment, Neuro2a cells were incubated with various concentrations of compounds (**2a–c**, **7a–c**, **8a**,**b** and **12**) in the absence or presence of TM (final concentration: 0.5 μg/mL) or TG (final concentration: 10 nM) for 24 h (Fig. 2).

The results show that the natural hericenes, specifically hericenes B (**2b**) and C (**2c**), exhibited moderate protective activity against TG-induced ER stress at concentrations above 30 μM. Their protective activities were less than that of **1a**, where significant protection against TG toxicity

was observed above 1 μg/mL (1.7 μM).[5] For **2a–c**, no significant protection was observed against TM-induced ER stress, in contrast to **1a**.[5] It is noteworthy that a significant increase in cell viability was observed when TM-induced ER stressed Neuro2a cells were incubated in the presence of the regioisomers of hericene B (**7b**) and C (**7c**). An approximately 20% increase in cell viability was observed at 10 μM, comparable to the activity of **1a** where an approximately 10% increase in cell viability was observed at 10 μg/mL (17 μM).[5] The repeated protection assay with the regioisomers (**7a**-**c**) indicated that they also possessed moderate protective activity against TG-induced cell death under the slightly different incubation conditions. [21] (Supplementary Fig. S1-S3). In contrast, the diesters (**8a** and **8b**) showed no protective activity, presumably due to the high hydrophobicity [calculated log P (clog P) = 19.7 for **8a** and 20.9 for **8b**, respectively][22] that was too large to dissolve in aqueous media and enter the cytoplasm. Hericenol A (**12**) (clog P = 3.2), which lacks the ester moiety, showed significant toxicity rather than protective activity, demonstrating the importance of one fatty ester component for protective activity.[23]

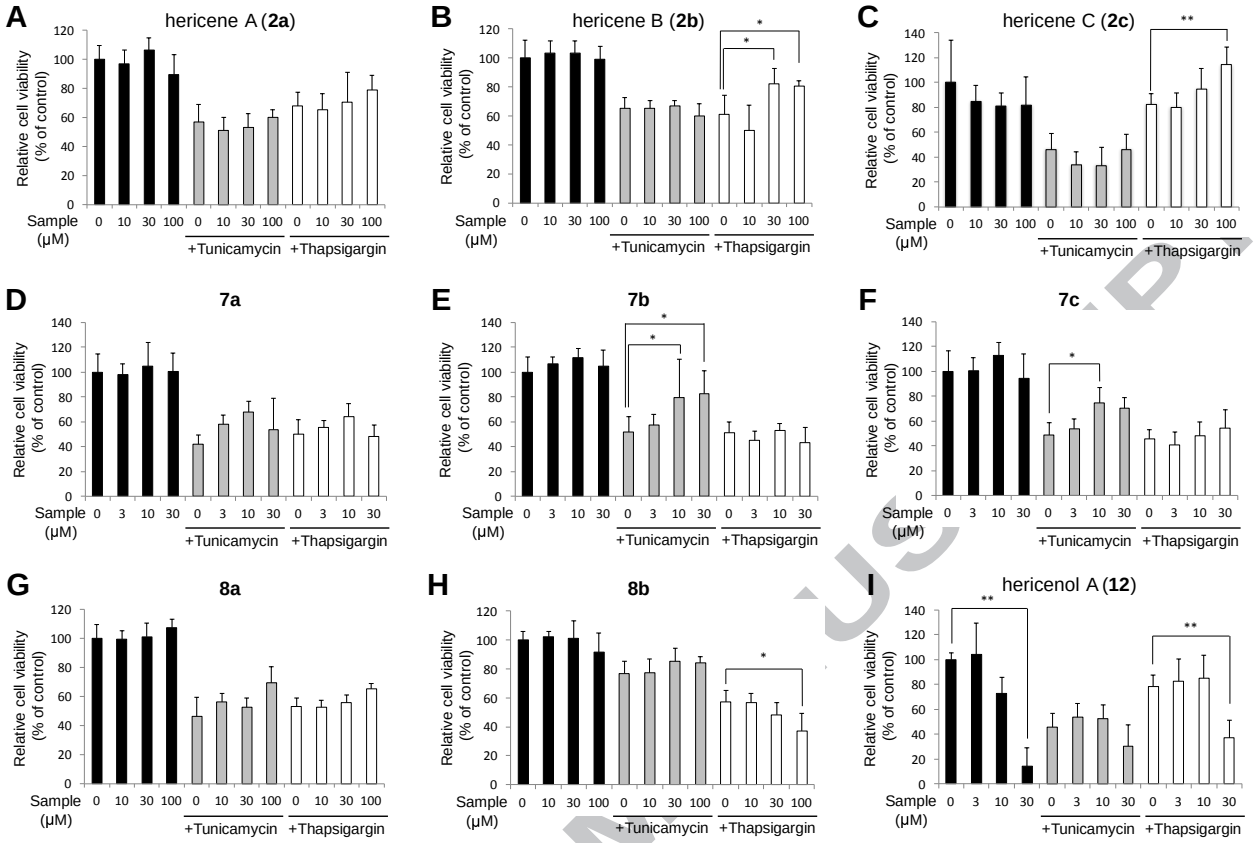


Fig. 2. Protective effects of (A) hericene A (**2a**), (B) hericene B (**2b**), (C) hericene C (**2c**), (D) the regioisomer of hericene A (**7a**), (E) the regioisomer of hericene B (**7b**), (F) the regioisomer of hericene C (**7c**), (G) the diester derivative of hericene A (**8a**), (H) the diester derivative of hericene B (**8b**), and (I) hericenol A, against ER stress-dependent cell death. The cell viabilities were analyzed by MTT assay, and the values were represented as means ± SEM of the relative percentage of surviving cells compared with the untreated cells (n=6 for A, B and D−I, and n=12 for C). * $p < 0.05$, ** $p < 0.01$, Tukey−Kramer multiple comparison tests.

In conclusion, we have shown the neuroprotective activities of hericenes and their synthetic derivatives for the first time. Hericenes B (**2b**) and C (**2c**) exhibited moderate protective activity against TG-induced ER stress-dependent cell death, whereas their regioisomers (**7b** and **7c**) exhibited significant protective activity against TM-induced ER stress at a concentration of 10 μM. Although details regarding the protective mechanisms presently remain unknown, these

preliminary SAR data clearly show the importance of the fatty chain: namely, the binding position of the fatty ester chain significantly affects protective activity. It is worth noting that the synthetic isomers exhibited stronger activity than the natural products. This finding will encourage synthetic chemists to design new ER stress-suppressive molecules aimed at the prevention and treatment of neurodegenerative disorders.

Acknowledgements

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A. Supplementary data

Supplementary data (synthetic procedures, spectral data for all synthetic compounds, and cell protection assay) associated with this article can be found, in the online version, at @@@.

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[13] Probably the differences of selectivity are not due to the type of acylating agents but to the minor differences of reaction conditions. Experimental procedures are presented in Supplementary data.

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[15] After some trials, oxidation with AZADO turned out to be better than other oxidation conditions such as Swern, Dess-Martin, and TEMPO/PhI(OAc)$_2$ oxidations regardless of the type of fatty esters.

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[19] Low yields are probably attributed to the esterification step, during which migration of the TBS group occurred to some extent. We also observed that diol **10** tended to degrade during extraction and purification. Further optimization of the synthetic route or the modification of the protective group was not investigated, as our initial purpose was to obtain preliminary SAR data.

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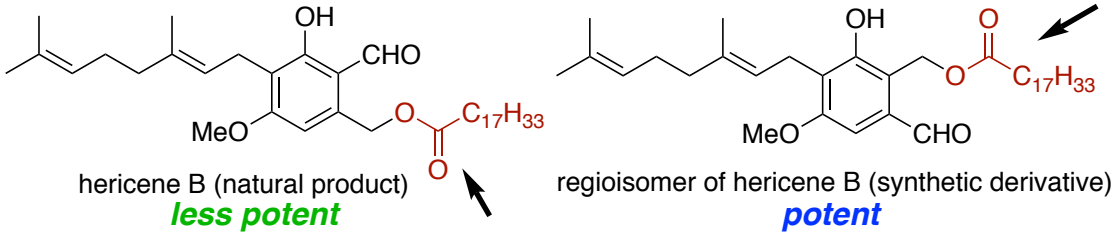
[21] We found that cell reactivity varied to some extent depending on the incubation time, cell passage number, cell density in medium, and other trivial factors. We repeatedly carried out experiments with modified conditions until significant and reliable results were observed, in consideration of inherent variability of cell reactivity. Figure 2 represents the results that were carried out under certain fixed optimal conditions. Variability of blank cell viability may attribute to the time when the data was collected. In practice, cell viability analyses were carried out in parallel to the syntheses in order to gain preliminary information on the structure-activity relationship. On the basis of the preliminary SAR data, we set up further experiments. As can be seen from synthetic schemes (Schemes 1 and 2), unexpectedly it took long time to synthesize the molecules. Therefore, the time when each data was collected is naturally different. The evidence that cell reactivity changes with cell passage number is reported in the following reference: Witek P, Korga A, Burdan F, Ostrowska M, Nosowska B, Iwan M, et al. *Cytotechnology*. 2016;68:2407–2415.

[22] The calculated log P (clog P) was obtained by a ChemDraw Professional 17.0 program.

[23] One reviewer pointed out the relevance between the activity and the clog P value. The clog P values of **2a**, **2b**, and **2c** are 12.3, 12.9, and 13.4, respectively, which are slightly higher than those of their regioisomers (11.9 for **7a**, 12.5 for **7b**, and 13.0 for **7c**, respectively). The clog P of 3-hydroxyhericenone F (**1a**) is 9.6 that is lower than those of **7a-c**, but the activity against TM-induced ER stress is in the same level as **7b** and **7c**. Given these results, it might be reasonable to consider that the protective activity is somewhat dependent on the hydrophobicity but not parallel to the clog P value.

- Structure-activity relationship of geranyl resorcylates derived from *Hericium erinaceum* was investigated.
- Four natural products and five derivatives were synthesized.
- Neuroprotective activities against endoplasmic reticulum (ER) stress-dependent cell death were evaluated.
- Synthetic derivatives exhibited higher neuroprotective activities than natural products.



hericene B (natural product)
less potent

regioisomer of hericene B (synthetic derivative)
potent

Neuroprotective effect against ER stress-dependent cell death