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Total synthesis of Herbarin A and B, determination of their antioxidant properties and toxicity in zebrafish embryo model

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ABSTRACT

Herbarin A and B were isolated from the fungal strains of *Cladosporium herbarum* found in marine sponges *Aplysina aerophoba* and *Callyspongia aerizusa*. Total synthesis of Herbarin A and B was achieved by carrying out a multi-step synthesis approach, and the antioxidant properties were evaluated using FRAP assay. Toxicity of these compounds was determined using a zebrafish embryo model.

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Herbarin A and B were recently isolated by Jadulco and co-workers[1] from two strains of the fungi *Cladosporium herbarum* found in sponges *Aplysina aerophoba* and *Callyspongia aerizusa*, respectively. Spectroscopic analysis of ethyl acetate extracts from these fungi revealed two new α-pyrone derived structures, Herbarin A and B. Various spectroscopic techniques such as, NMR and mass-spectrometry were used for structural elucidation. To determine the initial biological activity, a feeding assay was performed on polyphagous pest insect larvae and brine shrimp larvae.[1,2] As a consequence of their potent toxicity towards insect and brine shrimp larvae and unique chemical structure, we decided to pursue the laboratory syntheses of Herbarin A and B. A multistep synthetic approach provided the target compounds in multi-gram scale quantity, which is not possible from the natural source. Having the larger quantities allowed for further investigation of the potential toxicity and antioxidant properties of these natural products (Fig. 1).

Herein, we describe the synthesis of target compounds using a multi-step reaction sequence as described in Scheme 1. We identified an aldehyde (**7**) as the crucial intermediate that can afford both the target compounds. As a first step, our research efforts were directed towards the synthesis of 4-hydroxypyrone (**5**) by utilizing commercially available starting materials as basic building blocks and using a modified approach described by Wilcox and group.[3] 3-Methyl-2,4-pentanedione (**3**) was treated with $NaNH_2$ and then reacted with dry ice to provide diketo-acid (**4**) in 77% crude yield. Diketo-acid (**4**) without any purification was then treated with HF to afford the 4-hydroxypyrone (**5**) in about 39% yield. Additionally, etherification provided 4-methoxypyrone (**6**) and upon oxidation with SeO_2 selectively converted the methyl group at position C-6 to aldehyde (**7**) in quantitative yield. After successful execution of the synthetic scheme, aldehyde (**7**) was synthesized in multi-gram quantities to be utilized in further reactions.

Aldehyde (**7**) was then reacted with phosphonate ester (**8**) using Horner–Wadsworth–Emmons coupling strategy to obtain an exclusively *trans*-alkene (**9**) in about 90% yield.[4] Hydrolysis of the ester group with 3M HCl yielded Herbarin B (**2**) as a white solid. All products were purified using column chromatography and structure elucidation was carried out using various spectroscopic techniques including NMR and mass-spectrometry. Lastly, the spectroscopic data for Herbarin B was compared with literature data to confirm the product formation. Herbarin B was deliberately chosen as the first synthetic target considering it's lesser complexity compared to Herbarin A.

After successfully accomplishing the synthesis of Herbarin B, Herbarin A was targeted by using a similar synthetic scheme. The aldehyde intermediate (**7**) was treated with commercially available predominantly *trans*-phosphonate ester (**10**) to form an exclu-

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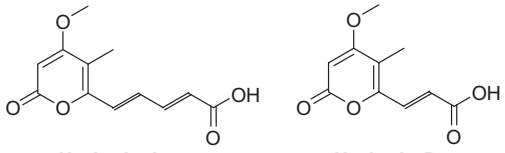


Figure 1. Structures of Herbarin A and Herbarin B.

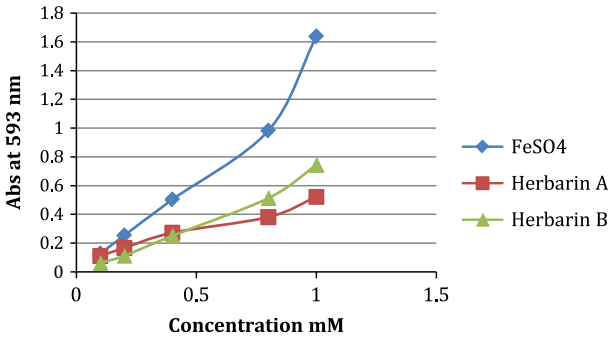
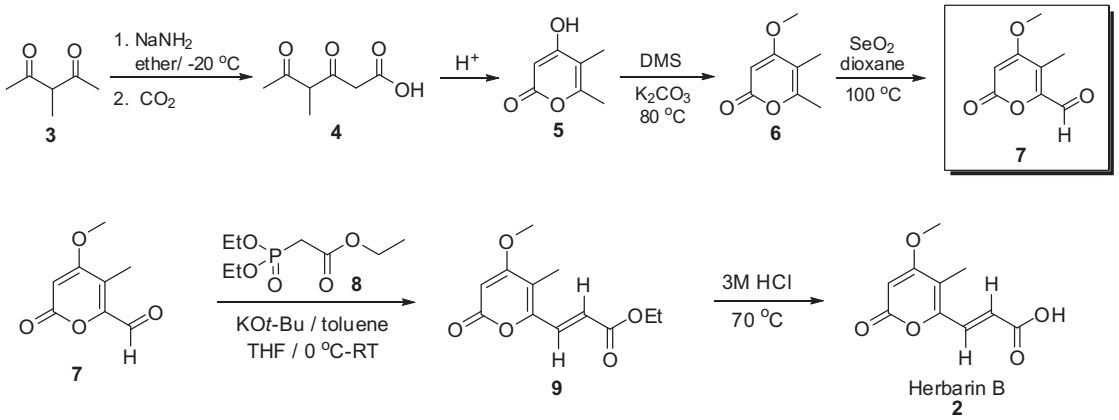


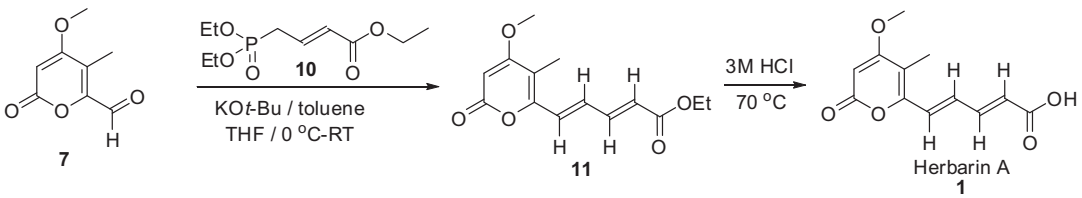
Figure 2. Antioxidant activity of Herbarin A and B.

sively *trans–trans*-ester (**11**) in 81% yield, which on further hydrolysis with 3M HCl provided Herbarin A in 67% yield (Scheme 2).

Determination of antioxidant properties: Ferric Reducing Ability of Plasma (FRAP) assay: A wide range of assays is known for measuring the reducing capacity of antioxidants. The assays are carried out at acidic (FRAP), neutral (TEAC), or basic (total phenols assay FCR) conditions. The pH values have an important effect on the reducing capacity of antioxidants.[5] Herbarin A and B both have carboxylic acid groups and to maintain the integrity of these molecules, we choose to use the acidic FRAP assay. Also FRAP assay has several advantages such as high reproducibility, simple, rapidly performed and showed the highest efficiency for acids and phenols.[6] Therefore, it would be an appropriate technique for determining antioxidant assay for Herbarin A and B. The FRAP assay, is presented as a novel method for assessing 'antioxidant power'. Ferric to ferrous ion reduction at low pH causes a colored ferrous–tripyridyltriazine complex to form. FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration. Absorbance changes are linear over a wide concentration range with antioxidant mixtures, including plasma, and with solutions containing one antioxidant in purified form.[7] The FRAP assay is inexpensive, reagents are simple to prepare, results are highly reproducible, and the procedure is straightforward and speedy. The FRAP assay offers a putative index of antioxidant, or reducing potential of organic compounds within the technological reach of every laboratory. The FRAP assay was employed as described in the literature.[8] The mechanism of this method is based on the reduction of ferric 2,4,6-tripyridyl-s-triazine complex (Fe^{3+}–TPTZ) to its ferrous form (Fe^{2+}–TPTZ) in the presence of antioxidants. A solution of acetate buffer 25 mL, 2.5 mL of Fe^{3+}–tripyridyl-triazine (Fe^{3+}–TPTZ) (10 mmol/L in 40 mmol/L of HCl, and 2.5 mL $FeCl_3$ (20 mmol/L) was then prepared. This solution (300 μL) was then mixed with Herbarin A and B (at 10, 20, 40, and 80 μg/mL) and 30 μL of distilled H_2O. The electron-donating capacity of the antioxidant was measured by the change in absorbance at 593 nm; the blue-colored Fe^{2+}–tripyridyl-s-triazine (Fe^{2+}–TPTZ) compound was formed from colorless oxidized Fe^{3+}–TPTZ. The absorbance of the reaction mixture was measured spectrophotometrically at 593 nm after incubation at room temperature for 1 h. Calibration curves were generated from aqueous solutions of $FeSO_4$ at different concentrations ranging from 0.1 to 1 mM. Both Herbarin A and B showed activity towards antioxidant assay at 0.1–1 mM concentrations (Fig. 2).



Scheme 1. Synthetic strategy for Herbarin B.



Scheme 2. Synthetic strategy for Herbarin A.

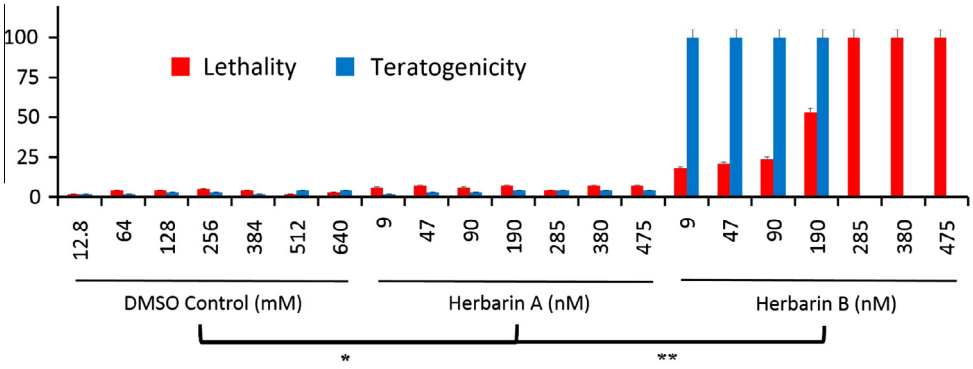


Figure 3. Quantitative summary of lethality and teratogenicity in zebrafish embryos.

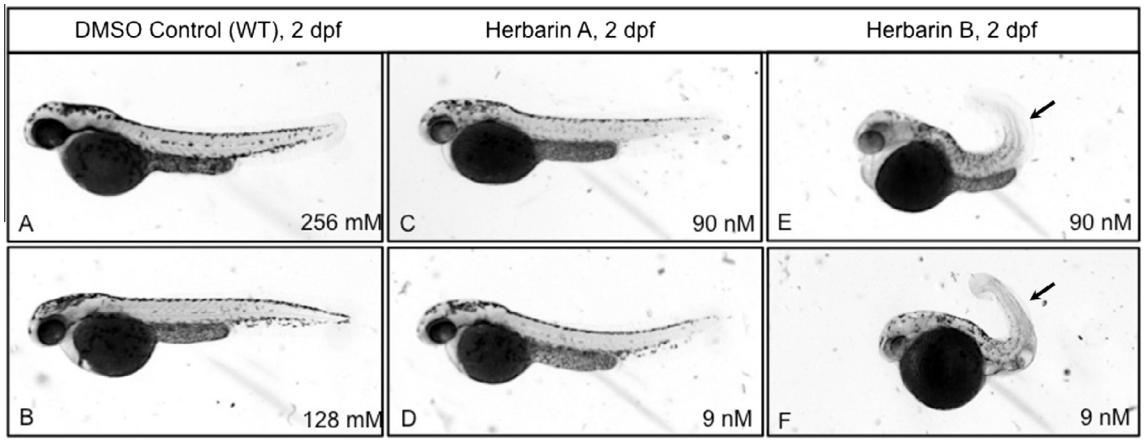


Figure 4. Zebrafish development in Herbarin A and Herbarin B treatment.

Toxicity assay: To assess the biological value of Herbarin A and B, we used a zebrafish embryo screening assay. Herbarin A and B were tested for toxicity and teratogenicity in zebrafish embryos.[9–13] These assays were performed using an established literature procedure.[9–13] Briefly, wild-type zebrafish embryos were collected from spawn tanks and allowed to grow until 6 hpf (hours post fertilization) at standard conditions.[14] At 6 hpf, embryos were observed under a microscope for embryonic development and only normally developing embryos were transferred to wells in 24-well plate and allowed to grow in 1 mL of treatment solution for up to 2 dpf (days post fertilization). Observations were made at 24 hpf and solutions were replaced as necessary. Herbarin A and B were dissolved in 100% DMSO after purification, further they were resuspended in E3 (embryonic medium) at various concentrations (Figs. 3 and 4) for zebrafish assay. Therefore our controls were WT (wild-type) embryos in E3 with same concentrations of DMSO (Fig. 4A and B) as in Herbarin A and B solutions. This study had identified that Herbarin B is more biologically active than Herbarin A (Figs. 3 and 4). Lethality assay was performed at concentrations from 9 nM to 475 nM and these studies revealed that Herbarin B has a LD50 (50% lethal dose) of 190 nM (Fig. 3). Herbarin B displayed dynamically increasing lethality from 9 nM to 190 nM concentrations beyond which it is completely lethal (Fig. 3). Herbarin B was also 100% teratogenic at concentrations of 9–190 nM (Fig. 4). Teratogenic phenotypes were specific showing a shortened body axis resembling gastrulation defects during early development, dorsal curvature of the trunk, tail malformations and pericardial edema (Fig. 4E and F; black arrows denote dorsal curvature of trunk and tail malformations). While Herbarin B was lethal and teratogenic, Herbarin A appears to be a docile compound with no observed toxicity and teratogenicity at similar concentrations (Fig. 4C and D). We performed at least 3 replicates of the zebrafish assay. Statistical analyses using student *t*-test revealed significant difference (Fig. 3; $^{*}p$ <0.001) between DMSO control and Herbarin B observations and no significant differences (Fig. 3; $^{**}p$ <0.001) between DMSO control and Herbarin A treatment. In summary, the zebrafish assays have identified that Herbarin B is more biologically active compared to Herbarin A.

In conclusion, we have successfully achieved the synthesis of Herbarin A and Herbarin B utilizing commercially available starting materials and following known literature synthetic protocols. All the products were characterized using analytical techniques. The antioxidant activity of target compounds was investigated. Furthermore, the toxicity assay using zebrafish embryo was also investigated. The bioassay results indicated the potent toxicity of Herbarin B.

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Supplementary data

Supplementary data (experimental procedure and NMR characterization data of all the products) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.01.065.

References and notes

1. Jadulco, R.; Brauers, G.; Edrada, R. A.; Ebel, R.; Sudarsono, V. W.; Proksch, P. *J. Nat. Prod.* **2002**, *65*, 730.
2. Lou, J.; Fu, L.; Peng, Y.; Zhou, L. *Molecules* **2013**, *18*, 5891.
3. Suh, H.; Wilcox, C. S. *J. Am. Chem. Soc.* **1988**, *110*, 470.
4. Teichert, A.; Jantos, K.; Harms, K.; Studer, A. *Org. Lett.* **2004**, *6*, 3477.
5. Huang, D.; Ou, B.; Prior, R. L. *J. Agric. Food Chem.* **2005**, *53*, 1841.
6. Thaipong, K.; Boonprakob, U.; Crosby, K.; Cisneros-Zevallos, L.; Byrne, D. H. J. *Food Comp. Anal.* **2006**, *19*, 669.
7. Benzie, I. F. F.; Strain, J. J. *Anal. Biochem.* **1996**, *239*, 70.
8. Kpegba, K.; Agbonon, A.; Petrovic, A. G.; Amouzou, E.; Gbeassor, M.; Proni, G.; Nesnas, N. *J. Nat. Prod.* **2011**, *74*, 455.
9. He, N.; Li, X.; Feng, D.; Wu, M.; Chen, R.; Chen, T.; Chen, D.; Feng, X. *Chem. Res. Toxicol.* **2013**, *26*, 89.
10. Fako, V. E.; Furgeson, D. Y. *Adv. Drug Deliv. Rev.* **2009**, *61*, 478.
11. Hill, A. J.; Teraoka, H.; Heideman, W.; Peterson, R. E. *Toxicol. Sci.* **2005**, *86*, 6.
12. Zhang, J. P.; Meng, J.; Li, Y. P.; Hu, C. Q. *Arch. Pharm.* **2010**, *343*, 553.
13. Zhang, J.; Qian, J.; Tong, J.; Zhang, D.; Hu, C. *Chem. Res. Toxicol.* **2013**, *26*, 1168.
14. Westerfield, M. *The Zebrafish Book. A Guide for the Laboratory Use of Zebrafish (Danio rerio)*, 4th ed.; University of Oregon Press: Eugene, 2000.