

2017

Efficient semisynthesis of (-)-pseudoirroratin A from (-)-flexicaulin A and assessment of their antitumor activities

Lei Guo

*Hong Kong Baptist University*

Siu Wai Tsang

*Hong Kong Baptist University*

Tong-Xin Zhang

*Sun Yat-sen University*

Kang-Lun Liu

*Hong Kong Baptist University*

Yi-Fu Guan

*Hong Kong Baptist University*

*See next page for additional authors*

This document is the authors' final version of the published article.

Link to published article: <http://dx.doi.org/10.1021/acsmchemlett.7b00033>

---

#### APA Citation

Guo, L., Tsang, S., Zhang, T., Liu, K., Guan, Y., Wang, B., Sun, H., Zhang, H., & Wong, M. (2017). Efficient semisynthesis of (-)-pseudoirroratin A from (-)-flexicaulin A and assessment of their antitumor activities. *ACS Medicinal Chemistry Letters*, 8 (3), 372-376. <https://doi.org/10.1021/acsmchemlett.7b00033>

---

**Authors**

Lei Guo, Siu Wai Tsang, Tong-Xin Zhang, Kang-Lun Liu, Yi-Fu Guan, Bo Wang, Han-Dong Sun, Hong-Jie Zhang, and Man Shing Wong

# Efficient Semi-Synthesis of (-)-Pseudoirroratin A from (-)-Flexicaulin A and Assessment of their Antitumor Activities

Lei Guo,<sup>1,2,†</sup> Siu Wai Tsang,<sup>3,†</sup> Tong-Xin Zhang,<sup>4,†</sup> Kang-Lun Liu,<sup>3</sup> Yi-Fu Guan,<sup>3</sup> Bo Wang,<sup>5</sup> Han-Dong Sun,<sup>6</sup> Hong-Jie Zhang,<sup>3,\*</sup> and Man Shing Wong<sup>1,\*</sup>

<sup>1</sup>Department of Chemistry and Institute of Advanced Materials, Hong Kong Baptist University, Kowloon Tong, Hong Kong SAR, China

<sup>2</sup>School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou 510006, P. R. China

<sup>3</sup>School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong SAR, China

<sup>4</sup>Department of Pharmacy, Liaocheng University, Shandong, China

<sup>5</sup>BioTools, Inc., 17546 Bee Line Hwy, Jupiter, FL 33458, USA

<sup>6</sup>State Key Laboratory of Phytochemistry and Plant Resources in West China, Chinese Academy of Sciences, Kunming, China

**KEYWORDS** (-)-pseudoirroratin A, (-)-flexicaulin A, apoptosis, antitumor activity

---

**ABSTRACT:** Accumulating evidence indicates that natural *ent*-kaurane diterpenoids show great potential for medical treatment of different pathological conditions including cytotoxicity, antibacterial and anti-inflammatory activity. Amongst a variety of diterpenoids tested, (-)-pseudoirroratin A displayed a promising antitumor property *in vitro* and *in vivo*. However, this diterpenoid could merely be isolated in a limited amount from a rare source of *Isodon pseudoirrorata*. To overcome a such scanty source, we developed a novel, facile and efficient semi-synthetic strategy to prepare (-)-pseudoirroratin A from natural (-)-flexicaulin A, which can be expediently obtained from *I. flexicaulis* in a great quantity. The three-dimensional structure and the absolute configuration of our synthetic diterpenoid have been determined and confirmed with the X-ray crystallographic analysis. More importantly, we demonstrated for the first time that pseudoirroratin A exerted significant cytotoxicity against human colorectal carcinoma cells via an induction of apoptosis, as well as a remarkable suppression on tumor growth in a colon cancer xenograft mouse model.

---

Natural products are regarded as the major source of lead compounds in drug discovery. Their complexity but unique structures represent a prime platform for structural modification complementary to the development and optimization of health-promoting drugs. In recent years, hundreds of natural *ent*-kaurane diterpenoids<sup>1</sup> isolated from *Isodon* (formerly *Rabdosia*) species (Lamiaceae) have been shown to have a broad range of biological activities including cytotoxic, antibacterial and anti-inflammatory effects.<sup>2-4</sup> The potent anti-cancer activity of this class of compounds has drawn considerable attention in recent years because of the steady unveiling of their novel mechanisms of actions.<sup>5-9</sup> Therefore, synthesis and structural studies of these *ent*-kaurane compounds have been intensely explored.<sup>10-12</sup> Among various types of *ent*-kaurane diterpenoids classified by Sun *et al.*,<sup>1</sup> pseudoirroratin A, one of the 11, 20-epoxy subtypes, is of particularly appealing (or particularly appealing) because it exerts remarkable cytotoxicity against a spectrum of cancer cell lines at low IC<sub>50</sub> values.<sup>13</sup> However, this diterpenoid could only be isolated in small quantity from the leaves of *I. pharicus* (Prain) Murata (Synonym: *I. pseudoirrorata* C. Y. Wu) growing in the high-altitude regions of southern Tibet and southwestern Sichuan Province in China.<sup>14-15</sup> In fact, *I. pseudoirrorata* has been using as pain-killer in traditional Tibetan medicine for centuries, as well as a treatment for tragus, trachoma, conjunctivitis

and parasite-induced gastrointestinal colic.<sup>16</sup> However, owing to the scarcity of pseudoirroratin A, a comprehensive exploration of pharmaceutical applications was hampered.

Interestingly, the natural *ent*-kaurane diterpenoid (-)-flexicaulin A,<sup>17</sup> which is obtained from the leaves of *I. flexicaulis* (C. Y. Wu et H. W. Li) H. Hara growing abundantly in southwestern Sichuan,<sup>18-19</sup> shows a structural similarity to (-)-pseudoirroratin A (Figure 1). The only structural difference between these two diterpenoids is the presence of a 5-member cyclic acetal group across the B and C rings in (-)-pseudoirroratin A. Thus, (-)-flexicaulin A is classified as the C-20 oxygenated subtype that does not have 11, 20-epoxy subtype functionality. Because of the structural similarity and easy accessibility, we used (-)-flexicaulin A as a scaffold to expedite the synthesis of (-)-pseudoirroratin A in order to obtain a sufficient quantity of the synthetic diterpenoid as well as the intermediate compounds for an investigation of the diverse biological activities of the new molecular entities.

Herein, we report a facile and efficient semi-synthesis of (-)-pseudoirroratin A from naturally rich (-)-flexicaulin A for the first time. The absolute configurations of the synthesized (-)-pseudoirroratin A and the natural scaffold (-)-flexicaulin A were determined and confirmed with the single-crystal X-ray diffraction and vibrational circular dichroism (VCD) techniques. The intermediates obtained from our synthetic path-

ways were established and analyzed by spectroscopic data. Furthermore, the biological activities of (-)-pseudoirroratin A were assessed in both cellular and animal models. The synthetic route to (-)-pseudoirroratin A from (-)-flexicaulin A is outlined in Scheme 1.

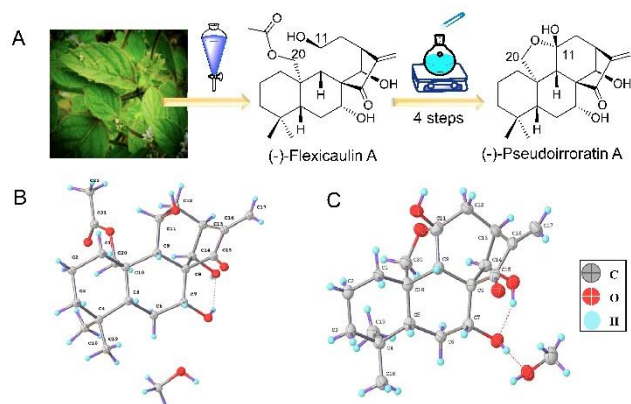
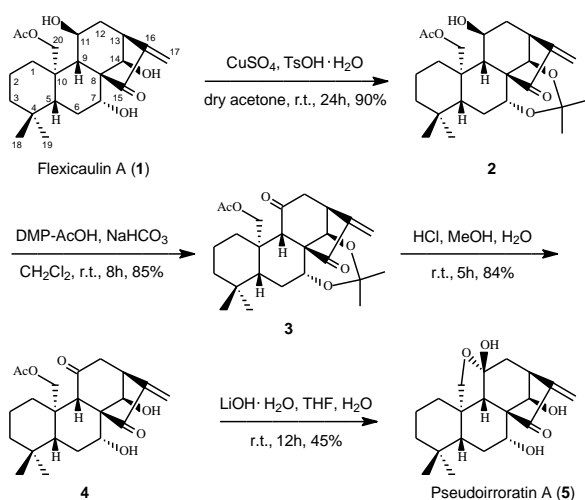


Figure 1. (A) An efficient approach to obtain a sufficient quantity of (-)-pseudoirroratin A from a natural source. Structures of the natural scaffold (-)-flexicaulin A (B) and the synthetic (-)-pseudoirroratin A (C) determined by the single crystal x-ray diffraction technique.



Scheme 1. The semi-synthesis of (-)-pseudoirroratin A from (-)-flexicaulin A.

Firstly, the hydroxyl groups at C-7 and C-14 in (-)-flexicaulin A (1),  $[\alpha]_D^{23} = -113.6$  (MeOH), were protected by a classical ketal functionality using anhydrous CuSO<sub>4</sub> as a catalyst. The mild Dess–Martin periodinane oxidation converting the hydroxyl group at C-11 to a carbonyl functionality afforded diketone 3 in good yield. The removal of the ketal protecting group by dilute acid yielded dihydroxyl intermediate 4. Finally, one-pot saponification and subsequent stereoselective cyclization of hemiketal formation under mild basic conditions afforded (-)-pseudoirroratin A,  $[\alpha]_D^{17} = -158.5$  (MeOH), in an overall yield of 29%, a higher optical rotation value was obtained manifesting a higher purity of the synthesized product than that isolated in plants.<sup>17</sup> The dilute and mild alkaline condition in the final step was crucial to obtain the desired product from the extremely sensitive  $\alpha$ ,  $\beta$ -unsaturated ketone. All the intermediates were fully characterized by <sup>1</sup>H

NMR, <sup>13</sup>C NMR, HRMS and optical activity, and found to be in good agreement with their structures.

The method of absolute configuration determination by VCD technique has been fully utilized to make the correct assignment of absolute configurations for the natural and semi-synthesized products.<sup>20</sup> As clearly illustrated in the absolute configuration determination reports by BioTools (Tables S1-S4 and Figures S1-S5), a high level of agreement was achieved between the measured and calculated spectra of the natural and synthetic (-)-pseudoirroratin A, indicating that both forms share the same absolute configurations. Since the experimental IR and VCD spectra of natural (-)-pseudoirroratin A matched one of the lowest energy conformation from the calculations, these agreements unambiguously established the absolute configurations of the molecule as (-)-(5R,7R,8R,9S,10S,11R,13S,14R)-pseudoirroratin A, assigned by the original C atom sequence in the skeleton shown in Figure 1. As the two natural products share the same skeleton, the same approach was also adopted to make the assignment of absolute configurations for (-)-(5R,7R,8R,9S,10S,11S,13S,14R)-flexicaulin A. To further confirm the structures and absolute configurations of the natural scaffold (-)-flexicaulin A (1) and the synthetic (-)-pseudoirroratin A (5), X-ray crystallographic analyses were pursued. Flexicaulin A was recrystallized in MeOH affording a crystal of the space group C<sub>2</sub> whilst pseudoirroratin A was also recrystallized in MeOH with space group P2<sub>1</sub> (Figure 1). The absolute configurations of (-)-flexicaulin A and (-)-pseudoirroratin A were determined by single-crystal X-ray diffraction analysis using an anomalous scattering of Cu K $\alpha$  radiation with the value of a Flack parameter less than 0.001, by which their absolute configurations were confirmed consistent with those predicted and previously reported (Figure 1).

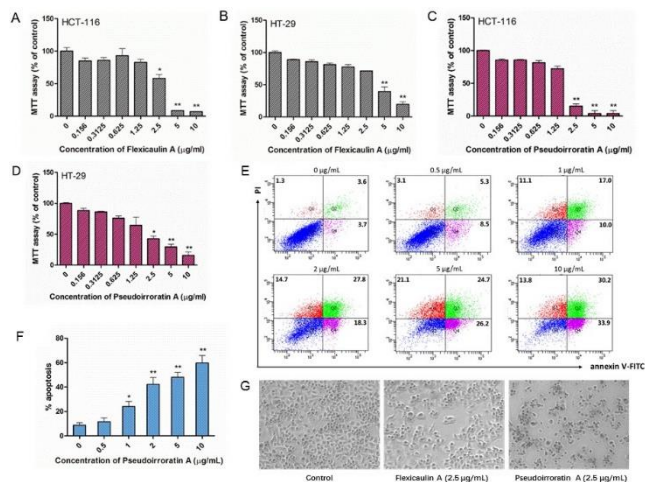


Figure 2. (-)-Flexicaulin A inhibits the proliferation of human colorectal carcinoma HCT-116 (A) and HT-29 (B) cells in a dose-dependent manner. (-)-Pseudoirroratin A inhibits the proliferation of colorectal carcinoma HCT-116 (C) and HT-29 (D) cells in a dose-dependent manner. Data are expressed as mean  $\pm$  S.D. (\* $p$ <0.05 and \*\* $p$ <0.001) of three separate experiments. (E) Apoptosis of HCT-116 cells treated with series concentrations of (-)-pseudoirroratin A (0–10  $\mu$ g/ml) was detected utilizing flow cytometry. Q1: necrosis; Q2: late apoptosis; Q3: live; Q4: early apoptosis. (F) The percentage of apoptotic HCT-116 cells (Q2+Q4) was calculated according to the flow cytometric analysis. Data are expressed as mean  $\pm$  S.D. (\* $p$ <0.05 and \*\* $p$ <0.001) of three separate experiments. (G) Untreated control, (-)-flexicaulin

A-treated and (-)-pseudoirroratin A-treated HCT-116 colorectal carcinoma cells were visualized using light microscopy (magnification: 10 $\times$ ).

With a sufficient amount of (-)-pseudoirroratin A synthesized, we explored its ability to treat cancer cell proliferation. A potent anticancer agent is characterized by the significant anti-proliferative effect on cancer cells. In this study, we examined the effect of (-)-flexicaulin A and (-)-pseudoirroratin A on cellular proliferation of two commonly used human colorectal carcinoma cell lines, i.e. HCT-116 and HT-29, by means of the MTT viability assay. The IC<sub>50</sub> values of (-)-flexicaulin A in HCT-116 cells (Figure 2A) and HT-29 cells (Figure 2B) were 2.9  $\mu$ g/mL (7.3  $\mu$ M) and 4.1  $\mu$ g/mL (10.5  $\mu$ M), respectively whereas the IC<sub>50</sub> values of (-)-pseudoirroratin A in HCT-116 (Figure 2C) and HT-29 cells (Figure 2D) were 1.7  $\mu$ g/mL (4.9  $\mu$ M) and 2.1  $\mu$ g/mL (5.9  $\mu$ M), respectively.

Apart from the MTT assay, the anti-proliferative effect of (-)-flexicaulin A and (-)-pseudoirroratin A on the aggressive colorectal carcinoma cells, which were cultured at a relative high density, was also evidenced in the light microscopy study (Figure 2G). From the cytotoxicity assay and microscopic study, we consistently noticed that (-)-pseudoirroratin A exhibited a more significant anti-proliferative potential than its natural counterparts. To this end, we further investigated the effect of (-)-pseudoirroratin A on the rate of programmed cell death, literally apoptosis, using the annexin V-FITC/propidium iodide (PI) double staining assay. The early (Q4) and late (Q2) apoptotic events were detected by means of flow cytometry. Our results showed that (-)-pseudoirroratin A treatment induced notable cellular apoptosis in HCT-116 cells in a concentration-dependent manner (Figure 2E and 2F).

To gain an insight into the molecular mechanism of (-)-pseudoirroratin A on the induction of apoptosis in colorectal carcinoma cells, we then examined the expression levels of several apoptosis-related markers, such as caspase-3, survivin and PARP as well as the phosphorylation of extracellular signal-regulated kinase (ERK) in the absence and presence of (-)-pseudoirroratin A. By means of Western blotting analysis, we demonstrated that the proteolytic cleavages of caspase-3 and PARP in HCT-116 cells were notably increased by the administration of (-)-pseudoirroratin A. The cleaved forms of caspase-3 and PARP are indeed their active forms, which are considered as the prime pro-apoptotic regulators.<sup>21</sup> For an induction of apoptotic cell death, the expression level of survivin, which is an important inhibitor of apoptosis protein (IAP), was markedly decreased upon (-)-pseudoirroratin A treatment with an enhanced phosphorylation of ERK. Our finding is in line with previous studies that ERK activity mediates the progression of cell death, explicitly apoptosis, *in vitro* and *in vivo*.<sup>22</sup> Interestingly, we also noticed that the expression level of histone deacetylase 1 (HDAC1) in the nuclear extract was significantly suppressed by (-)-pseudoirroratin A at doses higher than 0.625  $\mu$ g/mL (Figure 3A). The present study demonstrates for the first time that the anti-proliferative activity of *ent*-kaurane diterpenoid (-)-pseudoirroratin A in colorectal carcinoma cells is associated with an inhibition of HDAC1. The depletion of HDACs has been recently implicated as a promising therapeutic approach for anticancer treatment<sup>23</sup> suggesting the great potential of (-)-pseudoirroratin A in treating colorectal carcinoma.

To further validate the antitumor effect of our *ent*-kaurane diterpenoids *in vivo*, we performed some xenograft studies

using athymic nude mice. HCT-116 colon carcinoma cells were transplanted subcutaneously into the flanks of the nude mice.

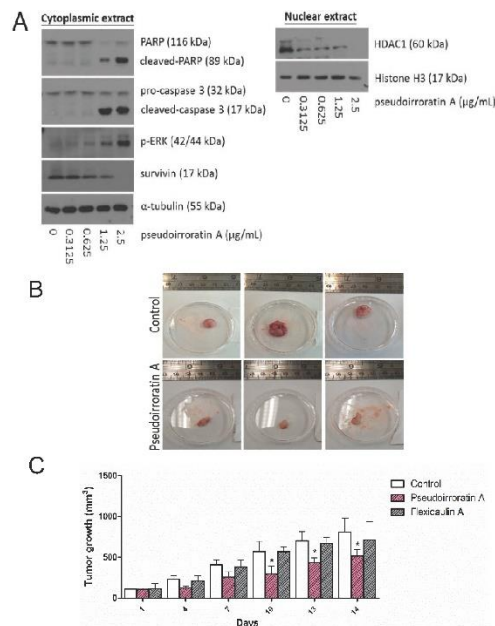


Figure 3. (A) Effects of (-)-pseudoirroratin A (0–2.5  $\mu$ g/mL) on the cleavage of PARP and caspase-3, ERK phosphorylation and survivin expression in the cytoplasmic extract as well as HDAC1 expression in the nuclear extract of HCT-116 cells were assessed by Western blotting. The probes of  $\alpha$ -tubulin and histone H3 served as the loading controls of the cytoplasmic and nuclear extracts, respectively. (B) Tumors obtained from the nude mice injected with HCT-116 carcinoma cells of the control and (-)-pseudoirroratin A treatment groups at the end of experiment. (C) The volume of tumor of each animal was measured at the indicated time point. Data are expressed as mean  $\pm$  S.D. (\* $p$ <0.05),  $n$ =10.

To the treatment groups, mice were either given (-)-flexicaulin A or (-)-pseudoirroratin A at 20 mg/kg every other day (pls insert the common as mentioned by) for 14 days whilst controls received vehicle treatment during the course of ex experiment. No statistically significant difference in body weight among all the experimental groups was obtained, indicating that both the natural (-)-flexicaulin A and the synthetic (-)-pseudoirroratin A did not exert toxic or adverse effects to the mice (Figure S6). By the end of experiment, tumors were obtained from all groups for further assessment. Three representative tumors of the control and the (-)-pseudoirroratin A treatment groups are shown in Figure 3B.

As compared to the untreated control, mice treated with (-)-pseudoirroratin A, but not (-)-flexicaulin A, showed a marked suppression on the growth of tumor with respect to tumor volume (Figure 3C) and tumor weight (Figure S7). These *in vivo* data revealed that the antitumor effect of the synthetic (-)-pseudoirroratin A was much more potent than its natural scaffold (-)-flexicaulin A, and consistent to the results of the biochemical analyses in the cellular experiments.

The enhanced antitumor activity of pseudoirroratin A was attributed to the synergism of the  $\alpha$ ,  $\beta$ -unsaturated ketone<sup>1</sup> and 11, 20-epoxy moiety in such a small molecule scaffold as these two moieties were considered as the crucial functional groups responsible for antitumor effect, disclosed by the widely accepted explanation of rational structure–activity relation-

ship (SAR).<sup>1</sup> Meanwhile, the 7 $\beta$ -OH and 14 $\beta$ -OH groups play a vital role in the binding to distinct enzymes in cancer cells; hence, leading to the deactivation of SH enzymes or SH coenzymes contributing to the antineoplastic mechanism.<sup>1,3</sup> Collectively, the potent antitumor activity of pseudoirroratin A appears to be a combined effect of the aforementioned functional groups.

In conclusion, a facile and efficient strategy for the semi-synthesis of (-)-pseudoirroratin A from natural (-)-flexicaulin A has been successfully developed for the first time. From the results of various biochemical assays, we demonstrate that (-)-pseudoirroratin A exhibits superior antitumor activity to (-)-flexicaulin A in inhibiting the proliferation of progressive colorectal adenocarcinoma cells via an effective induction of apoptosis, which was first found to be associated with a suppression of HDAC1. In the aspect of drug discovery, our current findings definitely provide new insights into the molecular actions underlying the antitumor effect of *ent*-kaurane diterpenoids, which would serve as potential chemotherapeutic candidates or anticancer drug leads in the future drug development.

## ASSOCIATED CONTENT

**Supporting Information.** Details of experimental procedures, characterization, X-ray crystallographic and VCD data of (-)-pseudoirroratin A and (-)-flexicaulin A, the *in vitro* and *in vivo* experimental protocols. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## AUTHOR INFORMATION

### Corresponding Author

E-mail: [mswong@hkbu.edu.hk](mailto:mswong@hkbu.edu.hk); [zhanghj@hkbu.edu.hk](mailto:zhanghj@hkbu.edu.hk)

### Author Contributions

<sup>1</sup>L. Guo, S.W. Tsang and T.X. Zhang contributed equally to this work.

### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENT

This work was supported by grants from the Hong Kong Baptist University (HKBU) Interdisciplinary Research Matching Scheme (RC-IRMS/12-13/03), the Research Grants Council of the Hong Kong Special Administrative Region, China (Project No. HKBU 12103014), and the Faculty Research Grants, Hong Kong Baptist University (FRG2/14-15/047 and FRG1/15-16/020).

## REFERENCES

- (1) Sun, H. D.; Huang, S. X.; Han, Q. B. Diterpenoids from *Isodon* species and their biological activities. *Nat. Prod. Rep.* **2006**, *23*, 673.
- (2) Wang, L.; Li, D.; Wang, C.; Zhang, Y.; Xu, J. Recent progress in the development of natural *ent*-kaurane diterpenoids with anti-tumor activity. *Mini-Rev. Med.Chem.* **2011**, *11*, 910.
- (3) Yang, J.; Wang, W. G.; Wu, H. Y.; Du, X.; Li, X. N.; Li, Y.; Pu, J. X.; Sun, H. D. Bioactive *ent*-type *ent*-kaurane diterpenoids from *Isodon phyllostachys*. *J. Nat. Prod.* **2016**, *79*, 132.
- (4) Luo, X.; Pu, J. X.; Xiao, W. L.; Zhao, Y.; Gao, X. M.; Li, X. N.; Zhang, H. B.; Wang, Y. Y.; Li, Y.; Sun, H. D. Cytotoxic *ent*-kaurane diterpenoids from *Isodon rubescens* var. *lushiensis*. *J. Nat. Prod.* **2010**, *73*, 1112.
- (5) Liu, C. X.; Yin, Q. Q.; Zhou, H. C.; Wu, Y. L.; Pu, J. X.; L. Xia, Liu, W.; Huang, X.; Jiang, T.; Wu, M. X.; He, L. C.; Zhao, Y. X.;

Wang, X. L.; Xiao, W. L.; Chen, H. Z.; Zhao, Q.; Zhou, A. W.; Wang, L. S.; Sun, H. D.; Chen, G. Q. Adenanthin targets peroxiredoxin I and II to induce differentiation of leukemic cells. *Nat. Chem. Biol.* **2012**, *8*, 486.

(6) Lu, Y.; Chen, B.; Song, J. H.; Zhen, T.; Wang, B. Y.; Li, X.; Liu, P.; Yang, X.; Zhang, Q. L.; Xi, X. D.; Chen, S. D.; Zuo, J. P.; Chen, Z.; Chen, S. J. Eriocalyxin B ameliorates experimental autoimmune encephalomyelitis by suppressing Th1 and Th17 cells. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 2258.

(7) Li, X. Y.; Pu, J. X.; Jiang, S. Y.; Su, J.; Kong, L. M.; Mao, B. Y.; Sun, H. D.; Li, Y. Henryin, an *ent*-kaurane diterpenoid, inhibits Wnt signaling through interference with  $\beta$ -catenin/TCF4 interaction in colorectal cancer cells. *PlosOne* **2013**, *8*, e68525.

(8) Gu, Z. M.; Wu, Y. L.; Zhou, M. Y.; Liu, C. X.; Xu, H. Z.; Yan, H.; Zhao, Y.; Huang, Y.; Sun, H. D.; Chen, G. Q. Pharicin B stabilizes retinoic acid receptor- $\alpha$  and presents synergistic differentiation induction with ATRA in myeloid leukemic cells. *Blood* **2010**, *116*, 5289.

(9) Wang, Z.; Xu, Z. J.; Niu, Z. C.; Liang, B. J.; Niu, J. Epierycalyxin A induces cell apoptosis through JNK and ERK1/2 signaling pathways in colon cancer cells. *Cell Biochem. Biophys.* **2015**, *73*, 559.

(10) Lazarski, K. E.; Moritz, B. J.; Thomson, R. J. The total synthesis of *Isodon* diterpenes. *Angew. Chem. Int. Ed.* **2014**, *53*, 10588.

(11) Xu, S. T.; Wang, G. Y.; Lin, Y.; Zhang, Y. J.; Pei, L. L.; Yao, H.; Hu, M.; Qiu, Y. Y.; Huang, Z. J.; Zhang, Y. H.; Xu, J. Y. Novel anticancer oridonin derivatives possessing a diazen-1-ium-1,2-diolate nitric oxide donor moiety: Design, synthesis, biological evaluation and nitric oxide release studies. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 2795.

(12) Zhang, W. B.; Lin, G.; Shao, W. B.; Gong, J. X.; Zhen Y. Total synthesis of maoecrystal V. *Chem. Asian J.* **2015**, *10*, 903.

(13) Zhang, H. J.; Fan, Z. T.; Tan, G. T.; Chai, H. B.; Pezzuto, J. M.; Sun, H. D.; Fong, H. H. S. Pseudoirroratin A, a new cytotoxic *ent*-kaurane diterpene from *Isodon pseudo-irrorata*. *J. Nat. Prod.* **2002**, *65*, 215.

(14) *Plectranthus pharicus* Prain, *J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist.* **1891**, *59*: 297.

(15) *Isodon pharicus* (Prain) Murata, *Acta Phytotax. Geobot.* **1955**, *16*: 15.

(16) Chinese Materia Medica-Tibetan Medicine: Shanghai Science and Technology Press, **2002** (Dec; first edition), item 235.

(17) Zhang, H. J.; Sun, H. D. Diterpenoids from *Rabdosia flexicaulis*. *Phytochemistry* **1989**, *28*, 3534.

(18) *Rabdosia flexicaulis* C. Y. Wu et H. W. Li, *Fl. Reipubl. Popularis Sin.* **1977**, *66*, 587.

(19) *Isodon flexicaulis* (C. Y. Wu et H. W. Li) H. Hara, *J. Jap. Bot.* **1985**, *60*, 234.

(20) Freedman, T. B.; Cao, X.; Dukor, R. K.; Nafie, L. A. Absolute configuration determination of chiral molecules in the solution state using vibrational circular dichroism. *Chirality*, **2003**, *15*, 743.

(21) Chaitanya, G. V.; Steven, A. J.; Babu, P. P. PARP-1 cleavage fragments: signatures of cell-death proteases in neurodegeneration. *Cell Commun Signal.* **2010**, *8*, 31.

(22) Cagnol, S.; Chambard, J. C. ERK and cell death: mechanisms of ERK-induced cell death--apoptosis, autophagy and senescence. *FEBS J.* **2010**, *277*, 2.

(23) Zhang, T.; Chen, Y.; Li, J.; Yang, F.; Wu, H.; Dai, F.; Hu, M.; Lu, X.; Peng, Y.; Liu, M.; Zhao, Y.; Yi, Z. Antitumor action of a novel histone deacetylase inhibitor, YF479, in Breast Cancer. *Neoplasia*. **2014**, *8*, 665.

TOC:

