Taylor & Francis
Taylor & Francis Group

Natural Product Research
Formerly Natural Product Letters

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/gnpl20

Synthesis and in vitro biological evaluation of novel dendrocandin analogue as potential anti-tumor agent

Jing-Yun Yan, Hao-Nan Yang, Ning Yang, Yin-Rong Xie, Xiu-Li Sun, Ye-Wei Huang, Cheng-Ting Zi, Xuan-Jun Wang & Jun Sheng

To cite this article: Jing-Yun Yan, Hao-Nan Yang, Ning Yang, Yin-Rong Xie, Xiu-Li Sun, Ye-Wei Huang, Cheng-Ting Zi, Xuan-Jun Wang & Jun Sheng (2021): Synthesis and in vitro biological evaluation of novel dendrocandin analogue as potential anti-tumor agent, Natural Product Research, DOI: 10.1080/14786419.2021.1901698

To link to this article: https://doi.org/10.1080/14786419.2021.1901698

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2021.1901698

Taylor & Francis
Taylor & Francis Group

Synthesis and in vitro biological evaluation of novel dendrocandin analogue as potential anti-tumor agent

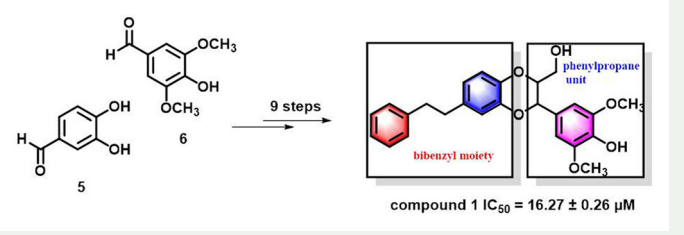
Jing-Yun Yan[a,b]*, Hao-Nan Yang[a,b]*, Ning Yang[a,b], Yin-Rong Xie[a,b], Xiu-Li Sun[a,b], Ye-Wei Huang[a], Cheng-Ting Zi[a], Xuan-Jun Wang[a] and Jun Sheng[a,b]

[a]Key Laboratory of Pu-er Tea Science, Ministry of Education, Yunnan Agricultural University, Kunming, China; [b]College of Food Science and Technology, Yunnan Agricultural University, Kunming, China

ABSTRACT

Dendrocandins are characteristic chemical structures of D. officinale and have strong physiological bioactivities. In this study, a dendrocandin analogue (1) has been prepared by total synthesis (9 steps, 12.6% overall yield) in which coupling reaction and Wittig reaction as the key steps. Compound 1 was also evaluated for its anticancer activity in vitro against six human cancer cells (MCF-7, A549, A431, SW480, HepG-2 and HL-60) using MTT assays. Compound **1** showed potent cytotoxicity, with the IC50 value 16.27 ± 0.26 μM. The expression levels of apoptotic proteins indicated that compound **1** can up-regulate the expression of apoptotic proteins, leading to apoptosis. This compound suggested that it's potential as anticancer agent for further development.

Graphical Abstract



ARTICLE HISTORY
Received 23 October 2020
Accepted 7 March 2021

KEYWORDS
Dendrocandin analogue; synthesis; anticancer activity; apoptosis

1. Introduction

Dendrobium officinale Kimura et Migo (*D. officinale*, Tiepi Shihu) is one of the most popular and valuable *Dendrobium* species and has been widely used as health supplements and traditional Chinese medicine (TCM) for antipyretic, eyes-benefiting, and tonic purposes et al. (China Pharmacopoeia Committee 2010). The main chemical components of *D. officinale* are polysaccharides, sesquiterpenoids, alkaloids, and phenolic compounds had the bioactivities of antioxidantion, antitumor, hypoglycemic and

CONTACT Cheng-Ting Zi zichengting@126.com; Xuan-Jun Wang wangxuanjun@gmail.com; Jun Sheng shengj@ynau.edu.cn
*These authors contributed equally to this work

Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2021.1901698.

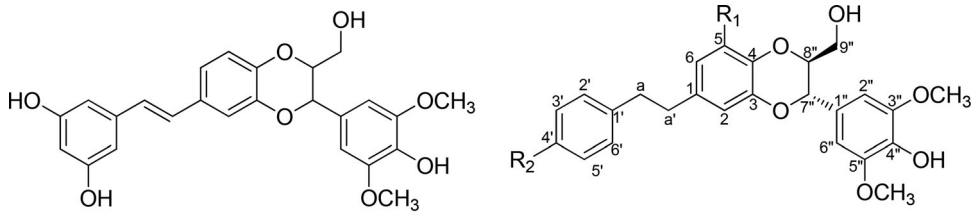


Figure 1. Structures of aiphanol and dendrocandin analogues (Dendrocandins V and compound **1**).

enhancing immune ability (Ma et al. 1994; Wu et al. 2004; Zha et al. 2007; Hsieh et al. 2008).

The natural dendrocandins were extracted from the stem of the *Dendrobium* species (Li et al. 2008, 2014; Yang et al. 2015; Zhang et al. 2017; Yang et al. 2018; Li et al. 2020). The structure of dendrocandin was elucidated as an unprecedented stilbenolignan skeleton in which a bibenzyl moiety is linked to a phenylpropane unit through a dioxane bridge. It has a wide range of biological activities, such as antioxidantive, anticancer, and anti-inflammatory activities (Kuboki et al. 2003; Li et al. 2008, 2014; Yang et al. 2015; Zhang et al. 2017; Yang et al. 2018; Li et al. 2020). Among the dendrocandin family, aiphanol (Figure 1) exerts a potent anticancer activity (Shou et al. 2017). Zhang et al. (2017) reported that Dendrocandin V (Figure 1) showed cytotoxic activities against five human cancer cell lines (HL-60, A-549, SMMC-7721, MCF-7 and SW-480). Thus, those compounds may serve as a new anticancer drug that utilized starvation tactics to attack solid tumors.

However, dendrocandins are found in the stem of the *Dendrobium* species have very low levels, and also isolated very difficult because they have very similar chemical structures. So, very little information regarded dendrocandins from *D. officinale* has been reported. To study the relationship between the structure of dendrocandins and the anti-proliferati effects in human cancer cells, we synthesized a novel dendrocandin (**1**) using the starting materials 4-hydroxy-3,5-dimethoxybenzaldehyde and 3,4-dihydroxybenzaldehyde.

Aiphanol has been synthesized through a [4 + 2]-cycloaddition reaction and Witing reaction (Wang et al. 2004; Banwell et al. 2005). Compound **1** was prepared by total synthesis using a similar method of aiphanol, except the oxidative coupling reaction was promoted by an Ag(I) species (Guz et al. 2001). In the retrosynthetic analysis (Figure 2), the stibene moiety of compound **1** would be obtained from aldehyde **2** and phosphonium salt **3** by Wittig reaction. The 1,4-benzodioxane skeleton might be available through a silver oxide promoted oxidative coupling of a benzylallylic alcohol **4** and 3,4-dihydroxybenzaldehyde **5**. Compound **4** would be prepared from 4-hydroxy-3,5-dimethoxybenzaldehyde **6** by Wittig olefination and reduction reaction with DIBAL-H.

Furthermore, we have evaluated cytotoxicity of compound **1** against human cancer cells in vitro using the MTT assay, and analysed cell apoptosis in SW480 cells by flow cytometry. The synthetic procedures and the biological assay results are also provided herein.

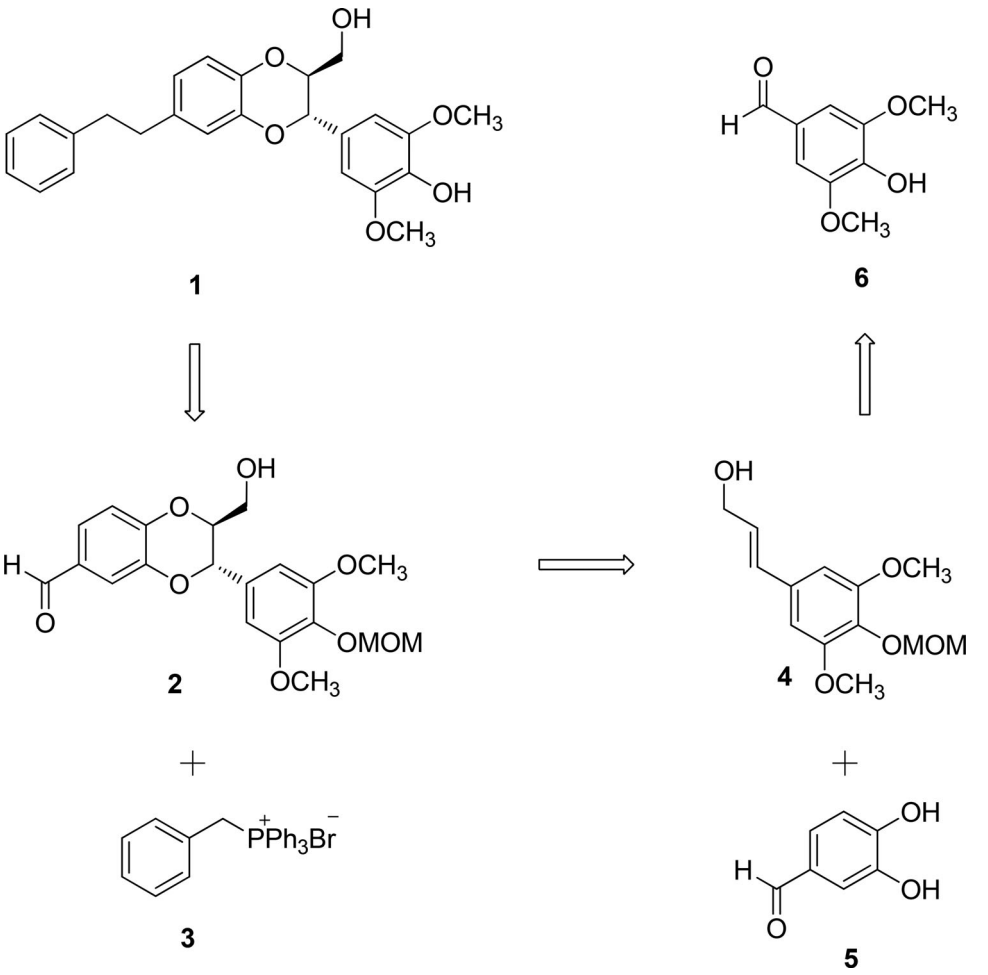


Figure 2. A retrosythetic analysis of compound **1**.

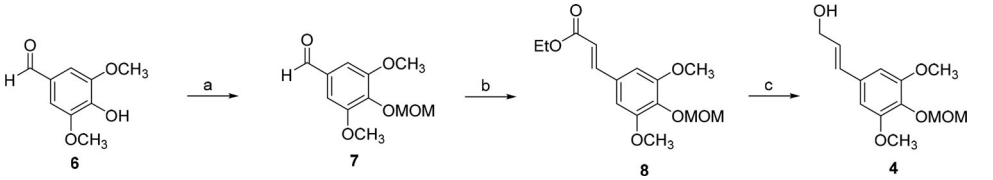
2. Results and discussion

2.1. Chemical synthesis

As shown in Scheme 1, the first half of the convergent synthesis required preparation of compound **4** Synthesis of compound **4** began from 4-hydroxy-3,5-dimethoxybenzaldehyde **6**. By following the reported method (Banwell et al. 2005), the phenolic unit of commercially available syringealdehyde **6** was protected as the corresponding MOM-ehter **7** in 95% yield. Aldehyde **7** was reacted with triethyl phosphonoacetate and sodium hydride (NaH) to afford the *E*-configured α,β-unsaturated ester **8** in 80% yield, which was reduced with diisobutyl aluminium hydride (DIBAL-H) in toluene to corresponding the cinnamyl alcohol **9** in 80% yield (Toshio et al. 1996).

Following the procedure of Merlini et al. (1980) the hetero-Diels–Alder reaction between the cinnamyl alcohol **9** and 3,4-dihydroxybenzaldehyde **5** gave the cycloadduct **2** in 56% yield (Scheme 2). The next step was construction of a stibene skeleton by Witting reaction using phosphonium salt **3** (Townsend et al. 1981), which was

prepared from benzyl alcohol in the 2 steps using a similar method has been reported



Scheme 1. Synthesis of compound **4**. Reagents and reaction conditions: (a) MOM-Cl, DIPEA, DCM, 0–18 °C, 6 h, 95%; (b) $EtOCOCH_2P(O)(OEt)_2$, NaH, THF, 0–18 °C, 4 h, 80%; (c) DIBAL-H, toluene, −10 °C, 1 h, 78%.

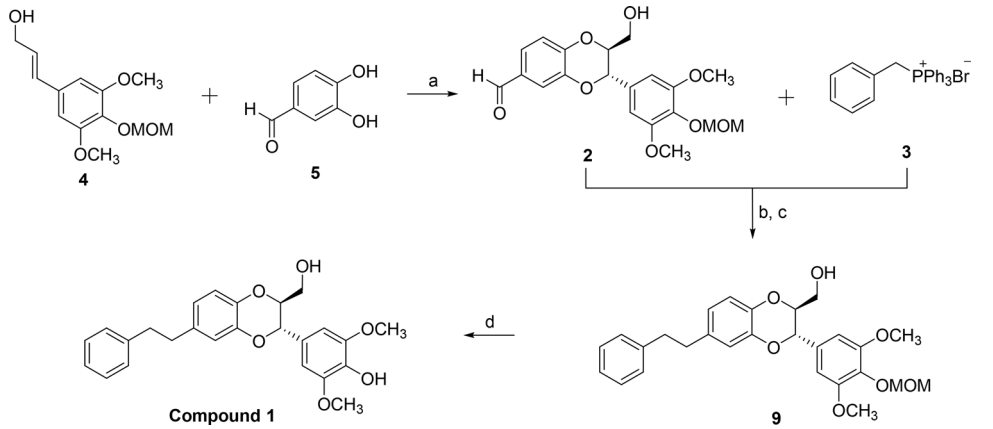
in the literature. Aldehyde **2** was treated with **3** in the presence of cesium fluoride (CsF) in toluene under reflux conditions to give protected the *E/Z* diastereomeric mixture in 60% yield, which was hydrogenated under hydrogen balloon conditions using 5% palladium-carbon (Pd-C) as a catalyst to afford protected dendrocandin **9** in 96% yield. Finally, the MOM group **9** was removed under acidic conditions to give compound **1** in 83% yield, which was further confirmed by the conducting of sets of 2D NMR experiments (1H-1H COSY, HSQC, HMBC, and ROESY spectra) (Supplementary material Figure S1). To determine the absolute configuration of compound **1**, the comparison between the experimental and calculated electronic circular dichroism (ECD) spectra of 1 was performed using the time dependent density functional theory (TDDFT) method with B3LYP/6-311++G(d, p)//B3LYP/6-31 G(d, p) level. The calculated ECD spectrum was comparable with the measured for compound 1 (Supplementary material Figure S2). From the above evidence, the structure of compound 1 was elucidated with 7′R, 8′S configurations.

2.2. In vitro antiproliferative activities

Combretastatin A-4P (CA-4P) was used as positive control. The activity of compound **1** was expressed by the IC_{50} value was presented in Table 1. As shown in Table S1 (Supplementary material), compound **1** displayed weak cytotoxicity to the A549, MCF-7 and HL-60 cell lines ($IC_{50} > 40\,\mu M$). However, compound **1** showed potent against SW480 cell line tested at IC_{50} value with $16.27 \pm 0.26\,\mu M$. Thus, we decided to study the effect of compound **1** on SW480 cells. Moreover, colony formation assays were performed to further validate the anti-proliferating effect of compound **1** when the concentration of **1** reached 15 μM (Supplementary material Figure S3).

2.3. Compound 1 induced apoptosis in the SW480 cells

Considering that compound **1** exhibits antiproliferative activity *in vitro*, we further studied the capacity of **1** to induce cell death through apoptosis. The apoptotic effect of compound 1 on SW480 cells was detected by using CA-4P as the reference compound. As shown in the Figure S4A (Supplementary material), the percentages of apoptotic cells were determined to be greater than those under control conditions, indicating that compound **1** induced apoptosis in SW480 cells. Caspase-3 and PARP (poy ADP ribose polymerase) are considered to be an important indicator of apoptosis



Scheme 2. Synthesis of compound **1**. Reagents and reaction conditions: (a) Ag_2O, benzene-acetone (2:1), reflux, 24 h, 56%; (b) CsF, toluene, 110 °C, 24 h, 60%; (c) H_2, Pd-C (5%), CH_2Cl_2, 8 h, 96%; (d) AcOH, CH_3OH, 18 °C, 20 h, 83%.

(Hensley et al. 2013). SW480 cell lines were treated with compound **1** at different concentrations (3.75, 7.5 and 15 μM) for 24 h and the expression level of cleaved caspase-3 and PARP was monitored using western blot. As shown in the Figure S4B (Supplementary material), the expression levels of apoptotic proteins (cl-PARP) were significantly up-regulated, indicating that compound **1** can up-regulate the expression of apoptotic proteins, leading to apoptosis.

3. Conclusions

In summary, the total synthesis of a dendrocandin analogue (**1**) was achieved using a convergent strategy (9 steps, 12.6% overall yield) using the starting materials 4-hydroxy-3,5-dimethoxybenzaldehyde and 3,4-dihydroxybenzaldehyde which included a silver oxide promoted oxidative coupling and Wittig reaction as the key steps, and screened for anticancer activity against six human cancer cells (MCF-7, A549, A431, SW480, HepG-2 and HL-60). Compound **1** showed potent cytotoxicity against SW480 cells, with the IC_{50} value 16.27 ± 0.26 μM. The colony formation assays were performed to further validate the anti-proliferating effect of compound **1**. Compound **1** also can up-regulate the expression of apoptotic proteins (cl-PARP). Zhang et al. (2017) reported the anti-cancer mechanism of Aiphanol. There were many autophagosomes appeared in the cytoplasm treated with Aiphanol. However, after the addition of autophagy inhibitors, autophagosomes were significantly reduced. The structure of Compound 1 is similar to Aiphanol. We speculate that 1 may induce cell death through autophagy.Thus, this compound suggested that its potential as anticancer agent for further development. Further refinement of the synthetic scheme and structure-function relationships (SARs) of dendrocandins will be reported in due course.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was financially supported by grants from National Nature Science Foundation of China (grant numbers 21602196, 31960075); the Science and Technology Project of Yunnan province (grant numbers 2017ZF003, 2017FG001-046, 2018FG001-035 and 2019ZG00904).

References

Banwell MG, Chand S, Savage GP. 2005. An enantioselective total synthesis of the stilbenolignan (-)-aiphanol and the determination of its absolute stereochemistry. Tetrahedron Asymmetr. 16(9):1645–1654.

China Pharmacopoeia Committee 2010. Chinese Pharmacopoeia, Part I. Beijing: Chemistry and Industry Press, p. 265.

Guz NR, Stermitz FR, Johnson JB, Beeson TD, Willen S, Hsiang J, Lewis K. 2001. Flavonolignan and flavone inhibitors of a staphylococcus aureus multidrug resistance pump: structure-activity relationships. J Med Chem. 44(2):261–268.

Hensley P, Mishra M, Kyprianou N. 2013. Targeting caspases in cancer therapeutics. Biol Chem. 394(7):831–843.

Hsieh SY, Chien C, Liao KS, Liao SF, Hung WT, Yang WB, Lin CC, Cheng TR, Chang CC, Fang JM, Wong CH. 2008. Structure and bioactivity of the polysaccharides in medicinal plant Dendrobium huoshanense. Bioorg Med Chem. 16(11):6054–6068.

Kuboki A, Yamamoto T, Ohira S. 2003. Total Synthesis of (±)-Aiphanol, a Novel Cyclooxygenase-inhibitory Stilbenolignan. Chem Lett. 32(5):420–421.

Li QM, Jiang H, Zha XQ, Wu DL, Pan LH, Duan J, Liu J, Luo JP. 2020. Anti-inflammatory bibenzyls from the stems of dendrobium huoshanense via bioassay guided isolation. Nat Prod Res. 34(4):563–566.

Li Y, Wang CL, Guo SX, Yang JS, Xiao PG. 2008. Two new compounds from dendrobium candidum. Chem Pharm Bull (Tokyo)). 56(10):1477–1479.

Li Y, Wang CL, Zhao HJ, Guo SX. 2014. Eight new bibenzyl derivatives from Dendrobium candidum. J Asian Nat Prod Res. 16(11):1035–1043.

Ma GX, Xu GJ, Xu LS. 1994. Inhibitory effects of Dendrobium chrysotoxum and its constituents on the mouse HePA and ESC. China Pharm Univ. 3:188–189.

Merlini L, Zanarotti A, Pelter A, Rochefort MP, Hänsel R. 1980. Benzodioxans by oxidative phenol coupling. synthesis of silybin. J Chem Soc Perk T. 1(0):775–778.

Shou CC, Feng JN, Zhao CK. inventors; 2017. Feb 27. New application of Aiphanol in antitumor therapy. China Patent. CN201710110342.2

Toshio W, Takatoshi F, Setsuya O, Seiitsu M. inventors; 1996. Japan Patent JP08113567.

Townsend CA, Davis SG, Christensen SB, Link JC, Lewis CP. 1981. Methoxymethyl-directed aryl metalation. Total synthesis of (.+.)-averufin. J Am Chem Soc. 103(23):6885–6888.

Wang XL, Feng JP, Xie XG, Cao XP, Pan XF. 2004. First total synthesis of (±)-aiphanol. Chinese Chem Lett. 15:1036–1038.

Wu HS, Xu JH, Chen LZ, Sun JJ. 2004. Studies on anti-hyperglycemic effect and its mechanism of Dendrobium candidum. China J Chin Mater. Med. 2:69–72.

Yang D, Cheng ZQ, Yang L, Hou B, Yang J, Li XN, Zi CT, Dong FW, Liu ZH, Zhou J, et al. 2018. Seco-dendrobine-type alkaloids and bioactive phenolics from dendrobium findlayanum. J Nat Prod. 81(2):227–235.

Yang L, Liu SJ, Luo HR, Cui J, Zhou J, Wang XJ, Sheng J, Hu JM. 2015. Two new dendrocandins with neurite outgrowth-promoting activity from dendrobium officinale. J Asian Nat Prod Res. 17(2):125–131.

Zha XQ, Wang JH, Pan LH, Luo JP, Lu MM. 2007. Study on antioxidant activity of polysaccharides from dendrobium species. Food Ence. 12:90–93.

Zhang C, Liu SJ, Yang L, Yuan MY, Li JY, Hou B, Li HM, Yang XZ, Ding CC, Hu JM. 2017. Sesquiterpene amino ether and cytotoxic phenols from dendrobium wardianum warner. Fitoterapia. 122:76–79.