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Insights into the structure–activity relationship of the anticancer compound ZJ-101, a derivative of marine natural product superstolide A: A critical role played by the conjugated trienyl lactone moiety

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ABSTRACT

Compound ZJ-101, a structurally simplified analog of the marine natural product superstolide A, was previously developed in our laboratory. In the subsequent structure–activity relationship study, two new analogs, ZJ-105 and ZJ-106, were designed and synthesized to probe the importance of the conjugated trienyl lactone moiety of the molecule by replacing the C2–C3 double bond in ZJ-101 with a single bond and switching the geometry of the C4–C5 double bond in ZJ-101 from *Z* to *E*, respectively. Biological evaluation showed that ZJ-105 completely loses antiproliferative activity whereas ZJ-106 is significantly less active against cancer cells in vitro than ZJ-101, suggesting that the conjugated trienyl lactone moiety of the molecule is critical for its anticancer activity.

Natural products have been a major source of drugs for centuries.[1] Since ziconotide, the first drug from the sea, was approved in the United States in 2004 for the treatment of chronic pain in spinal cord injury, biologically active marine natural products have been playing an increasingly important role in drug discovery and development.[2] In fact, we are likely experiencing a 21st century renaissance of drug discovery based on marine natural products.[3] In addition, potent anticancer natural products play very important roles in both antibody-drug conjugates (ADCs) and small molecule-drug conjugates (SMDCs) therapies because natural products can be used as cytotoxic payloads.[4]

Superstolide A (**1**) (Fig. 1), isolated in minute amounts from the deep-water marine sponge *Neosiphonia superstes*, exhibits potent antiproliferative activity against several tumor cell lines with IC_{50} values ranging from 4.8 to 64 nM.[5] Its potent anticancer activity suggests a potential use of superstolide A as a payload for an antibody-drug conjugate (ADC) or a small molecule-drug conjugate (SMDC). However, the lack of adequate compound supply from natural resources coupled with the overwhelming difficulty in the development of a practical total synthesis approach[6] severely impeded the preclinical evaluation of this group of structurally distinct and mechanistically unique marine natural products.

We recently designed and synthesized a truncated superstolide A (named as ZJ-101) that maintains the potent anticancer activity of the original natural product (Fig. 1).[7] Because our synthetic approach is very efficient and the synthesis can be scaled up, we have for the first time successfully developed a synthetic strategy that enabled sufficient amounts of material for additional biological and mechanistic evaluation. A possible novel mechanism of action[8] plus the potential use as a payload for an antibody-drug conjugate (ADC) or a small molecule-drug conjugate (SMDC) demands careful understanding the structure–activity relationships of ZJ-101.[9]

While the potent anticancer activity of ZJ-101 partially confirmed our original hypothesis that the 16-membered macrolactone is likely the pharmacophore responsible for interacting with its putative target,[7] it is imperative to precisely characterize the role of the conjugated trienyl lactone moiety in its anticancer activity. Therefore, we designed two analogs ZJ-105 and ZJ-106 (Fig. 2) where the C2–C3 double bond is replaced with a single bond and the geometry of the C4–C5 double bond is switched from *Z* to *E*, respectively. These two analogs would not only enable us to gain insights into the structure–activity relationship of ZJ-101 but also

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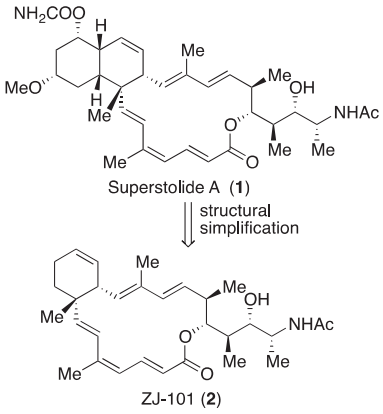


Figure 1. Superstolide A and truncated superstolide A (ZJ-101).

reveal the significance of the conjugated trienyl lactone moiety on the anticancer activity. Herein, we report our synthesis and biological evaluation of compounds ZJ-105 and ZJ-106.

The retrosynthetic analyses of ZJ-105 and ZJ-106 are analogous to that of ZJ-101 and are outlined in Scheme 1. These synthetic routes are advantageous as they are flexible and convergent.

The commercially available butane-1,4-diol **8** was converted to compound **9** using reported procedures (Scheme 2).[10] Compound **9** reacted with TBAF to give alcohol **10** in 57% yield. Swern oxidation of the primary alcohol **10** to aldehyde **11** followed by Pinnick oxidation provided the carboxylic acid **6** in excellent yield. Compound **6** was then converted to anhydride **12** in 91% yield.

Regioselective esterification reaction of diol **5**[7] and anhydride **12** gave ester **13** in 80% yield (Scheme 3). Compound **13** was treated with Ti(O-*i*-Pr)$_4$ to obtain the desired ester **14**, which underwent intramolecular Stille coupling to provide compound ZJ-105 (**3**) in 53% yield.[7]

But-2-yn-1-ol **15** was converted to vinyl stannane **16** using published literature procedures (Scheme 4).[11] Vinyl stannane **16** reacted with iodine gave vinyl iodide **17** in 91% yield. MnO_2-mediated allylic oxidation of compound **17** followed by Horner–Wadsworth–Emmons olefination provided ester **18** in 73% yield with complete *E*-stereoselectivity. Ester **18** was hydrolyzed to carboxylic acid **7**, which was converted to anhydride **19** in excellent yield.

Regioselective esterification reaction of diol **5** and anhydride **19** gave ester **20** in 91% yield (Scheme 5). Compound **20** was treated with Ti(O-*i*-Pr)$_4$ to obtain the desired ester **21**, which underwent intramolecular Stille coupling to provide compound ZJ-106 (**4**) in 65% yield.[7]

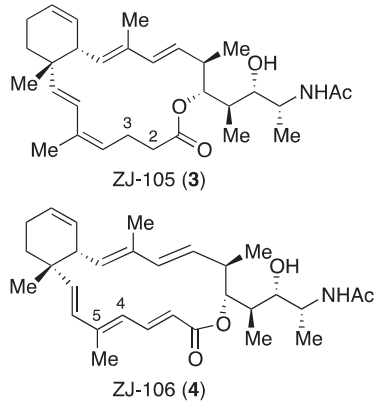
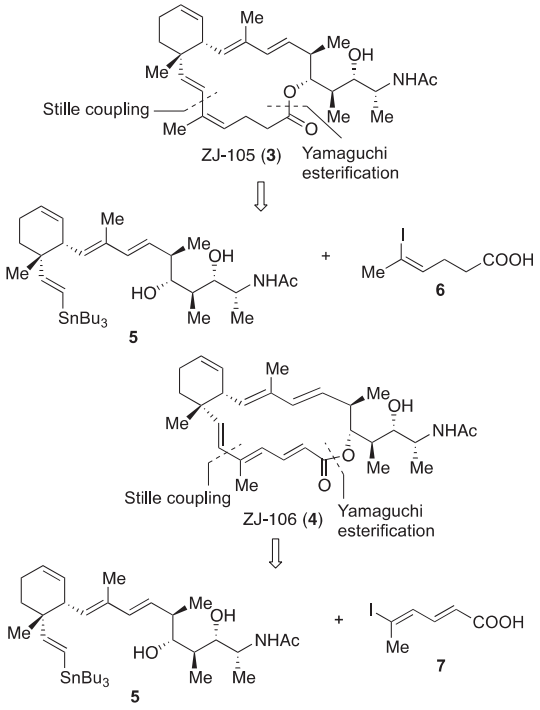
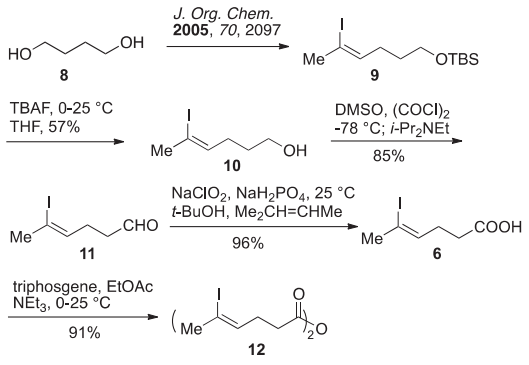


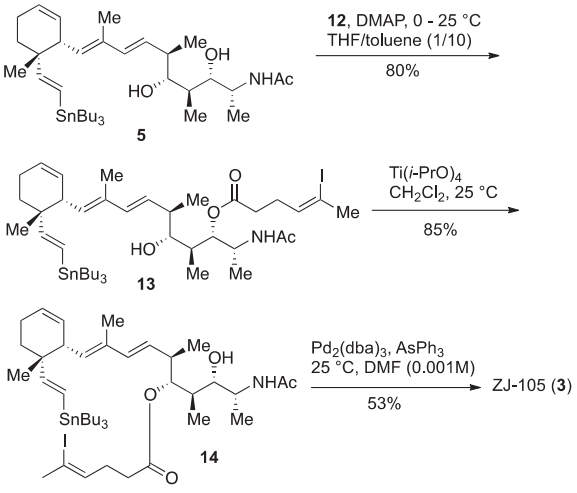
Figure 2. Designed analogs ZJ-105 and ZJ-106.



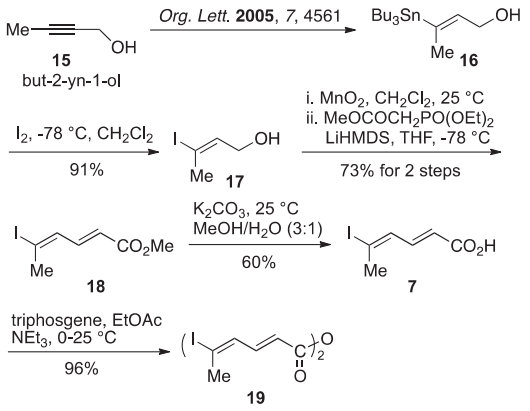
Scheme 1. Retrosynthetic analysis of ZJ-105 and ZJ-106.



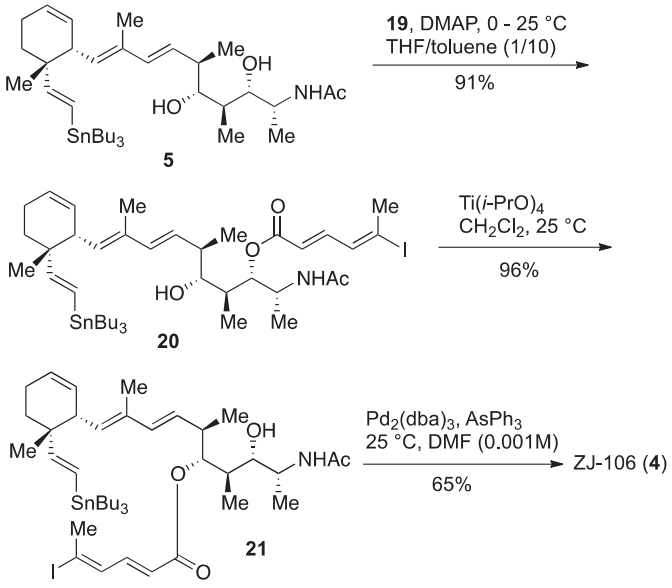
Scheme 2. Synthesis of compound **12**.



Scheme 3. Synthesis of ZJ-105.



Scheme 4. Synthesis of compound **19**.



Scheme 5. Synthesis of ZJ-106.

The antiproliferative effects of ZJ-105 and ZJ-106 were determined in MDA-MB-231 (human breast cancer), SF-295 (human glioblastoma), MCF-7 (human breast cancer) and RKO (human colon cancer) cell lines using the Alamar Blue viability assay, along with ZJ-101 as the positive control.[12] The IC_{50} values are shown in Table 1. ZJ-105 completely lost its antiproliferative activity in these four cell lines, suggesting that the C2–C3 double bond is absolutely essential for its biological activity. In addition, the activity of ZJ-106 dropped by up to 10 folds, suggesting the *Z*-geometry of the C4–C5 double bond is also important.

In conclusion, we have designed and synthesized compounds ZJ-105 and ZJ-106 by replacing the C2–C3 double bond in ZJ-101 with a single bond and switching the geometry of the C4–C5 double bond in ZJ-101 from *Z* to *E*, respectively. The biological testing has confirmed our original hypothesis that the trienyl conjugated lactone moiety of the molecule is indeed the pharmacophore and future structural optimization should keep the chemical structure of this region intact. Research in this direction is currently underway and will be reported in due course.

Table 1
Antiproliferative effect of ZJ-105 and ZJ-106 on four malignant tumor cells (Alamar Blue assay)

Entry	Cell Line	ZJ-105 IC_{50} (nM)	ZJ-106 IC_{50} (nM)	ZJ-101 IC_{50} (nM)
1	MDA-MB-231	>5000	384.3	63.0
2	SF-295	>5000	610.6	63.5
3	MCF-7	>5000	376.0	36.5
4	RKO	>5000	1068	213.8

Conflict of interest statement

Dr. Zhendong Jin is the founder and a shareholder of InnoBioPharma, LLC that sponsored the project, 'Development of novel anticancer agents based on natural products' at the University of Iowa.

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