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# Semisynthesis and biological evaluation of a cotylenin A mimic derived from fusicoccin A

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ABSTRACT

In an effort to overcome the unavailability of cotylenin A (CN A), an anticancer agent and a stabilizer of protein–protein interactions (PPIs) mediated by 14-3-3 proteins, ISIR-050 was designed as a CN A mimic. The synthesis was accomplished via a semisynthetic approach starting from fusicoccin A. ISIR-050 showed interferon-α (IFNα)-dependent growth inhibitory activity and a PPI stabilization effect similar to those of CN A. The biochemical analysis suggested that ISIR-050 and CN A induce the same pharmacological response to IFNα-treated cancer cells and that 14-3-3 proteins play a role in the mode of action.
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Fusicoccins (FCs) and cotylenins (CNs) are structurally similar diterpene glycosides, with a fusicoccane (5-8-5 tricyclic ring system) core skeleton linked to a functionalized glucose unit at the 9-position (Fig. 1). Although FCs and CNs have been independently isolated from different fungus strains,[1–4] they show similar phytohormone-like activities *via* a unique mode of action. They activate the plant plasma membrane $H^+$-ATPase (PMA) through stabilization of the protein–protein interaction (PPI) between a plant 14-3-3 protein and PMA, in which the penultimate threonine in the C-terminus is phosphorylated.[5,6] 14-3-3 proteins are a family of highly conserved proteins in all eukaryotes that regulate diverse intracellular signal transductions by forming complexes with numerous phosphorylated client proteins in a phosphorylation-dependent manner.[7,8] Previously, we showed that FCs and CNs (FC/CNs) are able to bind to human 14-3-3 proteins and stabilize their PPIs in the same binding site and manner as in the case of plant 14-3-3 proteins and PMA. 14-3-3 binding motifs in client phosphoproteins are classified into a C-terminal motif (X-pS-$X_{COOH}$, mode 3) and internal motifs (-RXXpSXP-, mode 1 or 2), although many noncanonical binding motifs have been identified.[7] PPI stabilization of FCs is well characterized in binary complexes of 14-3-3 and mode 3 clients.[5,6,9] Further, some internal motifs in which the interaction with 14-3-3 is stabilized by FCs have been found recently.[10–12] As the α-faces of FC/CNs interact with 14-3-3 clients, the α-facial functionalities of FC/CN derivatives are considered important in terms of the preference for X residues in the 14-3-3 binding motifs.[13]

The biological activities of FC/CNs against vertebrates have been reported over the past two decades.[14,15] For example, FC/CN derivatives are known to have anticancer activities.[16–21] Among them, CN A shows differentiation-inducing activity toward some leukemia cells,[16,20] inhibits tumor growth in combination with interferon-α (IFNα)[17,18] and cancels the cetuximab-resistance associated with the HRAS G12V mutation[11] not only *in vitro* but also *in vivo*. Thus, although CN A has potential as a useful anticancer drug, the fungal strain producing CNs, an unidentified *Cladosporium* species,[3,4] has lost its proliferative ability. Some research groups including ours, have attempted to provide CN A *via* organic synthesis[22–25] or biogenetic methods.[26] However, no practical method to produce CN A has been found. Therefore, it is difficult to conduct further biological studies on CN A aimed at drug development.

To overcome this problem, a CN A analogue was synthesized in this study to serve as an alternative to CN A as a potent anticancer agent. As 14-3-3 PPI stabilizers, the 5-8-5 ring of a FC/CN faces 14-3-3-binding phosphoproteins, and the individual functionality

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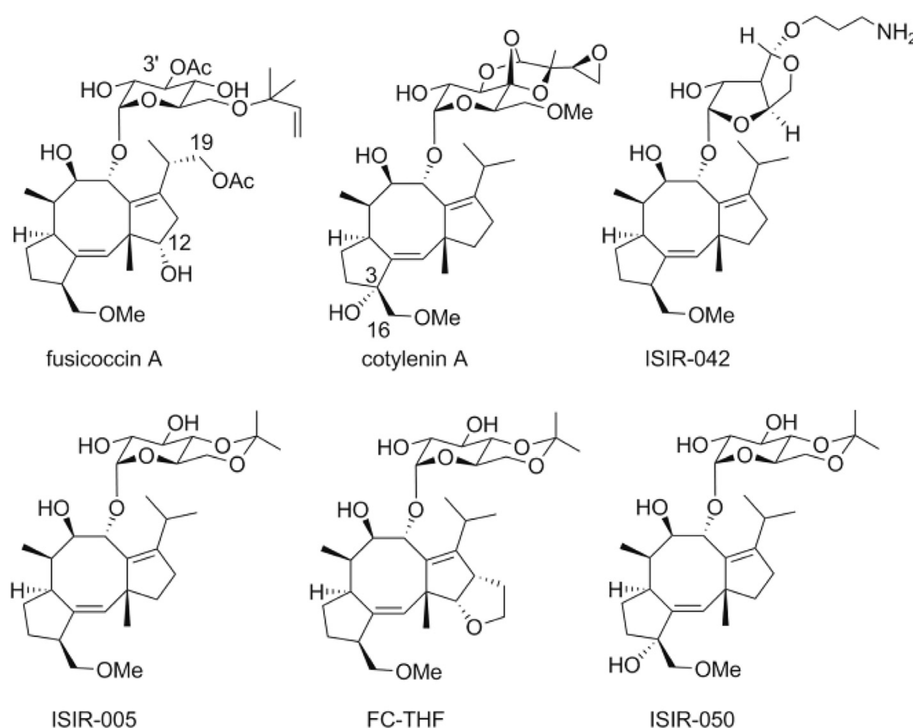


**Fig. 1.** Natural and semisynthetic fusicoccin analogues.

on the aglycone affects its preference for 14-3-3 client proteins in ternary complexes. Therefore, anticipating that the entire CN aglycone structure will be necessary to mimic the biological activity of CN A, we designed ISIR-050 (Fig. 1). Previously, we developed various FC/CN analogues such as ISIR-005, ISIR-042, and FC-THF,[9,19,27] in which aglycones are not exactly the same as CN A. ISIR-050 possesses the same aglycone structure as CN A and shares the sugar moiety with ISIR-005. In other words, the presence of the 3α-hydroxy group is the only structural difference between ISIR-050 and ISIR-005.

The semisynthesis of ISIR-050 from FC A, a major secondary metabolite of *Phomopsis amygdali* that is commercially available,[28] is summarized in Scheme 1. First, FC A was treated with $NaHCO_3$ to selectively hydrolyze the acetoxy group at the 3′ position to afford 3′-deacetyl-FC A. Then, bisacetonide formation utilizing methoxypropene and camphorsulfonic acid (CSA) was carried out to protect the four hydroxy groups at the 2′, 3′, 4′, and 8 positions, affording **1**.[9] Hydrolysis of the acetoxy group at the 19 position of **1** afforded a diol. Subsequent methanesulfonylation and removal of the acetonides and the 2-methyl-1-butenyl group in the sugar moiety by acid treatment gave dimesylate **2**. The primary sulfonyl ester at the 19 position of **2** was reductively eliminated by $LiBH_4$ in diglyme. The remaining 12-mesylate was β-eliminated using *tert*-BuOK/dimethyl sulfoxide (DMSO) to give a conjugated diene in the C ring; the less-hindered double bond of the diene was then selectively hydrogenated by Rh-catalyzed hydrogenation. Subsequent acetal formation at the 4′ and 6′ hydroxy groups with 2,2-dimethoxypropane and pyridinium *p*-toluenesulfonate (PPTS) provided ISIR-005. Previously, we synthesized ISIR-005 from FC H, a biosynthetic intermediate of FC A.[19] As FC A is more easily available than FC H, using this method, we can prepare ISIR-005 on the gram scale. After protecting all the hydroxy groups of ISIR-005 as triethylsilyl (TES) ethers (**3**), the 16-methoxy group was eliminated using 10 equiv. of *tert*-butyllithium at −10 to −15 °C to give exomethylene **4** in moderate yield with 11% of **3** recovered. The reaction temperature and the excess of *tert*-butyllithium are important factors in this elimination reaction. Only 7% yield was achieved when the reaction was started at −80 °C and the temperature was slowly raised to −10 °C. The C(3)–C(16) exomethylene of **4** was dihydroxylated using $OsO_4$/*N*-methyl-morpholine-*N*-oxide (NMO). The reaction proceeded stereoselectively and gave the product as a single diastereomer. The synthesis of ISIR-050 was completed by methylation and deprotection of the TES groups. Dimethyl ether **5** was also obtained as a minor byproduct. The stereochemistry at the C(3) position in ISIR-050 was confirmed by NOE experiments, as an NOE correlation was observed between the C(17) methyl protons and the C(16) methylene proton (see supplementary material). Overall, starting from FC A, ISIR-050 was semisynthesized in 12% yield (14 steps).

Following the successful semisynthesis of ISIR-050, we performed MTS-dye-based cell growth inhibition assays. A human ovarian carcinoma cell line, OVCAR-3, and a human lung carcinoma cell line, A549, which were used in previous studies,[17,21] were treated with FC derivatives in the presence or absence of IFNα. The dose response curves are shown in Fig. 2. Treatment with 400 U/mL of IFNα reduced the viability of OVCAR-3 cells to approximately 70% compared with the control, whereas it had no influence on the viability of A549 cells. The $EC_{50}$ values calculated from each dose response curve are summarized in Table 1. As we anticipated, ISIR-050 showed IFNα-dependent growth inhibitory activity similar to or slightly better than that of CN A. In the absence of IFNα, ISIR-050 was less toxic than CN A. Thus, the toxicity of CN A is likely associated with the chemical structure of the sugar moiety. ISIR-005 showed the highest toxicity for both cell lines when FC derivatives were used without IFNα, and the $EC_{50}$ values were somewhat decreased by IFNα. In contrast to the other derivatives, dimethoxy derivative **5** did not induce a reduction of cell viability below 40 μM. Instead, **5** caused IFNα-treated OVCAR-3 cells to recover their viability. Despite their structural similarity, the pharmacological responses induced by these three derivatives are quite different. This very limited but interesting structure–activity relationship (SAR) indicates that small structural differences at the C(3) position have a great influence on the biological characteristics of FC/CN derivatives.

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