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Critical role of a methyl group on the γ-lactone ring of annonaceous acetogenins in the potent inhibition of mitochondrial complex I

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ABSTRACT

C34-*epi* and C34-*epi*-C35-trifluoro analogues of solamin, a mono-THF annonaceous acetogenin, were synthesized. Their inhibitory activity, along with previously synthesized analogues (C35-fluoro, C35-difluoro, and C35-trifluorosolamins), against bovine mitochondrial NADH–ubiquinone oxidoreductase (complex I) was determined. The present study revealed that the methyl group on the γ-lactone moiety is critical to the potent inhibition of complex I by natural acetogenins.

More than 500 annonaceous acetogenins have been isolated from the plant family *Annonaceae* since the discovery of uvaricin in 1982.[1–3] Acetogenins have very potent and diverse biological effects such as antitumor, antimalarial, and pesticidal activities.[2,3] Annonacin, the major acetogenin in the tropical plant *Annona muricata*, is highly toxic to cultured neurons and may play a role in some neurodegeneration in humans.[4,5] The inhibitory effect of acetogenins on mitochondrial NADH–ubiquinone oxidoreductase (complex I) is of particular importance since their diverse biological activities are thought to be attributable to this effect.

The chemical structure of most natural acetogenins is characterized by four segments: (i) an α,β-unsaturated γ-lactone ring, (ii) one to three tetrahydrofuran (THF) rings with flanking OH group(s), (iii) a long alkyl tail, and (iv) an alkyl spacer linking the two pharmacophores (i.e., γ-lactone and THF moieties) (Fig. 1). On the basis of structure–activity relationship studies carried out by ourselves and other groups, the roles of each segment in the inhibitory action against bovine complex I have been elucidated as follows: (i) a natural γ-lactone ring itself is not crucial for the activity and is substitutable with other structures,[6–10] (ii) neither the number of THF rings nor the stereochemistry around the THF ring moiety with the flanking hydroxy group is an essential factor,[11–14] and the presence of either of two OH groups adjacent to the THF ring(s) sufficiently sustains the potent activity,[15] and (iii) a long alkyl tail is preferable, but not essential since even a methyl derivative elicited strong inhibition at the nanomolar level.[16] Thus, the crucial structural factors of acetogenins are ambiguous, suggesting that complex I recognizes each of the multiple functional groups of the inhibitors in a fairly loose way. It is however noteworthy that acetogenins act as strong inhibitors only when the γ-lactone and THF moieties are directly linked by a long alkyl spacer:[17] the optimal length of the spacer for exhibiting the inhibition is approximately 13 carbon atoms.[18,19]

Kojima et al. synthesized three C35-fluorinated solamin analogues (C35-fluoro, C35-difluoro, and C35-trifluorosolamins **1**–**3**, Fig. 2) and investigated their growth inhibitory activities against human cancer cell lines.[20,21] Interestingly, the activity decreased as the number of fluorine atoms increased, though it remained to be clarified whether the decrease is due to a reduction of the activity at the target enzyme level (i.e., complex I) or to other factors such as differences in metabolic stability. If the former is a cause,

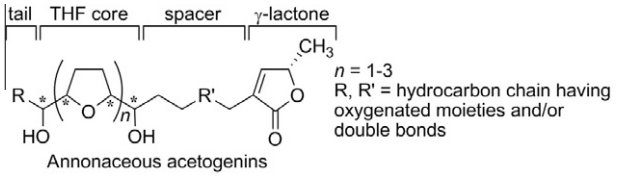

Figure 1. Representative structure of natural acetogenins.

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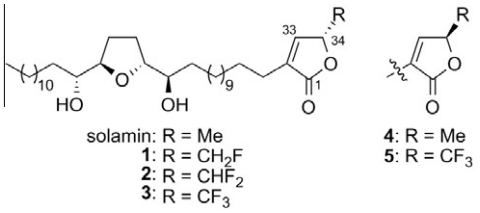


Figure 2. C35-fluorinated solamins and their epimer.

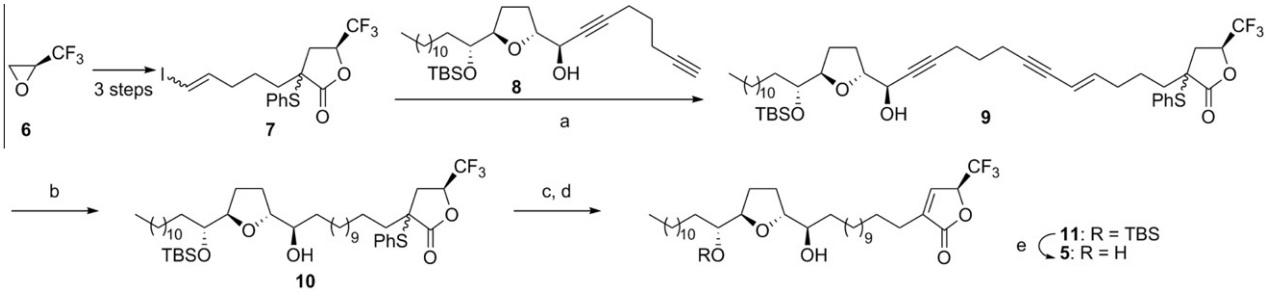
it means that the γ-lactone moiety is strictly recognized by the enzyme, and hence a critical structural factor required for the inhibition, but we did not examine the inhibition of complex I in that report. To further elucidate the inhibitory action of acetogenins, we here synthesized C34-*epi*- and C34-*epi*-C35-trifluorosolamins, and determined the inhibitory activity of all fluorinated analogues, C34-*epi*-solamin, and solamin against bovine heart mitochondrial complex I.

To clarify the effect of stereochemistry of the methyl group in the γ-lactone on the biological activity,[22] we planned the synthesis of C34-*epi*-solamin **4** and C34-*epi*-C35-trifluorosolamin **5**. Derivative **5** was synthesized using the same procedure as that for C35-trifluorosolamin **3**,[20] as shown in Scheme 1. A commercially available (*S*)-3,3,3-trifluoro-1,2-epoxypropane **6** was converted to iodide **7** by reported procedures. The Sonogashira coupling of **7** and the THF-ring fragment **8** followed by hydrogenation with Wilkinson's catalyst gave sulfide **10**. Oxidation of **10**, followed by thermal elimination of the resulting sulfoxide, afforded α,β-unsaturated γ-lactone **11**. The synthesis of C34-*epi*-C35-trifluorosolamin **5** was completed via deprotection of TBS ether with acidic conditions.

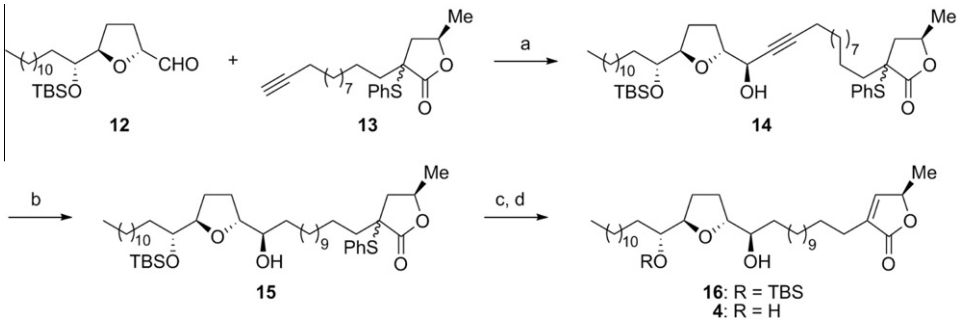
We previously reported the total synthesis of solamin by asymmetric alkynylation of α-tetrahydrofuranyl aldehyde **12**, which was stereoselectively synthesized by our method,[23,24] with methyl 13-tetradecynoate followed by the construction of α,β-unsaturated γ-lactone moiety.[25] When synthesizing C34-*epi*-solamin **4**, a more efficient method was examined (Scheme 2). Direct asymmetric alkynylation[26] of **12** with the alkyne **13**,[27] which has a γ-lactone moiety, proceeded smoothly to give propargyl alcohol **14** in good yield with high diastereoselectivity (95:5). Hydrogenation of **14** with Pd–C in EtOAc afforded a saturated alcohol **15** in 64% yield. Oxidation of **15** followed by thermal elimination of the resulting sulfoxide afforded a crude product including alcohol **16**. Deprotection of TBS ether was proceeded by purification of the reaction mixture by column chromatography over silica gel giving C34-*epi*-solamin **4**.

We determined the inhibitory activity of all fluorinated analogues (**1**–**3**, **5**), C34-*epi*-solamin **4**, and solamin against complex I using bovine heart submitochondrial particles (Table 1). The activity decreased in the following order: C35-fluoro > C35-difluoro > C35-trifluorosolamin. This tendency is consistent with that of the growth inhibitory activity against human cancer cell lines.[20] A structural 'bulkiness' of fluorine, which often replaces hydrogen in organic molecules but the size and stereoelectronic influences of the two atoms are quite different,[28,29] may disturb the intermolecular interaction between the γ-lactone ring and the enzyme. It is worth noting that both C34-*epi*- and C34-*epi*-C35-trifluorosolamins remarkably lost the activity; the loss due to epimerization was greater for the former. These results unambiguously indicate that both the bulkiness and the stereochemistry of C34–CH_3 are very important structural factors for interacting with the enzyme: in other words, the γ-lactone moiety is strictly recognized by the enzyme.

The present results are inconsistent with our previous work. We had performed a structure–activity relationship study concerning the γ-lactone moiety using bis-THF acetogenin derivatives (not mono-THF derivatives), indicating that a natural γ-lactone ring itself is not crucial for the activity and can be substituted with other structures.[6] We concluded therefore that the enzyme might not



Scheme 1. Reagents and conditions: (a) $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, rt, 87%; (b) $Rh(PPh_3)_3Cl$, H_2 (1 atm), benzene–MeOH (1:1), rt, 55%; (c) *m*CPBA, CH_2Cl_2, 0 °C; (d) toluene, 60–65 °C, 57% in two steps; (e) 48% HF aq, MeCN–THF (1:2), rt, 93%.



Scheme 2. Reagents and conditions: (a) $Zn(OTf)_2$, (1*R*,2*S*)-*N*-methylephedrine, *i*-Pr_2NEt, toluene, rt, 81%, *dr* = 95:5; (b) Pd–C, H_2 (3 atm), EtOAc, rt, 64%; (c) *m*CPBA, CH_2Cl_2, 0 °C; (d) toluene, 80 °C then silicagel column chromatography, 68% in two steps.

Table 1
Inhibition of bovine heart mitochondrial complex I by solamin analogues

Compounds	IC_{50} (nM)
Natural solamin (C35-CH_3)	2.1 ± 0.20
C35-Fluorosolamin **1**	2.9 ± 0.22
C35-Difluorosolamin **2**	31 ± 1.9
C35-Trifluorosolamin **3**	310 ± 28
C34-*epi*-Solamin **4**	270 ± 20
C34-*epi*-C35-Trifluorosolamin **5**	570 ± 49

The IC_{50} values, which is the molar concentration (nM) needed to reduce the control NADH oxidase activity (0.63–0.75 μmol NADH/min/mg of protein) in bovine heart submitochondrial particles by half. Values are means ± SD of three independent experiments.

recognize this moiety in a strict sense. To further elucidate the inhibitory mechanism of acetogenins as well as important structural factors required for the inhibitory action, a discussion of possible causes of the discrepancy is needed.

There is a point to be made before the discrepancy is discussed however. We produced 'Δlac-acetogenins' by deleting the γ-lactone ring from natural bis-THF acetogenins.[30–32] It is worth noting that Δlac-acetogenins elicit an inhibitory effect on complex I as strongly as natural acetogenins do, whereas the binding site of the inhibitors differs from that of natural acetogenins;[31,32] in other words, deletion of the γ-lactone ring converts natural acetogenins to a different type of complex I inhibitor. By contrast, deletion of the γ-lactone ring from mono-THF acetogenins results in an almost complete loss of the activity.[30] Thus, Δlac-acetogenin-like inhibitory behavior occurs only in the case of bis-THF derivatives. This complicates the profile of structure–activity relationship for natural bis-THF acetogenins: a decrease in the inhibitory activity due to structural modifications of the γ-lactone moiety is apparently masked by the inhibitory activity elicited as Δlac-acetogenin since the two separate events cannot be distinguished.[33] To overcome this problem and to examine the effects of structural modifications of the γ-lactone moiety on the inhibitory activity, mono-THF acetogenins are better than bis-THF derivatives as control compounds. Altogether, the present study unambiguously indicates that the γ-lactone moiety, which binds to the region spanning the fourth to fifth transmembrane helices (Val144-Glu192) in the ND1 subunit of bovine complex I,[34] is strictly recognized by the enzyme, and hence a critical structural factor of natural acetogenins required for the potent inhibition.

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