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Synthesis of C4-fluorinated solamins and their growth inhibitory activity against human cancer cell lines

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ABSTRACT

C4-Fluorinated analogues of solamin, an antitumor acetogenin, were synthesized and investigated for their antitumor activities against 39 tumor cell lines. C4-Fluorinated solamins showed more potent growth inhibitory activity against cancer cell lines than solamin.

Annonaceous acetogenins are polyketides isolated from *Annonaceae* species growing in the tropics. Most acetogenins are characterized by one to three tetrahydrofuran (THF) ring(s) with various stereochemistries at the center of a long hydrocarbon chain having an α,β-unsaturated γ-lactone moiety at the end. More than 400 acetogenins have been isolated and they exhibit a broad range of biological activities, including immunosuppressive, pesticidal, and antitumor activities.[1] Regarding their antitumor activity, they have been shown to function by blocking NADH-ubiquinone oxidoreductase (complex I) in mitochondria. This leads to depletion of ATP and subsequent apoptosis.[2] Many researchers are engaged in the synthesis of natural acetogenins due to their attractive biological activities and unique structural features.[3] The synthesis and evaluation of acetogenin analogues have also been reported.[4] Fluorination of biologically active natural products is known to sometimes enhance the activity. Because of this, in medicinal chemistry, the fluorination of such compounds is often designed.[5] However, to our knowledge, the synthesis of fluorinated acetogenins has not been reported so far. Because we are very interested in the bioactivity of fluorinated acetogenins, we planned the synthesis of C4-fluorinated solamin (**1**). Solamin, a *threo*/*trans*/*threo*-type mono-THF ring acetogenin, was isolated from *Annona muricata* (Fig. 1).[6] Murisolin, a C4-hydroxyl solamin, was also isolated from the same plant. Interestingly, their growth inhibitory activity against human cancer cell lines greatly differs in spite of their structural similarity. We have also examined in detail the growth inhibitory activity of our synthetic samples against 39 human cancer cell lines. Murisolin showed 400 times stronger growth inhibitory activity against the human lung cancer cell line, DMS114, than solamin. It is well known that fluorine atom mimics hydrogen atom. Substitution of the hydroxyl group of biologically active compounds with an isoelectronic function, fluorine, produced more potent analogues.[7] Therefore, we were interested in whether C4-fluorinated solamin (**1**) showed similar activity to solamin or murisolin. Herein we describe the synthesis of C4-fluorinated

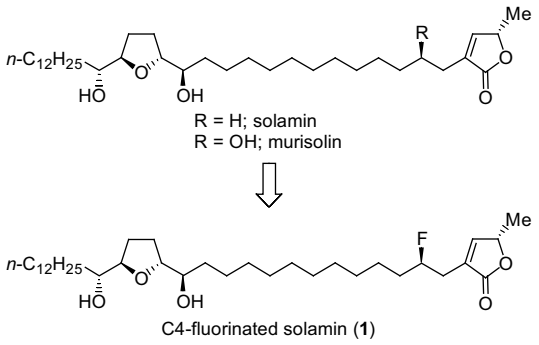

Figure 1. Design of C4-fluorinated solamin.

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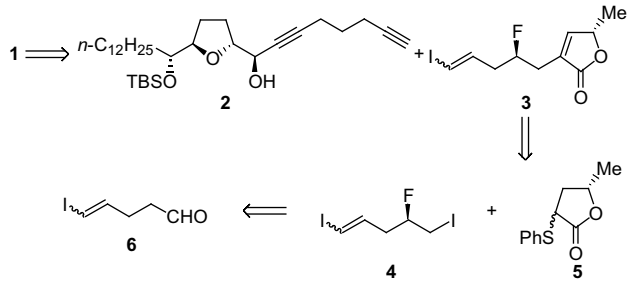
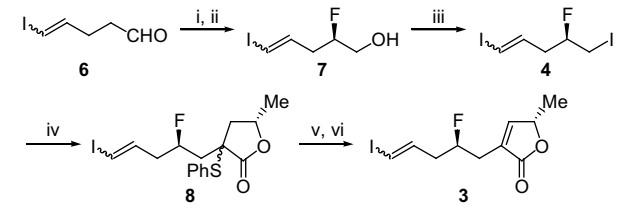
solamins and the evaluation of their growth inhibitory activity against 39 tumor cell lines.

C4-Fluorinated solamin (**1**) was prepared by Sonogashira coupling of THF-ring segment (**2**)[8] with fluorinated γ-lactone segment (**3**). Fluorinated segment (**3**) was synthesized by α-alkylation of known α-sulfenyl-γ-lactone (**5**)[9] with iodide (**4**) prepared by enantioselective α-fluorination of known aldehyde (**6**) (Scheme 1).[10]

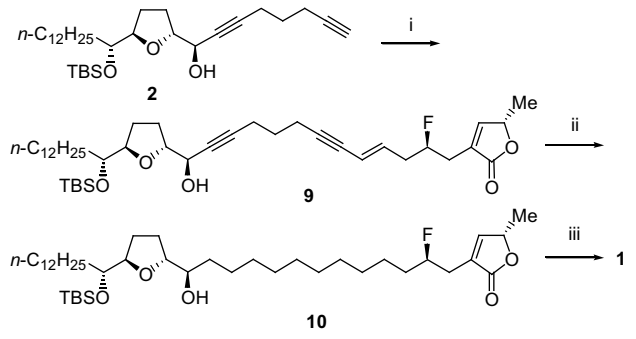


Scheme 1. Retrosynthesis of C4-fluorinated solamin (**1**).

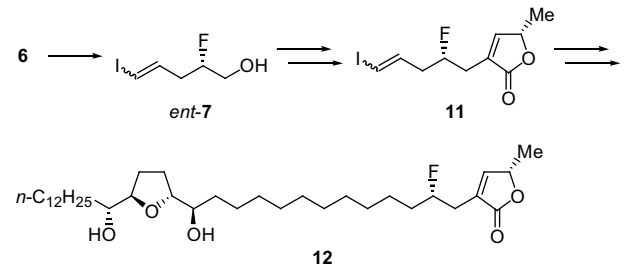
The preparation of fluorinated γ-lactone segment (**3**) is summarized in Scheme 2. Enantioselective α-fluorination of known aldehyde (**6**) was accomplished with MacMillan's conditions (*N*-fluorobenzenesulfonimide (NFSI), (*R*)-5-benzyl-2,2,3-trimethylimidazolidin-4-one dichloroacetic acid salt),[11] and the resulting α-fluoroaldehyde was immediately reduced to give β-fluoroalcohol



Scheme 2. Reagents and conditions: (i) NFSI, (*R*)-5-benzyl-2,2,3-trimethylimidazolidin-4-one dichloroacetic acid salt, THF–*i*-PrOH, −10 °C; (ii) $NaBH_4$, CH_2Cl_2–EtOH, rt, 53% (>98% *ee*) in two steps; (iii) I_2, PPh_3, DMF, 60 to 100 °C, 90%; (iv) **5**, *t*-BuOK, DMSO, rt, 74%; (v) *m*-CPBA, CH_2Cl_2, 0 °C; (vi) toluene, reflux, 70% in two steps.



Scheme 3. Reagents and conditions: (i) **3**, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, rt, 84%; (ii) $TsNHNH_2$, NaOAc, 1,2-dimethoxyethane–H_2O, reflux, 56%; (iii) 48% HF aq., CH_3CN–THF, rt, 88%.



Scheme 4. Synthesis of C4-*epi*-fluorinated solamin **12**.

Table 1
GI_{50} values of C4-fluorinated solamins (**1** and **12**), solamin, and murisolin against 39 human tumor cell lines.

Human tumor cell line	Compound/Growth inhibitory activity (GI_{50}[a] in μM)			
	1	**12**	Solamin	Murisolin
Breast				
HBC-4	>100	>100	>100	>100
BSY-1	>100	69	>100	8.3
HBC-5	>100	>100	>100	9.7
MCF-7	56	37	71	3.9
MDA-MB-231	>100	>100	>100	>100
CNS				
U251	>100	>100	>100	>100
SF-268	>100	>100	>100	>100
SF-295	37	31	40	7.3
SF-539	>100	>100	>100	88
SNB-75	84	58	>100	39
SNB-78	>100	>100	>100	78
Colon				
HCC2998	>100	>100	>100	>100
KM-12	>100	>100	>100	>100
HT-29	>100	92	>100	>100
HCT-15	>100	>100	>100	51
HCT-116	2.0	27	>100	3.7
Lung				
NCI-H23	23	5.1	73	0.22
NCI-H226	58	40	>100	29
NCI-H522	25	16	58	3.6
NCI-H460	>100	>100	>100	>100
A549	>100	>100	>100	96
DMS273	>100	>100	>100	72
DMS114	0.26	0.46	4.3	<0.01
Melanoma				
LOX-IMVI	6.5	97	29	1.9
Ovarian				
OVCAR-3	>100	>100	>100	15
OVCAR-4	>100	>100	>100	6.9
OVCAR-5	>100	>100	>100	>100
OVCAR-8	62	>100	>100	38
SK-OV-3	>100	>100	>100	>100
Renal				
RXF-631L	>100	>100	>100	40
ACHN	>100	>100	>100	>100
Stomach				
St-4	74	>100	>100	41
MKN1	>100	>100	>100	20
MKN7	0.96	19	13	0.70
MKN28	41	>100	14	2.8
MKN45	>100	43	>100	>100
MKN74	>100	>100	>100	4.6
Prostate				
DU-145	>100	>100	>100	>100
PC-3	>100	84	>100	17
MG-MID[b]	54	63	76	20
Delta[c]	2.33	2.14	1.24	3.3
Range[d]	2.59	2.34	1.36	4.00

[a] Concentration for 50% inhibition of cell growth relative to control. Cell growth was determined according to the sulforhodamine B assay.
[b] Mean GI_{50} value in all cell lines tested.
[c] Difference between log GI_{50} value of the most sensitive cell line and the MG-MID value.
[d] Difference in log GI_{50} value between the most and least sensitive cell lines.

(**7**, >98% *ee*[12]) due to instability of the α-fluoroaldehyde. Iodination of **7** with iodine and PPh_3 gave iodide (**4**) in 90% yield. Alkylation of known γ-lactone (**5**) with **4** in the presence of *t*-BuOK[13] gave sulfide (**8**) in 74% yield. Oxidation of sulfide (**8**) into the sulfoxide, followed by thermal elimination, afforded fluorinated γ-lactone segment (**3**).

THF-ring segment (**2**) was prepared with our developed synthetic method[14] with high stereoselectivity. Assembly of fragments **2** and **3** was performed with the Sonogashira reaction.[15] Selective reduction of resulting enediyne **9** with diimide, followed by deprotection of TBS ether, gave C4-fluorinated solamin (**1**) (Scheme 3).

Epimer (**12**) at C4 position was synthesized from *ent*-**7** prepared by α-fluoriation of **6** with (*S*)-MacMillan's catalyst (Scheme 4).

Synthesized C4-fluorinated solamin (**1**) and C4-*epi*-fluorinated solamin (**12**) were tested for in vitro antiproliferative activity against a panel of 39 human cancer cell lines.[16] Table 1 shows the 50% growth inhibitory concentration relative to control (GI_{50}). The GI_{50} values of analogues **1** and **12** were lower than those of solamin for a large number of cell lines, which means that the two analogues have stronger growth inhibitory activity against cancer cell lines than solamin. The human lung carcinoma cell line, DMS114, was most sensitive to synthesized **1** and **12** (GI_{50}: 0.26 and 0.46 μM, respectively), and to natural solamin (4.3 μM) and murisolin (0.01 μM). We also noted some features in the fingerprints of the two fluorinated analogues. For example, C4-fluorinated solamin (**1**) exhibited approximately 20 times higher cytotoxicity to MKN7 than C4-*epi*-fluorinated solamin (**12**). Compound (**1**) showed at least 50 times higher cytotoxicity to HCT-116 than solamin. Together, the results suggest that the existence and stereochemistry of fluorine atom at C4-position are recognized by the cancer cell lines.

Using COMPARE analysis,[17] we compared the fingerprints of **1** and **12** with those of more than 60 conventional anticancer drugs currently in use, and found that the fingerprints of **1** and **12** did not show any significant correlation with those of conventional anticancer drugs. This suggests that **1** and **12** have a unique mode of action. In addition, COMPARE analysis indicated that **1**, **12**, and solamin were very similar (**1** vs solamin: $r = 0.81$; **12** vs solamin: $r = 0.69$; **1** vs **12**: $r = 0.79$). This may indicate that these three compounds share the same mode of action.

Further synthesis of other fluorinated analogues of acetogenins and investigation of their growth inhibitory activity are under way.

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