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Synthesis of dansyl-labeled probe of thiophene analogue of annonaceous acetogenins for visualization of cell distribution and growth inhibitory activity toward human cancer cell lines

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ABSTRACT

The convergent synthesis of the dansyl-labeled probe of the thiophene-3-carboxamide analogue of annonaceous acetogenins, which shows potent antitumor activity, was accomplished by two asymmetric alkynylations of the 2,5-diformyl THF equivalent with an alkyne having a thiophene moiety and another alkyne tagged with a dansyl group. The growth inhibitory profiles toward 39 human cancer cell lines revealed that the probe retained the biological function of its mother compound, and would be useful for studying cellular activity.

1. Introduction

The development of novel antitumor agents, particularly ones with hitherto unexplored modes of action, is strongly advocated because no effective treatment for all kinds of cancers exists and the number of cancer patients continues to rise with the accelerated aging of the world's population.

Our group has been engaged in the syntheses of the analogues of annonaceous acetogenins,[1] which are polyketides isolated from the *Annonaceae* plant growing in tropical and subtropical regions, for use as novel anticancer agents. In Figure 1, the structure of solamin, a mono-THF acetogenin, is shown as an example of acetogenins.[2] Annonaceous acetogenins are long-chain fatty acids (C32 or C34) whose terminal carboxylic acid combines with a 2-propanol unit at the C-2 position to form methyl-substituted α,β-unsaturated γ-lactones.

Most of the annonaceous acetogenins have one to three 2,5-disubstituted tetrahydrofuran (THF) systems with one or two flanking hydroxy groups at the center of a long hydrocarbon chain. It was revealed that acetogenins potently suppressed the growth of human cancer cell lines by inhibiting NADH ubiquinone oxidoreductase (complex I) in the mitochondrial electron transport system.[3] Many total syntheses of natural acetogenins[4] and their analogues[5] have been reported.

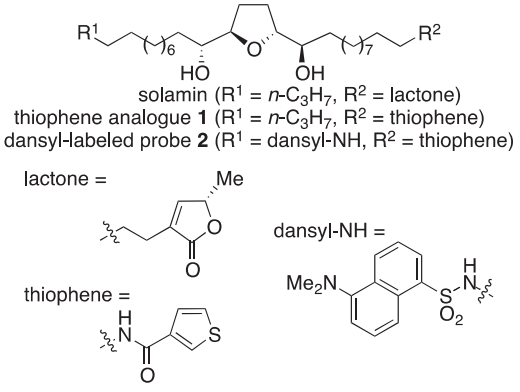

Figure 1. Structures of solamin and its analogues.

We have previously reported the synthesis and biological evaluation of the analogues of solamin,[6] a mono-THF acetogenin. Our analogues are characterized by the presence of various heterocycles instead of an α,β-unsaturated γ-lactone,[7] and most of them

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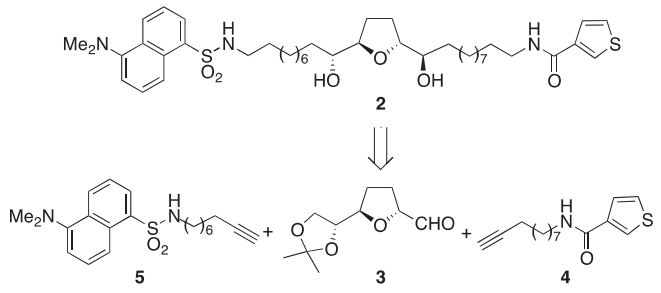
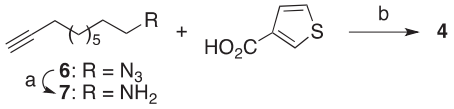


Scheme 1. Retrosynthetic analysis of dansyl-labeled probe **2** of thiophene-3-carboxamide analogue.



Scheme 2. Reagents and conditions: (a) PPh_3, H_2O, Et_2O, 0 °C to rt; (b) EDC, DMAP, THF, 0 °C to rt, 77% in two steps.

show stronger inhibitory activity toward human cancer cell lines than solamin. One of them, thiophene-3-carboxamide **1**, potently inhibited the growth of NCI-H23, a human lung cancer cell line, without critical toxicity in a mouse xenograft assay.[8] To elucidate the mode of action of lead compound **1**, we attempted to visualize its cell distribution.[9] Herein we describe the synthesis of dansyl-labeled analogue **2** and its growth inhibitory activity toward human cancer cell lines.

2. Results and discussion

2.1. Synthesis of dansyl-labeled probe of thiophene analogue of annonaceous acetogenins

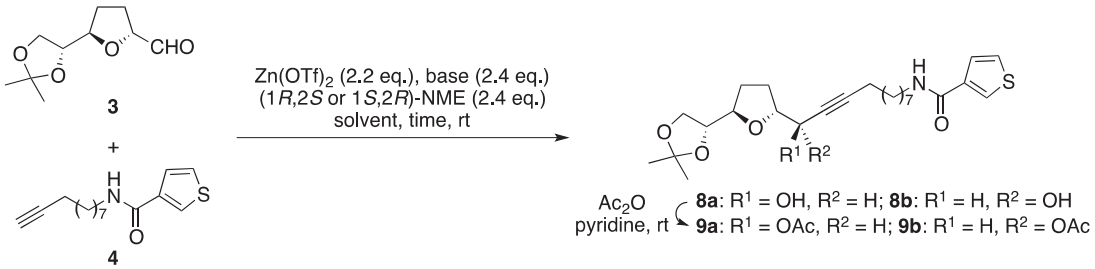
We designed fluorescent-labeled probe **2**, which has a dansyl group as the fluorescent group at the left end of the hydrocarbon chain, because an analogue of solamin, which has a dansyl group at the same position, gave good results in the elucidation of the mode of action of natural acetogenins.[9d–f] Scheme 1 outlines the synthesis of dansyl-labeled probe **2**. Probe **2** is divided into three fragments retrosynthetically: THF fragment **3**, thiophene fragment **4**, and dansyl fragment **5**. A convergent synthesis involving the sequential asymmetric alkynylations of 2,5-diformyl THF equivalent **3** with two alkynes **4** and **5** was planned. The direct alkynylation with **4** or **5** is a challenging approach because the amide proton may hinder the reaction.

Thiophene fragment **4** was synthesized by reducing known azide **6**[7a] into primary amine **7** followed by condensation of commercially available 3-thiophenecarboxylic acid with EDC and DMAP in THF in good yield (Scheme 2).

We examined the applicability of Carreira's asymmetric alkynylation[10] to the reaction of α-tetrahydrofuranyl aldehyde **3**, which was prepared with our procedure,[11] with alkyne **4** (Table 1). Our first attempted reaction with Et_3N in the presence of (1*R*,2*S*)-*N*-methylephedrine (NME) in toluene gave propargyl alcohol **8a** in low yield with moderate diastereoselectivity (entry 1).[12] The diastereoselectivity was determined by analyzing the ^1H NMR spectra of acetate **9** after acetylation with Ac_2O in pyridine. The use of *i*-Pr_2NEt instead of Et_3N accelerated the reaction, but the decomposition of aldehyde **3** was observed (entry 2). It was revealed that this asymmetric alkynylation gave a higher yield when it was conducted in the presence of a higher solvent concentration.[11f] However, the reaction hardly proceeded when the solvent concentration exceeded 0.2 M because of the low solubility of alkyne **4** in toluene. Therefore, the solvent was changed to CH_2Cl_2 as **4** showed good solubility in it. As a result, the yield was improved to 65% with good diastereoselectivity (85:15) when the reaction was carried out in 0.4 M CH_2Cl_2 solution (entry 4). When the solvent concentration was increased further, the diastereoselectivity was slightly reduced (entry 5). Diastereomer **8b** was obtained in good yield with moderate diastereoselectivity by use of the antipode of NME (entry 6).

Separation of the two diastereomers (**8a**–**b** or **9a**–**b**) was difficult by silica gel chromatography. As mentioned above, acetylation of the mixture of **8a** and **8b** with Ac_2O in pyridine gave acetates **9a** and **9b** while retaining the diastereomeric ratio. Interestingly, we found that acetylation of the mixture of **8a** and **8b** (88:12) by using NaH as the base in THF at 0 °C afforded acetate **9a** with high diastereoselectivity (96:4), although the yield was low (Table 2, entry 1). By increasing the equivalent of Ac_2O and the reaction time, the

Table 1
Asymmetric alkynylation of α-tetrahydrofuranyl aldehyde **3** with alkyne bearing thiophene **4**



Entry	NME	Solvent[a] (concn, M)	Base	Time (h)	Yield[b] (%, **8a**:**8b**)
1	1*R*,2*S*	Toluene (0.2)	Et_3N	16	47 (80:20)
2	1*R*,2*S*	Toluene (0.2)	*i*-Pr_2NEt	4	49 (86:14)
3	1*R*,2*S*	CH_2Cl_2 (0.3)	*i*-Pr_2NEt	3	63 (88:12)
4	1*R*,2*S*	CH_2Cl_2 (0.4)	*i*-Pr_2NEt	2	65 (85:15)
5	1*R*,2*S*	CH_2Cl_2 (0.8)	*i*-Pr_2NEt	2.5	70 (79:21)
6	1*S*,2*R*	CH_2Cl_2 (0.4)	*i*-Pr_2NEt	3.5	85 (12:88)

[a] Concentration of aldehyde in reaction solvent.
[b] Isolated yield. Diastereomer ratio (dr) was determined by ^1H NMR measurement after acetylation of resulting secondary alcohol with Ac_2O and pyridine.