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Note

Synthesis of cinnamoyl glucoside derivatives and their antiproliferation activities against murine melanoma B16-F10 cell line

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ABSTRACT

Twelve cinnamoyl glucoside derivatives were prepared by glycosylation of glucosyl trichloroacetimidate and cinnamic acid derivatives, followed by dechloroacetylation with a pyridine/H_2O mixture. Their structures were characterized by ^1H and ^{13}C NMR, as well as mass analysis. All the products were tested for their antiproliferation activities against murine melanoma B16-F10 cell line. Compounds **4e-4j** were able to inhibit the proliferation of murine melanoma B16-F10 cell line with IC_{50} values of 17.38 ± 0.07, 9.87 ± 0.09, 9.69 ± 0.12, 29.42 ± 0.04, 32.95 ± 0.08, 25.68 ± 0.09 μM, respectively.

1. Introduction

Melanoma, a kind of malignant skin tumor, poses a great threat to the quality of human life due to its high malignancy, easy spreading, and poor prognosis [1]. As one of the highest mortality tumors in the world, its incidence has been rising at a consistent rate of 3% over the past thirty years [2]. Despite molecular targeted therapy and immunotherapy have achieved some positive results for treatments of metastatic melanoma, these methods can hardly be widely used in clinical practice due to their high prices, serious side effects and obvious individual variations [3]. Therefore, developing new treatment strategies or agents is still urgent to further improve melanoma prognosis.

Cinnamoyl glucoside derivatives consist of cinnamic acid derivatives and glucose. They are widely spread in plants [4–7], fruits [8–11] and vegetables [12,13] and have antioxidant [14], antiglycative [15], phytotoxic [16] and inhibition of antigen-stimulated degranulation [9] activities. Recent study showed that cinnamoyl glucoside derivatives could inhibit the proliferation of tumor cells; for example, 1-*O*-*p*-hydroxycinnamoylglucose can significantly inhibit the proliferation of human HCT116 colorectal cancer cell line in a dose-dependent manner [17].

In this work, a series of cinnamoyl glucoside derivatives **4a-4l** were synthesized and screened as lead compounds for melanoma treatment by evaluating their antiproliferation activities against murine melanoma B16-F10 cell line. Among them, the hit compounds **4e-4j** showed potential on inhibiting the proliferation of B16-F10 cell line (IC_{50} values: 17.38 ± 0.07 μM for **4e**, 9.87 ± 0.09 μM for **4f**, 9.69 ± 0.12 μM for **4g**, 29.42 ± 0.04 μM for **4h**, 32.95 ± 0.08 μM for **4i**, 25.68 ± 0.09 μM for **4j**), being comparable with the positive control doxorubicin (IC_{50} value: 2.18 ± 0.04 μM). Herein, the synthesis, structure elucidation and antiproliferation activity evaluation of the twelve cinnamoyl glucoside derivatives were reported.

2. Results and discussion

2.1. Chemistry

Reports recommended that the cinnamoyl moiety posed obvious influence on the bioactivities of cinnamoyl glucosides (Scheme 1). For example, in DPPH radical scavenging activity assay (DPPH assay), 1-*O*-(*E*)-caffeoyl-β-D-glucopyranose exhibited stronger activity than 1-*O*-(*E*)-cinnamoyl-β-D-glucopyranose, while in superoxide radical scavenging assay (NBT assay) the latter compound showed more effective than the former [14]. In the caspase 3/7 activity inhibition tests, cinnamoyl glucosides had an order of cinnamoyl- > caffeoyl- > *p*-coumaroyl- [15] (Scheme 1). Therefore, considering the structural diversity of synthesis, we set the cinnamoyl moiety as aglycone for **4a-4h**, with electron-donating, electron-withdrawing, and neutral substituents at the

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ortho-, *meta*- or *para*-position of the benzene ring. Additionally, we designed **4i-4l** by small modifications of the structures, for example, replacing the six-membered aromatic ring with the less bulky five-membered heterocyclic ring (**4i**, **4j**), or further introducing a methyl onto the β-position of double bond (**4k**, **4l**).

Given the base-sensitive groups in the target compounds **4a-4l**, chloroacetyl was chosen as the key protecting group for glycosyl donor since it could be smoothly removed under mild conditions and keep the base-sensitive 1-*O*-acyl intact [18]. The general synthesis route consisted of two steps as shown in Scheme 2. Initially, glucopyranosyl trichloroacetimidate **1** and various cinnamic acid derivatives **2a-2l** were stirred at room temperature with 10% TMSOTf as catalyst to obtain protected cinnamoyl glycosides **3a-3l** (81–94% yield). Afterwards, **3a-3l** were dechloroacetylation with a pyridine/H_2O mixture, resulting in the corresponding target compounds **4a-4l** (90–95% yield). Under mild, concise reaction conditions, all of the synthetic compounds were obtained in good yields and gave satisfactory analytical data, which coincided with the structures in Scheme 2. Although the synthesis approach of cinnamoyl glucoside derivatives **4a** and **4b** have been reported [18], **4c-4l** were synthesized for the first time.

2.2. Anti-proliferation assay

For developing novel anti-melanoma agents, the *in vitro* anti-proliferation effects of compounds **4a-4l** against murine melanoma B16-F10 cell line were evaluated. The results were shown in Table 1. A general tendency was that when the substituent group of benzene ring changed from electron-donating (**4b**-**4d**) to electron-withdrawing (**4e**-**4h**), the anti-proliferation effect became more potent. Further replacing the benzene ring (**4a**) with the five-membered heterocyclic ring (**4i**, **4j**), the anti-proliferation effect increased obviously. However, introducing a methyl onto the β-position of double bond (**4k**) led to a sharp decrease of its activity against the growth of B16-F10 cell line. Over all, the density of the double bond and electron density of ring could make big differences on the anti-proliferation effects of cinnamoyl glucoside derivatives. When looking at the most potential compounds **4e** (17.38 μM), **4f** (9.87 μM), **4g** (9.69 μM), **4h** (29.42 μM), **4i** (32.95 μM), **4j** (25.68 μM), the structures are quite similar but a simple structure-activity relationship could be got which can guide us towards the preparation of other more effective analogs in the future. When the electron-withdrawing effects of substituent groups on benzene ring became stronger, the antiproliferation activities of cinnamoyl glucosides increased greatly. In order to see whether these compounds were selectively toxic to cancer cells, normal human dermal fibroblast (HDF) cell line was evaluated in parallel. All tested compounds showed no significant cytotoxicity at a concentration of 40 mM (Table 2), indicating that they were all nontoxic to the healthy cell line. Those first attempts to try to understand bioactivity of these compounds using simple *in vitro* methods need obviously more *in vivo* studies to clearly identify their mode or mechanism of action. The anti-proliferation activities of **4a-4l** were tested for the first time and the results above would shed light on the design and development of novel anti-melanoma agents.

3. Materials and methods

3.1. General information

All reactions were monitored by thin-layer chromatography over silica-gel-coated TLC plates (Yantai Chemical Industry Research Institute). The spots on TLC were visualized by warming 10% H_2SO_4 (10% H_2SO_4 in ethanol) sprayed plates on a hot plate. Column chromatography was performed using silica gel (Qingdao Marine Chemical Inc., China). NMR spectra were recorded on a Bruker AM-400 spectrometer (400 MHz), and the ^1H and ^{13}C NMR chemical shifts were referenced to the solvent or solvent impurity peaks for $CDCl_3$ at δ_H 7.24 and δ_C 77.23, for CD_3OD at δ_H 3.31 and δ_C 49.15, for $(CD_3)_2CO$ at δ_H 2.05 and δ_C 29.84. High-resolution electrospray ionization mass spectra (HRESIMS) were carried out on a Bruker micrOTOF II spectrometer. Compounds **2a-2j** were purchased from Adamas and used without further purification. Compounds **2k** and **2l** were synthesized following literature procedures [19].

3.2. Preparation of compounds 3a-3l

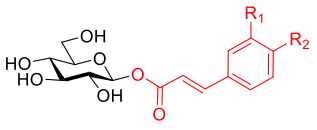
General glycosylation procedure: A solution of the glycosyl donor **1** (0.12 mmol, 1.2 equiv) and acceptor **2** (0.1 mmol, 1.0 equiv) in dry CH_2Cl_2 (2.0 mL) in the presence of 4 Å MS (100 wt%) was stirred for 15 min at 0 °C. After addition of TMSOTf (0.01 mmol, 0.1 equiv), the mixture was slowly warmed to room temperature and stirred for 5 h. Molecular sieves were removed by filtration and the solution was purified by silica gel chromatography to give the desired product **3**.

*Compound **3a***. 14.8 mg, 0.1 mmol of **2a** to yield **3a** (56.0 mg, 0.09 mmol, 91%) as a white powder. ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.73 (1H, d, J = 16.0 Hz), 7.53–7.49 (2H, m), 7.40–7.38 (3H, m), 6.38 (1H, d, J = 16.0 Hz), 5.89 (1H, d, J = 8.0 Hz, H-1), 5.43 (1H, t, J = 9.2 Hz), 5.29 (1H, dd, J = 9.2, 8.0 Hz), 5.24 (1H, t, J = 9.6 Hz), 4.39 (1H, dd, J = 12.8, 4.4 Hz), 4.28 (1H, dd, J = 12.8, 2.0 Hz), 4.10 (2H, s), 4.01 (2H, d, J = 3.2 Hz), 3.99 (2H, s), 3.98 (2H, s), 3.97 (1H, m) [18].

*Compound **3b***. 17.8 mg, 0.1 mmol of **2b** to yield **3b** (60.1 mg, 0.09 mmol, 93%) as a white powder. ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.67 (1H, d, J = 16.0 Hz), 7.45 (2H, d, J = 8.8 Hz), 6.87 (2H, d, J = 8.8 Hz), 6.22 (1H, d, J = 16.0 Hz), 5.88 (1H, d, J = 8.4 Hz, H-1), 5.42 (1H, t, J = 9.2 Hz), 5.30 (1H, dd, J = 9.2, 8.4 Hz), 5.24 (1H, t, J = 9.6 Hz), 4.38 (1H, dd, J = 12.8, 4.0 Hz), 4.28 (1H, dd, J = 12.8, 2.4 Hz), 4.09 (2H, s), 4.00 (2H, d, J = 3.6 Hz), 3.99 (2H, s), 3.98 (2H, s), 3.97 (1H, m), 3.81 (3H, s, OCH_3) [18].

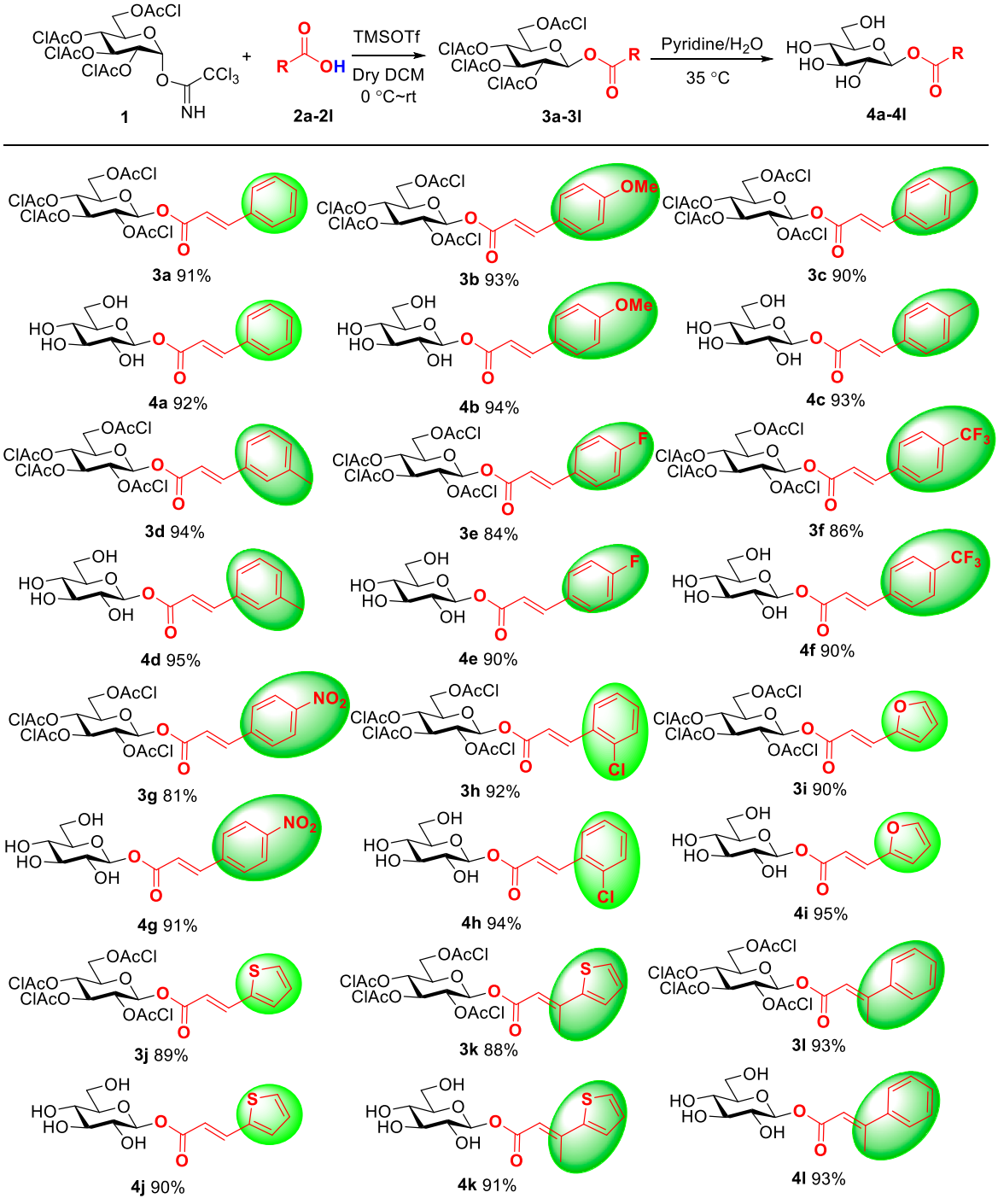
*Compound **3c***. 16.2 mg, 0.1 mmol of **2c** to yield **3c** (56.7 mg, 0.09 mmol, 90%) as a white powder. ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.72 (1H, d, J = 16.0 Hz), 7.41 (2H, d, J = 8.8 Hz), 7.19 (2H, d, J = 8.8 Hz), 6.33 (1H, d, J = 16.0 Hz), 5.88 (1H, d, J = 8.0 Hz, H-1), 5.42 (1H, t, J = 9.2 Hz), 5.30 (1H, dd, J = 9.2, 8.4 Hz), 5.24 (1H, t, J = 10.0 Hz), 4.39 (1H, dd, J = 12.8, 4.4 Hz), 4.28 (1H, dd, J = 12.8, 2.4 Hz), 4.10 (2H, s), 4.02 (2H, d, J = 2.8 Hz), 3.99 (2H, s), 3.97 (1H, m), 3.98 (2H, s), 2.36 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.2, 167.1, 166.5, 166.3, 164.8, 148.4, 142.0, 131.2, 130.0, 130.0, 128.7, 128.7, 114.7, 91.5 (C-1), 74.1, 72.3, 71.6, 69.4, 63.0, 40.7, 40.4, 40.4, 40.4, 21.7. HRMS calc. for $C_{24}H_{24}Cl_4O_{11}Na$ $[M+Na]^+$: 652.9941, found: 652.9943.

*Compound **3d***. 16.2 mg, 0.1 mmol of **2d** to yield **3d** (59.2 mg, 0.09 mmol, 94%) as a white powder. ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.71 (1H,



	DPPH assay IC_{50} (μM)	NBT assay IC_{50} (μM)	Inhibition of caspase 3/7 activity (%) at 3 μg/mL
1-*O*-(*E*)-cinnamoyl-β-D-glucopyranose $R_1 = R_2 = H$	-	-	37.6
1-*O*-(*E*)-*p*-coumaroyl-β-D-glucopyranose $R_1 = H$ $R_2 = OH$	25.6	18.8	7.1
1-*O*-(*E*)-caffeoyl-β-D-glucopyranose $R_1 = R_2 = OH$	8.0	34.9	19.7

Scheme 1. Cinnamoyl glucosides with different cinnamoyl moieties and their bioactivities.



Scheme 2. Synthesis of cinnamoyl glucoside derivatives **4a-4l**.

d, $J = 16.0$ Hz), 7.33 (1H, d, $J = 1.6$ Hz), 7.32 (1H, d, $J = 8.0$ Hz), 7.27 (1H, t, $J = 8.0$ Hz), 7.21 (1H, d, $J = 8.0$ Hz), 6.37 (1H, d, $J = 16.0$ Hz), 5.89 (1H, d, $J = 8.0$ Hz, H-1), 5.42 (1H, t, $J = 9.2$ Hz, H-1), 5.31 (1H, dd, $J = 9.2$, 8.4 Hz), 5.25 (1H, t, $J = 9.6$ Hz), 4.40 (1H, dd, $J = 12.8$, 4.0 Hz), 4.30 (1H, dd, $J = 12.8$, 2.4 Hz), 4.10 (2H, s), 4.02 (2H, d, $J = 3.2$ Hz), 3.99 (2H, s), 3.99 (1H, m), 3.99 (2H, s), 2.35 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.2, 167.0, 166.5, 166.3, 164.7, 148.5, 139.0, 133.8, 132.2, 129.3, 129.1, 125.9, 115.6, 91.5 (C-1), 74.0, 72.3, 71.6, 69.3, 62.9, 40.7, 40.4, 40.4, 40.4, 21.5. HRMS calc. for $C_{24}H_{24}Cl_4O_{11}Na$ $[M+Na]^+$: 652.9941, found: 652.9945.

*Compound **3e***. 16.6 mg, 0.1 mmol of **2e** to yield **3e** (53.3 mg, 0.08 mmol, 84%) as a white powder. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.67 (1H, d, $J = 16.0$ Hz), 7.50 (1H, d, $J = 8.0$ Hz), 7.49 (1H, t, $J = 8.0$ Hz), 7.06 (1H, d, $J = 8.0$ Hz), 7.04 (1H, t, $J = 8.0$ Hz), 6.28 (1H, d, $J = 16.0$ Hz), 5.88 (1H, d, $J = 8.0$ Hz, H-1), 5.43 (1H, t, $J = 9.6$ Hz), 5.29 (1H, t, $J = 9.6$ Hz), 5.23 (1H, t, $J = 9.6$ Hz), 4.38 (1H, dd, $J = 12.8$, 4.0 Hz), 4.28 (1H, dd, $J = 12.8$, 2.4 Hz), 4.08 (2H, s), 4.01 (2H, s), 3.99 (2H, s), 3.99 (1H, m), 3.98 (2H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.1, 167.0, 166.5, 166.3, 164.5, 146.9, 130.7, 130.6, 130.1, 130.1, 116.5, 116.3, 115.5, 91.5 (C-1), 73.9, 72.2, 71.5, 69.2, 62.9, 40.7, 40.4, 40.4, 40.4. HRMS calc. for $C_{23}H_{21}O_{11}NaCl_4F$ $[M+Na]^+$: 654.9720, found: 654.9724.

*Compound **3f***. 21.6 mg, 0.1 mmol of **2f** to yield **3f** (58.8 mg, 0.09 mmol, 86%) as a white powder. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.74 (1H, d, $J = 16.0$ Hz), 7.64–7.60 (4H, overlap), 6.45 (1H, d, $J = 16.0$ Hz), 5.90

Table 1
Anti-proliferation activities of compounds **4a-4l** against murine melanoma B16-F10 cell line.[a]

Compounds	IC_{50} (μM)	Compounds	IC_{50} (μM)
4a	195.1 ± 0.15	**4h**	29.42 ± 0.04
4b	90.48 ± 0.09	**4i**	32.95 ± 0.08
4c	91.92 ± 0.10	**4j**	25.68 ± 0.09
4d	112.0 ± 0.08	**4k**	299.7 ± 0.16
4e	17.38 ± 0.07	**4l**	44.13 ± 0.06
4f	9.87 ± 0.09	**Doxorubicin**[b]	2.18 ± 0.04
4g	9.69 ± 0.12		

[a] Results were expressed as means ± SEMs.
[b] Positive control.

Table 2
Inhibitory effects of compounds **4a-4l** on cell viability of HDF cell line at 40 mM.[a]

Compounds	Viability (%)	Compounds	Viability (%)
4a	100.11 ± 0.15	**4h**	100.36 ± 0.21
4b	101.95 ± 1.66	**4i**	100.65 ± 0.32
4c	100.46 ± 0.37	**4j**	102.13 ± 0.98
4d	101.46 ± 1.07	**4k**	100.10 ± 0.38
4e	101.18 ± 0.16	**4l**	99.72 ± 0.38
4f	102.96 ± 1.12	**Doxorubicin**[b]	101.23 ± 0.98
4g	100.79 ± 0.34		

[a] Results were expressed as means ± SEMs.
[b] Positive control.

(1H, d, J = 8.4 Hz, H-1), 5.44 (1H, t, J = 8.4 Hz), 5.31 (1H, t, J = 8.0 Hz), 5.25 (1H, d, J = 9.6 Hz), 4.40 (1H, dd, J = 12.8, 4.0 Hz), 4.30 (1H, dd, J = 12.8, 1.2 Hz), 4.09 (2H, s), 4.02 (2H, s), 4.01 (1H, m), 3.99 (2H, s), 3.99 (2H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.2, 167.0, 166.5, 166.3, 164.1, 146.2, 137.2, 133.0 (q, J = 32.3 Hz), 128.8, 128.8, 128.8, 126.2 (d, J = 4.0 Hz), 126.2 (d, J = 4.0 Hz), 118.5, 91.7 (C-1), 73.9, 72.4, 71.5, 69.3, 62.9, 40.7, 40.4, 40.4, 40.4. HRMS calc. for $C_{24}H_{22}O_{11}Cl_4F_3$ $[M+H]^+$: 682.9868, found: 682.9861.

*Compound **3g***. 19.3 mg, 0.1 mmol of **2g** to yield **3g** (53.6 mg, 0.08 mmol, 81%) as a white powder. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.25 (2H, d, J = 8.4 Hz), 7.76 (1H, d, J = 16.0 Hz), 7.68 (2H, d, J = 8.4 Hz), 6.51 (1H, d, J = 16.0 Hz), 5.89 (1H, d, J = 8.0 Hz, H-1), 5.43 (1H, t, J = 9.6 Hz), 5.32 (1H, dd, J = 9.6, 8.0 Hz), 5.25 (1H, d, J = 9.6 Hz), 4.40 (1H, dd, J = 12.4, 4.0 Hz), 4.31 (1H, dd, J = 12.8, 2.0 Hz), 4.10 (2H, s), 4.02 (2H, d, J = 2.8 Hz), 3.99 (1H, m), 3.99 (2H, s), 3.99 (2H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.1, 167.0, 166.5, 166.3, 163.7, 149.2, 145.1, 139.8, 129.3, 129.3, 124.5, 124.5, 120.2, 91.9 (C-1), 73.9, 72.5, 71.5, 69.3, 62.9, 40.7, 40.4, 40.4, 40.3. HRMS calc. for $C_{23}H_{21}NO_{13}NaCl_4$ $[M+Na]^+$: 681.9665, found: 681.9659.

*Compound **3h***. 18.3 mg, 0.1 mmol of **2h** to yield **3h** (59.9 mg, 0.09 mmol, 92%) as a white powder. ^{1}H NMR ($(CD_3)_2CO$, 400 MHz) δ: 8.12 (1H, d, J = 16.0 Hz), 7.93 (1H, d, J = 7.6 Hz), 7.53 (1H, d, J = 8.0 Hz), 7.48 (1H, t, J = 8.0 Hz), 7.42 (1H, t, J = 8.0 Hz), 6.60 (1H, d, J = 16.0 Hz), 6.18 (1H, d, J = 8.0 Hz, H-1), 5.71 (1H, t, J = 9.6 Hz), 5.35 (1H, t, J = 9.2 Hz), 5.33 (1H, d, J = 9.2 Hz), 4.46–4.37 (3H, overlap), 4.32–4.20 (8H, overlap); ^{13}C NMR ($(CD_3)_2CO$, 100 MHz) δ: 168.4, 168.2, 168.0, 167.8, 165.4, 143.6, 136.2, 133.8, 133.5, 131.7, 129.9, 129.3, 120.8, 93.0 (C-1), 74.8, 73.5, 72.9, 71.0, 64.5, 42.2, 42.2, 42.1, 42.0. HRMS calc. for $C_{23}H_{21}O_{11}Cl_5Na$ $[M+Na]^+$: 670.9424, found: 670.9424.

*Compound **3i***. 13.8 mg, 0.1 mmol of **2i** to yield **3i** (54.6 mg, 0.09 mmol, 90%) as a white powder. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.49 (1H, m), 7.45 (1H, d, J = 16.0 Hz), 6.68 (1H, m), 6.47 (1H, m), 6.23 (1H, d, J = 16.0 Hz), 5.87 (1H, d, J = 8.0 Hz, H-1), 5.41 (1H, t, J = 9.2 Hz), 5.28 (1H, t, J = 9.2 Hz), 5.23 (1H, d, J = 9.2 Hz), 4.38 (1H, dd, J = 12.4, 4.0 Hz), 4.28 (1H, dd, J = 12.4, 2.0 Hz), 4.08 (2H, s), 4.00 (2H, d, J = 2.8 Hz), 3.99 (1H, m), 3.97 (2H, s), 3.97 (2H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.2, 167.0, 166.5, 166.3, 164.7, 150.5, 145.9, 133.9, 116.9, 113.2, 112.8, 91.5 (C-1), 74.0, 72.2, 71.5, 69.3, 62.9, 40.7, 40.4, 40.4, 40.4. HRMS calc. for $C_{21}H_{20}O_{12}Cl_4Na$ $[M+Na]^+$: 626.9607, found: 626.9606.

*Compound **3j***. 15.4 mg, 0.1 mmol of **2j** to yield **3j** (55.4 mg, 0.09 mmol, 89%) as a white powder. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.82 (1H, d, J = 16.0 Hz), 7.42 (1H, d, J = 4.8 Hz), 7.29 (1H, d, J = 3.6 Hz), 7.05 (1H, dd, J = 4.8, 3.6 Hz), 6.15 (1H, d, J = 16.0 Hz), 5.88 (1H, d, J = 8.0 Hz, H-1), 5.41 (1H, t, J = 9.2 Hz), 5.29 (1H, dd, J = 9.2, 8.0 Hz), 5.23 (1H, t, J = 9.2 Hz), 4.38 (1H, dd, J = 12.4, 4.4 Hz), 4.28 (1H, dd, J = 12.4, 2.4 Hz), 4.09 (2H, s), 4.00 (2H, d, J = 3.2 Hz), 3.99 (2H, s), 3.99 (2H, s), 3.98 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.2, 167.0, 166.5, 166.3, 164.5, 140.5, 139.0, 132.6, 130.2, 128.5, 114.3, 91.5 (C-1), 74.0, 72.2, 71.5, 69.2, 62.9, 40.7, 40.4, 40.4, 40.4. HRMS calc. for $C_{21}H_{20}O_{11}NaSCl_4$ $[M+Na]^+$: 642.9378, found: 642.9370.

*Compound **3k***. 16.8 mg, 0.1 mmol of **2k** to yield **3k** (56.0 mg, 0.09 mmol, 88%) as a white powder. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.37 (1H, d, J = 4.8 Hz), 7.35 (1H, d, J = 3.6 Hz), 7.04 (1H, dd, J = 4.8, 3.6 Hz), 6.18 (1H, s), 5.86 (1H, d, J = 8.4 Hz, H-1), 5.40 (1H, t, J = 9.6 Hz), 5.26 (1H, dd, J = 9.6, 8.4 Hz), 5.23 (1H, t, J = 9.6 Hz), 4.38 (1H, dd, J = 12.4, 4.0 Hz), 4.28 (1H, dd, J = 12.4, 2.4 Hz), 4.10 (2H, s), 4.01 (2H, d, J = 3.6 Hz), 3.99 (2H, s), 3.98 (2H, s), 3.97 (1H, m), 2.59 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.2, 167.1, 166.5, 166.3, 164.1, 152.6, 144.8, 128.7, 128.4, 128.1, 111.5, 91.0 (C-1), 74.2, 72.2, 71.5, 69.2, 62.9, 40.7, 40.4, 40.4, 40.4, 18.0. HRMS calc. for $C_{22}H_{22}O_{11}NaSCl_4$ $[M+Na]^+$: 656.9535, found: 656.9537.

*Compound **3l***. 16.2 mg, 0.1 mmol of **2l** to yield **3l** (57.3 mg, 0.09 mmol, 93%) as a white powder. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.47–7.45 (2H, overlap), 7.39–7.35 (3H, overlap), 6.10 (1H, d, J = 1.6 Hz), 5.86 (1H, d, J = 8.4 Hz, H-1), 5.40 (1H, t, J = 9.6 Hz), 5.27 (1H, dd, J = 9.6, 8.4 Hz), 5.24 (1H, t, J = 9.6 Hz), 4.39 (1H, dd, J = 12.4, 4.0 Hz), 4.30 (1H, dd, J = 12.4, 2.4 Hz), 4.11 (2H, s), 4.01 (2H, d, J = 3.2 Hz), 3.99 (2H, s), 3.98 (2H, s), 3.97 (1H, m), 2.58 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.2, 167.1, 166.5, 166.3, 164.1, 160.9, 141.5, 130.0, 128.9, 128.9, 126.6, 126.6, 114.8, 91.1 (C-1), 74.2, 72.3, 71.6, 69.3, 62.9, 40.8, 40.4, 40.4, 40.4, 18.7. HRMS calc. for $C_{24}H_{24}O_{11}NaCl_4$ $[M+Na]^+$: 650.9970, found: 650.9967.

3.3. Preparation of compounds 4a-4l

General dechloroacetylation procedure: Compound **3** (0.08 mmol) was suspended in a 1:1 mixture of pyridine and H_2O (1.0 mL). After stirring at 35 °C for 5 h, TLC showed the reaction was complete. The solvents were removed under vacuum and the residue was purified by flash column chromatography to yield **4**.

*Compound **4a***. 49.3 mg, 0.08 mmol of **3a** to yield **4a** (22.8 mg, 0.07 mmol, 92%) as a white powder. ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.80 (1H, d, J = 16.0 Hz), 7.64–7.62 (2H, m), 7.43–7.40 (3H, m), 6.58 (1H, d, J = 16.0 Hz), 5.59 (1H, d, J = 7.6 Hz, H-1), 3.87 (1H, dd, J = 12.4, 2.0 Hz), 3.70 (1H, dd, J = 12.4, 5.2 Hz), 3.52–3.40 (4H, overlap) [18].

*Compound **4b***. 51.7 mg, 0.08 mmol of **3b** to yield **4b** (25.6 mg, 0.08 mmol, 94%) as a white powder. ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.75 (1H, d, J = 16.0 Hz), 7.56 (2H, d, J = 8.4 Hz), 6.96 (2H, d, J = 8.4 Hz), 6.42 (1H, d, J = 16.0 Hz), 5.59 (1H, d, J = 7.6 Hz, H-1), 3.86 (1H, dd, J = 12.0, 2.0 Hz), 3.83 (3H, s), 3.70 (1H, dd, J = 12.0, 4.0 Hz), 3.48–3.36 (4H, overlap) [18].

*Compound **4c***. 50.4 mg, 0.08 mmol of **3c** to yield **4c** (24.1 mg, 0.07 mmol, 93%) as a white powder. ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.77 (1H, d, J = 16.0 Hz), 7.50 (2H, d, J = 8.0 Hz), 7.23 (2H, d, J = 8.0 Hz), 6.51 (1H, d, J = 16.0 Hz), 5.59 (1H, d, J = 7.6 Hz, H-1), 3.85 (1H, dd, J = 12.0, 1.6 Hz), 3.70 (1H, dd, J = 12.0, 4.4 Hz), 3.47–3.36 (4H, overlap), 2.36 (3H, s); ^{13}C NMR (CD_3OD, 100 MHz) δ: 167.5, 147.8, 142.7, 133.0, 130.9, 130.9, 129.6, 129.6, 117.2, 96.0 (C-1), 79.0, 78.1, 74.2, 71.2, 62.5, 21.6. HRMS calc. for $C_{16}H_{20}O_7Na$ $[M+Na]^+$: 347.1107, found: 347.1109.

*Compound **4d***. 50.4 mg, 0.08 mmol of **3d** to yield **4d** (24.6 mg, 0.08 mmol, 95%) as a white powder. ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.77 (1H, d, J = 16.0 Hz), 7.44 (1H, d, J = 1.6 Hz), 7.40 (1H, d, J = 7.6 Hz), 7.30 (1H, t, J = 7.6 Hz), 7.24 (1H, d, J = 7.6), 6.55 (1H, d, J = 16.0 Hz), 5.60

(1H, d, $J = 7.6$ Hz, H-1), 3.86 (1H, dd, $J = 12.0$, 1.6 Hz), 3.70 (1H, dd, $J = 12.0$, 4.4 Hz), 3.50–3.37 (4H, overlap), 2.37 (3H, s); ^{13}C NMR (CD_3OD, 100 MHz) δ: 167.3, 148.0, 140.1, 135.7, 132.7, 130.1, 130.1, 126.7, 118.1, 96.1 (C-1), 79.0, 78.1, 74.2, 71.2, 62.5, 21.4. HRMS calc. for $C_{16}H_{20}O_7Na$ $[M+Na]^+$: 347.1107, found: 347.1102.

Compound ***4e***. 50.7 mg, 0.08 mmol of **3e** to yield **4e** (23.6 mg, 0.07 mmol, 90%) as a white powder. ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.78 (1H, d, $J = 15.6$ Hz), 7.69 (1H, d, $J = 8.0$ Hz), 7.67 (1H, t, $J = 8.0$ Hz), 7.17 (1H, d, $J = 8.0$ Hz), 7.15 (1H, t, $J = 8.0$ Hz), 6.54 (1H, d, $J = 16.0$ Hz), 5.59 (1H, d, $J = 7.2$ Hz, H-1), 3.85 (1H, dd, $J = 12.0$, 1.2 Hz), 3.69 (1H, dd, $J = 12.0$, 4.8 Hz), 3.49–3.38 (4H, overlap); ^{13}C NMR (CD_3OD, 100 MHz) δ: 165.6, 144.9, 130.7, 130.7, 130.3, 130.2, 116.8, 115.7, 115.5, 94.6 (C-1), 77.4, 76.6, 72.6, 69.8, 61.0. HRMS calc. for $C_{15}H_{17}O_7FNa$ $[M+Na]^+$: 351.0856, found: 351.0856.

Compound ***4f***. 54.7 mg, 0.08 mmol of **3f** to yield **4f** (27.2 mg, 0.07 mmol, 90%) as a white powder. ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.84 (1H, d, $J = 16.0$ Hz), 7.81 (2H, d, $J = 8.0$ Hz), 7.71 (2H, d, $J = 8.0$ Hz), 6.71 (1H, d, $J = 16.0$ Hz), 5.62 (1H, d, $J = 7.2$ Hz, H-1), 3.86 (1H, dd, $J = 12.0$, 1.2 Hz), 3.70 (1H, dd, $J = 12.0$, 4.8 Hz), 3.51–3.37 (4H, overlap); ^{13}C NMR (CD_3OD, 100 MHz) δ: 166.7, 145.7, 139.5, 133.0 (q, $J = 32.2$ Hz), 130.0, 130.0, 130.0, 127.1 (d, $J = 3.6$ Hz), 127.0 (d, $J = 3.6$ Hz), 121.4, 96.2 (C-1), 79.0, 78.1, 74.2, 71.2, 62.5. HRMS calc. for $C_{16}H_{18}O_7F_3$ $[M+H]^+$: 379.1005, found: 379.1003.

Compound ***4g***. 52.9 mg, 0.08 mmol of **3g** to yield **4g** (25.9 mg, 0.07 mmol, 91%) as a white powder. ^{1}H NMR (CD_3OD, 400 MHz) δ: 8.28 (2H, d, $J = 8.0$ Hz), 7.88 (2H, d, $J = 8.0$ Hz), 7.86 (1H, d, $J = 16.0$ Hz), 6.78 (1H, d, $J = 16.0$ Hz), 5.62 (1H, d, $J = 8.0$ Hz, H-1), 3.86 (1H, dd, $J = 12.0$, 1.2 Hz), 3.70 (1H, dd, $J = 12.0$, 4.0 Hz), 3.49–3.37 (4H, overlap); ^{13}C NMR (CD_3OD, 100 MHz) δ: 166.4, 150.3, 144.8, 141.9, 130.5, 130.5, 125.2, 125.2, 122.8, 96.3 (C-1), 79.1, 78.1, 74.2, 71.3, 62.5. HRMS calc. for $C_{15}H_{17}NO_9Na$ $[M+Na]^+$: 378.0796, found: 378.0791.

Compound ***4h***. 52.0 mg, 0.08 mmol of **3h** to yield **4h** (25.9 mg, 0.08 mmol, 94%) as a white powder. ^{1}H NMR (CD_3OD, 400 MHz) δ: 8.15 (1H, d, $J = 16.0$ Hz), 7.78 (1H, d, $J = 7.6$ Hz), 7.46 (1H, d, $J = 8.0$ Hz), 7.39 (1H, t, $J = 8.0$ Hz), 7.35 (1H, t, $J = 8.0$ Hz), 6.60 (1H, d, $J = 16.0$ Hz), 5.64 (1H, d, $J = 7.6$ Hz, H-1), 3.87 (1H, dd, $J = 12.0$, 1.2 Hz), 3.71 (1H, dd, $J = 12.0$, 4.4 Hz), 3.51–3.39 (4H, overlap); ^{13}C NMR (CD_3OD, 100 MHz) δ: 166.8, 142.9, 136.0, 133.6, 133.0, 131.3, 129.2, 128.7, 121.3, 96.2 (C-1), 79.0, 78.1, 74.1, 71.2, 62.5. HRMS calc. for $C_{15}H_{17}O_7NaCl$ $[M+Na]^+$: 367.0561, found: 367.0562.

Compound ***4i***. 48.5 mg, 0.08 mmol of **3i** to yield **4i** (22.8 mg, 0.08 mmol, 95%) as a white powder. ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.65 (1H, m), 7.57 (1H, d, $J = 16.0$ Hz), 6.81 (1H, m), 6.56 (1H, m), 6.31 (1H, d, $J = 16.0$ Hz), 5.58 (1H, d, $J = 8.0$ Hz, H-1), 3.85 (1H, dd, $J = 12.0$, 1.2 Hz), 3.69 (1H, dd, $J = 12.0$, 4.8 Hz), 3.48–3.34 (4H, overlap); ^{13}C NMR (CD_3OD, 100 MHz) δ: 167.3, 152.2, 147.1, 133.9, 117.3, 115.5, 113.8, 96.1 (C-1), 79.0, 78.1, 74.1, 71.2, 62.5. HRMS calc. for $C_{13}H_{17}O_8$ $[M+H]^+$: 301.0923, found: 301.0928.

Compound ***4j***. 49.8 mg, 0.08 mmol of **3j** to yield **4j** (22.8 mg, 0.07 mmol, 90%) as a white powder. ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.93 (1H, d, $J = 16.0$ Hz), 7.58 (1H, d, $J = 4.8$ Hz), 7.41 (1H, d, $J = 3.6$ Hz), 7.12 (1H, dd, $J = 4.8$, 3.6 Hz), 6.30 (1H, d, $J = 16.0$ Hz), 5.57 (1H, d, $J = 7.6$ Hz, H-1), 3.85 (1H, dd, $J = 12.0$, 1.2 Hz), 3.69 (1H, dd, $J = 12.0$, 4.4 Hz), 3.49–3.37 (4H, overlap); ^{13}C NMR (CD_3OD, 100 MHz) δ: 167.1, 140.6, 140.3, 133.2, 130.8, 129.6, 116.7, 96.1 (C-1), 79.0, 78.1, 74.1, 71.2, 62.5. HRMS calc. for $C_{13}H_{16}O_7NaS$ $[M+Na]^+$: 339.0514, found: 339.0508.

Compound ***4k***. 50.9 mg, 0.08 mmol of **3k** to yield **4k** (24.0 mg, 0.07 mmol, 91%) as a white powder. ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.50 (1H, d, $J = 5.2$ Hz), 7.47 (1H, d, $J = 3.6$ Hz), 7.10 (1H, dd, $J = 5.2$, 3.6 Hz), 6.32 (1H, s), 5.55 (1H, d, $J = 8.0$ Hz, H-1), 3.85 (1H, dd, $J = 12.0$, 1.2 Hz), 3.69 (1H, dd, $J = 12.0$, 4.4 Hz), 3.48–3.34 (4H, overlap), 2.62 (3H, s); ^{13}C NMR (CD_3OD, 100 MHz) δ: 166.7, 151.9, 146.2, 129.5, 129.3, 129.0, 113.9, 95.5 (C-1), 78.9, 78.1, 74.1, 71.2, 62.5, 17.8. HRMS calc. for $C_{14}H_{18}O_7NaS$ $[M+Na]^+$: 353.0665, found: 353.0662.

Compound ***4l***. 49.3 mg, 0.08 mmol of **3l** to yield **4l** (24.1 mg, 0.07 mmol, 93%) as a white powder. ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.54–7.45 (2H, overlap), 7.42–7.35 (3H, overlap), 6.21 (1H, d, $J = 1.6$ Hz), 5.57 (1H, d, $J = 8.0$ Hz, H-1), 3.86 (1H, dd, $J = 12.4$, 2.4 Hz), 3.70 (1H, dd, $J = 12.4$, 4.8 Hz), 3.49–3.37 (4H, overlap), 2.59 (3H, s); ^{13}C NMR (CD_3OD, 100 MHz) δ: 166.7, 159.7, 143.2, 130.7, 129.9, 129.9, 127.5, 127.5, 117.1, 95.5 (C-1), 78.9, 78.2, 74.1, 71.3, 62.5, 18.5. HRMS calc. for $C_{16}H_{20}O_7Na$ $[M+Na]^+$: 347.1107, found: 347.1113.

3.4. Anti-proliferation assay

Anti-proliferation assay was conducted using the method described in the literature [20] with slight modifications. B16-F10 murine melanoma cell line and normal human dermal fibroblast (HDF) cell line were obtained from ATCC. B16-F10 cells were cultured in DMEM containing 10% FBS, 1 g/L glucose, 1 mmol/L glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. Murine fibrosarcoma L929 cells were used for cytotoxicity analysis, and were cultured in ATCC medium (McCoy's 5A medium with 1.5 mM L-glutamine, 90%; fetal bovine serum, 10%). Incubation was carried out at 37 °C in a humidified atmosphere of 95% air and 5% CO_2.

Cell viability was assessed by the MTT assay. B16-F10 and HDF cells were seeded at a density of 8×10^3 cells/well on 96-well plates. After 24 h incubation, **4a**-**4l** at different concentrations (1.25, 2.5, 5, 10, 20 and 40 μM) were dissolved in PBS containing 0.5% DMSO and added to cells in each well and then incubated for 48 h. Doxorubicin was used as the positive control. After incubation, 20 μL of MTT (5 mg/mL in PBS) was added to each well and incubated for 4 h at 37 °C. After the supernatant was removed, 160 μL of DMSO was added to each well to solubilize the produced fromazan crystals, and measurements were taken at 490 nm using a Bio-Rad 680 Microplate reader. Results were expressed as a percentage of the control, and half maximal inhibitory concentration (IC_{50}) values were calculated. Cell viability was expressed as a percentage of the control culture value, which was considered as 100% viable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

NMR spectra of compounds **3a**–**3l** and **4a**–**4l**. This material is available free of charge via the Internet at XXX.

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