



Synthesis of Cytotoxic Fluorinated Quassinoids

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Abstract—The C-15 senecioid side chain of brusatol was interchanged with fluorinated acyl groups, and the C-3 hydroxy group of bruceolide was esterified with fluorinated acyl chlorides. These fluorinated quassinoids **11**, **12**, **13**, and **17** showed significant cytotoxic activity against eight human cancer cell lines including small and non-small cell lung, colon, CNS, ovarian and renal cancers, leukemia, and melanoma with **17** being about 100 times more potent than **11**, **12**, and **13**. The activity of **17** was similar to that of bruceantin (**1**) in this in vitro cell line panel. © 1997 Elsevier Science Ltd.

Introduction

Bruceantin (**1**) has been tested in phase II clinical trials as an antitumor agent; however, it has not yet progressed to drug development. Oxidation of the side chain may cause deactivation of **1** and limit its efficacy.¹ Accordingly, substituting the side chain with a moiety more stable to oxidation may prove beneficial. In this investigation, we have introduced a fluorinated moiety into the quassinoid side chain. This moiety was chosen based on several interesting properties: (1) the C–F bond energy (105.4 kcal/mol) is higher than the C–H bond energy (98.7 kcal/mol), (2) generally, the activities and properties of fluorinated compounds mimic those of hydrogenated compounds, and (3) fluorinated compounds show increased hydrophobicity in comparison with analogous non-fluorinated compounds.

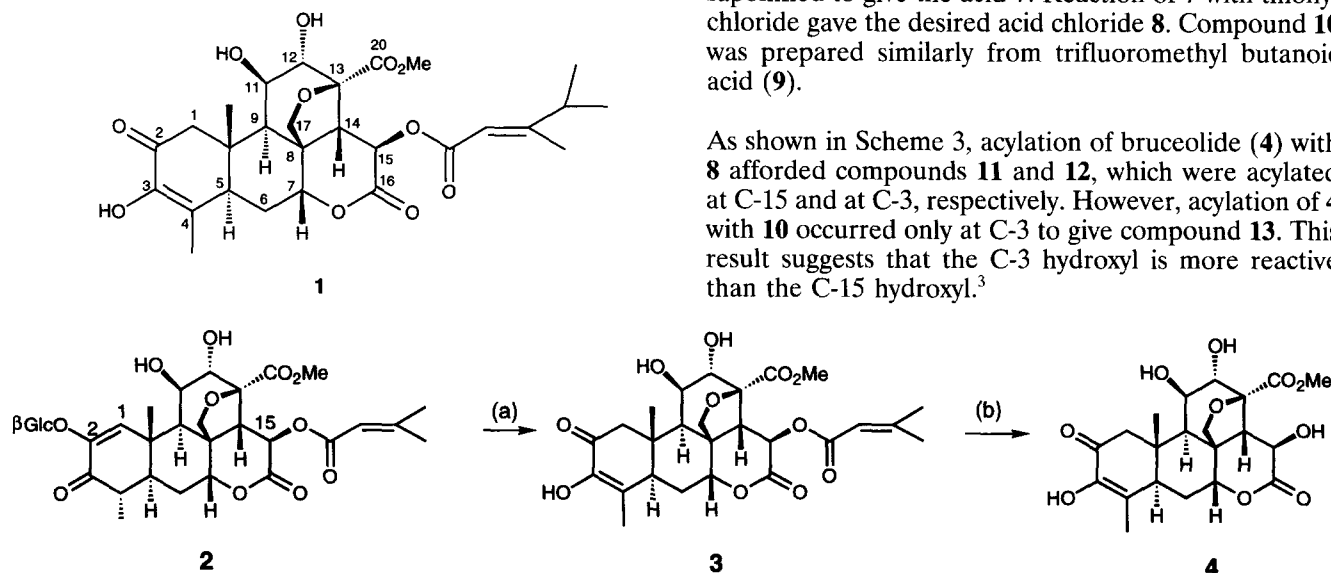
Results

Chemistry

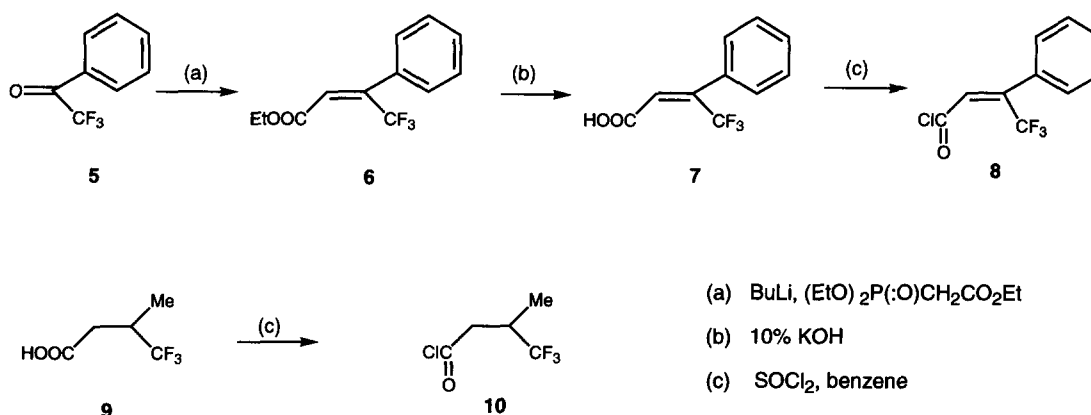
The target fluorinated quassinoids were synthesized from brusatol (**3**), which differs from bruceantin (**1**) only at the C-15 side chain, or bruceolide (**4**). Acid hydrolysis of bruceoside-A (**2**), which was isolated from *Brucea javanica*, gave brusatol (**3**) as shown in Scheme 1.² Bruceolide (**4**) was obtained by alkaline hydrolysis of **3**.

Scheme 2 shows the preparation of the fluorinated acyl chlorides, 4,4,4-trifluoro-3-phenyl-2-butenoyl chloride (**8**) and 4,4,4-trifluoro-3-methylbutanoyl chloride (**10**). In the former, a Wittig reaction with trifluoroacetophenone (**5**) gave the unsaturated ester **6**, which was saponified to give the acid **7**. Reaction of **7** with thionyl chloride gave the desired acid chloride **8**. Compound **10** was prepared similarly from trifluoromethyl butanoic acid (**9**).

As shown in Scheme 3, acylation of bruceolide (**4**) with **8** afforded compounds **11** and **12**, which were acylated at C-15 and at C-3, respectively. However, acylation of **4** with **10** occurred only at C-3 to give compound **13**. This result suggests that the C-3 hydroxyl is more reactive than the C-15 hydroxyl.³



Scheme 1. (a) 10% H₂SO₄. (b) 0.5 N KOH.



Scheme 2.

Therefore, the C-3 OH group of brusatol (**3**) was first protected with *tert*-butyldimethylsilyl chloride (TBDMS-Cl) to afford compound **14** (Scheme 4). Then, the C-15 side chain was removed by hydrolysis using MeOK to afford compound **15**. Acylation of compound **15** with **10** gave compound **16**, which was treated with Bu_4NF to remove the protecting group and afford the final compound **17**.⁴

Structural elucidation

Table 1 shows the ^1H NMR data for compounds **4** and **11–17**. All compounds are colorless amorphous solids.

MS analysis shows that both compounds **11** and **12** have the same molecular formula, $\text{C}_{31}\text{H}_{31}\text{F}_3\text{O}_{11}$, and contain one fluorinated moiety. The position of this moiety at C-15 in **11** and at C-3 in **12** was confirmed by comparing the chemical shifts of Me-4 in **11** and **12** (δ 1.96 and 1.72, respectively) with that (δ 1.96) of the starting material, bruceolide (**4**), which is esterified at C-15.

Compound **13** has a molecular formula of $\text{C}_{26}\text{H}_{31}\text{F}_3\text{O}_{11}$ and again, contains one fluorinated moiety. The upfield

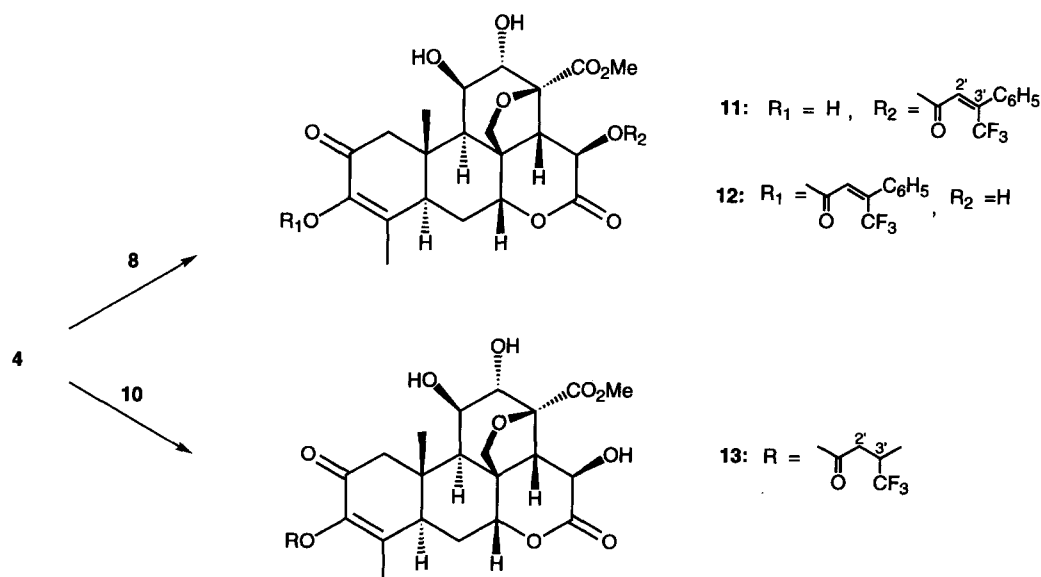
shift of Me-4 (δ 1.81 in **13**; δ 1.96 in **4**) confirmed the esterification of C-3.

Compounds **14**, **15**, and **16** contain the appropriate Me-Si and *t*Bu-Si signals in their NMR spectra (Table 1). The senecioid moiety in **14** shows signals at δ 5.86 for H-2' and at δ 1.63 and 2.15 for Me-2'3'. These signals were not observed in the spectrum of compound **15**. However, signals for the 4,4,4-trifluoro-3-methyl-butanoyl moiety were present in the spectrum of compound **16** [δ 2.91d (H-2'), 2.53m (H-3'), and 1.20d (Me-3')].

High-resolution MS of compound **17** shows a molecular formula of $\text{C}_{26}\text{H}_{31}\text{F}_3\text{O}_{11}$. The Me-4 ^1H NMR signals were identical in **17** (δ 1.97) and **4** (δ 1.96), and established the position of the fluorinated moiety at C-15.

Biological results

Recently, the NCI preclinical antitumor drug discovery screen has given results as IC_{50} mean graphs⁵ [IC_{50} = 50% Inhibition Concentration (M)] or GI_{50} mean graphs⁶ [GI_{50} = 50% Growth Inhibition (M)]. These mean graphs provide a characteristic fingerprint,



Scheme 3.

Table 1. ^1H NMR data of compounds **4** and **11–17** (ppm in $\text{C}_5\text{D}_5\text{N}$, J in Hz)

Proton	4 ^a	11 ^b	12 ^b	13 ^b	14 ^a	15 ^a	16 ^c	17 ^a
H-1 α	2.57d (11)	2.58d (10)	2.58d (16)	2.66	2.45d (15)	2.45d (16)	2.47	2.50
H-1 β	3.31d (16)	3.31d (16)	3.30d (16)	3.35d (16)	3.22d (15)	3.23d (16)	3.22d (16)	3.28d (16)
H-5	3.09d (13)	3.06d (13)	3.16d (13)	3.06brs	3.09s (5)	3.09d (13)	NA ^d	3.00brs (5)
H-6 α	2.29d (11)	2.15dd (13, 13)	2.21d (7)	2.33d (13)	2.31d (15)	2.28d (15)	2.32dd (14)	2.32d (15)
H-6 β	1.71dd (12, 12)	1.74 (13, 13)	1.72dd (12, 12)	1.66dd (13, 13)	1.76dd (12, 12)	1.72dd (16, 16)	1.78dd (14, 14)	1.77dd (13, 13)
H-7	NA ^d	NA ^d	NA ^d	NA ^d	5.1	NA ^d	NA ^d	5.10s
H-9	2.63d (5)	2.60	2.63s (7)	2.69	2.59d (5)	2.60d (5)	NA ^d	2.61d (5)
H-11	NA ^d	4.89s	4.79s	4.83s	4.78s	NA ^d	4.89s	4.78s
H-12	NA ^d	NA ^d	NA ^d	NA ^d	NA ^d	NA ^d	NA ^d	NA ^d
H-14	3.63d (12)	3.96	3.66d (13)	3.68d (13)	4.03brs	3.63d (13)	3.96s	3.95d (13)
H-15	6.06d (13)	6.94s	6.07d (13)	6.09d (13)	6.59brs	6.06d (13)	6.65s	6.58brs
H-17 α	3.95d (8)	NA ^d	3.95d (7)	3.99d (7)	3.94d (7)	3.95d (7)	3.98	3.95
H-17 β	NA ^d	NA ^d	NA ^d	NA ^d	5.10	NA ^d	5.13	5.10
Me-4	1.96s	1.96s	1.72s	1.81s	1.86s	1.86s	1.88s	1.97s
Me-10	1.64s	1.63s	1.62s	1.81s	1.63s	1.63s	1.62s	1.63s
OMe-20	3.81s	3.84s	3.81s	3.83s	3.76s	3.81s	3.82s	3.82s
H-2'	—	NA ^d	NA ^d	3.18d (19)	5.86s	—	2.91d (12)	2.95d (13)
H-3'	—	—	—	2.78m	—	—	2.53m	2.50m
Me-3'	—	—	—	1.27d (7)	1.63s, 2.15s	—	1.20d (7)	1.21d (6)
Ph-3'	—	7.45m	7.45m	—	—	—	—	—
Me-Si	—	—	—	—	0.36s, 0.38s	0.35s, 0.36s	0.37s, 0.39s	—
tBu-Si	—	—	—	—	1.06s	1.05s	1.07s	—

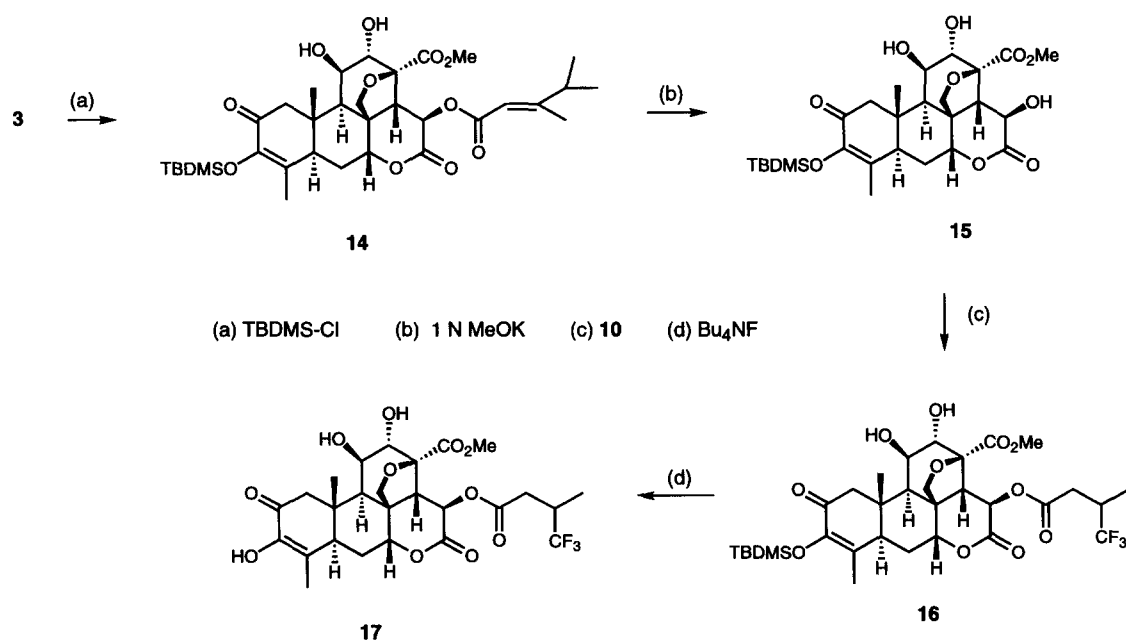
^aMeasured at 500 MHz.^bMeasured at 200 MHz.^cMeasured at 270 MHz.^dNA = Not assignable.**Scheme 4.**

Table 2. Log GI₅₀ values from the human disease-oriented cancer cell line screening panel for compounds **11**, **12**, **13**, and **17** [GI₅₀ = 50% growth inhibition (mol/L)]

Panel/Cell Line	11 (Log GI ₅₀)	12 (Log GI ₅₀)	13 (Log GI ₅₀)	17 (Log GI ₅₀)	1 (Log GI ₅₀)
Leukemia					
CCRP-CEM	-5.41	-5.06	-5.39	-7.92	-8.68
HL-60 (TB)	-6.44	-5.94	-6.57	< -8.43	-8.73
K-562	-5.31	-5.03	-5.22	-7.80	-8.42
MOLT-4	-6.00	-5.67	-6.11	-8.33	-8.42
RPMI-8226	-5.81	-5.69	-6.23	-7.94	-8.58
SR	-6.18	-5.48	-5.56	-8.37	-8.74
Non-Small cell lung cancer					
A549/ATCC	-5.36	-4.78	-4.76	-7.58	-8.33
EKVX	-4.92	-4.41	-4.58	-6.89	—
HOP-18	-5.20	-4.52	-5.70	-6.51	—
HOP-62	-5.22	-4.91	-4.82	-7.01	-7.97
HOP-92	-5.67	-5.23	-6.05	-8.02	-7.74
NCI-H226	-5.69	-5.19	-5.69	-7.97	-8.69
NCI-H23	-5.83	-5.34	-5.86	-7.90	-8.41
NCI-H322M	-4.94	-4.52	-4.65	-6.40	-7.95
NCI-H460	-5.99	-5.61	-5.75	-8.27	-8.48
NCI-H522	-5.62	-5.20	-5.46	-8.05	-8.60
LXFL 529	-5.58	-5.13	-5.42	-8.14	—
Small cell lung cancer					
DMS 114	-5.38	-5.37	-5.84	-8.02	—
DMS-273	-6.01	-5.46	-6.42	-8.16	—
Colon cancer					
COLO 205	-5.18	-4.95	-5.17	-7.00	-8.61
DLD-1	-5.27	-5.13	-5.56	-7.06	—
HCC-2998	-5.75	-5.46	-5.70	-7.98	—
HCT-116	-5.61	-5.06	-5.61	-8.00	-8.49
HCT-15	-5.10	-4.62	> -4.20	-6.78	—
HT29	-5.66	-5.17	-5.16	-7.88	-8.49
KM 12	-5.24	-4.84	-4.95	-7.49	—
KM20L2	-5.41	-5.07	-5.42	-7.71	—
SW-620	—	—	-5.92	-7.66	-7.86
CNS cancer					
SP-268	-5.06	-4.95	-5.16	-7.06	-7.71
SF-295	-5.38	-5.10	-5.29	-7.35	-7.76
SF-539	-6.00	-5.42	-5.82	-7.98	-8.07
SNB-19	-5.44	-5.33	-6.09	-8.10	-8.29
SNB-75	-5.11	-5.40	-5.68	-7.35	-7.84
SNB-78	-5.04	-4.95	-4.53	-6.69	—
U251	-5.36	-5.18	-5.32	-7.69	—
XP498	-5.65	-5.01	-6.89	-8.28	—
Melanoma					
MALME-3M	-5.94	-5.10	-6.67	-8.00	-8.47
M14	-5.45	-5.06	-5.70	-7.46	-7.92
M19-MEL	-5.92	-5.29	-6.36	-7.79	—
SK-MEL-2	-5.08	-5.10	-5.53	-7.11	-7.58
SK-MEL-28	-5.33	-5.27	-5.82	-7.92	-8.14
SK-MEL-5	-5.48	-5.32	-5.79	-7.90	-8.42
UACC-257	-5.15	-5.06	-5.53	-7.12	—
UACC-62	-5.69	-5.63	-5.95	-8.13	-7.89
Ovarian cancer					
IGROV1	-5.67	-5.41	-5.65	-7.77	-8.26
OVCAR-4	-5.20	-4.75	-4.83	-7.17	-8.38
OVCAR-5	-5.06	-4.66	-4.71	-6.84	-7.78
OVCAR-8	-4.98	-4.92	-5.33	-7.24	-7.90
SK-OV-3	-5.14	-5.10	-5.28	-7.29	-8.01
Renal cancer					
786-0	-5.10	-5.04	-5.72	-7.19	-7.57
A498	-5.74	-6.28	-6.81	-7.67	-7.78
ACHN	-5.38	-4.86	-5.08	-7.64	-8.39
CAKI-I	-5.12	-4.70	-5.03	-7.25	-7.65
RXF-393	-5.22	-4.77	-5.33	-7.04	—
SN12C	-5.06	-5.10	-5.70	-6.84	—
TK-10	-5.11	-5.00	-5.26	-7.18	—
UO-31	-5.17	-4.45	-4.97	-6.83	—
MG-MID	-5.44	-5.13	-5.54	-7.56	-8.10

displaying the individual cell lines that are proportionally more sensitive than average or proportionally less sensitive than average to the test compound.

Log GI_{50} values from the human disease-oriented cancer cell line screening panel for compounds **11**, **12**, **13**, and **17** are shown in Table 2, together with the data for bruceantin (**1**). The values of 'MG-MID' are mean values of all log GI_{50} values, which for **11**, **12**, **13**, and **17** are -5.44 , -5.13 , -5.54 , and -7.56 , respectively, compared with -8.10 for **1**. On the other hand, mean log IC_{50} values for 5-fluorouracil, 5-fluorodeoxyuridine, and bleomycin are -3.5 , -4.7 , and -5.2 , respectively.⁵ These data indicate that the fluorinated quassinoids are potent cytotoxic agents. Notably, the cytotoxic activity of **17** is about 100-times higher than that of **11**, **12**, and **13**. The position of the 4,4,4-trifluoro-3-phenyl-2-butenoyl group did not affect the potency (compare **11** to **12**). However, with a 4,4,4-trifluoro-3-methylbutanoyl group, the C-15 esterified compound (**17**) was much more active than the C-3 substituted analogue (**13**). In this in vitro cell line panel, compound **17** was approximately as active as bruceantin (**1**). Therefore, this fluorinated component is an ideal lead compound for further study.

Experimental

General experimental procedures and materials

Melting points were measured on an MRK air-bath-type melting point apparatus and are uncorrected. Specific rotations were obtained on a Jasco DIP-370 polarimeter ($L = 0.5$ dm). IR and UV spectra were recorded on a Jasco IR-810 spectrometer and a Hitachi 320-S spectrometer, respectively. 1H and ^{13}C NMR spectra were determined on a Varian XL-200 (200.6 MHz for 1H NMR), a Varian VXR-500 (499.8 MHz for 1H NMR and 125.7 MHz for ^{13}C NMR) and a Jasco GSX-500 (500.1 MHz for 1H NMR and 125.7 MHz for ^{13}C NMR) instrument in C_5D_5N or $CDCl_3$, using TMS as an internal standard. Mass spectra were recorded on a Hitachi M80 or a Hitachi M-2500 instrument.

Analytical HPLC was performed on a Tosoh liquid chromatograph equipped with a UV detector at 254 nm and a reverse-phase column (TSK-gel ODS-80T_M, 4.6×150 mm), using a mixed solvent of MeOH–H₂O or MeOH–H₂O–HOAc. Preparative HPLC was carried out on a Waters or a Tosoh liquid chromatograph equipped with a reverse-phase column (Lichrosorb RP-18, 10×250 mm or Dynamax-60A, 21.4×250 mm) and a UV monitor set at 254 nm, using the same solvents as those used in the analytical HPLC. The final compounds **11**, **12**, **13**, and **17** were homogeneous as determined by analytical HPLC.

Silica gel (Merck, type 60, 70–230 mesh) was used for column chromatography. Precoated Si gel plates (Merck, 60F₂₅₄) of 0.25 mm thickness were used for analytical TLC, and plates of 1 mm and 2 mm thickness

were used for preparative TLC. Components were detected by using a UV lamp (254 nm). Bruceoside-A (**2**), was obtained from the fruits of *Brucea javanica*, as previously described in the literature.² Compounds **5** and **9** used in this investigation were commercially available.

Brusatol (3). Bruceoside-A (**2**, 209 mg, 0.306 mmol) was dissolved in MeOH (40 mL), and 10% H₂SO₄ (20 mL) was added to the solution. The mixture was refluxed for 6 h. The reaction product was extracted with CHCl₃ and purified by preparative HPLC to afford brusatol (**3**, 137 mg, 0.263 mmol, 86% yield) as a colorless amorphous solid. It was identified by comparing its TLC, HPLC, and IR spectra with those of an authentic sample.

Bruceolide (4). Brusatol (**3**, 39.2 mg, 0.075 mmol) was dissolved in MeOH (4 mL), and 0.5 N KOH–MeOH solution (1.2 mL) was added to the solution. The mixture was stirred at room temperature for 8.5 h. The reaction mixture was neutralized with anion exchange resin (Dowex 50W-X2) and extracted with MeOH to afford crude bruceolide (**4**, 37.6 mg). This compound was purified by preparative HPLC (Lichrosorb RP-18, MeOH–H₂O, 7:3, v/v) to afford pure bruceolide (**4**, 14.5 mg, 0.033 mmol, 44% yield) as a colorless amorphous solid. It was identified by comparing its TLC, HPLC, and IR spectra with those of an authentic sample.

Ethyl-3-phenyl-4,4,4-trifluoro-2-butenic acid (6). Triethyl phosphonoacetate (4.93 g, 22 mmol) was dissolved in dry Et₂O (40 mL) with stirring at 0 °C under N₂. Butyl lithium (15% hexane solution, 15 mL, 24 mmol) was added dropwise to the solution at 0 °C over 10 min, and the mixture was stirred at room temperature for 30 min. 2,2,2-Trifluoroacetophenone (**5**, 3.49 g, 20 mmol) was dissolved in dry Et₂O (6 mL) and added to the reaction mixture. Stirring was then continued at room temperature for 17 h. The reaction product was extracted with Et₂O; evaporation of the solvent afforded compound **6** (4.70 g, 19 mmol, 95% yield) as a yellow liquid; IR (film) cm^{-1} 1730 (α,β -unsaturated C=O), 1650 (C=C); 1H NMR ($CDCl_3$) δ 7.41 (brs, 5H), 6.36 (brs, 1H), 4.32 (q, $J = 7$ Hz, 2H), 1.33 (t, $J = 7$ Hz, 3H). HPLC showed the compound to be a mixture of the *E* and *Z* isomers.

(Z)-3-Phenyl-4,4,4-trifluoro-2-butenic acid (7). Compound **6** (*E*, *Z* mixture, 1.35 g, 5.5 mmol) was dissolved in MeOH (15 mL), and 10% KOH aqueous solution (15 mL) was added to the solution, and the mixture was stirred at 0 °C for 3 h. Trifluoroacetic acid (2 mL) was added to the reaction mixture, and the product was extracted with Et₂O to afford compound **7** (*E*, *Z* mixture, 1.08 g, 5.00 mmol, 90.4% yield). The *E* and *Z* isomers of **7** were separated by preparative TLC (EtOAc:Et₂O, 1:1, v/v) to afford the pure *Z* isomer (440 mg, 2.04 mmol, 63.8% yield) as pale-yellow needles; IR (KBr) cm^{-1} 3000 (carboxylic OH), 1720 (α,β -unsaturated C=O), 1650 (C=C); 1H NMR ($CDCl_3$) δ 7.42 (brs, 5H), 6.59 (s, 1H).

(Z)-3-Phenyl-4,4,4-trifluoro-2-butenoyl chloride (8). A mixture of **7** (*Z* isomer, 186 mg, 0.860 mmol), SOCl₂ (660 μ L), and dry benzene (2 mL) was refluxed for 11 h under N₂. Excess SOCl₂ and benzene were removed by evaporation to afford compound **8** (194 mg, 0.826 mmol, 96% yield) as a brown liquid: IR (film) cm⁻¹ 1800 (C=O), 1645 (C=C); ¹H NMR (CHCl₃) δ 7.53 (brs, 5H), 6.80 (brs, 1H).

3-Trifluoromethylbutanoyl chloride (10). Compound **10** (583 mg, 3.3 mol, 50% yield) was obtained from 3-trifluoromethylbutanoic acid (**9**, 1.05 g, 6.7 mmol) in an identical manner to that of compound **8** from compound **7**. Colorless liquid: IR (CHCl₃) cm⁻¹ 1800 (C=O); ¹H NMR (CDCl₃) δ 3.33 (d, *J* = 12 Hz, 2H), 2.53 (m, 1H), 1.24 (d, *J* = 7 Hz, 3H).

15- and 3-(4',4',4'-Trifluoro-3'-Phenyl-2'-butenoyl)-bruceolide (11 and 12). Bruceolide (**4**, 26.5 mg, 0.061 mmol) was dissolved in dry pyridine (400 μ L), and compound **8** (64.7 mg, 0.276 mmol) was dissolved in dry CHCl₃ (500 μ L). The solution of **8** was added to the solution of **4** at 0 °C, and the mixture was stirred at 57 °C for 48 h under N₂. Excess **8** was decomposed with EtOH (500 μ L), and the acylation product was extracted with CHCl₃ to afford a brown oil (56 mg) that contained compounds **11** and **12**. Pure compounds **11** (2.1 mg, 0.0033 mmol, 5.5% yield) and **12** (3.7 mg, 0.0058 mmol, 9.6% yield) were obtained by preparative HPLC (Lichrosorb RP-18, H₂O:MeOH:AcOH, 49.5:49.5:1, v/v).

15-(4',4',4'-Trifluoro-3'-phenyl-2'-butenoyl)-bruceolide (11). Colorless amorphous solid; mp 154–156 °C; [α]_D²⁷ –13.3° (*c* 0.030, EtOH); UV (EtOH) nm 270 (ϵ 1900); IR (KBr) cm⁻¹ 3400 (OH), 1740 (δ -lactone and ester C=O), 1680 (α,β -unsaturated C=O), 1645 (C=C); ¹H NMR see Table 1; EIMS *m/z* 636 (M⁺, 6.5%), 437 ([M - side chain C₁₀H₆F₃O]⁺, 47.9%), 199 ([side chain C₁₀H₆F₃O]⁺, 100%); CIMS *m/z* 637 ([M + H]⁺, 6.7%), 437 ([M - side chain C₁₀H₆F₃O]⁺, 16.4%), 199 ([side chain C₁₀H₆F₃O]⁺, 100%).

3-(4',4',4'-Trifluoro-3'-phenyl-2'-butenoyl)-bruceolide (12). Colorless amorphous solid; mp 147–149 °C; [α]_D²⁶ +13.3° (*c* 0.030, EtOH); UV (EtOH) nm 243 (ϵ 3600); IR (KBr) cm⁻¹ 3400 (OH), 1740 (δ -lactone and ester C=O), 1680 (α,β -unsaturated C=O), 1645 (C=C); ¹H NMR see Table 1; EIMS *m/z* 636 (M⁺, 1.0%), 437 ([M - side chain C₁₀H₆F₃O]⁺, 47.9%), 199 ([side chain C₁₀H₆F₃O]⁺, 38.6%); CIMS *m/z* 637 ([M + H]⁺, 3.2%), 199 ([side chain C₁₀H₆F₃O]⁺, 100%).

3-[3'-(Trifluoromethyl)butanoyl]-bruceolide (13). Bruceolide (**4**, 30.0 mg, 0.068 mmol) was reacted with compound **10** (70.4 mg, 0.403 mmol) in an identical manner to the reaction of **4** and **8**. Pure compound **13** (9.9 mg, 0.017 mmol, 25% yield) was obtained by preparative HPLC (Lichrosorb RP-18, H₂O:MeOH:AcOH, 49.5:49.5:1, v/v). Colorless amorphous solid; mp 168–170 °C; [α]_D²⁷ +16.3° (*c* 0.049, EtOH); UV (EtOH) nm 243 (ϵ 9200); IR (KBr) cm⁻¹ 3450 (OH), 1740 (δ -

lactone and ester C=O), 1680 (α,β -unsaturated C=O); ¹H NMR see Table 1; CIMS *m/z* 577 ([M + H]⁺, 1.3%), 139 ([side chain C₅H₆F₃O]⁺, 55.2%).

3-(tert-Butyldimethylsilyl)-brusatol (14). A mixture of **3** (105 mg, 0.202 mmol), *tert*-butyldimethylsilyl chloride (TBDMS-Cl, 103 mg, 0.685 mmol), and imidazole (104 mg, 1.52 mmol) was stirred at room temperature for 3.5 h. Water (1 mL) was added to the reaction mixture, and colorless crystals (119 mg) were obtained. The crystals were subjected to preparative HPLC (Dynamax-60A, H₂O:MeOH, 1:4) to afford pure compound **14**. Colorless amorphous solid; mp 230–233 °C; [α]_D²⁶ +14.6° (*c* 0.123, EtOH); UV (EtOH) nm 220 (ϵ 12100), 270 (ϵ 7300); IR (KBr) cm⁻¹ 3450 (OH), 1740 (δ -lactone and ester C=O), 1680 (α,β -unsaturated C=O), 1645 (C=C); ¹H NMR see Table 1; EIMS *m/z* 577 ([M - C₄H₉]⁺, 100%), 83 ([side chain C₅H₇O]⁺, 19.7%); CIMS *m/z* 577 ([M - C₄H₉]⁺, 100%), 83 ([side chain C₅H₇O]⁺, 100%). (57.0 mg, 0.090 mmol, 45% yield).⁷

3-(tert-Butyldimethylsilyl)-bruceolide (15). Compound **14** (54.7 mg, 0.086 mmol) was dissolved in dry MeOH (1 mL). Then, 1 N-MeOK solution (MeOH, 1 mL) was added dropwise at 0 °C, and the mixture was stirred at 0 °C for 6 h. The reaction mixture was treated with cation exchange resin (AMBERLITE IRC-50) and extracted with CHCl₃ to afford crude crystals (40 mg). Purification by preparative HPLC (Lichrosorb RP-18, H₂O:MeOH, 3:7, v/v) afforded compound **15** (19.0 mg, 0.035 mmol, 40% yield). Colorless amorphous solid; mp 238–240 °C; [α]_D²⁷ –11.1° (*c* 0.072, EtOH); UV (EtOH) nm 270 (ϵ 6400); IR (KBr) cm⁻¹ 3450 (OH), 1740 (δ -lactone and ester C=O), 1670 (α,β -unsaturated C=O), 1610 (C=C); ¹H NMR see Table 1; EIMS *m/z* 495 ([M - C₄H₉]⁺, 100%); CIMS *m/z* 495 ([M - C₄H₉]⁺, 100%).

3-(tert-Butyldimethylsilyl)-15-[3'-(trifluoromethyl)-butanoyl]-bruceolide (16). Compound **15** (16.0 mg, 0.029 mmol) and compound **10** (32.2 mg, 0.184 mmol) were reacted as described for the reaction of **4** and **8**. Pure compound **16** (6.8 mg, 0.0099 mmol, 34% yield) was obtained by preparative HPLC. Colorless amorphous solid; mp 174–177 °C; [α]_D²⁵ +23.5° (*c* 0.068, EtOH); UV (EtOH) nm 270 (ϵ 6400); IR (KBr) cm⁻¹ 3450 (OH), 1740 (δ -lactone and ester C=O), 1680 (α,β -unsaturated C=O), 1645 (C=C); ¹H-NMR see Table 1; EIMS *m/z* 633 ([M - C₄H₉]⁺, 100%), 139 ([side chain C₅H₆F₃O]⁺, 55.2%); CIMS *m/z* 633 ([M - C₄H₉]⁺, 100%).

15-[(3'-Trifluoromethyl)-butanoyl]-bruceolide (17). Tetrabutyl ammonium fluoride (1.0M, Bu₄NF–THF solution, 40 μ L, 0.04 mmol) was added to a solution of compound **16** (5.7 mg, 0.008 mmol, dry THF 500 μ L), and the mixture was stirred at room temperature for 15 min. The reaction mixture was poured into ice–water and extracted with CHCl₃ to afford crude crystals (8.0 mg), which were purified by preparative HPLC (Lichrosorb RP-18, H₂O:MeOH, 1:1, v/v) to afford pure compound **17** (2.5 mg, 0.0043 mmol, 53% yield).

Colorless amorphous solid; mp 146–148 °C; $[\alpha]_D^{28} +4.0^\circ$ (c 0.025, EtOH); UV (EtOH) nm 277 (ϵ 6600); IR (KBr) cm^{-1} 3450 (OH), 1740 (δ -lactone and ester C=O), 1680 (α,β -unsaturated C=O); ^1H NMR see Table 1; EIMS m/z 576 ($[\text{M}]^+$, 5.7%), 139 ([side chain $\text{C}_5\text{H}_6\text{F}_3\text{O}]^+$, 49.3%); High-resolution MS m/z 576.1842 (M^+) (Calcd for $\text{C}_{26}\text{H}_{31}\text{F}_3\text{O}_{11}$: 576.1817).

Cytotoxicity assay

Compounds **11**, **12**, **13**, and **17** were tested against 60 cell lines of eight human cancers including leukemia, non-small cell lung cancer, small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, and renal cancer according to literature methods^{8,9} at the National Cancer Institute.

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