Semi-Synthesis of Kaurenoic Acid Derivatives and Their *In Vitro* Cytotoxic Activities

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Abstract

The cytotoxic activities of the diterpene kaurenoic acid (**1**) and its 15 semi-synthesis derivatives were assessed on human cell cultures. The human tumor cells used comprised colon (SW620 and SW480), pancreatic (PANC-1 and BxPC-3), stomach (SGC-7901), esophageal (Eca-109), and leukemia (K562 and HL-60). Kaurenoic acid was inactive against the tumor cell lines; however, its derivatives which contain α,β-unsaturated ketone rendered compounds with cytotoxic activity. Compounds **5**–**14, 17**–**19**, and **24** with a substitution at the C-4 position showed significant inhibitory activity against the tested cell lines, while compound **3**, without a substitution at the C-4 position, was slightly less active in these cell lines. The SW620 colon cancer cell was highly susceptible to all of the tested derivatives.

Key words

Wedelia prostrata · Asteraceae · kaurenoic acid derivatives · semi-synthesis · cytotoxic activity · cancer cell lines

Abbreviations

BrR:	alkyl bromide
Br-R-Br:	dibromoalkanes
DCC:	dicyclohexylcarbodiimide
DMF:	dimethylformamide
DMSO:	dimethylsulfoxide
EI-MS:	electron impact mass spectrometry
EtBr:	bromine ethane
EtOH:	ethanol
HOBt:	hydroxybenzotriazole
HR-MS:	high resolution mass spectrometer
IR:	infrared spectrum
KI:	potassium iodide
MTT:	3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide
OD:	optical density
PDC:	pyridinium dichromate
t-BuOOH:	peroxide tert-butyl alcohol
THF:	tetrahydrofuran
TMS:	tetramethylsilane
TLC:	thin layer chromatography

Supporting information available online at http://www.thieme-connect.de/products

Kaurenoic acid (**1**) [1,2] is one of the active constituents in *Wedelia prostrata* (Hook. et Arn.) Hemsl. (Asteraceae) [3], a traditional Chinese herbal medicine [4]. It is an ent-kaurane diterpenoid [5], which is claimed to have important biological activities, mainly antimicrobial [6], antibacterial [7], cytotoxic [8,9], anti-inflammatory [10], and anticonvulsant properties [11]. In the present paper, we describe the semi-synthesis of kaurenoic acid derivatives and their preliminary cytotoxic activities. A conversion of the 15-hydroxy group of kaurenoic acid to a ketone is made in order to incorporate an α,β-unsaturated ketone into the ent-kaurane skeleton. It is well know that a main structural determinant for cytotoxicity is present in an α,β-unsaturated ketone system [12,13], which likely serves as an alkylating center and can be part of an ester, ketone, or lactone moiety. We also report several transformations on the carboxylic acid group at the C-4 position. Compound **2** was obtained from **1** after treatment with SeO_2/t-BuOOH in THF. Oxidation of the 15-hydroxy group of compound **2** using PDC produced α,β-unsaturated ketone **3** (**Fig. 1 S**, Supporting Information). Compound **2** was amidated with RNH_2/DCC/HOBt in THF/DMF to yield **4a**–**4d** and oxidized with PDC to yield the corresponding amide derivatives **5**–**8**. Amide derivatives **9**–**10** were synthesized from **2** under treatment with pyridine/DCC and then oxidized to ketone. Ester derivatives of compounds **11**–**14** were obtained directly from **3** with BrR/K_2CO_3/KI in DMF. Treatment of **2** with K_2CO_3 and Br-R-Br in DMF formed **15a**–**15c**, which subsequently converted into compounds **16a**-**16c** under treatment with piperidine and K_2CO_3 in THF. The oxidation of **16a**–**16c** with PDC yielded **17**–**19**. Compound **1** was esterfied with EtBr to give **20**, which was reduced with $LiAlH_4$ to form the alcohol **21**. The reaction of **21** with Ac_2O formed the corresponding ethyl ester **22**. The reaction of **22** with SeO_2 and t-BuOOH obtained compound **23** and the oxidation of **23** yielded **24** (**Fig. 1**). Compound **3** has been reported previously [14], while the 14 derivatives (**5**–**14, 17**–**19, 24**) were reported here for the first time. The structures of the derivatives were confirmed by ^{1}H-NMR, ^{13}C-NMR, IR, HR-MS, EI-MS, and ESI-MS data (see Supporting Information).

The cytotoxic activities of compound **1** and its 15 semi-synthesis derivatives were assessed on eight human cell lines (**Table 1**). The results of the cytotoxicity assays indicated that compound **1**, without the α,β-unsaturated ketone, was inactive, while the 15 derivatives, which do contain this moiety, were active against all or some of the cell lines. Thus, as proposed in the literature [12, 13], the α,β-unsaturated ketone is the active center, possibly acting as an alkylation site. Compound **3** without a substitution at the C-4 position had moderate activities to SGC-7901 and K562 with IC_{50} values ranging from 7.37 to 7.53 μM, while compounds **5**–**14, 17**–**19**, and **24** with a substitution at the C-4 position showed stronger inhibitory activity than compound **3** to those tumor cell lines with IC_{50} values ranging from 0.25 to 2.47 μM. This indicated that the substitution of the acid moiety at the C-4 position led to significant changes in the cytotoxic activity. Compounds **5**–**7** with amide groups at C-4 displayed more potent cytotoxicity than compounds **11**–**14** with ester groups at C-4 against the K562 cell line but were less potent to the SGC-7901 cell line. Meanwhile, compounds **11**–**14** with only ester groups at C-4 showed higher cytotoxic activity than compounds **17**–**19** with piperidine groups conjunct to them at C-4 to SGC-7901. Therefore, different kinds of substituted groups would cause different effects to different cell lines. The cytotoxicity of compounds **11**–**13** with ethyl, propyl, and butyl ester groups at C-4 implied that the elongated aliphatic chain length did not influ-

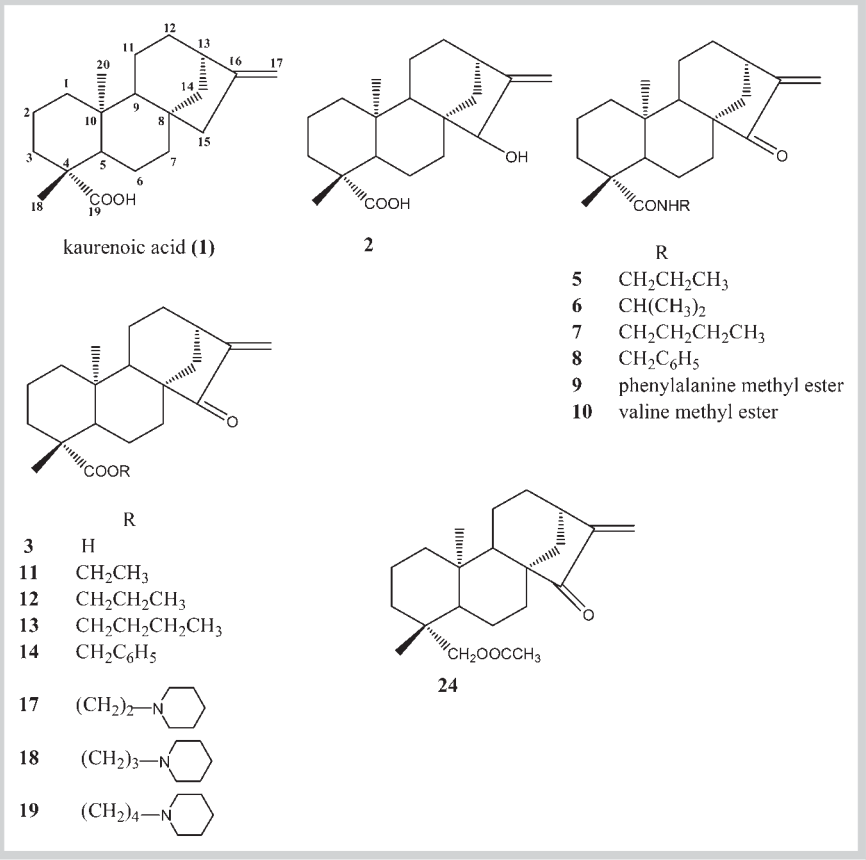

Fig. 1 Structure of kaurenoic acid (**1**) and its derivatives.

Table 1 *In vitro* cytotoxicitives of **1, 3, 5–14, 17–19**, and **24** against selected tumor cell lines as IC_{50} (μM).

Compound	SW620	SW480	PANC-1	BxPC-3	SGC-7901	Eca-109	K562	HL-60
1	> 100	NT	> 100	NT	> 100	> 100	> 100	> 100
3	1.01	NT	5.60	NT	7.37	NT	7.53	7.91
5	0.98	NT	3.36	3.28	4.51	1.85	0.53	1.68
6	0.81	NT	0.90	1.46	2.46	1.23	0.25	1.40
7	0.97	NT	1.64	1.48	2.53	1.24	0.38	1.40
8	0.99	1.16	1.97	NT	1.95	1.26	2.47	0.42
9	0.73	NT	NT	NT	1.15	NT	1.09	NT
10	0.58	NT	NT	NT	2.65	NT	0.93	NT
11	0.70	1.13	1.22	NT	1.63	1.92	2.09	0.93
12	1.06	NT	NT	NT	1.28	NT	0.98	NT
13	0.89	NT	NT	NT	1.59	NT	1.21	NT
14	0.87	NT	NT	NT	1.23	NT	0.96	NT
17	0.96	1.48	1.64	NT	3.14	2.46	1.64	1.48
18	0.73	1.29	1.45	NT	2.59	1.93	1.29	1.09
19	0.70	1.14	1.45	NT	2.19	1.91	1.60	1.25
24	1.10	NT	NT	NT	2.47	NT	1.77	NT
Cisplatin	13.26	15.58	10.78	5.17	8.92	2.76	4.98	1.92

α Cell line: SW620 = colon; SW480 = colon; PANC-1 = pancreatic; BxPC-3 = pancreatic; SGC-7901 = stomach; Eca-109 = esophageal; K562 = leukemia; HL-60 = leukemia; NT: not tested

ence their activities. This was yet again evidenced by the cytotoxicity of compounds **17–19** with piperdinethyl, piperdinepropyl, and piperdinebutyl ester groups at the C-4 position. The activity of compounds **11** and **24** indicated that inversion of the ester bond had little affect on the activity. The SW620 colon cancer cell was highly susceptible to all tested derivatives, and the standard deviation of their average IC_{50} from compound **3** to compound **24** was 0.16 μM.

This semi-synthesis of kaurenoic acid derivatives has revealed several compounds with increased cytotoxic activity. Further studies are required to lower toxicity against normal cells and enhance the effect against cancer cell lines.

Materials and Methods

Isolation: The staring material kaurenoic acid [ent-kaur-16-en-19-oic acid, (**1**; **Fig. 1**)] was isolated from the mangrove-associated plant of *W. prostrata* as previously described [3].

General: IR spectra were measured on a Nicolet FT-IR spectrometer with KBr pellets. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker AVANCE III spectrometer using TMS as the internal standard and $CDCl_3$ as the solvent, reported in Supporting Information. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. Column chromatography was performed on silica gel (100–200 mesh and 200–300 mesh; Qingdao Marine Chemical Group Corporation), ESI-MS data were obtained using a Bruker APEX II FT-MS, and EI-MS data was obtained with a ThermoFinnigan DECAX-30000 mass spectrometer. HR-ESI-MS data were obtained with a Bruker APEX II mass spectrometer. Optical rotations were obtained using a JASCO-20 polarimeter. TLC was carried out on silica gel GF254 on glass plates (Qingdao Marine Chemical, Inc.) using various solvent systems. The spots were visualized under UV light or by spraying with 5% H_2SO_4 in EtOH followed by heating. All other reagents were purchased from Aladdin Reagent Company in analytic grade.

Cytotoxic activities against human tumor cell lines including SW620, SW480, PANC-1, BxPC-3, SGC-7901, Eca-109, K562, and HL-60 were evaluated with the MTT assay method [15].

15-Oxo-kaurenoic acid (**3**) [14]: amorphous solid (36% yield); m. p. 179.8–180.9 °C; IR (KBr): ν_{max} = 3106, 2926, 1682, 1731 cm^{-1}; ESI-MS: *m/z* (rel. int.) = 316 [M]$^+$ (95), 317 (26), 301 (27), 148 (60), 91 (53); anal. C 75.94, H 8.86, calcd for $C_{20}H_{28}O_3$, C 75.91, H 8.92.

15-Oxo-kaurenoic acid propanamide (**5**): white foamy solid (19% yield); m. p. 156.4–157.6 °C; $[\alpha]_D^{24}$: − 13.5 ($CHCl_3$, *c* 0.10); IR (KBr): ν_{max} = 3380, 2926, 2860, 1720, 1638, 1517, 1463 cm^{-1}; HR-ESI-MS: [M + Na]$^+$ *m/z* = 380.2568 (calcd. for $C_{23}H_{35}NO_2Na$: 380.2566); EI-MS: *m/z* (rel. int.) = 357 [M]$^+$ (90), 358 (23), 329 (95), 228 (76).

15-Oxo-kaurenoic acid isopropyl amide (**6**): white foamy solid (24% yield); $[\alpha]_D^{24}$: − 12.9 ($CHCl_3$, *c* 0.10); IR (KBr): ν_{max} = 3418, 2958, 2860, 1724, 1632, 1518, 1448 cm^{-1}; HR-ESI-MS: [M + Na]$^+$ *m/z* = 380.2568 (calcd. for $C_{23}H_{35}NO_2Na$: 380.2566); ESI-MS: *m/z* (rel. int.) = 357 [M]$^+$ (100), 358 (26), 329 (97) 228 (48).

15-Oxo-kaurenoic acid butyramide (**7**): white foamy solid (21% yield); $[\alpha]_D^{24}$: − 13.9 ($CHCl_3$, *c* 0.10); IR (KBr): ν_{max} = 3375, 2915, 2854, 1726, 1632, 1512, 1452 cm-1; HR-ESI-MS: [M + Na]$^+$ *m/z* = 394.2722 (calcd. for $C_{24}H_{37}NO_2Na$: 394.2723); EI-MS: *m/z* (rel. int.) = 371 [M]$^+$ (56), 343 (60), 228 (45), 209 (48) 142 (100).

15-Oxo-kaurenoic acid benzoylamide (**8**): white foamy solid (20% yield); $[\alpha]_D^{24}$: − 14.9 ($CHCl_3$, *c* 0.10); m. p. 210.2–211.6 °C; IR (KBr): ν_{max} = 3386, 2920, 2849, 1720, 1638, 1517, 1452, 1249 cm^{-1}; HR-ESI-MS: [M + Na]$^+$ *m/z* = 428.2567 (calcd. for $C_{27}H_{35}NO_2Na$: 428.2566); ESI-MS: *m/z* (rel. int.) = 405 [M]$^+$ (52), 377 (34), 243 (38), 228 (41).

15-Oxo-kaurenoic acid phenylalanine methyl ester amide (**9**): white amorphous solid (20% yield); $[\alpha]_D^{24}$: − 11.7 ($CHCl_3$, *c* 0.10); IR (KBr): ν_{max} = 3408, 2942, 2855, 1726, 1643, 1517 cm^{-1}; HR-ESI-MS: [M + Na]$^+$ *m/z* = 500.2771 (calcd. for $C_{30}H_{39}NO_4Na$: 500.2777); ESI-MS: *m/z* (rel. int.) = 477 [M]$^+$ (100), 423 (5), 380 (6).

15-Oxo-kaurenoic acid valine methyl ester amide (**10**): white amorphous solid (22% yield); $[\alpha]_D^{24}$: − 15.2 ($CHCl_3$, *c* 0.10); IR (KBr): ν_{max} = 3413, 2936, 2866, 1736, 1720, 1660, 1506, 1194 cm^{-1}; HR-ESI-MS: [M + Na]$^+$ *m/z* = 452.2783 (calcd. for $C_{26}H_{39}NO_4Na$: 452.2777); ESI-MS: *m/z* (rel. int.) = 429 [M]$^+$ (100), 398 (8), 380 (6).

15-Oxo-kaurenoic acid ethyl ester (**11**): amorphous solid (27% yield), m. p. 148.5–149.6 °C; $[\alpha]_D^{24}$: − 14.5 ($CHCl_3$, *c* 0.10); IR: (KBr) ν_{max} = 3426, 2950, 2926, 1728, 1692, 1452, 1245 cm^{-1}; HR-ESI-MS: [M + Na]$^+$ *m/z* = 367.2253 (calcd. for $C_{22}H_{32}O_3Na$: 367.2249); EI-MS: *m/z* (rel. int.) = 344 [M]$^+$ (100), 329 (11), 271 (48), 91 (37).

15-Oxo-kaurenoic acid propyl ester (**12**): amorphous solid (23% yield); $[\alpha]_D^{24}$: − 6.4 ($CHCl_3$, *c* 0.10); IR: (KBr) ν_{max} = 3426, 2955, 2926, 1727, 1692, 1456, 1245 cm^{-1}; HR-ESI-MS: [M + Na]$^+$ *m/z* = 382.2400 (calcd. for $C_{23}H_{34}O_3Na$: 381.2406); EI-MS: *m/z* (rel. int.) = 359 [M + H]$^+$ (100), 343 (16), 318 (5).

15-Oxo-kaurenoic acid butyl ester (**13**): amorphous solid (25% yield); $[\alpha]_D^{24}$: − 14.1 ($CHCl_3$, *c* 0.10); IR (KBr): ν_{max} = 3428, 2955, 2926, 1729, 1692, 1456, 1244 cm^{-1}; HR-ESI-MS: [M + Na]$^+$ *m/z* = 395.2565 (calcd. for $C_{24}H_{36}O_3Na$: 395.2562); EI-MS: *m/z* (rel. int.) = 373 [M + H]$^+$ (100), 357 (6), 318 (5).

15-Oxo-kaurenoic acid benzyl ester (**14**): amorphous solid (31% yield); $[\alpha]_D^{24}$: − 17.8 ($CHCl_3$, *c* 0.10); IR (KBr): ν_{max} = 3418, 2942, 2855, 1721, 1638, 1522, 1241 cm^{-1}; HR-ESI-MS: [M + Na]$^+$ *m/z* = 429.2403 (calcd. for $C_{27}H_{34}O_3Na$: 429.2406); EI-MS: *m/z* (rel. int.) = 407 [M + H]$^+$ (100), 389 (13), 338 (33), 315 (50).

15-Oxo-kaurenoic acid piperdinethyl ester (**17**): yellow oily liquid (17% yield); $[\alpha]_D^{24}$: − 11.9 ($CHCl_3$, *c* 0.10); IR (KBr): ν_{max} = 2931, 2854, 1721, 1638, 1457, 1221, 1162 cm^{-1}; HR-ESI-MS: [M + Na]$^+$ *m/z* = 450.2979 (calcd. for $C_{27}H_{41}NO_3Na$: 450.2984); ESI-MS: *m/z* (rel. int.) = 427 [M]$^+$ (5), 111 (34), 98 (100).

15-Oxo-kaurenoic acid piperdinepropyl ester (**18**): yellow oily liquid (18% yield); $[\alpha]_D^{24}$: − 12.2 ($CHCl_3$, *c* 0.10); IR (KBr): ν_{max} = 2931, 2849, 1721, 1643, 1457, 1222, 1156 cm^{-1}; HR-ESI-MS: [M + H]$^+$ *m/z* = 442.3322 (calcd. for $C_{28}H_{44}NO_3$: 442.3321); ESI-MS: *m/z* (rel. int.) = 441 [M]$^+$ (8), 413 (4), 149 (15), 98 (100).

15-Oxo-kaurenoic acid piperdinebutyl ester (**19**): yellow oily liquid (21% yield); $[\alpha]_D^{24}$: − 12.7 ($CHCl_3$, *c* 0.10); IR (KBr): ν_{max} = 2926, 2849, 1720, 1671, 1469, 1436, 1227, 1151 cm^{-1}; HR-ESI-MS: [M + Na]$^+$ *m/z* = 478.3293 (calcd. for $C_{29}H_{45}NO_3Na$: 478.3297); EI-MS: *m/z* (rel. int.) = 455 [M]$^+$ (8), 156 (10), 98 (100).

ent-15-Oxo-kaur-16-en-19-acetoxy (**24**): white crystals (19% yield); m. p. 156.4–157.6 °C; $[\alpha]_D^{24}$: − 12.0 ($CHCl_3$, *c* 0.10); IR (KBr): ν_{max} = 2932, 2834, 1732, 1638, 1446, 1391, 1238, 1024 cm^{-1}; HR-ESI-MS: [M + Na]$^+$ *m/z* = 367.2244 (calcd. for $C_{22}H_{32}O_3Na$: 367.2249); EI-MS: *m/z* (rel. int.) = 344 [M]$^+$ (100), 318 (83), 304 (42).

The MTT assay was performed in 96-well plates. Test cells at the log phase of their growth cycle (3×10^4 cell/mL) were added to each well (100 µL/well), then treated in three replicates at various concentrations of the samples (0.1–100 µg/mL), and incubated for 24 h at 37 °C in a humidified atmosphere of 5% CO_2. After 48 h, 20 µL of MTT solution (5 mg/mL) per well were added to each cultured medium, which were then incubated for a further 4 h. Then, DMSO was added to each well (150 µL/well). After 15 min at room temperature, the OD of each well was measured on a microplate reader at a wavelength of 570 nm. IC_{50} values were obtained by a linear regression analysis of percent absorbance versus log drug concentration.

Supporting information

The ^{1}H-NMR, ^{13}C-NMR, EI-MS, ESI-MS, and HR-MS data of compounds **3, 5–14, 17–19**, and **24**, and the general procedures for the synthesis of them are available as Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

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