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New substituted C-19-andrographolide analogues with potent cytotoxic activities

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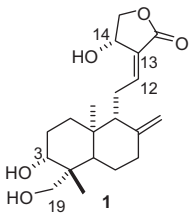
ABSTRACT

Andrographolide, the major diterpenoid lactone from *Andrographis paniculata*, is toxic against cancer cells. In the present study, we investigated the structure–activity relationships (SARs) of 19 andrographolide analogues which were synthesized by modification at the three hydroxyl groups. A number of the andrographolide analogues showed much higher cytotoxic activities than that of the parent compound on cancer cells including P-388, KB, COL-2, MCF-7, LU-1 and ASK cells. SAR studies of the synthetic analogues indicated that the introduction of silyl ether or triphenylmethyl ether group into C-19 of the parent compound led to increase in toxicity against the cancer cells. The 19-*O*-triphenylmethyl ether analogue **18** showed higher cytotoxic activity than the potent anticancer drug ellipticine, and this analogue may serve as a potential structure lead for the development of new anticancer drugs.

Andrographolide (**1**), the major labdane diterpene was isolated in a large quantity (2%) from the methanol extract of the aerial parts of *Andrographis paniculata*.[1] This natural substance has therapeutic potential for a wide range of diseases.[2] Its chemically modified analogues are also reported to be effective as TNF-α, IL-6[3] and α-glucosidase inhibitor[4] as well as antibacterial[2a,5] and cytotoxic[6,7] agents. However, among the modified andrographolide analogues that have been synthesized so far, only a few cases of the possible analogues that could hold therapeutic potential over the parent compound. Therefore, simple synthetic structures with strong bioactivity as potential drug by convenient modification method will be much more attractive in pharmaceutical industry.

Herein, we report the synthesis of a series of andrographolide analogues derived from the natural substrate **1**. Since compound **1** bears cytotoxic activity[2k,m] and is available from nature, the synthesis of analogues from this compound could be of a great value in discovering potential anticancer semisynthetic drug.



In our design of the synthetic andrographolide analogues, the core structural scaffold of **1** was kept intact to maintain the key biological activities.[2k] Indeed, the double bond between C-12 and C-13 was reported to be crucial for cytotoxicity,[6,7] possibly is associated with their ability to promote alkylation of biological nucleophiles such as enzyme through Michael addition to the *exo*-alkene of α-alkylidene-χ-butyrolactone moiety.[6] Our strategy was to keep this functional group and conversions of the three hydroxyl groups in **1** to a series of acetyl, silyl and triphenylmethyl ether groups at the C-19 position, and acetyl groups at the C-3 and C-14 positions, have been undertaken.[8]

The first series of silyl-andrographolide analogues were prepared as shown in Scheme 1. Compounds **2**, **6** and **8** were synthesized by silylation at C-19 position in basic condition using pyridine to obtain *tert*-butyldimethylsilyl-(TBS), triisopropylsilyl-(TIPS) and *tert*-butyldiphenylsilyl-(TBDPS) ether derivatives, respectively, in moderate to high yields. Acetylation of the resulting silyl ether at C-3 and C-14 in the presence of acetic anhydride at 70 °C afforded the mono- and di-acetylsilane products **3**–**5**, **7** and **9**–**11**.

Compounds **13**–**16** were obtained from the reaction of **1** in 22%, 46%, 7% and 18% yields (total 93%), respectively, after heating in the excess acetic anhydride at 70 °C for 5 h (Scheme 2). In order to afford the mono-acetyl derivative **12**, compound **3** was employed as a starting material and *tert*-butyldimethylsilyl ether group at C-**19** was de-protected using formic acid/water in THF. Compound **17** was obtained in moderate yield under the same reaction condition from **5**.

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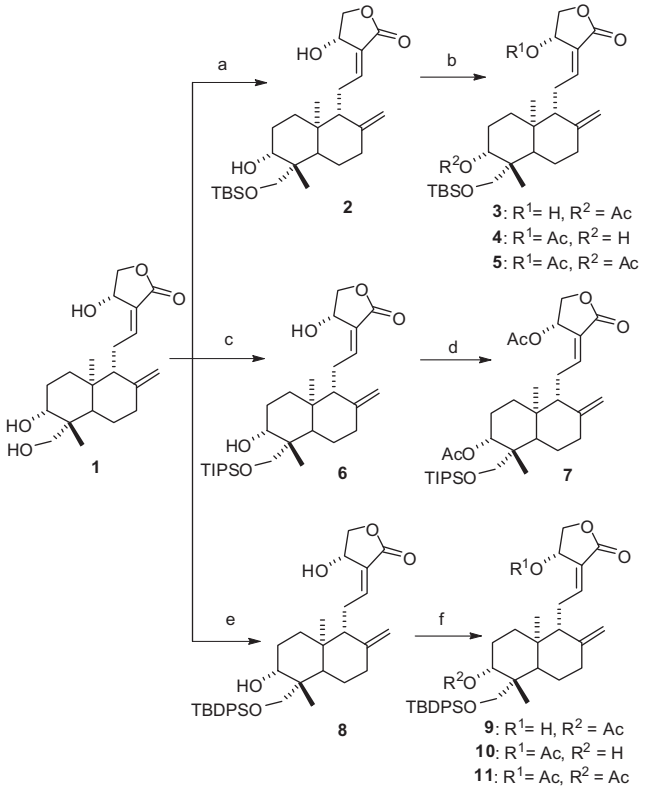
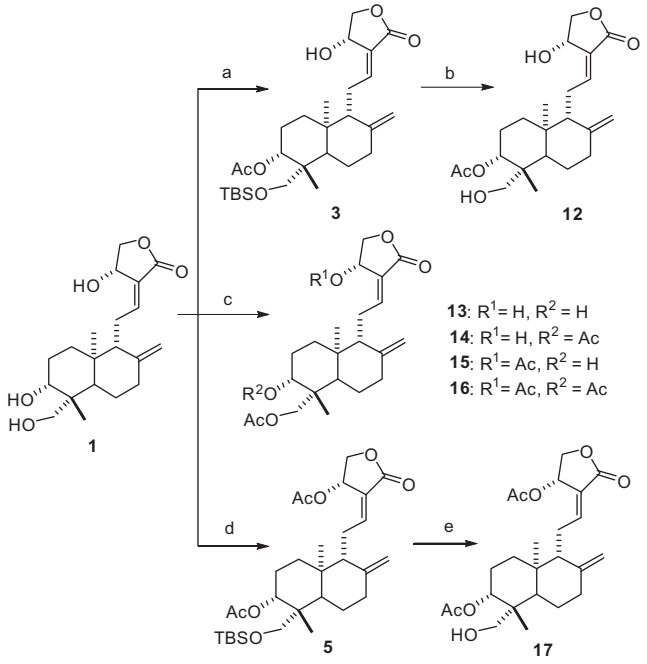
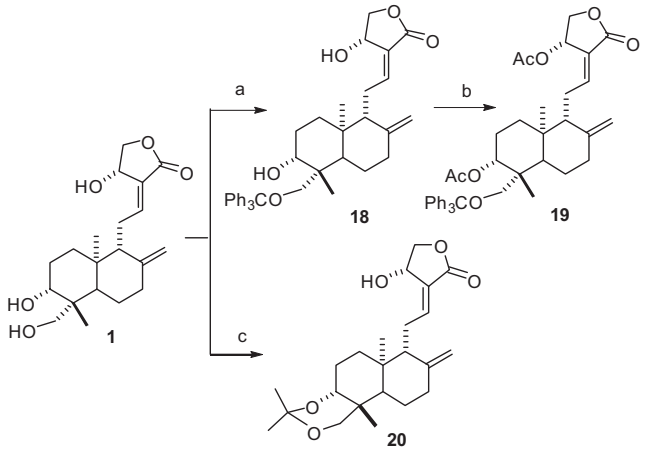


Scheme 1. Reagents and conditions: (a) TBSCl, pyridine, rt, 1 h, 92%; (b) Ac_2O, 70 °C, 18 h, total yields of **3**, **4** and **5** = 93%; (c) TIPSCl, pyridine, rt, 4 h, 65%; (d) Ac_2O, 140 °C, 3 h, 80%; (e) TBDPSCl, pyridine, rt, 1 h, >99%; (b) Ac_2O, 70 °C, 6 h, total yields of **9**, **10** and **11** = 76%.



Scheme 2. Reagents and conditions: (a) (i) TBSCl, pyridine, rt, 1 h, 88%, (ii) Ac_2O, 70 °C, 18 h, 33%; (b) $HCOOH/H_2O$ (9:1), THF, 0 °C, 30 min, 51%; (c) Ac_2O, 80 °C, 1 h, total yields of **14**, **15** and **16** = 93%; (d) (i) TBSCl, pyridine, rt, 1 h, 88%, (ii) Ac_2O, 145 °C, 1 h, 94%; (e) $HCOOH/H_2O$ (9:1), THF, 0 °C, 30 min, 48%.



Scheme 3. Reagents and conditions: (a) TrCl, pyridine, 70 °C, 2.5 h, 99%; (b) Ac_2O, 140 °C, 1 h, 33%; (c) 2,2-dimethoxypropane, PPTS, acetone, 97%.

To increase structural diversities, the third series of ether analogues were prepared as shown in Scheme 3. An excellent yield of 19-*O*-triphenylmethylandrographolide **18** was obtained by heating the parent compound **1** in the presence of trityl chloride and pyridine. Subsequent acetylation of the remaining two hydroxyl groups led to the analogue **19**. Finally, the protection of C-3 and C-19 hydroxyl groups as acetonide yielded compound **20**.

The cytotoxic activity of **1** and its synthetic analogues were evaluated using in vitro sulforhodamine B (SRB) assay against six cancer cell lines including P-388 (murine leukaemia cell line), KB (human epidermoid carcinoma of the mouth), COL-2 (human colon cancer), MCF-7 (human breast cancer), LU-1 (human lung cancer), and ASK (rat glioma). All tested samples were dissolved in DMSO (0.1%). Ellipticine was used as a positive control. It is a potent anticancer agent which exhibits several modes of mechanisms against cancer cells.[9] The results were expressed as ED_{50} values (drug concentration causing 50% growth inhibition) in μM, are shown in Table 1.

Ten out of 19 synthetic analogues (compounds **3**–**6**, **8**–**10** and **16**–**18**) displayed cytotoxic activities stronger than that of the parent compound **1** in all cancer cell lines. Comparison on the cytotoxic activities of C-19 mono-substituted analogues **2**, **6**, **8**, **13**, and **18** revealed the importance of the protecting group of the hydroxyl function at C-19 on the andrographolide core.

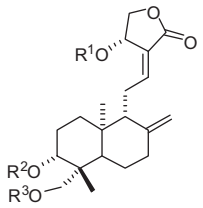
The introduction of a *tert*-butyldimethylsilyl (TBS) group on the tetrahydropyran ring[10] has previously been reported to result in a significant enhancing cytotoxic activity against HL60 (human leukaemia cells) and MCF7. Evaluation of the cytotoxic activity of **1** and TBS-derivative **2** against a series of cancer cell lines did not show any enhancement in the present work. The differences in sensitivity of cell lines may account for the discrepancy of results.

However, changing of the silyl-TBS group at C-19 to other silyl derivatives such as the more bulky triisopropylsilyl (TIPS) **6** led to 6- to 15-fold increase in cytotoxic activity. Conversion to the TBDPS **8** resulted in a moderate increase in activity, and this compound showed selectivity against P-388.

These bulky functional groups containing silicon might provide lipophilicity to the compound, allowing it to pass the cell membrane by passive diffusion. This strategy has been successfully applied in the antitumor drug analogues silatecans[11] (silicon-containing camptothecins) and silaplatins[12] (cisplatin analogues).

Compound **13**, containing acetyl group at C-19 position, exhibited similar activity to the parent **1**. However, this analogue was also highly toxic against P-388. Our novel synthetic analogue **18** bearing triphenylmethoxyl group at C-19 was found to be the most potent analogue against the tested cancer cells (ED_{50} 0.45–2.86 μM)

Table 1
Cytotoxic activity against six human cancer cells



Entry	Compound	ED_{50} (μM)[a] (SRB assay)								
		R^1	R^2	R^3	P-388	KB	COL-2	MCF-7	LU-1	ASK
1	**1**	H	H	H	2.25	27.37	13.60	15.40	12.98	16.18
2	**2**	H	H	TBS	8.39	40.71	17.10	46.96	48.81	15.51
3	**3**	H	Ac	TBS	1.71	11.15	11.31	9.79	9.62	8.17
4	**4**	Ac	H	TBS	1.03	10.74	8.94	10.44	10.44	3.41
5	**5**	Ac	Ac	TBS	0.34	3.62	2.23	2.84	2.92	1.22
6	**6**	H	H	TIPS	0.88	2.73	2.73	2.88	3.01	2.85
7	**7**	Ac	Ac	TIPS	1.41	7.39	13.59	13.95	18.79	7.56
8	**8**	H	H	TBDPS	0.68	11.13	9.65	8.15	11.47	5.54
9	**9**	H	Ac	TBDPS	0.50	9.86	3.80	7.53	10.93	10.47
10	**10**	Ac	H	TBDPS	0.33	10.54	10.70	9.63	11.86	11.96
11	**11**	Ac	Ac	TBDPS	1.67	29.04	12.28	45.70	52.92	32.81
12	**12**	H	Ac	H	4.78	35.65	27.21	26.43	30.59	58.13
13	**13**	H	H	Ac	2.04	15.26	12.94	18.25	13.99	16.71
14	**14**	H	Ac	Ac	2.65	35.22	15.26	19.69	34.72	22.39
15	**15**	Ac	H	Ac	1.62	26.38	15.23	18.47	19.77	16.31
16	**16**	Ac	Ac	Ac	1.40	11.62	10.82	10.02	12.41	11.41
17	**17**	Ac	Ac	H	0.69	11.66	10.16	10.95	8.75	11.48
18	**18**	H	H	CPh_3	0.45	2.42	2.73	2.72	0.88	2.86
19	**19**	Ac	Ac	CPh_3	1.92	9.77	22.79	22.68	8.37	15.29
20	**20**	H			3.78	66.45	39.80	42.80	52.98	52.22
21	Ellipticine				2.44	2.46	2.72	2.71	1.62	3.56

[a] Cell lines used are P-388 (murine leukaemia cell line); KB (human epidermoid carcinoma of the mouth); COL-2 (human colon cancer); MCF-7 (human breast cancer); LU-1 (human lung cancer); and ASK (rat glioma). Ellipticine (Ellipt) was used as a positive control. The results were expressed as ED_{50} values (drug concentration causing 50% growth inhibition) in μM. See Supplementary Table 1 for the detail of each value which represents mean ± SE from three different experiments performed in triplicate.

and, most importantly, it was more potent than the reference drug ellipticine. A variety of compounds containing the trityl motif have been report to possess anticancer properties because of the size and hydrophobic nature of the trityl pharmacophore and function through different mechanisms of action.[13] It might be suspected that anticancer compounds possessing this functional group share a common mode of cell death induction.

With the success of the modified silyl and triphenylmethyl analogues (**6**, **8** and **18**), addition of the acetyl groups to C-3 and C-14 of the resulting silyl and triphenylmethyl analogues were investigated, including the variations of hydroxyl groups either at C-3 or C-14 of other compounds.

Interestingly, introduction of acetyl groups to C-3 and C-14 of the silyl analogue **2** to give the analogue **5** resulted in 7- to 24-fold increase in cytotoxicity; it was potent against P-388, COL-2 and ASK with ED_{50} values of 0.34, 2.23 and 1.22 μM, respectively, and was more potent than ellipticine. On the other hand, the diacetyl analogues **7**, **11**, and **19** showed sharp decreases in cytotoxic activities in comparison with the analogues **6**, **8**, and **18**, respectively, which did not bear the acetyl groups at C-3 and C-14.

The activities of compounds bearing acetyl group at C-3 and C-14 positions were compared among those of the 3-acetyl analogues **3** with the C-14 acetyl analogues **4**, and the analogue **9** with **10**. The presence of one acetyl group at C-3 or C-14 of **4** and **9** led to a dramatic increase in the cytotoxic activity as compared with compound **2**. However, the introduction of acetyl group at either C-3 or C-14 gave similar activities.

In addition, the introduction of mono-, di-, tri-acetyl, and acetonide groups to the parent compound **1** to give the analogues **12**–**17** and **20** did not alter the cytotoxic activity, except that the analogues **16** and **17** exhibited better activities than the parent **1**.

In conclusion, we have successfully modified the hydroxyl groups at C-3, C-4 and C-19 of andrographolide (**1**) to 19 analogues. To our knowledge, this is the first report on the silyl and triphenylmethyl ethers at C-19 of **1** as the essential structural features for cytotoxic activities against a series of cancer cell lines. The most potent analogue **18** was simply prepared in one step with excellent yield (99%) by using the starting material **1** which was isolated in a large quantity from *A. paniculata*. Owing to the less synthetic step involved and the commercially available inexpensive reagents, this new synthetic compound may serve as a potential lead compound for the development of new anticancer drugs.

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Supplementary data

Supplementary data (General procedures and spectral data.) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.11.085.

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