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Synthesis and in vitro cytotoxicity of andrographolide-19-oic acid analogues as anti-cancer agents

Dongsheng Chen, Yaping Song, Yunlong Lu, Xiaowen Xue *

Department of Medicinal Chemistry, China Pharmaceutical University, Nanjing 210009, China

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ABSTRACT

The synthesis of a series of andrographolide-19-oic acid derivatives was described and their in vitro anti-tumor activity against two human cell lines was evaluated. Most compounds were found to exhibit significant cytotoxicity, better than andrographolide, and compounds **9d** and **9b** were identified as the most potent with IC_{50} values of 1.18 and 6.28 μm against HCT-116 and MCF-7 cell lines, respectively. The preliminary results indicated that the oxidation of C-19-hydroxyl group of andrographolide to corresponding carboxyl group and the subsequent esterification of the formed carboxylic acid led to considerable improvement in cytotoxicity against the cancer cells.

Andrographolide **1**, chemically named as 2(3*H*)-furanone, 3-[2-[decahydro-6-hydroxy-5- (hydroxymethyl)-5,8a-dimethyl-2-methylene-1-napthalenyl]ethylidene]dihydro-4-hydroxy (Fig. 1), is the major labdane diterpenoidal constituent isolated from the plant *Andrographis paniculata* (family Acanthaceae),[1,2] which is used extensively as the traditional Chinese medicine. Andrographolide exhibits a wide spectrum of biological activities including antibacterial,[3] antiinflammatory,[4] antimalarial,[5] immunomodulation,[6] antithrombotic,[7] and hepatoprotective effect.[8] In recent past, the compound is reported for its anti-tumor activity.[9,10] Several mechanisms have been proposed for its anti-cancer activity, such as cytotoxicity against cancer cells,[11] induction of cell-cycle arrest,[12] and induction of apoptosis.[13]

The promising anti-cancer activity of andrographolide makes it a good lead. Various semi-synthetic analogues have been synthesized and evaluated in order to find out a better candidate holding therapeutic potential over the parent compound.[14–16] Most structure modifications were focused on the functionallization of 3,14,19-hydroxy groups of andrographolide. Stanslas reported that 14-acetylandrographolide is more potent from 60 NCI human cancer cell lines in vitro screen compared to andrographolide.[17] In another report, the α-alkylidene-γ-butyrolactone moiety of andrographolide was believed to play a major role in the activity profile.[18] He et al. recently studied the biotransformed products from andrographolide by *Rhizopus stolonifer* ATCC 12939 and *Aspergillus ochraceus*, and found that the antiproliferative activities against MCF-7 and HCT-116 cell lines were decreased after the C-3-hydroxyl group of parent compound has been oxidized to the keto group,[19] while increased significantly after the C-19-hydroxyl group has been oxidized to the carboxylic group.[20] Unfortunately, the α-alkylidene-γ-butyrolactone core moiety of andrographolide was destroyed during the bioconversion of C-19-hydroxyl group into carboxyl group. Therefore, it is natural for us to envisage that the andrographolide-19-oic acid analogues with the α-alkylidene-γ-butyrolactone core moiety unbroken may have much better anti-tumor activity than andrographolide (Fig. 2). To our surprise, no attempt was made to design the andrographolide analogues bearing these structural characters. In this Letter, we report the synthesis and biological evaluation of new andrographolide-19-oic acid analogues as potent cytotoxic agents.

The first series of andrographolide-19-oic acid analogues were prepared as shown in Scheme 1. Andrographolide was used as starting material since it is readily available from nature. The

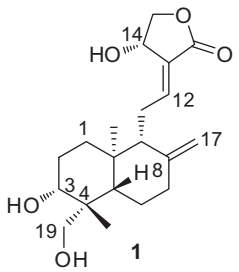

Figure 1. Andrographolide.

* Corresponding author. Tel./fax: +86 25 83199600.
E-mail address: xwenxue@cpu.edu.cn (X. Xue).

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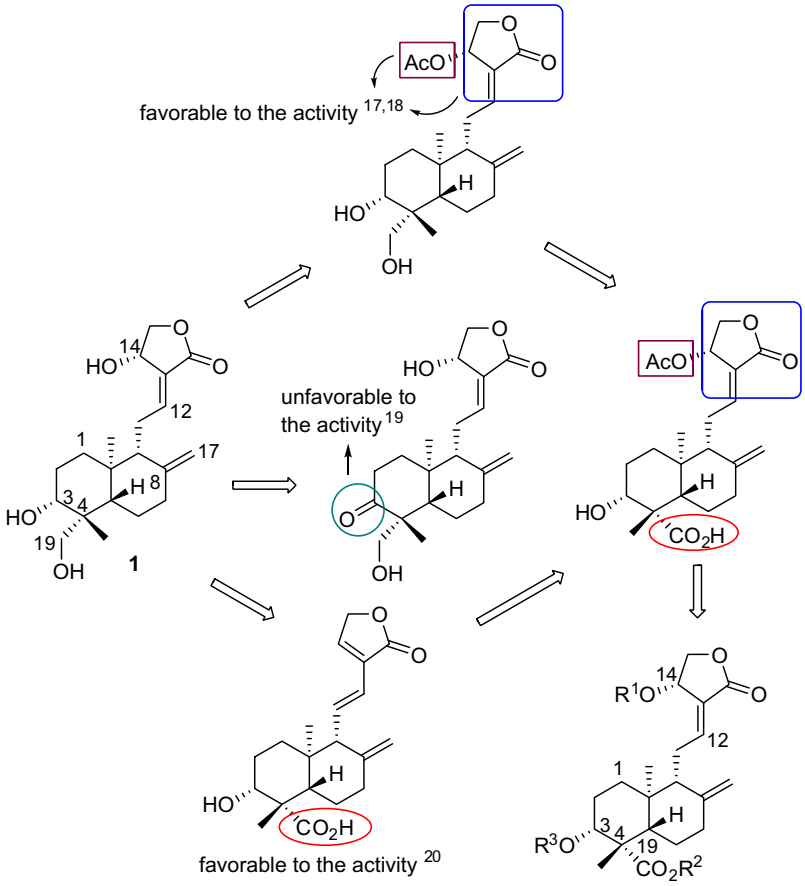
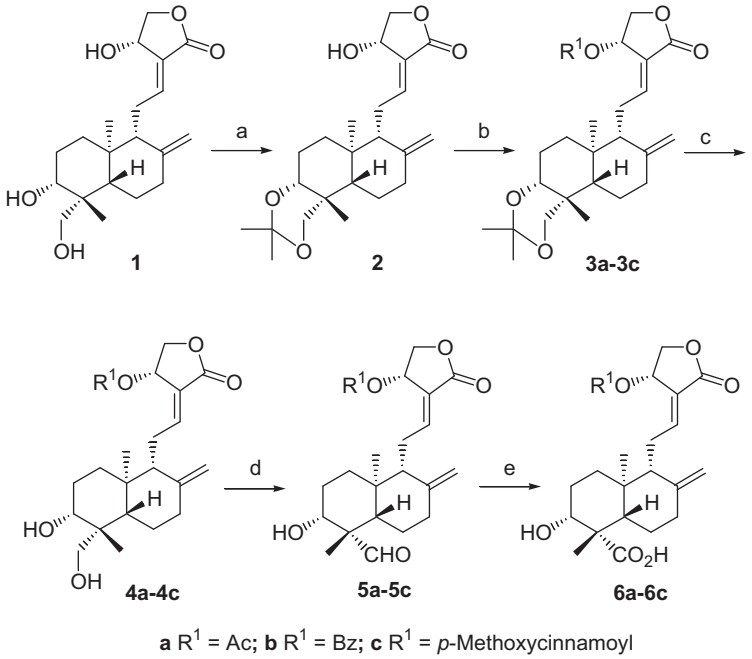


Figure 2. Design strategy for the new andrographolide-19-oic acid analogues.



a R^1 = Ac; **b** R^1 = Bz; **c** R^1 = *p*-Methoxycinnamoyl

Scheme 1. Reagents and conditions: (a) 2,2-dimethoxypropane, *p*-TsOH (cat.), Tol/DMSO, 80 °C, 1.5 h, 93%; (b) for **3a**: Ac_2O, reflux 1.5 h; for **3b**: BzCl, TEA, DMAP, CH_2Cl_2, rt, 4 h; for **3c**: *p*-methoxycinnamic acid/Tf_2O, TEA, CH_2Cl_2, 0 °C; then **2**, 0 °C to rt; (c) HOAc–H_2O (7:3), rt, 42–85% for two-steps; (d) TEMPO, TBAI, NCS, CH_2Cl_2, K_2CO_3–$KHCO_3$ buffer, 0 °C, 9 h, 83-91%; (e) $NaClO_2$–NaH_2PO_4, isopantene, *t*-BuOH, DMSO, 0 °C to rt,48 h, 42–59%.

hydroxyl groups at C-3 and C-19 of andrographolide were protected by reacting with 2,2-dimethoxypropane at the presence of catalytic *p*-TsOH according to a modified literature approach,[21] to furnish 3,19-isopropylideneandrographolide **2**. The hydroxyl group at C-14 was then acylated with corresponding acylating agents (acetic anhydride, benzoyl chloride, and *p*-methoxycinnamic

trifluoromethanesulfonic anhydride respectively) to provide **3a**–**3c**. Here, aliphatic acetyl, aromatic benzoyl and conjugated *p*-methoxycinnamoyl were chosen to investigate the influence of different types of acyl groups on the anti-tumor activity. The isopropylidene protecting group of **3a**–**3c** was removed by stirring with aqueous acetic acid at room temperature, followed by selective oxidation of primary hydroxyl group at C-19 position with TEMPO–NCS system[22] to yield corresponding aldehydes **5a**–**5c**. The formed aldehyde group of **5a**–**5c** was further oxidized to carboxyl group with $NaClO_2$–NaH_2PO_4 and isopentene,[23] affording the first series target compounds **6a–6c**.

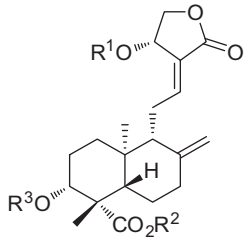
The second and third series of andrographolide analogues **8a**–**8c** and **9a**–**9f** were synthesized as shown in Scheme 2. Compounds **5a**–**5c** were acetylated with acetic anhydride under reflux to furnish 3-acetylated products **7a**–**7c**. The aldehyde group of **7a**–**7c** was then oxidized to carboxyl group with the same method described in Scheme 1, to afford the second series targets **8a–8c**. The carboxyl group of **8a**–**8c** was esterificated with MeI or BnBr at the presence of K_2CO_3 in DMF at room temperature to provide the third series targets **9a–9f**.

The structure of the target compounds was identified using spectroscopic techniques (IR, ^{1}H NMR, ^{13}C NMR, and mass spectroscopic analyses).[24]

The cytotoxic activity of **1** and its synthetic analogues were evaluated by MTS method,[25] using in vitro assay against two cancer cell lines: HCT-116 (human colon cancer) and MCF-7 (human breast cancer). The reason why these two cell lines were selected is that andrographolide was found most effective against colon cancer cell lines, followed by breast cancer cell lines.[12,17] All tested samples were dissolved in DMSO (0.1%). *cis*-Platin was selected as positive control. The concentrations that cause 50% inhibition of cancer cell growth against various cell lines are expressed as IC_{50} values and are summarized in Table 1.

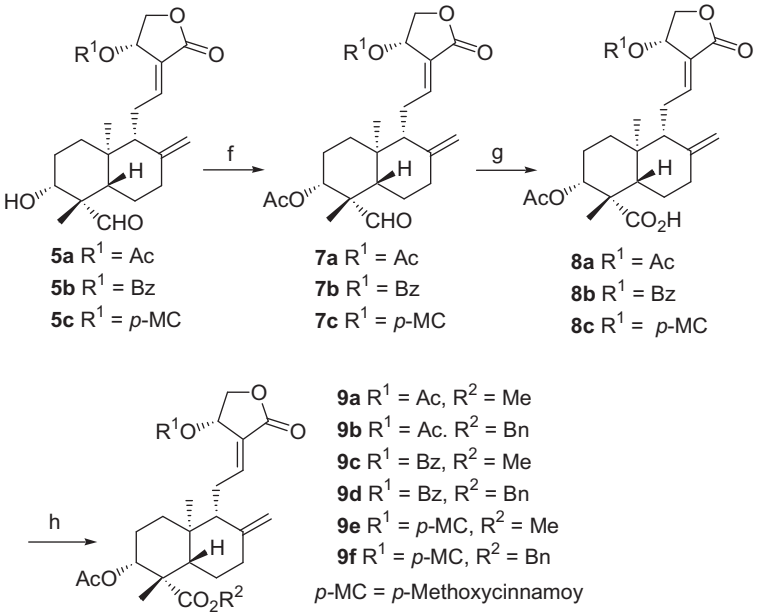
Almost all 12 synthesized analogues exhibited cytotoxic activities stronger than that of parent compound **1** in both cancer lines (except for the compound **8a**, which displayed almost no activity against MCF-7). The cytotoxicity of these compounds might be attributed to their ability to inhibit proliferation and induce apoptosis in cancer cells. Seven compounds **8c** and **9a**–**9f** even showed better anti-tumor activities against these two cell lines than positive control *cis*-platin, with **9d** and **9b** displaying the strongest activities with IC_{50} values of 1.18 and 6.28 μm against HCT-116 and MCF-7, respectively. All test compounds were found more effective against HCT-116 than MCF-7, which could be accounted for by the difference in sensitivity between these two cell lines. The esterification of andrographolide-19-oic acid derivatives contributes significantly to the potent cytotoxicity (compounds **8a** vs **9a**–**9b**, **8b** vs **9c**–**9d**, and **8c** vs **9e**–**9f**), and more interestingly,

Table 1
Evaluation of in vitro cell growth inhibitory effects of andrographolide analogues in against two cell lines



Entry	Compound	Cell growth inhibition in terms of IC_{50} (μM)[a]				
		R^1	R^2	R^3	HCT-116	MCF-7
1	**6a**	Ac	H	H	29.24	35.66
2	**6b**	Bz	H	H	36.85	40.20
3	**6c**	*p*-MC[b]	H	H	32.39	95.00
4	**8a**	Ac	H	Ac	20.90	>100
5	**8b**	Bz	H	Ac	17.81	44.57
6	**8c**	*p*-MC	H	Ac	9.73	43.31
7	**9a**	Ac	Me	Ac	2.29	11.80
8	**9b**	Ac	Bn	Ac	2.48	6.28
9	**9c**	Bz	Me	Ac	3.01	10.62
10	**9d**	Bz	Bn	Ac	1.18	8.37
11	**9e**	*p*-MC	Me	Ac	3.01	19.73
12	**9f**	*p*-MC	Bn	Ac	3.00	8.92
13	**1**				24.91	>100
14	*cis*-Platin				13.36	89.83

[a] IC_{50} was expressed as an average value of two experiments.
[b] *p*-MC = *p*-methoxycinnamoyl.



Scheme 2. Reagents and conditions: (f) Ac_2O, reflux 1.5 h, 60–78%; (g) $NaClO_2$–NaH_2PO_4, isopantene, *t*-BuOH, DMSO, 0 °C to rt,48 h, 50–76%; (h) for **9a**, **9c** and **9e**: MeI, K_2CO_3, DMF, rt, 1 h, 60–62%; for **9b**, **9d** and **9f**: BnBr, K_2CO_3, DMF, rt, 1 h, 49–55%.

the size of ester group has an important influence on the activity against MCF-7: the compounds with benzyl ester group (**9b**, **9d** and **9f**) express a better activity than those with methyl ester group (**9a**, **9c** and **9e**). However, acetylation of C-3-hydroxyl group seems unfavorable to the activity against MCF-7, while favorable to the activity against HCT-116 (compounds **6a** vs **8a**, **6b** vs **8d**, and **6c** vs **8c**). Moreover, for the structure modification of C14-hydroxyl group of andrographolide-19-oic acid analogues with acyl group, what should be noted is that the type of the acyl group has no obvious effect on the activity: whatever the acyl group is, aliphatic acetyl, aromatic benzoyl or conjugated *p*-methoxycinnamoyl, the activity keeps almost unchangeable.

In summary, we have successfully synthesized twelve andrographolide-19-oic acid analogues and biologically evaluated their in vitro anti-tumor activities against human cancer cell lines HCT-116 and MCF-7. To our knowledge, this is the first attempt to oxidize the C-19-hydroxyl group of andrographolide to corresponding carboxyl group with its α-alkylidene-γ-butyrolactone core moiety intact meanwhile. The preliminary results indicated that the oxidation of C-19-hydroxyl of the parent compound to carboxylic acid group, especially the subsequent esterification of the formed carboxylic acid, indeed led to a big increase in activity against the tested cancer cells. The most potent analogue **9d**, whose preparation was relatively simply achieved in seven-steps from andrographolide, showed 11-fold higher cytotoxic activity against HCT-116 than the anticancer drug *cis*-platin, which made it a promising structure lead for the development of new anticancer drugs. Studies in this direction are in progress.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.04.010.

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24. The structure of the compounds **6a–c**, **8a–c**, and **9a–f** was confirmed by spectroscopic techniques. For example, methyl 3-acetyl-14-acetyl-andrographolide-19-oate (**9a**): IR (KBr): 3449, 3127, 1763, 1735, 1724, 1676, 1647, 1396, 1251, 1234, 1218, 1155, 1025 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.01 (1H, t, J = 6.5 Hz, H-12), 5.92 (1H, d, J = 5.8 Hz, H-14), 4.96 (1H, s, H-17b), 4.61–4.52 (3H, m, H-3, H-15b and H-17a), 4.27–4.23 (1H, d, J = 11.2 Hz, H-15a), 3.66 (3H, s, CO_2CH_3), 2.52–2.29 (4H, m), 2.12 (3H, s, CH_3CO), 2.07 (3H, s, CH_3CO), 2.01–1.94 (1H, m), 1.86–1.80 (3H, m), 1.60–1.30 (4H, m), 1.25 (3H, s, H-18), 0.65 (3H, s, H-20); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 173.8, 170.9, 170.4, 169.0, 150.1, 146.4, 124.0, 109.0, 79.0, 71.5, 67.8, 55.6, 55.3, 51.1, 48.4, 39.3, 37.6, 37.1, 25.2, 25.2, 24.5, 24.0, 21.2, 20.6, 12.4; HRMS (ESI) m/z calcd for $C_{25}H_{34}O_8Na$ $[M+Na]^+$ 485.2151, found 485.2152.
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