Research Article

Synthesis of New Andrographolide Derivatives and Their Cytotoxic Activity

Virohit Patil, Narendra Sing Chauhan, RP. Venkat, Ajith Singhai and Ravindra Patil*

Department of Pharmaceutical Sciences, Allana College of Pharmacy, Pune, Maharashtra, India-411038.

ABSTRACT

A new series of andrographolide derivatives were synthesized from andrographolide, the cytotoxic constituent of the plant *Andrographis paniculata*. The derived analogs (**4a-4g**) were evaluated for their cytotoxic activity against human small lung cancer (NCI-H187), leukemia K562, breast cancer (MCF-7/ADR) and lung adenocarcinoma (A549) cell lines. Most of the analogues show significant cytotoxic activity against tested cell lines. The methyl malonyl derivative **4a** had higher activity than parent compound andrographolide **1**, and reduced activity than standard drug cisplatin against tested cell lines**.**

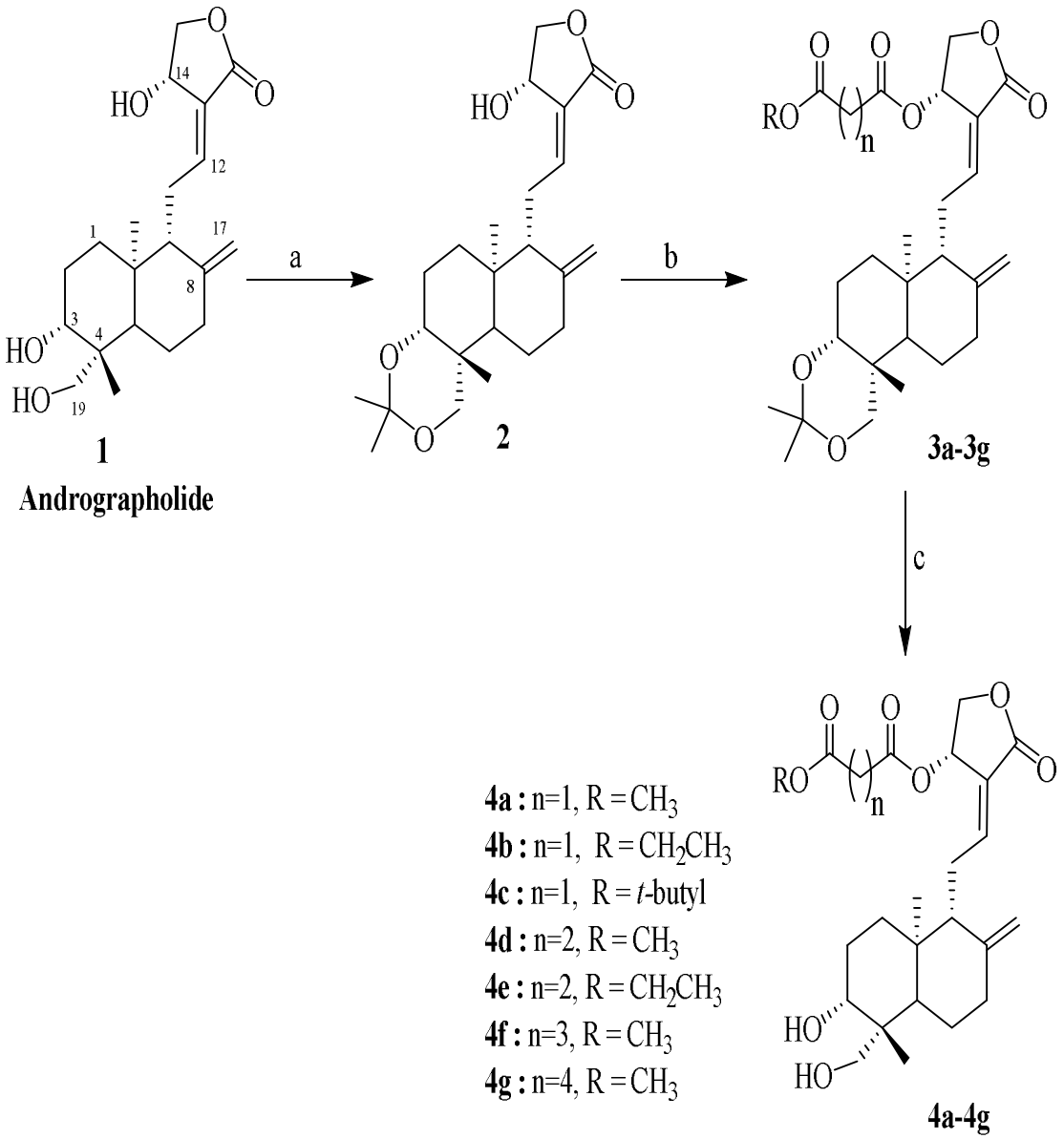
Keywords: Andrographolide, *Andrographis paniculata,* cytotoxic activity.

INTRODUCTION

The labdane diterpenoid andrographolide (**1**) isolated from the whole plant of *Andrographis paniculata* (family Acanthaceae), is extensively used in the traditional system of medicine in south east Asia since antiquity.[1] Extracts of plants and their constituents including androgarpholide (**1)** have been reported to exhibit a wide range of biological activities[2-18] of therapeutic importance that include anti-inflammatory, hepatoprotective, antimalarial, antibacterial, antithrombotic, immune stimulant, antidepressive, antiallergic, central nervous system disorders, anti HIV, and anticancer. Since its discovery of plethora of activities, a large number of andrographolide (**1**) analogs have been prepared by semi-synthesis for the modification of the biological activities which are available in the literature.[19-28] Presuming that incorporation of dicarboxylic esters at C-14 in andrographolide might generate some bioactive molecules, herein, we report the synthesis of a new series of dicarboxylic ester andrographolide derivatives and their cytotoxic activity against human small lung cancer (NCI-H187), leukemia K562, breast cancer (MCF-7/ADR) and lung adenocarcinoma (A549) cell lines.

Chemistry

Andrographolide (**1**) was isolated in high yields from the plant of *Andrographis paniculata* and used as the starting material for the preparation of the C(14)-modified alkoxy analogue library **4a-4g** (Scheme 1). Initially, Andrographolide **1** was treated with 2, 2-dimethoxy propane in the presence of pyridinium *p*-toulenesulfonate (PPTS) in CH_2Cl_2 at 40^{o}C to yield 87% of compound **2**. Compound **2** was treated with appropriate acid halides in the presence of diisopropylethyl amine base in DCM to give compounds **3a-3g**. Derivatives **4a-4g** were prepared in yields of 69-73% by reacting compounds **3a-3g** with acetic acid in water to remove isopropylidene (Scheme 1).



Scheme. 1: Synthesis of dicarboxylic ester-type andrographolide analogs 4a-4i. Reagents and conditions: (a) 2,2-dimethoxypropane, PPTS, DCM, reflux at 40°C, 1h; (b) appropriate acid chloride, Et_3N, dry DCM, N_2, r.t, 3-4 h; (c) Acetic acid, H_2O, r.t, 30 min

Biological activity

Andrographolide (**1**) and its dicarboxylic ester type analogs (**4a-4g)** were evaluated for their *in vitro* cytotoxic activity against human small lung cancer (NCI-H187), leukemia K562, breast cancer (MCF-7/ADR) and lung adenocarcinoma (A549) cell lines. The *in vitro* cytotoxic activity assays were conducted using classical MTT method.[29] The cytotoxicity data of **1** and its analogs are collated in Table 1. For comparison purpose, IC_{50} values of positive control, cisplatin against cell lines are included in the Table 1. Most of the synthesized dicarboxylic ester derivatives showed appreciable cytotoxic activity compared to the parent compound Andrographolide **1** against tested cell lines. Analogs **4a** and **4b** have also shown potent activity than the standard cisplatin and parent compound Andrographolide **1**.

Table 1: Cytotoxicity effects of C(14)-dicarboxylic ester-derived andrographolide analogues (4a-4g) against cancer cell lines

	Cell lines (IC_{50} µM)[a]			
Compound	**NCI-H187**	**K562**	**MCF-7/ADR**	**A549**
1	17.85±3.50	16.18±3.35	13.82±2.56	4.17±1.15
4a	6.24±1.65[b]	5.97±2.20	11.30±3.45	3.98±1.63
4b	10.83±2.17	12.98±1.85	15.63±3.64	7.50±2.19
4c	>130	76.55±12.75	>165	NT
4d	11.15±2.30	13.90±2.55	22.85±5.45	7.96±1.85
4e	16.20±4.30	15.76±5.36	29.74±4.94	8.95±2.73
4f	29.56±6.85	33.85±7.50	23.80±6.50	11.85±3.20
4g	44.85±7.85	51.18±8.80	36.54±5.45	17.65±4.60
Cisplatin[c]	2.79±0.50	3.76±0.85	9.55±1.25	0.86±0.35

[a] Concentration of compound required to inhibit cell growth by 50% as determined by MTT assay;
[b]data are expressed as mean±standard deviation; [c]Cisplatin was used as positive control;
NA- not active; NT- not tested;

As demonstrated in table 1, among all derivatives methyl malonyl derivative **4a** and ethyl malonyl analog **4b** have significant cytotoxic activity against tested cell lines. The methyl malonyl derivative **4a** had higher activity than parent compound andrographolide **1** (IC_{50}= 6.26 *vs* 17.85 µM against NCI-H187; 5.97 *vs* 16.18 µM against K562; 11.30 *vs* 13.82 µM against MCF-7; 3.98 *vs* 4.17 µM against A549 respectively), and reduced activity than standard drug cisplatin against tested cell lines (IC_{50}= 6.24 *vs* 2.79 µM against NCI-H187; 5.97 *vs* 3.76 µM against K562; 11.30 *vs* 9.55 µM against MCF-7; 3.98 *vs* 0.86 µM against A549 respectively) (Table 1). The ethyl malonyl derivative **4b** had higher activity than **1** against NCI-H187 and K562 cell lines (IC_{50}= 10.83 *vs* 17.85 µM; 12.98 *vs* 16.18 µM respectively) (Table 1), and reduced activity than cisplatin. Similarly, the methyl succinyl derivative **4d** had higher activity than **1** against NCI-H187 and K562 cell lines (IC_{50}= 11.15 *vs* 17.85 µM; 13.90 *vs* 16.18 µM respectively); and also ethyl succinyl derivative **4e** had higher activity than **1** against NCI-H187 and K562 cell lines (IC_{50}= 16.20 *vs* 17.85 µM; 15.76 *vs* 16.18 µM respectively) (Table 1). Compounds **4f** and **4g** have reduced activity than standard cisplatin, but still show appreciable activity compared to the parent andrographolide **1** (Table 1); this reducing activity against cell lines may be due to increase of chain length between two carbonyls in their structures at C-17 position. Analog **4c** had no activity against tested cell lines; presence of bulkier group (*t*-butyl in analog **4c**) may reduce the cytotoxic activity.

In summary, a series of new dicarboxylic ester-type analogs of andrographolide were synthesized in an effort to explore the cytotoxic effects of C-14 substitution against human small lung cancer (NCI-H187), leukemia K562, breast cancer (MCF-7/ADR) and lung adenocarcinoma (A549) cell lines. Most of the analogs showed significant cytotoxic activity against tested cell lines compared to the parent andrographolide. Analogs methyl malonyl derivative **4a** and ethyl malonyl derivative **4b** have higher activity than parent compound andrographolide against NCI-H187, K562 and MCF-7 cell lines.

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^{1}H-NMR, ^{13}C-NMR and MS data for all products

Methyl andrographolide-14-*O*-malonate (4a)

White amorphous powder, ^{1}H NMR (400 MHz, CDC_{l3}): δ 7.04 (t, *J* = 6.8 Hz, 1H), 5.98 (d, *J* = 5.8 Hz, 1H), 4.91 (s, 1H), 4.57-4.51 (m, 2H), 4.24-4.15 (m, 2H), 3.91 (d, *J* = 11.6 Hz, 1H), 3.71(s, 3H), 3.51-3.48 (m, 1H), 3.31 (d, *J* = 10.6 Hz, 1H), 3.19 (s, 2H), 2.51-2.31 (m, 4H), 1.99-1.94 (m, 1H), 1.80-1.71 (m, 5H), 1.32-1.15 (m, 6H), 0.69 (s, 3H). ^{13}C NMR (100 MHz, CDC_{l3}): δ 174.9, 168.6, 165.1, 152.2, 148.6, 124.2, 109.1, 80.9, 72.6, 70.3, 63.9, 62.1, 57.2, 55.9, 52.6, 43.9, 39.9, 38.2, 37.2, 29.4, 26.3, 25.7, 23.4, 16.1. HRESIMS *(m/z)*: $[M+H]^+$ calculated for $C_{24}H_{34}O_8$, 451.2287; found, 451.2274.

Ethyl andrographolide-14-*O*-malonate (4b)

White amorphous powder, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.03 (t, *J* = 6.8 Hz, 1H), 5.99 (d, *J* = 5.8 Hz, 1H), 4.90 (s, 1H), 4.57-4.52 (m, 2H), 4.26-4.11 (m, 4H), 3.92 (d, *J* = 11.6 Hz, 1H), 3.51-3.46 (m, 1H), 3.32 (d, *J* = 10.6 Hz, 1H), 3.19 (s, 2H), 2.51-2.31 (m, 4H), 1.99-1.94 (m, 1H), 1.79-1.71 (m, 5H), 1.34-1.12 (m, 9H),

0.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.1, 169.7, 165.3, 152.9, 148.7, 124.5, 109.2, 80.8, 72.8, 70.4, 63.7, 61.3, 58.2, 55.7, 52.3, 43.8, 39.8, 38.1, 37.3, 29.5, 26.4, 25.3, 23.8, 14.6, 16.4. HRESIMS (*m/z*): $[M+H]^+$ calculated for $C_{25}H_{36}O_8$, 465.2444; found, 465.2437.

_t_-Butyl andrographolide-14-_O_-malonate (4c)
White amorphous powder, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.03 (t, *J* = 6.8 Hz, 1H), 5.96 (d, *J* = 5.8 Hz, 1H), 4.90 (s, 1H), 4.57-4.52 (m, 2H), 4.26-4.11 (m, 4H), 3.92 (d, *J* = 11.6 Hz, 1H), 3.51-3.46 (m, 1H), 3.32 (d, *J* = 10.6 Hz, 1H), 3.21 (s, 3H), 2.51-2.31 (m, 4H), 1.99-1.94 (m, 1H), 1.79-1.71 (m, 5H), 1.36 (s, 9H), 1.34-1.12 (m, 9H), 0.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.8, 169.3, 164.9, 152.2, 148.1, 124.4, 109.1, 82.3, 80.8, 72.9, 70.6, 63.6, 58.1, 55.6, 52.3, 43.8, 39.9, 38.2, 37.4, 28.9 (3×*t*-$\underline{C}H_3$), 29.4, 26.4, 25.3, 23.6, 16.8. HRESIMS (*m/z*): $[M+H]^+$ calculated for $C_{27}H_{40}O_8$, 493.2797; found, 493.2789.

Methyl andrographolide-14-_O_-succinate (4d)
White amorphous powder, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.03 (t, *J* = 6.8 Hz, 1H), 5.96 (d, *J* = 5.8 Hz, 1H), 4.90 (s, 1H), 4.57-4.52 (m, 2H), 4.26-4.11 (m, 4H), 3.92 (d, *J* = 11.6 Hz, 1H), 3.67 (s, 3H), 3.51-3.46 (m, 1H), 3.32 (d, *J* = 10.6 Hz, 1H), 2.84-2.69 (m, 4H), 2.51-2.31 (m, 4H), 1.99-1.94 (m, 1H), 1.79-1.71 (m, 5H), 1.36 (s, 9H), 1.34-1.12 (m, 9H), 0.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.1, 169.1, 165.3, 151.9, 148.9, 123.3, 108.9, 80.7, 72.5, 70.2, 63.8, 62.2, 57.3, 55.8, 51.8, 43.8, 39.8, 38.2, 37.1, 29.5, 29.2, 26.4, 25.4, 23.6, 16.3. HRESIMS (*m/z*): $[M+H]^+$ calculated for $C_{25}H_{36}O_8$, 465.2444; found, 465.2438.

Ethyl andrographolide-14-_O_-succinate (4e)
White amorphous powder, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.03 (t, *J* = 6.8 Hz, 1H), 5.96 (d, *J* = 5.8 Hz, 1H), 4.90 (s, 1H), 4.57-4.52 (m, 2H), 4.26-4.09 (m, 6H), 3.92 (d, *J* = 11.6 Hz, 1H), 3.67 (s, 3H), 3.51-3.46 (m, 1H), 3.32 (d, *J* = 10.6 Hz, 1H), 2.83-2.68 (m, 4H), 2.51-2.31 (m, 4H), 1.99-1.94 (m, 1H), 1.79-1.71 (m, 5H), 1.29 (t, 3H), 1.34-1.12 (m, 9H), 0.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.1, 169.1, 165.3, 151.9, 148.9, 123.3, 108.9, 80.7, 72.5, 70.2, 63.8, 61.7, 62.2, 57.3, 55.8, 43.8, 39.8, 38.2, 37.1, 29.6, 29.4, 26.4, 25.4, 23.6, 16.3, 14.1. HRESIMS (*m/z*): $[M+H]^+$ calculated for $C_{26}H_{38}O_8$, 479.2600; found, 479.2595.

Methyl andrographolide-14-_O_-glutarate (4f)
White amorphous powder, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.03 (t, *J* = 6.8 Hz, 1H), 5.96 (d, *J* = 5.8 Hz, 1H), 4.90 (s, 1H), 4.57-4.52 (m, 2H), 4.26-4.09 (m, 6H), 3.92 (d, *J* = 11.6 Hz, 1H), 3.63 (s, 3H), 3.51-3.46 (m, 1H), 3.32 (d, *J* = 10.6 Hz, 1H), 2.83-2.68 (m, 4H), 2.55-2.29 (m, 10H), 1.99-1.94 (m, 1H), 1.79-1.71 (m, 5H), 1.29 (t, 3H), 1.34-1.12 (m, 9H), 0.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.1, 171.1, 168.3, 151.9, 148.9, 123.3, 108.9, 80.7, 72.5, 70.2, 63.8, 62.2, 57.3, 55.8, 51.9, 43.8, 39.8, 38.2, 37.1, 29.5, 29.2, 26.4, 25.4, 33.9, 33.4, 20.1, 16.3. HRESIMS (*m/z*): $[M+H]^+$ calculated for $C_{26}H_{38}O_8$, 479.2602; found, 479.2598.

Methyl andrographolide-14-_O_-adipate (4g)
White amorphous powder, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.03 (t, *J* = 6.8 Hz, 1H), 5.96 (d, *J* = 5.8 Hz, 1H), 4.90 (s, 1H), 4.57-4.52 (m, 2H), 4.26-4.09 (m, 6H), 3.92 (d, *J* = 11.6 Hz, 1H), 3.64 (s, 3H), 3.51-3.46 (m, 1H), 3.32 (d, *J* = 10.6 Hz, 1H), 2.83-2.68 (m, 4H), 2.55-2.29 (m, 10H), 1.99-1.94 (m, 1H), 1.79-1.71 (m, 5H), 1.65-1.61 (m, 4H), 1.29 (t, 3H), 1.34-1.12 (m, 9H), 0.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.1, 171.1, 168.3, 151.9, 148.9, 123.3, 108.9, 80.7, 72.5, 70.2, 61.9, 62.2, 57.3, 55.8, 43.8, 39.8, 38.2, 37.1, 29.5, 29.2, 26.4, 25.4, 34.4, 34.1, 24.3, 24.1, 16.3. HRESIMS (*m/z*): $[M+H]^+$ calculated for $C_{27}H_{40}O_8$, 493.2757; found, 493.2747.

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