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Research Article

DESIGN, SYNTHESIS OF NEW C(14)-ANDROGRAPHOLIDE ANALOGUES AND THEIR CYTOTOXIC ACTIVITY

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ABSTRACT: A new series of andrographolide analogues were synthesized from andrographolide, the cytotoxic constituent of the plant *Andrographis paniculata*. The derived analogs (**4a-4e**) were evaluated for their cytotoxic activity against lung cancer (H522), leukemia K562, breast cancer (MCF-7/ADR) and prostate cancer (DU145) cell lines. Most of the analogues display significant cytotoxic activity against tested cell lines. The allyl derivative **4c** had higher activity than parent compound andrographolide **1**, and standard drug cisplatin against tested cell lines**.**

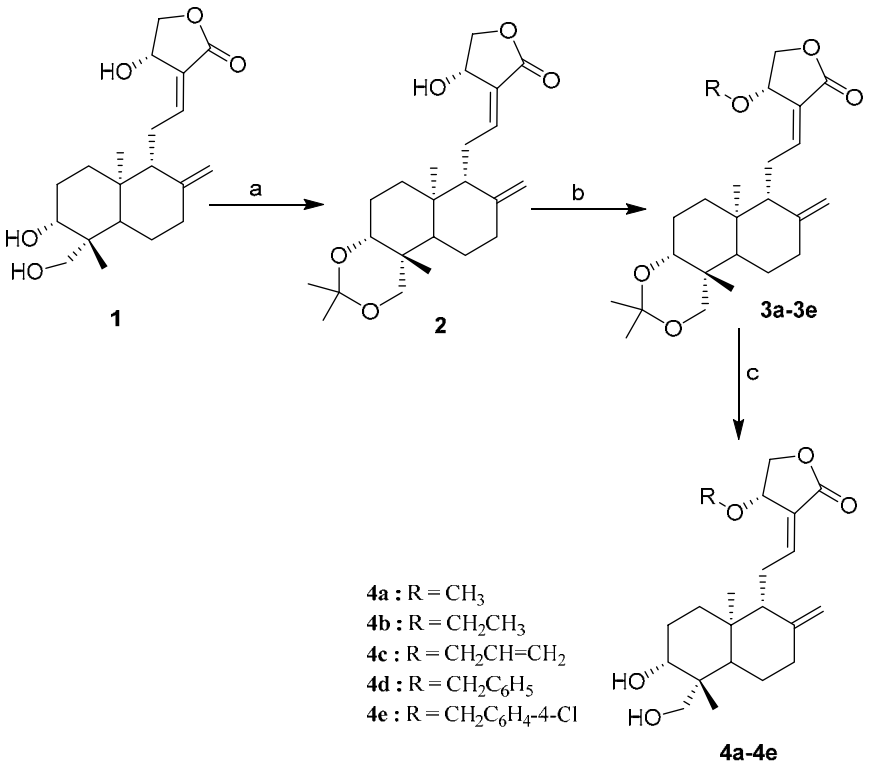
Key words: Andrographolide, *Andrographis paniculata,* cytotoxic activity, Andrographolide analogues.

INTRODUCTION

Natural products play an important role in drug discovery, serving as either a source or motivation for approximately half of all approved small-molecule drugs (Newman *et al*., 2012). Although a large number of these drugs are naturally occurring substances, derivatives of natural products are often essential to improve pharmacokinetic properties. These derivatives have traditionally been accessed through total synthesis and mutasynthesis (Wender *et al*., 2002). In cases where the natural product is readily available from the natural source, semi-synthesis is an attractive approach. Due to the large and often complex scaffolds nature develops, semi-synthesis requires highly selective transformations. Perhaps the best example of natural product derivatives that have been developed into drugs for the treatment of plethora of biological activities is andrographolide. Andrographolide (**1**) is a labdane diterpenoid, isolated from the whole plant of *Andrographis paniculata* (family Acanthaceae), it is extensively used in the traditional system of medicine in south east Asia since antiquity (Chakravarti *et al*., 1951). Extracts of plants and their phytochemical constituents together with andrographolide (**1)** have been reported to display a broad range of biological activities of therapeutic importance that include antimalarial (Najib *et al*., 1999, Li *et* al., 2007), antibacterial (Li *et al*., 2007), anti-inflammatory (Shen *et al*., 2002, Madav *et al*., 1995, Shen *et al*., 2000, Reddy *et al*., 2008, Salaga *et al.,* 2014), hepatoprotective (Handa *et al.,* 1990), antithrombotic (Li *et al*., 2007), immune stimulant (Kumar *et al*., 2004), antidepressive (White *et al*., 2014), antiallergic (Gupta *et al*., 1998), central nervous system disorders (White *et al*., 2014, Fajemiroye *et al.,* 2014, Polepally *et al*., 2013, Prabhakar *et al.,* 2014, Zjawiony *et al*., 2011), anti HIV (Li *et al*., 2007, Raju *et al*., 2008), and anticancer (Kumar *et al*., 2004, Nanduri *et al*., 2004). Andrographolide (**1**) has also been widely used in clinics for the treatment of fever, cold, inflammation, diarrhea and infectious diseases, so it has aroused the interest of pharmacologists. 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 (He *et* al., 2003, Li *et al*., 2006, Nanduri *et al*., 2004). Presuming that incorporation of alkoxy at C-14 in andrographolide might generate some bioactive molecules, herein, we report the synthesis of a new series of alkoxy andrographolide derivatives and their cytotoxic activity against lung cancer (H522), leukemia K562, breast cancer (MCF-7/ADR) and prostate cancer (DU145) 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-4e** (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^oC to yield 87% of compound **2**.



Scheme 1. Synthesis of alkoxy-type andrographolide analogs (**4a-4e**). Reagents and conditions: (a) 2,2-dimethoxypropane, PPTS, DCM, reflux at 40°C, 1h; (b) appropriate alkyl halide, Et_3N, CBr_4, dry DCM, N_2, r.t, 3-4 h; (c) Acetic acid, H_2O, r.t, 30 min.

Compound **2** was treated with appropriate acid halides in the presence of diisopropylethyl amine base in DCM to give compounds **3a-3e**. Derivatives **4a-4e** were prepared in yields of 69-73% by reacting compounds **3a-3e** with acetic acid in water to remove isopropylidene (Scheme 1).

Biological activity:

Andrographolide (**1**) and its dicarboxylic ester type analogs (**4a-4e)** were evaluated for their *in vitro* cytotoxic activity against lung cancer (H522), leukemia K562, breast cancer (MCF-7/ADR) and prostate cancer (DU145) cell lines. The *in vitro* cytotoxic activity assays were conducted using classical MTT method (Anne *et al*., 1991). 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 alkoxy andrographolide derivatives showed appreciable cytotoxic activity compared to the parent compound Andrographolide **1** against tested cell lines. Analogs **4c** had shown potent activity than the standard cisplatin and parent compound Andrographolide **1**.

As demonstrated in table 1, among all derivatives allyloxy derivative **4c** had significant cytotoxic activity against tested cell lines. The allyl derivative **4c** had higher activity than parent compound andrographolide **1** (IC_{50}= 4.35 *vs* 17.85 µM against H522; 3.98 *vs* 16.15 µM against K562; 10.23 *vs* 13.82 µM against MCF-7; 5.50 *vs* 8.17 µM against DU145 respectively), and significant activity than standard drug cisplatin against tested cell lines (IC_{50}= 4.35 *vs* 4.74 µM against H522; 3.98 *vs* 3.76 µM against K562; 10.23 *vs* 9.55 µM against MCF-7; 5.50 *vs* 5.54 µM against DU145 respectively) (Table 1). The methoxy derivative **4a** had higher activity than parent compound andrographolide against H522, K562 and MCF-7 cell lines (IC_{50}= 7.56 *vs* 17.85 µM; 9.55 *vs* 16.15 µM; 8.30 *vs* 13.82 µM respectively) (Table 1), and reduced activity than cispatin.

Similarly, The ethoxy derivative **4b** also had higher activity than parent compound andrographolide against H522, K562 and MCF-7 cell lines (IC_{50}= 7.56 *vs* 17.85 µM; 9.55 *vs* 16.15 µM; 8.30 *vs* 13.82 µM respectively) (Table 1), and reduced activity than cisplatin (Table 1). Compounds **4e** and **4f** 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 presence of bulkier phenyl ring in their structures at C-14 position.

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 lung cancer (H522), leukemia K562, breast cancer (MCF-7/ADR) and prostate cancer (DU145) cell lines. All the synthesized analogs showed significant cytotoxic activity against tested cell lines compared to the parent andrographolide. Analogs allyl derivative **4c** had higher activity than parent compound andrographolide and standard cisplatin against H522, K562, MCF-7 and DU145 cell lines.

Table 1. Cytotoxicity effects of C(14)- alkoxy derived andrographolide analogues (4a-4e) against cancer cell lines

Cell lines (IC_{50} μM)[a]				
Compound	H522	K562	MCF-7/ADR	DU145
1	17.85±3.50	16.15±3.35	13.82±2.56	8.17±1.15
4a	7.56±2.14[b]	9.55±2.95	8.30±2.75	10.56±2.75
4b	9.85±2.45	11.98±2.85	10.65±3.65	17.50±2.89
4c	4.35±1.45	3.98±2.12	10.23±2.65	5.50±2.75
4d	20.15±3.30	15.90±3.55	23.85±5.45	10.96±2.85
4e	16.20±4.30	15.76±5.36	29.74±4.94	8.95±2.73
cisplatin[c]	4.74±0.50	3.76±0.85	9.55±1.25	5.54±1.35

[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;

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

Methyl-14-*O*-andrographolide **(4a)**. White amorphous powder, ^{1}H NMR (400 MHz, CDC_{l3}): δ 7.02 (t, *J* = 6.8 Hz, 1H), 5.93 (d, *J* = 5.8 Hz, 1H), 4.92 (s, 1H), 4.55-4.49 (m, 2H), 4.23-4.14 (m, 2H), 3.89 (d, *J* = 11.6 Hz, 1H), 3.53-3.47 (m, 1H), 3.32 (d, *J* = 10.6 Hz, 1H), 3.29 (s, 3H), 2.52-2.32 (m, 4H), 1.98-1.92 (m, 1H), 1.81-1.69 (m, 5H), 1.23-1.15 (m, 6H), 0.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.5, 153.4, 148.7, 124.3, 109.3, 80.8, 72.8, 70.4, 63.9, 62.1, 57.1, 55.9, 52.6, 43.9, 39.9, 38.2, 37.2, 29.4, 26.3, 25.7, 23.4, 16.1. ESIMS *(m/z)*: $[M+H]^+$ calculated for $C_{21}H_{32}O_5$, 365.29; found, 365.31.

Ethyl-14-*O*-andrographolide (**4b**). White amorphous powder, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.04 (t, *J* = 6.8 Hz, 1H), 5.95 (d, *J* = 5.8 Hz, 1H), 4.93 (s, 1H), 4.53-4.48 (m, 2H), 4.21-4.13 (m, 2H), 3.87 (d, *J* = 11.6 Hz, 1H), 3.54-3.45 (m, 1H), 3.45 (q, 2H), 3.31 (d, *J* = 10.6 Hz, 1H), 2.51-2.31 (m, 4H), 1.97-1.92 (m, 1H), 1.80-1.69 (m, 5H), 1.21-1.13 (m, 6H), 1.11 (t, 3H), 0.72 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.5, 153.4, 148.7, 124.3, 109.3, 80.8, 72.8, 70.4, 66.1, 63.9, 62.1, 55.9, 52.6, 43.9, 39.9, 38.2, 37.2, 29.4, 26.3, 25.7, 23.4, 16.1, 15.3. ESIMS *(m/z)*: $[M+H]^+$ calculated for $C_{22}H_{34}O_5$, 379.24; found, 379.18.

Allyl-14-*O*-andrographolide (**4c**). White amorphous powder, ^{1}H NMR (400 MHz, $CDCl_3$): , ^{1}H NMR (400 MHz, CDC_{l3}): δ 7.03 (t, *J* = 6.8 Hz, 1H), 6.12 (m, 1H (olefin proton)), 5.91 (d, *J* = 5.8 Hz, 1H), 5.46 (d, *J* = 12.6 Hz, 1H), 5.32 (d, *J* = 6.2 Hz, 1H), 4.91 (s, 1H), 4.55-4.49 (m, 2H), 4.23-4.04 (m, 4H), 3.89 (d, *J* = 11.6 Hz, 1H), 3.53-3.47 (m, 1H), 3.32 (d, *J* = 10.6 Hz, 1H), 2.52-2.32 (m, 4H), 1.98-1.92 (m, 1H), 1.81-1.69 (m, 5H), 1.23-1.15 (m, 6H), 0.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.5, 153.4, 148.7, 134.8, 124.3, 117.3, 109.3, 80.8, 72.8, 72.1, 70.4, 63.9, 62.1, 55.9, 52.6, 43.9, 39.9, 38.2, 37.2, 29.4, 26.3, 25.7, 23.4, 16.1. ESIMS *(m/z)*: $[M+H]^+$ calculated for $C_{23}H_{34}O_5$, 391.24; found, 391.19.

Benzyl-14-*O*-andrographolide (**4d**). White amorphous powder, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29-7.26 (m, 5H), 7.03 (t, *J* = 6.8 Hz, 1H), 5.93 (d, *J* = 5.8 Hz, 1H), 4.92 (s, 1H), 4.63 (s, 2H), 4.55-4.49 (m, 2H), 4.23-4.14 (m, 2H), 3.89 (d, *J* = 11.6 Hz, 1H), 3.53-3.47 (m, 1H), 3.32 (d, *J* = 10.6 Hz, 1H), 2.52-2.32 (m, 4H), 1.98-1.92 (m, 1H), 1.81-1.69 (m, 5H), 1.23-1.15 (m, 6H), 0.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.5, 153.4, 148.7, 137.5, 129.6, 128.6, 127.5, 124.3, 109.3, 80.8, 72.8, 72.5, 70.4, 63.9, 62.1, 55.9, 52.6, 43.9, 39.9, 38.2, 37.2, 29.4, 26.3, 25.7, 23.4, 16.1. ESIMS *(m/z)*: $[M+H]^+$ calculated for $C_{27}H_{36}O_5$, 441.26; found, 441.29.

Para-Chlorobenzyl-14-*O*-andrographolide (**4e**). White amorphous powder, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39 (d, *J* = 7.6 Hz, 2H), 7.22 (d, *J* = 7.8 Hz, 2H), 7.02 (t, *J* = 6.8 Hz, 1H), 5.93 (d, *J* = 5.8 Hz, 1H), 4.69 (s, 2H), 4.92 (s, 1H), 4.55-4.49 (m, 2H), 4.23-4.14 (m, 2H), 3.89 (d, *J* = 11.6 Hz, 1H), 3.53-3.47 (m, 1H), 3.32 (d, *J* = 10.6 Hz, 1H), 2.52-2.32 (m, 4H), 1.98-1.92 (m, 1H), 1.81-1.69 (m, 5H), 1.23-1.15 (m, 6H), 0.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.5, 153.4, 148.7, 133.4, 128.7, 125.3, 124.3, 109.3, 80.8, 72.8, 70.4, 63.9, 62.1, 57.1, 55.9, 52.6, 43.9, 39.9, 38.2, 37.2, 29.4, 26.3, 25.7, 23.4, 16.1. ESIMS *(m/z)*: $[M+H]^+$ calculated for $C_{27}H_{35}O_5Cl$, 476.21; found, 476.29.

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