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RESEARCH ARTICLE

SEMISYNTHESIS OF NEW ANDROGRAPHOLIDE DERIVATIVESWITH CYTOTOXIC ACTIVITY

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ABSTRACT

Andrographolide is a major metabolite of the ayurvedic medicinal plant of *Andrographis paniculata*. In an effort to develop potent anti-cancer drugs, a new series of andrographolide derivatives (4a-4e) were designed, synthesized, and evaluated for their *cytotoxic* activity against lung cancer, leukemia, breast cancer and prostate cancer cell lines. The screening results revealed that the analogues display significant cytotoxic activity against tested cell lines. The dimethylacryloyl derivative 4c had higher activity than parent compound andrographolide1, and standard drug cisplatin against tested cell lines.

INTRODUCTION

Andrographolide (**1**) is a bicyclic diterpenoid lactone, is the major bioactive compound of the ayurvedic medicinal plant of *Andrographis paniculata* (family Acanthaceae). Itis extensively used in the traditional system of medicine in India, China, Japan and other Asian countries. Extracts of medicinal plants and their phytochemical constituents especially diterpenoids and their derivatives have been reported to display a broad range of biological activities of therapeutic importance that include antimalarial (Gupta *et al,* 1990; Najib *et al*., 1999; Li *et al*., 2007; Polepally *et al*., 2012, 2013, 2014), antibacterial (Li *et al*., 2007), anti-inflammatory, antioxidant, anticancer (Husen *et al.,* 2004; Shen *et al*., 2002; Rao *et al.,*2009, 2012; Madav *et al*., 1995; Shen *et al*., 2000; Reddy *et al*., 2008, 2009, 2010; Suresh *et al.,* 2010; 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, 2015; Fajemiroye *et al.,* 2014; Polepally *et al*., 2012, 2013, 2014; Salga *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; Reddy *et al*., 2009). Andrographolide (**1**) has also been widely used in clinics for the treatment of fever, respiratory infection, antihyperglycaemia, inflammation, diarrhea and infectious diseases, so it has aroused the interest of pharmacologists. Since its discovery of plethora of activities, a huge number of andrographolide (**1**) derivatives 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 insertion of michaelacceptor-type of groups at C-14 in andrographolide might generate significant bioactive molecules, herein, we report the synthesis of a new series of andrographolide derivatives and their cytotoxic activity against lung cancer (H522), leukemia K562, breast cancer (MCF-7/ADR) and prostate cancer (DU145) cell lines.

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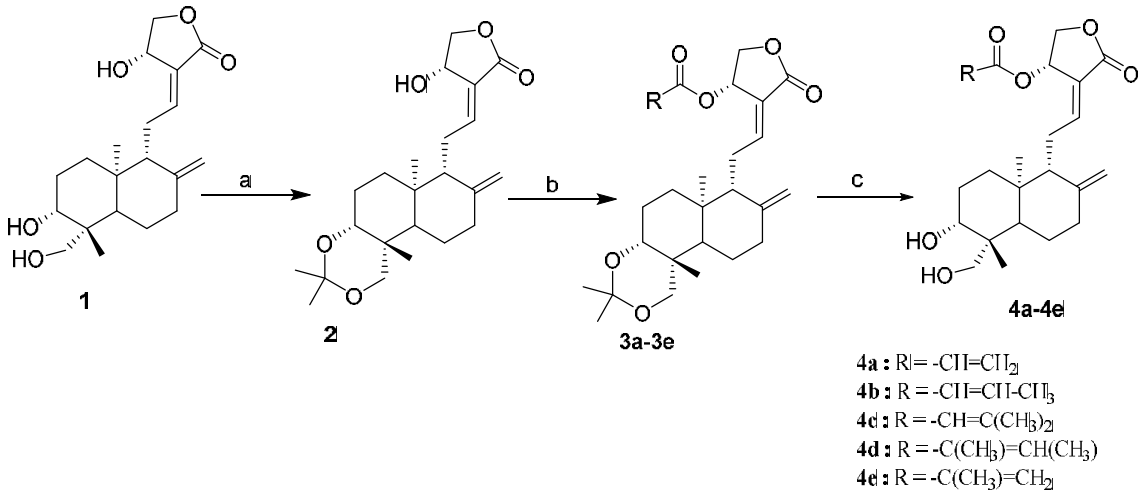
RESULTS AND DISCUSSION

Andrographolide (1) was isolated in great yields from the leaves of *Andrographis paniculata* and used as the starting material for the preparation of the C(14)-modified 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°C to yield 90% of compound 2.

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



Scheme 1. Synthesis of andrographolide analogs (4a-4e). Reagents and conditions: (a) 2,2-dimethoxypropane, PPTS, DCM, reflux at 40°C, 1h; (b) appropriate acid halide, Et_3N, CBr_4, dry DCM, N_2, r.t, 4-6 h; (c) Acetic acid, H_2O, r.t, 30 min

Biological activity

Andrographolide (1) and its derivatives (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 andrographolide derivatives showed appreciable cytotoxic activity compared to the parent compound Andrographolide1 against tested cell lines. Analog4chad shown potent activity than the standard cisplatin and parent compound Andrographolide1. As demonstrated in table 1, among all derivatives allyloxy derivative 4chad significant cytotoxic activity against tested cell lines. The allyl derivative 4chad higher activity than parent compound andrographolide1 (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 4ahad 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.

Table 1. Cytotoxicity effects of C(14)- 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;

Similarly, The crotonyl derivative 4balsohad 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 4eand 4fhave reduced activity than standard cisplatin, but still show appreciable activity compared to the parent andrographolide1 (Table 1); this reducing activity against cell lines may be due to presence of methyl group at α-position in their structures at C-14 position. In summary, a series of new Michaelacceptor-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 dimethyl acryloyl derivative 4c had higher activity than parent compound andrographolide and standard cisplatin against H522, K562, MCF-7 and DU145 cell lines.

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

Derivative (**4a**). White amorphous powder, ^{1}H NMR (400 MHz, CDC_{l3}): δ 7.11 (t, *J* = 6.8 Hz, 1H), 5.95 (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.

Derivative (**4b**). White amorphous powder, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.09 (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.

Derivative (**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.

Derivative (**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.

Derivative (**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.

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