NOVEL ANDROGRAPHOLIDE DERIVATIVES AND THEIR IN VITRO CYTOTOXIC ACTIVITY

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Abstract: A new series of sulfonyl-type 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 sulfonyl derivative 4a had higher activity than parent compound andrographolide **1**, and reduced activity than standard drug cisplatin against tested cell lines**.**

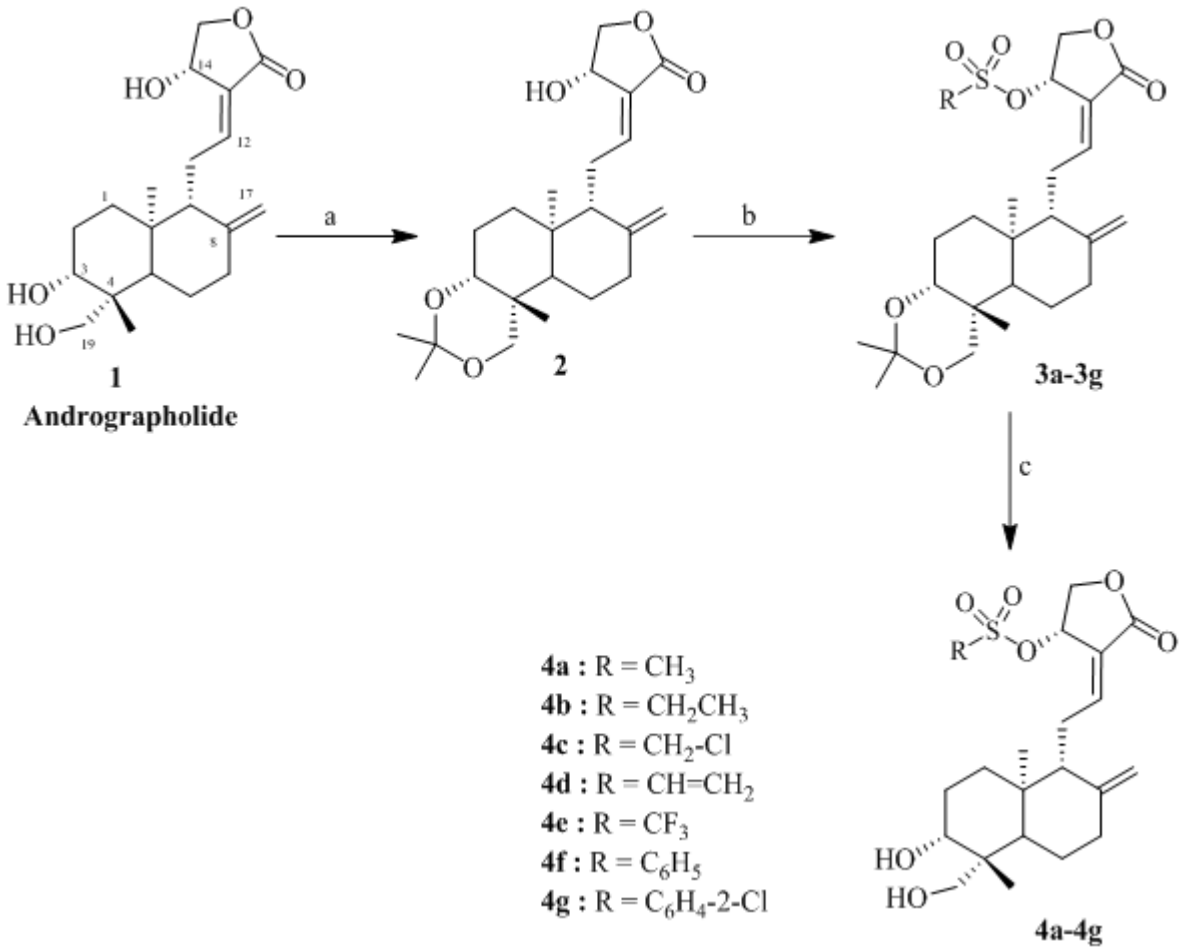
Key words: Andrographolide, *Andrographis paniculata,* cytotoxic activity, sulfonyl type of analogues.

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 andrographolide (**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 sulfonyl esters at C-14 in andrographolide might generate some bioactive molecules, herein, we report the synthesis of a new series of sulfonyl 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 sulfonyl 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^oC to yield 87% of compound 2. Compound 2 was treated with appropriate sulfonyl 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 sulfonylester-type andrographolide analogs **4a-4i**. Reagents and conditions: (a) 2,2-dimethoxypropane, PPTS, DCM, reflux at 40^{o}C, 1h; (b) appropriate sulfonyl 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 sulfonyl 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 sulfonyl 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)-sulfonyl 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 sulfony derivative 4a and ethyl sulfony analog 4b have significant cytotoxic activity against tested cell lines. The methyl sulfonyl 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 sulfony 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 vinyl sulfonyl 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 trifluoromethyl sulfony 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 the presence of bulkier aromatic ring in their structures at C-14 position. Analog 4c had no activity against tested cell lines; presence of chloro group may reduce the cytotoxic activity.

In summary, a series of new sulfonyl 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 sulfonyl derivative 4a and ethyl sulfonyl 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:

Methylsulfonyl-14-*O*-andrographolide **(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 $C_{21}H_{32}O_7S$, 429.1941; found, 429.1936.

Ethylsulfonyl-14-*O*-andrographolide (**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 $C_{22}H_{34}O_7S$, 443.2154; found, 443.2143.

Chloromethylsulfonyl 14-*O*-andrographolide (**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 $C_{21}H_{31}ClO_7S$, 464.1419; found, 464.1403.

Vinylsulfonyl 14-*O*-andrographolide (**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 $C_{22}H_{32}O_7S$, 441.1913; found, 441.1904.

Trifloromethylsulfonyl-14-*O*-andrographolide (**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 $C_{21}H_{29}F_3O_7S$, 483.1612; found, 483.1608.

Phenylsulfonyl 14-*O*-andrographolide (**4f**). White amorphous powder, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73-7.42 (m, 5H), 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, 134.5, 128.3, 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 $C_{26}H_{34}O_7S$, 491.2132; found, 491.2127.

Ortho-phenylsulfonyl 14-*O*-andrographolide -14-*O*-adipate (**4g**). White amorphous powder, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79-7.46 (m, 4H), 7.02 (t, *J* = 6.8 Hz, 1H), 5.97 (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, 128.1, 126.1, 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 $C_{26}H_{33}ClO_7S$, 526.1645; found, 526.1639.

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