Research Article

Oleanolic acid Derivatives and their Cytotoxic Activity

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ABSTRACT

A new series of C(3) alkoxy-type of oleanolic acid derivatives were synthesized from oleanolic acid, the cytotoxic constituent of the plant *Aerva lanata*. The derived analogs (**2-6**) were evaluated for their cytotoxic activity against human small lung cancer (H522), leukemia K562, breast cancer (MCF-7/ADR) and prostate cancer (DU145) cell lines. Most of the analogues show significant cytotoxic activity against tested cell lines. The methyl sulfonyl derivative **2** had higher activity than parent compound oleanolic acid **1**, and reduced activity than standard drug cisplatin against tested cell lines**.**

Keywords: Oleanolic acid, *Aerva lanata,* cytotoxic activity, alkoxy- type of analogues.

INTRODUCTION

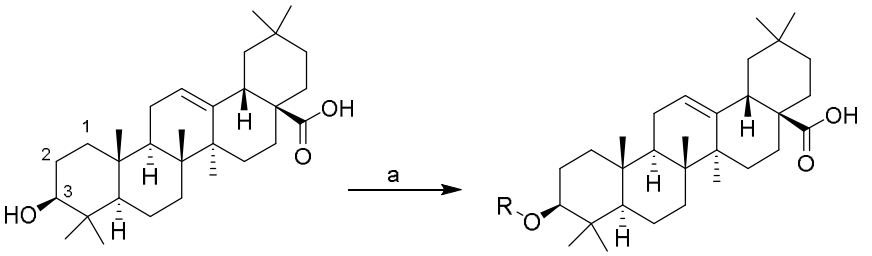
Oleanolic acid is the major mtabolite from the most of indian medicinal plants. Triterpenes, especially oleanolic acid, ursolic acid, and betulinic acid exist in large quantities in the plant kingdom. These triterpenes and their derivatives have been reported to have interesting bioactivity, such as anti-HIV,[1] inhibition of HIV protease[2] and cytotoxicity to tumor cell lines.[3] New interest in the cytotoxic activity of triterpenes has come from the findings that betulinic acid exhibited selective cytotoxicity against melanoma and had apoptosis induction properties.[4] Considerable structural modification has been performed on betulinic acid and potentially important derivatives, which may be developed as anti-tumor drugs, have been produced.[5] Ursolic acid and its esterified derivatives have also been reported to show signiÞcant cytotoxicity against some tumor cell lines.[3] Extracts of plants and their phytochemical constituents have been reported to display a broad range of biological activities of therapeutic importance that include antimalarial, antibacterial, anti-inflammatory, hepatoprotective, antithrombotic, immune stimulant, antidepressive, antiallergic, central nervous system disorders, anti HIV, and anticancer.[7-22] Some oleanane and ursane triterpenoids with modified rings A and C have been reported to have high inhibitory activity against nitric oxide production. This suggests the potential of these compounds as cancer chemopreventive drugs, as excessive production of NO, which is closely related mechanistically to carcinogenesis can destroy functional normal tissues.[6]

However, when compared to ursolic acid derivatives, oleanolic acid derivatives have not been thoroughly explored for their cytotoxic activity. In order to find biologically more active derivatives of this naturally occurring triterpene, we selected *Aerva lanata* extract as the source of these triterpenes. The present article describes the isolation and cytotoxicity of triterpene from *Aerva lanata* and the structural modification of oleanolic acid.

Presuming that incorporation of alkoxy at C-3 in oleanolic acid might generate some bioactive molecules, herein, we report the synthesis of a new series of alkoxy oleanolic acid derivatives and their cytotoxic activity against lung cancer (H522), leukemia K562, breast cancer (MCF-7/ADR) and prostate cancer (DU145) cell lines.

Chemistry

The ethyl acetate extract of the *Aerva lanata* was subjected to column chromatography over Silica gel to obtain oleanolic acid in large quantity. Oleanolic acid structure was confirmed by analyzing and comparing their spectral data with the reported in literature. Oleanolic acid was isolated in high yields from the plant of *Aerva lanata* and used as the starting material for the preparation of the C(3)-modified alkoxy analogue library **2-6** (Scheme 1).



Oleanolic acid (1)

C(3)-oleanolic acid derivatives

2 : R = CH_3
3 : R = CH_2CH_3
4 : R = $CH_2CH{=}CH_2$
5 : R = $CH_2C_6H_5$
6 : R = $CH_2C_6H_4$-4-Cl

Biological activity

Oleanolic acid (**1**) and its alkoxy analogs (**2-6)** 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.[31] 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 oleanolic acid derivatives showed appreciable cytotoxic activity compared to the parent compound oleanolic acid against tested cell lines. Analogs **4** had shown potent activity than the standard cisplatin and parent compound oleanolic acid.

As demonstrated in table 1, among all derivatives allyloxy derivative **4** had significant cytotoxic activity against tested cell lines. The allyl derivative **4** had higher activity than parent compound oleanolic acid **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 **2** had higher activity than parent compound oleanolic acid 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(3)- alkoxyderived oleanolic acid analogues (2-6) 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.

Similarly, the ethoxy derivative **3** also had higher activity than parent compound oleanolic acid 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). Compounds **5** and **6** have reduced activity than standard cisplatin, but still show appreciable activity compared to the parent oleanolic acid (Table 1); this reducing activity against cell lines may be due to presence of bulkier phenyl ring in their structures at C-3 position.

Experimental protocols

Aerva lanata, (3 kg, whole plant) was grinded and extracted with ethyl acetate by supersonication three times (2 h per time). After being concentrated in vacuo, the extract was defatted by petroleum ether (125 ml × 8) under supersonication and the white precipitate was collected to get the triterpene mixture (25 g). The triterpene mixture was separated by silica gel column chromatography, eluted with hexane-dichloroform-acetone to get 4 fractions. Fr. 1-4 was purified with ODS eluted with H_2O-MeOH to get oleanolic acid **1** (500 mg).

NMR data of compound **2**: ^{1}H NMR ($CDCl_3$) δ 0.81 (3H, s), 0.84 (3H, d, *J* = 6.6 Hz), 0.93 (3H, d, *J* = 6.6 Hz), 1.01 (3H, s), 1.03 (3H, s), 1.07 (6H, s), 2.18 (1H, d, *J* = 12.0 Hz), 2.36 (1H, m), 2.50 (1H, m), 5.24 (1H, *t*-like).

NMR data of compound **3**: ^{1}H NMR ($CDCl_3$) δ 0.80 (3H, s), 0.87 (3H, d, *J* = 6.6 Hz), 0.95 (3H, d, *J* = 6.6 Hz), 1.04 (3H, s), 1.05 (3H, s), 1.08 (6H, s), 2.25 (1H, d, *J* = 12.0 Hz,), 2.38 (1H, m), 2.55 (1H, m,), 3.63 (3H, s), 5.28 (1H, *t*-like).

NMR data of compound **4**: ^{1}H NMR ($CDCl_3$): δ 0.78 (6H, s), 0.89 (6H, d, *J* = 6.0 Hz), 0.92 (12H, br.), 0.96 (6H, s), 1.00 (6H, s), 1.11 (6H, s), 2.83 (4H, m), 3.22 (4H, m), 3.48 (2H, m), 5.34 (2H, br), 6.49 (2H, s).

NMR data of compound **5**: ^{1}H NMR ($CDCl_3$) δ 0.81 (3H, s), 0.89 (3H, d, *J* = 6.6 Hz), 0.98 (3H, d, *J* = 6.6 Hz), 1.06 (3H, s), 1.05 (3H, s), 1.08 (6H, s), 2.25 (1H, d, *J* = 12.0 Hz,), 2.38 (1H, m), 2.55 (1H, m,), 3.63 (3H, s), 5.27 (1H, *t*-like).

NMR data of compound **6**: ^{1}H NMR ($CDCl_3$) δ 0.82 (3H, s), 0.89 (3H, d, *J* = 6.6 Hz), 0.93 (3H, d, *J* = 6.6 Hz), 1.01 (3H, s), 1.03 (3H, s), 1.05 (6H, s), 2.25 (1H, d, *J* = 12.0 Hz,), 2.38 (1H, m), 2.55 (1H, m,), 3.63 (3H, s), 5.28 (1H, *t*-like), 7.58-7.42 (5H, m).

CONCLUSION

In summary, a series of new alkoxy-type analogs of oleanolic acid were synthesized in an effort to explore the cytotoxic effects of C-3 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 oleanolic acid. Analogs allyl derivative **4** had higher activity than parent compound oleanolic acid and standard cispatin against H522, K562, MCF-7 and DU145 cell lines.

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