European Journal of Medicinal Chemistry 74 (2014) 398–404

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis of novel ring-A fused hybrids of oleanolic acid with capabilities to arrest cell cycle and induce apoptosis in breast cancer cells

Uppuluri Venkata Mallavadhani [a,*], Nagi Reddy Vanga [a], Manish Kumar Jeengar [b], V.G.M. Naidu [b]

[a] Natural Products Chemistry Division, CSIR-Indian Institute of Chemical Technology, Hyderabad 500007, Andhra Pradesh, India
[b] Department of Pharmacology & Toxicology, National Institute of Pharmaceutical Education & Research, Hyderabad 5000 037, Andhra Pradesh, India

ARTICLE INFO

Article history:
Received 5 November 2013
Received in revised form
29 December 2013
Accepted 31 December 2013
Available online 8 January 2014

Keywords:
Oleanolic acid
Oleanolic acid hybrids
Anti-cancer activity
Apoptosis
Cell cycle arrest

ABSTRACT

Six novel oleanolic acid ring-A fused hybrids (**5**–**10**) have been synthesized by employing a four step protocol with the introduction of benzylidene functionality at C-2 as the key step. Their structures were established by high resolution NMR and Mass spectral data. The synthesized compounds have been screened against seven human cancer cell lines including ME-180 & HeLa (cervix), MCF-7, MDA-MB-453 & MDA-MB-231 (breast), PC-3 (prostate) and HT-29 (colon) using MTT assay. Most significantly, compound **10** showed potent activity against the three breast cancer cell lines. The IC_{50} value (10.60 μM) of compound **10** against MCF-7 found to be much lower than that of the standards and parent compound. Flow cytometric analysis reveals that compound **10** arrests cell cycle in S phase and induces apoptosis in MCF cells.

1. Introduction

Oleanolic acid [3β-hydroxy-olean-12-ene-28-oic acid] (**1**), a natural pentacyclic triterpenic acid, has been found widespread in plant kingdom [1]. It has been in clinical use as an anti-hepatitis drug in China for more than 20 years [2]. In addition to its excellent hepatoprotective effects, oleanolic acid has also been found to exhibit highly potent anti-inflammatory, antitumour, anti-HIV, cardiovascular and glycogen phosphorylase inhibitory activities [3,4]. In view of high natural abundance, significant bio-activities and relatively non-toxic nature, oleanolic acid has become a highly useful lead compound. It has three active sites such as C-3 hydroxyl, ring-C double bond and C-28 carboxylic acid, which are amenable for a wide range of chemical transformations. A number of oleanolic acid analogues have been reported so far by transforming these functionalities. The C-28 carboxylic acid has been utilised to prepare different types of esters, alcohols, amides, nitriles, and triazoles [5,6]. In case of ring-C, variety of functional groups like enones, oximes and amides have been introduced [7]. When it comes to ring-A, simple oxidation or alkylation of the C-3 hydroxyl group has been reported [8,9]. Most of the synthesized analogues have been reported to exhibit newer or enhanced activities than the parent oleanolic acid. Of the various synthesized analogues of oleanolic acid, ring-A modified analogues have been reported to be much more potent than the other analogues. The most important example of this class of analogues is Bardoxolone methyl (CDDO-Methyl ester), which is in advanced stage of clinical development for the treatment of cancer and chronic kidney disease [10]. Ring-A of oleanolic acid is still the attractive site to perform chemical modifications to generate diverse hybrid structures. The 3β-hydroxyl functionality, through its oxidation product, can be utilised to introduce a benzylidene functionality at C-2. The resultant exocyclic enone system can then be exploited to synthesize a range of hybrid structures by condensing with appropriate amino functionalities. This concept is novel and the resultant hybrid structures are expected to exhibit potent biological activities. With this background and in continuation to our research programs on synthesizing triterpenic acid based new chemical entities [6,8,11], we have synthesized some novel 2,3 ring-A fused heterocyclic hybrids of oleanolic acid and screened them against a panel of seven human cancer cell lines. The synthesis, anti-cancer

* Corresponding author. Tel./fax: +91 4027191882.
E-mail addresses: uvmavadani@yahoo.com, mallavadhani@iict.res.in (U.V. Mallavadhani).

0223-5234/$ – see front matter © 2014 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2013.12.040

activity and mode of action of the synthesized compounds are presented in this paper.

2. Results and discussion

2.1. Chemistry

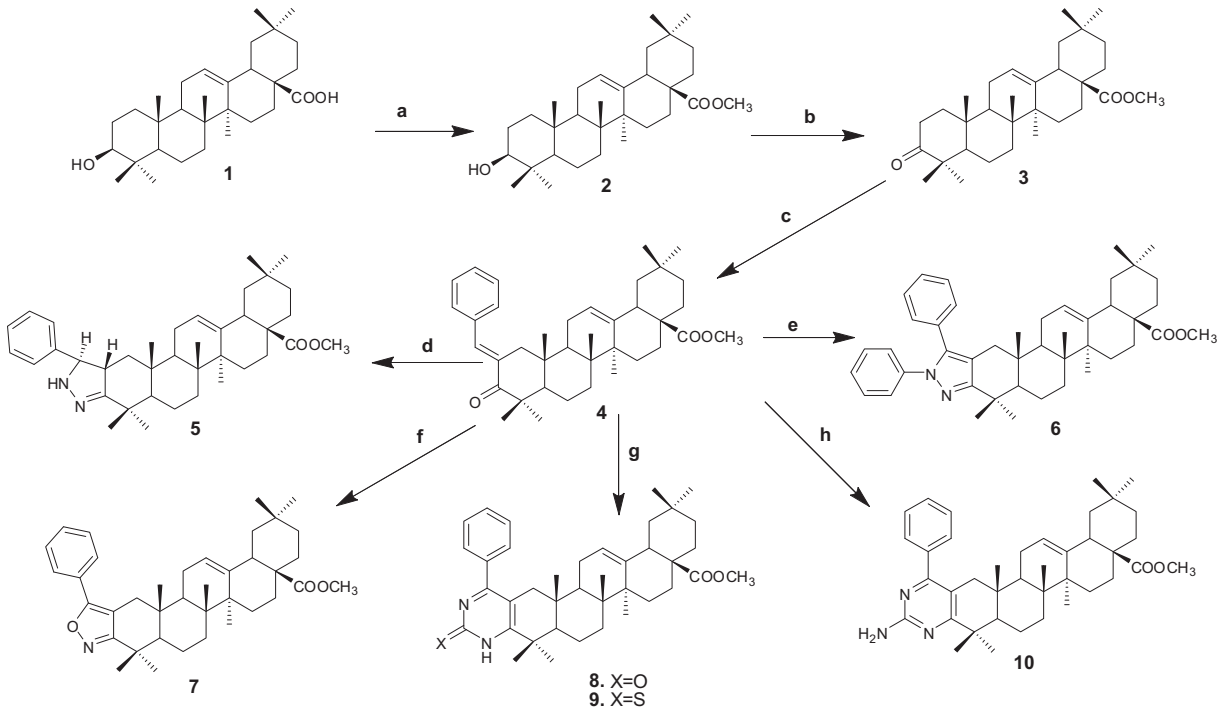
The synthetic protocols employed for the synthesis of the novel hybrid structures are presented in Scheme 1. Oleanolic acid (**1**) was treated with methyl iodide and potassium carbonate in acetone to form compound **2** in 90% yield, which was then oxidized with PCC in dichloromethane to afford compound **3** in 88% yield. The benzylidene compound **4**, which is the key precursor for the synthesis of the title compounds, was prepared by Claisen Schmidt condensation of compound **3** with benzaldehyde in presence of ethanolic potassium hydroxide in 92% yield. Compound **4** showed a sharp peak at 1677 cm^{-1} in IR spectrum indicating the presence of α,β unsaturated ketone functionality, which was further confirmed by the presence of olefinic proton signal at δ 7.53 in ^1H NMR and carbonyl & olefinic carbon signals at δ 207.80, 137.38 & 133.66 in ^{13}C NMR spectra. This benzylidene compound (**4**) with an exocyclic enone system is a versatile precursor. It can form fused heterocyclic ring systems such as pyrazolines, pyrimidines, isoxazoles on treatment with dinucleophiles. With this objective, compound **4** was treated with hydrazines, ureas and hydroxylamine hydrochloride to prepare a range of ring-A fused hybrids of oleanolic acid. Interestingly, compound **4** reacted differently with hydrazine and phenylhydrazine and yielded pyrazoline (**5**, 72%) and pyrazole (**6**, 58%) hybrids respectively. Compound **6** showed the characteristic carbon signals of pyrazole ring at δ 174.31, 161.98 and 113.65 in addition to the expected oleanane carbon signals in its ^{13}C NMR spectrum. Whereas, compound **5** showed a clean doublet at δ 4.28 (J = 11.042 Hz) in its ^1H NMR spectrum and diagnostic signals at δ 164.86, 70.03 & 38.33 in ^{13}C NMR spectrum confirming a pyrazoline structure to the compound with H-2 and H-2′ in anti-periplanar orientation. The stereo chemistry of compound **5** was further confirmed by its NOESY spectrum, which reveals that the proton H-2 correlates with 24-CH_3 and 25-CH_3 groups. The above observations suggest that H-2 is β proton and H-2′ is α proton (Fig. 1).

The benzylidene compound (**4**), when reacted with hydroxylamine hydrochloride yielded an isoxazole (**7**) in 56% yield. Its structure was confirmed by its ^{13}C NMR, which showed the characteristic carbon signals of isoxazole ring at δ 109.14, 166.24 and 169.46. Compound **4** when treated separately with urea, thiourea and guanidine yielded the corresponding 2-oxo-pyrimidine (**8**), pyrimidine-2-thione (**9**) and 2-amino-pyrimidine (**10**) in 73%, 76% and 82% yields respectively. The structures of compounds **8**–**10** were confirmed by their respective ^{13}C NMR spectra, which showed the characteristic C-2 carbon signals at δ 100.32, 105.69 and 128.32 respectively.

2.2. Biology

2.2.1. Anti-cancer activity

The anti-cancer activity of the synthesized compounds (**3**–**10**) was studied using ME-180 & HeLa (cervix), MCF-7, MDA-MB-453 & MDA-MB-231 (breast), PC-3 (prostate) and HT-29 (colon) cancer cell lines by employing MTT assay [15]. Doxorubicin and etoposide along with compound **1** were taken as reference standards in this study. From the close analysis of the IC_{50} values (Table 1), it is evident that the synthesized compounds showed selectivity in exhibiting significant anti-cancer activity against the 3 breast cancer cell lines. Especially, the synthesized compounds found active against MCF-7 cell line. Among the synthesized compounds, the pyrimidine compounds (**8**–**10**) showed much higher activity than pyrazoline (**5**), pyrazole (**6**) and isoxazole (**7**) analogues. Most significantly, 2-amino-pyrimidine (**10**) showed potent activity against all the three breast cancer cell lines with IC_{50} values ranging from 10.60 to 16.27 μM. Compound **10** showed highest anti-cancer



Scheme 1. Reagents and conditions **a**. CH_3I, K_2CO_3, acetone, rt, 90%, **b**. PCC, DCM, rt, 88% **c**. Ph–CHO, KOH, ethanol, rt, 92% **d**. $N_2H_4 \cdot H_2O$, Ethanol, reflux, 72% **e**. $PhNHNH_2 \cdot HCl$, KOH, ethanol, reflux, 58% **f**. $NH_2OH \cdot HCl$, KOH, ethanol, reflux, 56% **g**. Urea or thiourea, KOH, ethanol, reflux, 73–76% **h**. $NH_2C(NH)NH_2 \cdot HCl$, KOH, ethanol, reflux, 82%.