Phytochemistry Letters 18 (2016) 99–104

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Short communication

Synthesis and cytotoxic activity of boswellic acid analogues

Peihong Fan[a], Tao Li[b,c,1], Yaqing Ye[a], Qian Luo[a], Huiqing Yuan[d], Hongxiang Lou[a,*]

[a] Department of Natural Product Chemistry, Key Lab of Chemical Biology of Ministry of Education, School of Pharmaceutical Sciences, Shandong University, Jinan 250012, China
[b] Shandong Food and Drug Administration, Jinan 250014, Shandong, China
[c] Shandong Pharmaceutical Association, Jinan 250014, China
[d] Department of Biochemistry & Molecular Biology, School of Medicine, Shandong University, Jinan 250100, China

ARTICLE INFO

Article history:
Received 2 August 2016
Received in revised form 9 September 2016
Accepted 20 September 2016
Available online xxx

Keywords:
Boswellic acid analogues
AKBA
Biological evaluation
Human tumor cell lines
Cytotoxic activity

ABSTRACT

Fifteen stereoisomers of boswellic acid analogues bearing 2-OH, 24-OH, 3-keto or 2-OH, 3-OH, 24-OH groups were synthesised and their structures were confirmed using ^{1}H NMR, ^{13}C NMR, 2D NMR and HRMS. The cytotoxic activities of these compounds toward three human tumor cell lines, K562, PC3 and A549, were evaluated. Preliminary biological evaluation indicated most of these compounds exhibited cytotoxic activity comparable to that of 3-*O*-acetyl-11-keto-β-boswellic acid (AKBA). Notably, several analogues exhibited relatively stronger cytotoxicity, with IC_{50} values less than 10 μM against A549 and PC3 cell lines. For the 24-OH series of BAs analogues, structure-activity relationship (SAR) analysis indicated that the stereochemical configurations of compounds incorporating 2-OH, 3-keto or 2-OH, 3-OH group pairs could not predictably or markedly impact cytotoxic activity, except when 2β-OH and/or 3β-OH were present. Esterification of 2-OH, 3-OH and 24-OH groups tended to decrease cytotoxicity.

1. Introduction

Boswellia serrata Roxb. ex Colebr. (family *Burseraceae*, Syn. *B. glabra*) plant extracts have been used widely as medicinal treatment for various conditions such as arthritis, asthma, ulcers and inflammatory disorders (Shah et al., 2009). Recently, anti-tumor, anti-carcinogenic, anti-proliferative and apoptotic activities of plant extracts have also been demonstrated (Hostanska et al., 2002; Huang et al., 2000). Boswellic acids (BAs), major constituents of the gum resin of Boswellia trees, are comprised of mainly β-boswellic acid (BA), 3-*O*-acetyl-β-boswellic acid (ABA), 11-keto-β-boswellic acid (KBA) and 3-*O*-acetyl-11-keto-β-boswellic acid (AKBA). Previous studies of natural BAs have demonstrated potent inhibitory activities toward human neuroblastoma cells (Akihisa et al., 2006), malignant glioma cells (Glaser et al., 1999), colon cancer cells (Liu et al., 2002) and human leukaemia cells (Shao et al., 1998; Jing et al., 1999; Hoernlein et al., 1999). Due to their anticancer activities, BAs are receiving much attention from the research community.

In order to optimize the functionality of natural bioactive compounds, analogues are often generated using chemical modifications of key structural constituents and assessed for changes in bioactivity. Consequently, increasing numbers of studies focusing on semi-synthetic modifications of BAs have appeared in the literature, such as synthesis of 3-epi BAs, 3-acyl (or formyl, acetyl, propyl, butyl, etc.) analogues, diene analogues, nor-analogues, 12-keto analogues, 4-amino analogues (Shah et al., 2009), cyanoenone analogues (Rao et al., 2008; Kaur et al., 2011) and endoperoxide analogues (Csuk et al., 2010). Notably, several of these semi-synthetic analogues have exhibited significant cytotoxicity toward various human cancer cell lines *in vitro* or *in vivo*. While several other structurally related semi-synthetic molecules, including oleanolic acid and betulinic acid derivatives, have exhibited promising bioactivities and are currently in various stages of clinical assessment, research and development of BAs still lag far behind (Shah et al., 2009; Liu, 2007).

In our previous work, we synthesised multiple series of ring A-modified BA derivatives, some of which exhibited much higher cytotoxicity against various human cancer cell lines *in vitro* than did AKBA (Li, 2015). During ring A modification, the intermediate 24-hydroxy-urs-12-en-3,11-dione (**2**) was synthesised from AKBA though successive deacetylation, reduction, hydroxyl group protection and selective oxidation (Li, 2015). After surveying the literature, we found that 2-OH, 3-ketone and 2,3-dihydroxytriterpene derivatives exhibited notable cytotoxic activity. For example, a maslinic acid derivative bearing a 2,3-*trans*-OH group

* Corresponding author.
E-mail address: louhongxiang@sdu.edu.cn (H. Lou).
[1] The author contributed equally to the first author.

http://dx.doi.org/10.1016/j.phytol.2016.09.009
1874-3900/

exhibited the potential to provide an effective natural defence against colon cancer (Reyes-Zurita et al., 2009); 3α-Hydroxy-D:A-friedoolean-3-en-2-one, isolated from the stem bark of *Mallotus philippensis*, inhibited mouse skin tumor promotion (Tanaka et al., 2008). In this work, an extension of our previous studies focusing on regarding compound (**2**), we designed and synthesised BAs analogues bearing 2-OH, 3-ketone or 2,3-dihydroxy groups and evaluated their cytotoxic activities toward three tumor cell lines (K562, A549 and PC3).

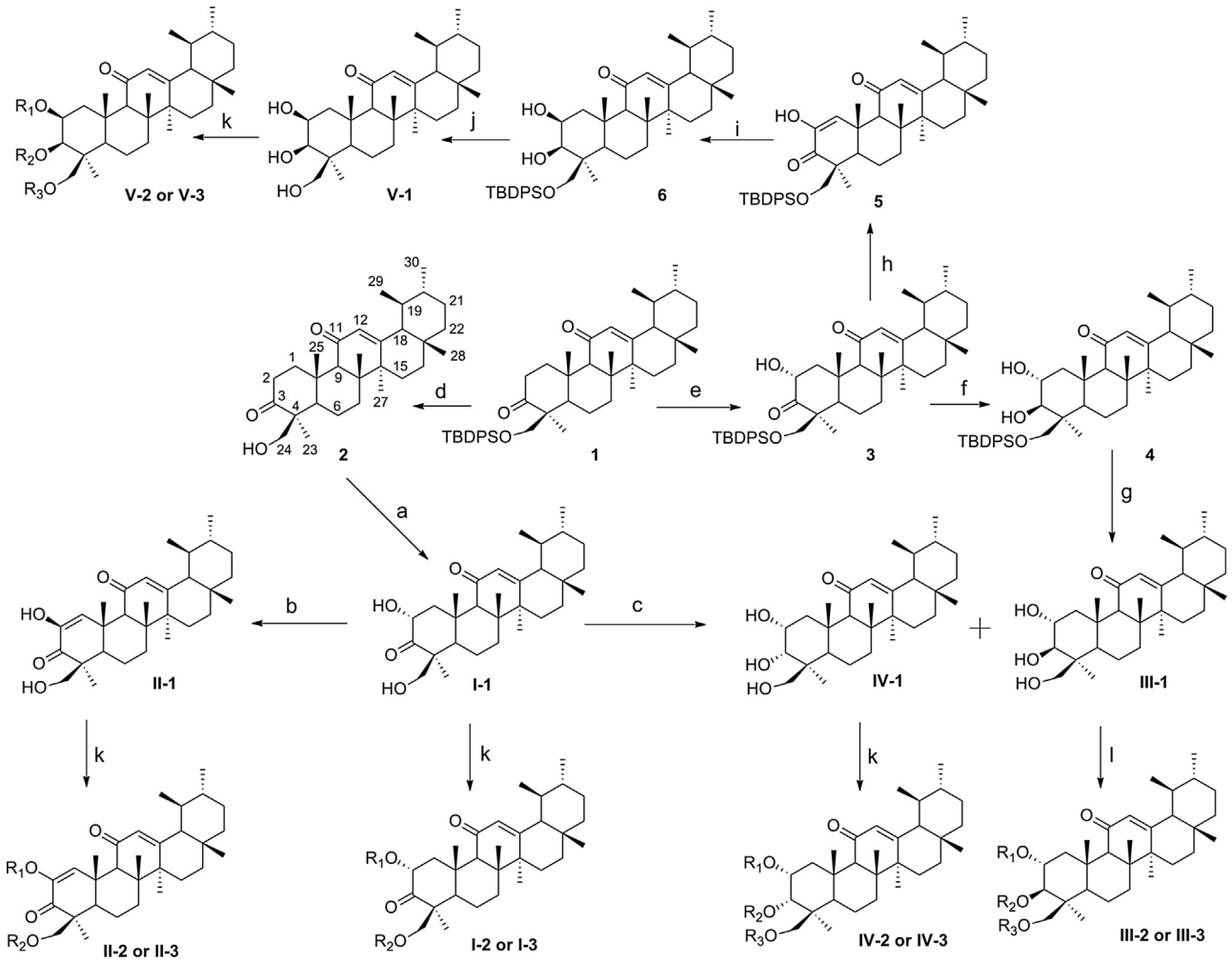
2. Results and discussion

24-*O*-*tert*-Butyldiphenylsilyl-urs-12-en-3,11-dione derivatives (**1**) and (**2**) were synthesised from AKBA though successive deacetylation, reduction, hydroxyl group protection and selective oxidation (Li, 2015). Depending on their structures, we introduced either a 2-OH and/or 3-OH group. As shown in Scheme 1, compounds **I-1**, **II-1**, **III-1**, **IV-1** and **V-1** were obtained by controlled oxidation or reduction reactions. **III-1**, **IV-1** and **V-1** are stereoisomers bearing OH groups at positions 2, 3, and 24. Notably, the synthetic route exhibited marked stereoselectivity. The configurations of 2-OH or 3-OH were confirmed by nuclear overhauser effect (NOE) correlation analysis between H-2 and H-24, H-25 (see NOESY spectra for **I-1**, **III-1**, **IV-1** and **V-1** in Supplementary file), as well as the multiplicity and coupling constants of H-2 and H-3 signals (see Table S in Supplementary file).

During the synthesis of **I-1**, 3-chloroperoxybenzoic acid (mCPBA) tended to form 2α-OH, due to the influence of large amounts of steric hindrance (Wen et al., 2008). **I-1** was further oxidised to form the 2-keto, 3-keto derivative, **II-1**, which actually existed as an α-hydroxyl ketene isomer after reaction using a precisely defined amount of the extremely mild oxidant $CuCl_2$ (Lokhande et al., 2012).

Due to a lack of stereoselective bias, after reduction of the 3-keto group of **I-1** using $NaBH_4$, both **III-1** and **IV-1** were generated. However, a high yield of **III-1** could alternatively be obtained from **1** by induction of steric hindrance before addition of 2α-OH to **1** to produce **3**, followed by selective reduction of the 3-keto group of **3** to yield compound **4** bearing 2α- and 3β- OH groups, followed by deprotection of **4** to yield **III-1**. The products of **III-1** produced via the above two synthetic routes were analysed using HPLC. The chromatograph shows that synthesis via the reduction of **I-1** produced **III-1** and **IV-1** in a ratio of about 2:1, while the second synthetic route involving reduction of **3** resulted in a 90% yield of **III-1** (see the HPLC chromatograph in the Supplementary data).

To obtain the derivative **V-1** containing 2β-OH and 3β-OH groups, $CuCl_2$, a very mild oxidant was used to first synthesise **5**



Scheme 1. Synthetic routes of analogues of BAs. a. mCPBA/H_2SO_4, MeOH-DCM, r.t., darkness; b. $CuCl_2$, THF, 80 °C; c. $NaBH_4$, THF-EtOH, r.t.; d. 1 M TBAF, THF, r.t.; e. mCPBA/H_2SO_4, MeOH-DCM, r.t., darkness; f. $NaBH_4$, THF-EtOH, r.t.; g. 1 M TBAF, THF, r.t.; h. $CuCl_2$, THF, 80 °C; i. $NaBH_4$, THF-EtOH, r.t.; j. 1 M TBAF, THF, r.t.; k. AcCl(C_2H_5COCl)/Py, DCM, ice bath; l. Ac_2O (($C_2H_5CO)_2O$)/Py, reflux.