European Journal of Medicinal Chemistry 82 (2014) 68–81

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

Antiproliferative and quinone reductase-inducing activities of withanolides derivatives

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ARTICLE INFO

Article history:
Received 5 September 2013
Received in revised form
15 May 2014
Accepted 20 May 2014
Available online 20 May 2014

Keywords:
Withanolide derivatives
Jaborosa runcinata
Antiproliferative activity
Quinone-reductase induction
Keap1 protein

ABSTRACT

Two new and five known withanolides (jaborosalactones 2, 3, 4, 5, and 24) were isolated from the leaves of *Jaborosa runcinata* Lam. We also obtained some derivatives from jaborosalactone 5, which resulted to be the major isolated metabolite. The natural compounds as well as derivatives were evaluated for their antiproliferative activity and the induction of quinone reductase 1 (QR1; NQ01) activity. Structure–activity relationships revealed valuable information on the pharmacophore of withanolide-type compounds. Three compounds of this series showed significantly higher antiproliferative activity than jaborosalactone 5. The effect of these compounds on the cell cycle was determined. Furthermore, the ability of major compounds to induce QR1 was evaluated. It was found that all the active test compounds are monofunctional inducers that interact with Keap1. The most promising derivatives prepared from jaborosalactone 5 include (23*R*)-4β,12β,21-trihydroxy-1,22-dioxo-12,23-cycloergostan-2,5,17,24-tetraen-26,23-olide (**18**) and (23*R*)-21-acetoxy-12β-hydroxy-1,22-dioxo-12,23-cycloergostan-2,5,17,24-tetraen-26,23-lactame (**20**).

1. Introduction

Withanolides comprise a group of naturally occurring C28 steroids based on an ergostane skeleton in which C-26 and C-22, or C-26 and C-23, are oxidized in order to form a δ- or γ-lactone. They are known for the diversity of structures involving the steroid nucleus and the side chain, including the formation of additional rings. Their chemistry and occurrence has been the subject of several reviews [1–4]. Among the nearly 100 genera included in Solanaceae, ca. 50% have been investigated [4]. *Jaborosa* is an interesting South American genus growing from southern Peru to Argentina in very diverse habitats [5]. *Jaborosa* Juss. represents one of the four major contributors of withanolide structures.

A family of withanolides spiranic at C-23 has been isolated from *Jaborosa runcinata* Lam., *Jaborosa odonelliana*, and *Jaborosa araucana* [6], and many of these compounds exhibit interesting biological activities such as antiproliferative [7] and cancer chemopreventive properties [8]. In addition, some studies have reported structural modifications of withanolides and structure–activity relationships. Fundamentally, the ring A of withanolides has been modified with various nucleophiles and the derivatives have been evaluated for antiproliferative activity [9,10].

Continuing our search for bioactive compounds, we evaluated the chemical content of *J. runcinata* based on its high content of withanolides. One objective was to obtain enough jaborosalactone 5 to synthesize derivatives and introduce changes at various positions of the molecule. Two new and five previously reported withanolides were isolated. From jaborosalactone 5, a set of derivatives was synthesized, and their antiproliferative and chemopreventive activities were assessed. Preliminary mechanistic studies were performed, and the effects on the cell cycle were

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http://dx.doi.org/10.1016/j.ejmech.2014.05.045
0223-5234/

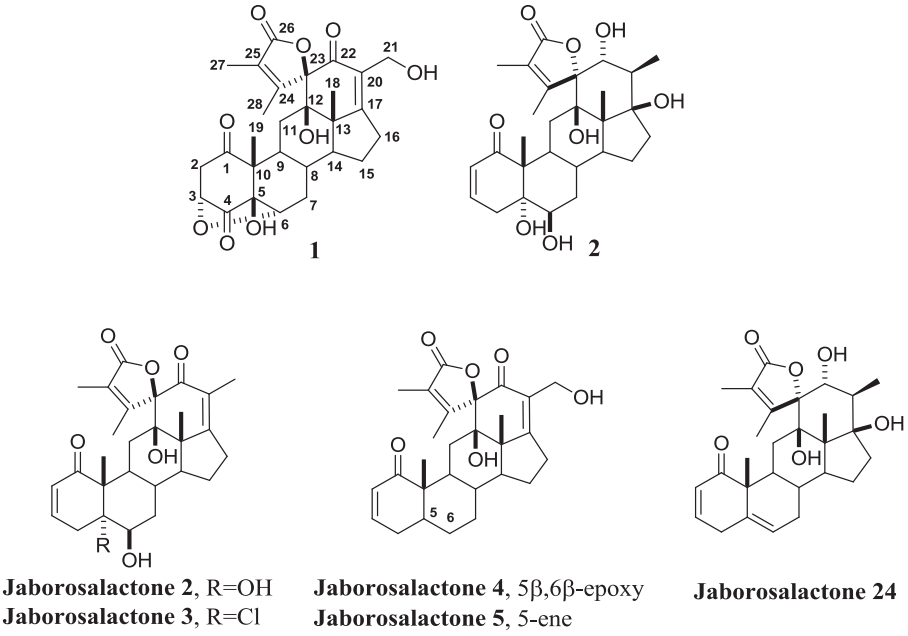

Fig. 1. Structures of isolated withanolides.

examined. With respect to quinone reductase (QR1; NQ01)-inducing compounds, their potential was determined for regulating gene expression through interaction with Keap1 protein.

The antioxidant response element (ARE) controls the expression of cytoprotective enzymes including QR1, UDP-glucuronyl transferases and glutathione *S*-transferases and is regulated, at least in part, by levels of the transcription factor NrF2 [11]. The cytosolic protein Keap1 binds Nrf2 and the enzyme, Cul3, which attaches ubiquitin to Nrf2 thereby marking it for hydrolysis by the proteosome. Ubiquitination of Nrf2 can be reduced or prevented by covalent modification of one or more of the 37 cysteine residues on Keap1 [12]. By blocking ubiquitination of Nrf2, cytosolic levels of Nrf2 increase leading to activation of the ARE. Most ARE inducers acting through modification of Keap1 cysteine sulfhydryl residues are electrophilic in nature and include Michael acceptors and isothiocyanates [13,14]. We have developed a mass spectrometry-based screening assay suitable for the identification of these Keap1 modifying agents [15].

2. Results and discussion

2.1. Chemistry

2.1.1. Natural products

The aerial parts of *J. runcinata* were air-dried and extracted with ethanol. After concentration and defatting, the residue was fractionated by a combination of several chromatographic techniques, ultimately giving two new withanolides, compounds **1** and **2**, and the known spiranic-type withanolides, jaborosalactones 2, 3, 4, 5 and 24 (Fig. 1).

The molecular formula of compound **1** was determined by high resolution electrospray mass spectrometry (HRESIMS) as $C_{28}H_{32}O_9Na$ (*m/z* 535.1946 $[M+Na]^+$). In its ^1H NMR spectrum, the presence of two methyl signals at δ 1.82 and δ 2.16 and the absence of a lactonic hydrogen in the 4–5 ppm region corresponding to the carbynolic hydrogen H-22 indicated a spiranoid dimethyl-substituted α,β-insaturated γ-lactone ring comprising C-23 to C-28. The typical pattern of the spiranoid arrangement was confirmed from the resonances of carbons 23–28; a similar arrangement has been described previously [6]. The absence of a singlet at the high field region of the ^1H NMR spectrum and the appearance of two doublets at 4.15 and 4.24 ppm suggested the presence of an isolated C-21 hydroxymethylene group. The ^{13}C NMR spectrum showed only four methyl groups at 14.6, 17.2, 8.6, and 15.9 ppm corresponding to C-18, C-19, C-27, and C-28, respectively. The methylene signal at 58.4 ppm (C-21) confirmed the presence of a hydroxyl group at C-21. The presence of a tetrasubstituted double bond at C-17-C-20 was ratified by the downfield shift for the signals at δ 166.1 and δ 127.5, respectively. The CH_2-21 signal was correlated with a signal of conjugated carbonyl carbon at 193.0 ppm in the HMBC experiment, confirming a 17(20)-en-22-keto functionality. The configuration at the spiranoid center (C-23*R*) was established by comparison with spectral data of jaborosalactones 1–6 and 25 [6].

Regarding the A/B rings, the ^{13}C NMR spectrum showed two carbonyl groups at δ 209.2 (C-1) and δ 207.4 (C-4), three carbynolic carbons, two signals of methine carbons at δ 74.7 and δ 72.6 (assigned to C-3 and C-6, respectively) and one signal of quaternary carbon at δ 77.6 (C-5). These data provided evidence for an oxygen ether bridge between the A/B rings through the C-3 and C-6 positions. This assumption was supported by the following cross-correlation peaks in the HMBC experiment: between H_3-19 (δ 0.77) and C-5 (δ 77.6) and C-10 (δ 50.6), confirming an oxygenated function at C-5 position; between H_2-2 (δ 3.04 and δ 2.42) and C-1 (δ 209.2), C-3 (δ 74.7), and C-4 (δ 207.4), indicating the presence of an oxygenated methine located at C-3 and two non-conjugated ketone groups located at C-1 and C-4; and finally the weak but diagnostic HMBC correlation observed between H-3 (δ 4.20) with C-6 (δ 72.2) validating C-3/C-6 epoxy functionality. The epoxy group orientation was established by analysis of the coupling constants of H_2-2, H-3, and H-6 and confirmed by comparison with spectral data of subtrifloralactone K, withanolide reported from *Deprea subtriflora* having the same A/B ring substitution pattern [16]. Compound **1** (jaborosalactone 47) was finally established as (23*R*)-3α,6α-epoxy-5β,12β,21-trihydroxy-1,4,22-trioxo-12,23-cycloergostan-17,24-dien-26,23-olide.