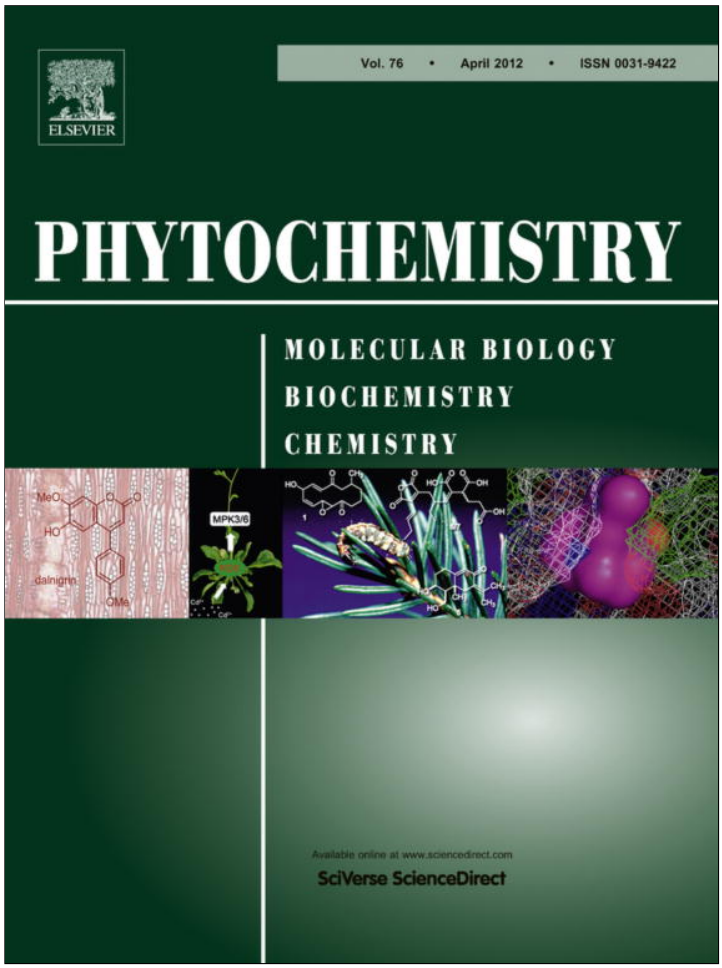

Phytochemistry 76 (2012) 150–157

Contents lists available at SciVerse ScienceDirect

Phytochemistry

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

Antiproliferative activity of withanolide derivatives from *Jaborosa cabrerae* and *Jaborosa reflexa*. Chemotaxonomic considerations

Manuela E. García [a], Gloria E. Barboza [a], Juan C. Oberti [a], Carla Ríos-Luci [b], José M. Padrón [b], Viviana E. Nicotra [a,b,*], Ana Estévez-Braun [b,c,*], Angel G. Ravelo [b,c,*]

[a] Facultad de Ciencias Químicas, Instituto Multidisciplinario de Biología Vegetal (IMBIV-CONICET), Universidad Nacional de Córdoba, Casilla de Correo 495, 5000 Córdoba, Argentina
[b] Instituto Universitario de Bio-Orgánica "Antonio González" IUBO-AG, Universidad de La Laguna, Avda. Astrofísico Francisco Sánchez 2, 38206 La Laguna, Spain
[c] Instituto Canario de Investigación del Cáncer (ICIC), Avda. Astrofísico Francisco Sánchez 2, 38206 La Laguna, Spain

ARTICLE INFO

Article history:
Received 26 July 2011
Received in revised form 20 December 2011
Accepted 28 December 2011
Available online 8 February 2012

Keywords:
Jaborosa reflexa
Jaborosa cabrerae
Solanaceae
Phytochemistry
Chemotaxonomy
Antiproliferative activity
Withanolides

ABSTRACT

Three withanolides were isolated from the aerial parts of *Jaborosa reflexa* Phil. *Jaborosa cabrerae* Barboza yielded five sativolide withanolides (including jaborosalactones R, S, 38, and 39) and two trechonolide withanolides epimeric at C-23 (trechonolide A and jaborosalactone 32). In addition, five derivatives were obtained by chemical derivatization of jaborosalactone 38, and all compounds were fully characterized by 1D and 2D NMR spectroscopic studies. The in vitro antiproliferative activities of the major natural withanolides and the semisynthetic derivatives were examined against HBL-100, HeLa, SW1573, T-47D, and WiDr human solid tumor cancer cell lines. Some chemotaxonomic considerations are discussed.

1. Introduction

Withanolides constitute a group of natural C-28 steroids isolated mainly from several genera of Solanaceae. They have an ergostane-type skeleton with oxidation at positions C-1, C-22, and C-26. Biogenetic transformations of withanolides can produce highly modified compounds in both the steroid nucleus and side-chain, including formation of additional rings. Their chemistry and occurrence have already been reviewed (Chen et al., 2011; Misico et al., 2011; Ray and Gupta, 1994; Veleiro et al., 2005). Many of these compounds exhibit interesting biological activities such as antifeedant (Bado et al., 2004), antiproliferative (Machín et al., 2010), cancer chemopreventive (Kennelly et al., 1997; Misico et al., 2002; Su et al., 2002), and phytotoxic properties (Nicotra et al., 2007).

Jaborosa Juss. is one of the five largest genera of South American Solanaceae (Hunziker, 2001), growing from southern Peru to Argentina with the main center of speciation in the Andean region of Argentina (Barboza and Hunziker, 1987). The genus comprises of twenty-three species of perennial herbs from semiarid or arid habits, and five from humid regions (e.g. *Jaborosa. oxipetala* Speg., *Jaborosa sativa* (Miers) Hunz. & Barboza, *Jaborosa integrifolia* Lam.). So far, more than 50% of the species have been phytochemically analyzed, withanolides being the compounds most extensively studied. *Jaborosa reflexa* is a Patagonian species (Chile and Argentina) with greenish corolla, whereas *Jaborosa cabrerae*, a rosette-like species with white corolla, is found in a small area of the province of Catamarca (ca. 3000 m); both grow in very sandy soils.

As part of our program aimed at the discovery of novel withanolides from *Jaborosa* species, reported herein the isolation of a set of both new and known withanolides from *J. reflexa* Phil. and *J. cabrerae* Barboza. Additionally, some chemotaxonomic considerations are discussed. To get insight into structure–activity relationships, five semi-synthetic derivatives were prepared from jaborosactone 38. The antiproliferative activities of semisynthetic and naturally occurring withanolides were evaluated in human solid tumor cell lines.

2. Results and discussion

The aerial parts of *J. reflexa* were air-dried and extracted with ethanol. After elimination of the solvent, the resulting residue was defatted and repeatedly chromatographed to yield three new withanolides (**1**–**3**, Fig. 1).

* Corresponding authors. Address: Facultad de Ciencias Químicas, Instituto Multidisciplinario de Biología Vegetal (IMBIV-CONICET), Universidad Nacional de Córdoba, Casilla de Correo 495, 5000 Córdoba, Argentina (V.E. Nicotra). Tel.: +54 351 4334170x147; fax: +54 351 4333030.
E-mail address: vnicotra@fcq.unc.edu.ar (V.E. Nicotra).

0031-9422/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2011.12.018

Jaborosalactol 28 **(1)** had molecular formula $C_{30}H_{40}O_7$ as determined by HRESIMS. Its ^{1}H NMR spectrum showed characteristic signals of the H-2, H-3, and H-6 hydrogens for a 1-oxo-2,5-diene-withanolide at δ 5.90 (*dd*, *J* = 10.0, 2.4 Hz), 6.81 (*ddd*, *J* = 10.0, 4.9, 2.4 Hz), and 5.60 (*d*, *J* = 5.5 Hz), respectively (Ramacciotti and Nicotra, 2007). Olefinic resonances at δ 127.8, 145.5, 135.9, and 124.2, and a carbonyl carbon at δ 202.8 (C-1) were observed in the ^{13}C NMR spectrum. The correlations found between the H-2/H-4β, H-3/H-4α, and H-3/H-4β pairs in the COSY spectrum led to the assignment of H-4 α and H-4 β at δ 2.90 and 3.30, respectively. Regarding C and D rings, the presence of an α-acetoxy group at C-16 was evidenced by the oxymethine hydrogen at δ 5.04 (*brt*, *J* = 7.3 Hz) in the ^{1}H NMR spectrum and the respective signals for an acetoxy group (δ 2.07 (3H); δ 21.2 (CH_3); δ 170.9 (C)). COSY correlations of H_3-21 (δ 0.87) with H-20 (δ 1.75), of H-20 with H-17 (δ 2.30), and of H-17 with H-16, clearly placed this acetoxy group at C-16. The α orientation of the acetoxy group at C-16 was confirmed from the NOE effect observed between H-16 and H_3-18 in the NOESY spectrum. In addition, the carbonyl resonance at δ 212.6 in the ^{13}C NMR spectrum was assigned to the keto group at C-12 (Ramacciotti and Nicotra, 2007). The correlations observed in the HMBC spectrum for H-11α (δ 3.08), H-11β (δ 2.69), H-17 (δ 2.30), and H_3-18 (δ 1.11), with C-12, confirm this assumption. The resonances shown in the ^{13}C NMR spectrum at δ 57.4, δ 54.9, δ 34.2, δ 78.3, and δ 49.3 were attributed to C-13, C-14, C-15, C-16, and C-17, respectively. Regarding the side-chain, evidence for the presence of an epoxy lactol was provided by the signal corresponding to the oxymethine proton at δ 4.97 (*d*, *J* = 8.1 Hz) assigned to the hydrogen H-26, and the resonances of two methyl groups at C-24 and C-25 at δ 1.41 (H_3-27) and 1.42 (H_3-28), respectively. H-22 was observed as a double triplet at δ 3.58, and H_3-21 as a doublet indicating the absence of a hydroxyl group at C-20. Signals at δ 65.0 (C-22), δ 65.3 (C-24), δ 63.9 (C-25), δ 91.8 (C-26), δ 16.5 (C-27), and δ 18.9 (C-28) in the ^{13}C NMR spectrum are in agreement with the epoxy lactol side chain (Nicotra et al., 2003). The side-chain β-orientation was confirmed by a NOESY experiment; a key cross-correlation peak was observed between H-17 (δ 2.30) and the cresonance at δ 1.68 corresponding to H-14 and H-15α (Bessalle and Lavie, 1992). Thus the structure of **1** was established as (17*R*,20*S*,22*R*,24*S*,25S,26R)-16α-acetoxy-22,26:24,25-diepoxy-26-hydroxyergost-2,5-dien-1-one.

The molecular formula of jaborosalactone 45 (**2**) was determined by HRESIMS as $C_{28}H_{36}O_6$. Comparison of its ^{1}H and ^{13}C NMR spectra with those of compound **1** showed signals almost identical for all the carbons and protons of rings A and B, indicating a 1-oxo-2,5-diene system. On the other hand, the resonances observed for rings C and D and the side-chain closely resemble those of sativolides, jaborosalactones *R*, *S*, and *T* previously isolated from *J. sativa* (Bonetto et al., 1995), jaborosalactone 37 isolated from *Jaborosa rotacea* (Nicotra et al., 2006), and jaborosalactones 38 and 39 isolated from *Jaborosa caulescens* (Nicotra et al., 2007). Sativolides have an oxygen bridge between C-21 and C-12 in the steroid nucleus. The additional six-membered hemiketal (or ketal) ring is presumably formed by intramolecular reaction between a carbonyl group at C-12 and a hydroxyl group at C-21. This unique arrangement is characterized by the absence of the methyl-21 ^{1}H NMR doublet at high field and by appearance of resonances at δ_H 3.95 and 3.63 (H_2-21), δ_C 100.7 (C-12), and δ_C 59.9 (C-21). The heterocyclic ring was confirmed by cross-correlation peaks observed for H_2-21 with C-12 in the HMBC spectrum.

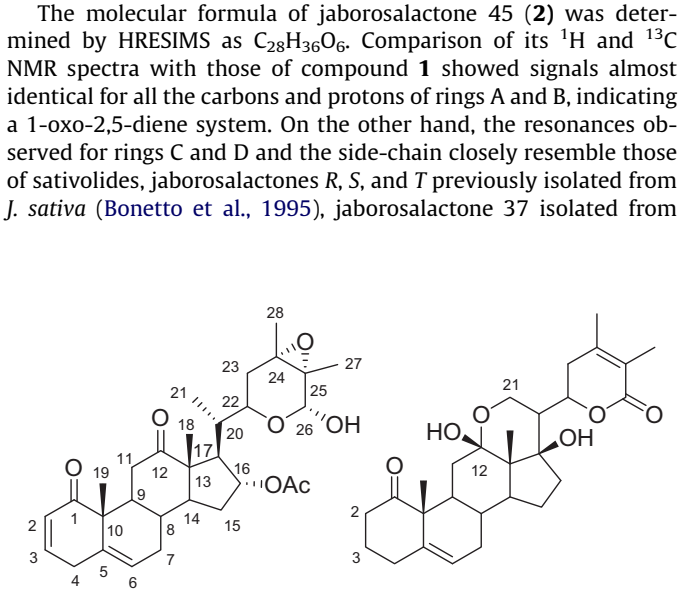

Fig. 1. New withanolides isolated from *Jaborosa reflexa*.

Jaborosalactone 47 (**3**) ($C_{28}H_{36}O_6$) could not be obtained pure through either normal phase TLC or reversed phase TLC. This compound was characterized from 4 mg of a mixture of compounds **2** and **3** in a 3:2 ratio. The ^{1}H and ^{13}C NMR spectra were closely related to those of compound **2**, differing only in the substitution pattern of rings A and B. A 1-oxo-3,5-diene substitution was inferred from the following observations: (i) two mutually coupled olefinic signals resonating at δ 5.65 (*m*) and 6.06 (*brd*, *J* = 9.6 Hz) assigned to H-3 and H-4, respectively; (ii) the downfield shift of the carbonyl carbon in the ^{13}C NMR spectrum from δ 203.9 for compound **2** to δ 210.2 for compound **3**, assigned to C-1; (iii) the downfield shift of H_3-19 in the ^{1}H NMR spectrum from δ 1.23 for **2** to δ 1.37 for **3**. The ^{13}C NMR signals corresponding to A and B rings were in agreement with the proposed structure (Pan et al., 2007). Since compound **3** could not be obtained in pure form and it was characterized as a mixture with compound **2**, its structure was tentatively established as (20*R*,22*R*)-12α,21-epoxy-12iβ,17β-dihydroxy-1-oxowitha-3,5,24-trien-26,22-olide.

The full and unambiguous proton and carbon NMR assignments for compounds **1**–**3** were made using a combination of DEPT, COSY, HSQC, and HMBC experiments.

Following a similar procedure, the EtOH extract of *J. cabrerae* was analyzed and the sativolide withanolides **2**, jaborosalactones R (**4**), S (**5**) (Bonetto et al., 1995), 38 (**6**) and 39 (**7**) (Nicotra et al., 2007), together with two trechonolide withanolides epimeric at C-23 [trechonolide A (**8**) (Fajardo et al., 1987; Lavie et al., 1987) and jaborosalactone 32 (**9**) (Nicotra et al., 2006)] were isolated.

The sativolide withanolides isolated from *J. reflexa* and *J. cabrerae* are closely interrelated. Compound **2**, the main withanolide isolated from *J. reflexa*, was also isolated from *J. cabrerae* and could be considered a biosynthetic precursor of compound **3** (isolated from *J. reflexa*) and jaborosalactones *R*, *S*, 38, and 39 isolated from *J. cabrerae*. With respect to rings A and B, it has been well established that the 1-oxo-2,5-diene system is a biosynthetic precursors of 1-oxo-3,5-diene; 1-oxo-5β,6β-epoxy; 1-oxo-5α,6β-dihydroxy; and 1-oxo-2,4-diene-6β-hydroxy systems (Glotter, 1991).

Some genera of the Solanaceae family contain withanolides with exclusive interesting arrangements which can be considered at a generic level as chemotaxonomic markers (Misico et al., 2011). Sativolide and trechonolide withanolides belong particularly to *Jaborosa* sect. *Lonchestigma* (Dunal) Wettstein in which *J. reflexa* and *cabrerae* form part of (Barboza and Hunziker, 1987). The results obtained in our study agree with this statement since both species have sativolide withanolides (jaborosalactones *R*, *S*, 38, 39, 45, and 46), whereas *J. cabrerae* also contains trechonolide withanolides (trechonolide A and jaborosalactone 32). *J. cabrerae* is closely related to another Andean species, *J. caulescens* Gillies & Hook. Phytochemically, both species are similar in composition; they not only have withanolides with the same skeleton (sativolide- and trechonolide-type withanolides), but also share several metabolites (jaborosalactones R, S, 38, 39 and trechonolide A). In addition, both are unique among their congeners with trechonolide-type withanolide epimeric at C-23 (trechonolide A and jaborosalactone 32 in *J. cabrerae*, and jaborosalactones 40 and 41 in *J. caulescens*). These similarities are shared with the morphological and ecological

features and with the phylogenetical evidence found. Both species have a similar habitat (sandy mountainous areas above 2500 m); the same habit with a strong root and underground stems (rhizomes); the long petiole and pinnatisect leaves; and the funnel-shaped corolla (Fig. 2 D–G) (Barboza, 1986; Barboza and Hunziker, 1987). A molecular analysis, based on nuclear and chloroplast DNA, indicates *J. cabrerae* as sister to *J. caulescens* with very high support (bootstrap 99%) (Moré et al., 2009). On the other hand, *J. reflexa* neither forms a sister group with *J. cabrerae* nor has a close chemical relationship with this species.

Some withanolides have exhibited antiproliferative activity against human breast cancer cell lines (Machín et al., 2010). Thus, jaborosalactone 38, previously isolated from *J. caulescens* var. *caulescens*, was able to induce growth inhibition in all cell lines tested (GI_{50} = 2.6–4.1 μM). Considering the activity of jaborosalactone 38 and the amount isolated from *J. cabrerae* (438 mg), a small set of derivatives (**10–14**, Fig. 3) was prepared. Chemical modifications have been introduced on A and B rings (jaborosalactone 38) since, in previous research, where the in vitro antiproliferative activity of a series of twenty-two naturally occurring withanolides was examined, the results underlined the relevance of the substitution pattern on A and B rings on the antiproliferative activity; the type of side-chain demonstrated no affect on activity (Machín et al., 2010).

Compound **10** was obtained in low yield by epoxide opening using NH4Ac/AcH. The Michael type-addition of several nucleophiles such as MeOH, BzNH2 and PhSH yielded the corresponding derivatives **11**, **12**, and **13**. Compounds **11** and **12** were obtained together with jaborosalactone *R* (4), while compound **13** was obtained together with methoxy derivative **14**.

The in vitro antiproliferative activities of natural compounds **1**, **2**, jaborosalactones 38 (**6**), 39 (**7**), R (**4**), and S (**5**), and semisynthetic derivatives 10–14 were evaluated using the well-established protocol of the National Cancer Institute (NCI) of the United States (Skehan et al., 1990). As a model, the representative panel of human solid tumor cell lines HBL-100 (breast), HeLa (cervix), SW1573 (non-small cell lung), T-47D (breast), and WiDr (colon), was used. Table 1 summarizes the results expressed as 50% growth inhibition (GI_{50}). The remaining compounds showed GI_{50} values larger than 50 μM. For comparison, the antiproliferative data of the standard anticancer drugs cisplatin, 5-fluorouracil and camptothecin against the same panel of solid tumor cell lines are included. Viewed as a whole; these results have allowed us to classify the compounds under three groups. A first group is comprised of jaborosalactone 38 (**6**) and derivatives **13** and **14**, which caused significant growth inhibition in all the lines tested with GI_{50} values in the range 1.4–8.3 μM, 4.4–17 μM, and 5.3–19 μM, respectively. The second set of compounds, involving jaborosalactones R (**4**) and 45 (**2**), jaborosalactol 28 (**1**), and derivative 12, exhibited moderate activity with GI_{50} values in the range 18–33 μM, 17–24 μM, 24–43 μM, and 33–50 μM, respectively. The third group includes jaborosalactones 39 (**7**) and S (**5**), and derivatives **10** and **11**, which failed to show any growth inhibition.

The analysis of the antiproliferative activity has allowed us to establish the following structure–activity relationships: (i) 1-oxo-2-ene-5β,6β-epoxy system in rings A/B is relevant for the biological activity jaborosalactone 38 being the most active compound; (ii) a decrease in or loss of activity was observed for 5-oxygenated (jaborosalactone S and 10), 5,6-unsaturated (2), or 1-oxo-2,4-diene-6β-hydroxylated (jaborosalactone R) compounds; (iii) Regarding compounds having a 1-oxo-2,3 dihidro-5β,6β-epoxy system in rings A/B, such as jaborosalactone 39 and 11–14 derivatives, in all cases the antiproliferative activity was lower than that shown by jaborosalactone 38 with a 1-oxo-2-ene-5β,6β-epoxy system in

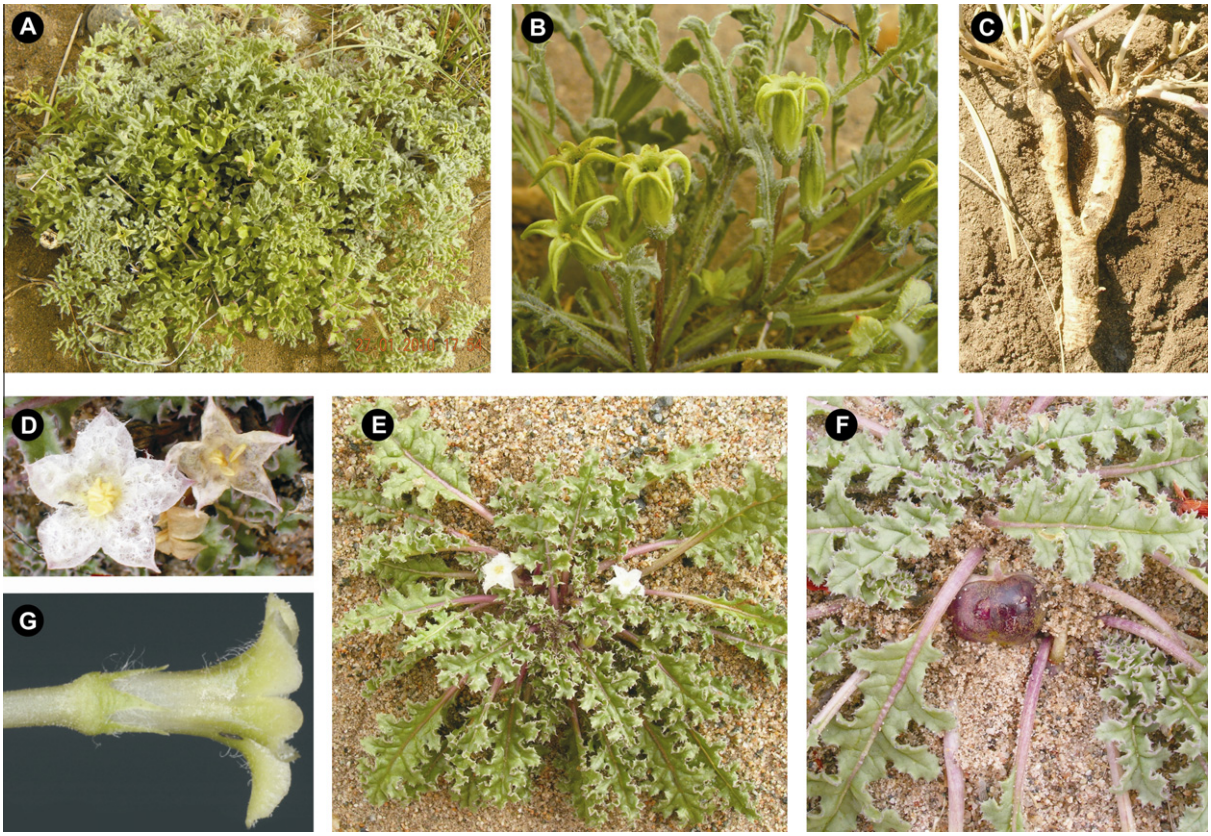

Fig. 2. *Jaborosa* species. A–C: *J. reflexa*. A: plant; B: branch with flowers; C: root; D–F: *J. cabrerae*. D: flower; E: plant; F: leaves (note the long purple petiole) and fruit; G: Flower of *J. caulescens*.

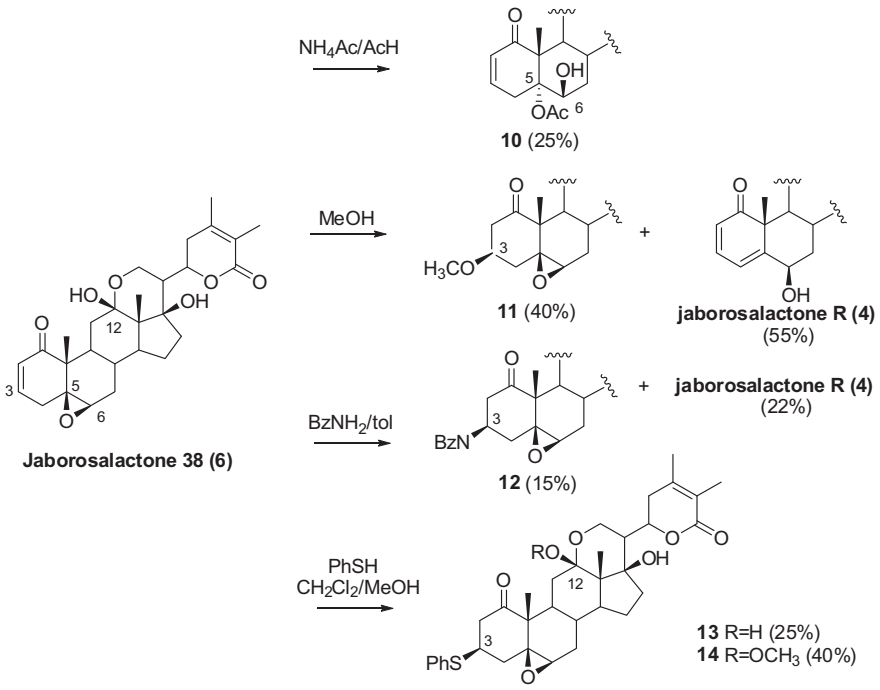

Fig. 3. Withanolide derivatives obtained from jaborosalactone 38 (**6**). Yields given in parentheses.

Table 1
In vitro Antiproliferative Activity of Withanolides and Derivatives against Human Solid Tumor Cell Lines.[a].

Compound	HBL-100	HeLa	SW1573	T-47D	WiDr
Jaborosalactol 28 (**1**)	34 ± (6.4)	24 ± (7.4)	33 ± (8.7)	43 ± (8.1)	40 ± (3.5)
Jaborosalactone R (**4**)	35 ± (1.5)	18 ± (1.7)	33 ± (1.3)	15 ± (2.9)	30 ± (2.8)
Jaborosalactone 38 (**6**)	4.0 ± (0.4)	8.3(± 1.4)	4.4(± 0.1)	8.1 ± (0.7)	1.4(± 0.1)
Jaborosalactone 45 (**2**)	24 ± (5.4)	17 ± (3.8)	23 ± (8.1)	29 ± (5.0)	20 ± (2.4)
11	90 ± (11)	>100	53 ± (12)	>100	>100
12	33±(12)	48 ± (2.0)	50 ± (1.3)	25 ± (5.6)	40 ± (1.0)
13	4.7 ± (1.3)	4.4 ± (1.6)	4.6 ± (0.8)	17 ± (3.2)	14 ± (4.3)
14	6.0 ± (2.2)	5.6 ± (1.0)	5.3 ± (0.1)	19 ± (0.1)	16 ± (0.7)
Cisplatin	1.9 ± (0.16)	2.0 ± (0.3)	3.4 ± (0.7)	15 ± (2.3)	26 ± (5.6)
5-fluorouracil	5.5 ± (2.3)	15 ± (4.7)	4.3 ± (1.6)	47 ± (18)	49 ± (6.7)
Campthotecin	0.2 ± (0.05)	0.5 ± (0.2)	0.3 ± (0.1)	2.0 ± (0.5)	1.8 ± (0.7)

[a] Expressed as GI_{50} values given in M and determined as means of three to five experiments. Standard deviations are given in parentheses.

rings A/B. However, the activity of 11–14 derivatives was subject to the electronegativity of 3β-substituents, with an activity increasing with decreasing electronegativity of the heteroatom at C-3; (iv) all compounds tested are sativolides except jaborosalactol 28 (1), an unmodified skeleton withanolide bearing a six-membered epoxy lactol ring. However, jaborosalactol 28 (1) showed a similar activity to jaborosalactone 45 (2). Both withanolides have different skeletons but the same substitution pattern in rings A/B. Our findings demonstrate the relevance of the substitution pattern in A/B rings to the resulting antiproliferative activity. These results are in line with those previously reported on the structure–activity relationship of withanolides (Kennelly et al., 1997; Machín et al., 2010; Misico et al., 2002; Su et al., 2002).

3. Conclusions

With this phytochemical information, the number of *Jaborosa* species studied increased to 15 out of 23 species of the genus. The metabolites isolated here agree with the exclusive withanolide arrangements of *Jaborosa* which can be considered chemotaxonomic markers at generic level. The chemical information provided by these compounds is of particular interest not only at specific or generic level but also at higher range (tribes, subtribes, subfamily) and may contribute to supporting phylogenetic relationships among the taxa.

With respect to relationships of structure-antiproliferative activity indicated, most of them agree with those already established. However, the activity of withanolides with a 1-oxo-3β-XR system in ring A has not yet been extensively studied so far. 3β-S withanolide derivatives showed marked activity (4.4–19 μM) similar to that reported by 3β-S withaferin A derivatives (Yousuf et al., 2011). Accordingly, this type of functionalization should be studied in depth to assess the biological activity and mechanism of action of such derivatives.

4. Experimental

4.1. General

Melting points were measured on a mercury thermometer apparatus and were uncorr, whereas optical rotations were recorded using a JASCO P-1010 polarimeter. UV spectra were obtained with a Shimadzu-260 spectrophotometer, whereas IR spectra employed a Nicolet 5-SXC spectrophotometer. NMR experiments were performed on Bruker AVANCE II 400 MHz and AMX 500 MHz instruments. Multiplicity determinations (DEPT) and 2D spectra (COSY, HSQC, HMBC, and NOESY) were obtained using standard Bruker software. Chemical shifts are given in ppm (δ) downfield from TMS internal standard. ESIMS and HRESIMS were measured on an LCT premier XE Micromass spectrometer, and EIMS was determined at 70 eV on a Finnigan 3300 F-100 mass spectrometer. UPLC-ESI-QTOF/MS was performed using a system: UPLC equipment Agilent 1200 l Series equipped with binary pump. Tandem ESI source (Bruker Daltonics, MA, USA), operated in positive mode 2 l/min nebulizer gas (Nitrogen) and drying gas 6 l/min at 200 °C (nitrogen), needle voltage (4500), shield voltage (600 V). Mass spectrometer Micro TOFQ II Bruker Daltonics (MA,

USA), operating in positive scan mode (from *m/z* 100 to *m/z* 800) calibrated using NaCOOH (10 mM). Software for data analysis: Hystar 3.2 (Bruker Daltonics, MA, USA). Chromatographic separations were performed by vacuum–liquid chromatography (VLC), column chromatography (CC) on silica gel 60 (0.063–0.200 mm), radial chromatography with a radial Chromatotron Model 7924 T on silica gel 60 PF_{254} Merck (1 mm thick), and prep. TLC on silica gel 60 F_{254} (0.2 mm thick) plates.

4.2. Plant material

Voucher specimens of *J. cabrerae* and *J. reflexa* are deposited at the herbarium of Museo Botánico Córdoba (CORD), Universidad Nacional de Córdoba. *J. reflexa* was collected in Departament Loncopué, Neuquén, Argentina, in February 2007 (Barboza et al. 1805). *J. cabrerae* was collected in Departament Belén, Catamarca, Argentina, in February 2008 (Barboza et al. 1990).

4.3. Extraction and isolation of compounds from J. reflexa and J. cabrerae

The air-dried powdered aerial parts of *J. reflexa* (85 g) were exhaustively extracted with EtOH, and the solvent evaporated under reduced pressure. The resulting residue was defatted by partition in *n*-hexane-EtOH–H_2O (10:3:1) with the EtOH–H_2O phase washed with *n*-hexane (3 × 100 ml), and the EtOH evaporated under reduced pressure. The residue was diluted with H_2O and extracted with CH_2Cl_2 (3 × 200 ml). The combined CH_2Cl_2 extracts were dried (Na_2SO_4), filtered, and evaporated to dryness under reduced pressure. The residue (2.6 g) was fractionated initially by VLC. Elution with *n*-hexane–EtOAc mixtures of increasing polarity (100:0–0:100) and EtOAc–MeOH (100:0–95:05) afforded six fractions containing withanolides. Fractions I-II (380 mg) were applied to silica gel 60 G using *n*-hexane–EtOAc mixtures of increasing polarity to afford a mixture that was further fractionated by prep. TLC (CH_2Cl_2–MeOH, 96:4), yielding a mixture (4 mg) which could not be separated by either normal phase TLC or reversed phase TLC. The ^{1}H NMR spectrum of this mixture indicated that this mixture consisted of compounds **2** and **3** in a 3:2 ratio. Fractions III–V (560 mg) were pooled and applied to a silica gel 60 G column. Elution with *n*-hexane–EtOAc mixtures of increasing polarity (90:10–0:100) afforded a mixture (70 mg) that was further processed by prep. TLC (CH_2Cl_2-MeOH, 98:02), yielding compound **1** (8.4 mg). From fraction VI (EtOAc 100%), compound **2** (88 mg) precipitated.

The air-dried powdered aerial parts of *J. cabrerae* (288 g) were exhaustively extracted with EtOH; the solvent was evaporated at reduced pressure. The residue obtained (36 g) was defatted by partition in *n*-hexane-EtOH–H_2O (10:3:1), the resulting EtOH–H_2O phase was washed with *n*-hexane (3 × 600 ml), and the EtOH evaporated at reduced pressure. The residue was diluted with H_2O and extracted with CH_2Cl_2 (4 × 200 ml). The CH_2Cl_2 extract was dried (Na_2SO_4), filtered, and evaporated to dryness under reduced pressure. The residue (4.9 g) was initially fractionated by VLC. Elution with *n*-hexane–EtOAc mixtures of increasing polarity (100:0–0:100) and EtOAc–MeOH (100:0–80:20) afforded five fractions containing withanolides. These fractions (1.2 g) were pooled and subjected to silica gel 60 G CC. Elution with *n*-hexane–EtOAc mixtures of increasing polarity (100:0–0:100) and EtOAc–MeOH (100:0–80:20) afforded eleven fractions containing withanolides. Fraction I (20 mg) was fractionated by prep. TLC with CH_2Cl_2-MeOH, 94:6 yielding (in order of elution) compound **2** (2.8 mg), trechonolide A (**8**) (2 mg), and jaborosalactone 32 (**9**) (1.5 mg). From fraction II jaborosalactone 38 (**6**) (438 mg) precipitated. Fraction III (22 mg) was fractionated by prep. TLC with CH_2Cl_2–MeOH, 94:6, yielding jaborosalactone 39 (**7**) (3.5 mg). Fractions IV–XI were pooled and processed by radial chromatography with CH_2Cl_2–MeOH mixtures of increasing polarity, yielding jaborosalactone R (**4**) (6.6 mg), and jaborosalactone S (**5**) (6.4 mg).

The mass spectrum of **3**, was measured using UPLC-ESI-QTOF/MS from the compounds **2** and **3** mixture. Detection was carried out using a PDA detector scanning between 200–800 nm, monitoring at 244 nm. A combination of three columns were used: Eclipse XDB-C18 (i.d. 3.0 mm × 100 mm; 1.8 μm), Zorbax Eclipse XDB-C18 (i.d 3.0 mm × 50 mm; 1.8 μm), and Zorbax Eclipse Plus Phenyl–Hexyl (i.d. 2.0 mm × 150 mm; 2 μm). The columns oven operated at 40 °C. Mobile phase and elution conditions were as followed: (A) 0.5% HCOOH in ultrapure H_2O; (B) 0.5% HCOOH in CH_3CN (HPLC grade, Merck). Flow 0.3 ml/min; program: from 90% A (*t* = 0 min) to 90% B (*t* = 20 min); 90% A (*t* = 21 min) and stabilization during 10 min before next injection. Injection volume was 40 μl (MeOH).

4.3.1. Jaborosalactol 28 (17R,20S,22R,24S,25S,26R)-16α-acetoxy-22,26:24,25-diepoxy-26-hydroxyergost-2,5-dien-1-one **(1)**

White amorphous powder; $[\alpha_D^{21}]$ + 2.7 (c 0.37, MeOH); UV (MeOH) λ_{max} (log ε) 244 (3.18) nm; IR (dry film) ν_{max} 3432 (s), 2961 (s), 2852 (s), 1704 (s), 1665 (s), 1680 (s), 1459 (s), 1381 (s), 1237 (s), 1069 (s), 848 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$–D_2O (95:05), 400.13 MHz) δ 6.81 (1H, *ddd*, *J* = 10.0,4.9,2.4 Hz, H-3), 5.90 (1H, *dd*, *J* = 10.0,2.4 Hz, H-2), 5.60 (1H, *d*, *J* = 5.5 Hz, H-6), 5.04 (1H, *brt*, *J* = 7.3 Hz, H-16), 4.97 (1H, *s*, H-26), 3.58 (1H, *dt*, *J* = 11.5,2.9 Hz, H-22), 3.30 (1H, *brd*, *J* = 21.3 Hz, H-4β), 3.08 (1H, *dd*, *J* = 12.9,5.3 Hz, H-11α), 2.90 (1H, *dd*, *J* = 21.3,4.9 Hz, H-4α), 2.69 (1H, *t*, *J* = 12.9 Hz, H-11β), 2.30 (1H, *dd*, *J* = 11.7,6.8 Hz, H-17), 2.16 (1H, *td*, *J* = 12.0,5.3 Hz, H-9), 2.07 (3H, *s*, H-CH_3CO-16), 2.01 (1H, *m*, H-7β), 1.97 (1H, *m*, H-15β), 1.96 (1H, *m*, H-23α), 1.84 (1H, *m*, H-8), 1.78 (1H, *m*, H-23β), 1.75 (1H, *m*, H-20), 1.68 (2H, *m*, H-14, H-15α), 1.67 (1H, *m*, H-7α), 1.42 (3H, *s*, H-28), 1.41 (3H, *s*, H-27), 1.30 (3H, *s*, H-19), 1.11 (3H, *s*, H-18), 0.87 (3H, *d*, J = 6.9 Hz, H-21); ^{13}C NMR ($CDCl_3$, 100.63 MHz) δ 212.6 (C, C-12), 202.8 (C, C-1), 170.9 (C, CH_3*C*O-16), 145.5 (CH, C-3), 135.9 (C, C-5), 127.8 (CH, C-2), 124.2 (CH, C-6), 91.8 (CH, C-26), 78.3 (CH, C-16), 65.3 (C, C-24), 65.0 (CH, C-22), 63.9 (C, C-25), 57.4 (C, C-13), 54.9 (CH, C-14), 50.9 (C, C-10), 49.3 (CH, C-17), 46.5 (CH, C-9), 39.3 (CH_2, C-11), 37.7 (CH, C-20), 34.2 (CH_2, C-15), 33.3 (CH_2, C-4), 32.5 (CH, C-8), 30.4 (CH_2, C-7), 29.7 (CH_2, C-23), 21.2 (CH_3, *C*H_3CO-16), 18.9 (CH_3, C-28), 18.6 (CH_3, C-19), 16.5 (CH_3, C-27), 12.8 (CH_3, C-21), 12.6 (CH_3, C-18); ESIMS *m/z* 535 $[M + Na]^+$ (100), 454 (28), 413 (13), 342 (20), 315 (9), 277 (5), 105 (64); HRESIMS *m/z* $[M + Na]^+$ 535.2675 (calcd. for $C_{30}H_{40}O_7Na$, 535.2672).

4.3.2. Jaborosalactone 45 (20R,22R)-12α,21-epoxy-12β,17β-dihydroxy-1-oxo-witha-2,5,24-trien-26,22-olide **(2)**

Colorless crystals (*n*-hexane–EtOAc), mp 243–244 °C; $[\alpha_D^{21}]$ + 31.9 (c 0.67, MeOH); UV (MeOH) λ_{max} (log ε) 244 (3.94) nm; IR (dry film) ν_{max} 3432 (s), 3058 (w), 2965 (s), 1692 (s), 1661 (s), 1385 (s), 1299 (s), 731 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ 6.79 (1H, *ddd*, *J* = 10.0,5.0,2.5 Hz, H-3), 5.87 (1H, *dd*, *J* = 10.0,2.5 Hz, H-2), 5.58 (1H, *d*, *J* = 5.9 Hz, H-6), 4.36 (1H, *ddd*, *J* = 12.4,8.3,3.4 Hz, H-22), 3.95 (1H, *dd*, *J* = 11.2,5.2 Hz, H-21β), 3.63 (1H, *t*, *J* = 11.2 Hz, H-21α), 3.28 (1H, *brd*, *J* = 21.3 Hz, H-4β), 2.94 (1H, *ddd*, *J* = 11.2,8.3,5.2 Hz, H-20), 2.85 (1H, *dd*, *J* = 21.3,5.0 Hz, H-4α), 2.58 (1H, *m*, H-23β), 2.52 (1H, *dd*, *J* = 12.1,3.2 Hz, H-11α), 2.21 (1H, *dd*, *J* = 17.5,2.0 Hz, H-23α), 2.12 (1H, *m*, H-16β), 2.05 (1H, *m*, H-7β), 1.94 (3H, *s*, H-28), 1.91 (1H, *m*, H-9), 1.88 (1H, *m*, H-11β), 1.87 (3H, *s*, H-27), 1.83 (1H, *m*, H-16α), 1.63 (1H, *m*, H-15α), 1.62 (1H, *m*, H-8), 1.60 (1H, *m*, H-7α), 1.52 (1H, *m*, H-15β), 1.48 (1H, *m*, H-14), 1.23 (3H, *s*, H-19), 1.07 (3H, *s*, H-18); ^{13}C NMR ($CDCl_3$, 100.63 MHz) δ 203.9 (C, C-1), 165.2 (C, C-26), 149.3 (C, C-24), 145.4 (CH, C-3), 135.9 (C, C-5), 127.9 (CH, C-2), 124.5 (CH, C-6), 121.9 (C, C-25), 100.7 (C, C-12), 79.0 (C, C-17), 76.5 (CH, C-22), 59.9 (CH_2, C-21), 50.8 (C, C-10), 50.6 (CH, C-14), 50.2 (C, C-13), 47.5 (CH, C-20), 41.1 (CH, C-9),

37.9 (CH_2, C-11), 35.4 (CH_2, C-16), 35.1 (CH_2, C-23), 33.3 (CH_2, C-4), 31.6 (CH, C-8), 30.6 (CH_2, C-7), 24.3 (CH_2, C-15), 20.4 (CH_3, C-28), 18.9 (CH_3, C-19), 12.4 (CH_3, C-27), 12.2 (CH_3, C-18); EIMS *m/z* 451 (26), 281 (43), 207 (34), 125 (100), 67 (56), 43 (92); HRESIMS *m/z* $[M + Na]^+$ 491.2408 (calcd. for $C_{28}H_{36}O_6Na$, 491.2410).

*4.3.3. Jaborosalactone 46 (20R,22R)-12α,21-epoxy-12,17-dihydroxy-1-oxowitha-3,5,24-trien-26,22-olide (**3**)[a]*

^1H NMR ($CDCl_3$, 400.13 MHz) δ 6.06 (1H, *brd*, *J* = 9.6 Hz, H-4), 5.65 (1H, *m*, H-3), 5.62 (1H, *m*, H-6), 4.35 (1H, *m*, H-22), 3.85 (1H, *dd*, *J* = 11.4,5.4 Hz, H-21β), 3.65 (1H, *t*, *J* = 11.4 Hz, H-21α), 3.28 (1H, *m*, H-2a), 2.95 (1H, *m*, H-20), 2.76 (1H, *dd*, *J* = 20.5,5.4 Hz, H-2b), 2.59 (1H, *m*, H-23β), 2.11 (1H, *m*, H-7β), 2.20 (1H, *dd*, *J* = 18.0,3.4 Hz, H-23α), 2.09 (1H, *m*, H-11α), 2.08 (1H, *m*, H-9), 1.95 (3H, *s*, H-28), 1.88 (3H, *s*, H-27), 1.80 (1H, *m*, H-7α), 1.71 (1H, *m*, H-8), 1.69 (1H, *m*, H-11β), 1.65 (1H, *m*, H-15α), 1.54 (1H, *m*, H-15β), 1.51 (1H, *m*, H-14), 1.37 (3H, *s*, H-19), 1.07 (3H, *s*, H-18); ^{13}C NMR ($CDCl_3$, 100.63 MHz) δ 210.2 (C, C-1), 165.1 (C, C-26), 149.3 (C, C-24), 140.8 (C, C-5), 129.1 (CH, C-4), 126.7 (CH, C-3), 121.8 (C, C-25), 121.6 (CH, C-6), 100.6 (C, C-12), 78.8 (C, C-17), 76.4 (CH, C-22), 59.9 (CH_2, C-21), 51.7 (C, C-10), 51,0 (CH, C-14), 50.7 (C, C-13), 47.4 (CH, C-20), 39.5 (CH_2, C-2), 39.2 (CH, C-9), 36.9 (CH_2, C-11), 35.4 (CH_2, C-7), 35.4 (CH_2, C-16), 35.3 (CH_2, C-23), 30.1 (CH, C-8), 24.1 (CH_2, C-15), 20.4 (CH_3, C-28), 20.3 (CH_3, C-19), 12.4 (CH_3, C-27), 12.2 (CH_3, C-18). HRESI-QTOFMS *m/z* $[M + Na]^+$ 491.2409 (calcd. for $C_{28}H_{36}O_6Na$, 491.2410).[a] These data were obtained from a mixture of **2** and **3** compounds.

4.4. Preparation of jaborosalactone 38 derivatives

*4.4.1. Preparation of (20R,22R)-5α-Acetoxy-12α,21-epoxy-6,12,17-trihydroxy-1-oxowitha-2,5,24-trien-26,22-olide (**10**)*

To a soln. of jaborosalactone 38 (**6**) (20 mg, 0.04 mmol) in AcOH (3 ml), $(NH_4)OAc$ (67 mg, 0.80 mmol) was added. The reaction mixture was heated until reflux began, this being maintained for 48 h until disappearance of the starting material had occurred. Once the solvent was removed, the residue was purified by prep. TLC using EtOAc to obtain compound **10** (5.6 mg, 25%) as an amorphous solid.

White amorphous powder; $[\alpha_D^{21}]$ + 33.6 (c 0.56, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 239 (3.71), 220 (3.73) nm; IR (dry film) ν_{max} 3440, 2932, 1688, 1382, 1255, 1023 cm^{-1}; ^1H NMR ($CDCl_3$, 500.13 MHz) δ 6.56 (1H, *ddd*, *J* = 10.1,5.4,1.7 Hz, H-3), 5.85 (1H, *dd*, *J* = 10.1,2.7 Hz, H-2), 4.82 (1H, *brs*, H-6), 4.36 (1H, *ddd*, *J* = 11.8,8.2,3.2 Hz, H-22), 3.97 (1H, *dd*, *J* = 11.3,5.3 Hz, H-21β), 3.70 (1H, *t*, *J* = 11.3 Hz, H-21α), 3.51 (1H, *dd*, *J* = 20.4,5.4 Hz, H-4α), 3.10 (1H, *dt*, *J* = 20.4,2.5 Hz, H-4β), 2.91 (1H, *ddd*, *J* = 11.3,8.2,5.3 Hz, H-20), 2.66 (1H, *m*, H-23β), 2.61 (1H, *dd*, *J* = 13.3,4.1 Hz, H-11α), 2.22 (1H, *brd*, *J* = 17.7 H, H-23α), 2.16 (1H, *m*, H-16β), 2.09 (1H, *m*, H-9), 2.00 (1H, *m*, H-8), 1.94 (3H, *s*, H_3-28), 1.92 (3H, *s*, $COCH_3$), 1.88 (3H, *s*, H_3-27), 1.82 (1H, *m*, H-16α), 1.76 (1H, *d*, *J* = 13.3 Hz, H-11β), 1.68 (1H, *dt*, *J* = 14.0,3.2 Hz, H-7β), 1.63 (2H, *m*, H_2-15), 1.52 (1H, *m*, H-14), 1.38 (1H, *m*, H-7α), 1.33 (3H, *s*, H_3-19), 1.09 (3H, *s*, H_3-18); ^{13}C NMR ($CDCl_3$, 100.63 MHz) δ 202.6 (C, C-1), 170.4 (C, $COCH_3$), 165.1 (C, C-26), 149.2 (C, C-24), 140.7 (CH, C-3), 128.3 (CH, C-2), 121.9 (C, C-25), 100.7 (C, C-12), 89.5 (C, C-5), 78.9 (C, C-17), 76.4 (CH, C-22), 67.4 (CH, C-6), 60.0 (CH_2, C-21), 51.9 (C, C-10), 51.2 (C, C-13), 50.0 (CH, C-14), 47.3 (CH, C-20), 39.4 (CH, C-9), 37.5 (CH_2, C-11), 35.3 (CH_2, C-16), 35.1 (CH_2, C-23), 33.3 (CH_2, C-7), 28.2 (CH_2, C-4), 27.8 (CH, C-8), 24.2 (CH_2, C-15), 22.3 (CH_3, $COCH_3$), 20.3 (CH_3, C-28), 15.4 (CH_3, C-19), 12.5 (CH_3, C-18), 12.3 (CH_3, C-27); ESIMS *m/z* 567 $[M + Na]^+$ (100), 507(8), 413 (9), 301(13); HRESIMS *m/z* $[M + Na]^+$ 567.2571 (calcd. for $C_{30}H_{40}O_9Na$, 567.2570).

*4.4.2. Preparation of (20R,22R)-5,6:12α,21-diepoxy-12,17-dihydroxy-3-methoxy-1-oxowitha-24-en-26,22-olide (**11**)*

A soln. of jaborosalactone 38 (**6**) (20 mg, 0.04 mmol) and MeOH (5 ml) was heated until reflux began, this being maintained for 24 h. After completion of the reaction, the reaction mixture was conc. and the residue subjected to prep. TLC using EtOAc to afford **11** (8.5 mg, 40%) and jaborosalactone R (**4**) (11.0 mg, 55%).

White amorphous solid; $[\alpha_D^{21}]$ -3.4 (c 1.46, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 240 (3.62)nm; IR (dry film) ν_{max} 3423, 2931, 1702, 1458, 1303 cm^{-1}; ^1H NMR ($CDCl_3$, 400.13 MHz) δ 4.33 (1H, *ddd*, *J* = 12.1,8.4,3.4 Hz, H-22), 3.85 (1H, *dd*, *J* = 11.1,5.3 Hz, H-21β), 3.74 (1H, *qd*, *J* = 5.9,2.1 Hz, H-3α), 3.57 (1H, *t*, *J* = 11.4, H-21α), 3.28 (3H, *s*, OCH_3), 3.23 (1H, *brs*, H-6), 2.93 (1H, *ddd*, *J* = 11.7,8.4,5.3 Hz, H-20), 2.75 (2H, *d*, *J* = 6.0 Hz, H-2), 2.59 (1H, *t*, *J* = 14.0 Hz, H-23β), 1.94 (3H, *s*, H-28), 1.87 (3H, *s*, H-27), 1.16 (3H, *s*, H-19), 0.99 (3H, *s*, H-18); ^{13}C NMR ($CDCl_3$, 100.63 MHz) δ 210.4 (C, C-1), 165.0 (C, C-26), 149.2 (C, C-24), 122.0 (C, C-25), 100.4 (C, C-12), 78.7 (C, C-17), 76.3 (CH, C-22), 72.6 (CH, C-3), 62.1 (C, C-5), 61.3 (CH, C-6), 60.2 (CH_2, C-21), 56.1 (CH_3, OCH_3), 51.6 (C, C-13), 51.2 (C, C-10), 50.5 (CH, C-14), 47.2 (CH, C-20), 42.7 (CH_2, C-2), 40.8 (CH, C-9), 36.7 (CH_2, C-11), 36.2 (CH_2, C-4), 35.5 (CH_2, C-16), 35.2 (CH_2, C-23), 31.3 (CH_2, C-7), 27.6 (CH, C-8), 24.2 (CH_2, C-15), 20.4 (CH_3, C-28), 13.7 (CH_3, C-19), 12.4 (CH_3, C-27), 12.1 (CH_3, C-18); ESIMS *m/z* 539 $[M + Na]^+$ (100), 499 (5), 301 (6); HRESIMS *m/z* $[M + Na]^+$ 539.2629 (calcd. for $C_{29}H_{40}O_8Na$, 539.2621).

*4.4.3. Preparation of (20R,22R)-5,6:12α,21-diepoxy-12,17-dihydroxy-3-N-benzyl-1-oxowitha-24-en-26,22-olide (**12**)*

To a soln. of jaborosalactone 38 (**6**) (20 mg, 0.04 mmol) in dry toluene (3 ml), benzylamine (10 μL, 0.048 mmol) was added. The reaction mixture was heated until reflux began, this being maintained for 72 h until all starting material was consumed. The solvent was then removed and the residue purified by prep.TLC using EtOAc to render jaborosalactone R (**4**) (3.0 mg, 15%) and compound **12** (5.4 mg, 22%).

White amorphous solid; $[\alpha_D^{21}]$ + 15.0 (c 0.52, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 239 (3.71) nm; IR (dry film) ν_{max} 3437, 2929, 1706, 1190, 1014 cm^{-1}; ^1H NMR ($CDCl_3$, 400.13 MHz) δ 7.32 (2H, *m*, H-3′/H-5′), 7.27 (3H, *m*, H-2′/H-6′ and H-4′), 4.34 (1H, *ddd*, *J* = 12.0,8.4,3.4 Hz, H-22), 3.81 (1H, *m*, H-21β), 3.75 (1H, *d*, *J* = 13.2 Hz, $PhCH_2N$ a), 3.71 ((1H, *d*, *J* = 13.2 Hz, $PhCH_2N$ b), 3.53 (1H, *dd*, *J* = 11.3,8.5 Hz, H-21α), 3.24 (1H, *m*, H-3α), 3.23 (1H, *brs*, H-6), 2.84 (1H, *m*, H-20), 2.72 (1H, *dd*, *J* = 13.3,6.0 Hz, H-2α), 2.55 (1H, *dd*, *J* = 13.3,6.2 Hz, H-2β), 2.52 (1H, *m*, H-23β), 2.29 (1H, *dd*, *J* = 14.6,5.0 Hz, H-4α), 2.26 (1H, *m*, H-11α), 2.20 (1H, *m*, H-23α), 2.17 (1H, *m*, H-7β), 2.12 (1H, m, H-16β), 1.95 (3H, *s*, H-28), 1.88 (3H, *s*, H-27), 1.87 (1H, *m*, H-11β), 1.75 (1H, m, H-16α), 1.67 (1H, *m*, H-8), 1.58 (2H, *m*, H-15), 1.55 (1H, *m*, H-9), 1.41 (1H, *m*, H-4β), 1.34 (1H, *m*, H-14), 1.33 (1H, *m*, H-7α), 1.17 (3H, *s*, H-19), 1.01 (3H, *s*, H-18); ^{13}C NMR ($CDCl_3$, 100.63 MHz) δ 211.1 (C, C-1), 165.0 (C, C-26), 149.2 (C, C-24), 139.8 (C, C-1′), 128.5 (x2, CH, C-3′/C-5′), 128.0 (x2, CH, C-2′/C-6′), 127.2 (CH, C-4′), 122.0 (C, C-25), 100.5 (C, C-12), 78.8 (C, C-17), 76.5 (CH, C-22), 62.8 (C, C-5), 62.0 (CH, C-6), 60.1 (CH_2, C-21), 51.2 (C, C-10), 51.1 (CH_2, $PhCH_2N$), 51.0 (C, C-13), 50.4 (CH, C-14), 49.6 (CH, C-3), 47.3 (CH, C-20), 43.4 (CH_2, C-2), 41.0 (CH, C-9), 37.7 (CH_2, C-11), 36.9 (CH_2, C-4), 35.5 (CH_2, C-16), 35.2 (CH_2, C-23), 31.2 (CH_2, C-7), 27.6 (CH, C-8), 24.2 (CH_2, C-15), 20.4 (CH_3, C-28), 14.4 (CH_3, C-19), 12.4 (CH_3, C-27), 12.1 (CH_3, C-18);); ESIMS *m/z* 614 $[M + Na]^+$ (38), 592 (100), 593 (40), 507 (21), 413 (5), 301 (8); HRESIMS *m/z* $[M + Na]^+$ 614.3093 (calcd. for $C_{35}H_{45}NO_7Na$, 614.3094).

*4.4.4. Preparation of (20R,22R)-5,6:12α,21-diepoxy-12,17-dihydroxy-1-oxo-3-thiophenyl-witha-24-en-26,22-olide (**13**) and (20R, 22R)-5, 6:12α,21-diepoxy-17-hydroxy-12-methoxy-1-oxo-3-thiophenyl-witha-24-en-26,22-olide (**14**)*

To jaborosalactone 38 (**6**) (20 mg, 0.04 mmol) dissolved in a mixture of CH_2Cl_2/ MeOH (4:1) (6 ml), 8 μL of thiophenol (0.08 mmol) was added. The reaction mixture was stirred for 5 h at room temperature. Once the reaction was completed, the reaction mixture was conc. and the residue subjected to prep.TLC purification using EtOAc to yield compound **13** (6.1 mg, 25%) and compound **14** (10.0 mg, 40%).

Compound **13**: White amorphous solid; $[\alpha_D^{21}]$ + 33.1 (c 0.61, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 240 (3.64) nm; IR (dry film) ν_{max} 3440, 2937, 1708, 1302, 1099 cm^{-1}; ^1H NMR ($CDCl_3$, 400.13 MHz) δ 7.37 (2H, *dt*, *J* = 7.7,1.7 Hz, H-2′), 7.31 (2H, *brt*, *J* = 7.7 Hz, H-3′), 7.27 (1H, *brd*, *J* = 7.7 Hz, H-4′), 4.34 (1H, *ddd*, *J* = 12.0,8.2,3.2 Hz, H-22), 3.86 (1H, *dd*, *J* = 11.3,5.2 Hz, H-21β), 3.73 (1H, *qd*, *J* = 7.0,2.2 Hz, H-3α), 3.60 (1H, *t*, *J* = 11.3 Hz, H-21α), 3.28 (1H, *brs*, H-6), 2.94 (1H, *m*, H-20), 2.77 (1H, *dd*, *J* = 14.2,7.0 Hz, H-2α), 2.68 (1H, *dd*, *J* = 14.2,7.2 Hz, H-2β), 2.60 (1H, *brt*, *J* = 14.3 Hz, H-23β), 2.47 (1H, *dd*, *J* = 14.8, 6.3 Hz, H-4α), 2.25 (1H, *d*, *J* = 14.2 Hz, H-7β), 1.94 (3H, *s*, H-28), 1.87 (3H, *s*, H-27), 1.16 (3H, *s*, H-19), 1.00 (3H, *s*, H-18); ^{13}C NMR ($CDCl_3$, 100.63 MHz) δ 210.0 (C, C-1), 165.0 (C, C-26), 149.2 (C, C-24), 133.5 (C, C-1′), 132.2 (CH, C-2′), 129.2 (CH, C-3′), 127.7 (CH, C-4′), 122.0 (C, C-25), 100.3 (C, C-12), 78.7 (C, C-17), 76.3 (CH, C-22), 62.2 (C, C-5), 61.5 (CH, C-6), 60.2 (CH_2, C-21), 51.5 (C, C-13), 51.2 (C, C-10), 50.5 (CH, C-14), 47.2 (CH, C-20), 42.0 (CH_2, C-2), 41.0 (CH, C-9), 39.3 (CH, C-3), 36.7 (CH_2, C-11), 35.9 (CH_2, C-4), 35.5 (CH_2, C-16), 35.2 (CH_2, C-23), 31.1 (CH_2, C-7), 27.6 (CH, C-8), 24.2 (CH_2, C-15), 20.4 (CH_3, C-28), 13.6 (CH_3, C-19), 12.4 (CH_3, C-27), 12.1 (CH_3, C-18);); ESIMS *m/z* 617 $[M + Na]^+$ (100), 521 (43), 507 (20), 413 (5), 301 (4); HRESIMS *m/z* $[M + Na]^+$ 617.2546 (calcd. for $C_{34}H_{42}O_7SNa$, 617.2549).

Compound **14**: White amorphous solid; $[\alpha_D^{21}]$ + 3.6 (c 0.98, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 240 (3.80) nm; IR (dry film) ν_{max} 3465, 2939, 1714, 1306, 1102 cm^{-1}; ^1H NMR ($CDCl_3$, 500.13 MHz) δ 7.39 (2H, *dt*, *J* = 7.7, 1.6 Hz, H-2′), 7.32 (2H, *brd*, *J* = 7.7 Hz, H-3′), 7.29 (1H, *brd*, *J* = 7.7 Hz, H-4′), 4.34 (1H, *ddd*, *J* = 11.4,7.9,3.2 Hz, H-22), 3.77 (1H, *dd*, *J* = 11.3,5.5 Hz, H-21β), 3.73 (1H, *qd*, *J* = 7.2,1.9 Hz, H-3α), 3.62 (1H, *t*, *J* = 11.3 Hz, H-21α), 3.30 (1H, *brs*, H-6), 3.22 (3H, *s*, OCH_3), 2.81 (1H, *m*, H-20), 2.77 (1H, *dd*, *J* = 14.0,7.0 Hz, H-2α), 2.71 (1H, *dd*, *J* = 14.0,7.4 Hz, H-2β), 2.60 (1H, *brt*, *J* = 14.5 Hz, H-23β), 2.46 (1H, *dd*, *J* = 14.7,6.4 Hz, H-4α), 2.26 (1H, *brd*, *J* = 14.4 Hz, H-7β), 2.19 (1H, *m*, H-23α), 2.15 (1H, *m*, H-16β), 1.95 (3H, *s*, H-28), 1.88 (3H, *s*, H-27), 1.82 (1H, *m*, H-11α), 1.80 (1H, *m*, H-16α), 1.67 (1H, *m*, H-8), 1.65 (1H, *m*, H-15β), 1.54 (1H, *m*, H-15α), 1.52 (1H, *m*, H-4β), 1.46 (1H, *m*, H-7α), 1.45 (1H, *m*, H-9), 1.40 (1H, *m*, H-11β), 1.38 (1H, *m*, H-14), 1.16 (3H, *s*, H-19), 0.95 (3H, *s*, H-18); ^{13}C NMR ($CDCl_3$, 100.63 MHz) δ 210.1 (C, C-1), 165.1 (C, C-26), 149.1 (C, C-24), 133.6 (C, C-1′), 132.3 (CH, C-2′), 129.2 (CH, C-3′), 127.8 (CH, C-4′), 122.0 (C, C-25), 102.7 (C, C-12), 78.9 (C, C-17), 76.4 (CH, C-22), 62.3 (C, C-5), 61.4 (CH, C-6), 60.2 (t, C-21), 51.8 (C, C-13), 51.4 (C, C-10), 50.5 (CH, C-14), 47.9 (CH_3, OCH_3), 47.1 (CH, C-20), 42.0 (CH_2, C-2), 40.8 (CH, C-9), 39.3 (CH, C-3), 35,9 (CH_2, C-4), 35.4 (CH_2, C-16), 35.3 (CH_2, C-23), 31.2 (CH_2, C-7), 30.4 (CH_2, C-11), 27.8 (CH, C-8), 24.1 (CH_2, C-15), 20.4 (CH_3, C-28), 13.4 (CH_3, C-19), 12.4 (CH_3, C-27), 12.1 (CH_3, C-18); ESIMS *m/z* 631 $[M + Na]^+$ (100), 617 (5), 413 (4), 301 (5); HRESIMS *m/z* $[M + Na]^+$ 631.2709 (calcd. for $C_{35}H_{44}O_7SNa$, 631.2705).

4.5. Biological Assays

4.5.1. Materials

All starting materials were commercially available research-grade chemicals and used without further purification. RPMI 1640 medium was purchased from Flow Laboratories (Irvine, UK), fetal calf serum (FCS) was from Gibco (Grand Island, NY), trichloroacetic acid (TCA) and glutamine were from Merck (Darmstadt, Germany), and penicillin G, streptomycin, dimethyl sulfoxide (DMSO) and sulforhodamine B (SRB) were from Sigma (St Louis, MO).

4.5.2. Cells, culture and plating

The human solid tumor cell lines HBL-100 (breast), HeLa (cervix), SW1573 (non-small cell lung), T-47D (breast) and WiDr (colon) were used in this study. These cell lines were a kind gift from Prof. Godefridus J. Peters (VU Medical Center, Amsterdam, The Netherlands) and Dr. Rubén P. Machín (HUGC Dr. Negrín, Las Palmas de Gran Canaria, Spain). Cells were maintained in 25 cm^2 culture flasks in RPMI 1640 supplemented with 5% FBS and 2 mM L-glutamine at 37 °C, 5% CO_2, 95% humidified air incubator. Exponentially growing cells were trypsinized and resuspended in antibiotic containing medium (100 units penicillin G and 0.1 mg of streptomycin per ml). Single cell suspensions displaying > 97% viability by trypan blue dye exclusion were subsequently counted. After counting, dilutions were made to establish the appropriate cell densities for inoculation onto 96-well microtiter plates. Cells were inoculated in a volume of 100 μL per well at densities of 10 000 (HBL-100 and SW1573), 15 000 (HeLa and T-47D), and 20 000 (WiDr) cells per well, based on their doubling times.

4.5.3. Chemosensitivity testing

Chemosensitivity tests were performed using the SRB assay of the NCI with slight modifications. Briefly, pure compounds were initially dissolved in DMSO at 400 times the desired final maximum test concentration. Control cells were exposed to an equivalent concentration of DMSO (0.25% v/v, negative control). Each agent was tested in triplicate at different dilutions in the range 1–100 μM. The drug treatment was started on day 1 after plating. Drug incubation times were 48 h, after which cells were precipitated with 25 μL ice-cold Cl_3CO_2H-H_2O (1:1, v/v) and fixed for 60 min at 4 °C. The SRB assay was then performed. The optical density (OD) of each well was measured at 492 nm, using BioTek's PowerWave XS Absorbance Microplate Reader. Values were corrected for background OD from wells only containing medium. The percentage growth (PG) was calculated with respect to untreated control cells (C) at each drug concentration level based on the difference in OD at the beginning (T_0) and end of drug exposure (T), according to NCI formulas (Monks et al., 1991). Therefore, if T is greater than or equal to T_0, the calculation is $100 \times [(T - T_0)/(C - T_0)]$. If T is lower than T_0 denoting cell killing, the calculation is $100 \times [(T - T_0)/(T_0)]$. The effect is defined as percentage of growth, where 50% growth inhibition (GI_{50}) represents the concentration at which PG is +50. With these calculations a PG value of 0 corresponds to the amount of cells present at the beginning of drug exposure, while negative PG values denote net cell kill.

Acknowledgement

This work was supported by Grants from CONICET (Argentina), Ministerio de Ciencia y Tecnología de Córdoba (Argentina), Myndel Botanica Foundation, SeCyT-UNC, EU-FEDER: the Spanish MICINN (SAF2009-13296-C02-01 and CTQ2008-06806-C02-01/BQU), MSC (RTICC RD06/0020/1046), Canary Islands' ACIISI (PI 2007/021), FUNCIS (PI 43/09), and ICIC. Thanks are also given to Dr. A. A. Cocucci (IMBIV-UNC) for providing the photographs of *J. caulescens* and L. Ribulgo for preparing the color figures. M.E.G. thanks CONICET (Argentina) for a fellowship. NMR assistance by G. Bonetto is gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2011.12.018.

References

Bado, S., Mareggiani, G., Amiano, N., Burton, G., Veleiro, A.S., 2004. Lethal and sublethal effects of withanolides from *Salpichroa origanifolia* and analogues on *Ceratitis capitata*. J. Agric. Food Chem. 52, 2875–2878.

Barboza, G., 1986. Una nueva especie de *Jaborosa* (Solanaceae). Kurtziana 18, 89–92.

Barboza, G.E., Hunziker, A., 1987. Estudios sobre Solanaceae XXV. Revisión de *Jaborosa*. Kurtziana 19, 77–153.

Bessalle, R., Lavie, D., 1992. Withanolide C, a chlorinated withanolide from *Withania somnifera*. Phytochemistry 31, 3648–3651.

Bonetto, G.M., Gil, R.R., Oberti, J.C., Veleiro, A.S., Burton, G., 1995. Novel withanolides from *Jaborosa sativa*. J. Nat. Prod. 58, 705–711.

Chen, L., He, H., Qiu, F., 2011. Natural withanolides: an overview. Nat. Prod. Rep. 28, 705–740.

Fajardo, V., Freyer, A., Minard, R., Shamma, M., 1987. (+)-Jaborol, an unusual phenolic withanolide from *Jaborosa magellanica*. Tetrahedron 43, 3875–3880.

Glotter, E., 1991. Withanolides and related ergostane-type steroids. Nat. Prod. Rep. 8, 415–440.

Hunziker, A.T., 2001. The Genera of Solanaceae Illustrated, Arranged According to a New System, Gantner, A.R.G. Verlag, K.-G. (Eds.), Ruggell, p. 500.

Kennelly, E.J., Gerhäuser, C., Song, L.L., Graham, J.G., Beecher, C.W., Pezzuto, J.M., Kinghorn, A.D., 1997. Induction of quinone reductase by withanolides isolated from *Physalis philadelphica* (tomatillos). J. Agric. Food Chem. 45, 3771–3777.

Lavie, D., Besalle, R., Pestchanker, M.J., Gottlieb, H.E., Frolow, F., Giordano, O.S., 1987. Trechonolide A, a new withanolide type from *Trechonaetes laciniata*. Phytochemistry 26, 1791–1795.

Machín, R.P., Veleiro, A.S., Nicotra, V.E., Oberti, J.C., Padrón, J.M., 2010. Antiproliferative activity of withanolides against human breast cancer cell lines. J. Nat. Prod. 73, 966–968.

Misico, R.I., Song, L.L., Veleiro, A.S., Cirigliano, A.M., Tettamanzi, M.C., Burton, G., Bonetto, G.L., Nicotra, V.E., Silva, G.L., Gil, R.R., Oberti, J.C., Kinghorn, A.D., Pezzuto, J.M., 2002. Induction of quinone reductase by withanolides. J. Nat. Prod. 65, 677–680.

Misico, R.I., Nicotra, V., Oberti, J.C., Barboza, G., Gil, R.R., Burton, G., 2011. Withanolides and related steroids. Progress in the Chemistry of Organic Natural Products 94, 127–229.

Monks, A., Scudiero, D., Skehan, P., Shoemaker, R., Paul, K., Vistica, D., Hose, C., Langley, J., Cronise, P., Vaigro-Wolff, A., Gray-Goodrich, M., Campbell, H., Mayo, J., Boyd, M., 1991. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. J. Natl. Cancer Inst. 83, 757–766.

Moré, M., Cocucci, A.A., Barboza, G., Raguso, R.A., 2009. Evolución de caracteres florales mediada por polinizadores en el género *Jaborosa* (Solanaceae). XXXII Jornadas Argentinas de Botánica, Huerta Grande. Córdoba, Argentina.

Nicotra, V.E., Gil, R.R., Vaccarini, C., Oberti, J.C., Burton, G., 2003. 15, 21-Cyclowithanolides from Jaborosa bergii. J. Nat. Prod. 66, 1471–1475.

Nicotra, V.E., Ramacciotti, N.S., Gil, R.R., Oberti, J.C., Feresin, G.E., Guerrero, C.A., Baggio, R.F., Garland, M.T., Burton, G., 2006. Phytotoxic withanolides from *Jaborosa rotacea*. J. Nat. Prod. 69, 783–789.

Nicotra, V.E., Gil, R.R., Oberti, J.C., Burton, G., 2007. Withanolides from *Jaborosa caulescens* var. *Caulescens* and *J. caulescens* var. *bipinnatifida* with phytotoxic activity. J. Nat. Prod. 70, 808–812.

Pan, Y., Wang, X., Hu, X., 2007. Cytotoxic withanolides from the flowers of *Datura metel*. J. Nat. Prod. 70, 1127–1132.

Ramacciotti, N.S., Nicotra, V.E., 2007. Withanolides from *Jaborosa kurtzii*. J. Nat. Prod. 70, 1513–1515.

Ray, A.B., Gupta, M., 1994. Withasteroids, a growing group of naturally occurring steroidal lactones. Prog. Chem. Org. Nat. Prod. 63, 1–106.

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J.T., Bokesch, H., Kenney, S., Boyd, M.R., 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer Inst. 82, 1107–1112.

Su, B., Misico, R.I., Santarsiero, B.D., Mesecar, A.D., Fong, H.H.S., Pezzuto, J.M., Kinghorn, A.D., 2002. Isolation and characterization of bioactive principles of the leaves and stems of *Physalis philadelphica*. Tetrahedron 58, 3453–3466.

Veleiro, A.S., Oberti, J.C., Burton, G., 2005. Chemistry and bioactivity of withanolides from South American Solanaceae, in: Atta-ur-Rahman (Ed.), Stud. Nat. Prod. Chem. Elsevier Science Publishers, Amsterdam, 32, pp 1019–1052.

Yousuf, S.K., Majeed, R., Ahmad, M., Sangwan, P.I., Purnima, B., Saxsena, A.K., Suri, K.A., Mukherjee, D., Taneja, S.C., 2011. Ring A structural modified derivatives of withaferin A and evaluation of their cytotoxic potential. Steroids 76, 1213–1222.