In vitro Cytotoxicity of Norditerpenoid Alkaloids

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Forty-three norditerpenoid alkaloids isolated from *Aconitum*, *Delphinium* and *Consolida* species have been evaluated for their cytotoxic effects on the tumor cell lines CT26 (murine colon adenocarcinoma), SW480 (human colon adenocarcinoma), HeLa (human cervical adenocarcinoma), SkMel25 (human melanoma) and SkMel28 (human malignant melanoma) with several multidrug resistance mechanisms and the non-tumor cell line CHO (Chinese hamster ovary cells). Neoline (**5**), 8-*O*-methylcolumbianine (**6**), 1,14-diacetylcardiopetaline (**9**), 18-*O*-demethylpubescenine (**13**), 14-deacetylpubescenine (**14**), pubescenine (**15**), 14-deacetylajadine (**25**), lycoctonine (**26**), browniine (**28**), delphatine (**29**), dehydrotakaosamine (**34**), and ajadelphinine (**37**) exhibited selective cytotoxicity to cancerous *versus* non-cancerous cells. Some of these compounds had an irreversible effect on SW480 (**5**, **15**, **25**, **26**, and **34**), HeLa (**15**, **34**, and **37**) and SkMel25 (**15** and **34**) cell lines. In order to gain insights into the mechanism of irreversible cytotoxic action of these compounds we compared the cell viability by means of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) and the acid phosphatase (AP) methods. Our results suggest that the effects of these compounds could be related to the inhibition of ATP production.

Key words: Norditerpenoid Alkaloids, Cytotoxicity, Tumor Cells

Introduction

Plant species of the genera *Aconitum*, *Delphinium*, and *Consolida* are known sources of C_{19}-norditerpene (NDAs) and C_{20}-diterpene alkaloids (DAs) and they are of pharmacological and economic importance (Atta-ur-Rahman and Choudhary, 1999; Panter *et al.*, 2002). NDAs act as potent nicotinic cholinergic receptor (nAcChR) agonists and antagonists in invertebrates, including insects, and vertebrates (see Panter *et al.*, 2002; Seitz and Ameri, 1998). The insecticidal and antifeedant activity of NDAs (Jennings *et al.*, 1986; Ulubelen *et al.*, 2001; González-Coloma *et al.*, 2004a) suggest a plant defensive role played by these compounds. NDAs are well known pharmacologically for their anti-inflammatory, analgesic, anti-arrythmia and antifungal actions (Atta-ur-Rahman and Choudhary, 1999). The biological actions of DAs are less known. There are a few reports on their plant defensive and pharmacological properties, including their effects on *Trypanosoma cruzi* epimastigote forms and anti-leishmanial properties (Bessonova and Shaidkhozaeva, 2000; González-Coloma *et al.*, 1998, 2004b; González *et al.*, 2005; Li *et al.*, 2002a, b; Ulubelen *et al.*, 2001), however, their neurotoxic effects are unknown. The selective cytotoxic effects of some of these structures indicate that NDAs and DAs can act on biological targets other than neuroreceptors with strong molecular selectivity (González-Coloma *et al.*, 2004a, b). However, little is known on their effects on human tumor cells.

Here we report on the cytotoxic effects of 43 NDAs isolated from *Aconitum*, *Delphinium* and *Consolida* species (Figs. 1–4) against mammalian CHO cells and the tumor cell lines CT26 (murine colon adenocarcinoma), SW480 (human colon adenocarcinoma), HeLa (human cervical adenocarcinoma), SkMel25 (human melanoma) and SkMel28 (human malignant melanoma). These cell lines express different resistance mechanisms including the multidrug resistance (MDR) phenotype, due to the overexpression of any of the energy-dependent drug efflux transmembrane proteins, such as the *P*-glycoprotein (Pgp), or the

multidrug resistance protein (MRP1) (Higgins, 1992; Ling, 1997; Cole and Deeley, 1998; Klein *et al.*, 1999), and the intracellular glutathione/glutathione *S*-transferase detoxification system (GSH/GST) which protects and detoxifies cells from highly reactive free radicals and organic peroxides and metabolizes xenobiotics (Zhang *et al.*, 1998). Among the cell lines used, HeLa expresses intermediate levels of GSH-conjugate export activity; CT26 expresses low levels of Pgp and MRP1 and murine GSTs; SkMel28 expresses GSHpx and GSTs and low levels of MRP1; SW480 has elevated levels of Pgp with low levels of MRP1 and the γ-glutamylcysteine synthetase (γ-GCS) responsible for *de novo* synthesis of GSH (for references see De Inés *et al.*, 2004). The SkMel25 cell line was included in this study for comparative purposes since these cells have a low invasive and metastatic potential (Suter *et al.*, 1985; Rass *et al.*, 2001) as opposite to SkMel28 cells. Additionally, in order to gain insight into the cytotoxic action of the active compounds we compared the cell viability by means of the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] and the AP (acid phosphatase) methods.

Materials and Methods

Materials

Compounds **1**–**43** (Figs. 1–4) were isolated from *Aconitum, Consolida* and *Delphinium* species (González-Coloma *et al.*, 2004a). RPMI 1640, fetal bovine serum (FBS), L-glutamine and penicillin/streptomycin were from GIBCO-BRL. Rotenone, MTT and *p*-nitrophenylphosphate were from Sigma-Aldrich. Taxol was a gift from Dr. L. Gunatilaka (University of Arizona, USA). The compounds were dissolved freshly and diluted in culture medium before their addition to the cell cultures.

Cell lines and culture conditions

Mammalian Chinese hamster ovary cells (CHO) (a gift from Dr. Pajares, ICB, CSIC, Spain), murine colon adenocarcinoma (CT26), human colon adenocarcinoma (SW480), human cervical adenocarcinoma (HeLa), human melanoma (SkMel25) and human malignant melanoma (SkMel28) (from Deutsches Krebsforschungszentrum, DKFZ, Heidelberg, Germany) cells were grown as previously described (De Inés *et al.*, 2004).

Cytotoxicity assays

Cell viability was analyzed by means of an adaptation of the MTT colorimetric assay method (Mossman, 1983) as previously described (De Inés *et al.*, 2004). In brief, cells in the logarithmic growth phase were added to 96-well flat-bottom microtiter plates (Falcon) and incubated for 6 d with different concentrations of the compounds dissolved in absolute ethanol. For reversibility experiments, cells were incubated with the minimal cytotoxic concentration (MIC) of each compound, washed three times with fresh culture medium and cultured in compound-free medium for different periods of time. Three independent experiments were carried out in duplicate.

Acid phosphatase (AP) method

In order to gain insights about the mechanism of action of the irreversibly cytotoxic compounds, cell viability was also measured by the AP method which determines the cellular acid phosphatase activity (Martin and Clynes, 1991). Cells were incubated for 6 d according to De Inés *et al.* (2004). To validate the experiment, the sensitive SkMel25 cells were incubated with Taxol® (dissolved in absolute ethanol) and rotenone (dissolved in dimethyl sulfoxide, DMSO). The viability of SkMel25 cells treated under the same conditions with the residual concentration of DMSO was $\geq$ 95%. Three independent experiments were carried out in duplicate.

Results and Discussion

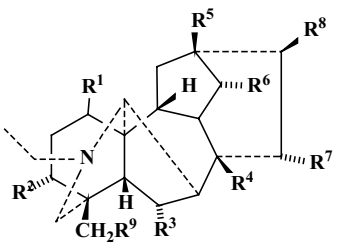
Table I shows the minimal inhibitory concentration (MIC) of the test compounds required to produce a cytotoxic effect on the different cell lines. CHO cells were sensitive to a few compounds randomly distributed among the chemical classes with MIC values ranking as follows: **36** > **30** > **35**, **41** > **24** > **11**. Overall CT26 and SW480 cells were sensitive to the largest number of compounds (33%) followed by SkMel25 (31%), HeLa (24%) and SkMel 28 (12%) cells. HeLa cells showed the lowest MIC value. The different cellular range of action of these compounds could be related to factors such as intracellular transportation, metabolism, inactivation and receptor geometry.

The cytotoxicity of the test alkaloids followed different patterns for each chemical class. Among the aconitine-type alkaloids (Fig. 1), **5** was the most active compound (HeLa, SW480, CT26,

Table I. Minimal inhibitory concentration (MIC) of the active test compounds, classified by chemical type, on several mammalian cell lines.

Compound[a]		MIC [μg/ml]					
		CHO	CT26	SW480	HeLa	SkMel25	SkMel28
5	Aconitine-type	> 100	25	12.50	6.25	25	> 100
6		> 100	50	50	> 100	50	> 100
9		> 100	100	100	> 100	> 100	> 100
11	Lycoctonine-type	100	100	100	> 100	100	100
13		> 100	> 100	> 100	25	50	50
14		> 100	> 100	> 100	> 100	50	> 100
15		> 100	100	25	50	50	> 100
24		50	50	50	> 100	> 100	50
25		> 100	> 100	100	50	100	> 100
26		> 100	50	50	> 100	> 100	> 100
29		> 100	> 100	> 100	100	> 100	> 100
30		12.50	12.50	50	50	100	100
34	Gadesine-type	> 100	6.25	6.25	0.40	6.25	25
35		25	50	25	25	25	> 100
36		6.25	12.50	12.50	12.50	25	6.25
37	Miscellaneous type	> 100	50	25	12.50	25	> 100
41		25	50	100	> 100	100	> 100

[a] Compounds **1**–**4**, **7**, **8**, **10**, **12**, **16**–**23**, **27**, **28**, **31**–**33**, **38**–**40**, **42** and **43** had MIC values > 100 μg/ml for all the cell lines tested.

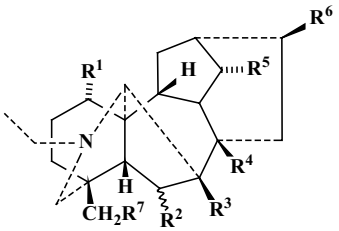


	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	R^9
Aconitine (**1**)	OMe	OH	OMe	OAc	OH	OBz	OH	OMe	OMe
3-Acetylaconitine (**2**)	OMe	OAc	OMe	OAc	OH	OBz	OH	OMe	OMe
8-*O*-Ethyl-14-benzoylaconine (**3**)	OMe	OH	OMe	OCH_2CH_3	OH	OBz	OH	OMe	OMe
8-*O*-Ethylaconine (**4**)	OMe	OH	OMe	OCH_2CH_3	OH	OH	OH	OMe	OMe
Neoline (**5**)	OH	H	OMe	OH	H	OH	H	OMe	OMe
8-*O*-Methylcolumbianine (**6**)	OH	H	H	OMe	H	OH	H	OMe	OH
Karakoline (**7**)	OH	H	H	OH	H	OH	H	OMe	H
Cardiopetaline (**8**)	OH	H	H	OH	H	OH	H	H	H
1,14-Diacetylcardiopetaline (**9**)	OAc	H	H	OH	H	OAc	H	H	H

Fig. 1. Aconitine-type structures.

SkMel25) followed by **6** (CT26, SW480, SkMel25) and **9** with low-moderate activity (CT26, SW480). Methylation at C-6 plus hydroxylation/methylation at C-8 (**5** and **6**) determined the activity of these compounds. Acetylation at C-1 and C-14 resulted in a moderate-low increase of activity (**9** in contrast to the inactive **7** and **8**). Compound **9** was cytotoxic to insect Sf9 cells (González-Coloma *et al.*, 2004a).

Among the lycoctonine-type alkaloids (Fig. 2), compound **11**, acetylated at C-1 and bearing OAc/H groups at C-14/C-18, moderately affected CHO, CT26, SW480, SkMel25 and SkMel28 cells, in contrast to the inactive compound **10**, hydroxylated at C-1 and C-14. The C-6α epimers **13**, **14**, and **15**, with OAc/OH, OMe/OH, OMe/OMe C-14/C-18 combinations, affected a lower number of cell lines (CT26, **15**; SW480, **15**, **11**; HeLa, **13**, **15**; SkMel25,



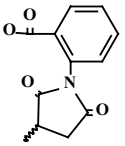
	R^1	R^2	R^3	R^4	R^5	R^6	R^7
Cardiopetalidine (**10**)	OH	H	OH	OH	OH	H	H
1,14-*O*-Acetylcardiopetalidine (**11**)	OAc	H	OH	OH	OAc	H	H
8-*O*-Methylconsolarine (**12**)	OH	αOH	OH	OMe	OH	OMe	H
18-*O*-Demethylpubescenine (**13**)	OH	αOH	OH	OMe	OAc	OMe	OH
14-Deacetylpubescenine (**14**)	OH	αOH	OH	OMe	OH	OMe	OMe
Pubescenine (**15**)	OH	αOH	OH	OMe	OAc	OMe	OMe
Consolidine (**16**)	OH	αOH	OH	OMe	OMe	OMe	OMe
18-*O*-Benzoyl-18-*O*-demethyl-14-*O*-deacetylpubescenine (**17**)	OH	αOH	OH	OMe	OH	OMe	OBz
14-*O*-Acetyldeltatsine (**18**)	OH	βOMe	OH	OMe	OAc	OMe	OMe
14-*O*-Acetyldelcosine (**19**)	OH	βOMe	OH	OH	OAc	OMe	OMe
Delsoline (**20**)	OH	βOMe	OH	OH	OMe	OMe	OMe
Takaosamine (**21**)	OH	βOMe	OH	OH	OH	OMe	OH
Gigactonine (**22**)	OH	βOMe	OH	OH	OMe	OMe	OH
Delcosine (**23**)	OH	βOMe	OH	OH	OH	OMe	OMe
Ajadine (**24**)	OMe	βOMe	OH	OH	OAc	OMe	OCOPhNHAc
14-Deacetylajadine (**25**)	OMe	βOMe	OH	OH	OH	OMe	OCOPhNHAc
Lycoctonine (**26**)	OMe	βOMe	OH	OH	OMe	OMe	OH
14-*O*-Acetyldelectinine (**27**)	OMe	βOMe	OH	OH	OAc	OMe	OH
Browniine (**28**)	OMe	βOMe	OH	OH	OH	OMe	OMe
Delphatine (**29**)	OMe	βOMe	OH	OH	OMe	OMe	OMe
Methyllycaconitine (**30**)	OMe	βOMe	OH	OH	OMe	OMe	

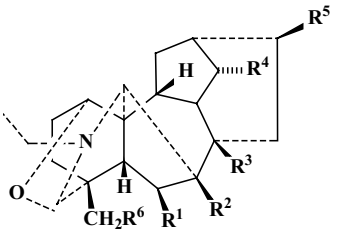
Fig. 2. Lycoctonine-type structures.

13, **14**, **15** and SkMel28, **13**) with varying potencies. Compounds **24**, **25**, **26**, **29** and **30**, methylated at C-1, β-methylated at C-6 and bearing OAc, OH/OCOPhNHAc, OMe/OMe and OMe/methylsuccinylantranoyl C-14/C-18 combinations, affected all the cell lines (CHO, **24**, **26**; CT26 and SW480, **30**, **24**, **26**; HeLa and SkMel25, **25**, **30** and SKMel28, **24**, **30**) with varying potencies. Among these lycoctonine-type alkaloids, **10**, **13**, **14**, **19** and **23** resulted cytotoxic to insect Sf9 cells (González-Coloma *et al.*, 2004a), indicating cell-dependent selectivity of action for these compounds except for **13** and **14**.

The most active alkaloids were found among the gadesine-type (Fig. 3). All the cell lines responded to **34**, **36** and **35** with varying potencies. Compound **36** was the most cytotoxic to CHO and SkMel28 while **34** was the most cytotoxic to CT26, SW480, HeLa and SkMel25 cells, indicating a selective structure-dependent cytotoxicity for this chemical class of compounds. Hydroxylation at both C-14/C-18 determined a strong cytotoxic effect (**34**) while complete or partial methylation at C-14/C-18 (**36** or **35**) reduced the potency of this effect for the sensitive cell lines. Compound **36** was also cytotoxic to Sf9 cells (González-Coloma *et al.*, 2004a).

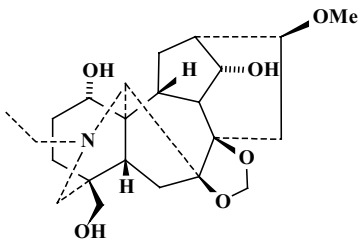
Among the miscellaneous compounds (Fig. 4), all the tumor cell lines, except SkMel28, responded to **37**, with a 7,8-methylenedioxy group, followed by **41** with a 19-oxo and C-1/C-18 diacetyl group (the structurally related **20** and **22** were not cytotoxic); this compound was also toxic to Sf9 cells (González-Coloma *et al.*, 2004a).

The selective cytotoxic effects of some structures indicate that these compounds can act on

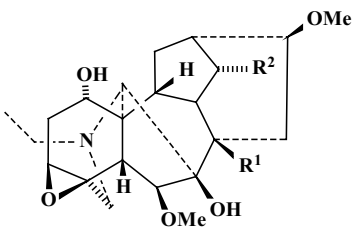


	R^1	R^2	R^3	R^4	R^5	R^6
Gadesine (**31**)	OMe	OH	OH	OH	OMe	H
14-*O*-Benzoylgadesine (**32**)	OMe	OH	OH	OBz	OMe	H
18-Hydroxy-14-*O*-methylgadesine (**33**)	OMe	OH	OH	OMe	OMe	OH
Dehydrotakaosamine (**34**)	OMe	OH	OH	OH	OMe	OH
18-*O*-Methoxygadesine (**35**)	OMe	OH	OH	OH	OMe	OMe
Dehydrodelsoline (**36**)	OMe	OH	OH	OMe	OMe	OMe

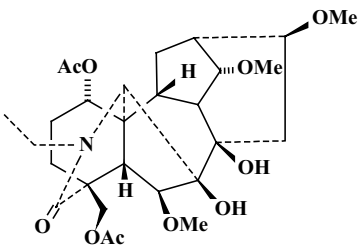
Fig. 3. Gadesine-type structures.



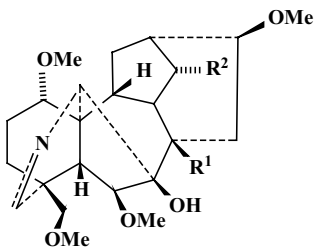
Ajadelphinine (**37**)



	R^1	R^2
Tuguaconitine (**38**)	OH	OMe
14-Demethyltuguaconitine (**39**)	OH	OH
14-Demethyldelboxine (**40**)	OMe	OH



1,18-*O*-Diacetyl-19-oxo-gigactonine (**41**)



Olividine (**42**); R^1 = OMe; R^2 = OAc
Olivimine (**43**); R^1 = OH; R^2 = OMe

Fig. 4. Miscellaneous structures.

biological targets other than neuroreceptors with strong molecular selectivity as previously demonstrated for several alkaloids belonging to different chemical classes (Wink *et al.*, 1998). The cytotoxic activity of the compounds studied here did not follow the expected structure-activity relationship from their reported receptor binding activity (Kukel and Jennings, 1994; Hardick *et al.*, 1996; Dobelis *et al.*, 1999; Panter *et al.*, 2002). The C-14 benzoyl group of nAcChR agonists **1** and **2** and related compounds **3**, **31** and **32** resulted in null cytotoxicity. The C-18 methylsuccinylanthranoyl substituent in the antagonist methyllycaconitine (**30**) resulted in a more potent cytotoxic action than that of the C-18 benzoyl (**24** or **25**).

To determine if the cytotoxic effects of the selective compounds (cytotoxic to tumoral cells vs. CHO cells) were reversible, the recovery of sensi-

Table II. Reversibility of the cytotoxic effect of selective compounds on cell viability. Cells were incubated with their respective MIC value for each compound (Table I).

Compound	Days	Reversibility (%)[a]					
		CHO	CT26	SW480	HeLa	SkMel25	SkMel28
5	0	61 ± 4	12 ± 1	2 ± 0	16 ± 1	17 ± 1	–
	3	91 ± 9	106 ± 15	1 ± 0	55 ± 12	17 ± 1	–
	6	104 ± 0	–	33 ± 1	113 ± 38	73 ± 9	–
6	0	55 ± 12	13 ± 1	19 ± 1	–	19 ± 7	–
	3	72 ± 4	115 ± 5	83 ± 0	–	30 ± 3	–
	6	111 ± 0	–	–	–	101 ± 2	–
9	0	100 ± 10	6 ± 0	2 ± 0	–	–	–
	3	–	38 ± 5	61 ± 6	–	–	–
	6	–	100 ± 1	93 ± 0	–	–	–
13	0	94 ± 0	–	–	2 ± 0	18 ± 3	20 ± 2
	3	–	–	–	108 ± 2	56 ± 3	98 ± 3
	6	–	–	–	–	85 ± 0	–
14	0	41 ± 6	–	–	–	21 ± 3	–
	3	98 ± 0	–	–	–	91 ± 8	–
	6	–	–	–	–	–	–
15	0	45 ± 6	–	15 ± 0	6 ± 2	28 ± 0	–
	3	60 ± 3	–	13 ± 1	4 ± 0	17 ± 3	–
	6	89 ± 0	–	3 ± 0	6 ± 1	14 ± 4	–
25	0	54 ± 8	–	8 ± 1	0	18 ± 1	–
	3	80 ± 9	–	4 ± 0	38 ± 3	32 ± 1	–
	6	–	–	8 ± 0	104 ± 5	94 ± 0	–
26	0	60 ± 3	20 ± 0	9 ± 1	–	–	–
	3	78 ± 5	114 ± 15	3 ± 1	–	–	–
	6	–	–	24 ± 1	–	–	–
28	0	106 ± 0	22 ± 4	6 ± 1	–	–	–
	3	–	100 ± 8	40 ± 5	–	–	–
29	0	59 ± 4	–	–	16 ± 6	–	–
	3	106 ± 1	–	–	59 ± 8	–	–
	6	–	–	–	89 ± 10	–	–
34	0	74 ± 8	17 ± 2	8 ± 1	5 ± 0	19 ± 0	20 ± 1
	3	97 ± 0	109 ± 13	1 ± 0	6 ± 0	18 ± 0	14 ± 0
	6	–	–	3 ± 0	23 ± 1	35 ± 2	68 ± 0
37	0	67 ± 1	21 ± 2	15 ± 0	3 ± 0	20 ± 1	–
	3	93 ± 1	79 ± 5	11 ± 0	13 ± 1	22 ± 0	–
	6	–	–	74 ± 0	40 ± 10	89 ± 13	–

[a] Percentage cell viability (percent absorbance of the respective untreated control cells). Represented are mean values ± SE.

tive tumoral cells was studied (Table II). Compound **15** had irreversible effects on all treated cell lines followed by **34** which affected 3 of 5 cell lines, with SW480 being the most sensitive of all. Alkaloids **25**, **26** and **5** had a selective strong effect on the recovery of SW480 cells with **25** being the most potent. Alkaloid **37** selectively acted on HeLa cells with moderate potency.

In order to gain insights about the mechanism of action of the irreversibly cytotoxic compounds, the sensitive cells were incubated with the active alkaloids for 6 d and then the viability was determined by using the MTT assay and the AP method (Table III). As a positive control, the sensitive SkMel25 cell line was incubated with rotenone and Taxol® at MIC values of 0.01 μg/ml. Rotenone interrupts mitochondrial electron transfer at the NADH dehydrogenase-ubiquinone junction of the respiratory chain (Palmer *et al.*, 1968) and Taxol® binds specifically to microtubules and blocks normal microtubule dynamics and cell division (Schiff and Horwitz, 1980). The viability of SkMel25 cells incubated with Taxol® was similar when measured by both methods, as expected for a compound that has no effect on cellular respiration and ATP generation. However, incubation of SkMel25 cells

Compound	MIC	Cell line	MTT	AP
	[μg/ml]		Viability (%)[a]	
Taxol®	0.01	SkMel25	3 ± 0	5 ± 1
Rotenone	0.01	SkMel25	10 ± 1	42 ± 1
5	12.50	SW480	5 ± 0	16 ± 2
15	25	SW480	10 ± 1	19 ± 2
25	100	SW480	na	na
26	50	SW480	7 ± 2	20 ± 1
34	6.25	SW480	5 ± 0	20 ± 4
37	12.50	HeLa	4 ± 0	22 ± 1

Table III. Comparative cytotoxicity of the irreversible compounds on the sensitive cell lines, determined by the AP and MTT methods.

[a] Percentage cell viability (percent absorbance of the respective untreated control cells). Represented are mean values + SE.
na, not enough compound available.

with rotenone resulted in significantly different results for cell viability when measured by both methods.

The incubation of the sensitive lines with **5**, **15**, **25**, **26**, **34** and **37** gave higher cell viability values when measured with the AP method (Table III). Therefore, the mode of action of these compounds could be related to the inhibition of ATP production. A decreased ATP production could compromise the tumor cell metabolism because of the high demand of energy needed for tumor growth and drug resistance. This will explain why SW480 (Pgp+) cells, with higher energy demand related to their resistance mechanism, were the most sensitive to most of these compounds (**5**, **15**, **25**, **26** and **34**). HeLa and SkMel25 were the following more sensitive lines, suggesting that these cells have a high ATP demand maybe related to their resistance mechanism (HeLa with intermediate levels of GSH-conjugate export activity) and/or metabolism.

Cervical cancer is the second most common cancer among women world wide in developing countries (Parkin *et al.*, 1988; Leminen *et al.*, 1990). Colon cancer is a leading cause of death in the Western world and one of the most untreatable and therapy-resistant cancers (Gryfe *et al.*, 1997). Melanoma is a major medical problem characterized by both rapidly rising incidence and growing lifetime risk (Marks, 1995). Therefore, the irreversible and selective cytotoxic compounds **5**, **15**, **25**, **26**, **34**, and **37** could be considered candidate leads for the design of new drugs combating chemoresistant human colon, cervical and melanoma cancers.

In summary, the present results indicate that neoline (**5**), 8-*O*-methylcolumbianine (**6**), 1,14-diacetylcardiopetaline (**9**), 18-*O*-demethylpubescenine (**13**), 14-deacetylpubescenine (**14**), pubescenine (**15**), 14-deacetylajadine (**25**), lycoctonine (**26**), browniine (**28**), delphatine (**29**), dehydrotakaosamine (**34**), and ajadelphinine (**37**) have greater cytotoxic activity to cancerous *versus* noncancerous cells. Considering the cell viability data obtained from the MTT and AP analysis as well as the non-neural cell lines used in this study, the mode of action of these cytotoxic compounds could be related to low ATP levels. To our knowledge, this is the first report on the potential antitumor activity of such norditerpenoid alkaloids.

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