In Vitro Antitumor Activity of Sesquiterpene Lactones from *Lychnophora trichocarpha*

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ABSTRACT: The sesquiterpene lactones lychnopholide and eremantholide C were isolated from *Lychnophora trichocarpha* Spreng. (Asteraceae), which is a plant species native to the Brazilian Savannah or Cerrado and popularly known as arnica. Sesquiterpene lactones are known to present a variety of biological activities including antitumor activity. The present paper reports on the evaluation of the *in vitro* antitumor activity of lychnopholide and eremantholide C, in the National Cancer Institute, USA (NCI, USA), against a panel of 52 human tumor cell lines of major human tumors derived from nine cancer types. Lychnopholide disclosed significant activity against 30 cell lines of seven cancer types with IC_{100} (total growth concentration inhibition) values between 0.41 μM and 2.82 μM. Eremantholide C showed significant activity against 30 cell lines of eight cancer types with IC_{100} values between 21.40 μM and 53.70 μM. Lychnopholide showed values of lethal concentration 50 % (LC_{50}) for 30 human tumor cell lines between 0.72 and 10.00 μM, whereas eremantholide C presented values of LC_{50} for 21 human tumor cell lines between 52.50 and 91.20 μM. Lychnopholide showed an interesting profile of antitumor activity. The α-methylene-γ-lactone present in the structure of lychnopholide, besides two α,β-unsaturated carbonyl groups, might be responsible for the better activity and higher cytotoxicity of this compound in relation to eremantholide C.

Keywords: Lychnopholide, eremantholide C, sesquiterpene lactones, *Lychnophora trichocarpha*, antitumor.

RESUMO: Atividade antitumoral *in vitro* de lactonas sesquiterpenicas de Lychnophora. As lactonas sesquiterpênicas licnofolida e eremantolida C foram isoladas de *Lychnophora trichocarpha* Spreng. (Asteraceae), espécie vegetal nativa do cerrado brasileiro e popularmente conhecida por arnica brasileira. As lactonas sesquiterpênicas são conhecidas por apresentarem variadas atividades biológicas, incluindo atividade antitumoral. O presente artigo relata a avaliação da atividade antitumoral *in vitro* de licnofolida e eremantolida C frente a um painel de 52 linhagens de células tumorais, provenientes de tumores humanos referentes a nove principais tipos de câncer. Os testes foram conduzidos no National Cancer Institute, USA (NCI, USA). Licnofolida apresentou atividade significativa frente a 30 linhagens de células tumorais referentes a sete tipos de câncer, com valores de CI_{100} (concentração que inibe 100% do crescimento celular) entre 0,41 μM e 2,82 μM. Eremantolida C mostrou atividade significativa frente a 30 linhagens de células tumorais referentes a oito tipos de câncer, com valores de CI_{100} entre 21,40 μM e 53,70 μM. Licnofolida apresentou valores de concentração letal 50 % (CL_{50}) para 30 linhagens de células tumorais humanas entre 0,72 e 10,00 μM, enquanto eremantolida C mostrou valores de CL_{50} para 21 linhagens entre 52,50 e 91,20 μM. Licnofolida apresentou um interessante perfil de atividade antitumoral. A presença na estrutura química da licnofolida de uma α-metileno-γ-lactona, além de dois grupos ésteres α,β-insaturados, podem ser responsáveis pela melhor atividade e maior citotoxicidade desta substância em relação à eremantolida C.

Palavras-chave: Licnofolida, eremantolida C, lactonas sesquiterpênicas, *Lychnophora trichocarpha*, antitumoral.

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INTRODUCTION

Sesquiterpene lactones are natural products occurring in many plant families, but most widely distributed within the Asteraceae (Bohlmann et al., 1980). These substances are known to present a variety of biological effects including antitumor activity (Rodrigues et al., 1976).

Lychnophora trichocarpha Spreng. (*Asteraceae*), popularly known as Brazilian arnica, is a bush native to Brazil. Ethanol extract of species of this genus are used in Brazilian folk medicine to treat bruise, pain, rheumatism and inflammatory diseases (Saúde et al., 1998).

The sesquiterpene lactones lychnopholide (**1**) and eremantholide C (**2**) were isolated from *L. trichocarpha* and occur also in other species of *Asteraceae* (Saúde et al., 1998; Bohlmann & Jakupovic, 1990). We have previously reported on the trypanocidal, antifungal and antibacterial activities of compounds **1** and **2** (Oliveira et al., 1996; Barrero et al., 2000; Saúde et al, 2002). Lychnopholide and eremantholide C were reported to show anti-hyperuricemic activity and were also found to inhibit monosodium urate crystals-induced paw oedema in mice (de Souza et al., 2012). Lychnopholide and eremantholide C were able to reduce the carrageenan-induced paw oedema when ointment formulations were administered topically to mice. The anti-inflammatory effect of lychnopholide seems to involve the inhibition of NO production and increase IL-10 production. The mechanism of the effect of eremantholide C on the reduction of carrageenan-induced paw oedema may be attributed to inhibition of TNF-α production and stimulation of IL-10 production. Lychnopholide increased production of TNF-α (Ferrari et al., 2013) and showed potent cytotoxicity against HT-29 human colon cancer cells line besides exhibiting NFκB (p65) inhibitory activity (Ren et al., 2012).

In this paper, we report on the *in vitro* antitumor activity evaluation of sesquiterpene lactones **1** and **2.**

MATERIAL AND METHODS

Plant material

Lychnophora trichocarpha Spreng. was collected at Minas Gerais State, Brazil, in August, 1991. A voucher specimen (N^0 20635) is deposited at Herbarium of the Instituto de Ciências Exatas e Biológicas, UFOP, Ouro Preto, MG, Brazil.

Preparation of plant extracts and 1 and 2 isolation. The aerial parts of the plant (14.0 Kg) were dried at room temperature, for 1 week, reduced to powder and extracted with ethanol, at room temperature, for 1 week. The solvent was removed under reduced pressure to afford the ethanol extract (900.0 g). The extract (900.0 g) was submitted to column chromatography on silica gel eluting with n-hexane, n-hexane:ethyl acetate (1:1), ethyl acetate and methanol. The fraction eluted with hexane:ethyl acetate (1:1) presented, in TLC (hexane:ethyl acetate 60:40; cerium sulfate), white spots characteristics of sesquiterpene lactones This fraction was submitted to column chromatography on silica gel and elution with n-hexane:ethyl acetate (80:20) afforded compound **1** (4.0 g, colorless solid, mp 128-129 ^{o}C, ethanol). Elution with n-hexane:ethyl acetate (70:30) yielded **2** (28.0 g, colorless solid, mp 214-215 ^{o}C, ethyl acetate). The compounds were identified by NMR spectroscopy and by comparison with spectral literature data (Le Quesne et al. 1978; Bohlmann et al., 1980; Vichnewiski et al., 1989; Saúde et al., 1998; Saúde-Guimarães et al., 2007).

Lychnopholide (1): 2'-metil-2'-butenoato de 2, 3, 3a, 4, 5, 6, 7, 11a-octaidro-6,10-dimetil-3-metileno-2,7-dioxo-6,9-epoxiciclodeca[b]-furan-4-ila [3aR*, 4S*(z), 6R*, 10Z, 11aR*]].

IR (KBr) $n_{máx.}$ (cm^{-1}): 2900 (CH), 1770 (C=O, γ-lactone), 1710 (C=O, ketone), 1660 (C=C), 1590 (C=COR, furanone), 1450, 1370, 1350, 1300, 1270, 1230, 1140, 1100, 1040, 1030, 950, 920, 880, 850, 820, 760. **EIMS, m/z (rel. int.)**: 358 (M^+, $C_{20}H_{22}O_6$, 5), 295 (2), 275 (M - COC_4H_7, 6), 258 (M - $C_4H_7CO_2H$, 2), 239 (2), 232 (10), 220 (5), 206 (5), 189 (5), 167 (10), 155 (5), 149 (34), 141 (5), 127 (7), 113 (12), 97 (20), 85 (41), 83 ($C_4H_7CO^+$, 81), 71 (66), 57 (**100**). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5,72 (s, H-2), 6.01-6.00 (m, H-5), 5.31-5.29 (m, H-6), 3.73-3.71 (m, H-7), 4.52 (ddd, *J* = 2.0, 2.4, 12.0 Hz, H-8), 2.30 (dd, *J* = 2.0, 14.0 Hz, H-9a), 2.49 (dd, *J* = 12.0, 14.0 Hz, H-9b), 6.21 (d, *J* = 2.8 Hz, H-13a), 5.44 (d, *J* = 2.8 Hz, H-13b), 1.53 (s, H-14), 2.08 (t, *J* = 2.0 Hz, H-15), 6.10 (qq, *J* = 1.6, 7.2 Hz, H-3'), 1,88 (dq, *J* = 1.4, 7.3 Hz, H-4'), 1.78 (t, *J* = 1.6 Hz, H-5'). **^{13}C NMR** ($CDCl_3$, 75 MHz): δ 204.94 (C-1), 104.82 (C-2), 186.98 (C-3), 130.43 (C-4), 135.14 (C-5), 81.77 (C-6), 51.27 (C-7), 73.08 (C-8), 44.12 (C-9), 89.78 (C-10), 133.84 (C-11), 168.98 (C-12), 124.35 (C-13), 20.78 (C-14), 20.41 (C-15), 167.16 (C-1'), 126.50 (C-2'), 140.80 (C-3'), 20.11 (C-4'), 15.78 (C-5').

Eremantholide C (2): 6,9-epoxi-2H-1,4-dioxaciclodeca [c,d] pentaleno-2,7(4aH)-dioxano, 2a, 3, 5, 6, 11a, 11b-hexaidro-3-hidroxi-2a, 6, 10-trimetil-3-(1'-metiletenil)-2aR*, 3S*, 4aR*, 6S*, 10Z, 11aS*, 11bS*).

IR (KBr) ν máx. (cm^{-1}): 3450 (OH), 2900 (CH), 1770 (C=O, γ-lactone), 1700 (C=O, ketone), 1660 (C=C), 1590 (C=C-OR, furanone), 1450, 1370, 1350, 1320, 1270, 1220, 1200, 1150, 1100, 1060, 1040, 1000, 960, 920, 810, 730. **EIMS, m/z (rel. int.)**: 346 (M^+, $C_{19}H_{22}O_6$, 9); 328 (M - H_2O, 5), 302

(M - CO_2, 5), 277 (2), 260 (3), 245 (1), 234 (46), 219 (8), 206 (6), 189 (7), 175 (5), 165 (25), 149 (4), 135 (21), 122 (26), 111 (6), 95 (72), 77 (8), 69 (100). **^{1}H** NMR ($CDCl_3$, 300 MHz): 5,63 (s, H-2); 6,04-6,03 (m, H-5); 5,02-4,98 (m, H-6); 2,82 (dd, *J* = 4,3; 7,1 Hz, H-7); 4,09 (ddd, *J* = 2,5; 4,3; 12,0 Hz, H-8); 2,48 (dd, *J* = 2,5; 13,5 Hz, H-9a); 2,00 (dd, *J* = 12,0; 13,5 Hz, H-9b); 1,18 (s, H-13); 1,45 (s, H-14); 2,05 (t, *J* = 1,9 Hz, H-15); 5,31 (br s, H-2'a); 5,07 (t, *J* = 1,6 Hz); 1,91 (s, H-3'); 3,79 (s, OH). **^{13}C** NMR ($CDCl_3$, 75 MHz): 205,89 (C-1); 104,54 (C-2); 187,27 (C-3); 130,00 (C-4); 134,77 (C-5); 81,46 (C-6); 62,53 (C-7); 78,37 (C-8); 43,46 (C-9); 90,24 (C-10); 59,88 (C-11); 175,72 (C-12); 21,94 (C-13); 20,48 (C-14); 20,30 (C-15); 106,09 (C-16); 142,22 (C-1'); 115,80 (C-2'); 19,00 (C-3'). NMR data were assigned with the aid of 2D NMR experiments ^{1}H-^{1}H homonuclear correlation (COSY), ^{1}H-^{13}C direct (HETCOR), and long-range (HMBC) heteronuclear correlations.

Biological assay

Antitumor activity screening was carried out in the National Cancer Institute (NCI, USA) according to an *in vitro* protocol, based on a panel of 52 cell lines of major human tumors, derived from nine cancer types including leukemia, lung, colon, prostate, breast, CNS, melanoma, ovarian and renal tumors. A description of the rationale and methodology for the 52-cell line assay has been presented elsewhere (NCI, USA). Approaches to the analysis and display of the data provided by these assays have been described previously (NCI, USA).

SRB assay

Viable human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well microtiter plates plates in 100 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. The plates were incubated for 24 h at 37° C, 5 % CO2, 95 % air and 100 % relative humidity for 24 h before addition of experimental compounds to ensure that no contamination of the medium had occurred. Frozen solutions in dimethyl sulfoxide of compounds to test are thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 µg/ml gentamicin. Following compound addition, the plates are incubated for an additional 48 h at 37°C, 5 % CO2, 95 % air, and 100 % relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA and incubated for 60 minutes at 4°C. The supernatant is discarded, and the plates are washed five times with tap water and air dried to remove TCA. The fixed plates were then left to dry at room temperature for at least 24 h, after which the Sulforhodamine B (SRB) SRB assay was performed. SRB solution (100 µl) at solution (100 µl) at 0.4 % (w/v) in 1 % acetic acid is added to each well, and plates are incubated for 10 minutes at room temperature. After staining, unbound dye is removed by washing five times with 1 % acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of compounds at the five concentration levels (Ti)], the percentage growth is calculated at each of the concentrations levels. Percentage growth inhibition is calculated as:

[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz

[(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz.

Three dose response parameters are calculated for each compound. Growth inhibition of 50 % (IC_{50}) is calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the compound concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the compound incubation. The compound concentration resulting in total growth inhibition (IC_{100}) is calculated from Ti = Tz. The LC_{50} (concentration of compound resulting in a 50% reduction in the measured protein at the end of the treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested (NCI, USA).

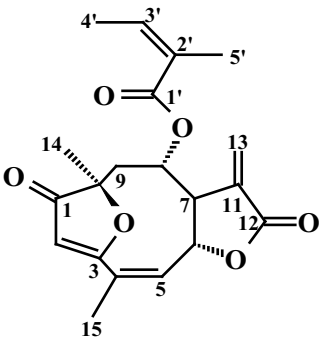
RESULTS AND DISCUSSION

Compounds **1** and **2** were evaluated in the National Cancer Institute (NCI, USA) by an *in vitro* disease-oriented antitumor screen, which determines antitumor effects against a panel of 52 humans tumor cell lines of major human tumors, derived from nine cancer types including leukemia, lung, colon, prostate, breast, CNS, melanoma, ovarian and renal tumors (NCI, USA).

Lychnopholide (**1**) total growth inhibition of 30 human tumor cell lines derived from eighth cancer types (Table 1), showing to be inactive only against prostate and CNS cancer. Lychnopholide showed significant activity against all leukemia human tumor cell lines tested with IC_{100} values between 0.43 µM and

1.51 µM and one lung (HOP-92, IC_{100} = 2.19 µM), five colon (COLO 205, HCT-116, HCT-15, KM-12, SW-620, 1.15 < IC_{100} < 2.63 µM), three melanoma (LOX-IMVI, SK-MEL-5, UACC-62, 0.41 < IC_{100} < 2.09 µM), four ovarian (IGROV1, OVCAR-3, OVCAR-4, OVCAR-8, 0.44 < IC_{100} < 2.75 µM), six renal (786-0, ACHN, CAKI-1, RXF-393, TK-10, UO-31, 0.65 < IC_{100} < 2.82 µM) and five breast (MCF7, MDA-MB-231/ATCC, MDA-MB-435, MDA-N, BT-549, 1.41 < IC_{100} < 2.19 µM) human tumor cell lines tested.

Eremantholide C (**2**) total growth inhibition of 30 human tumor cell lines and was inactive only against prostate cancer (Table 2). Compound **2** showed significant activity against all leukemia (22.40 < IC_{100} < 50.10 µM) and colon (21.90 < IC_{100} < 46.80 µM) human tumor cell lines tested, besides to present activity against one lung (HOP-92, IC_{100} = 41.70 µM), one CNS (U-251, IC_{100} = 37.10 µM), five melanoma (LOX-IMVI, M14, SK-MEL-2, SK-MEL-5, UACC-62, 21.40 < IC_{100} < 53.70 µM), two ovarian (OVCAR-3, OVCAR-5, 34.70 < IC_{100} < 38.90 µM), four renal (786-0, RXF-393, SN12-C, TK-10, 28.80 < IC_{100} < 37.10 µM) and four breast (MDA-MB-231/ATCC, MDA-N, BT-549, T-47D, 30.90 < IC_{100} < 53.70 µM) human tumor cell lines tested.



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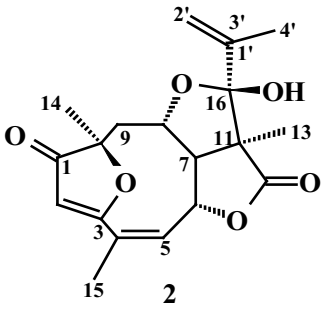

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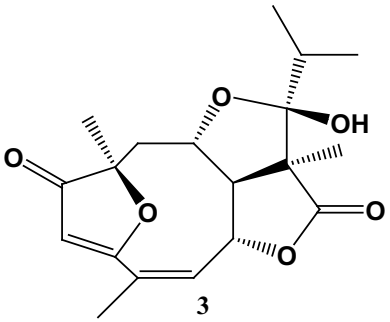

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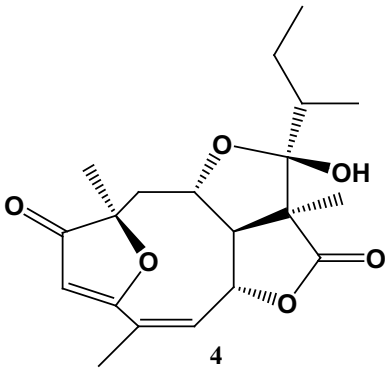

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Compound **1** showed higher cytotoxicity than **2** (Figure 1 and 2). Compound **1** presented LC_{50} values for 30 cell lines between 0.72 and 10.00 µM, whereas compound **2** showed values for 21 cell lines between 52.50 and 91.20 µM.

Kupchan and collaborators (1971) reported that cytotoxicity of sesquiterpene lactones is critically dependent upon the presence of the α-methylene-γ-lactone moiety. The endocyclic α,β– unsatured lactone appears not to confer cytotoxicity and the biological activity is enhanced by the presence of certain additional α,β-unsaturated carbonyl functions. The majority of the active germacranolides, except eremantholides, possess double bond conjugated to a lactone carbonyl group. On the basis of this study, the lower activity of **2** than **1** could be related to the absence of an α-methylene-γ-lactone unity in the structure of **2.** Compound **1** besides to possess an α-methylene-γ-lactone unity, to present others two α,β- unsaturated carbonyl groups. Nevertheless, the eremantholides were less cytotoxic (Mc Dougal et al., 1989).

Other eremantholides, such as eremantholide A (**3**) and eremantholide B (**4**), isolated from *Eremanthus efaeagnus* Schultz-Bip. (Asteraceae), showed significant *in vitro* inhibitory

TABLE 1. *In vitro* antitumor activity showed to lychnopholide (**1**) against humans tumor cell lines

	Concentrations (μM)		
Pannel / Cell Line	IC_{50}	IC_{100}	LC_{50}
Leukemia			
CCRF-CEM	0.14	0.56	4.79
HL-60 (TB)	0.12	0.43	4.90
K-562	**0.21**	**1.51**	49.00
MOLT-4	**0.21**	**0.98**	-
RPMI-8226	**0.11**	**0.48**	14.10
SR	0.13	0.54	0.72
Non-Small Cell Lung Cancer			
A549/ATCC	1.02	10.00	40.00
EKVX	3.72	17.40	> 100.00
HOP-62	4.27	15.80	40.00
HOP-92	0.54	2.19	6.46
NCI-H226	2.19	4.27	**8.13**
NCI-H23	0.87	3.02	**9.55**
NCI-H460	1.51	5.25	26.3
Colon cancer			
COLO 205	0.49	2.04	6.61
HCC-2998	1.66	3.09	**5.89**
HCT-116	0.42	1.74	4.57
HCT-15	0.24	1.02	3.98
HT-29	0.87	3.63	24.00
KM12	1.07	**2.63**	**6.46**
SW-620	0.23	1.15	5.75
CNS Cancer			
SF-295	2.63	6.46	29.5
SNB-19	13.8	28.80	60.3
U251	1.95	5.75	30.9
Melanoma			
LOX IMVI	0.17	0.41	1.00
M14	1.29	3.55	**10.00**
SK-MEL-2	1.35	3.16	**7.41**
SK-MEL-5	0.50	2.09	5.62
UACC-257	3.39	20.40	95.5
UACC-62	0.28	1.70	9.12
Ovarian cancer			
IGROV1	0.68	2.63	8.32
OVCAR-3	0.36	1.44	4.27
OVCAR-4	1.23	**2.75**	**6.17**
OVCAR-5	2.04	3.98	**7.59**
OVCAR-8	**0.18**	**0.44**	15.10
SK-OV-3	4.47	18.60	51.3
Renal Cancer			
786-0	0.36	1.48	3.89
ACHN	**0.39**	**2.82**	20.00
CAKI-1	0.23	0.65	3.02
RXF-393	0.08	1.02	4.79
SN12C	1.44	3.31	**7.76**
TK-10	0.59	1.91	4.37
UO-31	1.15	**2.45**	**5.37**
Prostate Cancer			

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TABLE 1. *In vitro* antitumor activity showed to lychnopholide (**1**) against humans tumor cell lines

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PC3	1.35	3.98	12.90
DU-145	3.63	14.40	41.70
Breast cancer			
MCF7	0.47	1.78	6.61
MCF/ADR-RES	1.44	12.9	> 100.00
MDA-MB 231/ATCC	0.47	1.78	4.57
HS 578T	3.80	20.00	93.30
MDA-MB-435	**0.28**	**2.19**	34.70
MDA-N	0.34	1.41	4,17
BT-549	0.48	2.19	7.41
T-47D	0.79	3.47	> 100.00

activity against KB cells (human carcinoma of the nasopharynx) (Raffauf et al., 1975; Le Quesne et al., 1978).

Alkylation of α,β- unsaturated carbonyl molecules by biological nucleophiles, in a Michael-type addition, is considered to be the general mechanism of action (Beekman et al., 1997) of compounds presenting this moiety. In the case of eremantholides, the eletrophilic center, that would be responsible for its antitumor activity, is the carbon five (Mc Dougal et al., 1989). Covalent binding of sesquiterpene lactones to free sulfydryl groups in proteins may interfere with the functions of these macromolecules. Consequently, sesquiterpene lactones would inhibit a large number of enzymes involved in key biological processes, namely DNA and RNA synthesis, protein and purine syntheses, glycolysis, citric acid cycle, and the mitochondrial

TABLE 2. *In vitro* antitumor activity showed to eremantholide C (**2**) against humans tumor cell lines

	Concentrations (μM)		
Pannel / Cell Line	**IC_{50}**	**IC_{100}**	**LC_{50}**
Leukemia			
CCRF-CEM	**5.25**	**26.30**	> 100.00
HL-60 (TB)	**4.79**	**22.40**	**89.10**
K-562	**6.76**	**33.90**	> 100.00
MOLT-4	**15.80**	**50.10**	> 100.00
RPMI-8226	**10.50**	**33.90**	> 100.00
SR	**7.94**	**41.70**	> 100.00
Non-Small Cell Lung Cancer			
A549/ATCC	31.60	> 100.00	> 100.00
EKVX	41.70	> 100.00	> 100.00
HOP-62	32.30	> 100.00	> 100.00
HOP-92	20.40	**41.70**	**83.20**
NCI-H226	38.90	58.90	**91.20**
NCI-H23	23.40	> 100.00	> 100.00
NCI-H460	**19.00**	58.90	> 100.00
Colon cancer			
COLO 205	20.40	**46.80**	> 100.00
HCC-2998	**12.30**	**25.70**	**52.50**
HCT-116	**13.20**	**26.90**	**53.70**
HCT-15	**11.50**	**27.50**	**66.10**
HT-29	24.00	**44.70**	**83.20**
KM12	**19.00**	**37.10**	**70.80**

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SW-620	**6.31**	**21.90**	**74.10**
CNS Cancer			
SF-295	20.90	64.60	> 100.00
SNB-19	> 100.00	> 100.00	> 100.00
U251	**17.40**	**37.10**	**81.30**
Melanoma			
LOX IMVI	**5.62**	**21.40**	**60.30**
M14	**19.00**	**53.70**	> 100.00
SK-MEL-2	**12.30**	**31.60**	**81.30**
SK-MEL-5	**13.80**	**32.20**	**75.90**
UACC-257	35.50	> 100.00	> 100.00
UACC-62	**14.50**	**38.90**	> 100.00
Ovarian cancer			
IGROV1	22.40	61.70	> 100.00
OVCAR-3	**15.80**	**34.70**	**77.60**
OVCAR-4	30.20	> 100.00	> 100.00
OVCAR-5	**19.50**	**38.90**	**77.60**
OVCAR-8	29.50	> 100.00	> 100.00
SK-OV-3	58.90	> 100.00	> 100.00
Renal Cancer			
786-0	**15.50**	**28.80**	**53.70**
ACHN	22.40	> 100.00	> 100.00
CAKI-1	24.50	58.90	> 100.00
RXF-393	**10.70**	**30.20**	**85.10**
SN12C	**14.80**	**30.90**	**63.10**
TK-10	20.40	**37.10**	**67.60**
UO-31	40.70	79.40	> 100.00
Prostate Cancer			
PC3	**19.50**	67.60	> 100.00
DU-145	34.70	> 100.00	> 100.00
Breast cancer			
MCF7	18,60	33,90	60.30
MCF/ADR-RES	20.00	> 100.00	> 100.00
MDA-MB 231/ATCC	**14.10**	**32.30**	**74.10**
HS 578T	44.70	> 100.00	> 100.00
MDA-MB-435	21.90	> 100.00	> 100.00
MDA-N	**10.50**	**40.73**	> 100.00
BT-549	**13.50**	**30.90**	**70.80**
T-47D	20.90	**53.70**	> 100.00

electron transport chain (Beekman et al., 1997).

CONCLUSIONS

Lychnopholide (**1**) showed an interesting profile of antitumor activity. The α-methylene-γ-lactone unity present in the molecule of lychnopholide, besides two α,β- unsaturated carbonyl groups, might be responsible for the better activity and higher citotoxicity of this compound than **2**. However, eremantholide C might be an interesting compound to be less cytotoxic.

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