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PRECLINICAL STUDIES

Modified ingenol semi-synthetic derivatives from *Euphorbia tirucalli* induce cytotoxicity on a large panel of human cancer cell lines

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Summary

The latex from *Euphorbia tirucalli* is used in Brazil as a folk medicine for several diseases, including cancer. Recently, we showed a cytotoxic activity of *E. tirucalli* euphol in a wide range of cancer cell lines. Moreover, we showed that euphol inhibits proliferation, motility and colony formation in pancreatic cancer cells, induces autophagy and sensitizes glioblastoma cells to temozolomide cytotoxicity. Herein, we report in vitro activity of three semi-synthetic ingenol compounds derived from *E. tirucalli*, IngA (ingenol-3-trans-cinnamate), IngB (ingenol-3-hexanoate) and IngC (ingenol-3-dodecanoate), against a large panel of human cancer cell lines. Antineoplastic effects of the three semi-synthetic compounds were assessed using MTS assays on 70 cancer cell lines from a wide array of solid tumors. Additionally, their antitumor potential was compared with known compounds of the same class, namely ingenol-3-angelate (Picato®) and ingenol 3,20-dibenzoate and in combination with standard chemotherapeutic agents. We observed that IngA, B, and C exhibited dose-dependent cytotoxic effects. Amongst the semi-synthetic compounds, IngC displayed the best activity across the tumor cell lines. In comparison with ingenol-3-angelate and ingenol 3,20-dibenzoate, IngC showed a mean of 6.6 and 3.6-fold higher efficacy, respectively, against esophageal cancer cell lines. Besides, IngC sensitized esophageal cancer cells to paclitaxel treatment. In conclusion, the semi-synthetic ingenol compounds, in particular, IngC, demonstrated a potent antitumor activity on all cancer cell lines evaluated. Although the underlying mechanisms of action of IngC are not elucidated, our results provide insights for further studies suggesting IngC as a putative therapy for cancer treatment.

Keywords Anticancer · Cytotoxic activity · Semi-synthetic derivative · Ingenol · *Euphorbia tirucalli*

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Introduction

Euphorbia tirucalli has an extensive use in traditional medicine [1–3]. The main constituent of *E. tirucalli* extract is euphol, a tetracyclic triterpene alcohol, which has shown anti-inflammatory, antiviral and analgesic properties [4, 5]. Recently, our group also reported euphol in vitro antineoplastic activity against several tumors types and elucidated its mechanism of action [6, 7]. In addition to euphol, *E. tirucalli* has numerous others secondary compounds such as ingenol diterpenoid [8].

Ingenol diterpenoid derivatives from natural products have been reported in critical biomedical activities, including antiviral [9, 10], thrombopoietic [11], anticancer [12, 13], and also a putative carcinogenic potential, due to the reported tumor-promoting activity of ingenol esters [14]. Many of these activities are associated with protein kinase C, an important protein in cellular signal pathways [15–17]. One of these compounds is

ingenol-3-angelate (I3A; PEP005; ingenol-3-mebutate; Picato® LEO Pharma A/S), which is derivated from the sap of the plant *Euphorbia peplus* [18, 19]. Recently, I3A was approved by the U.S. Food and Drug Administration as a topical gel formulation (PEP005) for actinic keratosis, a precancerous skin condition, and has been successfully evaluated in clinical trials for effective treatment of basal cell carcinoma and squamous cell carcinoma [2, 3, 20–23].

Recently, three novel ingenol semi-synthetic esters derived from natural isoforms isolated from *E. tirucalli* were revealed as potential candidates to adjuvant treatment for HIV eradication. Among the three derivatives, Ingenol B (IngB) effectively promoted reactivation of HIV LTR-induced gene expression through the activation of protein kinase C, exhibiting antiviral properties in both inflammatory and neuropathic pain of mouse and rat models [10, 24]. However, the antineoplastic therapeutic potential of ingenol esters derivatives from *E. tirucalli* has been little explored.

In this study, we evaluated the potential effect of ingenol esters derivatives from *E. tirucalli* as an antitumor agent against human cancer-derived cells, to provide insight into the tailoring designing of Ingenol-based therapies for cancer patients.

Material and methods

Cell lines and cell culture

Seventy immortalized human cancer cell lines, comprising 15 different solid tumor models were used in cytotoxic assays (Table 1) [6]. Cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM 1X, high glucose; Gibco, Invitrogen) or RPMI-1640 (Gibco, Invitrogen) supplemented with 10% fetal bovine serum (FBS) (Gibco, Invitrogen) and 1% penicillin/streptomycin solution (P/S) (Gibco, Invitrogen), at 37 °C and 5% CO_2. The identity of all cell lines was confirmed by short tandem repeat (STR) DNA typing, according to the International Reference Standard for Authentication of Human Cell Lines as previously described [25, 26] and was performed in the Center for Molecular Diagnostics of Barretos Cancer Hospital (São Paulo, Brazil) as previously reported [6, 7].

Modified ingenol derivatives

The natural ingenol from the sap of *Euphorbia tirucalli* was modified by the addition of specific ester chains at carbon 3 of the core ring to produce three new distinct molecules named IngA (ingenol-3-trans-cinnamate), IngB (ingenol-3-hexanoate) and IngC (ingenol-3-dodecanoate) (Fig. 1). The synthesis was performed by Kyolab Laboratories (Campinas, Brazil) and provided by Amazônia Fitomedicamentos, Brazil (patent) as previously described [10]. The ingenol synthetic derivatives were diluted in DMSO (10 mM stock) and stored at -20 °C.

Cell viability analysis and IC_{50} determination

The cytotoxic effect of ingenol synthetic derivatives (IngA, IngB and IngC) and their phorbol esters analogous, ingenol 3,20-dibenzoate (IDB) (Santa Cruz) and (ingenol-3-angelate (I3A) (Adipogen (Liestal, Switzerland)), was evaluated using the Cell Titer 96 Aqueous cell proliferation assay (MTS assay, Promega, Madison, WI, USA), following the manufacturer's instructions and plated as previously described [6, 27]. The cells were treated with increasing concentrations of the test compound diluted in DMEM (0.5% FBS) and incubated for 72 h. The untreated control groups received the same amount of vehicle (1% DMSO, final concentration). Absorbance was measured in the automatic microplate reader Varioskan (Thermo) at 490 nm. The half maximal inhibitory concentration (IC_{50}) was obtained by nonlinear regression using GraphPad PRISM version 5 (GraphPad Software, La Jolla California USA), as previously described [6, 7]. Since it was the most potent among the synthetic derivatives tested, growth inhibition (GI) to IngC was calculated as a percentage of untreated controls, and its values were determined at a fixed dose of 10 μM (concentration closer to the average IC_{50} value of all cell lines at screening) [28]. Samples exhibiting more than 60% growth inhibition in the presence of 10 μM ingenol C were classified as highly sensitive (HS), as moderately sensitive (MS) when they were between 40 and 60%, and as resistant when values were lower than 40% of inhibition as previous reported [28]. The assays were performed in triplicate and repeated at least three times for each cell line.

Drug combination studies

Combination studies were performed with fixed concentrations (determined by the IC_{50} value) of the standard chemotherapeutic agent paclitaxel (Sigma - T7402), exposed simultaneously to increasing concentrations of IngC. Drug interactions were evaluated by the combination index [5] that was calculated by the Chou–Talalay equation as previously described [29, 30], using CalcuSyn software version 2.0 (Biosoft; Ferguson, MO, USA). In CI analysis, synergy was defined as CI values significantly lower than 1.0; antagonism as CI values significantly higher than 1.0; and additivity as CI values equal to 1.0 [29, 30] at drug IC_{50} value for each cell line.

Statistical analysis

The results are expressed as the mean ± standard deviation (SD) of three independent experiments. We applied the Student's t test for comparing two different conditions whereas two-way analysis of variance (ANOVA) was used for assessing differences between more groups. *P*-values <0.05 were considered significant.

Table 1 The semi-synthetic ingenol derivatives (IngA, IngB, and IngC) values of half maximal inhibitory concentration (IC_{50}) against the cancer cell lines

Cell line	Ingenol A IC_{50} ± S.D. (μM)	Ingenol B IC_{50} ± S.D. (μM)	Ingenol C IC_{50} ± S.D. (μM)	Growth inhibition in % at 10uM	Growth inhibition score	S.D	Tumor type
MDA-MB-231	23.89 ± 7.64	31.58 ± 6.41	9.71 ± 1.83	47.55	MS	3.7	Breast
MDA-MB-468	21.24 ± 0.84	38.61 ± 5.95	8.2 ± 2.42	55.13	MS	13.45	
BT20	35.42 ± 5.36	56.31 ± 6.25	20.61 ± 4.72	4.41	R	13.29	
HS587T	25.69 ± 4.99	23.1 ± 6.45	6.64 ± 3.39	72.24	HS	12.65	
MCF7	33.72 ± 3.94	45.82 ± 0.9	15.85 ± 6.72	13.75	R	23.89	
MCF7/AZ	34.46 ± 3.72	37.52 ± 1.44	15.856 ± 6.72	17.41	R	5.61	
T47D	63.34 ± 5.15	51.11 ± 4.6	14.84 ± 3.22	14.73	R	3.85	
SW480	22.17 ± 2.66	43.63 ± 4.62	5.59 ± 1.1	85.82	HS	0.5	Colon
SW620	15.98 ± 1.92	39.79 ± 7.35	6.21 ± 1.85	78.06	HS	9.98	
CO115	9.41 ± 4.23	29.06 ± 9.89	8.645 ± 4.14	56.59	MS	18.57	
HCT15	11.36 ± 1.89	41.97 ± 6.15	3.64 ± 1.19	87.69	HS	5.8	
HT29	13.87 ± 3.52	41.83 ± 7.64	11.687 ± 2.45	28.24	R	25.38	
SK-CO-10	26.38 ± 3.87	50.21 ± 4.93	9.24 ± 4.01	64.99	HS	10.33	
DLD1	11.11 ± 3.52	18.21 ± 2.58	3.20 ± 0.35	85.08	HS	4.54	
LOVO	21.57 ± 2.54	47.56 ± 3.49	8.52 ± 1.88	54.88	MS	7.91	
DIFI	13.3 ± 2.19	43.99 ± 2.16	9.16 ± 3.54	50.22	MS	6.7	
Caco2	28.27 ± 5.34	50.87 ± 8.1	13.73 ± 2.89	38.11	R	15.91	
PC-3	29.76 ± 4.23	43.41 ± 5.36	8.749 ± 2.21	57.58	MS	18.21	Prostate
LNCaP	9.93 ± 0.03	25.98 ± 6.34	2.72 ± 0.41	82.33	HS	1.8	
JHU-022	21.6 ± 5.56	41.49 ± 4.21	10.51 ± 3.13	37.45	R	43.97	Head & Neck
HN13	26.09 ± 1.95	42.3 ± 2.86	16.93 ± 3.13	28.75	R	45.74	
SCC25	26.6 ± 2.14	41.56 ± 2.45	12.24 ± 1.46	44.93	MS	15.23	
SCC4	19.79 ± 9.74	49.55 ± 14.49	12.66 ± 2.98	51.52	MS	16.5	
SCC14	21.99 ± 5.93	46 ± 1.14	11.87 ± 1.75	46.54	MS	11.4	
FADU	7.86 ± 2.24	42.76 ± 4.95	10.80 ± 3.26	47.44	MS	14.61	
T24	23.61 ± 1.5	38.52 ± 8.67	4.99 ± 0.47	87.97	HS	2.97	Bladder
5637	23.99 ± 1.06	29.47 ± 6.4	4.33 ± 1.09	91.95	HS	0.51	
HT1376	18.56 ± 0.36	28.81 ± 6.3	8.32 ± 2.59	86.32	HS	0.79	
MCR	20.63 ± 2.71	42.02 ± 3.88	9.24 ± 1.18	63.1	HS	1.81	
U87-MG	29.44 ± 5.16	35.73 ± 7.07	4.02 ± 2.29	74.18	HS	10.46	Adult glioma
U251	24.66 ± 0.72	41.61 ± 2.14	5.95 ± 1.23	66.23	HS	7.01	
GAMG	0.3 ± 0.08	0.34 ± 0.25	0.19 ± 0.05	90.58	HS	1.32	
SW1088	25.42 ± 2.21	42.85 ± 1.66	7.48 ± 0.47	66.64	HS	10.18	
SW1783	14.71 ± 5.13	23.93 ± 0.8	7.4 ± 0.93	41.39	MS	0.63	
RES186	25.17 ± 2.95	51.7 ± 1.69	10.762 ± 2.6	60.4	HS	23.2	Pediatric glioma
RES259	12.83 ± 2.62	43.22 ± 7.03	5.28 ± 1.54	81.4	HS	5.51	
KNS42	29.7 ± 5.53	55.05 ± 9.26	8.10 ± 1.17	46.14	MS	5.07	
UW479	20.84 ± 2.56	56.85 ± 8.27	8.89 ± 0.86	57.57	MS	8.61	
SF188	11.72 ± 3.77	28.28 ± 5.21	3.38 ± 1.24	80.24	HS	2.8	
DAOY	22.8 ± 4.09	41.88 ± 1.9	10.39 ± 2.12	45.3	MS	11.76	Medulloblastoma
ONS76	25.87 ± 3.16	40.85 ± 9.7	13.85 ± 1.3	28.67	R	7.78	
JEG3	16.3 ± 4.75	31.48 ± 2.62	8.21 ± 2.87	66.29	HS	11.06	Choriocarcinome
SiHa	25.89 ± 0.31	42.82 ± 4.49	13.83 ± 2.52	26.98	R	3.24	Cervical
CaSki	23.29 ± 1.72	39.23 ± 4.03	9.06 ± 4.02	39.16	R	27.13	
C33	26.91 ± 1.34	44.75 ± 10.89	10.81 ± 7.33	58.35	MS	12.69	
HeLa	22.41 ± 4.35	41.76 ± 6.21	14.39 ± 5.5	3.38	R	4.74	
A431	20.17 ± 1.97	34.37 ± 7.51	9.72 ± 3.35	41.59	MS	21.38	Epidermoid
H292	15.2 ± 1.21	24.49 ± 5.82	4.29 ± 0.91	81.57	HS	3.03	Lung
A549	13.7 ± 0.88	35.22 ± 9.71	4.07 ± 1.45	44.9	MS	16.34	
SK-LU-1	20.3 ± 1.48	44.26 ± 3.25	6.52 ± 0.38	72.78	HS	1.77	
KYSE30	15.51 ± 1.92	34.34 ± 5.63	6.54 ± 0.34	73.18	HS	6.43	Oesophageal
KYSE70	11.23 ± 0.57	26.53 ± 2.32	3.58 ± 0.4	92.11	HS	3.27	
KYSE270	3.38 ± 0.58	7.77 ± 0.14	1.88 ± 0.67	77.6	HS	7.64	
KYSE410	10.78 ± 1.24	19.24 ± 4.35	3.49 ± 0.22	85.47	HS	8.03	
COLO858	12.95 ± 1.72	42.24 ± 6.49	5.76 ± 0.84	80.39	HS	5.72	Melanoma
COLO679	16.24 ± 2.89	38.93 ± 4.79	5.772 ± 1.9	53.51	MS	28.09	
A375	15.72 ± 3.47	30.97 ± 4.99	8.68 ± 1.32	59.65	MS	5.86	
WM1617	15.8 ± 2.37	45.01 ± 5.91	9.47 ± 0.25	53.22	MS	28.05	
WM9	12.31 ± 2.79	28.88 ± 7.82	3.76 ± 1	78.79	HS	1.94	
WM852	12.79 ± 1.5	26.79 ± 4.56	5.77 ± 0.04	81.52	HS	1.95	
WM278	17.37 ± 0.45	46.89 ± 3.2	6.53 ± 1.92	84.28	HS	4.83	
WM35	22.96 ± 2.72	49.74 ± 1.74	5.62 ± 1.45	82.02	HS	5.23	
WM793	14.26 ± 0.93	42.74 ± 5.64	6.35 ± 0.09	90.5	HS	1.54	
SKMEL-37	18.06 ± 4.24	41.88 ± 2.7	8.01 ± 0.95	68.58	HS	11.38	

Table 1 (continued)

Cell line	Ingenol A IC_{50} ± S.D. (μM)	Ingenol B IC_{50} ± S.D. (μM)	Ingenol C IC_{50} ± S.D. (μM)	Growth inhibition in % at 10uM	Growth inhibition score	S.D	Tumor type
Mia-Pa-Ca2	15.32 ± 1.25	44.73 ± 5.51	5.23 ± 2.04	85.68	HS	15.17	Pancreatic
PANC-1	28.44 ± 0.81	51.22 ± 5.22	14.44 ± 178	26.6	R	24.53	
PSN-1	14.19 ± 2.72	38.41 ± 4.6	5.647 ± 0.51	78.1	HS	3.72	
BXPC-3	16.6 ± 5.84	48.78 ± 4.24	5.98 ± 1.52	69.69	HS	16.02	
PA-1	31.51 ± 9.5	UD	12.85 ± 1.13	51.68	MS	19.52	Ovary
SW626	29.32 ± 0.26	50.24 ± 0.12	13.57 ± 2.16	36.11	R	23.67	

* IngA (ingenol-3-trans-cinnamate); IngB (ingenol-3-hexanoate); IngC (ingenol-3-dodecanoate). *(*HS* Highly Sensitive); Blue (*MS* Moderate Sensitive) and Red (*R* Resistant)

Results

The three semi-synthetic ingenol derivatives promote cytotoxicity in human cancer cell lines

The in vitro antitumor effect of semi-synthetic ingenol derivatives was assessed using MTS assay on 70 human cancer lines from 15 solid tumor models (breast, colon, bladder, prostate, lung, pancreas, esophageal, head and neck, cervical, epidermoid carcinoma, medulloblastoma, placental choriocarcinoma, ovarian carcinoma, glioblastoma, and melanoma) (Table 1). We generated complete dose-response curves and IC_{50} values for the treatment with these three semi-synthetic ingenol derivatives. The mean of IC_{50} values was 20.59 ± 2.90 μM (IngA), 38.24 ± 4.81 μM (IngB) and 8.72 ± 2.20 μM (IngC). Nevertheless, the distinct cell lines exhibited a heterogeneous profile of response to ingenols derivatives. Of the three compounds, IngC demonstrated the most marked effect on esophageal and lung cancer cell lines (IC_{50} mean value < 4.0 μM and < 6.0 μM, respectively). IngA showed the second most effective action and exhibited favorable activity on esophageal, melanoma, lung and colon cancer cell lines (IC_{50} mean value <18 μM), whereas IngB was the third most effective, and did not exhibit specific activity on any evaluated human cancer cells.

Since IngC presented the best cytotoxic results, we adopted its growth inhibition (GI) as the benchmark for classifying responsiveness of the cell lines to the ingenols tested (Table 1) (Fig. 2). Nearly 20% of the cell lines tested were resistant (14/70), 30% (21/70) were moderately sensitive, whereas 50% (35/70) were classified as sensitive. Cervical cancer (75%) and breast tumor type (50%) had the most cancer cell lines scored as resistant, with a lower percentage of cell lines considered as sensitive. Differently, esophageal (100%), bladder (100%), pancreatic cancer (75%) and melanoma (70%) all had more than 70% of cell lines sensitive to ingenols (Table 1) (Fig. 2).

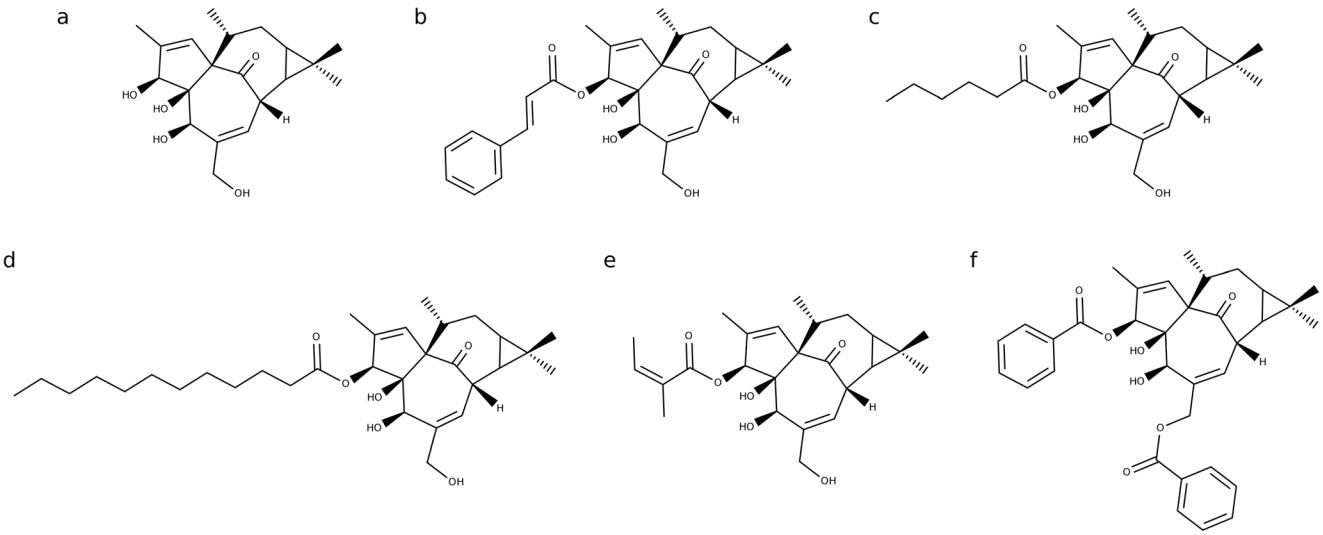

Fig. 1 Comparative chemical structures of modified ingenol derivatives and other esters class. **a** Ingenol core; **b** IngA (ingenol-3-trans-cinnamate); **c** IngB (ingenol-3-hexanoate); **d** IngC (ingenol-3-dodecanoate); **e** IDB (ingenol 3,20-dibenzoate) used as control; **f** I3A (ingenol-3-angelate) used as control. http://www.chemspider.com/Chemical-Structure.28533061.html

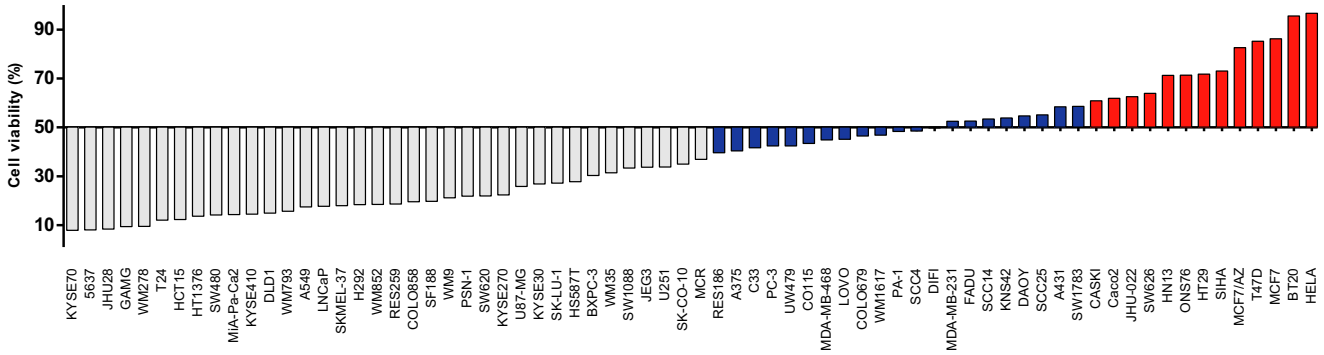

Fig. 2 Cytotoxicity profile of 70 human cancer cell lines exposed to ingenol C compound. Bars represent the cell viability at 10 μM of ingenol C. Colors represent the GI score classification: Grey (*HS* Highly Sensitive); Blue (*MS* Moderate Sensitive) and Red (*R* Resistant)

IngC is more cytotoxic than other ingenol-ester class on esophageal cancer cell lines

Next, we compared the potential antitumor activity of IngC with compounds of the same class, IDB and I3A, in sensitive esophageal cancer cell lines. The mean of IC_{50} values was 3.87 ± 0.41 μM for IngC, 14.09 ± 0.45 μM for IDB and 25.51 ± 0.99 μM for I3A. Therefore, IngC demonstrated the most marked effect in the majority of the esophageal cancer cell lines evaluated when compared with the other ingenol-ester class (Table 2).

IngC sensitize esophageal cancer cell lines to paclitaxel treatment

As ingenol C had a particularly high activity against esophageal cancer cell lines – a challenging disease – we tested IngC combined to paclitaxel, a chemotherapy commonly used to treat esophageal cancer. We demonstrated that IngC and paclitaxel combination treatment showed a synergistic effect (combination index (CI < 1) in 75% of the esophageal cancer cells lines investigated (mean CI values, range: 0.41–0.73; Table 2).

Discussion

In the current study, we evaluated three newly semi-synthetic ingenol diterpenes derivatives from *Euphorbia tirucalli* named IngA, IngB, and IngC, as antineoplastic drugs. We investigated the cytotoxic effects of ingenol derivatives on 70 cancer cell lines from 15 tumor models, and IngC stood as an exciting compound, with marked activity against chemo-resistant cancer lines, namely esophageal and lung cancer. The three derivatives (IngA, B, and C) exhibited dose-dependent cytotoxic effects. Among the tested compounds, IngC displayed the most potent cytotoxic activity across the tumor cell lines tested, mainly on esophageal and lung cancer cell lines. IngA showed the second most effective activity as well as favorable activity on esophageal, melanoma, lung and colon cancer cell lines. Ingenol B was less effective than the others semi-synthetic compounds with no particular activity against any cell line.

Most of the biological effects of ingenol derivatives are attributed to protein kinase C (PKC) [15, 17, 31]. Ingenol derivatives mimic endogenous diacylglycerol (DAG) promoting activation and translocation of PKC that is responsible for many cellular signal transduction pathways, including cancer

Table 2 The semi-synthetic ingenol derivative (IngC), IDB and I3A half maximal inhibitory concentration (IC_{50}) and drug combination studies against the esophageal cancer cell lines

Cell line	IngC IC_{50} ± S.D. (μM)	IDB IC_{50} ± S.D. (μM)	I3A IC_{50} ± S.D. (μM)	PC IC_{50} ± S.D. (μM)	Combination Index [CI]* PC + IngC
KYSE30	6.54 ± 0.34	41.02 ± 0.50	47.20 ± 1.08	0.015 ± 0.003	0.49
KYSE70	3.58 ± 0.40	6.01 ± 0.67	14.72 ± 0.56	0.009 ± 0.001	0.41
KYSE270	1.88 ± 0.67	0.10 ± 0.29	4.24 ± 0.78	0.018 ± 0.002	0.73
KYSE410	3.49 ± 0.22	9.26 ± 0.35	24.08 ± 1.56	0.023 ± 0.008	1.83

*IngC (ingenol-3-dodecanoate); IDB (ingenol 3,20 dibenzoate) and I3A (ingenol-3-angelate) used as control

*The CI was analyzed using CalcuSyn Software version 2.0. The CI value significantly lower than 1.0, indicates drug synergism; CI value significantly higher than 1.0, drug antagonism; and CI value equal to 1.0, additive effect. PC; paclitaxel

promoters and suppressors [15, 16]. These new ingenol PKC agonist derivatives (IngA, B, and C) up to now had only been evaluated in regarding to HIV-LTR activation context and presented low levels of cytotoxicity [32]. Several studies suggest that the efficiency in upregulating the HIV LTR as wells as PKC isoforms activation varies depending on the nature and position of the esters in the diterpenes ring [14, 33]. Therefore, the difference in antitumor potential demonstrated here could be related to the changes in carbon three that differs from these molecules and could be related to stability as well as their interaction with different isotypes of PKCs. Despite their structural and functional similarities to other PKC agonists such as PMA and DAG, future experiments could focus on addressing its PKCs mechanisms in the cancer context.

To gain more insight into the antitumoral properties of IngC, we compared its effects with other medicinal DAG ingenol diterpenes that can promote PKC activation and anticancer activity such as ingenol 3,20-dibenzoate (IDB) from *E. esula* L and ingenol-3-angelate from *E. peplus* (I3A) [2, 21, 34–36]. IDB has been identified as a promising antitumor compound in Jurkat cells and breast cancer cells by a relevant cell growth inhibition and apoptotic cell induction [14, 35]. Besides, I3A was approved as a topical gel formulation (Picato) and several reports have been demonstrating its activity in inducing primary necrosis in solid tumors such as human melanoma, cervical cancer, and prostate xenografts, apoptosis in colon cancer cells as well as senescence, anti-inflammatory, and antitumor immunomodulatory properties in in vitro and in vivo models [2, 12, 18, 21]. In the present study, we showed that IngC presented a higher efficacy compared to I3A and IDB on esophageal cancer cell lines. Although the cytotoxicity effect of I3A and IDB in esophageal tumor cells is unprecedent, both compounds demonstrate higher activity against leukemia cells breast and colon cancer cells [14, 21, 35, 36] suggesting IngC as a potential active semi-synthetic compound in oncology.

Moreover, it is worth noticing that IngC combinatory therapy with standard chemotherapy has a synergistic effect to paclitaxel, suggesting that IngC is an effective compound and its administration either alone or in combination with standard chemotherapy promoted increased cytotoxicity. We highlight that, even though our results provide critical evidence of IngC antitumour activity in vitro, further studies assessing its activity in normal counterpart cells as well as in vivo models are warranted, before moving to clinical trials [37].

To our knowledge, our study constitutes the first comparative assessment on a large panel of cell lines from different tumor sites to evaluate the specificity and efficacy of semi-synthetic formulation derivatives of *E. tirucali*. Our phytochemical screening ascertained the potential of IngC and constituted a first step forward in identifying the mechanisms of action of these compounds that shall allow the rational selection of clinical studies in the future.

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Compliance with ethical standards

Conflict of interest The authors confirm that this article content has conflicts of interest. This study was supported by grants from Amazônia Fitomedicamentos Ltda as part of the ingenol pre-clinical studies and Viviane A O Silva and Marcela N. Rosa received a scholarship from Amazônia Fitomedicamentos Ltda. to conduct the study.

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