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Synergistic Anti-proliferative Activity of Doxorubicin's Combination with Terpenoids Isolated from *Sarcophyton Glaucum*

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Abstract. Doxorubicin, a *Streptomyces* metabolite, is an anticancer drug that is effective against several solid cancers. The cytotoxicity of doxorubicin and five terpenoids, which were isolated from Sarcophyton glaucum was assessed. This was performed by employing SRB assays. Flow cytometry assay was used to detect apoptosis effect of the isolated compounds (1-5). The antiproliferative activity of the tested metabolites may be attributed to their capability to induce apoptosis. The combination index (CI) was employed to assess the synergism effect. Incubation with the reagents together at 1/4 and 1/2 of the IC50 dose of the isoprenoids and different concentrations of doxorubicin resulted in significant decreases of IC50 values in the Michigan Cancer Foundation (MCF-7) breast cancer cells.

Keywords: Sarcophyton glaucum, Cytotoxic, Combination index, MCF-7.

1. Introduction

Marine environment ecosystems are often characterized by the harsh atmosphere and high biological diversity which indicated the highly different archetype of metabolites other than those originated from terrestrial organisms. Huge number of compounds have been discovered from the marine origin (Aoki *et al*, 2004; Thomas *et al*., 2010; Kao *et al*., 2011).

Among marine creatures that are trove sources of biologically active chemical substances, soft corals come as one of the important sources of such materials. The marine substances showed potent inhibitors of physiological processes in the prey, predators or competitors. There are different targets, including ion channels, microtubule structures, and DNA. On this context, marine natural products play an important role in biomedical research and drug development, either directly as drugs or as lead structures for chemical drug synthesis specifically in the management of tumors (Pallela *et al*., 2010; Mayer *et al*., 2009; Hickford *et al*., 2009; El-Serag *et al*., 2011).

Breast cancer is considered as one of the wide distributed cancers among women (Boyle *et al*., 2003). For instances; in United States (2013), more than 232,000 (female) and 2,000 (male) were diagnosed with breast cancer, of which, 40,000 (Female) and 430 (Male) were died (Muir *et al*., 1991; Alarif, *et al*., 2013; Al-Lihaibi *et al*., 2014).

Flow cytometry is used for assesses the cell viability, relative cell size, DNA content and fluorescent protein expression in live cell populations. This method becomes a cornerstone of cell cycle analysis in model systems from yeast to mammals (Muir *et al*.,

1991; Alarif *et al*., 2013; Al-Lihaibi *et al*., 2014).

2. Results and Discussion

Extensive fractionation of organic extract of *Sarcophyton glaucum* yielded five terpenoidal metabolites; 10(14)-aromadendrene (**1**), deoxosarcophine (**2**); sarcotrocheliol acetate (**3**); sarcotrocheliol (**4**); sarcophytolide B (**5**) (Fig. 1) (Alarif *et al*., 2013; Al-Lihaibi *et al*., 2014).

Sarcophyton is a genus of rich cembrane-type diterpenoids, biscembranoids, sesquiterpenoids and steroids. Up to date more than 300 natural cembranoids were reported (Cheng, *et al*., 2010; Fahmy *et al*., 2006; Huang *et al*., 2006). *Sarcophyton glaucum* and its metabolites extract against MCF-7 (human breast carcinoma cells) and showed potent cytotoxicity (IC_{50} = 6.90 ± 0.032 µM). Five cembranoidal derivatives (**1**-**5**) were isolated and identified and subsequently, evaluated for their cytotoxicity against MCF-7 (Table 1). The antiproliferative activity of compounds **1**-**5** were evaluated against MCF-7 with reference to doxorubicin (Positive control), employing SRB assay (IC_{50}=20.0 ±0.06, 9.9±0.03, 23.9±0.07, 2.4±0.04 and 19.1±0.07 µM, respectively). These compounds were annoyed by the Flow cytometry assay aiming at detecting the effect of these compounds on the cell cycle of MCF-7 cells.

Wealthy is to study the mechanism of action of the significant antiproliferative effects. Thus, the cell cycle analysis for the potent and available metabolites **1**, **2**, **3**, **4** and **5** was processed. Compounds **1**, **2**, **3** and **5**, decreased the population in S phase from 31.99± 1.90 to 18.39 ± 0.091, 12.27 ± 0.081 and 21.97 ± 0.121, 19.54 ± 0.081%,while compound **4**, increased the population in S phase, from 31.99± 1.90 to 35.63± 0.081%. Compounds **1**, **3**, **4** and **5**, induced compensatory increase the population in the non-proliferating cell fraction G0/G1 from 55.42 ± 1.30 to 73.46± 0.081, 55.17 ± 0.031, 68.98 ± 0.011 and 56.16± 0.151, 72.79 ±0.091 %, while compound **5**, has no effect on the G0/G1 population. Compounds **1**, **2**, **3, 4** and **5** induced compensatory decreased the population of MCF-7 in G2/M phase from 10.82 ± 1.10 to 6.16± 0.031, 7.02, 13.50 ± 0.131, 6.28 ± 0.081, 7.63 ± 0.021 and 6.66± 1.30, 6.16± 0.031%, respectively. Compound **5**, increased the accumulation of the MCF-7 population in the Pre-G phase which indicates the apoptotic effect. The antiproliferative activity of the tested compounds can be attributed, at least partly, to their ability to induce intercellular apoptosis. Thus, the combination index (CI) was used to calculate the synergism potential, respectively. Incubation with the reagents together at 1/4 and 1/2 of the IC_{50} dose of the terpenoids and different concentrations of doxorubicin resulted in significant decreases of IC_{50} dose in MCF-7 cells.

Doxorubicin has been employed to treat early-stage breast cancer aiming at reducing the rate of mortality (Lomovskaya, *et al*., 1999). This resulted in increasing of the long-term survival rate among patients with breast cancer.

The major anthracycline chemotherapeutic reagent is doxorubicin (DOX), a *Streptomyces* metabolite. DOX-based chemotherapy is used against a wide range of cancers, including, soft-tissue sarcomas, lymphomas and various types of carcinomas and breast cancer. The main target is to decrease the toxicity of doxorubicin, which mainly appears as cardiotoxicity and the induction of multidrug resistance. A combination of DOX and isopernoids derivatives from marine sources led to decreasing the dose and toxicity.

The doxorubicin synergistically enhanced the terperenoidal derivatives in breast

cancer cells (MCF-7). To explore the impact of the terpenoidal compounds on the toxicity of doxorubicin, dose-response curves have been assessed and compared to those obtained after co-treatment with doxorubicin in MCF-7. The compounds **1**, **2**, **3**, **4** and **5** alone produced a significant inhibition of cell viability with IC_{50} values of 20.0± 0.054, 9.9±0.03, 2.4±0.04, 19.1±0.07 and 25 ±0.04 µM, in MCF-7 cell lines; respectively. Co-treatment doxorubicin with terpenoidal derivatives significantly reduced the IC_{50} in MCF-7 cell lines when combined the 1/4 IC_{50} of **1**, **3**, **4** and **5** to be 9.9 ±0.11, 0.8 ±0.09, 55.9±0.15, 18.5±0.07 µM and when combined with 1/2 IC_{50} of **1**, **3**, **4** and **5**, also reduced to be 5.0 ±0.08, 0.7±0.03, 52.0±0.14, 4.4±0.06 µM (Table 2). The combination index (CI) was calculated by using the method published by Talalay. The values indicated that compounds in MCF-7 are listed in Table 3. Compounds **1** and **4** showed synergistic activity when combined with the 1/4 of their IC_{50} with 100 µM of doxorubicin and CI was 0.02 and 0.184, while compounds **3** and **5** showed antagonistic activity. Comp # **1**, **4** and **5** showed synergistic activity when combined with the 1/2 of their IC_{50} with 100 µM of doxorubicin and CI was 0.114, 0.256 and 0.165, while compound **3** showed antagonistic activity.

3. Materials and Methods

The isolation and identification of the isolated compounds as well as taxonomical identification of the soft coral were previously reported (Al-Lihaibi *et al*., 2014).

Cytotoxicity assays and viability analysis

Compounds **1**-**5** were tested against the human breast adenocarcinoma (MCF-7), by SRB assay as previously described (Tolba *et al*., 2013).

***Analysis of cell cycle distribution was performed as published* by** Tolba *et al*., (2013).

The calculation of the combination Index was calculated as previously described (Chou and Talalay, 1984).

Statistical analysis of the data.

The results of experiments were expressed as means ± S.E.M. Statistical significance was acceptable to a level of $P<0.05$. All statistical analyses were performed using GraphPad Prism software, version 5.00 (GraphPad Software, La Jolla, CA).

4. Conclusion

Investigation of *Sarcophyton glaucum*, led to the identification of five compounds, which showed potent activity with $IC_{50} \leq 25$ µM against Breast cancer cell lines (MCF-7). Flow cytometry assay was used to detect apoptosis, especially for the potent compounds. The antiproliferative activity of the tested compounds can be attributed, at least partly, to their ability to induce intercellular apoptosis. The combination index (CI) was used to calculate the synergism potential. Incubation with the reagents together at 1/4 and 1/2 of the IC_{50} dose of the isoprenoids and different concentrations of doxorubicin resulted in significant decreases of IC_{50} dose in MCF-7 cells.

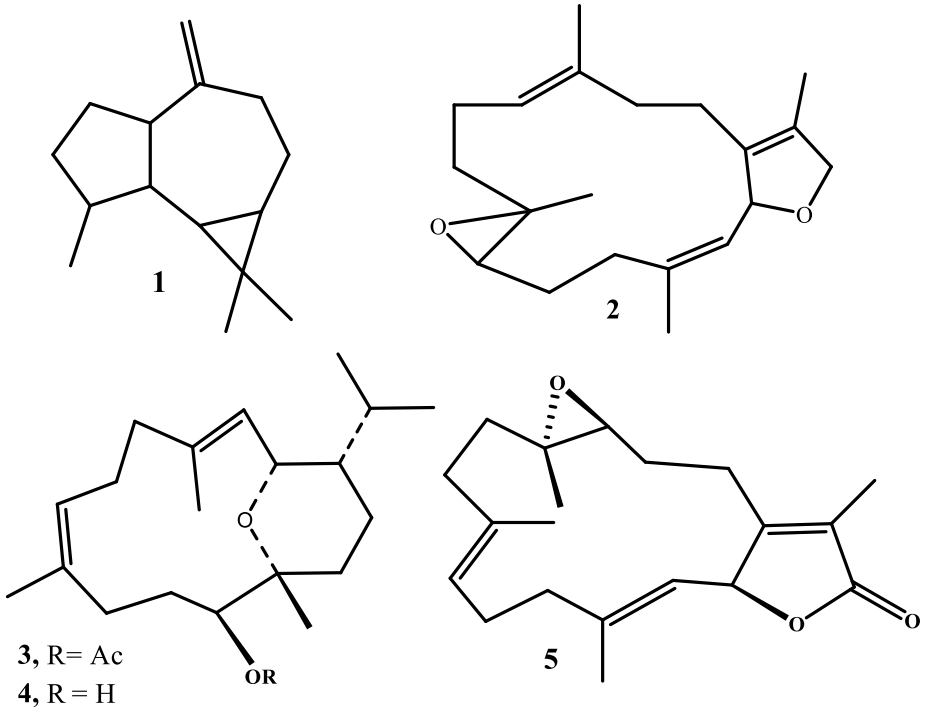

Fig. 1. Compounds isolated from *Sarcophyton glaucum*.

Table 1. Cytotoxic activities of the potent compounds (1-5) against MCF-7.

Comp. No.	IC_{50} (µM)		
	Pure compounds	**Doxorubicin + 1/4 IC_{50}**	**Doxorubicin + 1/2 IC_{50}**
1	20.0 ±0.06	9.9 ±0.11	5.0 ±0.08
2	9.9±0.03	18.6±0.03	11.8±0.17
3	2.4 ±0.04	0.8 ±0.09	0.7±0.03
4	19.1±0.07	55.9±0.15	52.0±0.14
5	25.0 ± 0.16	18.5±0.07	4.4±0.06
Doxorubicin	3.5 ±0.04	-	-

MCF-7 (Human breast adenocarcinoma). Data are presented as Mean ± standard error of the mean (SEM) (n=3).

Table 2. FACSCAN results of the marine samples in MCF-7.

	Cell cycle phase			
	Pre-G	**Go/G1** *	**S**	**G2/M**
Control	1.77 ± 0.20	55.42 ± 1.30	31.99 ± 1.90	10.82 ± 1.10
1	1.13 ± 0.091	73.46 ± 0.081	18.39 ± 0.091	7.02 ± 0.061
2	26.28 ± 0.061	55.17 ± 0.031	12.27 ± 0.081	6.28 ± 0.081
3	1.42 ± 0.031	68.98 ± 0.011	21.97 ± 0.121	7.63 ± 0.021
4	1.55 ± 0.051	56.16 ± 0.151	35.63 ± 0.081	6.66 ± 1.30
5	1.55 ± 0.111	72.79 ± 0.091	19.54 ± 0.081	6.16± 0.031

Data are presented as mean ± SEM, N = 3. * Significantly different from corresponding control value at $p < 0.05$.

Table 3. Synergistic results of isolated compounds with doxorubicin combinations in MCF-7.

Compounds	DOX (Dose) µM	Sample dose (µM)	Fa	CI
1	1	4.81[a]	0.40	35.53
		9.62 [b]	0.38	57.29
	10	4.81	0.80	0.15
		9.62	0.56	18.59
	100	4.81	0.92	0.02
		9.62	0.88	0.12
2	1	2.50	0.32	199.92
		5.00	0.35	109.13
	10	2.50	0.47	96.86
		5.00	0.56	19.44
	100	2.50	0.82	0.48
		5.00	0.84	0.26
3	1	4.80	0.45	16.97
		9.60	0.51	9.03
	10	4.80	0.46	130.21
		9.60	0.61	8.03
	100	4.80	0.72	7.14
		9.60	0.75	3.89
4	1	4.40	0.23	2247.18
		8.80	0.15	1110
	10	4.40	0.27	6312.75
		8.80	0.29	4527.33
	100	4.40	0.85	0.15
		8.80	0.87	0.08
5	1	4.21	0.45	17.03
		8.42	0.54	4.95
	10	4.21	0.50	58.52
		8.42	0.55	24.02
	100	4.21	0.71	09.66
		8.42	0.89	0.17

[a] 1/4 IC_{50} of the tested compound. [b] 1/2 IC_{50} of the tested compound. Abbreviation: Fa, fraction affected; CI, combination index.

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النشاط التآزري المضاد للتكاثر لمزيج دوكسوروبيسين مع التربينويدات المعزولة من *Sarcophyton glaucum*

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المستخلص. الدوكسوروبيسين، ناتج أيضي من *Streptomyces*، ويستخدم كدواء مضاد للسرطان والمعروف بفعاليته للعديد من الأورام الصلبة. تم تقييم السمية الخلوية للدوكسوروبيسين وخمسة تربينويد، والتي تم عزلها من *Sarcophyton glaucum*. وتم تنفيذ ذلك عن طريق استخدام فحوصات SRB. كما تم استخدام مقياس التدفق الخلوي للكشف عن موت الخلايا المبرمج للمركبات المعزولة (١–٥). ويمكن أن يعزى النشاط المضاد للتكاثر للنواتج الأيضية المستخدمة إلى قدرتها على تعزيز موت الخلايا المبرمج. كما تم استخدام مؤشر الجمع (CI) لتقييم تأثير التآزر. أعطت الحضانة مع الكواشف معًا عند ٤/١ و ٢/١ من جرعة IC50 من الأيزوبرينويدات وتركيزات مختلفة من دوكسوروبيسين انخفاضًا كبيرًا في قيم IC50 في خلايا سرطان الثدي في مؤسسة ميتشيغان للسرطان (MCF-7).