

Full Paper

Potent Skin Cancer Chemopreventing Activity of Some Novel Semi-synthetic Cembranoids from Marine Sources

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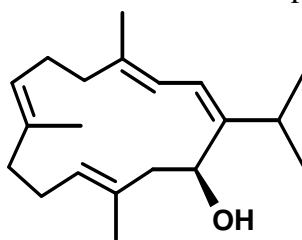
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Abstract: In the course of our continuing research in development and evaluation of novel skin cancer chemopreventive agents from marine sources, five semi-synthetic cembranoids derived from the marine natural product sarcophine, isolated from the soft coral *Sarcophyton glaucum*, were synthesized and shown to exhibit a remarkable chemopreventive activity in the *in-vitro* Epstein Barr Virus Early Antigen (EBV-EA) activation assay. These compounds were assayed *in vivo* using the two-stage carcinogenesis test of mouse skin tumors induced by 7,12-dimethylbenz[a]anthracene (DMBA) as an initiator, and 12-O-tetradecanoylphorbol-13-acetate (TPA) as a promoter by topical administration. They showed potent inhibition of both percentage incidence of skin tumor as well as the multiplicity of skin tumors per mouse compared to untreated controls.

Keywords: Cancer chemoprevention, Skin cancer, Cembranoids, Sarcophine, *Sarcophyton glaucum*.

Introduction

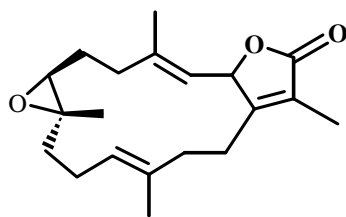
It is well-documented that marine natural products have great potential applications in the treatment of human diseases [1]. Many of these products are in preclinical and clinical trials, while many others are in the pipeline and showing promising pharmacological activities [1]. Of particular interest to our research are marine natural products of potential skin cancer chemopreventive activity. One of the most well-known marine natural products that stands out in this area is Sarcophytol A. Sarcophytol A is a cembranoid isolated from the soft coral *Sarcophyton glaucum*, and was shown to inhibit the development of several tumors in experimental animals [2,3], particularly skin cancer [2]. Sarcophytol A was extensively studied by the National Cancer Institute at a preclinical trials level [4].



Sarcophytol A

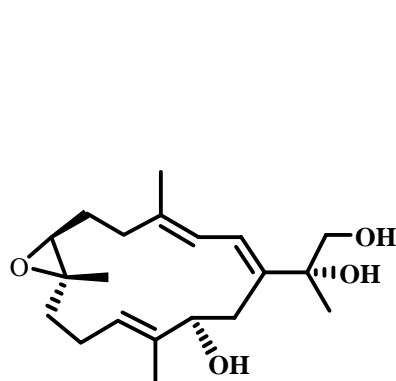
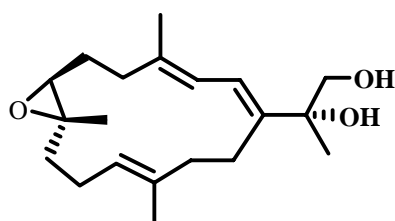
Cancer of the skin is the most common of all cancers [5]. Nonmelanoma skin cancers (NMSCs), usually basal cell and squamous cell cancers are the most common types of skin cancer [6]. Incidence of non-melanoma skin cancers (NMSCs) has been increasing worldwide at an alarming rate; more than one million new cases are reported annually in the United States alone where exposure to solar radiation, both occupational and recreational, is a major etiologic factor for this malignancy [7]. The alarming statistical information clearly suggests that the sunscreen usage as a primary preventive measure against NMSCs is not sufficient, and additional approaches and strategies are needed, which are far more efficacious and cost effective in preventing and controlling this malignancy. In light of these facts prevention of skin cancer becomes one of the most important issues in our society. One of the methods of cancer prevention is use of the non-toxic chemicals to inhibit cancer in its early stages of development [8]. Chemical and UVB radiation-induced carcinogenesis in murine skin and possibly in human skin is a stepwise process of at least three distinct stages: (a) initiation; (b) promotion; and (c) progression [9]. Chemoprevention of skin cancer involves the administration of chemical agents to prevent the initiation, and/or promotion, and/or progression events that occur during the multistage process of neoplastic development [10,11]. Classically, the mouse has been considered the laboratory animal most sensitive to skin carcinogenesis [12,13]. Mouse skin tumors can be induced using a multistage model that involves the process defined as initiation and promotion [12,13]. Initiation is accomplished by topical application of a single dose of a skin carcinogen, such as DMBA, and is essentially irreversible. An initiated dose of carcinogen may not produce visible tumors. Visible tumors result only after prolonged and repeated application of a tumor promoter, such as TPA to initiated skin [14].

In our previous work [15-18], we have reported a new class of compounds obtained by chemical modification of a marine natural product, sarcophine that is the main secondary metabolite of the soft coral *Sarcophyton glaucum*, and is much more abundant than sarcophytol A.

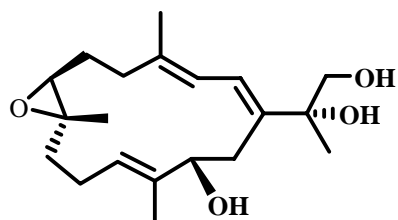
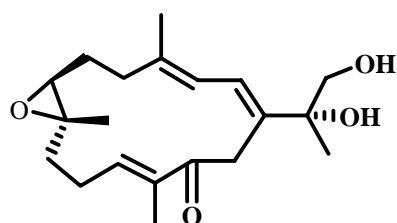


Sarcophine

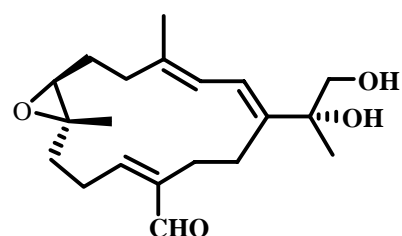
The most important results of our preliminary studies came from inhibition of Epstein-Barr virus early antigen (EBV-EA) activation induced by TPA. Some of the compounds showed more than 96% inhibition [15,16]. The inhibitory activity of these compounds were superior to Sarcophytol-A.

Sarcotriol α (2)

Sarcodiol (1)

Sarcotriol β (3)

Sarcodiolone (4)



Sarcodiolal (5)

In the present study, we continue our research by examining the *in vivo* chemopreventive activity of this newly-prepared series. We report the inhibitory effects of these compounds on the two-stage skin carcinogenesis model using DMBA as an initiator and TPA as a promoter [21].

Results and Discussion

All the five newly prepared semi-synthetic sarcophine derivatives exhibited significant inhibitory effects on the tumor promotion induced by DMBA and TPA. The control group, that received treatment with DMBA and TPA, showed 100% incidence of papillomas within 10 weeks of promotion. Mice treated with compounds 2-5 showed 26.6% incidence of papilloma in 10 weeks (fig. 1), and compound 1, the lead compound, showed only 20% incidence of papilloma after 10 weeks (fig. 2). After 15 weeks of promotion, mice treated with compounds 1-5 showed only 53.3-60 % incidence of papilloma. After 20 weeks of promotion, they showed 80-86.6 % papilloma formation.

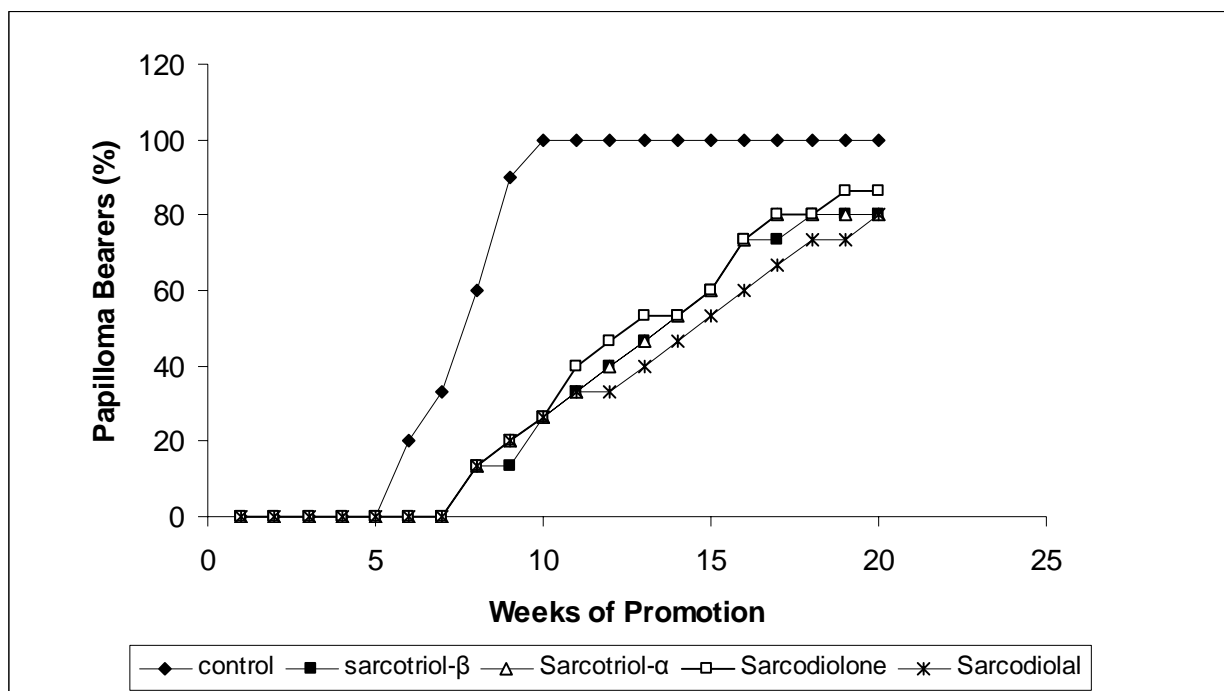


Figure 1. Inhibitory effects of compounds 2-5 on the percentage incidence of papilloma in the mouse skin carcinogenesis over 20 weeks of promotion.

The tumor inhibitory effects were also seen as a significant reduction in the multiplicity of papilloma. After 10 weeks of promotion, the control group showed an average of 5.4 papillomas per mouse, while in the treated groups with compounds 2-5 the average was 1.1-1.6 papillomas per mouse (fig 3), and was even only 0.6 per mouse for compound 1 (fig 4).

After 15 weeks of promotion, the average number of papillomas per mouse in the control group was 7.8 compared with 2.7-3.1 for compounds 2-5 and only 2.4 for compound 1. After 20 weeks of promotion, the average number of papillomas per mouse was 9.3 for the control group compared to 4.6-5.1 for groups treated with compounds 2-5, and 4.0 for the group treated with compound 1.

Conclusions

This significant reduction in both percentage of mice bearing papilloma and the multiplicity of papilloma per mouse in mice treated with this new series of compounds compared to the untreated controls, shows that this group of compounds has a very promising tumor chemopreventive activity. The results also confirmed that compound 1, sarcodiol, can serve as a lead compound since it is easier to prepare, obtained in almost 90-100% yield from sarcophine [17], and exhibit the strongest effects in both *in vitro* [16] and *in vivo* assays.

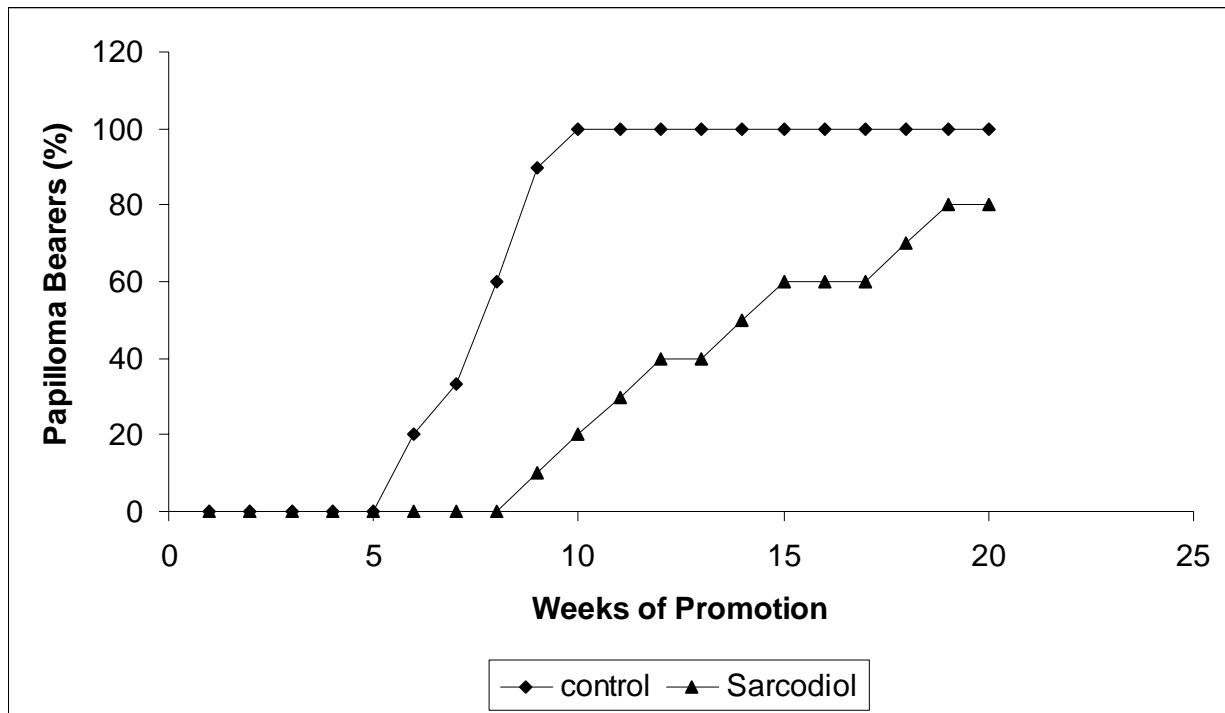


Figure 2. Inhibitory effects of the lead compound (1) on the percentage incidence of papilloma in the mouse skin carcinogenesis over 20 weeks of promotion.

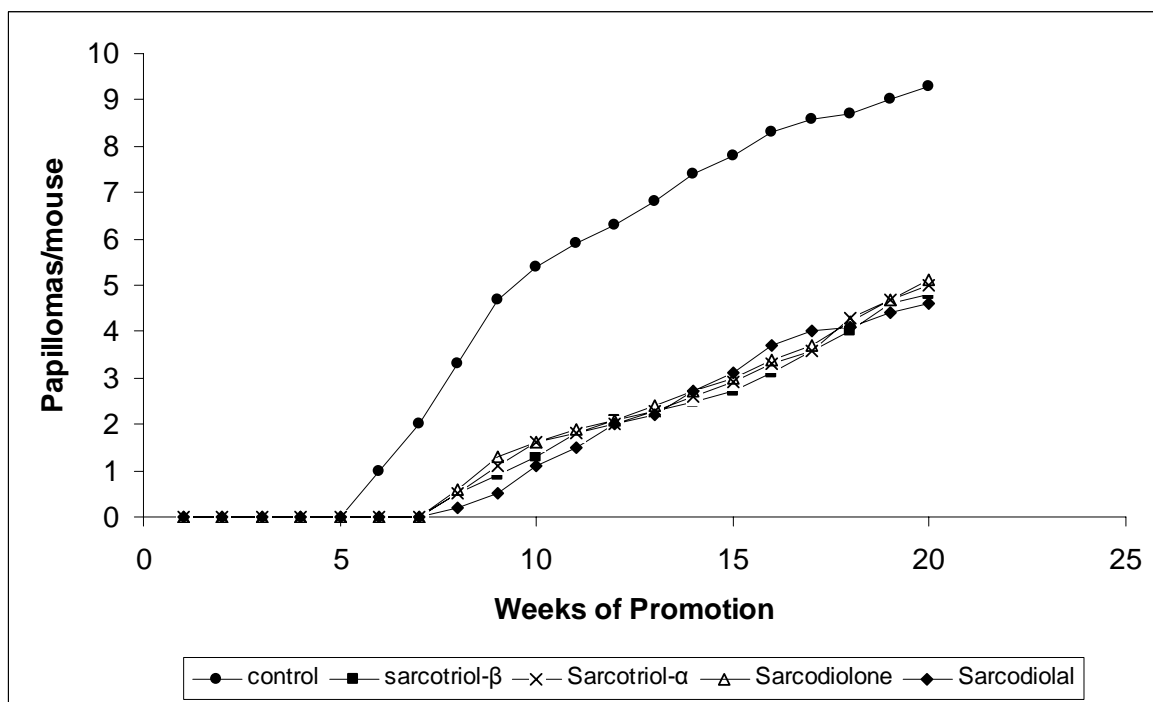


Figure 3. Inhibitory effects of compounds 2-5 on the multiplicity of papilloma in the mouse skin carcinogenesis test over 20 weeks of promotion.

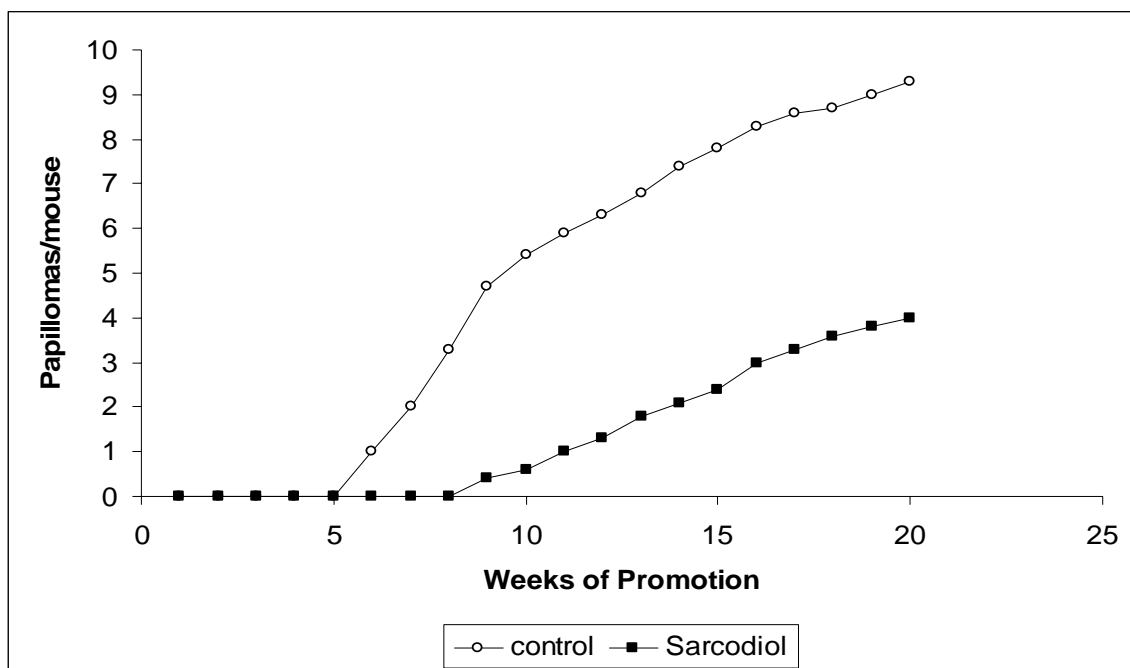


Figure 4. Inhibitory effects of the lead compound (1) on the multiplicity of papilloma in the mouse skin carcinogenesis test over 20 weeks of promotion.

Compound **1** also did not show any cytotoxic effects against healthy cells in the range of concentrations from 1 to 100 $\mu\text{g/mL}$. Its effects were similar to DMSO used as a negative control.

These results indicate that our compounds are valuable candidates for further studies in the search for new skin cancer chemopreventive agents from marine sources.

Experimental

General

TPA and DMBA were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sarcophine was isolated from the soft coral *Sarcophyton glaucum* collected from several locations of the Red Sea in Egypt.

Animals

Specific pathogen-free female ICR (6 weeks-old) were obtained from Japan SLC Inc. (Hamamatsu, Japan). These animals were housed, five per polycarbonate cage, in a temperature-controlled room at 24 ± 2 °C and given food and water as libitum.

Materials

Sarcophine was isolated from the soft coral by extraction several times with petroleum ether at room temperature following the reported procedure [22] in the laboratories of the Department of Pharmacognosy, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt. The dried extract was

evaporated under reduced pressure and chromatographed on silica gel column using hexane: ethyl acetate (1:2) as eluent. Pure sarcophine was obtained by crystallization from ethanol.

Synthesis of compounds

The five new cembranoids were prepared from sarcophine by the methods described previously [15-18]. The lactone ring of sarcophine was first opened by the reduction with lithium aluminum hydride, followed by selenium dioxide oxidation/allylic rearrangement to produce compounds **1-5**.

Two-stage mouse skin carcinogenesis model induced by DMBA/TPA

The animals (specific pathogen-free female ICR, 6-weeks-old) were divided into six experimental groups, 15 mice each. The back of each mouse was shaved with surgical clippers, and the mice were topically treated with DMBA (100 µg, 390 nmol) in acetone (0.1 ml) as an initiation treatment. For group I (control group), 1 week after initiation with DMBA, mice were treated with TPA (1 µg, 1.7 nmol) in acetone (0.1 ml) twice a week. Groups II-VI received a topical application of compounds **1-5** (85 nmol) 1 h before each TPA treatment, respectively. The incidence of papilloma was observed weekly for 20 weeks. The percentage of mice bearing papilloma and the average number of papillomas per mouse were recorded.

Statistical Analysis

ANOVA, χ^2 , and Student's *t* test were performed on sample means using INSTAT software (Graph Pad, San Diego, CA). χ^2 was used for analyzing the data in tumor incidence. ANOVA and student's *t* test were used for the data on tumor multiplicity. Significance was considered at $P < 0.05$.

Cytotoxicity studies

Compound **1** was selected for testing the general cytotoxicity of this series. Cytotoxicity was determined against healthy mammalian cells, i.e. Vero cells (monkey kidney fibroblasts). The assay was performed in 96-well culture-treated microplates according to modification of the neutral red staining procedure of Borenfreund and Puerner [23]. The cells were cultured in 75 cm² culture flasks in RPMI-1640 medium (Gibco™, Invitrogen Corp.) supplemented with bovine calf serum (10%) and amikacin (60 mg/L), at 37 C, 95% humidity, 5% CO₂, and sub-cultured every 2-3 days using standard cell culture technique.

Acknowledgements

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