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Garcinone E, a Xanthone Derivative, has Potent Cytotoxic Effect Against Hepatocellular Carcinoma Cell Lines

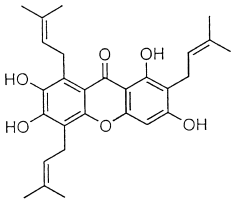
Abstract

Treatment of hepatocellular carcinomas (HCCs) with chemotherapy has generally been disappointing and it is most desirable to have more effective new drugs. We extracted and purified 6 xanthone compounds from the rinds (peel) of the fruits of *Garcinia mangostana* L., using partitioned chromatography and then tested the cytotoxic effects of these compounds on a panel of 14 different human cancer cell lines including 6 hepatoma cell lines, based on the MTT method. Several commonly used chemotherapeutic agents were included in the assay to determine the relative potency of the potential new drugs. Our results have shown that one of the xanthone derivatives which could be identified as garcinone E has potent cytotoxic effect on all HCC cell lines as well as on the other gastric and lung cancer cell lines included in the screen. We suggest that garcinone E may be potentially useful for the treatment of certain types of cancer.

Key words
Garcinone E · xanthones · hepatocellular carcinoma · chemotherapy · *Garcinia mangostana* · Guttiferae

Introduction

Xanthones are natural or synthetic compounds which are structurally related to anthraquinones and among these compounds, mitoxantrone is a well established anti-cancer drug. Xanthones could be found in relative abundance in the hulls, bark and dried latex of the guttiferaeous plants such as *Garcinia mangostana* L. [1], [2]. Extracts of the pericarb of the ripe fruits have been shown to have immunomodulating [3], [4], anti-bacterial [5], anti-mutagenic [6], [7], anti-cancer [8], [9] and other pharmacological [6] activities. In the present study, we report that one of the xanthones, garcinone E (Fig. **1**) has potent cytotoxic effects against a panel of different cancer cell lines. In comparison with some commonly used chemotherapeutic drugs, the cytotoxic potency of garcinone E is less effective only to taxol suggesting that garcinone E may be efficacious for use as an anti-cancer drug.



Garcinone E

Fig. **1** The basic chemical structure of the xanthone compound garcinone E extracted from the hulls of *Garcinia mangostana* L.

Materials and Methods

Cell culture

A panel of cancer cell lines from different tissue origins were used in the present study including those from the liver: HCC36, TONG [10], HA22T [11], HEp3B, HEpG2 and SK-HEp-1 (all from ATCC); the lung: NCI-Hut 125, CH27 LC-1, H2981 and Calu-1 (ATCC) and the stomach: AZ521 [12], NUGC-3 [13], KATO-III

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(ATCC) and AGS (ATCC). All other cell lines were obtained from the Cell Bank of the Veterans General Hospital, Taipei. The HEp3B, HCC36, TONG, HA22T and HEpG2 cells were cultured in DMEM supplemented with 10% non-essential amino acids and 10% fetal bovine serum (FBS). The SK-HEp-1 cells were in MEM + 10% FBS. The NCI-Hut 125, CH27LC-1, H2981 and Calu-1 cells were cultured in DMEM+ 5% FBS; AZ521, NUGC-3 and the KATO-III cells were cultured in RPMI-1640 + 10% FBS while the AGS cells were in F12 + 10% FBS. All cells were further supplemented with 2 mM glutamine and 30 μg/ml of gentamycin.

Extraction and isolation of compounds

The fruit hulls of *Garcinia mangostana* L., were collected at a local market in Taipei, Taiwan, and a voucher specimen (NRICM-98-010) was deposited in the herbarium of the National Research Institute of Chinese Medicine for future reference. Compounds were isolated using methods described in detail elsewhere [1], [3], [14]. In brief, the hulls were first dried at 60 °C in an oven for 24 h and 3 kg batches of the dried hulls were extracted with ethanol (25 l) at 50 °C for about 16 h. The extracts were pooled, concentrated by evaporation and then allowed to partition into aqueous and organic (EtOAc) phases. The EtOAc soluble fraction was then loaded onto a silica gel column (11 × 35 cm, 70 – 230 mesh) and eluted with gradient solvent systems consisting of: 10 l aliquots each of *n*-hexane-EtOAc (20 : 1, 10 : 1 and 5 : 1), 7 l aliquots each of CH_2Cl_2-Me_2CO (10 : 1, 5 : 1 and 0 : 1) and 7 l aliquots each of CH_2Cl_2-MeOH (10 : 1, 5 : 1 and 0 : 1) to yield 22 (~ 3 l) fractions. Fractions 4 and 5 eluted by *n*-hexane-EtOAc (20 : 1) were recrystallized with Me_2CO/*n*-hexane to yield 8-desoxygartanin [3] and gartanin [3] and the two compound could be separated on a Sephadex LH-20 column equilibrated and eluted with MeOH-H_2O (3 : 1). Unless otherwise stated, elution volumes for the silica gel column (6 × 50 cm) or the Sephadex LH-20 column (3.5 × 33 cm) used for further purification were 2.5 l and the fraction sizes collected from the two columns were 100 ml and 50 ml, respectively. The *n*-hexane-EtOAc (10 : 1) eluate (fraction 7), was sequentially fractionated in a silica gel column (in *n*-hexane-EtOAc, 5 : 1) and then in a Sephadex LH-20 column (in MeOH-H_2O, 3 : 1) to yield garcinone E [4] and tovophyllin A [2]. Fraction 13, the CH_2Cl_2-Me_2CO (10 : 1) eluted fraction, was further chromatographed on a silica gel column equilibrated and eluted with *n*-hexane-EtOAc (5 : 1) to yield α-mangostin [5], [6] or in a Sephadex LH-20 column equilibrated with MeOH-H_2O (5 : 1) to yield γ-mangostin [4], [6] respectively. All xanthone derivatives were identified based on physiochemical properties reported by us [14] and by other investigators [1], [2], [4], [5].

Cytotoxicity assay

Cells (1×10^5/ml) were seeded onto (1 ml/well) 24-well tissue culture plates (Falcon, Lincoln Park, NJ) and then treated or non-treated in triplcate with various concentrations (0 – 10 μM) of purified garcinone E or other purified compounds. All compounds were dissolved in 75% ethanol and the final concentration of ethanol added to the cells was ≤ 0.1%. The cells were then further incubated for 3 – 6 days at 37 °C in 5% CO_2 and cell viability was then assayed by the MTT method [15]. In some experiments, hepatoma cell lines were also treated in parallel with various concentrations of mitoxantrone, methrothrexate, vincristine, cisplatin, 5-fluorouracil and taxol under similar conditions. Cytotoxicity was expressed as 50% lethal dose (LD_{50}) based on extrapolation of adjacent doses above and below 50% killing. All experiments were repeated at least 5 times.

Cell cycle analysis

HEp G2, HEp 3B, SK-HEp-1, Calu-1 and AGS cells were cultured in p60 tissue culture dishes at 1×10^5/ml and then treated or non-treated with various concentrations (0 – 10 μM) of garcinone E. Following 12 to 48 h posttreatment, the cells were prepared for analysis of their cell cycle phase distribution by a CycleTest assay kit (Becton-Dickinson) using procedures recommended by the supplier. SK-HEp-1, Calu-1 and AGS cells were also treated with moscatilin (5 – 50 μm), a bibenzyl derivative from *Dendrobium moscatum* [16] which induced a G2 phase arrest in sensitive cells, to serve as positive controls. The treated cells were then analysed in a FACScan flow cytometer and for changes in cell cycle phases: Go/G1, diploid; S, intermediate; G2/M, polyploid and results were expressed as the mean percentages of cells at each phase. All test were performed at least 3 times and in duplicates.

Results

Purification of garcinone E

A total of 6 pure xanthone derivatives were obtained following extraction of the hulls of *Garcinia mangostana* L. These compounds could be identified as: 8-desoxygartanin (fraction 1): yellow needles from Me_2CO/*n*-hexane fractions; gartanin (fraction 2): yellow needles from Me_2CO/*n*-hexane fractions; garcinone E (fraction 3): yellow needles from MeOH/H_2O fractions; tovophyllin A (fraction 4): yellow needles from Me_2CO/*n*-hexane; α-mangostin (fraction 5): yellow needles from MeOH/H_2O; and γ-mangostin (fraction 6): yellow powder from Me_2CO/H_2O.

Identification of garcinone E

The physiochemical characteristics of garcinone E were identified based on infrared (IR) spectra, ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra and mass spectra, (EIMS) (20 eV) *m/z* 464 $[M]^+$, which were similar to those previously reported by Sakai et al. [17]. Identification of the other xanthone derivatives was based on comparison of the physiochemical properties of these compounds (not shown) with those described in published reports [1], [2], [4], [5], [14].

Cytotoxic effect of garcinone E

In our preliminary experiments, we have tested the cytotoxic effects of all six xanthone derivatives isolated from the hulls of the Guttiferaeous plant and we have found that the potency of garcinone E is by far the greatest. Thus, all subsequent experiments are focussed on garcinone E. As could be seen in Fig. **2**, six hepatoma cell lines including HEp 3B, HCC36, TONG, HA22T and SK-HEp-1 were all sensitive to the anti-proliferative effect of garcinone E. This cytotoxic effect was time- and dose dependent and at dosages ≦10 μM, total cell killing could be observed in all cell lines. Similarly, garcinone E is also effective in the killing of cancer cells derived from lung carcinoma (Fig. **3**) and the same holds true when tested on gastric carcinoma cell lines (Fig. **4**). As could be seen in Table **1**, all cancer cells tested, with the exception of the CH27LC-1 cells, invariably died at day 6 posttreatment with various concentrations (≦10 μM) of garcinone E. Based on LD_{50}, the relative potency of garcinone E against various hepatoma

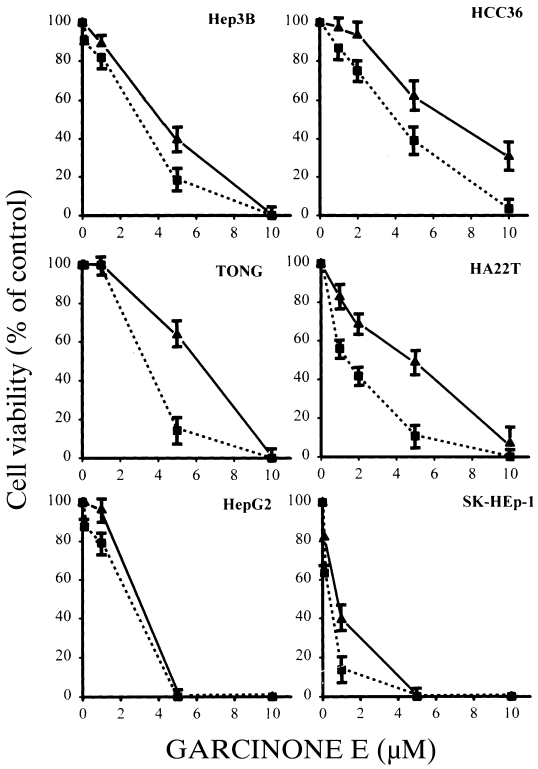

Fig. **2** The cytotoxic effect of garcinone E against 6 different hepatoma cell lines. Cell viability was assessed at day 3 (——) and day 6 (– – –) posttreatment and bars were standard error mean (SEM) of the mean data of 4 separate experiments.

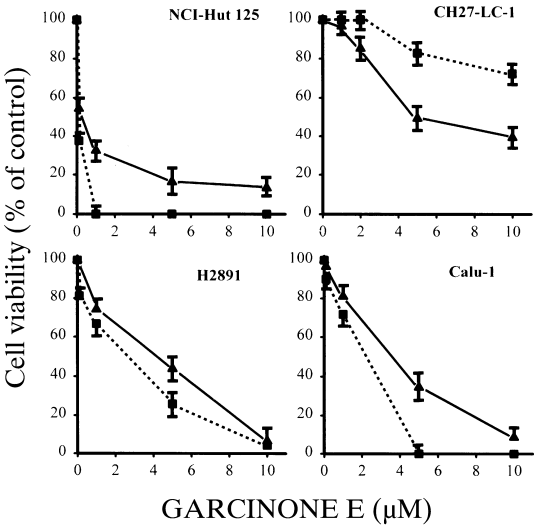

Fig. **3** The cytotoxic effect of garcinone E against 4 different lung carcinoma cell lines. Cell viability was assessed at day 3 (——) and day 6 (– – –) posttreatment and bars were standard error mean (SEM) of the mean data of 4 separate experiments.

cell lines was in the order of SK-HEp-1 > HA22T > HEp G2 > HEp 3B > HCC36. Furthermore, the potency against lung carcinoma cell lines was in the order of NCI-Hut 125 > Calu-1 > H2891 > CH27LC-1. Also shown in Table **1** was that the killing efficiency of garcinone E against stomach cancer cell lines was in the order of NUGC-3 > AZ521 > Kato III = AGS.

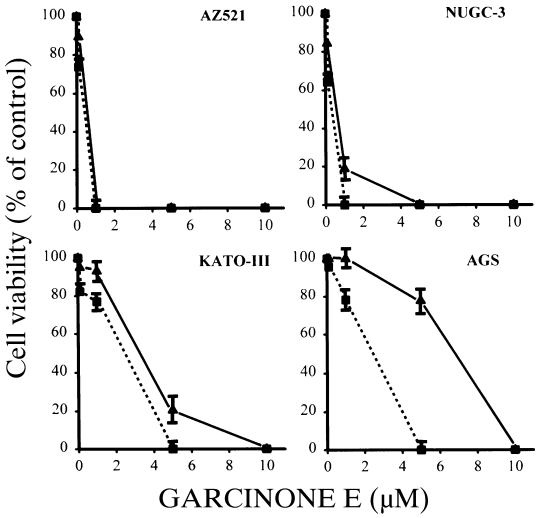

Fig. **4** The cytotoxic effect of garcinone E against 4 different gastric carcinoma cell lines. Cell viability was assessed at day 3 (——) and day 6 (– – –) posttreatment and bars were standard error mean (SEM) of the mean data of 4 separate experiments.

Table **1** The cytotoxic effect of garcinone E against different tumor cell lines

Cell Line	*Estimated mean Lethal Dose 50% (LD_{50}, μM)±SEM*	*Total Killing*
HEp3B	3.2 ± 0.8	+
HCC36	4.1 ± 1.0	+
TONG	5.4 ± 2.3	+
HA22T	1.6 ± 0.1	+
HEpG2	2.5 ± 1.2	+
SK-Hep-1	0.5 ± 0.2	+
NCI-Hut 125	0.1 ± 0.05	+
CH27 LC-1	> 10 ± 0	–
H2891	2.7 ± 0.1	+
Calu-1	2.3 ± 0.07	+
AZ521	0.5 ± 0.11	+
NUGC-3	0.4 ± 0.01	+
KATO-III	2.5 ± 0.08	+
AGS	2.5 ± 0.06	+

Data were estimated based on the results shown in Fig. **2 – 4** at day 6 post-exposure to garcinone E. SEM: standard error means. 100% killing (+) or < 100% killing (–) at ≦ 10 μM of garcinone E.

Relative cytotoxicity

In order to ascertain the efficacy of garcinone E as an anti-cancer drug, we compared the relative potency of garcinone E with six commonly used chemotherapeutic drugs against 4 hepatoma cell lines using LD_{50} and total killing as criteria. As could be seen in Table **2**, the LD_{50} values of garcinone E fall within a fairly narrow range between 0.5~5.4 μM and total killing could be achieved in all 4 hepatoma cell lines. Correspondingly, taxol has the lowest values of LD_{50} ranging from 0.06 ~ 0.76 μM and total killing could similarly be achieved in all cell lines (Table **2**). Our results also show that in terms of LD_{50}, mitoxantrone and methrothrexate are potent cytotoxic agents against all hepatoma cell lines. However these drugs are less effective when total killing is the criterion such that only 50~75% (2/4~3/4) and 25% (1/

4) of the hepatoma cell lines tested could be totally killed by mitoxantrone and methrothrexate respectively. Table **2** also shows that vincristine, 5-Fu and cisplatin are relatively inefficient in the killing of hepatoma cell lines. These results suggest that taxol is most effective in the killing of the hepatoma cells while the potency of cytotoxicity of garcinone E is equal to or more potent than that of mitoxantrone and should be a more effective drug than methothrexate, vincristine, 5-Fu and cisplatin in exerting cytotoxic/cytostatic effect against hepatoma cell lines (Table **2**).

Cell cycle analysis

In repeated experiments, we failed to observe any consistent change in the cell cycle phase distribution of cells (HEp G2, HEp 3B, Calu-1, AGS) treated with a wide range of doses of garcinone E for an extended observation period of 12–48 h (results not shown). In contrast, the positive control cell lines SK-HEp-1, Calu-1 and AGS treated with moscatilin (20 or 50 μm) were preferentially arrested at the G2 phase following treatment for ≥12 h. These results suggest that there is no apparent correlation between cytotoxicity and specific cell cycle arrest and the mechanisms of action of garcinone E remain to be determined.

Discussion

Xanthones are structurely related to anthraquinones and have been shown to have anti-bacterial [5] anti-mutagenic [6], [7] and cytotoxic activities [9]. However, unlike anthraquinone derivative such as mitoxantrone, there has been no record of xanthone derivatives being used as a chemotherapeutic agent for the treatment of cancers. The present study describes one candidate, garcinone E (Fig. **1**) which may have the potential of becoming an effective anticancer drug for the treatment of hepatocellular carcinoma (HCC) (Fig. **2**), lung cancer (Fig. **3**) and gastric cancer (Fig. **4**).

Garcinone E has a very broad spectrum of cytotoxic effects against various cancer cell lines and with the exception of the lung carcinoma cell line CH27LC-1, all cell lines tested could be totally killed (Table **2**). In addition, it is interesting that in the CH272C-1 cultures, there are more viable cells at day 6 than at day 3. Possibly, only a small fraction of the cells are sensitive to garcinone E which can be effectively removed at or before day 3 while the rest of the population resumes normal growth, resulting in a net increase in the number of viable cells as detectable at day 6. The LD_{50} values of garcinone E against the different cell lines range from 0.1 to 5.4 μM and it is particularly interesting that garcinone E exerts potent cytotoxic effect against hepatoma cell lines (Fig. **2** and Table **1**) since chemotherapy for the treatment of HCC is usually disappointing. Unlike other anti-cancer drugs such as methrothrexate and vincristine, the cytotoxic effect of garcinone E does not vary greatly among different cancer cell lines (Table **2**) and could achieve total killing of the target cells (Tables **1** and **2**). Comparing to other commonly used anti-cancer drugs, our estimate is that the potency of garcinone E against hepatoma cell lines is equal to or higher than that of mixtoxantrone but is less effective than taxol (Table **2**). In addition, garcinone E should be a more effective drug than methrothrexate, vincristine, 5-Fu and cisplatin (Table **2**) suggesting that it is potentially more effective than some of the commercially available anti-neoplastic agents presently in use. The ability to kill all cancer cells at a reasonably low dose is an important criterion in assessing the efficacy of an anti-cancer drug because even a very small fraction of surviving cells could lead to the emergence of a population of drug-resistant tumor cells resulting in a subsequent relapse and treatment failure. In this respect, garcinone E could be considered a potent anti-cancer drug against HCC.

Taiwan is one of the high incidence areas of HCCs which is also one of the major causes of death among cancer patients. Thus far, systemic chemotherapy using single agents or in combination for the treatment of HCCs is ineffective [18], [19], [20] and although regional chemotherapy has produced some objective responses, the efficacy is far from satisfactory [21]. Thus, the development of potential new drugs for the cure of HCC is most desirable. In addition, the same holds true for both lung cancers and stomach cancers which are also prevalent in Taiwan.

In addition to cytotoxic effects, some xanthones are also known to have antibacterial [5] and immunomodulating [3], [4] activities. These biological properties make garcinone E an ideal candidate for special treatment protocols such as pretransplant aggressive chemotherapy for the treatment of HCCs.

Table **2** The relative cytotoxic effects of garcinone E and other chemotherapeutic agents against various hepatoma cell lines

Agent	*Lethal Dose 50% (Total killing)(LD_{50}, μM)*							
	HEp G2		*TONG*		*HEp 3B*		*SK-HEp-1*	
garcinone E	2.5 ± 1.2	(+)	5.4 ± 2.3	(+)	3.2 ± 0.8	(+)	0.5 ± 0.2	(+)
taxol	0.06 ± 0.01	(+)	0.61 ± 0.31	(+)	0.76 ± 0.5	(+)	0.06 ± 0.03	(+)
mitoxantrone	0.41 ± 0.1	(+)	0.82 ± 0.32	(±)	0.6 ± 0.28	(–)	0.1 ± 0.05	(+)
methrothrexate	0.07 ± 0.03	(–)	0.97 ± 0.51	(+)	1.5 ± 0.45	(–)	0.07 ± 0.02	(–)
vincristine	> 50 ± 0	(–)	9.5 ± 3.5	(+)	30.9 ± 7.9	(–)	0.05 ± 0.03	(+)
5-fluorouracil	4.9 ± 1.0	(–)	> 50 ± 0	(–)	> 50 ± 0	(–)	> 50 ± 0	(–)
Cisplatin	25 ± 10.3	(–)	12 ± 4.8	(–)	> 50 ± 0	(–)	46 ± 2.5	(–)

Data were mean ± standard error mean (SEM) of results from 3 separate experiments performed under similar conditions. (+) Total killing of cells was observed at a doses ≦ 10 μM at day 6 following exposure to the drugs. (–) total killing was not observed. (±) total killing was observed in some but not all experiments.

In summary, the present study describes the efficacy of using garcinone E as a member of the chemotherapeutic regimen that may be useful for the treatment of HCC, lung cancers and gastric cancers.

Acknowledgements

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