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Research article

Xanthones from *Garcinia mangostana* inhibit the proliferation and migration of hepatocellular carcinoma cells

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Abstract

Hepatocellular carcinoma (HCC) accounts for more than 90% of liver cancer cases worldwide and is the most common cancer in men in Thailand. Here, we investigated the effect of five xanthones isolated from *Garcinia mangostana* root on the proliferation and migration of human HCC cell lines. We identified five xanthones, among which the prenylated xanthones **1–3** had significant anti-proliferative effects on both Hep-G2 and Huh-7 cell lines, with 50% inhibitor concentrations (IC_{50}) ranging from 5.9 µM to 15.0 µM. In contrast, the simple xanthones **4** and **5** were inactive (IC_{50} >50 µM), implying that the prenyl moiety is required for the inhibitory activity. Exposure of HCC cells to **1** and **2** (α- and β-mangostin) also suppressed cell migration, a crucial step in metastasis.

Keywords: xanthone, *Garcinia mangostana*, cell proliferation, cell migration

Introduction

Cancer is one of the leading causes of death globally. In Thailand, hepatocellular carcinoma (HCC) is the first and third most common cancer in men and women, respectively.[1] The risk factors for HCC include hepatitis B and C virus infection, aflatoxin exposure, heavy alcohol consumption, and type 2 diabetes.[2] Over the past several decades, the incidence and mortality of HCC have increased dramatically, driven in large part by the poor efficacy of conventional and targeted therapies, resulting from intrinsic or acquired drug resistance.[3] The ability of cancer cells to migrate and invade are crucial for metastasis, which is the main feature of malignant tumors and the main cause of death from cancer.[4,5] Thus, there is an urgent need to identify novel compounds that prevent and/or suppress cancer cell growth and metastasis.

Garcinia mangostana, also known as the "Queen of Fruit" in Thailand, is a rich source of xanthones, a unique class of compounds with a 9*H*-xanthen-9-one scaffold.[6] Most parts of *G. mangostana*, including the bark and root but especially the pericarp, have been used as herbal medicines in Southeast Asia

for hundreds of years. In Thai traditional medicine, *G. mangostana* pericarp is used to treat various disorders and infections, including inflammation, abdominal pain, leucorrhea, and gonorrhea. The xanthone α-mangostin is well-established as the major constituent of *G. mangostana* pericarp extracts and has broad biological activities, including potent cytotoxic properties against various cancer cells, which is also a property of other xanthones.[6] The structural and pharmacological diversity of xanthones thus makes them a topic of great interest for cancer researchers. In the current study, we investigated the effects of *G. mangostana* xanthones on the proliferation and migration of two human HCC cell lines, Hep-G2 and Huh-7. We focused our study on xanthone metabolites isolated from the roots of *G. mangostana* because there have been few studies on compounds isolated from this part of the plant.

Methods

General experimental procedures

NMR spectra were recorded on a Bruker AV400 (400 MHz for ^{1}H-NMR, 100 MHz for ^{13}C-NMR; Bruker, MA, USA). Column chromatography was performed on silica gel (230–400 mesh, Merck KGaA, Darmstadt, Germany), Sephadex LH-20 (18–111 µm, GE Healthcare, Tokyo, Japan) and ODS (63–212 µm, Wakogel® 100C18, Osaka, Japan). Analytical TLC was performed using precoated silica gel 60 GF254 plates (Merck KGaA).

Plant material

Roots of *G. mangostana* were collected in May 2019 from Nakhon Sri Thammarat Province, Thailand (GPS: 8.4330987,99.7802348) and were identified by staff of the Royal Forest Department, Nakhon Si Thammarat Province. A voucher specimen was assigned the code CUCHEM2019-003 and deposited at the Department of Chemistry, Faculty of Science, Chulalongkorn University.

Extraction and isolation

Air-dried and powdered *G. mangostana* roots (0.5 kg) were extracted with MeOH three times for 3 days (3 x 3 days, 1 L each) at room temperature and then concentrated under reduced pressure. The residue was partitioned between H_2O and EtOAc (1:1 v/v) three times, and the EtOAc extract (10 g) was separated by SiO_2 column chromatography using *n*-hexane:EtOAc (4:1) to give five fractions (A–E). Fractions B, C, and D were subjected to Sephadex LH-20 chromatography and eluted with MeOH to give two B subfractions (B1, B2) and three C and subfractions (C1–C3 and D1–D3). Subfraction B2 (62.1 mg) was further purified by SiO_2 column chromatography using *n*-hexane:EtOAc (4:1) to yield **4** (2.3 mg). Subfraction C1 (0.53 g) was recrystallized in MeOH to obtain **1** (68.0 mg). Subfraction C3 (0.37 g) was separated by open-column reverse-phase ODS (C-18) and eluted with H_2O:MeCN (1:4) to give **2** (51 mg). Subfraction D3 (82.1 mg) was purified by SiO_2 column chromatography and eluted with *n*-hexane:EtOAc (4:1) to afford **5** (5.4 mg). Fraction E (0.42 g) was fractionated by SiO_2 column chromatography with *n*-hexane:EtOAc (4:1) to provide three subfractions (E1–E3). Subfraction E3 (20.6 mg) was further purified by reverse-phase ODS column chromatography and eluted with H_2O:MeCN (3:7) to give **3** (0.7 mg).

α-Mangostin (**1**): yellow crystals; δ_H ($CDCl_3$) 13.70 (s, 1-OH), 6.80 (s, H-5), 6.31 (s, H-4), 5.27 (t, *J* = 8.0 Hz, H-12), 5.25 (t, *J* = 8.0 Hz, H-17), 4.08 (d, *J* = 8.0 Hz, H_2-11), 3.78 (s, 7-OCH_3), 3.41 (d, *J* = 8.0 Hz, H_2-16), 1.80 (s, H_3-20), 1.79 (s, H_3-15), 1.72 (s, H_3-14), 1.67 (s, H_3-19); δ_C ($CDCl_3$) 182.1 (C-9), 161.8 (C-3), 160.7 (C-1), 155.8 (C-6), 154.9 (C-10a), 152.9 (C-4a), 142.8 (C-7), 137.3 (C-8), 132.4 (C-18), 131.5 (C-13), 123.4 (C-17), 121.9 (C-12), 112.2 (C-8a), 103.5 (C-9a), 109.2 (C-2), 101.8 (C-5), 93.2 (C-4), 61.9 (7-OCH_3), 26.6 (C-11), 25.8 (C-19), 25.9 (C-14), 21.6 (C-16), 18.3 (C-20), 17.9 (C-15).

β-Mangostin (**2**): yellow crystals; δ_H ($CDCl_3$) 13.41 (s, 1-OH), 6.84 (s, H-5), 6.35 (s, H-4),

5.27 (t, *J* = 8.0 Hz, H-12), 5.23 (t, *J* = 8.0 Hz, H-17), 4.10 (d, *J* = 8.0 Hz, H_2-11), 3.90 (s, 3-OCH_3), 3.82 (s, 7-OCH_3), 3.35 (d, *J* = 8.0 Hz, H_2-16), 1.83 (s, H_3-20), 1.80 (s, H_3-15), 1.69 (s, H_3-19), 1.68 (s, H_3-14); δ_C ($CDCl_3$) 182.1 (C-9), 160.0 (C-1), 155.4 (C-6), 155.9 (C-4a), 154.5 (C-10a), 142.7 (C-7), 137.2 (C-8), 132.2 (C-18), 131.8 (C-13), 123.4 (C-17), 122.5 (C-12), 112.7 (C-8a), 104.0 (C-9a), 111.7 (C-2), 101.6 (C-5), 89.0 (C-4), 62.2 (7-OCH_3), 56.0 (3-OCH_3), 26.7 (C-11), 26.0 (C-14), 26.1 (C-19), 21.5 (C-16), 18.4 (C-20), 17.9 (C-15).

γ-Mangostin (**3**): light yellow powder; δ_H ($CDCl_3$) 13.85 (s, 1-OH), 6.83 (s, H-5), 6.40 (s, H-4), 5.28 (t, *J* = 8.0 Hz, H-12), 5.27 (t, *J* = 8.0 Hz, H-17), 4.13 (d, *J* = 8.0 Hz, H_2-11), 3.35 (d, *J* = 8.0 Hz, H_2-16), 1.83 (s, H_3-20), 1.78 (s, H_3-15), 1.66 (s, H_3-19), 1.64 (s, H_3-14); δ_C ($CDCl_3$) 182.2 (C-9), 161.5 (C-3), 161.0 (C-1), 155.6 (C-6), 155.0 (C-10a), 153.1 (C-4a), 142.8 (C-7), 137.2 (C-8), 132.4 (C-18), 131.9 (C-13), 123.0 (C-17), 121.8 (C-12), 112.0 (C-8a), 103.5 (C-9a), 109.1 (C-2), 101.8 (C-5), 93.2 (C-4), 27.0 (C-11), 25.8 (C-19), 25.9 (C-14), 21.6 (C-16), 18.0 (C-20), 17.6 (C-15).

Euxanthone (**4**): light yellow powder; δ_H ($CDCl_3$) 12.71 (s, 8-OH), 7.67 (t, *J* = 8.0 Hz, H-3), 7.49 (d, *J* = 8.0 Hz, H-5), 7.41 (d, *J* = 8.0 Hz, H-6), 7.41 (d, *J* = 8.0 Hz, H-8), 6.98 (d, *J* = 8.0 Hz, H-4), 6.74 (d, *J* = 8.0 Hz, H-2); δ_C ($CDCl_3$) 183.1 (C-9), 162.8 (C-1), 157.4 (C-4a), 155.1 (C-7), 150.6 (C-10a), 137.8 (C-3), 126.2 (C-6), 121.8 (C-8a), 120.2 (C-5), 110.6 (C-2), 109.2 (C-8), 109.1 (C-9a), 107.8 (C-4).

Norathyriol (**5**): yellow powder; δ_H ($CDCl_3$) 13.16 (s, 1-OH), 7.52 (s, H-8), 6.88 (s, H-5), 6.34 (s, H-4), 6.20 (s, H-2); δ_C ($CDCl_3$) 180.1 (C-9), 165.2 (C-3), 164.1 (C-1), 158.5 (C-4a), 153.9 (C-6), 152.2 (C-10a), 143.7 (C-7), 113.4 (C-8a), 108.9 (C-8), 102.8 (C-9a), 103.1 (C-5), 98.2 (C-2), 94.1 (C-4).

MTT viability assay

The effects of the isolated xanthones on cell viability were determined using an MTT assay.[7] Briefly, the two human HCC cell lines Hep-G2 and Huh-7 (purchased from Riken Bioresource Research Center, RBRC, Japan) were seeded in 96-well plates at a density of 4.5×10^4 cells/well in 200 μL/well of growth medium (DMEM with 10% fetal bovine serum and 1%penicillin/streptomycin, Hyclone, Logan, USA) and incubated for 24 h at 37°C in a 5% CO_2 atmosphere. The supernatant was removed, serial dilutions of test compounds in serum-free DMEM medium were added to the wells, and the plates were incubated for 48 h. Sorafenib at 3 μM and medium alone were included as positive and negative controls, respectively. Aliquots of MTT solution (10 μL/well of stock solution 0.5 mg/mL in PBS) were added to the cells and the plates were incubated for an additional 4 h. The supernatants were removed and 100 μL/well of 100% dimethylsulfoxide was added to dissolve the formazan crystals. Absorbance at 570 nm was measure using a microplate reader. The percentage of viable cells and the half-maximal inhibitory concentration (IC_{50}) of the compounds were calculated using Prism version 8 software (GraphPad, San Diego, CA, USA).

Wound-healing assay

Cell migration was assessed using a standard wound-healing assay.[7] In brief, monolayers of Huh-7 cells were prepared by seeding in 12-well plates at a density of 5.5×10^5 cells/well in DMEM medium. A micropipette tip (1 mm diameter) was used to scratch a line across the adherent cells to generate a gap. Growth medium was removed and replaced with serum-free DMEM containing test compounds at concentrations encompassing the IC_{50} values determined in the MTT assays. Sorafenib at 3 μM or medium alone were included as controls. The plates were examined under a microscope and the wound gap was photographed before incubation (0 h) and at 24 h and 48 h after incubation at 37°C. Cell migration was calculated as:

$$\%\text{cell migration} = \left[\frac{\text{gab closure at 0 h - gab closure at indicated time}}{\text{gab closure at 0 h}}\right] \times 100$$

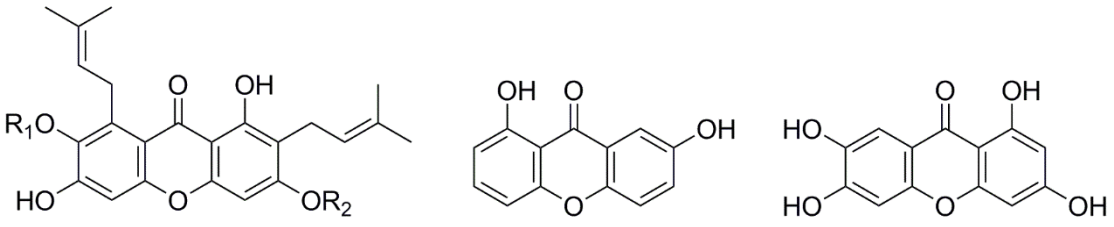
Results and Discussion

Isolation and identification of xanthones from *G. mangostana* root

The EtOAc extract of *G. mangostana* root was subjected to size exclusion, normal-phase, and reverse-phase column chromatgraphy to obtain five xanthones: α-mangostin (**1**), β-mangostin (**2**), γ-mangostin (**3**), euxanthone (**4**), and norathyriol (**5**). Compounds **1** and **2** were the major constituents. Compound structures (**Figure 1**) were determined from the NMR spectroscopic data and by comparison with data reported in the literature.[8–12]

Anti-proliferative effects of isolated xanthones on Hep-G2 and Huh-7 cells

Although xanthones are one of the largest constituents of most parts of *G. mangostana*, and some are known to possess anti-proliferative activity against various cancer cell lines[6], their effects on HCC growth and metastasis are unclear. To determine the effects of compounds **1–5** on HCC cells, we examined the proliferation of Hep-G2 and Huh-7 cells incubated with compounds **1–5** at 0–100 µM for 48 h. The tyrosine kinase inhibitor sorafenib, which is approved for the treatment of advanced HCC, was used as a positive control. As shown in **Table 1**, the three prenylated xanthones α-, β- and γ-mangostin (**1–3**) effectively inhibited HCC cell proliferation with IC_{50} values ranging from 5.9 µM to 12.4 µM for Hep-G2 cells and 6.8 µM to 15.0 µM for Huh-7 cells. In contrast, the simple xanthones **4** and **5** did not display any inhibitory effect up to the maximum concentrations tested (100 µM). These results indicate that the prenyl moiety is likely to be required for the inhibitory activity.



	R_1	R_2
α-mangostin (**1**)	Me	H
β-mangostin (**2**)	Me	Me
γ-mangostin (**3**)	H	H

euxanthone (**4**)

norathyriol (**5**)

Figure 1. Structures of xanthones **1–5**.

Table 1. Anti-proliferative effects of isolated xanthones on HCC cells

Compound	IC_{50} (μM)	
	Hep-G2	Huh-7
1	5.9 ± 1.1	6.8 ± 1.3
2	12.4 ± 1.0	15.0 ± 1.1
3	11.3 ± 0.8	12.0 ± 0.7
4	>100	>50
5	>100	>50
Sorafenib (3 μM)	2.7 ± 1.9	3.2 ± 1.2

Results are expressed as the mean ± SD of n=3 samples/point and are representative of 2 experiments.

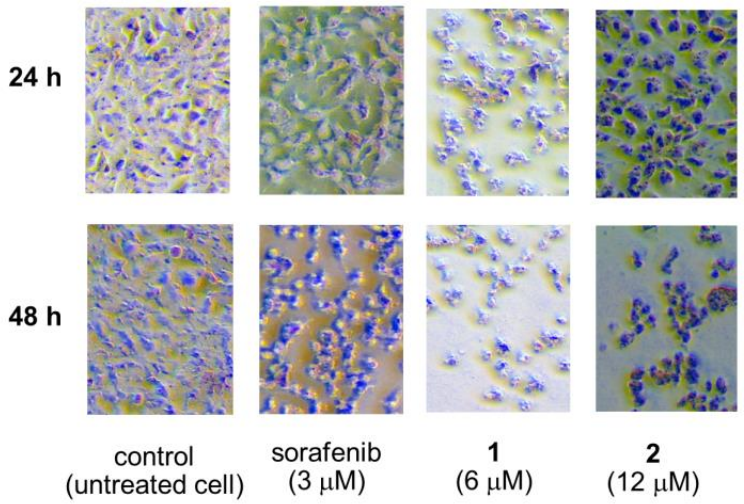


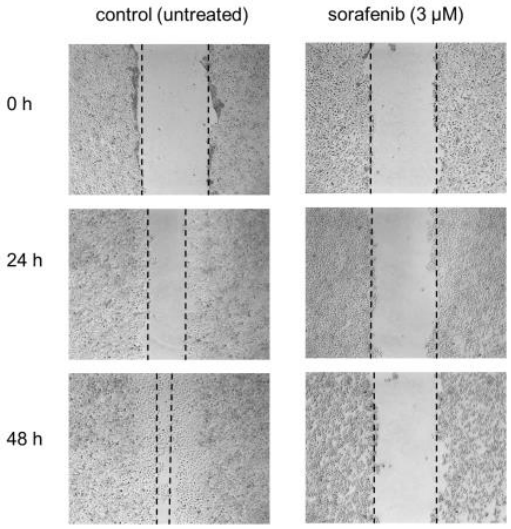
Figure 2. Effect of xanthones **1** and **2** on Huh-7 cell morphology.

We also examined the effects of prenylated xanthones **1** and **2** on HCC cell morphology. Compound **3** was not examined omitted due to limited quantities. Alterations in cell morphology may be indicative of rearrangement of the cellular cytoskeleton, which is a critical process necessary for the adhesion interactions and polarized cell migration required for cancer cell metastasis.[4] Huh-7 cells were treated with **1**, **2**, or sorafenib at their IC_{50} concentrations for 24 h and 48 h and then observed by phase contrast microscopy. As shown in **Figure 2**, untreated cells exhibited normal proliferative morphology, whereas **1**-, **2**-, and sorafenib-treated cells exhibited changes between 24 h and 48 h post-treatment that were characterized by a rounded cell morphology and loss of adhesion.

Effects of xanthones 1 and 2 on Huh-7 cell migration

Metastasis is the spread of primary tumor cells through the lymphatic and circulatory systems to other organs, and is responsible for the majority of cancer-related deaths.[13] The ability to migrate and invade tissues is coordinated by signals derived from cell–cell interactions with neighboring cells and with various factors in the surrounding tumor microenvironment.[4] Therefore, inhibition of this process may provide novel therapeutic agents to suppress metastasis. To evaluate the effects of the *G. mangostana* root xanthones on HCC cell migration, we employed a widely used wound-healing assay in which a gap is introduced into the cell monolayer,

a)



b)

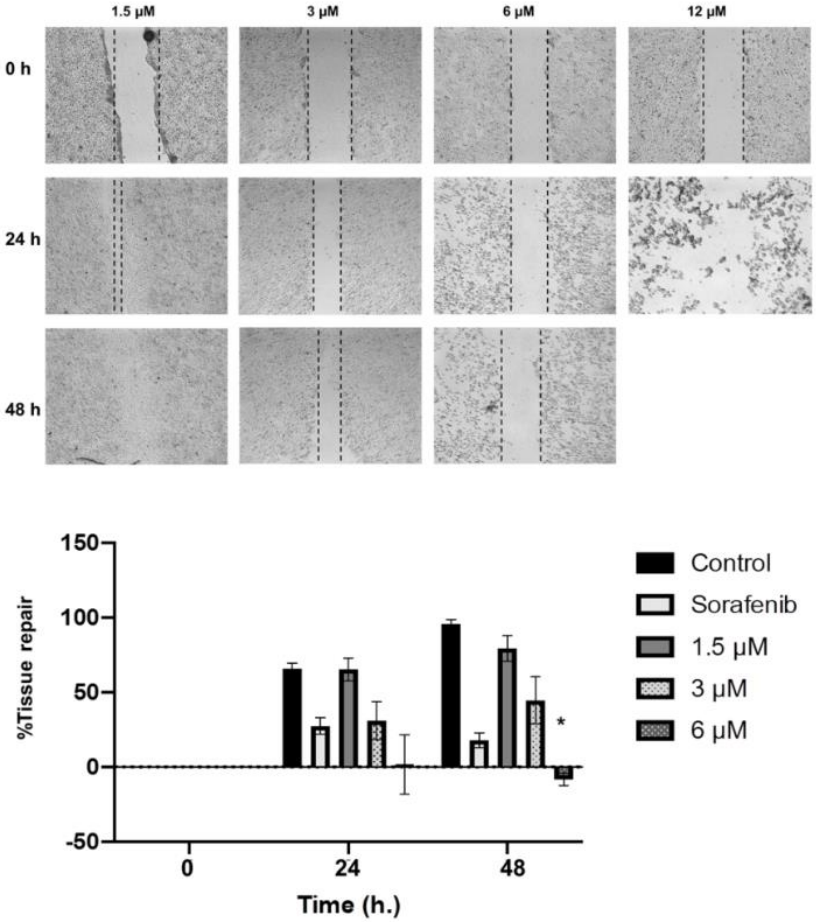


Figure 3. Wound-healing assays of Huh-7 cells with sorafenib and α-mangostin (**1**). (a) Images of control and sorafenib (3 μM)-treated cells. (b) Images of **1**-treated cells (upper panels) and quantification of cell migration (lower graph). Data are presented as the mean ± SD of n=3 samples/point and are representative of 2 experiments. * $P < 0.05$.

and the narrowing of the gap over time is quantified as cell migration. Huh-7 cells were treated with **1**, **2**, and sorafenib at concentrations encompassing the IC_{50} values, and incubated at 37°C. The wound was photographed and the gap measured before (0 h) and at 24 h and 48 h after incubation. As shown in **Figures 3** and **4**, xanthones **1** and **2** suppressed cell migration in a dose-dependent manner compared with untreated cells, which exhibited virtually 100% wound closure. Inhibition of cell migration was most striking after exposure to **1** and **2** at 6 μM for 24 h (**Figure 3b** and **4**). Sorafenib-treated cells also exhibited reduced cell migration, as expected, and underwent a morphology change. Treatment of cells with **1** and **2** at concentrations higher than 6 μM or for longer than 24 h induced cell damage (**Figure 3a**).

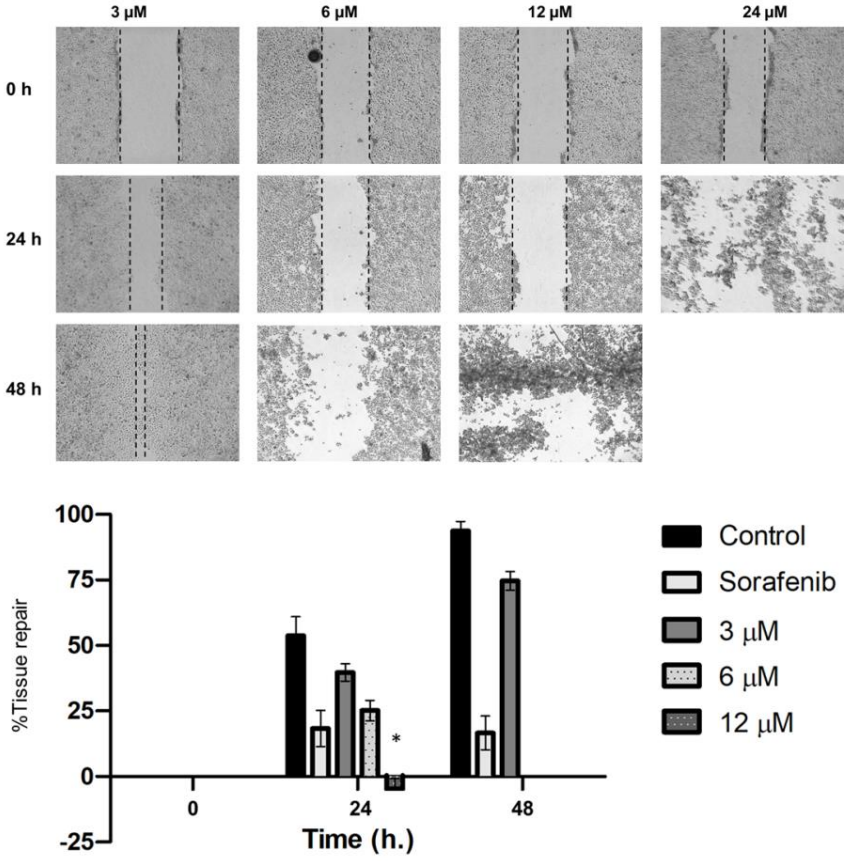


Figure 4. Wound-healing assays of Huh-7 cells with β-mangostin (**2**). Images of cells treated with **2** (upper panels) and quantification of cell migration (lower graph). Sorafenib was added at 3 μM. Data are presented as the mean ± SD of n=3 samples/point and are representative of 2 experiments. * $P < 0.05$.

Conclusions

We describe here the isolation and characterization of five xanthones, including three prenylated (**1–3**) and two simple (**4, 5**) xanthones, from the root of mangosteen (*G. mangostana*). *In vitro* testing revealed that only the prenylated xanthones **1**–**3** had detectable anti-proliferative efficacy against the two HCC cell lines tested. **1** and **2** also altered Huh-7 cell morphology and suppressed cell migration, which is a critical process for cancer invasion and metastasis. These results suggest that prenylated xanthones from *G. mangostana* may have potential utility as anti-metastatic agents for HCC and support further investigation into their potential clinical use. Given that mangosteen is a commonly consumed Thai fruit, further development of these pharmacologically active compounds may also have economic benefits.

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