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Fatty acid synthase inhibitors of phenolic constituents isolated from *Garcinia mangostana*

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ABSTRACT

Natural inhibitors of fatty acid synthase (FAS) are emerging as potential therapeutic agents to treat cancer and obesity. The bioassay-guided chemical investigation of the hulls of *Garcinia mangostana* led to the isolation of 13 phenolic compounds (**1–13**) mainly including xanthone and benzophenone, in which compounds **7**, **8**, **9**, **10**, and **11** were isolated from this plant for the first time and compound **9** was a new natural product. These isolates possess strong inhibitory activity of FAS with the IC_{50} values ranging from 1.24 to 91.07 μM. The study indicates that two types of natural products, xanthones and benzophenones, could be considered as promising FAS inhibitors.

The animal fatty acid synthase (EC 2.3.1.85, ab. FAS) is a key metabolic enzyme catalyzing the de novo synthesis of long chain saturated fatty acids from acetyl-CoA and malonyl-CoA in the presence of the reducing substrate NADPH.[1] It comprises two identical subunits, each containing seven discrete functional domains: malonyl/acetyltransferase (MAT), β-ketoacyl synthase (KS), β-ketoacyl reductase (KR), dehydrase (DH), enoyl reductase (ER), thioesterase (TE), and the acyl carrier protein (ACP).[2] A number of studies have recently reported that FAS may be a potential target for anti-obesity and anti-cancer drugs.[3–6] Central inhibition of FAS suppresses food intake and leads to dramatic weight-loss in mice, suggesting that FAS not only functions in providing metabolic substrates, but also plays a role in satiety signaling.[4] It has also been reported that inhibition of FAS, by either knockdown of FAS gene with siRNA or inactivation of FAS leads to caspase-8-mediated tumor cell apoptosis.[7] At the same time, many FAS inhibitors such as Cerulenin, C75, Orlistat, and EGCG, have joint effects of weight-loss and anti-tumor.[8,4,9–15] These prove that FAS may be a clinical and experimental target for scientific research and drug discovery. Therefore, it is important to find novel compounds especially from natural sources that reduce FAS activity or expressing levels, which may be useful for the treatment of obesity and cancer. In our preliminary work, we screened dozens of fruits extracts against FAS.

During our early screening of activity, the ethanol extract of the hulls of *Garcinia mangostana* L., family Guttiferae was shown strong capability of inactivating FAS with the IC_{50} value at 1.74 μg/ml. *G. mangostana* is widely cultivated in the Southeast Asia, which has been used for heeling skin infections and wounds and for the relief of diarrhea for a long time.[16,17] The fruit hull of *G. mangostana* has been used for hundreds of years around the world, mostly in Southeast Asia, as a medicine for the treatment of abdominal pain, dysentery, wound infections, eczema, suppuration, and chronic ulcer.[16–24] The constituents of *G. mangostana* have been reported mainly as flavonoids and xanthones.[25–27] Despite a number of studies on the chemical constituents and biological activities of the family Guttiferae, none of them investigates its FAS inhibitory activity. In order to screen FAS inhibitors, we isolated 13 known compounds from hulls of *G. mangostana* by column chromatographic (see Supplementary data), and assayed their FAS inhibitory activities. The structures of the isolated compounds **1–13** were identified as α-mangostin (**1**), β-mangostin (**2**), γ-mangostin (**3**), 9-hydroxycalabaxanthone (**4**), garcinone E (**5**), 1,5-dihydroxy-3-methoxy-2-(3-methylbuten-1-yl)-9*H*-xanthen-9-one (**6**), 1,3,7-trihydroxyxanthone (**7**), 2,4,6,7-tetrahydroxyxanthone (**8**), 3,4,5,3′-tetrahydroxybenzophenone (**9**), 2,4,6,3′,5′-pentahydroxybenzophenone (**10**), neosmitilbin (**11**), epicatechin (**12**), egonol (**13**), respectively, by analysis of MS and NMR

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data, and comparison with those in the literature (Fig. 1).[28–41] In this Letter, we report their FAS inhibitory activity.

The preparation, storage, and usage of FAS from chicken liver, which shares 63% identity with the amino acid sequence of human FAS, were performed as described as previous.[42] Briefly, the purified FAS were homogenized by polyacrylamide gel electrophoresis in the presence and absence of sodium dodecyl sulfate, respectively. The overall FAS activity was determined by decreasing of NADPH absorbance at 340 nm using a spectrophotometer at the constant temperature of 37 °C.[43]

All the isolates were assayed for their inhibitory activity against FAS, and the results are presented in Table 1. The known FAS inhibitor EGCG (IC_{50} = 51.97 μM) was used as positive controls in this assay. All of the isolates inhibited FAS in dose-dependent manners and most of these compounds were more potent than EGCG. Of the compounds tested, compounds **1**, **3**, **5**, and **10** exhibited the strongest inhibitory activity with lower IC_{50} values of 5.54, 1.24, 3.30, and 8.59 μM, respectively. In general, of these compounds **1**–**13** isolated from *G. mangostana*, those containing the conjugated system of phenyl and carbonyl (compounds **1**–**5** and **7**–**11**) showed the effective inhibitions of FAS. From the compounds of the same skeleton, γ-mangostin (**3**) was more active than α-mangostin (**1**) and β-mangostin (**2**), as well as compound **8** and compound **10** were better than

Table 1
The inhibitory activity of the compounds **1**–**13** isolated from *G. mangostana* against FAS

Compounds	FAS inhibitory activity IC_{50}[a] (μM)
1	5.54 ± 0.26
2	24.83 ± 1.35
3	1.24 ± 0.05
4	40.64 ± 1.69
5	3.30 ± 0.19
6	Nd[b]
7	40.98 ± 0.64
8	17.85 ± 0.49
9	14.76 ± 0.10
10	8.59 ± 0.28
11	49.16 ± 0.11
12	>1000[c]
13	91.07 ± 2.23
EGCG[d]	51.97 ± 1.65

[a] IC_{50} values were determined by regression analyzes and expressed as means ± SD for three distinct experiments.
[b] The IC_{50} value was not detected because of poor sample solubility.
[c] The number in parentheses represents a percentage of inhibition at a level of 1000 μM.
[d] Positive controls.

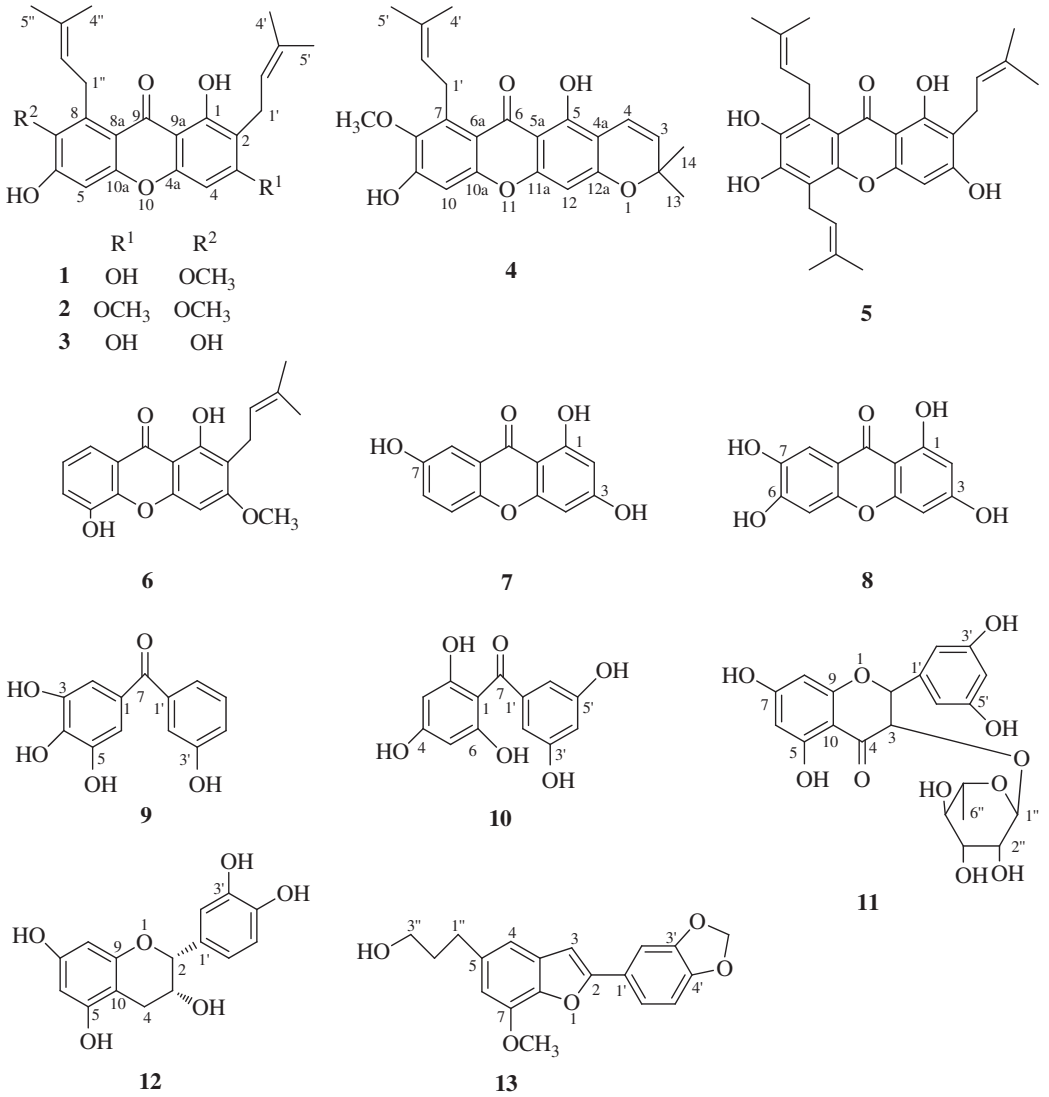

Figure 1. Structures of compounds **1**–**13** isolated from *G. mangostana*.

compounds **7** and **9**, respectively, indicating that the phenolic hydroxyl groups are required for the activity. The more phenolic hydroxyl groups in the compound, the higher activity. However, the acute function loss of compounds **4** and **7** compared to γ-mangostin (**3**) and compound (**8**) suggested that the number of the phenolic hydroxyl groups on the xanthone skeleton is the key factor of the inhibition of FAS. Another trend was shown in Table 1. The more methoxy-substituted on the benzene ring, the less active it appeared to be. Compounds **4** and **2** displayed much lower activity than compounds **3** and **1** both with less substituted methoxy. Isopentenyl group may not affect the activity because compound **3** showed slightly higher activity than compound **5** with one more isopentenyl group than former. Moreover, the higher activities of **3**, **5**, and **8** than **9** and **10** with the same number or one more of phenolic hydroxyl groups suggested the xanthones are superior to the benzophenones in inhibiting of FAS. Compounds **1**, **2**, and **4** were also isolated from the stem bark of *G. mangostana* and exhibited cytotoxic activity against HT-29 human colon cancer cell line,[44] which further confirmed that FAS was a potential target for anti-cancer drugs.

In conclusion, the phenolic constituents containing the conjugated system of phenyl and carbonyl, especially xanthones and benzophenones could be considered as a promising class of FAS inhibitors. Although xanthones have a wide known range of biological activities including anti-oxidant, anti-tumor, anti-bacterial, anti-malaria, anti-cyclooxygenase (COX), inducing apoptosis of cells, and against HIV-1.[45–54] The biological activities of antitumor and antibacterial also were found in benzophenones.[55–57] To our best knowledge, it is the first time to show the FAS inhibitory activity of the xanthones and benzophenones, which may give a clue to the underneath pharmacological mechanisms. Furthermore, as FAS plays an important role in obesity and cancer initiation and progression, the discovery of natural inhibitors has great potential in drug development for obesity and cancer treatment.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.061.

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