Antimycobacterial Activity of Prenylated Xanthones from the Fruits of *Garcinia mangostana*

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Prenylated xanthones, isolated from the fruit hulls and the edible arils and seeds of *Garcinia mangostana*, were tested for their antituberculosis potential. α- and β-Mangostins and garcinone B exhibited strong inhibitory effect against *Mycobacterium tuberculosis* with the minimum inhibitory concentration (MIC) value of 6.25 μg/ml. Tri- and tetra-oxygenated xanthones with di-C_5 units or with a C_5 and a modified C_5 groups are essential for high activities. Substitution in the A and C rings has been shown to modify the bioactivity of the compounds.

Key words antituberculosis; prenylated xanthone; *Garcinia mangostana*; *Mycobacterium tuberculosis*; structure–activity relationship

Plants have been used worldwide in traditional medicines for the treatment of diseases. It is estimated that even today approximately two-thirds to three-quarters of the world's population rely only on medicinal plants as their primary source of medicines. Mangosteen, *Garcinia mangostana* L. (Clusiaceae), is a tree fairly widespread in Southeast Asian countries, known for its medicinal properties. The edible fruit of this plant is considered to be one of the best of all tropical fruits. The fruit hulls have been in use in Thai folk medicine for the treatment of skin infections, wounds and diarrhea.[1)] Phytochemical studies have shown that this plant species are rich in a variety of prenylated xantones[2,3)] and the constituents have demonstrated a number of bioactivities.[4—11)] Tuberculosis continues to be an enormous global concern as it infects millions of people annually and the search for new drug leads is an urgent need due to the emergence of drug resistant strains of mycobacterial. A tuberculostatic effect has been noted in natural and synthetic non-prenylated xanthones,[12—14)] and quantitative structure–activity relationships investigations have established correlations between ^{13}C-NMR chemical shifts, lipophilicity and molar refractivities of the substituents of various synthetic non-prenylated xanthones and tuberculosis inhibition.[15,16)] However no study on the antimycobacterial potential of prenylated xanthones has been described. We have previously collected a number of prenylated xanthones isolated from the fruit hulls of this plant.[17)] We report here on the inhibitory activity of the prenylated xanthones obtained from the fruits of *G. mangostana* against *Mycobacterium tuberculosis* in *in vitro* experiments.

Prenylated xanthones **1**, **2**, **5**, **6**, **9**—**11**, **14**, and **15** were previously isolated from the green fruit hulls of *G. mangostana.*[17)] In order to obtain additional compounds for the testing, four known xanthones γ-mangostin (**3**),[11)] garcinone D (**4**),[18,19)] mangostanin (**8**)[20)] and 1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-methoxyxanthone (**12**)[1)] were isolated from a larger quantity of the MeOH extract of the fresh green fruit hulls. Compounds **7**,[21)] **13**,[1)] and more of compound **1**[17)] were obtained from the MeOH extract of the pulverized fresh arils and seeds (see Experimental).

The above prenylated xanthones **1**—**15**, the structures of which are shown in Chart 1, were examined in the present screening test. The inhibitory activity of **1**—**15** against *Mycobacterium tuberculosis* H_{37}Ra strain were determined using the Microplate Alamar Blue Assay (MABA).[22)] The minimum inhibitory concentration (MIC) data (Table 1) of the 15 xanthones suggested that, for a moderate to high antimycobacterial activity, the xanthones nucleus should contain tri- or tetra-oxygen functions with either di-C_5 units or with a C_5 and a modified C_5 groups in rings A and C. Among these, 1,3,6,7-tetraoxygenated xanthones bearing the C_5 units at C-2 and C-8 in α-mangostin (**1**), the major constituent, β-mangostin (**2**) and garcinone B (**6**) exhibited the most potent activities with the same MIC value of 6.25 μg/ml. γ-Mangostin (**3**), the second major constituent bearing C-3 and C-7 hydroxyls, exhibited lower inhibitory activity, suggesting that methylation of the 3-hydroxyl as well as the 7-hydroxyl groups resulted in increasing activity. A structure–activity comparison of **1** with **4** and mangostenol (**5**) revealed that modifications of the C_5 units in either C-8 or C-2 altered the activity. Furthermore, cyclization of the C_5 group at C-2 position resulted in decreasing inhibitory activity as exemplified by **7**, **8** and mangostanol (**9**). It is of interest to note that increment in polarity of the C_5 side chain reduced the activity, and addition of the third C_5 moiety in mangostenone A (**10**) and tovophyllin B (**11**), with respect to **6** and **7**, affected the inhibitory activity. In the case of 1,3,7-trioxygenated compounds, the inactivity of **12**, the xanthone with only one C_5 group located at C-2, compared with the moderately active demethylcalabaxanthone (**13**), indicated the essential of the di-C_5 side chains in the nucleus. For 1,3,5-trioxygenated xanthones, the more potent activity of trapezifolixanthone (**14**) than that of mangostinone (**15**) further supported this fact.

Experimental

General Procedures ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance 300 FT-NMR spectrometer, operating at 300 MHz (^{1}H) and 75 MHz (^{13}C). Column chromatography and TLC were carried out using Merck silica gel 60 (>230 mesh) and precoated silica gel 60 F_{254} plates, respectively. Plates of silica gel PF_{254}, thickness 1.25 mm, were utilized for preparative TLC. Spots on TLC were visualized under UV light and by spraying with anisaldehyde–H_2SO_4 reagent followed by heating.

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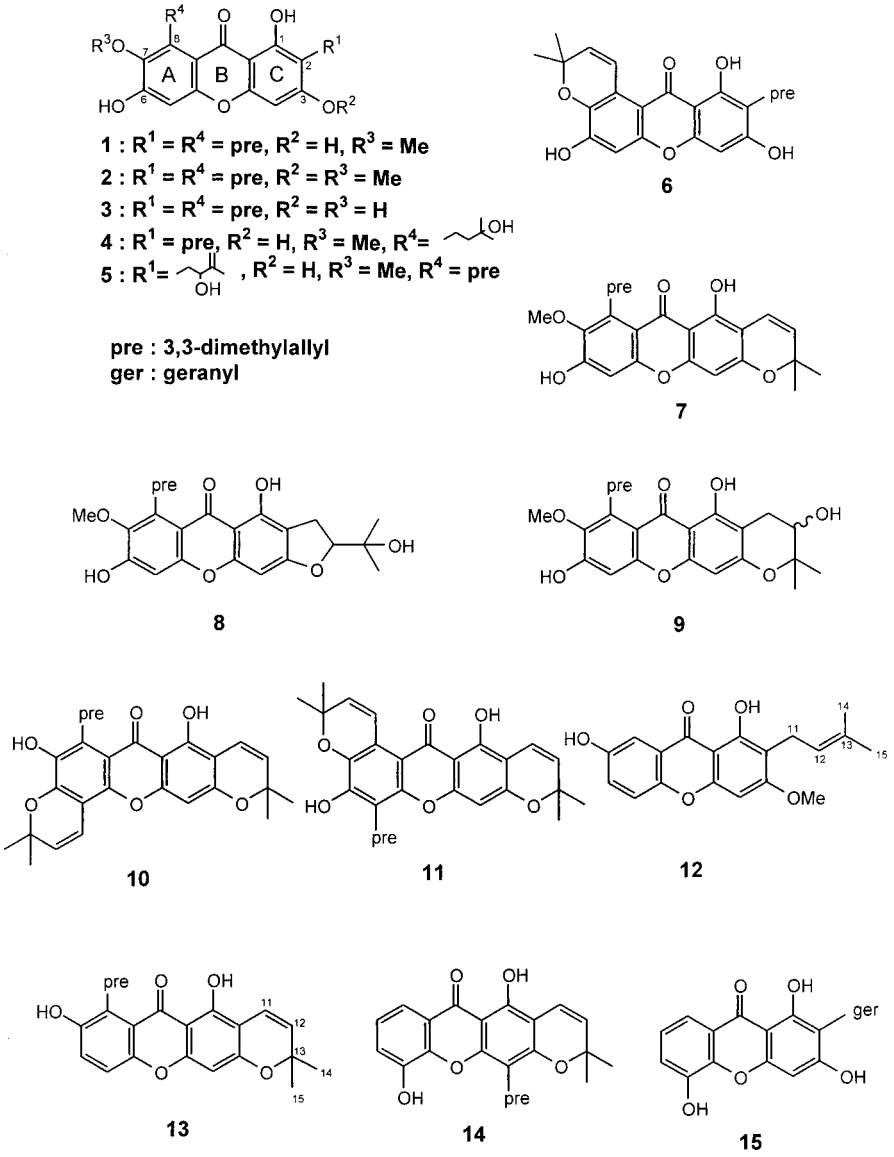

Chart 1. Structures of **1**—**15**

Table 1. MIC Value (μg/ml) for Compounds **1**—**15** against *Mycobacterium tuberculosis*

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
MIC	6.25	6.25	25	25	100	6.25	12.5	25	200	25	25	Inactive[a)]	12.5	12.5	200

a) Inactive at >200 μg/ml.

Plant Material The fruits of *G. mangostana* were collected from Bahnkai District, Chanthaburi Province, Thailand, in April 1999. A voucher specimen [voucher #0032(RU)] of this plant is deposited at the Faculty of Science, Ramkhamhaeng University, Bangkok, Thailand.

Extraction and Isolation On the larger scale investigation of the MeOH extract obtained from the fresh green fruit hulls of *G. mangostana*, four known xanthones γ-mangostin (**3**,[11] 100 mg), garcinone D (**4**,[18,19] 10 mg), mangostanin (**8**,[20] 7 mg) and 1,7-dihydroxy-2-(methylbut-2-enyl)-3-methoxyxanthone (**12**,[1] 2 mg) were further identified, in addition to those obtained previously.[17] Pulverized, fresh arils and seeds (4.7 kg) were extracted throroughly with MeOH and evaporation of the solvent gave crude extract (487.0 g). The crude extract was partitioned between $CHCl_3$ and H_2O to afford $CHCl_3$ extract (11.3 g). Repeated column chromatography of the $CHCl_3$ extract using $CHCl_3$, acetone and MeOH yielded demethylcalabaxanthone (**13**,[1] 80 mg), **7**[21] (180 mg) and α-mangostin (**1**,[17] 1.3 g) in order of polarity. All compounds were identified by comparison of their spectroscopic data (NMR and MS) with those reported in the literature. ^{13}C-NMR data of **12** and **13**, which have not previously been published, are also collected in this paper.

1,7-Dihydroxy-2-(3-methylbut-2-enyl)-3-methoxyxanthone (**12**): ^{13}C-NMR (acetone-d_6) δ: 181.5 (s, 9-C), 165.3 (s, 3-C), 160.0 (s, 1-C), 157.2 (s, 4a-C), 154.8 (s, 7-C), 150.7 (s, 8a-C), 131.7 (s, 13-C), 125.1 (d, 6-C), 123.0 (d, 12-C), 121.8 (s, 10a-C), 119.7 (d, 5-C), 111.8 (s, 2-C), 109.2 (d, 8-C), 104.2 (s, 9a-C), 90.6 (d, 4-C), 56.6 (q, OMe), 25.8 (q, 14-C), 21.8 (t, 11-C), 17.8 (q, 15-C).

1,7-Dihydroxy-8-(3-methylbut-2-enyl)-6′,6′-dimethylpyrano(2′,3′:3,2)-xanthone (**13**): ^{13}C-NMR ($CDCl_3$+CD_3OD) δ: 183.4 (s, 9-C), 160.1 (s, 3-C), 157.6 (s, 1-C), 156.6 (s, 4a-C), 151.5 (s, 10a-C), 151.1 (s, 7-C), 132.7 (s, 8-C), 128.2 (s, 18-C), 127.2 (d, 12-C), 123.0 (d, 6-C), 122.7 (d, 17-C), 118.9 (s, 8a-C), 116.1 (d, 5-C), 115.7 (d, 11-C), 104.1 (s, 2-C), 94.4 (s, 9a-C), 94.1 (d, 4-C), 78.0 (s, 13-C), 28.3 (q, 14-C, 15-C), 25.9 and 18.1 (2q, 19-C, 20-C), 25.7 (t, 16-C).

Bioassay Procedure The antimycobacterial activity was assessed against *M. tuberculosis* H_{37}Ra using the Microplate Alamar Blue Assay.[22]

Briefly, initial candidate compound dilutions were prepared in dimethyl sulfoxide (DMSO), and subsequent twofold dilutions were performed in 0.1 ml of 7H9GC medium in the microculture plates. 100 μl of 5×10^4 CFU/ml of *M. tuberculosis* in 7H9GC-Tween was added to each well of 96 well microculture plates containing of test compound. Plates were incubated at 37 °C for 7 d. To three control wells which contained drug and medium, bacteria and medium, and medium only, the Alarmar Blue dye solution (20 μl of Alarmar Blue solution and 12.5 μl of 20% Tween) was added daily until a color change from blue to pink occurred, at which time the dye was added to all remaining wells. Plates were incubated at 37 °C, and results were recorded at 24 h post-dye addition. Fluorescence was measured in a Cytofluoro Series 4000 Fluorescence Multi-Well Plate Reader (Per-Septive Biosystems, Framingham, MA, U.S.A.) in bottom-reading mode with excitation at 530 nm and emission at 590 nm. Percent inhibition was defined 1−(test well FU/mean FU of triplicate control wells)×100. The lowest drug concentration effecting an inhibition of ≥90% was considered the MIC. Experiments were usually completed within 10 d. Standard drugs rifampicin, isoniazid and kanamycin sulfate showed MIC of 0.003—0.0047, 0.025—0.05 and 1.25—2.5 μg/ml, respectively.

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