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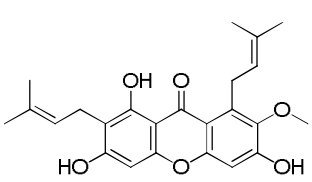
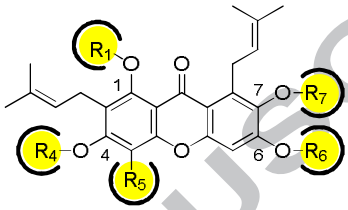


α-mangostin



α-mangostin analogs

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Synthesis of xanthone derivatives based on α-mangostin and their biological evaluation for anti-cancer agents

Xiang Fei[a], Minmi Jo[b], Bit Lee[a], Sang-Bae Han[c], Kiho Lee[b], Jae-Kyung Jung[c], Seung-Yong Seo[a,*], Young-Shin Kwak[b,*]

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ABSTRACT

A xanthone-derived natural product, α-mangostin is isolated from various parts of the mangosteen, *Garcinia mangostana* L. (Clusiaceae), a well-known tropical fruit. Novel xanthone derivatives based on α-mangostin were synthesized and evaluated as anti-cancer agents by cytotoxicity activity screening using 5 human cancer cell lines. Some of these analogs had potent to moderate inhibitory activities. The structure-activity relationship studies revealed that phenol groups on C3 and C6 are critical to anti-proliferative activity and C4 modification is capable to improve both anti-cancer activity and drug-like properties. Our findings provide new possibilities for further explorations to improve potency.

Mangosteen, *Garcinia mangostana* L. (Clusiaceae) is a well-known tropical fruit, indigenous to South East Asia, and has been used as a traditional medicine to treat inflammation, ulcer, skin infection, wound healing, amoebic dysentery and diarrhea.[1] The major secondary metabolites of mangosteen have found to be oxygenated and prenylated xanthones, among which α-mangostin is a yellow crystalline solid with a xanthone core structure (Figure 1). It was first isolated in 1855 by W. Schmid and its structure was correctly assigned in 1958 by Yates and Stout. So far α-mangostin has been investigated for biological properties including analgesic,[2] anti-inflammatory,[3] anti-oxidant,[4] anti-allergy,[5] anti-bacterial,[6] anti-tuberculosis,[7] anti-fungal,[8] anti-HIV,[9] and enhancement of immune system.[10] As various xanthones have been attracted as useful chemopreventive and therapeutic agents,[11] synthetic and medicinal chemistry studies of α-mangostin have been performed recently.[12] In contrast, there are a few reports that deal with chemical modification from them in order to discover improved analogs.[13] Since α-mangostin has limited aqueous solubility, we undertook an effort to identify analogs with greater aqueous solubility while retaining its excellent anti-tumor activity.

In this report, we disclose our effort to develop novel anti-cancer agents through the modification of polyphenolic natural product, α-mangostin. To improve the anti-cancer activity and the water-solubility, incorporation of polar solubility handles was mainly investigated.

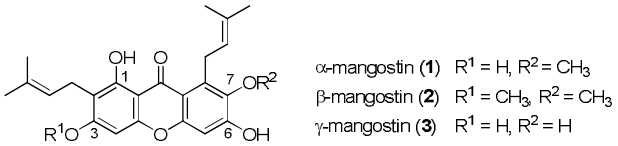

Figure 1. The structure of α-mangostin (**1**), β-mangostin (**2**) and γ-mangostin (**3**).

We first synthesized α-mangostin derivatives through various modifications of the phenols at the C1, C3 and C6 positions (Scheme 1). For the synthesis of acetates **4a** and **4b**, α-mangostin was treated with acetic anhydride, Et_3N and DMAP in CH_2Cl_2.[13c] The carboxyl groups of **5a** and **5b** were introduced utilizing methyl bromoacetate and K_2CO_3, followed by basic hydrolysis. As predicted from the precedent examples of selective alkylation on C-6 position prior to C-3, the allyl aryl ethers **6a** and **6b** were obtained by treating **1** with allyl bromide and K_2CO_3.[13a,14] Similarly, the triflates **7a** and **7b** were also prepared by trifluoromethanesulfonic anhydride, Et_3N and DMAP. The allyl group of **6a** and **6b** was used as a protecting group, while the triflates of **7a** and **7b** were replaced for the subsequent aromatic substitution with hydride or amines.

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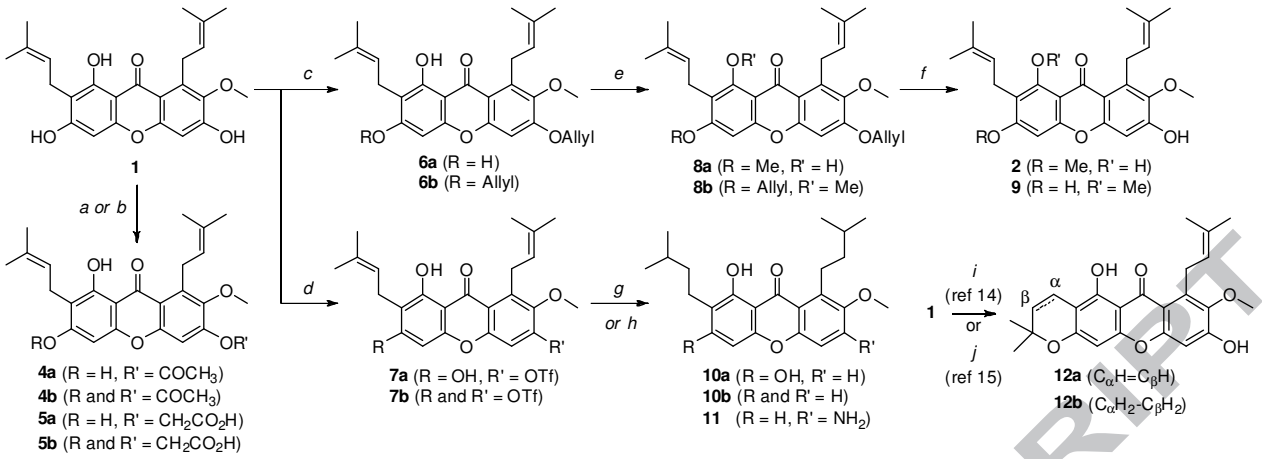
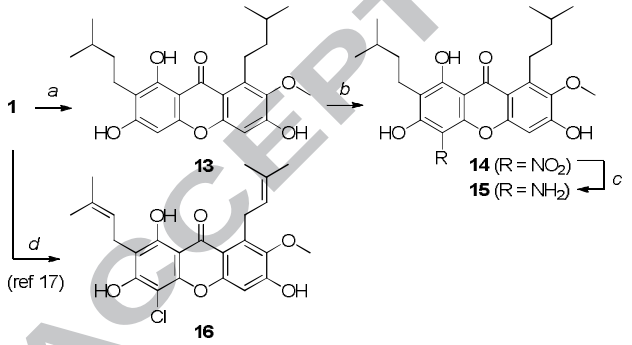


Scheme 1. Synthesis of α-mangostin analogs modified on C1, C3, and C6. Reagents and conditions: a) Ac_2O, pyridine, CH_2Cl_2, 30% for **4a**, 20% for **4b**; b) $BrCH_2CO_2CH_3$, K_2CO_3, acetone then aq. KOH, 7% for **5a**, 12% for **5b**; c) allyl chloride, K_2CO_3, acetone, 59% for **6a**, 20% for **6b**; d) Tf_2O, Et_3N, CH_2Cl_2 5% for **7a**, 18% for **7b**; e) CH_3I, K_2CO_3, acetone, 70% for **8a**, 80% for **8b**; f) cat. $Pd(PPh_3)_4$ and K_2CO_3, MeOH, 60 °C, for 91% for **2**, 51% for **9**; g) H_2, Pd/C, MeOH 41% for **10a**, 95% for **10b**; h) $BnNH_2$, $Pd(OAc)_2$, XPhos, Cs_2CO_3, DMF, μw, 160 °C, 23%, then H_2, Pd/C, MeOH, 34%; i) DDQ, benzene, reflux, 75%; j) TsOH, benzene, reflux, 70%.

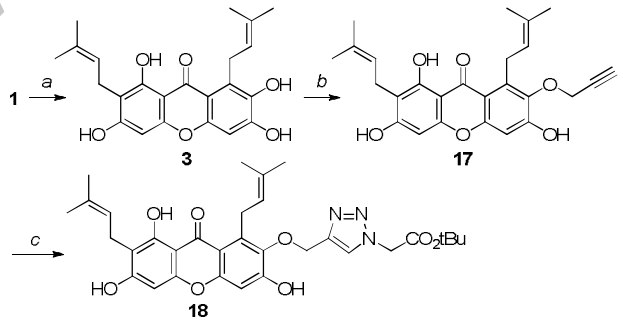
With **6a** and **6b** in hands, two methyl ether analogs were obtained through methylation at C3 and C1 phenols. The allyl protecting groups were removed using $Pd(PPh_3)_4$ and K_2CO_3 finally to give β-mangostin **2** and C1-methylated α-mangostin analog **9**. Two deoxygenated analogs **10a** and **10b** at C6 and C3/C6 position were obtained respectively from the mono-triflate **7a** and bis-triflate **7b**. During the reaction, two prenyl groups were reduced to isopentyl groups. The amine **11** was prepared from bis-triflate **7b** via a two-step sequence of a selective introduction of diphenylmethylamine at C6 position followed by an exhaustive hydrogenolysis under H_2, Pd/C. The known cyclized forms **12a** and **12b** were prepared by the treatment of DDQ[15] and TsOH,[16] respectively.



Scheme 2. Synthesis of α-mangostin analogs modified on C4. Reagents and conditions: : a) H_2, Pd/C, MeOH, 99%; b) HNO_3, AcOH, 19%; c) H_2, Pd/C, MeOH, 82%; d) NCS, CH_2Cl_2, 35%.

As shown in Scheme 2, analogs functionalized on C4 position were synthesized from **1**. First, the hydrogenated compound **13** was produced by catalytic hydrogenation under H_2 and Pd/C and subsequent nitration using HNO_3 and AcOH afforded the C4-nitro compound **14** in moderate yield.[17] Again, catalytic hydrogenation afforded the C4-amino compound **15**. By previous method,[13b] C4-chloride compound **16** was generated accompanied by a small amount of dichlorination.

We turned to the synthesis of γ-mangostin and analogs modified on C7 (Scheme 3). After screening some demethylation conditions,[18] we found that the methyl group can be removed most efficiently by using morpholine (neat) to give γ-mangostin. With γ-mangostin **3** in hands, the propargyl group was introduced by the mono-alkylation using propargylic bromide and potassium carbonate. Finally, with propargyl ether **17** in hands, the triazole **18** was synthesized via copper-catalyzed click chemistry, in which sodium ascorbate and copper sulfate in DMSO were treated with azidoacetate prepared in situ by the reaction of *t*-butyl bromoacetate and sodium azide. This developed synthetic process would be a very useful tool for a future chemical biology research to reveal α-mangostin's target protein and its biological significance.



Scheme 3. Synthesis of α-mangostin analogs modified on C7. Reagents and conditions: a) morpholine(neat), 32%; b) propargylic bromide, K_2CO_3, acetone, 34%; e) *t*-butyl bromoacetate, NaN_3, then, $CuSO_4$, sodium ascorbate, DMSO, 19%.

Newly synthesized xanthone analogs were screened for *in vitro* anti-proliferative activity against 5 human cancer cell lines, NCI-H460 (lung), SW-620 (colon), AsPC-1 (pancreas), MDA-MB-231 (breast), and B16F10 (skin) using a XTT kit, summarized as IC_{50} values in Tables 1~3. Adriamycin and α-mangostin were used as reference compounds and as expected, these compounds displayed potent cytotoxicity activity. This assay was performed under a standard assay condition by following a previously described assay protocol.[19]

The in vitro inhibition data for 15 compounds (**4**–**12**) modified at positions 1, 3, and 6 from α-mangostin are shown in Table 1. The effect of all phenol groups on the growth of cancer cells was examined. Among these compounds derived by acylation, alkylation and substitution, **4a**, **4b**, **6a**, **7b**, **9**, and **11** exhibited acceptable anti-proliferative activity within the range of 10–20 μM IC_{50}, whereas the other derivatives displayed very minimal inhibitory activity. This SAR data suggests that the phenol functional groups, especially at C3 and C6 position should play

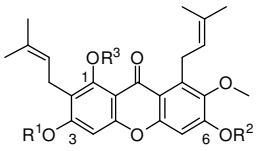
an important role in addressing the cytotoxicity of the xanthones.

Based on the finding of phenol group's importance for potency, a few different substituents were introduced at C4 position in order to modify C3 phenol's pK_a (Table 2). While the **13**, the reduced version of α-mangostin, has potent anti-proliferative activity, the nitro-analog **14** and amino-analog **15** also exhibited considerable activity (**15**, IC_{50} 6.24 μM for SW-620). Similarly, chloro-analog **16** showed a good potency (IC_{50} 9.59 μM for SW-620). To evaluate the significance of substitution on C7 position, compounds **3**, **17** and **18** were tested (Table 3). It turned out that while the synthetic γ-mangostin **3** showed the similar activity to that of natural α-mangostin, **17** and **18** completely lost cytotoxic activity.

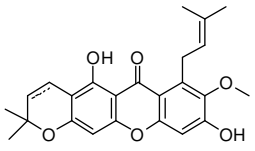
The kinetic solubility of these synthesized analogs was measured using 5% DMSO solution at pH 6.8.[20] The selected analogs **4a**, **6a**, **11**, **14**, and **16** which had moderate cytotoxicity showed markedly increased kinetic solubility by several times compared to α-mangostin.

We believe this structure-activity relationship studies as well as kinetic solubility offer a new window for development of more drug-like novel cytotoxic agents. Our future study is oriented towards identification of lead compounds for developing new anti-cancer agents derived from α-mangostin through animal studies and pharmacokinetic evaluation.

Table 1. *In vitro* cytotoxicity activity (IC_{50}, μM) of cancer cell lines of the C1, C3, and C6-substituted analogs



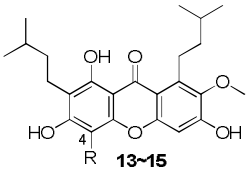
1 ~ 11



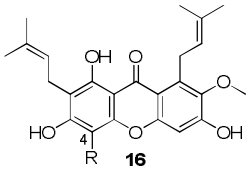
12

	NCI-H460	SW-620	AsPC-1	MDA-MB-231	B16F10
Adriamycin	0.08	0.12	1.87	0.98	0.12
1	3.23	2.97	4.02	3.04	3.23
4a	12.63	10.91	13.9	12.9	13.06
4b	12.49	22.73	14.25	17.45	15.08
5a	54.55	46.55	52.75	>100	28.6
5b	>100	>100	>100	>100	95.83
6a	12.21	12.13	21.33	17.88	16.52
6b	>100	>100	>100	>100	>100
7b	22.42	9.01	13.76	16.88	15.35
8a	>100	>100	>100	>100	>100
8b	14.59	44.64	25.78	28.00	40.99
9	10.82	18.17	13.9	20.78	16.6
10a	>100	>100	>100	>100	>100
10b	>100	>100	>100	>100	>100
11	17.59	15.88	31.34	30.9	14.38
12a	19.69	26.14	25.49	21.06	26.17
12b	>100	>100	>100	>100	>100

Table 2. *In vitro* cytotoxicity activity (IC_{50}, μM) of cancer cell lines of **12** and the C4-substituted analogs



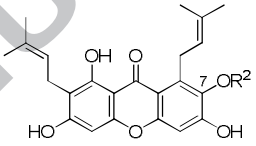
13~15



16

	NCI-H460	SW-620	AsPC-1	MDA-MB-231	B16F10
Adriamycin	0.08	0.12	1.87	0.98	0.12
1	3.23	2.97	4.02	3.04	3.23
13 (R = H)	4.17	6.42	4.64	4.91	6.54
14 (R = NO_2)	16.83	22.61	31.77	28.96	-
15 (R = NH_2)	15.1	6.24	12.96	17.57	12.34
16 (R = Cl)	9.96	9.59	19.26	10.11	12.44

Table 3. *In vitro* cytotoxicity activity (IC_{50}, μM) of cancer cell lines of the C7-substituted analogs



	NCI-H460	SW-620	AsPC-1	MDA-MB-231	B16F10
Adriamycin	0.08	0.12	1.87	0.98	0.12
1 (R = CH_3)	3.23	2.97	4.02	3.04	3.23
3 (R = H)	4.48	5.69	5.92	5.87	-
17	>100	>100	>100	>100	-
18	>100	>100	>100	>100	-

Table 4. Kinetic solubility (μM) of the xanthone analogs in 5% DMSO in water

compound	solubility	compound	solubility
1	20±0.9	**9**	104±4
3	94±3	**11**	138±6
4a	148±5	**13**	78±2
4b	122±2	**14**	339±5
6a	149±2	**16**	381±7

In summary, a series of novel xanthone analogs based on α-mangostin were synthesized and α-mangostin was efficiently converted to β-mangostin and γ-mangostin. Cytotoxicity activity screening using 5 human cancer cell lines identified potent cytotoxic agents such as **4a**, **6a**, **9**, **13**, **15**, and **16** Structure-activity relationship studies revealed that phenol groups on C3 and C6 are critical to inhibition activity to cancer cell lines and C4 modification is capable to improve activity and drug-like properties. These findings provide important information on the structural features that influence the biological activities within this class of compounds, and offer new possibilities for further explorations in analog design. Based on the SAR studies, further

study for mode of action of these prenylated xanthones is under progress.

Acknowledgement

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References and notes

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18. For the conversion of α-mangostin into γ-mangostin, we tried many demethylation conditions such as MeMgI, TMSI, BBr_3, $AlCl_3$/NaI/pyridine and piperidine/H_2O(2.5:1)
19. (a) Human cancer cells were plated at 1×104 cells/well in 96-well plates, incubated overnight and treated with compounds for 48 h. Cytotoxic assays were performed using a XTT kit (Roche Applied Science Mannheim, Penzberg, Upper Bavaria, Germany) in accordance with the manufacturer's instructions. The XTT labeling mixture was prepared by mixing 50 volumes of 1 mg/mL sodium 3′-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzenesulfonic acid hydrate with 1 volume of 0.383 mg/ml of N-methyldibenzopyrazine methyl sulfate. This XTT labeling mixture was subsequently added to the cultures and incubated for 2 h at 37 °C. Absorbance was measured at 490 nm, with 650 nm as a reference wavelength. (b) Kang, M.R.; Kang, J.S.; Yang, J.W.; Kim, B.G.; Kim, J.A.; Jo, Y.N.; Lee, K.; Lee, C.W.; Lee, K.H.; Yun, J.; Kim, H.M.; Han, G.; Kang, J.S.; Park, S.K. *Oncol. Lett.* **2012**, *3*, 113-118.
20. Stock solutions were prepared at 10 mM in 5% DMSO:95% PBS buffer. The stock solutions were diluted to decreasing molarity across the plate with 5% DMSO:95% PBS buffer. Each plate was read vertically, with a gain of 30 and a laser intensity of 90% at 635 nm to produce raw data of counts per well. All raw data was processed using the BMG LABTECH NEPHELOstar Galaxy Evaluation software. The solubility limit was determined by the significant increase in the signal/noise ratio.

Supplementary Material

Supplementary data associated with this article can be found, in the online version, at doi: