Xanthones from the Botanical Dietary Supplement Mangosteen (*Garcinia mangostana*) with Aromatase Inhibitory Activity

Marcy J. Balunas,[†,‡,§] Bin Su,[‡,#] Robert W. Brueggemeier,[‡] and A. Douglas Kinghorn*[,‡]

Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612, and Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210

Received January 11, 2008

Twelve xanthone constituents of the botanical dietary supplement mangosteen (the pericarp of *Garcinia mangostana*) were screened using a noncellular, enzyme-based microsomal aromatase inhibition assay. Of these compounds, garcinone D (**3**), garcinone E (**5**), α-mangostin (**8**), and γ-mangostin (**9**) exhibited dose-dependent inhibitory activity. In a follow-up cell-based assay using SK-BR-3 breast cancer cells that express high levels of aromatase, the most potent of these four xanthones was γ-mangostin (**9**). Because xanthones may be consumed in substantial amounts from commercially available mangosteen products, the consequences of frequent intake of mangosteen botanical dietary supplements require further investigation to determine their possible role in breast cancer chemoprevention.

Breast cancer is the most common cancer afflicting females worldwide, with over one million incident cases in the year 2000 and close to 400 000 deaths.[1] In the United States, it was estimated that in 2007 more than 178 000 women would be newly diagnosed with breast cancer with over 40 000 deaths occurring from the disease.[2] Estrogens and the estrogen receptors (ERs) are widely recognized to play an important role in the development and progression of hormone-dependent breast cancer, making estrogens and ERs widely studied molecular targets.[3,4] Estrogens have various effects throughout the body, including positive effects on the brain, bone, heart, liver, and vagina, with negative effects such as increased risk of breast and uterine cancers with prolonged estrogen exposure.[5,6]

One method to decrease estrogen production involves aromatase inhibition, with clinically available agents exhibiting almost complete estrogen ablation in postmenopausal women. Aromatase (estrogen synthase) is responsible for catalyzing the biosynthesis of estrogens from androgens and is a cytotochrome P450 enzyme complex that is encoded by the aromatase gene *CYP*19, for which the expression is regulated by tissue-specific promoters, implying that aromatase expression is regulated differently in various tissues.[4,7,8] Inhibition of the aromatase enzyme has been shown to reduce estrogen production throughout the body to nearly undetectable levels and is proving to have significant effects on the development and progression of hormone-responsive breast cancers. As such, aromatase inhibitors (AIs) can be utilized either as cancer chemotherapeutic agents or for cancer chemoprevention.[9,10]

Although synthetic AIs show a better side-effect profile when compared to tamoxifen, serious side effects still occur, generally related to estrogen deprivation. Synthetic AIs such as anastrozole (Arimidex), letrozole (Femara), and exemestane (Aromasin) may cause decreased bone mineral density and osteoporosis,[11] increases in cardiovascular events, and alterations in patient lipid profiles,[12] and can also affect cognition, decreasing the protective effects of estrogens on memory loss with aging.[13] Some of the side effects of synthetic AIs can be partially alleviated using available therapies, including osteoporosis and cholesterol medicines.[11,14]

With the clinical success of several synthetic AIs for the treatment of postmenopausal breast cancer, researchers have been investigating the potential of natural products as AIs.[15–17] Plants that have been used traditionally for nutritional or medicinal purposes (for example, botanical dietary supplements and ethnobotanically utilized species) may provide constituents having AI activity but with reduced side effects, possibly resulting from other compounds within the matrix that alleviate some of the side effects of estrogen deprivation (e.g., phytoestrogens).[18,19] As such, natural product AIs may be important for the translation of AIs from their current clinical uses as chemotherapy agents to future clinical uses in breast cancer chemoprevention. New natural product AIs may be clinically useful for treating postmenopausal breast cancer and may also act as chemopreventive agents for preventing secondary recurrence of breast cancer. Phase I clinical trials have recently begun on the botanical dietary supplement IH636 grape seed extract for the prevention of breast cancer in postmenopausal women who are at increased risk of developing breast cancer.[17,20]

As part of a screening program to identify natural product AIs, the methanol- and chloroform-soluble extracts of the pericarp of *Garcinia mangostana* L. (Clusiaceae) (mangosteen) were tested for their ability to inhibit aromatase in a noncellular, radiometric microsomal aromatase assay. Also tested for their ability to inhibit aromatase in this microsomal assay were 12 xanthones previously isolated from *G. mangostana*.[17] Following evidence of activity in this preliminary assay, these two extracts and four of the xanthones were evaluated in a follow-up cell-based aromatase inhibition assay. The results obtained will be discussed herein.

Results and Discussion

During the present study, the methanol- and chloroform-soluble extracts of *G. mangostana* were both found to be strongly inhibitory against aromatase in the microsomal assay (Table 1, Figure 1). A small library of 12 pure xanthones (**1**−**12**), isolated from *G. mangostana*,[21] were tested for aromatase inhibition in microsomes. Compounds were arbitrarily designated as strongly active if their percent control activity (PCA) was 0−10, moderately active if their PCA was >10−30, weakly active if their PCA was 30−50, and inactive if their PCA was greater than 50. Active compounds were then subjected to IC_{50} testing to determine if they acted in a dose-dependent manner (Figure 2). Two xanthones, γ-mangostin (**9**, 4.7 PCA, IC_{50} 6.9 μM) and garcinone D (**3**, 10.0 PCA, IC_{50} 5.2 μM), were found to be strongly active in microsomes (Table 1, Figures 1 and 2). Two other xanthones, α-mangostin (**8**, 22.2 PCA, IC_{50}

* To whom correspondence should be addressed. Tel: +1-614-247-8094. Fax: +1-614-247-8642. E-mail: kinghorn.4@osu.edu.
† University of Illinois at Chicago.
‡ The Ohio State University.
§ Current address: Smithsonian Tropical Research Institute, Unit 0948, APO, AA 34002.
# Current address: Beckman Research Institute, City of Hope, Duarte, CA 91010.

10.1021/np8000255 CCC: $40.75
Published on Web 06/18/2008

Chart 1

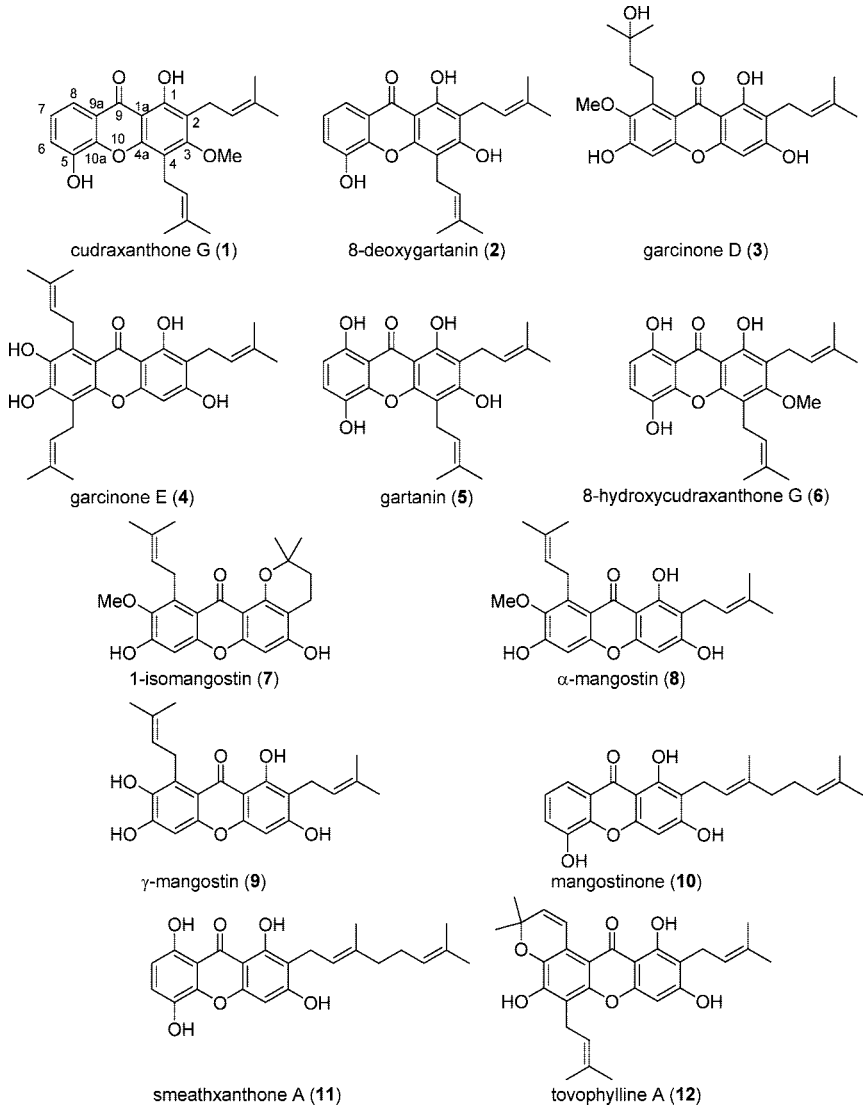

Table 1. Percent Control Activity (PCA) Values for Noncellular, Enzyme-Based and SK-BR-3 Cell-Based Aromatase Bioassays and IC_{50} Values for the Noncellular, Enzyme-Based Bioassay for Xanthones **1**−**12**

	noncellular bioassay			cell-based bioassay			
sample	PCA (20 μg/mL)	SEM	IC_{50} (μM)	PCA	SEM	cytotoxicity (% survival)	SEM
MeOH extract[a]	18.9	0.76		24.1[a]	3.06	65.7	1.23
$CHCl_3$ extract[a]	29.8	1.97		16.5[a]	2.39	58.5	1.18
1	57.8	0.47					
2	82.6	0.61					
3	10.0	0.87	5.2	50.7[b]	0.78	89.4	1.32
4	23.9	0.50	25.1	32.3[b]	3.23	52.3	0.73
5	75.9	2.84					
6	55.1	0.87					
7	52.6	1.06					
8	22.2	0.93	20.7	59.4[b]	3.49	18.4	0.70
9	4.7	1.20	6.9	−0.5[b]	1.45	30.5	0.54
10	78.8	1.07					
11	80.8	1.01					
12	74.7	1.62					
DMSO[c]	100.0	0.44		100.0	14.71	100.0	1.19
AG[d]	15.7[d]	0.55					
Let[e]				7.4[e]	1.05		

[a] Methanol and chloroform extracts of mangosteen (*G. mangostana*). Extracts were tested at 20 μg/mL in both noncellular and cell-based assays. [b] Compounds **3**, **4**, **8**, and **9** were tested at 50 μM in the cell-based assay. [c] Dimethyl sulfoxide (DMSO), blank/negative control for both noncellular and cell-based bioassay. [d] Aminoglutethimide (AG, 50 μM), positive control for noncellular bioassay. [e] Letrozole (Let, 10 nM), positive control for cell-based bioassay.

20.7 μM) and garcinone E (**4**, 23.9 PCA, IC_{50} 25.1 μM), were found to be moderately active in this microsomal assay. All other xanthones tested (**1**, **2**, **5**−**7**, and **10**−**12**) were inactive.

To determine if the *G. mangostana* MeOH and $CHCl_3$ extracts and compounds **3**, **4**, **8**, and **9** inhibited aromatase in a more biologically relevant, cell-based assay, these samples were then

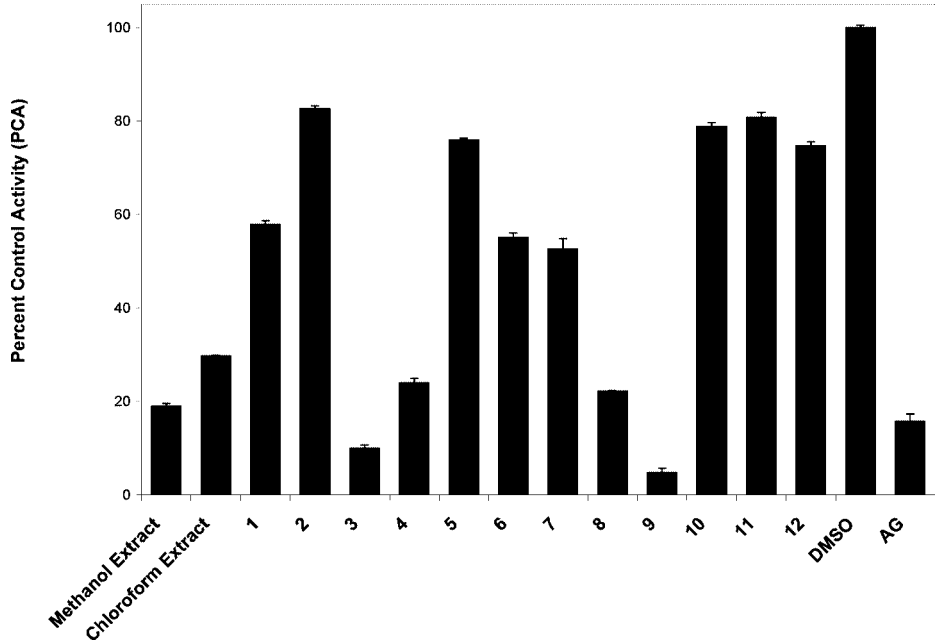

Figure 1. Percent control activity (PCA) of extracts (at 20 μg/mL) and compounds (at 20 μg/mL) from mangosteen (*G. mangostana*) tested in a noncellular, enzyme-based, microsomal aromatase bioassay (DMSO = dimethylsulfoxide, blank/negative control; AG = aminoglutethimide, positive control, 50 μM).

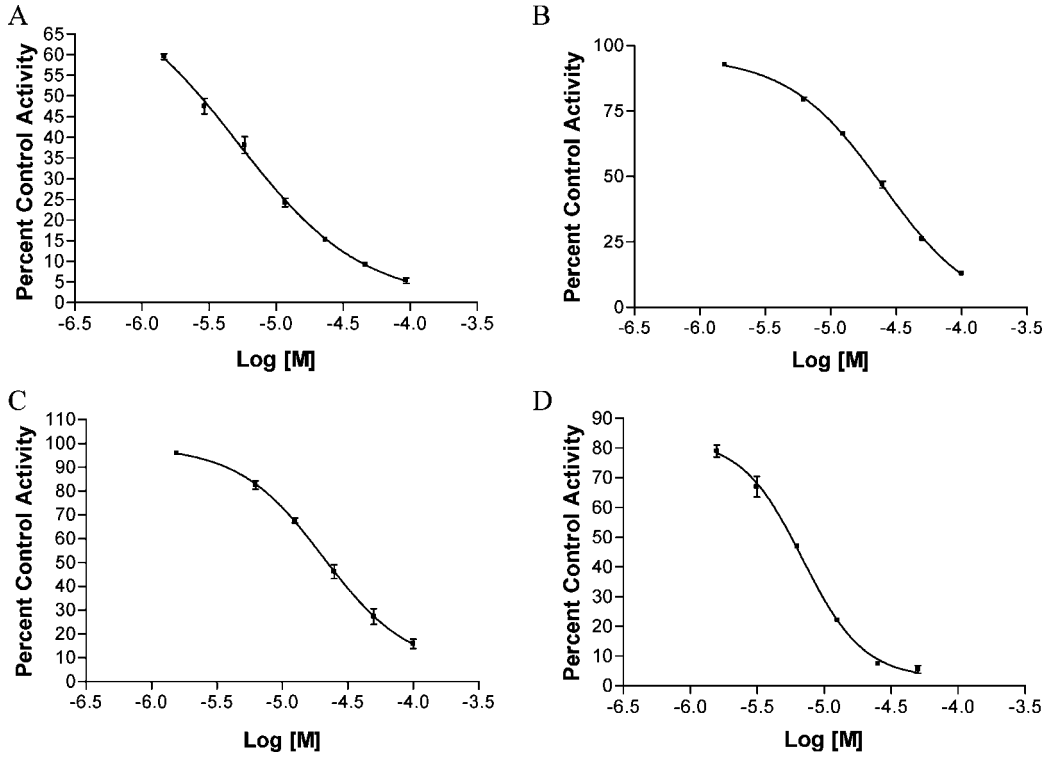

Figure 2. IC_{50} curves for active compounds from mangosteen: (A) garcinone D (**3**, IC_{50} = 5.16 μM), (B) garcinone E (**4**, IC_{50} = 25.14 μM), (C) α-mangostin (**8**, IC_{50} = 20.66 μM), and (D) γ-mangostin (**9**, IC_{50} = 6.88 μM).

tested at 50 μM in a secondary cell-based assay, using SK-BR-3 human breast cancer cells that overexpress the aromatase enzyme. Inhibitory activity was evident for both of the two mangosteen extracts, with γ-mangostin (**9**) being the only one of the four compounds found to strongly inhibit aromatase in cells (−0.5 PCA), garcinone E (**4**) being moderately inhibitory (32.3 PCA), and garcinone D (**3**) and α-mangostin less so (>50 PCA) (Table 1). However, γ-mangostin (**9**) was also found to be fairly cytotoxic in SK-BR-3 cells (Figure 4), complicating the determination of whether the aromatase inhibition was due to actual activity or the result of low cell survival. Therefore, γ-mangostin (**9**) was further subjected to IC_{50} testing in both the SK-BR-3 cell-based aromatase assay and SK-BR-3 cell-based cytotoxicity assay (Figure 5). The IC_{50} of γ-mangostin (**9**) in the cell-based AI assay was determined to be 4.97 ± 1.9 μM, while the IC_{50} in the cell-based cytotoxicity assay was found to be 25.99 ± 1.0 μM. Using the concept of a chemopreventive index (CI), which provides an indication of the therapeutic index and can be computed using the equation CI = cytotoxicity IC_{50}/aromatase inhibition IC_{50},[22] the CI for γ-mangostin (**9**) was calculated as 5.2, thus indicating over 5 times stronger

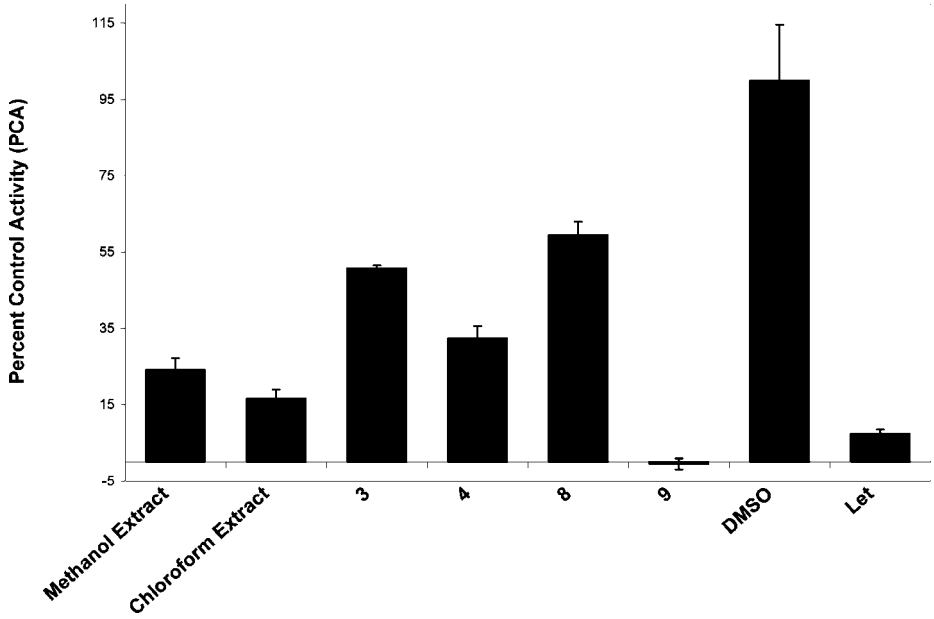

Figure 3. Percent control activity (PCA) of extracts (at 20 μg/mL) and compounds (at 50 μM) from mangosteen (*G. mangostana*) tested in a SK-BR-3 cell-based aromatase bioassay (DMSO = dimethylsulfoxide, blank/negative control; Let = letrozole, positive control, 10 nM).

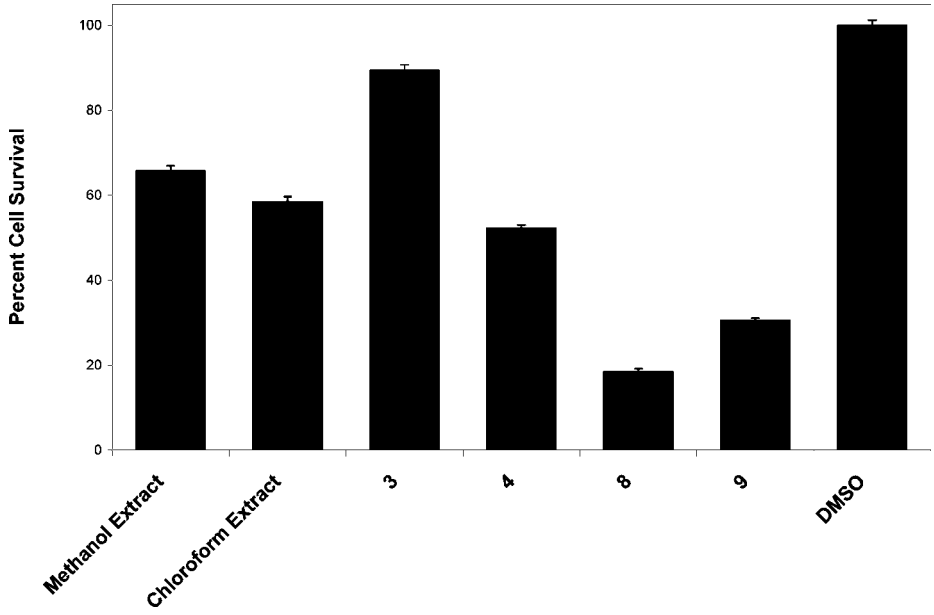

Figure 4. Percent cell survival of extracts and compounds from mangosteen (*G. mangostana*) tested in a SK-BR-3 cell-based cytotoxicity bioassay (DMSO = dimethylsulfoxide, blank/negative control).

inhibition of aromatase than cytotoxicity. As such, γ-mangostin (**9**) and the botanical dietary supplement mangosteen show considerable activity as aromatase inhibitors. Further molecular and in vivo studies should be performed to advance their development in this regard.

Xanthones **3**, **4**, **8**, and **9** are among the most potent natural products known to date in the microsomal AI assay. Apart from being based on a xanthone nucleus, these four compounds have several structural features in common. Of the 12 xanthones tested, **3**, **4**, **8**, and **9** are the only substances to bear hydroxy groups at C-1, C-3, and C-6, a prenyl group at C-2, and a five-carbon substituent at C-8. Furthermore, in the cell-based AI assay, hydroxylation at C-7 (compounds **9** and **4**) was shown to elicit more aromatase inhibition than C-7 methoxylation (compounds **3** and **8**) (Table 1), with methoxylation leading to higher cytotoxicity than hydroxylation. Xanthones have only recently been tested for their ability to inhibit aromatase, with several synthetic xanthones exhibiting activity in the nanomolar range,[23,24] but xanthones have not yet undergone extensive evaluation using additional in vitro, in vivo, or preclinical models. Further comparisons of the structure−activity relationship with both natural and synthetic xanthones should help to identify potential lead candidates for future development.

Xanthones are well-known for their numerous and varied pharmacological effects, including having antioxidant, antimicrobial, central nervous system (CNS) depressant or stimulant, antihypertensive, antidiabetic, anticancer, anti-inflammatory, hepatoprotective, and/or immunomodulation properties.[23] Xanthones used during the present study were isolated from *G. mangostana* L. (mangosteen), a slow-growing tropical tree with edible fruits.[21] Mangosteen is commonly referred to as the "queen of fruits," prized for its delicious fruits, and has been utilized in Southeast Asian traditional

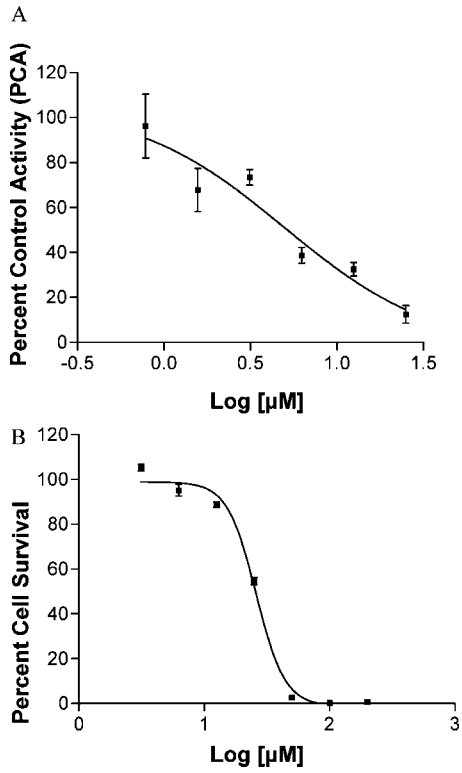

Figure 5. IC_{50} curves for γ-mangostin (**9**) in (A) SK-BR-3 aromatase bioassay ($IC_{50} = 4.97$ μM) and (B) SK-BR-3 cytotoxicity bioassay ($IC_{50} = 25.99$ μM).

medicine for stomach ailments (pain, diarrhea, dysentery, ulcers), as well as to treat infections and wounds.[25,26]

Owing to their activity as potent antioxidants,[21,27–29] some mangosteen-based botanical products are standardized to contain high levels of xanthones such as α- and γ-mangostin. Mangosteen products have recently become one of the top-selling botanical dietary supplements in the U.S. and in 2005 represented the sixth-ranked single-herb dietary supplement with sales of over $120 million, a substantial increase over the previous year.[30] The results from the present study, including the testing of both mangosteen extracts and compounds, indicate that certain xanthones from mangosteen fruits act as potent aromatase inhibitors in both noncellular and cell-based AI assays, especially γ-mangostin (**9**). Approximately two-thirds of postmenopausal women with breast cancer have the estrogen-dependent (hormone-dependent) form of this disease, in which estrogen is required for the growth of tumors.[31] Because of their relatively high yield of xanthones such as α- and γ-mangostin (**8** and **9**) in the pericarp of *G. mangostana*,[21] mangosteen botanical dietary supplements may be acting as aromatase inhibitors and might thus have a potential role in cancer chemoprevention for postmenopausal women with hormone-dependent breast cancer. However, before a definitive role of the mangosteen xanthones in this regard can be ascertained, additional work will need to be performed, including evaluation of the in vitro aromatase inhibitory xanthone constituents in an appropriate in vivo model.

Experimental Section

General Experimental Procedures. Methanol- and chloroform-soluble extracts of *Garcinia mangostana* L. (Clusiaceae) (mangosteen) were prepared, and individual xanthones were isolated as described in a previous publication.[21] Radiolabeled [1β-^{3}H]androst-4-ene-3,17-dione, scintillation cocktail 3a70B, and SK-BR-3 human breast cancer cells were obtained as described previously.[31] Radioactivity was counted on an LS6800 liquid scintillation counter (Beckman, Palo Alto, CA). All other chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO).

Noncellular, Enzyme-Based Aromatase Bioassay. This was carried out as described in earlier publications.[32–34] Human placental microsomes were obtained from human term placentas that were processed at 4 °C immediately after delivery from the OSU Medical Center [OSU Institutional Review Board (IRB) protocol number 2002H0105, last approved in December 2006]. Extracts and compounds were originally screened at 20 μg/mL in DMSO using a noncellular microsomal radiometric aromatase assay. Samples [extracts or compounds, DMSO as negative control, or 50 μM (±)-aminoglutethimide (AG) as positive control] were tested in triplicate. Each reaction mixture included sample, 100 nM [1β-^{3}H]androst-4-ene-3,17-dione (400 000−450 000 dpm), 0.1 M potassium phosphate buffer (pH 7.0), 5% propylene glycol, and an NADPH regenerating system (containing 2.85 mM glucose-6-phosphate, 1.8 mM $NADP^+$, and 1.5 units of glucose-6-phosphate dehydrogenase). Microsomal aromatase (50 μg) was added to initiate the reactions, which were then incubated in a shaking water bath at 37 °C and quenched after 15 min using 2 mL of $CHCl_3$. An aliquot of the aqueous layer was then added to 3a70B scintillation cocktail for quantitation of the formation of ^3H_2O. Percent control activity (PCA) and IC_{50} values were determined as previously indicated.[32–34]

Cell-Based Aromatase Bioassay. Extracts and compounds found to be active using the noncellular, enzyme-based radiometric aromatase inhibition assay were further tested at various concentrations in SK-BR-3 human breast cancer cells that overexpress aromatase, using previously described methodology.[34–36] Cells were treated with samples or 0.1% DMSO (negative control) or 10 nM letrozole (positive control) [in triplicate]. Results are initially determined as picomoles of ^3H_2O formed per hour incubation per million live cells (pmol/h/10^6 cells), with PCA calculated by comparison with negative control, DMSO.

Cell Viability Analysis. The effect of extracts and compounds on SK-BR-3 cell viability was assessed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium (MTT) bromide assay in six replicates, as previously described.[37] Results are expressed as percent cell survival as compared to the negative control, DMSO.

Acknowledgment. We are grateful for financial support through a University Fellowship and a Dean's Scholar Award from the Graduate College of the University of Illinois at Chicago, awarded to M.J.B. Other support was provided by NIH grant R01 CA73698 (P.I., R.W.B.), The Ohio State University Comprehensive Cancer Center (OSUCCC) Breast Cancer Research Fund (to R.W.B.), and the OSUCCC Molecular Carcinogenesis and Chemoprevention Program (to A.D.K.).

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NP8000255