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Tumor Cell Proliferation and Cyclooxygenase Enzyme Inhibitory Compounds in *Amaranthus tricolor*

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Amaranthus tricolor is consumed as a vegetable in Asia. Bioassay-directed isolation of leaves and stems of *A. tricolor* yielded three galactosyl diacylglycerols (**1**−**3**) with potent cyclooxygenase and human tumor cell growth inhibitory activities. The purified compounds were characterized by spectroscopic methods. In addition, the fatty acid moieties in diacyl galactosyl glyerols were characterized by GC-MS analyses. The galactosyl diacylglycerols **1**−**3** inhibited the cyclooxygenase-1 (COX-1) enzyme by 78, 63, and 93% and the cyclooxygenase-2 (COX-2) enzyme by 87, 74, and 95%, respectively. These compounds were tested for antiproliferative activity using human AGS (gastric), CNS (central nervous system; SF-268), HCT-116 (colon), NCI-H460 (lung), and MCF-7 (breast) cancer cell lines. Compound **1** inhibited the growth of AGS, SF-268, HCT-116, NCI-H460, and MCF-7 tumor cell lines with IC_{50} values of 49.1, 71.8, 42.8, 62.5, and 39.2 μg/mL, respectively. For AGS, HCT-116, and MCF-7 tumor cell lines, the IC_{50} values of compounds **2** and **3** were 74.3, 71.3, and 58.7 μg/mL and 83.4, 73.1, and 85.4, respectively. This is the first report of the COX enzyme inhibitory activity for galactosyl glycerols and antiproliferative activities against human colon, breast, lung, stomach, and CNS tumor cell lines.

KEYWORDS: ***Amaranthus tricolor*; diacylgalloylglycerols; cyclooxygenase inhibitory; antiproliferation**

INTRODUCTION

Cyclooxygenases-1 (COX-1) and -2 (COX-2) catalyze the rate-limiting step of the conversion of arachidonic acid to prostaglandins. Prostaglandins are responsible for mediating inflammation in the body. COX-1 is constitutively expressed in cells involved in normal physiological functions, whereas COX-2 is induced by various cytokines, growth factors, and carcinogens (*1*). The cyclooxygenases and lipoxygenases metabolize linoleic and arachidonic acids to eicosanoids, which cause mutagenesis (*2*). The COX-2 enzyme is overexpressed in several types of cancers, including colon, breast, lung, pancreas, and esophagus as well as squamous cell carcinoma of the head and neck (*1*, *3*). The prostaglandins play a major role in the growth of tumor blood vessels. Therefore, COX enzyme inhibitors may prevent the progression of carcinogenesis (*4*). The nonsteroidal anti-inflammatory drugs (NSAIDs) arrest the formation of prostanoids by blocking the COX enzymes. Several studies have shown that NSAIDs, in particular, selective COX-2 inhibitors, have anticancer activity. The long-term use of NSAIDs was reported to be beneficial in decreasing the incidence of cancer (*5*). Even though there are several positive findings about the use of NSAIDs in the treatment of cancer, the efficacy and safety remain major concerns (*5*).

Beneficial phytochemicals present in plant materials used as food are of great significance. They are of interest to the general population mainly due to anecdotal health claims and perceived safety. Many foods have not been investigated for health beneficial compounds. Therefore, a bioactivity-directed investigation of several foods may lead to the development of safe products for the prevention and treatment of diseases. For example, a number of *Amaranthus* species are being cultivated in Southeast Asia (*6*) for consumption as vegetables. The leaves of all *Amaranthus* spp. are high in calcium and iron, but the high oxalic acid content diminishes their bioavailability. Betacyanins, the coloring pigments in *Amaranthus tricolor*, have been reported to possess antioxidant activity (*7*−*10*). The leaves of *A. tricolor* have been used against external inflammations, as a diuretic, and as a treatment for bladder distress (*11*). However, only limited work has been carried out on the nonbetacyanin constituents of this plant. In this study, we report the isolation of three galactosyl glycerols that are present in the leaves of *A. tricolor* with COX and human tumor cell growth inhibitory activities.

MATERIALS AND METHODS

General Experimental. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR experiments were performed on an INOVA Varian VRX 500 instrument using standard pulse sequences. The chemical shifts were measured in CD_3OD plus $CDCl_3$ and are expressed in δ (parts per million). All solvents used for isolation and purification were of ACS grade. The silica gel used for MPLC was Merck silica gel 60 (35−70 μm particle size). Si gel PTLC plates (20 × 20, 500 μm) were purchased from Analtech, Inc. (Newark, DE). The COX-1 enzyme was prepared

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from ram seminal vesicles purchased from Oxford Biomedical Research, Inc. (Oxford, MI). The COX-2 enzyme was prepared from insect cells cloned with human PGHS-2 enzyme. Fetal bovine serum (FBS) and Roswell Park Memorial Institute 1640 (RPMI-1640) medium were obtained from Gibco BRL (Grand Island, NY). Human tumor cell lines MCF-7 (breast), SF-268 (central nervous system, CNS) and NCI-H460 (lung) were purchased from the National Cancer Institute (NCI, Bethesda, MD). HCT-116 (colon) and AGS (gastric) were purchased from American Type Culture Collection (ATCC, Rockville, MD). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). Concentrations are expressed in micrograms per milliliter.

Plant Material. The *A. tricolor* plants were grown in greenhouses at Michigan State University. *A. tricolor* seeds were germinated on Bacto soil, and seedlings were transplanted into clay pots. A mixture of loamy sand and Bacto soil (1:1) was used to grow the plants under a 12 h photoperiod at 75 °F. The plants were watered daily, harvested before flowering, and extracted immediately. The plants were grown for 6 weeks before harvesting.

Extraction and Isolation. *A. tricolor* leaves and stems are consumed only after boiling or cooking with other ingredients. Therefore, in our study we have boiled the plant material (leaves and stems, 1 kg) for 10 min with H_2O (2 × 2 L) and filtered it. The filtrate was frozen and lyophilized. The aqueous extract mostly contained betacyanins and an excess of inorganic salts. This extract was not active in COX-1 and -2 assays and hence was not analyzed further.

The plant material residue from hot water extraction was then soaked in ethyl acetate (1 L) for 6 h, and the extract was removed by filtration. The process was repeated three times, and the combined extract was evaporated under reduced pressure to yield a greenish residue (1 g). The extract showed both COX-1 and -2 enzyme inhibitory activities and was therefore purified further. The active EtOAc extract was fractionated by medium-pressure liquid chromatography (MPLC) with a model LBP-V pump operating at 10−15 psi (Sanki Engineering Ltd.) on a 40 cm × 30 mm i.d. silica gel column using *n*-hexane, $CHCl_3$, and MeOH under gradient conditions. A total of 70 fractions (20 mL each) were collected. Fractions 1−20, eluted with hexane, were similar, as indicated by thin-layer chromatography (TLC) and hence were combined and concentrated under reduced pressure to yield fraction 1 (100 mg). The hexane/$CHCl_3$ (1:1, v/v) elution gave fractions 21−35. These were combined to yield fraction 2 (150 mg). Evaporation of fractions 36−55, eluted with $CHCl_3$, gave a green residue, fraction 3 (40 mg). The column was then eluted with $CHCl_3$/MeOH (9:1) to yield fractions 56−70. These were similar by TLC analysis and hence combined to yield fraction 4 (300 mg). The pooled fractions, fractions 1−4, were evaluated for the inhibition of COX enzymes, and fraction 4 was found to be the most active. Therefore, it was further fractionated by silica gel column chromatography using $CHCl_3$, $CHCl_3$/MeOH (9:1), and $CHCl_3$/MeOH (8.5:1.5) as mobile phases and afforded fractions A (50 mg), B (100 mg), and C (50 mg), respectively. Only fractions B and C were active. Purification of B by preparative thin-layer chromatography (PTLC) using $CHCl_3$/MeOH (9:1) as the mobile phase gave compounds **1** (70 mg) and impure **2** (15 mg). Repeated purification of compound **2** by PTLC using $CHCl_3$/MeOH (9:1) as the mobile phase gave pure compound **2** (8 mg). Similarly, repeated purification of C by PTLC using $CHCl_3$/MeOH (93:7) as the mobile phase afforded compound **3** (9 mg).

Compound **1**: ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 5.29 (12H, m, H-9′,10′,12′,13′,15′,16′,9″,10″,12″,13″,15″,16″), 5.20 (1H, m, H-2), 4.29 (1H, dd, J = 3.5, 12.0 Hz, H-1a), 4.14 (1H, dd, J = 7.0, 11.0 Hz, H-1b), 4.13 (1H, d, J = 7.0 Hz, H-1‴), 3.87 (1H, dd, J = 4.0, 9.5 Hz, H-3a), 3.82 (1H, dd, J = 2.5, 1.0 Hz, H-4‴), 3.78 (1H, dd, J = 6.5, 12.0 Hz, H-6a‴), 3.70 (1H, d, J = 5.0, 11.5 Hz, H-6b‴), 3.64 (1H, dd, J = 6.0, 11.0 Hz, H-3b), 3.50 (1H, d, J = 7.0, 9.5 Hz, H-3‴), 3.44 (2H, dd, J = 7.0, 9.5 Hz, H-5‴, 2‴), 2.73 (8H, t, J = 5.5 Hz, 11′,11″,14′,14″), 2.24 (4H, t, J = 7.5 Hz, H-2‴,2‴), 1.99 (8H, m, C-8′,8″,17′,17″), 1.24 (CH_2s), 0.90 (6H, t, J = 8.0 Hz, CH_3s, 18′,18″); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 173.8 (C-1′), 173.5 (C-1″), 131.8−127.0 (C-9′,10′,12′,13′,15′,16′, 9″,10″,12″,13″,15″,16″), 103.8 (C-1‴), 74.7 (C-5‴), 73.2 (C-2‴), 71.1 (C-3‴), 70.2 (C-2), 68.7 (C-4‴), 67.8 (C-3), 62.6 (C-1), 61.5 (C-6‴), 34.1 (C-2′), 33.9 (C-2″), 29.5−28.9 (CH_2s), 27.0 (C-8′, 8″), 25.4 (11′,11″), 25.3 (14′,14″), 24.6 (3′,3″), 20.3 (17′,17″), 14.0 (C-18′,18″).

Compound **2**: ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 5.24 (6H, m, H-9′,10′,12′,13′,15′,16′), 5.15 (1H, m, H-2), 4.24 (1H, dd, J = 2.5, 12.0 Hz, H-1a), 4.11 (1H, dd, J = 7.0, 11.0 Hz, H-1b), 4.10 (1H, d, J = 7.0 Hz, H-1‴), 3.82 (1H, dd, J = 5.5, 11.0 Hz, H-3a), 3.78 (1H, d, J = 2.5 Hz, H-4‴), 3.74 (1H, dd, J = 6.5, 12.0 Hz, H-6a‴), 3.65 (1H, d, J = 5.0, 11.5 Hz, H-6b‴), 3.60 (1H, dd, J = 6.0, 11.0 Hz, H-3b), 3.42 (1H, dd, J = 7.0, 9.5 Hz, H-3‴), 3.38 (2H, d, J = 7.0, 9.5 Hz, H-5‴, 2‴), 2.69 (4H, t, J = 5.5 Hz, 11′, 14′), 2.22 (4H, t, J = 7.5 Hz, H-2′, 2″), 1.95 (4H, m, C-8′, 17′), 1.19 (CH_2s), 1.14 (CH_2s), 0.86 (6H, t, J = 8.0 Hz, CH_3s, 16″,18′); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 173.8 (C-1′), 173.2 (C-1″), 131.3−126.5 (C-9′,10′,12′,13′,15′,16′), 103.5 (C-1‴), 74.6 (C-5‴), 73.0 (C-2‴), 70.7 (C-3‴), 69.9 (C-2), 68.3 (C-4‴), 67.2 (C-3), 62.3 (C-1), 60.8 (C-6‴), 33.6 (C-2′), 33.5 (C-2″), 29.0−28.5 (CH_2s), 26.6 (C-8′), 25.0 (11′), 24.9 (14′), 24.3 (3′), 20.0 (17′), 13.4 (C-18′,16″).

Compound **3**: ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 5.24 (6H, m, H-9′,10′,12′,13′,15′,16′), 5.15 (1H, m, H-2), 4.30 (1H, dd, J = 2.5, 12.0 Hz, H-1a), 4.11 (1H, dd, J = 7.5, 11.0 Hz, H-1b), 4.10 (1H, d, J = 7.0 Hz, H-1‴), 3.88 (1H, dd, J = 5.5, 11.0 Hz, H-3a), 3.77 (1H, d, J = 2.5 Hz, H-4‴), 3.71 (1H, dd, J = 6.5, 12.0 Hz, H-6a‴), 3.66 (1H, d, J = 5.5, 13.0 Hz, H-6b‴), 3.64 (1H, dd, J = 6.0, 11.0 Hz, H-3b), 3.45 (1H, dd, J = 7.5, 9.5 Hz, H-3‴), 3.39 (2H, d, J = 7.0, 10.0 Hz, H-5‴, 2‴), 2.70 (4H, t, J = 6.0 Hz, 11′,14′), 2.23 (4H, t, J = 7.5 Hz, H-2′,2″), 1.97 (4H, m, C-8′,17′), 1.22 (CH_2s), 1.17 (CH_2s), 0.88 (6H, t, J = 8.0 Hz, CH_3s, 18″,18′).

Saponification of Compounds 1−3 and Methylation of Fatty Acids. Compounds **1**−**3** (1 mg each) were reacted with 5% methanolic KOH (0.5 mL) for 10 min at room temperature, and the solution was neutralized with 3 M HCl. The acidic solution was then extracted with EtOAc and the solvent evaporated under reduced pressure to yield fatty acids. Diazomethane for methylation of the fatty acids was prepared from *N*-nitroso-*N*-methyl urea (*12*) and kept over KOH pellets. The fatty acids obtained from the saponification of compounds **1**−**3** were dissolved in ether and methylated with an excess of diazomethane. After 30 min, the diazomethane was evaporated and the resulting fatty acid methyl esters were dissolved in *n*-hexane and analyzed by GC-MS.

GC-MS Analysis of Fatty Acid Moieties in Compounds 1−3. The GC-MS analyses were carried out on an HP-6890 GC-MS with a 30 m × 0.25 mm i.d. HP-5MS capillary column. An electron capture detector maintained at 250 °C was used for the analyses of fatty acid methyl esters. An aliquot of sample solution was injected by using an autoinjector at 250 °C. The carrier gas was helium at a flow rate of 0.8 mL/min. The mass spectrum was scanned from *m*/*z* 40 to 550. The fatty acids were identified by comparison of retention times with standards and molecular ions.

Tumor Cell Proliferation Inhibitory Assay. MCF-7 (breast), SF-268 (CNS), NCI-H460 (lung), HCT-116 (colon), and AGS (gastric) human tumor cells were cultured in RPMI-1640 medium containing penicillin−streptomycin (10 units/mL for penicillin and 10 μg/mL for streptomycin) and 10% fetal bovine serum (FBS). The cells were grown in a humidified incubator (37 °C, 5% CO_2), counted, and plated into 96-well plates. The number of cells for each cell line varied according to their doubling time. The samples were dissolved in DMSO and further diluted with RPMI medium to obtain a stock solution that gave the final desired concentration of 90 μg/mL and a DMSO concentration of 0.2%. Lower concentrations of test samples were prepared by serial dilution of the stock solutions with RPMI medium. After 24 h of incubation, test samples (100 μL) were added to the wells containing the appropriate tumor cells and incubated for 48 h. MTT (5 mg) was dissolved in 1 mL of phosphate-buffered saline (PBS, 1 mL), and aliquots of 25 μL were added to each well. The cell viability was determined according to the published procedure (*13*). Adriamycin was used as positive control in this assay. The samples were assayed in duplicate, and three independent experiments were carried out to calculate the IC_{50} values.

Cyclooxygenase Inhibitory Assay. COX enzyme inhibitory assay was performed in a microchamber at 37 °C by monitoring the initial rate of O_2 uptake using an oxygen electrode (Instech Laboratories, Plymouth Meeting, PA) attached to a biological oxygen monitor

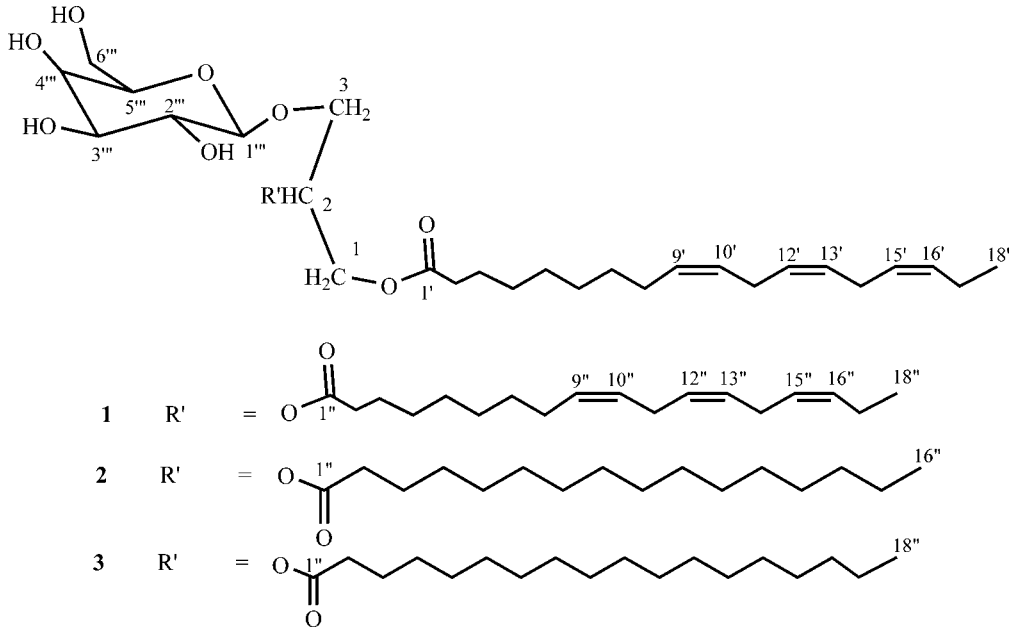

Figure 1. Structures of compounds **1**–**3**.

(Yellow Springs Instrument, Inc., Yellow Springs, OH) (*14*). The enzyme was diluted with Tris buffer (pH 7.0) to give a final concentration of 1.5 mg of protein/mL. The test compounds and positive controls were dissolved in DMSO. An aliquot of 10 μL of DMSO or test compounds or standards in DMSO was added to the reaction chamber containing 0.6 mL of 0.1 M Tris buffer (pH 7), 1 mM phenol, and hemoglobin (17 μg). COX-1 or -2 enzyme (10 μL) was added to the chamber and incubated for 3 min. The reaction was initiated by the addition of arachidonic acid (10 μL of a 1 mg/mL solution). Instantaneous inhibition was measured by using Quick Log Data acquisition and control computer software (Strawberry Tree Inc., Sunnyvale, CA). The percent inhibition was calculated with respect to DMSO control. Each sample was assayed two times, and the standard deviation was calculated for $n = 2$.

RESULTS AND DISCUSSION

The hot water extract of *A. tricolor* leaves and stems containing betacyanins and salt did not show COX-1 or -2 enzyme inhibitory activities and hence was not analyzed further. The purification of active EtOAc extract by repeated CC and PTLC yielded compounds **1**−**3**.

The ^{1}H NMR spectrum of compound **1** showed an anomeric proton signal at δ 4.13 and indicated it as a glycoside. A multiplet at δ 5.29 together with the signals at δ 2.73, 2.24, 1.99, and 0.90 suggested the presence of unsaturated fatty acid moieties in the molecule. The signals appearing at δ 5.20, 4.29, 4.14, 3.87, 3.82, and 3.64 were assigned to a glycerol backbone. The coupling constant of C-3 and C-4 protons in the sugar moiety indicated the presence of a galactosyl unit in compound **1**. The ^{13}C NMR spectrum showed the presence of two carbonyl signals at 173.8 and 173.5 ppm, assigned to two fatty acid residues. The presence of olefinic carbon signals between δ 131.8 and 127.0 supported the unsaturation in the fatty acid moieties. The anomeric carbon signal at δ 103.8 and the other eight oxygenated carbons between 74.6 and 61.5 ppm confirmed the galactosyl glycerol unit in the molecule. Therefore, the ^{1}H and ^{13}C NMR spectroscopic analyses confirmed the identity of compound **1** as galactosyl diacylglycerol.

Compounds **2** and **3** showed spectroscopic data similar to those of **1**. Therefore, compounds **1**−**3** were saponified with methanolic KOH separately. The resulting fatty acids were then methylated with diazomethane. The fatty acid methyl esters thus obtained were characterized by GC and GC-MS analyses and by comparison of the retention times with corresponding standards. The only fatty acid moiety present in compound **1** was found to be linolenic acid. Hence, the structure of compound **1** was confirmed as 1,2-dilinolenoyl-3-galactosylglycerol (*15*, *16*). Similarly, compound **2** contained palmitic and linolenic acids. A comparison of the literature data and compound **2** revealed that the glycerol backbone was esterified at the 1- and 2- positions, respectively (*16*, *17*). Therefore, the structure of compound **2** was determined as 1-linolenoyl-2-palmitoyl-3-galactosylglycerol (*18*). The GC-MS analysis of compound **3** confirmed the presence of stearic and linolenic acids in the molecule. It was therefore identified as 1-linolenoyl-2-steroyl-3-galactosylglycerol (*18*). The identity of compound **3** was further confirmed by comparison of its spectroscopic data with the published ^{1}H spectral data of 1-linolenoyl-2-steroyl-3-galactosylglycerol.

The galactosyl diacyl glycerols (**1**−**3**) (**Figure 1**) isolated from *A. tricolor* were evaluated for cyclooxygenase inhibitory activity (**Figure 2**) using COX-1 and -2 enzymes (*14*). Compound **1** was the most active and inhibited COX-1 and -2 enzymes by 78 and 87%, respectively, at 50 μg/mL concentration. However, compound **3** at 100 μg/mL showed 95 and 92% inhibition of COX-1 and -2 enzymes. The galactosyl diacylglycerol, **2**, inhibited COX-1 and -2 enzymes by 63 and 74%, respectively, at 100 μg/mL. The NSAIDs, aspirin (180 μg/mL), ibuprofen (2.52 μg/mL), and naproxen (2.06 μg/mL) inhibited COX-1 enzyme by 61, 53, 79, and COX-2 enzyme by 24, 59, 96, respectively. The selective COX-2 inhibitor, Vioxx, was tested at 1.67 μg/mL and showed 76% inhibition. Because the COX inhibitory activities of the NSAIDs vary, we have used NSAID concentrations to obtain an IC_{50} values in the range of 40−60% with respect to the solvent control.

On the basis of the COX-2 inhibitory activity of these galactosyl diacylglycerols, we evaluated compounds **1**−**3** for tumor cell antiproliferative activity using gastric (AGS), CNS (SF-268), colon (HCT-116), lung (NCI-H460), and breast (MCF-7) cancer cell lines (*13*). Compounds **1**−**3** were initially tested at 90 μg/mL and further assayed at 75, 45, and 30 μg/mL to obtain the 50% growth inhibition (IC_{50}) values. Compound **1** was the most active and inhibited the growth of gastric,

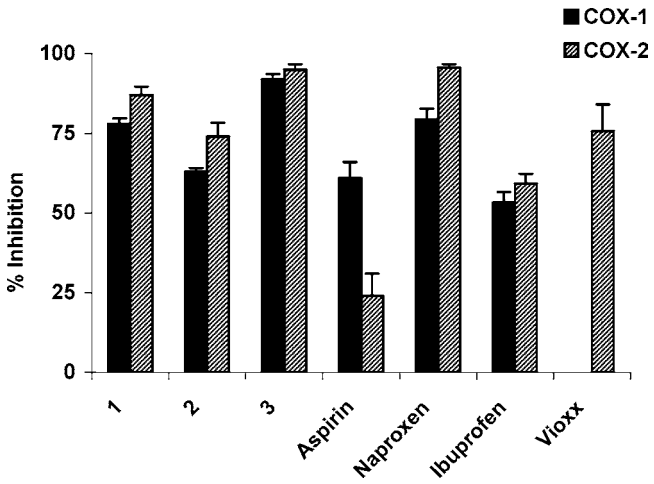

Figure 2. COX-1 and -2 enzyme inhibitory activities of compounds **1**–**3**. Compound **1** was tested at 50 μg/mL; compounds **2** and **3** were tested at 100 μg/mL. Aspirin, naproxen, and ibuprofen were assayed at 180, 2.52, and 2.06 μg/mL, respectively, and Vioxx was assayed at 1.67 μg/mL. NSAID concentrations were used to obtain IC_{50} values in the range of 40–60% with respect to the solvent control. Vertical bars represent standard deviation at each data point ($n = 2$). DMSO was used as solvent control. Results are expressed as percent inhibition with respect to solvent control.

CNS, colon, lung, and breast cancer cell lines with IC_{50} values of 49.1, 71.8, 42.8, 62.5, and 39.2 μg/mL, respectively (**Figure 3A**). The IC_{50} values observed for compound **2** (**Figure 3B**) were AGS (74.3 μg/mL), HCT-116 (71.3 μg/mL), and MCF-7 (58.7 μg/mL). Similarly, compound **3** inhibited the growth of colon tumor cell lines (**Figure 3C**) with an IC_{50} value of 73.1 μg/mL. It inhibited AGS and MCF-7 cells with an IC_{50} values of 83.4 and 85.4 μg/mL, respectively. Adriamycin was used as positive control and gave IC_{50} values of 0.44, 0.31, 0.23, 1.13, and 0.16 μg/mL for lung, breast, colon, CNS, and gastric tumor cell lines, respectively.

Among the galactosyl diacyl glycerols isolated from *A. tricolor*, compound **1** possessed two linolenic acid moieties in its structure and showed the highest inhibition of COX enzymes at test concentrations. Compounds **2** and **3** contained both saturated (palmitic/stearic) and unsaturated (linolenic) fatty acid moieties and were less active than compound **1**. A similar activity profile was observed in the tumor growth inhibitory assay. This indicated that the degree of unsaturation in the fatty acid moieties plays a role in both COX and tumor cell growth inhibitory activities of these compounds.

Several epidemiological and clinical studies show that NSAIDs have promising antitumor activity (*19*). It is well-known that the people who use aspirin and other NSAIDs reduce the risk of adenomatous polyps and colorectal cancer compared to nonusers (*6*). The glycolipids isolated from *A. tricolor* showed good anti-inflammatory activity as indicated by COX-1 and -2 enzyme inhibitions. The observed COX-2 inhibition of these compounds and their in vitro growth inhibitory activities of the gastric, colon, and breast cancer cell lines are probably related.

Although galactosyl diacyl glycerols are widely distributed in fruits and vegetables in substantial quantities, their health benefits are not fully explored. We have determined the total amount of these compounds present in *A. tricolor* leaves and stems as 100 mg/kg of fresh weight. Compounds **1** and **2** isolated from *Citrus hytrix* (*16*) inhibited 12-*O*-tetradecanoylphorbol 13-acetate (TPA) induced edema and dimethylbenz[α]anthracene (DMBA) induced tumor in vivo. The galactosyl diacyl glycerols

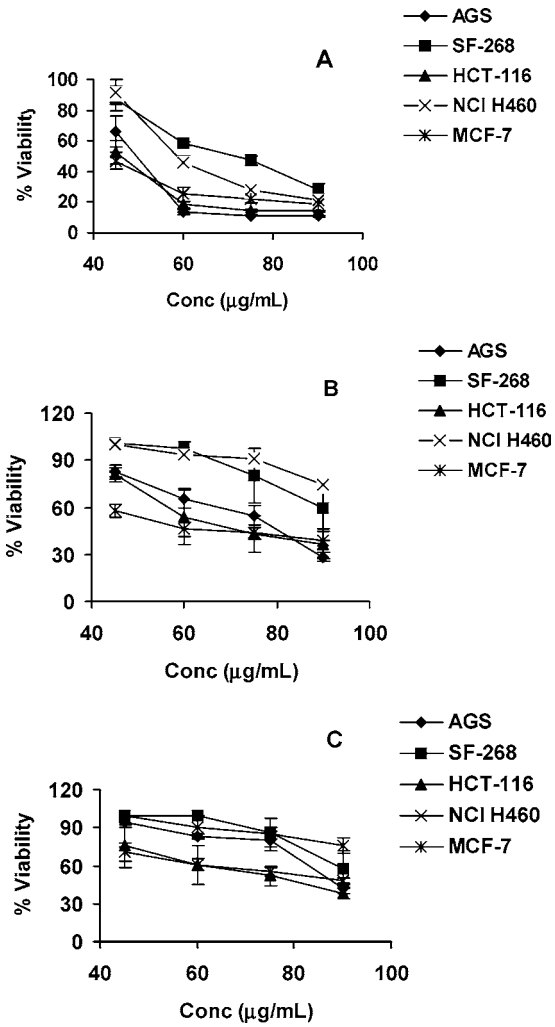

Figure 3. Percent AGS (gastric), SF-268 (CNS), HCT-116 (colon), NCI-H460 (lung), and MCF-7 (breast) human tumor cell survival when exposed to galactosyl glycerols: (**A**) compound **1**; (**B**) compound **2**; **(C)** compound **3**. Adriamycin was used as the positive control, and its IC_{50} values were calculated as NCIH-460 (0.44 μg/mL), MCF-7 (0.31 μg/mL), HCT-116 (0.23 μg/mL), SF-268 (1.13 μg/mL), and AGS (0.16 μg/mL). Percentage inhibition is represented as the mean obtained from three independent experiments performed in duplicate.

isolated from *A. tricolor* inhibited the COX enzymes and the growth of several cancer cell lines. Therefore, the consumption of *A. tricolor* may be beneficial in limiting COX enzyme mediated tumorogenesis and inflammation.

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