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The Cytotoxic Effects of Novel Persin Analogues on a Breast Cancer Cell Line

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Roberts, Gurisik, Biden, Sutherland, and Butt (2007) and Butt et al. (2006) previously found that persin, a compound isolated from avocado leaves, can induce apoptosis, or programmed cell death, in mammary epithelial cells of lactating mice in vivo and in certain human breast cancer cell lines in vitro. It has also been found that at higher doses, persin is cardiotoxic in mice and causes necrosis in mammary glands of lactating mammals (Oelrichs, 1995). Therefore, compounds with reduced mammary gland necrosis and cardiotoxicity but with the apoptotic effects of persin on breast cancer cells could be potential chemotherapeutic agents. Six novel analogues of persin have been synthesized to test their effects on MCF-7 breast cancer cells and MCF-10A normal breast epithelial cells. Cells cultured from each cell line were treated with each analogue at varying concentrations to determine potential cytotoxic doses. Cytotoxicity of the compounds was determined by a commercially available Cell Proliferation Assay. Compounds that were significantly cytotoxic were tested for apoptotic activity using an enzyme-linked immunosorbent assay. Three compounds were found to be cytotoxic to both cell lines, whereas the others had little to no impact on cell viability.

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INTRODUCTION

The widespread occurrence of breast cancer, especially among women, motivates the search for effective therapeutic agents. Chemotherapeutic properties of plant chemicals, or phytochemicals, have been studied and some found to have antitumor activity, providing effective therapeutic treatment. According to Butt et al. (2006), the most widely used phytochemicals are taxanes, such as paclitaxel and its derivatives, which are effective against some types of breast, ovary, and other cancers.

The aim of cancer drugs is to eliminate, or kill, cancerous cells but not harm the noncancerous cells of the body. Two mechanisms by which cell death can occur are apoptosis and necrosis. Many chemotherapeutic drugs are cytotoxic by inducing apoptosis in tumor cells. Apoptosis is commonly referred to as a form of programmed cell death or cell suicide. The process is a highly regulated method that removes cells without damage to the whole organism, such as in embryonic development, cell population maintenance in tissues, and immune system development. In contrast, necrosis occurs when an external stimulus damages cells, causing the cell to swell and burst, or lyse. Cell lysis releases intracellular contents, can damage neighboring cells, and can lead to an inflammatory response. Consequently, a necrotic mechanism of action is undesirable for potential drugs and an apoptotic mechanism is preferable.

Persin is a phytochemical that has been isolated from the avocado plant (Persea americana); persin is synthesized in the plant's idioblast oil cells from long-chain fatty acids, presumably as an insecticide and fungicide (Butt et al., 2006; Rodriguez-Saona and Trumble, 2000). It was previously shown that ingestion of avocado leaves by lactating livestock causes mastitis and decreased milk production, associated with necrosis of the secretory epithelium of mammary glands, as well as interstitial edema, congestion and hemorrhage (Kingsbury, 1964, pp. 124-5). This observation led to the isolation of persin, an unsaturated fatty acid, closely related in structure to the essential fatty acid linoleic acid and exhibiting necrotic activity in the R configuration (Oelrichs et al., 1995). One study found that treatment with 60-100 mg/kg of persin has the same effect on lactating mice as ingestion of avocado leaves: interstitial edema, congestion, hemorrhage, and coagulative necrosis and shedding of the mammary gland epithelium (Oelrichs et al., 1995). The epithelium did not appear to regenerate, and more severely damaged tissue was replaced by scar and adipose tissue (Oelrichs et al., 1995). These results, correlating with the effects of ingestion of avocado leaves, suggest that persin is likely the most notable toxin in avocado leaves (Oelrichs et al., 1995).

In addition to mammary gland necrosis and reduced milk production, apoptosis

has been shown to occur in the mammary glands of persin-treated lactating mice (Butt et al., 2006). In the estrogen receptor positive MCF-7 and T-47D human breast cancer cell lines, persin treatment inhibited cell proliferation, resulted in decreased expression of cyclins A, D1, and B1, and led to cell cycle arrest in G2-M phase (Butt et al., 2006). Taxane cancer drugs, such as paclitaxel, also induce G2-M phase arrest via microtubule stabilization and bundling, leading to apoptosis (Butt et al., 2006). Like paclitaxel, persin treatment resulted in rearrangement of cytoskeletal microtubules into thick, peripheral bundles instead of radiating from the center, as well as an overall increase in polymerized tubulin. Persin-induced apoptosis was found to be dependent on caspase activity and dependent on expression of BH3-only protein Bim (Butt et al., 2006). Bim is thought to monitor cytoskeletal processes and induce apoptosis when released from the microtubule-associated dynein motor complex in response to apoptotic stimuli (Puthalakath, Huang, O'Reilly, King, & Strasser, 1999). However, it was also shown that persin-induced apoptosis is independent of p53, Bcl-2, and estrogen receptor status, indicating its potential as a chemotherapeutic agent for estrogen receptor negative breast cancers that are nonresponsive to typical treatments (Butt et al., 2006; Roberts, Gurisik, Biden, Sutherland, & Butt , 2007).

In subsequent studies, simultaneous exposure of MCF-7 and T-47D cancer cells to persin and the antiestrogen drug tamoxifen decreased expression of estrogen receptor and enhanced Bim-dependent apoptosis. Apoptosis significantly increased in estrogen receptor negative cancer cell lines SK-Br3 and MDA-MB-157 when both persin and tamoxifen were used together, but no effect was seen in normal breast epithelial cells up to 40 µmol/L of persin alone or in combination with 10 µmol/L of tamoxifen (Roberts, Gurisik, Biden, Sutherland, & Butt, 2007).

Although the previously discussed studies suggest that persin or its derivatives may have significant potential as a chemotherapeutic drug, it was noted that persin treatment in mice damages not only lactating mammary glands, but also results in necrosis of myocardial fibers of the heart and potential hydrothorax and pulmonary edema (Oelrichs et al., 1995). These results prompt the search for other compounds that may be specific only to breast cancer cells.

Since persin appears to be uniquely independent of estrogen receptor status and to have no effect on normal breast epithelial cell lines at doses up to 40 µmol/L, persin analogues have been synthesized and tested for similar activity (Brooke et al., 2011). By altering the polar end, length, or saturation, Brooke et al. (2011) inferred a few moieties that may be significant. Bis-aroylated compounds were inactive, supporting that the presence of a free betahydroxyl group is important. An analogue with a short fatty acid

chain was inactive, indicating the significance of length. A fully saturated persin analogue appeared to be as cytotoxic as persin, suggesting the degree of saturation is not critical (Brooke et al., 2011).

To find compounds that may have a mechanism similar to persin but not cause mammary gland necrosis or cardiac cytotoxicity, six persin analogues were designed and synthesized from oleic acid and designated CG1-CG6 (Figure 1). Oleic acid is an eighteen-carbon, monounsaturated long-chain fatty acid similar to persin (Figure 1h). Since previous studies indicate the importance of a free betahydroxyl group in the activity of persin, each compound was structurally modified on the polar carboxylic end: CG1 is a secondary alcohol; CG2 is a derived acetate of the secondary alcohol; CG3 is a secondary ketone; CG4 is a primary alcohol; CG5 is a beta-keto ester; and CG6 is a beta-hydroxy ester. The compounds' effects were assessed by cell viability assays that estimate the number of cells that remain alive after exposure to each compound. Each compound was tested in two different cell lines: MCF-7 and MCF10-A cells. MCF-7 cells are human mammary gland epithelial cancer cells that served as a model for a breast tumor. MCF-10A cells are human mammary gland epithelial cells that grow rapidly in culture but are non-tumorigenic and thus served as a model for normal breast epithelial tissue.

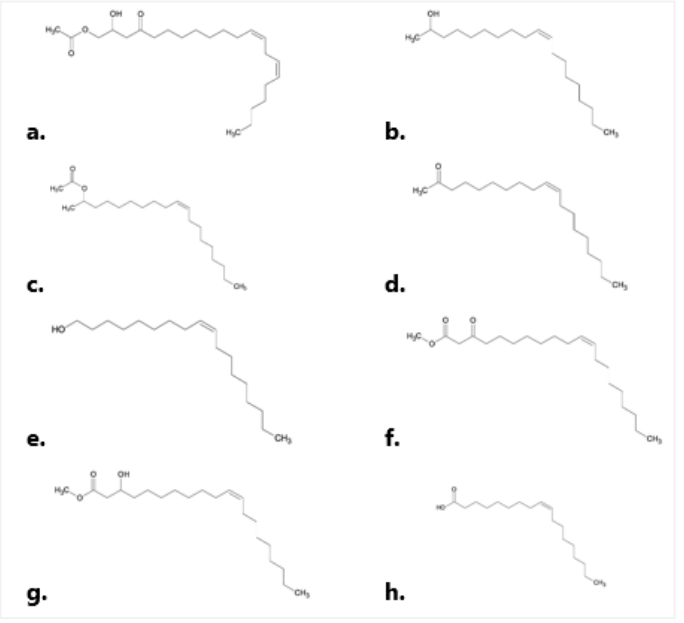


Figure 1. Persin analogues.
a.Persin **b.** CG1 **c.** CG2 **d.** CG3 **e.** CG4 **f.** CG5
g. CG6 **h.** oleic acid

MATERIALS AND METHODS

Cell Tissue Culture

MCF-7 and MCF-10A cells were maintained in a humidified incubator at 37°C with 5% CO2 to simulate human body conditions. Each cell line was grown in a 75 cm2 flask with their respective medium. The standard medium used for MCF-7 cells contains Dulbecco's Modified Eagle Medium (DMEM), 10% fetal bovine serum (FBS), and 100 units/ml each of penicillin and streptomycin. The standard medium used for MCF-10A cells contains DMEM, 5% horse serum, 20 ng/ml human recombinant EGF, 0.5 µg/ml hydrocortisone, 100 ng/ml cholera toxin, 10 ・g/ml bovine insulin, and 100 units/ml each of penicillin and streptomycin. Fresh media was given to the cells every couple of days as needed as they deplete the nutrients they metabolize to grow and divide. As they grow and divide, the cells eventually cover the surface of the flask. This leads to no room for new cells, therefore each new division leads to cells detaching from the plate and dying. To provide more space and maintain a continuous live culture, some cells are transferred to new flasks, a process called splitting.

To split a culture flask, the media was removed and cells were washed with 1X PBS (phosphate buffer saline). The PBS was removed and trypsin was added to digest the proteins that the cells use to adhere to the flask. After the cells detach from the flask, fresh media was added to inactivate the trypsin to prevent further protein digestion. The media and trypsin with suspended cells were collected in a centrifuge tube and centrifuged at 1000 rpm for five minutes to pellet the cells. Media and trypsin were removed from the centrifuge tube, leaving the cell pellet, which was then suspended in fresh media. Cells were counted with a hemocytometer to determine cell number, and the specific numbers of cells were added to a new flask or plates for experiments. All procedures were conducted under sterile conditions under Biosafety level 2 guidelines and standards in accordance with the Biological Safety Office of Georgia Regents University.

Compounds

This study was done in conjunction with the Georgia Regents University Department of Chemistry and Physics. Dr. Tom Crute, the chair of the department, designed and synthesized the persin analogues. The six persin analogues were synthesized from oleic acid following established methods and purified by column chromatography on silica gel; structures were confirmed by infrared spectroscopy and nuclear magnetic resonance. The purified compounds were diluted to 100x stock

concentrations in dimethyl sulfoxide (DMSO) and then diluted to the desired concentration in media appropriate for each cell line. The media was then added to the cells for treatment.

Cell Viability Experiments

MCF-7 and MCF-10A cells were plated at a density of 10,000 cells per well in 96-well plates with 100 µl of media and grown overnight prior to treatment. To assess cytotoxicity of the persin analogues, cells were treated in triplicates and incubated with the compounds diluted in media for 6, 24, and 48-hour time points. As a negative control, cells were incubated with media plus DMSO. Wells with media plus DMSO but no cells were included as a blank. After incubation, viability was assessed using the CellTiter 96® AQueous One Solution Cell Proliferation Assay, which is a colorimetric assay that uses the compound MTS to assess cell viability. The assay reagent with MTS (20 µl) was added to the cells. If cells are alive and metabolically active, enzymes present in the cells will reduce MTS to formazan, turning the yellow reagent to a dark red; therefore, the amount of formazan produced can be measured by the absorbance at 490 nm via spectrophotometry and is proportional to the number of living cells. Absorbance was measured in the microplate reader two hours after the reagent is added. Cell viabilities were then assessed as percentages of the negative control.

Apoptosis Assays

Assessment of apoptosis was conducted using the M30 CytoDeathTM Enzyme-Linked Immunosorbent Assay (ELISA) from Peviva. In the early stages of apoptosis, caspase enzymes are activated to begin protein degradation. Certain caspases cleave the protein cytokeratin 18 (K18), an intermediate filament present in epithelial cells. Cleavage of K18 exposes the M30 epitope. The amount of caspase-cleaved K18 can be measured using the ELISA. The assay uses a microstrip plate that is coated with mouse monoclonal K18 antibody M6. The M6 antibody binds the soluble K18 that is released from lysed sample cells. If the K18 is caspase-cleaved, a mouse monoclonal IgG2b antibody recognizes and binds the exposed M30 epitope (Figure 2). This antibody is conjugated, or linked, to the enzyme horseradish peroxidase (HRP). When HRP's substrate 3,3',5,5'-tetramethylbenzidine (TMB) is added, it is converted to a colored product that can be measured by a spectrophotometer. Absorbance is directly proportional to the amount of cleaved K18, thus absorbance is proportional to the number of apoptotic cells.

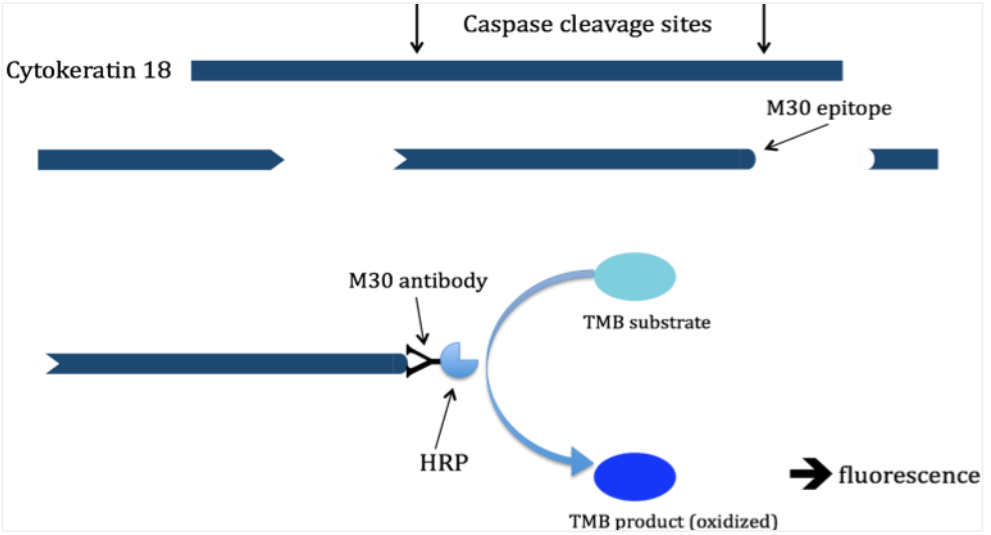


Figure 2. M30 Assay.

To assess MCF-7 and MCF-10A cells' apoptotic activity, cells were plated and treated just as for the cell viability assays. As a positive control, cells were treated with staurosporine, a microbial compound that induces apoptosis (Sanchez, Lucas, Sanz, & Goberna, 1992). Treatment was stopped by freezing the plate at -20°C. To perform the apoptosis assay, the plate was thawed to room temperature and a detergent was added to each well to disrupt the cell membranes. To help lysis occur, the plate was shaken for five minutes. Cells and media in each well were gently mixed and 25 µl was transferred to the M30 CytoDeathTM Coated Microstrips. Standard solutions (25 µl) were included to generate a standard curve for comparison. Diluted M30 CytoDeathTM HRP Conjugate solution (75 µl) was added to each well. The plate was shaken at 600 rpm for four hours. The plate was washed five times with 250 µl of the prepared wash solution provided by the assay kit. TMB substrate (200 µl) was added to each well and the plate was incubated in the dark for twenty minutes. Stop Solution (50 µl) was added to each well to stop the HRP-TMB reaction. The plate was shaken briefly (5-10 seconds) to ensure thorough mixing of the Stop Solution and TMB substrate. After five minutes, absorbance was read in a microplate reader at 450 nm.

Statistical Analysis

The data are presented as means + SE. Each separate experiment (n = 1) resulted from cells plated from each independent passaging. Statistical analysis was performed using SPSS. Data were analyzed by analysis of variance and multiple means were tested for significance by Fisher's Least Significant Difference test. The level of significance was

$p < 0.05$.

RESULTS

CG1 significantly decreased cell viability of MCF-7 but not MCF-10A cells.

CG1 (Figure 3) exhibited a time-dependent and dose-dependent cytotoxic effect on the MCF-7 cancer cells. Viability is expressed as percent of viability of DMSO-treated cells, which served as a negative control. As seen in Figure 4, at a concentration of 60 µM, a significant decrease in viability was not noted until 48 hours. At 90 µM, viability was decreased by about 20% within 6 hours and continued to decrease. At 120 µM, viability was notably reduced by over 60% within 6 hours, reducing to near zero after 24 hours. Over time, increasing CG1 concentrations had increasing cytotoxic effects on MCF-7 viability. Initial studies of the effects of CG1 on MCF-10A cells showed no decrease in cell viability following 6-hour exposure at doses up to 1 mM (data not shown).

Figure 3. CG1, a secondary alcohol.

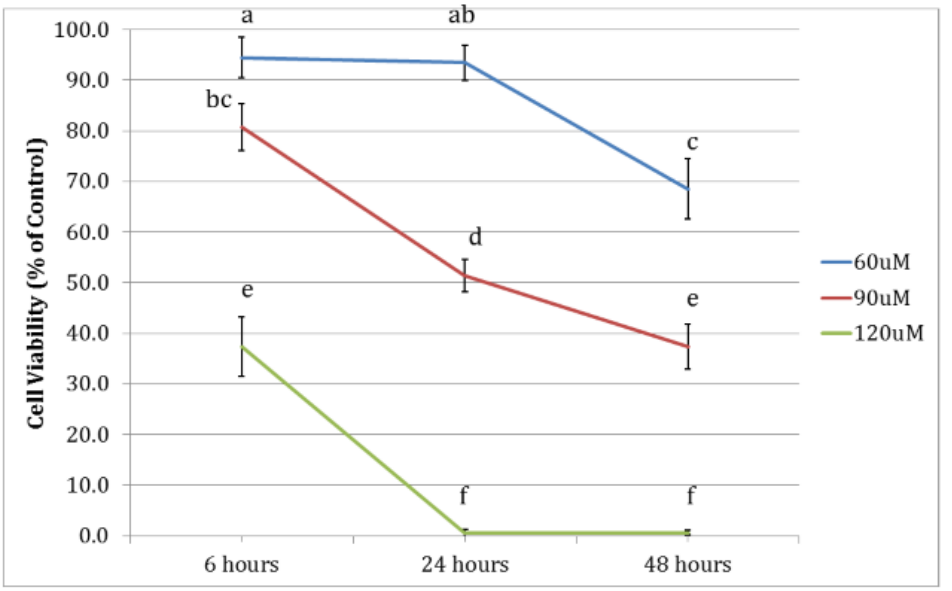


Figure 4. Effect of CG1 on MCF-7 cell viability over 6, 24, and 48 hour time points at various doses. $n \geq 4$. $p \leq 0.05$. Each letter corresponds to a statistically different data value.

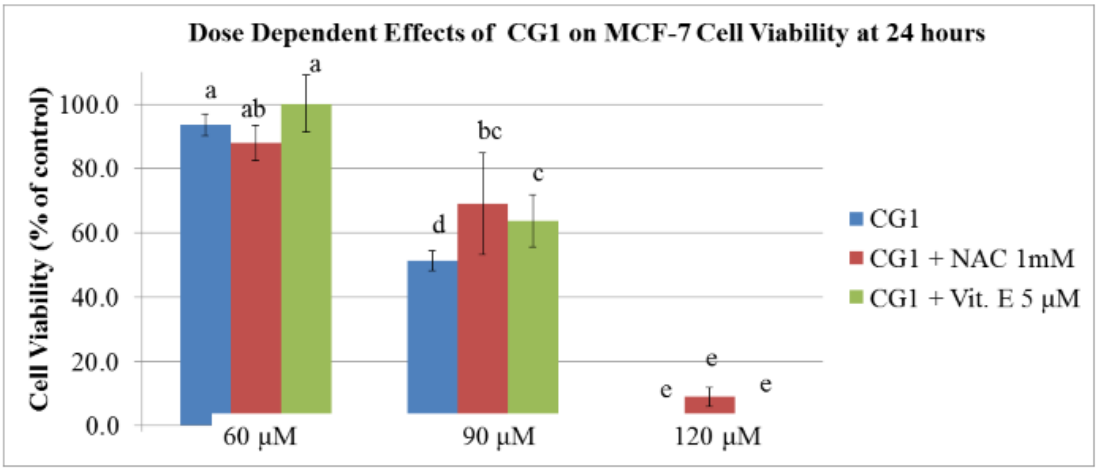


Figure 5. Dose dependent effects of CG1 on MCF-7 cell viability in the presence of tocopheryl acetate (vitamin E acetate) or N-acetylcysteine (NAC) at 24 hours. n = 4. $p \leq 0.05$. Each letter corresponds to a statistically different data value.

Antioxidants did not prevent reduced viability of CG1-treated MCF-7 cells.

To determine if CG1 exposure resulted in lipid peroxidation and oxidative stress in the MCF-7 cells, the cells were treated with CG1 and a water-soluble or lipid-soluble antioxidant. Tocopheryl acetate (vitamin E acetate) was chosen as the lipid-soluble antioxidant and N-acetylcysteine was chosen as the water-soluble antioxidant. Cell viability after treatment was assessed using the CellTiter assay and expressed as the percent of viability of DMSO-treated cells. Cancer cells grew prolifically when treated solely with tocopheryl acetate (50 µM) or N-acetylcysteine (10 mM) (data not shown). As previously shown, when treated with 90 µM or 120 µM of CG1, cell viability was greatly reduced within 24 hours. However, when treated simultaneously with CG1 and tocopheryl acetate or N-acetylcysteine, viability is greater with 90 ・M CG1 and not significantly different with 60 µM and 120 µM CG1 compared with CG1 alone ($p < 0.05$, Figure 5). This implies that the presence of these antioxidants do not inhibit the toxic effects of CG1, suggesting that CG1 does not result in oxidative stress in the MCF-7 cells and that the compound likely has a different mechanism of action.

CG1 significantly induced apoptosis in MCF-7 cells.

Apoptotic activity was determined using the M30 assay after 6 hours of treatment. Results are expressed as the absorbance at 450 nm, which is proportional to the number of apoptotic cells: greater absorbance indicates a greater number of cells that are going through or have already committed apoptosis. Staurosporine, a

compound known to induce apoptosis (Sanchez, Lucas, Sanz, & Goberna, 1992), was used as a positive control. DMSO-treated cells served as a negative control. As shown in Figure 6, 60 µM of CG1 significantly induced apoptosis after 6 hours, even more so than staurosporine ($p < 0.05$). CG1 at 30 ・M exhibited similar activity to staurosporine (Figure 6).

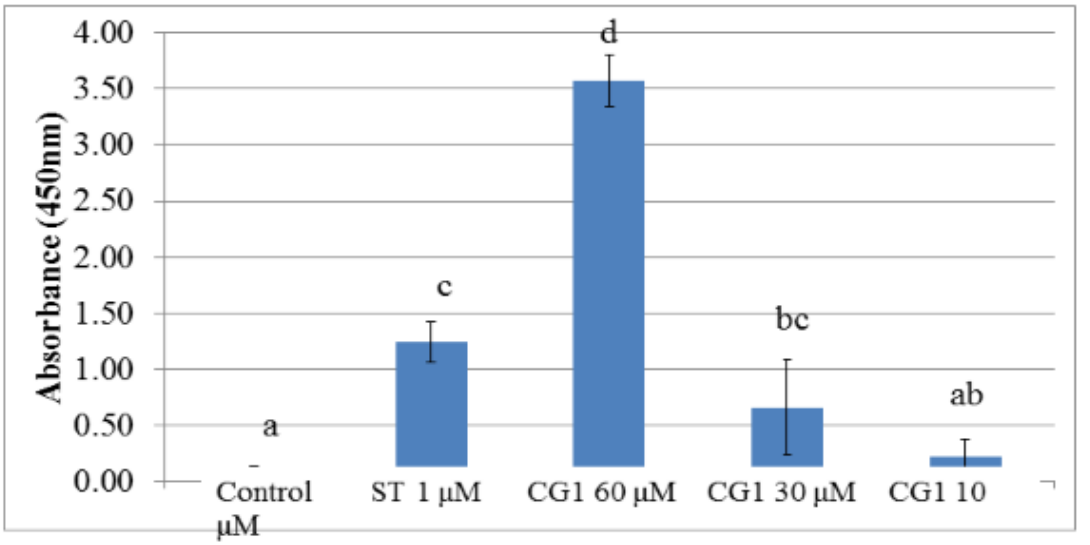


Figure 6. Absorbance as a measure of apoptotic activity of MCF-7 cells after treatment with CG1 for 6 hours. Staurosporine (ST) was used as a positive control. $n \geq 3$. $p \leq 0.05$. Each letter corresponds to a statistically different data value.

A second synthesized lot of CG1 is still cytotoxic to MCF-7 cells, but also to MCF-10A.

A new lot of CG1 was synthesized and tested for cytotoxic activity to ensure it had similar effects to the previous lot. Although different concentrations were tested, cell viability of MCF-7 cells continued to be dose- and time-dependent (Figure 7).

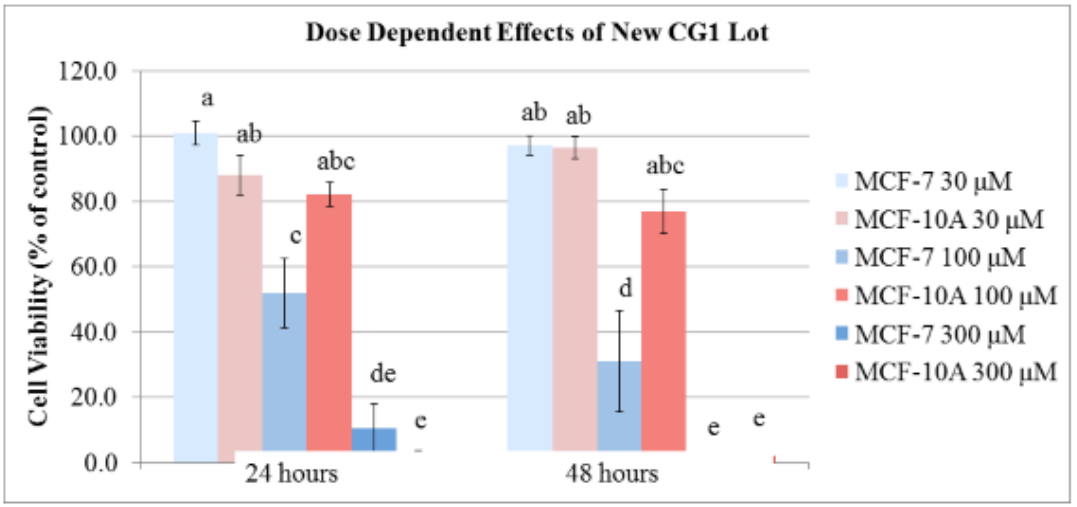


Figure 7. MCF-7 and MCF-10A cell viability after treatment with the newly synthesized CG1. $n \geq 4$. Blue bars correspond to the MCF-7 line; red bars correspond to the MCF-10A line. The lightest shade of each color correspond to 30 µM, the medium shade to 100 µM, and the darkest shade to 300 µM. $p \leq 0.05$. Each letter corresponds to a statistically different data value.

CG1 at 30 µM had little to no effect. Concentrations of 100 µM and 300 µM significantly reduced viability, resulting in a rounded morphology (Figure 8). At 48 hours, 100 ・M significantly reduced viability of MCF-7 more than MCF-10A, but 30 ・M did not reduce viability in either line ($p < 0.05$). Although the previous results showed that the normal MCF-10A cells were unaffected by CG1 up to 1 mM, the newly synthesized CG1 reduced MCF-10A cell viability to near zero at 300 µM within 24 hours.

a. **b.**

Figure 8. CG1-treated MCF-7 cells after 24 hours at 100X.
a. DMSO-treated MCF-7 cells (negative control). **b.** Cells treated with 300 µM.

CG3 reduces MCF-7 and MCF-10A cell viability.

CG3 (Figure 9) did not show a significant decrease of MCF-7 cell viability until a dose of 300 µM (Figure 10). It appeared to reduce viability of MCF-10A cells at an earlier time point than MCF-7 cells ($p < 0.05$).

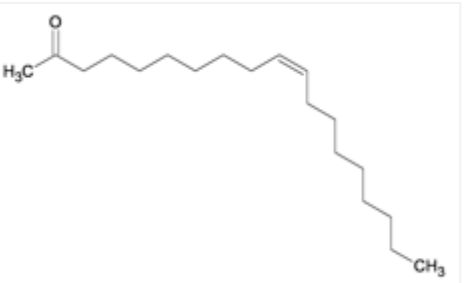


Figure 9. CG3, a secondary ketone.

CG4 is highly cytotoxic to both MCF-7 and MCF-10A cell lines.

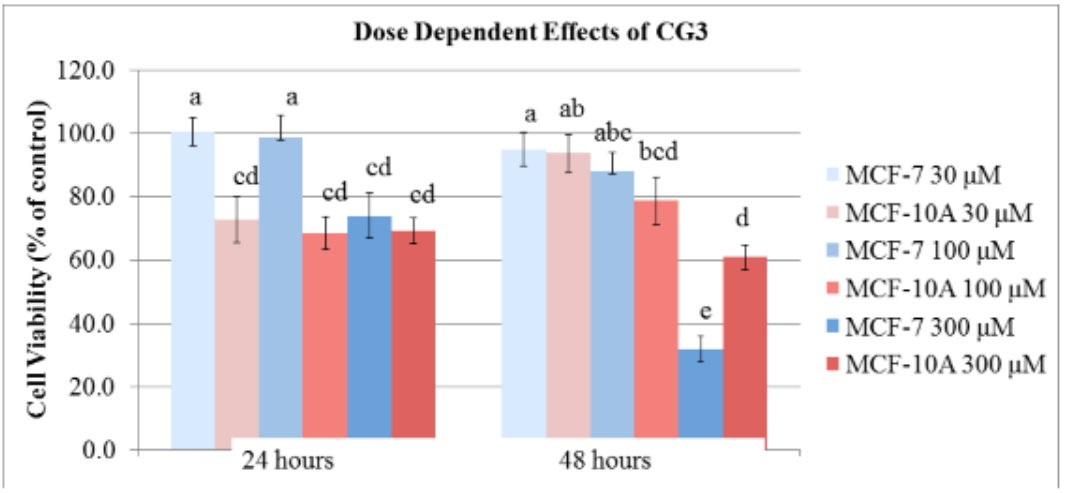


Figure 10. MCF-7 and MCF-10A cell viability after treatment with CG3. $n \geq 4$. Blue bars correspond to the MCF-7 line; red bars correspond to the MCF-10A line. The lightest shade of each color correspond to 30 µM, the medium shade to 100 µM, and the darkest shade to 300 µM. $p \leq 0.05$. Each letter corresponds to a statistically different data value.

The primary alcohol CG4 (Figure 11) exhibited greater toxicity than CG3 (Figure 12). However, it was toxic to both cell lines: viability was reduced to near zero within 24 hours of exposure at 100 µM in MCF-7 cells and 300 µM in MCF-10A cells. After another 24 hours of exposure to CG4, there is no further significant decrease in either cell line at any dose ($p < 0.05$).

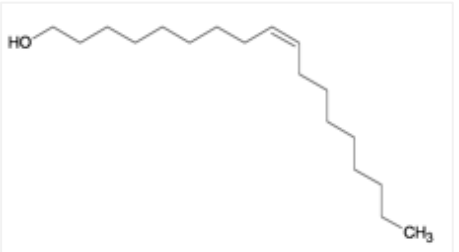


Figure 11. CG4, a primary alcohol.

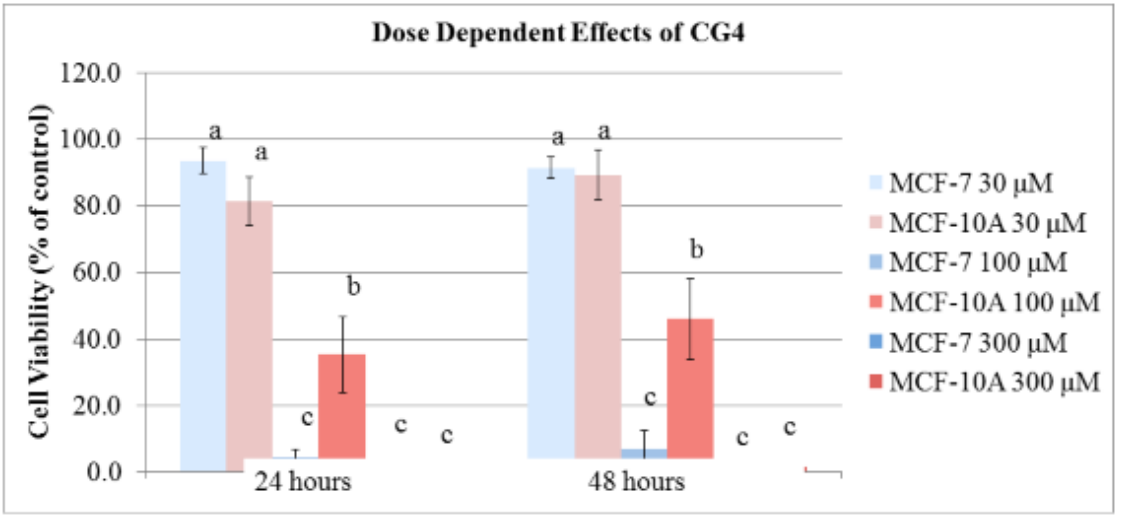


Figure 12. MCF-7 and MCF-10A cell viability after treatment with CG4. $n \geq 4$. Blue bars correspond to the MCF-7 line; red bars correspond to the MCF-10A line. The lightest shade of each color correspond to 30 µM, the medium shade to 100 µM, and the darkest shade to 300 µM. $p \leq 0.05$. Each letter corresponds to a statistically different data value.

CG5 reduced viability of MCF-10A cells more than MCF-7 cells.

CG5 (Figure 13) had a time- and dose-dependent effect on both MCF-7 and MCF-10A cells (Figure 14). Interestingly, it appeared to reduce viability of the MCF-10A cells more than the MCF-7 cells. Most MCF-10A viability values are significantly lower than the corresponding MCF-7 viability values ($p < 0.05$). In comparison, other compounds' effects were typically similar in both cell lines or reduced MCF-7 viability more than that of MCF-10A.

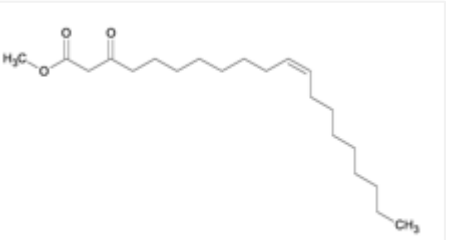


Figure 13. CG5, a beta-keto ester.

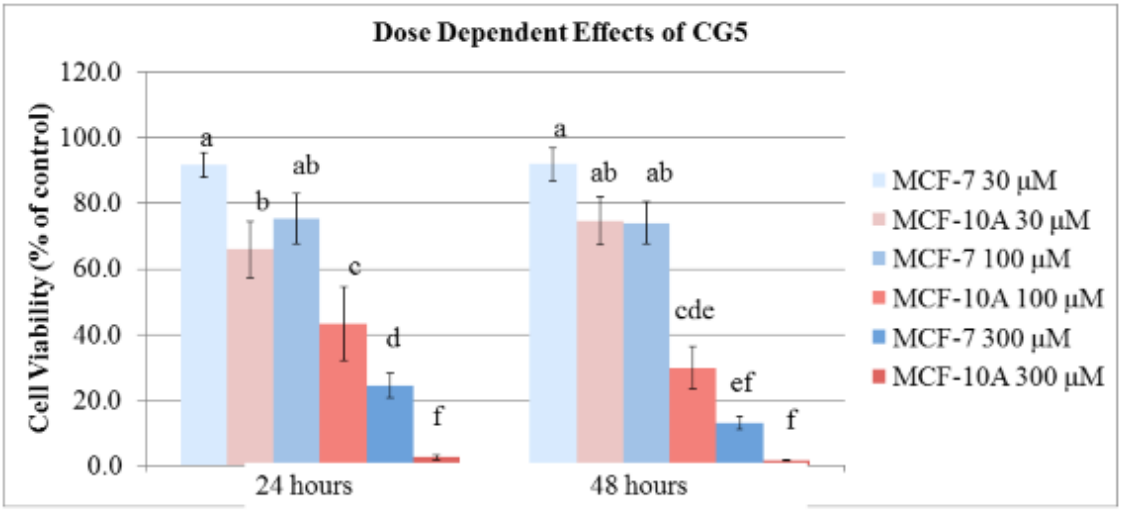


Figure 14. MCF-7 and MCF-10A cell viability after treatment with CG5. $n \geq 4$. Blue bars correspond to the MCF-7 line; red bars correspond to the MCF-10A line. The lightest shade of each color correspond to 30 µM, the medium shade to 100 µM, and the darkest shade to 300 µM. $p \leq 0.05$. Each letter corresponds to a statistically different data value.

CG6 did not substantially reduce cell viability, although cells have abnormal morphology.

Treatment of either cell line with CG6 (Figure 15) does not appear to result in significant differences between 24 and 48 hours or among different doses (Figure 16). Although cell viability decreased only 10-35% following 24-hour exposure, MCF-7 cells exhibited the rounded morphology characteristic of unhealthy or dead cells at as low as 30 µM of CG6 (Figure 17). MCF-10A cells also appeared rounded after 24-hour exposure at 100 and 300 µM (Figure 18). In each treatment, dark crystal-like structures were observed following CG6 exposure in both cell lines: in the MCF-7 line, they were seen after exposure to 30, 100, and 300 µM; in the MCF-10A line, they were seen after exposure to 300 µM. Despite the cells' abnormal appearance at 24 hours, their viability did not significantly decrease even after another 24 hours in either cell line ($p < 0.05$).

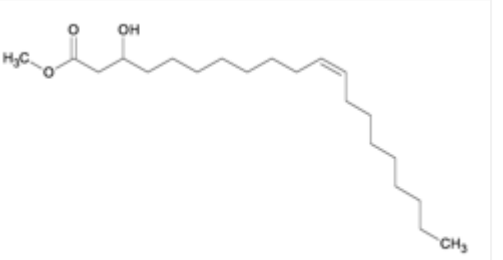


Figure 15. CG6, a beta-hydroxy ester.

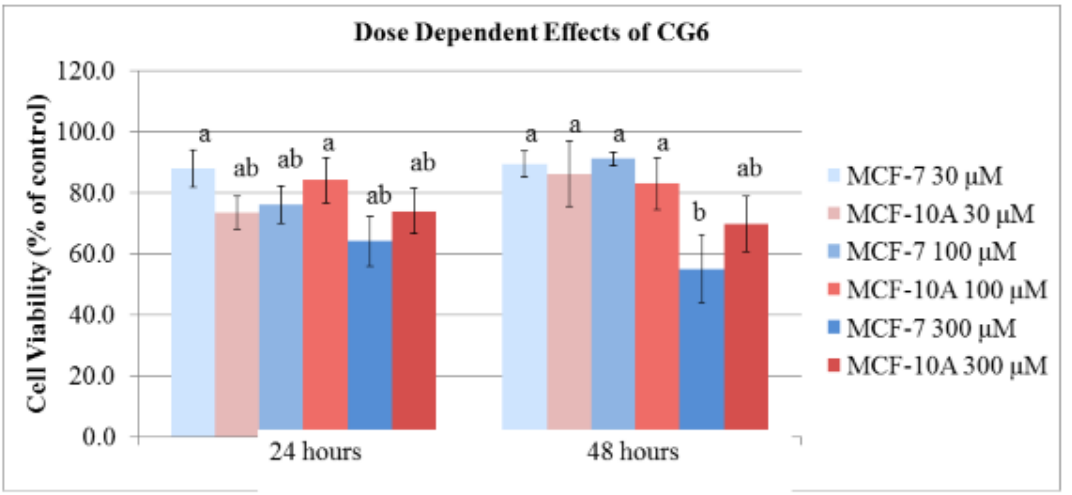


Figure 16. MCF-7 and MCF-10A cell viability after treatment with CG6. $n \geq 4$. Blue bars correspond to the MCF-7 line; red bars correspond to the MCF-10A line. The lightest shade of each color correspond to 30 µM, the medium shade to 100 µM, and the darkest shade to 300 µM. $p \leq 0.05$. Each letter corresponds to a statistically different data value.

Three of the six persin analogues exhibited dose- and time-dependent cytotoxicity to both cell lines.

In summary, CG1, CG4, and CG5 showed the greatest dose-dependent cytotoxicity to the cancerous MCF-7 cells, reducing viability to near zero at 300 µM (Figure 19). However, they were just as toxic or more toxic to the noncancerous MCF-10A cells.

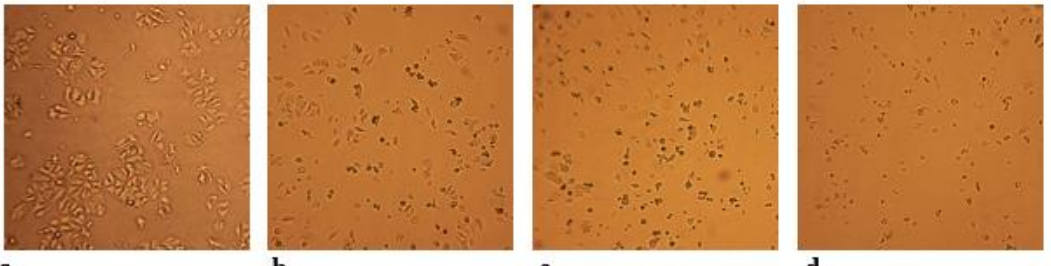
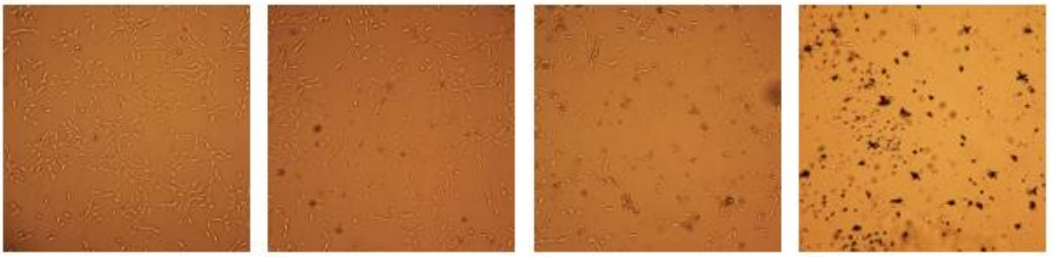


Figure 17. CG6-treated MCF-7 cells after 24 hours at 100X.
a. DMSO-treated cells (negative control). **b.** Cells treated with 30 µM. **c.** Cells treated with 100 µM. **d.** Cells treated with 300 µM.



a. **b.** **c.** **d.**

Figure 18. CG6-treated MCF-10A cells after 24 hours at 100X.
a. DMSO-treated cells (negative control). **b.** Cells treated with 30 µM. **c.** Cells treated with 100 µM. **d.** Cells treated with 300 µM.

The other two analogues, CG3 and CG6, were not as cytotoxic, reducing viability by only 10%-30% within 24 hours at the tested concentrations. There were no clear differences between the cancerous and noncancerous cell lines. CG2 showed no cytotoxicity following exposure to doses up to 1 mM in either cell line (data not shown).

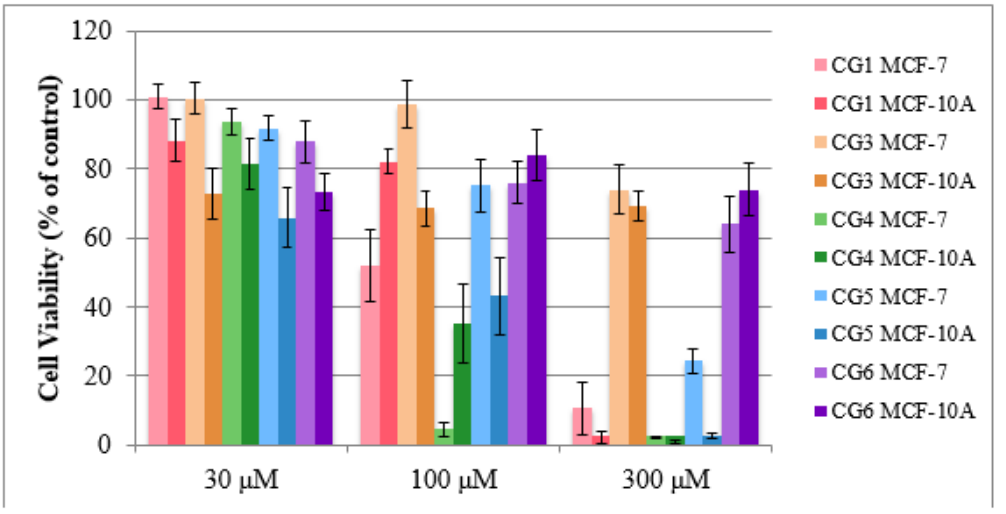


Figure 19. Cell viability of each cell line after 24-hour exposure to each compound at 30, 100, and 300 µM. Each color corresponds to each different compound. The lighter shade of each color corresponds to the MCF-7 line and each darker shade corresponds to the MCF-10A line.

DISCUSSION

As a potential chemotherapeutic drug, a compound would first need to show toxicity to cancer cells in vitro at a low concentration but not to noncancerous cells. Persin has been shown to be cytotoxic to breast epithelial cancer cell lines at 40 μM with no effect on normal breast epithelial cells at 40 μM (Roberts, Gurisik, Biden, Sutherland, & Butt, 2007).

Results from the early experiments with CG1 showed cytotoxicity to MCF-7 cells as low as 60 μM and no cytotoxicity to MCF-10A cells up to 1 mM. It was also determined that treated cells were not experiencing oxidative stress because simultaneous treatment with a water-soluble or lipid-soluble antioxidant was not beneficial. These results were intriguing, and apoptosis assays confirmed that CG1 at 60 μM induced apoptosis in MCF-7 cells. However, further studies with newly synthesized CG1 did not show the same results. A dose-dependent cytotoxic response was still seen in MCF-7 cells with viability reduced to about 50% at 100 μM and to near zero at 300 μM. Unlike the previous CG1 treatments, this new CG1 was just as cytotoxic to MCF-10A cells as to MCF-7 cells at 300 μM. In between the synthesis of each lot, the hexane solvents used in the chromatography purification were found to be contaminated. The hexanes were purified and CG1 was synthesized again. Contamination of the hexanes is the probable explanation of the discrepancy between the CG1 results. The promising results of the earlier experiments were not seen again. All other compounds tested were synthesized and purified after the hexane purification.

Experiments with CG6 resulted in dark crystals within 24-hour exposure of MCF-7 cells at as low as 30 μM and 24-hour exposure of MCF-10A cells at 300 μM. This could be due to the compound precipitating out of solution or causing a component of the media to precipitate. Formation of crystals at different concentrations of CG6 in the different cells lines could be due to the differences in their media.

CG1, CG4, and CG5 were the most potent to the cancerous MCF-7 cells at 100 and 300 μM. Unfortunately, they were also toxic to the noncancerous MCF-10A cells at the same concentrations. The others did not have substantial cytotoxicity to either cell line at the tested concentrations. Therefore, none appear to exhibit activity similar to persin and potential as a chemotherapeutic drug.

Differences in the media of the MCF-7 and MCF-10A cell lines are a possible cause for the differences, or lack of differences, in the results between the cell lines. MCF-7 media had twice as much serum and MCF-10A media had additional

supplements necessary for adequate growth of the culture that are absent in the MCF-7 media. The specific media was used because it was previously determined by ATCC (American Type Culture Collection) as the conditions under which each line grows best. A future study could be to test the effects of the compounds on each cell line grown in more similar media, or other experiments designed to test the role of the media.

Since there were no desirable differences between the cancerous and noncancerous cell lines with any of the synthesized compounds, no further antioxidant treatments or apoptotic assays were done. Future aims in this area of study include synthesis of additional analogues and testing their effects in vitro for similarity to persin.

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