Anticancer Activity of Branched-chain Derivatives of Oleic Acid

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Abstract. Background: A number of unsaturated fatty acids and a few saturated branched-chain fatty acids have been reported to exhibit anticancer activity. Materials and Methods: In previously reported research, several novel branched-chain derivatives (methyl, n-butyl, phenyl) of methyl oleate were produced by bromination in the allylic position and subsequent treatment with organocuprate reagents. These compounds and their free acid counterparts were tested in vitro for their antiproliferative activities against two cancer cell lines: MCF-7 (human breast) and HT-29 (human colon). In addition, two sets of isomeric tertiary alcohols obtained as side-products in the synthesis of the branched-chain derivatives were evaluated. Testing was performed at three concentration levels (50, 100, and 200 ppm) in dimethyl sulfoxide (DMSO). Results: The greatest growth inhibitory activity was exhibited by the branched phenyl derivative of oleic acid, with IC50 at 48 ppm against both MCF-7 and HT-29. The branched n-butyl derivative of oleic acid also exhibited significant antiproliferative activity, with IC50 at 82 ppm against MCF-7 and 77 ppm against HT-29. Conclusion: The observed potent anticancer activity of the n-butyl and phenyl derivatives indicates that certain synthetic branched-chain unsaturated fatty acids have potential in the treatment of cancer and further research is warranted.

Cancer is frequently a consequence of dysregulated cell cycle control and/or suppressed apoptosis (programmed cell death), as usually observed in colorectal cancer. Protective effects of bioactive compounds in cancer development should consequently be associated with inhibition of cell proliferation. Identification and development of new anticancer bioactive compounds, with fewer side-effects, from natural resources will play an important role in the advancement of cancer control.

A number of unsaturated fatty acids have been reported to inhibit cancer cell growth (1-5). In addition, a few saturated branched-chain fatty acids have been reported to exhibit anticancer activity. These specific compounds were determined to be benign to normal cells in animal studies (6-9). Furthermore, in human clinical studies of one month’s duration, therapeutic dosages of 13-methyltetradecanoic acid did not produce any side-effects (9).

Oleic acid is one of the most abundant fatty acids of many vegetable oils, such as cottonseed oil. As part of a project to develop new and expanded uses of oilseed products and by-products, methyl oleate was converted in a series of reactions to several different (methyl, n-butyl, phenyl) branched-chain derivatives (10-11). These compounds and their free acid counterparts (Figure 1) were tested in vitro for their antiproliferative activities against two cancer cell lines, MCF-7 (human breast) and HT-29 (human colon), at three concentrations. In addition, two tertiary alcohols (Figure 1) obtained as by-products in the synthesis of the branched-chain derivatives (10, 12) were also evaluated.

Materials and Methods

Materials. Rosewell Park Memorial Institute 1640 (RPMI-1640) medium, sodium pyruvate, sterile cell culture penicillin-streptomycin, sodium bicarbonate, non-essential amino acids, and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Tissue culture plates were purchased from Costar Corp. (Cambridge, MA, USA). Fetal bovine serum was purchased from Hyclone Laboratories, Inc. (Logan, UT, USA). Methyl oleate (99%) and Merck analytical thin layer chromatography (TLC) plates (silica gel 60 F254, 5×10 cm) were purchased from Aldrich Chemical Company (Milwaukee, WI, USA). Oleic acid was obtained from J. T. Baker Inc. (Phillipsburg, NJ, USA) and phosphomolybdic acid was supplied by Eastman Kodak Company (Rochester, NY, USA).

General conversion of methyl esters to free fatty acids. The syntheses and characterization (gas chromatography-mass spectroscopy, differential scanning calorimetry, and nuclear magnetic resonance spectroscopy) of the methyl esters 1, 2, and 3 have been reported (11-12). These esters were converted to the free
fatty acids by alkaline hydrolysis. The isolated product was confirmed to consist solely of fatty acid (no unreacted methyl ester) by TLC (developed in 5% ethyl acetate/hexanes). A 10% solution of phosphomolybdic acid in isopropanol was used as spray reagent for detection of individual compounds.

Preparation of the free fatty acids (6). The methyl esters (3) (32.0 mg; 0.086 mmol) were dissolved in 15 ml of 95% ethanol and 15 ml of 1 M sodium hydroxide (NaOH). The mixture was stirred at 80˚C under reflux for 16 h. After cooling, the reaction mixture was extracted with hexanes (2×25 ml). The combined organic layers were extracted with 15 ml of 1 M NaOH. Combined aqueous layers were acidified with 3 M HCl and extracted with ether (2×50 ml). Combined ether layers were washed with 50 ml of brine and dried (MgSO4). Removal of solvent in vacuo afforded 29.7 mg (96.4%) of free fatty acid.

Anticancer assay. MCF-7 (human breast) and HT-29 (human colon) cancer cell lines were purchased from the American Type Culture Collection (ATCC) (Rockville, MD, USA). The cells were cultured in RPMI-1640 with 2 mM L-glutamine, 1 mM sodium pyruvate, 100 units/ml penicillin, 0.1 mg/ml streptomycin, 0.1 mM non-essential amino acids, 2.0 g/l sodium bicarbonate and 10% fetal bovine serum. Both cell lines were incubated at 5% CO2 and 90-100% relative humidity at 37˚C. Medium renewal was carried out 2-3 times per week, and cells were subcultured when they achieved 80-90% confluence (13). Prior to chemical treatment, 1.5×104 cells/well (200 μl/well) were seeded into a 96-well tissue culture plate, then a defined concentration of the test compound dissolved in DMSO was added. As negative controls, cells were treated with DMSO only. After a 24-h incubation period, the old medium with the dead cells was removed, and the live cells were determined with the CellTiter 96® aqueous nonradioactivity cell proliferation assay (Promega, Madison, WI, USA). Results were recorded on a universal EL800 Bio-Tek microplate reader at 490 nm. The cell viability was determined by comparing the absorbance of the treated cells with that of the control. The 50% inhibition concentration (IC50) was defined as the chemical concentration causing 50% inhibition of cell growth and was calculated using linear regression analysis.

Statistics. Triplicates were performed for each concentration of the tested chemical. All the experiments were carried out in triplicates on different days. All data were subjected to statistical analyses of

![Figure 1. Structures of the synthetic compounds tested for anticancer activity.](image)
variances (ANOVA) to examine the differences between the means. Two-way ANOVA statistical analysis (14) was carried out in Excel 2003 (Microsoft, Redmond, WA, USA) followed by post-hoc testing using Tukey’s test (14).

Results and Discussion

Ten compounds were tested in vitro for their antiproliferative activities against two cancer cell lines, MCF-7 (human breast) and HT-29 (human colon) at three concentrations. Compounds 1-8 consist of four closely related isomers (designated a, b, c, and d in Figure 1), differing by position of the double bond and branched chain. The syntheses and characterizations of compounds 1, 2, 3, and 7 have been reported (10-11). The isomeric tertiary alcohols (8) were isolated as minor products in the synthesis of the esters (3) (12). Methyl oleate (9) and oleic acid (10) were tested for comparative purposes. The results are shown in Figure 2. Generally, all compounds showed increased inhibitory activity with increasing concentration for both cell lines. A two-way ANOVA statistical analysis using a significance level of 5% showed that there were significant differences between the means, both in relationship to the initial concentration, as well as to the compound type. This was true for both the MCF-7 and HT-29 cell lines. Pair-wise comparisons of means using Tukey’s test resulted in grouping of means according to the letters shown in Figure 2.

At 200 μg/ml, compounds 1, 4, 5, 6, and 8 showed the strongest antiproliferative activity against MCF-7 cells among all the compounds and concentrations tested; for HT-29, compounds 1, 4, 5, 6, 8, and 10 showed the strongest antiproliferative activity at 200 μg/ml. At the lowest test concentration of 50 μg/ml, compound 6 showed stronger anti-proliferative activity against MCF-7 cells than the other nine compounds tested, and compounds 5, 6 and 10 showed the strongest growth inhibition against HT-29 cells. With the exception of the branched methyl compounds 1 and 4, the free acids showed greater activity than their corresponding methyl ester counterparts. This observation was expected, since the free acid should exhibit greater water solubility, enhancing delivery to the cancer cells. In general, the methyl esters 1, 2, 3, and 9 were poor or fair inhibitors of cancer cell growth; however, compound 1 did reduce cell viability to approximately 30% for both cell lines at 200 μg/ml and 59% for MCF-7 cells at 100 μg/ml.

The greatest growth inhibitory activity was exhibited by the branched phenyl derivatives (6) (cell viability was below 50% for both cell lines at all three concentrations) with IC50 at 48 μg/ml (Table 1) against both MCF-7 and HT-29 cells. The growth activity of compound 6 against MCF-7 cells is comparable to that found for gossypol, an NIH-patented therapeutic agent for human cancer patients (13). The branched n-butyl derivatives (5) also exhibited highly significant activity, with IC50 at 82 μg/ml against MCF-7 cells and 77 μg/ml against HT-29 cells. The calculated IC50 values for all 10 compounds are shown in Table I. For compounds 1-9, the IC50 values determined for MCF-7 cells were comparable to those determined for HT-29 cells. The branched methyl compounds 1 and 4 exhibited virtually identical anticancer activity, with IC50 values ranging from 139 to 149 μg/ml.

In summary, for both MCF-7 and HT-29 cell lines, the phenyl-substituted fatty acids 6 exhibited antitumor activity superior to the alkyl-substituted derivatives 4 and 5. In addition, the phenyl-substituted tertiary alcohols (8) were more active than the n-butyl-substituted tertiary alcohols (7), particularly at the 200 μg/ml concentration level. These findings suggest that unsaturated fatty acids and related compounds containing aromatic branched chains could have potential as anticancer agents.

At all concentrations, oleic acid (10) was less active than compound 6 against both cell lines and less active than compound 5 against MCF-7 cells. However, oleic acid did show significant activity against HT-29 (IC50=50 μg/ml). Reports on the antitumor activity of oleic acid vary. It has been reported to be a poor inhibitor of HT-29 cells (15). On the other hand, there are reports on the inhibitory activity of oleic acid against tumor growth (16, 17). Finally, it has been reported that oleic acid suppresses the action of the cancer-causing HER-2/neu oncogene. Overexpression of this oncogene has been found in about 20% of breast carcinomas (18).

Conclusion

A number of specific branched-chain fatty acids have been reported to exhibit anticancer activity and yet not be harmful to normal cells (6-9). In addition, oleic acid is generally regarded as being nontoxic to normal cells. Accordingly, branched-chain fatty acids derived from oleic acid would be expected to have similar properties. The observed potent anticancer activity of the n-butyl derivatives (5) and phenyl derivatives (6) indicates that certain synthetic branched-chain derivatives of oleic acid and other unsaturated fatty acids have potential in the treatment of cancer and further research is warranted (with emphasis on aromatic substituents).

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Figure 2. Cell viability (%) with three different concentrations (50, 100, and 200 ppm) of compounds 1-10 against MCF-7 breast cancer cell line (A) and HT-29 colon cancer cell line (B). Error bars in the figure are standard deviations of triplicate experiments, and different letters represent significant difference at 95% confidence interval using Tukey’s test.
References


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