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New phenstatin–fatty acid conjugates: Synthesis and evaluation

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ABSTRACT

New phenstatin–fatty acid conjugates have been synthesized and tested against the KB-3-1, H460, MCF-7 and HEK293 cell lines, with an increase in anti-proliferative activity being observed at the micro-molar level paralleling an increase in un-saturation in the fatty acid component.

The quest for more potent and less toxic candidates in the treatment of various elusive forms of cancer remains unrelenting; and especially so, in light of the emergence of new multidrug resistance (MDR) mechanisms. It has been reported that this persistent MDR barrier is the most significant reason for treatment failures in excess of 90% of subjects with metastatic cancers.[1] Among the group of promising antineoplastic agents are new chemical entities which are hybrids of naturally occurring bioactive substances. These hybrids are often comprised of either the whole or partial structures of the natural product of interest. Indeed, reports indicate several cases of improved activity of the hybrids relative to the parent natural product.[2] Of direct relevance are the hybrids of recognized anti-cancer compounds and various unsaturated fatty acids, ranging from the monounsaturated systems (MUFAs) to different polyunsaturated systems (PUFAs).

The incorporation of unsaturated fatty acids provides a very promising alternative in addressing tumor-targeting delivery of drugs in an effort to improve the overall efficacy of these chemotherapeutic agents.[3] PUFAs such as linoleic acid (LA) and docosahexaenoic acid (DHA) fulfill significant roles in cell growth and development.[4]

Furthermore, studies have shown that PUFAs are readily taken up by tumor cells, and that certain PUFA-drug conjugates exhibit tumor specific accumulation through in vitro and in vivo studies.[5] For instance, Ojima et al. have synthesized conjugates of DHA or LNA (linolenic acid) with next generation taxoids that have demonstrated greatly improved efficacy.[6]

This study focuses on the effects of conjugating various fatty acids on the antiproliferative activity of the reported tubulin-binding compound phenstatin[7] **1**. Presently, intense efforts have been devoted to the development of novel analogues of phenstatin and its derivatives, largely based on the fact that being a benzophenone derivative, its structure is not prone to the (*Z*)/(*E*) isomerization associated with combretastatin A-4 (CA-4), the cause of decreased efficacy. We have also examined hybrids of the new compound isophenstatin **2** (Fig. 1).

Phenstatin, like CA-4, binds to the colchicine site of endothelial tubulin, thus interfering with the equilibrium dynamics associated with the cell division process.[8] Mitotic arrest typically follows leading to apoptosis and cell death.[9]

Herein we report the synthesis of some novel MUFA and PUFA-conjugates of phenstatin and isophenstatin.

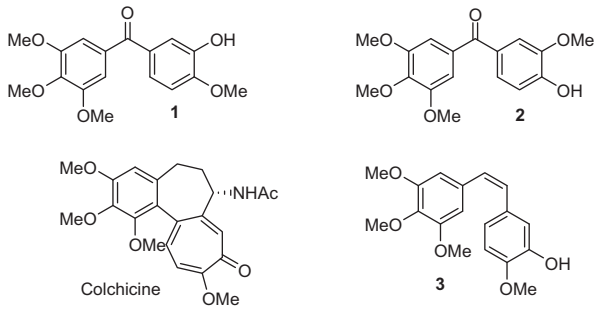

Figure 1. Structures of phenstatin, isophenstatin, CA-4 and cholchicine.

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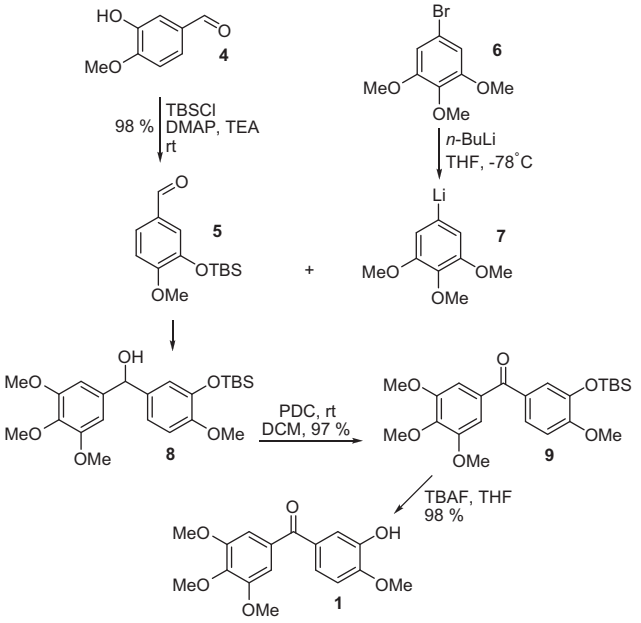
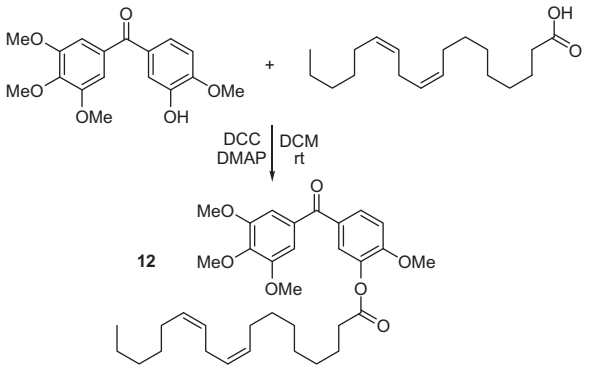


Scheme 1. Synthesis of phenstatin.



Scheme 2. Synthesis of phenstatin–linoleic acid conjugate.

Conjugates of oleic (OLA), linoleic (LA), and stearic acid (SA) have been prepared. Phenstatin was prepared in about 75% overall yield by the modified procedure outlined below.[10] Thus aryl bromide **6** was lithiated and reacted with aldehyde **5** to afford the alcohol **8**. Mild oxidation with pyridinium dichromate followed by desilylation afforded phenstatin **1** (Scheme 1). Isophenstatin was similarly prepared from vanillin.

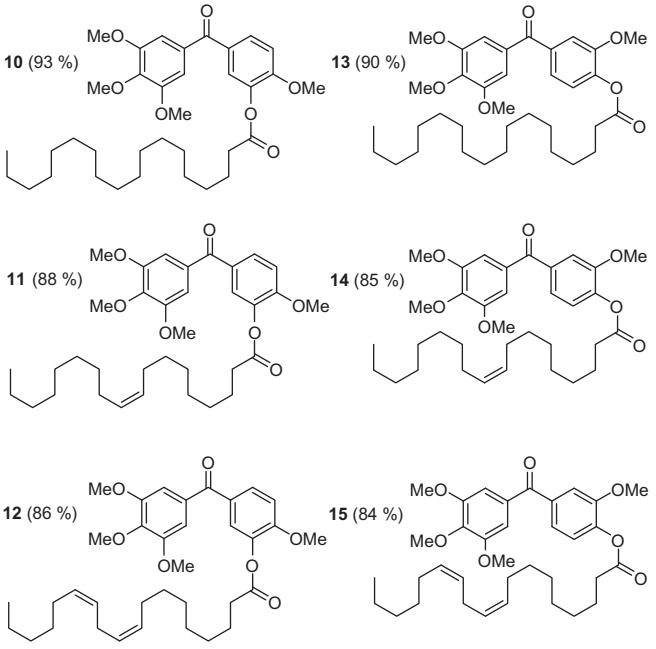


Figure 2. Fatty acid conjugates synthesized and isolated yields.

Conjugation of the fatty acids with phenstatin and isophenstatin to the hybrids of Figure 2, was accomplished at room temperature in DCM mediated by DCC and DMAP, Scheme 2.

IC_{50} values for compounds **10**–**15** (Fig. 2) in addition to values for phenstatin **1**, isophenstatin **2** and colchicine tested against the human epidermoid carcinoma cell line (KB-3-1), lung cancer cell line (H460), breast adenocarcinoma cell line (MCF-7), and human embryonic kidney cell line (HEK293) are summarized in Table 1.

Conjugates **12** and **15** (hybrids of linoleic acid) exhibited the greatest inhibitory effects against all four cell lines, while the conjugates of stearic acid (**10** and **13**) showed the least. For conjugate **12** average IC_{50} values of 0.163, 0.176, 0.181 and 0.210 μM against KB-3-1, H460, MCF-7 and HEK293 cells respectively were observed. Compound **15** gave average IC_{50} values of 0.547, 0.867, 0.745 and 0.903 μM, respectively, against the tested cell lines.

The data further reflects a consistent increase in anti-proliferative activity of the conjugate with increasing unsaturation in the fatty

Table 1
Summary of cytotoxicity data

Compounds	$IC_{50} \pm SD$[a] (μmol/L)			
	KB-3-1	H460	MCF-7	HEK293
Phenstatin + stearic acid **10**	2.777 ± 0.071	4.699 ± 0.736	3.353 ± 0.080	2.637 ± 0.356
Phenstatin + oleic acid **11**	0.786 ± 0.011	0.909 ± 0.030	0.943 ± 0.029	0.950 ± 0.004
Phenstatin + linoleic acid **12**	0.163 ± 0.032	0.176 ± 0.037	0.181 ± 0.062	0.210 ± 0.001
Iso-phenstatin + stearic acid **13**	16.932 ± 1.389	17.598 ± 6.940	26.914 ± 1.871	15.605 ± 0.649
Iso-phenstatin + oleic acid **14**	1.721 ± 0.288	2.195 ± 0.136	2.173 ± 0.464	2.363 ± 0.227
Iso-phenstatin + linoleic acid **15**	0.547 ± 0.148	0.867 ± 0.012	0.745 ± 0.052	0.903 ± 0.039
Phenstatin **1**	0.015 ± 0.004	0.020 ± 0.001	0.019 ± 0.004	0.023 ± 0.002
Iso-phenstatin **2**	0.146 ± 0.046	0.188 ± 0.004	0.199 ± 0.064	0.196 ± 0.008
Colchicine	0.018 ± 0.001	0.022 ± 0.002	0.019 ± 0.001	0.009 ± 0.001

The cytotoxic effect of phenstatin–PUFA hybrids on KB-3-1 (human epidermoid carcinoma cell line), H460 (lung cancer cell line), MCF-7 (breast adenocarcinoma cell line) and HEK293 (human embryonic kidney cell line).
Values in table are representative of at least three independent experiments performed in triplicate.
[a] IC_{50}: concentration that inhibited cell survival by 50% (means ± SD).

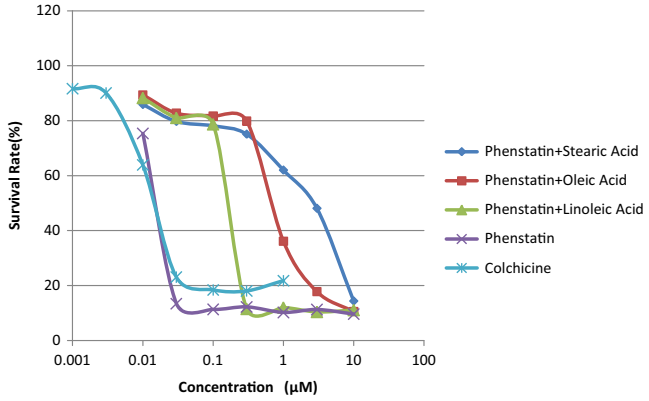


Figure 3. Inhibitory effects against KB-3-1 cells for compounds **1** and **10**–**12**.

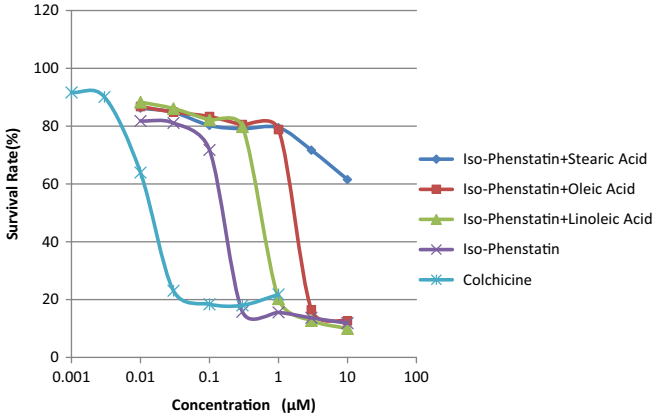


Figure 4. Inhibitory effects against KB-3-1 cells for compounds **2** and **13**–**15**.

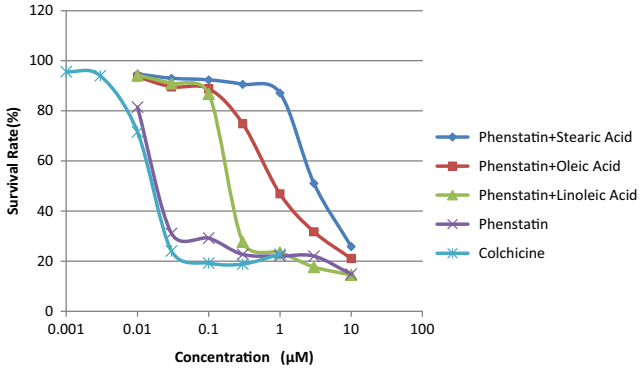


Figure 5. Inhibitory effects against MCF-7 cells for compounds **1** and **10**–**12**.

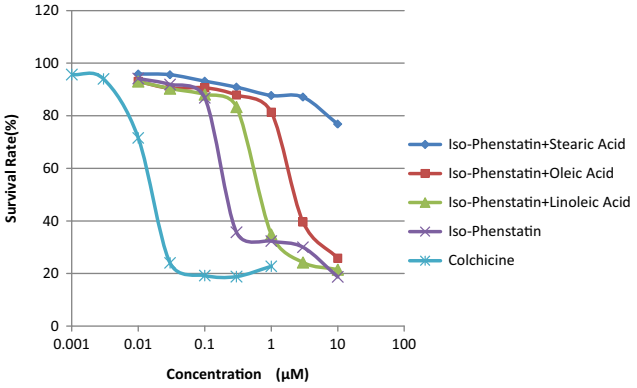


Figure 6. Inhibitory effects against MCF-7 cells for compounds **2** and **13**–**15**.

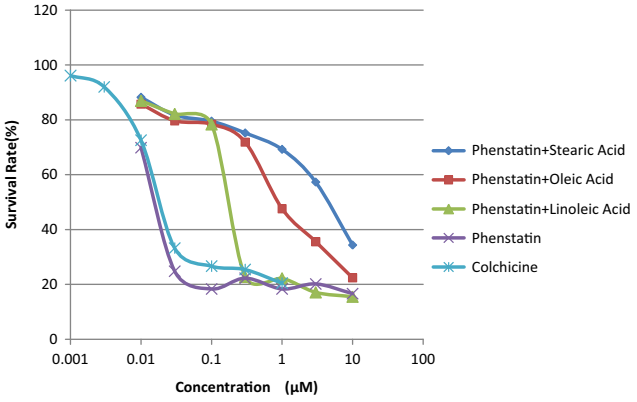


Figure 7. Inhibitory effects against H460 cells for compounds **1** and **10**–**12**.

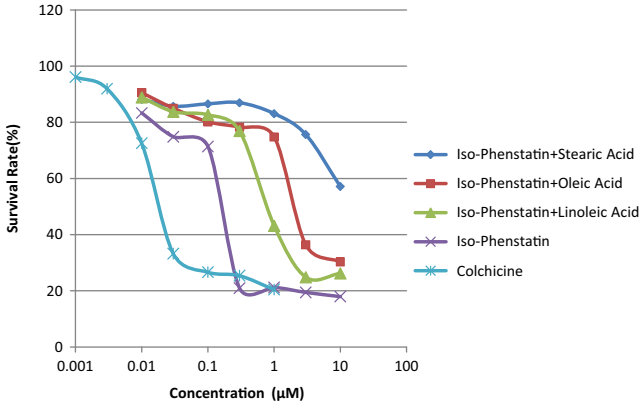


Figure 8. Inhibitory effects against H460 cells for compounds **2** and **13**–**15**.

acid structural component. At the 0.5 μM level, conjugate **12** was effective in decreasing the population of all four cell lines to about 20%. Compound **15** on the other hand exhibited similar cytotoxicity between 5.0 and 15.0 μM against the tested cell lines. These findings of diminished cytotoxicity with the isophenstatin systems and with conjugates of the more saturated fatty acids suggest that compound **13** is a good lead as an MDR reversal agent[11] and warrants further study.

Selected results of the activity screening against the four cell lines are depicted in Figures 3–10.

To further elucidate the effect of unsaturation in the fatty acid component, hybrids with up to six *cis* double bonds (DHA) are currently being investigated.

In summary, this correspondence reports the synthesis and evaluation of novel fatty acid conjugates as new antiproliferative compounds with levels of cytotoxicity directly related to the degree of unsaturation in the incorporated fatty acid. Since low cytotoxicity is a requirement for a drug reversal agent, the less active compounds will serve as leads in MDR reversal studies.[11]

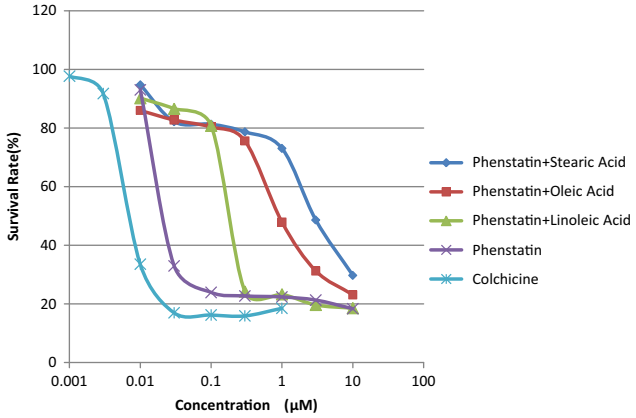


Figure 9. Inhibitory effects against HEK293 cells for compounds **1** and **10**–**12**.

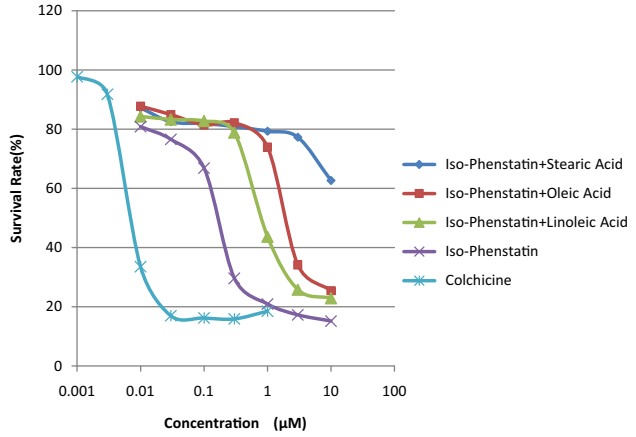


Figure 10. Inhibitory effects against HEK293 cells for compounds **2** and **13**–**15**.

Acknowledgments

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Supplementary data

Supplementary data (experimental procedure, selected NMR, IR and MS spectra of compounds **10**–**15**; testing data for all conjugates against the tested cell lines) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.07.025.

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