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Synthesis of 4′-ester analogs of resveratrol and their evaluation in malignant melanoma and pancreatic cell lines

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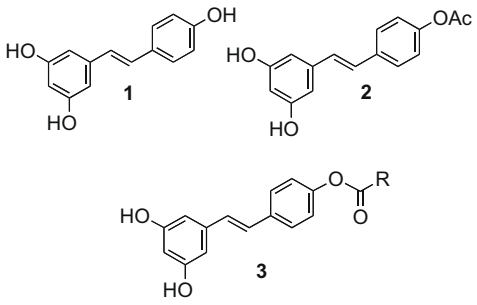
ABSTRACT

4′-Ester analogs of the disease preventative agent resveratrol were synthesized and evaluated for their potential as anti-melanoma and pancreatic cancer agents. A decarbonylative Heck coupling was used to assemble the protected stilbene core structure. The 4′-acetate and the palmitoate analogs demonstrated selective activity with DM443 and DM738 cells over normal NHDF cells.

Malignant melanoma is among the most lethal and chemo-resistant forms of cancer.[1] Resveratrol **1**, a dietary trihydroxy stilbene isolated from grapes and other sources known to possess significant disease preventative and general health promoting activity,[2] was found to bind and inhibit APE/Ref-I (12 μM, IC_{50}),[3] a protein involved in DNA repair that is up-regulated in melanoma cells. Previously we reported the synthesis of various 3, 5, and 4′-acetate ester analogs and fluoro variants of resveratrol together with their anticancer activity using HL-60 cell assays and longevity studies using yeast.[4] In this study the 4′-acetate analog **2** was found to be most potent at 17 μM compared to 22 μM for resveratrol. In addition, this analog was found to be more resistant to decomposition than resveratrol, remaining as a stable, pure white solid over extended time.[5] Longevity studies found that the 4′-acetate analog possesses enhanced metabolic stability with comparable activity to resveratrol by significantly extending the lifespan of yeast with reduced dosing required.[6] Resveratrol, as a polar triol stilbene has a short in vivo half life of 30 min and is rapidly cleared (30 min).[7] Based on previous findings, the synthesis and testing of extended 4′-ester variations **3** were pursued in an effort to discover new agents tailored to specific therapeutic applications including new types of cancer. A new set of 4′-resveratrol esters were produced and evaluated using DM443 and DM738 (Duke Melanoma) cell assays. This investigation also allowed for further exploration of the key decarbonylative Heck coupling reaction. The production of pro-drug analogs of this important dietary agent offers the possibility of new applications with compounds that possess enhanced stability and lipophilicity (Scheme 1).

The synthesis involves the original reported acid chloride intermediate with significant modifications and improvements to the key coupling conditions (Scheme 2). Inexpensive resorcylic acid **4** (3,5-dihydroxy benzoic acid) was converted to the bis-MOM (methoxymethyl ether) protected acid **5** now using potassium carbonate and MOMCl (3 equiv), prepared conveniently from bis-methoxymethane and hexanonyl chloride.[8] Previously sodium hydride was employed as base for this step.[4] The MOM-ester intermediate is hydrolyzed with aqueous NaOH followed by dilute HCl



Scheme 1. Resveratrol and 4′-ester analogs.

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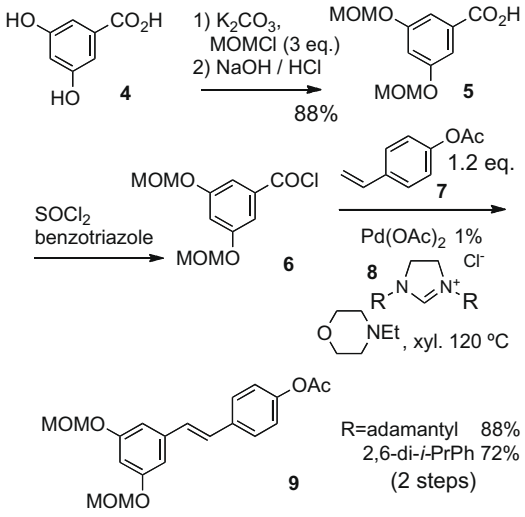


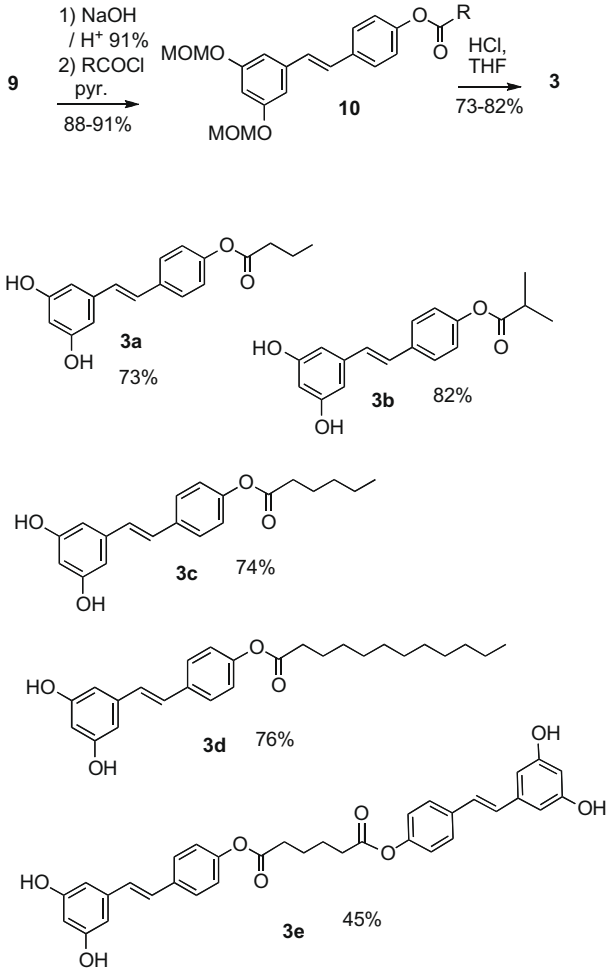
Scheme 2. Decarbonylative Heck coupling.

to access the neutral product **5**. Thionyl chloride with added benzotriazole was used to access acid chloride **6**, which was used without purification in the decarbonylative Heck coupling step with 4-acetoxystyrene **7**. Palladium acetate (1 mol %) with an N-heterocyclic carbene (NHC) ligand **8** (1 mol %) were employed with *N*-ethylmorpholine as base in xylenes at 120 °C. The acid chloride **6** and acetoxystyrene **7** were used at 1:1.25 stoichiometry. Previous results using *N,N*-bis-2,6-diisopropylphenyl-NHC (IPr) gave the product stilbene **9** in 72% overall yield.[4] The bulky commercially available *N,N*-bis-adamantyl-NHC ligand **8** (R = adamantyl) now produced the protected product in 88% yield for the two step sequence. Steric constraints of NHC ligands have been previously reported that improve related palladium catalyzed coupling reactions.[9]

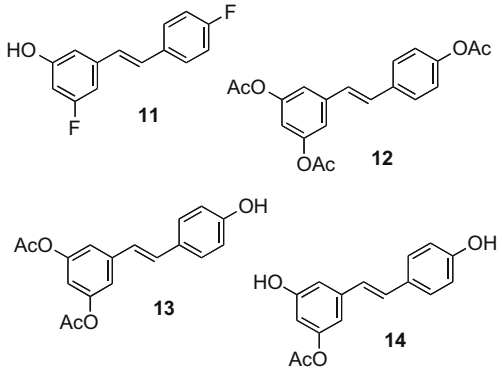
The acetate **9** was removed with base and the resulting 4′-phenol was acylated with various acid chlorides (Scheme 3). Sodium hydroxide followed by acidification gave the phenol in 91% yield and the acylations were efficiently performed individually with the corresponding acid chlorides with added pyridine to access the 4′-esters **10** (88–91%). Finally, the desired 4′-resveratrol esters **3a**–**e** were obtained by deprotecting the 3,5-bis-MOM **10** intermediates using anhydrous HCl (2.0 M) in ether.[10] Previous conditions for selective bis-MOM removal employed trimethylsilyl iodide, generated in situ from TMSCl and NaI.[4] These conditions were found to be problematic for larger scale production of 4′-acetate **2** and with the new 4′-esters. The new HCl approach for MOM removal requires extended time (36 h), however the product is now produced in reliable yield. Only the adipate dimer ester **3e** was obtained in lower yield, 45%. All the others, the *n*-butyrate, *i*-butyrate, *n*-hexanoate, and palmitoyl products were produced in moderate to good yield (73–82%).

In addition to the new 4′-esters, four other resveratrol analogs were included in the melanoma cell assays.[4] 3,4′-Difluoro-resveratrol **11**, triacetate **12**, 3,5-diacetate **13**, and 3-acetate-resveratrol **14**, reported previously (Scheme 4), were included to provide for a broad structure–activity profile in the cancer cell inhibition investigations.

With the set of new resveratrol analogs in hand, cell assays were performed to assess in vivo potential. DM443 and DM738, two previously characterized melanoma cell lines derived from human tumor tissue, were chosen for this study due to their chemoresistance properties to melphalan (LPAM) and temozolomide (TMZ) respectively.[11] DM443 and DM738 were plated and resveratrol or its analogs were then added and cells were incubated for 24, 48, or 72 h. Cell viability was then quantified using a colorimetric assay. Cell lines were treated with resveratrol and ten analogs of resveratrol at 10, 25, and 50 μM and evaluated at multiple time points. In comparing efficacies at the 50 μM dose, analogs **3b**, **3c**, **3a**, and **11** are shown to be significantly more cytotoxic than resveratrol at all time points in both cell lines (all p's <0.025), analog **3d** does not show any effect at any time point in either cell line ($0.34 < p < 0.83$), and analogs **2**, **3e**, **12**, **13** and **14** have varying efficacies between these two groups (Fig. 1). Analog **2** is shown to be



Scheme 3. Synthesis of 4′-resveratrol esters.



Scheme 4. Additional resveratrol-analogs.

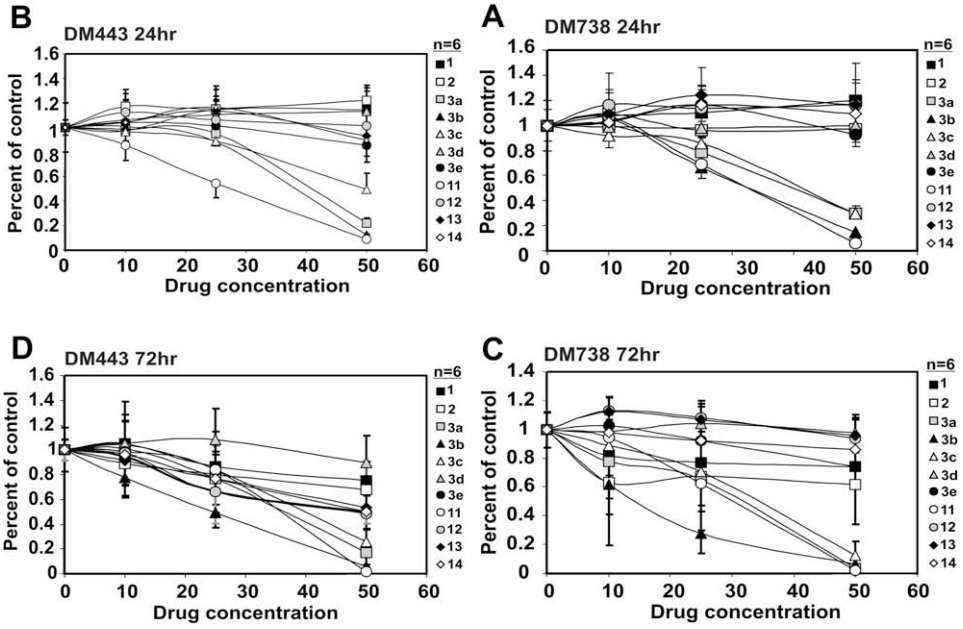


Figure 1. DM738 and DM443 treated with resveratrol and analogs for 24 (A and B) and 72 h (C and D) respectively. Data are expressed as mean ± SEM, $n = 6$.

equally effective as native resveratrol at every time point and dose in both cell lines.

Normal human dermal fibroblast (NHDF) cells were treated with resveratrol and analogs for 72 h at 50 μM to evaluate if the analogs were also selectively cytotoxic to malignant cells. We found that analogs **2** and **3e** relatively spared NHDF cells compared to their cytotoxic effect in melanoma cell lines (both p's <0.0001) (Fig. 2).

In order to evaluate whether the cytotoxic effects observed in melanoma cell lines would translate to other cancer types, a malignant line of pancreatic cells (Panc-1) was treated with either resveratrol, analog **2** or analog **12** at 50 μM for 72 h. Gemcitabine, a standard treatment of advanced pancreatic cancer,[12] was also added at 0, 1, or 10 μg/mL. Resveratrol and both analogs manifested significant cytotoxicity compared to both vehicle alone (p <0.0001) and to treatment with gemcitabine alone at all doses tested (p's <0.0001) (Fig. 3). We also found that the addition of gemcitabine did not enhance the cytotoxic response of resveratrol or its analogs (all p's > 0.05).

In summary, various 4′-resveratrol esters have been produced using a decarbonylative Heck coupling and a structure–activity profile has been produced from melanoma cell assays. Further studies with additional cells lines and in vivo investigations are needed to further establish the potential of compounds of this type.

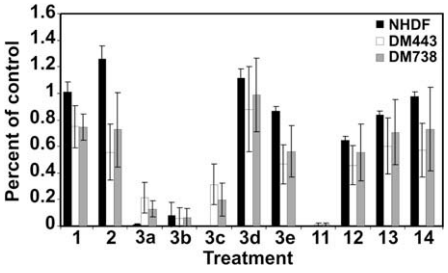


Figure 2. Cell lines DM738, DM443, and NHDF were treated with resveratrol or analog at 50 μM for 72 h. Data are expressed as mean ± SEM; $n = 6$.

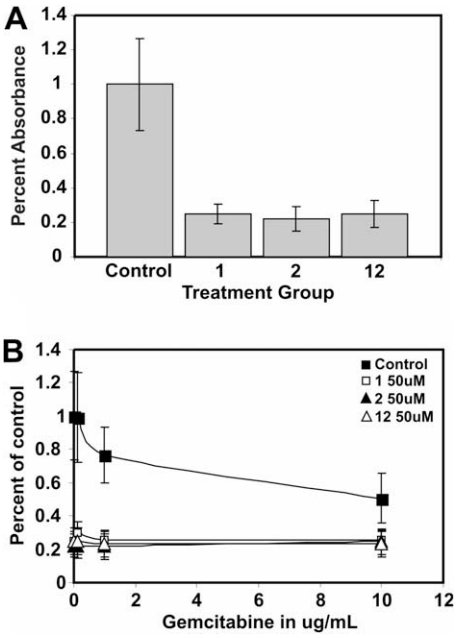


Figure 3. (A) Panc-1 treated with resveratrol or analog for 72 h. (B) Panc-1 treated with resveratrol or analog ± gemcitabine, or gemcitabine alone for 72 h. Data are expressed as mean ± SEM, $n = 6$.

Acknowledgments

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Supplementary data

Supplementary data (experimental procedures, characterization, NMR spectra, and cell assay methods) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.12.006.

References and notes

1. Eggermont, A.; Kirkwood, J. *Eur. J. Cancer* **2004**, *40*, 1825.
2. (a) Signorelli, P.; Ghidoni, R. *J. Nutr. Biochem.* **2005**, *16*, 449; (b) Garesveratrolin, S.; Ollinger, K.; Dabrosin, C. *Cancer Lett.* **2006**, *231*, 113.
3. Yang, S.; Irani, K.; Heffron, S. E.; Jirnak, F.; Meyskens, F. L., Jr. *Mol. Cancer Ther.* **2005**, *4*, 1923.
4. Andrus, M. B.; Liu, J. *Tetrahedron Lett.* **2006**, *47*, 5811.
5. Wong, Y.; Liu, J.; Andrus, M. B. unpublished results.
6. Yang, H.; Baur, J. A.; Chen, A.; Miller, C.; Sinclair, D. A. *Aging Cell* **2007**, *6*, 35.
7. (a) Kuhnle, G.; Spencer, J. P.; Chowrimootoo, G.; Schroeter, H.; Debnam, E. S.; Srai, S. K. *Biochem. Biophys. Res. Commun.* **2000**, *272*, 212; (b) Piver, B.; Fer, M.; Vitrac, X.; Merillon, J.-M.; Dreano, Y.; Berthou, F.; Lucas, D. *Biochem. Pharmacol.* **2004**, *68*, 773.
8. Linderman, R. J.; Jaber, M.; Griedel, B. D. *J. Org. Chem.* **1994**, *59*, 6499.
9. (a) Hadei, N.; Kantchev, E. A. B.; O'Brien, C. J.; Organ, M. G. *J. Org. Chem.* **2005**, *70*, 8503; (b) Scott, N. M.; Nolan, S. P. *Eur. J. Inorg. Chem.* **2005**, *10*, 1815; (c) Hadei, N.; Kantchev, E. A. B.; O'Brien, C. J.; Organ, M. G. *Org. Lett.* **2005**, *7*, 1991; (d) Song, C.; Ma, Y.; Chai, Q.; Ma, C.; Jiang, W.; Andrus, M. B. *Tetrahedron* **2005**, *61*, 7438; (e) Ma, Y.; Song, C.; Jiang, W.; Wu, Q.; Wang, Y.; Liu, X.; Andrus, M. B. *Org. Lett.* **2003**, *5*, 3317; (f) Altenhoff, G.; Goddard, R.; Lehmann, C. W.; Glorius, F. *Angew. Chem., Int. Ed.* **2003**, *42*, 3690.
10. Ding, H.; Zhang, C.; Wu, X.; Yang, C.; Zhang, X.; Ding, J.; Xie, Y. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4799.
11. (a) Yoshimoto, Y.; Augustine, C. K.; Yoo, J. S.; Zipfel, P. A.; Selim, M. A.; Pruitt, S. K.; Friedman, H. S.; Ali-Osman, F.; Tyler, D. S. *Mol. Cancer Ther.* **2007**, *6*, 1492; (b) Ueno, T.; Ko, S. H.; Grubbs, E.; Yoshimoto, Y.; Augustine, C.; Abdel-Wahab, Z.; Cheng, T.-Y.; Abdel-Wahab, O. I.; Pruitt, S. K.; Friedman, H. S.; Tyler, D. S. *Mol. Cancer Ther.* **2006**, *5*, 732.
12. Burris, H. A., III; Moore, M. J.; Andersen, J. *J. Clin. Oncol.* **1997**, *15*, 2403.