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Decoding Red Wine: Biological Activities of *Vitis vinifera* Phytochemicals in Mammalian Cells

The Cellular and Molecular Biology of Longevity



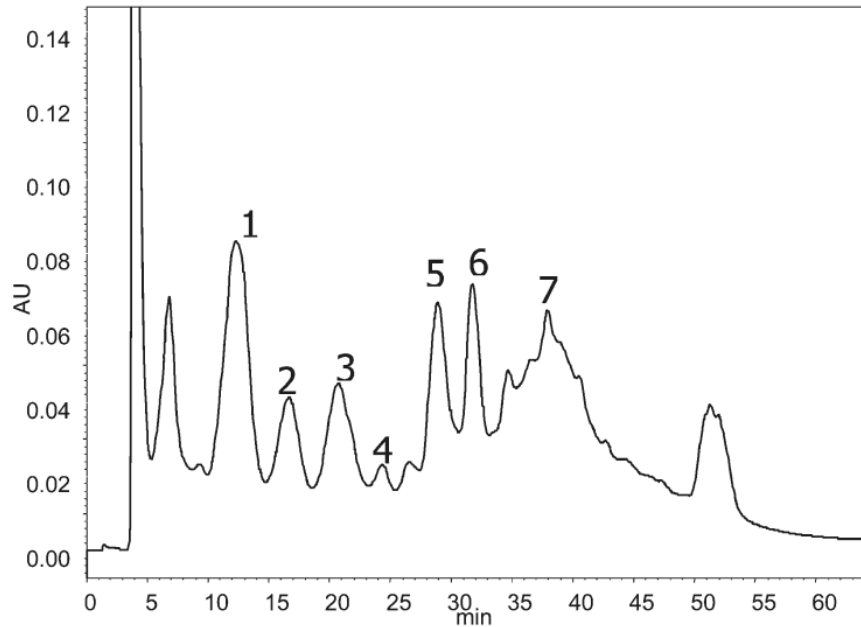
- Identification of pro-longevity traits
 - Cellular stress resistance
 - Tumour suppression
- Manipulation of pro-longevity genes using small molecule effectors
 - Anti-cancer activities
 - Anti-cell death activities

The Cellular and Molecular Biology of Longevity

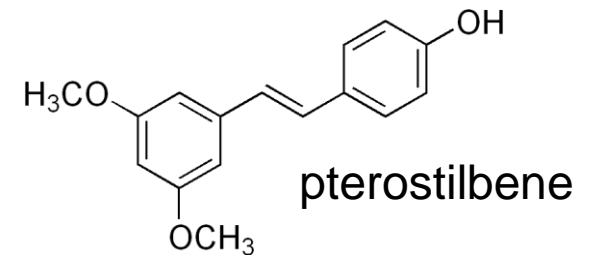
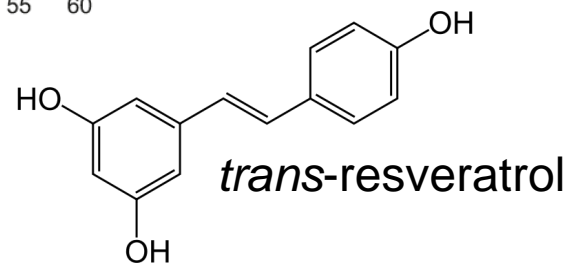
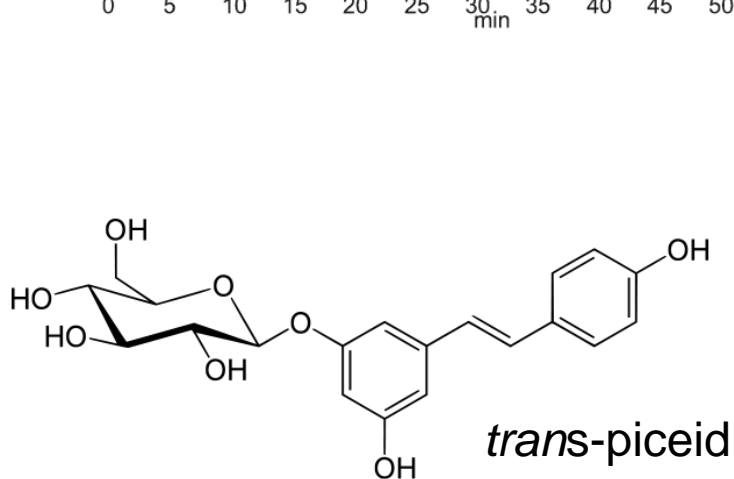


- Identification of pro-longevity traits
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Decoding Red Wine



Ellen Robb
PhD candidate



Phytoalexins



Plasmopara viticola (Downy Mildew) on *Vitis vinifera* leaf

Vitis vinifera responds to fungal infection by producing ‘phytoalexins’: resveratrol, pterostilbene, piceid, and others

Phytoalexins inhibit growth of parasitic fungi

Phytoalexins also inhibit growth of human cells

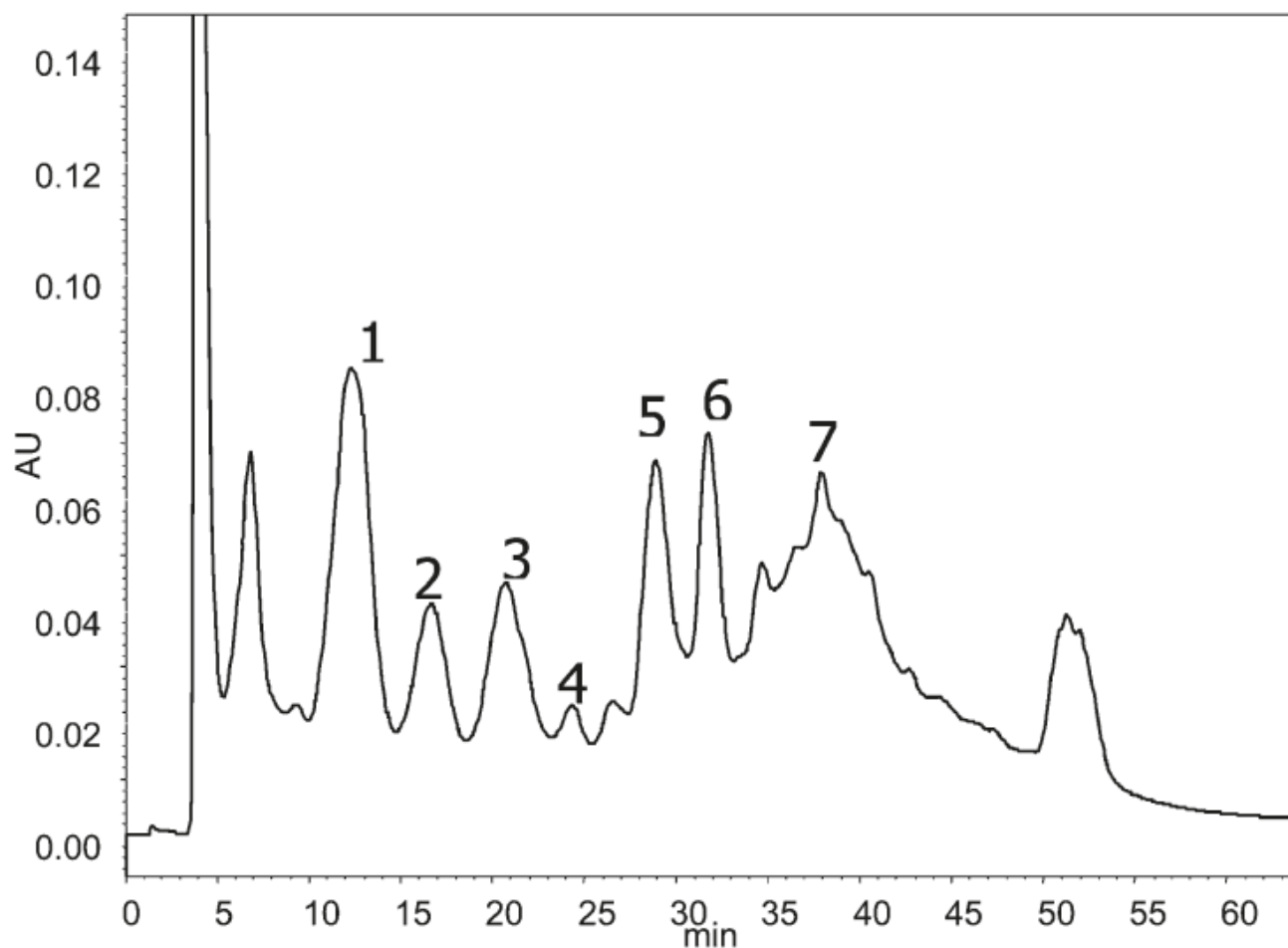
Stimulation of *Vitis vinifera* Phytoalexin Production by Fungal Infection



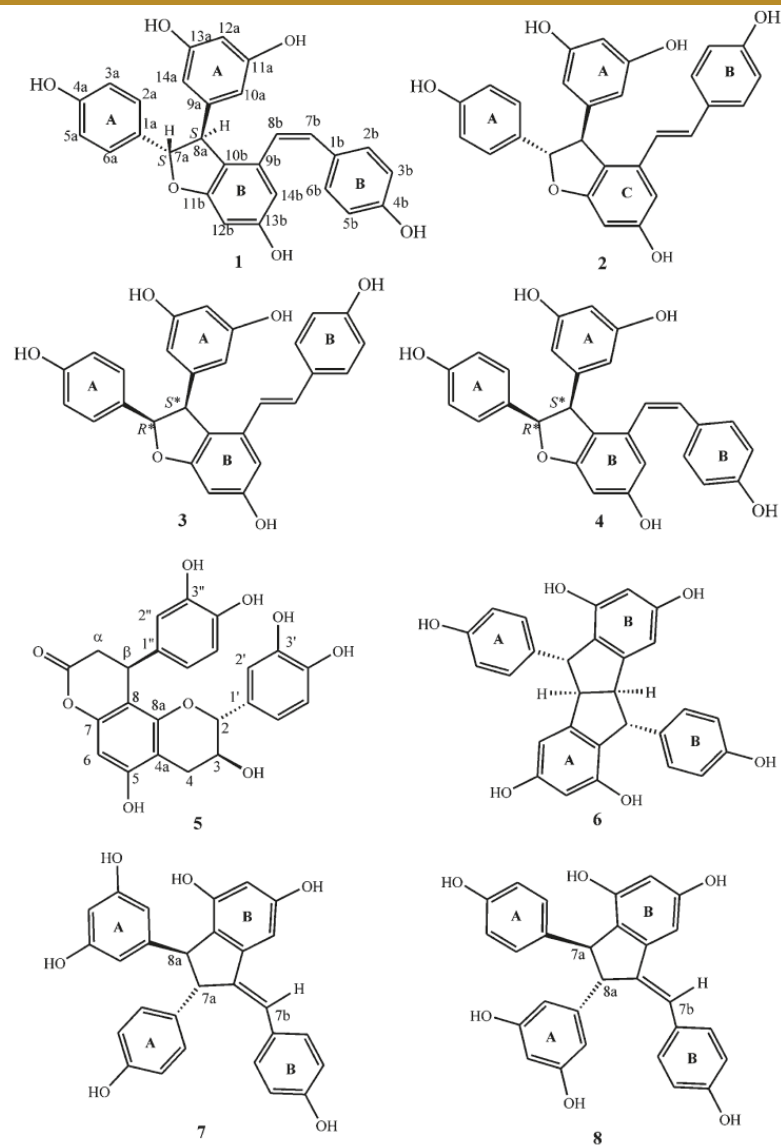
	0		2 dpi		6 dpi	
	Average	SD	Average	SD	Average	SD
<i>Trans</i> -piceid	ND	ND	ND	ND	2.7	0.4
<i>Trans</i> -resveratrol	ND	ND	ND	ND	5.7	3.6
Pallidol (ampelopsin H)	ND	ND	ND	ND	30.22	16.04
Ampelopsin D + quadrangularin A (1:1)	ND	ND	ND	ND	8.0	4.2
Isohopeaphenol	ND	ND	ND	ND	147.0	70.6
Ampelopsin H + vaticanol-C like isomer (ampelopsin H)	ND	ND	ND	ND	35.80	19.4
(+)- <i>E</i> - ϵ -viniferin	ND	ND	ND	ND	13.1	6.9
<i>Z</i> + <i>E</i> -miyabenol C (<i>Z</i> -miyabenol C)	ND	ND	ND	ND	10.5	2.9
<i>Z</i> + <i>E</i> - ω -viniferin (<i>E</i> - ω -viniferin)	ND	ND	ND	ND	9.5	2.7
α -viniferin	ND	ND	ND	ND	15.5	7.0
<i>E</i> - <i>cis</i> -miyabenol C	ND	ND	ND	ND	16.9	20.4
<i>Trans</i> -pterostilbene (<i>trans</i> -resveratrol)	ND	ND	ND	ND	6.0	4.0

ND, not detected.

Vitis vinifera Phytoalexins can be Isolated, Identified, and Characterized



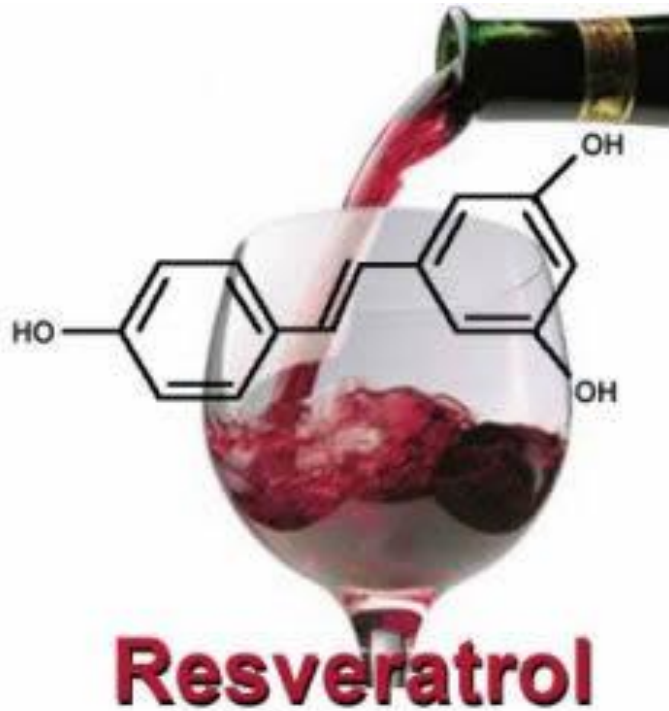
Structures of Viniferan Phytoalexins



Vinifera phytoalexins include resveratrol, analogues of resveratrol, and a number of resveratrol oligomers

Figure 4. Structures of stilbenoid dimers in grapevine leaves (*Z*-ε-viniferin (1), *E*-ε-viniferin (2), *E*-ω-viniferin (3), *Z*-ω-viniferin (4), pallidol (6), *E*-ampelopsin D (7), *E*-quadrangularin A (8)) and of a condensation product between (+)-catechin and *trans*-cafeic acid (5).

Resveratrol is Famous



Anti-Aging Breakthrough!
Key Ingredient Featured On
60 Minutes, FOX News & More.

60 MINUTES | FOX NEWS

ResVer XP

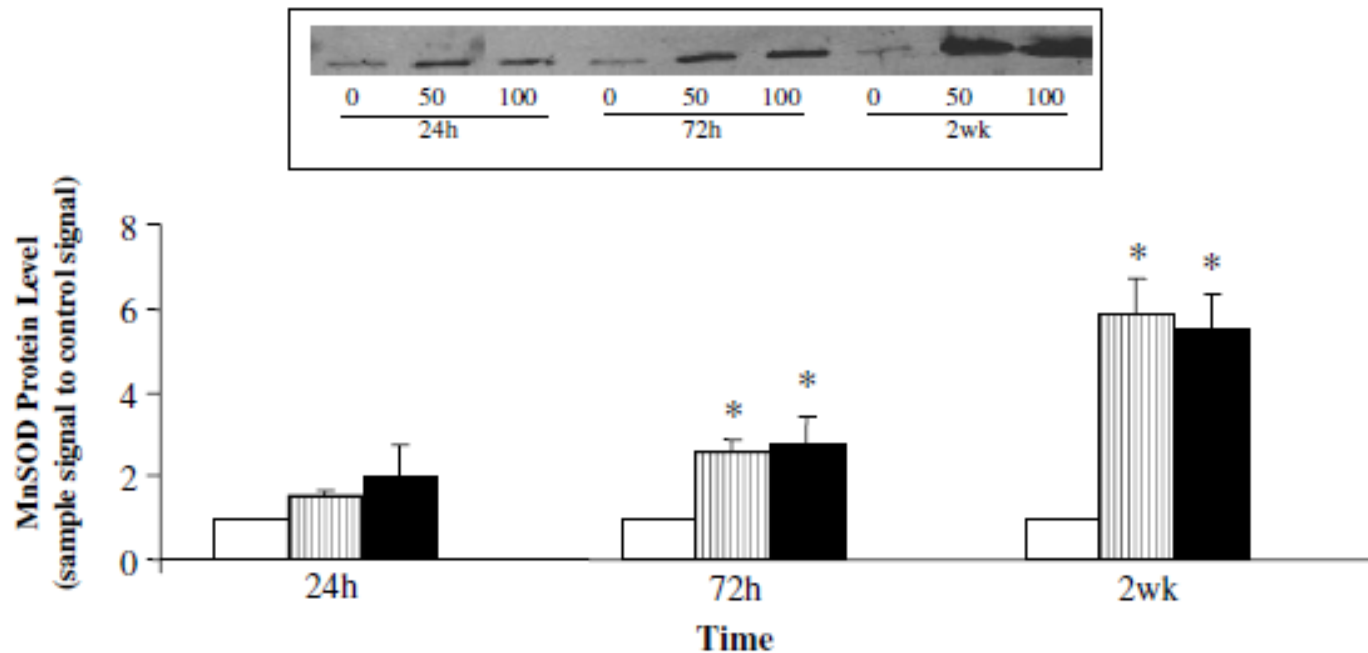
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Resveratrol: Mechanisms of Action in Mammalian Cells



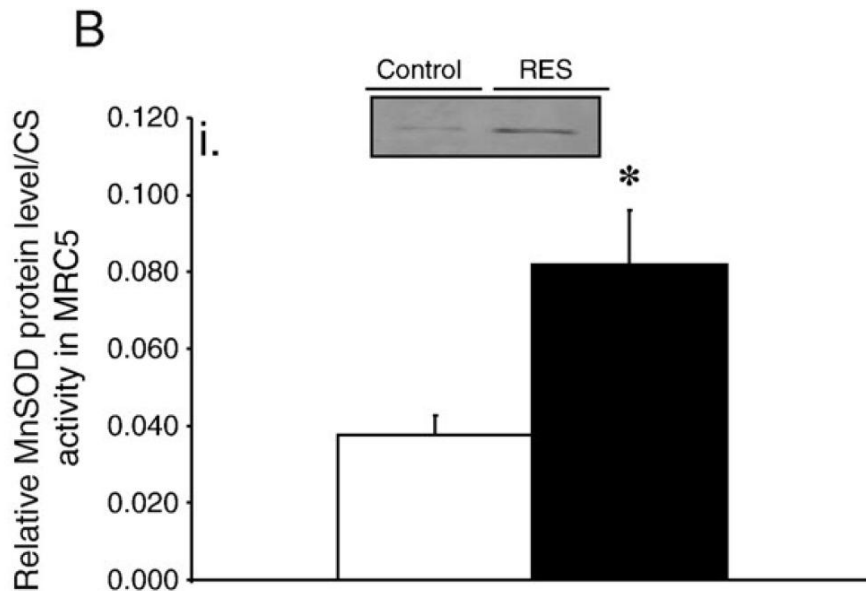
Resveratrol induces MnSOD expression in human cells



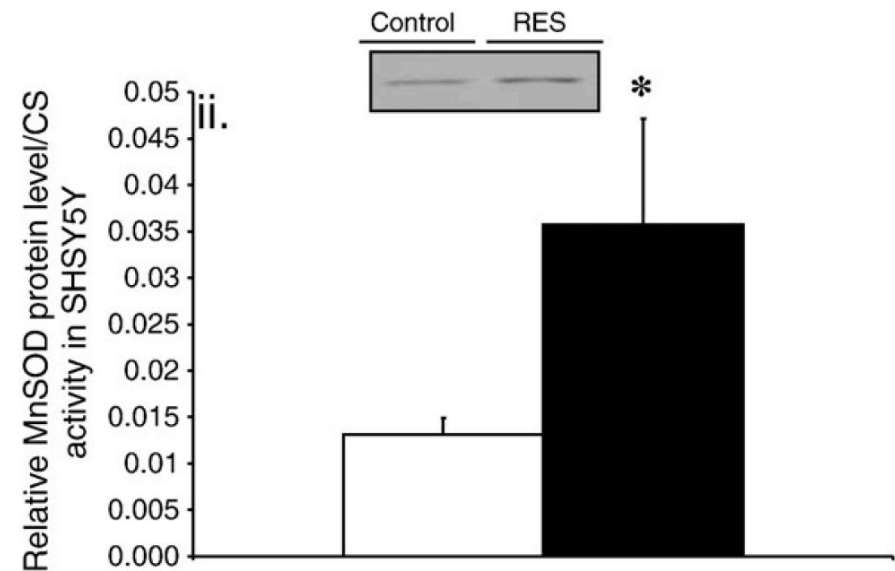
Resveratrol induces manganese superoxide dismutase expression in a wide variety of human cell types



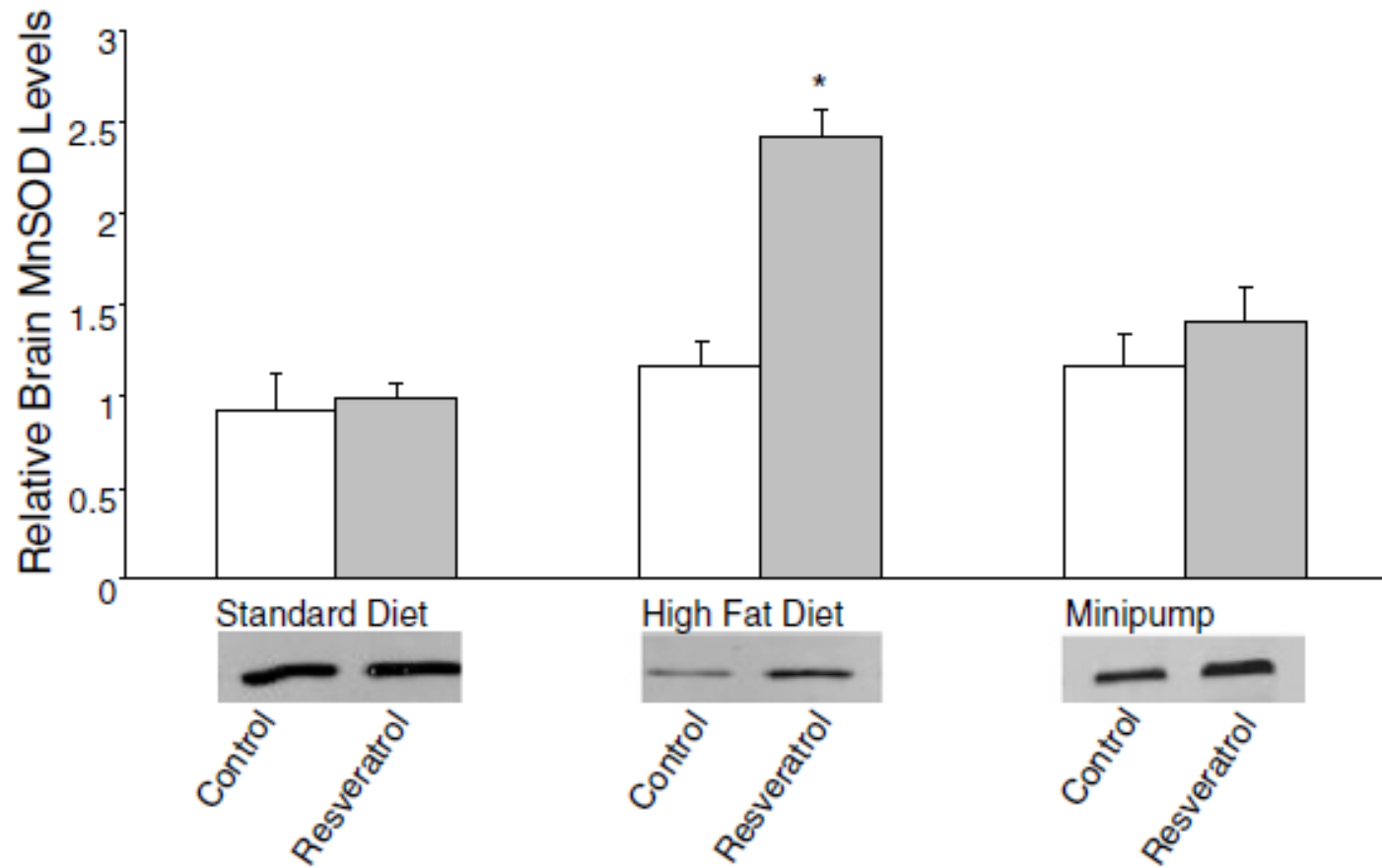
Human lung cells



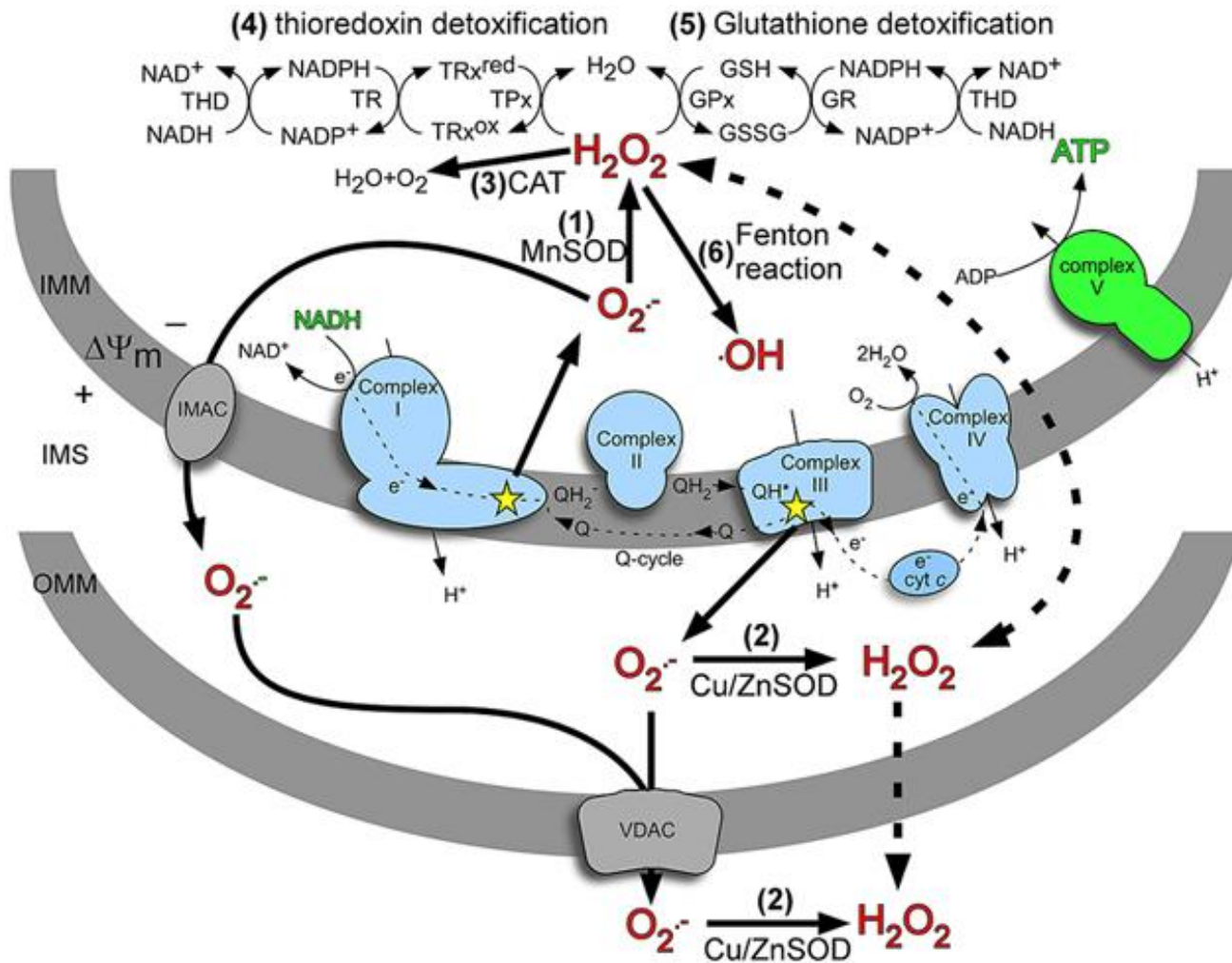
Human brain cells



Resveratrol provided as a diet supplement increases MnSOD expression in brain



What is MnSOD?



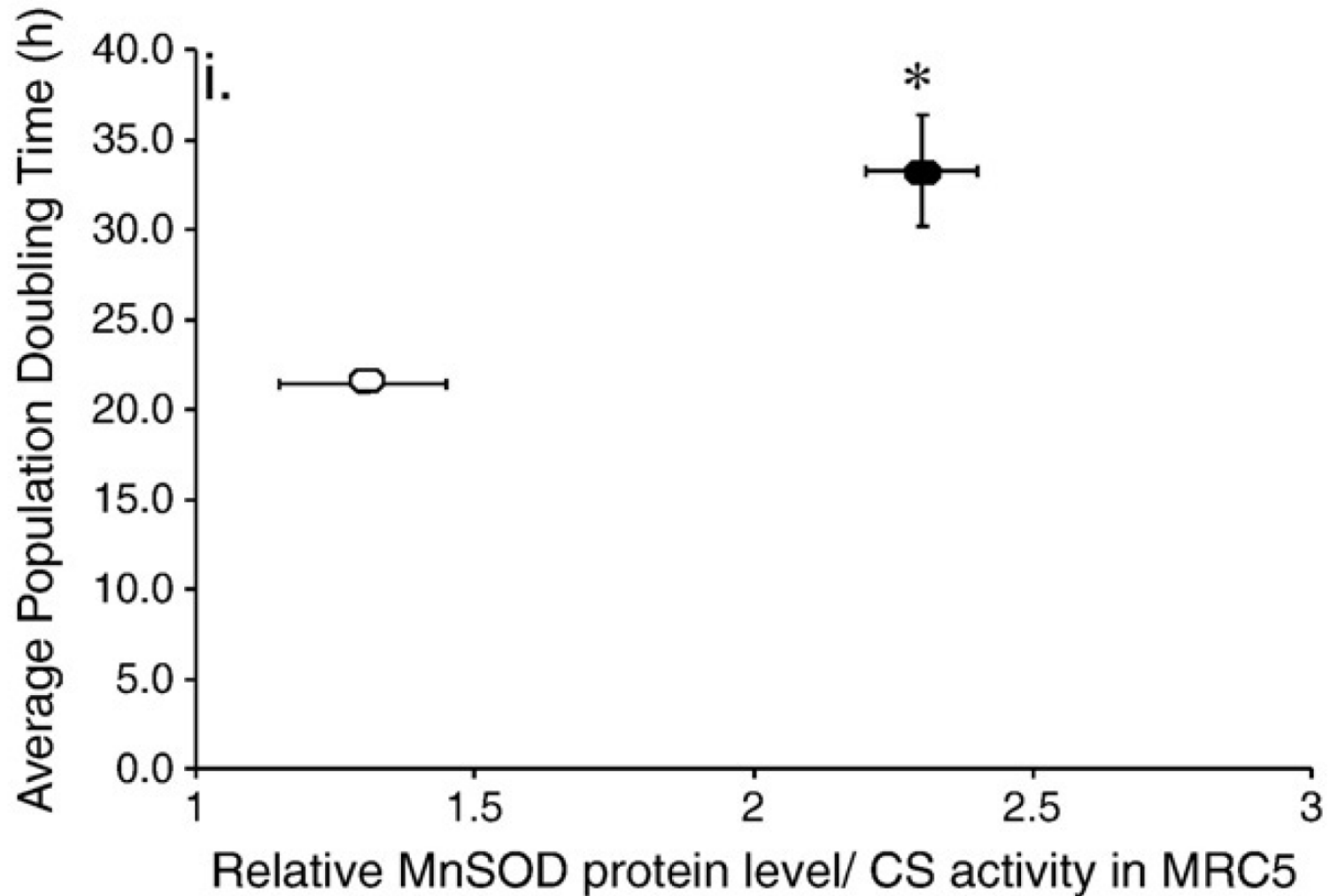
MnSOD: a
mitochondrial
superoxide
dismutase

MnSOD is a Cell Cycle Regulator

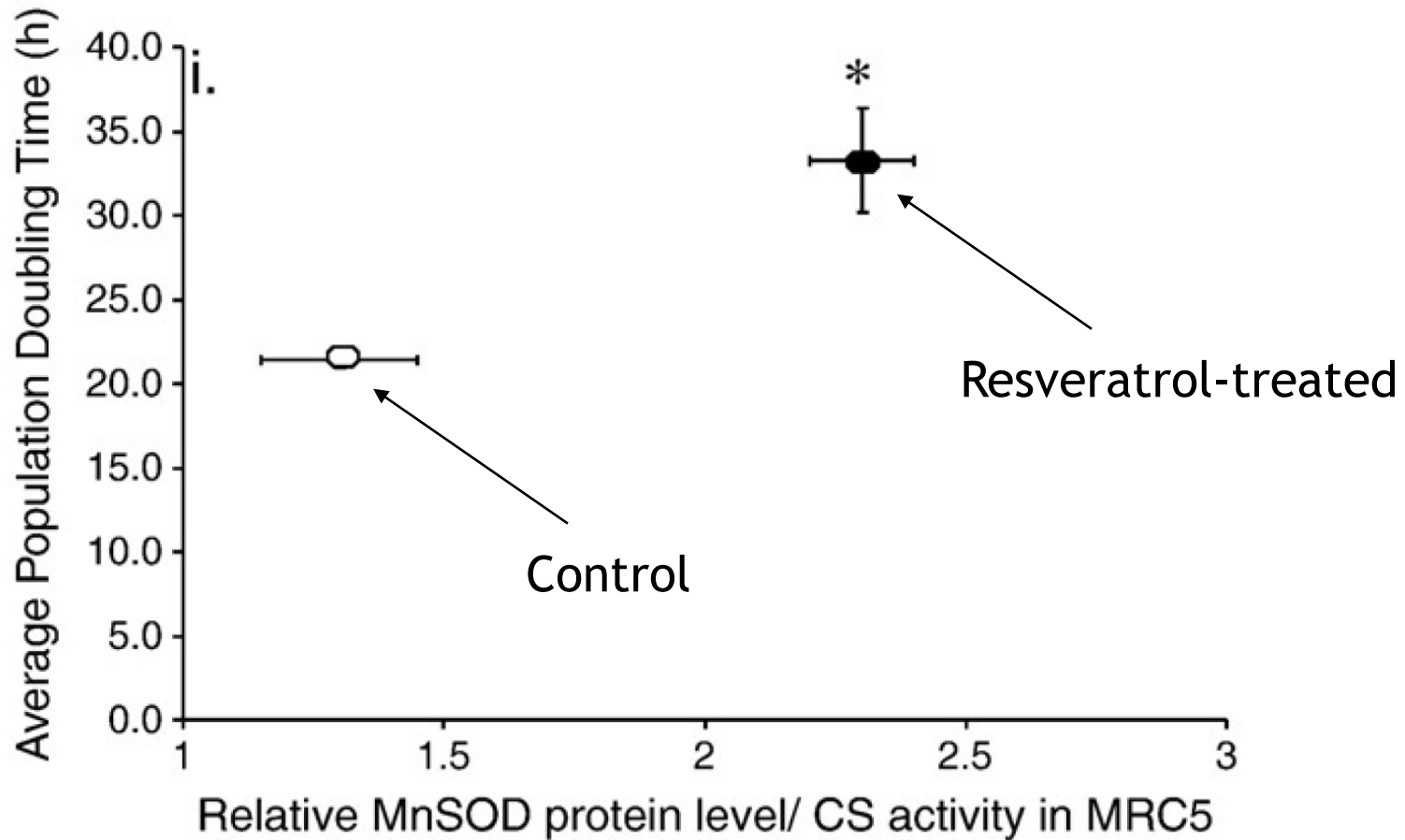


- Low MnSOD levels/activities → High proliferative growth rates
- High MnSOD levels/activities → Low proliferative growth rates

Resveratrol Effects on MnSOD and Growth in Human Cells



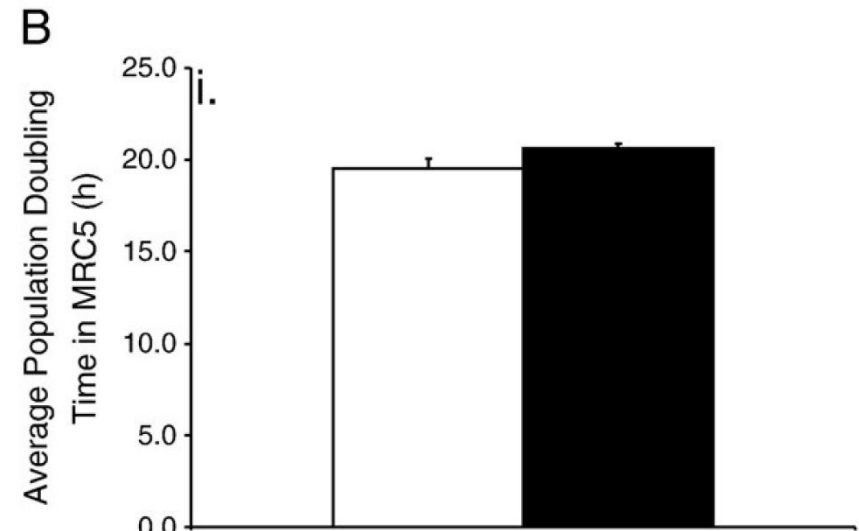
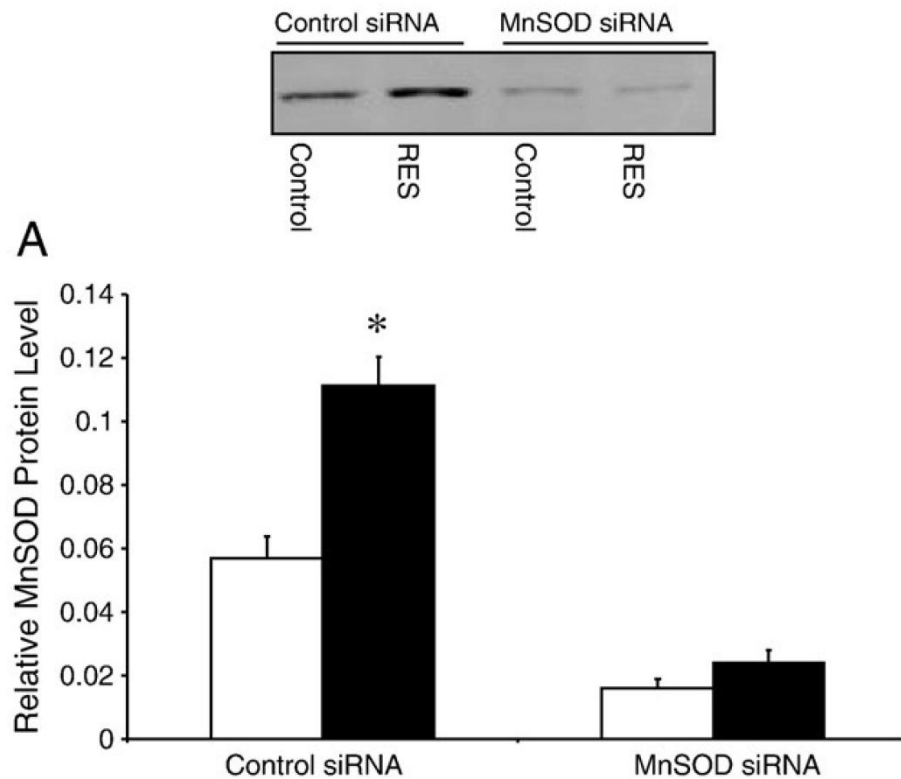
Resveratrol Effects on MnSOD and Growth in Human Cells



Resveratrol, MnSOD and proliferative growth



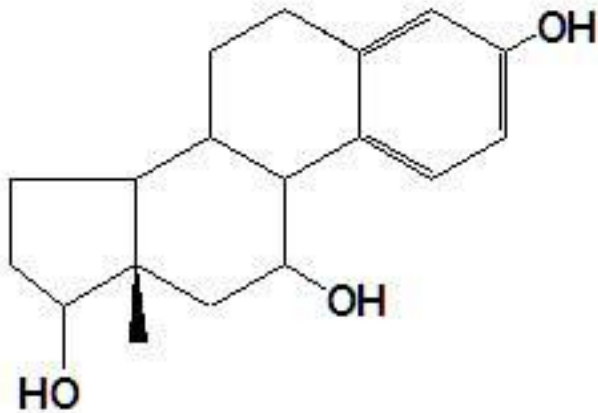
Induction of MnSOD is required for resveratrol to slow growth



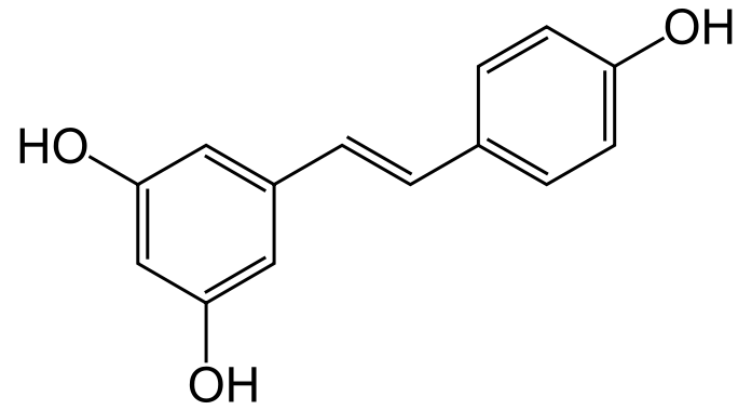
Resveratrol is a Phytoestrogen



Similar molecular structures of estrogens and resveratrol

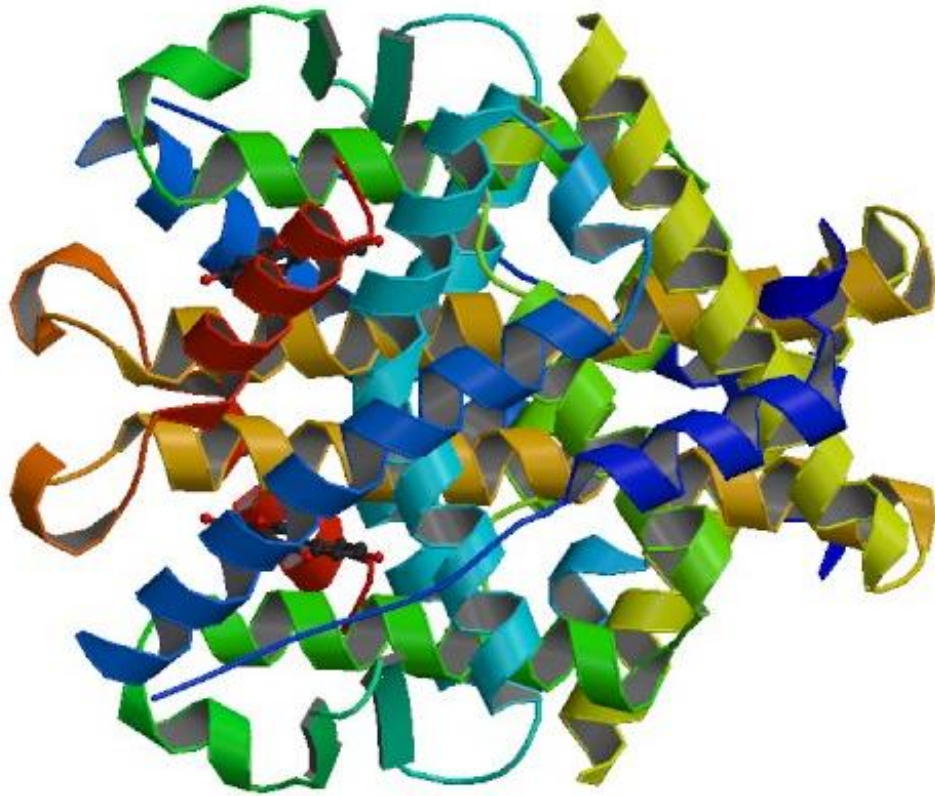


17β-Estradiol



trans-Resveratrol

Phytoalexins as Phytoestrogens



Resveratrol and related molecules are predicted to bind to ERβ

Estrogen receptor β (ERβ)
complexed to a related
phytoestrogen (genistein)

Estrogen Receptor β (ER β)

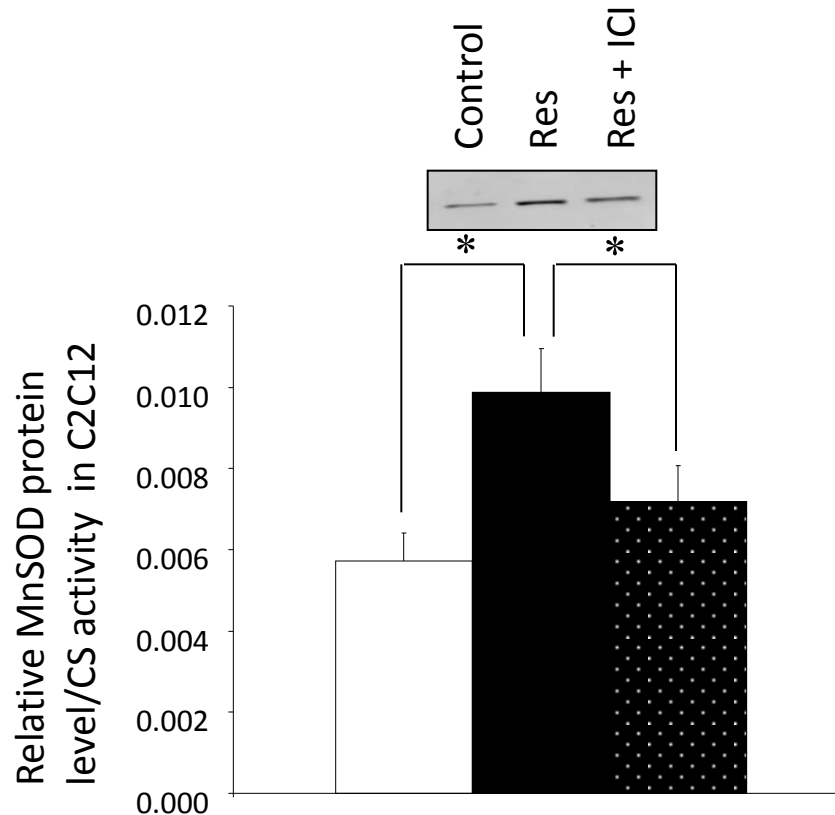


- A more recently discovered estrogen receptor (compared to ER α)
- Widely expressed (brain, skin, GI tract, many cancers)
- Associated with anti-proliferative effects

ER β and MnSOD



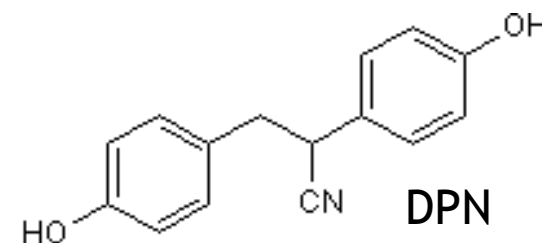
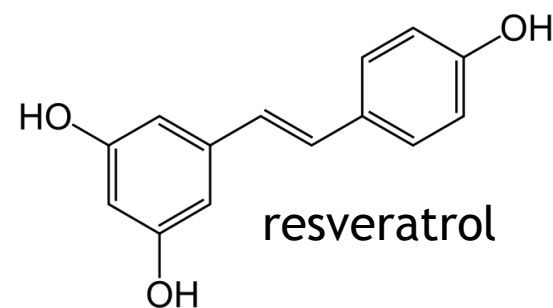
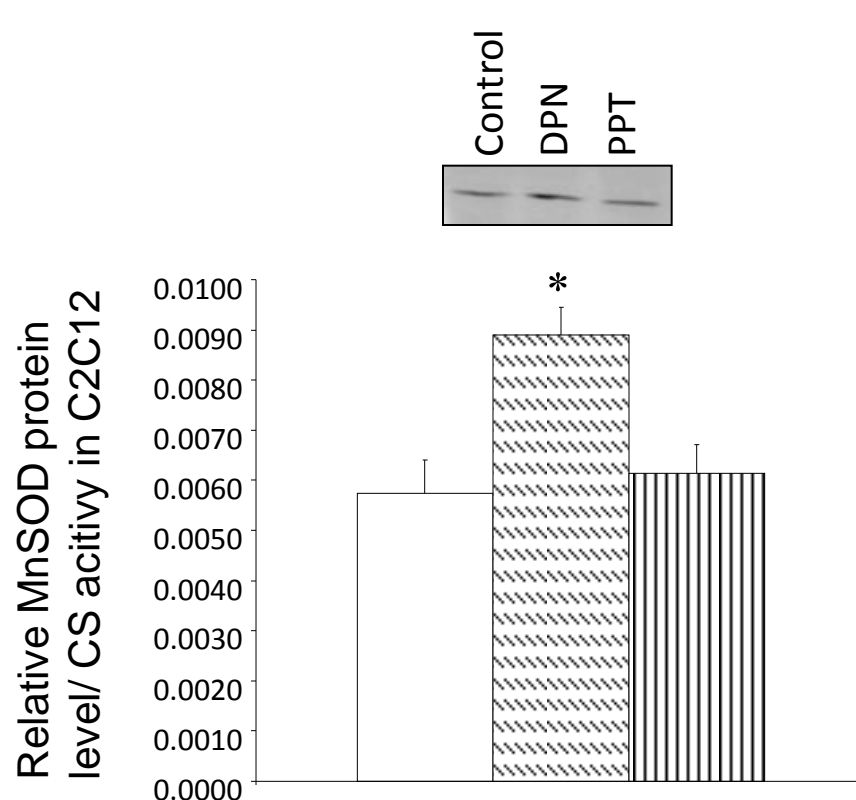
ER β antagonist ICI182780 prevents the resveratrol-mediated induction of MnSOD



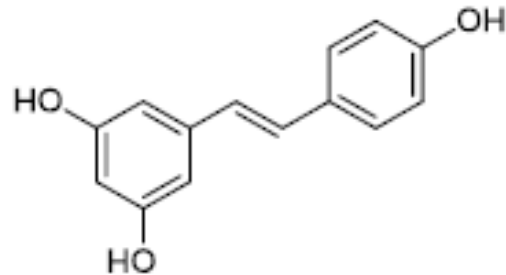
ER β and MnSOD



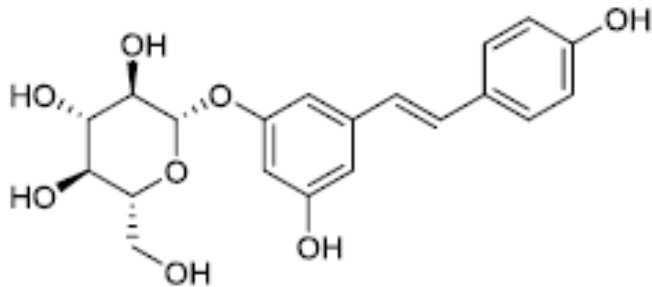
ER β -specific agonist DPN (but not ER α -specific agonist PPT) similarly induces MnSOD expression



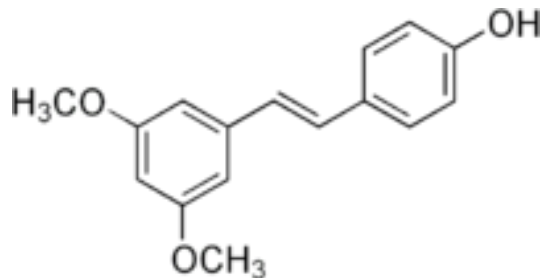
Other Vinifera Phytoalexins are Structurally Similar to Resveratrol....Do They Have Similar Effects?



Resveratrol

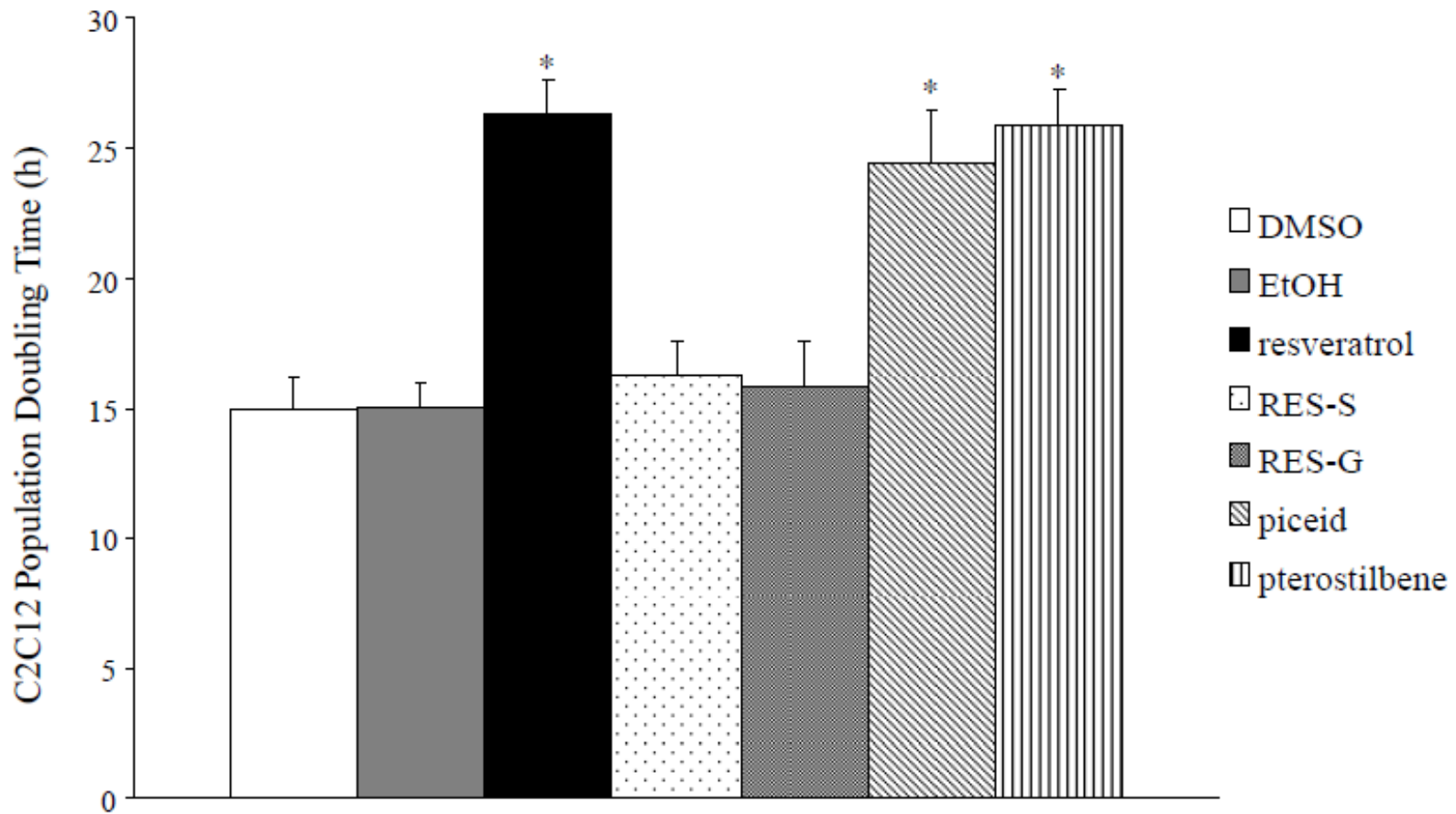


Piceid

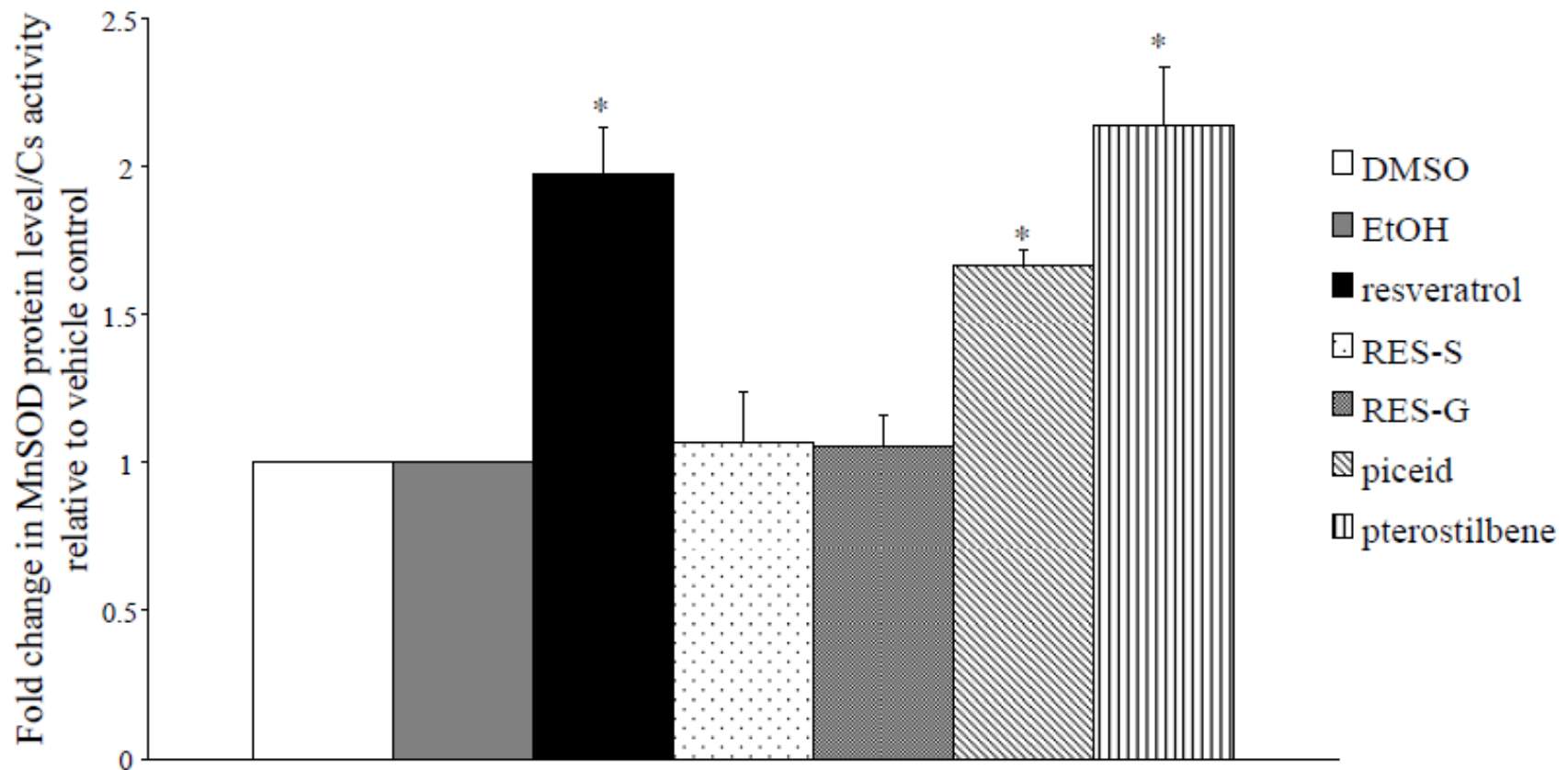


Pterostilbene

Other Vinifera Phytoalexins are Structurally Similar to Resveratrol....Do They Have Similar Effects?



Other Vinifera Phytoalexins are Structurally Similar to Resveratrol....Do They Have Similar Effects?

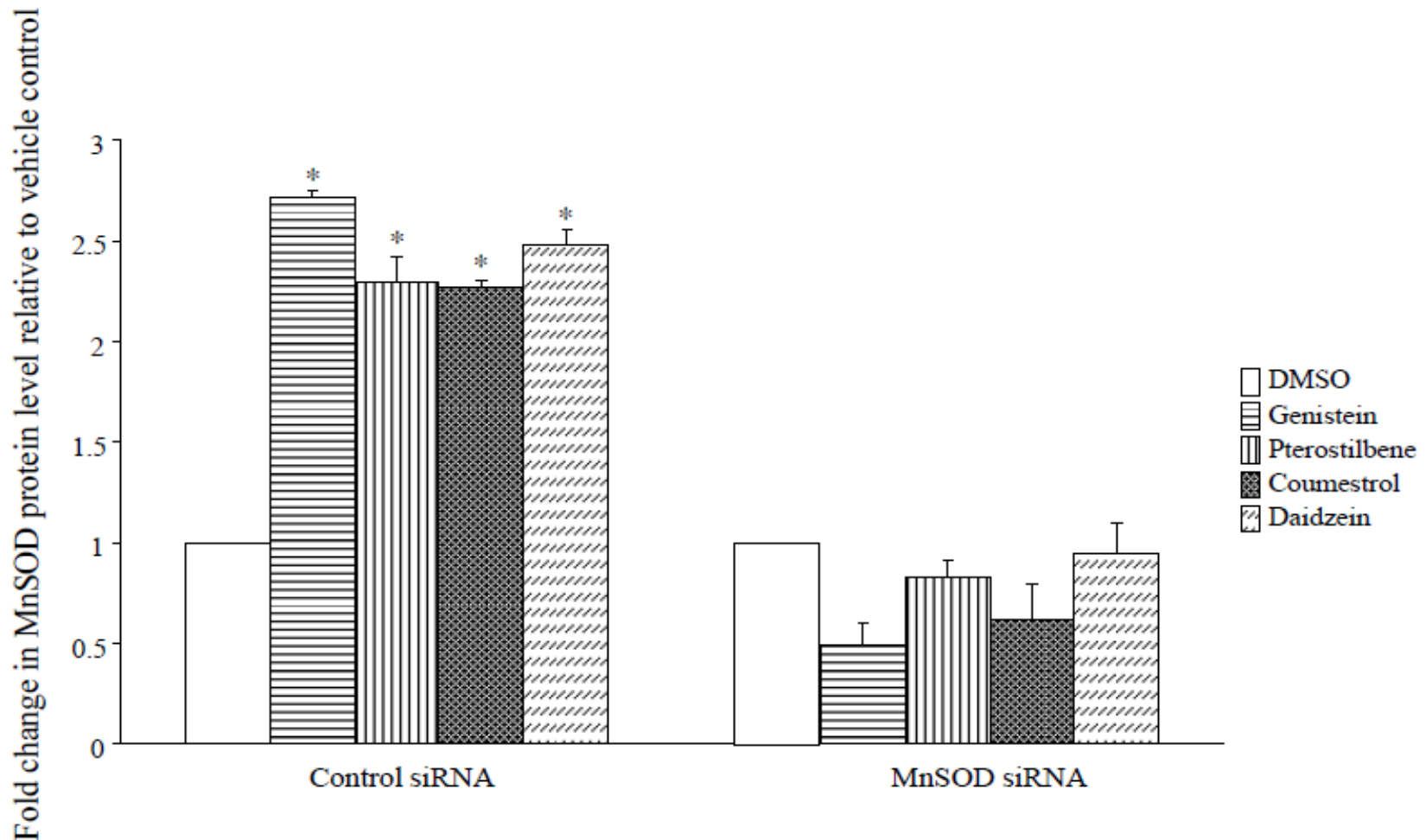


Other *Vinifera* Phytoalexins are Structurally Similar to Resveratrol....Do They Have Similar Effects?

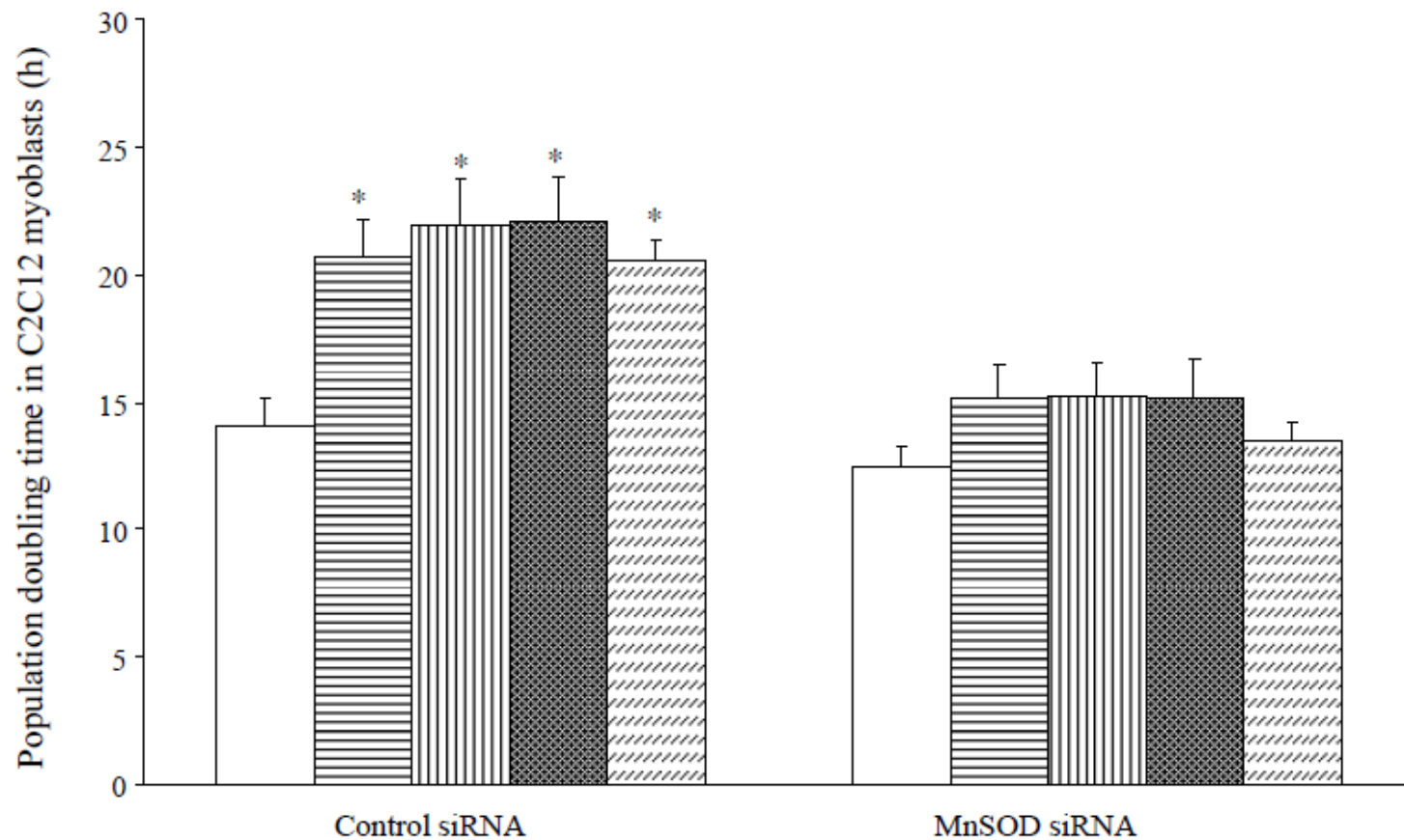


Yes, the structurally related *Vitis vinifera* molecules piceid and pterostilbene have similar effects to resveratrol on MnSOD expression and replicative growth

Effects of Pterostilbene on Proliferative Growth are Also Dependent Upon MnSOD Induction



Effects of Pterostilbene on Proliferative Growth are Also Dependent Upon MnSOD Induction



Conclusions & Applications



Resveratrol, piceid and pterostilbene inhibit proliferative growth of human cells via the induction of MnSOD mediated by ER β

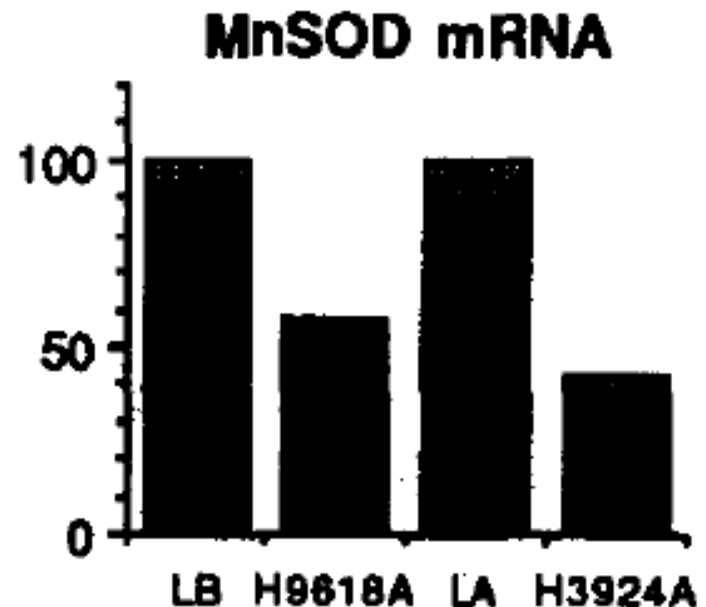
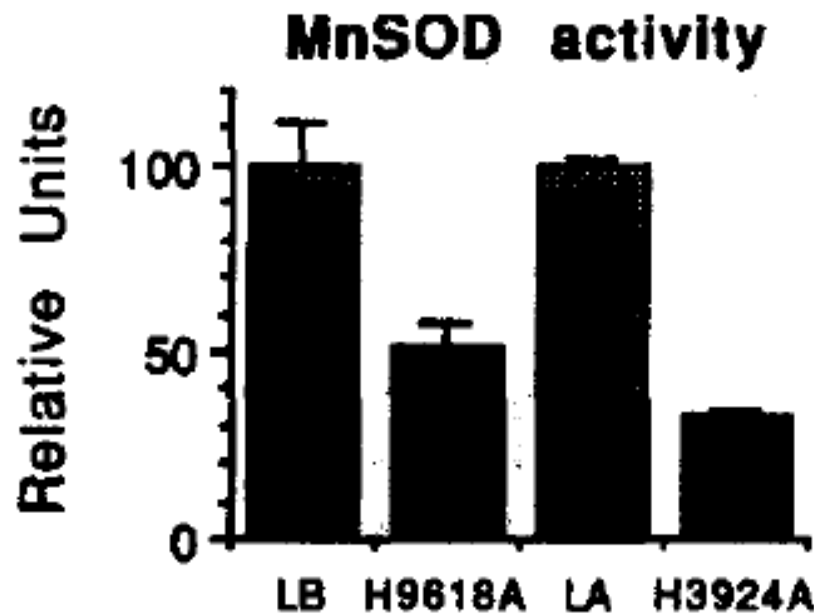
Conclusions & Applications



Resveratrol, piceid and pterostilbene inhibit proliferative growth of human cells via the induction of MnSOD mediated by ERβ

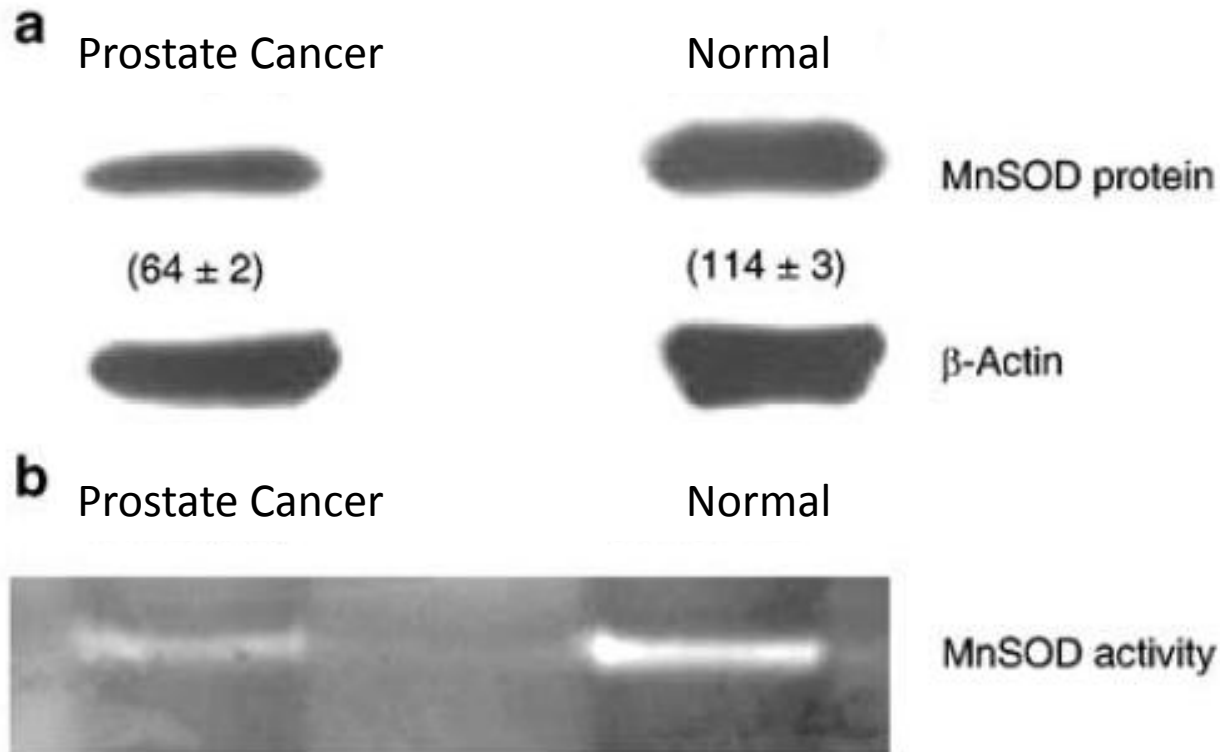
Can this be applied to cancer cell growth?

MnSOD is Transcriptionally Repressed in (many) Cancers



Molec. Aspects Med. Vol. 14, pp. 253–258, 1993

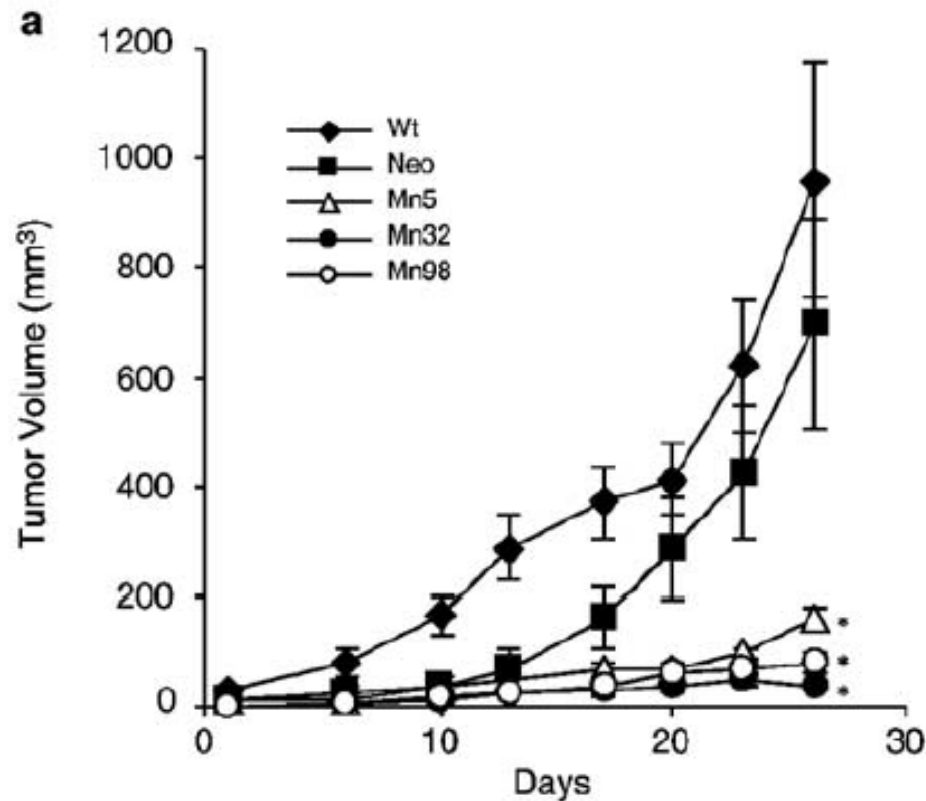
MnSOD is Transcriptionally Repressed in Prostate Cancer Cells



Transgenic Rescue of MnSOD Expression Slows Prostate Cancer Growth



Slowed growth of transplanted tumours by MnSOD



Prostate Cancer Cells Express High Levels of ER β



	VCaP	PC3	DU145
ER α	100 \pm 9	110 \pm 19	UD
ER β	100 \pm 14	206 \pm 18*	151 \pm 22*,§
Ratio ER α /ER β	1.00	0.48	0

A Strategy for Slowing Prostate Cancer Cell Growth



- Many cancers (including prostate) have reduced MnSOD expression

A Strategy for Slowing Prostate Cancer Cell Growth



- Many cancers (including prostate) have reduced MnSOD expression
- Transgenic rescue of MnSOD expression slows cancer cell growth

A Strategy for Slowing Prostate Cancer Cell Growth



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- ERB stimulates MnSOD expression

A Strategy for Slowing Prostate Cancer Cell Growth



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- ERβ stimulates MnSOD expression
- Prostate cancer cells have ERβ

A Strategy for Slowing Prostate Cancer Cell Growth



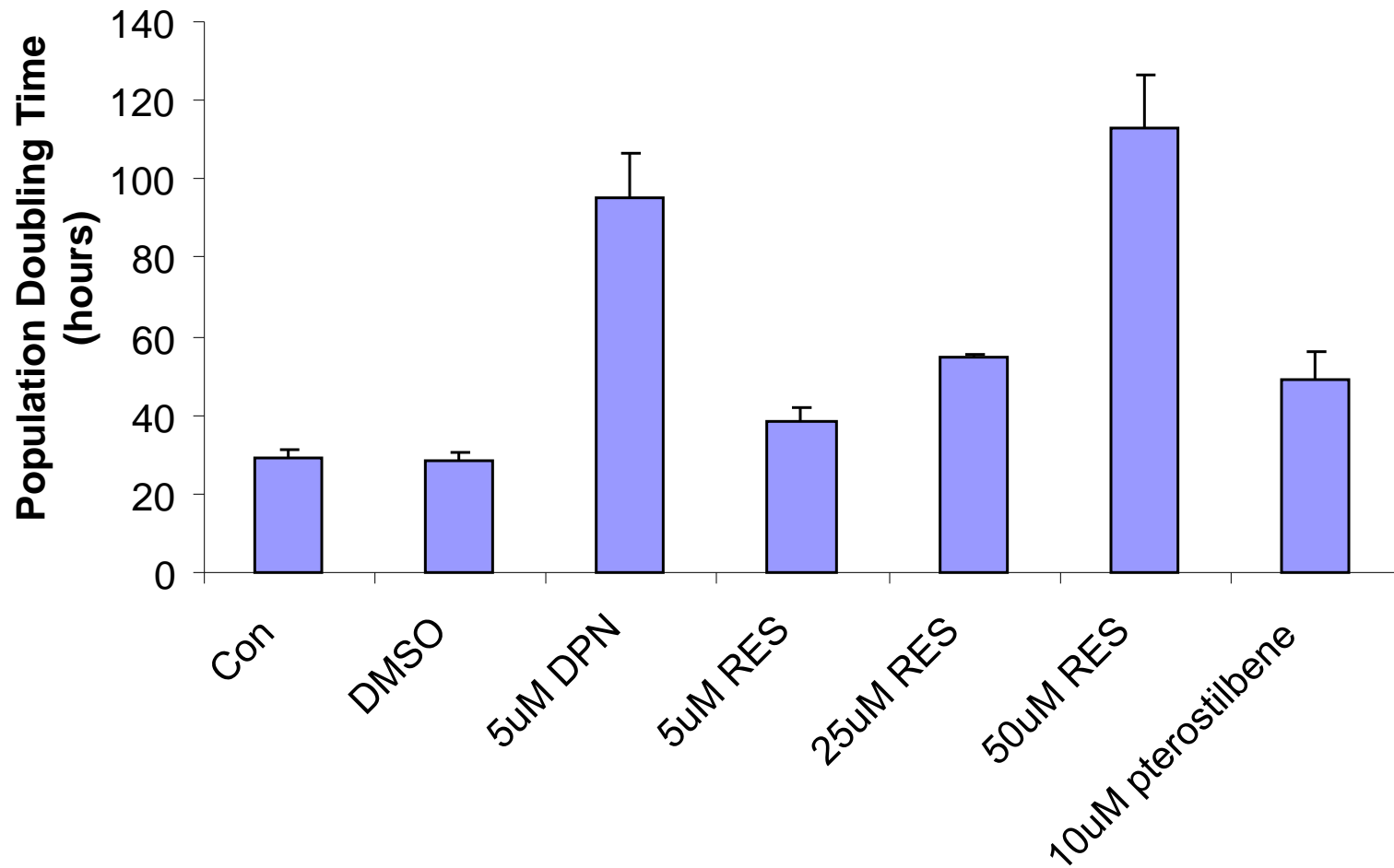
- Many cancers (including prostate) have reduced MnSOD expression
- Transgenic rescue of MnSOD expression slows cancer cell growth
- ERβ stimulates MnSOD expression
- Prostate cancer cells have ERβ
- Some *Vitis vinifera* phytoestrogens are ERβ agonists

Does phytoestrogen treatment of prostate cancer cells induce MnSOD and slow growth?

Resveratrol and Pterostilbene Similarly Inhibit Prostate Cancer Cell Growth



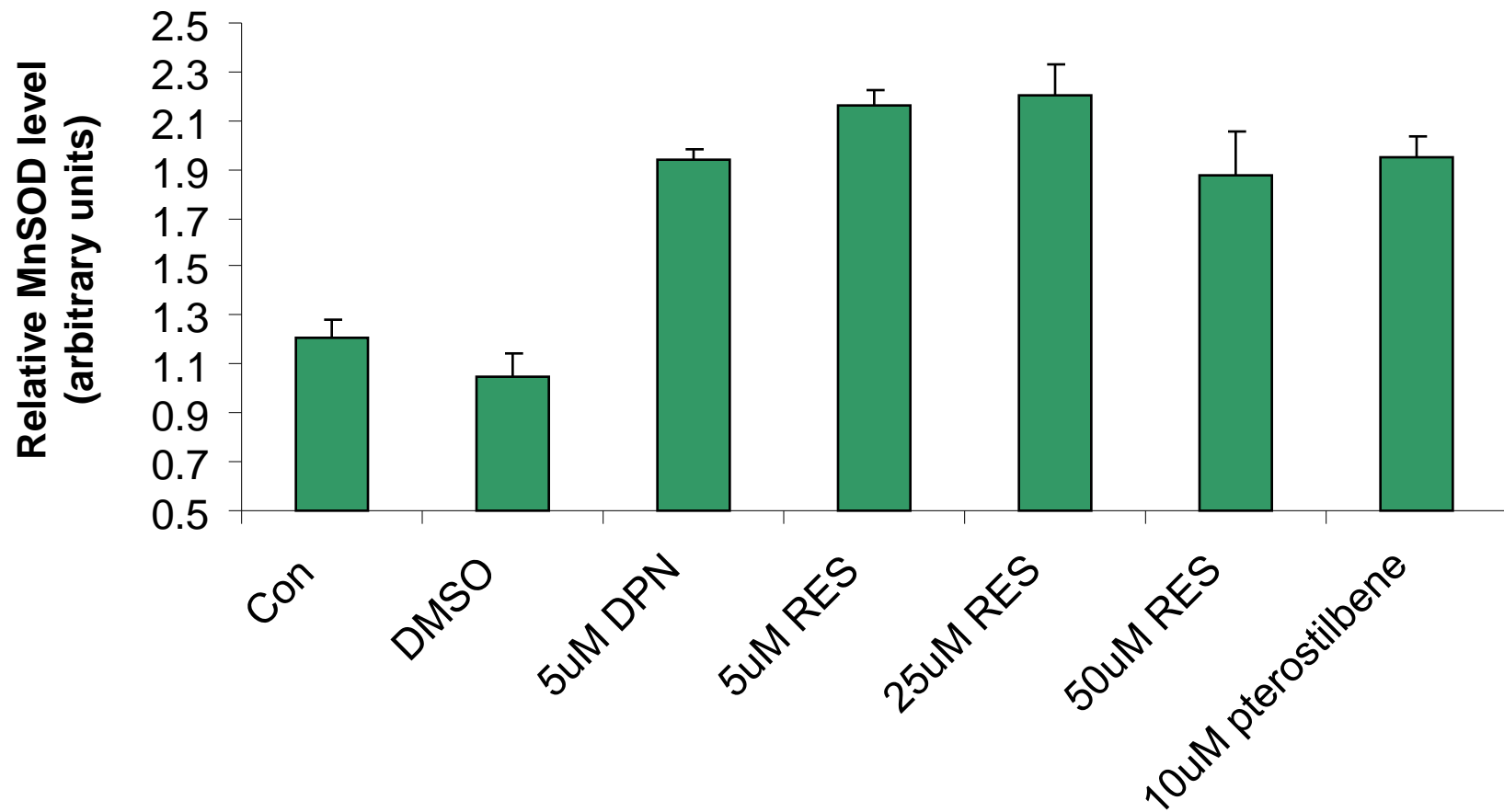
Effects of ERB agonists on proliferative growth



Resveratrol and Pterostilbene Similarly Stimulate MnSOD Expression in Prostate Cancer Cells



Effects of ERB agonists on MnSOD expression



A Strategy for Slowing Prostate Cancer Cell Growth



At least two *Vitis vinifera* phytoalexins (resveratrol and pterostilbene) inhibit prostate cancer growth, probably via the ER β -mediated induction of MnSOD expression

Ongoing Studies with Prostate Cancer



- Investigate other cancers, e.g. colon, breast
- Investigate efficacy of other viniferins
- In vivo studies with mice

Conclusions and Prospectus



- ERB agonists stimulate a significant induction of MnSOD, a tumour suppressor enzyme that negatively regulates cell proliferative growth
- Resveratrol, pterostilbene and piceid induce an ERB-dependent increase in MnSOD expression that inhibits proliferative growth in normal and cancerous cells
- Other *Vitis vinifera* phytoalexins with similar properties, e.g. resveratrol oligomers?



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