



Published in final edited form as:

*Aging Cell*. 2007 February ; 6(1): 35–43. doi:10.1111/j.1474-9726.2006.00259.x.

## Design and synthesis of compounds that extend yeast replicative lifespan

Hongying Yang, Joseph A. Baur, Allen Chen, Christine Miller, and David A. Sinclair  
Department of Pathology, Harvard Medical School, Boston, MA 02115, USA

### Summary

This past decade has seen the identification of numerous conserved genes that extend lifespan in diverse species, yet the number of compounds that extend lifespan is relatively small. A class of compounds called STACs, which were identified as activators of Sir2/SIRT1 NAD<sup>+</sup>-dependent deacetylases, extend the lifespans of multiple species in a Sir2-dependent manner and can delay the onset of age-related diseases such as cancer, diabetes and neurodegeneration in model organisms. Plant-derived STACs such as fisetin and resveratrol have several liabilities, including poor stability and relatively low potency as SIRT1 activators. To develop improved STACs, stilbene derivatives with modifications at the 4' position of the B ring were synthesized using a Horner-Emmons-based synthetic route or by hydrolyzing deoxyrhapontin. Here, we describe synthetic STACs with lower toxicity toward human cells, and higher potency with respect to SIRT1 activation and lifespan extension in *Saccharomyces cerevisiae*. These studies show that it is possible to improve upon naturally occurring STACs based on a number of criteria including lifespan extension.

### Keywords

lifespan; longevity; resveratrol; SIRT1; sirtuins; STACs

### Introduction

Dietary restriction (DR) delays aging and extends the maximum lifespan of a wide range of organisms (Koubova & Guarente, 2003). In mammals, DR delays numerous age-associated diseases including cancer, neurodegeneration, atherosclerosis and type II diabetes (Weindruch, 1996; Lane *et al.*, 2001; Roth *et al.*, 2001). The search for small molecule mimetics that can deliver the health benefits of DR is an active area of research, but no safe and effective DR mimetic has been found (Ingram *et al.*, 2004). An approach we have taken to finding DR mimetics has been to screen libraries for compounds that modulate the activity of longevity regulatory proteins, and to test these for their ability to extend lifespan in relatively simple organisms such as baker's yeast and the nematode *Caenorhabditis elegans* before moving on to mammals (Wood *et al.*, 2004).

There are a number of reasons for taking this approach. First, DR works on almost all organisms that have been tested. Therefore, we expect bona fide DR mimetics to extend the lifespans of species as different as yeast, worms, flies and mice. Second, simple organisms are far more amenable to genetic manipulation and their lifespans are relatively short – less than 10 days in the case of actively dividing yeast – therefore, the pathways through which lifespan-extending compounds are working can be identified more readily. Third, the

pathways that control the DR response are far better defined in these organisms than in mammals. Finally, small molecules can have effects that are more complex than simple activation or inhibition, such as altering substrate specificity, and understanding how they extend lifespan may reveal additional longevity pathways (Viswanathan *et al.*, 2005).

In *Saccharomyces cerevisiae*, *C. elegans*, and *Drosophila melanogaster*, increased copy number or expression of the *SIR2* gene extends lifespan 30–50% (Kaeberlein *et al.*, 1999; Tissenbaum & Guarente, 2001; Rogina & Helfand, 2004). With regards to *S. cerevisiae*, this refers to replicative lifespan, which is the number of daughter cells produced by a single mother before senescence, rather than the more recently defined chronological lifespan, which measures survival time in the absence of nutrients (Bitterman *et al.*, 2003). In wild-type *S. cerevisiae* and *Drosophila*, lifespan extension by DR requires *SIR2*, implying that the gene is intimately involved in mediating its effects. In the absence of the *FOB1* gene, which suppresses the rDNA hyper-recombination defect of a *sir2* mutant, glucose restriction can extend the lifespan of some *sir2* strains (Kaeberlein *et al.*, 2004; Lin & Guarente, 2006) revealing that there is redundancy in the DR pathway, which we have shown is mediated, at least in part, by the Sir2 homolog Hst2 (Lamming *et al.*, 2005). Although it is not known whether the closest mammalian Sir2 homolog, SIRT1, extends lifespan in mammals, this gene is known to modulate several distinct physiologies known to be important for lifespan including cell survival, energy metabolism, and resistance to stresses (Luo *et al.*, 2001; Vaziri *et al.*, 2001; Araki *et al.*, 2004; Brunet *et al.*, 2004; Cohen *et al.*, 2004; Yeung *et al.*, 2004; Moynihan *et al.*, 2005; Bordone *et al.*, 2006).

We recently reported the design, synthesis and characterization of SIRT1 inhibitors that are derivatives of splitomycin (Bedalov *et al.*, 2001; Mai *et al.*, 2005). Although SIRT1 inhibitors may be useful reagents in laboratory settings and possibly in the treatment of diseases such as cancer (Lim, 2006), we have placed most of our effort on identifying and optimizing molecules that activate Sir2/SIRT1, based on the hypothesis that they should extend lifespan and possibly mimic the health benefits of DR. To this end we performed *in vitro* screens for small molecules that could modulate human SIRT1 and identified 18 SIRT1-activating compounds or STACs, which were of three structural classes: chalcones, flavones and stilbenes (Howitz *et al.*, 2003). The most potent of these was 3,5,4'-hydroxystilbene (resveratrol). Resveratrol stimulated SIRT1 activity ~tenfold by lowering the  $K_m$  for both  $NAD^+$  and the peptide substrate. Resveratrol also provided the largest yeast lifespan extension, ~60%, and this was entirely dependent on the *SIR2* gene (Howitz *et al.*, 2003), as was the lifespan extension in *C. elegans* and *D. melanogaster* (Wood *et al.*, 2004), although we do not discount the possibility that the beneficial effects of resveratrol in mammals are mediated through a potentially large number of cellular factors in addition to SIRT1 (Baur & Sinclair, 2006).

Keijer and colleagues recently identified three additional STACs from plants, but none of them was more potent than resveratrol (de Boer *et al.*, 2006). Interestingly, resveratrol has been shown to provide protection from numerous diseases in rodent models including cancer and diabetes, but the pathways through which it exerts these effects are not known (Bhat & Pezzuto, 2002; Aggarwal *et al.*, 2004; Su *et al.*, 2006).

Activation of SIRT1 by STACs is detectable in a variety of *in vitro* assays including a fluorescent peptide-based assay (Howitz *et al.*, 2003), a high performance liquid chromatography (HPLC)-based assay (de Boer *et al.*, 2006) and a radiolabelled peptide assay (A. Suave & U. Cornell, personal communication). Activation of SIRT1 is not observed in some *in vitro* assays that use 4–20 amino acid peptide substrates, leading one group to conclude that activation is an *in vitro* artifact (Kaeberlein *et al.*, 2005). Although the reason for this discrepancy has not yet been fully elucidated, a likely explanation is that

not all peptide substrates fully mimic the endogenous situation, where the targets of deacetylation are full-length proteins (Borra *et al.*, 2005; de Boer *et al.*, 2006).

With regards the *in vivo* evidence for activation, STACs such as resveratrol and fisetin extend lifespan in yeast, *C. elegans* and *Drosophila*, and this is proportional to their level of activation *in vitro* (Howitz *et al.*, 2003; Bauer *et al.*, 2004; Jarolim *et al.*, 2004; Wood *et al.*, 2004; Viswanathan *et al.*, 2005). Importantly, STACs do not extend lifespan in the absence of *SIR2*. In *C. elegans*, resveratrol and fisetin can also mimic the protective effects of additional copies of Sir2, but they only work if the Sir2 gene is present (Parker *et al.*, 2005). Activation of mammalian SIRT1 by STACs has also been demonstrated in a variety of ways using a number of experimental systems, including showing increased deacetylation of a target peptide in HEK293 and HT29 cells (Howitz *et al.*, 2003; de Boer *et al.*, 2006), increased deacetylation of the SIRT1 target RelA/p65 (Yeung *et al.*, 2004), increased deacetylation and nuclear localization of the SIRT1 targets FOXO1 (Daitoku *et al.*, 2004; Yang *et al.*, 2005) and FOXO4 (Brunet *et al.*, 2004), the SIRT1-dependent mobilization of fat stores from cultured adipocytes (Picard *et al.*, 2004), suppression of axonal degeneration (Araki *et al.*, 2004), deacetylation of RelA/p65 and the protection of human neurons against A $\beta$  peptide (Chen *et al.*, 2005), sirtuin-dependent protection of striatal mouse neurons overexpressing mutant huntingtin (Wood *et al.*, 2004; Yeung *et al.*, 2004; Parker *et al.*, 2005), reduced neurotoxicity from mutant superoxide dismutase (an amyotrophic lateral sclerosis model) and mutant p35 (an Alzheimer's mouse model; L. H. Tsai & D. A. Sinclair, unpublished), SIRT1-dependent protection of cardiomyocytes from poly(ADP-ribose)polymerase (PARP) -induced apoptosis (Pillai *et al.*, 2005), and SIRT1-dependent neuroprotection following ischemia (Raval *et al.*, 2006).

Unfortunately, resveratrol has limitations as a laboratory tool and a proof-of-concept molecule, which include low bioavailability in mammals, low solubility, and sensitivity to light and to oxidation (Bhat *et al.*, 2001; Halliwell, 2003; Aggarwal *et al.*, 2004; Walle *et al.*, 2004; de Boer *et al.*, 2006). These limitations led us to design molecules with greater potency, stability, and a greater ability to extend the lifespan of model organisms. Here we describe the synthesis and characterization of a series of stilbene derivatives and show that it is possible to find STACs that are superior to resveratrol in terms of their stability in solution, toxicity, ability to activate SIRT1, and the extent to which they extend the lifespan of yeast cells.

## Results

Previous studies examining structure-activity relationship of SIRT1 activation by stilbenes from plant sources showed that hydroxyls on the 3 and 5 position of the A ring and the *trans* conformation of the rings are crucial structural elements, and that the 4' position is amenable to modification (Howitz *et al.*, 2003). We therefore focused our efforts on synthesizing resveratrol analogs with replacements at the 4' position of the stilbene backbone using the synthesis schemes outlined in Fig. 1. Modifications included thiomethyl, methyl, ethyl, methoxy, and acetoxy groups (Fig. 2). Between 5 and 10 mg of each compound was obtained. Because of the limiting amounts of each compound that were available, SIRT1 was chosen as a representative sirtuin to test activation, based on its large dynamic range and the previously demonstrated correlation between activation of SIRT1 and its orthologs of other species (Howitz *et al.*, 2003).

In agreement with previous reports (Howitz *et al.*, 2003; de Boer *et al.*, 2006), resveratrol (50  $\mu$ M) stimulated the activity of SIRT1 ~12-fold (Fig. 3). Derivative 1 (3,5-dihydroxy-4'-thiomethyl-*trans*-stilbene), in which the 4'-hydroxyl was replaced with a thiomethyl group, was the most effective, stimulating SIRT1 activity 18-fold, while Derivative 4 (3,5-

dihydroxy-4'-methoxy-*trans*-stilbene), bearing a chemically similar methoxy group activated SIRT1 11-fold (Fig. 3). Derivative 2 (3,5-dihydroxy-4'-methyl-*trans*-stilbene) and Derivative 3 (3,5-dihydroxy-4'-ethyl-*trans*-stilbene), substituted with hydrophobic methyl and ethyl groups at the 4' positions, activated SIRT1 16- and 14-fold, respectively. Derivative 5 (3,5-dihydroxy-4'-acetoxy-*trans*-stilbene), bearing an ester group with a relatively large volume at the 4' position was the least potent, stimulating SIRT1 only threefold. Based on this structure–activity relationship, it appears that small, hydrophobic substituents at the 4' position can enhance the potency of stilbenes.

### Solubility of resveratrol derivatives

The low solubility of resveratrol in aqueous buffers (approximately 400  $\mu\text{M}$  in distilled water or PBS) is a significant limitation to delivering high doses in animal studies. Using the limited quantities of derivatives that were available we were able to determine their relative solubility in aqueous solution. The solubility of Derivative 2 was threefold greater than resveratrol (> 1.2 mM). Thus, modification of the 4' position is a viable strategy for improving the solubility of resveratrol. In contrast, the solubility of Derivative 3 was not appreciably different, and those of Derivatives 1, 4, and 5 were lower than resveratrol (100–200  $\mu\text{M}$ ) (data not shown).

### Stability of resveratrol derivatives

Although resveratrol is stable when lyophilized and stored under nitrogen, it has a half-life of only ~4–5 days when dissolved in dimethyl sulfoxide (DMSO) or ethanol and stored at room temperature and exposed to ambient light. This presumably limits how effective it is in extending lifespan in yeast experiments that are conducted over a period of 2 weeks. As shown in Fig. 4, the half-life of Derivative 5 is increased, suggesting that its effects may last longer despite its low initial potency *in vitro*. This experiment demonstrates that modification of the 4' position of resveratrol is a feasible synthetic strategy to improve stability.

### Concentration-dependent toxicity of resveratrol derivatives to HEK 293 cells

Resveratrol has been reported to have a biphasic effect on cell survival. Lower concentrations are beneficial, whereas higher concentrations become toxic (Aggarwal *et al.*, 2004). A significant concern of ours was that improved SIRT1 activation might be counterbalanced by increased cytotoxicity, rendering them unsuitable for treatment of living cells or multicellular organisms. In order to test the cytotoxicity of our derivatives, we measured the survival of HEK 293 cells over a period of 96 h. At 25  $\mu\text{M}$ , resveratrol and all five of the synthesized derivatives had little to no effect on the growth of HEK 293 cells. At higher concentrations, Derivatives 3, 4 and 5 showed similar toxicity to resveratrol, but the other derivatives, 1 and 2, were less toxic (Fig. 5 and data not shown). After 48 h, at a concentration of 50  $\mu\text{M}$ , less than 10% of the cells were alive in the dishes containing resveratrol or Derivative 3, whereas 38% of the cells remained alive when treated with Derivative 2. Interestingly, 61% of the cells remained alive when treated with Derivative 1, the most potent activator, demonstrating that the replacement of the 4'-hydroxyl with a thiomethyl group reduces toxicity to cells while enhancing the ability of this compound to activate SIRT1. Thus, cytotoxicity can be uncoupled from the ability to stimulate SIRT1. Large-scale synthesis of this compound is in progress with a view to testing its pharmacokinetic properties and against cancer and age-related diseases in mouse models.

### Resveratrol derivatives extend yeast replicative lifespan

The ability of STACs to increase SIRT1 activity *in vitro* has been shown to correlate with yeast lifespan extension: resveratrol being the most potent activator and generating the

longest lifespan extension, and correspondingly lower activation potential and lifespan extension for compounds such as butein (3,4,2',4'-tetrahydrochalcone) and fisetin (3,7,3',4'-tetrahydroxyflavone) (Howitz *et al.*, 2003). We therefore sought to test whether our derivatives could extend yeast lifespan. For this experiment, we chose Derivatives 1, 3, and 5, which activated SIRT1 to varying extents. As shown in Fig. 6 (A,B), all of the three resveratrol derivatives tested extended yeast mean and maximum lifespan significantly ( $P$  value for Derivative 1 vs. no treatment  $< 0.05$ ;  $P$  values for Derivative 3 and 5 vs. no treatment  $< 0.001$ ). To our surprise, however, Derivative 5 extended lifespan the furthest (68%), despite its low *in vitro* potency. This may be attributable to the improved stability of Derivative 5 in solution (Fig. 4) or *in vivo* once it has been taken up by the yeast cells.

## Discussion

Our previous work with STACs demonstrated that the 3- and 5-hydroxyl groups of the A ring as well as the *trans* conformation are crucial structural elements for SIRT1 activation (Howitz *et al.*, 2003). In contrast, modification of the 4' position on the B ring, which is the residue that accounts for the majority of free radical scavenging ability (Stojanovic *et al.*, 2001), did not interfere with SIRT1 activation in five synthetic derivatives of resveratrol, although this activity was significantly diminished in Derivative 5, which bore a relatively bulkier acetoxy functional group at the 4' position.

Although there is no co-crystal structure for human SIRT1 in complex with a STAC, crystal structures of yeast Hst2, human SIRT2 and two bacterial Sir2 homologs have been solved. These Sir2 homologs possess a similar catalytic core composed of ~270 amino acids (Finnin *et al.*, 2001; Min *et al.*, 2001; Zhao *et al.*, 2003a,b, 2004a,b). In Hst2, the highly conserved  $\beta$ 1- $\alpha$ 2 loop in the N-terminus undergoes significant structural rearrangements to facilitate the ordered NAD<sup>+</sup> reactions of nicotinamide cleavage and ADP-ribose transfer to acetate (Marmorstein, 2004) and has been suggested as a candidate site to which sirtuin activators such as resveratrol might bind (Zhao *et al.*, 2004b). We speculate that resveratrol and its analogs might bind to the N-terminus of SIRT1 to induce a conformational change that lowers the  $K_m$  for the substrate. This model is supported by our studies showing that the mutations SIRT1-E230K and Sir2-D223K within the N-terminal sequences close to the catalytic cores of SIRT1 and Sir2, prevent activation by resveratrol without affecting basal activity (our unpublished data). The electronegativity of the substituted functionality at the 4' position in the derivatives may play a role in determining binding affinity since substitution of sulfur in Derivative 1 with a more electronegative oxygen atom in Derivative 4 decreased the *in vitro* sirtuin activating activity from 18-fold to 11-fold. In order to elucidate the exact binding site of STACs to sirtuins, co-crystallization of resveratrol with sirtuins is in progress.

The stability of a molecule is an important consideration when conducting lifespan studies in which the molecule is exposed to water, light and oxygen. According to Stojanovic and co-workers, the 4'-hydroxyl group in resveratrol plays a central role in its antioxidant activity against biologically generated free radicals (Stojanovic *et al.*, 2001). This implies that resveratrol could lose its activity due to redox reactions at this position. Substitution with different functionalities at the 4' position in resveratrol might lower the redox potentials of these derivatives, thereby increasing the half-life in solution. This hypothesis was supported by the enhanced stability of Derivative 5, substituted with a 4'-acetoxy group.

The activity of a STAC *in vivo* will depend on many factors such as its rate of uptake and metabolism. For this reason, *in vitro* activity may not always correlate with the ability to activate sirtuins in cells. For example, *in vivo* esterases are known to readily cleave off acetyl groups from acetyl-containing molecules to release acetate (White & Hope, 1984).



For this reason drugs are often acetylated in a prodrug form as a means of enhancing its properties, such as stability, without diminishing its biological activity (Takahashi *et al.*, 1992). Derivative 5, which is acetylated at the 4' position, was the least efficacious of the STACs in activating SIRT1 *in vitro*, although the lifespan extension it produced was the largest achieved by treatment with a STAC to date, possibly because it remains more stable in the yeast medium than other STACs and is cleaved by esterases once it enters the cell, releasing free resveratrol and acetate and providing a steady stream of the active compound, but clearly more experiments will be required to confirm this.

Although resveratrol has some liabilities as a pharmacological agent, one of its best attributes is its lack of toxicity. Cell toxicity is induced in mammalian cells upon prolonged exposure to doses typically in excess of 30  $\mu\text{M}$ , whereas serum resveratrol concentrations peak well below 10  $\mu\text{M}$  in rodents following a high dose of up to 50 mg kg<sup>-1</sup> and decay with a half-life of ~8–14 min (Asensi *et al.*, 2002; Marier *et al.*, 2002). No adverse effects have been detected in rats using doses as high as 300 mg kg<sup>-1</sup>. However, even minor changes to a non-toxic molecule can alter its toxicity so if any of these molecules are to be employed in higher animals, toxicity is an important consideration. At high concentrations, Derivative 1 was considerably less toxic than the other derivatives and even the parent compound. The observation that substitution of a less electronegative sulfur atom for the oxygen in the 4'-hydroxyl of resveratrol decreased toxicity while simultaneously increasing *in vitro* catalytic efficiency may be an important consideration in the design of STACs that achieve high *in vivo* concentrations.

In summary, we have explored the effects of altering the structure of resveratrol on stability, toxicity, and potency of SIRT1 activation. We show that it is possible to improve upon numerous characteristics of this molecule and extend yeast lifespan further than can be achieved using natural molecules. We consider Derivative 1 (with its high potency and lack of toxicity) and Derivative 5 with its improved stability and potential as a prodrug form of resveratrol as the two most promising candidates to explore in studies of lifespan extension in higher organisms.

## Experimental procedures

### Compound synthesis

Resveratrol Derivatives 1–3 and 5 were synthesized by a modified Horner-Emmons-based route using a diethyl phosphonate to generate stilbene (Andrus *et al.*, 2003). Briefly, 1-(bromomethyl)-3,5-dimethoxybenzene **1** purchased from Sigma-Aldrich (St. Louis, MO, USA) was treated with neat triethyl phosphate **2** by Arbuzov reaction to produce diethyl (3,5-dimethoxyphenyl) methylphosphonate **3** in high yield. Coupling with 4-(methylthio) benzaldehyde **4**; 4-methylbenzaldehyde **5**; 4-ethylbenzaldehyde **6**; 4-formylphenyl acetate **7** (from Sigma-Aldrich) using sodium hydride as base in tetrahydrofuran (THF) gave the protected stilbene **8**, **9**, **10**, **11** in 65% yield. Boron trichloride was then used to give resveratrol derivatives **12**, **13**, **14**, **15**. Derivative 4 (3,5-dihydroxy-4'-methoxy-*trans*-stilbene) was synthesized by hydrolysis of deoxyrhapontin (3-hydroxy-5[(E)-2-(4-methoxyphenyl)ethenyl]phenyl hexopyranoside) treated with 4-methyl benzenesulfuric acid in methanol. The numbers given in bold above are the identification numbers of structures in Figure 1.

### SIRT1 assays

SIRT1 assays were performed as previously described using a fluorescently labeled peptide substrate (Howitz *et al.*, 2003). To assess the stability of derivatives each compound was dissolved in ethanol at 2.5 mM and stored at room temperature exposed to ambient light. An

aliquot (1  $\mu\text{L}$ ) was added to a SIRT1 assay to give a final concentration of 50  $\mu\text{M}$  and the reaction was incubated for 60 min at 37  $^{\circ}\text{C}$  before addition of the developer reagent. Assays were incubated for an additional 20 min at room temperature to allow the development of fluorescence, and then read for 0.1 s using a Victor 3 fluorometer (PerkinElmer, Downers Grove, IL, USA) with excitation and emission wavelengths of 360 and 450 nm, respectively.

### Solubility assays

Resveratrol and derivatives were dissolved at 200 mM in DMSO, then serially diluted twofold in distilled water. Precipitation was judged by eye, then all samples were heated to 50  $^{\circ}\text{C}$  for 5 min and allowed to cool and precipitate a second time in order to confirm that the crystallization was not due to the dilution process. The final concentration of DMSO was less than 1% in all solutions. Identical results were obtained using PBS instead of distilled water.

### Toxicity assays

HEK293 cells were routinely cultured in Dulbecco's modified eagle medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum. Stock solutions of resveratrol and derivatives were made freshly in ethanol (at 1000 $\times$ ) at the start of each experiment. Cells grown in six-well dishes were treated with resveratrol or the indicated derivative in regular growth medium for the indicated periods of time. Images were captured using an RT Monochrome Spot camera attached to a Nikon Eclipse TE2000-U microscope with Spot (version 3.5.9) software. Cell counts were obtained by trypsinization and quantification using a Beckman-Coulter Z2 particle counter.

### Yeast lifespan analyses

Lifespan measurements were performed using PSY316AT $\alpha$  as previously described (Bitterman *et al.*, 2002). All compounds for lifespan analyses were dissolved in 95% ethanol. Plates were used within 24 h of preparation. Cells were pre-incubated on their respective medium for at least 15 h and equilibrated on the plates for a minimum of 4 h before micromanipulation. At least 30 cells were examined in each experiment. The statistical significance of lifespan differences were determined using JUMP 5.1 software by the Wilcoxon rank sum test. Differences are stated to be significant when the confidence was higher than 95%.

### Acknowledgments

We wish to thank BIOMOL for advice and assistance with compound synthesis. The Sinclair laboratory is supported by RO1 grants from National Institutes of Health and National Institute on Aging, and the Glenn Laboratories for the Molecular Biology of Aging. H. Yang was supported by a Harvard/Hartford Advanced Research Award. J. A. Baur was supported by American Heart Association, Award number 0425834T. David A. Sinclair is a cofounder and advisor to Sirtris Pharmaceuticals, Cambridge, MA.

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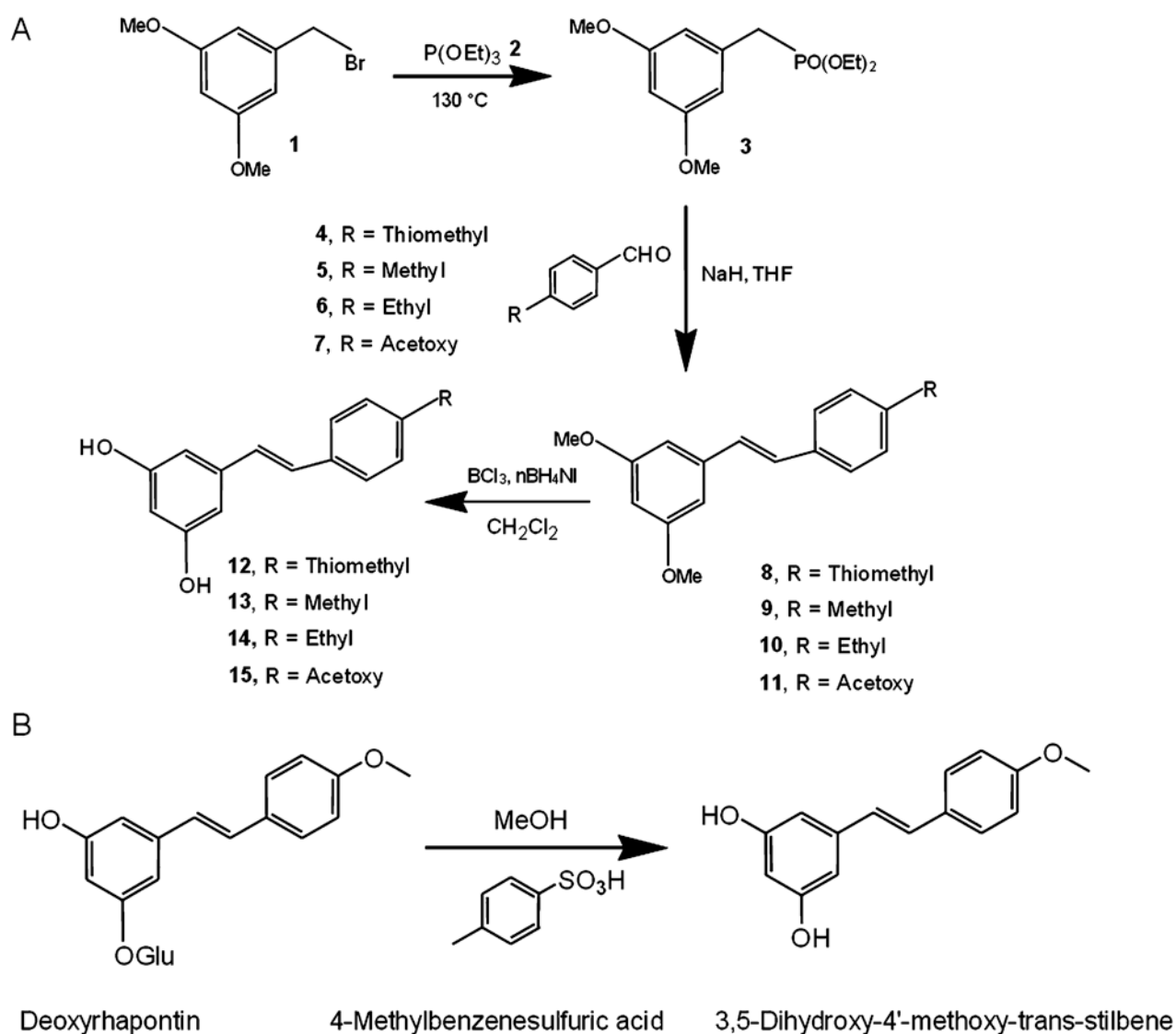
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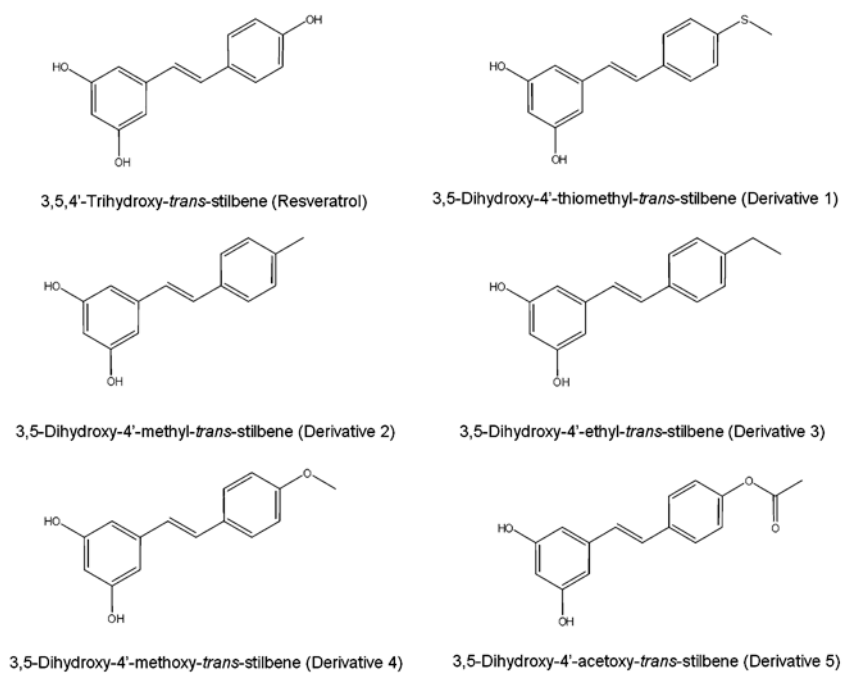


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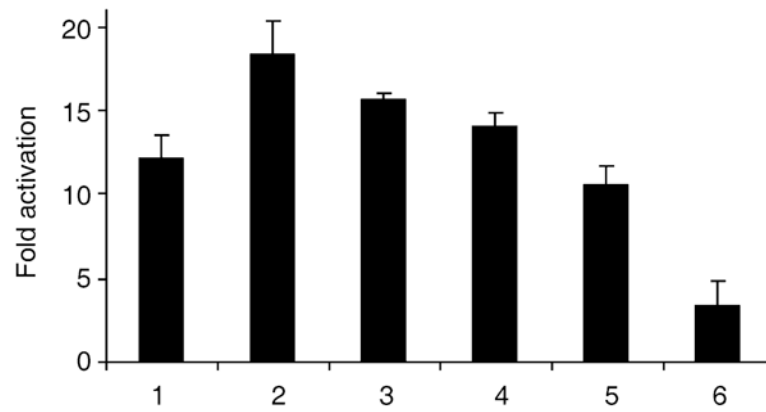
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**Fig. 1.** Synthesis steps for resveratrol derivatives. (A) Resveratrol Derivatives 1, 2, 3, and 5 were synthesized by a modified Horner-Emmons-based route using a diethyl phosphonate to generate stilbene (Andrus *et al.*, 2003). 1-(Bromomethyl)-3,5-dimethoxybenzene **1** was treated with neat triethyl phosphate **2** using an Arbuzov reaction to produce diethyl (3,5-dimethoxyphenyl)methylphosphonate **3** in high yield. Coupling with 4-(methylthio)benzaldehyde **4**; 4-methylbenzaldehyde **5**; 4-ethylbenzaldehyde **6**; 4-formylphenyl acetate **7** using sodium hydride as base in THF gave the protected stilbene **8**, **9**, **10**, **11** in 65% yield. Boron trichloride was then used to give resveratrol derivatives **12**, **13**, **14**, **15**. (B) Derivative 4 (3,5-dihydroxy-4'-methoxy-*trans*-stilbene) was synthesized by hydrolysis of deoxyrhapontin (3-hydroxy-5-[(*E*)-2-(4-methoxyphenyl)ethenyl]phenyl hexopyranoside) treated with 4-methyl benzenesulfuric acid in methanol.

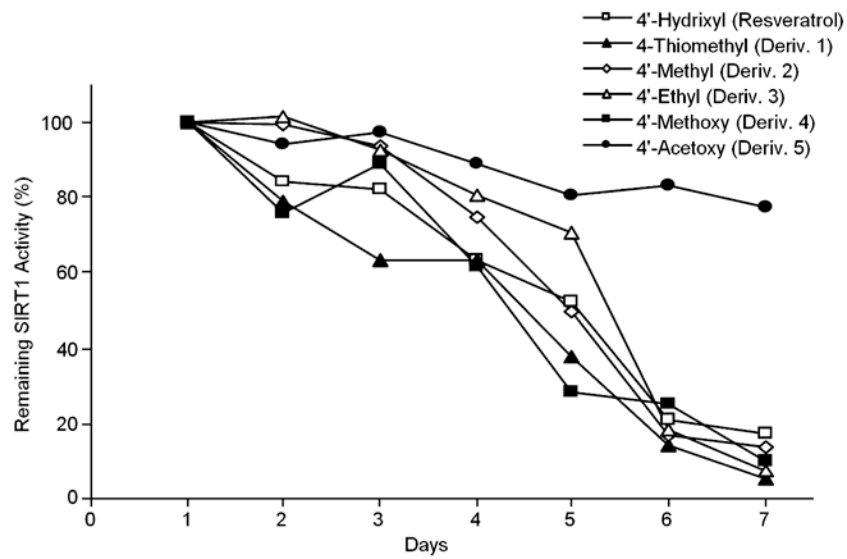


**Fig. 2.** Chemical structures of resveratrol and synthetic resveratrol derivatives used in this study.

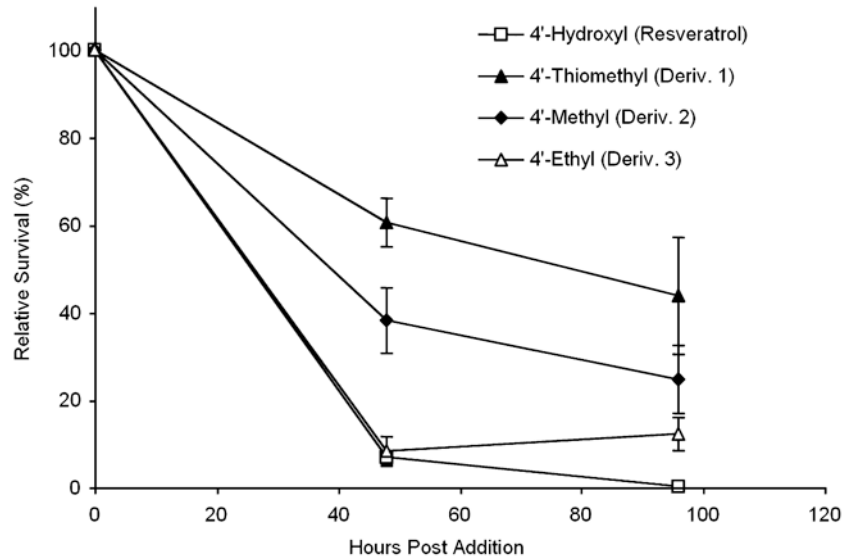


**Fig. 3.** Fold activation of SIRT1 by resveratrol and synthesized resveratrol derivatives. (1) Resveratrol; (2) 4'-thiomethyl (Derivative 1); (3) 4'-methyl (Derivative 2); (4) 4'-ethyl (Derivative 3); (5) 4'-methoxy (Derivative 4); (6) 4'-acetoxy (Derivative 5). Fold activations are calculated as the ratio of the deacetylation rate in samples containing the tested compounds to that of the samples with no activating compound added. Data from three independent experiments are shown  $\pm$  standard deviation.

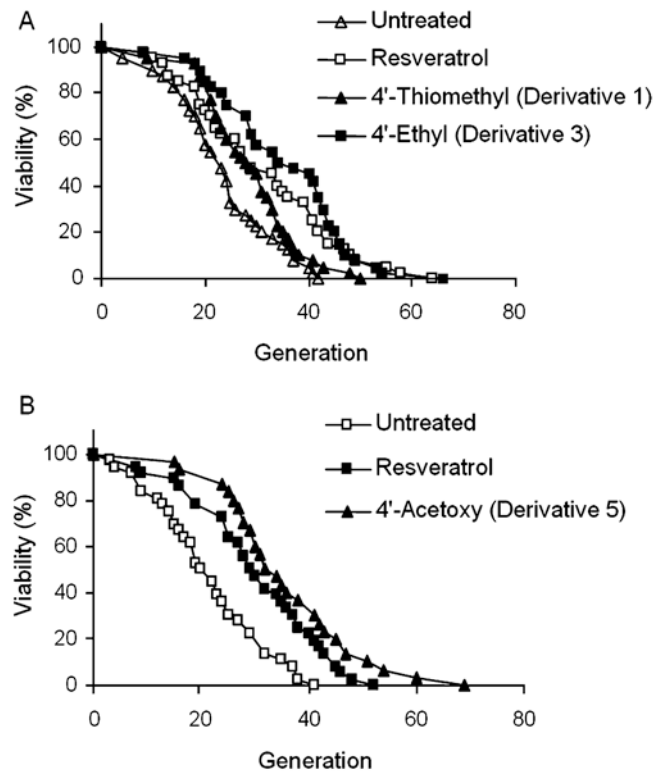




**Fig. 4.** Stability of resveratrol and synthesized derivatives. Resveratrol or derivatives were dissolved in ethanol at a concentration of 2.5 mM and stored on at room temperature with ambient lighting. Aliquots were taken daily and assessed for the ability to activate SIRT1. The parent compound decayed with a half-life of ~4.5–5 days and all derivatives were similar with the exception of Derivative 5 (3,5-hydroxy-4'-acetoxy-stilbene) which displayed significantly improved stability (half-life ~10 days).



**Fig. 5.** Toxicity of resveratrol derivatives to mammalian cells. HEK293 cells were seeded on six-well plates and allowed to attach overnight. The following day, resveratrol, Derivative 1 (3,5-hydroxy-4'-thiomethyl-stilbene), Derivative 2 (3,5-hydroxy-4'-methyl-stilbene), or Derivative 3 (3,5-hydroxy-4'-ethyl-stilbene) was added at a concentration of 50  $\mu\text{M}$ . Cells in replicate wells were periodically washed to remove any debris or detached cells, trypsinized, and counted using a Beckman-Coulter Z2 particle counter. The proportion surviving is plotted as a percentage of cells counted in the corresponding ethanol (vehicle)-treated wells.



**Fig. 6.** Lifespan analyses of *Saccharomyces cerevisiae* (PSY316AT $\alpha$ ) when grown in the presence of resveratrol derivatives. Replicative lifespan was performed on at least 30 cells by micromanipulating mother cells and counting how many daughters were produced. Resveratrol and the derivatives were dissolved in yeast glucose medium at a concentration of 10  $\mu$ M. Mean lifespans for treated and untreated cells were (A), 31.2 (Resveratrol); 28.6 (Derivative 1); 35.3 (Derivative 3); 23.3 (Untreated) and (B), 30.3 (Resveratrol); 36.0 (Derivative 5); 21.4 (Untreated).