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Resveratrol-Inspired Bridged Bicyclic Compounds: A New Compound Class for the Protection of Synaptic Function from Acute Oxidative Stress

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Abstract

While resveratrol protects organisms from the deleterious effects of oxidative stress, its multifarious mechanism of action limits its potential as a selective medicinal agent. To address this shortcoming, we have designed a molecular scaffold that we have termed a resveramorph. The structure of this compound class possesses much of the functional group characteristics of resveratrol but in a non-planar molecular arrangement and, in the present work, we probe the neuroprotective activities of two resveramorph analogs. These novel compounds were found to protect neurotransmission from hydrogen peroxide-induced oxidative stress. Our findings demonstrate that, at a subnanomolar level, one analog, resveramorph **1**, protects synaptic transmission from acute oxidative stress at the *Drosophila* neuromuscular junction. These results position resveramorphs as potential lead compounds in the development of new drugs for neurodegenerative diseases.

Graphical Abstract

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ASSOCIATED CONTENT

Supporting Information

Full experimental details for electrophysiological experiments and the synthesis of resveramorphs **1** and **2**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Conflict of Interest

The authors declare no competing financial interest.

Keywords

Resveramorphs; neuroprotection; oxidative stress; bridged bicyclic; *Drosophila*; synaptic function

Resveratrol has been shown to protect animals from the detrimental effects of aging,¹ a high fat diet,² traumatic brain injury,³ and ischemic stroke.⁴ Relevant to our current work, resveratrol also staves off age-related degeneration of neuromuscular junctions (NMJs)⁵ and generally protects biological systems against oxidative stress.⁶ However, as an oral drug, a recent study indicates that dietary resveratrol (500 μ M) does not protect fruit flies (*Drosophila melanogaster*) from oxidative stress (10% H₂O₂) administered ad libitum.⁷ It has been proposed that resveratrol mediates some of its neuroprotective effects by scavenging free radicals in biological tissues.⁸ More recent evidence indicates that resveratrol also confers neuroprotective effects by modulating the expression/activity of specific neuroprotective proteins during acute physiological stress.⁹

Despite these promising activities, resveratrol is poorly suited as a potential oral drug. This is due, in part, to its intense metabolism by human cytochrome P450 and intestinal microbiota¹⁰ and an especially low oral bioavailability (< 1%).¹¹ However, resveratrol is also deficient from a drug design perspective. Its planar chemical structure is at odds with recent trends that seek to incorporate more sp³ centers into a molecule to allow for placement of appendages in an out-of-plane orientation with respect to one another. This idealized “three dimensional” arrangement in turn potentially increases complementarity to a binding site leading to improved target selectivity.¹² The lack of saturated carbons in resveratrol may explain its promiscuity in modulating so many different biochemical pathways thus potentially limiting its neuroprotective utility.¹³

In resveratrol, the two oxygen-bearing aryl rings are connected to one another by a two-carbon bridge in a *trans* orientation. We envisioned a resveratrol-inspired design in which one of the rings, along with the two-carbon bridge, is replaced by a bridged bicycle. In a thought experiment, one could consider that an aryl unit of resveratrol twists to form a bond (no electrocyclization is intended here) with the ethylene bridge leading to a concave shape (Figure 1). In this conception, the two-carbon bridge of the bridged bicycle serves as the structural equivalent of the vinyl group in resveratrol. While our compounds are not perfect structural equivalents of resveratrol, the inspiration to model the two-dimensional structure of resveratrol onto molecules containing a significant amount of three-dimensional structure led us to term this new class of compounds “resveramorphs.” Among other advantages, replacing an aromatic ring with a bridged bicycle increases the number of sp³ centers without increasing molecular flexibility through rotatable bonds.¹⁴ The resulting compounds are also chiral (though studied here as racemic mixtures) and can be synthesized as single diastereomers. As will be discussed in the present article, compounds based on our resveramorph conception appear to protect neurotransmission in a fruit fly model under oxidative stress, ultimately offering a new scaffold for future drug development.

RESULTS AND DISCUSSION

Following our previously reported synthesis strategy,¹⁵ we were able to access racemic compounds **1** and **2** (Figure 2) bearing the all-carbon resveramorph scaffold in five to six synthetic steps.¹⁶

To quantify tolerance of neurotransmission to acute oxidative stress, electrophysiological recordings from *Drosophila* larval preparations were conducted in a hemolymph-like 3 (HL3) saline containing 2.25 mM hydrogen peroxide (H₂O₂). Under these conditions, the application of low doses (0.5 nM and 1 nM) of resveramorph **1** significantly increased the amount of time until synaptic failure compared to control larvae that were only treated with saline containing 2.25 mM H₂O₂ (Figure 3).¹⁷ Additionally, compound **1** exerted stronger neuroprotective effects compared to a similar dosage of resveratrol [*P* < 0.001; Figure 3]. These results establish that resveramorph **1** possesses a neuroprotective effect that is measurably more potent than resveratrol.

For further analysis, we compared resveramorph **1** to resveratrol across a broad range of dosage concentrations (Figure 4). These data reveal that **1** possesses stronger neuroprotective effects compared to resveratrol at almost all dosages tested (0.5 nM to 25 nM).

A determination of the EC₅₀, or half-maximal effective concentration of **1**, in protecting neurotransmission from H₂O₂ exposure is approximately 0.21 nM. Importantly, resveramorph **1** appears to confer its neuroprotective effects in a dose-dependent manner (Figure 5). By contrast, our measurements of resveratrol revealed an unusual dose curve, which could not reliably be used to determine its EC₅₀. These results indicate that unlike the resveramorphs, resveratrol does not act as a neuroprotectant at the chosen dosages.

In addition to its well-characterized neuroprotective effects, resveratrol has been shown to modulate neurotransmission at glutamatergic Shaffer collateral-CA1 synapses within the mammalian hippocampus.¹⁸ Specifically, resveratrol inhibits excitatory post-synaptic potentials (EPSPs), which are comparable to excitatory junction potentials (EJP) at the *Drosophila* NMJ, by desensitizing postsynaptic glutamate receptors.¹⁸ To ensure that the results obtained using resveramorph **1** and resveratrol were not due to changes in the EJP, we performed additional control experiments, as previously described.¹⁹ We found that resveratrol (1 nM) and resveramorph **1** (1 nM) do not have a significant acute effect on the shape or amplitude of the EJP in the presence and absence of 2.25 mM H₂O₂ (see Supplementary Figure 1). We also observed resting membrane potential (RMP) and EJP amplitude decline in larvae treated with H₂O₂ alone and H₂O₂ with 1 nM resveramorph **1** (see Supplementary Figure 2). Interestingly, the administration of resveramorph **1** significantly slowed the decline of RMP, potentially contributing to the protection of synaptic transmission (see Supplementary Figure 2A).

The current project does not encompass a traditional structure/activity relationship (SAR) study. However, we sought to determine if the activity of resveramorph **1** would be sensitive to a profound structural modification that was easily accomplished synthetically. To this end, the acetyl groups present in resveramorph **2** were removed to reveal hydroxyl groups, which

are capable of acting as potent hydrogen bond donors. Not surprisingly, resveramorph **2** exhibited substantially different activity from **1**. At low doses (0.1 nM and 0.5 nM), resveramorph **2** did not prolong time to failure during H₂O₂ exposure (Figure 3). However, at higher doses, resveramorph **2** significantly increased time to synaptic failure during H₂O₂ exposure compared to control preparations [$P < 0.001$; Figure 3]. In contrast, resveramorph **1** exhibited strong neuroprotective effects under these conditions when administered at doses as low as 500 pM (Figure 3). Also, the EC₅₀ of compound **2** was calculated to 2.99 nM, which is over ten-fold higher than resveramorph **1** (Figure 5).

In conclusion, the present findings suggest that the resveramorph scaffold may serve as a promising starting point in the development of novel neuroprotective compounds. These studies clearly indicate dose-dependent protection of neurotransmission from acute oxidative stress. The protective property also appears to depend on the functional groups that adorn the resveramorph scaffold, setting the stage for more detailed SAR analysis. Our current hypothesis is that the compound inhibits K⁺ channel conductance in *Drosophila* larvae, significantly increasing synaptic transmission tolerance to acute oxidative stress. This hypothesis is based on our previous studies of the natural product pseudopterosin A,¹⁹ which, as the aglycon, has a similar molecular weight and functional group composition to resveramorph **1**. Importantly, the studies reported here together with the highly potent neuroprotective activity of resveramorph **1** justify future attempts to identify its biological targets and potentially more potent analogs. These studies are currently underway.

METHODS

Animals

Wandering third instar larvae ($\approx$ 110 hours old) from the fruit fly, *Drosophila melanogaster*, were utilized for all electrophysiological experiments described throughout this study. Larvae were reared at 25°C on 12 h : 12 h light-dark (LD) cycles in an incubator. Only w¹¹¹⁸ larvae were utilized in this study; these were obtained from Dr. Gregory Macleod's lab.

Electrophysiology

All electrophysiological experiments were performed as previously described with the exception of the resistance of the intracellular electrodes (60–90 M Ω).¹⁹ Excitatory junction potentials (EJPs) were elicited via repetitive stimulation (0.3 ms pulses delivered suprathreshold at a frequency of 1 Hz) and measured until synaptic failure occurred, which was characterized by an EJP amplitude of < 1 mV being recorded. Electrophysiological recordings from larval preparations were conducted in hemolymph-like 3 (HL3) saline containing 2.25 mM H₂O₂. Preparations that took a greater amount of time to reach synaptic failure in HL3 saline containing 2.25 mM H₂O₂ possessed a higher tolerance for acute oxidative stress compared to preparations that took a lesser amount of time to reach stimulus-induced synaptic failure. HL3 saline containing 1.5 mM Ca²⁺ and 20 mM Mg²⁺ was used as the recording saline throughout this study. HL3 has been utilized by other research groups to record EJPs.²⁰ Intracellular recordings of resting membrane potential were taken from *Drosophila* larval muscle 6.

Pharmacology

Resveratrol, H₂O₂, and all other reagents utilized to make HL3 saline were obtained from Sigma-Aldrich Inc. (St. Louis, MO, USA). All test compounds and H₂O₂ were applied to the *Drosophila* larval NMJ preparation as previously described by Caplan et al.¹⁹ However, the compounds utilized in the Caplan work were dissolved in dimethyl sulfoxide (DMSO), whereas the compounds utilized in the present study are water-soluble at the concentrations used and thus were dissolved in ddH₂O.

Statistics

The statistics utilized in the vertical bar charts and line graphs were performed as previously described with the exception of the error bars being presented as mean $\pm$ SEM instead of mean $\pm$ SD.¹⁹ The EC₅₀, or half maximal effective concentration, of each test compound was determined by plotting the log₁₀(compound dose) against the corresponding average percentage of the maximal effectiveness, as previously described.²¹ A line was drawn to connect all of the data points for each compound; the point on the line at which a compound reached 50% efficacy was determined for each compound, and the inverse log of this value was calculated and determined to be the EC₅₀ dosage for resveramorph **1**, resveramorph **2**, and resveratrol (see Supplementary Equations 1).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
The resveramorph concept.

Figure 2.
Resveramorph compounds **1** and **2**.

Figure 3.

Resveramorph **1** protects synaptic function during acute oxidative stress at low doses. Resveramorph **1** significantly increased time to synaptic failure at the 0.5–5 nM dosage range compared to control and resveratrol-treated preparations [one-way ANOVA, $F(13, 62) = 25.04$, $P < 0.001$]. Resveramorph **2** similarly increased time to synaptic failure starting at the 5.0 nM dosage [$P < 0.001$]. $N = 5 - 9$ larvae per group. Letters in histogram bars represent statistical significance, where different letters indicate statistically significant differences (Holm-Šidák, $P < 0.001$) and identical letters indicate non-significance. The letter assignments start with “A” representing the highest mean, followed by “B” indicating the next highest, and so forth. All vertical bar charts are presented as mean $\pm$ SEM.

Figure 4.

Dose-response curve of resveramorphs **1**, **2**, and resveratrol. N = 6 – 21 larvae for each dosage tested.

Figure 5.

EC₅₀ determination of resveramorph 1, resveramorph 2, and resveratrol. The EC₅₀ of resveramorph 1 was calculated to be 0.21 nM, whereas the EC₅₀ of resveramorph 2 was calculated to be 2.99 nM. N = 6 – 21 larvae for each dosage tested.