A Scalable Biomimetic Synthesis of Resveratrol Dimers and Systematic Evaluation of their Antioxidant Activities**

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Abstract: An efficient synthetic route to the resveratrol oligomers quadrangularin A and pallidol is reported. It features a scalable biomimetic oxidative dimerization that proceeds in excellent yield and with complete regioselectivity. A systematic evaluation of the natural products and their synthetic precursors as radical-trapping antioxidants has revealed that, contrary to popular belief, this mode of action is unlikely to account for their observed biological activity.

The resveratrol (1) oligomers are a diverse set of polyphasic natural products, the stress-factor-induced biosynthesis of which constitutes an important chemical defense mechanism in many plants.1 Numerous reports of their health-promoting potential have generated widespread interest in these compounds,2 much of which has been focused on their capacity as radical-trapping antioxidants (RTAs). However, systematic studies of this activity are lacking, since investigators often assay only the position of their thermodynamic equilibrium with oxidants in solution, and not whether the relevant radical-trapping reactions are kinetically competitive under physiological conditions.3

With their intriguing biological activities and fascinating molecular architectures, the resveratrol oligomers have inspired a number of synthetic endeavors.4 Early work aimed to replicate nature’s approach, which is believed to involve single-electron oxidative coupling processes. While construction of these molecules in such a fashion would support their proposed biogenesis, efforts to date have resulted in low yields and/or complex mixtures of products. Recognizing this shortcoming, Snyder and co-workers developed powerful de novo strategies towards resveratrol dimers5a–c,d and higher-order oligomers.5e–h These impressive studies prompted a number of subsequent syntheses, including innovative approaches from the Nicolaou/Chen,6a Sarpong,6b and Studer6c,d groups. Following this precedent, we sought to rapidly access chemical diversity from common intermediates in a controlled fashion, and to address lingering questions regarding the role of resveratrol-derived natural products as bioactive small molecules. The total synthesis of pallidol (2)8 and quadrangularin A (3),9 and the systematic evaluation of their RTA activities, are reported herein.

Owing to the electron-rich nature of resveratrol (E_{ox} < 0.7 V vs Ag/AgCl),10 we reasoned that its oxidation by a visible-light photoredox catalyst would be feasible,11 thus enabling catalytic generation of the requisite phenoxyl radical for dimerization. In considering our reaction design, we drew inspiration from the seminal report of Hou and Li,5a in which they demonstrated that tert-butyl resveratrol derivative 4a (Scheme 1) could be dimerized regioselectively. However, as a result of apparent issues with oxidation and oligomerization of product dimers in the reaction mixtures, we instead elected to use the benzyl-protected resveratrol derivative 4b. Preliminary efforts using photoredox catalysis resulted in mixtures of the dimeric isomers 5, 6, and 8 (Scheme 1) in yields highly dependent on the solvent and the presence/absence of base.12 Extensive reaction optimization13 revealed that the phenoxyde of 4b14 undergoes a remarkably efficient and selective dimerization to bis-quinone methide 5 (60–80%) under aerobic conditions, but a significant decrease in selectivity and reproducibility was observed on scale-up. To overcome this limitation, we exchanged O2 for ferrocenium hexafluorophosphate ([FeCp2]PF6)15 as the oxidant, which reliably provided 5 in more than 95% yield on decagram scale. To the best of our knowledge, this represents the highest yield and largest scale yet reported for oxidative dimerization of a resveratrol derivative.14

The dimerization produces an inseparable mixture of meso and dl diastereomers 5/S5, and the relative configuration of the vicinal stereogenic centers has important (and likely biogenically relevant) consequences for the synthesis of pallidol (2) and quadrangularin A (3; Scheme 1). The dl diastereomer of 5 has the correct relative configuration to undergo two sequential cyclizations to provide the [3.3.0] ring system present in pallidol (2). By contrast, after the initial Friedel–Crafts reaction of meso-5, the anti/anti configuration of product indane 6 precludes a second cyclization event owing to the thermodynamically unfavorable formation of a trans-fused bicyclo[3.3.0]octane.16 Addition of BF3·OE3 to a dilute solution of 5/S at −78°C gave the desired pallidol derivative 7a in 43% yield of isolated product. The remaining mass balance was 6 (45%) and a complex mixture of oligomerized material. Hydrogenolysis of 7a followed by
removal of the tert-butyl groups through a retro-Friedel–Crafts reaction\(^\text{[7]}\) provided pallidol (2; 76%, 2 steps).

Note that in quadrangularin A (3), the stereochemical information at one of these centers is ultimately destroyed, which enabled the development of a one-pot dimerization/base-mediated isomerization that provides 8 in nearly quantitative yield from 4b (Scheme 1). The tautomerization is diastereoregovergent, giving exclusively the (E)-stilbene isomer of 8 as a racemic mixture. Quadrangularin A (3) could be accessed from 8 in one, two, or three steps in 51%, 73%, and 55% yields, respectively. Here, Lewis acids serve to promote cyclization, global debenzylation, and removal of the tert-butyl groups, while Me\(_5\)-benzene was essential in preventing oxygen-to-carbon transfer of the transiently formed benzylic cations.\(^\text{[23]}\)

Bis-quinone methides such as 5 have long been invoked as intermediates in the biosynthesis of resveratrol oligomers, but their existence remains unsubstantiated. Although seemingly trivial, the use of alkylated resveratrol derivative 4b proved to be crucial to the success of this strategy since it tempers the redox potential of the stilbene, prevents the formation of regioisomeric dimers or undesired oligomers, and improves substrate solubility in nonpolar solvents, an effect critical for substrate solubility in nonpolar solvents, an effect critical for regioisomeric dimers or undesired oligomers, and improves substrate solubility in nonpolar solvents, an effect critical for product chemoselectivity. Furthermore, despite the lability generally associated with quinone methides,\(^\text{[24]}\) which are typically generated and reacted in situ,\(^\text{[25]}\) dimers 5, 6, and 8 were found to be bench stable and could be purified by flash chromatography. This stability proved instrumental in the tautomerization of 5/5’ to 8 under strongly basic conditions while maintaining “switchable” Friedel–Crafts reactivity under Lewis acidic conditions.

With these compounds in hand, we compared their peroxyl radical trapping activities in homogenous organic solution, lipid bilayers, and cell culture. Peroxyl radicals mediate lipid peroxidation, a process that has been linked to myriad degenerative diseases that resveratrol has been suggested to prevent.\(^\text{[20]}\) Our studies in solution (chlorobenzene) were carried out with the peroxyl radical clock methodology,\(^\text{[21]}\) a kinetic competition method by which the rate constant for the reaction of a given antioxidant with a peroxyl radical (\(k_{inh}\)) can be obtained from the dependence of lipid autoxidation products on antioxidant concentration.\(^\text{[22]}\) While the poor solubility of the natural products precluded direct determinations of their \(k_{inh}\) values, the benzylated synthetic precursors were soluble and values of \(k_{inh}\) were readily determined (Table 1, see footnote [i]). These results reveal that \(k_{inh}\)-quadrangularin A 9 (6.8 \times 10^4 m^{-1} s^{-1}) is slightly more reactive than \(k_{inh}\)-resveratrol 4b (5.9 \times 10^4 m^{-1} s^{-1}), while \(k_{inh}\)-pallidol 7a (2.5 \times 10^4 m^{-1} s^{-1}) is less reactive. The \(k_{inh}\) value for 7a, which lacks an activating alkene, is indistinguishable from that of the common synthetic antioxidant BHT (once the two-fold statistical advantage of pallidol is considered). The reactivities of the authentic natural products could be estimated from the relative rate constants given above and the known rate constant for \(k_{inh}\) value for \(\alpha\)-tocopherol (\(k_{inh}\)-\(\alpha\)-TOH, \(k_{inh}\)-\(\alpha\)-TOH, \(k_{inh}\) = 3.2 \times 10^4 m^{-1} s^{-1}), a ubiquitious radical-trapping antioxidant in nature,\(^\text{[23]}\) thus suggesting that 1, 2, and 3 are unlikely to owe their biological activities to RTA activity.

The RTA activities of the corresponding dealkylated compounds were then determined in the lipid bilayers of unilammelar vesicles of egg phosphatidylcholine, again by using a competitive kinetics approach,\(^\text{[24]}\) but this time with a fluorogenic \(\alpha\)-TOH derivative.\(^\text{[25]}\) The relative rate constants determined this way (Table 1) clearly indicate an inversion in the relative reactivity between the natural products and their,

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**Scheme 1.** The total synthesis of pallidol (2) and quadrangularin A (3): a) KHMDS (1.05 equiv), [FeCp]PF\(_6\) (1.05 equiv), THF, 0°C, 30 min, 95%; b) BF\(_3\)·OEt\(_2\) (2 equiv), CH\(_2\)Cl\(_2\), –78°C, 40 min, 88%; 6/7a ca. 1:1; c) Pd/C (30 wt%), H\(_2\) (1 atm), 3:2 EtOAc/MeOH, RT, 12 h, 95%; d) AlCl\(_3\) (12 equiv, 2.25 M in CH\(_2\)NO\(_2\)), toluene, 60°C, 30 min, 80%; e) KHMDS (1.05 equiv), [FeCp]PF\(_6\) (1.05 equiv), THF, 0°C, 30 min, then KHMDS (1.25 equiv), 15 min; f) BF\(_3\)·OEt\(_2\) (2 equiv), CH\(_2\)Cl\(_2\), –78°C, 5 min, 90% over 3 steps; g) BCl\(_3\) (12 equiv, Me\(_5\)-benzene (20 equiv), CH\(_2\)Cl\(_2\), –78°C, 2 h, 82%; h) AlCl\(_3\) (12 equiv, 2.25 M in CH\(_2\)NO\(_2\)), toluene, 60°C, 30 min, 80%; i) Me\(_5\)-benzene (40 equiv, AlCl\(_3\) (25 equiv, 0.6 M in CH\(_2\)NO\(_2\)) slow addition, CH\(_2\)Cl\(_2\), 0°C, then 60°C, 1 h, 88%; 5:1 mixture of quadrangularin A (3) to its internal alkene isomer. Bn = benzyl, tBu = tert-butyl, taut. = tautomeration, KHMDS = potassium hexamethyldisilazide, [FeCp]PF\(_6\) = ferrocenium hexafluorophosphate, THF = tetrahydrofuran, Me\(_5\)-benzene = pentamethylbenzene.
Radical-trapping antioxidant activity of resveratrol, pallidol, and quadrangularin A and their synthetic precursors in solution (chlorobenzene), lipid bilayers (of egg phosphatidylcholine), and cell culture (human erythroblasts). Data are also given for the benchmark phenolic antioxidants α-tocopherol and BHT (2,6-di-tert-butyl-4-methylphenol).

<table>
<thead>
<tr>
<th>Solution</th>
<th>Lipid Bilayers</th>
<th>Cells</th>
<th>Cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>resveratrol (1)</td>
<td>k_{\text{obs}} \text{[PhCl]} [M^{-1} s^{-1}]</td>
<td>k_{\text{obs}} \text{[ROO_{\text{aq}}]} [M^{-1} s^{-1}]</td>
<td>EC_{50} [μM]</td>
</tr>
<tr>
<td>2.0 × 10^{-10}</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>12.6 ± 0.9</td>
</tr>
<tr>
<td>pallidol (2)</td>
<td>8.5 × 10^{-10}</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>quadrangularin A (3)</td>
<td>2.3 × 10^{-10}</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>α-tocopherol (α-TOH)</td>
<td>3.2 × 10^{-10}</td>
<td>1.8 ± 0.2</td>
<td>2.0^{[b]}</td>
</tr>
<tr>
<td>tBu-resveratrol (4a)</td>
<td>(5.9 ± 0.8) × 10^{-10}</td>
<td>17.9 ± 3.3</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>tBu-pallidol (7b)</td>
<td>(2.1 ± 0.3) × 10^{-10}</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>tBu-quadrangularin A (10)</td>
<td>(6.2 ± 0.9) × 10^{-10}</td>
<td>7.3 ± 0.6</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>BHT</td>
<td>(2.2 ± 0.1) × 10^{-10}</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

[a] Second-order rate constant for the reaction with (linoelyl) peroxyl radicals. [b] Second-order rate constant for the reaction with peroxyl radicals relative to the fluorescent probe H_{2}B-PMHC. [c] Number of peroxyl radicals trapped per molecule of test compound. [d] Initiated with MeOAMVN (lipid soluble) and AAPH (water soluble), respectively. [e] Determined at 30 °C from the inhibited autoxidation of styrene, see Ref. [22]. [f] Since two equivalent units exist on the same molecule, the observed rate constant has been divided by 2. [g] Estimated from the value for resveratrol and the relative reactivities of the corresponding synthetic precursors (tert-butylated analogues). [h] From Ref. [24b]. [i] Determined at 37 °C by using the peroxyl radical clock methodology on resorcinol ring protected (benzyl) compounds.

Interestingly, 4a is the most reactive radical-trapping antioxidant we have yet to study in cell culture (even more so than the naphthyridinols), and its EC_{50} value under the experimental conditions was 200-fold lower than the corresponding EC_{50} values for inducing cell death—a much larger margin than for the natural products. Curiously, although the tert-butyllated pallidol 7b was inefficient at trapping radicals in lipid bilayers, it was surprisingly potent against lipid peroxidation in cell culture, thus suggesting that it may operate through a different mechanism, possibly one involving the induction of the expression of genes controlled by the antioxidant response element (ARE). Further investigation is required to understand the mechanism of the comparatively poor antioxidant activity of the natural products, which is almost certainly not due to their RTA activity.

In summary, we have developed a highly efficient and scalable oxidative dimerization of tert-butyllated resveratrol derivative 4b to generate uniquely stable and synthetically versatile quinone methides. The “built-in” reactivity of these bioinspired intermediates was leveraged in the synthesis of dimeric natural products pallidol (2; 6 steps, 26% yield) and quadrangularin A (3; 5 steps, 54% yield), which represents the most efficient syntheses of these dimers to date. Through systematic evaluation of the antioxidant activities of the natural products and their synthetic precursors, we ascertained that the natural products examined in this study are not kinetically competitive as radical-trapping antioxidants under biologically relevant conditions. Preliminary data suggest that this biomimetic synthetic strategy will be applicable to the synthesis of higher-order resveratrol oligomers. Efforts to delineate these syntheses and to better understand the dichotomous behavior of the natural products and their tert-butyllated precursors in homogenous solution versus lipid bilayers and cultured human cells are underway.

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Table 1: Radical-trapping antioxidant activity of resveratrol, pallidol, and quadrangularin A and their synthetic precursors in solution (chlorobenzene), lipid bilayers (of egg phosphatidylcholine), and cell culture (human erythroblasts). Data are also given for the benchmark phenolic antioxidants α-tocopherol and BHT (2,6-di-tert-butyl-4-methylphenol).
Keywords: antioxidants - biomimetic synthesis - quinone methides - resveratrol - total synthesis


See Supporting Information for experimental details.

[12] For the synthesis of the stilbene in the absence of the photocatalyst suggested that the phenoxide of 4b may be acting as a singlet-oxygen sensitizer. When the reaction was run in the dark and open to air in the absence of the photocatalyst, the yield of the isolated dimer 5 increased to 55%. Taken together, these results suggest that the conjugate base of 4b was reducing O2 under the reaction conditions.

[26] It has been suggested that resveratrol can prevent lipid peroxidation by the same mechanism by which ascorbate does: the regeneration of α-TOH from α-TO. Valgimigli (Ref. [28]) has shown this equilibrium to be unfavourable, and we expect the same for 2 and 3 given their similar reactivities.
[28] Fang et al. (Ref. [30]) reported that resveratrol possesses only 3-fold lower inhibitory activity than α-TOH for the peroxidation of linoleic acid in CTAB and SDS micelles initiated with a water-soluble aza initiator. Obviously there is a difference between micelles composed of these detergents and liposomes composed of a single bilayer formed from physiological lipids.