

Development of a novel antioxidant based on a dimeric dihydroisocoumarin derivative

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Supplementary data	Supplementary data is available at https://doi.org/10.1016/j.tetlet.2021.153176 .
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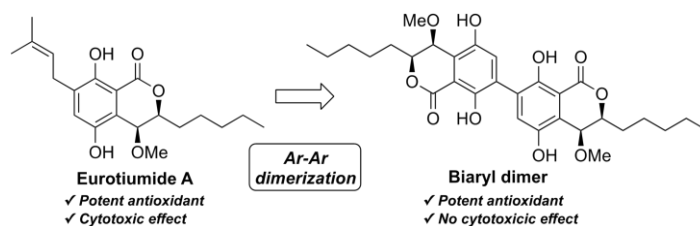
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This work is dedicated to Prof. Dale Boger on the occasion of his 2020 Tetrahedron Prize for Creativity in Organic Chemistry.

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ABSTRACT

A biaryl dimer based on eurotiumide A, a dihydroisocoumarin-type natural product possessing a 1,4-hydroquinone skeleton, was synthesized herein. The radical scavenging activity and cytotoxicity were evaluated. The prepared biaryl dimer and eurotiumide A displayed comparable potent antioxidant activities to those of α -tocopherol and edaravone. However, the biaryl dimer did not show any cytotoxicity against murine colon and human myeloma cells.

1. Introduction

In biological systems, oxidative stress is constantly induced by various reactive oxygen species (ROS). ROS play important roles in controlling and inducing various physiological events¹. While moderate amounts of ROS in living cells are beneficial to their function, excessive oxygen radicals lead to the oxidation of lipids, proteins, sugars, and nucleic acids, resulting in cellular damage². Thus, the disruption of adequately controlled oxidative stress by an imbalance between the generation and disappearance of ROS increases the risk for a variety of diseases such as carcinogenesis³, autoimmune disease⁴ as well as cardiovascular⁵, and neurodegenerative disorders⁶. As excessive ROS damages cellular constituents, numerous studies have aimed to develop radical scavenging agents for the rapid reduction of the generated radical species. For instance, an example of such a scavenging agent is the neuroprotective drug edaravone (**1**, 3-methyl-1-phenyl-2-pyrazolin-5-one)⁷ (Fig. 1A). This has been used for the treatment of acute cerebral infarction. The cerebral ischemia-reperfusion generates large amounts of ROS in the microenvironment of brain cells. Rapid treatment resulting in scavenging of ROS is crucial to prevent serious brain damage following the onset of ischemic stroke⁸. Edaravone (**1**) has been shown to suppress oxidation in the brain and trap the generated ROS. Hence, potent and fast-acting antioxidative agents are useful in clinical practice and have attracted considerable attention in the field of medicinal chemistry⁹.

1,4-Hydroquinone is a representative scaffold that has been found to exhibit potent antioxidative activity. It is present in important antioxidants, such as α -tocopherol (**2**). This functional scaffold displays antioxidative activity by accepting two radicals

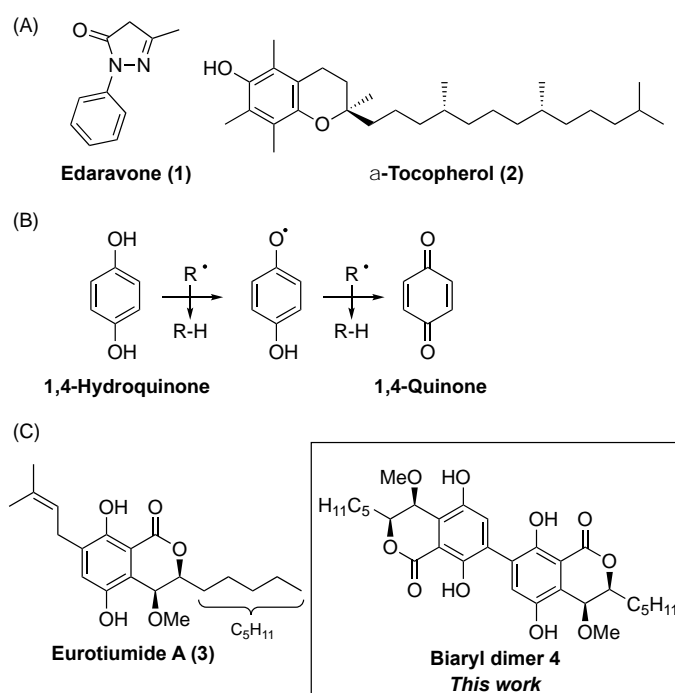
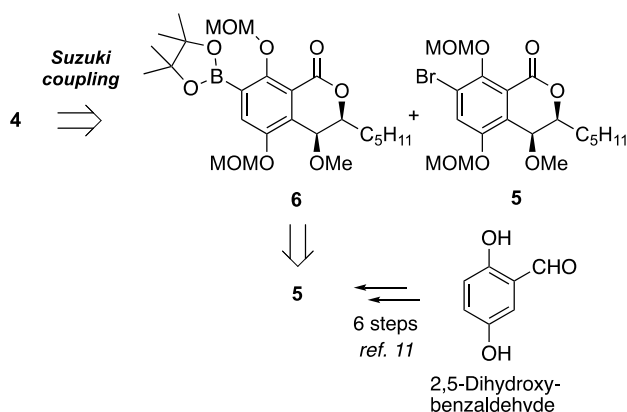


Figure 1. (A) Structures of general antioxidants. (B) Mechanism of antioxidant activity with 1,4-hydroquinone. (C) Structures of eurotiumide A (**3**) and biaryl dimer **4**.

(R-), which is followed by the formation of a quinone moiety (Fig. 1B). We have continued our research on the total synthesis and medicinal chemistry of eurotiumides, which are dihydroisocoumarin-type natural products isolated from Gorgonian-derived fungus, *Eurotium* sp. XS-200900E6¹⁰. We achieved the asymmetric total syntheses of eurotiumide A (**3**), B¹¹, F, and G¹². Moreover, we previously developed a novel antimicrobial agent¹³ based on **3**, which was effective against methicillin-resistant *Staphylococcus aureus* and demonstrated that eurotiumides are important drug seed molecules. Eurotiumides, specifically **3** and eurotiumide B, possess a 1,4-hydroquinone skeleton, indicating that the compounds should exhibit potent antioxidative activity. The above findings encouraged us to develop novel antioxidants based on eurotiumides. Although we previously established the asymmetric synthetic routes to both **3** and eurotiumide B, **3** was selected in the present work due to the availability of the raw material. In this study, we report the development of a novel antioxidative agent based on a dihydroisocoumarin-type natural product.

In developing the novel antioxidative agent, we focused on the dimerization of eurotiumide A (**3**). The dimerization of the dihydroisocoumarin skeleton would increase the functionality in a molecule. Furthermore, we speculated that the radical scavenging activity would be enhanced if the 1,4-quinone moiety produced after two oxidations could stabilize the phenoxy radical of another 1,4-hydroquinone, facilitating the formation of a phenoxy radical. Accordingly, we intended to connect the main skeletons of eurotiumide A to prepare the biaryl dimer **4** (Fig. 1C). The subsequent aim was to assess the antioxidant activity and cytotoxicity of **4** and **3** using 1,1-diphenyl-2-picrylhydrazyl (DPPH)¹⁴ and a highly water-soluble tetrazolium salt¹⁵.

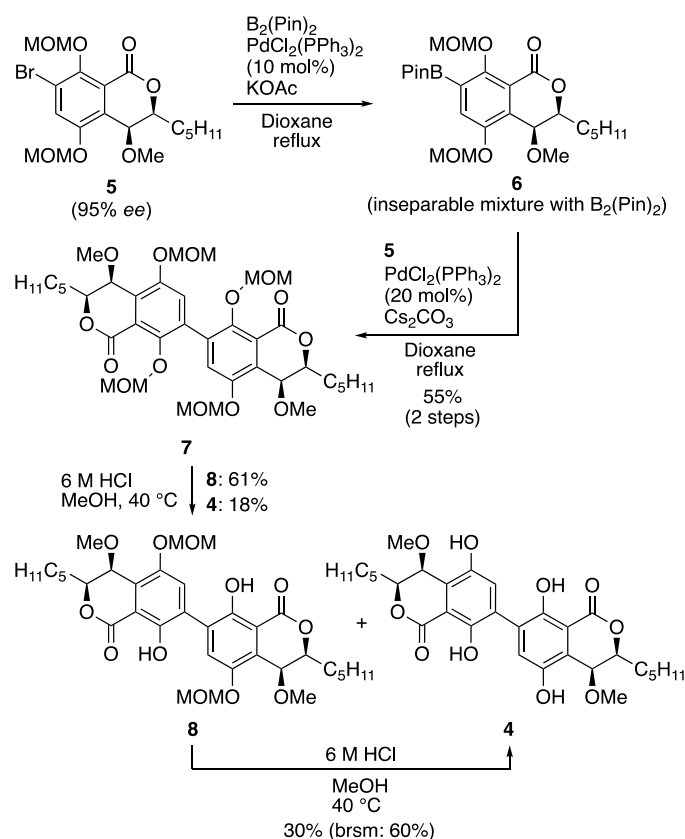
Our retrosynthetic analysis of **4** is shown in Scheme 1. Biaryl dimer **4** could be obtained by the Suzuki–Miyaura cross-coupling reaction between bromo intermediate **5** and boronic acid pinacol ester **6** followed by global deprotection of tetra methoxymethyl (MOM) group. The Miyaura–Ishiyama borylation of **5** would give **6**. Additionally, **5** could be easily prepared from commercially available reagents according to a previously reported procedure¹¹.



Scheme 1. Retrosynthesis of biaryl dimer **4**. MOM = methoxymethyl

Our synthesis commenced with the transformation of **5** to **6** (Scheme 2). The bromo intermediate (+)-**5** (95% ee) was obtained from commercially available 2,5-dihydroxybenzaldehyde in six sequential steps. Considering the presence of a lactone scaffold, we selected the Pd-catalyzed borylation instead of lithiation to install the boron moiety¹⁶. Treatment of **5** with an excess bis(pinacolate)diborane in the presence of PdCl₂(PPh₃)₂ and potassium acetate afforded the desired boronic acid pinacol ester **6** as the major product. However, it was hard to remove unreacted

bis(pinacolate)diboron completely. The inseparable mixture of **6** and the reagent was partially purified by silica gel column chromatography prior to conducting the Suzuki–Miyaura cross-coupling reaction. Intermediate **5** and the mixture of **6** were treated with cesium carbonate and a catalytic amount of PdCl₂(PPh₃)₂ in dioxane at reflux to afford the desired biaryl product **7** in 55% yield from **5**. Notably, all reagents were successfully separated from **7**. Subsequently, acidic treatment of **7** gave the desired biaryl compound **4** in a low yield of 18%.¹⁷ The predominant product of this reaction was the di MOM deprotected compound **8**. Following the separation of **4** and **8**, the remaining MOM moieties in **8** were deprotected by treatment with a 6 M aqueous solution of HCl at 40 °C to afford **4** (brsm: 60%).



Scheme 2. Total synthesis of biaryl dimer **4**. BPin = 4,4,5,5-tetramethyl-1,3,2-dioxaborolyl.

Having succeeded in the synthesis of the desired biaryl dimer **4**, we then conducted a DPPH-radical scavenging assay using eurotiumide A (**3**) and biaryl dimer **4** (Fig. 2). Edaravone (**1**) and α -tocopherol (**2**) were used as positive controls. As described above, since rapid administration of an adequate amount of an antioxidant is important in the treatment of cerebral ischemia-reperfusion, we measured the DPPH-radical scavenging ratio of each compound at different concentrations (5, 10, 20, and 40 μ M) after 15 min. The results of the DPPH-radical scavenging assay are summarized in Figure 2. The antioxidant activity of **3** has not been previously examined. Expectedly, **3** displayed antioxidant activity against DPPH radicals. The radical scavenging activity was concentration-dependent. Moreover, the potency of **3** was comparable to those of **1** and **2**. Biaryl dimer **4** also displayed DPPH-radical scavenging activity. Although after 15 min the antioxidant activity of **4** appeared to be more potent than those of **1**, **2**, and **3**, the formation of the biaryl moiety in the main skeleton of **3** did not enhance the antioxidant activity as much as we had initially expected. We consider that this was attributed to the high stability of a phenoxy radical of another 1,4-hydroquinone

scaffold containing a quinone moiety generated after three oxidation reactions. To investigate this further, we conducted density functional theory (DFT) calculations to compare the phenolic O–H dissociation energies as well as the stabilities of phenoxy radicals formed after the oxidation reactions of **3** and **4** (Fig. 3). The calculation results showed that the relative bond dissociation energy (BDE)¹⁸ of the first O–H dissociation step for dimer **4** (0.4 kcal/mol) was similar to that of **3**. We also considered further oxidation steps for dimer **4**, which displayed comparable BDEs even until the third oxidation step (1.2 kcal/mol) to generate a radical of **9**, having a quinone moiety. Moreover, the singly occupied molecular orbital (SOMO) energy of the radical of **9** significantly decreased (–12.1 kcal/mol compared to **3**), indicating that the radical is generated more easily showing its potential antioxidant ability due to the quinone moiety. Because of this enhanced stability of the radical of **9**, the fourth oxidation might not proceed well. Combining the experimental and DFT calculation results, we can modulate the antioxidant activity by adjusting the SOMO energy of the generated phenoxy radical through side chain conversion of 1,4-hydroquinone scaffold.

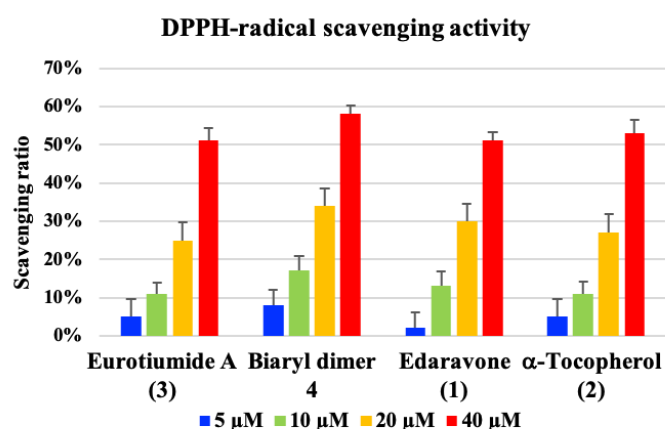


Figure 2. Concentration-dependent manner of DPPH-radical scavenging activities of four test compounds after 15 minutes. The data show the mean \pm S.D.

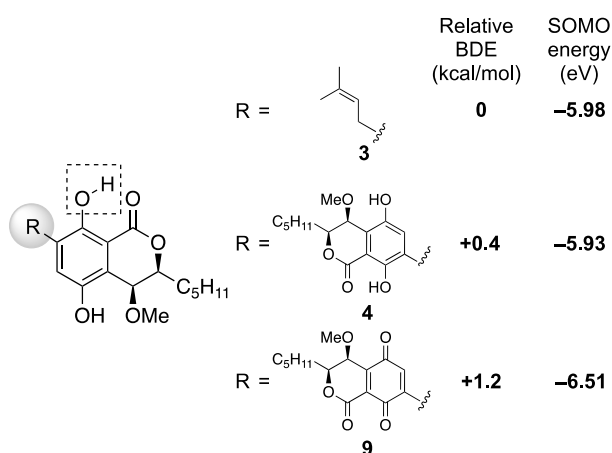


Figure 3. Relative Bond dissociation energies (BDEs) and SOMO energies of the phenolic O–H of compounds **3**, **4**, and **9** calculated using the density functional theory (B3LYP/6-31+g(d,p)).

We subsequently analyzed the cytotoxicity of eurotiumide A (**3**) and biaryl dimer **4** against a murine colon carcinoma cell line (Colon-26) and a human myeloma cell line (RPMI8226), respectively (Fig. 4). Etoposide was used as a positive control and displayed concentration-dependent cytotoxicity against the two

cell lines. Notably, **3** did not show the cytotoxicity against the Colon-26 cells up to 12.5 μ M, however, at 25 μ M, it exhibited cytotoxicity against almost half of analyzed Colon-26 cells. Moreover, at 12.5 μ M, almost all RPMI8226 cells were affected. In contrast, **4** did not display any cytotoxicity against the two cell lines even at the cytotoxic expression concentration of **3**. Specifically, all cells survived treatment with **4** at a concentration of 50 μ M. In general, the 1,4-hydroquinone structure is potentially toxic due to its transformation to 1,4-quinone, a strong Michael acceptor. In comparison with the prenyl side chain of eurotiumide A (**3**), the reactivity of biaryl dimer **4** might be reduced by the steric hindrance around the 1,4-hydroquinone scaffold. The mechanism of action for cytotoxicity of **4** needs to be further investigated.

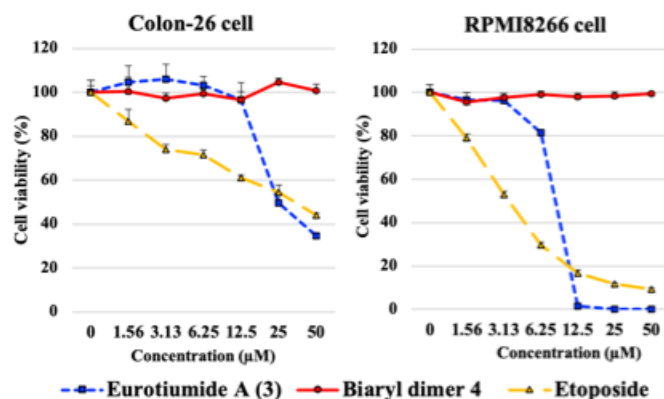


Figure 4. Comparison of the cytotoxicity of eurotiumide A (**3**) and biaryl dimer **4** against Colon-26 and RPMI 8226 cells. Etoposide was used as a positive control. The data show the mean \pm S.D.

Based on the results of the DPPH-radical scavenging assays, it was determined that both eurotiumide A (**3**) and biaryl dimer **4** showed potent antioxidant activity. However, the cytotoxic activity of **3** was observed at concentrations above 10 μ M. It is also noteworthy that there was only a slight difference between the concentration needed for the exertion of antioxidant effects and cytotoxic activity. This implied that eurotiumide A (**3**) exhibited low potential for the development of an effective antioxidant due to concerns regarding cellular toxicity. Conversely, even at large doses, **4** shows a rapid and potent antioxidant activity and does not exhibit cytotoxic activity. In particular, the antioxidant without the cytotoxicity has a great advantage for the treatment of brain in terms of brain protection. This is because non-specific cytotoxicity to brain cells in health and diseased areas of the brain can lead to undesired side effects. Biaryl dimer **4** has a potential to scavenge radicals generated in the brain without damaging the brain, suggesting that it could be applied as an effective brain treatment antioxidant.

In this work, we prepared biaryl dimer **4** by directly linking the aromatic ring moieties of a dihydroisocoumarin-type natural product, namely eurotiumide A (**3**). The antioxidant activity and cytotoxicity of both **3** and **4** were examined. We demonstrated that both the natural product **3** and its derivative **4** displayed potent antioxidant activity. However, **4** did not show any cytotoxicity against adherent and suspended cell lines even at high concentration. Further research is required to confirm the

sufficient antioxidant activity against other ROS, such as Super oxides.

Acknowledgments

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- Even using *t*BuLi at $-78\text{ }^{\circ}\text{C}$, nucleophilic attack to the lactone moiety happened prior to halogen-lithium exchange to afford the ring opening byproduct.
- In our previous total synthesis of eurotiumide A (**3**), we observed the C4 epimerization by extending the reaction time and higher concentration of HCl. Therefore, we stopped the reaction before occurring the C4 epimerization even that the MOM deprotection was uncompleted.
- The bond dissociation energy (BDE) was calculated by the following: $\text{BDE} = \text{enthalpy of } \text{O}^{\cdot} + \text{enthalpy of } \text{H}^{\cdot} - \text{enthalpy of neutral molecule}$