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# Enhanced radical scavenging activity of a procyanidin B3 analogue comprised of a dimer of planar catechin

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ABSTRACT

Proanthocyanidins are oligomers of catechins that exhibit potent antioxidative activity and inhibit binding of oxidized low-density lipoprotein (OxLDL) to the lectin-like oxidized LDL receptor (LOX-1), which is involved in the onset and development of arteriosclerosis. Previous attempts aimed at developing proanthocyanidin derivatives with more potent antioxidative activity and stronger inhibition for LOX-1 demonstrated the synthesis of a novel proanthocyanidin derivative (**1**), in which the geometry of one catechin molecule in procyanidin B3 was constrained to a planar orientation. The radical scavenging activity of **1** was 1.9-fold higher than that of procyanidin B3. Herein, we synthesized another procyanidin B3 analogue (**2**), in which the geometries of both catechin molecules in the dimer were constrained to planar orientations. The radical scavenging activity of **2** was 1.5-fold higher than that of **1**, suggesting that **2** may be a more effective candidate than **1** as a therapeutic agent to reduce oxidative stress induced in arteriosclerosis or related cerebrovascular disease.



Atherosclerosis is the major cause of cerebrovascular and cardiovascular diseases, which are among the most common causes of death in the industrial world. A number of studies aimed at reducing atherosclerosis have investigated the use of antioxidants, as well as anti-inflammatory and cholesterol-lowering agents in both animal models and human trials. While antioxidants such as vitamin C, vitamin E, and β-carotene demonstrated success in animal studies, their efficacy in human clinical studies remains inconclusive.[1] Atherosclerosis is usually characterized by endothelial dysfunction, accumulation of inflammatory and vascular smooth muscle cells, and extracellular deposition of lipids and fibrous tissue.[2] Among these, endothelial dysfunction, which is considered an early marker for atherosclerosis, contributes to the initiation and progression of atherosclerosis, and can also be considered an independent vascular risk factor.[3]

Endothelial dysfunction in the pathogenesis of atherosclerosis is caused by oxidized low-density lipoprotein (OxLDL), and is exerted via activation of the lectin-like oxidized low-density lipoprotein receptor (LOX-1).[4] LOX-1 is expressed at relatively high levels in pathological conditions such as hypertension,[5] hyperlipidaemia,[6] diabetes,[7] and arteriosclerosis.[8] LOX-1 is found not only on endothelial cells but also macrophages[9] and platelets,[10] among other areas. OxLDL is a LOX-1 binding ligand formed during the oxidation of LDL.[4] The binding of OxLDL to LOX-1 activates NADPH oxidase, causing rapid generation of reactive oxygen species (ROS),[11] and resulting in further production of OxLDL. Furthermore, OxLDL-mediated LOX-1 expression has been reported to stimulate the NF-κB signaling pathway, which is known to increase the expression of pro-inflammatory genes, to promote arteriosclerosis.[12]

Proanthocyanidins, oligomers of catechin, are natural compounds found in high concentrations in fruits, vegetables, wine, tea, nuts, and seeds.[13,14] The properties of proanthocyanidins are largely modulated by the degree of polymerization. That is, both the antioxidative activity and inhibitory activity of OxLDL binding to LOX-1 are enhanced with an increased degree of catechin polymerization.[15,16] Indeed, monomers of catechin and epicatechin do not exhibit any LOX-1 inhibitory activity.[16] When developing arteriosclerosis chemopreventive agents, however, highly polymerized proanthocyanidins are often inappropriate for targeting cerebrovascular disease, owing to high molecular masses, which do not allow them to pass through the blood-brain barrier.

*Abbreviations:* OxLDL, oxidized low-density lipoprotein; LOX-1, lectin-like oxidized low-density lipoprotein receptor; LDL, low-density lipoprotein; ROS, reactive oxygen species, NF-κB, nuclear factor-kappa B; DDQ, 2,3-dichloro-5,6-dicyano-*p*-banzoquinone, TMSOTf, trifluoromethanesulfonic acid trimethylsilyl ester; GO., galvinoxyl radical; MeCN, acetonerile.

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Previously, we synthesized a catechin analogue known as 'planar catechin,' in which the geometry of (+)-catechin is constrained in a planar orientation by a bridge between the 3-hydroxyl group and C'6 on the C-ring (Fig. 1).[17] Compared with (+)-catechin, planar catechin was found to exhibit not only more potent antioxidative activity, but also demonstrated additional bioactivities, such as α-glucosidase inhibition, and both anti-tumor and anti-virus activities.[17,18] Planar catechin, in particular, exhibits potent glucosidase inhibitory activity, suggesting a structural similarity to the glucosidic linkage that is hydrolyzed by α-glycosidase. That is, planar catechin is also expected to adopt a structure that facilitates affinity for the lectin-like domain of LOX-1.

In the context of prevention of cerebrovascular diseases, we focused on procyanidin B3, a dimer of (+)-catechin, which is likely to be capable of readily crossing the blood-brain barrier. When the geometries of one or both catechin molecules in procyanidin B3 are constrained to a planar orientation, the enhanced antioxidative activities are further improved, thus decreasing the formation of OxLDL. Introduction of a planar structure into the procyanidin B3 framework may also increase the LOX-1 inhibitory activity because of the expected high affinity for the C-type lectin-like recognition domain of LOX-1. Moreover, introduction of an alkyl moiety to allow for construction of such planar structures should further enhance the lipophilicity, allowing for the ability to more easily cross the blood-brain barrier.

To this end, we recently synthesized proanthocyanidin analogue **1**, in which the geometry of one catechin molecule was constrained to a planar orientation. The radical scavenging activity of **1** and procyanidin B3 (Cat-Cat) were evaluated in a non-aqueous system using galvinoxyl radical (GO**.**) as an oxyl radical species. Results demonstrated that the activity of **1** was 1.9-fold higher than that of Cat-Cat.[19] This finding led us to hypothesize that introduction of planar structures into catechin oligomers with a lower degree of polymerization may improve the antioxidative and LOX-1 inhibitory activities, leading to the development of chemopreventive agents for cerebrovascular diseases. In this study, we designed another novel proanthocyanidin derivative (**2**), in which the geometry of both catechin molecules in the catechin dimer were constrained to planar orientations, with the aim of further enhancing the potent antioxidative activity and binding affinity for LOX-1.

The procyanidin B3 analogue **2**, in which the geometry of the two catechin molecules in procyanidin B3 are constrained to planar orientations, was synthesized by two different methods. The first method involves the Oxa-Pictet–Spengler reaction with both catechin molecules in octa-*O*-benzyl procyanidin B3 (**9**), while the second method involves the same reaction with only one catechin molecule in compound **12**, the synthesis of which was accomplished via a condensation reaction between the catechin derivative **5** and the planar catechin derivative **7**.

The first method utilized octa-*O*-benzyl procyanidin B3 (**9**) as an intermediate in the synthesis of **2.** As shown in Scheme 1, the four phenolic hydroxyl groups of (+)-catechin were protected as benzyloxy moieties by reaction with benzyl bromide to yield **3**, and the alcohol hydroxyl group was subsequently protected by acetic anhydride. The desired product (**4**) formed in quantitative yield was then oxidized with DDQ in the presence of ethylene glycol to yield a C4-activated electrophile (**5**) with high stereoselectivity. Reaction between the nucleophilic compound **3** and the electrophilic derivative **5** in the presence of TMSOTf as a Lewis acid furnished the catechin dimer **8**, with a stereochemical preference for a 3,4-*trans* isomer in high yield. After removing the acetyl group with $K_2CO_3$, the geometries of both catechin molecules in **9** were constrained to planar orientations via the Oxa-Pictet–Spengler reaction using acetone in the presence of TMSOTf, to give **10** at a yield of 80%.

The second method employed for the synthesis of **2** used compound **12** as an intermediate, in which the geometry of one catechin molecule was constrained in a planar orientation. Synthesis of the unit of the planar catechin derivative (**7**) was readily accomplished via the Oxa-Pictet–Spengler reaction, in which (+)-catechin and acetone were treated with TMSOTf in THF, and the four hydroxyl groups were subsequently protected by benzyl bromide. The electrophilic catechin derivative **5** and the nucleophilic planar catechin **7** were then condensed using TMSOTf, resulting in the highly stereoselective formation of the 3,4-*trans* isomer (**11**) in high yield. The condensed product (**11**) was subjected to hydrolysis of the acetyl group with $K_2CO_3$ in methanol-toluene solvent, to yield **12**. The geometry of one catechin molecule was then constrained to a planar orientation via the Oxa-Pictet–Spengler reaction, in which **12** and acetone were treated with TMSOTf in THF, at a yield of 60% (**10**). Finally, **10** was subjected to deprotection of the benzyl groups by $Pd(OH)_2$-catalyzed hydrogenation, to yield the desired proanthocyanidin analogue **2** at a yield of 70%.

The radical scavenging activity of **2** was evaluated in a non-aqueous system using GO**.** as the oxyl radical species.[20] Because of its odd electron, GO**.** exhibits a strong absorption band at 428 nm, and a solution of GO**.** appears yellow in color. As the electron is paired, the absorption vanishes, and the resulting decolorization is stoichiometric with respect to the number of electrons taken up.

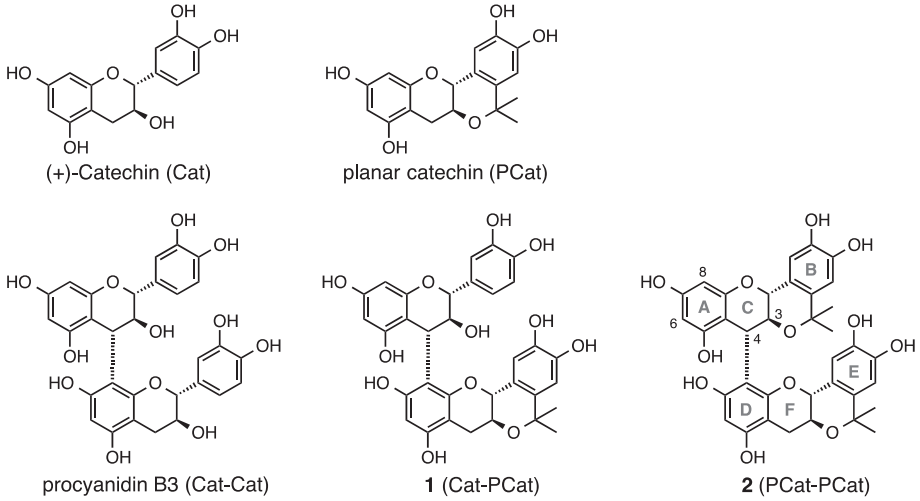
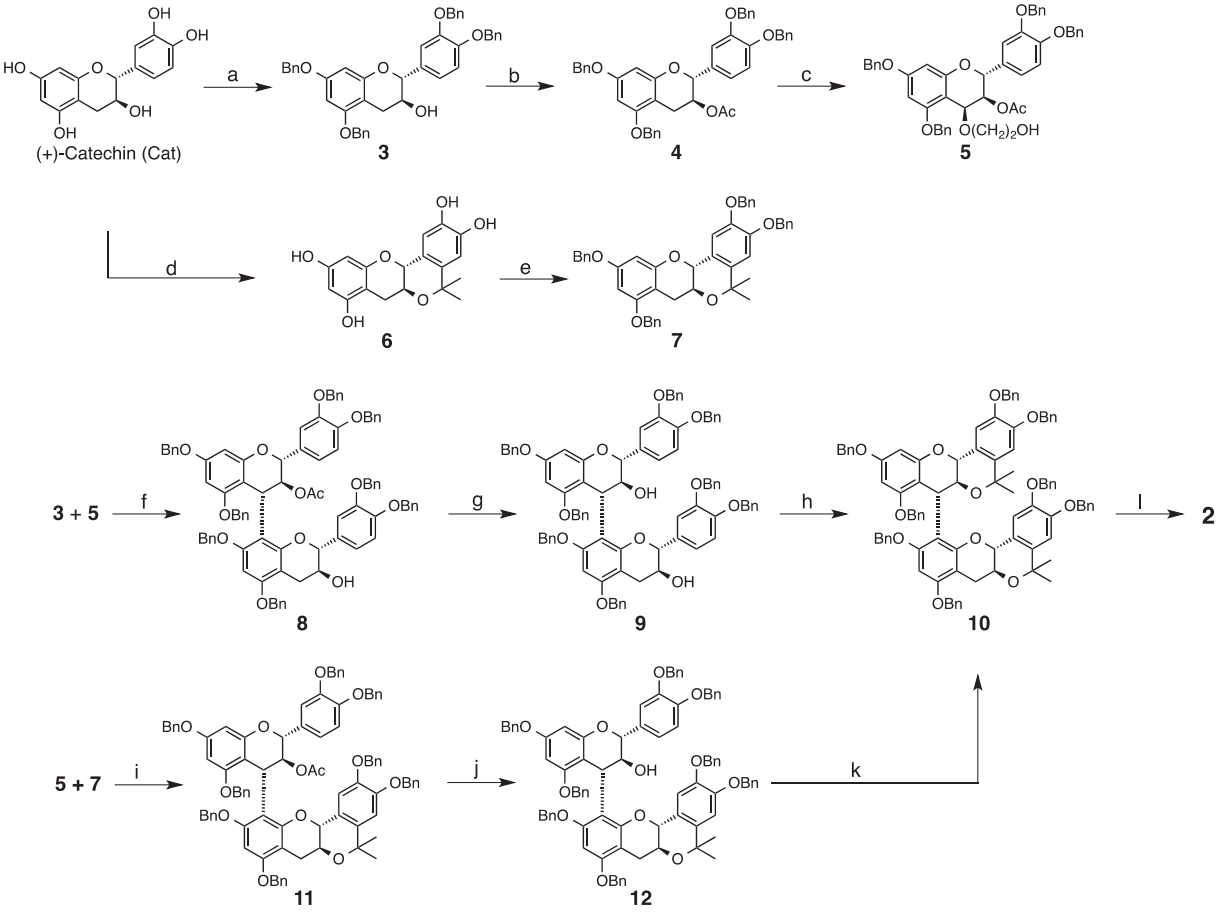



**Fig. 1.** Structures of (+)-catechin, planar catechin, procyanidin B3 (Cat-Cat), and procyanidin B3 derivatives **1** (Cat-PCat) and **2** (PCat-PCat).




**Scheme 1.** Synthesis of proanthocyanidin analogue **2**. Reagents and conditions: (a) BnBr, $K_2CO_3$, DMF, 0 °C → rt, 67%; (b) $Ac_2O$, pyridine, DMAP, rt, 97%; (c) ethylene glycol, DDQ, $CH_2Cl_2$, rt, 59%; (d) acetone, TMSOTf, THF, −20 °C, quant.; (e) BnBr, $K_2CO_3$, DMF, rt, 79%; (f) TMSOTf, $CH_2Cl_2$, −78 °C, 75%; (g) $K_2CO_3$, toluene/MeOH, rt, 97%; (h) acetone, TMSOTf, THF, −20 °C, 60%; (i) TMSOTf, $CH_2Cl_2$, −78 °C, 94%; (j) $K_2CO_3$, toluene/MeOH, rt, 60%; (k) acetone, TMSOTf, THF, −20 °C, 60%; (l) $H_2$, $Pd(OH)_2$, THF/MeOH/$H_2O$, rt, 70%.

Taking advantage of the color change of GO**.** in the presence of an antioxidant, the rate of radical scavenging of **2** was measured. As shown in Fig. 2, the concentrations of the compounds were maintained at more than 10-fold excess to that of GO**.**. The pseudo first-order rate constant ($K_{obs}$) exhibited first-order dependence with respect to the concentration of **2**, as shown in Fig. 3. From a linear plot of $K_{obs}$ vs **2**, the second-order rate constant ($k$) for the radical scavenging of GO**.** was determined to be $2.85 \times 10^2$ mol$^{-1}$ s$^{-1}$. We previously reported the $k$ values for (+)-catechin, PCat, Cat-Cat and Cat-PCat (**1**), determined under the same conditions, to be $2.6 \times 10^1$ mol$^{-1}$ s$^{-1}$, $1.5 \times 10^2$ mol$^{-1}$ s$^{-1}$, $1.00 \times 10^2$ mol$^{-1}$ s$^{-1}$, $1.89 \times 10^2$ mol$^{-1}$ s$^{-1}$, respectively.[19,21] Cat-Cat exhibited a radical

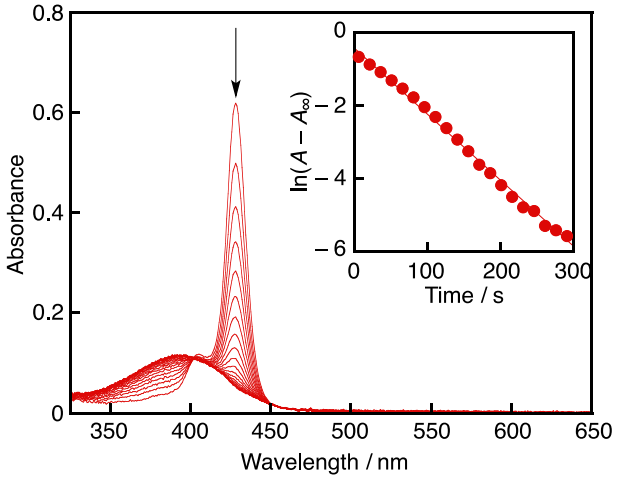



**Fig. 2.** Spectral changes observed during the reaction between PCat-PCat (**2**) (0.6 mM) and GO**.**(4.0 μM) in deaerated MeCN at 298 K. Inset: first-order plot of the change in absorbance at 428 nm due to reaction with GO**.**.

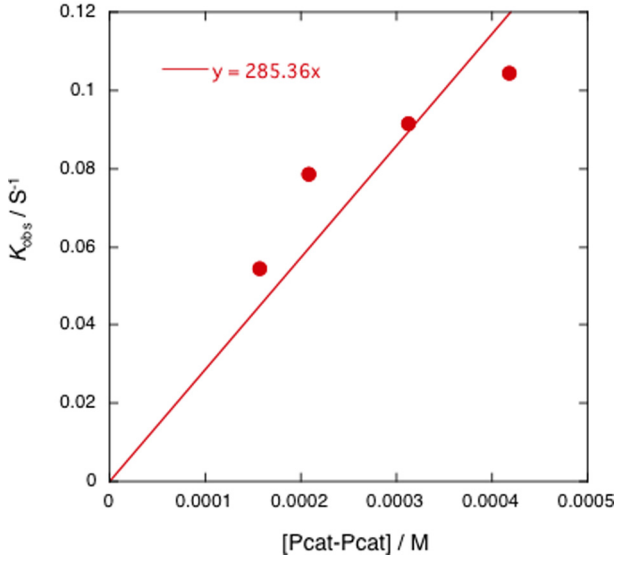



**Fig. 3.** Plot of the pseudo-first-order rate constants ($k_{obs}$) vs. [PCat-PCat (**2**)] for the reaction with GO**.** (4.0 μM) in deaerated MeCN at 298 K.

scavenging activity 3.8× that of the catechin monomer, suggesting that dimerization of catechin can synergistically enhance the antioxidant activity. The radical scavenging activity of **1** was further increased to 1.9× that of Cat-Cat, by constraining one of the catechin molecules to a planar orientation. In the present study, the radical scavenging activity of PCat-PCat (**2**) was 11× that of Cat, 1.9× that of PCat, 2.9× that of Cat-Cat and 1.5× that of Cat-PCat (**1**). These results demonstrate that constraining the geometry of the catechin molecule to be planar is highly efficacious in enhancing the radical scavenging activities of catechin dimer as well as that of catechin monomer. The enhanced radical scavenging activity of the planar structure is attributed to the improved reducing ability associated with the introduction of an alkyl group into the catechin framework. The stronger radical scavenging activity of 2 compared to PCat monomer is likely due to an additive effect of the radical scavenging activity of the two independent planar catechins.

In summary, we synthesized a novel proanthocyanidin analogue (**2**), in which the geometry of both catechin molecules in procyanidin B3 were constrained to planar orientations, and its antioxidative activity was evaluated. As expected, **2** exhibited more potent radical scavenging activity than both Cat-Cat and **1**. Moreover, both planar catechin analogues **1** and **2** demonstrated more potent radical scavenging activities than the catechin dimer, with that of **2** being the strongest, confirming the notion that constraining catechin to a planar orientation is effective at enhancing the radical scavenging activity of procyanidin B3. It is likely that these compounds (**1**, **2**) could exert their effects by scavenging ROS generated during the development of arteriosclerosis. The structure of compound **2** is also expected to facilitate enhanced affinity for LOX-1, by way of introduction of an alkyl moiety to improve lipophilicity, and also by exhibiting a structure specific for the substrate-binding site of alpha-glucosidase. Investigations are currently underway to better understand the effects of **1** and **2** on OxLDL binding to LOX-1, to assess how these compounds may be further modified to develop promising therapeutic agents for cerebrovascular disease.

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## A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.2017.10.007.

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20. Experimental procedure for analyzing the radical scavenging of **2**: The reaction of **2** with GO**.** was carried out under strictly deaerated condition. A continuous flow of argon gas was bubbled through an acetonitrile solution (3.0 mL) containing GO**.** ($4.0 \times 10^{-6}$ mol dm$^{-3}$) in a square quartz cuvette with a glass tube neck for 10 min. An aliquot of **2** ($6.0 \times 10^{-4}$ mol dm$^{-3}$) dissolved in deaerated acetonitrile was added to the cuvette with a microsyringe to initiate the reaction with GO**.**. UV–vis spectral changes associated with the reaction were monitored using an Agilent 8453 photodiode array spectrometer. The rate of the GO**.** scavenging reaction for each compound was determined by monitoring the absorbance change of GO**.** at 428 nm ($\varepsilon = 1.32 \times 10^5$ mol dm$^{-3}$ cm$^{-1}$) using a stopped flow technique on a UNISOKU RSP-1000-02NM spectrophotometer.
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