Wataru Hakamata, Masaaki Kurihara, Haruhiro Okuda, Toshiyuki Nishio, Tadatake Oku	Design and Screening Strategies for α-Glucosidase Inhibitor Based on Enzymological Information.	*Current Topics in Medicinal Chemis try,*	9	3-12	2009
Hideharu Akazaki, Fumihiro Kawai, Masahiro Hosokawa, Toshiyuki Hama, Hirotaka Chida, Takako Hirano, B. K. Lim, N Sakurai, Wataru Hakamata, Toshiyuki Nishio, sam-Yong Park, Tadatake Oku,	Crystallization and structural analysis of cytochrome *c6* from the diatom *Phaeodactylum tricornutum* at 1.5 Å resolution.	*Bioscience, Biotechnology, and Biochemistry*	73	189-191	2009
Wataru Hakamata, Yukiko Sato, Haruhiro Okuda, Shinobu Honzawa, Nozomi Saito, Seishi Kishimoto, Atsushi Yamashita, Takayuki Sugiura, Atsushi Kittaka, Masaaki Kurihara	(2S, 2'R)-Analogue of LG190178 is a major active isomer.	*Bioorganic and Medicinal Chemistry Letters*	18	120-123	2009
Kazunari Kadokura, Yusuke Sakamoto, Akiko Rokutani, Takanori Ikegami, Takako Hirano, Mahiro Yamamoto, Kaori Saito, Wataru Hakamata, Shiro Itoi, Haruo Sugita, Tadatake Oku, Toshiyuki Nishio	Purification, characterization, and cloning of *Vibrio parahaemolyticus* chitinolytic enzymes and application to oligosaccharide production.	*Bioscience, Biotechnology, and Biochemistry*	55	157-164	2008

Hideharu Akazaki, Fumihiro Kawai, Hirotaka Chida, Yuichirou Matsumoto, Mao Hirayama, Ken Hoshikawa, Satoru Unzai, Wataru Hakamata, Toshiyuki Nishio, sam-Yong Park, Tadatake Oku	Cloning, expression and purification of cytochrome *c6* from the brown alga *Hizikia fusiformis* and complete X-ray diffraction analysis of the structure.	*Acta Crystallographica F*	64	674-680	2008
Hideharu Akazaki, Yoshio Futami, Naoya Shibayama, Ikuko Shirasaki, Harumi Nakade, Hirotaka Chida, Wataru Hakamata, sam-Yong Park, Toshiyuki Nishio, Tadatake Oku,	Physicochemical properties of diheme cytochrome *c4* of unknown function from *Vibrio parahaemolyticus* strain RIMD2210633.	*Bioscience Biotechnology and Biochemistry*	72	2791-2794	2008
Shinji Kakuda, Kazuhisa Okada, Hiroshi Eguchi, Kazuya Takenouchi, Wataru Hakamata, Masaaki Kurihara, Midori Takimoto-Kamimura	Structure of the ligand-binding domain of rat VDR in complex with the nonsecosteroidal vitamin D_3 analogue YR301.	*Acta Crystallographica F*	64	970-974	2008
T. Satoh, N. P. Cowieson, W. Hakamata, H. Ideo, K. Fukushima, M. Kurihara, K. Kato, K. Yamashita, S. Wakatsuki	Structure Basis for Recognition of High Mannose Type Glycan Transport Lectin VIP36.	PF NEWS	25	17-22	2008
奥　忠武，千田浩隆，中沢愛子，赤崎秀治，西尾俊幸，袴田　航	藻類のタンパク質遺伝子導入による陸上植物の光合成増感	化学と生物	46	156-158	2008

S. Honzawa, Y. Yamamoto, A. Yamashita, T. Sugiura, M. Kurihara, M. A. Arai, S. Kato, A. Kittaka	The 2α-(3-hydroxypropyl) group as an active motif in vitamin D3 analogues as agonists of the mutant vitamin D receptor (Arg274Leu)	*Bioorg. Med. Chem*	16	3002-3024	2008
M. Kurihara, Y. Sato, F. Kaneko, H. Okuda, M. Nagano, Y. Demizu, M. Doi, M. Tanaka, H. Suemune	Computational Study on Secondary Structure of Oligopeptides Containing α,α-Disubstituted α-Amino Acids	*Peptide Science 2007*		137-138	2008
T. Hasegawa, K. Takahashi, M. Saijo, T. Ishii, T. Nagata, M. Kurihara, Y. Haishima, Y. Goda, N. Kawahara.	Isolation and structural elucidation of cyclopentynafil and N-octylnortadalafil found in a dietary supplement	*Chem. Pharm. Bull.,*	57	185-189	2009
Nagano, M., Tanaka, M., Doi, M., Demizu, Y., Kurihara, M., Suemune, H.	Helical-Screw Directions of Diastereoisomeric Cyclic a-Amino Acid Oligomers.,	*Org. Lett*	11	1135-1137	2009
M. Kurihara, Y. Sato, N. Yamagata, H. Okuda, M. Nagano, Y. Demizu, M. Doi, M. Tanaka, H. Suemune.	Computational Study on Helical Structure of a,a-Disubstituted Oligopeptides Containing Chiral a-Amino Acids	*Peptide Science 2008,*		149-150	2009
H. Takazaki, M. Tanaka, N. Kawabe, M. Nagano, M. Doi, M. Kurihara, H. Suemune	Design and synthesis of chiral cyclic a,a-disubstituted amino acid having azido functions and its oligopeptides.,	*Peptide Science 2008,*		159-160	2009

Sugiyama, T., Imamura, Y., Kurihara, M., Kittaka, A.	Cooperative Strand Invasion by Peptide Nucleic Acid.	*Peptide Science 2008,*		481-482	2009

研究成果の刊行物・別刷

1) Wataru Hakamata, Ikuo Nakanishi, Yu Masuda, Takehiko Shimizu, Hajime Higuchi, Yuriko Nakamura, Tadatake Oku, Shinichiro Saito, Shiro Urano, Toshihiko Ozawa, Nobuo Ikota, Naoki Miyata, Haruhiro Okuda, Kiyoshi Fukuhara. Planar catechin analogues with alkyl side chain, a potent antioxidant and a-glucosidase inhibitor. Journal of American Chemical Society, 128, 6524-6525 (2006).

2) Wataru Hakamata, Makoto Muroi, Toshiyuki Nishio, Tadatake Oku, Akira Takatsuki, Hiroyuki Osada, Kiyoshi Fukuhara, Haruhiro Okuda, Masaaki Kurihara, N-Linked oligosaccharide processing enzymes as molecular targets for drug discovery. Journal of Applied Glycoscience, 53 149-154, (2006).

3) Wataru Hakamata, Emiko Yamamoto, Makoto Muroi, Masataka Mochizuki, Masaaki Kurihara, Haruhiro Okuda, Kiyoshi Fukuhara. Design and synthesis of a-glucosidase inhibitor having DNA cleaving activity. Journal of Applied Glycoscience 53 (4), 255-260 (2006).

4) Masaaki Kurihara, Yukiko Sato, Wataru Hakamata, Haruhiro Okuda, Yosuke Demizu, Masanobu Nagano, Naomi Kawabe, Mitsunobu Doi, Masakazu Tanaka, Hiroshi Suemune. Computational study on conformation of oligopeptides containing chiral cyclic a, a-disubstituted a-amino acids. Peptide Science, 42, 371-372 (2006).

5) Toru Sugiyama, Yasutada Imamura, Wataru Hakamata, Masaaki Kurihara, Atsushi Kittaka. Sequence-specific recognition of double-stranded DNA by cooperatives strand invasion. Nucleic Acids Symp. Ser., 50,157-158 (2006).

6) Y. Suhara, M. Kurihara, A. Kittaka, M. Ichikawa, Y. Ichikawa. Efficient Synthesis of Carbopeptoid Oligomers and their Conformational Studies. Tetrahedron, 62, 8207-8217 (2006).

7) T. Fujishima, R. Tsutsumi, Y. Negishi, S. Fujii, H. Takayama,A.Kittaka, M. Kurihara, Methyl-introduced A-ring Analogues of 1a,25-Dihydroxyvitamin D3: Synthesis and Biological Evaluation Anticancer Res., 26, 2633-2636 (2006).

8) N. Kawabe, Y. Demizu, M. Tanaka, M. Kurihara, and H. Suemune, Synthesis of Various Chiral Cyclic a, a-Disubstituted Amino Acids and Conformational analysis of their Peptides, Peptide Science 2005, 347-348 (2006).

9) M. Nagano, Y Demizu, M. Tanaka, M. Kurihara, M. Doi, H. Suemune Chiral Cyclic a, a-Disubstituted a-Amino Acids Bearing Two Chiral Centers and Conformation of Their Peptides. Peptide Science 2005, 345-346 (2006).

10) Masaaki Kurihara, Wataru Hakamata, Yukiko Sato, Haruhiro Okuda, Yousuke Demizu, Kosuke Anan, Mitsunobu Doi, Masataka Tanaka, Hhiroshi Suemune. Computational study on helical structures of oligopeptides containing chiral cyclic a-amino acids. *Peptides 2006,* 546-547 (2007).

11) Kadokura, K.; Rokutani, A.; Yamamoto, M.; Ikegami, T.; Sugita, H.; Itoi, S.; Hakamata, W.; Oku, T.; Nishio, T. Purification and characterization of Vibrio parahaemolyticus extracellular chitinase and chitin oligosaccharide deacetylase involved in the production of heterodisaccharide from chitin. *Applied Microbiology and Biotechnology,* 75(2), 357-365 (2007).

12) Kadokura, Kazunari; Sakamoto, Yusuke; Saito, Kaori; Ikegami, Takanori; Hirano, Takako; Hakamata, Wataru; Oku, Tadatake; Nishio, Toshiyuki. Production of a recombinant chitin oligosaccharide deacetylase from Vibrio parahaemolyticus in the culture medium of Escherichia coli cells. Biotechnology Letters, 29(8), 1209-1215, (2007).

13) Kadokura, Kazunari; Sakamoto, Yusuke; Saito, Kaori; Ikegami, Takanori; Hirano, Takako; Hakamata, Wataru; Oku, Tadatake; Nishio, Toshiyuki. Production and secretion of a recombinant *Vibrio parahaemolyticus* chitinase by *Escherichia coli* and its purification from the culture medium. *Bioscience, Biotechnology, and Biochemistry,* 71(11), 2848-2851 (2007).

14) Satoh, Tadashi; Cowieson, Nathan P.; Hakamata, Wataru; Ideo, Hiroko; Fukushima, Keiko; Kurihara, Masaaki; Kato, Ryuichi; Yamashita, Katsuko; Wakatsuki, Soichi. Structural Basis for Recognition of High Mannose Type Glycoproteins by Mammalian Transport Lectin VIP36. *Journal of Biological Chemistry,* 282 (38), 28246-28255

(2007).

15) Chida, Hirotaka; Nakazawa, Aiko; Akazaki, Hideharu; Hirano, Takako; Suruga, Kohei; Ogawa, Masahiro; Satoh, Tadashi; Kadokura, Kazunari; Yamada, Seiji; Hakamata, Wataru; Isobe, Katsunori; Ito, Tei-ichiro; Ishii, Ryuichi; Nishio, Toshiyuki; Sonoike, Kintake; Oku, Tadatake. Expression of the algal cytochrome c6 gene in Arabidopsis enhances photosynthesis and growth. *Plant and Cell Physiology,* 48 (7), 948-957 (2007).

16) Sugiyama, T., Imamura, Y., Kurihara, M., Kittaka. A:, Recognition of Longer Duplex DNA by Cooperative Strand Invasion, *Nucleic Acids Symp. Ser.*, 51, 269-270 (2007).

17) Masakazu Tanaka, Yosuke Demizu, Masanobu Nagano, Mariko Hama, Yukio Yoshida, Masaaki Kurihara, and Hiroshi Suemune, Lipase-Catalyzed Kinetic Resolution of Cyclic trans-1,2-Diols Bearing a Diester Moiety: Synthetic Application to Chiral Seven-Membered-Ring α,α-Disubstituted α-Amino Acid, *J. Org. Chem.*, 72, 7750 -7756 (2007).

18) Y. Demizu, M. Tanaka, M. Nagano, M. Kurihara, M. Doi, T. Maruyama, H. Suemune, Controlling 3^{10}-Helix and α-Helix of Short Peptides in the Solid State, *Chem. Pharm. Bull.*, 55, 840-842 (2007).

19) M. Tanaka, M. Nagano, Y. Demizu, K. Anan, M. Kurihara, M. Doi, H. Suemune; Side-chain chiral centers of amino acids and helical-screw handedness of their peptides; *Peptides 2006*, 268-269(2007).

20) Nagano, M., Tanaka, M., Demizu, Y., Kurihara, M., Doi, M., Suemune, H, Secondary Structure of Heteropeptides Using Chiral Cyclic α,α-Disubstituted Amino Acids; Controlling the Helical; *Peptides 2006*, 476-477(2007).

21) Design and Screening Strategies for α-Glucosidase Inhibitor Based on Enzymological Information. Wataru Hakamata, Masaaki Kurihara, Haruhiro Okuda, Toshiyuki Nishio, Tadatake Oku, *Current Topics in Medicinal Chemistry*, 9 (1), 3-12 (2009).

22) Crystallization and structural analysis of cytochrome *c6* from the diatom *Phaeodactylum*

tricornutum at 1.5 Å resolution. Hideharu Akazaki, Fumihiro Kawai, Masahiro Hosokawa, Toshiyuki Hama, Hirotaka Chida, Takako Hirano, B. K. Lim, N Sakurai, Wataru Hakamata, Toshiyuki Nishio, sam-Yong Park, Tadatake Oku, *Bioscience, Biotechnology, and Biochemistry*, 73, 189-191 (2009).

23) (2S, 2'R)-Analogue of LG190178 is a major active isomer. Wataru Hakamata, Yukiko Sato, Haruhiro Okuda, Shinobu Honzawa, Nozomi Saito, Seishi Kishimoto, Atsushi Yamashita, Takayuki Sugiura, Atsushi Kittaka, Masaaki Kurihara, *Bioorganic and Medicinal Chemistry Letters*, 18, 120-123, (2008).

24) Purification, characterization, and cloning of *Vibrio parahaemolyticus* chitinolytic enzymes and application to oligosaccharide production. Kazunari Kadokura, Yusuke Sakamoto, Akiko Rokutani, Takanori Ikegami, Takako Hirano, Mahiro Yamamoto, Kaori Saito, Wataru Hakamata, Shiro Itoi, Haruo Sugita, Tadatake Oku, Toshiyuki Nishio, *Journal of Applied Glycoscience*, 55, 157-164, (2008).

25) Cloning, expression and purification of cytochrome *c6* from the brown alga *Hizikia fusiformis* and complete X-ray diffraction analysis of the structure. Hideharu Akazaki, Fumihiro Kawai, Hirotaka Chida, Yuichirou Matsumoto, Mao Hirayama, Ken Hoshikawa, Satoru Unzai, Wataru Hakamata, Toshiyuki Nishio, sam-Yong Park, Tadatake Oku, *Acta Crystallographica F.*, 64, 674-680, (2008).

26) Physicochemical properties of diheme cytochrome *c4* of unknown function from *Vibrio parahaemolyticus* strain RIMD2210633. Hideharu Akazaki, Yoshio Futami, Naoya Shibayama, Ikuko Shirasaki, Harumi Nakade, Hirotaka Chida, Wataru Hakamata, sam-Yong Park, Toshiyuki Nishio, Tadatake Oku, *Bioscience Biotechnology and Biochemistry*, 72, 2791-2794 (2008).

27) Structure of the ligand-binding domain of rat VDR in complex with the nonsecosteroidal vitamin D_3 analogue YR301. Shinji Kakuda, Kazuhisa Okada, Hiroshi Eguchi, Kazuya Takenouchi, Wataru Hakamata, Masaaki Kurihara, Midori Takimoto-Kamimura, *Acta Crystallographica F.*, 64, 970–973, (2008).

28) Structure Basis for Recognition of High Mannose Type Glycan Transport Lectin VIP36., T. Satoh, N. P. Cowieson, W. Hakamata, H. Ideo, K. Fukushima, M. Kurihara, K. Kato,

K. Yamashita, S. Wakatsuki., PF NEWS, 25, 17-22, (2008).

29) 奥　忠武，千田浩隆，中沢愛子，赤崎秀治，西尾俊幸，袴田　航、藻類のタンパク質遺伝子導入による陸上植物の光合成増感、46 (3), 156-158, (2008).

30) The 2α-(3-hydroxypropyl) group as an active motif in vitamin D3 analogues as agonists of the mutant vitamin D receptor (Arg274Leu) ; S. Honzawa, Y. Yamamoto, A. Yamashita, T. Sugiura, M. Kurihara, M. A. Arai, S. Kato, A. Kittaka; *Bioorg. Med. Chem.*, 16, 3002-3024 (2008)

31) Computational Study on Secondary Structure of Oligopeptides Containing α,α-Disubstituted α-Amino Acids; M. Kurihara, Y. Sato, F. Kaneko, H. Okuda, M. Nagano, Y. Demizu, M. Doi, M. Tanaka, H. Suemune; *Peptide Science 2007*, 137-138, (2008).

32) Isolation and structural elucidation of cyclopentynafil and N-octylnortadalafil found in a dietary supplement., T. Hasegawa, K. Takahashi, M. Saijo, T. Ishii, T. Nagata, M. Kurihara, Y. Haishima, Y. Goda, N. Kawahara., *Chem. Pharm. Bull.*, 57, 185-189 (2009).

33) Helical-Screw Directions of Diastereoisomeric Cyclic a-Amino Acid Oligomers., Nagano, M., Tanaka, M., Doi, M., Demizu, Y., Kurihara, M., Suemune, H., *Org. Lett.*, 11, 1135-1137 (2009).

34) Computational Study on Helical Structure of a,a-Disubstituted Oligopeptides Containing Chiral a-Amino Acids., M. Kurihara, Y. Sato, N. Yamagata, H. Okuda, M. Nagano, Y. Demizu, M. Doi, M. Tanaka, H. Suemune., *Peptide Science 2008*, 149-150 (2009).

35) Design and synthesis of chiral cyclic a,a-disubstituted amino acid having azido functions and its oligopeptides., H. Takazaki, M. Tanaka, N. Kawabe, M. Nagano, M. Doi, M. Kurihara, H. Suemune., *Peptide Science 2008*, 159-160 (2009).

36) Cooperative Strand Invasion by Peptide Nucleic Acid., Sugiyama, T., Imamura, Y., Kurihara, M., Kittaka, A., *Peptide Science 2008*, 481-482 (2009).

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Planar Catechin Analogues with Alkyl Side Chains: A Potent Antioxidant and an α-Glucosidase Inhibitor

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As mitochondrial oxidative damage[1] or oxidative modification of low-density lipoprotein (LDL)[2] contribute significantly to a range of degenerative diseases and further production of reactive oxygen species (ROS), it might be advantageous to develop lipophilic antioxidants which would be able to suppress mitochondrial ROS production or LDL oxidation due to their affinity to lipid particles or membrane. Recently, we synthesized planar catechin analogue (PC1), in which the catechol and chroman structure in (+)-catechin are constrained to be planar, by the reaction of (+)-catechin with acetone in the presence of $BF_3 \cdot Et_2O$.[3,4] The rate of hydrogen transfer from PC1 to galvinoxyl radical ($G^\bullet$), a stable oxygen-centered radical, is about 5-fold faster than that of hydrogen transfer from the native (+)-catechin to $G^\bullet$. PC1 also shows an enhanced protective effect against oxidative DNA damage induced by the Fenton reaction without the pro-oxidant effect, which is usually observed in the case of (+)-catechin. We also have found that PC1, as well as stilbene resveratrol[5] which is a typical cancer chemopreventive agent present in grapes, inhibits cell growth through induction of apoptosis in cancer cell lines (data not shown). Therefore, we envisioned that a conformationally constrained planar catechin might be valuable in the development of a new type of clinically useful antioxidant, if the hydrophobicity of PC1 could be controlled so as to fine-tune its membrane binding and penetration into the phospholipid bilayer. Here, we describe a synthetic method for planar catechin analogues (PCn), the lipophilicity of which was controlled by changing the length of the alkyl chains. Also described are their remarkable antioxidative potencies and α-glucosidase inhibitory activities.

The synthesis of PCn was carried out by reacting catechin with various ketones having alkyl chains of different lengths. However, the previously reported method for the synthesis of PC1[3] is inapplicable to other PCn synthesis. Because the original reaction is carried out in a solution of acetone, the synthesis of PCn is limited to using the corresponding ketone as a solvent. Therefore, it was necessary to improve the synthetic method of PC1 to be able to introduce various types of ketones into the catechin structure using a synthetic scheme applicable for any PCn production. We attempted to optimize the reaction using a combination of various acids and solvents, and finally, it was shown that the reaction using silyl Lewis acids such as TMSOTf, TESOTf, or TBSOTf gave the desired products in high yields. Typically, (+)-catechin and 1.2 equiv of ketone in THF was treated with 1.2 equiv of TMSOTf at -5 °C to form the desired PCn. This reaction was used to provide a series of PC1 ≈ PC6, 44−76% yield (Scheme 1), with slightly different lipophilicity.

PCn were evaluated for their radical scavenging activities against DPPH (2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl) radical and AAPH (2,2′-azobis(2-amidinopropane) dihydrochloride)-derived peroxyl radical (Scheme 2). The hydrogen abstraction of PCn by DPPH radical in deaerated acetonitrile solution was monitored using the decrease of the visible absorption band at 543 nm due to DPPH radical that obeyed pseudo-first-order kinetics. The second-order rate constant (k_{HT}) for hydrogen abstraction of PCn by DPPH radical was then determined (Table 1). Similar to what was found with hydrogen abstraction by galvinoxyl radical,[3] the k_{HT} value (533 $M^{-1} s^{-1}$) of PC1 is significantly larger than that of (+)-catechin (305 $M^{-1} s^{-1}$), indicating that the radical-scavenging activity of catechin using DPPH radical increased due to constraining the (+)-catechin in a planar configuration. In addition, it was found that the larger the number of carbon atoms there were in the alkyl chains, the greater the DPPH radical scavenging rates became, with the k_{HT} value of PCn plateauing at $n = 4$. The radical scavenging ability of PCn with longer side chains might be attributed to the $-I$ effect of the side chain that stabilizes the cation radical formed after electron transfer from PCn to DPPH. The radical scavenging activities of PCn in aqueous solution were investigated using AAPH as a source of free radicals in phosphate buffer (Table 1). AAPH-derived peroxyl radicals react with luminol to generate prolonged luminescence,[6] and the antioxidative activities of PCn were determined using the concentration of PCn where the luminescence is reduced to 50%. As a result, the antioxidative activity of planar catechin in phosphate buffer was again stronger than that of catechin as well as its antioxidative activity in acetonitrile. The alkyl side chains also affect the antioxidative activity; an increase ($n = 1-3$) in the length of the alkyl chains tends to increase the antioxidative activity, with PC3 showing the strongest antioxidative effect. However, further increase ($n = 4-6$) in the length of the side chain seems to weaken the antioxidative effects, which is consistent with the suggestion that longer alkyl side chains result in the formation of amphiphilic micelles in aqueous solvent.

For the evaluation of lipophilic PCn as antioxidants against biomolecular injury caused by ROS, the protecting effect of PCn on oxidative DNA damage induced by the Fenton reaction was

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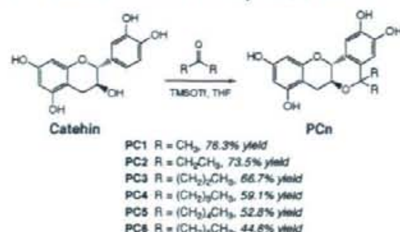
Scheme 1. Chemical Structure and Synthesis of PCn



Scheme 2. Radical Scavenging Reaction of PCn against DPPH• and AAPH

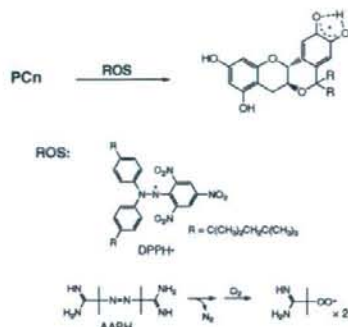


Table 1. Antioxidant Profile of Catechin and PCn Determined Using a DPPH and AAPH Scavenging Assay

compd	DPPH· k_{HT} (M^{-1} s^{-1})	AAPH IC_{50} (nM)
catechin	305	292
PC1	533	220
PC2	622	175
PC3	686	98
PC4	725	147
PC5	756	625
PC6	759	1700

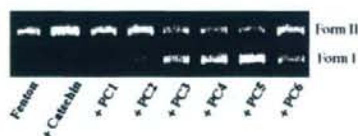


Figure 1. Effects of catechin and PCn on DNA breakage induced by the Fenton reaction (Fe^{3+}/H_2O_2). Assays were performed in 100 mM phosphate buffer, pH 7.0 containing 45 μM pBR322DNA, 10 mM H_2O_2, 100 μM $FeCl_3$, and 1 mM individual PCn for 1 h at 37 °C.

determined. Although PC1 showed an excellent protecting effect against oxidative DNA scission compared with catechin,[3] the antioxidative activity of the series of PCn was evaluated under conditions in which the protecting effect of PC1 appears to be weak. As shown in Figure 1, DNA cleaving activity induced by the Fenton reaction did not increase in the presence of PCn, and with an increase in the length of alkyl chains, the protecting effect of PCn on the oxidative DNA damage was greatly increased. The strong antioxidative activity might be attributed to a combination of radical scavenging activity and lipophilicity that tends to increase the binding between PCn and DNA. A small decrease in the protecting effect of PC6 might be responsible for the diminishing radical scavenging ability under aqueous solution.

In addition to the antioxidative ability, (+)-catechin is known to be an inhibitor against α-glucosidase[7] that catalyzes the final

Table 2. Inhibitory Activities of Catechin and PCn against α-glucosidases

compd	*S. cerevisiae* IC_{50} (μM)	*B. stearothermophilus* IC_{50} (μM)
catechin	>500	>500
PC1	1.2	0.7
PC2	47.5	26.8
PC3	37.5	28.4
PC4	2.1	14.2
PC5	5.3	6.8
PC6	0.9	1.1

step in the digestive process of carbohydrates. Therefore, the inhibitory effects of PCn on α-glucosidase from *Saccharomyces cerevisiae* and *Bacillus stearothermophilus* were evaluated (Table 2). Surprisingly, in contrast to the relative weak inhibitory effect of (+)-catechin with IC_{50} > 500 μM, PCn exhibited strong inhibitory effects with $IC_{50} = 0.7-47.5$ μM against both enzymes, with PC1 (IC_{50} = 1.2 μM for *S. cerevisiae* and 0.7 μM for *B. stearothermophilus*) and PC6 (IC_{50} = 0.9 μM for *S. cerevisiae* and 1.1 μM for *B. stearothermophilus*) showing especially high inhibition concentrations. The strong inhibitory effect of PCn on α-glucosidase suggested that these planar catechin analogues may be used as a lead compounds for the development of antidiabetic therapeutics, similar to acarbose and voglibose which are known to reduce postprandial hyperglycemia primarily by interfering with the carbohydrate digesting enzymes and delaying glucose absorption.

In summary, a practical method for the preparation of planar catechin analogues with various alkyl side chain lengths is described as well as the remarkable properties of these compounds as potent antioxidants and α-glucosidase inhibitors. In vivo studies to fully exploit these potential benefits of PCn are currently under way, and the results will be published in due time.

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Note Added after ASAP Publication. After this paper was published ASAP on May 3, 2006, Table 2 was corrected to show the *S. cerevisiae* IC_{50} value of 1.2 μM for PC1.

Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Raha, S.; Robinson, B. H. *Am. J. Med. Genet.* 2001, *106*, 62. (b) Kang, D.; Hamasaki, N. *Curr. Med. Chem.* 2005, *12*, 429.
(2) Tsimilas, S.; Witztum, J. L. In *Oxidative stress and vascular diseases*; Keaney, J. F., Jr., Ed.; Kluwer: Boston, 2000; pp 49−74.
(3) Fukuhara, K.; Nakanishi, I.; Kansui, H.; Sugiyama, E.; Kimura, M.; Shimada, T.; Urano, S.; Yamaguchi, K.; Miyata, N. *J. Am. Chem. Soc.* 2002, *124*, 5952.
(4) (a) Fukuhara, K.; Nakanishi, I.; Shimada, T.; Miyazaki, K.; Hakamata, W.; Urano, S.; Ikota, N.; Ozawa, T.; Okuda, H.; Miyata, N.; Fukuzumi, S. *Chem. Res. Toxicol.* 2003, *16*, 81. (b) Nakanishi, I.; Ohkubo, K.; Miyazaki, K.; Hakamata, W.; Urano, S.; Ozawa, T.; Okuda, H.; Fukuzumi, S.; Ikota, N.; Fukuhara, K. *Chem. Res. Toxicol.* 2004, *17*, 26.
(5) Joe, A. K.; Liu, H.; Suzui, M.; Vural, M. E.; Xiao, D.; Weinstein, I. B. *Clin. Cancer Res.* 2002, *8*, 893.
(6) Krasowska, A.; Rosiak, D.; Szkapiak, K.; Oswiecimska, M.; Witek, S.; Lukaszewicz, M. *Cell. Mol. Biol. Lett.* 2001, *6*, 71.
(7) Matsui, T.; Yoshimoto, C.; Osajima, K.; Oki, T.; Osajima, Y. *Biosci. Biotechnol. Biochem.* 1996, *60*, 2019.

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Supporting Information for

Planar Catechin Analogues with Alkyl Side Chains, a Potent Antioxidant and an α–Glucosidase Inhibitor

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General methods. The NMR spectra were recorded with a Varian AS 400 Mercury spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C). Chemical shifts were expressed in ppm downfield shift from Me_4Si. Low resolution mass spectra were obtained with a Waters Micromass ZQ instrument under positive and negative ESI conditions. Column chromatography was performed on silica gel 60 (0.063-0.200 mm, Merck). The progress of all reactions was monitored by thin-layer chromatography on silica gel 60 F_{254} (0.25 mm, Merck).

General method for synthesis of Planar Catechin Analogues (PCn). To the solution of purified (+)-catechin (0.5 g, 1.72 mmol) and ketone (5.16 mmol), in tetrahydrofuran at –5°C, Trimethylsilyl trifluoromethanesulfonate (TMSOTf, 1.72 mmol) was slowly added. The mixture was stirred for 12 h and poured into water. The product was extracted with diethylether and washed with satd. $NaHCO_3$aq, and brine, and then dried over Na_2SO_4.

Removal of solvent afforded the corresponding catechin derivatives.

(6a*S*, 12a*R*)-6a, 12a-*trans*-2, 3, 8, 10-Tetrahydroxy-5, 5-dimethyl-5, 6a, 7, 12a-tetrahydro-[1]benzopyrano[3, 2-c][2]benzopyran (PC1). After synthesis according to the general method using acetone as a ketone, the product was purified by column chromatography on silica gel (7:3:1 toluene–acetone-MeOH) to afford 0.39 g (76.3%) of PC1: ^{1}H NMR (CD_3OD) δ 1.48 (s, 3H, -$C\underline{H}_{3a}$), 1.53 (s, 3H, -$C\underline{H}_{3b}$), 2.44 (dd, 1H, *J* = 10.6 Hz, *J* = 15.4 Hz, 7ax), 2.93 (dd, 1H, *J* = 5.8 Hz, *J* = 15.4 Hz, 7eq), 3.84 (m, 1H, 6a), 4.44 (d, 1H, *J* = 9.2 Hz, 12a), 5.93 (d, 2H, *J* = 2.0 Hz, 11), 5.96 (d, 2H, *J* = 2.4 Hz, 9), 6.56 (s, 2H, 4), 7.02 (d, 2H, *J* = 0.8 Hz, 1); Lit.[1)] ^{1}H NMR (acetone-d_6) δ 1.45 (s, 3H, 5-$C\underline{H}_{3a}$), 1.51 (s, 3H, 5-$C\underline{H}_{3b}$), 2.49 (dd, 1H, *J* = 11.0 Hz, *J* = 15.0 Hz, 7ax), 2.96 (dd, 1H, *J* = 6.0 Hz, *J* = 15.0 Hz, 7eq), 3.82 (m, 1H, 6a), 4.45 (dd, 1H, *J* = 1.5 Hz, *J* = 9.5 Hz, 12a), 5.97 (d, 2H, *J* = 2.5 Hz, 11), 6.05 (d, 2H, *J* = 2.5 Hz, 9), 6.65 (s, 2H, 4), 7.07 (d, 2H, *J* = 1.0 Hz, 1); ^{13}C NMR (CD_3OD) δ 28.0 (4), 28.6 and 32.1 (-$\underline{C}H_3$), 68.0 (3), 74.5 (2), 77.0 (7'), 95.8 (6), 96.5 (8), 101.3 (10), 112.7 (2'), 112.8 (5'), 125.4 (1'), 135.4 (6'), 145.1 (3'), 146.1 (4'), 156.9 (5), 157.9(9), 157.9 (7); Lit. [1)] ^{13}C NMR (acetone-d_6) δ 28.1, 28.5, 32.0, 67.2, 74.1, 76.1, 96.0, 96.5, 101.1, 112.3, 113.0, 125.1, 135.3, 144.5, 156.3, 157.0, and 157.5; Lit. [2)] ^{13}C NMR (acetone-d_6) δ 27.2 (4), 28.7 and 31.4 (-$\underline{C}H_3$), 66.6 (3), 73.5 (2), 75.5 (7'), 95.2 (6), 95.9 (8), 100.1 (10), 111.8 (2'), 112.1 (5)), 124.7 (1'), 135.5 (6'), 143.9 (3'), 145.0 (4'), 156.7 (9), 156.7(7), 157.2 (5); $[M+H]^+$: 331; Lit. [2)] EI-MS $[M]^+$: 330.

S(6a12a*R*)-6a, 12a-*trans*-2, 3, 8, 10-Tetrahydroxy-5, 5-diethyl-5, 6a, 7, 12a-

tetrahydro-[1]benzopyrano[3, 2-c][2]benzopyran (PC2). After synthesis according to the general method using 3-pentanone as a ketone, the product was purified by column chromatography on silica gel (7:3:1 toluene-acetone-MeOH) to afford 0.41 g (73.5%) of PC2: ^{1}H NMR (CD_3OD) δ 0.63 (dd, 3H, J = 7.2 Hz, -$CH_2C\underline{H}_3$), 1.00 (dd, 3H, J = 7.2 Hz, -$CH_2C\underline{H}_3$), 1.61 (qd, 1H, J = 7.1 Hz, J = 14.4 Hz, -$C\underline{H}_2CH_3$), 1.69 (qd, 1H, J = 7.5 Hz, J = 14.8 Hz, -$C\underline{H}_2CH_3$), 1.92 (qd, 1H, J = 6.9 Hz, J = 14.0 Hz, -$C\underline{H}_2CH_3$), 2.07 (qd, 1H, J = 7.5 Hz, J = 15.2 Hz, -$C\underline{H}_2CH_3$), 2.45 (dd, 1H J =10.4Hz, J =15.2Hz, 7ax), 2.97 (dd, 1H J =6.0Hz, J =15.6Hz, 7eq), 3.77 (m, 1H, 6a), 4.41 (dd, 2H, J = 1,2 Hz, J = 9.2 Hz, 12a), 5.94 (d, 2H, J = 2.4 Hz, 11), 5.97 (d, 2H, J = 2.4 Hz, 9), 6.50 (s, 2H, 4), 7.05 (d, 2H, J = 0.8 Hz, 1); ^{13}C NMR (CD_3OD) δ 8.2 and 8.6 (-$\underline{C}H_3$), 27.9 (4), 33.5 and 34.1 (-$\underline{C}H_2CH_3$), 67.8 (3), 74.4 (2), 81.5 (7'), 95.8 (6), 96.5 (8), 101.3 (10), 112.5 (2'), 112.6 (5'), 127.2 (1'), 133.4 (6'), 144.9 (3'), 146.0 (4'), 156.8 (5), 157.7 (9), 157.8 (7); $[M+H]^+$: 359.

(6a*S*, 12a*R*)-6a, 12a-*trans*-2, 3, 8, 10-Tetrahydroxy-5, 5-dipropyl-5, 6a, 7, 12a-tetrahydro-[1]benzopyrano[3, 2-c][2]benzopyran (PC3). After synthesis according to the general method using 4-heptanone as a ketone, the product was purified by column chromatography on silica gel (7:3:1 toluene–acetone-MeOH) to afford 0.43 g (66.7%) of PC3: ^{1}H NMR (CD_3OD) δ 0.93 (dd, 3H, -$CH_2CH_2C\underline{H}_3$), 1.18 (dd, 3H, -$CH_2CH_2C\underline{H}_3$), 1.31 (m, 1H, -$CH_2C\underline{H}_2CH_3$), 1.47-1.70 (m, 5H, -$CH_2C\underline{H}_2CH_3$ and –$C\underline{H}_2CH_2CH_3$), 1.84 (m, 1H, -$C\underline{H}_2CH_2CH_3$), 1.97 (m, 1H, -$C\underline{H}_2CH_2CH_3$), 2.43 (dd, 1H, J = 10.4 Hz, J = 15.6 Hz, 7ax), 2.95 (dd, 1H, J = 6.0 Hz, J = 15.6 Hz, 7eq), 3.77 (m, 1H, 6a), 4.39 (dd, 1H, J = 1.2 Hz, J = 9.2 Hz, 12a), 5.94 (d, 2H, J = 2.4 Hz, 11), 5.97 (d, 2H, J = 2.4 Hz, 9), 6.52 (s, 2H, 4), 7.04

(d, 2H, J = 0.8 Hz, 1); ^{13}C NMR (CD_3OD) δ 14.7 and 15.1 ($-CH_2CH_2\underline{C}H_3$), 17.8 and 18.1 ($-CH_2\underline{C}H_2CH_3$), 27.9 (4), 44.2 and 44.3 ($-\underline{C}H_2CH_2CH_3$), 67.9 (3), 74.4 (2), 81.2 (7'), 95.8 (6), 96.5 (8), 101.3 (10), 112.4 (2'), 112.6 (5'), 126.9 (1'), 135.9 (6'), 144.8 (3'), 146.0 (4'), 156.8 (5), 157.7(9), 157.8 (7); $[M-H]^-$: 385.

(6a*S*, 12a*R*)-6a, 12a-*trans*-2, 3, 8, 10-Tetrahydroxy-5, 5-dibutyl-5, 6a, 7, 12a-tetrahydro-[1]benzopyrano[3, 2-c][2]benzopyran (PC4). After synthesis according to the general method using 5-nonanone as a ketone, the product was purified by column chromatography on silica gel (7:3:1 toluene-acetone-MeOH) to afford 0.37 g (59.1%) of PC4: ^1H NMR (CD_3OD) δ 0.81 (dd, 3H, $-CH_2CH_2CH_2C\underline{H_3}$), 0.92 (dd, 3H, $-CH_2CH_2CH_2C\underline{H_3}$), 1.16-1.37 (m, 6H, $-CH_2CH_2C\underline{H_2}CH_3$ and $-CH_2C\underline{H_2}CH_2CH_3$), 1.42-1.48 (m, 2H, $-CH_2C\underline{H_2}CH_2CH_3$ and $-C\underline{H_2}CH_2CH_2CH_3$), 1.59-1.71 (m, 2H, $-CH_2C\underline{H_2}CH_2CH_3$ and $-C\underline{H_2}CH_2CH_2CH_3$), 1.86 (m, 1H, $-C\underline{H_2}CH_2CH_2CH_3$), 1.99 (m, 1H, $-C\underline{H_2}CH_2CH_2CH_3$), 2.43 (dd, 1H, J = 10.4 Hz, J = 15.6 Hz, 7ax), 2.94 (dd, 1H, J = 6.0 Hz, J = 15.2 Hz, 7eq), 3.76 (m, 1H, 6a), 4.39 (d, 1H, J = 9.2 Hz, 12a), 5.93 (d, 2H, J = 2.4 Hz, 11), 5.96 (d, 2H, J = 2.0 Hz, 9), 6.51 (s, 2H, 4), 7.04 (d, 2H, J = 0.4 Hz, 1); ^{13}C NMR (CD_3OD) δ 14.5 and 14.5 ($-CH_2CH_2CH_2\underline{C}H_3$), 24.0 and 24.3 ($-CH_2CH_2\underline{C}H_2CH_3$), 26.9 and 27.2 ($-CH_2\underline{C}H_2CH_2CH_3$), 28.0 (4), 41.6 and 41.7 ($-\underline{C}H_2CH_2CH_2CH_3$), 67.9 (3), 74.5 (2), 81.2 (7'), 95.8 (6), 96.5 (8), 101.3 (10), 112.5 (2'), 112.6 (5'), 126.9 (1'), 136.0 (6'), 144.9 (3'), 146.1 (4'), 156.9 (5), 157.7 (9), 157.9 (7); $[M-H]^-$: 413.

(6a*S*, 12a*R*)-6a, 12a-*trans*-2, 3, 8, 10-Tetrahydroxy-5, 5-dipentyl-5, 6a, 7, 12a-

tetrahydro-[1]benzopyrano[3, 2-c][2]benzopyran (PC5). After synthesis according to the general method using 6-undecanone as a ketone, the product was purified by column chromatography on silica gel (15:3:1 toluene–acetone-MeOH) to afford 0.32 g (52.8%) of PC5: ^{1}H NMR (CD_3OD) δ 0.81 (dd, 3H, -$CH_2CH_2CH_2CH_2C\underline{H}_3$), 0.90 (dd, 3H, -$CH_2CH_2CH_2CH_2C\underline{H}_3$), 1.12-1.40 (m, 10H, -$CH_2CH_2CH_2C\underline{H}_2CH_3$, -$CH_2CH_2C\underline{H}_2CH_2CH_3$ and -$CH_2C\underline{H}_2CH_2CH_2CH_3$), 1.44-1.53 (m, 2H, -$CH_2C\underline{H}_2CH_2CH_2CH_3$ and -$C\underline{H}_2CH_2CH_2CH_2CH_3$), 1.58-1.70 (m, 2H, -$CH_2C\underline{H}_2CH_2CH_2CH_3$ and -$C\underline{H}_2CH_2CH_2CH_2CH_3$), 1.85 (m, 1H, -$C\underline{H}_2CH_2CH_2CH_2CH_3$), 1.98 (m, 1H, -$C\underline{H}_2CH_2CH_2CH_2CH_3$), 2.43 (dd, 1H, J = 10.4 Hz, J = 15.6 Hz, 7ax), 2.94 (dd, 1H, J = 6.0 Hz, J = 15.2 Hz, 7eq), 3.76 (m, 1H, 6a), 4.39 (dd, 1H, J = 0.8 Hz, J = 9.2 Hz, 12a), 5.93 (d, 2H, J = 2.4 Hz, 11), 5.96 (d, 2H, J = 2.4 Hz, 9), 6.51 (s, 2H, 4), 7.04 (d, 2H, J = 0.8 Hz, 1); ^{13}C NMR (CD_3OD) δ 14.4 and 14.4 (-$CH_2CH_2CH_2CH_2\underline{C}H_3$), 23.6 and 23.7 (-$CH_2CH_2CH_2\underline{C}H_2CH_3$), 24.3 and 24.6 (-$CH_2CH_2\underline{C}H_2CH_2CH_3$), 33.3 and 33.5 (-$CH_2\underline{C}H_2CH_2CH_2CH_3$), 28.0 (4), 41.8 and 41.9 (-$\underline{C}H_2CH_2CH_2CH_2CH_3$), 68.0 (3), 74.5 (2), 81.3 (7'), 95.8 (6), 96.6 (8), 101.3 (10), 112.5 (2'), 112.6 (5'), 126.9 (1'), 134.0 (6'), 144.9 (3'), 146.2 (4'), 156.8 (5), 157.7 (9), 157.9 (7); [M-H]$^-$: 441.

(6a*S*, 12a*R*)-6a, 12a-*trans*-2, 3, 8, 10-Tetrahydroxy-5, 5-dihexyl-5, 6a, 7, 12a-tetrahydro-[1]benzopyrano[3, 2-c][2]benzopyran (PC6). After synthesis according to the general method using 7-tridecanone as a ketone, the product was purified by column chromatography on silica gel (15:3:1 toluene-acetone-methanol) to afford 0.28 g (44.6%) of PC6: ^{1}H NMR (CD_3OD) δ 0.84 (dd, 3H, -$CH_2CH_2CH_2CH_2CH_2C\underline{H}_3$), 0.89 (dd, 3H, -

$CH_2CH_2CH_2CH_2CH_2C\underline{H}_3$), 1.16-1.34 (m, 14H, -$CH_2CH_2CH_2CH_2C\underline{H}_2CH_3$ and –$CH_2CH_2CH_2C\underline{H}_2CH_2CH_3$ and -$CH_2CH_2C\underline{H}_2CH_2CH_2CH_3$), 1.44-1.52 (m, 2H, –$CH_2C\underline{H}_2CH_2CH_2CH_2CH_3$ and -$C\underline{H}_2CH_2CH_2CH_2CH_2CH_3$), 1.59-1.70 (m, 2H, –$CH_2C\underline{H}_2CH_2CH_2CH_2CH_3$ and -$C\underline{H}_2CH_2CH_2CH_2CH_2CH_3$), 1.85 (m, 1H, –$C\underline{H}_2CH_2CH_2CH_2CH_2CH_3$), 1.98 (m, 1H, –$C\underline{H}_2CH_2CH_2CH_2CH_2CH_3$), 2.43 (dd, 1H, J = 10.4 Hz, J = 15.2 Hz, 7ax), 2.94 (dd, 1H, J = 6.0 Hz, J = 15.6 Hz, 7eq), 3.75 (m, 1H, 6a), 4.39 (d, 1H, J = 9.2 Hz, 12a), 5.94 (d, 2H, J = 2.4 Hz, 11), 5.97 (d, 2H, J = 2.4 Hz, 9), 6.51 (s, 2H, 4), 7.04 (d, 2H, J = 0.8 Hz, 1); ^{13}C NMR (CD_3OD) δ 14.4 and 14.4 (-$CH_2CH_2CH_2CH_2CH_2\underline{C}H_3$), 23.7 and 23.7 (-$CH_2CH_2CH_2CH_2\underline{C}H_2CH_3$), 24.6 and 24.9 (-$CH_2CH_2CH_2\underline{C}H_2CH_2CH_3$), 30.7 and 31.0 (-$CH_2CH_2\underline{C}H_2CH_2CH_2CH_3$), 32.9 and 33.0 (-$CH_2\underline{C}H_2CH_2CH_2CH_2CH_3$), 28.0 (4), 41.8 and 42.0 (-$\underline{C}H_2CH_2CH_2CH_2CH_2CH_3$), 68.0 (3), 74.5 (2), 81.3 (7'), 95.9 (6), 96.6 (8), 101.3 (10), 112.5 (2'), 112.6 (5'), 126.9 (1'), 134.0 (6'), 144.9 (3'), 146.1 (4'), 156.9 (5), 157.7 (9), 157.9 (7); $[M-H]^-$: 469.

Spectral and Kinetic Measurements.

Typically, an aliquot of catechin (1.4×10^{-4} M) in deaerated MeCN was added to a quartz cuvette (10 mm i.d.) that contained DPPH (4.8×10^{-6} M) in deaerated MeCN (3.0 mL). This led to a hydrogen-transfer reaction from catechin to DPPH. Changes in the UV-vis spectrum associated with this reaction were monitored using a Hewlett-Packard 8453 photo diode array spectrophotometer. The reaction rates were determined by following the change in absorbance at 543 nm due to DPPH. Pseudo-first-order rate constants (k_{obs}) were determined by a least-squares curve fitting using an Apple Macintosh personal

computer. The first-order plots of $\ln(A_\infty - A)$ vs. time (A_∞ and A denote the final absorbance and the absorbance at the reaction time, respectively) were linear for 3 or more half-lives, with $\rho > 0.999$.

Antioxidant activity measurements.

2,2'-Azobis(2-amidinopropane)dihydrochloride (AAPH) and 5-Amino-2,3-dihydro-1,4-phthalazineone (Luminol) were purchased from Wako Pure Chemical Industries, Ltd. and ICN Biomedicals, Inc. Antioxidant activity was assayed as follows: 200 μL of Compound solution (3% DMSO aqueous solution) was pre-incubated at 37°C for 2 min, and then the antioxidant activity measurement was initiated by the addition of 200 μL of AAPH solution (50 mM AAPH / 200 mM Na phosphate buffer, pH 7.0) in a borosilicate glass tube (disposable culture tubes, 9830-1007, ASAHI TECHENO GLASS). The reaction mixture was incubated at 37°C for 2 min, and then 200 μL of luminol solution (0.1 mM luminol / 50 mM Na borate buffer (pH 9.3) containing 2% MeOH) was added. The liberated photons were measured using a photon counter (AccuFLEX Lumi 400, ALOKA) with cumulative light emission being monitored for 2 min integrals.

Assay for DNA strand breaks.

The effects of catechin and planar catechin on DNA strand breakage were measured in terms of the conversion of supercoiled pBR322 plasmid DNA to the open circular and linear forms. Reactions were carried out in 20μL (total volume) of 50 mM Na cacodylate buffer (2.5 % DMF), pH 7.2, containing 45 μM pBR322 DNA, 10 μM $FeCl_3$, 10 mM H_2O_2,

and each flavonoid. The reaction was started by adding H_2O_2 and, after incubation at 37°C for 1hr, the reaction mixture was then treated with 5 μL of loading buffer (100 mM TBE buffer, pH 8.3, containing 30 % glycerol, 0.1 % bromophenol blue) and applied to 1% agarose gel. Horizontal gel electrophoresis was carried out in 50 mM TBE buffer, pH 8.3. The gels were stained with ethidium bromide (1 μg / ml) for 30 min, destained in water for 30 min, and photographed with UV translumination.

Assay for α-glucosidase inhibitory activity.

The α-glucosidase inhibitory activity was determined by a partial modification of the procedure reported by Matsui et al. (Biosci. Biotech. Biochem., 60, 2019-2022, 1996). The 3.0μL of α-glucosidase from *S. cerevisiae* (TOYOBO, AGH-211) and 10μL of α-glucosidase from *B. stearothermophilus* (Sigma, G-3651) were dissolved in 5mL of 100mM potassium phosphate buffer (pH7.0). The reaction for *S. cerevisiae* α-glucosidase consisted of 900μL of 1.11mM 4-nitrophenyl α–D-glucopyranoside (PNP-G) in 100mM potassium phosphate buffer (pH7.0), 50μL of flavonoid in DMSO and 50μL of enzyme solution. After incubation for 20 min at 37°C, 300μL of the reaction mixture was added to 1200μL of 0.3M K_2CO_3 to stop the reaction, and the absorbance of 4-nitrophenol released from PNP-G at 405nm was measured. In the case of α-glucosidase from *B. stearothermophilus*, the reaction consisted of 540μL of 1.11mM 4-nitrophenyl α–D-glucopyranoside (PNP-G) in 100mM potassium phosphate buffer (pH7.0), 30μL of flavonoid in DMSO and 30μL of enzyme solution. After incubation for 20 min at 37°C, 180μL of reaction mixture was added to 720μL of 0.3M K_2CO_3, and the absorbance of 4-

nitrophenol released from PNP-G at 405nm was measured. The concentration of inhibitors required for inhibiting 50% of α-glucosidase activity under the assay conditions was defined as the IC_{50} value. The IC_{50} value was measured graphically by a plot of percent inhibition versus log of the test compound.