J|A|C|S
COMMUNICATIONS

Published on Web 05/03/2006

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

Wataru Hakamata,[†] Ikuo Nakanishi,[‡,§] Yu Masuda,[∥] Takehiko Shimizu,[⊥] Hajime Higuchi,[⊥] Yuriko Nakamura,[#] Shinichi Saito,[#] Shiro Urano,[⊥] Tadatake Oku,[∥] Toshihiko Ozawa,[‡] Nobuo Ikota,[‡] Naoki Miyata,[◇] Haruhiro Okuda,[†] and Kiyoshi Fukuhara*[,†]

Division of Organic Chemistry, National Institute of Health Sciences, Setagaya-ku, Tokyo 158-8501, Japan, Redox Regulation Research Group, Research Center for Radiation Safety, National Institute of Radiological Sciences, Inage-ku, Chiba 263-8555, Japan, Graduate School of Engineering, Osaka University, SORST, Japan Science and Technology Agency, Suita, Osaka 565-0871, Japan, College of Bioresource Sciences, Nihon University, Fujisawa, Kanagawa 1866, Japan, Department of Applied Chemistry, Shibaura Institute of Technology, Minato-ku, Tokyo 108-8548, Japan, Faculty of Science, Tokyo University of Science, Shinjuku-ku, Tokyo 162-8601, Japan, and Graduate School of Pharmaceutical Sciences, Nagoya City University, Mizuho-ku, Nagoya, Aichi 467-8603, Japan

Received November 15, 2005; E-mail: fukuhara@nihs.go.jp

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·Et_2O.[3,4] The rate of hydrogen transfer from **PC1** to galvinoxyl radical (**G**•), a stable oxygen-centered radical, is about 5-fold faster than that of hydrogen transfer from the native (+)-catechin to **G**•. **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.

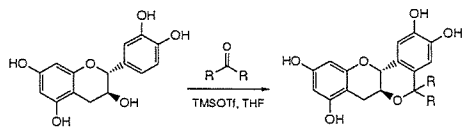
PCn were evaluated for their radical scavenging activities against DPPH (2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl) radical and AAPH (2,2′-azobis(2-amidinopropane) dihydrochloride)-derived peroxyl radical (Scheme 2). The hydrogen abstraction of **PCn** by DPPH radical in deaerated acetonitrile solution 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

† National Institute of Health Sciences, Japan.
‡ National Institute of Radiological Sciences, Japan.
§ Osaka University, SORST, Japan Science and Technology Agency.
∥ Nihon University.
⊥ Shibaura Institute of Technology.
Tokyo University of Science.
◇ Nagoya City University.

10.1021/ja057763c CCC: $33.50

Scheme 1. Chemical Structure and Synthesis of PCn



PC1 R = CH_3, *76.3% yield*
PC2 R = CH_2CH_3, *73.5% yield*
PC3 R = $(CH_2)_2CH_3$, *66.7% yield*
PC4 R = $(CH_2)_3CH_3$, *59.1% yield*
PC5 R = $(CH_2)_4CH_3$, *52.8% yield*
PC6 R = $(CH_2)_5CH_3$, *44.6% yield*

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

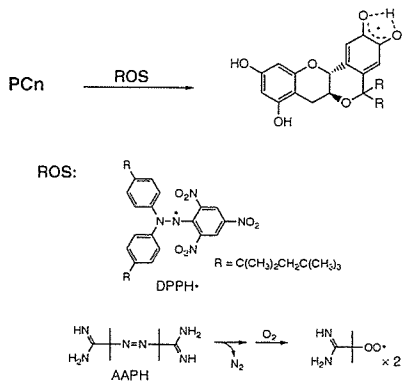


Table 1. Antioxidant Profile of Catechin and PCn Determined Using a DPPH and AAPH Scavenging Assay

compd	DPPH• k_{inh} (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

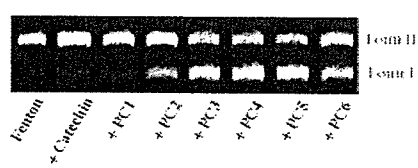


Figure 1. Effects of catechin and PCn on DNA breakage induced by the Fenton reaction (Fe^{3+}/H_2O_2). Assays were performed in 100 mM phosphate buffer, pH 7.0 containing 45 μM pBR322DNA, 10 mM H_2O_2, 100 μM $FeCl_3$, and 1 mM individual PCn for 1 h at 37 °C.

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 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.

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

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.

Acknowledgment. This work was supported partly by a Grant from the Ministry of Health, Labor and Welfare, and by a Grant-in-Aid for Research of Health Sciences focusing on Drug Innovation (KH51058) from the Japan Health Sciences Foundation, partly by Grant-in-Aids for Scientific Research (B) (No. 17390033) and for Young Scientist (B) (No. 17790044) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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.

JA057763C

Supporting Information for

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

Hakamata, Nakanishi, Masuda, Shimizu, Higuchi, Nakamura, Oku, Saito, Urano, Ozawa, Ikota, Miyata, Okuda, and Fukuhara*

General methods. The NMR spectra were recorded with a Varian AS 400 Mercury spectrometer (400 MHz for ^{1}H 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*(6a*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**: ^{1}H 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, -$CH_2CH_2C\underline{H}_2CH_3$ and -$CH_2C\underline{H}_2CH_2CH_3$), 1.42-1.48 (m, 2H, -$CH_2C\underline{H}_2CH_2CH_3$ and -$C\underline{H}_2CH_2CH_2CH_3$), 1.59-1.71 (m, 2H, -$CH_2C\underline{H}_2CH_2CH_3$ and –$C\underline{H}_2CH_2CH_2CH_3$), 1.86 (m, 1H, -$C\underline{H}_2CH_2CH_2CH_3$), 1.99 (m, 1H, -$C\underline{H}_2CH_2CH_2CH_3$), 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 (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.

(6*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 ($A\infty$ 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.

J. Appl. Glycosci., **53**, 149–154 (2006)

Proceedings of the Symposium on Amylases and Related Enzymes, 2005

N-Linked Oligosaccharide Processing Enzymes as Molecular Targets for Drug Discovery

(Received December 1, 2005)

Wataru Hakamata,[1,*] Makoto Muroi,[2] Toshiyuki Nishio,[3] Tadatake Oku,[3] Akira Takatsuki,[4] Hiroyuki Osada,[2] Kiyoshi Fukuhara,[1] Haruhiro Okuda[1] and Masaaki Kurihara[1]

[1]*Division of Organic Chemistry, National Institute of Health Sciences (NIHS) (1–18–1, Kamiyoga, Setagaya-ku, Tokyo 158–8501, Japan)*
[2]*Antibiotics Laboratory, The Institute of Physical and Chemical Research (RIKEN) (2–1, Hirosawa, Wako, Saitama 351–0198, Japan)*
[3]*Department of Biological Chemistry, College of Bioresource Sciences, Nihon University (1866, Kameino, Fujisawa, Kanagawa 252–8510, Japan)*
[4]*Department of Materials Chemistry, Faculty of Engineering, Hosei University (3–7–2, Kajino-cho, Koganei, Tokyo 184–8584, Japan)*

Abstract: *N*-Linked oligosaccharide processing enzymes are key enzymes in the biosynthesis of *N*-linked oligosaccharides. These enzymes are a molecular target for inhibition by anti-viral agents that interfere with the formation of essential glycoproteins required in viral assembly, secretion and infectivity. We think that the molecular recognition of three kinds of glucosidases (family 13 and family 31 α-glucosidases and endoplasmic reticulum glucosidases) are different. Therefore, glycon and aglycon specificity profiling of glucosidases was an important approach for the research of glucosidase inhibitors. We carried out the profiling of glucosidases using small molecules as a probe. Moreover, we designed and synthesized three types of glucosidase inhibitors. These compounds were evaluated with regard to their ability to inhibit glucosidases *in vitro*, and were also tested in a cell culture system. We found some compounds having glucosidase inhibitory activity and anti-viral activity.

Key words: α-glucosidase, ER glucosidase, inhibitor, anti-viral activity

α-Glucosidases (EC 3.2.1.20) are also exo-acting carbohydrases, catalyzing the release of α-D-glucopyranose from the non-reducing ends of various substrates,[1,2] and on the basis of amino acid sequence similarities, α-glucosidases are classified into two families, family 13 and family 31.[3,4] Endoplasmic reticulum (ER) glucosidases, glucosidase I (EC 3.2.1.106) and glucosidase II (EC 3.2.1.84), are key enzymes in the biosynthesis of asparagine-linked oligosaccharides that catalyze the first processing event after the transfer of $Glc_3Man_9GlcNAc_2$ to proteins. These enzymes are a target for inhibition by anti-viral agents that interfere with the formation of essential glycoproteins required in viral assembly, secretion and infectivity.[5] Many papers reported that inhibitors of α-glucosidases are potential therapeutics for the treatment of such diseases as viral diseases, cancer and diabetes.[5,6] However, many screenings of α-glucosidase inhibitors did not use enzymes from target tissues or organs. We think that the molecular recognitions of three kinds of glucosidases (family 13, family 31 α-glucosidases and ER glucosidases) are different. Therefore, the glycon and aglycon specificity profiling of glucosidases has been an important approach for the research of glucosidase inhibitors.

In this research, we first describe the glycon and aglycon specificity profiling of glucosidases using small molecules as probes. Next, compounds designed and synthesized as glucosidase inhibitor candidates were evaluated with regard to their ability to inhibit three kinds of glucosidases. Finally, the glucosidase inhibitor candidates were tested for their anti-viral activities in a cell culture system.

* Corresponding author (Tel. +81–3–3700–1141, Fax. +81–3–3707–6950, E-mail: hakamata@nihs.go.jp).

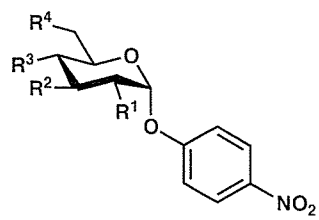
Glycon specificity profiling of glucosidases using chemically modified substrates.

Chemically modified substrates are effective methods in the study of substrate specificity profiling. We have applied this approach to family 13 and family 31 α-glucosidases,[7–10] ER glucosidases,[11,12] α-galactosidases[8,13] and α-mannosidases[8,14] using partially substituted monosaccharides. We used all of the monodeoxy analogs of *p*-nitrophenyl α-D-glucopyranoside (PNP α-Glc) **1–4** (Fig. 1) as chemically modified substrates for glycon specificity profiling. We investigated the hydrolytic activities of family 13 and family 31 α-glucosidases and ER glucosidase II of PNP α-Glc and its deoxy derivatives **1–4**, and checked the inhibitory activities of ER glucosidase I of PNP α-Glc and probes **1–4**, so that PNP α-Glc was not a substrate for ER glucosidase I. These results are shown in Table 1.[11,12] Clearly, of the four deoxy derivatives of PNP α-Glc **1–4**, family 31 α-glucosidases and ER glucosidase II hydrolyzed the 2-deoxy glucopyranoside (**1**); its activity with **1** appeared to be substantially higher than that with PNP α-Glc. Kinetic studies of the hydrolysis of PNP α-Glc, **1** and **2** were also carried out (Table 2).[9,11] The V_{max}/K_m or k_{cat}/K_m values of family 31 α-glucosidases

and ER glucosidase II for **1** was about twice as great as PNP α-Glc, which indicated that probe **1** was a good substrate for the enzymes. These reaction velocities to probe **1** increased to 3–28 fold that of PNP α-Glc. PNP Glc and probes **1–4** inhibited ER glucosidase I by 56.2, 71.7, 18.5, 22.2 and 32.3% at 5 mM, respectively. These results also indicated that ER glucosidase II might have properties similar to those found in family 31 α-glucosidases.

Aglycon specificity profiling and inhibition of glucosidases using heptitol derivatives.

For aglycon specificity profiling, we designed and synthesized eight probes, **5–12**, including 1-amino-2, 6-anhydro-1-deoxy-D-*glycero*-D-*ido*-heptitol, which might mimic to a great extent the topography of α-D-glucopyranoside and modified aglycon of α-glucopyranoside (Fig. 2).[11] These probes do not have the specific functional groups for glycosidase inhibition, electrostatic interactions (*e.g.* 1-deoxynojirimycine), transition state mimetic structure (*e.g.* D-gluconolactone), or covalent bond formation with the enzyme catalytic site (*e.g.* conduritol B epoxide). The structures of α-glucosidase inhibitors are summarized in Fig. 3. We investigated the inhibitory activities of family 13 and family 31 α-glucosidases, ER glucosidases, and other glycosidases (β-glucosidase, α- and β-mannosidase, α- and β-galactosidase) against probes



1: R^1=H, R^2=R^3=R^4=OH
2: R^1=R^3=R^4=OH, R^2=H
3: R^1=R^2=R^4=OH, R^3=H
4: R^1=R^2=R^3=OH, R^4=H

Fig. 1. Chemical structure of glycon profiling probes **1–4**.

5–12, and their aglycon specificity profiling was discussed. The values of the % inhibition and IC_{50} are summarized in Table 3.[12] Probe **8** indicated specific inhibitions of *Saccharomyces* (*S.*) *cerevisiae* (IC_{50}=55.5 μM) and *Bacillus* (*B.*) *stearothermophilus* (IC_{50}=415 μM) α-glucosidases. Probe **11** inhibited α-glucosidase from *S. cerevisiae* (IC_{50}=449 μM). Honey bee isozyme I (HBG I) was inhibited by probe **5** (IC_{50}=851 μM). Family 13 α-glucosidases and ER glucosidases were inhibited by the specific probes. On the other hand, family 31 α-glucosidases were broadly inhibited by probes **5–12**. All probes did not inhibit β-glucosidase, α- or β-mannosidases, or α- or β-galactosidases at a 5-fold concentration. These facts indicated that aglycon specificities of α-glucosidases differed greatly among family 13 α-glucosidases, family 31 α-glucosidases and ER glucosidases. Moreover, each aglycon specificity of family 13 α-glucosidases is different in spite of the highly conserved amino acid sequences in the catalytic site.[15] In the kinetic studies on the inhibitions of **8** and **11** and the hydrolysis of PNP α-Glc by *S. cerevisiae* and *B. stearothermophilus* α-glucosidases, the values of K_i and K_m (mM) were calculated from Dixon plots and Michaelis-Menten plots, respectively, and these values and inhibition types are summarized in Table 4.[12] Probes **8** and **11** were competitive type inhibitors of the *S. cerevisiae* enzyme (K_i=0.13 mM and 0.50 mM). Probe **8** was a mixed type inhibitor of *B. stearothermophilus* enzyme (K_i=0.58 mM). The affinities of **8** against both enzymes were higher than PNP α-Glc as a substrate. These results indicated that probe **8** formed a specific hydrogen bond between the primary hydroxyl group of aglycon moiety and *S. cerevisiae* enzyme, and that probe **11**, with a terminal phenyl group, formed a hydrophobic interaction with the *S. cerevisiae* enzyme.

Inhibition of α-glucosidase by reactive oxygen species.

The reactive oxygen species (ROS) generated com-

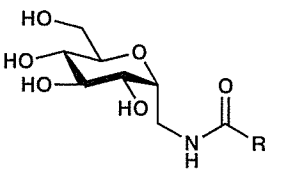
Table 1. Hydrolytic activities and inhibitory activities of probes **1–4** against glucosidases.[11,12]

Enzyme source	Relative rate of hydrolysis (%) / % Inhibition				
	PNP α-Glc	PNP 2D α-Glc (**1**)	PNP 3D α-Glc (**2**)	PNP 4D α-Glc (**3**)	PNP 6D α-Glc (**4**)
ER Processing glucosidase					
Rat microsome					
Glucosidase I	– / 56.2	– / 71.7	– / 18.5	– / 22.2	– / 32.3
Glucosidase II	100 / HD	189 / HD	– / –	– / –	– / –
	Relative rate of hydrolysis (%)				
α-Glucosidase family 13					
S. cerevisiae	100	–	–	–	–
B. stearothermophilus	100	–	–	–	–
Honey bee I	100	–	–	–	–
Honey bee II	100	–	–	–	–
Honey bee III	100	–	–	–	–
α-Glucosidase family 31					
Rice	100	175	–	–	–
Sugar beet	100	244	–	–	–
Flint corn	100	231	3.7	–	–
A. niger	100	259	11.9	–	–

Relative rate of hydrolysis was expressed by comparison with the amount of *p*-nitrophenol that was released from PNP α-Glc, which was taken as 100%. Assay of glucosidase I inhibitory activities used [^{3}H] glucose-labeled VSV glycoprotein as a substrate. –, Hydrolytic or inhibitory activity was not detected, HD, Hydrolyzing activity was observed.

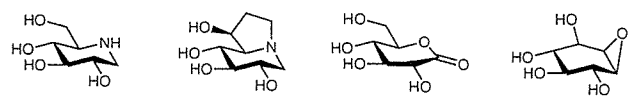
Table 2. Kinetic study of hydrolysis of family 31 α-glucosidases and ER glucosidase II.[9,11]

Enzyme / Substrate	K_m (mM)	V_{max} (μmol/min/U)	V_{max}/K_m
ER glucosidase II			
PNP α-Glc	0.92	1.12	1.23
PNP 2D α-Glc (**1**)	0.76	3.44	4.53
Enzyme / Substrate	K_m (mM)	k_{cat} (s^{-1})	k_{cat} / K_m
Rice α-glucosidase			
PNP α-Glc	2.62	43.8	16.7
PNP 2D α-Glc (**1**)	6.66	237	35.6
Sugar beet α-glucosidase			
PNP α-Glc	1.04	0.071	0.068
PNP 2D α-Glc (**1**)	5.70	0.64	0.11
Flint corn α-glucosidase			
PNP α-Glc	0.88	2.00	2.27
PNP 2D α-Glc (**1**)	7.38	17.0	2.30
PNP 3D α-Glc (**2**)	9.98	0.44	0.044
A. niger α-glucosidase			
PNP α-Glc	0.59	3.44	5.83
PNP 2D α-Glc (**1**)	6.09	96.9	15.9
PNP 3D α-Glc (**2**)	10.2	4.23	0.41



5: R=CH_2OH
6: R=$(CH_2)_2OH$
7: R=$(CH_2)_3OH$
8: R=$(CH_2)_4OH$
9: R=$(CH_2)_5OH$
10: R=Me
11: R=Ph
12: R=$C(CH_3)_3$

Fig. 2. Chemical structure of aglycon profiling probes **5**–**12**.



1-deoxynojirimycine castanospermine D-gluconolactone conduritol B epoxide

Fig. 3. Chemical structure of typical α-glucosidase inhibitor.

pounds, **13**–**24**, shown in Fig. 4, were assessed as inhibitors of glycoside hydrolase family 13 α-glucosidases and family 31 α-glucosidases,[16] and the results are listed in Table 5 (Preparation for publication). Compounds **18** and **24**, with a terminal α-naphthyl group, indicated inhibitions of α-glucosidases from *S. cerevisiae* (IC_{50}=51.7 μM and IC_{50}=74.1 μM) and *B. stearothermophilus* (IC_{50}=60.1 μM and IC_{50}=89.1 μM). We reasoned that the enzymatic liberation of the aglycon from compounds **18** and **24** might be followed by the ejection of a sulfinate anion with the concomitant formation of *p*-benzoquinone and *p*-benzoquinone imine, which would then generate ROS in the enzyme active site, leading to enzyme deactivation.[16,17] Therefore, the effects of compounds **18** and **24** on ROS-mediated DNA breakage were investigated. DNA strand scission in the super coiled pBR322DNA was induced by ROS in the presence of *p*-benzoquinone or *p*-benzoquinone imine, metal ion, and NADH.[17] Compound **24** induced DNA strand breakage condition in the above conditions (data not shown). We suggest that ROS-generated enzyme inhibition might be a new approach for the development of an enzyme inhibitor.

Inhibition of α-glucosidase by catechin derivatives.

The catechin derivatives **25**–**33** shown in Fig. 5 were assessed as inhibitors of family 13 and family 31 α-glucosidases, and the results are listed in Table 6.[18] A comparison of the results against family 13 and family 31 α-glucosidases shows that family 13 α-glucosidases were remarkably inhibited by catechin derivatives compared with family 31 α-glucosidases. The potent inhibition of family 13 α-glucosidases, *S. cerevisiae* and *B. stearothermophilus*, shown by catechin derivative **25** (IC_{50}=1.2 μM and IC_{50}=0.7 μM) and **30** (IC_{50}=0.9 μM and IC_{50}=1.1 μM), are in contrast to the weak activity shown by cate-

Table 3. Inhibitory activities of probes **5**–**12** against glycosidases.[12]

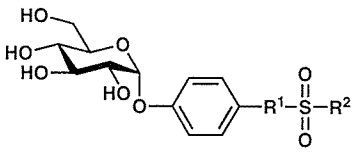
Enzyme source	% Inhibition (IC_{50})							
	5	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Family 13 α-glucosidase								
S. cerevisiae	<1.0	21.1	<1.0	100 (55.5 μM)	<1.0	<1.0	67.4 (449 μM)	6.1
B. stearothermophilus	<1.0	<1.0	<1.0	100 (415 μM)	<1.0	<1.0	<1.0	<1.0
Honey bee I	52.3 (851 μM)	<1.0	<1.0	37.5	<1.0	10.4	4.6	<1.0
Honey bee II	4.4	2.7	3.6	21.4	4.4	<1.0	12.3	<1.0
Honey bee III	<1.0	3.2	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Family 31 α-glucosidase								
Rice	10.7	8.5	7.6	18.3	26.0	21.8	16.0	3.8
Sugar beet	6.9	1.7	3.6	3.1	11.9	8.8	9.8	3.2
Flint corn	29.1	14.1	18.5	37.0	44.6	31.0	49.2	5.6
A. niger	6.6	2.6	<1.0	6.8	<1.0	23.3	14.0	1.2
ER processing glucosidase								
Glucosidase I	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	18.2	<1.0
Glucosidase II	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	5.9	<1.0
β-Glucosidase	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
α-Mannosidase	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
β-Mannosidase	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
α-Galactosidase	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
β-Galactosidase	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0

Probe concentrations (family 13 and 31 α-glucosidases: 1 μmol/mL, ER processing α-glucosidases: 2 μmol/mL, β-glucosidase, mannosidases and galactosidases: 5 μmol/mL). Substrate (family 13 and 31 α-glucosidases, ER glucosidase II: PNP α-Glc, ER glucosidases I: [^{3}H] glucose-labeled vesicular stomatitis virus glycoprotein, β-glucosidase: PNP β-Glc, α-mannosidase: PNP α-Man, β-mannosidase: PNP β-Man, α-galactosidase: PNP α-Gal, β-galactosidase: PNP β-Gal).

Table 4. Kinetic studies of the inhibition of family 13 α-glucosidases.[12]

Probe	*S. cerevisiae*		*B. stearothermophilus*	
	Ki (mM)	Inhibition type	*Ki* (mM)	Inhibition type
8	0.13	Competitive	0.58	Mixed
11	0.50	Competitive	–	–
PNP α-Glc	0.35[a]	–	1.16[a]	–

[a]*K*m value.

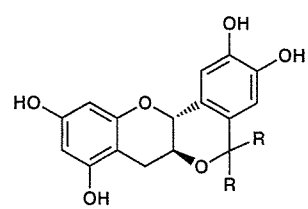


13: R^1=O, R^2=NO_2
14: R^1=O, R^2=Cl
15: R^1=O, R^2=CF_3
16: R^1=O, R^2=CH_3
17: R^1=O, R^2=$C(CH_3)_3$
18: R^1=O, R^2=α-naphthyl
19: R^1=NH, R^2=NO_2
20: R^1=NH, R^2=Cl
21: R^1=NH, R^2=CF_3
22: R^1=NH, R^2=CH_3
23: R^1=NH, R^2=$C(CH_3)_3$
24: R^1=NH, R^2=α-naphthyl

Fig. 4. Chemical structure of ROS-generated compounds **13**–**24**.

Table 5. Inhibitory activity of ROS-generated compounds **13**–**24** against α-glucosidases.

Compound	IC_{50} (μM)		
	Glycoside hydrolase family 13		Glycoside hydrolase family 31
	S. cerevisiae	*B. stearothermophilus*	Rice
13	499	>500	>500
14	437	>500	>500
15	407	>500	>500
16	499	>500	>500
17	391	>500	>500
18	51.7	60.1	>500
19	239	218	>500
20	200	254	>500
21	146	244	>500
22	231	325	>500
23	136	237	>500
24	74.1	89.1	>500



25: R=CH_3
26: R=CH_2CH_3
27: R=$(CH_2)_2CH_3$
28: R=$(CH_2)_3CH_3$
29: R=$(CH_2)_4CH_3$
30: R=$(CH_2)_5CH_3$
31: R=$(CH_2)_6CH_3$
32: R=$(CH_2)_7CH_3$
33: R=$(CH_2)_8CH_3$

Fig. 5. Chemical structure of catechin derivatives **25**–**33**.

chin derivative **26**, which has one methylene group long alkyl side chain compared with **25** (IC_{50}=47.5 μM and IC_{50}=26.8 μM) and catechin derivative **33** which has three methylene groups long alkyl side chain compared with **30** (IC_{50}=64.0 μM and IC_{50}=28.1 μM). From these results, it is thought that the inhibition mechanism of catechin derivative **25** and the inhibition mechanism of catechin derivative **30** are different. The IC_{50} values of typical α-glucosidase inhibitor 1-deoxynojirimycine (see Fig. 3) and catechin derivative **30** against *S. cerevisiae* α-glucosidase

Table 6. Inhibitory activity of catechin derivatives **25**–**33** against α-glucosidases.[18]

Compound	IC_{50} (μM)			
	Glycoside hydrolase family 13		Glycoside hydrolase family 31	
	S. cerevisiae	*B. stearothermophilus*	Rice	*A. niger*
Catechin	>500	>500	>500	>500
25	1.2	0.7	>500	>500
26	47.5	26.8	>500	>500
27	37.5	28.4	>500	>500
28	2.1	14.2	>500	>500
29	5.3	6.8	248	>500
30	0.9	1.1	>500	>500
31	4.9	21.1	>500	>500
32	33.2	13.8	>500	>500
33	64.0	28.1	>500	>500

were 3.3[19] and 0.9 μM, respectively. This result indicated that catechin derivative **30** is about 3.6 times more potent than 1-deoxynojirimycine when their IC_{50} values are compared.

Anti-viral activity of α-glucosidase inhibitors.

Compounds **1**–**33** were assayed with regard to their ability to inhibit glycoprotein processing at the cellular level. Vesicular stomatitis virus glycoprotein (VSV G) was prepared from VSV-infected and probe-treated baby hamster kidney (BHK) cells.[11] Analyses of the *N*-glycan structure of obtained VSV G using endo H, which is known to have hydrolytic activity against high-mannose type *N*-glycan, failed to confirm that compounds **1**–**24** except for catechin derivatives (**25**–**33**) inhibited processing glycosidases. The catechin derivatives had the possibility of inhibition of processing glycosidases (data not shown). Then, we assayed the anti-virus activities by effects of the catechin derivatives of processing glycosidases on virus glycoprotein synthesis and syncytium formation after newcastle disease virus (NDV) infection, and effects on synthesis and cell surface expression of NDV glycoprotein, hemagglutinin-neuraminidase (HANA) glycoprotein in whole cell lysates were quantified. Moreover, viral infectivity was determined by a plaque assay in BHK cells.[20] In the above assays, catechin derivative **30** showed potent inhibition of the viral infectivity (Table 7, Preparation for publication).

Conclusion and perspectives.

The discovery of glucosidase inhibitors may help us to understand the roles of the oligosaccharides of glycoproteins and glycolipids in cellular functions, and pharmaceutical applications. From this study, it is better to use enzymes of target tissues or organs for the screening of agents for viral diseases, cancer and diabetes. Moreover, in applying glucosidases as inhibitors of glycoprotein processing, inhibitory action of many inhibitors at the cellular levels is not so remarkable, as expected based on their action at the enzyme level. This was speculated to be caused by the difficulty for inhibitors to be able to access the site of action. We think that high throughput

Table 7. Anti-viral activity of catechin derivatives at the cellular level.

Compound	Conc. (μM)	% HAU	SF	% PFU	CPU
Catechin	500	100	+	95	+
	250	100	+	100	+
	125	100	+	NT	+
	63	100	+	NT	+
25	500	0	−	0	+
	250	6	−	14	+
	125	100	+	100	+
	63	100	+	100	+
26	500	0	−	0	−
	250	0	−	0	+
	125	100	+	24	+
	63	100	+	85	+
27	500	0	−	0	−
	250	0	−	0	−
	125	0	−	0	−
	63	100	+	90	+
28	500	0	−	0	−
	250	0	−	0	−
	125	0	−	0	−
	63	100	+	90	+
29	500	0	−	0	−
	250	0	−	0	−
	125	0	−	0	−
	63	100	+	50	−
	31	100	+	100	+
	16	100	+	100	+
30	500	0	−	0	−
	250	0	−	0	−
	125	0	−	0	−
	63	9	+/−	25	−
	31	100	+	95	+
	16	100	+	100	+
33	500	0	−	0	−
	250	0	−	0	−
	125	25	−	50	−
	63	100	+	100	+

screening assays using specific probes and enzymes of target tissues or organs and highly effective design and synthesis of inhibitors *in silico* are necessary for the development of new and potent glucosidase inhibitors.

The authors express sincere thanks to Dr. Seiya Chiba and Dr. Atsuo Kimura for their advice and for some of the α-glucosidases. We thank Ezaki Glico Co., Ltd. for the gift of α-arbutin. This work was performed at the Special Postdoctoral Researchers Program (RIKEN). This research was partially supported by a Ministry of Education, Science, Sports and Culture, Grant-in-Aid for Young Scientists (B), 17790097, 2005.

REFERENCES

1) S. Chiba: *Handbook of Amylases and Related Enzymes*, Amylase Research Society of Japan, eds., Pergamon, Oxford, pp. 104–116 (1988).
2) T.P. Frandesn and B. Svensson: Plant α-glucosidases of the glycoside hydrolase family 31. Molecular properties, substrate specificity, reaction mechanism, and comparison with family members of different origin. *Plant Molecul. Biol.*, **37**, 1–13 (1998).
3) G.J. Davies and B. Henrissat: Structural enzymology of carbohydrate-active enzymes: implications for the post-genomicera. *Biochem. Soc. Trans.*, **30**, 291–297 (2001).
4) Y. Bourne and B. Henrissat: Glycoside hydrolases and glycosyltransferases: families and functional modules. *Curr. Opin. Struct. Biol.*, **11**, 593–600 (2001).
5) A. Mehta, N. Zitzmann, P.M. Rudd, T.M. Block, R.A. Dwek: α-Glucosidase inhibitors as potential broad based anti-viral agents. *FEBS Lett.*, **430**, 17–22 (1998).
6) R.A. Dwek, T.D. Butters, F.M. Platt and N. Zitzmann: Targeting glycosylation as a therapeutic approach. *Nat. Rev. Drug Discovery*, **1**, 65–75 (2002).
7) W. Hakamata, T. Nishio and T. Oku: Synthesis of *p*-nitrophenyl 3- and 6-deoxy-α-D-glucopyranosides and their specificity to rice α-glucosidase. *J. Appl. Glycosci.*, **46**, 459–463 (1999).
8) W. Hakamata, T. Nishio, R. Sato, T. Mochizuki, K. Tsuchiya, M. Yasuda and T. Oku: Synthesis of monomethyl derivatives of *p*-nitrophenyl α-D-gluco, galacto, and mannopyranosides and their hydrolytic properties against α-glycosidase. *J. Carbohydr. Chem.*, **19**, 359–377 (2000).
9) T. Nishio, W. Hakamata, A. Kimura, S. Chiba, A. Takatsuki, R. Kawachi and T. Oku: Glycon specificity profiling of α-glucosidases using monodeoxy and mono-*O*-methyl derivatives of *p*-nitrophenyl α-D-glucopyranoside. *Carbohydr. Res.*, **337**, 629–634 (2002).
10) T. Nishio, W. Hakamata, M. Ogawa, K. Nakajima, Y. Matsuishi, R. Kawachi and T. Oku: Investigations of useful α-glycosidase for the enzymatic synthesis of rare sugar oligosaccharides. *J. Appl. Glycosci.*, **52**, 153–160 (2005).
11) W. Hakamata, M. Muroi, T. Nishio, T. Oku and A. Takatsuki: Recognition properties of processing α-glucosidase I and α-glucosidase II. *J. Carbohydr. Chem.*, **23**, 27–39 (2004).
12) W. Hakamata, M. Muroi, K. Kadokura, T. Nishio, T. Oku, A. Kimura, S. Chiba and A. Takatsuki: Aglycon specificity profiling of α-glucosidases using synthetic probes. *Bioorg. Med. Chem. Lett.*, **15**, 1489–1492 (2005).
13) W. Hakamata, T. Nishio and T. Oku: Hydrolytic activity of α-galactosidase against deoxy derivatives of *p*-nitrophenyl α-D-galactopyranoside. *Carbohydr. Res.*, **324**, 107–115 (2000).
14) T. Nishio, Y. Miyake, H. Tsujii, W. Hakamata, K. Kadokura and T. Oku: Hydrolytic activity of α-mannosidase against deoxy derivatives of *p*-nitrophenyl α-D-mannopyranoside. *Biosci. Biotechnol. Biochem.*, **60**, 2038–2042 (1996).
15) A. Kimura: Molecular anatomy of α-glucosidase, *Trends Glycosci. Glycotechnol.*, **12**, 373–380 (2000).
16) J.C. Briggs, A.H. Haines and R.J.K. Taylor: 4-(Sulfonylamino) phenyl α-D-glucopyranosides as competitive inhibitors of yeast α-glucosidase. *J. Chem. Soc., Chem. Commun.*, **18**, 1410–1411 (1993).
17) K. Fukuhara, I. Nakanishi, H. Kansui, E. Sugiyama, M. Kimura, T. Shimada, S. Urano, K. Yamaguchi and N. Miyata: Enhanced radical-scavenging activity of a planar catechin analogue. *J. Am. Chem. Soc.*, **124**, 5952–5953 (2002).
18) W. Hakamata, I. Nakanishi, Y. Masuda, T. Shimizu, H. Higuchi, Y. Nakamura, T. Oku, S. Saito, S. Urano, T. Ozawa, N. Ikota, N. Miyata, H. Okuda and K. Fukuhara: Planar catechin analogues with alkyl side chain, a potent antioxidant and α-glucosidase inhibitor. *J. Am. Chem. Soc.* (2006). (in press)
19) A.B. Hughes and A.J. Rudge: Deoxynojirimycin: synthesis and biological activity. *Nat. Prod. Rep.*, **11**, 135–162 (1994).
20) E. Tsujii, M. Muroi, N. Shiragami and A. Takatsuki: Nectrisine is a potent inhibitor of α-glucosidases, demonstrating activities similarly at enzyme and cellular levels. *Biochem. Biophys. Res. Commun.*, **220**, 459–466 (1996).

N-結合型糖鎖プロセッシング酵素を分子標的とした創薬

袴田 航[1]，室井 誠[2]，西尾俊幸[3]，奥 忠武[3]，
高月 昭[4]，長田裕之[2]，福原 潔[1]，奥田晴宏[1]，
栗原正明[1]
[1] 国立医薬品食品衛生研究所有機化学部
(158-8501 東京都世田谷区上用賀 1-18-1)
[2] 理化学研究所長田抗生物質研究室
(351-0198 和光市広沢 2-1)
[3] 日本大学生物資源科学部農芸化学科
(252-8510 藤沢市亀井野 1866)
[4] 法政大学工学部生命機能科学科
(184-8584 小金井市梶野町 3-7-2)

現在，新H5N1型インフルエンザやSARSなど続々と出現する新興ウイルス感染症や鳥インフルエンザのヒトへの伝播等，新興ウイルス感染症は人類の脅威となっている．しかし，ウイルス感染症に対する有効な薬剤の開発は，細菌感染症の抗生物質に比べ遅れている．そこで，ウイルス共通の感染機序に基づいた薬剤の開発が重要と考え，外被を有する多くのウイルスの感染・増殖には複合型の*N*-結合型糖鎖が関与している知見を基にして，小胞体*N*-結合型糖鎖プロセシング酵素を標的酵素とした分子標的薬の開発を目指して研究を行っている．分子標的薬の開発には，標的酵素である糖鎖プロセシング酵素の基質特異性の解明が必要であると考えた．そこで，合成プローブを用いて*N*-結合型糖鎖プロセシングの第1段階を担うプロセシンググルコシダーゼI (EC 3.2.1.106)と第2段階を担うプロセシンググルコシダーゼII (EC 3.2.1.84)のグリコンおよびアグリコン特異性を調べ，α-グルコシダーゼ (EC 3.2.1.20, GH13 and GH31) のそれと比較した．その結果，グルコシダーゼIのグリコン特性はGH13 α-グルコシダーゼと，グルコシダーゼIIのグリコン特性はGH31 α-グルコシダーゼと同様であった．またグルコシダーゼIとグルコシダーゼIIのアグリコン認識は同様であり，GH13およびGH31 α-グルコシダーゼとは異なっていた．そこで，プロセシンググルコシダーゼIおよびIIを標的として，酵素阻害剤候補化合物の設計と合成を行った．これら候補化合物の*in vitro*酵素阻害活性と細胞レベルでのウイルス外被糖タンパク質の合成・成熟・転送阻害およびプラーク法による感染性ウイルス数の測定を行った．その結果，*in vitro*においてヘプチトール誘導体，スルフォニル誘導体の一部にIC_{50}約50 μMの阻害活性を，カテキン誘導体の一部にIC_{50} 0.9 μMの強力な阻害活性を見いだした．さらに，細胞レベルではカテキン誘導体の一部にプロセシンググルコシダーゼ阻害を作用点とするとみられる比較的強い抗ウイルス活性を見いだした．今後，ウイルス外被糖タンパク質の糖鎖構造解析等により詳細な作用機序の解明を行う予定である．

＊＊＊＊＊

〔質 問〕 食総研 徳安

1）安全性の高いカテキン骨格をリード化合物として，カテキン骨格を含む阻害剤の設計と合成を行っていますが，その阻害剤の「安全性が高い」という理由はなにか．

2）ウイルスに対してカテキン誘導体の効果があったが，α-グルコシダーゼに対して作用した結果なのか．

〔答〕

1）誘導体合成前のカテキンの安全性が高いからといって，カテキン骨格を有する誘導体の安全性が高いということはできません．しかし，安全性の高い骨格を創薬リード化合物として用いることは，毒性を回避するという目的において理にかなっていると考えています．また，本誘導体はカテキン骨格をほぼ維持しているので，毒性発現の可能性を低く抑えられるのではないかと考えております．

2）α-グルコシダーゼに対する阻害効果なのかどうか，直接の証拠はありませんが，*in vitro*での強い阻害活性およびウイルスを感染させた培養細胞の形態からα-グルコシダーゼ阻害を作用機序とする抗ウイルス作用であると考えております．今後，ウイルス粒子を回収し，そのウイルス外被糖タンパク質の糖鎖解析を行うことにより，作用点を解明したいと考えております．

〔質 問〕 食総研 北岡

1）グルコシダーゼ阻害剤のリード化合物として，数ある安全性の高い物質の中から，カテキンを選択した理由はなにか．

2）グルコシダーゼ以外の糖質加水分解酵素の阻害剤になっている可能性はあるのでしょうか．

〔答〕

1）安全性の高い物質は他にもたくさんありますが，カテキンには弱いながらも血糖上昇抑制作用が報告されており，腸管グルコシダーゼ阻害が示唆されておりますので，リード化合物として選択いたしました．

2）グルコシダーゼ阻害以外の阻害活性があることは否定できません．現時点では，一部のα-マンノシダーゼに対する阻害活性がないことだけ確認しております．

J. Appl. Glycosci., **53**, 255–260 (2006)

Regular Paper

Design and Synthesis of α-Glucosidase Inhibitor Having DNA Cleaving Activity

(Received May 15, 2006; Accepted July 10, 2006)

Wataru Hakamata,[1,*] Emiko Yamamoto,[1,2] Makoto Muroi,[3] Masataka Mochizuki,[2] Masaaki Kurihara,[1] Haruhiro Okuda[1] and Kiyoshi Fukuhara[1]

[1]*Division of Organic Chemistry, National Institute of Health Sciences (NIHS) (1–18–1, Kamiyoga, Setagaya-ku, Tokyo 158–8501, Japan)*
[2]*Division of Organic and Bioorganic Chemistry, Kyoritsu University of Pharmacy (1–5–30, Shibakoen, Minato-ku, Tokyo 105–8512, Japan)*
[3]*Antibiotics Laboratory, The Institute of Physical and Chemical Research (RIKEN) (2–1, Hirosawa, Wako 351–0198, Japan)*

Abstract: Apoptosis, or programmed cell death, is a mechanism by which cells undergo death to control cell proliferation or in response to DNA damage. The present study was designed to explore small molecule apoptosis inducers for antitumor agents. The synthesis of 4-sulfonylphenyl α-D-glucopyranoside derivatives 1–6 and 4-(sulfonylamino)phenyl α-D-glucopyranoside derivatives 7–12, endoplasmic reticulum (ER)-targeted small molecules that were designed to induce apoptosis from ER stress by ER glucosidase inhibition and DNA damage is described. Compounds 6 and 12, with a terminal 2-naphthyl group, indicated inhibitions of α-glucosidases from *S. cerevisiae* (IC_{50}=51.7 μM and IC_{50}=74.1 μM) and *B. stearothermophilus* (IC_{50}=60.1 μM and IC_{50}=89.1 μM). Moreover, compound 12 strongly induced the DNA strand breakage condition. When compounds 1–12 were assayed for their ability to inhibit processing by glucosidases at the cellular level, no effects on glycoprotein processing were observed.

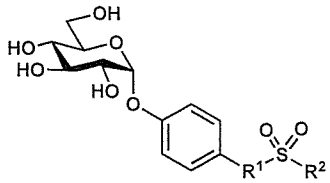
Key words: α-glucosidase, inhibitor, DNA cleavage, apoptosis, ER stress

The cell is perturbed by environmental stress conditions. In order to avoid cell death from the stress, cells must sense and respond to stress, including viral infection, genetic mutation, chemical insult, and nutrient depletion.[1)] In the ER, stress is a condition that accumulates misfolded or unfolded proteins by disturbing these ER circumstances. Specific response programs are activated to circumvent each type of stress. The ER stress induces a coordinated adaptive program called the unfolded protein response (UPR).[2)] The UPR is activated upon disruption of the ER environment by such events as the inhibition of *N*-linked oligosaccharide processing, which results in the accumulation of unfolded or misfolded proteins in the ER.[3)] *N*-Linked oligosaccharide processing is carried out by ER glucosidases I and II. Both enzymes are key enzymes in the biosynthesis of *N*-linked oligosaccharides that catalyze the first processing event after the transfer of $Glc_3Man_9GlcNAc_2$ to proteins.[4)] The inhibition of ER glucosidases induces the accumulation of unfolded proteins in the ER, and increases ER stress. The UPR caused by ER stress is insulted due to DNA damage, and the cell is led to apoptosis. Apoptosis targets are currently being explored for antitumor agent discovery, such as the tumor-necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) receptors, the BCL2 family of anti-apoptotic proteins, and inhibitor of apoptosis (IAP) proteins.[1,5)]

We think that the inhibition of ER glucosidases can be used to trigger ER stress, and that the ER stress may trigger the UPR. Further, following interruption of the UPR by DNA damage, the cell is led to apoptosis. We think that compounds that have α-glucosidase inhibitory activity and DNA breakage activity may be developed into an ER-targeted small molecule apoptosis inducer for use as an antitumor agent. We have already elucidated the molecular recognition properties[6–13)] and the inhibition[13,14)] of α-glycosidases necessary for the molecular design of glycosidase inhibitors using synthetic probes. Based on our knowledge, we designed compounds **1–12** to have α-glucosidase inhibitory activity and DNA breakage activity (Fig. 1). The enzymatic liberation of the aglycon from compounds **1–12** might be followed by the ejection of a R^2SO_2H with the concomitant formation of *p*-benzoquinone or *p*-benzoquinone imine,[15)] which would then generate reactive oxygen species (ROS), leading to DNA breakage,[16)] shown in Fig. 2. The group of Taylar *et al.* has developed a series of 4-(sulfonylamino)phenyl α-D-glucopyranosides.[15)] These compounds have been reported to act as competitive yeast α-glucosidase inhibitors. We suspect that these compounds may also be enhanced in their inhibitory activity by changing the sulfonamide of 4-(sulfonylamino)phenyl α-D-glucopyranoside to sulfonate, since the liberation of *p*-benzoquinone is easier than that of *p*-benzoquinone imine.

In this report, we first describe the design and synthesis series of 4-sulfonylphenyl α-D-glucopyranoside derivatives **1–6** and 4-(sulfonylamino)phenyl α-D-glucopyranoside derivatives **7–12**. These compounds **1–12** were evaluated with regard to their ability to inhibit three kinds of α-glucosidases, and the effects of α-glucosidase triggered

* Corresponding author (Tel. +81–3–3700–1141, Fax. +81–3–3707–6950; E-mail: hakamata@nihs.go.jp).



1: R^1=O, R^2=4-NO_2-C_6H_4
2: R^1=O, R^2=4-Cl-C_6H_4
3: R^1=O, R^2=4-CF_3-C_6H_4
4: R^1=O, R^2=4-CH_3-C_6H_4
5: R^1=O, R^2=4-$C(CH_3$-$C_6H_4)_3$
6: R^1=O, R^2=2-naphthyl
7: R^1=NH, R^2=4-NO_2-C_6H_4
8: R^1=NH, R^2=4-Cl-C_6H_4
9: R^1=NH, R^2=4-CF_3-C_6H_4
10: R^1=NH, R^2=4-CH_3-C_6H_4
11: R^1=NH, R^2=4-$C(CH_3)_3$-C_6H_4
12: R^1=NH, R^2=2-naphthyl

Fig. 1. Chemical structure of target compounds **1**–**12**.

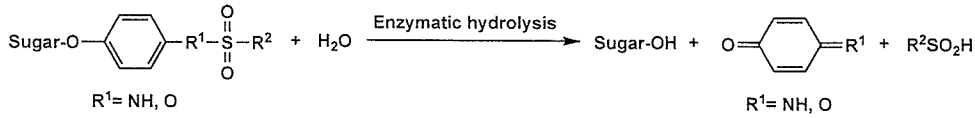


Fig. 2. Schematic diagram of enzymatic liberation of quinone derivatives.

ROS-mediated DNA breakage. Finally, these compounds were also tested in a cell culture system.

MATERIALS AND METHODS

General methods. Optical rotations were measured with a JASCO DIP-370 digital polarimeter at 25°C. The NMR spectra were recorded with a Varian Mercury 400 spectrometer (400 MHz for ^{1}H). Chemical shifts were expressed in ppm downfield relative to Me_4Si. Low resolution mass spectra were obtained with a Waters MicroMass ZQ instrument under positive and negative ion 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).

Method A. To the solution of α-arbutin (**1**, 0.5 g, 1.8 mmol) in 100 mL of dry acetone was added triethylamine (NEt_3, 10 mL) and the sulfonyl chloride derivative (2.8 mmol). After the mixture was stirred for 15 min, the resulting salt was removed by filtration through a cotton filter, and the solvent was concentrated.

Method B. To the solution of α-arbutin (**1**, 1.0 g, 3.7 mmol), in 50 mL of dry acetone was added dry potassium carbonate (K_2CO_3, 1.52 g, 11 mmol) and the sulfonyl chloride derivative (5.5 mmol). After the mixture was stirred overnight, the K_2CO_3 was removed by filtration through Celite, and the solvent was concentrated.

Method C. To the solution of *p*-nitrophenyl α-D-glucopyranoside (**14**, 1.0 g, 3.3 mmol), in pyridine (50 mL) at room temperature was added acetic anhydride (10 mL). The mixture was stirred overnight and poured into water. The product was extracted with AcOEt (3 × 50 mL) and washed with water, 1 M HClaq, satd. $NaHCO_3$aq and brine, and then dried over Na_2SO_4. The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford a quantitative yield of **15**. A mixture of compound **15** (1.6 g, 3.3 mmol) in ethanol (EtOH, 100 mL) was hydrogenated under H_2 with 20% palladium hydroxide on carbon (150 mg). After the mixture was stirred for 2 h, the palladium charcoal was removed by filtration through Celite and the solvent was concentrated. The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 1.5 g (93.9%) of **16**.

Method D. To the solution of compound **16** (0.5 g, 1.2 mmol), in pyridine (20 mL) at room temperature was added sulfonyl chloride derivative (1.36 mmol). The mixture was stirred for 10 min and poured into water. The product was extracted with AcOEt (3 × 50 mL) and washed with water, 1 M HClaq, satd. $NaHCO_3$aq and brine, and then dried over Na_2SO_4, and the solvent was concentrated.

Method E. A mixture of methanol (MeOH) : NEt_3 : H_2O (5:1:1, 60 mL) was added to a stirred solution of the sulfonyl derivative (0.85 mmol). After the mixture was stirred for 6 h at room temperature, the solvent was evaporated.

Synthesis of compounds 1–12.

4-(4-Nitrobenzenesulfonyl)phenyl α-D-glucopyranoside (1). According to method A, compound **1** was prepared from **13** (0.5 g, 1.8 mmol). The product was purified by column chromatography on silica gel (5:1 dichloromethane (CH_2Cl_2)-MeOH) to afford 0.46 g (54.8%) of **2**: $[\alpha]_D$ +14.9° (*c* 0.93, MeOH); ^{1}H NMR (CD_3OD) δ 3.38 (dd, 1H, J_{3-4}=8.8 Hz, J_{4-5}=10.0 Hz, H-4), 3.55 (dd, 1H, J_{1-2}= 3.8 Hz, J_{2-3}=9.8 Hz, H-2), 3.58 (ddd, 1H, J_{4-5}=10.0 Hz, J_{5-6a}=5.2 Hz, J_{5-6b}=2.4 Hz, H-5), 3.67 (dd, 1H, J_{5-6a}=5.0 Hz, J_{6a-6b}=11.8 Hz, H-6a), 3.73 (dd, 1H, J_{5-6b}=2.4 Hz, J_{6a-6b}=12.0 Hz, H-6b), 3.80 (dd, 1H, J_{2-3}=J_{3-4}=9.2 Hz, H-3), 5.42 (d, 1H, *J*=3.6 Hz, H-1), 6.95 (d, 2H, *J*=8.8 Hz, -OC_6H_4O-), 7.12 (d, 2H, *J*=9.2 Hz, -OC_6H_4O-), 8.07 (d, 2H, *J*=9.2 Hz, -$SO_2C_6H_4NO_2$-), 8.44 (d, 2H, *J*=9.2 Hz, -$SO_2C_6H_4NO_2$), MS: 480 $(M+Na)^+$.

4-(4-Chlorobenzenesulfonyl)phenyl α-D-glucopyranoside (2). According to method A, compound **2** was prepared from **13** (0.5 g, 1.8 mmol). The product was purified by column chromatography on silica gel (5:1 CH_2Cl_2-MeOH) to afford 0.59 g (72.5%) of **3**: $[\alpha]_D$ +13.8° (*c* 1.51, MeOH); ^{1}H NMR (CD_3OD) δ 3.40 (dd, 1H, J_{3-4}=9.0 Hz, J_{4-5}=9.8 Hz, H-4), 3.55 (dd, 1H, J_{1-2}=3.6 Hz, J_{2-3}=9.6 Hz, H-2), 3.59 (ddd, 1H, J_{4-5}=10.0 Hz, J_{5-6a}=5.4 Hz, J_{5-6b} =2.2 Hz, H-5), 3.67 (dd, 1H, J_{5-6a}=5.4 Hz, J_{6a-6b}=12 Hz, H-6a), 3.73 (dd, 1H, J_{5-6b}=2.4 Hz, J_{6a-6b}=12.0 Hz, H-6b), 3.81 (dd, 1H, J_{2-3}=9.6 Hz, J_{3-4}=9.2 Hz, H-3), 5.43 (d, 1H, *J*=3.6 Hz, H-1), 6.92 (d, 2H, *J*=9.6 Hz, -OC_6H_4O-), 7.12 (d, 2H, *J*=9.2 Hz, -OC_6H_4O-), 7.62 (d, 2H, *J*=8.8 Hz, -$SO_2C_6H_4Cl$), 7.78 (d, 2H, *J*=8.8 Hz, -$SO_2C_6H_4Cl$), MS: 469 $(M+Na)^+$.

4-(4-Trifluorobenzenesulfonyl)phenyl α-D-glucopyranoside (3). According to method A, compound **3** was prepared from **13** (0.5 g, 1.8 mmol). The product was purified by column chromatography on silica gel (5:1 CH_2Cl_2-MeOH) to afford 0.59 g (88.4%) of **3**: $[\alpha]_D$ +11.7° (*c* 1.25, MeOH); ^{1}H NMR (CD_3OD) δ 3.40 (dd, 1H, J_{3-4}=9.0 Hz, J_{4-5}=9.8 Hz, H-4), 3.55 (dd, 1H, J_{1-2}=3.6 Hz, J_{2-3}= 10.0 Hz, H-2), 3.58 (m, 1H, H-5), 3.66 (dd, 1H, J_{5-6a}=5.0

Hz, J_{6a-6b}=11.8 Hz, H-6a), 3.73 (dd, 1H, J_{5-6b}=2.4 Hz, J_{6a-6b}=12.0 Hz, H-6b), 3.81 (dd, 1H, J_{2-3}=J_{3-4}=9.4 Hz, H-3), 5.43 (d, 1H, J=3.6 Hz, H-1), 6.93 (d, 2H, J=9.2 Hz, -OC_6H_4O-), 7.12 (d, 2H, J=9.2 Hz, -OC_6H_4O-), 7.94 (d, 2H, J=8.0 Hz, -$O_2C_6H_4O$-), 8.02 (d, 2H, J=8.4 Hz, -$SO_2C_6H_4CF_3$), MS: 503 $(M+Na)^+$.

4-(4-Methylbenzenesulfonyl)phenyl α-D-glucopyranoside (**4**). According to method B, compound **4** was prepared from **13** (1.1 g, 5.5 mmol). The product was purified by column chromatography on silica gel (5:1 CH_2Cl_2-MeOH) to afford 1.03 g (63.4%) of **4**: $[\alpha]_D$ +18.4° (*c* 0.97, MeOH); ^1H NMR (CD_3OD) δ 2.44 (s, 3H, -CH_3), 3.39 (dd, 1H, J_{3-4}=8.8 Hz, J_{4-5}=10.0 Hz, H-4), 3.54 (dd, 1H, J_{1-2}=3.8 Hz, J_{2-3}=9.8 Hz, H-2), 3.58 (ddd, 1H, J_{4-5}= 10.0 Hz, J_{5-6a}=5.2 Hz, J_{5-6b}=2.4 Hz, H-5), 3.66 (dd, 1H, J_{5-6a}=5.2 Hz, J_{6a-6b}=12.0 Hz, H-6a), 3.73 (dd, 1H, J_{5-6b}= 2.6 Hz, J_{6a-6b}=11.8 Hz, H-6b), 3.80 (dd, 1H, J_{2-3}=9.8, J_{3-4} =9.0 Hz, H-3), 5.41 (d, 1H, J=3.6 Hz, H-1), 6.88 (d, 2H, J=9.6 Hz, -OC_6H_4O-), 7.09 (d, 2H, J=9.2 Hz, -OC_6H_4O-), 7.40 (d, 2H, J=8.0 Hz, -$SO_2C_6H_4CH_3$), 7.66 (d, 2H, J=8.4 Hz, -$SO_2C_6H_4CH_3$), MS: 449 $(M+Na)^+$.

4-(4-*tert*-Butylbenzenesulfonyl)phenyl α-D-glucopyranoside (**5**). According to method B, compound **3** was prepared from **13** (0.5 g, 1.8 mmol). The product was purified by column chromatography on silica gel (5:1 CH_2Cl_2-MeOH) to afford 0.39 g (43.9%) of **5**: $[\alpha]_D$ +13.6° (*c* 1.40, MeOH); ^1H NMR (CD_3OD) δ 1.36 (s, 9H, -C (CH_3)$_3$), 3.39 (dd, 1H, J_{3-4}=8.8 Hz, J_{4-5}=10.0 Hz, H-4), 3.54 (dd, 1H, J_{1-2}=3.6 Hz, J_{2-3}=9.6 Hz, H-2), 3.59 (ddd, 1H, J_{4-5}=10.0 Hz, J_{5-6a}=5.0 Hz, J_{5-6b}=2.5 Hz, H-5), 3.66 (dd, 1H, J_{5-6a}=5.0 Hz, J_{6a-6b}=11.8 Hz, H-6a), 3.73 (dd, 1H, J_{5-6b}=2.4 Hz, J_{6a-6b}=12.0 Hz, H-6b), 3.80 (dd, 1H, J_{2-3} =J_{3-4}=9.4 Hz, H-3), 5.41 (d, 1H, J=3.6 Hz, H-1), 6.89 (d, 2H, J=9.2 Hz, -OC_6H_4O-), 7.10 (d, 2H, J=9.2 Hz, -OC_6H_4O-), 7.64 (d, 2H, J=8.8 Hz, -$SO_2C_6H_4C(CH_3)_3$), 7.72 (d, 2H, J=8.8 Hz, -$SO_2C_6H_4C(CH_3)_3$), MS: 491 $(M+Na)^+$.

4-(2-Naphthalenesulfonyl)phenyl α-D-glucopyranoside (**6**). According to method B, compound **6** was prepared from **13** (0.5 g, 1.8 mmol). The product was purified by column chromatography on silica gel (5:1 CH_2Cl_2-MeOH) to afford 0.63 g (74.6%) of **6**: $[\alpha]_D$ +12.3° (*c* 1.32, MeOH); ^1H NMR (CD_3OD) δ 3.38 (dd, 1H, J_{3-4}=8.8 Hz, J_{4-5}=10.0 Hz, H-4), 3.52 (dd, 1H, J_{1-2}=3.6 Hz, J_{2-3}=9.6 Hz, H-2), 3.55 (ddd, 1H, J_{4-5}=10.0 Hz, J_{5-6a}=4.8 Hz, J_{5-6b} =2.8 Hz, H-5), 3.64 (dd, 1H, J_{5-6a}=4.8 Hz, J_{6a-6b}=12.0 Hz, H-6a), 3.68 (dd, 1H, J_{5-6b}=2.8 Hz, J_{6a-6b}=12.0 Hz, H-6b), 3.78 (dd, 1H, J_{2-3}=J_{3-4}=9.0 Hz, H-3), 5.38 (d, 1H, J=3.6 Hz, H-1), 6.89, 7.05 (d, 2H × 2, J=9.6 Hz, -OC_6H_4O-), 7.64–7.83, 8.00–8.11, 8.35 (m, 7H, -$SO_2C_{10}H_7$), MS: 485 $(M+Na)^+$.

4-(4-Nitrophenylsulfonylamino)phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**17**). According to methods C and D, compound **17** was prepared from **16** (0.5 g, 1.1 mmol). The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 0.67 g (94.6%) of **17**.

4-(4-Chlorophenylsulfonylamino)phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**18**). According to methods C and D, compound **18** was prepared from **16** (0.4 g, 1.0 mmol). The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 0.54 g (93.1%) of **17**.

4-(4-Trifluoromethylphenylsulfonylamino)phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**19**). According to methods C and D, compound **19** was prepared from **16** (0.4 g, 1.0 mmol). The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 0.65 g (99.9%) of **19**.

4-(4-Methlphenylsulfonylamino)phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**20**). According to methods C and D, compound **20** was prepared from **16** (0.5 g, 1.1 mmol). The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 0.64 g (93.1%) of **20**.

4-(4-tert-Butylphenylsulfonylamino)phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**21**). According to methods C and D, compound **20** was prepared from **16** (0.4 g, 1.0 mmol). The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 0.55 g (88.5%) of **21**.

4-(2-Naphthalenephenylsulfonylamino)phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**22**). According to methods C and D, compound **22** was prepared from **16** (0.4 g, 1.0 mmol). The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 0.53 g (92.5%) of **22**.

4-(4-Nitrophenylsulfonylamino)phenyl α-D-glucopyranoside (**7**). According to method E, compound **7** was prepared from **17** (0.7 g, 1.1 mmol). The product was purified by column chromatography on silica gel (5:1 CH_2Cl_2-MeOH) to afford 0.41 g (83.3%) of **7**: $[\alpha]_D$ +12.1° (*c* 1.23, MeOH); ^1H NMR (CD_3OD) δ 3.38 (dd, 1H, J_{3-4}=8.8 Hz, J_{4-5}=10.0 Hz, H-4), 3.53 (dd, 1H, J_{1-2}=3.6 Hz, J_{2-3}= 9.6 Hz, H-2), 3.59 (ddd, 1H, J_{4-5}=10.0 Hz, J_{5-6a}=5.0 Hz, J_{5-6b}=2.4 Hz, H-5), 3.65 (dd, 1H, J_{5-6a}=5.0 Hz, J_{6a-6b}=11.8 Hz, H-6a), 3.72 (dd, 1H, J_{5-6b}=2.4 Hz, J_{6a-6b}=12.0 Hz, H-6b), 3.80 (dd, 1H, J_{2-3}=J_{3-4}=9.2 Hz, H-3), 5.38 (d, 1H, J= 3.6 Hz, H-1), 7.0 (d, 2H, J=8.8 Hz, -OC_6H_4NH-), 7.05 (d, 2H, J=9.2 Hz, -OC_6H_4NH-), 7.91 (d, 2H, J=8.8 Hz, -$SO_2C_6H_4NO_2$), 8.31 (d, 2H, J=8.8 Hz, -$SO_2C_6H_4NO_2$), MS: 455 $(M-H)^-$.

4-(4-Chlorophenylsulfonylamino)phenyl α-D-glucopyranoside (**8**). According to method E, compound **8** was prepared from **18** (0.54 g, 0.9 mmol). The product was purified by column chromatography on silica gel (5:1 CH_2Cl_2-MeOH) to afford 0.38 g (95.2%) of **8**: $[\alpha]_D$ +13.4° (*c* 1.42, MeOH); ^1H NMR (CD_3OD) δ 3.39 (dd, 1H, J_{3-4} =9.2Hz, J_{4-5}=10.0 Hz, H-4), 3.53 (dd, 1H, J_{1-2}=3.8 Hz, J_{2-3}=9.8 Hz, H-2), 3.60 (ddd, 1H, J_{4-5}=10.0 Hz, J_{5-6a}=4.8 Hz, J_{5-6b}=2.6 Hz, H-5), 3.66 (dd, 1H, J_{5-6a}=4.8 Hz, J_{6a-6b} =11.9 Hz, H-6a), 3.72 (dd, 1H, J_{5-6b}=2.6 Hz, J_{6a-6b}=11.9 Hz, H-6b), 3.80 (dd, 1H, J_{2-3}=J_{3-4}=9.2 Hz, H-3), 5.38 (d, 1H, J=4.0 Hz, H-1), 6.98 (d, 2H, J=9.2 Hz, -OC_6H_4NH-), 7.05 (d, 2H, J=9.6 Hz, -OC_6H_4NH-), 7.48 (d, 2H, J=8.8 Hz, -$SO_2C_6H_4Cl$), 7.65 (d, 2H, J=8.8 Hz, -$SO_2C_6H_4Cl$), MS: 444 $(M-H)^-$.

4-(4-Trifluoromethylphenylsulfonylamino)phenyl α-D-glucopyranoside (**9**). According to method E, compound **9** was prepared from **19** (0.7 g, 1.0 mmol). The product was purified by column chromatography on silica gel (5:1 CH_2Cl_2-MeOH) to afford 0.44 g (90.9%) of **7**: $[\alpha]_D$ +13.4°