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2-Aminoresorcinol is a potent α-glucosidase inhibitor

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Abstract—A series of aminoresorcinols and related compounds were tested for rat intestinal α-glucosidase inhibition and these results suggested that the 2-aminoresorcinol moiety of 6-amino-5,7-dihydroxyflavone (**2**) is important to exert the intestinal α-glucosidase inhibitory activity and 2-aminoresorcinol (**4**), itself, is a potent α-glucosidase inhibitor and inhibited sucrose-hydrolyzing activity of rat intestinal α-glucosidase uncompetitively.

There is a marked increase in cardiovascular risk in individuals with postprandial hyperglycemia. Hence, there needs to be a new focus on suppression of postprandial hyperglycemia in treatment of not only diabetic patients but also individuals with impaired glucose tolerance. From this view, various targets as well as pharmacologic agents have been identified to manage this risk factor.[1] Mammalian α-glucosidase (α-D-glucoside glucohydrolase, EC 3.2.1.20), located in the brush-border surface membrane of intestinal cells, is the key enzyme which catalyzes the final step in the digestive process of carbohydrates. Hence, α-glucosidase inhibitors can retard the liberation of D-glucose of oligosaccharides and disaccharides from dietary complex carbohydrates and delay the glucose absorption, resulting in reducing postprandial plasma glucose levels and suppression of postprandial hyperglycemia.[2] In fact, α-glucosidase inhibitors like acarbose and miglitol effectively compensate for defective early phase insulin release by inhibiting postprandial absorption of monosaccharides, and have been approached for clinical use of the management of type 2 diabetes.

In the course of our screening study for rat intestinal α-glucosidase-inhibiting substances from natural resources, we previously reported that baicalein (5,6,7-trihydroxyflavone, **1**) and the related 6-hydroxyflavonoids were a new class of α-glucosidase inhibitors.[3,4] As a continuing study, we evaluated the influence of the A- and B-rings substitution for **1** on the α-glucosidase inhibitory activity.[5,6] A replacement of the hydroxyl group (–OH) at C-6 in **1** with an amino group (–NH_2) afforded 6-amino-5,7-dihydroxyflavone (**2**, IC_{50} = 2.4 μM), which is a more potent rat intestinal α-glucosidase inhibitor with sucrose as a substrate than **1** (IC_{50} = 52 μM) (Fig. 1). 6-Amino-5,7-dihydroxyflavone (**2**) is a unique flavone bearing a 6-amino group together with ordinary 5,7-dihydroxyl substituents. The initial SAR (structure–activity relationship) studies suggested that the 6-amino group and 7-hydroxyl group of **2** were crucial for the activity; 5-hydroxyl substitution was favorable to the activity, whereas the substitution of 8-amino group was unfavorable.[7] Therefore, we first address whether the 2-aminoresorcinol moiety of **2** is important to exert the α-glucosidase inhibitory activity as a minimal structure and then we evaluate the inhibitory effect of 2-aminoresorcinol (**4**) on α-glucosidase in this paper.

Compounds **5** and **6** were commercially available. 3-Amino-2,4-dihydroxyacetophenone (**3**),[8] 2-aminoresorcinol (**4**),[9] and 2-amino-1,3-cyclohexanediol (**7**)[10] were prepared as previously described. Structures of compounds **3**, **4**, and **7** were confirmed by spectroscopic data (MS and NMR).[11]

The crude enzyme solution prepared from rat intestinal acetone powder (Sigma Aldrich Japan Co., Tokyo, Japan), was used as the small intestinal α-glucosidases, sucrase, maltase and isomaltase. The reaction mixture consisted of crude enzyme solution (as sucrase,

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Keywords: α-Glucosidase inhibitor; Resorcinol; 6-Aminoflavones; 2-Aminoresorcinol.

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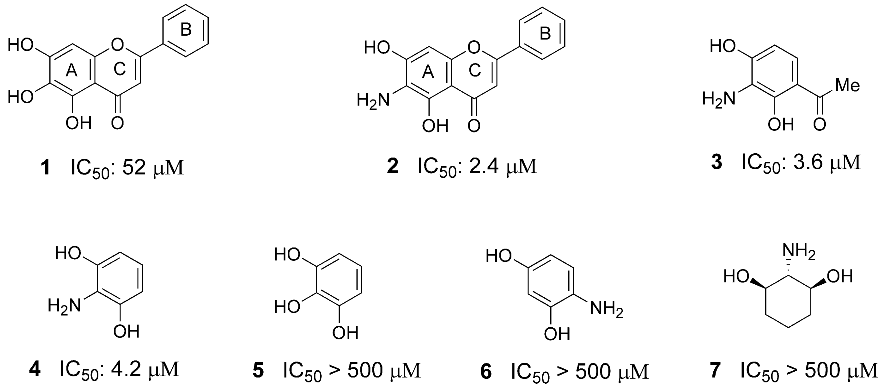

1 IC_{50}: 52 μM

2 IC_{50}: 2.4 μM

3 IC_{50}: 3.6 μM

4 IC_{50}: 4.2 μM

5 IC_{50} > 500 μM

6 IC_{50} > 500 μM

7 IC_{50} > 500 μM

Figure 1. Structures of baicalein (**1**), 6-amino-5,7-dihydroxyflavone (**2**), 3-amino-2,4-dihydroxyacetophenone (**3**), 2-aminoresorcinol (**4**), 1,2,3-trihydroxybenzene (**5**), 4-aminoresorcinol (**6**), 2-amino-1,3-cyclohexanediol (**7**), and IC_{50} values for inhibition of rat intestinal α-glucosidase. The crude enzyme solution prepared from rat intestinal α-glucosidase by using sucrose as a substrate, according to the reported method.[7] IC_{50} values were calculated from dose-dependent inhibition curves. At the concentration of 500 μM, sucrase inhibition for compounds **5**, **6**, and **7** were 42%, 46%, and 10%, respectively.

0.2 mL), substrate (56 mM sucrose, 0.2 mL) in 0.1 M potassium phosphate buffer (pH 6.3), and the test sample (0.1 mL). The α-glucosidase inhibitory activity was measured as described previously.[7] Dose–response curve of sucrase inhibition by 2-aminoresorcinol (**4**) is shown in Figure 2A. The IC_{50} value was calculated to be 4.2 μM. Compound **3** (IC_{50} = 3.6 μM) possessing a moiety of 2-aminoresorcinol also showed a strong sucrase inhibitory activity (Fig. 1). The activities of **3** and **4** were about 12–15 times higher than that of baicalein (**1**), and were comparable to that of **2**. Compared to **4**, compound **5** (42% inhibition at 500 μM) having a hydroxyl group at the place of the amino group at C-2 led to a dramatic drop in the activity. In addition, the IC_{50} value of **6** (having an amino group at C-4) was above 500 μM. It is interesting to note that compound (**7**), which has a cyclohexane ring, showed only 10% sucrase inhibition at the concentration of 500 μM, although **7** possesses a 2-amino-1,3-dihydroxyl substructure. Hence, these results suggested that the 2-aminoresorcinol moiety of **2** is important to exert the α-glcosidase inhibitory activity and **4**, itself, is a potent α-glucosidase inhibitor.

Having identified the importance of 2-aminoresorcinol moiety of **2**, we decided to evaluate the selectivity of compounds **2**–**4** for several selected sugar hydrolase inhibition (Table 1). As indicated above, compounds **3** and **4** showed, high activity against sucrase and maltase, like **2**. However, each of these compounds showed less than 40% inhibition against the other glycosidases such as isomaltase, β-glucosidase, α-mannosidase, α-galactosidase, β-galactosidase, and porcine pancreatic α-amylase. Therefore, compounds **3** and **4** would belong to specific rat intestinal sucrase and maltase inhibitors, like **2**.

The inhibitory mechanisms of glucosidases have been explained by two distinct mechanisms: reversible inhibitors that have a high affinity for the enzyme, and irreversible inhibitors that react with carboxylic acid of the active site of the enzyme.[12] To determine whether **4** is a reversible α-glucosidase inhibitor, we removed the un-reacted inhibitor from the enzyme solution by

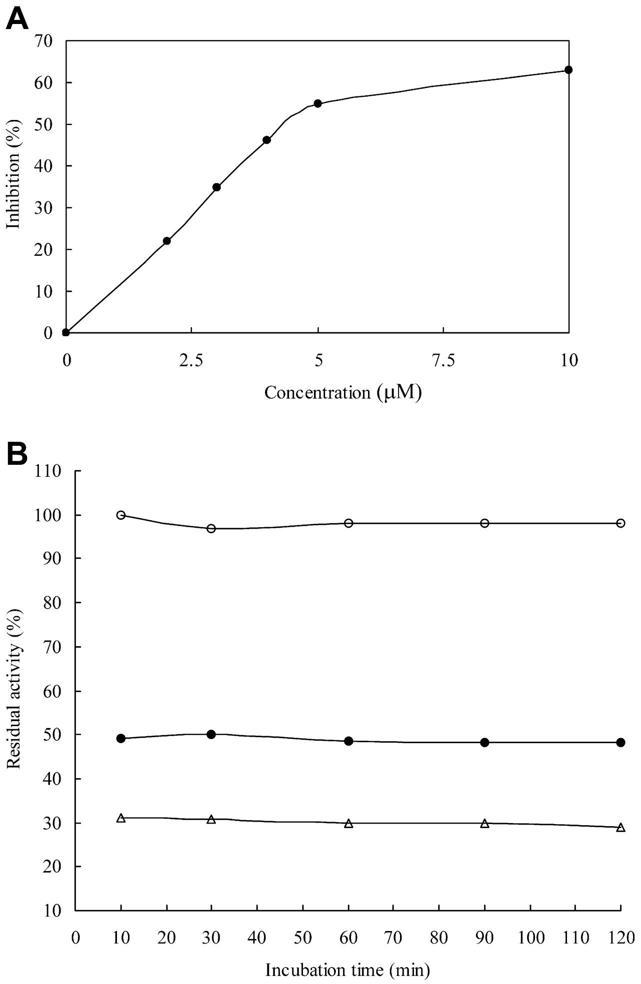

Figure 2. Dose- and time-dependent inhibition of rat intestinal α-glucosidase by 2-aminoresorcinol (**4**). Conditions: (A) assay was performed according to the reported method.[7] (B) rat intestinal α-glucosidase (sucrase specific activity: 0.38 U/mL) was incubated at 25 °C in 0.1 M potassium phosphate (pH 6.3) with various concentrations of **4** (●, 4 μM; △, 8 μM) or without **4** (○). At given time intervals, aliquots were withdrawn and assayed for remaining enzyme activity with sucrose as a substrate.

Table 1. Selectivity of compounds **2**–**4** for the inhibitory effect on eight sugar hydrolases

	IC_{50} (nM)		
	2	**3**	**4**
Sucrase (rat intestine)	2.4	3.6	4.2
Maltase (rat intestine)	4.4	5	8.5
Isomaltase (rat intestine)	32%[a]	28%	21%
β-Glucosidase (almonds)	19%	7%	12%
α-Mannosidase (jack bean)	7%	4%	3%
α-Galactosidase (green coffee been)	6%	10%	8%
β-Galactosidase (bovine liver)	10%	5%	4%
α-Amylase (porcine pancreas)	12%	15%	11%

The inhibitory activity of compounds **2**–**4** was tested on eight sugar hydrolases according to the previous methods.[7]

[a] Inhibition (%) at the concentration of 500 μM.

ultrafiltration, and examined whether the activity would be recovered or not by the method described previously.[12,13] When an enzyme solution containing **4** was subjected to ultrafiltration and then re-dissolved with the same concentration, the α-glucosidase activity was considerably recovered (Fig. 3). From this result, we can conclude that **4** is a reversible inhibitor against α-glucosidase.

To characterize the inhibitory mechanism by **4**, rat intestinal α-glucosidase preparation was incubated with **4** (4 μM and 8 μM, respectively) over a 120-min time period. As shown in Figure 2B, the decrease in sucrase activity induced by **4** was not observed and the sucrase activity remained constant when the reactions were carried out over various incubation times, indicating that **4** did not interfere with the activity of this free enzyme. Further, we analyzed the change in kinetic parameters of sucrase activity in response to different concentrations of sucrose ranging from 2.8 to 22.4 mM. The double reciprocal Lineweaver–Burk plot showed linear lines intercepting on $1/V$ axis in a parallel manner, with the increasing concentration of the inhibitor (**4**), decreasing values in maximum velocity (V_{max}), and Michaelis–Menten constant (K_m) recorded (Fig. 4). The data demonstrated that the mechanism of α-glucosidase inhibition of **4** was uncompetitive on sucrose-hydrolyzing activity with K_i value of 4.2 μM. These results indicate that **4** may reversibly combine with only the E–S (enzyme–substrate) complex and inhibit the activity of this enzyme. Especially, it is noted that uncompetitive inhibitors have an advantage over competitive inhibitors as therapeutic agents in that the inhibition is not overcome when the substrate concentration is saturation.[14] The precise explanation for how **4** interacts with the enzyme on a molecular basis is still unknown since the structure of α-glucosidase used for this study has not yet been established; however, this research may offer references and insights for designing and synthesizing some novel, safe, and effective α-glucosidase inhibitors containing 2-aminoresorcinol moiety.

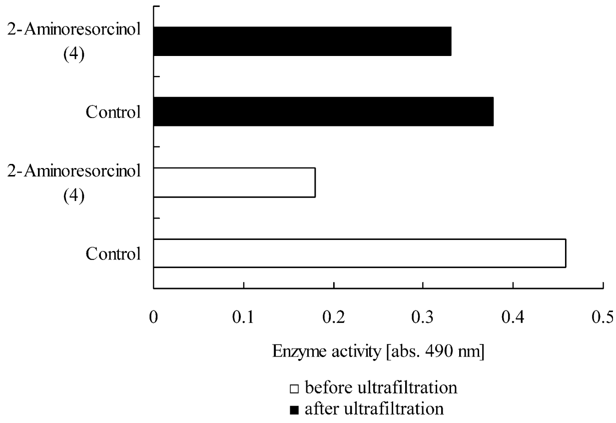

Figure 3. Recovery of enzymatic activity by elimination of 2-aminoresorcinol (**4**) by ultrafiltration. The enzyme solutions, in the presence or absence of **4** (final concentration, 20 μM), were treated by ultrafiltration. The unfiltered enzymes were re-dissolved and reacted with sucrose.

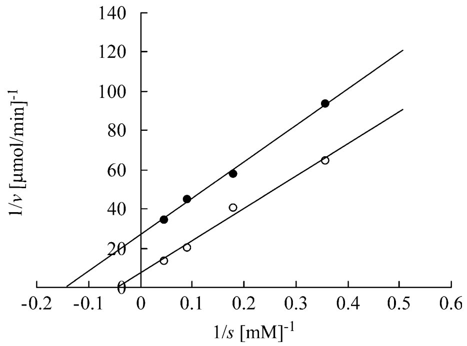

Figure 4. Lineweaver–Burk plots of rat intestinal α-glucosidase and sucrose without (○) and with (●) 5 μM 2-aminoresorcinol (**4**).

In summary, we documented that the 2-aminoresorcinol moiety of 6-amino-5,7-dihydroxyflavone (**2**) is important for α-glucosidase inhibition, and identified 2-aminoresorcinol (**4**), itself, as potent sucrase and maltase inhibitor in the present study.

Acknowledgments

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13. Effect of ultrafiltration on α-glucosidase inhibition by 2-aminoresorcinol (**4**). Rat intestinal α-glucosidase solution (0.49 mL) was treated with 0.01 mL of H_2O or **4** (1 mM) in H_2O. About 0.5 mL of each solution was applied to a USY-1 ultrafilter (10,000 nominal molecular weight limit) (Advance, Dublin, CA). An unfiltered high-molecular weight fraction was re-dissolved with 0.5 mL of 0.1 M phosphate buffer (pH 6.3), and the recovered enzymatic activity of 0.2 mL was measured by adding 56 mM of sucrose (0.2 mL) and the same buffer (0.1 mL). The enzyme solutions without ultrafiltration were also provided for measurement.
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