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Ligand compatibility of salacinol-type α-glucosidase inhibitors toward the GH31 family†

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We show that salacinol-type α-glucosidase inhibitors are ligand-compatible with the GH 31 family. Salacinol and its 3′-*O*-benzylated analogs inhibit human lysosomal α-glucosidase at submicromolar levels. Simple structure-activity relationship studies reveal that the salacinol side-chain stereochemistry significantly influences binding to GH31 α-glucosidases.

Introduction

GH31 α-glucosidases are retaining α-glucosidases that catalyze the hydrolysis of α-glycosidic linkages in oligosaccharides and glycoconjugates.[1,2] GH31 α-glucosidases are involved in several physiological processes, including the processing of newly biosynthesized glycoproteins in the endoplasmic reticulum, the breakdown of glycogen in the lysosome, and the hydrolysis of disaccharides in the gastrointestinal tract. GH31 α-glucosidases perform essential biological functions that have attracted considerable attention as therapeutic targets, with drugs for lysosomal storage disease,[3] diabetes,[4] obesity,[5] virus infections,[6] and tumors[7] having been developed. Furthermore, the therapeutic benefits of targeting GH31 α-glucosidases have facilitated the development of new inhibitor classes, including disaccharides,[8,9] iminosugars,[10] carbasugars,[11] peudoaminosugars,[12,13] and non-glycosidic derivatives.[14–20] However, there remains a major need to discover and design selective α-glucosidase inhibitors from the perspectives of both cellular tools and therapeutic agents.

Salacinol (**1**), a natural product, was isolated from the stems and roots of *Salacia reticulata*, which has been used to treat diabetes in Ayurvedic medicine (Fig. 1).[21,22] After the discovery of salacinol (**1**), related sulfonium sulfates, such as kotalanol,[23] ponkoranol,[24] and salaprinol,[24] as well as desulfonated analogs, including neosalacinol (**2**) (Fig. 1),[25] neokotalanol,[26] neoponkoranol,[27] and neosalaprinol,[27] were subsequently isolated from the same plant genus plant as the compounds responsible for antidiabetic activity. *In vitro* and *in vivo* activity studies revealed that the antidiabetic activity is due to the inhibition of intestinal α-glucosidases.[28] Furthermore, Lineweaver–Burk plots of the inhibition of intestinal α-glucosidases by **1** revealed a competitive type of inhibition on intestinal α-glucosidases.[22] On the basis of these results, clinical trials using the extract of *S. reticulata* on patients with type-2 diabetes showed promising therapeutic effects and minimal side effects.[29] Intensive structure-activity-relationship (SAR) studies around **1** have also been conducted around the world; indeed, we revealed the following important structural features of the side-chain

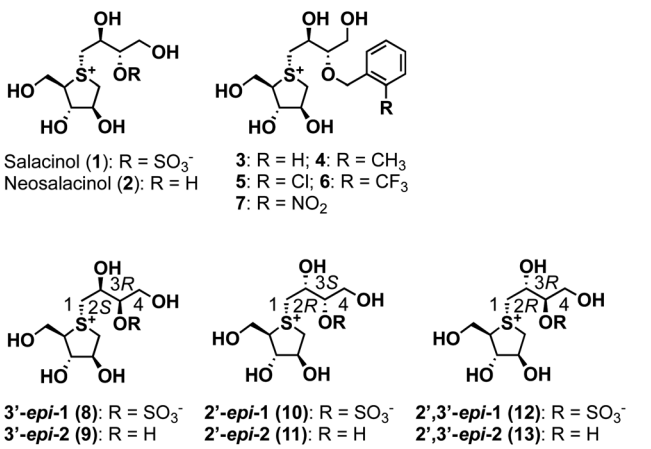

Fig. 1 Structures of naturally occurring salacinol (**1**), neosalacinol (**2**), and their derivatives **3**–**13** described in this study.

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Table 1 Apparent inhibitory constants (K_i^{app}) and IC_{50} values (μM) for **1–13**[a]

r	α-Glucosidase GAA[b]	*A. niger* β-glucosidase[c]
1	0.12 ± 0.02	>1000
2	3.6 ± 0.3	>1000
3 (H)	0.022 ± 0.007	>1000
4 (*o*-CH_3)	0.034 ± 0.009	>1000
5 (*o*-Cl)	0.030 ± 0.009	>1000
6 (*o*-CF_3)	0.017 ± 0.010	>1000
7 (*o*-NO_2)	0.17 ± 0.05	>1000
8 (3′-*epi*-**1**)	1.0 ± 0.1	>1000
9 (3′-*epi*-**2**)	25 ± 2	>1000
10 (2′-*epi*-**1**)	2794 ± 294	>1000
11 (2′-*epi*-**2**)	2742 ± 230	>1000
12 (2′,3′-*epi*-**1**)	3893 ± 262	>1000
13 (2′,3′-*epi*-**2**)	463 ± 36	>1000
Voglibose	7.6 ± 0.8	>1000
Acarbose	40 ± 2	>1000

[a] Mean ± SEM. [b] Apparent K_i. Assays conducted at pH 5.2 using α-*p*-NPG as the substrate. [c] Apparent IC_{50}. Assays conducted at pH 4.6 using β-*p*-NPG as the substrate.

structure of **1**: cooperativity between 2′S–OH and 4′-OH moieties is essential for the onset of the potent α-glucosidase inhibitory activity,[30] while the *O*-sulfonate anion moiety on the 3′-oxygen is not necessary.[31] We subsequently have developed an array of neosalacinols **3–7** bearing 3′-*O*-(*ortho*-substituted benzyl) groups through comprehensive SAR studies (Fig. 1).[32] The 3′-*O*-benzylated analogs **3–7** displayed *in vitro* inhibitory activities toward rat intestinal maltase (IC_{50} = 0.13–0.66 μM) (Table 1), which highlighted that they are the most potent thiosugar-based inhibitors synthesized to date.[32] Furthermore, *in vivo* activity studies involving **3–7** revealed the effective suppression of blood glucose levels in mice.[28] While salacinol (**1**) and its analogs **3–7** have been studied in detail using intestinal α-glucosidases, thiosugar-sulfonium salts remain an underexplored sector of lysosomal α-glucosidase chemical space. With this in mind, we sought to evaluate the compatibilities of thiosugar-based intestinal α-glucosidase inhibitors with lysosomal α-glucosidase GAA. To complete these SAR studies, we synthesized **8**, **10**, and **12**, and the related de-*O*-sulfonated versions **9**, **11**, and **13** (Scheme 1). We analyzed the abilities of these thiosugar sulfonium salts (**1–13**) to inhibit the enzymatic activities of recombinant human α-glucosidase GAA and rat intestinal disaccharidases, as determined by their inhibitory constants (K_i) or IC_{50} values, and also demonstrated their inhibitory properties toward β-glucosidase.

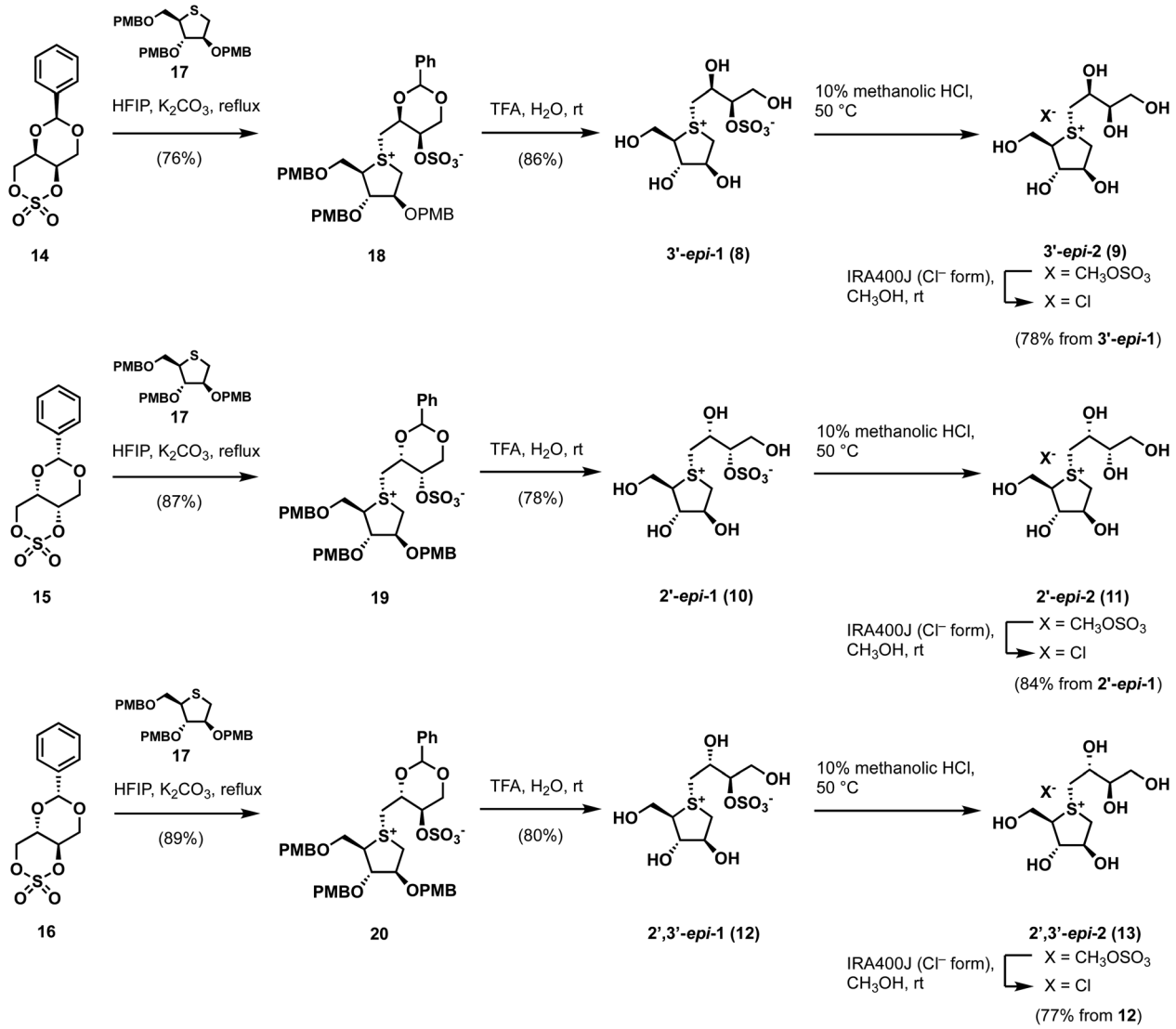
Results and discussion

By applying Ghavami's conditions for the synthesis of salacinol (**1**),[33] coupling reactions of thiosugar **17** was reacted with cyclic sulfates **14–16** in 1,1,3,3,3-hexafluoroisopropanol (HFIP); α-facial attack of **14–16** at the sulfur atom of **17** preferentially occurred to give coupled products **18–20** in yields of 76%, 87%, and 89%, respectively. Compounds **18–20** were subsequently treated with aqueous TFA to simultaneously remove each *p*-methoxybenzyl (PMB) group and benzylidene acetal moiety, which gave the desired sulfonium salts 3′-*epi*-**1**, 2′-*epi*-**1**, and 2′,3′-*epi*-**1** in good yields (Scheme 1). As shown in Table S1† (ESI), 3′-*epi*-**1**, 2′-*epi*-**1**, and 2′,3′-*epi*-**1** showed ^{13}C NMR spectral data that are similar to those of **1**, which confirms the formation of salacinol-type sulfonium inner-salt structures.

To remove the sulfo group at the C-3′ oxygen atom, 3′-*epi*-**1**, 2′-*epi*-**1**, and 2′,3′-*epi*-**1** were subjected to acidic methanolysis, which give the corresponding sulfonium salts, namely 3′-*epi*-**2**, 2′-*epi*-**2**, and 2′,3′-*epi*-**2** (X = CH_3OSO_3); their counterions were then exchanged using IRA400J (Cl^- form) to give the corresponding chlorides (3′-*epi*-**2**, 2′-*epi*-**2**, and 2′,3′-*epi*-**2**, X = Cl) in good yields. ^{13}C NMR spectroscopic data for 3′-*epi*-**2**, 2′-*epi*-**2**, and 2′,3′-*epi*-**2** are similar, with the exception of the C-3′ methine carbon signals ($\delta_{C3'}$ 74.2–75.1), which are significantly upfield shifted compared to those of 3′-*epi*-**1**, 2′-*epi*-**1**, and 2′,3′-*epi*-**1** ($\delta_{C3'}$ 82.0–82.8), confirming their de-*O*-sulfonated structures (Table S1†). The syntheses of cyclic sulfates **14–16** are described in Scheme S1.†

Our biochemical studies began by examining of the inhibitory properties of salacinol (**1**) and neosalacinol (**2**) toward α-glucosidase GAA (recombinant myozyme from genzyme, family GH31) at pH 5.2 using 4-nitrophenyl-α-D-glucopyranoside (α-*p*-NPG) as the substrate (Fig. S1† and Table 1). Prototypal **1** exhibited tight binding toward GAA, with a calculated K_i^{app} values of 0.12 ± 0.02 μM (Fig. S1† and Table 1). In contrast, the K_i^{app} value of the de-*O*-sulfonated analog **2** toward GAA was calculated to be 3.6 ± 0.3 μM, which is clearly inferior to that of **1** (Table 1 and Fig. S1†). In addition, voglibose and acarbose, which are widely used clinical intestinal α-glucosidase inhibitors, are interestingly less potent toward GAA than **1** and **2**, with K_i^{app} values of 7.6 ± 0.8 μM and 40 ± 2 μM, respectively (Table 1 and Fig. S2†). Based on these results, we conducted a comparative SAR study against GAA using potent intestinal α-glucosidase inhibitors **3–7**. Of the five, four analogs **3–6** showed strong inhibitory activities toward GAA, with calculated K_i^{app} values of 0.022 ± 0.007 μM, 0.034 ± 0.009 μM, 0.030 ± 0.009 μM, and 0.017 ± 0.010 μM, respectively (Fig. S3† and Table 1). Interestingly, the most potent intestinal α-glucosidase inhibitor (**7**)[32] was less potent inhibition (K_i^{app} = 0.17 ± 0.05 μM) (Table 1 and Fig. S3†) than the other 3′-*O*-benzylated analogs **3–6**. We conclude that *ortho*-substitution of the benzene ring and/or the electronic effects of the benzene-ring substituents appear to have no effect on GAA-inhibition characteristics, albeit with one exception. We therefore suggest that 3′-*O*-benzylation is an effective protocol for increasing binding affinity to GAA. Furthermore, *in silico* docking studies of salacinol (**1**), neosalacinol (**2**), and the 3′-*O*-benzylated analogs **3–7** with GAA strongly support the observed K_i^{app} trend (Fig. S4 and S5†). Although GAA and intestinal α-glucosidases are classified in family GH31, these results may highlight microenvironmental active-site differences between them.

We next evaluated the role of the stereochemistry of the salacinol side chain on inhibitory activity toward GH31 α-glucosidases (rat intestinal disaccharidases *vs.* GAA). As shown in Table 2, the 3′-*epi*-**1** (**8**) exhibited weak binding affinity toward



Scheme 1 Synthetic routes to side-chain stereo-derivatives of **1**.

rat intestinal sucrase and isomaltase (IC_{50}: 19 and 6.4 μM for sucrase and isomaltase, respectively); however, it completely lacked activity toward rat intestinal maltase (IC_{50} > 100 μM). In contrast, the de-*O*-sulfonated version of **8**, 3′-*epi*-**2** (**9**) exhibited stronger inhibitory activities toward three rat intestinal disaccharidases than **8** (**9**: IC_{50} = 0.69 μM for sucrase, IC_{50} 0.58 μM for isomaltase, and IC_{50} 4.3 μM for maltase). It was especially interesting that de-*O*-sulfonation significantly enhanced inhibitory activity toward rat intestinal maltase. Furthermore, by benchmarking against the inhibitory potency of **2** (IC_{50} 25 μM for maltase), we conducted that a combination of the 3′*R*-OH and 2′*S*-OH moieties is more suitable than 3′*S*-OH and 2′*S*-OH for the onset of potent maltase inhibitory activity. On the other hand, four stereo-derivatives **10–13**, each of which contains the 2′*R*-OH unit, exhibited virtually no binding to the rat intestinal disaccharidases, which highlights the importance of the cooperative roles of the 2′*S*-OH and 4′-OH moieties.

We next assessed the binding properties of **8–13** toward GAA (Table 1 and Fig. S6†); 3′-*epi*-**1** (**8**) and 3′-*epi*-**2** (**9**) are GAA-binding compounds, with K_i^{app} values determined to be 1.0 ± 0.1 μM and 25 ± 2 μM, respectively, which are nine- and seven-times higher than those of **1** and **2** (Table 1 and Fig. S6†). On the other hand, stereo-derivatives **10–13** exhibited no inhibitory activities was observed (Table 1 and Fig. S6†). As a result, the molecular recognizing abilities of GH31 α-glucosidases appear to be relatively tolerant of the stereochemistry at the 3′-position, but can strictly discriminate the stereochemistry at the 2′-position.

We next assessed the ability of **1–9** to specifically inhibit GH31 α-glucosidases. Hence, we examined their abilities to inhibit β-glucosidase from *Aspergillus niger*[34] at pH 4.6 using 4-nitrophenyl-β-D-glucopyranoside (β-*p*-NPG) as the substrate. None of these compounds displayed any inhibitory activity toward β-glucosidase from *A. niger* under our assay conditions, with apparent IC_{50} values > 1000 μM (Table 1 and Fig. S7†).

Table 2 Enzyme inhibiting efficacies of **1–13** toward rat intestinal disaccharidases[a]

Compound	Sucrase	Isomaltase	Maltase
1	1.6[b]	5.2[b]	5.2[b]
2	3.6	0.45	25
3 (H)	0.44[c]	0.14[c]	0.32[c]
4 (*o*-CH_3)	0.41[c]	0.48[c]	0.66[c]
5 (*o*-Cl)	0.090[c]	0.26[c]	0.31[c]
6 (*o*-CF_3)	0.15[c]	0.19[c]	0.33[c]
7 (*o*-NO_2)	0.042[c]	0.21[c]	0.13[c]
8 (3′-*epi*-**1**)	19	6.4	>100
9 (3′-*epi*-**2**)	0.69	0.58	4.3
10 (2′-*epi*-**1**)	>100	>100	>100
11 (2′-*epi*-**2**)	85	>100	>100
12 (2′,3′-*epi*-**1**)	>100	>100	>100
13 (2′,3′-*epi*-**2**)	84	34	>100
Voglibose	0.20[d]	2.1[d]	1.2[d]
Acarbose	1.5[e]	646[e]	1.7[e]

[a] Apparent IC_{50} in (μM). [b] Ref. 24. [c] Ref. 32. [d] Ref. 35. [e] Ref. 36.

These results demonstrate that thiosugar-based sulfonium salts **1–9** appear to be highly selective for GH31 α-glucosidases over *A. niger* β-glucosidase.

Conclusions

In summary, we demonstrated that salacinol-type α-glucosidase inhibitors exhibit ligand compatibility for the GH 31 family. Salacinol (**1**) and its 3′-*O*-benzylated analogs **3–7** displayed submicromolar-inhibitory activities toward human lysosomal α-glucosidase. Simple SAR studies demonstrated that the side-chain stereochemistry has a large effect on binding to GH31 α-glucosidases. We expect that the thiosugar skeleton may be valuable for the design of selective inhibitors that target glycosidases that recognize and process differently configured and substituted carbohydrates.

Author contributions

G. T. designed this project. A. H., Y. Y., K. Nishida, W. X., and G. T. synthesized compounds. F. I, K. Ninomiya, and T. M. conducted biochemical studies. S. N and I. N carried out *in silico* calculation. F. I., G. T., and O. M. evaluated the data. F. I. and G. T. wrote the manuscript.

Conflicts of interest

The authors declare no competing financial interests.

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