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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 tumors⁷ 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 pseudoaminosugars,^{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

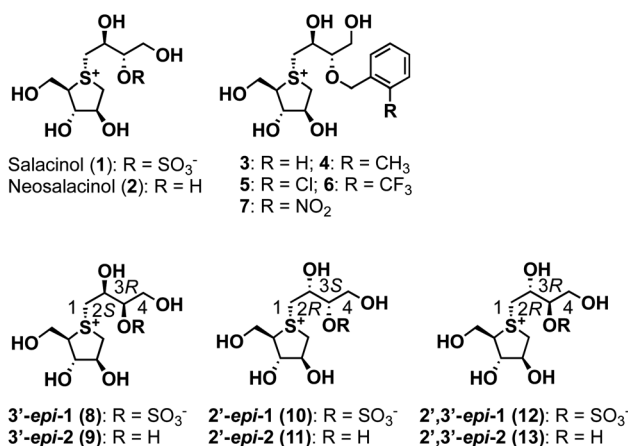


Fig. 1 Structures of naturally occurring salacinol (**1**), neosalacinol (**2**), and their derivatives **3**–**13** described in this study.

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

<i>r</i>	α -Glucosidase GAA ^b	<i>A. niger</i> β -glucosidase ^c
1	0.12 \pm 0.02	>1000
2	3.6 \pm 0.3	>1000
3 (H)	0.022 \pm 0.007	>1000
4 (<i>o</i> -CH ₃)	0.034 \pm 0.009	>1000
5 (<i>o</i> -Cl)	0.030 \pm 0.009	>1000
6 (<i>o</i> -CF ₃)	0.017 \pm 0.010	>1000
7 (<i>o</i> -NO ₂)	0.17 \pm 0.05	>1000
8 (3'- <i>epi</i> -1)	1.0 \pm 0.1	>1000
9 (3'- <i>epi</i> -2)	25 \pm 2	>1000
10 (2'- <i>epi</i> -1)	2794 \pm 294	>1000
11 (2'- <i>epi</i> -2)	2742 \pm 230	>1000
12 (2',3'- <i>epi</i> -1)	3893 \pm 262	>1000
13 (2',3'- <i>epi</i> -2)	463 \pm 36	>1000
Voglibose	7.6 \pm 0.8	>1000
Acarbose	40 \pm 2	>1000

^a Mean \pm 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 1,1,1,3,3,3-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

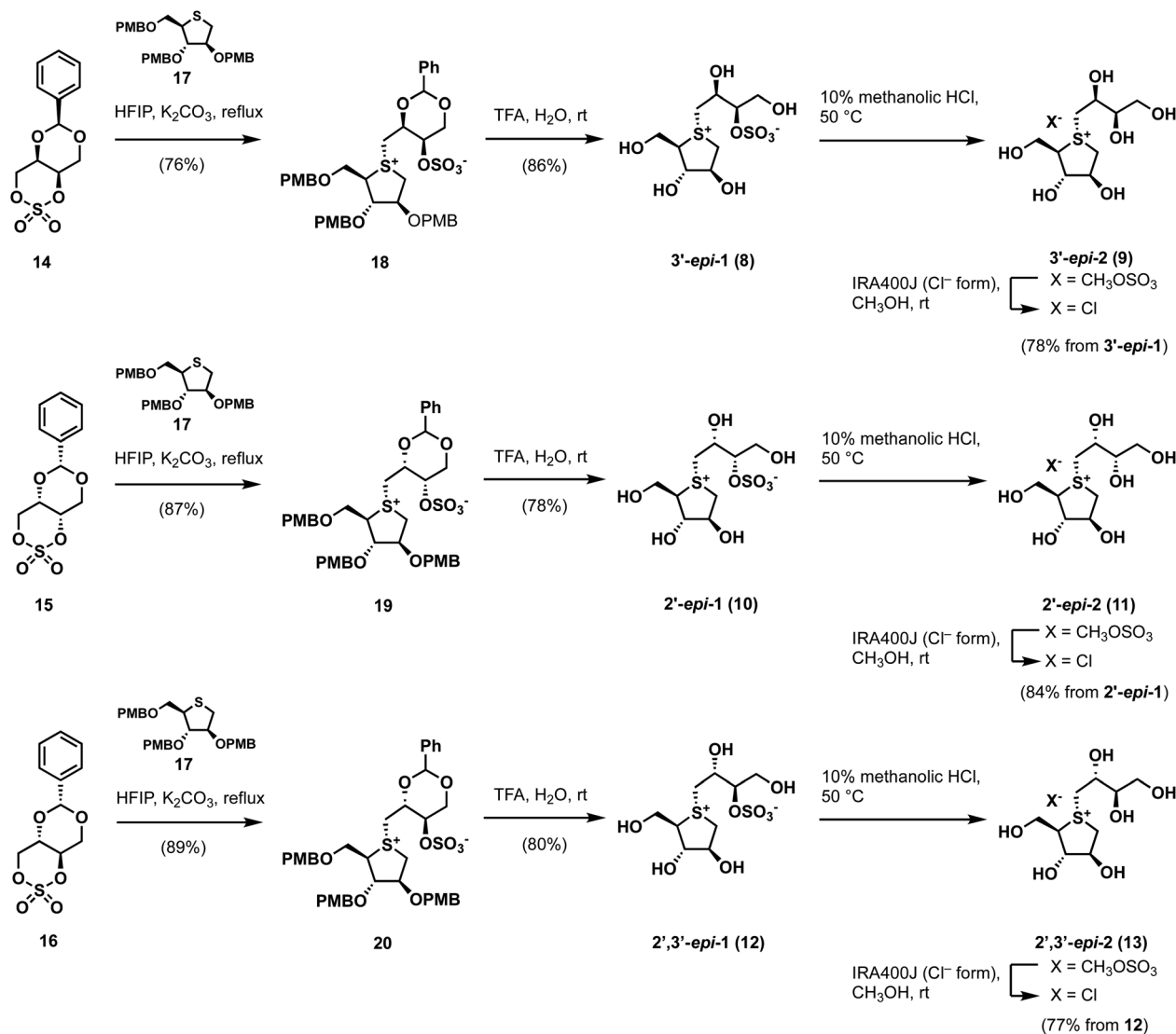
treated with aqueous TFA to simultaneously remove each *p*-methoxybenzyl (PMB) group and benzylidene acetal moiety, which gave the desired sulfonium salts 3'-*epi*-**1**, 2'-*epi*-**1**, and 2',3'-*epi*-**1** in good yields (Scheme 1). As shown in Table S1† (ESI), 3'-*epi*-**1**, 2'-*epi*-**1**, and 2',3'-*epi*-**1** showed ¹³C 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 = CH₃OSO₃); 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. ¹³C 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 ($\delta_{\text{C}3'}$ 74.2–75.1), which are significantly upfield shifted compared to those of 3'-*epi*-**1**, 2'-*epi*-**1**, and 2',3'-*epi*-**1** ($\delta_{\text{C}3'}$ 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 \pm 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 \pm 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 \pm 0.8 μM and 40 \pm 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 \pm 0.007 μM , 0.034 \pm 0.009 μM , 0.030 \pm 0.009 μM , and 0.017 \pm 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 \pm 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 ($\text{IC}_{50} > 100 \mu\text{M}$). 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: $\text{IC}_{50} = 0.69 \mu\text{M}$ for sucrase, $\text{IC}_{50} 0.58 \mu\text{M}$ for isomaltase, and $\text{IC}_{50} 4.3 \mu\text{M}$ 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 ($\text{IC}_{50} 25 \mu\text{M}$ 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_1^{APP} values determined to be $1.0 \pm 0.1 \mu\text{M}$ and $25 \pm 2 \mu\text{M}$, 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 $> 1000 \mu\text{M}$ (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 (<i>o</i> -CH ₃)	0.41 ^c	0.48 ^c	0.66 ^c
5 (<i>o</i> -Cl)	0.090 ^c	0.26 ^c	0.31 ^c
6 (<i>o</i> -CF ₃)	0.15 ^c	0.19 ^c	0.33 ^c
7 (<i>o</i> -NO ₂)	0.042 ^c	0.21 ^c	0.13 ^c
8 (3'- <i>epi</i> -1)	19	6.4	>100
9 (3'- <i>epi</i> -2)	0.69	0.58	4.3
10 (2'- <i>epi</i> -1)	>100	>100	>100
11 (2'- <i>epi</i> -2)	85	>100	>100
12 (2',3'- <i>epi</i> -1)	>100	>100	>100
13 (2',3'- <i>epi</i> -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 IC₅₀ 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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Notes and references

- S. A. K. Jongkees and S. G. Withers, Unusual enzymatic glycoside cleavage mechanisms, *Acc. Chem. Res.*, 2014, **47**(1), 226–235.
- V. Lombard, H. Golaconda Ramulu, E. Drula, P. M. Coutinho and B. Henrissat, The carbohydrate-active enzymes database (CAZy) in 2013, *Nucleic Acids Res.*, 2014, **42**(D1), D490–D495.
- T. D. Butters, R. A. Dwek and F. M. Platt, Imino sugar inhibitors for treating the lysosomal glycosphingolipidoses, *Glycobiology*, 2005, **15**(10), 43R–52R.
- A. Y. Y. Cheng and R. G. Josse, Intestinal absorption inhibitors for type 2 diabetes mellitus: prevention and treatment, *Drug Discov. Today*, 2004, **1**(2), 201–206.
- C. P. Kordik and A. B. Reitz, Pharmacological treatment of obesity: therapeutic strategies, *J. Med. Chem.*, 1999, **42**(2), 181–201.
- M. J. Papandreou, R. Barbouche, R. Guieu, M. P. Kieny and E. Fenouillet, The α -glucosidase inhibitor 1-deoxynojirimycin blocks human immunodeficiency virus envelope glycoprotein-mediated membrane fusion at the CXCR₄ binding step, *Mol. Pharmacol.*, 2002, **61**(1), 186–193.
- N. Asano, Glycosidase inhibitors: update and perspectives on practical use, *Glycobiology*, 2003, **13**(10), 93R–104R.
- R. A. Ugalde, R. J. Staneloni and L. F. Leloir, Microsomal glucosidases of rat liver. Partial purification and inhibition by disaccharides, *Eur. J. Biochem.*, 1980, **113**(1), 97–103.
- M. H. D. Postema, J. L. Piper, L. Liu, J. Shen, M. Foust and P. Andreama, Synthesis and partial biological evaluation of a small library of differentially-linked β -C-disaccharides, *J. Org. Chem.*, 2003, **68**(12), 4748–4754.
- K. Afarinkia and A. Bahar, Recent advances in the chemistry of azapyranose sugars, *Tetrahedron: Asymmetr.*, 2005, **16**(7), 1239–1287.
- O. Arjona, A. M. Gómez, J. C. López and J. Plumet, Synthesis and conformational and biological aspects of carbasugars, *Chem. Rev.*, 2007, **107**(5), 1919–2036.
- V. H. Lillelund, H. H. Jensen, X. Liang and M. Bols, Recent developments of transition-state analogue glycosidase inhibitors of non-natural product origin, *Chem. Rev.*, 2002, **102**(2), 515–554.
- T. Mahmud, The C₇N aminocyclitol family of natural products, *Nat. Prod. Rep.*, 2003, **20**(1), 137–166.
- S. Sou, S. Mayumi, H. Takahashi, R. Yamasaki, S. Kadoya, M. Sodeoka and Y. Hashimoto, Novel α -glucosidase inhibitors with a tetrachlorophthalimide skeleton, *Bioorg. Med. Chem. Lett.*, 2000, **10**(10), 1081–1084.
- T. Niwa, U. Doi and T. Ogasawara, Inhibitory activity of corn-derived bisamide compounds against α -glucosidase, *J. Agric. Food Chem.*, 2003, **51**(1), 90–94.
- K. Takada, T. Uehara, Y. Nakao, S. Matsunaga, R. W. M. van Soest and N. Fusetani, Schulzeines A-C, new α -glucosidase



- inhibitors from the marine sponge *Penares schulzei*, *J. Am. Chem. Soc.*, 2004, **126**(1), 187–193.
- 17 Y. Nakao, T. Maki, S. Matsunaga, R. W. M. van Soest and N. Fusetani, Penarolide sulfates A₁ and A₂, new α -glucosidase inhibitors from a marine sponge *Penares* sp., *Tetrahedron*, 2000, **56**(46), 8977–8987.
- 18 Y. Nakao, T. Maki, S. Matsunaga, R. W. M. van Soest and N. Fusetani, Penasulfate A, a new α -glucosidase inhibitor from a marine sponge *Penares* sp., *J. Nat. Prod.*, 2004, **67**(8), 1346–1350.
- 19 Y. Nakao, T. Uehara, S. Matsunaga, N. Fusetani and R. W. M. Soest, Callyspongynic Acid, a Polyacetylenic Acid Which Inhibits α -Glucosidase, from the Marine Sponge *Callyspongia truncate*, *J. Nat. Prod.*, 2002, **65**(6), 922–924.
- 20 J. Kawabata, K. Mizuhata, E. Sato, T. Nishioka, Y. Aoyama and T. Kasai, 6-Hydroxyflavonoids as α -glucosidase inhibitors from Marjoram (*Origanum majorana*) leaves, *Biosci., Biotechnol., Biochem.*, 2003, **67**(2), 445–447.
- 21 M. Yoshikawa, T. Murakami, H. Shimada, H. Matsuda, J. Yamahara, G. Tanabe and O. Muraoka, Salacinol, potent antidiabetic principle with unique thiosugar sulfonium sulfate structure from the Ayurvedic traditional medicine *Salacia reticulata* in Sri Lanka and India, *Tetrahedron Lett.*, 1997, **38**(48), 8367–8370.
- 22 M. Yoshikawa, T. Morikawa, H. Matsuda, G. Tanabe and O. Muraoka, Absolute stereostructure of potent α -glucosidase inhibitor, salacinol, with unique thiosugar sulfonium sulfate inner salt structure from *Salacia reticulata*, *Bioorg. Med. Chem.*, 2002, **10**(5), 1547–1554.
- 23 M. Yoshikawa, T. Murakami, K. Tashiro and H. Matsuda, Kotalanol, a potent α -glucosidase inhibitor with thiosugar sulfonium sulfate structure, from antidiabetic Ayurvedic Medicine *Salacia reticulata*, *Chem. Pharm. Bull.*, 1998, **46**(8), 1339–1340.
- 24 M. Yoshikawa, F. Xu, S. Nakamura, T. Wang, H. Matsuda, G. Tanabe and O. Muraoka, Salaprinol and ponkoranol with thiosugar sulfonium sulfate structure from *Salacia prnoides* and α -glucosidase inhibitory activity of ponkoranol and kotalanol desulfate, *Heterocycles*, 2008, **75**(6), 1397–1405.
- 25 Y. Minami, C. Kuriyama, K. Ikeda, A. Kato, K. Takebayashi, I. Adachi, W. J. G. Fleet, A. Kettawan, T. Okamoto and N. Asano, Effect of five-membered sugar mimics on mammalian glycogen-degrading enzymes and various glucosidases, *Bioorg. Med. Chem.*, 2008, **16**(6), 2734–2740.
- 26 O. Muraoka, W. Xie, G. Tanabe, F. A. M. Amer, T. Minematsu and M. Yoshikawa, On the structure of the bioactive constituent from ayurvedic medicine *Salacia reticulata*: revision of the literature, *Tetrahedron Lett.*, 2008, **49**(51), 7315–7317.
- 27 W. Xie, G. Tanabe, J. Akaki, T. Morikawa, K. Ninomiya, T. Minematsu, M. Yoshikawa, X. Wu and O. Muraoka, *Bioorg. Med. Chem.*, 2011, **19**(6), 2015–2022.
- 28 F. Ishikawa, K. Jinno, E. Kinouchi, K. Ninomiya, S. Marumoto, W. Xie, O. Muraoka, T. Morikawa and G. Tanabe, Diastereoselective synthesis of salacinol-type α -glucosidase inhibitors, *J. Org. Chem.*, 2018, **83**(1), 185–193.
- 29 M. H. S. Jayawardena, N. M. W. de Alwis, V. Hettigoda and D. J. S. Fernando, A double blind randomised placebo controlled cross over study of a herbal preparation containing *Salacia reticulata* in the treatment of type 2 diabetes, *J. Ethnopharmacol.*, 2005, **97**(2), 215–218.
- 30 O. Muraoka, K. Yoshikawa, T. Hatanaka, T. Minematsu, G. Lu, G. Tanabe, T. Wang, H. Matsuda and M. Yoshikawa, Synthesis and biological evaluation of deoxy salacinols, the role of polar substituents in the side chain on the α -glucosidase inhibitory activity, *Bioorg. Med. Chem.*, 2006, **14**(2), 500–509.
- 31 G. Tanabe, K. Yoshikai, T. Hatanaka, M. Yamamoto, Y. Shao, T. Minematsu, O. Muraoka, T. Wang, H. Matsuda and M. Yoshikawa, Biological evaluation of de-O-sulfonated analogs of salacinol, the role of sulfate anion in the side chain on the α -glucosidase inhibitory activity, *Bioorg. Med. Chem.*, 2007, **15**(11), 3926–3937.
- 32 G. Tanabe, S. Nakamura, N. Tsutsui, G. Balakishan, W. Xie, S. Tsuchiya, J. Akaki, T. Morikawa, K. Ninomiya, I. Nakanishi, M. Yoshikawa and O. Muraoka, *In silico* design, synthesis and evaluation of 3'-O-benzylated analogs of salacinol, a potent α -glucosidase inhibitor isolated from an Ayurvedic traditional medicine "*Salacia*", *Chem. Commun.*, 2012, **48**(69), 8646–8648.
- 33 A. Ghavami, K. S. Sadalapure, B. D. Johnston, M. Lobera, B. B. Snider and B. M. Pinto, Improved syntheses of the naturally occurring glycosidase inhibitor salacinol, *Synlett*, 2003, **2003**(9), 1259–1262.
- 34 T. Watanabe, T. Sato, S. Yoshioka, T. Koshijima and M. Kuwahara, Purification and properties of *Aspergillus niger* β -glucosidase, *Eur. J. Biochem.*, 1992, **209**(2), 651–659.
- 35 G. Tanabe, T. Otani, W. Cong, T. Minematsu, K. Ninomiya, M. Yoshikawa and O. Muraoka, Biological evaluation of 3'-O-alkylated analogs of salacinol, the role of hydrophobic alkyl group at 3' position in the side chain on the α -glucosidase inhibitory activity, *Bioorg. Med. Chem. Lett.*, 2011, **21**(10), 3159–3162.
- 36 O. Muraoka, T. Morikawa, S. Miyake, J. Akaki, K. Ninomiya, Y. Pongpiriyadacha and M. Yoshikawa, Quantitative analysis of neosalacinol and neokotalanol, another two potent α -glucosidase inhibitors from *Salacia* species, by LC-MS with ion pair chromatography, *J. Nat. Med.*, 2011, **65**(1), 142–148.

