Bioorganic & Medicinal Chemistry Letters 21 (2011) 3159–3162

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

Genzoh Tanabe [a], Tetsu Otani [a], Wenying Cong [a], Toshie Minematsu [a], Kiyofumi Ninomiya [b], Masayuki Yoshikawa [b,c], Osamu Muraoka [a,*]

[a] School of Pharmacy, Kinki University, 3-4-1 Kowakae, Higashi-osaka, Osaka 577-8502, Japan
[b] Pharmaceutical Research and Technology Institute, Kinki University, 3-4-1 Kowakae, Higashi-osaka, Osaka 577-8502, Japan
[c] Kyoto Pharmaceutical University, 1 Shichono-cho, Misasagi, Yamashina-ku, Kyoto 607-8412, Japan

ARTICLE INFO

Article history:
Received 2 November 2010
Revised 24 February 2011
Accepted 26 February 2011
Available online 11 March 2011

Keywords:
α-Glucosidase inhibitor
3′-*O*-Alkylated neosalacinol
Salacinol
Neosalacinol
Thiosugar

ABSTRACT

Four analogs with 3′-*O*-alkyl groups (**9a**: CH_3, **9b**: C_2H_5, **9c**: $C_{13}H_{27}$ or **9d**: CH_2Ph) instead of the 3′-*O*-sulfate anion in salacinol (**1**), a naturally occurring potent α-glucosidase inhibitor, were synthesized by the coupling reaction of 1,4-dideoxy-1,4-epithio-D-arabinitols (**18a** and **18b**) with appropriate epoxides (**10a–10d**). These analogs showed equal or considerably higher inhibitory activity against rat small intestinal α-glucosidases than the original sulfate (**1**), and one of them (**9d**) was found more potent than currently used α-glucosidase inhibitors as antidiabetics. Thus, introduction of a hydrophobic moiety at the C3′ position of this new class of inhibitor was found beneficial for onset of stronger inhibition against these enzymes.

Glycosidases are involved in several important biological processes (e.g., digestion, the biosynthesis of glycoproteins, and the lysosomal catabolism of glycoconjugates), thus glycosidase inhibitors have many potential therapeutic applications and have been implicated in several disease states,[1] such as diabetes, cancers and viral infections. Many naturally occurring and synthesized azasugars, which are believed to carry a positive charge at physiological pH and hence are postulated to bind in the active sites of glucosidase enzymes, are effective inhibitors of various glycosidases.[1] These evidences indicated that a potent glucosidase inhibitor might include an atom that carries a permanent positive charge at a suitable position that mimic the oxacarbenium ion-like transition state of the enzyme-catalyzed reaction.[1b]

In late 1990s′, salacinol (**1**) was isolated by the authors as one of the physiologically active components of an ayurvedic medicinal plant *Salacia reticulata*, which have traditionally been used for the treatment of diabetes in Sri Lanka and south region of India.[2] The structure of **1** was quite unique, bearing the permanent positive charge as the thiosugar sulfonium sulfate inner salt comprised of 1-deoxy-4-thio-D-arabinofranosyl cation and 3′-sulfate anion as shown in Figure 1. Its α-glucosidase inhibitory activity was revealed to be as potent as those of voglibose and acarbose which are widely used clinically these days.[2] Since the discovery of **1**, related sulfonium sulfates, kotalanol[3] (**2**), ponkoranol[4] (**3**) and salaprinol[4] (**4**) were subsequently isolated, and the stereostructure of these sulfonium sulfates (**2**, **3**, **4**) were elucidated by total syntheses or other means.[5] (Fig. 1)

Thereafter, their desulfonated versions, neosalacinol[6] (**5**), neokotalanol[4,7] (**6**) neoponkoranol[8] (**7**) and neosaraprinol[8] (**8**) were also isolated subsequently, and found as potent as their original sulfonates. The mode of action of **1** was also proved to be competitive inhibition against α-glucosidase, and K_i values of **1** against rat intestinal α-glucosidases, that is, maltase, sucrase, and isomaltase were revealed as 0.31, 0.32, and 0.47 μg/mL, respectively.[2b] Because of their intriguing structure and high α-glucosidase inhibitory activities, much attention has been focused on them, and intensive structure–activity relationships (SAR) studies on this new class of α-glucosidase family have been reported, determinants required for the activity being revealed to a certain extent.[5b,9] By the recent intensive X-ray crystallographic studies on N-terminal catalytic domain of maltase–glucoamylase (ntMGAM) in complex with kotalanol (**2**) and neokotalanol (**6**),[9f] and also by an in silico docking studies of salacinol (**1**) with an α-glucosidase,[9g] it was reported that the 3′-*O*-sulfate anion seemed to be constrained by hydrophobic residues of the enzymes, and made no hydrogen bonding interactions with them.

* Corresponding author. Tel.: +81 6 6721 2332; fax: +81 6 6721 2505.
E-mail address: muraoka@phar.kindai.ac.jp (O. Muraoka).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.02.109

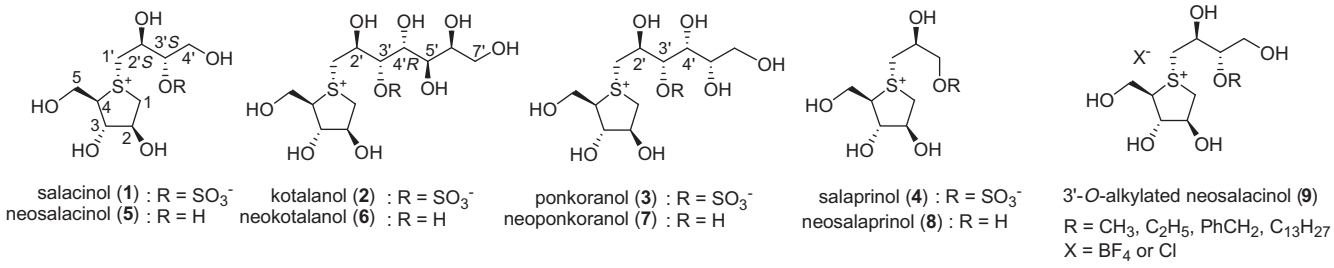


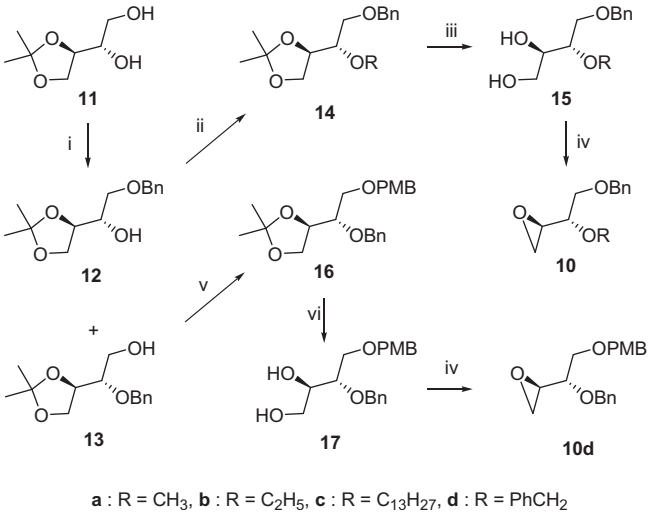
Figure 1. Sulfonium families as a new class of α-glucosidase inhibitors.

In this Letter, with an expectation of increment of the hydrophobic interactions of the molecule at this hydrophobic residue of the enzymes, four analogs (**9a**–**9d**) of salacinol (**1**), in which the 3′-*O*-sulfate moiety was replaced by OCH_3, OC_2H_5, $OC_{13}H_{27}$ or OCH_2Ph groups, were synthesized and their inhibitory activities were evaluated. Considerable enhancements of the inhibitory activity have been observed as was anticipated for the synthesized compounds.

Synthesis of **9** was carried out by applying the regioselective ring-opening reaction of appropriate epoxides with a thiosugar. Thus, epoxides **10a**, **10b**, and **10c** were first prepared in the following manner. 3,4-*O*-isopropylidene-D-erythritol (**11**), easily obtained via four steps starting from D-isoascorbic acid,[10] was subjected to selective benzylation of the primary hydroxyl to give 1-*O*-benzyl-3,4-*O*-isopropylidene-D-erythritol[11] (**12**) and 2-*O*-benzyl-3,4-*O*-isopropylidene-D-erythritol[12] (**13**) in 91% and 7% yields, respectively. Alkylation of the major alcohol **12** with CH_3I, C_2H_5I or $C_{13}H_{27}Br$ in DMF in the presence of NaH, and subsequent deacetonization of the resulting D-erythritol derivatives (**14a**, **14b**, and **14c**) by action of hydrochloric acid gave 2-*O*-alkyl-1-*O*-benzyl-D-erythritol (**15a**, **15b**, and **15c**), respectively, in good yields. Glycols **15a**, **15b**, and **15c** were then subjected to the Mitsunobu reaction by treatment with DEAD and Ph_3P in refluxing toluene to give the desired epoxides **10a**, **10b**, and **10c** in 77%, 76%, and 81% yields, respectively. Meanwhile, deacetonization of 2-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-3,4-*O*-isopropylidene-D-erythritol (**16**), which was prepared by alkylation of minor alcohol **13** with PMBCl, in aqueous acetic acid gave 2-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-D-erythritol (**17**) in 87% yield. Epoxydation of **17** under the Mitsunobu conditions gave the desired 1-*O*-*p*-methoxybenzylated epoxide **10d** in 70% yield (Scheme 1).

With epoxides **10a**, **10b**, **10c**, and **10d** in hand, perbenzylated thiosugar **18a** was treated with them in the presence of tetrafluoroboric acid dimethyl ether complex ($HBF_4{\cdot}Me_2O$) at −60 °C. By the regioselective attack of the thiosugar to the less hindered side of the epoxides, from epoxide **10a** was obtained predominantly the corresponding coupled product (**19a**) with BF_4^- as the counter anion, which was then exchanged with Cl^- by treatment with ion exchange resin IRA-400 J (Cl^- form) to give the corresponding chloride **19a**[13] in 88% yield. Finally, hydrogenolysis of chloride **19a** with palladium on carbon was carried out in 80% aqueous acetic acid at 50–60 °C, where concomitant formation of partially acetylated products was observed. Therefore, the crude product was subjected to acidic methanolysis to give the target compound **9a**[14] in 72% yield from **18a**. In a similar manner, epoxides **10b** and **10c** were converted to **9b** and **9c**, respectively, in good yields. On the other hand, the coupling reaction between epoxide **10d** with PMB ether of thiosugar (**18b**) resulted in a formation of a complex mixture, by the careful chromatography of which the target compound **9d** could be obtained in 15% yield.

The FAB-MS spectra of **9a**, **9b**, **9c**, and **9d** run in a positive mode showed peaks at *m*/*z* 269, 283, 345, and 437, respectively, due to the corresponding sulfonium cations. As shown in Table 1, ^{13}C



Scheme 1. Reagents and conditions: (i) Bu_2SnO, toluene, reflux, then BnBr, CsF, DMF, 60 °C; (ii) CH_3I, C_2H_5I, or $C_{13}H_{27}Br$, NaH, DMF, 0 °C to rt; (iii) 0.5% aq HCl, EtOH, reflux; (iv) Ph_3P, DEAD, toluene, reflux; (v) PMBCl, NaH, DMF, 0 °C; (vi) $AcOH/H_2O$ (2:1, v/v), rt.

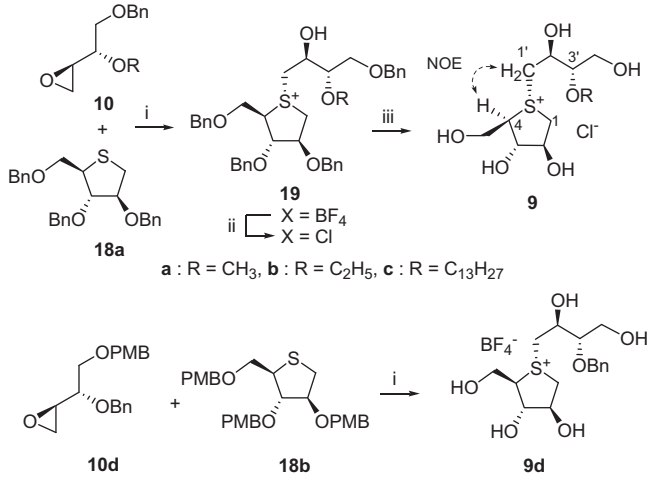
NMR spectra of **9a**, **9b**, **9c** and **9d** were similar with each other, and the significant down field shift of signals due to the C′3 methine carbon corresponded to the 3′-*O*-alkylated neosalacinol-type structure. The anti relationship between the side chain and the hydroxymethyl moiety on C4 of **9a**, **9b**, **9c**, and **9d** was confirmed by means of nuclear Overhauser effect (NOE) experiments as was shown in Scheme 2.

Finally, α-glycosidase inhibitory activities of synthesized compounds **9a**, **9b**, **9c**, and **9d** were tested for small intestinal α-glucosidases in vitro,[15] and compared with those of salacinol (**1**), neosalacinol (**5**) and currently used antidiabetics (voglibose and acarbose). (Table 2) All the molecules synthesized showed

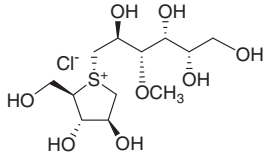
Table 1
^{13}C NMR data of neosalacinol[6b] (**5**) and compounds **9a**–**9d**

	5	**9a**[a]	**9b**[b]	**9c**[b]	**9d**[a]
C1	52.1	52.1	52.1	52.2	52.1
C2	79.4	79.4	79.4	79.5	79.4
C3	79.5	79.5	79.5	79.6	79.6
C4	73.7	73.7	73.7	73.8	73.7
C5	61.0	61.0	61.1	61.1	61.0
C1′	51.8	51.8	51.8	51.8	51.8
C2′	69.6	68.6	68.7	68.8	68.7
C3′	75.3	85.1	83.5	83.7	82.8
C4′	64.0	60.0	60.8	60.8	60.7

All spectra were measured in CD_3OD ([a]125 MHz, [b]175 MHz). Other signals: **9a**: 58.5 (OCH_3), **9b**: 15.8 (OCH_2CH_3), 67.2 (OCH_2CH_3), **9c**: 14.4 [$OCH_2(CH_2)_{11}CH_3$], 23.7/27.2/30.4/30.6/30.7/31.0/33.0 [$OCH_2(CH_2)_{11}CH_3$], 72.0 [$OCH_2(CH_2)_{11}CH_3$], **9d**: 73.5 (OCH_2Ph), 129.0/129.4/129.5 (d, arom.), 139.4 (s, arom.).



Scheme 2. Reagents and conditions: (i) $HBF_4{\cdot}(CH_3)_2O$, CH_2Cl_2, −60 °C; (ii) IRA-400 J (Cl^- form), MeOH–H_2O, rt; (iii) H_2, Pd–C, 80% AcOH, 50–60 °C, then 10% aq HCl–CH_3OH (1:100, v/v), rt.



3′-*O*-methylneoponkoranol (**20**)

Table 2
IC_{50} Values (μM) of salacinol (**1**), neosalacinol (**5**), compounds **9a**–**9d**, and two antidiabetics against disaccharidases

Entry	Compound	Sucrase	Maltase	Isomaltase
1	**1**	1.6[a]	5.2[a]	1.3[a]
2	**5**	1.3[a]	8.0[a]	0.3[a]
3	**9a**	0.46	5.3	0.39
4	**9b**	0.12	1.7	0.27
5	**9c**	1.3	1.0	0.95
6	**9d**	0.32	0.44	0.14
7	Voglibose	0.2	1.2	2.1
8	Acarbose	1.5[b]	1.7[b]	646[b]

[a] Lit. [4]
[b] Lit. [16]

equal or considerably higher inhibitory activity than the references. Against sucrase, all of them were found more active than original sufonate (**1**), and **9b** showed the highest inhibitory activity of ca. ten times as potent as **1**. It is noteworthy that, against maltase, **9d** was ca. ten times more potent than **1**, and exerted superior inhibitory activity to both acarbose and voglibose. The molecule is the most potent inhibitor among this type of molecules so far. Thus, it was concluded that higher hydrophobic property was preferred as the substituent on C3′ for higher inhibitory activity. The enhanced inhibitory activity encountered in the case of **9d** would be ascribed to π/π or CH/π interactions[17] of the phenyl ring at C3′ with surrounding aromatic residues of the active site in the enzyme.

Eskandari et al. have reported very recently the synthesis and evaluation of 3′-*O*-methylneoponkoranol (**20**), and mentioned that replacement of the sulfonate moiety with methyl group did not contribute to the improvement of the inhibitory activity against ntMGAM.[9j] We presume that the more hydrophobic moieties than the methyl as were exemplified in the present study would increase the inhibitory activity of the molecule.

Further SAR studies in search for stronger α-glucosidase inhibitors of this sulfonium type of compounds are in progress.

Acknowledgments

This work was supported by 'High-Tech Research Center' Project for Private Universities: matching fund subsidy from MEXT (Ministry of Education, Culture, Sports, Science and Technology), 2007–2011, and also supported by a grant-in aid for scientific research by the Japan society for the promotion of science (JSPS).

References and notes

1. (a) de Melo, E. B.; Gomes, A.; Da, S.; Carvalho, I. *Tetrahedron* **2006**, *62*, 10277; (b) Lillelund, V. H.; Jensen, H. H.; Liang, X.; Bols, M. *Chem. Rev.* **2002**, *102*, 515; (c) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683; *Iminosugars as Glycosidase Inhibitors: Nojirimycin and Beyond*; Stütz, A. E., Ed.; Wiley-VCH: Weinheim Germany, 1999.
2. (a) Yoshikawa, M.; Murakami, T.; Shimada, H.; Matsuda, H.; Yamahara, J.; Tanabe, G.; Muraoka, O. *Tetrahedron Lett.* **1997**, *38*, 8367; (b) Yoshikawa, M.; Morikawa, T.; Matsuda, H.; Tanabe, G.; Muraoka, O. *Bioorg. Med. Chem.* **2002**, *10*, 1547.
3. Yoshikawa, M.; Murakami, T.; Yashiro, K.; Matsuda, H. *Chem. Pharm. Bull.* **1998**, *46*, 1339.
4. Yoshikawa, M.; Xu, F.; Nakamura, S.; Wang, T.; Matsuda, H.; Tanabe, G.; Muraoka, O. *Heterocycles* **2008**, *75*, 1397.
5. (a) Jayakanthan, K.; Mohan, S.; Pinto, B. M. *J. Am. Chem. Soc.* **2009**, *131*, 5621; (b) Johnston, B. D.; Jensen, H. H.; Pinto, B. M. *J. Org. Chem.* **2006**, *71*, 1111; (c) Tanabe, G.; Sakano, M.; Minematsu, T.; Matusda, H.; Yoshikawa, M.; Muraoka, O. *Tetrahedron* **2008**, *64*, 10080; (d) Muraoka, O.; Xie, W.; Osaki, S.; Kagawa, A.; Tanabe, G.; Amer, M. F. A.; Minematsu, T.; Morikawa, T.; Yoshikawa, M. *Tetrahedron* **2010**, *66*, 3717.
6. (a) Minami, Y.; Kuriyama, C.; Ikeda, K.; Kato, A.; Takebayashi, K.; Adachi, I.; Fleet, G. W. J.; Kettawan, A.; Okamoto, T.; Asano, N. *Bioorg. Med. Chem.* **2008**, *16*, 2734; (b) Tanabe, G.; Yoshikai, K.; Hatanaka, T.; Yamamoto, M.; Shao, Y.; Minematsu, T.; Muraoka, O.; Wang, T.; Matsuda, H.; Yoshikawa, M. *Bioorg. Med. Chem.* **2007**, *15*, 3926; (c) Tanabe, G.; Xie, W.; Ogawa, A.; Cao, C.; Minematsu, T.; Yoshikawa, M.; Muraoka, O. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2195.
7. (a) Ozaki, S.; Oe, H.; Kitamura, S. *J. Nat. Prod.* **2008**, *71*, 981; (b) Oe, H.; Ozaki, S. *Biosci. Biotechnol. Biochem.* **2008**, *72*, 1962; (c) Muraoka, O.; Xie, W.; Tanabe, G.; Amer, M. F. A.; Minematsu, T.; Yoshikawa, M. *Tetrahedron Lett.* **2008**, *49*, 7315.
8. Xie, W.; Tanabe, G.; Akaki, J.; Morikawa, T.; Ninomiya, K., Minematsu, T., Yoshikawa, M.; Wu, X.; Muraoka, O. *Bioorg. Med. Chem.* **2011**, *19*, 2015.
9. Recent review relevant to the work: (a) Mohan, S.; Pinto, B. M. *Carbohydr. Res.* **2007**, *342*, 1551; (b) Tanabe, G.; Matsuoka, K.; Minematsu, T.; Morikawa, T.; Ninomiya, K.; Matsuda, H.; Yoshikawa, M.; Murata, H.; Muraoka, O. J. *Pharm. Soc. Jpn.* **2007**, *127*(Suppl. 4), 129; (c) Nasi, R.; Patrick, B. O.; Sim, L.; Rose, D. R.; Pinto, B. M. *J. Org. Chem.* **2008**, *73*, 6172; (d) Tanabe, G.; Hatanaka, T.; Minematsu, T.; Matsuda, H.; Yoshikawa, M.; Muraoka, O. *Heterocycles* **2009**, *79*, 1093; (e) Mohan, S.; Jayakanthan, K.; Nasi, R.; Kuntz, D. A.; Rose, D. R.; Pinto, B. M. *Org. Lett.* **2010**, *12*, 1088; (f) Sim, L.; Jayakanthan, K.; Mohan, S.; Nasi, R.; Johnston, B. D.; Pinto, B. M.; Rose, D. R. *Biochemistry* **2010**, *49*, 443; (g) Nakamura, S.; Takahira, K.; Tanabe, G.; Morikawa, T.; Sakano, M.; Ninomiya, K.; Yoshikawa, M.; Muraoka, O.; Nakanishi, I. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4420; (h) Eskandari, R.; Kuntz, D. A.; Rose, D. R.; Pinto, B. M. *Org. Lett.* **2010**, *12*, 1632; (i) Eskandari, R.; Jayakanthan, K.; Kuntz, D. A.; Rose, D. R.; Pinto, B. M. *Bioorg. Med. Chem.* **2010**, *18*, 2829; (j) Eskandari, R.; Jones, K.; Rose, D. R.; Pinto, B. M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5686. and references cited therein.
10. Abushanab, E.; Vemishetti, P.; Leiby, R. W.; Singh, H. K.; Mikkilineni, A. B.; Wu, D. C.-J.; Saibaba, R.; Panzica, R. P. *J. Org. Chem.* **1988**, *53*, 2598.
11. For the enantiomer of 12, see: Marco, J. A.; Carda, M.; González, F.; Rodríguez, S.; Murga, J. *Liebigs Ann.* **1996**, 1801.
12. Vijayasaradhi, S.; Beedimane, M. N.; Aidhen, I. S. *Synthesis* **2005**, 2267.
13. *Synthesis of* **19a**: To a mixture of epoxide **10a** (100 mg, 0.48 mmol), thiosugar **18a** (168 mg, 0.4 mmol), and CH_2Cl_2 (2 ml) was added tetrafluoroboric acid dimethyl ether complex ($HBF_4{\cdot}(CH_3)_2O$, 63 μl, 0.52 mmol) at −60 °C. The reaction mixture was stirred for 3 h and concentrated in vacuo. The residue was treated with ion exchange resin IRA-400 J (Cl^- form) in methanol (3 ml) at room temperature. Removal of the solvent left an oil (290 mg), which on column chromatography ($CHCl_3$ → $CHCl_3$–MeOH, 100:1 → 50:1), gave 1,4-dideoxy-2,3,5-tri-*O*-benzyl-1,4-[(*R*)-[(2*S*,3*S*)-4-benzyloxy-2-hydroxy-3-methoxybutyl]episulfoniumylidene]-D-arabinitol chloride (**19a**, 234 mg, 88%) as a colorless oil, $[\alpha]_D^{24}$ −7.3 (*c* = 0.65, $CHCl_3$). IR (neat): 3174, 1454, 1404, 1365, 1261, 1095, 1072, 1030 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 3.41 (3H, s, OCH_3), 3.66–3.70 (2H, m, H-3′ and H-4′a), 3.76 (2H, d-like, *J* = ca. 8.0 Hz, H-5a and H-5b), 3.80 (1H, dd, *J* = 11.5, 3.8 Hz, H-4′b), 4.10 (1H, dd, *J* = 12.6, 3.7 Hz, H-1′a), 4.11–4.15 (2H, m, H-1a and H-4), 4.15 (1H, dd-like, *J* = ca. 12.6, 7.4 Hz, H-1′b), 4.17-4.18 (1H, m, H-3), 4.31 (1H, dd, *J* = 13.2, 3.8 Hz, H-1b), 4.34–4.39 (1H, m, H-2′), 4.39–4.41 (1H, m, H-2), 4.39 (1H, d, *J* = 11.7 Hz, OCH_2Ph), 4.47–4.61 (7H,

m, OC*H*$_2$Ph), 6.65 (1H, br s, OH), 7.13–7.37 (20H, m, arom.). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 48.4 (C-1), 51.9 (C-1′), 57.9 (O*C*H$_3$), 66.1 (C-4), 66.9 (C-5), 67.4 (C-4′), 68.1 (C-2′), 71.9/72.3/73.6(2xC) (O*C*H$_2$Ph), 82.0 (C-3′), 82.3 (C-3), 82.4 (C-2), 127.7/127.8/127.96/127.99/128.2/128.3/128.4/128.5/128.56/128.6/128.7/128.8 (d, arom.), 135.8/136.0/136.7/137.9 (arom.). FAB-MS *m*/*z*: 629 [M−Cl]$^+$ (pos.).

14. *Compound* **9a**: colorless oil. $[\alpha]_D^{26}$ + 2.1 (*c* = 1.97, CH_3OH). IR (neat): 3333, 1651, 1408, 1261, 1084, 1053, 1026 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD) δ: 3.29 (1H, ddd-like, *J* = ca. 6.0, 4.0, 4.0 Hz, H-3′), 3.47 (3H, s, OC*H*$_3$), 3.66 (1H, dd, *J* = 12.3, 4.0 Hz, H-4′a), 3.73 (1H, dd, *J* = 12.9, 9.2 Hz, H-1′a), 3.80 (1H, dd, *J* = 12.3, 4.0 Hz, H-4′b), 3.82 (1H, dd, *J* = 12.9, 3.4 Hz, H-1′b), 3.85 (2H, d-like, *J* = ca. 2.3 Hz, H-1a and H-1b), 3.92 (1H, dd, *J* = 10.9, 9.5 Hz, H-5a), 3.99 (1H, br dd-like, *J* = ca. 9.5, 4.9 Hz, H-4), 4.05 (1H, dd, *J* = 10.9, 4.9 Hz, H-5b), 4.20 (1H, ddd, *J* = 9.2, 6.0, 3.4 Hz, H-2′), 4.36 (1H, dd, *J* = 2.3, 1.1 Hz, H-3), 4.62 (1H, dt, *J* = 2.3, 2.3 Hz, H-2). ^{13}C NMR data are listed in Table 1. FAB-MS *m*/*z*: 269 [M−Cl]$^+$ (pos.), FAB-HRMS *m*/*z*: 269.1059 ($C_{10}H_{21}O_6S$ requires 269.1059).
15. Rat small intestinal brush border membrane vesicles were prepared[18] and its suspension in 0.1 M maleate buffer (pH 6.0) was used as small intestinal α-glucosidase of maltase, sucrase, and isomaltase. Test compound was dissolved in dimethylsulfoxide (DMSO), and the resulting solution was diluted with 0.1 M maleate buffer to prepare the test compound solution (concentration of DMSO:10%). The substrate solution in maleate buffer (maltose, 74 mM, sucrose, 74 mM, isomaltose, 7.4 mM, 50 μL), the test compound solution (25 μL), and the enzyme solution (25 μL) were mixed and incubated at 37 °C for 30 min. After incubation, the solution was immediately heated by boiling water for 2 min to stop the reaction, and was mixed with water (150 μL). Glucose concentration was determined by the glucose–oxidase method. Final concentration of DMSO in the test solution was 2.5% and no influence of DMSO was detected on the inhibitory activity.
16. Muraoka, O.; Morikawa, T.; Miyake, S.; Akaki, J.; Ninomiya, K.; Pongpiriyadacha, Y.; Yoshikawa, M. J. *Nat. Med.* **2011**, *65*, 142.
17. *The CH/π Interaction. Evidence, Nature, and Consequences*; Marchard, A. P., Ed.; Wiley-VCH: New York, 1998.
18. Kessler, M.; Acuto, O.; Storelli, C.; Murer, H.; Müller, M.; Semenza, G. *Biochim. Biophys. Acta* **1978**, *506*, 136.