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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*"†

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With the aid of an *in silico* method, α-glucosidase inhibitors with far more potent activities than salacinol (1), a potent natural α-glucosidase inhibitor isolated from an Ayurvedic traditional medicine *Salacia reticulata*, have been developed.

In the late 1990s we isolated the highly potent α-glucosidase inhibitor, salacinol (**1**), from the roots and stems of *Salacia reticulata*, which has usually been used for the treatment of diabetes in traditional Ayurvedic medicine. The α-glucosidase inhibitory activity of **1** was revealed to be as potent as those of voglibose and acarbose, which have been widely used clinically.[1] After the discovery of **1**, the related sulfonium sulfates, kotalanol[2] (**2**) and ponkoranol[3] (**3**), and their desulfonated analogs, neosalacinol[4] (**4**), neokotalanol[5] (**5**), and neoponkoranol[6] (**6**) were subsequently isolated from the same genus plant as the compounds responsible for the antidiabetic activity. On this basis, human clinical trials on patients with type-2 diabetes, conducted with the extract of *Salacia reticulata*, have shown effective treatment of type-2 diabetes with minimal side effects.[7] On the other hand, owing to both the high inhibitory activity and the intriguing structure of the constituents (**1–6**), much attention has been focused on them, and intensive structure–activity relationship (SAR) studies on this new class of α-glucosidase family have been conducted. Through these studies several inhibitors with measurably better activity have been developed.[8]

Recent intensive X-ray crystallographic studies[9a] on the human N-terminal catalytic domain of maltase-glucoamylase (hNtMGAM) in complex with **2** and **5** and/or *in silico* docking studies[9b] of **1** with hNtMGAM indicated that the 3′-*O*-sulfate anion of **1** was constrained by the hydrophobic residues of the enzyme, and made no hydrogen bonding interactions with them. Actually, the introduction of a hydrophobic group at the 3′ position in **1** enhanced the activity to some extent.[9c] In the present study, with the aid of an *in silico* docking study, a series of 3′-*O*-benzylated analogs (**7b–7m**), in which the characteristic sulfate moiety of **1** was replaced by mono-substituted benzyl groups, were designed as more potent inhibitors. The synthesis and evaluation led to a compound (**7k**) *ca.* forty times as potent as the natural inhibitor (**1**) (Fig. 1).

From the intensive *in silico* docking studies on salacinol derivatives, it was predicted that the introduction of chloro or nitro substituents onto the benzyl moiety of 3′-*O*-benzyl-neosalacinol (**7a**) would increase the inhibitory activity [see Table 1, E_{bind} (kcal mol^{-1}) of the predicted binding affinities calculated by the MM/GBVI method].[10] In this study, to examine the effect of the position of the substitution in the

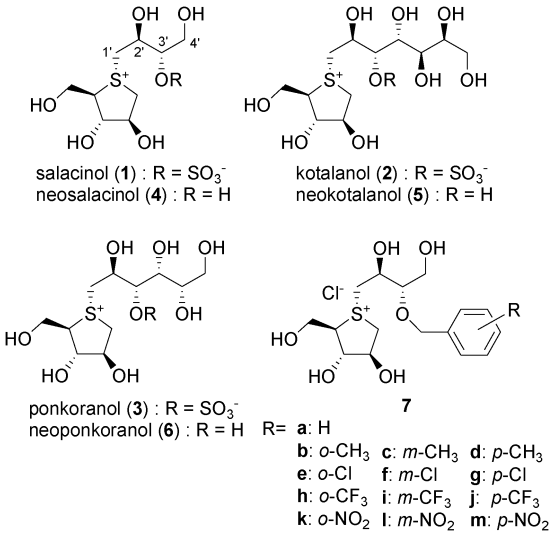

Fig. 1 Cyclic sulfoniums as a new class of α-glucosidase inhibitors.

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Table 1 E_{bind} (kcal mol^{-1}) of **7a–7m**, **1**, and **4** to hNtMGAM, and their IC_{50} (μM) values against rat intestinal disaccharidases

Entry	Compound	E_{bind}[a]	Maltase	Sucrase	Isomaltase
1	**7a** (H)	−37.2	0.32[c]	0.44[c]	0.14[c]
2	**7b** (*o*-CH_3)	−37.2	0.66	0.41	0.48
3	**7c** (*m*-CH_3)	−36.7	0.84	1.3	0.35
4	**7d** (*p*-CH_3)	−35.5	0.86	1.1	0.68
5	**7e** (*o*-Cl)	−41.6	0.31	0.09	0.26
6	**7f** (*m*-Cl)	−42.6	0.53	0.80	0.31
7	**7g** (*p*-Cl)	−40.0	0.89	0.72	0.48
8	**7h**(*o*-CF_3)	−37.5	0.33	0.15	0.19
9	**7i** (*m*-CF_3)	−35.4	0.98	0.82	0.25
10	**7j** (*p*-CF_3)	−40.5	0.98	0.72	0.38
11	**7k** (*o*-NO_2)	−38.9	0.13	0.042	0.21
12	**7l** (*m*-NO_2)	−37.4	0.94	0.49	0.23
13	**7m** (*p*-NO_2)	−42.2	0.68	0.38	0.23
14	**1**	−37.0[b]	5.2[d]	1.6[d]	1.3[d]
15	**4**	−36.4[b]	8.0[d]	1.3[d]	0.3[d]
16	Voglibose		1.2	0.2	2.1
17	Acarbose[c]		1.7[e]	1.5[e]	646[e]

[a] Calculation has been performed using the reported scheme.[9b] [b] Ref. 9*b*. [c] Ref. 9*c*. [d] Ref. 3. [e] Ref. 17.

benzene ring and/or the electronic effects of the substituents on the benzene ring, both of which were difficult to be precisely evaluated by simulation, three regioisomers (*ortho*, *meta*, and *para*) with respect to the four kinds of substituents (CH_3, Cl, CF_3, and NO_2) were synthesized and evaluated to confirm the computational SAR.

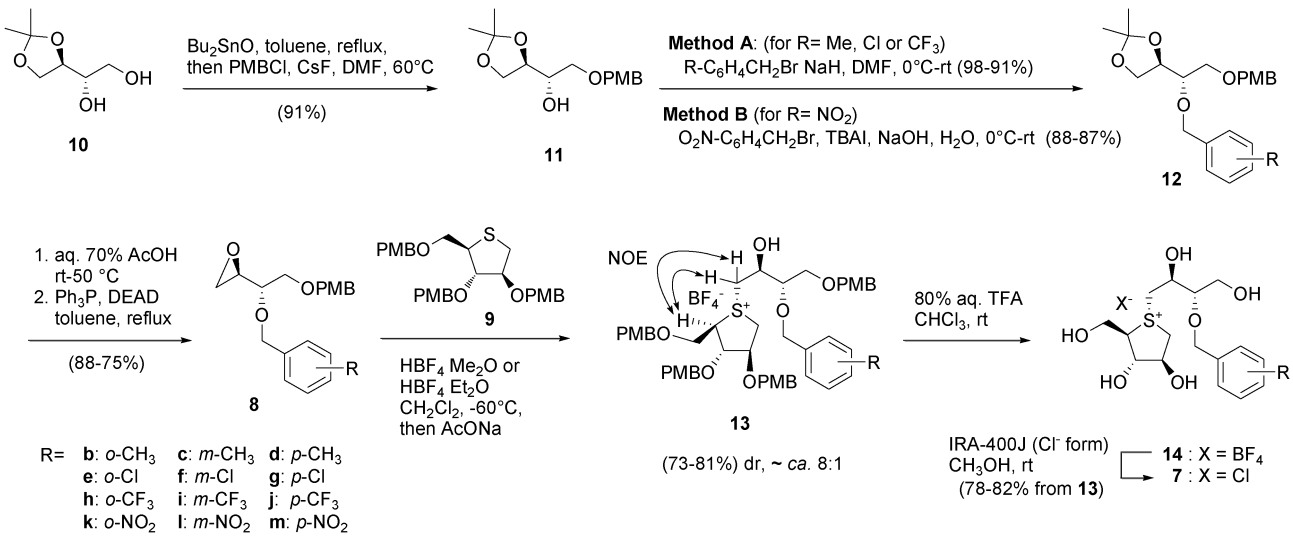
Syntheses of the twelve derivatives (**7b–7m**) were carried out by applying the regioselective ring-opening reaction of the appropriate epoxides **8b–8m** with a thiosugar, 2,3,5-tri-*O*-(*p*-methoxybenzyl)-1,4-dideoxy-1,4-epithio-D-arabinitol[11] (**9**). Firstly, the primary hydroxyl in **10**[12] was selectively protected with *p*-methoxybenzyl (PMB) chloride to give the corresponding PMB ether (**11**) in 91% yield. The preparation of methyl-, chloro-, and trifluoromethyl benzyl derivatives (**12b–12j**) was accomplished in the usual manner, whereas nitrobenzyl ethers (**12k–12m**) were obtained by employing NaOH as the base.[13] The deacetalization of **12b–12m** followed by epoxidation of the resulting glycols under the Mitsunobu conditions gave the desired epoxides (**8b–8m**) in 88–75% yields.

The obtained epoxides **8b–8m** (1.2 eq.) were treated with thiosugar **9** (1.0 eq.) in the presence of $HBF_4{\cdot}Me_2O$ or $HBF_4{\cdot}Et_2O$ (1.3 eq.) at −60 °C to give the corresponding coupling products (**13b–13m**) in good yields. In all cases, the ^{1}H NMR spectra showed the formation of a small amount of diastereoisomers at the sulfonium center (dr ∼*ca.* 8/1), which was consistent with our previous works.[4c] The *anti* relation between the side chain and the hydroxymethyl moiety on C4 of the major isomer (**α-13**) was confirmed by means of nuclear Overhauser effect (NOE) experiments as shown in Scheme 1.

Then the PMB groups of **13b–13m** were removed by aqueous trifluoroacetic acid at rt to give the corresponding sulfonium salts with the BF_4^- anion, which was finally exchanged with the Cl^- anion (**7b–7m**) by treating with the ion exchange resin IRA-400J (Cl^- form).

α-Glucosidase inhibitory activities of **7b–7m** were tested for rat small intestinal α-glucosidases *in vitro*, and compared with those of salacinol (**1**), neosalacinol (**4**) and currently used antidiabetics (voglibose and acarbose), as shown in Table 1. All the sulfonium salts (**7b–7m**) showed superior inhibitory activities regardless of the substitution position and of the substituent species. It is interesting to note that the inhibitory activities of *ortho*-substituted compounds (**7b**, **7e**, **7h**, and **7k**) against maltase and sucrase were stronger than those of the corresponding *meta*- and *para*-substituted analogs, although the results could not be predicted by the *in silico* docking studies. Among the twelve derivatives evaluated in this study, **7k** was found to be the most potent, and against maltase, the strongest inhibitor with approximately a 40-fold activity relative to **1**.

The differences between the computational prediction and the experimental results in this study might be caused by the different origin of the enzymes, although rat intestinal maltase is known to be homologous to hNtMGAM.[14] The contribution of other catalytic domains such as C-terminal maltase-glucoamylase (CtMGAM) and/or sucrase-isomaltase (CtSI),



Scheme 1 Synthesis of compounds **7b–7m**.

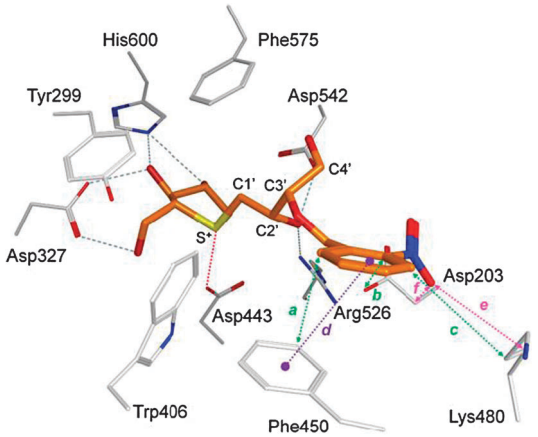


Fig. 2 Superposition of **7k** in the hNtMGAM active site. Dotted lines show hydrogen bonding (gray) and salt bridge (red). Double-headed green arrows show the van der Waals interactions of the phenyl ring with the amino acid residues (distances of a: 3.42 Å, b: 4.04 Å, c: 4.28 Å). Van der Waals interactions between an oxygen atom of the nitro group of **7k** and the amino acid residues are shown with the pink arrows (e: 3.69 Å, f: 4.05 Å). Distance between the π-planes shown with the purple dotted line was calculated to be 4.42 Å.

which have been proven to affect the hydrolysis of maltose,[15] would be another reason for these discrepancies.

By the hitherto conducted docking studies on salacinol (**1**) with hNtMGAM, it has been revealed that efficient hydrogen bonding interactions of C2′-OH and C4′-OH with Asp203 caused the strong inhibitory activity, which was essential for these inhibitors to exert their activity.[9b] It is interesting to note that in the present *in silico* docking study of **7k** with the enzyme, another binding mode different from that of **1** has been revealed as shown in Fig. 2, where instead of the hydrogen bonding interactions of C4′-OH of **1** with Asp203, van der Waals interactions were detected between the phenyl ring at C3′ and the hydrophobic parts of Asp203, Phe450 and Lys480 around the phenyl group (Fig. 2, green arrows a, b, and c). In addition, the phenyl ring of Phe450 and the C3′-phenyl were stacked almost in parallel, and the distance between these two π-planes was calculated to be *ca.* 4.4 Å, a sufficient distance for effective π–π interaction[16] (purple dotted line d). The nitro group introduced at the *ortho*-position on the benzene ring anchored in a concave pocket of the enzyme, and one of the oxygens of the nitro group affected the van der Waals interactions with the surrounding methylene chains of Asp203 and Lys480 (Fig. 2, pink arrows e and f), the distances of which were 3.7 and 4.1 Å, respectively. This anchoring effect on the binding could have been more effective than expected in the docking studies, and might be contributing to the inhibitory activity.

Thus, with the aid of the *in silico* method, more potent α-glucosidase inhibitors (**7b–7m**) than the seed compound salacinol (**1**) were effectively designed and developed. Finally, we comment that the sulfonium salt **7k** was found to be the most potent among the cyclic sulfonium family, a new class of α-glucosidase inhibitors, synthesized so far. On the basis of the present findings further studies to develop the more active and safe candidate for the new type of α-glucosidases are in progress.

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