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Synthesis and Biological Evaluation of Heteroanalogues of Kotalanol and De-*O*-Sulfonated Kotalanol

Sankar Mohan,[†] Kumarasamy Jayakanthan,[†] Ravindranath Nasi,[†] Douglas A. Kuntz,[‡] David R. Rose,[‡,§] and B. Mario Pinto*[,†]

Department of Chemistry, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6, Department of Medical Biophysics, University of Toronto and Division of Molecular and Structural Biology, Ontario Cancer Institute, Toronto, ON, Canada M5G 2M9, and Department of Biology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

bpinto@sfu.ca

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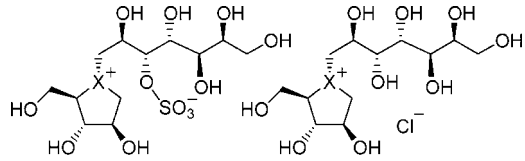
ABSTRACT



X = NH, Se

The synthesis of nitrogen and selenium analogues of kotalanol and de-*O*-sulfonated kotalanol, naturally occurring sulfonium-ion glucosidase inhibitors isolated from *Salacia reticulata*, and their evaluation as glucosidase inhibitors against the *N*-terminal catalytic domain of human maltase glucoamylase (ntMGAM) are described.

The aqueous extracts of *Salacia reticulata*, a climbing shrub native to Sri Lanka and Southern India, used in Indian Ayurvedic medicine, have been consumed by patients as a remedy for the treatment of type-2 diabetes.[1] The safety and efficacy of *Salacia* extracts have been studied in both rats[2] and human patients with type-2 diabetes and a placebo-control group.[3] These studies showed that the extract is an effective treatment for type-2 diabetes, with no serious acute toxicity and side effects comparable to the placebo control group. In recent years, we have focused our synthetic efforts on a novel class of sulfonium-ion glucosidase inhibitors, namely salacinol **1**,[4] kotalanol **2**[5] and de-*O*-sulfonated kotalanol **3**,[6] isolated from the aqueous extracts of *Salacia reticulata* (Figure 1). Along with salacinol **1** and kotalanol **2**, two other members of this class of compounds, namely salaprinol **4** and ponkoranol **5**, have also been isolated from *Salacia prinoides*, another medicinally useful plant that belongs to the *Salacia* genus (Figure 1).[7] The observed antidiabetic property of these herbal extracts is attributed, at least in part, to inhibition of the action of intestinal α-glucosidases by these sulfonium-ion active components.[4−6]

[†] Simon Fraser University.
[‡] University of Toronto and Ontario Cancer Institute.
[§] University of Waterloo.

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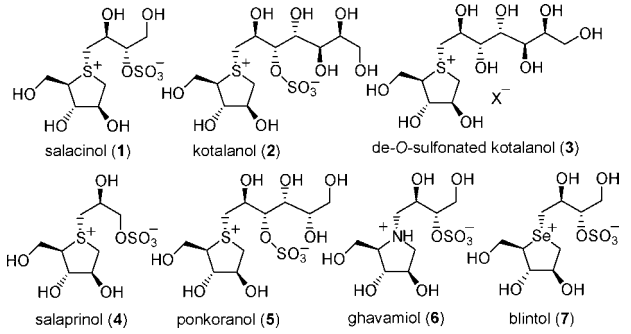

Figure 1. Sulfonium-ion glucosidase inhibitors isolated from *Salacia* species and related analogues.

We have synthesized several analogues of salacinol and studied their structure activity relationship (SAR) with human intestinal maltase glucoamylase (MGA).[8] Some of the modifications included: replacement of the ring-sulfur heteroatom by the cognate atoms nitrogen[9,10] and selenium;[11] change of the configurations of the stereogenic centers; and extension of the acyclic side chain.[12] Some of these compounds have shown higher or comparable inhibitory activities against MGA *in vitro* compared to acarbose and miglitol, two antidiabetic drugs that are currently in use for the treatment of type-2 diabetes.[13,14]

The acyclic side chain-extension studies of salacinol led us to predict the possible stereochemical pattern of the acyclic side chain in kotalanol **2**, for which the absolute stereostructure was not determined at the time of its isolation. Recently, we have proved the absolute stereostructure of kotalanol **2** and de-*O*-sulfonated kotalanol **3** by total syntheses.[15] In the case of salacinol, the substitution of the ring sulfur atom by nitrogen (ghavamiol, **6**,[9] IC_{50} = high mM range,[16] Figure 1) resulted in a dramatic decrease in inhibitory activity against MGA (compare the K_i value of salacinol, 0.19 μM[13]), whereas substitution by selenium (blintol, **7**, $K_i = 0.49$ μM,[13] Figure 1) did not affect its inhibitory activity appreciably.

It is of interest, therefore, to study the effect of heteroatom substitution on the inhibitory activities of kotalanol **2** and de-*O*-sulfonated kotalanol **3**, both having a 3-carbon-extended acyclic side chain compared to salacinol **1**. We report here the syntheses of the nitrogen **8** and **9** and selenium **10** and **11** congeners of kotalanol and de-*O*-sulfonated kotalanol (Figure 2) and their evaluation as glucosidase inhibitors against the amino terminal catalytic domain of human MGA (ntMGAM).[13] Since de-*O*-sulfonated kotalanol **3** was found to be more active than kotalanol **2** itself,[6] we have also converted two biologically active diastereomers **12** and **13** of kotalanol[17] into their corresponding de-*O*-sulfonated analogues **14** and **15**, respectively (Figure 2), and studied their inhibitory properties against ntMGAM.

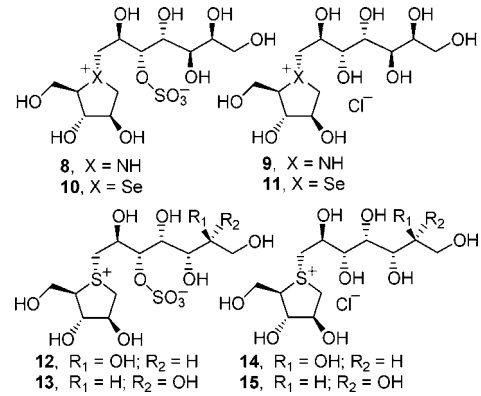

Figure 2. Heteroanalogues and stereoisomers of kotalanol and de-*O*-sulfonated kotalanol.

The required *para*-methoxybenzyl (PMB)-protected D-iminoarabinitol **16**[18] and D-selenoarabinitol **17**[19] were prepared by methods described in our earlier work. The required cyclic sulfate **18** was obtained from D-perseitol, as reported earlier.[15] The synthesis of the nitrogen analogue **8** of kotalanol was examined first. The coupling reaction of the iminoarabinitol **16** with the cyclic sulfate **18** proceeded smoothly under our optimized reaction conditions (sealed tube, acetone, K_2CO_3, 60 °C) as shown in Scheme 1.[18] The coupled product **19** was purified by short column chromatography but was deemed to be unstable, probably due to the partial removal of PMB protecting groups, as confirmed by the formation of a more polar spot on TLC. Hence, without any further characterization, the coupled product **19** was taken on to the next step, namely removal of the PMB and benzylidene protecting groups using TFA/CH_2Cl_2, as shown in Scheme 1.

Similarly, the selenium analogue **10** of kotalanol was obtained from selenoarabinitol **17** and the cyclic sulfate **18** using our optimized reaction conditions (sealed tube, HFIP, K_2CO_3, 70 °C).[18] As observed in previous work from our laboratory,[11] during the coupling reaction of D-selenoarabinitol **17** with the cyclic sulfate **18**, along with the desired coupled product **20** (40% yield), a considerable amount of the undesired diastereomer **21** (26% yield), with respect to the selenium center, was also formed (Scheme 1). The

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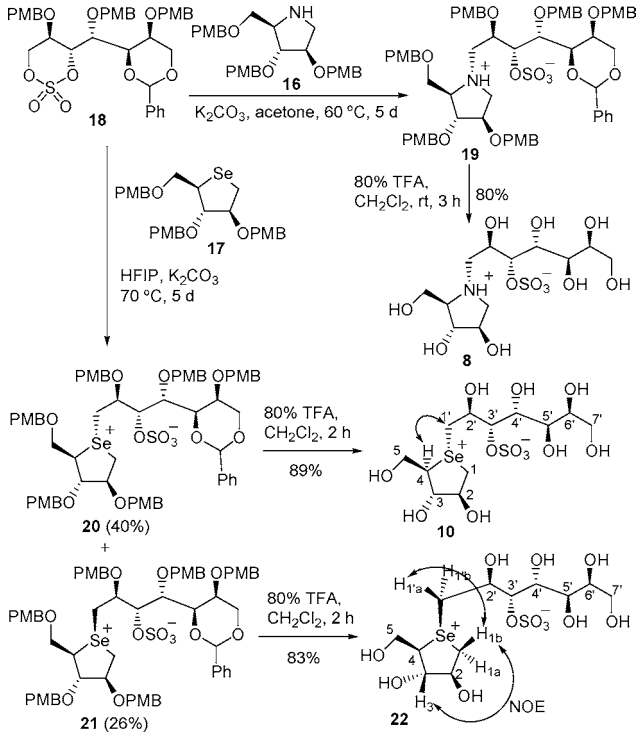
Scheme 1. Synthesis of **8** and **10**

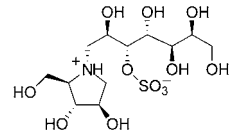
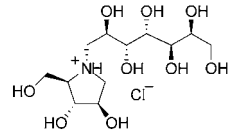
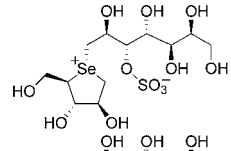
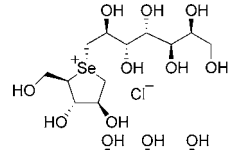
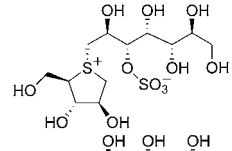
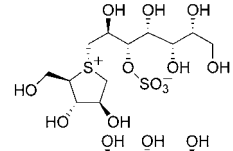
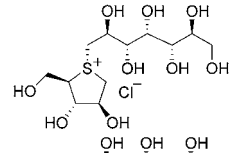
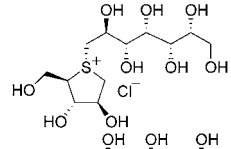
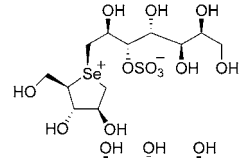
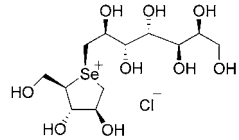
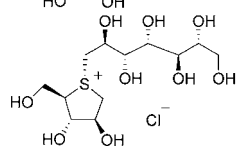


undesired diastereomer **21** was conveniently separated from the desired coupled product **20** by column chromatography. Once again, the removal of the PMB and benzylidene protecting groups was achieved in one pot using TFA/CH_2Cl_2. Thus, compounds **20** and **21** upon deprotection gave **10** and **22**, respectively, as final products.

The absolute configuration at the stereogenic selenium center in compound **10** was established by means of a 1D-NOESY experiment. A correlation between H-4 and H-1′a confirmed that they are syn-facial. In the case of compound **22**, correlation of H-1b with H-3 and also with H-1′a confirmed that they all are syn facial, thus establishing the absolute configuration at the selenium center as *S* (Scheme 1). Compound **22** differs from **10** only with respect to the configuration at the stereogenic selenium center. Hence, this compound **22** served as a probe of the importance of the *R* configuration at the positively charged ring heteroatom for inhibitory activity; all of the naturally occurring compounds **1**−**5** have the *R* configuration at the stereogenic sulfur center. In the case of the nitrogen analogue **8**, the absolute configuration at the ammonium center was assigned as *R* by analogy with our previous work,[9,18] since a NOESY experiment was not possible owing to the broad, overlapping signals at neutral pH.

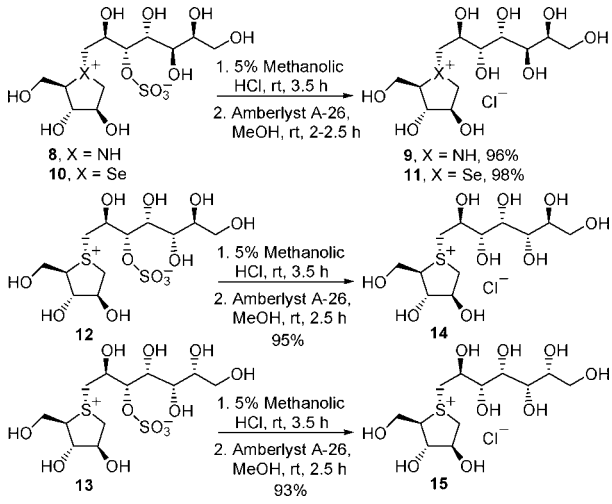
With the sulfated compounds in hand, we turned next to the synthesis of the corresponding de-*O*-sulfonated analogues. Compounds **8**, **10**, **12**,[17] and **13**[17] were converted into their corresponding de-*O*-sulfonated compounds **9**, **11**, **14**, and **15** respectively, in a two step process, first treatment with 5% methanolic HCl,[7] followed by treatment with Amberlyst-A26 (chloride resin) in MeOH, as shown in Scheme 2. Similarly, compound **22** was also converted into the corresponding de-*O*-sulfonated compound **23** (Table 1).

Table 1. Experimentally Determined K_i Values[a]

inhibitor		K_i (nM)
acarbose		62000 ± 13000[13]
kotalanol (**2**)		190 ± 30[14]
de-*O*-sulfonated kotalanol (**3**)		30 ± 10[14]
	**8**	90000 ± 6000
	**9**	61 ± 5
	**10**	80 ± 6
	**11**	20 ± 3
	**12**[17]	130 ± 20[17]
	**13**[17]	100 ± 20[17]
	**14**	24 ± 2
	**15**	26 ± 2
	**22**	7200 ± 700
	**23**	830 ± 70
	**24**[15]	17 ± 1

[a] Analysis of ntMGAM inhibition was performed using maltose as the substrate.

Scheme 2. Synthesis of De-*O*-sulfonated Compounds



The inhibitory activities of the synthesized compounds (**8**−**11**, **14**, **15**, **22**, and **23**) against the maltase activity of recombinant ntMGAM[13] are summarized in Table 1. In addition, we also report here the enzyme inhibitory activity of compound **24**[15] (Table 1), a diastereomer of de-*O*-sulfonated kotalanol, that was previously synthesized in our group. Except for the nitrogen analogue of kotalanol (**8**), all of the compounds synthesized in this study show greater inhibitory activities than acarbose, an antidiabetic agent that is currently approved for the treatment of type-2 diabetes (Table 1).[13] In general, de-*O*-sulfonation leads to an increase in inhibitory activity compared to the parent sulfated compounds. Interestingly, in the case of the nitrogen analogue of kotalanol **8**, de-*O*-sulfonation resulted in a very large increase in inhibitory activity (compare K_i values of compounds **8** and **9**, Table 1). Our results also indicate that the substitution of the ring sulfur atom by the cognate atom selenium does not confer any significant advantage (kotalanol, X = Se: K_i = 80 nM. X = S: K_i = 190 nM) and de-*O*-sulfonated kotalanol (X = Se: K_i = 20 nM. X = S: K_i = 30 nM)). Interestingly, substitution of the ring sulfur atom by nitrogen **8** is detrimental to inhibitory activity (K_i = 90 μM), whereas it does not have any significant change on the inhibitory activity of the nitrogen analogue of de-*O*-sulfonated kotalanol **9** (K_i = 61 nM).

The significant decrease in the inhibitory activity of the nitrogen analogue of kotalanol **8** relative to kotalanol **2** deserves comment. Interestingly, this trend was also observed with ghavamiol (the nitrogen analogue of salacinol) **6**[9] relative to salacinol **1**. We speculate, based on our recent crystallographic work with salacinol and kotalanol derivatives,[14] that the positioning of the sulfate anion of **8** in a hydrophobic pocket in the active site is more sterically compromised than in the sulfur congener **2**. Relief of this steric interaction by de-*O*-sulfonation to give **9** apparently relieves this interaction, and gives a compound that is just as active as its sulfur congener **3**. We note also that the *R* configuration at the stereogenic heteroatom center, as exhibited by all of the natural compounds **1**−**5** isolated so far, is essential for inhibitory activity; thus, the inhibitory activities of compounds **22** and **23**, bearing the *S* configuration at the stereogenic selenium center, are considerably less than those of their corresponding diastereomers with the *R* configuration, **10** and **11**, respectively. As predicted, the de-*O*-sulfonated compounds, **14** and **15** are found to be more active compared to the parent compounds, **12** and **13**, respectively. We note also that the compound **24**[15] is the most potent inhibitor of ntMGAM *in vitro* known to date.

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Supporting Information Available: Experimental procedures, characterization data, and ^{1}H, ^{13}C NMR spectra of compounds **8**−**11**, **14**, **15**, **22**, and **23**. This material is available free of charge via the Internet at http://pubs.acs.org.

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