



Cite this: *RSC Adv.*, 2017, 7, 28152

Aspects of extraction and biological evaluation of naturally occurring sugar-mimicking sulfonium-ion and their synthetic analogues as potent α -glucosidase inhibitors from *Salacia*: a review

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Human maltase-glucoamylase (MGAM) and sucrase-isomaltase (SI) are membrane bound small intestinal α -glucosidase enzymes which are responsible for the hydrolysis of terminal starch products and sugars into glucose. Each enzyme contains a catalytic N- and C-terminal subunit, ntMGAM, ctMGAM, ntSI, and ctSI, respectively. Inhibition of MGAM and SI is an important strategy for treatment of type-2 diabetes. In drug discovery, several plants from the *Salacia* species have been used for many years to treat diabetic conditions. Attempt to identify the source of the antidiabetic phytoconstituents in water-soluble fraction of this plant extract stimulated interest in its phytochemistry. This led to the identification of several novel class of sulfonium-ion. So far, eight sulfonium-ion glucosidase inhibitors have been isolated *i.e.* salacinol, kotalanol, ponkoranol, salaprinol and four of their corresponding de-*O*-sulfonated compounds. Due to both the intriguing structure and strong α -glucosidase inhibitory activity, much attention has been focused on salacinol and related compounds. Structural modification of sulfonium-ion represents a promising approach in the search for novel antidiabetic drugs. Several papers have described the synthetic route of sulfonium ion-based inhibitors. In this context, this review covers up to date information on sulfonium-ion from 1997 to till date and discusses the role of *Salacia* as antidiabetic drug as well as the extraction, isolation, structural elucidation, biological evaluation and structure–activity relationship studies of sulfonium-ion as potent α -glucosidase inhibitors coupled with crystallographic analysis and docking studies. We highlighted all the selective inhibitory activities of compounds against the intestinal glucosidases and also summarise the structural features which are important for its effective inhibition. The structural refinement of the sulfonium-ion generates inhibitors that selectively inhibit the activity of mucosal α -glucosidases. Further, the approach of toggling has been reviewed in aspects for controlling starch digestion and glucose release to the body and thus provides insight for designing most potent inhibitors for each subunit with low nanomolar inhibitory activities. This strategy will give a lead from the perspective of management of diabetes and associated metabolic disorders. The knowledge gained from this article will be helpful to a search for new potent inhibitors of human MGAM and SI. A summary of the inhibition data of all the compounds covered in this review have also been incorporated in tabular form as an Appendix.

Received 11th March 2017
 Accepted 26th April 2017

DOI: 10.1039/c7ra02955a

rsc.li/rsc-advances

1. Introduction

Glucosidases are considered to be an influential therapeutic target since they are the key enzymes involved in catalyzing the cleavage of glycosidic bonds in oligo- or polysaccharides to release free glucose. Glucosidases (α -glucosidase and β -glucosidase) causes hydrolysis of α and β glycosidic linkages of carbohydrates.¹ Among them, α -glucosidases increase the

postprandial blood glucose level and thus it is the major research topic of interest to pharmaceutical researchers.² Recently, glucosidase-inhibiting sugar mimics are arousing great interest in the field of chemical and medicinal research owing to their promising therapeutic potential against some diseases such as diabetes, metastatic cancer, hepatitis B and human immunodeficiency virus (HIV) infection.³ Inhibition of α -glucosidase enzymes provides a new approach in the management of diabetes as it helps to maintain normal blood glucose level by slowing down the carbohydrate digestion, extend the carbohydrate digestion time and thus reduces the rate of glucose absorption.

In humans, two major categories of enzymes namely α -amylase and α -glucosidase are responsible for the metabolism

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of dietary saccharides and starches into glucose. Maltase-glucoamylase (MGAM) and sucrase-isomaltase (SI) are the two human intestinal α -glucosidase enzymes, present in the brush

border epithelial cell of small intestine, involved in glucose formation. Catalytic C and N-terminal subunit are present in both enzymes and these are ctMGAM and ntMGAM for MGAM



Priyanka Bagri received her M. Pharmacy and Ph.D. degrees in Pharmacognosy and Phytochemistry from Jamia Hamdard University, New Delhi, India and completed her doctoral thesis on standardization, extraction and isolation of bioactive antidiabetic principles from natural drugs in 2010. In 2014 she joined bioactive natural product laboratory under the supervision of Dr Sayeed Ahmad as a women

scientist for postdoctoral researcher to work on the metabolomics and bioactivity guided isolation of antidiabetics phytoconstituents from herbal drugs. Her research interests concentrate on the phytochemical, metabolomics and chromatographic analysis of medicinal plants for quality control. She is also working on Chemoprofiling of oils using GC-MS as well as for in vitro/in vivo evaluation of plant drugs for antidiabetic, anti-inflammatory, antimicrobial and hepatoprotective activities.



Karishma Chester obtained her M. Pharmacy degree in Pharmaceutical Biotechnology from Jamia Hamdard University in 2011 which was followed as SRF in CCRUM project showing HIV potential of some herbal plants. She joined the Ph.D. program in 2013 in Pharmaceutical Sciences under the supervision of Prof. Sarvesh Paliwal, from Banasthali University, Rajasthan. She received P.D. Sethi

award for best paper. Her research has focused on molecular analysis mainly DNA isolation, RAPD, phytochemical, metabolomics and chromatographic analysis of herbal drugs. She is currently working on druggability of metabolite enriched fractions for in vitro/in vivo screening of hepatoprotective and antidiabetic drugs.



Mr Washim Khan received M. Pharm from Jamia Hamdard, and currently, he is doing Ph.D. under the Supervision of Dr Sayeed Ahmad, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi. He has been honoured with PD Sethi award in year 2016 for best publications in HPTLC, Best Poster award from Phytochem-

istry/Phytochemistry letters, awarded with Merit Scholarship from All India Council for Technical Education (AICTE) for the year 2010–2012 and from West Bengal Government for the year 2007–2010, qualified Graduate Pharmacy Aptitude Test (GPAT), Graduate Record Examinations (GRE). He has to his credit 23 research publications, participated in more than 30 conferences, presented 19 posters in India and Abroad. He was associated with the development of special issue of 'Journal of Pharmaceutical and Bioallied Sciences', published in October 2015 and of 'Drug Development and Therapeutics' published in January 2016 on Herbal Drugs and Botanicals edited by Dr Sayeed Ahmad. His field of interests in research is drug discovery from natural sources, targeted and untargeted metabolomics through HPTLC, HPLC, GC-MS, and LC-MS and with modern statistical tools. He is also working in the biological screening of Indian traditional formulation to support their claims.



Dr Sayeed Ahmad, a known researcher in the field of herbal drugs and natural products, received his Ph.D. degree in 2005 from Department of Pharmacognosy and Phytochemistry, Jamia Hamdard University, New Delhi, India. Since 2005 he has been working as a faculty member at the same university. He did post-doctoral research at Albert Einstein College of Medicine New York, USA (2011) and

was a Visiting Scientist in Sultan Qaboos University, Muscat, Oman (2012) His research interests span the study of quality control, chromatography and metabolomics analysis. Dr Ahmad has been associated with more than 26 sponsored research projects from different funding agencies in addition to some industry-based consultancy projects. He is a recipient of University Gold Medal (2002), DST Young Scientist Award (2007), CST-UP Young Scientist Award (2008–09), DST BOYSCAST (2010), AICTE Career Award (2010), UGC Research Award (2016) and Society for Ethnopharmacology Young Ethnopharmacologist award (2017) in addition to some Best Paper Awards such as P. D. Sethi Award (2013 and 2015), Al-Ameen College of Pharmacy Award (2014 and 2015) as well as Prof M. L. Khurana Memorial Award (2015).



Table 1 Key studies on antidiabetic research on *Salacia* sp.

Plant sp.	Biological activity	Extract type/compound used	Model system	References
<i>S. chinensis</i>	Antidiabetic activity	Aqueous extract of stem	KK-A ^y mice model (10–300 mg kg ⁻¹)	52
<i>S. chinensis</i>	α -Glucosidase inhibitory activity	Whole plant extract	<i>In vitro</i> enzyme inhibition assay (100 μ g mL ⁻¹)	53
<i>S. oblonga</i>	Antidiabetic activity	Polyherbal combo with <i>S. oblonga</i> (G-400)	Human (1000 mg day ⁻¹ for 8 weeks)	54
<i>S. chinensis</i>	Antidiabetic activity	Mangiferin from aqueous solution of root	Male adult Wistar rats (40 mg kg ⁻¹ body wt per day)	55
<i>S. chinensis</i>	Antidiabetic activity	Mangiferin from aqueous solution of root	Wistar male albino rats (40 mg kg ⁻¹)	56
<i>S. reticulata</i>	Antidiabetic activity	Aqueous extract from leaves and root bark	Human (500 mg day ⁻¹)	57
<i>S. oblonga</i>	Antidiabetic activity	Mehani (polyherbal formulation)	Wistar strains of male albino rats (2 g/60 kg)	58
<i>S. oblonga</i>	Antidiabetic activity	Hydroalcoholic root extract	Streptozotocin induced diabetic Wistar rat (50 and 100 mg kg ⁻¹)	59
<i>S. oblonga</i>	Antidiabetic activity	Lipo polysaccharide tea	KK-A ^y /Tajcl type-II diabetic mice model (200 and 300 mg)	60
<i>S. reticulata</i>	Antidiabetic and α -glucosidase inhibitory activity	Aqueous extracts of bark or core root	<i>In vitro</i> assay Wistar male alloxan (50 mg kg ⁻¹)	61
<i>S. oblonga</i>	Antidiabetic activity	Aqueous extract	L6 rat-myotubes model	62
<i>S. reticulata</i>	Antidiabetic activity	Aqueous extract	KK-A ^y genetically diabetic mice	63
<i>S. fruticosa</i>	Antihyperglycemic activity	Methanolic extract	Wistar albino adult male rats (125 mg kg ⁻¹ and 250 mg kg ⁻¹)	64
<i>S. reticulata</i>	Antidiabetic activity	Aqueous extract	Maltose or sucrose loaded mice (1.0 mg)	65
<i>S. reticulata</i>	Antidiabetic activity	Aqueous extract and salacinol	Maltose or sucrose loaded Wistar rats (50 and 75 mg kg ⁻¹ ; 2.50 and 5.00 mg kg ⁻¹)	66
<i>S. reticulata</i>	α -Glucosidase inhibitory activity	Water soluble fraction	Sucrose or malt sugar loaded rats (25–100 mg kg ⁻¹)	38
<i>S. oblonga</i>	Antidiabetic activity	Aqueous extract	Human (240 and 480 mg)	67
<i>S. reticulata</i>	Antihyperglycemic activity	Hydroalcoholic extract	Hydrocortisone induced hypoglycemic rat model (500 mg kg ⁻¹)	68
<i>S. reticulata</i>	Antidiabetic activity	Tea	Human (200 mg)	69
<i>S. reticulata</i>	Antidiabetic activity	Bark powder	Human (2 g per day for 90 days)	70
<i>S. oblonga</i>	Antidiabetic activity	Aqueous extract	Obese Zucker rats (100 mg kg ⁻¹)	71
<i>S. oblonga</i>	Antidiabetic activity	Aqueous extract	Obese Zucker rats (100 mg kg ⁻¹)	72
<i>S. chinensis</i>	α -Glucosidase inhibitory activity	Methanolic extract	Sucrose or malt sugar loaded rats (50, 100, 200 mg kg ⁻¹)	73
<i>S. reticulata</i>	α -Glucosidase inhibitory activity	Water soluble fraction	Sucrose or malt sugar loaded rats (25–100 mg kg ⁻¹)	74
<i>S. oblonga</i>	Antidiabetic and antioxidant activity	Petroleum ether extract of the root bark	Streptozotocin induced diabetic rats (250 mg kg ⁻¹)	45
<i>S. oblonga</i>	Antidiabetic activity	Methanolic extract	Sucrose and maltose loaded male Wistar rats (0, 100, 200 mg kg ⁻¹)	39
<i>S. oblonga</i>	Antidiabetic study	Petroleum ether extract of the root bark	Streptozotocin induced diabetic albino rats (250 mg kg ⁻¹ , orally)	40
<i>S. macrosperma</i>	Antidiabetic activity	Root extract	Rabbits and alloxan induced albino rats (200 mg kg ⁻¹)	51
<i>S. reticulata</i>	Antidiabetic activity	Aqueous extract	Sprague-Dawley rats	41
<i>S. prinoidea</i>	Antidiabetic activity	Root bark extract	Alloxan induced diabetic rats	50



of sulfonium family has been done in this paper. In this account, we summarize the role of *Salacia* as antidiabetic drug as well as the aspects of extraction, structural elucidation and biological evaluation of naturally occurring sulfonium-ion and their synthetic analogues with particular focusing on their selective inhibitory activities against the intestinal glucosidases along with crystal structure, docking and the toggling studies. The insight gathered in this review provides a wealth of information about sulfonium-ion α -glucosidase inhibitors. This knowledge will be useful in deriving more potent antidiabetic candidates from the bioactive natural molecule.

2. *Salacia*: a promising class of potential antidiabetic drug

Literature survey revealed that, *Salacia* sp. plays an important role in the management of diabetes. The genus *Salacia*, a member of Celastraceae family, are woody climbing plants which consist of 407 species and have almost 108 Generas.^{27,28} *Salacia* sp. are widely spread in India, China, Sri Lanka and Southeast Asian countries such as Thailand, Indonesia, and also in South American torrid zone areas such as Brazil.^{21,29}

Salacia has been officially listed in the Ayurvedic Pharmacopeia, its aerial parts and roots are extensively used in Ayurvedic system of medicine, traditional Indian medicine, and Unani as a precise remedy for early-stage diabetes and for treating asthma, ear diseases, gonorrhoea, rheumatism, itching, leukemia and inflammations.²⁹ Presently, *Salacia* sp. are extensively consumed in Japan and US and different countries as a food supplement formulation for the treatment of diabetes and have also been studied widely for their potential value in diabetes management.^{30,31} Ayurvedic medicine prescribes the herbal extract, obtained by storing water overnight in a mug made from the root of *S. reticulata* and other members of the *Salacia* genus, such as *S. oblonga*, *S. prinoidea*, and *S. chinensis*, as a remedy for type-2 diabetes. Earlier people with diabetes use to drink water from the mugs made from *Salacia* wood.^{24,32} Since 1990, *Salacia* sp. have been used in traditional system of medicine as effective drug targets for the management of diabetes. Inspired by this traditional and medicinal use, several types of research have been carried out by researchers to find out the antidiabetic phytoconstituents responsible for the activity. Earlier studies on this plant have resulted in the isolation of several potent sulfonium inner salts, salacinol,^{33,34} kotalanol,^{35–37} and ponkoranol,³⁸ responsible for the antidiabetic activity. These sulfonium phytoconstituents act as α -glucosidases inhibitor by competitively inhibiting α -glucosidase activity and thus helps to prevent the fast breakdown of oligosaccharides into monosaccharides and thereby control the normal blood sugar level (Fig. 2).³⁹

Literature information strongly validated the approach that the glucosidase inhibitory activities of the aforesaid sulfonium compounds are very potent and have been revealed to be as strong as those of acarbose (1) and voglibose (3).^{33,35,38} A number of reports on animal and human have been published that reinforced the reported reductions in glycemia as well.^{40–42} Various studies declare the role of *Salacia* sp. as an effective antidiabetic

drug.^{43,44} Table 1 illustrate the potential of *S. oblonga*,^{40,45–47} *S. reticulata*,^{41,48,49} *S. prinoidea*,⁵⁰ *S. macrosperma*⁵¹ and *S. chinensis*⁵² in diabetes therapy. Based on this evidence, it is apparent that *Salacia* sp., as a rich source for sulfonium ions, can be used as important nutraceuticals or as adjuvants for the management of diabetes.

3. Extraction, isolation and structural elucidation of sulfonium-ion from *Salacia* sp.

Salacinol, the first member of the naturally occurring sulfonium-ion class of glucosidase inhibitors, was originally isolated as one of the active principal constituents from the aqueous extracts of *S. reticulata* through bioassay guided separation using α -glucosidase inhibitory activities and afterward from other plant species of the *Salacia* genus, *S. oblonga*³⁹ and *S. chinensis*.⁷³ In the course of the search for antidiabetic phytoconstituents from *Salacia*, Yoshikawa *et al.* in 1997 discovered that a water-soluble fraction (25–100 mg kg⁻¹) prepared from the roots and stems of *S. reticulata* strongly inhibited elevations in serum glucose levels in rats after oral administration of sucrose or maltose, but not glucose. To authenticate this activity, they conducted a bioassay guided separation assay, in which they isolated a novel five-membered sugar analogue, salacinol having an internal sulfate counterion.^{33,75}

Fig. 3 summarizes the extraction and fractionation scheme of *Salacia* sp. Under reflux condition, the dried root and stems of *Salacia* sp. were extracted using methanol. The prepared methanolic extract was mixed with water and fractionated by ethyl acetate. The enzyme inhibitory effect and amelioration of serum glucose level in sucrose-loaded rats were found in water soluble fraction whereas the ethyl acetate fraction did not show any affect. Further, a bioactivity guided fractionation was carried out for the isolation of the α -glucosidase inhibitor from the water soluble fraction. This water soluble fraction was suspended in methanol and then filtered to give a methanol-soluble phase and a residue. The methanol-soluble phase was subjected to ordinary-phase silica gel

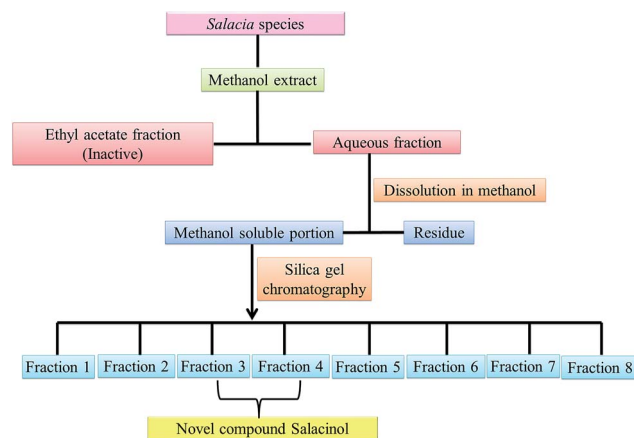


Fig. 3 Schematic representation of extraction of salacinol from *Salacia* species.



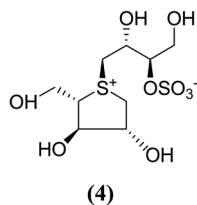


Fig. 4 Previously proposed structure of salacinol.

Table 2 α -Glucosidase inhibitory activity of salacinol for rat intestinal disaccharidases³³

Inhibitor	K_i ($\mu\text{g mL}^{-1}$)		
	Maltase	Sucrase	Isomaltase
Acarbose (1)	0.12	0.37	75
Salacinol (5)	0.31	0.32	0.47

chromatography to give eight fractions. The active fractions were subjected to ODS and NH column chromatography and finally HPLC to furnish salacinol. However, some modifications in extraction method can be possible for the isolation of salacinol.

The stereostructure of salacinol have been elucidated on the basis of various chemical reactions and spectroscopic analyses including 1D and 2D NMR experiments and a potassium rhodizionate test (a positive test that confirmed the presence of a sulfate moiety) and suggested the presence of an unusual zwitterionic sulfonium-sulfate structure. Based on the spectral and X-ray crystallographic analyses, Yoshikawa *et al.*³³ established the initial stereostructure of salacinol as an inner-salt sulfonium structure (4) that was composed of a 1,4-anhydro-4-thio-L-arabinitol unit alkylated at sulfur by a 1-deoxy-D-erythritol-3-sulfate unit (Fig. 4).³³ The isolated salacinol showed competitive inhibition as equivalent to acarbose against maltase and sucrase in *in vitro*. However, the inhibitory activity of salacinol against isomaltase was found to be greater than that of acarbose (Table 2).

Another potent sulfonium ion α -glucosidase inhibitor, kotalanol (1,2,3-trihydroxy-propyl-salacinol) was isolated from the same plant in 1998 by the same group by performing

another bioassay-guided separation study. They reported that this inhibitor possesses stronger inhibitory activity against certain glucosidase enzymes. Soon after, from *Salacia* genus, ponkoranol, salaprinol and de-O-sulfonated derivatives of all four parent compounds were also isolated.³⁵

4. Structural features and structure–activity relationship studies of naturally occurring sulfonium-ion and their synthetic analogues as potent α -glucosidase inhibitors

Several drug discovery research based on these natural sulfonium-ion glucosidase inhibitor compounds have been carried out. Thus far, researchers have isolated eight sulfonium-ion glucosidase inhibitors (Fig. 5) from *Salacia* sp.: salacinol (5), kotalanol (6), ponkoranol (7), salaprinol (8) and four of their corresponding de-O-sulfonated compounds such as de-O-sulfonated salacinol; neosalacinol (9), de-O-sulfonated kotalanol; neokotalanol (10), de-O-sulfonated ponkoranol; neoponkoranol (11) and de-O-sulfonated salaprinol; neosalaprinol (12).²² The salacinol structure was assigned to be the enantiomer (5) of the previous stereostructure (4) as the plant origin and biosynthetic pathways of salacinol (5) and kotalanol (6) are same.^{34,39} Yoshikawa *et al.*³⁵ revise the initial structure of salacinol (4) by performing degradation studies of kotalanol (6).

At the time of isolation, the absolute stereostructure for sulfonium compounds, except salacinol (5), was not resolved but synthetic work has shown the way to their stereochemical structure elucidation. In the case of parent compounds, salacinol (5), kotalanol (6), ponkoranol (7), and salaprinol (8), C-3' carry a sulfate group which provides a unique sulfonium-sulfate inner salt structure. This particular characteristic of sulfonium ion inhibitor, having permanent positive charge carried by the sulfur, is supposed to bind in the active site of glucosidase in the same manner as a protonated amine inhibitor.^{76–78} The sulfonium compounds vary only in the length of the polyhydroxylated side chain. For example, salacinol (5) has a side

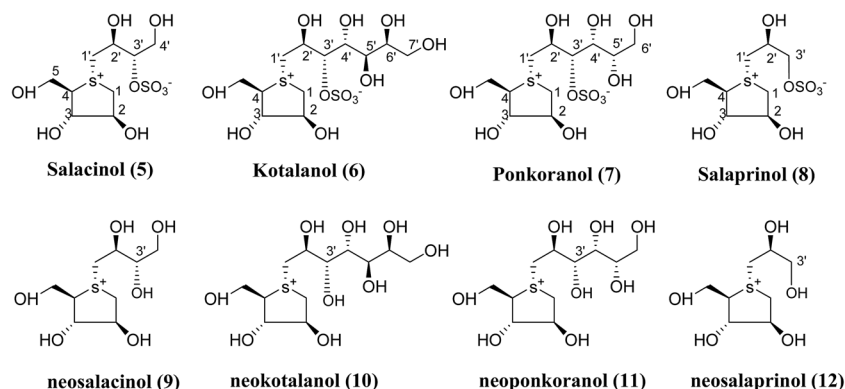


Fig. 5 Major sulfonium constituents (5–12) of *Salacia* species.



Table 3 α -Glucosidase inhibitory activities of sulfonium compounds (5–12) against rat intestinal disaccharidases^{38,80}

Inhibitor	IC ₅₀ (μ M)		
	Maltase	Sucrase	Isomaltase
Acarbose (1)	2.0	1.7	155
Voglibose (3)	1.2	0.2	2.1
Salacinol (5)	5.2	1.6	1.3
Kotalanol (6)	7.2	0.75	5.7
Ponkoranol (7)	3.2	0.29	2.6
Salaprinol (8)	>329 (42) ^a	>329 (23) ^a	15
Neosalacinol (9)	8.0	1.3	0.3
Neokotalanol (10)	4.8	4.5	1.8
Neoponkoranol (11)	5.1	1.0	1.4
Neosalaprinol (12)	>384 (34.5) ^a	90	6.5

^a Values in parentheses indicate inhibition (%) at the corresponding concentrations (μ M).

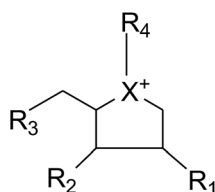
chain of four-carbon with defined stereochemistry, whereas, kotalanol (6) contains a seven-carbon side chain, with unspecified stereochemistry at the stereogenic centers and salaprinol (8) has a three-carbon side chain.

It is remarkable that the configurations up to the C-3' position in the side chain and configurations around the heterocyclitol moiety are preserved in all of these sulfonium compounds. Altering the configurations at any of these positions, including that at the stereogenic sulfur atom, give rise to complete or partial loss of inhibitory activities against the human intestinal α -glucosidase enzymes.²² Usually, the de-*O*-sulfonated analogues are more active than the corresponding original sulfates against the intestinal α -glucosidases. Various studies revealed that, on a comparison of the inhibitory activities of de-*O*-sulfonated salacinol (9) vs. salacinol (5) and de-*O*-sulfonated kotalanol (10) vs. kotalanol (6) against rat intestinal glucosidase (maltase, sucrase, and isomaltase), the desulfonated analogues were either equivalent or better inhibitors than the parent compounds.^{21,38,79} Data of inhibitory activity of these sulfonium compounds (5–12),^{38,80} tested against rat small intestinal α -glucosidase *in vitro* and compared with clinically used antidiabetics, acarbose (1) and voglibose (3), are given in Table 3.

Thus, because of the intriguing structure and high α -glucosidase inhibitory activities, great interest has been focused on sulfonium compounds and comprehensive studies on the structure–activity relationships of this new class of α -glucosidase inhibitors family has been reported.

4.1. Sulfonium-ion analogues as α -glucosidase inhibitors

Apart from extraction from natural sources, a non-naturally sulfonium-ion could be produced synthetically from readily



accessible starting materials. The compounds belong to this group are represented by the general formula: where X is selected from the group consisting of S, Se and NH. R₁, R₂ and R₃ are the same or different and are consist of H, OH, SH, NH₂ and halogens. Whereas, R₄ is chosen from the group consisting of:

(1) a polyhydroxylated acyclic alkyl chain comprising an anionic sulfate, carboxylate or phosphate moiety.

(2) A lipophilic alkyl chain between 2 and 20 carbons in length with an external counterion.

These derivatives display interesting selectivity for glucosidase enzymes from various sources and act as active glucosidase inhibitors.

4.1.1. Salacinol and its synthetic analogues. Broad research on the synthesis of salacinol (5) and higher homologues have been carried out. The extensive structure–activity relationship studies directed towards salacinol (5) and related compounds have been reported.³⁵ Through these studies, several inhibitors with measurably better activity have been developed. Initially, Minami *et al.*⁸¹ reported the isolation of a thiosugar sulfonium-alkoxide inner salt, neosalacinol from *S. reticulata*. However, Tanabe *et al.*⁸² have shown that this compound is de-*O*-sulfonated salacinol.

In a search for potential glucosidase inhibitors, various synthetic analogues of salacinol have been synthesized by different researchers for structure–activity studies with different glucosidase enzymes. Miglitol (2) and salacinol analogues selectively target intestinal glucosidases and is a poor inhibitor of α -amylases.⁸³ In contrast, α -amylase is inhibited more strongly by acarbose (1) ($K_i = 15$ nM).⁸⁴ Salacinol (5, Table 4) and some of its derivatives revealed an interesting selectivity for tested glucosidase enzymes from various sources like glucoamylase G2, porcine pancreatic α -amylase (PPA), barley α -amylase (AMY1), human pancreatic α -amylase (HPA), recombinant human maltase glucoamylase (MGAM), glucosidase from rice and baker's yeast and compared with salacinol (5).²²

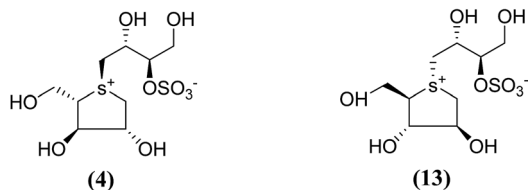
Total synthesis of salacinol (5) was accompanied by two groups.^{85–87} Yuasa *et al.*⁸⁵ and Ghavami *et al.*⁸⁶ independently reported the synthesis of salacinol (5) and its enantiomer (4) and diastereomers (13) (Fig. 6) and provided conclusive proof of structure of the natural product. Yuasa *et al.*⁸⁸ have also investigated the glucosidase inhibitory activities of compounds (5) and (13) and showed that although salacinol (5) is better inhibitor (IC₅₀ = 1.1 μ M) of rice α -mannosidase than its

Table 4 Inhibition of α -glucosidase enzymes from different sources by salacinol (5)²²

α -Glucosidase	Salacinol (5)
Glucoamylase G2 K_i (mM)	1.71
PPA K_i (mM)	0.01
AMY1 K_i (mM)	0.015
MGAM K_i (μ M)	0.19
HPA K_i (mM)	0.075
Rice IC ₅₀ (mM)	1.1×10^{-3}
Baker's yeast IC ₅₀ (mM)	NOI

NOI = no inhibition.





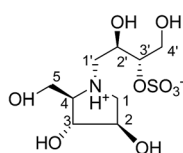
$K_i = 2.17\text{mM}$ for Glucoamylase G2
 $K_i > 5\text{mM}$ for PPA
 $K_i > 5\text{mM}$ for AMY1
 $K_i = \text{NOI}$ for MGAM and HPA

$K_i = 1.06\text{mM}$ for Glucoamylase G2
 $K_i > 5\text{mM}$ for PPA
 $K_i > 5\text{mM}$ for AMY1
 $K_i = \text{NOI}$ for MGAM and HPA
 $\text{IC}_{50} = 0.38\text{mM}$ for Rice
 $\text{IC}_{50} = \text{NOI}$ for Baker's yeast

Fig. 6 Stereoisomers of salacinol (4 and 13) and their inhibitory activity against α -glucosidases.

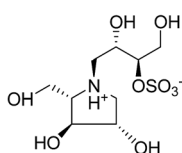
diastereomer (13, $\text{IC}_{50} = 0.38\text{mM}$), the inhibitory activities are comparable for almond α -glucosidase ($\text{IC}_{50} = 2.1\text{mM}$ for 5 and 3.6mM for 13). In the case of almond β -glucosidase, (13) ($\text{IC}_{50} = 3.4\text{mM}$) is a better inhibitor than (5) which showed no activity.

In order to develop more effective glucosidase inhibitors, the azacyclic version of salacinol was synthesized in which the thio-sugar sulfur was replaced by nitrogen to give compounds, ghavamiol (14) and (15) (Fig. 7), as potential glucosidase inhibitors.⁸⁹ Inhibition assays using intestinal α -glucosidase indicated that ghavamiol (14) was less potent against maltase, sucrase and isomaltase, compared to salacinol (5). Salacinol (5) inhibits maltase, sucrase and isomaltase with IC_{50} values of $9.6\text{ }\mu\text{M}$, $2.5\text{ }\mu\text{M}$ and $1.8\text{ }\mu\text{M}$, respectively, whereas compound (14) inhibits these enzymes with IC_{50} values of $306\text{ }\mu\text{M}$, $44\text{ }\mu\text{M}$ and $136\text{ }\mu\text{M}$, respectively. Thus, the inhibitory activities of ghavamiol (14) decreased considerably against these enzymes when compared to salacinol (5).⁹⁰ On



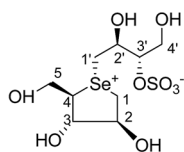
Ghavamiol (14)

$\text{IC}_{50} = 306\text{ }\mu\text{M}$ for Maltase
 $\text{IC}_{50} = 44\text{ }\mu\text{M}$ for Sucrase
 $\text{IC}_{50} = 136\text{ }\mu\text{M}$ for Isomaltase
 $\text{IC}_{50} > 315\text{ }\mu\text{M}$ for Trehalase
 $K_i > 2.5\text{mM}$ for Glucoamylase G2
 $K_i > 5\text{mM}$ for PPA
 $K_i > 5\text{mM}$ for AMY1
 $K_i = \text{NOI}$ for MGAM and HPA



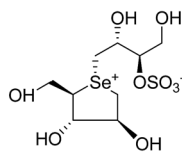
(15)

$K_i > 8\text{mM}$ for Glucoamylase G2
 $K_i > 5\text{mM}$ for PPA
 $K_i > 5\text{mM}$ for AMY1
 $K_i = \text{NOI}$ for MGAM
 $K_i = 0.4\text{mM}$ for HPA



Blintol (16)

$K_i = 0.72\text{mM}$ for Glucoamylase G2
 $K_i > 5\text{mM}$ for PPA
 $K_i > 5\text{mM}$ for AMY1
 $K_i = 0.49\text{mM}$ for MGA M
 $K_i > 5\text{mM}$ for HPA



(17)

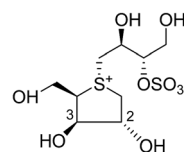
$K_i > 9\text{mM}$ for Glucoamylase G2
 $K_i > 5\text{mM}$ for PPA
 $K_i > 5\text{mM}$ for AMY1
 $K_i = \text{NOI}$ for MGAM
 $K_i = \text{NOI}$ for HPA

Fig. 7 Nitrogen (14 and 15) and selenium (16 and 17) heteroatom analogues of salacinol and their inhibition potential against α -glucosidase enzymes.

comparison of inhibitory activity of ghavamiol (14) and (15) against three different glucosidase enzymes, it was noticed that despite the low activity of the natural sulfonium ion, salacinol (5), towards glucoamylase ($K_i = 1.7\text{mM}$), nitrogen analogues (14) and (15) exhibited a 10-fold higher inhibition value than salacinol (5). Furthermore, the compounds (14) and (15) showed not active against AMY1 and PPA at concentrations of 5mM . In contrast, salacinol (5) showed stronger inhibition against AMY1 and PPA in the micromolar range, with K_i values of $15 \pm 1\text{ }\mu\text{M}$ and $10 \pm 2\text{ }\mu\text{M}$, respectively. Screening of ammonium compounds against HPA showed that ghavamiol (14) was not active against HPA whereas the compound (15) inhibited the enzyme with K_i value of 0.4mM compared to salacinol that showed stronger inhibition with a K_i value of $75\text{ }\mu\text{M}$.

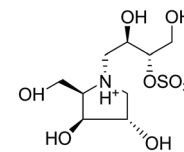
In the same manner, substitution of the sulfonium ion of salacinol (5) by selenium produced a derivative, blintol (16) (selenosalacinol) and its stereoisomers (17) (Fig. 7). After enzyme inhibition studies on blintol (16), it was found that blintol (16) was a weak inhibitor of glucoamylase G2 ($K_i = 0.72\text{mM}$) and showed no significant inhibition against AMY1, HPA, and PPA. The compound (17) showed no significant inhibition of glucoamylase, AMY1, HPA, and PPA. The blintol (16) was shown to be an effective treatment for regulating blood glucose levels in rats after a carbohydrate meal, thus act as a lead candidate for the treatment of type-2 diabetes.^{89,91-93}

The search for potent glucosidase inhibitors has led to the preparation of several salacinol analogues with varying stereochemistry at one or more stereogenic centers by modifying substituents and ring size or by replacing the sulfur in the sulfonium-ion by nitrogen or selenium. Ghavami *et al.*⁹⁴ explored five membered ring analogues of salacinol (5) with different sugar stereochemistry, an anhydro-thio-D-xylitol (18), its nitrogen congener (19) were synthesized (Fig. 8) in which stereochemistries at C-2 and C-3 were both inverted compared to salacinol (5). Compound (18) and (19) were tested for their inhibition of three glycosidase enzymes, namely glucoamylase G2, PPA and AMY1. Glucoamylase G2 was weakly inhibited by salacinol (5) ($K_i = 1.7\text{mM}$) whereas compound (18) and (19) showed no significant inhibition of glucoamylase. The sulfonium ion (18) inhibited AMY1 and PPA, with K_i values of 109 ± 11 and $55 \pm 5\text{ }\mu\text{M}$, respectively, as compared to salacinol (5), with K_i values of 15 ± 1 and $10 \pm 2\text{ }\mu\text{M}$, respectively. The ammonium ion analogue (19) showed no significant inhibition of either AMY1 or PPA. Thus,



(18)

$K_i > 5\text{mM}$ for Glucoamylase G2
 $K_i = 0.052\text{mM}$ for PPA
 $K_i = 0.109\text{mM}$ for AMY1



(19)

$K_i > 30\text{mM}$ for Glucoamylase G2
 $K_i > 5\text{mM}$ for PPA
 $K_i > 5\text{mM}$ for AMY1

Fig. 8 Salacinol analogues containing anhydrothio-D-xylitol (18) moiety, its nitrogen congener (19) and their inhibitory activity against glucosidase enzymes.





Fig. 9 Salacinol analogues containing anhydrothio-D-lyxitol (20) and anhydrothio-D-ribitol (21) moiety and their inhibitory activity against MGAM.

analogues (18) and (19) and salacinol (5) showed discrimination or selectivity for certain glucosidase enzyme.

Other analogues of salacinol, anhydrothio-D-lyxitol (20), and anhydrothio-D-ribitol (21) have also been reported (Fig. 9) in which the stereochemistries at C-3 and C-2, respectively, are inverted compared to salacinol (5).⁹⁵ Enzyme inhibition assay of (20) and (21) against MGAM indicated that they were not effective inhibitors against MGAM. While salacinol inhibited this enzyme with a K_i value of 0.19 μM .⁹⁶ Therefore, an important conclusion derived from the enzyme inhibition data that the D-arabinitol configuration in the heterocyclic ring as in salacinol is critical for its inhibitory activity.

To discuss the importance of the heterocyclic ring size on glucosidase inhibitory activity, several six membered ring analogues (22–26, Fig. 10) of salacinol have been reported. Szczepina *et al.*⁹⁷ synthesized a series of pyranosil compounds (22a–26a) and their corresponding isomers (22b–26b), having a cyclic alditol structure, in which an L-erythritol sulfated side chain was attached to the ring nitrogen, sulphur, or selenium atom. The objective was to investigate if changes to this group, acting as a counterion to ammonium or selenium salt analogues, might increase the *in vivo* stability or biomembrane permeability. The enzyme inhibition data indicated that these

salacinol analogues with six-membered rings against glucoamylase G2 were either weak or absent, indicating the importance of the five-membered ring incorporated in salacinol (5).⁹⁷ Hence, it could be inferred that the five-membered ring of salacinol (5) is necessary and key structural determinant of sulfonium sulfate glucosidase inhibitors and their analogues for this particular enzyme.^{86,94}

In order to provide additional information about the structure requirements for anti-glucosidase activity, Gallienne *et al.*⁹⁸ reported two-series of five (27–32, Fig. 11) and six-membered ring (33, 34, Fig. 12) analogues having thioanhydro alditol moieties with *erythro*, *D,L-threo*, *xylo*, *ribo*, *D-arabino*, and *D-manno* configurations (27–36), including previously reported



Fig. 11 Five membered ring analogues (27–32) of salacinol synthesized by Gallienne *et al.*⁹⁸ and their inhibitory profile against α -glucosidase enzymes.



(22a)	X=NH R=H	$K_i = \text{NOI}$ for glucoamylase G2	(22b)	X=NH R=H	$K_i = \text{NOI}$ for glucoamylase G2
(23a)	X=NH R=CH ₂ OH	$K_i = \text{NOI}$ for glucoamylase G2 and MGAM	(23b)	X=NH R=CH ₂ OH	$K_i = \text{NOI}$ for glucoamylase G2 and MGAM
(24a)	X=S R=H	$K_i = \text{NOI}$ for glucoamylase G2	(24b)	X=S R=H	$K_i = \text{NOI}$ for glucoamylase G2 and MGAM $K_i > 5\text{mM}$ for HPA $\text{IC}_{50} = 1.41\text{mM}$ for rice $\text{IC}_{50} = \text{NOI}$ for baker's yeast
(25a)	X=S R=CH ₂ OH	$K_i = \text{NOI}$ for glucoamylase G2	(25b)	X=S R=CH ₂ OH	–
(26a)	X=Se R=H	$K_i = \text{NOI}$ for glucoamylase G2	(26b)	X=Se R=H	$K_i = \text{NOI}$ for glucoamylase G2 and MGAM

Fig. 10 Structure of salacinol analogues having six-membered ring (22–26) and their inhibitory activity against glucosidase enzymes.



Several chain-modified analogues of salacinol have also been synthesized for evaluation as inhibitors of glucosidase enzymes. These modifications incorporate the extension of the acyclic chain, the change in the position or complete removal of the sulfate group, and the introduction of acyclic chains that are missing one or two hydroxyl groups when compared to salacinol (5). Some of these compounds displayed inhibitory activities in a low micromolar range against MGAM. The stereochemistries at the different stereogenic centres on the side chain play significant roles, and structure–activity studies revealed an interesting variation in the inhibitory power of these compounds (Table 5). Based on the information, kotalanol (6), with a longer polyhydroxylated side chain, has been reported to acquire greater inhibitory action against certain glucosidase enzymes when compared to salacinol (5).³⁵ So, a lot of interest has been directed toward the synthesis of acyclic chain modified analogues of salacinol.

To test this assumption and to afford new candidates for the treatment of type 2 diabetes, chain-extended homologues of salacinol (41–44, Fig. 14) containing polyhydroxylated, acyclic chains of 5- and 6-carbons differing in stereochemistry at the stereogenic centers were synthesized.^{96,100} The enzyme inhibition studies showed that the compound (41) did not inhibit the MGAM activity, while the other compounds (42–44) inhibited MGAM and showed K_i values of 0.25 ± 0.02 , 0.26 ± 0.02 , and $0.17 \pm 0.02 \mu\text{M}$, respectively. By comparison of the stereochemistry of the stereogenic centers in the extended acyclic chain and the observed inhibitory properties of the four compounds, it is clear that the *R* stereochemistry of the C-4' stereogenic center is important for activity.

A series of six chain extended analogues (45–50, Fig. 15) with heteroatom variations having polyhydroxylated, extended acyclic chains of 6-carbons, was also reported.¹⁰¹ The α -glucosidase inhibition data against MGAM revealed that the sulfur analogue (46) with the *D*-arabinitol configuration in the heterocyclic ring did not inhibit MGAM, while the corresponding selenium (45) and nitrogen (47) analogues inhibited MGAM with K_i values of 41.0 ± 7.0 and $26 \pm 0.02 \mu\text{M}$, respectively. It is pertinent to mention that the sulfur and nitrogen congeners with the *L*-arabinitol configuration in the heterocyclic ring, (49) and (50), were also active, with K_i values of 25 and 5 μM , respectively.

Another acyclic, chain-modified, sulfur (51) and selenium (52) analogues (Fig. 16), with an extended polyhydroxylated acyclic chain of 6-carbons, have also been reported.¹⁰² Enzyme inhibition observed from these compounds showed that the sulfonium ion (51) with the enantiomeric configuration at C-5' to (44) has a K_i

value of $0.65 \pm 0.10 \mu\text{M}$. In contrast, the corresponding selenonium ion (52) with the enantiomeric configuration at C-5' to (54) is just as active, with a K_i value of $0.14 \pm 0.03 \mu\text{M}$, respectively.

In another study, a different synthetic path to the active chain-extended analogues (42) and (43) have developed.¹⁰⁰ In addition, their corresponding selenium analogues (53) and (54) and nitrogen analogues (55) and (56) were also synthesized (Fig. 17) and the enzyme inhibition studies of above analogues showed that the selenium analogues (53) and (54) inhibited MGAM with K_i values of 0.10 ± 0.03 and $0.10 \pm 0.01 \mu\text{M}$, respectively.¹³ In this series, compounds (53) and (54) represent the most active glucosidase inhibitors. Both of the nitrogen analogues (55) and (56) were less active compared to the corresponding sulfur and selenium analogues, with K_i values of 35 ± 2 and $8 \pm 1 \mu\text{M}$, respectively. These data support the earlier conclusion with other derivatives (42–44 and 51, 52)^{100,102} that is, the configuration at C-3' does not appear to be critical for inhibitory activity since all pairs with enantiomeric configurations at C-3', (53) and (54); (55) and (56) shows similar K_i values in the same range.

In summary, the structure–activity relationships predict, comparison of the K_i values of the sulfonium and selenonium chain-extended analogues (41–44, 45, 46, 51, 52, 53, and 54)

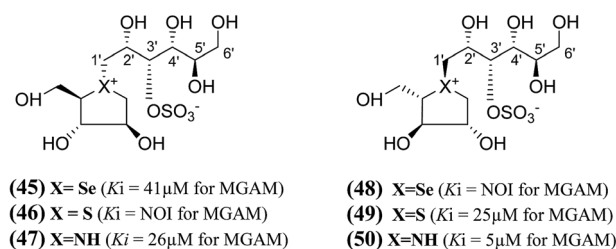


Fig. 15 Salacinol analogues (45–50) with an extended chain, heteroatom variations and polyhydroxylated, acyclic chain of 6-carbon and their inhibition profile against MGAM.

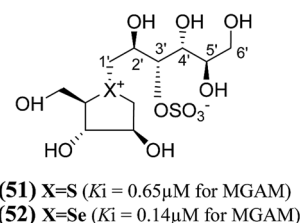


Fig. 16 Chain extended sulfur (51) and selenium (52), analogues of salacinol containing polyhydroxylated, acyclic chains of 6-carbons and their inhibition potential against MGAM.



Fig. 14 Chain extended salacinol homologues (41–44) containing polyhydroxylated, acyclic chain of 5- and 6-carbons and their inhibition profile against MGAM.





Fig. 17 Selenium (53 and 54) and nitrogen (55 and 56) analogues of chain extended salacinol and their inhibitory activity against MGAM.

against MGAM, that the *S* configuration at C-2' and the *R* configuration at C-4' are essential for activity (highlighted in bold in Table 5). The *S* configuration at C-3', bearing the sulfate group, was found to be unimportant. The configurational requirement at C-5' was not clear because the trend was found to depend on the heteroatom. Thus, it is clear that the stereochemistry at the different stereogenic centers on the side chain plays a significant role in biological activity. It appears that the compounds containing the *S* configuration at C-2', the *R* configuration at C-4', and the *S* configuration at C-5' are the most active in the sulfur series of compounds; however, that in the selenium series the activities of the selenium analogues, (52) and (53), suggest that the stereochemistry at C-5' could be *R*. The stereochemistry at C-3' was judged to be unimportant but can be fixed as *S* to reflect a presumed common biosynthetic pathway as salacinol. However, the overall conclusion was that the effect of the acyclic chain extension did not confer any dramatic change on inhibitory properties for this particular enzyme, because the K_i values of the active chain-extended analogues were in a similar range to those of salacinol (5) (0.19 μM) and blintol (16) (0.49 μM).

Three new class of deoxy-salacinols analogues lacking one (57 and 58) or two polar (59) substituents in the side chain of salacinol (5) were reported by Muraoka *et al.*¹⁰³ All these analogues showed less inhibitory activity as compared to salacinol (5), and proved the importance of cooperative role of the polar substituents for the α -glucosidase inhibitory activity. Compounds (58) and (59) having acyclic chains that lack the hydroxyl group at C-2' were not efficient inhibitors of intestinal α -glucosidases *in vitro* as compared to the acyclic chain of salacinol (5). However, compound (57), lacking a hydroxymethyl group at C-3', maintain some inhibitory activity against sucrase ($\text{IC}_{50} = 780\mu\text{M}$). These results recommended that both the hydroxymethyl group at C-3'



*Values in parentheses indicates inhibition (%) at 400 $\mu\text{g/ml}$

Fig. 18 Deoxy-salacinol analogues lacking one (57 and 58) or two polar (59) substituents in the side chain and their inhibitory activity against disaccharidases.



Fig. 19 Chain-modified frame-shifted analogues (60–63) of salacinol showing the position of the sulfate moiety which is shifted from C-3' to C-4' and their inhibitory activity against MGAM.

and the hydroxyl group at C-2' with an *S* configuration are critical for α -glucosidase inhibitory activity.

In order to develop more effective glucosidase inhibitors, additional salacinol derivatives were synthesized and tested for inhibitory activity including chain-extended and chain-modified embodiments, where the sulfate moiety is frame-shifted. Nasi *et al.*¹⁰⁴ described a frame shifted chain-extended and chain-modified (60–63, Fig. 19) analogues of salacinol (5) and blintol (16) where the sulfate moiety was shifted from C-3' to the C-4' location. The inhibitory activities of compounds (60–63) against recombinant human MGAM was determined. Compounds (60) and (61), with the same configuration at the stereogenic centers in the acyclic chain, have K_i values of 20 ± 4 and $53 \pm 5\mu\text{M}$, respectively. These compounds are less active than salacinol (5) and blintol, (16) with K_i values of 0.19 ± 0.02 and $0.49 \pm 0.05\mu\text{M}$, respectively.⁹⁶ Interestingly, the analogue (41), in which the sulfate moiety is located at the C-3' and not the C-4' position, is inactive.¹⁰⁰ A second 5-carbon chain-extended compound with the same configuration at C-3' and C-4' as (44), but with the opposite configuration at C-2' and in which the sulfate moiety is located at the C-3' and not the C-4' position, has a K_i value of $0.17 \pm 0.03\mu\text{M}$. In contrast, compounds (62) and (63) were not active against MGAM.¹⁰⁴

Tanabe *et al.*¹⁰⁵ have reported the synthesis of de-*O*-sulfonated analogues (64, X = CH_3OSO_3 and 65, X = Cl, Fig. 20) of salacinol with monomethyl sulfate and chloride as external counter anions and their inhibitory activity were examined to discover the role of the sulfate anion moiety in the acyclic chain of salacinol (5). These analogues (64 and 65) had almost equal inhibitory activities to salacinol (5) against intestinal α -glucosidase *in vitro*. These studies indicated that the internal sulfate counterion or *O*-sulfonate anion moiety of salacinol is not essential for inhibitory



activity as the de-*O*-sulfonated analogues (**64** and **65**) retained equal inhibitory activities against intestinal α -glucosidases when compared to salacinol (**5**).¹⁰⁵

An important new class of compounds comprising salacinol analogues containing sulfonium or ammonium ion-inner carboxylate structure were also discovered. The acyclic side chain of salacinol (**5**) and kotalanol (**6**) includes a sulfate group which is believed to be important to the inhibitory activity of these compounds. Exploring the glucosidase inhibitory activity, Chen *et al.*¹⁰⁶ prepared carboxylate analogues in which the sulfate moiety has been substituted with a carboxylate moiety (Fig. 21). Target compounds (**66**) and (**67**) comprise a thioarabinitol having a polyhydroxylated side chain containing a carboxylate residue. The inhibitory activity of target compounds (**66**) and (**67**) was assessed in respect of recombinant human MGAM. Only compound (**66**), with the *D*-arabinitol configuration in the heterocyclic ring displayed by salacinol (**5**), and was found to be active, with a K_i value of 10 μM whereas compound (**67**) did not show significant inhibition against MGAM.

To understand the role of the sulfate group present in the acyclic chain of ghavamiol (nitrogen analogues of salacinol **14**), an unusual class of amino acids (**68**) and (**69**) were synthesized (Fig. 21). These target analogues were considered to be the carboxylate analogues of ghavamiol (**14**), having a polyhydroxylated side chain containing a carboxylate residue.¹⁰⁷ Compound (**68**), inhibits recombinant human MGAM with a K_i value of 21 μM whereas salacinol (**5**) itself has a K_i value of 0.2 μM . In addition, this compound was also active against *Drosophila melanogaster* Golgi α -mannosidase II (dGMII) with an IC_{50} of 0.3 mM. This is a significant improvement (25-fold) over the inhibition measured for salacinol (**5**) and kotalanol (**6**) as well as other salacinol analogues such as blintol (**16**) which all inhibited dGMII with an IC_{50} of approximately 7.5 mM.¹⁰⁴ Compound (**69**) was not active on either enzyme MGAM or dGMII; similar results were obtained for other salacinol analogues derived from anhydro-*L*-heteroarabinitol moieties and presumably reflect minimal contacts of the enantiomeric five-membered rings in the enzyme active sites.¹⁰⁸ The crystal structure of (**68**) bound in the active site of dGMII indicated that the hydroxyl groups from the acyclic chain and also the carboxylic acid group form extensive contacts with both side chains and water molecules in the active site. Previously, the role of the sulfate group on glucosidase inhibitory activity was inferred by Yuasa *et al.*¹⁰⁹ It was reported that the docking of salacinol (**5**) into the binding site of glucoamylase indicated close contacts between the sulfate ion and Arg305.

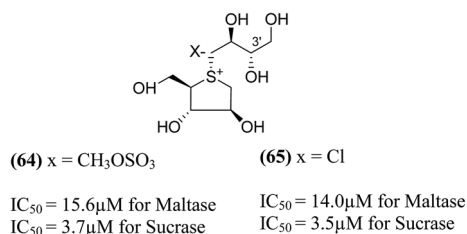


Fig. 20 De-*O*-sulfonated analogues (**64** and **65**) of salacinol and their inhibitory activity against disaccharidases.

The substitution of phosphate functional groups for sulfate groups in biologically important molecules also continues to attract much interest in bioorganic and medicinal chemistry. Therefore, in a same manner, phosphate derivatives (phosphorylated heteroalditols, **70** and **71**, Fig. 22) were prepared, in which the sulfate moiety on the acyclic side chain has been substituted with a phosphate moiety and after screening, it was found to not effective against MGAM.¹¹⁰

New salacinol analogues (**72–79**, Fig. 23) based on novel anhydroseleno- and anhydrothio-allitols, derived from *D*-gulono- γ -lactone and *L*-ascorbic acid, have also been synthesized. Enzyme inhibition assays of these analogues indicated that they were not active against MGAM.¹¹¹

In another synthetic approach, salacinol related compounds were synthesized bearing a permanent positive charge of the sulphur atom and including a lipophilic alkyl chain which may be necessary for substrate recognition. These target compounds (**80–88**, Fig. 24) include the 1,4-anhydro-4-thio-*D*-arabinitol moiety.¹¹² The inhibitory properties of target compounds were examined against recombinant human MGAM. All of the lipophilic compounds were inhibitors of MGAM with K_i values ranging from 6 to 75 μM . Increases in the alkyl chain lengths have significant, albeit not pronounced, effects on the enzyme inhibitory properties of these compounds. There is a 6.7-fold increase in inhibitory activity upon extension of the alkyl chain from four carbons to eighteen carbons. However, these

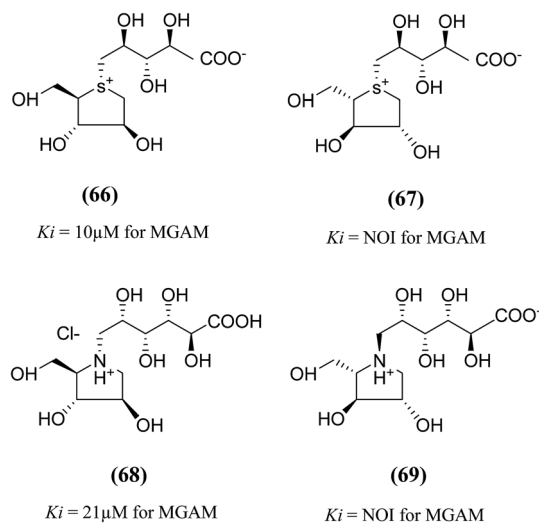


Fig. 21 Carboxylate analogues (**66** and **67**) of salacinol and ghavamiol (**68** and **69**) and their inhibitory activity against MGAM.

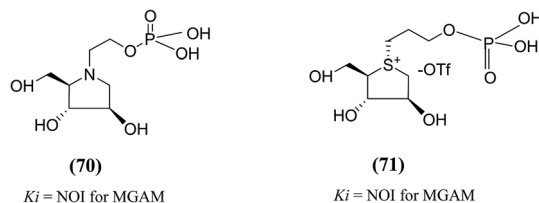


Fig. 22 Phosphate analogues (**70** and **71**) of salacinol and their inhibitory activity against MGAM.



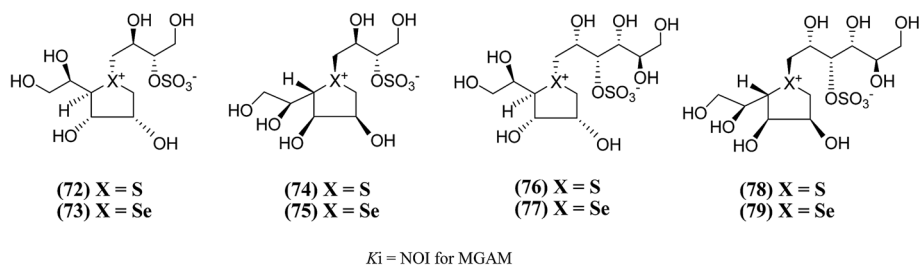


Fig. 23 Analogues of salacinol related to D-gulono- γ -lactone and L-ascorbic acid and their inhibitory activity against MGAM.

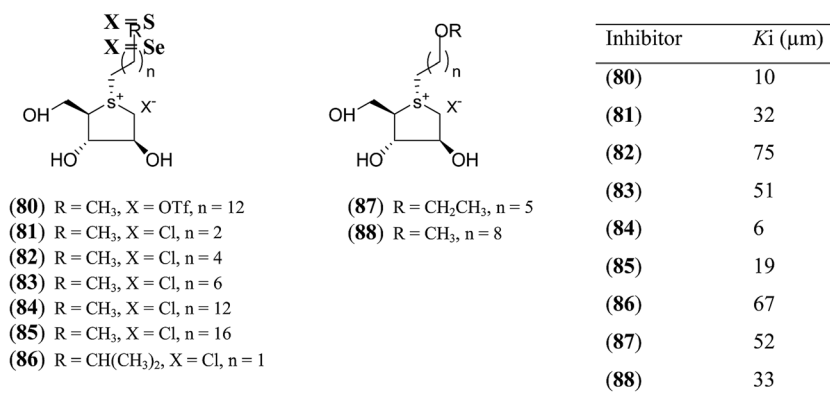


Fig. 24 S-Alkylated sulfonium ions with different alkyl chains (80–88) and their inhibitory activity against MGAM.

compounds are less active than salacinol (5) which was previously shown to have a K_i value of $0.19 \pm 0.02 \mu\text{M}$ against MGAM.

Tanabe *et al.*¹¹³ synthesized a series of four 3'-O-alkylated salacinol analogues (89–92, Fig. 25) having different hydrophobic groups (OCH₃, OCH₂H₅, OC₁₃H₂₇, or OCH₂Ph) at C-3' of the salacinol side chain. Inhibition assays against rat intestinal glucosidases revealed that all of these synthesized compounds showed equal or higher inhibitory activities as compared to salacinol (5). Compound (92) having benzyloxy group at C-3' was the most potent inhibitor of maltase ($\text{IC}_{50} = 0.44 \mu\text{M}$) and isomaltase ($\text{IC}_{50} = 0.14 \mu\text{M}$), and compound (90) with an ethoxy group at C-3' showed highest inhibitory activity against sucrase ($\text{IC}_{50} = 0.12 \mu\text{M}$).

With the aid of the *in silico* method, Tanabe *et al.*¹¹⁴ designed 3'-O-benzyl derivative of salacinol (92) and developed twelve analogues (93–103, Fig. 26) having four different substituents (methyl, chloro, trifluoromethyl, and nitro) at different positions (*meta* or *para* or *ortho*) of the phenyl ring. By comparison of enzyme inhibition activity, all the sulfonium salts (93–103)

showed better inhibitory activities than the salacinol (5). In general, *ortho* substituted compounds were more active than the corresponding *para* and *meta* substituted compounds. Compound (102), with an *ortho*-nitrophenyl group, was found to be the most potent inhibitor of all three rat intestinal glucosidases, maltase ($\text{IC}_{50} = 0.13 \mu\text{M}$), sucrase ($\text{IC}_{50} = 0.042 \mu\text{M}$), and isomaltase ($\text{IC}_{50} = 0.21 \mu\text{M}$).

Thus, structure–activity relationship studies towards salacinol (5) and related analogues enabled us to conclude the best inhibitors belonging to the salacinol (5) class have K_i values in the low micromolar range (*i.e.*, 0.10–0.19 μM). The salacinol inhibitors have been revealed to be a relatively poor inhibitor of HPA ($K_i = 75 \mu\text{M}$) and hence, they are selective against the intestinal glucosidases.²² Structural drawing of synthetic analogues of salacinol showing important structural features needed for the inhibitory activity is given in Fig. 27.

4.1.1.1. Crystallographic analysis and molecular docking studies of salacinol and its analogues. With the aim of gaining information on structure–activity relationships, various studies

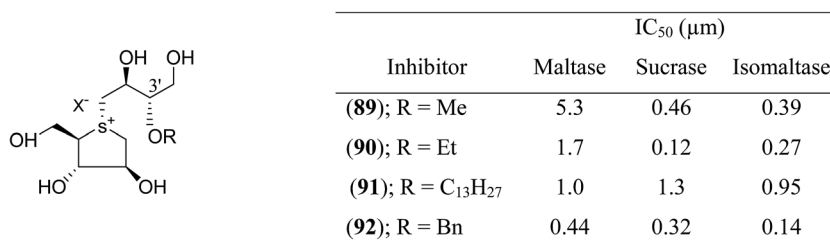
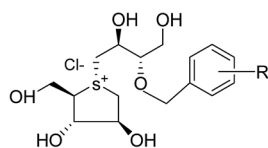


Fig. 25 3'-O-Alkylated analogues (89–92) of salacinol showing different hydrophobic groups and their IC_{50} (μM) values against disaccharidases.





Inhibitor	IC ₅₀ (μm)		
	Maltase	Sucrase	Isomaltase
(93); R = <i>O</i> -Me	0.66	0.41	0.48
(94); R = <i>m</i> -Me	0.84	1.3	0.35
(95); R = <i>p</i> -Me	0.86	1.1	0.68
(96); R = <i>O</i> -Cl	0.31	0.09	0.26
(97); R = <i>m</i> -Cl	0.53	0.80	0.31
(98); R = <i>p</i> -Cl	0.89	0.72	0.48
(99); R = <i>O</i> -CF ₃	0.33	0.15	0.19
(100); R = <i>m</i> -CF ₃	0.98	0.82	0.25
(101); R = <i>p</i> -CF ₃	0.98	0.72	0.38
(102); R = <i>O</i> -NO ₂	0.13	0.042	0.21
(103); R = <i>m</i> -NO ₂	0.94	0.49	0.23
(104); R = <i>p</i> -NO ₂	0.68	0.38	0.23

Fig. 26 Synthetic analogues (93–104) of 3'-*O*-benzyl derivatives of salacinol and their IC₅₀ (μm) values against disaccharidases.

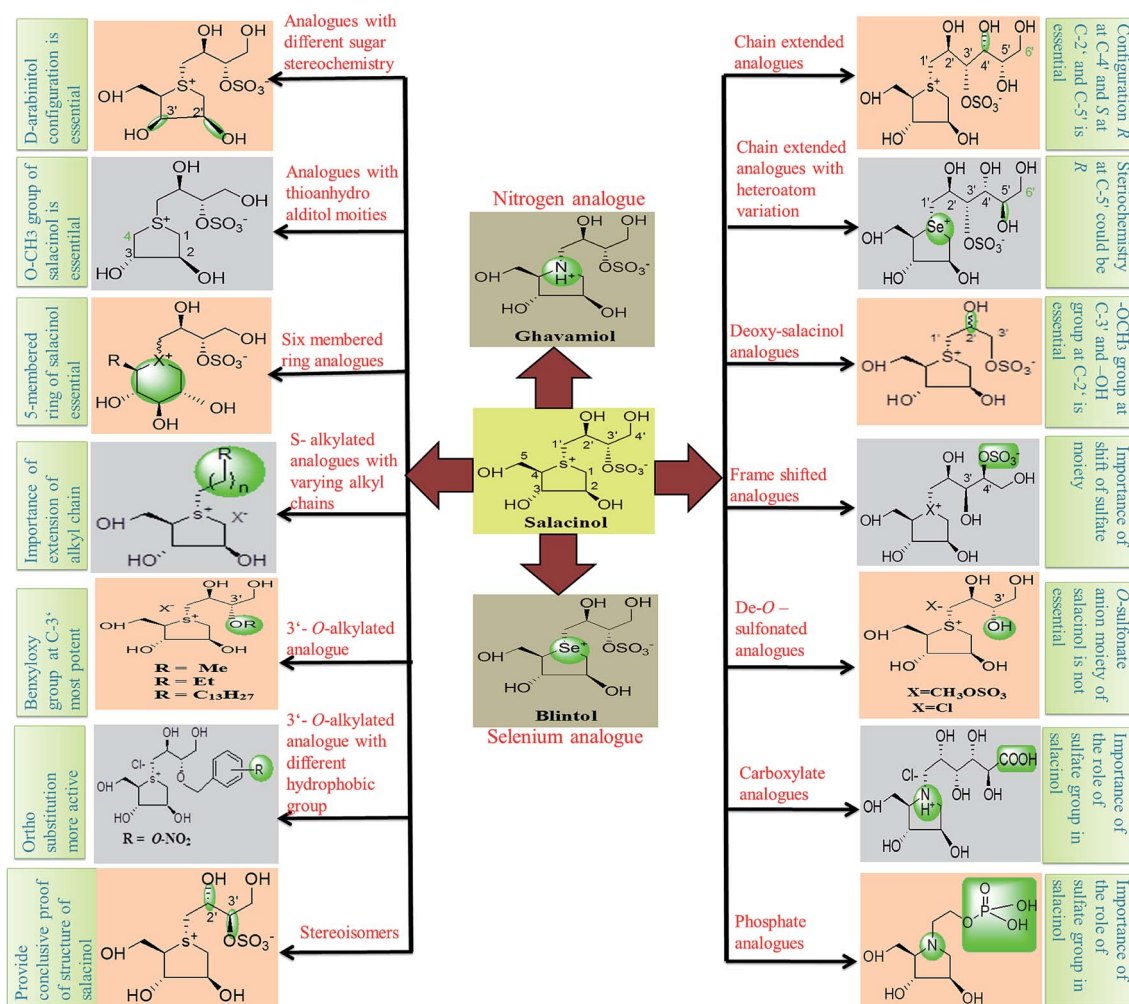


Fig. 27 Structural drawing of salacinol analogues showing important structural features required for the inhibitory activity.



isolated, and the absolute stereostructure of these compound were elucidated by the total synthesis or other means.^{36,100,120,121} Except salacinol (**8**), all these sulfonium sulfates possessed potent α -glucosidase inhibitory activity. Chemical degradation studies showed that the 1-deoxy-4-thiopentofuranosyl portion of kotalanol (**6**) is similar to that in salacinol (**5**).³⁵ Ozaki *et al.*⁷⁹ isolated another α -glucosidase inhibitor and assigned its structure to a 13-membered cyclic sulfoxide; the structure has been reassigned by Yoshikawa *et al.*³⁸ to be de-*O*-sulfonated kotalanol (**10**). The later compound was shown by Ozaki *et al.*⁷⁹ to be the most active compound against rat intestinal glucosidase (compare the K_i values for salacinol (**5**) = 0.97, 0.20 and 1.1 μM ; kotalanol (**6**) = 0.54, 0.42 and 42 μM and de-*O*-sulfonated kotalanol (**10**) = 0.11, 0.05 and 0.42 μM using maltase, sucrase and isomaltase as substrate, respectively). Absolute stereostructure of kotalanol (**6**) and de-*O*-sulfonated kotalanol (**10**) was proved by Jayakanthan *et al.*³⁶ by total synthesis.

Structure-activity-relationship studies of kotalanol analogues exposed that the inhibitory activities against ntMGAM improve significantly upon de-*O*-sulfonation.¹²² De-*O*-sulfonated kotalanol (**10**) ($K_i = 0.03 \pm 0.01 \mu\text{M}$) was found to be a \sim sevenfold better inhibitor of ntMGAM than kotalanol (**6**) ($K_i = 0.19 \pm 0.03 \mu\text{M}$).⁷⁸ The X-ray crystallographic studies of ntMGAM in complex with kotalanol (**6**), de-*O*-sulfonated kotalanol (**10**) and several salacinol derivatives revealed interesting features that de-*O*-sulfonated kotalanol (**10**) from the salacinol class of compounds is a \sim 30-fold stronger inhibitor compared to miglitol (**2**) and an \sim 2000-fold better inhibitor than the acarbose (**1**). The crystal structures revealed that in the case of kotalanol (**6**), the sulfate group at C-3' occupies a hydrophobic cavity causing a positional constraint, while in the case of de-*O*-sulfonated kotalanol (**10**), the positional constraint is removed and allows the rest of the polyhydroxylated side chain to make optimal contacts with the ntMGAM active site.⁷⁸

To discuss the role of the side chain stereochemistry to the inhibitory activity against MGAM, various synthetic studies directed towards the stereochemical determination of kotalanol (**6**), have been carried out.¹²³ The stereoisomer of kotalanol (**105**, Fig. 30), with the opposite stereochemistry at the C-6' stereogenic centre, inhibited the ntMGAM with a K_i value of $0.20 \pm 0.02 \mu\text{M}$,¹¹⁵ this compares to K_i value for kotalanol (**6**) of $0.19 \pm 0.03 \mu\text{M}$.⁷⁸ The results indicated that the configuration at C-6' is inconsequential for inhibitory activity against this enzyme as the C-6' epimer (**105**) of kotalanol showed equal inhibitory activity against ntMGAM to that of kotalanol (**6**).

The compound (**106**), diastereomers of de-*O*-sulfonated kotalanol (**10**), is the most potent inhibitor of ntMGAM ($K_i = 17 \pm 1 \text{ nM}$) *in vitro*.³⁶ The inhibitory values of (**105**) are comparable to the inhibitory activities of other diastereomers of kotalanol (**107**) and (**108**) with opposite configuration at C-5' ($K_i = 0.10 \pm 0.02 \mu\text{M}$ and $0.13 \pm 0.02 \mu\text{M}$, respectively, Fig. 30).¹²³ The de-*O*-sulfonated derivatives (**109**, **110**) derived from biologically active C-5' diastereomers of kotalanol, (**107**) and (**108**), were found to be more active against ntMGAM than the parent compounds, supporting the general proposition that de-*O*-sulfonation leads to an increase in the inhibitory activity against ntMGAM compared to the parent sulfated compounds.²²

To study the effect of heteroatom substitution on the inhibitory activities of kotalanol (**6**) and de-*O*-sulfonated kotalanol (**10**), Mohan *et al.*¹²² designed their nitrogen (**111–112**) and selenium analogues (**114–115**, Fig. 31). Screening of these analogues against ntMGAM indicated that, except for the nitrogen analogue of kotalanol (**111**), all of the compounds synthesized showed greater inhibitory activities than acarbose (**1**). As discussed above, 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, de-*O*-sulfonation resulted in a very large increase in inhibitory activity (compare K_i values of compound (**111**) and (**114**)). Additionally, selenium substitution doesn't confer any significant advantage on the inhibitory activities of both kotalanol (X = Se, $K_i = 80 \pm 6 \text{ nM}$; X = S, $K_i = 190 \pm 30 \text{ nM}$) and de-*O*-sulfonated kotalanol (X = Se, $K_i = 20 \pm 3 \text{ nM}$; X = S, $K_i = 30 \pm 10 \text{ nM}$). In contrast, nitrogen substitution (**111**)



Fig. 29 Interaction diagram of predicted binding mode of salacinol with amino acid residues.



Fig. 30 Diastereomers of kotalanol (**105**, **107**, **108**) and de-*O*-sulfonated kotalanol (**106**, **109**, **110**) and their inhibitory activity against ntMGAM.



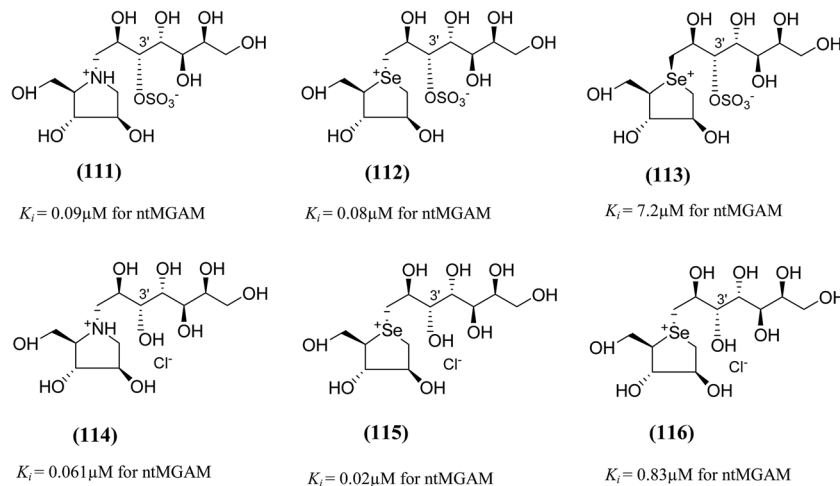


Fig. 31 Nitrogen, selenium and diastereomers of selenium analogues of kotalanol (**111**, **112** and **113**) and de-*O*-sulfonated kotalanol (**114**, **115** and **116**) and their inhibitory activity against ntMGAM.

drastically decreased the inhibitory activity in the case of kotalanol ($K_i = 90 \mu\text{M}$), whereas it does not have any significant change in the case of de-*O*-sulfonated kotalanol (**114**) ($K_i = 61 \pm 5 \text{ nM}$). This trend was also seen in the case of the nitrogen analogue of salacinol (**14**) (ghavamiol IC_{50} = high mM range) resulted in a remarkable decrease in inhibitory activity against MGAM (compare the K_i value of salacinol (**5**), $0.19 \mu\text{M}$), whereas substitution of the ring sulfur by selenium (**16**) (blintol $K_i = 0.49 \mu\text{M}$) didn't affect its inhibitory activity significantly.⁸⁹ Based on crystallographic work with salacinol and kotalanol derivatives,⁷⁸ that the positioning of the sulfate anion of nitrogen analogue of kotalanol (**111**) in a hydrophobic pocket in the active site is more sterically compromised than in the sulfur congener (**6**). Relief of this steric interaction by de-*O*-sulfonation to give (**114**) apparently relieves this interaction and gives a compound that is just active as its sulfur congener (**10**). Compound (**113**) bearing *S* configuration, differs from (**112**) only with respect to the configuration at the stereogenic selenium center, was converted into the corresponding de-*O*-sulfonated compound (**116**). Inhibitory activities of compounds (**112**) and (**115**) are considerably less than those of their corresponding diastereomers (**113** and **116**) with *R* configuration. As *R* configuration at the stereogenic heteroatom center, as exhibited by all of the natural compounds isolated so far, is essential for inhibitory activity. The undesired diastereomers (**113** and **116**, Fig. 31) bearing the *S* configuration at the stereogenic selenium center, showed significant decrease in inhibitory activities than those of their corresponding diastereomers with *R* configuration.¹²²

In order to investigate the active-site requirements of ntMGAM, two six-membered ring nitrogen analogues (**117** and **118**, Fig. 32) were synthesized.¹²⁴ The target compounds are hybrids of kotalanol (**6**) and miglitol (**2**) and consist of the six-membered ring of miglitol (**2**) and the 7-carbon side chains of kotalanol (**6**) and de-*O*-sulfonated kotalanol (**10**). The basis of these hybrid molecules was twofold: (1) to improve the binding interactions of miglitol (**2**) in the +1 binding site of ntMGAM by replacing the N-hydroxyethyl side chain with the polyhydroxylated side chain of kotalanol (**6**) or de-*O*-sulfonated kotalanol (**10**), and

(2) to probe the purported transition state mimicry by the kotalanol class of molecules. Inhibition studies of these hybrid compounds against ntMGAM indicated that they have comparable activities ($K_i = 2.3 \pm 0.6$ and $1.4 \pm 0.5 \mu\text{M}$, respectively) to that of miglitol (**2**) ($K_i = 1.0 \pm 0.1 \mu\text{M}$). However, they are less active compared to kotalanol (**6**) ($K_i = 0.19 \pm 0.03 \mu\text{M}$).^{78,124}

These outcomes undoubtedly signify the importance of the five-membered ring thiocyclitol moiety with a permanent positive charge and thus provide further support for transition state mimicry by these sulfonium-ion glucosidase inhibitors. The inhibitory activities of all synthesized kotalanol analogues (**105**–**118**) against the maltase activity of recombinant, ntMGAM are summarized in Table 6.

The synthesis, evaluation and structure–activity relationship studies on four kotalanol diastereomers (**119**–**122**, Fig. 33), having opposite configurations at C-3' and C-4', were described by Xie *et al.*⁸⁰ to understand the role of the side chain stereochemistry on the inhibitory activity. Out of the four analogues examined, compared to kotalanol (**6**) (IC_{50} values for sucrase = $0.75 \mu\text{M}$; maltase = $7.2 \mu\text{M}$; and isomaltase = $5.7 \mu\text{M}$), all of these diastereomers showed higher IC_{50} values against maltase, sucrase, and isomaltase (see Fig. 33 for comparison of IC_{50} values). Except compound (**120**), a considerable loss of inhibitory activity (IC_{50} values > $236 \mu\text{M}$) was observed against both maltase and sucrase, showing the importance of the *R* configuration at C-4'.

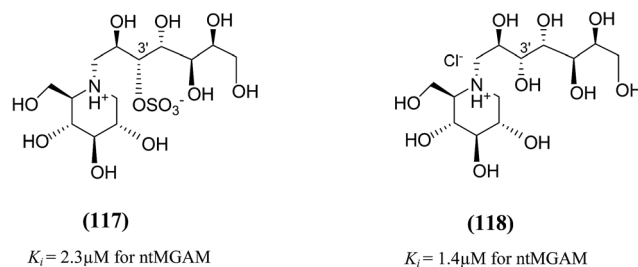


Fig. 32 Six-membered ring nitrogen analogues of kotalanol (**117**) and de-*O*-sulfonated kotalanol (**118**) and their inhibitory activity against ntMGAM.



Table 6 Inhibition of ntMGAM by kotalanol analogues

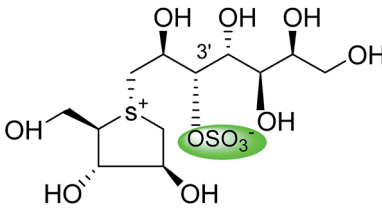
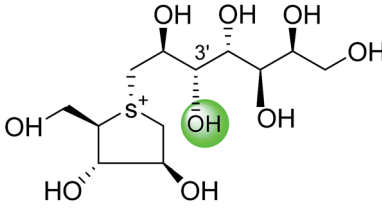
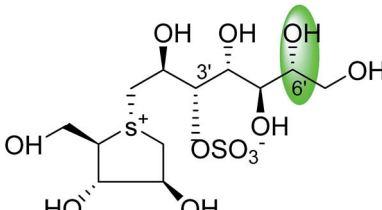
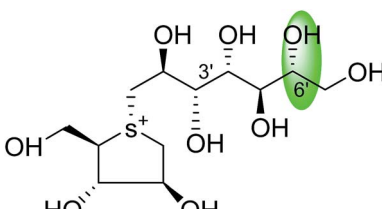
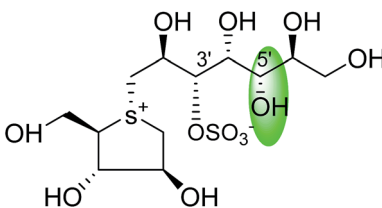
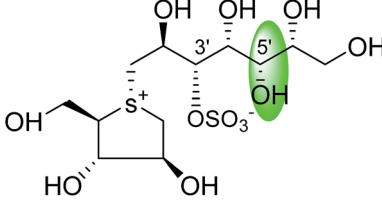
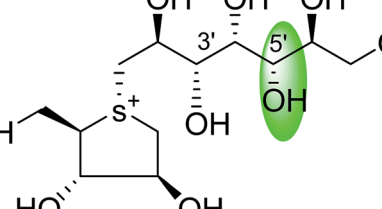
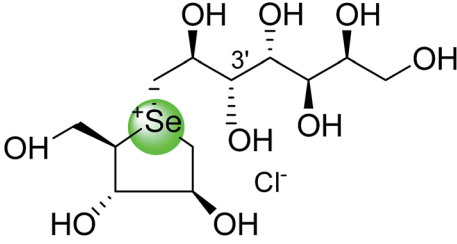
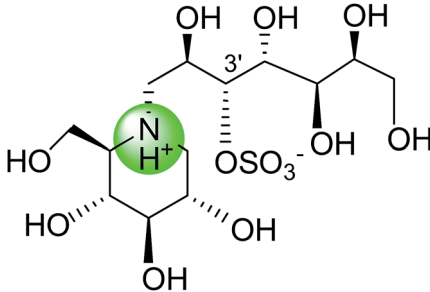
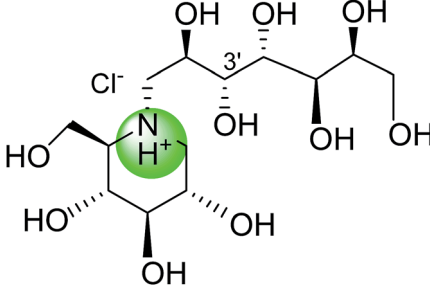
Inhibitor	Chemical structure	ntMGAM (μM)	References
Kotalanol (6)		0.19 ± 0.03	78
De-O-sulfonated kotalanol (10)		0.03 ± 0.01	78
Diastereomers of kotalanol and de-O-sulfonated kotalanol			
C-6' epimer of kotalanol (105)		0.20 ± 0.02	115
C-6' epimer of de-O-sulfonated kotalanol (106)		0.017 ± 0.01	36
C-5' diastereomers of kotalanol (107)		0.10 ± 0.02	123
C-5' diastereomers of kotalanol (108)		0.13 ± 0.02	123
C-5' diastereomers of de-O-sulfonated kotalanol (109)		0.024 ± 0.02	123



Table 6 (Contd.)

Inhibitor	Chemical structure	ntMGAM (μM)	References
Diastereomer of selenium analogue of de-O-sulfonated kotalanol (116)		0.83 ± 0.07 nm	122
Six membered ring analogues of kotalanol and de-O-sulfonated-kotalanol			
Six membered ring nitrogen analogue of kotalanol (117)		2.3 ± 0.6	124
Six membered ring nitrogen analogue of de-O-sulfonated-kotalanol (118)		1.4 ± 0.5	124

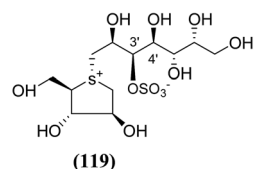
4.1.3. Ponkoranol, its synthetic analogues and de-O-sulfonated salaprinol. Ponkoranol (**7**), six-carbon-chain homologue of salacinol, was synthesized by Johnston *et al.*¹⁰⁰ Evaluation of the spectral data of the six-carbon, chain-extended synthetic analogues allowed to establish the absolute stereostructure of ponkoranol (**7**).¹⁰⁰ Unlike salacinol (**5**) and kotalanol (**6**), ponkoranol (**7**) and salaprinol (**8**) were first synthesized before to the information of their presence in *S. prinooides*.³⁸ Tanabe *et al.*¹²⁰ assigned the stereostructure of salaprinol (**8**) by its total synthesis.

An efficient synthetic route to de-O-sulfonated ponkoranol (**11**) and its 5'-stereoisomer (**123**) (Fig. 34) was reported by Eskandari *et al.*¹²⁵ and showed that they are very potent inhibitors of the ntMGAM. As observed previously with the de-O-sulfonated analogues of kotalanol (**10**), the de-O-sulfonated ponkoranol (**11**) and its C-5' epimer (**123**) showed significantly higher inhibitory activities with K_i values of 43 ± 3 and 15 ± 1 nM, respectively,¹²⁵ against ntMGAM compared to the parent sulfated compound, ponkoranol (**7**) ($K_i = 170 \pm 30$ nM).¹⁰⁰ Thus, it would appear that de-O-sulfonation is beneficial. Comparison of the K_i values for (**11**) and (**123**) to that of K_i value for de-O-sulfonated kotalanol (**10**) of 30 ± 1 nM,⁷⁸ led to the finale that the configuration at C-5' is not important for dictating enzyme inhibitory activity against

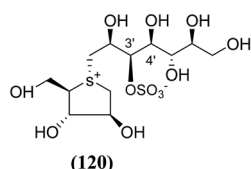
ntMGAM and further expansion of the acyclic side chain beyond six carbons is not favorable.

After applying the similar protocol, the selenium analogues of de-O-sulfonated ponkoranol (**124**) and its C-5' epimer (**125**) (Fig. 34) were synthesized¹²⁶ and tested against the four subunits, ntMGAM, ctMGAM, ntSI, and ctSI, and two splice-forms of ctMGAM, ctMGAM-N2, and ctMGAM-N20.^{118,127} The catalytic activities of the ctMGAM-N2 and ctMGAM-N20 are slightly different, as revealed by the kinetic analysis for maltose as a substrate; ctMGAM-N2 ($K_m = 1.59 \pm 0.18$ mM) and ctMGAM-N20 ($K_m = 1.91 \pm 0.23$ mM).¹²⁷ Compared to the parent compounds (**11**), (ntSI, $K_i = 302$ nM; ctSI, $K_i = 103$ nM) and (**123**) (ntSI, $K_i = 138$ nM; ctSI, $K_i = 132$ nM, respectively), substitution of the ring sulfur atom with selenium (**124** and **125**) increased the enzyme inhibitory activities against both ntSI ($K_i = 13$ and 10 nM, respectively) and ctSI ($K_i = 18$ and 19 nM, respectively), while it did not have much effect in the case of ntMGAM and ctMGAM-N20. Surprisingly, all four compounds, both sulfur (**11** and **123**) and selenium (**124** and **125**) analogues did not inhibit the ctMGAM-N2 spliceform.¹²⁸ These results suggested that only slight changes in the structure of these compounds could selectively inhibit one or a combination of subunits.

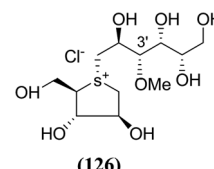




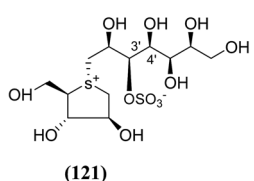
$IC_{50} = >236\mu\text{M}$ for Maltase
 $IC_{50} = >236\mu\text{M}$ for Sucrase
 $IC_{50} = 16\mu\text{M}$ for Isomaltase



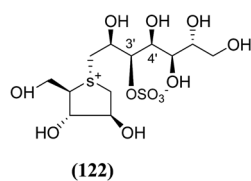
$IC_{50} = 57\mu\text{M}$ for Maltase
 $IC_{50} = 23\mu\text{M}$ for Sucrase
 $IC_{50} = 25\mu\text{M}$ for Isomaltase



$K_i = 7\text{nm}$ for ctSI
 $K_i = 35\text{nm}$ for ntSI
 $K_i = 500\text{nm}$ for ntMGAM
 $K_i = 60\text{nm}$ for ctMGAM-N2
 $K_i = 55\text{nm}$ for ctMGAM-N20



$IC_{50} = >236\mu\text{M}$ for Maltase
 $IC_{50} = >236\mu\text{M}$ for Sucrase
 $IC_{50} = 8.5\mu\text{M}$ for Isomaltase



$IC_{50} = >236\mu\text{M}$ for Maltase
 $IC_{50} = >236\mu\text{M}$ for Sucrase
 $IC_{50} = 9\mu\text{M}$ for Isomaltase

Fig. 33 Diastereomers of kotalanol (119–122) synthesized by Xie *et al.*⁸⁰ and their inhibitory activity against rat intestinal disaccharidases.

The installation of a hydrophobic methoxy group in place of a sulfate group might increase inhibitory activity against ntMGAM. A new synthetic analogue, 3'-*O*-methylponkoranol (126) (Fig. 35), was synthesized and screened against ntMGAM. Disappointingly, the K_i value of 3'-*O*-methylponkoranol (126) was higher than that of de-*O*-sulfonated ponkoranol (11) and was less active ($K_i = 0.50 \pm 0.04 \mu\text{M}$) against ntMGAM than de-*O*-sulfonated ponkoranol (11, $K_i = 43 \text{ nM}$)¹²⁹ showing that the hydrophobic interactions between the methyl group and the hydrophobic residues in the active site of ntMGAM are not as optimal as the interactions of the latter groups with the rest of the polyhydroxylated chain in the absence of the methyl ether. However, compound (126) showed some interesting selectivity for the four subunits and two spliceforms of ctMGAM.¹³⁰ It showed significant inhibitory activity against ctSI ($K_i = 0.007 \pm 0.002 \mu\text{M}$), which is approximately 70 times higher when compared to ntMGAM ($K_i = 0.50 \pm 0.04 \mu\text{M}$). The compound (126) also revealed an inhibitory activity against ntSI ($K_i = 0.035 \pm 0.013 \mu\text{M}$), ctMGAM-N2 ($K_i = 0.060 \pm 0.015 \mu\text{M}$), and ctMGAMN20 ($K_i = 0.055 \pm 0.014 \mu\text{M}$) in a nanomolar range. Comparison with the inhibition report of de-*O*-sulfonated ponkoranol (11) against all four subunits clearly support that the installation of the methoxy group at C-3' is beneficial except in the case of ntMGAM where the activity

Fig. 35 Chemical structure of 3'-*O*-methylponkoranol analogue and their inhibitory profile against MGAM and SI.

dropped by an order of magnitude. The favorable effect of a methoxy group at C-3' (126) is remarkable, giving the most active inhibitor of ctSI with a K_i value of $0.007 \pm 0.002 \mu\text{M}$ (de-*O*-sulfonated ponkoranol (11) had a K_i value of $0.103 \pm 0.037 \mu\text{M}$), and also showing an order of magnitude increased inhibitory activity against ntSI compared to (11). Based on the potent inhibitory activity of the 3'-*O*-methyl derivative (126) against ctSI, it is envisaged that ctSI will have a hydrophobic pocket in the catalytic site that better accommodates the methyl group and provides favorable hydrophobic interactions relative to the other subunits. Such information is essential in designing inhibitors against ctSI, particularly in the lack of its crystal structure.

Xie *et al.*¹³¹ reported the isolation, structure identification and structure–activity relationship studies of de-*O*-sulfonated ponkoranol (11), de-*O*-sulfonated salaprinol (12) and their epimers (Fig. 36) against rat small intestinal α -glucosidase *in vitro*. Neoponkoranol (11) showed potent inhibitory activity against three enzymes tested, while the neosalaprinol (12) inhibited both sucrase and isomaltase more effectively than its sulfate (8). As mentioned, upon de-*O*-sulfonation, all the resulting sulfonium salts showed improved inhibitory activities against isomaltase. The configuration of the chiral center at C'3 and/or C'5 positions showed no significant influence on the inhibitory activity while the 2'-epimerization caused drastic decrease in its inhibitory activity against isomaltase, all the four compounds in ponkoranol series *i.e.* ponkoranol (7), neoponkoranol (11), 3'-*epi*-ponkoranol (127), and 5'-*epi*-ponkoranol (128) showed potent inhibitory activities irrespective of existence of the sulfate moiety at C3' and also of stereochemistry at 3' and/or 5'. 3'-*epi*-ponkoranol (127) showed almost the same inhibitory abilities as voglibose (3) against all enzymes and was found to be the most potent inhibitor

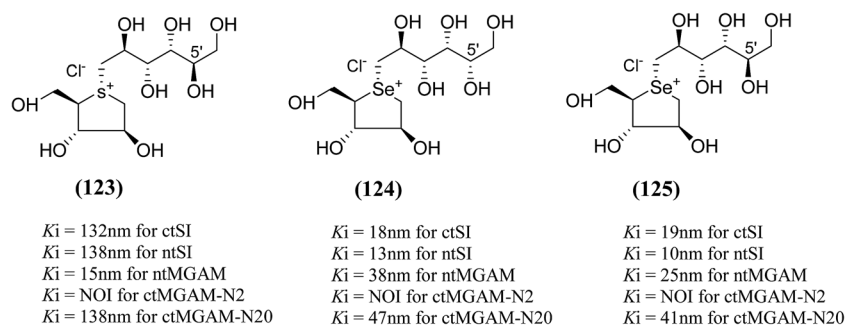


Fig. 34 5'-Stereoisomer (123) of de-*O*-sulfonated ponkoranol and selenium analogues of de-*O*-sulfonated ponkoranol (124) and its C-5' epimer (125) and their inhibitory profile against MGAM and SI.



against rat small intestinal α -glucosidases. Epimerization at C'2-position in 2'-episalaprinol (**130**) resulted in losing completely the activity even against isomaltase.

5. Toggling of the mucosal α -glucosidases by sulfonium-ion inhibitors

The concept of toggling of the enzymes *i.e.* differential inhibition of individual mucosal α -glucosidase activities is considered to be one of the effective approaches for monitoring blood glucose in type-2 diabetes. Through toggling of enzymes, the rate of starch digestion and glucose release to the body can be controlled and thus, slow glucose delivery is possible.²⁶ Natural molecules have been derivatized through structural activity relationship to make it more potent inhibitors that have the ability to inhibit activities of α -glucosidase enzymes four subunits together with two alternative spliceforms of ctMGAM. Thus, the optimization of structural changes yielded the most potent inhibitors, known to have for each subunit. Various synthetic inhibitors reported to block the activity of mucosal α -glucosidases and can be used to switch off selectively the different enzyme activities.

In a study, a series of inhibitors, including acarbose (**1**), miglitol (**2**), salacinol (**5**), kotalanol (**6**), de-*O*-sulfonated kotalanol (**10**) and blintol (**16**), were investigated to regulate the activities of four subunits including two spliceforms of ctMGAM.¹²⁷ This catalytic domain selectivity study helps to differentiate the active site requirements of each catalytic subunits. It was found that acarbose (**1**) was a poor inhibitor of ntMGAM ($K_i = 0.009 \mu\text{M}$) and ntSI ($K_i = 14 \mu\text{M}$) and a 1000-fold better inhibitor of two spliceforms ctMGAM-N2 and ctMGAM-N20 and 100-fold better inhibitor

of ctSI. Salacinol (**5**) and kotalanol (**6**) showed 4 to 6-fold selectivity for inhibiting ctMGAM and ctSI, as compared to N-terminal MGAM and SI units. In comparison to salacinol (**5**), blintol (**16**) effectively inhibited ct-MGAM-N2 with little effect on the other enzymes. Miglitol (**2**) showed a small difference between the catalytic subunits. This study reflects a small change in the structure of these compounds can have brought a significant difference in inhibiting the selectivity of one enzyme over the others and showed different biochemical and structural properties of these enzyme units, despite their overall similarity in amino acid sequence and tertiary structure.^{26,127} An example of toggling effect, as acarbose (**1**) is better inhibitor of C-terminal domains of MGAM and SI but a weak inhibitor of N-terminal domains of MGAM and SI. To understand the further role of ctMGAM in the terminal starch digestion process, X-ray crystallography of the ctMGAM in complex with acarbose (**1**) was studied by Ren *et al.*¹³²

To understand the individual roles of the MGAM and SI domains in the course of terminal starch digestion, an advance modification had proposed to the ponkoranol (**7**) scaffold to toggle their activities on and off with domain specific inhibitors. Two ponkoranol-based compounds, 3'- and 5'- β -maltose-extended de-*O*-sulfonated ponkoranol (**131** and **132**, Fig. 37), was described by Eskandari *et al.*¹³³ and their inhibitory activities were tested against all four subunits, using maltose as a substrate.

These sulfonium compounds have shown some striking selectivities on inhibition of all four subunits. Compound (**132**) inhibit all of the catalytic subunits very well and is the most potent inhibitor of ntMGAM ($K_i = 0.008 \mu\text{M}$) to date. In contrast, compound (**131**), showed selectivity and differentiated ctMGAM from the others is a poor inhibitor of ctMGAM-N20 ($K_i = 0.655 \mu\text{M}$) and showed no inhibition against ctMGAM-N2; while, the other subunits showed similar inhibition ($K_i = 0.039 \mu\text{M}$ for ntMGAM; $0.046 \mu\text{M}$ for ntSI and $0.062 \mu\text{M}$ for ctSI). As a result, with compound (**131**), it is feasible to maintain the ctMGAM activity on and dampen the others. The most potent inhibitor known to date for each subunit is shown in Fig. 38. Enzyme inhibition values and selectivity profile of some of the potent inhibitors, described in this review, against the four subunits *i.e.* ntMGAM, ctMGAM, ntSI and ctSI are given in Table 7.

In another study, Lee *et al.*¹³⁴ investigated the control of starch digestion through the concept of toggling of differential inhibition of MGAM and SI enzymes activities. Inhibitory profile of acarbose (**1**) and three sulfonium glucosidase inhibitors, de-*O*-sulfonated kotalanol (**10**) and the 3'- and 5'- β -maltose-extended de-*O*-sulfonated ponkoranol (**131** and **132**) were studied against all four subunits, using LM- α LDx (linear maltooligosaccharides, LM and α -limit dextrins, α LDx) as substrates, produced by the α -amylase reaction on waxy corn starch. Blood glucose level can be regulated by the regulation of activities of mucosal α -glucosidases, as MGAM and SI enzyme subunits are required to produce glucose from LM- α LDx mixture. Once again, this study showed that acarbose (**1**), effectively inhibited C-terminal enzymes ($\text{IC}_{50} = 0.12 \text{ nM}$ for ctMGAM and 0.4 nM for ctSI) as compared to N-terminal enzymes ($\text{IC}_{50} = 62 \text{ nM}$ for ctMGAM and 135 nM for ctSI). Inhibition of mucosal α -glucosidases by de-*O*-sulfonated kotalanol (**10**) showed 20% and 40% inhibition of ctMGAM

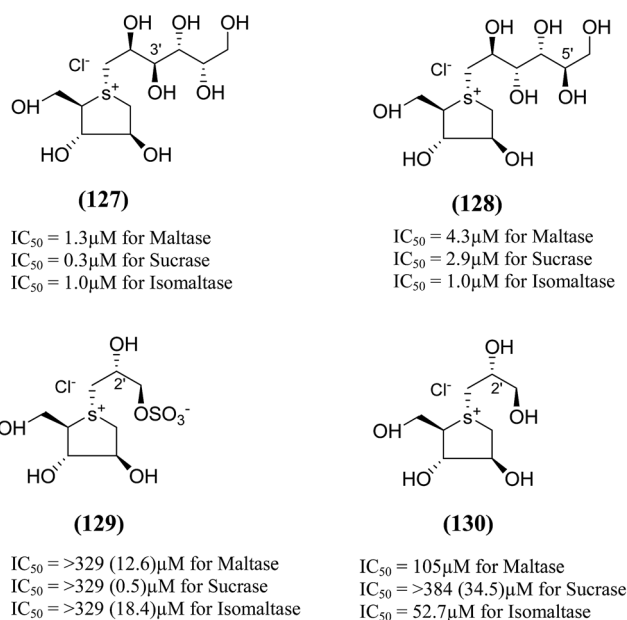


Fig. 36 Epimers of de-*O*-sulfonated ponkoranol (**127** and **128**), salaprinol (**129**) and de-*O*-sulfonated salaprinol (**130**) synthesized by Xie *et al.*¹³¹ and their inhibitory activity against rat intestinal disaccharidases.



Table 7 K_i values and selectivity profile of potent inhibitors of ctSI, ntSI, ntMGAM and ctMGAM^{26,127,133a}

Inhibitor	Structure	ctSI (nm)	ntSI (nm)	ntMGAM (nm)	ctMGAM-N2 (nm)	ctMGAM-N20 (nm)
Acarbose (1)		246	14 000	62 000	9	28
Selectively turn-off C-terminal enzymes						
Miglitol (2)		130	148	1000	211	230
Salacinol (5)		47	277	190	213	58
Kotalanol (6)		42	600	190	64	92
Neo-kotalanol (10)		26	600	30	26	78
Except ntSI, turn of all subunits						
Neo-ponkoranol (11)		103	302	43	NOI	96



Table 7 (Contd.)

Inhibitor	Structure	ctSI (nm)	ntSI (nm)	ntMGAM (nm)	ctMGAM-N2 (nm)	ctMGAM-N20 (nm)
3'- β -Maltose-extended de-O-sulfonated ponkoranol (131)		62	46	39	NOI	655
Except ctMGAM spliceforms, selectively turn-off all						
5'- β -Maltose-extended de-O-sulfonated ponkoranol (132)		45	19	8	77	67
Best inhibitor of ntMGAM						

^a NOI = no inhibition.

6. Conclusion

Glucosidase inhibitors from natural or synthetic sources can play biochemical tools for the evaluation of enzyme mechanism through kinetics parameters along with their variations in structural conformation. By virtue of this, a lead molecule can be discovered for therapeutic use. Now a days, α -glucosidase inhibitors have received a substantial attention for the development of antidiabetic drugs. This review highlighted the potent α -glucosidases inhibition potential by the sulfonium ion-based inhibitors, isolated from the various *Salacia* sp. and also describes the structural features which are important for effective inhibition of glucosidase enzyme. Mimicking in the design of sulfonium-ion could be a promising approach to the discovery of novel inhibitors. The intensive structure–activity relationship studies of naturally occurring sulfonium compounds, as well as their synthetic analogues, enable us to determine the more potent inhibitors. Subtle in structural modifications of molecules may improve

the selectivity of inhibitory potential. Such structural information will be helpful for the designing of main structural moiety needed for the inhibitory activity and can be used for future generation drug design and for the synthesis of specific inhibitors of the α -glucosidase enzymes subunits. The insights gathered in this review provides a wealth of information about sulfonium-ion α -glucosidase inhibitors, this knowledge will be useful in deriving more potent antidiabetic candidates from the bioactive natural molecule for the clinical development of antidiabetic therapeutics and can be used as a model for researchers in the field of natural product chemistry. Similar efforts in structural-activity relationship based evaluation can be devoted for other natural molecules to make it more potent. Furthermore, the crystal structure and the concept of toggling will give a lead in terms of treatment of diabetes and other metabolic syndrome-associated diseases. Inhibition data for the compounds listed in this article and the type of each glucosidase linked with a specific inhibitor are incorporated in a tabular form as an Appendix.



Appendix

Table 8 Inhibition data of compound (5–132) produced in various α -glucosidase and other enzymes associated with diabetes

Inhibitor	α -Glucosidase inhibition		Other enzyme inhibition	References
	K_i	IC ₅₀	IC ₅₀	
Salacinol (5)	0.31 ^a	9.6 ^a	NOI ^k	33, 86 and 93
	0.32 ^b	2.5 ^b	2.1 ^{l*,p} ~7.5 ^q	
	0.47 ^c	1.8 ^c		
	1.71 ^d	1.1 × 10 ⁻³ⁱ		
	0.01 ^e	NOI ^j		
	0.015 ^f			
	0.19 ^g			
	0.075 ^h			
Kotalanol (6)	0.23 ^a	—	—	35 and 78
	0.18 ^b			
	1.8 ^c			
	0.19 ^g			
Ponkoranol (7)	0.17 ^{g1}	—	—	100
De-O-sulfonated kotalanol (10)	0.03 ^{g1}	—	—	78
De-O-sulfonated ponkoranol (11)	0.043 ^{g1}	—	—	125
Diastereomers of salacinol				
(4)	2.17 ^d	—	—	81–84
	>5 ^e			
	>5 ^f			
	NOI ^g			
(13)	NOI ^h			85–88
	1.06 ^d	0.38 ⁱ	3.4 ^k	
	>5 ^e	NOI ^j	3.6 ^{l*} , ~7.5 ^q	
	>5 ^f			
	NOI ^g			
NOI ^h				
Heteroatom analogues of salacinol				
Ghavamiol (14)	>2.5 ^d	306 ^a	~7.5 ^{l,q}	89
	>5 ^e	44 ^b		
	>5 ^f	136 ^c		
	NOI ^g	>315 ^m		
	NOI ^h			
(15)	>8 ^d	—	—	89
	>5 ^e			
	>5 ^f			
	NOI ^g			
	NOI ^h			
Blintol (16)	0.4 ^h	—	~7.5 ^{l,q}	89 and 91–93
	0.72 ^d			
	>5 ^e			
	>5 ^f			
	0.49 ^g			
(17)	>5 ^h	—	~7.5 ^{l,q}	89 and 91–93
	>9 ^d			
	>5 ^e			
	>5 ^f			
	NOI ^g			
	NOI ^h			
Five-membered ring analogues of salacinol with different sugar stereochemistry				
(18)	>5 ^d	—	—	94
	0.052 ^c			
	0.019 ^f			
(19)	>30 ^d	—	—	94
	>5 ^e			
	>5 ^f			



Table 8 (Contd.)

Inhibitor	α -Glucosidase inhibition		Other enzyme inhibition	References
	K_i	IC_{50}	IC_{50}	
(20)	NOI^g	—	—	96
(21)	NOI^g	—	—	96
(27)	—	NOI^i NOI^j	NOI^k NOI^l $0.853^{n,r}$ NOI^o	98
(28)	—	NOI^i NOI^j	$2.47^{k,r,s}$ NOI^l $0.716^{n,r}$ NOI^o	98
(29 + 30)	—	NOI^i NOI^j	$1.87^{k,r,s}$ NOI^l NOI^n NOI^o	98
(31 + 32)	—	NOI^i NOI^j	NOI^k NOI^l $1.29^{n,r,s}$ NOI^o	98
Six-membered ring analogues of salacinol				
(22)	NOI^d NOI^g	—	—	97
(23)	NOI^d	—	—	97
(24a, 25, 26a)	NOI^d NOI^g	—	—	97
(24b)	NOI^d NOI^g $>5^h$	$1.41^{i,r,s}$ NOI^j	$0.016^{k,r}$ $0.467^{l,r}$ NOI^n NOI^o	97
(26b)	NOI^d NOI^g $>5^h$	—	—	97
(33)	—	NOI^i NOI^j	NOI^k $1.34^{l,r,s}$ $0.85^{n,r,s}$	98
(34)	—	$1.32^{i,r,s}$ NOI^j	— NOI^k $3.59^{l,r,s}$ NOI^n NOI^o	98
(37–40)	—	NOI^i NOI^j	NOI^k NOI^l NOI^n NOI^o	99
Seven-membered ring analogues of salacinol				
(35)	—	NOI^i NOI^j	NOI^k NOI^l NOI^n NOI^o	98
(36)	—	NOI^i	NOI^k $1.83^{l,s,t}$ NOI^n —	98
Chain-modified analogues of salacinol				
(41)	NOI^g	—	—	100
(42)	0.25^g	—	—	100
(43)	0.26^g	—	—	100



Table 8 (Contd.)

Inhibitor	α -Glucosidase inhibition		Other enzyme inhibition	References
	K_i	IC ₅₀	IC ₅₀	
(44)	0.17 ^g	—	—	100
(45)	41 ^g	—	—	101
(46)	NOI ^g	—	—	101
(47)	26 ^g	—	—	101
(48)	NOI ^g	—	—	101
(49)	25 ^g	—	—	101
(50)	5 ^g	—	—	101
(51)	0.65 ^g	—	—	102
(52)	0.14 ^g	—	—	102
(53)	0.10 ^g	—	—	13
(54)	0.10 ^g	—	—	13
(55)	35 ^g	—	—	13
(56)	8 ^g	—	—	13
Deoxy-salacinol analogues of salacinol				
(57)	—	>1320 ^a 780 ^b	—	103
(58)	—	>1260 ^a >1260 ^b	—	103
(59)	—	>1390 ^a > 1390 ^b	—	103
Frame-shifted analogues of salacinol				
(60)	20 ^g	—	—	104
(61)	53 ^g	—	—	104
(62)	NOI ^g	—	—	104
(63)	NOI ^g	—	—	104
De-O-sulfonated analogues of salacinol				
(64)	15.6 ^a 3.7 ^b	—	—	105
(65)	14.0 ^a 3.5 ^b	—	—	105
Carboxylate analogues of salacinol				
(66)	10 ^g	—	—	106
(67)	NOI ^g	—	—	106
(68)	21 ^g	—	0.3 ^{l,q}	107
(69)	NOI ^g	—	NOI ^{l,q}	107
Phosphate analogues of salacinol				
(70)	NOI ^g	—	—	110
(71)	NOI ^g	—	—	110
Anhydroseleno- and anhydrothio-allitols analogues of salacinol				
(72–79)	NOI ^g	—	—	111
Alkylated alkyl analogues with varying alkyl chain of salacinol				
(80–88)	6–7.5 ^g	—	—	112
3'-O-Alkylated analogues of salacinol				
(89)	—	5.3 ^a 0.46 ^b 0.39 ^c	—	113
(90)	—	1.7 ^a 0.12 ^b 0.27 ^c	—	113



Table 8 (Contd.)

Inhibitor	α -Glucosidase inhibition		Other enzyme inhibition	References
	K_i	IC ₅₀	IC ₅₀	
3'-And 5'- β -maltose-extended de-O-sulfonated ponkoranol (131)	0.039 ^{g1}	—	—	133
	0.062 ^{g2}			
	0.046 ^{g3}			
	NOI ^{g4}			
(132)	0.655 ^{g5}			133
	0.008 ^{g1}	—	—	
	0.045 ^{g2}			
	0.019 ^{g3}			
	0.077 ^{g4}			
	0.067 ^{g5}			

NOI: no inhibition. ^a α -glucosidase: rat intestinal maltase (K_i and IC₅₀ values in μ M); ^b α -glucosidase: rat intestinal sucrase (K_i and IC₅₀ values in μ M); ^c α -glucosidase: rat intestinal isomaltase (K_i and IC₅₀ values in μ M); ^d α -glucosidase: gluco-amylase G2 (K_i values in mM); ^e α -glucosidase: porcine pancreatic α -amylase (PPA) (K_i values in mM); ^f α -glucosidase: barley α -amylase (AMY1) (K_i values in mM); ^g α -glucosidase: recombinant human maltase glucoamylase (MGAM) (K_i values in μ M); ^{g1}nt-MGAM: n-terminal maltase glucoamylase (K_i values in μ M); ^{g2}ctSI: c-terminal sucrase isomaltase (K_i values in μ M); ^{g3}ntSI: n-terminal sucrase isomaltase (K_i values in μ M); ^{g4}ctMGAM-N2: spliceform of ctMGAM (K_i values in μ M); ^{g5}ctMGAM-N20: spliceform of ctMGAM (K_i values in μ M); ^h α -glucosidase: human pancreatic α -amylase (HPA) (K_i values in mM); ⁱ α -glucosidase: rice α -glucosidase (IC₅₀ values in mM); ^j α -glucosidase: baker's yeast α -glucosidase (IC₅₀ values in mM); ^k β -glucosidase: β -glycase from almond (IC₅₀ values in mM); ^l α -mannosidase: α -manase from jack beans (IC₅₀ values in mM); ^m α -mannosidase: α -manase from almond (IC₅₀ values in mM); ⁿ α -glucosidase: rat intestinal trehalase (K_i values in μ M); ^o β -galactosidase: β -Galase from *Aspergillus oryzae* (IC₅₀ values in mM); ^p α -galactosidase: α -galase from green coffee beans (IC₅₀ values in mM); ^q40% inhibition; ^r*Drosophila melanogaster* Golgi mannosidase II; ^s K_i values in mM; ^tpreliminary determined with K_m and one K'_m ; ^uIC₅₀ values in μ M.

Acknowledgements

The author, Dr Priyanka Bagri, is supported by a grant from University Grants Commission, New Delhi, India under Post-doctoral fellowship for women (PDFSS-2013-14-DEL-SC-5938).

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