REVIEW

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Glycosidase inhibitors and their chemotherapeutic value, part 3*

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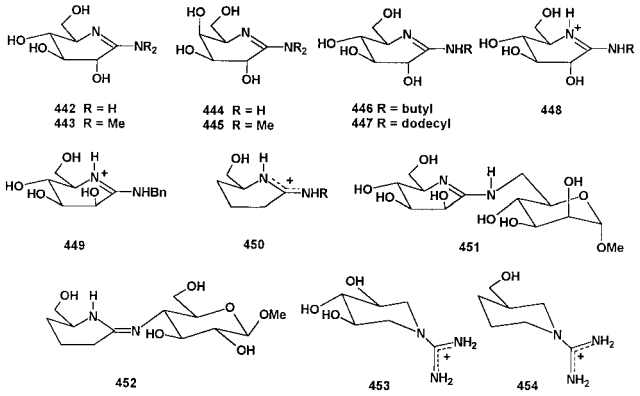
The various compounds that have been investigated as glycosidase inhibitors are reviewed. The last one of three parts of this review article covers the following classes of compounds: amidines, amidrazones, amidoximes, sugars with sulphur in the ring, carba sugars, pseudo oligosaccharides, cyclitols and trehazolamine analogues, fused heterocycles and trehazolin analogues, dioxane derivatives, open chain compounds, heterocyclic compounds with hydroxyalkyl residues, aromatic compounds, amino acids and other derivatives.

18. Amidines

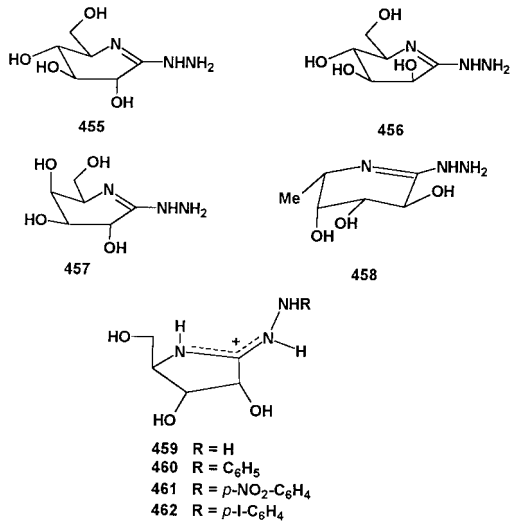
The amidines **442** and **443** possessing the D-gluco configurations, were competitive inhibitors of sweet almond β-glucosidase ($K_i = 1.0 \pm 0.2 \times 10^{-5}$ [400], $8.0 \pm 5.0 \times 10^{-6}$ [401] and $8.3 \pm 0.8 \times 10^{-5}$ [400] M, $8.3 \pm 0.5 \times 10^{-5}$ M [401], respectively). The inhibition was pH independent between 4.5 and 7.0. Amidine **442** was also a potent competitive inhibitor of jack bean α-mannosidase ($K_i = 9.0 \pm 1.0 \times 10^{-6}$ M, which was 4 to 5 fold better than **443**), and moderately inhibited the bovine liver β-galactosidase. The half-chair conformation of **442** might be responsible for its unusually broad spectrum of activity [46]. The D-galactoamidine **444** showed significant inhibition of almond β-glucosidase ($K_i = 2.5 \pm 0.5 \times 10^{-5}$ M), bovine liver β-galactosidase and green coffee bean α-galactosidase ($K_i = 8.5 \pm 0.4 \times 10^{-6}$ M, for both enzymes), but no inhibition of amyloglucosidase [400]. The *N,N*-dimethylamidine **445** was less potent than **444**. *N*-Butyl-D-gluconamidine (**446**) was found to be a good inhibitor of yeast α-glucosidase ($K_i = 9.0 \times 10^{-6}$ M) [28]. This inhibition was very similar to the inhibition by nojirimycin ($K_i = 6.3 \times 10^{-6}$ M) [24–27] and 1-deoxynojirimycin ($K_i = 2.5 \times 10^{-5}$ M) [402]. The data showed that for this enzyme the glucosyl cation-like character did not give any particular enhancement of the inhibitory potency. The activity against the *Asp. wentii* β-glucosidase by **446** increased with pH ($K_i = 1.3 \times 10^{-8}$ M at pH 5.0, 4.3×10^{-9} M at pH 6.0 and 3.0×10^{-9} M at pH 7.0), but this increase was less than that of other cationic glycons or substrate related inhibitors [129]. Compound **446** was a powerful inhibitor of bovine cytosolic and sweet almond β-glucosidases ($K_i = 1.3 \times 10^{-7}$, and 4.2×10^{-6} M, respectively). The kinetic studies showed that, in spite of the large K_s/K_i, compound **447** was not a transition state mimic but a cationic substrate analogue which has large inhibitory potency due to the formation of ion-pair with carboxylate groups of the active site. The inhibition of bovine cytosolic and bovine lysosomal β-glucosidase by *N*-dodecyl-D-gluconamidine (**447**) was very strong ($K_i = 2.0$ and 7.0×10^{-10} M, respectively) [28]. The amidinium ion **448** was active against β-galactosidase, α-mannosidase, α- and β-glucosidases and *N*-acetylglucosaminidase ($K_i = 8.0–10.0 \times 10^{-6}$ M). A strong competitive inhibition of jack bean α-mannosidase with amidine **449** was observed ($K_i = 5.0 \times 10^{-7}$ M) [403]. There was a prediction that a half-chair amidinium ion **450** should be an inhibitor of all glycosidases with K_i in the range $2.0–4.0 \times 10^{-5}$ M [116]. The comparison between the gluconamidines and the corresponding *N*-alky1-D-glucosylamines showed that the former compounds were slightly better inhibitors than the latter amines. Most glycosidases were inhibited much better by the unprotonated form of the inhibitor [24–27] provided that the protonated form has a pKa > 5.0. There were few enzymes like β-glucosidase A_3 from *Asp. wentii* which were strongly inhibited by the protonated or the permanently cationic glycon derivatives, e.g. glycosylpyridinium salts or *N,N*-dimethyliminoalditols. Nojirimycin (pKa 5.6) and its galacto-analogue were better inhibitors than the corresponding amidines and amidrazones. The advantage of the glycosyl cation-like structure of the amidine and amidrazone type inhibitors was due to their strong basicity which causes their full protonation and by the inability of the inhibitor cations to act as hydrogen bond acceptors [400]. The amidine pseudodimannoside **451** was a potent inhibitor of the enzyme involved in the processing of glycoproteins in mammalian cells. Thus, it showed competitive inhibition of jack bean α-mannosidase ($K_i = 2.6 \times 10^{-6}$ M, at pH 4.5) [404], however, low inhibition of snail β-mannosidase and sweet almond β-glucosidase ($K_i = 1.2$ and 1.0×10^{-4} M, respectively) was observed. The amidine derivative **452** was developed as a potential β-glucosidase inhibitor [405]. The kinetic measurements of meso-*N*-amidino-xylo-3,4,5-trihydroxypiperidine (**453**) and (*R*)-3-(hydroxymethyl)-1-amidino-piperidine (**454**) showed that, compound **453** exhibited strong competitive inhibition against almond β-glucosidase ($K_i = 2.5 \times 10^{-6}$ M) [404]. The inhibition of yeast α-D-glucosidase by **453** was of the mixed type and uncompetitive inhibition was observed with jack bean α-D-mannosidase. Compound **454** did not show any inhibitory effect towards yeast α-D-glucosidase or jack bean α-D-mannosidase. But



it showed mixed competitive inhibition towards emulsin β-D-glucosidase.

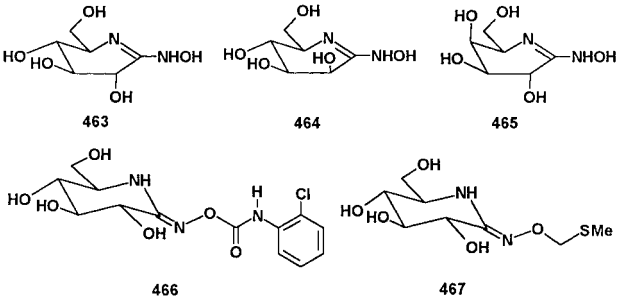
19. Amidrazones

The amidrazone **455** was a good competitive inhibitor of almond β-glucosidase ($K_i = 8.4 \pm 0.9 \times 10^{-6}$ M) [400, 406]. It displayed non-competitive inhibition against β-galactosidase ($K_i = 1.9 \pm 0.1 \times 10^{-5}$ M, *vide infra*) and potent competitive inhibition against jack bean α-mannosidase ($K_i = 3.1 \pm 0.6 \times 10^{-6}$ M) [400]. Amidrazone **456** inhibited jack bean α-mannosidase ($K_i = 1.7 \pm 0.2 \times 10^{-7}$ M, $IC_{50} = 4.0 \times 10^{-7}$ M) [400, 407]. It inhibited also mung bean α-mannosidase ($IC_{50} = 4.0 \times 10^{-7}$ M), fungal β-mannosidase ($IC_{50} = 1.5 \times 10^{-7}$ M), Golgi α-mannosidase I ($IC_{50} = 4.0 \times 10^{-6}$ M), and α-mannosidase II ($IC_{50} = 9.0$ to 10.0×10^{-8} M) [400, 407]. It was inactive against the purified plant processing glucosidases (glucosidase I and II) [337, 408], and against the fungal amyloglucosidase even at concentrations of 2.5×10^{-4} M. It was the first potent inhibitor of (soluble or endoplasmic reticulum) α-mannosidase ($IC_{50} = 1.0 \times 10^{-6}$ M) [407], and a strong inhibitor of both mannosidase I and mannosidase II of MDCK cells ($IC_{50} = 3.0$ to 4.0×10^{-6} M and 4.0×10^{-7} M, respectively) [407]. This inhibitor acted on the processing mannosidases, whereby the oligosaccharides were not processed beyond the high mannose structure. It also inhibited mannosidase I and the ER mannosidase *in vivo*, but at the levels used (1.0×10^{-5} g ml^{-1}) the inhibition of these enzymes, especially the ER mannosidase, was probably incomplete [407]. It had an effect on almond β-glucosidase. Amidrazone **456** exhibited a significant degree of linear, mixed, non competitive inhibition against bovine liver β-galactosidase ($K_i = 5.7 \pm 0.25 \times 10^{-5}$ M) [400]. Galactoamidrazone (**457**) showed a modest level of inhibition against jack bean α-mannosidase, and competitive inhibition against almond β-glucosidase ($K_i = 2.4 \pm 0.7 \times 10^{-6}$ M). It was three times more potent than glucoamidrazone (**455**) ($K_i = 8.4 \times 10^{-6}$ M) [400] against the latter's cognate enzyme. It was an active inhibitor of green coffee bean α-galactosidase ($K_i = 8.3 \pm 0.4 \times 10^{-6}$ M) and competitively inhibited bovine liver β-galactosidase ($K_i = 6.50.1 \times 10^{-6}$ M) [400]. In contrast to galactoamidrazone (**457**), gluco and mannoamidrazones (**455**) and (**456**) displayed non-competitive inhibition of bovine β-galactosidase, which has evolved both a galactose and glucose binding site to hydrolyze lactose [409, 410]. Amidrazones **455** and **456** apparently interacted strongly with the glucose site, causing weak non-competitive inhibition by binding with both the free enzyme and the enzyme substrate complex [46] whereas **457**, which possessed the natural D-galacto configuration, bound competively and selectively at the catalytic site. L-Fucoamidrazone **458** was a potent fucosidase inhibitor. Thus, it has displayed clean competitive inhibition against human α-L-fucosidase ($K_i = 8.2 \times 10^{-7}$ M) [411], under steady-state assay conditions [412]. It was found that the amidrazone **459** inhibited *C. fasciculata* nucleoside hydrolase with a dissociation constant of 1.0×10^{-6} M. *N*-Phenylamidrazone **460** was a slope-linear competitive inhibitor, for which full kinetic analysis revealed a $K_i = 2.1 \pm 0.13 \times 10^{-7}$ M. *p*-Nitrophenylamidrazone (**461**) exhibited slow-onset, tight binding inhibition, with an overall dissociation constant of 2.0×10^{-9} M, so it was the most potent nucleoside hydrolase inhibitor. *p*-Iodophenylamidrazone (**462**) was very potent inhibitor of nucleoside hydrolase ($K_i = 8.0 \times 10^{-9}$ M) [46].



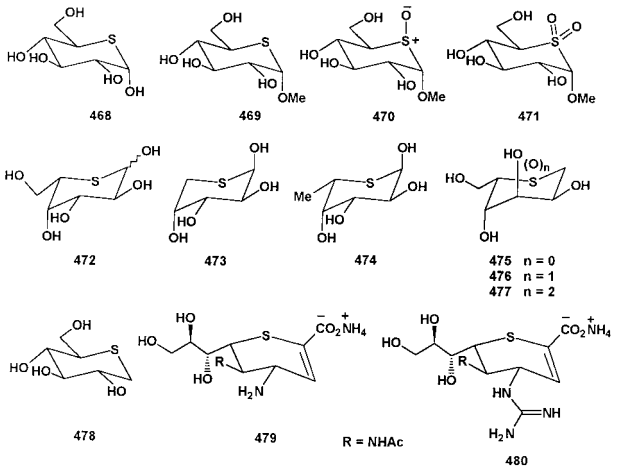
20. Amidoximes

The amidoxime **463** was a good competitive inhibitor of almond β-glucosidase ($K_i = 1.4 \pm 0.3 \times 10^{-5}$ M) due to its basic character [400, 406]. It strongly inhibited yeast α-glucosidase ($K_i = 2.9 \times 10^{-6}$ M) [79]. A very similar inhibition was found for the 2-chlorophenyl carbamate **466** ($K_i = 3.4 \times 10^{-6}$ M) [79]. The manno analogue **464** was a strong inhibitor of jack bean α-mannosidase ($K_i = 1.5 \times 10^{-7}$ M [65], $2.0 \pm 0.1 \times 10^{-6}$ M [400]), whereas glucohydroximolactam **463** inhibited this enzyme about eighty times more weakly ($K_i = 1.2 \times 10^{-5}$ M) [65]. The galacto analogue **465** showed significant inhibition of almond β-glucosidase, and competitively inhibited bovine β-galactosidase ($K_i = 1.0 \pm 0.05 \times 10^{-5}$ M) [400], but it was much weaker than expected (59% of control activity). It also inhibited green coffee bean α-galactosidase, but did not inhibit amyloglucosidase. The (methylthio)methyl ether **467** can bind to yeast α-glucosidase in a purely competitive mode ($K_i = 5.0 \times 10^{-5}$ M), and inhibited almond and *C. saccharolyticum* β-glucosidase ($K_i = 2.4 \times 10^{-5}$ and 2.4×10^{-6} M, respectively).



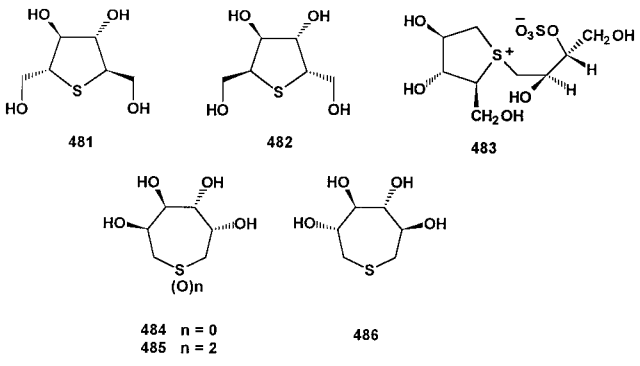
21. Sugars with sulfur in the ring

5-Thioglucose (**468**) was a moderate inhibitor of α-glucosidase from brewer's yeast ($K_i = 7.5 \times 10^{-4}$ M). It exhibited weak inhibition against β-glucosidase from sweet almond ($K_i > 1.0 \times 10^{-2}$ M) [116]. Compounds **469–471** showed some inhibition against α-glucosidase from brewer's yeast ($K_i > 5.0 > 5.5$ and $> 2.0 \times 10^{-3}$ M, respectively), but were not as potent as **468** and displayed no inhibition against β-glucosidase from sweet almond. 5-



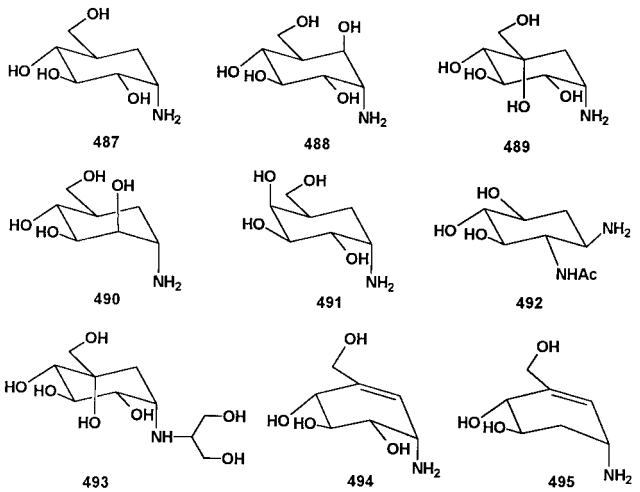
Thio-L-galactose (**472**) showed competitive inhibition towards α-L-fucosidase from the bovine kidney ($K_i = 9.6 \times 10^{-4}$ M) [413] as well as the 5-thio-D-arabinose (**473**) ($K_i = 7.7 \times 10^{-4}$ M), which was about 9 times larger than that of 5-thio-L-fucose (**474**) ($K_i = 8.4 \times 10^{-5}$ M) [414]. This difference in K_i values indicated that the α-L-fucosidase-5-thio-D-arabinose complex was destabilized by 1.4 Kcal/mol in comparison with its 5-thio-L-fucose complex. Thus, the 6-methyl group in L-fucose was crucial for binding with α-L-fucosidase from bovine kidney [415]. L-Gulo-tetrahydrothiopyrane (**475**) competitively inhibited the α-D-mannosidase ($K_i = 7.0 \times 10^{-4}$ M). Comparison of inhibitory activity of **475**–**477** showed that oxidation into sulfoxide and sulfone, respectively, reduce the inhibition. Like 5-thio-D-glucose [116] which was an inhibitor of α-D-glucosidase, oxidation into sulfoxide or sulfone weakened the inhibition [416]. 1-Deoxythionojirimycin (**478**) was less potent inhibitor of α- and β-glucosidases than its parent aza sugar. The same behaviour has been reported for 1-deoxythiomannojirimycin [417, 418] which was a weak inhibitor of α-D-glucosidase and inactive towards β-D-glucosidase, whereas 1-deoxymannojirimycin [181, 230] was a good inhibitor of α- and β-D-glucosidase and α-D-mannosidase. The thioisosteres of 4-amino-4-deoxy-Neu-5Ac2en **479** and 4-deoxy-4-guanidino-Neu-5Ac2en **480** were found to be as bioactive as their oxygen counterparts against influenza virus sialidase ($IC_{50} = 1.0 \times 10^{-6}$ and 5.0×10^{-9} M, respectively) [419].

Thiosugars with a tetrahydrothiophene or thiepane insiety (**481**–**486**) showed that they are weak inhibitors of several glycosidases (α- and β-D-glucosidases, α-D-mannosidase and α-L-fucosidase). For example, the tetrahydrothiophenes **481** and **482** were less potent inhibitors of α-D-glucosidase than the parent aza sugars (I = 20 and 18%, respectively). L-Ido-thiepane (**486**) showed a weak inhibition of α-D-glucosidase ($K_i = 3.9 \times 10^{-3}$ M). The comparison of the inhibitory activity of **484** and **485** showed that the oxidation of thiepane into sulfone reduced the inhibition of β-D-glucosidase [416]. The most potent natural α-glucosidase inhibitor named salacinol (**483**) has been isolated from an antidiabetic Ayurvedic traditional medicine, *Salacia reticulate* WIGHT, showed competitive inhibition for the intestinal α-glucosidase *in vitro* ($K_i = 3.1 \times 10^{-7}$ g ml^{-1}, $IC_{50} = 3.2 \times 10^{-6}$ g ml^{-1} to maltase, $K_i = 3.2 \times 10^{-7}$ g ml^{-1}, $IC_{50} = 8.4 \times 10^{-7}$ g ml^{-1} to sucrase and $K_i = 4.7 \times 10^{-7}$ g ml^{-1}, $IC_{50} = 5.9 \times 10^{-7}$ g ml^{-1} to isomaltase). The inhibitory activities of **483** against maltase and sucrase were almost equal to those of acarbose ($K_i = 1.2$ and 3.7×10^{-7} M, respectively) while its inhibitory activity against isomaltase was much stronger than that of acarbose ($K_i = 7.5 \times 10^{-5}$ g ml^{-1}). Salacinol inhibited more strongly the increase of serum glucose levels in sucrose-loaded rats than acarbose [420].



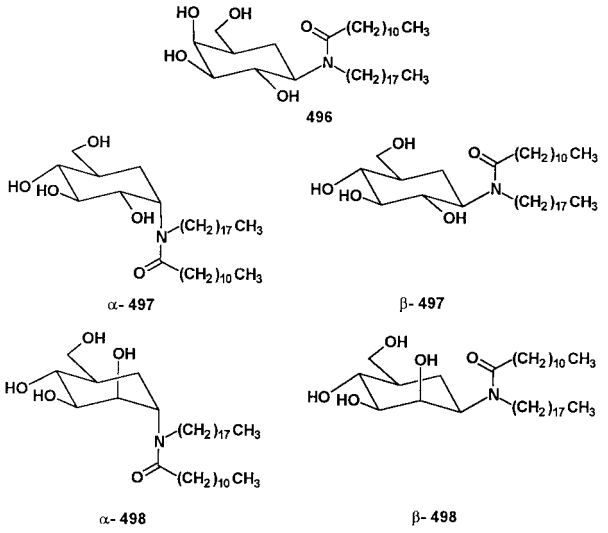
22. Carba sugars

It was anticipated that the close resemblance of carba sugars to the true sugars may lead to their acceptance by enzymes and incorporation into biological systems instead. Thus, the topological resemblance of some of these carba sugars to the postulated transition state glucosyl cation of the glucoside hydrolysis led to their anticipation as good inhibitors of α-glucosidases [421]. The validamycin complex, isolated from the fermentation broth of *Streptomyces hygroscopicus* subsp. *limoneus* [422], exhibited a strong effect in the control of sheath blight of rice plants. The carba-aminosugars isolated from this complex had inhibitory potency against α-glucosidases. The inhibitory activity against α-glucosidase I of validamine (**487**) ($IC_{50} = 7.8 \times 10^{-4}$ M), and valienamine (**494**) ($IC_{50} = 1.2 \times 10^{-5}$ M)



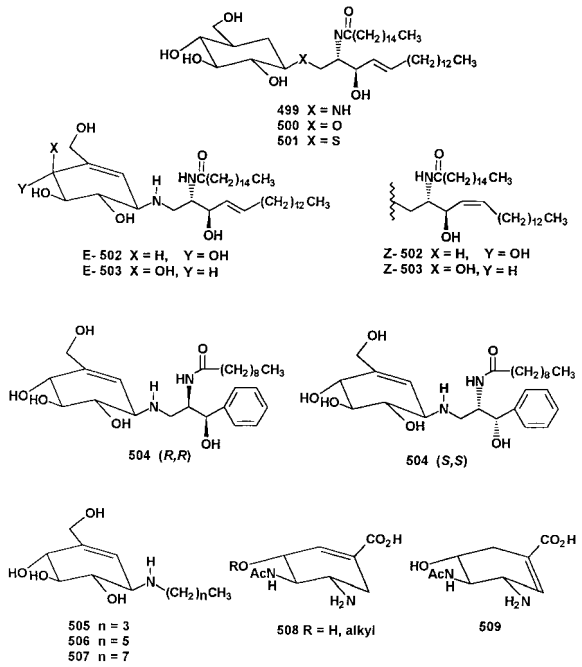
and hydroxyvalidamine (**488**) has been also evaluated. Thus, valiolamine (**489**) has the strongest inhibitory activity against porcine maltase ($IC_{50} = 2.2 \times 10^{-6}$ M) and sucrase ($IC_{50} = 4.9 \times 10^{-8}$ M) [423]. The inhibitory activities of manno- and galacto-validamines **490** and **491** were determined against several mannosidases and galactosidases, respectively. Compound **490** showed potent competitive inhibition of jack bean and almond α-mannosidases ($K_i = 4.6$ and 2.8×10^{-5} M, respectively) as well as endoplasmic reticulumal, lysosomal and Golgi α-mannosidases ($K_i = 1.2 \times 10^{-6}$, 1.7×10^{-5} and 2.8×10^{-5} M, respectively). While the galacto derivative **491** showed a moderate inhibition of the α- and β-galactosidases (coffee bean, *E. coli* and *Asp. niger*) with IC_{50} in the range of 10^{-4} M [424].

The pseudo sugar analogue **492** behaved as a weak inhibitor against *N*-acetylglucosaminidase from *Charonia lampas* [425] ($IC_{50} = 2.0 \times 10^{-3}$ M). Voglibose (**493**), which was a derivative of valiolamine was the first α-glucosidase inhibitor marketed in Japan and it was a potent sucrase ($IC_{50} = 4.6 \times 10^{-9}$ M) and maltase ($IC_{50} = 1.5 \times 10^{-8}$ M) inhibitors. It was considered an effective therapeutic agent for the treatment of diabetes [31, 426]. Administration of voglibose may improve hyperglycemia and hyperlipidemia *via* inhibition of digestion and absorption of carbohydrate in OLETF [427]. It significantly decreased the responses of plasma glucose and insulin in the meal tolerance test, and showed a significant decline of triglyceride levels and an elevation of high-d lipoprotein (HDL) cholesterol and apolipoprotein A-1 levels [428]. 2-Deoxyvalienamine (**495**) did not exhibit any inhibitory activity against β-glucosidase, α-mannosidase, isomaltase or glycogen phosphorylase [429]. The lack of a 2-hydroxy group in the cyclohexene ring was suggested to cause this lack of activity. It is, however, a weak inhibitor of α-glucosidase [429].



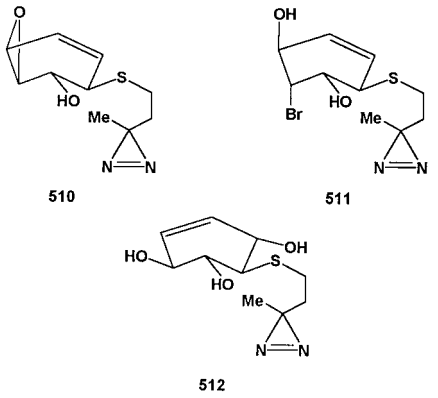
The 5a-carba-sugars analogues **496**–**498** possessed a comparable potency as an immunomodulator to the corresponding glycosylamide analogues [430–432].

5a-Carba-glucosylceramide (**499**) moderately inhibited the β-glucocerebrosidase and β-galactocerebrosidase [433], whereas the ether **500** and sulfide **501** analogues showed no marked inhibitory potencies against both enzymes. The unsaturated analogues *E*- and *Z*-**502** almost completely inhibited β-glucocerebrosidase at a concentration of 1.0×10^{-5} M; the IC_{50} values of the *E*- and *Z*-isomers were 3.0 and 1.0×10^{-7} M, respectively [434]. The *E*-isomer **502** inhibited the infection of normal human peripheral blood mononuclear cells with HIV-1 *in vitro* by 47%, taking the inhibition effect of AZT as 100% [435]. The β-galacto-analogues *E*- and *Z*-**503** showed potent and specific inhibitory activity against β-galactocerebrosidase ($IC_{50} = 2.7$ and 4.5×10^{-6} M, respectively), whereas both analogues did not inhibit glucosylceramide synthase at all. Preliminary bioassay of 1-phenyl-2-decanoylamino-3-(β-valienaminyl)-1-propanol (**504**) indicated that it was a strong β-glucocerebrosidase inhibitor ($IC_{50} = 7.0$ and 3.0×10^{-7} M, for the *R*,*R* and *S*,*S*-isomers, respectively) as well as it was a potent β-galactocerebrosidase [436]. The complex ceramide portion could be replaced by a simple aliphatic chain without affecting the activity. Thus



homologous series of *N*-alkyl-β-valienamines **505**–**507** were potential inhibitors of β-glucocerebrosidase [437] ($IC_{50} = 1.1 \times 10^{-5}$, 3.0×10^{-7} and 3.0×10^{-8} M, respectively), and α-glucosidase ($IC_{50} = 5.0 \times 10^{-5}$ M for **506** and 1.7×10^{-5} M for **507**). The structural activity of carbocyclic analogues **508** as transition-state-based inhibitors of influenza neuraminidase (NA) showed that the length, size of branching, and geometry of the alkyl groups in **508** profoundly influence NA inhibitory activity. A steady increase in enzyme inhibitory activity was observed up to the n-propyl analogue. The over 20-fold increase in NA inhibitory activity for **508** ($R = CH_3CH_2CH_2$) compared to **508** ($R = Me$) implicated a significant hydrophobic interaction of the n-propyl group with amino acids in the active site. The high antiviral potency observed for **508** ($R = (Et)_2CH$, $IC_{50} = 1.0 \times 10^{-9}$ M) appeared to be attributed to the high favorable hydrophobic interaction in this pocket. Replacing of the amino group in **508** ($R = (Et)_2CH$) with the guanidino moiety resulted in a significant increase in its enzymatic and cell culture activities. Thus, carbocyclic NA inhibitors represented by **508** ($R = (Et)_2CH$) and its guanidino analogue were more potent inhibitors than **148** and **149** [438]. The double bond position in the carbocyclic analogue played an important role in NA inhibition as demonstrated by the antiviral activity of **508** ($R = H$, $IC_{50} = 6.3 \times 10^{-6}$ M) *vs* **509** ($IC_{50} > 2.0 \times 10^{-4}$ M).

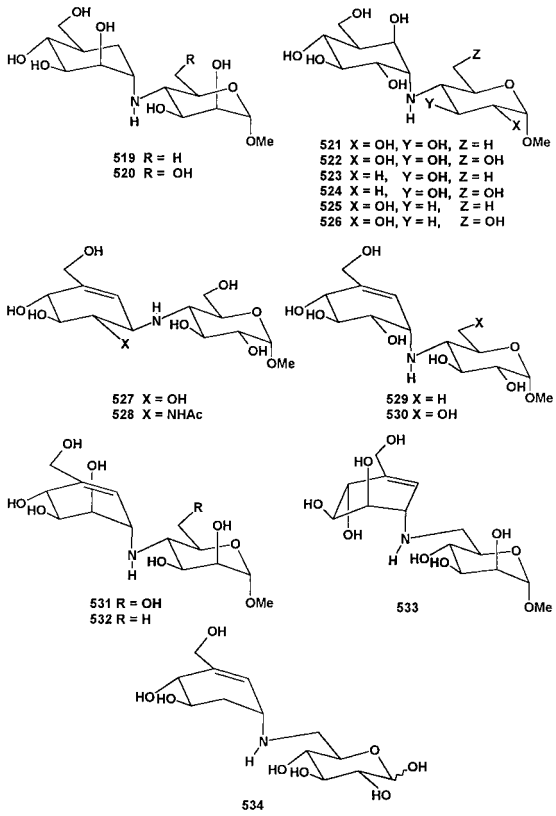
(1/2,5,6)-2-(3-Azibutylthio)-5,6-epoxy-3-cyclohexen-1-ol (**510**) was found to be an irreversible inactivator of β-D-galactosidase (*Escherichia coli*) [439]. Inactivation was prevented by the presence of isopropyl 1-thio-β-D-galactopyranoside (IPTG). Compound **510** showed a very weak competitive inhibition of *E. coli* β-D-galactosidase ($K_i = 2.9 \times 10^{-2}$ M). (1,2/3,6)-6-(3-Azibutylthio)-2-bromo-4-cyclohexene-1,3-diol (**511**) and (1,3/2,4)-3-(3-azibutylthio)-5-cyclohexene-1,2,4-triol (**512**) showed the same inhibition against the same enzyme ($K_i = 1.3$ and 2.3×10^{-2} M, respectively) [439]. There was an evidence of non-competitive inhibition in the case of compound **512**. Its structure (as an inositol) could explain its function as a good transferolytic acceptor in the β-D-galactosidase-catalysed transfer reaction [440]. This would result in non-competitive inhibition kinetics.



510 511 512

23. Pseudo oligosaccharides

Inhibition studies showed that **513** was stable against enzymic cleavage and competitively inhibited β-D-galactosidase from *Escherichia coli* with $K_i = 5.5 \times 10^{-3}$ M [441]. The unseparable mixture of the regioisomers (1,3/2,4)-1,2-diamino-3-*O*-(β-D-galactopyranosyl)-4-hydroxycyclohexane (**514**) and (1,3/2,4)-1,2-diamino-4-*O*-(β-D-galacto-pyranosyl)-3-hydroxycyclohexane (**515**) inhibited β-D-galactosidase competitively with $K_i = 9.0 \times 10^{-4}$ M [441]. The glucuronide derivative **516** showed an inhibitory activity to the hyaluronidase from beef testes up to 5.0×10^{-3} M [425]. The 4′-*O*-(β-D-galactopyranosyl)-β-valienamine derivative **517** has as strong inhibition properties as (*R*,*R*)-



519 R = H
520 R = OH

521 X = OH, Y = OH, Z = H
522 X = OH, Y = OH, Z = OH
523 X = H, Y = OH, Z = H
524 X = H, Y = OH, Z = OH
525 X = OH, Y = H, Z = H
526 X = OH, Y = H, Z = OH

527 X = OH
528 X = NHAc

529 X = H
530 X = OH

531 R = OH
532 R = H

533

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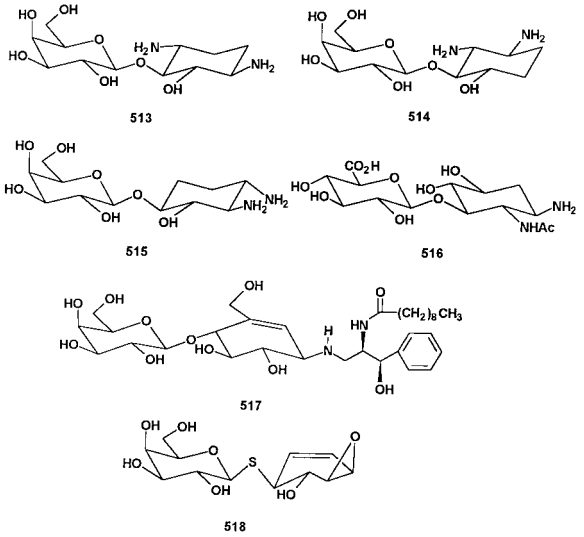


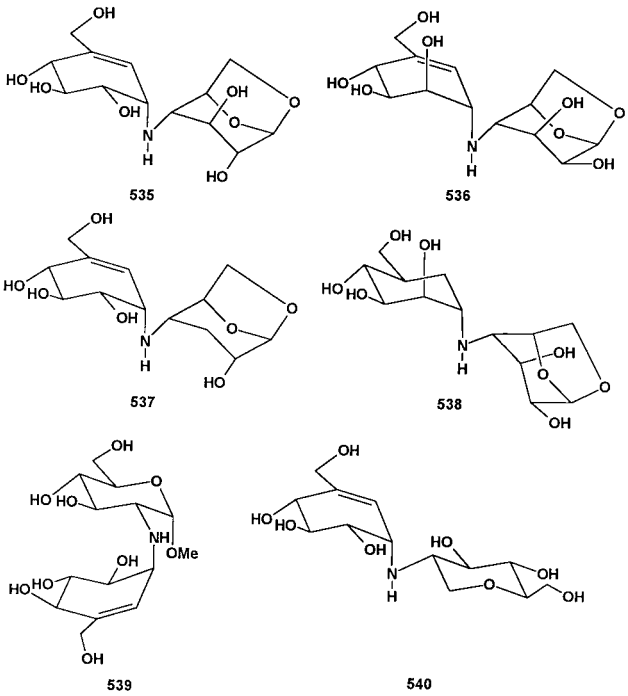
513 514 515 516 517 518

504. The pseudo oligosaccharide (3,4,6/5)-3,4-epoxy-6-(β-D-galactopyranosylthio)-5-hydroxycyclohexene (**518**) was a good competitive inhibitor of *E. coli* β-D-galactosidase ($K_i = 8.5 \times 10^{-5}$ M). Although **518** decomposed in buffer at pH 6.8 ($t_{½} \sim 3.3$ h), it deactivated the enzyme irreversibly at a high initial rate [442].

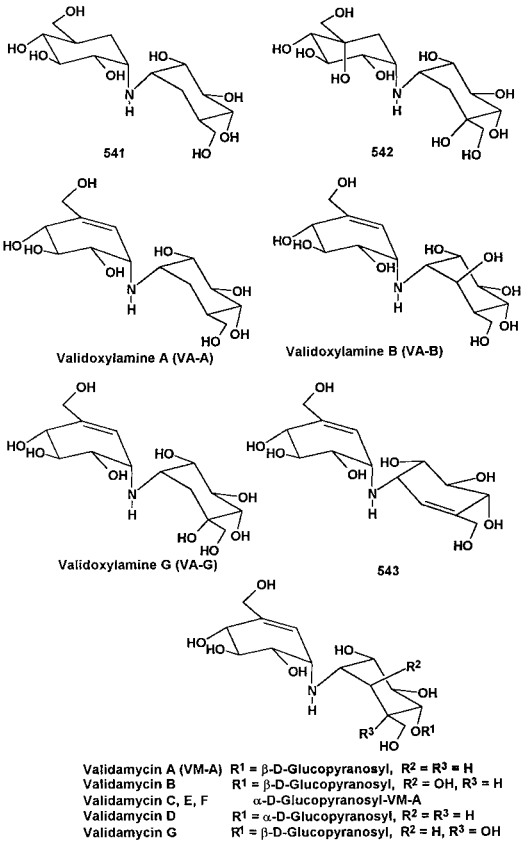
A very weak inhibition of both α- and β-D-glucosidase (9.5% and 9.8% respectively) was observed for compound **519**. It also inhibited α-D-mannosidase (34.7%). Compound **520** was a moderate inhibitor of jack bean α-D-mannosidase (48.5%), but it was a very weak inhibitor of α- and β-D-glucosidase (7.5% and 5.0%, respectively) [443]. The inhibitory activities of the pseudo disaccharides **521**–**526** against α- and β-D-glucosidases and α-D-mannosidase indicated that methyl oligobiosaminide **521**, the core structure of oligostatin has almost no inhibitory activity, whereas compounds **523** and **526** possess appreciable activity against α-D-glucosidase and α-D-mannosidase (65.4% and 43.9%, respectively) [444]. Compounds **527** and **528** did neither inhibition β-glucosidase nor chitinase [445, 446]. The potent α-glucosidase inhibitor methyl acarviosin (**529**) may be considered as an imino-linked carba maltose [447]. During the course of study on chemical modification of **529**, it was found that the 6-hydroxy moiety could be replaced without affecting the inhibitory potency ($IC_{50} = 3.0$ and 9.8×10^{-5} g ml^{-1} for **529** and **530**, respectively) [71, 445]. The carba disaccharide **531** was inactive against jack bean α-D-mannosidase and almond β-D-glucosidase although it exhibited moderate inhibitory activity against yeast α-D-glucosidase (43.5%), while the corresponding 6-deoxy analogue **532** had mild and moderate inhibitory activity against both of α-D-glucosidase and α-D-mannosidase (73.5 and 40.7%, respectively) [443], and was a very weak inhibitor of β-D-glucosidase (7.7%). The carba disaccharide **533** has a good competitive inhibitory activity and a high degree of selectivity against jack bean α-D-mannosidase ($K_i = 3.0 \times 10^{-5}$ M) [448], but it was inactive against yeast α-D-glucosidase, almond β-D-glucosidase, coffee bean α-D-galactosidase, jack bean β-D-galactosidase, and snail β-D-mannosidase. N-Acetylation of **533** removed its inhibitory activity against jack bean α-D-mannosidase completely, pointing to the importance of the basic NH group in the inhibitory process [448]. The glycosidase inhibition of 6-amino-6-deoxy-6-*N*-((1*R*,3*R*,4*R*)-3,4-dihydroxy-5-hydroxymethylcyclohex-5-enylamino)-D-glucose (**534**) showed that this compound moderately inhibited α-glucosidase at slightly acidic conditions. It inhibited α-glucosidase from baker's yeast with K_i values of 3.2 and 4.5×10^{-4} M at pH 6.8 and 6.2, respectively. But inhibition of this enzyme was weak at pH 7.5 ($K_i = 1.0 \times 10^{-3}$ M). Other glycosidases such as α-mannosidase from jack bean and β-glucosidase from almonds were weakly inhibited by the isomaltose analogue **534** ($K_i > 1.0 \times 10^{-3}$ M). A moderate inhibition

of glycogen phosphorylase ($IC_{50} = 3.6 \times 10^{-4}$ M) was observed for compound **534**. Very surprisingly, no inhibition of isomaltase was seen with **534** which may be due to the lack of a 2-hydroxy group in the cyclohexene ring [429].

A slight decrease in the inhibitory activity of the 1,6-anhydro analogues against α-glucosidase was observed as compared with their parent hydroxy compounds. Thus **535** and **537** showed the IC_{50} values 1.8 and 0.3×10^{-5} g ml^{-1}, respectively [71, 449]. Compound **536** weakly inhibited α-D-mannosidase (7.7%) and α-D-glucosidase (35.1%) but no inhibition of β-D-glucosidase was observed [443]. Compound **538** was a very weak inhibitor of β-D-glucosidase (2.3%) and α-D-glucosidase (8.4%), but it moderately inhibited α-D-mannosidase (44.3%) [443]. This compound has been introduced as an effective agent in diabetes therapy. The pseudo Kojibiose **539**, containing the unsaturated carba-aminosugar valienamine showed strong inhibition of baker's yeast α-glucosidase ($K_i = 3.1 \times 10^{-6}$ M) [450]. It did not possess any inhibitory activity against both maltase (porcine) and processing glucosidase I (rat liver microsomes) [451]. The trehalase inhibitor salbostatin (**540**) has recently been isolated [452].

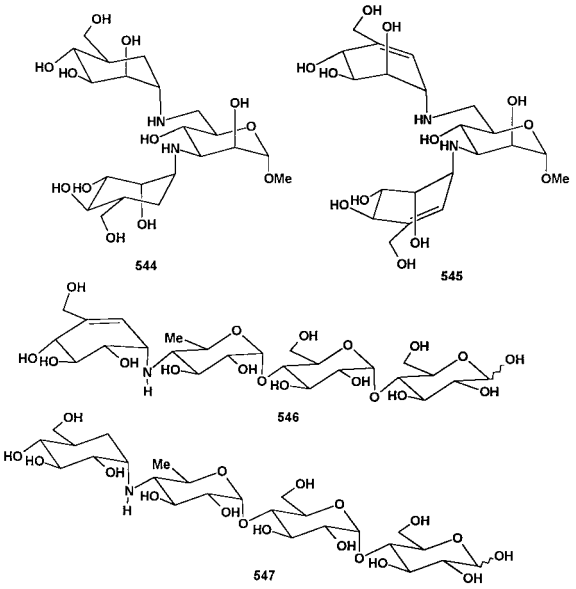


The carba disaccharides **541** and **542** composed of validamine **487** and valiolamine **489**, respectively possessed strong activity against trehalase from muscidae ($IC_{50} = 7.3 \times 10^{-6}$ and 1.5×10^{-5}g ml^{-1}, respectively) indicating that the valienamine part was not essential for a trehalase inhibitor, and symmetrical structures mimicking the substrate played a key role as trehalase inhibitors [453]. Validoxylamine A can be considered as a mimic of the substrate α,α-trehalose and was likely to bind to the active site of the enzyme trehalase which was inhibited in a competitive manner ($K_i = 1.9 \times 10^{-9}$ M, $IC_{50} = 1.4 \times 10^{-7}$ M). The validoxylamine B has an IC_{50} of 1.6×10^{-5} M and validoxylamine G has $IC_{50} = 7.4 \times 10^{-6}$ M. The carba disaccharide **543** which is composed of valienamine (**494**) strongly inhibited trehalase from muscidae ($IC_{50} = 1.3 \times 10^{-5}$g ml^{-1}) [453]. Some validamycins showed potent inhibitory activity against trehalase of *Rhizoctonia solani*, whereas no significant activity was exhibited against



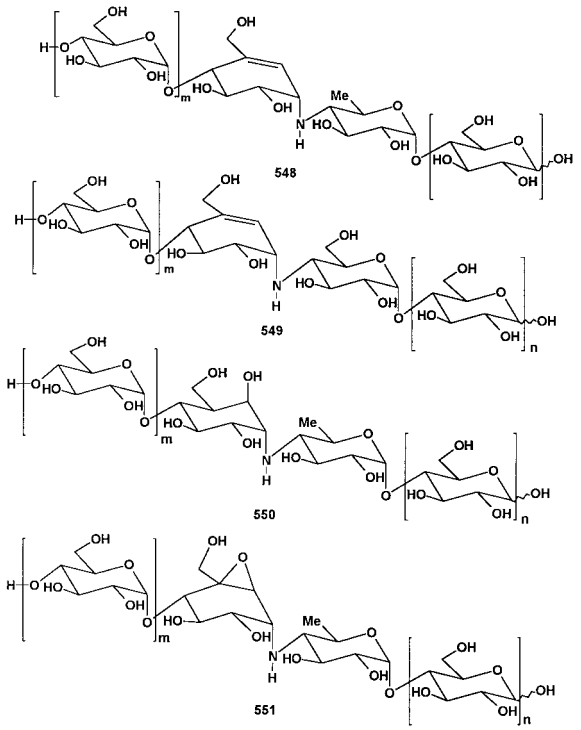
cellulase, pectinase, chitinase, α-amylase, α- and β-glucosidase [454]. Eight validamycins (A-H) and validoxylamines have been isolated from the fermentation broth. On the other hand, carba glycosylamines were found substantially as minor components in the fermentation broth [455]. The pseudo tetrasaccharides such as validamycins C, E, and F had no inhibitory effect on trehalase. Validamycin A was the major component of the complex and most active against sheath-blight disease caused by *Rhizoctonia solani*. Validamycin A was neither antimicrobial nor fungicidal, but controlled disease on host plants by retarding hyphal elongation of the fungus. It was found that validamycin A was transported into the cell and hydrolyzed therein by a β-glucosidase giving validoxylamine A with greater inhibitory activity. Validamycin A suppressed the *in vivo* degradation of the intracellular trehalase at very low concentrations [454].

The imino-linked trisaccharide **544** was a mild jack bean α-D-mannosidase inhibitor, showing 64% inhibition ($IC_{50} = 7.4 \times 10^{-5}$ M), whereas its C-5, C-5a unsaturated carba-sugar **545** was shown to be somewhat greater ($IC_{50} = 1.4 \times 10^{-8}$ M, 91% inhibition) [456]. Many validamycins were isolated from the culture broths of streptomycetes. The most important member was acarbose (precose, Glucobay®, **546**) which was the first α-glucosidase inhibitor to be used in the treatment of patients whose non-insulin dependent diabetes mellitus cannot be managed by diet alone. It is useful as an adjuvant therapy in diabetes [457–460]. Acarbose delays the carbohydrate metabolism in the gastrointestinal tract and consequently regulates the sugar level in blood. It competitively and reversibly inhibits pancreatic α-amylase and membrane bound intestinal α-glucosidase which were involved in carbohydrate digestion [30]. In its structure, a maltose unit is linked to acarviosin. Its homologues inactivate α-amylase and sucrase quite differently. The inhibition is maximum with homolo-



gues having four or five glucose units. The K_i values of acarbose towards hydrolysis of maltose, maltotriose, maltotetraose and maltoheptaose by human small glucoamylase-maltase are 8.4 ± 0.8, 4.7 ± 0.7, 4.2 ± 0.3 and 4.2 ± 0.4×10^{-7} M, respectively [215]. It inhibits glucose absorption in the rate jejunum by reducing postprandial hyperglycemia [461, 462]. Acarbose, competitively inhibited ($K_i = 1.1 \times 10^{-6}$ M) sucrase activity, and strongly suppressed glycemic responses in both sucrose loading ($ED_{50} = 1.1 \times 10^{-3}$ g/kg) as well as starch loading ($ED_{50} = 1.7 \times 10^{-3}$ g/kg) in mice [54]. Dihydroacarbose (**547**) was found to be a powerful, non-competitive inhibitor ($K_i = 1.1 \times 10^{-6}$ M) of sucrase (small-intestinal mucosa of rat) [463].

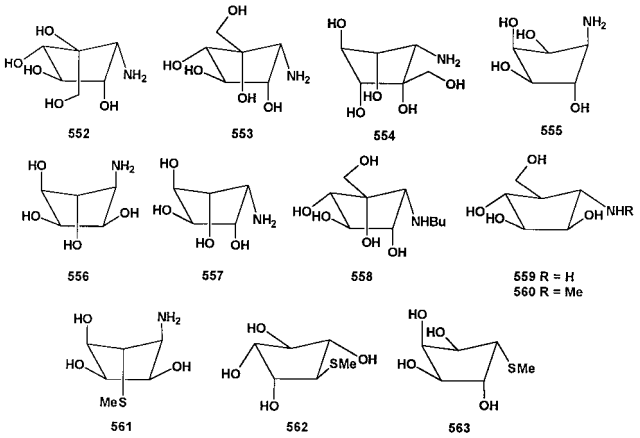
The homologous series of pseudo-oligosaccharides amylostatins (**548**), adiposins (**549**), oligostatins (**550**), trestatins, and NS-504 (**551**) have been isolated and characterized as α-glucosidase inhibitors. They exhibited potent



inhibition against intestinal α-glucosidase of mammals. They were found in the culture filtrate of *Actinoplanes* strains. Their essential core structure is a pseudo disaccharide, α-methyl acarviosin (**529**), obtained by the methanolysis of acarbose [31].

24. Cyclitols and trehazolamine analogues

The trehazolamine **552** has a moderate inhibitory potency against α-glucosidase ($IC_{50} = 2.6 \times 10^{-4}$ M) and a mild β-glucosidase inhibition ($IC_{50} = 2.1 \times 10^{-5}$ M). Compound **553** possessed a strong potency against baker's yeast α-glucosidase ($K_i = 5.0 \times 10^{-7}$ M, $IC_{50} = 9.5 \times 10^{-7}$ M), whereas **554** has moderate potency. The tetraol **555** but not **556** or **557**, which mimic the half-chair conformation of the galactosyl cation, showed a good inhibition against *E. coli* β-galactosidase ($IC_{50} = 7.8 \times 10^{-6}$ M) [31]. Interestingly, **555** and **557** possessed a weak potency against β-glucosidase. The N-butyl derivative **558** has a very strong activity against α-glucosidase (baker's yeast) [31]. The aminocyclopentane **559** was an α-mannosidase inhibitor ($IC_{50} = 1.0 \times 10^{-6}$ M) [464]. Molecular modeling studies of hydroxycyclopentylamine (**560**) show that all of the polar substituents on **560** overlap with analogous groups in the mannosyl cation, in addition the exocylic

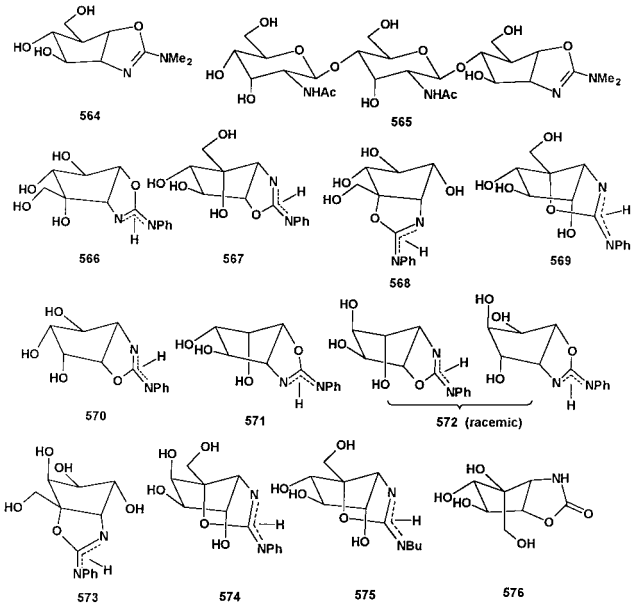


amine lies between the ring oxygen and C-1 of the mannosyl cation which was a favorable orientation to form hydrogen bonds with residues in the catalytic region of the enzyme active site. It was a potent inhibitor of jack bean α-mannosidase ($IC_{50} = 6.2 \times 10^{-8}$ M), consistent with its similarity to the proposed model [198]. Mannostatin A (**561**) was a potent α-mannosidase inhibitor ($IC_{50} = 1.0–1.5 \times 10^{-8}$ M) [465], and was completely inactive against β-mannosidase even at concentrations high as 2.0×10^{-3} M. Also it was fairly inactive against mannosidase I even at concentrations as of 2.5×10^{-4} M [407]. Compound **562** showed inhibition against almond β-glucosidase ($IC_{50} = 8.0 \times 10^{-6}$ g ml^{-1}) and was shown to be a topographical analogue of β-D-glucopyranoside, while compound **563** proved to be a specific inhibitor of jack bean α-mannosidase ($IC_{50} = 4.0 \times 10^{-8}$ g ml^{-1}) [466].

25. Fused heterocycles and trehazolin analogues

Allosamizoline (**564**) was a sugar mimic of the trisaccharide allosamidin (**565**) which was a chitinase inhibitor, inhibited *Streptomyces chitinase* with an IC_{50} value of 2.0×10^{-6} M, which was close to that reported earlier (3.7×10^{-6} [467], 3.5×10^{-7} M [369, 372]). Allosamidin

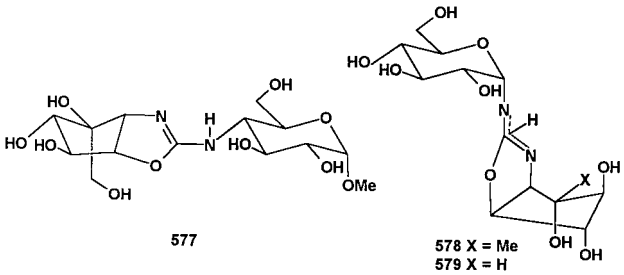
(**565**) had little or no inhibitory effects on other glycosidases. In contrast, allosamizoline (**564**) inhibited chitinase slightly even at 5.0×10^{-4} M (I = 20%), whereas, it inhibited rat intestinal disaccharidases with IC_{50} values ranging from 3.4×10^{-5} to 8.1×10^{-6} M much more potently than allosamidin [468]. *N*-Phenyl cyclic isoureas of 5-aminocyclopentanetetraol derivatives as modified aminocyclitols were prepared as glycosidase inhibitors [469]. This modification may change charge distributions and conformations. The inhibitory activities of the *N*-phenyl cyclic isourea derivatives **566**–**575** indicated that compound **567** exhibited a moderate inhibition against bovine liver β-galactosidase ($IC_{50} = 1.5 \times 10^{-4}$ M), whereas **569** strongly inhibited this enzyme ($IC_{50} = 5.2 \times 10^{-5}$ M). Both **567** and **569** had a specific and very potent inhibitory effect against baker's yeast α-glucosidase ($IC_{50} = 2.9$ and 1.0×10^{-8} M, respectively) [470]. Both **566** and **568** showed high inhibition activity against this enzyme with respective IC_{50} values of 2.3 and 1.0×10^{-6} M. These



compounds showed also potent inhibition against bovine liver β-galactosidase ($IC_{50} = 3.0$ and 1.9×10^{-6} M, respectively) [470], whereas **570** and **571** did not exhibit notable inhibition against α-glucosidase or β-galactosidase. The configurations of the hydroxyl groups, including the oxygen atoms of the isoureas seemed to be important for obtaining specificity on action. The *N*-phenyl isourea derivative **572** was a very powerful β-galactosidase inhibitor ($IC_{50} = 2.5 \times 10^{-7}$ M) as well as a moderate α-glucosidase inhibitor ($IC_{50} > 4.2 \times 10^{-4}$ M). Compound **573** inhibited bovine liver β-galactosidase ($IC_{50} = 5.7 \times 10^{-7}$ M) more potent than its isomer **574** ($IC_{50} = 2.4 \times 10^{-5}$ M). The *N*-butyl cyclic isourea derivative **575** had a strong potency against glucosidase I (yeast) ($K_i = 4.2 \times 10^{-6}$ M) almost comparable to *N*-butyl deoxynojirimycin (**220**). Recently, cyclitol **553** and the fused heterocycles **569** and **575** were tested as potent inhibitors of baker's yeast α-glucosidase *in vitro* ($IC_{50} = 4.0 \times 10^{-7}$, 7.6×10^{-9} and 7.0×10^{-14} M, respectively) [471, 472]. Oxazolidinone (**576**) weakly inhibited yeast α-glucosidase with ($K_i \approx 6.0 \times 10^{-3}$ M), and *Agrobacterium* β-glucosidase ($K_i \approx 7.0 \times 10^{-4}$ M).

Trehazoloid **577** was an example of a designed, linkage-spanning glycosidase inhibitor whose effective selectivity was attributed to the aglycon portion, suggesting that enzyme inhibition may be tunable by this kind of structural modification. Although its aglycon portion was designed to bind to an α-glucosidase. Nevertheless, **577** showed good competitive inhibition ($K_i = 4.8 \times 10^{-5}$ M) to *Agrobacterium* β-glucosidase. It was found to be a potent competitive inhibitor of yeast α-glucosidase ($K_i = 9.3 \times 10^{-6}$ M) [75]. The pseudo glucopyranosyl amino portion of **577,** trehalamine **552** bound about an order of magnitude more weakly ($K_i \approx 7.0 \times 10^{-5}$ M) than **577** [75]. The trehazolin analogues **578**–**580** completely lacked inhibitory activity against α-amylase, β-glucosidase, α-mannosidase, maltase and sucrase. The enantiomers **579** and **580** were strong inhibitors of trehalase indicating that the cyclopentane-1,2,3-triol structure with all-*trans* stereochemistry was essential for exhibiting the activity, i.e. the important sites for interaction with the enzymes. Interestingly, compound **580** with the unnatural absolute structure, possessed inhibitory activity against silkworm trehalase about 50 times more potent than the natural type **579** against both silkworm and porcine trehalase ($IC_{50} = 2.8 \times 10^{-6}$ and 2.6×10^{-7} g ml^{-1} for **579** and 5.9×10^{-8} and 5.0×10^{-9} g ml^{-1} for **580,** respectively). On the other hand, it may be assumed that, in compound **578** the presence of the C-methyl function at C-6 would hinder the cyclopentane part in binding the active site of the enzyme through stereoelectronic effects, leading to a significant decrease in activity ($IC_{50} > 1.0 \times 10^{-4}$ and 1.6×10^{-6} g ml^{-1} for the respective trehalases). The inhibitory activity of these analogues against isomaltase (rat) was measured ($IC_{50} = 2.3 \times 10^{-5}$, 1.7×10^{-5}, 7.0×10^{-6} and $> 1.0 \times 10^{-4}$ g ml^{-1}, respectively) [473]. The pseudoligosaccharides **581** and **582** having cyclic isourea moiety were very strong inhibitors of α-glucosidase (baker's yeast) ($K_i = 1.2 \times 10^{-8}$ and 1.8×10^{-7} M, respectively). They did not have any inhibitory potency against either sucrase, isomaltase (rat intestine) or processing α-glucosidase (rat liver microsomes) [450].

Among the trehazolin analogues **583**–**594** Compounds **584** and **585**, having aromatic rings instead of sugar residues, did not exhibit any inhibitory activity against silkworm trehalase at all. The two epimers, **587** and **589** and the two deoxy derivatives, **588** and **590**, had decreased potency ($IC_{50} = 3.7 \times 10^{-5}$, 1.1×10^{-4}, 1.2×10^{-6} and 7.9×10^{-6} M, respectively) compared to a specific slow, tight-binding inhibitor trehazolin (**586**) ($IC_{50} = 2.7 \times 10^{-8}$ M [468], 4.9×10^{-8} M [474] and $IC_{50} = 1.1 \times 10^{-8}$ g ml^{-1} [475]). However, 5a′-carbatrehazolin **591** has been shown to be a potent inhibitor with an IC_{50} value equal to 1.8×10^{-8} g ml^{-1} [476]. These results suggested that the four hydroxyl groups of the D-glucopyranosyl residue of trehazolin were topologically essential for its binding to the active site of the enzyme through hydrogen bonding. The unsaturated analogue **592** containing a valienamine moiety showed lower activity ($IC_{50} = 3.1 \times 10^{-7}$ M) owing to conformation deformation of the 5a′-carbahexopyranose ring. Compounds **584** and **585** have been demonstrated to be potent and specific α-glucosidase inhibitors ($IC_{50} = 1.3 \times 10^{-6}$ and 4.8×10^{-7} M (yeast), respectively). The analogue **593** seemed to hinder free interaction between its active core and the binding site of the enzyme, resulting in an appreciable decrease of both antifungal activity and trehalase inhibition ($IC_{50} = 5.7 \times 10^{-6}$ M). The cellobiose analogue **594** showed an increase in antifungal activity against *Rhizoctonia solani* as compared with that of trehazolin **586** and an excellent inhibitory activity ($IC_{50} = 1.9 \times 10^{-7}$ M) against trehalase [474]. The natural pseudodisaccharide trehazolin **586** bound poorly to yeast α-



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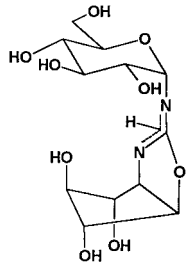
578 X = Me
579 X = H



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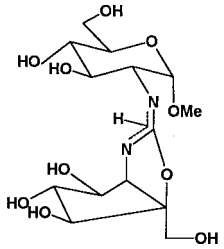

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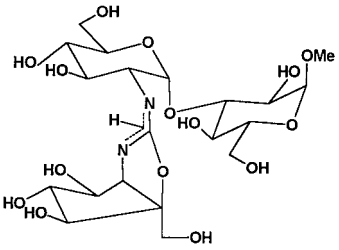

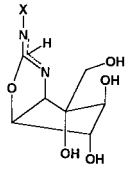

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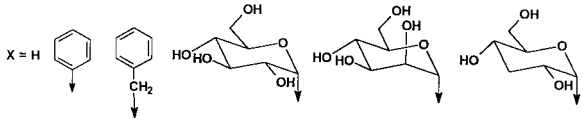

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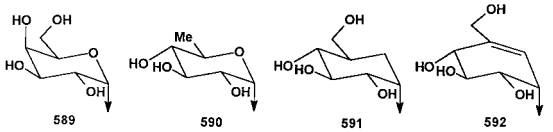

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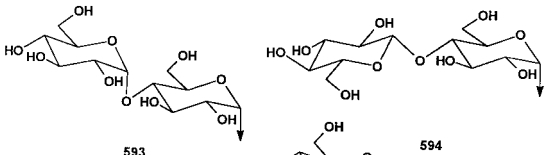

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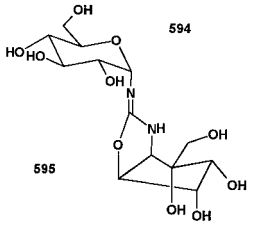

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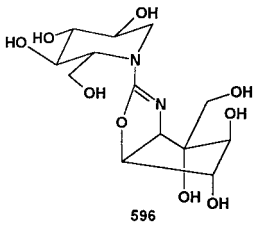

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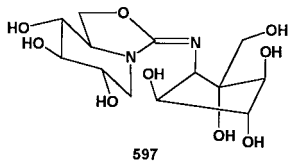

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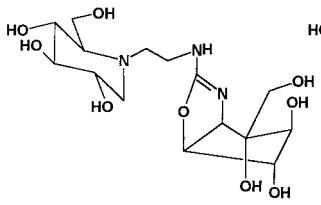

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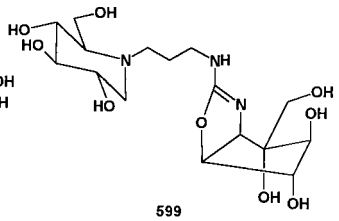

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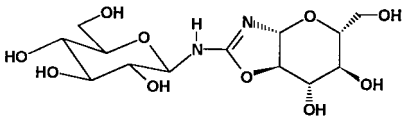

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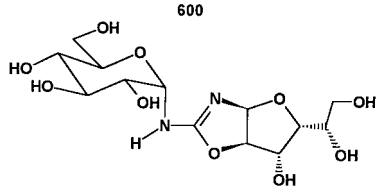

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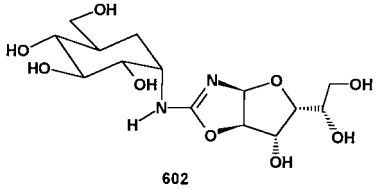

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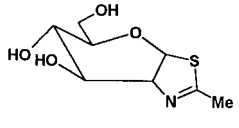

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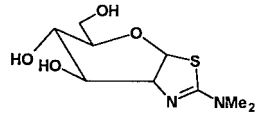

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glucosidase [469, 477], whereas its aglycon trehalamine **583** possessed a strong inhibitory activity towards yeast α-glucosidase ($K_i = 7.0 \times 10^{-5}$ M). Trehazolin **586** and variety of its analogues have been synthesized [75, 166, 473, 475, 478, 479] to find potential glycosidase inhibitors. It exhibited different inhibitory activities towards various α-glucosidases [475] such as trehalases (porcine) ($IC_{50} = 6.0 \times 10^{-9}$ g ml^{-1}), maltase (rat) ($IC_{50} = 7.6 \times 10^{-5}$ g ml^{-1}) and sucrase (rat) ($IC_{50} = 7.6 \times 10^{-5}$ g ml^{-1}). Trehazolin **586** also inhibited other *exo*-α-glucosidases such as rat isomaltase, but as indicated by its IC_{50} value of 7.6×10^{-6} M [468] and 3.9×10^{-6} g ml^{-1} [475], the concentrations required for inhibition were higher by a factor of 10^2 or 10^3 than those to inhibit the trehalases. Trehalamine, a deglucosylated form of trehazolin, inhibited porcine trehalase with an IC_{50} value of 2.9×10^{-7} M [468]. Trehalostatine **595** was a potent and specific inhibitor against blowfly (*Aldrichna grahami*) trehalase ($IC_{50} = 6.8 \times 10^{-10}$ g ml^{-1}) [480, 481].

The enzyme inhibitory activities of a mixture of **596** and **597** and the two homologues **598** and **599** exhibited IC_{50} values of 6.8×10^{-5}, 4.2 and 1.5×10^{-6} g ml^{-1}, respectively towards intestinal maltase [482].

The biological activity of tetrahydropyrano[2,3-*d*]oxazole **600** as well as furo[2,3-*d*]oxazole **601** indicated that both compounds were disappointingly void of activity towards the various tested glycosidases [483]. The carba analogue **602** showed $IC_{50} > 1.0 \times 10^{-4}$ and 6.5×10^{-5} g ml^{-1}, against silkworm and porcine trehalases respectively, [450]. The "NAG-thiazoline" **603** was found to be a powerful competitive inhibitor of jack bean NAGase ($K_i =$

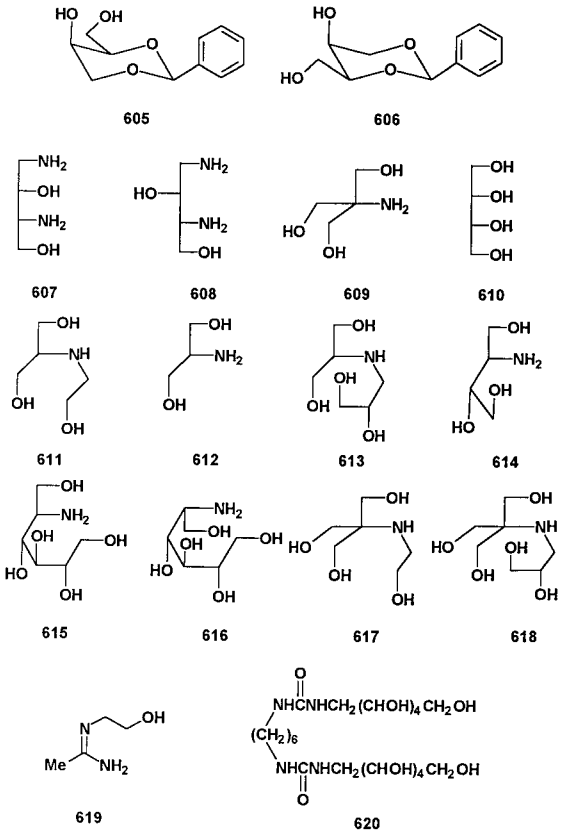
2.8×10^{-7} M) and it bound more tightly than its parent sugar *N*-acetyl-β-D-glucosamine ($K_i = 5.0 \times 10^{-3}$ M) [484]. The inhibition of the glucosamine isothiourea derivative (allosamizoline analogue) **604** which resembled the aminocyclitol isourea portion of chitinase inhibitor allosamidin (**565**), showed that it was a competitive inhibitor of jack bean NAGase ($K_i = 1.6 \times 10^{-4}$ M) [485]. Thus, **604** bound NAGase about 600-fold less tightly than NAG-thiazoline **603** and about 30-fold more tightly than its product, *N*-acetyl-β-D-glucosamine. Therefore, the (presumed) greater basicity of **604** relative to **603** did not improve its binding. However, the interaction of **604** with NAGase may be reduced by the greater steric bulk, and/or solvation effects, offered by the dimethylamino substituent relative to methyl [486].

26. Dioxane derivatives

It was found that 1,3-(*R*)-*O*-benzylidene-D-threitol (**605**) competitively inhibited the hydrolysis of *o*-nitrophenyl β-D-galactopyranoside by *E. coli* β-D-galactosidase with a remarkable K_i of $7.5 \pm 1.0 \times 10^{-4}$ M [487] which was comparable to a K_i of $4.5 \pm 0.3 \times 10^{-4}$ M for β-D-galactopyranosyl benzene (**160**) [176]. Even prolonged incubation of **605** in the presence of high concentrations of the enzyme did not cause any hydrolysis of the acetal **605**. Specificity of binding to β-D-galactosidase is demonstrated by the fact that **605** was ineffective inhibitor of green coffee bean α-D-galactosidase. The enantiomer 1,3-(*R*)-*O*-benzylidene-L-threitol (**606**) [488] competitively inhibited β-D-galactosidase, although to a lesser extent ($K_i = 3.5 \pm 0.5 \times 10^{-3}$ M) [487].

27. Open chain compounds

The hepatic lysosomal glycosidases, α-glucosidase and β-glucuronidase were inhibited *in vitro* and *in vivo* by mono- and diethanolamine. The treatment of the enzymes *in vitro* by ethanolamine exhibited a reversible inhibition of the mixed and competitive types for α-glucosidase and β-glucuronidase ($K_i = 3.5$ and 2.0×10^{-4} M, respectively). Diethanolamine showed a reversible inhibition of the competitive type for both enzymes ($K_i = 1.3 \times 10^{-4}$ and 5.0×10^{-5} M, respectively) [124]. 1,3-Diamino-1,3-dideoxytetritols **607** and **608** competitively inhibited both β-D-glucosidase from sweet almond emulsin and β-D-galactosidase from *E. coli* with K_i values ranging from 3.0 to 10.0×10^{-3} M [489]. *N*-Butyl(dodecyl) amines inhibited bovine cytosolic β-glucosidase moderatly (strongly) ($K_i = 2.8 \times 10^{-4}$ and 7.1×10^{-7} M, respectively), the inhibition constant of *N*-butylamine against bovine lysosomal β-glucosidase was 2.2×10^{-5} M [210]. Amines such as triethanolamine and 2-amino-2-hydroxymethylpropane-1,3-diol **609** have been found to inhibit intestinal carbohydrases (maltase and oligo-1,6-glucosidase). The inhibition by amines was pH-dependent, where it was reversed in the presence of excess substrate at alkaline pH. The inhibition of intestinal carbohydrases by amines such as **609**, 2-amino-2-methyl-1-propanol, 2-amino-2-methyl-1,3-propanediol, and tert-butylamine indicated that inhibition of both enzymatic activities decreased with increasing the pKa of amines. The pKa values of previous amines were 8.1, 8.9, 9.8 and 10.8, respectively [490]. The K_i values of **609** were 2.2 and 1.4×10^{-4} M, for maltase and oligo-1,6-glucosidase respectively, calculated as total amine concentration, but on the basis of free base present concentration K_i values were 4.0 and 2.1×10^{-5} M, respectively for both

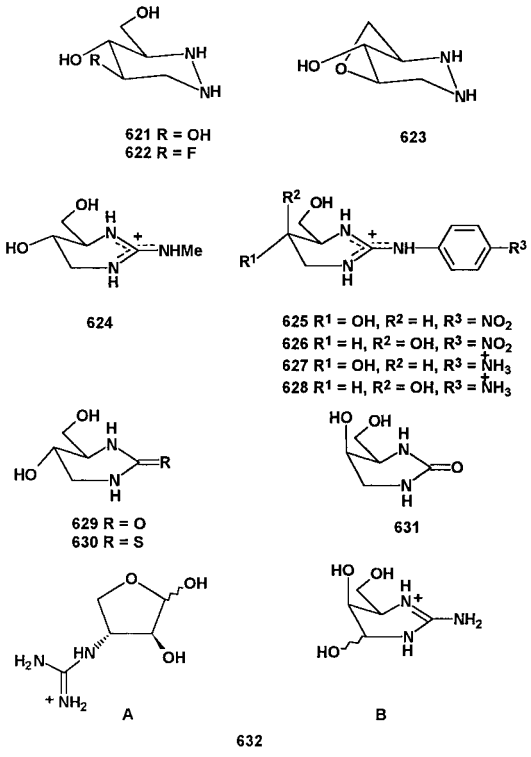


enzymes [47]. Neither maltase nor oligo-1,6-glucosidase was inhibited by diisopropyl fluorophosphate (DFP) or tetramethyl pyrophosphate (TMP). Several glycoside hydrolases were inhibited by **609** [47, 491–493] and polyols such as erythritol (**610**) [494]. The inhibition results indicated that malt α-glucosidase was competitively inhibited by **609** and **610** [495]. They, At pH 4.6, they compete with each other and with maltose for the enzyme. The results indicated that malt α-glucosidase existed in three ionized forms. The substrate will react with a negatively charged group in the enzyme (pKa = 3.9). Compound **609** was not a substrate analogue of maltose. Also, erythritol was not a true substrate analogue in whole pH range. The inhibition of yeast β-fructosidase by **609** at pH 5–7 can be explained in a similar manner as that for the inhibition of malt α-glucosidase by **609** [496]. The competitive **609** inhibition of maltose hydrolysis was explained by assuming that it caused a steric hinderance for maltose by complexing with the enzyme, or that complexing with **609** changed the conformation or the charge distribution of the enzyme. The competitive erythritol inhibition of maltose hydrolysis was explained as a competition for the isoelectric enzyme form $X\text{-}YH^+$, in which erythritol required both groups i.e the same group as maltose and the same group as **609**. The latter inhibited sucrase and isomaltase activity and was the strongest inhibitor of the ethanolamine derivative. Bis-**609** inhibited sucrase more than isomaltase in contrast to mono-, di-, and triethanolamine derivatives [497]. It was found that a series of acyclic analogues of nojirimycin and 1-deoxynojirimycin **609** and **611**–**618** were competitive inhibitors of yeast α-glucosidase, with varying degrees of effectiveness [74], but none of them, in contrast to 1-deoxynojirimycin showed significant anti-HIV activity. Serinol **612**, which possessed the smallest common structural unit found in deoxynojirimycin, was the least effective with a K_i value of 7.0×10^{-4} M compared with other simple analogues.

However, addition of an *N*-(2-hydroxyethyl) or an *N*-[(2*S*-2,3-dihydroxypropyl] substituent increased the efficiency of binding, compounds **611** and **613** having K_i values of 4.0×10^{-6} and 1.0×10^{-5} M, respectively. 2-Amino-2-deoxy-1-erythritol (**614**), which mimics C-3 to C-6 and the ring nitrogen of deoxynojirimycin was also effective as an inhibitor with K_i value of 8.2×10^{-6} M. It was interesting that 5-amino-5-deoxy-D-glucitol (**615**), an acyclic analogue of nojirimycin, was a remarkably good inhibitor of the enzyme ($K_i = 3.6 \times 10^{-6}$ M), whereas the ido isomer **616** was much less effective ($K_i = 2.2 \times 10^{-4}$ M). The greater effectiveness of **609** ($K_i = 3.8 \times 10^{-4}$ M) over serinol **612** could be attributed to the greater statistical likehood of the required stereo-arrangement for binding, which presumably involves 4-OH, 6-OH and N in deoxynojirimycin. However, compounds **617** and **618** showed poor binding to the enzyme ($K_i = 3.6 \times 10^{-3}$ and 6.5×10^{-4} M, respectively) [74]. The inhibition of sweet almond β-glucosidase with ethanolamine derivatives **611** and **619** was found to be uncompetitive ($K_i = 9.1$ and 3.8×10^{-4} M, respectively) [148, 498]. Compound **620** was found to be a competitive inhibitor of *Trichomonas vaginalis* N-acetyl-β-D-hexosaminidase ($K_i = 3.0 \times 10^{-5}$ M) [120].

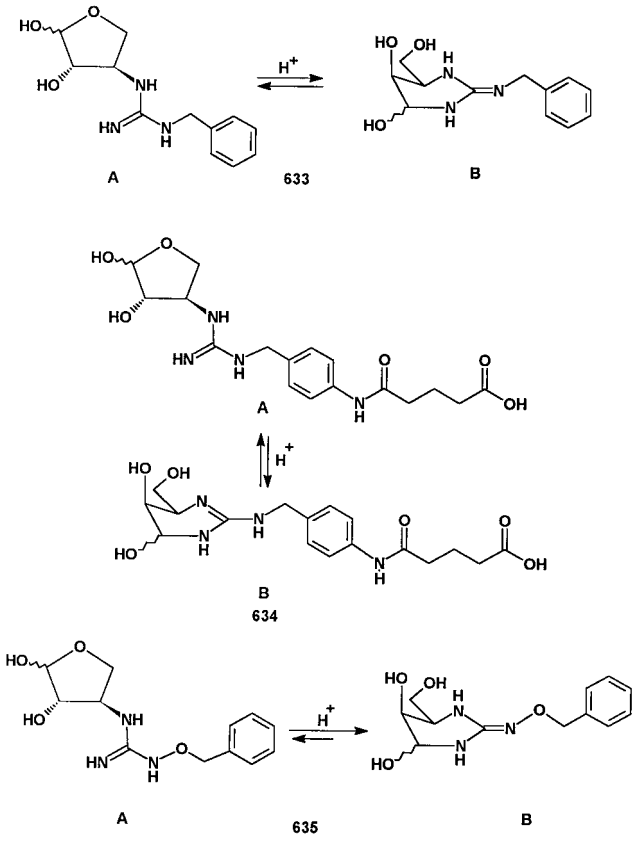
28. Pyridazine derivatives

1-Azafagomine (**621**) was a potent glycosidase inhibitor. It inhibited baker's yeast α-glucosidase and almond β-glucosidase with Ki values of 3.9×10^{-6} and 6.5×10^{-7} M, respectively. The most interesting result was that the fluoride **622** was a relatively poor inhibitor of both α- and β-glucosidases ($K_i > 7.0 \times 10^{-3}$ and 7.9×10^{-5} M, respectively). This suggested that **621** was bound to both enzymes in a manner where the action of the 5-OH as a hydrogen bond doner was an important contribution to the binding. The poor inhibition by **623** ($K_i > 1.0 \times 10^{-3}$ M and 8.9×10^{-4} M for both enzymes, respectively) could be anticipated, because this compound was forced to adopt a different conformation than the substrate [499, 500].



29. Cyclic guanidine derivatives

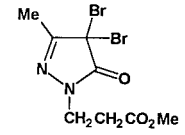
No inhibition could be detected for **624** against α-D-glucosidase, but a weak inhibition of β-D-glucosidase ($K_i = 1.8 \pm 0.2 \times 10^{-3}$ M) [122] was observed. The effects of disaccharide analogues were generally weaker as compared to the effect of the configurationally related nitrophenyl glycoside analogues **625** and **626**. Compounds **625** and **626** inhibited α-D-glucosidase with K_i values $2.0 \pm 1.0 \times 10^{-3}$ M and $6.0 \pm 1.0 \times 10^{-3}$ M, respectively [121]. Compound **626** showed a moderate inhibition against β-D-galactosidase ($K_i = 3.0 \pm 1.0 \times 10^{-4}$ M) [121]. The guanidinium derivatives **625** and **627** significantly inhibited emulsin β-D-glucosidase with K_i values of $3.0 \pm 1.0 \times 10^{-5}$ M and $2.0 \pm 1.0 \times 10^{-3}$ M, respectively [121]. This inhibitory effect may be due to the interaction of the aromatic nitrophenyl group with the aglycon binding-site. Compound **628** showed a very good inhibition against β-D-galactosidases ($K_i = 8.0 \pm 1.0 \times 10^{-5}$ M) [121]. The cyclic carbonamides **629** and **631** showed extremely weak competitive inhibition of β-D-glucosidase from sweet almond emulsin and β-D-galactosidase from *E. coli* with K_i values 1.3×10^{-1} M and 9.0×10^{-2} M, respectively, also thiocarbonamide **630** has $K_i = 9.0 \times 10^{-2}$ M with its corresponding first enzyme [489]. The poor affinities of compounds **629** and **631**, which conformationally resembled the envelope structure of the glycopyranosyl cation, compared to those of their corresponding lactames [24] demonstrated the importance of the hydroxyl groups at C-2 and C-3 in pyranoside derivatives. Inhibition studies of the guanidinosugar **632A** on green coffee bean α-galactosidase showed that the inhibition increased with an increase in pH. At pH 7.5 (IC_{50} reached 1.3×10^{-3} M), but when the inhibitor was in pH 10.7 and then added to the assay (still at pH 7.5), the IC_{50} dropped to 4.8×10^{-4} M. It was observed that the concentration of tetrahydropyrimidine **632B** increased as the pH of the solution was raised.



Therefore, the inhibition may be due to the interaction of **632B** as a transition-state inhibitor with the enzyme [317]. Guanidine compounds which existed solely as tetrahydropyrimidines should be better inhibitors of galactosidase.
The inhibition by **633**–**635** was pH dependent, indicating that the enzyme recognizes the tetrahydropyrimidine B-form which was the most potent form of the guanidino-sugars A at higher pH values. The furanose-tetrahydropyrimidine equilibrium can be modulated with an appropriate N-substituent which affects the guanidino-sugar pKa value [317]. The inhibition at a pH high enough shifted all the guanidino-sugar to the tetrahydropyrimidine. Although the inhibition potency was moderate (K_i range 4.0×10^{-6} to 5.0×10^{-5} M, at neutral pH), the use of cyclic guanidino-sugars with different pKa provided new insights into the mechanism of inhibition of glycosidases [501].

30. Heterocyclic compounds

The pyrazolone **636** showed an IC_{50} value of 1.0×10^{-6} g ml^{-1} against α-glucosidase from *Saccharomyces* sp. [502]. The cyclic phosphonamidate analogue of glucose **637** gave a moderate competitive inhibition of two inverting α-glycosidases (trehalase and glucoamylase) and the retaining α-glucosidase. The phosphonamide showed enhanced binding relative to a simple monosaccharide only with the inverting glycosidases. This enhanced binding was believed to be due to hydrogen bonding interactions between the phosphonamidate group and two active site carboxylate residues implicated in catalysis [503].



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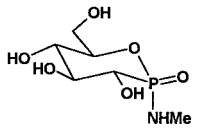

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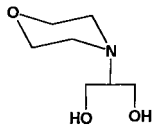

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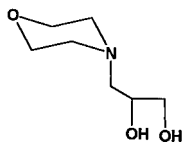

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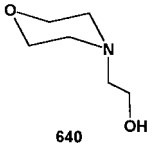

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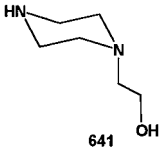

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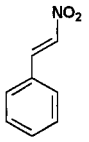
31. Heterocyclic compounds with hydroxyalkyl residues

The inhibitions of sweet almond β-glucosidase by the morpholino derivative **638** ($K_i = 7.3 \times 10^{-4}$ M), **639** ($K_i = 6.4 \times 10^{-4}$ M), and **640** ($K_i = 6.2 \times 10^{-4}$ M) as well as the piperazine **641** ($K_i = 7.1 \times 10^{-4}$ M) have been investigated. The compounds were found to be uncompetitive inhibitors [498].

32. Aromatic compounds

The nitro derivatives **642** and **643** were demonstrated to be irreversible inhibitors of *Escherichia coli* β-galactosidase with an inhibition reached to 94% at pH 8.3 for compound **643** [504]. Photooxidation of intestinal extracts (maltase and oligo-1,6-glucosidase) by addition of methylene blue (**644**) led to little or no effect on enzymatic



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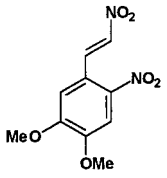

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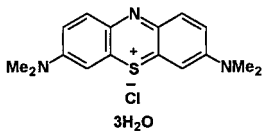

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activity. Both enzymatic activities decreased progressively during Photooxidation [47].

33. Amino acids and other acid derivatives

Maltase activity decreased less than oligo-1,6-glucosidase on a percent basis of amino acid histidine which was the most photosensitive amino acid in the intestinal extracts, while tyrosine and tryptophan decreased to a much smaller extent than did histidine. It was noted that the percent decrease in histidine was greater than percent decrease in enzymatic activity. With other enzymes studies, such as ribonuclease or chymotrypsin [505, 506], the decrease in histidine was smaller than the decrease in enzymatic activity. The inhibition studies of sulfhydryl inhibitors indicated that when *p*-chloromercuribenzoate, *o*-iodosobenzoate, or iodoacetic acid were preincubated with intestinal extracts under certain conditions, they led not to a decrease in enzymatic activity of oligo-1,6-glucosidase or maltase over controls preincubated under the same conditions with 1.0×10^{-3}M NaCl [47]. The inhibition studies of intestinal carbohydrases by heavy metals [47] showed that both enzymes maltase and oligo-1,6-glucosidase were moderately inhibited by Cu^{++}, Zn^{++} and slightly by Hg^{++}. Maltase was inhibited by Ag^{+}. Fungal lactase was inhibited by mercuric acetate [48], and it was not inhibited by *p*-chloromercuribenzoate, salyrgan, iodoacetate, or arsenite [48].

* For part 1 including chapters 1–15 and references 1–180 see PHARMAZIE **55**, 251 (2000); for part 2 including chapters 16–17 and references 181–399 see PHARMAZIE **55**, 331 (2000).

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