REVIEW

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# Glycosidase inhibitors and their chemotherapeutic value, part 1

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The various compounds that have been investigated as glycosidase inhibitors are reviewed. The first of three parts of this review article covers the following classes of compounds: sugar, lactones and hydroximolactones, glycosyl halides, oligosaccharides, glycosides and their derivatives, deoxy thiosugar derivatives, thioglycosides, deoxy amino and guanidino sugars, glycosylamines, anhydrosugars and their analogues, deoxysugars, glycals, C-glycosides and C-nucleosides.

## 1. Introduction

Enzyme inhibitors are compounds that slow down or prevent enzyme catalysis from occurring. Inhibitors can be classified into two groups namely, reversible and irreversible inhibitors [1]. The most common enzyme inhibitor drugs belong to the reversible type, particularly those that compete with the substrate for binding at the active-site (competitive reversible inhibitors). There are three types of the reversible inhibitors. The simple competitive reversible inhibitors are compounds which have a strong molecular similarity to the substrates for the target enzyme, which allows for tighter binding to the enzyme. The other type is the transition state analogues, which are stable compounds whose structures resemble that of the substrate at a postulated transition state of the reaction. These will bind to the enzyme much more tightly than that of the substrate in the ground state. They are slow tight-binding inhibitors which bind and interact very strongly either covalently or noncovalently [2–5] with the active site. On the other hand, irreversible enzyme inhibitors are specific enzyme inhibitors. There are two principal types; one of them is the affinity labeling agents which are reactive covalent inactivators that have a structural similarity to a substrate for a target enzyme, but they contain a reactive functional group. The other one is the mechanism-based enzyme inactivators which are unreactive compounds that bear a structural similarity to a substrate or product for the target enzyme [1].

Glycosidases are enzymes that are involved in several important biological processes such as digestion, biosynthesis of glycoproteins and catabolism of glycoconjugates. Glycosidase inhibitors have the potential to produce a number of beneficial therapeutic effects and are of biotechnological relevance [6]. They stimulated interest for the potential treatment of metabolic disorders such as diabetes [7–9] and lysosomal storage diseases [10–12].

α-Glucosidase inhibitors are effective in lowering the postprandial glycemic rise after carbohydrate ingestion. They lower the insulin release, insulin requirement and some lower plasma lipids [13]. They have been also used or suggested as inhibitors of tumor metastasis [14], antiobesity drugs, fungistatic compounds [15], insect antifeedants [16–18], antivirals [19–22], and immune modulators [23].

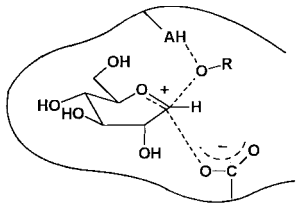
The enzymic glycoside hydrolysis takes place with retention of configuration. This is achieved by a two-step double displacement mechanism. Thus, there are two transition states to be passed on the pathway from substrate to the products. The transition state for the first step is described by a model, where the hexose is deformed from its ground state chair conformation towards a structure flattened at C-1 [24–27]. The breaking of the glycosidic bond is aided by a strong hydrogen bond to the glycosidic oxygen leading to proton transfer in the transition state and by the stabilization of a partial positive charge on C-1 by a closely positioned carboxylate group. Release of the aglycon, and formation of the glycosyl ester with the carboxylate would complete the first step. The catalytic cycle is completed by hydrolysis of the glycosyl-enzyme intermediate where the orientation and deprotonation of the water molecule in the second transition state also proceeds with inversion, causing the sugar to be released in the original anomeric configuration [24, 28] (Scheme).

Basicity and/or hydrophobicity of the aglycon are more important factors in binding the inhibitor to the enzyme than the conformation of the sugar moiety [29]. When the electrostatic and/or hydrophobic interactions have no influence, the change in the conformation *i.e.* "the transition state analogy" of the sugar ring is effective. Numerous reviews have been published dealing with certain aspects of the chemistry and biological activities of the glycosidase inhibitors [30–46]. Thus, the mechanistic information on the glycosidase inhibitors [24] as well as the synthesis of sugar shaped heterocycles [46], amino sugar derivatives as versatile tools for glycobiologists [40], and microbial α-glucosidase inhibitors [13] have been reported. Owing to the great deal of informations and the attraction of many investigators to the subject, it became interesting to review the variety of compounds that have been investigated as glycosidase inhibitors.

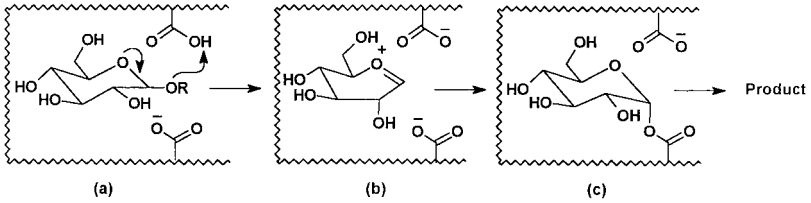
The glycosidase inhibitors are classified in the present review according to the structure of the inhibitor and not according to their modes of action.

## 2. Sugars

Sugars have been studied [24] as inhibitors since the early stage of developing the subject. Glucose has the ability to inhibit both maltase and oligo-1,6-glucosidase activities [47]. Intestinal lactase can be also inhibited by glucose and galactose [48]. Amylo-1,6-glucosidase was not inhibited by glucose at certain concentrations [49]. It has been reported that glucose is a weak competitive inhibitor of glycogen phosphorylase (GP) ($K_i = 1.7 \times 10^{-3}$ M), and in a self regulatory system it helps the lowering of blood glucose levels by inhibition of glycogen degradation and promotion of glycogen synthesis [50, 51]. Mannose is a very weak inhibitor of α-mannosidase jack bean *(Canavalia ensiformis)* with $IC_{50} = 2.0 \times 10^{-2}$ M [52].




A model for the first transition state of enzymatic β-glucoside hydrolysis



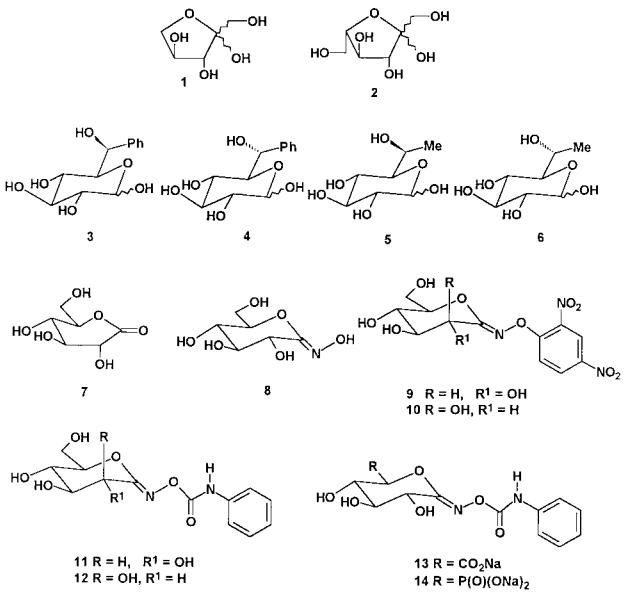

The mode of action of glycosidases

L-Xylulose (**1**) and L-fructose (**2**) are the best inhibitors of several arylglycosidases and of glucosidase I. The inhibition activity of **1** against yeast α-glucosidase showed an $IC_{50}$ value of $1.0 \times 10^{-5}$ M. Both **1** and **2** inhibited purified soybean glucosidase I ($IC_{50} \approx 1.0 \times 10^{-4}$ M), but showed no inhibitory activity against soybean glucosidase II. However, L-fructose was a poorer inhibitor than L-xylulose and required much higher concentrations for the same degree of inhibition. Neither of these compounds inhibited protein synthesis or the formation of Lipid-linked saccharides in cultured MDCK cells [53]. L-Arabinose inhibited the sucrase in the activity of intestinal mucosa in an uncompetitive manner ($K_i = 2.0 \times 10^{-3}$ M). D-Arabinose did not inhibit sucrase activity, whereas D-xylose was as potent as L-arabinose. L-Arabinose suppressed the increase of blood glucose after sucrose loading dose-dependently in mice ($ED_{50} = 3.5 \times 10^{-3}$ M/kg), but showed no effect after starch loading. The suppressive effect of D-xylose on the increase of blood glucose after sucrose loading was 2.4 times less than that of L-arabinose, probably due to intestinal absorption of the former. L-Arabinose suppressed the increase of plasma glucose and insulin in rats after sucrose loading [54]. The 6-C-aryl-D-glucose inhibited glucokinase in the order L-phenyl (**3**) > D-phenyl (**4**) ≈ L-methyl (**5**) > D-methyl (**6**). The latter analogue did not inhibit glucokinase at concentrations up to $1.0 \times 10^{-2}$ M. The inhibition was at least partly competitive with glucose since $1.0 \times 10^{-3}$ M of **3** (and $1.0 \times 10^{-2}$ M of **4**) inhibited glucokinase completly at $2.0 \times 10^{-3}$ M glucose, but only by one third at $5.0 \times 10^{-2}$ M glucose. Both of the 6-C-phenyl glucose derivatives **3** and **4** inhibited Glc-6-Pase, as did the corresponding methyl analogues **5** and **6** [55–60].

## 3. Lactones and hydroximolactones

The first known glycosidase inhibitors were the δ-aldonolactones [61]. They are competitive inhibitors against the glycosidases. D-Gluconolactone (**7**) is a moderately strong neutral inhibitor of sweet almond β-glucosidase ($K_i = 1.5 \times 10^{-5}$ [62], $2.0 \times 10^{-4}$ [63] and $4.0 \times 10^{-4}$ M [64]), and a strong inhibitor of *A. faecalis* β-glucosidase ($K_i = 1.4 \times 10^{-6}$ M) [65]. It exhibited certain degrees of inhibition against human liver cytosolic β-glucosidase ($K_i = 1.5 \times 10^{-5}$ M) [66, 67], but it was not as potent as the aza sugar, 1-deoxynojirimycin. It also inhibited emulsin β-glucosidase ($IC_{50} = 1.1 \times 10^{-5}$ M) [68] and bound weakly and non-competitively to yeast α-glucosidase ($K_i = 2.0 \times 10^{-3}$ M, $IC_{50} = 2.1 \times 10^{-3}$ M) [64, 68], but it did not exhibit any inhibition of insect trehalase, *Canavalia ensiformis* α-mannosidase, *Aspergillus niger* α- and β-galactosidases and *Helix pomatia* β-glucuronidase (at concentrations up to $10^{-2}$ M) [68]. Both the β-glucosidase and pyridoxine-β-D-glucoside hydrolase were inhibited by glucono-δ-lactone (**7**), with $K_i$ values of $1.0 \times 10^{-5}$ and $7.4 \times 10^{-6}$ M, respectively [69].

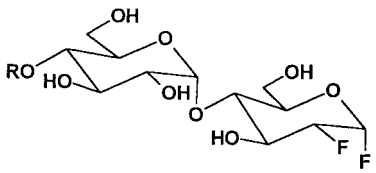
The hydroximolactone **8** is a weak inhibitior of sweet almond β-glucosidase ($K_i = 4.3 \times 10^{-3}$ M at pH 6.8 [65], $K_i = 1.0 \times 10^{-4}$ M at pH 4.5 [70]). It inhibited yeast α-glucosidase non-competitively ($K_i = 6.8 \times 10^{-3}$ M) and *A. faecalis* β-glucosidase ($K_i = 3.0 \times 10^{-5}$ M at pH 7.0). The selectivity of the inhibition of α-versus β-glycosidases by hydroximolactone derivatives may depend on the substituent on the hydroximo group. Thus, the 2,4-dinitrophenyl ether **9** is a strong competitive inhibitor of yeast α-glucosidase ($K_i = 5.0 \times 10^{-6}$ M) and a moderate inhibitor of almond β-glucosidase ($K_i = 8.0 \times 10^{-5}$ M) [71–78]. The phenyl carbamoyl derivative **11** inhibited almond β-glucosidase ($K_i = 4.3 \times 10^{-5}$ M) [79], yeast α-glucosidase ($K_i = 7.5 \times 10^{-5}$ M) [64, 78], and more strongly *A. faecalis* β-glucosidase ($K_i = 1.4 \times 10^{-6}$ M) [79]. It inhibited competitively emulsin β-D-glucosidase ($K_i = 2.3 \times 10^{-6}$ M, $IC_{50} = 5.8 \times 10^{-6}$ M) [70]. The manno analogues **10** and **12**



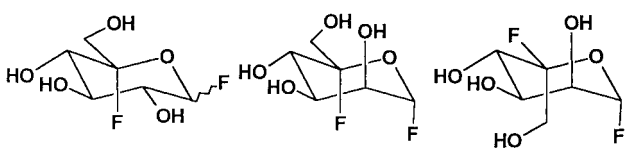
were found to be competitive inhibitors of jack bean α-mannosidase and snail β-mannosidase with comparable and modest activities on α-mannosidase ($K_i = 5.0$ and $8.0 \times 10^{-5}$ M, respectively) and with modest and strong activities on β-mannosidase ($K_i = 1.0 \times 10^{-5}$ and $2.5 \times 10^{-8}$ M, respectively). Thus, compound **12** was the best inhibitor of β-mannosidase so far reported. Mannonolactone itself is a good inhibitor of β-mannosidase ($K_i = 1.7 \times 10^{-5}$ M) [80]. The phenylcarbamate **13**, but not its phosphonate analogue **14**, is a good inhibitor ($K_i = 8.0 \times 10^{-6}$ M) of the *E. coli* β-glucuronidase. The bovine liver β-glucuronidase was inhibited strongly by **13** ($IC_{50} = 2.0 \times 10^{-7}$ M) and weakly by **14** ($IC_{50} = 2.0 \times 10^{-3}$ M) [81].

## 4. Glycosyl halides

2-Deoxy-2-fluoro-β-glucosyl- and β-mannosyl fluorides administered to rats in a single dose (10 mg/kg) inhibited the β-glucosidase and β-mannosidase activities after 1 h in brain, spleen, liver and kidney tissues. This inhibition was presumably caused by the accumulation of 2-deoxy-2-fluoroglycosyl-enzyme intermediates, indicating that intact 2-deoxy-2-fluoro-glycosyl fluorides were distributed to these organs and in the case of brain, that they crossed the blood/brain barrier. β-Glucosidase activity recovered completely or partially in brain, spleen, liver and kidney after 20–48 h. β-Mannosidase activity partially recovered in all tissues after 48 h. β-Galactosidase activity in brain and kidney was not significantly affected by administration of either gluco or manno analogues, indicating that these inhibitors were directed towards specific glycosidases [82]. 3,4,6-Tri-*O*-acetyl-2-deoxy-2-fluoro-β-glucopyranosyl fluoride exhibited a great chemoprotectant activity against ricin toxicity in chinese hamster ovary and macrophage J 774A-1 [83]. It was found that 2-deoxy-2-fluoro-β-galactosyl fluoride exhibited an inhibition *of E. coli* β-galactosidase with a $K_i$ value of $1.3 \times 10^{-3}$ M [84]. The two glycosyl halides, 2-deoxy-2-fluoro-α-maltosyl and α-maltotriosyl fluorides (**15** and **16**) failed to inactivate human pancreatic α-amylase and rabbit muscle glycogen debranching enzyme, respectively [85]. 5-Fluoro-α- and β-D-glucosyl fluorides (**17**) are potent mechanism-based inhibitors of yeast *and Agrobacterium faecalis* α- and β-glucosidases, respectively with steady state $K_i'$ values of $1.4 \times 10^{-6}$ M for the α-analogue and $3.0 \times 10^{-7}$ M for the β-analogue [77]. 5-Fluoro-α-D-mannosyl fluoride (**18**) and 5-fluoro-β-L-gulosyl fluoride (**19**) showed high affinity for jack bean α-mannosidase ($K_i' = 7.1$ and $8.6 \times 10^{-5}$ M, respectively) for the steady state traping intermediate [86].



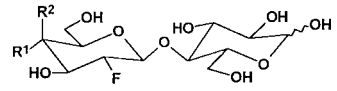
15 R = H
16 R = α-D-glucopyranosyl



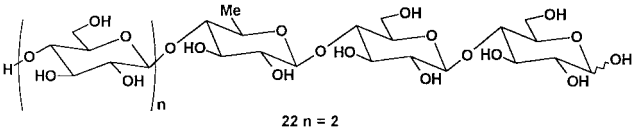
17 18 19

## 5. Oligosaccharides

The *Agrobacterium faecalis* β-glucosidase was inactivated by both 2′-deoxy-2′-fluorocellobiose (**20**) and lactose (**21**) ($K_i \approx 2.0 \times 10^{-2}$ and $9.0 \times 10^{-4}$ M, respectively) in a competitive manner [87]. $6^3$-Deoxymaltopentaose (**22**) and $6^3$-deoxymaltotetraose (**23**) showed strong inhibitory activities for human pancreastic α-amylase (HPA) and human salivary α-amylase (HSA). The $IC_{50}$ of **22** ($8.0 \times 10^{-5}$ M for HPA, $1.0 \times 10^{-4}$ M for HSA) and **23** ($2.0 \times 10^{-3}$ M for HPA, $2.0 \times 10^{-3}$ M for HSA) were lower than that of $6^3$-deoxymaltotriose (**24**) ($2.0 \times 10^{-3}$ M for HPA, $4.2 \times 10^{-2}$ M for HSA) [88].



20 $R^1$ =OH, $R^2$ = H
21 $R^1$ = H, $R^2$ = OH

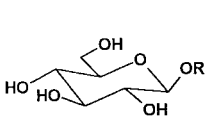


22 n = 2
23 n = 1
24 n = 0

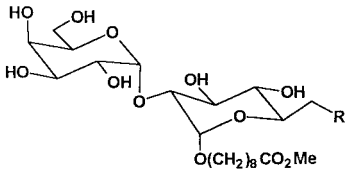
## 6. Glycosides and their derivatives

The inhibitory effects of alkyl β-D-glucosides increased in proportion to the alkyl chain length. Thus, n-octyl β-D-glucoside (**26**) inhibited the activities of both β-glucosidase and pyridoxine-β-D-glucoside hydrolase more effectively ($K_i = 1.2 \times 10^{-4}$ and $7.5 \times 10^{-3}$ M, respectively) than did n-amyl-β-**D**-glucoside (**25**) ($K_i = 1.1 \times 10^{-3}$ and $3.3 \times 10^{-2}$ M, respectively) [69]. The kojibioside **27** inhibited 20% of the glucosidases I activity at $1.0 \times 10^{-3}$ M, whereas **28** caused a 70% increase in the inhibition activity at $1.0 \times 10^{-3}$ M [89]. 6-Fluorolactosylceramide **29** at $5.0 \times 10^{-5}$ M inhibited 92% β-galactosidase *in vitro* [90]. The inhibition studies of a spacer-modified oligosaccharide derivatives indicated that they were essentially stable against enzyme catalysed hydrolysis except the azide and the amine **32** and **35** which showed $K_i$ values of $2.4 \times 10^{-3}$ M and $4.2 \times 10^{-4}$ M, respectively against porcine pancreatic α-amylase. The rate of hydrolysis of the amine **35** was two times slower than the azide **32** [91]. The inhibition constants of porcine pancreatic α-amylase by the azides **30** and **31** were $3.7 \times 10^{-2}$ M and $8.1 \times 10^{-3}$ M, respectively, and those of the amines **33** and **34** were $1.1 \times 10^{-2}$ M and $5.3 \times 10^{-4}$ M, respectively [91]. The difference in the competitive inhibition between a neutral spacer-modified oligosaccharide as **31** and its basic analogue **34** would be greatest when a maximal occupation of subsites is supplemented by the correct placing of the basic functionality.

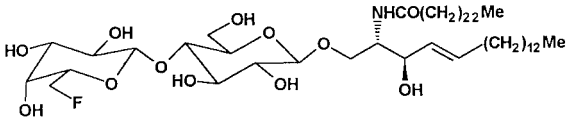
The kinetic studies of the hydrolysis of xylobiosides with *B. subtilis endo*-xylanase showed that 3,4-dinitrophenyl-β-xylobioside (**37**) and 2,5-dinitrophenyl-β-xylobioside (**38**) have a biphasic behaviour [92] but 2-nitrophenyl-β-xylobioside (**36**) has not. Thus the kinetic parameters are $K_m$ $3.4 \pm 0.3 \times 10^{-3}$ M, $k_{cat}$ 8.3 $s^{-1}$ for **37** and $K_m$ $2.2 \pm 0.1 \times 10^{-3}$ M, $k_{cat}$ 75.6 $s^{-1}$ for **38,** which were determined at low substrate concentrations and were due to the simple hydrolysis process, while those at high substrate concentration are $K_m$ $11.8 \pm 1.2 \times 10^{-3}$ M, $k_{cat}$ 17.9 $s^{-1}$ for **37** due to a transglycosylation process. The kinetic param-



25 R = n-amyl
26 R = n-octyl



27 R = OH
28 R = $NH_2$



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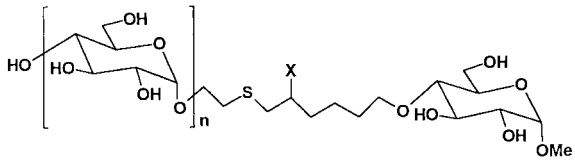
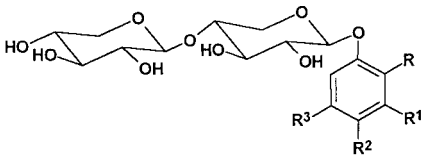


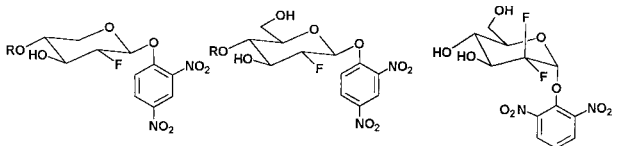
30 n = 1, X = $N_3$ 33 n = 1, X = $NH_2$
31 n = 2, X = $N_3$ 34 n = 2, X = $NH_2$
32 n = 3, X = $N_3$ 35 n = 3, X = $NH_2$



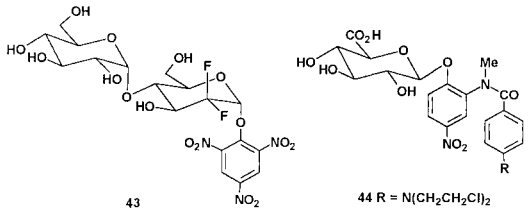
36 R = $NO_2$, $R^1 = R^2 = R^3 = H$
37 $R = R^3 = H$, $R^1 = R^2 = NO_2$
38 $R = R^3 = NO_2$, $R^1 = R^2 = H$

eters of **36** were $K_m$ 14.2 $\pm$ $0.5 \times 10^{-3}$ M, $k_{cat}$ 14.3 $s^{-1}$. Therefore **38** is the best substrate for assaying the xylanase, since it has the highest $k_{cat}$ and the lowest $K_m$ values, thus allowing reactions to be performed with a minimum concentration of the substrate at high sensitivity. Since **37** has high $K_m$ value, so it can be used in stopped assays.

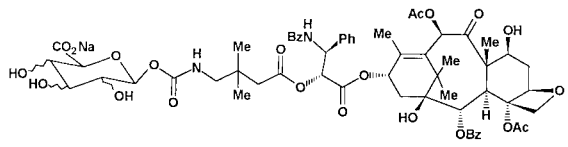
2-Deoxy-2-fluoro-xylobioside (**39**) showed an excellent time dependent inactivation of *Cellulomonas fimi exo*-xylanase/glucanase (Cex) ($K_i = 3.5 \times 10^{-6}$ M) [92], presumably functioning via the formation and accumulation of a relatively stable 2-deoxy-2-fluoro-xylobiosyl enzyme intermediate, analogous to that formed by Cex with the corresponding 2-deoxy-2-fluoro-glucoside **40** [93–97] and cellobioside **41** [85]. It was the best inactivator for this enzyme, on the basis of $k_i/K_i$ values, being some 26-fold better than the cellobioside and $10^5$-fold better than the glucoside [98]. It has been shown that 2′,4′-dinitrophenyl-2-deoxy-2-fluoro-β-cellobioside (**41**) was an effective inactivator ($K_i = 1.1 \times 10^{-4}$ M) [85], of the *exo*-glucanase from *Cellulomonas fimi*; the first instance of a disaccharide mechanism-based cellulase inactivator [85]. The corresponding 2′,4′-dinitrophenyl-2-deoxy-2-fluoro-β-D-glucopyranoside (**40**) showed a rapid time-dependent inhibition activity [93] against *A. faecalis* β-glucosidase with a dissociation constant ($K_i$) of $5.0 \times 10^{-5}$ M and a rate constant ($k_i$) of 25 $min^{-1}$. 2′,4′,6′-Trinitrophenyl-2-deoxy-2,2-difluoro-α-glucoside **(42)** inactivated yeast α-glucosidase with a second order rate constant of $k_i/K_i$ $2.5 \times 10^{-4}$ $min^{-1}$ $M^{-1}$. The difluoromaltoside **43** inactivated human pancreatic α-amylase with $k_i/K_i = 7.3 \times 10^{-6}$ $min^{-1}$ $M^{-1}$. They were considered as valuable therapeutic agents [99].



39 R = β-D-xylopyranosyl 40 R = H
41 R = β-D-glucopyranosyl 42



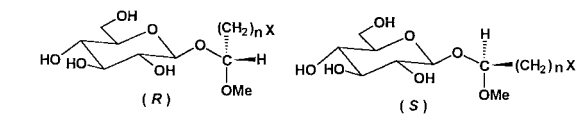
43 44 R = $N(CH_2CH_2Cl)_2$



45

The prodrug **44** was found to be toxic to LoVo tumor cells with an $IC_{50}$ value of $6.3 \times 10^{-6}$ M, in the presence of β-glucuronidase [100]. The paclitaxelglucuronyl carbamate prodrug **45** was relatively non-toxic and could be used in cancer treatment ($IC_{50} = 1.9 \times 10^{-8}$ g $ml^{-1}$ without β-glucuronidase and $IC_{50} = 6.0 \times 10^{-10}$ g $ml^{-1}$ with β-glucuronidase) [101].

The kinetic studies of ω-azido-1-methoxyalkyl β-D-glucopyranosides **46–49, 54** and **55** with β-D-glucosidase from sweet almond emulsin indicated that all azides were smoothly cleaved under standard conditions at a rate comparable to that for 2-nitrophenyl β-D-glucopyranoside and considerably faster than methyl β-D-glucopyranoside. When the ω-amino-1-methoxyalkyl-β-D-glucopyranosides **50–53, 56** and **57** were treated with very high concentrations of β-D-glucosidase from sweet almond emulsin, they exhibited significant differences in the rates of hydrolysis [102]. The expected decrease of protection by the amino group against enzyme-catalyzed hydrolysis with the length of the alkyl chain in the aglycon was observed when **50** and **56** (n = 1) and **53** (n = 4) were compared. However, the rates for **51** and **57** were much lower when compared with that for **50** (n = 1) and measurably lower when compared with that for **56** indicating that they are competitive inhibitors of the β-D-glucosidase activity with $K_i$ values around $6.0 \times 10^{-2}$ M, except for the isomer **51**. It is not



(R) (S)

46 n = 1, X = $N_3$ 50 n = 1, X = $NH_2$ 54 n = 1, X = $N_3$
47 n = 2, X = $N_3$ 51 n = 2, X = $NH_2$ 55 n = 2, X = $N_3$
48 n = 3, X = $N_3$ 52 n = 3, X = $NH_2$ 56 n = 1, X = $NH_2$
49 n = 4, X = $N_3$ 53 n = 4, X = $NH_2$ 57 n = 2, X = $NH_2$

Stereoisomeric mixture at aglycon carbon atom

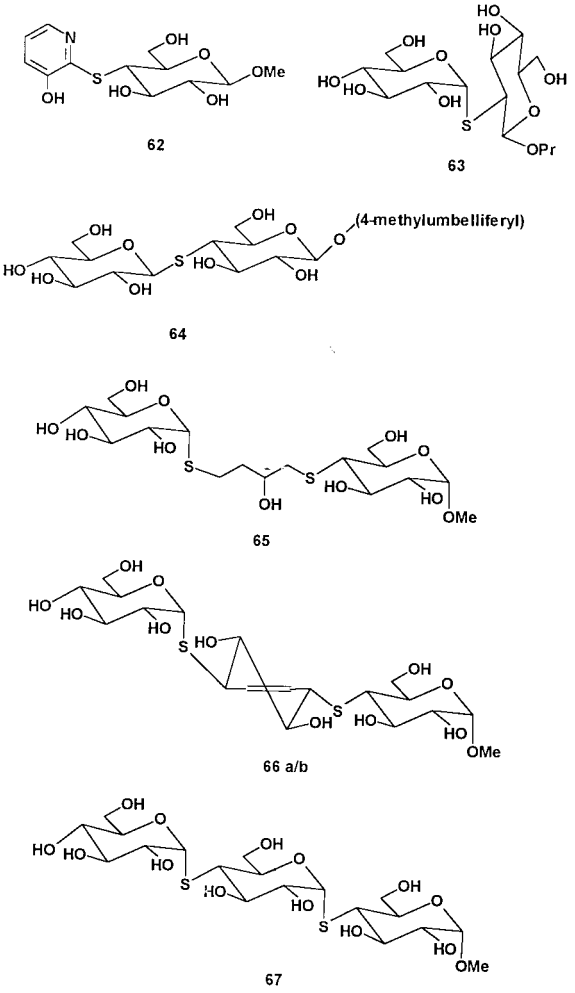
58 X = I, R = Me 60 X = I, R = Me
59 X = Br, R = Et 61 X = Br, R = Et

clear why **51** has an affinity 20 times larger than its diastereomer **57** and all other amines [102]. The inhibition of α-D-galactosidase from green coffee beans in the presence of diastereomeric mixtures of **58/60** or **59/61** together with β-D-glucosidase as sources of bromo and iodo-acetaldehyde showed a significant loss of galactosidase activity [103].

## 7. Deoxy thiosugar derivatives

The heteroaromatic ring in compound **62** was proposed to serve as a glycopyranosyl surrogate and this compound showed the best inhibition of β-glucosidase ($K_i = 8.0 \times 10^{-4}$ M) [104]. The thio-linked oligosaccharides were found to be potential competitive glucosidase inhibitors [105–113]**.** The disaccharide α-D-Glc-(1-S-2)-β-D-Glc-(1-OPr) (**63**), a thio analogue of α-D-Glc-(1 → 2)-α-D-Glc-(1-OPr) (n-Propyl kojibioside) has been synthesized for evaluation as a potential glucosidase inhibitor [114]. The methylumbelliferyl derivative **64** was found to be a competitive inhibitor of cellobiohydrolase I (CBH I) ($K_i = 6.7 \times 10^{-5}$ M) [113].

The inhibition of spacer-modified disaccharide **65** and the diastereomeric pseudo-trisaccharide methyl 4-*S*-[(1,5/4,6)- and (4,6/1,5)-4-α-D-glucopyranosylthio-5,6-dihydroxy-2-cyclohexen-1-yl]-4-thio-α-D-glucopyranoside (**66a/b**) that mimic maltotriose showed that all these compounds were competitive inhibitors for porcine pancreatic α-amylase [112]. The $K_i$ values for **65, 66a** and **66b** were (1.7, 1.1 and $1.6 \times 10^{-2}$ M, respectively). Also, methyl 4,4′-dithio-α-maltotrioside (**67**) competitively inhibited porcine pancreatic α-amylase with $K_i = 9.0 \times 10^{-3}$ M [112]. The spacer-modified disaccharide glycoside **65** as well as the two diastereomeric pseudo-trisaccharide glycosides **66a/b** have affinities for α-amylase slightly higher than that of maltose which has a $K_i$ value of $2.0 \times 10^{-2}$ M [115]. This situation demonstrated the clustering effect caused by the linking of two glucosyl units by a spacer of suitable length. The binding energy may be increased by the added polarity of the spacer, whereas **65** with the flexible spacer has the same affinity regardless of the stereochemistry of the butyl moiety, the diastereomers **66a/b,** with the fairly rigid spacer, showed significant differences in binding [112].




## 8. Thioglycosides

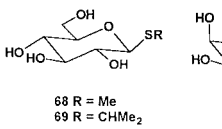
The thioglycosides **68** and **69** are weak inhibitors of β-glucosidase from brewer's yeast ($K_i > 5.0 \times 10^{-3}$ M) [116] and from *Agrobacterium faecalis* ($K_i = 4.0 \times 10^{-3}$ M) [93]. A competitive inhibition of cellobiohydrolase I (CBH I) by **70** ($K_i = 2.9 \times 10^{-5}$ M) [117], and by 4-acetamidophenyl 4-S-β-D-glucopyranosyl-1,4-dithio-β-D-glucopyranoside (**71**) ($K_i = 2.4 \times 10^{-5}$ M) [113] has been observed. A better competitive inhibition of CBH I ($K_i = 6.5 \times 10^{-6}$ M) [113] by 4-acetamidophenyl 1,4,4′-trithiocellotrioside (**72**) was reported. These results suggested that the sulfur exocyclic atom induces a preferential, nonproductive binding in the active side of CBH I.

## 9. Deoxy amino and guanidino sugars

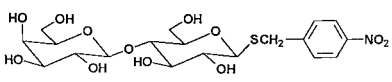
The inhibition studies of 2-acetamide-2-deoxy-D-glucose (**73**) showed that this compound has the ability to inhibit bovine β-*N*-acetyl glucosaminidase ($K_i = 4.0 \times 10^{-3}$ M) [118]. The activity of the mannosamine disaccharide **74** has been tested [119] against α-glucosidase I. The aminodisaccharide **75** inhibited purified porcine liver α-glucosidase I with an $IC_{50}$ value of $1.6 \times 10^{-5}$ M [119]. The urea derivative **76** showed a significant inhibition of *Trichomonas vaginalis N*-acetyl-β-D-hexosaminidase (I = 46%) [120] and it is a better inhibitor than tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucose itself (I = 31%).

Some analogues of disaccharides and glycosides containing a cyclic guanidinium structure such as **77a–80** showed varying inhibitory effects on glycoside hydrolases [121–124]. They showed either competitive or mixed competitive inhibition with α-D-glucosidase, β-D-glucosidase, α-D-galactosidase, and β-D-galactosidase. The guanidinium derivative **77a** moderately inhibited β-D-glucosidase ($K_i = 4.5 \pm 0.5 \times 10^{-4}$ M) [122]. Compound **77b** showed a weak inhibition of α- and β-galactosidases ($K_i = 5.0 \pm 0.5$ and $4.0 \pm 0.5 \times 10^{-3}$ M, respectively) [121]. The guanidinium derivative **78** exhibited very weak inhibition against β-D-glucosidase ($K_i = 3.5 \pm 0.5 \times 10^{-2}$ M) [122]. The low but almost equal inhibiting effect of the (1 → 4)-linked analogues **79** and **80** on β-glucosidase ($K_i = 1.0 \pm 0.2 \times 10^{-2}$ M for both) as well as β-D-galactosidase ($K_i = 7.5 \pm 1.0$, $5.5 \pm 1.0 \times 10^{-3}$ M, respectively) [122] indicated that the diastereomers **79** and **80** are bounded to the glycon binding sites of both enzymes through their reducing "aglyconic" ends which are, unlike their "glyconic" ends identical. The free lactol group could then mimic the Gal-configuration as α- and the Glc-configuration as β-anomer [122].

The inhibitory activity of the 6-deoxy-6-morpholino glycosides **81** and **82** indicated that the former ($K_i = 6.1 \times 10^{-5}$ M) has a better inhibition properties than **82**



68 R = Me
69 R = $CHMe_2$



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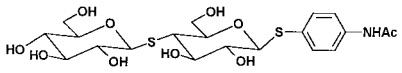

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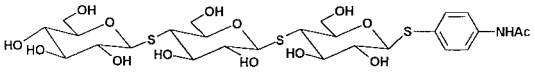
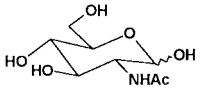


72



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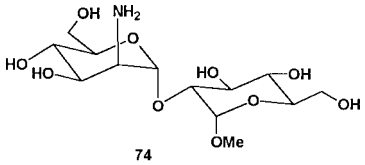

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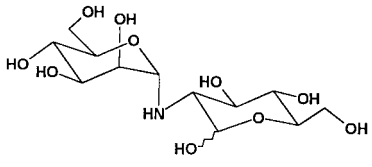

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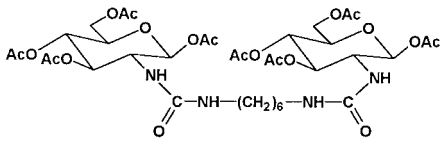
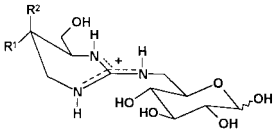
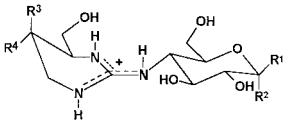


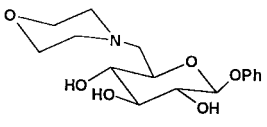
76



77a $R^1$ = OH, $R^2$ = H
77b $R^1$ = H, $R^2$ = OH



78 $R^1$ = OMe, $R^2$ = H, $R^3$ = H, $R^4$ = OH
79 (α) $R^1$ = H, $R^2$ = OH, $R^3$ = H, $R^4$ = OH
(β) $R^1$ = OH, $R^2$ = H, $R^3$ = H, $R^4$ = OH
80 (α) $R^1$ = H, $R^2$ = OH, $R^3$ = OH, $R^4$ = H
(β) $R^1$ = OH, $R^2$ = H, $R^3$ = OH, $R^4$ = H



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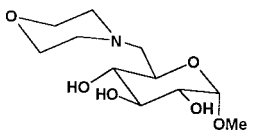

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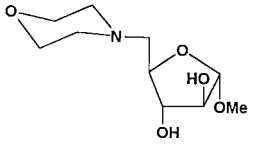

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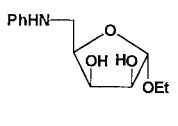
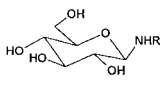


84

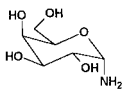
($K_i = 1.3 \times 10^{-4}$ M), while 5-deoxy derivatives **83** ($K_i = 7.2 \times 10^{-4}$ M) and **84** ($K_i = 3.4 \times 10^{-4}$ M), were found to be moderate competitive inhibitors of β-glucosidase from sweet almond. The phenyl glycoside **81** was found to be a non-competitive inhibitor for hepatic α-glucosidase with $K_i$ value of $1.6 \times 10^{-5}$ M [124].

## 10. Glycosylamines

Simple sugar analogues bearing a basic nitrogen attached to the anomeric position (glycosylamines) are strong glycosidase inhibitors [125]. The high affinity of glycosylamines and "imino sugars" for the corresponding glycosidases was due to the formation of an ion-pair between the protonated inhibitor and an active site function (typically, carboxylate) involved in the hydrolysis of the glycosidic bond of the substrates [25, 126]. Glycosylamines **85** and **87**–**89**, derived from D-glucose, D-galactose, D-mannose, and *N*-acetyl-D-glucosamine, inhibited the corresponding glycosidases up to $10^3$ times more strongly than their respective non-basic analogues, *e.g.* free sugars, thioglycosides, *N*-acetyl- or *N*-aryl-glycosylamines [118, 125, 127–129]. The glucosylamine **85** exhibited a potent inhibition of yeast β-glucosidase ($K_i = 2.2 \times 10^{-6}$ M, $IC_{50} = 2.0 \times 10^{-6}$ M), while galactosylamine **87** inhibited *Vicina faba* α-galactosidase and yeast β-galactosidase with $K_i$ values of 7.3 and $4.9 \times 10^{-5}$ M, respectively. The D-mannosylamine **88** was found to be a powerful inhibitor of hog kidney α-mannosidase ($K_i = 4.2 \times 10^{-6}$ M) [125]. Both **87** and **88** are known to mutarotate rapidly in solution [130]. It has been shown that the sweet almond enzyme possesses a hydrophobic domain at the aglycon binding site [126] that promotes a favorable interaction with nonpolar substituents such as the phenyl group of *N*-benzyl-β-D-glucopyranosylamine (**86**) which inhibited the almond β-glucosidase ($K_i = 3.2 \times 10^{-7}$ M) [127]. Although glycosylamines have good inhibitory activity and can be prepared easily, their susceptibility to spontaneous hydrolysis and instability of the α,β-anomeric configuration in aqueous solution prevented them from becoming practical inhibitors. 2-Acetamido-2-deoxy-β-D-glucosylamine (**89**) inhibited the bovine β-*N*-acetylglucosaminidase with a $K_i$ value of $4.3 \times 10^{-5}$ M [118]. *N*-Bromoacetyl-β-glycopyranosylamines **90** and **91** inactivated both β-glucosidase and *exo*-glucanase ($K_i = 8.3$ and $9.1 \times 10^{-3}$ M, respectively) according to pseudo-first order kinetics [131]. The diglycosylamines may be more stable than glycosylamines because of the combined electron-withdrawing effect of the two ring oxygens which may decrease the basicity of the nitrogen. The α-D-glucopyranosyl-β-D-glucopyr-



85 R = H
86 R = Bn



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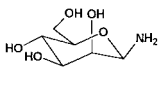
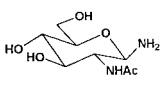


88



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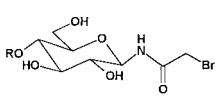
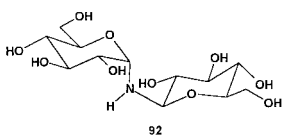


90 R = H
91 R = β-D-glucopyranosyl



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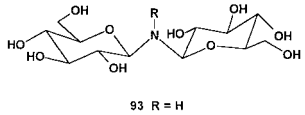
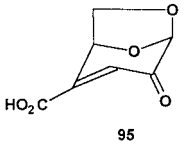


93 R = H
94 R = Ac

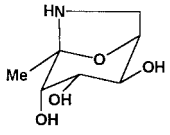
anosylamine (**92**) and di-β-D-glucopyranosylamine (**93**) competitively inhibited *Trichoderma* β-glucosidase [132] ($K_i = 2.8$ and $3.8 \times 10^{-5}$ M, respectively). The similarity of the $K_i$ values indicated that the enzyme recognized only the β-glucosyl unit in the diglucosylamine molecule, since β-glucosylamine (**85**) has a $K_i$ value of $2.4 \pm 0.4 \times 10^{-5}$ M. The introduction of an *N*-acetyl group as in *N*-acetyl-di-β-D-glycosylamine (**94**) led to a drastic increase in the $K_i$ value ($5.7 \pm 1.2 \times 10^{-2}$ M) which may be due to the decreased basicity of the nitrogen atom or to the steric hindrance by the *N*-acetyl group. The effect of pH on the inhibition constant ($K_i$) indicated that the neutral forms of **92** and **93** and not the respective ionized one were responsible for the inhibition where the maximum inhibitory capacity of both **92** and **93** was at pH 5, which coincident with the pH optimum for the enzyme activity [133]. Compounds **92** and **93** effectively inhibited β-glucosidases from *Aspergillus niger* and *Aspergillus phoenicis* [132].

## 11. Anhydrosugars and their analogues

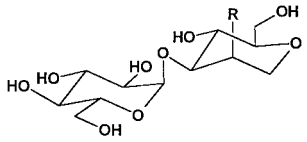
The enopyranose anhydrosugar derivative **95** *in vitro* showed an $IC_{50}$ value of $5.2 \times 10^{-6}$ g ml$^{-1}$ against α-glucosidase [134]. The hemiaminal **96** is a powerful and selective inhibitor of human liver α-fucosidase ($K_i = 6.0 \times 10^{-6}$ M) and human placenta α-fucosidase ($K_i = 6.8 \times 10^{-5}$ M) [135].



95



96



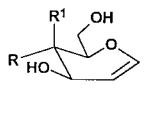
97 R = H
98 R = OH

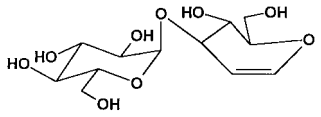
## 12. Deoxysugars

The 1,2-dideoxy derivative **97** inhibited *endo*-mannosidase 40 times stronger than the 1-deoxy derivative **98** [136, 137].

## 13. Glycals

The α-glucosidase from a species of *Candida* yeast has a high capacity to utilize D-glucal **99** as a substrate [138]. Kinetic analysis showed that the hydration of D-glucal by α-glucosidase [139] to give 2-deoxy-D-glucose is a dead-end inhibition, due to the formation of complexes of enzyme with two D-glucal molecules [140]. The acceptor site is a good place where a second D-glucal molecule might bind with high affnity to an enzyme associated with the substrate, and also cause inhibition. The inhibition of the hydration [141] of D-glucal with β-D-glucosidase from sweet almond and *Aspergillus wentii* was $1.0 \times 10^{-2}$ M and $1.9 \times 10^{-4}$ M, respectively. β-D-Galactosidases of bacterial, fungal and plant origins were inhibited specifically [142] by D-galactal (**100**) with $K_i$ values from 1.8 to $9.0 \times 10^{-5}$ M. The inhibition of β-D-galactosidase from *Escherichia coli* by D-galactal indicated that D-galactal itself was only a weak inhibitor ($K_i \geq 1.0 \times 10^{-2}$ M from presteady-state





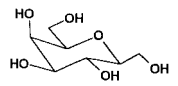
99 R = OH, R¹ = H
100 R = H, R¹ = OH

101

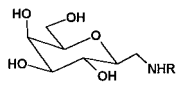
rates). Whereas that from *Aspergillus wentii* has strong inhibitory potency ($K_i = 1.3 \times 10^{-5}$ M) [143] due to the slow formation of a 2-deoxy-D-hexosyl-enzyme intermediate which was slowly hydrolyzed to regenerate the free enzyme and 2-deoxy-D-galactose. Glycals have been found in several cases to serve as pseudo substrates of some glycosidases to form, under hydroxylation, a 2-deoxyhexose derivative [24]. The glycal derivative **101** is a strong inhibitor of *endo*-mannosidase (74% relative potency to 1-deoxy-3-*O*-(α-D-glucopyranosyl)mannojirimycin) [136].

## 14. C-Glycosides

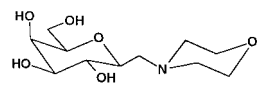
It has been shown that β-D-galactopyranosyl methanol (**102**) was a weak reversible inhibitor of *E. coli lacZ* β-D-galactosidase ($K_i = 1.1 \times 10^{-2}$ M) [144]. C-(β-D-Galactopyranosylmethyl) amine (**103**) is a weak mixed inhibitor of sweet almond β-glucosidase ($K_i = 1.7 \times 10^{-2}$ M) [145]. The inhibition of *E. coli lacZ* β-D-galactosidase with **103** at different pH values showed that its binding constant ($K_i = 1.2 \pm 0.34 \times 10^{-4}$ M) at pH 8.0 was one-fourth less than that at pH 7.3 ($5.1 \times 10^{-4}$ M) and at pH 6.0 ($K_i = 1.1 \times 10^{-2}$ M) [146] was over twenty times larger than the $K_i$ at pH 7.3. These results indicated that when the amine is protonated, it is not a good competitive inhibitor of *E. coli lacZ* β-galactosidase, perhaps either because a proton must be donated within the binding site ($AH \rightarrow A^-$) in order to stabilize an ion pair to be formed, or because of a barrier to the approach of a positively charged species. The *N*-(β-D-galactopyranosylmethyl)aniline (**104**) inhibited *E. coli lacZ* β-D-galactosidase with a binding constant of $K_i = 1.7 \times 10^{-4}$ M [146]. *N*-Acetyl-(β-D-galactopyranosylmethyl)amine (**105**) and *N*-benzoyl-(β-D-galactopyranosylmethyl)amine (**106**) are reversible inhibitors of *E. coli lacZ* β-galactosidase ($K_i$ =



102



103 R = H 104 R = Ph
105 R = Ac 106 R = Bz
107 R = Bn 108 R = $CH_2CF_3$
109 R = $(CH_2)_2OMe$



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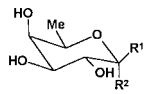
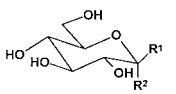
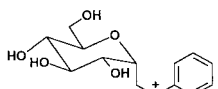


111 R¹ = $CH_2NH_2$, R² = H
112 R¹ = H, R² = $CH_2NH_2$



113 R¹ = $CH_2NH_2$, R² = H
114 R¹ = H, R² = $CH_2NH_2$



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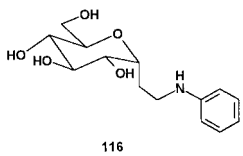
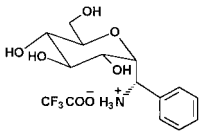


116

3.3 and $3.8 \times 10^{-3}$ M, respectively) [144]. *N*-Benzyl-(β-D-galactopyranosylmethyl)amine (**107**) was found to be a potent reversible inhibitor of *E. coli lacZ* β-galactosidase ($K_i = 2.3 \times 10^{-6}$ M) [146]. Compounds **108**–**110** showed a reversible inhibition against this enzyme with $K_i$ values of $1.6 \times 10^{-3}$, $1.9 \times 10^{-5}$ and $5.0 \times 10^{-4}$ M, respectively [146]. The *C*-(β-D-fucopyranosylmethyl)amine (**111**) exhibited competitive inhibition against sweet almond β-glucosidase ($K_i = 2.8 \times 10^{-3}$ M) [145], whereas *C*-(α-D-fucopyranosylmethyl)amine (**112**) did not exhibit any inhibitory activity against yeast α-glucosidase in accordance with a high glycon specificity of the yeast α-glucosidase [147]. *C*-(β-D-Glucopyranosylmethyl)amine (**113**) is a mixed inhibitor of sweet almond β-glucosidase ($K_i = 5.5 \times 10^{-3}$ M) [145]. *C*-(α-D-Glucopyranosylmethyl)amine (**114**) competitively inhibited yeast α-glucosidase with a $K_i$ value in the $10^{-3}$ M range [145]. The aniline salt **115** exhibited a strong inhibition against yeast α-D-glucosidase ($K_i = 1.1 \times 10^{-5}$ M) [72]. The homologue **116** inhibited β-D-glucosidase with $K_i$ value of $1.3 \times 10^{-4}$ M [148].

There is a clear distinction between the diastereomeric α-amino-substituted derivative **117** and its isomeric analogue **118** towards the inhibition of yeast α-glucosidase. Thus, **117** inhibits this enzyme with $K_i = 1.1 \times 10^{-3}$ M, whereas **118** shows a relatively strong inhibition ($K_i = 3.8 \times 10^{-5}$ M) [72]. The corresponding β-C-glycosides **119** and **120** competitively inhibited β-glucosidase with $K_i = 7.0 \times 10^{-5}$ M and $7.6 \times 10^{-3}$ M, respectively [149]. Also, α-D-glucosylmethylbenzene (**121**) exhibited only low inhibition of α-D-glucosidase ($K_i = 1.3 \times 10^{-3}$ M), whereas the β-C-glycosides **122** and **123** inhibited sweet almonds β-glucosidase with $K_i$ values in the same range as for **120** [149]. The C-glycoside **124** and **125** exhibited low competitive inhibition ($K_i = 3.8 \times 10^{-2}$ and $7.0 \times 10^{-3}$ M, respectively) against almond β-glucosidase [150]. *Bis-C*-(β-D-glucopyranosyl)methylamine (**126**) and C-β-β-trehalose (**127**) were tested as inhibitors of the sweet almond β-glucosidase [145] and they were found to be devoid of inhibitory activity, due to the hydrophilicity of their "aglycon". Compounds **128** and **129** are very weak inhibitors of porcine kidney trehalase with $IC_{50}$ values higher than $4.7 \times 10^{-2}$ M [151].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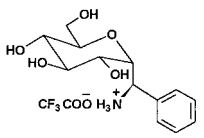
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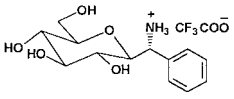
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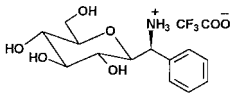
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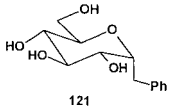
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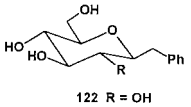
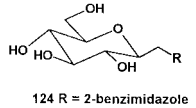
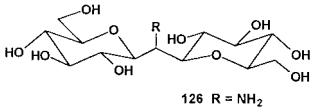
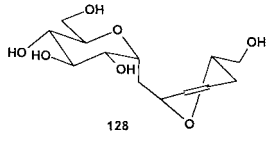

122 R = OH
123 R = H


124 R = 2-benzimidazole
125 R = 2-imidazole


126 R = $NH_2$
127 R = H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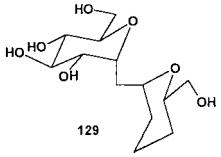
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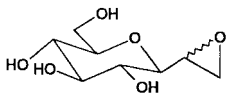
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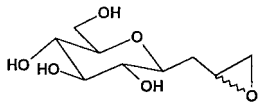
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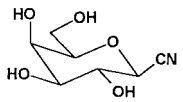
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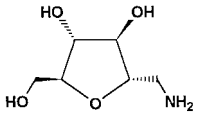
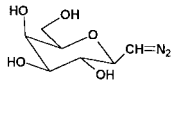

133

The epoxides **130** and **131** showed irreversible deactivation of sweet almond β-D-glucosidase [152]. The β-D-galactopyranosyl cyanide (**132**) showed a reversible inhibition against *E. coli lacZ* β-galactosidase ($K_i$ $2.0 \times 10^{-3}$ M) [146]. Compound **133** moderately inhibited β-D-glucosidase ($K_i = 1.3 \times 10^{-4}$ M) [148].

It has been shown that 2,6-anhydro-1-deoxy-1-diazo-D-glycero-L-mannoheptitol (**134**) was an efficient, active-site-directed, irreversible inhibitor (suicide substrate) of β-D-galactosidase from *Escherichia coli* [153]. The diazoketone **135** showed irreversible inhibition of yeast α-D-glucosidase, but it did not show any significant inhibition against sweet almonds β-glucosidase [34]. Studies on diazomethylglycosyl ketones **136** and **137** indicated that these compounds function as enzyme-activated irreversible inhibitors [129, 154–156]. The diazomethyl β-D-galactopyranosyl ketone (**136**) inactivated *Aspergillus oryzae* β-D-galactosidase *in vitro,* in the absence of light or transition-metal ions. This occurred in a time dependent, pseudo-first-order process which exhibited saturation kinetics [157]. Similarly, the diazomethyl β-D-glucopyranosyl ketone (**137**) inactivated sweet almond β-D-glucosidase *in vitro* [158]. It was proposed that compounds **136** and **137** might act as suicide substrates of glycosidases [157, 158]. Thus, active site-mediated protonation of the diazo carbon atom of these substrate analogues by the corresponding glycohydrolases would generate a highly reactive alkyl diazonium ion which could alkylate some active site nu-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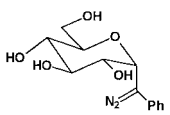
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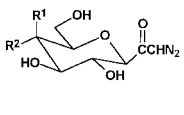
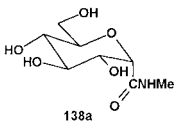

136 $R^1$ = OH, $R^2$ = H
137 $R^1$ = H, $R^2$ = OH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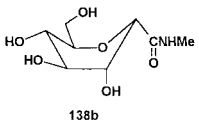
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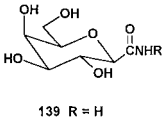
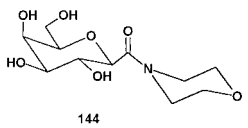

139 R = H
140 R = Ph
141 R = Bn
142 R = $CH_2CF_3$
143 R = $(CH_2)_2OMe$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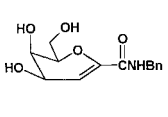
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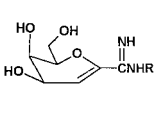
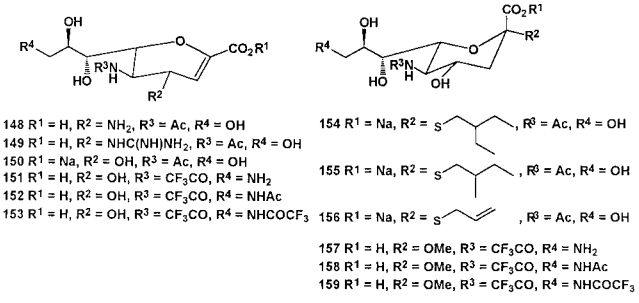

146 R = H
147 R = Bn

cleophile before dissociation occurred, resulting in suicide inactivation of the enzyme. The diazomethylglycosyl ketones are potential photoaffinity-labeling reagents [159, 160] and classical affinity labeling reagents for carbohydrate-binding proteins (enzymes, lectins, antibodies, *etc.*) [155] where the diazoacetyl functional group can be activated by photolysis and by certain transition-metal ions. 2,6-Anhydro-*N*-methyl-D-glycero-D-ido-heptonamide (**138**) which exists in a skew-boat conformation **138b** [161] was a poorer inhibitor than glucose towards glycogen phosphorylase ($K_i = 3.7 \times 10^{-2}$ M). Distortion of the chair conformation of **138a** to the skew-boat conformation **138b** leads to fewer hydrogen bonds between **138** and the enzyme than between glucose and the enzyme. The C-β-D-galactopyranosylformamide (**139**) is a competitive inhibitor of *E. coli lacZ* β-galactosidase ($K_i = 9.8 \times 10^{-3}$ M) [144, 158]. N-Substituted *C*-(β-D-galactopyranosyl)formamides **140, 142** and **144** were found to be reversible inhibitors of this enzyme with binding constants similar to those of the corresponding amines with $K_i$ values of $1.8 \times 10^{-3}$, $3.1 \times 10^{-3}$ and $4.3 \times 10^{-4}$ M, respectively [146]. Compounds **141** and **143** bound less tightly than did their amines counterparts with $K_i$ values of $8.2 \times 10^{-4}$ and $1.3 \times 10^{-2}$ M, respectively [146].

The *N*-benzylamide derivatives **141** and **145** are almost equally efficient inhibitors against β-D-galactosidase from *Escherichia coli* ($K_i = 8.2$ and $7.6 \times 10^{-4}$ M, respectively) [29, 146]. A strong inhibition of the free bases of the amidines **146** and **147** ($K_i = 4.3 \times 10^{-7}$ and $8.3 \times 10^{-9}$ M, respectively) were found for the same enzyme [63, 128, 146, 162].

The 4-amino- and 4-guanidino neu-5Ac2en, **148** and **149** are potent and selective influenza neuraminidase inhibitors with $K_i$ values $1.0 \times 10^{-8}$ and $1.0 \times 10^{-10}$ M, respectively [163–165]. They inhibited the replication of both influenza A and B viruses in cell culture and were efficacious in animal models [164–169]. Their design was based on protein structure data from a complex of influenza sialidase with an inhibitor [165, 170]. The C-glucoside analogues of sialic acid have been tested as potential influenza inhibitors [171]. The Neu-5Ac2en sodium salt **150** has a moderate $K_i$ value ($\approx 10^{-4}$ M) against *S. typhimurium* sialidase when compared with other bacterial or viral sialidases [167, 172]. The reaction catalyzed by *S. typhimurium* sialidases proceed through a single transition state that resembles the ground state $^2C_5$ chair conformation [173]. Compounds **154–156** exhibited weak inhibition for the last enzyme ($K_i \geq 1.0 \times 10^{-3}$ M) due to the nature of the hydrophobic aglycone [167]. It was found that 2,3-didehydro-2,3-dideoxy-D-glycero-D-galacto-2-nonulopyranosonic acid was a strong competitive inhibitor of deaminoneuraminic acid enzyme (KDNase Sm) ($K_i = 7.7 \times 10^{-6}$ M). Neu-5Ac2en (**150,** $R^1$ = H) and its *N*-glycolyl derivative were known to be strong competitive inhibitors for bacterial sialidases such as *Arthrobacter nreafaciens* sialidases.

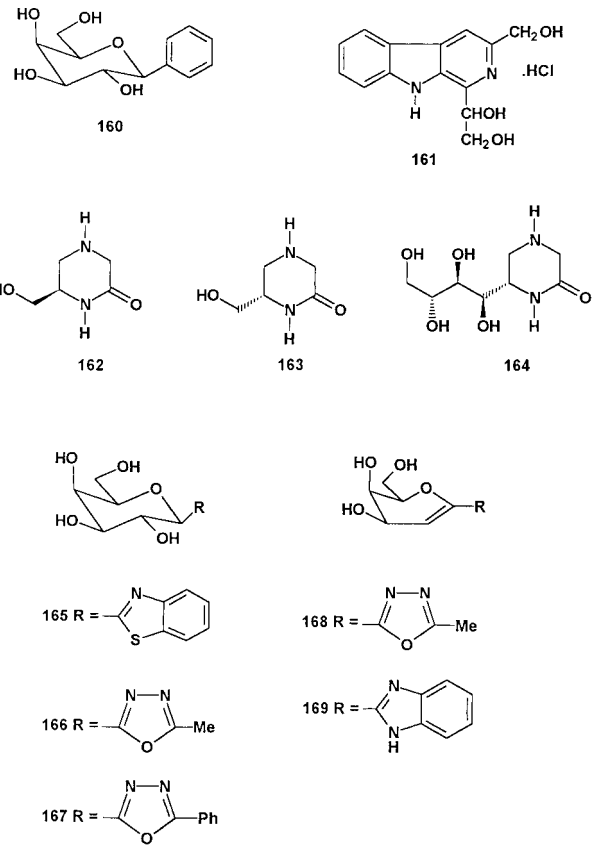



However, KDNase Sm activity is not significantly inhibited by these compounds. This observation suggests that the hydroxyl group at C-5 is important for recognition of the inhibitor by the enzyme [174]. The 2,3-didehydro analogues **152** and **153** inhibited influenza A sialidase ($IC_{50} = 7.8 \times 10^{-6}$ and $1.2 \times 10^{-5}$ M, respectively) more than did the 9-amino analogue **151** ($IC_{50} = 5.3 \times 10^{-4}$ M). However, the methyl α-glycoside types **157–159** exhibited poor inhibitory activity up to $1.0 \times 10^{-4}$ M concentration [175].

The C-phenyl galactoside **160** inhibited competitively the β-D-galactosidase from *Escherichia coli* ($K_i = 4.5 \times 10^{-4}$ M) [176].

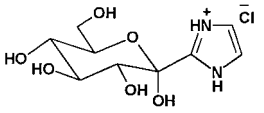
## 15. C-Nucleosides

The naturally occuring 1-[1*R*,2-dihydroxyethyl]-3-hydroxymethyl-9*H*-pyrido[3,4-*b*]indole (pyridindol) (**161**) is a specific inhibitor of β-galactosidase from bovine liver in acidic conditions at pH 4.2 ($IC_{50} = 2.0 \times 10^{-6}$ gm$1^{-1}$) [177, 178], but there is no inhibition at pH 7.0 ($IC_{50} >$ 250). The inhibition was non competitive by the formation of a complex with the enzyme. On the other hand, it has no effect on β-galactosidases from other sources. The piperazinones **162–164** that have a high degree of structural resemblance to isofagomine are weak competitive inhibitors of glycosidases having $K_i$ values above $1.0 \times 10^{-3}$ M, suggesting that the 3- and 4-OH groups were crucial for their binding to glycosidases and also indicating that 3-OH group acted as a hydrogen bond donor [179]. The competitive inhibition of *C*-(β-D-galactosyl) and *C*-(2-deoxy-D-lyxo-hex-1-enopyranosyl) C-nucleosides against β-D-galactosidase from *Escherichia coli* has been studied. The results showed that 2-(β-D-galactopyranosyl) benzothiazole (**165**, $K_i = 6.5 \times 10^{-4}$ M) was a similar inhibitor as β-D-galactopyranosylbenzene (**160**; $K_i = 4.5 \times 10^{-4}$ M),



while the 5-methyl-1,3,4-oxadiazole derivative (**166**, $K_i = 6.8 \times 10^{-3}$ M) was a weaker by one order of magnitude, which may be due to the less hydrophobic character of the oxadiazole ring [29]. The change of the 5-methyl in **166** to a 5-phenyl group as in **167** strengthened the inhibition ($K_i = 1.5 \times 10^{-5}$ M) significantly by more than two orders of magnitude indicating that the position of the hydrophobic group was very important. The comparison between the inhibition of 2-deoxy-D-lyxo-hex-1-enopyranosyl derivatives with that of β-D-galactopyranosyl compounds indicated that the unsaturated substances are generally weaker inhibitors [24]. Although, benzimidazole **169** has high basicity, it was less effiicient inhibitor ($K_i = 1.6 \times 10^{-2}$ M) than the 2-(β-D-galacto-pyranosyl)benzothiazole **165**. The pair of 5-methyl-1,3,4-oxadiazoles **166** and **168** ($K_i = 6.8 \times 10^{-3}$ M and $5.5 \times 10^{-4}$ M, respectively) were the examples which showed higher inhibitory activity for derivatives of the D-galactal type [29].

The imidazolyl C-glucopyranosides **170** and **171** are moderate competitive inhibitors of sweet almond β-glucosidase (pH 6.8, $K_i \approx 7.9$ and $6.4 \times 10^{-4}$ M, respectively), while **172** is a competitive inhibitor of yeast α-glucosidase (pH 6.8, $K_i \approx 2.6 \times 10^{-4}$ M) [180].

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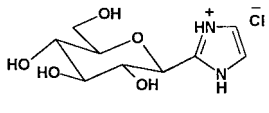


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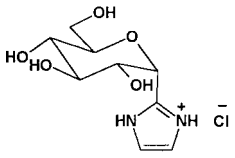


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