



Small molecule inhibitors of mammalian glycosylation

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Abstract

Glycans are one of the fundamental biopolymers encountered in living systems. Compared to polynucleotide and polypeptide biosynthesis, polysaccharide biosynthesis is a uniquely combinatorial process to which interdependent enzymes with seemingly broad specificities contribute. The resulting intracellular, cell surface, and secreted glycans play key roles in health and disease, from embryogenesis to cancer progression. The study and modulation of glycans in cell and organismal biology is aided by small molecule inhibitors of the enzymes involved in glycan biosynthesis. In this review, we survey the arsenal of currently available inhibitors, focusing on agents which have been independently validated in diverse systems. We highlight the utility of these inhibitors and drawbacks to their use, emphasizing the need for innovation for basic research as well as for therapeutic applications.

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Introduction

All cells synthesize complex glycans, also called sugars, carbohydrates, or polysaccharides. In mammals, glycoconjugates are broadly classified as (i) glycoproteins which bear O-linked glycans on serine/threonine residues or N-linked glycans on asparagine residues, (ii) proteoglycans which are proteins modified by long glycosaminoglycan chains, (iii) glycolipids, (iv) free polysaccharides, and (v) the recently reported glycosylated RNAs (Fig. 1A) [1–4].

Human glycans are constructed from nine monosaccharide units: fucose (Fuc), galactose (Gal), glucose (Glc), glucuronic acid (GlcA), mannose (Man), *N*-acetylgalactosamine (GalNAc), *N*-acetylglucosamine (GlcNAc), sialic acid (the most common of which is *N*-acetylneuraminic acid, Neu5Ac), and xylose (Xyl) (Fig. 1A). These monomers are converted to nucleotide sugar

donors which act as substrates for glycosyltransferases (glycosyltransferase biochemistry has been reviewed elsewhere [5–8]). The majority of the cellular glycan pool is composed of complex, branching glycans that are biosynthesized in the endoplasmic reticulum (ER) and the Golgi apparatus by hundreds of enzymes acting through interwoven metabolic pathways. Complex glycoconjugates are then secreted into the extracellular space or trafficked to the cell membrane. Intracellular proteins can be modified with unelaborated *O*-GlcNAc, which has been reviewed elsewhere [9–12]. All glycan classes can be modified post-synthetically, e.g. through sulfation and acetylation [13]. Taken together, glycosylation is both more abundant and more structurally diverse than other post-translational modifications such as phosphorylation and palmitoylation [14].

Complex glycoconjugates are a vital constituent of cell signalling [15–17]. Indeed, extracellular

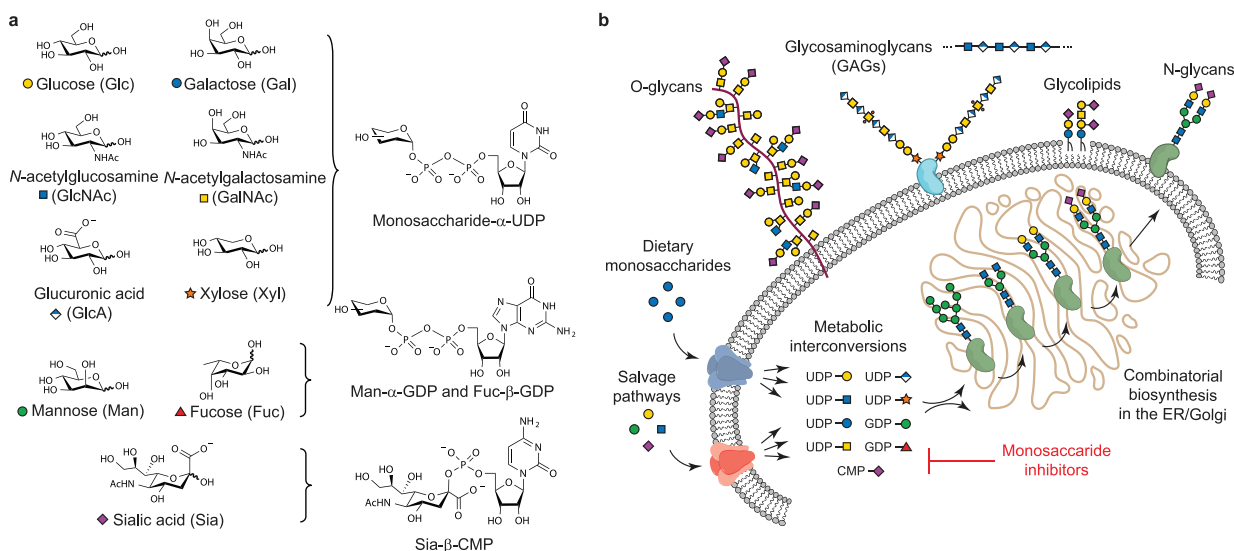


Fig. 1. Fundamentals of glycosylation. (a) Monosaccharides units used in human glycans and their nucleotide sugar donors, along with symbol nomenclature [292] (b) Overview of glycosylation pathways. Dietary monosaccharides and monosaccharides obtained from salvage pathways are internalized via membrane channels and transporters. Through complex metabolic pathways, they are converted to nucleotide sugars, and transported to the ER and Golgi apparatus where combinatorial biosynthesis is performed. The final glycosylated lipids and proteins are then shuttled to the cell membrane. Monosaccharide inhibitors (red text) are structural analogues of naturally occurring monosaccharides, and act as competitive inhibitors for downstream enzymes.

glycans are recognized as a key regulator of cell, tissue, and organismal processes including leukocyte adhesion, host–pathogen interaction, viral entry, embryonic development, cancer cell adhesion, and cellular differentiation [18–24]. Decades of biochemical and clinical evidence point not only to the significance of the complex glycans in health, but also to the consequences of altered glycosylation in disease, including vascular diseases, infectious diseases, autoimmune diseases, and cancer progression [15,16,25–35]. Cell-surface and secreted glycoconjugates have therefore attracted considerable attention as a point of therapeutic intervention [36].

Efforts to selectively target extracellular glycans have historically faced several technical challenges: (i) glycans are not directly encoded in the genome, meaning it is extremely difficult to manipulate a gene and observe an exclusive consequence on a single glycan structure [37]; (ii) robust technologies for labelling and sequencing complex glycans have historically lagged behind those of peer fields. As a result, despite promising recent efforts to, for example, prevent loss of cell surface glycans upon vascular injury or reverse upregulation of immune checkpoint binding sialic acids on tumors [1,38], progress in small molecule drug targeting of glycosylation pathways has trailed progress made for targeting of other fundamental biomolecules and post-translational modifications.

Here, we aim to describe and critically assess the current battery of small, drug-like molecules that

target the biosynthetic machinery responsible for complex, extracellular mammalian glycosylation (compiled in Table 2). We strive to provide a useful resource for experimentalists as well as highlight the need for innovation: many key molecular targets cannot be addressed at the moment. Thus, efforts from medicinal chemists and biochemists to accelerate the progress in this field are required, which we hope to inspire.

Monosaccharide inhibitors

Monosaccharide inhibitors are engineered sugars that mimic naturally occurring monosaccharides but cannot be readily used by cellular biosynthetic machinery (Fig. 1b, red text). Several deoxygenated and fluorinated analogues of glucose, mannose, glucosamine, and mannosamine have been developed, and the most commonly used ones are described below [39,40].

Note, if the experimenter's goal is to reduce levels of a single glycan class, these inhibitors may be inappropriate, as they will reduce the levels of all glycan types bearing the inhibited monosaccharide unit. If the experimenter's goal is to reduce total levels of a single monosaccharide unit, still caution is required as other precursors may be reduced through intermediary metabolism [41]. Therefore, it can be difficult to directly link observed phenotypes to loss of specific glycan classes using these inhibitors.

2-Deoxyglucose

2-Deoxyglucose (2-DG) was one of the first sugar analogues described [42,43]. 2-DG is a glucose and mannose analogue which features a hydrogen atom instead of the 2-hydroxyl group. Mimicking glucose, 2-DG enters the cell via glucose transporters, where it can act to inhibit glucose-dependent pathways. For example, 2-DG seems to preferentially inhibit protein *N*-glycosylation at normoxic conditions, whereas at hypoxic conditions it preferentially inhibits glycolysis [44,45]. The effects of 2-DG on *N*-glycoprotein synthesis are the best studied, and involve 2-DG metabolism to UDP-2-DG and GDP-2-DG. GDP-2-DG then gets transferred onto its lipid carrier, thus forming dolichol-P-2DG. The formation of dolichol-P-2DG terminates further extension of the oligosaccharide precursor and its transfer to the protein due to the lack of the hydroxyl group necessary for extension. Notably, the inhibitory effect of 2-DG is reversed in cells upon the addition of glucose as well as mannose [46]. The effective concentration range for 2-DG in cells is 1–5 mM, with some reports going as high as 50 mM [47]. As cancer cells exhibit increased glucose uptake and metabolism, several groups have investigated the use of 2-DG as an adjuvant to chemotherapeutic agents in several cancer types [48–50].

Several fluorinated sugar analogues that are similar to 2-DG, including 2-fluoro-D-mannose (2-FM) and 2-fluoro-2-deoxy-D-glucose (2-FG), have been developed as well. Both 2-FM and 2-FG inhibit protein *N*-glycosylation at an effective concentration range of 0.2–10 mM and 0.2–3 mM, respectively [47,51,52]. Kurtoglu, Lampidis, *et al.* showed the potential of these sugar analogues in blocking glycoprotein synthesis in cancer cells in the following order: 2-DG > 2-FM > 2-FG [45].

Glucosamine

Glucosamine is also referred to as 2-amino-2-deoxy-D-glucose. Its use as an inhibitor of *N*-glycosylation has been reported at a concentration of 1 mM in Madin-Darby Canine Kidney (MDCK) cells. At this concentration the cells exhibited a lipid carrier with an oligosaccharide precursor of the structure $\text{Man}_{6-8}(\text{GlcNAc})_2$ compared to $\text{Glc}_3\text{Man}_9(\text{GlcNAc})_2$ structures in control cells, which may be of interest in scenarios where truncated *N*-glycosylation is desired. This shift towards smaller precursors was more profound when the inhibitor concentration was increased to 10 mM [53]. This compound is usually used at a concentration range of 4–40 mM in cells [51]. It was reported that glucosamine also exhibits anti-cancer activity that is thought to arise from its ability to inhibit *N*-glycosylation [54,55]. Glucosamine is unlikely to act selectively on *N*-glycosylation pathways. For example, it can modulate lipid biosynthesis causing ER stress, [56] as well as *O*-glycan

biosynthesis resulting in differential response of cultured cells to TNF- α [57]. In addition, glucosamine is used as an over-the-counter remedy for joint pain [58].

4-F-GlcNAc

Woynarowska, Bernacki, *et al.* first reported 4-F-GlcNAc as a glycosylation inhibitor, demonstrating inhibition of glycoconjugate biosynthesis in human ovarian carcinoma cells (A-121) [59]. 4-F-GlcNAc has also been applied in mouse models of melanoma (B16) and lymphoma (EL-4), where 100 mg/kg intraperitoneal treatments were tolerated [60]. In those models, reduction in levels of T cell and B cell *N*-acetylglucosamines (LacNAcs) were observed by flow cytometry. Barthel, Dimitroff, *et al.* explored 4-F-GlcNAc's mechanism of action, concluding that 4-F-GlcNAc functions through inhibition of intracellular UDP-GlcNAc formation, not via direct incorporation into glycans [61]. They reported a decrease in the content and diversity of *N*- and *O*-glycans through lowering the abundance of tri- and tetra-antennary *N*-glycan structures, LacNAc extensions, and core 2 *O*-glycan and sialyl-Lewis X structures. RNA sequencing revealed that 4-F-GlcNAc had negligible effects on the expression of glycosylation-related genes, and glycosyltransferase assays in cell lysates showed no change in activity upon 4-F-GlcNAc treatment.

Inhibitors of *N*-glycans

N-glycosylation is a post-translational modification by which glycans are covalently linked to asparagine (Asn) (Fig. 2). This complex process initiates in the endoplasmic reticulum, where dolichol phosphate is embedded into the membrane. Analogous to a step-by-step assembly of a product on a conveyor belt, glycans are transferred to the dolichol, which is then flipped into the lumen of the ER. In the ER lumen, the glycolipid is further decorated by mannosyltransferases and glucosyltransferases. The oligosaccharyltransferase complex (OST) then mediates transfer of the glycan tree from the lipid carrier to the target protein. Next, α -glucosidases and α -mannosidases trim the glycan tree on the protein. The resulting glycoprotein (-Asn-GlcNAc₂Man₈) is then transferred to the Golgi apparatus where mannose units are further trimmed. Finally, a series of Golgi enzymes build the glycan tree back up, diverging into pathways responsible for synthesis of high mannose, complex, and hybrid *N*-glycan structures. *N*-glycosylation has been implicated in several diseases including inflammatory bowel disease, diabetes, viral infections, and cancer [62–66]. As one example, the functions of cancer-related adhesion molecules including integrins, TGF β receptor, and epithelial growth factor receptor, are influenced by their *N*-glycosylation

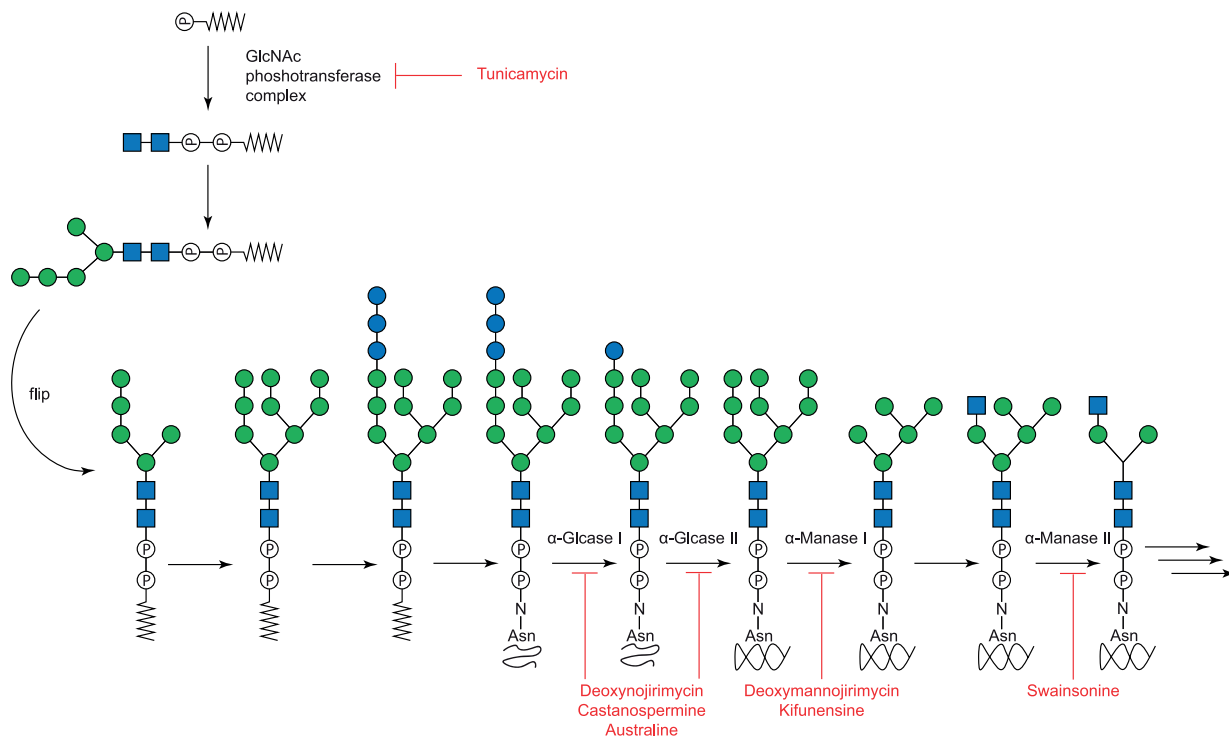


Fig. 2. Inhibitors of *N*-glycosylation. Canonical sequence of *N*-glycan biosynthesis. Key inhibitors are shown in red. Enzymes are shown only for steps for which an inhibitor is available.

status [67–69]. Modulating the biosynthetic pathways involved in this post-translational modification has emerged as a promising strategy for suppressing cancer progression [70–77].

Inhibitors of dolichol precursor assembly

One approach to reduce *N*-glycan levels is to block the glycan assembly onto dolichol phosphate. Several antibiotics have been shown to inhibit *N*-linked glycosylation in both animal and cell models via this route.

Tunicamycin. Over four decades ago, while screening for bacterially-derived antiviral drugs, Takatsuki, Tamura, *et al.* isolated tunicamycin from *Streptomyces lysosuperificus*, demonstrated its antiviral potential, and solved its structure [78,79]. Subsequently, Heifetz, Elbein, *et al.* discovered the *N*-glycosylation inhibitory effect of tunicamycin. Early pre-clinical studies exploiting a microsomal preparation of pig aorta demonstrated the potent inhibitory effect of tunicamycin on GlcNAc-phosphotransferase complex ($K_i \cong 5 \times 10^{-8}$ M compared to a K_m value of $\cong 3 \times 10^{-6}$ M for UDP-GlcNAc), thus revealing its mechanism of action [80–82]. Tunicamycin has been widely used on various cell culture models, and its effective inhibitory concentration range seems to vary between 0.1 and 10 $\mu\text{g/ml}$ [39]. In addition, tunicamycin-resistant mutant cells with elevated GlcNAc-phosphotransferase levels have been reported

[83]. As a note of caution, experiments with rat and chick hepatocytes have revealed that tunicamycin can also antagonize protein synthesis, a potential confound when interpreting results with this molecule [84].

Studies have shown that tunicamycin elevates the sensitivity of cancer cells to the anticancer drug trastuzumab *in vivo* and *in vitro* [85]. This implicates tunicamycin as a potential candidate for adjuvant cancer therapy. Unfortunately, tunicamycin did not make its way into clinic due to its poor selectivity and toxicity [86]. Today, tunicamycin is mainly used as a research tool in glycobiology and to induce ER stress [87].

Lipopeptide antibiotics. Following the discovery of tunicamycin, lipopeptide antibiotics which target peptidoglycans or lipopolysaccharides in the bacterial cell wall attracted attention as potential *N*-glycan inhibitors, the two main ones of which are amphomycin and tsushimycin [88–90]. Unfortunately, both antibiotics fail to inhibit *N*-glycoproteins in mammalian cells when added to cell culture media, owing to poor cell membrane permeability. Therefore, both antibiotics would have to be modified before use in a mammalian system. Mechanistically, amphomycin is reported to block the synthesis of dolichol-P-mannose by forming complexes with the carrier lipid dolichol-phosphate [91]. Reports have demonstrated that other types of antibiotics (e.g., showdomycin and diumycin) are also capable of interfering with dolichol precursor

assembly, however they all seem to do so in a less specific manner than tunicamycin [92,93]. Looking forward, inhibitors that can penetrate cell membranes, specifically target a single step in the dolichol precursor assembly pathway, and demonstrate potency *in vivo* are needed.

Inhibitors of glucosidases and mannosidases

Following the transfer of the oligosaccharide from the lipid carrier to the protein, the oligosaccharide then undergoes several processing reactions that are regulated by glucosidases and mannosidases. Therefore, drugs that target these enzymes are powerful pharmacological tools that can alter the formation of *N*-glycans.

Deoxynojirimycin. In 1966, Inouye, Nida, *et al.* discovered the first natural iminosugar, nojirimycin, originally isolated from *Streptomyces* strains [94]. Nojirimycin was a potent inhibitor of glucosidases, but its poor stability limited its applications. Deoxynojirimycin (DNJ) is a closely related iminosugar with improved potency and chemical stability. DNJ can inhibit both glucosidases I and II, and therefore the processing of *N*-glycoproteins [95]. The effective inhibitory concentration range of DNJ in cells is 0.5–20 mM, with 5 mM being the optimal concentration. Functionally, DNJ treatment can reduce antibody secretion [96] and protein secretion with accumulation in the rough ER. This highlights the effects *N*-glycosylation inhibition can have on protein stability, especially prior to protein folding. Since such early intervention removes or modifies the carbohydrate residues that are vital for the calnexin/calreticulin cycle, protein maturation, transport, and secretion are affected.

DNJ analogues have been explored as well. The methylated derivative of DNJ, *N*-methyl-1-deoxynojirimycin (MDNJ), demonstrated maximal inhibition at 1 mM compared to 5 mM achieved by DNJ in rat intestinal epithelial cells [98]. Additionally, in DNJ-treated cells 20% of the oligosaccharides exhibited three glucose units compared to 70% achieved by MDNJ [98]. Clinically, acarbose, an oral drug integrating a structural isomer of DNJ, is currently used as an antidiabetic due to its ability to prevent amylase breakdown in the intestine [99].

Castanospermine. In the early 1980s, a plant alkaloid known as castanospermine was isolated from the toxic seeds of Australian legume *Castanospermum australe* [100]. The toxicity of these plant's seeds arises from the ability of castanospermine to potently inhibit various α -glucosidases (e.g., maltase and sucrase) that regulate starch and sucrose metabolism, causing gastrointestinal irritation [101]. Additionally, pharmacological demonstrations in cells have revealed that castanospermine blocks glucosidase I and II, causing enrichment of immature *N*-glycans [102]. The effective inhibitory concentration range of castanospermine is 1–

50 μ g/ml for one to two hours [103]. Notably, castanospermine-treated cells exhibited a significant reduction in glycosylated receptors such as insulin receptor and low-density lipoprotein receptor, again highlighting effects on protein folding and secretion [104,105].

To increase the cellular uptake of castanospermine and decrease its gastrointestinal tract toxicity, researchers at Merrell Dow developed a butanoyl-modified castanospermine known as celgosivir (6-*O*-butanoyl-castanospermine) [106, 107]. Celgosivir is a prodrug that is less active against intestinal sucrase, maintains its inhibitory activity against α -glucosidase I, and is approximately 30 times more potent than castanospermine [107]. Celgosivir has been clinically evaluated for the treatment of several viruses including human Dengue virus and Hepatitis C virus, through modification of host glycans in a manner that reduces viral–host interactions [106]. Thus far, the prodrug has failed to demonstrate clinical anti-viral efficacy as a monotherapy [106,108,109]. Recently, demonstrations from Clarke, Bradfute, *et al.* have shown that castanospermine and celgosivir inhibit SARS-CoV-2 replication in a cell culture system [110].

Australine. One limitation to the use of deoxynojirimycin and castanospermine is that they act on both glucosidase I and II, which makes it difficult to distinguish the effects of these two enzymes. Another iminosugar, australine, fills this gap, by preferentially inhibiting glucosidase I. Similar to castanospermine, australine was also isolated from the seeds of *Castanospermum australe* [111]. Notably, australine-treated MDCK cells exhibited accumulated immature $\text{Glc}_3\text{Man}_{7-9}$ - $(\text{GlcNAc})_2$ structures [111,112]. However, australine is a rather weak inhibitor compared to castanospermine, since 500 μ g/mL of australine was required to achieve the same degree of inhibition produced by 10 μ g/mL of castanospermine, which might explain its rather rare use and furthermore emphasizes the need to develop more potent selective glucosidase inhibitors [102].

Swainsonine. Swainsonine was the first *N*-glycoprotein processing inhibitor discovered, originally isolated from the Australian plant, *Swainsona canescens*. Swainsonine is an iminosugar that inhibits Golgi mannosidase II, thus blocking the synthesis of glycoproteins with complex mannose content. Consequently, swainsonine treated cells expressed hybrid *N*-glycan structures that lack α -1,3 and α -1,6 mannose residues [113]. The effective concentration range in cells for swainsonine is 1–10 μ g/ml [103]. In mice and rats, administration is either through addition to drinking water (dose of 2.5–3 μ g/mL) or intraperitoneal injection (doses of 2–8 mg/kg), with which authors have observed reduction of tumour growth and metastasis [114–117]. A hydrochloride salt of swainsonine

(GD0039) exhibited adverse side effects and failed to demonstrate significant anti-tumour activity in 17 patients with metastatic renal cancer [118]. Nonetheless, this does not eliminate the potential of swainsonine in treating other types of cancer.

Deoxymannojirimycin. The successful development of deoxymannojirimycin as an *N*-glycosylation inhibitor prompted scientists to synthesize structural analogues that exhibit a mannose configuration. Both chemically synthesized and naturally-derived deoxymannojirimycin (DMJ; the 2-epimer of deoxynojirimycin) have been shown to inhibit 50% of Golgi-mannosidase I in rat liver lysate at a concentration of 1 μ M [119,120]. Notably, the treatment of MDCK cells with DMJ resulted in the accumulation of high mannose glycoproteins with structures $\text{Man}_{8-9}(\text{GlcNAc})_2$ [121]. The effective concentration range of DMJ in live cells is 1–5 mM. Since its discovery, DMJ has proven to be a powerful tool to study the function of glycans on proteins. For example, unlike deoxynojirimycin-treated rat hepatocytes, DMJ-treated hepatocytes fail to block IgM secretion. This observation suggested that the assembly of glucose on oligosaccharides is important for the intracellular transport of some glycoproteins, whereas a switch from high mannose structures to normal complex chains seems to be less significant for correct protein folding and secretion [39,96,122]. Remarkably, the mannosidase inhibitors DMJ and swainsonine aided the discovery of the calreticulin-calnexin cycle, thus highlighting the importance of glycosylation inhibitors for functional studies [123].

Kifunensine. Kifunensine is an alkaloid which was first isolated from *Kitasatosporia kifunense* by Iwami, Imanaka, et al. in 1987 [124]. Similar to deoxymannojirimycin, kifunensine is an inhibitor of Golgi mannosidase I, but is approximately 100 times more potent [39]. Kifunensine shifts the structure of complex glycan chains to $\text{Man}_9(\text{GlcNAc})_2$. In contrast to deoxymannojirimycin, which failed to fully inhibit complex glycosylation at 50 μ g/ml, kifunensine demonstrated complete inhibition of complex glycosylation at a concentration of 1 μ g/ml in MDCK cells [125]. Kifunensine has an IC_{50} of 20 nM measured in extracted plant mannosidase I, and its effective concentration range in cells is 5–20 μ M for 24 h [126]. The high potency of kifunensine in blocking glycoprotein processing makes it a valuable tool to study glycans. Recently, kifunensine and swainsonine aided the discovery of a novel glycosylated small non-coding RNA [3]. Clinically, kifunensine is currently used to construct recombinant glucocerebrosides for the treatment of Gaucher disease type 1 [127]. Additionally, kifunensine seems to be a promising drug for sarcoglycanopathy, which involves accumulation of *N*-glycosylated proteins [128,129].

Inhibitors of mucin-type O-glycosylation

O-Glycosylation refers to the attachment of sugar molecules to the hydroxyl groups of serine (Ser) or threonine (Thr) residues in a protein [130]. The most common initiating sugars are GalNAc and GlcNAc. GalNAc-linked glycans, also known as mucin-type O-glycans, are found on cell surfaces, while O-GlcNAc is an unelaborated intracellular post-translational modification. As the focus of this review is complex glycosylation, post-translational O-GlcNAcylation, which is vitally involved in diverse cellular processes such as protein–protein interactions, tuning of signalling pathway activity, and protein trafficking, is not surveyed here. We refer the reader to the extensive body of literature on the biological role of O-GlcNAcylation and its inhibition [9–12].

Mucin-type O-glycan biosynthesis is initiated by a family of polypeptide *N*-acetyl- α -galactosaminyl transferases (GALNTs) which transfer GalNAc from its donor, uridine diphosphate *N*-acetyl- α -galactosamine (UDP-GalNAc) to peptide backbone acceptors (Fig. 3) [131,132]. A glycopeptide with an α -GalNAc-Ser/Thr (Tn antigen) structure is therefore generated. Tn antigen is elaborated via addition of galactose, GlcNAc, sialic acid, and fucose by downstream glycosyltransferases to form more complex glycan structures. As is the case with *N*-glycans, O-glycans play key roles in both health and disease [133]. As one example, aberrated O-glycosylation is a common feature of human neoplasms [134–138]. For example, the metastasis of gastric cancer cells is decreased with the overexpression of GALNT2 and is increased with the expression of GALNT12 [139,140]. Other GALNTs such as GALNT3, GALNT6, and GALNT7 have also been associated with the exacerbation of tumor prognosis [141–147]. As such, GALNTs are therapeutic targets for cancer treatment and important

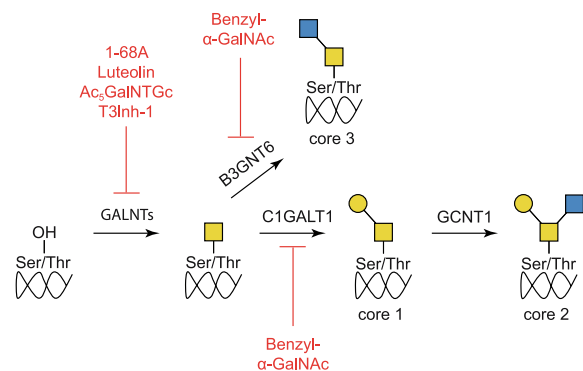


Fig. 3. Inhibitors of O-glycosylation. Initial steps of O-glycan biosynthesis. Key inhibitors are shown in red. Enzymes are shown only for steps for which an inhibitor is available.

markers for cancer diagnosis and prognosis [148–150].

2,3,4-trihydroxybenzene modified uridine

Inhibitors of GALNTs were described by Hang, Bertozzi, *et al.* in 2004 from screening of a uridine-based library [131]. The lead compound, 1-68A, is formed by a 2,3,4-trihydroxybenzene motif (68A) connected through an oxime linkage to uridine. 1-68A bound to GALNT1 in a competitive inhibition mode with respect to the native uridine diphosphate *N*-acetylgalactosamine (UDP-GalNAc) substrate. Its inhibition constant (K_i) of $7.8 \pm 0.1 \mu\text{M}$ was half as large as the Michaelis constant (K_m), $13.9 \pm 1.8 \mu\text{M}$ in the absence of inhibitor. K_i measurements with structurally similar compounds indicated that the 68A group and linker length were both involved in driving affinity for GALNT1. Beyond GALNT1, 1-68A exhibited *in vitro* micromolar inhibitory activity against GALNT1–5, –T7, –T10, and –T11, with negligible inhibitory activity on other glycosyltransferases and nucleotide sugar utilizing enzymes tested, such as α 1,3-galactosyltransferase and UDP-GlcNAc/GalNAc C₄-epimerase. Cell lines treated with 80–100 μM 1-68A for 24 h showed reduced binding to *O*-glycan selective lectins but not *N*-glycan selective lectins, alongside a significant induction of cell death, which alongside its commercial unavailability, is a drawback for use of this compound.

Luteolin

Luteolin extracted from the common weed *Reseda luteola* has been used as a fabric dye since prehistoric times [151]. Liu, Wu, *et al.* [152] reported in 2017 that luteolin inhibited GALNT2, –T3, –T6, –T10, and –T14 (subfamilies Ib, Ic, and IIb) but not GALNT1 or –T13 (subfamily Ia). Authors focused mechanistic work on GALNT2, where they measured competitive inhibition of catalytic activity on a peptide acceptor substrate with a K_i of $1.4 \pm 0.05 \mu\text{M}$ relative to a K_m of $160.2 \pm 11.5 \mu\text{M}$, indicating a ~ 100 -fold greater binding capacity for luteolin over the acceptor peptide. In contrast, luteolin displayed non-competitive inhibition with respect to UDP-GalNAc substrates (K_i $4.1 \pm 1.1 \mu\text{M}$ versus K_m $59.7 \pm 4.6 \mu\text{M}$). X-ray structures revealed that luteolin bound to GALNT2 via interactions with its peptide-binding groove. Treatment of cultured cells with 20–25 μM luteolin for 24 h resulted in loss of *O*-glycan selective lectin staining (Jacalin), without loss of *N*-glycan selective lectin staining (ConA). The same conditions resulted in inhibition of GALNT2 mediated, but not –T1 mediated, glycosylation of an exogenously expressed glycoprotein. In mice, doses of 50 mg/kg/day are tolerated [152]. Outside of its context as an *O*-glycan inhibitor, luteolin has not been investigated for its antioxidant, anticancer, anti-inflammatory, neuroprotective, and cardioprotective properties [153–159]. It remains to be seen

whether these effects arise from luteolin's activity on GALNTs or other, possibly pleiotropic mechanisms. Furthermore, luteolin has been reported to trigger topoisomerase II-mediated DNA damage, which can accumulate over time, causing off-target toxicity in the context of cancer treatment [160,161].

Peracetyl *N*-thioglycolyl-D-galactosamine (Ac₅GalNTGc)

In 2013, Agarwal, Sampathkumar, *et al.* identified peracetyl *N*-thioglycolyl-D-galactosamine (Ac₅GalNTGc) as an inhibitor of mucin-type *O*-glycosylation [162]. Ac₅GalNTGc enters cells through the GalNAc salvage pathway and is added to serine/threonine residues in place of GalNAc, preventing elaboration at those sites and *O*-glycan initiation at neighbouring sites. The same group further characterized Ac₅GalNTGc in a follow-up study published in 2021 [132]. Employing mass spectrometry, lectin blotting, and flow cytometry on cultured cells they quantified a 30–60% inhibition of *O*-glycan elaboration beyond Tn-antigen (GalNAc α -Ser/Thr) with typical treatment conditions of 50–80 μM for 16–40 h. Functionally, Ac₅GalNTGc treatment mediated loss of leukocyte sialyl-Lewis X (sLeX) expression and diminished L- and P-selectin dependent rolling adhesion. Ac₅GalNTGc showed no effects on cellular nucleotide sugars and negligible changes in *N*-glycan structures, but did reduce glycosphingolipids to some extent based on mass spectrometry analysis. Mice tolerated treatment with 100 mg/kg Ac₅GalNTGc daily for four days. Flow cytometry analysis of neutrophils in the peritoneum after 16 h revealed 2-fold higher *Vicia villosa* agglutinin (VVA) binding with respect to vehicle control, indicating inhibition of core 1 *O*-glycan elaboration by Ac₅GalNTGc *in vivo*.

T3Inh-1

The first isoform selective GALNT inhibitor was reported by Song and Linstedt in 2017 [163]. It was discovered through small molecule library screening for inhibition of a cell-based fluorescence sensor of GALNT3, with counter screening against compounds influencing an analogous sensor of GALNT2. The lead compound, T3Inh-1, bound to both free GALNT3 and enzyme-substrate complexes in a mode of mixed inhibition with K_i at 9.9 μM and 2.9 μM with respect to acceptor peptide and UDP-GalNAc substrates, respectively. It showed negligible activity against either GALNT2 or GALNT6. In various cancer cell lines, treatment with 5 μM T3Inh-1 for 5–10 h reduced migration and invasiveness. In mice, a single intraperitoneal injection of 25 mg/kg T3Inh-1 influenced the behaviour of a known GALNT3 target glycoprotein, while a GALNT2 substrate was unaffected. Notably, T3Inh-1 treatment of cell lines resulted in no detectable decrease in staining by *N*- and *O*-glycan selective lectins, possibly due to its activity against only a

small number of protein substrates. Isoform selective inhibitors of GALNTs will continue to grow in importance as GALNT substrate profiles are characterized [164,165].

Aryl- α -*N*-acetylgalactosamines

The most commonly used inhibitor of mucin-type *O*-glycosylation is benzyl- α -GalNAc, first described by Kuanm, Kim, *et al.* in 1989 [166]. They found that benzyl- α -GalNAc, along with other aryl versions such as phenyl and *p*-nitrophenyl, could block the activity of galactosyltransferases in human colon cancer cells in a dose-dependent manner by acting as a decoy substrate, analogous to β -D-xylosides (*vide infra*). The IC₅₀ for inhibition of glycosylated mucin production in these cells was ~ 1 mM, with a maximum observed reduction of 90% seen at 2 mM, for a treatment time of 24 h. Inhibitory activity on GALNTs and *N*-linked glycoprotein secretion was not observed. With respect to asialo-ovine submaxillary mucin acceptor substrates, benzyl- α -GalNAc bound to the galactosyltransferases in a competitive mode with an increase in the apparent K_m from 0.06 mg/mL in the absence of inhibitor to 0.4 mg/mL in the presence of 3 mM inhibitor. The many aryl- α -GalNAc analogues that have been synthesized and characterized as mucin-type *O*-glycosylation inhibitors since 1989 support the above mechanism of activity [167]. A drawback of benzyl- α -GalNAc and similar compounds are the millimolar doses at which they need to be used, which can be toxic to cell lines and animals.

Inhibitors of capping modifications

Two glycosylation types are typically viewed as “capping” modifications: fucosylation and sialylation. They can be found on *O*-glycans, *N*-glycans, and glycolipids and are not further modified with other types of sugars (the exception being long chains of 2,8-linked sialic acids, called polysialic acid, with diverse functions, e.g. in neuron function and cell adhesion) [168,169]. Sialic acid is almost always at the terminal end of glycans while fucose can be found both at the terminal ends of glycan trees as well as branching from core residues. Due to their exposed location on the glycocalyx (which is already the outermost part of the cell), it is not surprising that both fucose and sialic acids play roles in a broad range of cellular processes. Examples include immune system regulation, pathogen binding, cancer progression, angiogenesis, leukocyte adhesion, and host–microbiome interactions [29,170–176]. Although they fulfil different biological functions, we discuss them together, not only due to their capping function, but also because their unique structures have enabled development of high specificity inhibitors, summarized in Fig. 4.

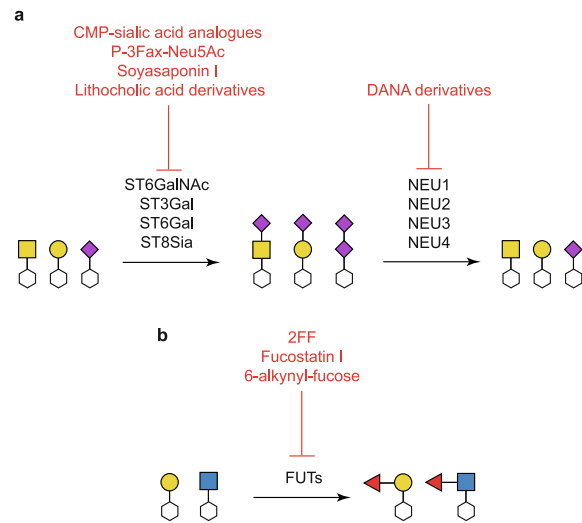


Fig. 4. Inhibitors of capping modifications. (a) Inhibitors of sialylation and desialylation. Empty hexagons indicate that sialylation can occur on a wide variety of monosaccharide units. The four human sialyltransferase families are indicated along with the four sialidases found in humans. (b) Inhibitors of fucosylation. The fucosyltransferase (FUT) family of enzymes catalyzes the transfer of fucose to a variety of monosaccharide units.

Sialylation

The most common form of sialic acid, Neu5Ac, is synthesized in the cytosol and then relocates to the nucleus where it is converted to cytosine 5'-monophosphate *N*-acetylneuraminic acid (CMP-Neu5Ac) by CMP-Neu5Ac synthetase. CMP-Neu5Ac is then transferred to the Golgi where sialyltransferases facilitate the transfer of sialic acids from the donor substrate to various glycoconjugates. Twenty sialyltransferases have been identified so far, which can be classified according to their acceptor substrates and their linkage specificities into four families (Table 1) [357]. Sialic acids modify a range of glycoconjugates and are involved in pathways relating to multicellular communication [4]. As one example, members of the sialic-acid binding Ig-like lectins (Siglec) family of cell surface receptors are expressed widely on immune cells, where they can bind various sialylated species presented to them [177]. Due to the presence of inhibitory intracellular domains in several family members, Siglecs can act as immune checkpoint receptors, analogous to the clinical targets PD-1 and CTLA-4 [133,178]. In the context of tumor progression, targeting either the Siglec receptors or their sialylated ligands has proved to be a promising strategy in model systems [38,179]. Indeed, an engineered human sialidase enzyme genetic fusion, recently received investigational new drug approval in the

Table 1 Sialyltransferase families.

Sialyltransferase family	Acceptor substrate	Linkage
ST3Gal	A terminal galactose of <i>N</i> - or <i>O</i> -glycans	α 2,3
ST6Gal	Galactose residues of <i>N</i> -glycans	α 2,6
ST8Sia	Another Neu5Ac residue in <i>N</i> - or <i>O</i> -glycans	α 2,8
ST6GalNAc	Terminal <i>N</i> -acetyl galactosamine (GalNAc) residues of glycoproteins and glycolipids	α 2,6

U.S. for testing in human clinical trials (E-602, Palleon Pharmaceuticals).

Several classes of molecule have been shown to inhibit sialyltransferases, including CMP-sialic acid analogues, cytidine analogues, sialic acid analogues, and several natural product inhibitors. Many of these inhibitors fail to penetrate the cell membrane, thus hampering their clinical potential. Sialyltransferase inhibitors have been exhaustively reviewed elsewhere [180–182], and here we focus on particularly promising sialyltransferase inhibitors in each category.

CMP-sialic acid analogues. The earliest sialyltransferase inhibitors are CMP-sialic acid analogues. This class of molecules was pioneered two decades ago by Richard Schmidt [183,184]. These molecules were designed based on the observation that sialyltransferase-catalysed reactions of the donor substrate CMP-Neu5Ac exhibit a transition state where CMP leaves prior to bond formation with the hydroxyl group of the nucleophile [185]. By mimicking this transition state, CMP-Neu5Ac analogues can inhibit sialyltransferase activity. Over the last decade, numerous CMP-analogues with a range of activities have been reported [180–182]. Among the most potent agents are aryl- ($K_i = 19$ nM, ST6Gal I), [186] benzamide- ($K_i = 16$ nM, hST6Gal I), [187] and cyclopentyl- ($K_i = 28$ nM, ST6Gal I) derivatives of CMP-sialic acid [188]. Despite their excellent potency, these compounds are associated with pharmacokinetic limitations, owing to their polar phosphodiester linker which hampers their ability to penetrate the cell and makes them unstable. Various groups are working toward overcoming these limitations by masking the charged linker with neutral ones (e.g., amide and carbamate) [189–192].

Cytidine analogues have also been developed [185]. Among them is 2'-*O*-methyl-CMP which demonstrated polysialyltransferase inhibitory effects. This is significant considering the role of polysialic acid in tumour progression and metastasis [193]. Treatment with 0.25 mM of this analogue strongly inhibited ST8Sia-II, ST8-Sia-III, and ST8Sia-IV when chinese hamster ovary (CHO) cells were treated for 24 h, thus suppressing polysialic acid levels on the cell surface [194]. Additionally, the same concentration also inhibited ST3Gal-III and ST3Gal-IV, but not ST6Gal-I.

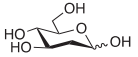
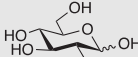
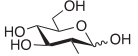
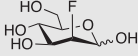
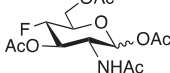
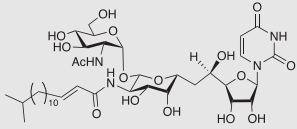
P-3Fax-Neu5Ac. As discussed earlier, exploiting fluorinated sugar analogues can provide an

effective strategy to inhibit glycosyltransferases [195]. Recently, Rillahan, Paulson, *et al.* developed a cell-penetrating peracetylated analogue known as P-3Fax-Neu5Ac. Upon cellular uptake, P-3Fax-Neu5Ac is metabolized to the active inhibitor CMP-3F_{ax}-Neu5Ac, which can inhibit the synthesis of various sialylated glycan epitopes [196]. When human myeloid cells (HL-60 cells) were treated with 30–500 μ M of P-3Fax-Neu5Ac for 3 days, the formation of sialyl Lewis X was substantially inhibited [196]. Moreover, P-3Fax-Neu5Ac blocked sialylation when 100 or 300 mg/kg was administered in mice, suppressing the levels of sialylated glycans in all cells of tissues tested including brain, kidney, lung, heart, and liver. Treated mice in these experiments developed kidney and liver dysfunction, thus highlighting the significance of sialosides in these tissues [197]. Furthermore, P-3Fax-Neu5Ac was able to induce long-term suppression in the levels of α 2,3-/ α 2,6-linked sialic acids in murine melanoma cells (B16F10 cells), thus impairing tumor adhesion and migration [198]. This implicates P-3Fax-Neu5Ac as a potential anticancer drug, limited by its systemic toxicity. In the light of this context, the same group formulated P-3Fax-Neu5Ac into tumor-targeted nanoparticles that reduced systemic toxicity [199]. A recent study showed that P-3Fax-Neu5Ac suppresses SARS-CoV-2 viral infectivity in cells, highlighting the role of sialylated glycans in facilitating SARS-CoV-2 entry and implicating them as a potential therapeutic target [200,201].

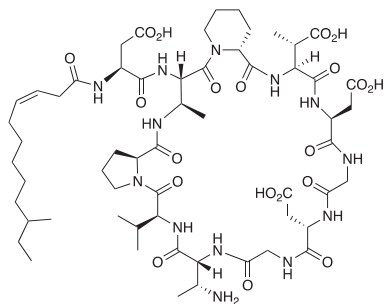
Soyasaponin I. Wu, Tsai, *et al.* identified soyasaponin I from screening of a library of microbial extracts and natural products [202]. Soyasaponin I is derived from soybean saponin and has been shown to competitively inhibit the binding of CMP-Neu5Ac to ST3GAL1 ($K_i = 2.3$ μ M), thus suppressing the levels of 2,3-linked sialic acid on the cell surface [202]. When breast cancer cells were incubated with soyasaponin I at a concentration of 50 μ M for 3 days, ~40% of the surface α 2,3-sialic acids were inhibited. Attenuation of sialic acid levels in these cells resulted in decreased cell migration and stimulated cell adhesion, altering the tumour's metastatic and invasive behaviour [203]. Moreover, soyasaponin I-treated mice exhibited suppressed lung metastasis compared to controls [204].

Lithocholic acid derivatives. Several steroidal compounds have been shown to inhibit sialyltransferases. One promising class of steroidal inhibitors

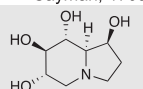
Table 2 Structures, commercial sources, and typical treatment conditions for inhibitors discussed in this review.

Drug	Structure and Commercial Availability	Drug Class	Molecular Weight	Reported Concentration	Model Used	References
2-Deoxy-D-glucose	 <p>Available from Sigma, D8375</p>	Monosaccharide inhibitor	164.16 g/mol	1–50 mM	Chick embryo fibroblasts Rabbit kidney cells Madin Darby kidney cells Human skin cells Monkey kidney cells	[42,293–295] [296] [297] [298] [299]
2-Deoxy-2-fluoro-D-glucose	 <p>Available from Sigma, F5006</p>	Monosaccharide inhibitor	182.15 g/mol	0.2–3 mM	Chick embryo fibroblasts Rabbit kidney cells Saccharomyces cerevisiae	[52,295,300] [52] [301]
Glucosamine	 <p>Available from Sigma, G4875</p>	Monosaccharide inhibitor	179.17 g/mol g/mol	4–40 mM	Chick embryo fibroblasts Human embryo lung cells Baby Hamster Kidney cells Madin Darby bovine kidney cells Mouse embryonic fibroblasts Human prostate cancer cell line (DU145)	[294,295,302] [303] [304] [297] [56] [54]
2-Deoxy-2-fluoro-D-mannose	 <p>Available from Sigma, CH6371389583</p>	Monosaccharide inhibitor	182.15 g/mol	0.2–10 mM	Chick embryo fibroblasts Rabbit kidney cells Saccharomyces cerevisiae	[52,295] [52] [305]
4-F-GlcNAc	 <p>Available from Toronto Research Chemicals, A158435</p>	Monosaccharide inhibitor	349.31 g/mol	0.05–0.5 mM 100 mg/kg 0.01–0.05 mM	Human ovarian carcinoma cell line (A-121) Mice Human sLe ^x (+) T cells and leukemic KG1a cells	[59] [60] [61]
Tunicamycin	 <p>Available from Sigma, T7765</p>	N-Glycan inhibitor; inhibits dolichol precursor assembly	844.94 g/mol	0.1–10 µg/ml 0.5 µg/mL	Chick embryo fibroblasts Human fibroblasts Baby hamster kidney cells Liver cells Plasmacytoma cells Human leukaemia cells Hen oviduct Madin Darby bovine kidney cells Sea urchin embryos Chick cornea Saccharomyces cerevisiae Trypanosoma brucei Human B-cell lymphocytes	[306–310] [311,312] [313–316] [84,97,317,318] [319–321] [322] [323] [297] [324,325] [326] [327,328–330] [331] [333]

Amphotericin

Available from
Cayman, 17091*N*-Glycan inhibitor; inhibits
dolichol precursor assembly1290.4 g/mol 500 µg/ml
0–200 µgCell-free system (Pig aorta)
Rabbit kidney cells[\[333\]](#)
[\[334\]](#)

Castanospermine

Available from
MedChemExpress,
HY-N2022*N*-Glycan inhibitor; inhibits
glucosidases I and II189.21 g/mol 10 µg/mL
250 µg/mLMadin-Darby canine kidney cells
Primate smooth muscle cells; human
skin fibroblasts[\[102\]](#)
[\[105\]](#)

100 µg/ml

Human B-cell lymphocytes

[\[104\]](#)

20 µg/ml

Feline embryo cells

[\[335\]](#)

100 µg/ml

Human T-lymphoblastoid cells

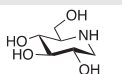
[\[336–338\]](#)

2 mM

Human B-cell lymphocytes

[\[332\]](#)

Deoxynojirimycin

Available from
Sigma, 08012*N*-Glycan inhibitor; inhibits
glucosidases I and II

163.17 g/mol 5 mM

Small intestinal epithelial cells

[\[95\]](#)

7.5 mM

Cultured human B-cell lymphocytes

[\[104\]](#)

5 mM

Small intestinal epithelial cells

[\[339\]](#)

1 mM

Daudi and Raji lymphoblastoid cell lines

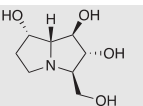
[\[96\]](#)

5 mM

Cultured human B-cell lymphocytes

[\[332\]](#)

Australine

Available from
Cayman, 30051*N*-Glycan inhibitor; inhibits
glucosidases I and II

189.21 g/mol 5.8 µM

Madin-Darby canine kidney cells

[\[113\]](#)

500 µM

Cultured human B-cell lymphocytes

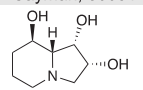
[\[332\]](#)

20 µM

Chinese hamster ovary (CHO) cells

[\[340\]](#)

Swainsonine

Available from
Sigma, S9263*N*-Glycan inhibitor; inhibits
mannosidase II

173.21 g/mol 100 µM

Cultured human B-cell lymphocytes

[\[332\]](#)

0.5 µM

Rat liver

[\[341\]](#)

0–10 µM

Purified rat liver mannosidases

[\[114\]](#)

1 µg/ml

Primary calf kidney cultures

[\[342\]](#)

3 µg/ml

Mice

[\[343\]](#)

(drinking

water)

0.3 µg/ml

Leukemia cell lines

[\[344\]](#)

20 µM

HEK293T cells

[\[345\]](#)

1 µg/ml

Chinese hamster ovary (CHO) cells

[\[346\]](#)

2–12 mg/kg

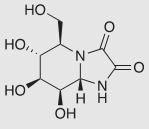
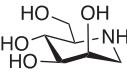
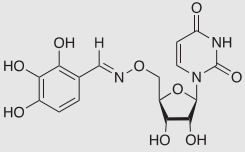
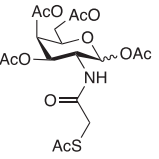
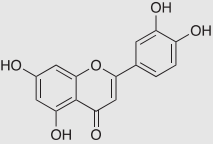
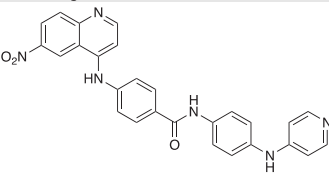
Rats

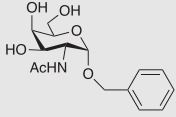
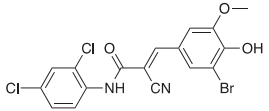
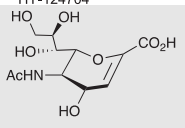
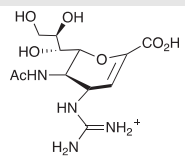
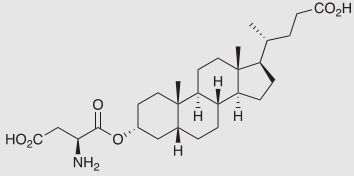
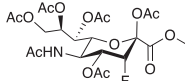
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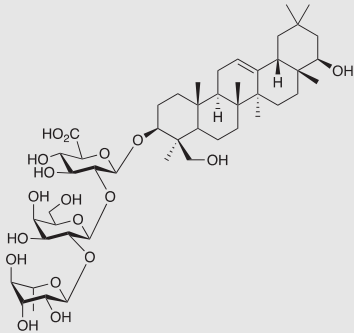
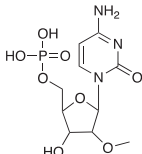
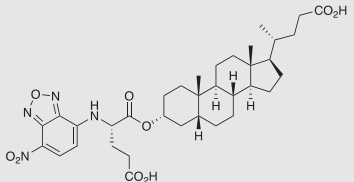
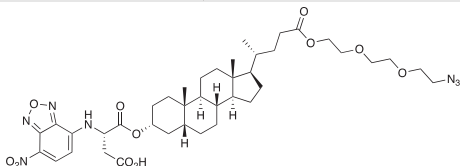
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
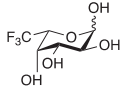
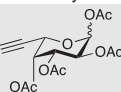
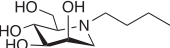
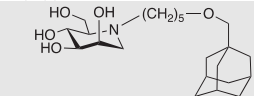
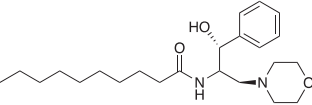
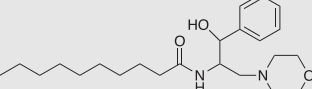
Drug	Structure and Commercial Availability	Drug Class	Molecular Weight	Reported Concentration	Model Used	References
Kifunensine	 <p>Available from Sigma, 422500</p>	N-Glycan inhibitor; inhibits mannosidase I	232.19 g/mol	≥1 µg/ml 50 µM 5 µM 2 µg/µl 5 µM 1 µg/ml	Madin-Darby canine kidney cells Human breast cancer cell lines Renal cell lines HeLa cervical cancer cell line HEK293T cells Chinese hamster ovary (CHO) cells	[124] [347] [348] [349] [345] [346]
Deoxymannojirimycin	 <p>Available from Sigma, D9160</p>	N-Glycan inhibitor; inhibits mannosidase I	163.17 g/mol	1 mM 150 µM 1 mM 0.5–4 mM	Human hepatocarcinoma cells Chinese hamster ovary (CHO) cells Hybridomas Primary cultures of rat hepatocytes	[350] [119] [120] [122]
1-68A	 <p>Not commercially available.</p>	O-Glycan inhibitor; inhibits GALNTs	395.32 g/mol	100 µM 100 µM	Human Jurkat T cell lymphoma cell line Human embryonic kidney (HEK) 293T cells	[131]
Ac ₅ GalNTGc	 <p>Quote available from Carbosynth, MT145185</p>	O-Glycan inhibitor; inhibits GALNTs	463.45 g/mol	0–100 µM 0–100 µM 0–200 µM 100 mg/kg/day	Human Jurkat T cell lymphoma cell line Various human leukemia cell lines Mouse peripheral blood neutrophils; human promyelocytic leukemia (HL-60) cells, human breast cancer (T47D and ZR-75-1) cells, human prostate cancer cells (PC-3) Mice	[162] [132]
Luteolin	 <p>Available from Sigma, L9283</p>	O-Glycan inhibitor; inhibits GALNTs	286.24 g/mol	0–30 µM 25 µM 50 mg/kg/day	Human embryonic kidney (HEK) 293T cells Chinese hamster ovary (CHO) cells Mice	[152]
T3Inh-1	 <p>Available from MolPort, 046-156-276</p>	O-Glycan inhibitor; inhibits GALNT3	476.50 g/mol	0–50 µM 0–50 mg/kg	Human embryonic kidney (HEK) 293T cells Mice	[163]

benzyl- α -GalNAc		O-Glycan inhibitor; decoy inhibitor for Ser/Thr- α -GalNAc	311.33 g/mol	0–10 mM	Human coloncancer (LS174T) cells	[166]
Available from Sigma, B4894						
3-(3-Bromo-4-hydroxy-5-methoxyphenyl)-2-cyano- <i>N</i> -(2,4-dichlorophenyl)acrylamide (Compound 34)		Inhibitor of glycosaminoglycan sulfotransferases.	442.09 g/mol	0–25 μ M 3.0 mg/kg	Neu7 astrocytes Mice	[249]
Quote available from MedChemExpress, HY-124704						
<i>N</i> -Acetyl-2,3-dehydro-2-Deoxyneuraminic Acid (DANA)		Sialidase inhibitor	291.26 g/mol	100 μ M 1 mM 1 mM 10 mg/kg 10 μ M 70–2100 μ mol/kg 1.6 mM	A549 human lung carcinoma epithelial cells U937 human lymphoma cells Rat brain slices Mice Rat pancreatic beta cell line C57BL/6J mice	[215] [210] [212] [213] [351]
Available from Cayman, 19939						
4-Guanidino-DANA (Zanamivir)		Sialidase inhibitor	332.32 g/mol	2.5 mM	Alligator primary pulmonary fibroblasts Human HeLa cervical cancer cell line	[211] [216]
Available from Sigma, SML0492						
Lith-O-Asp		Sialyltransferase inhibitor; inhibits ST3GAL1, ST3GAL3, and ST6GAL1	491.66 g/mol	15 μ M	Human lung cancer cell lines (H1299, A549, CL1-0, CL1-1, and CL1-5F4)	[205]
Available from MedChemExpress, HY-112415						
P-3Fax-Neu5Ac		Sialyltransferase inhibitor; inhibits α 2,3-sialylation	551.47 g/mol	3 mg/kg i.p. 32–512 μ M 10–300 mg/kg	Mice Human leukemia cell line (HL-60) C57BL/6J mice	[206] [196] [197]
Available from Sigma, 566224						

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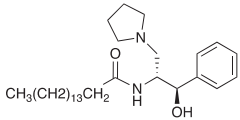
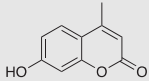
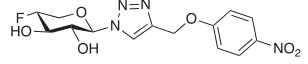
Table 2 (continued)

Drug	Structure and Commercial Availability	Drug Class	Molecular Weight	Reported Concentration	Model Used	References
Soyasaponin I	 <p>Available from Sigma, S9951</p>	Sialyltransferase inhibitor; inhibits ST3GAL1	943.1 g/mol	50 μM	Human breast cancer cell lines	[95,202]
2'-O-methyl-cytidine monophosphate	 <p>Available from Carbosynth, 18422-43-0</p>	Sialyltransferase inhibitor; Inhibits ST8Sia-IV, ST8-Sia-II, ST8Sia-III, ST3Gal-III and ST3Gal-IV	337.223 g/mol	250 μM	Chinese hamster ovary (CHO) cells	[194]
AL-10	 <p>Quote available from GlxxxLabs, LXC-02444</p>	Sialyltransferase inhibitor; inhibits ST3GAL1 and ST6GAL1	668.79 g/mol	~1 μM	Human lung cancer cells (CL1-0, CL1-5, A549) Human bronchial epithelial cells (BEAS-2B)	[207]
FCW34	 <p>Quote available from GlxxxLabs, GLXC-23461</p>	Sialyltransferase inhibitor; inhibits ST3GAL3 and ST6GAL1	811.93 g/mol	1.7 μM	Human breast cancer cell-lines	[208]

2-Deoxy-2-fluoro-D-fucose (2FF)	 Available from Carbosynth, MD06089	Fucosylation inhibitor; monosaccharide analog	166.15 g/mol	10–100 μ M 150 mg/kg i. p.; 50–100 mM oral (drinking water)	Human colon cancer (LS174T) cell lines, leukemia monocytes (THP-1), cervical cancer cells (HeLa), lung carcinoma cells (H1299) Mice	[225,227]
6,6,6-Trifluorofucose (Fucostatin I)	 No commercial availability	Fucosylation inhibitor; monosaccharide analog	218.13 g/mol	0.02–10 mM	Chinese Hamster Ovary (CHO) cells Murine hybridoma cells	[229] [230]
6-Alkynyl-fucose	 Available from Carbosynth, MT59558	Fucosylation inhibitor; monosaccharide analog	342.3 g/mol	10–50 μ M 60 pmol injection	Various cultured cell lines Zebrafish eggs	[240,242] [242]
N-butyldeoxymannojirimycin (NB-DNJ)	 Available from Sigma, B8299	Glycolipid inhibitor; inhibits glucosylceramide synthase	219.28 g/mol	0.5 mM	Human leukemia and lymphoma cell lines (HL-60, K-562, MOLT-4, H9)	[254]
Adamantane-pentyl-deoxymannojirimycin (AMP-DNJ)	 Available from Cayman, 10010332	Glycolipid inhibitor; inhibits glucosylceramide synthase	397.5 g/mol	10 μ M 2 nM	3T3-L1 adipocytes Human melanoma cells	[262] [261]
(+)-D- <i>threo</i> -PDMP	 Available from Matreya, 1756	Glycolipid inhibitor; inhibits glucosylceramide synthase	390.57 g/mol	40 μ M 50 μ M	Human liver cancer cell line (Hep2G cells) Rabbit skin fibroblasts	[262] [263]
PDMP (racemic mixture)	 Available from Matreya, 1719	Glycolipid inhibitor; inhibits glucosylceramide synthase	390.57 g/mol	25 μ M	Murine melanoma cell line (B16)	[267]

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Table 2 (continued)

Drug	Structure and Commercial Availability	Drug Class	Molecular Weight	Reported Concentration	Model Used	References
D-Threo-PPPP	 <p>Quote available from 001 Chemical, DY529262</p>	Glycolipid inhibitor; inhibits glucosylceramide synthase	511.2 g/mol	2 μ M 1 μ M	PC12 cells Mouse embryonic fibroblasts	[269] [272]
4-Methylumbelliferone (4-MU)	 <p>Available from Sigma, M1381</p>	Glycosaminoglycan inhibitor; competitively inhibits UDP-glucuronosyltransferases and depletes UDP-glucuronic acid	176.17 g/mol	1.0 mM 0.5 mM Oral dose of 3000 mg/kg oral 0–1 mM 0.125–0.5 mM 200 mg/kg/day 0–0.6 mM	Breast cancer cell lines (MDA-MB-231) and murine fibroblast cell line (NIH3T3) Murine melanoma cell line (B16F-10) Mice Human skin fibroblasts Mouse tumour cell line (CT26) Mice Prostate cancer cell lines (DU145, PC3-ML, LNCaP, C4-2B, and LAPC-4) Mice	[352] [353] [278] [282] [285]
Aryl β -D-xylosides	 <p>Example shown: 4-Deoxy-4-fluoro-aryl-xyloside</p> <p>Various options available commercially, such as Sigma, N2132</p>	Glycosaminoglycan inhibitor; decoy inhibitor for Ser/Thr- β -xylose	Variable; for shown structure: 354.29 g/mol	0.5–30 mM 1–25 mM	RG-C6, NB41A, and rat hepatoma cells Chinese hamster ovary (CHO) cells Human glioblastoma cells Chicken embryos	[354] [355] [277] [274,356]

are lithocholic acid derivatives, of which sixteen compounds were developed by Li, Chang, *et al.* [205]. Among these compounds, Lith-*O*-Asp was capable of inhibiting ST3GAL1, ST3GAL3, and ST6GAL1 with IC₅₀s of 15.9 μM, 12.2 μM, and 15.8 μM, respectively, when incubated with various lung and breast cancer cell lines for 48 h. Moreover, a 3 mg/kg intraperitoneal treatment of Lith-*O*-Asp suppressed metastasis formation of cancer models in mice [206]. AL-10, another lithocholic acid analogue developed by the same group, was also found to be capable of penetrating cell membranes and inhibiting ST3GAL1 and ST6GAL1 at IC₅₀s of 0.88 μM and 1.50 μM, respectively. Moreover a 3 mg/kg intraperitoneal treatment of AL-10 suppressed metastasis formation of lung cancer in mice without disturbing liver and kidney function [207]. Therefore, further testing of AL-10 as an anti-cancer drug is warranted.

Recently, Fu, Li, *et al.* reported a more selective lithocholic acid derivative known as FCW34 which inhibits ST3GAL3 and ST6GAL1 with IC₅₀s of 1.74 μM and of 3.60 μM, respectively. In contrast to other compounds, FCW34 exhibited no inhibition of ST3GAL1 at concentrations lower than 500 μM. Moreover, this molecule was able to inhibit breast cancer tumour growth *in vivo* in mice [208].

Sialidase inhibitors: DANA, zanamivir, and oseltamivir. Clinically, sialidase (also known as neuraminidase) inhibitors are primarily attractive for the inhibition of viral sialidases, which may reduce the attachment and cellular entry of viruses to host cells, thus providing a treatment strategy [209]. However, inhibition of sialidases is also valuable in mammalian systems, targeting the four canonical mammalian sialidases NEU1–4. The sialidase inhibitor 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid (DANA) inhibits mammalian sialidases in cultured human leukaemia cells, primary pulmonary fibroblasts, and primary rat hippocampal neurons at a concentration of 1 mM, in pancreatic beta cells at 100 μM, and at 10 mg/kg *i.p.* in mice [210–213]. However, DANA is still much more efficient in the inhibition of viral and bacterial sialidases [214].

Based on the DANA scaffold, derivatives have been developed which exhibit stronger or more specific inhibition. One of these derivatives is C9-butyl-amide-2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid (C9-BA-DANA), which is a specific inhibitor of neuraminidase-1 (NEU1) at 100 μM concentration in cells and at 15 μg/kg *i.p.* in mice [215]. Another derivative is 4-Guanidino-DANA, also known as Zanamivir, marketed under the trade name Relenza [216,217]. A functionally equivalent compound is GS4104, also called oseltamivir, and marketed under the name Tamiflu [218]. Both Zanamivir and Oseltamivir are FDA approved anti-influenza drugs [219]. They are designed to specif-

ically inhibit viral sialidases, however, activity of zanamivir against human NEU2 and NEU3 has been reported (K_i of ~ 4 μM and ~ 13 μM, respectively). Notably, oseltamivir showed no inhibition against human sialidases even at 1 mM concentration [220]. Nevertheless, considering the nanomolar concentrations at which oseltamivir and zanamivir inhibit viral sialidases, the affinity of zanamivir to NEU2 and NEU3 is comparably low.

Fucosylation

Fucosylation is a late stage step in glycan biosynthetic pathways catalyzed by fucosyltransferase (FUT) enzymes. Inhibitors of fucosylation have been explored in the context of their potential to combat cancer, chronic obstructive pulmonary disease, Crohn's disease, rheumatoid arthritis, and others [174,221–223].

2-Deoxy-fluoro-L-fucose. 2-Deoxy-fluoro-L-fucose (2FF) was synthesized by Murray, Wong, *et al.* in 1997 [224]. As with other fluorinated monosaccharides, 2FF is transported through the cell membrane then converted to its nucleotide sugar, in this case GDP-2FF. GDP-2FF inhibits fucosylation through both feedback inhibition of the native GDP-fucose biosynthesis pathway and competitive inhibition of fucosyltransferases ($K_i = \sim 4 \mu\text{M}$) [195,224]. 2FF is active *in vitro* and *in vivo*. Typical dosages are 100–200 μM for 7–10 days in cell culture, 50–100 μM orally for 21 days in mice, and 150 mg/kg injection for 10 days in mice [196,225]. No significant toxicity has been reported *in vitro* or in mice. It is worth noting that under some conditions 2FF treatment can result in sufficient depletion of GDP-fucose such that cells use GDP-2FF as a donor substrate. Promising results in tumor models have resulted in use of 2FF in a human clinical trial for patients with advanced solid tumors [226]. Recently, β- and α-GDP analogues of 2FF have been reported, which cross cell membranes and exhibit 4–7 fold higher inhibition potency, with no observed change in toxicity relative to 2FF [227].

6,6,6-Trifluorofucose. 6,6,6-Trifluorofucose (Fucostatin I) was first synthesized in 1991 [228]. Fucostatin I's mechanism of action relies on allosteric inhibition of GDP-mannose 4,6-dehydratase, which is necessary for cellular synthesis of GDP-fucose ($K_D = 11 \mu\text{M}$) [229]. In Chinese hamster ovary cells (CHO) Fucostatin I inhibited fucosylated glycoproteins with an EC₅₀ of 4 μM, with no changes in cell growth, viability or behaviour observed up to a dose of 20 μM. A possible point of caution is that Fucostatin I can be incorporated in protein glycans (at approximately 1%) due to its accumulation as a nucleotide sugar. A viable alternative is the α-fucose-1-phosphonate analog Fucostatin II which acts as an inhibitor with slightly less potency (EC₅₀ = 30 μM), and showed no incorporation in antibodies [229]. Fucostatin II shows some

toxicity at concentrations of 40 μM [229]. As shown by McKenzie, Goddard-Borger, *et al.*, work is ongoing to refine both the production and the characterization of Fucostatins as fucosylation inhibitors [230].

Indolizidine 21. Bastida, Garcíá-Junceda, *et al.* have demonstrated that a castanospermine stereoisomer (a polyhydroxylated indolizidine) inhibits α 1,6-Fucosyltransferase (FucT-VIII) with an IC_{50} of 45 μM in cell-free extracts [231]. As FucT-VIII is upregulated in several cancer types, [232–235] developing FucT-VIII inhibitors may serve as a strategy for anticancer therapy.

6-Alkynyl fucose. Various alkyne- and azide-modified fucose analogs have been developed as metabolic labeling tools for fucosylated glycans [236–239], and a subset of these exhibit inhibitory activity on cellular fucosylation. 5-alkynyl-fucose (5AF) was reported to have similar inhibition potential to 2FF *in vitro* (CHO cells, 50 μM concentration), though it exhibited a three-fold reduced oral bioavailability in mice when compared to 2FF [225].

Kizuka, Taniguchi *et al.* showed that 6-alkynyl-fucose (6AF) inhibits fucosylation in a variety of cultured cell lines at <10 μM concentration [240]. 6AF directly inhibits GDP-L-fucose synthase (also called GFUS, FX, or TST3), and does not appear to influence the GDP-fucose transporter FUT8 or GDP-mannose 4,6-dehydratase when used at low micromolar concentrations [240]. When compared to other inhibitors like 2FF, 6AF seems to have similar inhibition potential, although results vary in different cell lines and experimental conditions. For example, 6AF is reported to be more potent than 2FF in MEF, [240] Caco-2, and PNT2 cell lines, while 2FF performs better in A549 [241]. An additional complexity emerges from the analysis of the post-inhibition glycome, revealing that alkynylated fucose analogues are incorporated in the cell [241].

From a therapeutic standpoint, 6-alkynyl-fucose (as well as 6-alkenyl fucose) has been shown to inhibit notch signalling, which is an *O*-fucose-regulated cell-surface receptor that controls cell fate and has been targeted in the context of cancer progression. A recent study showed 6AF and 6-alkenyl fucose inhibit notch signalling in HEK293 cells (50 μM) and in zebrafish embryo (60 pmol injection in yolk at 1-cell stage) [242].

Inhibitors of postsynthetic glycan modifications

Postsynthetic modifications, as their name suggests, involve changes that occur to the glycoconjugates after the glycosylation process is completed, and the target chain is fully synthesized. There are four common types of postsynthetic modification to glycans: methylation, phosphorylation,

acetylation, and sulfation. Methylation has not been reported in humans to the best of our knowledge, although such modification is observed in other organisms, including bacteria, fungi, algae and worms [243]. Mannose-6-phosphate is the best characterized example of a phosphorylated glycan; it is critical for shuttling enzymes to the lysosome. The existing inhibitor to this pathway, PF-429242, [244] does not act directly on a glycan modifying enzyme, and is as such not discussed further here. Below, we focus on inhibitors of glycosaminoglycan sulfation and sialic acid *O*-acetylation. Functionally, *O*-acetylation and sulfation of glycans can influence their signaling, as is the case for modulation of sialic acid binding to host lectins [245] and the modulation of heparin activity in the coagulation pathway [246]. Furthermore, sulfated GAGs are key components of the extracellular matrix and are involved in the stabilization of tissue, with changes in GAG sulfation being implicated in pathological alterations of the skin, of joints, muscles, and bones, as well as diseases of aging such as Alzheimer's Disease and cancer [247].

Inhibitors of glycosaminoglycan sulfation

A cell-permeable small molecule glycosaminoglycan (GAG) sulfotransferase inhibitor, (*E*)-3-(3-bromo-4-hydroxy-5-methoxyphenyl)-2-cyano-*N*-(2,4-dichlorophenyl)acrylamide, was first reported by Cheung, Hsieh-Wilson, *et al.* in 2017 [248]. The molecule, designated compound 34 by authors, is a dichlorinated version of the lead scaffold identified in a screen for inhibition of the chondroitin sulfotransferase Chst15, with counter screening for the cytosolic sulfotransferase Sult1c1. Compound 34 inhibited Chst15 with respect to a chondroitin sulfate-A (CS-A) substrate in a mixed inhibition mode, with a K_i of 1.43 μM , K_i' of 2.45 μM , and an increase in K_m from 0.96 μM to 1.49 μM . Based on kinetic analysis of compound 34 and analogues, authors postulated that 34 binds the sulfate donor binding site competitively, while also interacting with the acceptor binding site to some degree. In addition to Chst15, compound 34 was effective against chondroitin 4-*O*-sulfotransferase Chst11, the chondroitin 2-*O*-sulfotransferase Ust, and the heparan 3-*O*-sulfotransferase Hs3st1 (IC_{50} values 2.0–2.5 μM). Activity against cytosolic sulfotransferases, such as Sult1e1, Sult2b1a, and Sult2b1b, had IC_{50} values of 19–42 μM . Treatment of cultured astrocytes with 25 μM compound 34 for 24 h resulted in \sim 60% increase in unsulfated chondroitin. The compound is tolerated by mice at an intravenous dose of 3 mg/kg, with a short half-life of 1.6 h, and is a promising initial foray into GAG sulfotransferase inhibition.

Inhibitors of sialic acid *O*-acetylation

Sialic acids can be *O*-acetylated at various positions, resulting in altered signaling behaviour

that influences the many pathways in which sialic acids are key players [245]. Inhibitors of sialic acid-specific *O*-acetyltransferases and *O*-acetyltransferases that add and remove *O*-acetyl groups, respectively, have been recently and thoroughly reviewed by Visser, Büll, *et al.* [249]. To our knowledge, compounds described to date were developed for and tested as inhibitors of influenza *O*-acetyltransferases, and none have achieved activity below millimolar concentrations [250]. Inhibitors for mammalian *O*-acetyltransferases and *O*-acetyltransferases are an unmet need.

Glycolipid inhibitors

Glycosphingolipids make up the majority of glycolipids that are found in cell membranes in animals, [251] and are therefore the focus of this section. Glycosphingolipids consist of one or more sugar residues that are connected to ceramide. They are classified based on common core structures, which in vertebrates are chiefly isoglobo-, globo-, ganglio-, lacto- and neolacto-series (Fig. 5). Gangliosides are especially abundant in nervous tissue, where they modulate neurite outgrowth and myelination, and are implicated in neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease [252]. In addition, pathological accumulation of glycosphingolipids in lysosomal storage disorders such as Gaucher type I and Tay-Sachs disease is functionally implicated in disease progression and severity [253].

N-Butyldeoxynojirimycin

N-Butyldeoxynojirimycin (NB-DNJ) is a derivative of the iminosugar nojirimycin and inhibits glucosylceramide synthase, which is essential for the synthesis of the vast majority of glycosphingolipids. Platt, Butters, *et al.* showed that glycosphingolipid synthesis in HL-60, K-562, H9 and MOLT-4 cell lines was inhibited by approximately 90% when incubated with NB-DNJ at 0.5 mM for three days [254]. NB-DNJ inhibits human immunodeficiency virus (HIV) replication *in vitro* [255–257], but despite demonstrating antiviral potential in humans, it has not been approved due to adverse side effects arising from off-target activity [258]. NB-DNJ (miglustat, Zavesca) is currently an approved drug for the treatment of Gaucher type I disease and Niemann-Pick disease type C, which are lysosomal storage disorders driven by pathological lipid accumulation [106,259,260].

In 1998, Overkleeft, Aerts, *et al.* reported the synthesis of a novel nojirimycin derivative *N*-(5'-adamantane-1'-yl-methoxy)-pentyl-1-deoxynojirimycin (AMP-DNJ) and its ability to inhibit glucosylceramide synthase in human melanoma cells with an IC_{50} value of 2 nM [261]. Furthermore, glucosylceramide synthase was inhibited in 3T3-L1

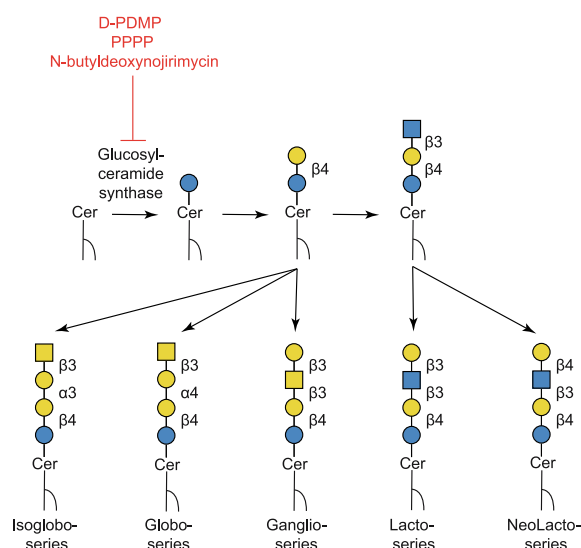


Fig. 5. Inhibitors of glycolipid synthesis. Initial steps of glycolipid biosynthesis. Key inhibitors are shown in red. Enzymes are shown only for steps for which an inhibitor is available. Linkage stereochemistry is shown next to the corresponding bond, in order to differentiate glycolipid series with identical monosaccharide sequences.

adipocytes by exposure to AMP-DNJ at 10 μ M (reported IC_{50} value of 150–220 nM) [262]. Administration of up to 100 mg/kg AMP-DNJ in mice and rats was tolerated. Note, at concentrations >1 μ M AMP-DNJ can also inhibit lysosomal glucocerebrosidase [262].

D-PDMP

(+)*D*-*threo*-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (D-PDMP, the active isomer of the four possible isomers) is a glucosylceramide analogue that inhibits glycosphingolipids in cultured cells at 50 μ M [263,264]. Owing to the role of glycosphingolipids in the aetiology of atherosclerosis, D-PDMP has attracted attention as a potential therapeutic candidate for this disease. In a mouse model of atherosclerosis, D-PDMP reduced lipid accumulation and vascular inflammation [265]. In a separate study using the same murine model of atherosclerosis, D-PDMP reversed hair loss and skin inflammation linked to western diet [266]. DL-*threo*-PDMP, the racemic mixture, is in use as well, and inhibits glycosphingolipids in B16 melanoma cells at an effective concentration of 25 μ M [267].

DL-*threo*-PPPP

DL-*threo*-1-Phenyl-2-hexadecanoylamino-3-pyrrolidino-1-propanol-HCl (DL-*threo*-PPPP) is a more potent inhibitor than DL-*threo*-PDMP [268]. DL-*threo*-PPPP was shown to inhibit glycosphingolipid synthesis in human fibroblasts and rat pheochromocytoma at 1–2 μ M [269,270].

Glycosaminoglycan inhibitors

Glycosaminoglycans (GAGs) are a diverse class of glycans in which long, linear chains of polysaccharides are attached to serine residues initially modified with xylose. GAGs have a wide range of roles in cell and tissue biology, including cell cycle progression, the inflammatory response, and joint lubrication [271,272]. GAG structures and biosynthetic pathways differ in various regions of the body, but follow the general steps outlined in Fig. 6. GAG biosynthetic enzyme inhibitors are of particular importance for their potential in targeting GAG expression in cancer, as well as being anti-inflammatory drug alternatives to corticosteroids [273].

Aryl β -D-xylosides

Robinson, Okayama, *et al.* made the initial observation in 1974 that administration of D-xylose β -linked to various hydrophobic groups primes GAG chain elongation on the exogenously added molecule [274]. β -D-Xylosides therefore compete with endogenous cellular proteins as substrates for GAG elongation, causing (i) secretion of free GAGs initiated on the exogenous molecule and (ii) inhibition of endogenous GAG chains. In the years since, β -D-xylosides, often termed xyloside primers, have found use in both cell and animal models. Note, though β -D-xylosides can be considered to be monosaccharide analogues, they act more specifically than the analogues discussed above, and we therefore discuss them separately here.

Concentrations and treatment times vary dramatically depending on the structure of the hydrophobic group as well as the abundances of endogenous enzymes and substrates (Table 2). Additionally, the choice of β -D-xyloside can affect the structure of the GAGs that are made on the xyloside [275]. Next generation inhibitors have been developed from screens of glycone structure, aglycone structure, and linkage chemistry. For example, treatment of cells for 24 h with 0.3–1 mM 4-deoxy-4-fluoro-xyloside linked through a triazole to naphthyl inhibited GAG biosynthesis by $\sim 80\%$ [276]. Efforts are ongoing to generate prodrugs to achieve higher potency [277]. Caution should be exercised when using xyloside primers, as depletion of monosaccharides used in GAG biosynthesis can in some cases influence other glycosylation pathways.

4-Methylumbelliferone

4-Methylumbelliferone (4-MU) is a coumarin derivative, a class of herbal-derived substances initially isolated in the seeds of *Dipteryx odorata* (cumaru) in Central and South America. In 1995, Nakamura, Endo, *et al.* first demonstrated that incubating human skin fibroblasts with 4-MU, at a concentration of 0.5 mM for 72 h, inhibits hyaluronic acid (HA) synthesis [278]. 4-MU appears to exert its inhibitory effects by suppressing the levels of HA synthases and depleting UDP-glucuronic acid, which is necessary for HA synthesis [279,280]. Recently, 4-MU has attracted attention as an anticancer drug candidate, owing to the roles it can play in regulating tumour behaviour, promoting tumour metastasis, and prompting tumour

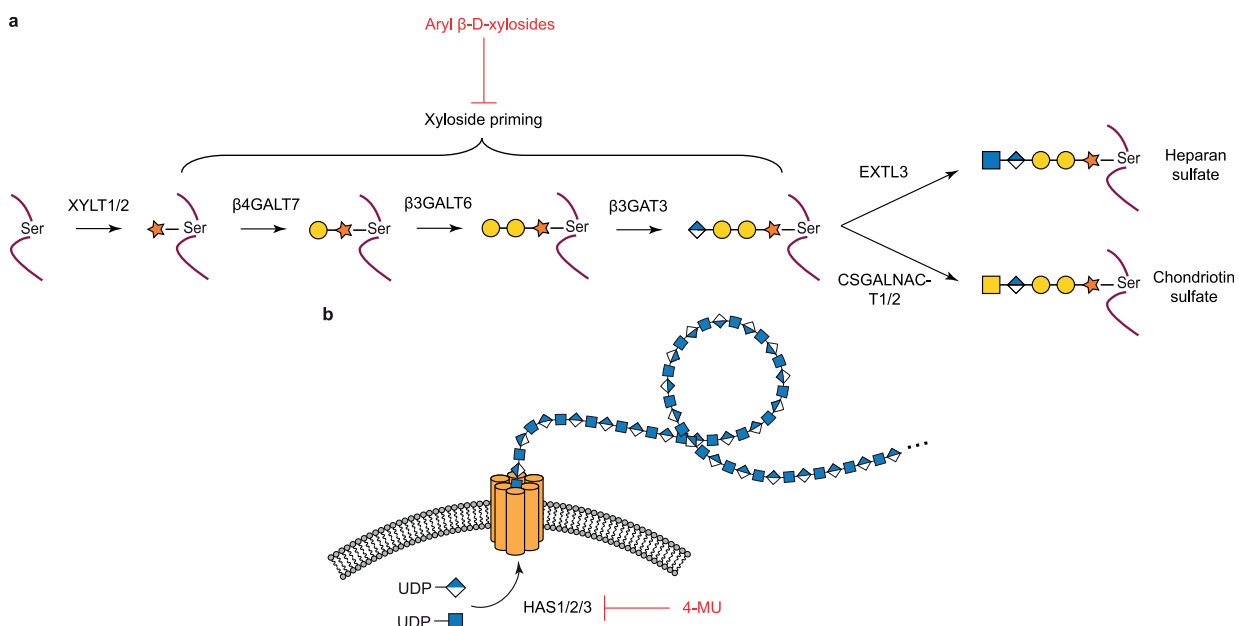


Fig. 6. Inhibitors of glycosaminoglycan synthesis. (a) Initial steps of glycosaminoglycan (GAG) biosynthesis. Phosphorylation of xylose is omitted for clarity. Key inhibitors are shown in red. Enzymes are shown only for steps for which an inhibitor is available. (b) Hyaluronan biosynthesis and key inhibitors.

immune evasion [281]. Indeed, 4-MU demonstrated antitumor effects in several cancer types, both *in vitro* and in mouse models, including colon, pancreatic, prostate, breast, and ovarian cancers [282–287]. In mice, its effective antitumor dose range is 1000–3000 mg/kg [284].

Clinically, 4-MU (hymecromone) is approved for the treatment of bile spasm [288]. Its choleric effect is induced at doses of 1500–2200 mg/day in humans. Unfortunately, its poor pharmacokinetic properties (e.g., short half-life and poor bioavailability) limit its applications outside the biliary tract [279]. Addressing the toxicological and pharmacological limitations of 4-MU would enhance its therapeutic potential.

Conclusion and outlook

In this review, we have surveyed small molecules that inhibit well-characterized glycosylation pathways. The number of available compounds might give the impression that the currently available toolbox is sufficient for precise intervention in a broad range of scenarios. However, the opposite is true. As we have discussed, many inhibitors suffer from significant limitations, among which low specificity for the target and cytotoxicity stand out in particular. We hope that future research, combining insights from glycobiology, organic chemistry, structural biology, and biochemistry, will close this gap and provide specific, high-affinity inhibitors for a broad range of glycosylation enzymes. Looking ahead, an analogy to another field that historically faced a similar challenge, protein kinase inhibitors, may be instructive. For decades, inhibitors for protein kinases were regarded as intractable due to their seemingly broad substrate profiles and structural overlap [289,290]. However, recent decades have seen the development of specific and high affinity inhibitors of single kinases, equipping the field with important tools. Moreover, dozens of small molecule kinase inhibitors have reached FDA approval, marking a step forward in cancer therapy [291]. There are many reasons to be optimistic that the near future will see a similar trend for inhibitors of glycosylation enzymes. Considering that aberrant glycosylation is established as a functional driver of cancer progression, autoimmunity, viral infection, and more, we are also optimistic that one day we might employ glycosylation-targeted small molecules widely in the clinic.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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