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# Beyond substrate analogues: new inhibitor chemotypes for glycosyltransferases

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**Abstract**

Glycosyltransferases (GTs) are a large family of carbohydrate-active enzymes, which act as nature's glycosylation agents. GTs catalyse the transfer of a mono- or oligosaccharide from a glycosyl donor to an individual acceptor, and play a central role in the biosynthesis of complex carbohydrates, glycans and glycoconjugates. Several GTs have emerged as potential drug targets in a range of therapeutic areas, including infection, inflammation and cancer. Small molecular GT inhibitors are therefore sought after not only as chemical tools for glycobiology, but also as potential lead compounds for drug discovery. Most existing GT inhibitors are donor or acceptor analogues with limited potential for further development due to intrinsic drawbacks, such as a lack of cell penetration and limited chemical stability. In this article, we review recent progress in the identification of alternative inhibitor chemotypes that are not structurally derived from GT donors or acceptors. This growing class of non-substrate-like GT inhibitors now includes several examples with drug-like properties, which provide exciting new starting points for medicinal chemistry and drug discovery. The increasing availability of such alternative GT inhibitor chemotypes represents a significant advance, which will help realise the considerable potential of this important enzyme family as therapeutic targets.

## Introduction

Glycosyltransferases (GTs) are nature's glycosylation reagents: a large family of enzymes that catalyse the transfer of a mono- or oligosaccharide from a glycosyl donor, e.g. a sugar-nucleotide, to an individual acceptor, e.g. another sugar, peptide, protein, lipid or small molecule (Fig. 1).[1-6] Depending on the stereochemistry of the glycosylation reaction, GTs can be grouped into retaining and inverting enzymes (Fig. 1), and our current understanding of the mechanistic details of the GT reaction has recently been summarised in several instructive reviews.[3-6] At the cellular and organismal level, GTs and their biosynthetic products play a key role in many fundamental processes underpinning human health and disease, including cellular adhesion,[7] cell signalling,[8] malignant transformation and metastasis,[9] and bacterial virulence.[10] As a result, several GTs have emerged as potential drug targets in a range of therapeutic areas, including infection, inflammation and cancer.[11] The identification and development of small molecular inhibitors for GTs is therefore of considerable interest for medicinal chemistry and drug discovery.[11-18]

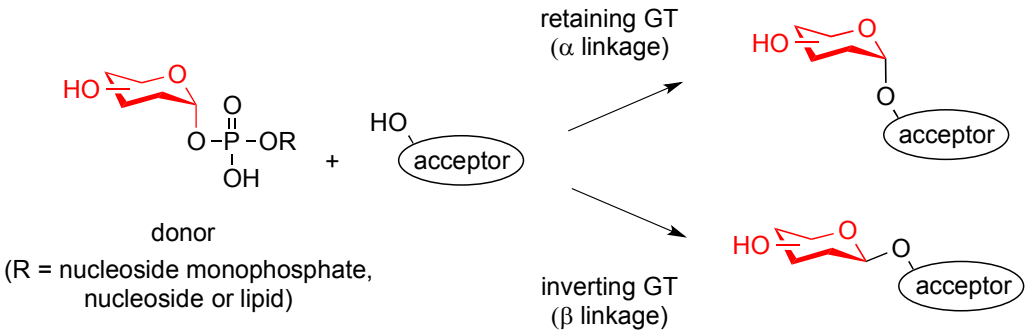
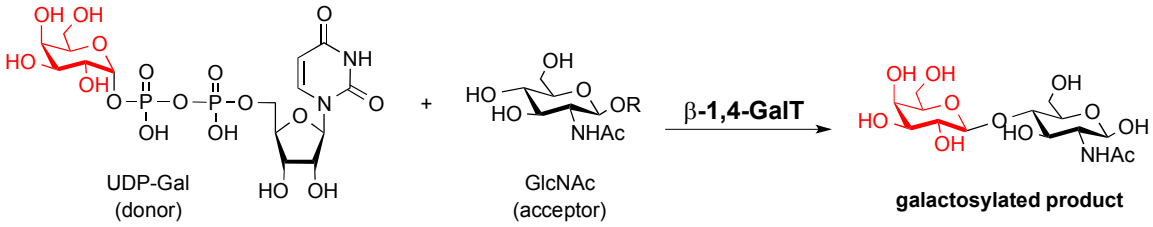



**Fig. 1** General scheme of the glycosyltransferase reaction.

Traditionally, the design of inhibitors for GTs has focussed mainly on the natural substrate(s) of these enzymes.[13-18] This general strategy usually involves either the

structural modification of the donor or acceptor, or the replacement of one or more of its structural features (e.g. the pyrophosphate linkage in the sugar-nucleotide donor) with a suitable mimic (Fig. 2). For example, the β-1,4-galactosyltransferase (β-1,4-GalT) that is involved in the biosynthesis of the cell surface antigen Sialyl Lewis X is of interest as a drug target in inflammation and cancer.[12] β-1,4-GalT catalyses the transfer of D-galactose from a UDP-galactose (UDP-Gal) donor to a GlcNAc acceptor, and a range of inhibitors derived directly from either substrate have been reported (Fig. 2). Such substrate-based inhibitors have proven highly useful as chemical tools for mechanistic and structural studies with recombinant enzymes, not only with β-1,4-GalT,[19] but with many different GTs.[20-24] However, substrate-based GT inhibitors frequently retain certain unfavourable properties of their parent compounds, such as a lack of cell penetration and limited chemical stability. These intrinsic drawbacks often reduce their suitability for cellular applications and drug development. In addition, many of these substrate-based GT inhibitors are only accessible via multi-step synthesis,[16] which further compromises their practical utility. Alternative inhibitor chemotypes, on the other hand, which are not structurally derived from either donor or acceptor and may offer advantages e.g. with regard to their physicochemical properties, have remained relatively rare in this enzyme family. Indeed, it can be argued that a lack of drug-like inhibitors has been one of the main reasons for the perception of GTs as “difficult” targets for drug discovery.

**(a) The β-1,4-GalT reaction**




**(b) Substrate-based β-1,4-GalT inhibitor classes**

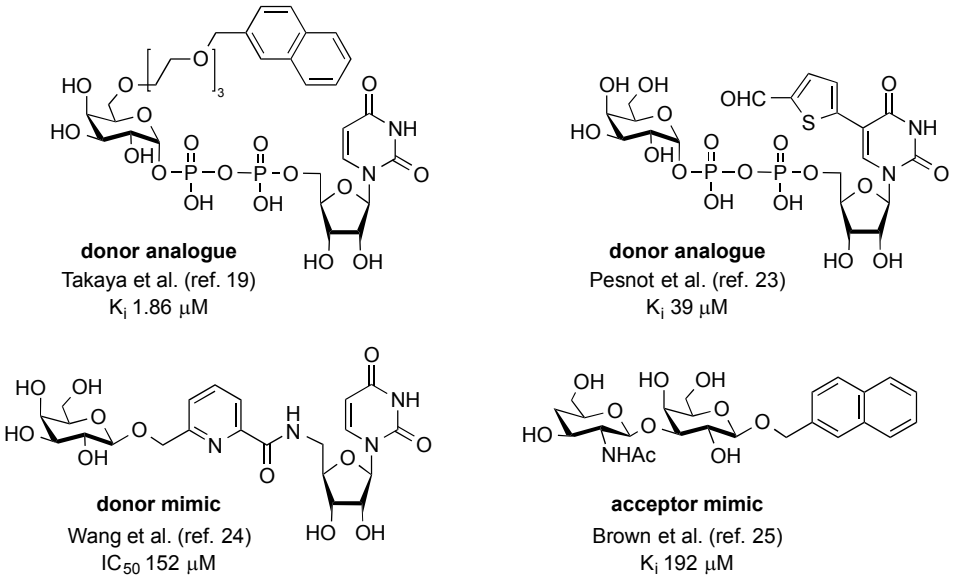



**Fig. 2** The β-1,4-GalT reaction, and selected substrate-based β-1,4-GalT inhibitors.[19, 23-25]

To date, more than 87,000 protein sequences with proven or putative GT activity have been reported, and it is estimated that 1% of open reading frames (ORFs) in the human genome code for GTs.[5] While this enzyme family therefore offers enormous opportunities for drug discovery, at present, only very few glycosyltransferase inhibitors are used in the clinic. Two prominent examples are miglustat and ethambutol, neither of which was originally designed as a GT inhibitor. The iminosugar miglustat is used for the treatment of the lysosomal storage disorders Morbus Gaucher and Niemann-Pick type C disease. It acts by blocking the glucosyltransferase glucosylceramide synthase (GCS), thus reducing the accumulation of glycosphingolipids in affected organs.[26] The molecular

target of ethambutol, an established treatment for tuberculosis, has recently been identified as an arabinosyltransferase responsible for the polymerisation of arabinose into the arabinan of arabinogalactan in the mycobacterial cell wall.[27]

While these examples demonstrate that GT inhibitors can be safe and effective medicines, the search for new, drug-like GT inhibitors with similar potential for clinical applications has been complicated by a number of factors. Although the majority of GTs belong to one of only two general fold types (GT-A or GT-B),[1, 3-6] many are highly dynamic proteins which follow a complex, multi-substrate reaction mechanism that involves several conformational changes.[28] This unusual conformational plasticity has complicated the rational de-novo design of inhibitors, especially as for a long time there had been a distinct lack of 3D structures.[1, 4-6] In the past, GT inhibitor discovery also suffered from a relative lack of operationally simple assays, and in particular of formats that could be adapted for HTS.[29] In both of these areas – structures and assays – there has been significant progress recently. The number of X-ray structures of GTs has increased from just two in 1999 to well over 100 in 2014. Over the same period, several HTS assays have been developed and exploited for GT inhibitor discovery.[29,30] Both of these developments, as well as the successful application of modern techniques in medicinal chemistry such as dynamic combinatorial chemistry,[31, 32] Click chemistry[33] and fragment-based strategies,[34, 35] have greatly facilitated GT inhibitor discovery and contributed to the growing number of new inhibitor chemotypes.

While several excellent reviews have been published on substrate-based GT inhibitors, including substrate mimics,[13-18] alternative inhibitor chemotypes, which are structurally distinct from GT donors and acceptors, have never been systematically reviewed. With this article, we aim to fill this gap. We have surveyed the literature from 2000-2013 and give an overview of the different non-substrate-based GT inhibitors that have been reported in this period, complemented by relevant older examples. With the

exception of a few “borderline cases”, we have deliberately not included inhibitors that are directly derived from GT donor or acceptor substrates, as these have been comprehensively reviewed elsewhere.[13-18] Innovative approaches based on metabolic precursors of donor analogues[36, 37] are also beyond the scope of this review. Structurally, the non-substrate-based GT inhibitors that have been identified to date represent a very diverse group. As the focus of this review is on inhibitor structures, we have organised the material by inhibitor chemotype rather than target enzyme. We have grouped the different inhibitors into the following categories, some of which are necessarily relatively broad:

(1) Iminosugars and pyrrolidines
(2) Ceramide analogues
(3) Amino acids and peptides
(4) Diamines
(5) Polyaromatics
(6) Steroids, terpenoids and substituted decalins
(7) Miscellaneous natural products and their derivatives
(8) Synthetic heterocycles
    a. Thiazolidinones
    b. Pyrazolones
    c. Uracil and uric acid derivatives
    d. Miscellaneous

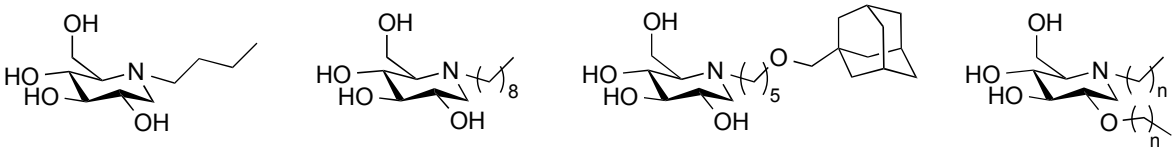
For each chemotype, we briefly introduce its target GT. At present, non-substrate-like inhibitors have been reported for only a relatively small number of GTs, including in particular glucosylceramide synthase (lysosomal storage diseases), fucosyl- and

sialyltransferases (cancer), OGT (cancer, diabetes) as well as several bacterial enzymes such as MurG, WaaC and LgtC. We highlight chemotypes that are active against several targets and show, where appropriate, the substrates of the respective GT reaction for direct comparison. A good number of these new inhibitor chemotypes are drug-like molecules, which highlights the considerable potential GTs still hold as targets for medicinal chemistry and drug discovery. We hope that by presenting these alternative inhibitor chemotypes in one place, this review will help in realising this potential.

**1) Iminosugars and pyrrolidines**

Morbus Gaucher is a lysosomal storage disease characterised by an accumulation, in multiple organs, of glycosphingolipids (GSLs).[26] The iminosugar Miglustat (Fig. 3) acts by inhibiting the glucosyltransferase glucosylceramide synthase (GCS). GCS catalyses the glucosylation of ceramide to glucosylceramide, the first committed step in the biosynthesis of more complex GSLs such as lactosylceramide and gangliosides. Pharmacological inhibition of GCS therefore effectively reduces the accumulation of glycosphingolipids in affected organs. The study of miglustat has suggested that the molecule behaves as a mimic of ceramide, its acceptor substrate (Fig. 3), through similarities between the N-acyl chain in ceramide and N-alkyl chain of the imino sugar.[38] This has been proposed as having a significant effect on the potency, and the addition of further alkyl chains was envisaged to improve the ceramide mimicry. However, the addition of a second alkyl chain did not enhance potency (Fig. 3). This was explained by the miglustat cyclic ring prohibiting the close alignment of the alkyl chains in the manner that is seen in ceramide.[39] Increasing the length and therefore inherent hydrophobicity of the alkyl chain does, however, increase potency and provides further improvement by virtue of membrane adsorption, persistence in tissues and greater brain penetration (Fig. 3).[40] Furthermore, the adamantyl-substituted iminosugar AMP-DNM (Fig. 3) shows

marked improvement in GCS inhibition over the simpler alkyl-substituted iminosugars, with an $IC_{50}$ of 90 nM.[41] Whilst an important treatment for Gaucher disease and other GSL storage diseases, Miglustat does suffer from reported tremor and gastrointestinal-based side effects.[42] Therefore, further development of the iminosugar scaffold will be of considerable interest, in order to find an even more effective treatment with fewer physiological side effects.




**Miglustat (NB-DNJ)**
$IC_{50}$ 20 μM

**NN-DNJ**
$IC_{50}$ 1.6 μM

**AMP-DNM**
$IC_{50}$ 90 nM

n = 3: no inhibition
n = 6: $IC_{50}$ 164 μM

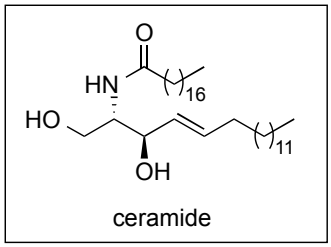



**Fig. 3** Iminosugar inhibitors of GCS, and the natural GCS acceptor substrate ceramide.

Iminosugars were originally developed as glycosidase inhibitors, leading to a variety of novel structures and biological activities.[43,44] Since glycosyltransferase reactions are thought to proceed through transition states similar to those of glycosidases, iminosugars and related compounds have recently been investigated for inhibition of glycosyltransferases. Various hydroxylated pyrrolidines derivatives have been screened and were found to be weak inhibitors of β-1,4-galactosyltransferase (Fig. 4).[45] Similarly, a small library of highly substituted pyrrolidines were also found to show inhibitory

activity against this enzyme, whilst no activity was observed against α-1,3-galactosyltransferase in this case (Fig. 4).[46] The highest inhibitory activities were achieved when R' was adamantyl or alkylhydroxyl. For further discussion of the GT inhibitory activity of simple iminosugars and iminosugar-nucleotide analogues, we refer the reader to excellent reviews of these inhibitor classes.[43,47]

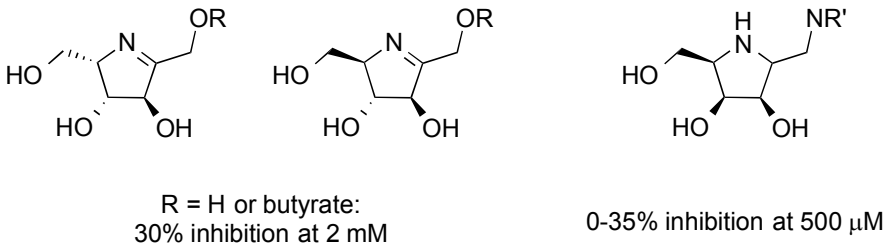


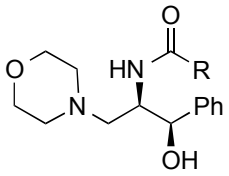

**Fig. 4** Hydroxylated pyrrolidines as inhibitors of β-1,4-galactosyltransferase.

**(2) Ceramide analogues**

GCS inhibitors loosely based on the natural GCS acceptor ceramide have also been investigated (Fig. 3). For example, US6916802[48] discloses that increasing the acyl chain of ceramide-based inhibitors from 10 to 16 carbons greatly enhances the inhibitory activity towards GCS (Fig.5, compare 1-phenyl-2-*decanoyl*amino-3-morpholino-1-propanol, PDMP, with 1-phenyl-2-*palmitoyl*amino-3-morpholino-1-propanol, PPMP). Further improvements were achieved by incorporation of a pyrrolidino head group as well as, in some cases, the installation of an unsaturated hydrocarbon chain (Fig. 5, BML-119 vs. IV-231B). One representative from this inhibitor class, eliglustat (Genz-112638, Fig. 5) is currently in clinical development for the treatment of type 1 Gaucher disease.[49]

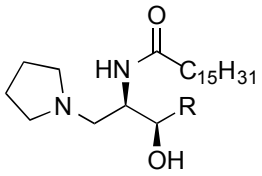
A close homologue of eliglustat is Genz-123346, which has a longer acyl chain and slightly better inhibitory activity (Fig. 5).[41] Derived from a series of GCS inhibitors described by Lee and co-workers in 1999,[50] Genz-123346 has since become a widely

used pharmacological tool, not the least because of its relative selectivity for GCS (e.g. over 1-*O*-acylceramide synthase, α-glucosidase and glucocerebrosidase), its ability to efficiently block the accumulation of glucosylceramide in cells, and its oral availability. Thus, Genz-123346 has been used successfully in a range of animal models, including type 2 diabetes,[51] polycystic kidney disease[52] and asthma.[53] This GCS inhibitor was also found to protect rats against the cytotoxic effects of shiga toxin 2.[54] Pro-drugs of the generic structure **1** have also been disclosed for this class of ceramide-based GCS inhibitors (Fig. 5).[48] Due to their increased hydrophobicity, these pro-drugs (R''' = acyl) are more readily transported across the cell membrane into the cytosol, where they are converted into their active form (R''' = H).



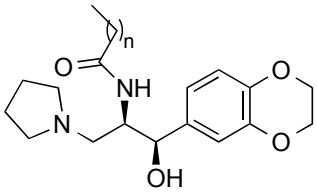
**PDMP (R = $C_9H_{18}$)**
16% inhibition at 5 μM

**PPMP (R = $C_{15}H_{31}$)**
32% inhibition at 5 μM



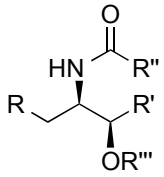
**BML-119 (R = Ph)**
50% inhibition at 5 μM

**IV-231B (R = CH=CH$C_{13}H_{27}$)**
87% inhibition at 5 μM



**Eliglustat (Genz-112638, n = 6)**,
$IC_{50}$ = 16 nM

**Genz-123346 (n = 7)**
$IC_{50}$ = 9 nM

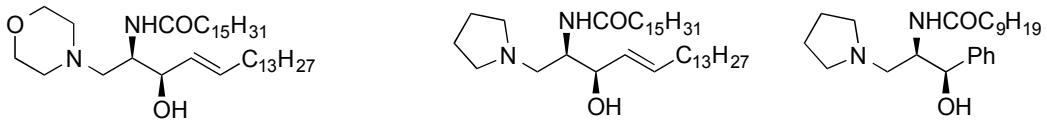


**cmpd 1**
(R''' = an enzymatically hydrolyzable group, usually acetyl)

**Fig. 5** Ceramide-based GCS inhibitors (I).

Separate investigations of morpholino-, piperidino- and pyrrolidino-derivatives of ceramide led to the discovery of several further GCS inhibitors (Fig. 6).[55,56] The most

potent compounds were the derivatives with the (*R,R*)-configuration in the morpholino[55] and pyrrolidino series.[56]




(2*R*,3*R*): 73% inhibition at 5 μM
(2*R*,3*S*), (2*S*,3*R*) and (2*S*,3*S*): 5-20% inhibition at 5 μM

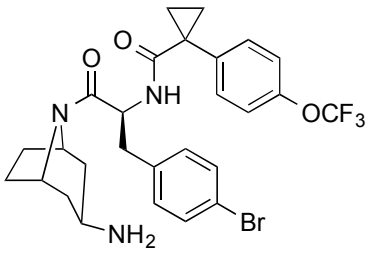
$IC_{50}$ = 32 μM

$IC_{50}$ = 4 μM

**Fig. 6** Ceramide-based GCS inhibitors (II).

**(3) Amino acids and peptides**

Through a high-throughput-screening strategy Richards et al. have identified a novel scaffold against GCS, which was developed from investigating L-amino acid core structures. In particular, EXEL-0346 has emerged as a promising inhibitor of GCS with an $IC_{50}$ as low as 2 nM (Fig. 7).[41] Importantly, this compound shows high exposure in liver, muscle and fat tissues, i.e. those tissues primarily responsible for glucose metabolism, whilst maintaining a low plasma exposure.[41]




**EXEL-0346**
$IC_{50}$ 2 nM

**Fig. 7** A novel inhibitor scaffold against GCS.

Inhibitors based on amino acids or short peptides have also been reported against other glycosyltransferases, including sialyltransferases (SialTs). SialTs catalyze the transfer of

sialic acid from CMP-*N*-Acetylneuraminic acid (CMP-Neu5Ac) to the terminal positions of growing oligosaccharide chains of glycoconjugates. Hypersialylation of cell-surface glycans is often a hallmark of tumor metastasis and inflammation, and SialTs are therefore of interest as potential anti-cancer and anti-inflammatory targets.[57] Following the discovery that rat α-1 microglobulin behaves as an inhibitor against rat Gal-β-1,4-GlcNAc-α-2,6-sialyltransferase,[58] 114 hexapeptide library 'pools' were screened by Lee et al., in order to determine if any specific or generic sialyltrasferase inhibition could be identified.[59] Six hexapeptides were finally identified as potential hits, comprising almost entirely of glycine, arginine and tryptophan. Upon further investigation, hexapeptide $NH_2$-GNWWWW was shown to have the best inhibition profile, and was demonstrated as a competitive inhibitor of CMP-Neu5Ac binding to the SialT ST3 Gal I. Furthermore, the hexapeptide was shown to strongly inhibit both the *N*-glycan specific ST3 Gal I and ST6 Gal I in vitro, suggesting that it may have potential for development for a broad range of sialyltransferases, regardless of their linkage specificity. The kinetic analysis of the optimal peptide $NH_2$-GNWWWW indicated that it could strongly compete with CMP-Neu5Ac binding to ST3 Gal I.[59] Possible explanations of this mode of inhibition were that the peptide could mimic the structural conformation of the endogenous substrate, or a part thereof, or that the peptide could mimic the structural pocket of the enzyme. It was further pointed out that the first two amino acids of the hexapeptide are well conserved in the sialyl motif of mammalian transferases, and that the Gly and Asn residues may provide a likely contact point to CMP-Neu5Ac. Further investigation into this intriguing mode of action is underway.

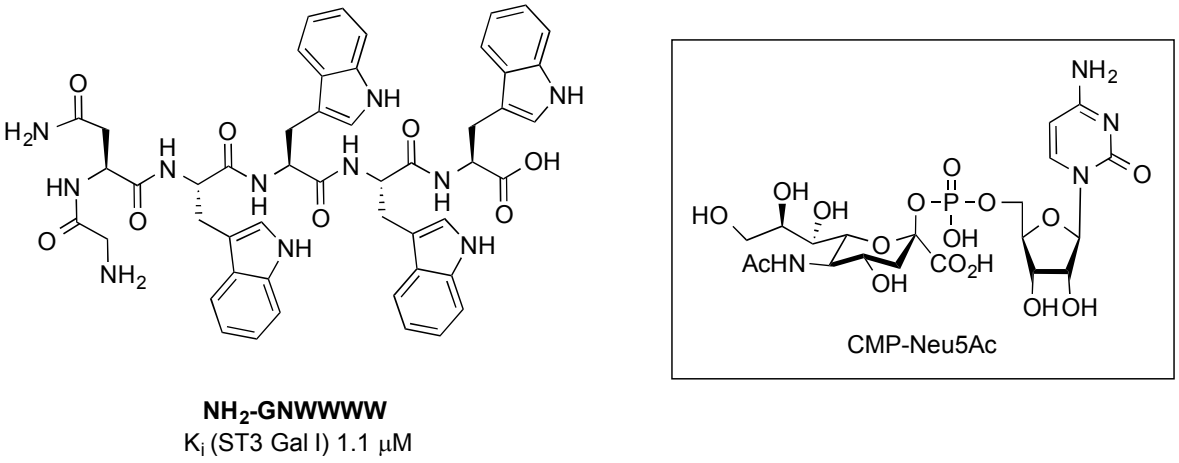



**Fig. 8** A hexapeptide sialyltransferase inhibitor, and the natural sialyltransferase donor CMP-*N*-Acetylneuraminic acid (CMP-Neu5Ac).

**(4) Diamines**

Ethambutol (Fig. 9) is an established treatment for tuberculosis, which was introduced in the clinic in the 1960s.[60,61] It has an excellent tolerability profile and the development of resistance in treatment-naive tuberculosis sufferers is very rare in most countries.[62] The molecular target of ethambutol has been identified as an arabinosyltransferase responsible for the polymerisation of arabinose into the arabinan of arabinogalactan in the mycobacterial cell wall. The endogenous donor of this arabinosyltransferase is decaprenylphosphoryl arabinofuranose (DPA, Fig. 9).[27] A combinatorial approach to finding analogues of ethambutol, based on the 1,2-ethylenediamine core was embarked upon.[63] This strategy led to the discovery of diamine SQ109, which showed good selectivity and efficacy in mouse models of tuberculosis,[64] and was advanced to early stage Phase II trials.[65] Interestingly, while it has been established that SQ109 is inhibiting a target that is involved in cell wall biosynthesis, interestingly, it appears that this target is not the same arabinosyltransferase as as for its parent ethambutol.[66] Instead, the primary target of SQ109 appears to be a membrane transporter of an important lipid involved in cell wall biosynthesis.[67]

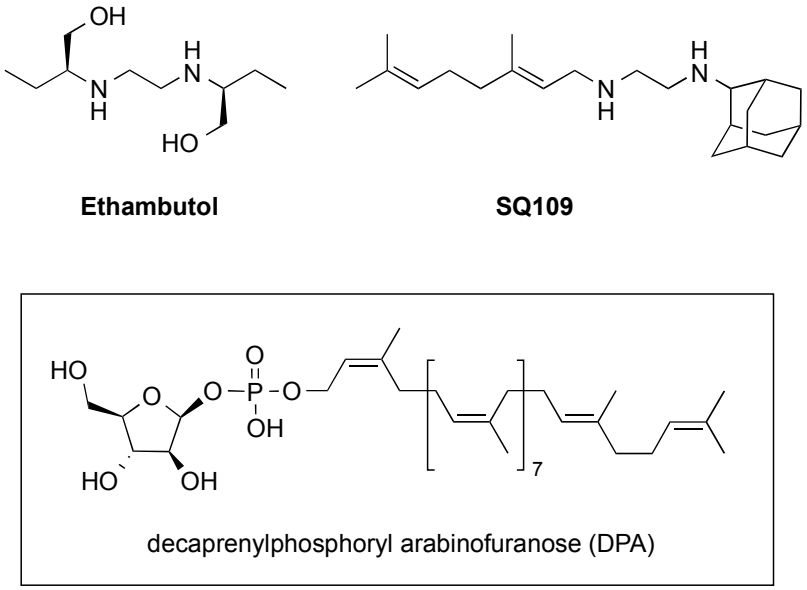
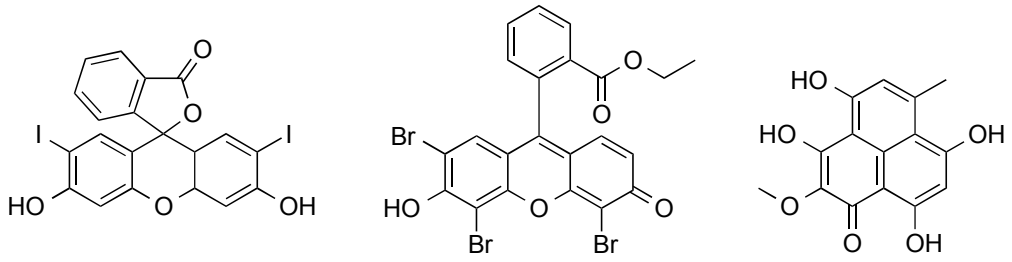



**Fig. 9** Ethambutol inhibits a mycobacterial arabinosyltransferase which uses DPA as its donor substrate. SQ109 was developed from ethambutol but has a different molecular target.

**(5) Polyaromatics**

Several bacterial glycosyltransferases and closely related carbohydrate-active enzymes have been identified as potential anti-microbial targets due to their critical role for bacterial viability or virulence. The MraY and MurG enzymes, for example, catalyse consecutive reactions in the final stages of cytoplasmic cell wall synthesis. MraY catalyses the transfer of muramylpentapeptide monophosphate from UDP-muramylpentapeptide (UDP-MurNAc-pp) to undecaprenol phosphate, to form undecaprenyl-pyrophosphoryl-MurNAc-pp (lipid I). The *N*-acetyl glucosamine (GlcNAc) transferase MurG then catalyses the transfer of GlcNAc from UDP-GlcNAc to lipid I, to yield lipid II. These essential enzymes are difficult to assay because the substrates are lipidic and challenging to prepare in large quantities. This has prompted the development of a so-called ‘two-for-one’ assay, which uses both enzymes in parallel. Inhibitory activity in this combination assay is therefore an indication of inhibition of

either, or both, of the enzymes. Several inhibitor scaffolds have been identified from this combination assay, and a representative subset is shown in Fig. 10.[68] The high-throughput screen described by Zawadzke et al.[68] allowed the use of the native UDP-MurNac-pp substrate without further modification. The hits found by the authors were recognised as suboptimal for further development of antimicrobials, due to their polyphenolic structures, but screening of new compound libraries is planned. Owing to the nature of the combination assay, the specific mode of action of these compounds has not been established, but it is a topic for further development.




$IC_{50}$ 166 μM (MraY/MurG)

$IC_{50}$ 18.4 μM (MraY/MurG)

$IC_{50}$ 54 μM (MraY/MurG)

**Fig. 10** Polyaromatic scaffolds identified from a MraY/MurG combination assay.

Fucosyltransferases (FucTs) catalyse the transfer of fucose from GDP-Fucose (Fig. 11) to individual glycoconjugate acceptors. The biosynthetic products of FucTs, such as the cell-surface glycan Sialyl Lewis X, are often involved in cell adhesion processes e.g. during cancer metastasis and leukocyte recruitment. FucTs have therefore been extensively studied as potential therapeutic targets, particularly in cancer and inflammation. A recent review of fucosyltranferases covers acceptor and donor mimics as well as some new inhibitor chemotypes.[69] Kaminska et al. have investigated a series of triazine dyes for their inhibitory activity of fucosyltransferases.[70] Reactive Yellow 86, Reactive Green 19, Reactive Blue 4, and Reactive Brown 10, Cibacron 3GA and

Reactive Red 120 were characterised as α-1,6 fucosyltransferase (FucT-VIII) inhibitors (Fig. 11). Interestingly, the latter two dyes also showed inhibitory activity against serum glycosyltransferases such as α-1,2-fucosyltransferase, β-1,4-galactosyltransferase and β-1,3-N-acetylglucosaminyltransferase.[70]

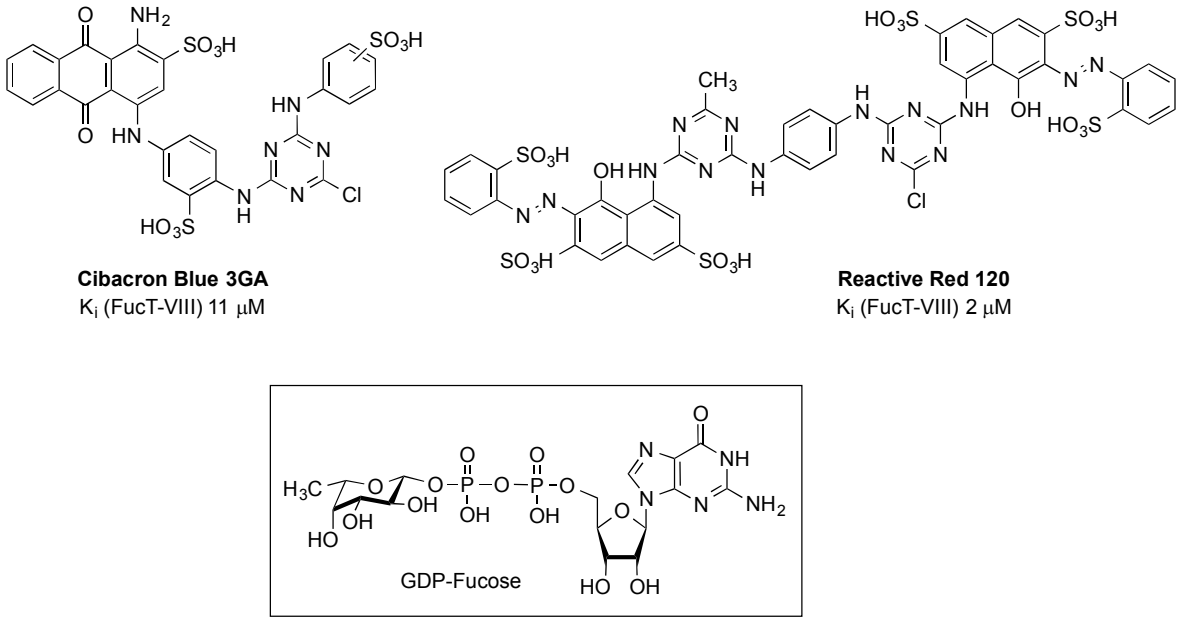
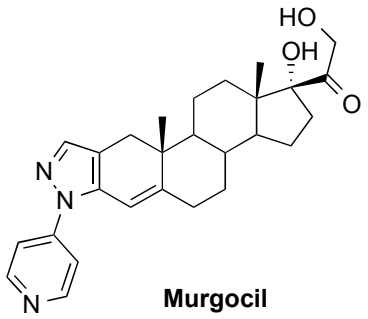



**Fig. 11** Triazine dye inhibitors of FucT-VIII, and the natural FucT donor GDP-Fucose.

**(6) Steroids, terpenoids and substituted decalins**

For several GTs, inhibitors based on a fused cycloalkane scaffold (e.g. steroids, terpenoids, decalins) have been reported. Murgocil is a steroidal inhibitor of the bacterial GlcNAc-transferase MurG, which was identified initially from meticillin-resistant *Staphylococcus aureus* (MRSA) phenotypic screening by Mann et al. (Fig. 12).[71] The target was thoroughly confirmed by applying Murgocil to MurG knock-down and upregulated bacterial strains, as well as the purified recombinant MurG GT.[71] The minimum inhibitory concentration (MIC) in bacterial cells was as low as 2-4 μg/mL, but

the $IC_{50}$ against the isolated GT was 115 μg/mL.[71] This discrepancy is attributed to the solubility problems encountered when using the buffer systems required for the recombinant protein as well as complex, off-target effects in the cell. Although selective for *Staphylococci*, Murgocil had a fast rate of spontaneous resistance. While murgocil therefore represents a good starting point for new antibiotics targeted at MurG, it will require further development in order to afford useful therapies.



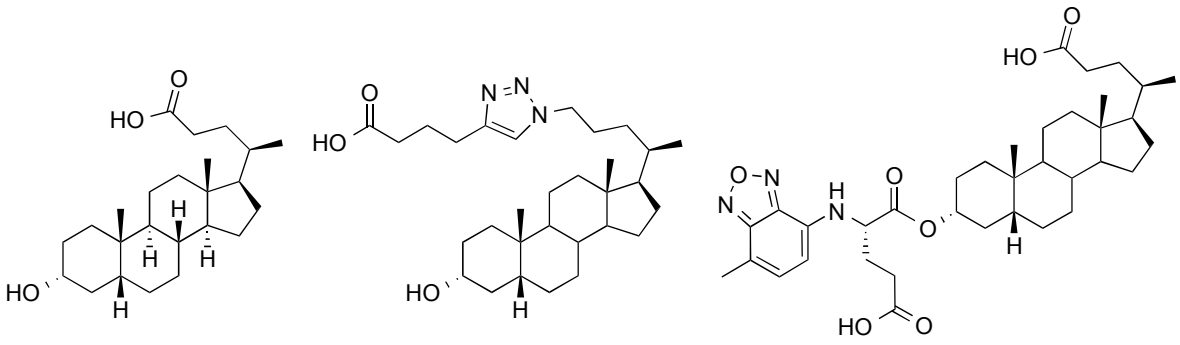

**Murgocil**

$IC_{50}$ (MurG) 115 μg/mL
MIC *Staphylococci* 2-4 μg/mL

**Fig. 12** Murgocil is an inhibitor of MurG.

Steroid-based inhibitors have also been reported for mammalian glycosyltransferases. From enzymatic assays, a series of lithocholic acid derivatives were identified with good inhibitory activity towards different sialyltransferases, which are expressed at high levels in metastatic cells (Fig. 13).[72,73] In enzyme kinetic experiments, the triazole-containing derivative **2** exhibited noncompetitive inhibition of α-2,3-sialyltransferase (ST3Gal I) relative to the natural donor CMP-Neu5Ac.[73] Promisingly, some of these inhibitors were also active in pharmacological assays. Compound AL10 inhibits adhesion, migration, actin polymerization and invasion of human lung cells that overexpress α-2,3-sialyltransferase (ST3Gal I), without causing significant cell death up to 10 μM.[72] AL10

therefore represents a particularly promising derivative from this series for further development as an anti-metastatic agent.



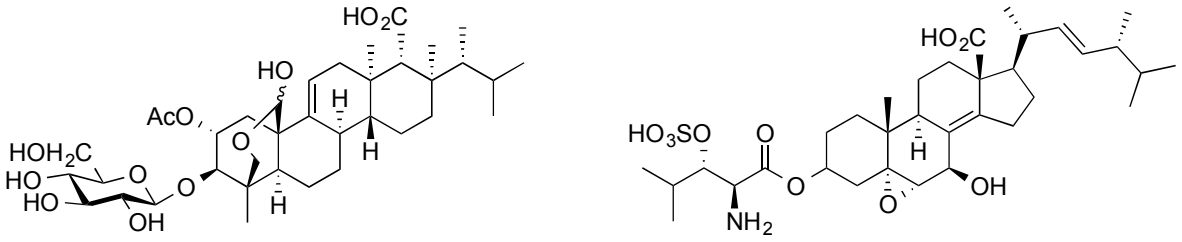
**Lithocholic acid**
$IC_{50}$ (ST3Gal I) 21 μM

**cmpd 2**
$IC_{50}$ (ST3Gal I) 5 μM
Ki (ST3Gal I) 2.2 μM

**AL10**
$IC_{50}$ (ST3Gal I) 0.88 μM
$IC_{50}$ (ST6Gal I) 1.5 μM

**Fig. 13** Sialyltransferase inhibitors derived from lithocholic acid.

Several terpenoid natural products, exemplified by Enfumafungin and Ergokonin A (Fig. 14), have been reported as inhibitors of the fungal β-1,3-glucan synthase,[74] a glucosyltransferase that is required for the biosynthesis of β-glucan structures in the fungal cell wall.[75] Test compounds were evaluated in a modified version of a β-1,3-glucan synthase assay, which measures the transfer of radiolabelled glucose from UDP-[3H]-glucose onto acceptor.[74] Although these terpenoids were less potent than the reference compound L-733,560 (see Fig. 23) against the target enzyme, interestingly, they achieved similar levels of inhibition against glucan biosynthesis in whole cells.

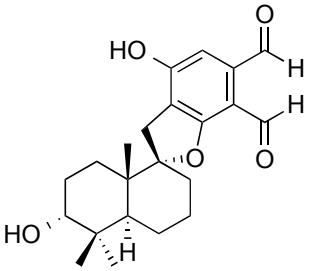



**Enfumafungin**
$IC_{50}$ (glucan synthase) 1.0 μM
MIC (glucan synthesis) 0.05 μg/mL

**Ergokonin A**
$IC_{50}$ (glucan synthase) 3.0 μM
MIC (glucan synthesis) 0.2 μg/mL

**Fig. 14** Terpenoid inhibitors of the fungal β-1,3-glucan synthase.

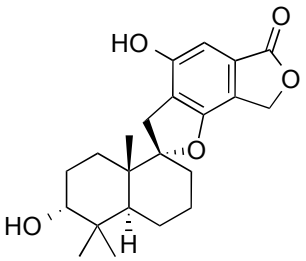
Strachbotrydial and related compounds containing a decalin ring system have been shown as active against a variety of glycosyltransferases involved in terminal glycosylation of cell surface glycoconjugates, including fucosyltransferases.[76] Interestingly, further cyclisation in place of the aldehydic aromatic groups changes the inhibition profile dramatically, particularly for the fucosyltransferases. Strachbotrydial was characterised as an uncompetitive inhibitor with respect to the donor GDP-fucose and noncompetitive with respect to the acceptor substrate LacNAc, with $K_i$ values of 10.7 ± 0.6 and 9.7 ±1.2 μM, respectively (Fig. 15).[76]



**Strachbotrydial**

$IC_{50}$

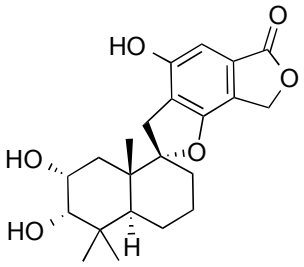
FucT-V 11.3 μM
FucT-VI 20.9 μM
ST3O 6.7 μM
ST3N 10 μM
ST6N 0.61 μM



**Strachbotrylactone**

$IC_{50}$

FucT-V >200 μM
FucT-VI >200 μM
ST3O 30.2 μM
ST3N 50 μM
ST6N 8 μM



**2α-Hydroxy-strachbotrylactone**

$IC_{50}$

FucT-V >200 μM
FucT-VI >200 μM
ST3O 168.4 μM
ST3N 110.5 μM
ST6N 18.5 μM

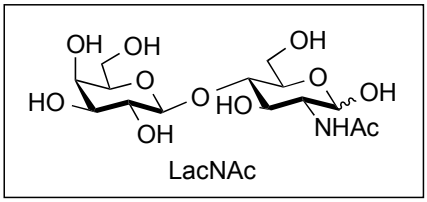



**Fig. 15** Strachbotrydial-based inhibitors of fucosyl- and sialyltransferases, and the natural acceptor substrate LacNAc.

**(7) Miscellaneous natural products and their derivatives**

The panosialins are naturally occuring alkyl benzene sulfates, which have been shown to inhibit two fucosyltransferases, FucT-VI and FucT-VII.[77] While both panosialins A and B inhibited FucT-VII more potently than FucT-VI, the mode of inhibition of the panosialins has not been determined (Fig. 16). Although alkyl benzene sulfates are well known detergents, it is thought that the inhibitory effect of the panosialins towards FucTs is not merely the result of their detergent properties, as the activity of the enzyme was not affected by the presence of the surfactants Triton X-100 or Tween 20.[77]

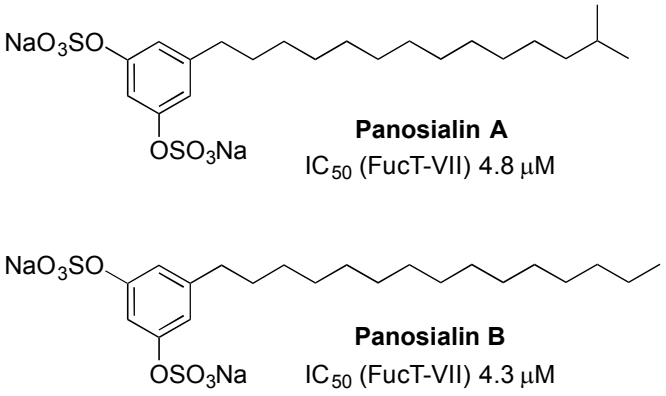
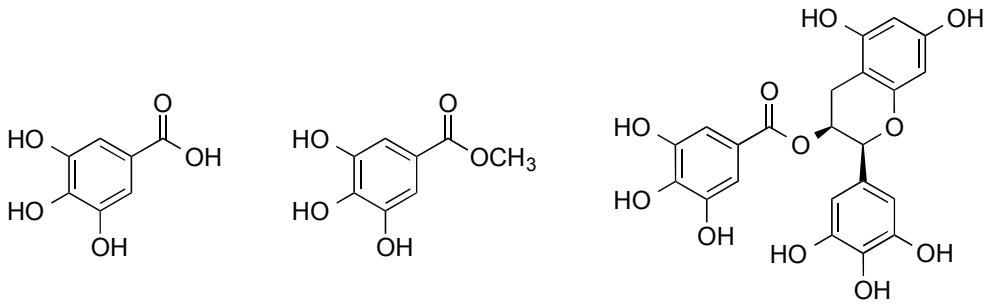



**Fig. 16** The panosialins are inhibitors of fucosyltransferases.

Gallic acid and a number of structurally related compounds were also tested against FucT-VII and found to have a range of inhibitory activity.[78] The strongest inhibitors included gallic acid itself, methyl gallate and (-)-epigallocatechin gallate (EGCG, Fig. 17). Further investigations indicated that gallic acid and EGCG also inhibit FucT-IV and α-2,3-(*N*)-sialyltransferase (ST) in the low micromolar range.[78] The gallate moiety itself (3,4,5-trihydroxybenzoate) appears to be important for activity and it was proposed that both GA and EGCG are likely able to bind to the active site, facilitated by the presence of $Mn^{2+}$.[78] This may potentially enable the cross-linking to susceptible amino acid residues nearby, although this hypothesis remains unsubstantiated.



**Gallic acid**

$IC_{50}$

FucT-VII 0.06 μM
FucT-IV 14 μM
ST 19 μM

**Methyl gallate**

$IC_{50}$ (FucT-VII) 8 μM

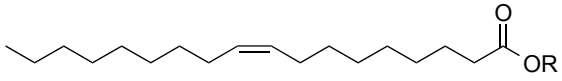
**EGCG**

$IC_{50}$

FucT-VII 0.7 μM
FucT-IV 1.8 μM
ST3N 2.7 μM

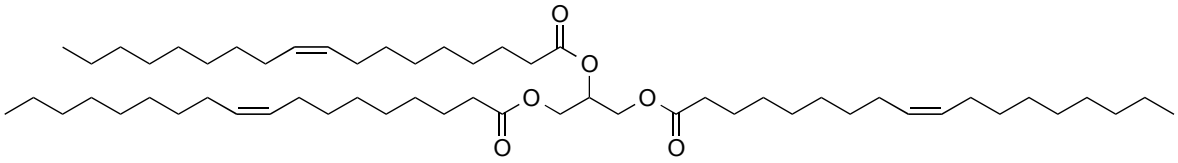
**Fig. 17** Gallic acid derivatives inhibit fucosyl- and sialyltransferases.

Sucrose 6-glucosyltransferase is expressed by bacteria, which are significantly involved in tooth decay. In attempts to develop an agent that could be used as a food additive or medicine to safely treat decaying teeth, Won et al. have investigated a series of fatty acids as potential inhibitors of sucrose 6-glucosyltransferase.[79] Of the fatty acids examined, it was found that oleic acid exhibited the best inhibitory activity.[79] Comparison with oleic acid methyl ester and triolein indicated that the carboxyl group of the fatty acid plays an important role in the glucosyltransferase inhibitory activity (Fig. 18).[79] No mechanism of action has been proposed thus far for oleic acid and its derivatives.




**Oleic acid (R = H)**
42.4% inhibition at 50 μM

**Oleic acid methyl ester (R = $CH_3$)**
16.8% inhibition at 50 μM

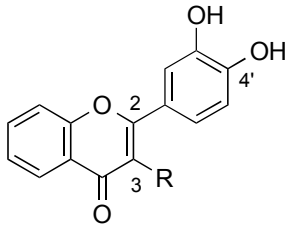



**Triolein**
26.3% inhibition at 50 μM

**Fig. 18** Inhibitors of sucrose 6-glucosyltransferase based on oleic acid.

A variety of flavones, flavonols and flavanones have been tested against human (ST6Gal I) and rat (ST6Gal I and ST3 Gal III) sialyltransferases.[80] Whilst the flavonols and flavanones were inactive, the two best flavones share several structural similarities (Fig. 19). The key C2-C3 double bond was required for activity as without it, no inhibition was observed, while sialyltransferase inhibition increased markedly with increasing number of hydroxyl groups on the phenyl ring.[80] It has been suggested that these observations indicate that hydrophilicity in this region of the molecule is crucial for

activity.[80] However, introduction of glucose into position 4 of the phenyl ring abolished activity, implying that larger substituents at this position are not tolerated, regardless of the hydrophilicity. Early investigations by Hidari et al. into the kinetic properties of the flavanoids strongly suggest interactions with two regions of the catalytic domain, which are known, respectively, as the sialyl L and sialyl S motif.[80] These motifs are conserved, to a varying degree, amongst most sialyltransferases. The authors suggest, however, that there may also be interactions with other amino acid residues, not directly related to the sialyl motifs.[80] These "remote" interactions may, in turn, lead to inhibition via the induction of conformational changes in the catalytic domain.




| R = H | R = OH |
|---|---|
| $IC_{50}$ | $IC_{50}$ |
| rST6 5.1 μM | rST6 1.9 μM |
| hST6 7.1 μM | hST6 7.5 μM |
| rST3 1.4 μM | |

**Fig. 19** Flavonoid inhibitors of human and rat sialyltransferases.

The natural product tunicamycin, a mixture of homologous nucleoside antibiotics, (Fig. 20), was one of the first chemical tools for the inhibition of N-glycosylation.[81,82] It has been shown over the years that tunicamycin interacts with a wide range of different molecular targets. In eukaryotes, tunicamycin blocks, for example, GlcNAc-1-phosphotransferase, the enzyme that catalyses the transfer of GlcNAc-1-phosphate from UDP-GlcNAc to dolichyl phosphate, the first step in the biosynthesis of the N-glycan precursor. Early studies indicated that over a wide range of concentrations,

tunicamycin is a non-competitive inhibitor of GlcNAc-1-phosphotransferase with respect to both the UDP-GlcNAc donor, and the dolichyl phosphate acceptor, and its inhibitory activity is unaffected by the addition of exogenous phospholipid.[81] Tunicamycin has also been shown to inhibit the glycosyltransferase oligosaccharyl transferase (OST) and thus the transfer of a 14-residue oligosaccharide core unit ($Glc_3Man_9GlcNAc_2$) from dolichol pyrophosphate to an asparagine moiety.[83]

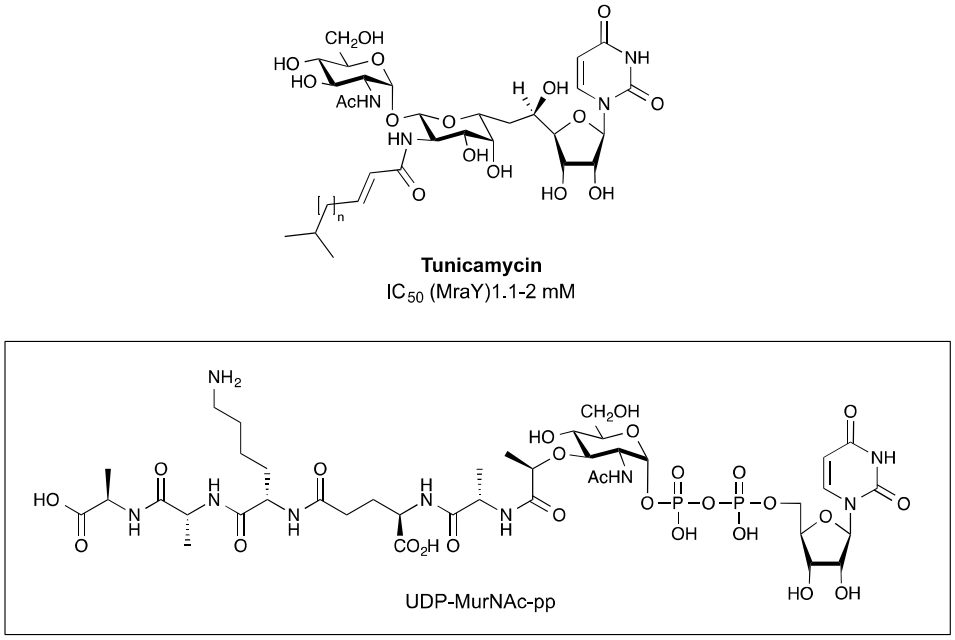



**Fig. 20** Tunicamycin and UDP-MurNAc-pp, the natural donor substrate of MraY.

Tunicamycin has also shown activity in non-eukaryotes. The natural product inhibits, for example, the incorporation of glycans into hepatitis C virus glycoproteins E1 and E2,[84] by blocking *N*-glycosylation of the translated peptide at a very early stage, without inhibiting core protein expression. Tunicamycin is also a known inhibitor of the bacterial enzyme MraY, with a low $IC_{50}$ of just 1.1 μM in a solid-phase extraction assay,[85] and 2 μM in a fluorescence-based assay.[86] This activity is perhaps not surprising, given the close structural similarity of tunicamycin to UDP-MurNAc-pp, the natural donor substrate

of MraY (Fig. 20). Unfortunately, tunicamycin is toxic at high concentrations and significantly decreases eukaryotic cell viability,[84,87] which can limit its practical application as a pharmacological tool. It has recently been established that tunicamycin is taken up into eukaryotic cells primarily through the major facilitator domain containing 2A (MFSD2A) transporter, and that this transporter is a critical determinant of tunicamycin toxicity.[87] Given the widespread use of tunicamycin as a chemical inhibitor of N-glycosylation, optimised tunicamycin derivatives with reduced toxicity would be highly desirable.

The reserve antibiotic vancomycin has been shown to act as an inhibitor both in a MurG assay, and in a MraY/MurG combination assay (Fig. 21).[88] Ravishankar et al. report that the inhibitory activity of vancomycin is suppressed by the substrate used in the assay (UDP-MurNac-pp) as it binds to vancomycin and neutralizes the inhibitory effect.[88] Vancomycin inhibits MurG by binding to the terminal region of the stem peptide.[88]

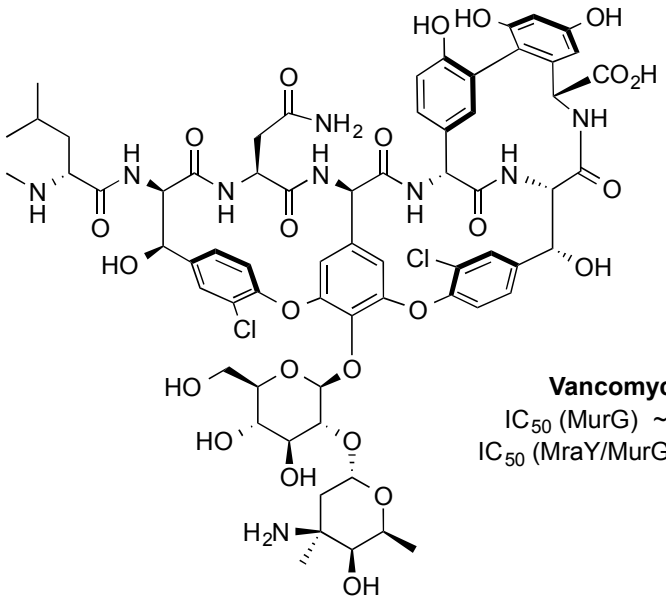
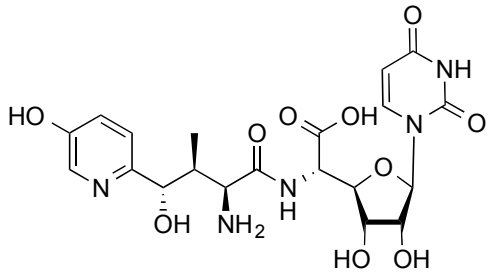



**Fig. 21** Vancomycin is active against MurG and MraY.

*Candida albicans* is one of the most prevalent fungal causing disease in patients weakened by cancer, immunosuppressive therapy, AIDS and other debilitating diseases.[89] Nikkomycins are potent inhibitors of the fungal GlcNAc-transferase chitin synthase (Fig. 22).[90] McCarthy et al. describe the inhibitory effect of nikkomycin as a result of competition with natural substrates such as UDP-GlcNAc (Fig. 25), owing to their structural similarity.[90] Chitin synthase occurs exclusively in arthropods and fungi, which makes it an attractive target for the development of new anti-fungals. In an early study carried out by Becker et al., mice infected with *C. albicans* died after 8 days, whilst mice treated with 200 µg nikkomycin per day for 20 days survived for 23 days.[89]

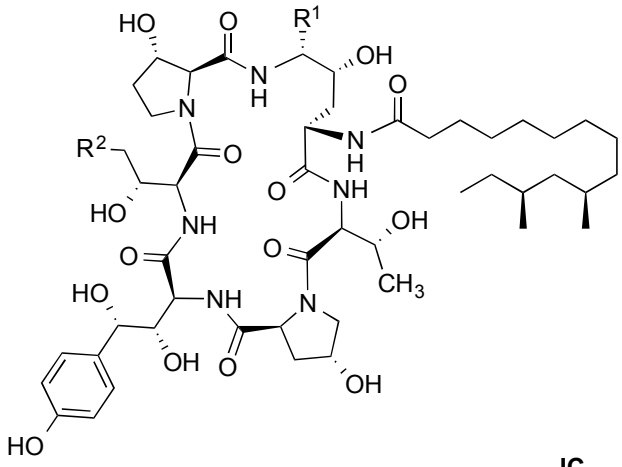



**Nikkomycin**
$K_i$ (chitin synthase) 0.16 µM

**Fig. 22** Nikkomycins are potent inhibitors of chitin synthase.

Another class of natural products that have received attention as potential treatments for fungal infections are the echinocandins, a family of macrocyclic lipopeptides exemplified by pneumocandin $B_O$ (Fig. 23). Pneumocandin $B_0$ showed nanomolar inhibition against a membrane preparation of the *C. albicans* β-(1,3)-D-glucan synthase as well as in vitro anti-*Candida* activity.[91] Structural modifications around the macrocyclic scaffold increased bioactivity even further and led to the discovery of L-733,560, which is around 70-fold more potent against β-(1,3)-D-glucan synthase than pneumocandin $B_0$.[91] Importantly, this strategy also improved the water solubility of these inhibitors,[92] which

greatly facilitated their development into clinically useful anti-fungal agents. Amongst many candidate molecules, caspofungin (L-743,872, MK-0991), a close analogue of L-733,560, ultimately emerged as the most promising one (Fig. 23). Caspofungin can be prepared semi-synthetically from pneumocandin $B_0$,[93] which in turn is a fermentation product of *Glarea lozoyensis*. Caspofungin is active against *Candida albicans* as well as other medically relevant *Candida* species,[91] although it is less effective against some other pathogenic fungi, e.g. *Cryptococcus neoformans*.[94] Despite this limitation, and its lack of oral availability, caspofungin has become an important weapon in our arsenal of anti-fungals, and it is now in clinical use for the treatment of *Aspergillus* and *Candida* infections.[95]



| | $R^1$ | $R^2$ | $IC_{50}$ β-1,3-glucan synthase |
|---|---|---|---|
| **Pneumocandin $B_0$** | OH | $CONH_2$ | 70 nM |
| **L-733,560** | $OCH_2CH_2NH_2$ | $CH_2NH_2$ | 1 nM |
| **Caspofungin** | $NCH_2CH_2NH_2$ | $CH_2NH_2$ | |

**Fig. 23** Echinocandins are inhibitors of the fungal β-1,3-glucan synthase.

## (8) Synthetic heterocycles

### (a) Thiazolidinones

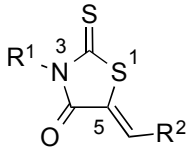
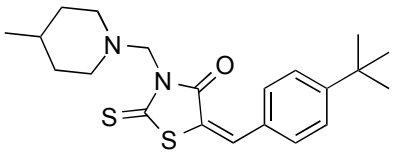



**Fig. 24** The general structure of thiazolidinones discussed in the text.

Thiazolidinones (Fig. 24) have been identified in various screens as inhibitors for different glycosyltransferases, including the bacterial GlcNAc-transferase MurG,[96,97] and the fungal enzyme Dol-*P*-Man:protein mannosyltransferase (PMT1).[98] There is evidence that at least in some cases, these thiazolidinones can bind at the donor binding site of GTs, and their inhibitory activity has been attributed to their ability to mimic the pyrophosphate fragment of the donor sugar-nucleotide.[97,99] For example, thiazolidinone **3** (Fig. 25) has been described as active against MurG, with several other molecules with the thiazolidinone core exhibiting greater than 50% inhibition of MurG at 2.5 μg/mL.[96,97] MurG catalyses the transfer of GlcNAc from UDP-GlcNAc (Fig. 25) to Lipid I, an *N*-acetylmuramic acid derivative.[100] Molecular docking of inhibitors of this scaffold into the UDP-GlcNAc binding pocket of MurG suggested that the five-membered ring is placed in, or near to, the diphosphate binding site, the N-1 substituent is pointed towards the GlcNAc binding pocket and the arylidene substituent placed in the uridine binding site.[96]




**cmpd 3**

$IC_{50}$ = 1.4 μM

$K_i$ = 1.3 μM

88% MurG inhibition at 2.5 μg/mL

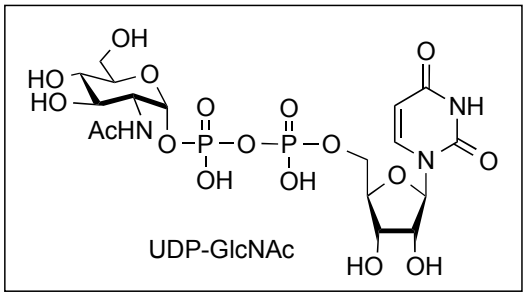



**Fig. 25** A representative thiazolidinone inhibitor of MurG, and UDP-GlcNAc, the natural donor substrate of MurG.

Thiazolidinones have also been shown to inhibit or bind to other GTs.[101,102] For example, thiazolidinones of the core structure **4** have been identified as inhibitors of the dolicholphosphate mannose synthase (DPMS) from *T. brucei* (Table 1),[101] a validated anti-trypanosomal drug target. The mannosyltransferase DPMS, which catalyses the synthesis of dolicholphosphate mannose (Dol-P-Man) from GDP-mannose (GDP-Man, Fig. 26) and dolichol phosphate, is a key enzyme for the biosynthesis of GPI anchors in *T. brucei*. Structurally related thiazolidinones have also been shown to bind in a donor-competitive manner to three different galactosyltransferases.[102] Inclusion of an aromatic moiety on the thiazolidinone nitrogen imparted some selectivity between α-1,3-galactosyltransferase, LgtC and GTB.[102]

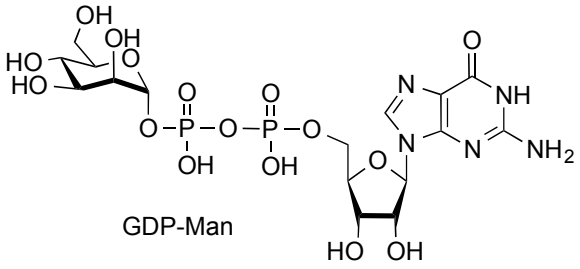
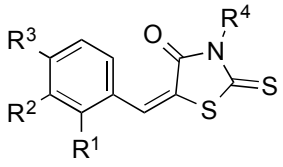



**Fig. 26** GDP-Mannose (GDP-Man), the natural donor substrate of dolicholphosphate mannose synthase (DPMS).

**Table 1** Thiazolidinone inhibitors of DPMS.



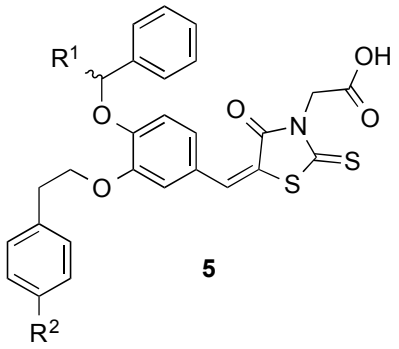
**4**

| $R^1$ | $R^2$ | $R^3$ | $R^4$ | % DPMS inhibition at 1 mM |
|---|---|---|---|---|
| H | BnO | H | $CH_2CO_2H$ | 77 |

| | | | | |
|---|---|---|---|---|
| H | BnO | BnO | $CH_2CO_2H$ | 80 |
| OH | H | H | $CH_2CO_2H$ | 77 |

Another mannosyltransferase, the fungal enzyme PMT1, has been investigated by Orchard and co-workers.[98] Their findings indicate that thiazolidinones decorated with additional substituents can show significant inhibitory activity, with $IC_{50}$ values as low as 0.17 μM (Table 2). PMTs are protein mannosyl transferases responsible for the transfer of mannose residues from dolicholphospho-β-D-mannose (DPM) onto serine or threonine residues with inversion of configuration to form an α-D-mannosyl bond.[98] A variety of substituted variants of compound **5** were synthesised, including trifluoromethoxylated phenyl rings and morpholino amides, but the compounds that showed the highest level of inhibition were much simpler.[98] A selection of the best inhibitors is given in Table 2.

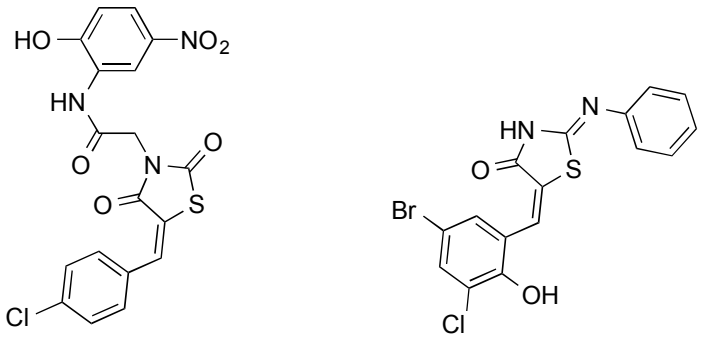
**Table 2** Thiazolidinone inhibitors of PMT1.




**5**

| **$R^1$** | **$R^2$** | **PMT1 $IC_{50}$ (μM)** |
|---|---|---|
| $CH_2OH$ | H | 0.17 |
| Me | H | 0.2 |
| H | H | 1.5 |
| H | F | 1.2 |
| H | Cl | 1.6 |

In addition to the thiazolidinones, structurally related compounds have also been identified as inhibitors of MurG by Hu et al.,[97] such as the thiazolidinediones **6** and iminothiazolidinones **7** (Fig. 27). Whilst all of these small molecules represent "drug-like" scaffolds with potential for further optimisation, thiazolidinones and related compounds are also known as frequent hitters in bioactivity screens.[103,104] Their potential target promiscuity and lack of selectivity may therefore complicate further development. This is a particularly pertinent point in an enzyme family as large as the GTs.



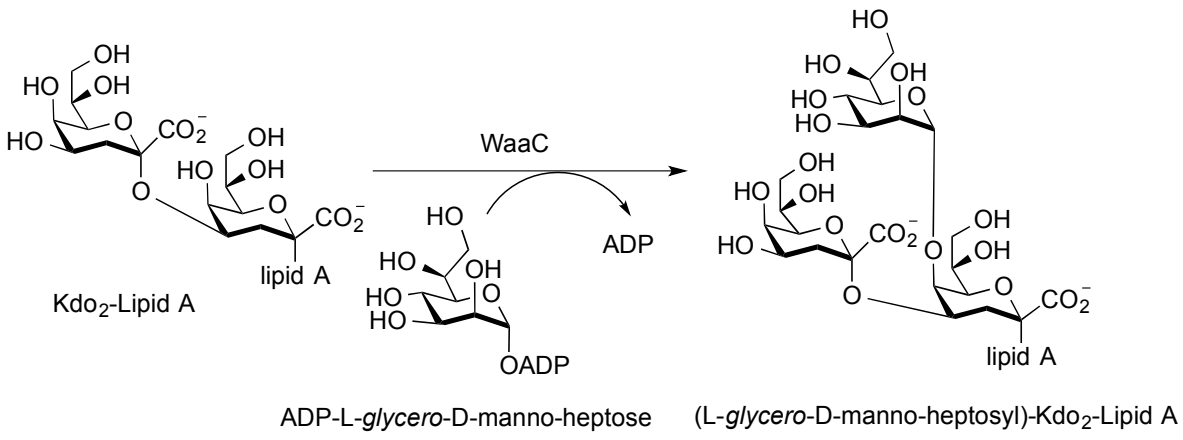

**6**
72% inhibition at 2.5 μg/mL

**7**
71% inhibition at 2.5 μg/mL

**Fig. 27** Thiazolidinedione- and iminothiazolidinone-based inhibitors of MurG.

**(b) Pyrazolones**

Drug-like inhibitors against WaaC, a bacterial heptosyltransferase, have recently been identified from screening.[105] In Gram-negative bacteria, WaaC, alongside WaaF and WaaQ, catalyses the sequential addition of a heptose from an ADP-L-*glycero*-D-manno-heptose donor to a 3-deoxy-D-manno-oct-2-ulosonic acid residue in the $Kdo_2$-Lipid A module (Fig. 28).[106]

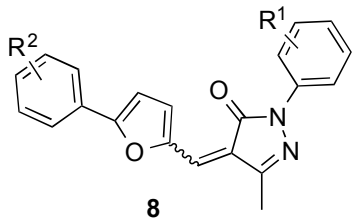



ADP-L-*glycero*-D-manno-heptose (L-*glycero*-D-manno-heptosyl)-$Kdo_2$-Lipid A

**Fig. 28** The WaaC reaction.

Structurally similar to the thiazolidinones, the aryl pyrazolones **8** designed by Moreau and co-workers[105] all contain the same 4-(5-arylfuran-2-ylidene) pyrazol-3-one core, decorated with two *meta*- or *para*-substituted phenyl substituents (Table 3). Depending on the substitution pattern, individual analogues varied considerably in their potency, with the best inhibitors showing $IC_{50}$ values against WaaC in the low micromolar range (Table 3).[105] Analysis of structure-activity relationships suggested that the aromatic group attached to the furan moiety is important for activity.[105] It appeared to act as a 'spacer' for $R^2$, leading to enhanced interaction with the protein, particularly when $R^2$ was a carboxylic acid. Mechanistic studies, in conjunction with molecular docking experiments, indicated that these inhibitors are competitive inhibitors relative to the acceptor, but not the donor substrate of WaaC (Fig. 28).

**Table 3** Pyrazol-3-one inhibitors of WaaC.

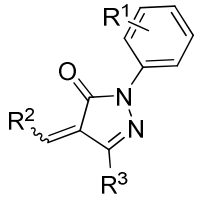


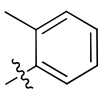
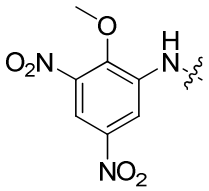
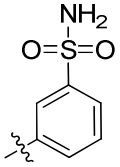
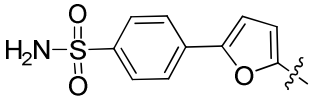
**8**

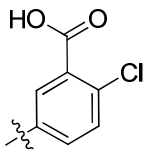
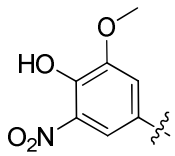
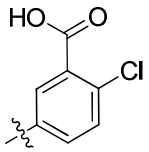
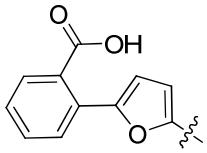
| $R^1$ | $R^2$ | WaaC $IC_{50}$ (μM) |
|---|---|---|
| *m*-$CO_2H$ | *m*-$CO_2H$ | 2 |
| *m*-$CO_2H$ | *m*-$CO_2H$, *p*-Cl | 1 |
| *m*-$CO_2H$ | *p*-$CO_2H$ | 3 |

From high throughput screening, some aryl pyrazolones with inhibitory activity against MurG have also been discovered (Table 4).[97] They were identified *via* their ability to displace a fluorescently labelled derivative of the native glycosyl donor UDP-GlcNAc (Fig. 25). Recently, structurally related aryl pyrazol-3-ones were found to inhibit another bacterial GT, the galactosyltransferase LgtC.[107] In Gram negative pathogens such as *Haemophilus influenzae*, LgtC[108] is a key enzyme for the expression of the digalactoside motif in the bacterial lipooligosaccharide coat, which significantly increases serum resistance.[10] LgtC inhibitors are therefore of interest as a new class of anti-virulence agents.

**Table 4** Pyrazol-3-one inhibitors of MurG.



| $R^1$ | $R^2$ | $R^3$ | % MurG inhibiton[a] |
|---|---|---|---|
|  |  | $CH_3$ | 66 |
|  |  | $CF_3$ | 66 |

| | | | |
|---|---|---|---|
|  |  | $CH_3$ | 54 |
|  |  | $CH_3$ | 48 |

[a]measured at 2.5 μg/mL

**(c) Uracil and uric acid derivatives**

Uracil (Fig. 29) is a recurring structural motif in a number of GT inhibitors, from complex natural products such as tunicamycin and nikkomycin (discussed in section 7) to much simpler inhibitor structures. Uracil-based inhibitors have been reported against various GTs, including OGT, an *O*-GlcNAc transferase which catalyses the transfer of *N*-acetylglucosamine (GlcNAc) from UDP-GlcNAc to serine or threonine residues on many nuclear and cytosolic proteins. *O*-GlcNAc-ylation is emerging as a signalling event comparable to protein phosphorylation in its importance for many fundamental cellular processes, from transcription and translation to nuclear transport and stress responses.[109,110] The identification of potent, specific and cellularly active OGT inhibitors would therefore provide valuable tools for the investigation of the many complex roles of this enzyme within the cell. In 2002, the uracil analogue alloxan (Fig. 29) was shown to completely block O-GlcNAcylation, possibly due to inhibition of OGT.[111] This was further corroborated when recombinant OGT was incubated with varying concentrations of alloxan, demonstrating an $IC_{50}$ of 0.1 mM.[111] This was the first OGT inhibitor described, and it was proposed that alloxan may act through interaction with the UDP-binding domain in OGT or, alternatively, by covalent modification of cysteine residues.[111] Unfortunately, the applicability of alloxan as a pharmacological tool is limited by its significant cell toxicity, especially against pancreatic cell lines.[112] Indeed, alloxan is

commonly used as an inducer in experimental animal models of diabetes due to this toxicity. This is particularly pertinent as OGT is a potential target in diabetes, and the identification of alternative, non-toxic OGT inhibitors is therefore of considerable interest.

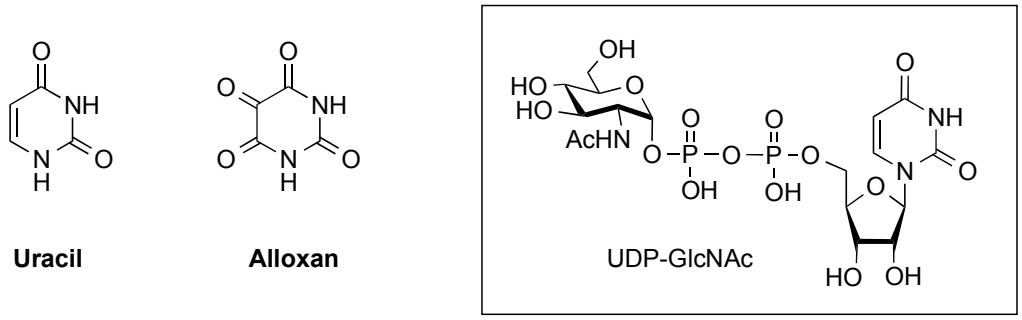



**Fig. 29** Alloxan was the first OGT inhibitor. The structures of uracil and of the OGT donor UDP-GlcNAc are shown for direct comparison.

Although itself not strictly a glycosyltransferases, the bacterial translocase MraY is closely associated with bacterial GTs as part of the biosynthetic machinery that is required for peptidoglycan biosynthesis. One recent study directed solely at MraY found inhibitors based on a uridine-triazole scaffold (Fig. 30).[113] The inhibitors, which were identified via "Click" chemistry, exhibited micromolar $IC_{50}$ values.

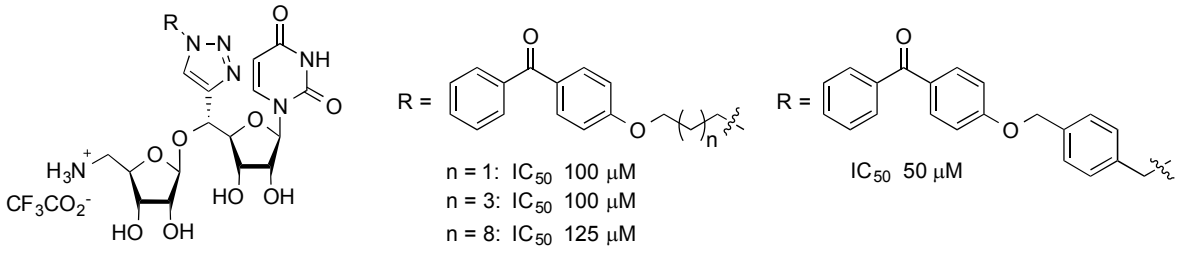
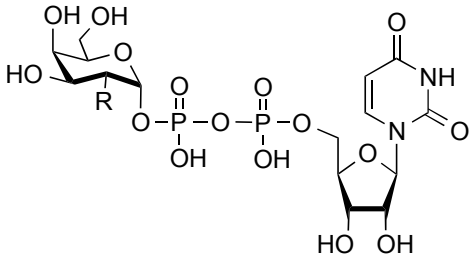



**Fig. 30** Uridine-based inhibitors of MraY, obtained from "Click" chemistry.

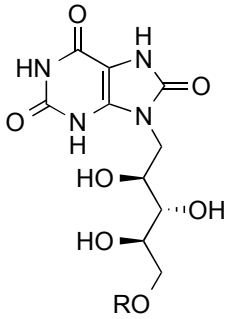
Schaefer et al. have shown that derivatives of uric acid are inhibitors of the human blood group B galactosyltransferase GTB (Table 5).[114] Inhibitors **9** and **10** were designed by molecular docking, and it is thought that their inhibitory activity arises from the ability of the pentitol-linked uric acid to mimic the uracil, the ribofuranoside and the pyrophosphate group of UDP-Gal, the natural donor substrate of GTB (Fig. 31).[115] Both **9** and **10** have shown activity, albeit at high micromolar concentrations, against GTB in the presence of the enzyme co-factor $Mg^{2+}$.[115] In order to confer specificity onto the uric acid derivatives, compound **10** was developed further by the addition of an α-D galactose at the terminal hydroxyl of the pentitol. The resulting galactose-modified analogue **11** showed selectivity for GTB over GTA, a closely related blood group enzyme, which transfers N-acetyl galactose (GalNAc) from a UDP-GalNAc donor. Compounds **9** and **10**, which lack the galactose motif, showed no such selectivity, bearing out the original design concept (Table 5).[114]



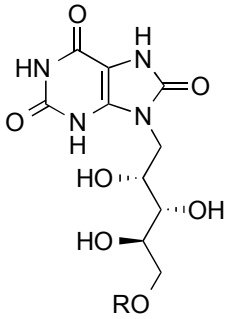

UDP-Gal (R = OH)
UDP-GalNAc (R = NHAc)

**Fig. 31** UDP-Gal and UDP-GalNAc, the natural donor substrates of the human blood group glycosyltransferases GTB and GTA.

**Table 5** Uric acid derivatives as inhibitors of GTA and GTB.



**9** (R = H)

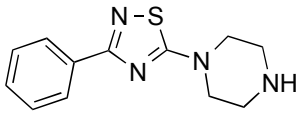


**10** (R = H)
**11** (R = galactose)

| Compound | GTA $K_i$ (μM) | GTB $K_i$ (μM) |
|---|---|---|
| **8** | 712 | 361 |
| **9** | 1100 | 571 |
| **10** | no inhibition | 565 |

### (d) Miscellaneous

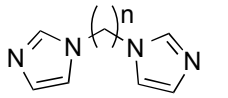
A structurally unrelated 1,2,4-thiadiazole (Fig. 32) has also been reported as a selective inhibitor for GTB versus a range of other galactosyltransferases. First identified from an STD NMR study on small molecule fragments,[35] subsequent structural studies revealed a previously unknown binding mode for this inhibitor.[34] Thus, this thiadiazole interferes with both the disaccharide acceptor and the UDP-gal donor binding sites, whilst displacing the $Mn^{2+}$ ion that is essential for GTB function.[34]



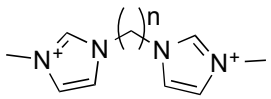
| enzyme | $K_i$ |
|---|---|
| GTB | 0.8 mM |
| GTA | 5.1 mM |
| AAGlyB | >15.0 mM |
| α-1,3-GalT | 6.3 mM |
| α-1,4-GalT | no inhibition |
| β-1,4-GalT | no inhibition |

**Fig. 32** A 1,2,4-thiadiazole inhibitor of GTB shows weaker activity against other glycosyltransferases.

Positively charged, bivalent imidazolium salts have been described as another interesting new class of GT inhibitors.[116] In a recent paper, Gao et al. report that when connected by a long alkyl chain ($C_{20}$-$C_{22}$), two imidazolium units become inhibitors of a range of galactosyltransferases.[116] Intriguingly, neither the charge-neutral versions of the molecules, nor the shorter alkyl chain analogues show any activity against the galactosyltransferases tested (Fig. 33). The three active compounds were tested against eight other tranferases, exhibiting a range of activites from complete inhibition of Human core 2 β-1,6-N-acetylglucosaminyl transferase 1 (C2GnT1) to a complete lack of inhibition of Human core 2 β-1,6-N-acetylglucosaminyl transferase 2 (C2GnT2).[116]



n = 4 to 20:
no activity

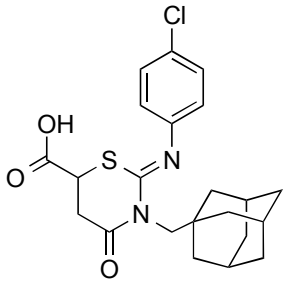


n = 4 to 18:
no activity

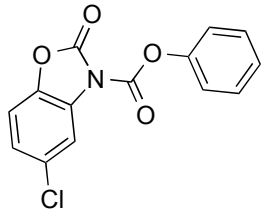
| | inhibition at 500 μM: β**3-GalT5** | β**4-GalT1** |
|---|---|---|
| n = 20 (chloride) | 84% | 54% |
| n = 20 (mesylate) | 79% | 41% |
| n = 22 (mesylate) | 95% | 78% |

**Fig. 33** Imidazolium inhibitors of galactosyltransferases

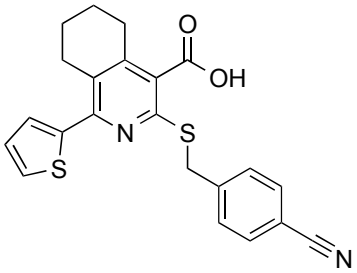
Structurally diverse heterocycles, usually containing a combination of sulphur, nitrogen or oxygen have also been identified as inhibitors of OGT.[30,117] The Walker research group have made significant headway in discovering novel inhibitors for OGT (Fig. 34), several of which showed no activity against MurG, which has been proposed as structurally similar.[118] Importantly, one of these inhibitors decreased the expression of the transcription factor FoxM1 in MCF-10A-ErbB2 cells and blocked breast cancer growth and invasion in cellular assays.[119] These impressive results demonstrate the great value small molecular GT inhibitors can have as chemical tools compounds for biological studies.



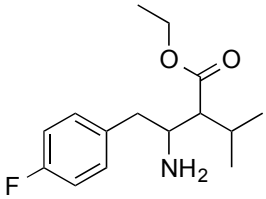
$IC_{50}$ (sOGT) 30 μM
$IC_{50}$ (ncOGT) 53 μM



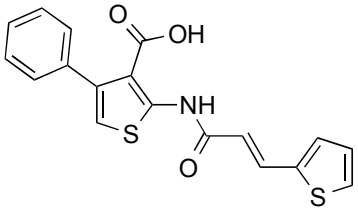
$IC_{50}$ (sOGT) 27 μM
$IC_{50}$ (ncOGT) 10 μM



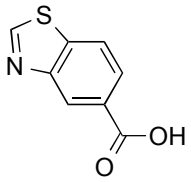
$IC_{50}$ (sOGT) 60 μM
$IC_{50}$ (ncOGT) ~100-150 μM



>30% inhibition



>30% inhibition



>30% inhibition

**Fig. 34** Heterocyclic OGT inhibitors.

The Walker group have also disclosed a new OGT inhibitor that mimics the diphosphate moiety of the endogenous ligand, UDP-GlcNAc, *via* a carbamate (Fig. 35).[120] The two carbonyls of the carbamate lead to complete inhibition of the enzyme by cross-linking a lysine and a cysteine residue in the active site.[120]

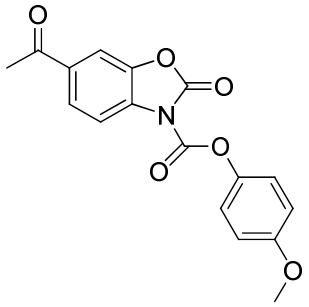
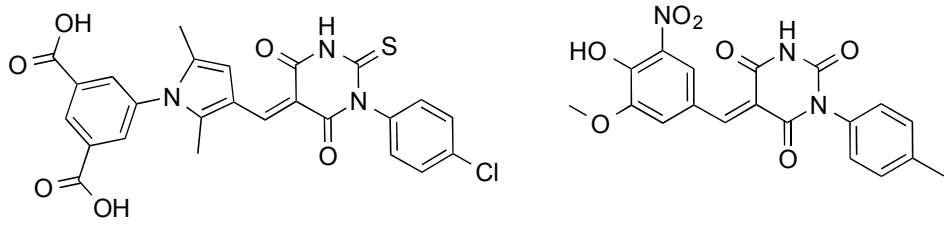


**Fig. 35** A cross-linking OGT inhibitor.

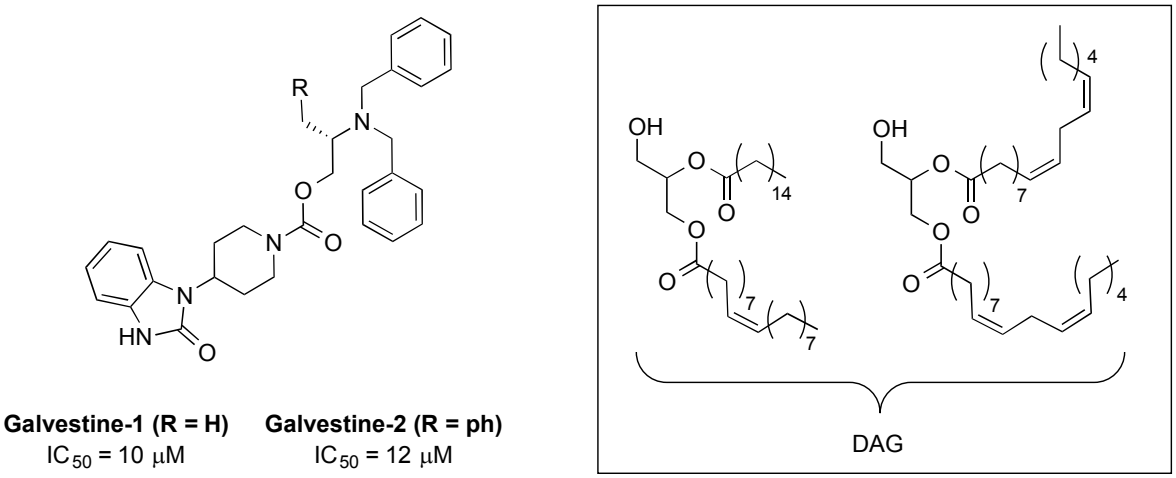
In addition to thiazolidinones and aryl pyrazolones, Hu et al. have also identified structurally more diverse pyrimidinetriones and thioxopyrimidinediones with inhibitory activity against MurG from their donor displacement assays.[97] Two of the structures that exhibited the highest inhibition are shown, whilst a variety of aryl-focussed variations showed inhibition above 45% at 2.5 μg/mL (Fig. 36).[97]




88% inhibition at 2.5 μg/mL 72% inhibition at 2.5 μg/mL

**Fig. 36** Pyrimidinetrione and thioxopyrimidinedione inhibitors of MurG.

Monogalactosyldiacylglycerol (MGDG) is one of the most abundant lipids of photosynthetic membranes in the chloroplasts of algae and plant cells. The biosynthesis of MGDG requires the galactosylation of diacylglycerol (DAG) by MGDG synthases, galactosyltransferases which use UDP-Gal as their donor. Botté et al. have disclosed the inhibitory activity of galvestines against these MGDG synthases (Fig. 37).[121] Through the study of a variety of synthetic analogues, they have ascertained that the central scaffold is critical for interaction with the enzyme, but only when a hydrophobic tertiary amine moiety is present. Using computational modelling, superposition of galvestine-1 with DAG showed that the scaffold can mimic the glycerol backbone of DAG and that oxygen atoms in galvestine-1 can be positioned with a geometry similar to that of oxygen atoms in DAG.[121] Galvestine-1 has also recently been used as a probe to identify candidate genes involved in MGDG synthesis in plant cells.[122]




**Galvestine-1 (R = H)** $IC_{50}$ = 10 μM

**Galvestine-2 (R = ph)** $IC_{50}$ = 12 μM

**Fig. 37** Galvestines are inhibitors of MGDG synthase. The natural diacylglycerol (DAG) acceptors of MGDG synthase are shown for comparison.

## Conclusion

Compared to other enzyme classes of similar size and biological and medical significance such as the protein kinases, glycosyltransferases (GTs) remain underexploited as a target class for therapeutics development. In part, this is due to the relative lack of small molecular inhibitors with drug-like properties. The recent discovery of several new, non-substrate-based inhibitor chemotypes for individual GTs is therefore a significant advance for this research area. While some of these chemotypes, such as the thiazolidinones, have a reputation as frequent hitters,[103] several others provide suitable starting points for medicinal chemistry and drug discovery efforts. In order to realise the potential of these new GT inhibitors, both as chemical tools for glycobiology and as potential lead compounds for drug discovery, several issues need to be addressed now:

(i) Cellular activity – for many of the new inhibitors only inhibitory data against recombinant or isolate enzymes has been reported to date. It will be an essential next step in these cases to assess if this in vitro activity translates into activity in relevant cellular and functional assays.

(ii) Target selectivity – the selectivity profile of individual inhibitors needs to be established against a panel of different GTs. This is particularly important in view of the close mechanistic and structural similarities in this enzyme family. While the development of inhibitors with selectivity for an individual target GT may be the ultimate goal, the identification of chemotypes with broad activity against different GTs will also be highly valuable. These chemotypes may represent privileged scaffolds against this enzyme class, whose activity can be tailored to individual GTs through appropriate structural modification. Such privileged scaffolds could

therefore also be exploited as templates for inhibitor design against GTs where no small molecular inhibitors exist to date.

(iii) Mode of action – a detailed understanding of the binding mode and mode of action of non-substrate like inhibitors, which at the moment are not always known, will be crucial. This will allow the rational optimisation of their potency and target selectivity. Structural studies with the new inhibitors, using e.g. crystallography or NMR approaches, will therefore be of great importance.

Addressing these questions, as well as the identification of further GT inhibitor chemotypes, should therefore be regarded as a priority for the field of GT inhibitor discovery. There can be little doubt that the availability of potent, selective and cellularly active GT inhibitors would create many new and exciting opportunities for glycobiology, medicinal chemistry and drug discovery. Establishing comprehensive biological profiles (target selectivity, cellular activity etc.) of at least some of the non-substrate-based GT inhibitors that have recently been described is a critical next step towards this goal. If further progress can be made in this direction, there is every possibility that the perception of glycosyltransferases as “difficult” targets for drug discovery will change.

**Acknowledgement**

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