Exploring the Potential of Chemical Inhibitors for Targeting Post-translational Glycosylation of Coronavirus (SARS-CoV-2)

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Cite This: ACS Omega 2022, 7, 27038−27051

ABSTRACT: The Spike (S) protein of SARS-CoV-2 expressed on the viral cell surface is of particular importance as it facilitates viral entry into the host cells. The S protein is heavily glycosylated with 22 N-glycosylation sites and a few N-glycosylation sites. During the viral surface protein synthesis via the host ribosomal machinery, glycosylation is an essential step in post-translational modifications (PTMs) and consequently vital for its life cycle, structure, immune evasion, and cell infection. Interestingly, the S protein of SARS-CoV-2 and the host receptor protein, ACE2, are also extensively glycosylated and these surface glycans are critical for the viral-host cell interaction for viral entry. The glycosylation pathway of both virus (hijacked from the host biosynthetic machinery) and target cells crucially affect SARS-CoV-2 infection at different levels. For example, the glycosaminoglycans (GAGs) of host cells serve as a cofactor as they interact with the receptor-binding domain (RBD) of S-glycoprotein and play a protective role in host immune evasion via masking the viral peptide epitopes. Hence, the post-translational glycan biosynthesis, processing, and transport events could be potential targets for developing therapeutic drugs and vaccines. Especially, inhibition of the N-glycan biosynthesis pathway amplifies S protein proteolysis and, thus, blocks viral entry. The chemical inhibitors of SARS-CoV-2 glycosylation could be evaluated for Covid-19. In this review, we discuss the current status of the chemical inhibitors (both natural and synthetically designed inhibitors) of viral glycosylation for Covid-19 and provide a future perspective. It could be an important strategy in targeting the various emerging SARS-CoV-2 variants of concern (VOCs), as these inhibitors are postulated to aid in reducing the viral load as well as infectivity.

1. INTRODUCTION

The coronaviruses, including the SARS-CoV-2 accountable for the ongoing global pandemic, are a family of enveloped positive-sense RNA viruses which cause acute and chronic respiratory illness in humans and animals.¹ Human coronavirus was first identified in the 1960s, and out of the seven known coronavirus species, only three are responsible for fatal respiratory diseases, i.e., SARS-CoV, MERS-CoV, and SARS-CoV-2.² The SARS-CoV caused a global pandemic in 2003, infecting thousands of people and claiming more than 700 lives across 26 countries. The MERS-CoV, first identified in 2012 in Saudi Arabia, affected more than 2500 people and claimed over 850 lives.³ The SARS-CoV-2, responsible for the new coronavirus disease (Covid-19), was reported in December 2019. The SARS-CoV-2 virus, with around 80% similarity with SARS-CoV-1 and 50% with MERS-CoV,⁴ has infected over 569 million people and claimed about 6.39 million deaths as of July 2022.

The genome of SARS-CoV-2 encodes 28 proteins, out of which three are known to be glycosylated, i.e., the Spike (S) glycoprotein, Membrane (M) protein, and Envelope (E) protein.⁵ Several proteins undergo post-translational modifications before executing their functions. These modifications are required for the development of their functionality, folding, stability, and interaction with cellular proteins; for example, the glycosylation of S and E proteins is critical for proper protein folding and viral infectivity.¹ The modifications occur in the endoplasmic reticulum (ER) or Golgi complex via proteolytic cleavage, disulfide bond formation, methylation, acetylation, and addition of functional groups such as lipidation (palmitoylation, myristoylation), phosphorylation, and glycosylation.⁶ SARS-CoV-2 relies on this machinery for entry, replication, immune evasion, pathogenesis, and attachment. N-Linked and O-linked glycosylation have prominent roles in the pathogenesis of the virus.¹ In N-linked glycosylation, the glycans attach to the amide nitrogen of asparagine residue, followed by trimming and

Received: April 14, 2022
Accepted: July 8, 2022
Published: July 28, 2022
remodeling of the oligosaccharides in the ER and Golgi complex resulting in the formation of glycoproteins.\textsuperscript{7}

The newly formed glycoprotein undergoes several modifications or “processing” in the ER and then it is transported through the Golgi complex to its final destination.\textsuperscript{6} Two ER membrane-bound glucosidases, i.e., glucosidase I and II, catalyze the initial processing reactions involving the removal of three glucose residues. Glucosidase I cleaves the outermost $\alpha$-1,2-linked glucose residue, and glucosidase II removes the remaining two $\alpha$-1,3-linked glucose. In the Golgi complex, the resulting glycoprotein may be acted upon by mannosidase I, which facilitates cleavage of remaining $\alpha$-1,2-linked mannose residues and mannosidase II catalyzes the removal of $\alpha$-1,3- and $\alpha$-1,6-linked mannose residues giving rise to the substrate that are acted upon by glycosyltransferases, forming the complex glycoproteins.\textsuperscript{9} Other than the viral glycosylated proteins, several viral cell receptors are also glycosylated. Thus, the ER glucosidase inhibition can disrupt the biosynthesis of both viral receptors and viral glycoproteins.\textsuperscript{1}

\section*{2. GLYCOSYLATED SARS COV-2 PROTEINS}

The surface $S$ glycoprotein of SARS-CoV-2 mediates entry into the host cells via the host’s angiotensin-converting enzyme 2 (ACE2) receptor.\textsuperscript{10} The S-protein comprises two functional subunits, S1 and S2. The S1 subunit possesses a receptor-binding domain (RBD) and is responsible for the initial binding of the virus with the peptide domain of ACE2, whereas the S2 subunit is responsible for fusion of the membrane that triggers the viral glycoproteins, giving rise to the substrate that are acted upon by glycosyltransferases, forming the complex glycoproteins.\textsuperscript{9} Other than the viral glycosylated proteins, several viral cell receptors are also glycosylated. The ER glucosidase inhibition can disrupt the biosynthesis of both viral receptors and viral glycoproteins.\textsuperscript{1}

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Besides, the proximal glycosylation sites, N282, N331, and N343, are found to shield the RBD in its “down” state.\textsuperscript{11,15} The O-glycosites (T323 and S325) in the RBD are conserved and may have an important role in the mediation of S-ACE2 binding, but this is not much explored. It was found that out of six mutations reported in the RBD of the S protein (compared with that of SARS-CoV), one is S494, and attachment of O-glycans to S494 could increase the binding affinity to ACE2.\textsuperscript{12} The entry, infectivity, and severity of SARS-CoV-2 varies from individual to individual, and this variation could be attributed to ACE2 expression and also glycosylation.\textsuperscript{13} Seven N-glycosylation and 2 O-glycosylation sites have been reported in recombinant ACE2 protein from HEK293 cells. The binding of ACE2 with S protein RBD is crucially affected by N-glycosylation at the N90, N322, and N546 residues of ACE2. The mutation in the N90 residue is found to increase the binding affinity of ACE2 to the S protein. The N322 residue is identified to bind at the core region of the RBD, and its interaction with the RBD is much stronger than that between the RBD and the N90 residue.\textsuperscript{7,16} In the S1 subunit, the glycan residues at N74 and N165 are also found to interact with the ACE2 receptor glycan residue at N546 and, hence, are involved in modulating the interaction of ACE2 with the S protein. This proposes that alterations in occupancy of glycans present on the host receptor and viral protein may alter the affinity as well as infectivity of the virus.\textsuperscript{17}

The M protein is the most profuse envelope protein in SARS-CoV-2, consisting of three N-terminal transmembrane domains, and is crucial for viral particle assembly. The glycosylation of the M protein is not broadly studied and characterized yet. The in silico studies have predicted eight N-glycosylation sites, which are N5, N21, N41, N43, N117, N121, N203, and N216.\textsuperscript{18} Though the glycosylation of the M protein is conserved in various coronaviruses, the functions of this N-linked glycosylation are yet to be explored.\textsuperscript{7,19}

The E protein is the smallest of the four major structural proteins and has a role in viral assembly, release, and pathogenesis. It consists of a short hydrophilic N-terminal domain, a hydrophobic transmembrane domain, and a long hydrophilic C-terminal region. The sequence prediction proposes two putative N-linked glycosylation sites that may exist in the transmembrane segment at N48 and N66 positions. The residue at N48 could not be glycosylated because of its proximity to the membrane. The N66 residue is found to be glycosylated with oligomannose-type glycans and its mutation could resemble the dimers and trimers of the E protein that is vital for virion assembly, whereas the monomer may function in disruption of the host secretory pathway.\textsuperscript{7,20}

\begin{figure}
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\includegraphics[width=\textwidth]{schematic_representation_of_n-glycosylation_sites_in_sars_cov-2_s-glycoprotein.png}
\caption{Schematic representation of N-glycosylation sites in SARS CoV-2 S-glycoprotein (protein backbone, light pink; SS, signal sequence; NTD, N-terminal domain; RBD, receptor-binding domain; FP, fusion peptide; HR1, heptad repeat 1; CH, central helix; CD, connector domain; HR2, heptad repeat 2; TM, transmembrane domain; CT, cytoplasmic tail). Reprinted from Lardone et al.\textsuperscript{12a} Copyright 2021 The Authors.}
\end{figure}
Like other viruses, coronaviruses also rely on the host protein synthesis machinery for replication, and the viral proteins are likewise modified by post-translational modification processes that impact viral replication and pathogenesis. Accumulating evidence suggests that given the importance of these PTMs, targeting them could be an impressive approach to inhibit viral replication, attenuate viral load, viral antigenicity, and pathogenicity. This review summarizes the chemical inhibitors targeting the glycosylation step of PTMs.

3. IMPORTANCE AND REGULATION OF GLYCOXYLATION

It is well-known that viruses have evolved to leverage host-cell machinery for their replication, translation, formation of new virions, etc. Glycosylation is also one such process where the virus steals host glycosylation machinery to hide the epitope of their crucial proteins by adorning its surface with host glycans, thus evading the host humoral immune response. Viral glycosylation is essential for viral pathogenesis as it aids in glycoprotein structure, folding, sorting, stability, and trafficking. During the glycosylation process, the glycans get structurally integrated into the protein fold, and the subsequent interactions between protein and glycan help in stabilizing the protein. N-Linked glycosylation is the commonest form of protein glycosylation. The viral structural proteins S, E, and M are translated and then undergo N- and O-glycosylation in the ER, the ERGIC (ER-Golgi intermediate compartment), and the Golgi apparatus using host glycosylation machinery. The viral glycoproteins have a retrieval signal that delays their trafficking. Hence, they get accumulated in the ER, ERGIC, and Golgi complex, followed by assembly of virus particles and subsequent budding. The viral glycoproteins are rich in high-mannose N-glycans because of incomplete maturation. In the glycosylation process, the performed N-linked glycan core (Glc₃Man₉GlcNAc₂) is attached to an asparagine residue in the sequence “asparagine-X-serine/threonine (N-X-S/T)” with the aid of oligosaccharyltransferase complex (OST) (“X” = any amino acid residue except proline). The glycosylation efficiency of N-X-S/T motifs is dependent on various factors; one such factor includes the ability of the OST complex to access sequon and sequence content. It is reported that the efficiency of glycosylation is generally higher if the target sequence has threonine in place of serine. The attached N-linked oligosaccharides are then subjected to processing that involves sequential cleavage of glucose residues attached at the terminal by ER α-glucosidases I and II. The α-glucosidases I cleave α-1,2-glucose (distal residue) to form Glc₂-Man₉GlcNAc₂, which may
undergo further processing by α-glucosidase II, which cleaves second and third glucose residues and forms Man\(_9\)GlcNAc\(_2\) (Figure 2). Before cleavage of the third glucose residue, calnexin and calreticulin bind to monoglucosidated glycan and help in protein folding and disulfide bond formation. Then the third residue is cleaved, and after cleavage, if some proteins are not adequately folded, they are transiently reglycosylated with the help of UDP glucotransferase 1. The properly folded proteins are transferred to the Golgi apparatus for further processing. To prevent the improperly folded protein from undergoing reglycosylation and reprocessing, the ER mannosidase I cleaves the mannose residues from the oligosaccharide and protein may subsequently undergo the degradation ER-associated degradation pathway.\(^{21,22}\)

4. COMPARATIVE ANALYSIS OF GLYCOSYLATION IN DIFFERENT VIRUSES

Viruses are solely dependent on host glycosylation machinery (Figure 3) for their protein glycosylation, which is reported to be critical for HIV, dengue virus (DENV), Hepatitis B virus (HBV), and SARS-CoV-2 for their infectivity, antigenicity, and stability. Glycosylation has a multifactorial role in viral pathogenesis as it is involved in receptor binding, proper protein folding, protein trafficking, masking antigenic sites from recognition by neutralizing antibodies, and most importantly in dodging immune recognition by the host immune system. An example is that HIV and DENV require a high-mannose-type glycosylation for binding to mannose receptors and DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Nonintegrin).\(^{23}\) Also, there are affirmations that enveloped viruses such as HIV and HBV are highly dependent on the post-translational processing of proteins via N-linked glycosylation in the ER and thus, their life cycle is significantly altered if it is inhibited.\(^{24}\)

The attachment of HIV-1 to host CD4 receptors is mediated by gp120 and gp41, which are heavily glycosylated envelope proteins and considered to have a role in syncytium formation and cytopathic effects of the virus. It is observed that N-glycosylation has a significant role in HIV-1 pathogenesis for its infectivity and cytopathicity. The glycosylation inhibitors, tunicamycin, castanospermine, 1-deoxymannojirimycin, and 1-deoxynojirimycin, are shown to reduce the infectivity and cytopathicity of HIV-1. They are also found to block the syncytium formation induced by HIV-1.\(^{25}\)

Three proteins of DENV, viz., prM (glycosylated precursor of membrane (M) protein), E (envelope protein), and NS1, are found to possess N-glycosylation sites and require a host glycosylation apparatus for the processing of their N-glycans. The N-glycosylation inhibitors are found to have antiviral effects on DENV by inhibiting enzymes associated with N-glycan processing.\(^{26}\) The α-glucosidase inhibitors (castanospermine and deoxynojirimycin) are reported to alter the pathways involved in the folding of envelope glycoproteins prM and E. The incomplete folding of these proteins results in the formation of an unstable complex between prM and E that ultimately leads to nonproductive pathways. Also, the blocking of α-glucosidase mediated N-linked oligosaccharide trimming at the initial stages of glycoprotein processing averts viral assembly.\(^{24}\)

The HBV contains a single N-glycosylation site at asparagine residue N146 in the antigenic loop, and this site is conserved and only partially (50%) glycosylated.\(^{27}\) The formation of the HBV is dependent on N-glycosylation and N-glycan processing by host ER glucosidases and inhibition of the same interferes with virion detachment. In a study conducted by Lambert and Prange, it was found that glucosidase inhibitors castanospermine and N-butyl-deoxynojirimycin significantly reduced the release of HBV from the host cell.\(^{28}\)

It is evident that the enveloped viruses including SARS-CoV-2 rely completely on the N-glycosylation machinery of the host to carry out their glycoprotein processing which is critical for virus pathogenesis. The host glycosylation apparatus involves various enzymes, viz., oligosyltransferase, ER-α-glucosidase I and II, and Golgi mannosidase I and II, which carry out N-glycosylation of the viral proteins. The glucosidase inhibitors like castanospermine, swainsonine, deoxynojirimycin, deoxymannojirimycin, etc. are found to inhibit these enzymes involved in glycosylation and, thus, interrupt viral protein glycosylation. Interestingly, the glycosylation inhibitors that have been tested against HIV,
DENV, and HBV could be potentially useful against SARS-CoV-2 also as it is a host-targeted approach.

5. POTENTIAL GLYCOSYLATION INHIBITORS FOR SARS-CoV-2

5.1. Natural Products as Glycosylation Inhibitors.

5.1.1. Tunicamycin. Tunicamycin is an antibiotic produced by Streptomyces clavuligerus and Streptomyces lyosuperficus bacteria and is an analogue of UDP-N-acetylgalactosamine. It has a unique structure composed of a rare C5′-C6′-linked undecosidolose core, called tunicamycin, along with uracil, d-N-acetylgalactosamine (GlcNAc) that is attached to the core via a 1,1-β,α-trehalose-type glycosidic bond, and an amide-linked fatty acid (Figure 4). Tunicamycin interferes with the glycoproteins glycosylation by inducing ER stress in cells via inhibition of N-linked glycans biosynthesis in proteins, which ultimately results in the complete absence of glycan residues. When the antibiotic blocks glycosylation of N-glycans, the cell cycle arrests at the G1 phase in human cells. It is reported that tunicamycin inhibits the formation of the E2 glycoprotein. Interestingly, it has been found that tunicamycin treatment resulted in the production of S protein-less, noninfectious virions devoid of the fully functional S protein because of tunicamycin driven trimming of the mannose residues. This allows the endoglycosidase H to carry out hydrolysis of mannose of the S protein causing the formation of spikeless viral particles. It is also reported that in the presence of tunicamycin, the M protein is normally produced and glycosylated, resulting in the formation of noninfectious virions containing normal amounts of N and M proteins but lacking the S protein completely. In a pilot study conducted by Sanchez et al. to find the effect of chemical inhibition of enzymes involved in the N-glycosylation pathway, tunicamycin was used to inhibit the N-glycan intermediate formation, i.e., GlcNAc2-dolichol phosphate. It was found that it provided high protection against SARS-CoV-2, which was 97.3%, 92.3%, and 87.5% against Vero cells, HEK cells, and Calu-3 cells, respectively. The high efficacy of tunicamycin could be attributed to the complete absence of N-glycans because of inhibition of GlcNAc2-dolichol formation. It was also found that incubation of HERK cells with tunicamycin resulted in reduction in the amount of N-glycan produced overall by 56.3%. Monensin.

5.1.2. Monensin. Monensin is a polyether metabolite produced by Streptomyces cinnamonensis. It is also referred to as monensic acid and is found in a monohydrate form complexed with a water molecule. It has six oxygen atoms, out of which five take part in the cation complexation. Monensin is a polyether metabolite produced by Streptomyces cinnamonensis and Streptomyces clavuligerus, and it is an analogue of UDP-N-acetylgalactosamine. It has a unique structure composed of a rare C5′-C6′-linked undecosidolose core, called tunicamycin, along with uracil, d-N-acetylgalactosamine (GlcNAc) that is attached to the core via a 1,1-β,α-trehalose-type glycosidic bond, and an amide-linked fatty acid (Figure 4). Monensin has great application as a glycoprotein transport inhibitor localized within the Golgi apparatus. Because the viral glycoproteins cannot reach the plasma membrane in the presence of the drug, the assembly process of the virus is inhibited. Also, as the coronaviruses are known to bud from the rough endoplasmic reticulum (RER) membrane and the canaliculi harbor a large number of viros, it is fascinating that infected cells did not fuse in the presence of the inhibitor, indicating that glycoprotein E2 carrying the fusion capacity of the virus does not reach the cell surface. Although monensin does not inhibit the budding of coronavirus, it interferes with virus release from the cell. The block in virus release could be because of substantial destruction of the cell structure that happens in the presence of monensin. However, virions may not be released from the cells either because of the absence of glycoproteins or their incomplete glycosylation. Monensin is reported to inhibit replication of SARS-CoV-2 in infected Vero-E6 cells [multiplicity of infection (MOI) of 0.01] with IC50 of 11 µM and CC50 of >50 µM. In the study, it was found to change the expression of mRNA levels of HMGCS1 (3-hydroxy-3-methylglutaryl-CoA synthase 1) in human lung primary small airway cells by 3–8-fold at 0.5 µM concentration. The anti-SARS-CoV-2 efficacy of monensin (EC50) was predicted to be 11 µM in Vero E6 cells. Mannostatin A. First isolated from the Streptoverticalium verticillatus var. quinque ME3-AQ3 bacterial culture filtrate, mannostatin (Figure 4) is a potent inhibitor of the rat epididymal α-mannosidase. It is the first nonalkoidal inhibitor of aryl-α-mannosidase, tested as a potential glycoprotein processing inhibitor and found to be a potent inhibitor of the Golgi processing mannosidase II but not mannosidase I. In cell culture, mannostatin A inhibited glycoprotein processing and increased hybrid types of oligosaccharides which is consistent with mannosidase II inhibition. Castanospermine. Castanospermine, a tetrahydroxyindolizidine alkaloid consisting of an octahydrodolizidine scaffold with four hydroxy substituents at positions 1, 6, 7, and 8 (15,6,7R,8R,8aR-diastereomer) (Figure 4), is a major constituent of seeds of the medicinal plant Castanospermum australe. It is also obtained from various other plants, viz., Primus prostrata, Cassina glauca, Morus bombycis, and Richteria pyrethroides. It has been shown to possess antiviral effects against several viruses in vitro and in vivo studies. It belongs to the class of intestinal α, β-glucosidase inhibitors and also plays a critical role in the inhibition of α-glucosidase I and II present in endoplasmic reticulum (ER). Castanospermine potently inhibits β-glucosidase and lysosomal α-glucosidase. Because these enzymes are involved in the trimming of the terminal sugar moieties present on N-linked glycans at the nascent proteins, this enzymatic process is pivotal for appropriate folding and function of the glycoproteins. The envelope proteins present in the virus also contain N-linked glycans, and thus, the ER α-glucosidase inhibitors can modify, alter, or destroy them. Castanospermine and N-methyl-1-deoxyojirimycin are reported to have similar biological effects. Clarke et al. tested castanospermine against SARS-CoV-2 at a concentration 100 µM and found a significant increase in live cells compared to untreated infected cells after 48 h, but it showed no significant difference in the number of live cells at either 10 or 1 µM concentration. The significant decrease in viral copies was only seen at the highest concentration of castanospermine, i.e., 100 µM. Celgosivir. Celgosivir is an iminosugar and an esterified 6-butanoyl derivative of castanospermine [(15,S,6S,7R,8R,8aR)-1,6,7,8-tetrahydroxy-octahydro-indolizine] (Figure 4). The integration of a lipophilic butanoyl side chain improved the absorption and toxicity of castanospermine. Its oral bioavailability was also higher than that of castanospermine, making it more relevant for long-term use as a therapeutic agent. The castanospermine prodrug, celgosivir, has been extensively explored as the dengue virus inhibitor, where it is more effective than castanospermine itself. Celgosivir has been demonstrated to be safe in human phase 1b trials, with an in vivo efficacy in mouse models of dengue virus. Recently, celgosivir was investigated in SARS-CoV-2 infected Vero E6 cells and was found to inhibit viral replication, reduce S protein levels as well as...
as prevent SARS-CoV-2 induced cell death. The treatment of SARS-CoV-2 Vero E6 cells with 10 and 100 \( \mu M \) celgosivir resulted in 1.5- and 2-fold increases in the relative number of live cells, respectively, when compared to results for untreated cells after 48 h (thus, reduced cytopathic effect of virus). Celgosivir was also found to significantly decrease viral replication at 100 and 10 \( \mu M \) concentration, but no such reduction was seen at 1 \( \mu M \) concentration. Thus, the action of celgosivir could be said to be dose dependent. It also lowered the levels of the S protein and lowest level was found at 100 \( \mu M \). The S protein produced was glycosylated, but alterations in glycan structure was proposed.

Casas-Sanchez et al. conducted a study where they preincubated Vero E6 cells, HEK293 ACE-2 cells, and Calu-3 cells with celgosivir, and it was observed that it is effective in reducing SARS-CoV-2 infection in these cells. Ceglosivir was found to show an overall reduction in these cells, a small cluster of infected cells, protection against cytopathic effects, and moderate reduction in viral titer. In all the studies reported, the potency of celgosivir was found to be higher than its parent drug, canstanospermine, which could be attributed to 30–50 times higher absorption of celgosivir than the later.

5.1.6. Australine. Australine is a tetrahydroxypyrrolizidine alkaloid isolated from Castanospermum australe, and interestingly, it is the only pyrrolizidine alkaloid possessing glucosidase inhibitory activity. It possesses a unique substitution pattern, bearing the hydroxymethyl group at the third position rather than first (Figure 4). It is a specific competitive inhibitor of amylglucosidase, an \( \alpha_{1-4}, \alpha_{1-6} \) exoglucosidase. Additionally, australine also displays good inhibitory activity against glucosidase I, but it is a very poor inhibitor of glucosidase II. It has been evaluated for inhibition of glycoprotein processing in vitro studies using an influenza virus-infected MDCK cell culture system. Australine disrupts the normal glycoprotein processing of viral glycoproteins. It has been found that a high concentration of australine is required to produce marked effects on glycoprotein processing compared to other inhibitors. For example, australine produced the same degree of inhibition at a concentration of 500 \( \mu g/mL \), as was observed at 10 \( \mu g/mL \) in

![Chemical Structures](Figure 5) Natural and synthetic iminosugar derivatives as glycosylation inhibitors.
the case of castanospermine. As far as the structure—activity is concerned, it appears that the characteristic six-membered ring structure of the indolizidine alkaloids (castanospermine, 6-epicastanospermine, and swainsonine) and other related inhibitors (deoxyoxojirimycin and deoxyoxomannojirimycin) is not necessary for a compound to be a glucosidase inhibitor. Accordingly, the ring nitrogen and the configuration of the hydroxyl groups relative to this nitrogen might be the only factors obligatory for such an inhibitor. It has been hypothesized that the critical requirement for these polyhydroxy alkaloids to possess glucosidase inhibitory activity is that at least three hydroxyl groups be located in a beta position relative to the nitrogen. All the known inhibitors follow this requirement, including australine.

5.1.7. Kifunensine. Kifunensine is an alkaloid produced by actinomycete Kitasatospora kifunensis 9482. It corresponds to a cyclic oxamide derivative of 1-amino mannojirimycin (Figure 4). It is a potent and selective inhibitor of ER mannosidase-I, complex N-glycosylation inhibitor and is also found to be an excellent glycprotein processing inhibitor in MDCK cell culture studies. It results in the production of glycoproteins that lack characteristic terminal sugar found on mature N-glycans. However, it is a 50–100-fold more active inhibitor of mannosidase I than deoxymannojirimycin. Albeit the indolizine ring structures of castanospermine and swainsonine are similar, kifunensine has a more intricate and heavily substituted structure with a bridgehead nitrogen, an additional nitrogen in the five-membered ring, four hydroxyl groups that are asymmetric, and two carbonyl groups. These other functional groups may be responsible for enhanced enzyme—inhibitor interactions. Additionally, the uncommon five-membered ring could lead the compound to assume a favorable conformation pertaining to the enzyme’s active site. It is reported that the use of kifunensine to block N-glycan biosynthesis significantly reduced viral entry into ACE2-expressing HEK293T cells. Kifunensine inhibited N-glycosylation in SARS-CoV-2 pseudovirus and resulted in widespread cleavage/shedding of the viral S protein because of enhanced proteolysis at the S1–S2 interface and reduced the viral entry into human ACE2 expressing cells by 85–90%. This water-soluble inhibitor reduced the production of complex N-glycans in cultured cells within 24–48 h while maintaining cell viability. Viruses produced in 15 μM kifunensine containing culture possessed ~85–90% reduced virulence. In a study conducted by Allen et al., for the ACE2 treated with 20 μM kifunensine, the K_50 increased by ~50%, which resulted in a reduced binding affinity of ACE2 with SARS-CoV-2. This could be because of conversion of N-linked glycans of ACE2 to the oligomannose-type.

5.1.8. Swainsonine. Swainsonine (8αβ-indolizidine-1α,2α,β-triol), an indolizidine alkaloid (Figure 4), isolated from the plant Swainsona canescens, inhibits the processing of asparagine-linked glycoproteins. It can also be synthesized chemically from α-glucose and β-mannose. It inhibits the α-mannosidase involved in glycoprotein processing. Inhibition of the α-mannosidase is expected to prevent the formation of the complex type glycoproteins. Swainsonine is found to inhibit the processing of oligosaccharides on influenza viral hemagglutinin. It acts as an active site-directed and reversible inhibitor of lysosomal α-mannosidase in a cell free system. It inhibits Golgi α-mannosidase-II in an irreversible and non-competitive way at concentrations <1 μM, a reaction similar to that with lysosomal enzyme. Swainsonine results in the production of viral glycoproteins with hybrid-type oligosaccharides rather than complex-type oligosaccharides. Swainsonine is tested for its inhibitory action on Golgi α-mannosidase in SARS-CoV-2 infected Vero cells and HEK cells. It was found to produce no effect on Vero cells but showed reduction in HEK cell infection.

5.2. Natural Iminosugars. These are carbohydrate mimetics with endocyclic nitrogen in the parent carbohydrate instead of oxygen. They exert their key antiviral mechanism via inhibition of ER-associated α glucosidases (I and II) involved in the glycosylation process resulting in misfolded proteins.

5.2.1. Nojirimycin. Nojirimycin is an antibiotic produced by Streptomyces species as well as the first 5-amino sugar found in nature. Its peculiar feature is the presence of a nitrogen atom in the pyranose ring instead of oxygen (Figure 5). Nojirimycin cause inhibition of both α- and β-glucosidases but inhibits β-glucosidases more potently (nonmammalian origin). The amino sugar substantially inhibits lysosomal α-glucosidase from the human liver. α-Glucosidases are inhibited more prominently in mammalian systems compared to β-glucosidases. Nojirimycin completely inhibited glucose release at a concentration of 5 μM in a microsomal glucosidase preparation from rat liver and using Glc3Man3GlcNAc (substrate). Thus, it is a potent processing glucosidase I inhibitor.

5.2.2. 1-Deoxynojirimycin (dMM). The catalytic or NaBH₄ reduction of nojirimycin results in 1-deoxynojirimycin (Figure 5). It was also isolated from some Bacillus strains, leaves of mulberry tree, and several Streptomyces strains. DNJ is also a natural carbohydrate mimic obtained from the roots and barks of Morus species, Morus alba, M. thau, and M. multicalulis. Like the parent compound, its biological activity is because of the competitive inhibition of glycosidic enzymes involved in glycoprotein processing. It mimics the normal carbohydrate substrates of enzymes like d-glucose and d-mannose.

However, in contrast to nojirimycin, it inhibits β-glucosidases of nonmammalian origin very weakly. Studies performed with β-glucosidases propose that both nojirimycin and DNJ bind to the enzyme active center in nonprotonated form and get protonated later, hence mimicking the transition state produced in catalysis. Also, DNJ inhibits the trimming glucosidases potently. A 50% inhibition was seen at 20 and 2 μM for glucosidase I and glucosidase II, respectively, in a yeast enzyme system. DNJ inhibits N-linked complex-type oligosaccharides synthesis in intact cells and, thus, acts on trimming glucosidase I as well as II, but principally glucosidase II. DNJ and its derivatives have low cytotoxicity even at relatively high concentrations and, thus, possess good therapeutic potential. When tested for N-glycosylation inhibitory effects, showed effects at high dose only, which was also not very significant. The overall glycanc level was reduced to 67.8% in the presence of DNJ.

5.2.3. 1-Deoxyoxojirimycin (dMM). dMM is a mannose analogue of DNJ (Figure 5) and is also found naturally in the seeds of Lonchocarpus sericeus and Lonchocarpus costaricensis. dMM cause 10^4 times more inhibition of α- and β-d-mannosidases than the mannose itself, but it is less noticeable than the glucosidases inhibition by nojirimycin or DNJ. dMM noncompetitively inhibits rat liver Golgi α-mannosidase I and brings about 50% inhibition at concentrations of 1–2 μM. dMM does not cause inhibition of RER and soluble α-mannosidase and affects the lysosomal α-mannosidase only slightly. As a mannosidase inhibitor, dMM (mannose analogue of DNJ) advocates that the reaction pathways for trimming glucosidases and mannosidases are probably the same and likely involve a cationic reaction intermediate. dMM prevented N-linked glycan biosynthesis in SARS-CoV-2 infected Vero cells and HEK cells. It was found to produce no effect on Vero cells but showed reduction in HEK cell infection.
complex type oligosaccharide synthesis in intact cells primarily via inhibiting Golgi α-mannosidase I activity. It is stated that the N-methyl-1-deoxynojirimycin is a better glucosidase I inhibitor than DNJ itself, but contrary to this, the N-methyl-1-deoxynojirimycin is far less potent in inhibiting Golgi α-mannosidase I than the dMM.385555b dMM is also tested for its N-glycosylation inhibitory effect by inhibition of Golgi α-mannosidase in SARS-CoV-2 infected Vero cells and HEK cells. It was found to produce no effect on Vero cells but showed reduction in HEK cell infection.31

5.3. Semisynthetic and Synthetic Iminosugar Derivatives. 5.3.1. N-Methyl-1-deoxynojirimycin. N-Methyl-1-deoxynojirimycin is a product of DNJ methylation (Figure 5). The methylation of nitrogen brings two crucial changes in activity: first, it does not inhibit lipid-linked oligosaccharide synthesis unlike nojirimycin and in some conditions DNJ, and second, it inhibits processing glucosidase I more efficiently than DNJ in a cell-free system.50 N-Methyl-1-deoxynojirimycin potentially inhibited the N-linked complex-type oligosaccharides synthesis in intact cells. In cultured rat intestinal epithelial cells, the N-methyl derivative inhibited the biosynthesis of complex type glycoprotein at only 1 mM, whereas DNJ produced similar effects at ~5 mM. Thus, N-methyl-1-deoxynojirimycin inhibited complex-type oligosaccharide synthesis in intact cells primarily by glucosidase I inhibition.38

5.3.2. Miglustat. Miglustat or N-butyldeoxynojirimycin (NBDNJ) (Figure 5) is a FDA-approved drug used in the treatment of Gaucher disease and Niemann-Pick disease type C because of its α-glucosidase inhibitory activity. The peculiar feature of SARS-CoV-2 pathogenesis is the avoidance of host immune recognition because of the protective glycans coat. The proposed antiviral mechanism of action of miglustat lies in the removal of the protective glycans coat because of inhibition of glycosyltransferase involved in SARS-CoV-2 S protein glycosylation. It subsequently exposes the S protein and results in exposure of the virus to immune system recognition. Thus, miglustat can be an effective treatment for SARS-CoV-2. The other postulated antiviral mechanism of miglustat is via induction of viral N-linked glycoproteins misfolding because of inhibition of host ER-resident α-glucosidase I and II enzymes which leads to reduced glycoprotein production and consequently lower virion production, both in vitro and in vivo.41 In a study performed by Sanchez et al., the miglustat preincubated Vero E6 cells, HEK293ACE2 cells, and Calu-3 cells showed reduction in SARS-CoV-2 infection. Miglustat showed overall reduction in infection in these cells, reduced cluster of infected cells, protected against cytopathic effects, and moderately reduced viral titer in supernatant.31 Miglustat was also found to be effective against SARS-CoV-2 with an IC50 value of 41 ± 2 μM in a plaque reduction assay on Vero E6 cells.7

5.3.3. N-Alkyl-1-deoxynojirimycin Derivatives. The N-alkyl derivatives of DNJ have also been synthesized and evaluated for α-glucosidase inhibitory, antiviral, and immunosuppressive activity.77 One of the derivatives, N-(7-oxadecyl)-1-deoxynojirimycin, was found to be the most potent compound of the series possessing an IC50 value of 0.28 μM against α-glucosidase I. It also inhibited HIV-1 induced syncytia formation, lymphocyte proliferation, and Ig production in vitro. Other iminosugar derivatives having N-alkyl side chains have been synthesized. One such derivative terminating in a cyclohexanol group, OSL-951II, was a potent antiviral agent and was far less cytotoxic. The hydroxyl group at the terminal cyclohexyl ring appeared to be responsible for reduced cytotoxicity.58 In the series, to further improve the biological profile of iminosugar derivatives, it was found that oxygenated alkyl imino sugar derivatives, CM-9-78 and CM-10-18 (Figure 5), potently inhibited α-glucosidases I and II in an in vitro study as well as in animal models. These compounds efficiently inhibited dengue virus infection in cultured human cells. Moreover, CM-10-18 in combination with antiviral drug ribavirin remarkably increased the antiviral activity of ribavirin.59

Further modification of the terminal ring structure of CM-9-78 resulted in a novel series of compounds represented by PBDNJ compounds that possessed dramatically improved antiviral activity, retaining low toxicity, with EC50 values at submicromolar concentrations. This study showed that incorporation of an oxygenated alkyl side chain combined with a substituted terminal side chain is essential for the antiviral activity of these compounds.60

Furthermore, 120 CM-10-18 derivatives were synthesized to improve the antiviral activity. Out of them, only 24 compounds were found to have superior antiviral activities and were
proceeded for pharmacokinetic studies. Finally, three novel derivatives, IHVR11029, IHVR17028, and IHVR19029 (Figure 5), having IC<sub>50</sub> values ranging from 0.09 to 0.48 μM, were identified as the lead compounds on the basis of enhanced α-glucosidase I and II inhibitory activity, antiviral activity, cytotoxicity, and ADME profiles. These studies suggested that iminosugar derivatives can be potential leads for the management of Covid-19.

5.3.4. N-(9-Methoxynonyl)-1-deoxyxojirimycin (UV-4). UV-4 causes inhibition of filoviruses in vitro and has antiviral effects against dengue virus and influenza virus in vivo (Figure 5). In an in vitro study, UV-4 significantly reduced SARS-CoV-2 viral replication in treatment groups 24 h postinfection. However, an opposite trend was observed at 48 h. Clarke et al. also tested UV-4 for inhibition of SARS-CoV-2 replication at concentrations from 100 to 1 μM at 24 and 48 h postinfection. At concentrations of 100 and 10 μM, a significant reduction in the number of viral copies was observed after 24 h of infection compared to results with untreated cells. However, after 48 h, this difference between treated UV-4 samples and untreated ones was found to be insignificant, implying that viral replication recovers after UV-4 is cleared.

5.3.5. Iminocyclitols. 5.3.5.1. 2,5-Dihydroxyxymethyl-3,4-dihydroxypyrrrolidine (DMDP). DMDP, a pyrrolidine alkaloid as well as a β-D-fructofuranose analogue (Figure 6), is a very potent α- and β-glucosidase inhibitor. Compared to results with DNJ, about 60 and 10 times lower concentrations of DMDP produce 50% inhibition of α- and β-glucosidase activities, respectively. In all the cases, inhibition is competitive and pH dependent in such a way that the nonprotonated form of DMDP is an active inhibitor. The addition of DMDP in influenza virus-infected MDCK cells inhibited the formation of N-linked complex-type oligosaccharides, demonstrating that DMDP inhibited the trimming glucosidase I. To further improve the glucosidase inhibition, several iminocyclitol derivatives were synthesized using combinatorial synthetic chemistry, and these derivatives were screened against different glucosidas. The active compounds were tested for antiviral activity (Japanese encephalitis virus, dengue virus serotype 2, and SARS coronavirus) using cell-based assay. It was found that compounds with bicyclic rings possessed greater activity, as shown by a derivative (1) with a K<sub>i</sub> value of 53 nM (Figure 4). The N-alkylated derivatives of (2) were also synthesized and screened for antiviral activity (Figure 4). The derivatives (3) (Figure 4) with a lipophilic alkyl group were the most active with IC<sub>50</sub> of 5–10 μM.

5.3.5.2. 1,4-Dideoxy-1,4-imino-o-mannitol (DIM). DIM is a furanos sugar analogue chemically synthesized by Fleet et al. from benzyl α-D-mannopyranoside (Figure 6) and inhibits the lysosomal α-mannosidase, although at higher concentrations. The active form of DIM that acts as an inhibitor is the one with the unprotonated ring nitrogen. DIM inhibits complex-type oligosaccharides synthesis in intact cells via inhibiting α-mannosidase I and possibly α-mannosidase II. However, compared to swainsonine, it does not inhibit the synthesis of N-linked complex oligosaccharides very effectively.

5.3.6. UDP (Uridine Diphosphate) Glucose Analogues. The nucleoside phosphate sugars are involved in the biosynthesis of glycoproteins by donating glycosyl residues. This process can be inhibited by altering sugar residues in nucleoside phosphate sugars, as proved by Camarasa et al. The group synthesized different analogues of UDP-glucose and UDP-glucosamine and evaluated their antiviral activity against HSV-1. Different analogues possessed antiviral activity as shown by their ability to protect against cytopathic effect induced by HSV-1 replication. Among the synthesized derivatives, the 2,3,4,6-tetra-O-benzyl-derivative (3) (Figure 6) was evaluated for protein glycosylation inhibitory activity and it completely blocked glycosylation of HSV-1 proteins at the tested concentration of 100 μM. Compound 3 reduced the production of new virus particles by 88% at the concentration of 12 μM, and it was completely inhibited at 100 μM. It was found that analogues devoid of protecting groups, or those with very low/high lipophilicity, were devoid of antiviral activity. Moreover, the presence of hexose, a five-atom bridge (diphosphate-like), and uridine were essential for their antiviral effects. In another work, the same compound possessed in vitro antiviral activity against other enveloped viruses as well.

5.3.7. Chloroquine. The possible benefit of the antimalarial drug chloroquine (Figure 6) in the treatment of SARS-CoV-2 infected patients has been brought to consideration. Touret and de Lamballerie evaluated chloroquine in vitro against a SARS-CoV-2 clinical isolate. The plausible antiretroviral effects of chloroquine could be attributed to the loss in a viral cell surface receptor, i.e., ACE2. Earlier, two in vitro studies confirmed that chloroquine might inhibit SARS-CoV-1 replication, where it inhibited replication with a 50% effective concentration of 8.8 μmol/L. The potent in vitro anti-SARS coronavirus effects of chloroquine are suggested to be because of decreased glycosylation of the host ACE2 receptors. It can also obstruct viral protein post-translational modifications, which is carried out by proteases and glycosyltransferases found in the ER and trans-Golgi network vesicles. Because both SARS-CoV-1 and SARS-CoV-2 utilize the same cell surface receptor ACE2, it could be postulated that chloroquine also interferes with ACE2 receptor glycosylation, thus inhibiting the binding of SARS-CoV-2 with target cells. Chloroquine also affects the virion assembly and budding by interfering with proteolytic processing of the M protein. In an in vitro study on the SARS coronavirus, it was reported that chloroquine treatment of Vero cells at 100 μM concentration resulted in reduction in biosynthesis of the S protein and processed viral glycoprotein levels. At concentrations less than 100 μM, chloroquine was not found to produce any significant effect on S protein biosynthesis or processing. Chloroquine was also found to terminate terminal glycosylation of ACE2 at a concentration 25 of μM.

5.3.8. Bromoconduritol. A synthetic molecule obtained from myo-D-inositol (Figure 6), bromoconduritol is an active site-directed covalent inhibitor of α-glucosidases. When bromoconduritol (6-bromo-3,4,5-trihydroxyxyclohex-1-ene) was added to yeast α-glucosidase, the reaction resulted in the modification of the histidine residue. It is postulated that the reaction involves formation of an epoxide at the active site of enzyme following dehydromobromination of bromoconduritol. Therefore, the trimming of the last glucose does not occur in the presence of bromoconduritol. Bromoconduritol and its derivatives have been tested for glucosidase inhibitory activity on influenza virus infected PR8 cells. It was found to strongly inhibit complex oligosaccharide formation and produce different high-mannose oligosaccharides at a concentration of 2 mM. Bromoconduritol inhibited the complex-type oligosaccharides synthesis by inhibiting the release of the innermost glucose residue from the Glc<sub>3</sub>Man<sub>9</sub>(GlcNAc)<sub>2</sub> precursor of high-mannose and complex oligosaccharides.

5.3.9. D-Mannopyranosylmethyl-p-nitrophenyltriazene. The α- and β-forms of this compound (Figure 6) inhibit
lyosomal β-glucosidase and α-mannosidase, respectively as suicide inhibitors that are irreversible and active-site directed. It is observed that incubation of cultured hepatocytes with 1 mM α-α-mannopyranosylmethyl-p-nitrophenyltriazene resulted in production of α-α-acid glycoprotein with high-mannose oligosaccharides rather than N-linked complex-type oligosaccharides. However, normal α-α-acid glycoprotein synthesis resumes after 24 h of removal of drug, most probably because of newly synthesized α-mannosidases.

5.3.10. NGI-1. NGI-1 is a small molecule inhibitor of N-linked glycosylation that directly inhibits STT3A and STT3B. The host oligosaccharyltransferase (OST) complex and E, M, and S proteins of SARS-CoV-2 are closely associated, and thus, it suggested that blocking of OST by NGI-1 could inhibit the SARS-CoV-2 infection significantly. Huang et al. conducted a study to determine if NGI-1 inhibits the N-glycosylation of E, M, and S proteins of SARS-CoV-2 in HEK293T cells. It was found that treatment with NGI-1 attenuated protein glycosylation in HEK293T cells that were previously overexpressed whereas there was a noticeable decrease of E and M proteins. It was also found that the proportion of N-glycosylated peptides at two highly abundant N-glycosomes, N1074 and N1194, was decreased from 12.30% to 2.10% and 87.39% to 65.92%, respectively, which suggests NGI-1 could reduce N-glycosylation of SARS-CoV-2 proteins. NGI-1 has been shown to strongly inhibit viral replication in Caco2 cells with IC50 of 0.860 and 438 μM, respectively.

In another study conducted by Casas-Sanchez et al., NGI-1 is effective in reducing infection by SARS-CoV-2 variants of concern, B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), and B.1.617.2 (Delta), in Vero cells and HEK293T cells. The NGI-1 treated cells were found to provide the highest degree of protection against SARS-CoV-2 in Vero cells (97%), HEK cells (92%), and Calu-3 cells (93.7%). It was also observed that NGI-1 combined with α-glucosidase inhibitor showed high protection rates. Incubation of HEK cells with NGI-1 reduced the total amount of N-glycans by 53.8%.

6. SAR FOR GLUCOSIDASE INHIBITORS

The key features for a molecule to be a glucosidase inhibitor include the following:

1. All inhibitors contain a nitrogen atom mostly present in a bridgehead form in the fused ring systems. The exception to this is mannostatin A, the only glycoprotein processing inhibitor having an exocyclic amino group instead of an endocyclic amine but still a potent mannosidase II inhibitor, activity comparable to that of swainsonine. Hence, in certain instances, an amino group outside the ring is enough for activity.

2. The inhibitors contain ring structures where some, like castanospermine and swainsonine, have a fused five-membered and six-membered ring system, whereas others, like australine, have two five-membered ring systems. Some natural as well as synthetic compounds have a single five-membered or six-membered ring system that might be good inhibitors. dMM is similar to mannose with nitrogen rather than oxygen in the ring, and it is a good mannosidase I inhibitor. An α-glucosidase inhibitor, 2,5-dihydroxymethyl-3,4-dihydroxypropirolidine (five-membered ring system) also causes inhibition of glycoprotein processing, possibly at the glucosidase I stage. A synthetic compound 1,4-dideoxy-1,4-imino-α-

mannotol possesses a five-membered ring structure where the nitrogen is present inside the ring, and it still causes inhibition of α-mannosidase as well as glycoprotein processing mannosidase I.

3. Lastly, the inhibitor should possess a minimum of two and possibly three hydroxyl groups having the configuration similar to that of sugar, which is a substrate for the concerned enzyme. Almost all inhibitors contain a minimum of three asymmetric centers, except lentinosine with an indolizidine ring system yet just two hydroxyl groups. However, it is much less effective as an amyloglucosidase inhibitor than castanospermine or australine.

7. LIMITATIONS AND CHALLENGES ASSOCIATED WITH GLYCOSYLATION INHIBITORS

The relying of virus on host glycosylation machinery and host-derived glycans opens a window of hope for employing a host-directed approach as therapy against SARS-CoV-2. Taking this into consideration, several carbohydrate mimetics such as iminosugars have been investigated in vitro settings to check their efficacy in combating SARS-CoV-2 as glycosylation inhibitors. Many among them, such as miglustat, have shown great efficacy in SARS-CoV-2 Vero E6 cells. Despite offering great hope, the practical issue associated with targeting these host-directed glycosylation inhibitors against SARS-CoV-2 is the dependency of the virus as well as the host on the glycosylation machinery (ER glucosidases and mannosidases) for processing of glycans. The potentially active compounds might impact the host in a negative way. Thus, these pragmatic concerns might pull off the glycosylation inhibitors from developing as host-directed therapy against SARS-CoV-2. However, this challenge could be overcome by focusing on and considering the tolerability and therapeutic window of these inhibitors. It might be possible that viral proteins may be more susceptible to glycosylation enzyme inhibition than the host, as it is known that during the proliferative phase, viral glycoproteins are synthesized at a very high rate. This acute viral infection phase provides a timeline where drug levels could be adjusted between those required to have a therapeutic effect against virus and those that could harness a negative impact on host.

The other hurdle for the use of glycosylation inhibitors against SARS-CoV-2 is the rate of viral replication and the viral load. These two parameters and severity of infection differ from individual to individual, and this might be attributed to variation in expression of the host receptor ACE2, the main human receptor for SARS-CoV-2. Thus, the schedule and dosage of glycosylation inhibitor would require tailoring depending upon the severity of infection in different individuals, which could be a costly and time-consuming process. Thus, determining a therapeutic window that would be acceptable to regulatory bodies for use of glycosylation inhibitors against SARS-CoV-2 remains a tough challenge.

8. CONCLUSION

Coronaviruses employ the host glycosylation machinery to augment their infectivity, antigenicity, and pathogenicity. The existing studies have shown that coronavirus proteins undergo several post-translational modifications in the host cell prior to release from the infected cell. This post-translational modification is a very crucial step as it is involved in the proper
function of viral proteins. The transmembrane structural proteins of SARS CoV-2, viz., S, E, and M (particularly the S glycoprotein), are found to be highly glycosylated, making them an attractive target for attenuating viral propagation. The glycosylation of the SARS CoV-2 S protein is principally N-linked, which facilitates folding as well as intracellular migration of the S glycoprotein. These N-linked glycans constitute a major part of the protein and greatly affect the conformation of the mature S protein as well as its binding to host surface receptors. The N-linked glycans are also proposed to have a role in the S

Table 1. Chemical Inhibitors of Glycosylation

| S.No. | Class | Compound Name | Mechanism of Action | Therapeutic potential | Clinical Status |
|-------|-------|---------------|---------------------|----------------------|-----------------|
| 1.    | Nucleosides | N-acetyl-D glucosamine | Anti-sialic acid | Experimental | Experimental |
| 2.    | Nucleotides | CMP-N-Ac-Man | Anti-sialic acid | Experimental | Experimental |
| 3.    | Nucleosides | CMP-N-Ac-Man | Anti-sialic acid | Experimental | Experimental |
| 4.    | Nucleotides | CMP-N-Ac-Man | Anti-sialic acid | Experimental | Experimental |
| 5.    | Nucleosides | CMP-N-Ac-Man | Anti-sialic acid | Experimental | Experimental |
| 6.    | Nucleotides | CMP-N-Ac-Man | Anti-sialic acid | Experimental | Experimental |
| 7.    | Nucleotides | CMP-N-Ac-Man | Anti-sialic acid | Experimental | Experimental |
| 8.    | Nucleosides | CMP-N-Ac-Man | Anti-sialic acid | Experimental | Experimental |
| 9.    | Nucleotides | CMP-N-Ac-Man | Anti-sialic acid | Experimental | Experimental |
| 10.   | Nucleosides | CMP-N-Ac-Man | Anti-sialic acid | Experimental | Experimental |
| 11.   | Nucleotides | CMP-N-Ac-Man | Anti-sialic acid | Experimental | Experimental |
| 12.   | Nucleosides | CMP-N-Ac-Man | Anti-sialic acid | Experimental | Experimental |
| 13.   | Nucleotides | CMP-N-Ac-Man | Anti-sialic acid | Experimental | Experimental |
| 14.   | Nucleosides | CMP-N-Ac-Man | Anti-sialic acid | Experimental | Experimental |
| 15.   | Nucleotides | CMP-N-Ac-Man | Anti-sialic acid | Experimental | Experimental |
| 16.   | Nucleotides | CMP-N-Ac-Man | Anti-sialic acid | Experimental | Experimental |
| 17.   | Nucleotides | CMP-N-Ac-Man | Anti-sialic acid | Experimental | Experimental |
protein antigenicity. The glycosylation might affect the viral pathogenesis via induction of innate immune response. Hence, finding therapeutic ways to combat the pandemic via targeting the host glycosylation assembly is under consideration. The glycosylation inhibitors discussed in this review offer interesting new opportunities for their repurposing for Covid-19, as well as being potential lead compounds for new anti-SARS-CoV-2 drug discovery (Table 1). At this crucial time, the repurposing of existing FDA-approved glycosylation inhibitors (such as miglustat and celgosivir) and others are in progress and a number of clinical trials are underway across the world. The authors believe that inhibiting viral post-translational processes/enzymes/transporters could be an effective approach for new therapeutic development for Covid-19.

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**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

N.T., B.G., and N.B. are thankful to IIT (BUH), Varanasi, for providing teaching assistance.

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