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# First round of a focused library of cholera toxin inhibitors

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**Abstract**—*C*-Galactosides have been used as scaffolds to design a library of non-hydrolysable inhibitors of cholera toxin (CT). Test elements from the library were synthesized and found to inhibit CT binding to an asialofetuin-coated SPR chip with micromolar affinity. Preliminary results are reported.


*Keywords:* Carbohydrate mimics; Cholera toxin; Glycomimetics; *C*-Glycosides; GM1 ganglioside

## 1. Introduction

*Vibrio cholerae* cholera toxin (CT) is the causative agent of cholera, a disease that is responsible for the death of hundreds of thousands of people each year in developing countries.[1] CT is a member of the AB5 holotoxin family. It consists of a single catalytically active component, A, and a non-toxic receptor-binding component, a pentamer of B subunits. The B pentamer (CTB5) is responsible for binding to the cell surface and this function is retained even in the absence of the A subunit.[2,3]

The mechanism of CT action is initiated by binding of the B subunits to the oligosaccharide head-group of the GM1 ganglioside, β-Gal*p*-(1→3)-β-Gal*p*NAc-(1→4)-(α-Neu*p*Ac-(2→3)-β-Gal*p*-(1→4)-β-Glc–OH, **1** (o-GM1, Fig. 1), on intestinal epithelial cell membranes. The A subunit is then translocated into the host cell, where it mono-ADP-ribosylates Gsα, a guanine nucleotide-binding regulatory protein, resulting in persistent activation of adenylyl cyclase.[3] The rising level of cAMP leads to massive loss of fluids, which in turn can lead to dehydration and shock.[4,5]

Small molecules acting as decoys for the toxin's GM1 binding site could work as toxin inhibitors by competing with GM1, possibly saturating the toxin, which would prevent it from binding to the epithelial cells and causing the symptoms of cholera. Natural and synthetic antagonists of CT are known, and they are under investigation as potential agents for the treatment of cholera and other toxin-caused enteropathies.[6]

The interactions of o-GM1 with CTB5, as seen in the X-ray structure of the complex, are highlighted in Figure 1.[7,8] The terminal galactose stacks on top of Trp88 and is linked through hydrogen bonds to Asn90, Lys91, Glu51, and Gln61 (Fig. 1). The terminal sialic acid (NeuAc) is exposed to solvent on the α-face and it is in van der Waals contact with Tyr12 on the β-face. Hydrogen bonds are formed between the sialic acid polar groups with crystallographic water molecules and with the backbone of Glu11 and Tyr12. The carboxyl group of NeuAc establishes a key interaction with Trp88, mediated by a key water molecule. The outer part of the binding pocket, with Glu11 and Arg35, constitutes a dipole that orients the *N*-acetyl fragment of the sialic acid (Fig. 1).

The terminal galactose and sialic acid residues contribute the most to GM1 binding. Although the two

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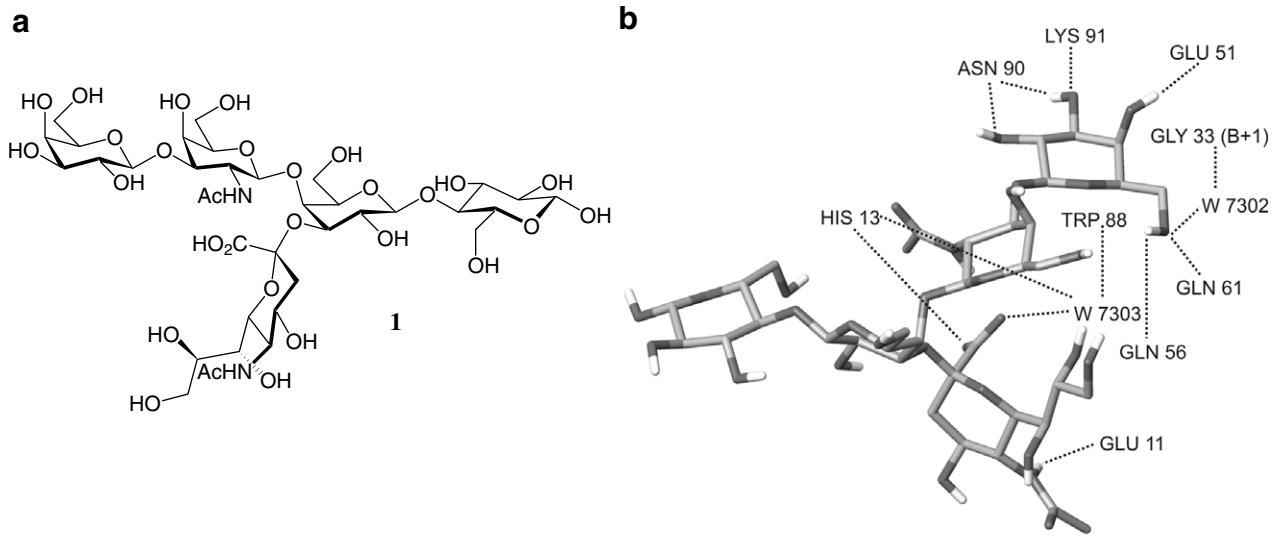


**Figure 1.** (a) The GM1 oligosaccharide o-GM1, and (b) its key interactions with CT binding site.

isolated monosaccharides show very low affinity for the protein (Gal 40 mM; NeuAc > 200 mM),[9] o-GM1 binds to CT with a dissociation constant $K_D = 43$ nM.[9] This appears to depend on the conformational preorganization of o-GM1,[9,10] which has been shown by NMR to adopt one major conformation[11] closely resembling the bound conformation observed in the CT complex.[7,12]

According to this interpretation, the pseudo-oligosaccharide **2** (Fig. 2), which reproduces the 3D structure of o-GM1, was found to display a similar affinity for CTB5.[10] A second group of pseudo-oligosaccharides rationally designed to replace the sialic acid moiety while retaining CT affinity have led to micromolar inhibitors of toxin (Fig. 2).[12–18] Similar results have been achieved by using galactose as a CT-binding anchor and developing several generations of galactoside libraries[3,6a,19–22] (Fig. 2). Some of the molecules discussed above display good affinity for CT and are structurally much simpler than the natural ligand GM1. However, they all are *O*-glycosides, and are unlikely to be metabolically stable to any significant extent. Furthermore, the synthetic methods used to connect the pharmacophoric sugar moieties are those of traditional carbohydrate chemistry, which are often laborious and not high yielding procedures.

To address these shortcomings, we are currently working toward the development of CT ligands starting from simple *C*-galactosides. *C*-Glycosides are well known metabolically stable analogs of *O*-glycosides.[23–29] Furthermore, some of us have recently shown that the functionalized *C*-galactosides **7** and **8** (Fig. 3) can be easily synthesized in a few steps directly from galact-

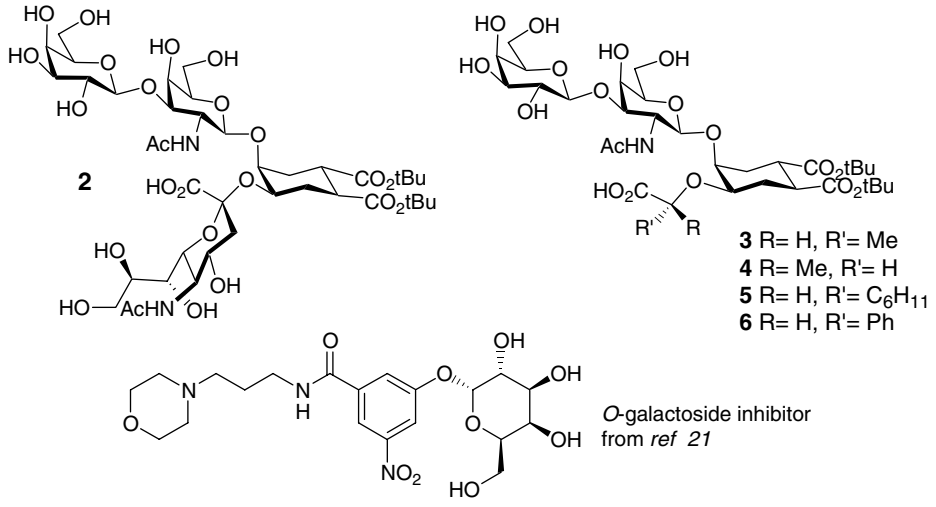


**Figure 2.** Some currently known CT inhibitors.