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Potent Glycomimetic Inhibitors of the Bacterial Pathogen, *Pseudomonas aeruginosa*: Novel Therapy Based on Targeting the Adhesion Molecule, PA-IIL Surface Lectin

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Many pathogens recognize, bind and colonize tissues by a direct binding to specific carbohydrate structures on the host cell surfaces. As an example, *Pseudomonas aeruginosa* is a ubiquitous pathogen that infects a wide variety of tissue sites in human patients. In particular, this bacterium is the major cause of morbidity and mortality in patients with Cystic Fibrosis (CF). Infection and colonization of CF lungs follows a cyclical course of infection and inflammation eventually resulting in destruction of the lungs and death. CF is a genetic disease caused by mutations that alter chloride channels but also affects the glycosylation of lung tissue. Several reports (1) demonstrate a net increase fucosylation and decrease in sialylation. *P. aeruginosa* binds strongly to these modified carbohydrates and colonizes CF lung tissue through the expression of surface lectins PA-IL and PA-IIL (2). Blockade of these lectins presents a novel therapeutic strategy for *P. aeruginosa* infection. By analyzing the precise carbohydrate epitope of the bioactive conformation required for binding PA-IIL lectin a potent glycomimetic inhibitor for the PA-IIL adhesion molecule was developed which has inhibitory activity in the nanomolar range.

An ELISA-based assay was developed to determine the direct binding of PA-IIL lectin to immobilized complex carbohydrate structures (conjugated to albumin for coating plates, GlycoTech Corp, Gaithersburg, MD USA). Bound PA-IIL lectin was detected by antisera to PA-IIL lectin followed by HRP labeled anti-antibodies. Using this assay a library of complex carbohydrates structures of the lacto/neolacto-series containing either sialylated or neutral oligosaccharides was screened. he results are shown in figures 1 and 2 below respectively.

Carbohydrate Structure	Name	PA-IIL binding
Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-R 4 Fucα1	SialylLea	++
Neu5Acα2 6 Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-R 4 Fucα1	DiSialylLea	+
Neu5Acα2 6 Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-R	DiSialylLNT	-
Neu5Acα2-3Galβ1-3GlcNAcb1-3Galβ1-4Glc-R	LSTa	-
Galβ1-3GlcNAcβ1-3Galβ1-4Glc-R 6 Neu5Acα2	LSTb	-
Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-R	LSTa	-
Neu5Acα2-3Galβ1-4Glc-R	3'SL	-
Neu5Acα2-6Galβ1-4Glc-R	6'SL	-

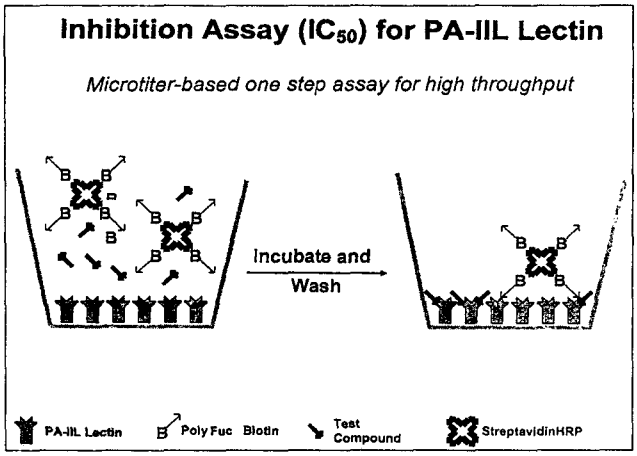
Figure 1. Screening Neutral Oligosaccharides for binding PA-IIL Lectin

Carbohydrate Structure	Name	PA-IIL binding
Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc-R	LNFI (H-type 1)	+
Galβ1-3GlcNAcβ1-3Galβ1-4Glc-R 4 Fucα1	Lea	++
Fucα1-2Galβ1-4GlcNAcβ1-3Galβ1-4Glc-R 3 Fucα1	Ley	+
Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-R 3 3 Fucα1 Fucα1	DiLex	+
Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-R 3 3 3 Fucα1 Fucα1 Fucα1	TriLex	+
Galβ1-4GlcNAcβ1-3Galβ1-4Glc-R	LNnT	-
Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-R 4 3 Fucα1 Fucα1	Lea/Lex	+++
Galβ1-3GalNAcβ1-4Galβ1-4Glc-R	Gangliotetraose	-

Figure 2. Structures of immobilized neutral oligosaccharides screened for binding to PA-IIL lectin.

Epitope analysis of the binding information from these experiments demonstrate a enhanced binding specificity of PA-IIL lectin to extended fucosylated type 2 chains. Arecent publication (3) predicted the bioactive conformation of these oligosaccarides required the stacking of the fucose under the galactose. A series of glycomimetic compounds were synthesized that retained this stacking conformation as determined by NOE/NMR.

An ELISA-based assay was developed to determine IC_{50} values for inhibitors of the PA-IIL lectin using specialized biotinylated polymers containing fucose (GlycoTech Corp., Gaithersburg, MD, USA). Purified PA-IIL lectin was immobilized in microtiter wells and inhibition of binding to fucosylated, biotinylated polymer conjugates with streptavidinHRP was tested. schematic representation of the assay is presented in below in Figure 3.



Glycomimetic compounds that mimic the bioactive conformation of the native carbohydrate ligands, but contain more drug-like properties, were found to be potent inhibitors of the bacterial adhesion molecule PA-IIL and displayed IC_{50} activity in the nanomolar range. These rationally designed glycomimetic inhibitors represent a novel class of therapeutic compounds that specifically target the molecules of the bacterial pathogen that are required for recognition, binding and colonization of host tissue.

References

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