Small Molecule Inhibitors of Glycosaminoglycan Biosynthesis as a Substrate Optimization Therapy for the Mucopolysaccharidoses

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Introduction and Goals

The primary goal of this research is to identify novel CNS penetrant inhibitors of heparan sulfate biosynthesis as a treatment for the Mucopolysaccharidoses.

The Mucopolysaccharidoses (MPS) are a family of lysosomal storage diseases caused by a deficiency in enzymes responsible for the degradation of glycosaminoglycans (GAGs). Despite progress in treating many of the peripheral symptoms, the central nervous system (CNS) aspect of the disease remains unaddressed.

To address this unmet need, we are developing a small molecule CNS penetrant therapy for MPS I, II, and III that works by altering the synthesis of the GAG implicated in the CNS disease, heparan sulfate. Our therapeutic approach, Substrate Optimization Therapy (SOT) is based on selectively modifying the synthesis of heparan sulfate to render it more easily degradable despite the presence of specific enzyme deficiencies. In vitro data with cultured human primary fibroblasts from MPS I, II, and III patients indicate that inhibiting the 2-O sulfation of heparan sulfate can reduce the lysosomal accumulation of this glycan.

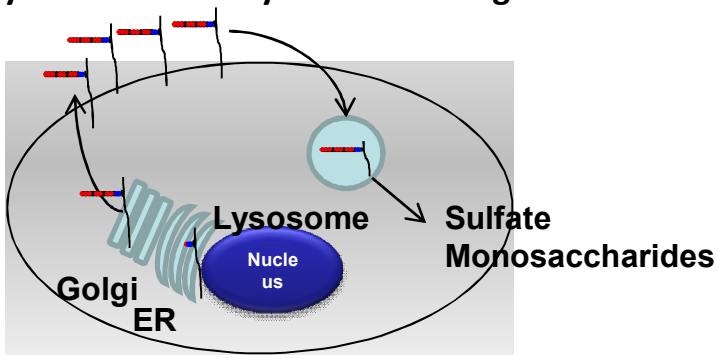
Using a cell-based high throughput screen, we have identified a set of compounds that inhibit the 2-O sulfation of heparan sulfate and reduce lysosomal accumulation of GAGs in MPS cells. From these compounds, we identified a blood-brain barrier penetrant compound that reduces lysosomal accumulation of GAGs in the central nervous system in the mouse model of MPS II and IIIA with no adverse tolerability observed.

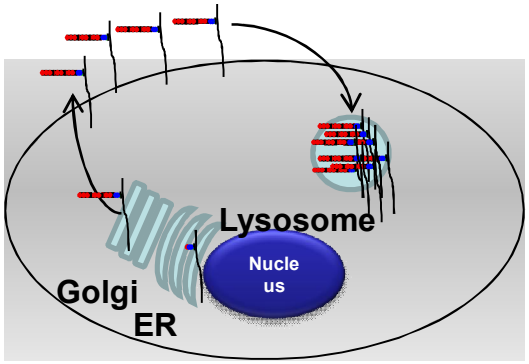
To optimize potency, PK, and other drug-like properties, we then synthesized and tested a number of analogs and identified compounds with improved in vitro potency in cultured human MPS fibroblasts while retaining excellent blood-brain barrier penetration. Ongoing lead optimization activities are aimed at identifying a clinical candidate drug for IND enabling activities and human clinical trials.

When successfully completed, this research will have enabled the first small molecule therapy capable of addressing the CNS and other unmet needs in patients with MPS I, II, and III.

The Mucopolysaccharidoses

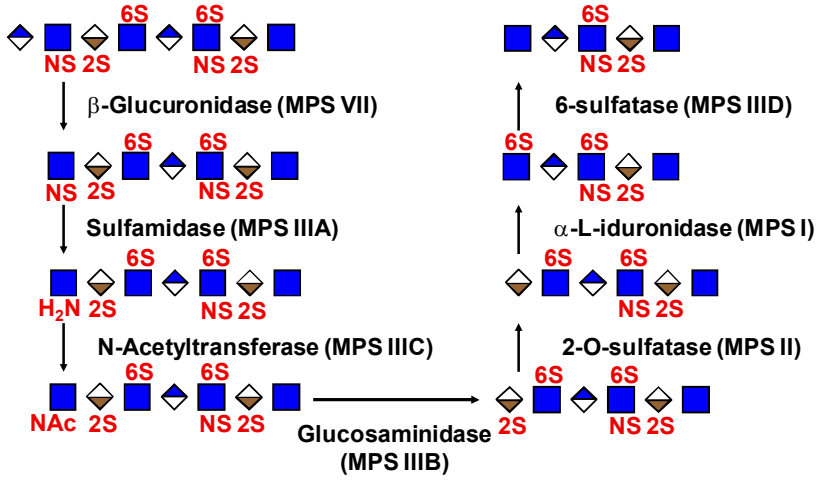
Ⓐ Mucopolysaccharidosis: Lysosomal Storage of GAGs





Panel 3A. Mucoploysaccharidoses: Lysosomal Storage of GAGs. The Mucopolysaccharidoses (MPS) are a collection of genetic disorders caused by mutations in genes encoding lysosomal enzymes required to degrade glycosaminoglycans (GAGs). The impaired degradation leads to accumulation of GAGs within lysosomes which in turn leads to serious multi-system disease. The disease generally strikes early in life with devastating consequences to development and function. The most serious consequences are severe neurological impairment.

Ⓑ The Mucopolysaccharidosis Define the Lysosomal GAG Degradation Enzymes

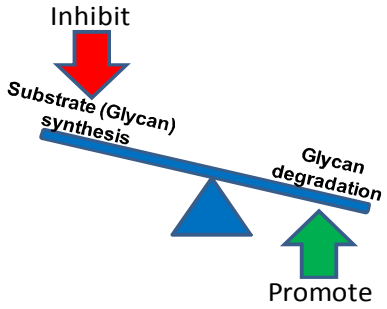


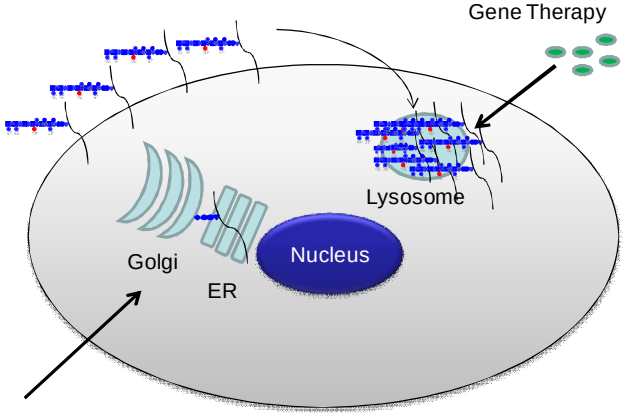
Panel 3B. The MPS Classes Define Lysosomal Degradation of GAGs. There are at least 11 unique classes of MPS, each caused by a mutation in a gene required for lysosomal degradation of GAGs. The lysosomal enzymes are exo-enzymes that degrade GAGs exclusively from the non-reducing end of the glycan. Due to this mechanism of action, a disruption in any step terminates further degradation by other enzymes. Seven of these MPS classes are deficient in enzymes required for the degradation of heparan sulfate.

Substrate Optimization Therapy (SOT)

Enzyme Targeted Therapies
Enzyme Replacement (ERT)
Aldurazyme, Elaprase, Naglazyme
Chaperone
Gene Therapy

Therapeutic Approaches to the Mucopolysaccharidoses





Substrate Targeted Therapies
Glycosaminoglycan synthesis inhibitors

Existing Therapeutic Strategies: Correcting the Enzyme Deficiency

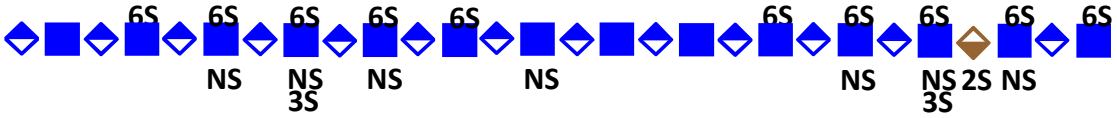
The most common therapy for MPS is enzyme replacement therapy (ERT) which is based on intravenous delivery of the deficient lysosomal enzyme. These products have shown that ERT can be effective at reducing the lysosomal accumulation in peripheral tissues such as the liver and spleen. Unfortunately, ERT fails to penetrate the CNS or reach other critical tissues such as the heart valves and cartilage. Additional therapeutic strategies which are designed to correct the enzyme deficiency include gene therapy, transplantation, and pharmaceutical chaperones. However, to date, these have not proven very useful in MPS.

A Novel Approach: Substrate Optimization Therapy (SOT)

SOT is based on selectively modifying heparan sulfate biosynthesis to favor the production of structures that can be degradable despite specific enzyme deficiencies. As shown below, an inhibitor of the 2-O sulfation of heparan sulfate would reduce the abundance of 2-O sulfated structures that cannot be degraded in an lysosomal 2-O sulfatase deficient MPS II patient. In vitro studies indicate that a minor reduction in the substrate can significantly reduce the lysosomal accumulation. Because this therapeutic approach is based on classic small molecules, it has the advantage of a greater chance of accessing the CNS and other difficult to penetrate tissues where it could have benefit on the devastating neurological aspects of the disease. Unfortunately, the development of substrate targeted therapies for MPS have been hindered by the lack of selective inhibitors of GAG biosynthesis.

Normal Heparan Sulfate Structure

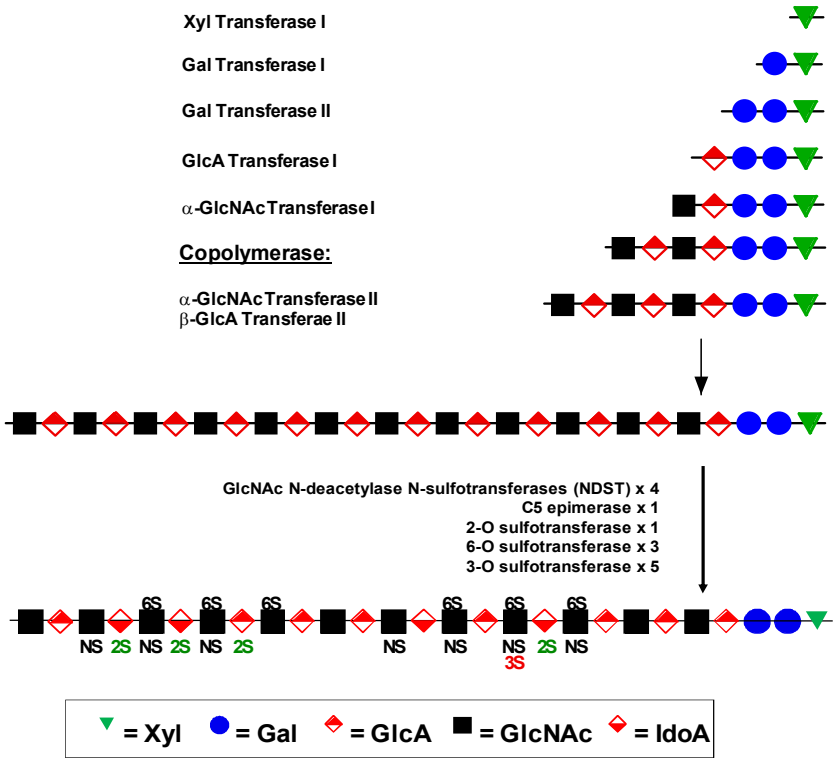
Substrate "Optimized" Heparan Sulfate Structure*



*Note reduced 2S and iduronic acid content

Heparan Sulfate Inhibitor Discovery

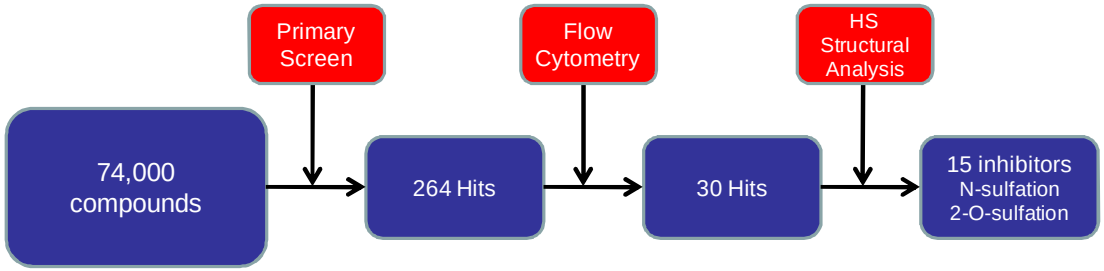
Ⓐ Heparan Sulfate Biosynthesis



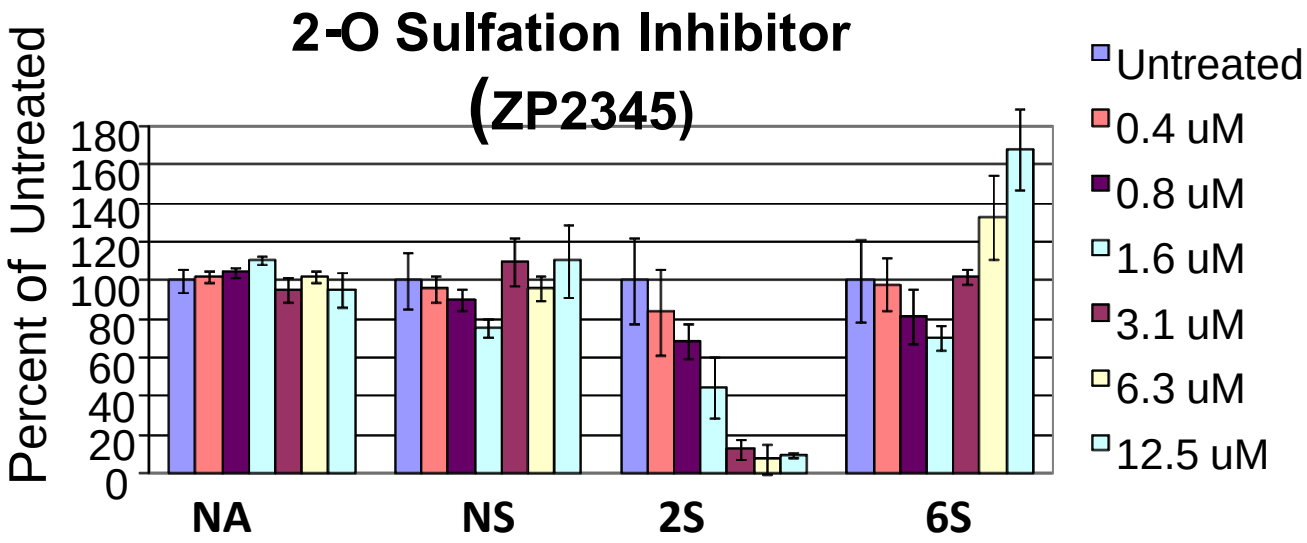
Panel 4A. Target of Inhibition: Heparan Sulfate Biosynthesis. Previous attempts to identify small molecule inhibitors of the key GAG biosynthetic enzymes using classic in vitro enzyme assays have failed. In order to overcome this limitation, we developed a cell-based assay able to identify inhibitors of any of the critical biosynthetic steps. Heparan sulfate is produced by a series of over 20 biosynthetic enzymes in the ER and Golgi. This cellular assay has the ability to identify inhibitors of any of these steps in addition to unknown targets that regulate HS synthesis. The cellular assay for heparan sulfate inhibitors was based on screening for compounds that reduce FGF2 binding in cultured human cell lines. Using this assay in a 384-well format, we screened 74,000 drug-like small molecules and identified 264 hit compounds that reduce FGF2 binding but had no effect on other lectins. As summarized below, 30 of these hit compounds actually altered the composition of heparan sulfate in cells grown in the presence of the compound.

Heparan Sulfate Inhibitor Discovery, Cont.

B Heparan Sulfate Inhibitor Discovery Overview



C Heparan Sulfate Compositional Analysis



Panel 4C. Heparan Sulfate Compositional Analysis. The compounds were tested for their ability to inhibit the biosynthesis in HeLa cells using heparan sulfate disaccharide analysis. This assay identified 15 compounds that altered N-sulfation, 2-O sulfation, or 6-O sulfation by >10%. One example is ZP2345 which was found to inhibit the 2-O sulfation of heparan sulfate.

Mechanism of Action

The Novel Inhibitors Do Not Directly Inhibit Key Heparan Sulfate Biosynthetic Enzymes

Sample No.	2-OST (%) Average	NDST-1 (%) average
ctrl	100	100
positive ctrl	3.0	4
2345	110	107
2356	99	105
2362	97	108
2376	105	113
2493	100	105
2497	106	100
2504	106	103
2527	97	93
2529	113	92
2536	109	26/31
2544	101	103
2549	108	103
2561	110	105
2565	105	114
2566	103	111
2567	102	111
2569	97	105
2571	93	102
2572	95	105
2575	98	98
2576	102	106
2577	93	93
2578	93	98
2579	92	89
2581	92	100
2582	91	97
2583	89	89
2585	93	96
2586	91	102
2589	90	92
PAP	11	92
PAPS	5	26

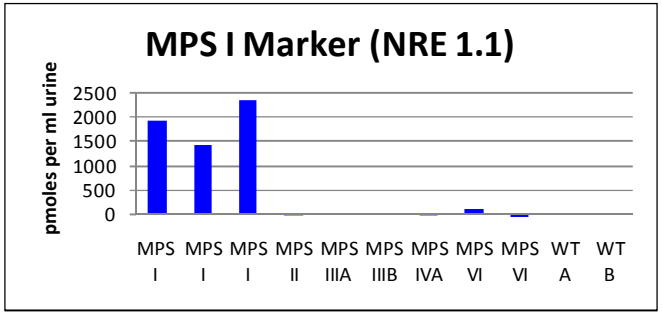
Panel 5. Investigations into the Mechanism of Action of the Heparan Sulfate Inhibitors. Based on the effects of the compounds on HS biosynthesis, we investigated the effect of the compounds on the 2-O sulfotransferase, N-sulfotransferase-1, and 6-O sulfotransferase-1 with with Dr. Jian Liu (UNC). These studies indicate that none of the compounds directly inhibit the active site of the sulfotransferase.

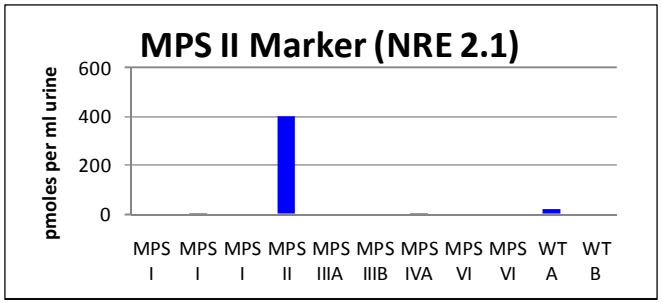
Methods: 2-O sulfotransferase assays were run using 5.0×10^6 cpm/mL of PAPS, 5 µg/mL of CDSNS-heparin and 0.6 µg/mL 2OST in 100 µL of MES buffer system. N-sulfotransferase assays were run using 9.0×10^6 cpm/mL of hot PAPS, 10 µM cold PAPS, 1 µg of NH_2-k5p and 1.2 µg/mL 2OST in 100 µL of MES buffer system. Compounds were tested at 30 µM.

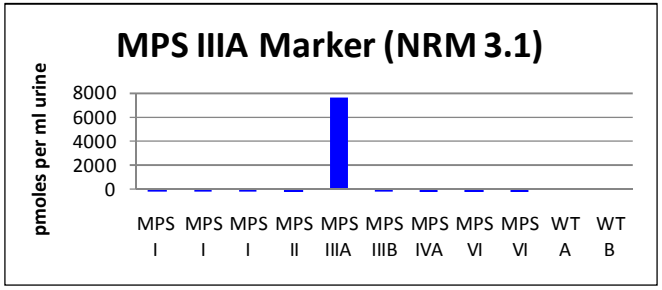
The Sensi-Pro Assay: A Lysosomal Storage Assay

Sensi-Pro Assay Overview – a sensitive and specific measure of lysosomal storage

Panel 6: The Sensi-Pro Assay – a Cellular Assay for MPS. In order to develop a small molecule inhibitor of heparan sulfate a high throughput cellular assay is required in order to test a large number of analogs for efficacy. To accomplish this, we developed the Sensi-Pro Assay as an in vitro model of MPS. This assay is able to sensitively quantify GAG accumulation in MPS cells by detecting the abundance of GAGs with the specific non-reducing end (NRE) structure that can not be degraded. For example, MPS II patients are lacking the lysosomal 2-O sulfatase which leads to an accumulation of a heterogeneous array of GAG fragments that terminate with a 2-O sulfated uronic acid on the NRE. By detecting the unique NRE structure, this can be measured as a direct reflection of the GAG lysosomal storage. The panel on the right illustrates the specificity and flexibility of the assay across distinct MPS classes. MPS I, II, and IIIA patients lack the lysosomal iduronidase, 2-O sulfatase, and N-sulfatase, respectively. Using this assay, we are able to quantify the lysosomal GAG accumulation in a 96-well format.

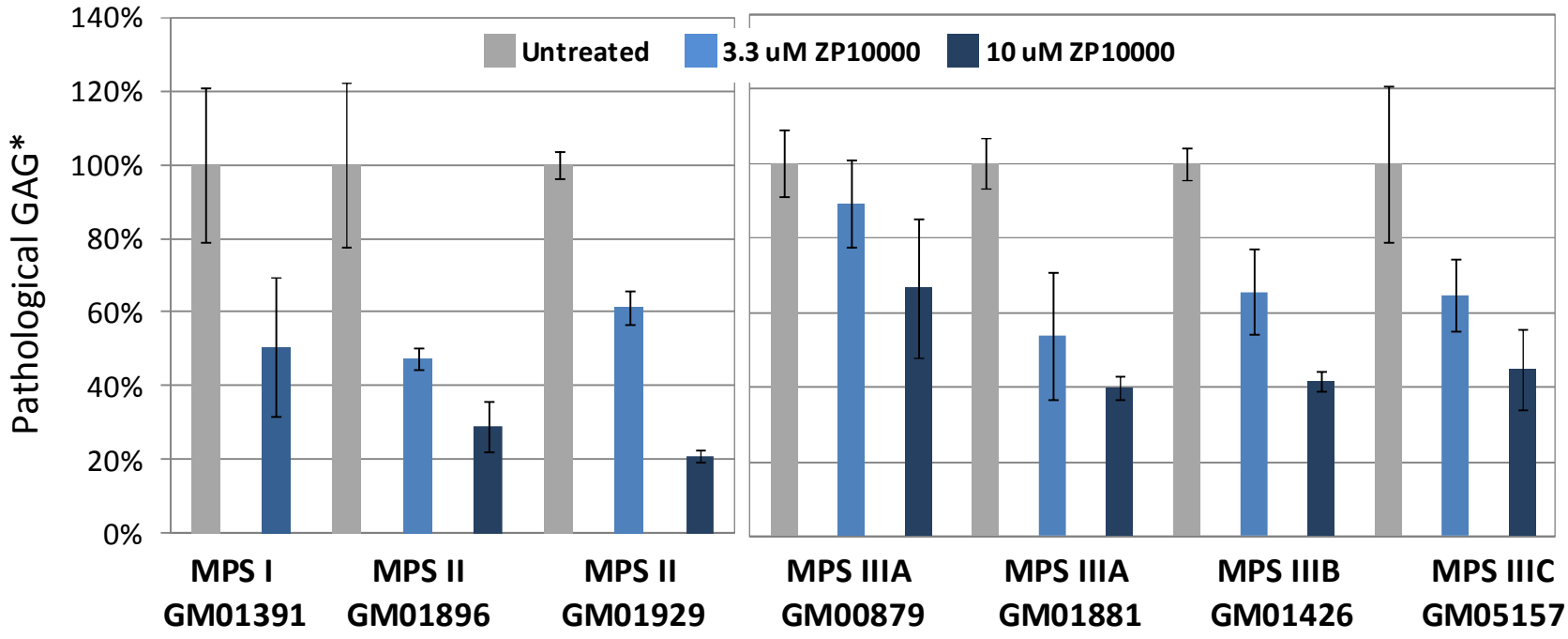






In Vitro Efficacy of HS Inhibitors

ZP10000 Reduces Lysosomal Accumulation in MPS I, II, and III

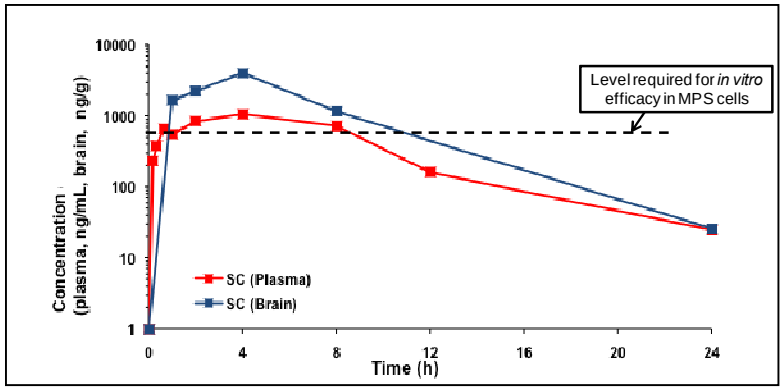


* Expressed as % of untreated cells

Panel 7. ZP10000 Reduces Lysosomal GAG Accumulation in MPS I, II, and III. Using the Sensi-Pro assay, we tested ZP10000 for its ability to reduce lysosomal accumulation in primary fibroblasts isolated from MPS patients. Through these studies we identified analogs of seven scaffolds that reduced GAG accumulation in MPS cells. Panel 7 shows the data for ZP10000 which reduced heparan sulfate accumulation in multiple classes of MPS, including MPS I, II, IIIA, IIIB, and IIIC.

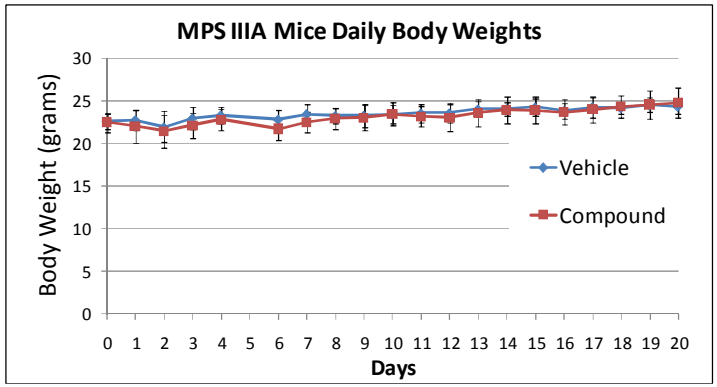
In Vivo PK and Tolerability Studies

A ZP10000 is CNS Penetrant and Has Acceptable PK Properties for In Vivo Testing



Panel 8A. Mouse PK Studies Indicate ZP10000 Penetrates the Blood-Brain Barrier. Penetration of the blood brain barrier is essential for a CNS active SOT for MPS. To determine if ZP10000 is CNS penetrant, we analyzed the serum and brain levels of ZP10000 following subcutaneous administration of in mice (50 mg/kg). This analysis revealed that ZP10000 penetrates the brain and obtains levels equal to or greater than the blood (~10 µM). This data suggests that ZP10000 is a good scaffold for further development to address the CNS aspect of these diseases. Based on the significant levels that were obtained in the brain, we decided to test this compound for efficacy in vivo.

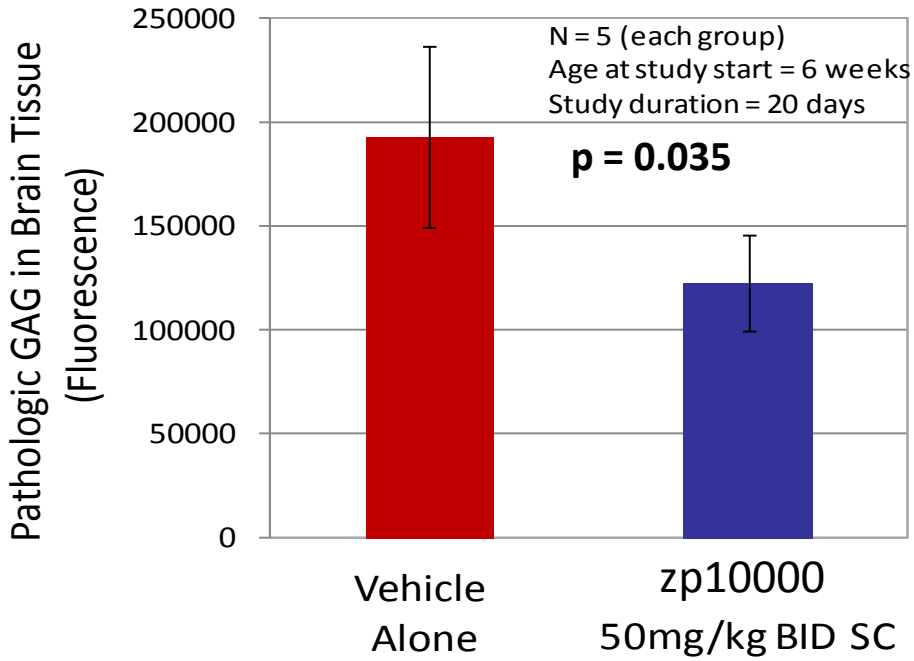
B Twice Daily Dosing of ZP10000 is Tolerated by MPS IIIA Mice



Panel 8B. ZP10000 is Tolerated in the MPS IIIA Mouse Model. Before testing the compound for efficacy in MPS mice, we investigated the in vivo tolerability of ZP10000. Two groups of five mice were dosed twice daily with 50 mg/kg ZP10000 by subcutaneous injection, or with vehicle alone for 20 days. The mice were monitored daily for behavior changes and weight gain. No changes in weight gain or behavior were observed over the 20 day dosing period.

Pilot In Vivo Efficacy Studies (MPS IIIA)

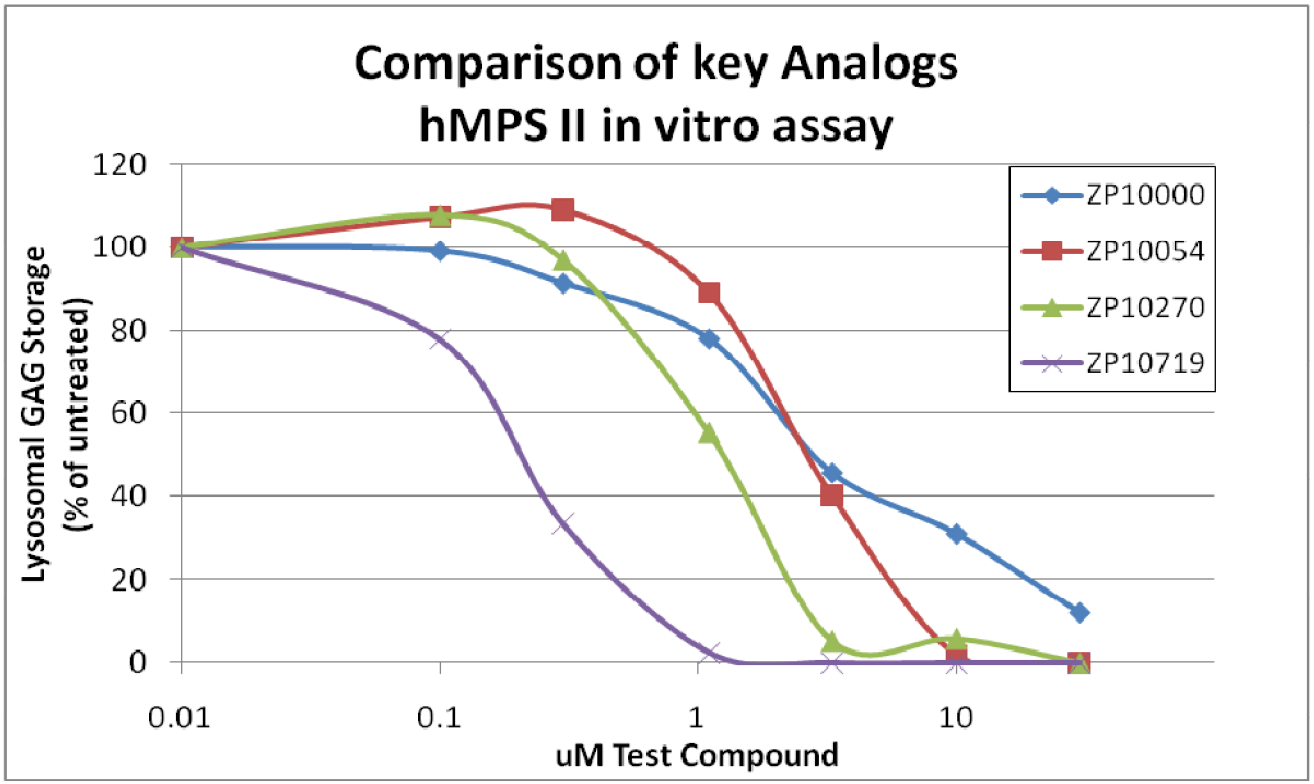
ZP10000 Reduces Lysosomal Accumulation in the Brain of MPS IIIA Mice



Panel 9. ZP10000 Reduces Lysosomal Accumulation of GAGs in the Brain of MPS IIIA Mice. Six week old MPS IIIA (N-sulfatase deficient) mice were dosed twice daily subcutaneously with 50 mg/kg ZP10000 or vehicle alone for 20 days. At the end of the study, the lysosomal accumulation of HS was measured using the Sensi-Pro assay. A significant reduction in lysosomal HS accumulation was observed in the brain. We are currently exploring longer studies with more potent analogs.

Medicinal Chemistry Program

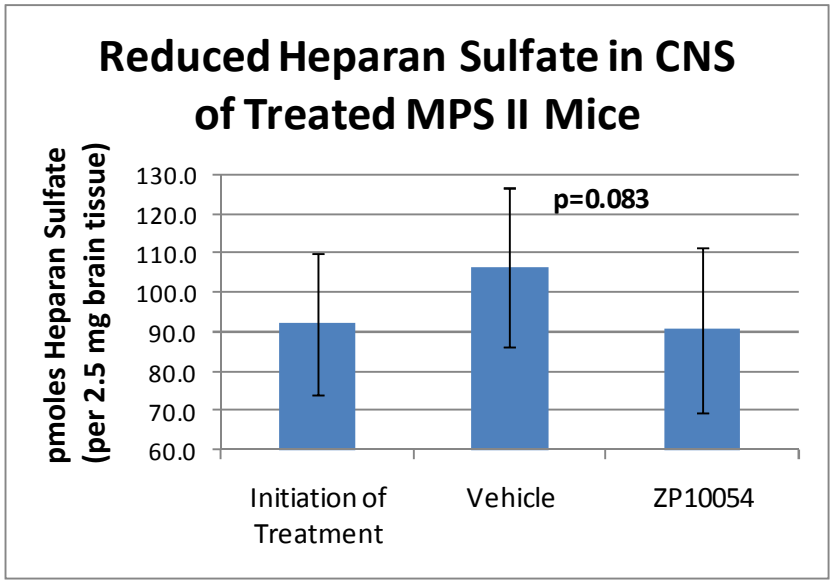
More Potent Analogs Have Been Identified Through the Medicinal Chemistry Program

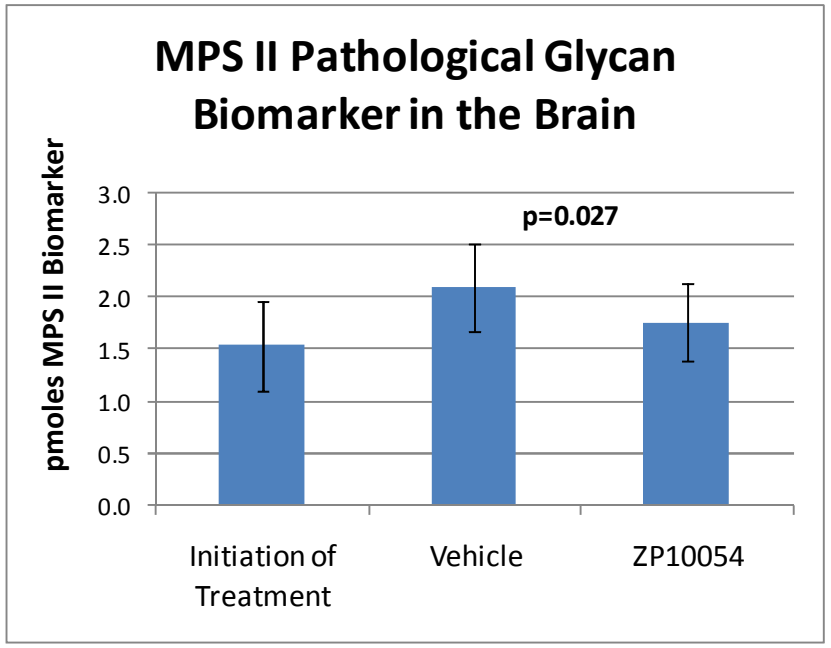


Panel 10. ZP10000 Medicinal Chemistry Program Has Identified More Potent Analogs. Our medicinal chemistry program is working to improve the potency and PK properties of ZP10000 while maintaining the CNS penetration. Compounds are tested in full dose ranges in the Sensi-Pro assay using human MPS II fibroblasts. The basic structure-activity relationship of the scaffold has been defined and we are currently focused on the synthesis and testing of analogs that combine structural features expected to increase potency. Through these studies, we have identified analogs with improved potency and PK properties (Ex: ZP10054, ZP10270, and ZP10719).

In Vivo Efficacy Studies (MPS II)

ZP10054 Demonstrates Efficacy in the Mouse MPS II Model





Panel 11. ZP10054 Reduces Lysosomal accumulation of GAGs in the MPS II Mouse Model. Four week old MPS II (2-sulfatase deficient) mice were dosed twice daily subcutaneously with 25 mg/kg ZP10054 (a more potent analog of ZP10000) or vehicle alone for 30 days. At the end of the study, the lysosomal accumulation of HS was measured using the Sensi-Pro assay. A significant reduction in lysosomal HS accumulation was observed in the brain. This study demonstrates the ability of a heparan sulfate inhibitor to have efficacy across distinct MPS classes that accumulate GAGs.

Conclusions and Acknowledgements

Development of a Novel Pan-MPS I, II, and III CNS Penetrant Therapy

These data demonstrate the potential for a novel substrate optimization therapy for MPS based on small molecules that selectively alter the structure of heparan sulfate in a way that makes it easier to degrade in MPS patients. Additional development activities are currently ongoing including:

1. The medicinal chemistry program is continuing to design, synthesize and test analogs of our best compounds to further improve the PK, solubility, and drug-like properties of the best compounds.
2. Further efficacy studies in MPS animal models including reduction in pathologic substrate in brain tissue (potentially several MPS classes) to determine the minimum effective dose.
3. Further preclinical safety and tolerability studies.
4. Investigation of the mechanism of action.

Upon identification of an effective and potent analog with the properties required for clinical development, a clinical drug candidate will be nominated for formal IND-enabling studies.

We would like to thank Dr. Jian Liu for testing the compounds in sulfotransferase assays, Dr. Jeff Esko for collaborating on the Sensi-Pro assay and general consulting. Funding for this project comes from Avalon Ventures, the NINDS, The National MPS Society, and Team Sanfilippo.