Discovery of novel inhibitors of O-GlcNAc transferase through structure-based virtual screening

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Introduction

- The O-GlcNAc modification (Figure 1) is a widespread and reversible post-translational modification in eukaryotes.[1,2]
- Hyperactivity of O-GlcNAc Transferase (OGT) is implicated in cancer cell progression and tumor growth.[3]
- The development of drug-like inhibitors of OGT for use in pre-clinical studies remains a major challenge. Despite recent advances, currently available inhibitors suffer from structural liabilities and selectivity issues.
- To discover new inhibitory scaffolds for OGT, we performed virtual screening using the AtomNet® model, a unique artificial intelligence platform that applies deep convolutional neural networks to drug discovery.

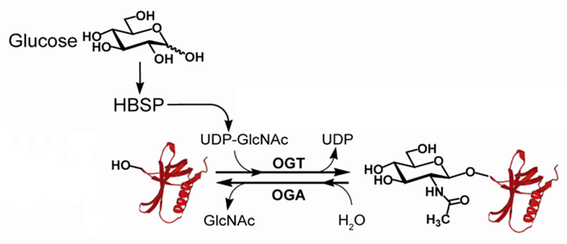


Figure 1: The *O*-GlcNAc modification. *O*-GlcNAc transferase (OGT) catalyzes addition of *O*-GlcNAc on serine and threonine residues of proteins using UDP-GlcNAc, which is derived biosynthetically from glucose. This modification impacts the stability and signalling activity of numerous proteins involved in cancer signalling and cell cycle control.

AtomNet® Model Virtual Screening Methodology

- The AtomNet® model uses a single global convolutional neural net trained to predict K_i and IC_{50} values from several million small molecule affinity labels and several thousand protein structures (Figure 2).
- Using this model, we screened a library of 3.5 million commercially-available compounds for potential small molecule inhibitors that bind in the active site of OGT. After clustering and filtering the top scoring 30,000 hits, we selected 96 potential inhibitors that were purchased for confirmation through in vitro assays.

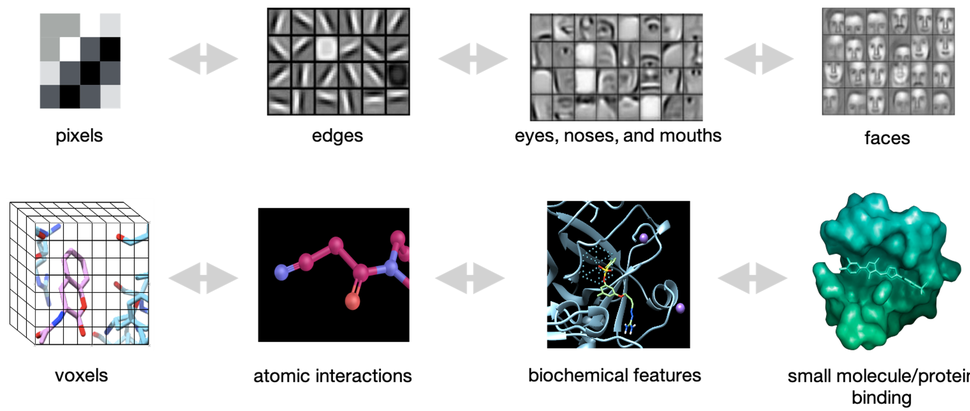


Figure 2: The AtomNet® virtual screening approach. AtomNet® technology uses deep convolutional neural networks (DCNNs) similar to those employed in image recognition and computer vision technologies but adapted for structure-based drug design and discovery. Trained on large data sets, comprising several thousand different protein structures and millions of protein-ligand binding affinity measurement, these DCNNs can systemically and efficiently learn optimal model parameters for performing robust predictions and finding small molecules that specifically bind to a protein of interest.

In vitro validation of inhibitors

Assay methods

- Our laboratory has developed a first-in-class direct fluorescent activity assay for OGT (Figure 3).
- This assay enables rapid evaluation of potential inhibitors in an automated 384-well microplate format.
- Top-scoring hit compounds from the AtomNet® model screen were commercially procured and evaluated against OGT using this assay.

Primary screening results

- 96 compounds were screened for inhibition towards OGT at a concentration of 100 µM. Screening was performed under balanced substrate conditions with robust signal/background and a Z' score of 0.64 (Figure 4A).
- Two compounds showing >50% inhibition were assessed in IC_{50} experiments and showed moderate inhibitory potency (Figure 4B, 4C).
- Inhibition from these compounds was confirmed using an orthogonal radioactivity-based assay (Figure 4D).

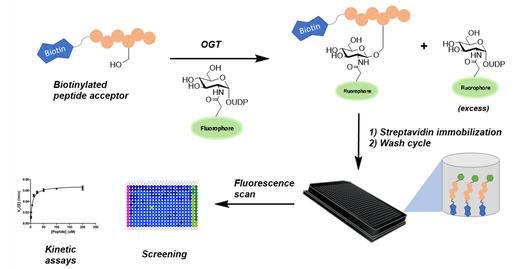


Figure 3: Methodology for fluorescent activity-based OGT assay and inhibitor screen.

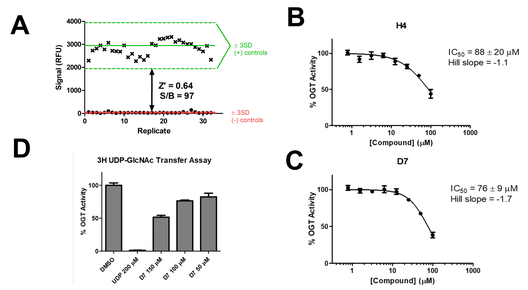
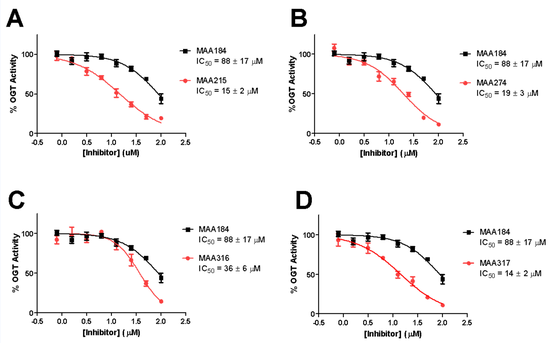


Figure 4: In vitro evaluation of inhibitors. A; screening assay provides robust signal under balanced conditions. B, C; concentration-response curves of hit compounds. D; Inhibition of OGT is observed in an orthogonal radioactivity assay.

SAR-by-catalog optimization

- A secondary SAR-by-catalog virtual screen was performed based around the inhibitory scaffolds identified
- Compounds in each structural family were scored and purchased for in vitro evaluation
- Four improved inhibitors with 3-5 fold increases in potency were identified
- Structure-activity relationships reveal the presence of conserved motifs and regions for further optimization with isosteric groups



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