Discovery of small molecule compounds interfering with YKL-40 carbohydrate binding as potential therapeutics for cancer

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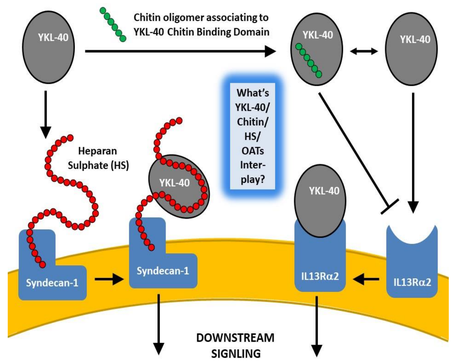
INTRODUCTION

YKL-40 is a chitinase-3-like 1 (CHI3L1) protein that unlike other chitinases (CHIT1 and AMCase) does not have enzymatic activity. Instead, YKL-40 is a carbohydrate-binding protein shown to bind chitin oligomers and suggested to bind heparin/heparan sulphate ligands.

Significantly elevated serum level of YKL-40 has been implicated in the pathology of cancer, inflammatory, and neurodegenerative diseases. The protein has been proposed to act through IL13Rα2 and Syndecan-1 receptors via respectively chitin-binding and heparan sulphate-binding properties, but its detailed biological functions are still poorly understood.



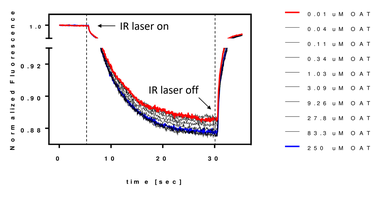
Herein, we demonstrate carbohydrate-binding properties of YKL-40. We also present the screening cascade of small molecule compounds capable of interfering with YKL-40 binding chitooligosaccharide ligands alone or chitooligosaccharide and heparan sulphate ligands concurrently.

HIT IDENTIFICATION USING MICROSCALE THERMOPHORESIS (MST)

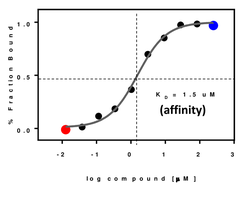
We identified YKL-40 binders including small molecule compounds (OATs), but also chitin oligomers, heparin (HEP), and heparan sulphate (HS) ligands using MicroScale Thermophoresis technology. We performed single-point screening on OAT's library of over 200 inhibitors of diversified structures and activities followed by K_D determination for promising hits. OAT compounds were confirmed to bind Chitin Binding Domain (CBD) of YKL-40 using X-Ray. Next, the most active OAT was biotinylated and its binding activity against CBD of YKL40 was confirmed with MST. The biotinylated OAT as well as biotinylated HS were necessary for setting up high-throughput competitive binding assays using AlphaScreen method (↗).

In MicroScale Thermophoresis direct binding assay, molecular diffusion through IR-induced temperature gradient (thermophoresis) is measured. Herein, thermophoresis of labeled YKL-40 changes upon ligand binding allowing for protein-ligand affinity (K_D) determination in titration experiments.

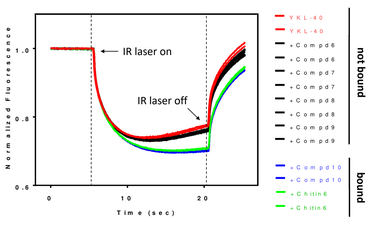
Example of thermophoretic traces of mYKL-40 at increasing OAT



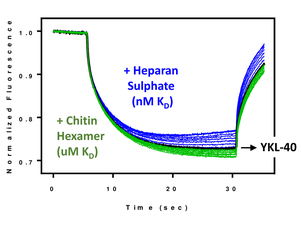
Resulting equilibrium binding curve of mYKL-40 and OAT



Example of MST single-point screening for YKL-40 binders



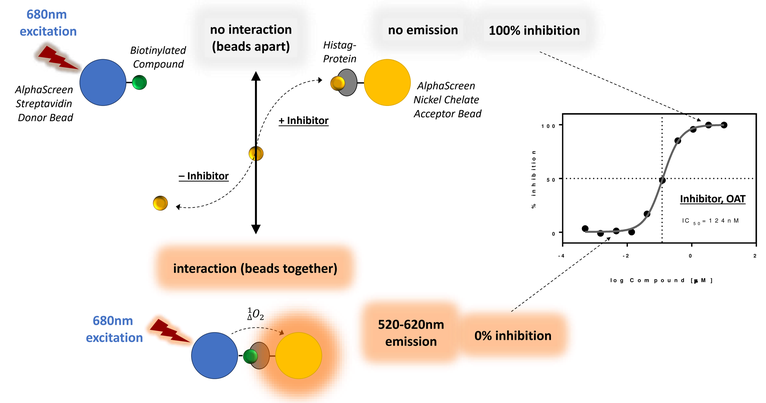
MST traces for YKL-40 binding different carbohydrates



ALPHASCREEN PRIMARY & COMPLEMENTARY SCREENING ASSAYS

We established two complementary indirect AlphaScreen assays for parallel small molecule compound screening against YKL-40. First AlphaScreen of YKL40-histag & OAT-biot to identify compounds capable to interfere with YKL-40: chitooligosaccharide interaction and second AlphaScreen of YKL40-histag & HS-biot to identify compounds capable to interfere with YKL-40: heparan sulphate (HS) interaction.

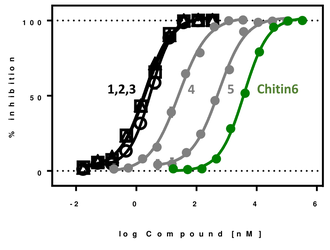
In AlphaScreen (AS) indirect competitive binding assay, the chemiluminescence signal generated due to AS beads bridging through interaction of biotinylated compound on 1st bead and histag protein on 2nd bead is decreased through biotinylated compound displacement by non-biotinylated compound (inhibitor, OAT ↓)



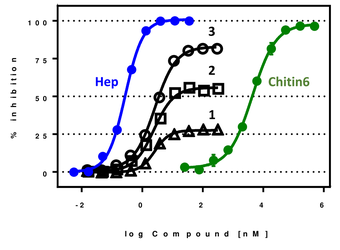
Data below demonstrate OAT compounds 1, 2, and 3 fully displacing biotinylated OAT from chitin binding site of YKL-40 with the same strength (affinity) via competitive mode, while simultaneously interfering with YKL-40: heparan sulphate interaction to a different degree (% inhibition) via non-competitive mode. Moreover, compounds of different affinity towards chitin binding site of YKL-40 can interfere similarly with YKL-40: heparan sulphate interaction, i.e. can interfere weakly (1), moderately (2) or strongly (3, 4, 5).

Natural YKL-40 carbohydrate ligands, chitin hexamer (Chitin6) and HS/HEP fully displace biotinylated OAT-biot and HS-biot in respective AlphaScreen tests. In addition Chitin6, unlike small molecule compounds can completely prohibit YKL40: heparan sulphate interaction.

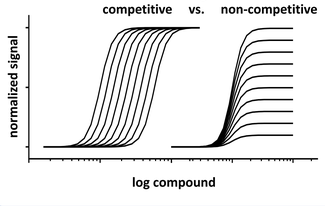
AlphaScreen of YKL40-histag & OAT-biot



AlphaScreen of YKL40-histag & HS-biot



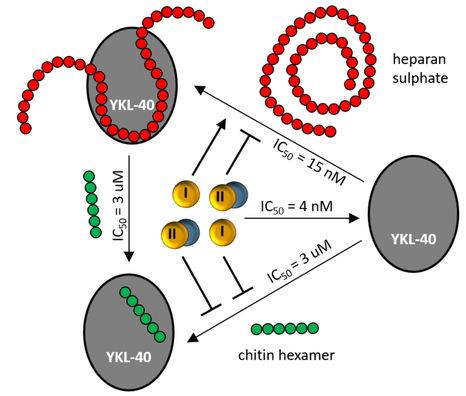
Binding mode: competitive vs. non-competitive



Ligand	AlphaScreen of YKL40-histag & OAT-biot affinity [nM]	% inh	AlphaScreen of YKL40-histag & HS-biot affinity [nM]	% inh
Chitin6	3000	100	3000	100
Hep	2.0	75	0.5	100
HS	15	50	15	100
Cmpd1 (I)	4.0	100	4.0	25
Cmpd2	4.0	100	4.0	50
Cmpd3 (II)	4.0	100	4.0	80
Cmpd4	40	100	6.0	80
Cmpd5	400	100	40	80

INTERPLAY OF YKL-40 INTERACTIONS WITH CARBOHYDRATES & OATS

Chitin oligomers can inhibit YKL-40 binding to heparan sulphate ligands. On the other hand, different OAT compounds bind to same chitin binding site of the protein and can solely interfere with YKL-40: chitin oligomer interaction (compound series I ↙→) or can collectively interfere with YKL-40: chitooligosaccharide and YKL-40: heparan sulphate interactions (compound series II ↙→). This also suggest that chitin binding site and heparan sulphate binding site on YKL-40 are not equivalent but may partially overlap.



CONCLUSIONS

- ✓ We established reliable high-throughput screening assays for identification of small molecule compounds binding chitin binding site of non-enzymatic YKL-40 protein
- ✓ We identified different groups of OAT compounds with distinct modes of action (series I and II) capable to inhibit any known YKL-40: carbohydrate binding activities
- ✓ Low nanomolar activities have been identified among both groups of compounds
- ✓ Parallel optimization of ADME and pharmacokinetic properties of compounds is underway
- ✓ *In vitro* cellular assays, and *in vivo* models to study OATs anti-cancer effects are under development

ACKNOWLEDGEMENTS

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