DISCOVERY OF SMALL MOLECULE COMPOUNDS THAT MODULATE BINDING OF YKL-40 WITH CARBOHYDRATES OR GALECTIN-3 AS POTENTIAL THERAPEUTICS FOR CANCER

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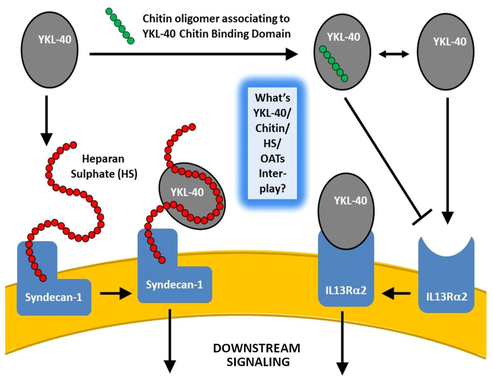
INTRODUCTION

Protein-carbohydrate interactions are important biological processes which include signaling, recognition and catalysis, and are dysregulated in diseases states. Specifically in cancer, important targets for therapeutic intervention are the binding processes mediated through multivalent protein-carbohydrate interactions. Among others, interaction between chitinase-3-like protein 1 (CHI3L1, YKL-40) and carbohydrates gains a particular interest in the field of immuno-oncology. YKL-40 is a secreted glycoprotein that, unlike other chitinase family members (CHIT1 and AMCase), does not have enzymatic activity. YKL-40 interacts and subsequently determines functionality of protein such as with IL13Rα2, Syndecan-1, p53, Galectin-3 *via* either chitin-binding or heparin-binding domains.

Herein, we report carbohydrate-binding properties of YKL-40. In addition, the screening cascade of small molecule compounds capable of interfering with YKL-40 binding chitooligosaccharide ligands alone or hexamer of chitin and heparin/heparan sulphate ligands concurrently is demonstrated. Finally, we provide evidence that the use of our compounds enables better understanding of YKL-40 interactions at the molecular level and present a profound approach for the development of novel, effective and highly selective therapeutics.

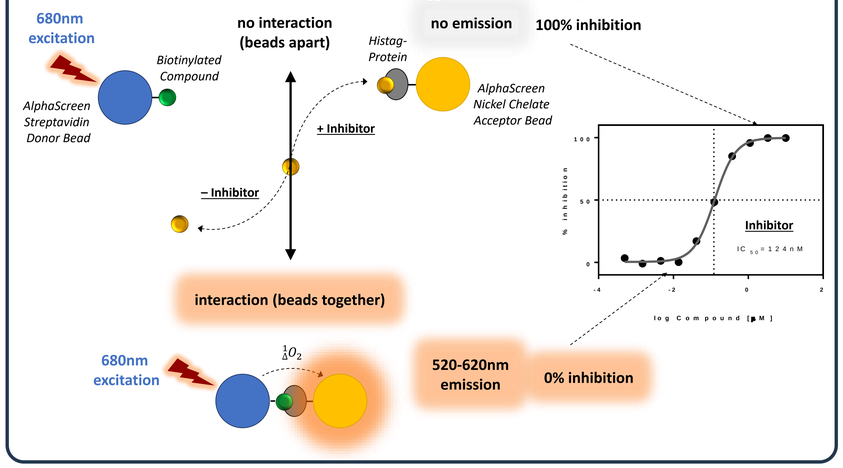


ALPHASCREEN AS A PRIMARY SCREENING ASSAY

To identify YKL-40 binders among our small molecule compounds library, we implemented an AlphaScreen method as a high-throughput primary screening assay. We established two complementary assays:

- **AlphaScreen of YKL40-histag & OAT-biot** to identify compounds capable to interfere with YKL-40: chitooligosaccharide interaction
- **AlphaScreen of YKL40-histag & HS-biot** to identify compounds capable to interfere with YKL-40: heparan sulphate (HS) interaction.

Firstly, using MicroScale Thermophoresis (MST) technology we found the promising hit with a nanomolar K_D value. Afterward this compound was biotinylated and immobilized on donor bead for binding to chitin-binding pocket of YKL-40 immobilized on the acceptor bead in AlphaScreen of YKL40-histag & OAT-biot. In turn, biotinylated HS was used in AlphaScreen of YKL40-histag & HS-biot. AlphaScreen assay was optimized to obtain IC_{50} values close to K_D.



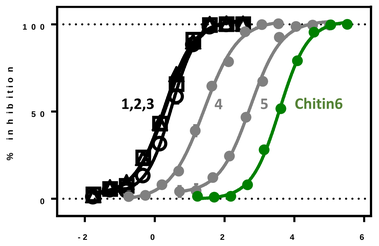
COMPOUNDS ACTIVITY

Our research showed that natural YKL-40 carbohydrate ligands: **chitin hexamer (Chitin6**) and **HS/HEP** fully displaced biotinylated compound (Cmpd-biot) and biotinylated heparane sulfate (HS-biot) in respective AlphaScreen tests. In addition, Chitin6, unlike small molecule compounds, can completely prohibit YKL40: heparan sulphate interaction.

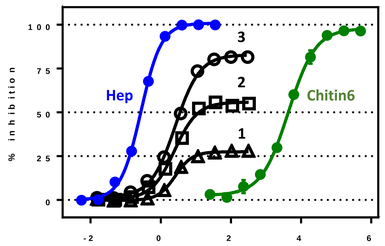
Among our small molecule library, we found compounds binding to the chitin binding site of YKL-40 with a different affinity. **Compounds 1**, **2** and **3** fully displaced Cmpd-biot from chitin-binding site of YKL-40 with the same strength (affinity, IC_{50} = 4 nM) *via* competitive mode, while **compounds 4** and **5** bound to the chitin-binding site of YKL-40 weaker.

Simultaneously **compounds 1**, **2** and **3** interfered with YKL-40: heparan sulphate interaction to a different degree (% inhibition) via non-competitive mode. In turn, **compounds 4** and **5**, although are weaker binder to YKL-40, can strongly interfere with YKL-40: heparan sulphate interaction.

AlphaScreen of YKL40-histag & Cmpd-biot



AlphaScreen of YKL40-histag & HS-biot



Ligand	AlphaScreen of YKL-40-histag & Cmpd-biot affinity [nM]	% inh	AlphaScreen of YKL-40-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
Cmpd6	3.2	100	n.d.	-30

% inh at the compound's concentration 100 nM; HEP – heparin, HS- heparane sulfate, n.d. – not determined

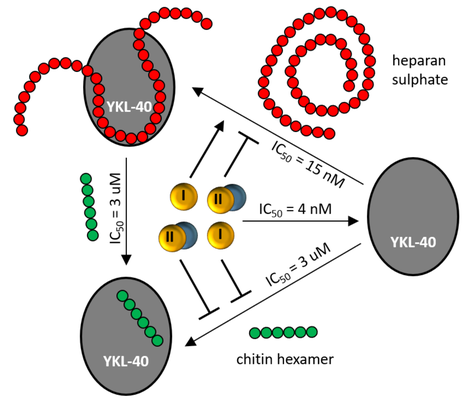
CONCLUSIONS & PERSPECTIVES

Our investigations resulted in:

- establishment of reliable high-throughput screening assay for identification of small molecule compounds interacting with chitin-binding site of YKL-40
- characterization of chitooligosaccharides binding and heparins/heparan sulphates binding to YKL-40 and determination of interplay between both types of these interactions
- development of different groups of compounds with nanomolar activities and unique modes of action that inhibit any known binding of YKL-40 and carbohydrates
- identification of lead compound, for which optimization of ADME and PK properties as well as evaluation of anti-cancer effect *in vivo* is currently under investigation

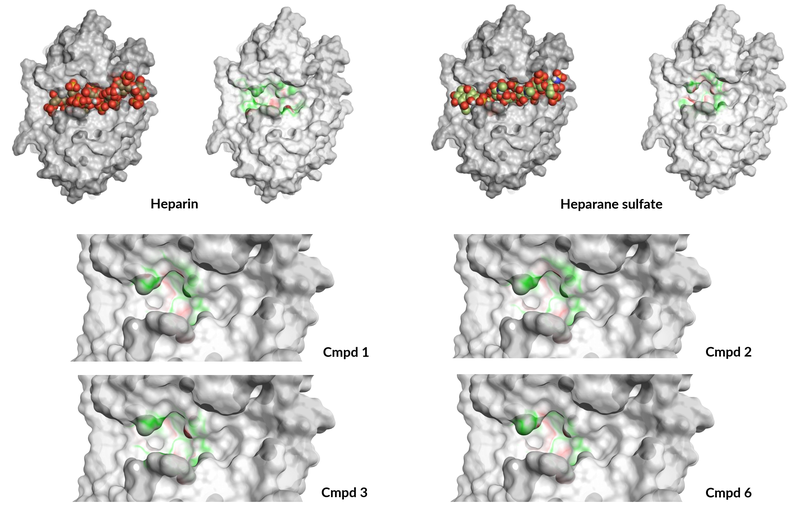
INTERPLAY OF YKL-40 INTERACTIONS WITH CARBOHYDRATES & OUR COMPOUNDS

Chitin oligomers can inhibit YKL-40 binding to heparan sulphate ligands. On the other hand, different compounds bind to the 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.



MOLECULAR MODELING

Docking experiments confirmed very high affinity of heparin and heparan sulfate toward YKL -40. In line with AlphaScreen results, compounds with higher percent of inhibition showed better electrostatic complementarity projected on the surface representations of YKL-40 structure.



The docking poses of heparin and heparan sulfate in YKL-40 have been obtained on the basis of template docking, using LeadFinder scoring function by Cresset. Electrostatic complementarity between compounds Heparin, Heparan sulfate (HS), Cmpd 1, Cmpd 2, Cmpd 3 and Cmpd 6 projected on the surface representations of the YKL-40 were calculated using Flare v6 (Cresset) software. Green and red represent good and bad complementarity, respectively.

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European Union
European Regional Development Fund

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Smart Growth

The research was carried out as part of the Project: "Development of a first-in-class small molecule drug candidate for cancer treatment through YKL-40 inhibition", co-financed by the European Union under the European Regional Development Fund.

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