Determination of the affinity and kinetic rate constants of galectin inhibitors using surface plasmon resonance

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Introduction

The galectins are a family of 15 soluble cytosolic proteins found in mammals that are defined by structurally similar carbohydrate recognition domains (CRD) that recognize sugar residues on the surface of cells.

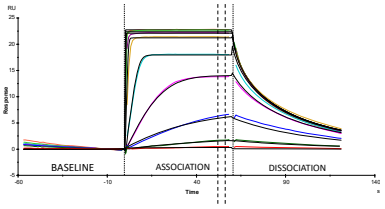
For the development of small molecule inhibitors of galectins, historically the standard approaches for determining affinity and selectivity have been fluorescence polarization or homogenous time-resolved fluorescence. In this study we investigated surface plasmon resonance (SPR) as an alternative approach for defining mouse/human galectin-1 (Gal-1) and galectin-3 (Gal-3) affinity and selectivity with the additional focus on association & dissociation kinetics for these interactions.

Here we present a study to rapidly screen compounds for affinity to Gal-1 and Gal-3 CRD sites in both human and mouse systems to rank and select lead compounds.

Results

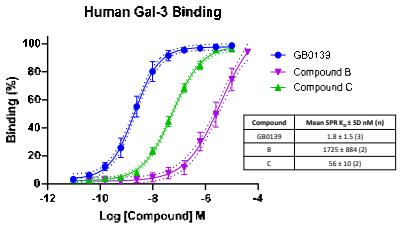
Compound Screening Assay

Steady state measurement of binding



Steady state measurement of response was taken 3 seconds prior to end of association in 'late binding phase'

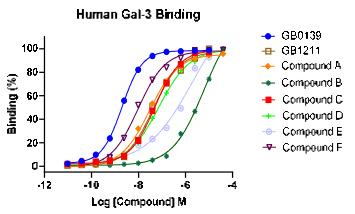
Reproducibility of K_D determination



Compound	Mean SPR K_D ± SD nM (n)
GB0139	1.8 ± 1.5 (3)
B	1725 ± 884 (2)
C	56 ± 10 (2)

K_D determined by SPR was reproducible between experimental replicates.

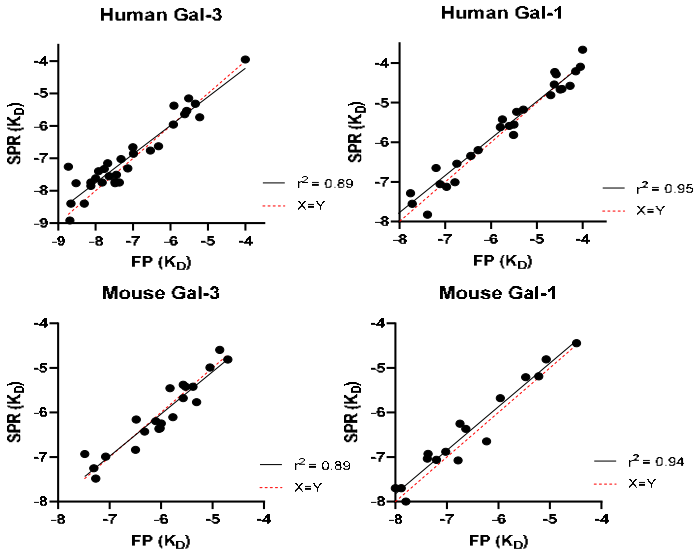
Ranking of compounds



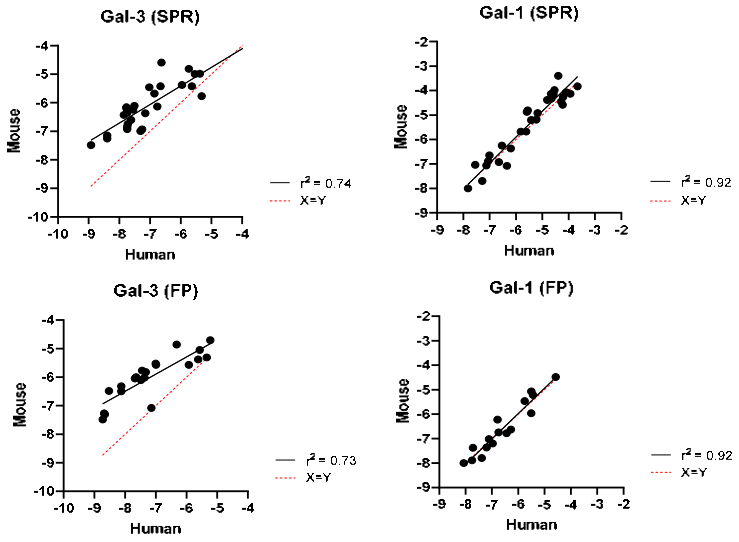
From the steady state response, compounds could be ranked based on competition binding IC_{50} values

Dissociation Constant Screen of Compounds Binding Human and Mouse Galectin-1 and Galectin-3

Comparison of K_D values between FP and SPR binding assay



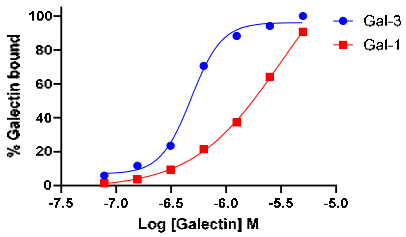
Comparison of K_D values between human and mouse Gal-1 and Gal-3 binding assays



Galectins and SARS-CoV-2

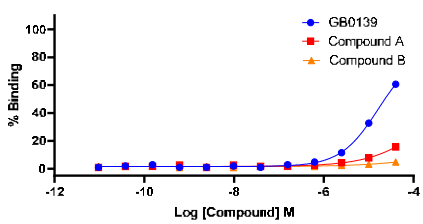
Gal-1 and Gal-3 have been implicated in COVID-19[1,2] as potential therapeutic targets and a clinical study investigating GB0139 (see also ELRIG poster 29) has recently been completed[3]. Therefore, the interaction between the SARS-CoV-2 RBD protein and these galectins was investigating. SARS-CoV-2 RBD protein was immobilised on a CM5 sensor chip using amine coupling. Galectin or inhibitor was then associated at varying concentrations, with steady state binding response plotted on the graphs below.

Galectins binding to SARS-CoV-2 RBD



Both galectins demonstrated binding to SARS-CoV-2 RBD (Gal-3 = 0.8μM, Gal-1 = 2.8 μM).

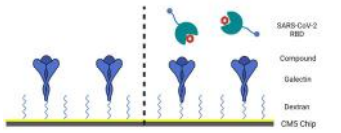
Galectin inhibitors binding to SARS-CoV-2 RBD



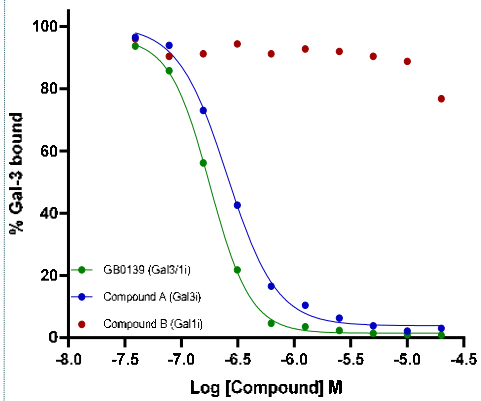
Galectin inhibitors bind weakly to the SARS-CoV-2 RBD.

Galectin-1 and Galectin-3 Binding to SARS-CoV-2 is CRD Dependent

Gal-1 (1 μM) or Gal-3 (0.6 μM) was associated over immobilised SARS-CoV-2 RBD protein in the presence of titrated inhibitor (GB0139 (Gal-3/1i), Compound A (selective Gal-3i) and Compound B (selective Gal-1i)). Steady state response was plotted for each concentration of compound with Gal-1 and Gal-3 CRD inhibitors shown to inhibit their respective galectin binding partner.

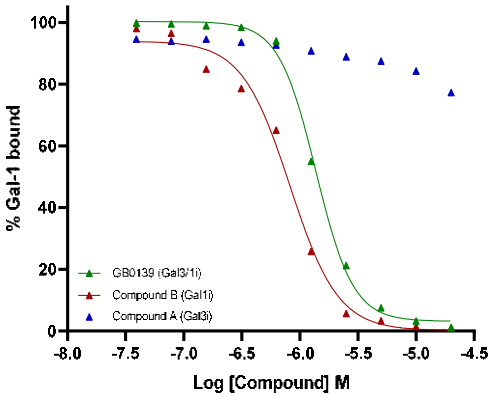


Inhibition of Gal-3:SARS-CoV-2 RBD binding



Compounds	IC_{50} (nM)
GB0139 (Gal3/1i)	179
Compound A (Gal3i)	255
Compound B (Gal1i)	>20,000

Inhibition of Gal-1:SARS-CoV-2 RBD binding



Compounds	IC_{50} (nM)
GB0139 (Gal3/1i)	1373
Compound A (Gal3i)	821
Compound B (Gal1i)	>20,000

Assays shows clear, target specific inhibition of the interactions between both Gal-1 and Gal-3 with SARS-CoV-2 RBD.

Conclusion

We have developed a high throughput affinity screening assay for small molecule galectin inhibitors of ranging affinities (1nM to 10μM) using SPR. In addition, we have demonstrated that Gal-1 and Gal-3 bind to the SARS-CoV-2 RBD and that the interaction is CRD binding site mediated.

References

1. Caniglia, J.L. *et al.*, *PeerJ* (2020)
2. Kazancioglu, S. *et al.*, *Jpn J Infect Dis* (2021)
3. ClinicalTrials Identifier: NCT04473053

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