The Efficacity of DCIR Inhibitors to Limit HIV-1 Infection

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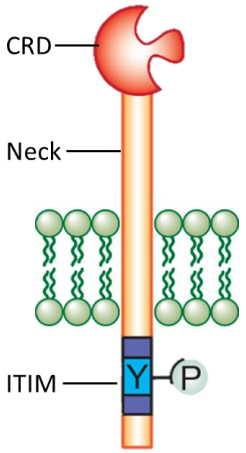
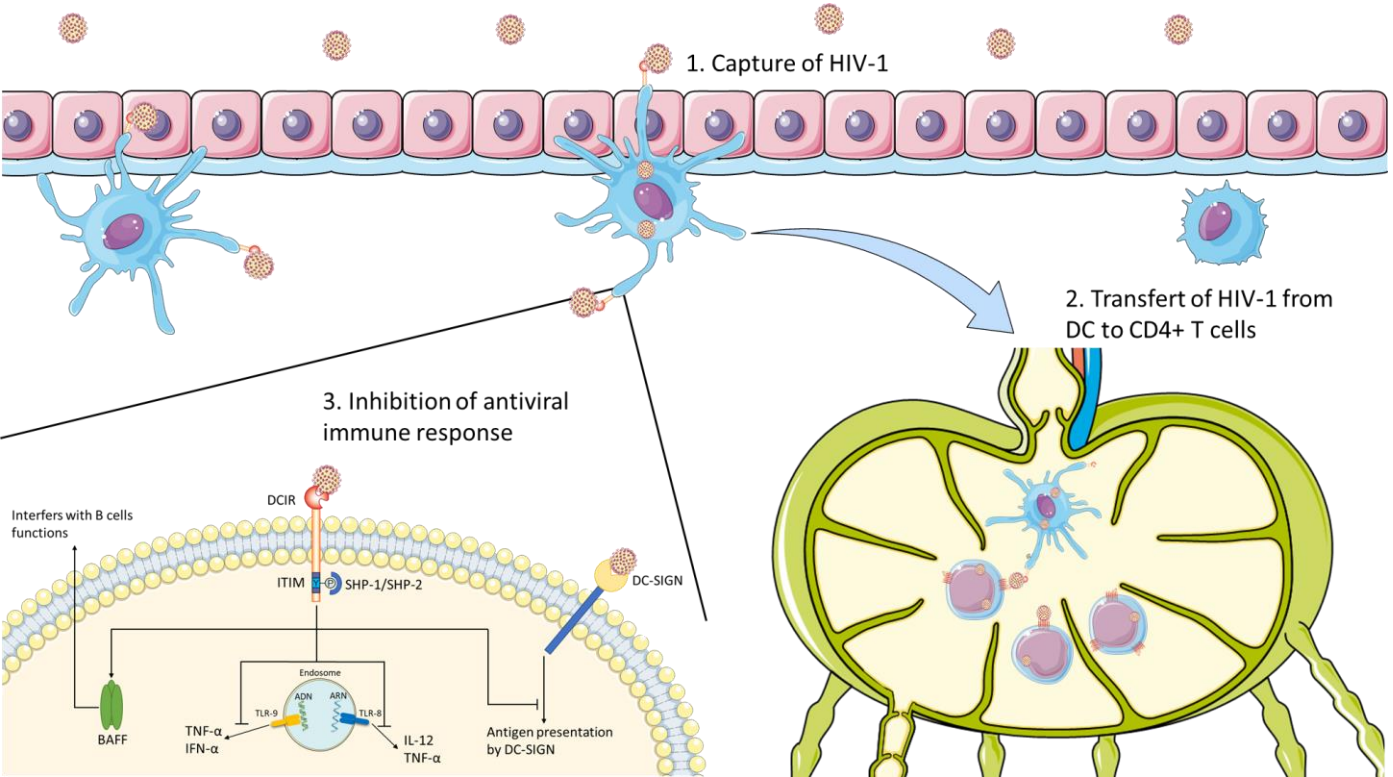
The Dendritic Cell Immunoreceptor (DCIR)



DCIR is a type C lectin composed of a Carbohydrate Recognition Domain (CRD) for interaction with ligands, a Neck domain potentially required for oligomerization of multiple DCIR and an Immunoreceptor Tyrosine-based Inhibitory Motif (ITIM) for the intracellular signalisation upon receptor activation.

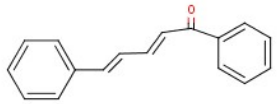
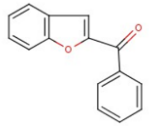
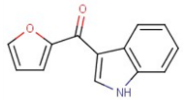
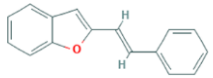
This lectin is constitutively expressed on neutrophils, macrophages, monocytes, dendritic cells and B cells, but its expression can also be induced on CD4+ and CD8+ T cells and natural killer cells.

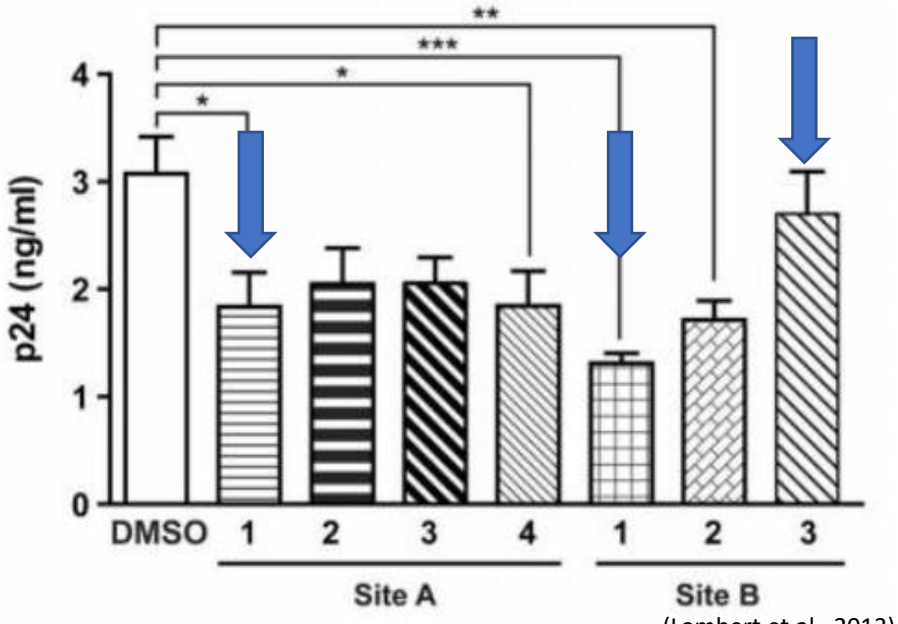
We are particularly interested in studying the DCIR on dendritic cells in the context of HIV-1 infection because of its early roles in HIV-1 pathogenesis.



1. DCIR interacts with gp120 and gp140 of HIV-1, resulting in the capture of the virus by dendritic cells.
2. Dendritic cells carry HIV-1 to the lymph nodes where DCIR is implicated in the transfer of the virus to CD4+ T cells.
3. Upon activation, the ITIM is phosphorylated and DCIR interferes with multiple antiviral immune responses such as DC-SIGN mediated antigen presentation, production of IFN-α, IL-12 and TNF-α from TLR activation and BAFF production by dendritic cells.

Therefore, we hypothesized that using DCIR inhibitors would prevent HIV-1 infection by limiting attachment between DCIR and gp120/gp140 of HIV-1, hence preventing the infection of CD4+ T cells by transfer of the virus from dendritic cells and avoiding the inhibition of the antiviral immune response.

Inhibitors	Name	Structure	anti-HIV activity
A1	1,5-diphenylpenta-2,4-dien-1-one		++
B1	1-benzofuran-2-yl(phenyl)methanone		+++
B3	2-furyl(1H-indol-3-yl)methanone		+
B4	2-(2-phenylvinyl)-1-benzofuran		?



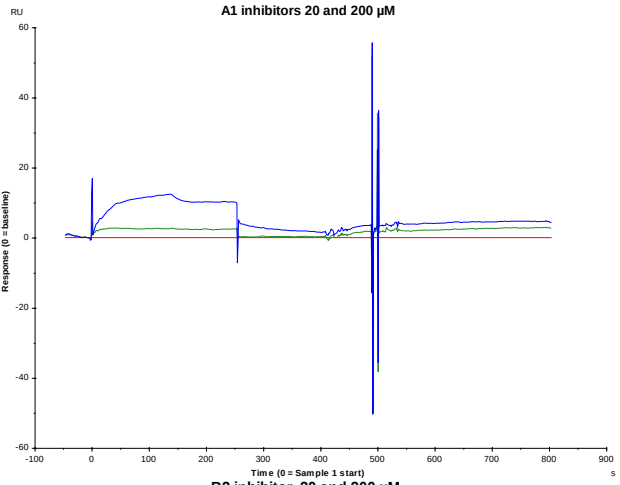
(Lambert et al., 2013)

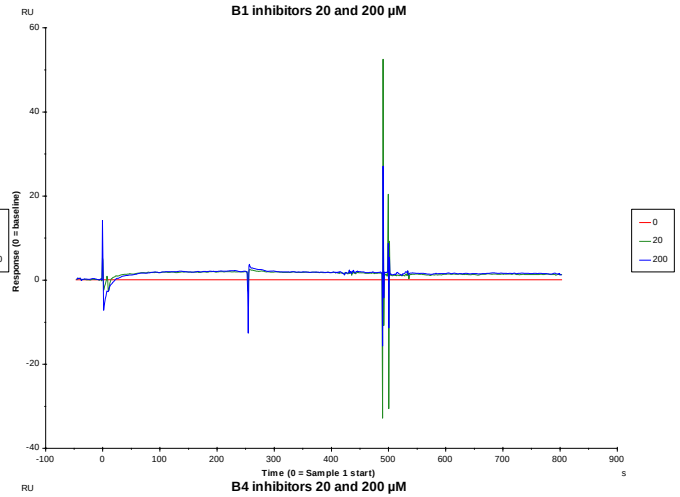
To identify DCIR inhibitors, the structure of the receptor was modeled *in silico* according to its sequence and multiple compounds were screened by virtual docking to this model. Sixteen inhibitors were selected for their potential ability to limit HIV-1 binding to cells expressing DCIR. The table above shows the 4 inhibitors that will be used in my project.

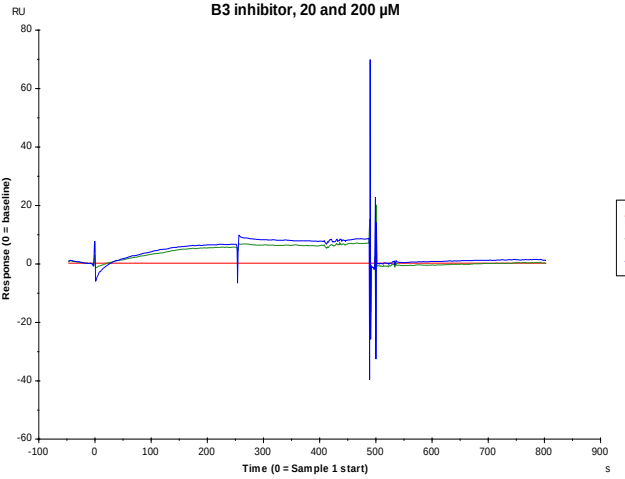
In order to measure the inhibitors' anti-HIV activity, Raji cells expressing DCIR were treated with an inhibitor at 10 µM for 10 minutes, then the cells were pulsed with NL4-3 for 1 hour. The cells were washed and their p24 content was determined by ELISA. The inhibitors A1 and B1 were able to reduce significantly HIV-1 binding to Raji-DCIR cells, but not B3. B4 was not tested in this experiment.

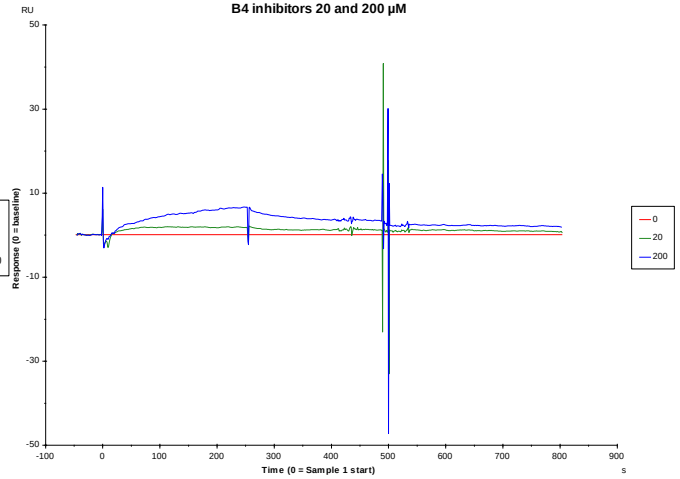
We wanted to know more about the molecular interactions between DCIR and the 4 inhibitors, so we studied them using Surface Plasmon Resonance (SPR).

Using SPR to detect molecular interactions between DCIR and the inhibitors

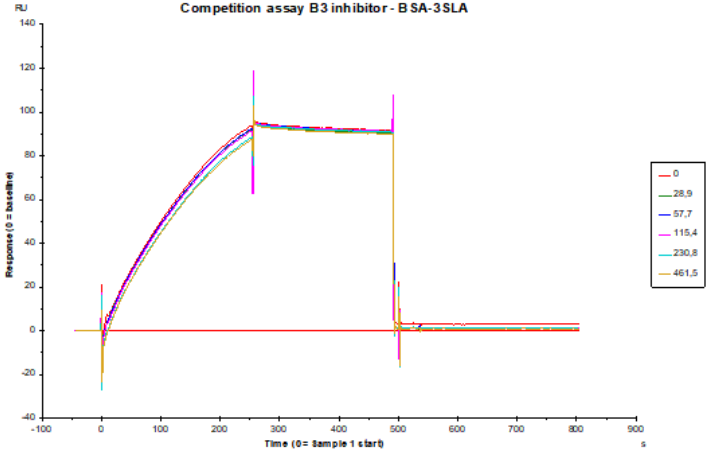


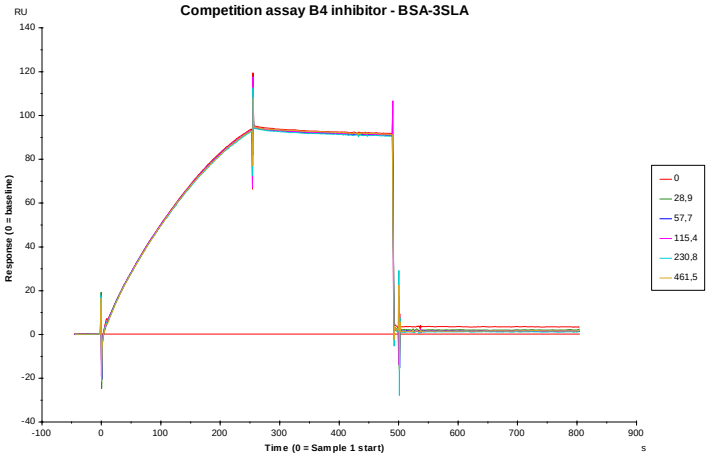






A chip was functionalized with DCIR and inhibitors were sequentially injected onto the surface at 20 and 200 µM. The inhibitors A1, B3 and B4 interacted with DCIR in a concentration-dependant manner. Interactions between B1 and DCIR were detected, but very weak.





A competition assay was performed where 3-sulfo-Lewis^a was injected onto the surface with increasing concentration of inhibitors. The inhibitors were not able to diminish interactions between DCIR and 3-sulfo-Lewis^a. Hence, the inhibitors interact with a different site than the CRD of DCIR.

Conclusion

DCIR plays multiple roles in HIV-1 infection, so we proposed that inhibiting this receptor could prevent HIV-1 infection.

DCIR inhibitors A1 and B1 prevented HIV-1 binding to Raji-DCIR cells.

DCIR inhibitors A1, B3 and B4 interacted directly with DCIR, but were not able to inhibit interactions between DCIR and its ligand 3-sulfo-Lewis^a.

The mechanism by which the inhibitors limit HIV-1 binding to DCIR has yet to be solved, but it is known that the inhibitors do not interact with DCIR in the same site as 3-sulfo-Lewis^a.

Perspectives

Further studies are required in order to fully understand the inhibitors' mechanism of action.

We are currently studying the use of the inhibitors in prophylaxis in humanised NSG mice infected with VIH-1.

The *in vitro* and *in vivo* results obtained will allow the development of a derivation program from the lead compound identified.

Acknowledgments

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