Looking for novel p24 multimerization inhibitors of FIV.

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Graphical Abstract

400 Compounds Tested
Abstract: Feline immunodeficiency virus (FIV) is a member of the retroviridae family of viruses. It causes an acquired immunodeficiency syndrome (AIDS) in domestic and non-domestic cats worldwide, representing an important veterinary issue. Genome organization of FIV and clinical characteristics of the disease caused by the virus are similar to those of human immunodeficiency virus (HIV). Both viruses infect T lymphocytes, monocytes and macrophages, and their replication cycle in infected cells is analogous. Thus, infection of cats with FIV is also a useful tool to study and develop novel drugs and vaccines against HIV. Anti-retroviral drugs studied extensively in HIV infection have targeted different steps of the virus replication cycle, like the inhibition of virion assembly and maturation. Despite much success of anti-retroviral therapy in slowing HIV progression in humans, similar therapy has not been thoroughly investigated for FIV infection in cats. FIV capsid protein (CA) drives the assembly of the viral particle, which is a critical step in the viral replication cycle. During this step, the CA protein oligomerizes to form a protective coat that surrounds the viral genome. In this article we perform a large screening of four hundred molecules from our in-house library. We used an in vitro assembly assay of p24, combined with microscale termophoresis to estimate binding affinity. This screening led to the discovery of around 5 novel hits to drug development and drug design for new antiviral drugs.

Keywords: Assembly inhibitors, immunodeficiency virus, microscale termophoresis
Introduction

• Feline Immunodeficiency Virus
  • Related to HIV
  • AIDS-like disease in felines (domestic cats, wild felines).

• Veterinary issue:
  • >50 million domestic cats infected worldwide.
  • 2.5% of domestic cats are seropositive for FIV in the United States.

=> Need for the rational development of therapeutic drugs against FIV
Genomic and viral organization of FIV:

- Enveloped virus (~100nm).
- Canonical retroviral genes (gag, pol, env) => structural proteins
- Regulatory/accessory genes.
Gag, motor of the viral particle assembly:

N-terminal myristoylated polyprotein (53kDa):

- Gag is targeted to the viral membrane through its N-myristoyl moiety.
- The oligomerization of the polyprotein leads to assembly, budding and maturation of viral particles.
- Proteolysis of the polyprotein by the viral protease leads to a reorganization of its subunits, creating the mature conical viral core which is necessary for infectivity.
The capsid sub-unit of Gag, the architect of the viral core:

- $\alpha$-helical protein with two domains separated by a flexible linker
- Hexameric or pentameric in the mature viral particle.
The recombinant capsid protein can spontaneously assemble \textit{in vitro} in presence of salt:

- Protein:protein interactions close to those observed in the viral particle.
Results and discussion

Screening in the polymerization assay of p24.

In a multiwall plate reader, using 384 well plates, at 39°C for 40 min.
Checking the interaction by thermophoresis.
New HITs
Docking analysis of the HIT-P24 interaction.

Surface accessible to solvent
Discussion

Docking experiments reveal a preferential position in the pocket between helices $\alpha_3$, $\alpha_4$ and $\alpha_7$ of FIV p24. In HIV-1, helices $\alpha_4$ and $\alpha_7$ are involved in the protein:protein interactions with neighbouring monomers, leading to the formation of p24 hexamers during capsid formation.
Conclusions

We tested in the assembly assay for p24 of FIV around 400 compounds from our in-house library. We found and checked by thermophoresis and docking 5 new hits for antiviral drug development as p24 assembly interrupters.