TOWARDS THE DESIGN OF MULTIVALENT FUSION PEPTIDE INHIBITORS OF HEMAGGLUTININ

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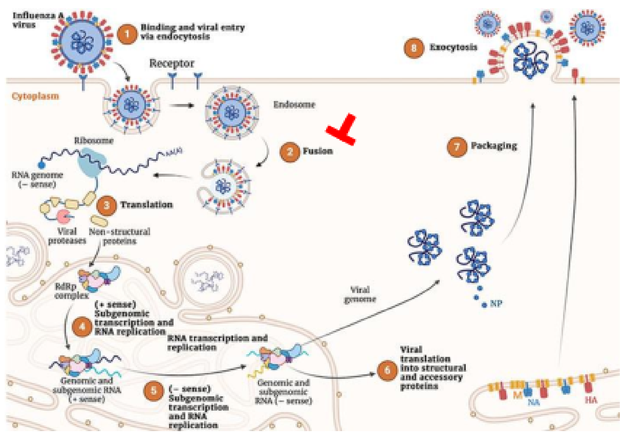
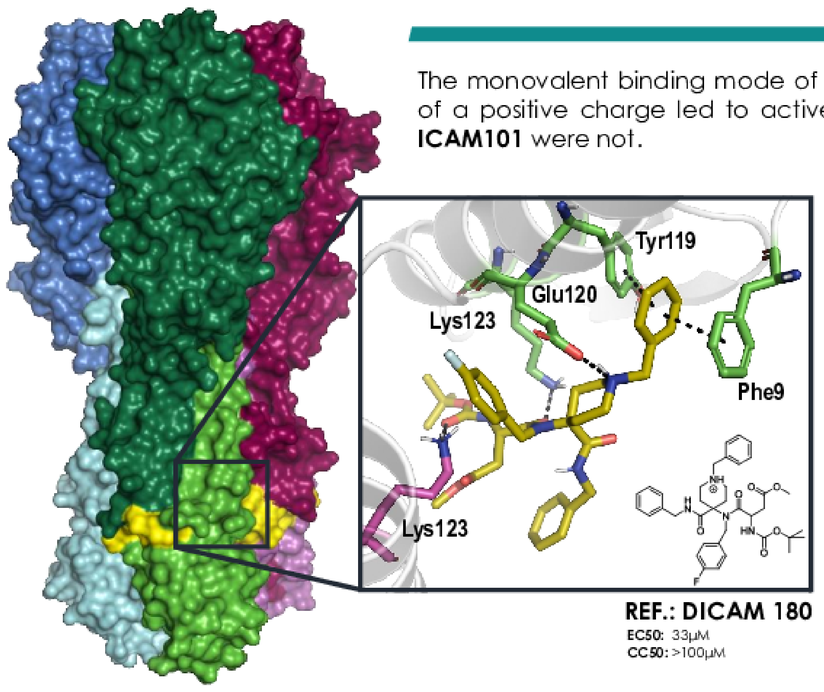
ABSTRACT

Hemagglutinin (HA) is one of the glycoproteins present in the viral envelope of Influenza A virus (IAV). This protein plays a key role in early stages of the infection and facilitates viral entry and fusion of viral and host membranes in the endosome [1]. Previous studies carried out by the IQM-CSIC members identified **DICAM180** as a potent fusion inhibitor that targets Phe9 within the fusion peptide and blocks the fusion process [2,3]. Based on the binding mode of **DICAM180**, several chemical and structural modifications have been explored to enhance the potency of **DICAM180**. These results highlight the relevant influence exerted by an additional positive charge in multivalent derivatives designed to bind simultaneously two protomers of HA.

OBJECTIVES

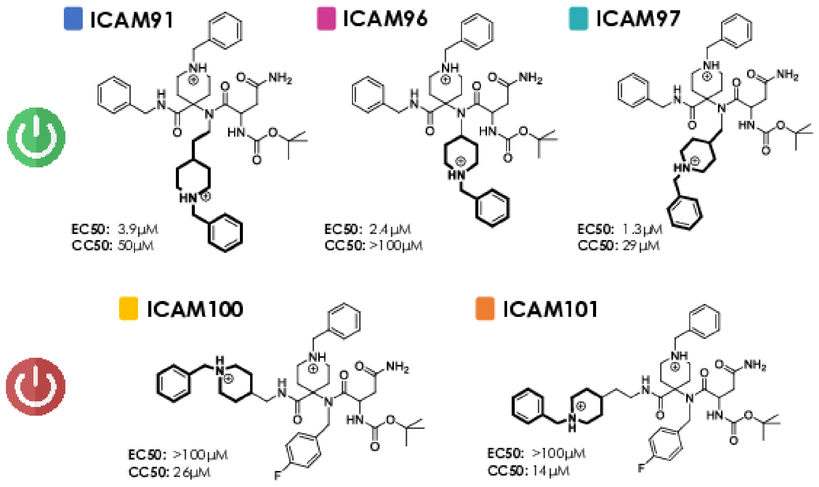
- Block the release of the fusion peptide (FP, yellow): inhibition of the fusion membrane process.
- Optimizing **DICAM180** to achieve a bivalent interaction with Phe9 (from FP) and enhance the antiviral potency.



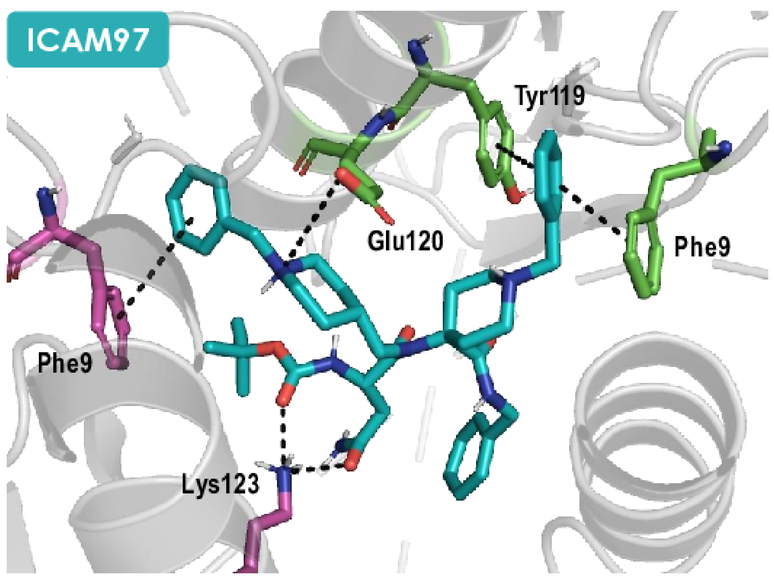


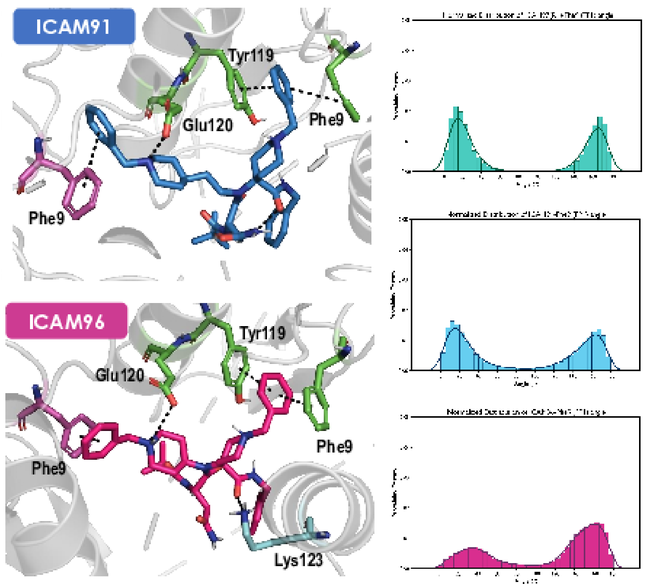
LIGANDS

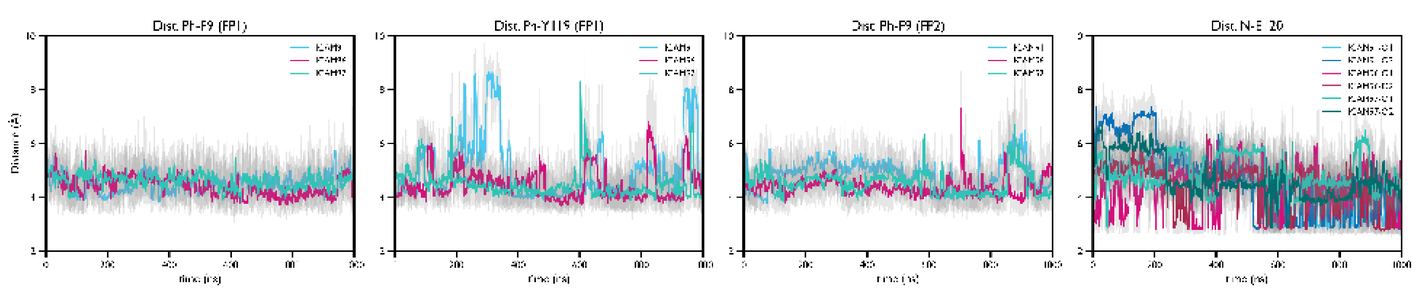
The monovalent binding mode of **DICAM180** was the template to design the novel ligands. The addition of a positive charge led to active compounds **ICAM91**, **ICAM96** and **ICAM97**, whereas **ICAM100** and **ICAM101** were not.

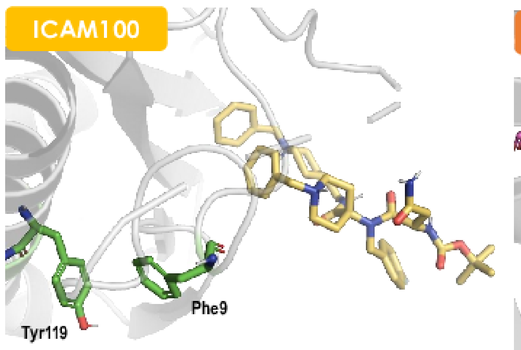


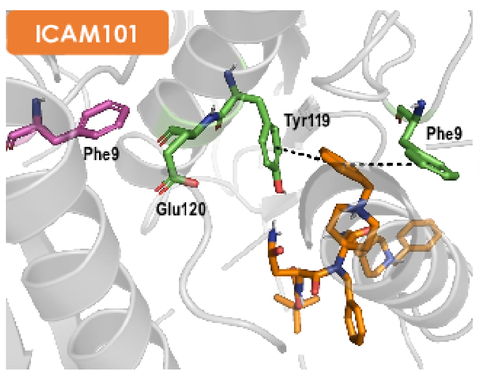
RESULTS

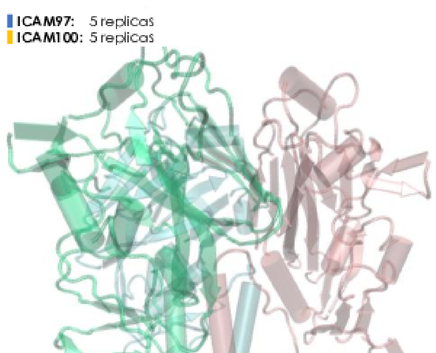


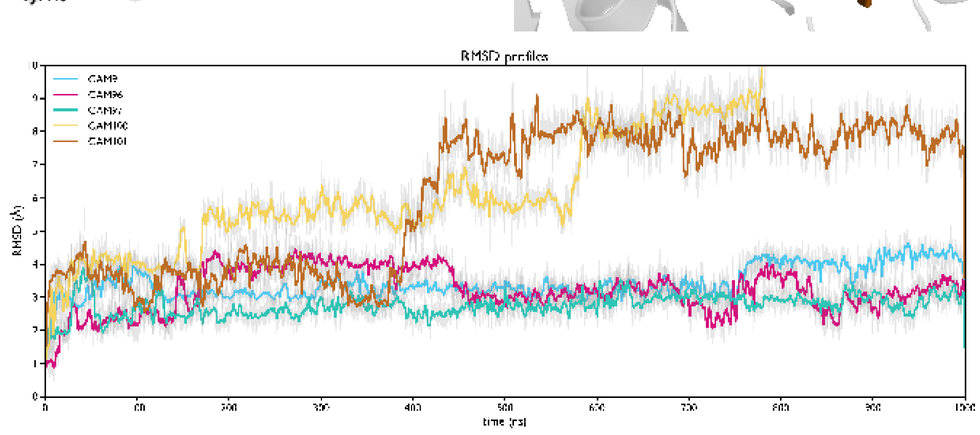






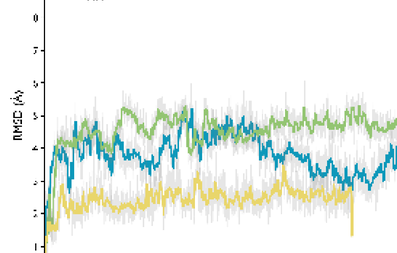


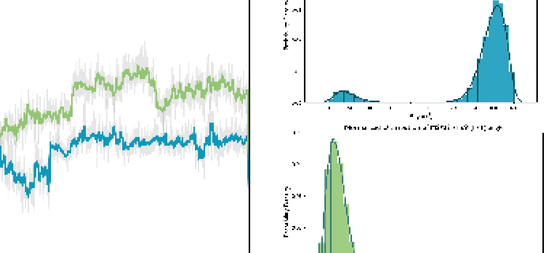




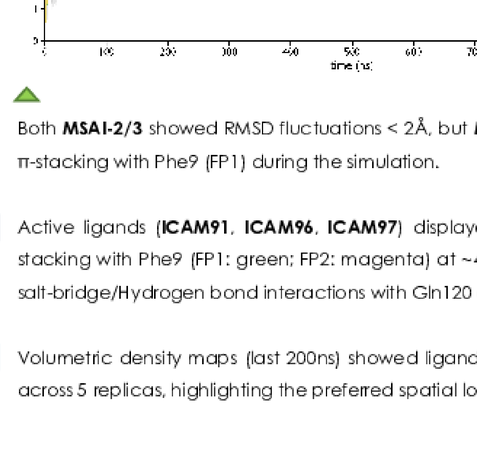
CYTOTOXICITY

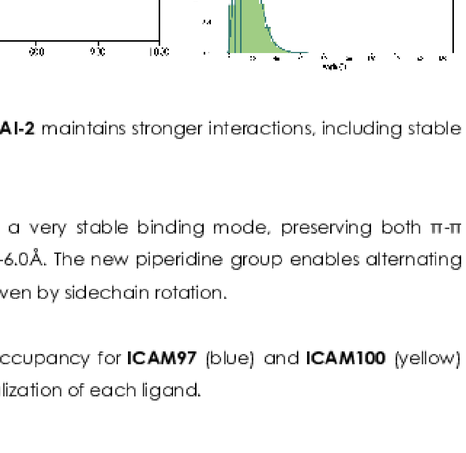
The cytotoxicity of the compounds with a single positive charge may, at least in part, be attributed to the formation of aggregation.
Conductivity measurements and Dynamic Light Scattering (DLS) suggest that the CMC of these compounds is around **85 μM**:

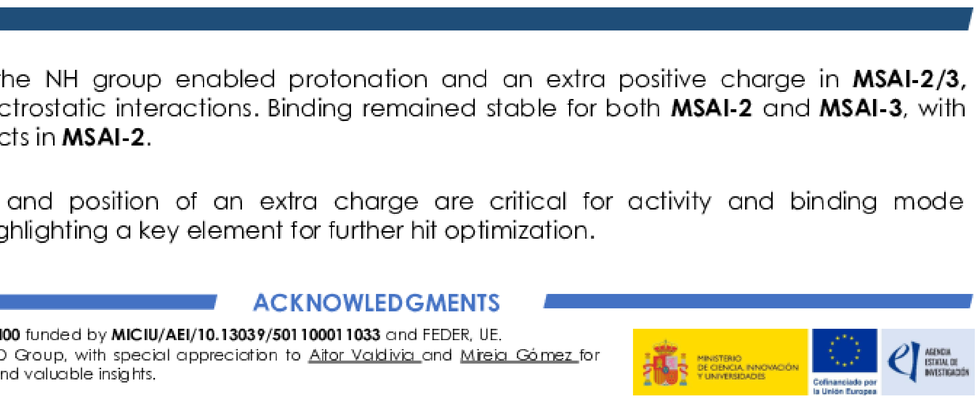




MAY THE POSITION OF THE NH AFFECT THE CYTOTOXICITY?







Both **MSAI-2/3** showed RMSD fluctuations < 2Å, but **MSAI-2** maintains stronger interactions, including stable π-stacking with Phe9 (FP1) during the simulation.

Active ligands (**ICAM91**, **ICAM96**, **ICAM97**) displayed a very stable binding mode, preserving both π-π stacking with Phe9 (FP1: green; FP2: magenta) at ~4.0-6.0Å. The new piperidine group enables alternating salt-bridge/Hydrogen bond interactions with Gln120 driven by sidechain rotation.

Volumetric density maps (last 200ns) showed ligand occupancy for **ICAM97** (blue) and **ICAM100** (yellow) across 5 replicas, highlighting the preferred spatial localization of each ligand.

DISCUSSION

- Adding a protonated benzylpiperidine at the central nitrogen (**ICAM91**, **ICAM96** and **ICAM97**) improved activity and stabilized binding via a dual interaction with Glu120, alternating between salt-bridge and hydrogen-bond-like contacts. In contrast, modifications in R2 (**ICAM100**, **ICAM101**) led to binding destabilization or ligand escape from the pocket.
- Despite favorable predictions for chemical modification that retain a single positive charge in the chemical skeleton, these ligands were inactive and cytotoxic. Self-aggregation was considered as a cause for the difference between predictions and inhibitory potency.
- Repositioning the NH group enabled protonation and an extra positive charge in **MSAI-2/3**, enhancing electrostatic interactions. Binding remained stable for both **MSAI-2** and **MSAI-3**, with stronger contacts in **MSAI-2**.
- The presence and position of an extra charge are critical for activity and binding mode stabilization, highlighting a key element for further hit optimization.

REFERENCES

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