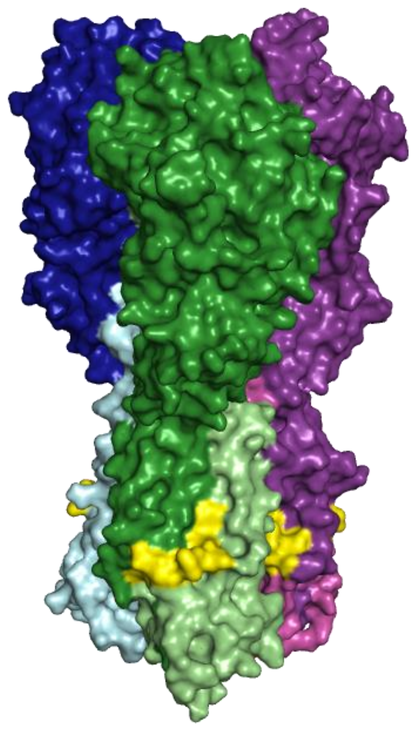
TOWARDS THE DESIGN OF MULTIVALENT FUSION PEPTIDE INHIBITORS OF HEMAGGLUTININ

F. J. Hermoso-Pinilla,[1] de la Cruz Potenciano, A.,[2] S. Velázquez,[2] M. J. Camarasa,[2] T. Ginex,[3] F. J. Luque[1]

[1] CBDD Group, Facultat de Farmàcia i Ciències de l'Alimentació, Institut de Biomedicina, Universitat de Barcelona, Sta. Coloma de Gramenet.
[2] Instituto de Química Médica (C.S.I.C.), Juan de la Cierva 3, E-28006 Madrid.
[3] Pharmacelera, Parc Científic de Barcelona (PCB), Baldiri Reixac 4-8, 08028 Barcelona, Spain

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 subsequently the 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 simulatenouly two protomers of HA.



STRUCTURE AND FUNCTION

HA: homotrimeric glycoprotein consisting of a globular head (GH) and a stem coil (SC).

It is involved in molecular recognition of host-cell receptors and the membrane fusion process in the endosome.

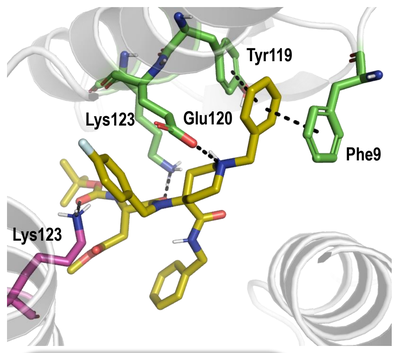
FUSION PEPTIDE (FP)

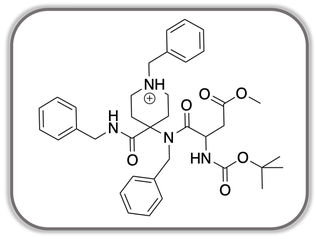
Due to pH acidification, the fusion peptide (yellow) is released and inserted into the endosome membrane, triggering the fusion process.

OBJECTIVES

The aim is to modify **DICAM180** to achieve a bivalent interaction with Phe9 from the FP in the protomers of HA, and hence enhance the antiviral potency.

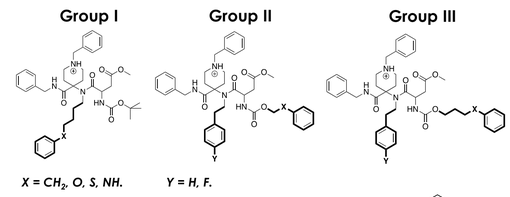
LIGANDS



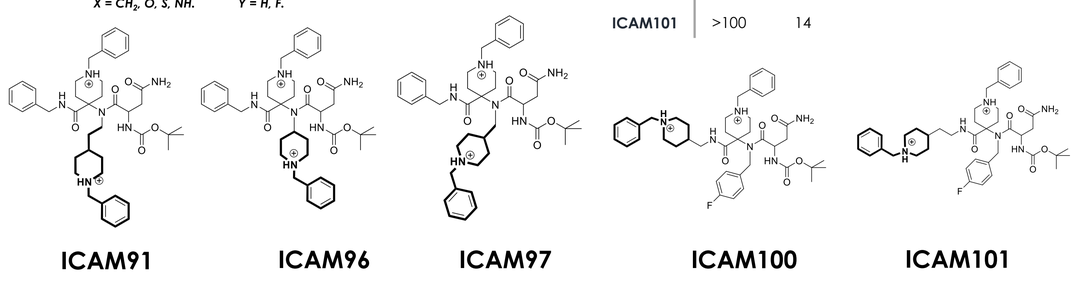


REFERENCE CMPD: DICAM 180

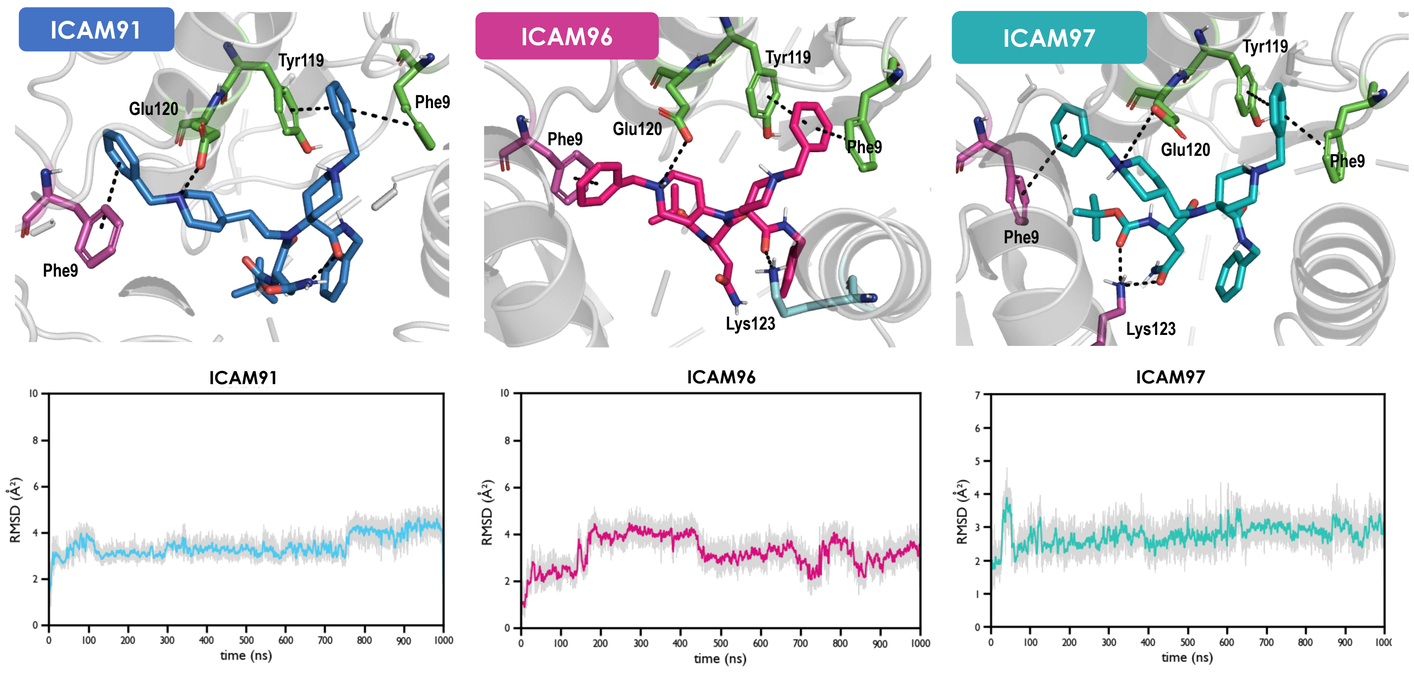
The monovalent binding mode of **DICAM180** was the template to design the novel ligands. Compounds in groups I, II and III were examined to gain an additional anchoring with Phe9 through modifications in sidechain length and inclusion of heteroatoms.
The addition of a positive charge led to active compounds **ICAM91**, **ICAM96** and **ICAM97**, whereas **ICAM100** and **ICAM101** were not.



#	EC50 (μM)	CC50 (μM)	#	EC50 (μM)	CC50 (μM)
GI-N	>100	50	ICAM91	3.9	50
GI-O	>100	4.1	ICAM96	2.4	>100
GI-C	>100	3.6	ICAM97	1.3	29
ICAM100	>100	26	DICAM 180	33	>100
ICAM101	>100	14			

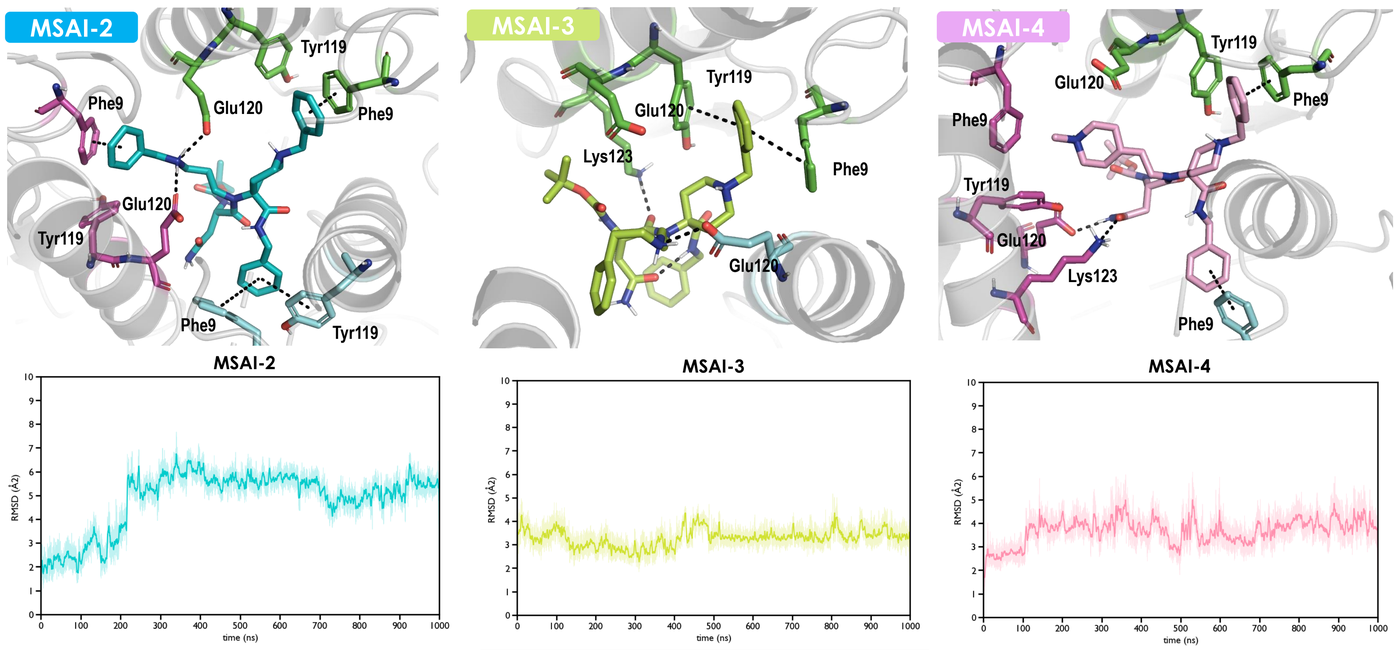


RESULTS



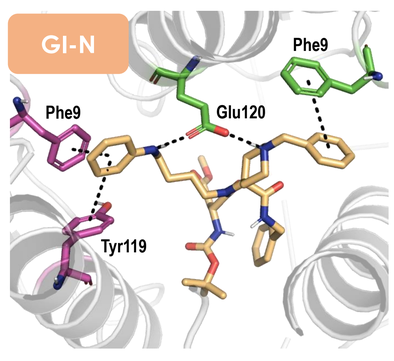
Active ligands showed a very stable binding mode where all main interactions were conserved.

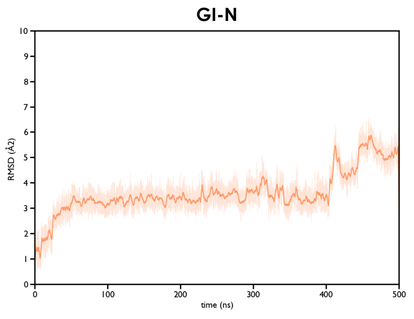
- Both π- π stacking interactions with Phe9 from FP1 (green) and FP2 (magenta) were about 4.0-6.0Å during all the simulation.
- The new piperidine moiety led to the direct interaction of Gln120 with the NH^+ showing an alternant behavior between a salt-bridge and a Hydrogen bond like interaction due to the rotation of its sidechain.
- Additional interactions with other charged residues (Lys) are found but not maintained over time.



The influence of the NH position through the sidechain of the ligand was explored. Fluctuations in RMSDs are lower than 2Å for **MSAI2/3**, but better interactions are found for the first case. In the case of **MSAI4**, not relevant interactions were found.

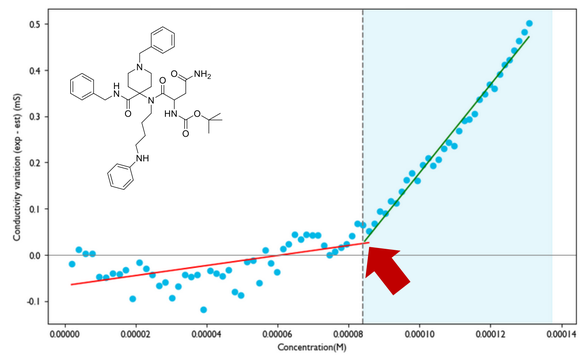
CYTOTOXICITY





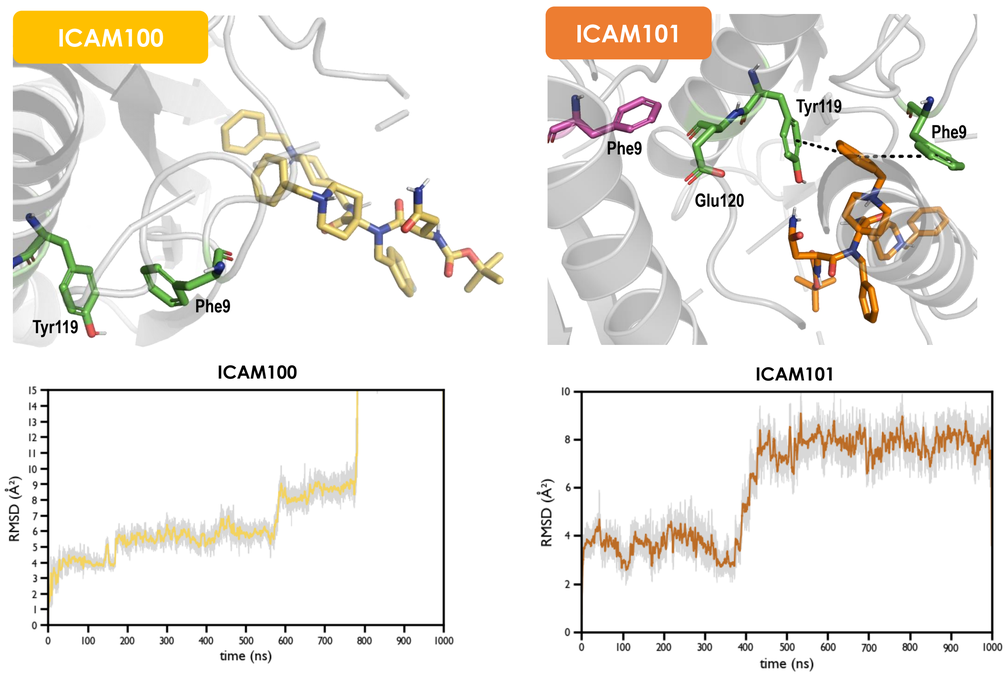
The cytotoxicity of the compounds with a single positive charge may, at least in part, be attributed to the formation of micelles.

Conductivity measurements and Dynamic Light Scattering (DLS) suggest that the CMC of these compounds is around **81-87μM**:



◀ Replacement of the benzylamide moiety in **DICAM180** by a protonated benzylpiperidine is not properly acommodated in the binding pocket:

- The inactive **ICAM100** was released from the binding pocket after 800 ns.
- The inactive **ICAM101** partly escapes from the binding pocket, though retains the Phe9/Tyr119 stacking.



DISCUSSION

- Compounds from Group I, despite good computational results were brought, resulted inactive and cytotoxic. Micelle formation was considered as a possible explanation to these values, but CMC experiments were not conclusive.
- For compound **GI-N**, the position of NH over the sidechain were explored. This change in the position enables the NH group to be protonated showing an additional charge. As well as it can be seen, the binding mode remains stable for both **MSAI-2** and **MSAI-3**, where the first shows better interactions than this last.
- The addition of a new piperidine moiety enhances the biological activity while it is added to the central N (**ICAM91**, **ICAM96**, **ICAM97**). If this inclusion is done at other position, the biological activity is suppressed as well as the binding mode (**ICAM100**, **ICAM101**).
- These studies confirm the presence of an additional charge as critical to improve the biological results and opens a new door to improve hit optimization deeply.

REFERENCES

[1] Hermoso-Pinilla FJ, Valdivia A, Camarasa MJ, Ginex T, Luque FJ. *Explor Drug Sci*, **2024**, *2*, 85-116.
[2] Caffrey M, Lavie A. *Front. Mol. Biosci.*, **2021**, *8*, 1-6.
[3] de Castro S, Ginex T, Vanderlinden E, Laporte M, Stevaert A, Cumella J, Gago F, Camarasa MJ, Luque FJ, Naesens L, Velazquez S. *Eur J Med Chem.*, **2020**, *194*, 1-16.

ACKNOWLEDGMENTS

Grant PRE2021-100418 funded by MICIU/AEI/10.13039/501100011033 and by ESF+.