MODULATING SARS-COV-2 INFLAMMASOME ACTIVATION THROUGH SMALL-MOLECULE INHIBITION OF ORF9b

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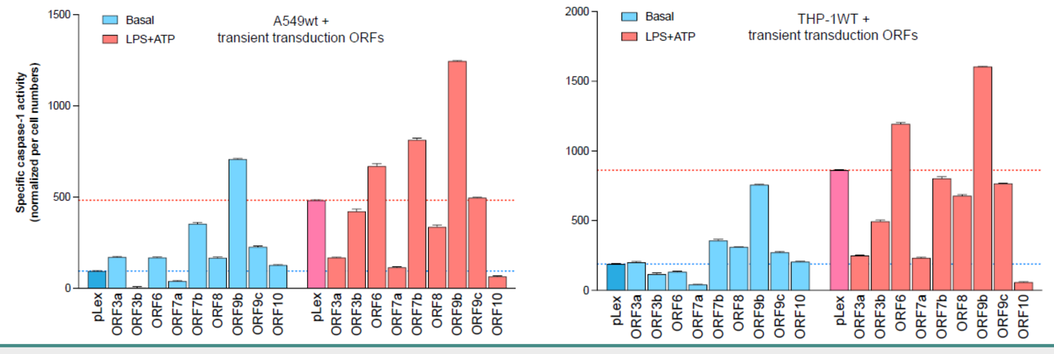
SARS-CoV-2 encodes several accessory proteins that impact immune responses and contribute to disease severity. **ORF9b** has been identified as a key player in activating the inflammasome, a protein complex that mediates inflammatory responses. Understanding how **ORF9b functions and developing inhibitors against it** can help mitigate the severe inflammatory responses seen in COVID-19.

1 ORF9b INDUCES CASPASE-1 ACTIVATION

A549 and THP-1 cells were transduced with ORF9b and other accessory proteins, followed by caspase-1 activity assays. ORF9b significantly increases caspase-1 activity compared to other accessory proteins, highlighting its role in inflammasome activation and its potential as a therapeutic target.

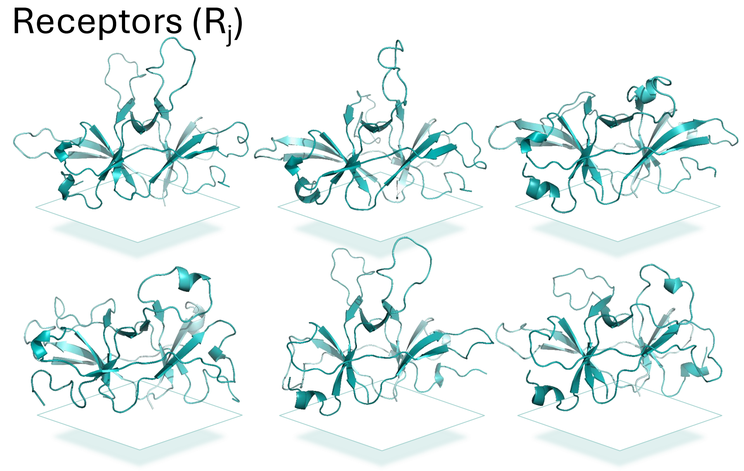


2 VIRTUAL SCREENING: ORF9B INHIBITION

Databases (L_i)

european chemical biology database	
fECBD	dECBD
1.056 ligands	*96.096 ligands*

Receptors (R_j)



DOCKING
AutoDock, QVina

L_1R_1	L_1R_2	...	L_1R_j
L_2R_1	L_2R_2	...	L_2R_j
...	...	...	...
L_iR_1	L_iR_2	...	L_iR_j

MINIMIZATION
AMBER [pmemd]

1. Ligand and protein restrained
2. Backbone atoms restrained
3. Unrestrained

Re-score

BINDING FREE ENERGY (MMPB/GBSA)
AMBER [mmgbsa]

$$\Delta G_{bind} = \langle G_{complex} \rangle - (\langle G_{receptor} \rangle + \langle G_{ligand} \rangle)$$

$$\Delta G_{bind} = \Delta E_{MM} + \Delta G_{solv} - T\Delta S$$

MOLECULAR DYNAMICS
AMBER [pmemd]

Iterative Process

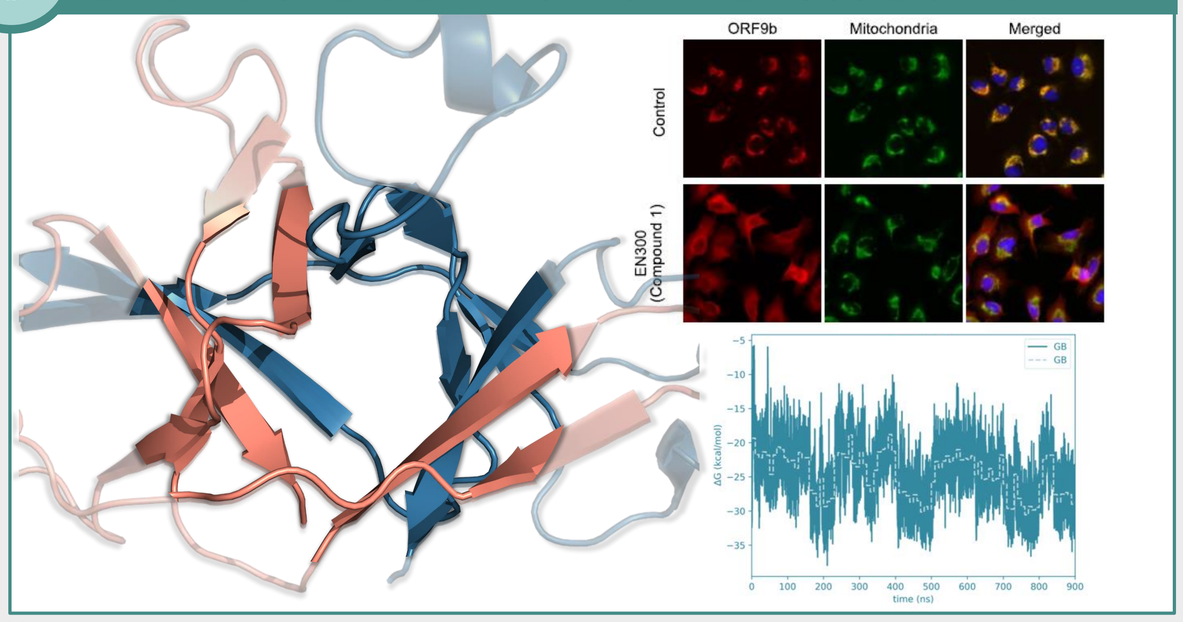
※ Heating in canonical ensemble (NVT)
※ Equilibration in isothermal-isobaric ensemble (NPT)
※ Production
- ⌗ Classical Molecular Dynamics
- ⌗ Gaussian Accelerated Molecular Dynamics

Re-score

ΔG_{GB}

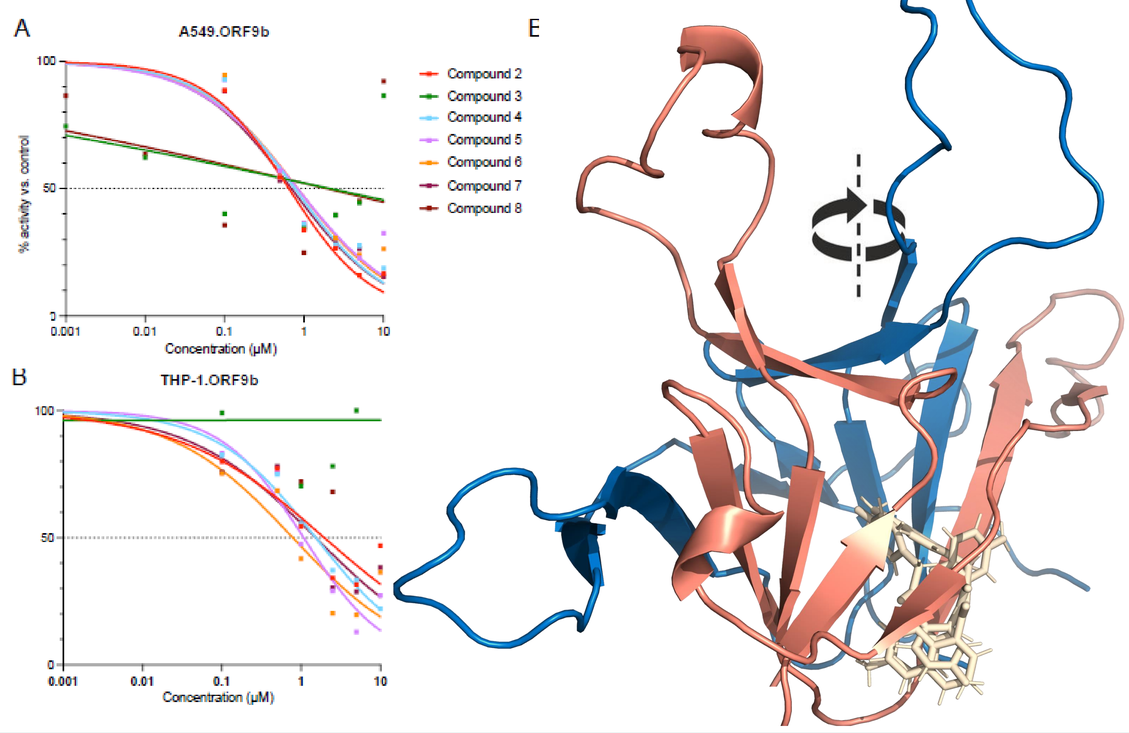
HITS

3 BINDING SITE IDENTIFICATION + EN300 LIGAND



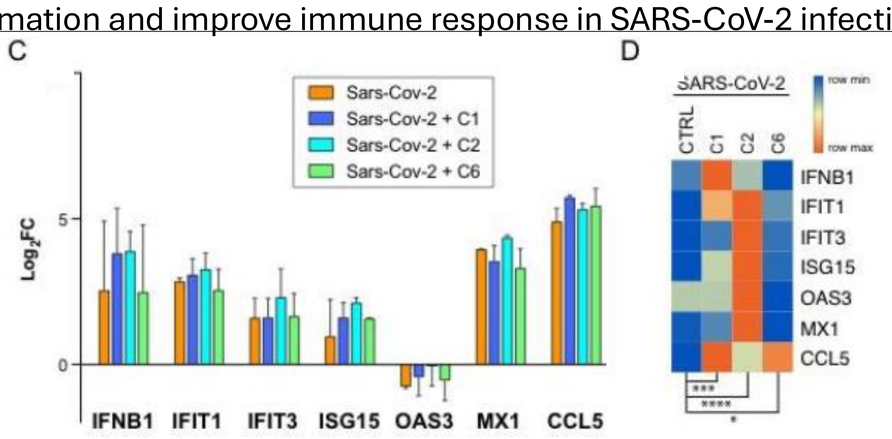
4 EN300 SIMILAR SEARCH

Search for structural analogs of EN300 using small molecule databases to identify compounds with enhanced properties. This analysis revealed several promising analogs that share structural similarity with EN300 and possess favorable physicochemical properties for ORF9b inhibition.



5 EXPERIMENTS ON TYPE I INTERFERON

Proposed inhibitors of ORF9b restored type I interferon (IFN-I) signaling in treated cells. This suggests a potential mechanism to reduce severe inflammation and improve immune response in SARS-CoV-2 infections.



References

1. Zodda, E., Pons, M., DeMoya-Valenzuela, N., Calvo-González, C., Benítez-Rodríguez, C., Díes López-Ayllón, B., Hibot, A., Cascante, M., Montoya, M., Pujol, M. D., Rubio-Martínez, J., & Thomson, T. M. (2024). *Dominant induction of the inflammasome by the SARS-CoV-2 accessory protein ORF9b, abrogated by small-molecule ORF9b homodimerization inhibitors*. bioRxiv. https://doi.org/10.1101/2024.05.31.596900

2. Rubio-Martinez, J., Wang, W., Raghunath, S., Kang, Y., Chen, W., Liu, H., & Tan, X. (2021). *Discovery of diverse natural products as inhibitors of SARS-CoV-2 M(pro) protease through virtual screening*. J Chem Inf Model, 61(12), 6094-6106. https://doi.org/10.1021/acs.jcim.1c00958