Inhibition of Influenza Virus Polymerase by Interfering with Its Protein-Protein Interactions

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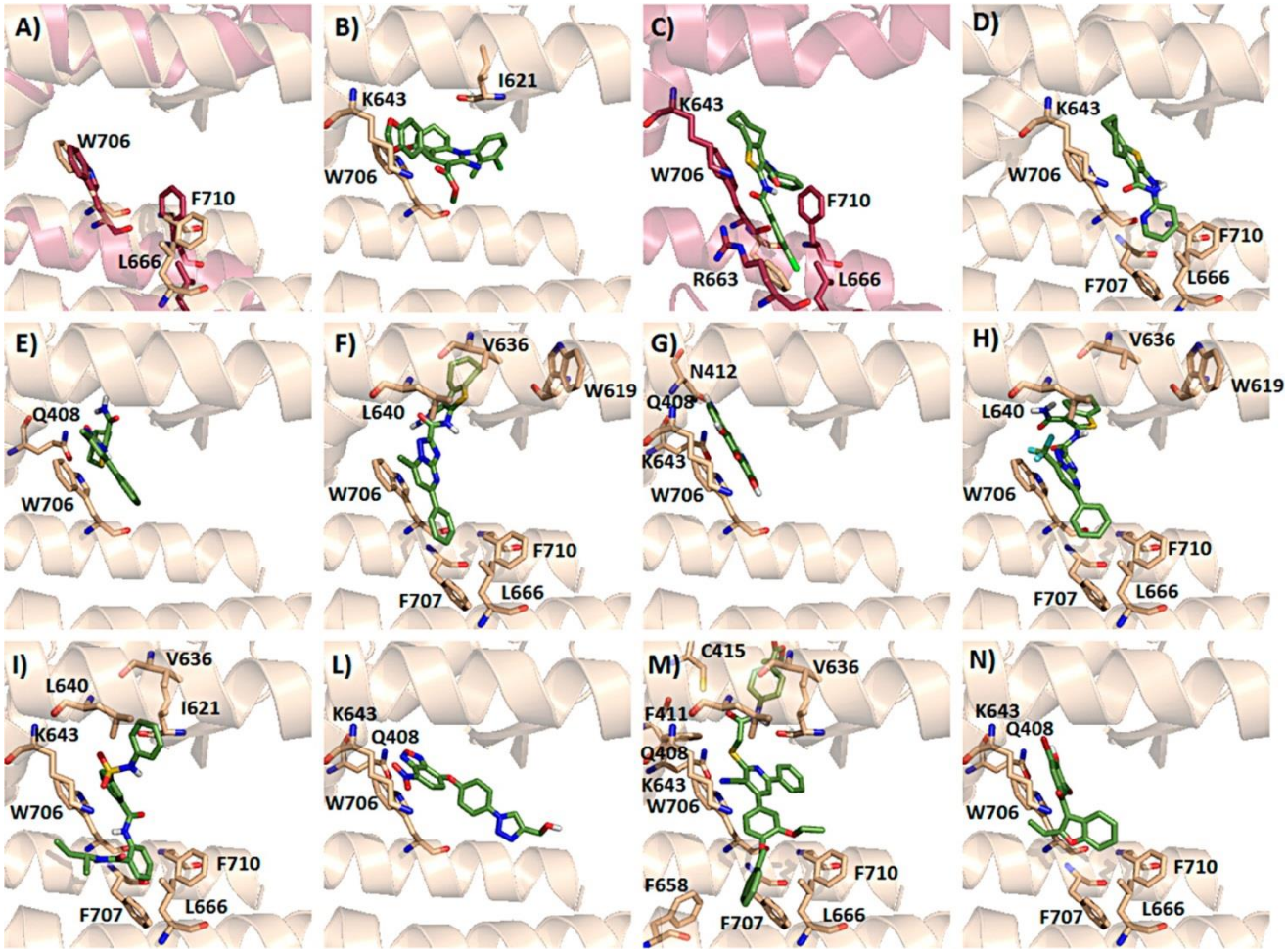


Figure S1. Key residues located in the PA cavity (A, pdb: 3CM8) and FLAP binding pose for compounds **1** (B), **2** (C), **3** (H), **4** (E), **5** (F), **7** (I), **8** (L), **9** (N), and **10** (G). (D) and (M) show the binding pose for two compounds not mentioned in this review. Reproduced from [Massari, S. Goracci, L.; Desantis, J.; Tabarrini, O. Polymerase Acidic Protein-Basic Protein 1 (PA-PB1) Protein-protein interaction as a target for next-generation anti-influenza therapeutics. *J. Med. Chem.* **2016**, *59*, 7699-7718].[1] Copyright [2016] American Chemical Society.

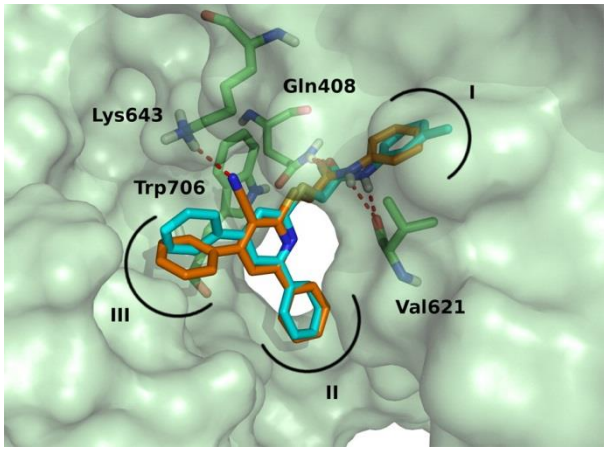


Figure S2. Docking pose for compound **6** (cyan sticks) within the PAc (pdb: from docking simulations. Reproduced from [Trist, I. M.; Nannetti, G.; Tintori, C.; Fallacara, A. L.; Deodato, D.; Mercorelli, B.; Palù, G.; Wijtmans, M.; Gospodova, T.; Edink, E.; Verheij, M.; de Esch, I.; Viteva, L.; Loregian, A.; Botta, M. 4,6-Diphenylpyridines as promising novel anti-influenza agents targeting the PA-PB1 protein-protein interaction: structure-activity relationships exploration with the aid of molecular modeling. *J. Med. Chem.* **2016**, *59*, 2688-26703].[2] Copyright [2016] American Chemical Society.

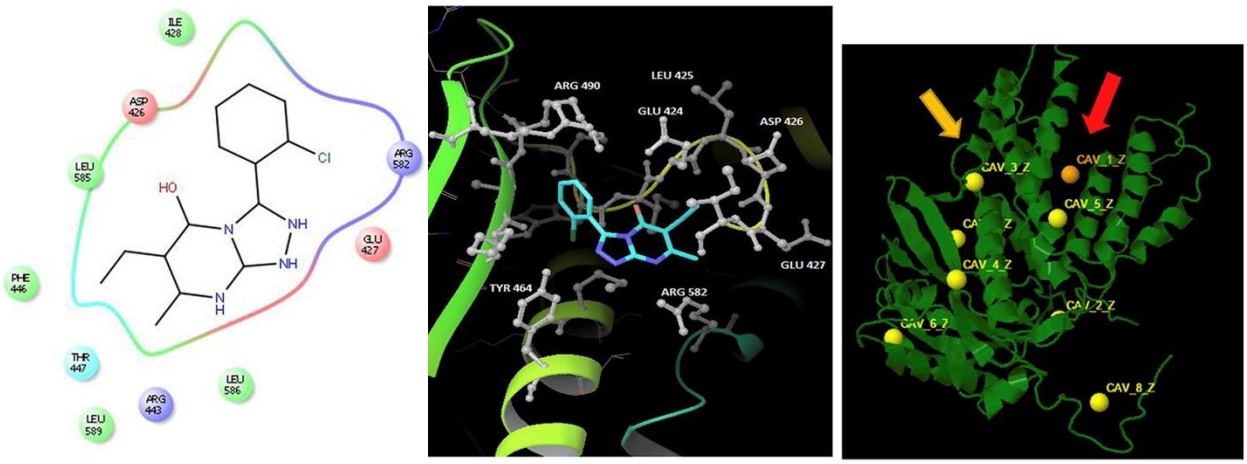


Figure S3. Docking pose for compound **14** (left and center) and binding site of compound **14** (yellow arrow) and PB1 binding site (red arrow) (right) within the PA_C (pdb: 3CM8). Reproduced with permission from [Yuan, S.; Chu, H.; Zhao, H.; Zhang, K.; Singh, K.; Chow, B. K. C.; Kao, R. Y. T.; Zhou, J.; Zheng, B.-J. Identification of a small-molecule inhibitor of influenza virus via disrupting the subunits interaction of the viral polymerase. *Antiviral Res.* **2016**, *125*, 34–42.][3] Copyright [2015] Elsevier B.V.

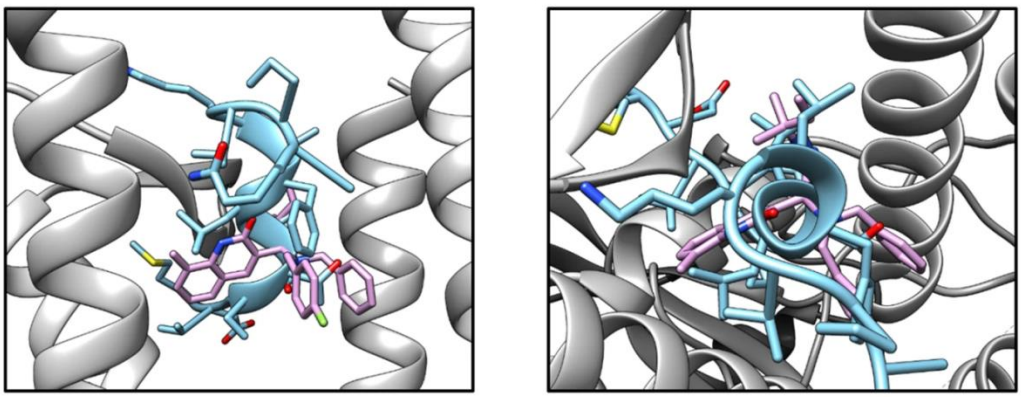


Figure S4. Docking pose for compound **15** within the PA_C based on in silico calculations, side view (left) and top view (right); crystal structure of PA (pdb: 2ZNL, grey) in complex with PB1 (light blue) and binding structure of compound **15** (pink) from docking simulation were overlaid. Reproduced with permission from [Watanabe, K.; Ishikawa, T.; Otaki, H.; Mizuta, S.; Hamada, T.; Nakagaki, T.; Ishibashi, D.; Urata, S.; Yasuda, J.; Tanaka, Y.; Nishida, N. Structure-based drug discovery for combating influenza virus by targeting the PA–PB1 interaction. *Sci. Rep.* **2017**, *7* (1), 9500.][4] Copyright [2017] Springer Nature.

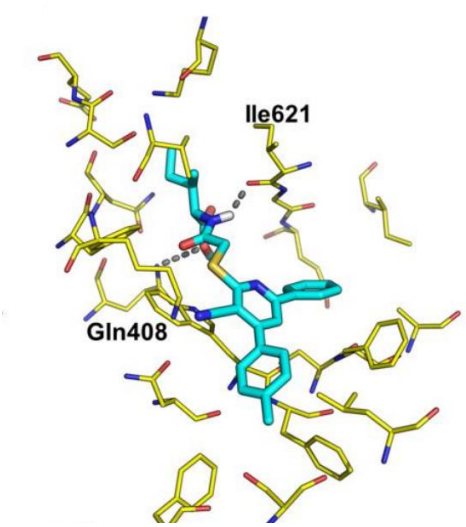


Figure S5. Docking pose for compound **19** (cyan stick). The crystallographic structure of PA in complex with PB1 (pdb: 4WSB) is shown as yellow lines. Polar interactions are highlighted by grey dashed lines. Only residues within 4 Å from the ligands are showed; non-polar H atoms are omitted. Reproduced with permission from [D'Agostino, I.; Giacchello, I.; Nannetti, G.; Fallacara, A. L.; Deodato, D.; Musumeci, F.; Grossi, G.; Palù, G.; Cau, Y.; Trist, I. M.; Loregian, A.; Schenone, S.; Botta, M. Synthesis and biological evaluation of a library of hybrid derivatives as inhibitors of influenza virus PA-PB1 interaction. *Eur. J. Med. Chem.* **2018**, *157*, 743-758].[5] Copyright [2018] Elsevier Masson SAS.

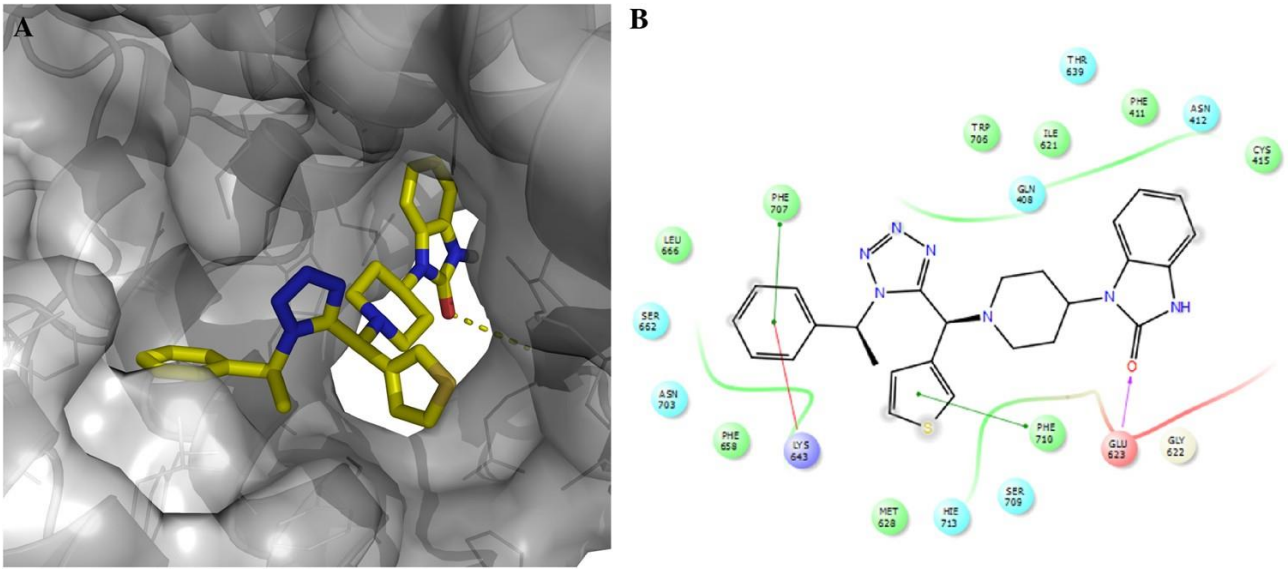


Figure S6. Surface view of docking pose for compound **21** in the $PB1_N$-binding pocket in PA_C (A) and its ligand interaction diagram with residues in the binding site (B). Reproduced with permission from [Zhang, J.; Hu, Y.; Foley, C.; Wang, Y.; Musharrafieh, R.; Xu, S.; Zhang, Y.; Ma, C.; Hulme, C.; Wang, J. Exploring Ugi-azide four-component reaction products for broad-spectrum influenza antivirals with a high genetic barrier to drug resistance. *Sci. Rep.* **2018**, *8* (1), 4653].[6] Copyright [2018] Springer Nature.

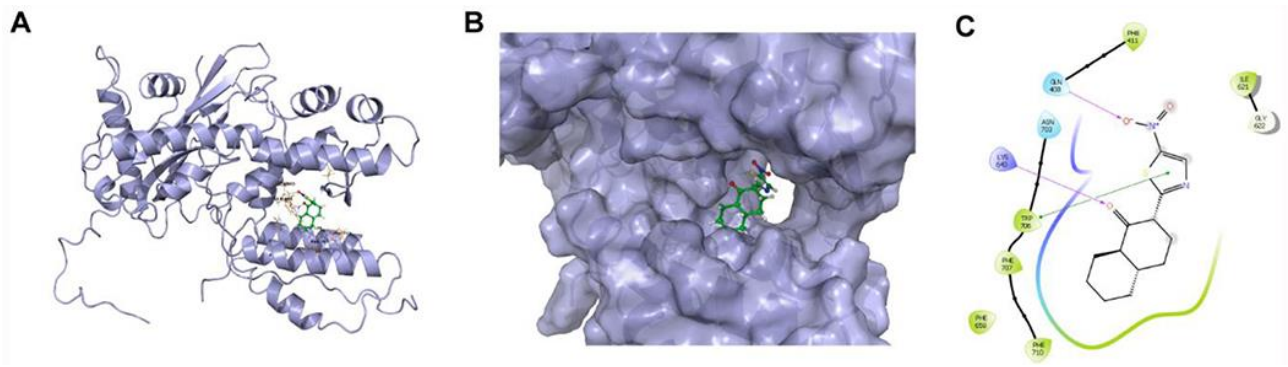


Figure S7. Best docking pose of compound **23** in PA_C cavity from PA–PB1 complex (pdb: 3CM8) (A), its surface view (B) and its ligand interaction diagram (C). Reproduced from [Zhang, J.; Hu, Y.; Wu, N.; Wang, J. Discovery of influenza polymerase PA-PB1 interaction inhibitors using an in vitro split-luciferase complementation-based assay. *ACS Chem. Biol.* **2020**, *15* (1), 74–82].[7] Copyright [2020] American Chemical Society.

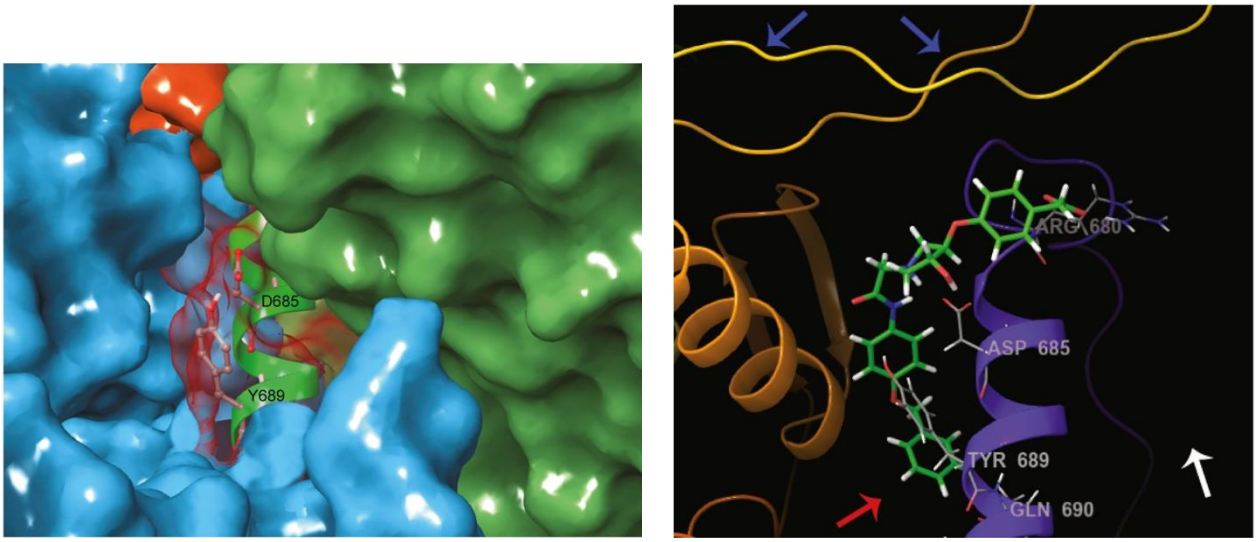
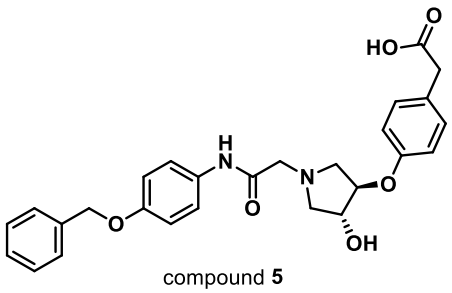


Figure S8. Proposed binding site of compound **5** (original number reported in the manuscript by Mohl et al., structure is reported below), a strict analogue of compound **32** (left): PA-PB1 complex (pdb: 4WSB, blue surface) in complex with RanBP5 homologue Kap121p (pdb: 3W3Z, green surface) and key residues D685 and Y689 of PB1 anchor helix (transparent red surface); view of the binding site of compound **5** docked (right): the bipartite NLS (blue arrows), RanBP5 binding interface (white arrow), and PB2 binding site (red arrow). Reproduced with permission from [Mohl, G., Liddle, N., Nygaard, J., Dorius, A., Lyons, N., Hodek, J., Weber, J., Michaelis, D. J., and Busath, D. D. (2019) Novel influenza inhibitors designed to target PB1 interactions with host importin RanBP5. *Antiviral Res. 164*, 81–90].[8] Copyright [2019] Elsevier B.V.



compound **5**

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