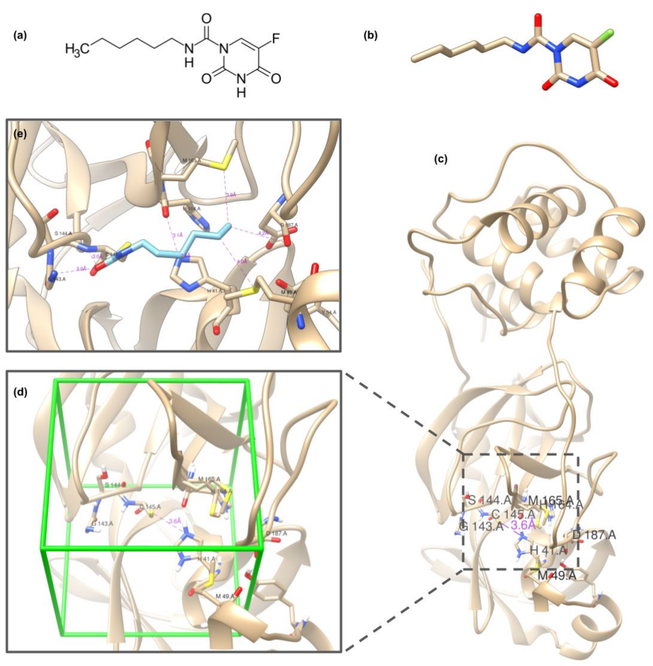
In silico screen and ^{19}F nuclear magnetic resonance spectroscopy enabled chemical synthesis of a library of carmofur analogs as potential inhibitors of the SARS-CoV-2 main protease (M^{pro})

Charissa Luk, Xina Wang, Neha Mandava, Emma Le, Udbhav Avadhani, Sarah Su, Jane Wu, Aishi Rao, Shloka Raghavan, Aashi Shah, Priya Chanda, Jeslyn Wu, Darshita Prathap, Anushka Peer, Julia Vu, Ananya Anand, Suhani Babu, *Edward Njoo*
Department of Chemistry, Biochemistry, and Physical Science | Aspiring Scholars Directed Research Program, Fremont, CA

Abstract

Carmofur, a 5-fluorouracil (5-FU) derivative and antineoplastic agent that inhibits acid ceramidase, has been widely studied as an anticancer agent but was more recently, through a drug repurposing screen, identified as a covalent inhibitor of the main protease of SARS-CoV-2. The ongoing severe acute respiratory syndrome coronavirus 2 pandemic has caused millions of fatalities worldwide, impacting all aspects of daily life. The SARS-CoV-2 main protease (M^{pro}) plays an essential role in the processing of the polyproteins that are translated from the viral RNA, therefore making it an attractive drug target for the treatment of COVID-19.
However, with new mutations arising in M^{pro}, designing drugs that retain efficacy against mutants is crucial. Here, we present the *in silico* evaluation and synthesis of carmofur and a library of carmofur analogs with aliphatic, amino acid, and aromatic fragments against mutations in M^{pro}. Homology modeling was used to determine the conformational change in the protein as a result of the mutations and their effects on the binding affinity of our analogs, revealing potential hit compounds to further develop for treating COVID-19. Furthermore, we detail how the application of benchtop ^{19}F NMR to monitor real-time kinetics of a reaction seemingly impossible to track quantitatively enables the synthesis of hit analogs for future *in-vivo* testing.

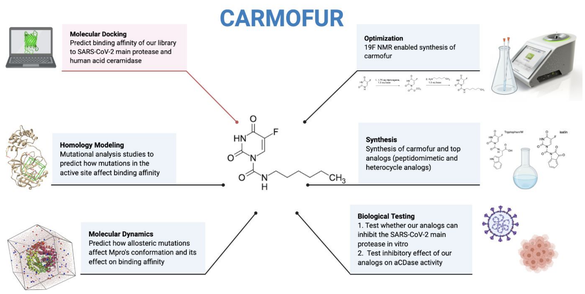
Docking Analysis



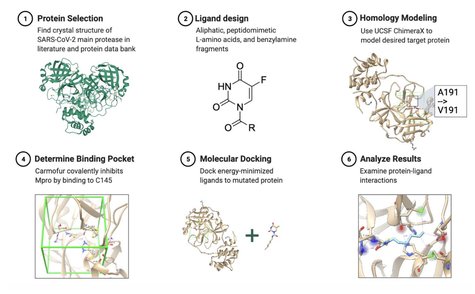
Carmofur binding interactions with M^{pro}

(a) The 2D skeleton structure of carmofur. (b) The 3D structure of carmofur. (c) The active site of M^{pro} to assess carmofur's binding affinity to it. (d) Interactions between amino acids comprising the M^{pro} active site. (e) The interactions between the aliphatic tail of carmofur (without 5-fluorouracil). The aliphatic tail engages in many hydrophobic interactions with the residues in the active site and hydrophobic S2 subsite, suggesting that such interactions help to stabilize carmofur in M^{pro}.

Project Overview

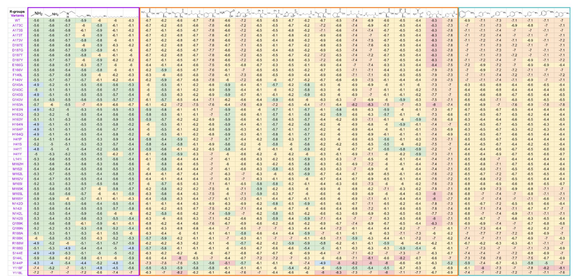


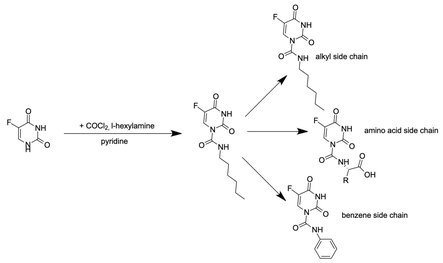
Homology Modeling

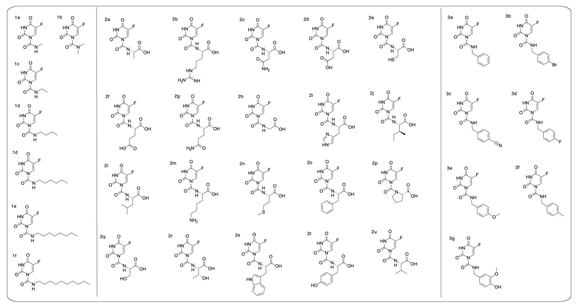


Analog Design

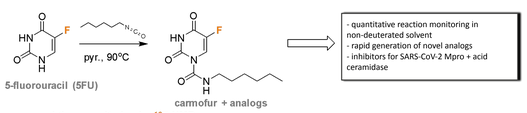
High Throughput Virtual Screen Heat Map



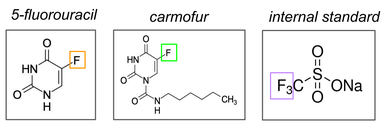


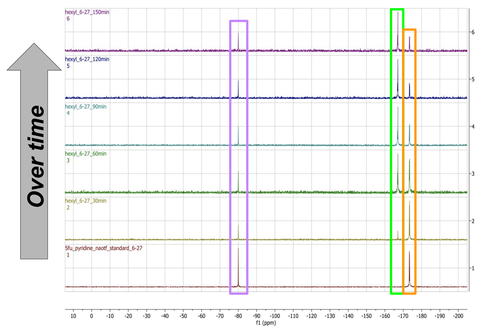


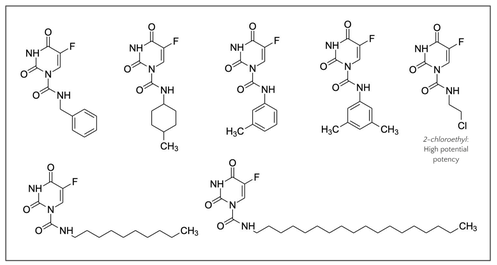
^{19}F Kinetics Monitoring



real-time monitoring by ^{19}F NMR

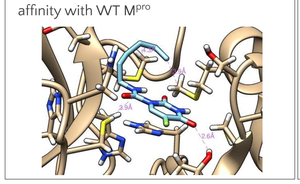




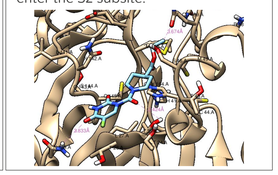


Conclusions

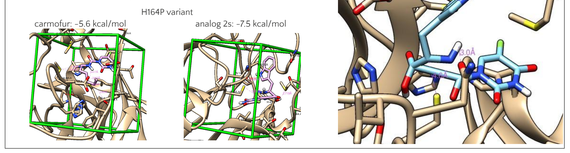
Aliphatic Analogs: increasing aliphatic tail length confers marginally better binding affinity with WT M^{pro}



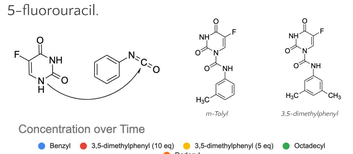
Benzyl Analogs: exhibit fewer hydrophobic interactions and do not enter the S2 subsite.

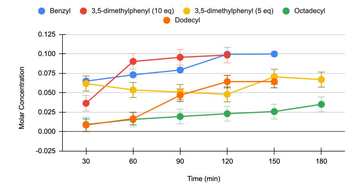


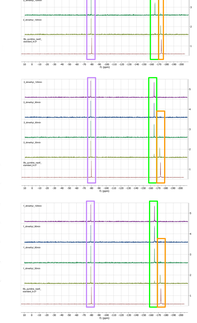
Amino Acid Analogs: Analog 2s consistently had high binding affinity with WT and its variants.



Aryl Isocyanates: We hypothesize that aryl isocyanates have more difficulty coupling with 5-fluorouracil.







References

1. WHO Coronavirus Disease (COVID-19) Dashboard. https://covid19.who.int/ (accessed May 31, 2021).
2. Morse, J. S., Lalonde, T., Xu, S., & Liu, W. R. (2020). Learning from the past: possible urgent prevention and treatment options for severe acute respiratory infections caused by 2019-nCoV. Chembiochem, 21(5), 730-738.
3. Naqvi, A. A. T., Fatima, K., Mohammad, T., Fatima, U., Singh, I. K., Singh, A., ... & Hassan, M. I. (2020). Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: Structural genomics approach. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 165878.
4. Jin, Z., Zhao, Y., Sun, Y., Zhang, B., Wang, H., Wu, Y., ... & Rao, Z. (2020). Structural basis for the inhibition of SARS-CoV-2 main protease by antineoplastic drug carmofur. Nature structural & molecular biology, 27(6), 529-532.
5. Shelton, J., Lu, X., Hollenbaugh, J. A., Cho, J. H., Amblard, F., & Schinazi, R. F. (2016). Metabolism, biochemical actions, and chemical synthesis of anticancer nucleosides, nucleotides, and base analogs. Chemical reviews, 116(23), 14379-14455.
6. Dementiev, A., Joachimiak, A., Nguyen, H., Corelik, A., Illes, K., Shabani, S., ... & Doan, N. (2018). Molecular mechanism of inhibition of acid ceramidase by carmofur. Journal of medicinal chemistry, 62(2), 987-992.
7. Pizzirani, D., Pagliuca, C., Realini, N., Branduardi, D., Bottegoni, G., Mor, M., ... & Bandiera, T. (2013). Discovery of a new class of highly potent inhibitors of acid ceramidase: Synthesis and structure–activity relationship (SAR). Journal of medicinal chemistry, 56(9), 3518-3530.
8. Li, Q., & Kang, C. (2020). Progress in developing inhibitors of SARS-CoV-2 3C-like protease. Microorganisms, 8(8), 1250.
9. Günther, S., Reinke, P. Y., Fernández-García, Y., Lieske, J., Lane, T. J., Ginn, H. M., ... & Meents, A. (2021). X-ray screening identifies active site and allosteric inhibitors of SARS-CoV-2 main protease. Science, 372(6542), 642-646.
10. Hanwell, M. D., Curtis, D. E., Lonie, D. C., Vandermeersch, T., Zurek, E., & Hutchison, G. R. (2012). Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. Journal of cheminformatics, 4(1), 1-17.
11. Neese, F. (2012). The ORCA program system. Wiley Interdisciplinary Reviews: Computational Molecular Science, 2(1), 73-78.
12. Trott, O., & Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of computational chemistry, 31(2), 455-461.
13. Owen, C. D., Lukacik, P., Strain-Damerell, C. M., Douangamath, A., Powell, A. J., Fearon, D., Brandao-Neto, J., Crawshaw, A. D., Aragao, D., Williams, M., Flaig, R., Hall, D. R., McAuley, K. E., Mazzorana, M., Stuart, D. I., von Delft, F., & Walsh, M. A. (2020). SARS-CoV-2 main protease with unliganded active site (2019-nCoV, coronavirus disease 2019, COVID-19).
14. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera--a visualization system for exploratory research and analysis. Journal of computational chemistry, 25(13), 1605-1612.
15. Pettersen, E. F., Goddard, T. D., Huang, C. C., Meng, E. C., Couch, G. S., Croll, T. I., ... & Ferrin, T. E. (2021). UCSF ChimeraX: Structure visualization for researchers, educators, and developers. Protein Science, 30(1), 70-82.
16. Goddard, T. D., Huang, C. C., Meng, E. C., Pettersen, E. F., Couch, G. S., Morris, J. H., & Ferrin, T. E. (2018). UCSF ChimeraX: Meeting modern challenges in visualization and analysis. Protein Science, 27(1), 14-25.
17. Shu, Y., & McCauley, J. (2017). GISAID: Global initiative on sharing all influenza data – from vision to reality. Eurosurveillance, 22(13). https://doi.org/10.2807/1560-7917.es.2017.22.13.30494