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FlICked ACE2 Mimics Inhibit SARS-CoV-2 Spike Protein-ACE2 Interaction

Mihajlo Todorovic, Antonio A.W.L Wong, Naysilla Dayanara, Taylor Navi, David Marc Perrin

[1] University of British Columbia

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Abstract: SARS-CoV-2 is the viral cause of the most significant global health crisis of our generation, causing over 144 million confirmed cases and 3 million deaths in the year following the declaration of a public health emergency of international concern by the WHO. While the first mRNA vaccines were deployed, the use of stapled peptides to investigate more synthetically accessible inhibitors has sparked rampant interest since as such peptides can have well-defined secondary structures and small molecule-like pharmacokinetic properties. Peptide stapling allows shorter amino acid sequences to mimic the interactions found at the surfaces of protein-protein interactions. In the environment of a rapidly growing field our lab has developed a unique stapling technology we coined Fluorescent Isoindole Crosslinking (FlICk) which employs ortho-phthalaldehyde (OPA) to staple an amine bearing side chain and a thiol bearing side chain in a dehydrative aromatization reaction to yield an isoindole, a chemical moiety which adds value beyond just rigidification through the emergent fluorescence of the staple itself, directly yielding lead molecules which do not have to be further derivatized with fluorophores for use as probes. The reaction conditions are highly benign, operating at room temperature and at ambient conditions close to physiological pH, thus lending themselves naturally to cyclization of unprotected peptides. In the process of developing this chemistry we were able to produce both monocycles and bicycles with biological activity (on melanocortin stimulating hormone and α-amanitin platforms, respectively.) With the emergence of the COVID-19 pandemic in 2020 we were compelled to apply our newly developed technology to what may be the most significant global health crisis of our time. The SARS-CoV-2 virus enters human cells via the interaction of the spike-RBD proteins on its surface with the Angiotensin Converting Enzyme 2 (ACE2) on the host cell surface. The viral protein interacts with the α-helical N-terminal domain of ACE2, a portion of the protein we sought to interrogate with stapled peptide surrogates as such molecules could act as decoys for virus, blocking attachment and thus entry to host cells. We developed an ELISA for testing new lead molecules and set off by examining linear sequences that were direct cut-outs from the ACE2 protein and found that none inhibited the ACE2-Spike interaction at the concentrations tested. We found that while we could smoothly apply our FlICk chemistry to produce stapled helical mimics, such monocyclic compounds were also inactive at the concentrations tested. Thus, we sought to expand the capabilities of FlICk and explore the possibility of introducing a double staple on an unprotected linear sequence and were pleased to find that the resulting double FlICked peptides were our first hits for successfully inhibiting the Spike-ACE2

interaction (at μM concentrations.) We are rapidly developing a pseudovirus neutralization assay (PNA) to validate these initial hits *in-vivo* while simultaneously pressing lead development in search of better inhibitors. Additionally, we are beginning work on examining the interactions of our ACE2 mimicking stapled peptides with COVID-19 variant spike proteins, which have an even greater affinity for ACE2 than the original viral protein. While this fact has grave implications for public health and has led to their increased transmissivity, it also offers the potential that peptide mimics of ACE2, such as ours, could be more effective treatments against these mutant strains.