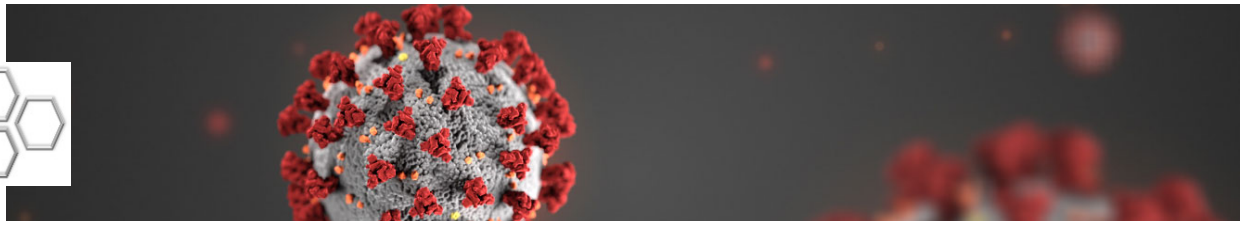
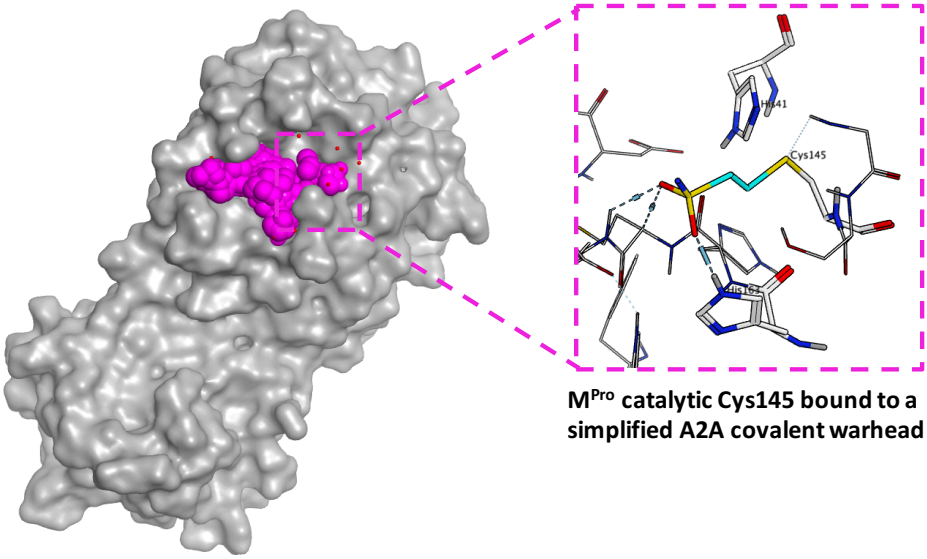


A2A Pharmaceuticals has designed selective covalent inhibitors of SARS-CoV-2 Main Protease (M^{Pro})

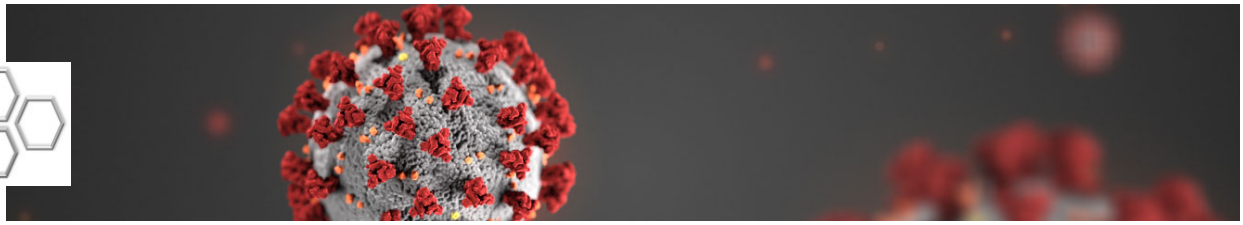
Executive Summary

- The pharmaceutical industry is focused on repurposing therapeutics for Covid19 that were developed for other diseases. At A2A, we designed highly selective small molecule M^{Pro} inhibitors with drug-like properties and moieties inspired by respiratory, antiviral and immunomodulating therapeutics to accelerate the timeframe to achieve molecules with clinical activity, favorable ADME properties, and a clean toxicological profile.
- The central innovation of our compounds is that they are covalent inhibitors that get activated exclusively in the active site of M^{Pro}, completely shutting down the viral enzyme activity and preventing viral replication.



M^{Pro} catalytic Cys145 bound to a simplified A2A covalent warhead

- Selective covalent SARS-CoV-2 M^{Pro} inhibitors could be used in early intervention for COVID-19 patients to reduce viral load and improve outcomes, or in more advanced COVID-19 in combination with antiviral antibodies such as those in development by Vir, Regeneron, Lilly, and Amgen
- M^{Pro} proteases across viral species share 40−60% sequence identity and 60−100% sequence similarity.[1] Therefore, targeting SARS-CoV-2 MPro is an important approach for the development of antiviral therapies that can be applied for broad viral infections, including for the potential emergence of novel related pathogens in the future.



Introduction

The COVID-19 pandemic caused by SARS-CoV-2 is a global health emergency. It highlights two immediate needs:

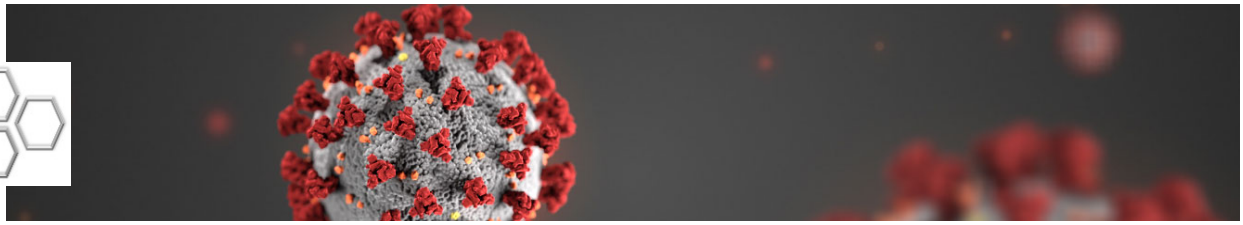
1) the development of a vaccine that immunizes people and prevents us from getting the virus, and 2) the development of therapeutics that can cure infected patients and thereby decrease both mortality rates and the average number of people such patients can infect, mitigating social and economic costs.

At A2A we have evaluated multiple protein targets essential to viral replication and cell entry central to COVID-19 pathogenesis and we selected SARS-CoV-2 Main Protease (M^{Pro}) as one of the best characterized drug targets among coronaviruses. M^{Pro} plays a pivotal role in mediating viral replication and transcription, making it an attractive drug target for COVID-19.

Recent M^{Pro} crystal structures allowed us to perform a detailed structural analysis of the catalytic site and the 3 domains of the dimer protein, using our SCULPT technology. SARS-CoV-2 M^{Pro} has a key catalytic cysteine residue (Cys145) that offers the possibility of designing covalent inhibitors that would completely block natural activity of the viral enzyme, and prevent replication. M^{pro} also presents key amino acid residues for the coordination of specific covalent warheads meaning they will only be activated in the specific environment of the active site, and will not have non-specific toxicity issues.

Using our SCULPT computational technology, we designed several series of covalent inhibitors with high specificity and high affinity for the SARS-CoV-2 M^{Pro} catalytic site. Given the high specificity, and since no human proteases with a similar cleavage specificity are known, inhibitors are unlikely to be toxic.[2] Compounds also have drug-like properties incorporated to improve their probability of success as drugs.

Analyzing the M^{Pro} ligand space, there are currently no targeted therapeutics and the search for effective treatment options has come from virtual high throughput screening (vHTS) and repurposing approaches,[3] meaning optimal designs for this novel target have not been discovered. Some peptide-based alpha-keto amide compounds have shown activity, however these are peptides highly susceptible to enzyme degradation and unlikely to be effective drug molecules in the environment of the infection in humans.[2, 4]



SARS-CoV-2 MPro - SCULPT Targeted Covalent Drug Design

The first objective of SCULPT design is to identify hotspots within the target protein important for ligand or substrate binding, using crystallographic and biochemical data, as well as site analysis tools (e.g., Diamond Therapeutics, and PanDDA SARS-CoV-2 group deposition). SCULPT then searches drug-like virtual fragments to interact specifically with these hotspots. We applied this analysis to the recently solved SARS-CoV-2 MPro crystal structures.[5]

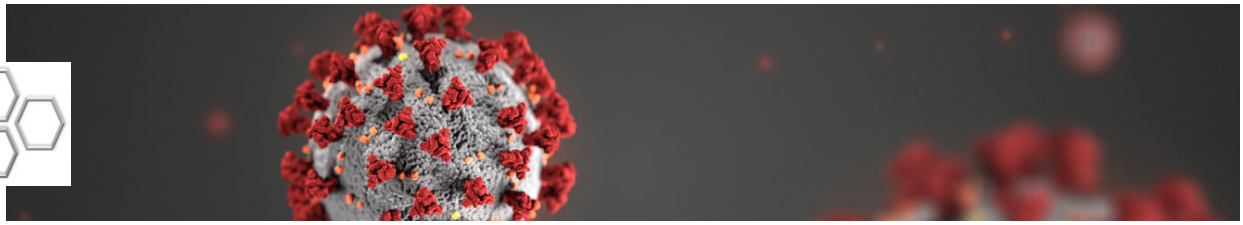
Fragments originate from a comprehensive pool of public databases, deconstructed clinical development and approved drugs (e.g., cardiovascular, respiratory, antiviral, immunomodulating) using a KNIME workflow, and internal knowledge-base, resulting in a comprehensive exploration of chemotypes. Specifically for M^{Pro}, we also explored a variety of covalent warheads positioned to bond to Cys145 when activated by coordination to surrounding Histidine residues (His41, His163). Importantly, these covalent warheads are not likely to be reactive with nucelophiles when not bound to the SARS-CoV-2 M^{Pro} active site.

The SCULPT process then combines the optimally placed fragments into full molecules enriched for specific interaction with the M^{Pro} active site. The result was a large number of molecules covalently attached to Cys145 with high predicted binding affinity, exploring the S2 – S3 – S4 (P2 – P3) regions of the M^{Pro} active site, as well as interactions not described in existing crystal structures. Promising molecules then go through iterative rounds of optimization to enhance binding affinity and ensure drug-like properties of the molecule are favorable.

molecule	warhead	TPSA	weight	clogP	....	....	....	....	mutagenic	....	....
A2A_CoV2_01	1	123.30	458.88	3.75	9	2	1	1.24	0	0.1290	2.8274
A2A_CoV2_02	1	108.05	401.49	1.06	8	2	1	1.19	0	0.3571	2.7887
A2A_CoV2_03	2	122.83	445.44	1.34	9	3	1	1.84	0	0.2333	1.0120
A2A_CoV2_04	2	122.83	481.48	1.99	9	3	1	2.82	0	0.4848	1.7929
A2A_CoV2_05	2	127.27	485.49	-1.14	10	4	1	1.62	0	0.3636	-0.6576
A2A_CoV2_06	3	165.87	540.46	2.62	12	3	0	0.20	0	0.4103	2.5307
A2A_CoV2_07	1	113.18	386.38	1.25	8	3	1	1.76	0	0.5769	2.3130
A2A_CoV2_08	1	101.30	395.39	3.05	7	2	1	2.10	0	0.4444	3.4793
A2A_CoV2_09	4	91.23	443.30	3.45	7	2	1	3.04	0	0.3571	3.5723
A2A_CoV2_10	4	91.23	432.40	3.51	7	2	1	3.17	0	0.2258	4.1401
A2A_CoV2_11	1	80.20	401.41	2.61	6	2	1	3.69	0	0.3333	2.9267
A2A_CoV2_12	1	97.19	389.48	1.76	8	1	1	1.08	0	0.3333	2.8435
A2A_CoV2_13	1	108.05	415.52	1.80	8	2	1	1.27	0	0.3103	3.1804
A2A_CoV2_14	5	134.55	426.35	1.44	10	3	1	-0.94	0	0.3000	0.2404
A2A_CoV2_15	4	124.43	512.45	4.09	10	2	1	2.16	0	0.3514	3.6894
A2A_CoV2_16	4	130.42	459.41	2.52	10	2	1	1.91	0	0.3333	2.7386
A2A_CoV2_17	5	122.52	453.42	2.70	9	2	1	0.52	0	0.2500	2.1096
A2A_CoV2_18	1	150.76	558.58	2.25	11	4	0	1.34	0	0.2632	2.0580
A2A_CoV2_19	5	96.33	381.44	2.21	8	2	1	1.17	0	0.6429	1.4411
A2A_CoV2_20	5	111.24	446.39	2.67	9	1	1	0.68	0	0.2813	2.7269
A2A_CoV2_21	5	105.98	429.43	2.62	8	2	1	1.24	0	0.3226	2.9178
A2A_CoV2_22	4	122.27	471.40	3.49	10	1	1	1.14	0	0.2941	3.6818

Table 1. Sample calculated properties for a subset of specifically designed SARS-CoV-2 MPro covalent inhibitors. Molecules chosen for synthesis and testing have drug-like properties incorporated.

The SCULPT process converged on multiple drug-like small molecules that incorporate specific binding to the SARS-CoV-2 M^{Pro} active site, and covalent warheads designed to be activated for binding to Cys145 after coordination to neighboring Histidine residues in the active site.



Additional A2A programs

A2A Pharmaceuticals' focus is to accelerate the development of novel drugs by leveraging proprietary computational systems including A.I. to treat life threatening diseases. Using deep learning multi-descriptor model generation and data analysis for compound activity and toxicity, A2A Pharma designs and builds pre-optimized molecules with chemical features uniquely enriched to be specific against each target it tackles.

Further validation of the SCULPT design approach to target difficult to drug targets comes from our programs in oncology. The personalized nature of oncology treatments and the ability to diagnose and tailor treatments to patient genetic factors means targeted therapeutics like MLL-Menin inhibitors, K-RAS and YAP-TEAD inhibitors have vast potential. These have been unsolved challenges in the industry for a long time. In our MLL-Menin program, one of our initial SCULPT compound designs is our current lead candidate, which is showing high promise in late-stage IND-enabling studies. See the table below for some other programs in which - with very small amounts of capital and time - we have been able to generate hit molecules in diverse diseases.

Delivering Proof of Concept for SCULPT

with less than $100,000 in 6 months

Program	SCULPT Design & Synthesis (# of compounds)	Screening	Hit!!
MLL- Menin (Leukemia)	12 synthesized	MLL-translocated AML cell lines	4 Highly potent compounds More than reference compound
YAP-TEAD (Solid tumors)	28 in 5 scaffolds synthesized (both allosteric site and YAP binding site (PPI))	HTRF assay PPI inhibition	4 Compounds - ~70% inhibition 5 Compounds - ~40% inhibition at a range of 20-0.2 µM
RAS (Cancer)	5 in 5 scaffolds synthesized (KRAS binders) & 32 KRAS degraders synthesized	SPR Binding Cellular degradation	5 compounds showed binding K_D sub-micromolar range KRAS degrader candidate identified
LpxC (Gram negative bacteria)	11 in 6 families synthesized (non-hydroxamate toxic group)	Bacterial panel of 30 strains	3 Distinctive families - MIC 16-32 µg/ml in *N. gonorrhoeae* (FA6140, FA19, 35/02)
CLK-1 (DMD)	20 in 10 scaffolds synthesized	CLK-1 Human CMGC Kinase enzymatic Radiometric Assay DMD exon skipping in human muscle cells	6 Highly potent compounds 80% inhibition of CLK-1 Exon 51 skipping inducer candidate

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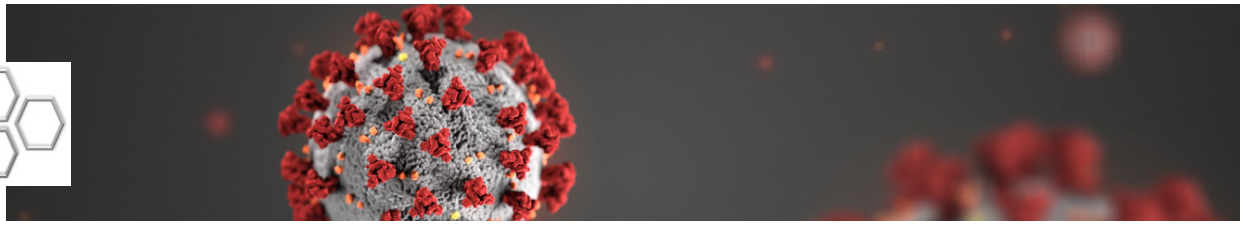

Additionally, literature suggests that bacterial infections combined with COVID-19 increase patients' risk of mortality in severe cases. Therefore, antibacterial alternatives should be of interest in addition to antivirals to improve patients' prognosis. A2A has a gram negative antibacterial program targeting LpxC, which has achieved positive antibacterial results in certain difficult to treat stains of gonorrhea.

Summarizing, SCULPT has the capability to generate novel and improved molecules to be able to tackle difficult-to-drug targets and aid efforts to flatten the curve in pandemics that jeopardize the health of the world population. In this presentation we described our design of highly specific targeted SARS-CoV-2 M^{Pro} covalent inhibitors with potential in the fight to treat COVID-19.

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2) L. Zhang et al., Science 10.1126/science.abb3405 (2020).
3) M. Kandeel et al., Life Sciences 251 (2020) 117627.
4) Jin, Z. et al. Structure of M^{pro} from COVID-19 virus and discovery of its inhibitors. Nature (2020). https://doi.org/10.1038/s41586-020-2223-y.
5) PDB Crystal Structures 6Y7M, 6YB7, 6W63, 6LU7, 6Y2F, 6Y2G.