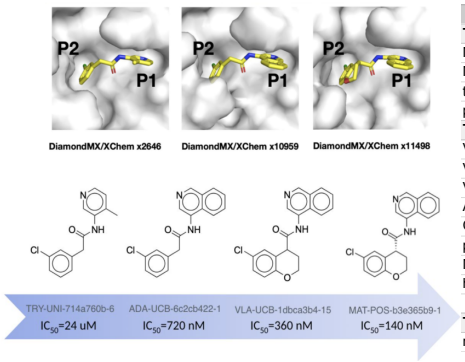
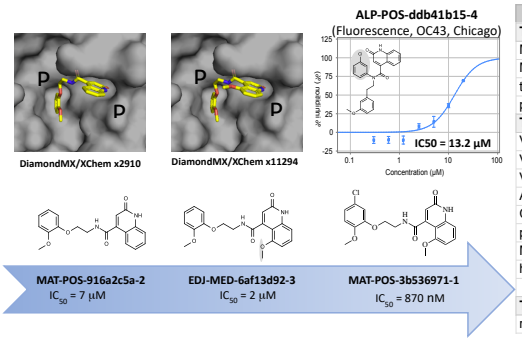
Since submitting R01 AI161245-01 on 13 Aug 2020, we have synthesized and assayed more than 509 new compounds, generated 135 new X-ray structures, and carried out additional assays that support the likelihood of achieving our Target Product Profile (TPP) for an orally bioavailable SARS-CoV-2 Mpro inhibitor.

Aim 1: Our main synthetic effort (189 new compounds) has been focused on the **aminopyridine** lead series, achieving >50-fold improvement in biochemical IC50, antiviral activity IC50 <5 μM with cytotoxicity CC50 >100 μM, 50-fold selectivity over host proteases, and measurable oral bioavailability in rats. The figure below reports progress on a high ligand efficiency P1-P2 scaffold, achieving IC50 < 300 nM before integrating P1' and P4 substituents. SAR (not shown) in P1' and P4 suggests potency goals of <50 nM are readily achievable, with a compound (MAT-POS-53907a1c-3) integrating the P1' and P4 substituents reported in **Figure 7** of the proposal demonstrating a biochemical IC50 of 58 nM with near-additive SAR. In addition, SAR around the P1-P2 scaffold suggests metabolic and PK goals will be attainable with further rounds of med chem.



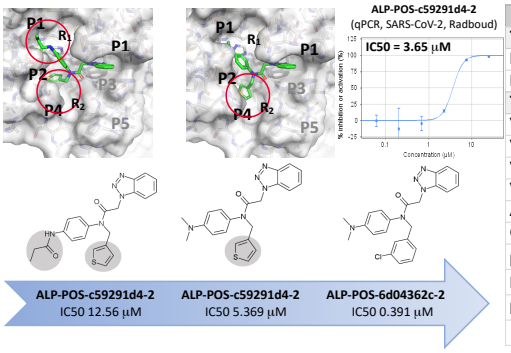
Assay	Type	August	December	December	TPP goal
Tier 1		**JOR-UNI-2fc98d0b-12**	**MAT-POS-b3e365b9-1**	**MAT-POS-53907a1c-3**	
Mpro inhibition (Fluorescence)	IC50	3.1 μM	141 nM	58 nM	<50 nM
Mpro inhibition (RapidFire)	IC50	3.3 μM	257 nM		<50 nM
thermodynamic solubility	solubility		34 μM		>10 μM
plasma protein binding	fraction unbound		12±2% unbound		>1% unbound
Tier 2					
VeroE6 antiviral activity (CPE)	IC50		1.57 μM		<5 μM
VeroE6 antiviral activity (qPCR)	IC50	7.31 μM	2.63 μM		<5 μM
VeroE6 cytotoxicity	CC50	25.5 μM	>500 μM		>100 μM
A549 cytotoxicity	CC50	14.1 μM	>100 μM		>100 μM
Calu-3 cytotoxicity	CC50	18.2 μM	>100 μM		>100 μM
protease selectivity at 100 μM	40 human protease panel		<12%		<40%
MDCK-MDR1	Papp		41±1 x10^-6 cm/s		>10 x10^-6 cm/s
human liver	CLint		98.3 μg/min/mg protein		<10 μg/min/mg protein
microsomal stability	t 1/2		14.1 min		>120 min
Tier 3					
rat oral bioavailability	t 1/2		1 h		>8 h

Aim 2: For the **quinolone** series, biochemical potency has progressed to <1 μM, with low oral exposure achieved in rat PK.



Assay	Type	August	December	December	TPP goal
Tier 1		**MAT-POS-916a2c5a-2**	**EDJ-MED-6af13d92-3**	**MAT-POS-3b536971-1**	
Mpro inhibition (Fluorescence)	IC50	7.5 μM	2.03 μM	870 nM	<50 nM
Mpro inhibition (RapidFire)	IC50	3.5 μM	2.08 μM		<50 nM
thermodynamic solubility	solubility		84 μM		>10 μM
plasma protein binding	fraction unbound		29.5±0.7% unbound		>1% unbound
Tier 2					
VeroE6 antiviral activity (fluorescence, OC43)	IC50		>20 μM		<5 μM
VeroE6 antiviral activity (CPE)	IC50		not active		<5 μM
VeroE6 cytotoxicity	CC50		>20 μM		>100 μM
A549 cytotoxicity	CC50		>10 μM		>100 μM
Calu-3 cytotoxicity	CC50		>100 μM		>100 μM
protease selectivity at 100 μM	40 human protease panel		<10%		<40%
MDCK-MDR1	Papp		2.0±0.1 x 10^-6 cm/s		>10 x 10^-6 cm/s
human liver	CLint		19.3 μg/min/mg protein		<10 μg/min/mg protein
microsomal stability	t 1/2		71.9 min		>120 min
Tier 3					
rat oral bioavailability	t 1/2		43 min		>8 h

Aim 3: The **benzotriazole** series has progressed biochemical IC50 from 12.5 μM to <500 nM.



Assay	Type	August	December	TPP goal
Tier 1		**ALP-POS-c59291d4-2**	**ALP-POS-6d04362c-2**	
Mpro inhibition (Fluorescence)	IC50	1.63 μM	497 nM	<50 nM
Mpro inhibition (RapidFire)	IC50	12.6 μM	391 nM	<50 nM
Tier 2				
VeroE6 antiviral activity (Fluorescence, OC43)	IC50	>20 μM		<5 μM
VeroE6 antiviral activity (CPE)	IC50	not active		<5 μM
VeroE6 antiviral activity (CPE)	IC50	3.65 μM		<5 μM
VeroE6 cytotoxicity	CC50	>100 μM		>100 μM
A549 cytotoxicity	CC50	>20 μM		>100 μM
Calu-3 cytotoxicity	CC50	>100 μM		>100 μM
protease selectivity at 100 μM		<35%		<40%
MDCK-MDR1	Papp			>10 x10^-6 cm/s
human liver	CLint	641 μg/min/mg protein		<10 μg/min/mg protein
microsomal stability	t 1/2	2.16 min		>120 min

Modifications to TPP goals: We have observed a smaller drop-off than initially expected from enzyme to cell activity---likely resulting from the non-peptidomimetic nature of our lead compounds---and have therefore relaxed the criteria for biochemical potency to <50 nM. Further benchmarking for rapidly generating a first-in-class agent also led us to reduce the hurdle for initial solubility to >10 μM, although formulation to higher solubility remains desirable.