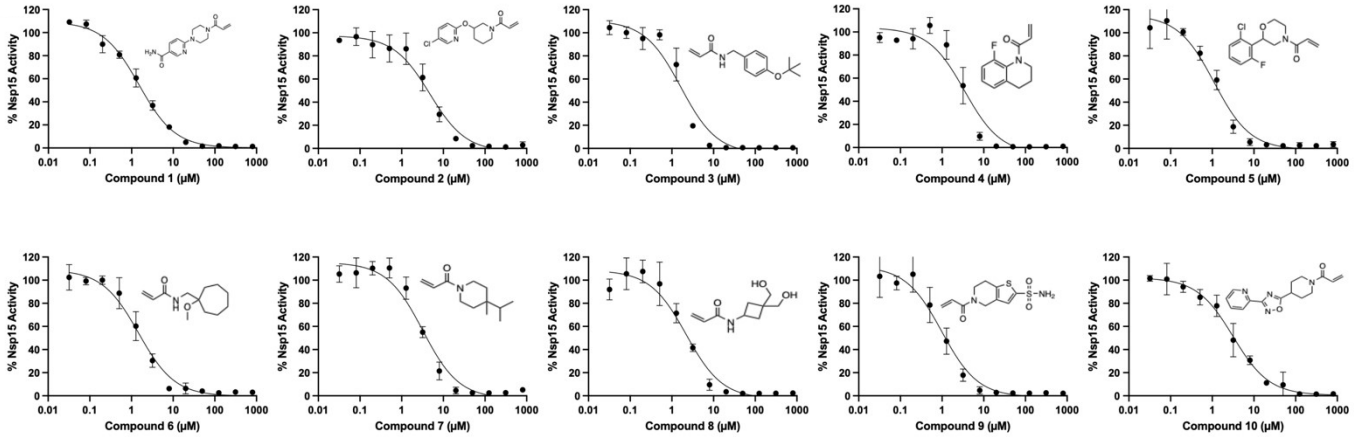

Fig S1. Dose response curves and chemical structures of ten compounds that were fished out from high throughput screening of acrylamide library of 2640 compound as covalent inhibitors of SCoV-2 Nsp15.

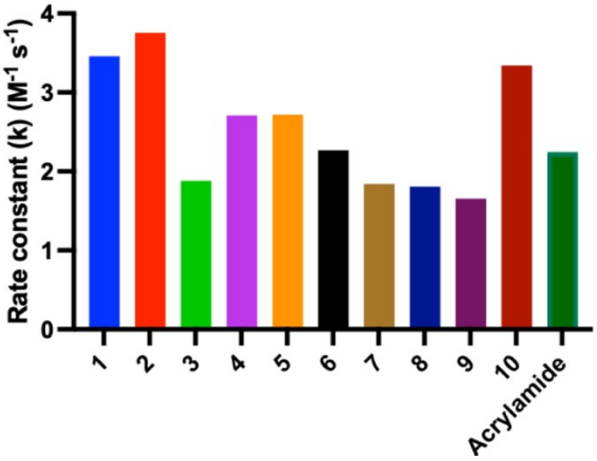

Fig S2. Calculated the rate constant (k) of reactivity of ten compounds towards thiols and compared it with acrylamide.

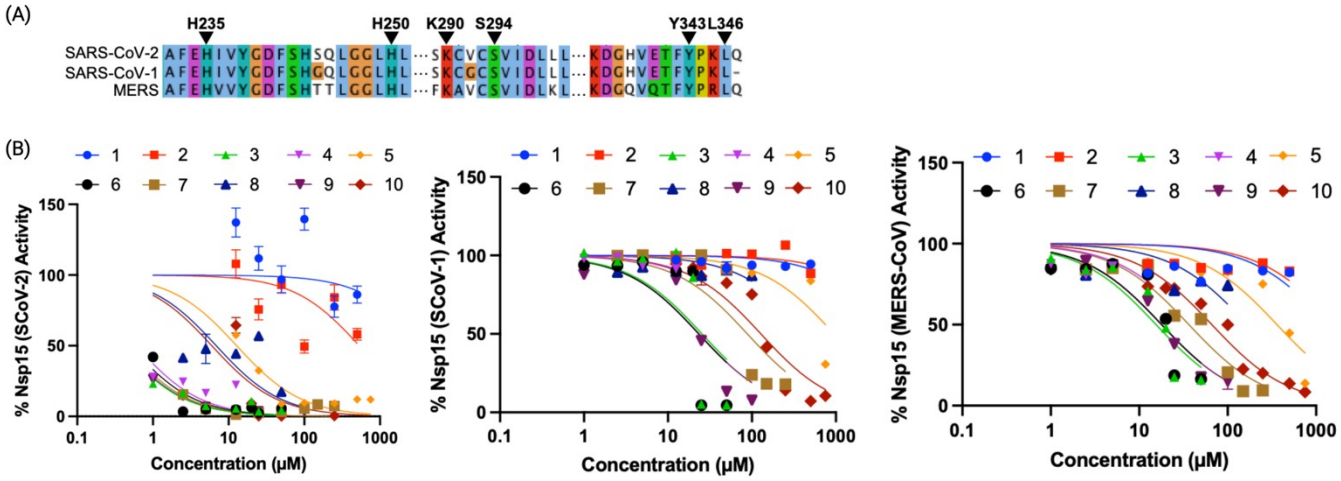

Fig S3. Efficacy of covalent Nsp15 inhibitors against related and unrelated enzymes. (A) Alignment of Nsp15 protein sequence amongst related homologs; key residues involved in catalysis are indicated with arrows. Alignment was performed with ClustalW; (B) Dose response assay of ten inhibitors at several concentrations against Nsp15 from SCoV-2, SCoV-1 and MERS-CoV.

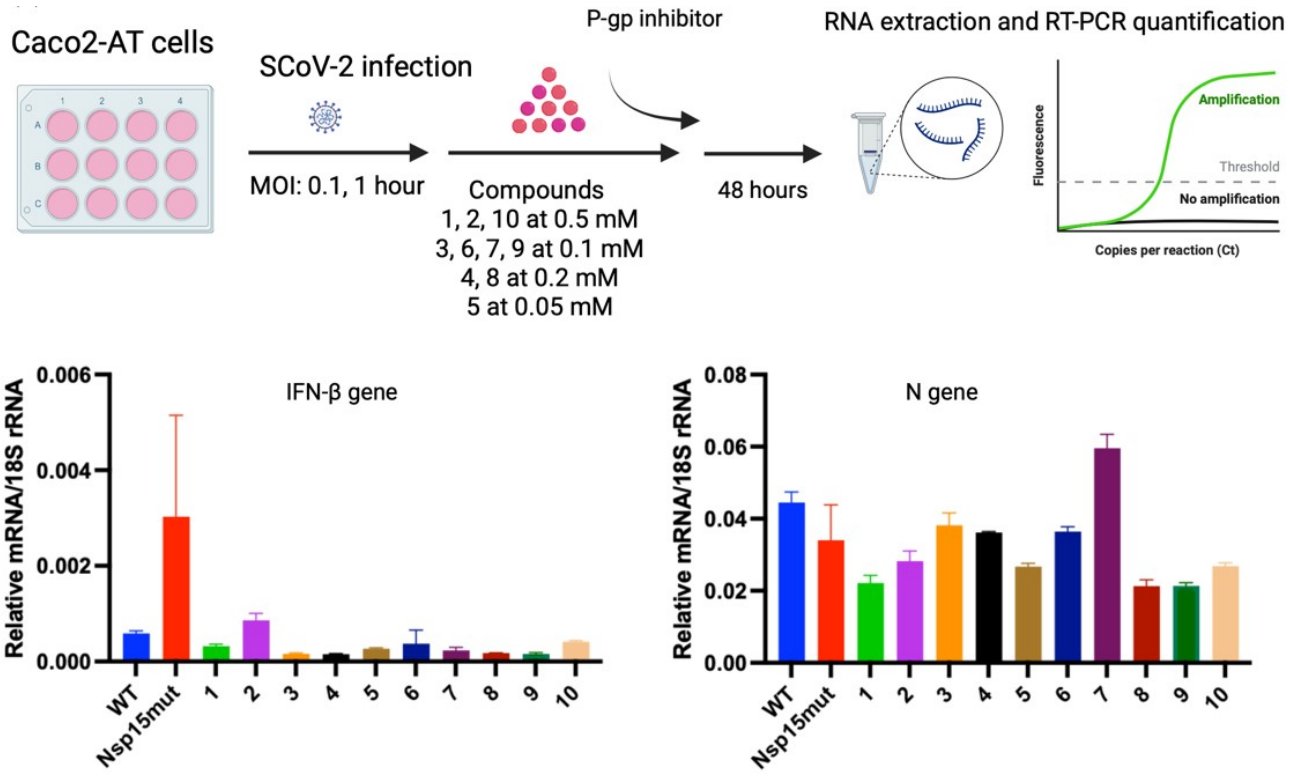

Fig. S4 Assessment of Nsp15 inhibitors in SCoV-2 infected Caco2-AT cells. (A) Scheme for evaluating inhibitory activity of compounds towards SCoV-2 Nsp15 through the expression of IFN-β and N gene; (B) evaluation of expression of N gene through RT-PCR suggesting the successful infection of SCoV-2 virus; (C) compounds did not upregulate the expression of IFN-β gene as catalytic inactive Nsp15 mutant.

(A)

	Physiochemical properties						Water	Lipophilicity	Medicinal Chemistry
Compound	MW	Fsp^3	RB	HB-A	HB-D	TPSA	LogS	LogP	Synthetic accessibility
1	260.29	0.31	4	3	1	79.53	-1.45	1.55	2.33
2	266.72	0.38	4	3	0	42.43	-3.01	2.63	2.76
3	233.31	0.36	6	2	1	38.33	-2.78	2.88	1.59
4	205.23	0.25	2	2	0	20.31	-2.76	2.17	2.02
5	269.7	0.31	3	3	0	29.54	-2.95	2.7	2.83
6	211.3	0.75	5	2	1	38.33	-2.17	2.57	2.07
7	195.3	0.75	3	1	0	20.31	-2.60	2.66	1.64
8	185.22	0.67	5	3	3	69.56	-0.30	1.29	1.71
9	272.34	0.30	3	4	1	68.90	1.84	0.70	2.93
10	284.31	0.33	4	5	0	72.12	-2.39	2.70	2.98

(B)

	Pharmacokinetics		Druglikeness		Medicinal Chemistry
Compound #	GI Absorption	BBB permeant	Lipinski violation	Bioavailability score	PAINS #alert
1	High	No	0	0.55	0
2	High	Yes	0	0.55	0
3	High	Yes	0	0.55	0
4	High	Yes	0	0.55	0
5	High	Yes	0	0.55	0
6	High	Yes	0	0.55	0
7	High	Yes	0	0.55	0
8	High	No	0	0.55	0
9	High	No	0	0.55	0
10	High	No	0	0.55	0

Fig. S5 Acrylamide based Nsp15 covalent inhibitors are predicted as drug-like compounds. (A) The physiochemical properties including molecular weight (MW), saturation referring fraction of carbon in sp3 (Fsp3), number of rotatable bonds (RB), hydrogen bond acceptor (HB-A) and donor (HB-D), polarity (TPSA) (Å) ranged optimal for effective drugs. LogS determining the water solubility is not higher than 6, lipophilicity (logP) ranged between -0.7 to +5.0; (B) the pharmacokinetics properties showed these inhibitors could be highly absorbed in GI tract and most of them are BBB permeant. All inhibitors follow the Lipinski's rule of five with a good chance of oral availability. Additionally, all inhibitors are very easy to synthesize in easy and do not show any promiscuity.

(A)

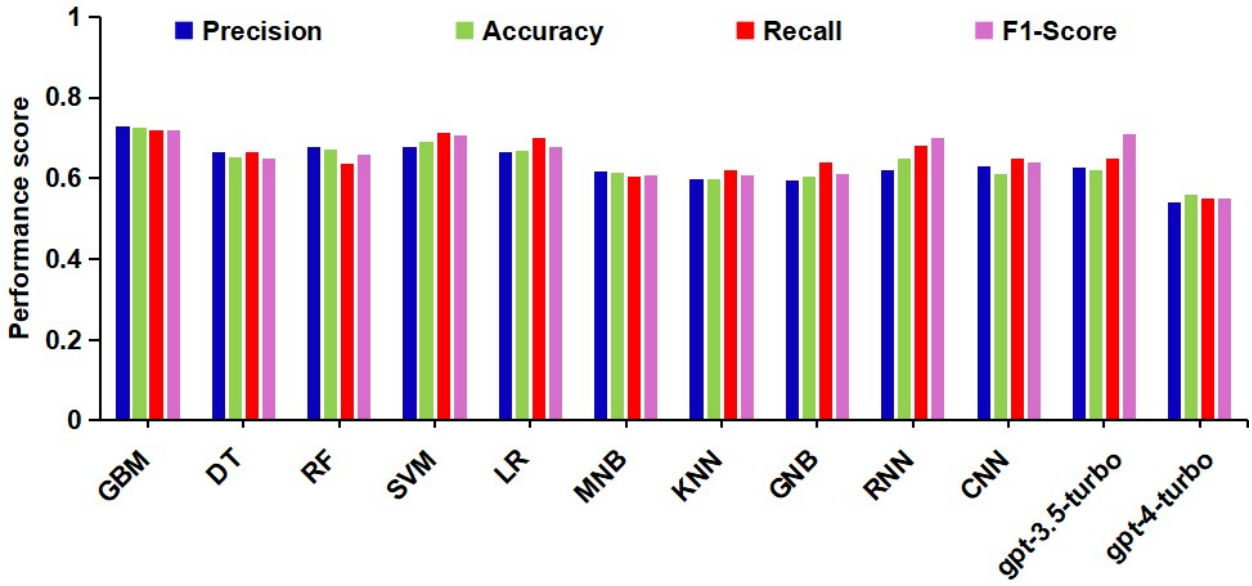

Fig. S6 Prediction of inhibitors against Nsp15 using AI model. (A) Performance of machine learning and AI models (SVM, random forest (RF), decision tree (DT),Gradient Boostin(GB), logistic regression (LR), naive bayes (NB), KNN, C4.5, RNN, CNN, ChemBERTa, GPT-3.5-turbo, GPT-4-turbo) based on different algorithms in terms of accuracy, F1-Score, precision and recall across the deep learning and LLM models; (B) the AI model was applied to 50 untrained compounds to predict as Nsp15 inhibitors or non-inhibitors and result showed correctly predicted positive and negative hits from unlabled, providing 73% accuracy.

(B)

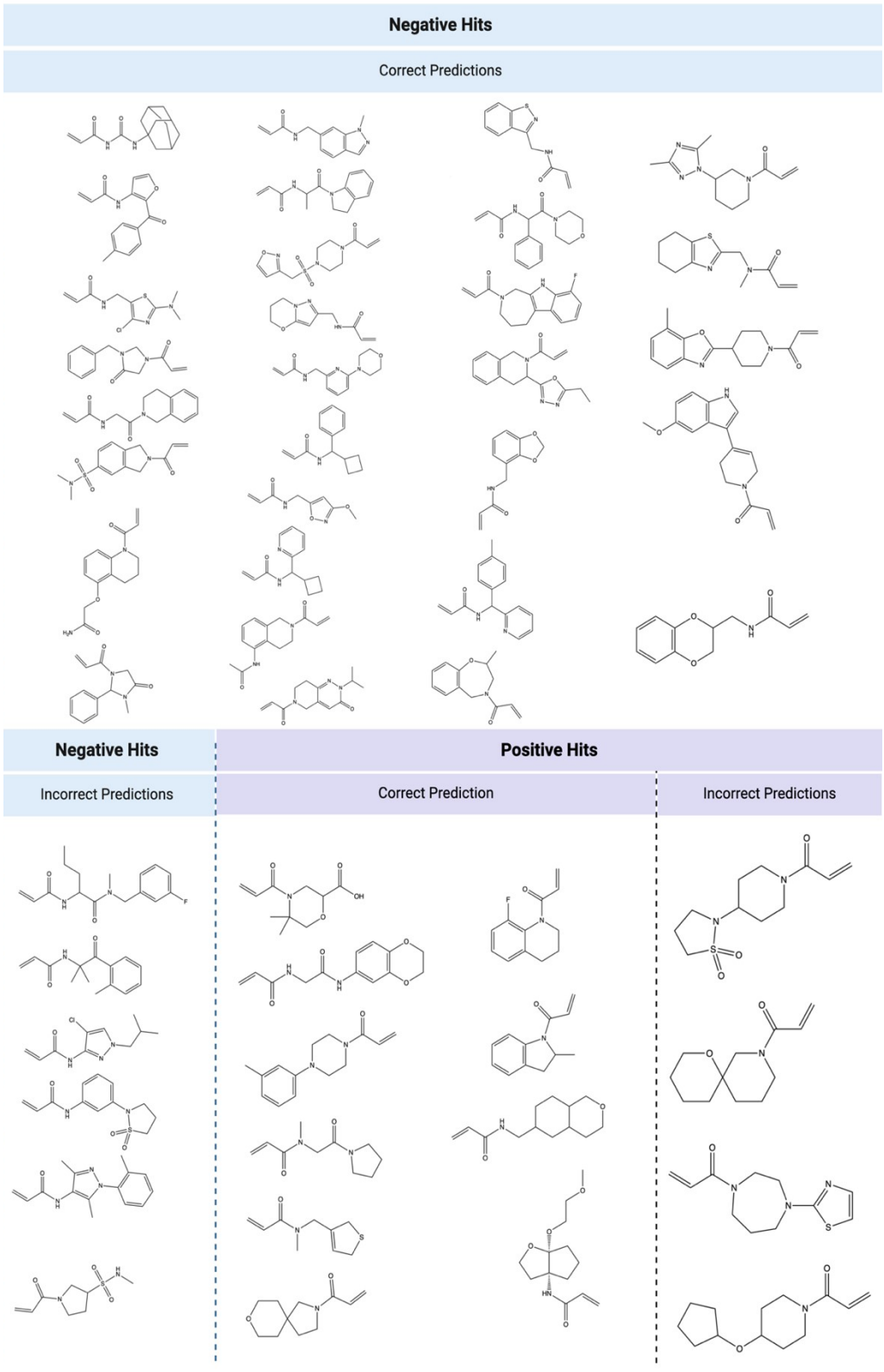

Fig. S6 Prediction of inhibitors against Nsp15 using AI model. (A) Performance of machine learning and AI models (SVM, random forest (RF), decision tree (DT), logistic regression (LR), naive bayes (NB), KNN, C4.5, RNN, CNN, FNN, GPT-3.5-turbo-0125, ChemBERTa, GPT-3.5-turbo, GPT-4-vision, GPT-4-turbo) based on different algorithms in terms of accuracy, F1-Score, precision and recall across the deep learning and LLM models; (B) the AI model was applied to 50 untrained compounds to predict as Nsp15 inhibitors or non-inhibitors and result showed correctly predicted positive and negative hits from unlabled, providing 80% accuracy.