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Evaluating the *In Vitro* Efficacy of Antiviral Therapeutics Against SARS-COV-2

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PURPOSE

By using artificial intelligence (AI) to identify marketed drugs exhibiting antiviral activity against SARS-CoV-2, the purpose of this study was to determine the potential antiviral activity of several AI-identified drugs against the Washington (USA-WA1/2020) variant of SARS-CoV-2 within human lung cancer (Calu-3) cells.

OBJECTIVE

The objective of this project is to test the *in vitro* efficacy of potential antiviral compounds against SARS-CoV-2, the causative agent of COVID-19.

METHODS

Our AI DeepDrug^{TM} software (**Table 1**) identified several potential antiviral drugs through analysis of host cell – SARS-CoV-2 virus, protein-protein interactions. The validation of the AI analysis was performed *in vitro* using Calu-3 cells seeded into 96 well plates, at 40,000 cells/well. The concentration where drug toxicity occurred was determined using MTT assay. For the infection assays, cells were inoculated with the SARS-CoV-2 USA-WA-1/2020 strain at a multiplicity of infection (MOI) of 0.01 $TCID_{50}$/cell and incubated for 60 – 90 minutes. Following the incubation, viral inoculum will be removed with cells washed. The cells were then provided with EMEM2 with 2% FBS containing test compounds and incubated at 37ºC in 5% CO_2. Cells are fixed and evaluated for the presence of virus *via* an immunostaining assay 48 hours post-inoculation. Absorbance readings for each well were collected by Softmax Pro software and imported into a Microsoft Excel spreadsheet. Outliers were detected by Grubbs' test in the built-in analysis of GraphPad Prism 9.

Table 1. DeepDrug^{TM} AI Platform.

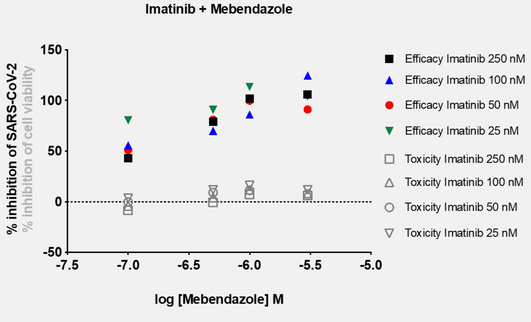
AI SYSTEM	DESCRIPTION	BENEFIT TO PLATFORM PARTNER
eSynth	New Molecule Synthesis Engine	Synthesize New Molecules and New Chemical Entities based on Protein Targets
eToxPred	Fragment based toxicity analysis (SVM, ET, Deep Belief Network)	Creates a 2/3/4 Drug Interaction Graph to let researchers analyze Pharmacological Index and if 2/3/4 drug combinations are toxic or not
eDrugRes	Graph Convolutional Neural Network based bacterial antibiotic susceptibility	AI Network with an entire volume known components of antibiotics to break down existing antibiotics to recompile to make new novel antibiotics
eVir	Peptide-based, protein-protein interaction led drug discovery (Graph Node Embeddings, MultiLayer Perceptron, Siamese Network) Dose-response based results	A Model of all known protein interactions from SARS COV 2003, SARS COV2, MERS and other Coronavirus which enables the syntheses of efficacy models from existing drugs or synthesizes new chemical entities from known protein targets
eComb	Drug combination synergy analysis and drug-drug interaction prediction (BLISS, MuSyC, PDDIs, Neural Network based)	A Next generation analysis engine that scores efficacy, synergy and toxicity of 2,3,4,5 or greater drug combinations to look at combination analysis for new indications

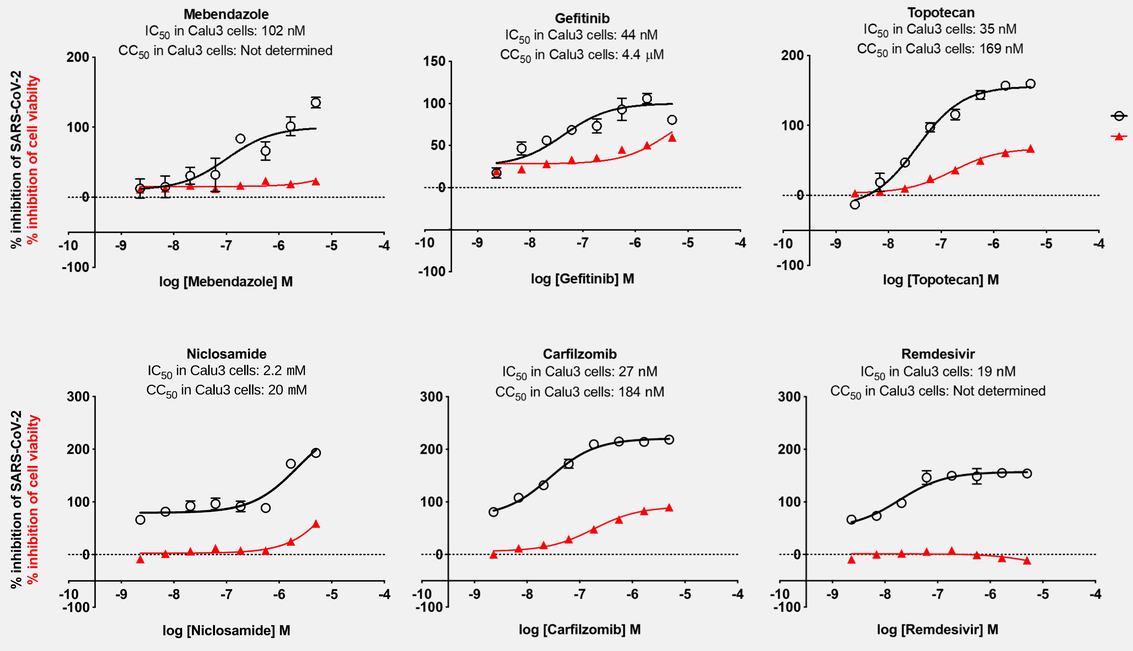
RESULTS

Test compounds were evaluated against SARS-CoV-2 in Calu-3 cells. The cellular toxicity (CC_{50}) and antiviral efficacy (IC_{50}) data are summarized in the Figures below. SM-4, gefitinib, topotecan, and – to a lesser extent – carfilzomib showed conventional drug-response curves, with IC_{50} values near or below that of remdesivir with excellent confidence that were all above $R^2 > 0.91$ with no cytotoxicity at the IC_{50} concentration (**Table 2**). Cyclosporine A showed antiviral activity, but also unconventional drug-response curves and low R^2, which are explained by the dose-independent toxicity of the compound. Additionally, niclosamide displayed a conventional drug-response curve and good confidence, although the inherent cytotoxicity produced an artifact antiviral efficacy curve. Remdesivir was used as a control compound and was evaluated in parallel with the submitted test articles and had conventional drug-response curves validating the overall results of the assay.

Table 2. Tabulated *in vitro* results in Calu-3 cells.

Treatment	Code	IC50	Units	R square
Mebendazole	SMRES-003	48.55	nM	0.9194
Gefitinib	SMRES-012	62	nM	0.9494
Topotecan	SMRES-040	9.362	nM	0.9815
Niclosamide	SMRES-014	982.5	nM	0.9047
Carfilzomib	SMRES-055	5.741	nM	0.9882
Remdesivir	-	115.7	nM	0.9811





CONCLUSIONS

Taken together, these preliminary findings suggest that several novel compounds may have significant *in vitro* activity against SARS-CoV-2. Mebendazole, gefitinib, topotecan, niclosamide, and carfilzomib have promising antiviral activity with little to no apparent cytotoxicity (as measured by inhibition of cell viability). Future directions will test other predicted compounds with good confidence scores according to the DeepDrug^{TM} AI.

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REFERENCES

1. Bess A, Berglind F, Mukhopadhyay S, Brylinski M, Griggs N, Cho T, Galliano C, Wasan KM. Artificial intelligence for the discovery of novel antimicrobial agents for emerging infectious diseases. Drug Discov Today. 2022 Apr;27(4):1099-1107.