Supporting Information

The Natural Products Withaferin A and Withanone From the Medicinal Herb *Withania somnifera* are Covalent Inhibitors of the SARS-CoV-2 Main Protease

Shayantani Chakraborty[‡], Dibyendu Mallick[†], Mausumi Goswami[¥], F. Peter Guengerich[¶], Anindita Chakrabarty[‡]*, and Goutam Chowdhury[#]*

[‡]Department of Life Science, Shiv Nadar University, Greater Noida, UP, India

[†]Department of Chemistry, Presidency University, Kolkata, India

[¥]Department of Chemistry, School of Advanced Sciences, Vellore Institute of Technology, Vellore, TN, India

[¶]Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN, USA

[#]Independent Researcher, Greater Noida, UP, India

Contents:

Figure S1: LC-HRMS spectra of win-Cys, win-GSH, wifA-Cys and wifA-GSH adducts

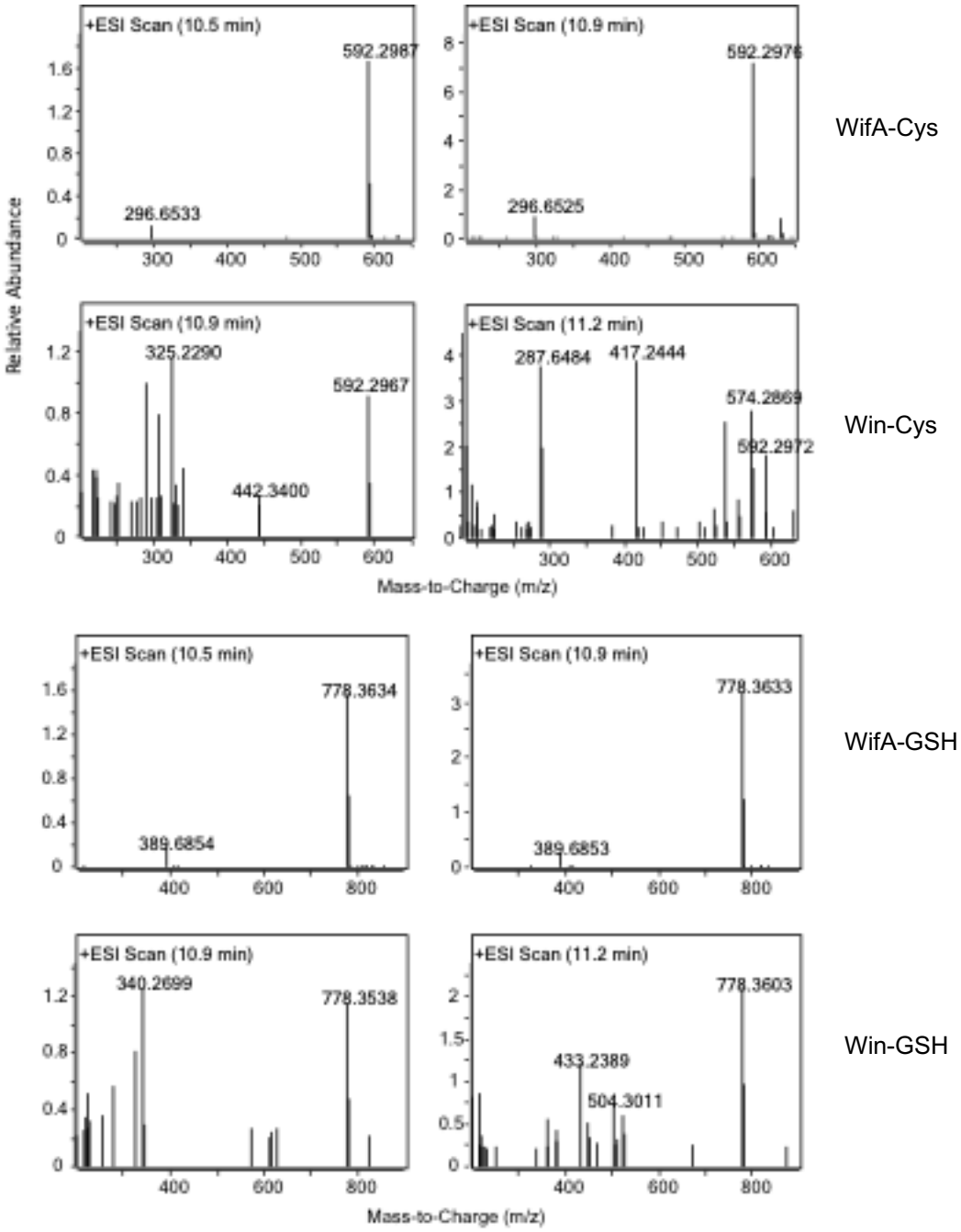


Figure S2: LC-MSMS spectra and fragmentation analysis of win-Cys, win-GSH, wifA-Cys and wifA-GSH adducts

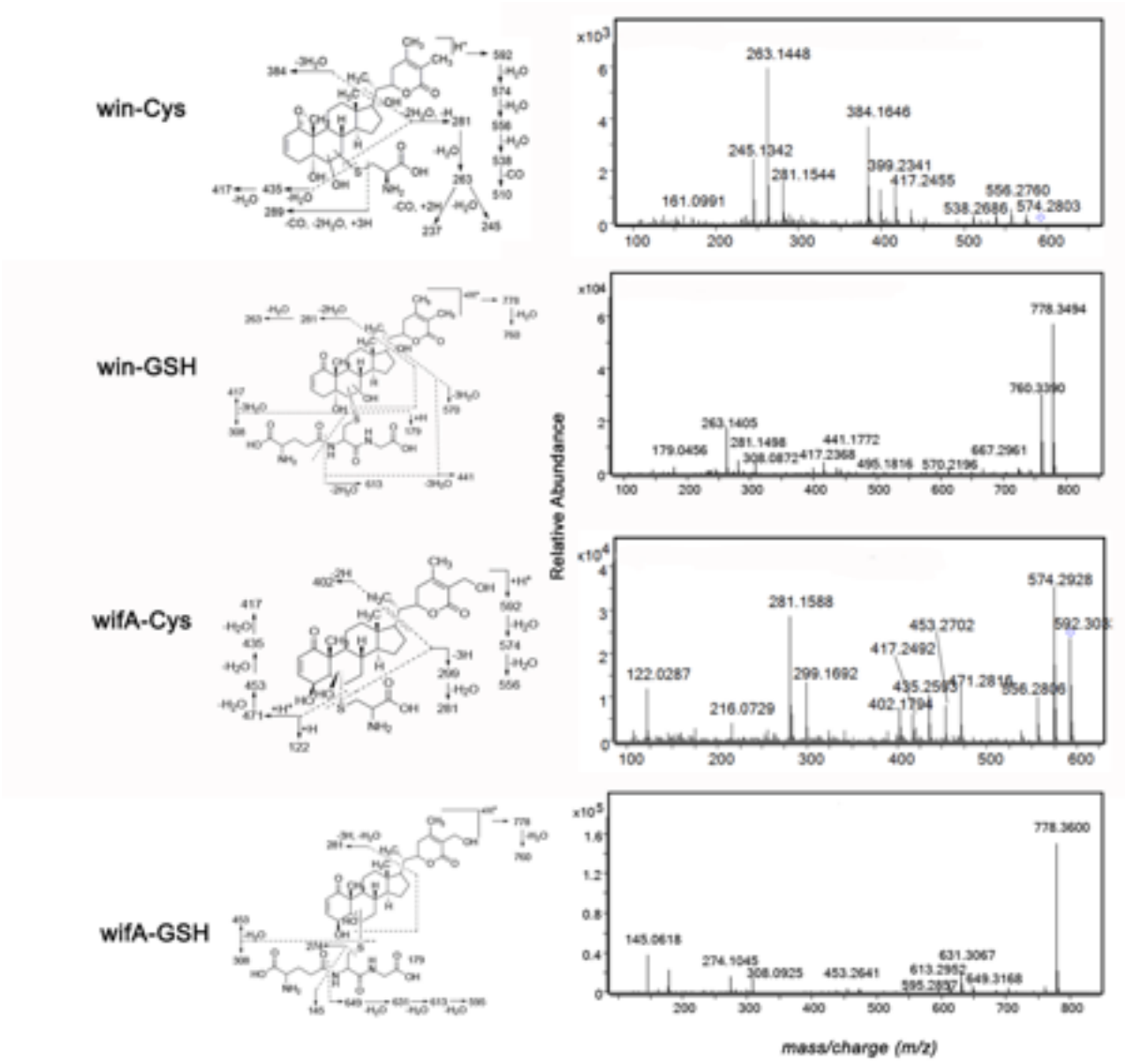


Figure S3: Effect of win and wifA on HepG2 and A549 cells. A) 2D culture B) Organoid like culture

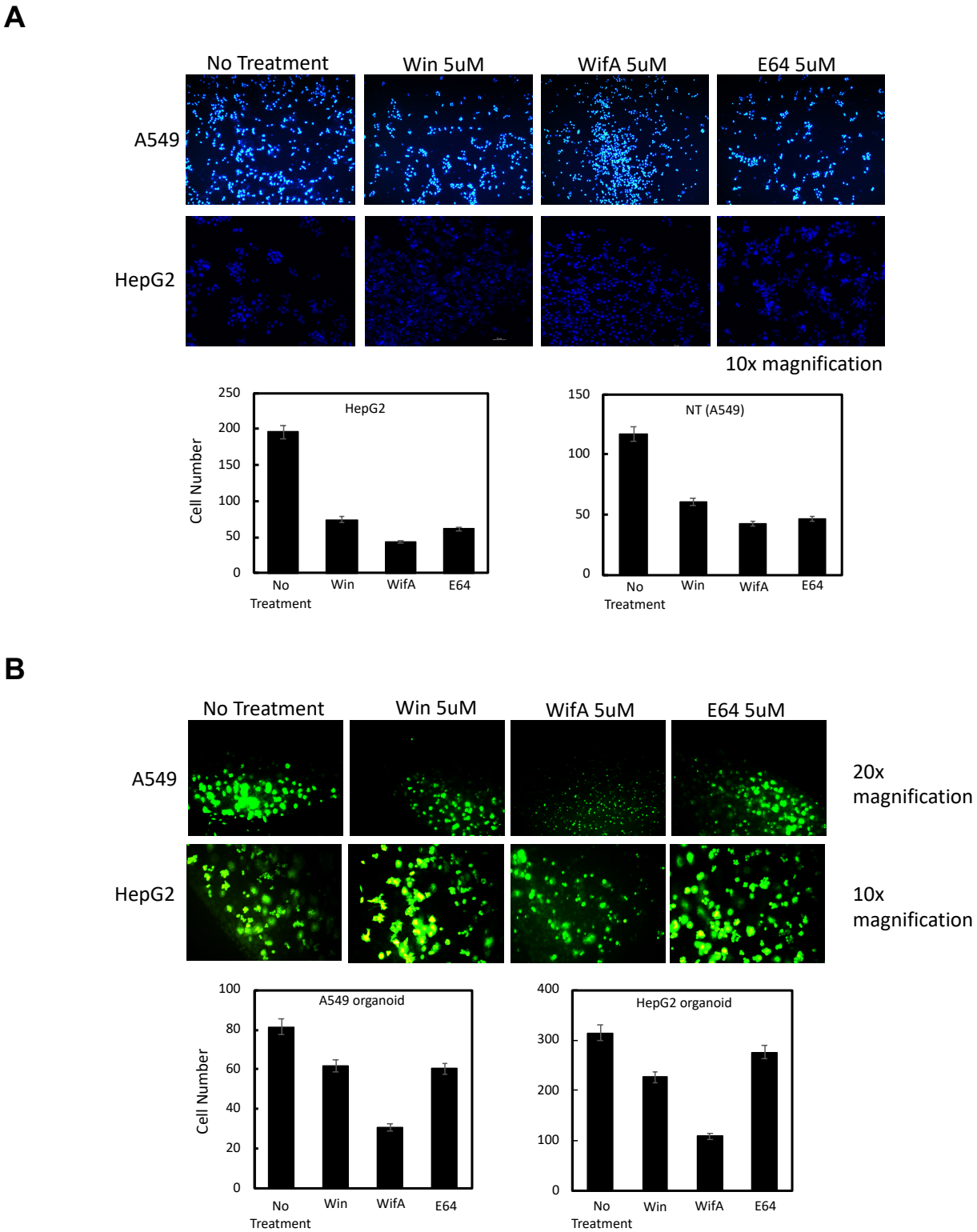


Figure S4: Expression and purification of M^{pro}. A) Image of a polyacrylamide gel showing the various purified fractions and the purity of isolated M^{pro} B) Image of western blot.

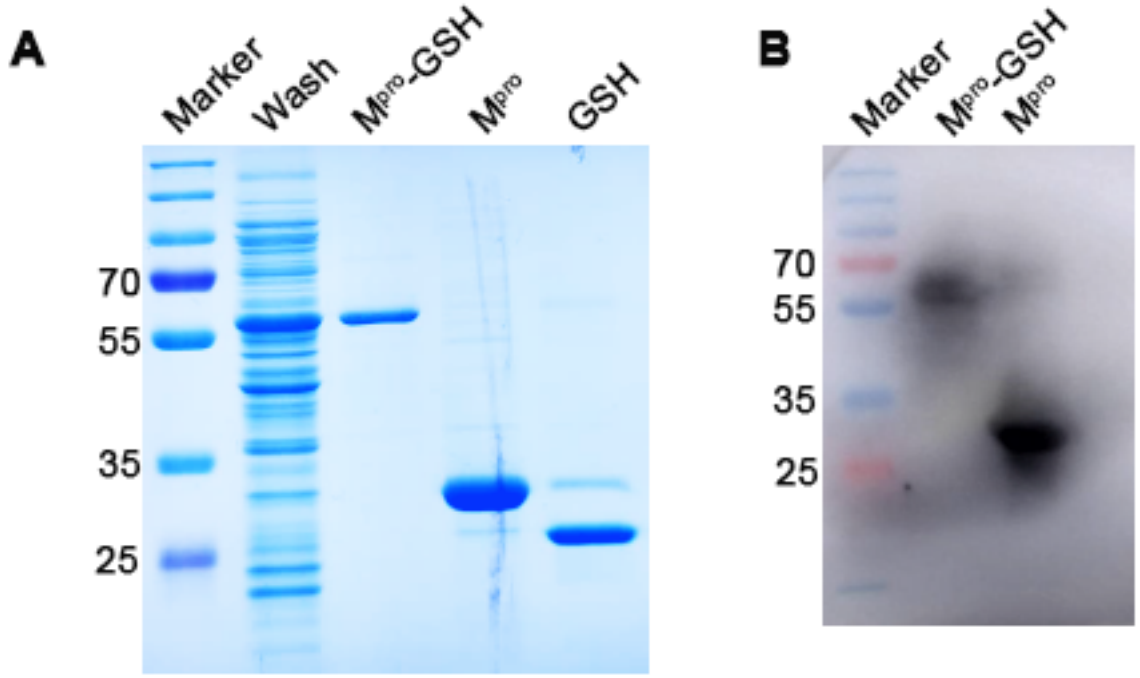
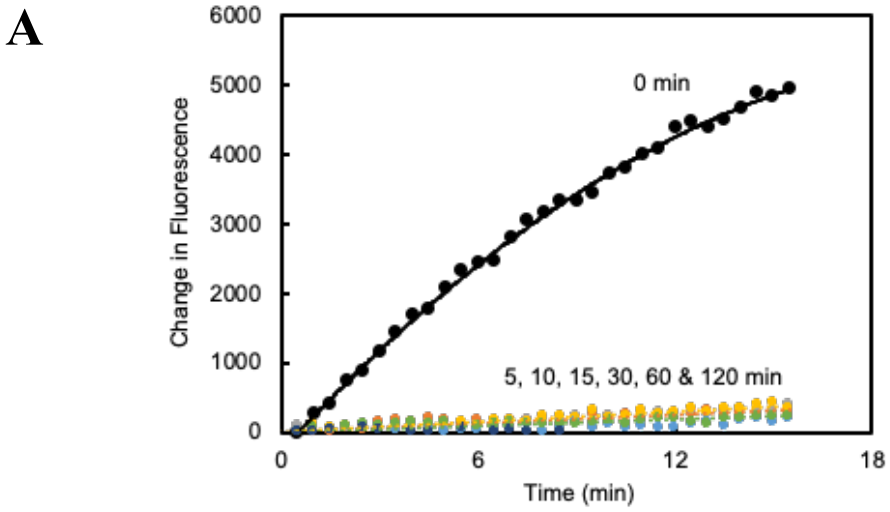
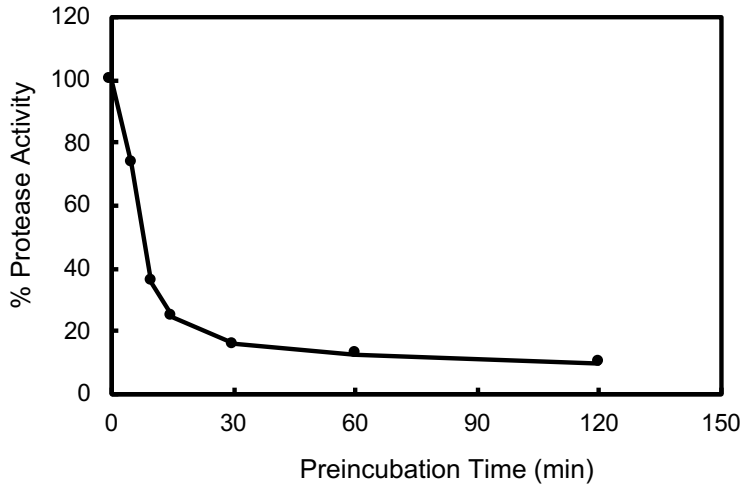


Figure S5: Inhibition of protease activity of M^{pro} by wifA. A. Purified M^{pro} (0.5 μM) was preincubated for 5-120 min with 5 μM of wifA before adding the substrate DABCYL-KTSAVLQSGFRKME-EDANS and measuring fluorescence over time B. Purified M^{pro} (0.25 μM) was preincubated for 5-120 min with 0.5 μM of wifA before adding the substrate DABCYL-KTSAVLQSGFRKME-EDANS and measuring fluorescence over time C. FRET-based kinetic assay demonstrating the percent inhibition of protease activity of the recombinant M^{pro} (0.5 μM) following preincubation with an increasing concentration of wifA in the presence of GSH (5 mM). The error bars represent standard deviation of two replicates.



B


% Protease Activity
120
100
80
60
40
20
0
0
30
60
90
120
150
Preincubation Time (min)

C

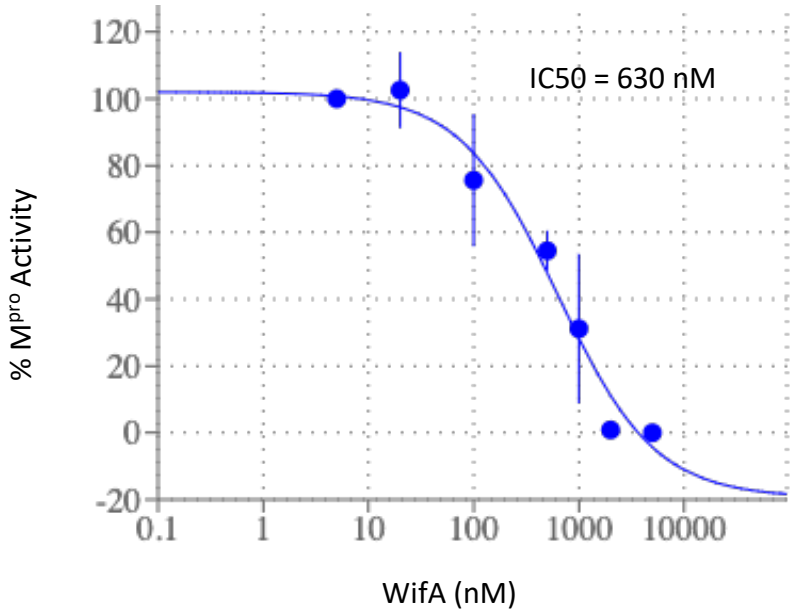
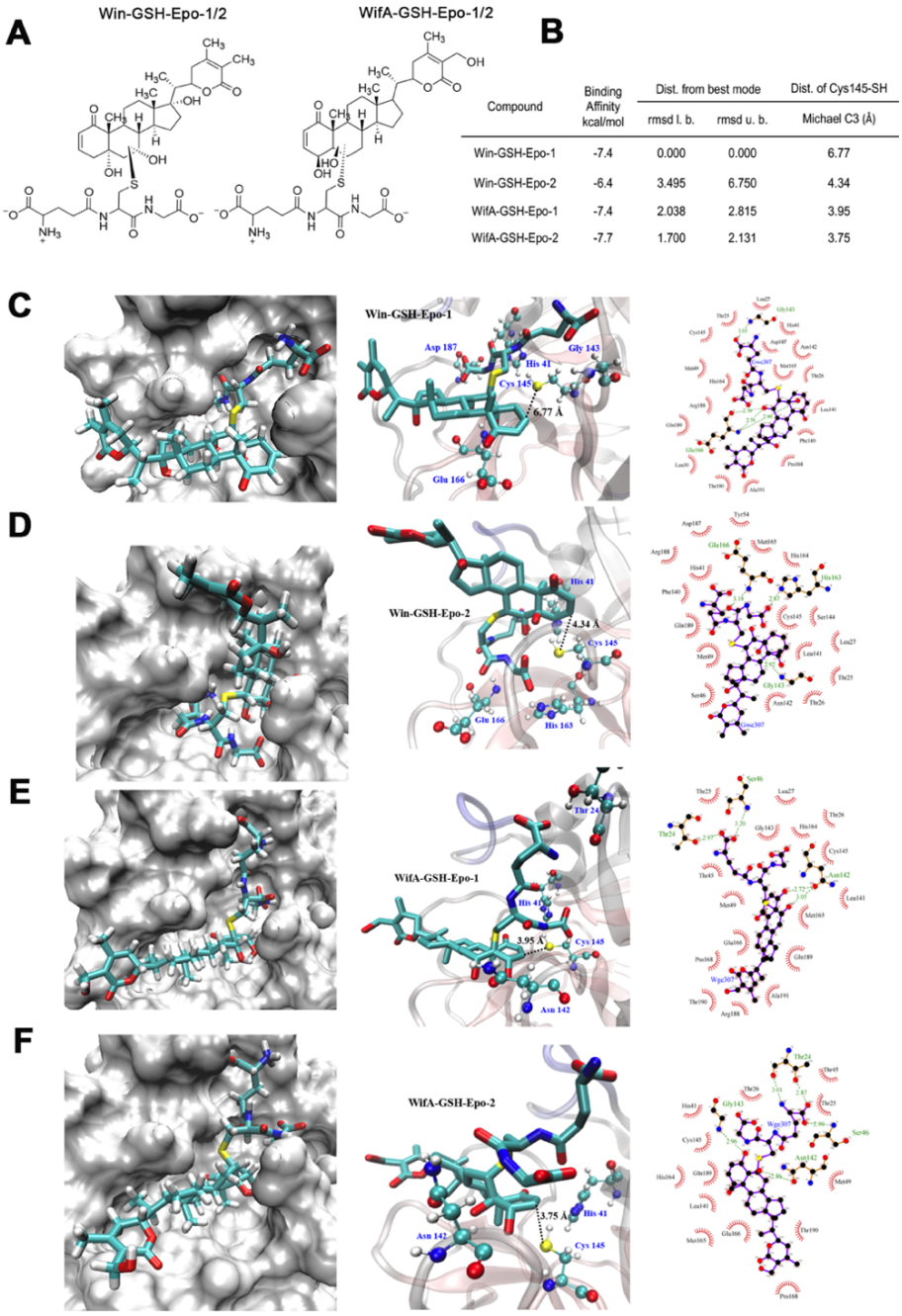

IC50 = 630 nM
% M^pro Activity
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
10000
WifA (nM)

Figure S6. Non-covalent binding of win-GSH-epoxide and wifA-GSH-epoxide at the active site of the SARS-CoV-2 cysteine protease M^{pro} using *in silico* docking studies. A) Structures of win-GSH-epoxide and wifA-GSH-epoxide B) Binding parameters of win-GSH-epoxide and wifA-GSH-epoxide at the active site of M^{pro} C-D) Binding of win-GSH-epo-1/2 at the active site of M^{pro} E-F) Binding of wifA-GSH-epo-1/2 at the active site of M^{pro}.



Compound	Binding Affinity kcal/mol	Dist. from best mode		Dist. of Cys145-SH
		rmsd l. b.	rmsd u. b.	Michael C3 (Å)
Win-GSH-Epo-1	-7.4	0.000	0.000	6.77
Win-GSH-Epo-2	-6.4	3.495	6.750	4.34
WifA-GSH-Epo-1	-7.4	2.038	2.815	3.95
WifA-GSH-Epo-2	-7.7	1.700	2.131	3.75