Protein production for Mass Spectrometry and Bio-Layer Interferometry (BLI)

The codon optimized wild type cDNA of SARS-CoV-2 receptor-binding domain (RBD) (residues 333–530) was synthesized by GENEWIZ. The fusion protein sequence with an C-terminal 8×His tag for purification was cloned into pACgp67 vector with *BamH* Ⅰ and *Not* Ⅰ restriction sites using the cloning primers. The sequences of the primers were: 5'-TCTCCTACATCTACGCCGACGGATCCACCAACCTCTGCCCTTTCGGT-3' (forward), 5'-GATGGTGATGGTGGTGATGATGTGCGGCCGCTTCATGCCACTCAATCTTCTGAGCCTCG AAAATATCATTAAGACC-3'(reverse). The accuracy of the insert was verified by sequencing.

The SARS-CoV-2 RBD was expressed using the Bac-to-Bac baculovirus system. The construct was transformed into bacterial DH5α component cells, and the extracted bacmid was then transfected into Sf9 cells using Cellfectin II Reagent (Invitrogen). The low-titer viruses were harvested and then amplified to generate high-titer virus stock. The viruses and Endo H, Kifunensine were co-infected Hi5 cells at a density of 2×10^6 cells/ml. The supernatant of cell culture containing the secreted removal of glycosylated RBD was harvested 72 h after infection, concentrated and RBD was captured by Ni-NTA resin (GE Healthcare). Resin was washed 5-6 times with 30 mL of wash buffer (25 mM Tris, 150 mM NaCl, 40 mM imidazole, pH 7.5), the target protein was eluted with elution buffer containing 25 mM Tris, 150 mM NaCl, 500 mM imidazole, pH 7.5. The protein was further purified on a Superdex S75 (GE Healthcare) column equilibrated with 25 mM Tris, 150 mM NaCl, pH 7.5. SDS-PAGE analysis revealed over 95% purity of the final purified recombinant protein. Fractions from the single major peak were pooled and concentrated. The RBD protein was biotinylated by using Biotin-Protein Ligase kit (GeneCopoeia™), further purified on the Superdex S75 column and concentrated to 15 mg/mL.

Protein production for Surface Plasmon Resonance

The codon-optimized genes encoding for S1-RBD of SARS-CoV-2 (residues 319-591) and its subdomain of SARS-CoV-2 (residues 333-530) containing human CD5 signal peptide sequence and mouse Ig Kappa Locus secretion signal sequence respectively, two purification tags (8xHistidine tag and HRV 3C tag) and TEV protease cleavage sites were cloned into the mammalian expression vector pCDNA3.4(Thermo Fisher Scientific). All constructs were confirmed by BioSune sequencing. Proteins were expressed in Expi293F cells using an ExpiFectamine expression system kit in accordance with the recommended protocol of the manufacturer (Thermo Fisher Scientific). Cells were grown in sterile TC flasks and ventedin a Climo Shaker ISF4-XC at 110 RPM (orbital diameter of 50 mm) at 37° C and 8% CO_2. When cells reached a density between 3.5 and 5x10^6 cells/mL, they were counted, and appropriately diluted into a shaker flask for transfections. The culture supernatant was harvested on day 7 post transfection unless otherwise specified, and then adjusted to 1× Ni-NTA buffer (50mM Tris-HCl/500mM NaCl/20mM Imidazole, pH 8) using stock solutions, and centrifuged at 30,000 g for 25 min before purification using Ni-NTA resin (GE Healthcare). Protein purity was estimated as > 95% by SDS-PAGE and protein concentration was measured spectrophotometrically (NanoVue, GE Healthcare).

BLI kinetic measurements of ACE2 binding to immobilized Fc-RBD

All interaction experiments were conducted at 30 °C in PBS,0.02% Tween 20,0.1% BSA, pH 7.4，using an Octet Red 96 instrument (Sarotirus, Fremont, California, USA). The final volume for all solutions was 200 µl. Assays were performed in black, solid 96-well, flat bottom plates and agitated, which was set to 1000 rpm. Sensors were loaded with 10 µg/ml ligand (Fc-RBD). A 120 s biosensor washing step was utilized before the analysis of the ligand association from the biosensor to the analyte in solution, which was performed for 180 s. The concentration of the analyte (ACE2) were 150nM, 75nM, 37.5nM, 18.75nM, 9.375nM, 4.68nM. Finally, the dissociation was allowed to proceed for 180 s. The dissociation wells were used only once to ensure the potency of the buffer. The correction of any systematic baseline drift was accomplished by subtracting the shift recorded for sensors loaded with ligand but no analyte.

To characterize whether MLN4760 could inhibit ACE2 binding to immobilized Fc-RBD, the proA sensors, which were coated with Fc-RBD, were exposed to 150nM, 75nM, 37.5nM, 18.75nM, 9.375nM, 4.68nM ACE2 with 1.5uM MLN4760. The proA sensors, which were coated with Fc, were exposed to 150nM, 75nM, 37.5nM, 18.75nM, 9.375nM, 4.68nM ACE2 with 1.5uM MLN4760. Sensors coated with Fc were as negative control. The correction of any systematic baseline drift was accomplished by subtracting the shift recorded for sensors loaded with ligand but no analyte.

ADMET properties prediction

To evaluate the ADMET properties of these identified natural products, we used the admetSAR webserver to make the predictions. The ADMET properties include Human Intestinal Absorption (HIA), Blood Brain Barrier, P-glycoprotein inhibition as well as substrate, CYPs substrate as well as inhibition (CYP3A4, CYP2C9, CYP2D6, CYP3A4, CYP2C19 and CYP1A2), ames

mutagenesis, and hepatotoxicity.