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(54) Title: ANTI-VIRAL THERAPEUTIC AGENTS AND USES THEREOF

(57) Abstract: The present invention is related to compositions and methods for the reduction of risk and treatment of viral infections.



ANTI-VIRAL THERAPEUTIC AGENTS AND USES THEREOF**BACKGROUND OF THE INVENTION**

Viruses represent a dire threat to human health. Viruses infect host cells, forcing the host cell to replicate the original virus. Viruses remain difficult to treat due to their ability to mutate resulting in reduced efficacy of treatments designed specifically to address the original variant. What is needed in the field is improved methods of reducing the risk of and treating viral infections that function with a mechanism of action that remain applicable for new variants.

SUMMARY OF THE INVENTION

The present invention is related to compositions and methods for the reduction of risk and the treatment of viral infections.

In one embodiment, the present invention provides methods of reducing the risk of viral infection in a subject at risk of a viral infection or treating a viral infection in an infected subject, the method including the step of administering to the subject a composition containing sodium 2-mercaptoethane sulfonate or 2-mercaptoethylamine particles. In one embodiment, the subject is administered a composition containing sodium 2-mercaptoethane sulfonate particles. In another embodiment, the subject is administering a composition comprising 2-mercaptoethylamine particles.

In some embodiments, the viral infection is coronavirus. In some embodiments, the viral infection is severe acute respiratory syndrome coronavirus (SARS-CoV). In some embodiments, the viral infection is severe acute respiratory syndrome coronavirus 2 (SARS-CoV2). In some embodiments, the viral infection is a SARS-CoV2 variant. In some embodiments, the variant is Delta (B.1.617.2), Alpha (B.1.1.7), Gamma (P.1), Beta (B.1.351), or Omicron (B.1.1.529).

In some embodiments, the viral infection is a rhinovirus.

In some embodiments, the viral infection is a respiratory syncytial virus (RSV).

In some embodiments, the viral infection is an infection of an avian influenza virus; influenza A, B, or C virus; an adenovirus; a herpesvirus; a human papillomavirus, such as HPV; a parvovirus; a reovirus; a picornavirus; a flavivirus; a togavirus; an orthomyxovirus; a bunyavirus; a rhabdovirus; a paramyxovirus; herpes simplex virus; varicella zoster virus; parvovirus B19; canine parvovirus; an orbivirus; a rotavirus (e.g., Rotavirus A; Rotavirus B; or Rotavirus C); an aquaracovirus; a coltivirus; an enterovirus (e.g., enterovirus 68, 70); a hepatovirus; a torovirus; a petsivirus; an alphavirus; a rubivirus; Thogoto virus; Hantavirus; Nairovirus; a phlebovirus; Punta toro phlebovirus; an adeno-associated virus; Aichi virus; Australian bat lyssavirus; BK polyomavirus; Banna virus; Barmah forest virus; Bunyamwera virus; Bunyavirus La Crosse; Bunyavirus snowshoe hare; Cercopithecine herpesvirus; Chandipura virus; Chikungunya virus; Cosavirus A; Cowpox virus; Coxsackievirus; Crimean-Congo hemorrhagic fever virus; Dengue virus; Dhori virus; Dugbe virus; Duvenhage virus; Eastern equine encephalitis virus; Ebolavirus; Echovirus; Encephalomyocarditis virus; Epstein-Barr virus; European bat lyssavirus; Hantaan virus; Hendra virus; Hepatitis A virus; Hepatitis B virus; Hepatitis C virus; Hepatitis E virus; delta virus; Horsepox virus; astrovirus; cytomegalovirus; herpesvirus 1; herpesvirus 2; herpesvirus 6; herpesvirus 7; herpesvirus 8; immunodeficiency virus; papillomavirus 1; papillomavirus 2; papillomavirus 16,18; parainfluenza; spumaretrovirus; T-lymphotropic virus; Isfahan virus; JC polyomavirus; Japanese encephalitis virus; Junin arenavirus; KI Polyomavirus; Kunjin virus; Lagos bat virus; Lake Victoria

marburgvirus; Langat virus; Lassa virus; Lordsdale virus; Louping ill virus; Lymphocytic choriomeningitis virus; Machupo virus; Mayaro virus; MERS coronavirus; Measles virus; Mengo encephalomyocarditis virus; Merkel cell polyomavirus; Mokola virus; Molluscum contagiosum virus; Monkeypox virus; Mumps virus; Murray valley encephalitis virus; New York virus; Nipah virus; Norwalk virus; O'nyong-nyong virus; 5 Orf virus; Oropouche virus; Pichinde virus; Poliovirus; Puumala virus; Rabies virus; Rift valley fever virus; Rosavirus A; Ross river virus; Rubella virus; Sagiyama virus; Salivirus A; Sandfly fever sicilian virus; Sapporo virus; SARS coronavirus 2; Semliki forest virus; Seoul virus; Simian foamy virus; Simian virus 5; Sindbis virus; Southampton virus; St. louis encephalitis virus; Tick-borne powassan virus; Torque teno virus; Toscana virus; Uukuniemi virus; Vaccinia virus; Variola virus; Venezuelan equine encephalitis virus; 10 Vesicular stomatitis virus; Western equine encephalitis virus; WU polyomavirus; West Nile virus; Yaba monkey tumor virus; Yaba-like disease virus; Yellow fever virus; Zika virus; an ephemerovirus; or a vesiculovirus.

In some embodiments, the subject is diagnosed with a medical condition. In some embodiments, the medical condition is lung disease, sinus disease, airway disease, ear disease, heart disease, high 15 blood pressure, diabetes, kidney disease, liver disease, gastrointestinal disease, central nervous system (CNS) disease, dementia, Alzheimer's disease, stroke, an immunocompromised state, cancer, or obesity.

In some embodiments, wherein the lung, sinus, airway, or ear disease is primary ciliary dyskinesia, cystic fibrosis, sinusitis, rhinosinusitis, bronchiolitis obliterans, plastic bronchitis, emphysema, influenza, avian influenza, bronchitis, bronchiectasis, pneumonitis, pneumonia, bronchopulmonary 20 dysplasia (BPD), chronic obstructive pulmonary disease (COPD), chronic obstructive airway disease (COAD), acute respiratory distress syndrome (ARDS), chronic respiratory diseases (CRDS), or COVID-19.

In some embodiments, the subject at risk is under the age of 5 or over the age of 60. In some 25 embodiments, the subject is immunocompromised. In some embodiments, the subject is hospitalized. In some embodiments, the subject is pregnant.

In some embodiments, the composition delivered to the subject contains between 0.1 and 600 mg of sodium 2-mercaptoethane sulfonate or 2-mercaptoethylamine.

In some embodiments, the composition contains between 5 and 600 mg of sodium 2-mercaptoethane sulfonate or 2-mercaptoethylamine.

30 In some embodiments, the composition contains between 20 and 95% w/w sodium 2-mercaptoethane sulfonate or 2-mercaptoethylamine.

In some embodiments, the composition is a dry powder. In some embodiments, the composition is a spray-dried powder.

In some embodiments, the composition is liquid.

35 In some embodiments, the sodium 2-mercaptoethane sulfonate or 2-mercaptoethylamine particles are encapsulated. In some embodiments, the sodium 2-mercaptoethane sulfonate or 2-mercaptoethylamine particles are encapsulated in liposomes, microspheres, engineered spray-dried particles, or nanoparticles.

In some embodiments, the composition further includes a second anti-viral.

40 In some embodiments, the second anti-viral is remdesivir, idoxuridine, trifluridine, brivudine, vidarabine, entecavir, telbivudine, foscarnet, zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, emtricitabine, nevirapine, delavirdine, efavirenz, etravirine, rilpivirine, saquinavir, ritonavir,

indinavir, nelfinavir, amprenavir, lopinavir-ritonavir, atazanavir, fosamprenavir, tipranavir, darunavir, telaprevir, boceprevir, simeprevir, asunaprevir, paritaprevir, grazoprevir, raltegravir, elvitegravir, dolutegravir, palivizumab, docosanol, enfuvirtide, maraviroc, varizig, acyclovir, ganciclovir, famciclovir, valacyclovir, penciclovir, valganciclovir, cidofovir, tenofovir disoproxil fumarate, or adefovir dipivoxil.

5 In some embodiments, the composition further includes one or more anti-inflammatory, corticosteroid, antihistamine, bronchodilator, short-acting beta-agonist, long-acting beta-agonist, short-acting muscarinic antagonist, long-acting muscarinic antagonist, immunosuppressant, antibiotic, antiviral, antifungal, anti-infective agent, or any combination thereof. In some embodiments, the composition further includes an anti-inflammatory. In some embodiments, the composition further includes a
10 corticosteroid. In some embodiments, the composition further includes a short-acting beta-agonist. In some embodiments, the composition further includes a long-acting beta-agonist. In some embodiments, the composition further includes a short-acting muscarinic antagonist. In some embodiments, the composition further includes a long-acting muscarinic antagonist. In some embodiments, the composition further includes an immunosuppressant. In some embodiments, the composition further includes an
15 antibiotic. In some embodiments, the composition further includes an antifungal. In some embodiments, the composition further includes an anti-infective agent. In some embodiments, the composition further includes an antihistamine. In some embodiments, the composition further includes a bronchodilator.

In some embodiments, the composition may further include mannitol, calcium chloride, magnesium stearate, edetate disodium (EDTA), sodium acetate, dipalmitoylphosphatidylcholine (DPPC),
20 soy lecithin, egg lecithin, hydrogenated soybean phosphatidylcholine (HSPC), cholesterol, PEG (polyethylene glycol); distearoyl-sn-glycero-phosphoethanolamine (DSPE); distearoylphosphatidylcholine (DSPC); dioleoylphosphatidylcholine (DOPC); egg phosphatidylcholine (EPC); DOPS (dioleoylphosphatidylserine); palmitoyl-oleoylphosphatidylcholine (POPC); sphingomyelin (SM); methoxy polyethylene glycol (MPEG); dimyristoyl phosphatidylcholine (DMPC); dimyristoyl phosphatidylglycerol
25 (DMPG); distearoylphosphatidylglycerol (DSPG); dioleoylphosphatidylcholine (DEPC); dioleoyl-sn-glycero-phosphoethanolamine (DOPE), triolein, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt (MPEG-DSPE), or 1,2-Dipalmitoyl-sn-glycero-3-phosphorylglycerol sodium salt (DPPG).

In another aspect, the invention provides methods of reducing extracellular disulfide bonds in a viral protein, the method comprising the step of contacting the viral protein with sodium 2-mercaptoethane
30 sulfonate or 2-mercaptoethylamine particles.

In another aspect, the invention provides methods of reducing extracellular disulfide bonds in a viral protein, the method comprising the step of contacting the viral protein with sodium 2-mercaptoethane sulfonate particles.

In another aspect, the invention provides methods of reducing extracellular disulfide bonds in a
35 viral protein, the method comprising the step of contacting the viral protein with 2-mercaptoethylamine particles.

In some embodiments, the viral protein is a coronavirus spike protein. In some embodiments, the coronavirus is SARS-CoV. In some embodiments, the coronavirus is SARS-CoV2. In some
40 embodiments, the SARS-CoV2 is a SARS-CoV2 variant. In some embodiments, the variant is Delta (B.1.617.2), Alpha (B.1.1.7), Gamma (P.1), Beta (B.1.351), or Omicron (B.1.1.529).

In some embodiments, the disulfide bonds are reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%.

In some embodiments, the size of the particles is from 0.01 microns to 15 microns.

In some embodiments, the particles are sized for predominant absorption at a target location of the lung, gastro-intestinal system, kidney, liver, heart, brain, blood-brain barrier (BBB), blood, tissues, central nervous system, lymph nodes, pancreas, gallbladder, diaphragm, reproductive organs, esophagus, colon, or bladder.

In some embodiments, the particles are sized for predominant absorption at a target location of the airways of the subject, wherein the target location is the upper respiratory track or the lower respiratory track.

In some embodiments, the bulk density of the composition is between 0.1 and 5 g/mL.

In some embodiments, the sodium 2-mercaptoethane sulfonate or 2-mercaptoethylamine particles have a median mass aerodynamic diameter between 1 and 8 μm . In some embodiments, the fine particle fraction of the composition less than or equal to a mass median aerodynamic diameter of 6 μm is between 10 and 90%. In some embodiments, the fine particle fraction of the composition less than or equal to a mass median aerodynamic diameter of 5 μm is between 10 and 90%. In some embodiments, the fine particle fraction of the composition less than or equal to a mass median aerodynamic diameter of 7 μm is between 10 and 90%.

In some embodiments, the composition is delivered through the oral, sublingual, parenteral, intravenous, transdermal, or subcutaneous route.

In some embodiments, the composition is delivered through the nose, mouth, trachea, or bronchia.

In some embodiments, the composition is administered nasally, orally, sublingually, intravenously, parenterally, intratracheally, rectally, transdermal, or subcutaneously.

In some embodiments, the composition is administered via inhalation, oral spray, nasal spray, instillation, or nebulization.

In some embodiments, the inhalation is dry powder inhalation.

In some embodiments, the subject is treated at least once per week. In some embodiments, the subject is treated at least once per day. In some embodiments, the subject is treated one to four times per day. In some embodiments, the subject is treated as an out-patient.

Definitions

To facilitate the understanding of this invention, a number of terms are defined below and throughout the disclosure. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The terminology herein is used to describe specific embodiments of the invention, but their usage does not limit the invention, except as outlined in the claims.

Terms such as "a", "an," and "the" are not intended to refer to only a singular entity but include the general class of which a specific example may be used for illustration.

As used herein, the term "about" refers to a value that is within 10% above or below the value being described.

The terms "administration" or "administering" refer to a method of giving a dosage of a compound or pharmaceutical composition to a subject.

As used herein, the terms "pharmacologically effective amount," "therapeutically effective amount," and the like, when used in reference to a therapeutic composition, refer to a quantity sufficient to, when administered to the subject, including a mammal, for example a human, effect beneficial or desired results, such as clinical results. For example, in the context of treating infection, described herein, these terms refer to an amount of the composition sufficient to achieve a treatment response as compared to the response obtained without administration of the composition. The quantity of a given composition described herein that will correspond to such an amount may vary depending upon various factors, such as the given agent, the pharmaceutical formulation, the route of administration, the type of disease or disorder, the identity of the subject (e.g., age, sex, weight) or host being treated, and the like. An "effective amount," "pharmacologically effective amount," or the like, of a composition of the present disclosure, also include an amount that results in a beneficial or desired result in a subject as compared to a control.

As used herein, the terms "treat," "treating," or "treatment" refer to administration of a compound or pharmaceutical composition for a therapeutic purpose. To "treat a disorder" or use for "therapeutic treatment" refers to administering treatment to a patient already suffering from a disease to ameliorate the disease or one or more symptoms thereof to improve the patient's condition (e.g., by reducing one or more symptoms of inflammation). The term "therapeutic" includes the effect of mitigating deleterious clinical effects of certain inflammatory processes (i.e., consequences of the inflammation, rather than the symptoms of inflammation). The methods of the invention can be used as a primary prevention measure, i.e., to prevent a condition or to reduce the risk of developing a condition. Prevention refers to prophylactic treatment of a patient who may not have fully developed a condition or disorder, but who is susceptible to, or otherwise at risk of, the condition. Thus, in the claims and embodiments, the methods of the invention can be used either for therapeutic or prophylactic purposes.

Other features and advantages of the invention will be apparent from the following Detailed Description, Examples, Figure, and Claims.

Brief Description of the Drawings

FIG. 1 shows mutation regions from spike protein amino acid sequences: SARS-CoV-2 and A) Alpha (B.1.1.7), B) Beta (B.1.351), C) Gamma (P1) and D) Delta (B.1.617.2) variants highlighting receptor binding domain (RBD) regions.

FIG. 2 shows the impact of the present invention on extent (%) of SARS-CoV2 spike protein binding with human ACE-2 receptor when with increasing concentrations of a present composition.

FIG. 3 shows the percentage of ACE-2 receptor binding with SARS-CoV2 spike protein when incubated with increasing concentrations of encapsulated sodium 2-mercaptoethane sulfonate, unencapsulated sodium 2-mercaptoethane sulfonate, 2-mercaptoethylamine, and N-acetylcysteine.

DETAILED DESCRIPTION OF THE INVENTION

The following description sets forth various examples along with specific details to provide a thorough understanding of claimed subject matter. It will be understood by those skilled in the art, however, that claimed subject matter may be practiced without one or more of the specific details disclosed herein. Further, in some circumstances, well-known methods, procedures, systems, and/or components have not been described in detail in order to avoid unnecessarily obscuring claimed subject

matter. The illustrative embodiments described in the detailed description and claims are not meant to be limiting. Other embodiments may be utilized, and other changes may be made, without departing from the spirit or scope of the subject matter presented here. It will be readily understood that the aspects of the present disclosure, as generally described herein, can be arranged, substituted, combined, and designed in a wide variety of different configurations, all of which are explicitly contemplated and make part of this disclosure.

Without limiting the scope of the invention, the present invention is related to anti-viral activity of therapeutic agents. International Patent Application No. PCT/US2021/047774, incorporated herein by reference, describes exemplary therapeutic agents which the inventors have now discovered may be used to reduce the risk of and/or treat viral infections.

Viral Infections

In some embodiments, the present invention is directed to a method of reducing the risk of a viral respiratory infection in a subject at risk of an infection or treating the subject after the onset of infection.

The present compositions reduce the risk of a viral infection in a subject at risk and treat a range of viral respiratory infections.

In some embodiments, the present invention is directed to the reduction of risk of and treatment of diseases caused or worsened by viral respiratory infections, e.g., coronavirus; severe acute respiratory syndrome (SARS); the Middle East Respiratory Syndrome (MERS); severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of COVID-19 disease; rhinovirus, the common cold virus; respiratory syncytial virus (RSV); avian influenza viruses; influenza A, B, and C viruses; adenoviruses; herpesviruses; human papillomaviruses; parvoviruses; reoviruses; picornaviruses; coronaviruses; flavivirus; togaviruses, orthomyxovirus: bunyaviruses; rhabdoviruses; paramyxoviruses; pneumonia; conjunctivitis; gastroenteritis; pharyngitis; acute haemorrhagic cystitis; herpes simplex virus; varicella zoster virus; Epstein- Barr virus; HPV types 1-65; parvovirus B 19; canine parvovirus; orbivirus; rotavirus; aquaracovirus; coltivirus; enterovirus; hepatovirus; coronavirus and torovirus; petsiviruses; hepatitis C-like viruses; alphavirus; rubivirus; Thogoto virus; Hantavirus; Nairovirus; phlebovirus; rabies virus; ephemerovirus; vesiculovirus; measles virus; mumps virus; etc. through the use of oral, inhalable, intranasal, parenteral, rectal, dermal, and/or nebulized anti-viral compositions.

In some embodiments, present invention is directed to the reduction of risk of and treatment of diseases caused or worsened by viral respiratory infections, wherein the viral infection is from a virus family selected from the group consisting of Herpesviridae, Papillomaviridae, Polyomaviridae, Poxviridae, Anelloviridae, Circoviridae, Genomoviridae, Parvoviridae, Hepadnaviridae, Retroviridae, Picobirnaviridae, Reoviridae, Coronaviridae, Astroviridae, Caliciviridae, Flaviviridae, Matonaviridae, Hepeviridae, Picornaviridae, Togaviridae, Filoviridae, Paramyxoviridae, Pneumoviridae, Rhabdoviridae, Arenaviridae, Hantaviridae, Nairoviridae, Peribunyaviridae, Phenuiviridae, and Orthomyxoviridae.

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2)

Coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2 infection, is a global pandemic with greater than 252 million confirmed cases and greater than five million deaths worldwide. In the US there have been more than 46 million confirmed cases and greater than 760,000 deaths. In addition to human loss, the pandemic has been described by International Monetary Fund (IMF) the cause of a

global decline on the scale of the Great Depression of the 1930s. The global economy contracted by 3.5% with specific region economy and unemployment rates increased dramatically.

The clinical presentation of COVID-19 is heterogeneous, ranging from asymptomatic to severe respiratory failure leading to mechanical ventilation and/or death. The pathology of serious SARS-CoV-2 lung infection is characterized by neutrophil inflammation, mucous plug with fibrinous exudate in the alveoli, alveolar damage and microthrombi.

Elderly individuals and patients with underlying lung diseases have a significantly increased risk of severe COVID-19 because of their diminished immune response and ability to repair the damaged epithelium. Reduced muco-ciliary clearance also contributes to viral spread to the gas exchange units more readily. The airway blockage with mucus produces regional hypoxia and necrosis of airway epithelial cells. Necrotic epithelial cells lead to neutrophilic inflammation even in the absence of infection.

In August of 2020 the Food and Drug Administration (FDA) approved the first COVID-19 vaccine from Pfizer-BioNTech for the prevention of COVID-19 disease in individuals 16 years of age and older. The approval of the Pfizer-BioNTech vaccine was followed by 3 other vaccines from Moderna, Astra Zeneca, and Johnson & Johnson. As the vaccination rate increased, a decline in COVID-19 cases occurred. However, there has been a rise in new variants of the virus, that are more contagious, causing widespread disease, hospitalization, and death, against which the currently available vaccines have less effectiveness, as well as breakthrough infections in vaccinated subjects.

Coronavirus is a family of the viruses and can cause illness such as the common cold, severe acute respiratory syndrome (SARS), the Middle East Respiratory Syndrome (MERS) and COVID-19 infection.

The SARS-COV2 genome encodes four structural proteins, Nucleocapsid (N) protein, Membrane (M) protein, spike (S) protein, and Envelop (E) protein, as well as several non-structural proteins. The S-protein is integrated over the surface of the virus and mediates attachment of the virus to the host cell surface receptors and fusion between the viral and host cell membranes to facilitate viral entry into the host cell. The virus utilizes the coronavirus spike (S) glycoprotein protein RBD, with S1 and S2 subunits in each spike monomer, to attach itself to a common human angiotensin converting enzyme 2 (ACE2) receptor that is found on the outer membrane of many human cells, including those in the respiratory apparatus, such as nose, throat, and lungs.

The binding between SARS-COV2 and the ACE2 receptor initiates a cascade of events resulting in the fusion between cell and viral membranes for cell entry. As binding to the ACE2 receptor is a critical first step for SARS-CoV2 to enter into target cells and, the vaccines and therapeutics discovery efforts are focused on blocking this binding.

The ACE2 receptor on the surface of epithelial cells also mediates the entry of severe acute respiratory syndrome coronavirus 1 (SARS-CoV), the virus resulting in severe acute respiratory syndrome commonly known as SARS. However, SARS-CoV-2 binding to ACE2 is 2-4 times stronger than that of SARS-CoV. The increase in binding strength is believed to be due to the differences in the sequence of spike protein resulting in a stabilized RBD that is readily recognized by the receptor.

Viruses often change through mutation. A variant of the original virus occurs when the virus has one or more new mutations in their amino acid sequence. Since March 2020, several new genetic variants of SARS-COV2 with varying degrees of pathogenicity have appeared. World Health Organization (WHO) has declared four of those as "variants of concern (VOC)". The Center for Disease

Control (CDC) defines the variant of concern as a variant with specific genetic markers that have been associated with changes to receptor binding, reduced neutralization by antibodies generated against previous infection or vaccination, reduced efficacy of treatments, potential diagnostic impact, or predicted increase in transmissibility or disease severity.

The SARS-COV2 variants that are currently in circulation and causing concern in the United States include:

- Delta (B.1.617.2): The delta variant was identified in India in October of 2020 and gained dominance quickly after it was first reported in the United States in March 2021. This variant is now the most common COVID-19 variant in the United States. The delta variant is nearly twice as contagious as earlier variants and may cause more severe illness. The greatest risk of transmission of the delta variant is among unvaccinated people. However, fully vaccinated individuals with breakthrough infections accompanied by symptoms can also spread the virus to others. The delta variant may also reduce the effectiveness of some monoclonal antibody treatments and the antibodies generated by a COVID-19 vaccine.
- Alpha. (B.1.1.7): The alpha strain first appeared in Great Britain. The alpha variant of COVID-19 appears to spread more easily, with about a 50% increase in transmission compared to previous circulating variants. The alpha variant also may have an increased risk of hospitalization and death.
- Gamma (P.1): The gamma strain first surfaced in Brazil. The gamma variant reduces the effectiveness of some monoclonal antibody medications and the antibodies generated by a previous COVID-19 infection or a COVID-19 vaccine.
- Beta (B.1.351): The beta strain first surfaced in South Africa. The beta variant appears to spread more easily, with about a 50% increase in transmission compared to previous circulating variants. It also reduces the effectiveness of some monoclonal antibody medications and the antibodies generated by a previous COVID-19 infection or COVID-19 vaccine.
- Omicron (B.1.1.529): The omicron strain was first detected in South Africa. The omicron variant includes a high number of variants, increasing the risk of spread, seriousness of disease, and reduction in effectiveness of some monoclonal antibody medications and the antibodies generated by a previous COVID-19 infection or COVID-19 vaccine.

FIG. 1 shows mutation regions from spike protein amino acid sequences: SARS-CoV-2 and A) Alpha (B.1.1.7), B) Beta (B.1.351), C) Gamma (P1) and D) Delta (B.1.617.2) variants highlighting RBD regions. FIG. 1 further shows the alignment of 60 consecutive amino acids residues from the spike protein where mutations have been occurred.

The mutations in the virus's spike protein are responsible for the degree of severity of the resulting disease of the variants. The mutations in the receptor binding domain of the spike protein may enable the virus to bind more tightly to human receptors, become more contagious and enabling the virus to re-infect someone who has previously been exposed or infected, and possibly evade the immune response in a subject who has been vaccinated. These infections are often described as break-through infections.

The present compositions are effective independent of virus mutation. Without being bound to theory, the present compositions disrupt both the virus's disulfide bridge hydrogen bond, as well as other non-valent interactions of the receptor binding domain of the virus spike protein and alter the three-

dimensional structure of the receptor binding domain. The present compositions may also alter the extracellular binding site of the receptor. The changes in the three-dimensional structure of the virus spike protein may lead to further reduction in binding affinity, and therefore a reduction of entry of the virus into the cell via the receptor mediated mechanism.

5 Variants with new mutations present a significant problem as they have shown to reduce the effectiveness of some antibody treatments. Given that mutations are likely to continue, therapeutics that are mutation agnostic and can inhibit the binding of spike protein RBD with host cell receptor for all variants are critically needed in the field and would benefit all patients.

10 Targeting the spike proteins by via small molecules and broad-neutralizing antibodies to reduce the binding of RBD with the host receptor offers a method of treating and reducing the risk of further infections of COVID-19. The objective of the present invention is to reduce the risk of and treat viral infections in subjects regardless of virus mutations. The present compositions achieve this objective by altering the structure of the viral protein irrespective of the mutation in specific amino acid residues.

15 In some embodiments, the present invention reduces the risk of a viral infection in a subject at risk or treats a viral infection of a subject, wherein the viral infection is coronavirus. In some embodiments, the coronavirus is SARS-CoV. In some embodiments, the coronavirus is SARS-CoV2. In some embodiments, the viral infection is a SARS-CoV2 variant. In some embodiments, the viral infection is a SARS-CoV2 variant, such as Delta (B.1.617.2), Alpha. (B.1.1.7), Gamma (P.1), Beta (B.1.351), or Omicron (B.1.1.529).

20 *Rhinovirus*

Multiple viruses including Rhinovirus and Respiratory Syncytial Virus (RSV) are responsible for pulmonary exacerbations, emergency room visits, and hospitalizations. Rhinovirus, the common cold virus, cause upper respiratory tract symptoms in both children and adults. Rhinovirus is also the most
25 identified virus associated with pulmonary exacerbations in patients with chronic respiratory disease, such as chronic obstructive pulmonary disease (COPD), asthma, cystic fibrosis. Pulmonary exacerbations are characterized by increased respiratory symptoms (cough, wheeze, sputum production, dyspnea) and can result in emergency room visits (COPD: 1.5 million per year, asthma: 1,725,000 per year in US) and hospitalization (COPD: 726,000 per year, asthma: 500,000 per year in US).

30 There are >160 types of rhinoviruses, with three genetically distinct groups: RV-A, RV-B, RV-C. Most respiratory disease is caused by RV-A and RV-C. Rhinovirus can also be classified by the cellular surface receptor used for entry into respiratory epithelial cells. The majority of RV-A and RV-B use intercellular adhesion molecule-1 (ICAM-1) as their receptor. Some RV-A use low density lipoprotein receptor (LDLR) family members. RV-C uses cadherin related family member 3 (CDHR3). The three-
35 dimensional structure of these receptor proteins is stabilized via intra-protein Cys-Cys disulfide linkages, hydrogen bonds and other electrostatic linkages formed between amino acid residue as result of specific protein folding. This three-dimensional structure enables the presentation of specific sites to be recognized and bind with the receptor and facilitate its transport into the host cell. All three rhinovirus receptors are characterized by extracellular domains. These domains are coiled and achieve their
40 configuration through Cys-Cys disulfide linkages. Viral surface proteins form specific binding sites that are recognized by these domains of the host cell receptors resulting in cell attachment. Changes in the three-dimensional structure of virus protein and/or the extracellular domains of the host cell receptor can

impact the ability of virus to enter the host cell and therefore affects the infectivity and pathogenicity of the virus.

Respiratory Syncytial Virus (RSV)

5 Respiratory syncytial virus (RSV) is a leading cause of upper and lower respiratory tract disease in young children and elderly people. Despite that the virus was identified in 1955, and efforts from public institutions and multiple biotechnology companies, an effective RSV vaccine is yet to be developed. Currently the only approved intervention is a humanized monoclonal antibody for passive immunoprophylaxis in premature and high-risk infants. RSV respiratory infection being the most common
10 cause of hospitalization of infants and children in the developed world. Globally, RSV is responsible for ~60,000 in-hospital deaths annually in children younger than 5 years of age. RSV can infect almost every child by the age of 2, with two percent of infants developing severe disease. Beyond childhood, the CDC estimates that 177,000 older adults are hospitalized each year due to RSV infection.

 RSV has an attachment protein G on its surface that binds to the host cell surface receptor
15 CX3CR1. A soluble form of the G protein is used for immune evasion. The G fusion protein has a critical hairpin configuration (supported via Cys-Cys disulfide bonds) that is necessary for RSV entry into the host cell. Another RSV surface protein, the F protein, facilitates host cell attachment (binding to ICAM-1 amongst other receptors) to the domains and plays an essential role in viral fusion with the host cell. Its configuration also depends upon critical Cys-Cys disulfide linkages. Changes to the three-dimensional
20 structure of the G and F proteins will impact the ability of RSV to enter host cells and therefore the pathogenicity of RSV.

 In some embodiments, the present invention reduces the risk of a viral infection in a subject at risk or treats a viral infection of a subject, wherein the viral infection is due to Rhinovirus. In some
25 embodiments, the present invention reduces the risk of a viral infection in a subject at risk or treats a viral infection of a subject, wherein the viral infection is due to RSV.

Subjects At Risk

 The present invention includes methods for reducing the risk of viral infections in “at risk” subjects. A subject at risk may be a subject more easily susceptible to acquiring a viral infection, or one
30 who may be more likely to get severely ill from a viral infection. Severe illness may mean person who may need hospitalization, intensive care, oxygen or a ventilator to help them breath, and can potentially result in death.

 In some embodiments, the subject at risk is diagnosed with a medical condition or a pre-existing condition. The subject at risk may be diagnosed with cancer, a kidney disease, a lung or respiratory
35 airway diseases, dementia or other neurological conditions, diabetes (type 1 or type 2), trisomy 21, hypertension, a heart condition, a human immunodeficiency virus (HIV) infection, acquired immunodeficiency syndrome (AIDS), an immunocompromised state, a liver disease, sickle cell disease or thalassemia, stroke or cerebrovascular disease. The subject at risk may be pregnant. The subject at risk may be overweight. The subject at risk may be obese, e.g., a body mass index (BMI) over 40. The
40 subject at risk may be current or former smoker. The subject at risk may be the subject of a solid organ or blood stem cell transplant. The subject at risk may have a substance use disorder. The subject at risk

may have a disability. The subject at risk may be diagnosed with a chronic disease or condition. The subject at risk can be a person being treated with immunosuppressant drugs for unrelated illness.

Subjects at risk may be diagnosed with a lung or respiratory airways diseases. Conditions and diseases that may benefit from the present invention include: asthma, primary ciliary dyskinesia (PCD), cystic fibrosis (CF), bronchiectasis (BE), bronchopulmonary Dysplasia (BPD), sinusitis, rhinosinusitis, bronchiolitis obliterans (BO), emphysema, bronchitis, pneumonia, pneumonitis, chronic obstructive pulmonary disease (COPD), other lung and airways diseases, other muco-obstructive diseases, chronic obstructive airway disease (COAD), acute respiratory distress syndrome, chronic respiratory distress, viral and bacterial infections of lungs, and sinus and ear diseases.

In some embodiments, the subject at risk may be classified at risk due to age. The Elderly are at higher risk than the overall population. The subject at risk may be over the age of 65. Children are at a higher risk than the overall population for acquiring some viruses, such as RSV. The subject at risk may be under the age of 5. The subject at risk may be premature neonate. Subjects with underlying diseases as listed above are at risk across the age spectrum.

In some embodiments, the subject at risk may be classified at risk due to housing conditions, professional exposure, or other environmental factors. For example, the subject at risk may be a nursing home resident or the subject at risk may work in the field of healthcare.

Anti-Viral Compositions

The present invention is directed to methods of reducing the risk of viral infection in a subject at risk of a viral infection or treating viral infection of a subject.

The present invention has stabilized therapeutic activity, reduced expected dose, potential for sustained release, and potential for combination with other molecules, without pre-delivery interactions. These advantages are explained in detail below.

The present invention provides methods of reducing the risk of viral infection in a subject at risk of a viral infection or treating a viral infection of a subject. The method includes the step of administering to the subject a composition containing sodium 2-mercaptoethane sulfonate or 2-mercaptoethylamine.

In some embodiments, the anti-viral agent is sodium 2-mercaptoethane sulfonate. Without being bound to theory, sodium 2-mercaptoethane sulfonate is advantageous in that it has a unique chemical structure that can alter the three-dimensional structure of viral proteins and diminish or block the binding and prevent the virus entry into host cell. Sodium 2-mercaptoethane sulfonate has an additional terminal charged sulfonate group with hydrophilic properties that can break the hydrogen bonds and disulfide linkages in viral proteins.

In the SARS-COV2, the RBD of spike protein includes four Cys-Cys pairs of disulfide linkages that stabilize the three-dimensional structure and binding site of RBD; three of those are in the core (Cys336–Cys361, Cys379–Cys432 and Cys391–Cys525), that serve to stabilize the β sheet structure. The fourth pair (Cys480–Cys488) in the contact region of RBD and connects the loops in the distal end of the receptor-binding motif (RBM).

Furthermore, there are also a number of electrostatic hydrogen bonds that help stabilize the structure of the RBD necessary for being recognized by the ACE-receptor. The N-terminal peptidase domain of ACE2 has two lobes, that form the binding site between them. The extended RBM in the

SARS-CoV2 RBD contacts the bottom side of the small lobe of ACE2, with a concave outer surface in the RBM that accommodates the N-terminal helix of the ACE2.

Without being bound to theory, sodium 2-mercaptoethane sulfonate, combining a terminal free thiol group and a terminal charged sulfonate group, offers dual action for altering the structure of the SARS-CoV2 spike protein. The free thiol group of sodium 2-mercaptoethane sulfonate may break the four disulfide Cys-Cys linkages in the RBD, and the charged sulfonate group may disrupt hydrogen bonds and other non-covalent links that help stabilize RBD structure to be recognized with ACE-2 receptor. This combination of actions may modify the three-dimensional structure of the RBD of spike protein and impact its ability to bind with the ACE-2 receptor.

The Cys-Cys pairs are conserved in all variants of SARS-CoV2. Therefore, sodium 2-mercaptoethane sulfonate may inhibit the binding in a mutation agnostic manner. Furthermore, and without being bound to theory, the ability of sodium 2-mercaptoethane sulfonate to scavenge the reactive oxygen species in the airways offers additional benefit of reducing oxidative stress.

The present invention provides methods of reducing the risk of viral infection in a subject at risk or treating viral infection of a subject, the method comprising administering to the subject a composition comprising sodium 2-mercaptoethane sulfonate particles. The present invention also provides methods of reducing the risk of viral infection in a subject at risk or treating viral infection of a subject, the method comprising administering to the subject a composition comprising sodium 2-mercaptoethane sulfonate particles.

In some embodiments, the anti-viral agent is 2-mercaptoethylamine, also known as cysteamine. Without being bound to theory, 2-mercaptoethylamine may also break the disulfide linkages and extracellular components of viral proteins, resulting in altered three-dimensional structure of the receptor binding domain. In the process, 2-mercaptoethylamine may form cysteine-cysteamine mixed disulfide linkages. 2-mercaptoethylamine may also promote the transport of L-cysteine into cells, which may be further used to synthesize glutathione, a potent intracellular antioxidant that serves to scavenge reactive oxygen species and serve as an anti-inflammatory. Thus, 2-mercaptoethylamine may simultaneously reduce the rate of viral entry while also reducing inflammation caused by reactive oxygen species. The present invention provides methods of reducing the risk of viral infection in a subject at risk or treating viral infection of a subject, the method comprising administering to the subject a composition comprising 2-mercaptoethylamine particles. The present invention also provides methods of reducing the risk of viral infection in a subject at risk or treating viral infection of a subject, the method comprising administering to the subject a composition comprising 2-mercaptoethylamine particles.

Composition Forms

In some embodiments, the present compositions are in dry powder form. Dry powder formulations offer several advantages, including improved stability (such as at room temperature) and ease of storage and transportation. Dry powder formulations are easy to inhale, rapid to administer, portable, and have low risk of infection. In some embodiments, delivery via inhalation may also reduce the risk of side effects. In some embodiments, the dry powder compositions are produced using spray-drying. In some embodiments, the present compositions are spray-dried powders. In some embodiments, the dry powder compositions may be reconstituted into a liquid form prior to administration.

In some embodiments, the present compositions can be in liquid form. In some embodiments, the present compositions are solubilized and reconstituted in solution for the administration, e.g., via nebulization or instillation to children under the age of 5 or ventilated patients. Nebulization and instillation are advantageous for administration to children and ventilated patients.

5

Excipients

In some embodiments, the present compositions include one or more excipients.

In some embodiments, the present invention is directed to inhalable and/or intranasal compositions including an anti-viral agent and one or more excipients, as well as methods of reducing the risk of viral infections in a subject at risk or treating viral infection of a subject, the method including administering via inhalation to the subject a composition including an anti-viral agent and one or more excipients.

Exemplary excipients include mannitol, magnesium stearate, sodium acetate, calcium chloride, soy lecithin; egg lecithin; hydrogenated soybean phosphatidylcholine (HSPC); cholesterol, polyethylene glycol (PEG); dipalmitoylphosphatidylcholine (DPPC); DSPE (distearoyl-sn-glycero-phosphoethanolamine); DSPC (distearoylphosphatidylcholine); DOPC (dioleoylphosphatidylcholine); EPC (egg phosphatidylcholine); DOPS (dioleoylphosphatidylserine); POPC (palmitoyl-oleoylphosphatidylcholine); SM (sphingomyelin); MPEG (methoxy polyethylene glycol); DMPC (dimyristoyl phosphatidylcholine); dimyristoyl phosphatidylglycerol (DMPG); DSPG (distearoylphosphatidylglycerol); dierycylphosphatidylcholine (DEPC); dioleoyl-sn-glycero-phosphoethanolamine (DOPE); triolein; 1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt (MPEG-DSPE); phosphatidylcholine (PC); and 1,2-Dipalmitoyl-sn-glycero-3-phosphorylglycerol sodium salt (DPPG). In some embodiments, the excipient is phospholipid based, and are part of lung surfactants and thus compatible with lung environment.

In some embodiments, excipients may make up less than 50% of the total composition, e.g., less than 50%, less than 40%, less than 30%, less than 20%, less than 10%, less than 5%, or less than 1%. In some embodiments, the excipients make up between 1 to 50% of the total composition, e.g., 1 to 10%, 10 to 20%, 20 to 30%, 30 to 40%, or 40 to 50%.

Administration

The present invention encompasses therapeutic agents and methods for reducing the risk of viral infection in a subject at risk or treating viral infection of a subject by administration of the present compositions. The present compositions may be administered nasally, orally, sublingually, intravenously, parenterally, intratracheally, rectally, transdermal, or subcutaneously.

In some embodiments, the present compositions are delivered to the subject through direct pulmonary administration through any one or more of the routes including the nasal, oral, intratracheal, and bronchial instillation. The present invention, an inhalable and/or intranasal anti-viral, offers multiple advantages, including delivery directly to the nasal and oral passages for certain viruses that use these routes to enter the upper and/or lower respiratory tract, the ability to reach viruses present in lower part of the respiratory tract, and the ability to reach the site of airway mucus plugs that may cause inflammation and airway blockage. In some embodiments, the present compositions are formulated for depositing on

the airway surfactant layer and can offer delayed absorption by the airway surface resulting in prolonged retention at the airway surface.

In some embodiments, the present compositions may be delivered by means of administering inhalable and/or intranasal liposomes, microspheres, engineered spray-dried particles, or nanoparticles directly to lungs and the respiratory tract.

In some embodiments, the present compositions may be deployed by intranasal route, direct instillation, dry powder inhalation, wet nebulized-inhalation, or aerosolized inhalation via airways route.

The methods for delivery by inhalation or the intranasal route envision the use of a dispersing apparatus such an inhaler, e.g., a dry powder inhaler, a low-resistance inhaler, such as a capsule-based monodose low-resistance inhaler device; a nasal spray device; a dropper; a nebulizer, such as a handheld portable nebulizer; or compressor-nebulizer inhaling device capable of delivering the drug encapsulated particles via the inhaled and/or intranasal route. In some embodiments, a dispersing apparatus is used such that the product is easy to inhale, even with advanced respiratory disease.

An exemplary device compatible with the present compositions is the RS01 Monodose inhaler, e.g., with size #2 and/or #3 capsule packaged individually. The monodose inhaler is advantageous in that the present drug particles will reach deep lungs even for the patients with breathing difficulties as the device offers both low resistance and works well with a flow rate of 15-20 L/min–100 L/min depending on a patient's ability to inhale. Particles of the present compositions may be filled into size #3 capsules as desired for potency and fill weight. Exemplary fill weights are less than 40 mg, e.g., less than 30 mg, less than 25 mg, less than 20 mg, less than 15 mg, less than 10 mg, less than 5 mg, or less than 1 mg. The above described may then be inhaled (e.g., using a Plastiap DPI (RS01) or other inhaler or nebulizer) at a flow rate of 15-60 LPM by the patient (slow and deep inhalation).

In some embodiments, the compositions are administered with an intratracheal device.

In some embodiments, the formulation is a prescription drug that is advantageous in that it does not require hospitalization or clinical setting for administration because of quick and convenient delivery anywhere, e.g., at home, via an inhaler for patient convenience.

In some embodiments, the compositions are administered at least once a week, e.g., every other day, once a day, twice a day, three times a day, etc. In some embodiments, the compositions are administered one to four times a day. In some embodiments, the compositions are administered at least once a day. In some embodiments, the compositions are administered at least twice a day. In some embodiments, the compositions are administered at least three times a day.

In some embodiments, the compositions are administered for at least three days, e.g., at least four days, at least five days, at least six days, at least seven days, at least eight days, at least nine days, at least ten days, at least eleven days, at least twelve days, at least thirteen days, or at least fourteen days.

In some embodiments, the invention includes a method of reducing the risk of a viral infection in a subject at risk, the method including administering to the subject a composition including sodium 2-mercaptoethane sulfonate or 2-mercaptoethylamine particles. In some embodiments, the method includes administering the composition at least once per day. In some embodiments, the method includes administering the composition at least twice per day. In some embodiments, the method includes administering the composition at least three times per day. In some embodiments, the method includes administering the composition for at least three days. In some embodiments, the method

includes administering the composition for at least five days. In some embodiments, the method includes administering the composition for at least seven days.

In some embodiments, the present invention includes a method of treating a viral infection in a subject, the method including administering to the subject a composition including sodium 2-mercaptoethane sulfonate or 2-mercaptoethylamine particles. In some embodiments, the method includes administering the composition at least once per day. In some embodiments, the method includes administering the composition at least twice per day. In some embodiments, the method includes administering the composition at least three times per day. In some embodiments, the method includes administering the composition for at least three days. In some embodiments, the method includes administering the composition for at least five days. In some embodiments, the method includes administering the composition for at least seven days.

Dosing

A multitude of doses of composition are included herein. In some embodiments, the composition delivered to the subject is may be from about 5 mg to about 600 mg, e.g., 5 to 50 mg, 5 to 100 mg, 50 to 100 mg, 100 to 150 mg, 100 to 300 mg, 100 to 400 mg, 100 to 500 mg, 150 to 200 mg, 200 to 250 mg, 200 to 400 mg, 200 to 500 mg, 250 to 300 mg, 300 to 350 mg, 350 to 400 mg, 400 to 450 mg, 400 to 500 mg, 400 to 600 mg, 450 to 500 mg, 500 to 550 mg, 500 to 600 mg, or 550 to 600 mg. Doses of anti-viral agent of greater than 5 mg are included herein, e.g., greater than 10 mg, greater than 15 mg, greater than 20 mg, greater than 25 mg, greater than 30 mg, greater than 35 mg, greater than 40 mg, greater than 45 mg, greater than 50 mg, greater than 60 mg, greater than 70 mg, greater than 80 mg, greater than 90 mg, greater than 100 mg, greater than 125 mg, greater than 150 mg, greater than 175 mg, greater than 200 mg, greater than 250 mg, greater than 300 mg, greater than 350 mg, greater than 400 mg, greater than 450 mg, greater than 500 mg, greater than 550 mg.

A multitude of anti-viral loadings in the compositions are included herein. Anti-viral loading in the present compositions may be from about 50-99% of the total composition, e.g., 50 to 60%, 50 to 70%, 50 to 75%, 50 to 80%, 50 to 90%, 60 to 70%, 60 to 80%, 60 to 90%, 70 to 80%, 70 to 90%, 80 to 90%, 90 to 95%, or 90 to 100%.

The present compositions may include greater than 50% by total weight anti-viral agent, e.g., greater than 50%, greater than 60%, greater than 70%, greater than 80%, greater than 90%, greater than 95%, greater than 99% or greater than 99.9%.

Target Locations

In some embodiments, the particles of the present compositions are sized for predominant absorption at a target location. The particles of the present invention may be formulated to absorb into a target location of a human body, thus targeting a viral infection.

In some embodiments, the particles are sized for predominant absorption at a target location of the lung, gastro-intestinal system, kidney, liver, heart, brain, blood-brain barrier (BBB), blood, tissues, central nervous system (CNS), lymph nodes, pancreas, gallbladder, diaphragm, reproductive organs, esophagus, colon, or bladder.

In some embodiments, the particles are sized for predominant absorption at a target location of the airways of the subject, wherein the target location is the upper respiratory track or the lower respiratory track.

5 *Encapsulation*

In some embodiments, the compositions are in the form of encapsulated particles.

In some embodiments, the formulations stabilize oxidative activity of thiol agents, such as the monomeric form of sodium 2-mercaptoethane sulfonate, preventing potential loss of therapeutic activity. In some embodiments, the present compositions are configured to stabilize the active monomeric state of sodium 2-mercaptoethane sulfonate.

In some embodiments, the thiol group of the sodium 2-mercaptoethane sulfonate is kept from dimerization via disulfide linkage. However, in some instances, and without being bound to theory, sodium 2-mercaptoethane sulfonate may autoxidize in oxygen rich environments to form a dimer, disodium 2-mercaptoethane sulfonate, resulting in potential loss of therapeutic activity.

Thus, the present invention offers the advantage of stabilized anti-viral activity without pre-delivery interaction. In some embodiments, sodium 2-mercaptoethane sulfonate may be stabilized with an encapsulation of the particles, such as microsphere, spray-dried, liposome, or nanoparticle encapsulations, in order to increase the ability of the sodium 2-mercaptoethane sulfonate to break disulfide linkages and other electrostatic interactions of viral proteins. Further, the stability of the encapsulated particles enables the use of standard supply chain, as opposed to requiring specialized and costly cold-chain supply methods.

Furthermore, encapsulation of active pharmaceutical ingredients may be useful method for targeted drug delivery. Without wishing to be bound by theory, liposome formulations can be used to deliver pharmaceuticals to specific locations, and release them at specific times, as demonstrated in U.S. patent number 4,797,285 herein incorporated by reference in its entirety. Liposomes, microspheres, engineered spray-dried particles, and nanoparticles represent unique drug carriers that can site-specifically deliver the drug directly to the respiratory tract while protecting it from interaction with environment (blood, metabolism, exposure to air, etc.).

As such the present encapsulation vehicles are advantageous for protecting the anti-viral agents, alone or when in combination with other therapies, from pre-delivery conversion to its metabolites, thus preserving its potency until the drug is released at the target site.

Microspheres encapsulating active pharmaceutical compounds may include polymers (e.g., polyethylene glycol, polylactide, polyhydroxybutyrate, or polycaprolactone), or copolymers (e.g., polyethylene glycol-polylactide block copolymer). An exemplary method to form microspheres involves dissolving the active pharmaceutical ingredient in a non-aqueous solvent, forming an oil-in-water emulsion with the solution, followed by volatilizing the organic solvent. In some embodiments, microsphere compositions are spray dried. U.S. patent number 7,011,776 demonstrates the use of microspheres and is incorporated herein by reference in its entirety.

Spray-dried particles, also known as engineered spray-dried particles, may be formed in a process in which a mixture of active pharmaceutical compound and carrier is injected into a hot gas stream, thereby forming fine droplets. The mixture may be a solution, suspension, slurry, or similar. As the solvent evaporates individual dry particles on the order of single micron diameter are formed. Further

details on the methods of formation of these engineered spray-dried particles are demonstrated in U.S. patent number 6,673,335 and U.S. patent number 5,993, 805, herein incorporated in its entirety.

Nanoparticles may be used as drug carriers for targeted delivery or timed-release of active pharmaceutical compounds. Polymer and co-polymer materials are among those used in preparing nanoparticles for encapsulation. An exemplary method in which active pharmaceutical ingredients may be encapsulated in nanoparticles is interfacial polymerization. In interfacial polymerization the active pharmaceutical ingredient and polymerizable monomer are dissolved in a non-aqueous solvent, which is added to an aqueous solvent. Polymerization and encapsulation occur at the organic-aqueous interface. Prominent examples of polymerizable monomers are the alkylcyanoacrylates (where alkyl is e.g., n-butyl, isobutyl, isohexyl, etc.). Nanoparticle encapsulation may be used to deliver small molecules as well as peptide pharmaceuticals. Further methods and compositions are detailed in U.S. patent number 8,3818,208 and 5,641,515, both incorporated by reference.

The advantages of encapsulation of sodium 2-mercaptoethane sulfonate include stabilization of the active monomer form, and ability to be delivered directly to the airway and lungs. Encapsulated formulations of sodium 2-mercaptoethane sulfonate may be delivered directly to the respiratory tract, thus having limited systemic exposure from absorption through the lung and reduced risk of systemic side effects.

Particle Properties

In some embodiments, the compositions may be formulated in particles of sizes appropriate to deposit in the upper and lower airways when inhaled.

The microspheres, liposomes, engineered spray-dried particles, and nanoparticles of the invention may be evaluated based on bulk density. Where density of a single solid object, or fluid, is the mass divided by the volume, bulk density is the mass divided by the volume of many particles. The bulk density of a substance is variable depending on the size of individual particles in the sample. Through micronization, spray-drying, or other processes that affect particle size of the present compositions, the bulk density of a sample may change without going through a chemical transformation. The distance through the respiratory tract that a sample may travel depends in part on bulk density. The bulk density of the present invention may vary between 0.1 and 5 g/mL, e.g., between 0.1 and 0.6, 0.2 and 0.7, 0.3 and 0.8, 0.4 and 0.9, 0.5 and 1, 1 and 1.5, 1.5 and 2, 2 and 3, 3 and 4, 4, and 5 g/mL.

The microspheres, liposomes, engineered spray-dried particles, and nanoparticles used may also be evaluated using the mass median aerodynamic diameter (MMAD). Particles of smaller mass median aerodynamic diameter travel further into the airways of a subject. Particles with sufficiently small aerodynamic diameters will permeate the lower respiratory tract. Particles with larger mass median aerodynamic diameters will settle into the upper respiratory tract. The present invention contemplates the use of a range of MMAD for targeted delivery to any portion of the respiratory tract necessary. In preferred embodiments, the MMAD for the particles of the invention are between 0.1 μm and 10 μm , e.g., between 0.1 and 0.2, 0.2 and 0.3, 0.3 and 0.4, 0.4 and 0.5, 0.5 and 0.6, 0.6 and 0.7, 0.7 and 0.8, 0.8 and 0.9, 0.9 and 1, 1 and 1.25, 1.25 and 1.5, 1.5 and 1.75, 1.75 and 2, 2 and 2.25, 2.25 and 2.5, 2.5 and 2.75, 2.75 and 3, 3 and 3.5, 3.5 and 4, 4 and 4.5, 4.5 and 5, 5 and 5.5, 5.5 and 6, 6 and 6.5, 6.5 and 7, 7 and 7.5, 7.5 and 8, 8 and 8.5, 8.5 and 9, 9 and 9.5, 9.5 and 10, 0.1 and 1, 1 and 3, 3 and 5, 5 and 7, or 7 and 10 μm .

Mass median aerodynamic diameter is the apparent aerodynamic particle size (i.e., the particle size of a water droplet falling at the same terminal velocity as the particle of sample being measured. The Mass median aerodynamic diameter (of a population) = geometric particle size (of a population) * square root of the bulk density.

5 In a preferred embodiment, the mass median aerodynamic diameter of the particle is 3.0 - 4.5 μm

In a preferred embodiment, the particle distribution of various geometric sizes are as follows: 10% of particles $e < 1.2 \mu\text{m}$, 50% of particles between 3-4.5 μm , and 40% of particles between 4.0-7.9 μm .

The microsphere, liposome, engineered spray-dried particle, freeze dried particles and nanoparticle compositions used may be evaluated using the fine particle fraction (FPF). FPF is the
10 fraction of particles in a composition that have a mass median aerodynamic diameter equal to or below the desired a mass median aerodynamic diameter for targeted drug delivery, e.g., 2 μm , 3 μm , 4 μm , 5 μm , 6 μm , or 7 μm . The desired aerodynamic diameter varies according to the target location in the respiratory tract. When the method employed targets the upper respiratory tract the desired aerodynamic diameter will be larger, and when the method employed targets the lower respiratory tract the desired a
15 mass median aerodynamic diameter will be smaller. Acceptable ranges of FPF in a composition are between 10 and 90% of the composition, e.g., between 10 and 20%, 20 and 30%, 30 and 40%, 40 and 50%, 50 and 60%, 60 and 70%, 70 and 80%, 80 and 90%, 10 and 40%, 20 and 50%, 30 and 60%, 40 and 70%, 50 and 80%, 60 and 90% or 70 and 90% of the composition.

The mass in the FPF that is delivered to a subject is the fine particle dose (FPD), wherein the
20 FPD is the percent of particles with a diameter under 5 μm . Acceptable ranged of FPD in the methods described herein are between 0.1 and 600 mg, e.g., between 0.1 and 1 mg, 0.1 and 250 mg, 1 and 2 mg, 2 and 3 mg, 3 and 4 mg, 4 and 5 mg, 5 and 6 mg, 5 to 50 mg, 5 to 100 mg, 6 and 7 mg, 7 and 8 mg, 8 and 9 mg, 9 and 10 mg, 1 and 7 mg, 3 and 6 mg, 1 and 10 mg, 10 and 20 mg, 20 and 30 mg, 30 and 40 mg, 40 and 50 mg, 50 to 100 mg, 100 to 150 mg, 100 to 300 mg, 100 to 400 mg, 100 to 500 mg, 150 to
25 200 mg, 200 to 250 mg, 200 to 400 mg, 200 to 500 mg, 250 to 300 mg, 300 to 350 mg, 350 to 400 mg, 400 to 450 mg, 400 to 500 mg, 400 to 600 mg, 450 to 500 mg, 500 to 550 mg, 500 to 600 mg, or 550 to 600 mg.

The microspheres, liposomes, engineered spray-dried particles, and nanoparticles used may also be evaluated using the mass median aerodynamic diameter (MMAD). Particles of smaller mass median
30 aerodynamic diameter travel further into the airways of a subject. Particles with sufficiently small aerodynamic diameters will permeate the lower respiratory tract. Particles with larger mass median aerodynamic diameters will settle into the upper respiratory tract. The present invention contemplates the use of a range of MMAD for targeted delivery to any portion of the respiratory tract necessary. In preferred embodiments, the MMAD for the particles of the invention are between 0.1 μm and 10 μm , e.g.,
35 between 0.1 and 0.2, 0.2 and 0.3, 0.3 and 0.4, 0.4 and 0.5, 0.5 and 0.6, 0.6 and 0.7, 0.7 and 0.8, 0.8 and 0.9, 0.9 and 1, 1 and 1.25, 1.25 and 1.5, 1.5 and 1.75, 1.75 and 2, 2 and 2.25, 2.25 and 2.5, 2.5 and 2.75, 2.75 and 3, 3 and 3.5, 3.5 and 4, 4 and 4.5, 4.5 and 5, 5 and 5.5, 5.5 and 6, 6 and 6.5, 6.5 and 7, 7 and 7.5, 7.5 and 8, 8 and 8.5, 8.5 and 9, 9 and 9.5, 9.5 and 10, 0.1 and 1, 1 and 3, 3 and 5, 5 and 7, or 7 and 10 μm .

40 Mass median aerodynamic diameter is the apparent aerodynamic particle size (i.e., the particle size of a water droplet falling at the same terminal velocity as the particle of sample being measured. The

Mass median aerodynamic diameter (of a population) = geometric particle size (of a population) * square root of the bulk density.

In some embodiments, the mass median aerodynamic diameter of the particle is 3.0 - 5.5 μm .

In a preferred embodiment, the particle distribution of various geometric sizes are as follows: 10% of particles $\leq 1.2 \mu\text{m}$, 50% of particles between 3-4.5 μm , and 40% of particles between 4.0-10 μm .

The microsphere, liposome, engineered spray-dried particle, freeze dried particles and nanoparticle compositions used may be evaluated using the fine particle fraction (FPF). FPF is the fraction of particles in a composition that have a mass median aerodynamic diameter equal to or below the desired a mass median aerodynamic diameter for targeted drug delivery, e.g., 2 μm , 3 μm , 4 μm , 5 μm , 6 μm , or 7 μm . The desired aerodynamic diameter varies according to the target location in the respiratory tract. When the method employed targets the upper respiratory tract the desired aerodynamic diameter will be larger, and when the method employed targets the lower respiratory tract the desired a mass median aerodynamic diameter will be smaller. Acceptable ranges of FPF in a composition are between 10 and 90% of the composition, e.g., between 10 and 20%, 20 and 30%, 30 and 40%, 40 and 50%, 50 and 60%, 60 and 70%, 70 and 80%, 80 and 90%, 10 and 40%, 20 and 50%, 30 and 60%, 40 and 70%, 50 and 80%, 60 and 90% or 70 and 90% of the composition.

Disulfide Bond Reduction

The present invention provides methods of reducing extracellular disulfide bonds in a viral protein, the method comprising the step of contacting the viral protein with the present compositions.

A reduced level is, for example, a reduction of about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95% and / or about 100%.

Storage and Stability

In some embodiments, the present compositions may be stored in a dry powder form (such as capsule, mono, or multiple dose dry powder inhaler) or in a liquid form (such as ampoules for a nebulizer). In some embodiment the dry powder form will be reconstituted as solution prior to administration.

In some embodiments, the present compositions are formulated for stability and resistance to oxidation or other kind of molecular disintegration. Therefore, and without being bound to theory, more of the drug reaches the site of action in its active, reduced monomeric form. The reach and stabilized activity together may make the product more efficacious, for example at reduced doses.

In some embodiments, the present compositions are stored at room temperature. In some embodiments, the present compositions are refrigerated, e.g., at 40°F. In some embodiments, the present compositions are stored at temperatures below freezing.

Combination Therapies

The present invention is directed to both single anti-viral treatments, as well as combination therapies. As such, in some embodiments, the present invention includes a combination therapy for the reduction of risk of a viral infection in a subject at risk or the treatment of a viral infection of a subject.

In some embodiments, the present compositions further include anti-inflammatory, corticosteroid, short-acting beta-agonist, long-acting beta-agonist, short-acting muscarinic antagonist, long-acting muscarinic antagonist, immunosuppressant, antibiotic, antifungal, antihistamine, bronchodilator, anti-infective agent, additional anti-viral agent, or any combination thereof.

5 In some embodiments, the additional antiviral is a reverse-transcriptase inhibitor (e.g., remdesivir, abacavir, adefovir, delavirdine, descovy, didanosine, doravirine, efavirenz, emtricitabine, entecavir, etravirine, lamivudine, loviride, nevirapine, rilpivirine, stavudine, tenofovir alafenamide, tenofovir disoproxil, zalcitabine, or zidovudine), RNA or DNA polymerase inhibitor (e.g., acyclovir, cidofovir, penciclovir, famciclovir, foscarnet, ganciclovir, valganciclovir, idoxuridine, ribavirin, taribavirin, sofosbuvir, 10 telbivudine, trifluridine, valaciclovir, or vidarabine), protease inhibitor (e.g., boceprevir, atazanavir, darunavir, fosamprenavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, simeprevir, telaprevir, or tipranavir), integrase inhibitor (e.g., bictegravir, elvitegravir, dolutegravir, or raltegravir), neuraminidase inhibitor (e.g., oseltamivir, zanamivir, laninamivir, or peramivir), ledipasvir, amprenavir, amantadine, umifenovir, baloxavir marboxil, daclatasvir, docosanol, edoxudine, enfuvirtide, fomivirsen, ibacitabine, 15 ibalizumab, letermovir, maraviroc, metisazone, moroxydine, nexavir, nitazoxanide, pleconaril, rimantadine, tromantadine, or vicriviroc.

In some embodiments, the present compositions include an anti-infective agent. In some embodiments, an anti-infective agent may include quinolones (such as Nalidixic Acid, Cinoxacin, Ciprofloxacin and Norfloxacin and the like), sulfonamides (e.g., Sulfanilamide, Sulfadiazine, 20 Sulfamethoxazole, Sulfisoxazole, Sulfacetamide, and the like), aminoglycosides (e.g., Streptomycin, Gentamicin, Tobramycin, Amikacin, Netilmicin, Kanamycin, and the like), tetracyclines (such as Chlortetracycline, Oxytetracycline, Methacycline, Doxycycline, Minocycline and the like), para-aminobenzoic acid, diaminopyrimidines (such as Trimethoprim, often used in conjunction with Sulfamethoxazole, pyrazinamide, and the like), penicillins (such as Penicillin G, Penicillin V, Ampicillin, 25 Amoxicillin, Bacampicillin, Carbenicillin, Carbenicillin indanyl, Ticarcillin, Azlocillin, Mezlocillin, Piperacillin, and the like), penicillinase resistant penicillin (such as Methicillin, Oxacillin, Cloxacillin, Dicloxacillin, Nafcillin and the like), first generation cephalosporins (such as Cefadroxil, Cephalexin, Cephadrine, Cephalothin, Cephapirin, Cefazolin, and the like), second generation cephalosporins (such as Cefaclor, Cefamandole, Cefonicid, Cefoxitin, Cefotetan, Cefuroxime, Aefuroxime axetil, Cefinetazole, Cefprozil, 30 Loracarbef, Ceforanide, and the like), third generation cephalosporins (such as Cefepime, CefoperaZone, Cefotaxime, Ceftizoxime, Ceftriaxone, Ceftazidime, Cefixime, Cefpodoxime, Ceftibuten, and the like), other beta-lactams (such as Imipenem, Meropenem, Aztreonam, Clavulanic acid, Sulbactam, Tazobactam, and the like), beta-lactamase inhibitors (such as Clavulanic acid), Chloramphenicol, macrolides (such as Erythromycin, Azithromycin, Clarithromycin, and the like), Lincomycin, Clindamycin, 35 Spectinomycin, Polymyxin B, polymyxins (such as Polymyxin A, B, C or D, E1. Colistin A), or E2, Colistin B or C, and the like) colistin, Vancomycin, Bacitracin, Isoniazid, Rifampin, Ethambutol, Ethionamide, Aminosalicic Acid, Cycloserine, Capreomycin, sulfones (such as Dapsone, Sulfoxone Sodium, and the like), Clofazimine, Thalidomide, or any other antibacterial agent that can be lipid encapsulated. Anti-infectives can include antifungal agents, including polyene antifungals (such as Amphotericin B, 40 Nystatin, Natamycin, and the like), Flucytosine, imidazole (such as Miconazole, Clotrimazole, Econazole, Ketoconazole, and the like), triazoles (such as Itraconazole, Fluconazole, and the like), Griseofulvin, Terconazole, Butoconazole, Ciclopirox, CiclopiroX Olamine, Haloprogin, Tolnaftate, Naftifine, Terbinafine,

or any other antifungals that can be lipid encapsulated or complexed and pharmaceutically acceptable salts thereof and combinations thereof.

In some embodiments, the present compositions may include a mucolytic. In some embodiments, the mucolytic is DNase, eNaC inhibitors, N-acetylcysteine, L α ureido mercaptopropionic acid, Bromhexine, ascorbic acid, vitamin E, tris(2-carboxyethyl)phosphine hydrochloride, N-butylcysteine, reduced glutathione, N-derivatives and C-derivatives of amino acid cysteine, di-peptide of cysteine and glutamic acid, di-peptide of aspartic acid, or Ambroxol Hydrochloride.

In some embodiments, the present compositions may include a corticosteroid. In some embodiments, the corticosteroid may include any inhalable and/or intranasal steroid. In some embodiments, the corticosteroid is flunisolide, fluticasone, flucortolone, triamcinolone, beclomethasone, budesonide, mometasone, ciclesonide, prednisolone, betamethasone, dexamethasone, hydrocortisone, methylprednisolone, or delfazacort.

In some embodiments, the present compositions include a bronchodilator. In some embodiments, the bronchodilator is a long-acting bronchodilator such as long-acting β -agonist (LABA), long-acting muscarinic antagonist (LAMA), short-acting bronchodilator such as long-acting β -agonist (SABA), short-acting muscarinic antagonist (SAMA), or any combination thereof. In some embodiments, the bronchodilator is a beta-2 agonist, such as salbutamol, salmeterol, formoterol and vilanterol anticholinergics, such as ipratropium, tiotropium, aclidinium and glycopyrronium; theophylline; muscarinic antagonist; or methylxanthines.

In some embodiment, the present compositions include nitric oxide.

In some embodiment, the present compositions include glycopyrrolate, formoterol fumarate, budesonide, glycopyrronium, fluticasone furoate, vilanterol trifenate, umeclidinium bromide, or any combination thereof.

In some embodiments, the present compositions include a drug for treatment of Asthma and COPD. In some embodiments, the present compositions includes aclidinium bromide (Tudorza Pressair), albuterol sulfate inhalation aerosol (Proventil HFA), indacaterol and glycopyrronium inhalation aerosol (Ultibro), bromide inhalation aerosol of budesonide (Pulmicort), mometasone furoate (Nasonex), tiotropium bromide inhalation aerosol (Spiriva), budesonide and formoterol (Symbicort), albuterol sulfate inhalation aerosol (Ventolin HFA), albuterol sulfate inhalation aerosol (ProAir HFA), albuterol sulfate inhalation powder (ProAir Respiclick), albuterol sulfate and ipratropium bromide (Combivent Respimat), albuterol sulfate and ipratropium bromide (Duoneb Inhalation Solution), arformoterol tartrate (Brovana Inhalation Solution), beclomethasone dipropionate inhalation aerosol (QVAR), budesonide inhalation powder (Pulmicort Turbohaler), budesonide inhalation powder (Pulmicort Flexhaler), budesonide and formoterol fumarate (Symbicort Inhalation Aerosol), ciclesonide (Alvesco Inhalation Aerosol), flunisolide (Aerospan HFA), fluticasone furoate and vilanterol trifenate (Breo Ellipta), fluticasone propionate HFA inhalation aerosol (Flovent HFA), fluticasone propionate inhalation powder (Flovent Diskus), formoterol fumarate inhalation powder (Foradil Aerolizer), formoterol fumarate (Perforomist Inhalation Solution), indacaterol inhalation powder (Arcapta Neohaler), ipratropium bromide HFA inhalation aerosol (Atrovent HFA), levalbuterol sulfate HFA inhalation aerosol (Xopenex), mometasone furoate (Asmanex Inhalation Aerosol HFA), mometasone furoate inhalation powder (Asmanex Twisthaler), mometasone furoate and formoterol fumarate (Dulera Inhalation Aerosol), olodaterol (Striverdi Respimat Inhalation Spray), salmeterol xinafoate powder for inhalation (Serevent Diskus), salmeterol xinafoate/fluticasone propionate

powder for inhalation (ADVAIR Diskus), salmeterol xinafoate/fluticasone propionate (ADVAIR HFA), tiotropium bromide (Spiriva Respimat Inhalation Spray), tiotropium bromide inhalation powder (Spiriva Handihaler), umeclidinium inhalation powder (Incruse Ellipta), or umeclidinium and vilanterol inhalation powder (Anoro Ellipta).

5 In some embodiments, the present compositions include an immunosuppressant. In some embodiments, immunosuppressant is cyclosporin.

In some embodiments, the present compositions include an anti-fungal agent. In some embodiments, the anti-fungal agent is polyene, amphotericin B, nystatin, natamycin, flucytosine, imidazole, miconazole, clotrimazole, econazole, ketoconazole, triazole, itraconazole, fluconazole, 10 griseofulvin, terconazole, butoconazole, ciclopirox, ciclopirox olamine, haloprogin, tolnaftate, naftifine, or terbinafine.

In some embodiments, the present compositions include a short-acting beta-agonist. In some embodiments, the short-acting beta-agonist is bitolterol, fenoterol, isoprenaline, levosalbutamol, orciprenaline, pirbuterol, procaterol, ritodrine, salbutamol, terbutaline, or albuterol.

15 In some embodiments, the present compositions include a long-acting beta-agonist. In some embodiments, the long-acting beta-agonist is formoterol, bambuterol, clenbuterol, formoterol, salmeterol, indacaterol, olodaterol, or vilanterol.

In some embodiments, the present compositions include a short-acting muscarinic antagonist. In some embodiments, the short-acting antagonist is ipratropium.

20 In some embodiments, the present compositions include a long-acting muscarinic antagonist. In some embodiments, the long-acting muscarinic antagonist is aclidinium, glycopyrronium, glycopyrrolate, tiotropium, or umeclidinium.

25 EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the methods and compounds claimed herein are performed, made, and evaluated, and are intended to be purely exemplary of the invention and are not intended to limit the scope of what the inventors regard as their invention.

30 **Example 1: Assessment of Sodium 2-Mercaptoethane Sulfonate on Binding of Virus Spike Protein Receptor Binding Domain with ACE-2 Receptor of Human Cell**

COVID-19 Spike-ACE2 Binding Assay Kit (CODE: CoV-ACE2S2-1) from RayBiotech was used to determinate percentage (%) of binding between SARS-CoV-2 Receptor Binding Domain (RBD) and 35 Angiotensin Converting Enzyme 2 (ACE2). First, 100 μ L of sodium 2-mercaptoethane sulfonate and a composition including an encapsulated sodium 2-mercaptoethane sulfonate in a formulation with excipients 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and calcium chloride at concentrations ranging from 0 to 20 mM diluted in 0.9% sodium chloride solution (saline) and ddH₂O respectively were incubated in appropriated wells for 1.5 hours at 37°C under gentle shake. Steps following the incubation 40 were performed as directed in RayBiotech kit's protocol. Briefly, once the incubation was over; wells were washed then incubated in HRP-conjugated IgG antibody solution. After the incubation, wells were

washed again. Then, they were incubated in TMB One-Step Substrate Reagent. Lastly, Stop Solution was added to each well. The absorbance (A) was read at 450 nm on a spectrophotometer.

Blanks used ddH₂O for the composition. Negative control contained Assay Diluent from the kit and no RBD. Positive control contained assay reagent and 0 mM set as 100 percent binding (% of binding was calculated with the following equation: ((A of test reagent with test concentration of the composition – A of test reagent with no drug(blank)) / A of positive control – A of Blank)) x 100.

An 85% loading of API was used for testing the COVID-19 spike protein binding.

FIG. 2 shows the percent of SARS-CoV2 spike protein binding with ACE receptor while being treated with increasing concentration of the composition and control.

Table 1:

Concentration of Composition (mM)	% Of Binding			STD (Standard Deviation)
	Composition	Control (+ve)	Control (-ve)	
0	100	100	0.2	0
0.625	64.5	99.0	0.2	1.6
1.25	57.8	99.0	0.2	1.5
2.5	43.6	99.0	0.2	1.7
5	31.9	99.0	0.2	1.0
10	20.7	99.0	0.2	1.5
20	5.7	99.0	0.2	1.0

Table 2 shows the stability of one embodiment of the present invention.

Table 2:

Stability of Therapeutic Activity MMAD of Composition @ T0, T1, (7-day 40°C and 75% RH) T2 (6 months storage)						
	85% API Loading			49% API Loading		
@	T0	T1	T2	T0	T1	T2
MMAD (µm)	5.2	5.1	5.2	4.5	4.9	5.5
% Recovery of Activity	98	92	94	97	93	94

Table 3 shows an example of composition and particle size of one embodiment of the present invention.

Table 3:

Composition and Particle Size				
Formulations	X ₁₀	X ₅₀	X ₉₀	MMAD
High & Low API Conc.	(µm)			

Sodium 2-Mercaptoethane Sulfonate, 85.8%, w/w	1.08	3.76	7.5	< 5.2
Sodium 2-Mercaptoethane Sulfonate, 49.5%, w/w	1.07	3.43	7.1	< 5.5

COVID-19 Spike-ACE2 Binding Assay Kit (CODE: CoV-ACE2S2-1) from RayBiotech was used to determinate percentage (%) of binding between SARS-CoV-2 Receptor Binding Domain (RBD) and Angiotensin Converting Enzyme 2 (ACE2). The Inhibitory concentration reducing binding by 50% (IC₅₀) was 1.8 mM for the composition. For comparison, for sodium 2-mercaptoethane sulfonate intravenous solution, the IC₅₀ was 2.7 mM.

The result of the experiment demonstrates that the present invention inhibits the binding of SARS-COVID spike protein with human ACE-2 receptors in a dose dependent manner.

Example 2: Assessment of Binding Between Human ACE-2 Receptor and SARS-CoV2 Spike Protein when Incubated with Increasing Concentrations of Different Drug Candidates

COVID-19 Spike-ACE2 Binding Assay Kit (CODE: CoV-ACE2S2-1) from RayBiotech was used to determinate percentage (%) of binding between SARS-CoV-2 Receptor Binding Domain (RBD) and Angiotensin Converting Enzyme 2 (ACE2).

First, encapsulated sodium 2-mercaptoethane sulfonate (MESNA), unencapsulated sodium 2-mercaptoethane sulfonate, 2-mercaptoethylamine, and N-acetylcysteine were resuspended in ddH₂O at 60 mM. A sample of encapsulated sodium 2-mercaptoethane sulfonate at 60 mM was sonicated for 60 sec (CIL-05B-S). 60 mM solutions were then diluted to 20 mM. A serial dilution by a factor of 2 was used to get following concentration; 10 mM, 5 mM, 2.5 mM, 1.25 mM, and 0.625 mM. 250 μ L was pipetted from the first solution tube (20 mM) into the second serial dilution tube (10 mM) and the final volume was completed to 500 μ L. This step was repeated for each serial dilution, using 250 μ L of the prior concentration until the final concentration was reached.

Next, 100 μ L each of encapsulated dry-powder sodium 2-mercaptoethane sulfonate, a solution of encapsulated dry-powder sodium 2-mercaptoethane sulfonate, unencapsulated sodium 2-mercaptoethane sulfonate IV, 2-mercaptoethylamine, and N-acetylcysteine (NAC) at concentrations ranging from 0 to 20 mM were incubated in appropriated wells for 1.5 hours at 37°C under gentle shake.

Steps following the incubation were performed as directed in RayBiotech kit's protocol. Briefly, once the incubation was over; wells were washed then incubated in HRP-conjugated IgG antibody solution. After the incubation, wells were washed again. Then, the wells were incubated in TMB One-Step Substrate Reagent. Lastly, Stop Solution was added to each well. The absorbance (A) was read at 450 nm on a spectrophotometer.

The blank was ddH₂O. Negative control contains Assay Diluent from the kit and no RBD. Wells containing 0 mM of drug was set as 100 percent binding (positive control). The percent of binding was calculated with the following equation: $((A \text{ of Test Reagent} - A \text{ of Blank}) / A \text{ of positive control} - A \text{ of Blank}) \times 100$.

FIG. 3 shows the percentage of ACE-2 receptor binding with SARS-CoV2 spike protein when incubated with increasing concentrations of encapsulated sodium 2-mercaptoethane sulfonate, unencapsulated sodium 2-mercaptoethane sulfonate, 2-mercaptoethylamine, and N-acetylcysteine.

Other Embodiments

Various modifications and variations of the described disclosure will be apparent to those skilled in the art without departing from the scope and spirit of the disclosure. Although the disclosure has been
5 described in connection with specific embodiments, it should be understood that the disclosure as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the disclosure that are obvious to those skilled in the art are intended to be within the scope of the disclosure.

Other embodiments are in the claims.

CLAIMS

1. A method of reducing the treating or risk of viral infection in a subject, the method comprising administering to the subject a composition comprising sodium 2-mercaptoethane sulfonate or 2-mercaptoethylamine particles.
2. The method of claim 1, wherein the viral infection is coronavirus.
3. The method of claim 2, wherein the viral infection is severe acute respiratory syndrome coronavirus 2 (SARS-CoV2).
4. The method of claim 3, wherein the SARS-CoV2 is a SARS-CoV2 variant, and the SARS-CoV2 variant is Omicron (B.1.1.529), Delta (B.1.617.2), Alpha (B.1.1.7), Gamma (P.1), or Beta (B.1.351).
5. The method of claim 1, wherein the viral infection is rhinovirus or the common cold.
6. The method of claim 1, wherein the viral infection is respiratory syncytial virus (RSV).
7. The method of claim 1, wherein the viral infection is Middle East Respiratory Syndrome (MERS).
8. The method of claim 1, wherein the viral infection is avian influenza viruses; influenza A, B, and C viruses; adenoviruses; herpesviruses; human papillomaviruses, such as HPV; parvoviruses; reoviruses; picornaviruses; coronaviruses; flavivirus; togaviruses, orthomyxovirus; bunyaviruses; rhabdoviruses; paramyxoviruses; pneumonia; conjunctivitis; gastroenteritis; pharyngitis; acute haemorrhagic cystitis; herpes simplex virus; varicella zoster virus; types 1-65; parvovirus B19; canine parvovirus; orbivirus; rotavirus; Rotavirus A; Rotavirus B; Rotavirus C; aquarcovirus; coltivirus; enterovirus; enterovirus 68, 70; hepatovirus; torovirus; petsiviruses; alphavirus; rubivirus; Thogoto virus; Hantavirus; Nairovirus; phlebovirus; Punta toro phlebovirus; Adeno-associated virus; Aichi virus; Australian bat lyssavirus; BK polyomavirus; Banna virus; Barmah forest virus; Bunyamwera virus; Bunyavirus La Crosse; Bunyavirus snowshoe hare; Cercopithecine herpesvirus; Chandipura virus; Chikungunya virus; Cosavirus A; Cowpox virus; Coxsackievirus; Crimean-Congo hemorrhagic fever virus; Dengue virus; Dhori virus; Dugbe virus; Duvenhage virus; Eastern equine encephalitis virus; Ebolavirus; Echovirus; Encephalomyocarditis virus; Epstein-Barr virus; European bat lyssavirus; Hantaan virus; Hendra virus; Hepatitis A virus; Hepatitis B virus; Hepatitis C virus; Hepatitis E virus; delta virus; Horsepox virus; astrovirus; cytomegalovirus; herpesvirus 1; herpesvirus 2; herpesvirus 6; herpesvirus 7; herpesvirus 8; immunodeficiency virus; papillomavirus 1; papillomavirus 2; papillomavirus 16,18; parainfluenza; spumaretrovirus; T-lymphotropic virus; Isfahan virus; JC polyomavirus; Japanese encephalitis virus; Junin arenavirus; KI Polyomavirus; Kunjin virus; Lagos bat virus; Lake Victoria marburgvirus; Langat virus; Lassa virus; Lordsdale virus; Louping ill virus; Lymphocytic choriomeningitis virus; Machupo virus; Mayaro virus; MERS coronavirus; Measles virus; Mengo encephalomyocarditis virus; Merkel cell polyomavirus; Mokola virus; Molluscum contagiosum virus; Monkeypox virus; Mumps virus; Murray valley encephalitis virus; New York virus; Nipah virus; Norwalk virus; O'nyong-nyong virus; Orf virus; Oropouche virus; Pichinde virus; Poliovirus;

Puumala virus; Rabies virus; Rift valley fever virus; Rosavirus A; Ross river virus; Rubella virus; Sagiya virus; Salivirus A; Sandfly fever sicilian virus; Sapporo virus; SARS coronavirus 2; Semliki forest virus; Seoul virus; Simian foamy virus; Simian virus 5; Sindbis virus; Southampton virus; St. Louis encephalitis virus; Tick-borne powassan virus; Torque teno virus; Toscana virus; Uukuniemi virus; Vaccinia virus; Variola virus; Venezuelan equine encephalitis virus; Vesicular stomatitis virus; Western equine encephalitis virus; WU polyomavirus; West Nile virus; Yaba monkey tumor virus; Yaba-like disease virus; Yellow fever virus; Zika virus; ephemero virus; or vesiculovirus.

9. The method of any one of claims 1 to 8, wherein the subject is diagnosed with a medical condition.

10. The method of claim 9, wherein the medical condition is lung disease, sinus disease, airway disease, ear disease, heart disease, high blood pressure, diabetes, kidney disease, liver disease, gastrointestinal disease, central nervous system disease, dementia, Alzheimer's disease, stroke, an immunocompromised state, cancer, or obesity.

11. The method of claim 10, wherein the lung, sinus, airway, or ear disease is primary ciliary dyskinesia, cystic fibrosis, sinusitis, rhinosinusitis, bronchiolitis obliterans, plastic bronchitis, emphysema, influenza, avian influenza, bronchitis, bronchiectasis, pneumonitis, pneumonia, bronchopulmonary dysplasia (BPD), chronic obstructive pulmonary disease (COPD), chronic obstructive airway disease (COAD), acute respiratory distress syndrome (ARDS), chronic respiratory diseases (CRDS), or COVID-19.

12. The method of any one of claims 1 to 11, wherein the subject at risk is under the age of 5 or over the age of 60.

13. The method of any one of claims 1 to 12, wherein the subject is immunocompromised.

14. The method of any one of claims 1 to 13, wherein the subject is hospitalized.

15. The method of any one of claims 1 to 14, wherein the subject is pregnant.

16. The method of any one of claims 1 to 15, wherein the composition delivered to the subject is between 0.1 and 600 mg.

17. The method of any one of claims 1 to 16, wherein the composition comprises between 5 and 600 mg of sodium 2-mercaptoethane sulfonate or 2-mercaptoethylamine.

18. The method of any one of claims 1 to 17, wherein the composition comprises between 20 and 95% w/w sodium 2-mercaptoethane sulfonate or 2-mercaptoethylamine.

19. The method of any one of claims 1 to 18, wherein the composition is a dry powder.

20. The method of any one of claims 1 to 19, wherein the composition is a spray-dried powder.
21. The method of any one of claims 1 to 18, wherein the composition is liquid.
22. The method of any one of claims 1 to 21, wherein the sodium 2-mercaptoethane sulfonate or 2-mercaptoethylamine particles are encapsulated.
23. The method of claim 22, wherein the sodium 2-mercaptoethane sulfonate or 2-mercaptoethylamine particles are encapsulated in liposomes, microspheres, engineered spray-dried particles, or nanoparticles.
24. The method of any one of claims 1 to 23, wherein the composition further comprises a second anti-viral.
25. The method of claim 24, wherein the second anti-viral is Remdesivir, Idoxuridine, Trifluridine, Brivudine, Vidarabine, Entecavir, Telbivudine, Foscarnet, Zidovudine, Didanosine, Zalcitabine, Stavudine, Lamivudine, Abacavir, Emtricitabine, Nevirapine, Delavirdine, Efavirenz, Etravirine, Rilpivirine, Saquinavir, Ritonavir, Indinavir, Nelfinavir, Amprenavir, Lopinavir-ritonavir, Atazanavir, Fosamprenavir, Tipranavir, Darunavir, Telaprevir, Boceprevir, Simeprevir, Asunaprevir, Paritaprevir, Grazoprevir, Raltegravir, Elvitegravir, Dolutegravir, Palivizumab, Docosanol, Enfuvirtide, Maraviroc, VariZIG, Acyclovir, Ganciclovir, Famciclovir, Valacyclovir, Penciclovir, Valganciclovir, Cidofovir, Tenofovir disoproxil fumarate, or Adefovir dipivoxil.
26. The method of any one of claims 1 to 25, wherein the composition further comprises one or more anti-inflammatory, corticosteroid, antihistamine, bronchodilator, short-acting beta-agonist, long-acting beta-agonist, short-acting muscarinic antagonist, long-acting muscarinic antagonist, immunosuppressant, antibiotic, antiviral, antifungal, anti-infective agent, or any combination thereof.
27. The method of any one of claims 1 to 26, wherein the composition may further comprises mannitol, calcium chloride, magnesium stearate, edetate disodium (EDTA), sodium acetate, dipalmitoylphosphatidylcholine (DPPC), soy lecithin, egg lecithin, hydrogenated soybean phosphatidylcholine (HSPC), cholesterol, PEG (polyethylene glycol); distearoyl-sn-glycero-phosphoethanolamine (DSPE); distearoylphosphatidylcholine (DSPC); dioleoylphosphatidylcholine (DOPC); egg phosphatidylcholine (EPC); DOPS (dioleoylphosphatidylserine); palmitoyl dioleoylphosphatidylcholine (POPC); sphingomyelin (SM); methoxy polyethylene glycol (MPEG); dimyristoyl phosphatidylcholine (DMPC); dimyristoyl phosphatidylglycerol (DMPG); distearoylphosphatidylglycerol (DSPG); dieryucoylphosphatidylcholine (DEPC); dioleoyl-sn-glycero-phosphoethanolamine (DOPE), triolein, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt (MPEG-DSPE), or 1,2-Dipalmitoyl-sn-glycero-3-phosphorylglycerol sodium salt (DPPG).

28. The method of any one of claims 1 to 27, wherein the size of the particles is from 0.01 microns to 15 microns.
29. The method of any one of claims 1 to 28, wherein the particles are sized for predominant absorption at a target location of the lung, gastro-intestinal system, kidney, liver, heart, brain, blood-brain barrier (BBB), blood, tissues, central nervous system (CNS), lymph nodes, pancreas, gallbladder, diaphragm, reproductive organs, esophagus, colon, or bladder.
30. The method of any one of claims 1 to 29, wherein the particles are sized for predominant absorption at a target location of the airways of the subject, wherein the target location is the upper respiratory track or the lower respiratory track.
31. The method of any one of claims 1 to 30, wherein the bulk density of the composition is between 0.1 and 5 g/mL.
32. The method of any one of claims 1 to 31, wherein the sodium 2-mercaptoethane sulfonate or 2-mercaptoethylamine particles have a median mass aerodynamic diameter between 1 and 8 μm .
33. The method of any one of claims 1 to 32, wherein the fine particle fraction of the composition less than or equal to a mass median aerodynamic diameter of 6 μm is between 10 and 90%.
34. The method of any one of claims 1 to 33, wherein the fine particle fraction of the composition less than or equal to a mass median aerodynamic diameter of 5 μm is between 10 and 90%.
35. The method of any one of claims 1 to 34, wherein the fine particle fraction of the composition less than or equal to a mass median aerodynamic diameter of 7 μm is between 10 and 90%.
36. The method of any one of claims 1 to 35, wherein the composition is delivered through the oral, sublingual, parenteral, intravenous, transdermal, or subcutaneous route.
37. The method of any one of claims 1 to 35, wherein the composition is delivered through the nose, mouth, trachea, or bronchia.
38. The method of claim 37, wherein the composition is administered nasally, orally, sublingually, intravenously, parenterally, intratracheally, rectally, transdermal, or subcutaneously.
39. The method of claim 38, wherein the composition is administered via inhalation, oral spray, nasal spray, instillation, or nebulization.
40. The method of any one of claims 1 to 39, wherein the inhalation is dry powder inhalation.
41. The method of any one of claims 1 to 40, wherein the subject is treated at least once per week.

42. The method of claim 41, wherein the subject is treated at least once per day.
43. The method of claim 42, wherein the subject is treated one to four times per day.
44. The method of any one of claims 1 to 43, wherein the subject is treated as an out-patient.
45. The method of any one of claims 1 to 44, wherein the subject is treated with a co-therapy.
46. A method of reducing extracellular disulfide bonds in a viral protein, the method comprising the step of contacting the viral protein with sodium 2-mercaptoethane sulfonate or 2-mercaptoethylamine particles.
47. The method of claim 46, wherein the viral protein is coronavirus protein.
48. The method of claim 47, wherein the coronavirus is severe acute respiratory syndrome coronavirus (SARS-CoV).
49. The method of claim 48, wherein the severe acute respiratory syndrome coronavirus (SARS-CoV) is severe acute respiratory syndrome coronavirus 2 (SARS-CoV2).
50. The method of claim 49, wherein the severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) is a severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) variant.
51. The method of claim 50, wherein the variant is Delta (B.1.617.2), Alpha. (B.1.1.7), Gamma (P.1), Beta (B.1.351), or Omicron (B.1.1.529).
52. The method of any one of claims 46 to 51, wherein the SARS-COV2 protein is the spike protein.
53. The method of claim 46, wherein the viral protein is rhinovirus protein or the common cold protein.
54. The method of claim 46, wherein the viral protein is respiratory syncytial virus (RSV) protein.
55. The method of claim 46, wherein the viral protein is severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) protein, respiratory syncytial virus (RSV) protein, rhinovirus protein, avian influenza viruses protein; influenza A, B, and C viruses protein; adenoviruses protein; herpesviruses protein; human papillomaviruses protein, such as an HPV protein; parvoviruses protein; reoviruses protein; picornaviruses protein; coronaviruses protein; flavivirus protein; togaviruses, orthomyxovirus: bunyaviruses protein; rhabdoviruses protein; paramyxoviruses protein; pneumonia protein; conjunctivitis protein; gastroenteritis protein; pharyngitis protein; acute haemorrhagic cystitis protein; herpes simplex virus protein; varicella zoster virus protein; types 1-65 protein; parvovirus B19 protein; canine parvovirus

protein; orbivirus protein; rotavirus protein; Rotavirus A protein; Rotavirus B protein; Rotavirus C protein; aquaracovirus protein; coltivirus protein; enterovirus protein; enterovirus 68, 70 protein; hepatovirus protein; torovirus protein; petsivirase protein; alphavirus protein; rubivirus protein; Thogoto virus protein; Hantavirus protein; Nairovirus protein; phlebovirus protein; Punta toro phlebovirus protein; Adeno-associated virus protein; Aichi virus protein; Australian bat lyssavirus protein; BK polyomavirus protein; Banna virus protein; Barmah forest virus protein; Bunyamwera virus protein; Bunyavirus La Crosse protein; Bunyavirus snowshoe hare protein; Cercopithecine herpesvirus protein; Chandipura virus protein; Chikungunya virus protein; Cosavirus A protein; Cowpox virus protein; Coxsackievirus protein; Crimean-Congo hemorrhagic fever virus protein; Dengue virus protein; Dhori virus protein; Dugbe virus protein; Duvenhage virus protein; Eastern equine encephalitis virus protein; Ebolavirus protein; Echovirus protein; Encephalomyocarditis virus protein; Epstein-Barr virus protein; European bat lyssavirus protein; Hantaan virus protein; Hendra virus protein; Hepatitis A virus protein; Hepatitis B virus protein; Hepatitis C virus protein; Hepatitis E virus protein; delta virus protein; Horsepox virus protein; astrovirus protein; cytomegalovirus protein; herpesvirus 1 protein; herpesvirus 2 protein; herpesvirus 6 protein; herpesvirus 7 protein; herpesvirus 8 protein; immunodeficiency virus protein; papillomavirus 1 protein; papillomavirus 2 protein; papillomavirus 16,18 protein; parainfluenza protein; spumaretrovirus protein; T-lymphotropic virus protein; Isfahan virus protein; JC polyomavirus protein; Japanese encephalitis virus protein; Junin arenavirus protein; KI Polyomavirus protein; Kunjin virus protein; Lagos bat virus protein; Lake Victoria marburgvirus protein; Langat virus protein; Lassa virus protein; Lordsdale virus protein; Louping ill virus protein; Lymphocytic choriomeningitis virus protein; Machupo virus protein; Mayaro virus protein; MERS coronavirus protein; Measles virus protein; Mengo encephalomyocarditis virus protein; Merkel cell polyomavirus protein; Mokola virus protein; Molluscum contagiosum virus protein; Monkeypox virus protein; Mumps virus protein; Murray valley encephalitis virus protein; New York virus protein; Nipah virus protein; Norwalk virus protein; O'nyong-nyong virus protein; Orf virus protein; Oropouche virus protein; Pichinde virus protein; Poliovirus protein; Puumala virus protein; Rabies virus protein; Rift valley fever virus protein; Rosavirus A protein; Ross river virus protein; Rubella virus protein; Sagiyama virus protein; Salivirus A protein; Sandfly fever sicilian virus protein; Sapporo virus protein; SARS coronavirus 2 protein; Semliki forest virus protein; Seoul virus protein; Simian foamy virus protein; Simian virus 5 protein; Sindbis virus protein; Southampton virus protein; St. Louis encephalitis virus protein; Tick-borne powassan virus protein; Torque teno virus protein; Toscana virus protein; Uukuniemi virus protein; Vaccinia virus protein; Variola virus protein; Venezuelan equine encephalitis virus protein; Vesicular stomatitis virus protein; Western equine encephalitis virus protein; WU polyomavirus protein; West Nile virus protein; Yaba monkey tumor virus protein; Yaba-like disease virus protein; Yellow fever virus protein; Zika virus protein; ephemerovirus protein; or vesiculovirus protein.

56. The method of any one of claims 46 to 55, wherein the disulfide bonds are reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%.

57. The method of any one of claims 46 to 56, wherein the sodium 2-mercaptoethane sulfonate or 2-mercaptoethylamine particles are encapsulated.

58. The method of claim 57, wherein the sodium 2-mercaptoethane sulfonate or 2-mercaptoethylamine particles are encapsulated in liposomes, microspheres, engineered spray-dried particles, or nanoparticles.
59. The method of any one of claims 46 to 58, wherein the size of the particles is from 0.01 microns to 15 microns.
60. The method of any one of claims 46 to 59, wherein the sodium 2-mercaptoethane sulfonate or 2-mercaptoethylamine particles have a median mass aerodynamic diameter between 1 and 8 μm .
61. The method of any one of claims 46 to 60, wherein the fine particle fraction of the composition less than or equal to a mass median aerodynamic diameter of 6 μm is between 10 and 90%.
62. The method of any one of claims 46 to 61, wherein the fine particle fraction of the composition less than or equal to a mass median aerodynamic diameter of 5 μm is between 10 and 90%.
63. The method of any one of claims 46 to 62, wherein the fine particle fraction of the composition less than or equal to a mass median aerodynamic diameter of 7 μm is between 10 and 90%.
64. The method of any one of claims 46 to 63, wherein the sodium 2-mercaptoethane sulfonate particles are in a composition.
65. The method of claim 64, wherein the bulk density of the composition is between 0.1 and 5 g/mL.
66. The method of claim 64 or 65, wherein the composition is a dry powder.
67. The method of any one of claims 64 to 66, wherein the composition is a spray-dried powder.
68. The method of claim 64 or 65, wherein the composition is liquid.
69. The method of any one of claims 64 to 68, wherein the composition further comprises a second anti-viral.
70. The method of claim 69, wherein the second anti-viral is Remdesivir, Idoxuridine, Trifluridine, Brivudine, Vidarabine, Entecavir, Telbivudine, Foscarnet, Zidovudine, Didanosine, Zalcitabine, Stavudine, Lamivudine, Abacavir, Emtricitabine, Nevirapine, Delavirdine, Efavirenz, Etravirine, Rilpivirine, Saquinavir, Ritonavir, Indinavir, Nelfinavir, Amprenavir, Lopinavir-ritonavir, Atazanavir, Fosamprenavir, Tipranavir, Darunavir, Telaprevir, Boceprevir, Simeprevir, Asunaprevir, Paritaprevir, Grazoprevir, Raltegravir, Elvitegravir, Dolutegravir, Palivizumab, Docosanol, Enfuvirtide, Maraviroc, VariZIG, Acyclovir, Ganciclovir, Famciclovir, Valacyclovir, Penciclovir, Valganciclovir, Cidofovir, Tenofovir disoproxil fumarate, or Adefovir dipivoxil.

71. The method of any one of claims 64 to 70, wherein the composition further comprises one or more anti-inflammatory, corticosteroid, antihistamine, bronchodilator, short-acting beta-agonist, long-acting beta-agonist, short-acting muscarinic antagonist, long-acting muscarinic antagonist, immunosuppressant, antibiotic, antiviral, antifungal, anti-infective agent, or any combination thereof.

72. The method of any one of claims 64 to 71, wherein the composition may further comprises mannitol, calcium chloride, magnesium stearate, sodium acetate, dipalmitoylphosphatidylcholine (DPPC), soy lecithin, egg lecithin, hydrogenated soybean phosphatidylcholine (HSPC), cholesterol, PEG (polyethylene glycol); distearoyl-sn-glycero-phosphoethanolamine (DSPE); distearoylphosphatidylcholine (DSPC); dioleoylphosphatidylcholine (DOPC); egg phosphatidylcholine (EPC); DOPS (dioleoylphosphatidylserine); palmitoyllecithin (POPC); sphingomyelin (SM); methoxy polyethylene glycol (MPEG); dimyristoyl phosphatidylcholine (DMPC); dimyristoyl phosphatidylglycerol (DMPG); distearoylphosphatidylglycerol (DSPG); dioleoylphosphatidylcholine (DEPC); dioleoyl-sn-glycero-phosphoethanolamine (DOPE), triolein, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt (MPEG-DSPE), or 1,2-Dipalmitoyl-sn-glycero-3-phosphorylglycerol sodium salt (DPPG).

A) Alpha (B.1.1.7)

Seqs: C107-2	120	NDVEGSPNCFYPLQSYGFQPTNGVGVGPIRVVLSPELLNAPATVGGPKSTLWKNKCVN
Alpha Variant		NDVEGSPNCFYPLQSYGFQPTNGVGVGPIRVVLSPELLNAPATVGGPKSTLWKNKCVN
Seqs: C107-2	144	PNPAGLTGTGVALTEGSHKFLPPQDFSPKADDTDAVRDPDLELRITPDSFGQVSVTP
Alpha Variant		PNPAGLTGTGVALTEGSHKFLPPQDFSPKADDTDAVRDPDLELRITPDSFGQVSVTP
Seqs: C107-2	168	ECDFPGAGICASYQTGTNSPRKARSVASQSHAYTMSLGAENSVAYSNNSAIPNTT
Alpha Variant		ECDFPGAGICASYQTGTNSPRKARSVASQSHAYTMSLGAENSVAYSNNSAIPNTT
Seqs: C107-2	192	TLVKQLSBNFGASVLANDESRLLDKYEAHVQDNLTQRLQSLQTYVYKGLPAAEIRA
Alpha Variant		TLVKQLSBNFGASVLANDESRLLDKYEAHVQDNLTQRLQSLQTYVYKGLPAAEIRA
Seqs: C107-1	216	QKQGNFFRLREFPYFNIDGYFNTYSHHTPNLVRDLPOGFSALEPLVQLPQINTRFGT
Alpha Variant		QKQGNFFRLREFPYFNIDGYFNTYSHHTPNLVRDLPOGFSALEPLVQLPQINTRFGT

B) Beta (B.1.351)

Seqs: C107-2	120	NDVEGSPNCFYPLQSYGFQPTNGVGVGPIRVVLSPELLNAPATVGGPKSTLWKNKCVN
Beta Variant		NDVEGSPNCFYPLQSYGFQPTNGVGVGPIRVVLSPELLNAPATVGGPKSTLWKNKCVN
Seqs: C107-2	144	PNPAGLTGTGVALTEGSHKFLPPQDFSPKADDTDAVRDPDLELRITPDSFGQVSVTP
Beta Variant		PNPAGLTGTGVALTEGSHKFLPPQDFSPKADDTDAVRDPDLELRITPDSFGQVSVTP
Seqs: C107-1	168	ECDFPGAGICASYQTGTNSPRKARSVASQSHAYTMSLGAENSVAYSNNSAIPNTT
Beta Variant		ECDFPGAGICASYQTGTNSPRKARSVASQSHAYTMSLGAENSVAYSNNSAIPNTT
Seqs: C107-2	192	TLVKQLSBNFGASVLANDESRLLDKYEAHVQDNLTQRLQSLQTYVYKGLPAAEIRA
Beta Variant		TLVKQLSBNFGASVLANDESRLLDKYEAHVQDNLTQRLQSLQTYVYKGLPAAEIRA
Seqs: C107-1	216	QKQGNFFRLREFPYFNIDGYFNTYSHHTPNLVRDLPOGFSALEPLVQLPQINTRFGT
Beta Variant		QKQGNFFRLREFPYFNIDGYFNTYSHHTPNLVRDLPOGFSALEPLVQLPQINTRFGT

C) Gamma (P.1)

Seqs: C107-2	120	NDVEGSPNCFYPLQSYGFQPTNGVGVGPIRVVLSPELLNAPATVGGPKSTLWKNKCVN
Gamma Variant		NDVEGSPNCFYPLQSYGFQPTNGVGVGPIRVVLSPELLNAPATVGGPKSTLWKNKCVN
Seqs: C107-2	144	PNPAGLTGTGVALTEGSHKFLPPQDFSPKADDTDAVRDPDLELRITPDSFGQVSVTP
Gamma Variant		PNPAGLTGTGVALTEGSHKFLPPQDFSPKADDTDAVRDPDLELRITPDSFGQVSVTP
Seqs: C107-1	168	ECDFPGAGICASYQTGTNSPRKARSVASQSHAYTMSLGAENSVAYSNNSAIPNTT
Gamma Variant		ECDFPGAGICASYQTGTNSPRKARSVASQSHAYTMSLGAENSVAYSNNSAIPNTT
Seqs: C107-2	192	TLVKQLSBNFGASVLANDESRLLDKYEAHVQDNLTQRLQSLQTYVYKGLPAAEIRA
Gamma Variant		TLVKQLSBNFGASVLANDESRLLDKYEAHVQDNLTQRLQSLQTYVYKGLPAAEIRA
Seqs: C107-1	216	QKQGNFFRLREFPYFNIDGYFNTYSHHTPNLVRDLPOGFSALEPLVQLPQINTRFGT
Gamma Variant		QKQGNFFRLREFPYFNIDGYFNTYSHHTPNLVRDLPOGFSALEPLVQLPQINTRFGT

D) Delta (B.1.617.2)

Seqs: C107-2	120	NDVEGSPNCFYPLQSYGFQPTNGVGVGPIRVVLSPELLNAPATVGGPKSTLWKNKCVN
Delta Variant		NDVEGSPNCFYPLQSYGFQPTNGVGVGPIRVVLSPELLNAPATVGGPKSTLWKNKCVN
Seqs: C107-2	144	PNPAGLTGTGVALTEGSHKFLPPQDFSPKADDTDAVRDPDLELRITPDSFGQVSVTP
Delta Variant		PNPAGLTGTGVALTEGSHKFLPPQDFSPKADDTDAVRDPDLELRITPDSFGQVSVTP
Seqs: C107-1	168	ECDFPGAGICASYQTGTNSPRKARSVASQSHAYTMSLGAENSVAYSNNSAIPNTT
Delta Variant		ECDFPGAGICASYQTGTNSPRKARSVASQSHAYTMSLGAENSVAYSNNSAIPNTT
Seqs: C107-2	192	TLVKQLSBNFGASVLANDESRLLDKYEAHVQDNLTQRLQSLQTYVYKGLPAAEIRA
Delta Variant		TLVKQLSBNFGASVLANDESRLLDKYEAHVQDNLTQRLQSLQTYVYKGLPAAEIRA

FIG. 1

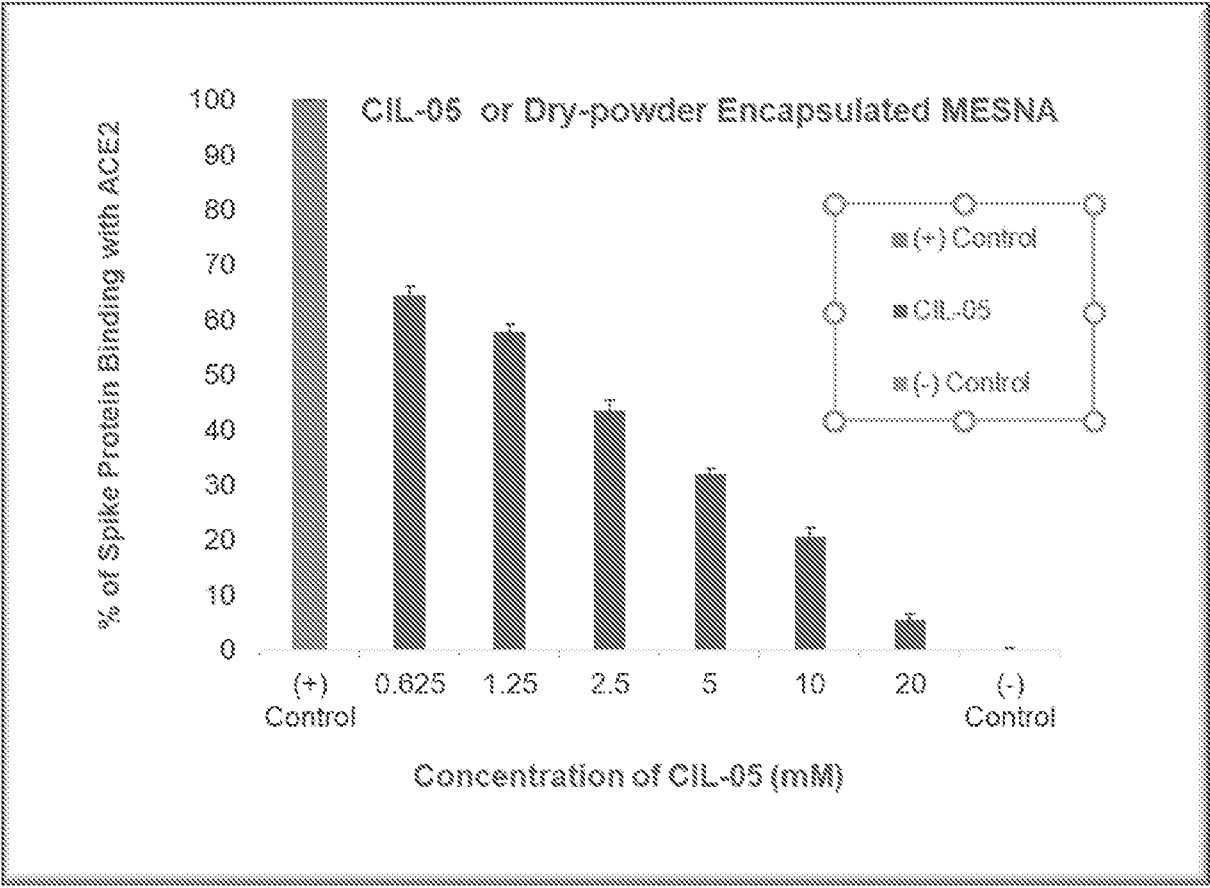


FIG. 2

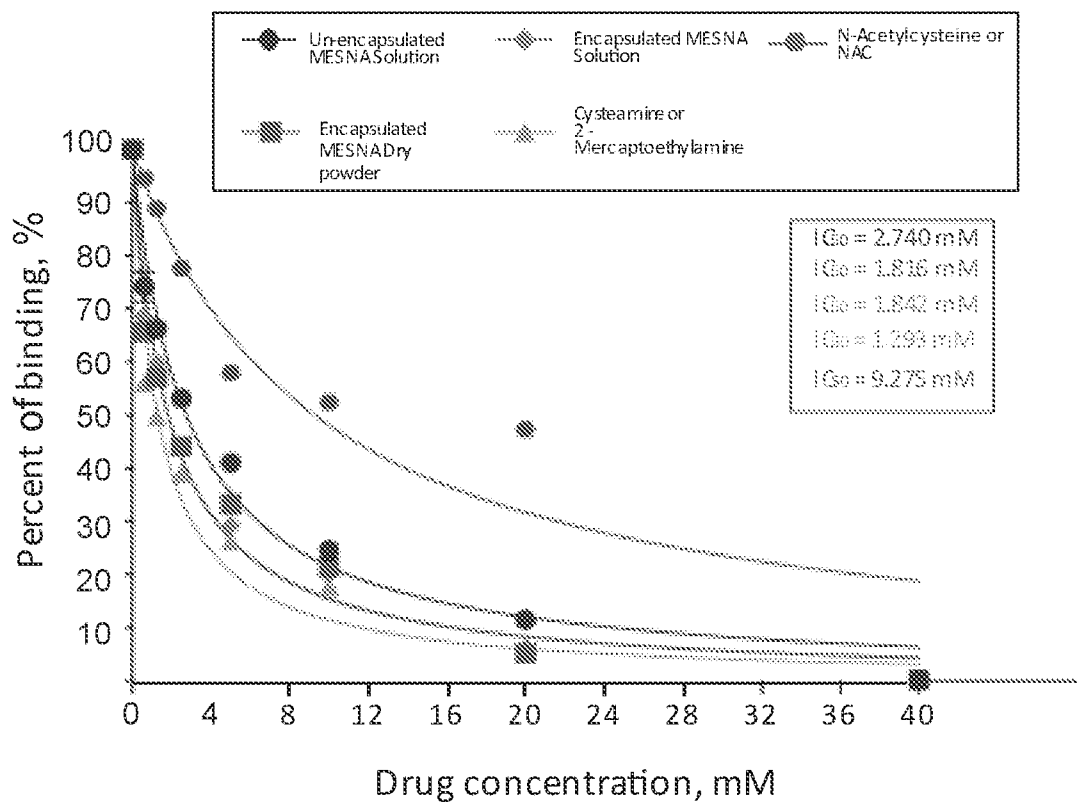
Binding of SARS-CoV-2RBD to ACE2 is inhibited by thiol-based drugs

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 22/81081

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. A61K 35/12 (2023.01)
 ADD. A61K 35/17 (2023.01)

CPC - INV. A61K 35/12
 ADD. A61K 35/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2021/231421 A1 (The Regents Of The University Of California) 18 November 2021 (18.11.2021); para[0006] para[0066] para[0074] para[0098] para[0144] para[0148]	1-4
X	US 2007/0135525 A1 (Liang et al.) 14 June 2007 (14.06.2007); Abstract para[0016] para[0021] para[0052] para[0059]	1, 5, 8-11
X	WO 2021/194890 A1 (Atossa Therapeutics, Inc.) 30 September 2021 (30.09.2021); para[0012] para[0014] para[0016] para[0034] para[0158]	1, 6-7
A	US 2006/0189688 A1 (Piazza et al.) 24 August 2006 (24.08.2006); entire document	1-11
A	WO 2021/231570 A1 (The Regents Of The University Of Michigan) 18 November 2021 (18.11.2021); entire document	1-11

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

21 February 2023

Date of mailing of the international search report

MAY 08 2023

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 22/81081

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 12-45, 56-72
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
See Supplemental Box

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-11

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 22/81081

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I: Claims 1-11 drawn to a method of reducing the treating or risk of viral infection in a subject, the method comprising administering to the subject a composition comprising sodium 2-mercaptoethane sulfonate or 2-mercaptoethylamine particles

Group II: Claims 46-55 drawn to a method of reducing extracellular disulfide bonds in a viral protein, the method comprising the step of contacting the viral protein with sodium 2-mercaptoethane sulfonate or 2-mercaptoethylamine particles

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Group I requires a method of reducing the treating or risk of viral infection in a subject, the method comprising administering to the subject a composition, not required by Group II

Group II requires a method of reducing extracellular disulfide bonds in a viral protein, not required by Group I

Shared Common Features

Groups I-II share technical feature of a method comprising administering to the subject or contacting the viral protein sodium 2-mercaptoethane sulfonate or 2-mercaptoethylamine particles.

However, these shared technical feature do not represent a contribution over prior art, because the shared technical feature as being anticipated by US 2006/0189688 A1 to Piazza et al. (hereinafter 'Piazza'). Piazza discloses a method comprising administering to the subject sodium 2-mercaptoethane sulfonate (Claim 4. "A method of treatment or prevention of viral diseases in a subject, administering to said subject an effective amount of sodium 2-mercaptoethanesulfonate").

As the technical features were known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups. Groups I-II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Continuation of Item 4: claims 12-45, and 56-72 are determined unsearchable because they are not drafted in accordance with the second and third sentences of Rule 6.4(a).