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(54) **Title:** COMPOUNDS AND METHODS FOR PREVENTION AND TREATMENT OF CORONAVIRUS INFECTIONS

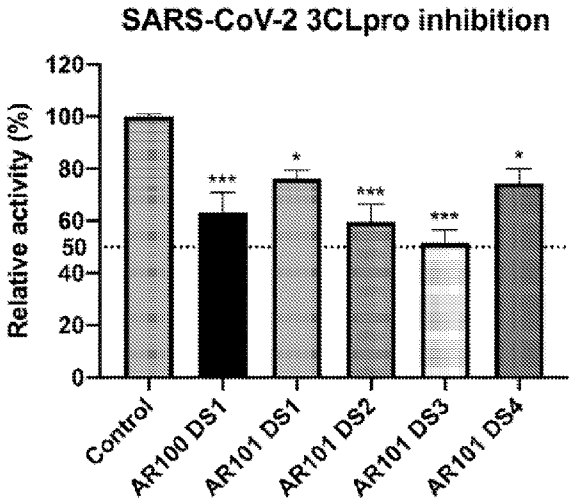


Figure 1

(57) **Abstract:** The present invention pertains to anti-coronaviral compounds. The disclosure includes a method for preventing and/or treating a coronavirus infection through the inhibition of a cysteine protease in a virus, particularly SARS-COV-2. Also provided includes the composition/pharmaceutical composition for preventing and/or treating a coronavirus infection comprising any of the compounds, pharmaceutically acceptable salt thereof, or its mixture, and the use of the compounds.

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TITLE OF THE INVENTION

COMPOUNDS AND METHODS FOR PREVENTION AND TREATMENT OF CORONAVIRUS INFECTIONS

CROSS-REFERENCE TO RELATED APPLICATION

【0001】 This application claims the benefit of and priority to U.S. Provisional Application Ser. Nos. 63/018,690, filed May 1, 2020, which is incorporated herein by reference in entirety.

FIELD OF THE INVENTION

【0002】 The present invention provides some anti-coronaviral compounds and the method and composition/pharmaceutical composition for prevention and treatment of coronavirus infections, particularly the disease caused by SARS-COV-2.

BACKGROUND OF THE INVENTION

【0003】 Severe acute respiratory syndrome (SARS) outbreak in November 1st, 2002 to June 18th, 2003 led to 801 deaths in over 29 countries and 8465 probable cases around the world according to the World Health Organization (WHO) (Chen *et al.*, 2005). SARS, an enveloped β coronavirus containing positive-sense, single-stranded RNA, has a genome size of about 30 kb, in which open reading frame (ORF) 1a and 1b encode for two respective polyproteins (pps), pp1a and pp1ab (Hegyi *et al.*, 2002; Needle *et al.*, 2015). To complete its lifecycle, successful replication and proteolytic processing are imperative (Herold *et al.*, 1998). Indeed, the consensus functions of these virus-encoded proteolytic proteins are found in all coronaviruses, specifically papline-like protease (PLpro) and chymotrypsin-like protease (3CLpro) (Herold *et al.*, 1998). In proteolytic processing of pp1a and pp1ab, PLpro and 3CLpro cleave the first three sites and the remaining 11 locations, respectively, yielding a total of 16 nonstructural proteins (nsp1–16) (Hegyi *et al.*, 2002; Needle *et al.*, 2015). Thus, 3CLpro inhibition has been regarded as a molecular approach in anti-SARS drug discovery and developments (Chen *et al.*, 2005; Jo *et al.*, 2020).

【0004】 SARS-COV-2 is a novel coronavirus that spreads rapidly since its identification in patients with severe pneumonia in Wuhan, China (named as COVID-19), has been reported in 25 countries, with nearly 72000 laboratory-confirmed cases and a death toll of

1775 worldwide as of February 17th, 2020 (*Coronavirus disease 2019 (Covid-19) Situation Report – 28*, 2020; Li & De Clercq, 2020). Devastatingly, no drug or vaccine has yet been approved to treat human coronaviruses (Li & De Clercq, 2020). Concerning the current outbreak of SARS-CoV-2 and the therapeutic experience of SARS and MERS (another β coronavirus), many studies extensively investigate the possibility of using the existing antiviral agents used for HIV, hepatitis B virus, hepatitis C virus and influenza infections for the treatment or intervention of SARS-COV-2 (De Clercq & Li, 2016; Li & De Clercq, 2020). In the meantime, SARS-CoV-2 has been characterized as an enveloped, positive-sense, single-stranded RNA β coronavirus, similar to SARS and MERS (Li & De Clercq, 2020). Consistent with the characteristics of coronaviruses, SARS-CoV-2 genome encodes structural proteins (e.g., spike glycoproteins), nonstructural proteins (e.g., 3CLpro, PLpro, helicase, RNA-dependent RNA polymerase), and accessory proteins. Regarding the available genomic sequence of SARS-COV-2, SARS and MERS, a high-level conservation of the proteolytic sites and proteolytic enzymes was found, whence repurposing SARS and MERS protease inhibitors for treatment of SARS-COV-2 is worth considering (Liu *et al.*, 2020). As 3CLpro plays a pivotal role in SARS, it is reasonable to approach protease inhibition by targeting the 3CLpro of SARS-COV-2 instead of its PLpro to intercept its lifecycle (Chen *et al.*, 2005; Jo *et al.*, 2020; Liu *et al.*, 2020).

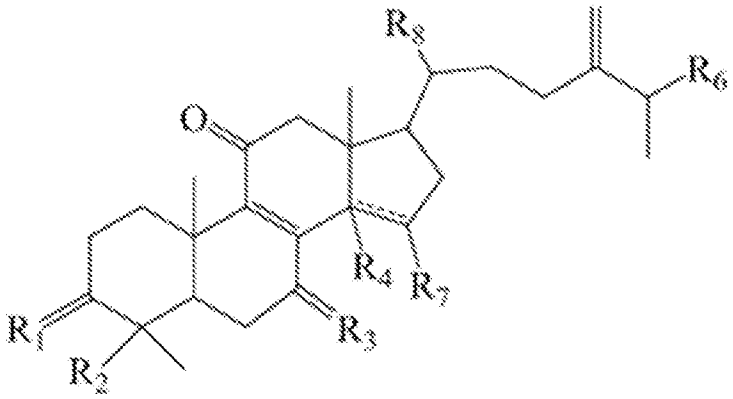
【0005】 Currently, disulfiram, an approved drug to treat alcohol dependence, has been reported to inhibit the PLpro of MERS and SARS in cell cultures but has yet been evaluated clinically (Li & De Clercq, 2020). In addition, clinical trials of HIV protease inhibitors (lopinavir and ritonavir) in SARS-CoV-2 patients have also commenced, yet it is uncertain if it can effectually inhibit those of SARS-CoV-2, as HIV and β coronavirus proteases belong to the aspartic protease family and the cysteine protease family, respectively (Li & De Clercq, 2020; Zumla *et al.*, 2016). On the other hand, remdesivir, a nucleotide analog of RNA dependent RNA polymerase inhibitor approved for HIV treatment, is currently under clinical trials in SARS-CoV-2 patients with estimated completion dates in April, 2020; galidesivir, another nucleotide analog of RNA dependent RNA polymerase inhibitor in early-stage clinical studies for HCV treatment, has shown broad-spectrum antiviral activities against severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS) in preclinical studies (Wang *et al.*, 2020; Zumla *et al.*, 2016). However, one might expect that a nucleoside analog can elicit toxicity that are still beyond our knowledge (Feng, 2018).

【0006】 There are yet to find vaccines or antiviral drugs to prevent or treat human coronavirus infections. There is an urgent need for exploring and developing a safe anti-coronavirus therapy, particularly against SARS-COV-2.

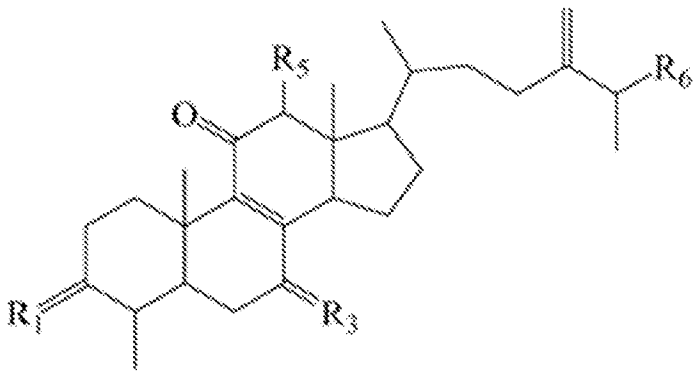
BRIEF SUMMARY OF THE INVENTION

【0007】 It is unexpectedly found in the present invention that some triterpenes are effective in inhibition of coronavirus infections, especially SARS-COV-2.

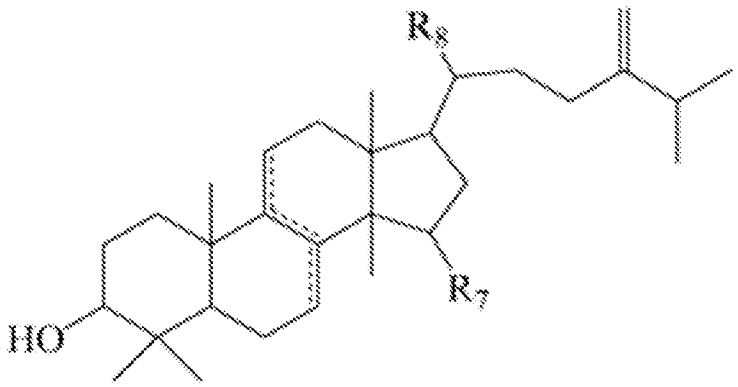
【0008】 In one object, the present invention provides a method for preventing and/or treating a coronavirus infection, comprising administering to a subject in need thereof a compound or a pharmaceutically acceptable salt thereof, in which the compound is selected from the group consisting of:



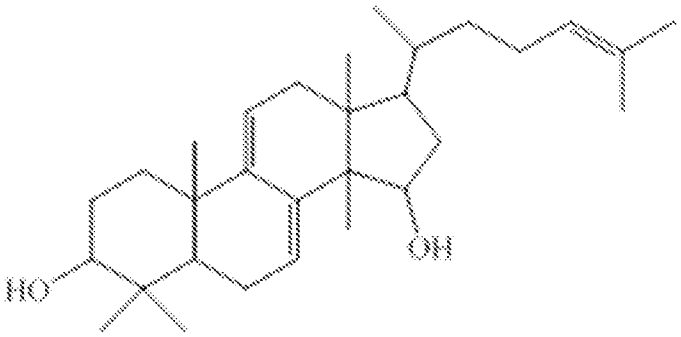
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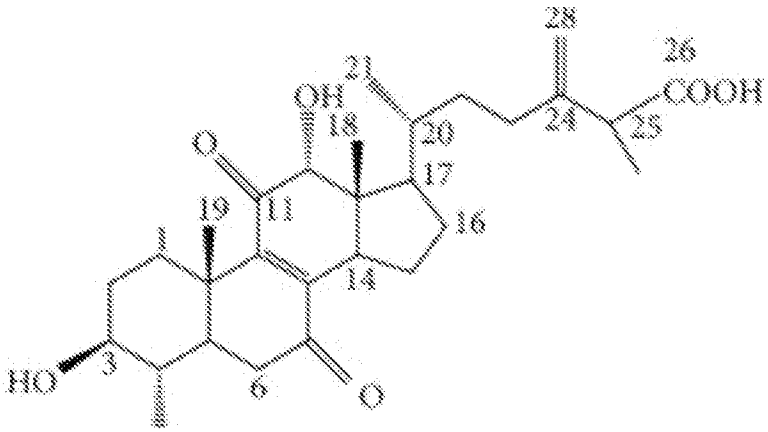
(Ib),



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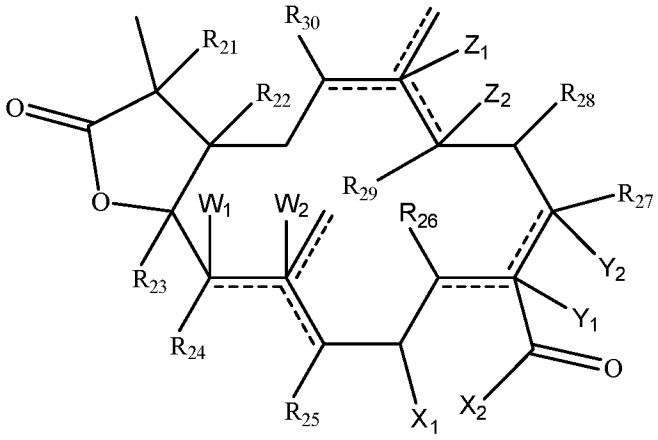


(Id),



(Ie),

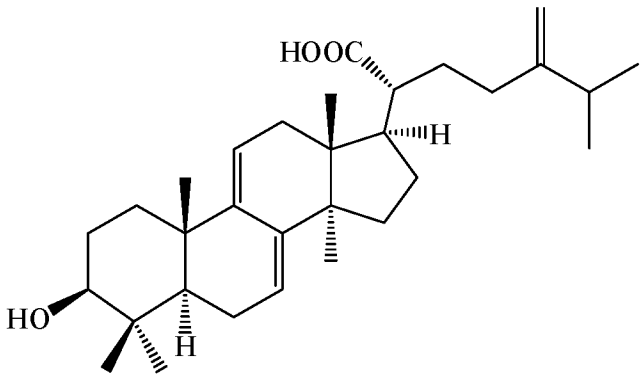
wherein R_1 is O, α-OH or β-H; R_2 is H or OH; R_3 is O, α-H, β-OH, β-OAc or H_2; R_4 is H or OH; R_5 is H, OH or ORx; R_6 is COORx or $COO(CH_2)n\text{-}CH_3$; n is an integer from 0-3; R_7 is H, Oh, ORx or OAc; R_8 is CH_3 or COORx; R_{21} is CH_3, COORx, or $COO(CH_2)n\text{-}CH_3$; n is an integer from 0-3; the dotted line represents a single bond or a double bond, Rx is H or a $C_{1\text{-}8}$ alkyl; and



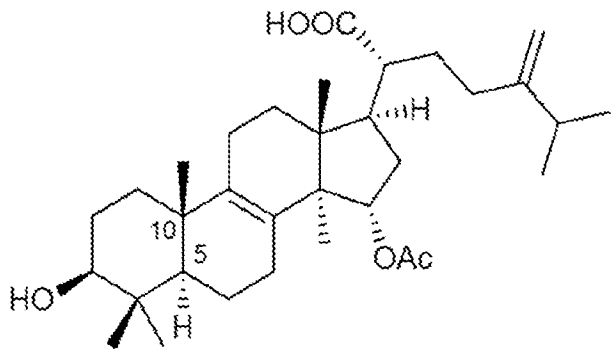
(II);

wherein each of R_{21}, R_{22}, R_{23}, R_{24}, R_{25}, R_{26}, R_{27}, R_{28}, R_{29}, R_{30}, X_1 and X_2 is H, OH, C_{1-8} alkyl, NRx, SRx, ORx, pyrazoline, cysteine, glutathione, halogen, COORx, or $COO(CH_2)n\text{-}CH_3$; n is an integer from 0-3; each of Y_1, Y_2, Z_1, Z_2, W_1 and W_2 is H, OH, C_{1-8} alkyl, or X_1 and X_2 together form -O-, or Y_1 and Y_2, Z_1 and Z_2, or W_1 and W_2 together form an epoxy.

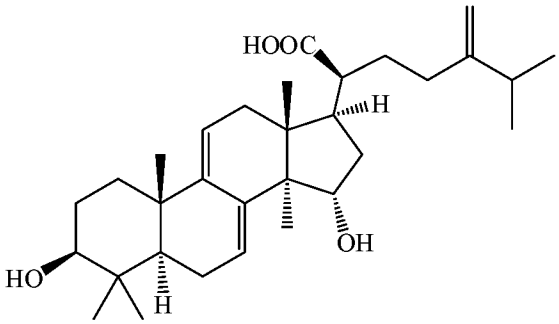
【0009】 In some particular examples of the present invention, the compound is selected from the group consisting of:



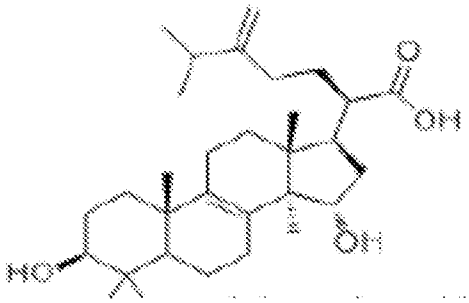
(dehydroeburicoic acid),



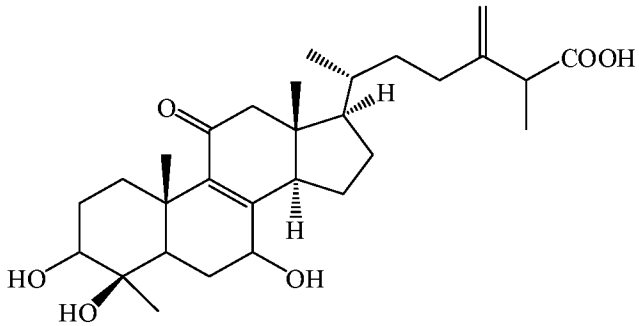
(versisponic acid D),



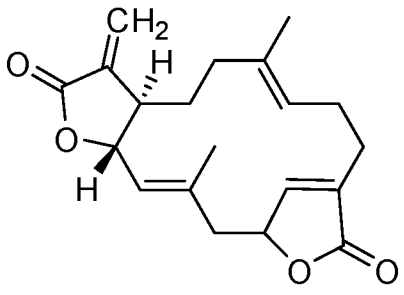
(dehydrosulphurenic acid),



(sulphurenic acid),



(antcin K), and



(ovatodiolide).

【0010】 In one further aspect, the present invention provides a composition/pharmaceutical composition for preventing and/or treating a coronavirus infection, particularly SARS-COV-2 comprising a therapeutically effective amount of any of

the compounds disclosed herein or pharmaceutically acceptable thereof, or its mixture, in combination of a pharmaceutically acceptable carrier.

【0011】 Optionally, the composition/pharmaceutical composition according to the invention may comprise at least one additional anti-viral therapeutic agent.

【0012】 In one yet aspect, the present invention provides a use of any of the compounds disclosed herein or pharmaceutically acceptable salts, or its mixture for manufacturing a medicament for preventing and/or treating a coronavirus infection, particularly SARS-COV-2.

【0013】 It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

【0014】 The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiments which are presently preferred.

【0015】 In the drawings:

【0016】 Figure 1 shows the inhibitory profiles of AR100-DS1, AR101 DS1, AR101 DS2, AR101 DS3 and AR101 DS4 at the concentration of 20 μM. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

【0017】 Figure 2A shows the relative 3CLpro activity (%) of AR101 DS2 (0.5p/5FP), and IC50=39 μM.

【0018】 Figure 2B shows the relative 3CLpro activity (%) of AR101 DS2 (0.125p/1.25FP), and IC50=15.82 μM.

【0019】 Figure 2C shows the relative 3CLpro activity (%) of AR101 DS3 (0.5p/5FP), and IC50=14.61 μM.

【0020】 Figure 2D shows the relative 3CLpro activity (%) of AR101 DS3 (0.5p/5FP), and IC50=11.06 μM.

【0021】 Figure 2E shows the relative 3CLpro activity (%) of AR101 DS3 (0.125p/1.25FP), and IC50=11.7 μM.

【0022】 Figure 3 shows the relative 3CLpro activity (%) of AR100 DS1 (0.125p/1.25FP), and IC50=21.31 μM.

DETAILED DESCRIPTION OF THE INVENTION

【0023】 The above summary of the present invention will be further described with reference to the embodiments of the following examples. However, it should not be understood that the content of the present invention is only limited to the following embodiments, and all the inventions based on the above-mentioned contents of the present invention belong to the scope of the present invention.

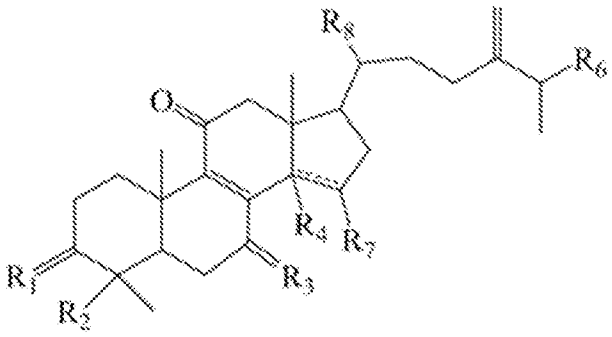
【0024】 Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by a person skilled in the art to which this invention belongs.

【0025】 As used herein, the singular forms “a”, “an”, and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a sample” includes a plurality of such samples and equivalents thereof known to those skilled in the art.

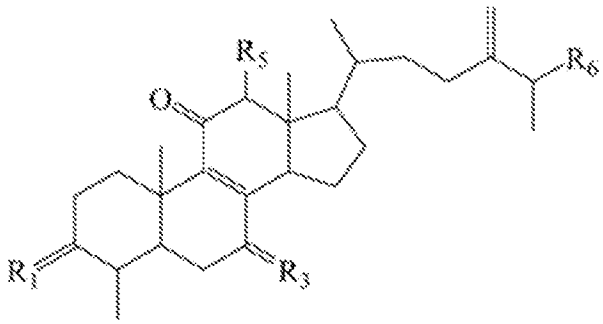
【0026】 In the present invention, to evaluate the effect of prospecting drugs on proteolytic processing inhibition in high-throughput, the synthetic peptides labelled fluorescence resonance energy transfer (FRET) pairs were employed as those used in the previous studies, in which the quenched fluorophore is released upon cleavage of the FRET-labelled peptides, generating fluorescent signals that can be monitored in real-time (Chen et al., 2005; Jean et al., 1995; Jo et al., 2020). It is confirmed in the present invention that any or its mixture of the compounds disclosed herein is effective in inhibition of a cysteine protease, particularly 3CLpro of SARS-CoV-2.

【0027】 The present invention provides a method for preventing and/or treating a coronavirus infection, particularly SARS-COV-2 comprising administering to a subject in need thereof a compound or pharmaceutically acceptable salt thereof, or its mixture, in which the compound is selected from the group consisting of:

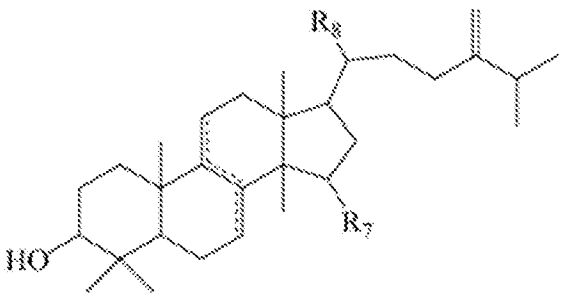
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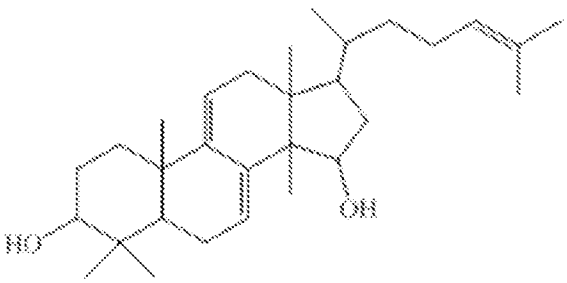
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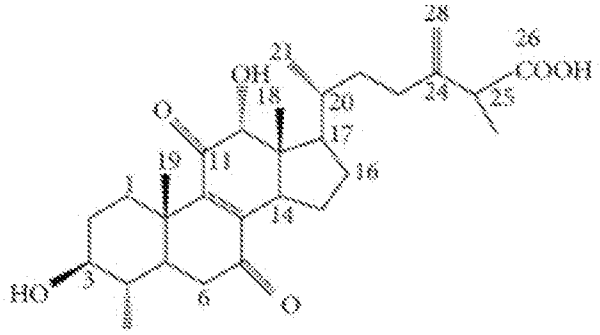
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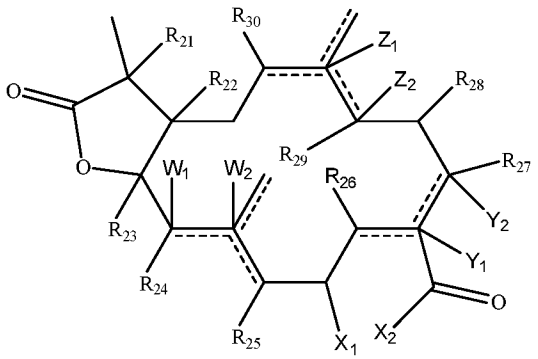
(Id),



(Ie),

wherein R_1 is O, α-OH or β-H; R_2 is H or OH; R_3 is O, α-H, β-OH, β-OAc or H_2; R_4 is H or OH; R_5 is H, OH or ORx; R_6 is COORx or $COO(CH_2)n\text{-}CH_3$; n is an integer from 0-3; R_7 is H, Oh, ORx or OAc; R_8 is CH_3 or COORx; R_{21} is CH_3, COORx, or $COO(CH_2)n\text{-}CH_3$; n is an integer from 0-3; , the dotted line represents a single bond or a double bond, Rx is H or a $C_{1\text{-}8}$ alkyl; and

(2)



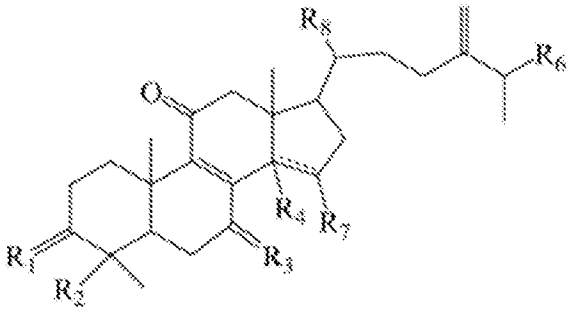
(II);

wherein each of R_{21}, R_{22}, R_{23}, R_{24}, R_{25}, R_{26}, R_{27}, R_{28}, R_{29}, R_{30}, X_1 and X_2 is H, OH, $C_{1\text{-}8}$ alkyl, NRx, SRx, ORx, pyrazoline, cysteine, glutathione, halogen, COORx, or $COO(CH_2)n\text{-}CH_3$; n is an integer from 0-3; each of Y_1, Y_2, Z_1, Z_2, W_1 and W_2 is H, OH, $C_{1\text{-}8}$ alkyl, or X_1 and X_2 together form -O-, or Y_1 and Y_2, Z_1 and Z_2, or W_1 and W_2 together form an epoxy.

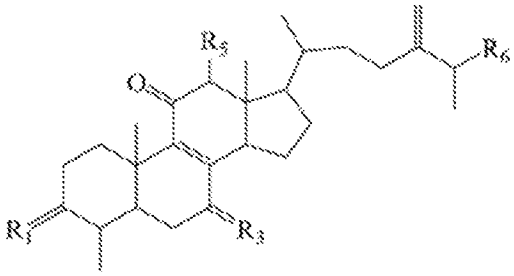
【0028】 The present invention also provides a composition/pharmaceutical composition for preventing and/or treating a coronavirus infection, particularly SARS-COV-2 infection, which comprises a therapeutically effective amount of a compound as disclosed herein or mixture thereof and a pharmaceutically acceptable carrier.

【0029】 In one embodiment, the compound is

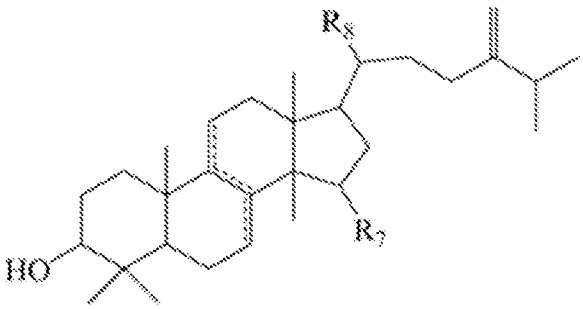
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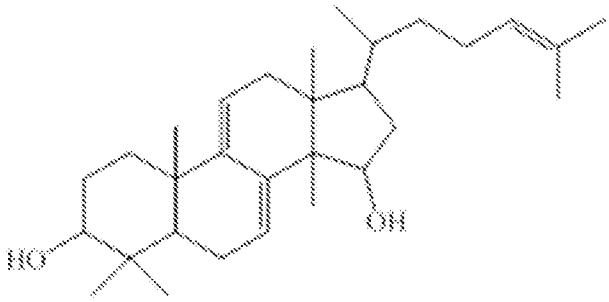
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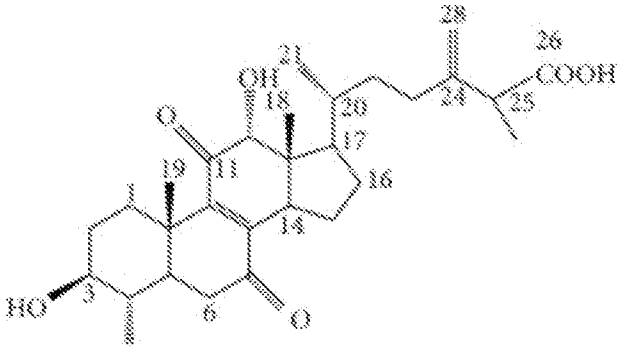
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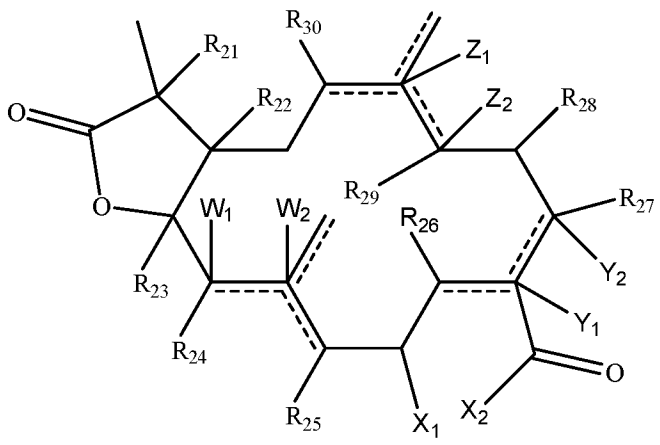
(Id), or



(Ie);

wherein R_1 is O, α-OH or β-H; R_2 is H or OH; R_3 is O, α-H, β-OH, β-OAc or H_2; R_4 is H or OH; R_5 is H, OH or ORx; R_6 is COORx or $COO(CH_2)n\text{-}CH_3$; n is an integer from 0-3; R_7 is H, Oh, ORx or OAc; R_8 is CH_3 or COORx; R_{21} is CH_3, COORx, or $COO(CH_2)n\text{-}CH_3$; n is an integer from 0-3; the dotted line represents a single bond or a double bond, Rx is H or a $C_{1\text{-}8}$ alkyl; or

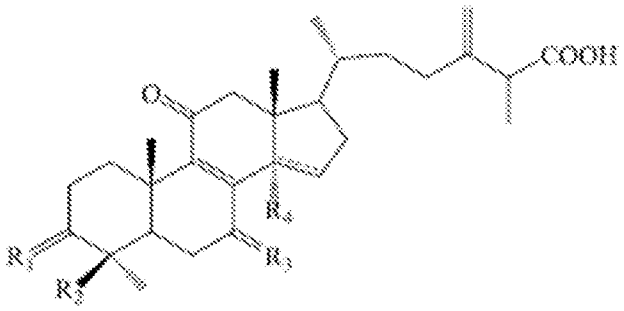
(2)



(II);

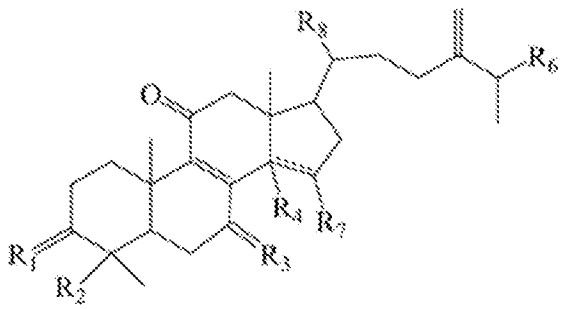
wherein each of R_{21}, R_{22}, R_{23}, R_{24}, R_{25}, R_{26}, R_{27}, R_{28}, R_{29}, R_{30}, X_1 and X_2 is H, OH, $C_{1\text{-}8}$ alkyl, NRx, SRx, ORx, pyrazoline, cysteine, glutathione, halogen, COORx, or $COO(CH_2)n\text{-}CH_3$; n is an integer from 0-3, Rx is H or a $C_{1\text{-}8}$ alkyl; each of Y_1, Y_2, Z_1, Z_2, W_1 and W_2 is H, OH, $C_{1\text{-}8}$ alkyl , X_1 and X_2 together form -O-, or Y_1 and Y_2, Z_1 and Z_2, or W_1 and W_2 together form an epoxy.

【0030】 In the example of the invention, the compound of formula (I) may be:



	R_1	R_2	R_3	R_4	Δ
Antcin A	O	H	H_2	H	
Antcin B	O	H	O	H	
Antcin C	O	H	β-OH	H	
Antcin D	O	H	O	OH	
Antcin E	O	H	H_2		14
Antcin F	O	H	β-OH		14
Antcin K	α-OH	OH	β-OH	H	

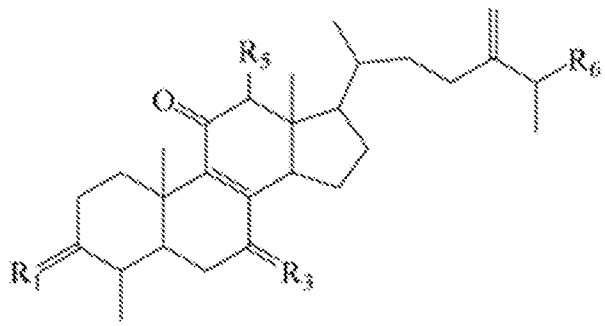
【0031】 In another example of the invention, the compound of formula (I) may be:



R_7=H; R_8=CH_3

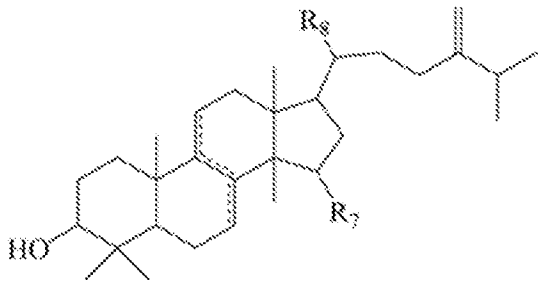
	R_1	R_2	R_3	R_4
Zhankuic acid B	β-H α-OH	H	H	COOH
Zhankuic acid C	β-H α-OH	H	OH	COOH
Zhankuic acid D	O	H	H	COOEt
Zhankuic acid E	β-H α-OH	H	OH	COOEt

【0032】 In one yet example of the invention, the compound of formula (I) may be:



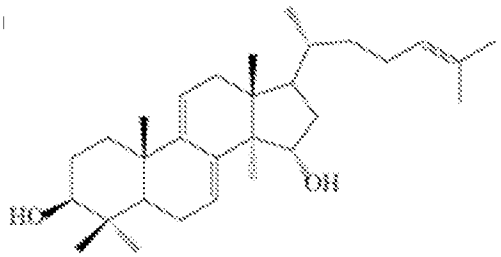
	R_1	R_3	R_5
methyl antcinate B	O	O	H
methyl antcinate G	O	β-OAc α-H	H
methyl antcinate H	β-H α-OH	O	OH
	O	H_2	H

【0033】 In further example of the invention, the compound of formula (I) may be:

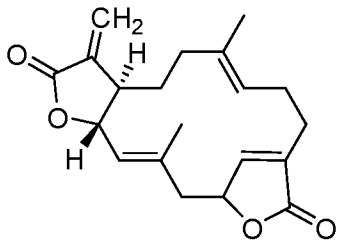


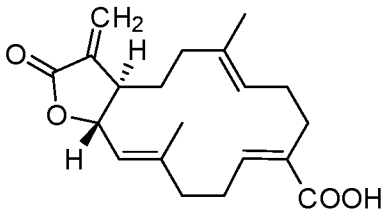
	R_7	R_8	Δ
dehydroeburicoic acid	H	COOH	7,9 (11)
eburicol	H	CH_3	8
15a-acetyl-dehydrosulphurenic acid	OAc	COOH	7,9 (11)
dehydrosulphurenic acid	OH	COOH	7,9 (11)
eburicoic acid	H	COOH	8
Versisponic acid	OAc	COOH	8
sulphurenic acid	OH	COOH	8

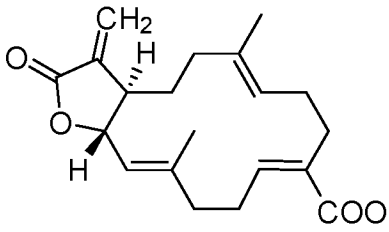
【0034】 In one particular example of the invention, the compound of formula (I) may be lanostane:

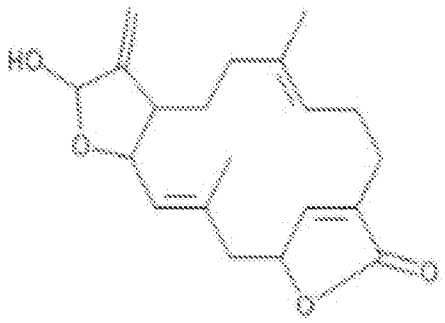


【0035】 In addition, the compound of formula (II) may be

 (ovatodiolide),

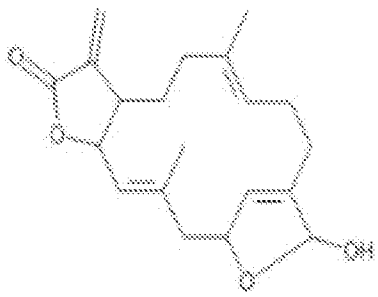
 (ovatodiolide acid),

 (anisomelic acid),



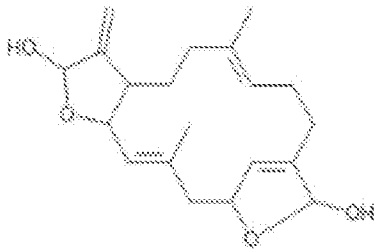
Modification 1

,



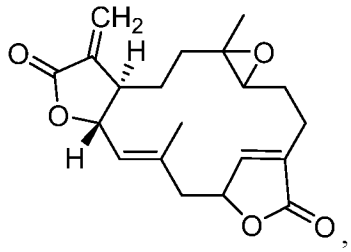
Modification 2

,

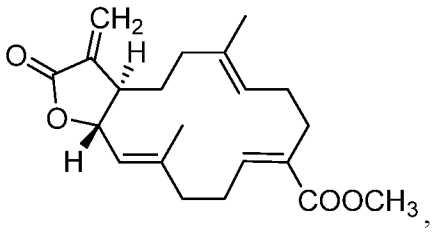


Modification 3

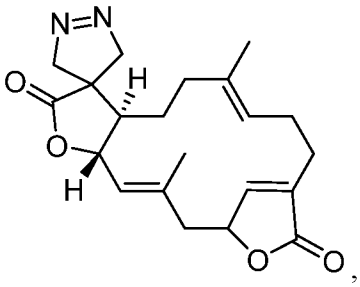
,



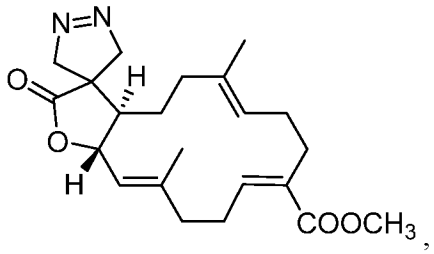
,

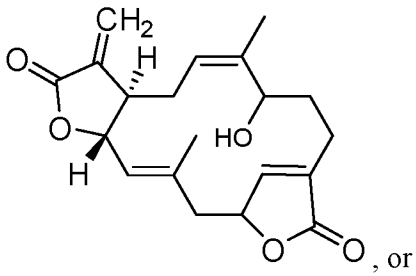


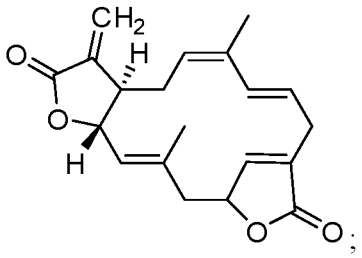
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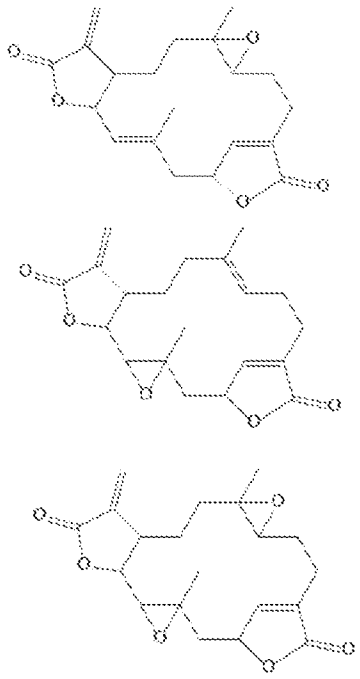
,



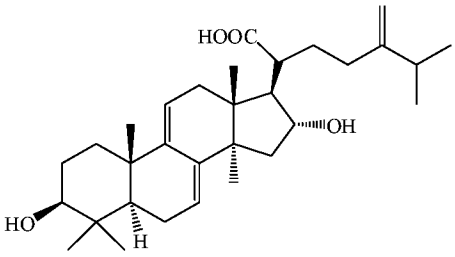




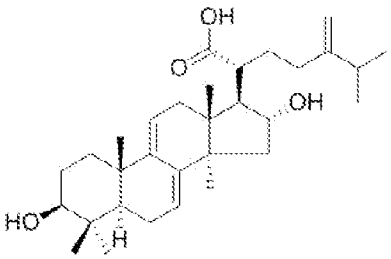
or its derivatives, such as



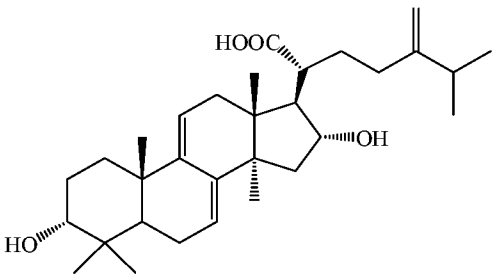
【0036】 Accordingly, the preferred compound is selected from the group consisting of:



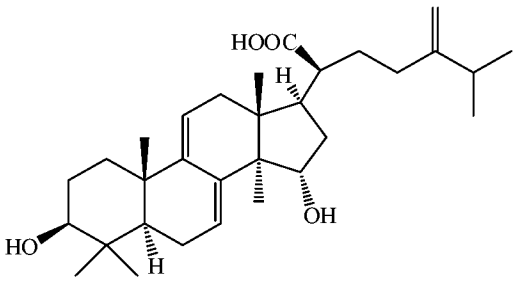
(dehydrotumolosaeure),



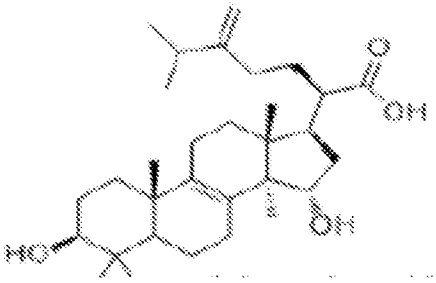
(dehydrotumulosic acid),



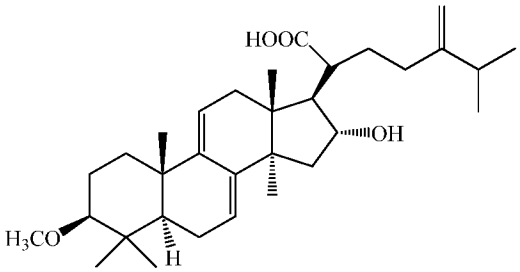
(3-epi-dehydrotumulosic acid),



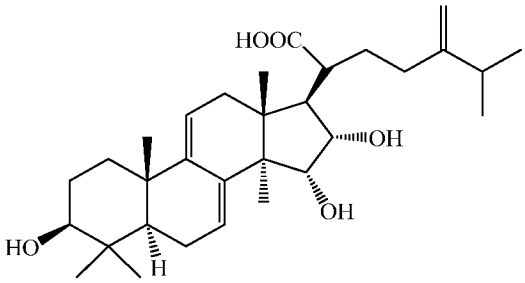
(dehydrosulphurenic acid),



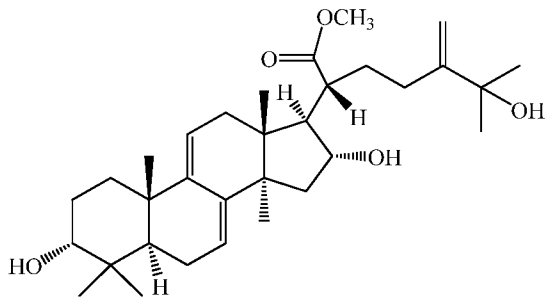
(sulphurenic acid),



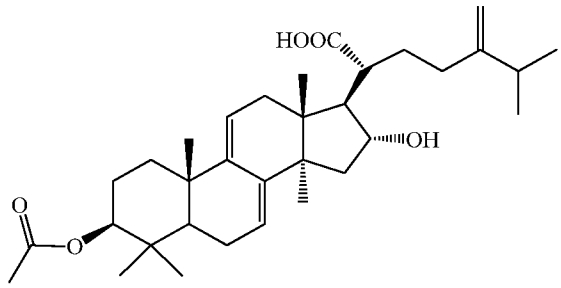
(dehydrotumolosaeure-methylester),



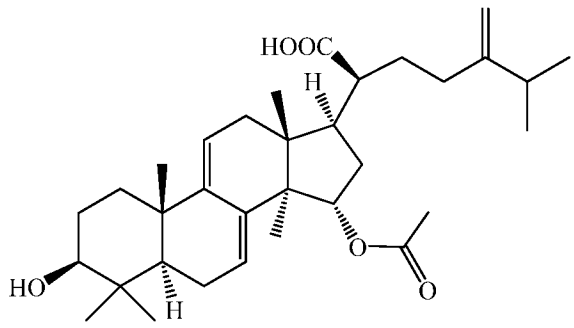
((20ξ)-3β,15α,16α-trihydroxy-24-methyllanosta-7,9(11),24(241)-trien-21-oic acid; 15α-hydroxydehydrotumulosic acid),



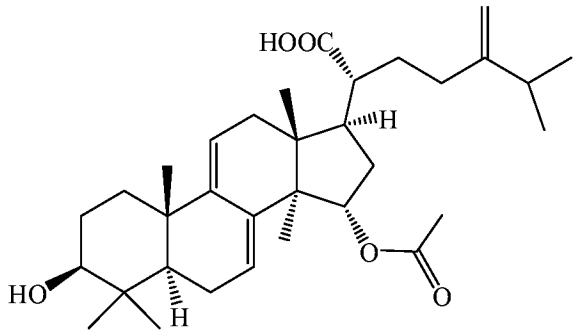
(methyl 25-hydroxy-3-epidehydrotumulosate(methyl)),



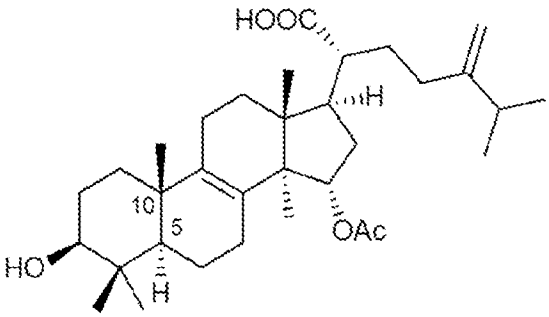
(dehydropachymic acid),



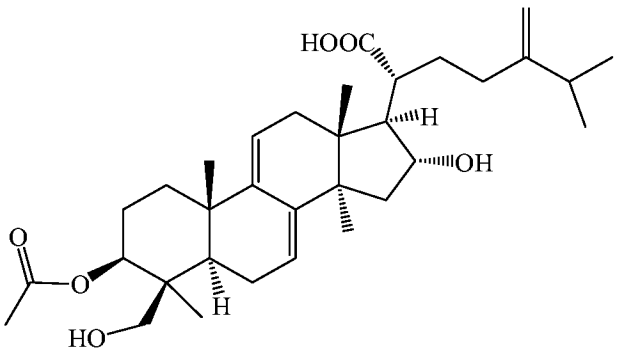
(15α-acetyldehydrosulfurenic acid),



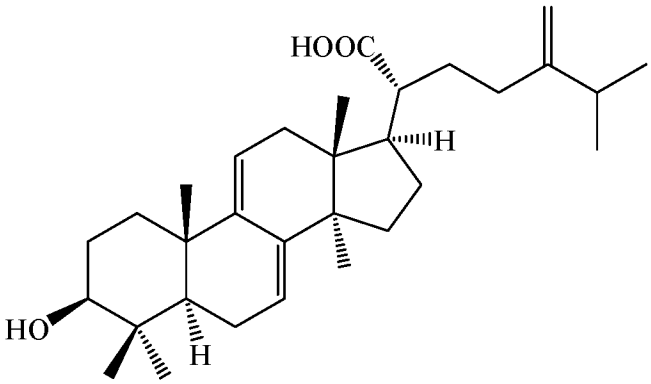
(15α-acetyldehydrosulphurenic acid),



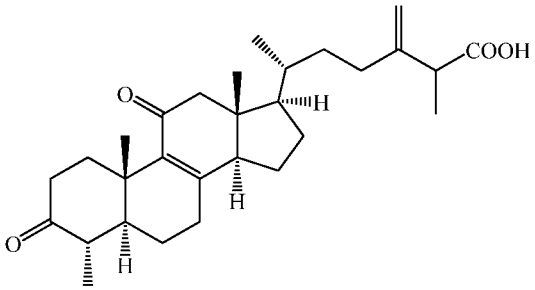
(versisponic acid D),



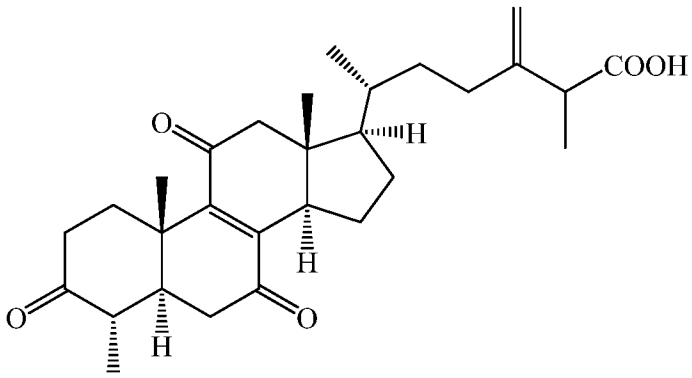
(29-hydroxydehydropachymic acid; (3β,16α)-3-(acetyloxy)-16,29-dihydroxy-24-methylidenelanosta-7,9(11)-dien-21-oic acid),



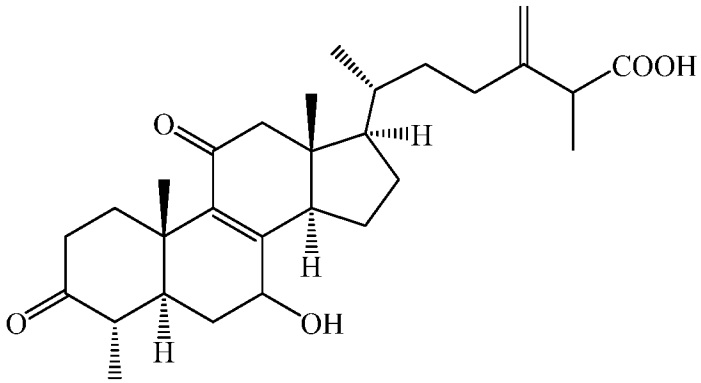
(dehydroeburicoic acid),



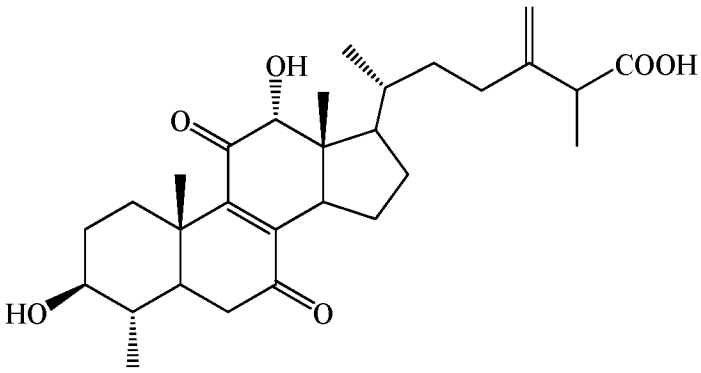
(antcin A),



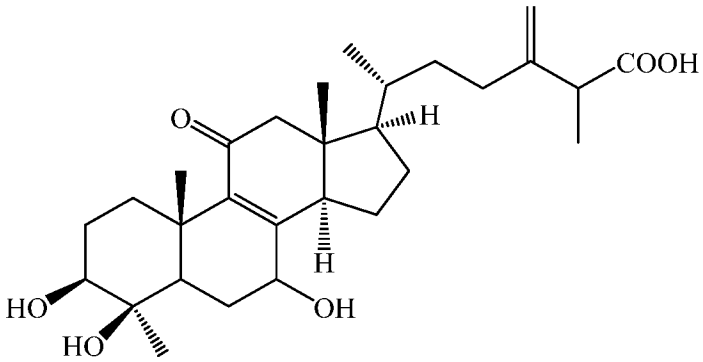
(antcin B),



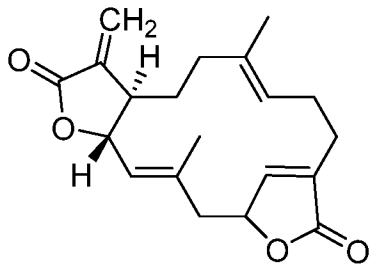
(antcin C),



(antcin H),

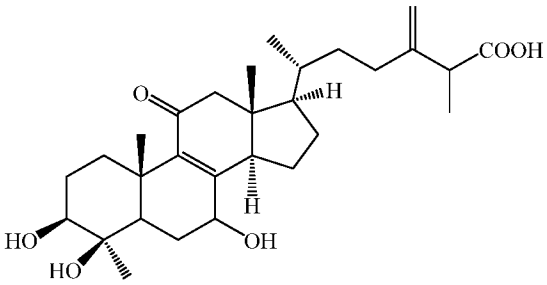


(antcin K), and

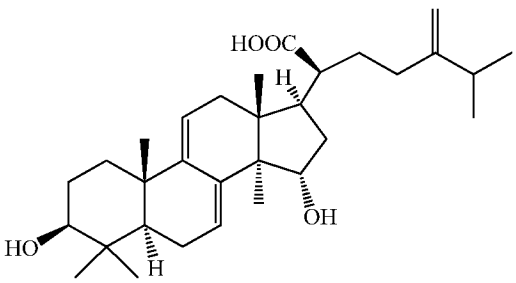


(ovatodiolide).

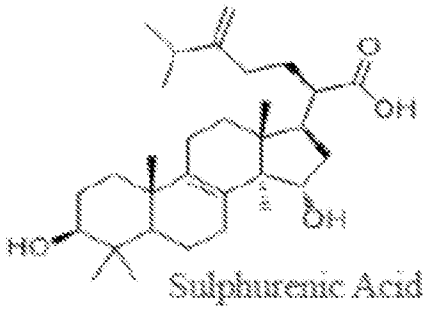
【0037】 According to the invention, the most preferred compound is selected from the group consisting of:



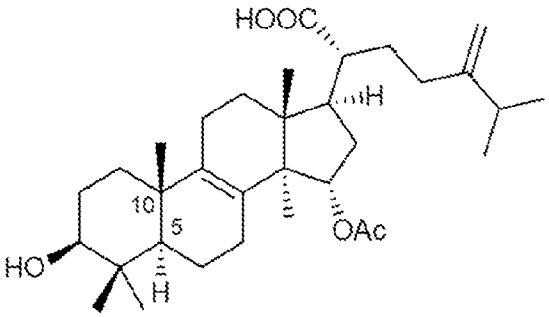
(antcin K, AR101 DS01);



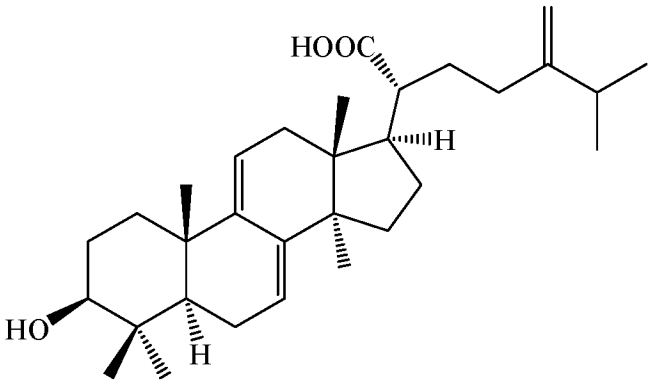
(dehydrosulphurenic acid, AR101 DS2),



(sulphurenic acid, AR101 DS2),

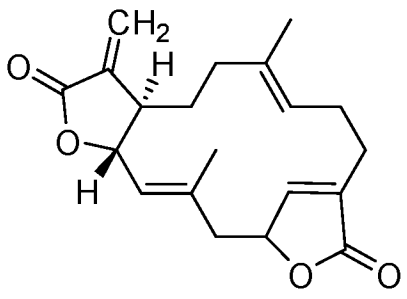


(versisponic acid D, AR101 DS3), and



(dehydroeburicoic acid, AR101 DS4).

【0038】 In one particular example of the invention, the compound of formula (II) is



(ovatodiolide, AR100 DS1).

【0039】 The term "coronavirus" as used herein refers to a Coronaviruse in the subfamily *Orthocoronavirinae*, the family *Coronaviridae*, order *Nidovirales*, and realm *Riboviria*, which is enveloped viruses with a positive-sense single-stranded RNA genome and a nucleocapside of helical symmetry. They have characteristic club-shaped spikes that project from their surface, which in electron micrographs create an image reminiscent of the solar corona from which their name derives. Coronaviruses cause diseases in mammals and birds, including humans. In humans, coronaviruses cause respiratory tract infections, including common cold, severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and SARS-COV-2.

【0040】 The term "cysteine protease" as used herein refers to thiol proteases, are enzymes that degrade proteins, sharing a common catalytic mechanism that involves a nucleophilic cysteine thiol in a catalytic triad or duad. One example of cysteine protease in a virus is 3CLpro in SARS-COV-2.

【0041】 The term "treat," "treating" or "treatment" as used herein refers to the application or administration of a composition including one or more active agents to a subject afflicted with a disease, a symptom or conditions of the disease, or a progression of the disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disease, the symptoms or conditions of the disease, the disabilities induced by the disease, or the progression of the disease.

【0042】 The term "prevent," "prevention" or "preventing" as used herein refers to the prevention of the recurrence, onset, or development of a virus infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by, or potentiating a coronavirus infection in a subject.

【0043】 The term "subject" as used herein includes human or non-human animals, such as companion animals (e.g. dogs, cats, etc.), farm animals (e.g. cattle, sheep, pigs, horses, etc.), or experimental animals (e.g. rats, mice, guinea pigs, etc.).

【0044】 The term "therapeutically effective amount" as used herein refers to an amount of a pharmaceutical agent which, as compared to a corresponding subject who has not received such amount, results in an effect in treatment, healing, prevention, or amelioration of a disease, disorder, or side effect, or a decrease in the rate of advancement of a disease or disorder. The term also includes within its scope amounts effective to enhance normal physiological function.

【0045】 For use in therapy, the therapeutically effective amount of the compound is formulated as a pharmaceutical composition for administration. Accordingly, the invention further provides a pharmaceutical composition comprising a therapeutically effective amount of any or its mixture of these compounds disclosed herein, and one or more pharmaceutically acceptable carriers.

【0046】 For the purpose of delivery and absorption, a therapeutically effective amount of the active ingredient according to the present invention may be formulated into a pharmaceutical composition in a suitable form with a pharmaceutically acceptable carrier. Based on the routes of administration, the pharmaceutical composition of the present invention comprises preferably from 0.1% to 100% in weight of the total weight of the active ingredient.

【0047】 The term "pharmaceutically acceptable carrier" used herein refers to a carrier(s), diluent(s) or excipient(s) that is acceptable, in the sense of being compatible with the other ingredients of the formulation and not deleterious to the subject to be administered with the pharmaceutical composition. Any carrier, diluent or excipient commonly known or used in the field may be used in the invention, depending to the requirements of the pharmaceutical formulation. Said carrier may be a diluent, vehicle, excipient, or matrix to the active ingredient. Some examples of appropriate excipients include lactose, dextrose, sucrose, sorbose, mannose, starch, Arabic gum, calcium phosphate, alginates, tragacanth gum, gelatin, calcium silicate, microcrystalline cellulose, polyvinyl pyrrolidone, cellulose, sterilized water, syrup, and methylcellulose. The composition may additionally comprise lubricants, such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preservatives, such as methyl and propyl hydroxybenzoates; sweeteners; and flavoring agents.

【0048】 The composition of the present invention can provide the effect of rapid, continued, or delayed release of the active ingredient after administration to the patient. According to the invention, the pharmaceutical composition may be adapted for administration by any appropriate route, including but not limited to oral, rectal, nasal, topical, vaginal, or parenteral route (such as intramuscular, intravenous, subcutaneous, and intraperitoneal), transdermal, suppository, and intranasal methods.

【0049】 Regarding parenteral administration, it is preferably used in the form of a sterile water solution, which may comprise other substances, such as salts or glucose sufficient to make the solution isotonic to blood. The water solution may be appropriately buffered

(preferably with a pH value of 3 to 9) as needed. Preparation of an appropriate parenteral composition under sterile conditions may be accomplished with standard pharmacological techniques well known to persons skilled in the art.

【0050】 In one particular example of the invention, the pharmaceutical composition is formulated for oral administration. Such formulations may be prepared by any method known in the art of pharmacy. According to the present invention, the form of said composition may be tablets, pills, powder, lozenges, packets, troches, elixers, suspensions, lotions, solutions, syrups, soft and hard gelatin capsules, suppositories, sterilized injection fluid, and packaged powder.

【0051】 In the invention, the method and composition/pharmaceutical composition are effective in treating a virus infection through an inhibition of a cysteine protease in a virus, particularly an RNA-dependent virus. Accordingly, the invention also provides a method and composition/pharmaceutical composition for treatment and/or prevention of a virus infection through inhibition of a cysteine protease in a virus, comprising using the compounds disclosed herein or pharmaceutically acceptable salt thereof.

【0052】 Exemplified viruses which are responsive include, without limitation, a coronavirus, and a HIV. Preferably, the viral infection is a coronavirus. More preferably, the viral infection is a SARS, MERS and SARS-COV-2.

【0053】 In another aspect, the present invention provides a method for treating or preventing an RNA-dependent virus infection through inhibiting a cysteine protease in a virus. One example of the virus is an RNA-dependent virus, such as SARS, MERS and SARS-COV-2; particularly SARS-COV-2.

【0054】 In one further aspect, the present invention provides a composition/pharmaceutical composition for treating and/or preventing a virus infection through inhibiting a cysteine protease in a virus, which comprises any of the compounds disclosed herein, pharmaceutically acceptable salt thereof, or its mixture. Optionally, the composition/ pharmaceutical composition may comprise at least one additional anti-viral therapeutic agent.

【0055】 In one further aspect, the present invention provides a use of any of the compounds disclosed herein for manufacturing a medicament for treating or preventing a virus infection through inhibiting a cysteine protease in a virus.

【0056】 The present invention is further illustrated by the following examples, which are provided for the purpose of demonstration rather than limitation.

【0057】 **Examples**

【0058】 Materials and Methods

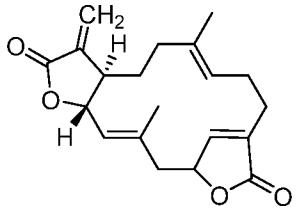
【0059】 **I. FRET protease assays with the SARS-CoV-2 3CLpro**

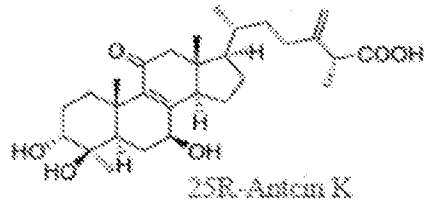
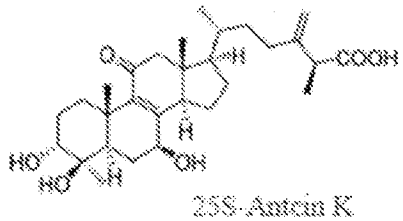
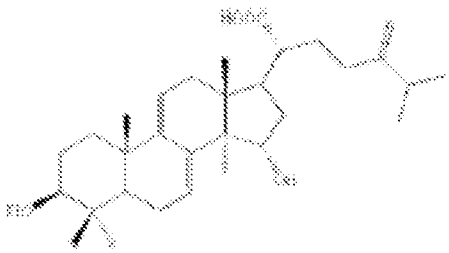
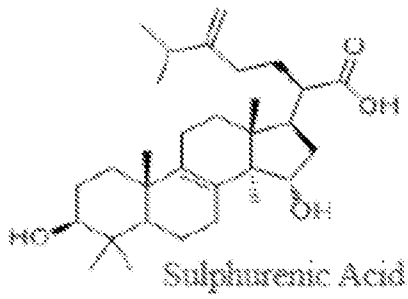
【0060】 The establishment of an ED-FRET platform follows the protocol given by Jo *et al.* (2020). Briefly, a custom proteolytic, fluorogenic peptide with DABCYL and EDANS on ends, DABCYL-TSAVLQSGFRKMG-EDANS (Genomics, Taiwan), contains the consensus nsp4/nsp5 cleavage sequence that can be recognized by 3CLpro of SARS-CoV-2. The peptide is dissolved in distilled water and incubated with 3CLpro of SARS-CoV-2. Measurements of the spectral-based fluorescence are determined by a SPARK® multimode microplate reader provided by TECAN. The proteolytic activity is determined at 37°C by fluorescent intensity of EDANS upon peptide hydrolysis as a function of time, in which $\lambda_{excitation}$ = 340 nm, $\lambda_{emission}$ = 490 nm, bandwidths = 9, 15 nm, respectively. Prior to the assay, the emission wavelength of the testing drugs at 340 nm excitation is examined to ensure that it does not overlap with the emission spectrum of EDANS.

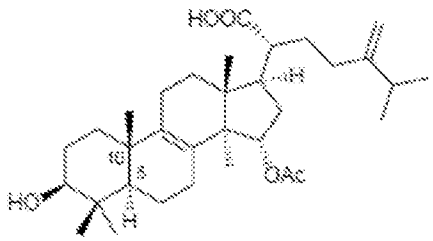
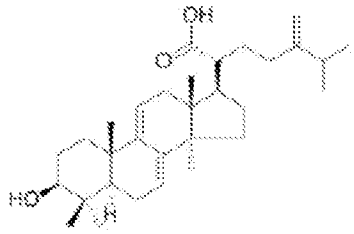
【0061】 Assays are conducted in triplicate in black 96-well microplates (Greiner) in 100 μL assay buffers containing 3CLpro of SARS-CoV-2 and the customized peptide. In SARS 3CLpro assay, 1 μM SARS-CoV-2 3CLpro containing 50 mM Tris pH 6.5 is incubated with 5 μM fluorescent substrate at 37°C for 3 h before measuring Relative Fluorescence Unit (RFU).

【0062】 **II. Inhibition assays in the presence of the compounds according to the invention**

【0063】 There are five example compounds (AR100-DS1, AR101-DS1, AR101-DS2, AR101-DS3, and AR101-DS4) in the first screening process at 20 μM (n=3). The five compounds are given in the table below.

Compound No.	Compound Name and Structure
AR100-DS1	

	Ovatodiolide
AR101-DS1	 25R-Antcin K  25S-Antcin K
AR101-DS2	 dehydrosulphurenic acid  Sulphurenic Acid

AR101-DS3	 Verticipoxic acid D
AR101-DS4	 Dehydrobetulonic Acid

【0064】 At first, the SARS-CoV-2 3CLpro and Arjil drugs will be mixed and pre-incubate at 37°C for 1 h. Those manifesting inhibitory activity against 3CLpro of SARS-CoV-2 will be investigated further at different concentrations to characterize their IC50 values, using GraphPad Prism 7.03 (GraphPad Software, San Diego, CA, USA).

【0065】 Based the knowledge and sequence-based SARS-CoV-2 3CLpro, the efficacy of 3CLpro inhibiting the compounds according to the invention were assessed *in vitro* to determine their therapeutic potential in SARS-CoV-2 treatment. Concerning that no drug or vaccine has yet been approved to treat human SARS-CoV-2 infection, developing a broad-spectrum antiviral agent to combat against SARS-CoV-2 is of utmost importance and urgency. Enactment of ED-FRET technology and its workflow provides a robust, high-throughput drug discovery in the lab. Meanwhile, identification of SARS-CoV-2 3CLpro inhibiting agents from the 5 example compounds according to the invention acts as guidelines of probable therapeutic doses in clinical assessment and prompts patent application in the future, contributing to antiviral library construction.

【0066】 **III. Results**

【0067】 **1. Identification of SARS-CoV-2 3CLpro inhibitors**

【0068】 To determine the efficacy of the five example compounds according to the invention, SARS-CoV-2 3CLpro, IQF peptide substrate, and the example compounds were incubated in the constructed ED-FRET platform at 0.5 μM, 5 μM and 20 μM, respectively. As shown in Figure 1, all five of the example compounds substantially inhibited the peptide-cleaving activity of SARS-CoV-2 3CLpro. Among these five example compounds, AR101 DS2 and AR101 DS3 manifested the utmost inhibitory effect upon at 20 μM. In particular, the SARS-CoV-2 3CLpro inhibition at the percentage of about 50% was found after three-hour treatment of AR101 DS3, while AR101 DS2 demonstrated an inhibition by 40% in the same condition, marking their potentials in combating the polyprotein processing cycle of SARS-CoV-2.

【0069】 **2. Characterization of the half maximal inhibitory concentration of inhibitors**

【0070】 The inhibitory profiles of AR100-DS1, AR101 DS1, AR101 DS2, AR101 DS3 and AR101 DS4 at the concentration of 20 μM were determined and given in Figure 1. As given in Figure 1, the half maximal inhibitory concentration (IC) toward SARS-CoV-2 3CLpro was characterized by treating the compounds at the indicated concentrations ranging from 0 μM to 200 μM. The IC50 values of AR101 DS2 and AR101 DS3 were given in Figures 2A-2E. As shown in Figure 2A, AR101 DS2 had an IC50 value of 39 μM in the presence of 0.5 μM SARS-CoV-2 3CLpro and 5 μM IQF peptide substrate (FP). Meanwhile, the inhibition of 0.125 μM AR101 DS2 on SARS-CoV-2 3CLpro and 1.25 μM IQF peptide substrate was determined (see Figure 2B), the IC50 value of AR101 DS2 against SARS-CoV-2 situated at 15.82 μM. As for the IC50 value of AR101 DS3, 50% SARS-CoV-2 3CLpro inhibition was reached at 14.61 μM in 0.5 μM SARS-CoV-2 3CLpro and 5 μM IQF peptide substrate (see Figure 2C), at 11.06 μM in 0.25 μM SARS-CoV-2 3CLpro and 2.5 μM IQF peptide substrate (see Figure 2D), or at 11.7 μM in 0.125 μM SARS-CoV-2 3CLpro and 1.25 μM IQF peptide substrate (see Figure 2E).

【0071】 The IC50 values of AR100 DS1 were given in Figure 3. AR100 DS1 had an IC50 value of 21.31 μM in the presence of 0.125 μM SARS-CoV-2 3CLpro and 1.25 μM IQF peptide substrate.

【0072】 Given the above, all the example compounds including AR100 DS1, AR101 DS1, AR101 DS2, AR101 DS3 and AR101 DS4 were confirmed to have the inhibitory effect on SARS-CoV-2 3CLpro highlights their therapeutic potentials against SARS-CoV-2, particularly AR100 DS1, AR101 DS2 and AR101 DS3.

【0073】 All publications, patents, and patent documents cited herein above are incorporated by reference herein, as though individually incorporated by reference.

【0074】 The invention has been described with reference to various specific and preferred embodiments and techniques. However, one skilled in the art will understand that many variations and modifications may be made while remaining within the spirit and scope of the invention.

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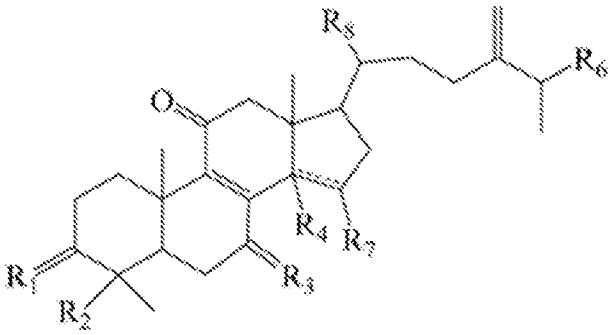
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CLAIMS

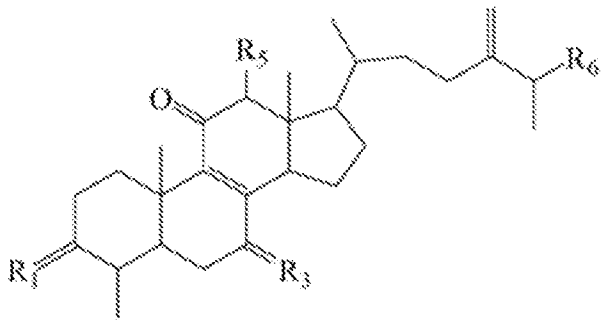
What is claimed is:

1. A method for inhibiting a coronavirus infection comprising administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of a compound or pharmaceutically acceptable salt thereof, or its mixture, in which the compound is selected from the group consisting of:

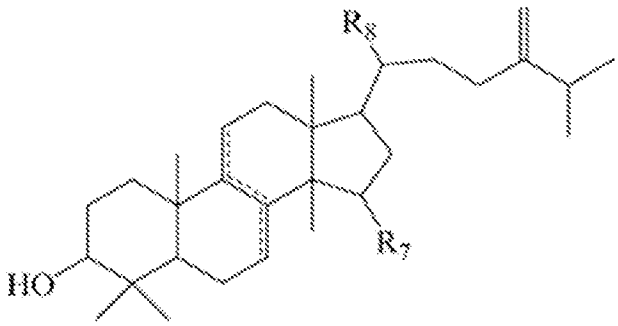
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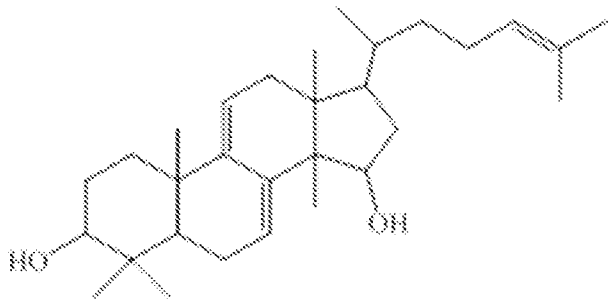
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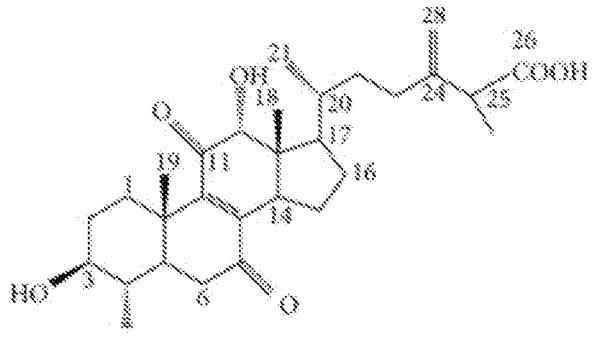
(Ib),



(Ic),



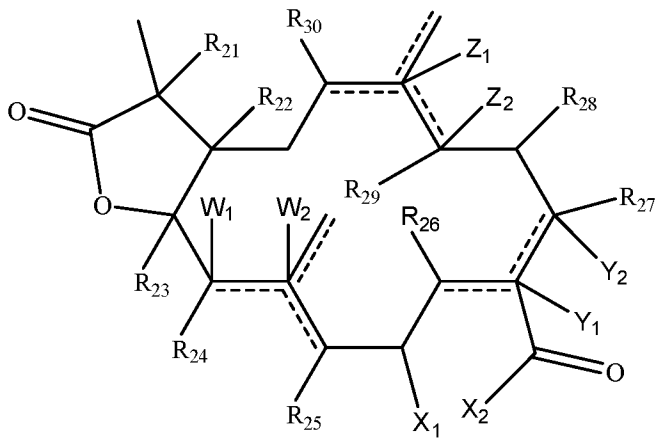
(Id), or



(Ie);

wherein R_1 is O, α-OH or β-H; R_2 is H or OH; R_3 is O, α-H, β-OH, β-OAc or H_2; R_4 is H or OH; R_5 is H, OH or ORx; R_6 is COORx or $COO(CH_2)n\text{-}CH_3$; n is an integer from 0-3; R_7 is H, Oh, ORx or OAc; R_8 is CH_3 or COORx; R_{21} is CH_3, COORx, or $COO(CH_2)n\text{-}CH_3$; n is an integer from 0-3; the dotted line represents a single bond or a double bond, Rx is H or a $C_{1\text{-}8}$ alkyl; and

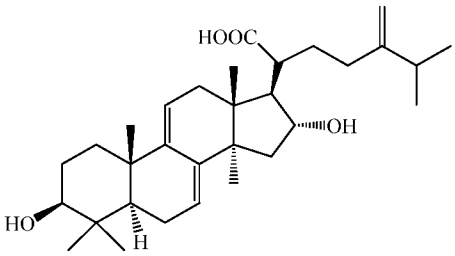
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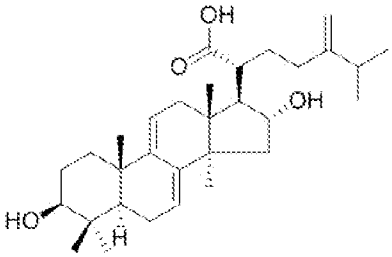
(II);

wherein each of R_{21}, R_{22}, R_{23}, R_{24}, R_{25}, R_{26}, R_{27}, R_{28}, R_{29}, R_{30}, X_1 and X_2 is H, OH, $C_{1\text{-}8}$ alkyl, NRx, SRx, ORx, pyrazoline, cysteine, glutathione, halogen, COORx, or $COO(CH_2)n\text{-}CH_3$; n is an integer from 0-3; each of Y_1, Y_2, Z_1, Z_2, W_1 and W_2 is H, OH, $C_{1\text{-}8}$ alkyl, or X_1 and X_2 together form -O-, or Y_1 and Y_2, Z_1 and Z_2, or W_1 and W_2 together form an epoxy.

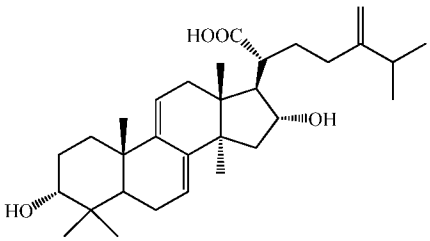
2. The method of claim 1, wherein the compound of formula (I) is selected from the group consisting of:



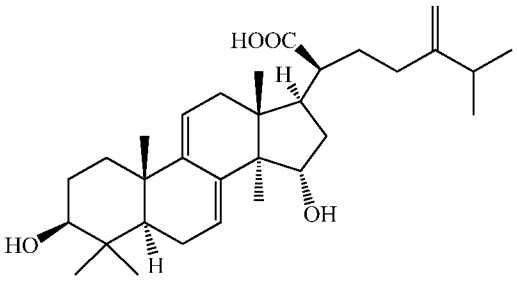
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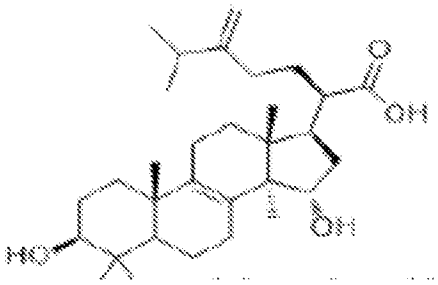
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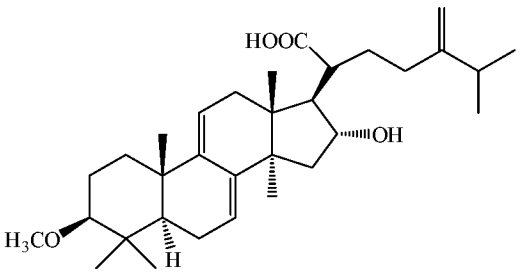
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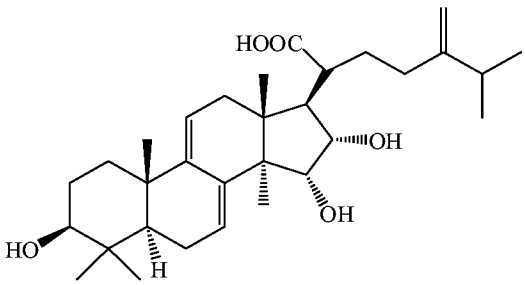
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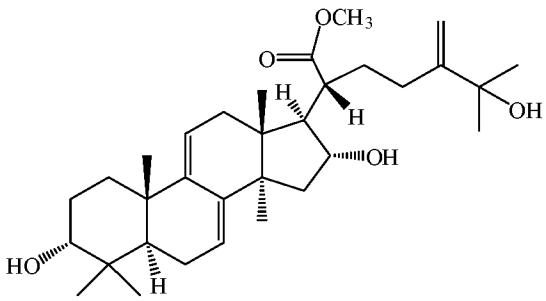
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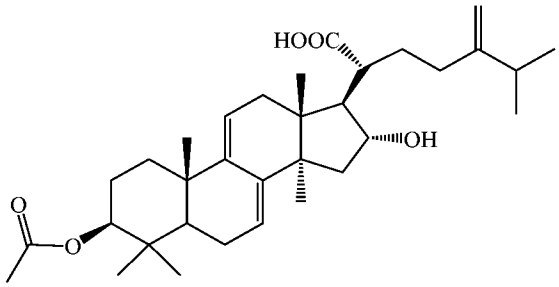
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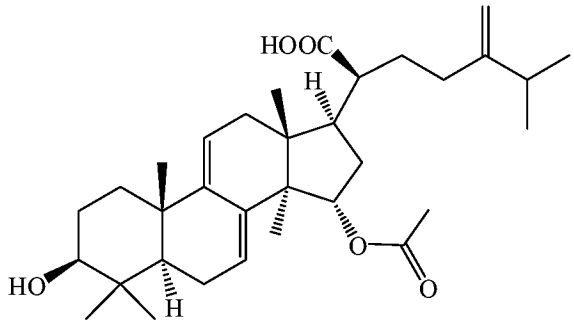
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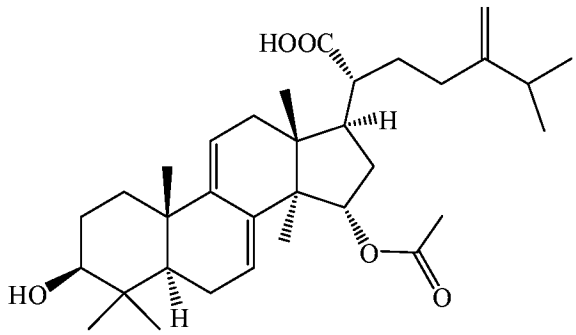
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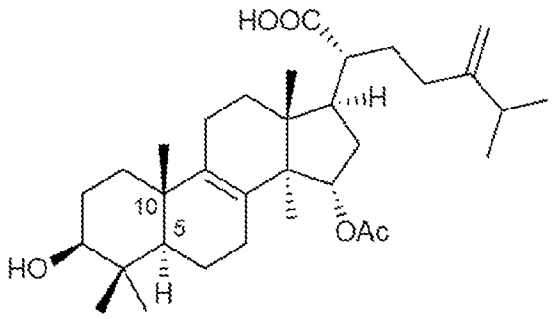
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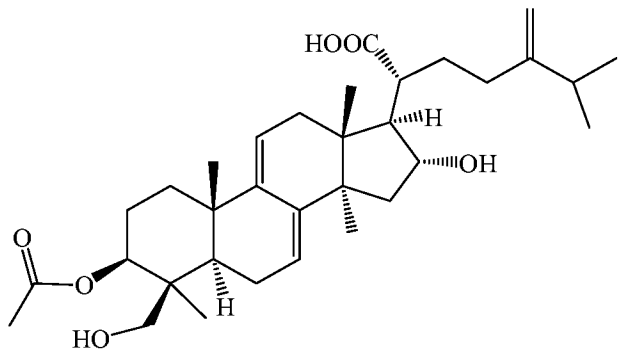
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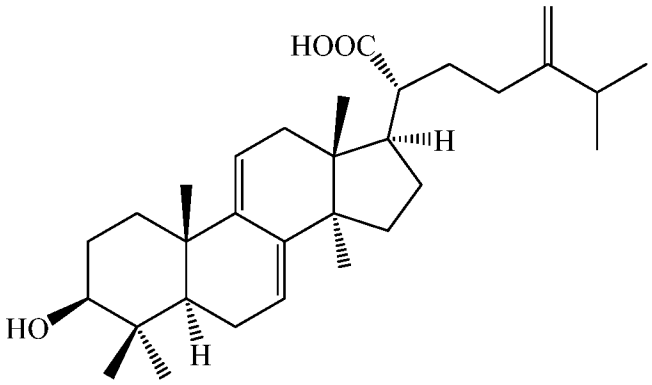
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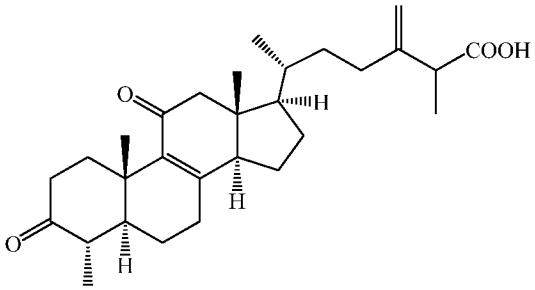
(versisponic acid D),



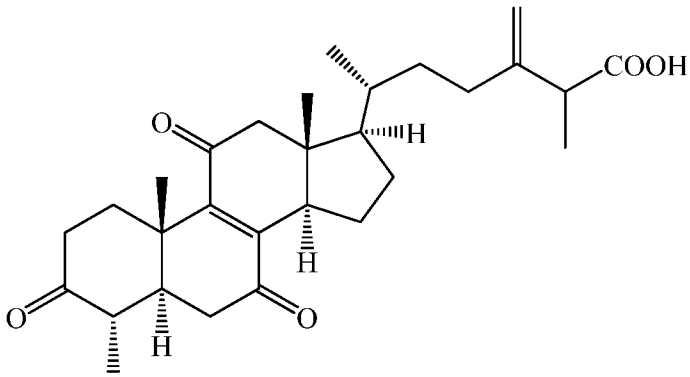
(29-hydroxydehydropachymic acid; (3β,16α)-3-(acetyloxy)-16,29-dihydroxy-24-methylidenelanosta-7,9(11)-dien-21-oic acid),



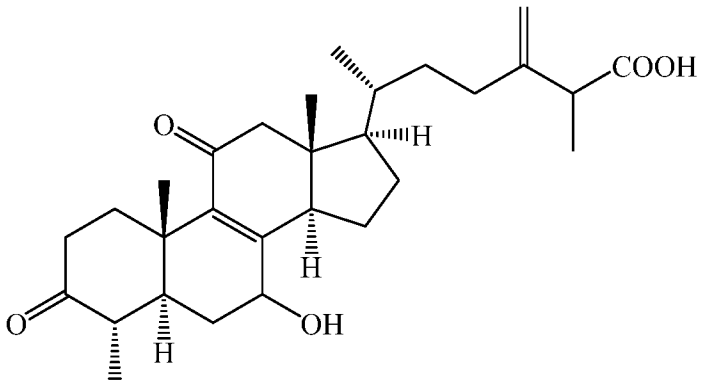
(dehydroeburicoic acid),



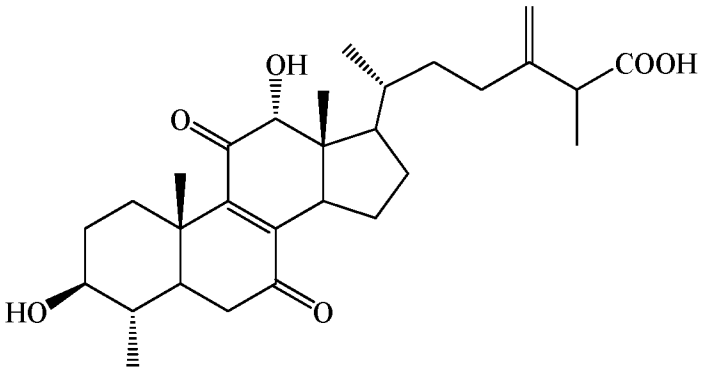
(antcin A),



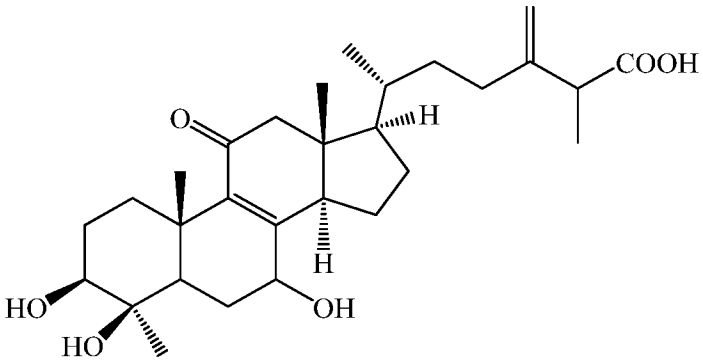
(antcin B),



(antcin C),

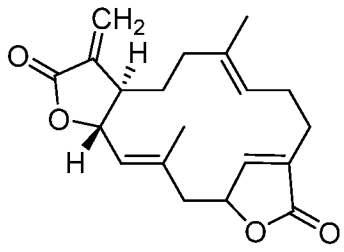


(antcin H), and

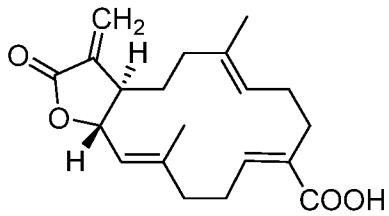


(antcin K).

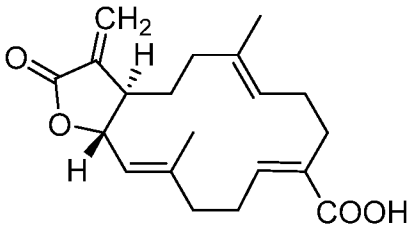
3. The method of claim 1, in which the compound of formula (II) is selected from the group consisting of



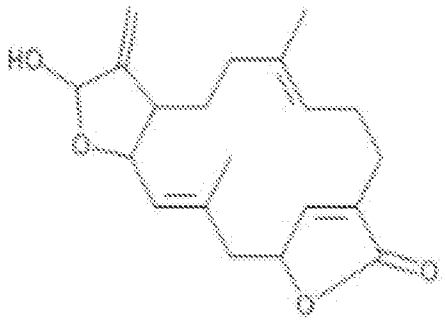
(ovatodiolide),



(ovatodiolide acid),

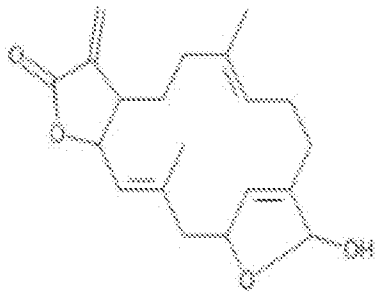


(anisomelic acid),



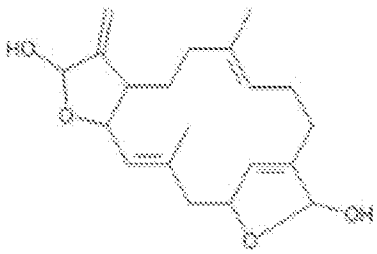
Modification 1

,



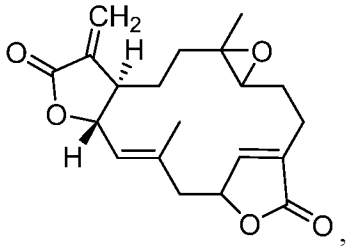
Modification 2

,

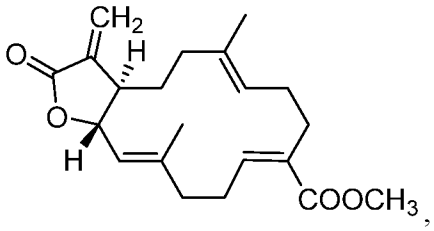


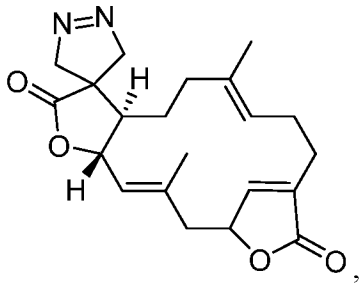
Modification 3

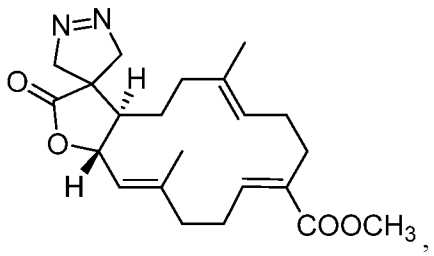
,

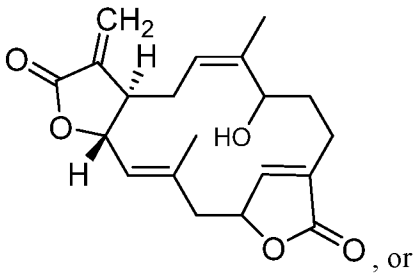


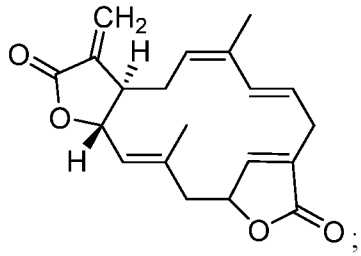
,

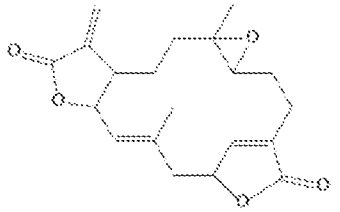




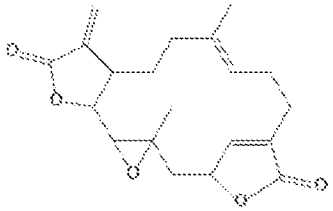



, or

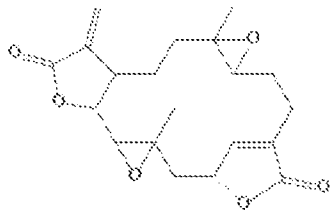

;



;

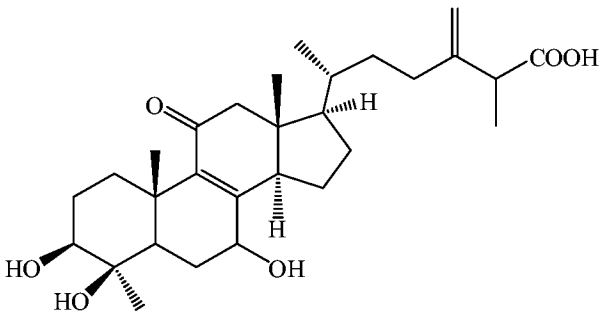


; and

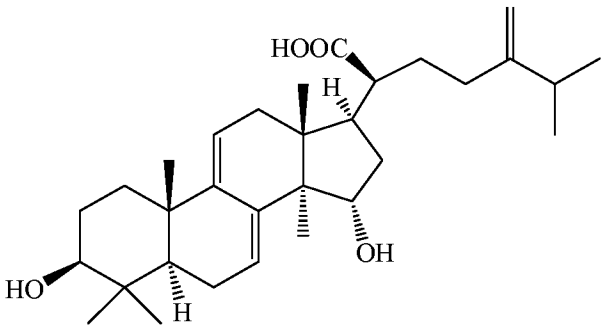


.

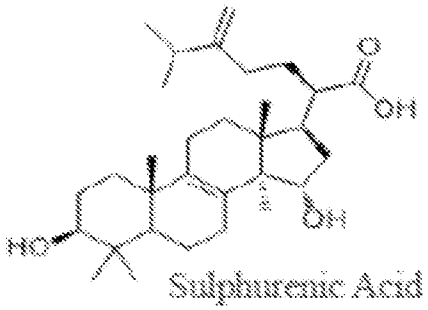
4. The method of claim 1, in which the compound is selected from the group consisting of



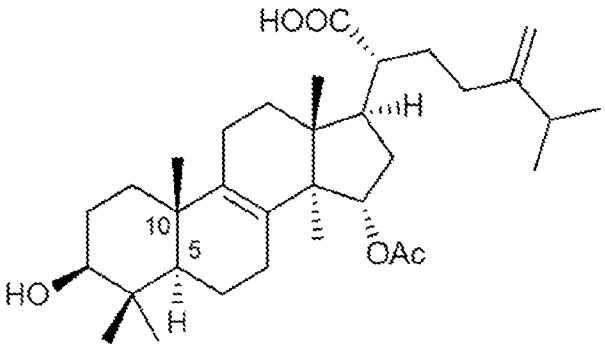
(antcin K);



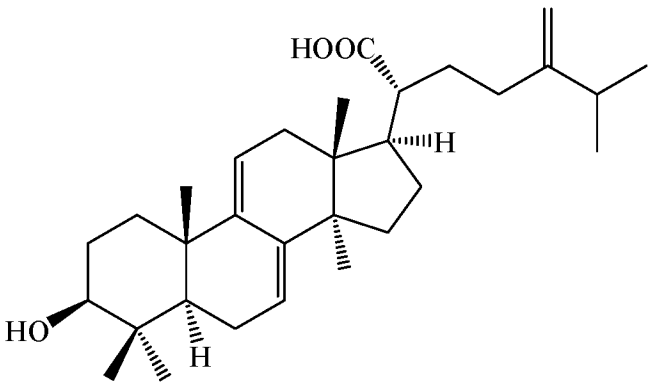
(dehydrosulphurenic acid),



(sulphurenic acid),



(versisponic acid D), and



(dehydroeburicoic acid).

5. The method of claim 1, wherein the coronavirus is selected from the group consisting of severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and SARS-COV-2.

6. The method of claim 1, wherein the coronavirus is SARS-COV-2.

7. The method of claim 1, in which the compound is effective in inhibiting a cysteine protease in a virus.

8. The method of any of claims 1 to 7, further comprising administering at least one additional anti-virus therapeutic agent.

9. A method for treating or preventing a virus infection through inhibiting a cysteine protease in a virus comprising administering to a subject in need thereof a composition/pharmaceutical composition comprising a compound or pharmaceutically acceptable salt, or its mixture, in which the compound is the compound as defined in any of claims 1 to 4, or pharmaceutically acceptable salt, or its mixture at the effective amount to inhibiting a cysteine protease in a virus.

10. A composition/pharmaceutical composition for treating or preventing a virus infection comprising a compound as defined in any of claims 1 to 4, or pharmaceutically acceptable salt, or mixture thereof, at the effective amount to inhibiting a cysteine protease in a virus.

11. The composition / pharmaceutical composition of claim 10, wherein the virus is an RNA-dependent virus.

12. The composition / pharmaceutical composition of claim 11, wherein the RNA-dependent virus is a coronavirus.

13. The composition / pharmaceutical composition of claim12, wherein the coronavirus is SARS, MERS or SARS-CoV-2.

14. The composition / pharmaceutical composition of claim12, wherein the coronavirus is SARS-CoV-2.

15. A use of the compound as defined in any of claims 1 to 4, pharmaceutically acceptable salt, or its mixture for manufacturing a medicament for preventing or treating a virus infection through inhibiting a cysteine protease in a virus.

16. The use of claim 15, wherein the virus is an RNA-dependent virus.

17. The use of claim 16, wherein the RNA-dependent virus is a coronavirus.

18. The use of claim17, wherein the coronavirus is SARS, MERS or SARS-CoV-2.

19. The use of claim17, wherein the coronavirus is SARS-CoV-2.

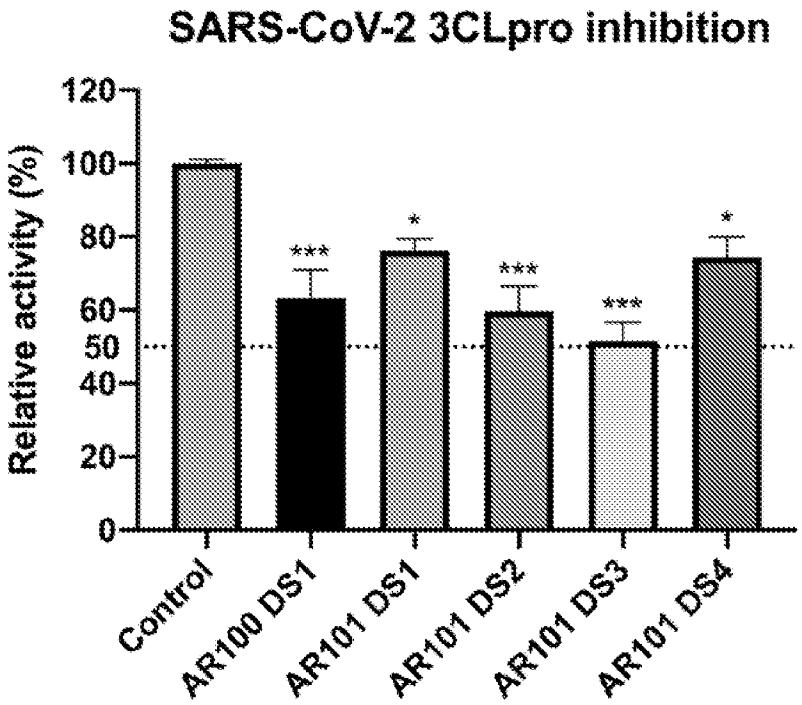


Figure 1

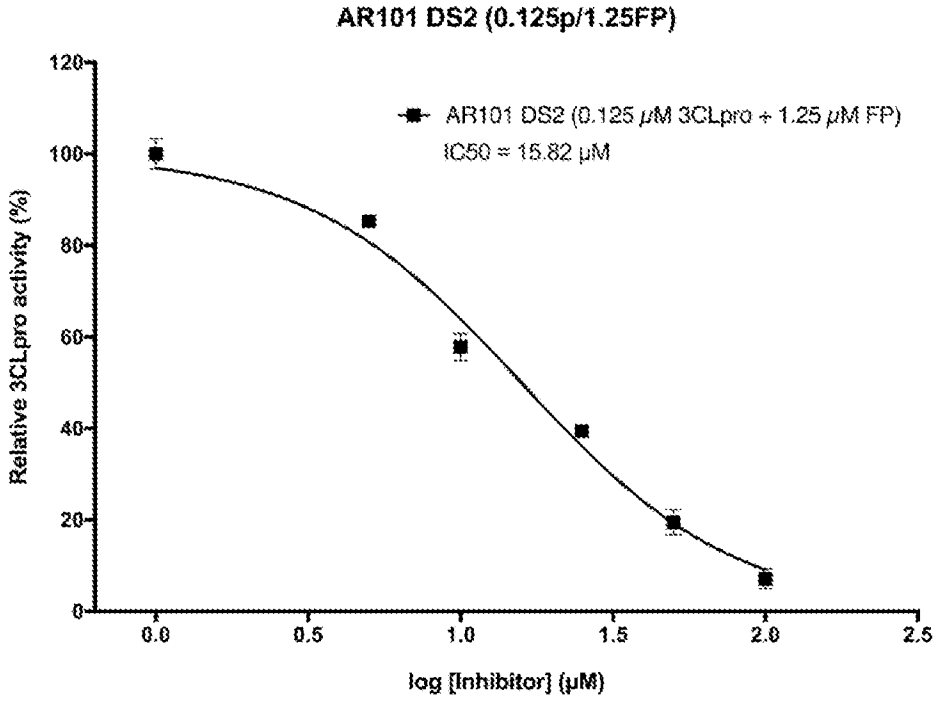


Figure 2A

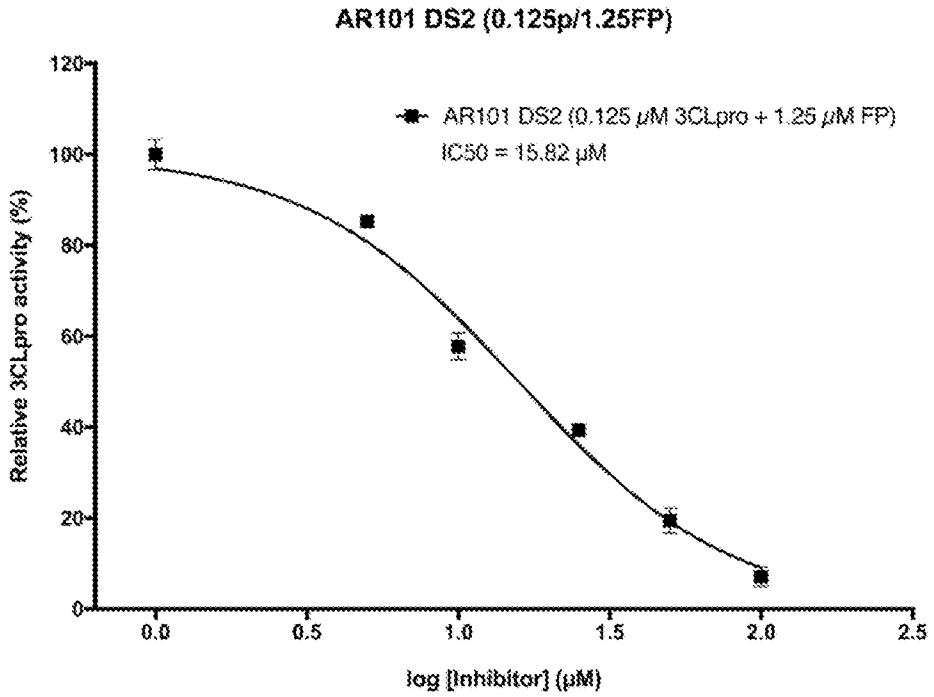


Figure 2B

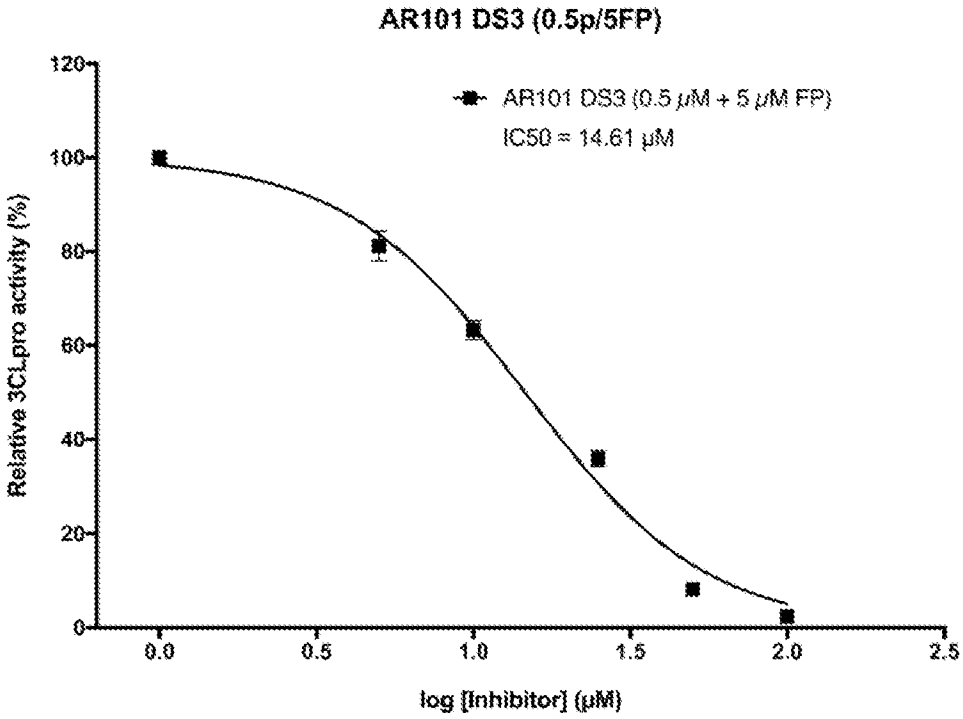


Figure 2C

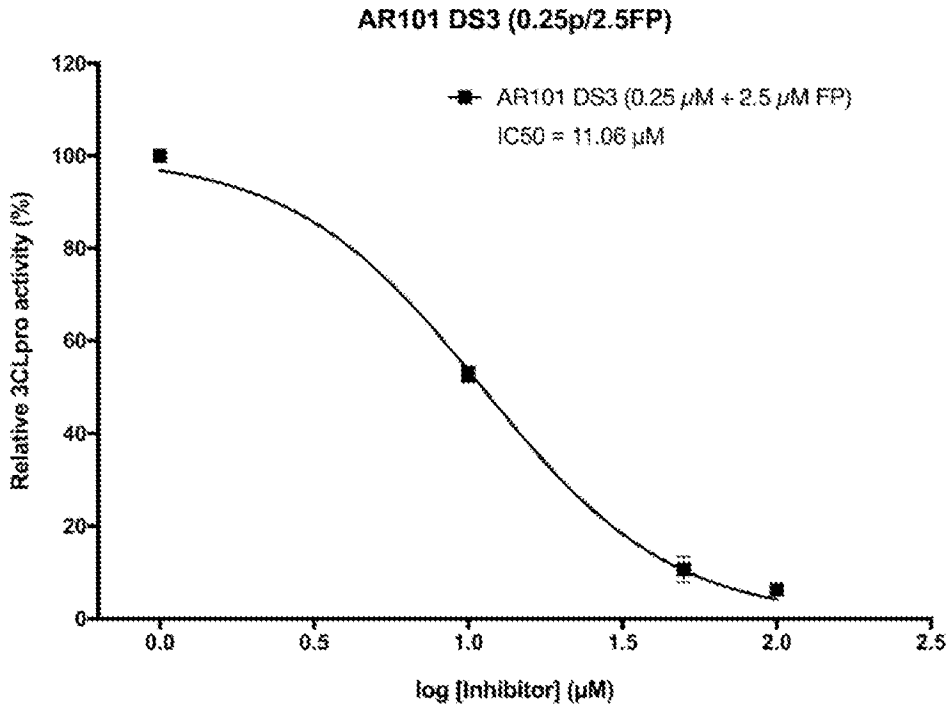


Figure 2D

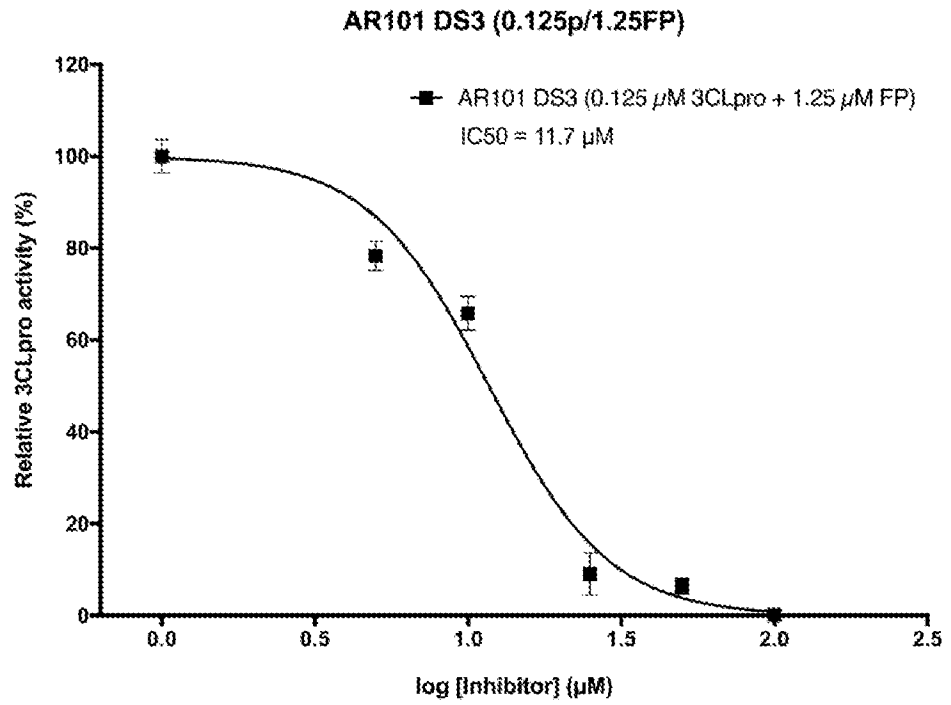


Figure 2E

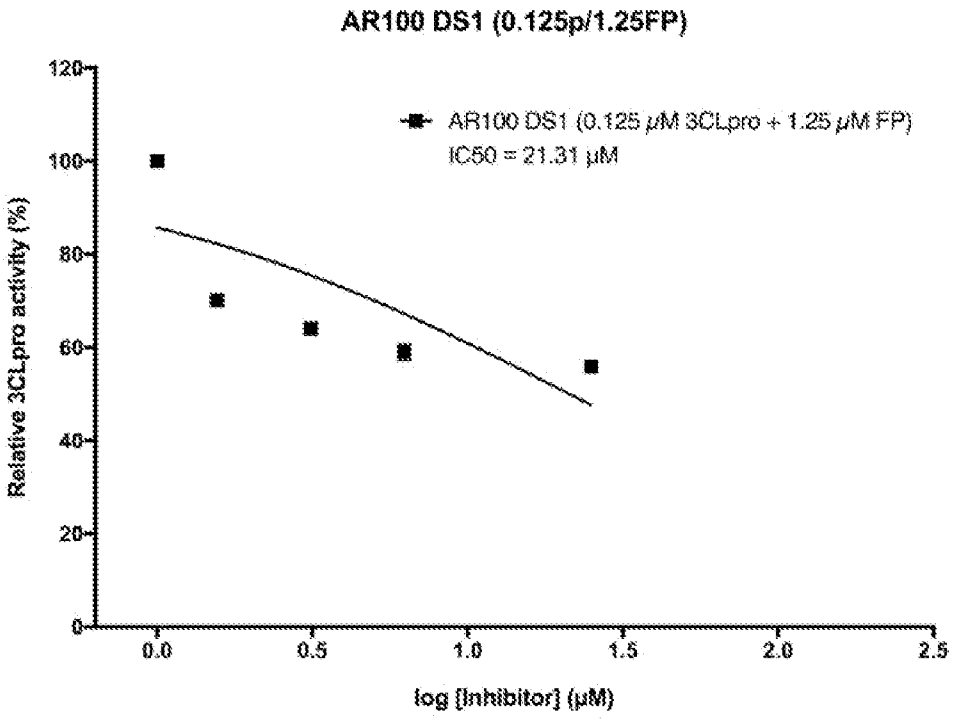

AR100 DS1 (0.125p/1.25FP)
AR100 DS1 (0.125 μM 3CLpro + 1.25 μM FP)
IC50 = 21.31 μM
Relative 3CLpro activity (%)
120
100
80
60
40
20
0
0.0
0.5
1.0
1.5
2.0
2.5
log [Inhibitor] (μM)

Figure 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/029840

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/357; A61K 31/575; A61P 31/14; C07J 9/00 (2021.01)
CPC - A61K 31/357; A61K 31/575; A61P 31/14; C07J 9/00; C07J 9/005 (2021.08)

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.
Y	US 2014/0105928 A1 (STAMETS) 17 April 2014 (17.04.2014) entire document	1, 2, 5-19
Y	US 2010/0210869 A1 (WU et al) 19 August 2010 (19.08.2010) entire document	1, 2, 5-19
Y	WU et al., Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods, Acta Pharm Sin B, PMC 27 February 2020 [retrieved on 07 June 2021]. Retrieved from the Internet: <URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7102550/> entire document	6, 14, 19
A	YANG et al., Antrodia camphorata Potentiates Neuroprotection against Cerebral Ischemia in Rats via Downregulation of iNOS/HO-1/Bax and Activated Caspase-3 and Inhibition of Hydroxyl Radical Formation, Evidence-Based Complementary and Alternative Medicine, Vol. 2015, 2015, Pgs. 1-8	1, 2, 5-19
A	US 2017/0226150 A1 (KAOHSIUNG MEDICAL UNIVERSITY) 10 August 2017 (10.08.2017) entire document	1, 2, 5-19
A	US 2005/0148504 A1 (KATUNUMA et al) 07 July 2005 (07.07.2005) entire document	1, 2, 5-19
P, X	US 2020/0268771 A1 (ARJIL BIOTECH HOLDING COMPANY LIMITED) 27 August 2020 (27.08.2020) entire document	1, 2, 5-19

☐ 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	Date of mailing of the international search report
06 August 2021	SEP 01 2021
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Harry Kim Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/029840

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.:
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 extra sheet(s).

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, 2, 5-19

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/US2021/029840

Continued from Box No. III Observations where unity of invention is lacking

Claims 1, 2, and 5-19 have been analyzed subject to the restriction that the claims read on a compound having formula (Ia) or pharmaceutically acceptable salt thereof, or its mixture, wherein R1 is O; R2 is H; R3 is O; R4 is H; R6 is COORX, wherein RX is H; R7 is H; and R8 is CH3.

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 examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-19 are drawn to compounds, or pharmaceutically acceptable salt thereof, or its mixture, comprising formula (Ia), (Ib), (Ic), (Id), (Ie), or (II), methods for inhibiting a coronavirus infection thereof, methods for treating or preventing a virus infection through inhibiting a cysteine protease in a virus thereof, compositions for treating or preventing a virus infection thereof, and uses of a compound for manufacturing a medicament thereof.

The first invention of Group I+ is restricted to a compound having formula (Ia) or pharmaceutically acceptable salt thereof, or its mixture, wherein R1 is O; R2 is H; R3 is O; R4 is H; R6 is COORX, wherein RX is H; R7 is H; and R8 is CH3; methods for inhibiting a coronavirus infection thereof; methods for treating or preventing a virus infection through inhibiting a cysteine protease in a virus thereof; compositions for treating or preventing a virus infection thereof; and uses of a compound for manufacturing a medicament thereof. It is believed that claims 1, 2, and 5-19 read on this first named invention and thus these claims will be searched without fee to the extent that they read on the above embodiment.

Applicant is invited to elect additional formula(e) for each additional compound to be searched in a specific combination by paying an additional fee for each set of election. Each additional elected formula(e) requires the selection of a single definition for each compound variable. An exemplary election would be a compound having formula (Ia) or pharmaceutically acceptable salt thereof, or its mixture, wherein R1 is O; R2 is OH; R3 is O; R4 is H; R6 is COORX wherein RX is H; R7 is H; and R8 is CH3; methods for inhibiting a coronavirus infection thereof; methods for treating or preventing a virus infection through inhibiting a cysteine protease in a virus thereof; compositions for treating or preventing a virus infection thereof; and uses of a compound for manufacturing a medicament thereof. Additional formula(e) will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the " + " group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ 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:

The Groups I+ formulae do not share a significant structural element requiring the selection of alternatives for the compound variables R1, R2, R3, R4, R6, R7, R8, and accordingly these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features of a compound having the core structure of formula (Ia), (Ib), (Ic), (Id), (Ie), or (II), a method for inhibiting a coronavirus infection thereof, a method for treating or preventing a virus infection through inhibiting a cysteine protease in a virus thereof, a composition for treating or preventing a virus infection thereof, and use of a compound for manufacturing a medicament thereof, these shared technical features do not represent a contribution over the prior art as disclosed by US 2017/0226150 A1 to Kaohsiung Medical University (hereinafter, " Kaohsiung ") and US 2005/0148504 A1 to Katunuma et al. (hereinafter, " Katunuma ").

Kaohsiung teaches a compound having the core structure of formula (Ia) (Pg. 4, compound of formula IX).

Katunuma teaches a method for inhibiting a coronavirus infection comprising administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of a compound (Paras. [0093]-[0094], diseases associated with cysteine protease are treated by administration of the cysteine protease inhibitor of the present invention to a subject, wherein the cysteine protease inhibitor is administered in an amount between 0.1-1200 mg/kg/day and the disease is a coronavirus infection); a method for treating or preventing a virus infection through inhibiting a cysteine protease in a virus comprising administering to a subject in need thereof (Paras. [0093]-[0094], diseases associated with cysteine protease are treated by administration of the cysteine protease inhibitor of the present invention to a subject, wherein the cysteine protease inhibitor is administered in an amount between 0.1-1200 mg/kg/day and the disease is a coronavirus infection); a composition/pharmaceutical composition comprising a compound at the effective amount to inhibiting a cysteine protease in a virus (Paras. [0093]-[0094], diseases associated with cysteine protease are treated by administration of a pharmaceutical composition comprising the cysteine protease inhibitor of the present invention to a subject, wherein the cysteine protease inhibitor is administered in an amount between 0.1-1200 mg/kg/day and the disease is a coronavirus infection); and use of a compound for manufacturing a medicament for preventing or treating a virus infection through inhibiting a cysteine protease in a virus (Paras. [0093]-[0094], diseases associated with cysteine protease are treated by administration of a pharmaceutical composition (medicament) comprising the cysteine protease inhibitor of the present invention to a subject, wherein the cysteine protease inhibitor is administered in an amount between 0.1-1200 mg/kg/day and the disease is a coronavirus infection).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.