

Compounds for use in inflammatory conditions

FIELD OF THE INVENTION

5 The present invention relates to the treatment of inflammation, and in particular, inflammation associated with or as a result of activation of Toll-like receptors. The invention also relates to the treatment of pathogen-induced inflammation.

BACKGROUND TO THE INVENTION

10 Inflammation is one of the main responses of the immune system to tissue damage and infections, and involves the major immune cells and extracellular mediators and regulators, such as cytokines.

In the case of infection, inflammation is triggered when the innate immune system is activated. Innate immune cells, such as macrophages, dendritic cells and neutrophils are all important players of innate immunity and infection. These cells express PRRs (pattern recognition receptors) that detect microbial components, the so-called pathogen-associated molecular patterns (PAMPS). One class of PRRs are the Toll-like receptors (TLRs, of which there are 10 sub-types TLR1-10), which can detect various pathogens from viruses, bacteria, protozoa and fungi. For example, TLRs can detect bacterial lipopolysaccharide, lipoproteins, flagellin, bacterial CpG-DNA and viral single and double-stranded RNA. After recognition of PAMPS, PRRs are activated and trigger an intracellular signalling cascade ultimately resulting in the expression of pro-inflammatory cytokine molecules. One signalling cascade results in the activation of the canonical NF- κ B pathway, which is the pivotal regulator of inflammation and the central mediator of pro-inflammatory gene induction. Activation of NF- κ B transduction is responsible for the transcriptional induction of pro-inflammatory cytokines, chemokines and additional inflammatory mediators in different types of immune cells. These inflammatory mediators can both directly engage in the induction of inflammation and act indirectly through promoting the differentiation of inflammatory T cells.

30 However, although the generation of a potent immune response is a crucial part of a host's response to a pathogen infection, excessive or inappropriate inflammation – e.g. hyperinflammation can be harmful. Indeed, excessive inflammation plays an important role in the pathogenesis of many infectious diseases. In one example, excessive inflammation following pathogen infection leads to hypercytokinemia, commonly known as a cytokine storm. A cytokine storm results from an uncontrolled and excessive release of pro-inflammatory cytokines. This sudden and excessive release of cytokines is particularly harmful to the host, and can lead to multi-system organ failure and death. In particular, acute lung injury (ALI) is a common consequence of a cytokine storm in the lung alveolar environment and systemic circulation. Pathogen induced lung injury can progress into ALI or the more severe form – called acute respiratory distress syndrome (ARDS), which is seen with SAR-CoV-2 infections. Hypercytokinemia can also result from a number of infectious agents, as well as non-infectious hosts.

40 In addition, pathogen infection also leads to the activation of macrophages. Activated macrophages are twice the size of resting macrophages and are more “aggressive”, having

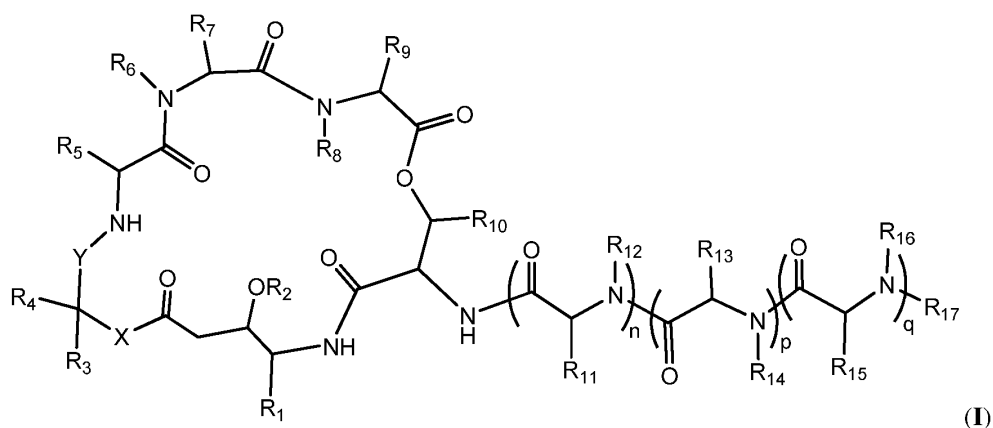
increased levels of lysosomal proteins and a greater ability to phagocytose. Classically activated macrophages also release proteases, neutrophil chemotactic factors, reactive oxygen species and pro-inflammatory cytokines (such as IL-1 beta/IL-1F2, IL-6, and TNF-alpha/TNFSF1A), leading to inflammation and tissue destruction. As such, macrophage effector function significantly affects the quality, duration and magnitude of an inflammatory response, and while this response is important for host defence, when uncontrolled, activated macrophages can cause significant tissue damage. As such, over-activation of macrophages are a key contributor to disorders characterised by excessive inflammation. Most notably, in Wang et al., 2020 it was concluded that activated alveolar macrophages are central to the cytokine storm caused by SARS-CoV-2 infection. For this reason alone, methods to reduce levels of macrophage activation and/or recruitment are also key to preventing and treating inflammation arising from pathogen infection, and in particular, hypercytokinemia.

As such, inflammation and cytokine storms are a common clinical feature in serious and lethal infections by a number of viral infections. These include respiratory viruses such as Orthomyxoviridae. They also include infections by single stranded (+) RNA viruses, including members of the families Flaviviridae, Picornaviridae, Togaviridae, Caliciviridae, Roniviridae, Retroviridae and Coronaviridae. In the majority of deadly infections, the agent responsible for the lethality is not the cytolytic activity of the virus, but the immunopathological response of the host. Among the ss (+) RNA viruses that cause serious illnesses in human and animals of economic importance: Flaviviridae, Picornaviridae, Togaviridae, Caliciviridae, Retroviridae, Coronaviridae, Orthomyxoviridae, Phlebovirus, Arenaviridae and Herpesviridae are particularly associated with inflammation and cytokine storms.

As such, there is a need to provide new treatments for inflammation, and in particular, excessive inflammation or hyperinflammation such as that which arises as a result of pathogen infection. The present invention address this need.

SUMMARY OF THE INVENTION

In one aspect, the present invention is directed to a compound of general formula I, or a pharmaceutically acceptable salt or stereoisomer thereof,



wherein X is selected from O and NH;

Y is selected from CO and $-\text{COCH}(\text{CH}_3)\text{CO}-$;

5 each n and p is independently selected from 0 and 1, and q is selected from 0, 1 and 2;

each R1, R3, R5, R9, R11, and R15 is independently selected from hydrogen, substituted or unsubstituted C1-C6 alkyl, substituted or unsubstituted C2-C6 alkenyl, and substituted or unsubstituted C2-C6 alkynyl;

10 R2 is selected from hydrogen, CORa, COORa, substituted or unsubstituted C1-C6 alkyl, substituted or unsubstituted C2-C6 alkenyl, and substituted or unsubstituted C2-C6 alkynyl;

each R4, R8, R10, R12, and R16 is independently selected from hydrogen and substituted or unsubstituted C1-C6 alkyl;

15 each R7 and R13 is independently selected from hydrogen, substituted or unsubstituted C1-C6 alkyl, substituted or unsubstituted C2-C6 alkenyl, and substituted or unsubstituted C2-C6 alkynyl; each R6 and R14 is independently selected from hydrogen and substituted or unsubstituted C1-C6 alkyl; or R6 and R7 and/or R13 and R14 together with the corresponding N atom and C atom to which they are attached may form a substituted or unsubstituted heterocyclic group;

20 R17 is selected from hydrogen, CORa, COORa, CONHRb, COSRc, $(\text{C}=\text{NRb})\text{ORa}$, $(\text{C}=\text{NRb})\text{NHRb}$, $(\text{C}=\text{NRb})\text{SRc}$, $(\text{C}=\text{S})\text{ORa}$, $(\text{C}=\text{S})\text{NHRb}$, $(\text{C}=\text{S})\text{SRc}$, SO2Rc, SO3Rc, substituted or unsubstituted C1-C12 alkyl, substituted or unsubstituted C2-C12 alkenyl, substituted or unsubstituted C2-C12 alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group, with the proviso that when n, p, and q are 0 then R17 is not hydrogen; and

25 each Ra, Rb, and Rc is independently selected from hydrogen, substituted or unsubstituted C1-C12 alkyl, substituted or unsubstituted C2-C12 alkenyl, substituted or unsubstituted C2-C12 alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group;

for use in the treatment of inflammation.

In one aspect, the present invention is directed to a compound as defined above (and herein), for use in the treatment of inflammation associated with activation of Toll-like receptors.

30 In another aspect, the present invention is directed to a compound as defined above (and herein), for use in the treatment of a disorder selected from acute respiratory syndrome (ARDS), pneumonia and immunopathology, and in particular hypercytokinemia (cytokine storm syndrome), sepsis and graft-versus-host disease.

35 In one aspect, the present invention is directed to a compound as defined above (and herein), for use in the treatment of pathogen-induced inflammation. In one embodiment, the pathogen is a bacteria or virus.

In one aspect, the present invention is directed to a compound as defined above (and herein), for

use in the combined treatment of inflammation associated with activation of Toll-like receptors or inflammation associated with pathogen-induced inflammation and in the treatment of a viral infection.

5 In one aspect, the present invention is directed to a compound as defined above (and herein), for use as an anti-inflammatory and an antiviral.

In a particular aspect, the compound of general formula I is PLD, or a pharmaceutically acceptable salt or stereoisomer thereof.

In a particular aspect, the compound of general formula I is DidemninB, or a pharmaceutically acceptable salt or stereoisomer thereof.

10 In another aspect, the present invention is also directed to a pharmaceutical composition comprising a compound as defined herein, and a pharmaceutically acceptable carrier, for use according to the present invention.

In another aspect, the present invention is directed to the use of a compound as defined herein, in the manufacture of a medicament for the treatment of inflammation.

15 In another aspect, the present invention is directed to the use of a compound as defined herein, in the manufacture of a medicament for the treatment of inflammation associated with activation of Toll-like receptors.

20 In another aspect, the present invention is directed to the use of a compound as defined herein, in the manufacture of a medicament for the treatment of a disorder selected from pneumonia, acute respiratory syndrome (ARDS), hypercytokinemia (cytokine storm syndrome), sepsis and graft-versus-host disease.

In another aspect, the present invention is directed to the use of a compound as defined herein, in the manufacture of a medicament for the treatment of pathogen-induced inflammation.

25 In another aspect, the present invention is directed to the use of a compound as defined herein, in the manufacture of a medicament for the combined treatment of inflammation associated with activation of Toll-like receptors or inflammation associated with pathogen-induced inflammation and in the treatment of a viral infection.

In another aspect, the present invention is directed to the use of a compound as defined herein, in the manufacture of a medicament for use as an anti-inflammatory and an antiviral.

30 In another aspect, the present invention is directed to a method for treating inflammation in any mammal, preferably a human, wherein the method comprises administering to an individual in need thereof a therapeutically effective amount of a compound as defined herein.

35 In another aspect, the present invention is directed to a method for treating any mammal, preferably a human, for inflammation associated with activation of Toll-like receptors, wherein the method comprises administering to an individual in need thereof a therapeutically effective amount of a compound as defined herein.

In another aspect, the present invention is directed to a method for treating any mammal, preferably a human, for a disorder selected from pneumonia, acute respiratory syndrome (ARDS),

hypercytokinemia (cytokine storm syndrome), sepsis and graft-versus-host disease, wherein the method comprises administering to an individual in need thereof a therapeutically effective amount of a compound as defined herein.

5 In another aspect, the present invention is directed to a method for treating any mammal, preferably a human, for pathogen-induced inflammation, wherein the method comprises administering to an individual in need thereof a therapeutically effective amount of a compound as defined herein.

10 In another aspect, the present invention is directed to a method of combined treatment of any mammal, preferably a human, for inflammation associated with activation of Toll-like receptors or inflammation associated with pathogen-induced inflammation and a viral infection, wherein the method comprises administering to an individual in need thereof a therapeutically effective amount of a compound as defined herein.

15 In another aspect, the present invention is directed to a method of anti-inflammatory and antiviral treatment, wherein the method comprises administering to an individual in need thereof a therapeutically effective amount of a compound as defined herein.

20 In a further aspect of the invention, there is provided a kit comprising the compound as defined herein, together with instructions for treating inflammation; for treating inflammation associated with activation of Toll-like receptors; for treating a disorder selected from pneumonia, hypercytokinemia (cytokine storm syndrome), sepsis and graft-versus-host disease; for treating pathogen-induced inflammation; for the combined treatment of inflammation associated with activation of Toll-like receptors or inflammation associated with pathogen-induced inflammation and in the treatment of a viral infection; or for use as an anti-inflammatory and an antiviral. In a further embodiment, the kit may also comprise instructions for treating a viral infection, preferably a SARS-CoV or SARS-CoV-2 infection.

25 The following embodiments apply to all aspects of the present invention.

The pathogen may be a virus. The Toll-like receptors may be activated by the virus. Preferably, the virus is a RNA virus. More preferably, the virus is selected from. Flaviviridae, Picornaviridae, Togaviridae, Caliciviridae, Retroviridae, Coronaviridae, Orthomyxoviridae, Phlebovirus and Arenaviridae. Alternatively, the virus is a dsDNA virus, preferably selected from Herpesviridae.

30 Alternatively or additionally, the virus is a respiratory virus.

R₃ and R₄ may be independently selected from hydrogen and substituted or unsubstituted C₁-C₆ alkyl. R₃ may be isopropyl and R₄ may be hydrogen. R₃ and R₄ may be methyl (this compound is also designated a compound of general formula **II**).

35 R₁₁ may be selected from hydrogen and substituted or unsubstituted C₁-C₆ alkyl. R₁₁ may be methyl or isobutyl. R₁₁ may be methyl and n=1 (this compound is also designated a compound of general formula **III**).

R₁, R₅, R₉, and R₁₅ may be independently selected from hydrogen and substituted or unsubstituted C₁-C₆ alkyl. R₁ may be selected from sec-butyl and isopropyl, R₅ may be isobutyl, R₉ may be p-methoxybenzyl, and R₁₅ may be selected from methyl and benzyl.

R₈, R₁₀, R₁₂, and R₁₆ may be independently selected from hydrogen and substituted or unsubstituted C₁-C₆ alkyl. R₈, R₁₀ and R₁₂ may be methyl, and R₁₆ may be hydrogen.

R₆ and R₁₄ may be independently selected from hydrogen and substituted or unsubstituted C₁-C₆ alkyl. R₆ may be selected from hydrogen and methyl, and R₁₄ may be hydrogen.

- 5 R₇ and R₁₃ may be independently selected from hydrogen and substituted or unsubstituted C₁-C₆ alkyl. R₇ may be methyl and R₁₃ may be selected from hydrogen, methyl, isopropyl, isobutyl, and 3-amino-3-oxopropyl.

R₆ and R₇ and/or R₁₃ and R₁₄ together with the corresponding N atom and C atom to which they are attached may form a substituted or unsubstituted pyrrolidine group.

- 10 R₂ may be selected from hydrogen, substituted or unsubstituted C₁-C₆ alkyl, and COR_a, and wherein R_a may be a substituted or unsubstituted C₁-C₆ alkyl. R₂ may be hydrogen.

- 15 R₁₇ may be selected from hydrogen, COR_a, COOR_a, CONHR_b, (C=S)NHR_b, and SO₂R_c, and wherein each R_a, R_b, and R_c may be independently selected from substituted or unsubstituted C₁-C₆ alkyl, substituted or unsubstituted C₂-C₆ alkenyl, substituted or unsubstituted C₂-C₆ alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group. R₁₇ may be selected from hydrogen, COObenzyl, CObenzo[b]thiophen-2-yl, SO₂(p-methylphenyl), COCOCH₃ and COOC(CH₃)₃.

X may be NH. X may be O. Y may be CO. Y may be -COCH(CH₃)CO-.

- 20 The compound may be PLD, or pharmaceutically acceptable salts or stereoisomers thereof. The compound may be PLD.

The compound may be didemninB, or pharmaceutically acceptable salts or stereoisomers thereof. The compound may be didemninB.

The inflammation may be due to COVID-19.

The CoV infection may be mild infection; and/or moderate infection; and/or severe infection.

- 25 The CoV infection may be acute CoV infection, preferably wherein the CoV infection is acute COVID-19 infection; and/or may be ongoing symptomatic CoV infection, preferably wherein the CoV infection is ongoing symptomatic COVID-19 infection; and/or may be post-CoV syndrome, CoV persistent or long CoV; preferably wherein the CoV infection is post-COVID-19 syndrome, COVID persistent or long COVID. The post-CoV syndrome, CoV persistent or long CoV may include one or more symptoms arising from the cardiovascular, respiratory, gastrointestinal, neurological, musculoskeletal, metabolic, renal, dermatological, otolaryngological, haematological and autonomic systems; psychiatric problems, generalised pain, fatigue and/or persisting fever.

- 35 The use may include use in the treatment of a patient with signs and symptoms of CoV infection (preferably COVID-19) for up to 4 weeks; and/or from 4 weeks to 12 weeks; and/or for more than 12 weeks.

The use may include use in the prophylaxis, reduction or treatment of COVID persistent, long COVID or post-COVID syndrome; preferably wherein the prophylaxis, reduction or treatment

minimises the likelihood that a patient suffers from COVID persistent, long COVID or post-COVID syndrome symptoms; and/or reduces the severity of such symptoms; further preferably wherein the treatment minimising the symptoms of CoV infection.

- 5 The use may reduce the infectivity of CoV patients; including wherein the patient is asymptomatic or not very symptomatic yet has a high viral load. The use may reduce the occurrence of supercontagators (asymptomatic or not very symptomatic patients with high viral loads (e.g. TC <25)).

The use may reduce complications associated with pathogen infection, including hospitalization, ICU and death. The pathogen is preferably a CoV infection.

- 10 The use may be in the prophylaxis, reduction or treatment of COVID persistent (also known as long COVID or post-COVID syndrome).

The compound may be administered in combination with a corticosteroid, preferably dexamethasone. The compound and corticosteroid may be administered concurrently, separately or sequentially.

- 15 The compound may be administered according to a regimen of a once daily dose for 10 days, 9 days, 8 days, 7 days, 6 days, 5 days, 4 days, 3 days, 2 days or 1 day; preferably 2-5 days, 3-5 days, or 3, 4 or 5 days; most preferably 3 days or 5 days; most preferably 3 days.

- 20 The compound may be administered at a dose of 5mg a day or less, 4.5mg a day or less, 4mg a day or less, 3.5mg a day or less, 3mg a day or less, 2.5mg a day or less or 2mg a day or less; 0.5 mg/day, 1 mg/day, 1.5 mg/day, 2 mg/day, 2.5 mg/day, 3 mg/day, 3.5 mg/day, 4 mg/day, 4.5 mg/day, or 5mg/day; preferably 1 mg/day, 1.5 mg/day, 2 mg/day or 2.5 mg/day; preferably 1.5-2.5 mg/day; further preferably 1.5 mg/day, 2 mg/day or 2.5 mg/day.

- 25 The compound may be administered at a total dose of 1-50 mg, 1-40 mg, 1-30 mg, 1-20 mg, 1-15 mg, 3-15 mg, 3-12 mg, 4-12 mg, 4-10 mg, or 4.5-10 mg; 4mg, 4.5mg, 5mg, 5.5mg, 6mg, 6.5mg, 7mg, 7.5mg, 8mg, 8.5mg, 9mg, 9.5mg or 10mg; preferably 4.5mg, 5mg, 6mg, 7.5mg, 8mg, 9mg or 10mg; more preferably 4.5-7.5 mg/day. The total dose may be split over 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 days, preferably 3 days or 5 days; most preferably 3 days.

The compound may be administered at a once daily dose for 3 days at a dose of 1.5-2.5mg/day. The dose may be 1.5mg/day. The dose may be 2.5mg/day.

- 30 The compound may be PLD administered as a 1.5-hour infusion, once a day for 3 consecutive days. 1.5 mg of PLD may be administered as a 1.5-hour infusion, once a day for 3 consecutive days. 2 mg of PLD may be administered as a 1.5-hour infusion, once a day for 3 consecutive days. 2.5 mg of PLD may be administered as a 1.5-hour infusion, once a day for 3 consecutive days. 1 mg of PLD may be administered as a 1.5-hour infusion, once a day for 5 consecutive days. 2 mg of PLD may be administered as a 1.5-hour infusion, once a day for 5 consecutive days.
- 35

- 40 The regimen may be a single dose (1 day). The compound may be administered as a single dose of 1-10 mg, 4-10 mg, 4.5-10 mg; 4mg, 4.5mg, 5mg, 5.5mg, 6mg, 6.5mg, 7mg, 7.5mg, 8mg, 8.5mg, 9mg, 9.5mg or 10mg; preferably 4.5mg, 5mg, 6mg, 7.5mg, 8mg, 9mg or 10mg; more preferably 5-9 mg, 6.5-8.5 mg, 7-8 mg or 7.5 mg. The compound may be PLD administered as a single dose 1.5-hour infusion.

The single dose regimen may be utilised with all therapies set out in the present invention. The combination use with corticosteroids (including subsequent corticosteroid administration) may in embodiments be used with the single dose regimen. The multi-day regimen may be utilised with all therapies set out in the present invention.

- 5 The corticosteroid may be administered daily on the same day(s) as administering a compound according to the present invention. The corticosteroid may be administered on one or more subsequent days. The corticosteroid may be administered on 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more subsequent days. The corticosteroid may be administered at a higher dose when administered on the same day(s) as a compound according to the present invention and at a lower dose on
10 subsequent days. The corticosteroid may be dexamethasone.

- The compound according to the present invention may be administered at a dose according to the present invention on days 1-3 of the dosage regimen. The corticosteroid may be administered intravenously on days 1-3 of the dosage regimen. The corticosteroid may thereafter be administered by oral administration or IV from Day 4 and up to Day 10 (as per physician
15 judgement according to patient clinical condition and evolution). The corticosteroid may be dexamethasone. The dose may be 6.6 mg/day IV on Days 1 to 3 (for example 8 mg dexamethasone phosphate), followed by dexamethasone 6 mg/day (for example 7.2 mg dexamethasone phosphate or 6 mg dexamethasone base) oral administration or IV from Day 4 and up to Day 10.

- In embodiments, dexamethasone is dexamethasone phosphate and is administered at a dose of 8
20 mg/day IV on Days 1 to 3, followed by dexamethasone 7.2 mg/day oral administration or IV from Day 4 and up to Day 10.

The compound according to the present invention may be administered as an infusion, preferably a 1 hour infusion, a 1.5 hour infusion, a 2 hour infusion, a 3 hour infusion or longer; particularly preferably a 1.5 hour infusion.

- 25 The regimen may be 1.5 mg of plitidepsin administered as a 1.5-hour infusion, once a day for 3 consecutive days; or 2 mg of plitidepsin administered as a 1.5-hour infusion, once a day for 3 consecutive days; or 2.5 mg of plitidepsin administered as a 1.5-hour infusion, once a day for 3 consecutive days; or 1 mg of plitidepsin administered as a 1.5-hour infusion, once a day for 5 consecutive days; or 2 mg of plitidepsin administered as a 1.5-hour infusion, once a day for 5
30 consecutive days.

The regimen may be 7.5 mg of plitidepsin administered as a 1.5-hour infusion, as a single dose on day 1.

The compound according to the present invention may be administered using a loading dose and a maintenance dose.

- 35 The regimen according to the present invention may be:

a loading dose of 2.5 mg for day 1, and followed by a maintenance dose of 2 mg/day for subsequent days;

a loading dose of 2.5 mg for day 1, and followed by a maintenance dose of 1.5 mg/day for subsequent days;

- a loading dose of 2.5 mg for day 1, and followed by a maintenance dose of 1 mg/day for subsequent days;
- a loading dose of 2.5 mg for day 1, and followed by a maintenance dose of 0.5 mg/day for subsequent days;
- 5 a loading dose of 2 mg for day 1, and followed by a maintenance dose of 1.5 mg/day for subsequent days;
- a loading dose of 2 mg for day 1, and followed by a maintenance dose of 1 mg/day for subsequent days;
- 10 a loading dose of 2 mg for day 1, and followed by a maintenance dose of 0.5 mg/day for subsequent days;
- a loading dose of 1.5 mg for day 1, and followed by a maintenance dose of 1 mg/day for subsequent days;
- a loading dose of 1.5 mg for day 1, and followed by a maintenance dose of 0.5 mg/day for subsequent days; or
- 15 a loading dose of 1 mg for day 1, and followed by a maintenance dose of 0.5 mg/day for subsequent days.
- The compound according to the present invention may be administered in combination with a corticosteroid. The corticosteroid may be administered on the same day(s) as administration of the compound.
- 20 The corticosteroid may also be administered on one or more subsequent days. For example, the corticosteroid is administered with the compound on days 1-3 and the corticosteroid is further administered on one or more of days 4-10.
- The corticosteroid may be administered intravenously on days when the compound is administered but administered by oral administration or IV on subsequent days.
- 25 The corticosteroid may be dexamethasone. Dexamethasone may be administered at a dose of 6.6 mg/day IV on days when the compound is administered.
- Dexamethasone may be administered at a dose of 6 mg/day oral administration or IV on subsequent days, preferably one or more of days 4, 5, 6, 7, 8, 9 and 10.
- 30 The dexamethasone dose as defined herein refers to the base weight. The dose can therefore be adjusted if used in salt form. For example, the dexamethasone may be dexamethasone phosphate such that 8 mg/day is equivalent to 6.6 mg of dexamethasone base, and 7.2 mg/day is equivalent to 6 mg of dexamethasone base.
- 35 The compound according to the present invention, particularly PLD, may be administered 1.5 mg/day intravenous (IV) combined with dexamethasone 6.6 mg/day IV on Days 1 to 3, followed by dexamethasone 6 mg/day oral administration (PO)/IV from Day 4 and up to Day 10 (as per physician judgement according to patient clinical condition and evolution).

The compound according to the present invention, particularly PLD, may be administered 2.0 mg/day intravenous (IV) combined with dexamethasone 6.6 mg/day IV on Days 1 to 3, followed by dexamethasone 6 mg/day oral administration (PO)/IV from Day 4 and up to Day 10 (as per physician judgement according to patient clinical condition and evolution).

- 5 The compound according to the present invention, particularly PLD, may be administered 2.5 mg/day intravenous (IV) combined with dexamethasone 6.6 mg/day IV on Days 1 to 3, followed by dexamethasone 6 mg/day oral administration (PO)/IV from Day 4 and up to Day 10 (as per physician judgement according to patient clinical condition and evolution).

- 10 The corticosteroid may be administered 20 to 30 minutes prior to starting treatment with the compound as defined herein.

In regimens according to the present invention, the patient may additionally receive the following medications, preferably 20 to 30 minutes prior to starting treatment with the compound according to the present invention:

Ondansetron 8 mg IV (or equivalent);

- 15 Diphenhydramine hydrochloride 25 mg IV (or equivalent); and

Ranitidine 50 mg IV (or equivalent).

In regimens according to the present invention, on Days 4 and 5, patients may receive ondansetron (or equivalent) 4 mg twice a day PO.

- 20 When administered as a single dose, patients may receive the following prophylactic medications 20-30 minutes prior to plitidepsin infusion:

- Diphenhydramine hydrochloride 25 mg i.v;
- Ranitidine 50 mg i.v;
- Dexamethasone 6.6 mg intravenously;
- Ondansetron 8 mg i.v. in slow infusion of 15 minutes.

- 25 Ondansetron 4 mg orally may be given every 12 hours for 3 days after plitidepsin administration to relieve drug-induced nausea and vomiting. If plitidepsin is administered in the morning the patient may receive the first dose of ondansetron in the afternoon.

DESCRIPTION OF THE FIGURES

The invention is further described in the following non-limiting figures:

- 30 **Figure 1** shows that NF- κ B transactivation in response to the activation of Toll-like receptors is inhibited by PLD. Human monocytic cells (THP-1) were stably transfected with a NF- κ B-Luc plasmid and (A) levels of NF- κ B transactivation measured in the presence and absence of PLD. (B) Compound-induced cytotoxicity was tested by the MTT cell proliferation assay. Cultures were exposed to PLD at 100nM for 6 hours. RQ – Resiquimod at 10 μ g/mL. LPS-B5 –

Lipopolysaccharide from Escherichia coli 055:B5 (LPS-B5) at 10µg/mL. Poly-C – Polyinosinic-polycytidylic at 500µg/mL. TNF-α was used as a positive control. *** p<0.001; ** p<0.01

Figure 2 shows that Plitidepsin negatively regulates TLR-triggered cytokine secretion. NF-κB transactivation in response to the activation of Toll-like receptors leads to increased secretion of the pro-inflammatory cytokines: IL-1(a), IL-6 (b), IL-8 (c) and TNF-α (d). Cultures were exposed to PLD at 100nM or DMSO for 6 hours. At 6 hours post-treatment secreted cytokines were analysed by ELISA. TNF-α was used as a positive control. *** p<0.001; ** p<0.01

Figure 3 shows the ex-vivo down-regulation of cytokines IL-6, IL-10 and TNF-α by PLD.

Figure 4 shows a decrease in classically activated macrophages in LPS-challenged mice.

Figure 5 shows the effects of plitidepsin on alveolar macrophage recruitment in LPS treated mice. Concentration-time curves (mean±SD) of plitidepsin in plasma and lung of mice(a), rats (b) and hamsters (c) after a single intravenous dose at 1.0, 0.2 and 0.2 mg/kg respectively.

Figure 6 - Graphical representation of the antiviral activity (-◆- RLUs) and toxicity (-●- Viability) of several concentrations (µM) of compound 3 in MT-2 cells (**Fig. 6A**) and in preactivated PBMCs (**Fig. 6B**), both infected with a recombinant virus (NL4.3 Luc). Graphical representations are at least mean of two independent experiments for MT-2 cells and four for PBMCs.

Figure 7 - Graphical representation of the antiviral activity (-◆- RLUs) and toxicity (-●- Viability) of several concentrations (µM) of compound 8 in MT-2 cells (**Fig. 7A**) and in preactivated PBMCs (**Fig. 7B**), both infected with a recombinant virus (NL4.3 Luc). Graphical representations are at least mean of two independent experiments for MT-2 cells and four for PBMCs.

Figure 8 - Graphical representation of the antiviral activity (-◆- RLUs) and toxicity (-■- Viability) of several concentrations (µM) of compound 9 in MT-2 cells (**Fig. 8A**) and in preactivated PBMCs (**Fig. 8B**), both infected with a recombinant virus (NL4.3 Luc). Graphical representations are at least mean of two independent experiments for MT-2 cells and four for PBMCs.

Figure 9 - Graphical representation of the antiviral activity (-◆- RLUs) and toxicity (-■- Viability) of several concentrations (µM) of compound 10 in MT-2 cells (**Fig. 9A**) and in preactivated PBMCs (**Fig. 9B**), both infected with a recombinant virus (NL4.3 Luc). Graphical representations are at least mean of two independent experiments for MT-2 cells and four for PBMCs.

Figure 10 - Graphical representation of the antiviral activity (-◆- RLUs) and toxicity (-■- Viability) of several concentrations (µM) of compound 11 in MT-2 cells (**Fig. 10A**) and in preactivated PBMCs (**Fig. 10B**), both infected with a recombinant virus (NL4.3 Luc). Graphical representations are at least mean of two independent experiments for MT-2 cells and four for PBMCs.

Figures 11-15 show fluorescence images showing a) cell growth and b) antiviral activity for DMSO 24 hpi against HCoV-229E infected Huh-7 cells (A1, A2, A3, A4, A5 from Table 2). It can be seen that cells remain viable but that no antiviral effect is seen.

5 **Figures 16-19** show fluorescence images showing a) cell growth and b) antiviral activity for Compound 240 (DidemninB) 24 hpi against HCoV-229E infected Huh-7 cells at 50nM, 5nM and 0.5nM concentrations (B1, B2, B3, B4 from Table 2 respectively). It can be seen that cells remain viable at all concentrations, including high concentrations; and that remarkable antiviral properties are seen across all concentrations, even at sub nano-molar concentrations.

10 **Figures 20-23** show fluorescence images showing a) cell growth and b) antiviral activity for PLD 24 hpi against HCoV-229E infected Huh-7 cells at 50nM, 5nM and 0.5nM concentrations (C1, C2, C3, C4 from Table 2 respectively). Again, it can be seen that cells remain viable at all concentrations, including high concentrations; and that remarkable antiviral properties are seen across all concentrations, even at sub nano-molar concentrations.

15 **Figures 24-26** show fluorescence images showing a) cell growth and b) antiviral activity for Compound 9 24 hpi against HCoV-229E infected Huh-7 cells at 50nM, 5nM and 0.5nM concentrations (D1, D2, D3, from Table 2 respectively). Again, it can be seen that cells remain viable at all concentrations, including high concentrations; and that remarkable antiviral properties are seen across all concentrations, even at sub nano-molar concentrations.

20 **Figures 27-29** show fluorescence images showing a) cell growth and b) antiviral activity for Compound 10 24 hpi against HCoV-229E infected Huh-7 cells at 50nM, 5nM and 0.5nM concentrations (E1, E2, E3, from Table 2 respectively). Again, it can be seen that cells remain viable at all concentrations, including high concentrations; and that remarkable antiviral properties are seen across all concentrations, even at sub nano-molar concentrations.

25 **Figures 30-33** show fluorescence images showing a) cell growth and b) antiviral activity for PLD (second run) 24 hpi against HCoV-229E infected Huh-7 cells at 50nM, 5nM and 0.5nM concentrations (F1, F2, F3, F4 from Table 2 respectively). Again, it can be seen that cells remain viable at all concentrations, including high concentrations; and that remarkable antiviral properties are seen across all concentrations, even at sub nano-molar concentrations.

Figures 34 and 35 show total plasma concentration profiles vs. time predicted for dosing schedules and administration according to the present invention.

Figure 36 shows total plasma concentration profiles vs. time predicted for further dosing schedules and administration according to the present invention.

30 **Figure 37** shows dose response curves showing the antiviral effect of PLD on SARS-CoV-2 in vero cells.

Figure 38 shows dose response curves showing the antiviral effect of PLD on SARS-CoV-2 in vero cells.

35 **Figure 39** shows x-rays showing the effects of PLD administration on a patient with bilateral pneumonia.

Figure 40 shows x-rays showing the effects of PLD administration on a patient with unilateral pneumonia.

Figure 41 shows C-reactive protein tests for patients treated with PLD.

Figure 42 shows the viral load log of Patient 4 (Fig. 42a), Patient 5 (Figure 42b), Patient 6 (Figure

42c) and Patient 7 (Figure 42d). Patients were administered PLD as a 90 minute IV infusion daily for 3 consecutive days (day 1-3) with viral load assessed by PCR at baseline, day 4, day 7, day 15 and day 31.

5 **Figure 43** shows the inflammatory profile in the BALF of mice infected with influenza virus with (PR8) or without (PC) treatment with PLD.

Figure 44 shows the titre of influenza virus in the lung of mice with or without treatment with PLD. The antiviral activity of PLD was quantified by qPCR measuring the mRNA level of viral NP (NP- PR8).

10 **Figure 45** shows a quantitative measurement of the level of immune cell infiltration, particularly AMs (alveolar macrophages) in the BALF of influenza infected mice treated with (PR8) or without (PC) PLD.

15 **Figure 46** shows WNV-GFP infection efficiency in VeroE6 cells. Plitidepsin dose-response curves for infection efficiency (GFP; green line) and cell biomass (DAPI; blue line). Data are shown as average and SEM of two independent experiments performed with four biological replicates (n=8).

Figure 47 shows WNV-GFP infection efficiency in Huh-7 cells. Plitidepsin dose-response curves for infection efficiency (GFP; green line) and cell biomass (DAPI; blue line). Data are shown as average and SEM of two independent experiments performed with four biological replicates (n=8).

20 **Figure 48** shows the impact of plitidepsin on WNV extracellular infectivity titers. Vero-E6 (a) or Huh-7 cells (b) were inoculated with WNV/NY99 at MOI 0.01. Forty-eight hours later, cell supernatants were collected to determine the extracellular infectivity titers by endpoint dilution and immunofluorescence microscopy. Data are expressed in infectious units per volume of supernatant (focus forming units (FFU) /ml) in logarithmic scale and are shown as average and SEM (N=4). Statistical significance was tested using one-way ANOVA and a Dunnet's post hoc test (*p<0.05).

30 **Figure 49** shows the impact of plitidepsin on intracellular WNV RNA levels. Vero-E6 (a) or Huh-7 (b) cells were inoculated with WNV/NY99 at MOI 0.01. Forty-eight hours later, total cellular RNA was subjected to RT-qPCR. Data are expressed as number of genome copies/60ng of total RNA normalized in logarithmic scale using 28S RNA as house-keeping gene and are shown as average and SEM (N=4). Statistical significance was tested using one-way ANOVA and a Dunnet's post hoc test (*p<0.05). n.d., not detected (<1000 copies/reaction).

35 **Figure 50** shows the effect of plitidepsin (APL) pre-treatment at 1nM, 10nM and 50 nM on secretion of the pro-inflammatory cytokines IL6 (a), IL8 (b), IL1 β (c) and TNF- α (d). (e) shows the effect of 1nM, 10nM and 50 nM PLD treatment on cell viability (as a percent of control). At 0 time THP-1 cells were treated with 1nM, 10nM or 50nM APL or DMSO (0.2%) followed by stimulus with Resiquimod at 2.5 or 5 μ g/mL at 8 hours. At 24 hours cytokines or cell viability was measured.

40 **Figure 51** shows the effect of plitidepsin treatment on the production of the pro-inflammatory cytokines, IL-6 (c), IL-10 (d) and TNF- α (e) mediated by LPS-B5 in CD45⁺ cells isolated from broncho-alveolar lavages (BALF). (a) shows the percent of CD45⁺ live cells in control, LPS-B5

and LPS-B5 and PLD treated cells. (b) shows cell survival as a percent of control in LPS-B5 and LPS-B5 and PLD treated cells.

Figure 52 shows the effect of plitidepsin treatment on the production of the pro-inflammatory cytokines TNF- α mediated by Resiquimod.

- 5 **Figure 53** shows total vs. plasma concentration profiles for single dose plitidepsin 7.5 mg and 1.5, 2.0 and 2.5 mg on day 1 to 3, using a 1.5 hour infusion.

DETAILED DESCRIPTION OF THE INVENTION

The following embodiments apply to all aspects of the present invention.

- 10 The present invention will now be further described. In the following passages, different aspects of the invention are defined in more detail. Each aspect so defined may be combined with any other aspect or aspects or embodiment or embodiments unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous.

- 15 In the present application, a number of general terms and phrases are used, which should be interpreted as follows.

- 20 “Treat”, “treating”, and “treatment” in the context of a viral infection may refer to one or more of the following: 1) reduction in the number of infected cells; 2) reduction in the number of virions present in the serum, including reduction in viral titre (which can be measured by qPCR); 3) inhibition (i.e., slowing to some extent, preferably stopping) the rate of viral replication; 4) reduction in the viral RNA load; 5) reduction in the viral infectivity titre (the number of virus particles capable of invading a host cell); and 6) relieving or reducing to some extent one or more of the symptoms associated with the viral infection. This may include inflammation associated with viral infection.

- 25 “Patient” includes humans, non-human mammals (e.g., dogs, cats, rabbits, cattle, horses, sheep, goats, swine, deer, and the like) and non-mammals (e.g., birds, and the like). The patient may require hospitalisation for management of inflammation and/or infection.

- 30 Plitidepsin (PLD) is a cyclic depsipeptide originally isolated from the marine tunicate *Aplidium albicans*. PLD is also known as Aplidin or Aplidine. Such terms are used interchangeably herein. PLD analogues are those analogues as defined herein as compounds of Formula I, II or III. In a preferred embodiment, the present invention relates to the use of PLD.

We have found that PLD

- 35 (i) inhibits transactivation of NF- κ B particularly when induced by activation of Toll-like receptors;
 (ii) inhibits secretion of pro-inflammatory cytokines, such as, but not limited to, IL-1, IL-6, IL-8, and TNF- α both *in vivo* and *ex vivo*; and
 (iii) inhibits activation and/or recruitment of macrophages.

These properties means that PLD has particular efficacy in the treatment of inflammation. In particular, we have found that PLD has particular efficacy in treating inflammation as a result of

pathogen infection. As shown in the Examples, we have found that PLD can inhibit the secretion of a number of key pro-inflammatory cytokines. In particular, we have found that PLD can inhibit the transactivation of NF- κ B through the Toll-like receptors (TLR) and subsequent secretion of pro-inflammatory cytokines. As explained herein, the Toll-like receptors are activated in response to a number of infectious stimuli. Binding of a TLR ligand (i.e. a stimuli) to a Toll-like receptor (TLR) triggers a downstream signalling cascade that ultimately leads to the activation of the transcription factor nuclear factor-kappa B (NF- κ B), which controls induction of pro-inflammatory cytokines and chemokines. We have found that PLD significantly blocks this cascade, consequently leading to a reduction in the release or secretion of pro-inflammatory cytokines. As a result, in one example, PLD can be used to prevent inflammation following activation of the Toll-like receptors. In another example, PLD can be used to inhibit NF- κ B transactivation or NF- κ B-induced expression or secretion of pro-inflammatory cytokines. In another example, PLD can be used to inhibit expression and/or secretion of pro-inflammatory cytokines.

We have also found that PLD significantly reduces levels of macrophage activation and/or recruitment, particularly in response to a pathogen stimulus. Activated macrophages are a key mediator of inflammation and inhibition of activation is central to treating inflammation. Accordingly, in a further example, PLD can also be used to treat pathogen-induced inflammation, particularly by reducing macrophage activation and/or recruitment.

PLD has also been shown to bind to the human translation elongation factor eEF1A (eukaryotic translation elongation factor 1 alpha 1) with a high-affinity and a low rate of dissociation. FLIM-phasor FRET experiments on tumor cells demonstrate that PLD localises in tumor cells sufficiently close to eEF1A to suggest the formation of a drug-protein complex in living cells. PLD-resistant cell lines also show reduced levels of eEF1A protein and ectopic expression of eEF1A in these resistant cells restores the sensitivity to PLD, demonstrating that eEF1A is directly involved in the mechanism of action of PLD.

The N protein of a number of viruses, such as SARS-CoV and SARS-CoV-2, also bind to eEF1A, and this binding is essential for viral replication. However, administration and subsequent binding of PLD to eEF1A prohibits or reduces the binding of the viral N-protein to eEF1A. This in turn prevents virus replication. The interaction between PLD and EF1A could therefore reduce the efficiency of de novo viral capsid synthesis and consequently cause a decrease in viral load.

In addition to the above, PLD binding to eEF1A prevents eEF1A from interacting with its usual binding partners. One such binding-partner is the dsRNA-activated protein kinase (PKR or eIF2AK2). Binding of PLD to eEF1A releases PKR from a complex with eEF1A leading to the activation of PKR. PKR is a key player in anti-viral immune responses. Specifically,

- (i) activated PKR phosphorylates the alpha subunit of initiation factor eIF2, leading to the formation of an inactive eIF2 complex;
- (ii) activation of PKR induces apoptosis through a mechanism involving Fas clustering and NF- κ B translocation leading to the elimination of infected cells.

Of note, protein 4a of viruses such as CoVs potently suppresses the activation of PKR through the sequestration of dsRNA. PLD bypasses this viral response, leading to activation of PKR by releasing PKR from the eEF1A complex, as can be seen from the activation of PKR in the absence of viral infection.

Finally – and in addition to the above, binding of PLD to eEF1A also activates the ER-stress induced unfolded protein response (UPR), which in turn leads to a number of anti-viral responses, including again the phosphorylation of eIF2 α .

5 Through a combination of these mechanisms – (i) inhibition or reduction of the N-protein/ eEF1A interaction; (ii) activation of PKR and (iii) activation of the UPR; PLD prevents viral replication and causes the activation of host responses that lead to the elimination of the virus. Both of which contribute to an effective viral therapy. An additional advantage of targeting eEF1A is that it is a human target and as such will not mutate to evade PLD the way viral proteins do.

10 Accordingly, compounds of the present invention (including PLD) can be used to treat both a viral infection and any inflammation arising from the viral infection. In other words, compounds of the present invention (including PLD) as a single therapy can be used to treat two indications that arise from a viral infection – the infection itself (inhibition of viral synthesis and elimination of virus) and any (subsequently) excessive host inflammatory response – i.e. hypercytokinemia.

15 Accordingly, compounds of the present invention (including PLD and DidemninB), particularly PLD, can be used in the treatment inflammation, and in particular inflammation associated with either the activation of Toll-like receptors and/or inflammation as a result of pathogen infection.

The present invention may be useful in relation to the following viruses:

Flaviviridae family

20 Flaviviridae viruses are responsible for many important diseases that affect public health worldwide. The Flaviviridae viruses include yellow fever virus (YFV), Zika virus (ZIKV), Japanese encephalitis virus (JEV), West Nile Virus (WNV), hepatitis C virus (HCV) and dengue virus (DENV). *Flaviviridae* pathogens usurp cellular pathways in infected cells, generating an environment that is permissive for viral replication. Through its interaction with the 3'(+)-stem loop of the viral RNA and with several replication complex proteins, eEF1A has an important
25 role in minus-strand RNA synthesis and viral replication. Elongation factor eEF1A interacts with the 3'-terminal stem loop of the RNAs of several divergent flaviviruses, including a tick-borne flavivirus, and colocalized with dengue virus replication complexes in infected cells. These results suggest that eEF1A plays a similar role in RNA replication for all flaviviruses. Also characteristic of all flavivirus-caused diseases is that a fraction of the patients suffer from the exacerbation of
30 the inflammatory response to the viral infection (cytokine storm), which eventually generates tissue damage and worsen the pathological process caused by the virus.

1. Dengue virus

Dengue is caused by any of the four dengue viruses (DENV-1–4), belonging to the Flaviviridae family. DENV are small enveloped viruses containing a single-stranded (+) RNA approximately
35 10 kilobases in length that encodes a single polyprotein that is cleaved to produce 10 viral proteins. DENV infection is a major health problem in the tropical and subtropical regions of the world. Initial DENV infection can be asymptomatic or produce dengue fever (DF), an acute febrile disease accompanied by headache, arthralgia and rash, from which patients usually recover without complications. A second infection with a heterologous dengue virus serotype can produce
40 the Dengue Hemorrhagic Fever/Dengue Shock Syndrome (DHF/DSS) characterized by plasma leakage and abnormal bleeding that can lead to shock and death. The hallmark of severe dengue is a transient perturbation in blood vessel integrity and coagulation. Recovery is usually rapid and

complete, suggesting that the key mechanisms are functional rather than structural changes in the vasculature, most likely due to effects of locally produced cytokines. Studies demonstrating exaggerated immune activation in severe dengue strongly suggest a critical role of the immune response in the pathogenesis of dengue. Both innate and adaptive immunity (and their interactions) are involved in this process. Early gene expression in peripheral blood cells of dengue patients is dominated by type I IFN-mediated response genes, in addition to type I IFN, dendritic cells and monocyte/macrophages also produce proinflammatory cytokines that can increase vascular permeability. This tsunami of cytokines over a short period induces the disruption of vascular endothelial cells and deregulation of the coagulation system, leading to plasma leakage, hemorrhage and shock.

Eukaryotic elongation factor 1A (eEF1A) is a direct activator of SphK1. DENV-2 RNA co-localizes and co-precipitates with eEF1A from infected cells. The reduction in SphK1 activity late in DENV-2-infected cells could be a consequence of DENV-2 out-competing SphK1 for eEF1A binding and hijacking cellular eEF1A for its own replication strategy.

2. Zika virus

Zika virus (ZIKV), the causative pathogen of Zika fever, belongs to the Flaviviridae family. ZIKV is a small enveloped ss (+) RNA virus with a genome of around 11 kilobases in length that encodes a single polyprotein that is cleaved to produce 10 viral proteins. ZIKV infections in humans were sporadic before emerging in the Pacific and the Americas in the last decade. Indeed, ZIKV infection was associated with only mild illness prior to the large French Polynesian outbreak in 2013 and 2014, when severe neurological complications were reported, and the emergence in Brazil of a dramatic increase in severe congenital malformations (microcephaly) associated with ZIKV infection during pregnancy. The majority of cases (around 80%) are asymptomatic. When symptoms occur, they are typically mild, self-limiting, and similar to other arbovirus infections (e.g., DENV and CHIKV). Commonly reported symptoms include rash, fever, arthralgia and headache. Rare deaths have been described in patients infected with Zika virus. ZIKV is now known to cause fetal infection and congenital Zika syndrome, which includes microcephaly, cerebral malformations, ophthalmological and hearing defects, and arthrogryposis.

Mild elevations in inflammatory markers have been described during ZIKV. Similarly to what happens in DENV infection, in ZIKV infection the interferon system is the central mediator of host defense and target of viral counterattack. A polyfunctional immune activation was seen during the acute phase of ZIKV infection, with elevated cytokine profiles associated with Th1 (IL-2), Th2 (IL-4, IL-13), Th17 (IL-17), and also Th9 (IL-9) responses (16). Increased IL-4, IL-6, IL-8, IL-10, and IP-10 levels are also observed in ZIKV infected patients. Although no “cytokine storm” is observed in ZIKV patients, in ZIKV-infected placentas a massive inflammation has been observed, that may hamper the success of pregnancy (17). ZIKV perturbs the pro-/anti-inflammatory equilibrium of the placenta leading to tissue damage and massive infiltration of the villous core by inflammatory Hofbauer cells.

3. Yellow fever virus

Yellow fever (YF) is a lethal viral hemorrhagic fever (VHF) caused by the Yellow Fever Virus (YFV) belonging to the Flaviviridae family. YFV is a small enveloped ss (+) RNA virus with a genome of around 11 kilobases in length that encodes a single polyprotein that is cleaved to produce 10 viral proteins. While most YFV infections are asymptomatic or have very mild symptoms, severe YF occurs in around 12% of patients, manifesting with jaundice, hemorrhage

and multi-organ failure. YFV infection is characterized by severe hepatitis, renal failure, hemorrhage, and rapid terminal events with shock and multi-organ failure.

5 There are three phases in the course of a Yellow fever (YF): the “infection” phase, a flu-like illness characterized by fever, headache, nausea and myalgia, starts after an incubation period of three to six days. The peak of viremia occurs during this infection phase. Then follows a “remission” period after which most patients recover. Finally, in the severe form of the disease, after this remission patients progress to the intoxication phase, in which the hemorrhagic and hepatic signs of illness occur, along with multi-organ dysfunction.

10 The early signs of infection are probably due to the innate immune response to infection, including the production of acute-phase reactants like interferon- α and TNF- α . In the severe form of the disease, a sudden systemic inflammatory response syndrome (‘cytokine storm’) contributes to terminal events and death. In monkeys, YF virus predominantly replicates in lymphoid tissues and this replication extends beyond the appearance of neutralizing antibodies. Lymphoid tissues undergo profound changes in YF infection and the activation of cells in these tissues contributes
15 to the systemic terminal features of YF, characterized by release of pro-inflammatory cytokines. The terminal events occur swiftly and are characterized clinically by cardiovascular shock and multi-organ failure. The features of this phase strongly suggest that they are mediated by an inflammatory cascade, although few patients have been directly studied. In naturally acquired disease, pro- and anti-inflammatory cytokines (IL-6, IL-8, TNF- α , monocyte chemoattractant protein-1, IL-1-receptor antagonist, IL-10), resembling bacterial sepsis were significantly
20 elevated in fatal cases. Immune clearance by antibodies and T cells result in the release of pro-inflammatory cytokines, GPCR signaling, NF κ B activation, and production of oxygen free radicals, contributing to pathogenesis and cytokine storm.

4. West Nile Virus

25 West Nile virus (WNV) is an important emerging neurotropic virus, responsible for increasingly severe encephalitis outbreaks in humans and horses worldwide. WNV is a member of the Flaviviridae family and is encoded by a ~11 kb positive-sense single-stranded RNA (ssRNA) genome. The genome is translated as a single polyprotein, and subsequent cleavage of this polyprotein by viral and host proteases generates 10 viral proteins. WNV pathogenesis follow
30 three phases, the early phase initial infection and spread (the early phase), peripheral viral amplification (the visceral-organ dissemination phase) and neuroinvasion (the central nervous system (CNS) phase). The innate immune response, including type I interferon (IFN) and innate cell-mediated responses is responsible for the early control of WNV, whereas the adaptive immune response, including humoral and adaptive immune cell mediated responses (CD4+,
35 CD8+ and regulatory T cells), is essential for WNV clearance and limiting possible immune response-mediated damage in the later stages of infection.

The early phase after subcutaneous infection is defined by WNV replication in keratinocytes and skin-resident DCs, followed by viral amplification within the draining lymph node, which results in viremia and spread to visceral organs. The specific target cells for WNV infection are not well
40 defined, but are thought to be subsets of DCs, macrophages and possibly neutrophils. WNV invasion of the CNS tissues (for example, the brain and spinal cord) constitutes the third phase of the infection. WNV may enter the brain through a combination of mechanisms that facilitates viral neuroinvasion, such as direct infection with or without a breakdown of the blood-brain barrier (BBB) and/or virus transport along peripheral neurons.

Innate antiviral defenses are essential for the control of WNV infection, including the production of type I IFNs and pro-inflammatory cytokines, the expression of antiviral genes and the subsequent activation of the adaptive immune response. In WNV infection the innate immune activity is mainly triggered by RIG-1 like receptor (RLR) signaling, although Toll-like receptors (TLRs) could also contribute to NF- κ B activation and the production of type I interferons and pro-inflammatory cytokines. DCs and macrophages, both of which are innate immune sentinel cells and target cells of WNV infection, are pivotal in linking innate and adaptive immune responses. Macrophages and DCs are readily activated by WNV, releasing pro-inflammatory cytokines and chemokines such as type I IFN, TNF, IL-1 β , CCL2, CCL3, CCL5 and IL-8. These cytokines are important in regulating innate cell-mediated responses (involving NK cells, neutrophils and $\gamma\delta$ T cells) as well as in developing adaptive immune responses. A major hallmark of WNV pathogenesis is neuroinflammation, which is caused by exaggerated innate and acquired immune response. Accumulation of inflammatory monocytes into the brain and their differentiation to macrophages and microglia can also worsen neuroinflammation and CNS injury, as demonstrated in a murine model of nonlethal WNV infection. Recognition of WNV nucleic acid in monocytes/microglia by TLRs may lead to the production of TNF- α , which results in a loss of tight junctions, allowing the entry of WNV and immune cells into the perivascular space of the brain in mice. Thus, activation of cells of the monocyte/macrophage system by WNV appears to result in important neuropathological consequences, and exaggerated innate responses may cause inflammation, altering the blood brain barrier permeability and allowing the virus to enter the CNS. Indeed, treatment of infected neuronal cells with antibodies blocking TNF- α and other pro-inflammatory mediators results in a significant reduction of WNV-mediated neuronal death, suggesting that such mediators play a major role in the pathogenesis of WNV infection in the CNS.

5. Hepatitis C Virus

Hepatitis C virus (HCV) infection causes a progressive liver disease that deteriorates from chronic inflammation to fibrosis, cirrhosis and even to hepatocellular carcinoma. HCV-caused hepatitis has an estimated prevalence of 71 million infected people and causes around 400000 deaths per year. Belonging to the Flaviviridae family, Hepatitis C Virus (HCV) is a small enveloped virus containing a single-stranded (+) RNA approximately 10 kilobases in length, which encodes a single polyprotein that is cleaved by cellular and viral proteases to generate 10 viral proteins. Upon HCV infection, hepatocytes and immune cells (macrophages, mast cells, dendritic cells and natural killer cells) recruited to the liver initiate the innate immune response, resulting in the spontaneous elimination of acute HCV infection. However, in 70%-80% of cases, the immune responses fail to eliminate the virus during the acute phase, leading to chronic infection. Persistent HCV replication in hepatocytes leads to uncontrolled inflammation and chemokine production. The excessive cytokines, as inflammatory agents, further cause inflammation in the liver, which eventually exacerbates tissue damage and liver disease progression.

Direct antiviral treatment is the first choice for HCV treatment, but antivirals alone are insufficient to block the severe inflammation and liver injury in HCV-infected individuals. HCV is just the trigger for pathophysiological processes, but persistent inflammatory cytokine storms and HCV-induced hepatocyte damage exacerbate the progression of severe liver diseases. HCV RNA elicits TLR-mediated NF- κ B activation and inflammatory cytokine release, while HCV proteins activate NLRP3. The released inflammatory factors bind to their corresponding receptors and then induce NF- κ B activation, leading to downstream inflammatory response. Therefore, a long-term, persistent and uncontrolled inflammatory response is a hallmark of these diseases and further leads to hepatic injury and more severe disease progression. Some patients who achieve a

sustained antiviral response after direct antiviral therapy are still at a long-term risk for progression to liver cirrhosis and hepatocellular carcinoma. Therefore, coupling direct antiviral therapies with anti-inflammatory/hepatoprotective agents is a promising therapeutic regimen for HCV patients.

5 6. Tick-Borne Encephalitis Virus

10 Tick-borne encephalitis virus (TBEV) causes a severe disease that can lead to permanent neurological conditions or even death. The severity of TBE varies depending on the viral subtype, the European (TBEV-Eu), the Siberian (TBEV-Sib), and the Far-Eastern (TBEV-FE). TBEV-Eu is the mildest variant (< 2% lethality), while TBEV-FE is the worst variant with important rates of neurological sequelae and up to 40% fatalities. TBEV is a small enveloped virus containing a single-stranded (+) RNA of approximately 11 kilobases, encoding a single polypeptide that is cleaved by cellular and viral proteases to generate 10 viral proteins. Tick-borne encephalitis (TBE) is a syndrome with a triphasic course, beginning with a flu-like illness (characterized by fever, fatigue and body pain) after which 65–70% of infected individuals clear the virus. Around one third of the patients follow with the asymptomatic disease phase, and finally the neuroinvasive phase with neurological symptoms of variable severity, ranging from meningitis to severe meningoencephalitis with or without myelitis.

20 Immune and non-immune mechanisms have been proposed to contribute to TBEV crossing through the blood-brain barrier (BBB) and invasion of the CNS. Cytokines may facilitate this process. Cytokines such as TNF- α and IL-6 have an impact on endothelial cell permeability that may induce a BBB disruption, leading to crossover of the virus into the CNS. TBEV-infected immune cells such as dendritic cells, neutrophils, monocytes, macrophages, and T cells could also migrate into the parenchymal compartment causing infection of neurons or other cells in the brain and the spinal cord.

25 TBEV infection activates type 1 IFN production through TLR3 recognition of viral dsRNA in the extracellular environment or in the cytoplasm. The IFN- α system appears to play a key role in activation of the innate immunity. It affects activation of immunocompetent cells and induction of other pro-inflammatory cytokines. In mice models, TBEV induces a cytokine storm during the terminal stage of infection, which could be considered as a cytokine storm.

30 Interestingly, during the phase of acute infection in TBEV-infected patients, the levels of S1P in blood and cerebrospinal fluid are highly elevated. This increase might promote a proinflammatory response. An increased production of extracellular S1P can be regulated by modulators of the S1P pathway. Indeed, elongation factor eEF1A binds to and activates SPHK to phosphorylate sphingosine and produce S1P. Plitidepsin targets eEF1A, inhibiting the production of S1P in treated cells.

7. Classical Swine Fever Virus

40 Classical swine fever (CSF) remains one of the most important transboundary viral diseases of swine worldwide. It has tremendous impact on animal health and pig industry and is therefore notifiable to the World Organization for Animal Health. Classical swine fever virus (CSFV) is a small enveloped virus belongs to the Flaviviridae family. It has a single-stranded (+) RNA genome of approximately 12.3 kb which is translated into one polypeptide. Co- and post-translational processing of the precursor protein by viral and cellular proteases results in 13 mature proteins. A putative receptor for the virus in porcine cells is CD46. Classical swine fever

can be divided into the following forms of the disease: an acute (transient or lethal), a chronic and a persistent course, which usually requires infection during pregnancy (34). During the first two weeks upon infection, the acute phase is characterized by unspecific (often referred to as “atypical”) clinical signs like high fever, anorexia, gastrointestinal symptoms, general weakness, and conjunctivitis. Around two to four weeks after infection neurological signs can occur including incoordination, paresis, paralysis and convulsions. At the same time, skin hemorrhages or cyanosis can appear in different locations of the body such as the ears, limbs, and ventral abdomen. These late signs are the textbook cases and are therefore referred to as “typical” CSF signs. In acute-lethal progressions, death usually occurs 2–4 weeks after CSFV infection and mortality can reach up to 100%. Chronic course occurs when an infected pig is not able to mount an adequate immune response. In general, only non-specific clinical signs are observed in infected animals like remittent fever, depression, wasting and diffuse dermatitis.

A hallmark of acute classical swine fever is the high interferon (IFN)- α levels found in the serum early after infection, followed by an inflammatory cytokine storm. Activated macrophages seem to play a crucial role in the immuno-pathogenesis of CSF. Indeed, direct damage by the virus could be almost excluded for many lesions occurring in the course of CSFV infection.

Retroviridae

Retroviridae are a family of positive-sense single stranded RNA viruses characterised by their expression of reverse transcriptase, an RNA-dependent DNA polymerase that generates DNA from their RNA genome, which is then subsequently integrated into the host genome of infected plants. Retroviridae viruses include HIV-1 and HIV-2 which cause AIDS and HTLV (human T-lymphotropic virus). HIV infection in particular triggers an immune response that manifests as acute retroviral syndrome (ARS) and in some individuals or advanced cases of infection, an inflammatory syndrome consistent with cytokine storm syndrome.

Picornaviridae Family

Picornaviruses are non-enveloped, small, single-stranded (+) RNA viruses. Their small genome spans over 8 kilobases, containing an open reading frame that is translated into a single polypeptide which is subsequently processed by viral proteases into 11–12 individual viral proteins. The family comprises enteroviruses (EVs), hepatoviruses, parechoviruses, rhinoviruses, aphthoviruses, and cardioviruses. One of the major features of severe pathogenic diseases as a result of several picornavirus infections (type 1 diabetes, myocarditis, or paralysis) is a strong association with autoimmunity. The simplest explanation may be that infection with cytopathic infectious agents results in cell death or injury, thus releasing either sequestered or cellular autoantigens which are present at low concentrations prior to infection, thus preventing autosensitization. Since several picornaviruses, including poliomyelitis virus (PV), coxsackievirus (CVB), and foot-and-mouth disease virus, can induce persistent viral infections, chronic activation of MDA5 and upregulation of IFN-I could produce the adjuvant effect attributed by some investigators as the major contributing factor in picornavirus infections to induce autoimmunity.

1. Foot and Mouth Disease Virus

Foot-and-mouth disease virus (FMDV) is the causative agent of an acute vesicular disease affecting pigs, cattle and other domestic and wild animals worldwide. FMDV, a small non-enveloped virus from the Picornaviridae family, has a single stranded (+) RNA genome of about

8.5 Kb in length encoding a single polyprotein which undergoes proteolytic processing by viral proteases to generate 14 viral proteins.

5 Viral infections can stimulate multiple pathways to induce type-I and type-III IFNs which have antiviral, antiproliferative, and immunomodulatory functions. Maturation of dendritic cells (DC) is promoted by IFN-I, influencing the efficacy of the adaptive immune responses induced. Some viral proteases can inhibit type-I IFN production and NF- κ B signaling through the cleavage of receptors, adaptors, and regulators participating in these pathways. The IFN response is then amplified and spread to surrounding uninfected cells by the expression of hundreds of IFN-stimulated genes. MDA5 is involved in recognizing picornaviruses. The relevance of the type 1
10 IFN induced double-stranded RNA-dependent protein kinase R (PKR) in the inhibition of FMDV replication has been well-documented in swine and bovine cells. Treatment with an inhibitor of PKR increased the virus yield for several folds compared with that of the untreated infected cells and PKR down-regulation by RNA interference also resulted in higher viral titers, further confirming a direct function of PKR in controlling FMDV replication. Elongation factor eEF1A2
15 binds PKR and maintains the kinase on hold. Plitidepsin binds to eEF1A2, releasing PKR from the complex with the elongation factor and activating the kinase (48). This way plitidepsin could enhance the response against FMDV infection.

2. Enterovirus A71

20 Hand, foot, and mouth disease (HFMD) is a contagious viral disease and mainly affects infants and young children. The main manifestations are fever, vesicular rashes on hand, feet and buttocks and ulcers in the oral mucosa. Usually, HFMD is self-limiting, with patients presenting fever, a maculopapular or papulovesicular rash on the hands and soles of the feet, and painful oral ulcerations that usually resolve in less than ten days. Nevertheless, a small proportion of children may experience severe complications including meningitis, encephalitis, acute flaccid paralysis
25 and neurorespiratory syndrome, and even death. HFMD is caused by two pathogens, the enterovirus 71 (EV-A71) and the coxsackievirus A16 (CV-A16). EV-A71 is a small, icosahedral, non-enveloped, single-stranded (+) RNA virus from the Picornaviridae family. The approximately 7.4-kb genome of EV71 encodes a single polyprotein that is proteolytically cleaved to various structural and nonstructural viral proteins.

30 Lethal EV-A71 infections course with extensive neuronal degeneration, severe CNS inflammation and necrosis, and pulmonary congestion and hemorrhaging. A systemic inflammatory response coupled with CNS inflammation and cytokine storm may play an important role in the development of EV71-related fulminant pulmonary edema. Brain-derived proinflammatory cytokines may enter the systemic circulation after the occurrence of
35 postencephalitis blood-brain barrier disruption to systemically activate an inflammatory cascade, thereby contributing to the development of postencephalitis systemic inflammatory response syndrome (SIRS) and subsequent cardiopulmonary failure and pulmonary edema.

3. Coxsackievirus

40 Coxsackieviruses (CVs) are relatively common enteroviruses associated with a number of serious human diseases, including myocarditis and meningoencephalitis. Coxsackieviruses are small, icosahedral, non-enveloped, single-stranded (+) RNA viruses from the Picornaviridae family, with a genome of 7-8 kilobases covalently linked to the viral protein VPg, which acts as a primer for RNA synthesis. Transmission is through the fecal-oral route. Coxackieviruses can be divided into two groups, A and B. Group A viruses infect preferentially the skin and mucous membranes,

causing diseases as hand, foot and mouth disease (HFMD). Group coxsackieviruses (CVB) tend to infect inner organs instead, causing pancreatitis, hepatitis, myocarditis, etc. In particular, serotype B3 viruses (CVB3) are associated with the development of myocarditis in humans.

- 5 CV is a relatively common pediatric virus, typically causing mild infections ranging from subclinical to flu-like symptoms and mild gastroenteritis. CV has been shown to infect the heart, pancreas, and CNS. In some cases CVs cause severe systemic inflammatory diseases such as meningoencephalitis, pancreatitis, and myocarditis, all of which can be fatal or result in lasting organ dysfunction, including dilated cardiomyopathy and encephalomyelitis, with a mortality rate as high as 10%.
- 10 Following uptake through the host gastrointestinal tract, CVB3 infects and replicates in lymphocytes and macrophages of Peyer's patches and the spleen. Subsequently, infectious virions are released into the bloodstream and disseminate into organs such as the heart and pancreas. The development of viral myocarditis is generally divided into three distinct phases. The first 3–4 days are the 'acute' phase, which is characterized by virus replication. Cell lysis produced by the virus
- 15 and the recognition of PAMPs by TLRs induce the expression of proinflammatory cytokines including IL-1b, IL-6, IL-18, TNF- α , and type I and type II interferons (IFNs). These cytokines are produced by cardiac resident cells, including myocytes, fibroblasts, endothelial cells, and dendritic cells (DCs). These cytokine signals activate local macrophages and upregulate endothelial adhesion molecules as well as chemokines and chemokine receptors to collectively
- 20 trigger the recruitment of innate immune cells. The subacute phase, from day 4 or 5 to approximately day 14, commences with infiltration of the heart by cells of the innate immune system. Monocytes engage in phagocytosis of dead cells and strongly augment the expression of proinflammatory cytokines. During the subacute phase, the immune response not only eliminates infected and dead cells but also significantly contributes to irreversible cardiac damage. The third
- 25 stage of viral myocarditis starts after complete elimination of the virus and is characterized by cardiac repair and replacement of dead tissue by a fibrotic scar, affecting cardiac function in the long term.

4. Rhinovirus

- 30 Human rhinoviruses (HRVs) are responsible for more than one-half of cold-like illnesses and cost billions of dollars annually in medical visits and missed days of work. HRVs are transmitted from person to person via contact (either direct or through a fomite) or aerosol (small or large particle). HRVs belong to the family *Picornaviridae* and are single stranded (+) RNA viruses with a genome of around 7,200 bp encompassing a single ORF which encodes a poly-protein that is cleaved by virally encoded proteases to produce the 11 viral proteins. There are three genetically
- 35 distinct HRV groups, designated groups A, B, and C.

- The majority of HRV-A and -B serotypes (the major receptor group) enter airway epithelial cells via ICAM-1, a member of the immunoglobulin superfamily. HRV is infrequently associated with cytopathology of the upper respiratory tract. In addition to a direct effect on respiratory epithelial cells, the innate and adaptive host responses also have a role in the pathogenesis of HRV infection.
- 40 The type I interferon (IFN) response is mediated by MDA-5 and RIG-1. These receptors maximize the induction of type I and II IFNs and proinflammatory cytokines, including RANTES, IP-10, IL-6, IL-8, and ENA-78. HRV stimulation of IL-8 production is mediated in part by an NF- κ B-dependent transcriptional activation pathway. Levels of IL-8 correlated with symptom severity (rhinorrhea and nasal obstruction) and peaked at 48 to 72 h after virus inoculation.

5. Hepatitis A

- Hepatitis A virus (HAV) causes around 1.4 million cases of enterically transmitted hepatitis per year (WHO Fact sheet N°328, Hepatitis A, 2013), being still a source of mortality despite the existence of a successful vaccine. Unlike the rest of picornaviruses, HAV has an envelope that makes it tremendously stable and cannot shut down host protein synthesis. The cell surface molecule TIM-1 acts as a receptor for HAV. Disease severity is age-dependent, being mild or asymptomatic in young children and presenting with acute hepatitis (jaundice, fatigue, general malaise, etc) and a higher incidence of fulminant hepatitis at older ages. Fulminant hepatitis affects those aged over 50, with mortality rates up to 5.4%.
- HAV is a single stranded (+) RNA pseudo-enveloped virus belonging to the Picornaviridae family. Its genome spans 7.5 kb, containing a single open reading frame that encodes for a polyprotein that is cut by viral proteases into 10 viral proteins. The RNA genome lacks a 5' cap structure but is bound to a VPg protein.
- Infection with HAV courses with initial symptoms, as fever or jaundice, in about 70% of cases, with about one third of patients remaining asymptomatic. HAV infection has no chronic carrier state and does not lead to chronic hepatitis or cirrhosis. The cytosolic RNA of picornaviruses such as HAV is sensed by MDA5 but, in contrast to HCV, HAV minimally stimulates IFN responses in the infected liver. HAV 3C protease cleaves NF- κ B essential modulator (NEMO), thereby attenuating nuclear factor- κ B (NF- κ B) activation downstream of both MAVS and TLR3. Liver injury could not be directly caused by HAV but caused by immune-mediated mechanisms instead. Treg cells from AHA patients undergo qualitative changes resulting in the production of TNF- α . Moreover, TNF- α -producing Treg cells from AHA patients exhibit Th17-like features in terms of their phenotype and have an attenuated suppression function. Inflammatory Treg cells are associated with the immunopathological liver injury in AHA.
- Togaviridae family
- Togaviridae family contains only one genus, Alphaviridae, with 31 species. Alphavirus are small enveloped icosahedral viruses with a single stranded (+) RNA genome of around 11-12 kilobases. This capped RNA genome is divided into a nonstructural domain encoding the nonstructural proteins (5'-terminal two-thirds of the genome) and a structural domain encoding the three structural proteins of the virus (3'-terminal one-third of the genome). The nonstructural proteins are translated as one or two polyproteins from the genomic RNA itself. These polyproteins are cleaved to produce nsP1, nsP2, nsP3, and nsP4, as well as a number of cleavage intermediates that possess important functions distinct from the final products. The structural domain is translated as a polyprotein from a capped subgenomic mRNA, the 26S mRNA.
- The alphaviruses are a serious or potential threat to human health in many areas. Eastern Equine Encephalitis Virus (EEEV) and Western Equine Encephalitis Virus (WEEV) regularly cause fatal encephalitis in humans in both North America and South America, although the number of cases is small. Venezuelan Equine Encephalitis Virus (VEEV) also causes human illness. Chikungunya Virus (CHIKV) and its close relative O'Nyong-Nyong Virus (ONNV) have caused millions of cases of serious, but not deadly, disease characterized by fever, rash, and a painful arthralgia. Ross River Virus (RRV), Barmah Forest Virus (BFV) and Sindbis Virus (SINV) cause epidemic polyarthritides in humans, with symptoms that last for years. Semliki Forest Virus (SFV) cause a disease characterized by exceptionally severe headache, fever, myalgia, and arthralgia.

1. Chikungunya Virus

Chikungunya virus (CHIKV) is the etiological agent of the mosquito-borne disease chikungunya fever, a debilitating arthritic disease that causes immeasurable morbidity and some mortality in humans, including newborn babies. CHIKV has a single-stranded (+) RNA genome of approximately 12 kilobases. This genome is organized in two open reading frames, the 5' ORF which is translated from genomic RNA and encodes four non-structural proteins, and the 3' ORF which is translated from a subgenomic 26S RNA and encodes for a polyprotein that is cleaved into 5 structural proteins.

Chikungunya fever (CF) has an incubation period of less than 10 days. Asymptomatic patients are less than 30 %, fewer than in other alphaviruses diseases. Symptomatic patients suffer from a sudden onset of high fever, polyarthralgia, headache and fatigue. Polyarthralgia represents the most characteristic symptom, especially peripheral joints pain. Myalgia is also frequent. Cutaneous manifestation happens in half of the patients, including macular rash. A variety of other clinical symptoms have been reported during the acute stage of chikungunya fever, such as conjunctivitis, neuroretinitis, iridocyclitis, myocarditis, pericarditis, pneumonia, dry cough, lymphadenopathy, nephritis, hepatitis and pancreatitis. The reported causes of death were heart failure, multiple organ failure syndrome, toxic hepatitis, encephalitis, bullous dermatosis, respiratory failure, renal failure, pneumonia, acute myocardial infarction, cerebrovascular disease, hypothyroidism or septicemia.

The CHIKV RNA genome can trigger host pattern recognition receptors (PRRs) including endosomal Toll-like (TLR3 and TLR7) and cytoplasmic RIG-I-like (RIG-I and MDA5) receptors, which activate downstream adaptor molecules (TRIF, MyD88, and MAVS) to induce nuclear translocation of interferon-regulatory (IRF1, IRF3, IRF5, and IRF7) and NF- κ B transcription factors, which induce expression of type I IFNs, IFN-stimulated genes (ISGs), and pro-inflammatory cytokines and chemokines. Inflammatory cytokines and chemokines are thought to be involved in the pathogenesis of CHIKV and are associated with severe clinical presentations as well as with development of abrupt and persistent arthralgia. A significant increase of IL-1 β and IL-6 levels in severe compared with non-severe CF cases has been reported. In a deadly case described in Italy, the analysis of inflammatory cytokines revealed a remarkable and strong increase of circulating type-I IFN, as well as of the IL-6 pro-inflammatory cytokine, consistent with a possible role of type-I IFN in the CHIKV-driven cytokine storm that, in turn, may have contributed to enhanced tissue injury and death.

2. Sindbis Virus

Sindbis virus (SinV) is the most widely distributed of all known arthropod-transmitted viruses. The Pogosta, Ockelbo and Karelian fever viruses belong to the Sindbis group. Ockelbo disease, characterized by arthritis, rash, and sometimes fever, was first recognized in Ockelbo village in central Sweden. Later on, the same disease was described in Finland as Pogosta disease. In Russia the disease was named Karelian fever. There is no evidence of any SinV-induced diseases in other vertebrates than humans. In general, the incubation time varies from 2 to 10 days, the symptomatic illness starts suddenly and the duration of the disease is short, less than 20 days. There are, however, a good proportion of SinV infections that may course with chronic illness. As with other alphaviruses, children are often infected but develop only subclinical diseases. Main symptoms are fever, muscle pain, headache, sore throat, maculopapular rash and joint pain. In human macrophages the infection promotes activation, leading to the release of macrophage migration inhibitor factor (MIF) from intracellular stores and inducing the secretion of TNF- α , IL-1 β , and

IL-6. In mice, SinV replication resulted in induction of high levels of TNF- α , IFN- γ , IFN- α/β , IL-6, and corticosterone in serum, two causes of Systemic Inflammatory Response Syndrome (SIRS, cytokine storm) (76). Therefore, there is a strong circumstantial case for involvement of SIRS in disease induced by virulent Sindbis virus.

5 3. Semliki Forest Virus

Semliki Forest virus (SFV) is a single stranded (+) RNA virus of the genus Alphavirus of the family Togaviridae. The genome of SFV is 13 kilobases in length, encoding for only 9 proteins in two open reading frames (ORF). Four non-structural proteins are encoded as a polyprotein in the first ORF, located in 5' region of the genome and directly translated from the genomic DNA.
10 The remaining 5 structural proteins are encoded as a polyprotein in a second ORF which is translated from a subgenomic RNA species called 26S and located in the 3' region of the genome. Polyproteins are then cut by viral proteases into individual proteins.

The original L10 isolate of SFV shows complete virulence in inbred mouse strains, causing lethal encephalitis by infection of the central nervous system (CNS). A critical difference between virulent and nonlethal strains could be the swift induction of neuronal damage in the CNS, causing
15 the death of infected mice before the immune system can intervene. Infection with nonlethal SFV strains induces inflammatory spongiform lesions in mouse brain. Those lesions are delayed in mice treated with the immunosuppressive drug cyclophosphamide (Berger ML, 1980). Therefore, direct viral cytolysis is not essential in the production of pathology. The fact that nude mice suffer
20 the same inflammatory spongiform lesions although lacking functional T cells indicates that the immunopathology does not depend on them. Instead, the lesions seem to be due to the presence of mononuclear cells from the monocyte-macrophage lineage, which can be recruited into the infected tissue and produce spongiform necrosis.

25 4. Eastern Equine Encephalitis Virus / Western Equine Encephalitis Virus / Venezuelan Equine Encephalitis Virus

Three viruses in the genus Alphavirus (Togaviridae family), Eastern, Western and Venezuelan equine encephalitisviruses (EEEV, WEEV and VEEV, respectively), can cause severe outbreaks of encephalitis in equines and humans. EEEV was first identified in humans in 1938 during an important outbreak affecting Massachusetts. Most EEEV infections in adult humans are
30 asymptomatic or produce a mild illness characterized by fever, chills, headache, nausea, vomiting and myalgia. The fatality rate in symptomatic patients approaches 80%, many survivors exhibiting important sequelae as mental retardation, convulsions, and paralysis. Lethality of WEEV ranges between 3 and 7% with 15–50% of the encephalitis survivors, especially young children, suffering permanent neurological damage. VEEV-associated encephalitis has caused
35 serious morbidity and mortality in outbreaks in Columbia and Venezuela, with more than 75000 affected people, over 3000 patients suffering neurologic conditions and over 300 deaths.

As the rest of alphaviruses, EEEV, WEEV and VEEV are single stranded (+) RNA viruses with a short, capped genome of around 11.7 kilobases. The genome has two ORFs, the 5' ORF encoding four non-structural proteins which are translated as a single polypeptide from genomic
40 RNA, and the 3' ORF which is translated from a subgenomic 26S RNA as a single polypeptide containing three structural proteins. Polypeptides are cut into individual proteins by viral proteases.

In experimentally infected laboratory mice, EEEV produces neurologic disease that resembles that following human and equine infections. Virus is detected in the brain on day 1 postinfection (PI), and signs of disease are evident on days 3 to 4 PI. Clinical signs of murine disease include ruffled hair, anorexia, vomiting, lethargy, posterior limb paralysis, convulsions, and coma. Pathological changes in infected mice include neuronal degeneration, cellular infiltration, and perivascular cuffing, similar to the CNS pathological changes described in naturally infected humans. VEEV infects dendritic cells (DCs) and macrophages in lymphoid tissues, fueling a serum viremia and facilitating neuroinvasion. In contrast, EEEV replicates poorly in lymphoid tissues.

5. Rubella virus

Rubella virus (RV) infection of children and adults is usually asymptomatic (up to 50% of cases) or characterized by a mild fever, sore throat, lymphadenopathy and a maculopapular rash. However, when infecting pregnant women, RV causes birth defects and fetal death. RV is a major public health concern since more than 100,000 cases of congenital rubella syndrome (CRS) occur every year. Rubella virus (RV), the sole member of the rubivirus genus within the Togaviridae family, has a single stranded (+) RNA genome of around 10 kilobases that encodes for five proteins. The genome contains two ORFs, 5' ORF which encodes two nonstructural proteins directly translated from the genomic RNA and a second 3' ORF which encodes three structural proteins translated from a subgenomic mRNA.

RV infection during critical stages of organ development in fetus (in the first trimester of pregnancy) can result in CRS, a syndrome characterized by severe defects in sensory organs (eyes and ears), central nervous system and cardiovascular system. In CRS, rubella virus is able to infect the placenta, spread to the fetus, and alter the function of multiple fetal systems by interfering with organ formation and causing systemic inflammation. Rubella virus-associated uveitis (RVU) is one of the inflammatory syndromes caused by RV. This inflammatory process is characterized by high intraocular levels of inflammatory cytokines, including IL-6, IL-6ra and MCP-1.

Caliciviridae Family

Caliciviridae family is composed by 11 genera, with seven genera (Lagovirus, Norovirus, Nebovirus, Recovirus, Sapovirus, Valovirus and Vesivirus) infecting mammals, two genera (Bavovirus and Nacovirus) infecting birds and two genera (Minovirus and Salovirus) infecting fish. Caliciviruses cause species-specific infections, with most noroviruses, sapoviruses and neboviruses restricted to the gastrointestinal tract, while lagoviruses, saloviruses and vesiviruses can cause severe systemic infections in their natural hosts. The family includes non-enveloped viruses with single stranded (+) RNA genomes of around 7-8 kilobases, organized in two, three or four ORFs. Caliciviruses are similar to picornaviruses in the presence of VPg protein attached to the 5' terminus of their genome and the in sequence of several proteins.

1. Norwalk virus

Norovirus (NoV) is a genus of the Caliciviridae family composed by a single species, Norwalk virus. Human norovirus is a major cause of epidemic and sporadic acute gastroenteritis worldwide, and can chronically infect immunocompromised patients. NoV infection generates typical gastroenteritis symptoms, including diarrhea, vomiting, and stomach pain, and may also provoke fever, headache and body aches. Noroviruses are non-enveloped viruses with a single stranded (+) RNA genome of around 7.5 kilobases. The genome is organized into three or four

ORFs. The 5' ORF1 encodes a large polyprotein that is cleaved by viral proteases into 6 nonstructural proteins. ORF2 encodes a single structural capsid protein. ORF3 encodes for a minor structural protein. A subgenomic RNA encodes for two additional structural proteins. Finally, ORF4 (which overlaps with ORF2) in murine novovirus encodes for an additional protein, the virulence factor 1. The 5' ends of NoV genomic and subgenomic RNAs are covalently linked to a small virus-encoded protein known as VPg and the 3' ends are polyadenylated.

Compelling evidence has accumulated in recent years demonstrating that a major target of NoVs is antigen presenting cells (APCs). MuNoVs efficiently replicate in primary dendritic cells and macrophages, as well as a number of macrophage-like cell lines, in vitro. The ability of NoVs to infect intestinal immune cells undoubtedly has a significant impact on NoV pathogenesis and the host immune response to NoV infection. Consistent with the short duration of NoV symptoms, innate immunity - and in particular type I IFNs - is critical for controlling acute NoV infections.

Coronaviridae Family

Coronaviruses are pathogens that can infect animal and human hosts, mostly causing mild to severe enteric or respiratory diseases that can be life threatening in some cases, as with MERS-CoV, SARS-CoV or the new SARS-CoV-2, this latter causing the COVID-19 disease which recently originated in Wuhan, Hubei province, China. Both SARS-CoV-2 and the closely related SARS-CoV belong to the subgenus Sarbecoviridae, within the genus Betacoronaviridae (to which also MERS-CoV belongs), one of the four genus of Coronaviridae. Coronaviruses are enveloped, single stranded (+) RNA viruses with genomes of around 26-32 kilobases in size, the largest known genome for an RNA virus. They were identified for the first time in 1964, by June Almeida. Their name comes from the similarity their electron microscope images share with the solar corona. Their genome encodes for 14 open reading frames, i.e., the replicase ORFs 1a and 1b (which encode for 15 or 16 mature nonstructural proteins also known as replicase proteins), the four common CoV structural protein genes (S, E, M, and N) and the ORFs encoding so-called accessory proteins. The replicase proteins seem to have multiple functions, being essential for the synthesis or processing of viral RNA, but also to establish virus-host interactions to facilitate viral entry, gene expression, RNA synthesis or virus release. The CoV N proteins are structural proteins of 350 to 450 amino acids, broadly phosphorylated in serine residues, highly basic, and its main function is to associate with viral RNA to form a long helical ribonucleoprotein. N proteins are also involved in viral RNA transcription, translation, and virus exit. The N protein of SARS-CoV shares a high homology with its SARS-CoV-2.

MERS-CoV, SARS-CoV and SARS-CoV-2 can cause acute respiratory distress syndrome (ARDS), the most acute and fatal stage of the disease, characterized by wide-spread inflammation in the lungs resulting from the aberrant immune response to the viral infection.

1. SARS-CoV-1

Severe Acute Respiratory Syndrome (SARS), caused by SARS-CoV, started in Guangdong province of China in 2002 and affected 8,096 people worldwide, resulting in 774 deaths (10 % mortality rate). The genome of SARS-CoV comprises 29.7 kilobases, containing 11 ORFs that encode for 23 proteins.

The most common symptoms of SARS are high fever, myalgia, dry cough, and lymphopenia. Around 30% of patients also developed an atypical form of pneumonia, with acute respiratory distress resulting from acute lung damage. Several factors including advanced age, male sex, a

high peak lactate dehydrogenase level, a high peak creatine kinase level, and a high initial absolute neutrophil count were significant predictive factors for intense care unit admission and death. An excessive inflammatory response in the lungs explains the development of acute lung injury, moreover when patients still manifest lung injury at a time when the viral load is falling.

- 5 Acute viral infection might produce damage to host cells by direct cytopathy or by indirect immunopathological damage. In the early stage, cytopathy is accompanied by viral amplification, time at which the treatment with antiviral drugs may be especially effective. In the later stage, when an adaptive immune response is mounted, viral clearance can be accompanied by severe inflammatory damage. An IFN- γ -related cytokine storm may happen in this latter phase of SARS
10 coronavirus infection, and this cytokine storm might be involved in the immunopathological damage in SARS patients.

2. MERS-CoV

- Middle East respiratory syndrome (MERS), caused by MERS-CoV, started in Saudi Arabia in 2012 and affected 2506 people, causing 862 deaths worldwide with a 35% mortality rate. The
15 genome of MERS-CoV is 30.1 kilobases in length, encompassing 10 ORFs that encode for 16 nonstructural proteins and 5 structural proteins.

- The most common symptoms of MERS are fever, cough, chills, sore throat, myalgia, and arthralgia, followed by dyspnoea and rapid progression to pneumonia within the first week, often requiring ventilatory and other organ support. While younger individuals experience mild-
20 moderate clinical illness, elderly individuals or those with comorbid conditions such as diabetes, obesity, heart failure, and renal failure among others experience severe disease after infection with MERS-CoV. In its most severe form, MERS causes an acute highly lethal pneumonia. Individuals with severe MERS show elevated levels of serum pro-inflammatory cytokines (IL-6 and IFN- α) and chemokines (IL-8, CXCL-10, and CCL5) compared to those with mild to
25 moderate disease. High serum cytokine and chemokine levels in MERS patients correlated with increased neutrophil and monocyte numbers in lungs and in the peripheral blood, being those cells the predominant source of cytokines and chemokines associated with the lethal acute respiratory distress syndrome that kills MERS patients.

3. SARS-CoV-2

- 30 COVID-19, caused by SARS-CoV-2, started in Wuhan city in China in December 2019, quickly spreading throughout the world and growing into a global pandemic. Mortality rate is still uncertain, although it seems smaller than in SARS and MERS diseases. SARS-CoV-2 genome encodes for 14 open reading frames, including the replicase ORFs 1a and 1b (which encode for 15 or 16 mature nonstructural proteins also known as replicase proteins), the four common CoV
35 structural protein genes (S, E, M, and N) and the ORFs encoding so-called accessory proteins. The replicase proteins seem to have multiple functions, being essential for the synthesis or processing of viral RNA, but also for other steps of the virus life cycle. The N protein is a broadly phosphorylated, highly basic structural protein, whose main function is to associate with viral RNA to form a long helical ribonucleoprotein. N protein is also involved in viral RNA
40 transcription, translation, and virus exit. The N protein of SARS-CoV and SARS-CoV-2 share a high homology. Several cellular proteins collaborate with viral proteins to support viral proliferation, among them eEF1A, which interacts with N protein to support viral replication and spreading.

Common mild symptoms of the early stage of infection are fever, dry cough, myalgia and fatigue. Less common are headache, hemoptysis and diarrhea. In the transition to the severe stage of the disease, symptoms such as dyspnea and hypoxia develop. Finally, the severe form of the disease manifests with Acute Respiratory Distress Syndrome (ARDS), together with severe lung inflammation and damage. Complications at the severe stage of disease associate with virus-induced hyperinflammation, similar to what has been previously seen in SARS-CoV and MERS-CoV infections.

In SARS-CoV-2, macrophages present CoV antigens to Th17 cells, leading to a massive release of pro-inflammatory cytokines such as IL-1, IL-6, IL-8, IL-21, TNF- α and MCP-1. This is responsible for immune amplification and is partly responsible for the tissue damage in the respiratory alveoli, bronchioles and pulmonary interstitial walls. In addition, the increased expression of the inflammatory mediators has a down regulatory effect on NK cells and CD8 cells, which are also important to clear the virus (Kaul. D (2020)). As such, in SARS-CoV-2 infections, hypercytokinemia has emerged as a main factor driving a more severe clinical outcome.

In relation to SARS-CoV-2, the present invention may be treating SARS-CoV-2 infection. The treatment may be treating COVID-19 infection. The treatment may be the treatment of COVID-19. The treatment may be the treatment of a disease that results from infection by CoV. The treatment may be the treatment of a disease that results from infection by SARS-CoV-2. The treatment may be the treatment of pneumonia caused by infection by CoV. The treatment may be the treatment of pneumonia caused by infection by SARS-CoV-2. The treatment may be the treatment of pneumonia caused by infection by COVID-19. The treatment may be the treatment of pneumonia caused by COVID-19. The treatment may be the treatment of acute respiratory syndrome (ARDS) caused by infection by SARS-CoV-2.

The infection may be moderate infection. The infection may be severe infection. The infection may be mild infection.

The treatment may be reducing complications associated with CoV infection, including hospitalization, ICU and death.

The present invention may be useful to treat acute COVID-19 infection (signs and symptoms of COVID-19 for up to 4 weeks); treat (or minimise) ongoing symptomatic COVID-19 (signs and symptoms of COVID-19 from 4 weeks up to 12 weeks); or treat or minimise post-COVID-19 syndrome (signs and symptoms that develop during or following an infection consistent with COVID-19, continue for more than 12 weeks and are not explained by an alternative diagnosis. It usually presents with clusters of symptoms, often overlapping, which can fluctuate and change over time and can affect any system in the body. Post-COVID-19 syndrome may be considered before 12 weeks while the possibility of an alternative underlying disease is also being assessed). The compounds of the present invention may treat a patient with signs and symptoms of COVID-19 for up to 4 weeks. The compounds of the present invention may treat a patient with signs and symptoms of COVID-19 from 4 weeks to 12 weeks. The compounds of the present invention may treat a patient with signs and symptoms of COVID-19 for more than 12 weeks.

The treatment may be prophylaxis, reduction or treatment of COVID persistent (also known as long COVID or post-COVID syndrome). The compounds according to the present invention can minimise the likelihood of a patient suffering from COVID persistent symptoms. The compounds according to the present invention may alternatively reduce the severity of such symptoms,

preferably may minimise the symptoms of CoV infection.

Post-COVID syndrome may be considered as signs and symptoms that develop during or following an infection consistent with COVID-19 which continue for more than 12 weeks and are not explained by an alternative diagnosis. The condition usually presents with clusters of symptoms, often overlapping, which may change over time and can affect any system within the body. Many people with post-COVID syndrome can also experience generalised pain, fatigue, persisting high temperature and psychiatric problems. Symptoms include (but are not limited to) symptoms arising in the cardiovascular, respiratory, gastrointestinal, neurological, musculoskeletal, metabolic, renal, dermatological, otolaryngological, haematological and autonomic systems, in addition to psychiatric problems, generalised pain, fatigue and persisting fever.

The treatment may be reducing the infectivity of CoV patients. The present invention achieves a rapid and significant reduction in the viral burden. Reducing the viral burden may reduce the infectiveness of patients. This is particular beneficial with patients who are asymptomatic or not very symptomatic yet have a high viral loads (e.g. TC <25). Such patients may be supercontagators or superspreaders. Administration of compounds according to the present invention upon detection of infection can reduce the viral burden and therefore reduce the infectiveness of the patient.

The treatment may result in a reduction of viral load. This may be expressed as a replication cycle threshold (Ct) value greater than 30 ($Ct > 30$), on day 6 after the administration. The treatment may reduce viral load from baseline. This may be expressed as a reduction in the percentage of patients requiring hospitalisation following administration. This may be expressed as a reduction in the percentage of patients requiring invasive mechanical ventilation and / or admission to the ICU following administration. This may be expressed as a reduction of patients who develop sequelae related to persistent disease. This may be expressed as an increase in the percentage of patients with normalization of analytical parameters chosen as poor prognosis criteria (including, for example, lymphopenia, LDH, D-dimer or PCR). This may be expressed as an increase in the percentage of patients with normalization of clinical criteria (disappearance of symptoms), including, for example: headache, fever, cough, fatigue, dyspnea (shortness of breath), arthromyalgia or diarrhoea.

Phlebovirus

Phleboviruses are negative or ambisense single-stranded, enveloped RNA viruses from the Bunyavirus family with genomes in the range of approximately 11-12kb. Phleboviruses can be broadly divided into two groups, the sandfly fever virus group (SFV) and the Uukuniemi-like virus (ULV) group. SFVs are transmitted by dipterans, including phlebotomines and mosquitoes, and are endemic in the Mediterranean and other regions. Symptoms of SFV infection include fever, headache, muscle and joint pain, fatigue, and abdominal pain. Patients typically recover within 7-14 days. In some cases, infection of the CNS can lead to encephalitis and meningitis. The ULV group is transmitted by ticks but they are non-pathogenic in humans.

The best-studied phlebovirus is Rift Valley fever (RVF) virus, which predominantly infects domesticated animals but it can also cause moderate-to-severe infection in humans. Human outbreaks of RVF have occurred in sub-Saharan Africa and Arabia via infected animals or mosquitoes. Symptoms are usually mild, flu-like and may include a biphasic fever. A small proportion of patients develop serious complications, such as hemorrhagic fever, encephalitis or

retinitis. Neurological disease is a delayed-onset, often fatal, complication, which may occur 5-30 days after the initial illness. During the 2000 Saudi outbreak, there was a 50% mortality rate for patients with CNS complications.

5 The interaction of viral proteins with the host immune response may play a role in pathogenicity and may explain why the clinical presentation of RVF varies between individuals. Chemokines, pro-inflammatory cytokines and anti-inflammatory cytokines were significantly increased (IL-8, CXCL9, MCP-1, IP-10, IL-10) or decreased (RANTES) in fatal cases during the 2010/2011 South African outbreak. In three Ugandan patients with severe hemorrhagic infection, levels of IL-8, IL-10, IP-10, MCP-2, MCP-3, fractalkine and GRO- α were increased, with MCP-2 and IP-10
10 levels correlating with viral load. The elevated levels of pro-inflammatory markers in the three Ugandan patients normalised as patients recovered. Furthermore, genetic polymorphisms in innate immune pathways, including IL-6, may be associated with the varying severity of RVFV symptoms. The host inflammatory response may affect the outcome of RVFV infection in humans and an uncontrolled inflammatory response may contribute to fatal outcomes.

15 Arenaviruses

Arenaviruses (AVs) are a family of enveloped, pleomorphic, single-stranded RNA viruses, with an approximate genome size of 11 kb spanning a small and large segment, with each segment encoding for two proteins in an ambisense manner. Transmission to humans occurs by exposure to rodents. Some arenaviruses can cause mild-to-severe illness in humans, including
20 haemorrhagic illness. After a long incubation period of 6–21 days, AV infection usually starts with fever, general weakness and malaise, followed by headache, sore throat, nausea, vomiting, diarrhea, and abdominal pain. In more severe cases, symptoms may include hemorrhaging, shock, seizures, coma and even death.

AVs can be divided into two serogroups, 'Old World' and 'New World'. 'Old World' viruses include Lassa fever and lymphocytic choriomeningitis virus. Lassa fever is endemic in Western African regions, causing approximately 5,000 deaths per year. Lassa fever causes systemic disease but the majority of infections are asymptomatic. Severe disease caused by viral haemorrhagic fever occurs in 20% of individuals. The overall fatality is about 1%. For patients admitted to hospital, the mortality rate is 15%- 25%. Fatal cases in humans are characterized by
30 necrosis in the liver, spleen and adrenal glands.

'New World' viruses include Pichinde virus and the Argentine, Bolivian and Venezuelan haemorrhagic fevers. Pichinde virus is non-pathogenic in humans. South American hemorrhagic fever has a 6–14-day prodromic phase. After 8–12 days, approximately 20–30% of patients progress to the neurologic and hemorrhagic phase. Symptoms include confusion, convulsions, coma and bleeding from body orifices. Case fatality rates within endemic regions can be 10–30%.
35

Arenaviruses initially target macrophages and dendritic cells in humans, which produce high levels of interferons and cytokines. Infection of these antigen-presenting cells is likely responsible for spreading the virus, establishing systemic infections and allowing for significant viral product and release. Infection with Lassa virus suppresses the type I interferon response, which is critical
40 for controlling the infection, leading to uncontrolled virus production. In contrast, infection with 'New World' arenaviruses is associated with high levels of interferons and cytokines in severe and fatal cases. Indeed, patients infected with Junin virus, the causative agent of Argentine haemorrhagic fever, can develop a cytokine storm and show increased levels of TNF- α , IFN- α ,

IL-6 and IL-10. Patients with severe illness and fatal cases consistently show elevated levels of TNF- α and IFN- α .

Herpesviruses

5 Herpesviruses (HSVs) are large, enveloped, double-stranded DNA viruses with complex genomes. HSV genomes range in size from 125-240kbp and encode for dozens of viral genes. Transmission of HSVs is via skin or oral contact between infected humans, with the main route being sexual transmission. Symptoms of herpes include painful blisters or ulcers at the site of infection, but most infections are asymptomatic. As such, HSVs are very prevalent in humans and animals, with an estimated 90% of the world's population is infected with either one or both of herpes simplex virus type-1 (HSV-1) and herpes simplex virus type-2 (HSV-2). HSV-1 is associated with orofacial infections, while HSV-2 can cause genital infections. Infants can also be infected with HSV-2 during childbirth, causing serious disease of the central nervous system or disseminated infection.

15 Infection with HSVs is incurable due to their ability to establish latent infection in host cells. Following uptake at the primary infection site, HSVs can establish latency in neuronal cells. For example, HSV-1 can establish latency in trigeminal ganglia, while HSV-2 can establish latency in sacral ganglia. During latency, the viral genome may become integrated into host cell DNA or it may remain extra-chromosomal. Latency-associated RNA transcripts are expressed which regulate the host cell genome and suppress host cell death mechanisms, preserving the viral infection of host cells and allowing subsequent recurrences of non-latent periods. The non-latent periods are usually symptomatic and allow the virus to infect a new host.

25 In some cases, HSV infection can lead to encephalitis. HSV encephalitis begins with flu-like symptoms and progresses into neurological deterioration that can be fatal if left untreated. Furthermore, in neonates with severe disseminated infection, the systemic inflammatory response to viral HSV infection can lead to organ damage and dysfunction, which may lead to death.

Orthomyxoviridae

The family Orthomyxoviridae is a family of negative-sense RNA viruses and contains significant pathogens of both humans and animals. There are seven genera in the family, including Influenzavirus A, Influenzavirus B, Influenzavirus C, Thogotovirus, Quaranjavirus, and Isavirus

30 1. Influenza virus

There are four classes of influenza viruses: A, B, C and D, with influenza virus A and B in particular causing winter epidemics of flu, causing around 300 to 650 thousand deaths a year. Influenza A viruses are of particular clinical significance as they have been the cause of a number of flu pandemics where many countries have been affected by large outbreaks. Influenza A viruses are negative-sense, single-stranded, segmented RNA viruses that are divided into subtypes based on two proteins on the surface of the virus: hemagglutinin (H) and neuraminidase (N). There are 18 different hemagglutinin subtypes and 11 different neuraminidase subtypes (H1 through H18 and N1 through N11, respectively), therefore there are many subtypes of influenza virus that may be circulating in the population at any given time, causing the disease known as flu.

5 Flu is characterized by a mild to severe disease which symptoms include high fever, runny nose, sore throat, muscle and joint pain, headache, coughing and tiredness, although vomiting and diarrhea can also occur in children infected with the virus. These symptoms typically appear one to four days after exposure and are generally self-limiting, however in some cases, particularly in those with weaker immune systems, complications such as pneumonia and sepsis can occur.

10 Severe influenza infection has been associated with significant pathological changes in pulmonary tissues associated with heightened levels of inflammatory cytokines and chemokines. This exuberant immune response, known as the cytokine storm, is associated with high levels of pro-inflammatory cytokines and widespread tissue damage. In fact, the term "cytokine storm" was first coined when describing the immune response to influenza-associated encephalopathy. It is thought that influenza infection of epithelial cells in the respiratory tract leads to a wave of inflammatory cytokine production from these cells, driving various interferon-regulated genes that go on to cause further downstream cytokine production through activation of innate immune cells such as macrophages, neutrophils and dendritic cells which, in severe cases, can proceed to cause tissue damage and chronic inflammation. It is this positive feedback loop of inflammation that can result in complications relating to influenza infection, and can ultimately result in death for patients most severely affected.

In compounds of the present invention; the groups can be selected in accordance with the following guidance:

20 Alkyl groups may be branched or unbranched, and preferably have from 1 to about 12 carbon atoms. One more preferred class of alkyl groups has from 1 to about 6 carbon atoms. Even more preferred are alkyl groups having 1, 2, 3 or 4 carbon atoms. Methyl, ethyl, n-propyl, isopropyl and butyl, including n-butyl, tert-butyl, sec-butyl and isobutyl are particularly preferred alkyl groups in the compounds of the present invention. As used herein, the term alkyl, unless otherwise stated, refers to both cyclic and noncyclic groups, although cyclic groups will comprise at least three carbon ring members.

30 Preferred alkenyl and alkynyl groups in the compounds of the present invention may be branched or unbranched, have one or more unsaturated linkages and from 2 to about 12 carbon atoms. One more preferred class of alkenyl and alkynyl groups has from 2 to about 6 carbon atoms. Even more preferred are alkenyl and alkynyl groups having 2, 3 or 4 carbon atoms. The terms alkenyl and alkynyl as used herein, unless otherwise stated, refer to both cyclic and noncyclic groups, although cyclic groups will comprise at least three carbon ring members.

35 Suitable aryl groups in the compounds of the present invention include single and multiple ring compounds, including multiple ring compounds that contain separate and/or fused aryl groups. Typical aryl groups contain from 1 to 3 separated or fused rings and from 6 to about 18 carbon ring atoms. Preferably aryl groups contain from 6 to about 10 carbon ring atoms. Specially preferred aryl groups include substituted or unsubstituted phenyl, substituted or unsubstituted naphthyl, substituted or unsubstituted biphenyl, substituted or unsubstituted phenanthryl, and substituted or unsubstituted anthryl.

40 Suitable heterocyclic groups include heteroaromatic and heteroalicyclic groups containing from 1 to 3 separated or fused rings and from 5 to about 18 ring atoms. Preferably heteroaromatic and heteroalicyclic groups contain from 5 to about 10 ring atoms, most preferably 5, 6 or 7 ring atoms. Suitable heteroaromatic groups in the compounds of the present invention contain one, two or three heteroatoms selected from N, O or S atoms and include, e.g., coumarinyl including 8-

coumarinyl, quinolyl including 8-quinolyl, isoquinolyl, pyridyl, pyrazinyl, pyrazolyl including pyrazol-3-yl, pyrazol-4-yl and pyrazol-5-yl, pyrimidinyl, furanyl including furan-2-yl, furan-3-yl, furan-4-yl and furan-5-yl, pyrrolyl, thienyl, thiazolyl including thiazol-2-yl, thiazol-4-yl and thiazol-5-yl, isothiazolyl, thiadiazolyl including thiadiazol-4-yl and thiadiazol-5-yl, triazolyl, tetrazolyl, isoxazolyl including isoxazol-3-yl, isoxazol-4-yl and isoxazol-5-yl, oxazolyl, imidazolyl, indolyl, isoindolyl, indazolyl, indoliziny, phthalazinyl, pteridinyl, purinyl, oxadiazolyl, thiadiazolyl, furazanyl, pyridazinyl, triazinyl, cinnoliny, benzimidazolyl, benzofuranyl, benzofurazanyl, benzothiophenyl including benzo[b]thiophen-2-yl and benzo[b]thiophen-3-yl, benzothiazolyl, benzoxazolyl, imidazo[1,2-a]pyridinyl including imidazo[1,2-a]pyridine-2-yl and imidazo[1,2-a]pyridine-3-yl, quinoxaliny, quinoxaliny, naphthyridinyl and furopyridyl. Suitable heteroalicyclic groups in the compounds of the present invention contain one, two or three heteroatoms selected from N, O or S atoms and include, e.g., pyrrolidinyl, tetrahydrofuranyl, dihydrofuranyl, tetrahydrothienyl, tetrahydrothiopyranyl, piperidinyl including piperidin-3-yl, piperidin-4-yl and piperidin-5-yl, morpholinyl, thiomorpholinyl, thioxanyl, piperazinyl, azetidiny, oxetanyl, thietanyl, homopiperidyl, oxepanyl, thiepanyl, oxazepinyl, diazepinyl, thiazepinyl, 1,2,3,6-tetrahydropyridyl, 2-pyrrolinyl, 3-pyrrolinyl, dihydropyrrolyl, indolinyl, 2H-pyranyl, 4H-pyranyl, dioxanyl, 1,3-dioxolanyl, pyrazolinyl, dithianyl, dithiolanyl, dihydropyranyl, dihydrothienyl, dihydrofuranyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, 3-azabicyclo[3.1.0]hexyl, 3-azabicyclo[4.1.0]heptyl, 3H-indolyl, and quinoliziny.

In the above mentioned groups one or more hydrogen atoms may be substituted by one or more suitable groups such as OR', =O, SR', SOR', SO₂R', NO₂, NHR', NR'R', =N-R', NHCOR', N(COR')₂, NHSO₂R', NR'C(=NR')NR'R', CN, halogen, COR', COOR', OCOR', OCONHR', OCONR'R', CONHR', CONR'R', substituted or unsubstituted C₁-C₁₂ alkyl, substituted or unsubstituted C₂-C₁₂ alkenyl, substituted or unsubstituted C₂-C₁₂ alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group, wherein each of the R' groups is independently selected from the group consisting of hydrogen, OH, NO₂, NH₂, SH, CN, halogen, COH, COalkyl, CO₂H, substituted or unsubstituted C₁-C₁₂ alkyl, substituted or unsubstituted C₂-C₁₂ alkenyl, substituted or unsubstituted C₂-C₁₂ alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group. Where such groups are themselves substituted, the substituents may be chosen from the foregoing list. When a substituent group terminates with a double bond (such as =O and =N-R') it replaces 2 hydrogen atoms in the same carbon atom.

Suitable halogen substituents in the compounds of the present invention include F, Cl, Br and I.

The term "pharmaceutically acceptable salts" refers to any salt which, upon administration to the patient is capable of providing (directly or indirectly) a compound as described herein. It will be appreciated that non-pharmaceutically acceptable salts also fall within the scope of the invention since those may be useful in the preparation of pharmaceutically acceptable salts. The preparation of salts can be carried out by methods known in the art. For instance, pharmaceutically acceptable salts of compounds provided herein are synthesized from the parent compound, which contains a basic or acidic moiety, by conventional chemical methods. Generally, such salts are, for example, prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent or in a mixture of the two. Generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol or acetonitrile are preferred. Examples of the acid addition salts include mineral acid addition salts such as, for example, hydrochloride, hydrobromide, hydroiodide, sulphate, nitrate, phosphate, and organic acid addition salts such as, for example, acetate, trifluoroacetate, maleate, fumarate, citrate,

oxalate, succinate, tartrate, malate, mandelate, methanesulfonate and *p*-toluenesulfonate. Examples of the alkali addition salts include inorganic salts such as, for example, sodium, potassium, calcium and ammonium salts, and organic alkali salts such as, for example, ethylenediamine, ethanolamine, *N,N*-dialkylenethanolamine, triethanolamine and basic amino acids salts.

The compounds of the invention may be in crystalline form either as free compounds or as solvates (e.g. hydrates, alcoholates, particularly methanolates) and it is intended that both forms are within the scope of the present invention. Methods of solvation are generally known within the art. The compounds of the invention may present different polymorphic forms, and it is intended that the invention encompasses all such forms

Any compound referred to herein is intended to represent such specific compound as well as certain variations or forms. In particular, compounds referred to herein may have asymmetric centres and therefore exist in different enantiomeric or diastereomeric forms. Thus any given compound referred to herein is intended to represent any one of a racemate, one or more enantiomeric forms, one or more diastereomeric forms, and mixtures thereof. Likewise, stereoisomerism or geometric isomerism about the double bond is also possible, therefore in some cases the molecule could exist as (*E*)-isomer or (*Z*)-isomer (*trans* and *cis* isomers). If the molecule contains several double bonds, each double bond will have its own stereoisomerism, that could be the same or different than the stereoisomerism of the other double bonds of the molecule. Furthermore, compounds referred to herein may exist as atropisomers. All the stereoisomers including enantiomers, diastereoisomers, geometric isomers and atropisomers of the compounds referred to herein, and mixtures thereof, are considered within the scope of the present invention.

In compounds of general formula **I** and **II**, particularly preferred R_1 , R_5 , R_9 , R_{11} , and R_{15} are independently selected from hydrogen and substituted or unsubstituted C_1 - C_6 alkyl. More preferred R_1 , R_5 , R_9 , R_{11} , and R_{15} are independently selected from hydrogen, substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl and substituted or unsubstituted butyl, including substituted or unsubstituted *n*-butyl, substituted or unsubstituted *tert*-butyl, substituted or unsubstituted isobutyl, and substituted or unsubstituted *sec*-butyl. Preferred substituents of said groups are OR' , $=O$, SR' , SOR' , SO_2R' , NO_2 , NHR' , $NR'R'$, $=N-R'$, $NHCOR'$, $N(COR')_2$, $NHSO_2R'$, $NR'C(=NR')NR'R'$, CN , halogen, COR' , $COOR'$, $OCOR'$, $OCONHR'$, $OCONR'R'$, $CONHR'$, $CONR'R'$, substituted or unsubstituted C_1 - C_{12} alkyl, substituted or unsubstituted C_2 - C_{12} alkenyl, substituted or unsubstituted C_2 - C_{12} alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group, wherein each of the R' groups is independently selected from the group consisting of hydrogen, OH , NO_2 , NH_2 , SH , CN , halogen, COH , $COalkyl$, CO_2H , substituted or unsubstituted C_1 - C_{12} alkyl, substituted or unsubstituted C_2 - C_{12} alkenyl, substituted or unsubstituted C_2 - C_{12} alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group. Where such groups are themselves substituted, the substituents may be chosen from the foregoing list. Hydrogen, methyl, *n*-propyl, isopropyl, isobutyl, *sec*-butyl, 4-aminobutyl, 3-amino-3-oxopropyl, benzyl, *p*-methoxybenzyl, *p*-hydroxybenzyl, and cyclohexylmethyl are the most preferred R_1 , R_5 , R_9 , R_{11} , and R_{15} groups. Specifically, particularly preferred R_1 is selected from *sec*-butyl and isopropyl, being *sec*-butyl the most preferred. Particularly preferred R_5 is selected from isobutyl and 4-aminobutyl, being isobutyl the most preferred. Particularly preferred R_{11} is methyl and isobutyl. Particularly preferred R_9 is selected from *p*-methoxybenzyl, *p*-hydroxybenzyl, and cyclohexylmethyl, being *p*-methoxybenzyl the most preferred. Particularly preferred R_{15} is selected from methyl, *n*-propyl, and benzyl, being methyl and benzyl the most preferred.

- In compounds of general formula **III**, particularly preferred R₁, R₅, R₉, and R₁₅ are independently selected from hydrogen and substituted or unsubstituted C₁-C₆ alkyl. More preferred R₁, R₅, R₉, and R₁₅ are independently selected from hydrogen, substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted n-propyl, substituted or unsubstituted isopropyl and substituted or unsubstituted butyl, including substituted or unsubstituted n-butyl, substituted or unsubstituted tert-butyl, substituted or unsubstituted isobutyl, and substituted or unsubstituted sec-butyl. Preferred substituents of said groups are OR', =O, SR', SOR', SO₂R', NO₂, NHR', NR'R', =N-R', NHCOR', N(COR')₂, NHSO₂R', NR'C(=NR')NR'R', CN, halogen, COR', COOR', OCOR', OCONHR', OCONR'R', CONHR', CONR'R', substituted or unsubstituted C₁-C₁₂ alkyl, substituted or unsubstituted C₂-C₁₂ alkenyl, substituted or unsubstituted C₂-C₁₂ alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group, wherein each of the R' groups is independently selected from the group consisting of hydrogen, OH, NO₂, NH₂, SH, CN, halogen, COH, COalkyl, CO₂H, substituted or unsubstituted C₁-C₁₂ alkyl, substituted or unsubstituted C₂-C₁₂ alkenyl, substituted or unsubstituted C₂-C₁₂ alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group. Where such groups are themselves substituted, the substituents may be chosen from the foregoing list. Hydrogen, methyl, n-propyl, isopropyl, isobutyl, sec-butyl, 4-aminobutyl, 3-amino-3-oxopropyl, benzyl, p-methoxybenzyl, p-hydroxybenzyl, and cyclohexylmethyl are the most preferred R₁, R₅, R₉, and R₁₅ groups. Specifically, particularly preferred R₁ is selected from sec-butyl and isopropyl, being sec-butyl the most preferred. Particularly preferred R₅ is selected from isobutyl and 4-aminobutyl, being isobutyl the most preferred. Particularly preferred R₉ is selected from p-methoxybenzyl, p-hydroxybenzyl, and cyclohexylmethyl, being p-methoxybenzyl the most preferred. Particularly preferred R₁₅ is selected from methyl, n-propyl, and benzyl, being methyl and benzyl the most preferred.
- In compounds of general formula **I**, **II** and **III**, particularly preferred R₈, R₁₀, R₁₂, and R₁₆ are independently selected from hydrogen and substituted or unsubstituted C₁-C₆ alkyl. More preferred R₈, R₁₀, R₁₂, and R₁₆ are independently selected from hydrogen, methyl, ethyl, n-propyl, isopropyl and butyl, including n-butyl, tert-butyl, isobutyl and sec-butyl, and even more preferred they are independently selected from hydrogen and methyl. Specifically, particularly preferred R₈, R₁₀ and R₁₂ are methyl, and particularly preferred R₁₆ is hydrogen.
- In compounds of general formula **I** and **III**, particularly preferred R₃ and R₄ are independently selected from hydrogen and substituted or unsubstituted C₁-C₆ alkyl. More preferred R₃ and R₄ are independently selected from hydrogen, substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted n-propyl, substituted or unsubstituted isopropyl, and substituted or unsubstituted butyl, including substituted or unsubstituted n-butyl, substituted or unsubstituted tert-butyl, substituted or unsubstituted isobutyl and substituted or unsubstituted sec-butyl. Preferred substituents of said groups are OR', =O, SR', SOR', SO₂R', NO₂, NHR', NR'R', =N-R', NHCOR', N(COR')₂, NHSO₂R', NR'C(=NR')NR'R', CN, halogen, COR', COOR', OCOR', OCONHR', OCONR'R', CONHR', CONR'R', substituted or unsubstituted C₁-C₁₂ alkyl, substituted or unsubstituted C₂-C₁₂ alkenyl, substituted or unsubstituted C₂-C₁₂ alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group, wherein each of the R' groups is independently selected from the group consisting of hydrogen, OH, NO₂, NH₂, SH, CN, halogen, COH, COalkyl, CO₂H, substituted or unsubstituted C₁-C₁₂ alkyl, substituted or unsubstituted C₂-C₁₂ alkenyl, substituted or unsubstituted C₂-C₁₂ alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group. Where such groups are themselves substituted, the substituents may be chosen from the foregoing list. Hydrogen, methyl, isopropyl, and sec-butyl are the most preferred R₃ and R₄ groups. Specifically, particularly preferred R₃ is selected from methyl and isopropyl and particularly preferred R₄ is

methyl or hydrogen.

In one embodiment of compounds of general formula **I**, **II** and **III**, particularly preferred R₆ and R₇ are independently selected from hydrogen and substituted or unsubstituted C₁-C₆ alkyl. More preferred R₇ is selected from hydrogen, substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted n-propyl, substituted or unsubstituted isopropyl and substituted or unsubstituted butyl, including substituted or unsubstituted n-butyl, substituted or unsubstituted tert-butyl, substituted or unsubstituted isobutyl, and substituted or unsubstituted sec-butyl. Preferred substituents of said groups are OR', =O, SR', SOR', SO₂R', NO₂, NHR', NR'R', =N-R', NHCOR', N(COR')₂, NHSO₂R', NR'C(=NR')NR'R', CN, halogen, COR', COOR', OCOR', OCONHR', OCONR'R', CONHR', CONR'R', substituted or unsubstituted C₁-C₁₂ alkyl, substituted or unsubstituted C₂-C₁₂ alkenyl, substituted or unsubstituted C₂-C₁₂ alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group, wherein each of the R' groups is independently selected from the group consisting of hydrogen, OH, NO₂, NH₂, SH, CN, halogen, COH, COalkyl, CO₂H, substituted or unsubstituted C₁-C₁₂ alkyl, substituted or unsubstituted C₂-C₁₂ alkenyl, substituted or unsubstituted C₂-C₁₂ alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group. Where such groups are themselves substituted, the substituents may be chosen from the foregoing list. More preferred R₆ is selected from hydrogen, methyl, ethyl, n-propyl, isopropyl and butyl, including n-butyl, tert-butyl, isobutyl and sec-butyl. Most preferred R₆ is selected from hydrogen and methyl and most preferred R₇ is methyl.

In another embodiment of compounds of general formula **I**, **II** and **III**, it is particularly preferred that R₆ and R₇ together with the corresponding N atom and C atom to which they are attached form a substituted or unsubstituted heterocyclic group. In this regard, preferred heterocyclic group is a heteroalicyclic group containing one, two or three heteroatoms selected from N, O or S atoms, most preferably one N atom, and having from 5 to about 10 ring atoms, most preferably 5, 6 or 7 ring atoms. A pyrrolidine group is the most preferred.

In one embodiment of compounds of general formula **I**, **II** and **III**, particularly preferred R₁₃ and R₁₄ are independently selected from hydrogen and substituted or unsubstituted C₁-C₆ alkyl. More preferred R₁₃ is selected from hydrogen, substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted n-propyl, substituted or unsubstituted isopropyl and substituted or unsubstituted butyl, including substituted or unsubstituted n-butyl, substituted or unsubstituted tert-butyl, substituted or unsubstituted isobutyl, and substituted or unsubstituted sec-butyl. Preferred substituents of said groups are OR', =O, SR', SOR', SO₂R', NO₂, NHR', NR'R', =N-R', NHCOR', N(COR')₂, NHSO₂R', NR'C(=NR')NR'R', CN, halogen, COR', COOR', OCOR', OCONHR', OCONR'R', CONHR', CONR'R', substituted or unsubstituted C₁-C₁₂ alkyl, substituted or unsubstituted C₂-C₁₂ alkenyl, substituted or unsubstituted C₂-C₁₂ alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group, wherein each of the R' groups is independently selected from the group consisting of hydrogen, OH, NO₂, NH₂, SH, CN, halogen, COH, COalkyl, CO₂H, substituted or unsubstituted C₁-C₁₂ alkyl, substituted or unsubstituted C₂-C₁₂ alkenyl, substituted or unsubstituted C₂-C₁₂ alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group. Where such groups are themselves substituted, the substituents may be chosen from the foregoing list. More preferred R₁₄ is selected from hydrogen, methyl, ethyl, n-propyl, isopropyl and butyl, including n-butyl, tert-butyl, isobutyl and sec-butyl. Most preferred R₁₃ is selected from hydrogen, methyl, isopropyl, isobutyl, and 3-amino-3-oxopropyl and most preferred R₁₄ is hydrogen.

In another embodiment of compounds of general formula **I**, **II** and **III**, it is particularly preferred

that R₁₃ and R₁₄ together with the corresponding N atom and C atom to which they are attached form a substituted or unsubstituted heterocyclic group. In this regard, preferred heterocyclic group is a heteroalicyclic group containing one, two or three heteroatoms selected from N, O or S atoms, most preferably one N atom, and having from 5 to about 10 ring atoms, most preferably 5, 6 or 7 ring atoms. A pyrrolidine group is the most preferred.

In compounds of general formula **I**, **II** and **III**, particularly preferred R₂ is selected from hydrogen, substituted or unsubstituted C₁-C₆ alkyl, and COR_a, wherein R_a is a substituted or unsubstituted C₁-C₆ alkyl, and even more preferred R_a is methyl, ethyl, n-propyl, isopropyl and butyl, including n-butyl, tert-butyl, sec-butyl and isobutyl. More preferably R₂ is hydrogen.

In compounds of general formula **I**, **II** and **III**, particularly preferred R₁₇ is selected from hydrogen, COR_a, COOR_a, CONHR_b, (C=S)NHR_b, and SO₂R_c, wherein each R_a, R_b, and R_c is preferably and independently selected from substituted or unsubstituted C₁-C₆ alkyl, substituted or unsubstituted C₂-C₆ alkenyl, substituted or unsubstituted C₂-C₆ alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group. Preferred substituents of said groups are OR', =O, SR', SOR', SO₂R', NO₂, NHR', NR'R', =N-R', NHCOR', N(COR')₂, NHSO₂R', NR'C(=NR')NR'R', CN, halogen, COR', COOR', OCOR', OCONHR', OCONR'R', CONHR', CONR'R', substituted or unsubstituted C₁-C₁₂ alkyl, substituted or unsubstituted C₂-C₁₂ alkenyl, substituted or unsubstituted C₂-C₁₂ alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group, wherein each of the R' groups is independently selected from the group consisting of hydrogen, OH, NO₂, NH₂, SH, CN, halogen, COH, COalkyl, CO₂H, substituted or unsubstituted C₁-C₁₂ alkyl, substituted or unsubstituted C₂-C₁₂ alkenyl, substituted or unsubstituted C₂-C₁₂ alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group. Where such groups are themselves substituted, the substituents may be chosen from the foregoing list. Hydrogen, COR_a, COOR_a, and SO₂R_c are the most preferred R₁₇ groups, and hydrogen, COObenzyl, CObenzo[b]thiophen-2-yl, SO₂(p-methylphenyl), COCOCH₃ and COOC(CH₃)₃ are even most preferred.

In another embodiment of compounds of general formula **I**, **II** and **III**, it is particularly preferred that Y is CO. In another embodiment, it is particularly preferred that Y is -COCH(CH₃)CO-.

In another embodiment of compounds of general formula **I**, **II** and **III**, it is particularly preferred that X is O. In another embodiment, it is particularly preferred that X is NH.

In another embodiment of compounds of general formula **I** and **II**, it is particularly preferred that n, p and q are 0. In another embodiment, it is particularly preferred that n is 1 and p and q are 0. In another embodiment, it is particularly preferred that n and p are 1 and q is 0. In another embodiment, it is particularly preferred that n, p, and q are 1. In another embodiment, it is particularly preferred that n and p are 1 and q is 2.

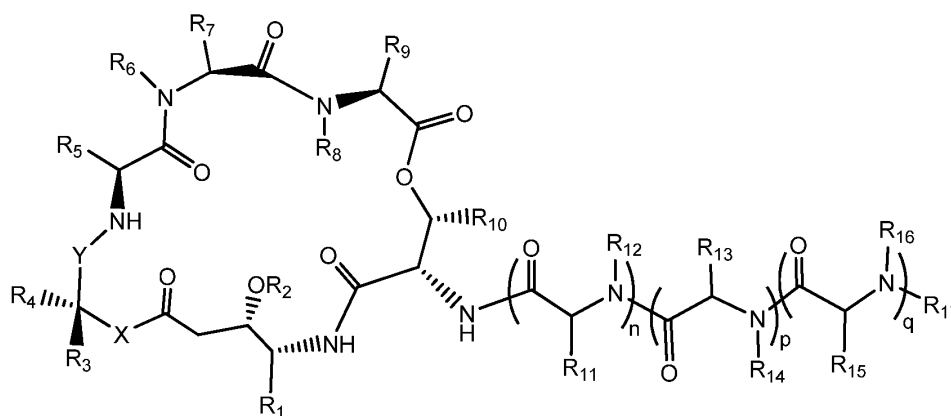
In another embodiment of compounds of general formula **III**, it is particularly preferred that p and q are 0. In another embodiment, it is particularly preferred that p is 1 and q is 0. In another embodiment, it is particularly preferred that p and q are 1. In another embodiment, it is particularly preferred that p is 1 and q is 2.

In additional preferred embodiments, the preferences described above for the different substituents are combined. The present invention is also directed to such combinations of preferred substitutions of formula **I**, **II** and **III** above.

In the present description and definitions, when there are several groups R_a , R_b , and R_c present in the compounds of the invention, and unless it is stated explicitly so, it should be understood that they can be each independently different within the given definition, i.e. R_a does not represent necessarily the same group simultaneously in a given compound of the invention.

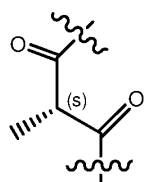
- 5 In compounds of general formula **I**, **II** and **III** when q takes a value of 2 there are two groups R_{15} and two groups R_{16} in the compound. It is hereby clarified that each R_{15} and each R_{16} group in a given compound may be independently selected among the different possibilities described above for such groups.

A particularly preferred stereochemistry for compounds of general formula **I** is

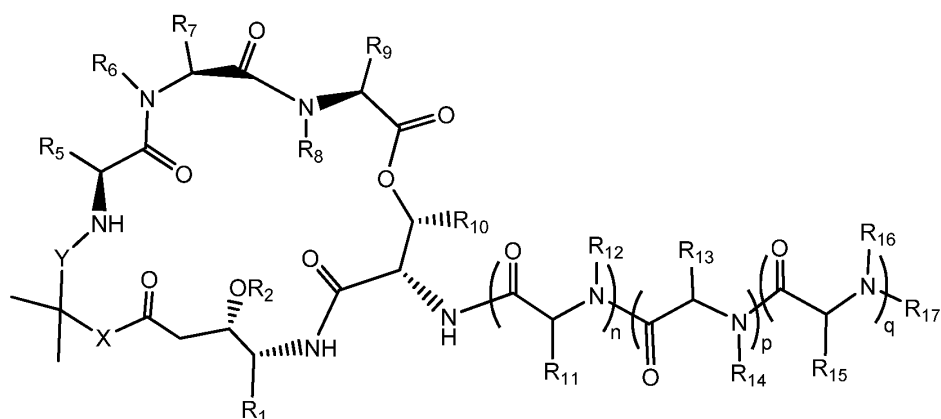


10

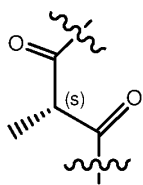
wherein X , Y , n , p , q , and R_1 - R_{17} are as defined above, and when Y is $-\text{COCH}(\text{CH}_3)\text{CO}-$ it has the following stereochemistry:



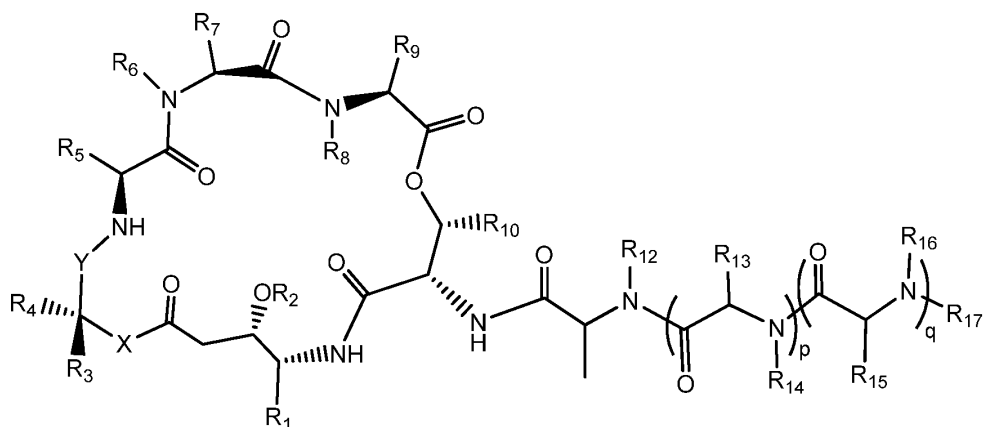
A particularly preferred stereochemistry for compounds of general formula **II** is



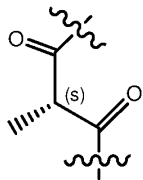
wherein X, Y, n, p, q, R₁, R₂, and R₅-R₁₇ are as defined above, and when Y is $-\text{COCH}(\text{CH}_3)\text{CO}-$ it has the following stereochemistry:



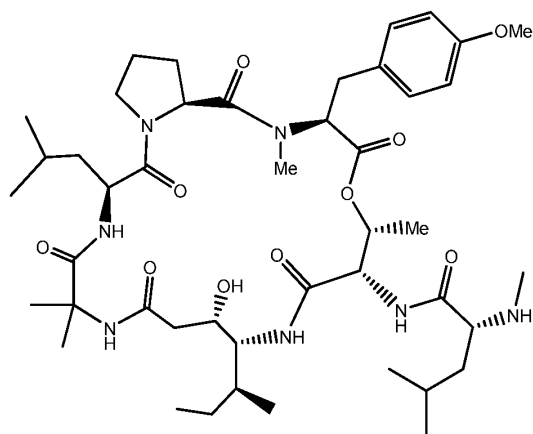
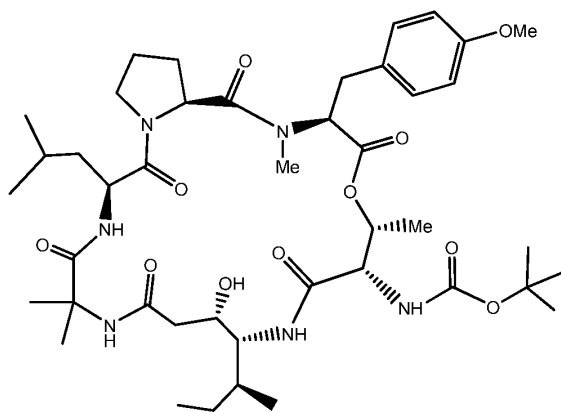
- 5 A particularly preferred stereochemistry for compounds of general formula **III** is

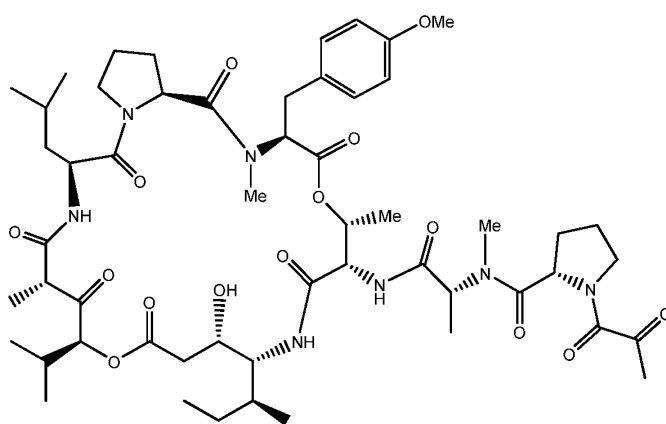
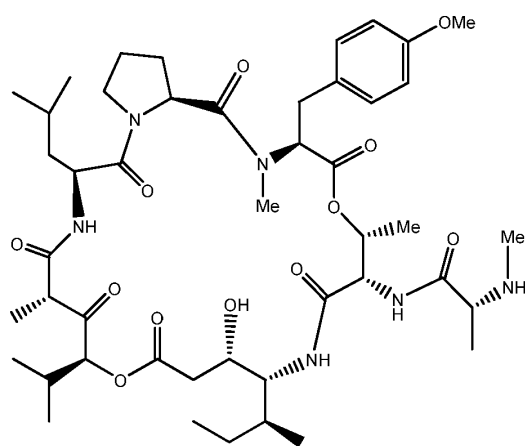
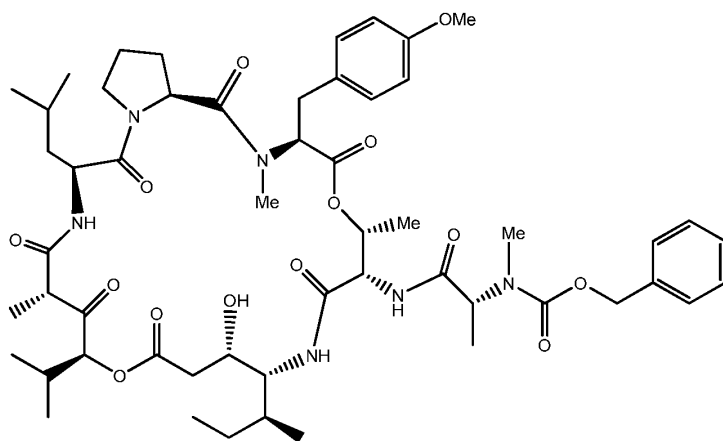


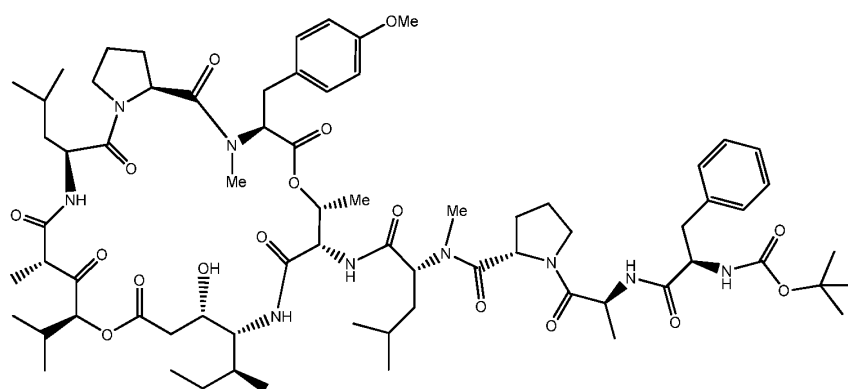
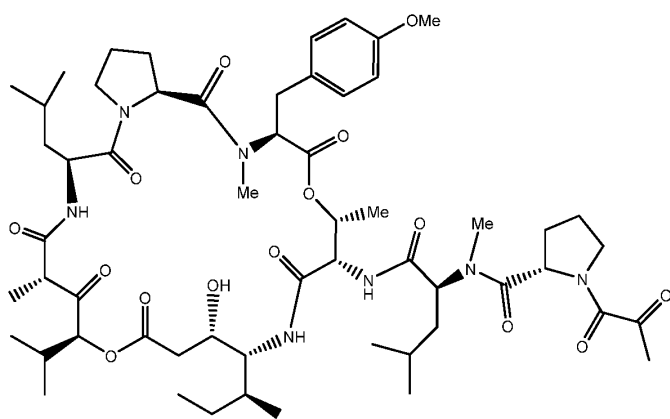
wherein X, Y, p, q, R₁-R₁₀, and R₁₂-R₁₇ are as defined above, and when Y is $-\text{COCH}(\text{CH}_3)\text{CO}-$ it has the following stereochemistry:



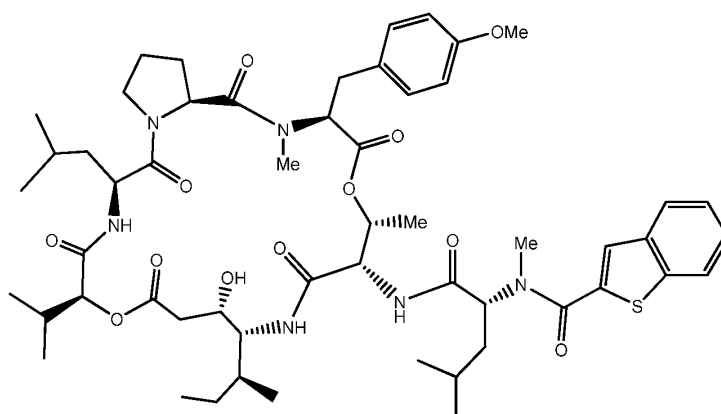
Particularly preferred compounds of the invention are the following:



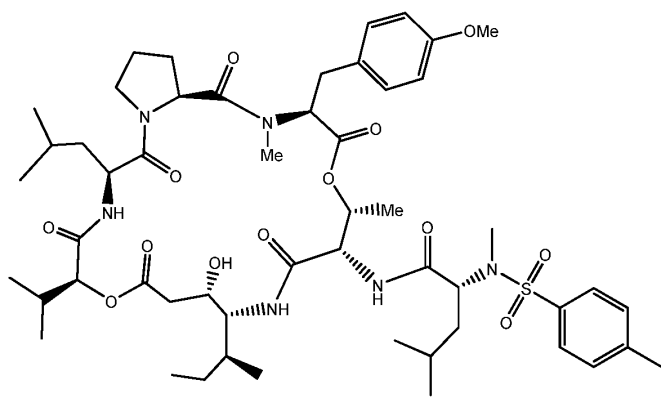




5



, and

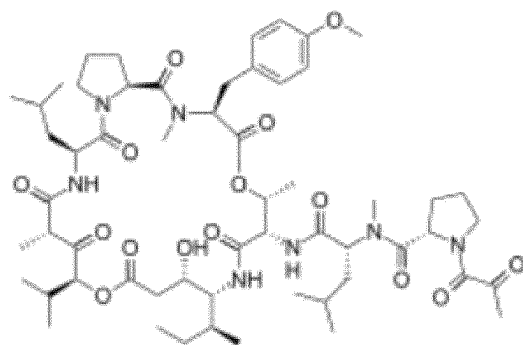


or pharmaceutically acceptable salts or stereoisomers thereof.

- 5 The compounds of general formula **I**, **II** and **III** may be prepared following any of the synthetic processes disclosed in Vera et al. Med. Res. Rev. 2002, 22(2), 102-145, WO 2011/020913 (see in particular Examples 1-5), WO 02/02596, WO 01/76616, and WO 2004/084812, which are incorporated herein by reference.

The preferred compound is PLD or pharmaceutically acceptable salts or stereoisomers thereof. Most preferred is PLD.

- 10 The chemical name of plitidepsin is (-)-(3S,6R,7S,10R,11S,15S,17S,20S,25aS)-11-hydroxy-3-(4-methoxybenzyl)-2,6,17-trimethyl-15-(1-methylethyl)-7-[[[(2R)-4-methyl-2-[methyl][(2S)-1-(2-oxopropanoyl)pyrrolidin-2-yl]carbonyl]amino]pentanoyl]amino]-10-[(1S)-1-methylpropyl]-20-(2-methylpropyl)tetradecahydro-15H-pyrrolo[2,1-f]-[1,15,4,7,10,20]dioxatetrazacyclotricosine-1,4,8,13,16,18,21(17H)-heptone corresponding to the
15 molecular formula $C_{57}H_{87}N_7O_{15}$. It has a relative molecular mass of 1110.34 g/mol and the following structure:



Plitidepsin is a cyclic depsipeptide originally isolated from a Mediterranean marine tunicate (*Aplidium albicans*) and currently manufactured by full chemical synthesis. It is licensed and marketed in Australia under the brand name plitidepsin for the treatment of multiple myeloma.

5 In eukaryotic cells, plitidepsin has been shown to target the eukaryotic elongation factor (eEF1A), which has a key role in modulating interaction with other proteins, some of which are believed to be essential in viral replication. It is noteworthy that one of the aforementioned proteins is the coronavirus N protein, which is produced abundantly within infected cells and is known to interact with elongation factor EF1A. As said above, the interaction between plitidepsin and EF1A could therefore reduce the efficacy of de novo viral capsid synthesis and consequently
10 lead to a decrease in viral load.

The present invention provides the use of a compound of the present invention in the treatment of inflammation. In particular, the invention provides the use of a compound of the present invention in the treatment of inflammation, and in particular inflammation associated with either the activation of Toll-like receptors and/or inflammation as a result of pathogen infection.

15 In one aspect of the invention, there is provided a compound of the present invention, for use in the treatment of inflammation. In another aspect of the invention, there is provided the use of a compound of the present invention, in the manufacture of a medicament for the treatment of inflammation. In another aspect of the invention, there is provided a method for the treatment of inflammation, the method comprising administering to an individual in need thereof a
20 therapeutically effective amount of a compound of the present invention.

In one embodiment, the inflammation is characterised by excessive or increased production or secretion of pro-inflammatory cytokines, and preferably at least one of IL-12, IL-10, IL-1, IL-6, IL-8, CCL-2 and TNF- α and more preferably at least one of IL-1, IL-6, IL-8 and TNF- α . In another embodiment, the inflammation is hyperinflammation. Hyperinflammation is
25 characterised by excessive cytokine production or secretion (also known as a cytokine storm). In one embodiment, hyperinflammation is defined by high levels (higher than normal levels) of at least one of the following cytokines: interleukin (IL)-1, IL-2, IL-6, GM-CSF, IFN- γ , and TNF, as well as the chemokines, C-C motif chemokine ligand (CCL)-2, CCL-3, and CCL-5. In another embodiment, the inflammation is chronic inflammation.

30 In particular embodiments, the virus is SARS-CoV-2 and the associated COVID-19 disease. Mortality associated with COVID-19 disease appears to be associated with a) severe respiratory failure secondary to respiratory distress and b) an inflammatory status caused by a cytokine storm. Thus, the proportion of patients with severe disease requiring hospitalisation with or without high-flow oxygen supplements and patients requiring mechanical ventilatory support was estimated to
35 be close to 15% and 5%, respectively, in the initial series from China. However, in Europe, the figures reported by the health authorities are higher, reaching 30% of serious cases requiring hospitalisation (in the city of Madrid) without the need for mechanical ventilation and close to 10% of patients requiring mechanical ventilation. Likewise, the duration of the need for mechanical ventilation in the Chinese series is much shorter than that reported in cities such as
40 Madrid, so the usual flow of patients to intensive care units is being altered by the prolonged stay of patients. This is putting an enormous burden on hospital services, which has made it necessary to take extraordinary, unprecedented measures. It is believed that the magnitude of the complications initially described can be avoided or reduced through the use of the present invention in patients with early-stage COVID-19 pneumonia, since once the cytokine storm and
45 respiratory distress take place, it is typically harder for an antiviral drug to have a beneficial

therapeutic effect. However, in embodiments, the compounds of the present invention are also useful at a later stage of the viral infection, for example in patients where cytokine storm and respiratory distress have taken place.

- 5 As mentioned above, in eukaryotic cells, FLIM-FRET experiments demonstrated that plitidepsin localises sufficiently close to eEF1A to suggest the formation of drug-protein complexes. A separate set of experiments carried out with ¹⁴C-plitidepsin and eEF1A purified from rabbit muscle showed that plitidepsin binds eEF1A with high affinity and a low rate of dissociation.

Plitidepsin Activity on SARS-CoV-2 in Vitro

- 10 Several *in vitro* experiments aimed at determining the effect of plitidepsin on SARS-CoV-2 were carried out and are disclosed herein. Two studies, each using Vero E6 cells infected with SARS-CoV-2 and direct quantitation of SARS-CoV-2 nucleocapsid (N) protein, which is clearly involved in the mechanism of plitidepsin-induced antiviral activity, showed that plitidepsin is a potent inhibitor of SARS-CoV-2 growth in vitro, with IC₅₀ of 0.7 to 3.0 nM. In another study, human stem cell-derived pneumocyte like cells were prophylactically exposed to 10 nm
15 plitidepsin for 1 hour and then infected with SARS-CoV-2 (4 x 10⁴ plaque forming units). After a 48 hour incubation period, both antiviral and cytotoxic plitidepsin effects were determined. Results showed that plitidepsin completely eliminated replication of SARS-CoV-2 with no observable cytotoxicity against the pneumocyte like cells.

Plitidepsin Effects on SARS-CoV-2 in Vivo

- 20 Plitidepsin demonstrated potent antiviral effects in vivo, using a previously described mouse model of adenovirus-mediated hACE2 infected with SARS-CoV-2. Plitidepsin also demonstrated potent antiviral effects in vivo using a previously described model of transgenic mice expressing hACE2 driven by the cytokeratin-18 gene promoter (K18-hACE2) infected with SARS-CoV-2.

Plitidepsin Effects on Host Inflammatory Reaction

- 25 Similar to SARS CoV, infection with SARS-CoV-2 also produces hypersecretion of several cytokines, with increasing plasma levels as the disease progresses, suggesting a possible relation between cytokine release and disease severity.

- Innate immunity is the first line of defence against invading pathogens. In the case of SARS-CoV-2, the entry of the virus into host epithelial cells is mediated by the interaction between the viral envelope spike (S) protein and the cell surface receptor ACE2. Viral RNAs, as pathogen associated molecular patterns, are then detected by the host pattern recognition receptors, which include the family of toll like receptors. Toll like receptors then upregulate antiviral and proinflammatory mediators, such as interleukin (IL) 6, IL 8, and interferon (IFN)- γ , through activation of the transcription factor nuclear factor kappa B (NF- κ B). The importance of NF- κ B in pro-inflammatory gene expression, particularly in the lungs, has been highlighted by studies
30 exploring SARS CoV infection in nonclinical species as well as in patients. In mice infected with SARS CoV, the pharmacologic inhibition of NF- κ B resulted in higher survival rates and reduced expression of tumour necrosis factor alpha (TNF α), CCL2, and CXCL2 in lungs.

- 40 As shown herein, plitidepsin induces down-regulation of NF κ B in tumour cells. Subsequently, both in vitro and ex vivo studies were also performed to assess the effects of plitidepsin on immune cells.

5 In vitro studies were performed using THP-1 cells, a spontaneously immortalised monocyte-like cell line derived from the peripheral blood of a childhood case of acute monocytic leukaemia, that is widely used for investigating monocyte structure and function. As shown in Figures 1 and 2 all the pathogen-associated molecular patterns-mimicking compounds induced the production of proinflammatory cytokines in THP-1 cells and the addition of plitidepsin significantly reduced the secretion of the proinflammatory cytokines.

10 An ex vivo study assessed the effect of plitidepsin on expression of the cytokines IL 6, IL 10, and TNF α in the lungs of mice. As shown in Figure 51 cluster of differentiation (CD)45⁺ cells from placebo treated mice were capable of producing IL 6, IL 10, and TNF α upon LPS-B5 stimulation. However, CD45⁺ cells from plitidepsin treated mice failed to show a marked increase in IL 6, IL 10, and TNF α compared with nonstimulated controls. These results suggest that the in vivo exposure to plitidepsin prevented the increased production of proinflammatory cytokines mediated by LPS-B5 in the CD45⁺ cells isolated from broncho-alveolar lavages.

15 In one aspect of the invention, there is provided a compound as defined herein, for use in the treatment of inflammation associated with (or caused by) activation of Toll-like receptors. In another aspect of the invention, there is provided the use of a compound as defined herein, in the manufacture of a medicament for the treatment of inflammation associated with activation of Toll-like receptors. In another aspect of the invention, there is provided a method for the treatment of inflammation associated with activation of Toll-like receptors, the method comprising
20 administering to an individual in need thereof a therapeutically effective amount of a compound as defined herein.

By "inflammation associated with activation of Toll-like receptors" is meant inflammation that arises from agonism (i.e. stimulation) of one or more Toll-like receptor (TLR) and activation of the toll-like receptor signalling cascade or increased signalling through at least one TLR (that may be caused by increased expression of at least one TLR). Methods for measuring activation of TLR signalling in response to a known or possible TLR agonist would be well-known to the skilled person, but in one example, levels of NF- κ B transactivation may be used as an indicator of TLR activation. As described herein NF- κ B transactivation may be measured using luciferase-tagged NF- κ B transactivation as described in the Examples. In another example, TLR activation can be
25 determined by measuring any one of IRAK1 (IL-receptor-associated kinase), IRAK4 phosphorylation and TAK1 activation (transforming growth factor- β -activated kinase-1). Other indicators of TLR activation would be known in the art (see for example, Kawai & Akira, 2007, which describes the TLR pathway). In one embodiment, the TLR is TLR-3, TLR4, TLR7 or TLR8.

35 Toll-like receptors or TLRs can be activated by microbial pathogens. In one embodiment, the microbial pathogen may be a bacteria, a virus, a parasite, fungus or other microorganism.

In a preferred embodiment, the Toll-like receptor is activated by a virus. In one embodiment, the virus is an RNA virus.

40 In one embodiment, the virus is a single stranded (+) RNA virus. In a further preferred embodiment, the virus is selected from the family: Flaviviridae, Picornaviridae, Togaviridae, Caliciviridae, Retroviridae and Coronaviridae. In an alternative embodiment, the virus is a respiratory virus.

In one embodiment, where the virus is a Flaviviridae virus, the virus is selected from Hepatitis C, Yellow fever virus (YFV), West Nile Virus (WNV), Dengue virus (DENV), Japanese encephalitis virus (JEV), Tick-borne encephalitis virus (TBEV), classical swine fever virus and Zika virus (ZIKV). In one embodiment, where the virus is a Picornaviridae virus, the virus is selected from foot and mouth disease virus, enterovirus A71, Coxsackieviruses, Rhinovirus and Hepatitis A. In another embodiment, where the virus is a Togaviridae virus, the virus is selected from Alphaviridae, and in particular, Chikungunya Virus, Sindbis Virus, Semliki Forest Virus, Eastern Equine Encephalitis Virus / Western Equine Encephalitis Virus / Venezuelan Equine Encephalitis Virus and Rubella virus. In another embodiment, where the virus is a Caliciviridae virus, the virus is selected from Lagovirus, Norovirus, Nebovirus, Recovirus, Sapovirus, Valovirus and Vesivirus, and in particular, Norwalk virus.

In one embodiment, the viral infection is a CoV infection. The term "CoV" infection means any infection from a virus in the family Coronaviridae and the sub-family *Orthocoronavirinae*. In one embodiment, the infection is from a virus in the genus *Betacoronavirus*, which includes Betacoronavirus 1, Human coronavirus HKU1, Murine coronavirus, Pipistrellus bat coronavirus HKU5, Rousettus bat coronavirus HKU9, Severe acute respiratory syndrome-related coronavirus (SARS-CoV), Tylonycteris bat coronavirus HKU4, Middle East respiratory syndrome-related coronavirus, Human coronavirus OC43 and Hedgehog coronavirus 1 (EriCoV). Preferably, the virus is selected from the α -type HCoV-229E, HCoV-NL63; the β -type HCoV-HKU1, SARS-CoV, MERS-CoV, and HCoV-OC43; and SARS-CoV-2. SARS-CoV-2 was previously called 2019-nCoV and such terms may be used interchangeably herein.

In another embodiment, where the virus is Retroviridae the virus is HIV (Human Immunodeficiency Virus).

In another embodiment, the virus is a negative sense single-stranded RNA virus. In one embodiment the virus is selected from the family Orthomyxoviridae, Phlebovirus and Arenaviridae.

In one embodiment, the Orthomyxoviridae virus is selected from Influenzavirus A, Influenzavirus B, Influenzavirus C, Thogotovirus, Quarantavirus, and Isavirus. In another embodiment, the Orthomyxoviridae virus is Influenza A, preferably selected from H1N1, H1N2 and H3N2. In another embodiment, the Orthomyxoviridae virus is influenza B, preferably selected from the Yamagata or Victoria lineages.

In one embodiment, where the virus is from the family Phlebovirus, the virus is Sandfly fever virus or Rift valley fever virus. In one embodiment, where the virus is selected from the family Arenaviridae, the virus is Pichinde virus.

In another embodiment, the virus is a dsDNA virus. In one embodiment the virus is selected from the family Herpesviridae, preferably, Herpes simplex virus 1 or 2.

In another embodiment, the virus is a respiratory virus. Respiratory viruses include rhinoviruses and enteroviruses (Picornaviridae), orthomyxoviridae, (influenza) parainfluenza, metapneumoviruses and respiratory syncytial viruses (Paramyxoviridae), coronaviruses (Coronaviridae), and several adenoviruses. With the exception of adenoviruses, all possess an RNA genome.

5 In a further embodiment, the compound of the invention may be further administered in combination with an anti-viral agent. The anti-viral agent may be administered concurrently, sequentially or separately to administration of a compound of the invention. In this example, the compound of the invention may be used as an anti-inflammatory to treat inflammation or hyperinflammation associated or as a consequence of the viral infection.

10 In another aspect of the invention, there is provided a compound as defined herein, for use in the treatment of a disorder selected from pneumonia and immunopathology, in particular hypercytokinemia (cytokine storm syndrome), sepsis and, graft-versus-host disease. In another aspect of the invention, there is provided the use of a compound as defined herein, in the manufacture of a medicament for the treatment of a disorder selected from pneumonia and immunopathology, in particular hypercytokinemia (cytokine storm syndrome), sepsis and graft-versus-host disease. In another aspect of the invention, there is provided a method for the treatment of a disorder selected from pneumonia and immunopathology, in particular hypercytokinemia (cytokine storm syndrome) sepsis and, graft-versus-host disease., the method comprising administering to an individual in need thereof a therapeutically effective amount of a compound as defined herein. In a particularly preferred embodiment, the disorder is immunopathology and in particular, hypercytokinemia.

20 In a further aspect of the invention, there is provided a compound as defined herein, for use in the treatment of pathogen-induced inflammation. In another aspect of the invention, there is provided the use of a compound as defined herein, in the manufacture of a medicament for the treatment of pathogen-induced inflammation. In another aspect of the invention, there is provided a method for the treatment of pathogen-induced inflammation, the method comprising administering to an individual in need thereof a therapeutically effective amount of a compound as defined herein.

25 In a further aspect of the invention, there is provided a compound as defined herein, for use in the combined treatment of a viral infection and inflammation associated with activation of Toll-like receptors or inflammation associated with pathogen-infection. In another aspect of the invention, there is provided the use of a compound as defined herein, in the manufacture of a medicament for the combined treatment of a viral infection and inflammation associated with activation of Toll-like receptors or inflammation associated with pathogen-infection. In another aspect of the invention, there is provided a method for the combined treatment of a viral infection and inflammation associated with activation of Toll-like receptors or inflammation associated with pathogen-infection, the method comprising administering to an individual in need thereof a therapeutically effective amount of a as defined herein.

In one embodiment, the inflammation may be hypercytokinemia.

35 By "pathogen-induced inflammation" is meant any inflammation or immunopathology caused by infection of a pathogen. Accordingly, in one aspect of the invention there is provided compounds and methods as described herein for the treatment of immunopathology. The pathogen may be a microbial pathogen. More preferably, the microbial pathogen may be a bacteria, a virus, a parasite, fungus or other microorganism. In one embodiment, the pathogen causes activation of the TLRs.

40 In an alternative embodiment, the pathogen does not cause activation of the TLRs. In a further embodiment, the pathogen causes macrophage activation. The inflammation may be chronic inflammation. In a preferred embodiment, the pathogen is a virus.

In a preferred embodiment, the Toll-like receptor is activated by a virus.

- In one embodiment, the virus is a positive sense single stranded (+) RNA virus. In a further preferred embodiment, the virus is selected from the family: Flaviviridae, Picornaviridae, Togaviridae, Caliciviridae, Retroviridae and Coronaviridae. In one embodiment, where the virus is a Flaviviridae virus, the virus is selected from Hepatitis C, Yellow fever virus (YFV), West Nile Virus (WNV), Dengue virus (DENV), Japanese encephalitis virus (JEV), Tick-borne encephalitis virus (TBEV), classical swine fever virus and Zika virus (ZIKV). In one embodiment, where the virus is a Picornaviridae virus, the virus is selected from foot and mouth disease virus, enterovirus A71, Coxsackieviruses, Rhinovirus and Hepatitis A. In another embodiment, where the virus is a Togaviridae virus, the virus is selected from Alphaviridae, and in particular, Chikungunya Virus, Sindbis Virus, Semliki Forest Virus, Eastern Equine Encephalitis Virus / Western Equine Encephalitis Virus / Venezuelan Equine Encephalitis Virus and Rubella virus. In another embodiment, where the virus is a Caliciviridae virus, the virus is selected from Lagovirus, Norovirus, Nebovirus, Recovirus, Sapovirus, Valovirus and Vesivirus, and in particular, Norwalk virus.
- 15 In one embodiment, the viral infection is a CoV infection. The term "CoV" infection means any infection from a virus in the family Coronaviridae and the sub-family *Orthocoronavirinae*. In one embodiment, the infection is from a virus in the genus *Betacoronavirus*, which includes Betacoronavirus 1, Human coronavirus HKU1, Murine coronavirus, Pipistrellus bat coronavirus HKU5, Rousettus bat coronavirus HKU9, Severe acute respiratory syndrome-related coronavirus (SARS-CoV), Tylonycteris bat coronavirus HKU4, Middle East respiratory syndrome-related coronavirus, Human coronavirus OC43 and Hedgehog coronavirus 1 (EriCoV). Preferably, the virus is selected from the α -type HCoV-229E, HCoV-NL63; the β -type HCoV-HKU1, SARS-CoV, MERS-CoV, and HCoV-OC43; and SARS-CoV-2. SARS-CoV-2 was previously called 2019-nCoV and such terms may be used interchangeably herein.
- 20 In another embodiment, where the virus is Retroviridae the virus is HIV (Human Immunodeficiency Virus).
- In another embodiment, the virus is a negative sense single-stranded RNA virus. In one embodiment the virus is selected from the family Orthomyxoviridae, Phlebovirus and Arenaviridae.
- 30 In one embodiment, the Orthomyxoviridae virus is selected from Influenzavirus A, Influenzavirus B, Influenzavirus C, Thogotovirus, Quarantavirus, and Isavirus. In another embodiment, the Orthomyxoviridae virus is Influenza A, preferably selected from H1N1, H1N2 and H3N2. In another embodiment, the Orthomyxoviridae virus is influenza B, preferably selected from the Yamagata or Victoria lineages.
- 35 In one embodiment, where the virus is from the family Phlebovirus, the virus is Sandfly fever virus or Rift valley fever virus. In one embodiment, where the virus is selected from the family Arenaviridae, the virus is Pichinde virus.
- In another embodiment, the virus is a dsDNA virus. In one embodiment the virus is selected from the family Herpesviridae, preferably, Herpes simplex virus 1 or 2.
- 40 In another embodiment, the virus is a respiratory virus. Respiratory viruses include rhinoviruses and enteroviruses (Picornaviridae), parainfluenza, metapneumoviruses and respiratory syncytial viruses (Paramyxoviridae), coronaviruses (Coronaviridae), and several adenoviruses. With the exception of adenoviruses, all possess an RNA genome.

Accordingly, in a specific embodiment, there is provided a compound as defined herein for use in the combined treatment of a SARS-CoV-2 infection and hypercytokinemia. The host inflammatory response to infection may also cause pneumonia or ARDS. Accordingly, in a further embodiment, there is provided a compound as defined herein for use in the combined treatment of a SARS-CoV-2 infection and (associated) pneumonia (also referred to as COVID-19 pneumonia) or associated ARDS. In another aspect of the invention, there is provided the use of a compound of as defined herein in the manufacture of a medicament for the combined treatment of a SARS-CoV-2 infection and hypercytokinemia. In another aspect, there is provided the use of a compound as defined herein in the manufacture of a medicament for the combined treatment of a SARS-CoV-2 infection and COVID-19 pneumonia. In another aspect of the invention, there is provided the use of a compound of as defined herein in the manufacture of a medicament for the combined treatment of a SARS-CoV-2 infection and ARDS. In another aspect of the invention, there is provided a method for the combined treatment of a SARS-CoV-2 infection and hypercytokinemia, the method comprising administering to an individual in need thereof a therapeutically effective amount of a compound as defined herein. In another embodiment, there is provided a method for the combined treatment of a SARS-CoV-2 infection and COVID-19 pneumonia, the method comprising administering to an individual in need thereof a therapeutically effective amount of a compound as defined herein. In another embodiment, there is provided a method for the combined treatment of a SARS-CoV-2 infection and ARDS, the method comprising administering to an individual in need thereof a therapeutically effective amount of a compound as defined herein. Current strategies to treat both the viral infection and the host inflammatory response to the infection (i.e. hypercytokinemia) (which may result in pneumonia or ARDS) require the presence of multiple agents to target each indication (i.e. viral replication and elimination) and inflammation separately. As shown in Example 12 and Figures 39-42, we have found that PLD can be used as a single agent to treat both the infection and subsequent hypercytokinemia or hyperinflammation (such terms may be used interchangeably herein).

Alternatively, the viral infection is a HIV (human immunodeficiency virus) infection. Accordingly, in a specific embodiment, there is provided a compound as defined herein use in the combined treatment of a HIV infection and hypercytokinemia. In another aspect of the invention, there is provided the use of a compound as defined herein in the manufacture of a medicament for the combined treatment of a HIV infection and hypercytokinemia. In another aspect of the invention, there is provided a method for the combined treatment of a HIV infection and hypercytokinemia, the method comprising administering to an individual in need thereof a therapeutically effective amount of a compound as defined herein.

Compounds of the invention may be used in pharmaceutical compositions having biological/pharmacological activity for the treatment of the above mentioned conditions. These pharmaceutical compositions comprise a compound of the invention together with a pharmaceutically acceptable carrier. The term "carrier" refers to a diluent, adjuvant, excipient or vehicle with which the active ingredient is administered. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin, 1995. Examples of pharmaceutical compositions include any solid (tablets, pills, capsules, granules, etc.) or liquid (solutions, suspensions, emulsions, etc.) compositions for oral, topical or parenteral administration. Pharmaceutical compositions containing compounds of the invention may be delivered by liposome or nanosphere encapsulation, in sustained release formulations or by other standard delivery means.

An exemplary composition is in the form of powder for solution for infusion. For example,

5 compositions as described in WO9942125. For example, a lyophilised preparation of a compound of the invention including water-soluble material and secondly a reconstitution solution of mixed solvents. A particular example is a lyophilised preparation of PLD and mannitol and a reconstitution solution of mixed solvents, for example PEG-35 castor oil, ethanol and water for injections. Each vial, for example may contain 2 mg of PLD. After reconstitution, each mL of reconstituted solution may contain: 0.5 mg of PLD, 158 mg of PEG-35 castor oil, and ethanol 0.15 mL/mL.

10 The specific dosage and treatment regimen for any particular patient may be varied and will depend upon a variety of factors, including the activity of the specific compound employed, the particular formulation being used, the mode of application, the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, reaction sensitivities, and the severity of the particular disease or condition being treated.

In embodiments of the invention, the compounds of the present invention may be administered according to a dosing regimen of a daily dose.

15 In embodiments of the invention, the compounds of the present invention may be administered according to a dosing regimen of a once daily dose.

20 In further embodiments, the compounds of the present invention may be administered according to a dosing regimen of a daily dose for 10 days, 9 days, 8 days, 7 days, 6 days, 5 days, 4 days, 3 days, 2 days or 1 day. Preferred regimen is 2-5 days, or 3-5 days, or 3, 4 or 5 days, most preferably 3 days or 5 days.

The dose may be a dose of 5mg a day or less, 4.5mg a day or less, 4mg a day or less, 3.5mg a day or less, 3mg a day or less, 2.5mg a day or less or 2mg a day or less.

25 Particular doses include 0.5 mg/day, 1 mg/day, 1.5 mg/day, 2 mg/day, 2.5 mg/day, 3 mg/day, 3.5 mg/day, 4 mg/day, 4.5 mg/day, or 5mg/day. Preferred doses are 1 mg/day, 1.5 mg/day, 2 mg/day and 2.5 mg/day.

30 In further embodiments, the compounds of the present invention may be administered according to a total dose of 1-50 mg, 1-40 mg, 1-30 mg, 1-20 mg, 1-15 mg, 3-15 mg, 3-12 mg, 4-12 mg, 4-10 mg, or 4.5-10 mg. Total doses may be 4mg, 4.5mg, 5mg, 5.5mg, 6mg, 6.5mg, 7mg, 7.5mg, 8mg, 8.5mg, 9mg, 9.5mg or 10mg. Preferred total doses are 4.5mg, 5mg, 6mg, 7.5mg, 8mg, 9mg or 10mg. The total dose may be split over 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 days, preferably 3 days or 5 days.

In a particular embodiment, the compounds of the present invention may be administered according to a dosing regimen of a once daily dose for 5 days, at a dose of 2.5mg a day or less.

35 In a further embodiment, the compounds of the present invention may be administered according to a dosing regimen of a once daily dose for 5 days, at a dose of 2mg a day or less.

In a further embodiment, the compounds of the present invention may be administered according to a dosing regimen of a once daily dose for 3 days, at a dose of 1.5mg a day or less.

In a further embodiment, the compounds of the present invention may be administered according to a dosing regimen of a once daily dose for 3 days, at a dose of 2mg a day or less.

In a further embodiment, the compounds of the present invention may be administered according to a dosing regimen of a once daily dose for 3 days, at a dose of 2.5mg a day or less.

In a further embodiment, the compounds of the present invention may be administered according to a dosing regimen of a once daily dose for 3 days, at a dose of 1.5mg a day.

- 5 In a further embodiment, the compounds of the present invention may be administered according to a dosing regimen of a once daily dose for 3 days, at a dose of 2.0mg a day.

In a further embodiment, the compounds of the present invention may be administered according to a dosing regimen of a once daily dose for 3 days, at a dose of 2.5mg a day.

- 10 In a further embodiment, the compounds of the present invention may be administered according to a dosing regimen of a once daily dose for 3 days, at a dose of 1.5 to 2.5mg a day.

An alternative regimen is a single dose on day 1. The single dose may be 1-10 mg, 4-10 mg, 4.5-10 mg; 4mg, 4.5mg, 5mg, 5.5mg, 6mg, 6.5mg, 7mg, 7.5mg, 8mg, 8.5mg, 9mg, 9.5mg or 10mg; preferably 4.5mg, 5mg, 6mg, 7.5mg, 8mg, 9mg or 10mg; more preferably 5-9 mg, 6.5-8.5 mg, 7-8 mg or 7.5 mg.

- 15 In a further embodiment, the compounds of the present invention may be administered according to the present invention, wherein the compounds of the present invention are administered with a corticosteroid. Preferably the corticosteroid is dexamethasone.

- 20 The corticosteroid may be administered daily with the compounds of the present invention. Administration may be sequential, concurrent or consecutive. The corticosteroid may be further administered on the days following administration of compounds according to the present invention. By way of example, with a 3 day dosing regimen, the corticosteroid may be administered on days 1-3 and then further administered daily for 3, 4, 5, 6, 7, 8, 9 or 10 or more further days.

- 25 In a particular embodiment, the corticosteroid may be administered is administered on days 1-3 as an intravenous administration and then on days 6-10 as an oral administration. In a further embodiment, the dosage of corticosteroid may be higher during the co-administration phase with the compounds of the present invention, and is lowered during the subsequent days.

Particular dosing schedules include:

- 30 • Plitidepsin 1.5 mg/day intravenous (IV) combined with dexamethasone 6.6 mg/day IV on Days 1 to 3, followed by dexamethasone 6 mg/day oral administration (PO)/IV from Day 4 and up to Day 10 (as per physician judgement according to patient clinical condition and evolution).
- 35 • Plitidepsin 2.0 mg/day intravenous (IV) combined with dexamethasone 6.6 mg/day IV on Days 1 to 3, followed by dexamethasone 6 mg/day oral administration (PO)/IV from Day 4 and up to Day 10 (as per physician judgement according to patient clinical condition and evolution).
- Plitidepsin 2.5 mg/day intravenous (IV) combined with dexamethasone 6.6 mg/day IV on Days 1 to 3, followed by dexamethasone 6 mg/day oral administration (PO)/IV from Day 4

and up to Day 10 (as per physician judgement according to patient clinical condition and evolution).

5 In an embodiment, to avoid administration-related infusion reactions, patients may receive the following medications 20 to 30 minutes prior to starting the infusion with a compound according to the present invention:

- Ondansetron 8 mg IV (or equivalent)
- Diphenhydramine hydrochloride 25 mg IV (or equivalent)
- Ranitidine 50 mg IV (or equivalent)
- Dexamethasone 6.6 mg IV (which is included in the schedule above)

10 Additionally, on Days 4 and 5 patients treated with compounds according to the present invention may receive ondansetron 4 mg twice a day PO.

Doses of dexamethasone, ondansetron and ranitidine are herein defined on the basis of the base form. The dose of diphenhydramine hydrochloride is given on the basis of the hydrochloride salt. Doses of compounds of the invention are given on the basis of the base form.

15 The daily doses may be administered as an infusion. The infusion may be a 1 hour infusion, a 1.5 hour infusion, a 2 hour infusion, a 3 hour infusion or longer. Preferably, the infusion is 1.5 hours.

In certain embodiments, the dose may be administered according to a regimen which uses a loading dose and a maintenance dose. Loading/maintenance doses according to the present invention includes:

20 a loading dose of 2.5 mg for day 1, and followed by a maintenance dose of 2 mg/day for subsequent days;

a loading dose of 2.5 mg for day 1, and followed by a maintenance dose of 1.5 mg/day for subsequent days;

25 a loading dose of 2.5 mg for day 1, and followed by a maintenance dose of 1 mg/day for subsequent days;

a loading dose of 2.5 mg for day 1, and followed by a maintenance dose of 0.5 mg/day for subsequent days;

a loading dose of 2 mg for day 1, and followed by a maintenance dose of 1.5 mg/day for subsequent days;

30 a loading dose of 2 mg for day 1, and followed by a maintenance dose of 1 mg/day for subsequent days;

a loading dose of 2 mg for day 1, and followed by a maintenance dose of 0.5 mg/day for subsequent days;

35 a loading dose of 1.5 mg for day 1, and followed by a maintenance dose of 1 mg/day for subsequent days;

a loading dose of 1.5 mg for day 1, and followed by a maintenance dose of 0.5 mg/day

for subsequent days; and

a loading dose of 1 mg for day 1, and followed by a maintenance dose of 0.5 mg/day for subsequent days.

5 According to a further embodiment, the daily dose may be reduced in the final day or days of the regimen.

According to a further embodiment, if the daily dose is 2 mg, the dose may be reduced to 1 mg on days 4 and 5.

Particular regimens include:

10 - 1mg of plitidepsin administered as a 1.5-hour infusion, once a day for 5 consecutive days. (total dose 5mg);

- 2mg of plitidepsin administered as a 1.5-hour infusion, once a day for 5 consecutive days. At the discretion of the researcher, the dose may be reduced to 1 mg / day on days 4 and 5 (total dose 8-10mg);

15 - 1.5mg of plitidepsin administered as a 1.5-hour infusion, once a day for 3 consecutive days. (total dose 4.5mg);

- 2mg of plitidepsin administered in 1.5 hour infusion, once a day for 3 consecutive days. (total dose 6mg); and

- 2.5mg of plitidepsin administered as a 1.5-hour infusion, once a day for 3 consecutive days. (total dose 7.5mg).

20 A single dose regimen includes:

- Plitidepsin administered as a 1.5-hour infusion, once on day 1 at a dose of 1-10 mg, 4-10 mg, 4.5-10 mg, 4mg, 4.5mg, 5mg, 5.5mg, 6mg, 6.5mg, 7mg, 7.5mg, 8mg, 8.5mg, 9mg, 9.5mg or 10mg, preferably 4.5mg, 5mg, 6mg, 7.5mg, 8mg, 9mg or 10mg, more preferably 5-9 mg, 6.5-8.5 mg, 7-8 mg or most preferably 7.5 mg.
- 25 • The single dose regimen may further include the following prophylactic medications 20-30 minutes prior to plitidepsin infusion:
 - Diphenhydramine hydrochloride 25 mg i.v.,
 - Ranitidine 50 mg i.v.
 - Dexamethasone 6.6 mg intravenously.
 - 30 - Ondansetron 8 mg i.v. in slow infusion of 15 minutes
- Ondansetron 4 mg orally may be given every 12 hours for 3 days after plitidepsin administration to relieve drug-induced nausea and vomiting. If plitidepsin is administered in the morning the patient may receive the first dose of ondansetron in the afternoon.

35 According to further embodiments, patients may be selected for treatment with compounds of the present invention based on clinical parameters and/or patient characteristics. Suitable parameters may be measurements disclosed in the present application.

The regimens and doses outlined above apply to both methods of treatment according to the

present invention, use, and use of a compound as defined herein in the manufacture of medicaments as defined herein.

5 In a further aspect of the invention, there is provided a method of decreasing the expression and/or secretion of one or more pro-inflammatory cytokine in a subject or in a sample obtained from a subject or in a cell culture, the method comprising administering a compound as defined herein.

In another aspect of the invention, there is provided a method of increasing macrophage activation in a subject or in a sample obtained from a subject or in a cell culture, the method comprising administering a compound as defined herein.

10 In embodiments, the present invention is directed to a compound for use according to the present invention, wherein the compound is administered in combination with one or more of the following prophylactic medications: diphenhydramine hydrochloride, ranitidine, dexamethasone, ondansetron. In particular, one or more of diphenhydramine hydrochloride 25 mg iv or equivalent; Ranitidine 50 mg iv or equivalent; Dexamethasone 8 mg intravenous; Ondansetron 8 mg i.v. in slow infusion of 15 minutes or equivalent. Patients may receive said prophylactic
15 medications 20-30 minutes before the infusion of plitidepsin. Dexamethasone 8 mg intravenous may be dexamethasone phosphate leading to 6.6 mg dexamethasone base.

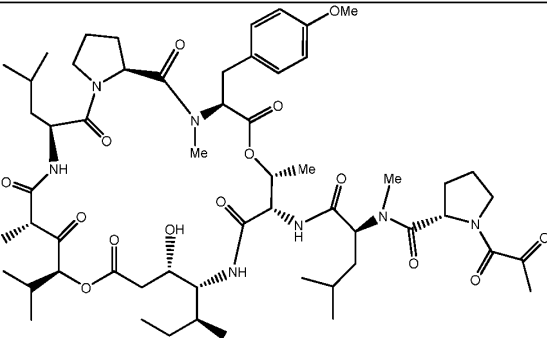
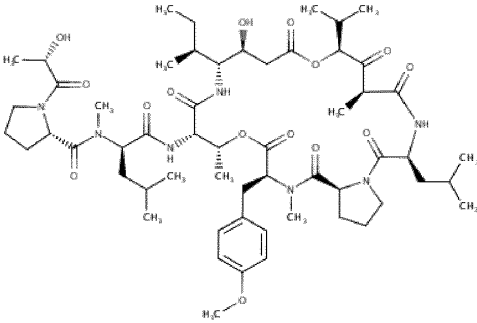
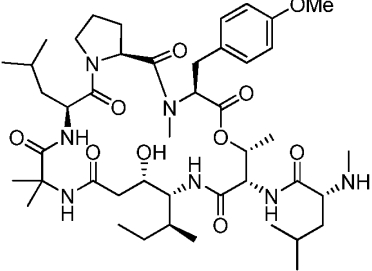
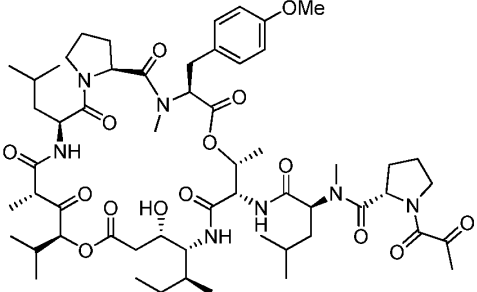
20 To provide a more concise description, some of the quantitative expressions given herein are not qualified with the term "about". It is understood that, whether the term "about" is used explicitly or not, every quantity given herein is meant to refer to the actual given value, and it is also meant to refer to the approximation to such given value that would reasonably be inferred based on the ordinary skill in the art, including equivalents and approximations due to the experimental and/or measurement conditions for such given value.

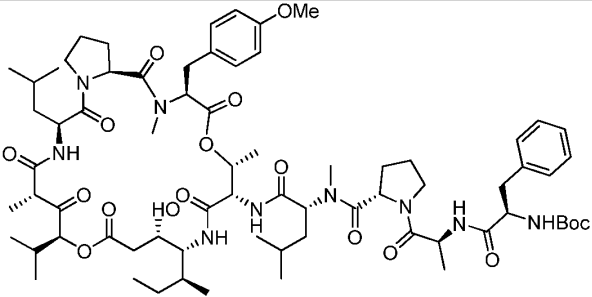
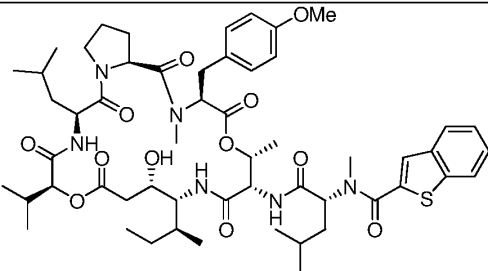
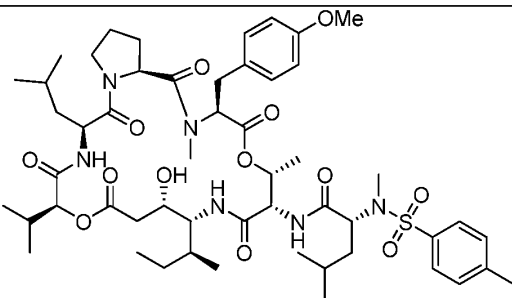
25 While the foregoing disclosure provides a general description of the subject matter encompassed within the scope of the present invention, including methods, as well as the best mode thereof, of making and using this invention, the following examples are provided to further enable those skilled in the art to practice this invention and to provide a complete written description thereof. However, those skilled in the art will appreciate that the specifics of these examples should not be read as limiting on the invention, the scope of which should be apprehended from the claims and equivalents thereof appended to this disclosure. Various further aspects and embodiments of
30 the present invention will be apparent to those skilled in the art in view of the present disclosure.

EXAMPLES

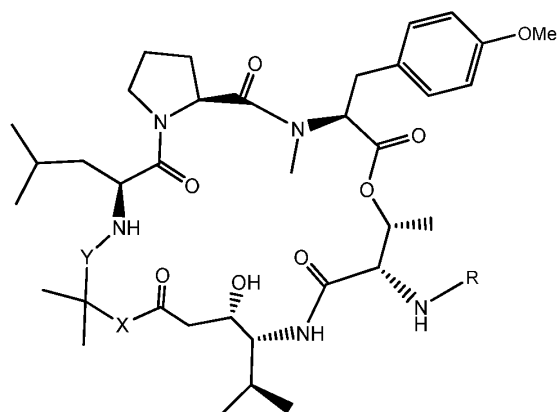
35 Compounds of the present invention can be obtained according to the processes set out in the literature, for example: Vera et al. Med. Res. Rev. 2002, 22(2), 102-145, WO 2011/020913 (see in particular Examples 1-5), WO 02/02596, WO 01/76616, and WO 2004/084812, the contents of which are incorporated herein by reference.

Particular compounds used in experiments of the present invention are:

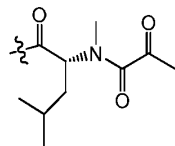
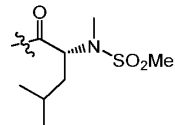
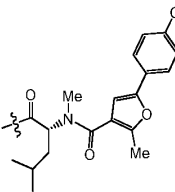
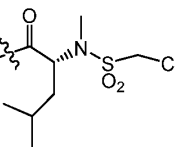
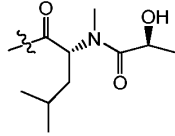
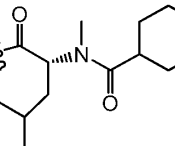
Compound	Structure
PLD	
DidemninB (compound 240)	
Compound 3	
Compound 8	

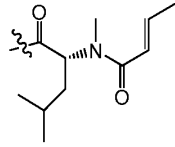
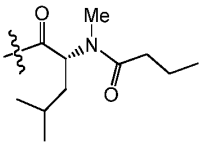
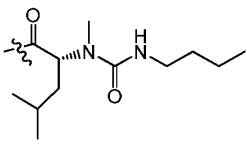
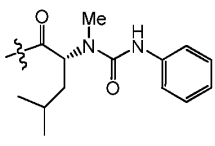
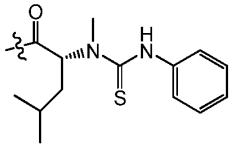
Compound 9	
Compound 10	
Compound 11	

Following the procedures described in WO 02/02596 and in the specification, and further disclosed in the previous examples, the following compounds are obtainable:

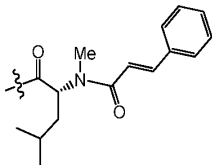
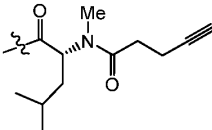
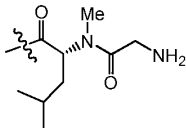
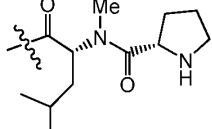
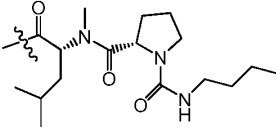
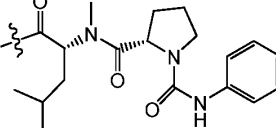


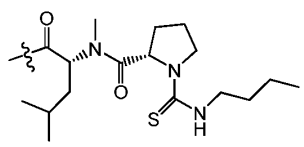
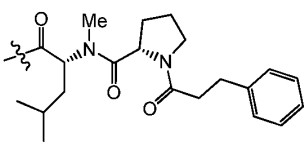
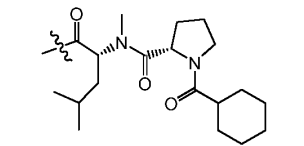
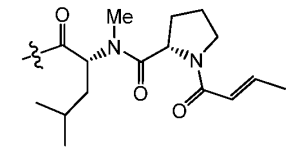
Compound	X	Y	R
12	O	CO	
13	NH	CO	
14	O	-COCH(CH ₃)CO-	
15	NH	-COCH(CH ₃)CO-	
16	O	CO	
17	NH	CO	
18	O	-COCH(CH ₃)CO-	
19	NH	-COCH(CH ₃)CO-	
20	O	CO	
21	NH	CO	
22	O	-COCH(CH ₃)CO-	
23	NH	-COCH(CH ₃)CO-	

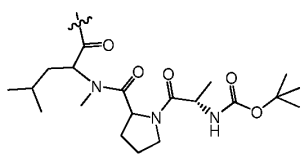
Compound	X	Y	R
24	O	CO	
25	NH	CO	
26	O	-COCH(CH ₃)CO-	
27	NH	-COCH(CH ₃)CO-	
28	O	CO	
29	NH	CO	
30	O	-COCH(CH ₃)CO-	
31	NH	-COCH(CH ₃)CO-	
32	O	CO	
33	NH	CO	
34	O	-COCH(CH ₃)CO-	
35	NH	-COCH(CH ₃)CO-	
36	O	CO	
37	NH	CO	
38	O	-COCH(CH ₃)CO-	
39	NH	-COCH(CH ₃)CO-	
40	O	CO	
41	NH	CO	
42	O	-COCH(CH ₃)CO-	
43	NH	-COCH(CH ₃)CO-	
44	O	CO	
45	NH	CO	
46	O	-COCH(CH ₃)CO-	
47	NH	-COCH(CH ₃)CO-	

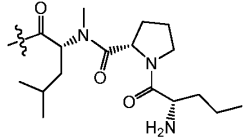
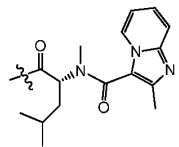
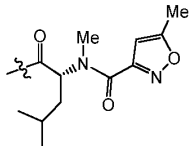
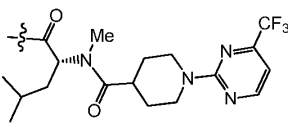
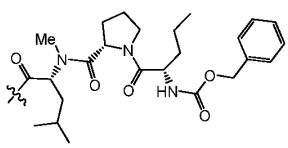
Compound	X	Y	R
48	O	CO	
49	NH	CO	
50	O	-COCH(CH ₃)CO-	
51	NH	-COCH(CH ₃)CO-	
52	O	CO	
53	NH	CO	
54	O	-COCH(CH ₃)CO-	
55	NH	-COCH(CH ₃)CO-	
56	O	CO	
57	NH	CO	
58	O	-COCH(CH ₃)CO-	
59	NH	-COCH(CH ₃)CO-	
60	O	CO	
61	NH	CO	
62	O	-COCH(CH ₃)CO-	
63	NH	-COCH(CH ₃)CO-	
64	O	CO	
65	NH	CO	
66	O	-COCH(CH ₃)CO-	
67	NH	-COCH(CH ₃)CO-	

Compound	X	Y	R
----------	---	---	---

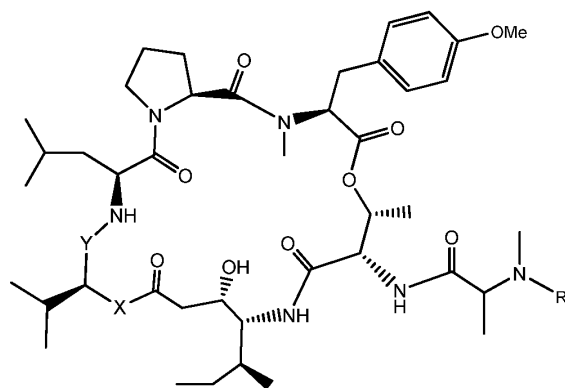
68	O	CO	
69	NH	CO	
70	O	-COCH(CH ₃)CO-	
71	NH	-COCH(CH ₃)CO-	
72	O	CO	
73	NH	CO	
74	O	-COCH(CH ₃)CO-	
75	NH	-COCH(CH ₃)CO-	
76	O	CO	
77	NH	CO	
78	O	-COCH(CH ₃)CO-	
79	NH	-COCH(CH ₃)CO-	
80	O	CO	
81	NH	CO	
82	O	-COCH(CH ₃)CO-	
83	NH	-COCH(CH ₃)CO-	
84	O	CO	
85	NH	CO	
86	O	-COCH(CH ₃)CO-	
87	NH	-COCH(CH ₃)CO-	
88	O	CO	
89	NH	CO	
90	O	-COCH(CH ₃)CO-	
91	NH	-COCH(CH ₃)CO-	

92	O	CO	
93	NH	CO	
94	O	-COCH(CH ₃)CO-	
95	NH	-COCH(CH ₃)CO-	
96	O	CO	
97	NH	CO	
98	O	-COCH(CH ₃)CO-	
99	NH	-COCH(CH ₃)CO-	
100	O	CO	
101	NH	CO	
102	O	-COCH(CH ₃)CO-	
103	NH	-COCH(CH ₃)CO-	
104	O	CO	
105	NH	CO	
106	O	-COCH(CH ₃)CO-	
107	NH	-COCH(CH ₃)CO-	

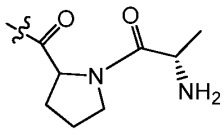
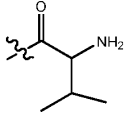
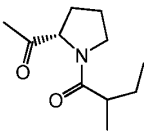
Compound	X	Y	R
108	O	CO	
109	NH	CO	
110	O	-COCH(CH ₃)CO-	
111	NH	-COCH(CH ₃)CO-	

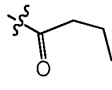
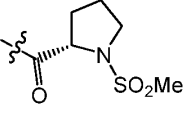
112	O	CO	
113	NH	CO	
114	O	-COCH(CH ₃)CO-	
115	NH	-COCH(CH ₃)CO-	
116	O	CO	
117	NH	CO	
118	O	-COCH(CH ₃)CO-	
119	NH	-COCH(CH ₃)CO-	
120	O	CO	
121	NH	CO	
122	O	-COCH(CH ₃)CO-	
123	NH	-COCH(CH ₃)CO-	
124	O	CO	
125	NH	CO	
126	O	-COCH(CH ₃)CO-	
127	NH	-COCH(CH ₃)CO-	
128	O	CO	
129	NH	CO	
130	O	-COCH(CH ₃)CO-	
131	NH	-COCH(CH ₃)CO-	

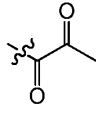
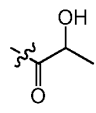
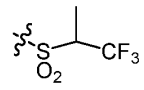
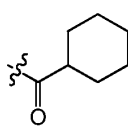
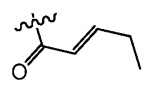
Following the procedures described in WO 02/02596 and in the specification, and further disclosed in the previous examples, the following compounds are obtainable:

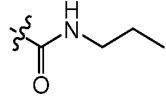
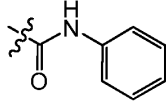


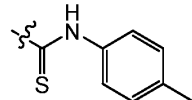
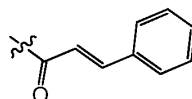
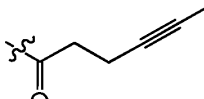
Compound	X	Y	R
132	O	CO	
133	NH	CO	
134	O	-COCH(CH ₃)CO-	
135	NH	-COCH(CH ₃)CO-	
136	O	CO	
137	NH	CO	
138	O	-COCH(CH ₃)CO-	
139	NH	-COCH(CH ₃)CO-	
140	O	CO	
141	NH	CO	
142	O	-COCH(CH ₃)CO-	
143	NH	-COCH(CH ₃)CO-	
144	O	CO	
145	NH	CO	
146	O	-COCH(CH ₃)CO-	
147	NH	-COCH(CH ₃)CO-	

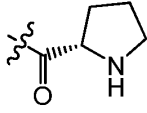
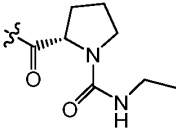
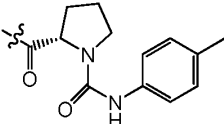
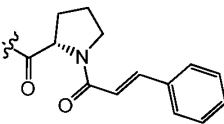
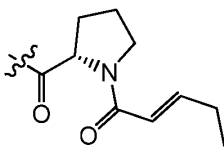
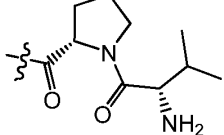
Compound	X	Y	R
148	O	CO	
149	NH	CO	
150	O	-COCH(CH ₃)CO-	
151	NH	-COCH(CH ₃)CO-	
152	O	CO	
153	NH	CO	
154	O	-COCH(CH ₃)CO-	
155	NH	-COCH(CH ₃)CO-	
156	O	CO	
157	NH	CO	
158	O	-COCH(CH ₃)CO-	
159	NH	-COCH(CH ₃)CO-	

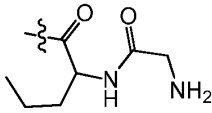
Compound	X	Y	R
160	O	CO	
161	NH	CO	
162	O	-COCH(CH ₃)CO-	
163	NH	-COCH(CH ₃)CO-	
164	O	CO	
165	NH	CO	
166	O	-COCH(CH ₃)CO-	
167	NH	-COCH(CH ₃)CO-	

168	O	CO	
169	NH	CO	
170	O	-COCH(CH ₃)CO-	
171	NH	-COCH(CH ₃)CO-	
Compound	X	Y	R
172	O	CO	
173	NH	CO	
174	O	-COCH(CH ₃)CO-	
175	NH	-COCH(CH ₃)CO-	
176	O	CO	-SO ₂ Me
177	NH	CO	
178	O	-COCH(CH ₃)CO-	
179	NH	-COCH(CH ₃)CO-	
180	O	CO	
181	NH	CO	
182	O	-COCH(CH ₃)CO-	
183	NH	-COCH(CH ₃)CO-	
184	O	CO	
185	NH	CO	
186	O	-COCH(CH ₃)CO-	
187	NH	-COCH(CH ₃)CO-	
188	O	CO	
189	NH	CO	
190	O	-COCH(CH ₃)CO-	
191	NH	-COCH(CH ₃)CO-	

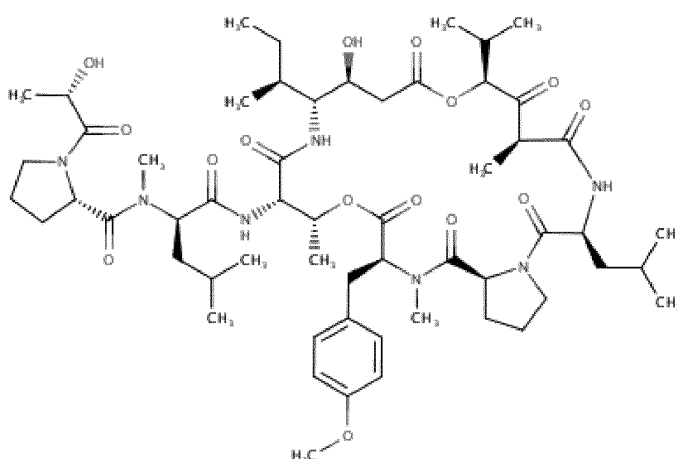
192	O	CO	
193	NH	CO	
194	O	-COCH(CH ₃)CO-	
195	NH	-COCH(CH ₃)CO-	
196	O	CO	
197	NH	CO	
198	O	-COCH(CH ₃)CO-	
199	NH	-COCH(CH ₃)CO-	

Compound	X	Y	R
200	O	CO	
201	NH	CO	
202	O	-COCH(CH ₃)CO-	
203	NH	-COCH(CH ₃)CO-	
204	O	CO	
205	NH	CO	
206	O	-COCH(CH ₃)CO-	
207	NH	-COCH(CH ₃)CO-	
208	O	CO	
209	NH	CO	
210	O	-COCH(CH ₃)CO-	
211	NH	-COCH(CH ₃)CO-	
Compound	X	Y	R

212	O	CO	
213	NH	CO	
214	O	-COCH(CH ₃)CO-	
215	NH	-COCH(CH ₃)CO-	
216	O	CO	
217	NH	CO	
218	O	-COCH(CH ₃)CO-	
219	NH	-COCH(CH ₃)CO-	
220	O	CO	
221	NH	CO	
222	O	-COCH(CH ₃)CO-	
223	NH	-COCH(CH ₃)CO-	
224	O	CO	
225	NH	CO	
226	O	-COCH(CH ₃)CO-	
227	NH	-COCH(CH ₃)CO-	
228	O	CO	
229	NH	CO	
230	O	-COCH(CH ₃)CO-	
231	NH	-COCH(CH ₃)CO-	
232	O	CO	
233	NH	CO	
234	O	-COCH(CH ₃)CO-	
235	NH	-COCH(CH ₃)CO-	

236	O	CO	
237	NH	CO	
238	O	-COCH(CH ₃)CO-	
239	NH	-COCH(CH ₃)CO-	

A further compound is Compound 240, known as DidemninB and shown by the structure below:



5 EXAMPLE 1

As shown in Figure 1, PLD inhibits *in vitro* the transactivation of NF-κB.

We checked whether the transcriptional activity of NFκB was regulated by plitidepsin. To that end, we took advantage of THP-1 cells stably transfected with an NFκB luciferase reporter plasmid. We treated the cells with 100 ng/mL TNFα (an activator of NF-κB), 500 μg/mL poly I:C (TLR3 ligand), 10 μg/mL LPS-B5 (TLR4 ligand) or 10 μg/mL Resiquimod (TLR-7/8 ligand). The compounds were used either alone (Fig. 1A grey bars) or combined with 100 nM of plitidepsin (Fig. 1A black bars) for 6 hours, and quantified the luciferase activity under each condition. In the presence of each one of the TLR ligands, plitidepsin clearly inhibited the production of luciferase indicating that transactivation from NF-κB was inhibited in the presence of the drug. Survival was analysed with the MTT assay (Fig. 1B grey bars (activators) and red bars (activators combined with 100 nM of plitidepsin)). No cytotoxic effect was detected.

As shown in Figure 2, PLD also inhibits *in vitro* the secretion of the pro-inflammatory cytokines IL-1, IL-6, IL-8 and TNF-alpha in human monocytes.

To investigate whether plitidepsin inhibits the TLR-triggered cytokine secretion, we treated THP-1 cells with 100 ng/mL TNF α (an activator of NF- κ B), 500 μ g/mL poly I:C (TLR3 ligand), 10 μ g/mL LPS-B5 (TLR4 ligand) or 10 μ g/mL Resiquimod (TLR-7/8 ligand). The compounds were used either alone (grey bars) or combined with 100 nM of plitidepsin (red bars) for 6 hours. We compared the variations in cytokine secretion in the cell culture supernatants between the different treatments by ELISA assays. As can be seen in Figure 2, poly I:C, LPS and Resiquimod induce the secretion of IL-1, IL-6, IL-8 and TNF- α . Furthermore, plitidepsin clearly inhibited the production of IL-1, IL-6, IL-8 and TNF- α . TNF- α failed to increase the secretion of IL-1 and IL-6. It is possible that THP-1 cells need other TNF- α exposure times to secrete these cytokines. Also, plitidepsin was unable to reduce IL-8 and TNF- α in TNF- α stimulated cells. That is because we measured TNF- α secreted and TNF α added to the cell culture medium. As such, the treatment with TNF- α masked the levels of secreted TNF- α .

In the presence of each one of the TLR ligands, plitidepsin clearly inhibited the secretion of pro-inflammatory cytokines IL-1, IL-6, IL-8.

In a further *in vitro* experiment, the effect of plitidepsin (APL) pre-treatment on THP-1 cells was studied. Using a THP-1 NF κ B luc line, 1, 10 or 50nM of APL or DMSO (0.2%) was added 8 hours before stimulus with Resiquimod (RQ) at 2.5 or 5 μ g/mL. RQ is a TLR7/8 agonist and mimics ssRNA. At 24 hours the level of cytokines or cell viability was measured. As shown in Figure 50, PLD pre-treatment inhibited secretion of the pro-inflammatory cytokines: IL6, IL8, IL1 β and TNF- α induced by RQ. Of note, the addition of PLD does not decrease viability over the untreated control at any of the 2 RQ concentrations assayed. While it is true that RQ itself displays some degree of dose-related toxicity, this effect is not increased by the presence of PLD in the culture and therefore, the changes in cytokine production is unrelated with this RQ induced slight cytotoxicity.

EXAMPLE 2

As shown in Figure 3, PLD inhibits *ex-vivo* secretion of the pro-inflammatory cytokines, IL-6, IL-8 and TNF-alpha in murine isolated from BAL (Bronchoalveolar lavage).

We checked whether plitidepsin inhibits the LPS-triggered cytokine secretion in alveolar macrophages. To that end mice were injected i.v. with plitidepsin (1 mg/kg) or vehicle and 12 hours after administration bronchoalveolar lavage fluid (BAL) was collected. Cells were plated and treated ex-vivo or not with 15 μ g/mL of LPS-B5 for 3 or 6 hours and secreted cytokines were measured. As can be seen LPS induce the secretion of IL-6, IL-10 and TNF- α (grey bars). Furthermore, in the animals treated with plitidepsin, the drug clearly inhibited the production of pro-inflammatory cytokines IL-6, and TNF- α induced by LPS (red bars) and led to an overall anti-inflammatory effect.

This is further shown again in Figure 51. In the animals treated with plitidepsin, plitidepsin was able to significantly reduce the secretion of IL-6, IL-10 and TNF α induced by LPS-B5 at 3 and 6 hours in CD45⁺ cells isolated from bronco-alveolar lavages. This effect was unrelated to cell viability as shown in Figure 51(b).

We further checked whether plitidepsin inhibits resiquimod (RQ)-triggered cytokine secretion in BALF. Mice were injected i.v. with plitidepsin (1 mg/kg) or vehicle 1 hour before a 50 μ g/mouse intranasal inoculation with resiquimod. At 1 or 3 hours after intranasal administration of RQ bronchoalveolar lavage fluid (BALF) was collected. Cells were plated and secreted cytokines

were measured. As can be seen, RQ induces the secretion of TNF α at both 1 and 3 hours following administration. *In vivo* administration of PLD prevented the increased production of TNF α . As can be seen in Figure 52, RQ induces the secretion of TNF α at both 1 and 3 hours following administration. *In vivo* administration of PLD prevented the increased production of TNF α .

- 5 Also we checked the effect of plitidepsin on alveolar macrophage recruitment. Activated monocyte-derived macrophages contribute to the COVID-19 cytokine storm by releasing massive amounts of pro-inflammatory cytokines. Bronchoalveolar lavage cells were stained and analyzed by flow cytometry. Plitidepsin decrease the percentage of macrophages presents on bronchoalveolar lavage without cytotoxic effects.

10 EXAMPLE 3

As shown in Figure 4, after a single iv administration in mice, PLD reduces the number of macrophages in BAL.

- To investigate whether plitidepsin decreases the percentage of alveolar macrophage in animals with acute inflammation, we treated mice with plitidepsin (1 mg/kg) i.v., with LPS (20 μ g/kg) i.p. in sterile saline or with plitidepsin (1 mg/kg; i.v.) in combination with LPS (20 μ g/kg, i.p.). Three hours later, bronchoalveolar lavages were collected. Bronchoalveolar lavage cells were obtained by centrifugation and analyzed by flow cytometry (Figure 4b). Upper panels show the strategy of analysis of macrophage population present in the samples. Lower right panel show the same result expressed as percentage of cells. Lower left panel show the percentage of CD45+ (leucocyte marker) alive cells. As can be seen LPS induce the recruitment of alveolar macrophages. The treatment with Plitidepsin decrease the percentage of macrophages presents on bronchoalveolar lavage without cytotoxic effects.

EXAMPLE 4

- As shown in Figure 5, PLD is distributed to the lungs in the mouse (which is the nonclinical species used in the pharmacological models). In addition, similar plasma exposures are achieved in non-clinical species and patients.

- The concentration of plitidepsin in lungs was consistently higher than that in plasma at any sampling time, with a lung-to-plasma ratio (calculated as $\frac{\text{lung AUC}_{0-\infty}}{\text{plasma AUC}_{0-\infty}}$) in mice, rats and hamsters of 133, 460 and 909, respectively, thus confirming the distribution of plitidepsin into the lung.

Table 1

Species (gender)	Strain	Dose [†] (mg/kg)	C _{max} (ng/mL)	AUC _{0-∞} (ng·h/mL)	t _{1/2} (h)	Cl (L/h/kg)	Vd _{ss} (L/kg)
Mouse (F)	C57BL6/J	1.0 ^a	50.7	225.3	18.2	4.4	101.8
Human (M/F)	-	0.135 ^b	29.1	256.0	20-80	0.7-0.9	29-33

-	0.02 ^c	8.5 ^d	174.0 ^d	-	-	-
---	-------------------	------------------	--------------------	---	---	---

F, female; M, male.

[†]Schedule:

- Nonclinical species: single intravenous bolus.
- Patients: 3-h intravenous infusion.

^a Maximum Tolerated Dose.

^b Calculated from the Recommended Dose of 5 mg/m², 3-h infusion or 9.5 mg/patient.

^c Equivalent to 1.5 mg/patient, this being a dose used in APLICOV (1-h infusion on days 1, 2 and 3).

^d Estimated from plitidepsin's population PK model (CPR/2016/01), following 3 daily doses of 1.5 mg/patient.

Human body surface area, 1.9.

Human body weight, 60 kg

Materials and Method

Transactivation luciferase assay.

NF-κB transactivation was assayed using the Bright-Glo™ Luciferase Assay System following the manufacturer's instructions. The NF-κB reporter (Luc)-THP-1 human monocytic cells stably transfected with NF-κB-Luc plasmid (containing four NF-κB binding sites, a minimal promoter and a luciferase gene), were exposed to 100 ng/mL TNFα (positive control), 500 μg/mL poly (I:C) (Polyinosinic-polycytidylic), 10 μg/mL LPS-B5 (Lipopolysaccharide from Escherichia coli 055:B5) or 10 μg/mL Resiquimod. The compounds were used either alone or combined with 100 nM plitidepsin for 6 hours. Luminescence was measured in a Perkin-Elmer EnVision reader. A MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell proliferation assay was simultaneously performed to control the cytotoxicity of the compounds. Cell survival was expressed as percentage of control cell growth. The data presented are the average of three independent experiments performed in triplicate.

ELISA assays for secreted cytokines

THP1-NFκB-LUC cell cultures were treated as described above, and the culture medium was sampled at 6 hours post-treatment to assay for secreted cytokines by ELISA. Media samples were stored at 4°C. IL-8, IL-1β, IL-6 and TNF-α protein secretion into culture medium was quantitated using highly specific and sensitive ELISA kits. Human IL-1b, human IL-6, human IL-8 and human TNF OptEIA™ ELISA kits were obtained from BD Biosciences and performed as described by the manufacturer. The data presented are the average of three independent experiments performed in triplicate.

MTT assay

Cells were seeded in 96 well microtiter plates and allowed to stand for 24 hours at 37°C and 5% CO₂ before treatment described above. After 6 hours of continuous treatment, cellular viability was estimated from conversion of MTT to its coloured reaction product, MTT formazan, which was dissolved to measure its absorbance at 540 nm. Data presented here are representative from a series of at three independent experiments performed in triplicate.

“In vivo” and “ex vivo” treatments.

Mice were randomized into groups of five animals to receive the treatments. Mice were injected intra venous (i.v.) with plitidepsin (1 mg/kg) and 12 hours after administration were euthanised. Control group received plitidepsin vehicle diluted with saline (Cremophor/Ethanol/Water). Bronchoalveolar lavage fluid (BAL) of each group was collected and centrifugated to obtain bronchoalveolar lavage cells. Cells underwent red blood cell lysis (Roche) and were plated and treated ex-vivo or not with 15 µg/mL of LPS-B5 for 3 or 6 hours. Secreted cytokines were measured using highly specific and sensitive ELISA kits. Mouse IL-6, mouse IL-10 and mouse TNF DuoSet ELISA kits were obtained from R&D Systems and performed as described by the manufacturer. Data presented here are representative from a series of at three independent experiments.

Animal inflammation model.

Mice were randomized into groups of two animals to receive the treatments. Mice were challenged with plitidepsin (1 mg/kg) intra venous (i.v.), with LPS (20 µg/kg) intra peritoneal (i.p.) in sterile saline or with plitidepsin (1 mg/kg; i.v.) in combination with LPS (20 µg/kg, i.p.). The control group received plitidepsin vehicle (Cremophor/Ethanol/Water) diluted with saline. Three hours later, animals were euthanised and bronchoalveolar lavage collected (a total of 1.2 ml, PBS). Bronchoalveolar lavage cells were obtained by centrifugation and analyzed by flow cytometry. Data presented here are representative from a series of at three independent experiments.

In another inflammation model, mice were randomized into groups of two animals to receive the treatments. Mice were challenged with plitidepsin (1 mg/kg) intra venous (i.v.) followed by Resiquimod (50 µg/mouse, intranasal;) 1 hour later. The control group received plitidepsin vehicle (Cremophor/Ethanol/Water) diluted with saline. One and 3 hours later, animals were euthanized, bronchoalveolar lavage collected (a total of 1.2 ml, PBS) and then, TNFα quantified by ELISA kits. Data presented here are representative from a series of at three independent experiments.

Analysis of macrophages by flow cytometry.

Bronchoalveolar lavage cells were stained with anti- F4/80-BV510, CD45-APC700, CD11b-BV650, CD11c-APC-Fire, CD24-PC7 and Ly6C-BV605 monoclonal antibodies (Biolegend) and a LIVE/DEAD™ Fixable Green Dead Cell Stain Kit, for 488 nm excitation (Thermofisher). Macrophages (F4/80+) were gated on alive immune cells (CD45+ LIVE/DEAD dye-), while alveolar macrophages (F4/80+ CD24-) were specifically gated on CD11c+ CD11b- population from alive immune cells. Isotype controls and compensation beads were used to set compensations and gating strategies.

EXAMPLE 5: Antiviral activity for compounds of the present invention have been demonstrated in HIV.

5 The recombinant virus assay was performed in both, MT-2 cells and PBMCs previously activated with PHA + IL-2. Cells were infected with supernatants obtained from 293t cells transfected with full-length infectious HIV-1 plasmids: pNL4.3-Luc (X4 tropic virus), pNL4.3-Renilla (X4 tropic virus able to develop more than one round of replication), pNL4.3- Δ env-Luc plus pVSV-env (HIV pseudotyped with the G protein of VSV) or pJR-Renilla (R5 tropic virus able to develop more than one round of replication). Resistant viruses were obtained cloning in NL4.3-Renilla the *pol* gene of viruses from different infected donors. Virus 9D carry the following mutations: 41L,67N,70R,98G,118I,184V,215F,219Q,74I and virus 4D: K65R, K70R, V75I, F77L, F116Y, Q151M, M184I, L10I. The assay was then performed in 96 well microplates seeded with 100 μ l containing 250.000 (PBMCs) or 100.000 (MT-2) cells/well. The compounds to be tested were added to the culture in concentrations ranging from 50 to 0.0016 μ g/ml (100 μ l/well). Finally, cell culture was infected with supernatants obtained from transfection of the different plasmids described above. After 48 hours, cell culture supernatant was removed and cells were lysed with Luciferase assay system or Renilla assay system (both from Promega) following the specifications of the manufacturer, and the luciferase-renilla activity was measured in a luminometre (Berthold Detection systems). All the experiments were controlled with cells treated with the vehicle (DMSO) and non-treated cells.

20 HIV-1 replication inhibition was evaluated by measuring the reduction of luciferase-renilla activity or RLUs (Relative light units) in a luminometre, being the 100% the infection of non-treated cells.

Compound 3 (Figure 6A and 6B) showed antiviral activity in both MT-2 cells and PBMCs (IC_{50} 1.39 μ M and 0.16 μ M, respectively). This compound was more toxic in PBMCs, as shown in Figure 6. Toxic concentrations were not reached at 57.3 μ M in MT-2 cells, while in PBMCs CC_{50} value was about 27 μ M.

25 Compound 8 (Figure 7A and 7B) showed also antiviral activity in both MT-2 cells and PBMCs. Although at concentrations of 50 μ M it was non-specific, at 10 μ M it was specific, with an IC_{50} value 100 fold lower.

30 Compounds 9 (Figure 8A and 8B), 10 (Figure 9A and 9B), and 11 (Figure 10A and 10B) were the most potent compounds of all tested compounds. Compounds 9, 10, and 11 showed IC_{50} s values in the nanomolar range in PBMCs (0.63, 0.86, and 69.4 nM, respectively), and they are among the most potent of the antiviral compounds in vitro existing in the literature.

EXAMPLE 6: Antiviral activity of the compounds of the invention has been demonstrated against Coronaviridae.

35 Antiviral activity of compounds of the invention were tested in Huh-7 cells (human hepatoma cell line) infected with HCoV-229E. HCoV-229E has a multiplication and propagation mechanism very similar to SARS-CoV-2. Indeed, the N protein of HCoV-229E has a protein homology greater than 90% with the homologous N protein in SARS-CoV-2. It is believed that all coronaviruses need their N (nucleocapsid) protein to bind to EF1A in order to replicate effectively and synthesise viral proteins. Reducing or abolishing the binding of N to EF1A reduces the viability for the spread of the virus.

40

Compounds of the invention as set out in Table 2 below were reconstituted in DMSO and stored at -20°C.

- 5 Huh-7 cells (human hepatoma cell line) grown to confluence in a M96 well plate, were infected with HCoV-229E-GFP virus at a moi (multiplicity of infection) of 0.01 pfu. Virus stock was HCoV-229E-GFP (from 31 Jan 2013) at 3×10^7 pfu/ml.
- At 8 hpi (hours post infection), media is replaced by media with the appropriated compound dilutions (DMSO final concentration 2%), following the scheme:

Table 2

	1	2	3	4	5
A (Figures 11-15)	50nM	5nM	0.5nM	5nM	0.5nM
B (Figures 16-19)	50nM	5nM	0.5nM	0.5nM	
C (Figures 20-23)	50nM	5nM	0.5nM	0.5nM	
D (Figures 24-26)	50nM	5nM	0.5nM		
E (Figures 27-29)	50nM	5nM	0.5nM		
F (Figures 30-33)	50nM	5nM	0.5nM	0.5nM	

- 10 A = DMSO (control); B = Compound 240 (DidemnB); C = PLD; D = Compound 9; E = Compound 10; F = PLD

Fluorescent cells were observed 24 hpi. Photos were obtained using an automated system. Cells were fixed for 30 min with PFA 4%, washed with PBS, and cell nuclei were stained with DAPI 1:200 in PBS 20 min RT. Images in green show GFP tagged viral particles. Images in blue show DAPI stained nuclei.

- 15 For a short while, confluent cultures of Huh-7 were infected at a multiplicity of infection (MOI) of 0.01 pfu/cell, with a viral inoculum of 3×10^7 pfu/ml and after 8 hours, plitidepsin was added at concentrations ranging from 0.5 nM to 50 μ M. The cultures with plitidepsin were incubated for 48 hours and then viral viability was measured by fluorescence. The results obtained showed an antiviral effect induced by plitidepsin at concentrations as low as 0.5 nM (0.555 μ g/l), much
- 20 lower than those reported with other antivirals.

It is shown that compounds of the invention are effective antiviral agents across a range of tested concentrations, whilst retaining cell viability.

EXAMPLE 7

- 25 A multicenter, randomized, parallel and proof of concept study was undertaken to evaluate the safety profile of three doses of Plitidepsin in patients with COVID-19 requiring hospitalization. Study details are available through ClinicalTrials.gov Identifier: NCT04382066.

The primary objective of the study was to determine the safety and toxicological profile of plitidepsin at each dose level administered according to the proposed administration scheme in patients admitted for COVID-19.

- 5 The secondary objectives were to assess the efficacy of plitidepsin in patients with COVID-19 at the proposed dose levels by reference to: change in SARS-CoV-2 viral load from baseline; time until negative detection of SARS-CoV-2 by PCR; cumulative incidence of disease severity (evaluation based on: mortality; need for invasive mechanical ventilation and/or ICU admission; need for non-invasive mechanical ventilation; need for oxygen therapy) and selection of the recommended dose levels of plitidepsin for a phase II/III efficacy study.

- 10 Patients included in the study were randomised in a 1:1:1 ratio to receive:

- Arm A) 1.5 mg of plitidepsin administered as a 1.5-hour infusion, once a day for 3 consecutive days (total dose 4.5 mg).
- Arm B) 2.0 mg of plitidepsin administered as a 1.5-hour infusion, once a day for 3 consecutive days (total dose 6.0 mg).
- 15 - Arm C) 2.5 mg of plitidepsin administered as a 1.5-hour infusion, once a day for 3 consecutive days (total dose 7.5 mg).

All patients received the following prophylactic medications 20-30 minutes before the infusion of plitidepsin:

- Diphenhydramine hydrochloride 25 mg iv or equivalent.
- 20 - Ranitidine 50 mg iv or equivalent.
- Dexamethasone 6.6 mg intravenous.
- Ondansetron 8 mg i.v. in slow infusion of 15 minutes or equivalent.

Patients included in the study received treatment for 3 days.

- 25 Plitidepsin is supplied as a powder for concentrate for solution for infusion at a concentration of 2 mg/vial. Before use, the vials are reconstituted with 4 ml of reconstitution solution to obtain a colourless to slightly yellowish solution containing 0.5 mg/ml of plitidepsin, 25 mg/ml of mannitol, 0.15 ml/ml of macroglycerol ricinoleate oil, 0.15 ml/ml of ethanol and 0.70 ml/ml of water for injection. An additional dilution should be made in any suitable intravenous solution prior to infusion.

- 30 Plitidepsin 2 mg is supplied in a Type I clear glass vial with a bromobutyl rubber stopper covered with an aluminium seal. Each vial contains 2 mg of plitidepsin.

The solvent for the reconstitution of macroglycerol ricinoleate (polyoxyl 35 castor oil)/absolute ethanol/water for injection, 15%/15%/70% (v/v/v) is supplied in a Type I colourless glass vial. The ampoules have a volume of 4 ml.

- 35 Plitidepsin will be labelled with the study protocol code, the batch number, the content, the expiry date, the storage conditions, the name of the investigator and the sponsor. The study drug will be

labelled in accordance with Annex 13 of the European Good Manufacturing Practices. Plitidepsin should be stored between 2°C and 8°C and the vials should be kept in the outer carton to protect them from light. The drug in these conditions is stable for 60 months.

- 5 After reconstitution of the 2 mg plitidepsin vial with 4 ml of the solution for reconstitution of macrogolglycerol ricinoleate/ethanol/water for injection, the reconstituted solution should be diluted and used immediately after preparation. If not used immediately, storage times and conditions until use are the responsibility of the user. The reconstituted concentrated solution of the drug product has been shown to be physically, chemically and microbiologically stable for 24 hours under refrigerated conditions (5°C±3°C) and for 6 hours when stored in the original vial under indoor light at room temperature. If storage is required before administration, solutions should be stored refrigerated and protected from light and should be used within 24 hours after reconstitution.

Plasma concentration

- 15 Figure 34 illustrates the simulation of the total plasma plitidepsin concentration profiles vs. time after a daily dose (D1-D5) of 1.0 mg and 2.0 mg. The horizontal black lines represent the total plasma concentrations associated with the concentrations in lung equivalent to IC₅₀, IC₉₀ and 3xIC₉₀ in vitro. With both dose levels (1.0 mg and 2.0 mg), plasma concentrations above IC₅₀ would be obtained throughout the treatment period, and would remain above IC₉₀ during most of the administration interval. Accumulation after five repeated administrations is minimal.

- 20 A further dosage regimen is 1.5mg daily for 5 days. A further regimen is illustrated in Figure 35 which simulates plitidepsin total plasma concentrations associated to an initial flat dose of 1 mg (Day 1) given as a 1-h i.v. infusion, followed by daily doses of 0.5 mg (D2-D5). With this dose regimen, plitidepsin plasma concentrations are above the IC₅₀ during the entire treatment period, and remains above IC₉₀ during 18 and 14 hours, after 1 mg and 0.5 mg dose infusion, respectively. Notably, minimal accumulation after repeated administration is foreseen. This regimen provides a loading dose of 1 mg of plitidepsin given as 1-h i.v. infusion on the first day of treatment, followed by a maintenance dose of 0.5 mg once daily for 4 days.

- 30 Figure 36 illustrates the simulation of the total plasma plitidepsin concentration profiles vs. time after a daily dose (D1-D3) of 1.5 mg, 2.0 mg and 2.5 mg. The horizontal black lines represent the total plasma concentrations associated with concentrations in lungs equivalent to IC₅₀, IC₉₀ and 3xIC₉₀ in vitro. With all three dosage levels (1.5 mg, 2.0 mg and 2.5 mg), plasma concentrations above IC₅₀ would be obtained throughout the treatment period and would remain above IC₉₀ during most of the administration interval. Accumulation after three repeated administrations is minimal.

Interim results

To date, data is available for nine patients. PLD was administered as a 90 minute IV infusion daily for 3 consecutive days (day 1-3) with viral load assessed by PCR at baseline, day 4, day 7 and day 15 and day 31.

- 40 **Patient 1** - 50 year old male, bilateral pneumonia. Received PLD 1.5 mg x 3. PCR COVID 19 test: POSITIVE at baseline, converted to NEGATIVE (no viral load) by day 4. Acute clinical improvement. Hospital discharge by day 7. As such, PLD 1.5 mg x 3 removed viral load by day

4. PLD achieved an acute clinical improvement, including removing all viral burden and treating bilateral pneumonia to enable hospital discharge by day 7.

Patient 2: 40 year old male, bilateral pneumonia. Received PLD 1.5 mg x 3. By day six, lack of improvement and cross over to Remdesivir + TOL + Corticoids + Opiates. PCR converted to negative by day 15, Hospital discharge by Day 19.

Patient 3: 53 year old male, bilateral pneumonia. Received PLD 1.5 mg x 3. PLD prevented clinical deterioration. Hospital discharge by day 10, PCR converted to negative by day 31.

Patient 4: 42 year old male, bilateral pneumonia. Received PLD 2.0 mg x 3. Corticoid therapy required. PCR COVID 19 test: POSITIVE at baseline, and still positive at day 7. By day 15 the patient was PCR negative, as shown in Figure 42a. Patient recovered sufficiently for hospital discharge by day 10.

Patient 5: 33 year old female, bilateral pneumonia at entry. Received PLD 1.5 mg x 3. PCR COVID 19 test: POSITIVE at baseline, converted to NEGATIVE (no viral load) by day 4 as shown in Figure 42b. Bilateral pneumonia resolved by day 6 (normal Rx Lung). Major clinical improvement. Hospital discharge by day 8. X-rays showing pneumonia resolution shown in Figure 39a-c. Bilateral pneumonia is evident in Figure 39a. After treatment with PLD, improvement was seen on day 6. Laminar atelectasis is evidenced Figure 39b. A follow up x-ray on day 15 showed return to normal Figure 39c. PLD 1.5 mg x 3 removed viral load by day 4. PLD achieved major clinical improvement, including removing all viral burden and treating bilateral pneumonia to enable hospital discharge by day 8.

Patient 6: 69 year old female, highly symptomatic COPD. Unilateral pneumonia on entry. Received PLD 1.5 mg x 3. PCR COVID 19 test: POSITIVE at baseline, converted to NEGATIVE (no viral load) by day 7 as shown in Figure 42c. Major clinical improvement seen. Patient discharged by day 8. X-rays showing pneumonia progression shown in Figure 40a-c. Unilateral pneumonia is evident in Figure 40a which progressed to bilateral pneumonia in Figure 40b. In Figure 40c, improvement is seen. PLD achieved major clinical improvement, including removing all viral burden and treating pneumonia as shown in Figure 40d to enable hospital discharge by day 8.

Patient 7: 39 year old female, pulmonary infiltrates. Received PLD 2.0 mg x 3. PCR COVID 19 test: POSITIVE at baseline, converted to NEGATIVE (no viral load) by day 7 as shown in Figure 42d. Following treatment with PLD, major clinical improvement. Hospital discharge by day 8.

Patient 8: 32 year old male. Received PLD 1.5 mg x 3. Not evaluable for efficacy, hospital discharge by day 4.

Patient 9: 34 year old male. Received PLD 2.0 mg x 3. PCR COVID 19 test: POSITIVE at baseline and still positive at day 7. However, major clinical improvement and hospital discharge by day 8.

C-reactive protein tests

The effect of PLD on inflammatory cytokines was also measured for patients 5, 7 and 9 and the results of C-reactive protein tests are shown in Figure 41. With patient 5 (Figure 41a), following

administration of PLD, an acute fall is seen by day 2. With patients 7 (Figure 41b) and 9 (Figure 41c), following administration of PLD, an acute fall is seen by day 3. These data demonstrate anti-inflammatory properties of PLD.

Discussion

- 5 The preliminary results of the PLD clinical trial on COVID-19 patients further demonstrate the remarkable properties of PLD in the treatment of SARS-CoV-2 infection and COVID-19.

6 out of 8 evaluable patients demonstrated SARS-CoV-2 PCR conversions to negative, with a median time for PCR conversion of 7 days (4-31). The antiviral properties of PLD on CoV infection, namely SARS-CoV-2, is clinically demonstrated with total removal of viral burden.

- 10 Remarkably, of the 9 patients currently tested, due to administration of PLD, none of the patients required mechanical ventilation or ICU admission, and there were no deaths on study. PDL induced disease control and major clinical improvements in 6 out of 8 evaluable patients with only 2 out of 8 requiring post PLD specific anti COVID 19 therapy. The median time to hospital discharge was 8 days (7 -19). These results demonstrate that PLD is an effective
15 therapy for SARS-CoV-2 infection and COVID-19, and pneumonia caused by SARS-CoV-2 infection.

Ad hoc analysis of the first cohort at 1.5mg and 2mg confirmed that PLD at the doses administered was active, achieved acute improvement and complete disease control. The complete removal of viral burden was noted in the vast majority of patients.

20 Results

- Upon completion of the study, 45 patients hospitalised for COVID 19 were randomised to treatment with plitidepsin at doses of 1.5, 2.0, and 2.5 mg daily for 3 days. Treatment was well tolerated in all 3 dose cohorts. Treatment outcomes, assessed by hospital discharge rate, were driven by disease severity and viral load at baseline. Across dose cohorts, 100% (9/9) patients
25 with mild disease, 82% (23/28) with moderate disease, and 57% (4/7) with severe disease were discharged by Day 15. Across dose cohorts, median viral load at baseline was 6.2 (0 to 10.6) log₁₀ copies/mL and a mean reduction in viral load of -3.1 log₁₀ copies/mL was achieved by Day 7 and -4.5 log₁₀ copies/mL by Day 15.

- The study was a Phase 1, multicentre, open-label study in which 45 patients hospitalised for management of COVID-19 were randomised into 3 dose groups, comprising 1.5, 2.0, and 2.5 mg plitidepsin administered as a 1.5-hour IV infusion once a day for 3 consecutive days. The primary objective of this study was to determine the safety and toxicological profile at each dose level, based on (1) frequency of Grade ≥ 3 treatment-emergent adverse events (TEAE) at Days 3, 7, 15, and 31 using National Cancer Institute (NCI) Common Terminology Criteria for
35 Adverse Events (CTCAE) version 5.0 criteria; (2) percentage of patients unable to complete treatment and reasons; (3) percentage of patients with TEAEs and SAEs at Days 3, 7, 15, and 31; (4) change from baseline haematologic and non-haematologic parameters on Days 3, 7, 15, and 31; and (5) percentage of patients with ECG abnormalities on Days 2, 3, 4, 5, 6, 7, 15, and 31. A secondary objective was to select a recommended dose for a pivotal study.

- 40 Findings for protocol-specified safety endpoints are summarised as follows:

- 5 • Grade ≥ 3 AEs: Only 31% (14/45) of patients experienced Grade ≥ 3 AEs and only 2 patients experienced a treatment-related Grade ≥ 3 AE: 1 case each of anaphylactic reaction during the first plitidepsin infusion that resulted in treatment discontinuation and a case of diarrhoea that had no impact on plitidepsin treatment. Consistent with Grade ≥ 3 AEs being

10 predominantly due to COVID-19 infection, the prevalence of Grade ≥ 3 AEs largely reflected the percentage of patients with severe disease in each cohort, with the highest prevalence in the 2.5-mg cohort (26.7% with severe disease, 40.0% Grade ≥ 3 AEs), a lower percentage in the 1.5-mg cohort (13.3% with severe disease, 33.3% Grade ≥ 3 AEs), and the lowest percentage in the 2.0-mg cohort (6.7% with severe disease, 20.0% Grade ≥ 3 AEs). None of

15 the events of special interest occurred, with the exception of 1 case of Grade ≥ 3 ALT elevation.
- 20 • Patients unable to complete treatment: Only 1 patient was unable to complete plitidepsin treatment.
- 25 • Patients with SAEs: A total of 10 patients experienced SAEs, including 6 dosed at 1.5 mg, 1 dosed at 2.0 mg, and 3 dosed at 2.5 mg. With the exception of 1 case of anaphylactic reaction, all these events were related to COVID-19 infection.
- 30 • Patients with AEs: No dose-related trends were noted for any reported AEs and the most commonly reported AEs (excluding infections) were consistent with the safety profile observed for patients with advanced haematologic malignancies and solid tumours receiving single-agent plitidepsin, including gastrointestinal disorders of constipation (29% vs. 18% for cancer and COVID-19 patients, respectively), diarrhoea (31% vs. 18%), nausea (64% vs. 42%), and vomiting (38% vs. 18%) and constitutional symptoms of asthenia/fatigue (83% vs. 13%) and pyrexia (28% vs. 47%).
- 35 • Changes in laboratory parameters: Although about half of patients (51%) showed ≥ 1 grade change in haematology parameters and most patients (89%) showed ≥ 1 grade change in chemistry parameters on study, few patients showed >1 grade shift. For haematology parameters, 6 patients had lymphopenia worsen by 2 to 3 grades and 2 had neutropenia worsen by 2 grades. For chemistry parameters, 5 patients had ALT increase by 2 to 3 grades, 2 patients had AST increase by 2 to 3 grades, 2 patients had GGT increase by 2 to 3 grades, and 1 patient had a 2 grade increase in CPK.

Based on these findings, it is concluded that plitidepsin treatment was well tolerated and it was not possible to detect a difference in safety between the 3 doses studied.

Efficacy in COVID-19 Patients

- 35 A total of 44 patients were evaluable for efficacy; 1 patient in the 1.5-mg cohort who experienced an anaphylactic reaction during the first infusion of plitidepsin had treatment discontinued and was not considered evaluable for efficacy. Results for protocol-specified efficacy endpoints showed equivalent results across the 3 dose cohorts (Table 3). Treatment outcomes were driven by baseline disease severity and viral load. Consistent with these findings, a recent study showed that SARS-CoV-2 viral load is associated with increased

40 disease severity and mortality. Across dose cohorts, 100% (9/9) patients with mild disease were discharged by Day 15, compared to 82% (23/28) with moderate disease and 57% (4/7) with severe disease.

Endpoint	Dose Cohort			
	1.5 mg N=14 ^A	2.0 mg N=15	2.5 mg N=15	Total N=44
Patients discharged from hospital by	n (%)			
Day 8	6 (42.9)	9 (60.0)	10 (66.7)	25 (56.8)
Day 15	11 (78.6)	14 (93.3)	11 (73.3)	36 (81.8)
Day 31	13 (92.9)	14 (93.3)	13 (86.7)	40 (90.9)
Mortality from Day 1 to				
Day 7	0	0	0	0
Day 15	0	0	0	0
Day 31	1 (7.1)	0	1 (6.7)	2 (4.5)
Patients requiring invasive mechanical ventilation and/or ICU admission from Day 1 to				
Day 7	2 (14.3)	1 (6.7)	2 (13.3)	5 (11.4)
Day 15	2 (14.3)	1 (6.7)	3 (20.0)	6 (13.6)
Day 31	2 (14.3)	1 (6.7)	3 (20.0)	6 (13.6)
Patients requiring noninvasive mechanical ventilation from Day 1 to				
Day 7	3 (21.4)	0	1 (6.7)	4 (9.1)
Day 15	3 (21.4)	0	2 (13.3)	5 (11.4)
Day 31	3 (21.4)	1 (6.7)	2 (13.3)	6 (13.6)
Patients requiring ICU admission from Day 1 to				
Day 7	2 (14.3)	1 (6.7)	2 (13.3)	5 (11.4)
Day 15	2 (14.3)	1 (6.7)	3 (20.0)	6 (13.6)
Day 31	2 (14.3)	1 (6.7)	3 (20.0)	6 (13.6)

Patients requiring oxygen therapy at				
Day 7	10 (71.4)	10 (66.7)	11 (73.3)	31 (70.5)
Day 15	4 (28.6)	1 (6.7)	4 (26.7)	9 (20.5)
Day 31	0	2 (13.3)	1 (6.7)	3 (6.8)
Mean change in viral load from baseline to ^B	log ₁₀ copies/mL			
Day 4	-1.58	-1.92	-2.16	-1.90
Day 7	-3.39	-2.69	-3.21	-3.07
Day 15	-5.47	-3.62	-4.40	-4.48
Day 31	-6.06	-4.71	-5.20	-5.27
Mean time from baseline until undetectable viral load, days	7	13	12	11

Table 3. Summary of Protocol-specified Efficacy Endpoints in Study APLICOV-PC

Abbreviations: ICU = intensive care unit

A: Patient who experienced an anaphylactic reaction during the first plitidepsin infusion had treatment discontinued and was not considered evaluable for efficacy

- 5 B: Results based on 39 patients: 1 patient missed baseline viral load assessment and 4 patients had baseline viral load below limit of quantitation despite having positive polymerase chain reaction test within 48 hours prior to enrolment

Benefit-risk Considerations

10 In the APLICOV-PC study, most patients (84%) had mild to moderate disease and 82% of patients were discharged by Day 15. As progressive deterioration of respiratory function and development of cytokine release syndrome typically occur at a mean of 10 days from onset of symptoms, the endpoint of hospital discharge rate at Day 15 is considered to reflect successful amelioration of life-threatening complications.

15 Post-hoc analyses showed that treatment response, assessed by hospital discharge rate, was correlated with baseline disease severity and viral load. Across dose cohorts, 100% (9/9) of patients with mild disease were discharged by Day 15, compared to 82% (23/28) with moderate disease and 57% (4/7) with severe disease.

20 In the APLICOV-PC study, median baseline viral load was 6.1 log₁₀ copies/mL and by Day 15 a mean -4.2 log₁₀ reduction in viral load was observed. These results support a conclusion that plitidepsin reduces viral replication.

Considering the low rate of drug-related Grade ≥ 3 AEs and the high discharge rate at Day 15, along with an average 4.2- $\log_{10}$ reduction in baseline viral load by Day 15 (-3.0- $\log$ reduction reported for those patients with moderate disease), demonstrates a positive benefit risk for plitidepsin for treatment of patients hospitalised for COVID 19 infection.

5 Example 8

The activity of plitidepsin against SARS-CoV-2 was further confirmed in an *in vitro* assay using vero cells.

Virus and cells

10 SARS-CoV-2 was obtained from Korea Centers for Disease Control and Prevention (KCDC). Vero cells were acquired from the American Type Culture Collection (ATCC CCL-81).

Dose-response curve (DRC) analysis by immunofluorescence

15 The compound was prepared a two-fold serial dilutions at 20-point concentrations with DMSO and Ampolla (Cremophor:Ethanol:Water (15:15:70)) respectively. 24 hours after cell seeding, the compound was treated in the cells with the top concentration at 5 μ M. After an hour, plates were transferred into the BSL-3 containment facility for viral infection and SARS-CoV-2 was added at a multiplicity of infection (MOI) of 0.0125. The plates were incubated at 37°C for 24 hours. The cells were fixed at 24 hpi with 4% paraformaldehyde (PFA) for permeabilization. 20 Anti-SARS-CoV-2 Nucleocapsid (N) 1st antibody and 488-conjugated goat anti-rabbit IgG 2nd antibody were treated to the cells and Hoechst 33342 were treated to dye the cells for the analysis by immunofluorescence. The acquired images with Operetta (Perkin Elmer) were analyzed using in-house software to quantify cell numbers and infection ratios, and antiviral activity was normalized to positive (mock) and negative (0.5% DMSO) controls in each assay plate.

25 DRCs were fitted by sigmoidal dose-response models, with the following equation: $Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + (\text{IC}_{50}/X)^{\text{Hillslope}})$, using XLfit 4 Software or Prism7. IC_{50} values were calculated from the normalized activity dataset-fitted curves. All IC_{50} and CC_{50} values were measured in duplicate, and the quality of each assay was controlled by Z'-factor and the coefficient of variation in percent (%CV).

30 Dose-response curves are shown in Figures 37A-C (three repeats). The blue squares represent inhibition of virus infection (%) and the red triangles represent cell viability (%). Means $\pm$ SD were calculated from duplicate experiments. Plitidepsin was able to inhibit viral-induced cytopathic effects (squares) at concentrations where no cytotoxic effects of the drug were observed (circles) in all experiments. In this experiment, plitidepsin had an IC_{50} of 0.0033-0.0039 μ M versus a CC_{50} of 0.178-0.431 μ M giving an SI of 49.95-129.92. 35

Example 9

The activity of plitidepsin against SARS-CoV-2 was further confirmed in a separate *in vitro* assay using vero cells.

Cell Cultures

Vero E6 cells (ATCC CRL-1586) were cultured in Dulbecco's modified Eagle medium, (DMEM; Lonza) supplemented with 5% fetal calf serum (FCS; EuroClone), 100 U/mL penicillin, 100 µg/mL streptomycin, and 2 mM glutamine (all ThermoFisher Scientific).

Virus isolation, titration and sequencing

5 SARS-CoV-2 virus was isolated from a nasopharyngeal swab collected from an 89-year-old male patient giving informed consent and treated with Betaferon and hydroxychloroquine for 2 days before sample collection. The swab was collected in 3 mL medium (Deltaswab VICUM) to reduce viscosity and stored at -80°C until use. Vero E6 cells were cultured on a cell culture flask (25 cm²) at 1.5×10^6 cells overnight prior to inoculation with 1 mL of the processed sample, for 1 h at 37°C and 5% CO₂. Afterwards, 4 mL of 2% FCS-supplemented DMEM were supplied and cells were
10 incubated for 48 h. Supernatant was harvested, centrifuged at 200 x g for 10 min to remove cell debris and stored at -80°C. Cells were assessed daily for cytopathic effect and the supernatant was subjected to viral RNA extraction and specific RT-qPCR using the SARS-CoV-2 UpE, RdRp and N assays (Corman et al., 2020). The virus was propagated for two passages and a virus stock was prepared collecting the supernatant from Vero E6.
15

Viral RNA was extracted directly from the virus stock using the Indimag Pathogen kit (Indical Biosciences) and transcribed to cDNA using the PrimeScript™ RT reagent Kit (Takara) using oligo-dT and random hexamers, according to the manufacturer's protocol. DNA library preparation was performed using SWIFT amplicon SARS-CoV-2 panel (Swift Biosciences).
20 Sequencing ready libraries were then loaded onto Illumina MiSeq platform and a 300bp paired-end sequencing kit. Sequence reads were quality filtered and adapter primer sequences were trimmed using trimmomatic. Amplification primer sequences were removed using cutadapt (Martin, 2011). Sequencing reads were then mapped against coronavirus reference (NC_045512.2) using bowtie2 tool (Langmead, B. and Salzberg, S, 2012). Consensus genomic sequence was called from the resulting alignment at a 18x1800x879 average coverage using samtools (Li et al., 2009). Genomic sequence was deposited at GISAID repository (<http://gisaid.org>) with accession ID EPI_ISL_510689.
25

Compound

Plitidepsin was used at a concentration ranging from 100 µM to 0.0512 nM at 1/5 serial dilutions, and also assayed from 10 µM to 0.5 nM at 1/3 dilutions.
30

Antiviral activity

Increasing concentrations of Plitidepsin was added to Vero E6 cells together with $10^{1.8}$ TCID₅₀/mL of SARS-CoV-2, a concentration that achieves a 50% of cytopathic effect. Non-exposed cells were used as negative controls of infection. In order to detect any drug-associated cytotoxic effect, Vero E6 cells were equally cultured in the presence of increasing drug concentrations, but in the
35 absence of virus. Cytopathic or cytotoxic effects of the virus or drugs were measured at 3 days post infection, using the CellTiter-Glo luminescent cell viability assay (Promega). Luminescence was measured in a Fluoroskan Ascent FL luminometer (ThermoFisher Scientific).

IC₅₀ calculation and statistical analysis

40 Response curves were adjusted to a non-linear fit regression model, calculated with a four-parameter logistic curve with variable slope. Cells not exposed to the virus were used as negative

controls of infection and set as 100% of viability, and used to normalize data and calculate the percentage of cytopathic effect. Statistical differences from 100% were assessed with a one sample t test. All analyses and figures were generated with the GraphPad Prism v8.0b Software.

- 5 The cytopathic effect on Vero E6 cells exposed to a fixed concentration of SARS-CoV-2 in the presence of increasing concentrations of plitidepsin is shown in Figure 38. Drug was used at a concentration ranging from 10 μ M to 0.5 nM at 1/3 dilutions. Non-linear fit to a variable response curve from one representative experiment with two replicates is shown (squares). The particular IC₅₀ value of this experiment is indicated in the figure. Cytotoxic effect on Vero E6 cells exposed to increasing concentrations of plitidepsin in the absence of virus is also shown (circles).
- 10 A constant concentration of SARS-CoV-2 was mixed with increasing concentrations of plitidepsin and added to Vero E6 cells. To control for drug-induced cytotoxicity, Vero E6 cells were also cultures with increasing concentrations of plitidepsin in the absence of SARS-CoV-2. Plitidepsin was able to inhibit viral-induced cytophatic effects (red squares) at concentrations where no cytotoxic effects of the drug were observed (grey circles). The mean IC₅₀ value and
- 15 standard deviation of plitidepsin in two experiments with two replicates each was 0.06 ± 0.02 μ M.

Example 10

- 20 The aim here was to evaluate *in vivo* the effects of plitidepsin in the treatment of severe pneumonia caused by the mouse-adapted A/H1N1 influenza virus infection (A/Puerto Rico/8/34), and also on viral titre levels.

- Experimental set-up: To achieve this objective we employed an *in vivo* model of viral pathogenesis based on the administration of high-dose of PR8 influenza virus (2x10⁵pfu), which generated a severe infection in the lungs. We then evaluated the therapeutic effect of plitidepsin on severe influenza virus infection in mice. Female mice at the age of 9 weeks were anesthetized
- 25 by intraperitoneal injection of ketamine-xylazine solution and infection was performed by intranasal administration of virus solution PBS into 20 μ l per nares.

- Mice that were receiving the treatment were injected subcutaneously with 0.3 mg/kg or 0.15mg/kg of plitidepsin. Subsequently, survival and body weight loss was monitored until day 3 p.i.. No death mice or mice with a weight loss of more than 30% of the starting body weight was recorded
- 30 during the time of the treatment.

- The control of influenza infection in the airways is mediated by enhanced inflammation in the bronchoalveolar lavage fluid (BALF). Figure 43 shows the inflammatory profile in the BALF of infected mice with or without treatment with plitidepsin. Among the major pro-inflammatory cytokines, plitidepsin strongly reduced the levels of IL-6, CCL2, IL-1 α , IFN- γ and TNF- α . Mice
- 35 that were receiving only half-dose of the drug were less protected and showed an intermediated phenotype.

The viral titer in the lungs was also assessed. As shown in Figure 44, plitidepsin reduced the viral titre in the lungs at both 0.3mg/kg and 0.15mg/kg.

- 40 The BALF cellular composition is defined as a marker of lung immune response viral infection. Quantitative measurement of infiltrating cells in correlation to inflammatory cytokine levels was assessed in influenza infected mice. Treatment with plitidepsin did not reduce the total cellular

composition of the BALF ($CD45^+ \times 10^6$). As shown in Figure 45 we also found an increase in alveolar macrophage (AM) infiltration suggesting that AMs contribute to viral clearance.

5 All together, these results confirmed that three subsequent administrations of (total dose of 0.9 mg/kg) of plitidepsin in influenza infected mice can positively reduce inflammation, as shown by the reduction of the early pro-inflammatory cytokines by the treatment. In addition, we detected an increase in alveolar macrophage absolute numbers, which could suggest that AMs play a critical role in viral spread and protection. We also saw a diminished viral titer in the lung of plitidepsin high-dose treated mice.

Example 11

10 In this example, the antiviral activity of plitidepsin on the spread of West Nile virus in cell culture was studied.

(a) Recombinant virus WNV-GFP (lineage 2; molecular clone WN956) in human hepatoma cells (Huh7) and African green monkey kidney cells (Vero-E6).

15 In order to determine the antiviral potential of plitidepsin against WNV-GFP, target cell lines Vero-E6 and Huh7 were inoculated with an infectious virus stock dilution in the presence of increasing concentrations of plitidepsin, starting at 2.3pM or 2.5pg/ml and using 4.5μM or 5μg/ml as the highest concentration. Infection efficiency was evaluated at 48 hours, after the virus has spread to a substantial fraction of the target cells in vehicle-treated cultures.

20 Relative infection efficiency was assessed by automated fluorescence microscopy in the green channel and overall cell biomass/well was estimated by nuclear staining with DAPI in the blue channel, as a preliminary assessment of the compound effective doses as well as overall compound cytotoxicity.

25 As shown in Figure 46, WNV-GFP infection efficiency rapidly decreased at doses around 5nM in VeroE6 cells, reaching background fluorescence levels at 36nM and above. The cell number remained unchanged up to 36nM, but significantly decreased at higher concentrations (180 nM; $p < 0.05$), suggesting that plitidepsin interferes with cell duplication capacity at concentrations above 36 nM. The estimated EC50 value for plitidepsin in VeroE6 cells is 4.9 nM and the EC90 is 9.5 nM. Based on the cell biomass the CC50 value is 2330 nM. Thus, the therapeutic index, expressed as the ratio CC50/EC50 in VeroE6 cells is 475.

30 In Huh7 cells, as shown in Figure 47, there is a dose-dependent reduction in infection efficiency with EC50 and EC90 values of 0.665 and 3.86 nM respectively. This reduction is associated with a parallel loss of cell biomass in the well, which is statistically significant at 7.2nM ($p < 0.05$) with a CC50 value of 14.7 and a therapeutic index of 22.

35 The above data shows that plitidepsin interferes with WNV-GFP propagation in cell culture infection models in both Vero-E6 and Huh-7 cells. In one example, antiviral activity in the absence of measurable interference with cell viability may be observed at 1.5 nM ($p < 0.05$) in Huh-7 cells and 7.2 nM ($p < 0.05$) in Vero-E6 cells.

40 (b) Recombinant virus with wild-type WNV-NY99 genome (lineage 1; molecular clone NY99) in Huh7 human hepatoma cells and African green monkey kidney cells (Vero-E6).

In this example, the impact of plitidepsin on WNV-NY99 propagation using viral RNA

load as readout of the infection efficiency was assessed. Vero-E6 or Huh-7 cells were inoculated (MOI 0.01) with a recombinant virus based on the NY99 WNV strain. Infection was performed in the presence of the vehicle or a dose range of plitidepsin

5 displaying bioactivity in the WNV-GFP model (45, 15, 5, 1.5 nM; see above). Inoculated cells were incubated for 48 hours, time at which samples of the supernatants were processed to determine infection efficiency by extracellular infectivity titration. In addition, total RNA was extracted from control and plitidepsin-treated cells to determine viral RNA load and independently assess overall viral infection efficiency.

10 Extracellular infectivity titers show that the presence of plitidepsin strongly interfered with WNV propagation as shown in Figure 48, with reductions >3 orders of magnitude at 45nM in VeroE6 and Huh-7 cells. This phenomenon is dose- dependent in both cell lines.

15 Intracellular WNV RNA load was determined by RT-qPCR in control and plitidepsin- treated cells. Remarkably, as shown in Figure 49, no RNA could be recovered from Huh-7 cells treated with 45nM plitidepsin. Nevertheless, viral RNA load was determined in the rest of the sample groups in both cell lines. In agreement with the trend exhibited by the extracellular infectivity titers, viral RNA load significantly decreased in a dose-dependent manner at 15 and 45nM in Vero-E6 cells, while the viral load at 5 and 1.5nM were not distinguishable from those in the control (Figure 49). Similarly, Huh-7 showed a marked reduction in viral load at 15nM, magnitude of which declined in a dose-dependent manner.

20 The WNV infection propagation efficiency data suggest that plitidepsin interferes with viral replication at doses above 5nM in both Vero-E6 and Huh-7 cells, although some degree of interference could be observed at lower concentrations in Huh-7 cells. The results measuring overall propagation efficiency using functional (infectivity) as well as molecular (RT-qPCR) approaches confirm that plitidepsin reduces virus propagation efficiency in the expected range of concentrations based on the data obtained with WNV-GFP.

25 In summary, supplementation of the cell culture medium with increasing doses of plitidepsin resulted in strong reduction in WNV propagation efficiency in the two infection (WNV-GFP and WNV/NY99) models and in both Vero-E6 and Huh-7 cells.

30 (c) Methods

Compound preparation: Pre-weighted solid was diluted to a final 1mg/ml solution in dimethylsulfoxide (DMSO) and aliquoted at -20°C until further use. Cell culture: Subconfluent Vero-E6 cells and Huh-7 cell cultures were maintained in complete media [(DMEM supplemented with 10 mM HEPES, 1x non-essential amino acids (Gibco), 100 U/ mL penicillin-streptomycin (Gibco) and 10% Fetal Bovine Serum (heat-inactivated at 56 °C for 30 min)].

Viruses: WNV (NY99) and WNV-GFP recombinant viruses were rescued from cloned cDNA as previously described. Stock infectivity titers were determined by plaque assay on Vero- E6 cells as previously described.

Part 1: WNV-GFP model

5 Infection experiments: Cells were seeded onto 96-well plates (2 X10⁴ cells/well). The day after, serial 5-fold dilutions of plitidepsin were prepared in 2% FCS-containing complete media to achieve the indicated final concentrations. WNV-GFP stock was diluted in complete media containing 2% FCS to achieve the required multiplicity of infection (MOI 0.01). Compound and virus dilutions were mixed 1:1 and added onto the target cells. Cells were incubated for 48 hours at 37°C; 5% CO₂ and 95% humidity.

10 Cells were fixed by addition of a 5X formaldehyde solution to achieve a 4% final concentration for 30 mins at room temperature. Cells were washed with PBS and stained with DAPI (4',6-diamidino-2-phenylindole) following manufacturer's recommendations. Relative infection efficiency was estimated by image analysis in an automated microscopy device (Tecan Spark Cyto). Uninfected cells and vehicle-treated controls were included in each plate.

Part 2: WNV (NY99) model

15 Cells were seeded onto a 24-well plate using 1.2 X10⁵ cells /well. The day after, cells were inoculated with a WNV/NY99 stock to achieve a multiplicity of infection of 0.01 (MOI 0.01) and the indicated plitidepsin concentration in a final volume of 1ml. Cultures were maintained at 37°C for 48 hours, time at which supernatants were collected and preserved at -80°C. Total RNA was collected from cells by adding Trizol™ reagent directly to the cells and following the manufacturer's instructions.

20 Infectivity titration: infectivity titers were determined using endpoint dilution and immunofluorescence microscopy using a monoclonal antibody against Flavivirus E protein (4G2; ATCC® HB-112™). Briefly, Huh-7 cells were inoculated with supernatant dilutions in a 96-well format. Forty-eight hours post infection, cells were fixed for 30 minutes at room temperature with a 4% formaldehyde solution in PBS, washed twice with PBS and incubated with binding buffer (0.3% Triton X100, 3%BSA in PBS) for 1 hour. Primary antibody was diluted in binding buffer and incubated with the cells for 1-hour, time after which the cells were washed with PBS and subsequently incubated with a 1:500 dilution of a goat anti-mouse conjugated to Alexa 488 (ThermoFisher). DAPI (4',6-diamidino-2-phenylindole; ThermoFisher) was used as nucleus staining reagent to evaluate cell number. Cells were washed with PBS and infection foci number was determined under a fluorescence microscope.

30 Reverse-transcription and qPCR: 60 ng of total cellular RNA were subjected to RT-qPCR using NZYSpeedy One-Step qPCR probe master mix, using manufacturer's recommendations and the primers.

Statistical Analysis: Means and SEM were calculated using Excel. Means were compared using one-way ANOVA and a Dunnet's post-hoc analysis (2-tails; alpha=0.05) using IBM- SPSS

35 EXAMPLE 12 A Phase 3, Multicentre, Randomised, Controlled Trial to Determine the Efficacy and Safety of Two Dose Levels of Plitidepsin Versus Control in Adult Patients Requiring Hospitalisation for Management of Moderate COVID 19 Infection

Indication: Treatment of patients hospitalised for management of moderate COVID 19 infection

40 **Objectives:**

Primary objective:

- 5 • Compare plitidepsin 1.5 or 2.5 mg versus control on the percentage of patients who achieve complete recovery by Day 8 (± 1), defined as (i) meeting categories 0 to 2 on the 11-point World Health Organization (WHO) Clinical Progression Scale below, (ii) having Barthel Index $>90/100$ at the time of discharge, and (iii) with no re-admission for COVID-19 infection through Day 31

10 0: uninfected, no viral RNA detected
 1: asymptomatic, viral RNA detected
 2: symptomatic, independent
 3: symptomatic, assistance needed
 4: hospitalised, no oxygen therapy (if hospitalised for isolation only, record status as for ambulatory patient)
 5: hospitalised, oxygen by mask or nasal prongs
 6: hospitalised, oxygen by noninvasive ventilation (NIV) or high flow
 7: intubation and mechanical ventilation. $pO_2/FIO_2 \geq 150$ or $SpO_2/FIO_2 \geq 200$
 8: mechanical ventilation $pO_2/FIO_2 < 150$ ($SpO_2/FIO_2 < 200$) or vasopressors
 9: mechanical ventilation $pO_2/FIO_2 < 150$ and vasopressors, dialysis, or extracorporeal membrane oxygenation (ECMO)
 10: dead

15 20 Key secondary objectives of this study are to compare plitidepsin 1.5 and 2.5 mg versus control on the following:

 - 25 • Time to complete recovery (in days), defined as the first day, from Day 1 through follow-up on Day 31, on which a patient (i) satisfies categories 0 to 2 on the 11-point WHO Clinical Progression Scale above, (ii) has Barthel Index $>90/100$ at the time of discharge, and (iii) has no subsequent re-admission for COVID-19 infection

 • Clinical status, as assessed by the 11-category WHO Clinical Progression Scale, at Day 8 (± 1)

Other secondary objectives of this study are:

- 30 • Safety and tolerability, based on treatment-emergent adverse events (TEAEs), Grade ≥ 3 TEAEs, serious adverse events (SAEs), and serious adverse reactions (SARs)

 • Compare the efficacy and safety/tolerability between plitidepsin arms (1.5 versus 2.5 mg) in case both arms are significantly better than the control arm on the primary endpoint

35 • Compare the percentage of patients who achieve complete recovery by Day 8 (± 1), as defined above, for the pooled plitidepsin arms versus control

 • Percentage of patients in each study arm requiring re-admission for COVID-19 infection

 • Clinical status in each study arm, as assessed by the 11-category WHO Clinical Progression Scale, at Days 4, 15, and 31

40 • Duration of oxygen therapy (in days) for each study arm

 • Percentage of patients in each study arm requiring high-flow oxygen at Days 4, 8, 15, and 31

 • Percentage of patients in each study arm requiring noninvasive mechanical ventilation at Days 4, 8, 15, and 31

- Percentage of patients in each study arm requiring invasive mechanical ventilation or ECMO at Days 4, 8, 15, and 31
- Percentage of patients in each study arm requiring intensive care unit (ICU) admission at Days 4, 8, 15, and 31
- 5 • Duration of hospitalisation in ICU for each study arm
- Percentage of patients receiving subsequent antiviral therapies or immunomodulatory drugs at Days 4, 8, 15, and 31
- Percentage of patients in each study arm with nosocomial infection
- 10 • Mortality in each study arm at Days 4, 8, 15, and 31
- Change in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral load in each study arm from Day 1 before administration of study drug to Day 8 as measured by quantitative polymerase chain reaction (qPCR) from samples of oro-nasopharyngeal exudate
- 15 • Percentage of patients in each study arm with undetectable SARS-CoV-2 viral load on Day 8, as measured by qPCR from samples of oro-nasopharyngeal exudate

Change in proinflammatory biomarkers (C-reactive protein [CRP], lactate dehydrogenase [LDH], ferritin, interleukin [IL]-6, IL-1 β , IL-10, and tumour necrosis factor alpha [TNF α]) in each study arm from baseline to Days 2, 3, 4, 8, 15, and 31

Methodology/study design:

- 20 This is a multicentre, open label, controlled Phase 3 study in which adults requiring hospital admission and O₂ supplementation for management of moderate COVID 19 infection will be randomised in 1:1:1 to:

- Plitidepsin 1.5 mg arm: Patients will receive plitidepsin 1.5 mg/day intravenous (IV) combined with dexamethasone 6.6 mg/day IV on Days 1 to 3, followed by dexamethasone 6 mg/day oral administration (PO)/IV from Day 4 and up to Day 10 (as per physician judgement according to patient clinical condition and evolution).
- 25 • Plitidepsin 2.5 mg arm: Patients will receive plitidepsin 2.5 mg/day IV combined with dexamethasone 6.6 mg/day IV on Days 1 to 3, followed by dexamethasone 6 mg/day PO/IV from Day 4 and up to Day 10 (as per physician judgement according to patient clinical condition and evolution).
- 30 • Control arm: Patients will receive dexamethasone 6.6 mg/day IV on Days 1 to 3, followed by dexamethasone 6 mg/day PO/IV from Day 4 and up to Day 10 (as per physician judgement according to patient clinical condition and evolution). Additionally, in accordance with local treatment guidelines, patients may receive remdesivir 200 mg IV on Day 1 followed by 100 mg/day IV on Days 2 to 5.
- 35 • Intention to administer remdesivir if the patient is randomised to the control arm (yes vs. no); and
- Charlson Comorbidity Index^{13,14} (0-1 vs. >1)

Randomisation will be stratified for 2 factors:

From treatment initiation on Day 1, patients will be followed in the hospital for at least 4 days and then through Day 31 or resolution/stabilisation of TEAEs that occurred through Day 31. Patients discharged from the hospital prior to Day 8 will return to an out-patient clinic for assessments on Days 8 and 31.

- 5 An Independent Data Monitoring Committee (IDMC) will oversee study conduct (safety and primary endpoint), including analysis of summary safety data as per the trial requirements.

Diagnosis and main criteria for inclusion and exclusion:

The following are the inclusion criteria:

- 10 1. Signed informed consent obtained prior to initiation of any study specific procedures and study treatment
2. Laboratory confirmed SARS-CoV-2 infection as determined by qualitative polymerase chain reaction (PCR) by local laboratory from oro/nasopharyngeal exudate (or other respiratory specimen) collected no more than 48 hours prior to study treatment on Day 1
- 15 3. Admitted to hospital as clinically indicated for management of moderate SARS-CoV-2 (COVID 19) infection, defined by the following criteria:
 - Positive PCR test for SARS-CoV-2
 - Symptoms of moderate illness with COVID 19, which could include any symptoms of mild illness or shortness of breath with exertion
 - 20 • Clinical signs suggestive of moderate illness with COVID 19 such as respiratory rate ≥ 20 breaths but < 30 breaths per minute, SpO₂ $> 93\%$ but $< 95\%$ on room air at sea level, heart rate ≥ 90 but < 125 beats per minute, and requiring O₂ supplementation
 - 25 • No clinical signs indicative of severe illness, which could include shortness of breath at rest or respiratory distress
4. Onset of COVID 19 symptoms no later than 6 days prior to initiation of study treatment on Day 1
5. Male or female aged ≥ 18 years
- 30 6. Adequate bone marrow, liver, kidney, and metabolic function, defined by the following tests performed at local laboratory:
 - Absolute neutrophil count $\geq 1000/\text{mm}^3$ ($1.0 \times 10^9/\text{L}$)
 - Lymphocyte count $\geq 500/\text{mm}^3$ ($0.5 \times 10^9/\text{L}$)
 - Platelet count $\geq 100\,000/\text{mm}^3$ ($100 \times 10^9/\text{L}$)
 - Haemoglobin $> 9.0 \text{ g/dL}$
 - 35 • Alanine transaminase (ALT), aspartate transaminase (AST) $\leq 3 \times$ upper limit of normal (ULN)
 - Serum bilirubin $\leq 1 \times \text{ULN}$ ($\leq 3 \times \text{ULN}$ if documented Gilbert's syndrome)
 - Calculated creatinine clearance $\geq 30 \text{ mL/min}$ (Cockcroft and Gault formula)
 - Creatine phosphokinase $\leq 2.5 \times \text{ULN}$
- 40 7. Agree not to participate in another interventional clinical trial through Day 31
8. Females of reproductive capacity must have a negative serum pregnancy test by local laboratory at study enrolment and must be non-lactating
9. Females and males with partners of child bearing potential must use effective contraception while on study treatment and for 3 months after last dose of plitidepsin.

- 45 The following are the exclusion criteria:

1. Subjects with a pre-baseline (ie, in the prior month) impairment in general health condition for whatever reason except COVID 19, requiring either assistance for daily living activities or chronic oxygen therapy
2. Participating in another clinical trial for treatment of COVID 19 infection or patients previously enrolled in clinical trials and currently in follow up, or patients previously vaccinated for COVID 19
3. Evidence of respiratory failure at the time of randomisation, based on resource utilisation requiring at least 1 of the following: endotracheal intubation and mechanical ventilation, oxygen delivered by high-flow nasal cannula, noninvasive positive pressure ventilation, ECMO, or clinical diagnosis of respiratory failure (ie, clinical need for 1 of the preceding therapies, but preceding therapies not able to be administered in setting of resource limitation)
4. Patients clinically indicated for management of SARS-CoV-2 (COVID 19), with baseline disease severity rated as severe (if positive testing by standard RT PCR assay or equivalent test, symptoms suggestive of severe illness with COVID 19, which could include any symptom of moderate illness or shortness of breath at rest, or respiratory distress, clinical signs indicative of severe systemic illness with COVID 19, such as respiratory rate ≥ 30 per minute, heart rate ≥ 125 per minute, $SpO_2 \leq 93\%$ on room air at sea level, or $PaO_2/FiO_2 < 300$)
5. Patients receiving treatment with antivirals, IL 6 receptor inhibitor, corticosteroids, or immunomodulatory drugs for COVID 19 infection within 4 weeks before enrolment
6. History of live vaccination within the last 4 weeks prior to study enrolment; subjects must not receive live, attenuated influenza vaccine within 4 weeks before enrolment or at any time during the study
7. Patients receiving treatment with chloroquine or derivatives within 8 weeks before enrolment or during the study
8. Receiving treatment with strong cytochrome P450 3A4 (CYP3A4) inhibitors or inducers
9. Viral illness (other than COVID 19) requiring therapy, except for patients with treated and adequately controlled (undetectable) human immunodeficiency virus infection are eligible
10. QT interval corrected using Fridericia's formula prolongation > 450 msec for males or > 470 msec for females, based on triplicate electrocardiogram (ECG) at screening
11. Pre-existing neuropathies of any type Grade ≥ 2
12. Hypersensitivity to the active ingredient or any of the excipients (mannitol, macrogolglycerol hydroxystearate, and ethanol).
13. Females who are pregnant (negative serum pregnancy test required for all females of child bearing potential at screening) or breast feeding
14. Females and males with partners of child bearing potential (females who are not surgically sterile or postmenopausal defined as amenorrhoea for > 12 months) who are not using at least 1 protocol-specified method of contraception
15. Any other clinically significant medical condition or laboratory abnormality that, in the opinion of the investigator, would jeopardise the safety of the patient or potentially impact patient compliance or the safety/efficacy observations in the study.

Test products, dose, and mode of administration:

Plitidepsin for injection is provided in vials containing 2 mg plitidepsin powder. For administration, vial contents are reconstituted by addition of 4 mL of solvent for plitidepsin to

obtain a slightly yellowish solution containing 0.5 mg/mL plitidepsin with mannitol, macrogolglycerol hydroxystearate, and ethanol excipients. The required amount of plitidepsin reconstituted solution is added to an IV bag containing 0.9% sodium chloride injection or 5% glucose for injection and administered as an IV infusion over 60 minutes.

5 For prevention of plitidepsin related infusion reactions, all patients must receive the following medications 20 to 30 minutes prior to starting the plitidepsin infusion:

- Ondansetron 8 mg IV (or equivalent; note: ondansetron is a prohibited medication for patients in the corrected QT interval [QTc] substudy during the QT evaluation [Days 1 to 3])
- 10 • Diphenhydramine hydrochloride 25 mg IV (or equivalent)
- Ranitidine 50 mg IV (or equivalent)
- Dexamethasone 6.6 mg IV

Additionally, on Days 4 and 5 patients treated with plitidepsin must receive ondansetron 4 mg twice a day PO.

15 Reference therapy, dose, dose form, and mode of administration:

Dexamethasone: Patients on both plitidepsin and control arms will receive dexamethasone 6.6 mg/day IV on Days 1 to 3, followed by dexamethasone 6 mg/day PO/IV from Day 4 and up to Day 10 (as per physician judgement according to patient clinical condition and evolution).

20 Remdesivir: Consistent with local treatment guidelines, patients randomised to the control arm may receive remdesivir 200 mg IV on Day 1 followed by 100 mg/day IV on Days 2 to 5.

Best Supportive Care (BSC): BSC consistent with National Institute of Health COVID 19 treatment guidelines (www.covid19treatmentguidelines.nih.gov) or other country guidelines will be provided to all study participants.

25 EXAMPLE 13 Open-label, randomized phase II study to evaluate the safety and reduction of viral load of a single-line treatment with plitidepsin in adult patients with COVID-19 at discharge from the Emergency Department

Indication: Treatment of patients with mild type COVID 19 infection.

30 Patients will be included in the study if presenting with acute clinical infection (onset of symptoms in the previous 5 days), in which the diagnosis of COVID-19 infection is reached through a diagnostic method that could be a positive antigen test or a positive PCR test.

The study comprises two arms:

- arm A) single dose of 7.5 mg plitidepsin administered as a slow infusion of 90 minutes ($\pm$ 10 minutes) plus symptomatic treatment according to routine clinical practice in the participating centers.
- 35 arm B) symptomatic treatment according to usual clinical practice in the participating centers.

All patients receive the following prophylactic medications 20-30 minutes prior to plitidepsin infusion:

- Diphenhydramine hydrochloride 25 mg i.v.,
- Ranitidine 50 mg i.v.
- Dexamethasone 6.6 mg intravenously.
- Ondansetron 8 mg i.v. in slow infusion of 15 minutes.

5

Ondansetron 4 mg orally is given every 12 hours for 3 days after plitidepsin administration to relieve drug-induced nausea and vomiting. If plitidepsin is administered in the morning the patient receives the first dose of ondansetron in the afternoon.

10

The study will show that a single dose of plitidepsin administered to patients results in a reduction of viral load. This may be expressed as a replication cycle threshold (Ct) value greater than 30 (Ct > 30), on day 6 after the administration. For example, the study will show that patients with COVID-19 infection who are to be discharged from the Emergency Department show a reduction in viral load on day 6 after discharge of emergencies expressed as a replication cycle threshold (Ct) value greater than 30 (Ct > 30), when administered with a single dose of plitidepsin. This may be expressed as a reduction in SARS-CoV-2 viral load from baseline. This may be expressed as a reduction in the percentage of patients requiring hospitalisation following administration. This may be expressed as a reduction in the percentage of patients requiring invasive mechanical ventilation and / or admission to the ICU following administration. This may be expressed as a reduction of patients who develop sequelae related to persistent disease. This may be expressed as an increase in the percentage of patients with normalization of analytical parameters chosen as poor prognosis criteria (including, for example, lymphopenia, LDH, D-dimer or PCR). This may be expressed as an increase in the percentage of patients with normalization of clinical criteria (disappearance of symptoms), including, for example: headache, fever, cough, fatigue, dyspnea (shortness of breath), arthromyalgia or diarrhea.

15

20

25

30

The study will show that single-dose treatment with plitidepsin can eliminate the SARS-CoV-2 viral load in the patient on day 6, which, according to several studies, leads to clinical improvement, and therefore a decrease in complications, understood as hospitalization, ICU and death. In addition to improving the prognosis of patients in the short term, the decrease in viral load is believed to be key to two other objectives. Firstly, reducing the infectivity of asymptomatic or not very symptomatic patients with high viral loads (TC <25), known as supercontagators. Secondly, decreasing viral load can be decisive to avoid long-term complications known as COVID persistent or long COVID.

35

A validated plitidepsin population pharmacokinetic model (Nalda-Molina R, et al. Population pharmacokinetics meta-analysis of plitidepsin in cancer subjects. *Cancer Chemother Pharmacol.* 2009 Jun;64(1):97-108. doi: 10.1007/s00280-008-0841-4) was used to confirm total plasma concentration will reach the estimated lung target concentrations. Figure 53 shows the results and it can be seen that plasma concentrations above IC50 and IC90 can be obtained for more than 6 days.

REFERENCES

- Almazan, F., Galan, C., Enjuanes, L., 2004. The nucleoprotein is required for efficient coronavirus genome replication. *J. Virol.* 78, 12683–12688. 10 Baric et al., **1988**
- 5 • Baric, R.S., Nelson, G.W., Fleming, J.O., Deans, R.J., Keck, J.G., Casteel, N., Stohlman, S.A. Interactions between coronavirus nucleocapsid protein and viral RNAs: implications for viral transcription. *J. Virol.* **1988**, 62, 4280–4287
- Escors, D., Camafeita, E., Ortego, J., Laude, H., Enjuanes, L. Organization of two transmissible gastroenteritis coronavirus membrane protein topologies within the virion and core. *J. Virol.* **2001**, 75, 12228–12240.
- 10 • Fang, S.G.; Shen, H.; Wang, J.; Tay, F.P.; Liu, D.X. Proteolytic processing of polyproteins 1a and 1ab between non-structural proteins 10 and 11/12 of Coronavirus infectious bronchitis virus is dispensable for viral replication in cultured cells. *Virology* **2008**, 379, 175–180.
- Graham, R.L.; Donaldson, E.F.; Baric, R.S. A decade after SARS: Strategies for controlling emerging coronaviruses. *Nat. Rev. Microbiol.* **2013**, 11, 836–848.
- 15 • Lai, M.M.; Cavanagh, D. The molecular biology of coronaviruses. *Adv. Virus Res.* **1997**, 48, 1–100.
- Losada A, Berlanga JJ, Molina-Guijarro JM, Jiménez-Ruiz A, Gaggo F, Avilés P, de Haro C, Martínez-Leal JF., Generation of endoplasmic reticulum stress and inhibition of autophagy by plitidepsin induces proteotoxic apoptosis in cancer cells. *Biochem Pharmacol.* **2020** Feb; 172:113744
- 20 • Losada A, Muñoz-Alonso MJ, García C, Sánchez-Murcia PA, Martínez-Leal JF, Domínguez JM, Lillo MP, Gago F, Galmarini CM., Translation Elongation Factor eEF1A2 is a Novel Anticancer Target for the Marine Natural Product Plitidepsin. *Sci Rep.* **2016** 7;6:35100
- 25 • Losada A, Muñoz-Alonso MJ, Martínez-Díez M, Gago F, Domínguez JM, Martínez-Leal JF, Galmarini CM., Binding of eEF1A2 to the RNA-dependent protein kinase PKR modulates its activity and promotes tumour cell survival. *Br J Cancer.* **2018**;119(11):1410-1420
- 30 • M. D. Vera and M. M. Joullié, Natural products as probes of cell biology: 20 years of didemnin research *Med. Res. Rev.*, **2002**, 22, 102
- Pirc, K.; Berkhout, B.; van der Hoek, L. The novel human coronaviruses NL63 and HKU1. *J. Virol.* **2007**, 81, 3051–3057.
- 35 • Rabouw HH, Langereis MA, Knaap RC, Dalebout TJ, Canton J, Sola I, Enjuanes L, Bredenbeek PJ, Kikkert M, de Groot RJ, van Kuppeveld FJ., Middle East Respiratory Coronavirus Accessory Protein 4a Inhibits PKR-Mediated Antiviral Stress Responses. *PLoS Pathog.* **2016**, 26;12(10):e1005982.
- Remington's Pharmaceutical Sciences, 17th Ed., Mack Publishing Company, Easton, Pa., **1995**
- 40 • Risco, C., Anton, I.M., Enjuanes, L., Carrascosa, J.L. The transmissible gastroenteritis coronavirus contains a spherical core shell consisting of M and N proteins. *J. Virol.* **1996**, 70, 4773–4777
- Snijder, E.J.; Bredenbeek, P.J.; Dobbe, J.C.; Thiel, V.; Ziebuhr, J.; Poon, L.L.; Guan, Y.; Rozanov, M.; Spaan, W.J.; Gorbalenya, A.E. Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineage. *J. Mol. Biol.* **2003**, 331, 991–1004
- 45 • Stark G.R., Kerr, I.M., Williams, B.R., Silverman, R.H., and Schreiber, R.D., How cells respond to interferons. *Annu. Rev. Biochem.* **1998**, 67: 227–264.

- Stohlman, S.A., Baric, R.S., Nelson, G.N., Soe, L.H., Welter, L.M., Deans, R.J. Specific interaction between coronavirus leader RNA and nucleocapsid protein. *J. Virol.* **1988**, 62, 4288–4295
- 5 • Sturman, L.S., Holmes, K.V., Behnke, J. Isolation of coronavirus envelope glycoproteins and interaction with the viral nucleocapsid. *J. Virol.* **1980**, 33, 449–462.
- Williams B.R. PKR; a sentinel kinase for cellular stress. *Oncogene.* **1999**; 18:6112–6120
- Xin Z, Hongyan S, Jianfei C, Da S, Changlong L, Li F., EF1A interacting with nucleocapsid protein of transmissible gastroenteritis coronavirus and plays a role in virus replication. *Vet Microbiol.* **2014**, 27;172(3-4):443-8
- 10 • Xu, Jiabao & Zhao, Shizhe & Teng, Tieshan & Abdalla, Abualgasim & Zhu, Wan & Xie, Longxiang & Wang, Yunlong & Guo, Xiangqian., Systematic Comparison of Two Animal-to-Human Transmitted Human Coronaviruses: SARS-CoV-2 and SARS-CoV. *Viruses.* **2020**, 12. 244.
- Zhou, B., Liu, J., Wang, Q., Liu, X., Li, X., Li, P., Ma, Q., Cao, C. The nucleocapsid protein of severe acute respiratory syndrome coronavirus inhibits cell cytokinesis and proliferation by interacting with translation elongation factor 1 alpha. *J. Virol.* **2008**, 82, 6962–6971.
- 15 • Ranieri VM, Rubenfeld GD, Thompson BT, et al. Acute respiratory distress syndrome: the Berlin Definition. *JAMA* 2012; 307: 2526-2533;
- 20 • Technical document. Clinical management of COVID-19: hospital care. Date: 03/19/2020 Source: Ministry of Health. Spain.
- Wiersinga WJ, Bonten MJ, Boersma WG, et al. Management of community-acquired pneumonia in adults: 2016 guideline update from the Dutch Working Party on Antibiotic Policy (SWAB) and Dutch Association of Chest Physicians (NVALT). *Neth J Med.* 2018 Jan; 76 (1): 4-13.
- 25 • Lim WS1, Baudouin SV, George RC, et al. BTS guidelines for the management of community acquired pneumonia in adults: update 2009. *Thorax.* 2009 Oct; 64 Suppl 3: iii1-55. doi: 10.1136 / thx.2009.121434.]
- Rodriguez-Morales AJ, Cardona-Ospina JA, Gutiérrez-Ocampo E, et al. Clinical, laboratory and imaging features of COVID-19: A systematic review and meta-analysis. *Travel Med Infect Dis.* 2020 Mar 13: 101623
- 30 • Contingency plan for intensive medicine services against the pandemic COVID-19 of the Spanish Society of Intensive Nursing and Coronary Units.
- Fleming TR. One-sample multiple testing procedure for phase II clinical trials. *Biometrics.* 1982 Mar; 38 (1): 143-51.
- 35 • Rinehart KL Jr, Gloer JB, Hughes RG et al. Didemnins: antiviral and antitumor depsipeptides from a caribbean tunicate. *Science.* 1981 May 22; 212 (4497): 933-5.
- Canonico PG, Pannier WL, Huggins JW, Rinehart KL. Inhibition of RNA viruses in vitro and in Rift Valley fever-infected mice by didemnins A and B. *Antimicrob Agents Chemother.* 1982 Oct; 22 (4): 696-7.
- 40 • Losada, A., et al., Translation Elongation Factor eEF1A2 is a Novel Anticancer Target for the Marine Natural Product Plitidepsin. *Sci Rep*, 2016. 6: p. 35100.
- Mateyak, M.K. and T.G. Kinzy, eEF1A: thinking outside the ribosome. *J Biol Chem*, 2010. 285 (28): p. 21209-13.
- 45 • Zhou, B., et al., The nucleocapsid protein of severe acute respiratory syndrome coronavirus inhibits cell cytokinesis and proliferation by interacting with translation elongation factor 1alpha. *J Virol*, 2008. 82 (14): p. 6962-71.

- Ma-Lauer, Y., et al., Influences of cyclosporin A and non-immunosuppressive derivatives on cellular cyclophilins and viral nucleocapsid protein during human coronavirus 229E replication. *Antiviral Res.* 2020. 173: p. 104620.
- 5 • Cervantes-Barragan, L., et al., Dendritic cell-specific antigen delivery by coronavirus vaccine vectors induces long-lasting protective antiviral and antitumor immunity. *mBio*, 2010. 1 (4).
- Wang M, Cao R, Zhang L, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Research* 2020; 30: 269–271.
- 10 • PharmaMar, data on file; VPT1992 / 2014.
- PharmaMar, data on file; VPT2678 / 2014.
- Maroun, J.A., et al., Phase I study of Aplidine in a dailyx5 one-hour infusion every 3 weeks in patients with solid tumors refractory to standard therapy. A National Cancer Institute of Canada Clinical Trials Group study: NCIC CTG IND 115. *Ann Oncol*, 2006. 17 (9): p. 1371-8.
- 15 • Nalda-Molina R, et al. Population pharmacokinetics meta-analysis of plitidepsin (Aplidin) in cancer subjects. *Cancer Chemother Pharmacol.* 2009 Jun; 64 (1): 97-108. doi: 10.1007 / s00280-008-0841-4.
- Kaul D. An overview of coronaviruses including the SARS-2 coronavirus - Molecular biology, epidemiology and clinical implications [published online ahead of print, 2020 Apr 9] [published correction appears in *Curr Med Res Pract.* 2020 May-Jun;10(3):135]. *Curr Med Res Pract.* 2020;10(2):54-64. doi:10.1016/j.cmrp.2020.04.001
- 20 • Wang C et al. Alveolar macrophage activation and cytokine storm in the pathogenesis of severe COVID-19; *Immunology Network*; 25 March 2020.
- 25 • Kawai T & Akira S (2007); Signaling to NF-κB by Toll-like receptors; *Trends in Molecular Medicine*; Volume 13, Issue 11, p.460-469.
- Pang T, Cardoso MJ, Guzman MG. Of cascades and perfect storms: the immunopathogenesis of dengue haemorrhagic fever-dengue shock syndrome (DHF/DSS). *Immunol Cell Biol.* 2007;85(1):43-5.
- 30 • Monath TP. Treatment of yellow fever. *Antiviral Res.* 2008;78(1):116-24.
- Rothman AL. Immunity to dengue virus: a tale of original antigenic sin and tropical cytokine storms. *Nat Rev Immunol.* 2011;11(8):532-43.
- Henchal EA, Putnak JR. The dengue viruses. *Clin Microbiol Rev.* 1990;3(4):376-96.
- Hayes EB, Gubler DJ. Dengue and dengue hemorrhagic fever. *Pediatr Infect Dis J.* 1992;11(4):311-7.
- 35 • Monath TP. Dengue: the risk to developed and developing countries. *Proc Natl Acad Sci U S A.* 1994;91(7):2395-400.
- Srikiatkachorn A, Mathew A, Rothman AL. Immune-mediated cytokine storm and its role in severe dengue. *Semin Immunopathol.* 2017;39(5):563-74.
- 40 • Rothman AL. Immunology and immunopathogenesis of dengue disease. *Adv Virus Res.* 2003;60:397-419.

- Leclercq TM, Moretti PA, Pitson SM. Guanine nucleotides regulate sphingosine kinase 1 activation by eukaryotic elongation factor 1A and provide a mechanism for eEF1A-associated oncogenesis. *Oncogene*. 2011;30(3):372-8.
- 5 • Carr JM, Kua T, Clarke JN, Calvert JK, Zebol JR, Beard MR, et al. Reduced sphingosine kinase 1 activity in dengue virus type-2 infected cells can be mediated by the 3' untranslated region of dengue virus type-2 RNA. *J Gen Virol*. 2013;94(Pt 11):2437-48.
- Kuno G, Chang GJ. Full-length sequencing and genomic characterization of Bagaza, Kedougou, and Zika viruses. *Arch Virol*. 2007;152(4):687-96.
- 10 • Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med*. 2009;360(24):2536-43.
- Rasmussen SA, Jamieson DJ, Honein MA, Petersen LR. Zika Virus and Birth Defects--Reviewing the Evidence for Causality. *N Engl J Med*. 2016;374(20):1981-7.
- 15 • Watrin L, Ghawche F, Larre P, Neau JP, Mathis S, Fournier E. Guillain-Barre Syndrome (42 Cases) Occurring During a Zika Virus Outbreak in French Polynesia. *Medicine (Baltimore)*. 2016;95(14):e3257.
- Tappe D, Perez-Giron JV, Zammarchi L, Rissland J, Ferreira DF, Jaenisch T, et al. Cytokine kinetics of Zika virus-infected patients from acute to convalescent phase. *Med Microbiol Immunol*. 2016;205(3):269-73.
- 20 • Chen Q, Gouilly J, Ferrat YJ, Espino A, Glaziou Q, Cartron G, et al. Metabolic reprogramming by Zika virus provokes inflammation in human placenta. *Nat Commun*. 2020;11(1):2967.
- ter Meulen J, Sakho M, Koulemou K, Magassouba N, Bah A, Preiser W, et al. Activation of the cytokine network and unfavorable outcome in patients with yellow fever. *J Infect Dis*. 2004;190(10):1821-7.
- 25 • Heim MH, Thimme R. Innate and adaptive immune responses in HCV infections. *J Hepatol*. 2014;61(1 Suppl):S14-25.
- Zeremski M, Petrovic LM, Talal AH. The role of chemokines as inflammatory mediators in chronic hepatitis C virus infection. *J Viral Hepat*. 2007;14(10):675-87.
- 30 • Li H, Li T, Crispe IN, Tu Z. HCV immune evasion and regulatory T cell activation: cause or consequence? *Cell Mol Immunol*. 2018;15(5):536-8.
- Lindquist L, Vapalahti O. Tick-borne encephalitis. *Lancet*. 2008;371(9627):1861-71.
- 35 • Blom K, Cuapio A, Sandberg JT, Varnaite R, Michaelsson J, Bjorkstrom NK, et al. Cell-Mediated Immune Responses and Immunopathogenesis of Human Tick-Borne Encephalitis Virus-Infection. *Front Immunol*. 2018;9:2174.

- Haller O, Kochs G, Weber F. The interferon response circuit: induction and suppression by pathogenic viruses. *Virology*. 2006;344(1):119-30.
- Shevtsova AS, Motuzova OV, Kuragina VM, Akhmatova NK, Gmyl LV, Kondrat'eva YI, et al. Lethal Experimental Tick-Borne Encephalitis Infection: Influence of Two Strains with Similar Virulence on the Immune Response. *Front Microbiol*. 2016;7:2172.
- Kulakowska A, Byfield FJ, Zendzian-Piotrowska M, Zajkowska JM, Drozdowski W, Mroczko B, et al. Increased levels of sphingosine-1-phosphate in cerebrospinal fluid of patients diagnosed with tick-borne encephalitis. *J Neuroinflammation*. 2014;11:193.
- Leclercq TM, Moretti PA, Vadas MA, Pitson SM. Eukaryotic elongation factor 1A interacts with sphingosine kinase and directly enhances its catalytic activity. *J Biol Chem*. 2008;283(15):9606-14.
- Moennig V, Floegel-Niesmann G, Greiser-Wilke I. Clinical signs and epidemiology of classical swine fever: a review of new knowledge. *Vet J*. 2003;165(1):11-20.
- Petrov A, Blohm U, Beer M, Pietschmann J, Blome S. Comparative analyses of host responses upon infection with moderately virulent classical swine fever virus in domestic pigs and wild boar. *Virology*. 2014;11:134.
- Blome S, Staubach C, Henke J, Carlson J, Beer M. Classical Swine Fever-An Updated Review. *Viruses*. 2017;9(4).
- Husser L, Ruggli N, Summerfield A. N(pro) of classical swine fever virus prevents type I interferon-mediated priming of conventional dendritic cells for enhanced interferon-alpha response. *J Interferon Cytokine Res*. 2012;32(5):221-9.
- Massilamany C, Koenig A, Reddy J, Huber S, Buskiewicz I. Autoimmunity in picornavirus infections. *Curr Opin Virol*. 2016;16:8-14.
- Baj A, Colombo M, Headley JL, McFarlane JR, Liethof MA, Toniolo A. Post-polio myelitis syndrome as a possible viral disease. *Int J Infect Dis*. 2015;35:107-16.
- Chapman NM, Kim KS. Persistent coxsackievirus infection: enterovirus persistence in chronic myocarditis and dilated cardiomyopathy. *Curr Top Microbiol Immunol*. 2008;323:275-92.
- Klein J. Understanding the molecular epidemiology of foot-and-mouth-disease virus. *Infect Genet Evol*. 2009;9(2):153-61.
- Fairweather D, Frisancho-Kiss S, Rose NR. Viruses as adjuvants for autoimmunity: evidence from Coxsackievirus-induced myocarditis. *Rev Med Virol*. 2005;15(1):17-27.
- Fensterl V, Chattopadhyay S, Sen GC. No Love Lost Between Viruses and Interferons. *Annu Rev Virol*. 2015;2(1):549-72.
- Schulz KS, Mossman KL. Viral Evasion Strategies in Type I IFN Signaling - A Summary of Recent Developments. *Front Immunol*. 2016;7:498.

- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15
20
25
30
35
- 5
10
15

- Slater L, Bartlett NW, Haas JJ, Zhu J, Message SD, Walton RP, et al. Co-ordinated role of TLR3, RIG-I and MDA5 in the innate response to rhinovirus in bronchial epithelium. *PLoS Pathog.* 2010;6(11):e1001178.
- 5 • Zhu Z, Tang W, Gwaltney JM, Jr., Wu Y, Elias JA. Rhinovirus stimulation of interleukin-8 in vivo and in vitro: role of NF-kappaB. *Am J Physiol.* 1997;273(4):L814-24.
- Turner RB, Weingand KW, Yeh CH, Leedy DW. Association between interleukin-8 concentration in nasal secretions and severity of symptoms of experimental rhinovirus colds. *Clin Infect Dis.* 1998;26(4):840-6.
- 10 • Deinhardt F. Prevention of viral hepatitis A: past, present and future. *Vaccine.* 1992;10 Suppl 1:S10-4.
- Wang X, Ren J, Gao Q, Hu Z, Sun Y, Li X, et al. Hepatitis A virus and the origins of picornaviruses. *Nature.* 2015;517(7532):85-8.
- 15 • Kaplan G, Totsuka A, Thompson P, Akatsuka T, Moritsugu Y, Feinstone SM. Identification of a surface glycoprotein on African green monkey kidney cells as a receptor for hepatitis A virus. *EMBO J.* 1996;15(16):4282-96.
- FitzSimons D, Hendrickx G, Vorsters A, Van Damme P. Hepatitis A and E: update on prevention and epidemiology. *Vaccine.* 2010;28(3):583-8.
- Wang D, Fang L, Wei D, Zhang H, Luo R, Chen H, et al. Hepatitis A virus 3C protease cleaves NEMO to impair induction of beta interferon. *J Virol.* 2014;88(17):10252-8.
- 20 • Choi YS, Jung MK, Lee J, Choi SJ, Choi SH, Lee HW, et al. Tumor Necrosis Factor-producing T-regulatory Cells Are Associated With Severe Liver Injury in Patients With Acute Hepatitis A. *Gastroenterology.* 2018;154(4):1047-60.
- Thiberville SD, Moyen N, Dupuis-Maguiraga L, Nougairede A, Gould EA, Roques P, et al. Chikungunya fever: epidemiology, clinical syndrome, pathogenesis and therapy. *Antiviral Res.* 2013;99(3):345-70.
- 25 • White LK, Sali T, Alvarado D, Gatti E, Pierre P, Streblow D, et al. Chikungunya virus induces IPS-1-dependent innate immune activation and protein kinase R-independent translational shutoff. *J Virol.* 2011;85(1):606-20.
- Ng KW, Chow A, Win MK, Dimatatac F, Neo HY, Lye DC, et al. Clinical features and epidemiology of chikungunya infection in Singapore. *Singapore Med J.* 2009;50(8):785-90.
- 30 • Colavita F, Vita S, Lalle E, Carletti F, Bordi L, Vincenti D, et al. Overproduction of IL-6 and Type-I IFN in a Lethal Case of Chikungunya Virus Infection in an Elderly Man During the 2017 Italian Outbreak. *Open Forum Infect Dis.* 2018;5(11):ofy276.
- 35 • Shirako Y, Niklasson B, Dalrymple JM, Strauss EG, Strauss JH. Structure of the Ockelbo virus genome and its relationship to other Sindbis viruses. *Virology.* 1991;182(2):753-64.

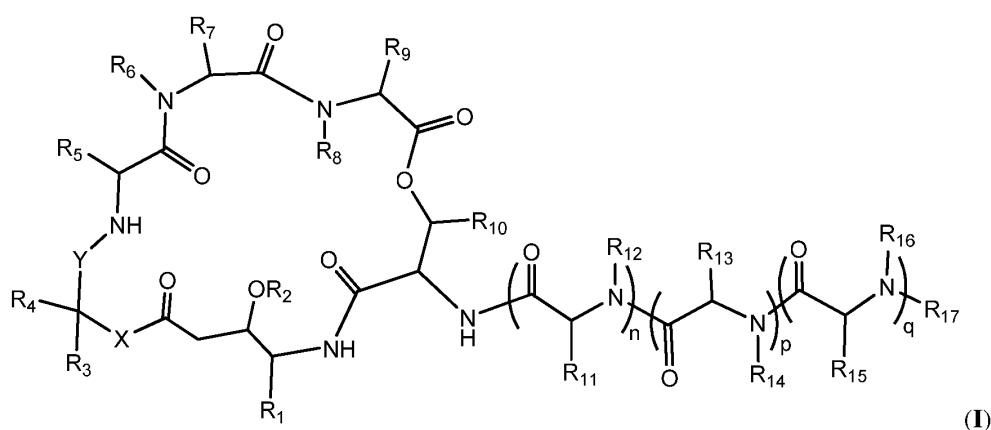
- Laine M, Luukkainen R, Toivanen A. Sindbis viruses and other alphaviruses as cause of human arthritic disease. *J Intern Med.* 2004;256(6):457-71.
- Laine M, Vainionpää R, Oksi J, Luukkainen R, Toivanen A. The prevalence of antibodies against Sindbis-related (Pogosta) virus in different parts of Finland. *Rheumatology (Oxford).* 2003;42(5):632-6.
- Assuncao-Miranda I, Bozza MT, Da Poian AT. Pro-inflammatory response resulting from sindbis virus infection of human macrophages: implications for the pathogenesis of viral arthritis. *J Med Virol.* 2010;82(1):164-74.
- Klimstra WB, Ryman KD, Bernard KA, Nguyen KB, Biron CA, Johnston RE. Infection of neonatal mice with sindbis virus results in a systemic inflammatory response syndrome. *J Virol.* 1999;73(12):10387-98.
- Davies MG, Hagen PO. Systemic inflammatory response syndrome. *Br J Surg.* 1997;84(7):920-35.
- Atkins GJ, Sheahan BJ, Liljestrom P. The molecular pathogenesis of Semliki Forest virus: a model virus made useful? *J Gen Virol.* 1999;80 (Pt 9):2287-97.
- Balluz IM, Glasgow GM, Killen HM, Mabruk MJ, Sheahan BJ, Atkins GJ. Virulent and avirulent strains of Semliki Forest virus show similar cell tropism for the murine central nervous system but differ in the severity and rate of induction of cytolytic damage. *Neuropathol Appl Neurobiol.* 1993;19(3):233-9.
- Berger ML. Humoral and cell-mediated immune mechanisms in the production of pathology in avirulent Semliki Forest virus encephalitis. *Infect Immun.* 1980;30(1):244-53.
- Aguilar PV, Adams AP, Wang E, Kang W, Carrara AS, Anishchenko M, et al. Structural and nonstructural protein genome regions of eastern equine encephalitis virus are determinants of interferon sensitivity and murine virulence. *J Virol.* 2008;82(10):4920-30.
- Painter GR, Bowen RA, Bluemling GR, DeBergh J, Edpuganti V, Gruddanti PR, et al. The prophylactic and therapeutic activity of a broadly active ribonucleoside analog in a murine model of intranasal Venezuelan equine encephalitis virus infection. *Antiviral Res.* 2019;171:104597.
- Weaver SC, Salas R, Rico-Hesse R, Ludwig GV, Oberste MS, Boshell J, et al. Re-emergence of epidemic Venezuelan equine encephalomyelitis in South America. VEE Study Group. *Lancet.* 1996;348(9025):436-40.
- Vogel P, Kell WM, Fritz DL, Parker MD, Schoepp RJ. Early events in the pathogenesis of eastern equine encephalitis virus in mice. *Am J Pathol.* 2005;166(1):159-71.
- Gardner CL, Burke CW, Tesfay MZ, Glass PJ, Klimstra WB, Ryman KD. Eastern and Venezuelan equine encephalitis viruses differ in their ability to infect dendritic cells and

- macrophages: impact of altered cell tropism on pathogenesis. *J Virol.* 2008;82(21):10634-46.
- George S, Viswanathan R, Sapkal GN. Molecular aspects of the teratogenesis of rubella virus. *Biol Res.* 2019;52(1):47.
 - 5 • Alford CA, Jr., Neva FA, Weller TH. Virologic and Serologic Studies on Human Products of Conception after Maternal Rubella. *N Engl J Med.* 1964;271:1275-81.
 - Fazil Z, Ten Berge JC, Langerak AW, Rothova A, Dik WA. An Intraocular Inflammatory Profile of Rubella Associated Uveitis. *Ocul Immunol Inflamm.* 2019;27(3):418-23.
 - 10 • Vinje J, Estes MK, Esteves P, Green KY, Katayama K, Knowles NJ, et al. ICTV Virus Taxonomy Profile: Caliciviridae. *J Gen Virol.* 2019;100(11):1469-70.
 - Karst SM, Wobus CE, Goodfellow IG, Green KY, Virgin HW. Advances in norovirus biology. *Cell Host Microbe.* 2014;15(6):668-80.
 - Wobus CE, Karst SM, Thackray LB, Chang KO, Sosnovtsev SV, Belliot G, et al. Replication of Norovirus in cell culture reveals a tropism for dendritic cells and macrophages. *PLoS Biol.* 2004;2(12):e432.
 - 15 • Weiss SR, Navas-Martin S. Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. *Microbiol Mol Biol Rev.* 2005;69(4):635-64.
 - Ruan YJ, Wei CL, Ee AL, Vega VB, Thoreau H, Su ST, et al. Comparative full-length genome sequence analysis of 14 SARS coronavirus isolates and common mutations associated with putative origins of infection. *Lancet.* 2003;361(9371):1779-85.
 - 20 • Huang KJ, Su IJ, Theron M, Wu YC, Lai SK, Liu CC, et al. An interferon-gamma-related cytokine storm in SARS patients. *J Med Virol.* 2005;75(2):185-94.
 - Zumla A, Hui DS, Perlman S. Middle East respiratory syndrome. *Lancet.* 2015;386(9997):995-1007.
 - 25 • Min CK, Cheon S, Ha NY, Sohn KM, Kim Y, Aigerim A, et al. Comparative and kinetic analysis of viral shedding and immunological responses in MERS patients representing a broad spectrum of disease severity. *Sci Rep.* 2016;6:25359.
 - Cardone M, Yano M, Rosenberg AS, Puig M. Lessons Learned to Date on COVID-19 Hyperinflammatory Syndrome: Considerations for Interventions to Mitigate SARS-CoV-2 Viral Infection and Detrimental Hyperinflammation. *Front Immunol.* 2020;11:1131.
 - 30 • Xu J, Yang X, Yang L, Zou X, Wang Y, Wu Y, et al. Clinical course and predictors of 60-day mortality in 239 critically ill patients with COVID-19: a multicenter retrospective study from Wuhan, China. *Crit Care.* 2020;24(1):394.
 - 35 • Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet.* 2020;395(10229):1033-4.

- Davis WG, Blackwell JL, Shi PY, Brinton MA. Interaction between the cellular protein eEF1A and the 3'-terminal stem-loop of West Nile virus genomic RNA facilitates viral minus-strand RNA synthesis. *J Virol.* 2007;81(18):10172-87.
- 5 • Suthar MS, Diamond MS, Gale M, Jr. West Nile virus infection and immunity. *Nat Rev Microbiol.* 2013;11(2):115-28.
- Rossi SL, Ross TM, Evans JD. West Nile virus. *Clin Lab Med.* 2010;30(1):47-65.
- Rossini G, Landini MP, Gelsomino F, Sambri V, Varani S. Innate host responses to West Nile virus: Implications for central nervous system immunopathology. *World J Virol.* 2013;2(2):49-56.
- 10 • Getts DR, Terry RL, Getts MT, Muller M, Rana S, Shrestha B, et al. Ly6c+ "inflammatory monocytes" are microglial precursors recruited in a pathogenic manner in West Nile virus encephalitis. *J Exp Med.* 2008;205(10):2319-37.
- Wang T, Town T, Alexopoulou L, Anderson JF, Fikrig E, Flavell RA. Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. *Nat Med.* 15 2004;10(12):1366-73.
- Kumar M, Verma S, Nerurkar VR. Pro-inflammatory cytokines derived from West Nile virus (WNV)-infected SK-N-SH cells mediate neuroinflammatory markers and neuronal death. *J Neuroinflammation.* 2010;7:73.
- 20

CLAIMS:

1. A compound of general formula I



wherein X is selected from O and NH;

5 Y is selected from CO and $-\text{COCH}(\text{CH}_3)\text{CO}-$;

each n and p is independently selected from 0 and 1, and q is selected from 0, 1 and 2;

each R_1 , R_3 , R_5 , R_9 , R_{11} , and R_{15} is independently selected from hydrogen, substituted or unsubstituted $\text{C}_1\text{-C}_6$ alkyl, substituted or unsubstituted $\text{C}_2\text{-C}_6$ alkenyl, and substituted or unsubstituted $\text{C}_2\text{-C}_6$ alkynyl;

10 R_2 is selected from hydrogen, COR_a , COOR_a , substituted or unsubstituted $\text{C}_1\text{-C}_6$ alkyl, substituted or unsubstituted $\text{C}_2\text{-C}_6$ alkenyl, and substituted or unsubstituted $\text{C}_2\text{-C}_6$ alkynyl;

each R_4 , R_8 , R_{10} , R_{12} , and R_{16} is independently selected from hydrogen and substituted or unsubstituted $\text{C}_1\text{-C}_6$ alkyl;

15 each R_7 and R_{13} is independently selected from hydrogen, substituted or unsubstituted $\text{C}_1\text{-C}_6$ alkyl, substituted or unsubstituted $\text{C}_2\text{-C}_6$ alkenyl, and substituted or unsubstituted $\text{C}_2\text{-C}_6$ alkynyl; each R_6 and R_{14} is independently selected from hydrogen and substituted or unsubstituted $\text{C}_1\text{-C}_6$ alkyl; or R_6 and R_7 and/or R_{13} and R_{14} together with the corresponding N atom and C atom to which they are attached may form a substituted or unsubstituted heterocyclic group;

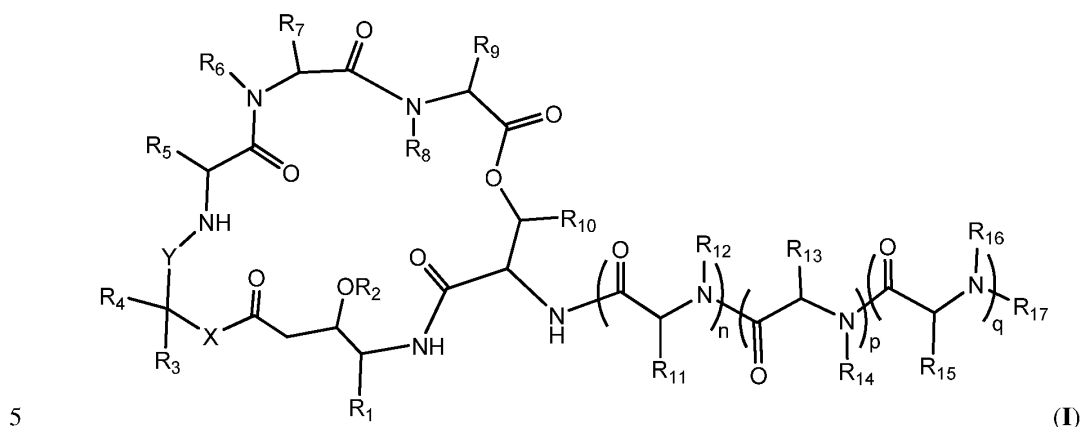
20 R_{17} is selected from hydrogen, COR_a , COOR_a , CONHR_b , COSR_c , $(\text{C}=\text{NR}_b)\text{OR}_a$, $(\text{C}=\text{NR}_b)\text{NHR}_b$, $(\text{C}=\text{NR}_b)\text{SR}_c$, $(\text{C}=\text{S})\text{OR}_a$, $(\text{C}=\text{S})\text{NHR}_b$, $(\text{C}=\text{S})\text{SR}_c$, SO_2R_c , SO_3R_c , substituted or unsubstituted $\text{C}_1\text{-C}_{12}$ alkyl, substituted or unsubstituted $\text{C}_2\text{-C}_{12}$ alkenyl, substituted or unsubstituted $\text{C}_2\text{-C}_{12}$ alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group, with the proviso that when n, p, and q are 0 then R_{17} is not hydrogen; and

25 each R_a , R_b , and R_c is independently selected from hydrogen, substituted or unsubstituted $\text{C}_1\text{-C}_{12}$ alkyl, substituted or unsubstituted $\text{C}_2\text{-C}_{12}$ alkenyl, substituted or unsubstituted $\text{C}_2\text{-C}_{12}$ alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group;

substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group;

or a pharmaceutically acceptable salt or stereoisomer thereof, for use in the treatment of inflammation associated with activation of Toll-like receptors.

3. A compound of general formula I



wherein X is selected from O and NH;

Y is selected from CO and $-\text{COCH}(\text{CH}_3)\text{CO}-$;

each n and p is independently selected from 0 and 1, and q is selected from 0, 1 and 2;

each R₁, R₃, R₅, R₉, R₁₁, and R₁₅ is independently selected from hydrogen, substituted or unsubstituted C₁-C₆ alkyl, substituted or unsubstituted C₂-C₆ alkenyl, and substituted or unsubstituted C₂-C₆ alkynyl;

R₂ is selected from hydrogen, COR_a, COOR_a, substituted or unsubstituted C₁-C₆ alkyl, substituted or unsubstituted C₂-C₆ alkenyl, and substituted or unsubstituted C₂-C₆ alkynyl;

each R₄, R₈, R₁₀, R₁₂, and R₁₆ is independently selected from hydrogen and substituted or unsubstituted C₁-C₆ alkyl;

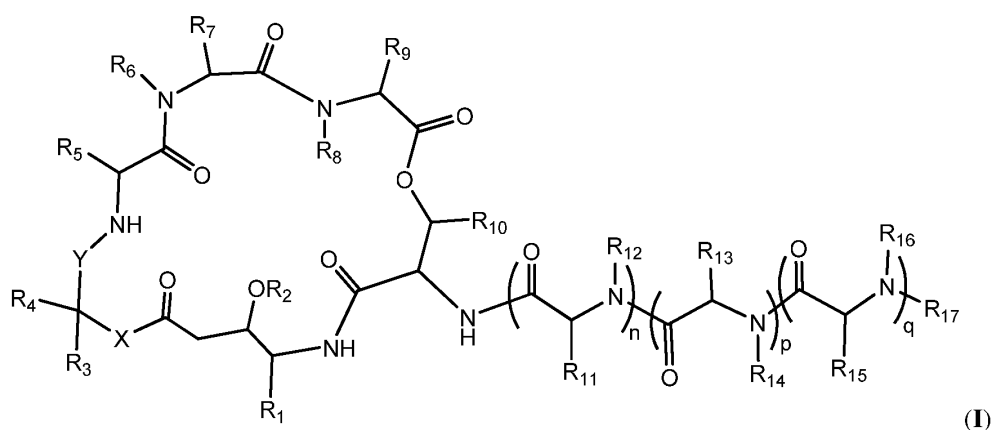
each R₇ and R₁₃ is independently selected from hydrogen, substituted or unsubstituted C₁-C₆ alkyl, substituted or unsubstituted C₂-C₆ alkenyl, and substituted or unsubstituted C₂-C₆ alkynyl; each R₆ and R₁₄ is independently selected from hydrogen and substituted or unsubstituted C₁-C₆ alkyl; or R₆ and R₇ and/or R₁₃ and R₁₄ together with the corresponding N atom and C atom to which they are attached may form a substituted or unsubstituted heterocyclic group;

R₁₇ is selected from hydrogen, COR_a, COOR_a, CONHR_b, COSR_c, (C=NR_b)OR_a, (C=NR_b)NHR_b, (C=NR_b)SR_c, (C=S)OR_a, (C=S)NHR_b, (C=S)SR_c, SO₂R_c, SO₃R_c, substituted or unsubstituted C₁-C₁₂ alkyl, substituted or unsubstituted C₂-C₁₂ alkenyl, substituted or unsubstituted C₂-C₁₂ alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group, with the proviso that when n, p, and q are 0 then R₁₇ is not hydrogen; and

each R_a , R_b , and R_c is independently selected from hydrogen, substituted or unsubstituted C_1 - C_{12} alkyl, substituted or unsubstituted C_2 - C_{12} alkenyl, substituted or unsubstituted C_2 - C_{12} alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group;

5 or a pharmaceutically acceptable salt or stereoisomer thereof, for use in the treatment of a disorder selected from pneumonia, acute respiratory distress (ARDS), immunopathology, preferably hypercytokinemia (cytokine storm syndrome), sepsis and graft-versus-host disease.

4. A compound of general formula I



wherein X is selected from O and NH;

10 Y is selected from CO and $-\text{COCH}(\text{CH}_3)\text{CO}-$;

each n and p is independently selected from 0 and 1, and q is selected from 0, 1 and 2;

each R_1 , R_3 , R_5 , R_9 , R_{11} , and R_{15} is independently selected from hydrogen, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_2 - C_6 alkenyl, and substituted or unsubstituted C_2 - C_6 alkynyl;

15 R_2 is selected from hydrogen, COR_a , COOR_a , substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_2 - C_6 alkenyl, and substituted or unsubstituted C_2 - C_6 alkynyl;

each R_4 , R_8 , R_{10} , R_{12} , and R_{16} is independently selected from hydrogen and substituted or unsubstituted C_1 - C_6 alkyl;

20 each R_7 and R_{13} is independently selected from hydrogen, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_2 - C_6 alkenyl, and substituted or unsubstituted C_2 - C_6 alkynyl; each R_6 and R_{14} is independently selected from hydrogen and substituted or unsubstituted C_1 - C_6 alkyl; or R_6 and R_7 and/or R_{13} and R_{14} together with the corresponding N atom and C atom to which they are attached may form a substituted or unsubstituted heterocyclic group;

25 R_{17} is selected from hydrogen, COR_a , COOR_a , CONHR_b , COSR_c , $(\text{C}=\text{NR}_b)\text{OR}_a$, $(\text{C}=\text{NR}_b)\text{NHR}_b$, $(\text{C}=\text{NR}_b)\text{SR}_c$, $(\text{C}=\text{S})\text{OR}_a$, $(\text{C}=\text{S})\text{NHR}_b$, $(\text{C}=\text{S})\text{SR}_c$, SO_2R_c , SO_3R_c , substituted or unsubstituted C_1 - C_{12} alkyl, substituted or unsubstituted C_2 - C_{12} alkenyl, substituted or unsubstituted C_2 - C_{12} alkynyl,

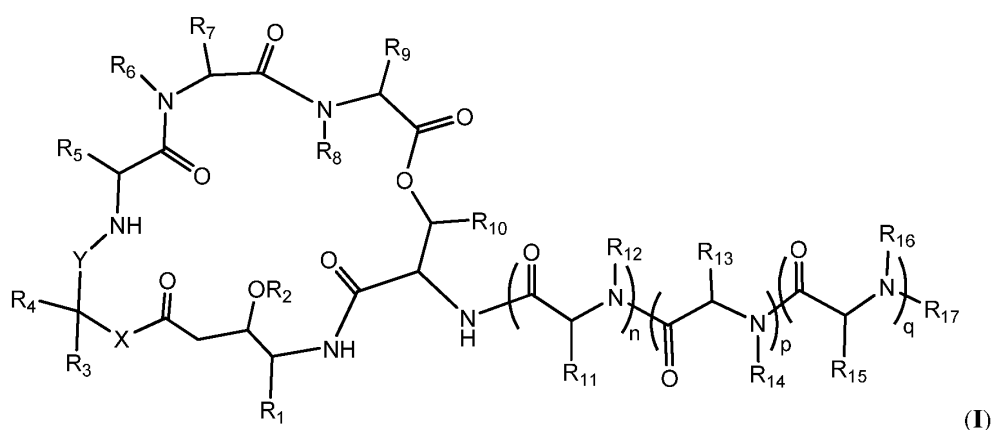
substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group, with the proviso that when n, p, and q are 0 then R₁₇ is not hydrogen; and

each R_a, R_b, and R_c is independently selected from hydrogen, substituted or unsubstituted C₁-C₁₂ alkyl, substituted or unsubstituted C₂-C₁₂ alkenyl, substituted or unsubstituted C₂-C₁₂ alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group;

or a pharmaceutically acceptable salt or stereoisomer thereof, for use in the treatment of pathogen-induced inflammation.

5. The compound of claim 4, wherein the pathogen is selected from a virus and bacteria.

6. A compound of general formula I



wherein X is selected from O and NH;

Y is selected from CO and -COCH(CH₃)CO-;

each n and p is independently selected from 0 and 1, and q is selected from 0, 1 and 2;

each R₁, R₃, R₅, R₉, R₁₁, and R₁₅ is independently selected from hydrogen, substituted or unsubstituted C₁-C₆ alkyl, substituted or unsubstituted C₂-C₆ alkenyl, and substituted or unsubstituted C₂-C₆ alkynyl;

R₂ is selected from hydrogen, COR_a, COOR_a, substituted or unsubstituted C₁-C₆ alkyl, substituted or unsubstituted C₂-C₆ alkenyl, and substituted or unsubstituted C₂-C₆ alkynyl;

each R₄, R₈, R₁₀, R₁₂, and R₁₆ is independently selected from hydrogen and substituted or unsubstituted C₁-C₆ alkyl;

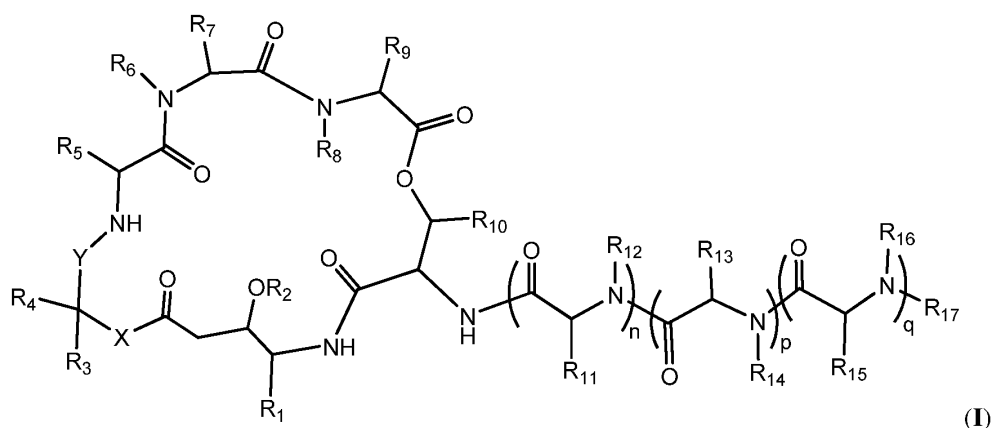
each R₇ and R₁₃ is independently selected from hydrogen, substituted or unsubstituted C₁-C₆ alkyl, substituted or unsubstituted C₂-C₆ alkenyl, and substituted or unsubstituted C₂-C₆ alkynyl; each R₆ and R₁₄ is independently selected from hydrogen and substituted or unsubstituted C₁-C₆ alkyl; or R₆ and R₇ and/or R₁₃ and R₁₄ together with the corresponding N atom and C atom to which they are attached may form a substituted or unsubstituted heterocyclic group;

5 R_{17} is selected from hydrogen, COR_a , $COOR_a$, $CONHR_b$, $COSR_c$, $(C=NR_b)OR_a$, $(C=NR_b)NHR_b$, $(C=NR_b)SR_c$, $(C=S)OR_a$, $(C=S)NHR_b$, $(C=S)SR_c$, SO_2R_c , SO_3R_c , substituted or unsubstituted C_1 - C_{12} alkyl, substituted or unsubstituted C_2 - C_{12} alkenyl, substituted or unsubstituted C_2 - C_{12} alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group, with the proviso that when n , p , and q are 0 then R_{17} is not hydrogen; and

each R_a , R_b , and R_c is independently selected from hydrogen, substituted or unsubstituted C_1 - C_{12} alkyl, substituted or unsubstituted C_2 - C_{12} alkenyl, substituted or unsubstituted C_2 - C_{12} alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group;

10 or a pharmaceutically acceptable salt or stereoisomer thereof, for use in the combined treatment of inflammation associated with activation of Toll-like receptors or pathogen-induced inflammation and in the treatment of a viral infection.

7. A compound of general formula I



wherein X is selected from O and NH ;

15 Y is selected from CO and $-COCH(CH_3)CO-$;

each n and p is independently selected from 0 and 1, and q is selected from 0, 1 and 2;

each R_1 , R_3 , R_5 , R_9 , R_{11} , and R_{15} is independently selected from hydrogen, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_2 - C_6 alkenyl, and substituted or unsubstituted C_2 - C_6 alkynyl;

20 R_2 is selected from hydrogen, COR_a , $COOR_a$, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_2 - C_6 alkenyl, and substituted or unsubstituted C_2 - C_6 alkynyl;

each R_4 , R_8 , R_{10} , R_{12} , and R_{16} is independently selected from hydrogen and substituted or unsubstituted C_1 - C_6 alkyl;

25 each R_7 and R_{13} is independently selected from hydrogen, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_2 - C_6 alkenyl, and substituted or unsubstituted C_2 - C_6 alkynyl; each R_6 and R_{14} is independently selected from hydrogen and substituted or unsubstituted C_1 - C_6 alkyl;

or R₆ and R₇ and/or R₁₃ and R₁₄ together with the corresponding N atom and C atom to which they are attached may form a substituted or unsubstituted heterocyclic group;

5 R₁₇ is selected from hydrogen, COR_a, COOR_a, CONHR_b, COSR_c, (C=NR_b)OR_a, (C=NR_b)NHR_b, (C=NR_b)SR_c, (C=S)OR_a, (C=S)NHR_b, (C=S)SR_c, SO₂R_c, SO₃R_c, substituted or unsubstituted C₁-C₁₂ alkyl, substituted or unsubstituted C₂-C₁₂ alkenyl, substituted or unsubstituted C₂-C₁₂ alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group, with the proviso that when n, p, and q are 0 then R₁₇ is not hydrogen; and

10 each R_a, R_b, and R_c is independently selected from hydrogen, substituted or unsubstituted C₁-C₁₂ alkyl, substituted or unsubstituted C₂-C₁₂ alkenyl, substituted or unsubstituted C₂-C₁₂ alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group;

or a pharmaceutically acceptable salt or stereoisomer thereof, for use as an anti-inflammatory and an antiviral.

8. A compound according to any preceding claim, wherein R₃ and R₄ are independently selected from hydrogen and substituted or unsubstituted C₁-C₆ alkyl; preferably wherein R₃ is isopropyl and R₄ is hydrogen.

9. A compound according to any preceding claim, of general formula **II**, wherein R₃ and R₄ are methyl.

10. A compound according to any preceding claim, wherein R₁₁ is selected from hydrogen and substituted or unsubstituted C₁-C₆ alkyl; preferably wherein R₁₁ is methyl or isobutyl.

20 11. A compound according to any preceding claim, of general formula **III**, wherein R₁₁ is methyl and n=1.

25 12. A compound according to any preceding claim, wherein R₁, R₅, R₉, and R₁₅ are independently selected from hydrogen and substituted or unsubstituted C₁-C₆ alkyl; preferably wherein R₁ is selected from sec-butyl and isopropyl, R₅ is isobutyl, R₉ is p-methoxybenzyl, and R₁₅ is selected from methyl and benzyl.

13. A compound according to any preceding claim, wherein R₈, R₁₀, R₁₂, and R₁₆ are independently selected from hydrogen and substituted or unsubstituted C₁-C₆ alkyl; preferably wherein R₈, R₁₀ and R₁₂ are methyl, and R₁₆ is hydrogen.

30 14. A compound according to any preceding claim, wherein R₆ and R₁₄ are independently selected from hydrogen and substituted or unsubstituted C₁-C₆ alkyl; preferably wherein R₆ is selected from hydrogen and methyl, and R₁₄ is hydrogen.

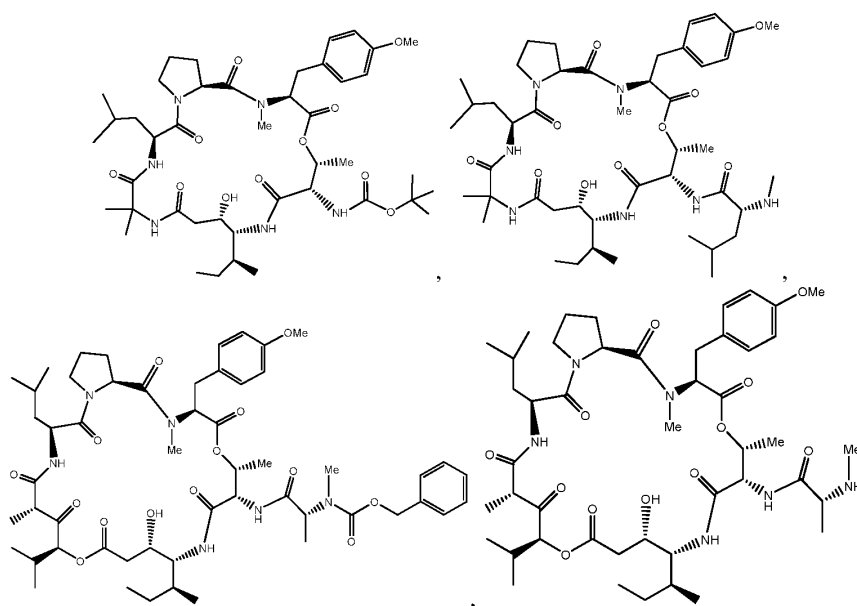
15. A compound according to any preceding claim, wherein R₇ and R₁₃ are independently selected from hydrogen and substituted or unsubstituted C₁-C₆ alkyl; preferably wherein R₇ is methyl and R₁₃ is selected from hydrogen, methyl, isopropyl, isobutyl, and 3-amino-3-oxopropyl.

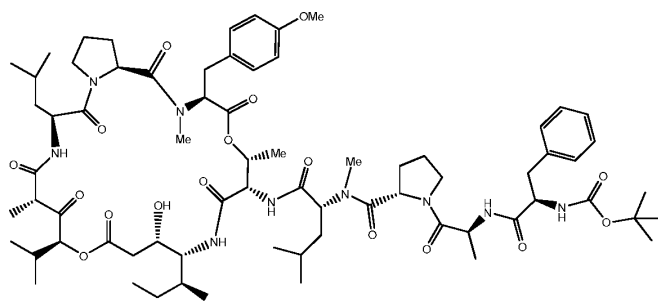
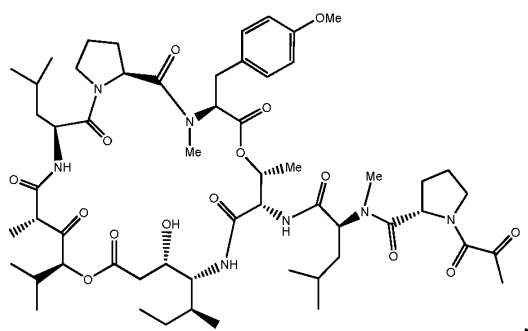
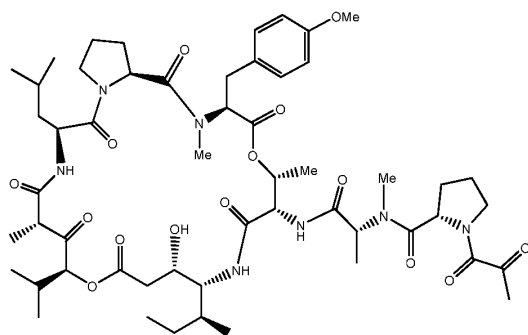
35 16. A compound according to any of claims 1 to 13, wherein R₆ and R₇ and/or R₁₃ and R₁₄ together with the corresponding N atom and C atom to which they are attached form a substituted or unsubstituted pyrrolidine group.

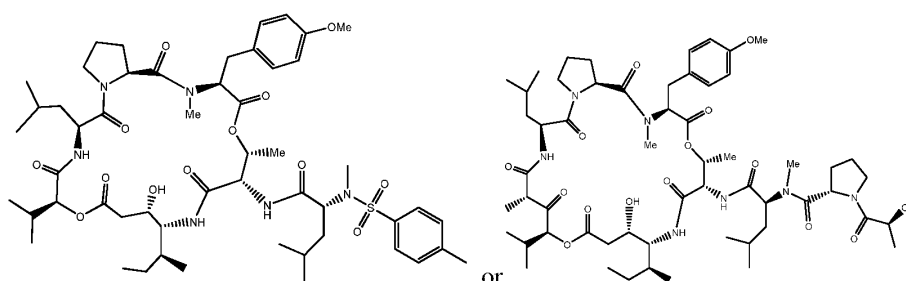
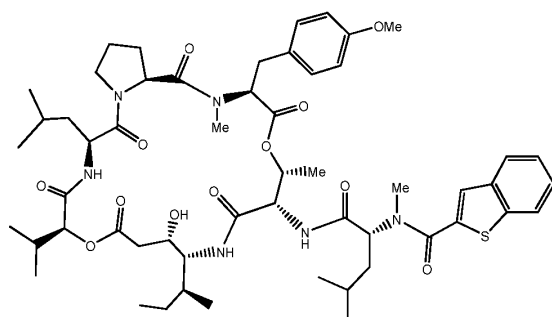
17. A compound according to any preceding claim, wherein R₂ is selected from hydrogen,

substituted or unsubstituted C₁-C₆ alkyl, and COR_a, and wherein R_a is a substituted or unsubstituted C₁-C₆ alkyl; preferably wherein R₂ is hydrogen.

18. A compound according to any preceding claim, wherein R₁₇ is selected from hydrogen, COR_a, COOR_a, CONHR_b, (C=S)NHR_b, and SO₂R_c, and wherein each R_a, R_b, and R_c is independently selected from substituted or unsubstituted C₁-C₆ alkyl, substituted or unsubstituted C₂-C₆ alkenyl, substituted or unsubstituted C₂-C₆ alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group; preferably wherein R₁₇ is selected from hydrogen, COObenzyl, CObenzo[b]thiophen-2-yl, SO₂(p-methylphenyl), COCOCH₃ and COOC(CH₃)₃.
19. A compound according to any preceding claim, wherein X is NH.
20. A compound according to any of claims 1 to 18, wherein X is O.
21. A compound according to any preceding claim wherein Y is CO.
22. A compound according to any of claims 1 to 20, wherein Y is -COCH(CH₃)CO-.
23. A compound according to any one of claims 1 to 7, having the following structure:







5 or pharmaceutically acceptable salts or stereoisomers thereof.

24. A compound according to any one of claims 1 to 7, wherein the compound is PLD, or pharmaceutically acceptable salts or stereoisomers thereof.

25. A compound according to any one of claims 1 to 7, wherein the compound is didemninB, or pharmaceutically acceptable salts or stereoisomers thereof.

10 26. The compound of claim 2, wherein the Toll-like receptor is activated by a single stranded RNA virus, wherein preferably the virus is selected from Flaviviridae, Picornaviridae, Togaviridae, Caliciviridae, Retroviridae, Coronaviridae, Orthomyxoviridae, Phlebovirus and Arenaviridae or a dsDNA virus, preferably Herpesviridae.

15 27. The compound of any one of claims 4 to 6, wherein the pathogen is a virus, preferably a single stranded RNA virus, wherein preferably the virus is selected from Flaviviridae, Picornaviridae, Togaviridae, Caliciviridae, Retroviridae, Coronaviridae, Orthomyxoviridae, Phlebovirus and Arenaviridae or a dsDNA virus, preferably Herpesviridae.

20 28. The compound of claim 5, 6 or 7, wherein the virus is a single stranded RNA virus, wherein preferably the virus is selected from Flaviviridae, Picornaviridae, Togaviridae, Caliciviridae, Retroviridae, Coronaviridae, Orthomyxoviridae, Phlebovirus and Arenaviridae or a dsDNA virus, preferably Herpesviridae.

29. The compound of any of claims 26 to 28, wherein the virus is a Flaviviridae virus; and is optionally selected from Yellow fever virus (YFV), Hepatitis C virus, West Nile Virus (WNV),

Dengue virus (DENV), Japanese encephalitis virus (JEV), Tick-borne encephalitis virus (TBEV), Classical swine fever virus and Zika virus (ZIKV).

30. The compound of any of claims 26 to 28, wherein the virus is a Coronavirinae; and is optionally SARS-CoV-2.
- 5 31. The compound of any of claims 26 to 28, wherein the virus is a Picornaviridae; and is optionally foot and mouth disease virus, enterovirus A71, Coxsackieviruses, Rhinovirus and Hepatitis A.
32. The compound of any of claims 26 to 28, wherein the virus is a Togaviridae; and is optionally Chikungunya Virus, Sindbis Virus, Semliki Forest Virus, Eastern Equine Encephalitis Virus / Western Equine Encephalitis Virus / Venezuelan Equine Encephalitis Virus and Rubella virus.
- 10 33. The compound of any of claims 26 to 28, wherein the virus is a Caliciviridae; and is optionally Lagovirus, Norovirus, Nebovirus, Recovirus, Sapovirus, Valovirus and Vesivirus, and in particular, Norwalk virus.
- 15 34. The compound of any of claims 26 to 28, wherein the virus is a Retroviridae; and is optionally HIV.
35. The compound of any of claims 26 to 28, wherein the virus is a Orthomyxoviridae, and is optionally influenza.
36. The compound of any of claims 26 to 28, wherein the virus is a Phlebovirus, and is optionally Sandfly fever virus or Rift Valley fever virus.
- 20 37. The compound of any of claims 26 to 28, wherein the virus is a Arenaviridae and is optionally the Pichinde virus.
38. The compound of any of claims 26 to 28, wherein the virus is a Herpesviridae preferably Herpes simplex virus 1 or 2.
- 25 39. The compound of any of claims 26 to 28, wherein the virus is a respiratory virus.
40. A compound according to any one of claims 1 to 39, wherein the virus is SARS-CoV-2, for use in the treatment of COVID-19 and/or for use in the treatment of pneumonia or ARDS caused by COVID-19.
- 30 41. A compound according to claim 30, wherein the CoV infection is mild infection; and/or wherein the CoV infection is moderate infection; and/or wherein the CoV infection is severe infection.
42. A compound according to claim 30, wherein the CoV infection is acute CoV infection, preferably wherein the CoV infection is acute COVID-19 infection; and/or wherein the CoV infection is ongoing symptomatic CoV infection, preferably wherein the CoV infection is ongoing symptomatic COVID-19 infection; and/or wherein the CoV infection is post-CoV syndrome, CoV persistent or long CoV; preferably wherein the CoV infection is post-COVID-19 syndrome, COVID persistent or long COVID.
- 35

43. A compound according to claim 42, wherein the post-CoV syndrome, CoV persistent or long CoV include one or more symptoms arising from the cardiovascular, respiratory, gastrointestinal, neurological, musculoskeletal, metabolic, renal, dermatological, otolaryngological, haematological and autonomic systems; psychiatric problems, generalised pain, fatigue and/or persisting fever.
44. A compound according to claim 30, for use in the treatment of a patient with signs and symptoms of CoV infection (preferably COVID-19) for up to 4 weeks; and/or for use in the treatment of a patient with signs and symptoms of CoV infection (preferably COVID-19) from 4 weeks to 12 weeks; and/or for use in the treatment of a patient with signs and symptoms of CoV infection, preferably COVID-19, for more than 12 weeks.
45. A compound according to claim 30, for use in the prophylaxis, reduction or treatment of COVID persistent, long COVID or post-COVID syndrome; preferably wherein the prophylaxis, reduction or treatment minimises the likelihood that a patient suffers from COVID persistent, long COVID or post-COVID syndrome symptoms; and/or reduces the severity of such symptoms; further preferably wherein the treatment minimising the symptoms of CoV infection.
46. A compound according to claim 30, wherein the treatment reduces the infectivity of CoV patients; including wherein the patient is asymptomatic or not very symptomatic yet has a high viral load.
47. A compound for use according to any preceding claim, wherein the compound is administered in combination with a corticosteroid, preferably dexamethasone.
48. A compound for use according to claim 47, wherein the compound and corticosteroid are administered concurrently, separately or sequentially.
49. A compound for use according to any preceding claim, wherein the compound is administered according to a regimen of a daily dose for 10 days, 9 days, 8 days, 7 days, 6 days, 5 days, 4 days, 3 days, 2 days or 1 day; preferably 2-5 days, 3-5 days, or 3, 4 or 5 days; most preferably 3 days or 5 days; most preferably 3 days.
50. A compound for use according to any preceding claim wherein the compound is administered at a dose of 5mg a day or less, 4.5mg a day or less, 4mg a day or less, 3.5mg a day or less, 3mg a day or less, 2.5mg a day or less or 2mg a day or less; 0.5 mg/day, 1 mg/day, 1.5 mg/day, 2 mg/day, 2.5 mg/day, 3 mg/day, 3.5 mg/day, 4 mg/day, 4.5 mg/day, or 5mg/day; preferably 1 mg/day, 1.5 mg/day, 2 mg/day or 2.5 mg/day; more preferably 1.5-2.5 mg/day; most preferably 1.5 mg/day, 2.0 mg/day or 2.5 mg/day.
51. A compound for use according to any preceding claim wherein the compound is administered at a total dose of 1-50 mg, 1-40 mg, 1-30 mg, 1-20 mg, 1-15 mg, 3-15 mg, 3-12 mg, 4-12 mg, 4-10 mg, or 4.5-10 mg; 4mg, 4.5mg, 5mg, 5.5mg, 6mg, 6.5mg, 7mg, 7.5mg, 8mg, 8.5mg, 9mg, 9.5mg or 10mg; preferably 4.5mg, 5mg, 6mg, 7.5mg, 8mg, 9mg or 10mg; more preferably 4.5-7.5 mg/day.
52. A compound for use according to any preceding claim, wherein the compound is administered by infusion.
53. A compound for use according to claim 52, wherein the infusion is a 1 hour infusion, a

1.5 hour infusion, a 2 hour infusion or a 3 hour infusion; preferably a 1.5 hour infusion.

54. A compound for use according to any one of claims 1 to 53, wherein 1.5 mg of plitidepsin is administered as a 1.5-hour infusion, once a day for 3 consecutive days; or wherein 2 mg of plitidepsin is administered as a 1.5-hour infusion, once a day for 3 consecutive days; wherein 2.5 mg of plitidepsin is administered as a 1.5-hour infusion, once a day for 3 consecutive days; or wherein 1 mg of plitidepsin is administered as a 1.5-hour infusion, once a day for 5 consecutive days; or wherein 2 mg of plitidepsin is administered as a 1.5-hour infusion, once a day for 5 consecutive days.
55. A compound for use according to any preceding claim, wherein the compound is administered using a loading dose and a maintenance dose.
56. A compound for use according to claim 55, wherein the dosage regimen is:
- a loading dose of 2.5 mg for day 1, and followed by a maintenance dose of 2 mg/day for subsequent days;
 - a loading dose of 2.5 mg for day 1, and followed by a maintenance dose of 1.5 mg/day for subsequent days;
 - a loading dose of 2.5 mg for day 1, and followed by a maintenance dose of 1 mg/day for subsequent days;
 - a loading dose of 2.5 mg for day 1, and followed by a maintenance dose of 0.5 mg/day for subsequent days;
 - a loading dose of 2 mg for day 1, and followed by a maintenance dose of 1.5 mg/day for subsequent days;
 - a loading dose of 2 mg for day 1, and followed by a maintenance dose of 1 mg/day for subsequent days;
 - a loading dose of 2 mg for day 1, and followed by a maintenance dose of 0.5 mg/day for subsequent days;
 - a loading dose of 1.5 mg for day 1, and followed by a maintenance dose of 1 mg/day for subsequent days;
 - a loading dose of 1.5 mg for day 1, and followed by a maintenance dose of 0.5 mg/day for subsequent days; or
 - a loading dose of 1 mg for day 1, and followed by a maintenance dose of 0.5 mg/day for subsequent days.
57. A compound for use according to any preceding claim, wherein the compound is administered in combination with a corticosteroid, and wherein the corticosteroid is administered on the same days as administration of the compound according to any one of claims 1 to 25.
58. A compound for use according to claim 57, wherein the corticosteroid may also be administered on one or more subsequent days; preferably wherein the corticosteroid is

administered with the compound on days 1-3 and the corticosteroid is further administered on one or more of days 4-10.

5 59. A compound for use according to claim 58, wherein the corticosteroid is administered intravenously on days when the compound is administered but is administered by oral administration or IV on subsequent days.

60. A compound for use according to any one of claims 57 to 59, wherein the corticosteroid is dexamethasone; preferably wherein dexamethasone is administered at a dose of 6.6 mg/day IV on days when the compound according to the present invention is administered.

10 61. A compound for use according to claim 60, wherein dexamethasone is administered at a dose of 6 mg/day oral administration or IV on subsequent days, preferably one or more of days 4, 5, 6, 7, 8, 9 and 10.

15 62. A compound for use according to any preceding claim, wherein PLD is administered 1.5 mg/day intravenous (IV) combined with dexamethasone 6.6 mg/day IV on Days 1 to 3, followed by dexamethasone 6 mg/day oral administration (PO)/IV from Day 4 and up to Day 10 (as per physician judgement according to patient clinical condition and evolution); or

wherein PLD is administered 2.0 mg/day intravenous (IV) combined with dexamethasone 6.6 mg/day IV on Days 1 to 3, followed by dexamethasone 6 mg/day oral administration (PO)/IV from Day 4 and up to Day 10 (as per physician judgement according to patient clinical condition and evolution); or

20 wherein PLD is administered 2.5 mg/day intravenous (IV) combined with dexamethasone 6.6 mg/day IV on Days 1 to 3, followed by dexamethasone 6 mg/day oral administration (PO)/IV from Day 4 and up to Day 10 (as per physician judgement according to patient clinical condition and evolution).

25 63. A compound for use according to any one of claims 57 to 62, wherein the corticosteroid is administered 20 to 30 minutes prior to starting treatment with the compound according to any one of claims 1 to 25.

64. A compound for use according to any preceding claim, wherein the patient additionally receives the following medications, preferably 20 to 30 minutes prior to starting treatment with the compound according to any one of claims 1 to 25:

30 Ondansetron 8 mg IV (or equivalent);

Diphenhydramine hydrochloride 25 mg IV (or equivalent); and

Ranitidine 50 mg IV (or equivalent).

65. A compound for use according to any preceding claim, wherein on Days 4 and 5, patients receive ondansetron (or equivalent) 4 mg twice a day PO.

35 66. A compound for use according to any one of claims 1 to 53, wherein the compound is administered as a single dose (on day 1).

67. A compound for use according to claim 66, wherein the single dose is 1-10 mg, 4-10 mg,

4.5-10 mg, 4mg, 4.5mg, 5mg, 5.5mg, 6mg, 6.5mg, 7mg, 7.5mg, 8mg, 8.5mg, 9mg, 9.5mg or 10mg, preferably 4.5mg, 5mg, 6mg, 7.5mg, 8mg, 9mg or 10mg, more preferably 5-9 mg, 6.5-8.5 mg, 7-8 mg or most preferably 7.5 mg.

5 68. A compound for use according to any one of claims 66 to 67, wherein the compound is administered as a 1.5-hour infusion.

69. A compound for use according to any one of claims 66 to 68, wherein a corticosteroid is administered according to the regimen according to any one of claims 57 to 63.

10 70. A compound for use according to any one of claims 66 to 69, wherein the following prophylactic medications are administered 20-30 minutes prior to administration with a compound of the present invention:

Ondansetron 8 mg IV (or equivalent), particularly in slow infusion of 15 minutes;

Diphenhydramine hydrochloride 25 mg IV (or equivalent);

Ranitidine 50 mg IV (or equivalent).

15 71. A compound for use according to any one of claims 66 to 70, wherein ondansetron 4 mg orally is given every 12 hours for 3 days after administration of a compound of the present invention.

20 72. A compound for use according to any preceding claim, wherein dexamethasone is dexamethasone phosphate and is administered at a dose of 8 mg if administered on days when a compound of the invention is administered (equating to a dose of 6.6 mg base) and is administered at a dose of 7.2 mg if administered thereafter (equating to a dose of 6 mg base).

73. A pharmaceutical composition comprising a compound as defined in any preceding claim, or a pharmaceutically acceptable salt or stereoisomer thereof, and a pharmaceutically acceptable carrier, for use in the treatment of inflammation.

25 74. A pharmaceutical composition comprising a compound as defined in any of claims 1 to 72, or a pharmaceutically acceptable salt or stereoisomer thereof, and a pharmaceutically acceptable carrier, for use in the treatment of inflammation associated with activation of Toll-like receptors.

30 75. A pharmaceutical composition comprising a compound as defined in any of claims 1 to 72, or a pharmaceutically acceptable salt or stereoisomer thereof, and a pharmaceutically acceptable carrier, for use in the treatment of a disorder selected from pneumonia, acute respiratory distress (ARDS) and immunopathology, in particular hypercytokinemia (cytokine storm syndrome), sepsis and graft-versus-host disease.

35 76. A pharmaceutical composition comprising a compound as defined in any of claims 1 to 72, or a pharmaceutically acceptable salt or stereoisomer thereof, and a pharmaceutically acceptable carrier, for use in the treatment of pathogen-induced inflammation.

77. A pharmaceutical composition comprising a compound as defined in any of claims 1 to 72, or a pharmaceutically acceptable salt or stereoisomer thereof, and a pharmaceutically acceptable carrier, for use in the combined treatment of inflammation associated with activation

of Toll-like receptors or pathogen-induced inflammation and in the treatment of a viral infection.

78. A pharmaceutical composition comprising a compound as defined in any of claims 1 to 72, or a pharmaceutically acceptable salt or stereoisomer thereof, and a pharmaceutically acceptable carrier, for use as an anti-inflammatory and an antiviral.

5 79. Use of a compound as defined in to any of claims 1 to 72, or a pharmaceutically acceptable salt or stereoisomer thereof, in the manufacture of a medicament for the treatment of inflammation.

80. Use of a compound as defined in to any of claims 1 to 72, or a pharmaceutically acceptable salt or stereoisomer thereof, in the manufacture of a medicament for the treatment of
10 inflammation associated with activation of Toll-like receptors.

81. Use of a compound as defined in to any of claims 1 to 72, or a pharmaceutically acceptable salt or stereoisomer thereof, in the manufacture of a medicament for the treatment of a disorder selected from pneumonia, acute respiratory distress (ARDS) and immunopathology, in particular hypercytokinemia (cytokine storm syndrome), sepsis and, graft-versus-host disease.

15 82. Use of a compound as defined in to any of claims 1 to 72, or a pharmaceutically acceptable salt or stereoisomer thereof, in the manufacture of a medicament for the treatment of pathogen-induced inflammation.

83. Use of a compound as defined in to any of claims 1 to 72, or a pharmaceutically acceptable salt or stereoisomer thereof, in the manufacture of a medicament for the combined
20 treatment of inflammation associated with activation of Toll-like receptors or pathogen-induced inflammation and in the treatment of a viral infection.

84. Use of a compound or a pharmaceutically acceptable salt as defined in to any of claims 1 to 72, or stereoisomer thereof, in the manufacture of a medicament as an anti-inflammatory and an antiviral.

25 85. A method of treating inflammation, wherein the method comprises administering to an individual in need thereof, a therapeutically effective amount of a compound as defined in any of claims 1 to 72, or a pharmaceutically acceptable salt or stereoisomer thereof.

86. A method of treating inflammation associated with activation of Toll-like receptors, wherein the method comprises administering to an individual in need thereof, a therapeutically
30 effective amount of a compound as defined in any of claims 1 to 72, or a pharmaceutically acceptable salt or stereoisomer thereof.

87. A method of treating a disorder selected from pneumonia, acute respiratory distress (ARDS), and immunopathology, in particular hypercytokinemia (cytokine storm syndrome), sepsis and graft-versus-host disease, wherein the method comprises administering to an individual
35 in need thereof, a therapeutically effective amount of a compound as defined in any of claims 1 to 72, or a pharmaceutically acceptable salt or stereoisomer thereof.

88. A method of treating pathogen-induced inflammation, wherein the method comprises administering to an individual in need thereof, a therapeutically effective amount of a compound as defined in any of claims 1 to 72, or a pharmaceutically acceptable salt or stereoisomer thereof.

89. A method of combined treatment of inflammation associated with activation of Toll-like receptors or pathogen-induced inflammation and in the treatment of a viral infection, wherein the method comprises administering to an individual in need thereof, a therapeutically effective amount of a compound as defined in any of claims 1 to 72, or a pharmaceutically acceptable salt or stereoisomer thereof.
90. A method of anti-inflammatory and antiviral treatment, wherein the method comprises administering to an individual in need thereof, a therapeutically effective amount of a compound as defined in any of claims 1 to 72, or a pharmaceutically acceptable salt or stereoisomer thereof.
91. A kit comprising the compound as defined in any of claims 1 to 72, together with instructions for treating inflammation; for treating inflammation associated with activation of Toll-like receptors; for treating a disorder selected from pneumonia, acute respiratory distress (ARDS) and immunopathology, in particular hypercytokinemia (cytokine storm syndrome), sepsis and graft-versus-host disease; for treating pathogen-induced inflammation; for the combined treatment of inflammation associated with activation of Toll-like receptors or pathogen-induced inflammation and in the treatment of a viral infection; or for use as an anti-inflammatory and an antiviral.
92. A method of decreasing the expression and/or secretion of one or more pro-inflammatory cytokine in a subject or in a sample obtained from a subject or in a cell culture, the method comprising administering a compound as defined in any of claims 1 to 72.
93. A method of increasing macrophage activation and/or recruitment in a subject or in a sample obtained from a subject or in a cell culture, the method comprising administering a compound as defined in any of claims 1 to 72.
94. A method of prophylaxis, reduction or treatment of inflammatory aspects of CoV persistent, long CoV or post-CoV syndrome, wherein the method comprises administering to an individual in need thereof, a therapeutically effective amount of a compound as defined in any of claims 1 to 72, or a pharmaceutically acceptable salt or stereoisomer thereof; preferably wherein the CoV is SARS-CoV-2; optionally wherein the treatment minimises the likelihood that a patient suffers from COVID persistent, long COVID or post-COVID syndrome symptoms; and/or reduces the severity of such symptoms.
95. A method of reducing the infectivity and inflammation of CoV patients, wherein the method comprises administering to an individual in need thereof, a therapeutically effective amount of a compound as defined in any of claims 1 to 72, or a pharmaceutically acceptable salt or stereoisomer thereof; preferably wherein the CoV is SARS-CoV-2; optionally wherein the patient is asymptomatic or not very symptomatic yet has a high viral load.
96. A method of minimising the inflammatory symptoms of CoV infection, wherein the method comprises administering to an individual in need thereof, a therapeutically effective amount of a compound as defined in any of claims 1 to 72, or a pharmaceutically acceptable salt or stereoisomer thereof; preferably wherein the CoV is SARS-CoV-2.
97. A corticosteroid for use in the treatment of inflammation, wherein the corticosteroid is administered in combination with a compound according to any one of claims 1 to 72.
98. A compound according to any one of claims 1 to 25 and a corticosteroid for use in the

treatment of inflammation; wherein the use is according to any one of claims 1 to 72.

5 99. A method of treatment of inflammation, the method comprising administering a combination therapy of compound according to any one of claims 1 to 25 or a pharmaceutically acceptable salt thereof and a corticosteroid to a patient in need thereof, thereby treating the inflammation; wherein said method is as defined in any one of claims 1 to 72.

100. Use of a compound as defined in any of claims 1 to 72 or a pharmaceutically acceptable salt or stereoisomer thereof, in the manufacture of a medicament for the treatment of inflammation; wherein said treatment includes administration of a corticosteroid.

10 101. Use of a corticosteroid in the manufacture of a medicament for the treatment of inflammation; wherein said treatment includes administration of a compound as defined in any of claims 1 to 72 or a pharmaceutically acceptable salt or stereoisomer thereof.

102. Use of a compound as defined in any of claims 1 to 72 or a pharmaceutically acceptable salt or stereoisomer thereof and a corticosteroid in the manufacture of a medicament for the treatment of inflammation.

15 103. A pharmaceutical package comprising a compound as defined in any of claims 1 to 25 and a corticosteroid, optionally further comprising instructions according to any one of claims 1 to 72.