There is therefore an interest in identifying other anti-inflammatory and analgesic agents for use in treating inflammation, pain and/or TG2, JAK, and/or COX-2 associated conditions. For example, anti-inflammatory and analgesic agents for use in treating gastrointestinal inflammation.

Honey is well-known for its anti-microbial activities. It is also suggested in the art that honey possesses anti-inflammatory activity, although the reason for this has not been well characterised. One patent publication, WO2015/030609, which is hereby incorporated by reference, explores the anti-inflammatory activity of a specific fraction of honey. This publication teaches that a low molecular weight fraction from honey has generalised anti-inflammatory effects and no immune-stimulatory effects. It does not discuss specific anti-inflammatory action.

It will be appreciated from the above that it would be useful to provide alternative methods of treating inflammatory conditions, including inflammatory conditions associated with the gastrointestinal tract.

It is an object of the invention to provide methods of treating inflammatory conditions, including inflammatory conditions associated with the gastrointestinal tract and/or to address on or more of the foregoing problems and/or at least to provide the public with a useful choice.

Further aspects and advantages of the products, compositions, methods and uses will become apparent from the ensuing description that is given by way of example only.

SUMMARY

Described herein are compositions comprising 3,6,7-trimethyllumazine, and methods of using the same for preventing, ameliorating or treating TG2, JAK, and/or COX-2 associated conditions, inflammation of the gastrointestinal tract, inflammatory conditions associated with gastrointestinal tract, and/or pain.

The inventors have identified that a pteridine from honey, 3,6,7-trimethyllumazine, has TG2, JAK, and/or COX-2 inhibitory activity. Being able to isolate the compound and characterise the anti-inflammatory and COX-2, TG2 and/or JAK inhibitory activity provides the ability to produce medicaments for various uses including the treatment, prevention and amelioration of conditions associated with TG2, JAK, and/or COX-2, including inflammatory conditions and pain. For example, inflammation and pain associated with the gastrointestinal tract.

The disclosure provides a method of preventing, ameliorating or treating a COX-2 associated condition in a subject, comprising administering to a subject in need thereof a composition comprising 3,6,7-trimethyllumazine. In one example, the COX-2 associated condition is an inflammatory condition. In one example, the inflammatory condition is associated with inflammation of the gastrointestinal tract.

In one example , the COX-2 associated condition is selected from the group consisting of; gastrointestinal inflammatory diseases, gastric ulcers, peptic ulcers, gastritis, inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, irritable bowel syndrome (IBS), digestive disease, gastroesophageal reflux disease (GERD), heartburn, acid reflux, *Helicobacter pylori* infection, mouth ulcers, stomatitis, pharyngitis, gingivitis, esophageal ulcers, inflammatory and degenerative nervous system disorders, neuropsychiatric illnesses, schizophrenia, bipolar mood disorder, neurodegenerative disorders, traumatic brain injury, multiple sclerosis, Alzheimer's disease, nervous system disorders, Parkinson's disease, seizures, brain hypoxia/ischemia, Creutzfeldt-Jakob disease, amyotrophic lateral sclerosis, arthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, ankylosing spondylitis, chronic inflammation, cardiovascular diseases, pain, cancer, colorectal cancer (CRC) and musculoskeletal diseases.

In one example, the COX-2 associated condition is pain. In one example, the pain is acute pain, chronic pain and/or dysmenorrhea.

The disclosure provides a method of preventing, ameliorating or treating COX-2 associated inflammation in a subject comprising administering to a subject in need thereof a composition comprising 3,6,7-trimethyllumazine. In one example, the inflammation is associated with the gastrointestinal tract of a subject.

The disclosure provides a method of preventing, ameliorating or treating COX-2 associated pain in a subject comprising administering to a subject in need thereof a composition comprising 3,6,7-trimethyllumazine.

The disclosure provides a method of preventing, ameliorating or treating a TG2 associated condition in a subject, comprising administering to a subject in need thereof a composition comprising 3,6,7-trimethyllumazine. In one example, the TG2 associated condition is selected from the group consisting of gastrointestinal inflammatory diseases, gastric ulcers (such as peptic ulcers),

gastritis, TG2 -associated inflammatory conditions, inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, irritable bowel syndrome (IBS), digestive diseases, gastroesophageal reflux disease (GERD), heartburn, acid reflux, *Helicobacter pylori* infection, mouth ulcers, stomatitis, pharyngitis, gingivitis and/or esophageal ulcers, neuropsychiatric illnesses (such as schizophrenia and bipolar mood disorder), multiple sclerosis, neurodegenerative disorders (such as traumatic brain injury, multiple sclerosis, and Alzheimer's disease), cardiovascular diseases, cancer, arthritis, celiac disease, Huntington's disease, fibrosis and cancer. TG2 also plays a role in wound healing.

The disclosure provides a method of preventing, ameliorating or treating a JAK associated condition in a subject, comprising administering to a subject in need thereof a composition comprising 3,6,7-trimethyllumazine. In one example, the JAK associated condition is selected from the group consisting of gastrointestinal inflammatory diseases, gastric ulcers (such as peptic ulcers), gastritis, JAK associated inflammatory conditions, inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, irritable bowel syndrome (IBS), digestive diseases, gastroesophageal reflux disease (GERD), heartburn, acid reflux, *Helicobacter pylori* infection, mouth ulcers, stomatitis, pharyngitis, gingivitis and/or esophageal ulcers, neuropsychiatric illnesses (such as schizophrenia and bipolar mood disorder), multiple sclerosis, neurodegenerative disorders (such as traumatic brain injury, multiple sclerosis, and Alzheimer's disease), cardiovascular diseases, cancer, arthritis, chronic inflammation, auto-immune conditions, ulcerative colitis, alopecia areata, atopic dermatitis, diffuse scleroderma, Crohn's disease, vitiligo, hemophagocytic syndrome, non-infectious uveitis and cutaneous lupus erythematosus.

In one example, the origin of the 3,6,7-trimethyllumazine is from *Leptospermum*. In one example, the 3,6,7-trimethyllumazine is derived substantially from plants selected from the group consisting of: *Leptospermum scoparium, Leptospermum polygalifolium, Leptospermum subtenue*, and/or combinations thereof. In one example, the 3,6,7-trimethyllumazine is from *Leptospermum scoparium*.

In one example, the origin of the 3,6,7-trimethyllumazine is honey.

In one example, the honey comprises honey of a floral origin substantially from the genus *Leptospermum*. In one embodiment, the honey

comprises honey of a floral origin substantially from: *Leptospermum scoparium*, *Leptospermum polygalifolium*, *Leptospermum subtenue*, and/or combinations thereof. In one example, the honey comprises honey of a floral origin substantially from *Leptospermum scoparium* (also referred to as Manuka).

In one example, the honey is of a floral origin substantially from the genus *Leptospermum*. In one example, the honey is of a floral origin substantially from: *Leptospermum scoparium*, *Leptospermum polygalifolium*, *Leptospermum subtenue*, and/or combinations thereof. In one example, the honey is of a floral origin substantially from *Leptospermum scoparium* (also referred to as Manuka).

In one example, the 3,6,7-trimethyllumazine is derived directly from a plant of the genus *Leptospermum*. In one example, the 3,6,7-trimethyllumazine is derived directly from the flowers, nectar, roots, fruit, seeds, bark, oil, leaves, wood, stems or other plant material of a plant of the genus *Leptospermum*. In one example, the 3,6,7-trimethyllumazine is substantially from plants selected from the group consisting of: *Leptospermum scoparium, Leptospermum polygalifolium, Leptospermum subtenue*, and/or combinations thereof.

In one example, the composition comprising 3,6,7-trimethyllumazine comprises honey. In one example, the composition comprising 3,6,7-trimethyllumazine consists of honey.

In one example, the composition comprising 3,6,7-trimethyllumazine comprises a honey extract. In one example, the composition comprising 3,6,7-trimethyllumazine comprises a honey extract, wherein the honey extract comprises a concentration of 3,6,7-trimethyllumazine that is higher than the concentration of 3,6,7-trimethyllumazine found naturally occurring in honey. In one example, the composition consists of a honey extract, wherein the honey extract comprises a concentration of 3,6,7-trimethyllumazine that is higher than the concentration of 3,6,7-trimethyllumazine found naturally occurring in honey. In one example, the honey extract comprises a concentration of 3,6,7-trimethyllumazine that is higher than the concentration of 3,6,7-trimethyllumazine found naturally occurring in the honey from which the extract was derived.

In one example, the honey from which the extract is derived comprises honey of a floral origin substantially from the genus

Leptospermum. In one example, the honey from which the extract is derived comprises honey of a floral origin substantially from: *Leptospermum scoparium, Leptospermum polygalifolium, Leptospermum subtenue*, and/or combinations thereof. In one example, the honey from which the extract is derived comprises honey of a floral origin substantially from *Leptospermum scoparium*. In one example, the composition further comprises honey.

In one example, the honey from which the extract is derived is of a floral origin substantially from the genus *Leptospermum*. In one example, the honey from which the extract is derived is of a floral origin substantially from: *Leptospermum scoparium, Leptospermum polygalifolium, Leptospermum subtenue*, and/or combinations thereof. In one example, the honey from which the extract is derived is of a floral origin substantially from *Leptospermum scoparium.* In one example, the composition further comprises honey.

In one example, the honey is raw honey, heat-treated honey or pasteurised honey.

In one example, the composition comprises 3,6,7-trimethyllumazine isolated from honey. In one example, the honey is of a floral origin substantially from the genus *Leptospermum*. In one example, the honey is of a floral origin substantially from: *Leptospermum scoparium, Leptospermum polygalifolium, Leptospermum subtenue*, and/or combinations thereof. In one example, the 3,6,7-trimethyllumazine is isolated by subjecting the honey to solid phase extraction, followed by normal-phase flash chromatography and preparative thin layer chromatography.

In one example, the 3,6,7-trimethyllumazine is synthetic. In one example, the composition further comprises honey.

In one example, the composition comprises about 2.5 µg/mL to about 1000 µg/mL 3,6,7-trimethyllumazine. In one example, the composition comprises 3,6,7-trimethyllumazine from about 2.5 µg/mL, about 5 µg/mL, about 10 µg/mL, about 20 µg/mL, about 40 µg/mL, about 50 µg/mL, about 60 µg/mL, about 70 µg/mL, about 80 µg/mL, about 90 µg/mL, about 100 µg/mL, 150 µg/mL, about 200 µg/mL, about 250 µg/mL, about 300 µg/mL, about 350 µg/mL, about 400 µg/mL, about 450 about 500 µg/mL, about 550 µg/mL, about 600 µg/mL, about 650 µg/mL, about 700 µg/mL, about 750 µg/mL, about 800 µg/mL, about 850 µg/mL, about 900 µg/mL or about 950 µg/mL, to about 1000

μg/mL, or the composition comprises about 2.5 to 5 μg/mL, about 5 to 10 μg/mL, about 10 to 20 μg/mL, about 20 to 40 μg/mL, about 40 to 50 μg/mL, about 50 to 60 μg/mL, about 60 to 70 μg/mL, about 70 to 80 μg/mL, about 80 to 90 μg/mL, about 90 to 100 μg/mL, about 100 to 150 μg/mL, 150 to 200 μg/mL, about 200 to 250 μg/mL, about 250 to 300 μg/mL, about 300 to 350 μg/mL, about 350 to 400 μg/mL, about 400 to 450 μg/mL, about 450 to 500 μg/mL, about 500 to 550 μg/mL, about 550 to 600 μg/mL, about 600 to 650 μg/mL, about 650 to 700 μg/mL, about 700 to 750 μg/mL, about 750 to 800 μg/mL, about 800 to 850 μg/mL, about 850 to 900 μg/mL, about 900 to 950 μg/mL or about 950 to 1000 μg/mL 3,6,7-trimethyllumazine.

In one example, the composition comprises 3,6,7-trimethyllumazine about 5 mg/kg to about 3000 mg/kg. In one example, the composition comprises 3,6,7-trimethyllumazine from about 5 mg/kg, about 10 mg/kg, about 15 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 35 mg/kg, about 40 mg/kg, about 45 mg/kg, about 50 mg/kg, about 55 mg/kg, about 60 mg/kg, about 70 mg/kg, about 80 mg/kg, about 90 mg/kg, about 100 mg/kg, about 150 mg/kg, about 200 mg/kg, about 250 mg/kg, about 300mg/kg, about 350 mg/kg, about 400 mg/kg, about 450 mg/kg, about 500 mg/kg, about 550 mg/kg, about 600 mg/kg, about 650 mg/kg, about 700 mg/kg, about 750 mg/kg, about 800 mg/kg, about 850 mg/kg, about 900 mg/kg, about 950 mg/kg, about 1000 mg/kg, about 1100 mg/kg, about 1200 mg/kg, about 1300 mg/kg, about 1400 mg/kg, about 1500 mg/kg, about 1600 mg/kg, about 1700 mg/kg, about 1800 mg/kg, about 1900 mg/kg, about 2000 mg/kg, about 2100 mg/kg, about 2200 mg/kg, about 2300 mg/kg, about 2400 mg/kg, about 2500 mg/kg, about 2600 mg/kg, about 2700 mg/kg, about 2800 mg/kg or about 2900 mg/kg to about 3000 mg/kg, or the composition comprises a concentration of 3,6,7-trimethyllumazine of about 5 to 10 mg/kg, about 10 to 15 mg/kg, about 15 to 20 mg/kg, about 20 to 25 mg/kg, about 25 to 30 mg/kg, about 30 to 35 mg/kg, about 35 to 40 mg/kg, about 40 to 45 mg/kg, about 45 to 50 mg/kg, about 50 to 55 mg/kg, about 55 to 60 mg/kg, about 60 to 70 mg/kg or about 70 to 80 mg/kg, about 90 to 100 mg/kg, about 100 to 150 mg/kg, about 150 to 200 mg/kg, about 200 mg/kg, about 250 to 300 mg/kg, about 300 to 350 mg/kg, about 350 to 400 mg/kg, about 400 to 450 mg/kg, about 450 to 500 mg/kg, about 500 to 550 mg/kg, about 550 to 600 mg/kg, about 600 to 650 mg/kg, about 650 to 700 mg/kg, about 700 to 750 mg/kg, about 750 to 800 mg/kg, about 800 to 850 mg/kg, about 850 to 900 mg/kg, about 900 to 950 mg/kg, about 950 to 1000 mg/kg, about 1000 to 1100 mg/kg, about 1100 to 1200 mg/kg, about 1200 to

1300 mg/kg, about 1300 to 1400 mg/kg, about 1400 to 1500 mg/kg, about 1500 to 1600 mg/kg, about 1600 to 1700 mg/kg, about 1700 to 1800 mg/kg, about 1800 to 1900 mg/kg, about 1900 to 2000 mg/kg, about 2000 to 2100 mg/kg, about 2100 to 2200 mg/kg, about 2200 to 2300 mg/kg, about 2300 to 2400 mg/kg, about 2400 to 2500 mg/kg, about 2500 to 2600 mg/kg, about 2600 to 2700 mg/kg, about 2700 to 2800 mg/kg, about 2800 to 2900 mg/kg or about 2900 to 3000 mg/kg.

In one example, the composition comprises a therapeutically effective amount of 3,6,7-trimethyllumazine.

In one example, the composition comprising 3,6,7-trimethyllumazine is formulated as a medicament, therapeutic product or health supplement. The composition comprising 3,6,7-trimethyllumazine may be formulated into a range of delivery systems, including but not limited to, liquid formulations, capsules, chewable tablets, tablets, suppositories, fast moving consumer goods, intravenous preparations, intramuscular preparations, subcutaneous preparations, solutions, food, beverages, dietary supplements, cosmetic formulations, gels, lotions, powders and sprays.

In one example, the method comprises administering the composition comprising 3,6,7-trimethyllumazine one, two, three, four or five times daily.

In one example, the method comprises administering the composition comprising 3,6,7-trimethyllumazine one, two, three, four, five, six or seven times weekly.

In one example, the composition comprising 3,6,7-trimethyllumazine is administered as a single dose or as a divided dose. In one example, the composition comprising 3,6,7-trimethyllumazine is administered as one, two, three or four separate doses.

In one example, the method comprises administration of the composition comprising 3,6,7-trimethyllumazine at a dose of about 1mg to about 3000mg. In one particular example, the method comprises administration of the composition comprising about 1mg, 10mg, 20mg, 30mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100mg, 150 mg, 200mg, 250 mg, 300mg, 350mg, 400mg, 450 mg, 500mg, 550 mg, 600mg, 650mg, 700mg, 750 mg, 800mg, 850 mg, 900mg, 950 mg, 1000mg, 1100mg, 1200mg, 1300mg,

1400mg, 1500mg, 1600mg, 1700mg, 1800mg, 1900mg, 2000mg, 2100mg, 2200mg, 2300mg, 2400mg, 2500mg, 2600mg, 2700mg, 2800mg, 2900mg or 3000mg 3,6,7-trimethyllumazine.

In one example, the method comprises administering the composition at a dose of about 5g to about 100g of the composition comprising 3,6,7-trimethyllumazine.

In one example, the composition comprising 3,6,7-trimethyllumazine has a standardised concentration of 3,6,7-trimethyllumazine obtained by:

a. selecting a first composition with a known concentration of 3,6,7-trimethyllumazine;
b. selecting at least one further composition with a known concentration of 3,6,7-trimethyllumazine; and
c. combining the first composition with the second composition to obtain a final composition with a standardised 3,6,7-trimethyllumazine concentration of about 5 mg/kg to about 3000 mg/kg.

In one example, the composition comprising 3,6,7-trimethyllumazine has a standardised concentration of 3,6,7-trimethyllumazine obtained by:

a. selecting a first composition with a known concentration of 3,6,7-trimethyllumazine;
b. combining the selected first composition with one or more of:
    - synthetic 3,6,7-trimethyllumazine;
    - isolated 3,6,7-trimethyllumazine;
    - a honey extract comprising 3,6,7-trimethyllumazine; and/or
    - 3,6,7-trimethyllumazine derived directly from a plant of the genus *Leptospermum*;

    to form a composition with a standardised 3,6,7-trimethyllumazine concentration of about 5 mg/kg to about 3000 mg/kg.

In one example, the composition comprises honey, a honey extract, isolated 3,6,7-trimethyllumazine and/or synthetic 3,6,7-trimethyllumazine.

In one example, the 3,6,7-trimethyllumazine derived directly from a plant is derived directly from the flowers, nectar, roots, fruit, seeds,

bark, oil, leaves, wood, stems or other plant material of a plant of the genus *Leptospermum*.

In one example, the standardised 3,6,7-trimethyllumazine concentration is about 5 mg/kg to about 3000 mg/kg. In one example, the standardised 3,6,7-trimethyllumazine concentration is: about 5 mg/kg, about 10 mg/kg, about 15 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 35 mg/kg, about 40 mg/kg, about 45 mg/kg, about 50 mg/kg, about 55 mg/kg, about 60 mg/kg, about 70 mg/kg, about 80 mg/kg, about 90 mg/kg, about 100 mg/kg, about 150 mg/kg, about 200 mg/kg, about 250 mg/kg, about 300mg/kg, about 350 mg/kg, about 400 mg/kg, about 450 mg/kg, about 500 mg/kg, about 550 mg/kg, about 600 mg/kg, about 650 mg/kg, about 700 mg/kg, about 750 mg/kg, about 800 mg/kg, about 850 mg/kg, about 900 mg/kg, about 950 mg/kg, about 1000 mg/kg, about 1100 mg/kg, about 1200 mg/kg, about 1300 mg/kg, about 1400 mg/kg, about 1500 mg/kg, about 1600 mg/kg, about 1700 mg/kg, about 1800 mg/kg, about 1900 mg/kg, about 2000 mg/kg, about 2100 mg/kg, about 2200 mg/kg, about 2300 mg/kg, about 2400 mg/kg, about 2500 mg/kg, about 2600 mg/kg, about 2700 mg/kg, about 2800 mg/kg or about 2900 mg/kg to about 3000 mg/kg of 3,6,7-trimethyllumazine.

In one example, the concentration of the 3,6,7-trimethyllumazine is determined by chromatography, analytical measurements, spectrophotometry and/or any other method known to a person skilled in the art. In one example, the concentration of 3,6,7-trimethyllumazine is determined by reverse-phase HPLC.

The disclosure provides a method of making a composition with anti-inflammatory, analgesic and/or TG2, JAK, and/or COX-2 inhibitory activity comprising:

a. testing a first composition comprising honey for 3,6,7-trimethyllumazine concentration;
b. testing at least one further composition comprising honey for 3,6,7-trimethyllumazine concentration;
c. selecting a composition comprising honey with a 3,6,7-trimethyllumazine concentration greater than about 5 mg/kg 3,6,7-trimethyllumazine;
d. selecting at least one further composition comprising honey with a 3,6,7-trimethyllumazine concentration greater than about 5 mg/kg;
e. combining the selected composition comprising honey to form a honey

composition with a 3,6,7-trimethyllumazine concentration of about 5mg/kg to about 80 mg/kg.

In one aspect, the disclosure provides a method of making a composition with anti-inflammatory, analgesic and/or COX-2 inhibitory activity comprising:

a. testing a first composition comprising honey for 3,6,7-trimethyllumazine concentration;
b. testing at least one further composition comprising honey for 3,6,7-trimethyllumazine concentration;
c. selecting a composition comprising honey with a 3,6,7-trimethyllumazine concentration greater than about 5 mg/kg;
d. selecting at least one further composition comprising honey with a 3,6,7-trimethyllumazine concentration greater than about 5 mg/kg 3,6,7-trimethyllumazine; and
e. combining the selected compositions comprising honey to form a honey composition with a 3,6,7-trimethyllumazine concentration of about 5 mg/kg to about 80 mg/kg.

In one example, a composition comprising honey is selected if it has a concentration of 3,6,7-trimethyllumazine greater than about 5 mg/kg, about 10 mg/kg, about 15 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 35 mg/kg, about 40 mg/kg, about 45 mg/kg, about 50 mg/kg, about 55 mg/kg, about 60 mg/kg, about 70 mg/kg or about 80 mg/kg.

In one example, the composition comprises, consists essentially of, or consists of honey.

In one example, the 3,6,7-trimethyllumazine concentration is determined by chromatography, analytical measurements, spectrophotometry and/or any other method known to a person skilled in the art. In one example, the concentration of 3,6,7-trimethyllumazine is determined by reverse-phase HPLC.

In one example, the composition with anti-inflammatory, analgesic and/or TG2, JAK and/or COX-2 inhibitory activity is suitable for use in the method as defined herein.

The disclosure provides a method of identifying a composition as having anti-inflammatory, analgesic and/or TG2, JAK, and/or COX-2 inhibitory activity comprising:

a. testing a composition for 3,6,7-trimethyllumazine concentration; and
    i. identifying the composition as having anti-inflammatory, analgesic, and/or TG2, JAK, and/or COX-2 inhibitory activity if it contains a 3,6,7-trimethyllumazine concentration greater than about 5 mg/kg; or
    ii. identifying the composition as not having anti-inflammatory, analgesic, and/or TG2, JAK, and/or COX-2 inhibitory activity if it contains a 3,6,7-trimethyllumazine concentration lower than about 5 mg/kg.

In one example, the composition comprises honey or a honey extract.

In one example, the composition comprising honey is determined as having anti-inflammatory activity if it contains greater than about 5 mg/kg, about 10 mg/kg, about 15 mg/kg, about 20 mg/kg, about 25 mg/kg, about

30 mg/kg, about 35 mg/kg, about 40 mg/kg, about 45 mg/kg, about 50 mg/kg, about 55 mg/kg, about 60 mg/kg, about 70 mg/kg or about 80 mg/kg.

In one example, the composition comprises, consists essentially of, or consists of honey or a honey extract.

In one example, the composition with anti-inflammatory activity is suitable for use in the methods as defined herein.

The disclosure provides a method of identifying a composition with anti-inflammatory, analgesic and/or TG2, JAK, and/or COX-2 inhibitory activity suitable for use as defined herein, comprising:

a. testing a composition for 3,6,7-trimethyllumazine concentration; and
    i. identifying the composition as suitable for use as defined herein if it contains a 3,6,7-trimethyllumazine concentration about 5 to about 80 mg/kg 3,6,7-trimethyllumazine; or
    ii. identifying the composition as not suitable for use as defined herein if it contains a 3,6,7-trimethyllumazine concentration lower than about 5 mg/kg 3,6,7-trimethyllumazine.

In another aspect, the disclosure provides a method of identifying a composition as having anti-inflammatory, analgesic and/or COX-2 inhibitory activity comprising:

a. testing a composition for 3,6,7-trimethyllumazine concentration; and
    i. identifying the composition as having anti-inflammatory, analgesic and/or COX-2 inhibitory activity if it contains a 3,6,7-trimethyllumazine concentration greater than about 5 mg/kg; or
    ii. identifying the composition as not having anti-inflammatory, analgesic and/or COX-2 inhibitory activity if it contains a 3,6,7-trimethyllumazine concentration lower than about 5 mg/kg,

optionally wherein the composition comprises honey.

In one example, the composition is identified as suitable for use in a method as defined herein if

it contains a 3,6,7-trimethyllumazine concentration greater than about 5 mg/kg, about 10 mg/kg, about 15 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 35 mg/kg, about 40 mg/kg, about 45 mg/kg, about 50 mg/kg, about 55 mg/kg, about 60 mg/kg, about 70 mg/kg or about 80 mg/kg.

In one example, the composition comprises, consists essentially of, or consists of honey or a honey extract.

In one example, the 3,6,7-trimethyllumazine concentration is determined by chromatography, analytical measurements, spectrophotometry and/or any other method known to a person skilled in the art. In one example, the concentration of 3,6,7-trimethyllumazine is determined by reverse-phase HPLC system.

The disclosure provides a composition comprising 3,6,7-trimethyllumazine suitable for use in the method as defined herein.

In one example, the origin of the 3,6,7-trimethyllumazine is from *Leptospermum*. In one example, the 3,6,7-trimethyllumazine is substantially from plants selected from the group consisting of: *Leptospermum scoparium, Leptospermum polygalifolium, Leptospermum subtenue*, and/or combinations thereof. In one example, the 3,6,7-trimethyllumazine is from *Leptospermum scoparium*.

In one example, the origin of the 3,6,7-trimethyllumazine is honey. In one example, the honey is of a floral origin substantially from the genus *Leptospermum*. In one example, the honey is of a floral origin substantially from: *Leptospermum scoparium, Leptospermum polygalifolium, Leptospermum subtenue*, and/or combinations thereof. In one example, the honey is of a floral origin substantially from *Leptospermum scoparium* (Manuka).

In one example, the 3,6,7-trimethyllumazine is derived directly from a plant of the genus *Leptospermum*. In one example, the 3,6,7-trimethyllumazine is derived directly from the nectar, roots, fruit, seeds, bark, oil, leaves, wood, stems or other plant material of a plant of the genus *Leptospermum*. In one example, the 3,6,7-trimethyllumazine is substantially from plants selected from the group consisting of: *Leptospermum scoparium, Leptospermum polygalifolium, Leptospermum subtenue*, and/or combinations thereof.

In one example, the composition comprising 3,6,7-trimethyllumazine comprises honey. In one example, the composition comprising 3,6,7-trimethyllumazine consists essentially of honey. In one example, the composition comprising 3,6,7-trimethyllumazine consists of honey.

In one example, the composition comprising 3,6,7-trimethyllumazine comprises a honey extract. In one example, the composition comprising 3,6,7-trimethyllumazine comprises a honey extract, wherein the honey extract comprises a concentration of 3,6,7-trimethyllumazine that is higher than the concentration of 3,6,7-trimethyllumazine found naturally occurring in honey. In one example, the composition comprising 3,6,7-trimethyllumazine consists essentially of a honey extract, wherein the honey extract comprises a concentration

of 3,6,7-trimethyllumazine that is higher than the concentration of 3,6,7-trimethyllumazine found naturally occurring in honey. In one example, the composition comprising 3,6,7-trimethyllumazine consists of a honey extract, wherein the honey extract comprises a concentration of 3,6,7-trimethyllumazine that is higher than the concentration of 3,6,7-trimethyllumazine found naturally occurring in honey. In one example, the honey extract comprises a concentration of 3,6,7-trimethyllumazine that is higher than the concentration of 3,6,7-trimethyllumazine found naturally occurring in the honey from which the extract was derived.

In one example, the honey from which the extract is derived is of a floral origin substantially from the genus *Leptospermum*. In one example, the honey from which the extract is derived is of a floral origin substantially from: *Leptospermum scoparium, Leptospermum polygalifolium, Leptospermum subtenue*, and/or combinations thereof. In one example, the composition further comprises honey.

In one example, the honey is raw honey, heat-treated honey or pasteurised honey.

In one example, the composition comprises 3,6,7-trimethyllumazine isolated from honey. In one example, the honey is of a floral origin substantially from the *Leptospermum*. In one example, the honey is of a floral origin substantially from: *Leptospermum scoparium, Leptospermum polygalifolium, Leptospermum subtenue*, and/or combinations thereof. In one example, the 3,6,7-trimethyllumazine is isolated by subjecting the honey to solid phase extraction, followed by normal-phase flash chromatography and preparative thin layer chromatography.

In one example, the composition comprises synthetic 3,6,7-trimethyllumazine. In one example, the composition further comprises honey.

In one example, the composition comprises about 2.5 µg/mL to about 1000 µg/mL 3,6,7-trimethyllumazine. In one example, the composition comprises from about 2.5 µg/mL, about 5 µg/mL, about 10 µg/mL, about 20 µg/mL, about 40 µg/mL, about 50 µg/mL, about 60 µg/mL, about 70 µg/mL, about 80 µg/mL, about 90 µg/mL, about 100 µg/mL, 150 µg/mL, about 200 µg/mL, about 250 µg/mL, about 300 µg/mL, about 350 µg/mL, about 400 µg/mL, about 450 about 500 µg/mL, about 550 µg/mL, about 600 µg/mL, about 650 µg/mL, about 700 µg/mL, about 750 µg/mL, about 800 µg/mL, about 850 µg/mL, about 900 µg/mL or about

950 µg/mL or to about 1000 µg/mL 3,6,7-trimethyllumazine, or the composition comprises about 2.5 to 5 µg/mL, about 5 to 10 µg/mL, about 10 to 20 µg/mL, about 20 to 40 µg/mL, about 40 to 50 µg/mL, about 50 to 60 µg/mL, about 60 to 70 µg/mL, about 70 to 80 µg/mL, about 80 to 90 µg/mL, about 90 to 100 µg/mL, about 100 to 150 µg/mL, 150 to 200 µg/mL, about 200 to 250 µg/mL, about 250 to 300 µg/mL, about 300 to 350 µg/mL, about 350 to 400 µg/mL, about 400 to 450 µg/mL, about 450 to 500 µg/mL, about 500 to 550 µg/mL, about 550 to 600 µg/mL, about 600 to 650 µg/mL, about 650 to 700 µg/mL, about 700 to 750 µg/mL, about 750 to 800 µg/mL, about 800 to 850 µg/mL, about 850 to 900 µg/mL, about 900 to 950 µg/mL or about 950 to 1000 µg/mL 3,6,7-trimethyllumazine.

In one example, the composition comprises 3,6,7-trimethyllumazine about 5 mg/kg to about 3000 mg/kg 3,6,7-trimethyllumazine. In one example, the composition comprises 3,6,7-trimethyllumazine from about 5 mg/kg, about 10 mg/kg, about 15 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 35 mg/kg, about 40 mg/kg, about 45 mg/kg, about 50 mg/kg, about 55 mg/kg, about 60 mg/kg, about 70 mg/kg, about 80 mg/kg, about 90 mg/kg, about 100 mg/kg, about 150 mg/kg, about 200 mg/kg, about 250 mg/kg, about 300mg/kg, about 350 mg/kg, about 400 mg/kg, about 450 mg/kg, about 500 mg/kg, about 550 mg/kg, about 600 mg/kg, about 650 mg/kg, about 700 mg/kg, about 750 mg/kg, about 800 mg/kg, about 850 mg/kg, about 900 mg/kg, about 950 mg/kg, about 1000 mg/kg, about 1100 mg/kg, about 1200 mg/kg, about 1300 mg/kg, about 1400 mg/kg, about 1500 mg/kg, about 1600 mg/kg, about 1700 mg/kg, about 1800 mg/kg, about 1900 mg/kg, about 2000 mg/kg, about 2100 mg/kg, about 2200 mg/kg, about 2300 mg/kg, about 2400 mg/kg, about 2500 mg/kg, about 2600 mg/kg, about 2700 mg/kg, about 2800 mg/kg or about 2900 mg/kg to about 3000 mg/kg or the composition comprises a concentration of 3,6,7-trimethyllumazine of 5 to 10 mg/kg, or about 10 to 15 mg/kg, or about 15 to 20 mg/kg, or about 20 to 25 mg/kg, or about 25 to 30 mg/kg, or about 30 to 35 mg/kg, or about 35 to 40 mg/kg, or about 40 to 45 mg/kg, or about 45 to 50 mg/kg, or about 50 to 55 mg/kg, or about 55 to 60 mg/kg, or about 60 to 70 mg/kg or about70 to 80 mg/kg, about 90 to 100 mg/kg, about 100 to 150 mg/kg, about 150 to 200 mg/kg, about 200 mg/kg, about 250 to 300 mg/kg, about 300 to 350 mg/kg, about 350 to 400 mg/kg, about 400 to 450 mg/kg, about 450 to 500 mg/kg, about 500 to 550 mg/kg, about 550 to 600 mg/kg, about 600 to 650 mg/kg, about 650 to 700 mg/kg, about 700 to 750 mg/kg, about 750 to 800 mg/kg, about 800 to 850 mg/kg, about 850 to 900 mg/kg, about 900 to 950

mg/kg, about 950 to 1000 mg/kg, about 1000 to 1100 mg/kg, about 1100 to 1200 mg/kg, about 1200 to 1300 mg/kg, about 1300 to 1400 mg/kg, about 1400 to 1500 mg/kg, about 1500 to 1600 mg/kg, about 1600 to 1700 mg/kg, about 1700 to 1800 mg/kg, about 1800 to 1900 mg/kg, about 1900 to 2000 mg/kg, about 2000 to 2100 mg/kg, about 2100 to 2200 mg/kg, about 2200 to 2300 mg/kg, about 2300 to 2400 mg/kg, about 2400 to 2500 mg/kg, about 2500 to 2600 mg/kg, about 2600 to 2700 mg/kg, about 2700 to 2800 mg/kg, about 2800 to 2900 mg/kg or about 2900 to 3000 mg/kg.

In one example, the composition comprises a therapeutically effective amount of 3,6,7-trimethyllumazine.

In one example, the composition comprising 3,6,7-trimethyllumazine is formulated as a medicament, therapeutic product or health supplement. The composition comprising 3,6,7-trimethyllumazine may be formulated into a range of delivery systems, including but not limited to, liquid formulations, fast moving consumer goods, capsules, chewable tablets, tablets, suppositories, intravenous preparations, intramuscular preparations, subcutaneous preparations, solutions, food, beverages, dietary supplements, cosmetic formulations, gels, lotions, powders or sprays.

In another aspect, the present disclosure provides a use of a composition comprising 3,6,7-trimethyllumazine in the manufacture of a medicament for preventing, ameliorating or treating a COX-2 associated condition.

In one example, the COX-2 associated condition is selected from the group consisting of; gastrointestinal inflammatory diseases, gastric ulcers, peptic ulcers, gastritis, inflammatory bowel disease (IBD), Crohn’s disease, ulcerative colitis, irritable bowel syndrome (IBS), digestive disease, gastroesophageal reflux disease (GERD), heartburn, acid reflux, *Helicobacter pylori* infection, mouth ulcers, stomatitis, pharyngitis, gingivitis, esophageal ulcers, inflammatory and degenerative nervous system disorders, neuropsychiatric illnesses, schizophrenia, bipolar mood disorder, neurodegenerative disorders, traumatic brain injury, multiple sclerosis, Alzheimer’s disease, nervous system disorders, Parkinson’s disease, seizures, brain hypoxia/ischemia, Creutzfeldt-Jakob disease, amyotrophic lateral sclerosis, arthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, ankylosing spondylitis, chronic inflammation, cardiovascular diseases, cancer, pain, colorectal cancer (CRC) and musculoskeletal diseases.

In one example, the COX-2 associated condition is pain. In one example, the pain is acute pain, chronic pain and dysmenorrhea.

In another aspect, the disclosure provides a use of a composition comprising 3,6,7-trimethyllumazine in the manufacture of a medicament for preventing, ameliorating or treating COX-2 associated inflammation. In one example, the inflammation is associated with the gastrointestinal tract.

In another aspect, the disclosure provides a use of a composition comprising 3,6,7-trimethyllumazine in the manufacture of a medicament for preventing, ameliorating or treating COX-2 associated pain. In one example, the pain is acute pain, chronic pain and/or dysmenorrhea.

The disclosure provides a use of a composition comprising 3,6,7-trimethyllumazine in the manufacture of a medicament for preventing, ameliorating or treating an TG2 and/or JAK associated condition.

In one example, the TG2 associated condition is selected from the group consisting of gastrointestinal inflammatory diseases, gastric ulcers (such as peptic ulcers), gastritis, TG2 associated inflammatory conditions, inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, irritable bowel syndrome (IBS), digestive diseases, gastroesophageal reflux disease (GERD), heartburn, acid reflux, *Helicobacter pylori* infection, mouth ulcers, stomatitis, pharyngitis, gingivitis and/or esophageal ulcers, neuropsychiatric illnesses (such as schizophrenia and bipolar mood disorder), multiple sclerosis, neurodegenerative disorders (such as traumatic brain injury, multiple sclerosis, and Alzheimer's disease), cardiovascular diseases, cancer, arthritis, celiac disease, Huntington's disease, fibrosis, cancer and a wound.

In one example, the JAK associated condition is selected from the group consisting of gastrointestinal inflammatory diseases, gastric ulcers (such as peptic ulcers), gastritis, JAK associated inflammatory conditions, inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, irritable bowel syndrome (IBS), digestive diseases, gastroesophageal reflux disease (GERD), heartburn, acid reflux, *Helicobacter pylori* infection, mouth ulcers, stomatitis, pharyngitis, gingivitis and/or esophageal ulcers, neuropsychiatric illnesses (such as schizophrenia and bipolar mood disorder), multiple sclerosis, neurodegenerative disorders (such as traumatic brain injury, multiple sclerosis, and Alzheimer's disease), cardiovascular diseases, cancer, arthritis, chronic inflammation, auto-immune conditions, ulcerative colitis, alopecia areata, atopic

dermatitis, diffuse scleroderma, Crohn's disease, vitiligo, hemophagocytic syndrome, non-infectious uveitis and cutaneous lupus erythematosus.

There is provided a method, use or composition as defined herein, wherein the composition further comprises a COX-2 inhibitor.

There is provided a method or use as defined herein, further comprising co-administration of a COX-2 inhibitor. Advantages of the above methods and uses may be varied. In some examples, the source of 3,6,7-trimethyllumazine is naturally occurring and able to be manufactured on a sustainable basis. 3,6,7-trimethyllumazine is not anticipated to have side effects and it may be formulated in a wide variety of ways for various methods of administration.

This disclosure may also be said broadly to consist in the parts, elements and features referred to or indicated in the specification of the application, individually or collectively, and any or all combinations of any two or more of said parts, elements and features, and where specific integers are mentioned herein which have known equivalents in the art to which this disclosure relates, such known equivalents are deemed to be incorporated herein as if individually set forth.

The disclosure, which should be considered as defined herein, will become apparent to those skilled in the art upon reading of the following description which provides at least one example of a practical application of the disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of the invention will now be described, by way of example only, with reference to the accompanying drawings in which:

Figure 1 is a graph illustrating the fluorescence intensity generated by MMP-9 activity over the course of 10 min.

Figure 2 is a graph illustrating the percentage inhibition of MMP-9 activity from 3,6,7-trimethyllumazine ranging from 2.5–40 µg/ml.

Figure 3 is a graph illustrating the correlation between 3,6,7-trimethyllumazine concentration and MMP-9 inhibition.

Figure 4 is a graph illustrating MMP-9 activity measured by absorbance at 412nm over 120 min.

Claims:

1. Use of a composition comprising 3,6,7-trimethyllumazine in the manufacture of a medicament for preventing, ameliorating or treating a COX-2 associated condition.

2. The use of claim 1 wherein the COX-2 associated condition is an inflammatory condition, optionally wherein the inflammatory condition is associated with inflammation of the gastrointestinal tract.

3. The use of claim 1 wherein the COX-2 associated condition is selected from the group consisting of; gastrointestinal inflammatory diseases, gastric ulcers, peptic ulcers, gastritis, inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, irritable bowel syndrome (IBS), digestive disease, gastroesophageal reflux disease (GERD), heartburn, acid reflux, *Helicobacter pylori* infection, mouth ulcers, stomatitis, pharyngitis, gingivitis, esophageal ulcers, inflammatory and degenerative nervous system disorders, neuropsychiatric illnesses, schizophrenia, bipolar mood disorder, neurodegenerative disorders, traumatic brain injury, multiple sclerosis, Alzheimer's disease, nervous system disorders, Parkinson's disease, seizures, brain hypoxia/ischemia, Creutzfeldt-Jakob disease, amyotrophic lateral sclerosis, arthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, ankylosing spondylitis, chronic inflammation, cardiovascular diseases, cancer, pain, colorectal cancer (CRC) and musculoskeletal diseases.

4. The use of claim 1 wherein the COX-2 associated condition is pain, optionally wherein the pain is acute pain, chronic pain and/or dysmenorrhea.

5. Use of a composition comprising 3,6,7-trimethyllumazine in the manufacture of a medicament for preventing, ameliorating or treating COX-2 associated inflammation.

6. The use of claim 5 wherein the COX-2 associated inflammation is associated with the gastrointestinal tract of a subject.

7. Use of a composition comprising 3,6,7-trimethyllumazine in the manufacture of a medicament for preventing, ameliorating or treating COX-2 associated pain.

8. The use of claim 7 wherein the COX-2 associated pain is acute pain, chronic pain and/or dysmenorrhea.

9. The use of any one of the preceding claims wherein the origin of the 3,6,7-trimethyllumazine is honey, optionally wherein the honey is of a floral origin substantially from *Leptospermum*.

10. The use of claim 9 wherein the honey is of a floral origin substantially from: Leptospermum *scoparium, Leptospermum polygalifolium, Leptospermum subtenue*, and/or combinations thereof.

11. The use of any one of claims 1 to 8 wherein the 3,6,7-trimethyllumazine is synthetic.

12. The use of any one of claims 1 to 8 wherein the composition comprising 3,6,7-trimethyllumazine comprises honey or a honey extract.

13. The use of any one of the preceding claims wherein the composition comprises:
about 2.5 µg/mL to about 1000 µg/mL 3,6,7-trimethyllumazine;
about 2.5 µg/mL to about 80 µg/mL 3,6,7-trimethyllumazine;
about 2.5 µg/mL, about 5 µg/mL, about 10 µg/mL, about 20 µg/mL, about 40 µg/mL, about 50 µg/mL, about 60 µg/mL, about 70 µg/mL or about 80 µg/mL 3,6,7-trimethyllumazine;
about 5 mg/kg to about 3000 mg/kg 3,6,7-trimethyllumazine;
about 5 mg/kg to about 80 mg/kg 3,6,7-trimethyllumazine; or
about 5 mg/kg, about 10 mg/kg, about 15 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 35 mg/kg, about 40 mg/kg, about 45 mg/kg, about 50 mg/kg, about 55 mg/kg, about 60 mg/kg, about 70 mg/kg or about 80 mg/kg of 3,6,7-trimethyllumazine.

14. The use of any one of the preceding claims wherein the medicament is formulated as a liquid formulation, fast moving consumer good, capsule, tablet, chewable tablet, gel, lotion, powder, suppository, cosmetic formulation, intravenous preparation, intramuscular preparation, subcutaneous preparation, solution, food, beverage, dietary supplement or spray.

15. The use of any one of the preceding claims wherein the composition comprising 3,6,7-trimethyllumazine has a standardised concentration of 3,6,7-trimethyllumazine obtained by:
    - selecting a first composition with a known concentration of 3,6,7-trimethyllumazine;
    - selecting at least one further composition with a known concentration of 3,6,7-trimethyllumazine; and
    - combining the first composition with the second composition to obtain a composition with a standardised 3,6,7-trimethyllumazine concentration of about 5 mg/kg to about 3000 mg/kg.

16. The use of any one of claims 1 to 14 wherein the composition comprising 3,6,7-trimethyllumazine has a standardised concentration of 3,6,7-trimethyllumazine obtained by:
    - selecting a first composition with a known concentration of 3,6,7-trimethyllumazine; and
    - combining the selected first composition with one or more of:
        - synthetic 3,6,7-trimethyllumazine;
        - isolated 3,6,7-trimethyllumazine;
        - a honey extract comprising 3,6,7-trimethyllumazine; and/or
        - 3,6,7-trimethyllumazine derived directly from a plant of the genus *Leptospermum*;

    to form a composition with a standardised 3,6,7-trimethyllumazine concentration of about 5 mg/kg to about 3000 mg/kg.

17. A method of making a composition with anti-inflammatory, analgesic and/or COX-2 inhibitory activity comprising:
    a. testing a first composition comprising honey for 3,6,7-trimethyllumazine concentration;
    b. testing at least one further composition comprising honey for 3,6,7-trimethyllumazine concentration;
    c. selecting a composition comprising honey with a 3,6,7-trimethyllumazine concentration greater than about 5 mg/kg;
    d. selecting at least one further composition comprising honey with a 3,6,7-trimethyllumazine concentration greater than about 5 mg/kg 3,6,7-trimethyllumazine; and

e. combining the selected compositions comprising honey to form a honey composition with a 3,6,7-trimethyllumazine concentration of about 5 mg/kg to about 80 mg/kg.

18. A method of identifying a composition as having anti-inflammatory, analgesic and/or COX-2 inhibitory activity comprising:
    a. testing a composition for 3,6,7-trimethyllumazine concentration; and
        i. identifying the composition as having anti-inflammatory, analgesic and/or COX-2 inhibitory activity if it contains a 3,6,7-trimethyllumazine concentration greater than about 5 mg/kg; or
        ii. identifying the composition as not having anti-inflammatory, analgesic and/or COX-2 inhibitory activity if it contains a 3,6,7-trimethyllumazine concentration lower than about 5 mg/kg,

    optionally wherein the composition comprises honey.

19. The use according to any one of claims 1 to 16 wherein:

a. the composition comprising 3,6,7-trimethyllumazine comprises a honey extract comprising a concentration of 3,6,7-trimethyllumazine that is higher than the concentration of 3,6,7-trimethyllumazine found naturally occurring in the honey from which the extract was derived;

b. the composition comprising 3,6,7-trimethyllumazine comprises synthetic 3,6,7-trimethyllumazine and/or 3,6,7-trimethyllumazine isolated from honey; and/or

c. the composition comprising 3,6,7-trimethyllumazine has a standardised concentration of 3,6,7-trimethyllumazine obtained by:

- selecting a first composition with a known concentration of 3,6,7-trimethyllumazine;
- selecting at least one further composition with a known concentration of 3,6,7-trimethyllumazine;
- combining the first composition with the at least one further composition to obtain a composition with a standardised 3,6,7-trimethyllumazine concentration of from about 5 to about 3000 mg/kg.