composition with a 3,6,7-trimethyllumazine concentration of about 5mg/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

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.

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 another aspect, the present disclosure provides 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, wherein the composition:

a. 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. comprises synthetic 3,6,7-trimethyllumazine and/or 3,6,7-trimethyllumazine isolated from honey;
c. comprises a 3,6,7-trimethyllumazine concentration of greater than 5 mg/kg; or
d. has a standardised concentration of 3,6,7-trimethyllumazine obtained by:

i. selecting a first composition with a known concentration of 3,6,7-trimethyllumazine;
ii. selecting at least one further composition with a known concentration of 3,6,7-trimethyllumazine;
iii. 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 5 mg/kg to 3000 mg/kg; and

wherein the preventing, ameliorating or treating comprises inhibiting COX-2 expression.

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.

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 use of a composition comprising 3,6,7-trimethyllumazine in the manufacture of a medicament for preventing, ameliorating or treating COX-2 associated inflammation, wherein the composition:

a. 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. comprises synthetic 3,6,7-trimethyllumazine and/or 3,6,7-trimethyllumazine isolated from honey;
c. comprises a 3,6,7-trimethyllumazine concentration of greater than 5 mg/kg; or
d. has a standardised concentration of 3,6,7-trimethyllumazine obtained by:
    i. selecting a first composition with a known concentration of 3,6,7-trimethyllumazine;
    ii. selecting at least one further composition with a known concentration of 3,6,7-trimethyllumazine;
    iii. 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 5 to 3000 mg/kg; and

wherein the preventing, ameliorating or treating comprises inhibiting COX-2 expression.

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.

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

a. 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. comprises synthetic 3,6,7-trimethyllumazine and/or 3,6,7-trimethyllumazine isolated from honey;
c. comprises a 3,6,7-trimethyllumazine concentration of greater than 5 mg/kg; or
d. has a standardised concentration of 3,6,7-trimethyllumazine obtained by:
    i. selecting a first composition with a known concentration of 3,6,7-trimethyllumazine;
    ii. selecting at least one further composition with a known concentration of 3,6,7-trimethyllumazine;
    iii. 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 5 to 3000 mg/kg; and

wherein the preventing, ameliorating or treating comprises inhibiting COX-2 expression.

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

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, wherein the composition:

    a. 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. comprises synthetic 3,6,7-trimethyllumazine and/or 3,6,7-trimethyllumazine isolated from honey;
    c. comprises a 3,6,7-trimethyllumazine concentration of greater than 5 mg/kg; or
    d. has a standardised concentration of 3,6,7-trimethyllumazine obtained by:

        i. selecting a first composition with a known concentration of 3,6,7-trimethyllumazine;
        ii. selecting at least one further composition with a known concentration of 3,6,7-trimethyllumazine;
        iii. 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 5 mg/kg to 3000 mg/kg; and

    wherein the preventing, ameliorating or treating comprises inhibiting COX-2 expression.

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, wherein the composition:

   a. 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. comprises synthetic 3,6,7-trimethyllumazine and/or 3,6,7-trimethyllumazine isolated from honey;
   c. comprises a 3,6,7-trimethyllumazine concentration of greater than 5 mg/kg; or
   d. has a standardised concentration of 3,6,7-trimethyllumazine obtained by:
      i. selecting a first composition with a known concentration of 3,6,7-trimethyllumazine;
      ii. selecting at least one further composition with a known concentration of 3,6,7-trimethyllumazine;
      iii. 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 5 to 3000 mg/kg; and

   wherein the preventing, ameliorating or treating comprises inhibiting COX-2 expression.

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, wherein the composition:

    a. 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. comprises synthetic 3,6,7-trimethyllumazine and/or 3,6,7-trimethyllumazine isolated from honey;
    c. comprises a 3,6,7-trimethyllumazine concentration of greater than 5 mg/kg; or
    d. has a standardised concentration of 3,6,7-trimethyllumazine obtained by:
        i. selecting a first composition with a known concentration of 3,6,7-trimethyllumazine;
        ii. selecting at least one further composition with a known concentration of 3,6,7-trimethyllumazine;
        iii. 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 5 to 3000 mg/kg; and

    wherein the preventing, ameliorating or treating comprises inhibiting COX-2 expression.

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:

5 mg/kg to 3000 mg/kg 3,6,7-trimethyllumazine;
5 mg/kg to 80 mg/kg 3,6,7-trimethyllumazine; or
5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, 50 mg/kg, 55 mg/kg, 60 mg/kg, 70 mg/kg or 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 5 mg/kg to 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 5 mg/kg to 3000 mg/kg.

17. 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 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 5 mg/kg,

optionally wherein the composition comprises honey.