(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) **World Intellectual Property Organization**
International Bureau

(43) **International Publication Date**
02 July 2020 (02.07.2020)

(10) **International Publication Number**
WO 2020/136221 A1

(51) **International Patent Classification:**
A61K 31/222 (2006.01) *A61K 36/63* (2006.01)
A61P 27/02 (2006.01)

(21) **International Application Number:**
PCT/EP2019/087044

(22) **International Filing Date:**
26 December 2019 (26.12.2019)

(25) **Filing Language:** English

(26) **Publication Language:** English

(30) **Priority Data:**
P201831296 28 December 2018 (28.12.2018) ES

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

WO 2020/136221 A1

(54) **Title:** USE OF SECOIRIDOIDS FOR THE TREATMENT OF OPTIC NEURITIS

(57) **Abstract:** The present invention relates to the use of the secoiridois oleacein and oleocanthal to prevent or treat neuropathies that conduct to optic nerve injury such as optic neuritis. Also, the present invention relates to pharmaceutical composition or nutraceutical composition comprising said secoiridois.

Use of secoiridoids for the treatment of optic neuritis

The present invention relates to the use of the secoiridoids, such as oleacein and oleocanthal, to prevent or treat neuropathies that conduct to optic nerve injury, such as optic neuritis (hereafter ON). The present invention also relates to pharmaceutical or nutraceutical compositions that contain said secoiridoids. Therefore, the present invention belongs to the technical field of medicine as well as to the food industry.

STATE OF ART

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Optic neuritis (ON) is demyelinating inflammation that damages the optic nerve, a bundle of nerve fibers that transmits visual information from your eye to your brain. Pathologic changes involving various retinal structures may precede this occurrence. ON is a common neuro-ophthalmologic inflammatory disease that results in persistent vision impairment and usually affects one eye. Symptoms might include: pain, vision loss in one eye, visual field loss, loss of color vision, flashing lights (Wu GF et al, Curr Immunol Rev. 2015;11:85-92; Beck RW et al, Am J Ophthalmol. 2004;137:77-83)

Ocular inflammation usually has an infectious or autoimmune etiology. ON with unilateral involvement is most commonly associated with multiple sclerosis (MS). In fact, at least 50% of MS patients developed ON, although not all ON patients develop MS. Atypical ON may, however, be associated with neuromyelitis optica spectrum disorders (with presence of anti-aquaporin-4 or anti-myelin oligodendrocyte glycoprotein, MOG, antibodies), acute disseminated encephalomyopathy and other autoimmune conditions such as sarcoidosis, systemic lupus erythematosus, Sjögren’s syndrome or Behçet’s disease. MOG has been shown to be present abundantly inside the optic nerve, and inflammatory cells presumably react to the MOG antigen of the optic nerve to cause tissue damage. Thus, MOG antigen has a high possibility of causing optic neuritis.

Methylprednisolone pulse therapy has been the mainstay of treatment for the acute phase of ON. Corticosteroids accelerate the recovery of vision; however, they do not improve the visual prognosis. Numerous undesirable side effects are associated with corticosteroids. One study even indicates that methylprednisolone accelerates neuron

apoptosis in the central nervous system. With the serious side effects of corticosteroids and their lack of neuroprotective effect, patients with ON urgently need a novel medication with anti-inflammatory properties, immune regulation, and neuroprotective effects.

5

The present invention relates to the search for new treatments for MS-related alterations, in particular, preferably optic neuritis and describes a new pharmacological application of the oleacein and oleocanthal as agents that markedly reduced the clinical and the immune/oxidative hallmarks of the experimental optic neuritis (EON).
10 Electroretinogram and visual-evoked potential studies have demonstrated that visual function is impaired in MS patients and in animals with experimental autoimmune encephalomyelitis (EAE), the animal model which mimicks many of the features of MS. Furthermore, since pathophysiological changes that occur in the EAE spinal cord also occur in optic nerve of EAE mice; the EAE animal model is considered an ideal model
15 for studying ON (Shields et al. Brain Res. 1998;784:299-304). Similar to MS, mice with EAE often develop ON. We used the well-established mouse model produced by immunization of female C57BL/6 mice with a peptide fragment of MOG, $MOG_{35–55}$. All C57BL/6 mice with clinical signs of EAE have also histologic evidence of EON. (Kuerten S et al., Ann Anat 2008, 190:1-15; Chaudhary P. et al., J Neuroimmunol.
20 2011; 233:90–96).

DESCRIPTION OF THE INVENTION

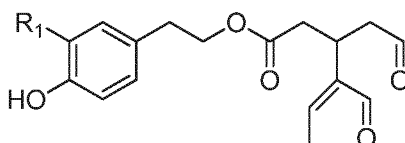
In the present invention, the inventors have found that secoiridoids, such as oleacein
25 and oleocanthal, have protective effects on the integrity and function of the blood-brain barrier (BBB) barrier as well as on the oxidative and immune-inflammatory events related to optic neuritis.

Secoiridoids are monoterpenoids, derived from iridoids in plants, based on the 7,8-
30 seco-cyclopenta[c]-pyranoid skeleton. Most of secoiridoids and iridoids have been isolated from plants and approximately 600 different structures are known. Almost all the secoiridoids are glycosides. This group of phytochemicals occur wide-spread in nature, and exhibit a wide range of biological and pharmacological activities, including antibacterial, anticancer, anticoagulant, antifungal, antioxidative, antiprotozoal and

hepatoprotective activities (Dinda B. et al., Chem Pharm Bull (Tokyo). 2009 Aug;57(8):765-96)

These products are natural substances that can be isolated from olives, these compounds could be used as a supplement to the diet or in nutraceutical preparations.

Thus, a first aspect of the present invention relates to a compound of formula (I):



(I)

its pharmaceutically acceptable salts, tautomers and/or solvates thereof
wherein R_1 is –OH or H,
for the treatment and/or prevention of optic neuritis.

The term "pharmaceutically acceptable salts or solvates thereof" relates to salts or solvates which, on being administered to the recipient, are capable of providing a compound such as that described herein. The preparation of salts and derivatives can be carried out by methods known in the state of the art. Preferably, "pharmaceutically acceptable" relates to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic reaction or a similar unfavourable reaction, such as gastric upset, dizziness and similar side effects, when administered to a human. Preferably, the term "pharmaceutically acceptable" means approved by a regulatory agency of a federal or state government or collected in the US Pharmacopoeia or other generally recognised pharmacopeia for use in animals and, more particularly, in humans.

The compounds used in the invention may be in crystalline form, either as free compounds or as solvates (e.g.: hydrates), and it is understood that both forms fall within the scope of the present invention. Solvation methods are generally known in the state of the art. Suitable solvates are pharmaceutically acceptable solvates. In a particular embodiment, the solvate is a hydrate.

"Tautomers" are understood to be the two isomers that differ only in the position of a functional group because between the two forms there is a chemical balance in which a migration of a group or atom occurs.

5 Unless otherwise stated, the compounds used in the invention are intended to include compounds that differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the substitution of a hydrogen atom for a deuterium atom or a tritium atom, or the substitution of a carbon atom for a carbon atom enriched in ^{13}C or ^{14}C or a nitrogen
10 atom enriched in ^{15}N fall within the scope of this invention.

In a particular embodiment, the compound of formula (I) is oleacein:

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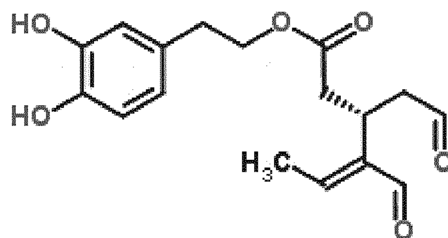
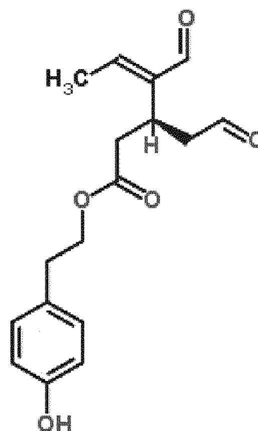


20 Oleacein

In other particular embodiment, the compound of formula (I) is oleocanthal:

25



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Oleocanthal

In a particular embodiment, the administered dose of the compound of formula (I) its
35 pharmaceutically acceptable salts, tautomers and/or solvates thereof (hereinafter, the

compounds of the present invention) varies between 5 mg/day and 20 mg/day, more particularly 10 mg per kg per day.

The compounds of the present invention can be administered by any suitable administration route, for example: oral, parenteral (subcutaneous, intraperitoneal, intravenous, intramuscular, etc.), intranasal inhaled etc.

The invention also relates to a composition comprising the compounds of the present invention for use in the treatment and/or prevention of optic neuritis.

The composition could be a pharmaceutical composition or a nutraceutical composition.

The term nutraceutical composition as used herein include food product, foodstuff, dietary supplement, nutritional supplement or a supplement composition for a food product or a foodstuff.

In a particular embodiment, the composition is a pharmaceutical composition which comprises pharmaceutically acceptable excipients, adjuvants and/or vehicles.

The term "excipients, adjuvants and/or carriers" relates to molecular entities or substances through which the active ingredient is administered. Such pharmaceutical excipients, adjuvants or carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and similar oils, excipients, disintegrating agents, humectants or dilutes. Suitable pharmaceutical excipients and carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin.

The pharmaceutical compositions can be administered by any suitable administration route, for example: oral, parenteral (subcutaneous, intraperitoneal, intravenous, intramuscular, etc.), etc.

In a particular embodiment, said pharmaceutical compositions may be in a pharmaceutical form of oral administration, either solid or liquid. Illustrative examples of pharmaceutical forms of oral administration include tablets, capsules, granules,

solutions, suspensions, etc., and may contain conventional excipients such as binders, dilutes, disintegrating agents, lubricants, humectants, etc., and may be prepared by conventional methods. The pharmaceutical compositions may also be adapted for parenteral administration, in the form of, for example, solutions, suspensions or lyophilised, sterile products in the suitable dosage form; in this case, said pharmaceutical compositions will include suitable excipients, such as buffers, surfactants, etc. In any case, the excipients are chosen according to the pharmaceutical form of administration selected. A review of the different pharmaceutical forms of drug administration and their preparation can be found in the book "Treatise on Galenic Pharmacy " by C. Faulí i Trillo, 10th Edition, 1993, Luzán 5, S.A. de Ediciones, or any book of similar characteristics in each country.

The compounds of the present invention may be used together with other additional drugs to provide a combined therapy. Said additional drugs may form part of the same pharmaceutical composition or, alternatively, may be provided as a separate composition for simultaneous administration or not with the pharmaceutical composition comprising the compounds of the present invention.

For therapeutic use, the compounds of the present invention are in a pharmaceutically acceptable form or are substantially pure, that is, that it has a pharmaceutically acceptable level of purity excluding the normal pharmaceutical additives, such as diluents and carriers, and is free from any materials considered toxic at normal dosage levels. The purity levels for the active substance are particularly above 50%, more particularly above 70%, and still more particularly above 90%. In a particular embodiment, the levels of the compound with formula (I), or its salts or solvates, are above 95%.

As said before, the invention relates to a compound of formula (I), its pharmaceutically acceptable salts, tautomers and/or solvates thereof or compositions for the treatment and/or prevention of optic neuritis. Alternatively, the invention relates to method to treat and/or prevent optic neuritis comprising administering a compound or a composition of the invention to a subject in need thereof. Alternatively, the invention relates to the use of a compound or a composition of the invention for the preparation of a medicament for the prevention and/or treatment of optic neuritis.

"Treatment" is understood widely, referring to reducing the potential for a certain disease, reducing the occurrence of a certain disease, and/or a reduction in the severity of a certain disease particularly, to an extent that the subject no longer suffers discomfort and/or altered function due to it. "Treatment" refers to provide a therapeutic benefit or desired clinical result, which it is not necessarily a cure for a particular disease or disorder, but rather encompasses a result which most typically includes alleviation of the disease, elimination of the disease, reduction or alleviation of a symptom associated with the disease, prevention of a secondary disease resulting from the occurrence of a primary disease, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable of the disease.

"Prevention" intends to avoid the appearance of said disease. The prevention can be complete (e.g. the total absence of a disease). The prevention can also be partial, such that for example the occurrence of a disease in a subject is less than that which would have occurred without the administration of the compounds of the present invention. Prevention also refers to reduced susceptibility to a clinical condition.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skilled in the art to which this invention belongs. Methods and materials similar or equivalent to those described herein can be used in the practice of the present invention. Throughout the description and claims the word "comprise" and its variations are not intended to exclude other technical features, additives, components, or steps. Additional objects, advantages and features of the invention will become apparent to those skilled in the art upon examination of the description or may be learned by practice of the invention. The following examples and drawings are provided by way of illustration and are not intended to be limiting of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1. Oleacein (OLE) treatment diminishes specific auto-antibody production in EAE mice. Titers of MOG-specific IgG1 in serum samples at 1/60 dilution. C, healthy mice. C+OLE, healthy mice treated with OLE. Experimental optic neuritis (hereafter,

EON), induced mice. EON+OLE, induced-mice treated with OLE. Bar graphs represent the mean ± SD of 5-8 animals. ‡$p < 0.001$ vs control and *$p < 0.001$ vs EAE.

Fig. 2. Oleacein (OLE) treatment reduces inflammation in the optic nerve during EON. To examine whether OLE treatment prevents from inflammatory cell infiltration, optic nerves were isolated from mice 21-23 days postimmunization and were stained with H&E (hematoxylin & eosin). Histological analysis of optic nerve from control mice, C; control mice treated with OLE, C+OLE; induced mice, EON; and induced-mice treated with OLE, EON+OLE, showed inflammatory infiltration. Oleacein treatment ameliorated all these parameters.

Fig. 3. Oleacein (OLE) treatment reduces inflammation in EON. Expression in serum of inflammatory-related parameters were used as measures of protective responses: levels of tumor necrosis factor-α,TNFα (A), granulocyte-macrophage colony-stimulating factor, GM-CSF (B), galectin-3, Gal-3 (C) and interleukin-1β, IL-1β (D) in serum were used as measures of protective responses. C, healthy mice. C+OLE, healthy mice treated with OLE. EON, induced mice. EON+OLE, induced-mice treated with OLE. Bar graphs represent the mean ± SD of 5-8 animals. ‡$p < 0.001$ and ‡‡$p<0.01$ vs control and **$p < 0.01$ and ***$p < 0.05$ vs EAE.

Fig. 4. Oleacein (OLE) treatment reduces demyelination in the optic nerve during EON. To examine whether OLE treatment prevents demyelination, optic nerves were isolated from mice 21-23 days postimmunization and were stained with LFB. Histological analysis of optic nerve from control mice, C; control mice treated with OLE, C+OLE; induced mice, EON; and induced-mice treated with OLE, EON+OLE, showed demyelination. Oleacein treatment ameliorated all these parameters.

Fig. 5. Oleacein (OLE) treatment reduces oxidative stress in the optic nerve during EON. To examine whether OLE treatment prevents generation of ion superoxide, optic nerves were isolated from mice 21-23 days postimmunization and were stained with the oxidative fluorescent dye, dihydroethidium, DHE. (A) Representative images of DHE staining , and (B) bar graph showing red fluorescence quantitation of sections of optic nerve from control mice, C; control mice treated with OLE, C+OLE; induced mice, EON; and induced-mice treated with OLE, EON+OLE,

showed anion superoxide buildup. Oleacein treatment ameliorated all these parameters.

Fig. 6. Oleacein (OLE) treatment increases neuroprotection in EON. Expression levels of malondialdehyde, MDA (A), advanced oxidation protein products, AOPP (B), the ferric reducing/antioxidant power, FRAP (C), and the ROS scavenger sestrin-3 (D), in serum were used as measures of protective responses. C, healthy mice. C+OLE, healthy mice treated with OLE. EON, induced mice. EON+OLE, induced-mice treated with OLE.

Fig. 7. Oleacein (OLE) treatment protects blood-brain barrier disruption and diminishes molecular extravasation in EON mice. Endogenous extravasation of IgG was used as a measurement of blood-brain barrier disruption and plasma proteins extravasation in brain. A) Representative immunofluorescence photomicrographs. B) Fluorescence intensity quantification. C, healthy mice. C+OLE, healthy mice treated with OLE. EON, induced mice. EON+OLE, induced-mice treated with OLE. Bar graphs represent the mean ± SD of 5 animals. ‡$p < 0.001$ vs control and ***$p < 0.05$ vs EAE.

Fig. 8. Oleacein (OLE) inhibits responses of activated microglia, including production of reactive oxygen species, ROS (A); induction of inflammatory regulators (B); and synthesis and release of inflammatory cytokines (C).

BV-2 microglial cells were pretreated for 30 min with the indicated doses of OLE: (A) After 24 h of stimulation with 0.1 µg/ml of LPS, intracellular ROS production was evaluated by Flow cytometry analysis. Panel shows quantification expressed in arbitrary units (A.U.), (‡$p < 0.001$ vs control, **$p < 0.01$ and *$p<0.001$ vs stimuli without OLE; n= 3). (B) After 4 or 24 h of stimulation with 0.1 µg/ml of LPS, COX-2, iNOS, p-p65-NFκB and NLRP3 expression was identified in cell lysates by Western blot; (C) the presence of TNFα and IL-1β in the 24 h cell culture medium was quantified by commercial ELISA (‡$p < 0.001$ vs control, *$p < 0.001$ vs stimuli without OLE; n= 3).

Fig. 9. Oleocanthal inhibits responses of activated microglia, including induction of inflammatory regulators (A); and synthesis and release of inflammatory cytokines (B). BV-2 microglial cells were pretreated for 30 min with the indicated doses of oleocanthal: (A) After 4 or 24 h of stimulation with 0.1 µg/ml of LPS, COX-2, iNOS, p-p65-NFκB and NLRP3 expression was identified in cell lysates by Western blot; and

(B) the presence of TNFα and IL-1β in the 24 h cell culture medium was quantified by commercial ELISA (‡p < 0.001 vs control, *p < 0.001 vs stimuli without OLE; n= 3).

EXAMPLES

5

The invention will be exemplified but not necessarily limited by the following experiments.

In the experiments the inventors used fifteen animals per group. Experimental optic neuritis (EON) was induced in C57BL mice as described (Quinn TA et al. Front Neurol.
10 2011;2:50; Chaudhary P. et al., J Neuroimmunol. 2011; 233:90–96).

The immunization was carried out with 100 µg of a partial peptide of myelin/oligodendrocyte glycoprotein (MOG33-55) in complete Freund's adjuvant containing 4 mg of Mycobacterium tuberculosis H37Ra in 1 ml. The mice were immunized by subcutaneous injection of this emulsion on day 0. In addition, on day 0
15 and 2, were administered intraperitoneally 300 ng/200 µl of *Bordetella pertussis* toxin. The administration of 10 mg/kg of oleacein (OLE) acid was performed intraperitoneally once a day, beginning on the immunization day. Oleacein were isolated from an olive oil extract prepared using the extraction method described by Karkoula E. (Karkoula E. et al., J Agric Food Chem. 2012;60:11696–11703). Briefly, pure oleacein, as well as
20 oleocanthal, were isolated from an olive oil extract prepared using the extraction method similar to that described below for sample preparation. Olive oil (5.0 g) was mixed with cyclohexane (20 mL) and acetonitrile (25 mL). The mixture was homogenized using a vortex mixer for 30 s and centrifuged at 4000 rpm for 5 min. A part of the acetonitrile phase (25 mL) was collected, mixed with 1.0 mL of a
25 syringaldehyde solution (0.5 mg/mL) in acetonitrile, and evaporated under reduced pressure using a rotary evaporator (Buchi, Flawil, Switzerland).

Mice were sacrificed 21-23 days following immunization with MOG. The induction was of MOG-IgG1 autoantibodies was quantified in serum of mice from the four experimental groups. As expected, the levels MOG-IgG1 autoantibodies were
30 significantly increased in serum from EON mice as compared to healthy-control group. In EON-mice treated with OLE the levels of autoantibodies were significantly lesser (Fig. 1).

To determine whether OLE could modulate the inflammatory response in EON, the inventors first assessed the occurrence of inflammatory cell infiltration in optic neuritis (ON) tissues collected at day 21-23 post-immunization (Fig. 2). Examination of hematoxylin & eosin (H&E) stained sections of optic nerve showed pronounced cell infiltrates in tissues from EON mice relatively to healthy-control mice. In contrast, the infiltrating cells in tissues from OLE-treated EON mice were notably reduced, being comparable to those observed in tissues of untreated-control mice. OLE treatment to healthy control mice had no noticeable effect.

The inventors also determine the circulating levels of the inflammatory proteins tumor necrosis factor-α (TNFα), galectin-3 (Gal-3) and granulocyte-macrophage colony-stimulating factor (GM-CSF) in serum samples. As expected, the expression of the inflammatory mediators was significantly increased in serum from EON mice as compared to healthy-control group. However, their overexpression was prevented in serum of mice with EAE treated with OLE: their levels were not significantly enhanced compared to those found in serum of both treated- or untreated-control mice. Further, high levels of active IL-1β, a mediator connecting innate and adaptive immunity, were detected in serum from EON mice and this increase was reduced by up to 80% in animals treated with OLE.

Next, it was assessed whether OLE protects optic nerve from demyelination. Optic nerve demyelination begins after onset of inflammation and can be detected by luxol fast blue (LFB) staining of myelin. Untreated EAE mice showed a notably increase in demyelination compared to control mice. Unstained regions indicating destruction of the myelin sheath was observed in optic nerve from EON mice. (Fig. 4). Optic nerves of OLE-treated EON mice showed increased LFB staining compared with optic nerves of untreated EAE mice, thus meaning a suppression in the demyelination in optic nerves.

It is well known that inflammation increase reactive oxygen species (ROS) levels leading to oxidative stress that mediates tissue damage. To investigate whether prophylactic administration of OLE to EON mice also resulted in reduced ROS accumulation, the inventors used as an initial gauge of ROS activity the redox-sensitive fluorescent probe DHE that detects superoxide anion (O2-.). Cryo-sections of optic nerve from mice of the different experimental groups were incubated with DHE stain and evaluated by fluorescence microscopy. The intensity of fluorescence signals was quantified using Image J software (NIH, Bethesda, MD, USA) (Fig. 5). In healthy-

control mice, DHE staining was basically undetectable in optic nerve. In contrast, optic nerve from EON mice showed an increased red fluorescence intensity throughout all tissue. However, OLE treatment inhibited EON-induced ROS production: ethidium red fluorescence was extensively attenuated, being similar to the observed in tissues from 5 healthy-control mice.

Given that superoxide anion has also been implicated on both lipid peroxidation and protein oxidation, the inventors assessed the serum levels of advanced oxidation protein products (AOPP) and malondialdehyde (MDA), as the end products of lipid 10 peroxidation (Fig. 6A and B). The EON group showed significantly increased serum levels of MDA and AOPP in comparison with the control group. Meanwhile, OLE treatment effectively prevented these increases. Likewise, the serum anti-oxidant capacity, evaluated by a ferric reducing/antioxidant power (FRAP) assay, as a marker of non-enzymatic antioxidant status, and Sestrin-3 levels, as a ROS disruptor, was 15 significantly reduced in EON mice in comparison with that of control group (Fig. 6C and D). Treatment with OLE showed a significant attenuation of this decrease compared to untreated EAE mice.

The optic nerve is considered a part of the CNS. During acute optic neuritis, blood-20 brain-barrier (BBB) breakdown, as well as brain-retinal-barrier (BRB) breakdown and activation of resident microglia in both the retina and optic nerve are pathological hallmarks of the disease. Consequently, the inventors characterized the EON-induced BBB damage in OLE treated and untreated mice by analysis of endogenous serum IgG leakage into brain using immunohistochemistry. The intensity of fluorescence signals 25 was quantified using Image J software (NIH, Bethesda, MD, USA). As shown in Fig. 7, brain from untreated EON-mice revealed a marked increase in IgG extravasation (c,g) compared to healthy-control animals (a,b). However, this enhanced permeability was prevented by the administration of OLE. The treatment with OLE did not modify BBB integrity in control animals.

30

Next, the inventors evaluate whether the protective effects found in vivo in OLE-treated EON mice involve direct actions on relevant CNS inflammatory cells, the inventors studied the effects of OLE on activated BV-2 microglia cells - to mimic responses observed on neuroinflammatory disorders (Fig. 8).

35

Given that ROS production may shape specific inflammatory programs the inventors investigated intracellular ROS accumulation. Flow cytometry analysis showed that intracellular ROS build up, which was significantly increased in LPS-treated BV-2 cells, was suppressed drastically in cells pretreated with different doses of OLE (Fig 8A).
5 To determine whether OLE was able to directly modulate inflammatory activity on microglia, BV-2 cells were pretreated with 1, 10 and 20 µM of OLE for 30 min and then stimulated with LPS (0.1 µg/ml) for 4 and 24 h. As shown in Fig. 8B, western blot analysis of LPS-activated BV2 cells, showed an increase expression of iNOS and COX-2 after 4 and 24 h in comparison to control unstimulated cells. However, cell
10 pretreatment with OLE led to a dose-dependent inhibition of LPS-induced iNOs and COX-2 production, whereas OLE per se did not affect their basal expression. The presence of OLE also reduced the ability of LPS to induce TNFα and mature IL-1β secretion in a dose-dependent manner (Fig. 8C). Likewise, the activation of the signaling mediators/mechanisms that influence these inflammatory responses such as
15 phosphorylation of p65-NFκB and overexpression of NLRP3 were also reduced (Fig. 8B).

This invention also relates to methods of using the natural compounds, oleacein and oleocanthal, to inhibit responses of activated microglia.

20

Finally, the inventors also evaluate the protective effects of other natural sercoiridoi: oleocanthal on relevant CNS inflammatory cells. The inventors studied the effects of oleocanthal on activated BV-2 microglia cells - to mimic responses observed on neuroinflammatory disorders (Fig. 9). Oleocanthal was isolated from an olive oil extract
25 prepared using the extraction method described by Karkoula E. (Karkoula E. et al., J Agric Food Chem. 2012;60:11696–11703) as described before.

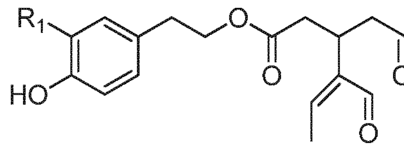
To determine whether oleocanthal was able to directly modulate inflammatory activity on microglia, BV-2 cells were pretreated with 1, 10 and 20 µM of oleocanthal for 30 min
30 and then stimulated with LPS (0.1 µg/ml) for 4 and 24 h. As shown in Fig. 9A, western blot analysis of LPS-activated BV2 cells, showed an increase expression of iNOS and COX-2 after 4 and 24 h in comparison to control unstimulated cells. However, cell pretreatment with oleocanthal led to a dose-dependent inhibition of LPS-induced iNOS and COX-2 production, whereas oleocanthal per se did not affect their basal
35 expression. The presence of oleocanthal also reduced the ability of LPS to induce

TNFα and mature IL-1β secretion in a dose-dependent manner (Fig. 9B). Likewise, the activation of the signaling mediators/mechanisms that influence these inflammatory responses such as phosphorylation of p65-NFκB and overexpression of NLRP3 were also reduced (Fig. 9A).

5

CLAIMS

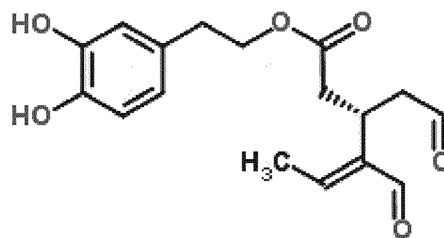
1. Compound of formula (I):



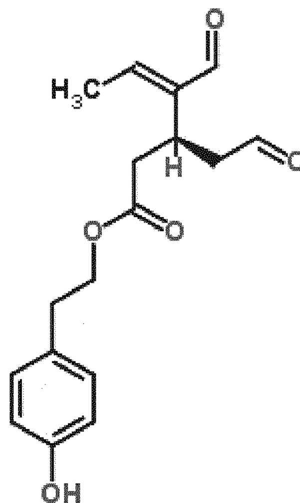
(I)

its pharmaceutically acceptable salts, tautomers and/or solvates,
wherein R_1 is –OH or H
for use in the treatment and/or prevention of optic neuritis.

2. Compound for use according to claim 1, wherein the compound of formula (I) is oleacein:



3. Compound for use according to claim 1, wherein the compound of formula (I) is oleocanthal:



4. Compound for use according to any of claims 1-3, wherein the compound is administered in oral, parenteral or intranasal route.

5. Compound for use according to any of claims 1-4, wherein the compound is administered at a dose between 5 mg/day and 20mg/day.

6. A composition comprising the compound defined in any of claims 1-5, for use in the treatment and/or prevention of optic neuritis.

7. The composition for use according to claim 6, wherein the composition is a pharmaceutical composition or nutraceutical composition.

8. The composition for use according to claim 6 or 7, further comprising other additional drugs to provide a combined therapy.

SERUM

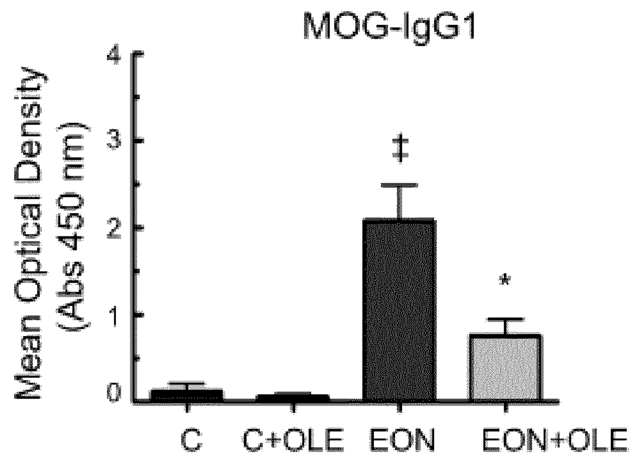


FIG. 1

OPTIC NERVE

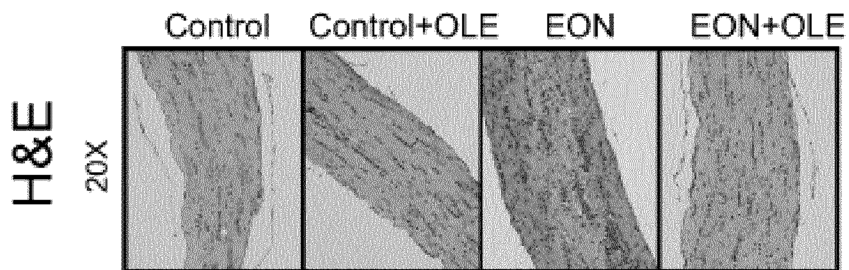


FIG. 2

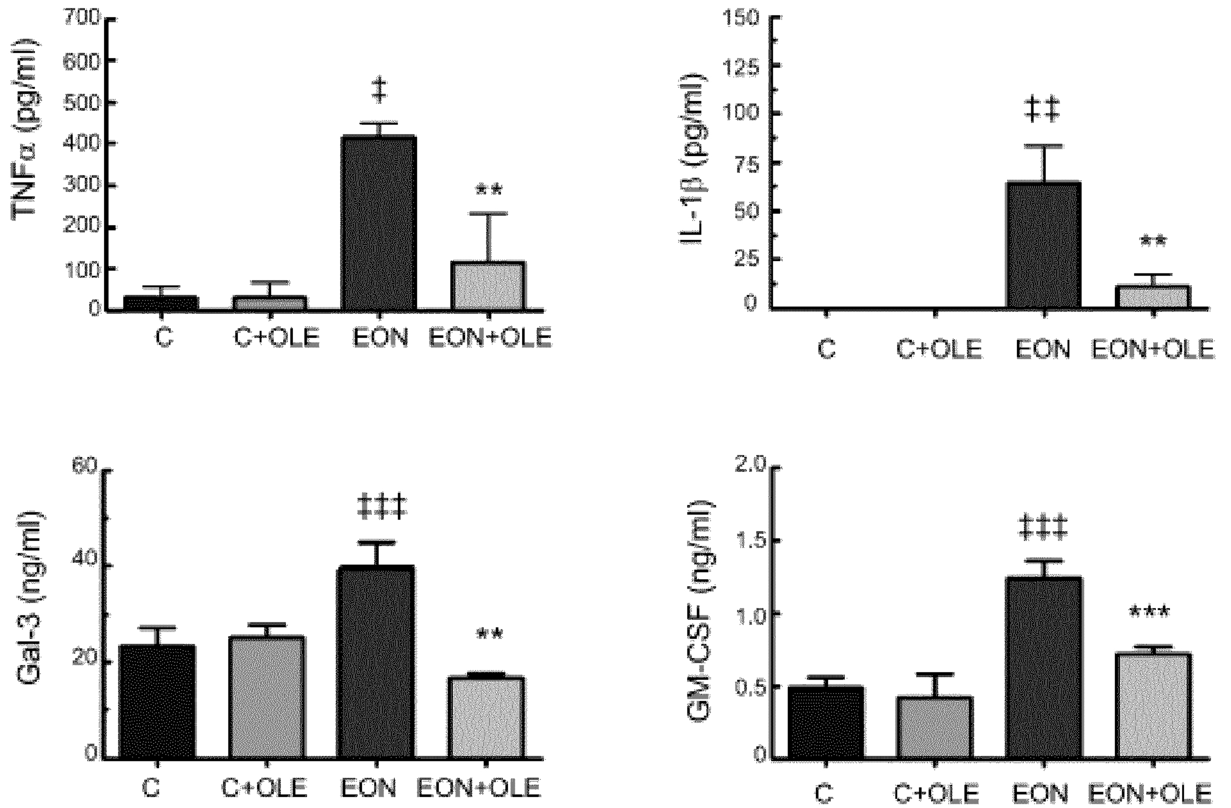


FIG. 3

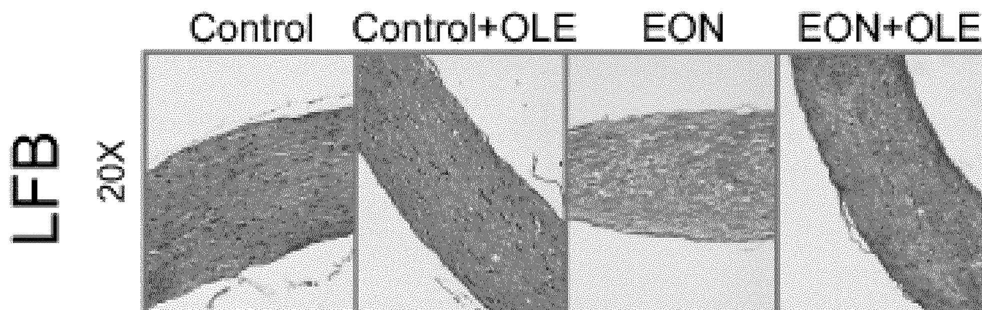
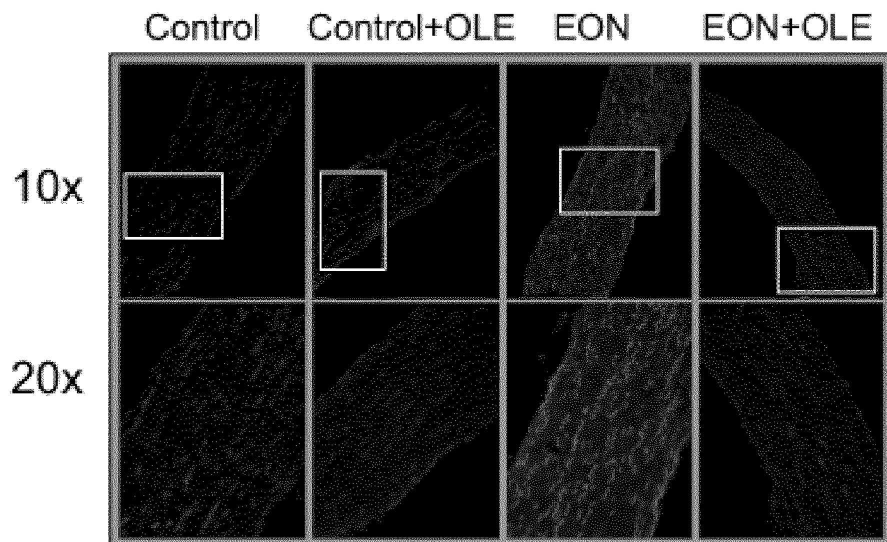


FIG. 4

OPTIC NERVE



B

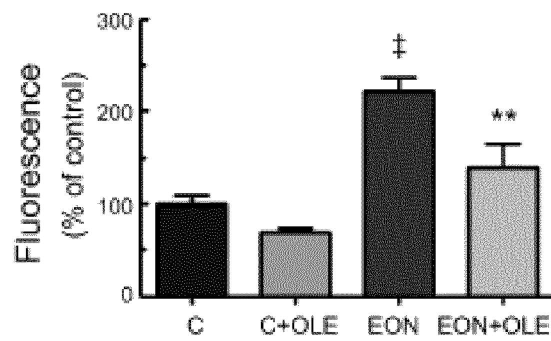


FIG. 5

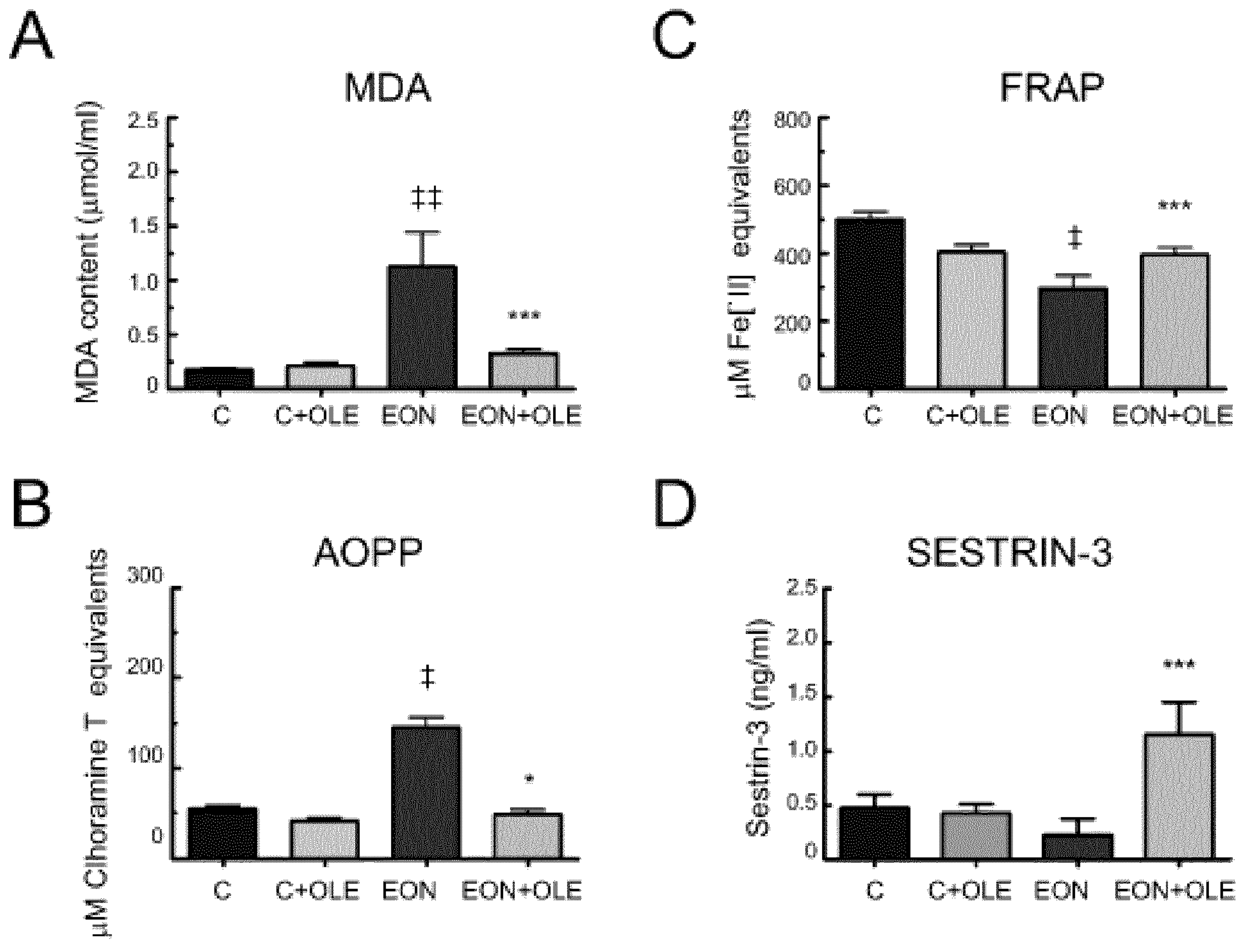
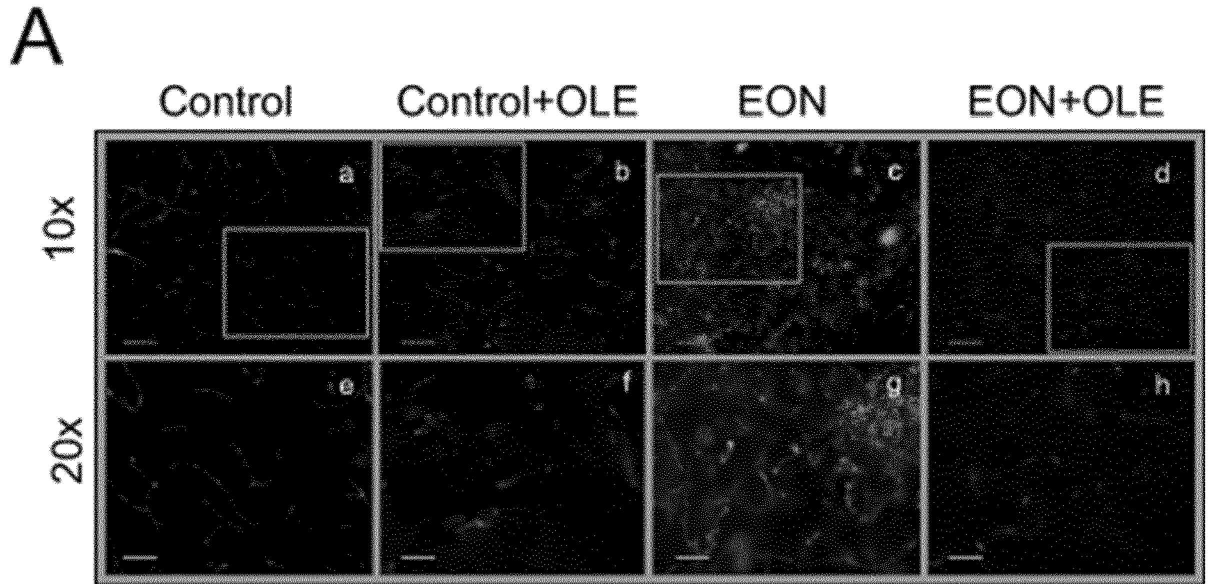


FIG. 6



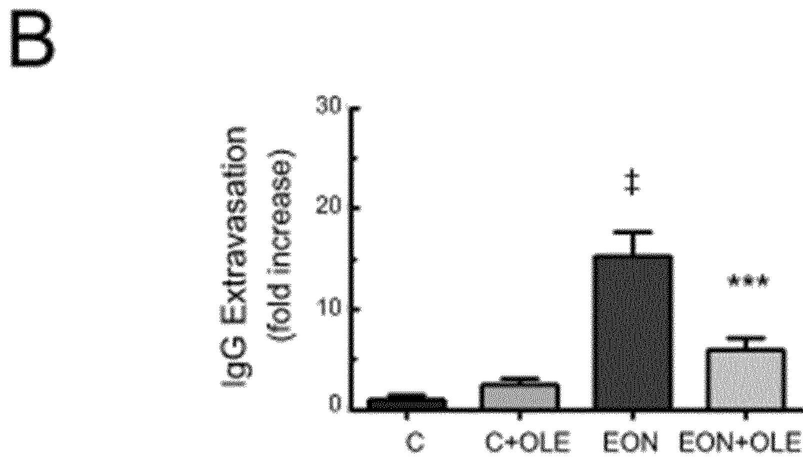
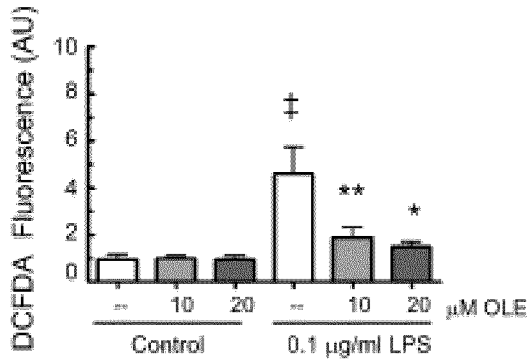
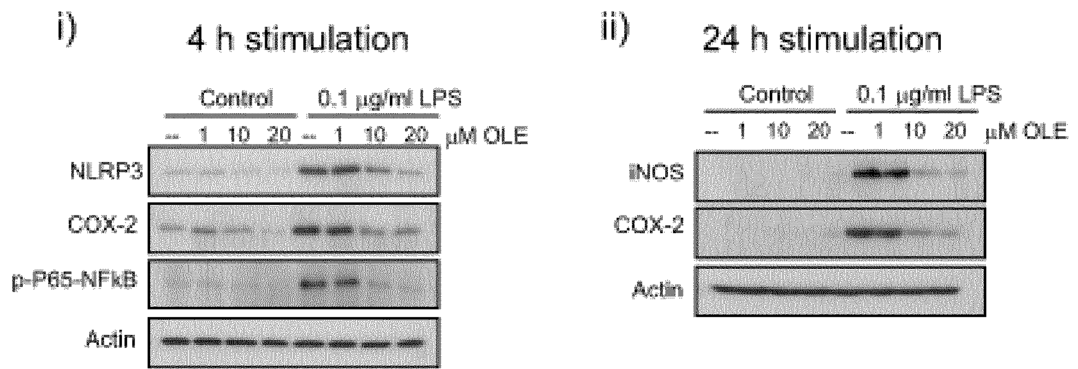


FIG. 7

A. ROS Production



B. Inflammatory Regulators



C: Inflammatory Cytokines

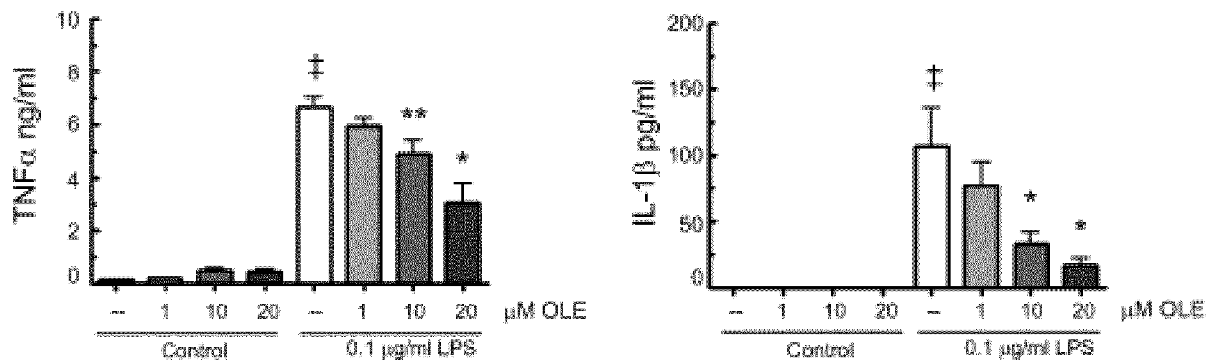


FIG. 8

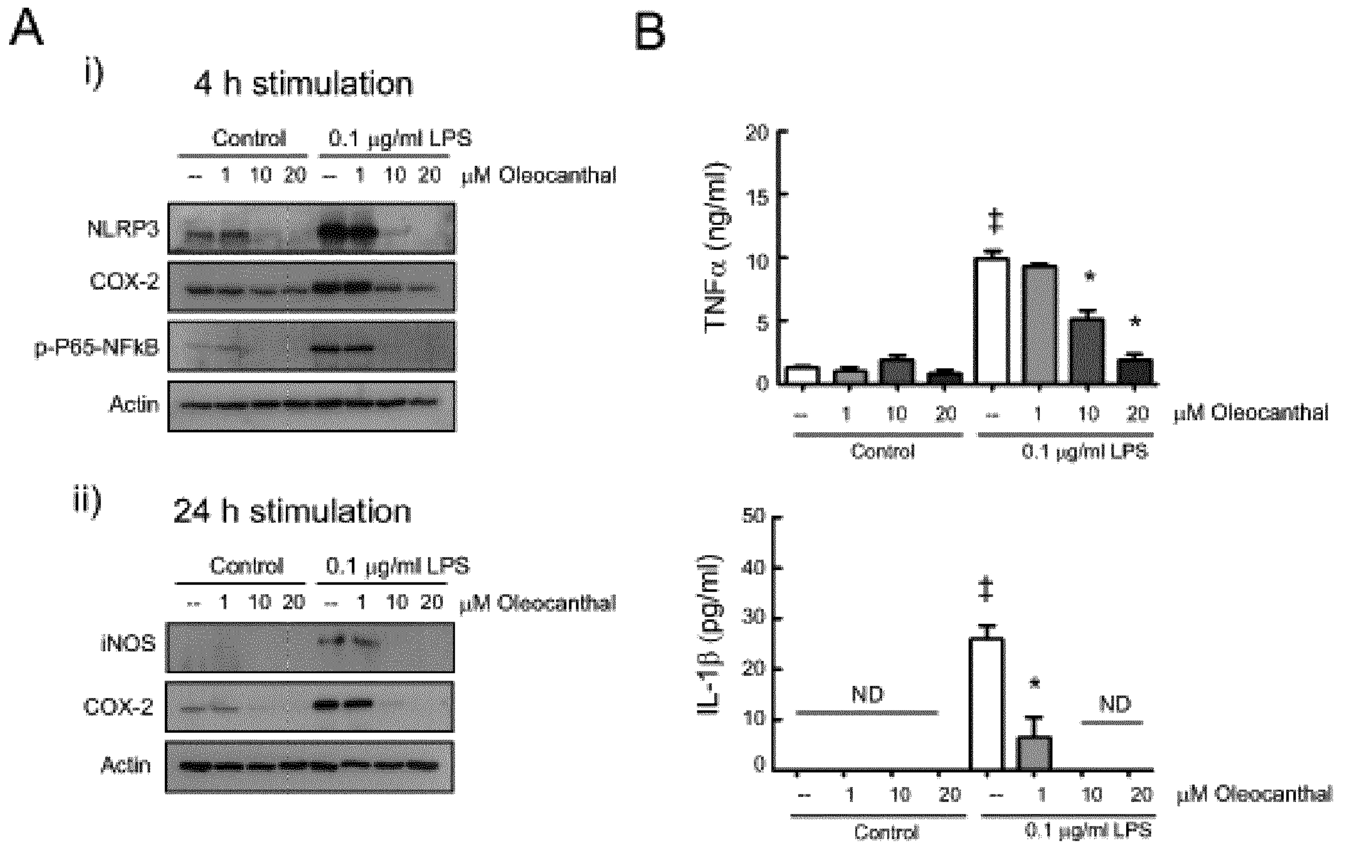


FIG. 9

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/087044

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/222 A61P27/02 A61K36/63
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

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

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

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	WO 2018/162769 A2 (UNIV SEVILLA) 13 September 2018 (2018-09-13) claims 1, 5, 6, 28	1-8
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A	WO 2004/028521 A2 (NOVARTIS AG [CH]; NOVARTIS PHARMA GMBH [AT] ET AL.) 8 April 2004 (2004-04-08) claims 1-3	1-8

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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

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

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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 March 2020	03/04/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Uryga-Polowy, V

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/087044

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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A	WO 2018/114557 A1 (NOVALIQ GMBH [DE]) 28 June 2018 (2018-06-28) claims 1-3 -----	1-8
A	WO 2008/001240 A2 (KIMBERLY CLARK CO [US]; FELDKAMP JOSEPH R [US] ET AL.) 3 January 2008 (2008-01-03) claim 1 page 22, line 32 - page 23, paragraph 60 -----	1-8
A	US 2009/082738 A1 (VAD VIJAY B [US]) 26 March 2009 (2009-03-26) claims 12, 15 -----	1-8

1

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International application No
PCT/EP2019/087044

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Information on patent family members

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PCT/EP2019/087044

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