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- (54) **Title:** METHOD OF TREATMENT OF CENTRAL NEUROPATHIC PAIN

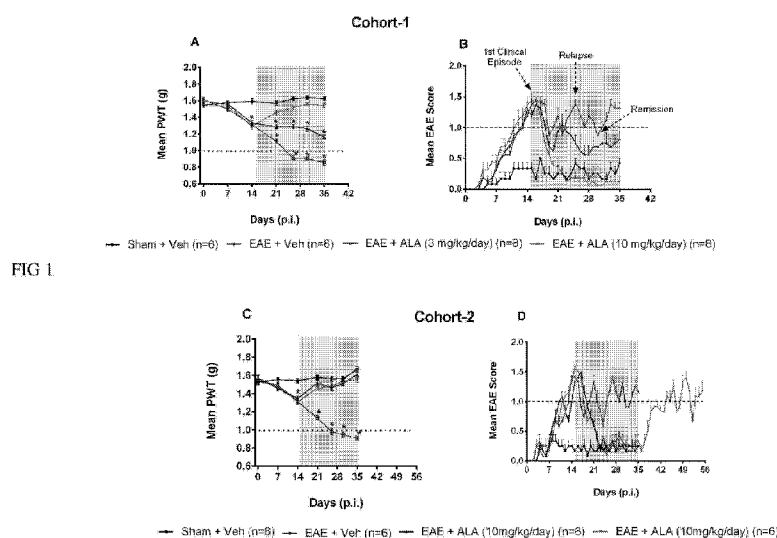


FIG 1

FIG 1 cont'd

- (57) **Abstract:** Provided is a method treating or preventing central neuropathic pain in a subject, which includes administering a 1,2-dithiolane carboxylic acid compound, in particular R-(+)-α-lipoic acid, or a pharmaceutically effective salt or ester thereof, to said subject. Also provided is said compound, or a pharmaceutically effective salt or ester thereof, for use in the treatment or prevention of central neuropathic pain in a subject.

METHOD OF TREATMENT OF CENTRAL NEUROPATHIC PAIN

FIELD OF THE INVENTION

[0001] The invention relates to the field of medical treatment. More particularly, this invention relates to the treatment of central neuropathic pain. Even more particularly, this invention relates to the treatment of central neuropathic pain associated with multiple sclerosis.

BACKGROUND TO THE INVENTION

[0002] Any reference to background art herein is not to be construed as an admission that such art constitutes common general knowledge in Australia or elsewhere.

[0003] Neuropathic pain is caused by damage to the nervous system resulting in pain signals being sent to the brain. The pain is often severe and, for some, can be debilitating. Neuropathic pain can be described as 'pins and needles', shooting, stabbing or burning pains and is often observed as a hypersensitivity to stimuli which would otherwise not invoke pain sensations (allodynia).

[0004] The root cause of the damage or dysfunction can be within the peripheral nervous system, resulting in peripheral neuropathic pain (PNP), or the central nervous system (the brain and spinal cord), resulting in central neuropathic pain (CNP). Neuropathic pain is recognised as being very difficult to treat in an effective and predictable way from patient to patient. The tricyclic antidepressants (TCAs), serotonin-norepinephrine reuptake inhibitors (SNRIs), voltage-gated calcium channel $\alpha 2\text{-}\delta$ subunit ligands (such as gabapentin and pregabalin) and topical lignocaine are generally regarded as first line treatments. These treatments have many drawbacks in terms of side-effects and their efficacy, typically tested against peripheral neuropathy, is questionable or at least does not often provide satisfactory long term relief.

[0005] PNP may occur as a result of physical trauma; diabetes (diabetic neuropathy) or other metabolic conditions; viral infections; toxins; immune-related disorders; and cancers, amongst others. CNP may result from certain forms of stroke, spinal cord injuries and multiple sclerosis (MS). Currently, the volume of research into the neurobiology of PNP greatly outweighs that for CNP.

[0006] MS is an inflammatory demyelinating disease of the central nervous system (CNS) resulting in motor, sensory and cognitive impairment. It is the most common neurological disease in young adults affecting more than 2 million people globally. Pain is a common disabling symptom of MS with estimates of its prevalence varying in the range 29–86 %. The incidence of chronic pain in MS is not correlated with disease severity. Patients may experience nociceptive pain such as muscular cramps, leg spasms, headaches and migraine concurrently with neuropathic pain. The neuropathic pain is more persistent in nature and is one of the most commonly distressing symptoms experienced by patients even in the early stages of the disease. Neuropathic pain associated with MS is inadequately relieved or not relieved at all with conventional analgesics such as non-steroidal anti-inflammatory drugs or opioid analgesics such as morphine.

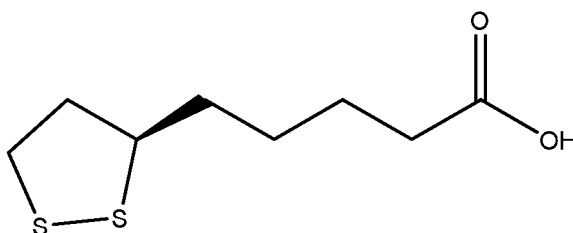
[0007] MS-induced neuropathic pain develops as a direct or indirect result of demyelinating lesions in the brain and spinal cord, and therefore is termed as CNP. Its clinical presentation can also be categorised as stimulus independent or dependent. The former includes persistent or paroxysmal pain, whereas evoked pain is characterised by hyperalgesia (exaggerated pain response to noxious stimuli) and allodynia.

[0008] There is a need to provide for further therapies which at least assist in reducing the severity of CNP, particularly when associated with MS.

SUMMARY OF INVENTION

[0009] The present invention is predicated, at least in part, on the finding that R(+)-alpha-lipoic acid ((*R*)-5-(1,2-dithiolan-3-yl)pentanoic acid referred to herein as R-ALA) has efficacy in the treatment of central neuropathic pain associated with MS.

[0010] α -Lipoic acid (alpha lipoic acid) is otherwise known as thiocctic acid or 6,8-dithiooctanoic acid and has a single chiral centre resulting in two enantiomers, (*R*)-(+)-lipoic acid (R-ALA) and (*S*)-(-)-lipoic acid (S-ALA), which together in the racemic mixture can be referred to as (*R/S*)-lipoic acid or Rac-ALA. α -Lipoic acid is commonly sold as a supplement based upon its supposed antioxidant properties. The structure of R-ALA is shown below:



R-ALA

[0011] U.S. 6,271,254 describes compositions comprising either R-ALA or S-ALA for use in combatting pain and inflammation and conferring certain cytoprotective effects. R-ALA is described as particularly demonstrating anti-inflammatory activity while S-ALA acts mainly as an analgesic. The only neuropathic syndromes mentioned are those of peripheral origin including polyneuropathy of diabetogenic, alcoholic, hepatic and uraemic origin. The compositions are not mentioned as being useful in treatment of CNP.

[0012] U.S. 7,858,655 recites the use of a combination of at least two substances selected from ALA, ambroxol and ACE inhibitors in the treatment of neurodegenerative diseases. The neuroprotective effect of the combination therapy is discussed as potentially being useful in treating MS due to the effect

on the cellular thiol/disulphide status. It is stated that any one of the substances alone is not considered sufficient to provide a therapeutic effect.

[0013] U.S. 8,722,013 discusses the use of R-ALA in treating cryptogenic neuropathy. Cryptogenic neuropathy is described as a disease characterised by clinical signs and symptoms of the sensory and autonomic peripheral nerves and so it is a condition relating only to PNP. The prior use of Rac-ALA in treating paresthesia associated with diabetic polyneuropathy is discussed in the context of the claim not being backed up by the clinical evidence. R-ALA itself is then described as being effective against cryptogenic neuropathy although it is notable that no experimental evidence of biomarkers or objective measurements are provided and rather the alleged efficacy is based purely, in each experiment, on statements of the relief from symptoms of a single patient.

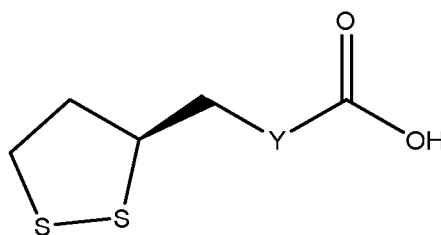
[0014] The person skilled in the art will be aware that there is a complete lack of predictability in terms of efficacious therapies between PNP and CNP. A recent review of clinical studies relating to the pharmacological management of MS-induced neuropathic pain (Khan and Smith, 2013) strongly indicates that drugs that are recommended for the treatment of peripheral neuropathic pain do not generally assist in alleviating MS-induced neuropathic pain, a CNP condition. In brief, the TCAs were found to be only equi-effective with TENS usage while duloxetine, a SNRI, was found to not reduce pain intensity beyond that level observed with a placebo. This document is considered to be incorporated herein in its entirety.

[0015] The underlying pathophysiology of MS-associated CNP is poorly understood but there are very significant apparent underlying differences between PNP and CNP such that not only is it currently impossible to pin point a single effective CNP pathway target for drug therapy but it is also pointless to compare drugs effective in one condition as potentially being efficacious in the other. Each individual therapy must be tested against each condition; otherwise no prediction of efficacy can be made.

[0016] A further complication in this landscape is that the traditional experimental autoimmune encephalomyelitis (EAE) rodent models of MS were developed to study the mechanisms that underlie symptoms of MS that were widely recognized decades ago, such as motor impairment, fatigue, and cognitive dysfunction. These models have provided invaluable insights on the pathobiology of MS clinical disease itself that have led to the development of new treatments for patients with MS. However, for investigation of MS-associated neuropathic pain, the commonly used EAE-mouse models need to be refined so that they are not confounded by hind limb paralysis. A new, less severe mouse model of MS-associated neuropathic pain had to be developed to enable the pathobiology of this CNP condition to be studied. This new model of relapsing-remitting EAE is ideal for investigation of the pathophysiology of MS-associated neuropathic pain as mice are able to move their hind paws away from the applied stimuli (e.g. graded von Frey filaments) so that mechanical hypersensitivity in the hind paws, a hallmark feature of CNP, can be observed and assessed. For traditional EAE mouse models of MS exhibiting hind limb paralysis, these mice are unable to move their hind paws in response to applied mechanical and/or thermal stimuli, and hence CNP cannot be assessed and also are unable to provide any indication of the successful, or otherwise, pharmacological management of CNP. Animal models employed for studying MS focused on monitoring clinical disease progression without consideration of the effects of the substance being tested on pain. Particularly, MS animal models would only observe the onset of neuropathy and then hind limb paralysis of the animal would obscure any signs of CNP progression or management. It is only with the use of an optimised RR-EAE mouse model, as described in the experimental section herein that does not result in hind limb paralysis, that observations on the effect on CNP of a substance can be objectively measured.

[0017] Therefore, given the lack of efficacy of a range of existing drugs in treating CNP, although they are known to be useful in treating PNP, and the dearth of information on the underlying causes of CNP coupled with the traditional use of animal models which completely obscured any potential success in CNP treatment it could not have been reliably predicted that R-ALA would have the efficacy, as shown herein, against CNP and, particularly, against CNP associated with MS.

[0018] In a first aspect, the invention provides a method of treatment or prevention of central neuropathic pain in a subject including the step of administering an effective amount of a compound of formula I, or a pharmaceutically effective salt or ester thereof;



Formula I

wherein Y is 1 to 6 carbon atoms;

to the subject, to thereby treat or prevent the central neuropathic pain in the subject.

[0019] In one embodiment, the method of this aspect further includes the step of selecting a subject exhibiting one or a plurality of symptoms of central neuropathic pain.

[0020] Suitably, the method further includes the step of administering to the subject one or a plurality of additional agents. Preferably, the one or plurality of additional agents are selected from the group consisting of a tricyclic antidepressant (TCA), a serotonin-norepinephrine reuptake inhibitor (SNRI), a voltage-gated calcium channel $\alpha 2\text{-}\delta$ subunit ligand, an opioid analgesic, an

antiepileptic, a sodium channel antagonist, an N-methyl-d-aspartate receptor antagonist, topical capsaicin, a cannabinoid, an adenosine A1 agonist, a nicotinic acetylcholine receptor agonist, an immunomodulatory agent, an angiotensin II (AT2) receptor antagonist and any combination thereof.

[0021] In a second aspect, the invention provides a compound of formula I, or a pharmaceutically effective salt or ester thereof, for use in the treatment or prevention of central neuropathic pain in a subject.

[0022] With respect to the above aspects, Y is suitably selected from 2, 3 or 4 carbon atoms. Preferably, the compound of formula I is R-(+)- α -lipoic acid, or a pharmaceutically effective salt or ester thereof.

[0023] Suitably, for the first and second aspects, the central neuropathic pain is or comprises central neuropathic pain associated with multiple sclerosis. Preferably, the CNP associated with MS is or comprises CNP associated with relapsing-remitting MS (RR-MS).

[0024] In particular embodiments of the first and second aspects, the compound modulates the function and/or expression level of a marker selected from the group consisting of BDNF, TrkB and pERK in one or a plurality of cells, tissues or organs of the subject. Preferably, the one or plurality of cells, tissues or organs are located in the central nervous system of the subject.

[0025] In other embodiments of the first and second aspects, the compound modulates the function and/or level of CD3+ T-cells in one or a plurality of tissues or organs of the subject. Preferably, the one or plurality of tissues or organs are located in the central nervous system of the subject.

[0026] With respect to the aforementioned aspects, the subject is suitably a human.

[0027] The various features and embodiments of the present invention, referred to in individual sections above apply, as appropriate, to other sections,

mutatis mutandis. Consequently features specified in one section may be combined with features specified in other sections as appropriate.

[0028] Further features and advantages of the present invention will become apparent from the following detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] In order that the invention may be readily understood and put into practical effect, preferred embodiments will now be described by way of example with reference to the accompanying figures wherein:

[0030] FIG 1 is a series of graphical representations of hind paw hypersensitivity and clinical disease in an RR-EAE mouse model of MS-induced neuropathic pain showing it was progressively alleviated by once-daily administration of single s.c. bolus doses of R-ALA administered according to an intervention protocol at 15-35 d.p.i. (days post intervention);

[0031] FIG 2 shows immunohistochemical analysis of the extent of CD3+ T-cell infiltration into the dorsal horn of lumbar (L4-L6) spinal cord sections from RR-EAE mice administered R-ALA at 10 mg kg⁻¹day⁻¹ or vehicle, for 3-weeks (15-35 d.p.i.) relative to sham-mice administered vehicle by the same dosing schedule;

[0032] FIG 3A and B show immunohistochemical analysis of CD11b, BDNF, TrkB and pERK in the dorsal horn of lumbar (L4-L6) spinal cord sections from RR-EAE mice administered R-ALA at 10 mg kg⁻¹day⁻¹ or vehicle, for 3-weeks relative to the corresponding data for vehicle-treated sham-mice;

[0033] FIG 4 is a series of graphically represented western blot analyses of microglial activation (Iba-1), as well as expression levels of BDNF, TrkB, pERK and total ERK in lumbar (L4-L6) spinal cord of RR-EAE mice administered R-ALA at 10 mg kg⁻¹day⁻¹ or vehicle, for 3-weeks relative to the respective data for the lumbar spinal cord of vehicle-treated sham-mice;

[0034] FIG 5 shows expression levels of mRNA for BDNF and TrkB in lumbar (L4-L6) spinal cord of RR-EAE mice administered either ALA at 10 mg kg⁻¹day⁻¹ or vehicle for 3-weeks did not differ significantly ($F(2, 8) = 3.8$; BDNF and $F(2, 7) = 3.59$; $P > 0.05$) from the respective data for the lumbar spinal cord of vehicle-treated sham-mice;

[0035] FIG 6 is a series of images showing that, in the dorsal horn of lumbar (L4-L6) spinal cord of vehicle-treated RR-EAE mice, BDNF is co-localised predominantly with **(A)** CD3+ T-cells. It is also co-localized with a subset of **(B)** neurons (NeuN) and to a lesser extent with **(C)** microglia/macrophages (CD11b) and **(D)** minimally with astrocytes (GFAP). Scale bars represent 20 μ m;

[0036] FIG 7 is a series of images showing that, in the dorsal horn of lumbar (L4-L6) spinal cord of vehicle-treated RR-EAE mice, TrkB is co-localised predominantly with **(A)** BDNF and **(B)** CD3+ T-cells. It is also co-localized with a subset of **(C)** neurons (NeuN) and to a lesser extent with **(D)** microglia/macrophages (Iba-1) and **(E)** minimally with astrocytes (GFAP). Scale bars represent 20 μ m;

[0037] FIG 8 is a series of images showing that, in the dorsal horn of lumbar (L4-L6) spinal cord of vehicle-treated RR-EAE mice, pERK is co-localised predominantly with **(A)** CD3+ T-cells. It is also co-localized with a subset of **(B)** neurons (NeuN) and to a lesser extent with **(C)** microglia/macrophages (CD11b) and **(D)** a subset of astrocytes (GFAP). Scale bars represent 20 μ m;

[0038] FIG 9 is a series of images showing total BDNF (pro-, truncated- and mature-BDNF) expression by western blot in lumbar (L4-L6) spinal cord of RR-EAE mice administered ALA at 10 mg kg⁻¹day⁻¹ or vehicle, for 3-weeks relative to the respective data for the lumbar spinal cord of vehicle-treated sham-mice; and

[0039] FIG 10 is a series of images showing immunohistochemical (IHC) analysis of CD11b, BDNF, TrkB and pERK in the dorsal horn of lumbar (L4-L6) spinal cord sections from RR-EAE mice administered ALA at 10 mg kg⁻¹day⁻¹ or vehicle, for 3-weeks relative to the corresponding data for vehicle treated sham-mice. **(A)** A representative image of lumbar spinal cord showing the region (yellow dotted lines) of the dorsal horn targeted uniformly during all IHC analyses. **(B)** The extent of microglia/macrophage (CD11b) activation (~3 fold; $F(2, 33) = 45.57; P < 0.05$), as well as expression levels of **(C)** BDNF (~2.5 fold; $F(2, 33) = 15.84; P < 0.05$); **(D)** TrkB and **(E)** pERK was significantly increased in vehicle-treated RR-EAE mice *c.f.* the respective data for the lumbar spinal cord of vehicle-treated sham-mice. Scale bars: 200 μ m for panel A and 100 μ m for panels B-E.

DETAILED DESCRIPTION

DEFINITIONS

[0040] In this patent specification, the terms ‘comprises’, ‘comprising’, ‘includes’, ‘including’, or similar terms are intended to mean a non-exclusive inclusion, such that a method or composition that comprises a list of elements does not include those elements solely, but may well include other elements not listed.

[0041] As generally used herein, the terms “administering”, “administration” or “administered” describe the introduction of the compound or composition to a mammal such as by a particular route or vehicle. Routes of administration may include topical, parenteral and enteral which include oral, buccal, sub-lingual, nasal, anal, gastrointestinal, subcutaneous, intramuscular and intradermal routes of administration, although without limitation thereto.

[0042] By “treat”, “treatment” or “treating” is meant administration of the compound or composition to a subject to at least ameliorate, reduce or

suppress existing signs or symptoms of central neuropathic pain experienced by a mammal.

[0043] By “prevent”, “preventing” or “preventative” is meant prophylactically administering the compound or composition to a mammal who does not exhibit signs or symptoms of central neuropathic pain, but who is expected or anticipated to likely exhibit such signs or symptoms in the absence of prevention. Preventative treatment may at least lessen or partly ameliorate expected symptoms or signs or inhibit or delay the development or progression such symptoms.

[0044] As used herein, “effective amount” or “therapeutically effective amount” refers to the administration of an amount of the relevant active agent sufficient to prevent the occurrence of symptoms of the condition being treated, or to bring about a halt in the worsening of symptoms or to treat and alleviate or at least reduce the severity of the symptoms. The effective amount will vary in a manner which would be understood by a person of skill in the art with patient age, sex, weight etc. An appropriate dosage or dosage regime can be ascertained through routine trial.

[0045] As used herein, the terms “subject” or “individual” or “patient” may refer to any mammalian subject. Mammals may include, but are not restricted to, primates, livestock animals (e.g., sheep, cows, horses, donkeys, pigs), laboratory test animals (e.g., rabbits, mice, rats, guinea pigs, hamsters), companion animals (e.g., cats, dogs) and captive wild animals (e.g., foxes, deer, dingoes). A preferred subject is a human in need of treatment for a disease, disorder or condition as described herein. However, it will be understood that the aforementioned terms do not imply that symptoms are necessarily present.

[0046] The term “pharmaceutically acceptable salt”, as used herein, refers to salts which are toxicologically safe for systemic or localised administration

such as salts prepared from pharmaceutically acceptable non-toxic bases or acids including inorganic or organic bases and inorganic or organic acids. The pharmaceutically acceptable salts may be selected from the group including alkali and alkali earth, ammonium, aluminium, iron, amine, glucosamine, chloride, sulphate, sulphonate, bisulphate, nitrate, citrate, tartrate, bitartrate, phosphate, carbonate, bicarbonate, malate, maleate, napsylate, fumarate, succinate, acetate, benzoate, terephthalate, palmoate, piperazine, pectinate and S-methyl methionine salts and the like. In one embodiment, the preferred pharmaceutically acceptable salt of any compound for administration to a subject is selected from the group consisting of sodium, potassium, ammonium, magnesium and calcium salts.

[0047] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as would be commonly understood by those of ordinary skill in the art to which this invention belongs.

[0048] The term "*alkyl*" refers to a straight-chain or branched alkyl substituent containing from, for example, 1 to about 6 carbon atoms, preferably 1 to about 5 carbon atoms, more preferably 1 to about 4 carbon atoms, even more preferably from 1 to about 3 carbon atoms. Such ranges include within their scope 2 to about 6 carbon atoms, 2 to about 5 carbon atoms, 2 to about 4 carbon atoms, 2 to about 3 carbon atoms, 3 to about 6 carbon atoms, 3 to about 5 carbon atoms and 3 or 4 carbon atoms. Examples of such substituents may be selected from the group consisting of methyl, ethyl, propyl, isopropyl, *n*-butyl, *sec*-butyl, isobutyl, *tert*-butyl, pentyl, isoamyl, 2-methylbutyl, 3-methylbutyl, hexyl and the like. The number of carbons referred to relates to the carbon backbone only and does not include carbon atoms belonging to carbon chain branches or any substituents, for example the carbon atoms of an alkoxy substituent branching off the main carbon chain. Substituted alkyl includes alkyl substituted with one or more moieties selected from the group consisting of halo (*e.g.*, Cl, F, Br, and I); halogenated alkyl (*e.g.*, CF₃, 2-Br-ethyl, CH₂F,

CF₂H, CH₂Cl, CH₂CF₃, or CF₂CF₃); hydroxyl; amino; carboxylate; carboxamido; alkylamino; alkoxy; thio; sulfonyl, and sulfate.

[0049] The term "*ester*" refers to a 1 to 6 carbon ester moiety formed by reaction of the carboxyl group of a compound of formula I, or R-ALA in one embodiment, with an appropriate alcohol. The ester group formed may have 1 to 6, 1 to 5, 1 to 4, 1 to 3, 2 or 1 carbon atoms which may be in a straight chain or branched form and may be optionally substituted as described for substituted alkyl. In one embodiment, the ester formed may be selected from methyl ester, ethyl ester, propyl ester, isopropyl ester, *n*-butyl ester, *sec*-butyl ester, isobutyl ester, *tert*-butyl ester, pentyl ester, isoamyl ester, 2-methylbutyl ester, 3-methylbutyl ester, hexyl ester and the like. The ester group may alternatively be a phosphate or benzoate ester. Appropriate guidance on choice of ester formation is widely available to the person of skill in the art and such literature as "Prodrugs - from Serendipity to Rational Design"; K M. Huttunen et al; Pharmacological Reviews; September 2011, vol. 63, no. 3, pp. 750-771 and "Textbook of Drug Design and Discovery"; Kristian Stromgaard, Povl Krogsgaard-Larsen, Ulf Madsen; CRC Press, 7 Oct 2009, may be appropriate.

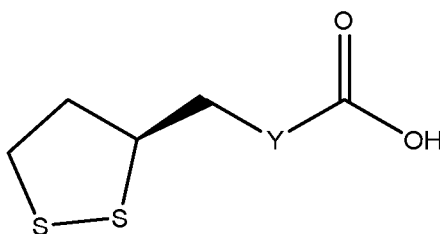
[0050] It has been unexpectedly found that the administration of R-ALA can attenuate the severity of CNP in a mammal. The findings discussed herein demonstrate that R-ALA modulates a number of key biomarkers, and in particular inflammatory biomarkers, related to CNP. It is understood that CNP may affect patients with MS even early in the disease course, is mostly persistent in nature and unaffected by the clinical disease course and has been found that the time course for development of CNP behaviours in the RR-EAE-rodent model used herein is discordant with that for development of EAE-induced motor impairments (Khan *et al.*, 2014a) thereby introducing a further order of complication in predicting the efficacy of drugs for PNP against CNP. EAE-induced motor deficits are predominantly correlated with pathobiologic changes in the ventral horn of the spinal cord and/or dorsal funicular white

matter (Wu *et al.*, 2008). By contrast, neuropathic pain behaviours in rodent models of CNP are associated with pathobiologic mechanisms in sensory neurons in the dorsal horn (laminae I-II) of the spinal cord, as has been found and presented herein (our data). Due, presumably, to at least some of these underlying pathobiological differences, recommended drug treatments for the relief of peripheral neuropathic pain conditions (Dworkin *et al.*, 2010) are often ineffective for alleviating MS-associated CNP (Khan *et al.*, 2014a).

[0051] The results shown and discussed herein indicate that R-ALA was extremely effective in attenuating hind paw hypersensitivity in an RR-EAE mouse model of MS induced CNP. The mechanical allodynia that developed in the bilateral hind paws of these RR-EAE mice was unaffected by the remitting-relapsing clinical disease progression again demonstrating that discordance. The experimental evidence herein also shows that (i) CD3+ T-cell infiltration into the lumbar spinal dorsal horn was markedly reduced relative to that for non-R-ALA mice exhibiting mechanical allodynia in their bilateral hind paws; (ii) BDNF and TrkB expression levels in vehicle-treated RR-EAE mice exhibiting robust mechanical allodynia are significantly elevated compared with vehicle-treated sham mice but this over expression can be inhibited by administration of R-ALA; and pERK expression levels in vehicle-treated RR-EAE mice exhibiting robust mechanical allodynia are significantly elevated compared with vehicle-treated sham mice but this over expression can be inhibited by administration of R-ALA.

[0052] According to a first aspect of the invention, there is provided a method of treatment or prevention of central neuropathic pain in a subject including the step of administering an effective amount of a compound of formula I, or a pharmaceutically effective salt or ester thereof;

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Formula I

wherein Y is 1 to 6 carbon atoms;

to the subject, to thereby treat or prevent the central neuropathic pain in the subject.

[0053] In one embodiment, the method of this aspect further includes the step of selecting a subject exhibiting one or more symptoms of central neuropathic pain. In this regard, the subject may be diagnosed as exhibiting symptoms of central neuropathic pain by any means known in the art. By way of example, central neuropathic pain may be diagnosed by identifying symptoms and neurological signs compatible with a lesion in the CNS, and excluding other possible causes of pain. Nonlimiting examples of neurological findings that may indicate a central neurological lesion in a subject may include a positive Babinski sign, accelerated tendon reflexes, and spasticity.

[0054] A second aspect of the invention provides for a compound of formula I, or a pharmaceutically effective salt or ester thereof, for use in the treatment or prevention of central neuropathic pain in a subject.

[0055] With respect to the second aspect, the compound of formula I, or a pharmaceutically effective salt or ester thereof, may be for use in the manufacture of a medicament for the treatment or prevention of central neuropathic pain in a subject.

[0056] The statements which follow apply equally to the first and second aspects of the invention.

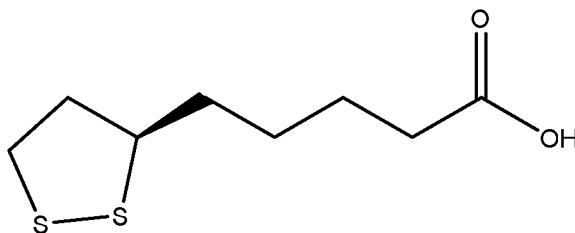
[0057] In one embodiment, Y is selected from 2, 3 or 4 carbon atoms.

[0058] In a preferred embodiment, the compound of formula I is R- α -lipoic acid (R-ALA), or a pharmaceutically effective salt or ester thereof.

[0059] In an alternative embodiment, the compound of formula I is a prodrug of R-ALA, or a pharmaceutically effective salt or ester thereof. It would be readily understood, that the term "prodrug" or "prodrugs" as generally used herein, refer to a compound or compounds which upon administration to a subject in need thereof undergo cleavage in vivo such as by enzymatic or chemical processes to release the parent drug from which the prodrug is derived.

[0060] In a particularly preferred embodiment, the compound of formula I, including R-ALA, is substantially enantiopure.

[0061] R-ALA may be otherwise known or referred to as (*R*)-(+)-lipoic or (*R*)-5-(1,2-dithiolan-3-yl)pentanoic acid and is shown below:



[0062] Suitably, the compound modulates the function and/or expression level of a marker selected from the group consisting of BDNF, TrkB and pERK in one or a plurality of cells, tissues or organs of the subject. Preferably, the one or plurality of cells, tissues or organs are located in the central nervous system, such as a lumbar spinal dorsal horn, of the subject.

[0063] The term "marker" or "biomarker" as used herein, refers to nucleic acid sequences or proteins or polypeptides or fragments thereof to be used for associating a disease state, such as CNP, with the marker. Levels of gene expression and protein levels are quantifiable and a variation in quantification or the mere presence or absence of the expression may serve as markers. With

respect to the present invention, the presence or altered expression (e.g., increased expression) of one or more markers described hereinafter may also be utilised to indicate whether a subject is suitable for treatment and/or responding to treatment with the compound of formula I.

[0064] As would be understood by the skilled person, the expression level of a marker, such as a gene or protein, may be deemed to be “*altered*” or “*modulated*” when the expression level is higher/increased or lower/decreased when compared to a control or reference sample or expression level, such as a threshold level. In this regard, a reference sample may be a biological sample taken from the subject prior to administration of the compound of the invention or one or a plurality of further subjects exhibiting symptoms of CNP.

[0065] As used herein a “gene” is a nucleic acid which is a structural, genetic unit of a genome that may include one or more amino acid-encoding nucleotide sequences and one or more non-coding nucleotide sequences inclusive of promoters and other 5’ untranslated sequences, introns, polyadenylation sequences and other 3’ untranslated sequences, although without limitation thereto. In most cellular organisms a gene is a nucleic acid that comprises double-stranded DNA.

[0066] By “protein” is meant an amino acid polymer. The amino acids may be natural or non-natural amino acids, D- or L- amino acids as are well understood in the art. As would be appreciated by the skilled person, the term “*protein*” also includes within its scope phosphorylated forms of a protein (*i.e.*, a phosphoprotein) and/or glycosylated forms of a protein (*i.e.* a glycoprotein).

[0067] As used herein, an expression level may be an absolute or relative amount of an expressed gene or gene product thereof inclusive of nucleic acids such as RNA, mRNA and cDNA and protein, including phosphoproteins. Accordingly, in particular embodiments, the expression level of a gene and/or a product thereof is compared to a control level of expression, such as the level of gene and/or protein expression of one or a plurality of “housekeeping” genes

in one or more cells, tissues or organs of the subject.

[0068] In one embodiment, the administration of the compound of formula I results in an inhibition or decrease in the function and/or expression level of the marker.

[0069] Determining, assessing, evaluating, assaying or measuring nucleic acids such as RNA, mRNA and cDNA may be performed by any technique known in the art. These may be techniques that include nucleic acid sequence amplification, nucleic acid hybridization, nucleotide sequencing, mass spectroscopy and combinations of any these.

[0070] Nucleic acid amplification techniques typically include repeated cycles of annealing one or more primers to a “template” nucleotide sequence under appropriate conditions and using a polymerase to synthesize a nucleotide sequence complementary to the target, thereby “amplifying” the target nucleotide sequence. Nucleic acid amplification techniques are well known to the skilled addressee, and include but are not limited to polymerase chain reaction (PCR); strand displacement amplification (SDA); rolling circle replication (RCR); nucleic acid sequence-based amplification (NASBA), Q- β replicase amplification; helicase-dependent amplification (HAD); loop-mediated isothermal amplification (LAMP); nicking enzyme amplification reaction (NEAR) and recombinase polymerase amplification (RPA), although without limitation thereto. As generally used herein, an “amplification product” refers to a nucleic acid product generated by a nucleic acid amplification technique.

[0071] PCR includes quantitative and semi-quantitative PCR, real-time PCR, allele-specific PCR, methylation-specific PCR, asymmetric PCR, nested PCR, multiplex PCR, touch-down PCR and other variations and modifications to “basic” PCR amplification.

[0072] Nucleic acid amplification techniques may be performed using DNA or RNA extracted, isolated or otherwise obtained from a cell or tissue source. In

other embodiments, nucleic acid amplification may be performed directly on appropriately treated cell or tissue samples.

[0073] Nucleic acid hybridization typically includes hybridizing a nucleotide sequence, typically in the form of a probe, to a target nucleotide sequence under appropriate conditions, whereby the hybridized probe-target nucleotide sequence is subsequently detected. Non-limiting examples include Northern blotting, slot-blotting, in situ hybridization and fluorescence resonance energy transfer (FRET) detection, although without limitation thereto. Nucleic acid hybridization may be performed using DNA or RNA extracted, isolated, amplified or otherwise obtained from a cell or tissue source or directly on appropriately treated cell or tissue samples.

[0074] It will also be appreciated that a combination of nucleic acid amplification and nucleic acid hybridization may be utilized.

[0075] Determining, assessing, evaluating, assaying or measuring protein levels may be performed by any technique known in the art that is capable of detecting cell- or tissue-expressed proteins whether on the cell surface or intracellularly expressed, or proteins that are isolated, extracted or otherwise obtained from the cell or tissue source. These techniques include antibody-based detection that uses one or more antibodies which bind the protein, electrophoresis, isoelectric focussing, protein sequencing, chromatographic techniques and mass spectroscopy and combinations of these, although without limitation thereto. Antibody-based detection may include flow cytometry using fluorescently-labelled antibodies that bind the protein, ELISA, immunoblotting, immunoprecipitation, in situ hybridization, immunohistochemistry and immunocytochemistry, although without limitation thereto. Suitable techniques may be adapted for high throughput and/or rapid analysis such as using protein arrays such as a TissueMicroArray™ (TMA), MSD MultiArrays™ and multiwell ELISA, although without limitation thereto.

[0076] In a further embodiment, the compound modulates the function and/or level of CD3+ T-cells in one or a plurality of tissues or organs of the subject. Preferably, the one or plurality of tissues or organs are located in the central nervous system, such as a lumbar spinal dorsal horn, of the subject.

[0077] As used herein, the terms "CD3+ T-cell" or "CD3+ T-lymphocyte" means a T-cell or lymphocyte cell which expresses CD3 cell surface antigens. It would be understood that the level of CD3+ T-cells may be deemed to be "*altered*" or "*modulated*" when that level is higher/increased or lower/decreased when compared to a control or reference sample or level, such as those previously described herein.

[0078] In one embodiment, the administration of the compound of formula I results in a decrease or prevents an increase in the level of CD3+ T-cells in the one or plurality of tissues or organs of the subject.

[0079] With respect to marker expression, such as BDNF, TrkB and pERK, or CD3+ T-cell levels, "higher", "enhanced", "increased" or "up regulated" as used herein, refer to an increase in and/or amount or level of one or more of said markers, including gene products thereof, or CD3+ T-cells in a biological sample when compared to a control or reference sample or value or a further biological sample from a subject. In some embodiments, the marker expression levels or CD3+ T-cell levels are increased if this level is more than about 0.5%, 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400% or at least about 500% greater than the level of expression of the corresponding marker or the CD3+ T-cell level in a control sample, reference value or further biological sample from a subject.

[0080] The terms, "lower", "reduced", "decreased" and "down regulated", in regard to marker expression, such as BDNF, TrkB and pERK, or CD3+ T-cell levels, refer to a reduction in and/or amount or level of one or more of the

markers, including gene products thereof, or CD3+ T-cells, in a biological sample when compared to a control or reference sample or value or further biological sample from a subject.

[0081] In some embodiments, the expression of a marker is decreased if its level of expression is less than about 95%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20% or 10%, or even less than about 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.01%, 0.001% or 0.0001% of the level of expression of the corresponding marker in a control sample, reference value or further biological sample from a subject.

[0082] In some embodiments, a CD3+ T-cell level is decreased if said level is less than about 95%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20% or 10%, or even less than about 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.01%, 0.001% or 0.0001% of the CD3+ T-cell level in a control sample, reference value or further biological sample from a subject.

[0083] In one embodiment, the compound of formula I, which may be R-ALA, may be administered as part of a dietary supplement or nutraceutical composition.

[0084] In this regard, R-ALA is generally widely available as a dietary supplement, typically being sold based on its supposed anti-oxidant activity. It is expected that in some instances these commercially available formulations may be appropriate for use in the method of the present invention. It may be preferred, however, to produce a composition better suited to pharmaceutical demands and/or for chronic usage.

[0085] Suitably, the subject is a mammal exhibiting symptoms of CNP. Preferably, the subject is a human exhibiting symptoms of CNP.

[0086] Non-limiting examples of CNP symptoms may include allodynia, including dynamic mechanical allodynia and cold allodynia, paresthesia and dysesthesia (e.g., burning, tingling, pins and needles, cold, and pressing

sensations). The pain associated with CNP may be described in terms such as burning, pricking, shooting, squeezing, and painful cold. Additionally, CNP can be spontaneous or stimulus-evoked and may be acute or chronic.

[0087] Central neuropathic pain may be associated with lesions due to infarction, a compressive tumor or abscess, such as in the thalamus or brainstem, Parkinson's disease, a spinal cord injury, such as due to injury or operation, MS, myelitis, syphilis, ischaemia, haemorrhage, arteriovenous malformation and/or syringomyelia, but without limitation thereto.

[0088] In a preferred embodiment of the invention, the CNP is CNP associated with MS. It would be appreciated by the skilled artisan that MS is generally a chronic inflammatory-demyelinating disease of the CNS typically characterised by motor impairment. In many patients, MS can also be associated with central neuropathic pain (CNP) which is unaffected by the clinical disease course. The occurrence of MS-associated pain has been estimated at about 29% of the patient population.

[0089] It would be appreciated that the form or type of MS may be any known in the art. In this regard, there are generally considered to be three different types of MS. Relapsing-remitting MS (RR-MS) is typically the most frequently observed form of MS and is characterized by clearly defined relapses of increased disease activity and worsening symptoms. These are followed by remissions in which the disease doesn't progress. Approximately 85% of patients are diagnosed with RR-MS at onset. Primary-progressive MS is generally diagnosed in about 10% of MS patients at onset. Progressive-relapsing MS is considered the rarest form of MS, representing about 5% of MS patients. In this type of MS, patients typically have clear relapses combined with a steady progression of the disease.

[0090] In one embodiment, the CNP is CNP associated with relapsing-remitting MS (RR-MS).

[0091] The compound of formula I and compositions containing same may be administered by any suitable route or dose form, (including modified release and/or slow release dose forms) and the person skilled in the art will readily be able to determine the most suitable route and dose for the condition to be treated. Dosage will be at the discretion of the attendant physician or veterinarian, and will depend on the nature and state of the disease, disorder or condition to be treated, the age and general state of health of the subject to be treated, the route of administration, and any previous treatment which may have been administered.

[0092] Any carrier or diluent, or other excipients, will depend on the route of administration or dose form, and again the person skilled in the art will readily be able to determine the most suitable formulation for each particular case.

[0093] Frequently used carriers or auxiliaries include but are not limited to magnesium carbonate, magnesium aluminium silicate, titanium dioxide, silicon dioxide, lactose, mannitol and other sugars, talc, milk protein, gelatin, starch, vitamins, cellulose and its derivatives, animal and vegetable oils, polyethylene glycols and solvents, such as sterile water, alcohols, glycerol and polyhydric alcohols. Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobials, anti-oxidants, chelating agents and inert gases. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like, as described, for instance, in Remington's Pharmaceutical Sciences, 20th ed. Williams & Wilkins (2000) and The British National Formulary 43rd ed. (British Medical Association and Royal Pharmaceutical Society of Great Britain, 2002; <http://bnf.rhn.net>), the contents of which are hereby incorporated by reference. The pH and exact concentration of the various components of the pharmaceutical composition are adjusted according to routine skills in the art. See Goodman and Gilman's The Pharmacological Basis for Therapeutics (7th ed., 1985).

[0094] Diluents may include one or more of microcrystalline cellulose, lactose, mannitol, calcium phosphate, calcium sulfate, kaolin, dry starch, powdered sugar, and the like. Binders may include one or more of povidone, starch, stearic acid, gums, hydroxypropylmethyl cellulose and the like. Disintegrants may include one or more of starch, croscarmellose sodium, crospovidone, sodium starch glycolate and the like. Solvents may include one or more of ethanol, methanol, isopropanol, chloroform, acetone, methylethyl ketone, methylene chloride, water and the like. Lubricants may include one or more of magnesium stearate, zinc stearate, calcium stearate, stearic acid, sodium stearyl fumarate, hydrogenated vegetable oil, glyceryl behenate and the like. A glidant may be one or more of colloidal silicon dioxide, talc or cornstarch and the like. Buffers may include phosphate buffers, borate buffers and carbonate buffers, although without limitation thereto. Fillers may include one or more gels inclusive of gelatin, starch and synthetic polymer gels, although without limitation thereto. Coatings may comprise one or more of film formers, solvents, plasticizers and the like. Suitable film formers may be one or more of hydroxypropyl methyl cellulose, methyl hydroxyethyl cellulose, ethyl cellulose, hydroxypropyl cellulose, povidone, sodium carboxymethyl cellulose, polyethylene glycol, acrylates and the like. Suitable solvents may be one or more of water, ethanol, methanol, isopropanol, chloroform, acetone, methylethyl ketone, methylene chloride and the like. Plasticizers may be one or more of propylene glycol, castor oil, glycerin, polyethylene glycol, polysorbates, and the like.

[0095] Reference is made to the Handbook of Excipients 6th Edition, Eds. Rowe, Sheskey & Quinn (Pharmaceutical Press), which provides non-limiting examples of excipients which may be useful according to the invention.

[0096] It will be appreciated that the choice of pharmaceutically acceptable carriers, diluents and/or excipients will, at least in part, be dependent upon the mode of administration of the formulation. By way of example only, the

composition may be in the form of a tablet, capsule, caplet, powder, an injectable liquid, a suppository, a slow release formulation, an osmotic pump formulation or any other form that is effective and safe for administration.

[0097] The pharmaceutical compositions are preferably prepared and administered in dosage units. Solid dosage units include tablets, capsules and suppositories. For treatment of a subject, depending on activity of the compound, manner of administration, nature and severity of the disorder, age and body weight of the subject, different daily doses can be used. Under certain circumstances, however, higher or lower daily doses may be appropriate. The administration of the daily dose can be carried out both by single administration in the form of an individual dose unit or else several smaller dose units and also by multiple administration of subdivided doses at specific intervals or may be given in an extended, depot or slow release format.

[0098] The pharmaceutical compositions according to the invention may be administered locally or, preferably, systemically in a therapeutically effective dose. Amounts effective for this use will, of course, depend on the severity of the disease and the weight and general state of the subject. Typically, dosages used in vitro may provide useful guidance in the amounts useful for in situ administration of the pharmaceutical composition, and animal models may be used to determine effective dosages for treatment of the cytotoxic side effects, if any. Formulations for oral use may be in the form of hard gelatin capsules, in which the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin. They may also be in the form of soft gelatin capsules, in which the active ingredient is mixed with water or an oil medium, such as peanut oil, liquid paraffin or olive oil.

[0099] Aqueous suspensions normally contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients may be suspending agents such as sodium carboxymethyl cellulose, methyl cellulose, hydroxypropylmethylcellulose, sodium alginate,

polyvinylpyrrolidone, xanthan gum, gum tragacanth and gum acacia, magnesium silicate; dispersing or wetting agents, which may be (a) a naturally occurring phosphatide such as lecithin; (b) a condensation product of an alkylene oxide with a fatty acid, for example, polyoxyethylene stearate; (c) a condensation product of ethylene oxide with a long chain aliphatic alcohol, for example, heptadecaethylenoxycetanol; (d) a condensation product of ethylene oxide with a partial ester derived from a fatty acid and hexitol such as polyoxyethylene sorbitol monooleate, or (e) a condensation product of ethylene oxide with a partial ester derived from fatty acids and hexitol anhydrides, for example polyoxyethylene sorbitan monooleate.

[00100] The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents such as those mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents which may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed, including synthetic mono-or diglycerides. In addition, fatty acids such as oleic acid may be used in the preparation of injectables.

[00101] Dosage levels of the compound of formula I will usually be of the order of about 10mg to about 3000mg per day, with a preferred dosage range between about 50mg to about 2500mg per day. The lower limit for both of these ranges (10mg to 3000mg and 50mg to 2500mg) may in one embodiment be selected from 50mg, 100mg, 200mg, 300mg, 400mg, 500mg, 600mg, 700mg, 800mg, 900mg or 1000mg. Similarly, the upper limit to be combined with any of these values or with either of the recited ranges may be selected from 2400mg,

2200mg, 2000mg, 1800mg, and 1600mg. A range of 100mg to 1000mg per day may be typical.

[00102] It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

[00103] In addition, some of the compounds of formula I may form solvates with water or common organic solvents. Such solvates are encompassed within the scope of the invention.

[00104] In one embodiment, the method of the present invention further includes the step of administering to the subject one or a plurality of additional agents. In this regard, the compounds of formula I may additionally be combined with the one or plurality of additional agents to provide an operative combination or co-treatment. It is intended to include any chemically compatible combination of pharmaceutically-active agents, as long as the combination does not negatively impact upon the activity of the compound of this invention.

[00105] To this end, the compound of the present invention and the additional agent may be administered together, i.e. in a single dose form, or may be administered separately, i.e. in a separate dose form. Thus, the method of the present invention may include the administration of a compound of the present invention as defined above and may further comprise administration of an additional agent for the prevention, alleviation or/and treatment of CNP. The pharmaceutical composition derived therefrom may comprise a single dose form or may comprise a separate dose form comprising a first composition comprising a compound of the present invention as defined above and a second composition comprising the additional agent.

[00106] In particular embodiments, the additional agent may be selected from the group consisting of a tricyclic antidepressant (TCA) (e.g., nortriptyline and amitriptyline), a serotonin-norepinephrine reuptake inhibitor (SNRI) (e.g., duloxetine and venlafaxine), a voltage-gated calcium channel $\alpha 2\text{-}\delta$ subunit ligand (e.g., gabapentin and pregabalin), an opioid analgesic (e.g. morphine, oxycodone, methadone, fentanyl, tramadol), an antiepileptic (e.g., carbamazepine, lamotrigine, oxcarbazepine, topiramate, valproic acid), a sodium channel antagonist (e.g., mexiletine, lignocaine, bupivacaine), an N-methyl-d-aspartate receptor antagonist (e.g. ketamine, memantine), topical capsaicin, a cannabinoid, an adenosine A1 agonist, a nicotinic acetylcholine receptor agonist, an immunomodulatory agent (e.g., thalidomide), an angiotensin II (AT2) receptor antagonist (e.g., losartan, candesartan, irbesartan, telmisartan, valsartan, fimasartan) and any combination thereof. The

[00107] Furthermore, the compounds of formula I may be combined with nonpharmacological approaches to the treatment of CNP, including, but not limited to, cognitive-behavioral therapy, hypnosis, and neurostimulation therapies.

[00108] The compounds of formula I may be utilized per se or in the form of a pharmaceutically acceptable ester, salt, solvate or prodrug. For example, the compound of formula I may be provided as a pharmaceutically acceptable salt. If used, a salt of the drug compound should be both pharmacologically and pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare the free active compound or pharmaceutically acceptable salts thereof and are not excluded from the scope of this invention. Such pharmacologically and pharmaceutically acceptable salts can be prepared by reaction of the drug with an organic or inorganic acid, using standard methods detailed in the literature.

[00109] Examples of pharmaceutically acceptable salts of the compounds useful according to the invention include acid addition salts. Salts of non-

pharmaceutically acceptable acids, however, may be useful, for example, in the preparation and purification of the compounds. Suitable acid addition salts according to the present invention include organic and inorganic acids. Preferred salts include those formed from hydrochloric, hydrobromic, sulfuric, phosphoric, citric, tartaric, lactic, pyruvic, acetic, succinic, fumaric, maleic, oxaloacetic, methanesulfonic, ethanesulfonic, p-toluenesulfonic, benzenesulfonic, and isethionic acids. Other useful acid addition salts include propionic acid, glycolic acid, oxalic acid, malic acid, malonic acid, benzoic acid, cinnamic acid, mandelic acid, salicylic acid, and the like. Particular examples of pharmaceutically acceptable salts include, but are not limited to, sulfates, pyrosulfates, bisulfates, sulfites, bisulfites, phosphates, monohydrogenphosphates, dihydrogenphosphates, metaphosphates, pyrophosphates, chlorides, bromides, iodides, acetates, propionates, decanoates, caprylates, acrylates, formates, isobutyrate, caproates, heptanoates, propiolates, oxalates, malonates, succinates, suberates, sebacates, fumarates, maleates, butyne-1,4-dioates, hexyne-1,6-dioates, benzoates, chlorobenzoates, methylbenzoates, dinitrobenzoates, hydroxybenzoates, methoxybenzoates, phthalates, sulfonates, xylenesulfonates, phenylacetates, phenylpropionates, phenylbutyrates, citrates, lactates, γ -hydroxybutyrates, glycolates, tartrates, methanesulfonates, propanesulfonates, naphthalene-1-sulfonates, naphthalene-2-sulfonates, and mandelates.

[00110] An acid addition salt may be reconverted to the free base by treatment with a suitable base. Preparation of basic salts of acid moieties which may be present on a compound or prodrug useful according to the present invention may be prepared in a similar manner using a pharmaceutically acceptable base, such as sodium hydroxide, potassium hydroxide, ammonium hydroxide, calcium hydroxide, triethylamine, or the like.

[00111] Esters of the compounds of formula I may be prepared through functionalization of the carboxyl group present on the compound. Prodrugs may

also be prepared using techniques known to those skilled in the art. Moreover, esters of compounds of the invention can be made by reaction with a carbonylating agent (*e.g.*, ethyl formate, acetic anhydride, methoxyacetyl chloride, benzoyl chloride, methyl isocyanate, ethyl chloroformate, methanesulfonyl chloride) and a suitable base (*e.g.*, 4-dimethylaminopyridine, pyridine, triethylamine, potassium carbonate) in a suitable organic solvent (*e.g.*, tetrahydrofuran, acetone, methanol, pyridine, N,N-dimethylformamide) at a temperature of 0 °C to 60 °C. Prodrugs are typically prepared by covalent attachment of a moiety, which results in a compound that is therapeutically inactive until modified by an individual's metabolic system. Examples of pharmaceutically acceptable solvates include, but are not limited to, compounds of formula I in combination with water, isopropanol, ethanol, methanol, DMSO, ethyl acetate, acetic acid, or ethanolamine.

[00112] In the case of solid compositions, it is understood that the compounds of formula I used in the methods of the invention may exist in different forms. For example, the compounds may exist in stable and metastable crystalline forms and isotropic and amorphous forms, all of which are intended to be within the scope of the present invention.

[00113] If a compound useful as an active agent according to the invention is a base, the desired salt may be prepared by any suitable method known to the art, including treatment of the free base with an inorganic acid, such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, or with an organic acid, such as acetic acid, maleic acid, succinic acid, mandelic acid, fumaric acid, malonic acid, pyruvic acid, oxalic acid, glycolic acid, salicylic acid, pyranosidyl acids such as glucuronic acid and galacturonic acid, alpha-hydroxy acids such as citric acid and tartaric acid, amino acids such as aspartic acid and glutamic acid, aromatic acids such as benzoic acid and cinnamic acid, sulfonic acids such as p-toluenesulfonic acid or ethanesulfonic acid, or the like.

[00114] If a compound described herein as an active agent is an acid, the desired salt may be prepared by any suitable method known to the art, including treatment of the free acid with an inorganic or organic base, such as an amine (primary, secondary or tertiary), an alkali metal or alkaline earth metal hydroxide or the like. Illustrative examples of suitable salts include organic salts derived from amino acids such as glycine and arginine, ammonia, primary, secondary and tertiary amines, and cyclic amines such as piperidine, morpholine and piperazine, and inorganic salts derived from sodium, calcium, potassium, magnesium, manganese, iron, copper, zinc, aluminum and lithium.

[00115] Suitably, the pharmaceutically acceptable carrier, diluent and/or excipient may be or include one or more of diluents, solvents, pH buffers, binders, fillers, emulsifiers, disintegrants, polymers, lubricants, oils, fats, waxes, coatings, viscosity-modifying agents, glidants and the like.

[00116] The various features and embodiments of the present invention, referred to in individual sections above apply, as appropriate, to other sections, *mutatis mutandis*. Consequently features specified in one section may be combined with features specified in other sections as appropriate.

[00117] The following example is provided by way of illustration and is in no way limiting upon the scope of the invention.

EXAMPLE 1

MATERIALS AND METHODS

Animals

[00118] Female C57BL/6 mice aged 4-6 weeks were from The University of Queensland Biological Resources (UQBR). Mice were housed in groups of 6-8 per cage in a temperature-controlled facility (22-23°C) with a 12 h/12 h light/dark cycle. Rodent chow and water were available *ad libitum*. Ethics

approval was from the Animal Ethics Committee of The University of Queensland. Experiments complied with the requirements of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC, 2004; NHMRC, 2013) and the ARRIVE guidelines (Kilkenny *et al.*, 2010).

EAE induction and clinical disease scoring

[00119] An optimised novel RR-EAE mouse model of MS-associated neuropathic pain that is devoid of confounding motor deficits in the hind paws, was employed (*Khan et al.*, 2014b). Briefly, mice were immunised with 200 µg of MOG₃₅₋₅₅ (Mimotopes, Clayton, VIC, Australia) mixed with a solution of Quil A (45 µg) in 100 µl of phosphate-buffered saline (PBS) (Sigma-Aldrich, Sydney, NSW, Australia). Mice then received an intraperitoneal (i.p.) injection of pertussis toxin at 250 ng (Sigma-Aldrich) in PBS (1 ng µl⁻¹) and this was repeated 48 h later (*Khan et al.*, 2014b). Sham-mice (control group) received adjuvants only (Quil A and pertussis toxin). Clinical disease scoring of RR-EAE and sham-mice was undertaken once-daily in a blinded manner using a 5-point scale with half-point gradations (Table 1) (*Khan et al.*, 2014b; *Peiris et al.*, 2007). RR-EAE clinical disease was classified as present by clinical scores ≥ 1 whereas clinical scores ≤ 0.5 were regarded as disease remission or absence. General health and body weights of all mice were assessed prior to immunisation and once-daily thereafter in a blinded manner until study completion.

Test compound dosing regimen

[00120] ALA was from GeroNova Research Inc. (Richmond, CA, USA) and was supplied and used as the water-soluble sodium salt of the R-enantiomer of ALA (R-ALA). Dosing solutions of ALA were prepared in sterile water for injection (Pfizer, West Ryde, NSW, Australia). RR-EAE mice received once-daily s.c. injections of ALA at 3 or 10 mg kg⁻¹day⁻¹, or vehicle for 21

consecutive days commencing at 15 days post-immunisation (d.p.i.), as the first episode of clinical disease had occurred in all EAE-mice by day 15. Sham control mice received once-daily s.c. vehicle injections for the same treatment period. Solutions of ALA or vehicle were prepared freshly each day and masked before administration to animals in a blinded manner. All drug treatments were administered between 09.00 am and 11.00 am. The total number of sham- and RR-EAE mice (in Cohorts 1 and 2) treated with vehicle or ALA is shown in FIG 1. To maintain blinding, equal numbers of animals were included in each group. Animals were randomised in a blinded manner and received masked solutions of ALA or vehicle by the first person. The drug and molecular target nomenclature used herein is in accordance with that recommended by Alexander *et al.*, (2013).

Anti-allodynic affects evoked by chronic ALA in RR-EAE mice

[00121] Calibrated von Frey filaments (Stoelting Co., Wood Vale, IL, USA) were used according to the up-down method (Chaplan *et al.*, 1994) to assess paw withdrawal thresholds (PWTs) in the bilateral hind paws of RR-EAE mice relative to sham-mice by a blinded tester. Briefly, baseline PWTs in the bilateral hind paws were measured prior to immunisation on day-0 and once-weekly thereafter to define the time course for temporal development of mechanical allodynia. For assessment of the anti-allodynic effects of ALA (or vehicle) in RR-EAE mice, bilateral hind paw PWTs were measured once-weekly at 0.5-0.75 h post-dosing. This was the time of peak effect observed in pilot experiments and it is in agreement with the time of peak antinociception produced by single bolus doses of ALA in a rat model of CIPN (Trevisan *et al.*, 2013). Hindpaw PWTs for sham-mice were assessed concurrently with the corresponding cohort of RR-EAE mice by a blinded tester.

Chronic ALA treatment: EAE clinical disease profiling

[00122] The effect of ALA on EAE clinical disease progression was assessed by measuring clinical disease scores once-daily at 0.5-0.75 h post-dosing. Clinical disease scores for sham-mice administered the chronic vehicle dosing regimen were also assessed concurrently with the corresponding cohort of RR-EAE mice in a blinded manner.

Ex-vivo analysis: Tissue collection and preparation

[00123] RR-EAE mice administered the chronic ALA ($10 \text{ mg kg}^{-1}\text{day}^{-1}$) or vehicle treatments, as well as sham-mice (administered the chronic vehicle dosing regimen $n=4/\text{group}$ for each technique), were euthanized with an overdose ($\sim 300 \text{ mg kg}^{-1}$; i.e. 1 mL/kg of 325 mg/mL , i.p.) of pentobarbitone (Lethabarb®, Virbac, Milperra, NSW, Australia) at 0.5-0.75 h post-dosing on the last treatment day (35 d.p.i.). Lumbar (L4-L6) spinal cord tissues were removed for ex-vivo mode of action analyses using immunohistochemistry (IHC), western blotting and quantitative real-time PCR (RTqPCR). For IHC, mice were perfused with 4% paraformaldehyde (Sigma-Aldrich, Sydney, NSW, Australia) in ice cold $1 \times \text{PBS}$ (pH 7.4; $\sim 100 \text{ mL/mouse}$) prior to removal of lumbar spinal cord tissues. Transverse cryosections ($10\text{-}12 \mu\text{m}$) were prepared and mounted on Superfrost Plus® slides (Thermo Fisher Scientific, Scoresby, VIC, Australia) prior to immunostaining, as per our previous report (Khan *et al.*, 2014b). For western blot and RTqPCR, lumbar (L4-L6) spinal cord tissues were collected without perfusion and immediately stored at -80°C prior to further processing as described below.

Immunohistochemistry

[00124] Sections of lumbar spinal cord were permeabilised with ice-cold acetone and incubated in blocking buffer containing 10% normal goat serum (Invitrogen, Mulgrave, VIC, Australia), 0.2% Triton-X (Sigma-Aldrich) and 0.05% Tween-20 in PBS solution prior to immunostaining (Khan *et al.*, 2014b). Briefly, sections were incubated overnight with the relevant primary antibody at

~4-8°C and on the following day, were incubated with the corresponding secondary antibody for ~1 h at room temperature. Later, sections were stained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI, Invitrogen) for 5 min and mounted with ProLong® Gold anti-fade reagent (Invitrogen) prior to imaging. Following staining, sections were washed with PBST (2 × 10 min). Primary antibodies used were: monoclonal rat anti-CD3 (1:200, Serotec, Kidlington, UK); monoclonal rat anti-mouse CD11b (1:200, Serotec); polyclonal rabbit anti-BDNF (1:200, Millipore, Kilsyth, VIC, Australia); monoclonal rat anti-TrkB (1:50, R&D Systems, Minneapolis, MN, USA) and monoclonal rabbit anti-pp44/pp42 MAPK (Cell Signaling Technology, Inc., Danvers, MA, USA). Additional antibodies used for co-localisation experiments were: monoclonal rabbit anti-CD3 (1:200, Abcam, Cambridge, MA, USA); polyclonal rabbit anti-Iba-1 (1:600, Wako, Osaka, Japan) and monoclonal mouse anti-NeuN Alexa Flour 488 conjugated (1:50, Millipore). Secondary antibodies used included: Cy3-goat anti-rat (1:800, Jackson ImmunoResearch, Westgrove, PA, USA); Cy3-goat anti rabbit (1:1000, Jackson); Alexa Flour 488 goat anti-rat (1:500, Invitrogen) or Alexa Flour 488 goat anti-rabbit (1:1000, Invitrogen). Primary and secondary antibodies were diluted in PBST containing 2% normal goat serum. Omission of primary antibodies on negative control sections during each staining procedure resulted in a complete absence of immunofluorescence (IF).

Western blotting

[00125] Lumbar spinal cord tissues were homogenised individually for each animal and protein extracted using radio-immunoprecipitation assay (RIPA) buffer. From each extract, 40 µg of total protein was loaded onto 4–20% gradient precast polyacrylamide gels (Bio-rad, Gladesville, NSW, Australia). Each gel was transferred to a 0.2 µm nitrocellulose membrane (Bio-rad) and blocked in Odyssey™ blocking buffer (Li-cor, Lincoln, NE, USA). For phospho-specific antibodies, blocking buffer comprised 5% bovine serum albumin (BSA)/0.1% Tween-20. Membranes were incubated overnight at ~4°C with

primary antibodies against BDNF (1:500), TrkB (1:300), Iba-1 (1:1000), pp44/pp42 MAPK (pERK) (1:1000) or p44/p42 MAPK (total ERK) (1:1000). Primary antibodies were the same as listed in the preceding section except for BDNF (N-20) from Santa Cruz Biotechnology (Texas, USA) that has been validated to identify all three isoforms of BDNF (Tongiorgi *et al.*, 2012). Monoclonal mouse anti-GAPDH (1:8000, Novus, Littleton, CO, USA) was used as loading control. Membranes were washed using PBST (3 × 5 min) and incubated in appropriate infrared dye-conjugated secondary antibodies (Li-cor) (1:8000) for ~1 h at room temperature. Membranes were visualised using an Odyssey infrared scanner (Li-cor).

Quantitative real-time PCR

[00126] Absolutely RNA Miniprep Kits (Agilent, Santa Clara, CA, USA) were used to isolate total RNA individually from lumbar spinal cord tissue from each mouse. Complementary DNA (cDNA) was reverse-transcribed from total RNA using high capacity cDNA reverse transcription kits (Applied Biosystems, Mulgrave, VIC, Australia). Quantitative BDNF and TrkB messenger RNA (mRNA) expression levels were analysed using SYBR[®] Green PCR master mix (Applied Biosystems) and specific primers. The specificity of the real time PCR reaction was confirmed using melting curve analysis. Primers used were (i) 18S-forward: CCCTCCAATGGATCCTCGTT; 18S-reverse: TCGAGGCCCTGTAATTGGAA (ii) BDNF QuantiTect[®] primer assay, Mm_Bdnf_1_SG (Qiagen, Pty Ltd., VIC, Australia) and (iii) TrkB QuantiTect[®] primer assay, Mm_Ntrk2_vb.1_SG (Qiagen) containing a mixture of forward and reverse primers.

Mouse plasma biochemistry and organ weights

[00127] At 35 d.p.i., blood samples were collected by cardiac puncture from euthanised RR-EAE or sham-mice in each treatment group (n=4-5/group), and immediately centrifuged at 3000 × g for 15 min. The separated plasma

(~0.5 mL) samples were transferred to clean pre-labelled tubes and stored at -20°C. Subsequently, plasma samples were thawed and analysed using a Roche-Cobas Integra 800 auto-analyser (Roche Diagnostics GmbH, Mannheim, Germany) for concentrations of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin, urea and creatinine (Lee *et al.*, 2014). After blood sample collection, these euthanized mice (n=6/group) underwent gross necropsy examination of the brain, liver, kidney, lungs, heart, and spleen. The liver and kidneys were also weighed individually.

DATA ANALYSES

Behavioural data

[00128] Graphical data are presented as mean $\pm$ standard error of the mean ($\pm$ SEM). Clinical scores were the mean ($\pm$ SEM) score for each group of mice at each time point. Hindpaw PWT values are the mean ($\pm$ SEM) of three measurements taken at least 5 min apart for both hind paws combined.

Immunohistochemistry data

[00129] Immunohistochemical (IHC) images of at least 3-4 lumbar spinal dorsal horn sections ($>100\ \mu\text{m}$ apart) per animal from each treatment group (n=3-4/group) were used for quantitative analysis or co-localisation studies in vehicle-treated RR-EAE mice as appropriate. Laminae I-II of the spinal dorsal horn was targeted for all IHC analyses (FIG 10). Densitometric counts were quantified using Axiovision Rel. v4.8 software (Carl Zeiss, Göttingen, Germany) in a blinded manner (Khan *et al.*, 2014b). Data are expressed as fold-changes in fluorescence intensity for sections from RR-EAE mice treated with ALA (10 mg kg⁻¹ day⁻¹) or vehicle, relative to the corresponding data for vehicle-treated sham-mice.

Western blot data

[00130] Image Studio Lite v4.0 software (Li-cor, Lincoln, NE, USA) was used to quantify immunoreactive band intensities in western blots for each treatment group (n=3/group). For each protein of interest, band intensity was normalised relative to GAPDH (Jain *et al.*, 2012) to give relative expression levels and fold-changes between ALA- and vehicle-treated RR-EAE mice compared with the vehicle-treated sham-group.

RTqPCR data

[00131] For RTqPCR, mRNA expression levels were normalised relative to 18S, for each treatment group (n=3-4/group). For the ALA- and vehicle-treated RR-EAE mice, mRNA levels were quantified relative to that for vehicle-treated sham-mice using the $\Delta\Delta C_T$ method (Schmittgen *et al.*, 2008). The fold-changes for each target of interest were determined using the $2^{-\Delta\Delta C_T}$ method (Schmittgen *et al.*, 2008).

Mouse plasma biochemistry and organ weights

[00132] Mouse plasma biochemistry parameters are reported as mean ($\pm$ SEM). Organ weights (kidneys and liver) are presented as relative to respective body weights on the day of euthanasia (Lee *et al.*, 2014) (Table 2).

Statistical analyses

[00133] Statistical analyses were performed using GraphPad Prism™ v6.04 (GraphPad Software, La Jolla, USA). Repeated measures two-way analysis of variance (ANOVA) followed by the Bonferroni test was used to analyse pain behavioural end-points. The Kruskal–Wallis test with the multiple comparison post-hoc Dunn's test, was used to analyse clinical scores for the various groups of mice, consistent with previous work by others (Amor and Baker, 2012; Fleming *et al.*, 2005). The one-way ANOVA followed by Tukey's multiple comparison test, was used to compare inter-groups differences for IHC, western blot and RTqPCR data, plasma biochemistry data and organ weights. The statistical significance criterion was $P < 0.05$.

RESULTS

RR-EAE mice: Mechanical hypersensitivity in the bilateral hind paws

[00134] Mean ($\pm$ SEM) hind paw PWTs for vehicle-treated RR-EAE mice of Cohorts 1 and 2 were significantly decreased *c.f.* PWTs for vehicle-treated sham-mice from 14 d.p.i. until study completion at 35 d.p.i. ($F_{(3, 6, 18/336)} = 142.2, 73.43, 43.66$; Cohort-1 and $F_{(3, 6, 18/264)} = 239.1, 25.11, 34.32$; Cohort-2; $P < 0.05$). Additionally, mechanical allodynia was fully developed (PWTs ≤ 1 g) in the bilateral hind paws of vehicle-treated RR-EAE mice from 26 d.p.i. ($P < 0.05$) which persisted until study completion at 35 d.p.i. (FIG 1).

Chronic ALA treatment alleviates hind paw hypersensitivity in RR-EAE mice

[00135] In RR-EAE mice, once-daily subcutaneous (s.c.) administration of ALA at 3 or 10 mg kg⁻¹day⁻¹ for 21 consecutive days (15-35 d.p.i.) evoked dose-dependent alleviation of mechanical hypersensitivity in the bilateral hind paws. Specifically, mean ($\pm$ SEM) hind paw PWTs for RR-EAE mice (Cohorts 1 and 2) administered the chronic dosing regimen of ALA at 10 mg kg⁻¹day⁻¹, did not differ significantly ($P > 0.05$) from the corresponding groups of vehicle-treated sham-mice at 35 d.p.i.. However, by 3-weeks after cessation of ALA at 10 mg kg⁻¹day⁻¹ in Cohort-2 RR-EAE mice at 55 d.p.i., mechanical allodynia was again fully developed in the bilateral hind paws of these mice (FIG 1).

RR-EAE disease course

[00136] For RR-EAE mice from Cohorts 1 and 2, disease onset (clinical scores ≥ 1 arbitrary units [a.u.]) was between 8 and 14 d.p.i.. This was followed by mild relapsing-remitting disease with mean ($\pm$ SEM) peak clinical scores of 1.4 (± 0.06) a.u. during the chronic vehicle treatment regimen (15-35 d.p.i.). Importantly, clinical disease progression in vehicle-treated RR-EAE-mice differed significantly from the corresponding cohorts of vehicle-treated sham-mice ($F_{(4, 144)} = 34.89$; Cohort-1 and $F_{(4, 164)} = 33.05$; Cohort-2; $P < 0.05$) that did

not develop clinical disease (mean $\pm$ SEM $\leq$ 0.5 a.u.) over the study period (FIG 1).

Chronic ALA treatment arrests clinical disease relapse in RR-EAE mice

[00137] RR-EAE mice (Cohorts 1 and 2) treated with ALA at 3 or 10 mg kg⁻¹day⁻¹ for 21 consecutive days commencing on day 15 d.p.i did not exhibit clinical disease relapses. Specifically, the clinical disease patterns in Cohorts 1 and 2 of RR-EAE mice treated with ALA at 10 mg kg⁻¹day⁻¹ were comparable ($P > 0.05$) to those from the corresponding vehicle-treated sham-mice at the end of treatment (35 d.p.i.) (FIG 1). However, by 9 days after cessation of ALA treatment at 10 mg kg⁻¹day⁻¹ in Cohort 2 RR-EAE mice (44 d.p.i.), there was a recurrence of relapsing-remitting clinical disease that persisted until study completion at 55 d.p.i. (FIG 1).

CD3+ T-cell infiltration

[00138] As the role of reactive T-cells in MS-associated neuropathic pain is unknown, it was decided to examine CD3+ T-cell immunofluorescence (IF) in the lumbar spinal dorsal horn of RR-EAE mice. Specifically, CD3+ T-cell IF was ~4.7-fold higher ($F_{(3, 44)} = 26.44$; $P < 0.05$) in vehicle-treated RR-EAE mice exhibiting neuropathic pain behaviour at 35 d.p.i. *c.f.* the respective levels in vehicle-treated sham-mice. For RR-EAE mice administered ALA at 10 mg kg⁻¹day⁻¹ for 21-days, CD3+ T-cell IF in the lumbar spinal dorsal horn did not differ significantly ($P > 0.05$) from that for vehicle-treated sham mice. However, by 3-weeks after cessation of ALA treatment at 10 mg kg⁻¹day⁻¹ in Cohort-2 RR-EAE mice at 55 d.p.i., CD3+ T-cell IF in the spinal dorsal horn was again increased to ~2.5 fold higher ($F_{(3, 44)} = 26.44$; $P < 0.05$) than in vehicle-treated sham mice (FIG 2).

Microglial activation

[00139] Next, the RR-EAE mice were examined for microglial activation, an important player in MS-induced neuropathic pain (Khan *et al.*, 2014a). For

vehicle-treated RR-EAE mice at 35 d.p.i., microglial activation, as measured by CD11b or Iba-1 expression, in the lumbar spinal dorsal horn was ~3 fold higher ($F_{(2, 33)} = 45.57$; $P < 0.05$; FIG 3) *c.f.* vehicle treated sham-mice, with a similar difference between the two groups when assessed by western blot ($F_{(2, 6)} = 29.82$; $P < 0.05$; FIG 4). However, for RR-EAE mice administered ALA at 10 mg kg⁻¹day⁻¹ for 21 days, levels of microglial expression in the spinal dorsal horn did not differ significantly *c.f.* vehicle-treated sham-mice when assessed by IHC ($F_{(2, 33)} = 45.57$; $P > 0.05$) or western blot ($F_{(2, 6)} = 29.82$; $P > 0.05$).

BDNF and TrkB expression

[00140] BDNF-TrkB signalling in the spinal cord has been observed in peripheral neuropathic pain conditions (Vanelderen *et al.*, 2010). Hence, expression levels of BDNF and TrkB in vehicle-treated RR-EAE mice exhibiting robust mechanical allodynia at 35 d.p.i. were investigated. The mean IF levels for BDNF and TrkB in lumbar spinal dorsal horn of vehicle-treated RR-EAE mice were ~2.5- ($F_{(2, 33)} = 15.84$; $P < 0.05$) and ~3.2- ($F_{(2, 33)} = 71.52$; $P < 0.05$) fold higher *c.f.* their respective levels in vehicle-treated sham-mice. By contrast, for RR-EAE mice treated chronically with ALA at 10 mg kg⁻¹day⁻¹, mean levels of BDNF and TrkB IF in lumbar spinal dorsal horn did not differ significantly ($P > 0.05$) from the respective mean levels of BDNF and TrkB IF for vehicle-treated sham-mice (FIG 3).

[00141] Using western blot, differential changes were observed in lumbar spinal cord expression levels of the three BDNF isoforms, viz pro-BDNF (precursor of mature-BDNF), truncated-BDNF (intermediate protein) and mature-BDNF between treatment groups (Mowla *et al.*, 2001). Specifically, for vehicle-treated RR-EAE mice, pro-BDNF and truncated-BDNF expression levels were increased significantly by ~2.0- ($F_{(2, 6)} = 20.31$; $P < 0.05$) and ~2.2- ($F_{(2, 6)} = 24.90$; $P < 0.05$) fold respectively *c.f.* the corresponding levels for vehicle-treated sham-mice. For RR-EAE mice, chronic treatment with ALA at 10 mg kg⁻¹day⁻¹ normalised lumbar spinal cord expression levels of pro-BDNF

and truncated-BDNF to match the corresponding levels for vehicle-treated sham-mice. By contrast, lumbar spinal cord expression levels of mature-BDNF did not differ significantly between the treatment groups ($F_{(2, 6)} = 1.79$; $P > 0.05$; FIG 4).

[00142] Additionally, western blot analysis showed that expression levels of TrkB isoforms including truncated-TrkB and full length-TrkB were increased by ~2.3- ($F_{(2, 6)} = 164.7$; $P < 0.05$) and ~4.0- ($F_{(2, 6)} = 125.2$; $P < 0.05$) fold respectively in lumbar spinal cord of vehicle-treated RR-EAE mice *c.f.* the respective levels in vehicle-treated sham mice. Chronic treatment of RR-EAE mice with ALA at $10 \text{ mg kg}^{-1}\text{day}^{-1}$ normalised lumbar spinal cord expression levels of both TrkB isoforms to match the corresponding levels for vehicle-treated sham-mice ($P > 0.05$; FIG 4).

Phospho-ERK (pERK)

[00143] pERK was then examined in RR-EAE mice as a marker of neuronal and/or glial cell activation (Gao *et al.*, 2009; Yamamoto *et al.*, 2013). For vehicle-treated RR-EAE mice exhibiting neuropathic pain behaviour, mean pERK IF in sections of lumbar spinal dorsal horn (laminae I-II) was ~3.6-fold higher ($F_{(2, 33)} = 42.49$; $P < 0.05$; Figure 3) *c.f.* the respective levels in vehicle-treated sham-mice, with a similar difference between the two groups when assessed by western blot ($F_{(2, 6)} = 16.43$; $P < 0.05$; FIG 4). By contrast, mean pERK expression levels in lumbar spinal dorsal horn of RR-EAE mice treated with ALA ($10 \text{ mg kg}^{-1}\text{day}^{-1}$) did not differ significantly from that for vehicle treated sham-mice when assessed by IHC ($F_{(2, 33)} = 42.49$; $P > 0.05$; Figure 3) and western blot ($F_{(2, 6)} = 16.43$; F ; $P > 0.05$; FIG 4). Also, there were no significant between-group differences ($F_{(2, 6)} = 4.06$; $P > 0.05$) in lumbar spinal cord expression levels of either total ERK or GAPDH (FIG 4).

Immunohistology: Co-localisation of BDNF, TrkB and pERK with CD3+ T-cells

[00144] Using specific IF antibodies, it was found that BDNF, TrkB and pERK were highly co-localised with CD3+ T-cells in the dorsal horn of lumbar spinal cord sections from RR EAE mice (FIGs 6-8). These markers were also co-localised with subsets of neurons (NeuN) and microglia/macrophages (CD11b or Iba1) (Figures 6-8). BDNF and TrkB were minimally co-localised with astrocytes (GFAP), whereas pERK was co-localised with a subset of astrocytes (Figures 6-8). BDNF was highly co-localised with its receptor, TrkB, in the dorsal horn of lumbar spinal cord sections from these animals (FIGs 6-8).

Chronic ALA treatment: No signs of hepatic or renal toxicity

[00145] Mean ($\pm$ SEM) plasma concentrations of various biochemical markers of hepatic and renal function (Table 2) did not differ significantly ($F_{(2, 9)} = 0.11$, ALT; $F_{(2, 9)} = 0.37$, AST; $F_{(2, 12)} = 0.26$, ALP; $F_{(2, 9)} = 2.43$, total bilirubin; $F_{(2, 12)} = 1.68$, Urea; ($P > 0.05$) between RR-EAE mice administered ALA ($10 \text{ mg kg}^{-1}\text{day}^{-1}$) or vehicle for 21 consecutive days *c.f.* vehicle-treated sham-mice. Similarly, mean ($\pm$ SEM) liver and kidney weights did not differ significantly $F_{(2, 15)} = 0.2$, liver; $F_{(2, 15)} = 2.7$, kidneys; $P > 0.05$) between these three groups (Table 2). At necropsy, there were no gross abnormalities observed in the brain, lungs, heart, spleen, liver and kidneys, consistent with the absence of treatment-related adverse behavioural effects in either group of RR-EAE mice or sham-mice (Table 2).

DISCUSSION

[00146] The results set out in the above experimental section indicate that once-daily chronic treatment with R-ALA (3 or $10 \text{ mg kg}^{-1}\text{day}^{-1}$) for 3-weeks attenuated the development of bilateral hind paw hypersensitivity in an optimised RR-EAE mouse model of MS-induced neuropathic pain in a dose-dependent manner. Specifically, at 35 d.p.i., mean ($\pm$ SEM) PWTs for RR-EAE mice administered chronic ALA at $10 \text{ mg kg}^{-1}\text{day}^{-1}$ did not differ significantly ($P > 0.05$) from the corresponding PWTs for vehicle-treated sham-mice. By

contrast, for RR-EAE mice administered vehicle for 3-weeks, mechanical allodynia was fully developed in the bilateral hind paws at 26 d.p.i. ($P < 0.05$), that persisted until study completion (FIG 1).

[00147] Additionally, it has been shown that the efficacious chronic ALA dosing regimen at $10 \text{ mg kg}^{-1}\text{day}^{-1}$ in RR-EAE mice was well-tolerated. There were no gross abnormalities at necropsy and biochemical indices of hepatic and renal function were within the normal ranges (Table 2). Interestingly, 3-weeks after cessation of the chronic ALA dosing regimen at $10 \text{ mg kg}^{-1}\text{day}^{-1}$ in Cohort 2 RR-EAE mice, mechanical allodynia recurred in the bilateral hind paws at 55 d.p.i. in a manner similar to that exhibited by vehicle-treated RR-EAE mice from 26 d.p.i. onwards. These findings clearly suggest that on-going chronic R-ALA treatment is required to prevent development of persistent MS associated neuropathic pain (FIG 1)

[00148] Importantly, mechanical allodynia that developed in the bilateral hind paws of these RR-EAE mice was unaffected by the remitting-relapsing clinical disease progression, in agreement with previous studies in this field (Aicher *et al.*, 2004., Khan *et al.*, 2014b., Olechowski *et al.*, 2009). Together, these findings suggest that distinct pathophysiological mechanisms underpin motor and sensory deficits in rodent models of EAE (Aicher *et al.*, 2004., Khan *et al.*, 2014).

[00149] The observations herein on the cellular and molecular mechanisms contributing to the progressive alleviation of hind paw hypersensitivity in RR-EAE mice treated with ALA ($10 \text{ mg kg}^{-1}\text{day}^{-1}$), show that CD3+ T-cell infiltration into the lumbar spinal dorsal horn was markedly reduced relative to that for vehicle-treated RR-EAE mice exhibiting mechanical allodynia in their bilateral hind paws (FIG 2). The augmented CD3+ T-cell infiltration into the lumbar spinal dorsal horn of vehicle-treated RR-EAE mice was accompanied by microglial activation, mirroring previous reports of activated microglia in the CNS of EAE-mice exhibiting clinical disease (Murphy *et al.*, 2010). Importantly,

relief of hind paw hypersensitivity in RR-EAE mice by the efficacious chronic ALA dosing regimen ($10 \text{ mg kg}^{-1}\text{day}^{-1}$) was accompanied by marked attenuation of microglial activation in the spinal dorsal horn (FIGs 3-4).

[00150] The results demonstrate that for vehicle-treated RR-EAE mice with fully developed mechanical allodynia in the bilateral hind paws, lumbar spinal dorsal horn expression levels of BDNF and TrkB were markedly increased compared with the corresponding expression levels in vehicle-treated sham-mice (FIGs 3-4). However, lumbar spinal cord expression levels of mRNA for BDNF and TrkB in our RR-EAE-mouse model of MS neuropathic pain did not differ significantly ($P > 0.05$) from the respective levels for vehicle-treated sham-mice (FIG 5).

[00151] Elevated expression levels of BDNF and TrkB in the lumbar spinal cord of RR EAE mice may be due to increased translation of BDNF and TrkB from mRNA in spinal cord neurons and/or glia. Alternatively, elevated spinal cord expression levels of BDNF and TrkB may be via a mechanism not involving enhanced local synthesis (Tonra *et al.*, 1998). This latter notion is supported by reports of upregulated BDNF expression by infiltrating T-cells due to antigen stimulation and/or secondary to demyelination of CNS neurons (Gielen *et al.*, 2003; Stadelmann *et al.*, 2002). Although infiltrating T-cell derived BDNF was originally proposed to be neuroprotective in the CNS of EAE-mice (Moalem *et al.*, 2000; Stadelmann *et al.*, 2002), more recent work suggests that T-cell derived BDNF is not critical for neuroprotective effects in the CNS of EAE-mice (Lee *et al.*, 2012; Xin *et al.*, 2012).

[00152] The present findings of differential changes in expression levels of BDNF isoforms in the lumbar spinal cord of vehicle-treated RR-EAE mice *c.f.* vehicle-treated sham-mice, are previously unknown (FIG 4). Specifically, there was significant upregulation of pro-BDNF and truncated-BDNF expression in lumbar spinal cord of vehicle-treated RR-EAE mice exhibiting neuropathic pain behaviour. Notably, the dysregulated expression of BDNF as well as TrkB

isoforms was normalised in RR-EAE mice administered chronic ALA treatment at $10 \text{ mg kg}^{-1}\text{day}^{-1}$ for 3-weeks (FIG 4).

[00153] The finding of upregulated total BDNF (FIGs 3 and 9) in the lumbar spinal dorsal horn of vehicle-treated RR-EAE mice is interesting. The findings herein of differential changes in BDNF isoforms in the lumbar spinal cord of RR-EAE mice are aligned with a recent report of dysregulated serum levels of BDNF isoforms in patients with RR-MS (Tongiorgi *et al.*, 2012). Hence, future investigation on the extent to which differential changes in serum levels of the various BDNF isoforms is a marker for MS502 associated neuropathic pain in humans, is warranted.

[00154] The series of images in FIG 9 show total BDNF (pro-, truncated- and mature-BDNF) expression by western blot in lumbar (L4-L6) spinal cord of RR-EAE mice administered ALA at $10 \text{ mg kg}^{-1}\text{day}^{-1}$ or vehicle, for 3-weeks relative to the respective data for the lumbar spinal cord of vehicle-treated sham-mice. Specifically, for vehicle-treated RR-EAE mice there was ~ 1.8 fold ($F(2, 6) = 7.27$; $P < 0.05$) increase in total BDNF *c.f.* the respective data for lumbar spinal cord of vehicle-treated sham-mice. For RR-EAE mice treated with ALA ($10 \text{ mg kg}^{-1}\text{day}^{-1}$) for 21 days, there were no significant differences ($P > 0.05$) in expression levels of total BDNF from the respective data for the lumbar spinal cord of vehicle-treated sham-mice (one-way ANOVA followed by Tukey's multiple comparison test).

[00155] FIG 10 shows TrkB (~ 3.2 fold; $F(2, 33) = 71.52$; $P < 0.05$) and pERK (~ 3.6 fold; $F(2, 33) = 42.49$; $P < 0.05$) were significantly increased in vehicle-treated RR-EAE mice *c.f.* the respective data for the lumbar spinal cord of vehicle-treated sham-mice. For RR-EAE mice treated with ALA ($10 \text{ mg kg}^{-1}\text{day}^{-1}$), lumbar spinal cord expression levels of CD11b, BDNF, TrkB and pERK did not differ significantly ($P > 0.05$) from the respective data for the lumbar spinal cord of vehicle-treated sham-mice (one-way ANOVA followed by Tukey's multiple comparison test).

[00156] The results herein show concurrent increases in pERK and pro-BDNF expression levels in vehicle-treated RR-EAE mice (FIGs 3-4). pERK may have a pathobiologic role in CNP conditions such as MS-associated neuropathic pain. In vehicle-treated RR-EAE mice herein, upregulated expression levels of BDNF, TrkB and pERK were co-localised primarily with infiltrated CD3+ T-cells in the lumbar spinal dorsal horn. These markers were also co-localised with subsets of neurons and to a lesser extent with microglia/macrophages. However, there was minimal co-localisation with astrocytes for BDNF and TrkB (FIGs 6-8). Hence, it may be that augmented levels of pro-BDNF signalling, at least in part, via full-length TrkB to increase pERK expression levels in the spinal dorsal horn of vehicle-treated RR-EAE mice, may contribute to the development of hind paw hypersensitivity in these mice.

TABLES

Score	Description
0	Normal behaviour
0.5	Limpness of the distal tail region and hunched appearance
1	Completely limp tail or developing weakness in the hindlimbs
1.5	Limp tail and distinct hindlimbs weakness recognized by unsteady gait and poor grip of hindlimbs during hanging on cage underside
2	Limp tail with unilateral partial hind limb paralysis
2.5	Limp tail and partial paralysis of bilateral hindlimbs
3	Complete paralysis of bilateral hindlimbs
3.5	Complete bilateral hindlimbs paralysis and unilateral forelimb paralysis
4	Quadriplegia

Table 1: EAE clinical disease scoring paradigm

	Sham + Veh	EAE + Veh	EAE + ALA (10 mg/kg/day)
Biochemical parameters (mean $\pm$ SEM)			
ALT (U/L)	29.25 $\pm$ 4.6	24.75 $\pm$ 9.1	27.5 $\pm$ 5.9
AST (U/L)	394.8 $\pm$ 53.3	324.5 $\pm$ 73.3	313.0 $\pm$ 85.7
ALP (U/L)	31.0 $\pm$ 10.9	38.8 $\pm$ 9.9	38.8 $\pm$ 3.3
Total bilirubin μ mol/L	2.5 $\pm$ 0.5	6.3 $\pm$ 2.5	2.0 $\pm$ 0.0
Urea mmol/L	4.4 $\pm$ 0.2	3.7 $\pm$ 0.5	4.6 $\pm$ 0.1
Creatinine* μ mol/L	BLOQ	BLOQ	BLOQ
Organ weights (mg; mean $\pm$ SEM)			
Body weights (at euthanasia)	20.2 $\pm$ 0.4	19.6 $\pm$ 0.3	19.5 $\pm$ 0.3
Liver (absolute)	947 $\pm$ 40.0	925 $\pm$ 35.9	894.5 $\pm$ 37.9
	4.68 $\pm$ 0.14	4.71 $\pm$ 0.15	4.57 $\pm$ 0.19
Kidneys (absolute)	247.6 $\pm$ 10.1	262.1 $\pm$ 8.1	260.1 $\pm$ 10.0
	1.22 $\pm$ 0.03	1.33 $\pm$ 0.02	1.33 $\pm$ 0.05

Table 2: Mean ($\pm$ SEM) plasma biochemical parameters and organ weights in mice. (Abbreviations: ALA, alpha lipoic acid; ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; EAE, experimental autoimmune encephalomyelitis; kg, kilogram; L, litre; μ mol, micromole; mg, milligram; U/L, units per litre; SEM, standard error mean; Veh, vehicle. *below lower limit of quantification (BLOQ) which was $< 20 \mu\text{mol/L}$; the normal range for C57BL6 mice is $\leq 20 \mu\text{mol/L}$ (Zhou *et al.*, 2004)).

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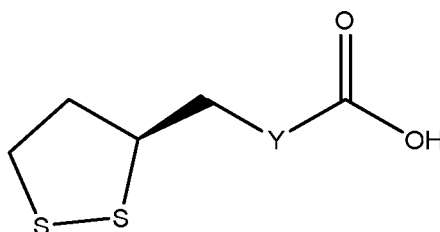
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CLAIMS

1. A method of treatment or prevention of central neuropathic pain in a subject including the step of administering an effective amount of a compound of formula I, or a pharmaceutically effective salt or ester thereof;



Formula I

wherein Y is 1 to 6 carbon atoms;

to the subject, to thereby treat or prevent the central neuropathic pain in the subject.

2. The method of claim 1, further including the step of selecting a subject exhibiting one or a plurality of symptoms of central neuropathic pain.
3. A compound of formula I, or a pharmaceutically effective salt or ester thereof, for use in the treatment or prevention of central neuropathic pain in a subject.
4. The method of claim 1 or claim 2 or the compound of claim 3, wherein Y is selected from 2, 3 or 4 carbon atoms.
5. The method or compound of any one of the preceding claims, wherein the compound of formula I is R-(+)- α -lipoic acid, or a pharmaceutically effective salt or ester thereof.
6. The method or compound of any one of the preceding claims wherein the central neuropathic pain is or comprises central neuropathic pain associated with multiple sclerosis.

7. The method or compound of any one of the preceding claims, wherein the CNP associated with MS is or comprises CNP associated with relapsing-remitting MS (RR-MS).
8. The method or compound of any one of the preceding claims, wherein the compound modulates the function and/or expression level of a marker selected from the group consisting of BDNF, TrkB and pERK in one or a plurality of cells, tissues or organs of the subject.
9. The method or compound of claim 8, wherein the one or plurality of cells, tissues or organs are located in the central nervous system of the subject.
10. The method or compound of any one of the preceding claims, wherein the compound modulates the function and/or level of CD3⁺ T-cells in one or a plurality of tissues or organs of the subject.
11. The method or compound of claim 10, wherein the one or plurality of tissues or organs are located in the central nervous system of the subject.
12. The method of any one of the preceding claims, further including the step of administering to the subject one or a plurality of additional agents.
13. The method of claim 12, wherein the one or plurality of additional agents are selected from the group consisting of a tricyclic antidepressant (TCA), a serotonin-norepinephrine reuptake inhibitor (SNRI), a voltage-gated calcium channel $\alpha 2\text{-}\delta$ subunit ligand, an opioid analgesic, an antiepileptic, a sodium channel antagonist, an N-methyl-d-aspartate receptor antagonist, topical capsaicin, a cannabinoid, an adenosine A1 agonist, a nicotinic acetylcholine receptor agonist, an immunomodulatory agent, an angiotensin II (AT2) receptor antagonist and any combination thereof.
14. The method or compound of any one of the preceding claims wherein the subject is a human.

Cohort-1

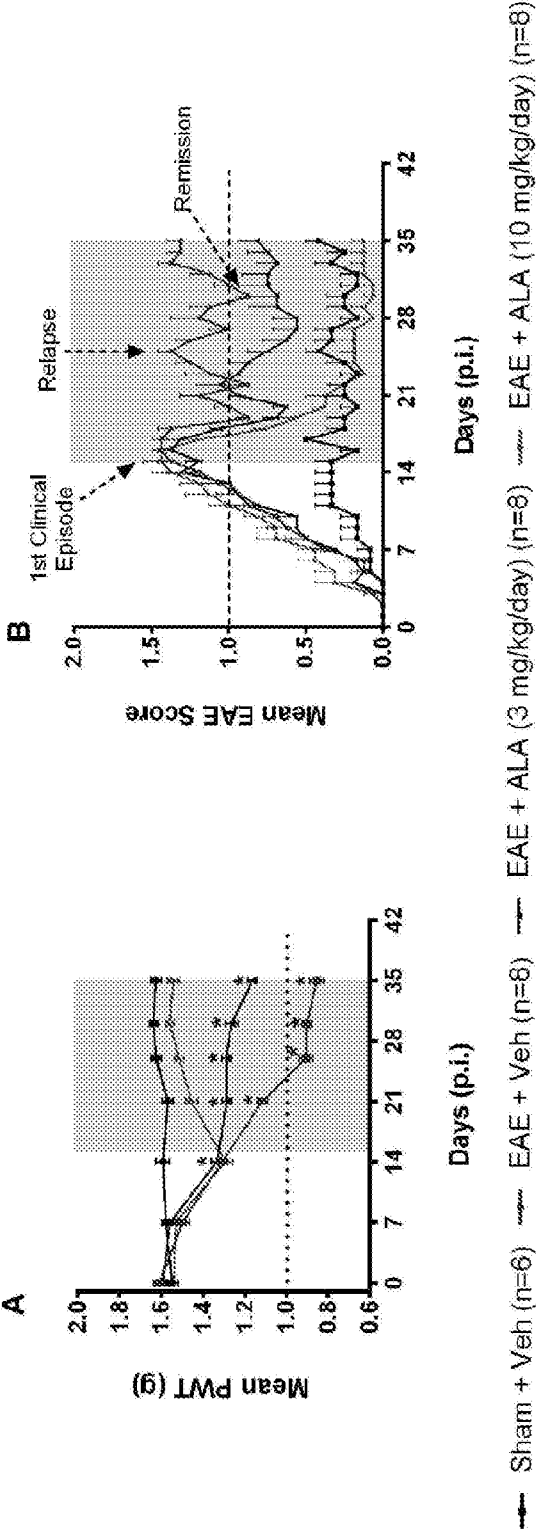


FIG 1

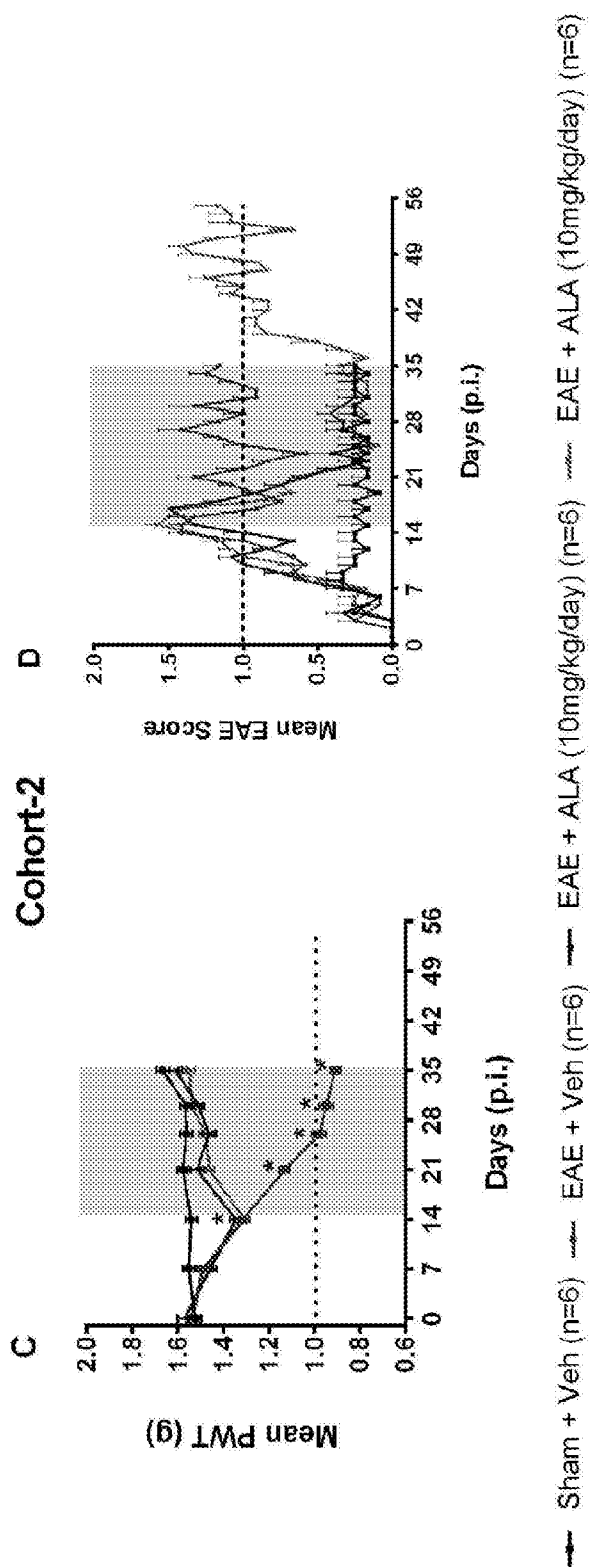


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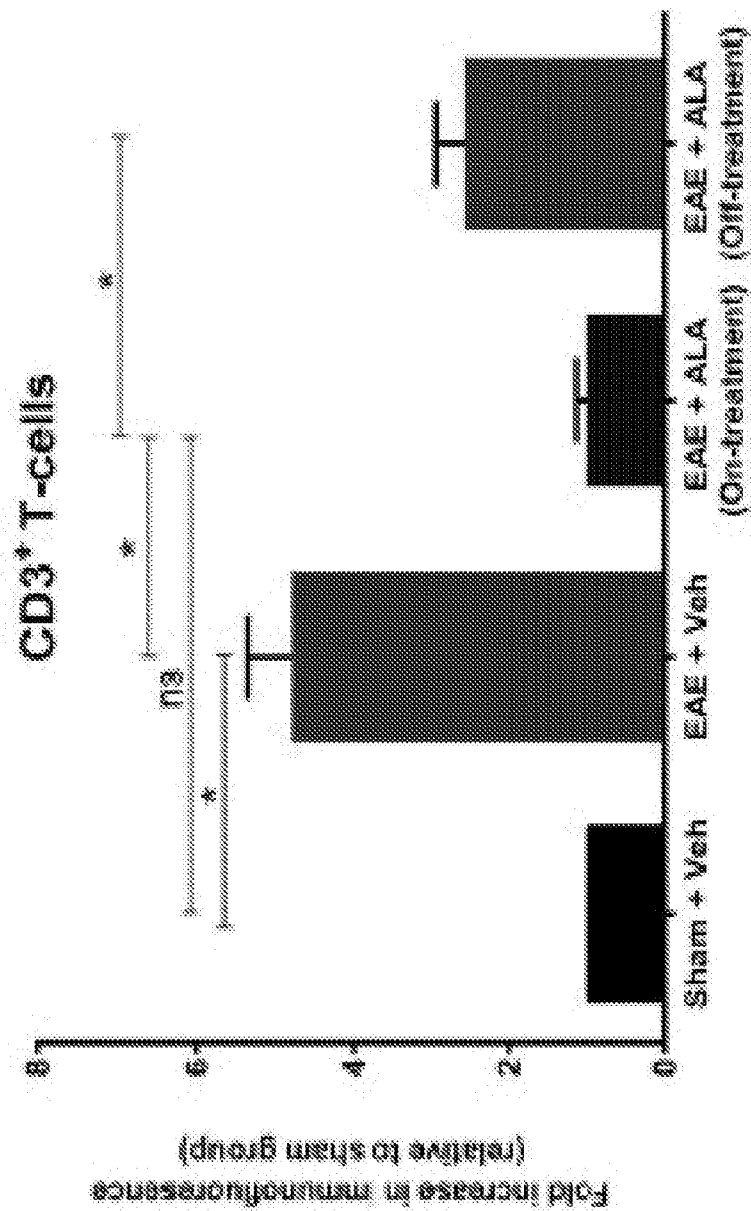
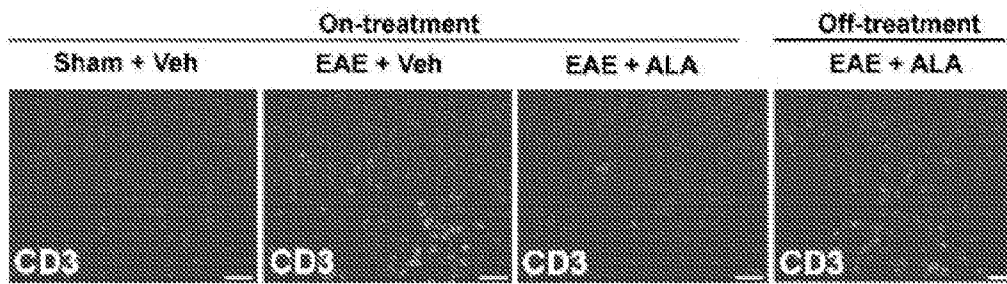


FIG 2

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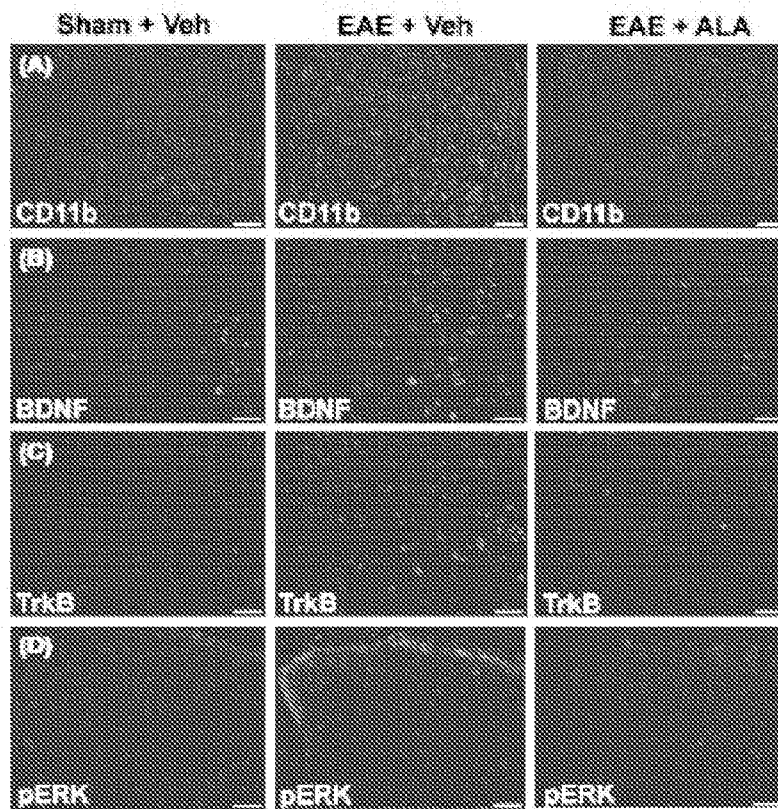


FIG 3A

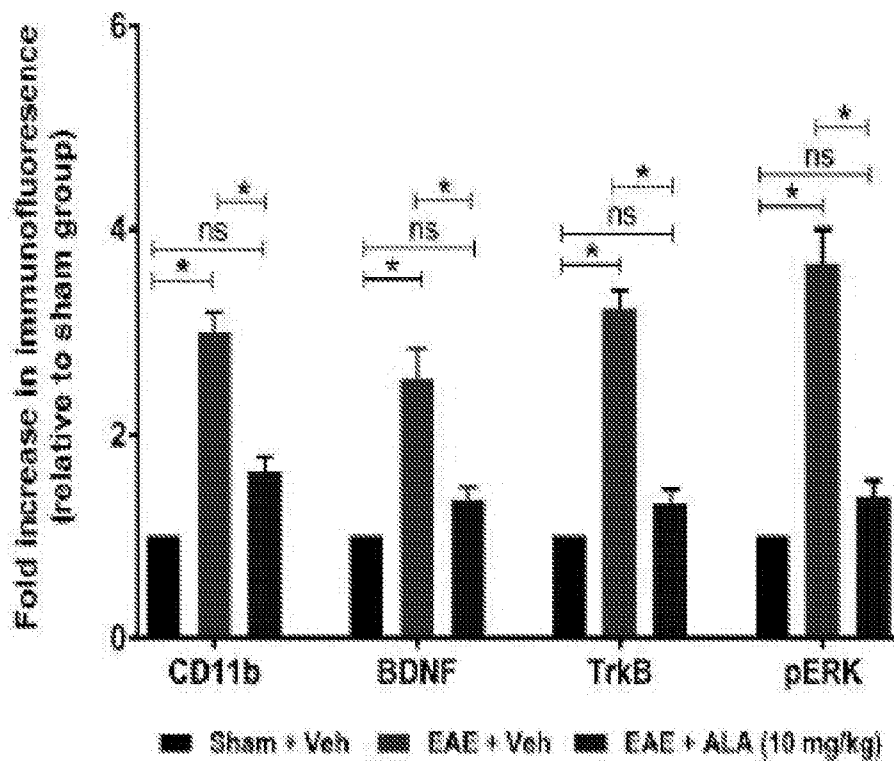


FIG 3B

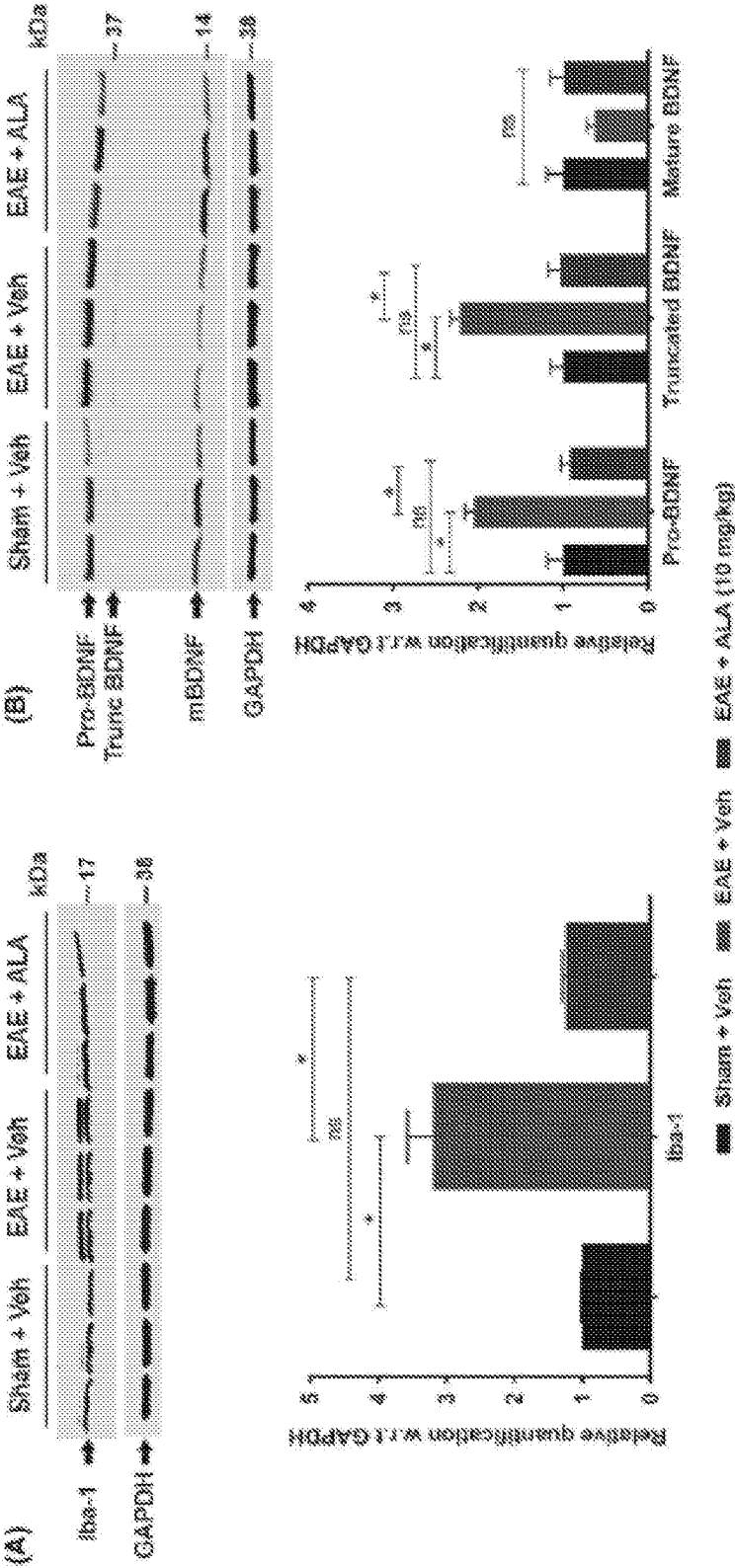


FIG 4

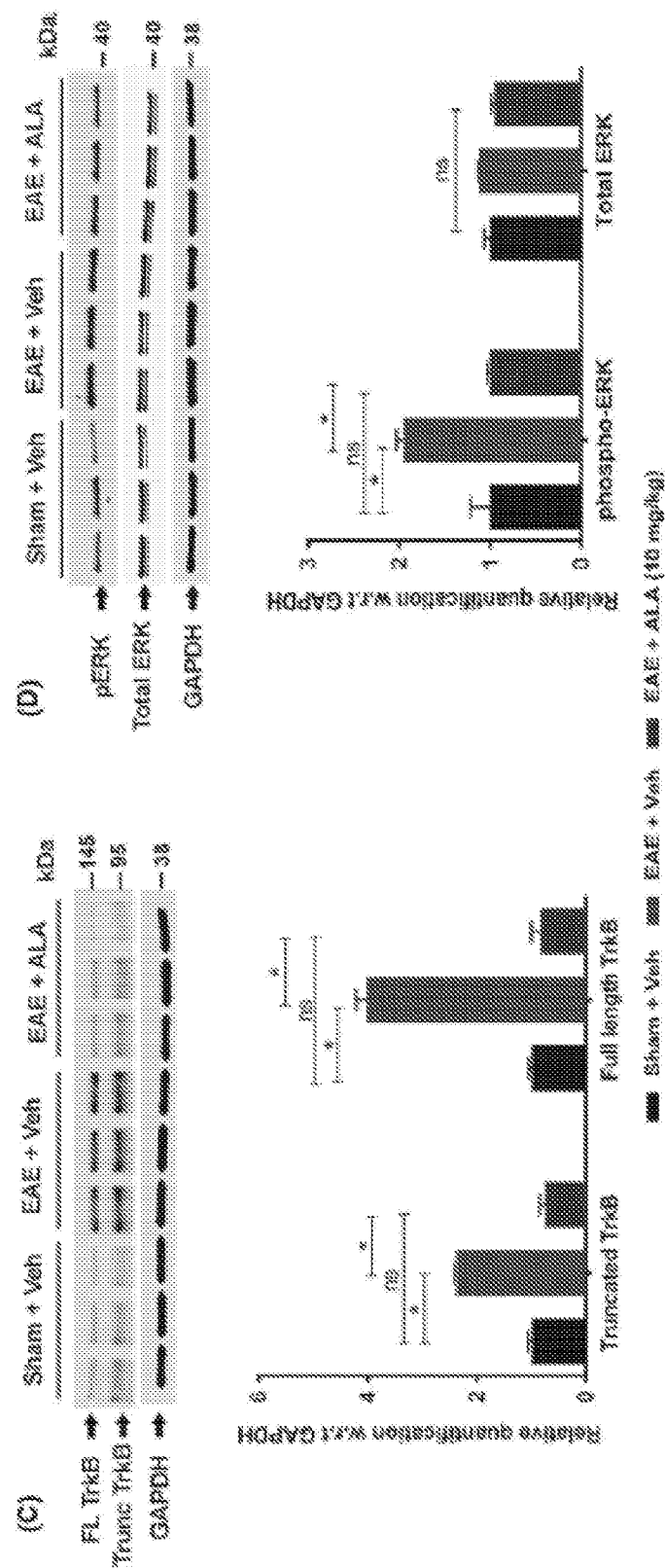


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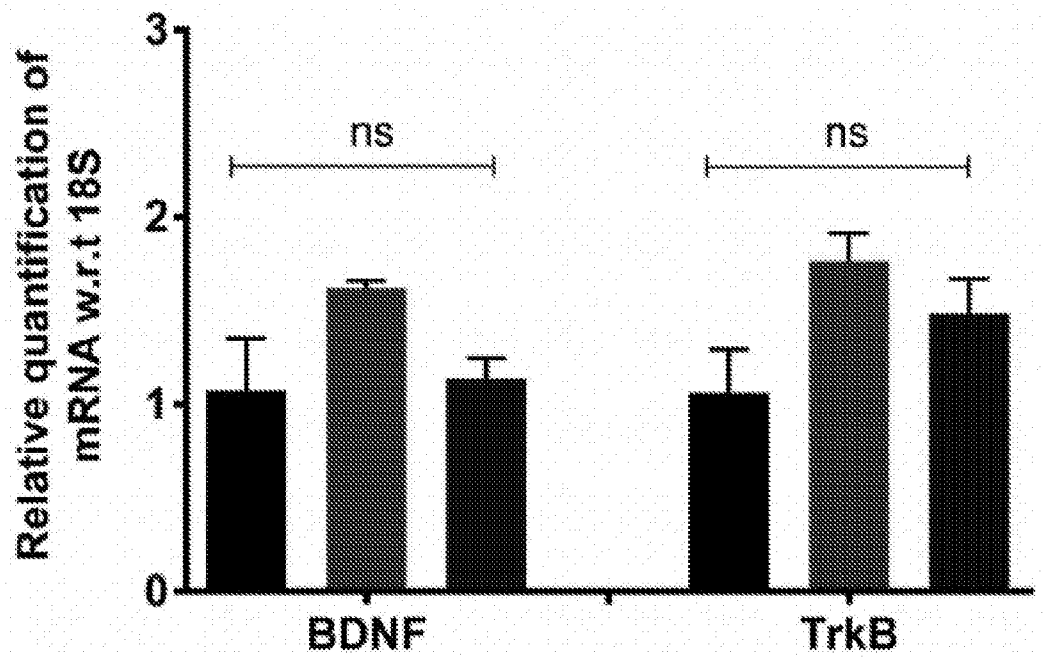


FIG 5

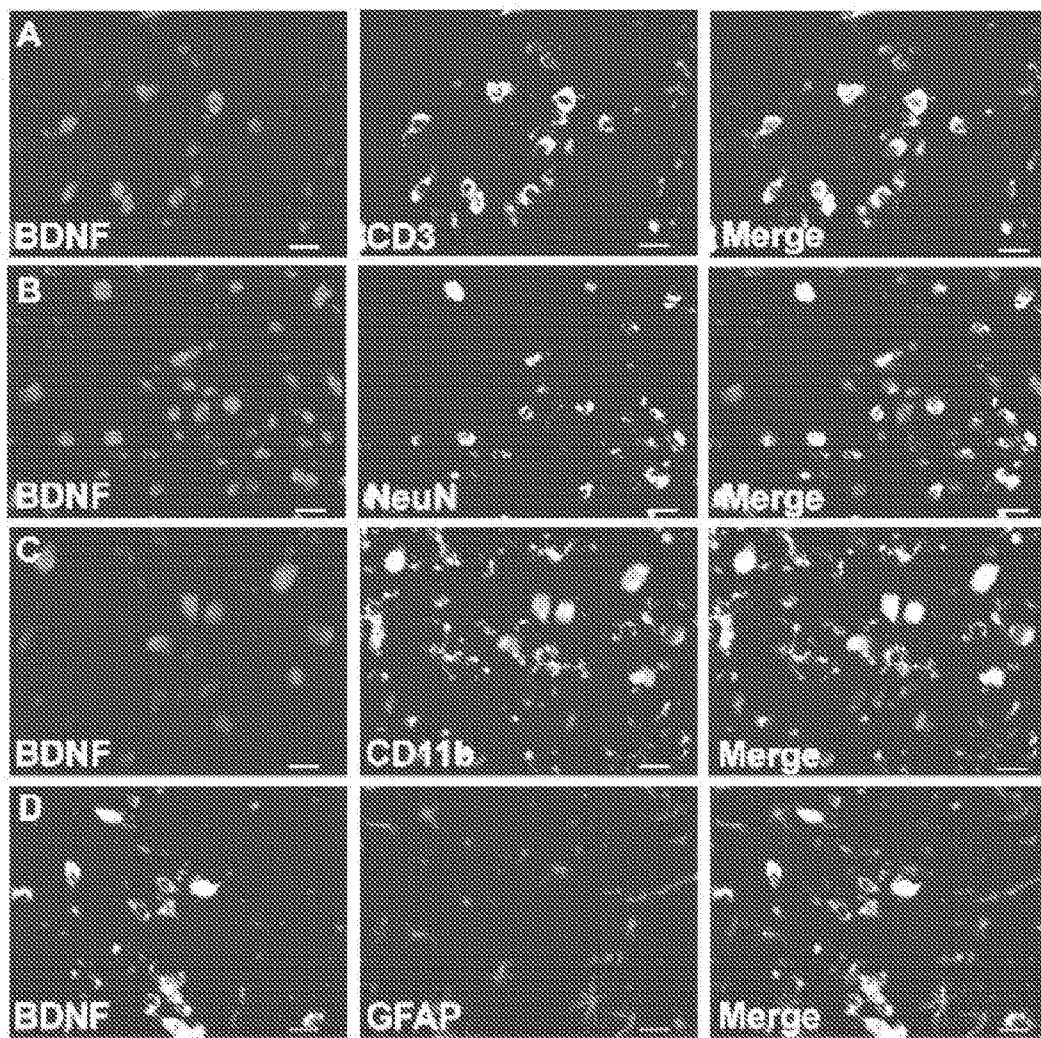


FIG 6

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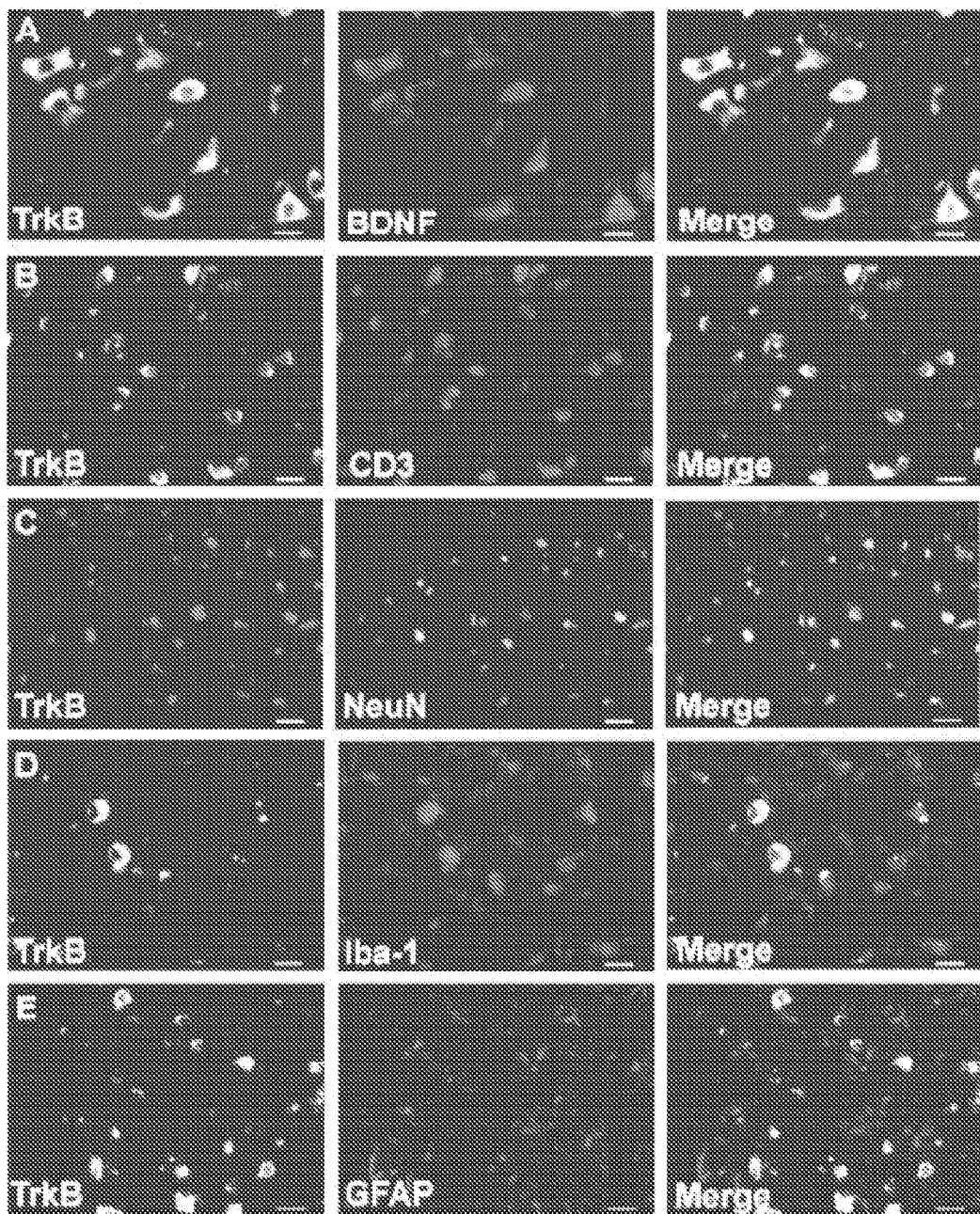


FIG 7

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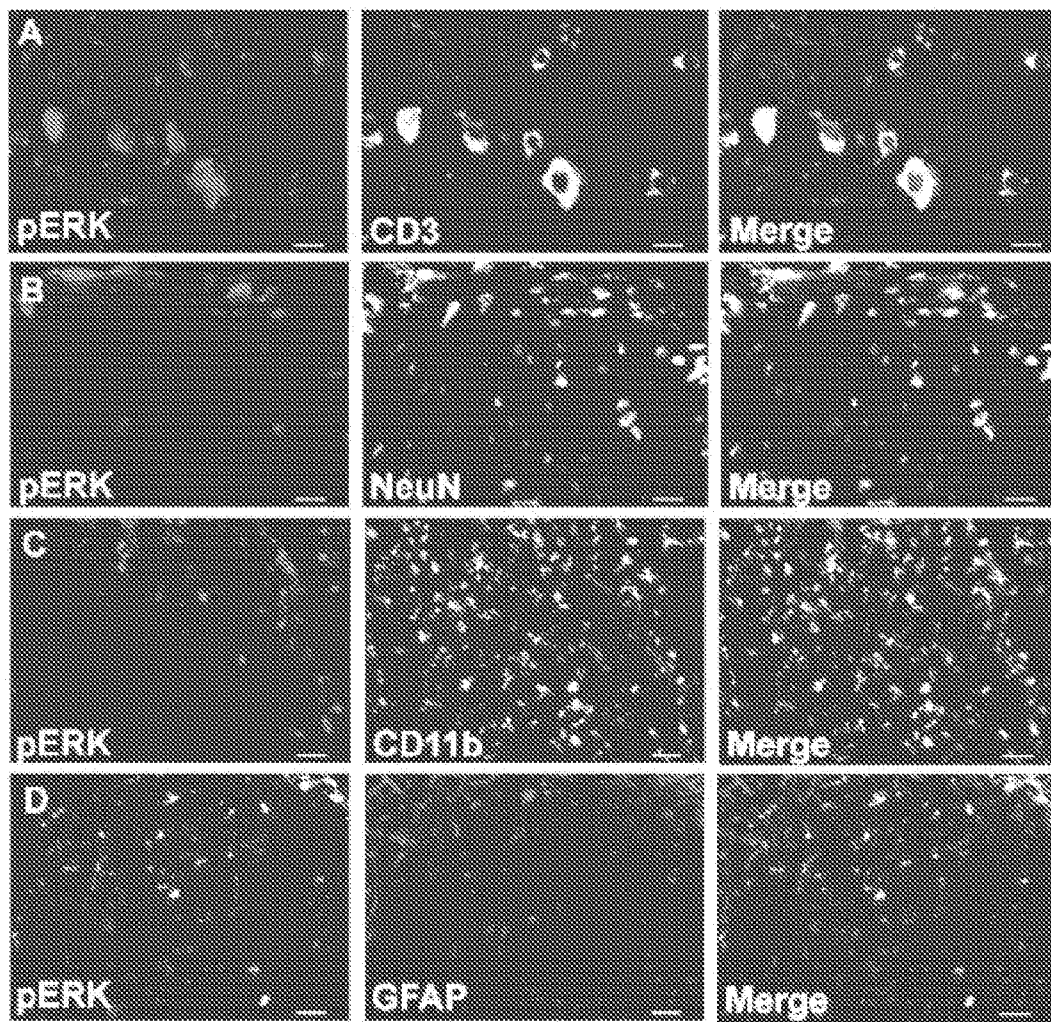


FIG 8

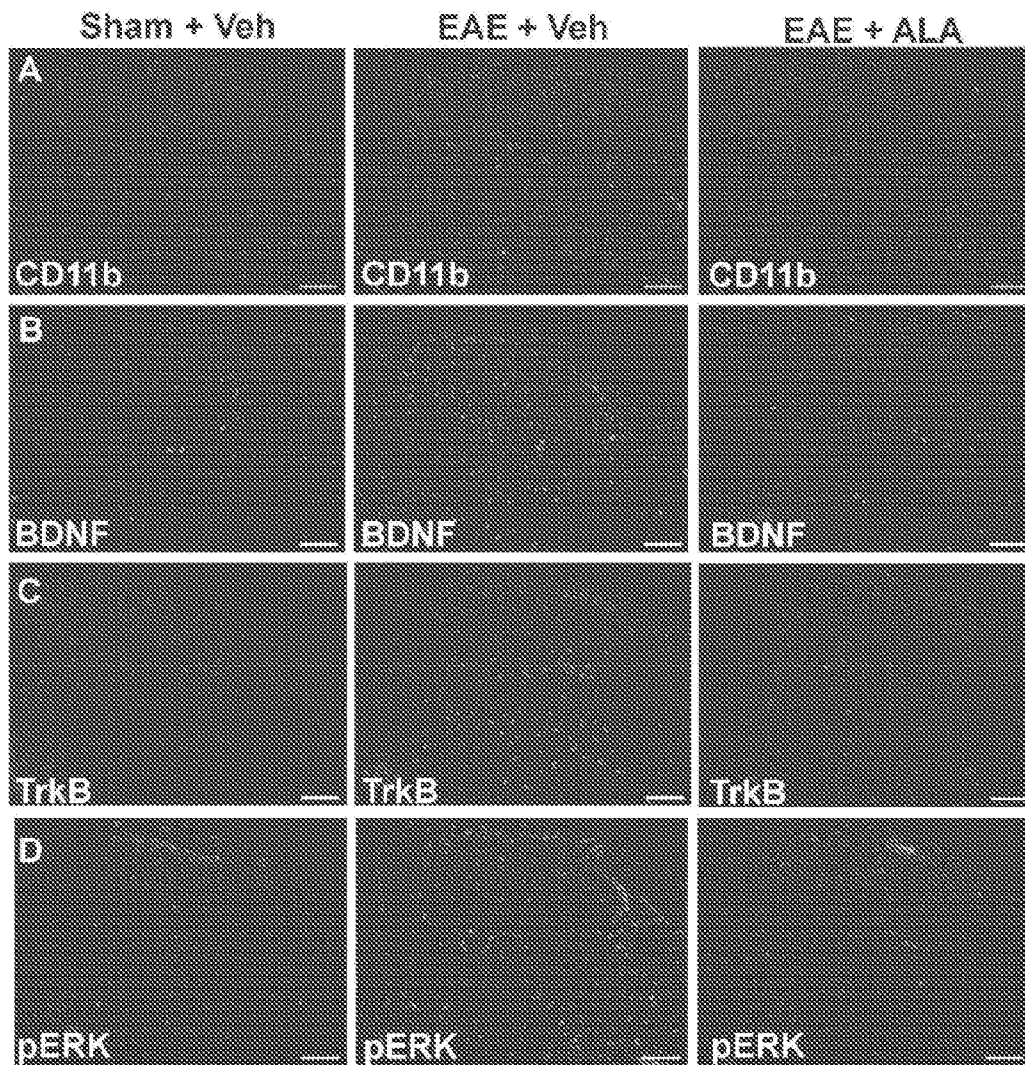


FIG 9

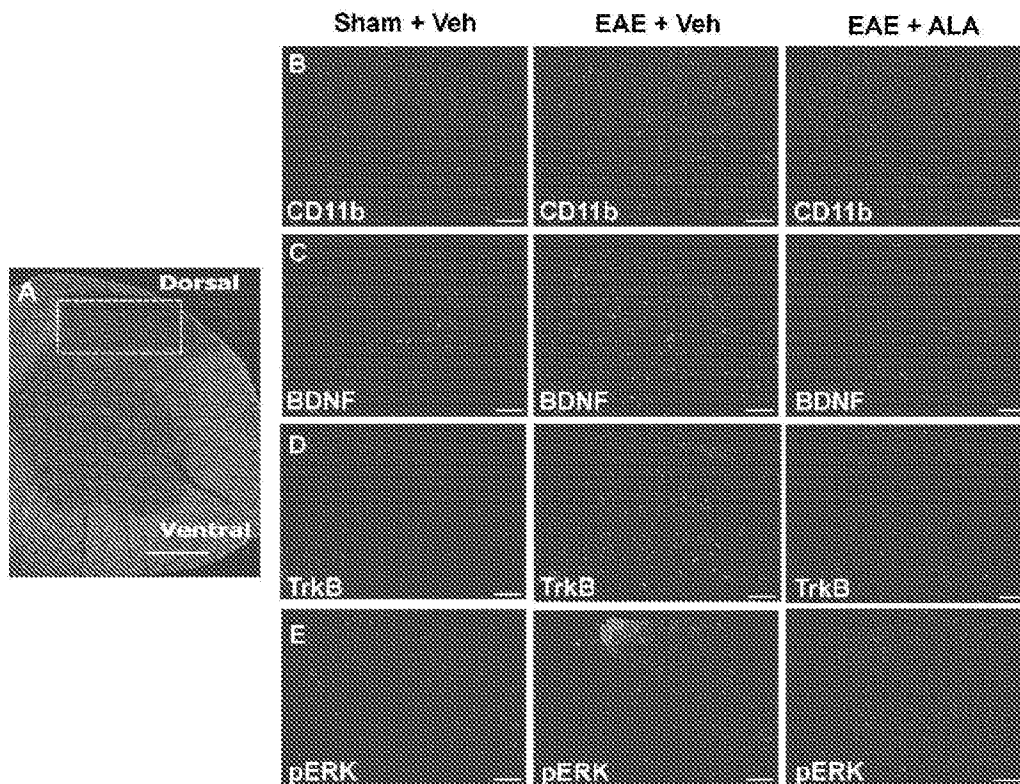


FIG 10

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2016/050150

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/385 (2006.01) A61P 25/04 (2006.01)

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)

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)

EPODOC, WPIAP, MEDLINE, CAPLUS, BIOSIS, EMBASE; Keywords: lipoic, lipoate, thioctic, thioctate, dithiolane, neuropathic, brain, spine, dorsal horn, spinothalamic tract, multiple sclerosis, stroke, pain, ache, neuralgia, analgesia, central neuropathic pain, centrally acting, central nervous system, brain derived neurotrophic factor, BDNF, tropomyosin receptor kinase B, TrkB, pERK & like terms. IPC: A61P 25/04

REGISTRY, CAPLUS; Structure search based on formula I of claim 1 with keywords based on above

PATENTSCOPE, ESPACENET, GOOGLE PATENTS, IP AUSTRALIA internal databases: Inventor and Applicant search, and with keywords based on above

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

☒ Further documents are listed in the continuation of Box C☒ See patent family annex

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent but published on or after the international filing date	"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
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search 9 May 2016		Date of mailing of the international search report 09 May 2016	
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au		Authorised officer Ross Heisey AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262833185	

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/AU2016/050150
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5728735 A (ULRICH et al.) 17 March 1998 Abstract; col. 1, lines 9-14 and 51-59; Examples 1-2; claims 1-10	3-11, 14
X	SALINTHONE, S., et al., "Lipoic acid stimulates cAMP production via G-protein coupled receptor-dependent and -independent mechanisms", Journal of Nutritional Biochemistry, 2011, vol. 22, pages 681-690 Whole document, in particular Abstract; Section 3.2 at page 684; Fig. 2; Discussion at page 687	3-11, 14
X	WO 2013/168008 A1 (KANDULA, Mahesh) 14 November 2013 Abstract; para. 0005, 0019, 0025, 0028, 0036, 0092; compound 11 at para. 0108-0109; claims 4, 6	1-14
X	WO 2013/168004 A2 (KANDULA, Mahesh) 14 November 2013 Abstract; para. 0004, 0006, 0017, 0025, 0028; compound (1-1) at para. 0011; claims 5-6	1-14
X	WO 2013/168011 A1 (KANDULA, Mahesh) 14 November 2013 Abstract, para. 0007, 0028, 0031, 0096; compound 4 at para. 0110-0111; claims 10, 16	1-14
X	WO 2013/168010 A1 (KANDULA, Mahesh) 14 November 2013 Abstract, para. 0006, 0020, 0026, 0029, 0093; compound 9 at para. 0103-0104; claims 5, 6	1-14
A	HUTTUNEN, K.M., et al., "Prodrugs-from Serendipity to Rational Design", Pharmacological Reviews, 2011, vol. 63, pages 750-771 Whole document, in particular Section III.B. Prodrug Design: Enhancing Permeability and Absorption, pages 755-758; Fig. 3	1-14
P,X	KHAN, N., et al., "Antiallodynic effects of alpha lipoic acid in an optimized RR-EAE mouse model of MS-neuropathic pain are accompanied by attenuation of upregulated BDNF-TrkB-ERK signaling in the dorsal horn of the spinal cord", Pharmacology Research & Perspectives, 2015, vol. 3, e00137, pages 1-16. Epub: 04 May 2015 Whole document, in particular Abstract, Discussion, Conclusions	1-14
Form PCT/ISA/210 (fifth sheet) (July 2009)		

INTERNATIONAL SEARCH REPORT		International application No.	
Information on patent family members		PCT/AU2016/050150	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
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Publication Number	Publication Date	Publication Number	Publication Date
US 5728735 A	17 March 1998	US 5728735 A	17 Mar 1998
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		JP H03169813 A	23 Jul 1991
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		US 6271254 B1	07 Aug 2001
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		US 2015141500 A1	21 May 2015
WO 2013/168004 A2	14 November 2013	WO 2013168004 A2	14 Nov 2013
		US 2015111966 A1	23 Apr 2015
WO 2013/168011 A1	14 November 2013	WO 2013168011 A1	14 Nov 2013
		US 2015141451 A1	21 May 2015
		US 9273061 B2	01 Mar 2016
WO 2013/168010 A1	14 November 2013	WO 2013168010 A1	14 Nov 2013
		US 2015087674 A1	26 Mar 2015
End of Annex			
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. Form PCT/ISA/210 (Family Annex)(July 2009)			