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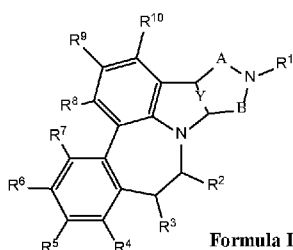
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(54) Title: PREVENTION AND TREATMENT OF NEURONAL DAMAGE WITH PYRIDOINDOLOBENZ[B, D] AZEPINE COMPOSITIONS



Formula I

(57) Abstract: A method of preventing or treating neuronal damage in a patient being administered a therapy that can cause nerve damage, comprising administering to said patient an effective amount of a neuroprotective compounds for Formula 1, (I) wherein Y is a single bond or a double bond; A and B are independently  $-(CH_2)_n-$ ; subscript 'n' varies from 0 to 3.



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PREVENTION AND TREATMENT OF NEURONAL DAMAGE WITH  
PYRIDOINDOLOBENZ[*b,d*]AZEPINE

This application claims benefit of priority based on provisional application No. 63/210,063 filed on June 13, 2021, and said application is incorporated herein by reference in its entirety.

CROSS-REFERENCE TO RELATED APPLICATIONS

Not applicable.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR  
DEVELOPMENT

Not applicable.

THE NAMES OF THE PARTIES TO A JOINT RESEARCH AGREEMENT

Not applicable.

INCORPORATION-BY-REFERECE OF MATERIAL SUBMITTED ON A COMPACT  
DISC

Not Applicable.

BACKGROUND OF THE INVENTION

Field of the Invention.

**[0001]** This invention relates to the suppression, prevention, or treatment of nerve and glial cell damages induced by physical (e.g., injury, trauma, or drug abuse), pathological (e.g., cancer, diabetes, or microbial infection), or clinical (e.g., chemo- or radiation therapy).

Description of the Related Art.

**[0002]** Various prior art references in the specification are indicated by italicized Arabic numerals in brackets. Full citation corresponding to each reference number is listed at the end of the specification and is herein incorporated by reference in its entirety to describe fully and clearly the state of the art to which this invention pertains.

**[0003]** Unless otherwise specified, all technical terms and phrases used herein conform to standard organic and medicinal chemistry nomenclature established by International Union of Pure and Applied Chemistry (IUPAC), the American Chemical Society (ACS), and other international professional societies. The rules of nomenclature are described in various publications, including, "Nomenclature of Organic Compounds," [1], and "Systematic Nomenclature of Organic Chemistry" [2], which are herein incorporated by reference in their entireties. For the purpose of this application, the word 'neuropathy' signifies inflammation of

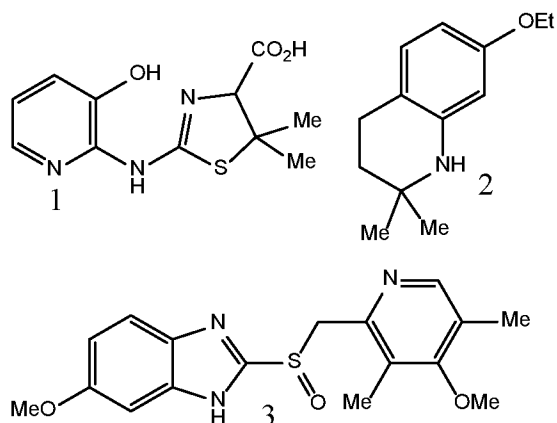
the nervous system; the word, 'neurotoxicity' or 'neuronal damage' signifies damages to central or peripheral nervous system that includes both neurons and glial cells; the word, 'neuroprotection' or 'neuroprotective' signifies prevention of damages to the structure and/or function of neurons or glial cells, or restoration of structure and function after said damages had occurred; the phrase, 'disease modifying,' signifies alleviation of root cause(s) of pain, namely, nerve damage or inflammation. The term "prevent or preventing" is meant either the reduction of or the complete blocking of a symptom and/or damage. The term "treatment" signifies the repair or restoration of damaged cells to their normal or near normal condition.

**[0004]** Pain is essentially a defense mechanism to protect the body from further damage, and typically persists until the healing process is completed. However, neuropathic pain is pathological and is only one among many cause of pain. Neuropathy is a consequence of nerve damage caused by various ailments including injuries, trauma, infections, cancer, cancer therapies, and metabolic disorders. It is a progressive, enduring, and often irreversible condition featuring pain, numbness, tingling, and sensitivity to cold in the hands and feet, which sometimes progresses to the arms and legs. It is a serious medical condition, affecting a significant portion of cancer and 25 % of diabetic patients. Chemotherapy induced neuropathy (CIN) is a painful and a major dose-limiting side effect of cancer treatment that can interrupt, delay, or compel premature cessation of therapy [3, 4]. Likewise, diabetic peripheral neuropathy (DPN) is also a debilitating condition that impairs quality of life. Chemotherapeutic drugs include paclitaxel, alumin-bound paclitaxel, docetaxel, doxorubicin, doxil, avastin, epirubicin, bortezomib, 5-fluorouracil, cyclophosphamide, cisplatin, oxaliplatin, carboplatin, vinorelbine, capecitabine, gemcitabine, ixabepilone, eribulin, irinotecan, etoposide, vinblastine, vincristine, tamoxifen, methotrexate, and pemetrexed.

**[0005]** Neuropathy is a highly complex event involving degradation of both neurons and glial cells, and is mediated by numerous receptors, enzymes, transporters, cell signaling molecules, and other factors [5]. Mika [6] demonstrated that modulation of glial cells can attenuate neuropathic pain symptoms. In necrosis, proinflammatory cytokines are released which produces neural inflammation and pain [7]. Chemotherapeutic drugs produce reactive species such as reactive oxygen species (hydroxyl radicals, hydroperoxyl radicals, and superoxide), nitric oxide, and the like that are injurious to both neurons and glial cells. Chronic neuropathic pain along with comorbid depression and anxiety are debilitating consequences of cancer, cancer therapy, diabetes, and viral infection [8].

**[0006]** Nerve degradation and pain are independent events. Although nerve damage leads to neuropathic pain, the perception of pain does not imply the existence of nerve damage.

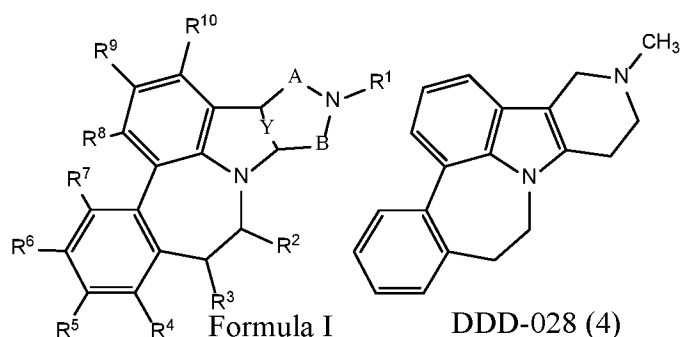
Further, in drug-induced neuropathies, some of the drugs are known to degrade mitochondria whereas others are known to damage axons, dendrites, or glial cells ultimately leading to necrotic cell death. At present, neuropathic pain is, in general, being managed with diverse CNS drugs such as opioids, cannabinoids (medical marijuana), gabapentin/pregabalin (epilepsy), duloxetine (anxiety), and amitriptyline (depression). Currently prescribed medications, oxycodone and duloxetine only relieve pain symptoms of CIPN; they do not block or suppress underlying nerve or glial damage. Scott et al. [9] disclose non-opioid analgesic 1 for neuropathic pain by activating the reactive species decomposition accelerant (RSDAx) pathway, but this compound has not demonstrated to be neuroprotective. Hoke et al. [10] disclose ethoxyquin (2) as a neuroprotective analgesic against cisplatin-induced CIPN that functions via the modulation of heat shock protein (Hsp). Melbrandt et al. [11] disclose neuroprotective agent 3 that prevents axonal degradation by inhibiting the SARM1 NADase



enzyme. However, no neuroprotective drugs are currently available for the treatment of neuropathic pain, including CIN and DPN. Hence, there is a considerable need for drugs that not only alleviate pain, but, more importantly, prevent neuron and glial cell damages.

## SUMMARY

**[0007]** It is well recognized in the art that relief of pain and prevention/treatment of nerve damage are independent, but related events. Pain sensations depend on specific physical, clinical, or pathological causes, and the perception of pain does not necessarily imply the existence of nerve damage, i.e., an individual may still experience non-neuropathic pain even after treating the nerve damage. Rajagopalan [12], which is incorporated herein by reference in its entirety, discloses pridoidolobenz[*b,d*]azepine derivatives of Formula 1 for the treatment of CNS disorders. One of the benzazepine derivatives, DDD-028 (4) has been shown to be a potent non-opioid analgesic in various pain models [13], viz., chronic



constriction injury (CCI), spinal nerve ligation (SNL), complete Freund's adjuvant (CFA) induced inflammation, and monosodium iodoacetate (MIA) induced osteoarthritis. However, Rajagopalan neither demonstrated nor disclosed the use of said benzazepine derivatives for the prevention and treatment of nerve or glial cell damage. Clearly, prevention of neuronal damage cannot be predicted from their analgesic effect. Therefore, the present invention relates to the method of treating or preventing neuronal damage in patients afflicted with or potentially encounter neuropathic condition by administering effective amount of pharmaceutically acceptable compositions of Formula 1, wherein Y is a single bond or a double bond; A and B are independently  $-(CH_2)_n-$ ; and subscript 'n' varies from 0 to 3. Each of the substituents,  $R^1$  to  $R^{10}$ , is independently selected to optimize efficacy and/or safety.

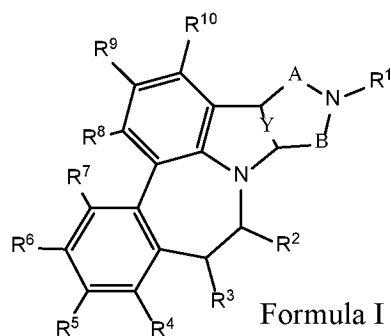
**[0008]** The present invention further relates to the method of treating or preventing neuronal damage comprising administering an effective amount of compounds of Formula I in combination with other neuroprotective agents (e.g. RXDAx accelerants), analgesics (e.g. opioids), anti-epileptics (pregabalin), or antidepressants (e.g., duloxetine) to elicit additive or synergistic effect.

#### BRIEF DESCRIPTION OF FIGURES

- [0009]** FIG. 1. Acute analgesic effect in paclitaxel CIN model.
- [0010]** FIG. 2. Prophylactic analgesic effect in CIN pain model.
- [0011]** FIG. 3. Neuroprotective effect: oxidative stress.
- [0012]** FIG. 4. Neuroprotective effect: reactive oxygen species (ROS).
- [0013]** FIG. 5. Neuroprotective effect: glial cell proliferation and morphology, spinal cord.
- [0014]** FIG. 6. Neuroprotective effect: glial cell proliferation, brain.
- [0015]** FIG. 7. Neuroprotective effect: intraepidermal nerve fiber (iENF) density.
- [0016]** FIG. 8 Neuroprotective effect: neurofilament heavy and light chain assays.
- [0017]** FIG. 9 Neuroprotective effect: myelin degradation.
- [0018]** FIG. 10 Pharmacodynamic mechanism of DDD-028.

## DETAILED DESCRIPTION

[0019] The present invention relates to the method of preventing or treating neuronal damage comprising administering an effective dose of Formula 1,



wherein Y is a single bond or a double bond; A and B are independently  $-(CH_2)_n-$ ; subscript 'n' varies from 0 to 3; and each of  $R^1$  to  $R^{10}$  is independently selected from the group consisting of hydrogen,  $C_1$ - $C_{10}$  alkyl,  $C_1$ - $C_{10}$  acyl, hydroxyl, amino, halo, cyano, carboxyl,  $C_1$ - $C_{10}$  alkoxy,  $C_1$ - $C_{10}$  hydroxyalkyl,  $C_1$ - $C_{10}$  alkoxy carbonyl,  $C_1$ - $C_{10}$  alkoxy carbonylalkyl;  $C_1$ - $C_{10}$  carbamoylalkyl;  $C_5$ - $C_{10}$  aryl unsubstituted or substituted with electron donating groups (EDG) or electron withdrawing groups (EWG),  $C_5$ - $C_{10}$  arylalkyl wherein the aryl portion is unsubstituted or substituted with EDG or EWG);  $C_5$ - $C_{10}$  aroylalkyl wherein the aryl portion is unsubstituted or substituted with EDG or EWG). The phrase, 'electron donating group (EDG)' and 'electron withdrawing group (EWG)' are well understood in the art. EDG comprises alkyl, hydroxyl, alkoxy, amino, acyloxy, acylamino, mercapto, alkylthio, and the like. EWG comprises halogen, acyl, nitro, cyano, carboxyl, alkoxy carbonyl, and the like.

[0020] The present invention further relates to the method of preventing or treating neuronal damage comprising administering an effective dose of a combination of Formula 1 and another neuroprotective and/or analgesic agent, wherein said neuroprotective agents comprise RXDAx accelerants, SARM1 NADase inhibitors, Hsp modulators, and the like; and analgesic agents comprise opioids such as, but not limited to, morphine or oxycodone; cannabinoids such as, but not limited to, cannabidiol; anti-epileptic agents such as, but not limited to, pregabalin or gabapentin, anti-depressants such as, but not limited to duloxetine.

[0021] One embodiment of the present invention is related to the prevention or treatment of neuronal damage by administering an effective amount of Compound 5 of Formula I, wherein,

$R^1$  is selected from the group consisting of hydrogen,  $C_1$ - $C_{10}$  alkyl,  $C_1$ - $C_{10}$  hydroxyalkyl,  $C_5$ - $C_{10}$ ,  $C_1$ - $C_{15}$  aroylalkyl,  $C_1$ - $C_{10}$  alkoxy carbonylalkyl,  $C_5$ - $C_{10}$  arylalkyl, and  $C_1$ - $C_{10}$  carbamoylalkyl;

$R^2$  is selected from the group consisting of hydrogen,  $C_1$ - $C_{10}$  alkyl,  $C_5$ - $C_{10}$  aryl, and  $C_5$ - $C_{10}$  arylalkyl;

each of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^8$ ,  $R^9$ , and  $R^{10}$  is independently selected from the group consisting of hydrogen,  $C_1$ - $C_{10}$  alkyl, hydroxyl,  $C_1$ - $C_{10}$  alkoxy,  $-NR^{11}R^{12}$ ,  $C_1$ - $C_{10}$  hydroxyalkyl, halogen, trihaloalkyl, cyano, carboxyl,  $C_1$ - $C_{10}$  acyl,  $C_1$ - $C_{10}$  alkoxyalkyl;  $C_1$ - $C_{10}$  alkoxy carbonyl,  $C_5$ - $C_{10}$  aryl, and  $C_5$ - $C_{10}$ ; and

$R^{11}$  and  $R^{12}$  are independently hydrogen or  $C_1$ - $C_{10}$  alkyl.

**[0022]** Another embodiment is related to the prevention or treatment of neuronal damage by administering an effective amount of Compound 6 of Formula I, wherein

Y is a double bond;

A is  $-CH_2-$ ; and

B is  $-CH_2CH_2-$ .

**[0023]** Another embodiment is related to the prevention or treatment of neuronal damage by administering an effective amount of Compound 7 of Formula I, wherein

Y is a double bond;

A is  $-CH_2-$ ;

B is  $-CH_2CH_2-$ ;

$R^1$  is selected from the group consisting of hydrogen,  $C_1$ - $C_{10}$  alkyl,  $C_1$ - $C_{10}$  hydroxyalkyl,  $C_5$ - $C_{10}$  arylalkyl, and  $C_1$ - $C_{10}$  carbamoylalkyl;

$R^2$  and  $R^3$  are hydrogens; and

each of  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^8$ ,  $R^9$ , and  $R^{10}$  is independently selected from the group consisting of hydrogen,  $C_1$ - $C_{10}$  alkyl, hydroxyl,  $C_1$ - $C_{10}$  alkoxy, and halogen.

**[0024]** Another embodiment is related to the prevention or treatment of neuronal damage by administering an effective amount of Compound 8 of Formula I, wherein

Y is a double bond;

A is  $-CH_2-$ ;

B is  $-CH_2CH_2-$ ;

each of  $R^4$ ,  $R^5$ ,  $R^6$ , and  $R^7$  is independently selected from the group consisting of hydrogen,  $C_1$ - $C_{10}$  alkyl, hydroxyl,  $C_1$ - $C_{10}$  alkoxy, and halogen;

$R^8$ ,  $R^9$ , and  $R^{10}$  are hydrogens;

optionally wherein

$R^1$  is hydrogen,  $C_1$ - $C_{10}$  alkyl,  $CH_2Ph$ ,  $CH_2CH_2OH$ , or  $CH_2CONH_2$ ; and

each of  $R^4$ ,  $R^5$ ,  $R^6$ , and  $R^7$  is hydrogen,  $CH_3$ , hydroxyl,  $OCH_3$ , F, or Cl.

**[0025]** Another embodiment is related to the prevention or treatment of neuronal damage

by administering an effective amount of Compound 9 of Formula I, wherein

Y is a double bond;

A is  $-\text{CH}_2-$ ;

B is  $-\text{CH}_2\text{CH}_2-$ ;

$\text{R}^4$ ,  $\text{R}^5$ ,  $\text{R}^6$ , and  $\text{R}^7$  are hydrogens; and

each of  $\text{R}^8$ ,  $\text{R}^9$ , and  $\text{R}^{10}$  is independently selected from the group consisting of hydrogen,  $\text{C}_1$ - $\text{C}_{10}$  alkyl, hydroxyl,  $\text{C}_1$ - $\text{C}_{10}$  alkoxy, and halogen, optionally wherein

$\text{R}^1$  is hydrogen,  $\text{C}_1$ - $\text{C}_{10}$  alkyl,  $\text{CH}_2\text{Ph}$ ,  $\text{CH}_2\text{CH}_2\text{OH}$ , or  $\text{CH}_2\text{CONH}_2$ ; and

each of  $\text{R}^8$ ,  $\text{R}^9$ , and  $\text{R}^{10}$  is hydrogen,  $\text{CH}_3$ , hydroxyl,  $\text{OCH}_3$ , F, or Cl, in particular optionally wherein  $\text{R}^1$  is hydrogen or  $\text{CH}_3$ ; and

$\text{R}^8$ ,  $\text{R}^9$ , and  $\text{R}^{10}$  are hydrogens.

**[0026]** Another embodiment is related to the prevention or treatment of neuronal damage by administering an effective amount of Compound 10 of Formula I, wherein

Y is a single bond with *cis*-fused B|C rings;

A is  $-\text{CH}_2-$ ;

B is  $-\text{CH}_2\text{CH}_2-$ ;

**[0027]** Another embodiment is related to the prevention or treatment of neuronal damage by administering an effective amount of Compound 11 of Formula I, wherein

Y is a single bond with *cis*-fused B|C rings;

A is  $-\text{CH}_2-$ ;

B is  $-\text{CH}_2\text{CH}_2-$ ;

$\text{R}^1$  is selected from the group consisting of hydrogen,  $\text{C}_1$ - $\text{C}_{10}$  alkyl,  $\text{C}_1$ - $\text{C}_{10}$  hydroxyalkyl,  $\text{C}_5$ - $\text{C}_{10}$  arylalkyl, and  $\text{C}_1$ - $\text{C}_{10}$  carbamoylalkyl;

$\text{R}^2$  and  $\text{R}^3$  are hydrogens; and

each of  $\text{R}^4$ ,  $\text{R}^5$ ,  $\text{R}^6$ ,  $\text{R}^7$ ,  $\text{R}^8$ ,  $\text{R}^9$ , and  $\text{R}^{10}$  is independently selected from the group consisting of hydrogen,  $\text{C}_1$ - $\text{C}_{10}$  alkyl, hydroxyl,  $\text{C}_1$ - $\text{C}_{10}$  alkoxy, and halogen.

**[0028]** Another embodiment is related to the prevention or treatment of neuronal damage by administering an effective amount of Compound 12 of Formula I, wherein

Y is a single bond with *cis*-fused B|C rings;

A is  $-\text{CH}_2-$ ;

B is  $-\text{CH}_2\text{CH}_2-$ ;

each of  $\text{R}^4$ ,  $\text{R}^5$ ,  $\text{R}^6$ , and  $\text{R}^7$  is independently selected from the group consisting of hydrogen,  $\text{C}_1$ - $\text{C}_{10}$  alkyl, hydroxyl,  $\text{C}_1$ - $\text{C}_{10}$  alkoxy, and halogen; and



$R^8$ ,  $R^9$ , and  $R^{10}$  are hydrogens,

optionally wherein

$R^1$  is hydrogen,  $C_1$ - $C_{10}$  alkyl,  $CH_2Ph$ ,  $CH_2CH_2OH$ , or  $CH_2CONH_2$  and

each of  $R^4$ ,  $R^5$ ,  $R^6$ , and  $R^7$  is hydrogen,  $CH_3$ , hydroxyl,  $OCH_3$ , F, or Cl,

optionally wherein

$R^4$ ,  $R^5$ ,  $R^6$ , and  $R^7$  are hydrogens; and

each of  $R^8$ ,  $R^9$ , and  $R^{10}$  is independently selected from the group consisting of hydrogen,  $C_1$ - $C_{10}$  alkyl, hydroxyl,  $C_1$ - $C_{10}$  alkoxy, and halogen,

optionally wherein

$R^1$  is hydrogen,  $C_1$ - $C_{10}$  alkyl,  $CH_2Ph$ ,  $CH_2CH_2OH$ , or  $CH_2CONH_2$ ; and

each of  $R^8$ ,  $R^9$ , and  $R^{10}$  is hydrogen,  $CH_3$ , hydroxyl,  $OCH_3$ , F, or Cl, in particular

optionally wherein  $R^1$  is hydrogen or  $CH_3$ ; and

$R^8$ ,  $R^9$ , and  $R^{10}$  are hydrogens.

**[0029]** Another embodiment is related to the prevention or treatment of neuronal damage by administering an effective amount of Compound 13 of Formula I, wherein

Y is a single bond with *trans*-fused B|C rings;

A is  $-CH_2-$ ;

B is  $-CH_2CH_2-$ .

**[0030]** Another embodiment is related to the prevention of neuronal damage by administering an effective amount of Compound 14 of Formula I, wherein

Y is a single bond with *trans*-fused B|C rings;

A is  $-CH_2-$ ;

B is  $-CH_2CH_2-$ .

$R^1$  is selected from the group consisting of hydrogen,  $C_1$ - $C_{10}$  alkyl,  $C_1$ - $C_{10}$  hydroxyalkyl,  $C_5$ - $C_{10}$  arylalkyl, and  $C_1$ - $C_{10}$  carbamoylalkyl;

$R^2$  and  $R^3$  are hydrogens; and

each of  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^8$ ,  $R^9$ , and  $R^{10}$  is independently selected from the group consisting of hydrogen,  $C_1$ - $C_{10}$  alkyl, hydroxyl,  $C_1$ - $C_{10}$  alkoxy, and halogen.

**[0031]** Another embodiment is related to the prevention or treatment of neuronal damage by administering an effective amount of Compound 15 of Formula I, wherein

Y is a single bond with *trans*-fused B|C rings;

A is  $-CH_2-$ ;

B is  $-CH_2CH_2-$ .

each of R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, and R<sup>7</sup> is independently selected from the group consisting of hydrogen, C<sub>1</sub>-C<sub>10</sub> alkyl, hydroxyl, C<sub>1</sub>-C<sub>10</sub> alkoxy, and halogen; and

R<sup>8</sup>, R<sup>9</sup>, and R<sup>10</sup> are hydrogens,

optionally wherein

R<sup>1</sup> is hydrogen, C<sub>1</sub>-C<sub>10</sub> alkyl, CH<sub>2</sub>Ph, CH<sub>2</sub>CH<sub>2</sub>OH, or CH<sub>2</sub>CONH<sub>2</sub>; and

each of R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, and R<sup>7</sup> is hydrogen, CH<sub>3</sub>, hydroxyl, OCH<sub>3</sub>, F, or Cl;

wherein

R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, and R<sup>7</sup> are hydrogens; and

each of R<sup>8</sup>, R<sup>9</sup>, and R<sup>10</sup> is independently selected from the group consisting of hydrogen, C<sub>1</sub>-C<sub>10</sub> alkyl, hydroxyl, C<sub>1</sub>-C<sub>10</sub> alkoxy, and halogen,

optionally wherein

R<sup>1</sup> is hydrogen, C<sub>1</sub>-C<sub>10</sub> alkyl, CH<sub>2</sub>Ph, CH<sub>2</sub>CH<sub>2</sub>OH, or CH<sub>2</sub>CONH<sub>2</sub>; and

each of R<sup>8</sup>, R<sup>9</sup>, and R<sup>10</sup> is hydrogen, CH<sub>3</sub>, hydroxyl, OCH<sub>3</sub>, F, or Cl, in particular

optionally wherein

R<sup>1</sup> is hydrogen, C<sub>1</sub>-C<sub>10</sub> alkyl, CH<sub>2</sub>Ph, CH<sub>2</sub>CH<sub>2</sub>OH, or CH<sub>2</sub>CONH<sub>2</sub>; and

each of R<sup>8</sup>, R<sup>9</sup>, and R<sup>10</sup> is hydrogen, CH<sub>3</sub>, hydroxyl, OCH<sub>3</sub>, F, or Cl, in particular

optionally wherein R<sup>1</sup> is hydrogen or CH<sub>3</sub>; and

R<sup>8</sup>, R<sup>9</sup>, and R<sup>10</sup> are hydrogens.

**[0032]** Another embodiment is related to the prevention or treatment of neuronal damage by administering an effective amount of Compound 16 of Formula I, wherein

R<sup>1</sup> is hydrogen, methyl, n-propyl, iso-propyl, n-butyl, sec-butyl, tert-butyl, or iso-butyl, preferably hydrogen, methyl, n-propyl, or iso-propyl, more preferably hydrogen or methyl, and most preferably methyl;

each of R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, R<sup>8</sup>, R<sup>9</sup>, and R<sup>10</sup> is hydrogen;

Y is a double bond;

A is -CH<sub>2</sub>-; and

B is -CH<sub>2</sub>CH<sub>2</sub>-.

**[0033]** Another embodiment is related to the prevention or treatment of neuronal damage by administering an effective amount of Compound 4 (DDD-028) of Formula I, wherein

R<sup>1</sup> is methyl;

each of R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, R<sup>8</sup>, R<sup>9</sup>, and R<sup>10</sup> is hydrogen;

Y is a double bond;

A is -CH<sub>2</sub>-; and

B is -CH<sub>2</sub>CH<sub>2</sub>-.

**[0034]** Another embodiment is related to the prevention of CIN and DPN by administering an effective amount of a combination of compound Formula I and a neuroprotective agent comprising RXDAx accelerants, SARM1 NADase inhibitors, or Hsp modulators.

**[0035]** Another embodiment is related to the prevention of CIN and DPN by administering an effective amount of a combination of compound Formula I and an analgesic agent comprising opioids, cannabinoids, antiepileptics, or antidepressants.

**[0036]** Another embodiment is related to the prevention of CIN and DPN by administering effective amounts of DDD-028 (compound 4) to patients undergoing or about to start chemotherapy.

**[0037]** Another embodiment is related to the prevention of CIN and DPN by administering effective amounts of DDD-028 (compound 4) and a neuroprotective agent comprising RXDAx accelerants, SARM1 NADase inhibitors, or Hsp modulators to patients undergoing or about to start chemotherapy.

**[0038]** Another embodiment is related to the prevention of CIN and DPN by administering effective amounts of DDD-028 (compound 4) and an analgesic agent comprising opioids, cannabinoids, antiepileptics, or antidepressants.

**[0039]** We had previously reported that DDD-028 potent, non-opioid analgesic in several models of neuropathic pain [13]. We now demonstrate that DDD-028 is not only a potent analgesic in the CIN model, but, more importantly, it prevents both glial and nerve cell damages. In all the three well-established tests for analgesic activity (viz., Paw Pressure for mechanical hyperalgesia, Von Frey for mechanical allodynia, and Cold Plate for Thermal allodynia), DDD-028 was able to recover, dose dependently, paclitaxel-induced hypersensitivity as well as to prevent the development of neuropathic symptoms when it was co-administered with the chemotherapeutic drug (Fig. 1). Moreover, in the sub-chronic administration of DDD-028 (Fig. 2), indicated that the animals did not develop tolerance to the antinociceptive effect exerted in paclitaxel-treated mice, in contrast to other known antinociceptive or analgesic drugs, such as morphine, which induces tolerance after repeated administration both in naïve animals and in mice and rats treated with paclitaxel representing one of the most limiting side effects of opioids.

**[0040]** The neuroprotective activity of one of the compounds of Formula 1, viz., DDD-028, in rat model of CIN has been demonstrated using many well-accepted assays using the brain and spinal cord and plasma: these test comprise glial cell proliferation, glial cell damage, protein degradation, (i.e., oxidative stress), peroxisome activity, nerve conduction, and nerve fiber density. DDD-028 prevented oxidative stress induced by paclitaxel (Figs. 3, 4). DDD-

028 blocks the proliferation of glial cells both in the peripheral regions as well in the brain (Fig. 5), and prevents astrocyte damage (Fig. 5, 6). DDD-028 blocks palitaxel induced nerve degeneration as shown by three immunohistopathological studies (Fig. 7-9). Thus, collectively these data firmly demonstrate that DDD-028 is both an analgesic and a neuroprotective agent. It is believed that dual action of DDD-028 is likely mediated via nicotinic acetylcholine pathway, particularly the  $\alpha 7$ NACChR (Fig. 10).

**[0041]** Early toxicity studies indicated that DDD-028 is well tolerated, does not induce sedation, and appears to be safe at the expected dose regimen. Finally, the Lipinski-Veber rules for drug-like properties, and the CNS multiparameter optimization (CNS MPO) value of 4.2 places DDD-028 in the high desirability range for CNS drugs. In addition to the fact the compounds of the present invention are structurally different from those disclosed by others, the neuroprotective activity of DDD-028 is also unlike those of the prior art in that DDD-028 elicits neuroprotective effect by inhibiting the damage to both nerve and glial cells.

**[0042]** The compounds of the present invention represented by Formula I, commonly referred to as ‘active pharmaceutical ingredient (API)’ or ‘drug substance’ is typically formulated as pharmaceutically acceptable salts. The phrase "pharmaceutically acceptable" means those formulations which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts include, but are not limited to acetate, adipate, citrate, tartarate, benzoate, phosphate, glutamate, gluconate, fumarate, maleate, succinate, oxalate, chloride, bromide, hydrochloride, sodium, potassium, calcium, magnesium, or ammonium.

**[0043]** The final formulated product, commonly referred to as ‘drug product,’ may be administered enterally, parenterally, or topically. Enteral route includes oral, rectal, topical, buccal, ophthalmic, and vaginal administration. Parenteral route includes intravenous, intramuscular, intraperitoneal, intrasternal, and subcutaneous injection or infusion. The drug product may be delivered in solid, liquid, or vapor forms, or can be delivered through a catheter for local delivery at a target. Topical delivery may also include transdermal patches or transdermal microneedles for subcutaneous delivery. Transdermal patch and microneedle technology described by Tran et al. [14] and is incorporated by reference in entirety.

**[0044]** The dosage levels of API in the drug product can be varied so as to achieve the desired therapeutic response for a particular patient. The phrase "effective amount" or “effective dose” of the compound of the invention means a sufficient amount of the

compound is administered to treat disorders, at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated, the severity of the disorder; activity of the specific compound employed; the specific composition employed, age, body weight, general health, sex, diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed, and the duration of the treatment. The total daily dose of the compounds of this invention administered may range from about 0.0001 to about 1000 mg/kg/day. For oral administration, preferable doses can be in the range from about 0.001 to about 5 mg/kg/day. If desired, the effective daily dose can be divided into multiple doses for optimal therapeutic effect.

**[0045]** The compositions of the present invention may be administered to any patient experiencing or expected to encounter neuropathy regardless of its cause. The term “chemotherapy co-treatment” means giving the anti-cancer compound to the patient before, during, or after administration of a dose of chemotherapy, in a manner that prevents CIN. The phrase “before administration” of a dose of chemotherapy may include a several seconds to several hours (such as 24 hours) before the chemotherapy. “During” administration of a dose of chemotherapy may include a specific administration given at any time during the chemotherapy, or co-administration for the duration of the chemotherapy administration (such as by combining the two drugs in the same infusion bag). “After” administration of a dose of chemotherapy may include administration several minutes or hours after the chemotherapy, and may include administration on a schedule for a number of days after the dose of chemotherapy.

**[0046]** Formulations for oral administration include capsules (soft or hard), tablets, pills, powders, and granules. Such formulations may comprise the API along with at least one inert, pharmaceutically acceptable ingredients selected from the following: (a) buffering agents such as sodium citrate or dicalcium phosphate; (b) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid; (c) binders such as carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose and acacia; (d) humectants such as glycerol; (e) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates and sodium carbonate; (f) solution retarding agents such as paraffin; (g) absorption accelerators such as quaternary ammonium

compounds; (h) wetting agents such as cetyl alcohol and glycerol monostearate; (i) absorbents such as kaolin and bentonite clay and (j) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; (k) coatings and shells such as enteric coatings, flavoring agents, and the like.

**[0047]** Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups and elixirs. In addition to the API, the liquid dosage forms may contain inert diluents, solubilizing agents, wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents used in the art.

**[0048]** Compositions suitable for parenteral injection may comprise physiologically acceptable, sterile aqueous or nonaqueous isotonic solutions, dispersions, suspensions or emulsions, and sterile powders for reconstitution into sterile injectable solutions or dispersions. The compositions may also optionally contain adjuvants such as preserving; wetting; emulsifying; dispensing, and antimicrobial agents, buffers, diluents, carriers, adjuvants, preservatives, and excipients. Examples of suitable carriers, diluents, solvents, vehicles, or adjuvants include, but are not limited to water; ethanol; polyols such as propyleneglycol, polyethyleneglycol, glycerol, and the like; vegetable oils such as cottonseed, groundnut, corn, germ, olive, castor and sesame oils, and the like; organic esters such as ethyl oleate and the like; phenol, parabens, sorbic acid, and the like.

**[0049]** Injectable formulations may also be suspensions that contain suspending agents such as ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, or mixtures of these substances, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin. Proper fluidity can be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants. In some cases, in order to prolong the effect of the drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This can be accomplished by the use of a liquid suspension. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

**[0050]** Injectable depot forms are made by forming microencapsulated matrices of the drug in biodegradable polymers. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, these compositions release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Thus,

the rate of drug release and the site of delivery can be controlled. Examples of embedding compositions include, but are not limited to polylactide-polyglycolide poly(orthoesters), and poly(anhydrides), and waxes. The technology pertaining to controlled release formulations are described in “Design of Controlled Release Drug Delivery Systems,” [15] incorporated herein by reference in its entirety.

**[0051]** Formulations for topical administration include powders, sprays, ointments and inhalants. These formulations include the API along with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at room temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

**[0052]** Compounds of the present invention can also be administered in the form of liposomes. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain, in addition to a compound of the present invention, stabilizers, preservatives, excipients and the like. The preferred lipids are natural and synthetic phospholipids and phosphatidyl cholines (lecithins) used separately or together. Methods to form liposomes are known in the art and are described in “Liposomes,” [16], which is incorporated herein by reference in its entirety.

**[0053]** The compounds of the present invention can also be administered to a patient in the form of pharmaceutically acceptable ‘prodrugs.’ Prodrugs are generally used to enhance the bioavailability, solubility, in vivo stability, or any combination thereof of the API. They are typically prepared by linking the API covalently to a biodegradable functional group such as a phosphate that will be cleaved enzymatically or hydrolytically in blood, stomach, or GI tract to release the API. A detailed discussion of the prodrug technology is described in “Prodrugs: Design and Clinical Applications,” [17] incorporated herein by reference.

**[0054]** The following examples illustrate specific embodiments and utilities of the invention, and are not meant to limit the invention. As would be apparent to skilled artisans, various modifications in the composition, operation, and method are possible, and are contemplated herein without departing from the concept and scope of the invention as defined in the claims. For all the experiments described in the following paragraphs, male Sprague–Dawley rats weighing approximately 200 – 250 g at the beginning of the experimental procedure were used. Animals were housed in CeSAL and used at least one week later after their arrival. Four rats were housed per cage (size 26×41 cm<sup>2</sup>), kept at 23±1 °C with a 12 h light/dark cycle, light at 7 a.m., and were fed with standard laboratory diet and tap water *ad libitum*. Experiments involving animals have been reported according to

ARRIVE guidelines. All efforts were made to minimize animal suffering and to reduce the number of animals used. Paclitaxel was dissolved in a mixture of 10% saline solution and Chremophor EL, a derivative of castor oil and ethylene oxide that is clinically used as paclitaxel vehicle. Control animals received an equivalent volume of the vehicle. The results were expressed as mean  $\pm$  S.E.M. Statistical analysis was performed using one-way ANOVA followed by post-hoc Bonferroni's significant difference procedure. The 'p' values of less than 0.05, 0.01 or 0.001 were considered significant. All data were collected by an observer who was blinded to the treatments.

#### Example 1

##### Acute Analgesic Effect of DDD-028 in CIN – Mechanical Hyperalgesia

**[0055]** The nociceptive threshold in the rat was determined with an analgesimeter (Ugo Basile, Varese, Italy) according to the method described by Leighton et al. [18]. Briefly, a constantly increasing pressure was applied to a small area of the dorsal surface of the hind paw using a blunt conical mechanical probe. Mechanical pressure was increased until vocalization or a withdrawal reflex occurred while rats were lightly restrained. Vocalization or withdrawal reflex thresholds were expressed in grams. These limits assured a more precise determination of mechanical withdrawal threshold in experiments aimed to determine the effect of treatments. An arbitrary cut-off value of 100 g was adopted. For these studies, DDD-028 was acutely *per os* administered when neuropathy was well established (day 10), 48 h after the last chemotherapeutic drug injection. On day 10, paclitaxel-treated rats showed a significantly reduction of the weight tolerated on posterior paws with respect to the control animals ( $43.2 \pm 0.5$  g vs  $66.5 \pm 0.7$  g, respectively). Increasing doses of DDD-028 (1 - 25 mg  $\text{kg}^{-1}$ ) reduced mechanical hypersensitivity in a dose-dependent manner starting 30 min after treatment. The highest dose of DDD-028 (25 mg  $\text{kg}^{-1}$ ) completely abrogated paclitaxel-induced mechanical hyperalgesia with maximum analgesic effect occurring at 30 min (Fig. 1a). The effect persisted for at least 90 min and then vanished 120 min after treatment. Strong, albeit slightly reduced, analgesic effect was also observed at the doses of 10, 5, and 1 mg  $\text{kg}^{-1}$ , but the duration of the analgesic effect was reduced from about 30 - 90 min at 25 mg  $\text{kg}^{-1}$  to about 30 - 60 min at 1 mg  $\text{kg}^{-1}$  (Fig. 1).

#### Example 2

##### Acute Analgesic Effect of DDD-028 in CIN – Mechanical Allodynia

**[0056]** In the same way, acute administration of DDD-028 counteracted paclitaxel-induced mechanical allodynia in a dose-dependent manner in the Von Frey test (Fig. 1b). The highest dose again showed a complete reversal of paclitaxel-induced neuropathy with a long-lasting



effect starting from 30 min up to 90 min after treatment. All the lower doses of DDD-028 displayed a shorter anti-hyperalgesic efficacy (Fig. 1b). Finally, DDD-028 also counteracted paclitaxel-induced thermal allodynia in a dose-dependent manner in the Cold Plate test (Fig. 1c). As shown in Fig. 1c, paclitaxel alone significantly enhanced the sensitivity to cold after 10 days of treatment. Thermal allodynia was fully alleviated by DDD-028 (25 mg kg<sup>-1</sup>) administration. The result obtained with the higher dose was more effective and long-lasting with respect to the lower doses that were capable anyway to reach the statistical significance peaking 45 min after administration.

### Example 3

#### Acute Analgesic Effect of DDD-028 in CIN – Thermal Allodynia

[0057] Thermal allodynia was assessed using the Cold-plate test. With minimal animal-handler interaction, rats were taken from home-cages, and placed onto the surface of the cold-plate maintained at a constant temperature of  $4 \pm 1$  °C. Ambulation was restricted by a cylindrical Plexiglas chamber (diameter: 10 cm, height: 15 cm), with open top. A timer controlled by foot pedal began timing response latency from the moment the mouse was placed onto the cold-plate. Pain-related behavior (licking of the hind paw) was determined by recording the time (seconds) of the first sign of licking of the hind paw. The cut-off time of the latency of paw lifting or licking was set at 30 s. DDD-028 was able to recover, dose dependently, paclitaxel-induced hypersensitivity and prevent the development of neuropathic symptoms when it was co-administered with the chemotherapeutic drug (Fig. 1c).

### Example 4

#### Prophylactic Analgesic Effect of DDD-028 in CIN

[0058] Thereafter, to assess the protective profile of DDD-028, the compound was subjected to repeated administrations over an 18-day period. Paclitaxel-treated animals were administered daily with DDD-028 (10 mg kg<sup>-1</sup>, p.o.) starting from the same day of paclitaxel injection. The response to mechanical noxious stimulus was measured on days 10, 12 and 18, 24 h after the last treatment. As shown in Fig. 2a, DDD-028 significantly increased the pain threshold of paclitaxel-injected rats at all time points considered without development of tolerance to the anti-hypersensitivity effect. Repeated administration of DDD-028 induced similar results in reducing paclitaxel-induced mechanical and thermal allodynia and mechanical hyperalgesia (Figs. 2a-c) at all time points as evidenced by the Paw Pressure, Von Frey, and the Cold plate tests. The efficacy of DDD-028 was not different among 30 min and 24 h after treatment suggesting a stable improvement of the pain threshold.

### Example 5

## Neuroprotective Effect of DDD-028 in CIN – Oxidative Stress

[0059] Among the multiplicity of the pathophysiological mechanisms, this oxidative stress plays a crucial role in the generation of paclitaxel-induced neuropathy. On day 18, (i.e., the end of the experiments on sub-chronic analgesic effect of DDD-028), animals were sacrificed by decapitation. L4-L5 dorsal root ganglia (DRG), sciatic nerve, lumbar spinal cord and brain were collected, frozen using liquid nitrogen or fixed by immersion in 4% neutral buffered formalin. Sciatic nerve and DRGs protein extracts were quantified by bicinchoninic acid. Five µg of each sample were denatured by 6% SDS and derivatized by 15-minute incubation with 2-4 dinitrophenyl hydrazine (DNPH; Sigma-Aldrich, Italy) at room temperature. Samples were separated on a 4–12% sodium dodecyl sulfate (SDS)-polyacrylamide gel by electrophoresis and transferred onto nitrocellulose membranes (Biorad, Italy). Membranes were blocked with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) containing .1% Tween 20 (PBST) and then probed overnight with primary antibody specific versus DNPH (Sigma-Aldrich, Italy) 1:5000 in PBST/1% BSA. After washing with PBST, the membranes were incubated for 1 hour in PBST containing the appropriate horseradish peroxidase-conjugated secondary antibody (1:5000; Cell Signalling, USA) and again washed. ECL (Pierce, USA) was used to visualize the peroxidase-coated bands. Densitometric analysis was performed using the “Image J” analysis software and the density of all bands displayed in the lane is reported as a mean. Ponceau-stained membranes were used as loading control [4]. Formalin fixed cryostat sections (10 µm for brain and 5 µm for spinal cord) were incubated for 1 h in blocking solution (Bio-Optica; Italy) at room temperature, and thereafter sections were incubated for 24 h at 4 °C in PBST containing primary antisera and 5% normal donkey serum. The primary antibody was directed against Iba1 (rabbit antiserum, 1:500; Wako Chemicals, USA; [23]) for microglial staining and against glial fibrillary acidic protein (GFAP; rabbit antiserum, 1:500; Dako, USA; [28]) for astrocyte staining. After rinsing in PBST, sections were incubated in donkey anti-rabbit IgG secondary antibody labelled with Alexa Fluor 488 or 568 (1:1000, Invitrogen, USA) at room temperature for 1 h. For all immunohistochemical studies, negative control sections (no exposure to the primary antisera) were processed concurrently with the other sections. A single optical density value for the dorsal horns in each rat was obtained by averaging the two sides, and this value was compared to the homologous average values from the vehicle-treated animals. Images were acquired using a motorized Leica DM6000B microscope equipped with a DFC350FX camera. Morphological examination of microglia and astrocytes was assessed by inspection of at least three fields (40 × 0.75NA objective) in the dorsal horn of the spinal cord and brain

areas per section. The full specimen thicknesses were acquired as z-stack series, deconvolved using Huygens Professional software (SVI, The Netherlands) and displayed using ImageJ software. To evaluate the capability of DDD-028 to intervene against direct damages and against the maladaptive plasticity of the nervous system induced by paclitaxel, nervous tissues (brain, spinal cord, DRGs and sciatic nerve) were collected and analyzed at the end of the repeated treatment with the compound (day 18). Oxidative stress, a typical signature of chemotherapy-induced neurotoxicity, was measured in the peripheral nervous system. As shown in Figure 5, paclitaxel induced an oxidative damage of dorsal root ganglia (DRG) as indicated by three-fold increase in carbonylation of proteins. Treatment with DDD-028 resulted in a significant prevention of the damage as evidenced by protein carbonylation values similar to the control group (Fig. 3).

#### Example 6

##### Neuroprotective Effect of DDD-028 in CIN – Peroxisome Functionality

**[0060]** Enzymatic activity in both DRGs and sciatic nerve was measured in PBS using the homogenated tissues: the suspension was sonicated in ice using three 10 s bursts at high intensity with a 10 s cooling period between each burst and then centrifuged (13.000 x g for 15 min at 4°C). Catalase activity was measured in the supernatant by Amplex Red Catalase Assay Kit (Invitrogen, Monza, Italy) following the manufacturer's instructions. Protein concentration was quantified by bicinchoninic acid assay (Sigma-Aldrich, Milan, Italy). Catalase activity for each sample was normalized to protein concentration. Control conditions in the absence of treatment were set as 100%. DDD-028 was able to prevent the development of hypersensitivity, and to reduce paclitaxel-related damage to PNS and CNS when repeatedly administered with the anticancer drug. DDD-028 demonstrated detoxifying properties as indicated by the enhancing the activity of the peroxisomal enzyme, catalase, and reducing the protein oxidation in DRGs (Fig. 4).

#### Example 7

##### Neuroprotective Effect of DDD-028 in CIN – Astrocyte Proliferation, Spinal Cord

**[0061]** To determine whether neurochemical reorganization in the spinal cord occurs following DDD-028 repeated treatment, lumbar spinal cord sections were examined by immunohistochemistry using antibodies against GFAP and Iba1 to label astrocytes and microglia, respectively, which are non-neuronal cells strongly involved in chemotherapy-induced neuropathic pain. Astrocyte activation was measured as an increase in the number of GFAP-expressing cells in the dorsal horn of the spinal cord of treated rats. GFAP-positive cell number in superficial laminae of paclitaxel-treated rats was significantly greater than

vehicle-treated number at day 18 (Fig. 5a). Animals treated with paclitaxel + DDD-028 showed a significantly lower number of astrocytes characterized by a reactive phenotype. Moreover, spinal astrocytes presented altered morphology showing hypertrophy of the cell *body* and processes (Fig. 5c). Microglia activation was measured by the quantification of Iba1 positive cells in the spinal cord of treated rats. On day 18, paclitaxel treatment produced increased density of Iba1 positive cells in the dorsal horns of the lumbar spinal cord (Fig. 5b). Animals treated with paclitaxel + DDD-028 showed a significant prevention of microglia activation (Figure 5b). However, no hypertrophy of this type of glia cells was observed, and microglia possessed a highly ramified morphology similar to those in saline-treated rats. On the other hand, day 18 can be considered a late phase for microglia activation that is, according to the literature, strongly involved in the first days of treatment.

#### Example 8

##### Neuroprotective Effect of DDD-028 in CIN – Astrocyte Proliferation, Brain

**[0062]** To probe the effect of DDD-028 in the brain regions involved in pain sensation, a topographic analysis of microglia and astrocyte cells in three regions, the periaqueductal gray (PAG; involved in endogenous pain modulatory system), the thalamus and the somatosensory cortex (S1) were examined. Paclitaxel induced a significant numerical increase of both glial cell populations in brain areas, but a higher astrocyte activation was observed (Figs. 6a,b). DDD-028 reduced both microglia and astrocyte cell number increase (numerical activation) in PAG, thalamus and S1.

#### Example 9

##### Neuroprotective Effect of DDD-028 in CIN – Intraepidermal Nerve Fiber Density

**[0063]** Mice hind limb paw skin was placed overnight at 4 °C in 4% paraformaldehyde in PBS 1X, transferred to 30% sucrose overnight, frozen and cryosectioned at 50 µm transversal to long paw axis. Free floating sections were incubated in PBS containing 0.3% Triton X-100 (TBS) 1 hour at room temperature, then in the primary antibody panaxonal marker PGP9.5 (ab108986, rabbit monoclonal [EPR4118] 1:600, Abcam) overnight at room temperature. Afterward, sections were rinsed in PBS 1X and placed in a goat anti-rabbit IgG secondary antibody labelled with Alexa Fluor 488 (1:500), 2 hours at room temperature in the dark. To stain nuclei, sections were incubated with DAPI in PBS for 10 min. After three washes in PBS and a final wash in distilled water, slices were mounted using ProLong Gold (Life Technologies-ThermoFisher Scientific, Milan, Italy) as mounting medium. Digitalized images were collected at 200× total magnification by a motorized Leica DM6000B microscope equipped with a DFC350FX. Quantitative analysis of intraepidermal nerve fibers

(IENF) density (fibers/mm) was performed by collecting 6 independent fields in the skin of each animal and counting the number of single PGP9.5-positive fibers crossing the epidermis-dermis boundary (basal membrane) by using the software ImageJ (NIH, Bethesda, MD, USA). Secondary branching is excluded from quantification. The administration of paclitaxel in mice determined a significant reduction of the intraepidermal nerve fibers (IENF) density, calculated as the number of PGP9.5 positive fibers per mm of skin (Fig. 7b). DDD-028 was able to effectively counteract the loss of IENF caused by paclitaxel (Fig. 7d). Indeed, the number of nerve fibers crossing the epidermis resulted significantly increased in paclitaxel-treated mice as a result of DDD-028 (10 mg kg<sup>-1</sup>) repeated administration. In contrast, the loss of IENF observed after paclitaxel administration in mice was unaffected by pregabalin (30 mg kg<sup>-1</sup>) treatment (Fig. 7c).

#### Example 10

##### Neuroprotective Effect of DDD-028 in CIN – NF-H Density

**[0064]** Paclitaxel also induced a significantly reduction of the neurofilament heavy chain (NF-H) expression in the sciatic nerve (Fig. 8b) measured as a percent of fluorescence intensity in comparison to the control group (vehicle + vehicle-treated animals). Repeated daily administrations of DDD-028 were able to restore the loss of NF-H expression in both tissues analyzed (Fig. 8c). Pregabalin did not prevent the loss of NF-H expression in the sciatic nerve (Fig. 8d). Moreover, paclitaxel treatment determined a derangement of the nerve fibers disposition in the sciatic nerve that was restored by DDD-028 treatment.

#### Example 11

##### Neuroprotective Effect of DDD-028 in CIN – Plasma NF-L Levels

**[0065]** Neurofilament light chain (NF-L), a well-accepted marker of neurotoxicity, was increased in plasma of paclitaxel-treated mice. In DDD-028 treated mice, the plasma NF-L concentration is substantially lower than in paclitaxel-treated mice (Fig. 9), whereas in pregabalin treated animals NF-L level was not significantly different from paclitaxel-treated animals.

#### Example 12

##### Neuroprotective Effect of DDD-028 in CIN – Myelin Degradation

**[0066]** Effect of DDD-028 on morphology and morphometry of DRGs sciatic nerves of paclitaxel-treated mice. Paclitaxel 2.0 mg kg<sup>-1</sup> was dissolved in a mixture of 10% Cremophor EL and saline solution and intraperitoneally (i.p.) administered on four alternate days (1, 3, 5 and 8). DDD-028 (10 mg kg<sup>-1</sup>) was suspended in 1% carboxymethylcellulose sodium salt (CMC) and daily administered per os starting the first day of paclitaxel treatment and

continuing 1 week after the end of treatment (from day 1 to day 17). Animals were sacrificed on day 18, 24h after the last treatment. Sciatic nerves and DRGs were fixed in 2.5% glutaraldehyde in cacodylate buffer, pH 7.4, for 24 hours. Tissues were embedded in Epoxy resin; sections (0.8  $\mu\text{m}$ ) were cut using an ultramicrotome, stained with toluidine blue. DRGs were studied measuring the number of neurons showing multinucleolated nuclei as sign of toxic alteration; the soma area was also assessed stratifying neurons in small (<600 microm diameter), medium (>600<1200 microm diameter) and large (>1200 microm diameter). In the sciatic nerve, myelin area, number of fibers (separated by axon diameter) and axon diameters were analyzed. The results indicate that DDD-028 prevents axon degradation induced by paclitaxel (Fig. 10).

### Example 13

#### Possible Pharmacodynamic Mechanism of Compounds of Formula 1

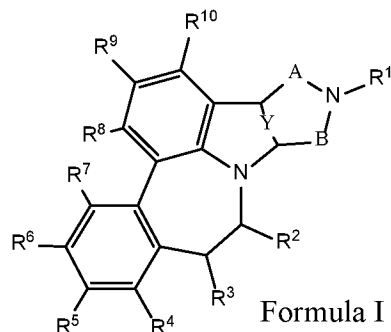
**[0067]** To study the pharmacodynamics of DDD-028, three possible targets were evaluated: the nicotinic receptor (nAChR) of the cholinergic system, the voltage-gated potassium channel subtype Kv7, and sigma 1 ( $\sigma_1$ ) and 2 ( $\sigma_2$ ) receptors. The relevance of the Kv7 was studied by using the selective Kv7 antagonist XE991 (1 mg/kg, i.p.). XE991 administered 15 min before DDD-028 was not able to alter the efficacy of DDD-028 over the time of observation (Fig. 11a). B on previous studies that  $\sigma_1$  receptor is involved in the pain pathway, DDD-028 was subjected to the modulation of the  $\sigma$  receptor-mediated neck dystonia. Accordingly, the  $\sigma$  agonist DTG was injected in the red nucleus. DTG induced postural changes characterized by a marked deviation in the head angle (neck dystonia), peaking at 25-30 min after microinjection. DDD-028 (25 mg kg<sup>-1</sup>) was administered *per os* 15 min before DTG infusion without preventing DTG-induced neck dystonia (Fig. 11b). Finally, in the Cold plate test, DDD-028 (25 mg kg<sup>-1</sup>, *per os*) increased the licking latency of paclitaxel treated animals starting 15 min after administration and lasting up to 105 min (Fig. 11c). Pre-treatment of the animals with the nAChR antagonist MECA (2 mg kg<sup>-1</sup>, i.p.) 15 min before DDD-028 administration completely abolished the pain-relieving effect of the compound up to 60 min (Fig. 11c). However, when MECA was administered the second time at 45 min after DDD-028 treatment, the effect of DDD-028 was entirely blocked for all the times observed. The anti-hyperalgesic effect induced by DDD-028 was also substantially reduced by the pre-treatment with the  $\alpha_7$  nAChR antagonist MLA (6 mg kg<sup>-1</sup>, i.p.) (Fig. 11c), which suggests that this receptor subtype is very likely involved in the mechanism of action of DDD-028.

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We claim:

1. A method of preventing or treating neuronal damage in a patient being administered a therapy that can cause neuronal damage, comprising administering to said patient an effective amount of a neuroprotective composition of Formula 1, wherein



Y is a single bond or a double bond;

A and B are independently  $-(CH_2)_n-$ ;

subscript 'n' varies from 0 to 3;

$R^1$  is selected from the group consisting of hydrogen,  $C_1$ - $C_{10}$  alkyl,  $C_1$ - $C_{10}$  hydroxyalkyl,  $C_5$ - $C_{10}$  aryl,  $C_1$ - $C_{15}$  aroylalkyl,  $C_1$ - $C_{10}$  alkoxyalkyl,  $C_5$ - $C_{10}$  arylalkyl, and  $C_1$ - $C_{10}$  carbamoylalkyl;

$R^2$  is selected from the group consisting of hydrogen,  $C_1$ - $C_{10}$  alkyl,  $C_5$ - $C_{10}$  aryl, and  $C_5$ - $C_{10}$  arylalkyl;

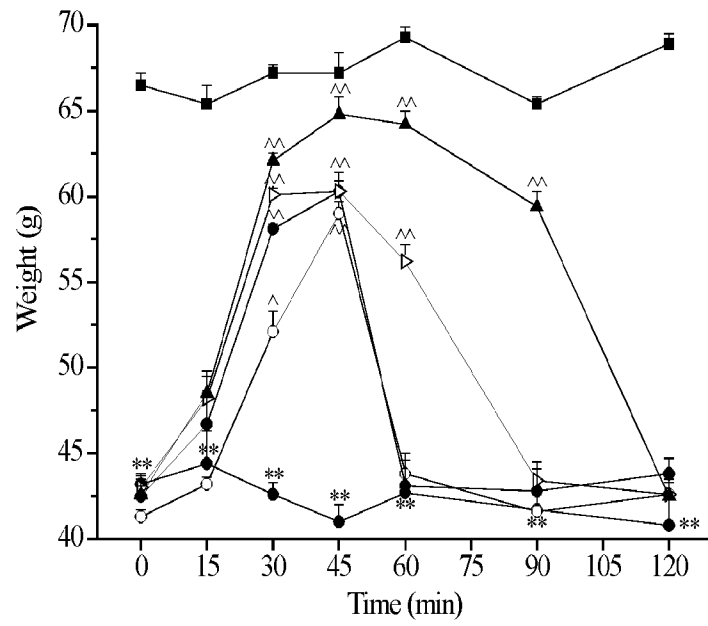
each of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^8$ ,  $R^9$ , and  $R^{10}$  is independently selected from the group consisting of hydrogen,  $C_1$ - $C_{10}$  alkyl, hydroxyl,  $C_1$ - $C_{10}$  alkoxy,  $-NR^{11}R^{12}$ ,  $C_1$ - $C_{10}$  hydroxyalkyl, halogen, trihaloalkyl, cyano, carboxyl,  $C_1$ - $C_{10}$  acyl,  $C_1$ - $C_{10}$  alkoxyalkyl;  $C_1$ - $C_{10}$  alkoxyalkyl,  $C_5$ - $C_{10}$  aryl, and  $C_5$ - $C_{10}$  arylalkyl; and

$R^{11}$  and  $R^{12}$  are independently hydrogen or  $C_1$ - $C_{10}$  alkyl.

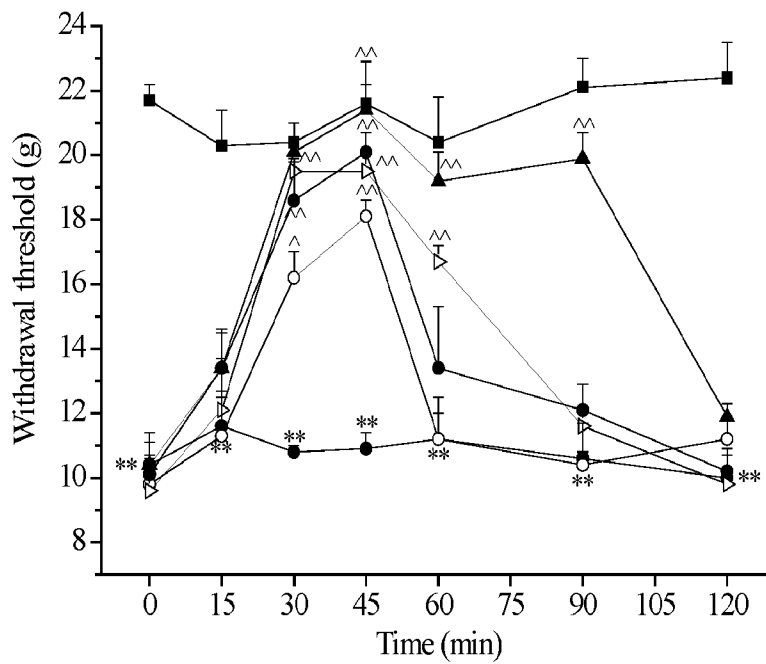
2. The method of claim 1, wherein Y is a double bond; A is  $-CH_2-$ ; and B is  $-CH_2CH_2-$ .
3. The method of claim 2, wherein said neuroprotective compound is selected from the group consisting of compounds 4, 6, 7, 8, and 9.
4. The method of claim 3, wherein said neuroprotective compound is 4, DDD-028.
5. The method of claim 1, wherein Y is a single bond; A is  $-CH_2-$ ; and B is  $-CH_2CH_2-$ .
6. The method of claim 5, wherein said neuroprotective compound is selected from the group consisting of compounds 10, 11, 12, 13, 14, and 15
7. The method of claim 1, wherein the neuronal damage is caused by cancer.
8. The method of claim 1, wherein the neuronal damage is caused by the administration of a chemotherapeutic agent to a patient undergoing cancer chemotherapy.



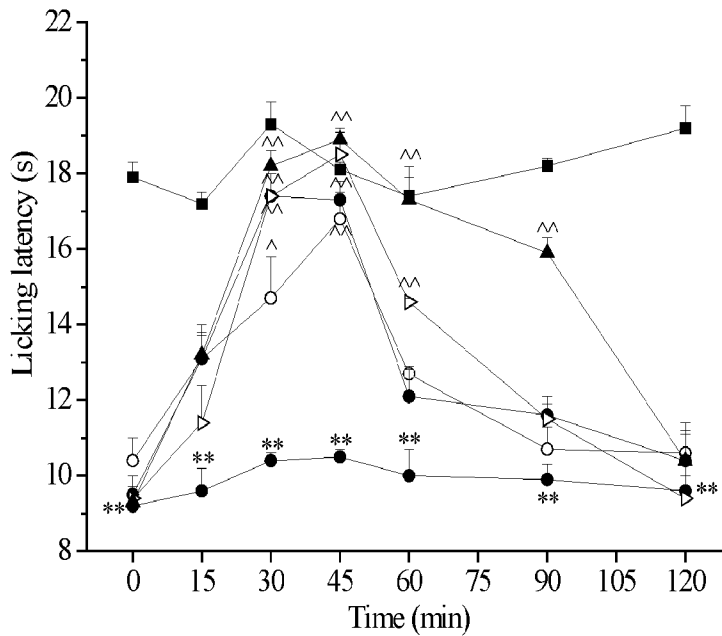
9. The method of claim 8, wherein the chemotherapeutic agent is paclitaxel, albumin-bound paclitaxel, doxorubicin, cisplatin, or carboplatin.
10. The method of claim 1, wherein the neuronal damage is caused by diabetes.
11. The method of claim 1, wherein the neuronal damage is caused by microbial infection.
12. The method of claim 1, wherein said neuroprotective composition comprises compounds Formula 1 and a second neuroprotective compound is selected from the group consisting of RXDAx accelerants, SARM1 NADase inhibitors, and Hsp modulators.
13. The method of claim 12, wherein said second neuroprotective compound is selected from the group consisting of compounds 1, 2, and 3.
14. The method of claim 13, wherein said neuroprotective composition consists of compound 4 and compound 1.
15. The method of claim 13, wherein said neuroprotective composition consists of compound 4 and compound 2.
16. The method of claim 13, wherein said neuroprotective composition consists of compound 4 and compound 3.
17. The method of claim 1, wherein said neuroprotective composition comprises compounds Formula 1 and an analgesic compound selected from the group consisting of opioids, cannabinoids, antiepileptics, or antidepressants.
18. The method of claim 17, wherein said analgesic compound is selected from the group consisting of morphine, oxycodone, pregabalin, gabapentin, and duloxetine.
19. The method of claim 18, wherein said neuroprotective composition comprises compound 4 and oxycodone.
20. The method of claim 18, wherein said neuroprotective composition comprises compound 4 and pregabalin.



1a: Mechanical Hyperalgesia



1b: Mechanical Allodynia



1c: Thermal Allodynia

- vehicle + vehicle
- paclitaxel + vehicle
- paclitaxel + DDD-028 1 mg kg<sup>-1</sup>
- paclitaxel + DDD-028 5 mg kg<sup>-1</sup>
- ▷— paclitaxel + DDD-028 10 mg kg<sup>-1</sup>
- ▲— paclitaxel + DDD-028 25 mg kg<sup>-1</sup>

Figure 1a-c. Acute Analgesic Effect in CIN model. Effect of single DDD-028 administrations on pain behavior induced by paclitaxel. Sensitivity to a noxious mechanical stimulus as measured by the Paw Pressure test (a). Pain threshold to a non-noxious mechanical stimulus as measured by the Von Frey test (b). Pain threshold to a non-noxious thermal stimulus as measured by the Cold Plate test (c). Paclitaxel (2.0 mg kg<sup>-1</sup>, i.p.) was administered on four days (1, 3, 5 and 8). Starting from day 10, DDD-028 was acutely *per os* administered (1 - 25 mg kg<sup>-1</sup>) and measurements assessed before treatment and 15, 30, 45, 60, 90 and 120 min after injection. Results were expressed as mean ± S.E.M. of 8 rats analyzed in 2 different experimental sets. \*\*p < 0.01 vs vehicle + vehicle; ^p < 0.01 vs paclitaxel + vehicle.

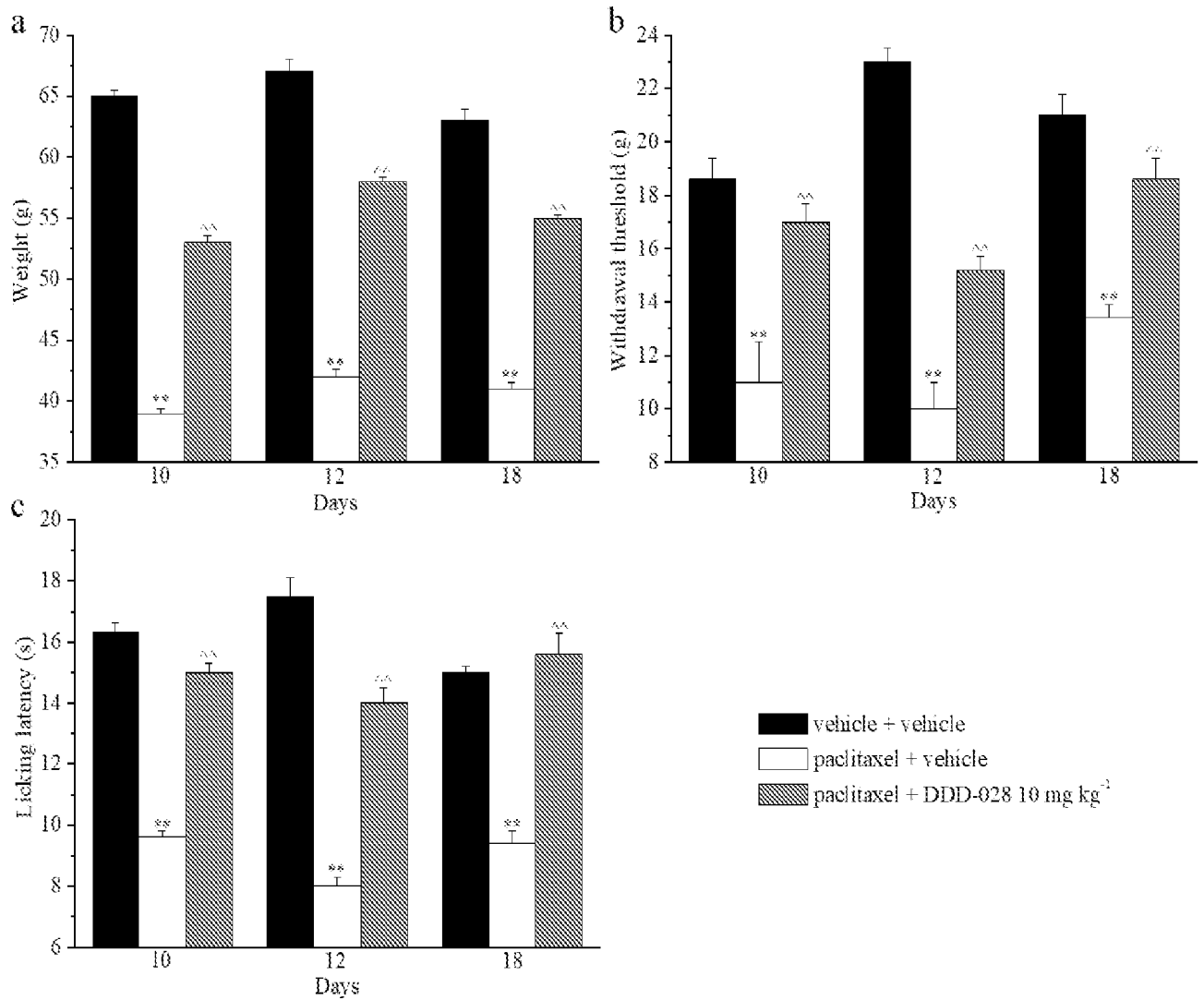


Figure 2a-c. Prophylactic Analgesic Effect in CIN model. Effects of repeated administration of DDD-028 on pain behavior induced by paclitaxel. Sensitivity to a noxious mechanical stimulus as measured by the Paw Pressure test (a). Pain threshold to a non-noxious mechanical stimulus as measured by the Von Frey test (b). Pain threshold to a non-noxious thermal stimulus as measured by the Cold Plate test. Behavioral tests were performed on days 10, 12 and 18 after the beginning of paclitaxel and DDD-028 administrations, 24 h after the last treatment. Paclitaxel (2.0 mg kg<sup>-1</sup>, i.p.) was administered on four days (1, 3, 5 and 8) while DDD-028 (10 mg kg<sup>-1</sup>, p.o.) was daily administered, starting from day 1 of paclitaxel injection. Results were expressed as mean ± S.E.M. of 8 rats analyzed in 2 different experimental sets. \*\*p < 0.01 vs vehicle + vehicle; ^^p < 0.01 vs paclitaxel + vehicle.

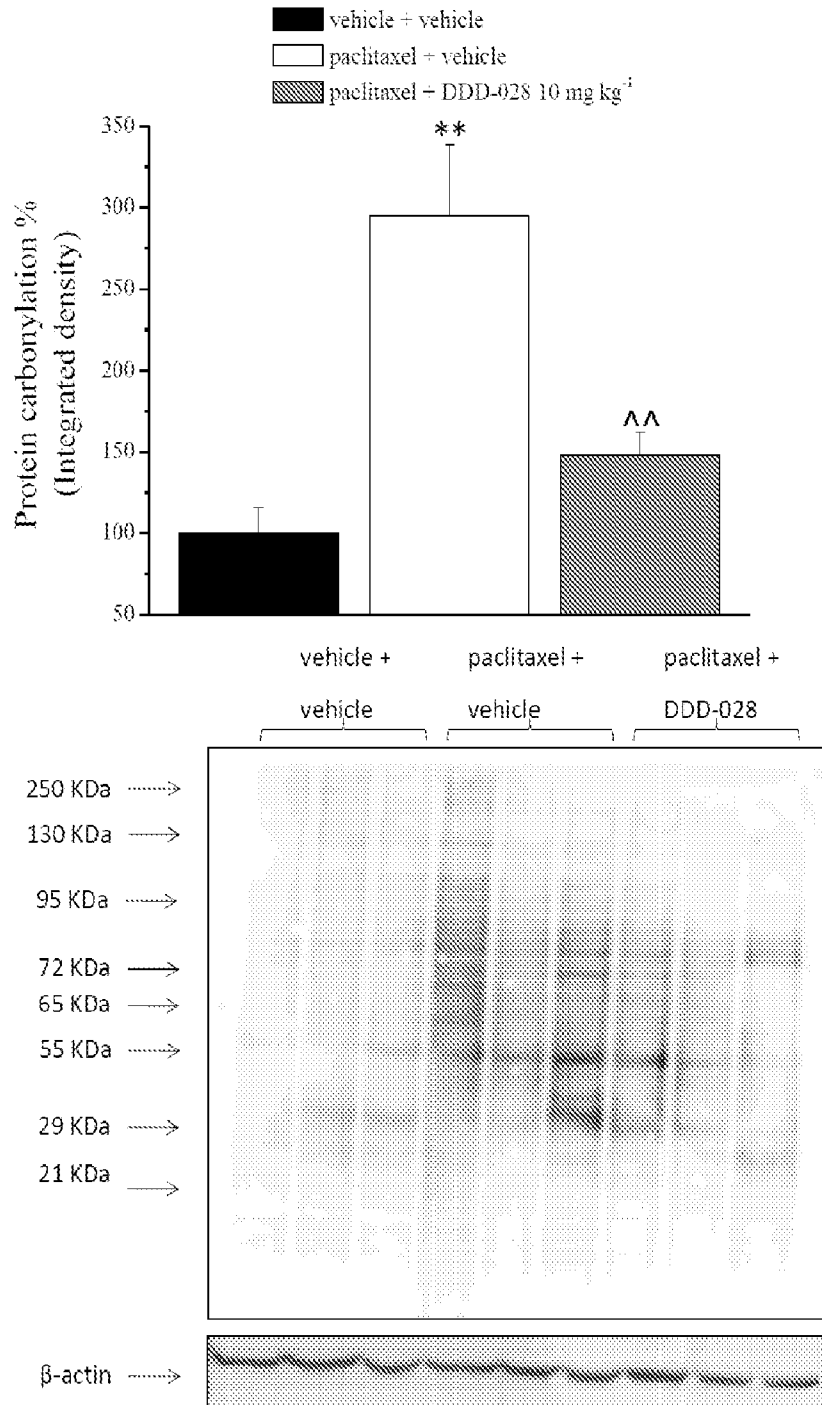
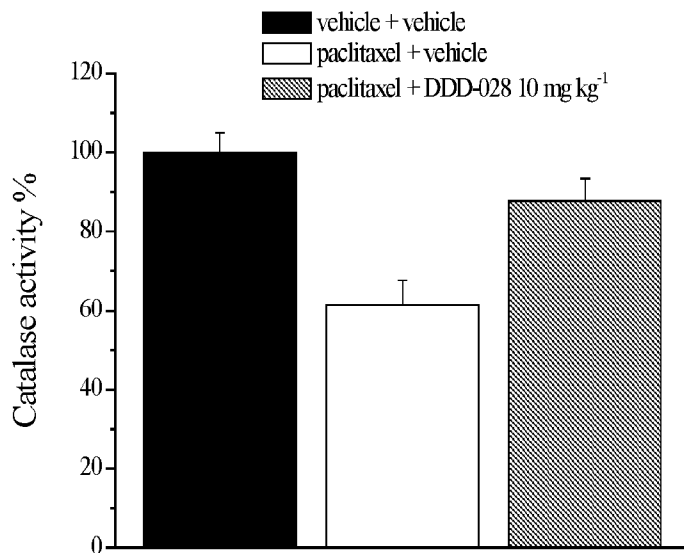
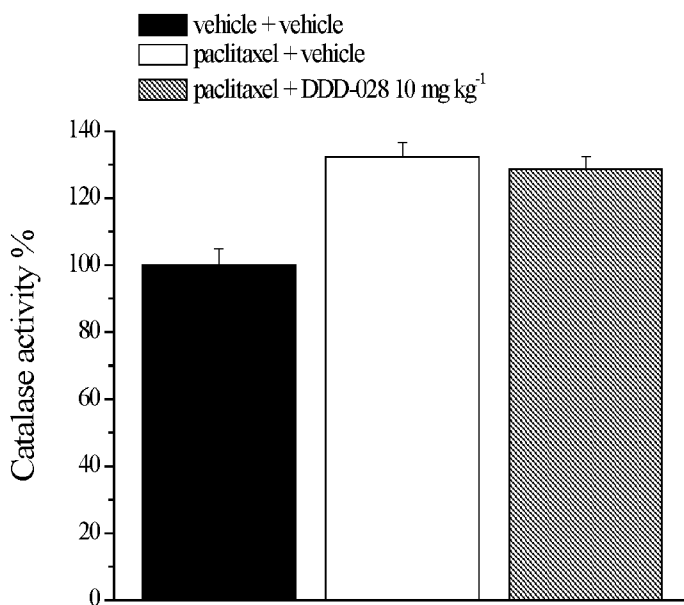


Figure 3. Neuroprotective Effect – Oxidative Stress. Carbonylated protein. Dorsal root ganglia. Densitometric analysis, data were normalized on the expression of beta-actin as housekeeping and expressed as mean ± S.E.M. of 6 samples from 6 different animals analyzed twice. Representative western blot was also showed (3 samples of each treatment are shown). \*\*p < 0.01 vs vehicle + vehicle; ^^p < .01 vs paclitaxel + vehicle.

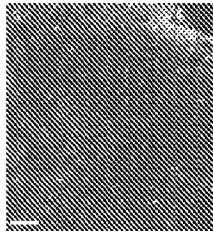


4a. Dorsal Root Ganglion (DRG)

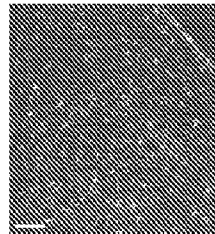


4b. Sciatic Nerve

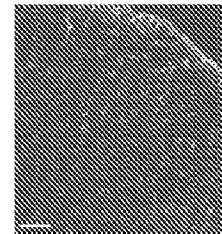
Figure 4. Neuroprotective Effect – Reactive Oxygen Species (ROS). Catalase activity. Dorsal root ganglia (a) and sciatic nerve (b) were analyzed. Enzymatic activity was expressed as percentage of control (vehicle + vehicle was considered as 100%). Data were expressed as mean ± S.E.M. of 6 samples from 6 different animals analyzed in triplicate. \*p < 0.05 vs vehicle + vehicle; ^p < 0.05 vs paclitaxel + vehicle.



Vehicle

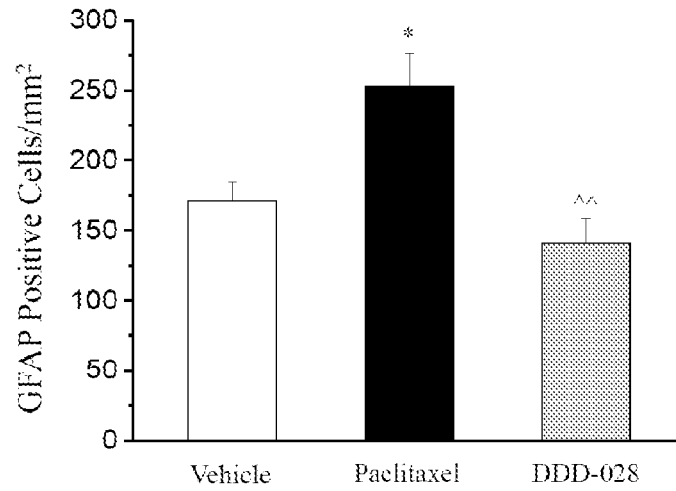


PCTX



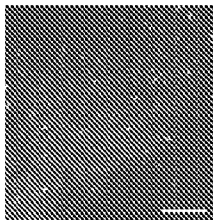
DDD-028

5a. Astrocytes

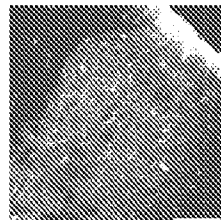


D. Astrocyte Cell Count

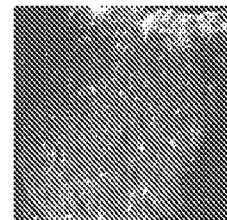
5a. Astrocyte proliferation in DRG



Vehicle

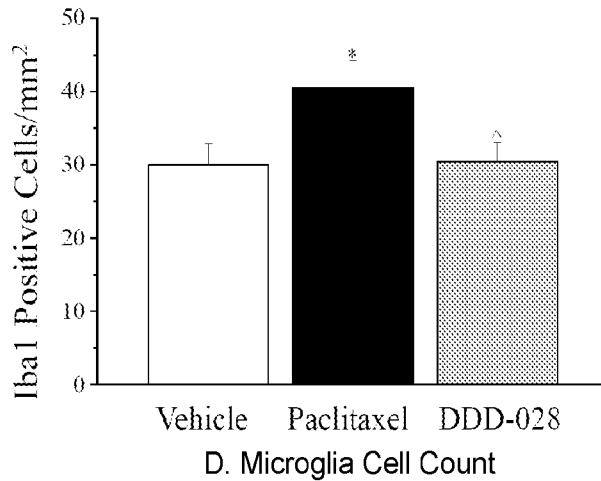


PCTX

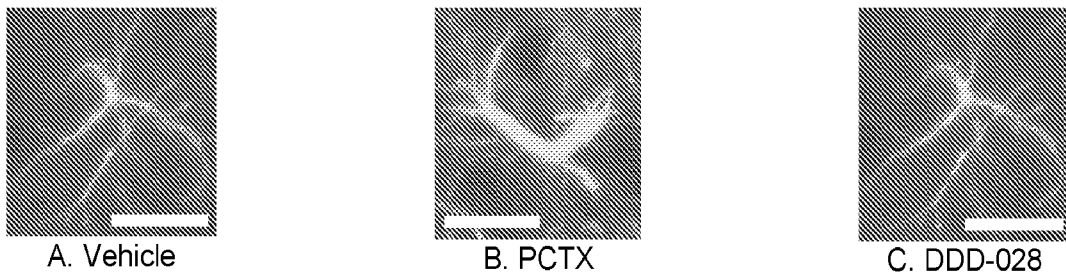


DDD-028

5b. Microglia



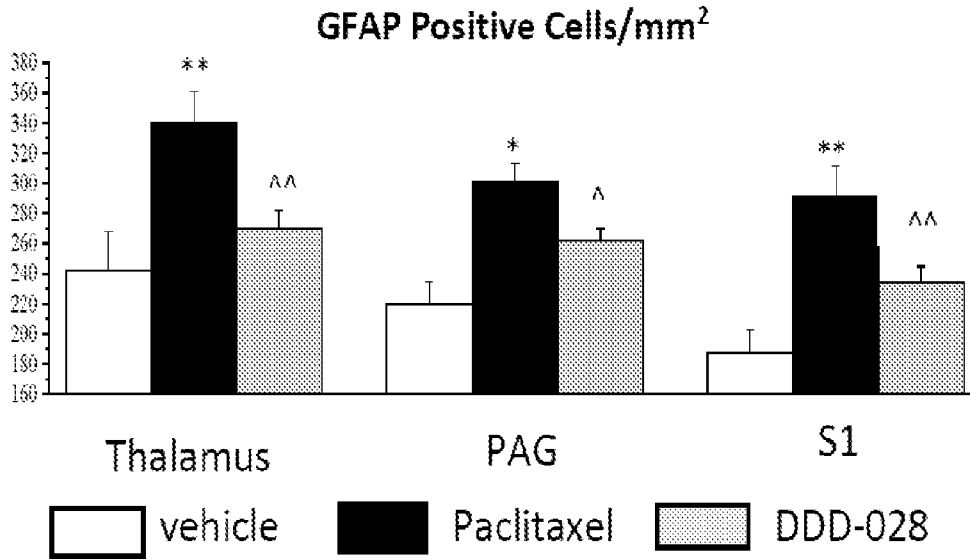
5b. Microglia proliferation in DRG



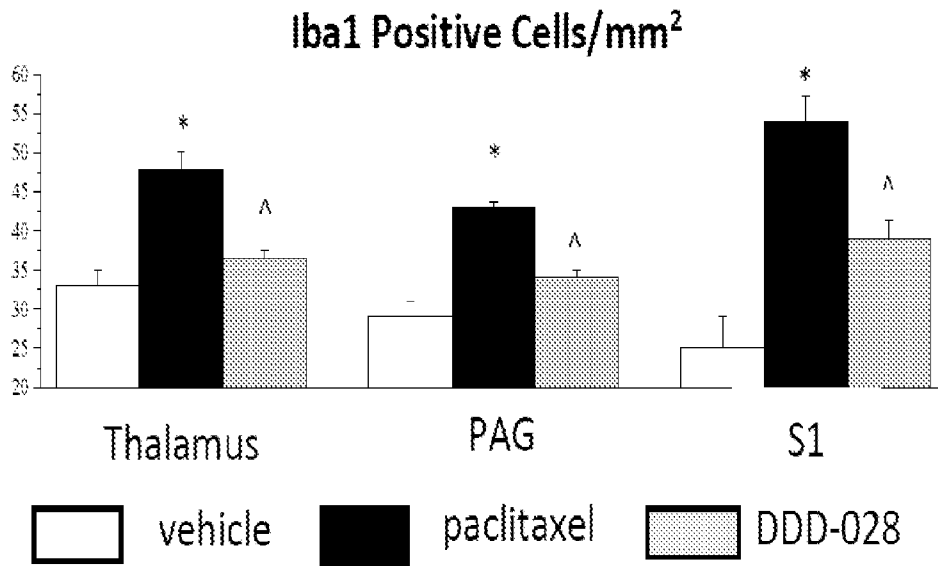
5c. Astrocyte morphology. (A) Vehicle; (B) PCTX; (C) DDD-028.

Figure 5a-c. Neuroprotective Effect – Glial Cell Proliferation and Morphology. Spinal cord. Astrocytes (a) were studied by immunohistochemistry performed with a GFAP antibody. Representative image of the dorsal horn (lumbar level) at 20X magnification. 40X images were shown to highlight morphological alterations. Quantitative analysis was reported as number of GFAP-positive cells, data were expressed as mean ± S.E.M. of 3 different fields of 3 specimens for each of 6 samples from 6 different animals. \*p < 0.05 vs vehicle + vehicle; ^p < 0.05 and ^^p < 0.01 vs paclitaxel + vehicle. Microglia (b) was studied by immunohistochemistry performed with an Iba1 antibody. Representative image of the dorsal horn (lumbar level) at 20X magnification. Quantitative analysis was reported as number of Iba1-positive cells, data were expressed as mean ± S.E.M. of 3 different fields of 3 specimens for each of 6 samples from 6 different animals. \*p < 0.05 vs vehicle + vehicle; ^p < 0.05 vs paclitaxel + vehicle.



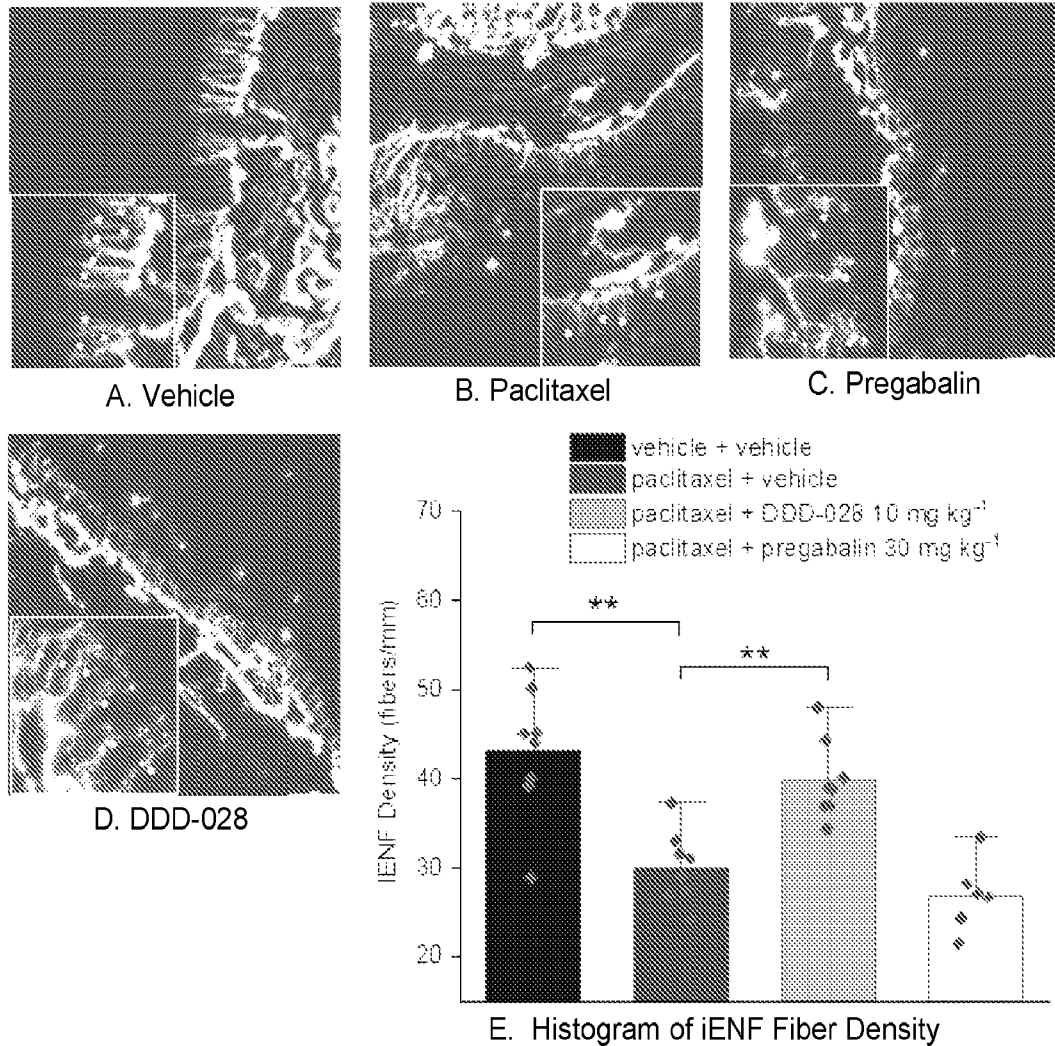


6a. Astrocyte Proliferation



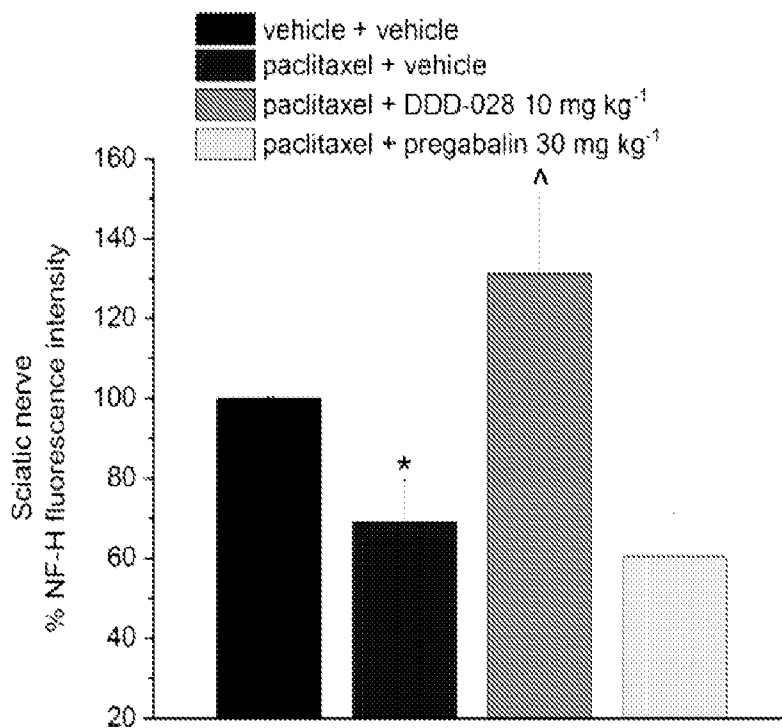
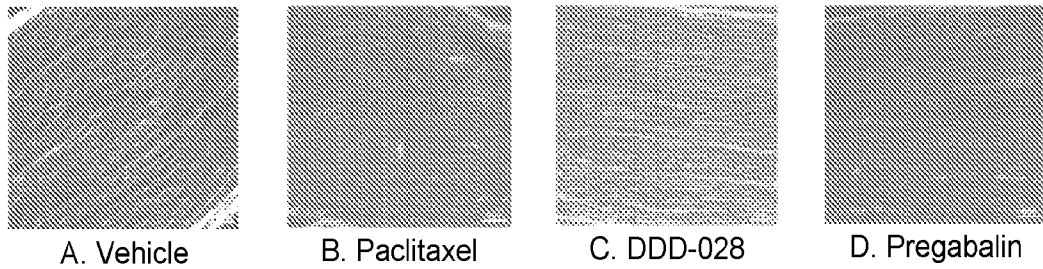
6b. Microglia Proliferation

Figure 6. Neuroprotective Effect – Glial Cell Proliferation, Brain. Brain. Astrocytes (c) and microglia (d) were studied by immunohistochemistry performed with GFAP and Iba1 antibodies, respectively. Analysis were performed on periaqueductal grey (PAG), thalamus and somatosensory area 1 (S1). Quantitative analysis was reported as number of GFAP- and Iba1-positive cells, data were expressed as mean ± S.E.M. of 3 different fields of 3 specimens for each of 6 samples from 6 different animals. \*p < 0.05 vs vehicle + vehicle; ^p < 0.05 vs paclitaxel + vehicle.



Intraepidermal fiber density in skin, indicated by PGP9.5 positive fibers: (A) Vehicle; (B) PCTX; (C) Pregabalin; (D) DDD-028; (E) iENF fiber density histogram.

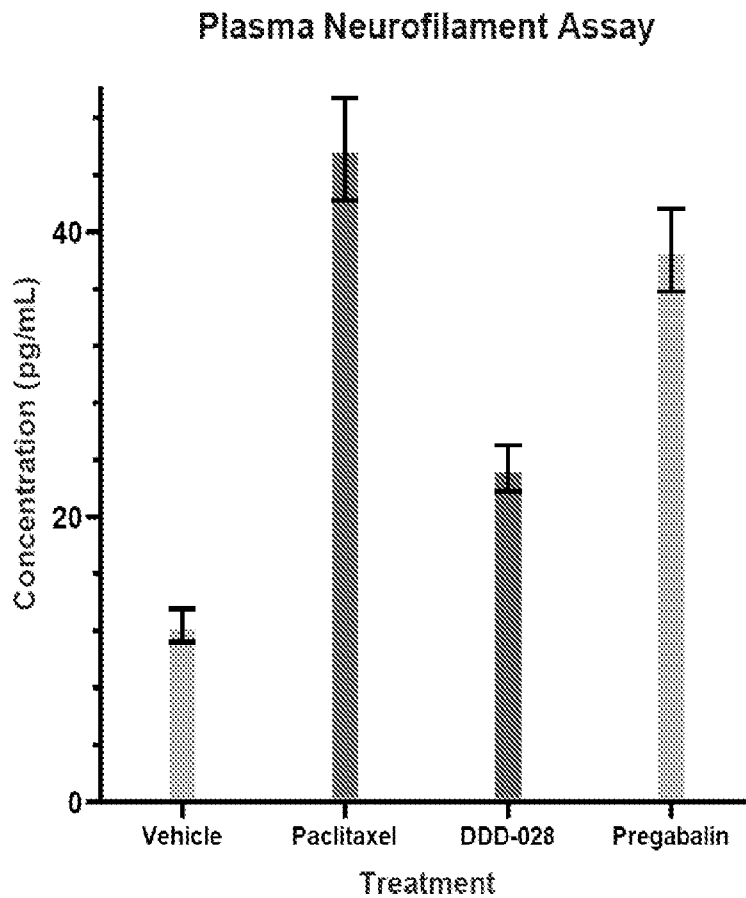
Figure 7. Neuroprotective Effect – Intraepidermal Nerve Fiber Density. Intraepidermal nerve fibers (IENF) density (fibers/mm); B) Representative photomicrograph of PGP9.5-positive intraepidermal nerve fibers (white arrows) in mice paw skin. Each value represents the mean ± S.E.M of 6-8 mice performed in two different experimental sets. \*\*p < 0.01 and \*p < 0.05.



E. Histogram of NF-H expression in sciatic Nerve

Figure 8a. NF-H density in sciatic nerve.

Figure 8. Neuroprotective Effect – Neurofilament Heavy Chain Expression. A) Neurofilament heavy chain (NF-H) fluorescence intensity; B) Representative photomicrograph of NF-H-positive fibers (green) in mice sciatic nerve. Each value represents the mean ± S.E.M of 6-8 mice performed in two different experimental sets. \*p < 0.05 vs vehicle + vehicle group; ^p < 0.05 vs paclitaxel + vehicle group.



**Figure 8b.** Plasma NF-L level

Figure 9. Neuroprotective Effect – Plasma Neurofilament Light Chain Level. On day 18, after behavioral assessments, blood was collected by exsanguination (from vena cava). Plasma was obtained by centrifugation, NF-L was dosed in triplicate by ELISA method. Each value represents the mean  $\pm$  S.E.M of 8 mice performed in two different experimental sets. \* $p < 0.05$  vs vehicle + vehicle;  $^{\wedge}p < 0.05$  vs paclitaxel + vehicle.

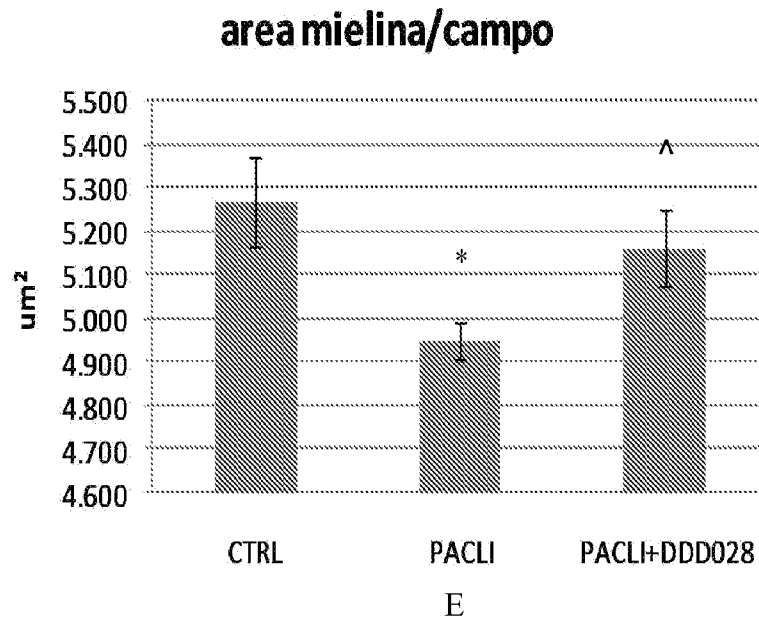
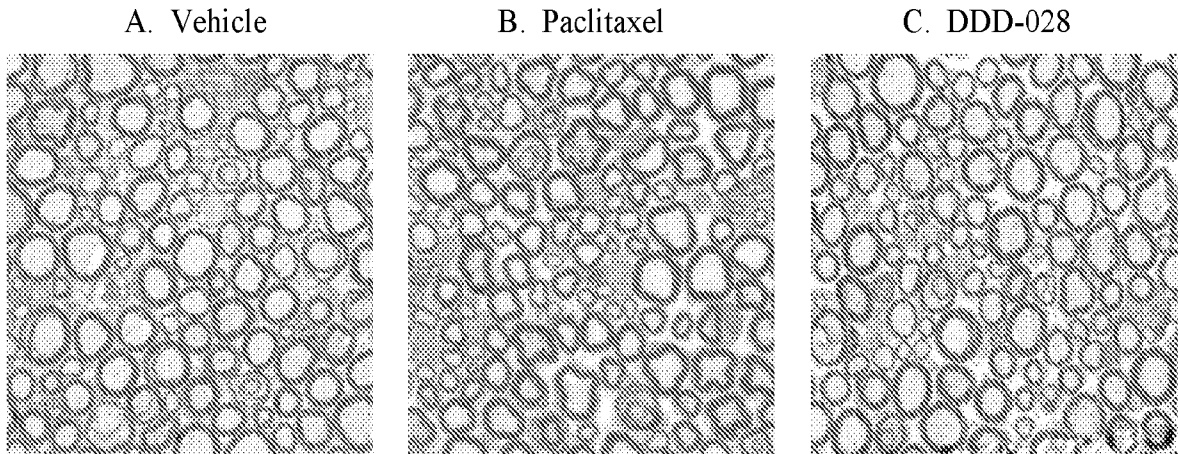
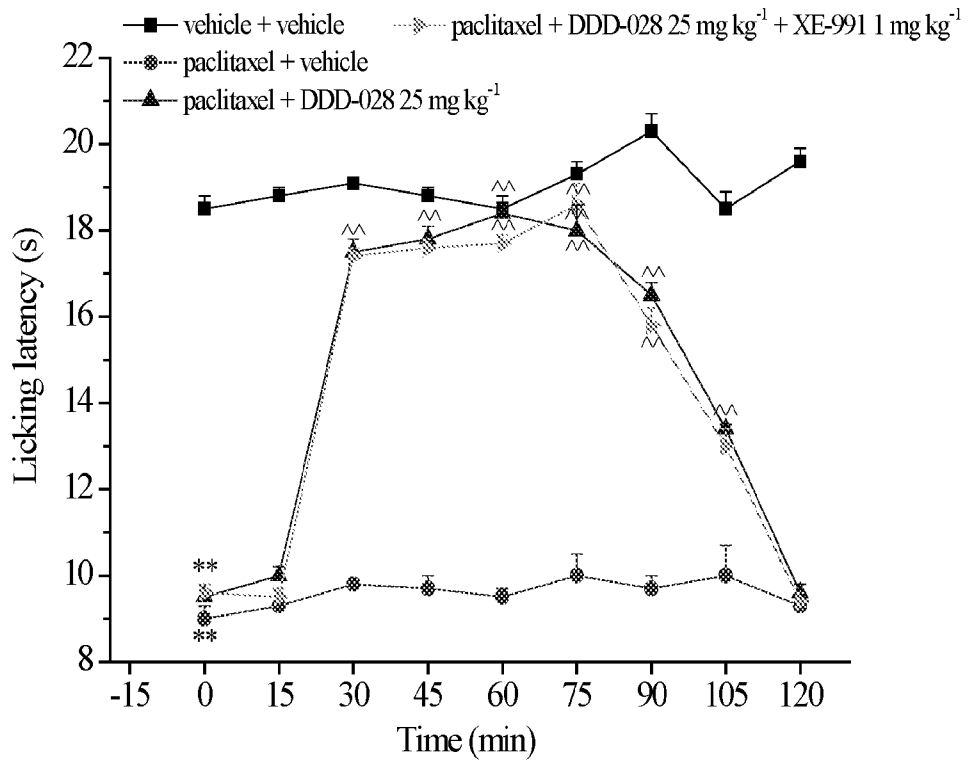
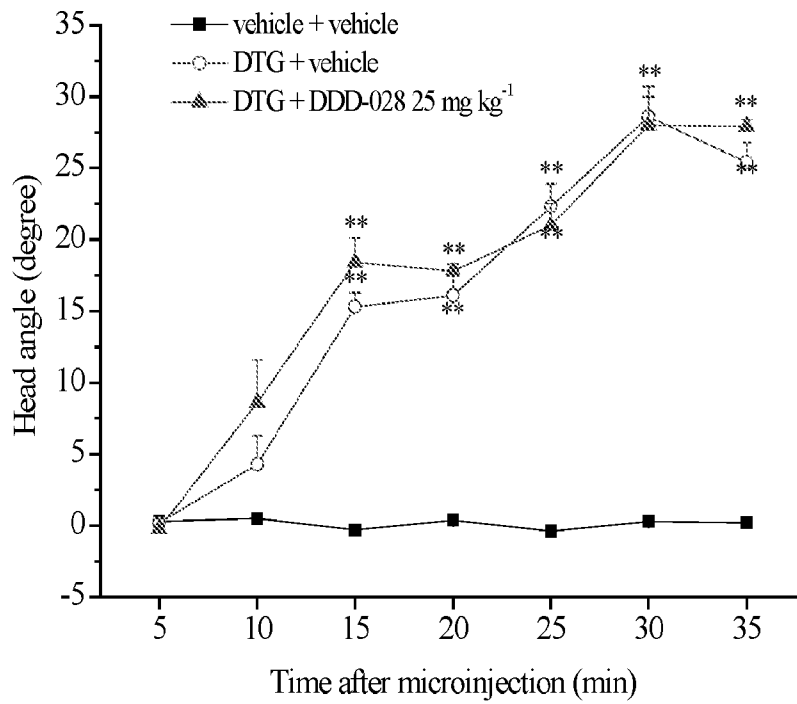


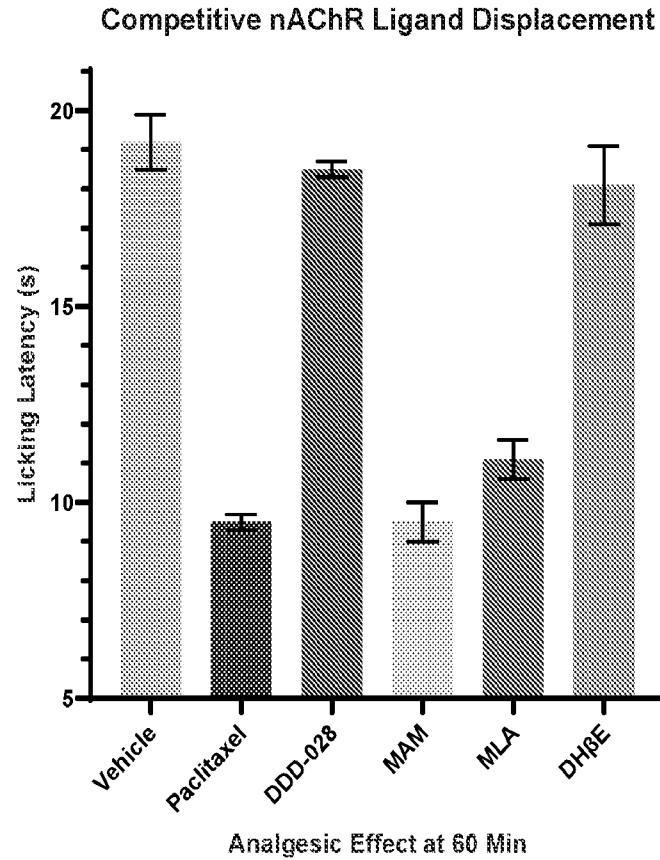
Figure 10a-e. Neuroprotective Effect – Myelin Degradation. Number of axons with reduced diameter due to degradation of myelin sheath induced by paclitaxel in a given area. Each value represents the mean ± S.E.M of 6 mice. \*p < 0.05 paclitaxel vs vehicle; ^p < 0.05 DDD-028 vs paclitaxel. DDD-028 prevents axonal degradation.



a. Ligand displacement (Kv.7)



b. Ligand displacement ( $\sigma$ )



c. Ligand displacement (nAChR)

Figure 11a-c. Pharmacodynamic Mechanism. (a) Study of the Kv7 potassium channel involvement. The Kv7 antagonist XE991 ( $1 \text{ mg kg}^{-1}$ ) was administered intraperitoneally 15 min before DDD-028 administration. Results were expressed as mean  $\pm$  S.E.M. of 8 rats analyzed in 2 different experimental sets. (b) Study of the  $\sigma$  receptors involvement. Neck dystonia was induced by the microinjection of the  $\sigma$  receptors agonist DTG ( $0.5 \text{ nmol}/1 \mu\text{l}$ ) in the red nucleus. DDD-028 ( $25 \text{ mg kg}^{-1}$ ) was administered per os 15 min before DTG infusion. The head angle deviation was measured over time. (c) The nAChRs (a) and  $\alpha 7$  nAChR (b) involvement. The nAChR antagonist MECA ( $2 \text{ mg kg}^{-1}$ ) was administered intraperitoneally 15 min before DDD-028 administration (triangle pointing down). In a separated experiment mecamlamine was administered for a second time 45 min after DDD-028 (triangle pointing left). The  $\alpha 7$  nAChR antagonist MLA ( $6 \text{ mg kg}^{-1}$ ) was administered intraperitoneally 15 min before DDD-028 injection.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 22/33189

## A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. C07D 223/14, C07D 223/16, A61K 31/33; ADD. A61K 31/00 (2022.01)

CPC - INV. C07D 401/00, C07D 401/14, C07D 223/14, C07D 223/16, A61K 31/33; ADD. A61K 31/00

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)

See Search History document

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

See Search History document

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

See Search History document

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	Rajagopalan et al. 'DDD-028: A potent potential non-opioid, non-cannabinoid analgesic for neuropathic and inflammatory pain', Bioorganic & Medicinal Chemistry Letters, 14 May 2014 (14.05.2014), Vol.24, pages3088-3091; p3088 p3089 p3090	1-4, 7-11 ----- 5-6, 12-20
Y	WO 2014/137848 A1 (Daya Drug Discoveries, Inc.) 12 September 2014 (12.09.2014); para[9] para[31]	5-6
Y	US 2020/0129493 A1 (Washington University) 30 April 2020 (30.04.2020); para[0007] p12	12-13, 16
Y	US 2019/0367499 A1 (Cersci Therapeutics Inc) 05 December 2019 (05.12.2019); para[0040] para[0041] para[0052]	12-14
Y	US 9,527,817 B2 (Hoke et al.) 27 December 2016 (27.12.2016); Abstract Fig.3	12-13, 15
Y	US 2020/0121651 A1 (Stc.Unm) 23 April 2020 (23.04.2020); para[0007] para[0008] para[0010]	17-20
A	US 2016/0039820 A1 (Daya Drug Discoveries, Inc.) 11 February 2016 (11.02.2016); entire document	1-20

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

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"E" earlier application or patent but published on or after the international filing date

"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)

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

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

"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

"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

"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

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05 August 2022

Date of mailing of the international search report

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