



- (51) **International Patent Classification:**
A61K 31/7076 (2006.01) *A61P 25/00* (2006.01)
A61K 45/06 (2006.01)
- (21) **International Application Number:**
PCT/US2012/029059
- (22) **International Filing Date:**
14 March 2012 (14.03.2012)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
61/477,964 21 April 2011 (21.04.2011) US
13/420,111 14 March 2012 (14.03.2012) US
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- (81) **Designated States** (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

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

(54) **Title:** USE OF ADENOSINE A3 RECEPTOR AGONISTS FOR TREATMENT OF NEUROPATHIC PAIN

(57) **Abstract:** A method of treating neuropathic pain in a subject is provided. The method comprises administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an A₃AR agonist.



WO 2012/145098 A1

USE OF ADENOSINE A₃ RECEPTOR AGONISTS FOR TREATMENT OF NEUROPATHIC PAIN

BACKGROUND

[001] The A₃ adenosine receptor (A₃AR) belongs to the Gi-protein-associated cell membrane receptors. Activation of these receptors inhibits adenylate cyclase activity, inhibiting cAMP formation, leading to the inhibition of PKA expression and initiation of a number of downstream signaling pathways [1]. A variety of agonists to this receptor subtype have been synthesized, with IB-MECA (*N*⁶-(3-iodobenzyl)-adenosine-5'-*N*-methyluronamide) and its chlorinated form Cl-IB-MECA (2-chloro-*N*⁶-(3-iodobenzyl)-adenosine-5'-*N*-methyluronamide), believed to be among the most potent and specific presently known A₃AR agonists [2, 3]. Such compounds have shown efficacy in several animal models of inflammation, ischemia, reperfusion injuries, and cancer [1] and have advanced to clinical trial studies for rheumatoid arthritis and cancer.

[002] Subjects with breast cancer, lung cancer, cervical cancer, ovarian cancer, germ cell tumors, acute leukemias and multiple myeloma who receive taxanes, platinum agents, vinca alkaloids and/or bortezomib as part of their initial therapy are at high risk of developing painful chemotherapy-induced peripheral neuropathy (CIPN) which can prematurely limit therapy and adversely impact quality of life. Thus, CIPN is a very serious complication of cancer chemotherapy and a major public health concern. It is estimated that the incidence of CIPN is as high as 70-90% in subjects receiving vincristine, cisplatin, oxaliplatin, and paclitaxel; 60% in subjects receiving docetaxel; 36-55% in subjects receiving bortezomib; and 40% in subjects receiving carboplatin [4, 5]. The development of CIPN with these agents appears not to be based on one single mechanism, as each of these drug classes possesses distinct anti-tumor mechanism of action [6]. There are currently no target-directed therapeutic approaches to treat CIPN. Consider the case of paclitaxel (Taxol[®]): Paclitaxel is a widely used chemotherapeutic agent indicated for the treatment of ovarian, breast, non-small cell lung carcinomas and Kaposi's sarcoma. Unfortunately, the dose-limiting side-effect of this highly efficacious antitumor drug is the precipitation of peripheral neuropathy accompanied by a chronic neuropathic pain syndrome that may resolve within weeks or months of drug termination, or it may last for years [7, 8]. The clinical management of these

subjects is very difficult as current pain drugs are only marginally effective for treating the symptoms of CIPN, and they also display additional unacceptable side effects [9]. The tragedy here is that paclitaxel-evoked neuropathic pain is a leading cause of discontinuation of an otherwise successful therapy and paclitaxel doses are often restricted to levels that are suboptimal for killing tumor cells [7, 8]. The very same problem is seen in chemotherapeutics of other classes.

[003] Chemotherapeutic strategies to treat various cancers are short-circuited by the numerous systemic side-effects observed. Pain, which is arguably the most debilitating and feared side-effect, greatly reduces the success of such strategies by limiting doses and imparting psychological distress. New methodologies to prevent or even reverse chemotherapy-induced chronic neuropathic pain would be transformative; indeed, the future development of a therapeutic of this nature is significant in two ways. First, the impact on quality of life for subjects would be enormous. The ability to reduce/eliminate CIPN amongst cancer survivors would result in lower costs related to the current chronic narcotic dependence needed to manage the pain. In addition, improved productivity in the work place would result, as many subjects with CIPN are unable to work and can no longer operate vehicles. Secondly, more lives may be saved. Subjects who currently would not be candidates for treatment (or continued treatment) with drugs such as paclitaxel due to the impending (or worsening) neuropathy, would instead benefit from full power anti-tumor dosages, if such dosages were to be made tolerable.

BRIEF DESCRIPTION OF THE FIGURES

[004] Figure 1. A₃AR agonists reverse mechano-allodynia in the CCI model. (A) IB-MECA (i.p.; 0.2, □; 0.5, ●; or 2 μmol/kg; △), but not its vehicle (○), on D7 after CCI (arrow) reversed mechano-allodynia in ipsilateral. (B) IB-MECA did not affect contralateral PWT (grams). (C) When compared to vehicle (○), daily i.p. injections (D8-D15, arrows) of IB-MECA (0.5 μmol/kg, ●) reversed mechano-allodynia to the same extent as D7. Results are expressed as mean ± SD, n=5 mice, analyzed by ANOVA with Bonferroni comparisons. *P<0.001 (D7 or vehicle vs. D0); †P<0.05 or ††P<0.001 (IB-MECA at each time point post treatment vs. D7); °P<0.001 (agonist + antagonist vs. agonist alone).

[005] Figure 2. IB-MECA reverses CCI-induced neuropathic pain through an apparently A₃AR-mediated mechanism(s). Mechano-allodynia developed by D7 after CCI of the sciatic nerve (○) in ipsilateral paws (A, C), but not contralateral paws (B, D), which was reversed by i.p. administration of IB-MECA (0.5 μmol/kg; ●; arrow). The A₃AR antagonist, MRS1523 (i.p.; 5 μmol/kg; ◆; A), but not the A₁AR antagonist, DPCPX (2 μmol/kg; ▲; C) or the A_{2A}AR antagonist, SCH-442416 (i.p.; 0.2 μmol/kg; ▼; C) prevented the anti-allodynic effect of IB-MECA. Neither MRS1523 (◇), DPCPX (△) nor SCH-442416 (▽), when given alone, had any effect on allodynia on ipsilateral (A, C) or contralateral (B, D) paws. Antagonists were given 15 minutes before IB-MECA or its vehicle. Results are expressed as mean ± SD for n=5 mice and analyzed by ANOVA with Bonferroni comparisons. **P*<0.001 for D7 vs. D0; †*P*<0.001 for IB-MECA at *t_h* vs. D7; and °*P*<0.001 for IB-MECA + antagonist vs. IB-MECA.

[006] Figure 3. CI-IB-MECA and MRS1898 reverse CCI-induced neuropathic pain through an apparently A₃AR-mediated mechanism. When given i.p. on D7 and compared to vehicle (○), administration (arrow) of CI-IB-MECA (0.6 μmol/kg; ●; A,B) or MRS1898 (0.5 μmol/kg; ●; C,D) reversed mechano-allodynia in ipsilateral (A, C), with no effects on contralateral paws (B, D). The A₃AR antagonist, MRS1523 (5 μmol/kg; ◆), blocked the ability of CI-IB-MECA (A) or MRS1898 (C) to reverse mechano-allodynia. The A₁AR antagonist, DPCPX (2 μmol/kg; ▲) or the A_{2A}AR antagonist, SCH-442416 (i.p.; 0.2 μmol/kg; ▼) did not prevent the anti-allodynic effects of MRS1898 (C). Neither MRS1523 (◇), DPCPX (△) nor SCH-442416 (▽), when given alone, had any effect on allodynia on ipsilateral (A, C) or contralateral (B, D) paws. Antagonists were given 15 min before CI-IB-MECA and MRS1898 or its vehicle. Results are expressed as mean ± SD for n=5 mice and analyzed by ANOVA with Bonferroni comparisons. **P*<0.001 for D7 vs. D0; †*P*<0.001 for A₃AR agonists ± antagonists at *t_h* vs. D7; and °*P*<0.001 for A₃AR agonists + antagonist vs. agonists.

[007] Figure 4. Naloxone does not block anti-allodynic effects of A₃AR agonists. (A) In ipsilateral paws, the reversal of mechano-allodynia by IB-MECA or MRS1898 (0.5 μmol/kg) was not prevented by naloxone (25 μmol/kg). (B) No differences in PWT (grams) were observed in contralateral paws. Results are expressed as mean

$\pm$ SD, n=5 mice, analyzed by ANOVA with Dunnett's comparisons. $*P<0.001$ (D7 or vehicle vs. D0); $^{\dagger}P<0.001$ (IB-MECA at 1 hour post treatment vs. D7).

[008] Figure 5. A₃AR agonists have no effect on acute nociception and Rotarod test. (A) Unlike morphine (35 μ mol/kg, s.c., $\blacktriangle$), IB-MECA (0.5 μ mol/kg, $\circ$) and MRS1898 (0.5 μ mol/kg, $\square$) lacked effect on mouse tail flick latency. (B) Mouse Rotarod Latency (s) was similar with IB-MECA (0.5 μ mol/kg, *black bar*), MRS1898 (0.5 μ mol/kg, *grey bar*) or vehicle (white bar). Results are expressed as mean $\pm$ SD, n=5 mice, analyzed by ANOVA with Bonferroni comparisons. $^{\dagger}P<0.001$ (morphine vs. t_{0h}).

[009] Figure 6. Morphine, gabapentin, or amitriptyline reverse mechano-allodynia in CCI-induced neuropathic pain. The development of mechano-allodynia observed on D7 after CCI in the ipsilateral paw ($\square$, n=6) was reversed in a dose and time-dependent manner by morphine (0.11, $\circ$; 0.35, $\bullet$; 1.05, $\blacksquare$; 3.5, $\blacktriangle$; 11, $\blacktriangledown$; or 35 μ mol/kg, $\blacklozenge$; A), gabapentin (18, $\blacksquare$; 58, $\blacktriangle$; 175, $\blacktriangledown$; or 584 μ mol/kg, $\blacklozenge$; C) or amitriptyline (3.2, $\bullet$; 9.6, $\blacksquare$; 32, $\blacktriangle$; 96, $\blacktriangledown$; or 191 μ mol/kg, $\blacklozenge$; E) in ipsilateral paws. These agents had no effect in contralateral paws (B, D, F). Results are expressed as mean $\pm$ SD for n=5 mice and analyzed by ANOVA with Bonferroni comparisons. $*P<0.001$ for D7 vs. D0; $^{\dagger}P<0.05$ or $^{\dagger\dagger}P<0.001$ for morphine, gabapentin or amitriptyline at t_h vs. D7.

[0010] Figure 7. Relative potencies of IB-MECA, morphine, gabapentin and amitriptyline in CCI. As tested on D7 and at time of peak reversal, IB-MECA was >5-, >350-, and >75-fold, respectively, more potent in reversing established mechano-allodynia when compared to morphine ($\blacktriangledown$), gabapentin ($\blacksquare$) or amitriptyline ($\blacktriangle$). In addition, IB-MECA was more efficacious than morphine but equiefficacious with gabapentin or amitriptyline. Results expressed as mean $\pm$ SD, n=5 mice, difference between curves were analyzed by extra sum-of-squares F-test comparisons. $*P<0.001$ (morphine, gabapentin or amitriptyline vs. IB-MECA); $^{\dagger}P<0.001$ (morphine, gabapentin or amitriptyline vs. gabapentin, amitriptyline or morphine + IB-MECA).

[0011] Figure 8. IB-MECA augments the anti-allodynic effects of morphine, gabapentin or amitriptyline in CCI. When compared to morphine (0.11 – 35 μ mol/kg, s.c., $\blacktriangledown$, A), gabapentin (18-584 μ mol/kg, i.p., $\blacksquare$, B) or amitriptyline (3-191

$\mu\text{mol/kg}$, oral, $\blacktriangle$, C) alone on D7, co-administration of a low dose of IB-MECA ($0.2 \mu\text{mol/kg}$) significantly increased their anti-allodynic effects as revealed by a shift to the left in the dose-response of morphine (∇ , A), gabapentin ($\square$, B) and amitriptyline ($\triangle$, C). Moreover, IB-MECA ($0.2 \mu\text{mol/kg}$) increased the efficacy of morphine (A). Results expressed as mean $\pm$ SD, $n=5$ mice, difference between curves were analyzed by extra sum-of-squares F-test comparisons. $*P<0.001$ (morphine, gabapentin or amitriptyline vs. IB-MECA); $^{\dagger}P<0.001$ (morphine, gabapentin or amitriptyline vs. gabapentin, amitriptyline or morphine + IB-MECA).

[0012] Figure 9. IB-MECA blocks chemotherapy-induced neuropathic pain. When compared to the vehicle group ($\circ$), paclitaxel ($\bullet$) or oxaliplatin ($\bullet$) led to a time-dependent development of mechano-allodynia (A, E) and mechano-hyperalgesia (B, F), which was blocked by daily i.p. injections (D0-D15/D17) with IB-MECA (0.02 , $\blacksquare$; 0.05 , $\blacktriangle$; or $0.2 \mu\text{mol/kg/d}$, $\blacktriangledown$). Effects of IB-MECA ($0.2 \mu\text{mol/kg/d}$) in paclitaxel-induced neuropathic pain were antagonized by co-administration of MRS1523 ($5 \mu\text{mol/kg/d}$; $\blacklozenge$, C,D). At the highest dose, IB-MECA ($0.2 \mu\text{mol/kg}$, ∇ , A-F) or MRS1523 ($5 \mu\text{mol/kg/d}$, $\diamond$, C,D) alone lacked effect in vehicle groups. Results expressed as mean $\pm$ SD, $n=6$ rats, analyzed by ANOVA with Bonferroni comparisons. $*P<0.001$ (chemotherapeutic agent vs. vehicle); $^{\dagger}P<0.01$ or $^{\dagger\dagger}P<0.001$ (chemotherapeutic agent + IB-MECA vs. chemotherapeutic agent); and $^{\circ}P<0.05$, $^{\circ\circ}P<0.01$ or $^{\circ\circ\circ}P<0.001$ (paclitaxel + IB-MECA + MRS1523 vs. paclitaxel + IB-MECA).

SUMMARY

[0013] In a first aspect, a method of treating neuropathic pain in a subject is provided. The method comprises administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an A_3AR agonist.

[0014] In a second aspect, a method of treating neuropathic pain in a subject is provided. The method comprises administering to the subject a first amount of an A_3AR agonist and a second amount of an analgesic, wherein the first and second amounts together comprise a therapeutically effective amount.

[0015] In a third aspect, a pharmaceutical composition for treating neuropathic pain is provided. The pharmaceutical composition comprises a first amount of an A_3AR

agonist and a second amount of an analgesic, wherein the first and second amounts taken together comprise a pharmaceutically effective amount.

DEFINITIONS

[0016] As used in the specification and claims, the forms "a" and "an" include singular as well as plural references unless the context clearly dictates otherwise. For example, the term "an A₃AR agonist" can include one or more such agonists.

[0017] As used herein, the term "neuropathic pain" means a type of pain which is usually caused by damage to or dysfunction of the nervous system. Neuropathic pain may result from disorders of the peripheral nervous system or the central nervous system (brain and spinal cord). Thus, neuropathic pain may be divided into peripheral neuropathic pain, central neuropathic pain, or mixed (peripheral and central) neuropathic pain. Neuropathic pain may be the result of a number disease processes and may be due to damage in a number of locations. Central neuropathic pain is usually found in spinal cord injury, multiple sclerosis, and some strokes. Aside from diabetes and other metabolic conditions, the common causes of painful peripheral neuropathies include herpes zoster infection, HIV-related neuropathies, nutritional deficiencies, toxins, remote manifestations of malignancies, genetic, and immune mediated disorders or physical trauma to a nerve trunk. Neuropathic pain is common in cancer as a direct result of cancer on peripheral nerves (e.g., compression by a tumor), or as a side effect of chemotherapy, radiation injury or surgery.

[0018] "Treatment" as used herein includes the alleviation, prevention, reversal, amelioration or control of a pathology, disease, disorder, process, condition or event, including pain. In this context, the term "treatment" is further to be understood as embracing the use of a drug to inhibit, block, reverse, restrict or control progression of any type of pain.

[0019] As used herein, the term "chemotherapy" refers to the treatment of a disease by chemotherapeutic drugs. Example chemotherapeutic drugs include taxanes (e.g. paclitaxel), platinum-based agents (e.g. cisplatin, oxaliplatin, carboplatin), vinka alkaloids (e.g. vincristine), proteasome inhibitors (e.g. bortezomib), alkylating agents, antimetabolites, anthracyclines, plant alkaloids, topoisomerase inhibitors, and other antitumor agents. Other types of chemotherapy include the use of

chemotherapeutic drugs in the treatment of autoimmune diseases such as multiple sclerosis, dermatomyositis, polymyositis, lupus, rheumatoid arthritis and the suppression of transplant rejections.

[0020] As used herein, the term "pharmaceutical composition" refers to compositions of matter comprising at least one pharmaceutical compound.

[0021] As used herein, the term "pharmaceutical compound" or "drug" refers to a free compound, its therapeutically suitable salts, solvates such as hydrates, specific crystal forms of the compound or its salts, or therapeutically suitable prodrugs of the compound.

[0022] The term "therapeutically suitable salt," refers to salts or zwitterions of pharmaceutical compounds which are water or oil-soluble or dispersible, suitable for treatment of disorders and effective for their intended use. The salts may be prepared, for instance, during the final isolation and purification of the compounds or separately by reacting an amino group of the compounds with a suitable acid. For example, a compound may be dissolved in a suitable solvent, such as but not limited to methanol and water, and treated with at least one equivalent of an acid, for instance hydrochloric acid. The resulting salt may precipitate out and be isolated by filtration and dried under reduced pressure. Alternatively, the solvent and excess acid may be removed under reduced pressure to provide the salt. Representative salts include acetate, adipate, alginate, citrate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, camphorate, camphorsulfonate, digluconate, glycerophosphate, hemisulfate, heptanoate, hexanoate, formate, isethionate, fumarate, lactate, maleate, methanesulfonate, naphthylenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, oxalate, maleate, pivalate, propionate, succinate, tartrate, trichloroacetate, trifluoroacetate, glutamate, para-toluenesulfonate, undecanoate, hydrochloric, hydrobromic, sulfuric, phosphoric, and the like. The amino groups of a compound may also be quaternized with alkyl chlorides, bromides, and iodides such as methyl, ethyl, propyl, isopropyl, butyl, lauryl, myristyl, stearyl, and the like.

[0023] Basic addition salts may be prepared, for instance, during the final isolation and purification of pharmaceutical compounds by reaction of a carboxyl group with a suitable base such as the hydroxide, carbonate, or bicarbonate of a metal cation

such as lithium, sodium, potassium, calcium, magnesium, or aluminum, or an organic primary, secondary, or tertiary amine. Quaternary amine salts may derived, for example, from methylamine, dimethylamine, trimethylamine, triethylamine, diethylamine, ethylamine, tributylamine, pyridine, *N,N*-dimethylaniline, *N*-methylpiperidine, *N*-methylmorpholine, dicyclohexylamine, procaine, dibenzylamine, *N,N*-dibenzylphenethylamine, 1-phenamine, and *N,N'*-dibenzylethylenediamine, ethylenediamine, ethanolamine, diethanolamine, piperidine, piperazine, and the like.

[0024] The term "therapeutically suitable prodrug," refers to those prodrugs or zwitterions which are suitable for use in contact with the tissues of subjects and are effective for their intended use. The term "prodrug" refers to compounds that are transformed *in vivo* to a pharmaceutical compound, for example, by hydrolysis in blood. The term "prodrug," refers to compounds that contain, but are not limited to, substituents known as "therapeutically suitable esters." The term "therapeutically suitable ester," refers to alkoxycarbonyl groups appended to the parent molecule on an available carbon atom. More specifically, a "therapeutically suitable ester," refers to alkoxycarbonyl groups appended to the parent molecule on one or more available aryl, cycloalkyl and/or heterocycle groups. Compounds containing therapeutically suitable esters are an example, but are not intended to limit the scope of compounds considered to be prodrugs. Examples of prodrug ester groups include pivaloyloxymethyl, acetoxymethyl, phthalidyl, indanyl and methoxymethyl, as well as other such groups known in the art. Other examples of prodrug ester groups are found in T. Higuchi and V. Stella, Pro-drugs as Novel Delivery Systems, Vol. 14 of the A.C.S. Symposium Series, and in Edward B. Roche, ed., Bioreversible Carriers in Drug Design, American Pharmaceutical Association and Pergamon Press, 1987, both of which are incorporated herein by reference.

[0025] The terms "pharmaceutically effective amount" and "effective amount", as used herein, refer to an amount of a pharmaceutical formulation that will elicit the desired therapeutic effect or response when administered in accordance with the desired treatment regimen.

DETAILED DESCRIPTION

[0026] The present disclosure is based on the discovery that A₃AR agonists can be used as pharmaceutical compounds in treatments against pain. In particular, A₃AR

agonists have been found to be effective in the treatment of neuropathic pain, especially with regard to blocking and/or reversing the development of chemotherapy-induced neuropathic pain (CIPN) and nerve-injury-derived neuropathic pain. Thus, A₃AR agonists may be used in shielding cancer patients from the pain due to chemotherapeutic agents and other causes. Moreover, A₃AR agonists and analgesics have been found to exhibit a synergistic effect in the treatment of neuropathic pain.

[0027] Thus, in a first aspect, a method of treating neuropathic pain is provided. The method comprises administering to a subject a pharmaceutical composition comprising a therapeutically effective amount of an A₃AR agonist. Various types of compounds having an A₃AR agonist activity are known, but no report in the past has discussed an analgesic effect of such a compound or a combination of such compounds in a neuropathic pain model. Without being bound to any particular theory, it is believed that the A₃AR is highly expressed in pathological cells; A₃AR agonists are therefore believed to tend to bind exclusively to the pathological cells, thereby inducing a specific therapeutic effect.

[0028] It can be confirmed that a compound has an A₃AR activity by known methods [76-84]. Examples of A₃AR agonists that may be used in the treatment of neuropathic pain include, but are not limited to, N⁶-benzyladenosine-5'-N-methyluronamides such as N⁶-(3-iodobenzyl)-adenosine-5'-N-methyluronamide, also known as IB-MECA [17], and 2-Chloro-N⁶-(3-iodobenzyl)-adenosine-5'-N-methyluronamide (also known as 2-Cl-IB-MECA; (N)-methanocarba nucleosides such as (1*R*,2*R*,3*S*,4*R*)-4-(2-chloro-6-((3-chlorobenzyl)amino)-9*H*-purin-9-yl)-2,3-dihydroxy-N-methylbicyclo[3.1.0]hexane-1-carboxamide (also known as CF502, Can-Fite Biopharma, MA); (2*S*,3*S*,4*R*,5*R*)-3-amino-5-[6-(2,5-dichlorobenzylamino)purin-9-yl]-4-hydroxytetrahydrofuran-2-carboxylic acid methylamide (also known as CP-532,903); (1'*S*,2'*R*,3'*S*,4'*R*,5'*S*)-4-(2-chloro-6-(3-chlorobenzylamino)-9*H*-purin-9-yl)-2,3-dihydroxy-N-methylbicyclo [3.1.0] hexane-1-carboxamide (also known as MRS-3558), 2-(1-Hexynyl)-N-methyladenosine; (1*S*,2*R*,3*S*,4*R*)-2,3-dihydroxy-4-(6-((3-iodobenzyl)amino)-4*H*-purin-9(5*H*)-yl)-N-methylcyclopentanecarboxamide (also known as CF101, Can-Fite), (1*S*,2*R*,3*S*,4*R*)-4-(2-chloro-6-((3-iodobenzyl)amino)-4*H*-purin-9(5*H*)-yl)-2,3-dihydroxy-N-methylcyclopentanecarboxamide (also known as CF102, Can-Fite); (1'*R*, 2'*R*, 3'*S*,

4'R, 5'S)-4-{2-chloro-6-[(3-iodophenylmethyl)amino]purin-9-yl}-1-(methylaminocarbonyl)-bicyclo[3.1.0]hexane-2,3-diol (also known as MRS1898); and 2-Dialkynyl derivatives of (N)-methanocarba nucleosides [82]. Preferred compounds include, but are not limited to, IB-MECA, CF101, and CF102.

5 [0029] Also included are A₃AR allosteric modulators which enhance the receptor activity in the presence of the native ligand [18], such as 2-cyclohexyl-N-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine (also known as CF602, Can-Fite). However, the above-listed A₃AR agonists are by no means exclusive and other such agonists may also be used. The administration of A₃AR agonists
10 covalently bound to polymers is also contemplated. For example, A₃AR agonists may be administered in the form of conjugates where an agonist is bound to a polyamidoamine (PAMAM) dendrimer [83, 84].

[0030] The administration of a pharmaceutical composition comprising an A₃AR agonist has been found to alleviate the symptoms of neuropathic pain regardless of
15 the cause of the pain or location of the bodily pain, and treats pain of varying severity, *e.g.* mild, moderate and severe pain in acute and/or chronic modes. Example causes of neuropathic pain include, but are not limited to, spinal cord injury, multiple sclerosis, stroke, diabetes, herpes zoster infection, HIV-related neuropathies, nutritional deficiencies, toxins, remote manifestations of malignancies,
20 genetic, immune mediated disorders or physical trauma to a nerve trunk, cancer, chemotherapy, radiation injury or surgery.

[0031] It is contemplated that the administration of an A₃AR agonist will be especially suited to the treatment of CIPN induced by a chemotherapeutic drug. Example types of chemotherapeutic drugs include podophyllotoxins, taxanes,
25 platinum complexes, vinca alkaloids, proteasome inhibitors, colchicines, eribulin, lenolidamide, ixabepilone, interferons, thalidomide, etoposide, ifosfamide, procarbazine, cytarabine, gemcitabine, and arsenic. Example chemotherapeutic drugs include, but are not limited to, one or more of the following: anti-cancer alkylating or intercalating agents (*e.g.*, Mechlorethamine, Chlorambucil,
30 Cyclophosphamide, Melphalan, and Ifosfamide); antimetabolites (*e.g.*, Methotrexate); purine antagonists and pyrimidine antagonists (*e.g.*, 6-Mercaptopurine, 5-Fluorouracil, Cytarabine, Capecitabine and Gemcitabine); spindle poisons (*e.g.*, Vinblastine, Vincristine, Vinorelbine and Paclitaxel); podophyllotoxins

(e.g., Etoposide, Irinotecan, Topotecan); antibiotics (e.g., Doxorubicin, Bleomycin and Mitomycin); nitrosoureas (e.g., Carmustine, Lomustine); inorganic ions (e.g., Cisplatin, Carboplatin, Oxaliplatin or Oxiplatin); enzymes (e.g., Asparaginase); hormones (e.g., Tamoxifen, Leuprolide, Flutamide and Megestrol); proteasome inhibitors (such as Velcade); other kinase inhibitors (e.g., inhibitors of Src, BCR/Abl, kdr, flt3, aurora-2, glycogen synthase kinase 3 ("GSK-3"), EGF-R kinase (e.g., Iressa, Tarceva, VEGF-R kinase, PDGF-R kinase); antibodies, soluble receptor or other receptor antagonists against a receptor or hormone implicated in a cancer (including receptors such as EGFR, ErbB2, VEGFR, PDGFR, and IGF-R); and agents such as Herceptin (or other anti-Her2 antibodies), Avastin, and Erbitux. For a more comprehensive discussion of updated cancer therapies see, <http://www.nci.nih.gov/>, a list of the FDA approved oncology drugs at <http://www.fda.gov/cder/cancer/druglistframe.htm>, and The Merck Manual, Seventeenth Ed. 1999, the entire contents of which are hereby incorporated by reference.

[0032] A₃AR agonists may also be used to treat pain associated with CIPN induced by one or more combinations comprising a chemotherapeutic drug as part of a treatment regimen. Example combinations include, but are not limited to: CHOPP (cyclophosphamide, doxorubicin, vincristine, prednisone, and procarbazine); CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone); COP (cyclophosphamide, vincristine, and prednisone); CAP-BOP (cyclophosphamide, doxorubicin, procarbazine, bleomycin, vincristine, and prednisone); m-BACOD (methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, dexamethasone, and leucovorin); ProMACE-MOPP (prednisone, methotrexate, doxorubicin, cyclophosphamide, etoposide, leucovorin, mechloethamine, vincristine, prednisone, and procarbazine); ProMACE-CytaBOM (prednisone, methotrexate, doxorubicin, cyclophosphamide, etoposide, leucovorin, cytarabine, bleomycin, and vincristine); MACOP-B (methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin, and leucovorin); MOPP (mechloethamine, vincristine, prednisone, and procarbazine); ABVD (adriamycin/doxorubicin, bleomycin, vinblastine, and dacarbazine); MOPP (mechloethamine, vincristine, prednisone and procarbazine) alternating with ABV (adriamycin/doxorubicin, bleomycin, and vinblastine); MOPP (mechloethamine, vincristine, prednisone, and procarbazine)

alternating with ABVD (adriamycin/doxorubicin, bleomycin, vinblastine, and dacarbazine); ChIVPP (chlorambucil, vinblastine, procarbazine, and prednisone); IMVP-16 (ifosfamide, methotrexate, and etoposide); MIME (methyl-gag, ifosfamide, methotrexate, and etoposide); DHAP (dexamethasone, high-dose cytarabine, and cisplatin); ESHAP (etoposide, methylpredisolone, high-dose cytarabine, and cisplatin); CEPP(B) (cyclophosphamide, etoposide, procarbazine, prednisone, and bleomycin); CAMP (lomustine, mitoxantrone, cytarabine, and prednisone); CVP-1 (cyclophosphamide, vincristine, and prednisone), ESHOP (etoposide, methylpredisolone, high-dose cytarabine, vincristine and cisplatin); EPOCH (etoposide, vincristine, and doxorubicin for 96 hours with bolus doses of cyclophosphamide and oral prednisone), ICE (ifosfamide, cyclophosphamide, and etoposide), CEPP(B) (cyclophosphamide, etoposide, procarbazine, prednisone, and bleomycin), CHOP-B (cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin), CEPP-B (cyclophosphamide, etoposide, procarbazine, and bleomycin), and P/DOCE (epirubicin or doxorubicin, vincristine, cyclophosphamide, and prednisone).

[0033] For use in accordance with this first aspect, the appropriate dosage is expected to vary depending on, for example, the particular A₃AR agonist employed, the mode of administration, and the nature and severity of the condition to be treated as well as the specific condition to be treated and is within the purview of the treating physician. Usually, an indicated administration dose may be in the range between about 0.1 to about 1000 µg/kg body weight. In some cases, the administration dose of the A₃AR agonist may be less than 400 µg/kg body weight. In other cases, the administration dose may be less than 200 µg/kg body weight. In yet other cases, the administration dose may be in the range between about 0.1 to about 100 µg/kg body weight. The dose may be conveniently administered once daily, or in divided doses up to, for example, four times a day or in sustained release form.

[0034] A₃AR agonists may be administered by any conventional route, in particular: enterally, topically, orally, nasally, *e.g.* in the form of tablets or capsules, via suppositories, or parenterally, *e.g.* in the form of injectable solutions or suspensions, for intravenous, intra-muscular, sub-cutaneous, or intra-peritoneal injection.

[0035] Suitable formulations and pharmaceutical compositions of A₃AR agonists will include those formulated in a conventional manner using one or more physiologically acceptable carriers or excipients, and any of those known and commercially available and currently employed in the clinical setting. Thus, the compounds may be formulated for oral, buccal, topical, parenteral, rectal or transdermal administration or in a form suitable for administration by inhalation or insufflation (either orally or nasally).

[0036] For oral administration, pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.* pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.* lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.* magnesium stearate, talc or silica); disintegrants (*e.g.* potato starch or sodium starch glycollate); or wetting agents (*e.g.* sodium lauryl sulphate). Tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.* sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.* lecithin or acacia); non-aqueous vehicles (*e.g.* almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.* methyl or propyl-p-hydroxybenzoates or sorbic acid). Preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

[0037] Preparations for oral administration may also be suitably formulated to give controlled-release or sustained release of the active compound(s) over an extended period. For buccal administration the compositions may take the form of tablets or lozenges formulated in a conventional manner known to the skilled artisan.

[0038] A₃AR agonists may also be formulated for parenteral administration by injection *e.g.* by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form *e.g.* in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain

additives such as suspending, stabilizing and/or dispersing agents. Alternatively, the A₃AR agonists may be in powder form for constitution with a suitable vehicle, e.g. sterile pyrogen-free water, before use. A₃AR agonists may also be formulated for rectal administration as suppositories or retention enemas, e.g. containing conventional suppository bases such as cocoa butter or other glycerides.

[0039] In a second aspect, a method of treating neuropathic pain in a subject, comprising administering to the subject an A₃AR agonist in conjunction with an analgesic, is provided. This second aspect is based on the discovery that A₃AR agonists and analgesics exhibit a synergistic effect increasing the potency of the analgesics. In other words, the administration of these compounds exhibits synergistic effects that exceed the mere additive contribution of the individual components. As a result, synergistically effective amounts of A₃AR agonist and analgesic taken together may be less than the effective amount of the A₃AR agonist or analgesic administered as monotherapies.

[0040] The method may involve administering to a subject a first amount of an A₃AR agonist in combination with a second amount of analgesic, wherein the first and second amount together comprise a pharmaceutically effective amount. Because of the above synergistic effect, the first amount, the second amount, or both may be less than effective amounts of each compound administered as monotherapies.

Therapeutically effective amounts of the A₃AR and analgesic are co-administered to the subject, *i.e.*, are administered to the subject simultaneously or separately, in any given order and by the same or different routes of administration. It may be advantageous to initiate administration of the A₃AR agonist first, for example one or more days or weeks prior to initiation of administration of the analgesic. Moreover, additional drugs may be given in conjunction with the above combination therapy.

[0041] The method of this second aspect may be used to alleviate the symptoms of neuropathic pain regardless of the cause of the pain, for example, but not limited to, spinal cord injury, multiple sclerosis, stroke, diabetes, herpes zoster infection, HIV-related neuropathies, nutritional deficiencies, toxins, remote manifestations of malignancies, genetic, immune mediated disorders or physical trauma to a nerve trunk, cancer, chemotherapy, radiation injury or surgery.

[0042] Examples of A₃AR agonists that may be used in conjunction with an analgesic include, but are not limited to, *N*⁶-benzyladenosine-5'-*N*-methyluronamides such as IB-MECA and 2-CI-IB-MECA; (N)-methanocarba nucleosides such as CF502; CP-532,903; MRS-3558; CF101; CF102; MRS1898, and 2-Dialkynyl derivatives of (N)-methanocarba nucleosides. Preferred compounds include, but are not limited to, IB-MECA, CF101, and CF102. Also included are A₃AR allosteric modulators which enhance the receptor activity in the presence of the native ligand, such as CF602. However, the above-listed A₃AR agonists are by no means exclusive and other such agonists may also be used.

The administration of A₃AR agonists covalently bound to polymers is also contemplated. For example, A₃AR agonists may be administered in the form of conjugates where an agonist is bound to a polyamidoamine (PAMAM) dendrimer.

[0043] The analgesic administered in conjunction with an A₃AR agonist may be selected in relation to the particular condition being treated, and preferably has proven efficacy in the treatment of pain without significant potential for addiction. Currently known analgesics include, but are not limited to, opioids, morphinomimetics, antidepressants, antiepileptics, NMDA receptor antagonists, fatty acid amine hydrolyase inhibitors, anticonvulsives, non-steroidal anti-inflammatory drugs (NSAIDs), COX-2 inhibitors, NOS inhibitors, and calcium channel subunit $\alpha_2\delta$ ligands.

[0044] Example opioids include any natural or synthetic opioid analgesic, such as morphine, fentanyl, codeine, thebaine, diacetylmorphine (heroin), dihydrocodeine, hydrocodone, hydromorphone, nicomorphine, oxycodone, oxymorphone, alphamethylfentanyl, alfentanil, sufentanil, remifentanil, carfentanyl, ohmefentanyl, nocaine, pethidine (meperidine), ketobemidone, MPPP, allylprodine, prodine, PEPAP, propoxyphene, dextropropoxyphene, dextromoramide, bezitramide, piritramide, methadone, dipipanone, levoalphacetylmethadol (LAAM), loperamide, diphenoxylate, pentazocine, phenazocine, buprenorphine, etorphine, butorphanol, nalbuphine, levorphanol, levomethorphan, dezocine, lefetamine, tilidine, tramadol, propoxyphene, and oxycodone. As intended herein, an opioid also encompasses any natural or synthetic narcotic antagonist such as nalmefene, naloxone or naltrexone as well as any natural or synthetic mixed opioid agonist/antagonist such as nalbuphine, butorphanol, buprenorphine and pentazocine.

[0045] Example non-steroidal anti-inflammatory drugs (NSAIDs) include aspirine, ibuprofen, acetaminophen, naproxen, fenoprofen, ketoprofen, flurbiprofen, oxaprozin, indomethacin, sulindac, etodolac, ketorolac, diclofenac, nabumetone, piroxicam, meloxicam, tenoxicam, droxicam, lornoxicam, isoxicam, mefenamic acid, meclofenamic acid, flufenamic acid, tolafenamic acid, celecoxib, rofecoxib, valdecoxib, parecoxib, lumiracoxib, etoricoxib, firocoxib, nimesulide, and licofelone. Example antidepressants include tricyclic antidepressants such as: amitriptyline, amitriptylinoxide, butriptyline, clomipramine, demexiptiline, desipramine, dibenzepin, dimetacrine, dosulepin, doxepin, imipramine, imipraminoxide, lofepramine, melitracen, metapramine, nitroxazepine, nortriptyline, noxiptiline, pipofezine, propizepine, protriptyline, and quinupramine; amineptine, norepinephrine, iprindole, opipramol, tianeptine, trimipramine, carbamezapine, and flupirtine.

[0046] It is contemplated that A₃AR agonists will be especially suited to the treatment of pain when co-administered with an opioid, a tricyclic antidepressant, or an analgesic believed to bind the calcium channel subunit $\alpha_2\delta$, *i.e.* a calcium channel subunit $\alpha_2\delta$ ligand. Examples of such ligands include GABA analogs, such as gabapentin (2-[1-(aminomethyl)cyclohexyl]acetic acid) and pregabalin ((*S*)-3-(aminomethyl)-5-methylhexanoic acid).

[0047] The relative amounts of the compounds may be selected to provide for synergistic pain relief. For example, a suitable ratio of IB-MECA to gabapentin may be in the range of from about 0.1 part by weight of the IB-MECA to from about 3 to about 30 parts by weight of the gabapentin. A suitable ratio of IB-MECA to morphine may be in the range of from about 0.1 part by weight of the IB-MECA to from about 1 to about 5 parts by weight of the morphine. While these ratios are calculated with respect to the free compounds (non-salt forms), it should be understood that the equivalent ratios can also readily be determined for pharmaceutically acceptable salts or prodrugs of the compounds by using a ratio of the molecular weights of the salts.

[0048] In some cases, co-administration of the A₃AR agonist and analgesic is achieved by formulating the compounds together in a combination composition. Accordingly, in a third aspect, a combination composition for treating neuropathic pain is provided.

[0049] Examples of A₃AR agonists that may be used in a combination composition together with an analgesic include, but are not limited to, *N*⁶-benzyladenosine-5'-*N*-methyluronamides such as IB-MECA and 2-CI-IB-MECA; (N)-methanocarba nucleosides such as CF502; CP-532,903; MRS-3558; CF101; CF102; MRS1898, and 2-Dialkynyl derivatives of (N)-methanocarba nucleosides. Preferred compounds include, but are not limited to, IB-MECA, CF101, and CF102. Also included are A₃AR allosteric modulators which enhance the receptor activity in the presence of the native ligand, such as CF602. However, the above-listed A₃AR agonists are by no means exclusive and other such agonists may also be used.

The administration of A₃AR agonists covalently bound to polymers is also contemplated. For example, A₃AR agonists may be administered in the form of conjugates where an agonist is bound to a polyamidoamine (PAMAM) dendrimer.

[0050] The analgesic administered in the combination composition may be selected in relation to the particular condition being treated, and preferably has proven efficacy in the treatment of pain without significant potential for addiction. Currently known analgesics include, but are not limited to, opioids, morphinomimetics, antidepressants, antiepileptics, NMDA receptor antagonists, fatty acid amine hydrolyase inhibitors, anticonvulsives, non-steroidal anti-inflammatory drugs (NSAIDs), COX-2 inhibitors, NOS inhibitors, and calcium channel subunit $\alpha_2\delta$ ligands.

[0051] In some cases, the combination composition comprises a first pharmaceutically acceptable composition containing a first amount of an A₃AR agonist, and a second pharmaceutically acceptable composition comprising a second amount of an analgesic, wherein the first and second amounts taken together comprise a pharmaceutically effective amount. The first amount, the second amount, or both may be less than effective amounts of each compound administered as monotherapies.

[0052] In other cases, the combination composition is a pharmaceutically acceptable composition comprising a first amount of an A₃AR agonist and a second amount of an analgesic, wherein the first and second amounts taken together comprise a pharmaceutically effective amount. The first amount, the second amount, or both may be less than effective amounts of each compound administered as monotherapies.

[0053] Suitable combination compositions will include those formulated in a conventional manner using one or more physiologically acceptable carriers or excipients, and any of those known and commercially available and currently employed in the clinical setting. Thus, the compounds of a combination composition may be formulated for oral, buccal, topical, parenteral, rectal or transdermal administration or in a form suitable for administration by inhalation or insufflation (either orally or nasally).

[0054] For oral administration, combination compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g. pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g. lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g. magnesium stearate, talc or silica); disintegrants (e.g. potato starch or sodium starch glycollate); or wetting agents (e.g. sodium lauryl sulphate). Tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g. sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g. lecithin or acacia); non-aqueous vehicles (e.g. almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g. methyl or propyl-p-hydroxybenzoates or sorbic acid). Preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

[0055] Preparations for oral administration may also be suitably formulated to give controlled-release or sustained release of the active compound(s) over an extended period. For buccal administration the compositions may take the form of tablets or lozenges formulated in a conventional manner known to the skilled artisan.

[0056] Combination compositions may also be formulated for parenteral administration by injection e.g. by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form e.g. in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and

may contain additives such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredients may be in powder form for constitution with a suitable vehicle, e.g. sterile pyrogen-free water, before use.

[0057] The combination compositions for use according to this aspect may also be formulated for rectal administration as suppositories or retention enemas, e.g. containing conventional suppository bases such as cocoa butter or other glycerides.

[0058] While formulation of the A₃AR agonist and analgesic has been described with regards to the combination of the compounds into a combination composition, it should also be understood that the compounds may be co-administered in separate preparations, such as a first unit dosage form comprising an A₃AR agonist, and a second unit dosage form suitable for co-administration with the first unit dosage form comprising an analgesic. Other methods or modes of co-administration not specifically described herein should also be understood to be encompassed.

[0059] The invention has been described with reference to various illustrative embodiments and techniques. However, it should be understood that many variations and modifications, as are known in the art, may be made while remaining within the scope of the claims of the present application. The examples that follow are illustrative and are not intended to be limiting.

EXAMPLES

[0060] Materials and Methods:

[0061] Materials: IB-MECA, CI-IB-MECA, DPCPX (8-cyclopentyl-1,3-dipropylxanthine) and SCH-442416 [2-(2-furanyl)-7-[3-(4-methoxyphenyl)propyl]-7Hpyrazolo [4,3-e] [1,2,4]triazolo[1,5-C]pyrimidin-5-amine] were purchased from Tocris (Ellisville, MO, USA). MRS1898 ((1'R, 2'R, 3'S, 4'R, 5'S)-4-{2-chloro-6-[(3-iodophenylmethyl)amino]purin-9-yl}-1-(methylaminocarbonyl)-bicyclo[3.1.0]hexane-2,3-diol) was synthesized as described previously [45, 46]. Morphine was a kind gift from Mallinckrodt (St. Louis, MO, USA). Paclitaxel, oxaliplatin and bortezomib were purchased commercially and respectively from Parenta Pharma (Yardley, PA, USA), Oncology Supply (Dothan, AL, USA) and Selleck Chemicals (Houston, TX, USA).

For cell culture: the media was purchased from Mediatech (Dulbecco's Minimal Essential Media and McCoy's 5A; Manassas, VA, USA) or Sigma-Aldrich (L12; St. Louis, MO, USA); fetal bovine serum from Thermo Scientific Hyclone (Waltham,

MA, USA); and the penicillin/streptomycin from Invitrogen (Carlsbad, CA, USA). Cell lines were kind gifts from colleagues: SKBR3 (Dr. Joseph Baldassare, Saint Louis University), SW480 (Stephanie Knebel, Saint Louis University), and RPMI 8226 (Jaki Kornbluth, Saint Louis University). MRS1523 (3-propyl-6-ethyl-5[(ethylthio)carbonyl]-2-phenyl-4-propyl-3-pyridine-carboxylate), gabapentin, amitriptyline, and all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

[0062] Experimental animals. Male Sprague Dawley rats (200-220 g) or mice (25-30 g) from Harlan (Indianapolis, IN, USA) were housed 3-4 (for rats) and 5 (for mice) per cage in a controlled environment (12 hours light/dark cycles) with food and water available *ad libitum*. Experiments were performed in accordance with International Association for the Study of Pain, NIH guidelines on laboratory animal welfare and Saint Louis University Institutional Animal Care and Use Committee recommendations. Experimenters were blinded to treatment conditions in all experiments.

[0063] CCI model of neuropathic pain. CCI to the sciatic nerve of the left hind leg in mice was performed under general anaesthesia using the well-characterized Bennett model [47]. Briefly, mice (weighing 25-30g at the time of surgery) were anesthetized with 3% isoflurane/100% O₂ inhalation and maintained on 2% isoflurane/100% O₂ for the duration of surgery. The left thigh was shaved, scrubbed with Nolvasan[®] and a small incision (1-1.5 cm in length) was made in the middle of the lateral aspect of the left thigh to expose the sciatic nerve. The nerve was loosely ligated around the entire diameter of the nerve at three distinct sites (spaced 1 mm apart) using silk sutures (6.0). The surgical site was closed with a single muscle suture and a skin clip. Pilot studies established that under our experimental conditions peak mechano-allodynia develops by day 5 – day 7 (D5-D7) following CCI. Test substances or their vehicles were given subcutaneously (s.c.), intraperitoneally (i.p.) or orally by gavage (0.1 ml) at peak mechano-allodynia (D7).

[0064] Induction of chemotherapy-induced neuropathic pain in rats: Paclitaxel or vehicle (Cremophor EL and 95% anhydrous ethanol in 1:1 ratio) were injected i.p. on four alternate days (2 mg/kg on D0, 2, 4 and 6 with a final cumulative dose of 8 mg/kg) [48]. Oxaliplatin or vehicle (5% dextrose) was injected i.p. in rats on five consecutive days (D0-D4) for a final cumulative dose of 10 mg/kg (31). Bortezomib

or vehicle (5% Tween 80, 5% ethanol) was injected i.p. in rats on five consecutive days (D0-D4; 0.2 mg/kg) for a final cumulative dose of 1 mg/kg (G.J. Bennett and W.H. Xiao, personal communication). Test substances or their vehicle were given i.p. (0.2 ml) 30 min before the chemotherapeutic agent or its vehicle on D0

(baseline) and then daily until D15 or D17. Behavioral responses (mechano-allodynia and mechano-hyperalgesia) were measured on D0 prior to the first i.p. injection of the chemotherapeutic agent and subsequently at various time points. If testing coincided with a day when rats received test substance, behavioral measurements were taken always before the injection of the test substance.

Chemotherapeutic treatments result in bilateral allodynia and hyperalgesia without differences in left and right paw withdrawal threshold (PWT, grams) in any group at any time point, thus values from both paws were averaged. None of the animals exhibited signs of observable toxicities; they exhibited normal posture, grooming, locomotor behavior, hair coat was normal without signs of piloerection or porphyrin, and body weight gain was normal and comparable to vehicle-treated rats.

[0065] Behavioral testing. Mechano-allodynia was measured in CCI and paclitaxel studies after first acclimating the animals to elevated cages with a wire mesh floor for 15 minutes. The plantar aspect of hindpaws were probed three times with calibrated von Frey filaments (Stoelting, mice: 0.07 – 2.00 grams; rats: 0.407 – 26 grams) according to the “up-and-down” method [50]. In oxaliplatin or bortezomib studies, allodynia was assessed with an electronic version of the VF test (dynamic plantar aesthesiometer, model 37450; Ugo Basile, Milan, Italy). Briefly, each rat was placed in a Plexiglas chamber (28 x 40 x 35-cm, wire mesh floor) and after its acclimation, a servo-controlled mechanical stimulus was applied to the plantar surface that exerted a progressively increasing punctate pressure up to 50 grams within 10 seconds. Mechanical threshold was assessed three times at each time point to yield a mean value, reported as PWT (grams). The development of mechano-allodynia is evidenced by a significant ($P < 0.05$) reduction in mechanical mean PWT (grams) at forces that failed to elicit withdrawal responses on D0 before CCI or chemotherapeutic/vehicle treatment. Mechano-hyperalgesia was assessed in rats by the Randall and Sellitto paw pressure test [51] using an analgesiometer (Ugo Basile). The nociceptive threshold was defined as the force (grams) at which the rat withdrew its paw (cut off set at 250 grams).

[0066] Tail flick and hot plate assay in mice for acute nociception. The tail flick test, which measures latency(s) of tail withdrawal from a noxious radiant heat source (Ugo Basile; model number 37360) was used to measure thermal nociceptive sensitivity in mice with baseline latencies of 3-5 seconds and a cut-off time of 15 seconds to prevent tissue injury [52]. Tail flick latencies were taken before and at 30, 60 and 120 minutes after i.p. injection of IB-MECA, MRS1898 (0.5 μ mol/kg) or its vehicle (0.1% DMSO in saline). For the hot plate test, nociceptive thresholds were determined as previously reported by our group by measuring latencies (in seconds) of mice placed in a transparent glass cylinder on a hot plate maintained at 52°C [53, 54]. Responses indicative of nociception included intermittent lifting and/or licking of the hindpaws or escape behavior. Hot plate latencies were taken in mice from all groups before and after drug administration. A cut-off latency of 15 seconds was employed to prevent tissue damage.

[0067] Rotarod test in mice: Mice were trained before experimentation for their ability to remain for 60 seconds on a revolving Rotarod for mice apparatus (Ugo Basile; accelerating units increasing from 5 to 40 rpm in 60 sec). Mice were injected i.p. with IB-MECA, MRS1898 (0.5 μ mol/kg) or vehicle (0.1% DMSO in saline) and then examined at 30, 60 and 120 minutes after administration for motor impairments on the Rotarod. The latency time to fall off the Rotarod was determined with a cut-off time of 120 seconds.

[0068] Effects of IB-MECA on antitumor activity of paclitaxel, oxaliplatin and bortezomib. Cells were cultured and assayed in DMEM (SKBR3; human breast cancer cells [55]), L15 (SW480; human colon cancer cells [56]) or RPMI 1620 (RPMI 8226; human multiple myeloma cells [57]) supplemented with heat-denatured 10% FBS and penicillin/streptomycin at 37°C, 0% (SW480) or 5% CO₂ (SKBR3 and RPMI 8226), 95% humidity. The antitumor activities of these agents were measured in cells (6.25 x 10⁴ cells/well) cultured overnight in 24-well plates (SKBR3 and SW480; BD Biosciences) or 12 mm x 7.5 mm culture tubes (RPMI 8226; BD Biosciences) in complete media. This plating regiment yielded 60% confluent plate cultures for testing. After equilibrating in fresh media (5 hours), cells were treated for 48 hours with IB-MECA (10 nM) or its vehicle (PBS) and paclitaxel (1-100 nM) or its vehicle (1% Cremophor EL: 0.9% ethanol); oxaliplatin (1-100 μ M) or its vehicle (0.01% DMSO in PBS); or bortezomib (1-100 nM) or its vehicle (0.05% dextrose).

Two naïve control wells were included as a control for 100% survival. Cell survival was determined using a MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay adapted from a previously described assay [58, 59]. Cells were incubated with MTT (500 µg/ml) for 75 minutes at 37°C, 0% or 5% CO₂, 95% humidity, removing the media and dissolving the tetrazolium crystals in isopropanol. The tetrazolium absorption (A_{560-570 nm}) was measured from paclitaxel experiments using a Unicam UV1 spectrophotometer (ThermoFisher Scientific, Waltham, MA) and from oxaliplatin or bortezomib experiments using a Glomax[®] Multi-Detection system (Promega, Madison, WI). The antitumor effects of IB-MECA alone were determined as %Survivability = (A_{560-570 nm} of chemotherapeutic vehicle + IB-MECA)/(mean A_{560-570 nm} of the naïve control wells) x 100. The LD₅₀ of each chemotherapeutic agent + IB-MECA or its vehicle was calculated using three-parameter non-linear analysis using %Survivability = (A_{560-570 nm} of chemotherapeutic + IB-MECA or its vehicle)/(mean A_{560-570 nm} chemotherapeutic vehicle + vehicle of IB-MECA) x 100. The top and bottom plateaus were constrained using GraphPad Prism v5.03 (GraphPad Software, Inc.).

[0069] Determining ED₅₀ values. Dose response data were curve-fitted using the least sum of square method by a normalized 4-parameter, variable slope non-linear analysis of the %Reversal of Mechano-allodynia in CCI and %Prevention of Mechano-allodynia or Mechano-hyperalgesia in CIPN using GraphPad Prism (Release 5.03, GraphPad Software, Inc.) from which the ED₅₀ was determined and reported with the 95% confidence interval (95% CI). In the CCI model, the %Reversal of Mechano-allodynia = (PWT_{1h}-PWT_{D7})/(PWT_{D0}-PWT_{D7}) x 100, where PWT_{D0}= Paw Withdrawal Threshold (g) at D0, PWT_{1h}= Paw Withdrawal Threshold (g) at 1 hour after the administration of IB-MECA, gabapentin, amitriptyline, or combinations of IB-MECA with gabapentin or amitriptyline, and PWT_{D7}= Paw Withdrawal Threshold (grams) at D7 prior to drug administration. In the chemotherapeutic-induced neuropathic pain models, the %Prevention = (PWT_{IB-MECA} - mean PWT_{chemo})/(mean PWT_{Veh} - mean PWT_{chemo}) x 100, where PWT = Paw Withdrawal Threshold, Chemo = paclitaxel or oxaliplatin and Veh = vehicle.

[0070] Statistical Analysis. Data are expressed as mean ± SD for n animals. Behavioral data were analyzed by two-way repeated measures ANOVA with Bonferroni comparisons (full time course studies) or one-way ANOVA Dunnett's

comparisons (1 hour behavioral data). The dose response curves were compared to a globally fitted curve using the extra sum-of-squares F-test comparisons to determine whether the data represented distinct curves between treatments. Significant differences were defined at a $P < 0.05$. All statistical analysis was performed using GraphPad Prism (v5.03, GraphPad Software, Inc.).

[0071] Results

[0072] A_3AR agonists block the development of neuropathic pain following chronic constriction injury (CCI) via a mechanism believed to be mediated by A_3AR . When peak mechano-allodynia develops (at D7) following CCI of the mouse sciatic nerve [47], i.p. administration of IB-MECA, but not vehicle (0.1% DMSO in saline), rapidly (≤ 30 min) and dose-dependently ($0.2\text{--}2\text{ }\mu\text{mol/kg}$, $n=5$) reversed allodynia, with maximal effect within 1 hour (ED_{50} , $0.4\text{ }\mu\text{mol/kg}$; 95% CI= $0.23\text{--}0.66$) (Figure 1A). IB-MECA lacked effect on Paw Withdrawal Thresholds (PWT, grams) in contralateral paws (Figure 1B). Noteworthy, when compared to D7, consecutive daily injections ($0.5\text{ }\mu\text{mol/kg}$ IB-MECA, $n=5$) on D8-D15 reversed mechano-allodynia to the same degree as observed on D7 following the first injection (Figure 1C). Without being bound to any particular theory, this suggests that A_3AR s do not become tolerant to agonist activation, at least over this dosing paradigm.

[0073] The anti-allodynic effect of IB-MECA ($0.5\text{ }\mu\text{mol/kg}$, $n=5$) was prevented by a 15 minutes pretreatment with a potent A_3AR antagonist, MRS1523 ($5\text{ }\mu\text{mol/kg}$, $n=5$; Figure 2A) [60]. MRS1523 is a 1,4-dihydropyridine derivative that binds to murine and rat A_3AR s with high affinity and has moderate selectivity against A_1AR , but excellent selectivity against A_2AAR and A_2BAR (at least 1000-fold) [61]. In contrast, the anti-allodynic effect of IB-MECA ($0.5\text{ }\mu\text{mol/kg}$, $n=5$) was not affected by the potent A_1AR antagonist, DPCPX ($2\text{ }\mu\text{mol/kg}$, $n=5$) or by the potent $A_{2A}AR$ antagonist, SCH-442416 ($0.2\text{ }\mu\text{mol/kg}$, $n=5$) (44) (Figure 2C). When given alone on D7, MRS1523, DPCPX or SCH-442416 lacked effect on ipsilateral (Figure 2A, C) or contralateral (Figure 2B, D) PWT. Agonist and antagonist doses were chosen from previous studies showing selectivity for their respective receptor subtype [62-64].

[0074] To prove that the benefit of A_3AR agonists in neuropathic pain is independent of which A_3AR agonist is used to treat the pain, two additional selective A_3AR agonists were tested: Cl-IB-MECA, the 2-chlorinated analogue of IB-MECA, and the

structurally distinct MRS1898. MRS1898 is a well-characterized, potent A₃AR agonist containing a rigid bicyclic ring substitution of ribose that is believed to maintain a receptor-preferred conformation [45]. CI-IB-MECA (0.6 µmol/kg, n=5; Figure 3A) and MRS1898 (0.5 µmol/kg, n=5; Figure 3C) rapidly and maximally (≤ 1 h) reversed mechano-allodynia, effects blocked by MRS1523 (5 µmol/kg, n=5) (Figure 3A) but not by DPCPX (2 µmol/kg, n=5) or SCH-442416 (0.2 µmol/kg, n=5) (Figure 3C). Doses of CI-IB-MECA and MRS1898 were selected from previous studies [45]. CI-IB-MECA and MRS1898 had no effect on PWT in contralateral paws (Figure 3B, D). Without being bound to any particular theory, such results indicate that A₃AR agonists of two distinct classes reverse mechano-allodynia through an A₃AR-mediated mechanism and without involving other ARs.

[0075] Anti-allodynic effects of A₃AR agonists are naloxone-independent. A high dose (i.p.) of non-selective opioid receptor antagonist naloxone (25 µmol/kg, n=5) was administered 15 minutes before IB-MECA (0.5 µmol/kg, n=5) or MRS1898 (0.5 µmol/kg, n=5). Naloxone did not interfere with the ability of the A₃AR agonists to reverse established mechano-allodynia in the CCI model (Figure 4A), supporting the exclusion of an opioid-dependent mechanism. Given alone on D7, naloxone did not affect ipsilateral (Figure 4A) or contralateral (Figure 4B) PWT.

[0076] A₃AR agonists have no effect on acute nociception. IB-MECA or MRS1898 (0.5 µmol/kg, n=5) tested at 30, 60 and 120 minutes lacked effect on acute nociception in the mouse tail flick (Figure 5A). On the other hand, morphine injected s.c. (35 µmol/kg; n=5) and used as a positive control elicited potent acute antinociceptive effects with a significant (P<0.001) increase in tail flick latencies (Figure 5A). Similarly, IB-MECA or MRS1898 (0.5 µmol/kg, n=5) had no effect when tested on the hot plate (not shown), supporting the lack of a role for A₃AR agonists in modulating normal nociception.

[0077] A₃AR agonists have no effect on the Rotarod test. IB-MECA or MRS1898 (0.5 µmol/kg, n=5) did not induce Rotarod deficits, thereby providing evidence countering potential motor function impairment in mice (Figure 5B). In addition, A₃AR agonists when tested at the highest dose lacked observable signs of lethargy or sedation *i.e.* the specimens exhibited normal posture, no loss of normal activity such as grooming, no effect on alert and exploratory behavior, no effect on

spontaneous locomotor activity and no loss of motor coordination, paw dragging or their ability to remain upright.

[0078] IB-MECA increases the potency the analgesic effects of morphine, gabapentin and amitriptyline in CCI. Morphine (0.11-35 $\mu\text{mol/kg}$, $n=5$), but not its vehicle (saline), given s.c. on D7 led to a rapid peak (≤ 0.5 h) and dose-dependent reversal of mechano-allodynia in ipsilateral paws (Figure 6A), but not in contralateral paws (Figure 6B). Morphine at the time of maximal reversal (0.5 hours) displayed an ED_{50} of 2.1 $\mu\text{mol/kg}$ (95% CI= 1.5-3.0), which was 5-fold less potent than IB-MECA (Figure 7). Moreover, IB-MECA ($E_{\text{max}} = 100\%$) was more efficacious than morphine alone ($E_{\text{max}} = 62 \pm 3\%$, Figure 7). Gabapentin (18-584 $\mu\text{mol/kg}$, i.p., $n=5$; Figure 6C) or amitriptyline (3-191 $\mu\text{mol/kg}$, oral, $n=5$; Figure 6E), but not their vehicle (saline), on D7 led to a rapid (≤ 0.5 h) and dose-dependent reversal of mechano-allodynia in ipsilateral paws that peaked at 1 hour. The ED_{50} of gabapentin and amitriptyline at maximal reversal (1 hour) was, respectively, 140 $\mu\text{mol/kg}$ (95% CI= 122-162) and 31 $\mu\text{mol/kg}$ (95% CI= 22-43) (Figure 7). Therefore, the gabapentin and amitriptyline were shown to be >350- and 75-fold less potent than IB-MECA. Gabapentin and amitriptyline had no effect on contralateral PWT (Figure 6D, F).

[0079] It is noteworthy that an IB-MECA dose devoid of anti-allodynic effects (0.2 $\mu\text{mol/kg}$, $n=5$) augmented the ability of morphine (0.11-35 $\mu\text{mol/kg}$, $n=5$; Figure 8A), gabapentin (1.8-175 $\mu\text{mol/kg}$, $n=5$; Figure 8B) or amitriptyline (1-96 $\mu\text{mol/kg}$, $n=5$; Figure 8C) to reverse established mechano-allodynia, as evidenced by a significant ($P < 0.001$) leftward dose response shift. To this end, the ED_{50} at peak reversal for morphine decreased from 2.1 $\mu\text{mol/kg}$ (95% CI= 1.5-3.0) to 0.98 $\mu\text{mol/kg}$ (95% CI= 0.66-1.5) when combined with IB-MECA, whereas the ED_{50} at peak reversal for gabapentin or amitriptyline decreased from 140 $\mu\text{mol/kg}$ (95% CI= 122-162) and 31 $\mu\text{mol/kg}$ (95% CI= 22-43) to 27 $\mu\text{mol/kg}$ (95% CI= 21-34) and 13 $\mu\text{mol/kg}$ (95% CI= 11-16) respectively when combined with IB-MECA. This combined treatment lacked effect on contralateral PWT (not shown). Collectively, IB-MECA increased the potency of morphine by >2-fold and that of gabapentin and amitriptyline by >5- and 2-fold, respectively. Moreover, IB-MECA also enhanced the efficacy of morphine by 1.6-fold (Figure 8A).

[0080] A₃AR agonists block the development of chemotherapy-induced neuropathic pain without interfering with antitumor effects. A₃AR were tested in models of neuropathic pain induced by widely used chemotherapeutics in distinct classes and with well known, distinct antitumor mechanisms of action: paclitaxel, oxaliplatin and bortezomib. Although chemotherapeutic dosing in each model is completed within several days, A₃AR agonists were administered until the time when pain typically occurs (usually between 15-17 days). When compared to vehicle, paclitaxel administration led to neuropathic pain (mechano-allodynia and mechano-hyperalgesia) that peaked by D16, plateaued through D25 and was dose-dependently attenuated by daily (D0-D15) administration of IB-MECA (0.02-0.2 μmol/kg/d, i.p., n=6; Figure 9A, B) but not vehicle (0.1% DMSO in saline). The D16 ED₅₀ values for prevention of paclitaxel-induced mechano-allodynia and mechano-hyperalgesia were 0.02 and 0.03 μmol/kg/d (95% CI= 0.018-0.024 and 0.024-0.033).

[0081] It is noteworthy that following discontinuation of IB-MECA treatment on D15, neuropathic pain did not emerge through D25 (Figure 9A, B). The effects of IB-MECA (0.2 μmol/kg/d, n=6) were prevented by co-administration with MRS1523 (5 μmol/kg/d, n=6; Figure 9C, D). Without being bound to any particular theory, this indicates that A₃AR agonism is involved, since CI-IB-MECA (0.2 μmol/kg/d, n=5) or MRS1898 (0.2 μmol/kg/d, n=3) completely blocked paclitaxel-induced neuropathic pain (not shown) eliminating the possibility that a pharmacological action particular to IB-MECA causes the protective effects.

[0082] The beneficial effects of IB-MECA were not restricted to paclitaxel. Indeed, the development of oxaliplatin-induced neuropathic pain (peaking by D17 and plateauing through D25) was attenuated dose-dependently by daily (D0-D17) administration of IB-MECA (0.02-0.2 μmol/kg/d, n=6; Figure 9E, F) and did not emerge upon drug termination. IB-MECA prevented mechano-allodynia and mechano-hyperalgesia with D17 with ED₅₀ values of 0.05 and 0.06 μmol/kg/d, respectively (95% CI= 0.039-0.053 and 0.048-0.07). Finally, IB-MECA (0.2 μmol/kg/d, n=5) blocked the development of bortezomib-induced mechano-allodynia and mechano-hyperalgesia, which did not emerge when treatment was discontinued on D17 (not shown). IB-MECA administered alone (0.2 μmol/kg/d, n=6) did not affect PWT in any of the chemotherapy models used (Figure 9).

[0083] Additional experiments confirming an A₃AR-dependent mechanism in oxaliplatin- or bortezomib-induced neuropathic pain were unnecessary as the IB-MECA doses matched those used with paclitaxel. None of the drugs tested affected body weight, and all animals gained weight to the same extent over the course of the experiment (not shown). The potent antitumor effects of A₃AR agonists are well documented [65]. The effects of IB-MECA on the antitumor activity of paclitaxel in human breast cancer cells (SKBR3) [45], oxaliplatin in human colon cancer cells (SW480) [46], or bortezomib in human multiple myeloma cells (RPMI 8226) [57] were assessed using a MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay adapted from a previously described assay [58, 59]. At a dose yielding <20% decrease in cell survival when used alone, IB-MECA (10 nM) did not diminish the antitumor effects of paclitaxel, oxaliplatin or bortezomib on human breast, colon and multiple myeloma cancer cells (Table 1, below). Higher doses of IB-MECA were not used, because these doses had direct antitumor effects on all three cell lines tested and so could interact positively with antitumor effects of the chemotherapeutic to provide benefit.

Treatment	LD ₅₀	n-value	<i>P</i> -value
<i>SKBR3 breast cancer cells</i>			
Paclitaxel	7.0 nM	5	1.0
Paclitaxel +	7.1 nM	5	
IB-MECA (10 nM)			
<i>SW480 colon cancer cells</i>			
Oxaliplatin	3.8 μM	6	0.89
Oxaliplatin +	3.2 μM	6	
IB-MECA (10 nM)			
<i>RPMI 8226 multiple myeloma cells</i>			
Bortezomib	27 nM	5	0.25
Bortezomib +	29 nM	5	
IB-MECA (10 nM)			

Table 1

REFERENCES

1. Fishman, P. & Bar-Yehuda, S. Pharmacology and therapeutic applications of A3 receptor subtype. *Curr Top Med Chem* 3, 463-469 (2003).
- 5 2. Jacobson, K.A. Adenosine A3 receptors: novel ligands and paradoxical effects. *Trends Pharmacol Sci* 19, 184-191 (1998).
3. Kim, H.O., *et al.* 2-Substitution of N6-benzyladenosine-5'-uronamides enhances selectivity for A3 adenosine receptors. *J Med Chem* 37, 3614-3621 (1994).
- 10 4. De Grandis, D. Acetyl-L-carnitine for the treatment of chemotherapy-induced peripheral neuropathy: a short review. *CNS Drugs* 21 Suppl 1, 39-43; discussion 45-36 (2007).
- 5 5. Cata, J.P., Weng, H.R., Lee, B.N., Reuben, J.M. & Dougherty, P.M. Clinical and experimental findings in humans and animals with chemotherapy-induced peripheral neuropathy. *Minerva Anesthesiol* 72, 151-169 (2006).
- 15 6. Farquhar-Smith, P. Chemotherapy-induced neuropathic pain. *Curr Opin Support Palliat Care* 5, 1-7 (2011).
7. Polomano, R.C. & Bennett, G.J. Chemotherapy-evoked painful peripheral neuropathy. *Pain Med* 2, 8-14 (2001).
- 20 8. Dougherty, P.M., Cata, J.P., Cordella, J.V., Burton, A. & Weng, H.R. Taxol-induced sensory disturbance is characterized by preferential impairment of myelinated fiber function in cancer subjects. *Pain* 109, 132-142 (2004).
9. Lynch, J.J., 3rd, Wade, C.L., Zhong, C.M., Mikusa, J.P. & Honore, P. Attenuation of mechanical allodynia by clinically utilized drugs in a rat chemotherapy-induced neuropathic pain model. *Pain* 110, 56-63 (2004).
- 25 10. Polomano, R.C., Mannes, A.J., Clark, U.S. & Bennett, G.J. A painful peripheral neuropathy in the rat produced by the chemotherapeutic drug, paclitaxel. *Pain* 94, 293-304 (2001).
11. Fishman, P., Bar-Yehuda, S., Madi, L. & Cohn, I. A3 adenosine receptor as a target for cancer therapy. *Anticancer Drugs* 13, 437-443 (2002).
- 30

12. Flatters, S.J. & Bennett, G.J. Studies of peripheral sensory nerves in paclitaxel-induced painful peripheral neuropathy: evidence for mitochondrial dysfunction. *Pain* 122, 245-257 (2006).

13. Jin, H.W., Flatters, S.J., Xiao, W.H., Mulhern, H.L. & Bennett, G.J.
5 Prevention of paclitaxel-evoked painful peripheral neuropathy by acetyl-L-carnitine: effects on axonal mitochondria, sensory nerve fiber terminal arbors, and cutaneous Langerhans cells. *Exp Neurol* 210, 229-237 (2008).

14. Bennett, G.J. & Xie, Y.K. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* 33, 87-107
10 (1988).

15. Randall, L.O. & Selitto, J.J. A method for measurement of analgesic activity on inflamed tissue. *Arch Int Pharmacodyn Ther* 111, 409-419 (1957).

16. Dixon, W.J. Efficient analysis of experimental observations. *Annu Rev Pharmacol Toxicol* 20, 441-462 (1980).

17. Taliani, L., Pugliesi, I., Bellandi, M., La Motta, C. & Da Settimo, F. A₃ receptor ligands: past, present and future trends.
15

18. Göblyös, A. & Izjerman, P. Allosteric modulation of adenosine receptors.

19. Torrance, N., Smith, B. H., Bennett, M. I., and Lee, A. J. (2006) The
20 epidemiology of chronic pain of predominantly neuropathic origin. Results from a general population survey. *J Pain* 7, 281-289.

20. Farquhar-Smith, P. (2011) Chemotherapy-induced neuropathic pain. *Curr Opin Support Palliat Care* 5, 1-7.

21. Cata, J. P., Weng, H. R., Lee, B. N., Reuben, J. M., and Dougherty, P.
25 M. (2006) Clinical and experimental findings in humans and animals with chemotherapy-induced peripheral neuropathy. *Minerva Anesthesiol* 72, 151-169.

22. Finnerup, N. B., Sindrup, S. H., and Jensen, T. S. (2010) The evidence for pharmacological treatment of neuropathic pain. *Pain* 150, 573-581.

23. Fredholm, B. B., AP, I. J., Jacobson, K. A., Linden, J., and Muller, C.
30 E. (2011) International Union of Basic and Clinical Pharmacology. LXXXI.

Nomenclature and classification of adenosine receptors--an update. *Pharmacol Rev* 63, 1-34.

24. Fredholm, B. B., AP, I. J., Jacobson, K. A., Klotz, K. N., and Linden, J. (2001) International Union of Pharmacology. XXV. Nomenclature and classification
5 of adenosine receptors. *Pharmacol Rev* 53, 527-552.

25. Kumar, V., and Sharma, A. (2009) Adenosine: an endogenous modulator of innate immune system with therapeutic potential. *Eur J Pharmacol* 616, 7-15.

26. Fishman, P., and Bar-Yehuda, S. (2003) Pharmacology and
10 therapeutic applications of A3 receptor subtype. *Curr Top Med Chem* 3, 463-469.

27. Fishman, P., Bar-Yehuda, S., Madi, L., and Cohn, I. (2002) A3 adenosine receptor as a target for cancer therapy. *Anticancer Drugs* 13, 437-443.

28. Kiesman, W. F., Elzein, E., and Zablocki, J. (2009) A1 adenosine receptor antagonists, agonists, and allosteric enhancers. *Handb Exp Pharmacol*,
15 25-58.

29. Taliani, S., Pugliesi, I., Bellandi, M., La Motta, C., and Da Settimo, F. (2010) A3 receptor ligands: past, present and future trends. *Curr Top Med Chem* 10, 942-975.

30. Zylka, M. J. (2011) Pain-relieving prospects for adenosine receptors
20 and ectonucleotidases. *Trends Mol Med* 17, 188-196.

31. Loram, L. C., Harrison, J. A., Sloane, E. M., Hutchinson, M. R., Sholar, P., Taylor, F. R., Berkelhammer, D., Coats, B. D., Poole, S., Milligan, E. D., Maier, S. F., Rieger, J., and Watkins, L. R. (2009) Enduring Reversal of Neuropathic Pain by a Single Intrathecal Injection of Adenosine 2A Receptor Agonists: A Novel
25 Therapy for Neuropathic Pain. *J Neurosci* 29, 14015-14025.

32. Jacobson, K. A., Gao, Z. G., Goblyos, A., and Ijzerman, A. P. (2011) Allosteric modulation of purine and pyrimidine receptors. *Adv Pharmacol* 61, 187-220.

33. Jacobson, K. A. (1998) Adenosine A3 receptors: novel ligands and
30 paradoxical effects. *Trends Pharmacol Sci* 19, 184-191.

34. Kim, H. O., Ji, X. D., Siddiqi, S. M., Olah, M. E., Stiles, G. L., and Jacobson, K. A. (1994) 2-Substitution of N6-benzyladenosine-5'-uronamides enhances selectivity for A3 adenosine receptors. *J Med Chem* 37, 3614-3621.

35. Silverman, M. H., Strand, V., Markovits, D., Nahir, M., Reitblat, T., Molad, Y., Rosner, I., Rozenbaum, M., Mader, R., Adawi, M., Caspi, D., Tishler, M., Langevitz, P., Rubinow, A., Friedman, J., Green, L., Tanay, A., Ochaion, A., Cohen, S., Kerns, W. D., Cohn, I., Fishman-Furman, S., Farbstein, M., Yehuda, S. B., and Fishman, P. (2008) Clinical evidence for utilization of the A3 adenosine receptor as a target to treat rheumatoid arthritis: data from a phase II clinical trial. *J Rheumatol* 35, 41-48.

36. Poulsen, S. A., and Quinn, R. J. (1998) Adenosine receptors: new opportunities for future drugs. *Bioorg Med Chem* 6, 619-641.

37. Ochaion, A., Bar-Yehuda, S., Cohen, S., Barer, F., Patoka, R., Amital, H., Reitblat, T., Reitblat, A., Ophir, J., Konfino, I., Chowers, Y., Ben-Horin, S., and Fishman, P. (2009) The anti-inflammatory target A3 adenosine receptor is over-expressed in rheumatoid arthritis, psoriasis and Crohn's disease. *Cell Immunol* 258, 115-122.

38. Abbracchio, M. P., Rainaldi, G., Giammarioli, A. M., Ceruti, S., Brambilla, R., Cattabeni, F., Barbieri, D., Franceschi, C., Jacobson, K. A., and Malorni, W. (1997) The A3 adenosine receptor mediates cell spreading, reorganization of actin cytoskeleton, and distribution of Bcl-XL: studies in human astrogloma cells. *Biochem Biophys Res Commun* 241, 297-304.

39. Ru, F., Surdenikova, L., Brozmanova, M., and Kollarik, M. (2011) Adenosine-induced activation of esophageal nociceptors. *Am J Physiol Gastrointest Liver Physiol* 300, G485-493.

40. Zhang, M., Hu, H., Zhang, X., Lu, W., Lim, J., Eysteinnsson, T., Jacobson, K. A., Laties, A. M., and Mitchell, C. H. (2010) The A3 adenosine receptor attenuates the calcium rise triggered by NMDA receptors in retinal ganglion cells. *Neurochemistry international* 56, 35-41.

41. Lopes, L. V., Rebola, N., Pinheiro, P. C., Richardson, P. J., Oliveira, C. R., and Cunha, R. A. (2003) Adenosine A3 receptors are located in neurons of the rat hippocampus. *Neuroreport* 14, 1645-1648.

42. Jacobson, K. A., Nikodijevic, O., Shi, D., Gallo-Rodriguez, C., Olah, M. E., Stiles, G. L., and Daly, J. W. (1993) A role for central A3-adenosine receptors. Mediation of behavioral depressant effects. *FEBS Lett* 336, 57-60.

43. Giannaccini, G., Betti, L., Palego, L., Fabbrini, L., Schmid, L., Castagna, M., Giusti, L., Mascia, G., and Lucacchini, A. (2008) Species comparison of adenosine receptor subtypes in brain and testis. *Neurochem Res* 33, 852-860.

44. Yoon, M. H., Bae, H. B., and Choi, J. I. (2005) Antinociception of intrathecal adenosine receptor subtype agonists in rat formalin test. *Anesth Analg* 101, 1417-1421.

45. Tchilibon, S., Joshi, B. V., Kim, S. K., Duong, H. T., Gao, Z. G., and Jacobson, K. A. (2005) (N)-methanocarba 2,N6-disubstituted adenine nucleosides as highly potent and selective A3 adenosine receptor agonists. *J Med Chem* 48, 1745-1758.

46. Gao, Z. G., Teng, B., Wu, H., Joshi, B. V., Griffiths, G. L., and Jacobson, K. A. (2009) Synthesis and pharmacological characterization of [125I]MRS1898, a high-affinity, selective radioligand for the rat A3 adenosine receptor. *Purinergic Signal* 5, 31-37.

47. Bennett, G. J., and Xie, Y. K. (1988) A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* 33, 87-107.

48. Polomano, R. C., Mannes, A. J., Clark, U. S., and Bennett, G. J. (2001) A painful peripheral neuropathy in the rat produced by the chemotherapeutic drug, paclitaxel. *Pain* 94, 293-304.

49. Zheng, H., Xiao, W. H., and Bennett, G. J. (2011) Functional deficits in peripheral nerve mitochondria in rats with paclitaxel- and oxaliplatin-evoked painful peripheral neuropathy. *Exp Neurol* 232(2):154-61.

50. Dixon, W. J. (1980) Efficient analysis of experimental observations. *Annu Rev Pharmacol Toxicol* 20, 441-462.

51. Randall, L. O., and Selitto, J. J. (1957) A method for measurement of analgesic activity on inflamed tissue. Arch Int Pharmacodyn Ther 111, 409-419.

52. D'Amour, F. (1941) A method for determining loss of pain sensation. J Pharmacol xp Ther 72, 74-79.

53. Ndengele, M. M., Cuzzocrea, S., Masini, E., Vinci, M. C., Esposito, E., Muscoli, C., Petrusca, D. N., Mollace, V., Mazzon, E., Li, D., Petrache, I., Matuschak, G. M., and Salvemini, D. (2009) Spinal ceramide modulates the development of morphine antinociceptive tolerance via peroxynitrite-mediated nitroxidative stress and neuroimmune activation. J Pharmacol Exp Ther 329, 64-75.

54. Wang, Z. Q., Porreca, F., Cuzzocrea, S., Galen, K., Lightfoot, R., Masini, E., Muscoli, C., Mollace, V., Ndengele, M., Ischiropoulos, H., and Salvemini, D. (2004) A newly identified role for superoxide in inflammatory pain. J Pharmacol Exp Ther 309, 869-878.

55. Yi, D., Smythe, G. A., Blount, B. C., and Duncan, M. W. (1997) Peroxynitrite-mediated nitration of peptides: characterization of the products by electrospray and combined gas chromatography-mass spectrometry. Arch Biochem Biophys 344, 253-259.

56. Dahan, L., Sadok, A., Formento, J. L., Seitz, J. F., and Kovacic, H. (2009) Modulation of cellular redox state underlies antagonism between oxaliplatin and cetuximab in human colorectal cancer cell lines. Br J Pharmacol 158, 610-620.

57. Pellat-Deceunynk, C., Amiot, M., Bataille, R., Van Riet, I., Van Camp, B., Omede, P., and Boccadoro, M. (1995) Human myeloma cell lines as a tool for studying the biology of multiple myeloma: a reappraisal 18 years after [letter]. Blood 86, 4001-4002.

58. Shah, M. R., Kriedt, C. L., Lents, N. H., Hoyer, M. K., Jamaluddin, N., Klein, C., and Baldassare, J. (2009) Direct intra-tumoral injection of zinc-acetate halts tumor growth in a xenograft model of prostate cancer. J Exp Clin Cancer Res 28, 84.

59. Kriedt, C. L., Baldassare, J., Shah, M., and Klein, C. (2010) Zinc functions as a cytotoxic agent for prostate cancer cells independent of culture and growth conditions. J Exp Ther Oncol 8, 287-295.

60. Li, A. H., Moro, S., Melman, N., Ji, X. D., and Jacobson, K. A. (1998) Structure-activity relationships and molecular modeling of 3, 5-diacyl-2,4-dialkylpyridine derivatives as selective A₃ adenosine receptor antagonists. *J Med Chem* 41, 3186-3201.

5 61. Kreckler, L. M., Wan, T. C., Ge, Z. D., and Auchampach, J. A. (2006) Adenosine inhibits tumor necrosis factor-alpha release from mouse peritoneal macrophages via A_{2A} and A_{2B} but not the A₃ adenosine receptor. *J Pharmacol Exp Ther* 317, 172-180.

10 62. Zheng, J., Wang, R., Zambraski, E., Wu, D., Jacobson, K. A., and Liang, B. T. (2007) Protective roles of adenosine A₁, A_{2A}, and A₃ receptors in skeletal muscle ischemia and reperfusion injury. *Am J Physiol Heart Circ Physiol* 293, H3685-3691.

15 63. Cohen, S., Stemmer, S., Zozulya, G., Ochaion, A., Patoka, R., Barer, F., Bar-Yehuda, S., Rath-Wolfson, L., Jacobson, K., and Fishman, P. (2010) CF102 an A₃ adenosine receptor agonist mediates anti-tumor and anti-inflammatory effects in the liver. *J Cell Physiol*, 226, 2438-2447.

20 64. Ge, Z. D., Peart, J. N., Kreckler, L. M., Wan, T. C., Jacobson, M. A., Gross, G. J., and Auchampach, J. A. (2006) Cl-IB-MECA [2-chloro-N⁶-(3-iodobenzyl)adenosine-5'-N-methylcarboxamide] reduces ischemia/reperfusion injury in mice by activating the A₃ adenosine receptor. *J Pharmacol Exp Ther* 319, 1200-1210.

65. Fishman, P., Bar-Yehuda, S., Liang, B. T., and Jacobson, K. A. (2012) Pharmacological and therapeutic effects of A₃ adenosine receptor agonists. *Drug Discov Today*, in press.

25 66. Foley, K. M. (1995) Misconceptions and controversies regarding the use of opioids in cancer pain. *Anticancer Drugs* 6 Suppl 3, 4-13.

67. Joseph, E. K., and Levine, J. D. (2004) Caspase signalling in neuropathic and inflammatory pain in the rat. *Eur J Neurosci* 20, 2896-2902.

30 68. Watkins, L. R., Milligan, E. D., and Maier, S. F. (2001) Glial activation: a driving force for pathological pain. *Trends Neurosci* 24, 450-455.

69. Bennett, G. J. (2010) Pathophysiology and animal models of cancer-related painful peripheral neuropathy. *Oncologist* 15 Suppl 2, 9-12.

70. Shen, H., Chen, G. J., Harvey, B. K., Bickford, P. C., and Wang, Y. (2005) Inosine reduces ischemic brain injury in rats. *Stroke* 36, 654-659.

5 71. Von Lubitz, D. K., Lin, R. C., Boyd, M., Bischofberger, N., and Jacobson, K. A. (1999) Chronic administration of adenosine A3 receptor agonist and cerebral ischemia: neuronal and glial effects. *Eur J Pharmacol* 367, 157-163.

72. Fedorova, I. M., Jacobson, M. A., Basile, A., and Jacobson, K. A. (2003) Behavioral characterization of mice lacking the A3 adenosine receptor: sensitivity to hypoxic neurodegeneration. *Cell Mol Neurobiol* 23, 431-447.

73. Chen, G. J., Harvey, B. K., Shen, H., Chou, J., Victor, A., and Wang, Y. (2006) Activation of adenosine A3 receptors reduces ischemic brain injury in rodents. *J Neurosci Res* 84, 1848-1855.

74. Hentschel, S., Lewerenz, A., and Nieber, K. (2003) Activation of A3 receptors by endogenous adenosine inhibits synaptic transmission during hypoxia in rat cortical neurons. *Restor Neurol Neurosci* 21, 55-63.

75. Wunderlich, J. E., Needleman, B. J., Chen, Z., Yu, J. G., Wang, Y., Grants, I., Mikami, D. J., Melvin, W. S., Cooke, H. J., and Christofi, F. L. (2008) Dual purinergic synaptic transmission in the human enteric nervous system. *Am J Physiol Gastrointest Liver Physiol* 294, G554-566.

76. Rubaj, A., Zgodzinski, W., and Sieklucka-Dziuba, M. (2003) The influence of adenosine A3 receptor agonist: IB-MECA, on scopolamine- and MK-801-induced memory impairment. *Behav Brain Res* 141, 11-17.

77. Tchilibon, S., *et al.* (2005) (N)-methanocarba 2, N⁶-disubstituted adenine nucleosides as highly potent and selective A₃ adenosine receptor antagonists. *J. Med. Chem.* 48, 1745-1758.

78. Kim, H.O., *et al.* (1994) Substitution of N⁶-benzyladenosine-5'-uronamides enhances selectivity for A₃ adenosine receptors. *J. Med. Chem.* 37, 3614-3621 (1994).

79. Gao, Z.G., *et al.* (2009) Synthesis and pharmacological characterization of [125 I]MRS 1898, a high-affinity, selective radioligand for the rat A₃ adenosine receptor. *Purine Signal* 5, 31-37.

80. Ge, Z.D., *et al.* (2006) Cl-IB-MECA [2-Chloro-N⁶-(3-iodobenzyl)adenosine-5'-N-methylcarboxamide] reduces ischemia/reperfusion injury in mice by activating the A₃ adenosine receptor. *J Pharmacol Exp Ther* 319, 1200-1210 (2006).

81. Tosh, D.K. *et al.* (2009) Functionalized congeners of A₃ adenosine receptor-selective nucleosides containing a bicycle[3.1.0]hexane ring system. *J Med Chem* 52, 7580-7592.

82. Tosh, D.K., *et al.* (2010) 2-Dialkynyl derivatives of (N)-methanocarba nucleosides: "clickable" A₃ adenosine receptor-selective agonist. *Bioorg Med Chem* 18, 508-517.

83. Chanyshhev, B., *et al.* (2012) Anti-ischemic effects of multivalent dendrimeric A₃ adenosine receptor agonists in cultured cardiomyocytes and in the isolated rat heart. *Pharmacol Res* 65, 338-346.

84. Wan, T.C. *et al.* (2011) Polyamidoamine (PAMAM) dendrimer conjugate specifically activates the A₃ adenosine receptor to improve post-ischemic/reperfusion function in isolated mouse hearts. *BMC Pharmacol* 11, 11.

CLAIMS

1. A method of treating neuropathic pain in a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an A₃AR agonist.

5

2. The method of claim 1, wherein the A₃AR agonist is an A₃AR allosteric modulator.

3. The method of claim 1, wherein the A₃AR agonist is selected from the group consisting of: *N*⁶-(3-iodobenzyl)-adenosine-5'-*N*-methyluronamide; 2-Chloro-*N*⁶-(3-iodobenzyl)-adenosine-5'-*N*-methyluronamide; (1*R*,2*R*,3*S*,4*R*)-4-(2-chloro-6-((3-chlorobenzyl)amino)-9*H*-purin-9-yl)-2,3-dihydroxy-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide; (2*S*,3*S*,4*R*,5*R*)-3-amino-5-[6-(2,5-dichlorobenzylamino)purin-9-yl]-4-hydroxytetrahydrofuran-2-carboxylic acid methylamide; (1'*S*,2'*R*,3'*S*,4'*R*,5'*S*)-4-(2-chloro-6-(3-chlorobenzylamino)-9*H*-purin-9-yl)-2,3-dihydroxy-*N*-methylbicyclo [3.1.0] hexane-1-carboxamide; 2-(1-Hexynyl)-*N*-methyladenosine; (1*S*,2*R*,3*S*,4*R*)-2,3-dihydroxy-4-(6-((3-iodobenzyl)amino)-4*H*-purin-9(5*H*)-yl)-*N*-methylcyclopentanecarboxamide; (1*S*,2*R*,3*S*,4*R*)-4-(2-chloro-6-((3-iodobenzyl)amino)-4*H*-purin-9(5*H*)-yl)-2,3-dihydroxy-*N*-methylcyclopentanecarboxamide; 2-cyclohexyl-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine; (1'*R*, 2'*R*, 3'*S*, 4'*R*, 5'*S*)-4-{2-chloro-6-[(3-iodophenylmethyl)amino]purin-9-yl}-1-(methylaminocarbonyl)-bicyclo[3.1.0]hexane-2,3-diol, and combinations thereof.

4. The method of claim 1, wherein the A₃AR agonist is *N*⁶-(3-iodobenzyl)-adenosine-5'-*N*-methyluronamide.

5. The method of claim 1, wherein the neuropathic pain is due to a cause selected from the group consisting of spinal cord injury, multiple sclerosis, stroke, diabetes, herpes zoster infection, HIV-related neuropathies, nutritional deficiencies, toxins, remote manifestations of malignancies, genetic, immune mediated disorders,

physical trauma to a nerve trunk, cancer, chemotherapy, radiation injury, surgery, and combinations thereof.

6. The method of claim 1, wherein the neuropathic pain is due to chemotherapy.

5

7. The method of claim 6, wherein the pain is due to the administration to the subject of a chemotherapeutic agent selected from the group consisting of podophyllotoxins, taxanes, platinum complexes, vinca alkaloids, proteasome inhibitors, colchicines, eribulin, lenolidamide, ixabepilone, interferons, thalidomide, etoposide, ifosfamide, procarbazine, cytarabine, gemcitabine, arsenic, and combinations thereof.

10

8. The method of claim 6, wherein the neuropathic pain is due to the administration of paclitaxel to the subject.

15

9. A method of treating neuropathic pain in a subject, comprising administering to the subject a first amount of an A₃AR agonist and a second amount of an analgesic, wherein the first and second amounts together comprise a therapeutically effective amount.

20

10. The method of claim 9, wherein the A₃AR agonist is an A₃AR allosteric modulator.

11. The method of claim 9, wherein the analgesic is selected from the group consisting of opioids, morphinomimetics, antidepressants, antiepileptic, NMDA receptor antagonists, fatty acid amine hydrolyase inhibitors, NMDA receptor antagonists, anticonvulsants, antidepressants, non-steroidal anti-inflammatory drugs, calcium channel subunit $\alpha_2\delta$ ligands, COX-2 inhibitors, NOS inhibitors, and combinations thereof.

30

12. The method of claim 9, wherein the analgesic is selected from the group consisting of an opioid, a tricyclic antidepressant, a calcium channel subunit $\alpha_2\delta$ ligand, and combinations thereof.

13. The method of claim 9, wherein the analgesic is selected from the group consisting of morphine, gabapentin, pregabalin, amitriptyline, and combinations thereof.

14. The method of claim 9, wherein the A_3AR agonist is selected from the group consisting of: N^6 -(3-iodobenzyl)-adenosine-5'-*N*-methyluronamide; 2-Chloro- N^6 -(3-iodobenzyl)-adenosine-5'-*N*-methyluronamide; (1*R*,2*R*,3*S*,4*R*)-4-(2-chloro-6-((3-chlorobenzyl)amino)-9*H*-purin-9-yl)-2,3-dihydroxy-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide; (2*S*,3*S*,4*R*,5*R*)-3-amino-5-[6-(2,5-dichlorobenzylamino)purin-9-yl]-4-hydroxytetrahydrofuran-2-carboxylic acid methylamide; (1'*S*,2'*R*,3'*S*,4'*R*,5'*S*)-4-(2-chloro-6-(3-chlorobenzylamino)-9*H*-purin-9-yl)-2,3-dihydroxy-*N*-methylbicyclo [3.1.0] hexane-1-carboxamide; 2-(1-Hexynyl)-*N*-methyladenosine; (1*S*,2*R*,3*S*,4*R*)-2,3-dihydroxy-4-(6-((3-iodobenzyl)amino)-4*H*-purin-9(5*H*)-yl)-*N*-methylcyclopentanecarboxamide; (1*S*,2*R*,3*S*,4*R*)-4-(2-chloro-6-((3-iodobenzyl)amino)-4*H*-purin-9(5*H*)-yl)-2,3-dihydroxy-*N*-methylcyclopentanecarboxamide; 2-cyclohexyl-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine; (1'*R*, 2'*R*, 3'*S*, 4'*R*, 5'*S*)-4-{2-chloro-6-[(3-iodophenylmethyl)amino]purin-9-yl}-1-(methylaminocarbonyl)-bicyclo[3.1.0]hexane-2,3-diol, and combinations thereof.

15. The method of claim 9, wherein the neuropathic pain is induced due to a cause selected from the group consisting of spinal cord injury, multiple sclerosis, stroke, diabetes, herpes zoster infection, HIV-related neuropathies, nutritional deficiencies, toxins, remote manifestations of malignancies, genetic, immune mediated disorders, physical trauma to a nerve trunk, cancer, chemotherapy, radiation injury, surgery, and combinations thereof.

16. A pharmaceutical composition for treating neuropathic pain, comprising a first amount of an A₃AR agonist and a second amount of an analgesic, wherein the first and second amounts taken together comprise a pharmaceutically effective amount.

17. The pharmaceutical composition of claim 16, comprising: a first pharmaceutically acceptable composition comprising the first amount of the A₃AR agonist, and a second pharmaceutically acceptable composition comprising the second amount of the analgesic.

18. The pharmaceutical composition of claim 16, wherein the A₃AR agonist is selected from the group consisting of: *N*⁶-(3-iodobenzyl)-adenosine-5'-*N*-methyluronamide; 2-Chloro-*N*⁶-(3-iodobenzyl)-adenosine-5'-*N*-methyluronamide; (1*R*,2*R*,3*S*,4*R*)-4-(2-chloro-6-((3-chlorobenzyl)amino)-9*H*-purin-9-yl)-2,3-dihydroxy-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide; (2*S*,3*S*,4*R*,5*R*)-3-amino-5-[6-(2,5-dichlorobenzylamino)purin-9-yl]-4-hydroxytetrahydrofuran-2-carboxylic acid methylamide; (1'*S*,2'*R*,3'*S*,4'*R*,5'*S*)-4-(2-chloro-6-(3-chlorobenzylamino)-9*H*-purin-9-yl)-2,3-dihydroxy-*N*-methylbicyclo [3.1.0] hexane-1-carboxamide; 2-(1-Hexynyl)-*N*-methyladenosine; (1*S*,2*R*,3*S*,4*R*)-2,3-dihydroxy-4-(6-((3-iodobenzyl)amino)-4*H*-purin-9(5*H*)-yl)-*N*-methylcyclopentanecarboxamide; (1*S*,2*R*,3*S*,4*R*)-4-(2-chloro-6-((3-iodobenzyl)amino)-4*H*-purin-9(5*H*)-yl)-2,3-dihydroxy-*N*-methylcyclopentanecarboxamide; 2-cyclohexyl-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine; (1'*R*, 2'*R*, 3'*S*, 4'*R*, 5'*S*)-4-{2-chloro-6-[(3-iodophenylmethyl)amino]purin-9-yl}-1-(methylaminocarbonyl)-bicyclo[3.1.0]hexane-2,3-diol, and combinations thereof.

19. The pharmaceutical composition of claim 16, wherein the analgesic is selected from the group consisting of opioids, morphinomimetics, antidepressants, antiepileptic, NMDA receptor antagonists, fatty acid amine hydrolyase inhibitors, NMDA receptor antagonists, anticonvulsants, antidepressants, non-steroidal anti-inflammatory drugs, calcium channel subunit $\alpha_2\delta$ ligands, COX-2 inhibitors, NOS inhibitors, and combinations thereof.

20. The pharmaceutical composition of claim 16, wherein the analgesic is selected from the group consisting of morphine, gabapentin, pregabalin, amitriptyline, and combinations thereof.

5

21. Use of an A₃AR agonist in the manufacture of a medicament for the treatment of neuropathic pain.

22. The use according to claim 21, wherein the A₃AR agonist is selected from the group consisting of selected from the group consisting of: *N*⁶-(3-iodobenzyl)-adenosine-5'-*N*-methyluronamide; 2-Chloro-*N*⁶-(3-iodobenzyl)-adenosine-5'-*N*-methyluronamide; (1*R*,2*R*,3*S*,4*R*)-4-(2-chloro-6-((3-chlorobenzyl)amino)-9*H*-purin-9-yl)-2,3-dihydroxy-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide; (2*S*,3*S*,4*R*,5*R*)-3-amino-5-[6-(2,5-dichlorobenzylamino)purin-9-yl]-4-hydroxytetrahydrofuran-2-carboxylic acid methylamide; (1'*S*,2'*R*,3'*S*,4'*R*,5'*S*)-4-(2-chloro-6-(3-chlorobenzylamino)-9*H*-purin-9-yl)-2,3-dihydroxy-*N*-methylbicyclo [3.1.0] hexane-1-carboxamide; 2-(1-Hexynyl)-*N*-methyladenosine; (1*S*,2*R*,3*S*,4*R*)-2,3-dihydroxy-4-(6-((3-iodobenzyl)amino)-4*H*-purin-9(5*H*)-yl)-*N*-methylcyclopentanecarboxamide; (1*S*,2*R*,3*S*,4*R*)-4-(2-chloro-6-((3-iodobenzyl)amino)-4*H*-purin-9(5*H*)-yl)-2,3-dihydroxy-*N*-methylcyclopentanecarboxamide; 2-cyclohexyl-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine; (1'*R*, 2'*R*, 3'*S*, 4'*R*, 5'*S*)-4-{2-chloro-6-[(3-iodophenylmethyl)amino]purin-9-yl}-1-(methylaminocarbonyl)-bicyclo[3.1.0]hexane-2,3-diol, and combinations thereof.

23. The use according to claim 21, wherein the A₃AR agonist is *N*⁶-(3-iodobenzyl)-adenosine-5'-*N*-methyluronamide.

Sheet 1 of 9

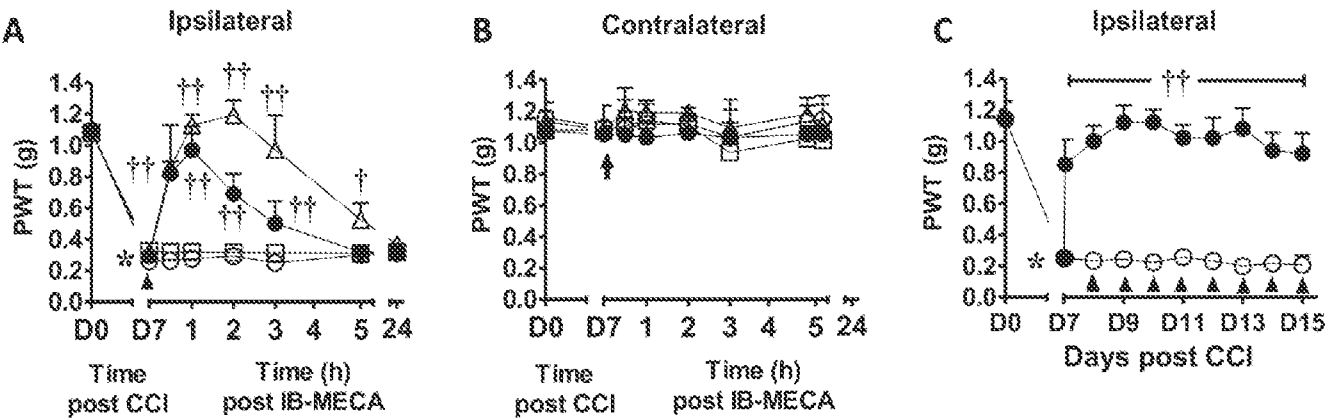


FIGURE 1

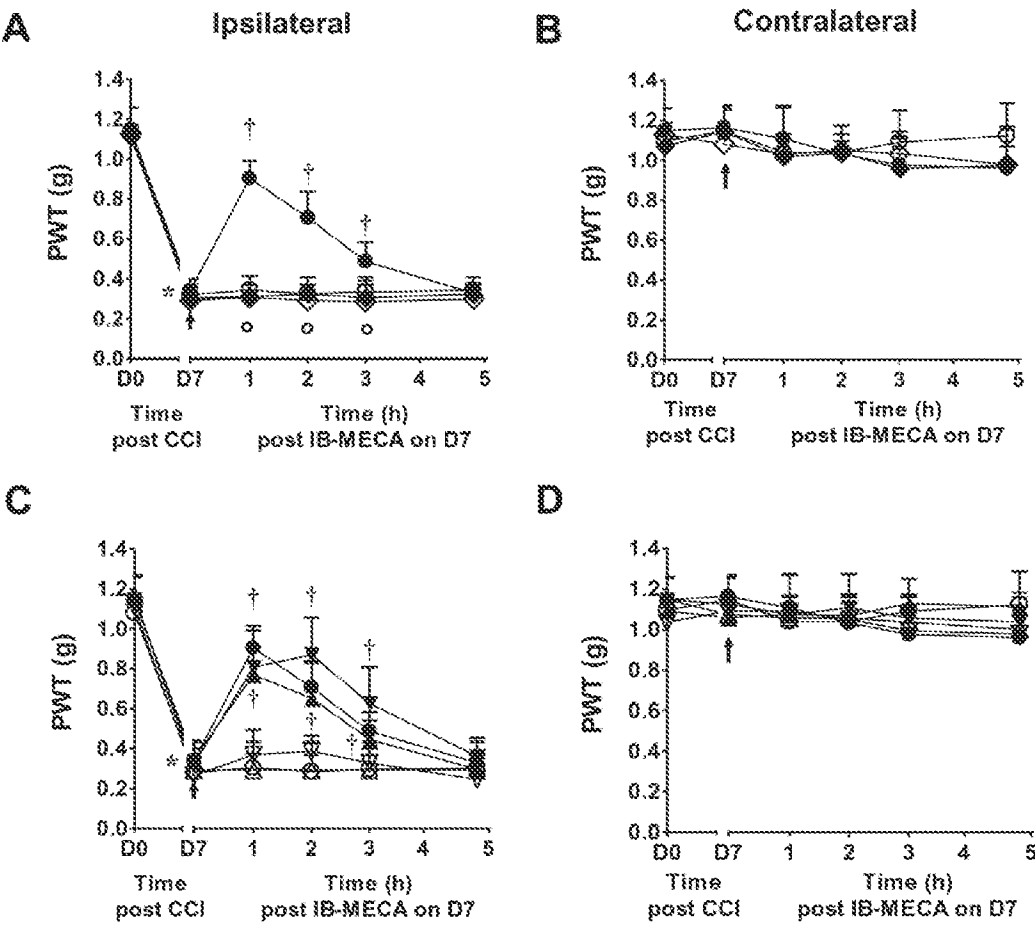


FIGURE 2

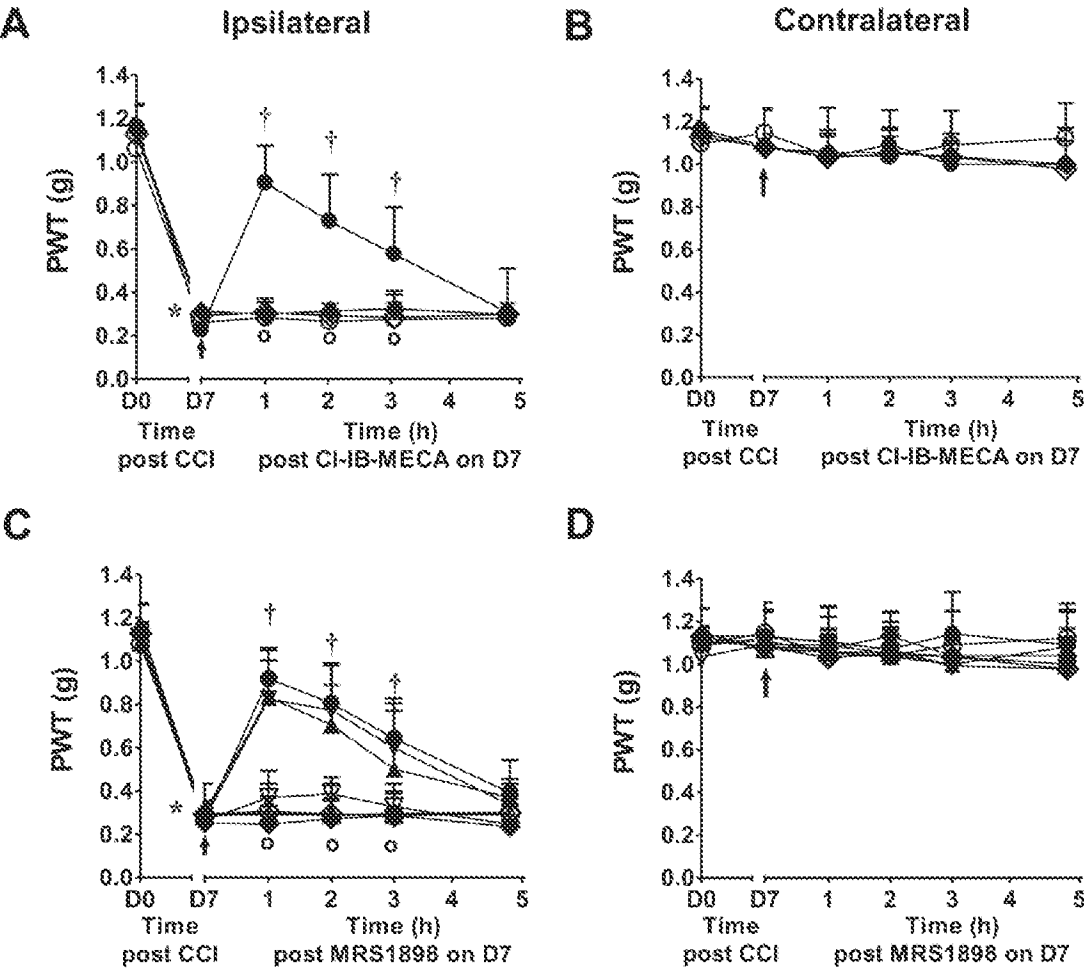


FIGURE 3

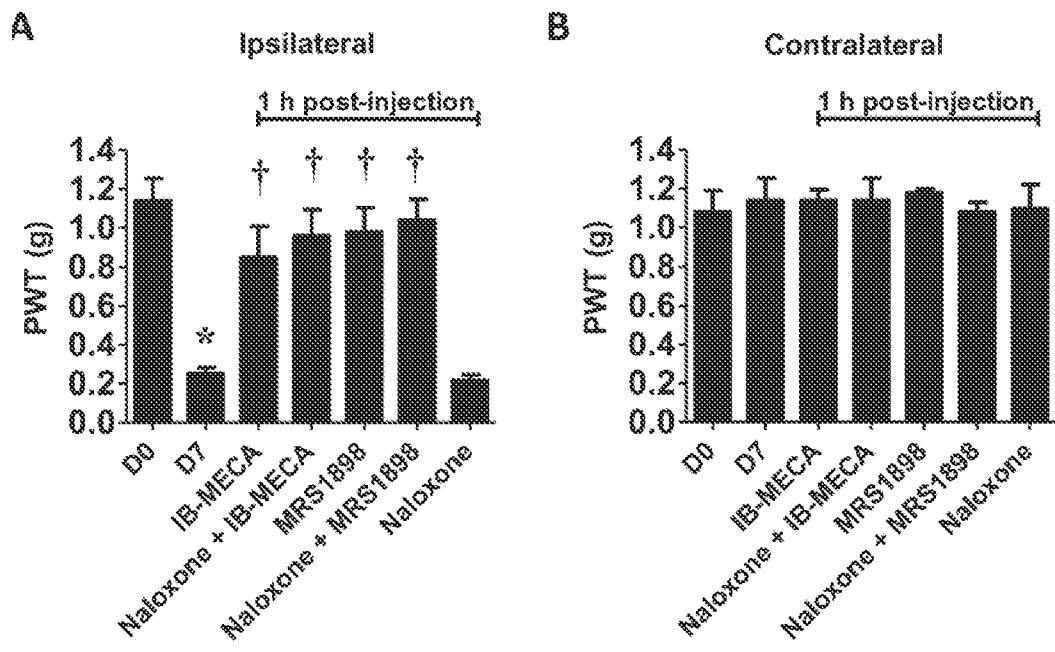


FIGURE 4

Sheet 5 of 9

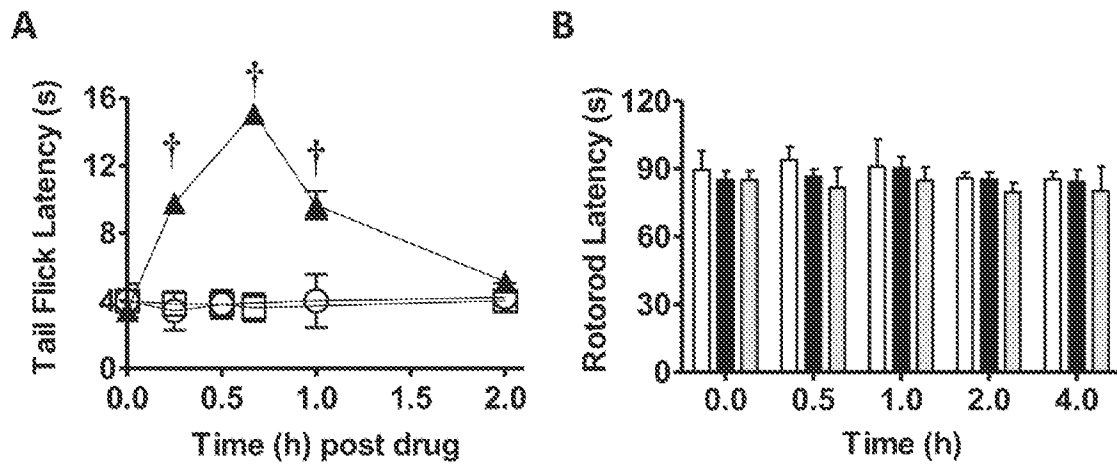


FIGURE 5

Sheet 6 of 9

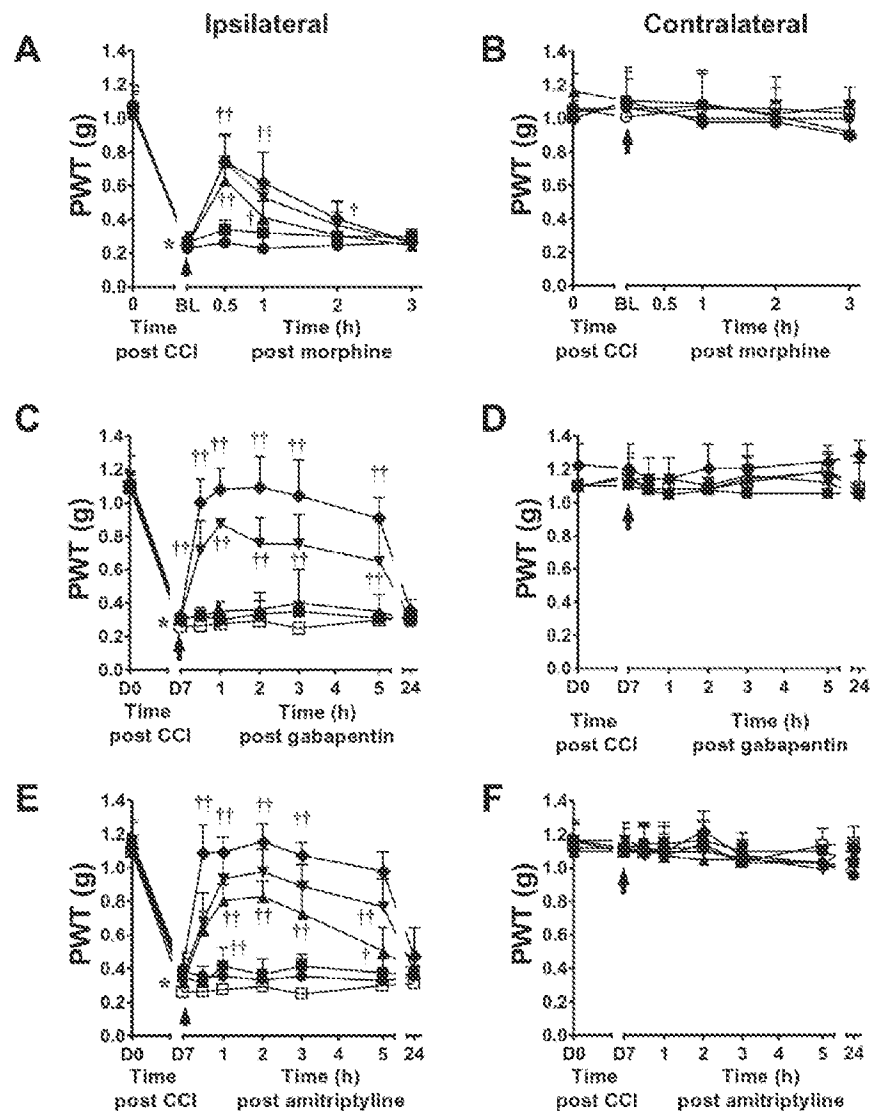


FIGURE 6

Sheet 7 of 9

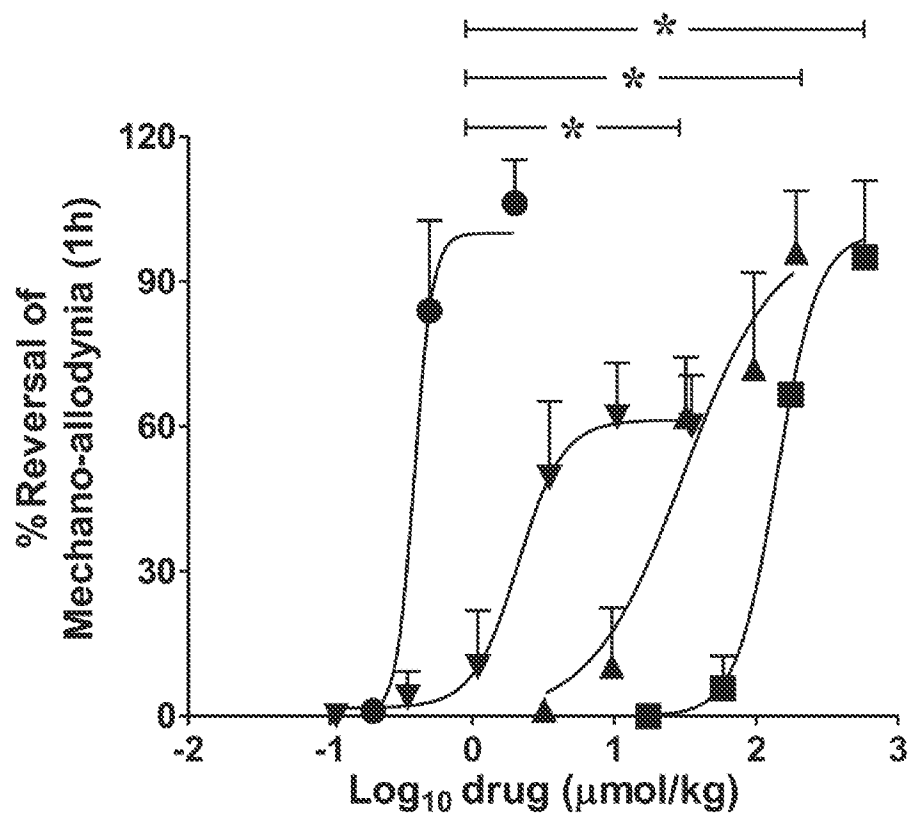


FIGURE 7

Sheet 8 of 9

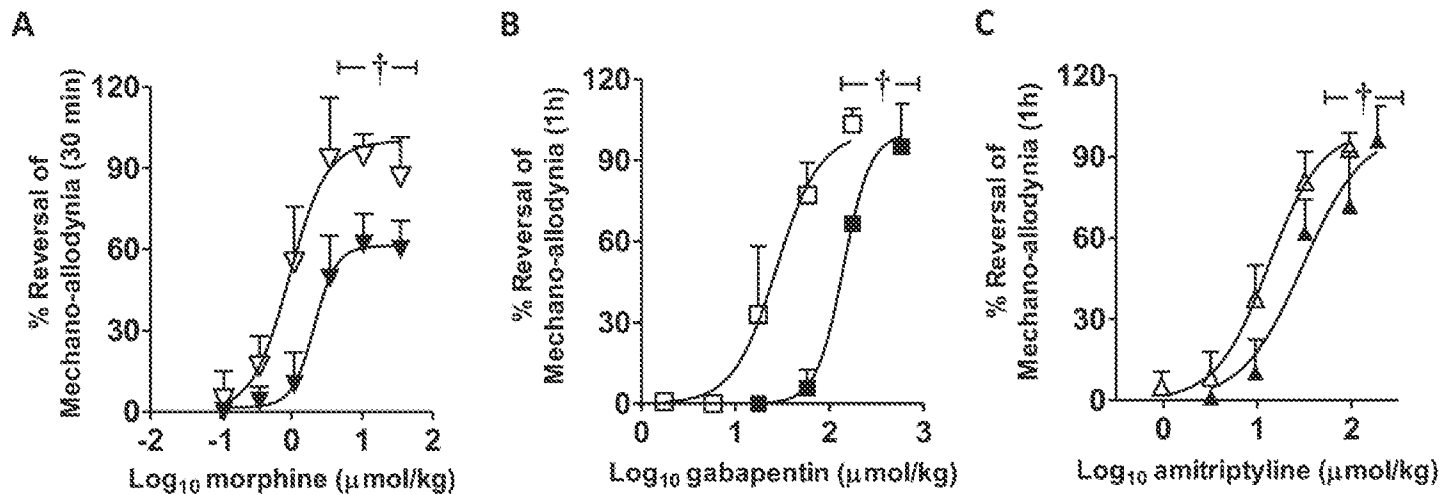


FIGURE 8

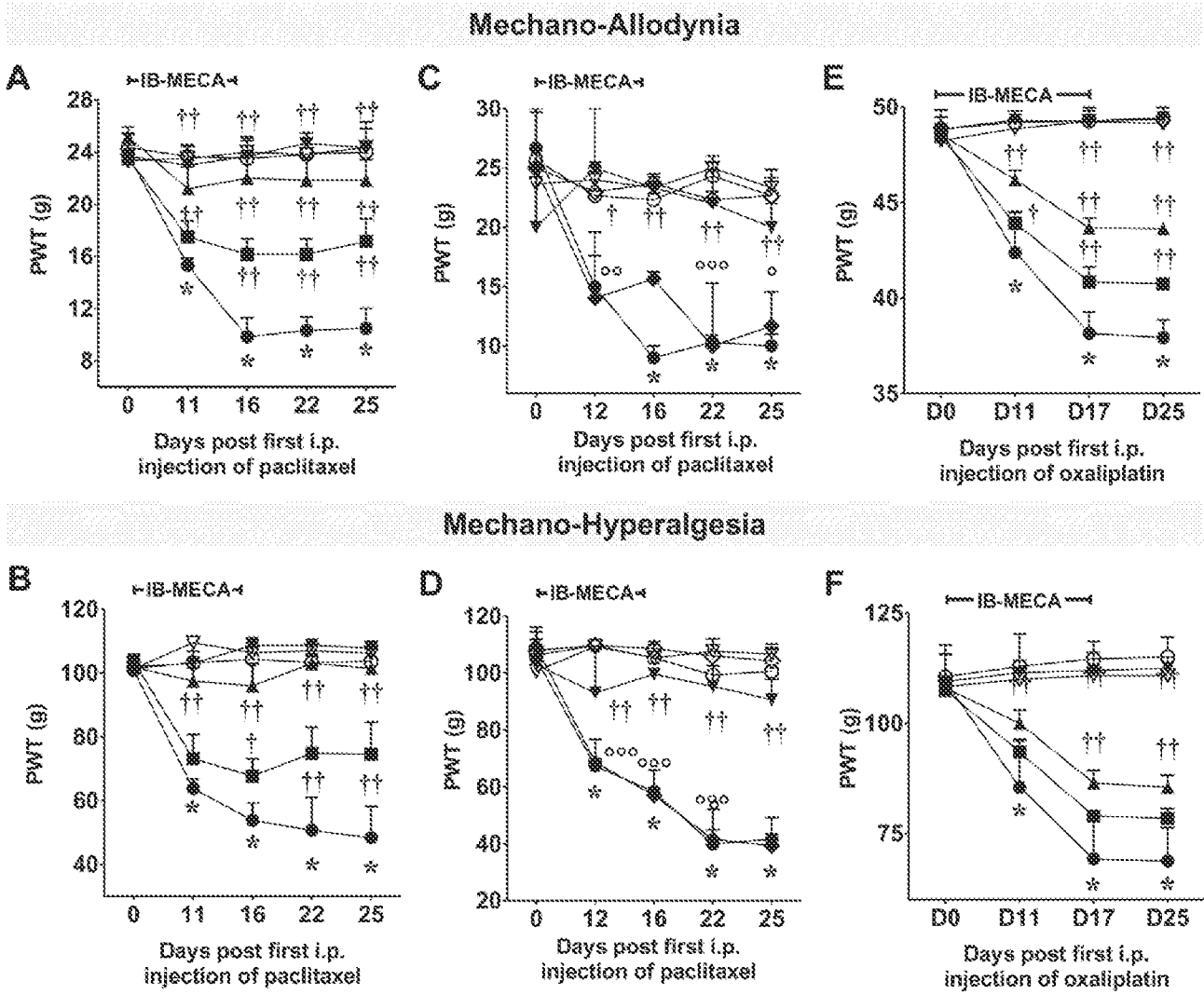


FIGURE 9

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/029059

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/7076 A61K45/06 A61P25/00
ADD.

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

B. FIELDS SEARCHED

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

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

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

EPO-Internal, WPI Data, BIOSIS, EMBASE, FSTA, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MASAKAZU HAYASHIDA ET AL: "Clinical application of adenosine and ATP for pain control", JOURNAL OF ANESTHESIA, SPRINGER-VERLAG, TO, vol. 19, no. 3, 1 August 2005 (2005-08-01) , pages 225-235, XP019373367, ISSN: 1438-8359, DOI: 10.1007/S00540-005-0310-8 page 226, column 1, paragraph 1 page 228, column 1, paragraph 3 - column 2, paragraph 3 ----- -/--	1,21



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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"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

"&" document member of the same patent family

Date of the actual completion of the international search

16 May 2012

Date of mailing of the international search report

06/06/2012

Name and mailing address of the ISA/

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Authorized officer

Peris Antoli, Berta

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/029059

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BALASUBRAMANYAN SRIDHAR ET AL: "Protective effect of adenosine in diabetic neuropathic pain is mediated through adenosine A1-receptors", INDIAN JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY, ASSOCIATION OF PHYSIOLOGISTS AND PHARMACOLOGISTS OF INDIA, IN, vol. 52, no. 3, 1 July 2008 (2008-07-01), pages 233-242, XP009146454, ISSN: 0019-5499 abstract -----	1,5,21
X	US 2009/181920 A1 (WATKINS LINDA [US] ET AL) 16 July 2009 (2009-07-16) claims 1,15 paragraphs [0222], [0227] examples 1-3 -----	1,3-5, 14,18, 21-23
X	LAVAND'HOMME P M ET AL: "Exogenous and endogenous adenosine enhance the spinal antiallodynic effects of morphine in a rat model of neuropathic pain.", PAIN MAR 1999 LNKD- PUBMED:10204715, vol. 80, no. 1-2, March 1999 (1999-03), pages 31-36, XP002676048, ISSN: 0304-3959 abstract page 33, column 1, paragraph 4 - column 2, paragraph 2 page 34, column 1, paragraph 2 -----	1,5-9, 11-13, 15-17, 19-21
X	BOROWICZ K K ET AL: "N<6>-2-(4-Aminophenyl)ethyl-adenosine enhances the anticonvulsive action of conventional antiepileptic drugs in the kindling model of epilepsy in rats", EUROPEAN NEUROPSYCHOPHARMACOLOGY, ELSEVIER SCIENCE PUBLISHERS BV, AMSTERDAM, NL, vol. 10, no. 4, 1 July 2000 (2000-07-01), pages 237-243, XP027358350, ISSN: 0924-977X [retrieved on 2000-07-01] abstract -----	16,17,19
X	SEGERDAHL M ET AL: "The influence of adenosine, ketamine, and morphine on experimentally induced ischemic pain in healthy volunteers.", ANESTHESIA AND ANALGESIA OCT 1994 LNKD- PUBMED:7943793, vol. 79, no. 4, October 1994 (1994-10), pages 787-791, XP002676049, ISSN: 0003-2999 abstract -----	16,17, 19,20
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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/029059

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DOUPEUX L ET AL: "Measurement of the analgesic effects of remifentanyl-adenosine combinations", ACTA ANAESTHESIOLOGICA BELGICA 200703 BE, vol. 58, no. 1, March 2007 (2007-03), page 67, XP009159406, ISSN: 0001-5164 the whole document	16,17, 19,20
X	----- US 6 004 945 A (FUKUNAGA ATSUO F [US]) 21 December 1999 (1999-12-21) claims 1,3	16,17,19
X	----- US 2003/139371 A1 (GORNYPHILIPPE [FR] ET AL) 24 July 2003 (2003-07-24) claims 1-3, 5 paragraph [0027]	16,17,19
X	----- INBAR S ET AL: "Effects of adenosine in combination with calcium channel blockers in patients with primary pulmonary hypertension.", JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY FEB 1993 LNKD- PUBMED:8426006, vol. 21, no. 2, February 1993 (1993-02), pages 413-418, XP002676051, ISSN: 0735-1097 abstract	16,17,19
X	----- PAREEK S S ET AL: "Adenosine enhances analgesic effect of tricyclic antidepressants", INDIAN JOURNAL OF PHARMACOLOGY 1994 IN, vol. 26, no. 2, 1994, pages 159-161, XP009159403, ISSN: 0253-7613 abstract	16,17,19
A	----- LORAM LISA C ET AL: "Enduring reversal of neuropathic pain by a single intrathecal injection of adenosine 2A receptor agonists: a novel therapy for neuropathic pain.", THE JOURNAL OF NEUROSCIENCE : THE OFFICIAL JOURNAL OF THE SOCIETY FOR NEUROSCIENCE 4 NOV 2009 LNKD- PUBMED:19890011, vol. 29, no. 44, 4 November 2009 (2009-11-04), pages 14015-14025, XP002676052, ISSN: 1529-2401 abstract ----- -/--	1-23

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/029059

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SAWYNOK J: "Adenosine receptor activation and nociception.", EUROPEAN JOURNAL OF PHARMACOLOGY 17 APR 1998 LNKD- PUBMED:9650842, vol. 347, no. 1, 17 April 1998 (1998-04-17), pages 1-11, XP002676053, ISSN: 0014-2999 abstract page 2, column 2, paragraph 1 page 5, column 1, paragraph 1 table 1	1-23
A	----- BOREA P A ET AL: "A3 adenosine receptor: Pharmacology and role in disease", HANDBOOK OF EXPERIMENTAL PHARMACOLOGY, SPRINGER VERLAG, BERLIN, DE, vol. 193, 1 January 2009 (2009-01-01), pages 297-327, XP001539889, ISSN: 0171-2004 the whole document	1-23
A	----- JOSHI BHALCHANDRA V ET AL: "Purine derivatives as ligands for A(3) adenosine receptors", CURRENT TOPICS IN MEDICINAL CHEMISTRY, BENTHAM SCIENCE PUBLISHERS LTD, NETHERLANDS, vol. 5, no. 13, 1 October 2005 (2005-10-01), pages 1275-1295, XP008089968, ISSN: 1568-0266, DOI: 10.2174/156802605774463079 abstract table 1	1-23
A	----- DEVINE S M ET AL: "Synthesis and evaluation of new N<6>-substituted adenosine-5'-N-methylcarboxamides as A3 adenosine receptor agonists", BIOORGANIC & MEDICINAL CHEMISTRY, PERGAMON, GB, vol. 18, no. 9, 1 May 2010 (2010-05-01), pages 3078-3087, XP027030038, ISSN: 0968-0896 [retrieved on 2010-04-26] page 3078, column 1, paragraph 1 figure 1 ----- -/--	1-23

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/029059

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	CHEN ZHOUMOU ET AL: "Controlling murine and rat chronic pain through A3 adenosine receptor activation.", FASEB JOURNAL : OFFICIAL PUBLICATION OF THE FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY MAY 2012 LNKD-PUBMED:22345405, vol. 26, no. 5, May 2012 (2012-05), pages 1855-1865, XP009159205, ISSN: 1530-6860 the whole document	1-23
X,P	----- HOFER MICHAL ET AL: "Inhibition of cyclooxygenase-2 promotes the stimulatory action of adenosine A3 receptor agonist on hematopoiesis in sublethally [gamma]-irradiated mice.", BIOMEDICINE & PHARMACOTHERAPY = BIOMÉDECINE & PHARMACOTHÉRAPIE SEP 2011 LNKD- PUBMED:21719245, vol. 65, no. 6, September 2011 (2011-09), pages 427-431, XP002676054, ISSN: 1950-6007 abstract -----	16,18,19

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2012/029059

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2009181920 A1	16-07-2009	AU 2009204084 A1	16-07-2009
		CA 2711495 A1	16-07-2009
		CN 101938904 A	05-01-2011
		EA 201001135 A1	28-02-2011
		EP 2240020 A1	20-10-2010
		JP 2011509305 A	24-03-2011
		US 2009181920 A1	16-07-2009
		WO 2009089425 A1	16-07-2009

US 6004945 A	21-12-1999	US 5679649 A	21-10-1997
		US 6004945 A	21-12-1999

US 2003139371 A1	24-07-2003	AU 8412501 A	18-02-2002
		AU 8412601 A	18-02-2002
		BR 0112830 A	24-06-2003
		BR 0112908 A	24-06-2003
		CA 2419042 A1	14-02-2002
		CA 2420066 A1	14-02-2002
		CN 1468104 A	14-01-2004
		CN 1496265 A	12-05-2004
		EP 1309331 A2	14-05-2003
		EP 1311273 A2	21-05-2003
		FR 2812812 A1	15-02-2002
		JP 2004505897 A	26-02-2004
		JP 2004505921 A	26-02-2004
		NZ 523906 A	24-09-2004
		US 2003139368 A1	24-07-2003
		US 2003139371 A1	24-07-2003
		WO 0211665 A2	14-02-2002
		WO 0211735 A2	14-02-2002
		ZA 200300966 A	09-02-2004
		ZA 200300967 A	25-02-2004
