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(54) Title: IBUDILAST FOR INHIBITING MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) ACTIVITY

(57) Abstract: Methods of antagonizing MIF activity using ibudilast are described. Also described are methods of screening for MIF antagonists. These agents can be used for treating addictions, including drug and behavioral addictions, as well as for treating neuropathic pain.



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## IBUDILAST FOR INHIBITING MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) ACTIVITY

**FIELD OF THE INVENTION**

The present invention relates generally to activity exhibited by ibudilast (also  
5 termed "AV411"). In particular, the present invention pertains to methods for  
antagonizing macrophage migration inhibitory factor (MIF) activity using ibudilast. The  
invention also relates to methods for identifying agents for treating and/or preventing  
neuropathic pain, methods for identifying agents useful for opiate withdrawal and for  
treating other addictions and dependence, as well as methods for identifying agents  
10 useful for treating and/or preventing other disorders wherein MIF activity and/or glial  
activation are implicated.

**BACKGROUND OF THE INVENTION**

The small molecule, ibudilast, (3-isobutyryl-2-isopropylpyrazolo[1,5-a]pyridine),  
15 is a non-selective inhibitor of cyclic nucleotide phosphodiesterase (PDE) (Fujimoto et  
al., (1999) *J. of Neuroimmunology* 95:35-92). Ibudilast displays glial attenuating  
properties, differentiating it from some other PDE inhibitors (Suzumuar et al., *Brain Res.*  
(1999) 837:203-212). Glial cell activation may have multiple, diverse neurological  
consequences including contributions to neuropathic pain, opiate withdrawal/addiction  
20 and Alzheimer's (Narita et al., *Nihon Shinkei Seishin Yakurigaku Zasshi* (2006) 26:33-  
39). Ibudilast also acts as an LTD4 antagonist, an anti-inflammatory, a PAF antagonist,  
and a vasodilatory agent (Thompson Current Drug Reports). Ibudilast is thought to  
exert a neuroprotective role in the central nervous system of mammals, presumably via  
suppression of the activation of glial cells (Mizuno et al., (2004) *Neuropharmacology* 46:  
25 404-411).

Ibudilast has been widely used in Japan for relieving symptoms associated with  
ischemic stroke or bronchial asthma. Marketed indications for ibudilast in Japan include  
its use as a vasodilator, for treating allergy, eye tissue regeneration, ocular disease, and  
treatment of allergic ophthalmic disease (Thompson Current Drug Reports). In recent  
30 clinical trials, its use in the treatment of multiple sclerosis, an inflammatory disease of  
the central nervous system, has been explored (News.Medical.Net; Pharmaceutical  
News, 2 Aug 2005).

The cytokine macrophage migration inhibitory factor (MIF) has been shown to play a role in multiple inflammatory processes, primarily by influencing macrophage function (Bloom and Bennett, *Science* (1966) 153:80; and Calandra and Roger, *Nat. Rev. Immunol.* (2003) 3:791-800). Neutralizing antibodies to MIF have been demonstrated to  
5 be effective therapeutics in preclinical models of rheumatoid arthritis, endotoxemia and septic shock (Calandra et al., *Nat. Med.* (2000) 6:164-170; and Santos and Morand, *Wein. Med. Wochenschr.* (2000) 156:11-18). MIF mRNA is upregulated in microglia three days post-spinal cord injury and may act as a modulator to inflammatory cytokines (Koda et al., *Acta Neuropathol.* (2004) 108:31-36).

10 While the use of ibudilast for a number of varying indications, including the regulation of mononuclear and glial cell response (Kawanokuchi et al., *Neuropharmacology* (2004) 46:734-742; Feng et al., *Mult. Scler.* (2004) 10:494-498) has been reported to date, to the best of applicants' knowledge, its activity as an inhibitor of the cytokine macrophage migration inhibitory factor (MIF) has heretofore remained  
15 unexplored.

There remains a need for identifying improved compounds and compositions that inhibit MIF.

### SUMMARY OF THE INVENTION

The present invention is based on the discovery that ibudilast acts as an antagonist of MIF activity. As glial cells (astrocytes, microglia, oligodendrocytes) have cell-type functional similarities to monocytes/macrophages, MIF may influence glial cell activity. Therefore, antagonism of MIF binding to or activity on blood mononuclear and/or glial cells may account for its anti-inflammatory activity, as well as for the beneficial properties it exerts in neurological and other disorders. The glial attenuating activity displayed by ibudilast may be central to the mechanism for this small molecule's efficacy in neuropathic pain and opiate withdrawal and dependence syndromes. (See, U.S. Patent Publication No. 2006/0160843 for a description of the use of ibudilast to treat neuropathic pain; and U.S. Patent Publication No. 2007/0072899, for a description of the use of ibudilast to treat opiate withdrawal and other dependence syndromes.

These findings not only provide a potential molecular mechanistic link to ibudilast's pharmacological activities, but also provide evidence that other selective MIF antagonists may represent a new therapeutic approach for the treatment of neuropathic pain, opiate withdrawal and dependence, and for the treatment of other disorders where MIF activity and/or glial activation are implicated. In addition, there are diagnostic implications related to MIF binding and MIF antagonism.

In one aspect, then, the invention provides a method for attenuating MIF activity in a vertebrate subject. In certain aspects, MIF activity is inhibited by providing ibudilast.

In certain embodiments, the subject is a human. In certain embodiments, ibudilast is administered systemically, for example, via intravenous, subcutaneous, oral, intranasal, sublingual or other systemic routes. In other embodiments, ibudilast is administered centrally, for example, intrathecally. In certain embodiments, multiple therapeutically effective doses of ibudilast are administered to the subject. In certain embodiments, ibudilast is administered according to a daily dosing regimen. In certain embodiments ibudilast is administered twice a day. In certain embodiments, ibudilast is administered intermittently.

In additional embodiments, the invention is directed to a method for identifying a compound that modulates neuropathic pain. In certain embodiments, the method comprises screening a compound library to identify a small molecule that inhibits neuropathic pain.

In further embodiments, the invention is directed to a method of selecting a compound useful for treating neuropathic pain. The method comprises:

(a) exposing a peripheral blood mononuclear (PBMC) cell culture to a putative compound for treating neuropathic pain;

5 (b) providing MIF to the exposed cells in an amount and under conditions that normally provide for expression of intracellular adhesion molecule-1 (ICAM-1) and/or vascular cell adhesion molecule-1 (VCAM-1);

(c) comparing expression of ICAM-1 and/or VCAM-1 by the cells in step (b), to expression of ICAM-1 and/or VCAM-1 in a PBMC cell culture treated with MIF as in  
10 step (b) in the absence of the putative compound for treating neuropathic pain; and

(d) selecting a compound from step (c) that inhibits expression of ICAM-1 and/or VCAM-1 relative to expression of ICAM-1 and/or VCAM-1 in the absence of the compound.

In certain embodiments, the above method further comprises testing the  
15 compound selected in step (d) in an acceptable model of neuropathic pain.

In additional embodiments, the neuropathic pain is selected from postherpetic neuralgia, trigeminal neuralgia, neuropathic pain associated with herpes, HIV, traumatic nerve injury, stroke, post-ischemia, fibromyalgia, reflex sympathetic dystrophy, complex regional pain syndrome, spinal cord injury, sciatica, phantom limb pain, multiple  
20 sclerosis, or cancer chemotherapeutic-induced neuropathic pain.

In other embodiments, the invention is directed to a method for identifying a compound that is useful for treating addictions, such as drug or behavioral addictions. In certain embodiments, the method comprises screening a compound library to identify a small molecule that is useful for treating addictions.

25 In additional embodiments, the invention is directed to a method of selecting a compound useful for treating addiction. The method comprises:

(a) exposing a peripheral blood mononuclear (PBMC) cell culture to a putative compound for treating addiction;

(b) providing macrophage migration inhibitory factor (MIF) to the exposed cells  
30 in an amount and under conditions that normally provide for expression of intracellular adhesion molecule-1 (ICAM-1) and/or vascular cell adhesion molecule-1 (VCAM-1);

(c) comparing expression of ICAM-1 and/or VCAM-1 by the cells in step (b), to expression of ICAM-1 and/or VCAM-1 in a PBMC cell culture treated with MIF as in

step (b) in the absence of the putative compound for treating addiction; and  
(d) selecting a compound from step (c) that inhibits expression of ICAM-1 and/or VCAM-1 relative to expression of ICAM-1 and/or VCAM-1 in the absence of the compound.

5 In certain embodiments, the method above further comprises testing the compound selected in step (d) in an acceptable model of addiction.

In additional embodiments, the addiction is a drug addiction. In certain embodiments, the drug addiction is selected from an opiate addiction, a cocaine addiction, an amphetamine addiction, a methamphetamine addiction, a cannabinoid  
10 addiction, an alcohol addiction, or a nicotine addiction.

In further embodiments, the addiction is a behavioral addiction. In certain embodiments, the behavioral addiction is selected from an eating addiction, a drinking addiction, a smoking addiction, a shopping addiction, a gambling addiction, a sex addiction, or a computer use addiction.

15 These and other embodiments of the subject invention will readily occur to those of skill in the art in view of the disclosure herein.

#### DETAILED DESCRIPTION OF THE INVENTION

The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, and pharmacology, within the skill of  
20 the art. Such techniques are explained fully in the literature. See, e.g.; A.L. Lehninger, *Biochemistry* (Worth Publishers, Inc., current addition); Morrison and Boyd, *Organic Chemistry* (Allyn and Bacon, Inc., current addition); J. March, *Advanced Organic Chemistry* (McGraw Hill, current addition); *Remington: The Science and Practice of Pharmacy*, A. Gennaro, Ed., 20<sup>th</sup> Ed.; *Goodman & Gilman The Pharmacological Basis  
25 of Therapeutics*, J. Griffith Hardman, L. L. Limbird, A. Gilman, 10<sup>th</sup> Ed.

#### I. DEFINITIONS

In describing and claiming the present invention, the following terminology will be used in accordance with the definitions described below.

30 It must be noted that, as used in this specification and the intended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a drug " includes a single drug as

well as two or more of the same or different drugs, reference to "an optional excipient" refers to a single optional excipient as well as two or more of the same or different optional excipients, and the like.

The term "addiction" is defined herein as compulsively using a drug or  
5 performing a behavior repeatedly that increases extracellular dopamine concentrations in the nucleus accumbens. An addiction may be to a drug including, but are not limited to, psychostimulants, narcotic analgesics, alcohols and addictive alkaloids such as nicotine, cannabinoids, or combinations thereof. Exemplary psychostimulants include, but are not limited to, amphetamine, dextroamphetamine, methamphetamine, phenmetrazine,  
10 diethylpropion, methylphenidate, cocaine, phencyclidine, methylenedioxymethamphetamine and pharmaceutically acceptable salts thereof. Exemplary narcotic analgesics include, but are not limited to, alfentanil, alphaprodine, anileridine, bezitramide, codeine, dihydrocodeine, diphenoxylate, ethylmorphine, fentanyl, heroin, hydrocodone, hydromorphone, isomethadone, levomethorphan,  
15 levorphanol, metazocine, methadone, metopon, morphine, opium extracts, opium fluid extracts, powdered opium, granulated opium, raw opium, tincture of opium, oxycodone, oxymorphone, pethidine, phenazocine, piminodine, racemethorphan, racemorphan, thebaine and pharmaceutically acceptable salts thereof. Addictive drugs also include central nervous system depressants, such as barbiturates, chlordiazepoxide, and alcohols,  
20 such as ethanol, methanol, and isopropyl alcohol. The term addiction also includes behavioral addictions, for example, compulsive eating, drinking, smoking, shopping, gambling, sex, and computer use.

A subject suffering from an addiction experiences addiction-related behavior, cravings to use a substance in the case of a drug addiction or overwhelming urges to  
25 repeat a behavior in the case of a behavioral addiction, the inability to stop drug use or compulsive behavior in spite of undesired consequences (*e.g.*, negative impacts on health, personal relationships, and finances, unemployment, or imprisonment), reward/incentive effects associated with dopamine release, and dependency, or any combination thereof.

30 Addiction-related behavior in reference to a drug addiction includes behavior resulting from compulsive use of a drug characterized by dependency on the substance. Symptomatic of the behavior is (i) overwhelming involvement with the use of the drug, (ii) the securing of its supply, and (iii) a high probability of relapse after withdrawal.

By "pathological pain" is meant any pain resulting from a pathology, such as from functional disturbances and/or pathological changes, lesions, burns and the like. One form of pathological pain is "neuropathic pain" which is pain thought to initially result from nerve damage but extended or exacerbated by other mechanisms including  
5 glial cell activation. Examples of pathological pain include, but are not limited to, thermal or mechanical hyperalgesia, thermal or mechanical allodynia, pain arising from irritable bowel or other internal organ disorders, endometriosis pain, low back pain, pain arising from infection, inflammation or trauma to peripheral nerves or the central nervous system, multiple sclerosis pain, entrapment pain, neuropathic pain associated  
10 with certain syndromes such as viral neuralgias (e.g., herpes, AIDS), diabetic neuropathy, phantom limb pain, stump/neuroma pain, post-ischemic pain (stroke), fibromyalgia, reflex sympathetic dystrophy (RSD), complex regional pain syndrome (CRPS), cancer pain, vertebral disk rupture, and trigeminal neuralgia, cancer-chemotherapy-induced neuropathic pain, among others.

15 By "peripheral blood mononuclear cells" or "PBMC" is meant a population of cells isolated from peripheral blood of a mammal, such as a human, using, e.g., density centrifugation. Generally, a PBMC population includes mostly lymphocytes and monocytes and lacks red blood cells and most polymorphonuclear leukocytes and granulocytes.

20 "Pharmaceutically acceptable excipient or carrier" refers to an excipient that may optionally be included in the compositions of the invention and that causes no significant adverse toxicological effects to the patient.

"Pharmaceutically acceptable salt" includes, but is not limited to, amino acid salts, salts prepared with inorganic acids, such as chloride, sulfate, phosphate,  
25 diphosphate, hydrobromide, and nitrate salts, or salts prepared with an organic acid, such as malate, maleate, fumarate, tartrate, succinate, ethylsuccinate, citrate, acetate, lactate, methanesulfonate, benzoate, ascorbate, para-toluenesulfonate, palmoate, salicylate and stearate, as well as estolate, gluceptate and lactobionate salts. Similarly salts containing pharmaceutically acceptable cations include, but are not limited to, sodium, potassium,  
30 calcium, aluminum, lithium, and ammonium (including substituted ammonium).

"Active molecule" or "active agent" as described herein includes any agent, drug, compound, composition of matter or mixture which provides some pharmacologic, often beneficial, effect that can be demonstrated *in-vivo* or *in vitro*. This includes foods, food

supplements, nutrients, nutraceuticals, drugs, vaccines, antibodies, vitamins, and other beneficial agents. As used herein, the terms further include any physiologically or pharmacologically active substance that produces a localized or systemic effect in a patient.

5 "Substantially" or "essentially" means nearly totally or completely, for instance, 95% or greater of some given quantity.

"Optional" or "optionally" means that the subsequently described circumstance may or may not occur, so that the description includes instances where the circumstance occurs and instances where it does not.

10 The term "central nervous system" or "CNS" includes all cells and tissue of the brain and spinal cord of a vertebrate. Thus, the term includes, but is not limited to, neuronal cells, glial cells, astrocytes, cerebrospinal fluid (CSF), interstitial spaces and the like.

The terms "subject", "individual" or "patient" are used interchangeably herein and refer to a vertebrate, preferably a mammal. Mammals include, but are not limited to, 15 murines, rodents, simians, humans, farm animals, sport animals and pets.

The term "about", particularly in reference to a given quantity, is meant to encompass deviations of plus or minus five percent.

The terms "effective amount" or "pharmaceutically effective amount" of a 20 composition or agent, as provided herein, refer to a nontoxic but sufficient amount of the composition to provide the desired response, such as to suppress MIF activity in a subject, and optionally, a corresponding therapeutic effect. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the condition being treated, the particular drug or drugs 25 employed, mode of administration, and the like. An appropriate "effective" amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation.

By "therapeutically effective dose or amount" of ibudilast is intended an amount that, when ibudilast is administered as described herein, brings about a positive 30 therapeutic response.

## II. MODES OF CARRYING OUT THE INVENTION

Before describing the present invention in detail, it is to be understood that this invention is not limited to particular formulations or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

Although a number of methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred materials and methods are described herein.

The present invention is based on the discovery of a previously unrecognized activity of ibudilast, the antagonism of MIF activity. As explained above, ibudilast also displays glial cell activation and is useful for treating neuropathic pain and addictive disorders. Based on the discoveries presented herein, it appears that MIF antagonism may contribute to the glial attenuating activity demonstrated by ibudilast. This activity may be central to the mechanism for ibudilast's efficacy in neuropathic pain and opiate withdrawal and dependence syndromes. Thus, other antagonists of MIF may also display similar activities.

Accordingly, the present invention provides methods for identifying such antagonists.

Additionally, based on the fact that ibudilast antagonizes MIF activity, ibudilast may be useful for treating a large number of disorders where MIF activity is implicated. Thus, ibudilast can be provided in compositions, as described further below, to antagonize MIF activity in a vertebrate subject, such as a human, to treat a whole host of disorders associated with MIF. MIF antagonists other than ibudilast for treating such disorders, can also be discovered using the screening methods described herein and subsequently used in compositions for treating these MIF-associated disorders.

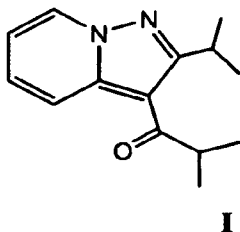
Such disorders include, but are not limited to, various inflammatory disorders such as rheumatoid arthritis (see, e.g., Onodera et al., *Arthritis. Rheum.* (2004) 50:1437-1447; Lubetsky et al., *J. Biol. Chem.* (2002) 277:24976-24982); filariasis (Maizels et al., *Int. J. Parasitol.* (2001) 31:889-898); pancreatitis (Sakai et al., *Gastroenterol.* (2003) 124:725-736); dermal photoaging of human skin (Watanabe et al., *J. Biol. Chem.* (2004) 279:1676-1683); obesity (Dandona et al., *J. Clin. Endocrinol. Metab.* (2004) 89:5043-5047); drug resistance in cancer treatment (Lin et al., *Oncol. Rep.* (2005) 13:983-988); diabetes (Cvetkovic et al., *Endocrinol.* (2005) 146:2942-2951); invasiveness/metastasis

of cancer cells (Hagemann et al., *J. Immunol.* (2005) 175:1197-1205); Guillain-Barre syndrome (Micolette et al., *J. Neuroimmunol.* (2005) 168:168-174; severe sepsis (Al-Abed et al., *J. Biol. Chem.* (2005) 280:36541-36544); asthma (Rossi et al., *J. Clin. Invest.* (1998) 101:2869-2874); Neuro-Behcet's Disease (NBD) and conventional-form multiple sclerosis (C-MS) ((Ninno et al., *J Neurol Sci.* (2000) 179:127-131); spinal cord injury (Fujimoto, S., *Hokkaido Igaku Zasshi.* (1997) 72:409-430 and Koda et al., *Acta Neuropathol (Berl).* (2004) 108:31-36); bladder inflammation (Meyer-Seigler et al., *J Interferon Cytokine Res.* (2004) 24:55-63); nephropathy (Kim et al., *Mol. Med.* (2000) 6:837-848); cutaneous lymphoproliferative diseases such as Sezary Syndrome and mycosis fungoides (Umbert et al., *Brit. J. Dermatol.* (1976) 95:475-480); allergic neuritis (Breborrowicz et al., *Scand J Immunol.* (1981) 14:15-20); atopic dermatitis, tumor progression and neoplasia, cell proliferation and tumor progression and angiogenesis (Orita et al., *Curr. Pharm. Des.* (2002) 8:1297-1317); anemia caused by malaria (McDevitt et al., *J Exp Med.* (2006) 203:1185-1196); and colitis (Morand, E.F., *Intern Med J.* (2005) 35:419-426). MIF antagonists are also useful as tautomerase inhibitors (see, e.g., Orita et al., *Curr. Pharm. Des.* (2002) 8:1297-1317).

In order to further an understanding of the invention, a more detailed discussion is provided below regarding ibudilast, compositions including ibudilast and screening methods for finding agents useful for treating neuropathic pain and addictions.

### IBUDILAST

Ibudilast is a small molecule drug (molecular weight of 230.3) having the structure shown below.



Ibudilast is also found under ChemBank ID 3227, CAS # 50847-11-5, and Beilstein Handbook Reference No. 5-24-03-00396. Its molecular formula corresponds to [C<sub>14</sub>H<sub>18</sub>N<sub>2</sub>O]. Ibudilast is also known by various chemical names which include 2-

methyl-1-(2-(1-methylethyl)pyrazolo(1,5-a)pyridin-3-yl)1-propanone; 3-isobutyryl-2-isopropylpyrazolo(1,5-a)pyridine]; and 1-(2-isopropyl-pyrazolo[1,5-a]pyridin-3-yl)-2-methyl-propan-1-one. Other synonyms for ibudilast include Ibudilastum (Latin), BRN 0656579, KC-404, and the brand name Ketas<sup>®</sup>. Ibudilast, as referred to herein, is meant to include any and all pharmaceutically acceptable salt forms thereof, prodrug forms (e.g., the corresponding ketal), and the like, as appropriate for use in its intended formulation for administration.

Ibudilast is a non-selective nucleotide phosphodiesterase (PDE) inhibitor (most active against PDE-3 and PDE-4), and has also been reported to have LTD4 and PAF antagonistic activities. Its profile appears effectively anti-inflammatory and unique in comparison to other PDE inhibitors and anti-inflammatory agents. PDEs catalyze the hydrolysis of the phosphoester bond on the 3'-carbon to yield the corresponding 5'-nucleotide monophosphate. Thus, they regulate the cellular concentrations of cyclic nucleotides. Since extracellular receptors for many hormones and neurotransmitters utilize cyclic nucleotides as second messengers, the PDEs also regulate cellular responses to these extracellular signals. There are at least eight classes of PDEs: Ca<sup>2+</sup>/calmodulin-dependent PDEs (PDE1); cGMP-stimulated PDEs (PDE2); cGMP-inhibited PDEs (PDE3); cAMP-specific PDEs (PDE4); cGMP-binding PDEs (PDE5); photoreceptor PDEs (PDE6); high affinity, cAMP-specific PDEs (PDE7); and high affinity cGMP-specific PDEs (PDE9).

As stated previously, a reference to any one or more of the herein-described drugs, in particular ibudilast, is meant to encompass, where applicable, any and all enantiomers, mixtures of enantiomers including racemic mixtures, prodrugs, pharmaceutically acceptable salt forms, hydrates (e.g., monohydrates, dihydrates, etc.), different physical forms (e.g., crystalline solids, amorphous solids), metabolites, and the like.

### **SCREENING METHODS**

One aspect of the invention provides methods of screening for compounds that modulate neuropathic pain. In other embodiments, the invention is directed to methods for identifying compounds useful for treating addictions, such as drug or behavioral addictions. In certain embodiments, the addiction is an opiate, cocaine, amphetamine, methamphetamine, cannabinoid, alcohol, or nicotine addiction. In other embodiments,

the addiction is a behavioral addiction, for example, an eating, drinking, smoking, shopping, gambling, sex, or computer use addiction.

Molecules screened for the activities described above include but are not limited to small organic compounds, combinatorial libraries of organic compounds, nucleic acids, nucleic acid derivatives, saccharides or oligosaccharides, peptoids, soluble  
5 peptides, peptides tethered on a solid phase, peptides displayed on bacterial phage surface proteins, bacterial surface proteins or antibodies, and/or peptides containing non-peptide organic moieties.

For example, libraries of diverse molecular species can be made using  
10 combinatorial organic synthesis. See, e.g., Gordon et al. (1994) *J. Med. Chem.* 37:1335. Examples include but are not limited to pyrrolidines; oligocarbamates (Cho et al. (1993) *Science* 261:1303); peptoids such as N-substituted glycine polymers (Simon et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:9367); and vinylogous polypeptides (Hagihara et al. (1992) *J. Am. Chem. Soc.* 114:6568).

15 A variety of approaches, known in the art, can be used to track the building blocks as they are added during synthesis so that the history of individual library members can be determined. These approaches include addressable location on a photolithographic chip (oligocarbamates), a deconvolution strategy in which "hits" are identified through recursive additions of monomers to partially synthesized libraries  
20 (peptoids, pyrrolidines, peptides) (Zuckermann et al. (1994) *J. Med. Chem.* 37:2678), and coding combinatorial libraries by the separate synthesis of nucleotides (Nielsen et al. (1993) *J. Am. Chem. Soc.* 115: 9812) or other organic moieties (Ohlmeyer et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:10922) ("tags"). The coded tags associated with each library member can then be decoded after an agent has been selected. For example,  
25 nucleic acid tags can be decoded by DNA sequencing. Other methods for identifying active compounds in pools of small molecules include fractionating the pool by reverse phase HPLC or affinity selection/mass spectroscopy (Nedved et al., (1996) *Anal. Chem.* 68:4228).

Peptoid combinatorial libraries can also be used for identifying MIF antagonists.  
30 Peptoids are oligomers of N-substituted glycine (Simon et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:9367) and can be used to generate chemically diverse libraries of novel molecules. The monomers may incorporate *t*-butyl-based side-chain and 9- fluorenyl-methoxy-carbonyl  $\alpha$ -amine protection. The assembly of monomers into peptoid

oligomers can be performed, for example, on a solid phase using the "submonomer method" of Zuckermann et al. (1992) *J. Am. Chem. Soc.* 114:10646. In this method, syntheses are conducted with Rink amide polystyrene resin (Rink et al. (1987) *Tetrahedron Lett.* 28:3787). Resin-bound amines are bromoacetylated by *in situ* activation of bromoacetic acid with diisopropyl-carbodiimide. Subsequently, the resin-bound bromoacetamides are displaced by addition of an amine. The amines may incorporate t-butyl-based protection of additional reactive groups. This two-step cycle is repeated until the desired number of monomers is added. The oligopeptide is then released from the resin by treatment with 95% trifluoroacetic acid/5% water. The syntheses are performed, preferably, using a robotic synthesizer. See, e.g., Zuckermann et al. (1992) *Pept. Protein Res.* 40:498; and Zuckermann et al. (1996) *Methods in Enzymology* 267:437. In the alternative, oligomerization of the peptoid monomers may be performed by *in situ* activation by either benzotriazol-1-yloxytris (pyrrolidino)phosphonium hexafluorophosphate or bromotris(pyrrolidino) phosphonium hexafluorophosphate. In this alternative method, the other steps are identical to conventional peptide synthesis using  $\alpha$ -(9- fluorenyl methoxycarbonyl) amino acids (see, e.g., Simon et al. (1992), *supra*).

Compounds and libraries of compounds can be screened for their ability to antagonize MIF activity by treating peripheral blood mononuclear cells (PBMC) with a test compound or library of compounds as described above, and stimulating the cells with MIF. PBMCs for stimulation can be isolated from whole blood using techniques well known in the art, such as by using Ficoll-Hypaque density gradients. After centrifugation, adherent mononuclear cells can be, but need not be, separated from nonadherent mononuclear cells (NAMNC) by successive cycles of adherence to plastic for, e.g., 45 min. at 37 degrees C. In order to prepare stimulated cells, the therapeutic agent in question and PBMCs are combined. The amount of agent to be added will depend on the particular substance being tested. One of skill in the art can easily determine the appropriate concentration for use. MIF is then added approximately 30 minutes to 5 hours later, preferably about 45 minutes to 2 hours later, and more preferably about 1 hour after the test compound has been added. The plates are incubated for approximately 2-24 hours, or longer, after MIF is added, preferably 5 to 15 hours, and more preferably 7 to 10 hours, such as 9 hours.

The expression of intracellular adhesion molecule-1 (ICAM-1) and/or vascular cell adhesion molecule-1 (VCAM-1) are markers of MIF-stimulation. Thus, the expression of these molecules can be measured and compared to expression using MIF alone, without the test compound. The protein expression of VCAM-1 and ICAM-1 can be measured using standard techniques, such as using a cell surface enzyme linked immunosorbent assay (ELISA) or by flow cytometry. Alternatively, VCAM-1 and ICAM-1 mRNA expression can be measured by reverse transcription polymerase chain reaction (RT PCR). See, e.g., Zapolska-Downar et al., *Atherosclerosis* (2001) 155:123-130.

Compounds identified as having MIF-antagonistic activity will be candidates for use as drugs in the treatment of neuropathic pain and addictive behaviors. These compounds can be tested in accepted models of neuropathic pain, such as, but not limited to the tail-flick model (D'Amour et al., *J. Pharmacol. Exp. and Ther.* (1941) 72:74-79); the rat tail immersion model; the carrageenan paw hyperalgesia model; the formalin behavioral response model; the von Frey filament test (Chaplan et al., *J. Neurosci. Methods* (1994) 53:55-63); the chronic constriction injury test (CCI); the Hargreaves test (Hargreaves et al., *Pain* (1998) 32:77-88); and the cold allodynia model (Gogas et al., *Analgesia* (1997) 3:111-118). For a detailed description of these models, see, e.g., U.S. Patent Publication No. 2006/0008446.

Similarly, MIF antagonists can be tested in any of the several known models for addictive behavior, including but not limited to rat models for alcohol and drug addiction (May et al., *J. Pharmacol. Exp. Ther.* (1995) 275:1195-1203); a rat model for amphetamine addiction (Hayne and Wolffgramm, *Psychopharmacol. (Berl)* (1998) 140:510-518); a rat model for methadone addiction (Flahery and Sadava, *Arch. Int. Pharmacodyn. Ther.* (1974) 212:103-107; the *C. elegans* model of addiction (Schafer, W.R., *Neuropharmacol.* (2004) 47:123-131); the weaver mutant mouse model of addiction (Maharajan et al., *Prog. Neurobiol.* (2001) 64:269-276); and a model for sugar addiction (Wideman et al., *Nutr. Neurosci.* (2005) 8:269-276).

Agents that have the desired properties are appropriate for further use, for example, in compositions, such as compositions described below.

**FORMULATION COMPONENTS****Excipients/Carriers**

As explained above, ibudilast and/or compounds identified using the screening methods described herein can be provided in pharmaceutical compositions to antagonize MIF activity. Optionally, in addition to the active agent, the compositions may further comprise one or more pharmaceutically acceptable excipients or carriers. Exemplary excipients include, without limitation, carbohydrates, starches (*e.g.*, corn starch), inorganic salts, antimicrobial agents, antioxidants, binders/fillers, surfactants, lubricants (*e.g.*, calcium or magnesium stearate), glidants such as talc, disintegrants, diluents, buffers, acids, bases, film coats, combinations thereof, and the like.

A composition may also include one or more carbohydrates such as a sugar, a derivatized sugar such as an alditol, aldonic acid, an esterified sugar, and/or a sugar polymer. Specific carbohydrate excipients include, for example: monosaccharides, such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol, sorbitol (glucitol), pyranosyl sorbitol, myoinositol, and the like.

Also suitable for use in the compositions are potato and corn-based starches such as sodium starch glycolate and directly compressible modified starch.

Further representative excipients include inorganic salt or buffers such as citric acid, sodium chloride, potassium chloride, sodium sulfate, potassium nitrate, sodium phosphate monobasic, sodium phosphate dibasic, and combinations thereof.

A pharmaceutical composition may also include an antimicrobial agent, *e.g.*, for preventing or deterring microbial growth. Non-limiting examples of antimicrobial agents suitable for the present invention include benzalkonium chloride, benzethonium chloride, benzyl alcohol, cetylpyridinium chloride, chlorobutanol, phenol, phenylethyl alcohol, phenylmercuric nitrate, thimersol, and combinations thereof.

A composition may also contain one or more antioxidants. Antioxidants are used to prevent oxidation, thereby preventing the deterioration of the drug(s) or other components of the preparation. Suitable antioxidants for use in the compositions include, for example, ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, hypophosphorous acid, monothioglycerol, propyl gallate, sodium

bisulfite, sodium formaldehyde sulfoxylate, sodium metabisulfite, and combinations thereof.

Additional excipients include surfactants such as polysorbates, *e.g.*, "TWEEN 20" and "TWEEN 80," and pluronics such as F68 and F88 (both of which are available  
5 from BASF, Mount Olive, New Jersey), sorbitan esters, lipids (*e.g.*, phospholipids such as lecithin and other phosphatidylcholines, and phosphatidylethanolamines), fatty acids and fatty esters, steroids such as cholesterol, and chelating agents, such as EDTA, zinc and other such suitable cations.

Further, a composition may optionally include one or more acids or bases. Non-  
10 limiting examples of acids that can be used include those acids selected from the group consisting of hydrochloric acid, acetic acid, phosphoric acid, citric acid, malic acid, lactic acid, formic acid, trichloroacetic acid, nitric acid, perchloric acid, phosphoric acid, sulfuric acid, fumaric acid, and combinations thereof. Examples of suitable bases include, without limitation, bases selected from the group consisting of sodium  
15 hydroxide, sodium acetate, ammonium hydroxide, potassium hydroxide, ammonium acetate, potassium acetate, sodium phosphate, potassium phosphate, sodium citrate, sodium formate, sodium sulfate, potassium sulfate, potassium fumarate, and combinations thereof.

The amount of any individual excipient in the composition will vary depending  
20 on the role of the excipient, the dosage requirements of the active agent components, and particular needs of the composition. Typically, the optimal amount of any individual excipient is determined through routine experimentation, *i.e.*, by preparing compositions containing varying amounts of the excipient (ranging from low to high), examining the stability and other parameters, and then determining the range at which optimal  
25 performance is attained with no significant adverse effects.

Generally, however, the excipient will be present in the composition in an amount of about 1% to about 99% by weight, preferably from about 5% to about 98% by weight, more preferably from about 15 to about 95% by weight of the excipient. In general, the amount of excipient present in an ibudilast composition is selected from the  
30 following: at least about 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or even 95% by weight.

These foregoing pharmaceutical excipients along with other excipients are described in "Remington: The Science & Practice of Pharmacy", 19<sup>th</sup> ed., Williams &

Williams, (1995), the "Physician's Desk Reference", 52<sup>nd</sup> ed., Medical Economics, Montvale, NJ (1998), and Kibbe, A.H., Handbook of Pharmaceutical Excipients, 3<sup>rd</sup> Edition, American Pharmaceutical Association, Washington, D.C., 2000.

5           **Other Actives**

A formulation (or kit) in accordance with the invention may contain, in addition to ibudilast and/or a MIF antagonist identified as described herein, one or more additional active agents. Preferably, the active agent is one that possesses a mechanism of action different from that of ibudilast and/or the identified MIF antagonist. Such  
10 actives include naltrexone, metoclopramide, loperamide, diazepam, clonidine, and paracetamol.

**Sustained Delivery Formulations**

Preferably, the compositions are formulated in order to improve stability and  
15 extend the half-life of ibudilast and/or another MIF antagonist. For example, ibudilast and/or the MIF antagonist may be delivered in sustained-release formulations. Controlled or sustained-release formulations are prepared by incorporating the active agent into a carrier or vehicle such as liposomes, nonresorbable impermeable polymers such as ethylenevinyl acetate copolymers and HytreI® copolymers, swellable polymers  
20 such as hydrogels, or resorbable polymers such as collagen and certain polyacids or polyesters such as those used to make resorbable sutures. Additionally, the active agent can be encapsulated, adsorbed to, or associated with, particulate carriers. Examples of particulate carriers include those derived from polymethyl methacrylate polymers, as well as microparticles derived from poly(lactides) and poly(lactide-co-glycolides),  
25 known as PLG. See, e.g., Jeffery et al., *Pharm. Res.* (1993) 10:362-368; and McGee et al., *J. Microencap.* (1996).

**DELIVERY FORMS**

The compositions described herein encompass all types of formulations, and in  
30 particular, those that are suited for systemic or intrathecal administration. Oral dosage forms include tablets, lozenges, capsules, syrups, oral suspensions, emulsions, granules, and pellets. Alternative formulations include aerosols, transdermal patches, gels, creams, ointments, suppositories, powders or lyophilates that can be reconstituted, as

well as liquids. Examples of suitable diluents for reconstituting solid compositions, e.g., prior to injection, include bacteriostatic water for injection, dextrose 5% in water, phosphate-buffered saline, Ringer's solution, saline, sterile water, deionized water, and combinations thereof. With respect to liquid pharmaceutical compositions, solutions and suspensions are envisioned.

In turning now to oral delivery formulations, tablets can be made by compression or molding, optionally with one or more accessory ingredients or additives. Compressed tablets are prepared, for example, by compressing in a suitable tableting machine, the active ingredients in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g., povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (e.g., sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose) and/or surface-active or dispersing agent.

Molded tablets are made, for example, by molding in a suitable tableting machine, a mixture of powdered compounds moistened with an inert liquid diluent. The tablets may optionally be coated or scored, and may be formulated so as to provide slow or controlled release of the active ingredients, using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with a coating, such as a thin film, sugar coating, or an enteric coating to provide release in parts of the gut other than the stomach. Processes, equipment, and toll manufacturers for tablet and capsule making are well-known in the art.

Formulations for topical administration in the mouth include lozenges comprising the active ingredients, generally in a flavored base such as sucrose and acacia or tragacanth and pastilles comprising the active ingredients in an inert base such as gelatin and glycerin or sucrose and acacia.

A pharmaceutical composition for topical administration may also be formulated as an ointment, cream, suspension, lotion, powder, solution, paste, gel, spray, aerosol or oil.

Alternatively, the formulation may be in the form of a patch (e.g., a transdermal patch) or a dressing such as a bandage or adhesive plaster impregnated with active ingredients and optionally one or more excipients or diluents. Topical formulations may additionally include a compound that enhances absorption or penetration of the ingredients through the skin or other affected areas, such as dimethylsulfoxidem

bisabolol, oleic acid, isopropyl myristate, and D-limonene, to name a few.

For emulsions, the oily phase is constituted from known ingredients in a known manner. While this phase may comprise merely an emulsifier (otherwise known as an emulgent), it desirably comprises a mixture of at least one emulsifier with a fat and/or an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier that acts as a stabilizer. Together, the emulsifier(s) with or without stabilizer(s) make up the so-called emulsifying wax, and the wax together with the oil and/or fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of cream formulations. Illustrative emulgents and emulsion stabilizers include TWEEN 60, SPAN 80, cetostearyl alcohol, myristyl alcohol, glyceryl monostearate and sodium lauryl sulfate.

Formulations for rectal administration are typically in the form of a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

Formulations suitable for vaginal administration generally take the form of a suppository, tampon, cream, gel, paste, foam or spray.

Formulations suitable for nasal administration, wherein the carrier is a solid, include a coarse powder having a particle size, for example, in the range of about 20 to about 500 microns. Such a formulation is typically administered by rapid inhalation through the nasal passage, e.g., from a container of the powder held in proximity to the nose. Alternatively, a formulation for nasal delivery may be in the form of a liquid, e.g., a nasal spray or nasal drops.

Aerosolizable formulations for inhalation may be in dry powder form (e.g., suitable for administration by a dry powder inhaler), or, alternatively, may be in liquid form, e.g., for use in a nebulizer. Nebulizers for delivering an aerosolized solution include the AERx™ (Aradigm), the Ultravent® (Mallinkrodt), and the Acorn II® (Marquest Medical Products). A composition of the invention may also be delivered using a pressurized, metered dose inhaler (MDI), e.g., the Ventolin® metered dose inhaler, containing a solution or suspension of a combination of drugs as described herein in a pharmaceutically inert liquid propellant, e.g., a chlorofluorocarbon or fluorocarbon.

Formulations suitable for parenteral administration include aqueous and non-aqueous isotonic sterile solutions suitable for injection, as well as aqueous and non-aqueous sterile suspensions.

Parenteral formulations are optionally contained in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the types previously described.

A formulation may also be a sustained release formulation, such that each of the drug components is released or absorbed slowly over time, when compared to a non-sustained release formulation. Sustained release formulations may employ pro-drug forms of the active agent, delayed-release drug delivery systems such as liposomes or polymer matrices, hydrogels, or covalent attachment of a polymer such as polyethylene glycol to the active agent.

In addition to the ingredients particularly mentioned above, the formulations may optionally include other agents conventional in the pharmaceutical arts and particular type of formulation being employed, for example, for oral administration forms, the composition for oral administration may also include additional agents as sweeteners, thickeners or flavoring agents.

The compositions may also be prepared in a form suitable for veterinary applications.

20

#### **METHOD OF ADMINISTRATION**

Methods of delivery of ibudilast-based or other MIF antagonistic therapeutic formulations include systemic and localized delivery, *i.e.*, directly into the central nervous system. Such routes of administration include but are not limited to, oral, intra-arterial, intrathecal, intramuscular, intraperitoneal, intravenous, intranasal, and inhalation routes.

More particularly, a formulation may be administered for therapy by any suitable route, including without limitation, oral, rectal, nasal, topical (including transdermal, aerosol, buccal and sublingual), vaginal, parenteral (including subcutaneous, intramuscular, intravenous and intradermal), intrathecal, and pulmonary. The preferred route will, of course, vary with the condition and age of the recipient, the particular neuralgia-associated syndrome being treated, and the specific combination of drugs employed.

One preferred mode of administration for delivery of ibudilast and/or another MIF antagonist is directly to neural tissue such as peripheral nerves, the retina, dorsal root ganglia, neuromuscular junction, as well as the CNS, *e.g.*, to target spinal cord glial cells by injection into, *e.g.*, the ventricular region, as well as to the striatum (*e.g.*, the caudate nucleus or putamen of the striatum), spinal cord and neuromuscular junction, with a needle, catheter or related device, using neurosurgical techniques known in the art, such as by stereotactic injection (see, *e.g.*, Stein et al., *J. Virol.* 73:3424-3429, 1999; Davidson et al., *PNAS* 97:3428-3432, 2000 ; Davidson et al., *Nat. Genet.* 3:219-223, 1993; and Alisky and Davidson, *Hum. Gene Ther.* 11:2315-2329, 2000).

A particularly preferred method for targeting spinal cord glia is by intrathecal delivery, rather than into the cord tissue itself.

Another preferred method for administering the ibudilast-based compositions is by delivery to dorsal root ganglia (DRG) neurons, *e.g.*, by injection into the epidural space with subsequent diffusion to DRG. For example, a composition can be delivered via intrathecal cannulation under conditions where ibudilast is diffused to DRG. See, *e.g.*, Chiang et al., *Acta Anaesthesiol. Sin.* (2000) 38:31-36; Jain, K.K., *Expert Opin. Investig. Drugs* (2000) 9:2403-2410.

Yet another mode of administration to the CNS uses a convection-enhanced delivery (CED) system. In this way, the agent can be delivered to many cells over large areas of the CNS. Any convection-enhanced delivery device may be appropriate for delivery of the desired agent. In a preferred embodiment, the device is an osmotic pump or an infusion pump. Both osmotic and infusion pumps are commercially available from a variety of suppliers, for example Alzet Corporation, Hamilton Corporation, Alza, Inc., Palo Alto, California). Typically, a composition is delivered via CED devices as follows. A catheter, cannula or other injection device is inserted into CNS tissue in the chosen subject. Stereotactic maps and positioning devices are available, for example from ASI Instruments, Warren, MI. Positioning may also be conducted by using anatomical maps obtained by CT and/or MRI imaging to help guide the injection device to the chosen target. For a detailed description regarding CED delivery, see U.S. Patent No. 6,309,634.

A composition, when comprising more than one active agent, may be administered as a single combination composition comprising a combination of a ibudilast and/or the MIF antagonist and at least one additional active agent of interest. In

terms of patient compliance and ease of administration, such an approach is preferred, since patients are often adverse to taking multiple pills or dosage forms, often multiple times daily, over the duration of treatment. Alternatively, albeit less preferably, the combination of the invention is administered as separate dosage forms. In instances in  
5 which the drugs comprising the therapeutic composition are administered as separate dosage forms and co-administration is required, the desired agent and each of the additional active agents may be administered simultaneously, sequentially in any order, or separately.

10           **KITS**

Also provided herein is a kit containing at least one combination composition of the invention, accompanied by instructions for use.

For example, in instances in which each of the drugs themselves are administered as individual or separate dosage forms, the kit comprises ibudilast and/or another MIF  
15 antagonist in addition to each of the drugs making up the composition of the invention, along with instructions for use. The drug components may be packaged in any manner suitable for administration, so long as the packaging, when considered along with the instructions for administration, clearly indicates the manner in which each of the drug components is to be administered.

For example, for an illustrative kit comprising ibudilast and naltrexone, the kit may be organized by any appropriate time period, such as by day. As an example, for Day 1, a representative kit may comprise unit dosages of each of ibudilast and  
20 naltrexone. If each of the drugs is to be administered twice daily, then the kit may contain, corresponding to Day 1, two rows of unit dosage forms of each of ibudilast and  
25 naltrexone, along with instructions for the timing of administration. Alternatively, if one or more of the drugs differs in the timing or quantity of unit dosage form to be administered in comparison to the other drug members of the combination, then such would be reflected in the packaging and instructions. Various embodiments according to the above may be readily envisioned, and would of course depend upon the particular  
30 combination of drugs, in addition to ibudilast, employed for treatment, their corresponding dosage forms, recommended dosages, intended patient population, and the like. The packaging may be in any form commonly employed for the packaging of pharmaceuticals, and may utilize any of a number of features such as different colors,

wrapping, tamper-resistant packaging, blister paks, dessicants, and the like.

### **DOSAGES**

5 Therapeutic amounts can be empirically determined and will vary with the particular condition being treated, the subject, and the efficacy and toxicity of each of the active agents contained in the composition. The actual dose to be administered will vary depending upon the age, weight, and general condition of the subject as well as the severity of the condition being treated, the judgment of the health care professional, and particular combination being administered.

10 Therapeutically effective amounts can be determined by those skilled in the art, and will be adjusted to the requirements of each particular case. Generally, a therapeutically effective amount of ibudilast or another MIF antagonist will range from a total daily dosage of about 0.1 and 200 mg/day, more preferably, in an amount between 0.1 and 100 mg/day, 0.1-60 mg/day, 0.1 and 40 mg/day, or 0.1 and 10 mg/day.

15 Administration can be one to three times daily for a time course of one day to several days, weeks, months, and even years, and may even be for the life of the patient.

Practically speaking, a unit dose of any given composition of the invention or active agent can be administered in a variety of dosing schedules, depending on the judgment of the clinician, needs of the patient, and so forth. The specific dosing  
20 schedule will be known by those of ordinary skill in the art or can be determined experimentally using routine methods. Exemplary dosing schedules include, without limitation, administration five times a day, four times a day, three times a day, twice daily, once daily, every other day, three times weekly, twice weekly, once weekly, twice monthly, once monthly, and so forth.

25

### **III. EXPERIMENTAL**

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

30 Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

**EXAMPLE 1**

**EFFICACY OF IBUDILAST IN ANTAGONIZING MIF ACTIVITY**

In order to determine whether ibudilast antagonized MIF activity, a peripheral blood mononuclear cell (PBMC) culture model was used. Human PBMCs were isolated by Ficoll gradient. Cells were plated in a 96-well tissue culture plate in RPMI medium without serum and incubated overnight to achieve quiescence. They were then treated with 0.1% DMSO (vehicle) or ibudilast at 10µM, one hour prior to stimulation with recombinant human MIF (0.8, 8, or 80 nM) or LPS (10 ng/mL). 9 hours post-stimulation, the adherent cells were fixed and analyzed for expression of intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) via cell surface enzyme linked immunosorbent assay (ELISA).

The results observed (Table 1) indicate that ibudilast antagonizes the recombinant MIF-induced expression of adhesion molecules ICAM-1 and VCAM-1. Importantly, the ibudilast inhibition of the adhesion molecules appeared to be specific to MIF, as LPS induction of adhesion molecules was not significantly affected by ibudilast.

**Table 1: Effect of 10µM ibudilast on rMIF-induced cell surface ICAM-1 or VCAM-1 expression in PBMCs**

% change in ICAM-1 expression			% change in VCAM-1 expression		
Stimulant	without ibudilast	with ibudilast	Stimulant	without ibudilast	with ibudilast
MIF 80nM	38.8	-1.3	MIF 80nM	54.9	5.4
MIF 8nM	4.1	-0.95	MIF 8nM	26	4.4
MIF 0.8nM	16.1	-2.5	MIF 0.8nM	13.2	-2.9
No Stimulant	0	30.9	No Stimulant	0	95.1
Vehicle for ibudilast	-1.60		Vehicle for ibudilast	16.2	
LPS 10ng/mL	65.6	74.5	LPS 10ng/mL	7.4	-1.5

Values represent mean percent change from n=2 replicates per condition normalized to no stimulant levels.

ICAM-1 and VCAM-1 are adhesion molecules primarily involved in leukocyte trafficking (Hamann and Syrbe, *Rheumatology* (Oxford) (2000) 39(7):696-699). Increased expression of these adhesion molecules is correlated with inflammation and autoimmune diseases and antagonists may have clinical benefit (Yusuf-Makagiansar et al., *Med. Res. Rev.* (2002) 22(2):146-167). Expression of ICAM-1 and VCAM-1 is elevated in diabetes-related neuropathy (Jude et al., *Diabetologia* (1998) 41(3):330-336), and rheumatoid arthritis-related

peripheral neuropathy (El et al., *J. Rheumatol.* (2002) 29(1):57-61. ICAM-1 is implicated in the process of neuro- degeneration in Alzheimer's disease (Pola et al., *Neurobiol. Aging* (2003) 24(2):385-387), indicating that adhesion molecules play a role in neurological disorders. Thus antagonism of these adhesion molecules may have clinical benefit in  
5 inflammatory and neurological disorders.

Potential regulation of ICAM-1 and VCAM-1 by ibudilast in quiescent cells is of uncertain consequence in human neuropathic pain. It may not be relevant to ibudilast's attenuation of neuropathic pain or other neurological disorders as those syndromes may present partly as a result of factor (e.g. MIF) activation of inflammatory cells (e.g. glia,  
10 monocytes). Hence, the dominant outcome of therapeutic administration of ibudilast in disorders of glial (or monocyte) activation would likely be reduced adhesion molecule expression and related inflammatory effects.

In summary, the results indicate that ibudilast antagonizes MIF activity by abrogating recombinant MIF-induced expression of adhesion molecules ICAM-1 and VCAM-1. Ibudilast  
15 may exert its pharmacological effects including its anti-inflammatory activity and the ability to attenuate neuropathic pain through antagonism of MIF.

Thus, methods for identifying agents for treating neuropathic pain and addiction are described. Also described are methods for utilizing ibudilast for antagonizing MIF activity. Although preferred embodiments of the subject invention have been described in some detail,  
20 it is understood that obvious variations can be made without departing from the spirit and the scope of the invention as defined herein.

**CLAIMS**

What is claimed is:

1. A method for inhibiting macrophage migration inhibitory factor (MIF) activity  
5 in a vertebrate subject in need thereof, comprising providing a therapeutically effective amount of ibudilast to said vertebrate subject.
2. The method of claim 1, wherein the subject is human.
- 10 3. The method of either of claims 1 or 2, wherein the ibudilast is administered systemically.
4. The method of claim 3, wherein the ibudilast is administered intravenously, subcutaneously, orally, intranasally, or sublingually.
- 15 5. The method of either of claims 1 or 2, wherein the ibudilast is administered centrally.
6. The method of claim 5, wherein the ibudilast is administered intrathecally.
- 20 7. The method of any one of claims 1-6, wherein multiple therapeutically effective doses of ibudilast are administered to the subject.
8. The method of claim 7, wherein ibudilast is administered according to a daily  
25 dosing regimen.
9. The method of claim 8, wherein ibudilast is administered twice a day.
10. The method of any one of claims 1-6, wherein ibudilast is administered  
30 intermittently.

11. A method of selecting a compound useful for treating neuropathic pain, said method comprising:

(a) exposing a peripheral blood mononuclear (PBMC) cell culture to a putative compound for treating neuropathic pain;

5 (b) providing macrophage migration inhibitory factor (MIF) to said exposed cells in an amount and under conditions that normally provide for expression of intracellular adhesion molecule-1 (ICAM-1) and/or vascular cell adhesion molecule-1 (VCAM-1);

(c) comparing expression of ICAM-1 and/or VCAM-1 by the cells in (b), to expression of ICAM-1 and/or VCAM-1 in a PBMC cell culture treated with MIF as in

10 (b) in the absence of the putative compound for treating neuropathic pain; and

(d) selecting a compound from (c) that inhibits expression of ICAM-1 and/or VCAM-1 relative to expression of ICAM-1 and/or VCAM-1 in the absence of the compound.

15 12. The method of claim 11, wherein the method further comprises testing the compound selected in (d) in an acceptable model of neuropathic pain.

20 13. The method of either of claims 11 or 12, wherein the neuropathic pain is selected from postherpetic neuralgia, trigeminal neuralgia, neuropathic pain associated with herpes, HIV, traumatic nerve injury, stroke, post-ischemia, fibromyalgia, reflex sympathetic dystrophy, complex regional pain syndrome, spinal cord injury, sciatica, phantom limb pain, multiple sclerosis, or cancer chemotherapeutic-induced neuropathic pain.

25 14. A method of selecting a compound useful for treating addiction, said method comprising:

(a) exposing a peripheral blood mononuclear (PBMC) cell culture to a putative compound for treating addiction;

30 (b) providing macrophage migration inhibitory factor (MIF) to said exposed cells in an amount and under conditions that normally provide for expression of intracellular adhesion molecule-1 (ICAM-1) and/or vascular cell adhesion molecule-1 (VCAM-1);

(c) comparing expression of ICAM-1 and/or VCAM-1 by the cells in (b), to

expression of ICAM-1 and/or VCAM-1 in a PBMC cell culture treated with MIF as in step (b) in the absence of the putative compound for treating addiction; and

5 (d) selecting a compound from (c) that inhibits expression of ICAM-1 and/or VCAM-1 relative to expression of ICAM-1 and/or VCAM-1 in the absence of the compound.

15 15. The method of claim 14, wherein the method further comprises testing the compound selected in (d) in an acceptable model of addiction.

10 16. The method of either of claims 14 or 15, wherein the addiction is a drug addiction.

15 17. The method of claim 16, wherein the drug addiction is selected from an opiate addiction, a cocaine addiction, an amphetamine addiction, a methamphetamine addiction, a cannabinoid addiction, an alcohol addiction, or a nicotine addiction.

18. The method of either of claims 14 or 15, wherein the addiction is a behavioral addiction.

20 19. The method of claim 18, wherein the behavioral addiction is selected from an eating addiction, a drinking addiction, a smoking addiction, a shopping addiction, a gambling addiction, a sex addiction, or a computer use addiction.

25 20. Use of ibudilast in the manufacture of a medicament for inhibiting macrophage migration inhibitory factor (MIF) activity in a vertebrate subject in need thereof.

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2007/012656

## A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/437 A61P25/00 A61P29/00 A61P25/30 A61P25/32  
A61P25/34 A61P25/36

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

## B. FIELDS SEARCHED

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

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

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

EPO-Internal, WPI Data, EMBASE, BIOSIS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	WO 2006/108671 A (NOVARTIS AG; NOVARTIS PHARMA GMBH; BILlich ANDREAS [AT]; GSTACH HUBERT) 19 October 2006 (2006-10-19) page 3, line 12 - page 4, line 26 page 12, lines 24-30 page 13, lines 31-33 page 17, line 22 - page 18, line 11 claims 8,9	1-20
A	WO 2006/045505 A (NOVARTIS AG [CH]; NOVARTIS PHARMA GMBH [AT]; BILlich ANDREAS [AT]; LEH) 4 May 2006 (2006-05-04) page 1, lines 2-6 page 3, line 8 - page 4, line 15 page 9, lines 10,11 page 10, lines 7-16 page 12, lines 4,22-32; claims 8,9 ----- -/--	1-20



Further documents are listed in the continuation of Box C.



See patent family annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*&\* document member of the same patent family

Date of the actual completion of the international search

16 October 2007

Date of mailing of the international search report

29/10/2007

Name and mailing address of the ISA/

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## INTERNATIONAL SEARCH REPORT

International application No

PCT/US2007/012656

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>WO 2005/058304 A (CORTICAL PTY LTD [AU]; MORAND ERIC FRANCIS [AU]; ISKANDER MAGDY NAGUIB) 30 June 2005 (2005-06-30)</p> <p>page 1, lines 2-5  page 4, lines 5-22  page 14, lines 2,24  page 15, lines 8,18</p>	1-20
A	<p>WO 03/104203 A (CORTICAL PTY LTD [AU]; MORAND ERIC FRANCIS [AU]; ISKANDER MAGDY NAGUIB) 18 December 2003 (2003-12-18)</p> <p>page 1, lines 5-9  page 7, line 29 - page 8, line 11  page 9, line 12  page 38, line 17 - page 39, line 17  page 40, line 18  page 41, line 17  page 42, line 12; claims 19,21</p>	1-20
A	<p>WO 03/104178 A (CORTICAL PTY LTD [AU]; MORAND ERIC FRANCIS [AU]; ISKANDER MAGDY NAGUIB) 18 December 2003 (2003-12-18)</p> <p>page 1, lines 5-9  page 7, line 28 - page 8, line 10  page 9, line 11  page 39, line 21 - page 40, line 10  page 41, line 11  page 42, line 10  page 43, line 5  page 55, line 7</p>	1-20
A	<p>WO 2004/058713 A (IRM LLC [US]; KING FRED [US]; CHYBA JASON [US]; HAMPTON GARRET [US]; D) 15 July 2004 (2004-07-15)</p> <p>page 8, lines 3-25  page 10, lines 1-11</p>	1-20

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2007/012656

## Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
Although claims 1-10 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2.  Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2007/012656

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