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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 September 2007 (27.09.2007)

PCT

(10) International Publication Number
WO 2007/109324 A2

(51) International Patent Classification:
C12Q 1/68 (2006.01)

(21) International Application Number:
PCT/US2007/007025

(22) International Filing Date: 21 March 2007 (21.03.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/784,535 21 March 2006 (21.03.2006) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, 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, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, 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, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

Published:
— *without international search report and to be republished upon receipt of that report*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) **Title:** POTENT AND SELECTIVE NAV 1.7 SODIUM CHANNEL BLOCKERS

(57) **Abstract:** The present invention relates to the discovery that mutations in Nav1.7 are causative of Congenital Indifference to Pain (CIP) in humans. The invention also relates to methods of utilizing the Nav1.7 gene and expression products thereof for the screening and identification of therapeutic agents, including small organic compounds, which are selective for Nav1.7, and are useful in the treatment of pain and other Nav1.7-mediated disorders. The invention discloses potent and selective small molecule inhibitors of Nay1.7 and also relates to methods of using these compounds to treat or otherwise ameliorate such disorders.

WO 2007/109324 A2

POTENT AND SELECTIVE $Na_V1.7$ SODIUM CHANNEL BLOCKERS

This application claims priority of U.S. Provisional Application 60/784,535, filed 21 March 2006, the disclosure of which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

This invention relates to method of treating pain by selectively inhibiting the $Na_V1.7$ and other $Na_V1.7$-mediated diseases using compounds that are potent blockers of $Na_V1.7$ and highly selective for $Na_V1.7$ over other sodium channels.

BACKGROUND OF THE INVENTION

Congenital Indifference To Pain (also referred to herein as "C-I-P"), an extremely rare autosomal recessive disorder that interferes with the normal perception of pain, is an hereditary pain disorder (see, Landrieu, P. S. G., and Allaire, C. Ann. Neurology, **27** (5):574-58 (1990) and Comings DE and Amromin GD, 1974. Neurology. Sep;24(9):838-48).

Patients with C-I-P are essentially completely indifferent to sensations that would cause pain in most individuals; yet at the same time they are able

to distinguish between other sensations, such as thermal (hot/cold) and tactile (sharp/dull) stimuli. Tendon reflex, deep sensation and vibration recognition are normal but discrimination between "touch" and "pinprick" may be attenuated. Patients do not perceive inflammatory pain or dental pain even though there is a normal flare response to intradermal histidine injection, indicating that the peripheral arc reflex is intact. (Landrieu et al, 1990) Intelligence and neurological exams prove normal and no differences are identified in autonomic factors such as sweating, blood-pressure, heart rate and sympathetic nervous system responses. Remarkably, women having C-I-P do not experience labor pain. A general description of C-I-P can be found at Online Mendelian Inheritance in Man™ (OMIM™) reference *243000 and at Guillermo A. and A. Grinspan, Rev Neurol (Paris), 1970. 123(6): 434-5; Comings et al (1974), above; and at Hirsch et al. South Med J, 88(8):851-857 (1995)). C-I-P is compared to other hereditary pain disorders in Nagasako, EM et al. (2003) Pain 101:213-219.

The mutant gene associated with the disorder provides a therapeutic target against which novel therapeutic agents can act. Therapeutic agents that are highly selective for this therapeutic target, and that mimic the effect of the mutation, have the potential to induce the same kind of analgesia, in the general population, as experienced by C-I-P patients.

Drug therapy is the mainstay of management for acute and cancer pain in all age groups, including neonates, infants and children. The pain drugs are classified by the American Pain Society into three main categories; 1) non-opioid analgesics- acetaminophen, and non-steroidal anti-inflammatory drugs (NSAIDs), including salicylates (e.g. aspirin), 2) opioid analgesics and 3) co-analgesics.

Non-opiod analgesics such as acetaminophen and NSAIDs are useful for acute and chronic pain due to a variety of causes including surgery, trauma, arthritis and cancer. NSAIDs are indicated for pain involving

inflammation because acetaminophen lacks anti-inflammatory activity. Opioids also lack anti-inflammatory activity. All NSAIDs inhibit the enzyme cyclooxygenase (COX), thereby inhibiting prostaglandin synthesis and reducing the inflammatory pain response. There are at least two COX isoforms, COX-1 and COX-2. Common non-selective COX inhibitors include, ibuprofen and naproxen. Inhibition of COX-1, which is found in platelets, GI tract, kidneys and most other human tissues, is thought to be associated with adverse effects such as gastrointestinal bleeding. The development of selective COX-2 NSAIDs, such as Celecoxib, Valdecoxib and Rofecoxib, have the benefits of non-selective NSAIDs with reduced adverse effect profiles in the gut and kidney. However, evidence now suggests that chronic use of certain selective COX-2 inhibitors can result in an increased risk of stroke occurrence.

The use of opioid analgesics is recommended by the American Pain Society to be initiated based on a pain-directed history and physical that includes repeated pain assessment. Due to the broad adverse effect profiles associated with opiate use, therapy should include a diagnosis, integrated interdisciplinary treatment plan and appropriate ongoing patient monitoring. It is further recommended that opiods be added to non-opiods to manage acute pain and cancer related pain that does not respond to non-opiods alone. Opioid analgesics act as agonists to specific receptors of the mu and kappa types in the central and peripheral nervous system. Depending on the opioid and its formulation or mode of administration it can be of shorter or longer duration. All opioid analgesics have a risk of causing respiratory depression, liver failure, addiction and dependency, and as such are not ideal for long-term or chronic pain management.

A number of other classes of drugs may enhance the effects of opioids or NSAIDSs, have independent analgesic activity in certain situations, or counteract the side effects of analgesics. Regardless of which of these actions the drug has, they are collectively termed "coanalgesics". Tricyclic antidepressants, antiepileptic drugs, local anaesthetics, glucocorticoids,

skeletal muscle relaxants, anti-spasmodil agents, antihistamines, benzodiazepines, caffeine, topical agents (e.g. capsaicin), dextroamphetamine and phenothizines are all used in the clinic as adjuvant therapies or individually in the treatment of pain. The antiepeileptic drugs in particular have enjoyed some success in treating pain conditions. For instance, Gabapentin, which has an unconfirmed therapeutic target, is indicated for neuropathic pain. Other clinical trials are attempting to establish that central neuropathic pain may respond to ion channel blockers such as blockers of calcium, sodium and/or NMDA (N-methyl-D-aspartate) channels. Currently in development are low affinity NMDA channel blocking agents for the treatment of neuropathic pain. The literature provides substantial pre-clinical electrophysiological evidence in support of the use of NMDA antagonists in the treatment of neuropathic pain. Such agents also may find use in the control of pain after tolerance to opioid analgesia occurs, particularly in cancer patients.

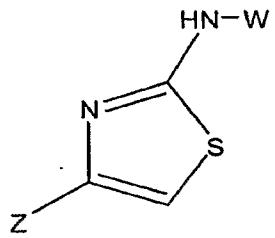
Systemic analgesics such as NSAIDs and opioids are to be distinguished from therapeutic agents which are useful only as local analgesics/anaesthetics. Well known local analgesics such as lidocaine and xylocaine are non-selective ion channel blockers which are generally fatal if administered systemically. A good description of non-selective sodium channel blockers is found in Madge, D. et al. 2001. J Med. Chem. 44(2):115-37

In accordance with the present invention, the identification of the hereditary basis for Congenital Indifference To Pain will be a key step for developing novel therapeutic agents because the underlying gene/protein has the potential to be a novel therapeutic target. This therapeutic target can be used to identify and discover more effective analgesics. Discovery of the target will also provide new methods and compositions for diagnosis of C-I-P and for distinguishing between types of inherited pain disorders.

BRIEF SUMMARY OF THE INVENTION

The invention relates to high potency compounds that selectively inhibit $Na_V1.7$ over other sodium channels are useful therapeutic agents, temporarily reproducing some aspects of congenital indifference to pain in normal animals. In accordance with this, two classes of high potency compounds which selectively inhibit $Na_V1.7$ ahead of other sodium channels are disclosed.

Accordingly, in one aspect this invention is directed to compounds of Formula (Ia):



(Ia)

wherein:

Z is selected from optionally substituted straight or branched chain C_1 to C_5 alkyl, optionally substituted straight or branched chain C_1 to C_5 alkoxy, optionally substituted cycloalkyl, optionally substituted cycloalkylalkyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl or optionally substituted heteroaralkyl,

wherein said substitutions are each independently selected from hydrogen, straight or branched chain C_1 to C_5 alkyl, halo, haloalkyl, $-OR^2$, $-S(O)_tR^2$ (where t is 0, 1 or 2), -CN, $-C(O)R^2$, $-C(O)OR^2$, $-C(O)N(R^2)R^3$, $-N(R^2)R^3$ and $-NO_2$;

W is $-R^4-C(O)-R^4-X-R^1$;

R^1 is optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

wherein said substitutions are each independently selected from hydrogen, straight or branched chain C_1 to C_5 alkyl, halo, haloalkyl, -

OR^2, $-S(O)_tR^2$ (where t is 0, 1 or 2), -CN, $-C(O)R^2$, $-C(O)OR^2$, $-C(O)N(R^2)R^3$, $-N(R^2)R^3$ and $-NO_2$;

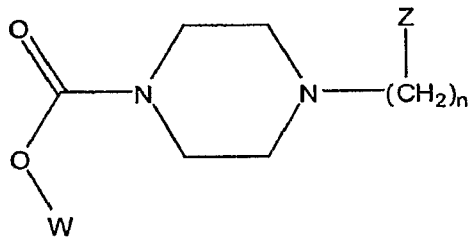
R^2 and R^3 are each independently hydrogen or optionally substituted straight or branched chain C_1 to C_5 alkyl, wherein said substitutions are each independently selected from hydrogen, straight or branched chain C_1 to C_5 alkyl, halo, haloalkyl, $-OR^5$, $-S(O)_tR^5$ (where t is 0, 1 or 2), -CN, $-C(O)R^5$, $-C(O)OR^5$, $-C(O)N(R^5)R^6$, $-N(R^5)R^6$ and $-NO_2$;

R^4 is a direct bond or a straight or branched C_1 to C_5 alkylene;

X is -O-, -S- or $-N(R^2)-$;

as a stereoisomer, enantiomer, tautomer thereof or mixtures thereof; or a pharmaceutically acceptable salt, solvate or prodrug thereof.

In another aspect, this invention is directed to compounds of Formula (IIa):



(IIa)

wherein:

n is 0, 1, 2 or 3;

Z is selected from $-R^7-N(R^6)R^7$, $-OR^7$, $-S(O)_tR^7$ (where t is 0, 1 or 2), optionally substituted straight or branched chain C_1 to C_5 alkyl, optionally substituted straight or branched chain C_1 to C_5 alkoxy, optionally substituted cycloalkyl, optionally substituted cycloalkylalkyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl or optionally substituted heteroaralkyl,

wherein said substitutions are each independently selected from hydrogen, C_1 to C_5 alkyl, halo, haloalkyl, $-OR^5$, $-S(O)_tR^5$ (where t is 0, 1 or 2), -CN, $-C(O)R^5$, $-C(O)OR^5$, $-C(O)N(R^5)R^6$, $-N(R^5)R^6$ and $-NO_2$;

W is selected from $-R^8-X-R^8-R^9$, optionally substituted straight or branched chain C_1 to C_5 alkyl, optionally substituted straight or branched chain

C_1 to C_5 alkoxy, optionally substituted aryl, optionally substituted heteroaryl,

wherein said substitutions are each independently selected from hydrogen, straight or branched chain C_1 to C_5 alkyl, halo, haloalkyl, -OR^5, -$S(O)_tR^5$ (where t is 0, 1 or 2), -CN, -$C(O)R^5$, -$C(O)OR^5$, -$C(O)N(R^5)R^6$, -$N(R^5)R^6$ and -NO_2;

X is -O-, -S- or -$N(R^5)$-;

R^5 and R^6 are each independently hydrogen or optionally substituted straight or branched chain C_1 to C_5 alkyl, wherein said substitutions are each independently selected from hydrogen, straight or branched chain C_1 to C_5 alkyl, halo, haloalkyl, -OR^{10}, -$S(O)_tR^{10}$ (where t is 0, 1 or 2), -CN, -$C(O)R^{10}$, -$C(O)OR^{10}$, -$C(O)N(R^{10})R^{11}$, -$N(R^{10})R^{11}$ and -NO_2;

R^7 is optionally substituted aryl or optionally substituted heteroaryl,

wherein said substitutions are each independently selected from hydrogen, straight or branched chain C_1 to C_5 alkyl, halo, haloalkyl, -OR^5, -$S(O)_tR^5$ (where t is 0, 1 or 2), -CN, -$C(O)R^5$, -$C(O)OR^5$, -$C(O)N(R^5)R^6$, -$N(R^5)R^6$ and -NO_2;

each R^8 is independently a direct bond or a straight or branched C_1 to C_5 alkylene;

R^9 is optionally substituted aryl or optionally substituted heteroaryl,

wherein said substitutions are each independently selected from hydrogen, straight or branched chain C_1 to C_5 alkyl, halo, haloalkyl, -OR^5, -$S(O)_tR^5$ (where t is 0, 1 or 2), -CN, -$C(O)R^5$, -$C(O)OR^5$, -$C(O)N(R^5)R^6$, -$N(R^5)R^6$ and -NO_2; and

R^{10} and R^{11} are each independently hydrogen or optionally substituted straight or branched chain C_1 to C_5 alkyl;

as a stereoisomer, enantiomer, tautomer thereof or mixtures thereof; or a pharmaceutically acceptable salt, solvate or prodrug thereof.

In another aspect, the invention is directed to pharmaceutical compositions comprising a pharmaceutically acceptable excipient and a therapeutically effective amount of a compound of formula (Ia) or formula (IIa) as set forth above.

In another aspect, the present invention relates to compounds having activity in a screening assay wherein the $Na_V1.7$ gene or protein is incorporated and whereby said compounds (potential therapeutic agents) modulate $Na_V1.7$ gene expression or $Na_V1.7$ protein activity.

In another aspect, the present invention relates to a method for treating pain comprising administering to an animal in need thereof a therapeutically or analgesically effective amount of an agent that modulates the activity of a polypeptide encoded by a polynucleotide as disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the pedigree for a family designated CIP-10 showing family relationships and haplotypes. Squares and circles represent males and females, respectively. Filled symbols indicate individuals with CIP. Symbols with an "N" indicate individuals diagnosed as normal, and clear symbols with a question mark indicate individuals who have not been diagnosed

Figure 2 shows the pedigree for a family designated CIP-14. Symbols as in Figure 1.

Figure 3 shows the pedigree for a family designated CIP-08. Symbols as in Figure 1.

Figure 4a. Nucleotide sequence of the 5067G>A $Na_V1.7$ mutation in CIP patient CIP-10-503 (SEQ ID NO: 23). The patient is homozygous for a G>A substitution at nt 5067 (numbered relative to the initiator methionine in Genbank record NM_002977). This corresponds to a change to a stop codon at tryptophan 1689. The wild-type amino acid sequence from an unaffected patient is shown on top (SEQ ID NO: 22) and the wild-type nucleotide sequence as SEQ ID NO: 21. The amino acid sequence for the mutant is given as SEQ ID NO: 24. Figure 4b. Nucleotide sequence of the 984C>A $Na_V1.7$ mutation in CIP patient CIP-14-A005 (SEQ ID NO: 27). The patient is

homozygous for a C>A substitution at nt 984. This corresponds to a change to a stop codon at tyrosine 328. The wild-type amino acid sequence from an unaffected patient is shown on top (SEQ ID NO: 26) with the wild-type nucleotide sequence above it (SEQ ID NO: 25). The mutant amino acid sequence is SEQ ID NO: 28. Figure 4c. Nucleotide sequence of the 2488C>T $Na_V1.7$ mutation in CIP patient CIP-08-II:01 (SEQ ID NO: 31). The patient is homozygous for a C>T substitution at nt 2488. This corresponds to a change to a stop codon at arginine 830. The wild-type sequence from an unaffected patient is shown on top (SEQ ID NO: 30) with the wild-type nucleotide sequence above it (SEQ ID NO: 29). The mutant amino acid sequence is SEQ ID NO: 32.

Figure 5a. Genomic arrangement of alternatively spliced exon 5N and 5A of the human $Na_V1.7$ gene. The neonatal (N) and adult (A) isoforms are generated by a mutually exclusive alternative splicing mechanism. The length in base pairs of the introns and exons are shown. Figure 5b. Formation of $Na_V1.7$ 5N (neonatal) isoform generated by splicing. Figure 5c. Formation of $Na_V1.7$ 5A (adult) isoform generated by splicing. Bent lines indicated portions of the pre-mRNA which are removed to generate the mature mRNA for translation.

Figure 6. Comparison of the alternatively spliced exons of other sodium channels with the two variants of human $Na_V1.7$. The cDNA sequence and the predicted amino acid sequence of the alternatively spliced exon 5 of the A and N isoforms of the human (SEQ ID NO: 33 and 35, respectively) and rabbit (SEQ ID NO: 36 and 37, respectively) $Na_V1.7$, human (SEQ ID NO: 42 and 43, respectively) and rat (SEQ ID NO: 44 and 45, respectively) SCN3A, human SCN2A2 (SEQ ID NO: 38 and 39, respectively), and rat (SEQ ID NO: 40 and 41, respectively) SCN2A1 are shown. Isoform-specific differences in the amino acid sequence are indicated below the nucleotide sequence (SEQ ID NO: 34 is the human $Na_V1.7$ isoform A).

Figure 7. The proposed transmembrane topography of the first domain of a sodium channel protein. The 30 amino acids specified by these exon 5 variants encode a short extracellular portion of the S3 transmembrane helix, the short extracellular loop between S3 and S4, and most of the S4 transmembrane helix, which is thought to be involved in sensing changes in membrane potential. The + symbols refer to the repeating basic (positively charged) residues within the S4 transmembrane segment. Amino acid 206 of $Na_V1.7$ is shown as an aspartate residue (A isoforms); it would be an uncharged residue in the N isoforms (i.e. asparagine in $Na_V1.7$ and SCN2A; and serine in SCN3A).

Figure 8. Illustration of nucleotide and amino acid sequence of alternative exon 11B. Alternative splice donor sites for exon 11 found in $Na_V1.7$ isoform are indicated as upstream site and downstream site. Arrow indicates the approximate position of the additional amino acids in the secondary structure of the sodium channel protein.

Figure 9. Evolutionary conservation of the additional amino acids of Exon 11B in other members of the sodium channel gene family ($Na_V1.7$, SCN1A, SCN8A).

Figure 10. Expression of $Na_V1.7$ mRNA in various tissues of the central nervous system (CNS) and other organs. A representative gel is shown. To control for difference in the amounts of RNA among samples, pixel density relative to an internal β-actin standard was calculated. The graph shows the results of 3 relative quantitative RT-PCR experiments relative to a dorsal root ganglia (DRG) positive control.

Figure 11. Graphic representation of mutations of $Na_V1.7$ found in (a) patients having Congenital Indifference to Pain versus mutations of $Na_V1.7$ found in patients having (b) erythermalgia and (c) familial rectal pain.

Figure 12. Graphic depiction of the analgesic activity of the test compounds at 5 mg/kg, 10 mg/kg and 30 mg/kg expressed as a percentage of the maximum possible effect (%MPE) over 160 minutes, as described in the tail flick test.

Figure 13. Graphic depiction of weighted pain score for rats injected with 10% formalin administered at 30 minutes after treatment with the test compound, as described in the formalin test.

Figure 14. Full sequences showing normal and mutant nucleotides for the mutations in Table 2.

DEFINITIONS

'Selectivity' and 'selective' as used herein is a relative measure of the tendency for a compound of the invention to preferentially associate with one thing as opposed to another (or group of others), as between or among sodium channels. The tendency of a compound to associate with a sodium channel can be measured by many different techniques, and many types of association are known to those skilled in the art, as disclosed elsewhere herein. Selectivity means that in a particular type of association, measured in a specific way, a compound demonstrates a tendency or preference to associate with one sodium channel as opposed to another. This association is reproducible, though it may be different for different types of assays or different ways of measurement.

"High potency" is defined as having an IC-50 for $Na_V1.7$ of less than 10 μM in a 2 hour guanidine flux assay. As such, high potency compounds of the invention have an IC-50 for $Na_V1.7$ of 10, 7.5, 5, 2.5, 2, 1.0, 0.90, 0.80. 0.75, 0.60, 0.40, 0.30, 0.25, 0.20 μM or less.

This specification employs the nomenclature 'IC-50' or 'IC50' or 'IC_{50}' to mean 'Inhibitory Concentration 50' which is the concentration of a test agent estimated to inhibit the biological endpoint of interest (e.g., inhibition of sodium ion flux) by 50%. IC-50 measurements are generally employed on a population of ion channels dispersed across an area of membrane (see Examples). IC-50 measurements are a suitable way to evaluate selectivity of compounds of the invention, though many other measurements are possible. A preferred IC-50 measure is a 2 hour guanidine flux assay, explained elsewhere herein. The IC-50 of a compound for a specific channel (e.g. $Na_v1.7$) in a specific assay (e.g. 2 hour guanidine flux assay) is sometimes reported herein as $IC\text{-}50_{NaV1.7}$.

The parameter "R_{IC}" means the measure of ($IC\text{-}50_{(other\ sodium\ channel)}$ /$IC\text{-}50_{NaV1.7}$) and in specific examples herein is the ratio of the "other sodium channel may be such as ($IC\text{-}50_{NaV1.8}$/$IC\text{-}50_{NaV1.7}$).

"On-rate" is a measure of how quickly a compound tends to bind to, and block, the activity of a given target. Compounds with a fast on-rate bind quickly to their targets, but these often also display a fast off-rate, leading to only modest reductions in overall ion flux. Compounds with a slow on rate demonstrate limited early blockage of channels, but over a 2 hour assay, such as the guanidine flux assay, provide a substantial inhibition of ion flux.

The term "agent" is used interchangeably with the term "compound" and likewise the term "test agent" is used interchangeably with the term "test compound."

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a novel therapeutic target related to pain response and methods of using this target to identify useful analgesic agents. The invention also provides compounds of formulas (I), (Ia), (II) and

(IIa), and their pharmaceutical compositions which modulate this target, and methods for treating pain in human patients using this target and these compounds and compositions.

Description of $Na_V1.7$

In one aspect, the present invention ~~identifies~~ relates to a gene and its corresponding protein related to pain indifference, one such embodiment being the $Na_V1.7$ gene. The $Na_V1.7$ gene and protein may be known to those skilled in the art under a variety of names, including voltage-gated sodium channel type IX alpha polypeptide, SCN9A, neuroendocrine sodium channel, peripheral sodium channel 1, Schwann cell sodium channel, NENA, NE-Na, hNE-Na, hNE, PN1, hPN1, NaS, Nas, $Na_v1.7$, hNav1.7, NaV1.7 and Na(v)1.7. Preliminary versions of the cDNA and amino acid sequences are found at GenBank Accession No. NM_002977.1 or XM_011955; The gene is described further at OMIM (On-line Mendelian Inheritance in Man) reference *603415.

The correct wild type nucleic acid sequence of the two isoforms of $Na_V1.7$ (herein called "neo-natal" and "adult") is herein provided for the first time. The neo-natal $Na_V1.7$ nucleic acid sequence is shown in SEQ ID NO: 1 with the correct neo-natal wild type amino acid sequence set forth at SEQ ID NO: 2. Table 1 identifies where the correct wild type sequence of neo-natal $Na_V1.7$ set forth in SEQ ID NO: 1 diverges from the previously available public sequences of neo-natal $Na_V1.7$. The distinction between neo-natal and adult forms is a tentative description based on alternate Exon 5 (i.e. Exon 5N (for neo-natal) and Exon 5A (for adult)) further described, below. A nucleic acid sequence encoding a predicted adult splice variant of $Na_V1.7$ employing exon 5A is found in SEQ ID NO: 3. The corresponding amino acid sequence of the predicted splice variant of $Na_V1.7$ employing exon 5A is found in SEQ ID NO: 4. A nucleic acid sequence employing an alternate splice donor site at the end of Exon 11, herein called Exon 11B, is set forth at SEQ ID No. 5. The

corresponding amino acid sequence of the predicted splice variant of $Na_V1.7$ employing Exon 11B is found at SEQ ID No. 6.

The sequences listed in Table 1, shown below, represent SEQ ID NO: 46 to 111, respectively.

The present invention further provides at SEQ ID No. 7, a first mutation in the gene encoding this protein found in a first family (CIP-10) at G5067A (herein shown in the 5N/11 isoform, although 5A and 11B isoforms are also expected at different developmental stages). The mutation results in a truncation described as W1689X (SEQ ID NO: 8).

The present invention also provides a second mutation in the gene encoding $Na_V1.7$ which is responsible for Congenital Indifference to Pain in a different family (CIP-14). The nucleic acid sequence of this second mutation, C984A, is set out in SEQ ID NO: 9 (herein shown in the 5N/11 isoform) and the corresponding mutant protein, Y328X, is described in SEQ ID NO: 10.

Table 1

Sequence variations (numbered relative to initiator methionine)	Exon/Intron	Variation in relation to a cDNA sequence	Variation in relation to a protein sequence		SEQ ID NO.	Genomic sequence differences
Differences between all samples or genomic sequence and Genbank entry NM_002977.1	26	6182_6185delGATT	not applicable	Public sequence:	46	AAAAGTGATTGATTCAGTTTTTTG
				Correct sequence:	47	AAAAGTGATTCAGTTTTTTG
Mutations	8	984C>A	Y328X	More common:	48	CAGAGGGGTACACCTGTGTGA
				Less common:	49	CAGAGGGGTAAACCTGTGTGA
	15	2488C>T	R830X	More common:	50	CTTTTAGCTCCGAGTCTTCAA
				Less common:	51	CTTTTAGCTCTGAGTCTTCAA
	26	5067G>A	W1689X	More common:	52	CTGCTGGCTGGGATGGATTGC
				Less common:	53	CTGCTGGCTGAGATGGATTGC
Polymorphisms	1	174A>G	not applicable	More common:	54	CTGGCAAACAACTGCCCTTCA
				Less common:	55	CTGGCAAACAGCTGCCCTTCA
	3	444G>A	not applicable	More common:	56	TGAATAACCCGCCGGACTGGA
				Less common:	57	TGAATAACCCACCGGACTGGA
	9	1119C>T	not applicable	More common:	58	CGCTGCGTGCCGCTGGCAAAA
				Less common:	59	CGCTGCGTGCTGCTGGCAAAA
	9	1266G>A	not applicable	More common:	60	AAGAATTAGAGTTTCAACAGA
				Less common:	61	AAGAATTAGAATTTCAACAGA
	9	1287A>T	not applicable	More common:	62	TGTTAGACCGACTTAAAAAAG
				Less common:	63	TGTTAGACCGTCTTAAAAAAG
	18	3448C>T	R1150W	More common:	64	GTTGTGTACGGAGGTTCTC
				Less common:	65	GTTGTGTATGGAGGTTCTC
	26	4779G>T	not applicable	More common:	66	CGTATTTTGTGTCCCCTACCC
				Less common:	67	CGTATTTTGTTTCCCCTACCC
	Intron 2	377+93_94delTGinsGA	not applicable	More common:	68	TTTGCACCTTTGAAGACTCTGG
				Less common:	69	TTTGCACCTTGAAAGACTCTGG
	Intron 2	378-90A>T	not applicable	More common:	70	ATTTTTTTCTAAGGAAAAGTT
				Less common:	71	ATTTTTTTCTTAGGAAAAGTT
	Intron 3	478-219T>C	not applicable	More common:	72	ATATTCTTAGTTATTTCAAGT
				Less common:	73	ATATTCTTAGCTATTTCAAGT
	Intron 4	596+25T>C	not applicable	More common:	74	CTTTTTGAAATGGCAAATTTA
				Less common:	75	CTTTTTGAAACGGCAAATTTA
	Intron 4	596+591T>G	not applicable	More common:	76	TCAGAAAAATTGATTTTTACA
				Less common:	77	TCAGAAAAATGGATTTTTACA

Table 1 (Cont'd)

Intron 6	902-118T>C	not applicable	More common:	78	GGATGCATATTGCCTGGGACC
			Less common:	79	GGATGCATATCGCCTGGGACC
Intron 8	1107+41A>G	not applicable	More common:	80	TTTGAATGGCATATGTACCTG
			Less common:	81	TTTGAATGGCGTATGTACCTG
Intron 8	1107+48T>C	not applicable	More common:	82	GGCATATGTATCTGGTGTATG
			Less common:	83	GGCATATGTACCTGGTGTATG
Intron 9	1314+76C>T	not applicable	More common:	84	GCGGTATATGCTTGGCCTTCT
			Less common:	85	GCGGTATATGTTTGGCCTTCT
Intron 9	1314+199C>T	not applicable	More common:	86	CCCATAATCACCTCACTGCAT
			Less common:	87	CCCATAATCATCTCACTGCAT
Intron 10	1603-368T>C	not applicable	More common:	88	TTTGTGAAGCTTGGGGATTGA
			Less common:	89	TTTGTGAAGCCTGGGGATTGA
Intron 11	c.1942-2_3insT	not applicable	More common:	90	ATTTTTTTTTTAGGGCACGACC
			Less common:	91	ATTTTTTTTTTAGGGCACGACC
Intron 12	2072-14T>C	not applicable	More common:	92	ATGTTCTCTGTTTTTTTCTCC
			Less common:	93	ATGTTCTCTGCTTTTTTCTCC
Intron 19	3769-70T>C	not applicable	More common:	94	TAGTGAGTTTTAGAATTGACT
			Less common:	95	TAGTGAGTTTCAGAATTGACT
Intron 19	3769-4A>G	not applicable	More common:	96	TGTTATTTTTATAGGTTTCTT
			Less common:	97	TGTTATTTTTGTAGGTTTCTT
Intron 21	4173+58_59delAA	not applicable	More common:	98	CGAAGGATATAAGTTATTCTTT
			Less common:	99	CGAAGGATATGTTATTCTTT
Intron 21	4173+77C>A	not applicable	More common:	100	TTTAAATAGTCTATTAATTAT
			Less common:	101	TTTAAATAGTATATTAATTAT
Intron 21	4174-146insA	not applicable	More common:	102	TTTAAAAAAATCTTTACATT
			Less common:	103	TTTAAAAAAAATCTTTACATT
Intron 21	4174-196A>G	not applicable	More common:	104	ATAATTAACTAGGACTAAGAT
			Less common:	105	ATAATTAACTGGGACTAAGAT
Intron 23	4365+99A>G	not applicable	More common:	106	TTCATGATTAATTTTATTAGA
			Less common:	107	TTCATGATTAGTTTTATTAGA
Intron 23	4366-13_14insGTTT	not applicable	More common:	108	TTTTTTGTTTCTTTACCTTG
			Less common:	109	TTTTTTGTTTGTTTCTTTACCTTG
Intron 25	4741+16A>T	not applicable	More common:	110	AATATTTATTATTCAGATTTT
			Less common:	111	AATATTTATTTTCAGATTTT

The present invention also provides a third mutation in the gene encoding $Na_V1.7$ which is responsible for Congenital Indifference to Pain in a third family (CIP-08). The nucleic acid sequence of this second mutation, C2488T, is set out in SEQ ID NO: 11 (herein shown in the 5N/11 isoform) and the corresponding mutant protein, R830X, is described in SEQ ID NO: 12.

The present invention provides a total of seven mutations which are responsible for C-I-P, as identified from families having this condition. These mutations are set out in Table 2.

Table 2: $Na_V1.7$ mutations identified in families having C-I-P

Mutation - DNA	Mutation – protein	Family	SEQ ID NO.
G5067A	W1689X	CIP-10	8
C984A	Y328X	CIP-14	9
C2488T	R830X	CIP-08	11
C877T	R277X		
2076_77insT	E693X		
3600delT	F1200fsX1232		
C4462T	Y1488X		

Numbering scheme in this table and throughout this specification is based on $Na_V1.7$ nucleotide and amino acid sequences at Genbank accession number NM_002977.1, unless otherwise noted. Full sequences of normal and mutant nucleotides are provided in Figure 14.

A novel feature of mutation 3600delT is the short frameshift polypeptide sequence that runs from the point of mutation for 32 amino acids. This polypeptide, either on its own or in association with the normal upstream sequence, provides a unique epitope for identification of this mutation. Those skilled in the art are familiar with techniques to identify antibodies that will

specifically bind to such an epitope, and thus be useful for diagnostic purposes, etc. Compositions according to the present invention comprise such antibodies.

All mutations lead to premature stop in the translation of the $Na_V1.7$ transcript, thus producing a truncated, non-functional protein andproviding a strong mechanistic basis for the observed conditions of C-I-P patients. A comparison of the types of $Na_V1.7$ mutations found in painful conditions such as familial rectal pain and primary erythermalgia is set out in Figure 11. The striking difference in the size of the translated protein between mutation type and phenotype has been recognized by the inventors.

This invention also provides human BAC clone RP11-437H3 (AC108146) that carries the complete human genomic fragment bearing the $Na_V1.7$ gene. This BAC may be obtained commercially from well known sources (including Invitrogen Corp.). A short part of this BAC bearing the nucleotide sequence from the 3' end of exon 4 to the 5' end of exon 6, including exon 5A and exon 5N, is set forth at SEQ ID No. 13. The promoter region of $Na_V1.7$, including both genomic sequence and the 5'UTR of $Na_V1.7$ is set forth at SEQ ID No. 14.

The sequence of SEQ ID NO: 14 up to the ATG initiating codon represents the promoter and is also useful in generating probes of the genome to find similar sequences, which probes may be drawn from sequences having at least 95% identity to the sequence of the promoter portion of SEQ ID NO: 14, more preferably at least 98% identity and most preferably have the sequence of the promoter portion of SEQ ID NO: 14. The most useful probes will have segments at least 15 nucleotides in length, more preferably at least 20 nucleotides in length, even more preferably at least 30 nucleotides in length, even more preferably at least 50 nucleotides in length, even more preferably at least 80 nucleotides in length, and most preferably at least 100 nucleotides in length.

This invention also provides $Na_V1.7$ from mouse (M. musculus) and various isoforms in Table 3 below.

Table 3: Isoforms of Mouse $Na_V1.7$ disclosed

SEQ ID No.	Mouse $Na_V1.7$ isoform
15	Mouse 11B nt
16	Mouse 11B a.a.
17	Mouse 5A nt
18	Mouse 5A a.a.
19	Mouse 5N nt
20	Mouse 5N a.a.

Thus the invention thus discloses several novel isoforms and mutants of human and mouse $Na_V1.7$.

In aspects of the invention relating to the use of $Na_V1.7$, such as in screening assays, or for the development of antibodies, etc., etc., the inventors recognize that those skilled in the art may prefer to use forms of $Na_V1.7$ corresponding to the sequences disclosed herein, although not necessarily the same. For example, screening assays may utilize $Na_V1.7$ from a different organism, preferably a vertebrate, and most preferably from a mammalian species. The shared technical features of these forms of $Na_V1.7$, are that, when expressed, they have sodium channel activity, and that they share functional similarity with $Na_V1.7$, such as may be determined by those skilled in the art. Thus the invention encompasses the use of, for example, sheep, dog or horse $Na_V1.7$, for the same purposes as set out more specifically herein for human or mouse $Na_V1.7$.

Because of the processing that may take place in transforming the initial RNA transcript into the final mRNA, the sequences disclosed herein may represent less than the full genomic sequence. They may also represent sequences derived from alternate splicing of exons, ribosomal and/or transfer

RNAs. Consequently, the genes present in the cell (and representing the genomic sequences) and the sequences disclosed herein, which are mostly cDNA sequences, may be identical or may be such that the cDNAs contain less than the full genomic sequence. Such genes and cDNA sequences are still considered corresponding sequences because they both encode similar RNA sequences. Thus, by way of non-limiting example only, a gene that encodes an RNA transcript, which is then processed into a shorter mRNA, is deemed to encode both such RNAs and therefore encodes an RNA complementary to (using the usual Watson-Crick complementarity rules), or that would otherwise be encoded by, a cDNA (for example, a sequence as disclosed herein). (Those skilled in the art understand that the word "encode" and its derivatives mean, in this field "can be transcribed into".) Thus, the sequences disclosed herein correspond to genes contained in the cells and are used to determine relative levels of expression because they represent the same sequences or are complementary to RNAs encoded by these genes. Such genes also include different alleles and splice variants that may occur in the cells used in the processes of the invention.

Thus, the polynucleotides, such as the genes disclosed herein, for use in the screening assays of the invention "correspond to" the polynucleotide encoding $Na_V1.7$ mRNA (processed or unprocessed, including naturally occurring splice variants and alleles) at least 60%, preferably at least 70%, even more preferably at least 80%, or even at least 85%, most preferably at least 90%, or even at least 95%, or most especially at least 98%, with the especially preferred embodiment of identical to, and especially having the sequence of, an RNA that would be encoded by, or be complementary to, such as by hybridization under reasonably stringent conditions, with a $Na_V1.7$ polynucleotide (SEQ ID NO: 1). In addition, sequences encoding the same polypeptides and proteins as any of these sequences, regardless of the percent identity of such sequences, are also specifically contemplated by any of the methods of the present invention that rely on any or all of said sequences, regardless of how they are otherwise described or limited. Thus, any such sequences are available for use in carrying out any of the methods

disclosed according to the invention. Such sequences also include any open reading frames, as defined herein, present within an $Na_V1.7$ polynucleotide.

Identification of potent and selective $Na_V1.7$ sodium channel blockers

The present invention readily affords different means for identification of $Na_V1.7$ modulating agents that are useful as therapeutic agents. One such protocol involves the screening of chemical agents for ability to modulate the activity of $Na_V1.7$ thereby identifying a $Na_V1.7$ modulating agent.

$Na_V1.7$ modulating agents so identified are then tested in a variety of *in vivo* models so as to determine if they alleviate pain, especially chronic pain or other disorders. In an embodiment the agent modulates the activity of $Na_V1.7$ downwards, inhibits the activity of $Na_V1.7$, and/or reduces or prevents sodium ion flux across a cell membrane by preventing a $Na_V1.7$ activity such as ion flux. Any such modulation, whether it be partial or complete inhibition or prevention of ion flux, is sometimes referred to herein as "blocking" and corresponding compounds as "blockers". The effectiveness of the methods of the invention is demonstrated below with the identification of two classes of potent and selective small molecule $Na_V1.7$ sodium channel blockers.

Thus, in one aspect the present invention relates to a method for identifying an agent that modulates the activity of a polynucleotide whose expression contributes to pain sensation or whose non-expression contributes to lack of pain sensation, comprising:

a) contacting under physiological conditions a test compound or other chemical agent with a polynucleotide corresponding to a promoter of the $Na_V1.7$ gene, preferably having the sequence of SEQ. ID NO. 14, under conditions promoting such contacting; and

b) detecting a change in the expression of said polynucleotide as a result of said contacting;

wherein said change in expression indicates that the test compound is an agent that modulates said polynucleotide activity.

Such modulation is preferably a decrease in expression. In various embodiments, such expression is measured by measuring the amount of an expression product encoded by said polynucleotide, most preferably an RNA or a polypeptide. In an embodiment the promoter sequence is operably linked to a reporter gene, and the assay measures relative expression of the reporter gene or its gene product.

In various embodiments, the polynucleotide whose expression is to be measured or monitored is present in an intact cell, preferably a mammalian cell, most preferably a neuronal cell, and may include a recombinant cell. In various embodiments, such an intact cell is a cell that has been engineered to comprise said polynucleotide, such as by genetic engineering, most preferably wherein the cell does not express the subject gene or polynucleotide absent having been engineered to do so.

Agents that alter the activity of $Na_V1.7$ or active fragments or portions of said gene, or that may modulate the activity of polypeptides encoded by $Na_V1.7$, or polypeptides that act as transcription factors to modulate the activity of such genes, or other related gene segments, such as enhancers or other regulatory genetic elements that modulate the activity of $Na_V1.7$, acting either in cis or trans fashion, are thereby identified and may prove useful in treating chronic and other types of pain. Related genes might include those with high sequence homology, perhaps at least 98%, to the sequences disclosed herein and would, preferably, have sequences identical to the sequences disclosed herein.

In another aspect, the present invention relates to a method for identifying an agent that modulates the activity of a polypeptide encoded by a polynucleotide as disclosed herein, comprising:

a) contacting under physiological conditions a test compound or other chemical agent with a polypeptide encoded by a polynucleotide corresponding

to a $Na_V1.7$ gene, preferably having the sequence of SEQ. ID NO. 1, 3, 5, 15, 17, 19; and

b) detecting a change in the activity of said polypeptide as a result of said contacting;

wherein a change in activity indicates that said test compound is an agent that modulates said polypeptide activity.

In an embodiment, the observed change in activity in step (b) is a decrease in activity, most preferably wherein said change in activity is the result of binding to or interacting with said polypeptide by said chemical agent of step (b), especially where the polypeptide is an ion channel, and the ion channel is blocked by said binding. In a further embodiment, the polypeptide is a sodium channel, and said blocking is voltage dependent. A therapeutic agent may be an irreversible binding agent or it may be a reversible binding agent.

In additional embodiments, the polypeptide is part of a lipid bilayer, such as an intact cell, preferably a mammalian cell, such as a neuronal cell, most preferably a recombinant cell. In one such embodiment, a cell that has been engineered to comprise said polypeptide, including by genetic engineering, especially where the cell does not possess the polypeptide absent said engineering.

In another embodiment, the polypeptide is part of a cell, tissue, cell-line, immortalized cell or the like, which carries the gene and that expresses it, either naturally or upon induction. In this embodiment, the expressed gene is non-recombinant. As a non-limiting example, the PC-12 cell line derived from a transplantable rat pheochromocytoma responds reversibly to nerve growth factor (NGF) by induction of the neuronal phenotype and $Na_V1.7$ expression. See Toledo-Aral JJ, et al. Proc Natl Acad Sci U S A. 1997 Feb 18;94(4):1527-32.

In a preferred embodiment of such method, the $Na_V1.7$ polypeptide is a polypeptide comprises the amino acid sequence of SEQ ID NO: 2, 4, 6, 16, 18, or 20.

Thus, the present invention specifically contemplates embodiments in which the cell is engineered by other than genetic engineering, such as where the activity of a polypeptide is to be enhanced and the cell has been engineered to contain, or have on its surface, said polypeptide but wherein the polypeptide is present due to physical insertion of the polypeptide into the membrane or cytoplasm of the cell and not through expression of a gene contained in the cell. Such engineering includes direct insertion of the polypeptide into the lipid bilayer, such as where a lipid bilayer comprising the polypeptide is fused to a membrane, such as that of an intact cell, resulting in the cell membrane comprising the polypeptide. Methods well known in the art, such as use of polyethylene glycol, viruses, and the like, are available to effect such insertions and the details of such procedures need not be further described herein.

In one embodiment of such method, the polypeptide is a polypeptide that reacts with an antibody that reacts with, or is specific for, a polypeptide having an amino acid sequence having least 95% identity to, more preferably at least 98% identity to, the sequence of SEQ ID NO: 2 and where any difference in amino acid sequence is due only to conservative amino acid substitutions. In an embodiment, the polypeptide has the amino acid sequence of SEQ ID NO: 2.

In a further aspect, the present invention relates to a method for identifying an analgesic agent, comprising:

a) administering to an animal an agent found to have activity using an assay or screening method as disclosed herein, and

b) detecting in said animal a decrease in response to a pain stimulus following said administering and due thereto,

wherein said decrease indicates that said test compound is an an analgesic agent.

Preferably, the animal is a mammal, such as a human being. In specific embodiments, the pain stimulus is a heat stimulus and reaction or sensitivity to hot and/or cold may be measured. In another embodiment, an electrical stimulus may be used. In all cases, the stimulus may be represented as a sharp or dull sensation. In some cases, the animal may otherwise react normally to such

stimulus so that a decrease in normal response due to the test agent is being measured whereas in other cases the animal may initially possess a heightened sensitivity to the stimulus prior to administering the test agent. In all cases, observation of an analgesic effect need not necessarily involve a reduced sensitivity or response to pain but may involve simply a reduced sensation of a particular stimulus. The analgesics identified by the methods of the invention may induce general analgesia in an animal or may have more localized analgesic or anesthetic effects.

Further embodiments of the assays useful for identifying modulators of $Na_V1.7$ are set out further below in this specification.

Selectivity for $Na_V1.7$ over other sodium channels

The present invention specifically contemplates the identification of chemical agents, especially small organic molecules, that inhibit the expression of a $Na_V1.7$ gene or the activity of a $Na_V1.7$ polypeptide, with high potency and selectivity and that therefore have limited effect on other sodium channel genes and/or expression products. The inventors recognize on the basis of their discovery in Congenital Indifference to Pain, that selective inhibition of $Na_V1.7$ as opposed to any of the other sodium channels leads to the temporary induction in subjects of the therapeutically desirable outcomes observed in C-I-P. Because the other sodium channels are implicated in other essential physiological processes, such as heart activity ($Na_V1.5$), muscle contraction ($Na_V1.4$), various CNS neurological processes ($Na_V1.1$, 1.2 and 1.3) and PNS activity ($Na_V1.8$ and 1.9), it is desirable to avoid modulation of these other sodium channels.

In an embodiment, the invention relates to a method, and a screening assay relating thereto, for identifying a selective inhibitor of $Na_V1.7$ comprising,

a) detecting the concentration of a test compound or agent required to inhibit human $Na_V1.7$ polypeptide activity; and

b) detecting the concentration of a test compound or agent required to inhibit the activity of a human sodium channel polypeptide other than $Na_V1.7$,

wherein if the ratio of the detected concentration of step b) over the detected concentration of step a) is greater than the same ratio observed for lamotrigine and carbamazepine, then the test agent is said selective inhibitor.

In this embodiment, selectivity is determined based on concentration dependent inhibition of polypeptide activity. The measure of 'greater than the same ratio observed for lamotrigine and carbamazepine' has been employed in this embodiment to indicate that the compound has a selectivity for $Na_V1.7$ that is greater than the selectivity for $Na_V1.7$ demonstrated by certain known compounds.

A feature of this assay method, is that it may also identify blockers of $Na_V1.7$ having a high potency in the first screening assay. "High potency" is defined elsewhere herein.

A variety of selectivity measures can be employed, as are known to those in the art, and as detailed elsewhere in this specification. A preferred selectivity measure is 'Inhibitory Concentration-50%' (IC-50) in a 2 hour guanidine flux assay, explained elsewhere herein. This assay may be reproducibly performed using the methods disclosed herein. An alternative biological activity of the $Na_V1.7$ channel could be used as the basis for determining selectivity, as long as the polypeptide activity can be measured.

Selectivity is a desirable aspect of the instant invention because the inventors recognize that among the many advantages of this invention, is the striking clarity it provides around the $Na_V1.7$ target. The invention establishes that $Na_V1.7$ is an important selective target for pain and related disorders. Current dogma holds the tetrodotoxin insensitive channels $Na_V1.8$ and $Na_V1.9$ are the preferred pain targets (see Wood JN, Boorman JP, Okuse K, Baker MD. Voltage-gated sodium channels and pain pathways.J Neurobiol. 2004

Oct;61(1):55-71. Review. Lai J, Porreca F, Hunter JC, Gold MS. Voltage-gated sodium channels and hyperalgesia. Annu Rev Pharmacol Toxicol. 2004;44:371-97. Review. Lai J, Gold MS, Kim CS, Bian D, Ossipov MH, Hunter JC, Porreca F. Inhibition of neuropathic pain by decreased expression of the tetrodotoxin-resistant sodium channel, $Na_V1.8$. Pain. 2002 Jan;95(1-2):143-52).

To date, human $Na_V1.7$ has not be seen as a realistic target for the treatment of pain, as it was originally described as being a sodium channel solely expressed in neuroendocrine cells associated with the autonomic system (Klugbauer N, et al Structure and functional expression of a new member of the tetrodotoxin-sensitive voltage-activated sodium channel family from human neuroendocrine cells. EMBO J. (1995) Mar 15;14(6):1084-90) and as such has been implicated in autonomic processes, thus making it an unsuitable therapeutic target for inhibition. Recently it was shown that in mice, deleting Nav1.7 in all sensory and sympathetic neurons causes a lethal perinatal phenotype. (Nassar et al. (2004). Proc Natl Acad Sci U S A. 101(34):12706-11.) Those skilled in the art recognize that this mouse $Na_V1.7$ knock-out data suggests $Na_V1.7$ is not a suitable target for inhibition by drugs, and in fact, should likely be avoided. The instant disclosure of the role of $Na_V1.7$ in C-I-P provides a sharp contrast with this previous line of thinking.

In addition, recent clinical reports indicate that mutations in human $Na_V1.7$ are associated with severe pathological conditions, thus further undermining its utility as a therapeutic target. Primary Erythermalgia, a condition resulting in burning sensations of the extremities, has been attributed to missense mutations T2573A and T2543C in $Na_V1.7$ (Yang Y, Wang Y, Li S, Xu Z, Li H, Ma L, Fan J, Bu D, Liu B, Fan Z, Wu G, Jin J, Ding B, Zhu X, Shen Y. Mutations in SCN9A, encoding a sodium channel alpha subunit, in patients with primary erythermalgia. J Med Genet. 2004 Mar;41(3):171-4). These mutations in $Na_V1.7$ produce a hyperpolarizing shift in activation and slow deactivation and cause an increase in amplitude of the

current produced by $Na_V1.7$ in response to slow, small depolarizations. Thus, the $Na_V1.7$ channel in affected individuals is hyper sensitive and hyper-active. [Cummins TR, Dib-Hajj SD, Waxman SG. Electrophysiological properties of mutant Nav1.7 sodium channels in a painful inherited neuropathy. J Neurosci. 2004 Sep 22;24(38):8232-6.]. Another inherited form of chronic pain, familial rectal pain is a rare disorder which was shown to be caused by missense mutations M1627K, T1464I and I1461T located in the inactivation gate area of $Na_V1.7$. . [Abstract: Saturday Oct 30, 2004 Platform session American Society of Human Genetics (ASHG) conference, Toronto. *Identification of the* gene underlying an inherited disorder of pain sensation CR Fertleman, M Rees, KA Parker, E Barlow, RM Gardiner Pediatrics and Child Health, RFUCMS, London, UK Funded by Wellcome Trust]. Another mutation, R996C located in the "Large intracellular loop," where the function is unclear, has also been associated with familial rectal pain. [Abstract: Saturday Oct 30, 2004 Platform session American Society of Human Genetics (ASHG) conference, Toronto. Identification of the gene underlying an inherited disorder of pain sensation CR Fertleman, M Rees, KA Parker, E Barlow, RM Gardiner Pediatrics and Child Health, RFUCMS, London, UK Funded by Wellcome Trust].

The instant invention, however, demonstrates that a comprehensive inhibition of $Na_V1.7$ (e.g. via a deletion mutation) will provide comprehensive analgesia without the apparently serious side effects of blocking autonomic processes, primary erythermalgia or familial rectal pain. Figure 11 provides a visual comparison of the mutations of $Na_V1.7$ that lead to C-I-P versus those that lead to painful conditions. Clearly, the deletion mutations of the instant invention, which are comparable to a comprehensive block of the $Na_V1.7$ channel, provide desirable patient outcomes, whereas the point substitutions known in the art lead only to undesirable and painful conditions.

This invention thus sets forth, for the first time, a scientifically justified rationale for the pursuit and discovery of sodium channel blockers that are

highly selective for $Na_V1.7$ for use as analgesics and to induce other symptomatic responses observed in C-I-P. The invention herein provides a solution to the problems posed in Anger et al. (2001. J. Med. Chem. 44(2):115-137) about the lack of information concerning which sodium channel sub-type to pursue and about the significant uncertainty in the art regarding the extent of selectivity required.

Also, by identifying $Na_V1.7$ as the therapeutic target of choice in humans, the invention overcomes prior art teachings which suggested, but did not prove, that $Na_V1.7$ may be involved in essential autonomic system bodily functions. $Na_V1.7$ was believed to be the primary sodium channel of unmyelinated sensory and autonomic nerve cells. This prior art work led the field to believe that $Na_V1.7$ was to be avoided as a target for therapeutic modulation because such modulation would negatively effect these essential bodily functions. However, the instant invention teaches, for the first time, that in humans, modulation, especially inhibition, of $Na_V1.7$ can provide a highly desirable result, namely the treatment of pain, evidently without other physiological or autonomic impacts.

Characteristics of Human Sodium Channels

The sodium channel family of proteins are involved in a number of vital body functions. $Na_V1.1$ and $Na_V1.2$ are highly expressed in the brain (Raymond CK, et al., . Expression of alternatively spliced sodium channel alpha-subunit genes. Unique splicing patterns are observed in dorsal root ganglia. J Biol Chem. 2004 Oct 29;279(44):46234-41. E-pub 2004 Aug 09.) and are vital to normal brain function. In humans it has been shown that mutations in $Na_V1.1$ and $Na_V1.2$ result in severe epileptic states and in some cases mental decline (Rhodes TH, Lossin C, Vanoye CG, Wang DW, George AL Jr. Noninactivating voltage-gated sodium channels in severe myoclonic epilepsy of infancy.Proc Natl Acad Sci U S A. 2004 Jul 27;101(30):11147-52; Kamiya K, et al., A nonsense mutation of the sodium channel gene SCN2A in a patient with intractable epilepsy and mental decline. J Neurosci. 2004 Mar 17;24(11):2690-8; Pereira S, et al., Severe epilepsy, retardation, and

dysmorphic features with a 2q deletion including SCN1A and SCN2A. Neurology. 2004 Jul 13;63(1):191-2).

Nav1.3, which has been shown to be broadly expressed throughout the body (Raymond CK, et al., Expression of alternatively spliced sodium channel alpha-subunit genes. Unique splicing patterns are observed in dorsal root ganglia. J Biol Chem. 2004 Oct 29;279(44):46234-41. E-pub 2004 Aug 09.) has been implicated in peripheral neuropathic pain. For example, it has been demonstrated in rats that Nav1.3 is unregulated in second-order dorsal horn sensory neurons after nervous system injury. The authors concluded that this showed that spinal cord injury could trigger changes in sodium channel expression, and suggested a functional link between Nav1.3 expression and neuronal hyperexcitability associated with central neuropathic pain (Hains BC, et al., Upregulation of sodium channel Nav1.3 and functional involvement in neuronal hyperexcitability associated with central neuropathic pain after spinal cord injury. J Neurosci. 2003 Oct 1;23(26):8881-92). Many other experts in the field have also considered $Na_V1.3$ a likely target over $Na_V1.7$ for pain therapeutics (Lai J, Hunter JC, Porreca F.The role of voltage-gated sodium channels in neuropathic pain. Curr Opin Neurobiol. 2003 Jun;13(3):291-7; Wood JN, Boorman JP, Okuse K, Baker MD. Voltage-gated sodium channels and pain pathways. J Neurobiol. 2004 Oct;61(1):55-71; Chung JM, Chung K. Sodium channels and neuropathic pain. Novartis Found Symp. 2004;261:19-27; discussion 27-31, 47-54).

$Na_V1.4$ expression is essentially limited to muscle (Raymond CK, et al., Expression of alternatively spliced sodium channel alpha-subunit genes. Unique splicing patterns are observed in dorsal root ganglia. J Biol Chem. 2004 Oct 29;279(44):46234-41. Epub 2004 Aug 09.). Mutations in this gene have been shown to have profound effects on muscle function. For example, gain-of-function mutations in $Na_V1.4$ cause delayed channel inactivation and a persistent sodium current, resulting in hyper-(and hypo-)kalemic periodic paralysis, paramyotonia (Tamaoka A. Paramyotonia congenita and skeletal sodium channelopathy. Intern Med. 2003 Sep;42(9):769-70). In individuals with a myasthenic syndrome associated with fatigable generalized weakness

and recurrent attacks of respiratory and bulbar paralysis since birth, two heteroallelic mutations involving conserved residues not present in 400 normal alleles: S246L in the S4/S5 cytoplasmic linker in domain I, and V1442E in the S3/S4 extracellular linker in domain IV of $Na_V1.4$ were detected. (Tsujino A, et al., Myasthenic syndrome caused by mutation of the SCN4A sodium channel. Proc Natl Acad Sci U S A. 2003 Jun 10;100(12):7377-82).

The cardiac sodium channel, $Na_V1.5$, is expressed mainly in the heart ventricles and atria (Raymond CK, et al., Expression of alternatively spliced sodium channel alpha-subunit genes. Unique splicing patterns are observed in dorsal root ganglia. J Biol Chem. 2004 Oct 29;279(44):46234-41. E-pub 2004 Aug 09.), though they may also be found in the sinovial node, ventricular node and possibly Purkinje cells. The rapid upstroke of the cardiac action potential and the rapid impulse conduction through cardiac tissue is due to the opening of $Na_V1.5$. As such, $Na_V1.5$ is central to the genesis of cardiac arrhythmias. Mutations in human $Na_V1.5$ result in multiple arrhythmic syndromes, including long QT3 (LQT3), Brugada syndrome (BS), an inherited cardiac conduction defect, sudden unexpected nocturnal death syndrome (SUNDS) and sudden infant death syndrome (SIDS; Liu H, Clancy C, Cormier J, Kass R. Mutations in cardiac sodium channels: clinical implications. Am J Pharmacogenomics. 2003;3(3):173-9. Review).

$Na_V1.6$ encodes an abundant, widely distributed voltage-gated sodium channel found throughout the central and peripheral nervous systems, clustered in the nodes of Ranvier of neural axons (Caldwell JH, et al., Sodium channel Na(v)1.6 is localized at nodes of ranvier, dendrites, and synapses. Proc Natl Acad Sci U S A. 2000 May 9;97(10):5616-20). Although no mutations in humans have been detected, $Na_V1.6$ is thought to play a role in the manifestation of the symptoms associated with multiple sclerosis (Craner MJ, et al., Molecular changes in neurons in multiple sclerosis: altered axonal expression of Nav1.2 and Nav1.6 sodium channels and Na+/Ca2+ exchanger. Proc Natl Acad Sci U S A. 2004 May 25;101(21):8168-73). Null mutations of $Na_V1.6$ in mice produce motor neuron failure, loss of

neuromuscular transmission, and lethal paralysis. Less severe mutations result in ataxia, tremor, muscle weakness, and dystonia. (Meisler MH, et al., Allelic mutations of the sodium channel SCN8A reveal multiple cellular and physiological functions. Genetica. 2004 Sep;122(1):37-45; Kearney JA, et al., Molecular and pathological effects of a modifier gene on deficiency of the sodium channel Scn8a (Na(v)1.6). Hum Mol Genet. 2002 Oct 15;11(22):2765-75).

The expression of $Na_V1.8$ is essentially restricted to the DRG (Raymond CK, et al., Expression of alternatively spliced sodium channel alpha-subunit genes: Unique splicing patterns are observed in dorsal root ganglia. J Biol Chem. 2004 Oct 29;279(44):46234-41. Epub 2004 Aug 09.). There are no identified human mutations for $Na_V1.8$. However, $Na_V1.8$-null mutant mice were viable, fertile and normal in appearance. A pronounced analgesia to noxious mechanical stimuli, small deficits in noxious thermoreception and delayed development of inflammatory hyperalgesia suggested to the researchers that $Na_V1.8$ is involved in pain pathways. (Akopian AN, et al., The tetrodotoxin-resistant sodium channel SNS has a specialized function in pain pathways. Nat Neurosci. 1999 Jun;2(6):541-8). The relevance to humans of this mouse evidence has not been established.

Finally, $Na_V1.9$, a novel tetrodotoxin insensitive, peripheral sodium channel was disclosed by Dib-Hajj SD, Tyrrell L, Black JA, Waxman SG. NaN, a novel voltage-gated Na channel, is expressed preferentially in peripheral sensory neurons and down-regulated after axotomy. Proc Natl Acad Sci U S A. 1998 Jul 21;95(15):8963-8. The function of this channel is largely unknown. However, it has been demonstrated that this channel underlies neurotrophin (BDNF)-evoked depolarization and excitation, and is the only member of the voltage gated sodium channel superfamily to be shown to be ligand mediated (Blum R, Kafitz KW, Konnerth A. Neurotrophin-evoked depolarization requires the sodium channel Na(V)1.9. Nature. 2002 Oct 17;419(6908):687-93.

The benefits of the present invention and the value of selectivity for $Na_V1.7$ over other sodium channels for the treatment of pain is further illustrated in Table 4 below. Table 4 sets out the known phenotypic or disease consequences of mutations in various known sodium channels in rodents and humans. As can be seen, modulation of the activity of almost all the other sodium channels results in severe and undesirable physiological consequences. For example, inhibitory mutations in human SCN1A result in a type of epilepsy. Gain of function mutations in rodent SCN2A result in behavioral disorders. Loss of function mutations in rodent SCN2A result in severe neurological disorders. SCN2A mutations in humans also result in a type of epilepsy. The list leads to the conclusion that a compound which selectively inhibits $Na_V1.7$ will be a useful treatment for pain, and will not cause the undesirable consequences of modulation of the other sodium channels.

Table 4.

Name/Alternative Gene/Protein Name	Rodent Phenotype	Human Disease or Phenotype	Reference
Na(v)1.1 SCN1A	Unknown	Generalized epilepsy with febrile seizures plus type II (OMIM 604233); severe myoclonic epilepsy of infancy (OMIM 607208)	1, 2, 3,
Na(v)1.2 SCN2A2, SCN2A	Transgenic Mouse - seizures, focal motor abnormalities, behavioral arrest and stereotyped repetitive behaviors; Knock-Out Mouse - perinatal lethal with severe hypoxia and massive neuronal apoptosis in the brainstem	Generalized epilepsy with febrile seizures plus (OMIM 604233)	4, 25
Na(v)1.3 SCN3A	Unknown	Unknown	

Na(v)1.4 SCN4A	Unknown	Hyper- (and hypo-) kalemic periodic paralysis (OMIM 170500, 170400 paramyotonia congenita (OMIM 168300, 168350)	10, 11
Na(v)1.5 SCN5A	Knock-In Mice - arrhythmias; Knock-Out mice (-/-) – intrauterine lethality with defective ventricular morphogenesis; Knock-Out mice (+/-) – arrhythmias	Long QT syndrome-3 (OMIM 603830), Brugada syndrome (601144); heart block (OMIM 113900; paroxysmal familial ventricular fibrillation (OMIM 603829)	12, 13, 14, 15, 16, 17, 26
Na(v)1.6 SCN8A	Various spontaneous mutations result in: motor endplate disease - progressive paralysis and juvenile lethality in C57BL/6J mice; Viable adults with dystonia in C3H mice; Jolting mice - cerebellar ataxia; DMU mice - skeletal and cardiac muscle degeneration; heterozygotes unaffected	Unknown	5, 6, 7, 8, 9
Na(v)1.8 SCN10A	Knock-out mice mechanical analgesia, reduced thermal hyperalgesia, and delayed inflammatory hyperalgesia , but normal neuropathic pain perception (heterozygotes like wild-type); Knock-down mice - reduced inflammatory hyperalgesia and neuropathic pain (note discrepancy with above)	Unknown	18, 19, 20, 21,22
Na(v)1.9 SCN12A, SCN11A	Knock-down in rat: No effect on nerve-injury induced behavior responses	Unknown	23
Na(x) SCN6A, SCN7A	Knock-out: Altered salt intake	Unknown	24
Na(v)1.7 SCN9A	KO is lethal after birth. Conditional KO is viable, and demonstrates increased latency in the Randell-Sellitto and CFA VonFrey filament tests when expression is	Congenital indifference to pain (OMIM 243000)	This work

	eliminated in $Na_V1.8$ expressing cells.		

Thus, in an embodiment of the invention, the compound identified which modulates $Na_V1.7$ gene expression or $Na_V1.7$ polypeptide activity is selective for $Na_V1.7$ as opposed to other sodium channel alpha subunits (i.e. SCN1A to SCN8A, SCN10A to SCN12A). Such selectivity of modulation is preferably at least 10%, 50%, 100%, 10 times, 20 times, 100 times, 1000 times, 10,000 times or higher for $Na_V1.7$ over any other sodium channel alpha subunit. Alternatively, a compound which is selective for $Na_V1.7$ may not demonstrate an absolute preference for $Na_V1.7$, but the compound may show a preference for modulating $Na_V1.7$ compared to any other sodium channel which preference is greater than any other prior art sodium channel blocker.

In another embodiment, the methods disclosed herein for identifying an agent that modulates, preferably inhibits, expression or activity of a gene or polypeptide corresponding to $Na_V1.7$ comprise first identifying such agent and then testing such agent for effects on expression or activity of at least one other sodium channel gene or polypeptide, as the case may be, preferably at least two other such genes, or polypeptides, with little or no effect.

In another embodiment, a compound identified as having $Na_V1.7$ inhibiting activity by an assay of the invention is further tested to identify whether it also blocks activity of other sodium channels, other ion channels and/or other proteins. Such testing may be performed by a wide variety of methods, including systematic *in vitro* evaluations.

According to the invention, preferred compounds are those that inhibit $Na_V1.7$ at a lower concentration than any other ion channel protein. In particular, for a compound of the invention, the IC-50 of $Na_V1.7$ is lower than the IC-50 of the next closest ion channel by a multiple of at least 1.1, 1.2, 1.5, 1.7, 2, 3, 4, 5, 10, 20, 25, 50, 75, 100, 200, 500, 1000, 2000, 5000, 10000 or more.

Thus, in a preferred embodiment, the ratio of IC-50 of said next closest ion channel to the IC-50 of $Na_V1.7$ is at least 1.1, 1.2, 1.5, 1.7, 2, 3, 4, 5, 10, 20, 25, 50, 75, 100, 200, 500, 1000, 2000, 5000, 10000 or more.

In an alternative embodiment, a compound of the invention has a ratio of IC-50 of a sodium channel selected from among $Na_V1.1$, $Na_V1.2$, Nav1.3, $Na_V1.4$, $Na_V1.5$, $Na_V1.6$, $Na_V1.8$, and $Na_V1.9$ to the IC-50 for Na $_V1.7$ that is at least 1.1, 1.2, 1.5, 1.7, 2, 3, 4, 5, 10, 20, 25, 50, 75, 100, 200, 500, 1000, 2000, 5000, or 10000.

Stated in the alternative, the measure of $IC\text{-}50_{(other\ sodium\ channel)}$ /$IC\text{-}50_{NaV1.7}$ is at least 1.1 The results demonstrate that a useful therapeutic agent of the invention is selective for $Na_V1.7$ over other sodium channels by a multiple of at least 1.1, that is, the measure of $IC\text{-}50_{(other\ sodium\ channel)}$ /$IC\text{-}50_{NaV1.7}$ in a 2 hour guanidine flux assay is at least 1.1. More specifically, the therapeutic agent has $IC\text{-}50_{NaV1.7}$ of less than 6 μM and $IC\text{-}50_{(other\ sodium\ channel)}$ of greater than 6.6 μM.

In an alternative embodiment, the compound's IC-50 of $Na_V1.7 < Na_V1.5 \leq Na_V1.8$. In an alternative embodiment, the compound's IC-50 of $Na_V1.7 < Na_V1.8 \leq Na_V1.5$. In an alternative embodiment, the compound's IC-50 of $Na_V1.7$ is less than its IC-50 for CNS sodium channels ($Na_V1.1$, 1.2 and 1.3). In an alternative embodiment the compound's IC-50 of $Na_V1.7$ is less than its IC-50 for peripheral sodium channels ($Na_V1.4$, $Na_V1.5$, $Na_V1.6$, $Na_V1.8$, $Na_V1.9$).

Those skilled in the art may also employ other kinds of measurements to identify agents which selectively reduce ion flux activity of Na_V 1.7 as compared to flux activity of another ion channel, especially another sodium ion channel. *In vitro* assays, for example, may use measurements of concentrations which inhibit ion flux from 10 – 100% (e.g. IC-10 to IC-100), including 10, 20, 25, 40, 50, 60, 75, 90, 99 and 100%. Additionally, blocking

of ion channels may be measured under adjusted conditions, such as under increased or decreased membrane potentials (voltage), as to evaluate voltage dependence of inhibition (a measure of the strength of membrane depolarization necessary to open the channels), as to evaluate frequency dependence of inhibition, as to evaluate voltage dependence of steady state inactivation or the time course of inactivation, in the presence or absence of known inhibitors (competitive or otherwise), and under many other conditions as known to those skilled in the art. The inventors recognize that any of these techniques may be employed in the assays of the invention to identify compounds of the invention.

In one example, those skilled in the art may prefer to measure EC_{50} (or 'EC50'), the effective concentration for a 50% change in polypeptide activity (e.g. a measure not limited to 'inhibition' activity alone). This may be useful when measuring electrical conductivity of a membrane which is mediated by a plurality of sodium channels. In this case, the EC50 measures the amount or concentration of a substance required to effect the measured change in electrical conductivity, or stated differently, the dose producing 50% maximum current inhibition (*See for example* Chevrier, P. et al. (2004) B. J. Pharmacol. 142:576-584).

In an alternative measure, an investigator may prefer to use a measure of *in vivo* selectivity, such as 'Effective Dose – 50%' (ED-50, ED50 or ED_{50}) which is the dose required to produce the desired effect in 50% of a population of animals tested.

A selective $Na_V1.7$ inhibitor compound of the invention may be distinguished from known sodium channel inhibitors (some of which are listed in Table 5 and Table 6 below) based on its relative ability to block other ion channels, including other sodium ion channels. Thus it is possible to compare the relative selectivity of test agents to known sodium channel blocking compounds of tables 5 and 6. Thus a preferred therapeutic agent of the

invention is relatively more selective for $Na_V1.7$ (compared to other sodium channels) than any known compound.

A compound of the invention normally demonstrates a ratio of IC-50 of a sodium channel selected from among $Na_V1.1$, $Na_V1.2$, Nav1.3, $Na_V1.4$, $Na_V1.5$, $Na_V1.6$, $Na_V1.8$, and $Na_V1.9$ to the IC-50 against $Na_V1.7$ of at least 1.1, 1.2, 1.5, 1.7, 2, 3, 4, 5, 10, 20, 25, 50, 75, 100, 200, 500, 1000, 2000, 5000, 10000 or more. Preferably the comparative sodium channel is $Na_V1.3$ and/or $Na_V1.8$, and the ratio is at least 2. For clarity, an agent's IC-50 'against' a specific channel is used herein interchangeably with the agent's IC-50 'of" that channel.

Thus, the agents contemplated by the present invention are highly selective for the $Na_V1.7$ gene or protein and administration of such an agent to a human or other animal in need thereof generates a temporary condition which mimics the Congenital Indifference to Pain phenotype to the highest degree possible, or aspects thereof. For example, it mimics the effects of one or more of the mutated forms of the gene as disclosed herein. In an embodiment, the agent generates indifference to pain, but does not diminish sensations which are available to patients with CIP, such as but not limited to heat/cold sensing, pressure sensing, sharp/dull sensations, and does not diminish physiological responses such as sweating, heart rate, muscle activity and the like. For example, indifference to pain without any concomitant numbness or loss of sensation would represent a useful manifestation of the results to be achieved.

It is one embodiment of this invention to use the screening assays of the invention to identify potential therapeutic agents, or analogs thereof, that are selective for the $Na_V1.7$ sodium channel ahead of other sodium channels, in humans and other animals. It will therefore be apparent to those skilled in the art that a series of assays for measuring differential interaction with other sodium channels would provide the tools necessary to identify selective

agents for $Na_V1.7$. In this embodiment, a first screening assay employing $Na_V1.7$ is used to identify compounds which inhibit $Na_V1.7$ activity. A suite of secondary assays are then employed, each containing one or more different sodium channels selected from among $Na_V1.1$ (SCN1A); $Na_V1.2$ (SCN2A), $Na_V1.3$ (SCN3A), $Na_V1.4$ (SCN4A), $Na_V1.5$ (SCN5A), $Na_V1.6$ (SCN8A), $Na_V1.8$ (SCN10A), $Na_V1.9$ (SCN11A), and Na_x (SCN6A, SCN7A) (for consensus nomenclature see Goldin, AL. 2001. Ann. Rev. Physiol. 63:871-894). Compounds which were found to inhibit $Na_V1.7$ expression or activity, are systematically tested against the assays for the other sodium channels. Those compounds which are more selective for $Na_V1.7$ over other channels than current sodium channel blocking compounds used in the art are preferred compounds.

The invention recognizes further that not all sodium channels need to be distinguished equally. In fact, some useful compounds of the invention demonstrate some inhibitory activity against channels other than $Na_V1.7$ as long as other channels are substantially not inhibited at reasonable physiological concentrations. For example, compounds of the invention include agents where the IC-50 of $Na_V1.7 < Na_V1.5 \leq Na_V1.8$. In an alternative embodiment, the compound's IC-50 of $Na_V1.7 < Na_V1.8 \leq Na_V1.5$. In an alternative embodiment the compound's IC-50 of $Na_V1.7 < Na_V1.3 \leq Na_V1.5$. In an alternative embodiment, the compound's IC-50 of $Na_V1.7 < Na_V1.5 \leq Na_V1.3$.

The instant invention thus recognizes a hierarchy of selectivity, in which the agent's IC-50 of $Na_V1.7$ is lower than all other ion channels, especially sodium ion channels. All such agents are compounds of the invention.

For one example, the channels to be avoided in developing a therapeutic compound antagonising or blocking Nav1.7, may be ranked in order of potential liability as follows

$Na_V1.5>Na_V1.1 \geq Na_V1.2> Na_V1.4>Na_V1.6> Na_V1.3>Na_V1.8 \geq Na_V1.9$.

The other sodium channels can be incorporated into screening assays according to methods disclosed herein, or as disclosed elsewhere in the art or as later discovered or invented. As such, the $Na_V1.1$, $Na_V1.2$ and $Na_V1.3$ screening assays set out in WO 01/38564 (Rouleau et al, published 31 May 2001) may be used to effectively distinguish compounds which are broadly selective for sodium channels from those that are specific and selective exclusively for $Na_V1.7$.

Some of the standard screening assays for a protein like $Na_V1.7$ are set out in US 6,110,672 (a US counterpart of WO 96/14077), incorporated herein by reference in its entirety. These assays provide for basic analysis of agents which interact with a protein like $Na_V1.7$ (although the reference provides no guidance on achieving selectivity among sodium channels). The genetic and phenotypic information provided in the instant patent application now provides therapeutic agents which effectively mimic the Congenital Indifference to Pain phenotype, by selecting those compounds which are selective for $Na_V1.7$ over other sodium channel family members. The invention provides a compound which, while not necessarily being selective for the peripheral nervous system over the central nervous system (as prescribed in WO 96/14077), the compound is specific for $Na_V1.7$ ahead of all other sodium channels. Such a compound provides analgesic relief without side-effects such as nausea, tremor and irritability.

The emphasis noted herein on the selectivity for $Na_V1.7$ over other sodium channels, should also be understood to include selectivity over other ion channels and related proteins and genes. Preferred selective $Na_V1.7$ modulating agents have limited or no effect on the activity of ion channels such as potassium, calcium, ion co-transporters and the like, nor does it effect the hERG channel or other physiologically relevant channels. The emphasis on sodium channel selectivity is based on the high degree of similarity among

sodium channels among which selectivity is technically more challenging to achieve than with less closely related proteins/genes.

On another note, this invention also establishes that improved therapeutic agents for the treatment of pain must take advantage of the dynamic state of a sodium channel. Therapeutic agents can now be identified with entirely novel modes of action against the channel. A sodium channel cycles from the open (activated) state to the closed (inactivated) state, then to the resting (closed but capable of being activated) state, where it waits for an electrical signal to convert to the open state and allow passage of sodium ions. The transition from open to closed may be by fast inactivation or slow inactivation. Previous knowledge of the $Na_V1.7$ channel did not allow for the identification of which state or transition state of the channel was desireable for therapeutic intervention.

The instant invention now establishes that the Y328X, R830X, W1689X and the other mutations identified in Table 2, cause the desired channel behavior to generate the desirable indifference to pain in humans. An irreversible binding agent may be as suitable for use as a reversible binding agent. This invention therefore provides mutant proteins which may be used in the screening assays to assist in the identification of therapeutic agents or their analogs which mimic the truncation mutations of the invention. It also teaches a functional description about what the binding agent/blocking agent must do to achieve analgesia.

Further assays

Stated broadly, the screening assays of the invention simplify the identification, evaluation and development of classes of compounds which are suitable for use as analgesic agents and for the reduction of adverse pain responses to disease, such as chronic disease, for example cancer. In general, these screening methods provide a ready means for selecting either natural product extracts or synthetic compounds of interest from a large

population (i.e. a chemical library, for example, one produced by combinatorial means). As stated previously, an assay is first designed which measures $Na_V1.7$ expression or activity. Exemplary methods useful for the identification of such compounds are detailed herein, although those skilled in the art will be aware of alternative means. In a first step, compounds are sequentially tested against the assay to determine whether they influence a measurable biological activity of the assay.

Functional assays may be based one or more of the diverse measurable biological activities of a gene or polypeptide corresponding to $Na_V1.7$. "$Na_V1.7$ activity" or "$Na_V1.7$ polypeptide activity" or "$Na_V1.7$ biological activity" as used herein, especially relating to screening assays, is to be interpreted broadly and contemplates all directly or indirectly measurable and identifiable biological activities of the $Na_V1.7$ gene and protein. Relating to the purified $Na_V1.7$ protein, $Na_V1.7$ activity includes, but is not limited to, all those biological processes, interactions, binding behavior, binding-activity relationships, pKa, pD, enzyme kinetics, stability, and functional assessments of the protein. Relating to $Na_V1.7$ activity in cell fractions, reconstituted cell fractions or whole cells, these activities include, but are not limited to the rate or amount of $Na_V1.7$ mediated ion flux across a membrane, which ion may be sodium, guanidine, lithium or other ion, or the dynamics of this flux (such as voltage dependence, rate of transition between states) and all measurable consequences of these effects, including cell growth, development or behavior and other direct or indirect effects of $Na_V1.7$ activity. A useful $Na_V1.7$ activity used in some embodiments of this invention is guanidine ion flux activity, as detailed in the examples. Relating to $Na_V1.7$ genes and transcription, $Na_V1.7$ activity includes the rate, scale or scope of transcription of genomic DNA to generate RNA; the effect of regulatory proteins on such transcription, the effect of modulators of such regulatory proteins on such transcription; plus the stability and behavior of mRNA transcripts, post-transcription processing, mRNA amounts and turnover, and all measurements of translation of the mRNA into polypeptide sequences. Relating to $Na_V1.7$ activity in organisms, this includes but is not limited biological activities which

are identified by their absence or deficiency in disease processes or disorders caused by aberrant $Na_V1.7$ biological activity in those organisms. Broadly speaking, $Na_V1.7$ biological activity can be determined by all these and other means for analyzing biological properties of proteins and genes that are known in the art.

The invention therefore provides numerous assays which measure an activity of $Na_V1.7$ and are useful for the testing of chemical compounds to identify which ones effect such activity. The invention also invites those skilled in the art to develop further $Na_V1.7$ activity assays which go beyond those disclosed herein, for use in the screening compound libraries.

A typical assay uses patch-clamp techniques to study the behavior of channels. Such techniques are known to those skilled in the art, and may be developed, using current technologies, into low or medium throughput assays for evaluating compounds for their ability to modulate sodium channel behavior.

A competitive binding assay with known sodium channel toxins such as tetrodotoxin, alpha-scorpion toxins, BTX and the like, may be suitable for identifying potential therapeutic agents with high selectivity for $Na_V1.7$.

In still another assay, uptake of radioactive isotopes into or out of a vesicle can be measured. The vesicles are separated from the extra-vesicular medium and the radioactivity in the vesicles and in the medium is quantitated and compared.

These techniques can be carried out in a cell expressing the channel of interest in a natural endogenous setting or in a recombinant setting. The assays that can be used include plate assays which measure Na influx through surrogate markers such as ^{14}C-guanidine influx or determine cell depolarization using fluorescent dyes such as the FRET based and other fluorescent assays or a radiolabelled binding assay employing radiolabelled

aconotine, BTX, TTX or STX.. More direct measurements can be made with manual or automated electrophysiology systems. The guanidine influx assay is explained in more detail in the Examples.

As applied to measurement of ion flux activity of $Na_V1.7$, the invention relates to a method for identifying an agent that modulates the activity of $Na_V1.7$ polypeptide, comprising:

a) contacting a chemical agent with an $Na_V1.7$ polypeptide in a membrane under conditions supporting ion transport across said membrane by said polypeptide; and

b) detecting a change in ion transport activity of said polypeptide as a result of said contacting;

wherein said change in ion transport activity identifies said test compound as an agent that modulates $Na_V1.7$ polypeptide activity.

"Conditions promoting or supporting ion transport across said membrane" include those conditions disclosed in the guanidine influx assay detailed in the Examples below. Those skilled in the art are familiar with other conditions which will promote ion transport across a membrane containing $Na_V1.7$, including other assay types.

Other typical assays employ drug screening technology such as (but not limited to) radioactive, colorimetric or fluorescent based measurements. A classic colorimetric assay measures the ability of a dye to change color in response to changes in assay conditions resulting from the activity of the polypeptides. A useful instrument for the study of ion channels, including sodium channels is the ICR 8000 available from Aurora Biomed Inc. (Vancouver, BC).

Functional drug screening assays can also be based upon the ability of $Na_V1.7$ polypeptides to interact with other proteins. Such interacting proteins can be identified by a variety of methods known in the art, including, for example, radioimmunoprecipitation, co-immunoprecipitation, co-purification,

and yeast two-hybrid screening. Such interactions can be further assayed by means including but not limited to fluorescence polarization or scintillation proximity methods. Drug screens can also be based upon functions of the polypeptides deduced upon X-ray crystallography of the protein and comparison of their 3-D structure to that of proteins with known functions. Drug screens can be based upon a function or feature apparent upon creation of a transgenic or knockout mouse, or upon overexpression of the protein or protein fragment in mammalian cells *in vitro*. Moreover, expression of mammalian (e.g., human) polypeptides in yeast or *C. elegans* allows for screening of candidate compounds in wild-type and mutant backgrounds, as well as screens for mutations that enhance or suppress a low pain sensitivity phenotype. Modifier screens can also be performed in transgenic or knock-out mice.

Additionally, drug screening assays can also be based upon polypeptide functions deduced upon antisense interference with the gene function. Intracellular localization of $Na_V1.7$ polypeptides, or effects which occur upon a change in intracellular localization of such proteins, can also be used as an assay for drug screening.

Polypeptides encoded by the polynucleotides disclosed herein can be used as an antigen to raise antibodies, including monoclonal antibodies. Such antibodies will be useful for a wide variety of purposes, including but not limited to functional studies and the development of drug screening assays and diagnostics. Monitoring the influence of agents (e.g., small organic compounds) on the expression or biological activity of the pain-related polypeptides identified according to the invention can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase or decrease gene expression, protein levels, or biological activity can be monitored in clinical trails of subjects exhibiting symptoms of chronic or persistent pain due to inadequate gene expression, protein levels, or biological activity (for example, the individuals studied herein. Alternatively, the effectiveness of an agent determined by a screening assay to modulate

expression of $Na_V1.7$, as well as structurally and functionally related genes, including genes with high homology thereto, and including protein levels, or biological activity can be monitored in clinical trials of subjects exhibiting decreased altered gene expression, protein levels, or biological activity. In such clinical trials, the expression or activity of the genes or polypeptides disclosed herein and, preferably, other genes that have been implicated in, for example, Congenital Indifference to Pain stimuli, can be used to ascertain the effectiveness of a particular analgesic drug.

As a non-limiting example, genes that are modulated in cells by treatment with an agent (e.g., compound, drug or small molecule) that modulates the activity of the $Na_V1.7$ gene, or any expression products thereof, or polypeptides that modulate the activity of $Na_V1.7$ (e.g., identified in a screening assay as described herein) can be identified. Preferably, such cells are recombinant cells engineered to express a polynucleotide or polypeptide as disclosed herein. Thus, such recombinant cells are prepared and RNA isolated and analyzed for the levels of expression of $Na_V1.7$ after contacting said cells with agents that may have analgesic properties. The levels of gene expression can be quantified by Northern blot analysis or RT-PCR, or, alternatively, by measuring the amount of protein produced, by one of a number of methods known in the art, or by measuring the levels of biological activity of polypeptides encoded thereby or other genes. In this way, the gene expression can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

In another aspect, the invention provides a method for computationally identifying a compound having analgesic properties. The method involves (a) determining crystal structure and preferably the active site of a $Na_V1.7$ protein (i.e. through x-ray crystallography or other techniques); and (b) through computational modeling, identifying a compound which interacts with the active site, thereby identifying a compound, or its analog, as a compound which is useful for modulating the activity of such a polypeptide. Useful

screening assays may also be performed *in silico* using available computerized databases for the identification of such compounds.

In an embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) including the steps of (i) determining that a patient exhibits discomfort due to a disease or disorder that causes some type of painful stimulus; (ii) administering an effective amount of an agent identified using one of the screening assays disclosed herein; (iii) ascertaining a reduction to pain or other stimuli following said administration and (iv) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to decrease the expression or activity of gene or encoded polypeptide, i.e., to increase the effectiveness of the agent.

Where the patient is non-human, biopsy samples can be taken to show a decrease in gene expression, such as by measuring levels of protein, mRNA, or genomic DNA post-administration samples and comparing the level of expression or activity of said protein, mRNA, or genomic DNA in the pre-administration sample with that of the corresponding post administration sample or samples, thereby showing the effects of drug administration on one or more of the genes disclosed herein and concomitant reduction in pain response and/or sensitivity.

The gene disclosed herein as being involved in Congenital Indifference to Pain in an animal can be used, or a fragment thereof can be used, as a tool to express a protein, where such genes encode a protein, in an appropriate cell *in vitro*, or can be cloned into expression vectors which can be used to produce large enough amounts of protein to use in *in vitro* assays for drug screening. Expression systems which may be employed include baculovirus, herpes virus, adenovirus, adeno-associated virus, bacterial systems, and

eucaryotic systems such as CHO cells, HEK cells and other cells commonly available. Naked DNA and DNA-liposome complexes can also be used.

Candidate modulators may be purified (or substantially purified) molecules or may be one component of a mixture of compounds (*e.g.*, an extract or supernatant obtained from cells). In a mixed compound assay, $Na_V1.7$ activity is tested against progressively smaller subsets of the candidate compound pool (*e.g.*, produced by standard purification techniques, *e.g.*, HPLC or FPLC; Ausubel et al.) until a single compound or minimal compound mixture is demonstrated to modulate gene or protein activity or expression in a manner having analgesic effects.

Specific compounds which will modulate the gene expression or gene transcript levels in a cell of $Na_V1.7$ include antisense nucleic acids, ribozymes and other nucleic acid compositions which specifically hybridize with $Na_V1.7$ mRNA (including exons or introns of such genes, promoters, 3'tails, etc.). These specific compounds are compounds of the invention, and are useful for treating the diseases discussed previously. Design and manufacturing of such compounds are well known to those skilled in the art.

Specific compounds which will modulate the gene expression or gene transcript levels of $Na_V1.7$ in a cell include small-interfering RNAs (siRNA) molecules. $Na_V1.7$ siRNA are short double stranded RNA sequences with short overhanging ends designed to hybridize to mRNA or pre-mRNA sequences for $Na_V1.7$, leading to the destruction of both by cellular enzymes. siRNA strategies are well known in the art, and explained in more detail at Bantounas I, Phylactou LA, Uney JB. RNA interference and the use of small interfering RNA to study gene function in mammalian systems.J Mol Endocrinol. 2004 Dec;33(3):545-57; and Zhang J, Hua ZC.Targeted gene silencing by small interfering RNA-based knock-down technology.Curr Pharm Biotechnol. 2004 Feb;5(1):1-7. Review.. Suitable siRNA sequences for compounds of the invention include the following compounds:

Table 7 – siRNA compounds for inhibiting $Na_V1.7$ expression

Nav1.7 siRNA Oligonucleotides

name	nucleotide start (5' sense)	sequence (5'-3')	SEQ ID NO.
1.7 D3S	1560	gcauaggcgagcacaugaauu	112
1.7 D3AS		uucguauccgcucguguacuu	113
1.7 I3S	3452	gguucucaugcugccaaguuaacauuu	114
1.7 I3AS		uuccaagaguacgacgguucaauugua	115
1.7 I4S	3465	ccaaguuaacauagagucagggaaauu	116
1.7 I4AS		uugguucaauuguaucucagucccuuu	117
1.7 I8S	4578	gggucaaagucaacauaugacugaauu	118
1.7 I8AS		uucccaguuucaguuguauacugacuu	119

Specific compounds which modulate the activity of a $Na_V1.7$ polypeptide include antibodies (polyclonal or monoclonal) which specifically bind to an epitope of said polypeptide. These specific compounds are compounds of the invention, and are useful for inducing resistance or tolerance to pain stimuli. Design and manufacturing of such compounds are well known to those skilled in the art.

Specific compounds which modulate the activity of $Na_V1.7$ in the body include gene therapy vectors comprising all or a part of the $Na_V1.7$ gene sequence or mutant $Na_V1.7$ sequence. As is well known to those skilled in the art, gene therapy allows the delivery of the $Na_V1.7$ gene in an organism to cells where it is taken up and expressed, thus changing the level or amount of $Na_V1.7$ protein in such cell. These vectors thereby modulate the activity of $Na_V1.7$ in the body and are useful for the therapeutic indications disclosed herein.

In accordance with the foregoing, the present invention provides the amino acid sequence of a protein, designated $Na_V1.7$, that is a known ion channel structure found in neuronal cells (SEQ ID NO: 2) and which is

associated with hereditary transmission of indifference to pain. In addition, a mutation, here a truncation, has been found in this sequence derived from individuals found to have such indifference to pain. Thus, agents that mimic the phenotypic effects of this truncation, such as aberrant protein structure and decreased, or absent, function represent candidate compounds for evaluation as analgesic agents. In addition, agents that have the effect of reducing the half-life of such polypeptide in cells would also act to mimic indifference to pain and thereby achieve analgesia.

By way of non-limiting example, cells expressing a wild-type $Na_V1.7$ polypeptide are transiently metabolically labeled during translation, contacted with a candidate compound, and the half-life of the polypeptide is determined using standard techniques. Compounds that decrease the half-life of the polypeptide are useful compounds in the present invention.

In other embodiments, treatment with an antagonist of the invention may be combined with other analgesics to achieve a combined, possibly even synergistic, effect.

The ability of analgesic compounds to modulate polypeptides as disclosed herein, such as $Na_V1.7$, can be determined by any number of different binding assays, including use of a solid support, either as part of a column or as a batch procedure. Such support may be composed of plastic or glass, and includes standard resins and resin beads. Such assays are also available to test the ability of polypeptides, such as $Na_V1.7$, including mutated and/or truncated forms thereof, to bind to such test compounds or to other proteins present in cells, thereby identifying modulators of $Na_V1.7$ activity according to the invention.

In one such assay for which the polypeptides encoded by genes disclosed herein are useful, the polypeptide (or a polypeptide fragment thereof or an epitope-tagged form or fragment thereof) is bound to a suitable support (e.g., nitrocellulose or an antibody or a metal agarose column in the case of,

for example, a his-tagged form of said polypeptide). Binding to the support is preferably done under conditions that allow proteins associated with the polypeptide to remain associated with it. Such conditions may include use of buffers that minimize interference with protein-protein interactions. If desired, other proteins (e.g., a cell lysate) are added, and allowed time to associate with the polypeptide. The immobilized polypeptide is then washed to remove proteins or other cell constituents that may be non-specifically associated with it the polypeptide or the support. The immobilized polypeptide can then be used for multiple purposes. In a compound screening embodiment, compounds can be tested for their ability to interfere with interactions between $Na_V1.7$ and other bound molecules (which are presumably $Na_V1.7$ interacting proteins). Compounds which can successfully displace interacting proteins are thereby identified as $Na_V1.7$ modulating agents of the invention.

In an alternative embodiment designed to identify the $Na_V1.7$ interacting proteins, the immobilized polypeptide is dissociated from its support, and proteins bound to it are released (for example, by heating), or, alternatively, associated proteins are released from the polypeptide without releasing the latter polypeptide from the support. The released proteins and other cell constituents can be analyzed, for example, by SDS-PAGE gel electrophoresis, Western blotting and detection with specific antibodies, phospho-amino acid analysis, protease digestion, protein sequencing, or isoelectric focusing. Normal and mutant forms of such polypeptide can be employed in these assays to gain additional information about which part of the polypeptide a given factor is binding to. In addition, when incompletely purified polypeptide is employed, comparison of the normal and mutant forms of the protein can be used to help distinguish true binding proteins. Such an assay can be performed using a purified or semipurified protein or other molecule that is known to interact with a polypeptide encoded by a $Na_V1.7$ polynucleotide.

This assay may include the following steps.

1. Harvest the encoded polypeptide and couple a suitable fluorescent label to it;

2. Label an interacting protein (or other molecule) with a second, different fluorescent label. Use dyes that will produce different quenching patterns when they are in close proximity to each other vs. when they are physically separate (i.e., dyes that quench each other when they are close together but fluoresce when they are not in close proximity);

3. Expose the interacting molecule to the immobilized polypeptide in the presence or absence of a compound being tested for its ability to interfere with an interaction between the two; and

4. Collect fluorescent readout data.

An alternative assay for such protein interaction is the Fluorescent Resonance Energy Transfer (FRET) assay. This assay can be performed as follows.

1. Provide the encoded protein or a suitable polypeptide fragment thereof and couple a suitable FRET donor (e.g., nitro-benzoxadiazole (NBD)) to it;

2. Label an interacting protein (or other molecule) with a FRET acceptor (e.g., rhodamine);

3. Expose the acceptor-labeled interacting molecule to the donor-labeled polypeptide in the presence or absence of a compound being tested for its ability to interfere with an interaction between the two; and

4. Measure fluorescence resonance energy transfer.

Quenching and FRET assays are related. Either one can be applied in a given case, depending on which pair of fluorophores is used in the assay.

One or more of the genes disclosed herein may act by altering membrane permeability, such as the permeability of membranes to ions, an example being the $Na_V1.7$ gene disclosed herein. Such activity may be assayed for using vesicles, such as liposomes or intact cells, wherein such structures comprise one or more of the polypeptides of the invention, which

polypeptides are expressed in such vesicle, preferably an intact cell, such as a mammalian recombinant cell, and the permeability of the membrane of the cell is determined in the presence or absence of such expression. In the same way, such permeability can then be assayed in the presence and absence of chemical agents known to modulate the activity of one or more of the genes disclosed herein. Thus, the utility of these agents in enhancing the activity of proteins known to affect such membrane transport can be readily determined. In the same way, the ability of these agents to affect the transport of other molecules, such as lipids, amino acids, and the like, across such membranes is readily determined.

In performing assays, the test cell, such as a mammalian recombinant cell expressing $Na_V1.7$ protein or a non-recombinant cell which expresses $Na_V1.7$ protein (either naturally or when specifically induced, such as at PC-12 cell or TT cell), is loaded with a reporter molecule (such as a fluorescent ion indicator whose fluorescent properties change when it binds a particular ion) that can detect ions (to observe outward movement), or alternatively, the external medium is loaded with such a molecule (to observe inward movement). A molecule which exhibits differential properties when it is inside the vesicle compared to when it is outside the vesicle is preferred. For example, a molecule that has quenching properties when it is at high concentration but not when it is at another low concentration would be suitable. The movement of the charged molecule (either its ability to move or the kinetics of its movement) in the presence or absence of a compound being tested for its ability to affect this process can be determined.

Throughput of test compounds is an important consideration in the choice of screening assay to be used. In some strategies, where hundreds of thousands of compounds are to be tested, it is not desirable to use low throughput means. In other cases, however, low throughput is satisfactory to identify important differences between a limited number of compounds. Often it will be necessary to combine assay types to identify $Na_V1.7$ modulating compounds

In brief, electrophysiology using patch clamp techniques is accepted as a gold standard for detailed characterization of sodium channel compound binding. There is a manual low-throughput screening (LTS) method which can compare 2-10 compounds per day; a recently developed system for automated medium-throughput screening (MTS) at 20-50 patches (i.e. compounds) per day; and a technology reportedly in development from Molecular Devices Corporation (Sunnyvale, CA) which permits automated high-throughput screening (HTS) at 1000-3000 patches (i.e. compounds) per day.

One automated patch-clamp system utilizes planar electrode technology to accelerate the rate of drug discovery. Planar electrodes are capable of achieving high-resistance, cells-attached seals followed by stable, low-noise whole-cell recordings that are comparable to conventional recordings. A suitable instrument is the PatchXpress 7000A (Axon Instruments Inc, Union City, CA). A variety of cell lines and culture techniques, which include adherent cells as well as cells growing spontaneously in suspension are ranked for seal success rate and stability. HEK cells stably expressing high levels of the relevant sodium ion channel can be adapted into high-density suspension cultures.

The choice of assay will be influenced by whether the investigator seeks to identify compounds with a fast or slow on-rate.

Other assays can be selected which allow the investigator to identify compounds which block specific states of the channel, such as the open state, closed state or the resting state, or which block transition from open to closed, closed to resting or resting to open. Those skilled in the art are generally familiar with such assays.

Binding Assays are also available, however these are of only limited functional value and information content. Designs include traditional

radioactive filter based binding assays or the confocal based fluorescent system available from Evotec OAI group of companies (Hamburg, Germany), both of which are HTS.

Radioactive flux assays can also be used. In this assay, channels are stimulated to open with veratridine or aconotine and held in a stabilized open state with scorpion toxin, and channel blockers are identified by their ability to prevent ion influx. The assay can use radioactive ^{22}[Na] and ^{14}[C] guanidinium ions as tracers. FlashPlate & Cytostar-T plates in living cells avoids separation steps and are suitable for HTS. Scintillation plate technology has also advanced this method to HTS suitability. Because of the functional aspects of the assay, the information content is reasonably good.

Yet another format measures the redistribution of membrane potential using the FLIPR system membrane potential kit (HTS) available from Molecular Dynamics (a division of Amersham Biosciences, Piscataway, NJ). Again, in this assay channels are stabilized in an open state with veratridine, and channel blockers are identified. The assay can use radioactive 22[Na] and 14[C] guanidinium ions as tracers. This method is limited to slow membrane potential changes. Some problems may result from the fluorescent background of compounds. Test compounds may also directly influence the fluidity of the cell membrane and lead to an increase in intracellular dye concentrations. Still, because of the functional aspects of the assay, the information content is reasonably good.

Sodium dyes can be used to measure the rate or amount of sodium ion influx through a channel. This type of assay provides a very high information content regarding potential channel blockers. The assay is functional and would measure Na influx directly. CoroNa Red, SBFI and/or sodium green (Molecular Probes, Inc. Eugene OR) can be used to measure Na influx; all are Na responsive dyes. They can be used in combination with the FLIPR instrument. The use of these dyes in a screen has not been previously

described in the literature. Calcium dyes may also have potential in this format.

In a further embodiment, FRET based voltage sensors are used to measure the ability of a test compound to directly block Na influx. Commercially available HTS systems include the VIPR™ II FRET system (Aurora Biosciences Corporation, San Diego, CA, a division of Vertex Pharmaceuticals, Inc.) which may be used in conjunction with FRET dyes, also available from Aurora Biosciences. This assay measures sub-second responses to voltage changes. There is no requirement for a modifier of channel function. The assay measures depolarization and hyperpolarizations, and provides ratiometric outputs for quantification. A somewhat less expensive MTS version of this assay employs the FLEXstation™ (Molecular Devices Corporation) in conjunction with FRET dyes from Aurora Biosciences.

The present invention also relates to assays that may employ transcription factors for one or more of the genes disclosed herein. The effect of a test compound on the relative ability of such transcription factor to modulate transcription of the $Na_V1.7$ gene is assessed by means of such an assay. In accordance with the disclosure herein, untranslated regions and promoter regions of $Na_V1.7$ are provided or readily obtained. Such genomic or untranslated regions may be included in plasmids comprising the identified gene, such as in assays to identify compounds which modulate expression thereof. In one such assay, 1 – 5 kilobases of upstream genomic region of $Na_V1.7$ is operably linked (i.e. ligated) to a reporter gene, and incorporated into an expression plasmid. The plasmid is transfected into a cell, and the recombinant cell is exposed to test compound(s). Those compounds which increase or decrease the expression of the reporter gene are then modulators of the $Na_V1.7$ gene/protein, and are considered therapeutic agents of the invention.

Those skilled in the art are familiar with typical and easily measured reporter genes, such as luciferase, chloramphenicol acetyl-transferase (CAT), and other luminescent or fluorescent assays.

Thus, in one aspect the present invention relates to a method for identifying an agent that modulates the activity of a polynucleotide whose expression contributes to pain sensation or whose non-expression contributes to lack of pain sensation, comprising:

a) contacting under physiological conditions a chemical agent with a promoter of the $Na_V1.7$ gene, preferably having the sequence of SEQ. ID NO. 14, operably linked to a reporter gene, including an $Na_V1.7$ polynucleotide under conditions supporting such contacting; and

b) detecting a change in the expression of said polynucleotide as a result of said contacting;

wherein said change in expression of said reporter gene indicates modulation of said promoter

thereby identifying an agent that modulates said polynucleotide activity.

Medicinal Chemistry and Lead Optimization

While this invention discloses a wide variety of assays for measuring the effect of a compound on $Na_V1.7$ expression or activity, it is important to note that this compound detection is merely the first step in the industrial process of identification of an approvable therapeutic agent. The library screening accomplished with the first screening assay may be low, medium or high-throughput screening. It identifies "hits" or individual compounds from the library which cause the desired modulation of $Na_V1.7$ expression or activity. The hits are further evaluated at a chemical structure level, and, if possible, are organized according to shared core structures, which presumably define chemical features required to achieve the desired modulation of $Na_V1.7$. This process, sometimes called lead identification,

may involve a structure-activity relationship (or SAR) analysis. Various cycles of medicinal chemistry or focused library generation may then be employed to generate multiple analogs of such core structures in a process called lead optimization. Finally, those skilled in the art know how to identify and test those preferred analogs (optimized leads) which have improved characteristics as therapeutic agents, generally through a series of *in vitro* and *in vivo* analyses.

In general, novel agents having $Na_V1.7$ modulating properties are identified from large libraries of both natural product or synthetic (or semi-synthetic) extracts or chemical libraries according to methods known in the art. Those skilled in the field or drug discovery and development will understand that the precise source of test extracts or compounds is not critical to the screening procedure(s) of the invention. Accordingly, virtually any number of chemical extracts or compounds can be screened using the exemplary methods described herein. Examples of such extracts or compounds include, but are not limited to, plant-, fungal-, prokaryotic- or animal-based extracts, fermentation broths, and synthetic compounds, as well as modification of existing compounds. Numerous methods are also available for generating random or directed synthesis (*e.g.*, semi-synthesis or total synthesis) of any number of chemical compounds, including, but not limited to, saccharide-, lipid-, peptide-, and nucleic acid-based compounds. Synthetic compound libraries are commercially available from Brandon Associates (Merrimack, NH) and Aldrich Chemical (Milwaukee, WI). Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant, and animal extracts are commercially available from a number of sources, including Biotics (Sussex, UK), Xenova (Slough, UK), Harbor Branch Oceangraphics Institute (Ft. Pierce, FL), and PharmaMar, U.S.A. (Cambridge, MA). In addition, natural and synthetically produced libraries are produced, if desired, according to methods known in the art, *e.g.*, by standard extraction and fractionation methods. Furthermore, if desired, any library or compound is readily modified using standard chemical, physical, or biochemical methods.

In addition, those skilled in the art of drug discovery and development readily understand that methods for dereplication (*e.g.*, taxonomic dereplication, biological dereplication, and chemical dereplication, or any combination thereof) or the elimination of replicates or repeats of materials already known for their analgesic and/or anesthetic activities should be employed whenever possible.

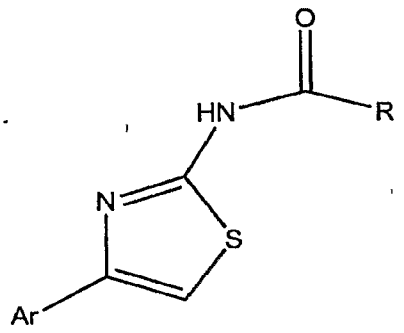
When a crude extract is found to have $Na_V1.7$ modulating activity, further fractionation of the positive lead extract is necessary to isolate chemical constituent responsible for the observed effect. Thus, the goal of the extraction, fractionation, and purification process is the careful characterization and identification of a chemical entity within the crude extract having potential therapeutic activities. The same assays described herein for the detection of activities in mixtures of compounds can be used to purify the active component and to test derivatives thereof. Methods of fractionation and purification of such heterogeneous extracts are known in the art.

In an alternative strategy, if a compound is identified which is known to modulate expression or activity of a polynucleotide corresponding to $Na_V1.7$, or polypeptide encoded by such polynucleotide, analogs of that compound can be developed and tested for improved selectivity, potency, binding affinity or the like against the target gene/protein. According to the invention, such analogs will also be tested against other sodium channels (such as Na_V 1.1 to 1.6 and Na_V 1.8 to 1.9) to identify analogs which are preferentially selective towards blocking Na_V 1.7 and which do not interact with the others. Such improved analogs, which are compounds of the invention, are expected to demonstrate reduced side effects and improved analgesic effects in human patients as compared to their parent compounds.

Compositions of the Invention

This invention provides $Na_v1.7$ selective sodium channel blockers. The screening assays and methods of the instant invention have been employed to identify these compounds and compositions.

In one aspect, this invention is directed to the compounds of Formula (I):



(I)

wherein:

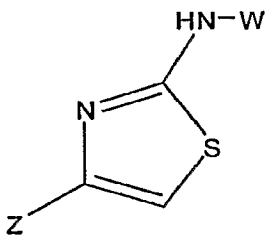
R is $-(CH_2)_n(X)_mAr$, wherein n is 0, 1, 2 or 3; m is 0 or 1; and

Ar is selected from the group consisting of phenyl, substituted phenyl, naphtyl, substituted naphtyl, heteroaryl, and substituted heteroaryl;

X is CH_2, O, S or NH;

including a stereoisomer, enantiomer or tautomer thereof, a pharmaceutically acceptable salt thereof, a pharmaceutical composition thereof or a prodrug thereof.

In another aspect this invention is directed to compounds of Formula (Ia):



(Ia)

wherein:

Z is selected from optionally substituted straight or branched chain C_1 to C_5 alkyl, optionally substituted straight or branched chain C_1 to C_5 alkoxy,

optionally substituted cycloalkyl, optionally substituted cycloalkylalkyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl or optionally substituted heteroaralkyl,

wherein said substitutions are each independently selected from hydrogen, straight or branched chain C_1 to C_5 alkyl, halo, haloalkyl, -OR^2, -$S(O)_tR^2$ (where t is 0, 1 or 2), -CN, -$C(O)R^2$, -$C(O)OR^2$, -$C(O)N(R^2)R^3$, -$N(R^2)R^3$ and -NO_2;

W is -R^4- C(O)-R^4-X-R^1;

R^1 is optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

wherein said substitutions are each independently selected from hydrogen, straight or branched chain C_1 to C_5 alkyl, halo, haloalkyl, -OR^2, -$S(O)_tR^2$ (where t is 0, 1 or 2), -CN, -$C(O)R^2$, -$C(O)OR^2$, -$C(O)N(R^2)R^3$, -$N(R^2)R^3$ and -NO_2;

R^2 and R^3 are each independently hydrogen or optionally substituted straight or branched chain C_1 to C_5 alkyl, wherein said substitutions are each independently selected from hydrogen, straight or branched chain C_1 to C_5 alkyl, halo, haloalkyl, -OR^5, -$S(O)_tR^5$ (where t is 0, 1 or 2), -CN, -$C(O)R^5$, -$C(O)OR^5$, -$C(O)N(R^5)R^6$, -$N(R^5)R^6$ and -NO_2;

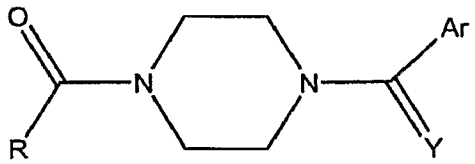
R^4 is a direct bond or a straight or branched C_1 to C_5 alkylene;

X is -O-, -S- or -$N(R^2)$-;

as a stereoisomer, enantiomer, tautomer thereof or mixtures thereof; or a pharmaceutically acceptable salt, solvate or prodrug thereof.

Compounds of Formulas (I) and (Ia) selectively reduce the ion flux activity of the $Na_V1.7$ polypeptide more than the ion flux activity of $Na_V1.3$.

In another aspect of the present invention, compounds are disclosed of Formula (II):



(II)

wherein:

R is $-(CH_2)_n(X)_m(CH_2)_n(X)_m(CH_2)_nZ$ wherein n are each independently 0, 1, 2 or 3; and m are each independently 0 or 1;

Ar is selected from the group consisting of phenyl, substituted phenyl, naphtyl, substituted naphtyl, heteroaryl, substituted heteroaryl, and substituted heteroarylaminoheteroaryl;

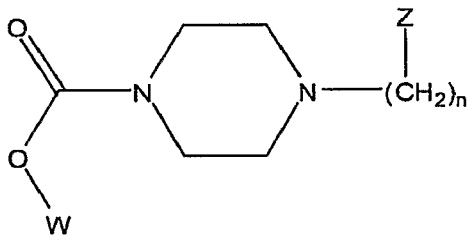
X are each independently CH_2, O, S or NH;

Y is H or O; and

Z is alkyl, Ar, amino, alkylamino or dialkyamino;

including a stereoisomer, enantiomer or tautomer thereof, a pharmaceutically acceptable salt thereof, a pharmaceutical composition thereof or a prodrug thereof.

In another aspect, this invention is directed to compounds of Formula (IIa):



(IIa)

wherein:

n is 0, 1, 2 or 3;

Z is selected from $-R^7-N(R^6)R^7$, $-OR^7$, $-S(O)_tR^7$ (where t is 0, 1 or 2), optionally substituted straight or branched chain C_1 to C_5 alkyl, optionally substituted straight or branched chain C_1 to C_5 alkoxy, optionally substituted cycloalkyl, optionally substituted cycloalkylalkyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally

substituted aralkyl, optionally substituted heteroaryl or optionally substituted heteroaralkyl,

wherein said substitutions are each independently selected from hydrogen, C_1 to C_5 alkyl, halo, haloalkyl, $-OR^5$, $-S(O)_tR^5$ (where t is 0, 1 or 2), -CN, $-C(O)R^5$, $-C(O)OR^5$, $-C(O)N(R^5)R^6$, $-N(R^5)R^6$ and $-NO_2$;

W is selected from $-R^8-X-R^8-R^9$, optionally substituted straight or branched chain C_1 to C_5 alkyl, optionally substituted straight or branched chain C_1 to C_5 alkoxy, optionally substituted aryl, optionally substituted heteroaryl,

wherein said substitutions are each independently selected from hydrogen, straight or branched chain C_1 to C_5 alkyl, halo, haloalkyl, $-OR^5$, $-S(O)_tR^5$ (where t is 0, 1 or 2), -CN, $-C(O)R^5$, $-C(O)OR^5$, $-C(O)N(R^5)R^6$, $-N(R^5)R^6$ and $-NO_2$;

X is -O-, -S- or $-N(R^5)-$;

R^5 and R^6 are each independently hydrogen or optionally substituted straight or branched chain C_1 to C_5 alkyl, wherein said substitutions are each independently selected from hydrogen, straight or branched chain C_1 to C_5 alkyl, halo, haloalkyl, $-O\ R^{10}$, $-S(O)_t\ R^{10}$ (where t is 0, 1 or 2), -CN, $-C(O)\ R^{10}$, $-C(O)O\ R^{10}$, $-C(O)N(R^{10})R^{11}$, $-N(R^{10})R^{11}$ and $-NO_2$;

R^7 is optionally substituted aryl or optionally substituted heteroaryl,

wherein said substitutions are each independently selected from hydrogen, straight or branched chain C_1 to C_5 alkyl, halo, haloalkyl, $-OR^5$, $-S(O)_tR^5$ (where t is 0, 1 or 2), -CN, $-C(O)R^5$, $-C(O)OR^5$, $-C(O)N(R^5)R^6$, $-N(R^5)R^6$ and $-NO_2$;

each R^8 is independently a direct bond or a straight or branched C_1 to C_5 alkylene;

R^9 is optionally substituted aryl or optionally substituted heteroaryl,

wherein said substitutions are each independently selected from hydrogen, straight or branched chain C_1 to C_5 alkyl, halo, haloalkyl, $-OR^5$, $-S(O)_tR^5$ (where t is 0, 1 or 2), -CN, $-C(O)R^5$, $-C(O)OR^5$, $-C(O)N(R^5)R^6$, $-N(R^5)R^6$ and $-NO_2$; and

R^{10} and R^{11} are each independently hydrogen or optionally substituted straight or branched chain C_1 to C_5 alkyl;

as a stereoisomer, enantiomer, tautomer thereof or mixtures thereof; or a pharmaceutically acceptable salt, solvate or prodrug thereof.

Compounds of Formulas (II) and (IIa) selectively reduce the ion flux activity of the $Na_V1.7$ polypeptide more than the ion flux activity of $Na_V1.8$.

In an embodiment, selective sodium channel blocking compounds of the invention demonstrate little or no inhibiting activity against $Na_V1.5$, $Na_V1.4$ or hERG. Further characteristics of selectivity of these compounds are set out in the Examples.

In another embodiment, selective sodium channel blocking compounds of the invention may optionally inhibit the ion flux activity of a sodium channel polypeptide selected from among $Na_V1.1$, $Na_V1.3$ and $Na_V1.8$. Further characteristics of selectivity of these compounds are set out in the Examples.

Chemical Definitions

Certain chemical groups named herein are preceded by a shorthand notation indicating the total number of carbon atoms that are to be found in the indicated chemical group. For example; C_7-C_{12}alkyl describes an alkyl group, as defined below, having a total of 7 to 12 carbon atoms, and C_4-C_{12}cycloalkylalkyl describes a cycloalkylalkyl group, as defined below, having a total of 4 to 12 carbon atoms. The total number of carbons in the shorthand notation does not include carbons that may exist in substituents of the group described and therefor relates only to the carbon backbone of the substituent group.

Accordingly, as used in the specification and appended claims, unless specified to the contrary, the following terms have the meaning indicated:

"Amino" refers to $-NH_2$.

"Cyano" refers to the -CN radical.

"Nitro" refers to the $-NO_2$ radical.

"Alkyl" refers to a straight or branched hydrocarbon chain radical consisting solely of carbon and hydrogen atoms, containing no unsaturation, having from one to twelve carbon atoms, preferably one to eight carbon atoms or one to five carbon atoms, and which is attached to the rest of the molecule by a single bond, *e.g.*, methyl, ethyl, *n*-propyl, 1-methylethyl (*iso*-propyl), *n*-butyl, *n*-pentyl, 1,1-dimethylethyl (*t*-butyl), and the like. Unless stated otherwise specifically in the specification, an alkyl group may be optionally substituted.

"Alkenyl" refers to a straight or branched hydrocarbon chain radical group consisting solely of carbon and hydrogen atoms, containing at least one double bond, having from two to twelve carbon atoms, preferably one to eight carbon atoms and which is attached to the rest of the molecule by a single bond, *e.g.*, ethenyl, prop-1-enyl, but-1-enyl, pent-1-enyl, penta-1,4-dienyl, and the like. Unless stated otherwise specifically in the specification, an alkenyl group may be optionally substituted.

"Alkylene" refers refers to a straight or branched divalent hydrocarbon chain linking the rest of the molecule to a radical group, consisting solely of carbon and hydrogen, containing no unsaturation and having from one to twelve carbon atoms, *e.g.*, methylene, ethylene, propylene, *n*-butylene, and the like. The alkylene chain is attached to the rest of the molecule through a single bond and to the radical group through a single bond.

"Alkyamino" refers to –NH-Alkyl.

"Dialkyamino" refers to –N-Alkyl-Alkyl.

"Aryl" refers to aromatic monocyclic or multicyclic hydrocarbon ring system consisting only of hydrogen and carbon and containing from 6 to 19 carbon atoms, preferably 6 to 10 carbon atoms, where the ring system may be partially or fully saturated. Aryl groups include, but are not limited to groups such as fluorenyl, phenyl and naphthyl. Unless stated otherwise specifically in the specification, the term "aryl" or the prefix "ar-" (such as in "aralkyl") is meant to include aryl radicals optionally substituted.

"Aralkyl" refers to a radical of the formula $-R_aR_b$ where R_a is an alkyl radical as defined above and R_b is one or more aryl radicals as defined above, *e.g.*, benzyl, diphenylmethyl and the like. The aryl part of the aralkyl radical

may be optionally substituted as described above for an aryl group. The alkyl part of the aralkyl radical may be optionally substituted as defined above for an alkyl group.

"Heteroaryl" refers to a 5 to 18 membered aromatic ring radical which consists of carbon atoms and from one to five heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur. For purposes of this invention, the heteroaryl radical may be a monocyclic, bicyclic, tricyclic or tetracyclic ring system, and the nitrogen, carbon or sulfur atoms in the heteroaryl radical may be optionally oxidized; the nitrogen atom may be optionally quaternized. Examples include, but are not limited to, azepinyl, acridinyl, benzimidazolyl, benzthiazolyl, benzindolyl, benzothiadiazolyl, benzonaphthofuranyl, benzoxazolyl, benzodioxolyl, benzodioxinyl, benzopyranyl, benzopyranonyl, benzofuranyl, benzofuranonyl, benzothienyl (benzothiophenyl), benzotriazolyl, benzo[4,6]imidazo[1,2-a]pyridinyl, carbazolyl, cinnolinyl, dibenzofuranyl, furanyl, furanonyl, isothiazolyl, imidazolyl, indolyl, indazolyl, isoindolyl, indolinyl, isoindolinyl, indolizinyl, isoxazolyl, naphthyridinyl, oxadiazolyl, 2-oxoazepinyl, oxazolyl, oxiranyl, phenazinyl, phenothiazinyl, phenoxazinyl, phthalazinyl, pteridinyl, purinyl, pyrrolyl, pyrazolyl, pyridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, quinazolinyl, quinoxalinyl, quinolinyl, quinuclidinyl, isoquinolinyl, thiazolyl, thiadiazolyl, triazolyl, tetrazolyl, triazinyl, and thiophenyl.

"Cycloalkyl" refers to a stable non-aromatic monocyclic or bicyclic hydrocarbon radical consisting solely of carbon and hydrogen atoms, having from three to fifteen carbon atoms, preferably having from three to twelve carbon atoms, and which is saturated or unsaturated and attached to the rest of the molecule by a single bond, *e.g.*, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, decalinyl and the like. Unless otherwise stated specifically in the specification, the term "cycloalkyl" is meant to include cycloalkyl radicals which are optionally substituted.

"Cycloalkylalkyl" refers to a radical of the formula $-R_aR_d$ where R_a is an alkyl radical as defined above and R_d is a cycloalkyl radical as defined above. The cycloalkyl part of the cycloalkyl radical may be optionally substituted as

defined above for an cycloalkyl radical. The alkyl part of the cycloalkyl radical may be optionally substituted as defined above for an alkyl radical.

"Halo" refers to bromo, chloro, fluoro or iodo.

"Haloalkyl" refers to an alkyl radical, as defined above, that is substituted by one or more halo radicals, as defined above, *e.g.*, trifluoromethyl, difluoromethyl, trichloromethyl, 2,2,2-trifluoroethyl, 1-fluoromethyl-2-fluoroethyl, 3-bromo-2-fluoropropyl, 1-bromomethyl-2-bromoethyl, and the like. The alkyl part of the haloalkyl radical may be optionally substituted as defined above for an alkyl group.

"Haloalkenyl" refers to an alkenyl radical, as defined above, that is substituted by one or more halo radicals, as defined above, e.g., 2-bromoethenyl, 3-bromoprop-1-enyl, and the like. The alkenyl part of the haloalkenyl radical may be optionally substituted.

"Heterocyclyl" refers to a stable 3- to 18-membered non-aromatic ring radical which consists of carbon atoms and from one to five heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur. For purposes of this invention, the heterocyclyl radical may be a monocyclic, bicyclic, tricyclic or tetracyclic ring system, which may include fused or bridged ring systems; and the nitrogen, carbon or sulfur atoms in the heterocyclyl radical may be optionally oxidized; the nitrogen atom may be optionally quaternized; and the heterocyclyl radical may be partially or fully saturated. Examples of such heterocyclyl radicals include, but are not limited to, dioxolanyl, decahydroisoquinolyl, imidazolinyl, imidazolidinyl, isothiazolidinyl, and isoxazolidinyl. Unless stated otherwise specifically in the specification, the term "heterocyclyl" is meant to include heterocyclyl radicals as defined above which are optionally substituted.

"Heterocyclylalkyl" refers to a radical of the formula $-R_aR_e$ where R_a is an alkyl radical as defined above and R_e is a heterocyclyl radical as defined above, and if the heterocyclyl is a nitrogen-containing heterocyclyl, the heterocyclyl may be attached to the alkyl radical at the nitrogen atom. The alkyl part of the heterocyclylalkyl radical may be optionally substituted as defined above for an alkyl group. The heterocyclyl part of the

heterocyclylalkyl radical may be optionally substituted as defined above for a heterocyclyl group.

"Heteroaryl" refers to a 5- to 18-membered aromatic ring radical which consists of carbon atoms and from one to five heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur. For purposes of this invention, the heteroaryl radical may be a monocyclic, bicyclic, tricyclic or tetracyclic ring system, which may include fused or bridged ring systems; and the nitrogen, carbon or sulfur atoms in the heteroaryl radical may be optionally oxidized; the nitrogen atom may be optionally quaternized. Examples include, but are not limited to, azepinyl, acridinyl, benzimidazolyl, benzthiazolyl, and benzindolyl. Unless stated otherwise specifically in the specification, the term "heteroaryl" is meant to include heteroaryl radicals as defined above which are optionally substituted.

"Heteroarylalkyl" refers to a radical of the formula $-R_aR_f$ where R_a is an alkyl radical as defined above and R_f is a heteroaryl radical as defined above. The heteroaryl part of the heteroarylalkyl radical may be optionally substituted as defined above for a heteroaryl group. The alkyl part of the heteroarylalkyl radical may be optionally substituted as defined above for an alkyl group.

"Heteroarylaminoheteroaryl" refers to a radical of the formula $-R_kR_lR_m$ where R_k and R_m are heteroaryl radicals as defined above and R_l is a amino radical as defined above. The heteroaryl part of the Heteroarylaminoheteroaryl radical may be optionally substituted as defined above for an heteroaryl group.

"Optionally substituted" means that the subsequently described event of circumstances may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not. For example, "optionally substituted aryl" means that the aryl radical may or may not be substituted and that the description includes both substituted aryl radicals and aryl radicals having no substitution.

"Substituted" refers to the radical as defined herein, unless stated otherwise specifically in the specification, which is substituted by one or more substituents selected from the group consisting of alkenyl, halo, haloalkenyl,

cyano, nitro, aryl, cycloalkyl, heterocyclyl, heteroaryl, $-OR^{14}$, $-OC(O)-R^{14}$, $-N(R^{14})_2$, $-C(O)R^{14}$, $-C(O)OR^{14}$, $-C(O)N(R^{14})_2$, $-N(R^{14})C(O)OR^{16}$, $-N(R^{14})C(O)R^{16}$, $-N(R^{14})(S(O)_tR^{16})$ (where t is 1 to 2), $-S(O)_tOR^{16}$ (where t is 1 to 2), $-S(O)_tR^{16}$ (where t is 0 to 2), and $-S(O)_tN(R^{14})_2$ (where t is 1 to 2) where each R^{14} is independently hydrogen, alkyl, haloalkyl, cycloalkyl, cycloalkylalkyl, aryl (optionally substituted with one or more halo groups), aralkyl, heterocyclyl, heterocylylalkyl, heteroaryl or heteroarylalkyl; and each R^{16} is alkyl, haloalkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heterocyclyl, heterocylylalkyl, heteroaryl or heteroarylalkyl, and where each of the above substituents is unsubstituted unless otherwise indicated. For example, "substituted heteroaryl" means that the heteroaryl radical is substituted with one or more substituents selected from the group described herein.

A "stereoisomer" refers to a compound made up of the same atoms bonded by the same bonds but having different three-dimensional structures, which are not interchangeable. The present invention contemplates various stereoisomers and mixtures thereof and includes "enantiomers", which refers to two stereoisomers whose molecules are nonsuperimposeable mirror images of one another.

A "tautomer" refers to a proton shift from one atom of a molecule to another atom of the same molecule. The present invention includes tautomers of any said compounds.

The term "prodrug" is also meant to include any covalently bonded carriers which release the active compound of the invention in vivo when such prodrug is administered to a mammalian subject. Prodrugs of a compound of the invention may be prepared by modifying functional groups present in the compound of the invention in such a way that the modifications are cleaved, either in routine manipulation or in vivo, to the parent compound of the invention.

"Pharmaceutically acceptable salt" includes both acid and base addition salts.

"Pharmaceutically acceptable acid addition salt" refers to those salts which retain the biological effectiveness and properties of the free bases, which are not biologically or otherwise undesirable, and which are formed with

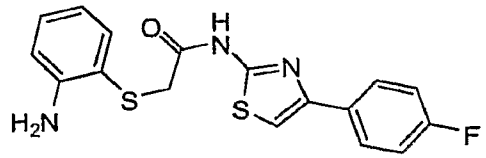
inorganic acids such as, but not limited to, hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids.

"Pharmaceutically acceptable base addition salt" refers to those salts which retain the biological effectiveness and properties of the free acids, which are not biologically or otherwise undesirable. These salts are prepared from addition of an inorganic base or an organic base to the free acid. Salts derived from inorganic bases include, but are not limited to, the sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like.

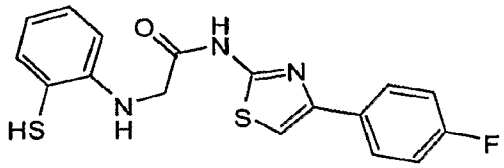
A "pharmaceutical composition" refers to a formulation of a compound of the invention and a medium generally accepted in the art for the delivery of the biologically active compound to mammals, e.g., humans. Such a medium includes all pharmaceutically acceptable carriers, diluents or excipients therefor.

In one embodiment of the invention, compounds of Formula (I), as set forth above in the Summary of the Invention, are directed to compounds wherein n and m are each 1; X is S or NH; and Ar is phenyl or phenyl substituted with halo, Br or S.

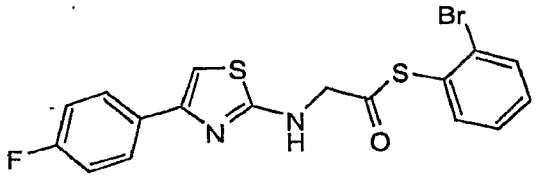
In further embodiments of the invention, compounds of Formula (I) or Formula (Ia) include:



Example XA: 2-(2-aminophenylthio)-*N*-(4-(4-fluorophenyl)thiazol-2-yl)acetamide.



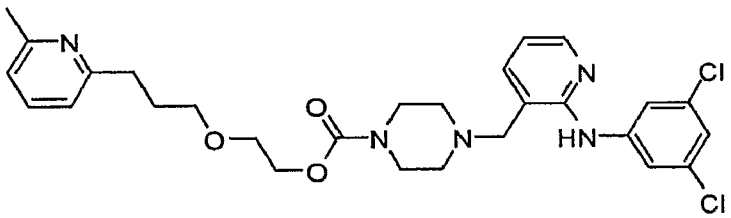
Example XB: *N*-(4-(4-fluorophenyl)thiazol-2-yl)-2-(2-mercaptophenylamino)acetamide.



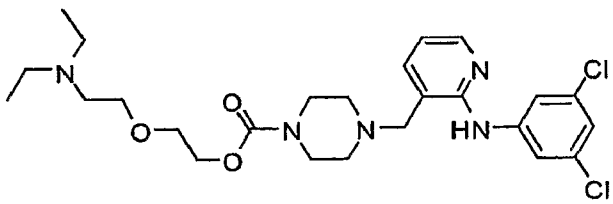
Example XC: *S*-2-bromophenyl 2-(4-(4-fluorophenyl)thiazol-2-ylamino)ethanethioate.

In another embodiment of the invention, compounds of Formula (II), as set forth above in the Summary of the Invention, are directed to compounds wherein n are each independently 0, 2 or 3; m are each independently 0 or 1; X is O; Z is alkyl, heteroaryl, or dialkyamino; Y is H; and Ar is heteroaryl.

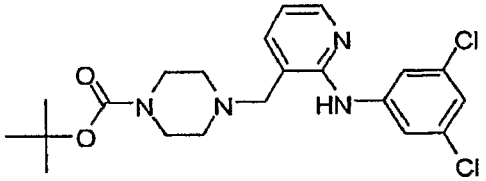
In another embodiment, compounds of Formula (II) or Formula (IIa) include:



Example XD: 2-(3-(6-methylpyridin-2-yl)propoxy)ethyl 4-((2-(3,5-dichlorophenylamino)pyridin-3-yl)methyl)piperazine-1-carboxylate



Example XE: 2-(2-(diethylamino)ethoxy)ethyl 4-((2-(3,5-dichlorophenylamino)pyridin-3-yl)methyl)piperazine-1-carboxylate.



Example XF: tert-butyl 4-((2-(3,5-dichlorophenylamino)pyridin-3-yl)methyl)piperazine-1-carboxylate

In an embodiment of this invention, an investigator may desire to initiate research with known sodium channel blockers and to develop analogs of the blocker which are more selective for $Na_V1.7$. Known sodium channel modulators that may be selectively improved to increase specificity for $Na_V1.7$ include those in Table 5:

Table 5: Known sodium channel blocking compounds

Drug/clinical candidate	**Company**
AWD-140-190	ASTA Medica AG
AWD-33-173	ASTA Medica AG
BIA-2-024	Boehringer Ingelheim
BIA-2-093	Boehringer Ingelheim
Carbamazepine	Novartis AG
Co-102862	University of Saskatchewan
Conopeptides	Cognetix Inc
DCUKA	Lohocla Research Corp
felbamate	Carter-Wallace Inc
fosphenytoin	InterX Research Corp
GW-273293	Glaxo Wellcome plc
Lamotrigine	Glaxo Wellcome plc
mexilitene (or mexiletine)	generic
Novocaine	generic
OROS (phenytoin)	ALZA Corp
oxcarbazepine	Novartis AG
rufinamide	Novartis AG
safinamide	Pharmacia & Upjohn AB
topiramate	Johnson & Johnson

valproate semisodium	Abbott Laboratories
vinpocetine	Richter Gedeon VG
Xylocaine	generic
Xylocaine	generic
ZM-227189	Zeneca Group plc
zonisamide	Dainippon Pharm. Co Ltd.

Improved analogs may also include compounds with improved stability, biodistribution, pharmacokinetics or other desirable features for therapeutic agents which are not directly related to modulation of the therapeutic target.

In an embodiment, the improved analog of the invention is effectively delivered, either by physiological means or assisted means, to cells of the body expressing the $Na_V1.7$ protein.

Some known sodium channel blocking compounds which can be used for comparison purposes include the following:

1. Amiloride hydrochloride (3,5-Diamino-N-(aminoiminomethyl)-6-chloro-pyrazinecarboxamide) M.W 266.11, Store at RT, Soluble in water [2016-88-8]

Na^+ channel blocker. Affinity for, or lack of, defines the I_{2A}-amiloride sensitive and I_{2B}-amiloride insensitive imidazoline binding sites. Merck Index *12* 426. Kleyman *et al* (1988) Amiloride and its analogues as tools in the study of ion transport. J.Membr.Biol. *105* 1. Ernsberger *et al* (1992) A second generation of centrally acting antihypertensive agents act on putative I_1-imidazoline receptors. J.Cardiovasc.Pharmacol. *20* S1. Hamill and McBride (1996) The pharmacology of mechanogated membrane ion channels. Pharmacol.Rev. *48* 231.

2. Flecainide acetate (*N*-(2-Piperidylmethyl)-2,5-*bis*-(2,2,2-trifluoroethoxy)benzamide acetate), M.W. 474.40, Desiccate at +4°C, Soluble to 50 mM in water [54143-56-5]

Open Na^+ channel blocker that inhibits fast Na^+ current in cardiac muscle in a use- and concentration-dependent manner. Orally-active class Ic antiarrhythmic agent. Banitt *et al* (1977) Antiarrhythmics. 2. Synthesis and antiarrhythmic activity of *N*-(piperidylalkyl) trifluoroethoxybenzamides. J.Med.Chem. *20* 821. Singh *et al* (1984) The electrophysiology and pharmacology of verapamil, flecainide, and amiodarone: correlations with clinical effects and antiarrhythmic actions. Ann.N.Y.Acad.Sci.USA *17* 251. Rouet and Ducouret (1994) Use- and concentration-dependent effects of flecainide in guinea pig right ventricular muscle. J.Cardiovasc.Pharmacol. *24* 177.

3. Flunarizine dihydrochloride, ((E)-1-[Bis(4-fluorophenyl)methyl]-4-(3-phenyl-2-propenyl)piperazine), M.W. 477.43, Store at RT, Soluble to 5 mM in ethanol
[30484-77-6]

Dual Na^+/Ca^{2+} channel blocker; a cerebral and peripheral vasodilator. Neuroprotective.

Pauwels *et al* (1991) Ca^{2+} and Na^+ channels involved in neuronal cell death - protection by flunarizine. Life.Sci. *48* 1881. Eichler *et al* (1994) The ability of diphenylpiperazines to prevent neuronal death in dorsal root ganglion neurons *in vitro* after axotomy. J.Neurochem. *62* 2148. Urenjak and Obrenovitch (1996) Pharmacological modulation of voltage gated Na^+ channels: a rational and effective strategy against ischemic brain damage. Pharmacol.Rev. *48* 21.

4. Pompilidotoxin, PMTX, M.W. 1557.90, Desiccate at -20°C, Solubility: see Peptides
[216064-36-7]

Novel wasp neurotoxin that slows Na^+ channel inactivation. Facilitates neuromuscular synaptic transmission and discriminates between rat neuronal and cardiac Na^+ channel □-subunits. Konno *et al* (1998) Isolation and structure of pompilidotoxins, novel peptide neurotoxins in solitary wasp venoms. Biochem.Biophys.Res.Commun. *250* 612. Kinoshita *et al* (2001) Novel wasp

toxin discriminates between neuronal and cardiac sodium channels. Mol.Pharmacol. *59* 1457. Miyawaki *et al* (2002) Differential effects of novel wasp toxin on rat hippocampal interneurons. Neurosci.Lett. *328* 25.

5. QX 222 (2-[(2,6-Dimethylphenyl)amino]-N,N,N-trimethyl-2-oxoethanaminium chloride), M.W. 256.78, store at RT, Soluble to 100 mM in water, [21236-55-5]

Sodium channel blocker. Cuevas and Adams (1994) Local anaesthetic blockade of neuronal nicotinic ACh receptor-channels in rat parasympathetic ganglion cells. Br.J.Pharmacol. *11* 663. Hanck *et al* (1994) Kinetic effects of quaternary lidocaine block of cardiac sodium channels: a gating current study. J.Gen.Physiol. *103* 19.

6. QX 314, (N-(2,6-Dimethylphenyl carbamoylmethyl)triethylammonium bromide) M.W. 343.31, Desiccate at +4°C, Soluble to 100 mM in water, [21306-56-9]

Membrane impermeable quaternary derivative of lidocaine, a blocker of voltage-activated Na^+ channels. Stichartz *et al* (1973) The inhibition of sodium currents in myelinated nerve by quaternary derivatives of lidocaine. J.Gen.Physiol. Alreja and Aghajanian (1994) QX-314 blocks the potassium but not the sodium dependent components of the opiate response in locus coeruleus neurons. Brain Res. *639* 320. Perkins and Wong (1995) Intracellular QX-314 blocks the hyperpolarization activated inward current Iq in hippocampal CA1 pyramidal cells. J.Neurophysiol. *72* 911.

7. Riluzole hydrochloride (2-Amino-6-trifluoromethoxy benzothiazole), M.W. 270.66
Store at RT, Soluble to 100 mM in DMSO, [1744-22-5]

Novel psychotropic agent with anticonvulsant, hypnotic, anxiolytic, anti-ischaemic and anaesthetic properties. Riluzole is able to act as a glutamate

release inhibitor, blocks voltage dependent Na^+ channels and inhibits GABA uptake by striatal synaptosomes.

Benazzouz *et al* (1995) Riluzole prevents MPTP-induced parkinsonism in the rhesus monkey: a pilot study. Eur.J.Pharmacol. *284* 299. Umeniya and Berger (1995) Inhibition by riluzole of glycinergic postsynaptic currents in rat hypoglossal motoneurones. Br.J.Pharmacol. *116* 3227. Taylor and Meldrum (1995) Na+ channels as targets for neuroprotective drugs. TiPS. *16* 309. Song *et al* (1997) Differential action of riluzole on tetrodotoxin-sensitive and tetrodotoxin-resistant sodium channels. J.Pharmacol.Exp.Ther. *282* 707.

8. Tetrodotoxin (Octahydro-12-(hydroxymethyl)-2-imino-5,9:7,10a-dimethano-10aH-[1,3]dioxocino[6,5-d]pyrimidine-4,7,10,11,12-pentol), M.W. 319.27
Desiccate at +4°C, Soluble in acidic buffer (pH 4.8) [4368-28-9]

Selective inhibitor of Na^+ channel conductance. Binding is reversible and of high affinity (K_d = 1-10 nM). Blocks in a use-dependent manner. Merck Index *12* 9382. Kao (1972) Pharmacology of tetrodotoxin and saxitoxin. Fed.Proc. *31* 1117. Kao (1986) Structure-activity relations of tetrodotoxin, saxitoxin, and analogues. Ann.N.Y.Acad.Sci. *479 52.* Gleitz *et al* (1996) The protective action of tetrodotoxin and (±)-kavain on anaerobic glycolysis, ATP content and intracellular Na^+ and Ca^{2+} of anoxic brain vesicles. Neuropharmacology *35* 1743.

9. Tetrodotoxin citrate (Octahydro-12-(hydroxymethyl)-2-imino-5,9:7,10a-dimethano-
10aH-[1,3]dioxocino[6,5-d]pyrimidine-4,7,10,11,12-pentol), M.W. 511.40
Desiccate at +4°C, Soluble in water
[18660-81-6]

Highly selective, reversible sodium channel blocker; citrate salt of tetrodotoxin.

Merck Index *12* 9382. Kao (1972) Pharmacology of tetrodotoxin and saxitoxin. Fed.Proc. *31* 1117. Kao (1986) Structure-activity relations of tetrodotoxin,

saxitoxin, and analogues. Ann.N.Y.Acad.Sci. *479 52*. Gleitz *et al* (1996) The protective action of tetrodotoxin and (±)-kavain on anaerobic glycolysis, ATP content and intracellular Na^+ and Ca^{2+} of anoxic brain vesicles. Neuropharmacology *35* 1743.

10. Vinpocetine (Eburnamenine-14-carboxylic acid ethyl ester), M.W. 350.46 Store at RT, Soluble in DMSO and ethanol [42971-09-5]

Phosphodiesterase inhibitor, selective for PDE1 (IC_{50} = 21 μM). Also blocks voltage-gated Na^+ channels.Merck Index *12* 10128. Moln? and Erd?(1995) Vinpocetine is as potent as phenytoin to block voltage-gated Na^+ channels in rat cortical neurons. Eur.J.Pharmacol. *273* 303.

Those skilled in the art are aware that improved compounds of the invention will not only demonstrate selectivity towards $Na_v1.7$, but they will not cause the toxicity associated with known sodium channel blocking agents. This toxicity, takes two major forms: neurotoxicity and cardiotoxicity.

Lignocaine, bupivacaine and ropivacaine are all more likely to cause neurotoxicity than cardiac toxicity, indicating a lack of selectivity and an ability to inhibit $Na_v1.1$, 1.2, 1.3 and probably 1.6, the sodium channels associated with CNS function. This relative risk has been called the cc:cns ratio. The dose in (mg/kg) that cause cardiovascular collapse versus the dose in (mg/kg) that cause central nervous system collapse is the CC/CNS ratio. Neurotoxicity can be measured by changes in mentation, followed by perioral paraesthesia, flushing, tinnitus and other neurological symptoms culminating in generalised seizures. Cardiotoxicity is measured by peripheral arteriolar and venous dilatation, decreased myocardial contractility (inhibition of Ca2+ channels), a decrease in cardiac rate; quinidine like action on the action potential with an increase in the refractory period, an increased firing threshold, increased conduction time and Bradycardia with a long p-r interval,

widened QRS complex and an increased Q-Tc leading on to forms of dysrhythmias (blocks, re-entry, ventricular ectopics).

A well known drawback of existing sodium channel blocking compounds, besides their relative non-selectivity, is their low potency. Potency in this context, is a measure of the amount of compound required to modulate the behaviour of a protein (in vitro) or provide a therapeutic effect (in vivo). The relationship between the therapeutic effect of a drug and the dose necessary to achieve that effect; a drug with a higher potency will require a smaller dose to produce a given effect. Potency is often used as a means to compare the relative activities of pharmaceutic agents.

Well known sodium channel blockers typically have low potency, that is, their IC-50 is substantially over 15 μM. Lamotrigine, the most potent blocker of $Na_v1.7$ has an IC-50 of 14.5 μM in the 2 hour guanidine flux assay. By contrast, compounds of the invention demonstrate high potency.

Thus, in an embodiment of the invention, a compound which is a potent and selective blocker of $Na_v1.7$ sodium channel has an IC-50 for $Na_v1.7$ of less than 10μM (when measured in a 2 hour guanidine flux assay) and wherein said therapeutic agent selectively reduces ion flux activity of $Na_v1.7$ more than ion flux activity of $Na_v1.3$, $Na_v1.5$, and/or $Na_v1.8$.

Compounds identified as having potential therapeutic value are subsequently analyzed using any standard *in vitro* assay or *in vivo* animal model for the disease indication known in the art.

Method of Treatment

In a further aspect, the present invention relates to a method for treating a condition in an animal afflicted with a $Na_v1.7$-mediated disease,

such as but not limited to chronic pain, comprising administering to said animal an effective amount of a composition of the invention, or a composition first identified by an assay method of the invention.

As used in this specification '$Na_V1.7$-mediated disease' includes the wide range of disorders, conditions and diseases that are caused by, or treatable with, modulation, preferably inhibition, of $Na_V1.7$ channel activity. Such diseases include but are not limited to pain (whether chronic, acute, inflammatory, etc.), neuralgia, neuropathic pain, eudynia, visceral pain, trauma pain, post-operative pain, heat sensitivity, irritable bowel syndrome, Crohns disease, multiple sclerosis, diabetic neuropathy, arthritic pain, rheumatoid arthritis, paroxysmal dystonia, myasthenia syndromes, myotonia, malignant hyperthermia, cystic fibrosis, pseudoaldosteronism, rhabdomyolysis, sodium channel toxin related Illnesses, hypothyroidism, familial erythermalgia, primary erythermalgia, familial rectal pain, cancer, trigeminal neuralgia, migraine headache and other headaches. In addition, $Na_V1.7$-mediated disease includes such pathologies as inflammatory diseases, neuropathies (e.g., diabetic neuropathy), dystrophies (e.g., reflex sympathetic dystrophy, post-herpetic neuralgia); trauma (tissue damage by any cause); central nervous conditions such as epilepsy, anxiety, depression and bipolar disease; cardiovascular conditions such as arrhythmias, atrial fibrillation and ventricular fibrillation; neuromuscular conditions such as restless leg syndrome and muscle paralysis or tetanus; neuroprotection against stroke, neural trauma and multiple sclerosis; focal pain by any cause. Inflammatory diseases can include, but are not limited to, chronic inflammatory pathologies and vascular inflammatory pathologies. Chronic inflammatory pathologies include, but are not limited tosarcoidosis, chronic inflammatory bowel disease, ulcerative colitis, and Crohn's pathology and vascular inflammatory pathologies, such as, but not limited to, disseminated intravascular coagulation, atherosclerosis, and Kawasaki's pathology. In addition, $Na_V1.7$-mediated disease also includes benign prostatic hyperplasia (BPH), hypercholesterolemia, cancer and pruritis (itch). Selective $Na_V1.7$

antagonists can be used to treat one or more of these $Na_V1.7$-mediated diseases. $Na_V1.7$-mediated diseases also include but are not limited to those diseases which would benefit from comprehensive blockade of the $Na_V1.7$ sodium channel, as observed in patients having congential indifference to pain.

As used herein, 'disease' includes disorder, condition, symptoms of a disease, incipient disease, anticipated disease or anticipated symptom, and the like.

Therapeutic Use of Selective $Na_V1.7$ Modulators

In an embodiment, the agent generates indifference to pain, but does not diminish sensations which are available to patients with CIP, such as but not limited to hot/cold sensing, pressure sensing and sharp/dull sensations. For example, indifference to pain without any concomitant numbness or loss of sensation would represent an important use of the compounds of the invention. In an alternative embodiment, a therapeutic agent is useful in humans or other animals for treating pain, inducing analgesia or anesthesia, or another disorder which is connected to the C-I-P phenotype.

Compounds first identified as useful in reducing sensitivity to pain stimuli using one or more of the assays of the invention may be administered with a pharmaceutically-acceptable diluent, carrier, or excipient, in unit dosage form. Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer such compositions to patients. Although oral administration is preferred, any appropriate route of administration may be employed, for example, intravenous, parenteral, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intrathecal, epidural, intracisternal, intraperitoneal, intranasal, or aerosol administration. A compounds or

pharmaceutical composition of the invention, can be administered by any means that achieve its intended purpose. Therapeutic formulations may be in the form of liquid solutions or suspension; for oral administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

Methods well known in the art for making formulations are found in, for example, Remington: The Science and Practice of Pharmacy, (19th ed.) ed. A.R. Gennaro AR., 1995, Mack Publishing Company, Easton, PA. Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for agonists of the invention include ethylenevinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, or example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel.

The methods of the invention simplify the evaluation, identification and development of active agents for the treatment of conditions of chronic or other types of pain while not necessarily treating the causative condition. Of course, both may be treated simultaneously as contemplated by the invention. For example, an analgesic agent identified by one of the screening methods disclosed herein may be administered along with an agent intended to treat a coincident conditions, such as where analgesic and antitumor agents are given together or contemporaneously.

As used herein, 'treatment' includes preventative treatment, prophylaxis, treatment of symptoms (if not the underlying disease), and the like. Preventative

treatment can be used to mitigate the severity or rapidity of onset of symptoms of the disease.

For topical applications, it is preferred to administer an effective amount of a pharmaceutical compound or composition according to the invention to target area, e.g., skin surfaces, mucous membranes, and the like, which are adjacent to peripheral neurons which are to be treated. This amount will generally range from about 0.0001 mg to about 1 g of a compound of the invention per application, depending upon the area to be treated, whether the use is diagnostic, prophylactic or therapeutic, the severity of the symptoms, and the nature of the topical vehicle employed. A preferred topical preparation is an ointment, wherein about 0.001 to about 50 mg of active ingredient is used per cc of ointment base.

A typical regimen for treatment of $Na_V1.7$-mediated disease comprises administration of an effective amount over a period of one or several days, up to and including between one week and about six months, or it may be chronic. It is understood that the dosage of a diagnostic/pharmaceutical compound or composition of the invention administered in vivo or in vitro will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the diagnostic/ pharmaceutical effect desired. The ranges of effective doses provided herein are not intended to be limiting and represent preferred dose ranges. However, the most preferred dosage will be tailored to the individual subject, as is understood and determinable by one skilled in the relevant arts. (see, e.g., Berkowet al., eds., The Merck Manual, 16th edition, Merck and Co., Rahway, N.J., 1992; Goodmanetna., eds.,Goodman and Cilman's The Pharmacological Basis of Therapeutics, 10th edition, Pergamon Press, Inc., Elmsford, N.Y., (2001); Avery's Drug Treatment: Principles and Practice of Clinical Pharmacology and Therapeutics, 3rd edition, ADIS Press, LTD., Williams and Wilkins, Baltimore, MD. (1987), Ebadi, Pharmacology, Little, Brown and Co., Boston, (1985); Osolci al., eds.,Remington's Pharmaceutical

Sciences, 18th edition, Mack Publishing Co., Easton, PA (1990); Katzung, Basic and Clinical Pharmacology, Appleton and Lange, Norwalk, CT (1992)).

The total dose required for each treatment can be administered by multiple doses or in a single dose. The diagnostic pharmaceutical compound or composition can be administered alone or in conjunction with other diagnostics and/or pharmaceuticals directed to the pathology, or directed to other symptoms of the pathology. Effective amounts of a diagnostic pharmaceutical compound or composition of the invention are from about 0.1ug to about 100 mg/kg body weight, administered at intervals of 4-72 hours, for a period of 2 hours to 1year, and/or any range or value therein, such as 0.0001-1.0, 1-10,10-50 and 50-100, 0.0001-0.001, 0.001-0.01, 0.01-0.1, 0.1-1.0,1.0-10, 5-10, 10-20, 20-50 and 50-100 mg/kg, at intervals of 1-4,4-10, 10-16, 16-24,24-36, 24-36,36-48, 48-72 hours, for a period of 1-14, 14-28, or 30-44 days, or 1-24 weeks, or any range or value therein. The recipients of administration of compounds and/or compositions of the invention can be any vertebrate animal, such as mammals. Among mammals, the preferred recipients are mammals of the Orders Primate (including humans, apes and monkeys), Arteriodactyla (including horses, goats, cows, sheep, pigs), Rodenta (including mice, rats, rabbits, and hamsters), and Carnivora (including cats, and dogs). Among birds, the preferred recipients are turkeys, chickens and other members of the same order. The most preferred recipients are humans.

Process of Data Transfer

The present invention also relates to a process that comprises a method for producing a product comprising identifying an agent according to one of the disclosed processes for identifying such an agent (i.e., the therapeutic agents identified according to the assay procedures disclosed herein) wherein said product is the data collected with respect to said agent as a result of said identification process, or assay, and wherein said data is

sufficient to convey the chemical character and/or structure and/or properties of said agent. For example, the present invention specifically contemplates a situation whereby a user of an assay of the invention may use the assay to screen for compounds having the desired enzyme modulating activity and, having identified the compound, then conveys that information (i.e., information as to structure, dosage, etc) to another user who then utilizes the information to reproduce the agent and administer it for therapeutic or research purposes according to the invention. For example, the user of the assay (user 1) may screen a number of test compounds without knowing the structure or identity of the compounds (such as where a number of code numbers are used the first user is simply given samples labeled with said code numbers) and, after performing the screening process, using one or more assay processes of the present invention, then imparts to a second user (user 2), verbally or in writing or some equivalent fashion, sufficient information to identify the compounds having a particular modulating activity (for example, the code number with the corresponding results). This transmission of information from user 1 to user 2 is specifically contemplated by the present invention.

In one embodiment of the foregoing, the present invention relates to a method for producing test data with respect to the $Na_V1.7$ polynucleotide modulating activity of a compound, comprising

a) contacting a chemical agent with a polynucleotide corresponding to a $Na_V1.7$ polynucleotide, or corresponding to a $Na_V1.7$ promoter sequence polynucleotide and under conditions promoting expression of such polynucleotide;

b) detecting a change in the expression of said polynucleotide as a result of said contacting; and

(c) producing test data with respect to the $Na_V1.7$ polynucleotide modulating activity of said compound based on a change in the expression of the determined $Na_V1.7$ polynucleotide modulating activity indicating such modulating activity.

Diagnostics and Pharmacogenomics

In a further embodiment, the invention relates to diagnostic and pharmacogenomic compounds, kits and methods. This aspect relates to analysis $Na_V1.7$ for the diagnosis of insensitivity or indifference to pain, primary erythermalgia, familial rectal pain, other pain disorder, or in the selection of a therapeutic agent for a patient (i.e. pharmacogenomics).

For example, nucleic acid analysis can be used to identify the mutations in Table 2 (e.g. G5067A or the C984A mutation) thus confirming the diagnosis of Congenital Indifference to Pain. Many nucleic acid diagnostic techniques are well known to those skilled in the art. Such techniques include DNA sequencing, hybridization probing, single stranded conformational analysis, PCR based techniques such as mismatch amplification, and myriad other well known methods. All such analysis can be performed on a small sample of blood, saliva, urine or other tissue provided by the patient.

Alternatively, protein based analyses such as antibody based assays (Elisa, Radioimmunoassay and the like) can be employed to identify the expression, amount or presence or absence of a mutant $Na_V1.7$ protein (for example, those mutations identified in Table 2), such as the W1689X or Y328X mutant.

Gene expression, both comparable and absolute, as well as biological activity, and mutational analysis can each serve as a diagnostic tool for pain disorders; thus determination of the amount of $Na_V1.7$ mRNA can be used to diagnose the presence or absence of a mutation correlated with such pain disorder.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons (Eichelbaum, M., Clin. Exp. Pharmacol. Physiol., 23:983-

985, 1996; Linder, M. W., Clin. Chem., 43:254-266, 1997). In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). Altered drug action may occur in a patient having a polymorphism (e.g., an single nucleotide polymorphism or SNP) in promoter, intronic, or exonic sequences of $Na_V1.7$. Thus by determining the presence and prevalence of polymorphisms allow for prediction of a patient's response to a particular therapeutic agent.

This pharmacogenomic analysis can lead to the tailoring of drug treatments according to patient genotype, including prediction of side effects upon administration of therapeutic agents, particularly therapeutic agents for treating disorders disclosed in this specification. Pharmacogenomics allows for the selection of agents (e.g., drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (e.g., the genotype of the individual is examined to determine the ability of the individual to respond to a particular agent).

Diagnostics employing a gene or protein corresponding to $Na_V1.7$ can also be useful in selecting patients for clinical trials of a potential therapeutic agent. Patients can be stratified according to the DNA or protein sequence of $Na_V1.7$ and their response to drug treatment can be evaluated. Such stratification can greatly reduce the number of patients required to establish efficacy for a potential therapeutic agent.

Sequences 1 to 20 have the following identifications:

SEQ ID NO: 1 - Homo sapiens sodium channel, voltage-gated, type IX, alpha polypeptide ($Na_V1.7$), mRNA sequence

SEQ ID NO: 2 - Homo sapiens sodium channel, voltage-gated, type IX, alpha polypeptide ($Na_V1.7$), protein sequence

SEQ ID NO: 3 - Homo sapiens sodium channel, voltage-gated, type IX, alpha polypeptide ($Na_V1.7$), mRNA sequence, predicted splice variant

SEQ 4 - $Na_V1.7$ predicted splice variant

SEQ 5 - Homo sapiens sodium channel, voltage-gated, type IX, alpha polypeptide ($Na_V1.7$), mRNA, alternately spliced transcript using exon 5N and alternate splice donor site for exon 11 (11B)

SEQ 6 - Homo sapiens sodium channel, voltage-gated, type IX, alpha polypeptide ($Na_V1.7$), protein sequence, alternately spliced isoform using exon 5N and alternate splice donor site for exon 11 (11B)

SEQ 7 - Homo sapiens sodium channel, voltage-gated, type IX, alpha polypeptide ($Na_V1.7$), mRNA sequence, CIP-10 mutant

SEQ 8 - $Na_V1.7$ W1689X protein sequence

SEQ 9 - Homo sapiens sodium channel, voltage-gated, type IX, alpha polypeptide ($Na_V1.7$), mRNA sequence, CIP-14 mutant

SEQ ID NO: 10 - $Na_V1.7$ Y328X protein sequence

SEQ ID NO: 11 - Homo sapiens sodium channel, voltage-gated, type IX, alpha polypeptide ($Na_V1.7$), mRNA sequence, c.2488C>T mutation in CIP-08

SEQ ID NO: 12 - Homo sapiens sodium channel, voltage-gated, type IX, alpha polypeptide ($Na_V1.7$), protein sequence, R830X CIP-08 mutation

SEQ ID No. 13 - Partial Sequence from human BAC clone RP11-437H3 (AC108146) showing genomic arrangement of Exon 5A and Exon 5N. The first exon is the 3' end of Exon 4 (residues 1 – 16). Next Exon is Exon 5N

(residues 1479-1570). The one following is Exon 5A (residues 1686-1777). The final exon is the 5' end of Exon 6 (residues 2506-2521).

SEQ ID NO: 14 - human genomic sequence containing the $Na_V1.7$ promoter region ending with the ATG of the initiator methionine codon; a partial cDNA sequence appears as residues 9913-9963.

SEQ ID NO: 15 - predicted mouse $Na_V1.7$ 11B isoform

SEQ ID NO: 16 - predicted mouse $Na_V1.7$ 11B isoform

SEQ ID NO: 17 - predicted mouse $Na_V1.7$ 5A isoform

SEQ ID NO: 18 - predicted_1 mouse $Na_V1.7$ 5A isoform

SEQ ID NO: 19 - predicted mouse $Na_V1.7$ 5N isoform

SEQ ID NO: 20 - predicted mouse $Na_V1.7$ 5N isoform

In carrying out the procedures of the present invention it is of course to be understood that reference to particular buffers, media, reagents, cells, culture conditions and the like are not intended to be limiting, but are to be read so as to include all related materials that one of ordinary skill in the art would recognize as being of interest or value in the particular context in which that discussion is presented. For example, it is often possible to substitute one buffer system or culture medium for another and still achieve similar, if not identical, results. Those of skill in the art will have sufficient knowledge of such systems and methodologies so as to be able, without undue experimentation, to make such substitutions as will optimally serve their purposes in using the methods and procedures disclosed herein.

The invention is described in more detail in the following non-limiting examples. It is to be understood that these methods and examples in no way limit the invention to the embodiments described herein and that other

embodiments and uses will no doubt suggest themselves to those skilled in the art.

Example 1

Identification of the genetic mutation responsible for Congenital Indifference to Pain (CIP) in humans.

We collected a multigenerational family with 3 affecteds (CIP-14) **Figure 1** and a smaller family with 1 affected (CIP-10) **Figure 2** reported previously (Guillermo A. and A. Grinspan. 1970. Rev Neurol (Paris) 123(6): p. 434-5.)

31 family members from CIP-14 were genotyped at 763 autosomal markers and at 48 X markers. A genome-wide two-point analysis identified 8 regions with LOD scores >1.0. A region on chromosome 2 had two consecutive markers - D2S2330-D2S335 - spanning 6cM, with positive scores. A LOD score of 1.51 at zero recombination was obtained at D2S2330. Multi-point analysis of this region was consistent with linkage to CIP. The CIP-14 pedigree contains 3 affecteds, one issued from a consanguineous marriage, and two expected to share one allele identical by descent with the first case and one allele identical by descent between themselves. Because the pedigree comes from an isolated population, it can be expected that there would be linkage disequilibrium at the disease gene, and that all 3 affected would share alleles. However, as there was only one allele that could be assumed to be inherited identically by descent to the 3 affected, a scoring method was designed to assign scores to allele sharing taking that into consideration. The allele sharing algorithm identified 4 regions as having two-point LOD scores >1.0, and identified 3 additional regions that had not been excluded in two-point linkage analysis.

Haplotype construction using 12 polymorphic markers in candidate regions identified by 2-point linkage analysis and allele-sharing methods suggested that only the D2S2330-D2S335 region was consistent with linkage to CIP. All 3 affected shared one chromosome in common inherited from 3006/3009. Individuals 0000 and 0002 share another chromosome in common inherited from a 3004/3005. A005 has a chromosome introduced through 1020 that appears to be identical by state (and potentially identical by descent) to the one inherited from 3006/3009, which may be explained by the consanguinity of the population. The homozygous haplotypes in the 3 affected could represent multiple copies of a single ancestral chromosome. Recombination with the proximal marker D2S306, and the distal marker, D2S2188, is seen in 2002 and 0002/A006, respectively, provided centromeric and telomeric boundaries for the disease gene at the 2q24-31 locus.

Definition of the CIP minimal genomic region

The interval between D2S306 and D2S2188 corresponds to a genetic size of ~16cM. To increase the resolution of the interval, we performed fine mapping with 32 additional markers, including several novel dinucleotide-repeat markers identified from genomic sequence. Haplotype construction using 41 polymorphic markers revealed one proximal and 5 distal recombinations. Of the 6 recombinant individuals, one was affected and 5 were carriers. The additional data narrowed the boundaries defined in 2002 and A006 to approximately 11.2MB, between CA1AC010876 and D2S1267.

To assess whether the disease in CIP-10 maps to the 2q24-31 locus, five family members were genotyped using the 41 polymorphic markers described above. The proband was homozygous for markers between D2S2299 and D2S2177, and the genotypic data was consistent with linkage to CIP. Haplotype construction revealed a candidate interval of ~19cM defined by inferred recombination events that must have occurred in the untyped generations.

Use of genetic data from CIP-14 and CIP-10 together provided a centromeric boundary at CA1AC010876 (based a meiotic recombination in CIP14-2002) and a telomeric boundary at D2S2177 (based on the haplotype sharing data of CIP-10).

Mutation Analysis

We next embarked on a positional cloning effort. According to the UCSC August 2001 genomic sequence assembly, the CIP genomic region contains 26 known genes, five of which code for alpha polypeptides of voltage-gated sodium channels. We initiated sequencing of the coding regions of $Na_V1.7$ and SCN3A in CIP-14-A005 and CIP-10-503, theorizing that selective expression in dorsal root ganglia and upregulation in models of neuropathic pain, respectively, made them interesting candidate genes. In CIP-10-503, we detected a homozygous nucleotide change of G>A at nt 5067 in $Na_V1.7$ that resulted in a change to a stop codon at tryptophan 1689 (**Figure 4a**). The stop codon results in deletion of 289 amino acids at the C-terminal of $Na_V1.7$, including the last transmembrane domain of the fourth channel domain. In CIP-14-A005 we detected a homozygous nucleotide change of 984C>A in $Na_V1.7$ that resulted in a change to a stop codon at tyrosine 328 (**Figure 4b**). We developed PCR-RFLP assays and showed that both mutations cosegregated perfectly with the disease and the affected haplotypes. Both mutations were absent in 142 control chromosomes from individuals of similar ancestral background. A third mutation was identified in a CIP proband descended from first cousins in another previously identified family. **Figure 3** (*See* Lievre JA, et al. 1968. Bull Mem Soc Med Hop Paris. 1968 Mar 15;119(5):447-56.) Patient CIP-08-II:1 is homozygous for a C>T substitution at nt 2488 that corresponds to a change to a stop codon at arginine 830 (**Figure 4c**).

Alternative Exons 5N and 5A

The rabbit $Na_V1.7$ gene (NaS), the rat and human SCN2A and SCN3A genes, and the human SCN8A gene contain two alternatively spliced exons encoding segments S3 and S4 of channel domain I. (*See* Belcher SM, et al. Proc Natl Acad Sci U S A. 1995 Nov 21;92(24):11034-8; Sarao R, et al. Nucleic Acids Res. 1991 Oct 25;19(20):5673-9; Gustafson TA, et al. J Biol Chem. 1993 Sep 5;268(25):18648-53; Lu CM, and Brown GB. J Mol Neurosci 1998 Feb; 10(1):67-70; and Plummer NW, et al. Genomics. 1998 Dec 1;54(2):287-96.) The two exons are separated by introns of ~90-150 bp. The isoforms are believed to be developmentally regulated, and without being bound to theory, it is suggested that the upstream exon (N) is predominantly expressed during the neonatal period, while the downstream exon (A) is expressed in adult brain. To determine whether this organization is conserved in human $Na_V1.7$, we aligned the genomic sequence from BAC clone RP11-437H3 (Genbank accession # AC108146) SEQ ID No. 13, with the human and rabbit coding sequences, and found that two potential copies of exon 5 are separated by 115 bp (**Figure 5**). The exons contain two predicted amino acid differences. Alternative splicing for these exons is illustrated in **Figure 5**. Residue 201 in the second exon is valine rather than leucine and residue 206 (corresponding to 209 in SCN2A and 208 in SCN3A) is aspartic acid rather than asparagine. A species and channel comparison in **Figure 6** shows the conserved relationship between isoforms. Because their physical arrangement and amino acid sequence correspond to those in other sodium channel genes, they are designated exons 5N and 5A. It has been suggested that the proximity of the residue 206 in these exons and the positively charged residues of the voltage sensor in transmembrane segment IS4 (see **Figure 7**) may alter the voltage dependence or permeability of the neonatal and adult channels. Plummer NW, supra.

We used PCR to amplify the region of human $Na_V1.7$ described above from total RNA of adult dorsal root ganglia (DRG) to examine exon usage within intact cDNAs. Primers designed to specifically bind cDNAs containing either exon 5A or exon 5N produced fragments of the expected size and sequencing confirmed mutually exclusive use of both exons.

Alternative Splice Donor for Exon 11

Two clones containing exons 1-13 of $Na_V1.7$ amplified from human DRG were sequenced. One clone contained an additional 33 nucleotides located between the sequences of exon 11 and exon 12. To identify the origin of the extra 33 bp in the novel transcript, we examined the genomic sequence of the human $Na_V1.7$ gene. Two alternative splice donor sites separated by 33 bp were identified. Splicing at the downstream site generates the novel transcript that encodes a protein with 11 additional amino acids in cytoplasmic loop 1 (**Figure 8**). The sequence of the additional amino acids is conserved in rat and rabbit $Na_V1.7$ and additional members of the channel gene family (**Figure 9**)

Example 2

RT-PCR analysis of relative $Na_V1.7$ expression.

cDNAs from a variety of human tissues were used to perform relative quantitative RT-PCR with primers and conditions that specifically amplified the $Na_V1.7$ transcript (**Figure 10**). Experiments to determine the tissue distribution of $Na_V1.7$ were performed with three separate primer sets. Dorsal root ganglia was included as a positive control and β-actin was included as an internal standard. We found highest levels of $Na_V1.7$ relative to the β-actin standard in dorsal root ganglia and medulla oblongata. Lower levels of $Na_V1.7$ product were detected in the samples from temporal lobe, thymus, hippocampus, cerebral cortex, amygdala, frontal lobe, spinal cord, occipital lobe, cerebellum, thalamus, and ovary. $Na_V1.7$ expression was not detected in cDNA from parietal lobe, corpus callosum, cerebral peduncles, pons, thyroid, or placenta. The sequences of the RT-PCR products amplified from dorsal root ganglia and medulla oblongata were identical to human $Na_V1.7$ cDNA. Previous studies have reported low levels of $Na_V1.7$ in the CNS

relative to the PNS, but we have detected significant levels in the CNS, and particularly high levels in the medulla oblongata.

This expression profile teaches those skilled in the art where compounds which modulate $Na_V1.7$ gene expression or $Na_V1.7$ protein activity are likely to have effect. For example, expression of $Na_V1.7$ in the CNS may support a role for this gene in central perception of pain, although alternative theories are suggested. It also is strongly suggestive of the other therapeutic indications for $Na_V1.7$ modulating agents.

Example 3

Assaying for Potent and Selective $Na_V1.7$ Blockers and Determination of IC-50

This example describes an *in vitro* assay for testing and profiling test agents against human sodium channels stably expressed in human embryonic kidney (HEK293) cells. The assay is also useful for determining the IC-50 of a sodium channel blocking compound.

The assay is based on the guanidine flux assay (also called the guanidine influx assay) described by Reddy NL, Fan W, Magar SS, Perlman ME, Yost E, Zhang L, Berlove D, Fischer JB, Burke-Howie K, Wolcott T, Durant GJ. Synthesis and pharmacological evaluation of N,N'-diarylguanidines as potent sodium channel blockers and anticonvulsant agents. J Med Chem. 1998 Aug 13;41(17):3298-302.

The guanidine influx assay is a radiotracer flux assay used to determine ion flux activity of sodium channels in a high-throughput microplate-based format. The assay uses ^{14}C-guanidine hydrochloride in combination with various known sodium channel modulators, to assay the potency of test agents. Potency is determined by an IC-50 calculation. Selectivity is determined by comparing potency of the compound for $Na_V1.7$ to its potency against other sodium channels (also called 'selectivity profiling').

Each of the test agents is assayed against cells that express the channels of interest. The following table summarizes endogenous cell lines and the sodium channels they express.

Table 6.

CELL LINE	mRNA Expression	Functional Characterisation
TT Cells (Human Medulary thyroid carcinoma) ATTC number **CRL-1803**	• Strong $Na_V1.7$ expression • The $hNa_V1.7$ cDNA was originally cloned from TT cells (Hoffman et al) • Weak expression of $Na_V1.6$, 1.4	• Stimulation of TT cells with Aconitine and inhibition of inactivation with scorpion toxin resulted in a ten-fold increase in [^{14}C] Guanidine influx above background. • The influx was completely blocked using TTX. ($Na_V1.7$ is a TTX sensitive channel)
CHO-K1 (Chinese Hamster Ovary; recommended host cell line) ATTC accession number CCL-61	• $Na_V1.4$ expression has been shown by RT-PCR • amplicon sequence confirmed $Na_V1.4$. • No other Na_V expression has been detected	• Stimulation of CHO cells with Aconitine and inhibition of inactivation with scorpion toxin resulted in a 18-20-fold increase in [^{14}C] Guanidine influx above background. • The influx was completely blocked using TTX. ($Na_V1.4$ is a TTX sensitive channel)

L6 (rat myoblast cell) ATTC Number CRL-1458	• Expression of Nav1.4 and 1.5	• Stimulation of L6 cells with Aconitine and inhibition of inactivation with scorpion toxin resulted in a 10-15 fold increase in [^{14}C] Guanidine influx above background. • The influx was only partially blocked by TTX ($Na_V1.5$ is TTX resistant)
SH-SY5Y (Human neuroblastoma) ATTC Number CRL-2266	• Expression of $Na_V1.9$, $Na_V1.8$ and $Na_V1.7$ (Blum et al)	• Stimulation of SH-SY5Y cells with Aconitine and inhibition of inactivation with scorpion toxin resulted in a 16-fold increase in [^{14}C] Guanidine influx above background. • The influx was partially blocked by TTX ($Na_V1.9$ is TTX resistant) • *See* Canc. Res. 1978. 38:3751; J. Nat. Canc. Inst. 1983. 71:741.
PC12 (rat pheochromocytoma) ATTC Number CRL-1721	• Expression of $Na_V1.2$	•
BE(2)-C (ECACC No. 95011817) clonal sub-line of neuroblastoma cell line AK-N-BE(2) (ECCAC No. 95011815)	• Expression of $Na_V1.8$	•
ND7-23 (dorsal root ganglion neuroblastoma cell line	• Expression of $Na_V1.8$	• John et al., Neuropharmacology, 46:425-38 (2004)

It is also possible to employ recombinant cells expressing these sodium channels. Cloning and propagation of recombinant cells are known to those skilled in the art (see for example Klugbauer N, Lacinova L, Flockerzi V, Hofmann F. Structure and functional expression of a new member of the tetrodotoxin-sensitive voltage-activated sodium channel family from human

neuroendocrine cells. EMBO J. 1995 Mar 15;14(6):1084-90). Neuron. 2002 Jun 13;34(6):877-84.)

To improve the assay, each of these sodium channels are modulated in an agonist-dependent manner, consisting of aconitine (a site 2 modulator that opens the channel) and *Leiurus quinquestriatus hebraeus* scorpion venom (a site 3 modulator that serves to destabilize the inactivated state of the channel).

Further details of this protocol are as follows:

Cells expressing Nav1.1, 1.3, 1.4, 1.5, and 1.7 are grown to 70-80% confluency in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum 1X Penicillin/ Streptomycin/ Glutamine (PSG) and 1200 μg/ml G418 (Gibco/Invitrogen) at 37 °C/5 % CO_2.

Cells expressing Nav1.8 are grown to 70-80% confluency in DMEM supplemented with 10% FBS, 1 X PSG, 600 μg/ml G418 and 90 μg/ml hygromycin B (Gibco/Invitrogen).

The cells are disassociated from the culture dishes with an enzymatic solution (1X) Trypsin/EDTA (Gibco/Invitrogen) and analyzed for density and viability using haemocytometer (Neubauer). Disassociated cells are washed and resuspended in their culture media at a density of approximately 1,000,000 cells/mL. The cells are plated using the Biomex FX liquid handler (Beckman Coulter Inc.) into custom-coated PDL Scintiplates (Beckman Coulter Inc.) at a density of approximately 125,000 cells/ well (150μl/well) and incubated at 37 °C/5 % CO_2.

At the end of the 20-24 hours, the cells are extensively washed with Low sodium HEPES-buffered saline solution (LNHBSS) (150 mM Choline Chloride, 20 nM HEPES (Sigma), 1mM Calcium Chloride, 5mM Potassium Chloride, 1 mM Magnesium Chloride, 10 mM Glucose). To each well are added 10 μl of test agent and 10 μl of activation/radiolabel mixture, both as described below. Test agent is obtained in dry DMSO films containing 0.4 mmols of compound. On the day of the test, films are reconstituted in 40 –

200 µl of DMSO (to give a final concentration of 10 – 2 mM) for 20 mins at room temperature. Agents are then diluted with LNHBSS to give a 10X starting concentration for subsequent use in dose response experiments (i.e. 20 µM initial concentration if desired final plate concentration is 2 µM). (Varying concentrations of test agent may be used, although 4 mM/well was selected for a standard reaction.)The activation/radiolabel mixture for all cell lines except Nav1.8 contains 10 µM aconitine (Sigma), 10 µg/ml scorpion venom (*L. quinquestriatus hebraeus*) (Sigma), and 0.05 µCi ^{14}C-guanidine hydrochloride (ARC), The activation/radiolabel mixture for cells expressing Nav1.8 contains 25 µg/ml scorpion venom, 40 µM tefluthrin, 25 µM veratridine (Sigma), and 0.05 µCi ^{14}C-guanidine hydrochloride.

After loading with cells, test agent and activation/radiolabel mixture, the Scintiplates are incubated at room temperature for 2 hours.

Following the incubation, the Scintplates are extensively washed with LNHBSS supplemented with 10 mM guanidine (Sigma). The Scintiplates are dried either at room temperature overnight or at 55°C for 45 – 60 minutes and cooled and then counted using a Wallac MicroBeta TriLux (Perkin-Elmer Life Sciences). The ability of the test agent to block sodium channel activity is determined by comparing the amount of ^{14}C-guanidine present inside the cells expressing the different sodium channels. Based on this data, a variety of calculations, as set out elsewhere in this specification, may be used to determine whether a test agent is selective for $Na_V1.7$.

IC-50 value of a test agent for a specific sodium channel may be determined using the above general method. IC-50 may be determined using a 3, 8, 10, 12 or 16 point curve in duplicate or triplicate with a starting concentration of 1, 5 or 10µM diluted serially with a final concentration reaching the sub-nanomolar, nanomolar and low micromolar ranges . Typically the mid-point concentration of test agent is set at 1 µM, and sequential concentrations of half dilutions greater or smaller are applied (e.g. 0.5 µM; 5 µM and 0.25 µM; 10 µM and 0.125 µM; 20 µM etc.). The IC-50

curve is calculated using the 4 Parameter Logistic Model or Sigmoidal Dose-Response Model formula (fit = (A+((B-A)/(1+((C/x)^D))))).

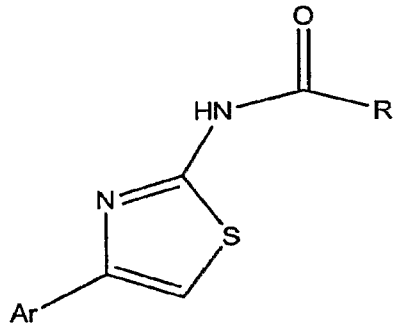
The fold selectivity, factor of selectivity or multiple of selectivity, is calculated by dividing the IC-50 value of the test sodium channel by the reference sodium channel ($Na_V1.7$).

Example 4

Compounds of Formula (I)

1. Effect of Compounds of Formula I on sodium channels.

The present invention provides thiazole derivatives that selectively inhibit ion flux activity of $Na_V1.7$ over $Na_V1.3$. Derivatives that preferably inhibit $Na_V1.7$ more effectively than any of the other sodium channels are also encompassed. Accordingly, in one aspect, the invention provides compounds of formula (I):



(I)

wherein:

R is $-(CH_2)_n(X)_mAr$; n is 0, 1, 2 or 3; m is 0 or 1; and

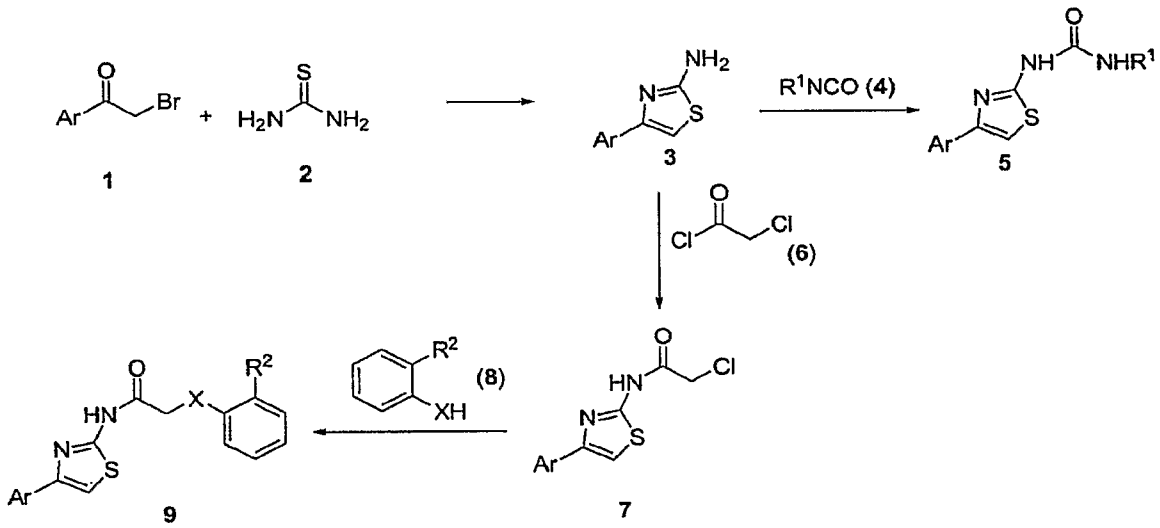
Ar is selected from the group consisting of phenyl, substituted phenyl, naphtyl, substituted naphtyl, heteroaryl, and substituted heteroaryl;

X is CH_2, O, S or NH;

including a stereoisomer, enantiomer or tautomer thereof, a pharmaceutically acceptable salt thereof, a pharmaceutical composition thereof or a prodrug thereof.

The following Reaction Schemes illustrate methods to make compounds of this invention. It is understood that one of those skilled in the art would be able to make these compounds by similar methods or by methods known to one skilled in the art. In general, starting components may be obtained from sources such as Sigma Aldrich, Lancaster Synthesis, Inc., Maybridge, Matrix Scientific, TCI, and Fluorochem USA, etc. or synthesized according to sources known to those skilled in the art (see, e.g., Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, 5th edition (Wiley, December 2000)) or prepared as described in this invention.

Scheme 1



The compounds of Formula (I) can be prepared as illustrated in Scheme 1. Reaction of bromoketone **1** with thiourea **2** provides 2-aminothiazole **3**. Treatment of **3** with isocyanate **4** affords the urea product **5** of Formula (I). On the other hand, the aminothiazole **3** can react with chloroacetyl chloride (**6**) to form amide **7**, which leads to the formation of **9** of Formula (I) by reacting with a reagent **8**.

Table 8 lists the fold selectivity for $Na_V1.7$ over other sodium channels obtained for test compounds of Formula (I), which were tested for its inhibitory effects on sodium channels selected from among $Na_V1.1$, $Na_V1.3$, $Na_V1.4$,

$Na_V1.5$, $Na_V1.7$, and $Na_V1.8$ as in example 3 described above. The results of the studies demonstrate directly that the test compounds selectively inhibit $Na_V1.7$ over $Na_V1.3$ by a multiple of up to 100 times.

Stated in the alternative, the measure of $IC\text{-}50_{NaV1.3}/IC\text{-}50_{NaV1.7}$ is up to 100. More specifically, the therapeutic agent has $IC\text{-}50_{NaV1.7}$ of less than 6 μM and $IC\text{-}50_{NaV1.3}$ of greater than 10 μM. Further, the results demonstrate that the compound is selective for $Na_V1.7$ over sodium channels other than $Na_V1.3$ by a multiple of up to 55, that is, the measure of $IC\text{-}50_{(other\ sodium\ channel)}/IC\text{-}50_{NaV1.7}$ is up to 55.

Compounds of Formula (I) also have a much greater potency and selectivity for $Na_V1.7$ than the known compounds lamotrigine and carbamazepine, Table 8 records that potency of Formula I compounds is 3 to 14 fold higher than known compounds. In addition, Compound 1, for example has higher fold selectivity for $Na_V1.7$ ahead of every other tested sodium channel than any other tested compound.

TABLE 8 INHIBITORY EFFECT OF TEST COMPOUND OF FORMULA (I) ON SODIUM CHANNELS

	Fold Selectivity for $Na_V1.7$ (Potency in μM)				
Sodium Channel assayed	**Compound XA**	**Compound XB**	**Compound XC**	**Lamotrigine**	**Carbamazepine**
$Na_V1.7$	1 (4.816)	1 (3.896)	1 (1.852)	1 (14.5)	1 (26.7)
$Na_V1.1$	55	1	3	>6.89	>3.74
$Na_V1.3$	>100	>100	5	>6.89	>3.74
$Na_V1.4$	29	1	1	1.26	>3.74
$Na_V1.5$	37	1	0.5	0.724	0.79
$Na_V1.8$	N/D	3	3	2.16	>3.74

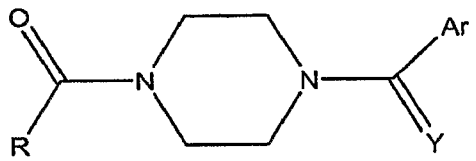
Test agents were also tested against the hERG channel. Inhibition of hERG activity after incubation with 10 μM of the test compounds in a standard hERG assay was measured. Results of the experiments demonstrated the representative test compounds had limited activity as inhibitors of hERG.

Example 5

Compounds of Formula (II)

1. Effect of Compounds of Formula (II) on sodium channels.

The present invention provides aminopyridinyl piperidine derivatives that selectively inhibit ion flux activity of $Na_V1.7$ over $Na_V1.8$. Derivatives that preferably inhibit $Na_V1.7$ more effectively than any of the other sodium channels are also encompassed. Accordingly, in one aspect, the invention provides compounds of formula (II):



(II)

wherein:

R is $-(CH_2)_n(X)_m(CH_2)_n(X)_m(CH_2)_nZ$ wherein n are each independently 0, 1, 2 or 3; m are each independently 0 or 1;

Ar is selected from the group consisting of phenyl, substituted phenyl, naphtyl, substituted naphtyl, heteroaryl, substituted heteroaryl, and substituted heteroarylaminoheteroaryl;

X are each independently CH_2, O, S or NH;

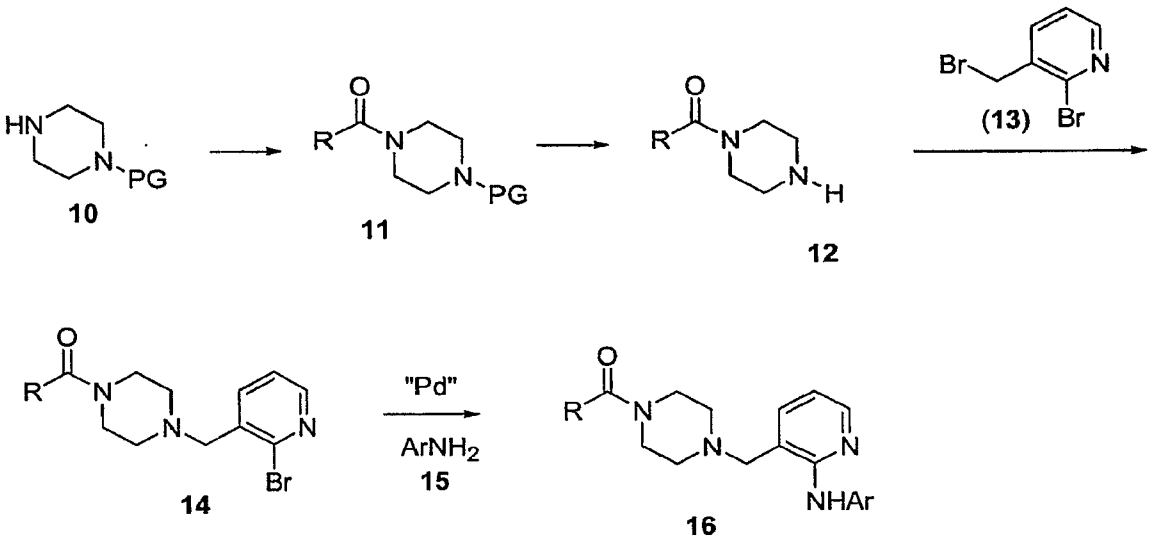
Y is H or O; and

Z is alkyl, Ar, amino, alkylamino or dialkyamino;

including a stereoisomer, enantiomer or tautomer thereof, a pharmaceutically acceptable salt thereof, a pharmaceutical composition thereof or a prodrug thereof.

The following Reaction Schemes illustrate methods to make compounds of this invention.

Scheme 2



The compounds of Formula (II) can be prepared as illustrated in Scheme 2. Reaction of protected piperazine **10** with either an alcohol in the presence of carbonyldiimidazole (CDI) or an acyl chloride in the presence of a base such as triethylamine provides carbamate or amide **11**, respectively. The protecting group can be removed using the method described in Green, T.W. and P.G.M. Wutz, *Protective Groups in Organic Synthesis* (1999), 3rd Ed., Wiley to give piperazine **12**, which upon treatment with a bromide such **13**, to form compound **14**. Under palladium catalysis (for example see Buchwald, S. L. et al *J. Org. Chem.* **2000**, 65, 1158), compound **14** can react with an aryl or heteroarylamine **15** to provide the final product **16** of Formula (II).

Table 9 lists the fold selectivity for $Na_V1.3$ over other sodium channels obtained for test compounds of Formula (II) tested for its inhibitory effects on sodium channels selected from among $Na_V1.1$, $Na_V1.3$, $Na_V1.4$, $Na_V1.5$, $Na_V1.7$, and $Na_V1.8$ as in example 3 described above. The results of the studies demonstrate directly that the test compounds selectively inhibit $Na_V1.7$ over $Na_V1.8$ by a multiple of up to 55 times. Further, the results demonstrate that the compound is selective for $Na_V1.7$ over other sodium channels by a multiple of up to 55 times. Stated in the alternative, the measure of $IC\text{-}50_{NaV1.8}/IC\text{-}50_{NaV1.7}$ is up to 55. More specifically, the therapeutic agent has $IC\text{-}50_{NaV1.7}$ of less than 6 μM and $IC\text{-}50_{NaV1.8}$ of greater than 10 μM. Further, the results demonstrate that the compound is selective for $Na_V1.7$ over sodium channels other than $Na_V1.8$ by a multiple of up to 55, that is, the measure of $IC\text{-}50_{(other\ sodium\ channel)}/IC\text{-}50_{NaV1.7}$ is up to 55.

Compounds of Formula (II) also have a much greater potency and selectivity for $Na_V1.7$ than the known compounds lamotrigine and carbamazepine, Table 9 records that potency of Formula I compounds is XX to XX fold higher than known compounds. In addition, Compound 6, for example has higher fold selectivity for $Na_V1.7$ ahead of $Na_V1.8$ than any other tested compound.

TABLE 9 INHIBITORY EFFECT OF TEST COMPOUND OF FORMULA II ON SODIUM CHANNELS

	Fold Selectivity for $Na_V1.7$ (Potency in μM)			
Sodium Channels	**Compound XE**	**Compound XF**	**Lamotrigine**	**Carbamazepine**
$Na_V1.7$	1 (1.584)	1 (0.188)	1 (14.5)	1 (26.7)
$Na_V1.1$	>10.	16	>6.89	>3.74
$Na_V1.3$	>10.	>55	>6.89	>3.74
$Na_V1.4$	6	2	1.26	>3.74

$Na_V1.5$	4	>55	0.724	0.79
$Na_V1.8$	N/D	>55	2.16	>3.74

Test agents were also tested on hERG Incubation with 10 μM of the test agents in a standard hERG assay demonstrated that the representative test compounds of Formula II have limited activity as inhibitors of hERG.

Example 6

Analgesia Induced by Selective $Na_V1.7$ Blockers

Tail Flick Latency Test

For the first part of this study, 65 animals underwent assessment of baseline tail flick latency once a day over two consecutive days. These animals were then randomly assigned to one of the 11 different treatment groups including a vehicle control, a morphine control, and 9 compounds at 30 mg/kg. Following dose administration, the animals were closely monitored for signs of toxicity including tremor or seizure, hyperactivity, shallow, rapid or depressed breathing and failure to groom. The tail-flick latency of the animals was determined prior to drug treatment, and at 40, 80, 120, and 160 minutes after treatment to assess analgesic activity of the test articles. The optimal incubation time for each compound was determined via regression analysis. The analgesic activity of the test compounds was expressed as a percentage of the maximum possible effect (%MPE) and was calculated using the following formula:

$$\%\ MPE = \frac{\text{Postdrug latency} - \text{Predrug latency}}{\text{Cut-off time (10 s)} - \text{Predrug latency}} \times 100\%$$

Where;

Postdrug latency= the latency time for each individual animal taken before the tail is removed (flicked) from the heat source after receiving drug

Predrug latency= the latency time for each individual animal taken before the tail is flicked from the heat source prior to receiving drug

Cut-off time (10 s)= is the maximum exposure to the heat source time in seconds for the tail

Figure 12 shows the analgesic activity of XEN-1, a compound of the invention, in 160 minute time course. Dosing at 5 mg/kg, 10 mg/kg and 30 mg/kg ip indicated that the drug effect was statistically evident for all doses from 80 to 120 minutes, with highest maximum possible effect (%MPE) value being reached at 80 min post-drug administration for all doses. Statistical significance compared to vehicle control ($p<0.05$) was also maintained at the 160 min period for the 30 mg/kg dose.

Formalin Test

In the formalin test, all animals from the tail-flick assay were reused after a washout period of one week. These animals were assigned to the same treatment groups as those in the tail-flick assay, as noted above. Animals involved in this test were briefly habituated to the plexiglass test chamber on the day prior to experimental day for 20 minutes. On the test day, animals were randomly injected with the test articles. At 30 minutes after drug administration, 50uL of 10% formalin was injected subcutaneously into the plantar surface of the left hind paw of the rats. Video data acquisition began immediately after formalin administration, for duration of 90 minutes.

The images were captured using the Actimetrix Limelight software which stores files under the *.lii extension, and then converts it into the MPEG-4 coding. The videos are then analyzed using behavior analysis software "The Observer 5.1", (Version 5.0, Noldus Information Technology, Wageningen, The Netherlands). The video analysis is done by watching the animal behavior and scoring each according to type, and defining the length of the behavior (Dubuisson and Dennis, 1977). Scored behaviors include: (1) normal behavior, (2) putting no weight on the paw, (3) raising the paw, (4) licking/biting or

scratching the paw. The pain score is calculated from a weighted scale as described above. The duration of the behaviour is multiplied by the weight (rating of the severity of the response), and divided by the total length of observation to determine a pain rating for each animal. The calculation is represented by the following formula:

$$\text{Pain rating} = [\, 0(T_o) + 1(T_1) + 2(T_2) + 3(T_3) \,] / (\, T_o + T_1 + T_2 + T_3 \,)$$

The analgesic properties of XEN 1 were further demonstrated in the second phase of the formalin test, where animals treated with XEN 1 demonstrated lower numerical MPIE (%) analgesic responses compared to vehicle ($p<0.05$), as shown in Figure 13.

No adverse effect for XEN-1 on the normal home cage activity was observed for the animals during the test period. In contrast, morphine dosing resulted in a significant increase in the activity shortly after administration. (data not shown)

Although the invention has been described with reference to illustrative embodiments, it is to be understood that the invention is not limited to these precise embodiments, and that various changes and modification are to be intended to be encompassed in the appended claims. All publications referred to in this specification are hereby incorporated by reference in their entireties, though no such publication is hereby admitted to be a prior art reference.

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WHAT IS CLAIMED IS:

1. A method for identifying an agent that modulates the activity of $Na_V1.7$ polynucleotide, comprising:

a) contacting a test compound with a polynucleotide corresponding to a $Na_V1.7$ polynucleotide, and under conditions supporting expression of such polynucleotide;

b) detecting a change in the expression of said polynucleotide as a result of said contacting;

wherein a change in expression identifies said test compound as an agent that modulates $Na_V1.7$ polynucleotide activity.

2. The method of claim 1 wherein said change in expression in step (b) is a decrease in expression.

3. The method of claim 1 wherein said agent demonstrates selectivity for $Na_V1.7$ over other sodium channel alpha subunits.

4. The method of claim 1 wherein said expression is measured by measuring the amount of an expression product encoded by said polynucleotide.

5. The method of claim 4 wherein said expression product is an RNA.

6. The method of claim 4 wherein said expression product is a polypeptide.

7. The method of claim 1 wherein said polynucleotide is part of an intact cell.

8. The method of claim 7 wherein said intact cell is a mammalian cell.

9. The method of claim 7 wherein said intact cell is a cell that has been engineered to comprise said polynucleotide.

10. The method of claim 7 wherein said cell is a recombinant cell that has been genetically engineered to express said polynucleotide.

11. The method of claim 10 wherein said cell does not express said polynucleotide absent said engineering.

12. The method of claim 7 wherein said cell is a neuronal cell.

13. A method for identifying an agent that modulates the activity of $Na_V1.7$ polynucleotide, comprising:

a) contacting a test compound with a $Na_V1.7$ promoter operably linked to a reporter gene and under conditions supporting expression of said reporter gene;

b) detecting a change in the expression of said reporter gene as a result of said contacting;

wherein said change in expression of said reporter gene identifies said test compound as an agent that modulates $Na_V1.7$ polynucleotide activity.

14. The method of claim 12A wherein said reporter gene is a $Na_V1.7$ polynucleotide.

15. A method for identifying an agent that modulates the activity of $Na_V1.7$ polypeptide, comprising:

a) contacting a test compound with a polypeptide corresponding to an $Na_V1.7$ polypeptide in a membrane under conditions promoting ion transport across said membrane by said polypeptide; and

b) detecting a change in ion transport activity of said polypeptide as a result of said contacting;

wherein a change in ion transport identifies said test compound as an agent that modulates $Na_V1.7$ polypeptide activity.

16. The method of claim 15 wherein said change in ion transport activity in step (b) is a decrease in activity.

17. The method of claim 15 wherein said agent demonstrates selectivity for $Na_V1.7$ over other sodium channel alpha subunits.

18. The method of claim 15 wherein said change in activity is the result of binding to said polypeptide by said test compound of step (b).

19. The method of claim 15 wherein said polypeptide is present in a lipid bilayer.

20 The method of claim 15 wherein said polypeptide is part of an intact cell.

21. The method of claim 20 wherein said intact cell is a cell that has been engineered to comprise said polypeptide.

22. The method of claim 19 wherein said intact cell is a recombinant cell that has been genetically engineered to express said polypeptide.

23. The method of claim 20 wherein said cell does not express said polypeptide absent said engineering.

24. The method of claim 20 wherein said cell is a neural cell.

25. The method of claim 15 wherein said polypeptide is a polypeptide that reacts with an antibody that is specific for a polypeptide having the amino acid sequence of SEQ ID NO: 2.

26. The method of claim 25 wherein said polypeptide has an amino acid sequence at least 98% identical to the sequence of SEQ ID NO: 2 and where

any difference in amino acid sequence is due only to conservative amino acid substitutions.

27. The method of claim 25 wherein said polypeptide has the amino acid sequence of SEQ ID NO: 2.

28. A method for identifying an analgesic agent, comprising:

a) administering to an animal an agent having activity in the method of claim 1 or 13, and

b) detecting in said animal a decrease in response to a pain stimulus due to said administering and wherein said animal retains ability to respond to non-nociceptive sensory stimuli,

wherein said decrease identifies said test compound as an analgesic agent.

29. The method of claim 28 wherein said mammal is a human being.

30. The method of claim 28 wherein said non-nociceptive sensory stimuli are selected from the group consisting of hot, cold, touch and point discrimination.

31. A method for treating an animal afflicted with pain comprising administering to said animal an effective amount of a selective, voltage dependent blocker of $Na_V1.7$.

32. The method of claim 31 wherein said animal is a human patient.

33. The method of claim 31 wherein said source of pain is a cancerous condition.

34. The method of claim 1 further comprising (c) contacting said chemical agent with at least one other sodium channel, wherein a compound that exhibits a selective preference for $Na_V1.7$ over said other sodium channel is identified.

35. A screening assay for identifying a selective inhibitor of $Na_V1.7$ comprising,

a) a first screening assay which detects the concentration of a test agent required to inhibit human $Na_V1.7$ polypeptide activity; and

b) a second screening assay which detects the concentration of a test agent required to inhibit the activity of a human sodium channel polypeptide other than $Na_V1.7$,

wherein if the ratio of the detected concentration of step b) over the detected concentration of step a) is greater than the same ratio observed for lamotrigine ($Na_V1.3$) and carbamazepine ($Na_V1.8$), then the test agent is said selective inhibitor.

36. The assay of claim 35 wherein the selective inhibitor of $Na_V1.7$ has a potency of IC50 < 10 μM in the assay of step (a).

37. A method for identifying a selective inhibitor of $Na_V1.7$ comprising the steps of,

a) detecting the concentration of a test agent required to inhibit human $Na_V1.7$ polypeptide activity; and

b) detecting the concentration of said test agent required to inhibit the activity of a human sodium channel polypeptide other than $Na_V1.7$,

wherein if the ratio of the detected concentration of step b) over the detected concentration of step a) is greater than the same ratio observed for lamotrigine ($Na_V1.3$) and carbamazepine ($Na_V1.8$), then said test agent is said selective inhibitor.

38. The method of claim 35 wherein the selective inhibitor of $Na_V1.7$ has a potency in the first screening assay of IC50 < 10 μM.

39. A method for treating a $Na_V1.7$-mediated disease in an animal, comprising administering to the animal an effective amount of a therapeutic agent to reduce the ion flux activity of the $Na_V1.7$ polypeptide, which therapeutic agent has an IC-50 for $Na_V1.7$ of less than 10μM as measured in a 2 hour guanidine flux assay and wherein said therapeutic agent selectively reduces ion flux activity of $Na_V1.7$ more than $Na_V1.3$.

40. The method of claim 39 wherein the ratio of IC-50 of $Na_V1.3$ to IC-50 of $Na_V1.7$ for the therapeutic agent is at least 1.1, 1.2, 1.5, 1.7, 2, 3, 4, 5, 10, 20, 25, 50, 75, 100, 200, 500, 1000, 2000, 5000, or 10000.

41. The method of claim 40 wherein the therapeutic agent selectively inhibits ion flux activity of $Na_V1.7$ over $Na_V1.3$ by a multiple which is greater than that achieved by lamotrigine.

42. The method of claim 39 wherein the therapeutic agent has IC-$50_{NaV1.7}$ less than 6 μM.

43. The method of claim 39 wherein the therapeutic agent has an IC-$50_{NaV1.3}$ of greater than 10 μM.

44. A method for treating a $Na_V1.7$-mediated disease in an animal, comprising administering to the animal an effective amount of a therapeutic agent to reduce the ion flux activity of the $Na_V1.7$ polypeptide, which therapeutic agent has an IC-50 for $Na_V1.7$ of less than 10μM as measured in a 2 hour guanidine flux assay and wherein said agent selectively reduces ion flux activity of Na_V 1.7 more than a different peripheral sodium channel.

45. The method of claim 44 wherein the peripheral sodium channel is selected from among $Na_V1.4$, $Na_V1.5$, $Na_V1.6$, $Na_V1.8$ and $Na_V1.9$.

46. The method of claim 45 wherein the peripheral sodium channel is $Na_V1.8$.

47. The method of claim 44 wherein the ratio of IC-50 of $Na_V1.8$ to IC-50 of $Na_V1.7$ for the therapeutic agent is at least 1.1, 1.2, 1.5, 1.7, 2, 3, 4, 5, 10, 20, 25, 50, 75, 100, 200, 500, 1000, 2000, 5000, or 10000.

48. The method of claim 45 wherein the therapeutic agent selectively inhibits ion flux activity of $Na_V1.7$ over $Na_V1.8$ by a multiple which is greater than that achieved by carbamazepine.

49. The method of claim 46 wherein the therapeutic agent has an $IC\text{-}50_{NaV1.8}$ of less than 6 μM.

50. The composition of claim 44 wherein the therapeutic agent has an $IC\text{-}50_{NaV1.3}$ of greater than 10 μM.

51. The method of claim 39 or 44 wherein said agent also selectively reduces ion flux activity of $Na_V1.7$ more than the ion flux activity of $Na_V1.5$.

52. The method of claim 51 wherein the ratio of IC-50 for $Na_V1.5$ to IC-50 against $Na_V1.7$ for the therapeutic agent is at least 1.1, 1.2, 1.5, 1.7, 2, 3, 4, 5, 10, 20, 25, 50, 75, 100, 200, 500, 1000, 2000, 5000, or 10000.

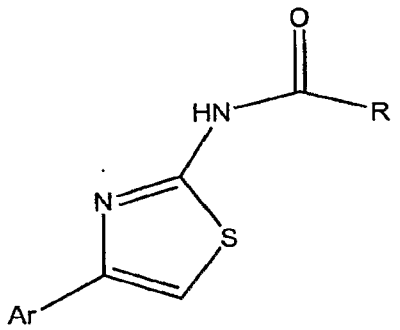
53. The method of claim 44 wherein said agent has an IC-50 for $Na_V1.7$ which is lower than its IC-50 for $Na_V1.1$, Na_V 1.2 or $Na_V1.3$. (CNS channels)

54. The method of claim 39 wherein said agent has an IC-50 for $Na_V1.7$ which is lower than its IC-50 for $Na_V1.1$, Na_V 1.2 or $Na_V1.8$.

55. The method of claim 39 or 44 wherein said agent has an IC-50 for $Na_V1.7$ which is lower than its IC-50 for $Na_V1.4$, $Na_V1.6$ or $Na_V1.9$ (Peripheral channels)

56. The method of claims 39 or 44 wherein the $Na_V1.7$-mediated disease is selected from the group consisting of: eudynia, visceral pain, acute pain, inflammatory pain, chronic pain, trauma pain, post-operative pain, neuralgia, trigeminal neuralgia, heat sensitivity, irritable bowel syndrome, crohns disease, multiple sclerosis, paroxysmal dystonia, myasthenia syndromes, myotonia, malignant hyperthermia, cystic fibrosis, pseudoaldosteronism, rhabdomyolysis, sodium channel toxin related Illnesses, hypothyroidism, familial erythermalgia, primary erythermalgia, migraines and headaches.

57. A method of treating a $Na_V1.7$-mediated disease in a mammal comprising administering to the mammal a therapeutically effective amount of a compound of Formula (I):



(I)

wherein:

R is $-(CH_2)_n(X)_mAr$, n is 0, 1, 2 or 3; m is 0 or 1; and

Ar is selected from the group consisting of phenyl, substituted phenyl, naphtyl, substituted naphtyl, heteroaryl, and substituted heteroaryl;

X is CH_2, O, S or NH;

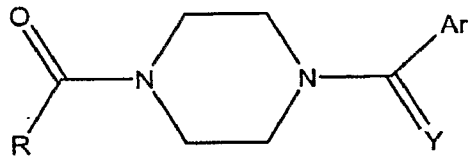
including a stereoisomer, enantiomer or tautomer thereof, a pharmaceutically acceptable salt thereof, a pharmaceutical composition or a prodrug thereof.

58. The composition of claim 57 wherein the therapeutic agent has $IC\text{-}50_{NaV1.7}$ of less than 6 μM.

59. The composition of claim 57 wherein the therapeutic agent has and $IC\text{-}50_{NaV1.3}$ of greater than 10 μM.

A method of treating a $Na_v1.7$-mediated disease comprising administering an effective amount of a composition of claim 57 to an animal in need thereof.

60. A method of treating a $Na_v1.7$-mediated disease in a mammal comprising administering to the mammal a therapeutically effective amount of a compound of of Formula (II):



(II)

wherein:

R is $-(CH_2)_n(X)_m(CH_2)_n(X)_m(CH_2)_nZ$, wherein n are each independently 0, 1, 2 or 3; and m are each independently 0 or 1;

Ar is selected from the group consisting of phenyl, substituted phenyl, naphtyl, substituted naphtyl, heteroaryl, substituted heteroaryl, and substituted heteroarylaminoheteroaryl;

X are each independently CH_2, O, S or NH;

Y is H or O; and

Z is alkyl, Ar, amino, alkylamino or dialkyamino;

including a stereoisomer, enantiomer or tautomer thereof, a pharmaceutically acceptable salt thereof, a pharmaceutical composition or a prodrug thereof.

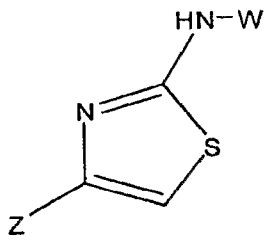
61. The composition of claim 61 wherein the therapeutic agent has $IC\text{-}50_{NaV1.7}$ of less than 6 μM

62. The composition of claim 61 wherein the therapeutic agent has $IC\text{-}50_{NaV1.8}$ of greater than 10 μM.

63. A method of treating a $Na_V1.7$-mediated disease comprising administering an effective amount of a composition of claim 57 or 60 to an animal in need thereof.

64. A method of treating a $Na_V1.7$-mediated disease comprising administering to an animal in need thereof an effective amount of a potent and selective inhibitor of $Na_V1.7$.

65. A method of treating a $Na_V1.7$-mediated disease in a mammal comprising administering to the mammal a therapeutically effective amount of a compound of Formula (Ia):



(Ia)

wherein:

Z is selected from optionally substituted straight or branched chain C_1 to C_5 alkyl, optionally substituted straight or branched chain C_1 to C_5 alkoxy, optionally substituted cycloalkyl, optionally substituted cycloalkylalkyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally

substituted aralkyl, optionally substituted heteroaryl or optionally substituted heteroaralkyl,

wherein said substitutions are each independently selected from hydrogen, straight or branched chain C_1 to C_5 alkyl, halo, haloalkyl, -OR^2, -$S(O)_tR^2$ (where t is 0, 1 or 2), -CN, -$C(O)R^2$, -$C(O)OR^2$, -$C(O)N(R^2)R^3$, -$N(R^2)R^3$ and -NO_2;

W is -R^4- C(O)-R^4-X-R^1;

R^1 is optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

wherein said substitutions are each independently selected from hydrogen, straight or branched chain C_1 to C_5 alkyl, halo, haloalkyl, -OR^2, -$S(O)_tR^2$ (where t is 0, 1 or 2), -CN, -$C(O)R^2$, -$C(O)OR^2$, -$C(O)N(R^2)R^3$, -$N(R^2)R^3$ and -NO_2;

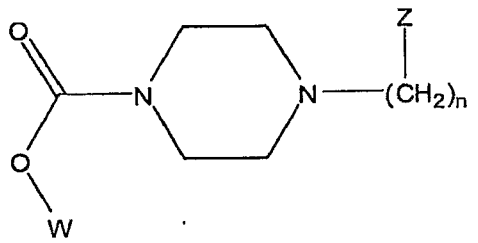
R^2 and R^3 are each independently hydrogen or optionally substituted straight or branched chain C_1 to C_5 alkyl, wherein said substitutions are each independently selected from hydrogen, straight or branched chain C_1 to C_5 alkyl, halo, haloalkyl, -OR^5, -$S(O)_tR^5$ (where t is 0, 1 or 2), -CN, -$C(O)R^5$, -$C(O)OR^5$, -$C(O)N(R^5)R^6$, -$N(R^5)R^6$ and -NO_2;

R^4 is a direct bond or a straight or branched C_1 to C_5 alkylene;

X is -O-, -S- or -$N(R^2)$-;

as a stereoisomer, enantiomer, tautomer thereof or mixtures thereof; or a pharmaceutically acceptable salt, solvate or prodrug thereof.

66. A method of treating a $Na_V1.7$-mediated disease in a mammal comprising administering to the mammal a therapeutically effective amount of a compound of Formula (IIa):



(IIa)

wherein:

n is 0, 1, 2 or 3;

Z is selected from $-R^7-N(R^6)R^7$, $-OR^7$, $-S(O)_tR^7$ (where t is 0, 1 or 2), optionally substituted straight or branched chain C_1 to C_5 alkyl, optionally substituted straight or branched chain C_1 to C_5 alkoxy, optionally substituted cycloalkyl, optionally substituted cycloalkylalkyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl or optionally substituted heteroaralkyl,

wherein said substitutions are each independently selected from hydrogen, C_1 to C_5 alkyl, halo, haloalkyl, $-OR^5$, $-S(O)_tR^5$ (where t is 0, 1 or 2), -CN, $-C(O)R^5$, $-C(O)OR^5$, $-C(O)N(R^5)R^6$, $-N(R^5)R^6$ and $-NO_2$;

W is selected from $-R^8-X-R^8-R^9$, optionally substituted straight or branched chain C_1 to C_5 alkyl, optionally substituted straight or branched chain C_1 to C_5 alkoxy, optionally substituted aryl, optionally substituted heteroaryl,

wherein said substitutions are each independently selected from hydrogen, straight or branched chain C_1 to C_5 alkyl, halo, haloalkyl, $-OR^5$, $-S(O)_tR^5$ (where t is 0, 1 or 2), -CN, $-C(O)R^5$, $-C(O)OR^5$, $-C(O)N(R^5)R^6$, $-N(R^5)R^6$ and $-NO_2$;

X is -O-, -S- or $-N(R^5)-$;

R^5 and R^6 are each independently hydrogen or optionally substituted straight or branched chain C_1 to C_5 alkyl, wherein said substitutions are each independently selected from hydrogen, straight or branched chain C_1 to C_5 alkyl, halo, haloalkyl, $-OR^{10}$, $-S(O)_tR^{10}$ (where t is 0, 1 or 2), -CN, $-C(O)R^{10}$, $-C(O)OR^{10}$, $-C(O)N(R^{10})R^{11}$, $-N(R^{10})R^{11}$ and $-NO_2$;

R^7 is optionally substituted aryl or optionally substituted heteroaryl,

wherein said substitutions are each independently selected from hydrogen, straight or branched chain C_1 to C_5 alkyl, halo, haloalkyl, $-OR^5$, $-S(O)_tR^5$ (where t is 0, 1 or 2), -CN, $-C(O)R^5$, $-C(O)OR^5$, $-C(O)N(R^5)R^6$, $-N(R^5)R^6$ and $-NO_2$;

each R^8 is independently a direct bond or a straight or branched C_1 to C_5 alkylene;

R^9 is optionally substituted aryl or optionally substituted heteroaryl,

wherein said substitutions are each independently selected from hydrogen, straight or branched chain C_1 to C_5 alkyl, halo, haloalkyl, -OR^5, -$S(O)_tR^5$ (where t is 0, 1 or 2), -CN, -$C(O)R^5$, -$C(O)OR^5$, -$C(O)N(R^5)R^6$, -$N(R^5)R^6$ and -NO_2; and

R^{10} and R^{11} are each independently hydrogen or optionally substituted straight or branched chain C_1 to C_5 alkyl;

as a stereoisomer, enantiomer, tautomer thereof or mixtures thereof; or a pharmaceutically acceptable salt, solvate or prodrug thereof.

67. A method for identifying a compound that selectively inhibits the activity of a $Na_V1.7$ polypeptide, comprising:

(a) determining a test agent's inhibitory concentration for inhibition of sodium ion flux by $Na_V1.7$ polypeptide across a membrane; and

(b) determining said test agent's inhibitory concentration for inhibition of sodium ion flux by a different human sodium channel polypeptide across a membrane; and

(c) comparing the inhibitory concentration determined in part (a) to the inhibitory concentration determined in part (b)

wherein the ratio of the inhibitory concentration from part (b) to the inhibitory concentration of part (a) is at least 1.1, 1.2, 1.5, 1.7, 2, 3, 4, 5, 10, 20, 25, 50, 75, 100, 200, 500, 1000, 2000, 5000, or 10000 is said compound.

68. The method of claim 66 wherein the inhibitory concentration is selected from among IC-40, IC-50 and IC-75.

69. The method of claim 66 wherein the sodium channel polypeptide of part b) is selected from among $Na_V1.1$, $Na_V1.2$, $Na_V1.3$, $Na_V1.4$, $Na_V1.5$, $Na_V1.6$, $Na_V1.8$. and $Na_V1.9$.

70. A method for selectively inhibiting a gene encoding Nav1.7 polypeptide, comprising contacting said gene with an siRNA complementary to said gene.

71. The method of claim 69, wherein said siRNA is selected from an siRNA in Table 7.

72. A method for treating CIP, comprising contacting said gene with an siRNA complementary to said gene.

73. The method of claim 71, wherein said siRNA is selected from an siRNA in Table 7.

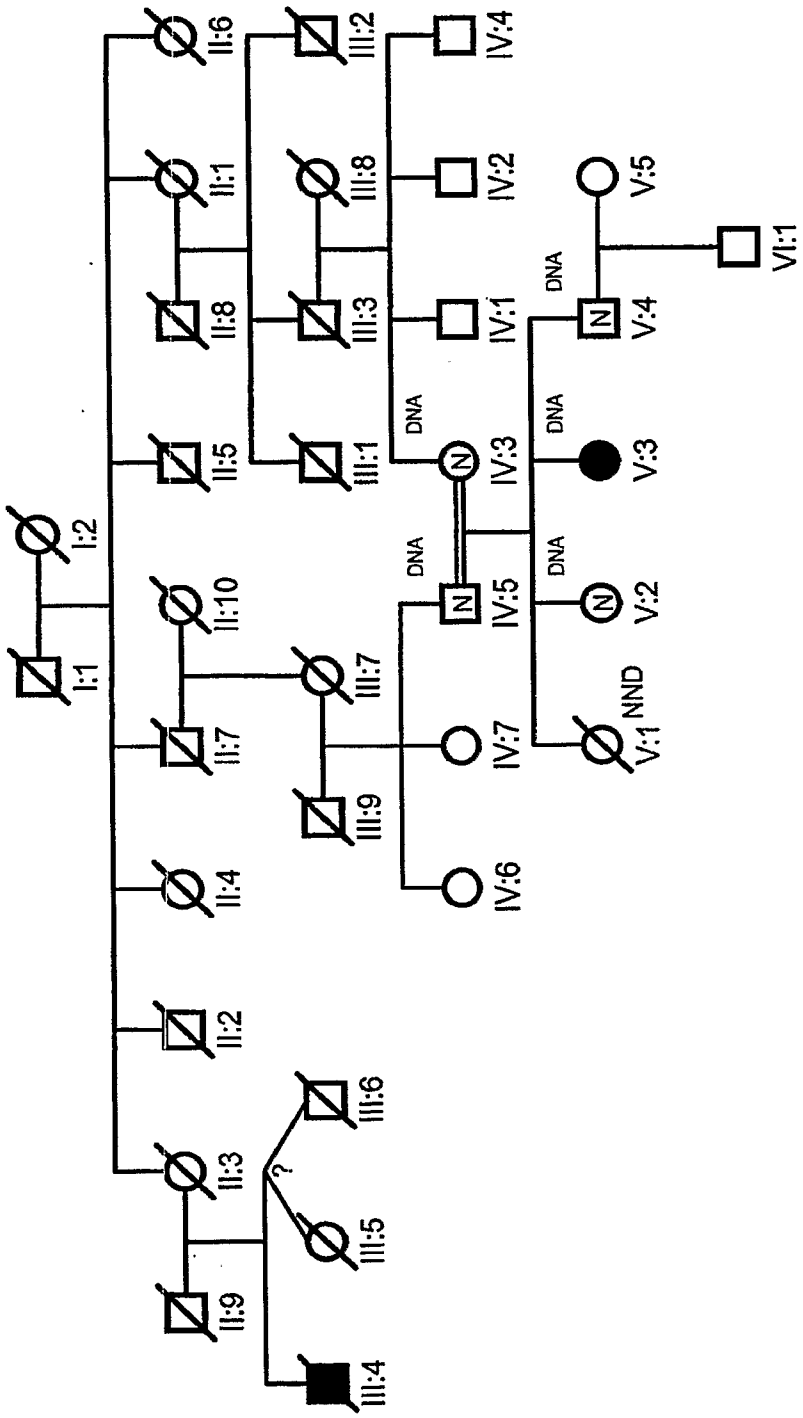
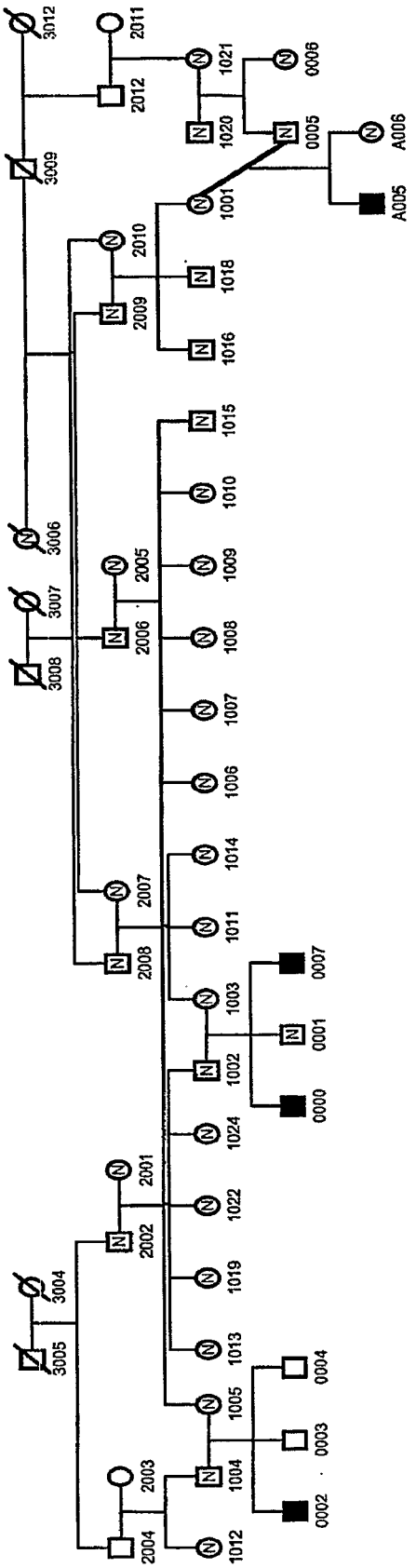

I:1
I:2
II:1
II:2
II:3
II:4
II:5
II:6
II:7
II:8
II:9
II:10
III:1
III:2
III:3
III:5
III:6
III:7
III:8
III:9
IV:1
IV:2
IV:3
IV:4
IV:5
IV:6
IV:7
V:1 NND
V:2
V:3
V:4
V:5
VI:1
DNA
N
?

Figure 1

Figure 2


3012
2011
2012
1021
0006
1020
0005
A006
3009
A005
1001
2010
1018
2009
1016
1015
3006
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2005
1009
3007
2006
1008
3008
1007
1006
1014
2007
1011
2008
1003
0007
0001
1002
0000
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2002
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3004
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3005
0004
1005
0003
2003
1004
0002
2004
1012

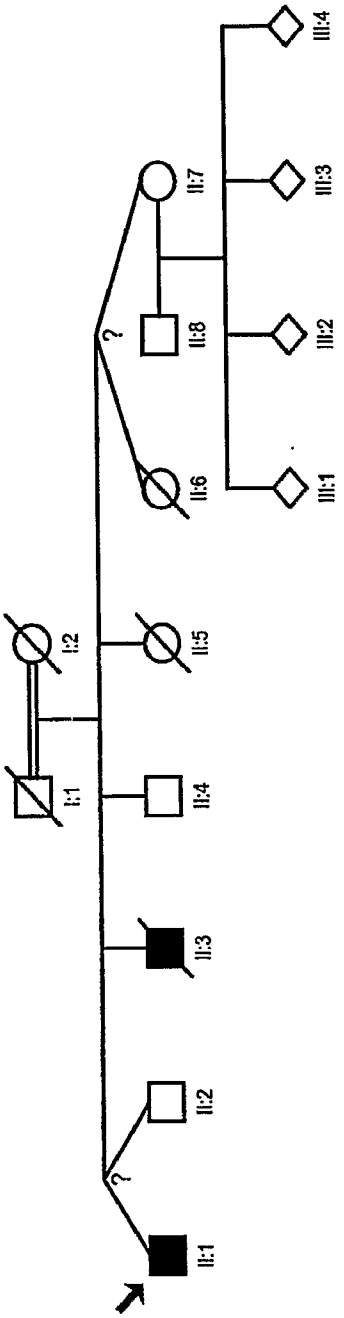


Figure 3

Figure 4a c.5067G>A, p.W1689X

Figure 4b c.984C>A, p.Y328X

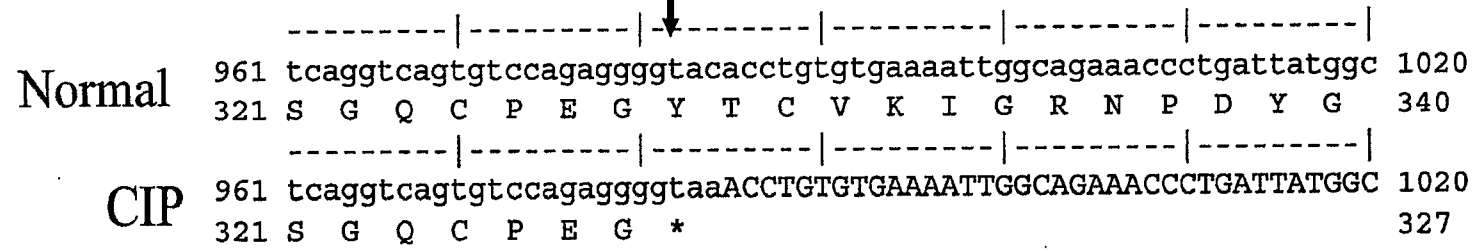


Figure 4c c.2488C>T, p.R830X

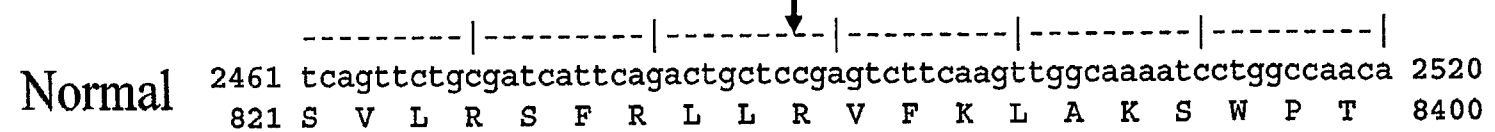


Figure 5

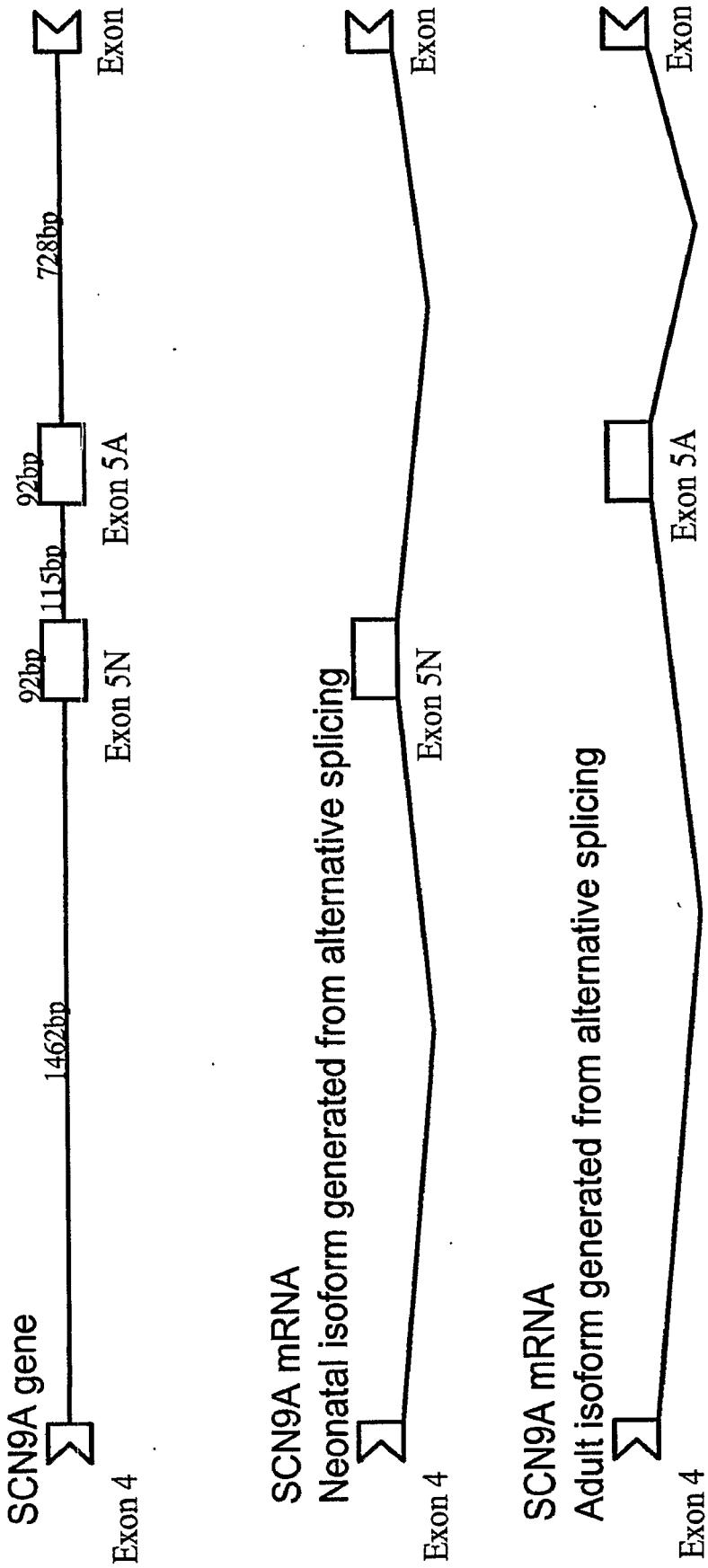

SCN9A gene
Exon 4
1462bp
92bp
Exon 5N
115bp
92bp
Exon 5A
728bp
Exon
SCN9A mRNA
Neonatal isoform generated from alternative splicing
Exon 4
Exon 5N
Exon
SCN9A mRNA
Adult isoform generated from alternative splicing
Exon 4
Exon 5A
Exon

Figure 6

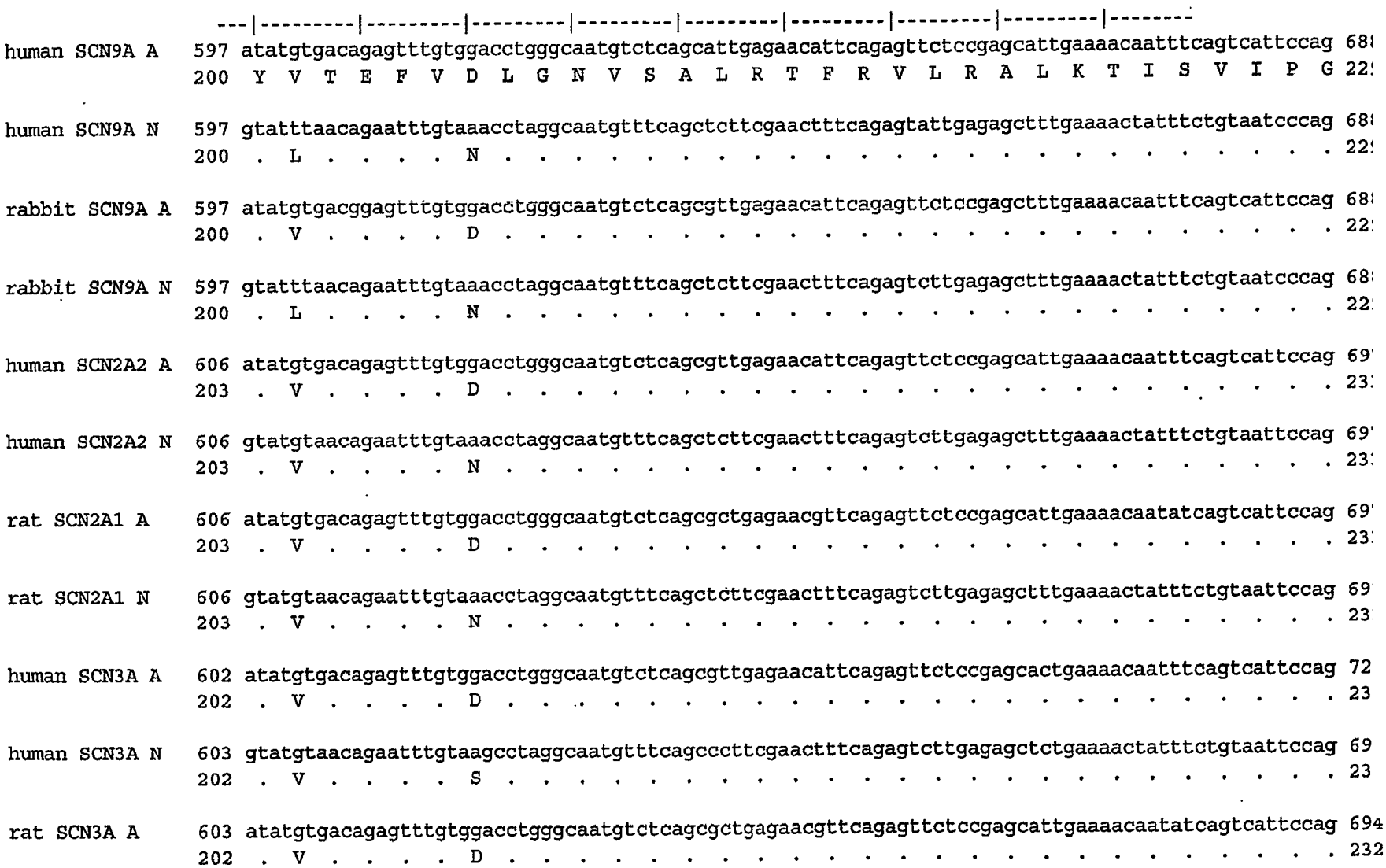

human SCN9A A 597 atatgtgacagagtttgtggacctgggcaatgtctcagcattgagaacattcagagttctccgagcattgaaaacaatttcagtcattccag 68
200 Y V T E F V D L G N V S A L R T F R V L R A L K T I S V I P G 22
human SCN9A N 597 gtatttaacagaatttgtaaacctaggcaatgtttcagctcttcgaactttcagagtattgagagctttgaaaactatttctgtaatcccag 68
200 . L . . . . N 22
rabbit SCN9A A 597 atatgtgacggagtttgtggacctgggcaatgtctcagcgttgagaacattcagagttctccgagctttgaaaacaatttcagtcattccag 68
200 . V . . . . D 22
rabbit SCN9A N 597 gtatttaacagaatttgtaaacctaggcaatgtttcagctcttcgaactttcagagtcttgagagctttgaaaactatttctgtaatcccag 68
200 . L . . . . N 22
human SCN2A2 A 606 atatgtgacagagtttgtggacctgggcaatgtctcagcgttgagaacattcagagttctccgagcattgaaaacaatttcagtcattccag 69
203 . V . . . . D 23
human SCN2A2 N 606 gtatgtaacagaatttgtaaacctaggcaatgtttcagctcttcgaactttcagagtcttgagagctttgaaaactatttctgtaattccag 69
203 . V . . . . N 23
rat SCN2A1 A 606 atatgtgacagagtttgtggacctgggcaatgtctcagcgctgagaacgttcagagttctccgagcattgaaaacaatatcagtcattccag 69
203 . V . . . . D 23
rat SCN2A1 N 606 gtatgtaacagaatttgtaaacctaggcaatgtttcagctcttcgaactttcagagtcttgagagctttgaaaactatttctgtaattccag 69
203 . V . . . . N 23
human SCN3A A 602 atatgtgacagagtttgtggacctgggcaatgtctcagcgttgagaacattcagagttctccgagcactgaaaacaatttcagtcattccag 72
202 . V . . . . D 23
human SCN3A N 603 gtatgtaacagaatttgtaagcctaggcaatgtttcagcccttcgaactttcagagtcttgagagctctgaaaactatttctgtaattccag 69
202 . V . . . . S 23
rat SCN3A A 603 atatgtgacagagtttgtggacctgggcaatgtctcagcgctgagaacgttcagagttctccgagcattgaaaacaatatcagtcattccag 69
202 . V . . . . D 232

Figure 7

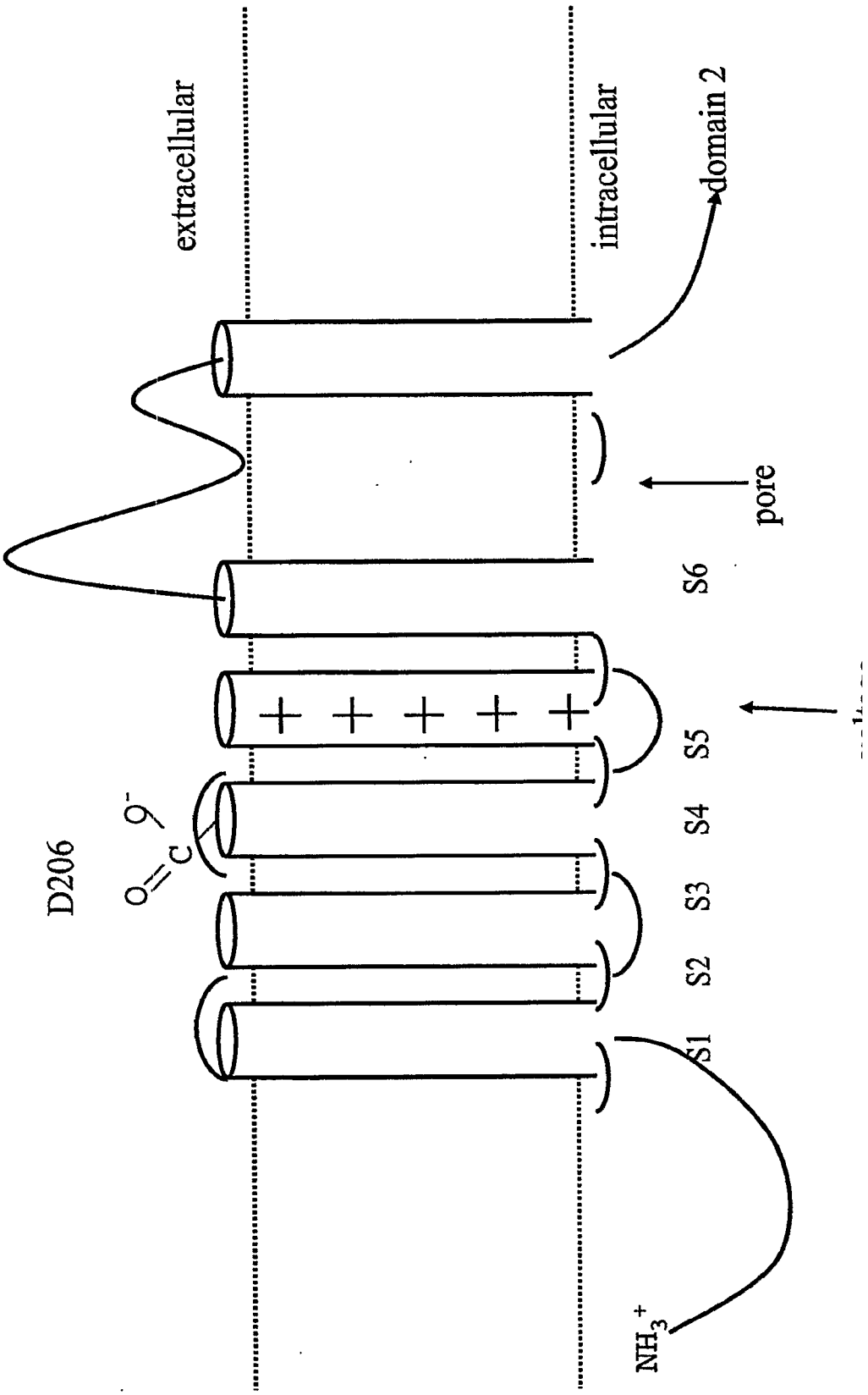
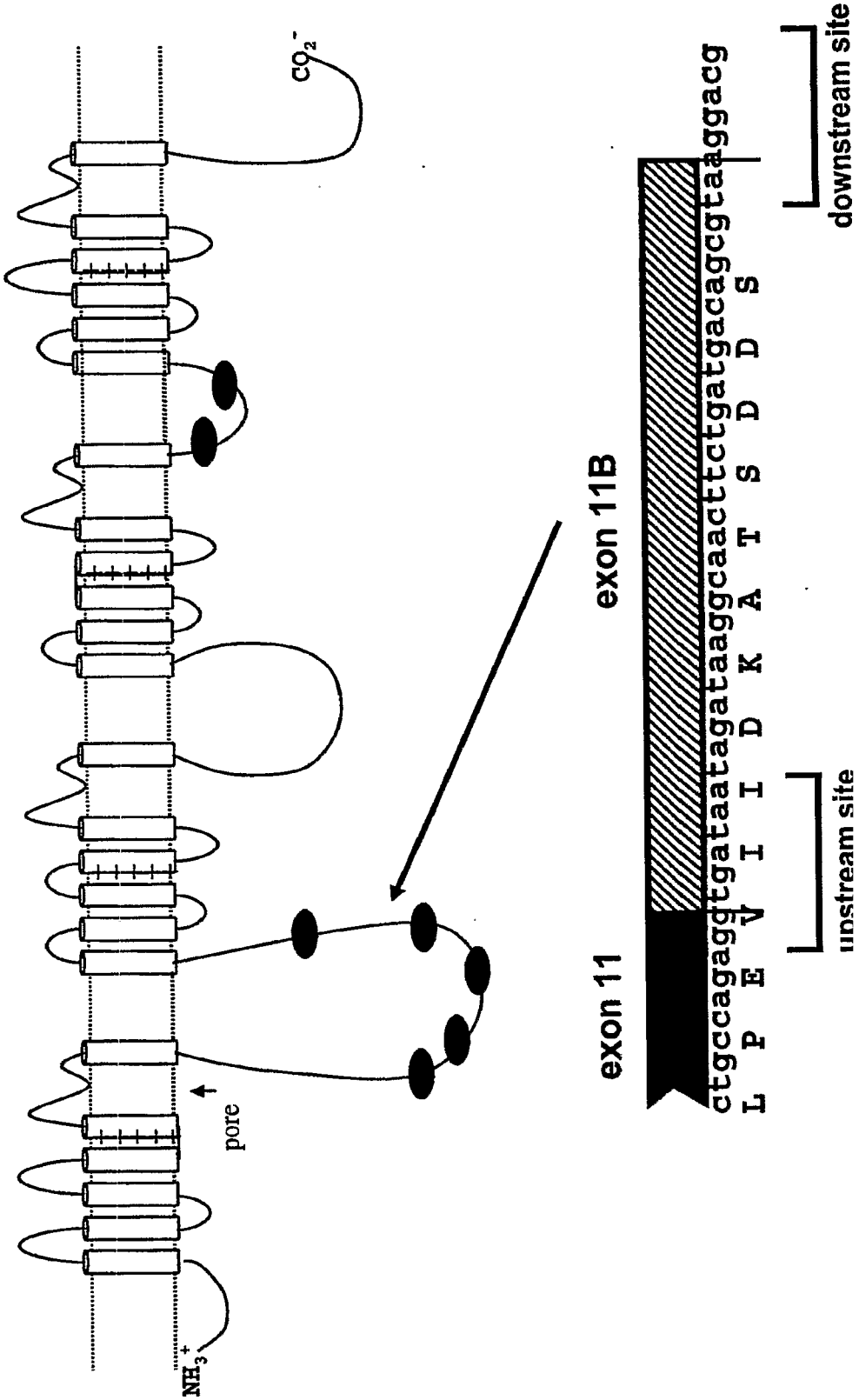

extracellular
intracellular
domain 2
pore
S6
S5
S4
S3
S2
S1
D206
O=C
O⁻
NH3+

Figure 8


CO_2^-
pore
NH_3^+
exon 11B
exon 11
ctgccagaggtgataaataggcaacttctgatgacagcgtaaggacg
L P E V I I D K A T S D D D S
downstream site
unstream site


human_SCN9A 597
rat_SCN9A 596
mouse_SCN9A 596
rabbit_SCN9A 595
human_SCN1A 617
rat_SCN1A 617
human_SCN8A 610
rat_SCN8A 610
mouse_SCN8A 610

Figure 9

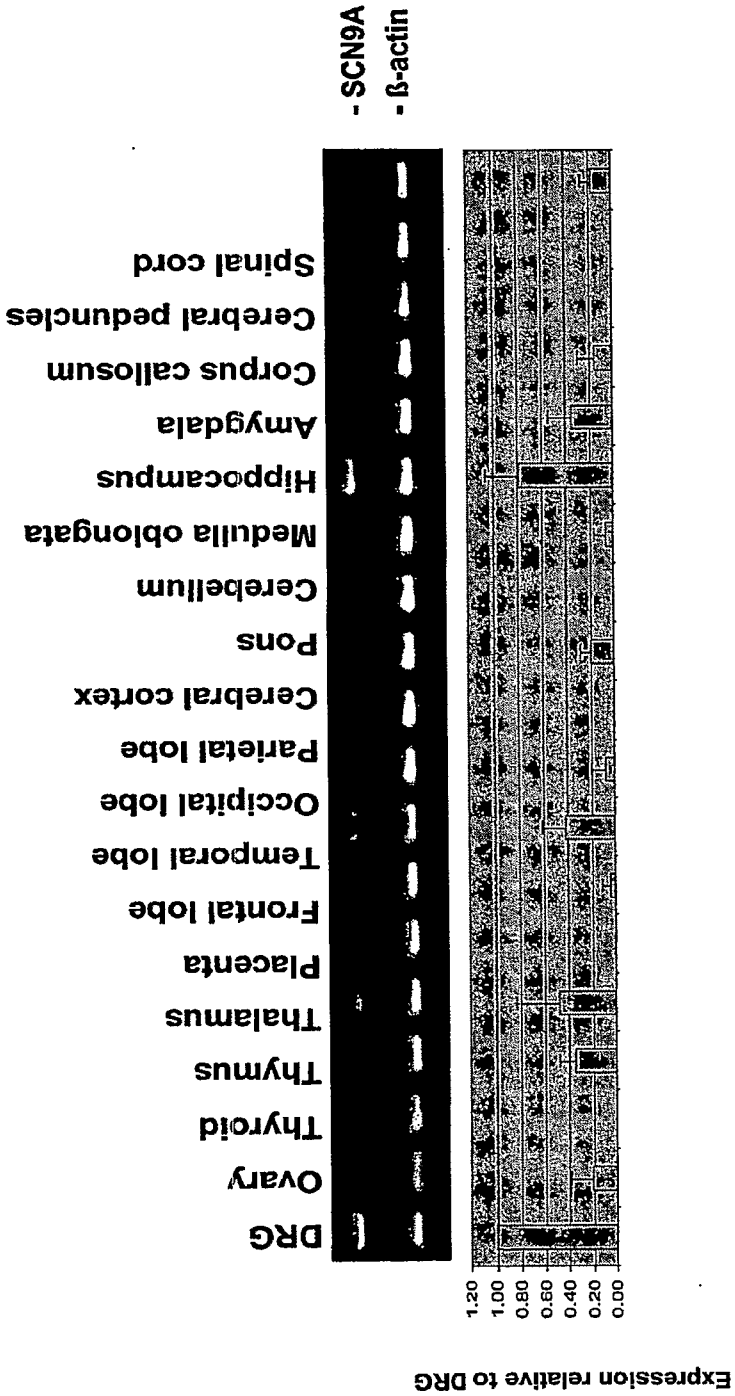
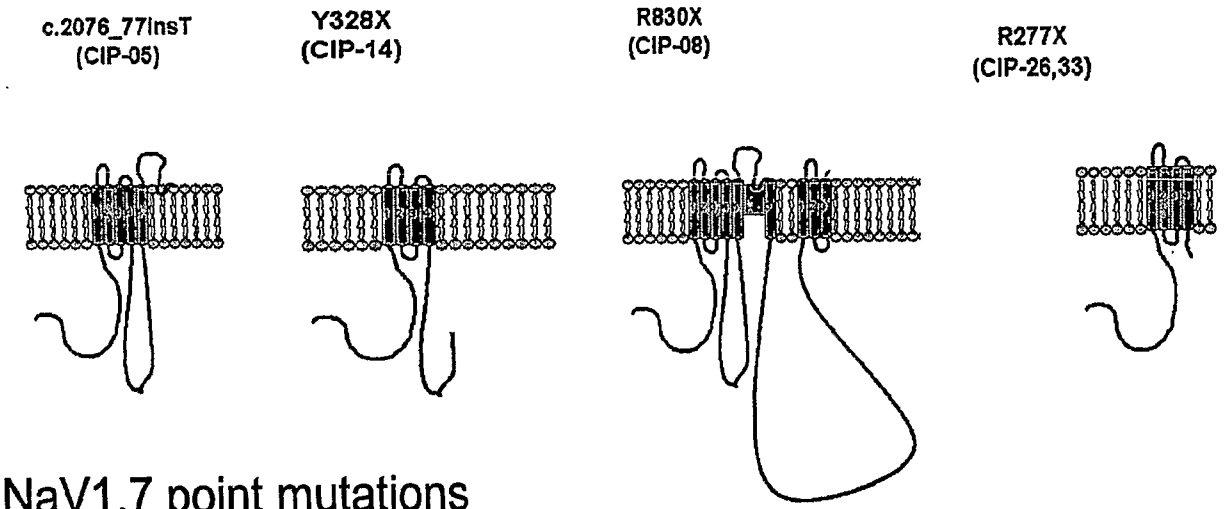

- SCN9A
- ß-actin
DRG
Ovary
Thyroid
Thymus
Thalamus
Placenta
Frontal lobe
Temporal lobe
Occipital lobe
Parietal lobe
Cerebral cortex
Pons
Cerebellum
Medulla oblongata
Hippocampus
Amygdala
Corpus callosum
Cerebral peduncles
Spinal cord
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Expression relative to DRG

Figure 10

Figure 11

NaV1.7 deletion mutations responsible for pain-free syndrome (C-I-P)



(Not shown W1689X, R1488X, 3600ΔT)

NaV1.7 point mutations responsible for painful syndromes

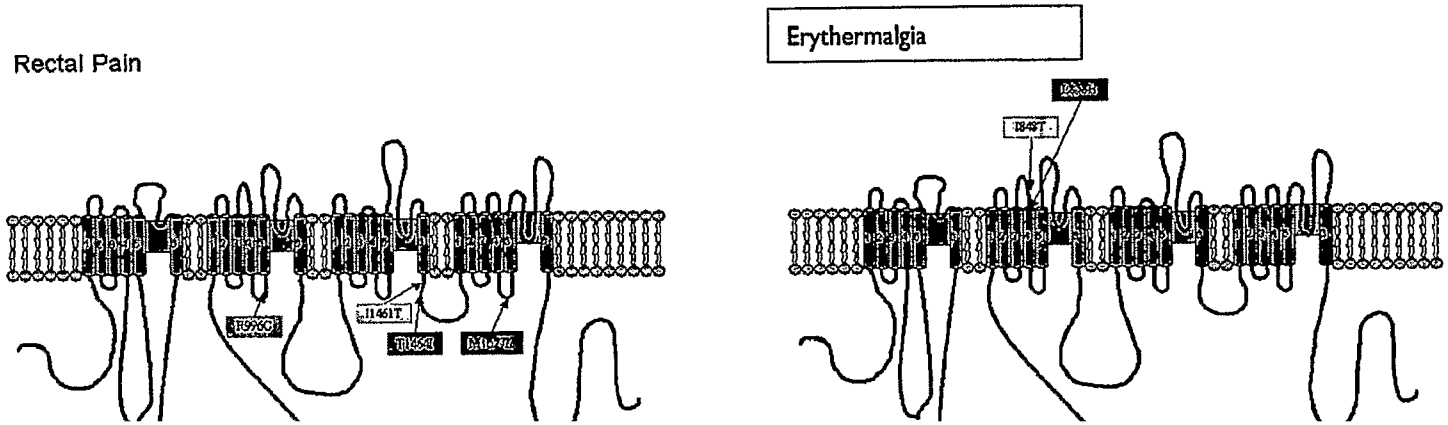


Fig. 12

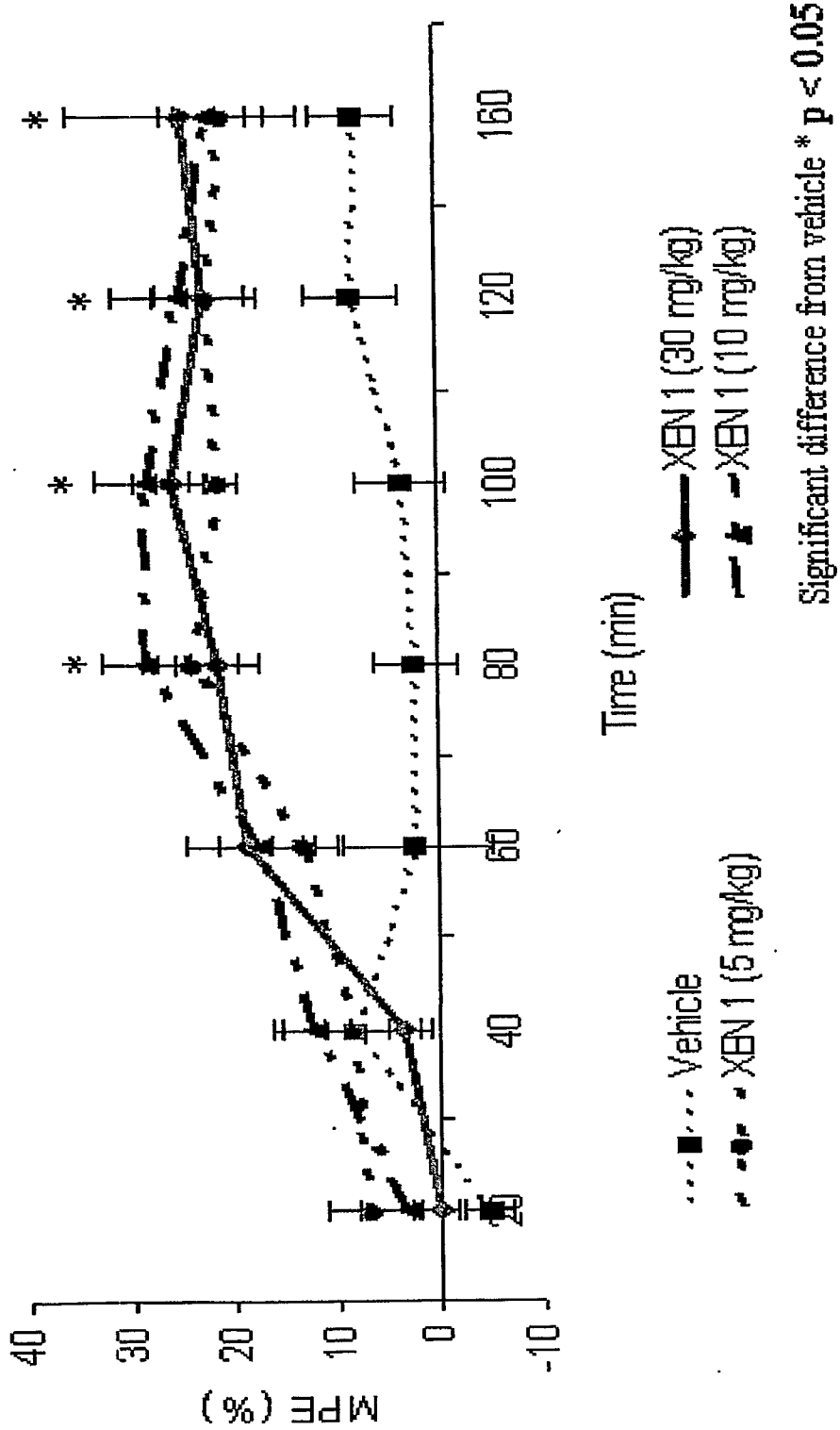

MPE (%)
40
30
20
10
0
-10
20
40
60
80
100
120
160
Time (min)
Vehicle
XEN 1 (5 mg/kg)
XEN 1 (30 mg/kg)
XEN 1 (10 mg/kg)
Significant difference from vehicle * p < 0.05

Fig. 13

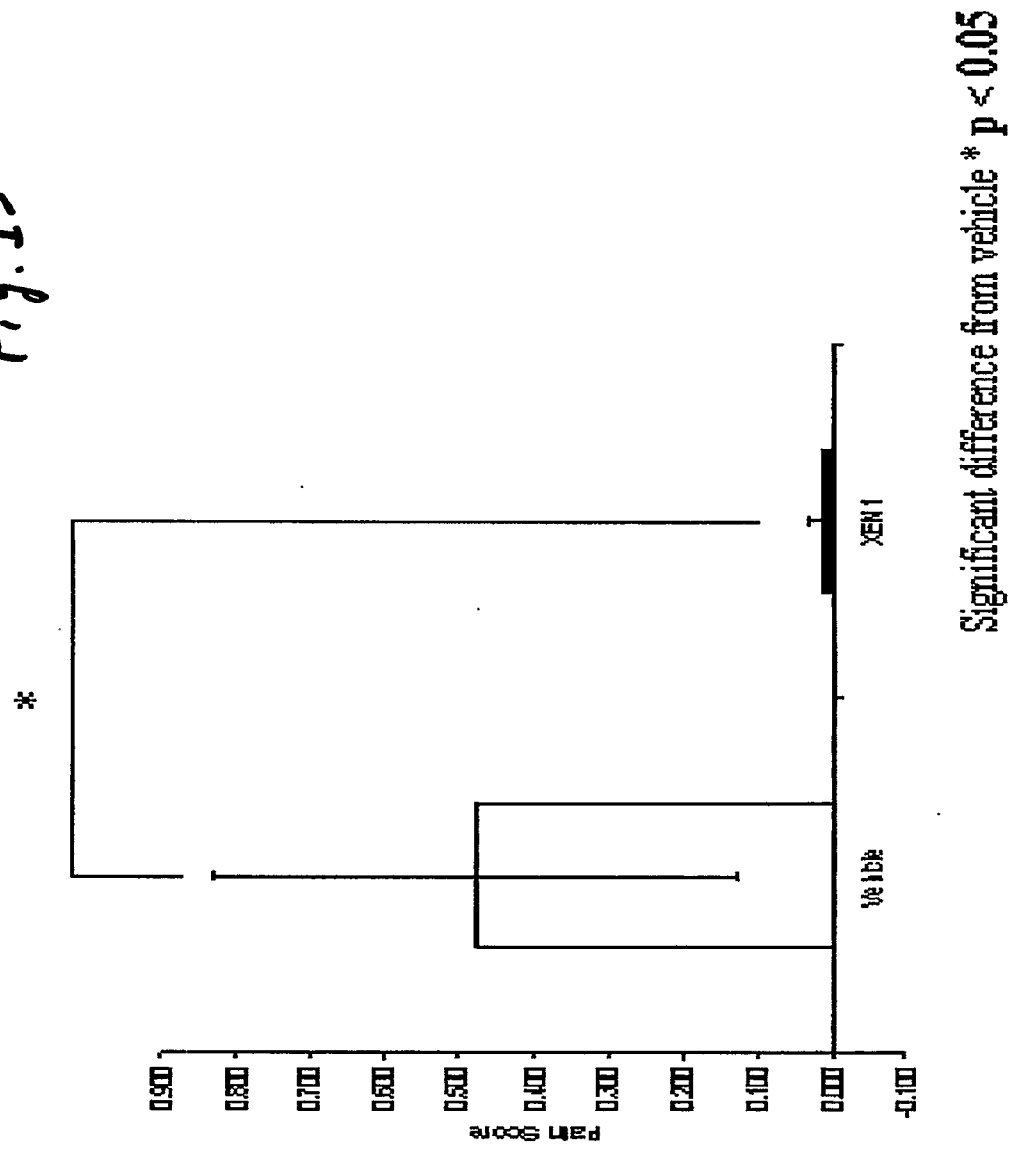
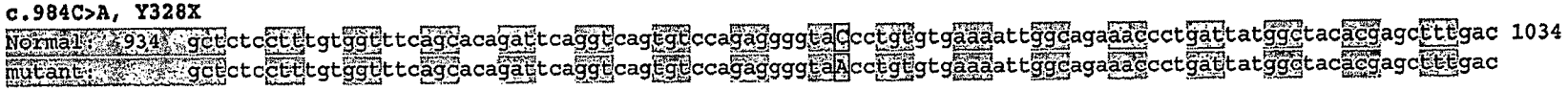

Significant difference from vehicle * p < 0.05
XEN1
Vehicle
Pain Score
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
-0.100
*

Figure 14

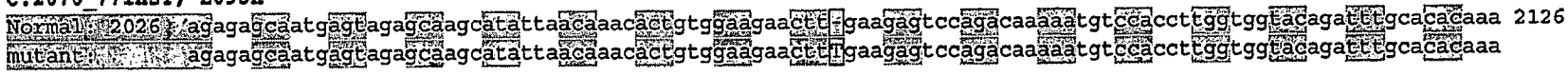
All sequences from NM_002977.1

c.877C>T, R277X

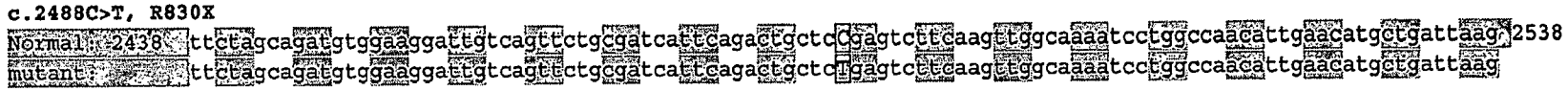
c.984C>A, Y328X



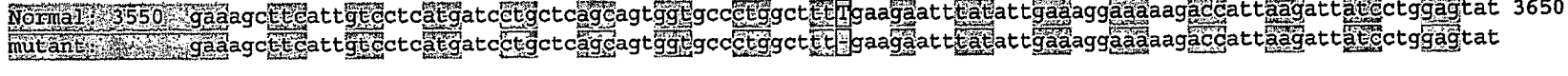
c.2076_77insT, E693X



c.2488C>T, R830X



c.3600delT, F1200fsX1232



c.4462C>T, Y1488X

c.5067G>A, W1689X