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(54) Title: N-TYPE CALCIUM CHANNEL ANTAGONISTS FOR THE TREATMENT OF PAIN

(57) Abstract: Compounds useful for the treatment of pain in accord with the following structuraldiagram,[Chemical formula should be inserted here. Please see paper copy]wherein A, R_1, b, R_3, d, R_4, R_5, R_6 and R_7 are any of a number of groups as defined in thespecification, and pharmaceutical compositions and methods of treatment utilizing suchcompounds.

N-TYPE CALCIUM CHANNEL ANTAGONISTS FOR THE TREATMENT OF PAIN

Field of the Invention

This invention relates to substituted quinoline compounds, methods of making such compounds and methods of using such compounds for the treatment or prevention of pain or nociception.

Related Art

Pain causes a great deal of suffering and is a sensory experience distinct from sensations of touch, pressure, heat and cold. It is often described by sufferers by such terms as bright, dull, aching, pricking, cutting or burning and is generally considered to include both the original sensation and the reaction to that sensation. This range of sensations, as well as the variation in perception of pain by different individuals, renders a precise definition of pain difficult. Where pain is "caused" by the stimulation of nociceptive receptors and transmitted over intact neural pathways, this is termed nociceptive pain. Pain may also be caused by damage to neural structures, and pain is often is manifested as neural supersensitivity; this type of pain is referred to as neuropathic pain.

The level of stimulation at which pain is perceived is referred to as the "pain threshold". Where the pain threshold is raised, for instance, by the administration of an analgesic drug, a greater intensity or more prolonged stimulus is required before pain is experienced. Analgesics are a class of pharmaceutical agent which, following administration to a patient in need of such treatment, relieve pain without loss of consciousness. This is in contrast to other pain-relieving drugs, for example, general anesthetics which obtund pain by producing a hiatus in consciousness, or local anesthetics which block transmission in peripheral nerve fibers thereby preventing pain.

Tachykinin antagonists have been reported to induce antinociception in animals, which is believed to be analogous to analgesia in man (for review see Maggi *et al*, J. Auton. Pharmacol. 13:23-93 (1993)). In particular, non-peptide NK-1 receptor antagonists have been shown to produce such analgesia, thus, for example, in classical tests of chemo-nociception (phenylbenzoquinone-induced writhing and formalin test) the NK-1 receptor antagonist RP 67,580 produced analgesia with potency comparable to that of morphine (Garret *et al*, Proc. Natl. Acad. Sci. USA 88:10208-10212 (1993)).

Opioid analgesics are a well-established class of analgesic agents. These compounds are generally accepted to include, in a generic sense, all drugs, natural or synthetic, with

morphine-like actions. The synthetic and semi-synthetic opioid analgesics are derivatives of five chemical classes of compound: phenanthrenes; phenylheptylamines; phenylpiperidines; morphinans; and benzomorphans. Pharmacologically these compounds have diverse activities, thus some are strong agonists at the opioid receptors (e.g. morphine); others are moderate to mild agonists (e.g. codeine); still others exhibit mixed agonist-antagonist activity (e.g. nalbuphine); and yet others are partial agonists (e.g. nalorphine). Whilst an opioid partial agonist such as nalorphine, (the N-alkyl analogue of morphine) will antagonize the analgesic effects of morphine, when given alone it can be a potent analgesic in its own right. Of all of the opioid analgesics, morphine remains the most widely used and is a suitable archetype compound. Unfortunately, apart from its useful therapeutic properties, morphine also has a number of drawbacks including respiratory depression, decreased gastrointestinal motility (resulting in constipation) and, in some individuals, nausea and vomiting may occur. Another characteristic is the development of tolerance and physical dependence which may limit the clinical use of such compounds.

Anti-inflammatory compounds directed at blocking or reducing synovial inflammation, and thereby improving function, and analgesics directed to reducing pain, are presently the primary method of treating the rheumatoid diseases and arthritis. Aspirin and other salicylate compounds are frequently used in treatment to interrupt amplification of the inflammatory process and temporarily relieve the pain. Other drug compounds used for these purposes include phenylpropionic acid derivatives such as Ibuprofen and Naproxin, Sulindac, phenyl butazone, corticosteroids, antimalarials such as chloroquine and hydroxychloroquine sulfate, and fenemates. For a thorough review of various drugs utilized in treating rheumatic diseases, reference is made to J. Hosp. Pharm., 36:622 (May 1979).

Calcium channels are membrane-spanning, multi-subunit proteins that allow controlled entry of Ca^{++} ions into cells from the extracellular fluid. Such channels are found throughout the animal kingdom, and have been identified in bacterial, fungal and plant cells. Commonly, calcium channels are voltage dependent. In such channels, the "opening" allows an initial influx of Ca^{++} ions into the cells which lowers the potential difference between the inside of the cell bearing the channel and the extracellular medium bathing the cell. The rate of influx of Ca^{++} ions into the cell depends on this potential difference. All "excitable" cells in animals, such as neurons of the central nervous system ("CNS"), peripheral nerve cells, and muscle cells, including those of skeletal muscles, cardiac muscles, and venous and arterial smooth muscles, have voltage-dependent calcium channels. Calcium channels are

physiologically important because the channels have a central role in regulating intracellular Ca^{++} ions levels. These levels are important for cell viability and function. Thus, intracellular Ca^{++} ion concentrations are implicated in a number of vital processes in animals, such as neurotransmitter release, muscle contraction, pacemaker activity, and secretion of hormones.

It is believed that calcium channels are relevant in certain disease states. A number of compounds useful in treating various cardiovascular diseases in animals, including humans, are thought to exert their beneficial effects by modulating functions of voltage-dependent calcium channels present in cardiac and/or vascular smooth muscle. Many of these compounds bind to calcium channels and block, or reduce the rate of, influx of Ca^{++} ions into the cells in response to depolarization of the cell membrane. An understanding of the pharmacology of compounds that interact with calcium channels in other organ systems, such as the central nervous system, and the ability to rationally design compounds that will interact with these specific subtypes of human calcium channels to have desired therapeutic, e.g., treatment of neurodegenerative disorders, effects have been hampered by an inability to independently determine how many different types of calcium channels exist or the molecular nature of individual subtypes, particularly in the CNS, and the unavailability of pure preparations of specific channel subtypes, i.e., systems to evaluate the specificity of calcium channel-effecting compounds.

Multiple types of calcium channels have been detected based on electrophysiological and pharmacological studies of various mammalian cells from various tissues (e.g., skeletal muscle, cardiac muscle, lung, smooth muscle and brain) Bean, B. P., Annu. Rev. Physiol. 51:367-384 (1989) and Hess, P., Annu. Rev. Neurosci. 56:337 (1990). These different types of calcium channels have been broadly categorized into four classes, L-, T-, N-, and P-type, distinguished by current kinetics, holding potential sensitivity and sensitivity to calcium channel agonists and antagonists. Four subtypes of neuronal voltage-dependent calcium channels have been proposed Swandulla, D. *et al.*, Trends Neurosci 14:46 (1991). The L-, N- and P-type channels have each been implicated in nociception, but only the N-type channel has been consistently implicated in acute, persistent and neuropathic pain. A synthetic version of ω-conotoxin MVIIA, a 25-amino acid peptide derived from the venom of the piscivorous marine snail, *Conus magus* has been used intrathecally in humans and has ~85% success rate for the treatment of pain with a greater potency than morphine.

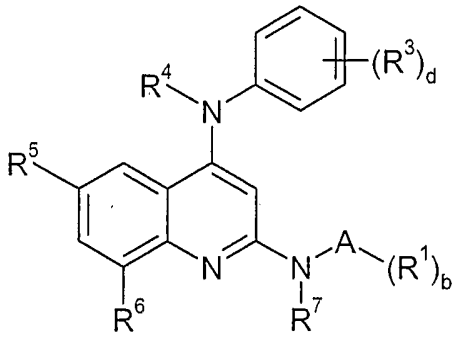
While known drug therapies have utility, there are drawbacks to their use. For

instance, it may take up to six months of consistent use of some medications in order for the product to have effect in relieving the patient's pain. Consequently, a particular subject may be receiving treatment and continuing to suffer for up to six months before the physician can assess whether the treatment is effective. Many existing drugs also have substantial adverse side effects in certain patients, and subjects must therefore be carefully monitored. Additionally, most existing drugs bring only temporary relief to sufferers and must be taken consistently on a daily or weekly basis for continued relief. Finally, with disease progression, the amount of medication needed to alleviate the pain may increase thus increasing the potential for side effects. Thus, there is still a need for an effective and safe treatment to alleviate pain.

Summary of the Invention

In one aspect the present invention provides compounds having selective action at N-type calcium channels that are useful for the treatment of pain.

Compounds of the present invention that show selective action at N-type calcium channels are compounds in accord with structural diagram I,



I

wherein:

A is selected from phenyl, heteroaryl or bicyclic heteroaryl;

R^1 at each occurrence is independently selected from halogen, $(C_1\text{-}C_6)$alkyl, heterocyclyl, OH, $(C_1\text{-}C_6)$alkoxy, or $NR^2{}_2$;

b is an integer selected from 0, 1, 2 or 3;

R^2 at each occurrence is independently selected from H or $(C_1\text{-}C_4)$alkyl;

R^3 at each occurrence is independently selected from halogen or $(C_1\text{-}C_4)$alkyl;

d is an integer selected from 0, 1, 2 or 3;

R^4 is selected from H or $(C_1\text{-}C_4)$alkyl;

R^5 is selected from the group consisting of H, halogen, $(C_1\text{-}C_3)$alkyl, $(C_1\text{-}C_3)$perfluoroalkyl, $(C_1\text{-}C_3)$alkoxy, hydroxy, NH_2 or NHR^2;

R^6 is selected from the group consisting of H, halogen, $(C_1$-$C_6)$alkyl, $(C_1$-$C_4)$perfluoroalkyl, $(C_1$-$C_6)$alkoxy, hydroxy, $(C_1$-$C_6)$alkanoyl, $C(=O)NR^2_2$ or NR^2_2; and

R^7 is selected from H or methyl.

Particular compounds of the present invention are those in accord with structural diagram I wherein;

A is selected from phenyl or bicyclic heteroaryl;

R^1 at each occurrence is independently selected from halogen, $(C_1$-$C_6)$alkyl, heterocyclyl, OH, $(C_1$-$C_6)$alkoxy, or NR^2_2;

b is an integer selected from 0, 1, 2 or 3;

R^2 at each occurrence is independently selected from H or $(C_1$-$C_4)$alkyl;

R^3 at each occurrence is independently selected from halogen or $(C_1$-$C_4)$alkyl;

d is an integer selected from 0, 1, 2 or 3;

R^4 is H;

R^5 is selected from H, halogen, , $(C_1$-$C_3)$alkyl, $(C_1$-$C_3)$perfluoroalkyl or $(C_1$-$C_3)$alkoxy;

R^6 is selected from H, halogen, $(C_1$-$C_6)$alkyl, $(C_1$-$C_4)$perfluoroalkyl, $(C_1$-$C_6)$alkoxy, hydroxy, $(C_1$-$C_6)$alkanoyl, $C(=O)NR_2$ or $-NR^2$; and

R^7 is H.

Most particular compounds of the invention are those exemplified herein.

In another aspect, the invention comprises a method for using compounds according to structural diagram I for the treatment of pain, said method comprising administering a pain-ameliorating effective amount of any such compound.

One embodiment of the method of the invention comprises administering a pain-ameliorating effective amount of a compound in accordance with structural diagram I to a subject in need of treatment for acute, persistent or neuropathic pain.

In a further aspect, the invention comprises methods for making compounds in accord with structural diagram I.

In yet another aspect, the invention comprises pharmaceutical compositions comprising compounds in accord with structural diagram I together with excipients, diluents or stabilizers, as further disclosed herein, useful for the treatment of acute, persistent and neuropathic pain.

Detailed Description of the Invention

Compounds of the invention are those within the scope of the generic description and particularly those compounds exemplified hereafter.

Suitable pharmaceutically-acceptable salts of compounds of the invention include acid addition salts such as methanesulfonate, fumarate, hydrochloride, hydrobromide, citrate, tris(hydroxymethyl)aminomethane, maleate and salts formed with phosphoric and sulfuric acid.

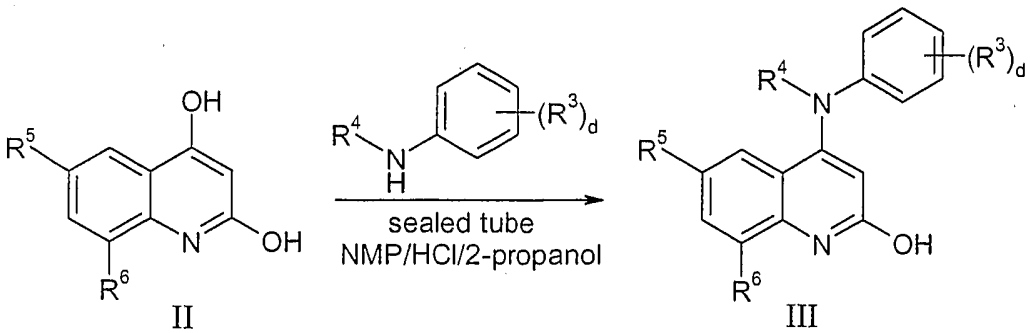
Where compounds of the present invention possess a chiral center it is to be understood that the invention encompasses all optical isomers and diastereoisomers of such compounds.

Where compounds of the present invention can tautomerize it is to be understood that the invention encompasses all tautomeric forms of such compounds.

Where compounds of the present invention can exist in unsolvated as well as solvated forms such as, for example, hydrated forms, it is to be understood that the invention encompasses all such solvated and unsolvated forms.

Another aspect of the invention provides processes for making compounds of the invention. Generally compounds of the present invention were prepared by reacting in an automated fashion substituted 2-chloro-4-aminoquinoline intermediates with aminoaryl precursors. The intermediate substituted 2-chloro-4-aminoquinolines were prepared by chlorination of substituted 2-hydroxy-4-aminoquinoline precursors, which were in turn prepared by amination of substituted 2,4-quinolinediol precursors.

a) 2-Hydroxy-4-aminoquinoline precursors in accord with structural diagram III were prepared by reacting a substituted 2,4-quinolinediol in accord with structural diagram II with three equivalents of an aryl amine in N-methylpyrrolidinone and 6N HCL in 2-propanol, in a sealed tube at a temperature of about 180 °C.

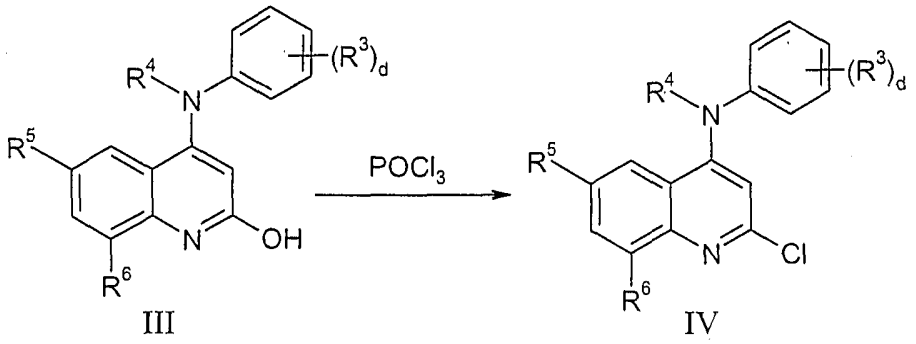


II III

wherein R^3, R^4, R^5, R^6 and d, are as heretofore defined;

a') 2-hydroxy-4-aminoquinoline precursors in accord with structural diagram III were alternatively prepared by reacting a substituted 2,4-quinolinediol in accord with structural diagram II with two equivalents of an aryl amine in N-methylpyrrolidinone and 4N HCL in dioxane, in a sealed teflon tube. The reaction was maintained at a temperature of about 200 °C using an Ethos 1600 Lab Microwave as the energy source.

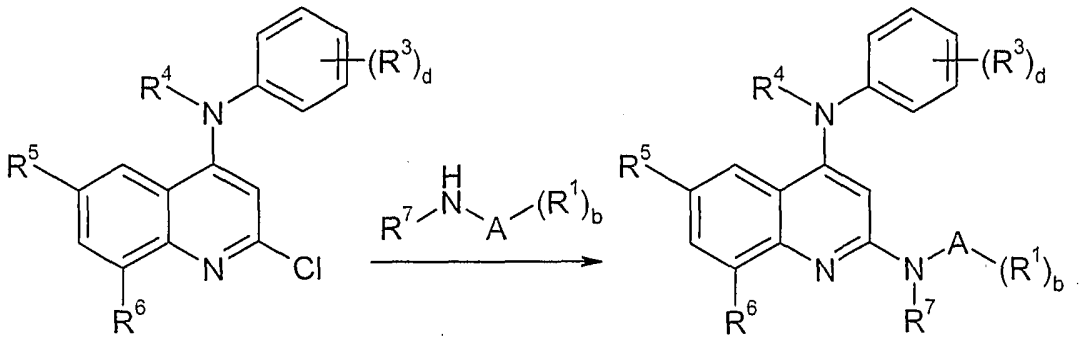
b) 2-chloro-4-aminoquinoline precursors were prepared by chlorinating a 2-hydroxy-4-aminoquinoline compound in accord with structural diagram III by refluxing with $POCl_3$ to form a compound according to structural diagram IV.



III IV

wherein R^3, R^4, R^5, R^6, b and d are as heretofore defined;

c) Compounds of the present invention in accord with structural diagram I were prepared by reacting 2-chloro-4-aminoquinoline precursors with aromatic amines in N-methylpyrrolidinone at temperatures of 100-180 °C.



IV I

wherein R^1, R^3, R^4, R^5, R^6, R^7, b and d are as heretofore defined.

The procedure of step c) may also be performed in a parallel fashion using a robotic instrumentality. A suitable robotic instrumentality for such a multiple parallel synthesis is a ChemSpeed robot.

To use a compound of the invention or a pharmaceutically-acceptable salt thereof for the therapeutic treatment or prophylactic treatment of pain in mammals, which may be

humans, the compound can be formulated in accordance with standard pharmaceutical practice as a pharmaceutical composition. Accordingly, a further aspect of the invention provides a pharmaceutical composition which contains a compound of the structural diagram I as defined herein or a pharmaceutically-acceptable salt thereof, in association with at least one pharmaceutically-acceptable additive such as an excipient or carrier.

In methods of the invention, treatment is contemplated to be administered in any physiologically-acceptable, such as by topical application, ingestion, inhalation insufflation or injection. Topical application, for example may be by a dermal, sublingual, nasal, vaginal or rectal route. Injection may be intradermal, subcutaneous, parenteral, intraperitoneal, intravenous, intramuscular or by infusion. Ingestion may be of a capsule a tablet, or a liquid. Suitable pharmaceutical compositions that contain a compound of the invention may be formulated by means known in the art in the form of, for example, tablets, capsules, aqueous or oily solutions or suspensions, emulsions, creams, ointments, gels, nasal sprays, suppositories, finely divided powders or aerosols for inhalation, and for injection sterile aqueous or oily solutions or suspensions or sterile emulsions. A preferred route of administration is orally by tablet or capsule.

In addition to a compound of the present invention, a pharmaceutical composition of this invention may also contain one or more other pharmacologically-active agents. Alternatively, a pharmaceutical composition comprising a compound of this invention may be co-administered simultaneously or sequentially with one or more other compatible pharmacologically-active agents.

Pharmaceutical compositions of this invention will normally be administered so that a pain-ameliorating effective daily dose is received by the subject. The daily dose may be given in divided doses as necessary, the precise amount of the compound received and the route of administration depending on the weight, age and sex of the patient being treated and on the particular disease condition being treated according to principles known in the art. A preferred dosage regime is once daily.

A yet further embodiment of the invention provide the use of a compound of the structural diagram I, or a pharmaceutically-acceptable salt thereof, in the manufacture of a medicament useful for binding to N-type calcium channels in a warm-blooded animal such as a human being.

Still another embodiment of the invention provides a method of binding a compound of the invention to N-type calcium channels of a warm-blooded animal, such as a human

being, in need of treatment for pain, which method comprises administering to said animal an effective amount of a compound of structural diagram I or a pharmaceutically-acceptable salt thereof.

A further aspect of the present invention provides a pharmaceutical composition which includes a compound of the present invention as defined herein or a pharmaceutically-acceptable salt thereof, in association with a pharmaceutically-acceptable additive such as an excipient or a carrier.

A still further aspect of the present invention is a method of treatment of the human or animal body that includes the administration of a compound of the present invention or a pharmaceutically-acceptable salt thereof.

Definitions:

When used herein "halo" or "halogen" means fluoro, chloro, bromo or iodo;

when substituents herein are stated to be "selected from" or "independently selected from" a group of moieties, it is to be understood that included compounds are those where all substituents are the same and compounds where each substituent may be different;

when used herein, the term heterocyclyl includes 5-7 membered rings containing 1, 2 or 3 heteroatoms selected from the group consisting of N, O and S and bicyclic rings containing such atoms and includes such groups as tetrahydrofuryl, dihydropyrrolynyl, tetrahydroisoquinolinyl, tetrahydrothiophene, oxiranyl, azidiridynl and oxetanyl

when used herein, the term heteroaryl includes such groups as pyridinyl, pyrrole, thiophenyl and furanyl;

when used herein the term "alkyl," as in for example $(C_1\text{-}C_6)$alkyl, unless otherwise defined, includes both straight, branched and cyclic chain alkyl groups. References to individual alkyl groups such as "propyl" mean the normal, straight chain form, that is, *n*-propyl;

when used herein, a term such as "$(C_1\text{-}C_6)$alkyl" means alkyl groups having 1, 2, 3, 4, 5 or 6 carbon atoms and collective groups such as $(C_1\text{-}C_4)$alkyl and includes straight, branched and cyclic moieties, straight chain moieties include for example methyl, ethyl and propyl, branched chain moieties include for example *iso*-propyl and *t*-butyl, cyclic moieties include for example cyclopentyl and cyclopropylmethyl; similarly, a term such as "$(C_1\text{-}C_3)$alkoxy" includes particular moieties such as methoxy, ethoxy and propoxy, and terms used herein that are not otherwise defined are intended to have their conventionally-understood meaning.

The Methods and Examples which follow are intended to illustrate but not limit the

invention. In the Methods and Examples, unless otherwise stated:

concentrations of solutions were carried out by rotary evaporation *in vacuo*;

operations were carried out at ambient temperature, that is in the range 18-26 °C and under a nitrogen atmosphere;

column chromatography (by the flash procedure) was performed on Merck Kieselgel silica (Art. 9385);

yields are given for illustrative purposes only and are not necessarily the maximum attainable;

the structure of compounds according to structural diagram I were generally confirmed by conventional NMR (Bruker Avance 300) and mass spectrometry techniques, peak multiplicities are shown thus: s, singlet; bs, broad singlet; d, doublet; AB or dd, doublet of doublets; t, triplet; dt, double of triplets; m, multiplet; bm, broad multiplet; FAB m/s data were obtained using a Platform spectrometer (supplied by Micromass) run in electrospray and, where appropriate, either positive ion data or negative ion data were collected, herein $(M+H)^+$ is quoted;

purity of intermediates were was in general assessed by LC/MS and/or NMR analysis; and where used the following abbreviations have meanings as follows:

DCM	is dichloromethane,
DMF	is *N,N*-dimethylformamide,
DMSO	is dimethylsulfoxide,
$CDCl_3$	is deuterated chloroform,
FAB	is fast atom bombardment,
LC/MS	is mass spectrometry linked to liquid chromatography instrumentation
m/s	is mass spectroscopy or mass spectral,
NMR	is Nuclear Magnetic Resonance,
NMP	is *N*-methylpyrrolidinone,
TFA	is trifluoroacetic acid, and
THF	is tetrahydrofuran.

Biological Methods:

I. N-channel FLIPR (Fluorescent Laser Imaging Plate Reader) assay.

The methods described herein provide a reliable FLIPR-based readout of the efficacy and potency with which test compounds inhibit calcium flux through the N-type calcium channel expressed in its native form in a human-derived neuroblastoma cell line differentiated chemically to a neuronal phenotype. The degree to which a compound at a particular concentration inhibited the N-channel calcium flux was determined by comparing the amplitude of peak calcium increase in the presence of the compound to a control 80 mM K^+ stimulus in wells without compound. Results obtained for this FLIPR assay were validated in two ways:

a) the N-channel specific peptide toxin, conotoxin MVIIA, showed an IC_{50} = 3 nM (determined from fit to five-point concentration response analysis), compatible with the known literature value; and

b) IC_{50} values were determined certain compounds of the invention (IC_{50} range: 2.37 - 10.54).

Potency of these same test compounds as inhibitors of the N-type calcium current was also determined by direct electrophysiological measurement either in neuronally differentiated IMR-32 cells, or in freshly-isolated rat superior cervical ganglion neurons. pIC_{50}'s yielded by the two methodologies for the compound set were closely comparable ($r = 0.91$; $p<0.001$).

A. Cell culture.

An immortalized cell line, IMR32, derived from human neuroblastoma cells obtained from the ATCC (product #CCL-127) was used for all experiments. Cells were grown in T75 flasks containing Eagle's minimum essential medium (MEM) w/ Earle's salts and non-essential amino acids without glutamine (Cat.#SLM-034-B, Specialty Media, Philipsburg, NJ), 10% FBS and 1% glutamine. Cells were grown to ~70-80% confluency (by visual microscopic estimation) before sub-culturing. To maintain a stock culture, cultures were split at a ratio of 1:3 - 1:4 by creating a cell suspension by trituration, and pipetting a volume of the cell suspension sufficient to yield this final ratio into new flasks containing ~20 mL of fresh media. Sub-culturing was generally performed two times per week. For preparation of 96 well plates (black-walled; Cat # 3603, Costar Co., Cambridge, MA), a T75 flask containing cells of desired confluency was brought up to 120 mL volume with media. Cells were then

freed by trituration, and the cell suspension was plated into 12-96 well plates to yield final well volume of 100 µL.

B. Cell differentiation to neuronal phenotype.

Cells were induced to differentiate in a differentiation medium consisting of: MEM, 10% FBS, 1% glutamine, 1µM 2-butyl-cAMP (49.1 mg/100 mL media (Cat. # D-0627, Sigma Corp., St Louis, MO), and 2.5 mM bromo-deoxy-uridine (stock: 30.7 mg/10 mL media, 25 mL of above stock/100 mL media; Sigma Cat .# B-9285). To induce differentiation, the cells were treated with differentiation media (by complete medium change) 2 days after an initial plating in 96 well plates. Confluency at this time was ~40%. A complete medium change with freshly prepared differentiating medium was subsequently performed every 2-3 days. Cells were exposed to these differentiation conditions for 6 to 11 days before being used in FLIPR experiments.

C. Standard experimental solutions.

Solutions of the following composition (in mM) were used in experiments (Buffers without probenicid purchased from Specialty Media (Buffers A and B: Cat. # BSS053A; Buffers C & D: Cat. # BSS056A).

Buffer A (first wash buffer): Krebs-Ringer-HEPES (KRH) buffer: NaCl: 125, KCl: 5, $MgSO_4$: 1.2, KH_2PO_4: 1.2, $CaCl_2$ $2H_2O$: 2, Glucose: 6, HEPES: 25, pH: 7.4 (pH adjusted with NaOH)

Buffer B (dye loading buffer) KRH buffer with 2.5 µM probenicid: same as buffer A, but probenicid added to final concentration of 2.5 µM. Probenecid (Cat. # P-8761, Sigma Chemical Co., St. Louis, MO) made as a stock solution at 250 mM.

Buffer C (dye washout buffer) KRH buffer with 0 mM K^+ and 2.5 µM probenicid: NaCl: 130, $MgSO_4$:1.2, NaH_2PO_4: 1.2, $CaCl_2$ $2H_2O$: 2, Glucose: 6, HEPES: 25, pH: 7.4 (pH adjusted with NaOH).

Buffer D (compound dilution buffer): Buffer C with 0.1% w/v bovine serum albumin (BSA; Sigma).

D. Pharmacological standards and compounds.

The following solutions were used to obtain the data disclosed herein.

Nitrendipine: (RBI Chemicals, Natick, MA): Stock: 10 mM in DMSO; Pipetting solution: 9 µM; pipette 20 µL into 120 µL volume in well for final well concentration: 1 µM.

w-Conotoxin MVIIA: (Cat. # H-8210; Bachem Inc., Torrance, CA): Stock: 1 mM in HPLC grade H_20 with 0.1% BSA; Pipetting solution: 4.5 μM; pipette 20 μl into 140 μl volume in well for final well concentration: 1 μM.

Test compound stock and solution preparation: Compounds prepared daily as stocks at 10 mM in 100% DMSO; Pipetting solution: 45 μM or serial dilutions thereof; pipette 20 μL into 140 μL volume in well for final well concentration: 1 μM or 10-fold dilutions thereof.

High potassium (depolarization) solution: Buffer C with 240 mM K^+ added; pipette 80 μL into 160 μL volume in well for final well concentration of 80 mM K^+.

E. Cell loading with fluorescent dyes.

Fluorescent dye solution preparation: A calcium indicator dye, Fluo-4 acetylmethylester (Fluo 4-AM; Cat. # F-124201; Molecular Probes, Eugene, OR) was used to measure changes in intracellular free calcium with FLIPR. 1 mM Fluo-4 AM stock solution was made by dissolution in DMSO. This stock was then diluted to 4.6 μM with Buffer B (Fluo-4 AM working solution).

Cell loading procedure: Plates containing cells were washed with Buffer A using an automated cell washer (Model #: 5161552, Labsystems Oy, Helsinki, Finland) with controls set to the following parameters: cell height: C/D; cell pulse: 4/5, washes: 3; volume: 5; DRY position setting. These settings resulted in a 70 μL residual depth of buffer over cells in each well. 100 μL of the Fluo-4 AM working solution was then added to each well resulting in a final Fluo-4 AM concentration of 2.7 μM Cells were incubated in this solution at 37 °C for 1-1.5 h. Cells were then washed with Buffer C five times using the cell washer with parameters the same as the pre-loading washes above with the exceptions of: washes: 5; WET position setting. A final wash was then conducted by changing the parameters as follows: washes: 1; volume: 2. This resulted in a final well volume of 120 μL. Cells were allowed to equilibrate under this condition for 10 min, and then used in the FLIPR protocol.

F. FLIPR protocol

Instrumentation: Real time changes in intracellular free calcium in response to potassium-induced depolarization in the absence or presence of putative N-channel inhibitors were measured by either a FLIPR I or FLIPR II (configured for 96-well format) instrument (Molecular Devices, Sunnyvale, CA). Identical settings and protocols were used with each

instrument, and results obtained from the two instruments were indistinguishable for a set of standard benchmark compounds.

FLIPR hardware settings: Laser power was set to about 0.3 watts. Excitation wavelength was set to a 488 nm peak, and the emission wavelength to 540 nm. Camera aperture was set to 2. All experiments were conducted at room temperature (20-22 °C).

Plate layout - reference signals: Certain wells on each plate were allocated to standards to determine minimum and maximum specific fluorescent signal against which inhibitory effects of compounds were normalized. The reference standards were distributed at plate locations including edge and interior wells

Maximum signal (N-channel + non-specific): 12 wells were incubated in nitrendipine (1 μM) solution and 80 mM K^+ added to determine maximal Ca^{2+} increase mediated by N-channels + non-specific (non-L-, non-N-channel mediated fluorescence increase). The coefficient of variation amongst these wells for the K^+-evoked peak increase in fluorescence units was typically less than 12%.

Minimum signal (non-specific): 6 wells were incubated in nitrendipine (1 μM) + w-conotoxin MVIIA and 80 mM K^+ added to determine background Ca^{2+} with all N-channels pharmacologically occluded. The peak non-specific signal component was typically less than 15% of the maximum signal peak amplitude.

N-channel reference small molecule: A compound that had been characterized extensively with respect to N-channel inhibitory activity in both FLIPR and patch clamp electrophysiology was included on each plate in triplicate at 1 μM (near IC_{50}) to establish a reference point.

Test compounds: 5 test compounds were evaluated for potency on each plate. Each compound was tested at 5 increasing concentrations spanning half-log units and typically reaching a maximal concentration of 10 μM. Each concentration was tested in triplicate wells.

Protocol structure: The FLIPR protocol was configured as three solution addition/sampling sequences (see below). Conotoxin (1 μM final conc.) was added to appropriate wells prior to placing the plate in the FLIPR instrument. Wells initially contained a total solution volume of 100 μl, and after all three solution additions contained 240 μl. The active mixing (by the pipette) option was not used in any sequence.

Nitrendipine addition sequence: 28 s total duration with fluorescence signal sampling at 1 Hz for 2 s, followed by addition of 20 µL nitrendipine standard solution at 10 µL/s, followed by sampling at 0.5 Hz for 24 s.

Test compound addition sequence: 64 s total duration with sampling at 0.5 Hz for 4 sec, test solution addition of 40 µL at 20 µL/s, followed by sampling at 0.2 Hz for 60 s.

Compound incubation, cell depolarization and calcium readout sequence: 1024 s total duration with sampling at 0.0167 Hz for 840 s, followed by solution addition 80 µL of high K^+ (depolarization) solution, followed by sampling at 1 Hz for 180 sec. This final 180 sec sampling interval thus represented the epoch where the peak increase in intracellular calcium due to flux through activated N-channels occurred.

G. Data analysis

FLIPR software: Prior to export, the data was normalized within the FLIPR software module for two effects.

Baseline correction: The baseline was corrected by "zeroing" at sample # 57 (immediately prior to KCl addition). This normalization served to correct the y axis offset of the fluorescent trace from each well so that all traces had a common point just prior to onset of the relevant evoked fluorescent increase.

Spatial uniformity correction factor: The data was normalized by a procedure which calculates a mean over the plate of fluorescent units from the first sample, and then multiplies the data from each well by a scalar that adjusts the value of the first sample to this average value, thus normalizing for differences in absolute baseline fluorescence amongst the wells caused by differences in cell densities or dye loading.

External software: Data were exported from FLIPR into Excel as "*.squ" extension files. Following export, operations were performed in Excel to calculate the maximal peak amplitude (relative to the zeroed baseline) of the fluorescence increase following potassium addition in each well. Measurements from wells where an test compound was added were then normalized as a percentage between the mean amplitudes from the reference wells providing the maximum (100%) and non-specific (0%) signal components, as described above. The resulting percent inhibition by test compounds was considered to reflect inhibition of calcium flux at the N-type channel.

II. **Formalin test.**

The Formalin test assesses the inhibitory effects of orally administered N-type calcium channel antagonists on formalin-induced nocifensive behaviours in rats. The formalin test is a well established pain test (Dubuisson and Dennis, 1977; Wheeler-Aceto *et al.*, 1990; Coderre *et al.*, 1993). This test consists of two distinct phases of formalin-induced behavior. The first phase response, occurring between 0 to 5 minutes, is caused by acute nociception to the noxious chemical (formalin) injected into the paw. This is followed by a quiescent period of between 5 to 15 min post injection. A second phase response, occurring after 15 minutes and lasting up to 60 minutes, is caused by sensitization of the central neurons in the dorsal horn. Central sensitization augments the noxious afferent input and a stronger pain barrage is transmitted into the brain. Inhibition of the second phase response is indicative of a central mechanism of drug action.

The procedure for the formalin test is as follows: male rats are placed in a Plexiglas chamber and observed for 30-45 min. to observe their baseline activity. Multiple groups of animals are pretreated with either vehicle or different doses of a test compound. Animals are dosed with the drug of interest either 40 min., if by the intraperitoneal route, or 90 min., if by the oral route, prior to injection of formalin into a hind paw (under the dorsal skin; 0.05 mL of sterile 5% formalin). The number of paw flinches and licks during first phase (0-5 min.) and second phase (20-35 min.) are scored and recorded. Flinch and lick responses are calculated as percentage of inhibition compared with the mean score of a saline control group. Drug potencies are expressed as the dose which causes 50% of the maximum inhibitory effect ("ID_{50}"). Student t-tests are used for statistical analysis to determine the significance of drug effects. Compounds are considered active based on their ability to inhibit the flinch response.

Chemical Methods:

2-hydroxyquinoline and 2-chloroquinoline intermediates of exemplary compounds disclosed herein, see Table 1, were prepared as described below.

Starting quinoline diols were made using a standard procedure (Fischer, M., R. Laschober, *et al.* (1996). "3,4,8-Trimethoxy-2-quinolone. Synthesis of a new alkaloid from Eriostemon gardneri." Sci. Pharm. **64**(3/4): 353-358; Patel, G. H. and C. M. Mehta (1960). "Synthesis of 2,4-Dihydroxyquinolines Using Polyphosphoric Acid as the Cyclizing Agent." J. Sci. Industrial Res. **19B**: 436). The methods and subject matter of these journal articles are

incorporated herein in their entirety by reference. All other reagents were purchased from Acros organics and used directly.

Intermediate 1: 6-methoxy-4-(3,4-dichlorophenyl)amino-2-hydroxyquinoline:

General procedure 1:

6-Methoxyquinolinediol (2.5 g, 13.08 mmol) and 3,4-dichloroaniline (4.2 g, 26.25 mmol) were placed in a 100 mL Teflon vessel for use with an Ethos 1600 Lab Microwave. NMP was then added (9 mL), followed by HCl in dioxane (5 mL, 4 M). The slurry was irradiated to achieve a 200 °C temperature using a 400 W upper setpoint for 30 min. The mixture was cooled, suspended in methanol (20 mL), and filtered to afford 1.049 g of the title compound. ^{1}H NMR (300MHz, DMSO): 3.84 (s, 3H), 5.84 (s, 1H), 7.23 (td, ?H, J=8.88, 8.48 Hz), 7.35(tt, ?H, J=8.88, 2.42 Hz), 7.56(d, ?H, J=2.42 Hz), 7.66 (~s, 2H), 8.71(s, 1H), 11.10(s, 1H).

Intermediate 2: 4-(3,4-dichlorophenyl)amino-2-hydroxyquinoline:

General procedure 2:

2,4-quinolinediol (10.0 g, 62.1 mmol) and 3,4-dichloroaniline (13.1 g, 80.7 mmol) were heated to 190 °C in 25 mL NMP for 48 h. After heating was discontinued, solids began to precipitate. These were collected, treated with 8.5 mL of HCl in isopropanol and sonicated in a 4:1 mixture of acetone:isopropanol. After 2.5 h of sonication, the solids were collected. The sonication cycle was repeated to afford 6.79 g of material (19.9 mmol, 32%). ^{1}H NMR (300MHz, DMSO): 12.7 (s, 1 H), 9.34 (s, 1 H), 8.41 (d, 1 H, J = 9 Hz), 7.71 (m, 3 H), 7.6 (d, 1 H, J = 8.1 Hz), 7.44 (m, 2 H), 6.25 (s, 1 H), 6.08 (broad s, 1 H).

Intermediate 3: 6-Bromo-4-(3,4-dichlorophenyl)amino-2-hydroxyquinoline:

Prepared using general procedure 1; 6-Bromo-2,4-quinolinediol (3g, 12.5 mmol), 3,4-dichloroaniline, 6.25 mL of 2 M HCl in Et_2O were irradiated to 180 °C (400W max) for 40 min. to afford 1.94 g (5.05 mmol, 40%). ^{1}H NMR (300MHz, DMSO): 11.36 (s, 1H), 8.83(s, 1H), 8.28(s, 1H), 7.70(dd, 1H, J=8.88, 1.61 Hz), 7.64(d, 1H, J=8.88 Hz), 7.55(d, 1H, J=2.02 Hz), 7.33(dd, 1H, J=8.88, 2.42 Hz), 7.25(d, 1H, J=8.88 Hz), 5.86(s, 1H).

Intermediate 4: 6-Fluoro-4-(3,4-dichlorophenyl)amino-2-hydroxyquinoline:

Prepared using general procedure 1; 6-Fluoro-2,4-dichloroquinolinediol (2.5 g, 13.95 mmol), 3,4-dichloroaniline (4.5 g, 27.91 mmol), 5 mL of 4 M HCl in dioxane (20 mmol) were irradiated to 180 °C (400W max) for 40 min. Pouring onto methanol followed by collection of the solids, afforded 2.136 g of material (6.61 mmol, 47%). ^{1}H NMR (300MHz, DMSO):

11.48(s, 2H), 8.74(s, 1H), 7.92(dd, 1H, J=10.70, 2.60 Hz), 7.65(d, 1H, J=8.88 Hz), 7.56(d, 1H, J=2.42 Hz), 7.49(d, 1H), 7.33(m, 2H), 5.89(s, 1H).

Intermediate 5: 8-Bromo-2-Hydroxy-4-(2,3-dichlorophenyl)aminoquinoline:

Prepared using general procedure 1; 8-Bromo-2,4-quinolinediol (4 g, 16.66 mmol), 3,4-dichloroaniline (8 g, 49.38 mmol), 2.78 mL of 6 M HCl (16.68 mmol) and 20 mL NMP were irradiated to achieve 200 °C (400W max) for 30 min. Cooling and precipitation by pouring onto 100 mL water afforded 3.52 g of product (9.16 mmol, 55%). ^{1}H NMR (300MHz, DMSO): 9.77(s, 2H), 8.95(s, 1H), 8.11(dd, 1H, J=8.48, 0.81 Hz), 7.89(dd, 1H, J=7.67, 0.81 Hz), 7.66(d, 1H, J=8.88 Hz), 7.58(d, 1H, J=2.42 Hz), 7.36(dd, 2H, J=8.48, 2.42 Hz), 7.20(t, 1H, J=8.07 Hz), 5.88(s, 1H).

Intermediate 6: 8-Fluoro-2-Hydroxy-4-(2,3-dichlorophenyl)aminoquinoline:

Prepared using general procedure 1; 8-Bromo-2,4-quinolinediol (4 g, 22.33 mmol), 3,4-dichloroaniline (10.88 g, 66.66 mmol), 3.7 mL of 6 M HCl (22.2 mmol) and 20 mL NMP were irradiated to achieve 200 °C (400W max) for 30 min. Cooling and precipitation by pouring onto 100 mL water afforded 3.2 g of product (9.90 mmol, 44%). ^{1}H NMR (300MHz, DMSO): 9.80(s, 2H), 8.94(s, 1H), 8.12(d, 1H, J=8.48 Hz), 7.90(d, 1H, J=7.67 Hz), 7.66(d, 1H, J=8.88 Hz), 7.58(d, 1H, J=2.42 Hz), 7.37(dd, 1H, J=8.88, 2.42 Hz), 7.12(t, ?H, J=7.87 Hz), 5.90(s, 1H).

Intermediate 7: 8-Bromo-2-chloro-4-(3,4-dichlorophenyl)aminoquinoline:

General procedure 3:

8-Bromo-2-hydroxy-4-(3,4-dichlorophenyl)aminoquinoline (3.52 g, 9.16 mmol) and 26 mL of $POCl_3$ were heated to 120 °C for 4 h. The bulk of the $POCl_3$ was distilled (~18 mL), and the reaction cooled. The solution was poured slowly onto warm water to get a gummy solid. The water was decanted and the solids washed with several portions of water. A final wash with toluene and drying under vacuum afforded 1.43 g of material (3.55 mmol, 38.8%). ^{1}H NMR (300MHz, DMSO): 9.58(s, 1H), 8.39(d, 1H, J=8.48 Hz), 8.15(d, 1H, J=7.67 Hz), 7.70(d, 1H, J=8.88 Hz), 7.66(d, 1H, J=2.42 Hz), 7.51(dd, 1H, J=8.48, 7.67 Hz), 7.44(d, 1H, J=8.88, 2.42 Hz), 6.90(s, 1H).

Intermediate 8: 8-Fluoro-2-chloro-4-(3,4-dichlorophenyl)aminoquinoline:

Prepared using general procedure 3; 8-Fluoro-2-hydroxy-4-(3,4-dichlorophenyl)amino-quinoline (3.2 g, 9.90 mmol) and 23 mL of $POCl_3$ were heated to 120 °C for 4 h. Isolation afforded 0.69 g of material (1.98 mmol, 20%). ^{1}H NMR (300MHz, DMSO): 9.50(s, 1H),

8.17(d, ?H, J=7.67 Hz), 8.04(d, 1H, J=5.25 Hz), 7.79(d, 1H, J=5.65 Hz), 7.71(d, 1H, J=8.88 Hz), 7.61(m, 1H), 7.45(dd, 1H, J=8.88, 2.42 Hz), 6.88(s, 1H).

Intermediate 9: 2-Chloro-4-(3,4-dichlorophenyl)aminoquinoline:

Prepared using general procedure 3; 2-Hydroxy-4-(3,4-dichlorophenyl)aminoquinoline (1.5 g, 4.9 mmol) and 9.2 mL of $POCl_3$ were heated to 120 °C for 4 h. Isolation afforded 1.075 g of material (3.32 mmol, 68%). ^{1}H NMR (300MHz, DMSO): 9.61(s, 1H), 8.40(d, 1H, J=8.07 Hz), 7.82(m, 2H), 7.65(m, 3H), 7.45(dd, 1H, J=8.68, 2.63 Hz), 6.87(s, 1H).

Intermediate 10: 6-Bromo-2-chloro-4-(3,4-dichlorophenyl)aminoquinoline:

Prepared using general procedure 3; 6-Bromo-2-hydroxy-4-(3,4-dichlorophenyl)amino-quinoline (1.94 g, 5.05 mmol) and 30 mL of $POCl_3$ were heated to 120 °C for 4 h. Isolation afforded 1.209 g of material (3.00 mmol, 60%). ^{1}H NMR (300MHz, DMSO): 9.69(s, 1H), 8.71(s, 1H), 7.91(d, 1H, J=8.88 Hz), 7.78(d, 1H, J=8.88 Hz), 7.69(d, 1H, J=8.88 Hz), 7.68(s, 1H), 7.44(dd, 1H, J=8.68, 2.22 Hz), 6.91(m, 1H).

Intermediate 11: 6-Fluoro-2-chloro-4-(3,4-dichlorophenyl)aminoquinoline:

Prepared using general procedure 3; 6-Fluoro-2-hydroxy-4-(3,4-dichlorophenyl)amino-quinoline (2.136 g, 6.6 mmol) and 30 mL of $POCl_3$ were heated to 120 °C for 4 h. Isolation afforded 0.654 g of material (1.914 mmol, 29%). ^{1}H NMR (300MHz, DMSO): 9.55(s, 1H), 8.27(dd, 1H, J=10.50, 2.02 Hz), 7.92(dd, 1H, J=8.88, 5.65 Hz), 7.70(m, 3H), 7.45(dd, 1H, J=8.48, 2.02 Hz), 6.90(s, 1H).

Intermediate 12: 6-Methoxy-2-chloro-4-(3,4-dichlorophenyl)aminoquinoline:

Prepared using general procedure 3; 6-Methoxy-2-hydroxy-4-(3,4-dichlorophenyl)amino-quinoline (1.05 g, 3.13 mmol) and 26 mL of $POCl_3$ were heated to 120 °C for 4 h. Isolation afforded 0.685 g of material (1.94 mmol, 61%).

Intermediate 13: 4-Chloro-2-(N-2-(4-morpholino)phenylamino)quinoline. 2,4-Dichloroquinoline (300 mg, 1.51 mmol), 2-morpholinoaniline (297 mg, 1.66 mmol), 1 M HCl in Et_2O (3 mL) and NMP (3 mL) were added to a 10 mL Schlenk Flask. The ether was removed with a stream of nitogen, and the flask heated to 130 °C for 16 h. The reaction was cooled and passed through a 500 mg tC18 Sep Pak© (Waters Corp), and purified on 3 x 100 Novapak HR RCM segments using methanol/water/0.1% TFA at 25 mL per min. Evaporation of the solvents afforded 326.2 mg of product as a TFA salt (0.718 mmol, 48%) along with the product arising from bis-addition (116 mg, 0.22 mmol, 15%). Bis-addition 1H NMR (DMSO, 300 mHz): 12.31(s, 1H), 9.90(s, 1H), 9.70(s, 1H), 8.49 (d, 1H, J=8.48 Hz),

7.84 (m, 2H), 7.57 (m, 1H), 7.33 (m, 4H), 7.14 (m, 4H), 5.85 (s, 1H), 3.38(s, 8H), 2.87 (s, 4H).

Intermediate 14: 4-(2-methoxyethylamino)-2-hydroxyquinoline: Prepared as in general procedure 1; Quinoline diol (1 g, 6.2 mmol), 2-methoxyethylamine (700 mg, 9.3 mmol) afforded 722 mg of product (53%) which was taken on directly.

Intermediate 15: 2-Chloro-4-(2-methoxyethylamino)quinoline: Prepared using general procedure 3; 4-(2-methoxyethylamino)-2-hydroxyquinoline (611 mg, 2.8 mmol) and 2.5 mL of $POCl_3$ were heated to 120 °C for 8 days. Isolation afforded 610 mg of material (92%).

Diversification Library: General Procedure 4:

2-Chloroquinoline intermediates (prepared above) (300 mg) were coupled with 2 equivalents of amine (from Table I) using a ChemSpeed robot. Each coupling was carried out in NMP for the time and temperature indicated in Table 2, and the isolated yield was calculated for >50% of the entries. The compounds were characterized with HPLC, HPLC-MS and purified by preparative HPLC.

Table 1: Amine reagents used in synthesis of compounds of the present invention.

A	2-hydroxyaniline	**B**	2-methoxyaniline
C	2-methylaniline	**D**	2-aminoaniline
E	2-fluoroaniline	**F**	Aniline
G	3,4-dichloroaniline	**H**	4-fluoro-3-chloroaniline
J	3,4-dimethoxyamine	**L**	2-morpholinoaniline
M	6-aminobenzothiazole	**N**	4-fluoroaniline
O	2-chloroaniline		

Examples:

Exemplary compounds 1 to 34 inclusive are illustrated in Table 2 which shows the name of each compound and the specifics of reaction time, reaction temperature, yield and molecular ion. Quinoline precursors were prepared by the general procedures 1, 2 and 3 as appropriate, reacted with an amine from Table 1 as indicated, using general procedure 4.

Table 2: Physical data for Examples 1-34.

Ex. #	amine	Compound Name	yield	time	temp.	MS (m+H)
1	L	N2,N4-bis-(2-morpholin-4-yl-phenyl)-quinoline-2,4-diamine	29	17:00	130	482

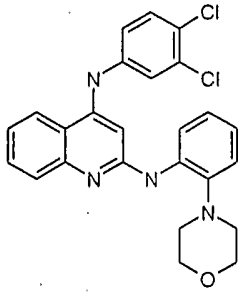
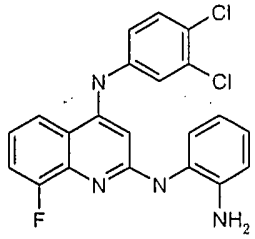
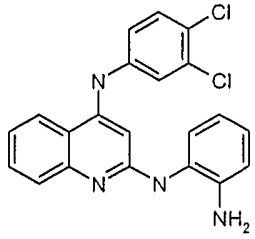
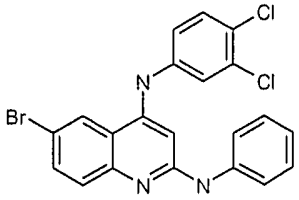
Ex. #	amine	Compound Name	yield	time	temp.	MS (m+H)
2	L	N4-(3,4-Dichloro-phenyl)-N2-(2-morpholin-4-yl-phenyl)-quinoline-2,4-diamine	74	24:00	107	465
3	B	8-Bromo-N4-(3,4-dichloro-phenyl)-N2-(2-methoxy-phenyl)-quinoline-2,4-diamine	45	16:00	180	488
4	C	8-Bromo-N4-(3,4-dichloro-phenyl)-N2-o-tolyl-quinoline-2,4-diamine	36	16:00	180	472
5	E	8-Bromo-N4-(3,4-dichloro-phenyl)-N2-(2-fluoro-phenyl)-quinoline-2,4-diamine	76	16:00	180	476
6	O	8-Bromo-N2-(2-chloro-phenyl)-N4-(3,4-dichloro-phenyl)-quinoline-2,4-diamine	58	16:00	180	492
7	A	2-[8-Bromo-4-(3,4-dichloro-phenylamino)-quinolin-2-ylamino]-phenol	46	16:00	180	474
8	D	N2-(2-Amino-phenyl)-8-bromo-N4-(3,4-dichloro-phenyl)-quinoline-2,4-diamine	34	16:00	180	473
9	A	2-[4-(3,4-Dichloro-phenylamino)-quinolin-2-ylamino]-phenol	56	18:00	150	396
10	B	N4-(3,4-Dichloro-phenyl)-N2-(2-methoxy-phenyl)-quinoline-2,4-diamine	54	18:00	150	410
11	C	N4-(3,4-Dichloro-phenyl)-N2-o-tolyl-quinoline-2,4-diamine	59	18:00	150	394
12	A	2-[4-(3,4-Dichloro-phenylamino)-8-fluoro-quinolin-2-ylamino]-phenol	54	16:00	180	414
13	B	N4-(3,4-Dichloro-phenyl)-8-fluoro-N2-(2-methoxy-phenyl)-quinoline-2,4-diamine	36	16:00	180	428
14	D	N2-(2-Amino-phenyl)-N4-(3,4-dichloro-phenyl)-8-fluoro-quinoline-2,4-diamine	54	16:00	180	413
15	F	N4-(3,4-Dichloro-phenyl)-8-fluoro-N2-phenyl-quinoline-2,4-diamine	32	16:00	180	398
16	E	N4-(3,4-dichloro-phenyl)-8-fluoro-N2-(2-fluoro-phenyl)-quinoline-2,4-diamine	22	16:00	180	416

Ex. #	amine	Compound Name	yield	time	temp.	MS (m+H)
17	C	6-bromo-N4-(3,4-dichloro-phenyl)-N2-o-tolyl-quinoline-2,4-diamine	57	16:00	150	472
18	D	N2-(2-amino-phenyl)-6-bromo-N4-(3,4-dichloro-phenyl)-quinoline-2,4-diamine	65	16:00	150	473
19	E	6-bromo-N4-(3,4-dichloro-phenyl)-N2-(2-fluoro-phenyl)-quinoline-2,4-diamine	67	16:00	150	476
20	F	6-bromo-N4-(3,4-dichloro-phenyl)-N2-phenyl-quinoline-2,4-diamine	89	16:00	150	458
21	A	2-[4-(3,4-dichloro-phenylamino)-6-fluoro-quinolin-2-ylamino]-phenol	65	16:00	150	414
22	E	N4-(3,4-dichloro-phenyl)-6-fluoro-N2-(2-fluoro-phenyl)-quinoline-2,4-diamine	36	16:00	150	416
23	F	N4-(3,4-dichloro-phenyl)-6-fluoro-N2-phenyl-quinoline-2,4-diamine	68	16:00	150	398
24	B	N4-(3,4-dichloro-phenyl)-6-methoxy-N2-(2-methoxy-phenyl)-quinoline-2,4-diamine	12	16:00	150	440
25	C	N4-(3,4-dichloro-phenyl)-6-methoxy-N2-o-tolyl-quinoline-2,4-diamine	30	16:00	150	424
26	B	6-bromo-N4-(3,4-dichloro-phenyl)-N2-(2-methoxy-phenyl)-quinoline-2,4-diamine	27	16:00	105	488
27	D	N2-(2-amino-phenyl)-N4-(3,4-dichloro-phenyl)-quinoline-2,4-diamine	34	16:00	150	395
28	E	N4-(3,4-dichloro-phenyl)-N2-(2-fluoro-phenyl)-quinoline-2,4-diamine	65	16:00	150	398
29	F	N4-(3,4-dichloro-phenyl)-N2-phenyl-quinoline-2,4-diamine	48	16:00	150	380
30	A	2-[6-bromo-4-(3,4-dichloro-phenylamino)-quinolin-2-ylamino]-phenol	82	16:00	150	474
31	F	8-bromo-N4-(3,4-dichloro-phenyl)-N2-phenyl-quinoline-2,4-diamine	15	16:00	180	458
32	D	N2-(2-amino-phenyl)-N4-(3,4-dichloro-phenyl)-6-methoxy-quinoline-2,4-diamine	45	16:00	150	425

Ex. #	amine	Compound Name	yield	time	temp.	MS (m+H)
33	E	N4-(3,4-dichloro-phenyl)-N2-(2-fluoro-phenyl)-6-methoxy-quinoline-2,4-diamine	63	16:00	150	428
34	F	N4-(3,4-dichloro-phenyl)-6-methoxy-N2-phenyl-quinoline-2,4-diamine	35	16:00	150	410

Biological data for selected compounds is shown in Table 3.

Table 3: Biological Data

Ex.#	Compound Structure	Formalin test % inhibition (dose, time route)	FLIPr IC_{50}(N)
2		42 (60, 40, IP)	10.00
14			4.01
27			9.82
33			10.00

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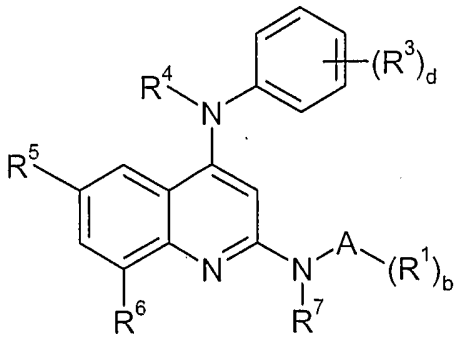
CLAIMS

1. Any compound in accord with structural diagram I;



I

wherein:

A is selected from phenyl, heteroaryl or bicyclic heteroaryl;

R^1 at each occurrence is independently selected from halogen, $(C_1\text{-}C_6)$alkyl, heterocyclyl, OH, $(C_1\text{-}C_6)$alkoxy or NR^2_2;

b is an integer selected from 0, 1, 2 or 3;

R^2 at each occurrence is independently selected from H or $(C_1\text{-}C_4)$alkyl;

R^3 at each occurrence is independently selected from halogen or $(C_1\text{-}C_4)$alkyl;

d is an integer selected from 0, 1, 2 or 3;

R^4 is selected from H or $(C_1\text{-}C_4)$alkyl;

R^5 is selected from the group consisting of H, halogen, $(C_1\text{-}C_3)$alkyl, $(C_1\text{-}C_3)$perfluoroalkyl, $(C_1\text{-}C_3)$alkoxy, hydroxy, NH_2 or NHR^2;

R^6 is selected from H, halogen, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_4)$perfluoroalkyl, $(C_1\text{-}C_6)$alkoxy, hydroxy, $(C_1\text{-}C_6)$alkanoyl, $C(=O)NR^2_2$ or $-NR^2_2$; and

R^7 is selected from H or methyl.

2. Compounds according to Claim 1, wherein:

A is selected from phenyl or bicyclic heteroaryl;

R^1 at each occurrence is independently selected from halogen, $(C_1\text{-}C_6)$alkyl, heterocyclyl, OH, $(C_1\text{-}C_6)$alkoxy or NR^2_2;

b is an integer selected from 0, 1, 2 or 3;

R^2 at each occurrence is independently selected from H or $(C_1\text{-}C_4)$alkyl;

R^3 at each occurrence is independently selected from halogen or $(C_1\text{-}C_4)$alkyl;

d is an integer selected from 0, 1, 2 or 3;

R^4 is H;

R^5 is selected from H, halogen, $(C_1$-$C_3)$alkyl, $(C_1$-$C_3)$perfluoroalkyl or $(C_1$-$C_3)$alkoxy;

R^6 is selected from H, halogen, $(C_1$-$C_6)$alkyl, $(C_1$-$C_4)$perfluoroalkyl, $(C_1$-$C_6)$alkoxy, hydroxy, $(C_1$-$C_6)$alkanoyl, $C(=O)NR^2_2$ or $-NR^2_2$; and

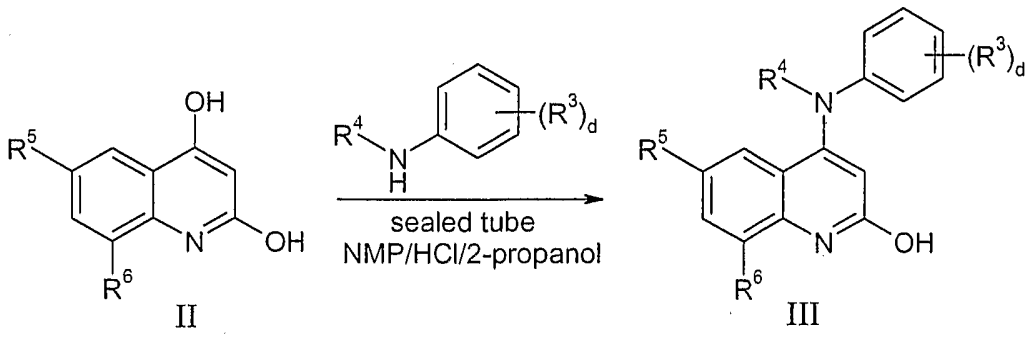
R^7 is H.

3. A method for the treatment of pain in a subject suffering therefrom, comprising: administering to said subject a pain-ameliorating effective amount of a compound according to Claim 1.

4. A pharmaceutical composition comprising a therapeutically-effective amount of a compound according to Claim 1 together with at least one pharmaceutically-acceptable excipient or diluent.

5. A method for preparing compounds according to Claim 1, said method comprising:

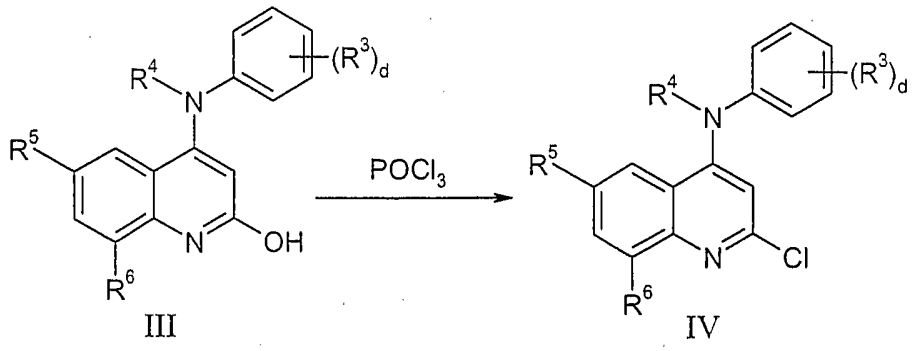
a) reacting a substituted 2,4-quinolinediol (structural diagram II) with three equivalents of an alkyl amine in N-methylpyrrolidinone and 6N HCL in 2-propanol, in a sealed tube at a temperature of about 180 °C;



II III

wherein R^3, R^4, R^5, R^6 and b are as defined as in claim 1;

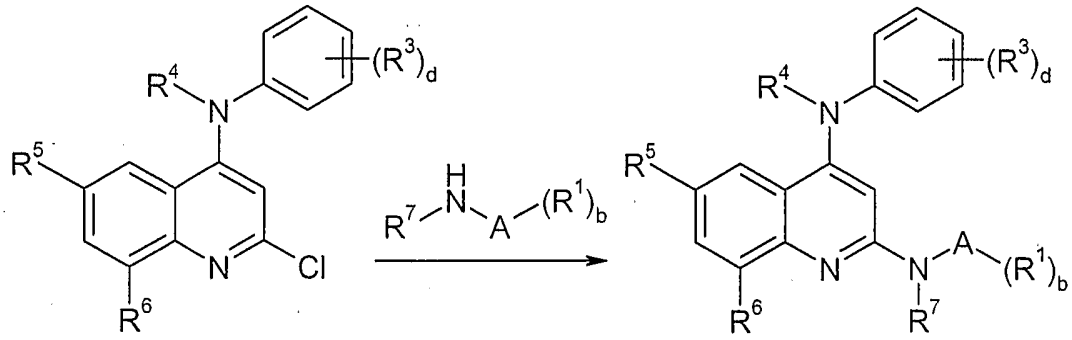
b) chlorinating the 2-hydroxy-4-aminoquinoline compound according to structural diagram III by refluxing with $POCl_3$ to form a compound in according to structural diagram IV;



III IV

wherein R^3, R^4, R^5, R^6 and d are as defined as in claim 1;

c) reacting 2-chloro-4-aminoquinoline precursors with aromatic amines in N-methylpyrrolidinone at temperatures of 100-180 °C.



IV I

wherein R^1, R^3, R^4, R^5, R^6, R^7, b and d are as defined as in claim 1;

6. The method according to claim 5 wherein step a) comprises;

reacting a substituted 2,4-quinolinediol in accord with structural diagram II with two equivalents of an aryl amine in N-methylpyrrolidinone and 4 M HCL in dioxane, in a sealed tube using microwave irradiation at a 400 W upper set point to maintain a 200 °C temperature for 30 minutes.

7. The method according to claim 5, wherein step c) is performed in a parallel fashion using robotic instrumentality.

8. The method according to claim 6, wherein step c) is performed in a parallel fashion using robotic instrumentality.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 02/01521

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C07D 215/46, C07D 413/12, A61K 31/4706, A61K 31/4709, A61K 31/5377, A61P 29/00, A61P 25/04

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C07D, A61K, A61P

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

SE,DK,FI,NO classes as above

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

EPO-INTERNAL, WPI DATA, CHEM. ABS DATA, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Tetrahedron Letters, Volume 33, 1977, E. B. Pedersen: "Synthesis of 2,4-bis(dimethylamino)quinolines by hmpt induced ring closure of anthranilates", pages 217-220 --	1-6
A	Chemica Scripta, Volume 28, No. 4, 1988, Jorgen A. Jensen et al: "New One-step Synthesis of 2,4-Bis(dialkylamino)quinoines and 4,6-Bis (dialkylamino)thieno[2,3-b]pyridines", pages 435-437 --	1-6
A	WO 064877 A1 (NEUROGEN CORPORATION), 2 November 2000 (02.11.00) --	1-6

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

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 November 2002	27-11-2002
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Viveca Norén/EÖ Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 02/01521

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4357333 A (JOHN L. ARCHIBALD ET AL), 2 November 1982 (02.11.82) -- --------	1-6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE02/01521

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 3
because they relate to subject matter not required to be searched by this Authority, namely:

see next sheet

2. ☒ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE02/01521

Claim 3 relates to a method of treatment of the human or animal body by surgery or by therapy/a diagnostic method practised on the human or animal body/Rule 39.1(iv). Nevertheless, a search has been executed for this claim. The search has been based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

28/10/02

International application No.
PCT/SE 02/01521

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 064877 A1	02/11/00	NONE	
US 4357333 A	02/11/82	AU 516318 B	28/05/81
		AU 3917178 A	28/02/80
		CA 1096870 A	03/03/81
		DE 2860620 D	00/00/00
		DK 149625 B,C	18/08/86
		DK 403878 A	15/03/79
		EP 0001175 A,B	21/03/79
		SE 0001175 T3	
		GB 1573186 A	20/08/80
		IE 47249 B	25/01/84
		IE 781728 L	14/03/79
		IT 1099075 B	18/09/85
		IT 7827698 D	00/00/00
		JP 54092974 A	23/07/79
		ZA 7804709 A	26/03/80