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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 October 2022 (27.10.2022)

WIPO | PCT

(10) International Publication Number
WO 2022/223590 A1

(51) International Patent Classification:
A61K 31/198 (2006.01) *A61P 29/02* (2006.01)
A61P 25/02 (2006.01)

(21) International Application Number:
PCT/EP2022/060375

(22) International Filing Date:
20 April 2022 (20.04.2022)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
102021000010181 21 April 2021 (21.04.2021) IT

(71) Applicant: **REDYNEUHEART S.R.L.** [IT/IT]; Via Santa Marta, 19, 20123 Milano (IT).

(72) Inventors: **FANTUCCI, Piercarlo**; Strada Ca' Intramonte 3, 15049 Vignale Monferrato (Alessandria) (IT). **GOVONI, Stefano**; Via Mascherpa 71, 27100 Pavia (IT). **PASCALE, Alessia Angela**; Via Baldo degli Ubaldi 136, 27100 Pavia (IT). **MARCHESI, Nicoletta**; Via N. Riccardi 36, 27040 Pinarolo Po (Pavia) (IT). **ALLEGRI, Massimo**; Via Pradello 2, 24121 Bergamo (IT). **VANOLI, Emilio**; Via Puccini 14, 22070 Guanzate (Como) (IT). **CALVILLO, Laura**; Via Bramante 2, 20812 Limbiate (Monza Brianza) (IT). **REGGIANI, Paolo**; Viale Parioli 50, 00197 Roma (IT). **PACE, Valentina**; Via del casale di Santa Maria 156, 00132 Roma (IT).

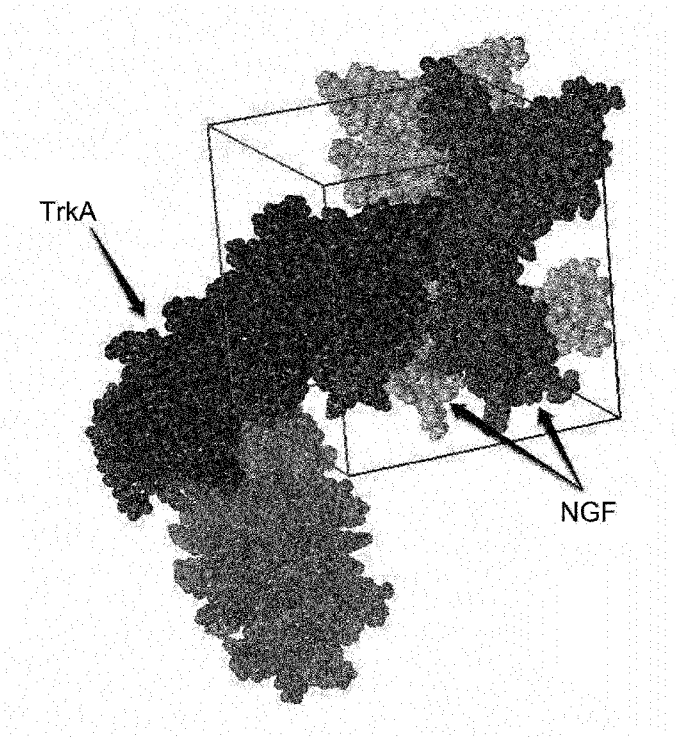
(74) Agent: **RAPISARDI, Mariacristina**; Via Serbelloni, 12, 20122 MILANO (IT).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM,

WO 2022/223590 A1

(54) Title: N-ACETYL CYSTEINE FOR NEURAXIAL USE AS A TRKA TYROSINE KINASE RECEPTOR INHIBITOR FOR THE TREATMENT OF ACUTE AND CHRONIC PAIN

FIG.1



(57) Abstract: N- Acetyl cysteine (NAC) for use as an antagonist of the TrkA tyrosine kinase receptor in the treatment and/or management of acute and chronic pain conditions in which there is hyperactivity of the nerve growth factor (NGF),, wherein said NAC is administered locally where the condition due to nerve growth factor (NGF) hyperactivity occurs.

[Continued on next page]

TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

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

N-ACETYL CYSTEINE FOR NEURAXIAL USE AS A TRKA TYROSINE KINASE RECEPTOR INHIBITOR FOR THE TREATMENT OF ACUTE AND CHRONIC PAIN

DESCRIPTION

FIELD OF THE INVENTION

The present invention relates to the field of medical preparations containing organic active ingredients, in particular it relates to a new use and route of administration of N-acetylcysteine (NAC) based on the discovery of a new mechanism of action of the molecule and the use thereof for the treatment of acute and chronic pain.

PRIOR ART

Nerve growth factor (NGF) is a protein which regulates the growth and survival of sympathetic and sensory nerve cells (neurons). Therefore, NGF belongs to a family of growth factors called neurotrophins.

NGF binds with great affinity and activates the TrkA tyrosine kinase receptor.

After activation, the TrkA receptor dimerizes and autophosphorylates, thus initiating an important signalling process for the growth and survival of sympathetic neurons.

The ability of NGF to activate the TrkA receptor and, consequently, to support the survival and stimulate the growth of neurons has made it of great interest in the field of research aimed at discovering new possibilities for the treatment of diseases and disorders of the central and peripheral nervous system. On the other hand, NGF is expressed and finds receptors not only on peripheral neurons and the central nervous system but also in other cell types and organs.

NGF has also been identified as an important pain mediator, in particular chronic pain, and as such is to be considered an important target in the search for new pharmacological strategies for treating pain. An approach in this regard employs the sequestration of NGF using anti-NGF antibodies such as Tanezumab (Pfizer), Fulranumab (Johnson and Johnson), REGN475/SAR164877 (Regeneron together with Sanofi Aventis) or medi578 (AstraZeneca).

For an analysis of pain control through NGF modulation, see for example McKelvey et al., J. Neurochm. (2013) 124, 276-289.

Therefore, there is a strong rationale for the development of new compounds with antagonistic activity against NGF.

Furthermore, severe clinical conditions such as acute and chronic pain should always be managed in polytherapy with the aim of minimizing the side effects associated with the use of any drug at maximum doses. In this sense, the antagonism of NGF is absolutely original, in particular in the manner envisaged in the present patent proposal.

Acetylcysteine (or N-acetylcysteine, NAC) is an N-acetylated derivative of the sulfur amino acid cysteine. The drug is used (in a dose of about 3- 9 mg/kg) orally or as an expectorant mucolytic and in case of tear deficiency or for its important systemic antioxidant action (an important patho-physiological element in the genesis of pain). The drug is also considered a lifesaver in the event of an overdose of paracetamol, especially to prevent the development of fulminant hepatitis which is often the result. In paracetamol intoxication, the intravenous administration route is used (150 mg/kg attack doses).

SUMMARY OF THE INVENTION

The inventors of the present invention have surprisingly discovered that NAC is capable of inhibiting the autophosphorylation of TrkA, acting at the level of the cysteine groups thereof including in particular, but not exclusively, the 300 and 345 cysteines.

According to the invention, NAC is used as an antagonist of the TrkA tyrosine kinase receptor in the treatment and/or management of acute and chronic pain conditions. According to the invention, NAC is used even more preferably in the treatment and/or management of pain by neuraxial infusion.

According to the invention, NAC is also used in the treatment and/or management of acute and chronic pain associated with high levels of NGF and chronicity after an episode of acute pain.

According to the invention, NAC is preferably administered at the neuraxial level, i.e., where the condition due to hyperactivity of the nerve growth factor (NGF) manifests itself.

According to the invention, the administration of NAC is neuraxial (intrathecal or intraspinal).

In particular, NAC according to the present invention is suitable for use in the treatment and/or management in single-administration and in co-administration with local anaesthetic (ropivacaine or bupivacaine) of acute postoperative and radiculopathic pain and chronic pain such as root, spine, spasticity central pain and as an adjuvant in osteoarthritic pain, rheumatoid arthritis, interstitial cystitis, chronic pelvic pain syndrome, and cancer pain.

According to the invention the effective dose of said NAC to be administered neuraxially in adult patients is comprised between 0.5 and 15 mg/Kg, preferably 12 mg/Kg, possibly in combination with the local anaesthetic.

In addition to man, NAC according to the present invention can be used in the treatment and prophylaxis of the above conditions in animals such as primates, pigs, ruminants (cows, sheep, goats), horses, cats, dogs, poultry (for example, chickens, ducks, geese, quails, pigeons, turkeys or ornamental birds), as well as productive and ornamental fish, reptiles and amphibians.

The term pain management refers to all treatment regimens which do not completely eliminate pain from the patient, but reduce pain to improve or significantly improve the patient's quality of life.

Pharmaceutical forms according to the invention are, among others, the preparations for injection and infusion in the form of sterile solutions, suspensions, emulsions, lyophilisates or powders.

In some embodiments, it can be preferred to combine NAC with other pharmaceutically active compounds, such as local anaesthetics (e.g., ropivacaine). Other analgesics suitable for use in combination with NAC include opioids such as morphine and fentanyl (opioids indicated for neuraxial use).

The minimum amount of NAC of the invention to be administered is a therapeutic amount. The term "therapeutically effective amount" means an amount of compound which prevents the onset of or alleviates symptoms, manages, stops the progression and/or eliminates a disease, a disorder or a condition by virtue of the inhibition of the TrkA receptor, acting in diseases linked to high levels of NGF.

In some embodiments of the invention, doses of NAC are administered as a single dose. In other embodiments of the invention, doses of NAC are administered every day or every 3, 5, 7 or 10 days.

In some cases of chronic pain, an increasing course of doses may be required to find the optimal dose for the individual patient. However, it may be necessary to deviate from the above amounts if required by the circumstances. Such deviations could be due to body weight, individual response to the active substance, severity of the condition, disease or disorder, type of preparation and time or interval in which the administration occurs. Therefore, in some cases it may be sufficient to use lower doses than the minimum amount above, while in other cases the upper limit above must be exceeded. In the case of administration of large amounts of NAC, it may be appropriate to distribute these in a plurality of single doses during the day.

The present invention can be better understood in the light of the following embodiment examples.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 – shows the NGF-TrkA complex.

Fig. 2 – shows the characteristic disulphide bridges between the 6 beta-sheets of TrkA.

Fig. 3 – shows the results of in vitro assays of inhibition of the phosphorylation of TrkA in the presence of 0.4 mM DTT (dithiothreitol, a non-specific reductant).

Fig. 4 – shows the results of in vitro TrkA phosphorylation inhibition assays

A) in the presence of 0.4 mM or 10 mM DTT or NAC;

B) at different concentrations of NAC.

Fig. 5 – shows the result of the NAC-TrkA in silico docking.

Fig. 6 – shows the result of the in silico covalent binding of NAC-TrkA.

Fig. 7 – shows the results of a formalin test on mice and in particular the effect of intrathecal NAC (5 microlitres, containing 0.3 mg of NAC) in the mouse against the pain induced by the administration of formalin in the hind paw.

EXPERIMENTAL PART

MATERIALS AND METHODS

A) Evaluation of biological activity

In-vitro

ELISA

The ability of NAC to act as an antagonist of the TrkA receptor was measured using an enzymatic immunosorbent assay (ELISA) for the phosphorylated TrkA receptor. This ELISA kit is commercially available, for example from *Cell Signalling Technologies* under the name PathScan® Phospho-TrkA (Tyr674/675) Sandwich ELISA Kit (catalogue number 7212).

The test is used according to the manufacturer's instructions by exposing the PC12 and/or SH-SY5Y cell lines, as cell types expressing TrkA receptors, to the compounds of the invention. The cells grow in a medium suitable for cell culture (for example: 60-mm Falcon©) at a certain cell density (example: 0.5-1x106 cells/ml) in a specific and complete culture medium, as for

example reported in Marchesi *et al.* (J Cell Physiol. 2014; 229(11):1776-86) for the SH-SY5Y cell line and Rossi *et al.* (Bioorg Med Chem. (2011); 19 (21):6210-24) for the PC12 cell line.

The procedure for the adhering cells is as follows: when the cell culture reaches a confluence of 80-90%, the culture medium is changed with a fresh medium containing a low percentage of bovine serum (Fetal Bovine Serum/FBS) and for a predetermined period of time. After washing with a phosphate buffered saline, 0.4/0.5 ml of cold 1X Cell Lysis Buffer (specific buffer name) containing protease inhibitors are added to each plate, in contact with the cells, for 5 minutes in ice. The cells are removed from the plate with a scraper and transferred to an appropriate tube stored in ice where they will be sonicated. The suspension is centrifuged for 10 minutes (at 13-14000 rpm) at 4°C and the supernatant is transferred to a new tube and stored at -80°C. The percentage of phosphorylated TrkA after exposure to the compounds of the invention can be compared to a calibration curve performed using NGF as a reference. The compounds having antagonistic activity prevent activation by NGF.

A) In-vivo

The ability of NAC to treat or manage pain can be demonstrated in a mouse or rat model. To test the analgesic properties of NAC, a nociceptive and peripheral neuropathic pain model is used. Such a model involves the intra-plantar injection of formalin (or possibly other substances), which induces an increase in the thermal and mechanical sensitivity at the injection site (primary hypersensitivity). Thereafter, the animals are treated one or more times with different doses of NAC. The ability of NAC to treat pain can be observed through behavioural and pain assessment tests.

EXAMPLE 1

It is known that during the action of NGF on the TrkA receptor, TrkA and NGF form a single complex consisting of two NGF molecules and two TrkA molecules, forming two homo-

dimers. The present inventors have mapped the short contact distances between the two TrkA molecules and the two NGF molecules to find the smallest example of TrkA-NGF complex. This research has shown that the largest number of short-range interactions occurs between the first NGF molecule and the second TrkA molecule (Fig. 1).

The specific distances between the nearest carbon atoms α in the first molecule of NGF and in the second TrkA molecule are shown in Table 1.

Table 1: Distances between the carbon atoms a of *NGF(1)* and *TrkA(2)*

Residue Nr. of NGF1	**Atom Nr. Cα of NGF1**	**Residue Nr. of TrkA2**	**Atom Nr. Cα of TrkA2**	**Distances (Å)**
12	84	297	114	4.20
13	95	297	114	4.59
2	2	291	68	5.65
11	75	297	114	5.79
6	31	344	497	6.14
20	141	379	753	6.19
21	148	380	761	6.66
19	135	379	753	6.82
5	24	343	487	6.91
23	169	382	777	6.93
10	71	296	106	7.09
4	14	343	487	7.37
24	173	382	777	7.96
22	162	382	777	8.07
9	60	333	411	8.15
7	39	296	106	8.17
18	128	295	97	8.19
14	101	297	114	8.33
3	8	291	68	8.49
16	114	297	114	8.82
17	122	297	114	9.39
15	108	297	114	9.60
8	50	333	411	10.05
52	387	382	777	10.29
54	410	352	558	10.32
56	430	350	542	10.57
50	369	382	777	10.65

EXAMPLE 2

Literature studies show that cysteine residues are important and necessary for the maintenance of the tertiary and quaternary structure of proteins; in particular, for the structure and function of receptors. Given these studies and the presence of cysteine residues within the sequences of the TrkA receptor considered, by the inventors of the present application, relevant for interaction with NGF, the latter decided to study the effect of known substances having reducing activity on the disulphide bridges of the TrkA receptor.

The investigations carried out show that a reagent known as dithiothreitol (DTT) is capable, in vitro, of inhibiting the phosphorylation of TrkA following stimulation with NGF. Different concentrations have been tested on the model in vitro and it was seen that there does not seem to be a concentration/effect relationship on phosphorylation; in particular, there is an inhibition of 24% with a treatment with 0.4 mM DTT with respect to the control with the NGF stimulus alone (Fig. 3). Following the in vitro tests, an in silico study was carried out with the reductive DTT where the amino acid sequence (residues 250-348) of TrkA was taken into account as the reference domain. In this sequence, two cysteines were observed at the binding interface between TrkA and NGF, and it was thus possible to verify that such

cysteine residues are engaged in a disulphide bridge (Fig. 2). The cysteines (cys) ascribed in this bond are in position 300 and 345.

The disulphide bridges have a structural role within the proteins, as mentioned above, i.e., they serve to maintain the correct three-dimensional structure of the protein. As can be seen from Figure 2, in this case the two cysteines form a well-characterized disulphide bridge between two loops of a beta-sheet; the cys in position 300 in sheet B and the cys in position 345 in sheet E. Therefore, it is possible to assume that such an interaction serves to maintain the receptor structure and its correct conformation in space intact.

Always analysing the 2IFG crystal (2IFG stands for the recognizable acronym of the crystallographic structure of the extracellular portion between TrkA and NGF at www.rcsb.org/structure/2ifg) it can be noted that these two cysteines do not interact directly with NGF. For the reasons listed above, the loss of NGF activity in stimulating the auto-phosphorylation of TrkA following a perturbation of the cysteines can be attributed to a conformational change of TrkA (modification induced by the loss of the disulphide bridge) and not to the loss of interactions between NGF and the cysteines.

It was further verified, by molecular docking, if the DTT can interact with TrkA. Since DTT can give polymerization reactions, the docking was conducted considering mono-DTT, bi-DTT and tri-DTT.

The docking highlighted an ever-increasing affinity of DTT for TrkA as the DTT chain grows (affinity for NGF tri-DTT > bi-DTT > mono-DTT).

It was possible to see how all the DTT polymers, listed above, are capable of binding in the same point as TrkA (see Fig.2). The DTT binding site is located near the two cysteines, 300 and 345, which form the disulphide bridge described above. This fact, combined with the reducing power of DTT, leads to the belief that the inhibitory activity of DTT is due to the reduction of the disulphide bridge with the consequent unfolding of the TrkA receptor.

Following this logic, the inventors evaluated the action of NAC, known in the scientific world as having reductant activity and action similar to that observed with the DTT described above in detail.

The NAC molecule was tested in vitro in the cell model and also in this case an inhibition of the phosphorylation activity of TrkA (pTrkA) occurred following response with NGF. In particular, it was possible to observe how the inhibition of pTrkA after NAC + NGF co-treatment with respect to NGF alone resulted in a 40% inhibition of phosphorylation at a NAC concentration

of 20mM (Fig. 4). This fact, combined with the reducing power of NAC comparable to that of DTT, leads to the belief that the inhibitory activity of the latter molecule is due to the reduction of the disulphide bridge as observed with the reducing molecule DTT.

EXAMPLE 3

NAC-TrkA in silico docking

NAC is capable of binding TrkA near the disulphide bridge formed by CYS_300 and CYS_345. The calculated binding ΔG is 12.8 kcal/mol.

Figure 5 shows the result of the docking, the NAC sulphur is located at a distance of 4.0 Å and 5.3 Å from the two sulphur atoms of the disulphide bridge.

Given the proximity of NAC to the disulphide bridge, the covalent binding of NAC was tested with one of the two cysteines of the bridge (see figure 6). The result of the covalent docking shows the real possibility of NAC to covalently bind one of the two cysteines, thus breaking the disulphide bridge, the binding ΔG is estimated at 31.1 kcal/mol.

EXAMPLE 4

Mouse formalin test (C57BL6J)

5-week old male C57BL6J mice of approximately 25 gr each were divided into 2 experimental groups:

1- Control group (8 animals): treated intrathecally with injectable H2O (vehicle), corresponding to the columns "CTRL" in figure 7;

2- Experimental group (8 animals): treated intrathecally with 12 mg/kg NAC corresponding to the columns "NAC" in figure 7.

Experimental protocol:

- Intraperitoneal administration of anaesthetic: 5 µl Zoletil 20 mg/ml
- After 15 minutes, intrathecal administration of NAC or vehicle: 5 µl NAC 12mg/kg (0.30 mg in 5 µl) or 5 µl injectable H2O.

The intrathecal administration was performed as follows:

- The animal was anaesthetized with Isoflurane and placed on a flat operating surface in the prone position, the back was disinfected with a skin antiseptic and shaved, then a nitrile glove (cut in half) was placed over the head and on the upper part of the animal's body in order to avoid stress.
- The animal was gripped firmly by the iliac crest using the thumb and index finger and the skin was stretched over the spine to identify on the back, by palpation, the spinal process L5 and L6 representing the cutaneous injection site.
- The column was gently curved to open the intervertebral space between the spinous process L5 and L6 and a 10 µl syringe was inserted vertically, connected to a sterile, disposable 30Gx1/2 needle. Once contact with the bone of the spine was detected, the angle of the syringe was reduced to about 30° and the syringe

was carefully inserted into the intervertebral space. The puncture of the dura mater is reliably indicated by a reflexive movement of the tail or by the formation of an "S" shape by the tail.

- Lastly, the solution containing the drug or vehicle was injected slowly in a volume of 5 µl.

- After 45 minutes, Formalin test: subcutaneous administration (right paw) of 20 µl Formalin 5% in 0.9% NaCl
- Start licking test (pain is estimated as the amount of time that the mouse spends licking its paw, index of the pain felt), for the duration of 1 hour with evaluation in two phases:
  - phase I: from 0 to 15 min (14.59);
  - phase II: from 15 to 60 min.

The results of the experiment are visible in figure 7.

The results of the experiment, as shown in fig. 7, show that intrathecal route was the only one way allowing to reach the concentration of NAC able to inhibit TrkA activation by NGF as indicated by the experiments performed on cultured cells. Literature data indicate that even at the highest tolerable intravenous doses NAC does not reach the CSF millimolar concentrations that may be attained by this route of administration and which are needed to modulate TrkA activation by NGF.

The results show an acute tolerability and an analgesic effect through the intrathecal route of administration of NAC.

Indeed in the reported experiment in vivo on mice, NAC was administered through intratechal admninistration that is exactly a neuroaxial administration. This route of administration allowed to deliver at spinal level amounts of NAC (300 micrograms) consenting to tribute the effect of

NAC to the inhibition of the NGF activation of TrkA through the interference with the recognized cysteine bond at the indicated residues. Our animal model demonstrates the clinical activity of neuraxial NAC in reducing pain.

The final understanding proposed by the authors brings about a mechanism of interaction between NAC and Trk which is not fully clarified. Our finding is based on a thorough in sylico study identifying the specific mechanisms of NAC inhibition of NGF binding with a precise and defined interaction with two cysteine residues of the TrkA sequence. In the present invention the intrathecal effect of administered NAC on pain in a rodent model was tested and the invention describes dose related pharmacological action allowing optimal route of administration and dosages.

CLAIMS

1. N-Acetyl cysteine (NAC) for use as an antagonist of the TrkA tyrosine kinase receptor in the treatment and/or management of acute and chronic pain conditions in which there is hyperactivity of the nerve growth factor (NGF), wherein said NAC is administered locally where the condition due to nerve growth factor (NGF) hyperactivity occurs, characterised in that an effective dose of said NAC to be administered locally in adult patients is comprised between 0.5 and 15 mg/Kg and in that said local administration is neuraxial.
2. N-Acetyl cysteine (NAC) for use according to the claim in the treatment and/or management of pain.
3. N-Acetyl cysteine (NAC) for use according to the preceding claim in the treatment and/or management of pain associated with high levels of NGF and the chronicity of other pain conditions.

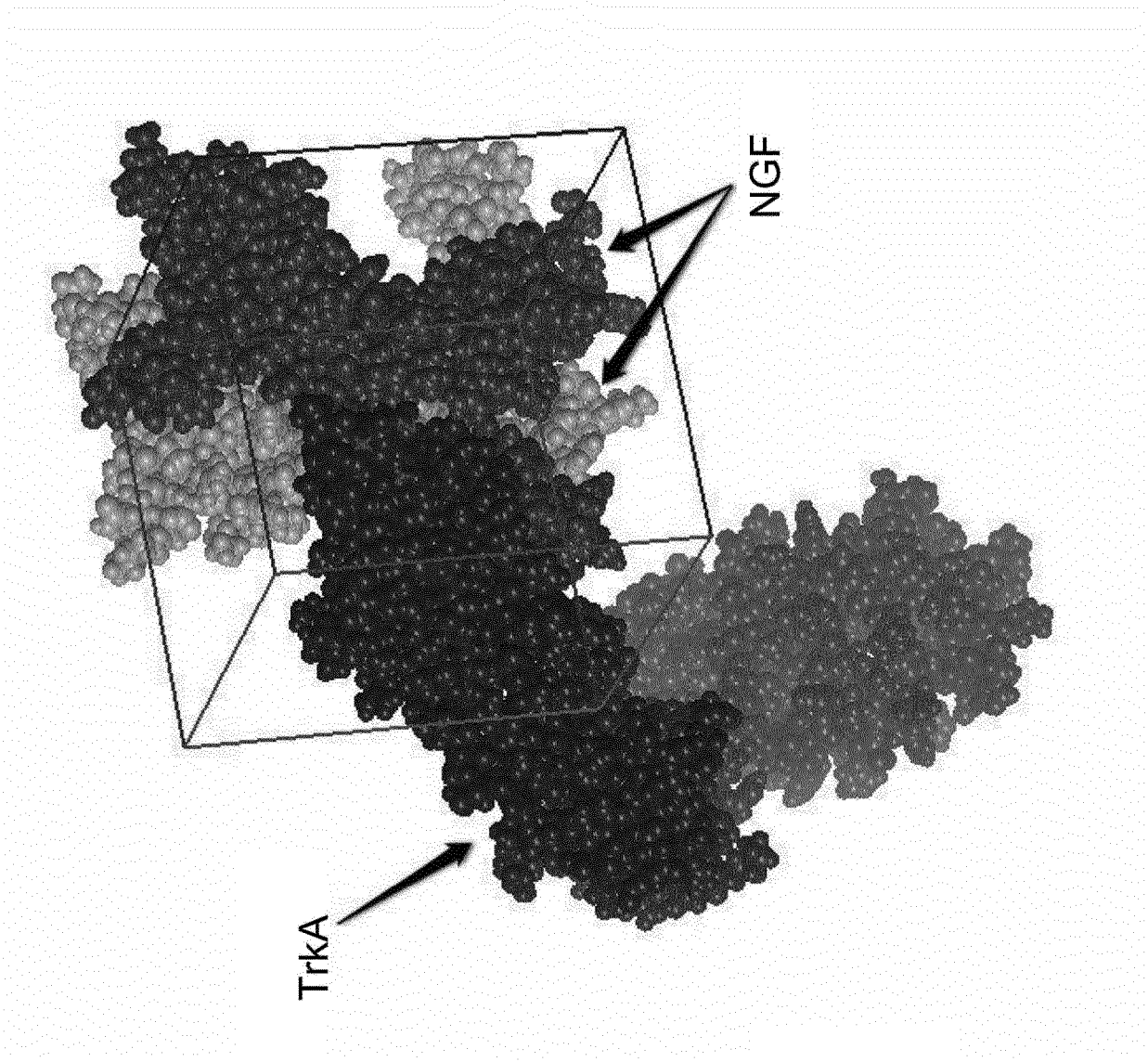


FIG.1

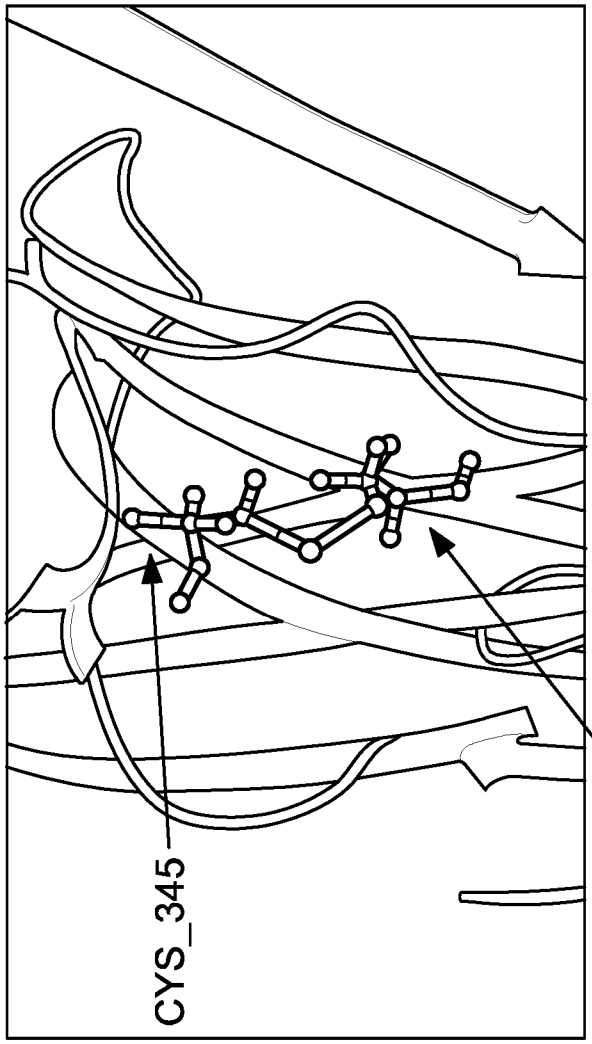


FIG.2

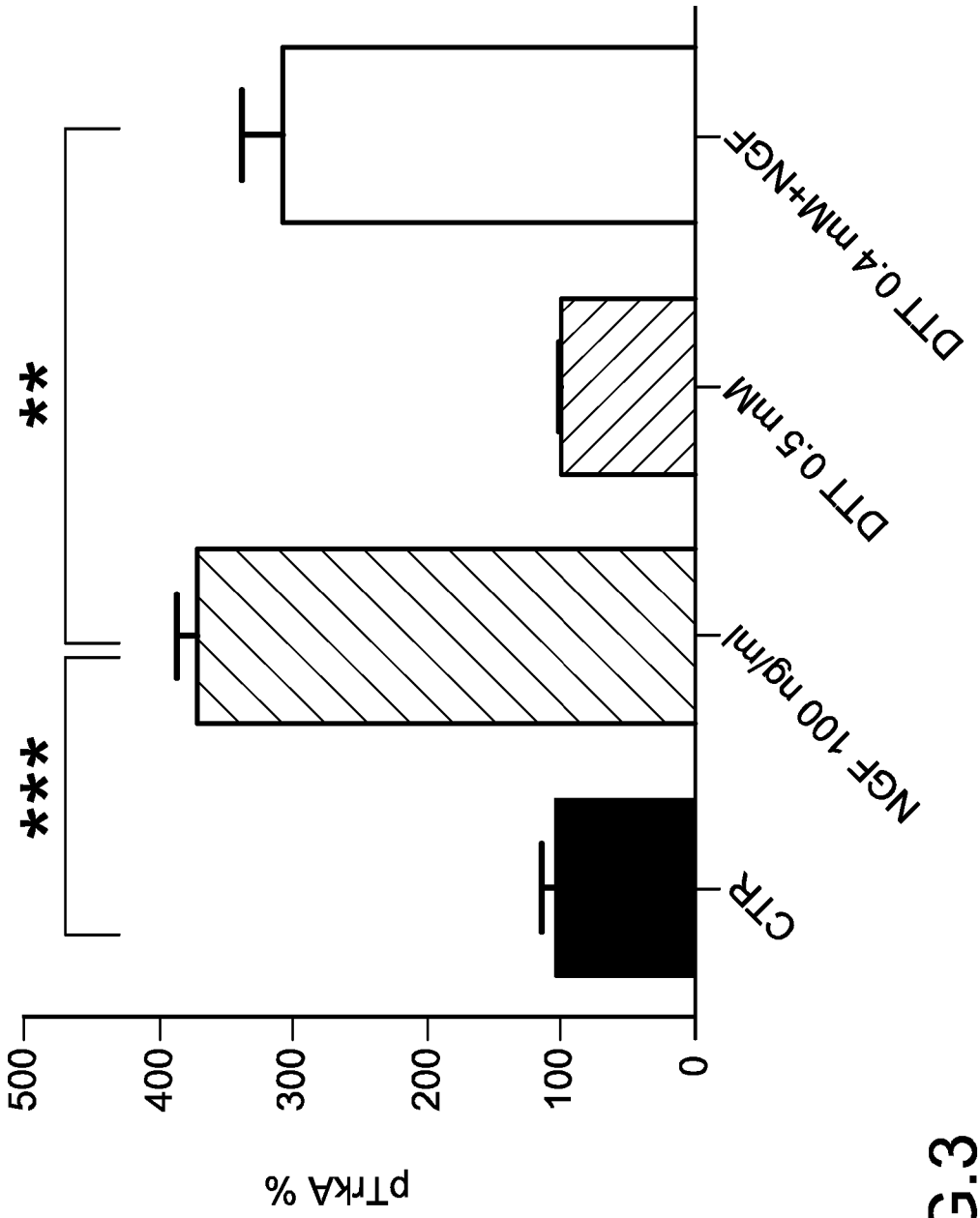

pTrkA %
0
100
200
300
400
500
CTR
NGF 100 ng/ml
DTT 0.5 mM
DTT 0.4 mM+NGF
****
**

FIG.3

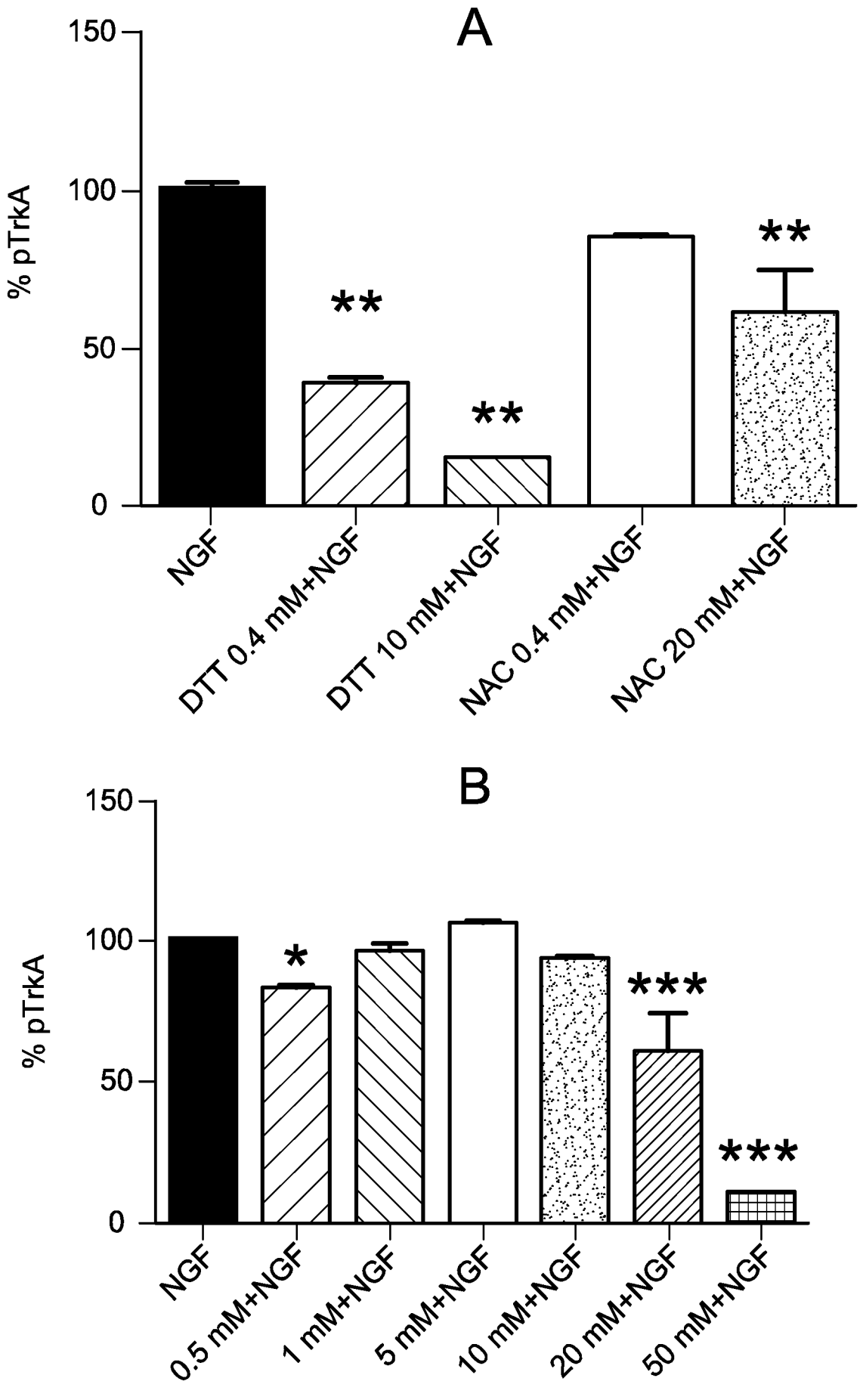

A
150
100
50
0
% pTrkA
**
**
**
NGF
DTT 0.4 mM+NGF
DTT 10 mM+NGF
NAC 0.4 mM+NGF
NAC 20 mM+NGF
B
150
100
50
0
% pTrkA
*
***
***
NGF
0.5 mM+NGF
1 mM+NGF
5 mM+NGF
10 mM+NGF
20 mM+NGF
50 mM+NGF

FIG.4

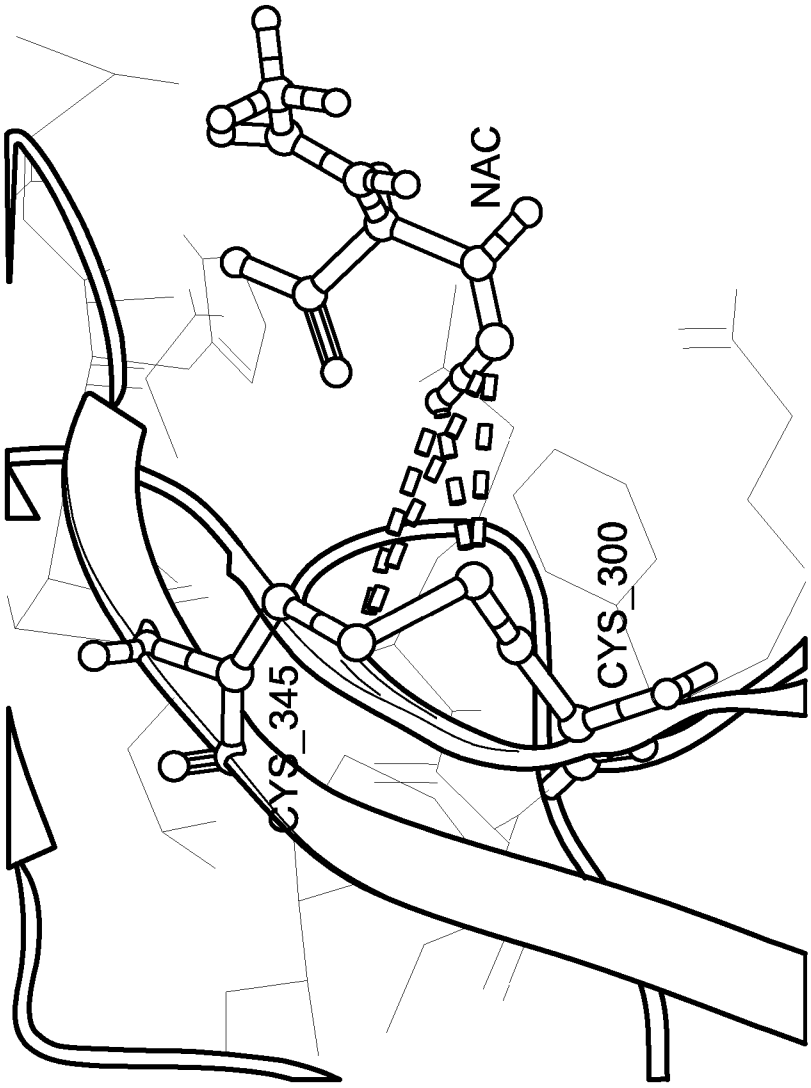


FIG.5

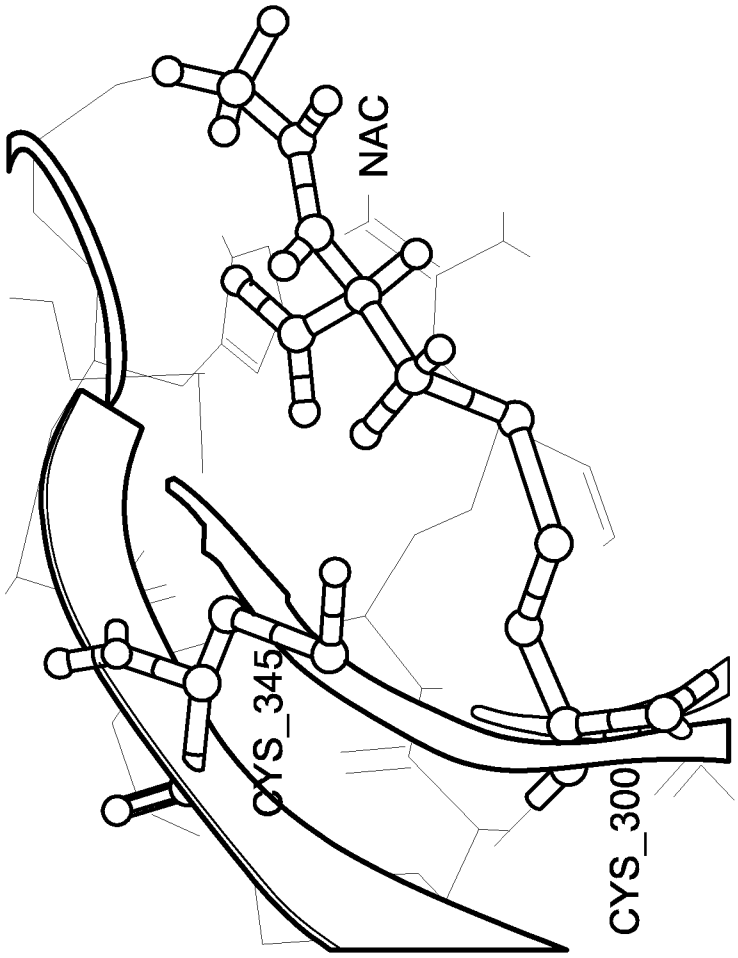


FIG.6

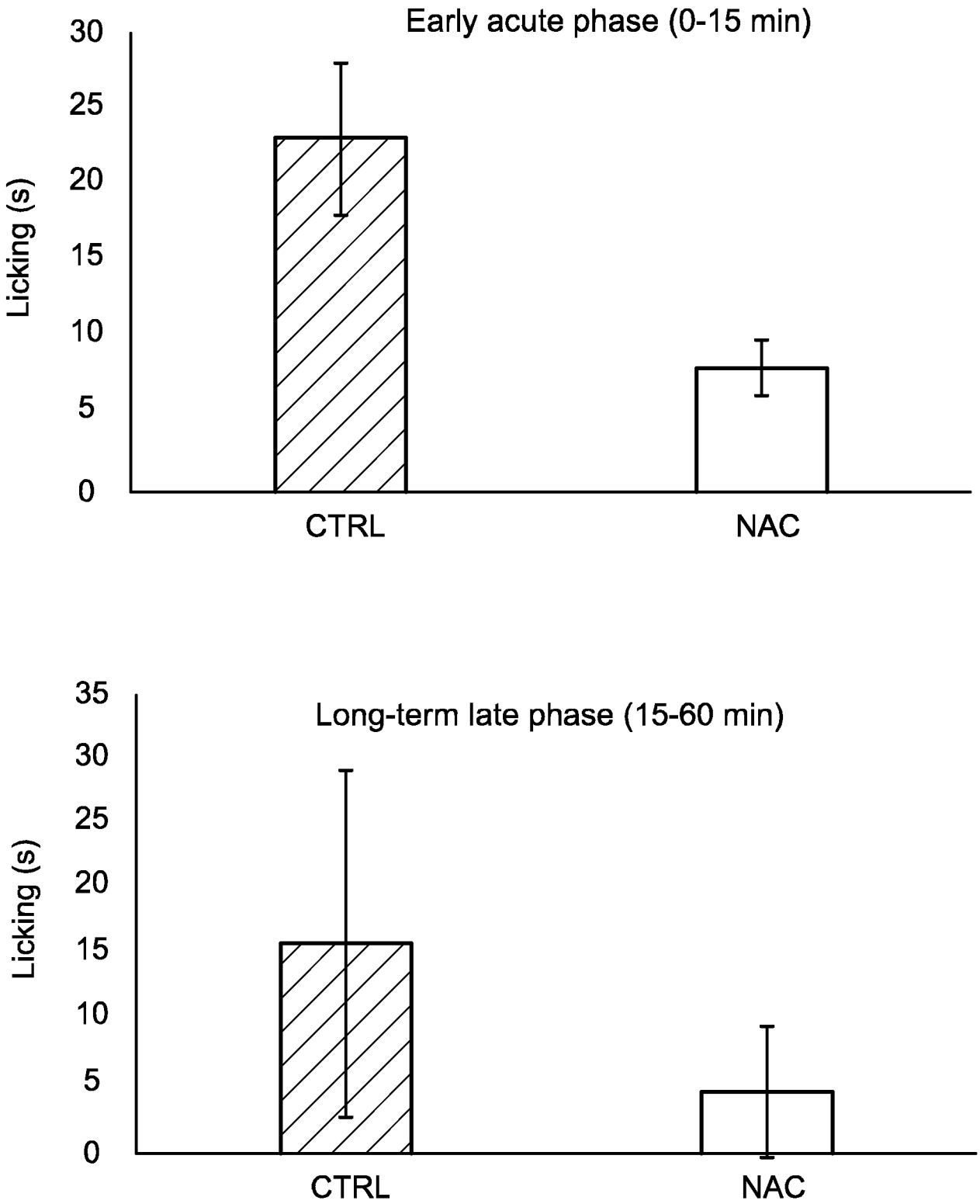

Early acute phase (0-15 min)
Licking (s)
30
25
20
15
10
5
0
CTRL
NAC
Long-term late phase (15-60 min)
Licking (s)
35
30
25
20
15
10
5
0
CTRL
NAC

FIG.7

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2022/060375

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/198 A61P25/02 A61P29/02
ADD.

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

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, WPI Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2012/157405 A1 (WHITE III JOHN B [US]) 21 June 2012 (2012-06-21) paragraph [0032] -----	1-3
Y	CARISSA CHU ET AL: "Mitochondrial dependence of nerve growth factor-induced mechanical hyperalgesia", PAIN, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 152, no. 8, 25 March 2011 (2011-03-25), pages 1832-1837, XP028380192, ISSN: 0304-3959, DOI: 10.1016/J.PAIN.2011.03.034 [retrieved on 2011-03-29] the whole document figures 2a, 2b page 1834, right-hand column ----- -/--	1-3

[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
30 June 2022	08/07/2022

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Collura, Alessandra

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LAURA MCKELVEY ET AL: "Nerve growth factor-mediated regulation of pain signalling and proposed new intervention strategies in clinical pain management", JOURNAL OF NEUROCHEMISTRY, vol. 124, no. 3, 1 February 2013 (2013-02-01), pages 276-289, XP055577227, GB ISSN: 0022-3042, DOI: 10.1111/jnc.12093 cited in the application the whole document page 280, left-hand column, paragraph 2 -----	1-3
Y	WO 2006/131951 A2 (LAY LINE GENOMICS SPA [IT]; PAVONE FLAMINIA [IT] ET AL.) 14 December 2006 (2006-12-14) claims -----	1-3
Y	KAMATA H ET AL: "Redox regulation of nerve growth factor-induced neuronal differentiation of PC12 cells through modulation of the nerve growth factor receptor, TrkA", ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, ACADEMIC PRESS, US, vol. 434, no. 1, 1 February 2005 (2005-02-01), pages 16-25, XP004699346, ISSN: 0003-9861, DOI: 10.1016/J.ABB.2004.07.036 page 20, left-hand column, paragraph 3 - page 22 -----	1-3
Y	WELIN D ET AL: "Effects of N-acetyl-cysteine on the survival and regeneration of sural sensory neurons in adult rats", BRAIN RESEARCH, ELSEVIER, AMSTERDAM, NL, vol. 1287, 1 September 2009 (2009-09-01), pages 58-66, XP026437575, ISSN: 0006-8993 [retrieved on 2009-06-23] page 64, paragraph 4.3 ----- -/--	1-3

1

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2022/060375

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KARALIJA AMAR ET AL: "Neuroprotective Effects of N-Acetyl-Cysteine and Acetyl-L-Carnitine after Spinal Cord Injury in Adult Rats", PLOS ONE, vol. 7, no. 7, 17 July 2012 (2012-07-17), page e41086, XP055937201, DOI: 10.1371/journal.pone.0041086 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3398872/pdf/pone.0041086.pdf> page 6, right-hand column, paragraph 3 -----	1-3

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2022/060375

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2012157405 A1	21-06-2012	US 2012157405 A1	21-06-2012
		WO 2012087726 A1	28-06-2012
WO 2006131951 A2	14-12-2006	AU 2006256387 A1	14-12-2006
		CA 2610596 A1	14-12-2006
		CA 2611433 A1	14-12-2006
		CN 101277718 A	01-10-2008
		DK 1890726 T3	16-02-2015
		EP 1890726 A1	27-02-2008
		EP 1893234 A2	05-03-2008
		EP 2484380 A1	08-08-2012
		HK 1121403 A1	24-04-2009
		JP 5133879 B2	30-01-2013
		JP 5730464 B2	10-06-2015
		JP 2008542439 A	27-11-2008
		JP 2008542440 A	27-11-2008
		JP 2012158604 A	23-08-2012
		JP 2013151509 A	08-08-2013
		JP 2014218513 A	20-11-2014
		KR 20080026593 A	25-03-2008
		NZ 564071 A	29-07-2011
		US 2009208490 A1	20-08-2009
		US 2010291083 A1	18-11-2010
		WO 2006131951 A2	14-12-2006
		WO 2006131952 A1	14-12-2006