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(54) Title: METHODS OF SCREENING FOR MODULATORS OF NERVE GROWTH FACTOR

(57) Abstract: The invention relates, in general, to a method of screening for agents that modulate NGF activity. More specifically this invention provides a method of assessing the ability of a candidate agent to modulate NGF activity comprising, measuring the level of expression of one or more or two or more NGF responsive genes in a culture of neurons expressing the high-affinity trk A receptor after contact with a candidate agent. The invention further provides methods of culturing primary cultures of neurons expressing the high-affinity trk A receptor and methods of isolating polynucleotides from such cultures.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/01609

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8): **C12Q 1/68**(2006.01);**G01N 33/567**(2006.01)

USPC: 435/6,7.21

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)

U.S. : 435/6, 7.21

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)

EAST, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WINSTON, J. et al. Nerve Growth Factor Regulates VR-1 mRNA in Cultures of Adult Dorsal Root Ganglion Neurons. Pain. 2001, Vol 89, pages 181-186, especially pages 181, 182, 184 and Figures 3 and 4.	1-5,7-9,11,15,27-31 ---------- 14,16-24,26
Y	US 6,218,531 (EKENBERG et al). 17 April 2001 (17.4.01), column 5, lines 38-41; column 6, lines 8-10; column 7, lines 13-15; Example 6.	16,17
Y	DICKENS, G. et al. Involvement of Protein Kinase C in Nerve Growth Factor- and K-252a-Stimulated Calcium Uptake Into PC12 Cells. Journal of Neuroscience Research. 1997, Vol 47, pages 271-276, especially Figure 4 on page 274.	18,19
Y	DEBEIR, T. et al. A Nerve Growth Factor Mimetic TrkA Antagonist Causes Withdrawal of Cortical Cholinergic Boutons in the Adult Rat. Proc Natl Acad Sci USA. March 1999, Vol 96, pages 4067-4072, especially Abstract on page 4067.	14,20-24,26

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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

"E" earlier application or patent 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

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/01609

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HUMBERT, S. et al. Toward Cell Specificity in SCA1. Neuron. May 30, 2002. Vol 34, pages 669-674.	10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/01609

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

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

2. ☒ Claims Nos.: 6
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:
Please See Continuation Sheet

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 No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

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 additional fees, this Authority did not invite payment of any additional fees.
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.: Please See Continuation Sheet

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Continuation of Box II Reason 2:
Claim 6 recites, "The method of claim 5, wherein the neurons in the culture are between about between about 3.5 cells per sequence millimeter to about 35 cells per square millimeter or 3.5 cells per square millimeter to about 35 cells per square millimeter". The repetition of the phases "between about" and "3.5 cells per square millimeter to about 35 cells per square millimeter" renders the claim so unclear that a meaningful search cannot be carried out for the claim.

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING
Group I, claims 1-32, drawn to a method comprising (1) contacting a culture of neurons expressing the trkA receptor with one or more candidate agents and (2) measuring the level of expression of one or more NGF responsive genes in said culture.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows:

(A) The first species of primary neuron culture is (1) nociceptive neurons of Dorsal Root ganglia and the 2^{nd}-6^{th} species are (2) trigeminal ganglion nociceptive neurons; (3) trigeminal ganglion non-nociceptive neurons; (4) sympathetic neurons; (5) NGF responsive subpopulations of the nodose ganglia; and (6) NGF responsive subpopulations of the basal forebrain cholinergic neurons.

The claims correspond to the species listed above in the following manner:
Claim 3 recites species 1-6 as a Markush-type group.
Claim 4 corresponds to species 1.
Claims 22 and 29 each correspond to species 1 and 2.
Claims 21 and 28 are generic with regard to nociceptive primary neuronal culture.
Claims 1, 2, 5-20, 23-27 and 30-32 are generic with regard to primary neuron culture.

(B) The first species of NGF responsive gene is (1) spinocerebellar ataxia type 1 (sca 1); and the 2^{nd}- species are (2) substance P; (3) lymphocyte antigen 86 (MD-1); (4) Hippocampus cDNA homologue to Microsomal Signal Peptidese; (5) Neuronal Leucine Rich Repeat Protein 1 (NLRR-1); (6) Synaptotagmin V; (7) Cadherin 1; (8) EST weakly similar to KIAA0982 protein; (9) EST weakly similar to RIKEN cDNA 2310042NO2; (10) small proline-rich repeat protein 1A; (11) Motopsin (Neurotrypsin); (12) Inhibin Beta B; (13) G protein coupled receptor 19; (14) Lipocalin 2; (15) Troponin C; (16) galanin; and (17) small proline rich repeat protein 1A (sprr1A).

The claims correspond to the species listed above in the following manner:
Claim 10 recites species 1-9 as a Markush-type group.
Claim 12 recites species 10-15 as a Markush-type group.
Claims 13, 25 and 32 each recite species 2, 16 and 17.
Claims 1-9, 11, 14-24 and 26-31 are generic with regard to the species of NGF responsive gene.

The fees previously paid cover the first species of (A) primary neuronal culture and the first species of (B) NGF responsive gene. In order for more than one species to be examined, the appropriate additional fees must be paid. Each additional species counts as an additional group for which fees must be paid. There are 5 additional species of (A) primary neuronal culture and 16 additional species of (B) NGF responsive genes. Therefore, for all of the groups and species to be searched, search fees for an additional 25 groups must be paid.
The inventions listed as Groups I-XIII do not relate to a single general inventive concept under PCT rule 12.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The species of (A) primary neuronal cultures listed above do not relate to a single general inventive concept under PCT Rule 13.1 because under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: the neuronal cell types are each structurally different on both cellular and molecular levels and therefore would be predicted to have different patterns of gene expression in response to a ligand such as NGF, and thus do not relate to a single general invention concept within the meaning of PCT Rule 13.1.

The species of (B) NGF responsive genes listed above do not relate to a single general inventive concept under PCT Rule 13.1 because under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: the NGF responsive genes are each structurally different nucleic acids, and therefore would be predicted to have different patterns of expression, and thus do not relate to a single general invention concept within the meaning of PCT Rule 13.1.

Continuation of Box III Item 4:
1-5,7-11,14-24 and 26-31, first species of neurons (dorsal root ganglion) and first species of NGF responsive gene (spinocerebellar ataxia type 1).