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(54) Title: NICOTINAMIDE RIBOSIDE KINASE COMPOSITIONS AND METHODS FOR USING THE SAME

(57) Abstract: The present invention relates to isolated nicotinamide riboside kinase (Nrk) nucleic acid sequences, vectors and cultured cells containing the same, and Nrk polypeptides encoded thereby. Methods for identifying individuals or tumors susceptible to nicotinamide riboside-related prodrug treatment and methods for treating cancer by administering an Nrk nucleic acid sequence or polypeptide in combination with a nicotinamide riboside-related prodrug are also provided. The present invention further provides screening methods for isolating a nicotinamide riboside-related prodrug and identifying a natural source of nicotinamide riboside.

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**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
- a. type of material
    - a sequence listing
    - table(s) related to the sequence listing
  - b. format of material
    - on paper
    - in electronic form
  - c. time of filing/furnishing
    - contained in the international application as filed
    - filed together with the international application in electronic form
    - furnished subsequently to this Authority for the purposes of search
2.  In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

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## 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.:  
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 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.:
- 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.

## INTERNATIONAL SEARCH REPORT

International application No.

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A. CLASSIFICATION OF SUBJECT MATTER IPC: C12N 9/12(2006.01),9/16(2006.01)  USPC: 435/194,196 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. : 435/194,196  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)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GENBANK Accession No. AX877238, (Ota T. et al) 07 February 2001	1-4, 6, 7
X	Bieganowski et al. Discoveries of nicotinamine riboside as a nutrient and conserved NRK genes establish a Preiss-Handler independent route to NAD+ in Fungi and in Humans. Cell October 2004, Vol. 117, pages 495-502.	16, 17
Y	Sasiak et al. Purification and properties of Human Nicotinamide Riboside kinase. Archives of Biochemistry and Biophysics (15 September 1996) Vol. 333, No. 2, pages 414-418.	12, 13, 20, 22
Y	Saunders et al Phosphorylation of 3-Deazaguanosine by nicotinamide riboside kinase in Chinese Hamster Ovary Cells. Cancer Research, 01 December 1989, Vol. 49, pages 6593-6599. entire document.	12, 13, 20, 22
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C.		<input type="checkbox"/> See patent family annex.
* Special categories of cited documents:		"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
"A" document defining the general state of the art which is not considered to be of particular relevance		"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
"E" earlier application or patent published on or after the international filing date		"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
"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)		"&" document member of the same patent family
"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		
Date of the actual completion of the international search 21 August 2006 (21.08.2006)	Date of mailing of the international search report <b>23 NOV 2007</b>	
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	Authorized officer Kagnev Gebreyesus Telephone No. 571-272-1600 <i>Janece Ford for</i>	

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**C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Farquhar et al Synthesis and antitumor evaluation of bis[(pivaloyloxy)methyl]2'-deoxy-5-fluorouridine 5'-monophosphate:a strategy to introduce nucleotides into cells. Journal of Medicinal Chemistry. 11 November 1994, Vol.11, No.37, pages 2902-2909.see abstract	25, 26

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### BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I- Claims 1-5 and 8 drawn to DNA and vectors and composition comprising the same.

Group II- Claims 6, 7, 9, 10 and 11 are drawn to nicotinamide riboside kinase polypeptides and compositions comprising the same.

Group III- Claims 12 and 13 are drawn to method of treating cancer with the composition comprising the DNA vector comprising nicotinamide riboside kinase gene or polypeptide.

Group IV- Claims 14-17 are drawn to a method of identifying a natural or synthetic source of nicotinamide riboside.

Group V- Claims 18, 19 are drawn to a method of preventing disease or condition associated with the nicotinamide ribonucleoside kinase pathway.

Group VI- Claims 20, 22 are drawn to identifying a nicotinamide riboside related pro-drug by determining whether a candidate agent is phosphorylated by the nicotinamide riboside kinase polypeptides.

Group VII Claim 21, 23 are drawn to a nicotinamide riboside-related prodrug identified by the ability of to be phosphorylated by a nicotinamide ribonucleotide kinase polypeptide or by a cell expressing the nicotinamide ribonucleoside kinase polypeptide.

VIII Claims 24 is drawn to a method of identifying an individual or a tumor susceptible to treatment with a nicotinamide riboside-related prodrug comprising detecting the presence of mutation or change in expression of a nicotinamide riboside kinase in an individual or tumor.

IX Claim 25 is drawn to a nicotinamide riboside composition comprising nicotinamide riboside derivatized with an L-valine L-phenylalanine ester.

X Claim 26 is drawn to a pharmaceutical composition comprising nicotinamide riboside in admixture with a pharmaceutically acceptable carrier.

The inventions listed as Groups I-VII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking the inventions in groups I-VII is the use of nucleic acids that encode nicotinamide riboside kinases in a method for treating cancer, identifying a natural or synthetic source of nicotinamide riboside, identifying a nicotinamide riboside related pro-drug, identifying a tumor caused by an aberration in nicotinamide riboside kinase in an individual. However, Sasiak et. al teach the purification and properties of a human ribonucleoside kinase and disclose two pro-drugs with potential clinical value. Therefore the only technical feature -i.e. nicotinamide riboside kinase encoded by the nucleic acid sequences disclosed is not a special technical feature as defined by PCT Rule 13.1