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(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, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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— *with international search report (Art. 21(3))*
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(88) **Date of publication of the international search report**:
27 December 2012

WO 2012/142245 A3

(54) **Title**: PEPTIDE MIMETIC LIGANDS OF POLO-LIKE KINASE 1 POLO BOX DOMAIN AND METHODS OF USE

(57) **Abstract**: Novel compounds are provided that bind to polo-like kinases through the polo -box domain. In certain embodiments, the novel compounds are PEGylated peptides. The PEGylated peptides in accordance with the invention demonstrate high PBD-binding affinity. In certain embodiments, the PEGylated peptides have also achieved activities in whole cell systems. The invention also provides compounds that bind polo- like kinases through the polo -box domain and possess reduced anionic charge. Further provided are methods of design and/or synthesis of the PEGylated peptides and methods of use thereof. The invention provides methods of use of the compounds and methods of synthesis of the compounds. The compounds of the invention have potential therapeutic activity in view of their binding and inhibitory activities towards Plkl. They are based on the amino acid sequence PLHSpT (phosphorylated Thr). The PEG moiety, when present, is covalently attached at the N- terminus. The invention also encompasses derivatives having substituents on the Pro and/or the His side chains, substituents on the phosphate group, the replacement of His with Gln, the replacement of Pro with a N-substituted Gly, the replacement of Ser with Ala, as well as HS-pT fragments with N-terminal alkoxycarbonyl group and PLHS fragments with a C-terminal glyco-imino-alkylxyalkylamide group.

INTERNATIONAL SEARCH REPORT

International application No
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A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/435
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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)
C12N C07K C12Y A61K

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, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	FA LIU ET AL: "Serendipitous alkylation of a Plk1 ligand uncovers a new binding channel", NATURE CHEMICAL BIOLOGY, vol. 7, no. 9, 17 July 2011 (2011-07-17), pages 595-601, XP055032617, ISSN: 1552-4450, DOI: 10.1038/nchembio.614 abstract; table 1 -----	1-49, 71-82
Y	WO 2010/132869 A2 (GOVERNMENT OF THE U S A AS REP [US]; BURKE TERRENCE R [US]; LIU FA [US) 18 November 2010 (2010-11-18) abstract; claims 1-36; figures 1,21 ----- -/--	1-49, 71-82

[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
18 July 2012	29/10/2012

Name and mailing address of the ISA/	Authorized officer
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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	YUN SANG-MOON ET AL: "Structural and functional analyses of minimal phosphopeptides targeting the polo-box domain of polo-like kinase 1", NATURE STRUCTURAL & MOLECULAR BIOLOGY,, vol. 16, no. 8, 1 August 2009 (2009-08-01) , pages 876-882, XP009137324, ISSN: 1545-9985, DOI: 10.1038/NSMB.1628 abstract; figures 2-4; tables 1-2	1-49, 71-82
Y	BHADRA D ET AL: "PEGNOLOGY: A REVIEW OF PEG-YLATED SYSTEMS", DIE PHARMAZIE, GOVI VERLAG PHARMAZEUTISCHER VERLAG GMBH, ESCHBORN, DE, vol. 57, no. 1, 1 January 2002 (2002-01-01), pages 5-29, XP001115789, ISSN: 0031-7144 abstract and paragraph 3.1.1	1-49, 71-82
Y	A. KHEDKAR ET AL: "A dose range finding study of novel oral insulin (IN-105) under fed conditions in type 2 diabetes mellitus subjects", DIABETES, OBESITY AND METABOLISM, vol. 12, no. 8, 1 August 2010 (2010-08-01) , pages 659-664, XP055032638, ISSN: 1462-8902, DOI: 10.1111/j.1463-1326.2010.01213.x abstract	1-49, 71-82
Y	MARK A. MILLER ET AL: "Amphiphilic Conjugates of Human Brain Natriuretic Peptide Designed for Oral Delivery: In Vitro Activity Screening", BIOCONJUGATE CHEMISTRY, vol. 17, no. 2, 1 March 2006 (2006-03-01), pages 267-274, XP055032637, ISSN: 1043-1802, DOI: 10.1021/bc0501000 abstract	1-49, 71-82
A	JOHNSON ERIC F ET AL: "Pharmacological and functional comparison of the polo-like kinase family: Insight into inhibitor and substrate specificity", BIOCHEMISTRY, AMERICAN CHEMICAL SOCIETY, US, vol. 46, no. 33, 27 July 2007 (2007-07-27) , pages 9551-9563, XP002540890, ISSN: 0006-2960, DOI: 10.1021/BI7008745 abstract; figure 1	1-49,71

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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.
A	LIU F ET AL: "Preparation of orthogonally protected (2S,3R)-2-amino-3-methyl-4-phosphonobutyric acid (Pmab) as a phosphatase-stable phosphothreonine mimetic and its use in the synthesis of polo-box domain-binding peptides", TETRAHEDRON, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 65, no. 47, 21 November 2009 (2009-11-21), pages 9673-9679, XP026696877, ISSN: 0040-4020, DOI: 10.1016/J.TET.2009.09.093 [retrieved on 2009-09-26] abstract; figure 2	1-49,71
L	KUI-THONG TAN ET AL: "Design, Synthesis, and Characterization of Peptide-Based Rab Geranylgeranyl Transferase Inhibitors", JOURNAL OF MEDICINAL CHEMISTRY, vol. 52, no. 24, 24 December 2009 (2009-12-24), pages 8025-8037, XP055032644, ISSN: 0022-2623, DOI: 10.1021/jm901117d page 8035, left-hand column, lines 9-10; table 2; compound 24	83
L	ROHITÂ?K. SHARMA ET AL: "New Antimicrobial Hexapeptides: Synthesis, Antimicrobial Activities, Cytotoxicity, and Mechanistic Studies", CHEMMEDCHEM, vol. 5, no. 1, 4 January 2010 (2010-01-04), pages 86-95, XP055032643, ISSN: 1860-7179, DOI: 10.1002/cmdc.200900330 figure 1; scheme 1	83

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2012/033259

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010132869 A2	18-11-2010	US 2012065146 A1	15-03-2012
		WO 2010132869 A2	18-11-2010

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/033259

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:

see additional 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 an additional fees, this Authority did not invite payment of 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.:

1-49, 72-82(completely); 71(partially)

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-49, 72-82(completely); 71(partially)

   N-terminally PEGylated Plk1 binding inhibitors, e.g. of formula I or II, which are derived from the physphorylated peptide PLHSpT, pharmaceutical compositions, chemical libraries and medical uses thereof.

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2. claims: 54-65(completely); 50-53, 71(partially)

   Plk1 binding inhibitors of formula III, i.e. N-terminally acetylated pentapeptides derived from the sequence PLHST.

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3. claims: 66-70(completely); 50-53, 71(partially)

   Plk1 binding inhibitors of formula IV, i.e. acylated or Alkyl-carbamates tripeptides derived from the sequence HST.

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4. claim: 71(partially)

   Plk1 binding inhibitors, which do not belong to any of the previous groups, as they are neither N-terminally PEGylated, nor of any of formulae III and IV, e.g. bearing longer amino acid sequences.

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5. claim: 83

   use of Fmoc-1-phenylalkyl-histidine in peptide synthesis
