

REVISED VERSION

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
07 June 2018 (07.06.2018)



(10) International Publication Number
WO 2018/102766 A9

(51) International Patent Classification:

A61K 38/08 (2006.01) *C07K 14/47* (2006.01)
A61K 38/17 (2006.01) *G01N 33/68* (2006.01)
C07K 7/06 (2006.01)

(US). PEARCE, Martin; 700 White Oak Circle, Apt. 326, Independence, OR 97351 (US).

(21) International Application Number:

PCT/US2017/064335

(22) International Filing Date:

01 December 2017 (01.12.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/428,864 01 December 2016 (01.12.2016) US

(71) Applicant: OREGON STATE UNIVERSITY [US/US];
312 Kerr Administration Building, Corvallis, OR 97331
(US).

(74) Agent: GALL, Anna, S.; Christensen O'Connor Johnson
Kindness PLLC, 1201 Third Avenue, Suite 3600, Seattle,
WA 98101 (US).

(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, 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, SM, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, 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,

(72) Inventors: KOLLURI, Siva, K; 4364 Northwest Honey-
sucke Drive, Corvallis, OR 97330 (US). KOPPARAPU,
Prasad, R.; 1558 1/2 Castle Court, Houston, TX 77006

(54) Title: SMALL MOLECULE BCL-2 FUNCTIONAL CONVERTERS AS CANCER THERAPEUTICS

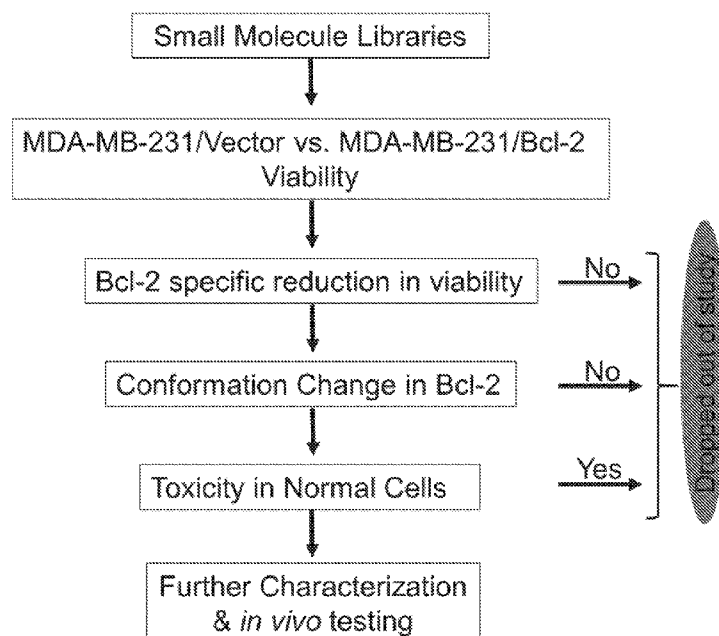


Fig. 3

(57) Abstract: Methods for inducing growth inhibition or apoptosis of Bcl-2-expressing cells and treatments of Bcl-2 expressing cancers are provided. Additionally, assays for agents that can induce apoptosis of Bcl-2 expressing cells are disclosed.

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:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
- *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:

12 July 2018 (12.07.2018)

Date of publication of the revised international search report:

20 September 2018 (20.09.2018)

(15) Information about Correction:

see Notice of 20 September 2018 (20.09.2018)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/064335

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/08; A61K 38/17; C07K 7/06; C07K 14/47; G01N 33/68 (2018.01)

CPC - A61K 38/00; C07K 14/4747; G01N 33/68; G01N 33/6893; G01N 2333/4704; G01N 2510/00; G01N 2800/52 (2018.05)

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)

See Search History document

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

USPC - 435/6.1; 436/501; 514/15; 514/18.9; 514/19.4; 530/324; 530/328; 530/350 (keyword delimited)

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2013/0157260 A1 (SATTERTHWAIT et al) 20 June 2013 (20.06.2013) entire document	1-3, 5-8, 10, 11
X	US 2009/0118135 A1 (REED et al) 07 May 2009 (07.05.2009) entire document	16
A	US 2015/0018285 A1 (CARMEL-HAIFA UNIVERSITY ECONOMIC CORPORATION LTD) 15 January 2015 (15.01.2015) entire document	1-3, 5-8, 10, 11, 16
A	KOLLURI et al. "A Short Nur77-Derived Peptide Converts Bcl-2 from a Protector to a Killer," Cancer Cell, 07 October 2008 (07.10.2008), Vol. 14, Pgs. 285-298. entire document	1-3, 5-8, 10, 11, 16
A	US 2008/0138847 A1 (SHI) 12 June 2008 (12.06.2008) entire document	1-3, 5-8, 10, 11, 16
A	US 2010/0286057 A1 (WALENSKY et al) 11 November 2010 (11.11.2010) entire document	1-3, 5-8, 10, 11, 16
P, A	YAN et al. "Methotrexate Induces Apoptosis of Postpartum Placental Cytotrophoblasts," Cells Tissues Organs, 25 January 2017 (25.01.2017), Vol. 203, No. 4, Pgs. 231-242. entire document	1-3, 5-8, 10, 11, 16

☐ 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 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

04 April 2018

Date of mailing of the international search report

30 JUL 2018

Name and mailing address of the ISA/IJS

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/064335

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

- a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/064335

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.: 12-15
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 Extra Sheets

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 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.:
1-3, 5-8, 10, 11, and 16 to the extent that they read on methotrexate.

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.

Continued from Box No. 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 need to be paid.

Group I+: claims 1-11 and 16 are drawn to agents that expose the BH3 domain of Bcl-2 thereby converting Bcl-2 into a pro-apoptotic protein and activate the intrinsic apoptosis pathway (hereinafter "Bcl-2 antagonists") and methods comprising the same.

The first invention of Group I+ is restricted to a Bcl-2 antagonist, and methods comprising the same, wherein the Bcl-2 antagonist is selected to be methotrexate. It is believed that claims 1-3, 5-8, 10, 11, and 16 read on this first named invention and thus these claims will be searched without fee to the extent that they read on methotrexate.

Applicant is invited to elect additional Bcl-2 antagonists, each with specified chemical structure, to be searched in a specific combination by paying an additional fee for each set of election. An exemplary election would be a Bcl-2 antagonist, and methods comprising the same, wherein the Bcl-2 antagonist is selected to be a compound of Formula 1 (Table 6). Additional Bcl-2 antagonists will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ 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 Groups I+ formulas do not share a significant structural element for inducing growth inhibition or apoptosis of a Bcl-2-expressing cell, requiring the selection of alternatives for the agent that exposes the BH3 domain of Bcl-2 thereby converting Bcl-2 into a pro-apoptotic protein and activating the intrinsic apoptosis pathway (Bcl-2 antagonist), where "the agent is a methotrexate or a methotrexate analog of Formula (IV)" and "the methotrexate analog is a compound having a structure of any one of Formulae 1-12" and "the agent is a compound of Table 1, Table 2, Table 3, Table 4, or Table 5, or a compound of Formulae (I), (II), or (III)".

Additionally, even if Groups I+ were considered to share the technical features of a method for inducing growth inhibition or apoptosis of a Bcl-2-expressing cell, comprising contacting a Bcl-2-expressing cell with an agent that exposes the BH3 domain of Bcl-2 thereby converting Bcl-2 into a pro-apoptotic protein and activating the intrinsic apoptosis pathway; a method of treating Bcl-2-expressing cancer in a subject, comprising administering to a subject in need of cancer treatment, a therapeutically effective amount of an agent that exposes the BH3 domain of Bcl-2 thereby converting Bcl-2 into a proapoptotic protein; an in vitro method of screening for an agent that converts Bcl-2 from an anti-apoptotic protein into a pro-apoptotic protein, comprising: (a) contacting a first population of cells with an agent, wherein the first population of cells expresses or overexpresses Bcl-2; (b) determining the cell viability of the first population of cells; (c) contacting a second population cells with the agent, wherein the second population of cells has no expression of Bcl-2 or expression of Bcl-2 lower than first cell population; and (d) determining the cell viability of the second population of cells; (e) comparing the cell viability of the first and second populations of cells to determine whether the agent converts Bcl-2 from an anti-apoptotic protein into a proapoptotic protein; these shared technical features do not represent a contribution over the prior art.

Specifically, US 2013/0157260 A1 Satterthwait et al. discloses a method for inducing growth inhibition or apoptosis of a Bcl-2-expressing cell (This finding provides novel and effective approaches to induce cancer cell apoptosis by targeting Bcl-2, Para. [0073]; a method of retarding the growth of the tumor by administering pro-apoptotic modulator of Bcl-2 to the tumor and subsequently administering a cytotoxic agent to the tumor, Para. [0214]), comprising contacting a Bcl-2-expressing cell with an agent that exposes the BH3 domain of Bcl-2 thereby converting Bcl-2 into a pro-apoptotic protein and activating the intrinsic apoptosis pathway (Third, the cell-permeable Nur77 peptide induces a conformational change in Bcl-2 in vivo as detected by exposure of the Bcl-2 BH3 domain to BH3 antibodies, Para. [0076]; NuBCP peptides induce Bcl-2 conformational change which is not the consequence of apoptosis. Bcl-2 fluorescence from peptide-treated cells, Para. [0061]; Embodiments of the invention relate to the discovery that Nur77 interaction with Bcl-2 converts Bcl-2 from an anti-apoptotic to a pro-apoptotic molecule by inducing a Bcl-2 conformational change, Para. [0073]); a method of treating Bcl-2-expressing cancer in a subject (This finding provides novel and effective approaches to induce cancer cell apoptosis by targeting Bcl-2, Para. [0073]; a method of retarding the growth of the tumor by administering pro-apoptotic modulator of Bcl-2 to the tumor and subsequently administering a cytotoxic agent to the tumor, Para. [0214]; administered to a patient in need of such treatment, Para. [0206]), comprising administering to a subject in need of cancer treatment (administered to a patient in need of such treatment, Para. [0206]), a therapeutically effective amount of an agent that exposes the BH3 domain of Bcl-2 thereby converting Bcl-2 into a proapoptotic protein (Third, the cell-permeable Nur77 peptide induces a conformational change in Bcl-2 in vivo as detected by exposure of the Bcl-2 BH3 domain to BH3 antibodies, Para. [0076]; NuBCP peptides induce Bcl-2 conformational change which is not the consequence of apoptosis. Bcl-2 fluorescence from peptide-treated cells, Para. [0061]; Embodiments of the invention relate to the discovery that Nur77 interaction with Bcl-2 converts Bcl-2 from an anti-apoptotic to a pro-apoptotic molecule by inducing a Bcl-2 conformational change, Para. [0073]); an in vitro method of screening for an agent that converts Bcl-2 from an anti-apoptotic protein into a pro-apoptotic protein (a method of screening for compounds capable of converting a Bcl-2 family protein from an antiapoptotic form to a proapoptotic form includes providing a Bcl-2 family protein, Para. [0015]; In vitro systems can be designed to identify compounds capable of interacting with (e.g., binding to) Bcl-2-family members, Para. [0188]), comprising: (a) contacting a first population of cells with an agent, wherein the first population of cells expresses or overexpresses Bcl-2 (Active compounds from the first screening are further tested at different concentrations (50, 25, 12.5, 6.25, 3.125 and 1.56 µM) in order to determine their IC50. Growth inhibition is tested using two methods: A. staining of viable cells with methylene blue, Para. [0251]; by overexpression of Bcl-2 in these cells, Para. [0265]); (b) determining the cell viability of the first population of cells (Growth inhibition is tested using two methods: A. staining of viable cells with methylene blue, B. incorporation of 3H-thymidine. For both methods cells are grown in 96-well plates for 24 hours, before tested compounds are added. The assays are done in triplicate for one to six days, Para. [0251]); (c) contacting a second population cells with the agent, wherein the second population of cells has no expression of Bcl-2 or expression of Bcl-2 lower than first cell

population (Fluorescence microscopy showed that the antibody failed to stain Bcl-2 in control cells, reflecting the anti-apoptotic conformation of Bcl-2, in which its BH3 domain epitope is buried, Para. [0266]); and (d) determining the cell viability of the second population of cells; (e) comparing the cell viability of the first and second populations of cells to determine whether the agent converts Bcl-2 from an anti-apoptotic protein into a proapoptotic protein (Bcl-2 fluorescence from peptide-treated cells (white histogram) was compared to that from the non-treated cells (shaded histogram). Numbers represent % of treated cells showing increased Bcl-2 immunofluorescence compared to the auto-fluorescence of the non-treated cells from the same experiment, Para. [0044]; Growth inhibition is tested using two methods: A. staining of viable cells with methylene blue, B. incorporation of 3H-thymidine. For both methods cells are grown in 96-well plates for 24 hours, before tested compounds are added. The assays are done in triplicate for one to six days, Para. [0251]).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.