# PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: KE YANG
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# PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

| Date of mailing *(day/month/year)* | 06 MAY 2020 |
|---|---|

| Applicant's or agent's file reference | FOR FURTHER ACTION |
|---|---|
| 1035795-000643 | See paragraph 2 below |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/US 19/64024 | 02 December 2019 (02.12.2019) | 30 November 2018 (30.11.2018) |

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

IPC - A61K 31/4439; C07D 401/04; C07D 401/10 (2020.01)

CPC - A61K 31/00; A61K 31/4439; A61P 35/00; C07D 213/56; C07D 213/82; C07D 401/04

Applicant THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

| Name and mailing address of the ISA/US | Date of completion of this opinion | Authorized officer |
|---|---|---|
| Mail Stop PCT, Attn: ISA/US<br>Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-8300 | 17 March 2020 | Lee Young<br>PCT Help Desk<br>Telephone No. 571-272-4300 |

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**Box No. I Basis of this opinion**

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(b)).

3. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established 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).

4. ☐ 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.

5. Additional comments:

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**Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 9-12, 15-23, 26-31, 34

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 9-12, 15-23, 26-31, 34 are so unclear that no meaningful opinion could be formed *(specify)*:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 9-12, 15-23, 26-31, 34

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

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**Box No. IV Lack of unity of invention**

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:

   ☐ paid additional fees.

   ☐ paid additional fees under protest and, where applicable, the protest fee.

   ☐ paid additional fees under protest but the applicable protest fee was not paid.

   ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

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 searched, the appropriate additional search fees must be paid.

Group I+: Claims 1, 3, 5, (6-8, 13-14, 24-25, 32-33, 35-36)(in part), 37-38, directed to a compound of claim 1, formula I, and methods comprising the same. The compound of claim 1 will be searched to the extent that it encompasses the first species of claim 1, represented by a compound of formula I wherein a dashed line indicates the presence of an optional double bond; X, Y and Z are C; R1, R2, R3 and R4 are no atom; R5, R6, R7, R8 and R10 are no atom; R9 and R11 are H. It is believed that claims 1, (6-8)/1, 13, 24 and 32 reads on this first named invention, and thus these claims will be searched without fee. Applicant is invited to elect additional compounds of claim 1 wherein each additional compound elected will require one additional invention fee. Applicants must specify the claims that encompass any additionally elected compound. Applicants must further indicate, if applicable, the claims which encompass 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. Additionally, an exemplary election wherein different actual variables are selected is suggested. An exemplary election would be a compound of formula I wherein a dashed line indicates the presence of an optional double bond; X, Y and Z are C; R1, R2, R3 and R4 are no atom; R5 is C1 alkyl; R6, R7, R8 and R10 are no atom; R9 is monocyclic group containing 6 carbon atoms and R11 is O (i.e., claims 1, 3, 6-8(in part), 13-14, 24-25, 32-33, 35-36).

Group II+: Claims 2, 4, (6-8, 13-14, 24-25, 32-33, 35-36)(in part), directed to a compound of claim 2, formula II, and methods comprising the same. The compound of claim 2 may be searched, for example, to the extent that it encompasses the first species of claim 2, represented by a compound according to Formula II wherein a dashed line indicates the presence of an optional double bond; R1 is H; R3, R4, R5, R6 and R8 are no atom; R7 and R9 are H (R2 is omitted as no specification is provided; and there are no valid substituents when R1 is H); for payment of additional fee and election as such. It is believed that claims 2, 6-8(in part), 13, 24, 32, read on this first named invention of Group II+. Applicant is invited to elect additional compounds of claim 2, wherein each additional compound elected will require one additional invention fee. Applicants must specify the claims that encompass any additionally elected compound. Applicants must further indicate, if applicable, the claims which encompass 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. Another exemplary election would be a compound of Formula II wherein a dashed line indicates the presence of an optional double bond; R1 is C1 alkyl; R2 is N(R)2, wherein R is H; R3 is C1 alkyl, R4, R5, R6 and R8 are no atom; R7 is monocyclic group containing 4 carbon atoms and 2 heteroatoms which are O and N and R9 is O (R2 is assumed to be selected from the group comprising R7 and R9 based on dependent claims) (i.e., 2, 4, 6-8(in part), 13-14, 24-25, 32-33, 35-36).

Group III: Claims 39-60, directed to a method for identifying a compound that targets a disease-related reactive oxygen species (ROS) regulator; comprising providing siRNA, gRNA, and/or cDNA libraries that target a plurality of ROS regulators.

Group IV: Claims 61-108, directed to a method for diagnosing a disease in a subject in need thereof, comprising: (a) administering to the subject a detectable probe that targets a reactive oxygen species (ROS) regulator, which is associated with the disease / a detectable probe for measuring protein disulfide isomerase (PDI).

--continued on supplemental page--

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1, (6-8)/1, 13, 24 and 32

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**Box No. V** **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Claims | 1, (6-8)/1, 13, 24, 32 | YES |
| | Claims | NONE | NO |
| Inventive step (IS) | Claims | 1, (6-8)/1, 13, 24, 32 | YES |
| | Claims | NONE | NO |
| Industrial applicability (IA) | Claims | 1, (6-8)/1, 13, 24, 32 | YES |
| | Claims | NONE | NO |

2. Citations and explanations:

Claims 1, (6-8)/1, 13, 24 and 32 meet the criteria set out in PCT Article 33(2)-(3) because the prior art does not teach or fairly suggest the subject matter claimed.

Regarding Claim 1, US 2016/0046651 A1 to Washington University (hereinafter Washington) discloses a compound of formula I wherein a dashed line indicates the presence of an optional double bond; X, Y and Z are C; R1, R2, R3 and R4 are no atom; R6, R7, R8 and R10 are no atom (claim 4, (pg. 14, right col, 3rd compound listed)), but does not disclose wherein R5 is no atom or R9 and R11 are H.

US 2018/0092908 A1 to THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK (hereinafter Columbia) discloses a compound of formula I wherein a dashed line indicates the presence of an optional double bond; X, Y and Z are C; R1, R2, R3 and R4 are no atom; R6, R7, R8 and R10 are no atom (para [0009]: 1st compound listed), but does not disclose wherein R5 is no atom or R9 and R11 are H or wherein the core aryl ring comprises a nitrogen atom.

A document entitled "Synthesis and Fluorescence Properties of New Ester Derivatives of Isothiazolo [4,5-b] Pyridine" to Krzyzak et al. (hereinafter Krzyzak) discloses a compound of formula I wherein a dashed line indicates the presence of an optional double bond; X, Y and Z are C; R2 and R4 are no atom; R5, R6, R7 and R8 are no atom; R9 is H (pg. 5, Fig. 1: 1st compound listed), but does not disclose wherein R1 and R3 are no atom; R10 is no atom and R11 is H.

Regarding Claims (6-8)/1, these are dependent claims depending from claim1 and therefore, meet the criteria set forth in PCT Article 33(2) and 33(3).

Regarding Claim 13, Washington discloses a compound of formula I wherein a dashed line indicates the presence of an optional double bond; X, Y and Z are C; R1, R2, R3 and R4 are no atom; R6, R7, R8 and R10 are no atom (claim 4, (pg. 14, right col, 3rd compound listed)), but does not disclose wherein R5 is no atom or R9 and R11 are H or a method for treating or ameliorating the effects of a neurodegenerative disease in a subject in need thereof comprising administering to the subject an effective amount of said compound.

Columbia discloses a compound of formula I wherein a dashed line indicates the presence of an optional double bond; X, Y and Z are C; R1, R2, R3 and R4 are no atom; R6, R7, R8 and R10 are no atom (para [0009]: 1st compound listed), but does not disclose wherein R5 is no atom or R9 and R11 are H or wherein the core aryl ring comprises a nitrogen atom or a method for treating or ameliorating the effects of a neurodegenerative disease in a subject in need thereof comprising administering to the subject an effective amount of said compound.

Krzyzak discloses a compound of formula I wherein a dashed line indicates the presence of an optional double bond; X, Y and Z are C; R2 and R4 are no atom; R5, R6, R7 and R8 are no atom; R9 is H (pg. 5, Fig. 1: 1st compound listed), but does not disclose wherein R1 and R3 are no atom; R10 is no atom and R11 is H or a method for treating or ameliorating the effects of a neurodegenerative disease in a subject in need thereof comprising administering to the subject an effective amount of said compound.

Regarding Claim 24, Washington discloses a compound of formula I wherein a dashed line indicates the presence of an optional double bond; X, Y and Z are C; R1, R2, R3 and R4 are no atom; R6, R7, R8 and R10 are no atom (claim 4, (pg. 14, right col, 3rd compound listed)), but does not disclose wherein R5 is no atom or R9 and R11 are H or a method for treating or ameliorating the effects of a condition associated with increased protein disulfide isomerase (PDI) activity in a subject in need thereof comprising administering to the subject an effective amount of said compound.

Columbia discloses a compound of formula I wherein a dashed line indicates the presence of an optional double bond; X, Y and Z are C; R1, R2, R3 and R4 are no atom; R6, R7, R8 and R10 are no atom (para [0009]: 1st compound listed), but does not disclose wherein R5 is no atom or R9 and R11 are H or wherein the core aryl ring comprises a nitrogen atom or ameliorating the effects of a condition associated with increased protein disulfide isomerase (PDI) activity in a subject in need thereof comprising administering to the subject an effective amount of said compound.

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**Box No. VIII Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claim 20 is improperly dependent on claim 8 as it further defines a mammal; for the purpose of this ISR; it is assumed to depend from claim 19.

Claim 2 lacks clarity as definitions for R2 are missing; for the purpose of this ISR, R2 is assumed to be selected from the group comprising R7 and R9 based on dependent claims or no atom wherein appropriate.

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**Supplemental Box**

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
--continued from Box No. IV--

The group of inventions listed above 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:

Special Technical Features:

Group I+ includes the technical feature of a unique compound of formula I, which is not required by any other invention of Group I+, II+, III or IV.

Group II+ includes the technical feature of a unique compound of formula II, which is not required by any other invention of Group I+, II+, III or IV.

Group III includes the technical feature of a method for identifying a compound that targets a disease-related reactive oxygen species (ROS) regulator; comprising providing siRNA, gRNA, and/or cDNA libraries that target a plurality of ROS regulators, which is not required by any other invention of Group I+, II+ or IV.

Group IV includes the technical feature of a method for diagnosing a disease in a subject in need thereof, comprising: (a) administering to the subject a detectable probe that targets a reactive oxygen species (ROS) regulator, which is associated with the disease / a detectable probe for measuring protein disulfide isomerase (PDI), which is not required by any other invention of Group I+, II+ or III.

Common technical features:

The inventions of Groups I+ share the technical feature of a compound of formula I.

These shared technical features, however, do not provide a contribution over the prior art, as being anticipated by US 2016/0046651 A1 to Washington University (hereinafter Washington). Washington discloses a compound of formula I wherein a dashed line indicates the presence of an optional double bond; X, Y and Z are C; R1, R2, R3 and R4 are no atom; R5 is C1 alkyl, R6, R7, R8 and R10 are no atom; R9 is a monocyclic group containing 6 atoms and R11 is O (claim 4, (pg. 14, right col, 3rd compound listed)).

The inventions of Groups II+ share the technical feature of a compound of formula II.

These shared technical features, however, do not provide a contribution over the prior art, as being anticipated by a document entitled "Pubchem CID 21526566" (hereinafter Pubchem-566). Pubchem-566 discloses a compound of formula II wherein a dashed line indicates the presence of an optional double bond; R1 is H; R3, R4, R5, R6 and R8 are no atom; R7 and R9 are H (R2 is omitted when R1 is H) (pg. 2, compound listed).

Groups I+ and II+ include the technical feature of methods for treating or ameliorating the effects of a neurodegenerative disease / condition associated with increased protein disulfide isomerase in a subject; comprising administering a compound of formula I/II.

Groups III and IV include the technical feature of compounds that targets a reactive oxygen species (ROS) regulator.

These shared technical features, however, do not provide a contribution over the prior art, as being anticipated by US 2018/0092908 A1 to THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK (hereinafter Columbia). Columbia discloses methods for treating or ameliorating the effects of a neurodegenerative disease (abstract) / condition associated with increased protein disulfide isomerase in a subject (para [0004]); comprising administering a compound of formula II wherein a dashed line indicates the presence of an optional double bond; R1 is H; R3, R4, R5, R6 and R8 are no atom; R7 is C1 alkyl and R9 is O (R2 is omitted as no specification is provided; and there are no valid substituents when R1 is H) (para [0029]: 9th compound listed).

Columbia discloses compounds that targets a reactive oxygen species (ROS) regulator (para [0206]: By oxidizing PDI with LOC14 , Ero1 can be bypassed , reducing the generation of ROS and hence providing neuroprotection).

As said compound was known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the inventions of Groups I+, II+, III or IV. The inventions of Group I+, II+, III or IV thus lack unity under PCT Rule 13.

Note: Claims 9-12, 15-23, 26-31, 34 have been found to be unsearchable because they are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Note: Claim 20 is improperly dependent on claim 8 as it further defines a mammal; for the purpose of this ISR; it is assumed to depend from claim 19.

Note: Claim 2 lacks clarity as definitions for R2 are missing; for the purpose of this ISR, R2 is assumed to be selected from the group comprising R7 and R9 based on dependent claims or no atom wherein appropriate.

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**Supplemental Box**

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
--continued from Box No. V--

Krzyzak discloses a compound of formula I wherein a dashed line indicates the presence of an optional double bond; X, Y and Z are C; R2 and R4 are no atom; R5, R6, R7 and R8 are no atom; R9 is H (pg. 5, Fig. 1: 1st compound listed), but does not disclose wherein R1 and R3 are no atom; R10 is no atom and R11 is H or a method for treating or ameliorating the effects of a condition associated with increased protein disulfide isomerase (PDI) activity in a subject in need thereof comprising administering to the subject an effective amount of said compound.

Regarding Claim 32, Washington discloses a compound of formula I wherein a dashed line indicates the presence of an optional double bond; X, Y and Z are C; R1, R2, R3 and R4 are no atom; R6, R7, R8 and R10 are no atom (claim 4, (pg. 14, right col, 3rd compound listed)), but does not disclose wherein R5 is no atom or R9 and R11 are H or a method of modulating PDI activity in a cell comprising administering to the subject in need thereof comprising administering to the subject an effective amount of said compound.

Columbia discloses a compound of formula I wherein a dashed line indicates the presence of an optional double bond; X, Y and Z are C; R1, R2, R3 and R4 are no atom; R6, R7, R8 and R10 are no atom (para [0009]: 1st compound listed), but does not disclose wherein R5 is no atom or R9 and R11 are H or wherein the core aryl ring comprises a nitrogen atom or a method of modulating PDI activity in a cell comprising administering to the subject in need thereof comprising administering to the subject an effective amount of said compound.

Krzyzak discloses a compound of formula I wherein a dashed line indicates the presence of an optional double bond; X, Y and Z are C; R2 and R4 are no atom; R5, R6, R7 and R8 are no atom; R9 is H (pg. 5, Fig. 1: 1st compound listed), but does not disclose wherein R1 and R3 are no atom; R10 is no atom and R11 is H or a method for treating or a method of modulating PDI activity in a cell comprising administering to the subject in need thereof comprising administering to the subject an effective amount of said compound.

Claims 1, (6-8)/1, 13, 24 and 32 have industrial applicability as defined by PCT Article 33(4) because the subject matter could be made or used in industry.