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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))*

(54) Title: LIGAND IONOPHORE CONJUGATES

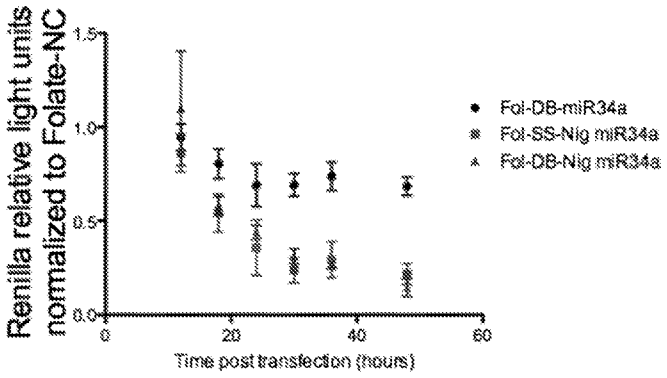

FIG. 1

(57) Abstract: The invention described herein pertains to ligand-ionophore conjugates, that may also comprise a linked therapeutic agent or imaging agent, and pharmaceutical compositions containing the conjugates. Also described are methods of using the conjugates for increasing the endosomal accumulation and escape of a therapeutic agent, or an imaging agent.

[Continued on next page]

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(88) Date of publication of the international search report:
28 June 2018 (28.06.2018)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/061997

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/08; A61K 47/48; A61P 37/06; G01N 33/533; G01N 33/84 (2018.01)
CPC - A61K 38/08; A61K 45/06; A61K 47/54; A61K 47/551; G01N 33/84 (2018.02)

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 - 514/44A; 514/44R; 536/24.5 (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.
P, X	WO 2016/183131 A1 (PURDUE RESEARCH FOUNDATION) 17 November 2016 (17.11.2016) entire document	1-12, 14-21
A	WO 2012/112440 A2 (ARIZONA BOARD OF REGENTS, A BODY CORPORATE OF THE STATE OF ARIZONA ACTING FOR AND ON BEHALF OF ARIZONA STATE UNIVERSITY et al) 23 August 2012 (23.08.2012) entire document	1-12, 14-21
A	WO 2015/130997 A1 (GDOVIN et al) 03 September 2015 (03.09.2015) entire document	1-12, 14-21
A	WO 2007/006041 A2 (PURDUE RESEARCH FOUNDATION et al) 11 January 2007 (11.01.2007) entire document	1-12, 14-21
A	US 8,969,543 B2 (JEONG et al) 03 March 2015 (03.03.2015) entire document	1-12, 14-21
A	FUCHS et al. "Salinomycin overcomes ABC transporter-mediated multidrug and apoptosis resistance in human leukemia stem cell-like KG-1a cells," Biochem Biophys Res Commun, 27 March 2010 (27.03.2010), Vol. 394, No. 4, Pgs. 1098-1104. entire document	1-12, 14-21
A	US 2016/0160220 A1 (UNIVERSITY OF CINCINNATI et al) 09 June 2016 (09.06.2016) entire document	1-12, 14-21

☐ 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	Date of mailing of the international search report
17 April 2018	14 MAY 2018
Name and mailing address of the ISA/US 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/US2017/061997

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 extra sheet(s).

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.:
1-12 and 14-21 to the extent that they read on the selected ligand-ionophore (see Extra Sheets for structure).

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.

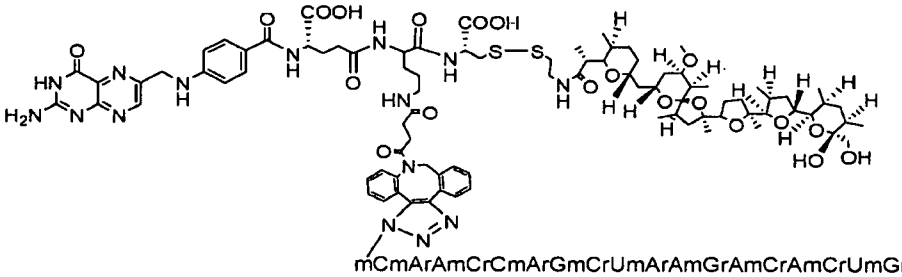
☐ No protest accompanied the payment of additional search fees.

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 1-23 are drawn to ligand-ionophore conjugates.

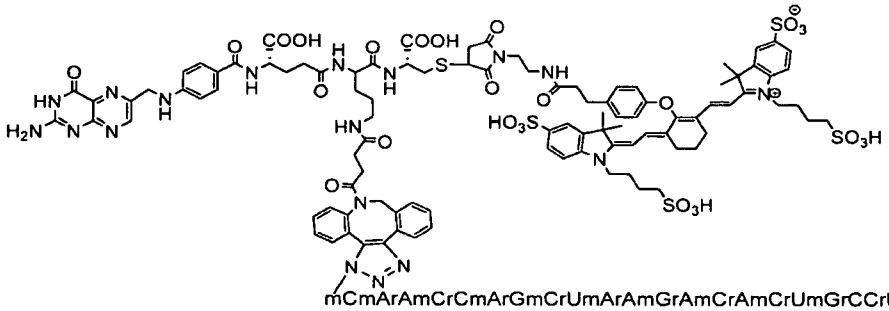
The first invention of Group I+ is restricted to a ligand-ionophore, wherein the ligand-ionophore is selected to be:



mCmArAmCrCmArGmCrUmArAmGrAmCrAmCrUmGrCC
rUmGmUrUrGrGrUrCrGrArUrUrCrUrGrUrGrArCrGrGrU/5Phos

It is believed that claims 1-12 and 14-21 read on this first named invention and thus these claims will be searched without fee to the extent that they read on the above embodiment.

Applicant is invited to elect additional ligand-ionophore conjugates, and their respective, corresponding chemical structures to be searched in a specific combination by paying an additional fee for each set of election. An exemplary election would be to a ligand-ionophore conjugate, wherein the ligand-ionophore conjugate is selected to be:



mCmArAmCrCmArGmCrUmArAmGrAmCrAmCrUmGrCCrU
mGmUrUrGrGrUrCrGrArUrUrCrUrGrUrGrArCrGrGrU/5Phos

Additional ligand-ionophore conjugates 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 increasing the endosomal accumulation and escape of a therapeutic agent or an imaging agent, requiring the selection of alternatives for the ligands (B), linkers (L), ionophores (A), and/or therapeutic agents (TA), where "(B) is a folate" and "(B) is a PSMA binding ligand" and "(A) is an inhibitor of the Na+/H+ exchanger" and "the ionophore (A) comprises nigericin or salinomycin" and "the therapeutic agent (TA) comprises an siRNA" and "the therapeutic agent (TA) comprises an iRNA" and "the therapeutic agent (TA) comprises a microRNA".

Additionally, even if Groups I+ were considered to share the technical features of a conjugate, or a pharmaceutically acceptable salt thereof, comprising: a ligand (B) targeted to a cell-surface receptor; one or more linkers (L); one or more ionophores (A) each of which couples efflux of protons (H+ ions) to influx of potassium ions (K+ ions); and/or a therapeutic agent (TA) comprising an siRNA, an iRNA, or a microRNA; (B) is covalently linked to (L); and each of (A) and/or (TA) is covalently linked to (L); a conjugate, or a pharmaceutically acceptable salt thereof, comprising: a ligand (B) targeted to a cell-surface receptor; one or more linkers (L); one or more of an ionophore (A) which couples efflux of protons (H+ ions) to influx of potassium ions (K+ ions); an RNA selected from an siRNA, an iRNA, and a microRNA; or an imaging agent (IA); wherein (L) comprises at least one releasable linker; (B) is covalently linked to (L); and each of (A), the RNA and/or (IA) is covalently linked to (L); these shared technical features do not represent a contribution over the prior art.

Specifically, WO 2015/130997 A1 to Gdovin et al. discloses a conjugate (Certain embodiments are directed to photo-activated compounds for the manipulation of pH in a cell, Abstract; may be conjugated to the nanoparticle, Para. [012]), comprising: a ligand (B) targeted to a cell-surface receptor (Targeting may also be accomplished by active targeting in which ligands are coupled to the nanoparticle that correspond to molecules found exclusively in cancer cells, Para. [013]; The wide array of possible targets for cancer include surface receptors such as human epidermal growth factor receptor 2, Para. [014]); one or more linkers (L) (Linkers used in context of the current invention can include bifunctional linkers, Para. [077]); one or more ionophores (A) each of which couples efflux of protons (H+ ions) (with the use of ratiometric pH-sensitive fluorescent dyes, that NBA can release a proton and induce acidosis inside of a cell, Para. [0123; Removal of NBA from the extracellular space does not result in an efflux of NBA, Para. [052]); and/or a therapeutic agent (TA) comprising an siRNA (The targeted delivery in vivo enhances the cellular uptake of these compounds or particles to enhance therapeutic efficacies, Para. [073]); (B) is covalently linked to (L); and each of (A) and/or (TA) is covalently linked to (L) (Targeting may also be accomplished by active targeting in which ligands are coupled to the nanoparticle that correspond to molecules found exclusively in cancer cells, Para. [013]; The wide array of possible targets for cancer include surface receptors such as human epidermal growth factor receptor 2, Para. [014]); a conjugate, or a pharmaceutically acceptable salt thereof (Certain embodiments are directed to photo-activated compounds for the manipulation of pH in a cell, Abstract; may be conjugated to the nanoparticle, Para. [012]), comprising: a ligand (B) targeted to a cell-surface receptor; one or more linkers (L) (Targeting may also be accomplished by active targeting in which ligands are coupled to the nanoparticle that correspond to molecules found exclusively in cancer cells, Para. [013]; The wide array of possible targets for cancer include surface receptors such as human epidermal growth factor receptor 2, Para. [014]); one or more of an ionophore (A) which couples efflux of protons (H+ ions) (with the use of ratiometric pH-sensitive fluorescent dyes, that NBA can release a proton and induce acidosis inside of a cell, Para. [0123]; Removal of NBA from the extracellular space does not result in an efflux of NBA, Para. [052]); wherein (L) comprises at least one releasable linker (Linkers used in context of the current invention can include bifunctional linkers. The bifunctional can be permanent or releasable linkers, Para. [077]).

Further, US 2016/0160220 A1 to University of Cincinnati et al. discloses coupling influx of potassium ions (K+ ions) (hyperactivity of T cells by selectively targeting memory T cells with the nanoparticles, which deliver their siRNA cargo into the cytosol of the TM cell, Abstract; The Kv1.3 channel is a voltage-activated potassium (K+) channel that shows a fast activation and slow C-type inactivation and recovery, Para. [0049]), and a conjugate to an siRNA, or a microRNA, (B) is covalently linked to (L); and each of (A), the RNA and/or (IA) is covalently linked to (L) (comprise antibody selective for a membrane protein unique to a target subset of immune system cells and bound to a surface of the lipid nanovesicle; and siRNA effective for inhibiting expression of an ion channel of the target subset cells upon transfection. The siRNA is encapsulated within the lipid nanovesicle, Para. [0011]).

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