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(54) Title: A SIMPLIFIED AND MORE ACCESSIBLE METHOD FOR PRODUCTION OF ^{111}ININ-XYIMSR-01 FOR PATIENT USE

(57) Abstract: A new simplified method for the production of the radiopharmaceutical agent ^{111}InIn-XYIMSR-01 is disclosed. The presently disclosed method significantly increases accessibility to the pharmaceutical as it can now be produced at any radiopharmacy without the need for specialized equipment.

A SIMPLIFIED AND MORE ACCESSIBLE METHOD FOR PRODUCTION OF ^{111}INIn-XYIMSR-01 FOR PATIENT USE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 63/600,329, filed November 17, 2023, which is incorporated herein by reference in its entirety.

BACKGROUND

A total of 73,750 new cases of kidney cancer, representing 5% of all diagnosed malignancies, are estimated to be diagnosed in the United States in 2020. Henley et al., 2020. Additionally, 14,830 deaths are estimated from this disease. The clear cell subtype of renal cell carcinoma (ccRCC) accounts for up to 70% of all kidney cancers. Zeng et al., 2019. Although computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound are useful noninvasive tools for detecting and staging kidney cancer, they are unable to reliably distinguish ccRCC from less aggressive tumor subtypes. Lindenberg et al., 2019. Positron emission tomography (PET) and single-photon emission computed tomography (SPECT) combined with new biomarkers have the potential to overcome those limitations.

Carbonic anhydrase IX (CAIX) is a membrane-associated member of the carbonic anhydrase (CA) family. CAIX is up-regulated in multiple different cancers including ccRCC. CAIX is overexpressed in 95% of ccRCC tumor specimens and has very low expression in normal tissues and other renal tumor subtypes. Supuran, 2008. Therefore, CAIX is a potential target for ccRCC imaging and therapy. Although anti-CAIX monoclonal antibodies (mAbs) radiolabeled with ^{124}I, ^{89}Zr, and ^{111}In for noninvasive PET and SPECT imaging of ccRCC have shown promising results, van Es et al., 2017; Divgi et al., 2013; Muselaers et al., 2013, they suffer from slow blood and nontarget tissue clearance and nonspecific organ uptake. Lindenberg et al., 2019; Yang et al., 2015.

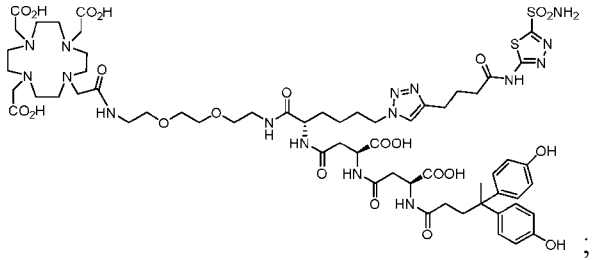
As opposed to mAbs, low-molecular-weight (LMW) CAIX inhibitors have rapid pharmacokinetics and the advantages of potential same-day imaging and lower radiation exposure to the patient. Lindenberg et al., 2019. Dual-motif targeting LMW CAIX ligands [^{111}In]In-XYIMSR-01, Yang et al., 2014, Yang et al., 2019, [^{64}Cu]Cu-XYIMSR-06, Minn et

al., 2016, and [^{177}Lu]Lu-XYIMSR-01, Minn et al., 2016, have been reported for SPECT imaging, PET imaging, and therapy of ccRCC, respectively. Those agents demonstrated high selectivity for CAIX *in vitro* and *in vivo*, with the potential to image both metastatic and localized ccRCC. There is a need, however, for simple methods for preparing such agents.

SUMMARY

In some aspects, the presently disclosed subject matter provides a method for preparing [^{111}In]In-XYIMSR-01, the method comprising:

(a) preparing a non-radiolabeled XYIMSR-01 precursor in a buffer solution:



; and

(b) add the non-radiolabeled XYIMSR-01 precursor in the buffer solution of step (a) to Indium Chloride In-111 ($^{111}InCl_3$) under heat for a period of time to provide [^{111}In]In-XYIMSR-01.

In certain aspects, the buffer solution comprises an ascorbate buffer solution. In particular aspects, the buffer solution has a pH of about 5.6.

In certain aspects, the period of time is about 30 minutes.

In certain aspects, step (b) comprises heating at about 75 °C.

In certain aspects, the method further comprises assaying the radioactivity of the [^{111}In]In-XYIMSR-01.

In certain aspects, the method comprises about 20 μg of the non-radiolabeled XYIMSR-01 precursor.

In particular aspects, step (b) does not include shaking of the non-radiolabeled XYIMSR-01 precursor in the buffer solution after it is added to the Indium Chloride In-111 ($^{111}InCl_3$).

In particular aspects, the method does not include purification of the [^{111}In]In-XYIMSR-01.

Certain aspects of the presently disclosed subject matter having been stated hereinabove, which are addressed in whole or in part by the presently disclosed subject

matter, other aspects will become evident as the description proceeds when taken in connection with the accompanying Examples and Figures as best described herein below.

BRIEF DESCRIPTION OF THE FIGURES

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

Having thus described the presently disclosed subject matter in general terms, reference will now be made to the accompanying figures, which are not necessarily drawn to scale, and wherein:

FIG. 1 is a scheme showing the synthesis of the XYIMSR-01 precursor;

FIG. 2 is a scheme showing the synthesis of [^{111}In]In-XYIMSR-01;

FIG. 3A shows *in vivo* imaging of In-111 XYIMSR using SPECT in rodent (see Yang et al., Imaging of carbonic anhydrase IX with an ^{111}In-labeled dual-motif inhibitor. *Oncotarget*. 2015; 6: 33733-33742; De Silva et al., Process validation, current good manufacturing practice production, dosimetry, and toxicity studies of the carbonic anhydrase IX imaging agent [111In]In-XYIMSR-01 for phase I regulatory approval. *J Label Compd Radiopharm*. 2021; 64: 243–250); and

FIG. 3B shows *in vivo* imaging of In-111 XYIMSR using SPECT in human.

DETAILED DESCRIPTION

The presently disclosed subject matter now will be described more fully hereinafter with reference to the accompanying drawings, in which some, but not all embodiments of the presently disclosed subject matter are shown. Like numbers refer to like elements throughout. The presently disclosed subject matter may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will satisfy applicable legal requirements. Indeed, many modifications and other embodiments of the presently disclosed subject matter set forth herein will come to mind to one skilled in the art to which the presently disclosed subject matter pertains having the benefit of the teachings presented in

the foregoing descriptions and the associated drawings. Therefore, it is to be understood that the presently disclosed subject matter is not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims.

[^{111}In]In-XYIMSR-01 is a promising single-photon emission computed tomography (SPECT) imaging agent for identifying tumors that overexpress carbonic anhydrase IX. To translate [^{111}In]In-XYIMSR-01 to phase I trials, animal toxicity and dosimetry studies have been conducted, the maximum dose for human use has been determined, and the chemistry, manufacturing, and controls component of a standard regulatory application have been completed. De Silva et al., 2021. The production process, quality control testing, stability studies, and specifications for sterile drug product release also are described in De Silva et al., 2021.

Methods known in the art for producing ^{111}InIn-XYIMSR-01 require the heating of 200 µg XYIMSR-01 precursor in 0.5M sodium acetate buffer (pH 5.6) over 31 mins at 61 °C, while being shaken at 350 rpm (rotations). The reaction mixture is then purified and the desired product collected using reverse phase, semi-preparative high performance liquid chromatography (HPLC). The collected product from the HPLC is purified again using solid phase extraction (Waters C18 Plus), prior to sterile filtration into a final product vial. The product then undergoes multiple quality control analysis procedures prior to being released for subsequent use. The radiochemical yield of the final compound with this known method was 50.5%. De Silva et al., 2021.

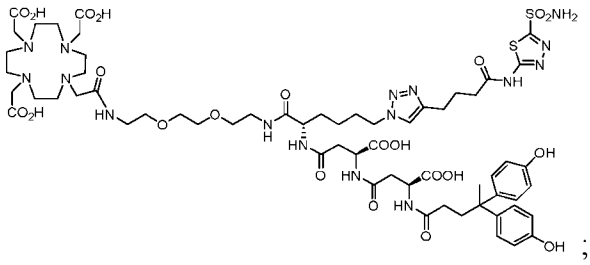
More particularly, the known process described by De Silva et al., 2021, for manufacturing [^{111}In]In-XYIMSR-01 includes the following four general steps: (1) radiolabeling XYIMSR-01 precursor with $^{111}InCl_3$; (2) RP-HPLC column purification of crude, radiolabeled [^{111}In]In-XYIMSR-01; (3) removal of RP-HPLC solvent via C-18 Sep-Pak with formulation solvent; and, finally, (4) aseptic filtration and dispensing. In the process reported by De Silva, 500 µL of 0.5 M NaOAc buffer (pH approximately 5.6) is added to a vial containing $^{111}InCl_3$. The activity in buffer is then transferred to a 2-mL reaction vial containing 200 µg of XYIMSR-01 precursor. A further 500 µL of buffer is added to the same 2-mL reaction vial. The 2-mL vial containing the reaction mixture is

transferred to a thermomixer and incubated at 61 °C while undergoing rotation at 350 rpm for a period of 31 ± 1 min. After cooling to room temperature, the reaction mixture containing crude [^{111}In]In-XYIMSR-01 is purified via chromatographic separation using a RP-HPLC column, eluted with 17% acetonitrile with 0.1% formic acid and 83% water at a flow rate of 10 mL/min. The column effluent is monitored using UV (254 nm) and radiometric detectors connected in series. The [^{111}In]In-XYIMSR-01 peak is collected in a clean glass bottle with 150 mL of sterile water for irrigation and passed through an activated Sep-Pak C-18 plus cartridge by N_2 flow attached to a 5-gang manifold to capture the product on the cartridge. The cartridge is washed with sterile water for injection (20 mL) to remove trace amounts of acetonitrile and formic acid (from the RP-HPLC mobile phase). The product is then eluted with 1 mL of ethanol and 14 mL of 0.9% saline solution into a 30-mL sterile vial. The drug product vial is transferred into the ISO Class 5 hot cell located in a cleanroom area. The product is processed aseptically through a 0.2-μm sterile filter into the final 30-mL sterile vial within the ISO Class 5 hot cell.

In contrast to the methods known in the art, the presently disclosed method for producing ^{111}InIn-XYIMSR-01 includes the heating of 20 μg of a XYIMSR-01 precursor (a 10-fold reduction compared to the method disclosed in De Silva et al., 2021, in 0.5-M ascorbate buffer (pH 5.6) over 30 mins. No shaking is required in the presently disclosed synthesis method, which eliminates the need for an elliptical shaker. The labeling yields of the presently disclosed methods are greater than 96%, which is significantly higher than previously reported methods.

More particularly, the presently disclosed method for synthesizing ^{111}InIn-XYIMSR-01 includes:

(a) preparing a non-radiolabeled XYIMSR-01 precursor in a buffer solution:



; and

(b) add the non-radiolabeled XYIMSR-01 precursor in the buffer solution of step (a) to Indium Chloride In-111 ($^{111}InCl_3$) under heat for a period of time to provide [^{111}In]In-XYIMSR-01.

In certain embodiments, the buffer solution comprises an ascorbate buffer solution. In particular embodiments, the ascorbate buffer solution comprises a 0.5-M ascorbate buffer. In more particular embodiments, the ascorbate buffer comprises a (+)-sodium L-ascorbate buffer solution. In particular embodiments, the buffer solution has a pH of about 5.6, including a pH of about 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, and 5.9.

In certain embodiments, the period of time is about 30 minutes, including about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, and 35 minutes.

In certain embodiments, step (b) comprises heating at about 75 °C, including about 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, and 80 °C.

In certain embodiments, the method further comprises assaying the radioactivity of the [^{111}In]In-XYIMSR-01.

In certain embodiments, the method comprises about 20 µg of the non-radiolabeled XYIMSR-01 precursor, including 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, and 25 µg of the non-radiolabeled XYIMSR-01 precursor.

In particular embodiments, step (b) does not include shaking of the non-radiolabeled XYIMSR-01 precursor in the buffer solution after it is added to the Indium Chloride In-111 ($^{111}InCl_3$).

In particular embodiments, the method does not include purification of the [^{111}In]In-XYIMSR-01.

Because the amount of precursor used is orders of magnitude less than the toxicity levels of the compound, and considering almost complete incorporation of starting $^{111}InCl_3$ into the precursor, the presently disclosed method also eliminates the need for both the HPLC and the solid phase extraction purification steps. Put together, this new methodology produces the desired radiopharmaceutical in much higher radiochemical yields, suitable for multiple patient studies with one production, while also eliminating the need for specialized equipment. Accordingly, the presently disclosed synthetic method makes this agent accessible to all radiopharmacies without the need for specialized staff expertise.

Following long-standing patent law convention, the terms "a," "an," and "the" refer to "one or more" when used in this application, including the claims. Thus, for example, reference to "a subject" includes a plurality of subjects, unless the context clearly is to the contrary (e.g., a plurality of subjects), and so forth.

Throughout this specification and the claims, the terms "comprise," "comprises," and "comprising" are used in a non-exclusive sense, except where the context requires otherwise. Likewise, the term "include" and its grammatical variants are intended to be non-limiting, such that recitation of items in a list is not to the exclusion of other like items that can be substituted or added to the listed items.

For the purposes of this specification and appended claims, unless otherwise indicated, all numbers expressing amounts, sizes, dimensions, proportions, shapes, formulations, parameters, percentages, quantities, characteristics, and other numerical values used in the specification and claims, are to be understood as being modified in all instances by the term "about" even though the term "about" may not expressly appear with the value, amount, or range. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are not and need not be exact, but may be approximate and/or larger or smaller as desired, reflecting tolerances, conversion factors, rounding off, measurement error and the like, and other factors known to those of skill in the art depending on the desired properties sought to be obtained by the presently disclosed subject matter. For example, the term "about," when referring to a value can be meant to encompass variations of, in some embodiments, in some embodiments ± 20%, in some embodiments ± 10%, in some embodiments ± 5%, in some embodiments ±1%, in some embodiments ± 0.5%, and in some embodiments ± 0.1% from the specified amount, as such variations are appropriate to perform the disclosed methods or employ the disclosed compositions.

Further, the term "about" when used in connection with one or more numbers or numerical ranges, should be understood to refer to all such numbers, including all numbers in a range and modifies that range by extending the boundaries above and below the numerical values set forth. For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9,

and for the range 6.0-7.0, the number 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

EXAMPLES

The following Examples have been included to provide guidance to one of ordinary skill in the art for practicing representative embodiments of the presently disclosed subject matter. In light of the present disclosure and the general level of skill in the art, those of skill can appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the scope of the presently disclosed subject matter. The synthetic descriptions and specific examples that follow are only intended for the purposes of illustration, and are not to be construed as limiting in any manner to make compounds of the disclosure by other methods.

EXAMPLE 1

Materials and Methods

Raw materials, reference standards, solvents, and media with valid manufacturer certificates of analysis (COA) were released for cGMP use as per standard operating procedures (SOPs) from the Center for Translational Molecular Imaging (CTMI), a Johns Hopkins cGMP facility. cGMP grade $^{111}InCl_3$ was purchased from Nordion, Inc. (Ottawa, ON, Canada), and identity testing was performed at CTMI before release. High purity XYIMSR-01 precursor was synthesized at CTMI according to the scheme provided in FIG. 1 and were characterized, Yang et al., 2015, by visual inspection for physical appearance, proton nuclear magnetic resonance (1H-NMR), and electrospray ionization (ESI) mass analysis for identity and reverse-phase high-performance liquid chromatography (RP-HPLC) for purity. Further details of the synthesis and characterization of XYIMSR-01 are provided in International PCT Patent Application No. WO2016196628 for Nuclear Imaging And Radiotherapeutics Agents Targeting Carbonic Anhydrase IX And Uses Thereof, to Yang et al., published December 8, 2016, which is incorporated herein by reference in its entirety.

EXAMPLE 2

Analytical Methods and Procedures for [^{111}In]In-XYIMSR-01 Product

Although not explicitly required for the presently disclosed methods, the HPLC and gas chromatography (GC) methods have been evaluated and validated in terms of linearity, accuracy, robustness, limit of detection (LOD), limit of quantification (LOQ), precision (repeatability and reproducibility conditions), and system suitability. De Silva et al., 2021.

The radiochemical purity, radiochemical identity, and chemical purity can be tested using an analytical Waters XBridge Peptide BEH C18 4.6 × 100 mm 5 µm. QC testing samples can be eluted with 10% acetonitrile/80% water/0.05% trifluoroacetic acid at a flow rate of 2 mL/min and a UV wavelength of 270 nm. A GC method was developed to analyze residual solvent in the final formulation of [^{111}In]In-XYIMSR-01 drug product. De Silva et al., 2021. The product identity can be determined by the percentage difference of peak retention times of the radiodetector chromatogram of [^{111}In]In-XYIMSR-01 and a referenced standard, [$^{113/115}$In]InIn-XYIMSR-01, from the UV chromatogram. Endotoxin can be quantified with an Endosafe-PTS instrument (Charles River Laboratories, Wilmington, MA) with 1:100 dilution of final drug product in endotoxin-free water. The gamma ray spectrum can be collected from a multichannel analyzer (MCA) with a NaI detector for ^{111}In radionuclidic identity and purity. In addition to the filter integrity test, a direct inoculation method can be used for sterility testing. Drug product samples can be incubated for 14 days at 20°C to 25 °C in tryptic soy broth (TSB) and at 30 °C to 35 °C in fluid thioglycollate medium (FTM) along with positive (BIOBALL [BioMérieux, Marcy-l'Étoile, FRA]) and negative (unopened TSB and FTM media tubes) controls. The pH of the final drug product can be measured by two pH indicator strips in the range of 4.0–7.0 and 5.0–10.0, respectively.

EXAMPLE 3

Representative Quality Control Criteria

Representative test and acceptance criteria for [^{111}In]In-XYIMSR-01 injectable drug product are provided in Table 1. These criteria, where possible, are based on existing current guidelines (USP monographs, ICH, and FDA) for radiopharmaceutical, dosimetry, and toxicity data. Sgouros et al., 2020; Mantel et al., 2919; Kolenc et al., 2019; Norenberg et al., 2011.

Table 1. Test and acceptance criteria for [^{111}In]In-XYIMSR-01 injectable drug product.

Test		**Method**	**Acceptance criteria**
Appearance	Visual inspection	USP <1790>	Clear, colorless, particulate-free
pH	pH strips (range of 4.0 - 7.0 and 5.0 - 10.0)	USP <791>	4.0-8.0 *
Radionuclidic ID	Gamma spectrometry	In house	Major peaks @ 245 (± 3) keV and 171 (±3) keV †
Radiochemical purity	Reverse phase HPLC with radiometric detector	In house	≥ 95.0 %
Chemical purity	Reverse phase HPLC with UV detector (wavelength 254 nm)	ICH Q3B(R)	Precursor and other impurities ≤ 10 ug/mL ŧ
Radiochemical ID	Reverse phase HPLC with [$^{113/115}$In]In-XYIMSR-01 reference standard	In house	% difference of retention time < 5%
Bacterial endotoxins	Endosafe-PTS (1:100 dilution)	USP <85>	< 175 EU/dose volume §
Residual solvents	GC method	USP <467> and ICH Q3C (R6)	Acetonitrile <0.25 mg/mL; Ethanol < 10% (v/v) #
Filter integrity test	Membrane filter bubble point test	USP <797>	> 50 psi
Retrospective Tests			
Sterility	Inoculating FTM and TSB nutrient media	USP <79>	Sterile (no growth after 14 days)

* pH range for small volume injectables as listed in USP monograph

† Characteristic energy peaks for ^{111}In

ŧ Set limit was based on acute toxicity data

§ Endotoxin limits for radiopharmaceuticals as listed in USP monograph

ICH limits for acetonitrile and ethanol residual solvents

EXAMPLE 4

Representative Protocol for the Radiochemical Synthesis of Indium-111 Labeled XYIMSR ([^{111}In]XYIMSR)

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Radiochemical Synthesis of Indium-111 Labeled XYIMSR ([^{111}In]XYIMSR)	
Step	Activity
1	Prepare final product enclosure/filter assembly
2	Prepare hot cell for synthesis
3	Prepare 0.5M (+)-sodium L-ascorbate solution – to a 50 mL bottle (EQ-038), add 4.95 ± 0.5 g of sodium ascorbate (RM-106) to 50 mL of HPLC water
4	Prepare 0.5M L-ascorbic acid solution – to a second 50 mL, add 4.04 ± 0.4 g of ascorbic acid (RM-107) to 50 mL of HPLC water
5	Prepare 0.5 M (+)-sodium L-ascorbate buffer – remove and discard 2.5 mL of the 0.5 M (+)-sodium L-ascorbate solution (step 3), then to the remaining 47.5 mL, add 2.5 mL of the 0.5 M L-ascorbic acid solution prepared in step 4.
6	Prepare XYIMSR precursor (0.2 mg/mL) in 0.5 M (+)-sodium L-ascorbate buffer: (a) provide XYIMSR precursor (RM-227; 1.0 ± 0.1 mg); (b) dissolve the precursor in a sufficient volume of 0.5 M ascorbate buffer (step 5) to make the concentration 0.2 mg/mL; (c) provide (+)-sodium L-ascorbate buffer solution (from step 5); and (d) calculate final concentration (mg of XYIMSR precursor/mL (+)-sodium L-ascorbate buffer).
7	Prepare In-111 for transfer –Record the calibrated activity (mCi) and the volume (mL) of the Indium Chloride In-111.
8	Perform radiolabeling: (a) add HPLC water (RM-019) for injection Indium-111 chloride to bring volume to 0.1 mL; (b) transfer the indium chloride (In-111) to a 2 mL screw top vial; (c) add 0.1 mL of precursor solution (prepared in step 6); (d) cap vial and heat at 75 °C for 30 minutes; and (e) cool reaction to room temperature.
9	Final formulation: (a) draw 9.8 mL of sodium chloride injection, 0.9% into a 10-mL polypropylene syringe; (b) add approximately 1 mL of sodium chloride

Radiochemical Synthesis of Indium-111 Labeled XYIMSR ([^{111}In]XYIMSR)	
Step	Activity
	injection, 0.9% to the reaction vial; (c) aspirate the reaction solution using the same 10-mL syringe; and (d) sterile filter the reaction solution into the sterile vial via the Millex GV sterile filter.
10	Assay final product radioactivity.

EXAMPLE 5

In Vivo Imaging of In-111 XYIMSR

FIG. 3A shows *in vivo* imaging of In-111 XYIMSR using SPECT in rodent (see Yang et al., Imaging of carbonic anhydrase IX with an ^{111}In-labeled dual-motif inhibitor. *Oncotarget*. 2015; 6: 33733-33742; De Silva et al., Process validation, current good manufacturing practice production, dosimetry, and toxicity studies of the carbonic anhydrase IX imaging agent [111In]In-XYIMSR-01 for phase I regulatory approval. *J Label Compd Radiopharm*. 2021; 64: 243–250).

FIG. 3B shows *in vivo* imaging of In-111 XYIMSR using SPECT in human.

REFERENCES

All publications, patent applications, patents, and other references mentioned in the specification are indicative of the level of those skilled in the art to which the presently disclosed subject matter pertains. All publications, patent applications, patents, and other references are herein incorporated by reference to the same extent as if each individual publication, patent application, patent, and other reference was specifically and individually indicated to be incorporated by reference. It will be understood that, although a number of patent applications, patents, and other references are referred to herein, such reference does not constitute an admission that any of these documents form part of the common general knowledge in the art.

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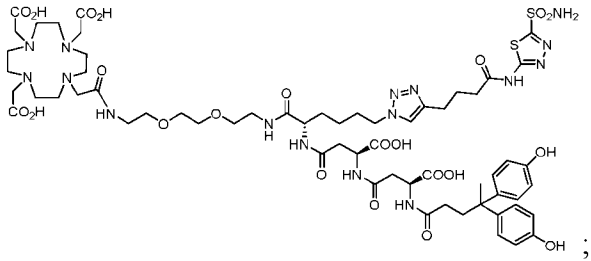
Although the foregoing subject matter has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be understood by those skilled in the art that certain changes and modifications can be practiced within the scope of the appended claims.

THAT WHICH IS CLAIMED:

1. A method for preparing [^{111}In]In-XYIMSR-01, the method comprising:

(a) preparing a non-radiolabeled XYIMSR-01 precursor in a buffer solution:



; and

(b) add the non-radiolabeled XYIMSR-01 precursor in the buffer solution of step (a) to Indium Chloride In-111 ($^{111}InCl_3$) under heat for a period of time to provide [^{111}In]In-XYIMSR-01.

2. The method of claim 1, wherein the buffer solution comprises an ascorbate buffer solution.

3. The method of claim 1 or claim 2, wherein the buffer solution has a pH of about 5.6.

4. The method of any one of claims 1 to 3, wherein the period of time is about 30 minutes.

5. The method of claim 1, wherein step (b) comprises heating at about 75 °C.

6. The method of claim 1, further comprising assaying the radioactivity of the [^{111}In]In-XYIMSR-01.

7. The method of any one of claims 1 to 6, comprising about 20 µg of the non-radiolabeled XYIMSR-01 precursor.

8. The method of claim 1, wherein step (b) does not include shaking of the non-radiolabeled XYIMSR-01 precursor in the buffer solution after it is added to the Indium Chloride In-111 ($^{111}InCl_3$).

9. The method of any one of claims 1 to 8, wherein the method does not include purification of the [^{111}In]In-XYIMSR-01.

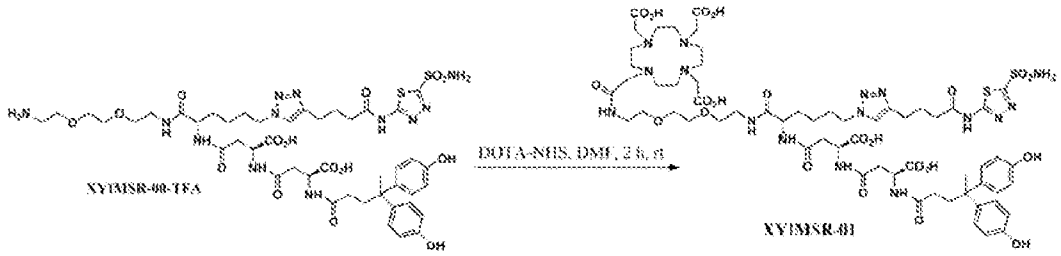


Fig. 1

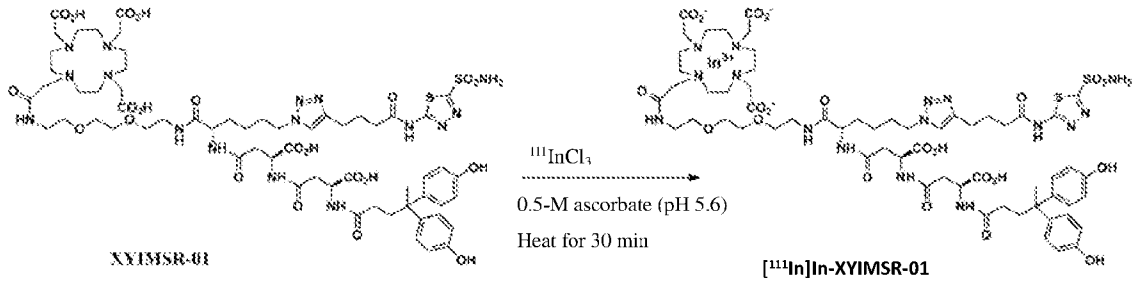
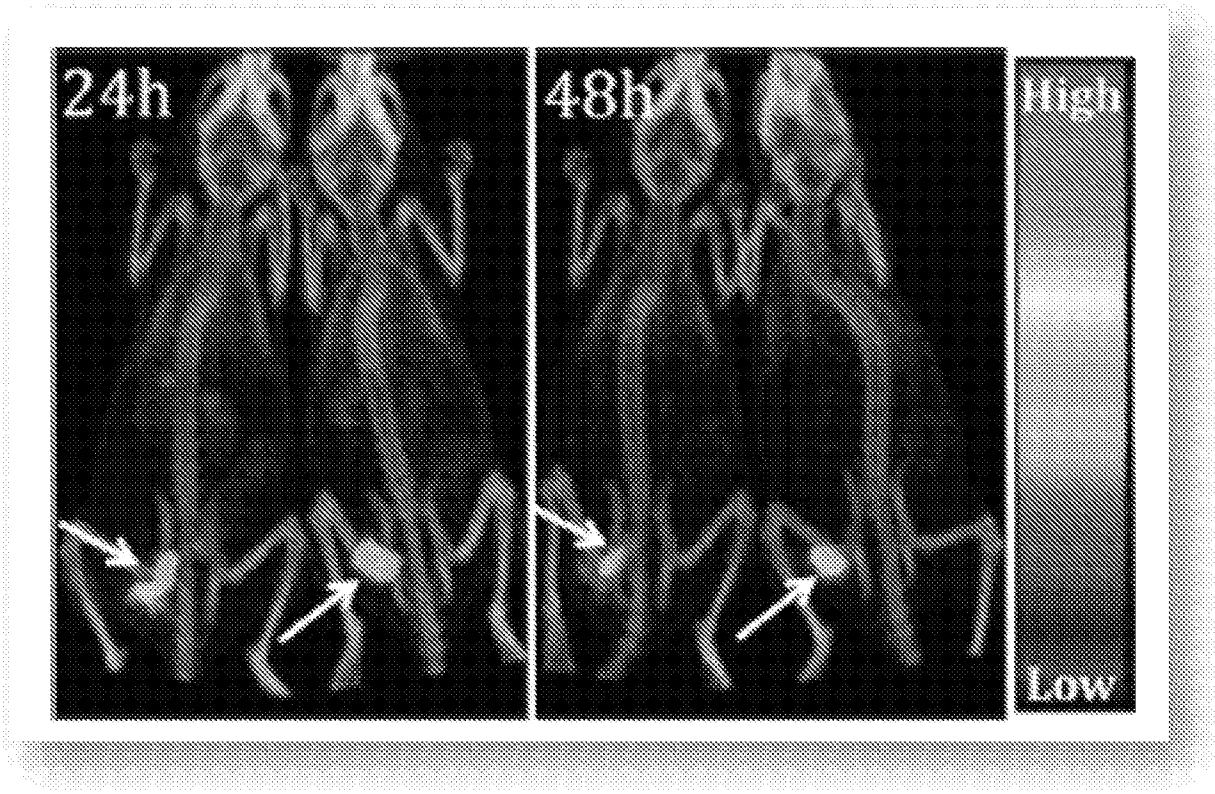

XYIMSR-01
$^{111}InCl_3$
0.5-M ascorbate (pH 5.6)
Heat for 30 min
[^{111}In]In-XYIMSR-01

Fig. 2



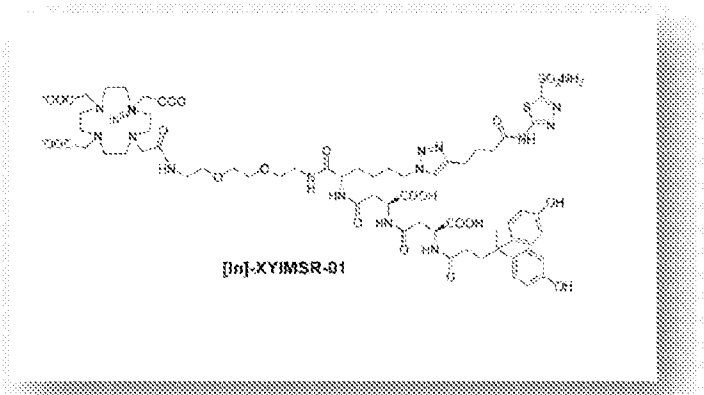


Fig. 3A

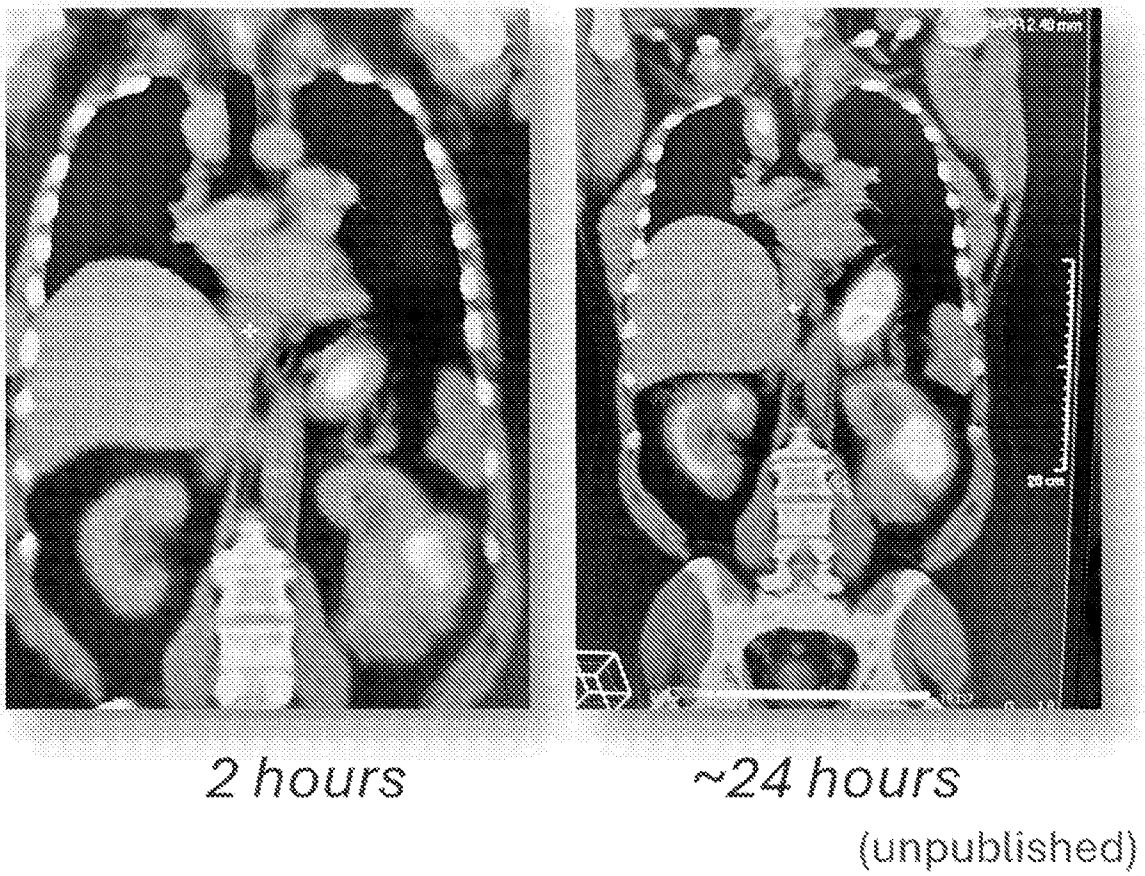


Fig. 3B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/056358

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***A61K 51/02*** (2024.01); ***C07D 417/14*** (2024.01); ***A61K 51/04*** (2024.01); *C07D 417/12* (2024.01)

CPC: ***A61K 51/02; C07D 417/14; A61K 51/04;*** *C07D 417/12*

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

See Search History Document

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.
A	US 2018/0133348 A1 (THE JOHNS HOPKINS UNIVERSITY) 17 May 2018 (17.05.2018) entire document	1-3, 5-6 and 8
A	US 2019/0192699 A1 (THE JOHNS HOPKINS UNIVERSITY) 27 June 2019 (27.06.2019) entire document	1-3, 5-6 and 8
X	Yang et al., "Imaging of carbonic anhydrase IX with an 111In-labeled dual-motif inhibitor, 16 September 2015 entire document especially page 33739	1-3, 5-6 and 8
X	Silva et al. "Process validation, cGMP production, dosimetry, and toxicity studies of the CAIX imaging agent [111In]XYIMSR-01 for phase I regulatory approval", J Labelled Comp Radiopharm. 2021 May 30; 64(6): 243–250. entire document	1-3, 5-6 and 8

☐ 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
"D" document cited by the applicant in the international application
"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
15 December 2024 (15.12.2024)	**21 January 2025 (21.01.2025)**

Name and mailing address of the ISA/US	Authorized officer
COMMISSIONER FOR PATENTS **MAIL STOP PCT, ATTN: ISA/US** **P.O. Box 1450** **Alexandria, VA 22313-1450** **UNITED STATES OF AMERICA**	**KARI RODRIQUEZ**
Facsimile No. **571-273-8300**	Telephone No. **PCT Help Desk: 571-272-4300**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/056358

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.: **4, 7 and 9**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).