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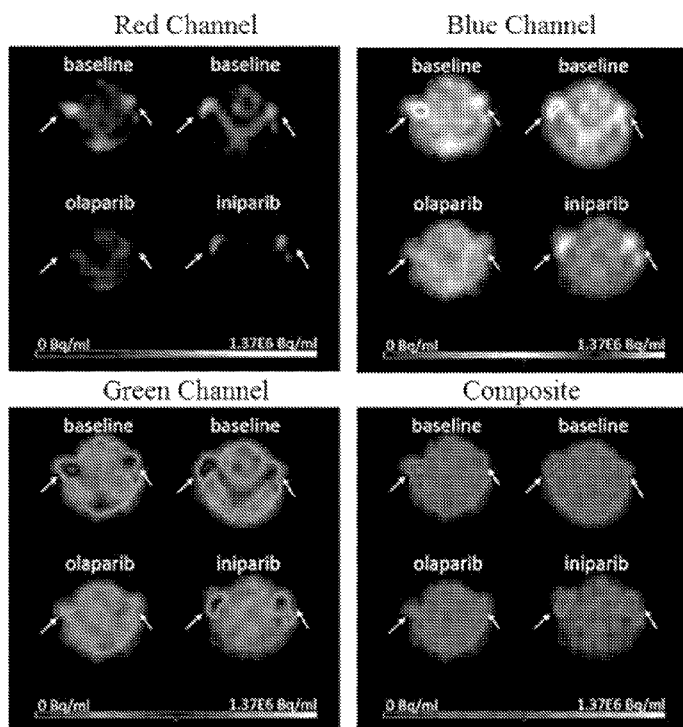
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[Continued on next page]

(54) Title: RADIOLABELED TRACERS FOR POLY (ADP-RIBOSE) POLYMERASE-1 (PARP-1), METHODS AND USES THEREFOR

FIG. 7



(57) Abstract: Disclosed are PARP-1 inhibitors, which can be ¹⁸F-labeled for use as tracers in positron emission tomographic (PET) imaging. Further disclosed are methods of synthesis. Of the compounds synthesized, 2-[p-(2-Fluoroethoxy)phenyl]-1.3.10-triazatricyclo[6.4.1.0^{4,13}]trideca-2,4(13),5,7-tetraen-9-one (12) had the highest inhibition potency for PARP-1 (IC₅₀= 6.3 nM). Synthesis of [¹⁸F]-12 is disclosed under conventional conditions in high specific activity with 40-50% decay-corrected yield. MicroPET imaging using [¹⁸F]-12 in MDA-MB-436 tumor-bearing mice demonstrated accumulation of [¹⁸F]-12 in a tumor. Binding can be blocked by olaparib. The compounds have utility for tumor imaging.



SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
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RADIOLABELED TRACERS FOR POLY (ADP-RIBOSE) POLYMERASE-1 (PARP-1), METHODS AND USES THEREFOR

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit of priority under 35 U.S.C. §119(e) to U.S. Provisional Application Serial No. 61/923,759 filed on January 5, 2014, which is incorporated by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with the support of Grants NIH P01 HL13851 and NIH R01 HL116389 from the National Institutes of Health. The government of the United States of America may have certain rights in this work.

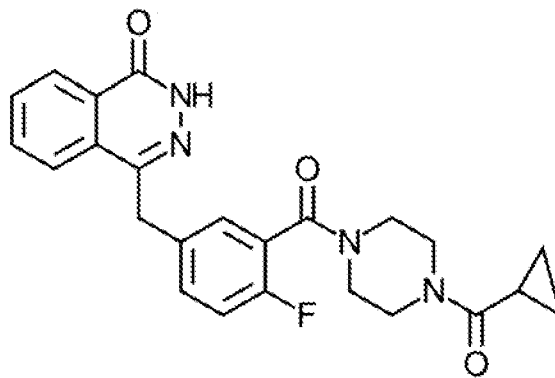
Technical Field

The present teachings are in the field of radiolabeled tracers for imaging PARP-1 distribution.

Introduction

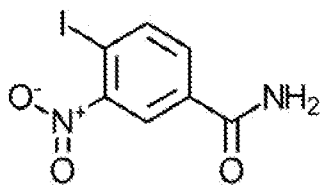
Poly (ADP-ribose) polymerase-1 (PARP-1) is one of the most abundant members of the PARP family of nuclear enzymes.¹ Its best characterized functions are sensing DNA damage and facilitating DNA repair, but PARP-1 also participates in many other DNA-related cellular processes, such as apoptosis regulation, cell division, differentiation, transcriptional regulation, and chromosome stabilization.²⁻⁵ PARP-1 may also play a central role in regulating inflammatory responses. PARP-1, a 113 kD protein, has three unique structural domains: the N-terminal DNA binding domain with two zinc fingers that specifically bind to damaged DNA strand breaks;¹⁻⁶ the central automodification domain; and the C-terminal catalytic domain that sequentially transfers ADP-ribose subunits from nicotinamide adenine dinucleotide (NAD⁺) to protein acceptors.⁷ Due to its critical role in DNA repair, PARP-1 has been actively pursued as a drug target over the past 20 years, with tremendous efforts invested to develop several generations of PARP-1 inhibitors (FIG. 1) for therapeutic purposes, especially in the area of ischemia-reperfusion injury and cancer.^{5,8} More recently, PARP1 inhibition has been demonstrated as an effective method for inducing synthetic lethality in cancers that depend on PARP1 activity for survival.⁹ Additionally, PARP inhibitors or the absence of PARP expression in transgenic mice reduces the degree of inflammation present and thus protects various organs, including the lungs, from the

detrimental effects of persistent inflammation. Therefore, a number of PARP inhibitors,



including olaparib (AZD2281), veliparib (ABT-888), rucaparib (PF-01367338, AG014699), and niraparib (MK4827), are now undergoing evaluation in clinical trials as anticancer drugs¹⁰⁻¹⁴. These PARP inhibitors effectively inhibit PARP1 activity as well as activity of other PARP-like enzymes such as PARP2 and tankyrase¹⁵. Other PARP inhibitors include benzimidazole carboxamide (NU1085)²¹ and its derivative (AG014361).²²

Despite the promising results from clinical trials related to progression-free survival, however, differences in the ability of the various PARP inhibitors to suppress tumoral PARP activity cannot be accurately determined by current assays. Additionally, the mechanism of



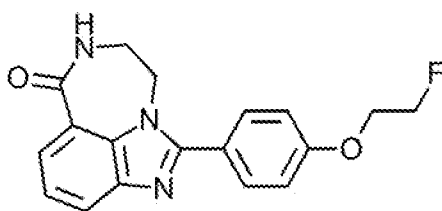
action for iniparib, initially developed as a PARP inhibitor, was more recently discovered to be most likely unrelated to inhibition of PARP activity¹⁶. Therefore, methods for quantifying tumoral PARP activity noninvasively *in vivo* are needed for demonstrating tumor-specific PARP inhibition as well as assessing the duration of effective PARP inhibition to guide dosing decisions.

Imaging with positron emission tomography (PET) could be an effective approach for noninvasively determining PARP activity levels. [¹³C]PJ-34, a PARP-1 targeted tracer, showed some potential in imaging PARP-1 expression in an animal model of diabetes.¹⁷ Recently, a ¹⁸F-labeled olaparib/AZD2281 derivative was synthesized by a two-step labeling strategy using a [4 + 2] cycloaddition between trans-cyclooctene and tetrazine and was used in *in vitro* cell studies and microPET tumor imaging.¹⁸⁻²⁰

SUMMARY

In various embodiments, the present teachings include radiolabeled PARP-1 inhibitors for measuring PARP-1 expression and imaging PARP-1 distribution in vivo with PET. In various embodiments, the present teachings include various compounds that bind PARP-1 and are labeled with a positron-emitting radionuclide such as ^{18}F . In various embodiments, methods of synthesis of the compounds are also disclosed. Also disclosed are the inhibition potencies against PARP-1 of compounds of the present teachings.

In some configurations, 2-[p-(2-Fluoroethoxy)phenyl]-1,3,10-triazatricyclo[6.4.1.0^{8,13}]trideca-2,4(13),5,7-tetraen-9-one



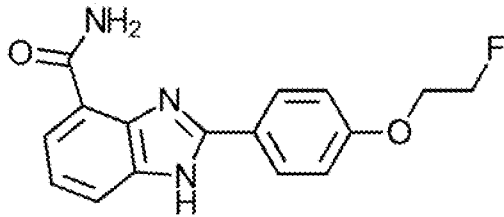
(**12**) ($\text{IC}_{50} = 6.3 \pm 1.3 \text{ nM}$) can be labeled with ^{18}F . In

various configurations, the ^{18}F labeling can be in one step and can provide high chemical and radiochemical purities. In some configurations, microPET imaging can be used to demonstrate increased uptake of [^{18}F]**12** in MDA-MB-436 tumors. In some configurations, this increased uptake can be blocked by both **12** and olaparib/AZD2281.

In various embodiments, a PARP-1 inhibitor **12** ($\text{IC}_{50} = 6.3 \text{ nM}$) has been developed. [^{18}F]**12** can be synthesized using conventional labeling methods with high purities and specific activity. MicroPET studies of [^{18}F]**12** in MBA-MD-436 tumor-bearing mice demonstrated accumulation of the radioactivity in these tumors which are known to overexpress PARP-1. Tumor uptake can be blocked by PARP-1 inhibitor olaparib and by **12**, supporting the specificity of [^{18}F]**12** uptake for PARP-1 activity. [^{18}F]**12** can thus be useful as a PET tracer for imaging PARP-1 expression in vivo.

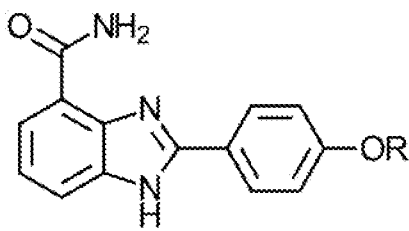
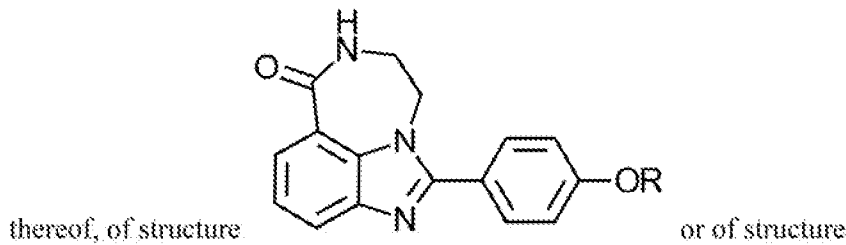
In various embodiments, incorporation of the radionuclide can be achieved either through a fluoroethoxy group via nucleophilic substitution²⁸ with [^{18}F]fluoride, or via Cu(I) catalyzed click reaction using 2-[^{18}F]fluoroethyl azide.^{29,30} The corresponding 2-fluoroethoxy and 2-fluoroethyl triazole analogs of NU1085 and AG014361 can be synthesized according to Schemes 1 and 2, respectively, in only a few steps. Although the propargyl and triazole groups slightly reduced the inhibition potency of the analogs, the most potent of these were

12 (IC₅₀ = 6.3 nM), with a 2-fluoroethoxy group, followed by

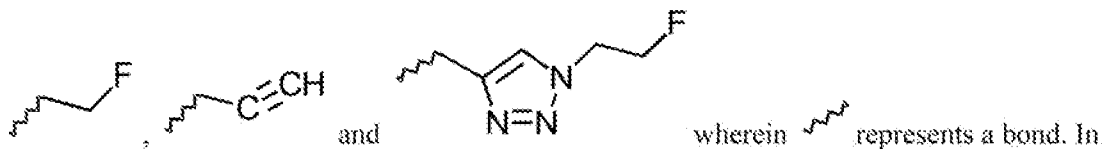



In some configurations, the one-step labeling of [¹⁸F]**12** under typical conditions can be automated for clinical production of this tracer. In some configurations, the final dose for injection can have high chemical and radiochemical purities and can have excellent specific activity. In various configurations, the injected mass of [¹⁸F]**12** in the microPET studies can be 0.0037-0.012 μg/dose (0.2 mCi/dose), which can be much lower than the blocking dose and therapeutic doses. This amount of mass can be unlikely to have a pharmacological effect. [¹⁸F]**12** can thus be useful as a PET tracer for imaging PARP-1 expression and tumors. In some configurations, a PARP-1 tracer such as [¹⁸F]**12** can also be useful to identify patients with chronic inflammatory diseases in whom PARP inhibitor treatments could potentially retard the progression of the inflammatory disease.

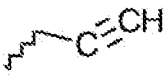
The present teachings thus include compounds and pharmaceutically acceptable salts

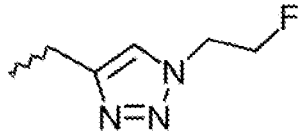


, wherein R can be selected from the group consisting of

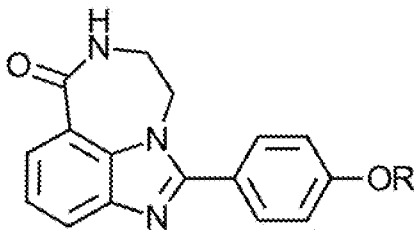


some configurations, R can be  . In some configurations, the F can be an ¹⁸F. In

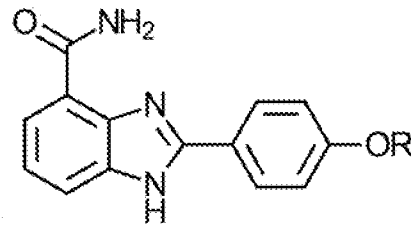
some configurations, R can be . In some configurations, R can be


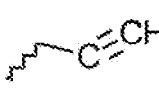


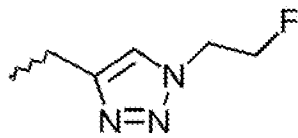
In some embodiments, the present teachings include methods of imaging a tumor in a subject, as well as methods of imaging inflammation in a subject. In various configurations, these methods can comprise administering to a subject a compound of structure




or of structure



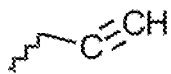
wherein R can be selected from the group consisting of ,  and



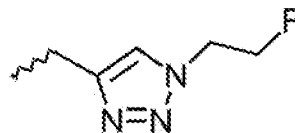
wherein  represents a bond. In some configurations, R can be



. In some configurations, the F can be an ¹⁸F. In some configurations, R can be



. In some configurations, R can be



, wherein the

compound comprises a positron-emitting radionuclide such as ¹⁸F. The methods can further include subjecting the subject PET scanning.

In various configurations of the present teachings, a subject can be a human having, or suspected of having, a tumor or inflammation. A subject can also be an animal having, or suspected of having, a tumor or inflammation. The animal can be a mammal, for example a companion animal such as, for example, a cat or dog, a laboratory animal such as, for example, a mouse, guinea pig, rabbit or rat, or a large animal, such as, for example, horse, a sheep, a cow, or a goat.

Brief description of the drawings.

FIG. 1 illustrates examples of PARP-1 inhibitors (prior art).

FIG. 2 illustrates analytical HPLC of [¹⁸F]**12** (WC-4-138), showing high chemical and radiochemical purities. (Top: UV, Bottom: radioactivity; specific activity = 11,500 mCi/μmol).

FIG. 3A-D illustrate microPET images of MDA-MB-231 and MDA-MB-436 tumors in mice at 60 min using [¹⁸F]**12** under baseline conditions and blocked with olaparib (i.p. 50 mg/kg 20 min pretreatment) and **12** (i.p. 1 mg/kg 20 min pretreatment). Top row: MDA-MB-231 tumor before and after treatment with olaparib (i.p. 50 mg/kg 20 min pretreatment). Bottom row: MDA-MB-436 (right) and MDA-MB-231 (left) tumors before and after **12** (i.p. 1 mg/kg 20 min pretreatment). FIG. 3A, FIG. 3B and FIG. 3C represent red, green, and blue RGB channels, respectively, each rendered in black-and-white. FIG. 3D represents a composite of RGB channels rendered in black-and-white.

FIG. 4 illustrates time-radioactivity curve of [¹⁸F]**12** in MDA-MB-436 tumors in mice under baseline conditions and blocked with olaparib (i.p. 50 mg/kg 20 min pretreatment) and **12** (i.p. 1 mg/kg 20 min pretreatment, top graph). The olaparib treated MDA-MB-231 tumor also demonstrated decreased [¹⁸F]**12** uptake (bottom graph).

FIG. 5 illustrates analysis of [¹⁸F]WC-4-138 (**12**) uptake and PARP activity in cancer cell lines.

FIG. 6 illustrates metabolic stability of the [¹⁸F]WC-4-138 tracer as evaluated in adult mice.

FIG. 7 illustrates MDA/MB-231 (breast cancer) tumor-bearing mice underwent dynamic imaging for 60 minutes after [¹⁸F]WC-4-138 tracer injection.

FIG. 8A-B illustrates MDA/MB-231 (breast cancer) or SCC1 (HNSCC) tumor-bearing mice that underwent dynamic imaging for 60 minutes after [¹⁸F]WC-4-138 tracer injection.

Detailed description

The present teachings in various embodiments include tracers that can be used for imaging of tumors or of inflammation by PET scanning. In various aspects, the tracers can bind PARP-1 with high affinity. In various embodiments, the present teachings also include methods of synthesis of the tracers. Tracers of the present teachings, when labelled with a positron-emitting radioisotope such as ¹⁸F, can be administered to a subject by intravenous or other suitable means.

The methods described herein utilize laboratory techniques well known to skilled artisans, and guidance can be found in laboratory manuals and textbooks such as Sambrook, J., et al., *Molecular Cloning: A Laboratory Manual*, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2001; Spector, D. L. et al., *Cells: A Laboratory Manual*, Cold

Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1998; Hedrickson et al., Organic Chemistry 3rd edition, McGraw Hill, New York, 1970; Carruthers, W., and Coldham, L., Modern Methods of Organic Synthesis (4th Edition), Cambridge University Press, Cambridge, U.K., 2004; Curati, W.L., Imaging in Oncology, Cambridge University Press, Cambridge, U.K., 1998; Welch, M.J., and Redvanly, C.S., eds. Handbook of Radiopharmaceuticals: Radiochemistry and Applications, J. Wiley, New York, 2003. Methods of administration of pharmaceuticals and dosage regimes, can be determined according to standard principles of pharmacology well known skilled artisans, using methods provided by standard reference texts such as Remington: the Science and Practice of Pharmacy (Alfonso R. Gennaro ed. 19th ed. 1995); Hardman, J.G., et al., Goodman & Gilman's The Pharmacological Basis of Therapeutics, Ninth Edition, McGraw-Hill, 1996; and Rowe, R.C., et al., Handbook of Pharmaceutical Excipients, Fourth Edition, Pharmaceutical Press, 2003.

In the experiments described herein, reagents and materials were purchased from commercial suppliers and used without further purification unless otherwise stated. Chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO USA) unless otherwise specified. All reactions were carried out by standard air-free and moisture-free techniques under an inert nitrogen atmosphere with dry solvents unless otherwise stated.

Flash column chromatography can be conducted using a variety of methods and instruments including Scientific Adsorbents, Inc. silica gel, 60A, "40 Micron Flash" (32-63 μm). Melting points can be determined using a variety of methods and instruments well known in the art, including the MEL-TEMP 3.0 apparatus. In some configurations, melting point data is uncorrected. ^1H and ^{13}C NMR spectra at 300 MHz can be recorded through a variety of routine methods on a variety of instruments, including a Varian Mercury-VX spectrometer. In some configurations, chemical shifts can be reported as parts per million (ppm) downfield from tetramethylsilane (TMS). All coupling constants (J) are given in Hertz (Hz). Splitting patterns are typically described as follows: s, singlet; d, doublet; t, triplet; m, multiplet.

Elemental analysis (C, H, N) can be determined by a variety of commercial contract organizations such as Atlantic Microlab, Inc., Norcross, GA. High performance liquid chromatography (HPLC) can be performed with an ultraviolet detector and a well-scintillation NaI (TI) detector and associated electronics for radioactivity detection. A Grace Altima C18 250 \times 10 mm 10 μ semi-preparative column (A) and an Altima C18 250 \times 4.6 mm 10 μ analytical column (B) can be used for preparation and analysis respectively.

[¹⁸F]Fluoride can be produced by the ¹⁸O(p,n)¹⁸F reaction through proton irradiation of enriched (95%) [¹⁸O] water in the RDS111 cyclotron. Radio-TLC can be accomplished using a Bioscan AR-2000 imaging scanner (Bioscan, Inc., Washington, DC). Published methods were used for the synthesis of compound **5**²⁷ and **11**²². All animal experiments were conducted under Washington University Animal Studies Committee IACUC-approved protocols in accordance with the recommendations of the National Research Council's 'Guide for the Care and Use of Laboratory Animals'.

EXAMPLES

The present teachings including descriptions provided in the Examples that are not intended to limit the scope of any claim. Unless specifically presented in the past tense, an example can be a prophetic or an actual example. The following non-limiting examples are provided to further illustrate the present teachings. Those of skill in the art, in light of the present disclosure, will appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the present teachings.

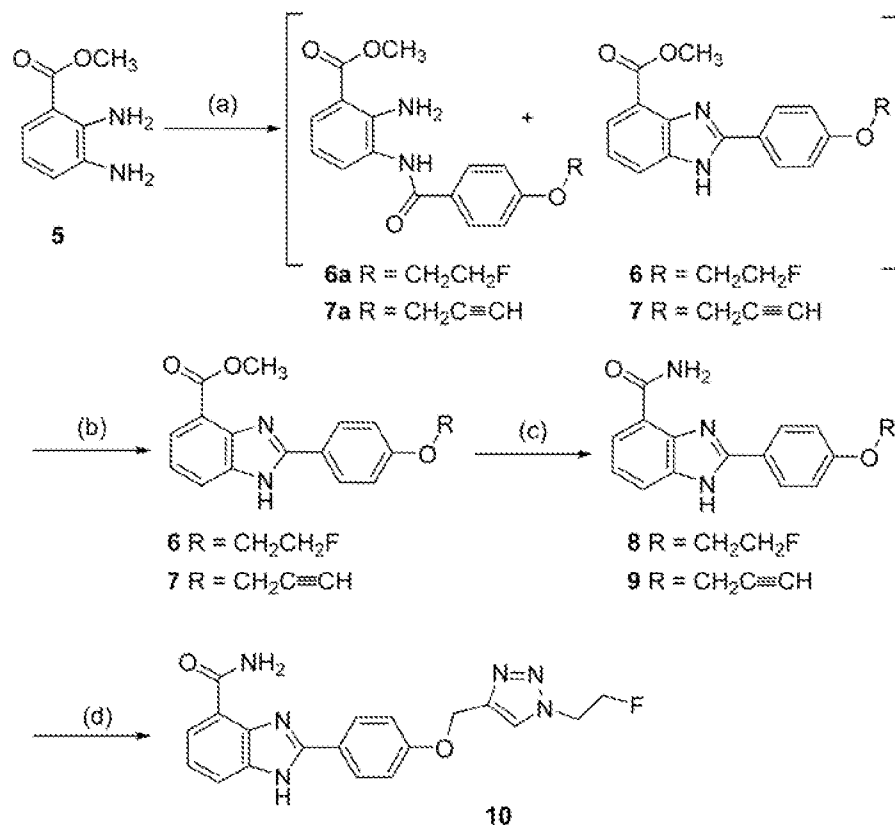
Example 1

This example illustrates synthesis of PARP-1 inhibitors. Compound numbers refer to Schemes 1 and 2, *infra*.

The syntheses of PARP-1 inhibitors of the present teachings are shown in Schemes 1 and 2. Methyl 2,3-diaminobenzate (**5**) was reacted with 4-(2-fluoroethoxy)benzoyl chloride in pyridine and dichloromethane to afford a mixture of intermediate **6a** and the benzimidazole compound **6**. After evaporation of the solvent, the mixture was refluxed with methanesulfonic acid in methanol to give **6**. Then the methyl ester of **6** was converted to the amide compound **8** using ammonium in methanol. Similarly, the alkyne analog **9** was synthesized starting from **5** and 4-(prop-2-ynyloxy)benzoyl chloride. The triazole compound **10** was prepared by the copper(I) catalyzed click reaction of 2-fluoroethylazide and **9** using CuSO₄·5H₂O and sodium ascorbate in DMF.

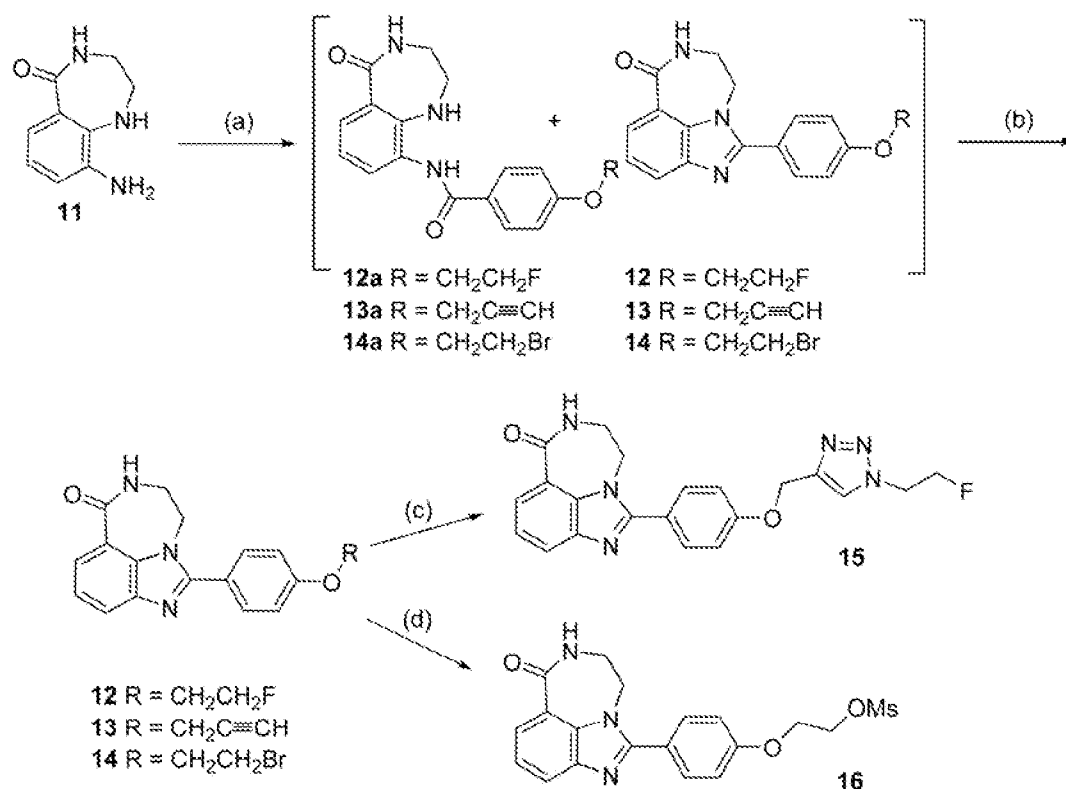
The tricycle compounds were synthesized from the diamine intermediate **11**. Compound **11** was reacted with 4-(2-fluoroethoxy)benzoyl chloride in pyridine and dichloromethane to afford a mixture of intermediate **12a** and the benzimidazole **12**. After evaporation of the solvent, the mixture was refluxed with methanesulfonic acid in methanol to give **12**. Similarly, **13** and **14** were made from the corresponding benzyl chlorides. Compound **15** was prepared by the click reaction under the same condition as for **10** using 2-fluoroethylazide.

and 13. The mesylate precursor 16 for the labeling of 12 with ^{18}F was synthesized by reflux of 14 and silver methanesulfonate in acetonitrile.



Scheme 1. Synthesis of derivatives of NU1085 (2)

Reagents and conditions: (a) $\text{ROC}_6\text{H}_4\text{COCl}$ (R = CH₂CH₂F for 6a and 6, R = CH₂C≡CH for 7a and 7) pyridine, CH₂Cl₂; (b) CH₃SO₃H, MeOH; (c) NH₃, MeOH; (d) 9, FCH₂CH₂N₃, CuSO₄, sodium ascorbate, DMF.

Scheme 2. Synthesis of derivatives of AG014361 (**3**)

Reagents and conditions: (a) $\text{ROC}_6\text{H}_4\text{COCl}$ (R = CH₂CH₂F for **12a** and **12**, R = CH₂C≡CH for **13a** and **13**, R = CH₂CH₂Br for **14a** and **14**), pyridine, CH₂Cl₂; (b) CH₃SO₃H, MeOH; (c) **13**, FCH₂CH₂N₃, CuSO₄, sodium ascorbate, DMF; (d) **14**, AgOMs, acetonitrile.

Example 2

This example illustrates the synthesis of Methyl 2-(4-(2-fluoroethoxy)phenyl)-1H-benzof[d]imidazole-4-carboxylate (**6**). Compound numbers refer to Schemes 1 and 2, supra.

A mixture of methyl 2,3-diaminobenzoate **5** (500 mg, 3 mmol) and 4-(2-fluoroethoxy)benzoyl chloride (638 mg, 3.15 mmol) in CH₂Cl₂ (10 mL) and pyridine (10 mL) was stirred overnight at 23 °C. After removal of the solvent under reduced pressure, the residue was dissolved in methanol (50 mL), and followed by addition of CH₃SO₃H (1 mL). After the mixture was refluxed for 3 h, methanol was removed under reduced pressure, and the residue was dissolved in ethyl acetate (75 mL). The solution was washed with saturated Na₂CO₃ (50 mL), water (50 mL) and saturated NaCl (50 mL), and dried over Na₂SO₄. After evaporation of the solvent, the crude product was purified by silica gel column chromatography eluting with hexane-ethyl acetate (1 : 1) to afford **6** as white solid (686 mg, 73 %), mp 134.2-134.6 °C. ¹H NMR (300 MHz, CDCl₃) δ 10.58 (s, 1H), 7.98 (d, J = 8.7 Hz, 2H), 7.95 (d, J = 8.7 Hz, 1H), 7.83 (d, J = 7.2 Hz, 1H), 7.26 (t, J = 7.8 Hz, 1H), 6.98 (d, J =

9.0 Hz, 2H), 4.75 (dt, $J = 47.1$ Hz, 4.2 Hz, 2H), 4.22 (dt, $J = 27.6$ Hz, 4.2 Hz, 2H), 3.96 (s, 3H). ^{13}C NMR (75 MHz, CDCl_3) δ 167.0, 160.2, 152.3, 144.8, 135.0, 128.2, 124.4, 124.2, 122.3, 121.7, 114.9, 113.0, 81.6 (d, $J = 169.7$ Hz), 67.1 (d, $J = 20.6$ Hz), 52.0.

Example 3

This example illustrates the synthesis of Methyl 2-(4-(prop-2-ynyloxy)phenyl)-1H-benzo[d]imidazole-4-carboxylate (**7**). Compound numbers refer to Schemes 1 and 2, supra.

Compound **7** was prepared according to the same procedure for compound **6** (Example 2), except using compound **5** (500 mg, 3 mmol) and 4-(prop-2-ynyloxy)benzoyl chloride (613 mg, 3.15 mmol) as starting materials. The crude product was purified by silica gel column chromatography eluting with hexane-ethyl acetate (1 : 1) to afford **7** as white solid (724 mg, 79 %), mp 176.0-176.8 °C. ^1H NMR (300 MHz, CDCl_3) δ 10.58, 8.03 (d, $J = 9.0$ Hz, 2H), 7.98 (d, $J = 8.1$ Hz, 1H), 7.86 (d, $J = 7.8$ Hz, 1H), 7.29 (t, $J = 8.1$ Hz, 1H), 7.10 (d, $J = 9.0$ Hz, 2H), 4.76 (d, $J = 2.4$ Hz, 2H), 4.00 (s, 3H), 2.57 (t, $J = 2.4$ Hz, 1H). ^{13}C NMR (75 MHz, CDCl_3) δ 167.1, 159.4, 152.4, 144.9, 135.1, 128.2, 124.6, 124.3, 122.8, 121.8, 115.4, 113.0, 77.9, 76.0, 55.8, 52.1.

Example 4

This example illustrates the synthesis of 2-(4-(2-Fluoroethoxy)phenyl)-1H-benzo[d]imidazole-4-carboxamide (**8**). Compound numbers refer to Schemes 1 and 2, supra.

A solution of Compound **6** (315 mg, 1 mmol) in 7 N NH_3 in methanol (10 mL) was stirred for 3 days at 23 °C. After evaporation of the solvent, the crude product was purified by silica gel column chromatography eluting with hexane-ethyl acetate (1 : 2) to afford **8** as white solid (245 mg, 82 %), mp 195.8-197.4 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 9.44 (s, 1H), 8.23 (d, $J = 9.0$ Hz, 2H), 7.89 (d, $J = 7.5$ Hz, 1H), 7.80 (s, 2H), 7.74 (d, $J = 7.8$ Hz, 1H), 7.34 (t, $J = 7.5$ Hz, 1H), 7.20 (d, $J = 8.4$ Hz, 2H), 4.81 (dt, $J = 47.7$ Hz, 3.6 Hz, 2H), 4.37 (dt, $J = 30.0$ Hz, 3.9 Hz, 2H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 166.4, 160.1, 152.0, 135.5, 128.6, 122.7, 122.0, 115.1, 82.1 (d, $J = 166.2$ Hz), 67.3 (d, $J = 19.4$ Hz). Anal. Calcd for $\text{C}_{16}\text{H}_{14}\text{FN}_3\text{O}_2 \cdot 0.5\text{H}_2\text{O}$: C, 62.33; H, 4.90; N, 13.63. Found: C, 62.54; H, 4.87; N, 13.67.

Example 5

This example illustrates the synthesis of 2-(4-(Prop-2-ynyloxy)phenyl)-1H-benzo[d]imidazole-4-carboxamide (**9**). Compound numbers refer to Schemes 1 and 2, supra.

Compound **9** was prepared according to the same procedure for compound **8**, except using compound **7** (460 mg, 1.5 mmol) as starting material. The crude product was purified by silica gel column chromatography eluting with hexane-ethyl acetate (1 : 2) to afford **9** as white solid (378 mg, 86 %), mp 183.4-183.9 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 9.39 (s,

1H), 8.21 (d, $J = 8.4$ Hz, 2H), 7.87 (d, $J = 7.8$ Hz, 1H), 7.78 (s, 2H), 7.72 (d, $J = 7.8$ Hz, 1H), 7.32 (t, $J = 7.8$ Hz, 1H), 7.20 (d, $J = 9.0$ Hz, 2H), 4.92 (d, $J = 1.8$ Hz, 2H), 3.63 (s, 1H). ^{13}C NMR (75 MHz, DMSO- d_6) δ 166.4, 159.0, 152.0, 128.5, 122.7, 122.3, 121.9, 115.4, 78.9, 78.6, 55.7. Anal. Calcd for $\text{C}_{17}\text{H}_{18}\text{N}_3\text{O}_2 \cdot 0.5\text{H}_2\text{O}$: C, 67.99; H, 4.70; N, 13.99. Found: C, 67.97; H, 4.72; N, 13.71.

Example 6

This example illustrates the synthesis of 2-(4-((1-(2-Fluoroethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[d]imidazole-4-carboxamide (**10**). Compound numbers refer to Schemes 1 and 2, supra.

A mixture of **9** (291 mg, 1.0 mmol), 1-azido-2-fluoroethane (1.68 mmol), sodium ascorbate (990 mg, 5.0 mmol), and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (125 mg, 0.5 mmol) in DMF (10 mL) was stirred overnight at 23 °C. The reaction mixture was diluted with ethyl acetate (75 mL), and washed with water (2×50 mL), and saturated NaCl (50 mL), dried over Na_2SO_4 . After evaporation of the solvent, the crude product was purified by silica gel column chromatography eluting with ethyl acetate to afford **10** as white solid (255 mg, 67%), mp 256.4-257.3 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 9.42 (s, 1H), 8.33 (s, 1H), 8.21 (d, $J = 8.7$ Hz, 2H), 7.87 (d, $J = 7.5$ Hz, 1H), 7.78 (s, 2H), 7.71 (d, $J = 7.8$ Hz, 1H), 7.32 (t, $J = 7.8$ Hz, 1H), 7.27 (d, $J = 8.7$ Hz, 2H), 5.28 (s, 2H), 4.85 (dt, $J = 46.8$ Hz, 4.5 Hz, 2H), 4.76 (dt, $J = 27.6$ Hz, 4.2 Hz, 2H). ^{13}C NMR (75 MHz, DMSO- d_6) δ 166.3, 159.9, 152.0, 142.5, 141.6, 135.3, 128.6, 125.1, 122.7, 122.1, 121.9, 115.3, 114.7, 99.5, 81.9 (d, $J = 167.3$ Hz), 61.3, 50.1 (d, $J = 20.5$ Hz). Anal. Calcd for $\text{C}_{19}\text{H}_{17}\text{FN}_6\text{O}_2$: C, 59.99; H, 4.50; N, 22.09. Found: C, 60.10; H, 4.67; N, 21.49.

Example 7

This example illustrates the synthesis of 5,6-Dihydro-2-(4-(2-fluoroethoxy)phenyl)-imidazo[4,5,1-jk][1,4]benzodiazepin-7(4H)-one (**12**). Compound numbers refer to Schemes 1 and 2, supra.

Compound **12** (WC-4-138) was prepared according to the same procedure for compound **6**, except using compound **11** (177 mg, 1 mmol) and 4-(2-fluoroethoxy)benzoyl chloride (213 mg, 1.05 mmol) as starting materials. The crude product was purified by silica gel column chromatography eluting with ethyl acetate-methanol (10: 1) to afford **12** as white solid (247 mg, 76%), mp 236.0-237.5 °C. ^1H NMR (300 MHz, DMSO- d_6) δ 8.44 (t, $J = 5.1$ Hz, 1H), 7.89-7.80 (m, 4H), 7.34 (t, $J = 7.8$ Hz, 1H), 7.17 (d, $J = 8.7$ Hz, 2H), 4.79 (dt, $J = 48.9$ Hz, 3.6 Hz, 2H), 4.44 (m, 2H), 4.35 (dt, $J = 31.2$ Hz, 3.9 Hz, 2H), 3.53 (m, 2H). ^{13}C NMR (75 MHz, DMSO- d_6) δ 167.8, 159.9, 154.1, 143.7, 132.9, 131.7, 125.5, 123.1, 122.5, 121.9, 118.1,

115.1, 82.5 (d, $J = 165.0$ Hz), 67.7 (d, $J = 19.3$ Hz), 50.9, 40.8. Anal. Calcd for $C_{13}H_{16}FN_3O_2$: C, 66.45; H, 4.96; N, 12.92. Found: C, 66.43; H, 5.03; N, 12.92.

Example 8

This example illustrates the synthesis of 5,6-Dihydro-2-(4-(prop-2-ynyloxy)phenyl)-imidazo[4,5,1-jk][1,4]benzodiazepin-7(4H)-one (**13**). Compound numbers refer to Schemes 1 and 2, *supra*.

Compound **13** was prepared according to the same procedure for compound **6**, except using compound **11** (177 mg, 1 mmol) and 4-(prop-2-ynyloxy)benzoyl chloride (204 mg, 1.05 mmol) as starting materials. The crude product was purified by silica gel column chromatography eluting with ethyl acetate-methanol (10: 1) to afford **13** as white solid (268 mg, 84 %), mp 258.0-259.1 °C. 1H NMR (300 MHz, DMSO- d_6) δ 8.47 (s, 1H), 7.85 (m, 4H), 7.34 (t, $J = 7.8$ Hz, 1H), 7.18 (d, $J = 6.9$ Hz, 2H), 4.93 (s, 2H), 4.46 (m, 2H), 3.64 (s, 1H), 3.54 (m, 2H). ^{13}C NMR (75 MHz, DMSO- d_6) δ 167.4, 158.5, 153.6, 143.3, 132.5, 131.1, 125.1, 122.7, 122.4, 121.5, 117.7, 115.0, 79.0, 78.5, 55.6, 50.5. Anal. Calcd for $C_{19}H_{15}N_3O_2$: C, 71.91; H, 4.76; N, 13.24. Found: C, 71.71; H, 4.82; N, 12.98.

Example 9

This example illustrates the synthesis of 2-(4-(2-Bromoethoxy)phenyl)-5,6-dihydro-imidazo[4,5,1-jk][1,4]benzodiazepin-7(4H)-one (**14**). Compound numbers refer to Schemes 1 and 2, *supra*.

Compound **14** was prepared according to the same procedure for compound **6**, except using compound **11** (177 mg, 1 mmol) and 4-(2-bromoethoxy)benzoyl chloride (277 mg, 1.05 mmol) as starting materials. The crude product was purified by silica gel column chromatography eluting with ethyl acetate-methanol (10: 1) to afford **14** as white solid (255 mg, 66 %), mp decomposed 280 °C. 1H NMR (400 MHz, DMSO- d_6) δ 8.44 (t, $J = 5.6$ Hz, 1H), 7.87 (d, $J = 8.0$ Hz, 1H), 7.85 (d, $J = 8.0$ Hz, 1H), 7.82 (d, $J = 8.4$ Hz, 2H), 7.34 (t, $J = 7.6$ Hz, 1H), 7.16 (d, $J = 8.4$ Hz, 2H), 4.44 (m, 4H), 3.86 (t, $J = 5.2$ Hz, 2H), 3.53 (m, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 167.8, 159.7, 154.1, 143.7, 132.9, 131.7, 125.5, 123.1, 122.6, 122.0, 118.1, 115.2, 68.4, 50.5, 40.8.

Example 10

This example illustrates the synthesis of 5,6-Dihydro-2-(4-((1-(2-fluoroethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-imidazo[4,5,1-jk][1,4]benzodiazepin-7(4H)-one (**15**).

Compound numbers refer to Schemes 1 and 2, *supra*.

Compound **15** was prepared according to the same procedure for compound **10**, except using compound **13** (159 mg, 0.5 mmol) as starting material. The crude product was purified

by silica gel column chromatography eluting with ethyl acetate-methanol (10: 1) to afford **15** as white solid (147 mg, 72 %), mp 226.5-227.6 °C. ¹H NMR (300 MHz, DMSO-d₆) δ 8.46 (t, J = 5.7 Hz, 1H), 8.33 (s, 1H), 7.89-7.81 (m, 4H), 7.34 (t, J = 7.8 Hz, 1H), 7.25 (d, J = 8.4 Hz, 2H), 5.28 (s, 2H), 4.85 (dt, J = 47.1 Hz, 4.2 Hz, 2H), 4.76 (dt, J = 27.6 Hz, 4.2 Hz, 2H), 4.45 (m, 2H), 3.54 (m, 2H). ¹³C NMR (75 MHz, DMSO-d₆) δ 167.4, 159.4, 143.3, 142.6, 131.2, 125.1, 125.0, 122.7, 122.0, 121.5, 117.7, 114.8, 81.9 (d, J = 167.3 Hz), 61.2, 50.5, 50.1 (d, J = 20.5 Hz), 40.4. Anal. Calcd for C₂₁H₁₉FN₆O₂·1.5H₂O: C, 58.19; H, 5.12; N, 19.39. Found: C, 57.89; H, 4.54; N, 18.92

Example 11

This example illustrates the synthesis of 5,6-Dihydro-2-(4-(2-(methylsulfonyloxy)ethoxy)phenyl)-imidazo[4,5,1-jk][1,4]benzodiazepin-7(4H)-one (**16**). Compound numbers refer to Schemes 1 and 2, supra.

A mixture of **14** (193 mg, 0.5 mmol) and AgOMs (508 mg, 2.5 mmol) was refluxed for 8 h. After evaporation of the solvent, the crude product was purified by silica gel column chromatography eluting with ethyl acetate-methanol (10 : 1) to afford **16** as white solid (129 mg, 64 %), mp 253.2-254.1 °C. ¹H NMR (400 MHz, CD₃OD) δ 7.97 (d, J = 8.0 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.76 (d, J = 8.8 Hz, 2H), 7.40 (t, J = 8.0 Hz, 1H), 7.17 (d, J = 8.8 Hz, 2H), 4.59 (t, J = 4.0 Hz, 2H), 4.49 (t, J = 4.0 Hz, 2H), 4.35 (t, J = 4.0 Hz, 2H), 3.65 (m, 2H), 3.12 (s, 3H). ¹³C NMR (100 MHz, CD₃OD) δ 160.2, 148.1, 142.7, 135.7, 132.2, 131.1, 131.0, 125.8, 122.6, 122.1, 121.4, 116.9, 114.6, 68.3, 66.0, 50.5, 40.6, 36.0.

Example 12

This example illustrates PARP-1 activity assays.

Newly synthesized PARP-1 inhibitors were assessed for their ability to inhibit active PARP-1 using the method described by Putt and Hergenrother.²³ The results are shown in Table 1. The tricycle benzimidazole compounds had higher inhibition potency than their respective benzimidazole analogs (e.g., **12** vs. **8**, **15** vs. **10**). In both benzimidazole and tricycle benzimidazole analogs, the analogs with fluoroethoxy substituent had three times higher inhibition potencies than the respective analogs with fluoroethyl triazole group (e.g., **8** vs. **10**, **12** vs. **15**). Therefore, the most potent inhibitor, **12**, was selected for ¹⁸F-labeling.

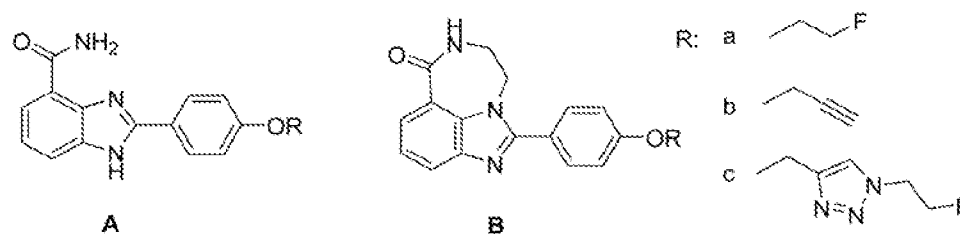


Table 1. Inhibition efficiency of PARP-1 inhibitors

Compound	Structure	R	IC ₅₀ (nM)
1 PJ34	/	/	170.2 ± 8.3 ^a
8	A	a	10.8 ± 0.4
9	A	b	25.8 ± 3.3
10	A	c	30.3 ± 5.6
12	B	a	6.3 ± 1.3
13	B	b	18.7 ± 2.7
15	B	c	22.1 ± 6.3

a. Reported values: IC₅₀ = 20 nM, EC₅₀ = 35 nM.^{1,24}

Example 13

This example illustrates a PARP-1 enzymatic activity assay.

This assay is based on the chemical quantification of NAD⁺, i.e. the amount of NAD⁺ consumed when the active PARP-1 C-terminal catalytic domain sequentially transfers ADP-ribose subunits from nicotinamide adenine dinucleotide (NAD⁺) to protein acceptors.⁷

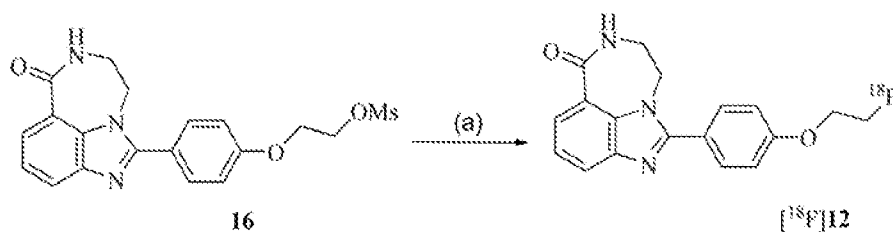
High-specific-activity PARP-1 and activated DNA were purchased from Trevigen (Gaithersburg, MD). All other reagents required for this assay including NAD⁺ were purchased from Sigma-Aldrich (St. Louis, MO). Known PARP-1 inhibitor PJ-34, used as a control in these experiments, was synthesized in-house. To test the compounds for PARP-1 inhibition, a solution of 250 nM NAD⁺ was first made in 50 mM Tris-HCl, 2 mM MgCl₂, at pH 8.0 (PARP assay buffer) and 20 μL transferred to each well of a 96-well black fluorescence plate. A solution of 50 μg/mL of activated DNA was made in PARP assay buffer and 10 μL was added to each well. Stock solutions of test compounds were first prepared in DMSO, diluted to varying concentrations in PARP assay buffer, and 10 μL was added to each well. To initiate the reaction, 10 μL of 10 μg/mL PARP-1 enzyme in PARP assay buffer was added to each well. The total volume was 50 μL, bringing the final concentrations to 2 μg/mL PARP-1 enzyme, 10 μg/mL activated DNA, and 100 nM NAD⁺ per well. The plate was then incubated at room temperature for 20 min. The amount of NAD⁺

present was then determined by first adding 20 μL 2 M KOH, followed by 20 μL of 20 % acetophenone (in ethanol) to each well. The plate was allowed to incubate at 4 $^{\circ}\text{C}$ for 10 min. Then 90 μL of 88 % formic acid was added, resulting in a final concentration of 222 mM KOH, 2.2% acetophenone, 44% formic acid, and varying concentrations of NAD^+ . The plate was incubated at 100 $^{\circ}\text{C}$ for 5 min., allowed to cool, and then read on a Perkin Elmer Victor microplate fluorometer (Waltham, MA) using 360 nm excitation and 450 nm emission filters. Dose-response curves were generated using GraphPad Prism version 5.04 for Windows (San Diego, CA) where control wells containing NAD^+ only were set at 0% PARP activity and control wells containing PARP-1 only were set at 100% PARP activity. IC_{50} values were calculated from the dose-response curves generated from at least three independent experiments and reported in Table 1 as mean \pm standard deviation (SD).

Example 14

This example illustrates the synthesis of radio-labeled PARP-1 inhibitors.

$[^{18}\text{F}]\mathbf{12}$ was synthesized by the nucleophilic substitution of the mesylate precursor **16** under conventional conditions ($\text{K}_{222}/\text{K}_2\text{CO}_3$) in DMF at 105 $^{\circ}\text{C}$ (Scheme 3), affording $[^{18}\text{F}]\mathbf{12}$ in 40-50 % yield (decay corrected) after reversed phase HPLC purification and solid phase extraction (FIG. 2). The total synthesis time was 90 min. The specific activity of the final dose in 10% ethanol/saline was 5500-18000 $\text{mCi}/\mu\text{mol}$.



Scheme 3. Radiosynthesis of $[^{18}\text{F}]\mathbf{12}$

Reagents and conditions: $[^{18}\text{F}]\text{KF}$, K_{222} , K_2CO_3 , DMF, 105 $^{\circ}\text{C}$, 10 min.

Example 15

This example further illustrates the synthesis $[^{18}\text{F}]\text{Compound 12}$ (WC-4-138).

$[^{18}\text{F}]\text{fluoride}$ (up to 50 mCi in 100-500 μL $[^{18}\text{O}]\text{water}$) was transferred to a BD vacutainer (13 \times 75 mm, 5 mL, glass, no additives) containing K_{222} (2.2 mg, 5.8 μmol) and K_2CO_3 (0.3 mg, 2.2 μmol). The mixture was then dried by azeotropic distillation at 105 $^{\circ}\text{C}$ using acetonitrile (3 \times 1 mL) under a gentle flow of N_2 gas. When the drying was nearly finished, the vacutainer was removed from the oil bath and the solvent residue (< 100 μL) was removed by a flow of N_2 at room temperature. A solution of **16** (0.65 mg, 1.6 μmol) in DMF (300 μL) was added to the vacutainer and then shaken and heated at 105 $^{\circ}\text{C}$ for 10 min. At

room temperature, the reaction mixture was diluted with water (2 mL) and then loaded onto a semi-preparative column (A) for purification (18% acetonitrile/82% water/0.1% TFA, 4 mL/min, 251 nm). The HPLC fraction containing [¹⁸F]**12** was collected, and [¹⁸F]**12** was obtained in ethanol using standard solid phase extraction method. The dose was diluted to 10% ethanol in saline. An analytical column (B) was used to analyze the dose (32% acetonitrile/68% 0.1 M ammonium formate buffer pH = 4.5, 2 mL/min, 251 nm). The total synthesis time was 90 min, the decay corrected yields 40-50%, the radiochemical purity 100%, and the specific activity ranged from 5500 to 18000 mCi/μmol at the end of synthesis.

Example 16

This example illustrates the visualization of tumor tissue in microPET in mice.

MDA-MB-436 human breast cancer xenograft tumors in immune-deficient mice were visualized by PET using [¹⁸F]**12**. These tumors demonstrated increased uptake that was distinguishable from background at 60 min post-tracer injection (FIG. 3). The same mice treated with olaparib (50 mg/kg i.p.) or **12** (1 mg/kg i.p.) 20 min prior to tracer injection decreased [¹⁸F]**12** uptake in the tumors to the background tissue activity levels (FIG. 3). The time-radioactivity curves of [¹⁸F]**12** in the tumors from 0-60 min of the microPET studies confirmed the visual assessment of the microPET images (FIG. 4), demonstrating significantly decreased [¹⁸F]**12** uptake as a result of treatment with either olaparib or **12**.

MDA-MB-436 is a human breast cancer cell line with innately high levels of PARP-1 activity¹⁹ and has been used in a mouse models to assess the efficacy of the ¹⁸F-labeled olaparib derivative for imaging PARP-1 activity with microPET. [¹⁸F]**12** progressively accumulated in the tumor during the 1 hour microPET acquisition, and [¹⁸F]**12** uptake was blocked in animals pretreated with either olaparib or **12**. Both olaparib and **12** are competitive PARP-1 inhibitors with high inhibition potencies (IC₅₀ = 5 nM³¹ and 6.3 nM, respectively). Thus, our data indicate that [¹⁸F]**12** uptake in the mouse model is due to PARP-1 expression, and that [¹⁸F]**12** is an effective PET tracer for in vivo imaging of PARP-1 expression specifically.

Example 17

This example illustrates the visualization of tumor tissue in mice using PET.

MDA-MB-436 and MDA-MB-231 human breast cancer cells were maintained in cell culture under standard conditions with 5% CO₂ atmosphere using Eagle's minimum essential medium (with Earle's balanced salt solution and 2 mM L-glutamine) supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids 2 % vitamins

for MEM, and 5 % fetal bovine serum. Cells in exponential growth were trypsinized and harvested for tumor implantation. After counting, cells were re-suspended in ice-cold 1:1 Matrigel and PBS to give the desired concentration and held on ice.

Mature female athymic nu/nu mice from Charles River Laboratories are allowed to acclimate in an AALAC accredited housing facility for at least 1 week prior to tumor implantation for these serial imaging studies. Female nu/nu mice were implanted in the mammary fat pads (near the axillary lymph nodes) with $\sim 8 \times 10^6$ MDA-MB-436 breast cancer cells in $\sim 100 \mu\text{L}$ of 1:1 Matrigel:PBS. Imaging studies were conducted 2-3 weeks post implantation.

Tumor-bearing mice were placed in an induction chamber containing $\sim 2\%$ isoflurane/oxygen and then secured to a custom double bed for placement of tail vein catheters; anesthesia was maintained via nose-cone at $\sim 1\%$ isoflurane/oxygen for the dynamic imaging procedure. The mice were injected with 150-200 μCi of [^{18}F]12 and scanned for 0-60 min using Focus 220 and Inveon PET/CT scanners. The standard uptake values (SUVs) were generated from manually drawn regions of interests for tumors and surrounding 'background' tissue. Visualization of specific uptake was determined by comparison of baseline scans with images acquired by tracer injection 20 minutes after pre-treatment with the blocking agents olaparib (50 mg/kg, IP) or 12 (1 mg/kg, IP).

Example 18

This example illustrates the uptake of [^{18}F]WC-4-138 (Compound 12) in cell culture assays.

In these experiments, the head and neck squamous cell carcinoma lines SCC1, SCC15, and SCC25 (ATCC) were propagated in Dulbecco's Modified Eagle's Medium (DMEM, Gibco) supplemented with 10% Fetal Bovine Serum (FBS, Gibco), 1% Penicillin-Streptomycin (P/S, Gibco), and 100 ng/ml hydrocortisone (Sigma-Aldrich). The small cell lung cancer lines NCI-h69 and NCI-h82 (ATCC) were propagated in RPMI Medium (Gibco) supplemented with 10% FBS and 1% P/S. The human breast cancer cell line MDA-MB-231 (ATCC) was propagated in Eagle's Minimum Essential Medium (Gibco) supplemented with 5% FBS, 2% Vitamins for MEM (Gibco), 1% 200 mM L-glutamine (Gibco), 1% 10 mM Non-Essential Amino Acid (NEAA, Gibco).

For each experimental replicate, approximately 1 μCi of [^{18}F]WC-4-138 was diluted in 1 ml of cell culture medium and added to 10^6 cells. After 5, 30, or 60 minutes, the medium was collected and cells were washed twice in 0.7 ml Phosphate Buffered Saline (PBS, Gibco). Adherent cells were collected by scraping the cell culture dish and transferring to a microfuge

tube. Radioactivity was measured in the collected medium, PBS, or cell pellets. Protein from the cell pellet was quantified using a standard chemiluminescent PARP ELISA kit (Trevigen #4520-096-K). All data were decay-corrected and normalized to total protein quantity in the cell pellet. For drug treatment studies, cells were incubated with 10 μ M Olaparib or Iniparib twenty-hours prior to incubation with [18 F]WC-4-138.

The head and neck cell lines SCC1 and SCC25 constitutively take up small amounts of tracer, registering as 0.001-0.002 μ Ci/ μ g in the cell pellet (FIG. 5A). Lung cell lines NCI-h69 and NCI-h82 take up [18 F]WC-4-138 registering 0.004-0.006 μ Ci/ μ g in the cell pellet (FIG. 5A). Radioactivity was measured in head and neck squamous cell cancer (HNSCC) lines (SCC1 and SCC25) or small cell lung cancer (SCLC) lines (NCI-h69 and NCI-h82) after incubation with [18 F]WC-4-138 for 5, 30, or 60 minutes (n=3 at each time point). These figures represent 1-2 units of PARP activity for the SCC cells and about 3.5 units of Parp activity for the NCI cells (FIG. 5B). PARP activity was measured in HNSCC or lung SCLC cells. (FIG. 5C-D) [18 F]WC-4-138 uptake was measured in HNSCC cell lines treated with olaparib or iniparib. Data are mean \pm SD for uptake after 30 or 60 min of incubation with tracer (*p<0.05). All data are presented as mean \pm SD, n=3, unless otherwise noted.

This activity is abolished by Olaparib (FIG. 5C) but not Iniparib (FIG. 5D).

Example 19

This example illustrates metabolic stability of Compound **12** ([18 F]WC-4-138).

In these experiments, the metabolic stability of [18 F]-WC-4-138 was assessed after injecting 400 μ Ci into the tail veins of adult male C57BL/6J mice (Jackson Laboratory). Mice were sacrificed by cervical dislocation 5 or 30 minutes post-injection. The inferior vena cava was lacerated and blood was collected from the abdominal cavity. The plasma was separated from the red blood cells by centrifugation at 14000 rpm. Plasma (100 μ l) was mixed with acetonitrile at a 1:1.5 ratio and centrifuged at 14000 rpm. Radioactivity associated with the red blood cell pellet, whole plasma, and acetonitrile-soluble and insoluble fractions was measured. The liver of each animal was also collected at the indicated times and frozen on dry ice. Livers were homogenized in 2 ml acetonitrile and 1 ml of the homogenized liver was centrifuged at 14000 rpm. Radioactivity associated with the supernatant and pellet was measured.

Acetonitrile-soluble plasma or liver supernatant (100 μ l) was mixed at a 1:1 ratio with water and separated by reverse phase HPLC. The parent compound was also separated by HPLC as a reference. The radioactivity associated with each HPLC fraction was measured.

The percent parent compound of each sample was calculated as the portion of radioactivity associated with the HPLC fractions expected to contain the parent compound.

Compound **12** ($[^{18}\text{F}]$ WC-4-138) was rapidly metabolized in the blood, with 50% relative to the reference compound present 5 minutes post-injection and only about 10% of the compound relative to the reference compound was observed 30 minutes post-injection (FIG. 6). In contrast, the compound had a much longer half-life in the liver, showing about 80% relative to the reference compound 5 minutes post injection and about 70% relative to the reference compound 30 minutes post-injection (FIG. 6).

Example 20

This example illustrates biodistribution of Compound **12** ($[^{18}\text{F}]$ WC-4-138) in mice. Eight week old, female, athymic nude mice (Harlan) were injected via tail vein with $[^{18}\text{F}]$ WC-4-138. Mice were sacrificed by cervical dislocation 5 or 30 minutes after IV tracer injection of 30 μCi , 1 or 2 hours after injection of 45 μCi , or 4 hours after injection of 60 μCi of $[^{18}\text{F}]$ WC-4-138. The acetonitrile-soluble fractions of plasma or liver and the control parent compound were separated via reverse-phase high performance liquid chromatography (HPLC) and the radioactivity of each fraction was quantified. The percent parent compound of each sample was calculated as the portion of radioactivity associated with the HPLC fractions that contained the parent compound. Data are presented as mean \pm SD, n=3.

Four mice were sacrificed at each timepoint, with the exception of the 2 hour timepoint (n=3). Blood, heart, lung, muscle, liver, spleen, fat, adrenal glands, kidney, uterus, ovaries, bone, bone marrow, pancreas, stomach, small intestine, and large intestine were collected from each animal. All organs were blotted to remove excess blood, weighed, and counted in a Beckmann 6000 gamma counter. The percent injected dose per gram (%ID/g) of tissue was determined for each organ. Results varied from 0.5 to 15% injected dose per gram of tissue and are reported in Tables 1 and 2.

Table 1 Organ biodistribution of [¹⁸F]WC-4-138 in athymic nude mice

	% ID/ g				
	5 min	30 min	60 min	120 min	240 min
Blood	1.60 +/- 0.21	2.07 +/- 0.16	2.30 +/- 0.28	2.54 +/- 0.13	1.61 +/- 0.05
Heart	6.57 +/- 0.45	3.44 +/- 0.30	2.80 +/- 0.23	2.68 +/- 0.09	1.67 +/- 0.06
Lung	11.88 +/- 0.89	5.33 +/- 0.48	3.99 +/- 0.29	3.17 +/- 0.28	2.03 +/- 0.43
Muscle	2.05 +/- 0.48	2.39 +/- 0.15	2.21 +/- 0.31	2.07 +/- 0.11	1.35 +/- 0.09
Liver	12.50 +/- 1.08	8.24 +/- 0.52	6.50 +/- 0.52	4.58 +/- 0.29	2.43 +/- 0.25
Spleen	12.47 +/- 6.69	26.85 +/- 3.52	25.75 +/- 1.95	17.19 +/- 1.08	9.80 +/- 1.01
Fat	1.16 +/- 0.49	1.32 +/- 0.25	1.16 +/- 0.45	1.07 +/- 0.11	0.62 +/- 0.44
Adrenals	11.80 +/- 0.46	5.18 +/- 0.94	3.51 +/- 1.08	2.69 +/- 0.44	1.74 +/- 0.34
Kidney	35.63 +/- 3.33	21.14 +/- 2.21	14.02 +/- 1.84	8.77 +/- 0.72	3.67 +/- 0.54
Uterus	3.44 +/- 2.01	4.53 +/- 1.45	5.51 +/- 0.79	4.13 +/- 0.20	2.05 +/- 0.31
Ovaries	4.71 +/- 1.21	5.31 +/- 0.82	4.75 +/- 0.36	4.25 +/- 0.52	2.00 +/- 0.41
Bone	3.40 +/- 0.74	4.49 +/- 0.34	5.21 +/- 0.40	6.56 +/- 0.80	9.29 +/- 1.17
Marrow	0.06 +/- 0.02	0.11 +/- 0.02	0.13 +/- 0.04	0.07 +/- 0.01	0.04 +/- 0.01
Pancreas	9.67 +/- 1.98	10.27 +/- 1.28	9.18 +/- 1.37	5.37 +/- 0.64	2.75 +/- 0.44
Stomach	3.17 +/- 0.66	3.77 +/- 0.73	3.62 +/- 0.37	2.56 +/- 0.27	1.20 +/- 0.08
Small Intestine	12.64 +/- 1.45	13.00 +/- 0.75	8.85 +/- 1.49	6.62 +/- 0.75	3.04 +/- 0.25
Upper Large Intestine	7.84 +/- 1.02	14.07 +/- 1.72	15.62 +/- 2.31	10.28 +/- 0.65	4.33 +/- 0.57
Lower Large Intestine	4.17 +/- 0.42	7.43 +/- 0.53	10.53 +/- 1.45	13.35 +/- 0.87	7.17 +/- 0.50
Thyroid	4.18 +/- 1.18	4.03 +/- 0.28	3.32 +/- 0.19	3.46 +/- 0.10	3.23 +/- 0.49
Brain	0.55 +/- 0.16	1.03 +/- 0.11	1.24 +/- 0.13	1.58 +/- 0.19	1.17 +/- 0.05

%ID/g = Percent injected dose per gram of tissue.

All data are mean \pm SD. n=4 for all groups, with the exception of n=3 at 120 min.

Table 2. Estimated human dosimetry from mouse biodistribution study

Organ	Male (rad/mCi)	Female (rad/mCi)
Adrenals	0.043	0.052
Brain	0.019	0.022
Breasts		0.019
Gallbladder Wall	0.028	0.033
LLI Wall	0.029	0.036
Small Intestine	0.026	0.030
Stomach Wall	0.024	0.030
ULI Wall	0.025	0.031
Heart Wall	0.038	0.047
Kidneys	0.083	0.093
Liver	0.050	0.065
Lungs	0.035	0.045
Muscle	0.026	0.035
Ovaries		0.045
Pancreas	0.055	0.064
Red Marrow	0.061	0.069
Osteogenic Cells	0.155	0.203
Skin	0.016	0.019
Spleen	0.110	0.133
Testes	0.022	
Thymus	0.020	0.026
Thyroid	0.033	0.038
Urinary Bladder Wall	0.220	0.301
Uterus		0.053
Total Body	0.031	0.039
Effective Dose Equivalent	0.062	0.075
Effective Dose (rem/mCi)	0.047	0.057

Estimates were obtained from the organ residence times calculated from the mouse biodistribution data, shown in Table 1, using standard MIRD methodology.

Example 21

This example illustrates uptake of Compound **12** ($[^{18}\text{F}]\text{WC-4-138}$) in mice. Mature female athymic nude mice (Charles River Laboratories) were implanted with 10^6 SCC1 cells or 10^7 MDA-MB-231 cells into the mammary fat pads. Mice bearing SCC1 tumors were imaged at 5 weeks and mice bearing MDA-MB-231 cells were imaged at 2.5

weeks after implant. Mice were anesthetized with 2% isoflurane/oxygen and maintained at 1% isoflurane/oxygen via nose cone throughout the imaging procedure. Whole animal microCT images were acquired on an Inveon PET/CT scanner. The mice were injected via tail vein with 11.36 ± 0.5 MBq (307 ± 13 μ Ci) of [18 F]WC(4)-138 and underwent a 60 minute dynamic scan using Focus 220 or Inveon PET/CT scanners. The microPET and microCT images were coregistered using Integrated Research Workflow software (Siemens). Regions of interest were drawn over tumors to determine time activity curves. For drug treatment studies, animals received intraperitoneal injections of 50 mg/kg olaparib or iniparib 30 minutes before imaging. Mice were imaged at baseline, or 30 minutes after IP injection of olaparib or iniparib. Transverse views of MDA-MB-231 tumor-bearing mice are shown in FIG. 7. Arrows indicate location of tumors as identified on CT. Tracer is abundantly taken up in tumors and other sections of the mouse in baseline (FIG. 7, top of each channel view, arrows), but tumor uptake of [18 F]12 is abolished by treatment with olaparib (FIG. 7, bottom left of each channel panel, arrows), but tracer is still present in other areas of the section. In contrast, iniparib treatment does not effect the uptake of [18 F]WC(4)-138 in tumor cells, but abolishes said uptake in other types of cells (FIG. 7, bottom right of each channel view, arrows).

Example 22

This example illustrates a quantitative analysis that shows significant decreases in injected dose per ml between baseline and drug treatment for both MDA-MB-231 tumors (FIG. 8A) and SCC tumors (FIG. 8B). In FIG. 8, mice were imaged at baseline, or 30 minutes after IP injection of olaparib or iniparib. (A) The percent of injected dose per ml was calculated for each MDA/MB-231 tumor at baseline or after drug treatment. Tracer uptake was significantly reduced in olaparib-treated mice ($*p=0.0038$ as determined by a paired t test, $n=6$ tumors). Tracer uptake was unchanged in iniparib-treated mice ($p=0.1098$ as determined by a paired t test, $n=6$ tumors). (B) The percent of injected dose per ml was calculated for each SCC1 tumor at baseline or after drug treatment. Tracer uptake was significantly reduced in olaparib-treated mice ($*p=0.001$ as determined by a paired t test, $n=8$ tumors). Tracer uptake was unchanged in iniparib-treated mice ($p=0.7216$ as determined by a paired t test, $n=5$ tumors).

Example 23

A subject presents with symptoms of potential PARP-1 associated breast cancer. A physician orders PET scanning, and a technician administers an effective amount of [18 F]WC(4)-138 and performs the PET scan. The tumor exhibits a large amount of tracer

uptake compared to the surrounding tissues of the subject. The physician diagnoses PARP-1 associated cancer and prescribes PARP-1 inhibitors as part of a treatment regime.

Example 24

A subject presents with abnormal inflammation of the lungs. A physician orders PET scanning, and a technician administers an effective amount of [¹⁸F]WC(4)-138 and performs the PET scan. The lung tissue exhibits large amounts of tracer uptake relative to control lung tissue, and the physician diagnoses PARP-1 related inflammation and prescribes PARP-1 inhibitors as part of a treatment regime.

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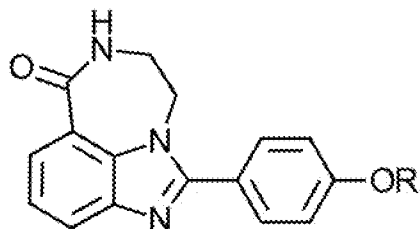
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All publications cited herein are incorporated by reference, each in its entirety. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context indicates otherwise.

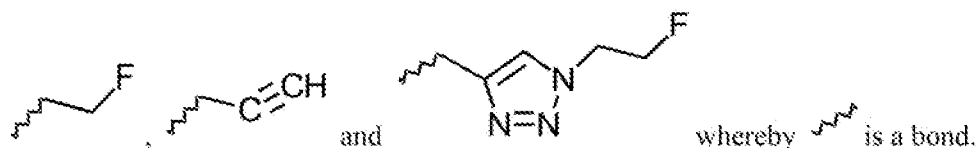
CLAIMS

What is claimed is:

1. A compound or pharmaceutically acceptable salt thereof of structure



wherein R is selected from the group consisting of

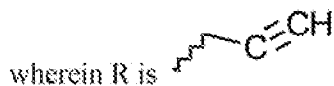


2. A compound or pharmaceutically acceptable salt thereof in accordance with claim 1,

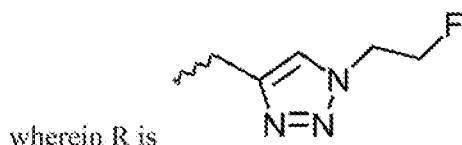


3. A compound or pharmaceutically acceptable salt thereof in accordance with claim 2 wherein the F is an ¹⁸F.

4. A compound or pharmaceutically acceptable salt thereof in accordance with claim 1,

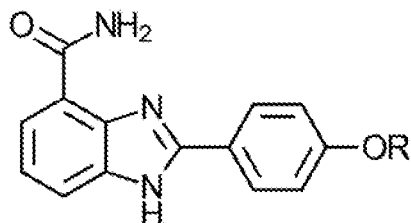


5. A compound or pharmaceutically acceptable salt thereof in accordance with claim 1

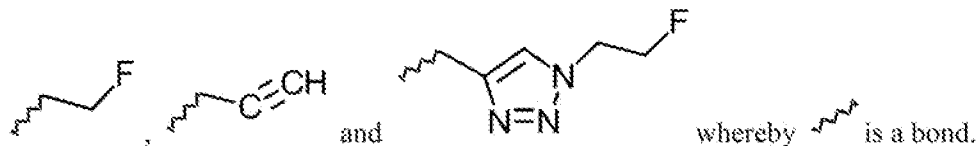


6. A compound or pharmaceutically acceptable salt thereof in accordance with claim 5, wherein the F is an ¹⁸F.

7. A compound or pharmaceutically acceptable salt thereof of structure



wherein R is selected from the group consisting of

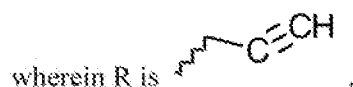


8. A compound or pharmaceutically acceptable salt thereof in accordance with claim 7,

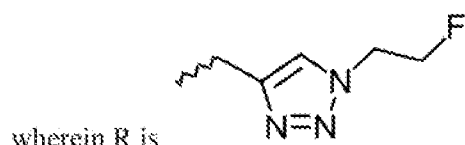


9. A compound or pharmaceutically acceptable salt thereof in accordance with claim 8,
wherein the F is an ^{18}F .

10. A compound or pharmaceutically acceptable salt thereof in accordance with claim 7,



11. A compound or pharmaceutically acceptable salt thereof in accordance with claim 7



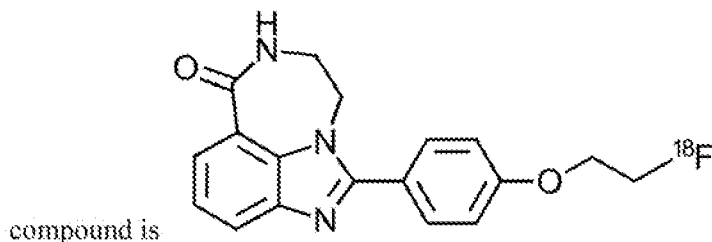
12. A compound or pharmaceutically acceptable salt thereof in accordance with claim 11,
wherein the F is an ^{18}F .

13. A method of imaging a tumor in a subject, comprising:

administering to a subject a compound or pharmaceutically acceptable salt thereof of claim 1; and

subjecting the subject PET scanning, wherein the compound comprises a positron-emitting radionuclide.

14. A method of imaging a tumor in a subject in accordance with claim 13, wherein the



15. A method of imaging a tumor in a subject, comprising:

administering to a subject a compound or pharmaceutically acceptable salt thereof of claim 7; and

subjecting the subject PET scanning, wherein the compound comprises a positron-emitting radionuclide.

16. A method of imaging inflammation in a subject, comprising:

administering to a subject a compound or pharmaceutically acceptable salt thereof of claim 1; and

subjecting the subject PET scanning, wherein the compound comprises a positron-emitting radionuclide.

17. A method of imaging inflammation in a subject, comprising:

administering to a subject a compound or pharmaceutically acceptable salt thereof of claim 7; and

subjecting the subject PET scanning, wherein the compound comprises a positron-emitting radionuclide.

18. A compound or pharmaceutically acceptable salt thereof of any one of claims 1-12 for use in the imaging of a tumor in a subject.

19. A compound or pharmaceutically acceptable salt thereof of any one of claims 1-12 for use in the imaging of inflammation in a subject.

20. A compound or pharmaceutically acceptable salt thereof of any one of claims 1-12 for use in the imaging of PARP-1 distribution.

FIG. 1 (prior art)

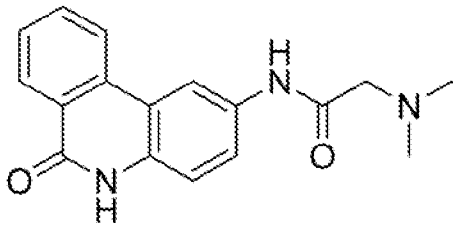
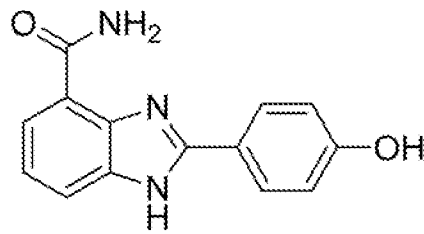
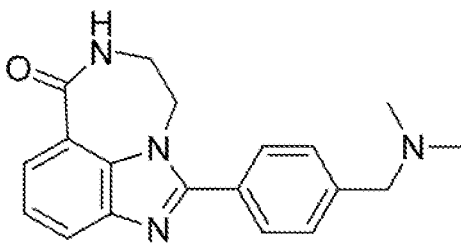
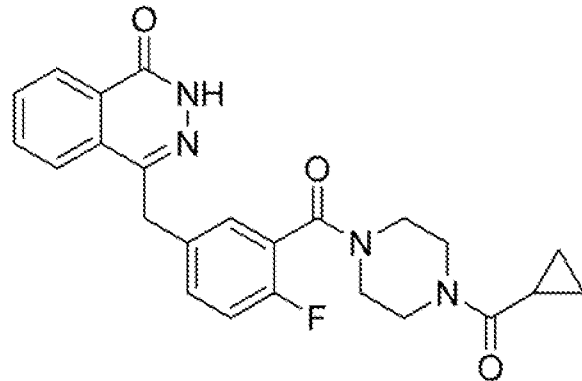
PJ-34 (1) ($IC_{50} = 20$ nM)NU1085 (2) ($K_i = 6$ nM)AG014361 (3) ($K_i = 5.8$ nM)AZD2281, olaparib (4) ($IC_{50} = 5$ nM)

FIG. 2

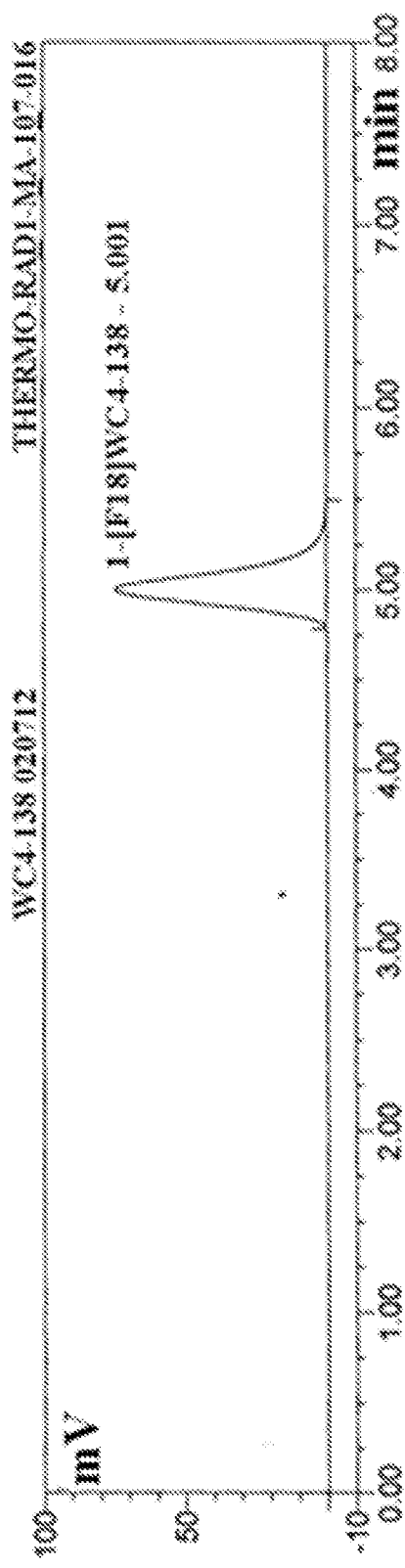
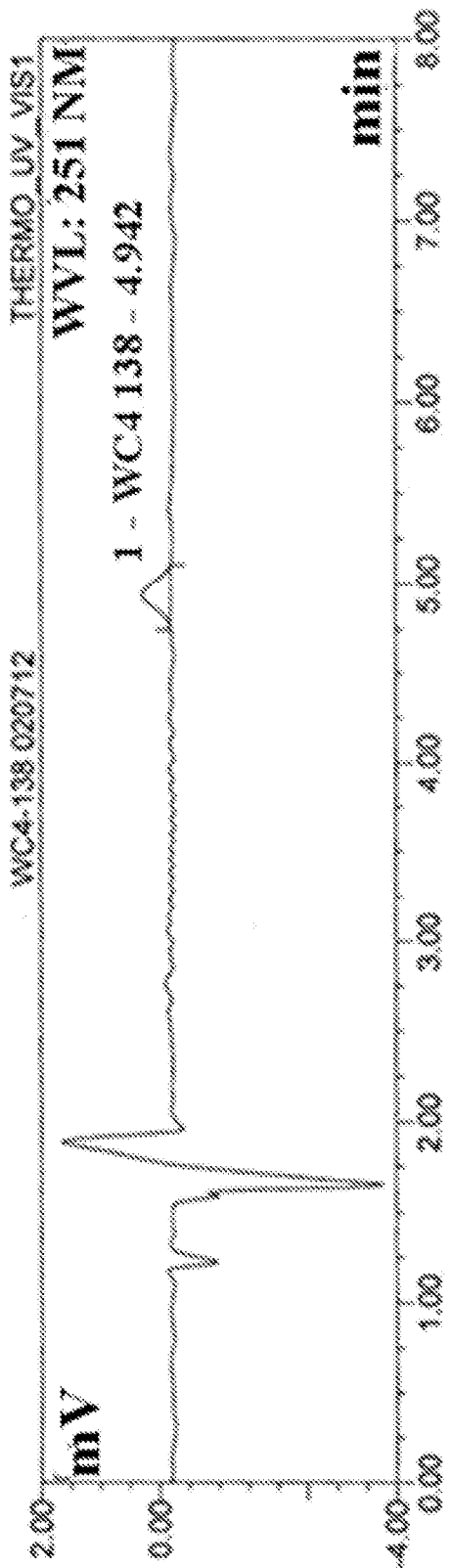


FIG. 3A Red Channel

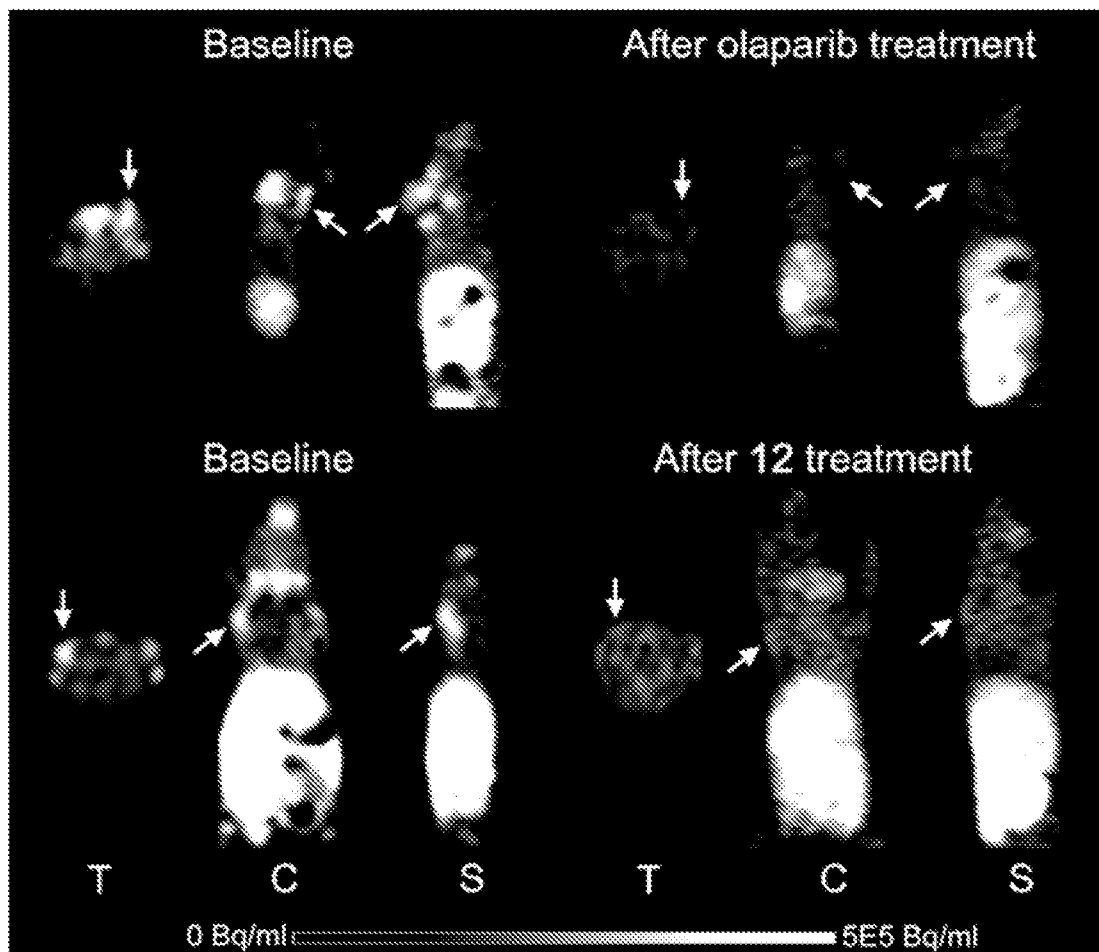


FIG. 3B Green Channel

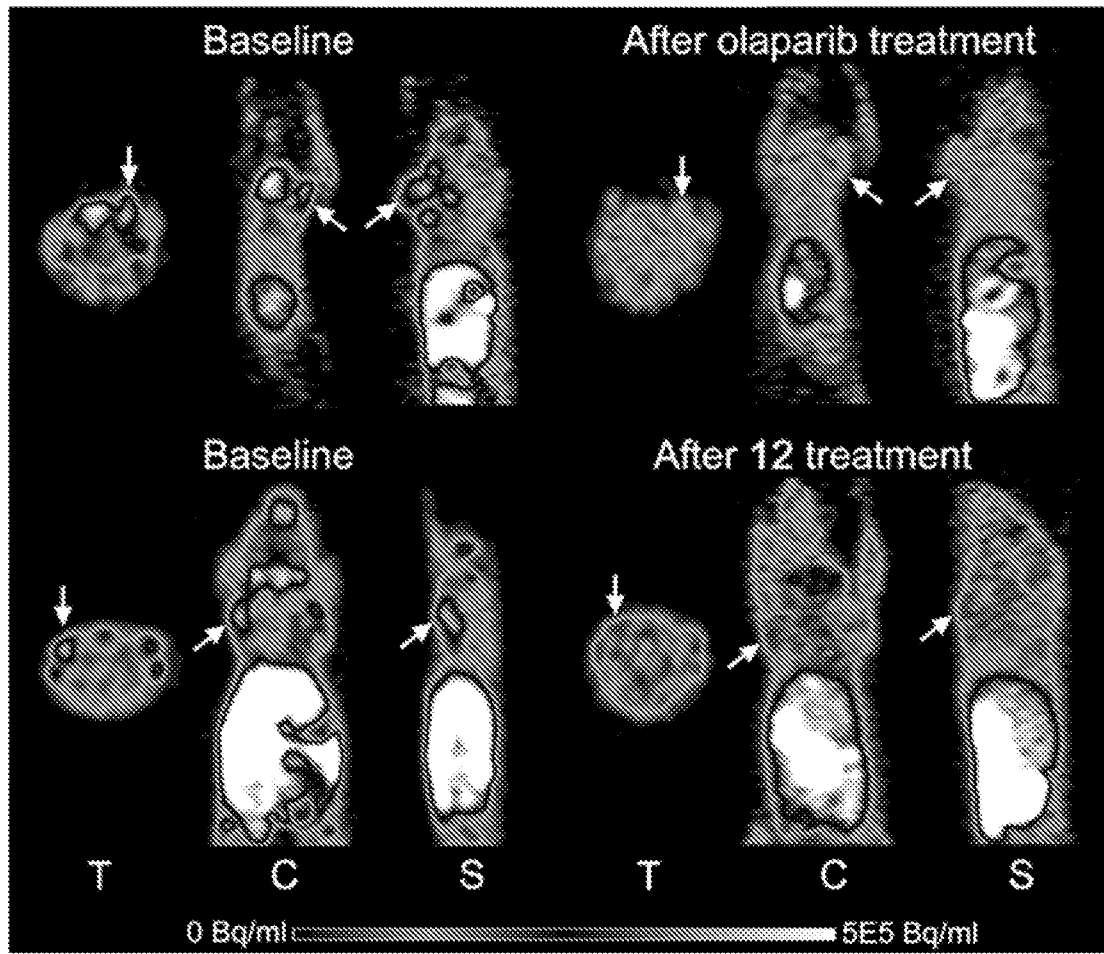


FIG. 3C Blue Channel

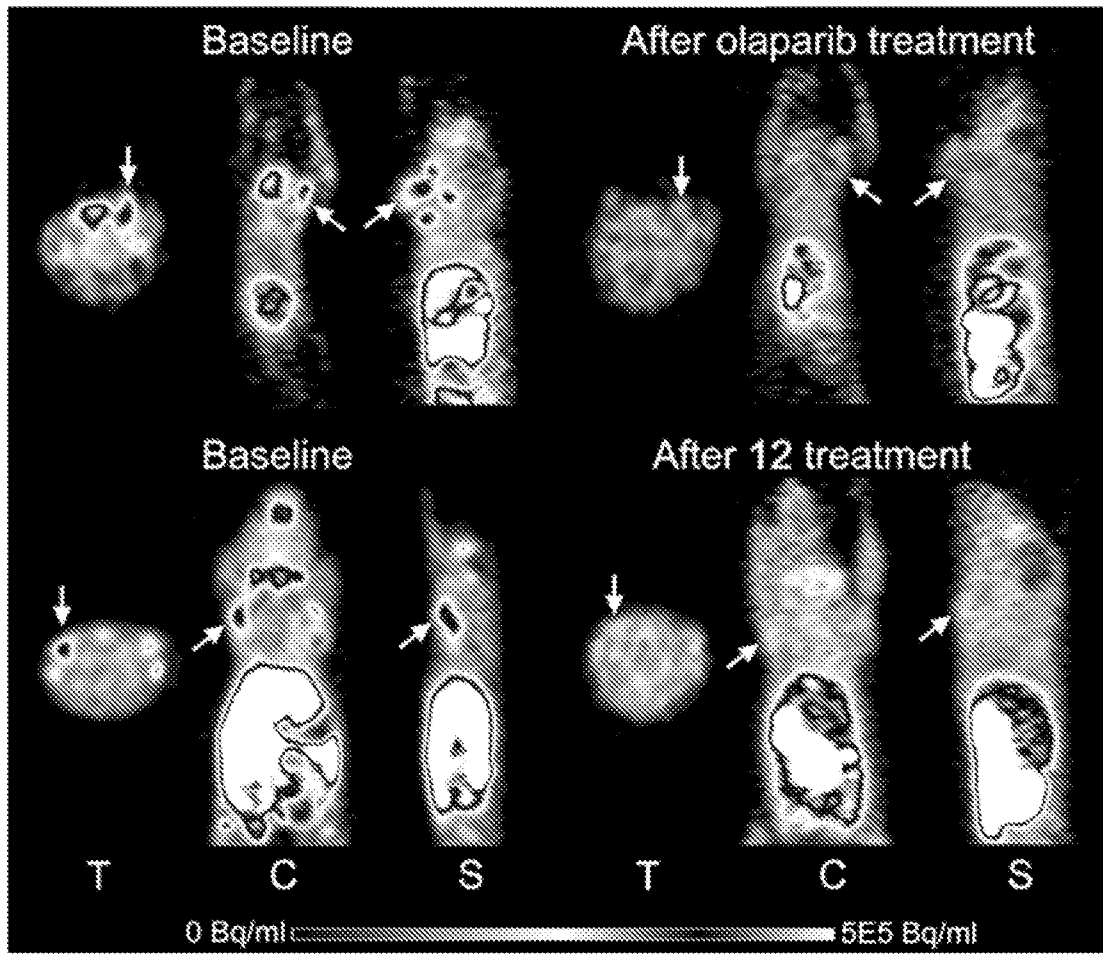


FIG. 3D Composite

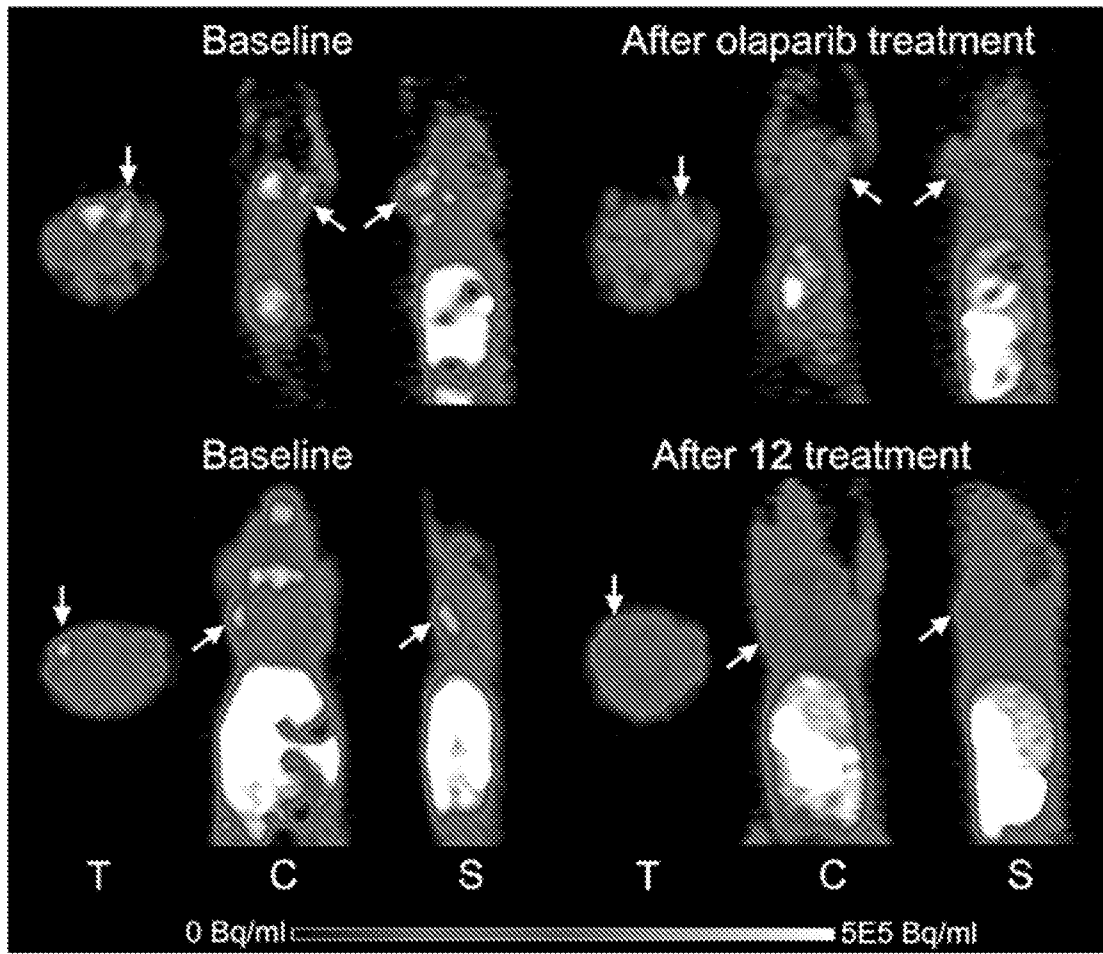


FIG. 4

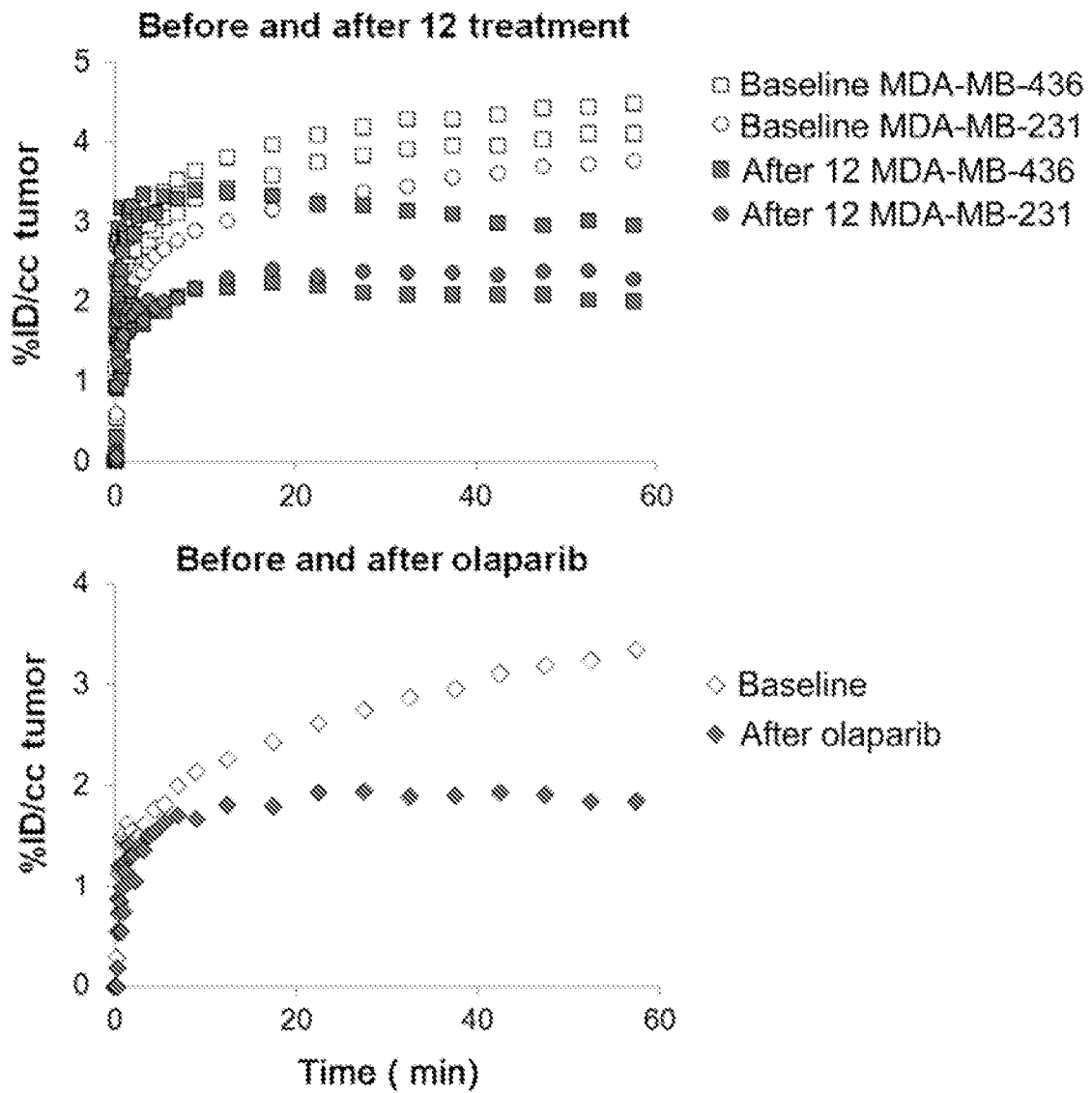
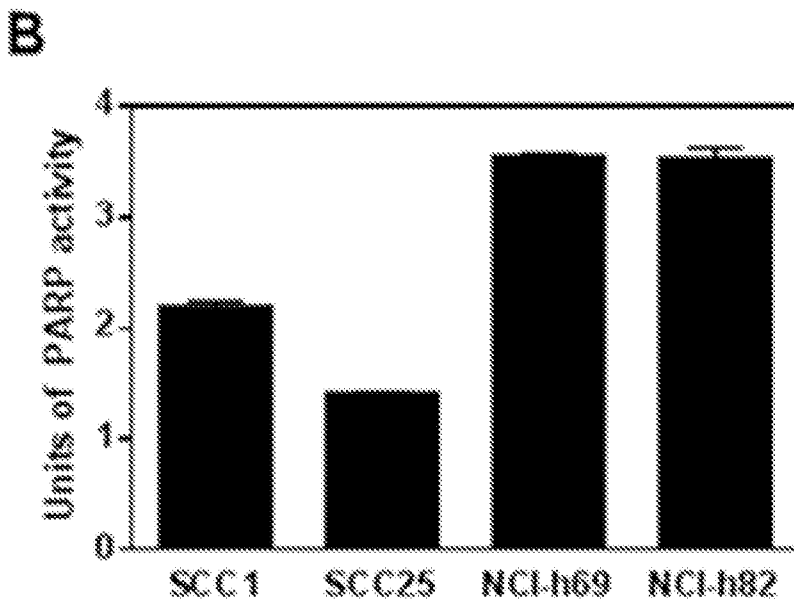
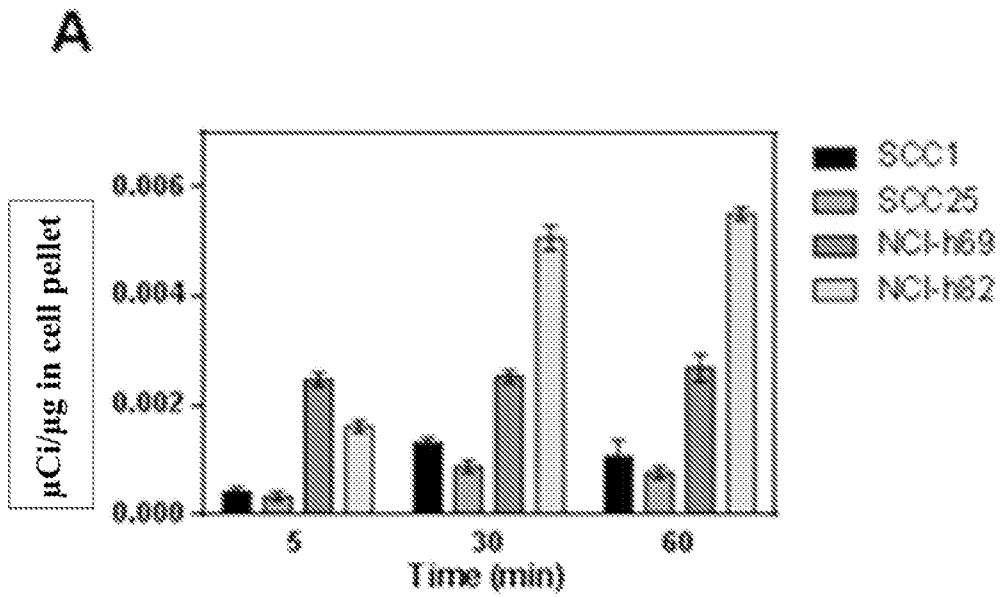
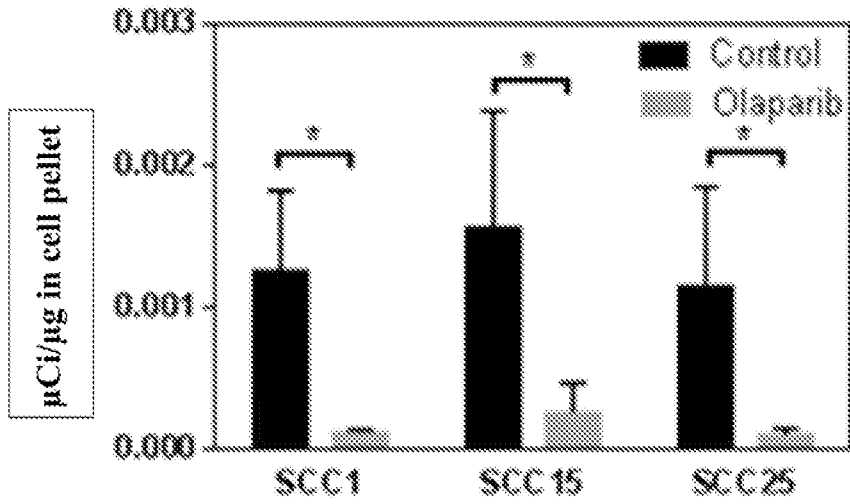


FIG. 5



C FIG. 5 continued



D

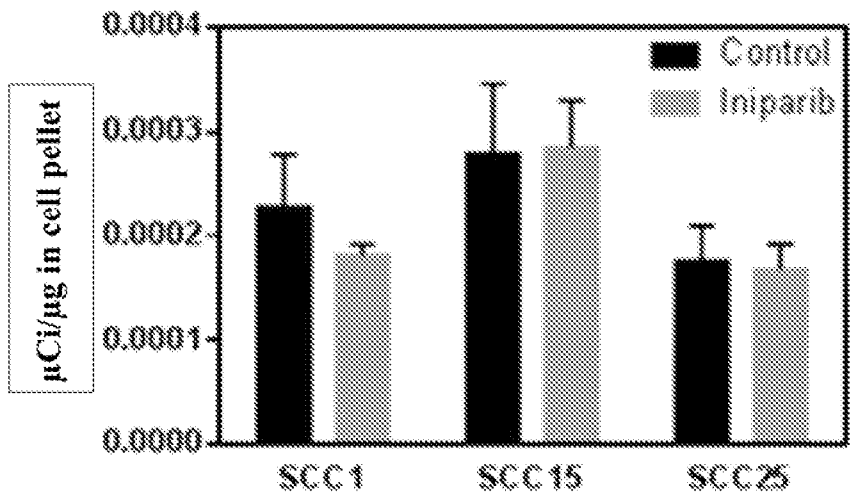


FIG. 6

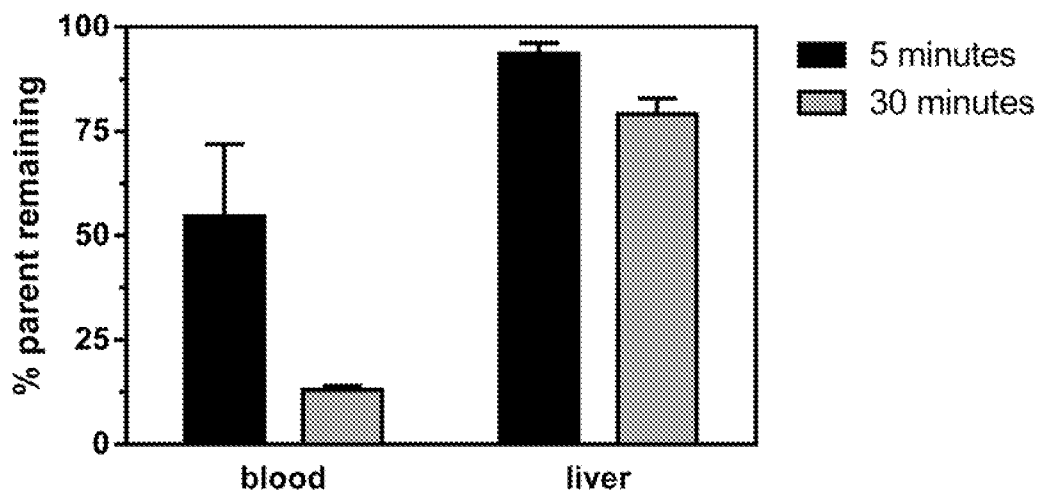


FIG. 7

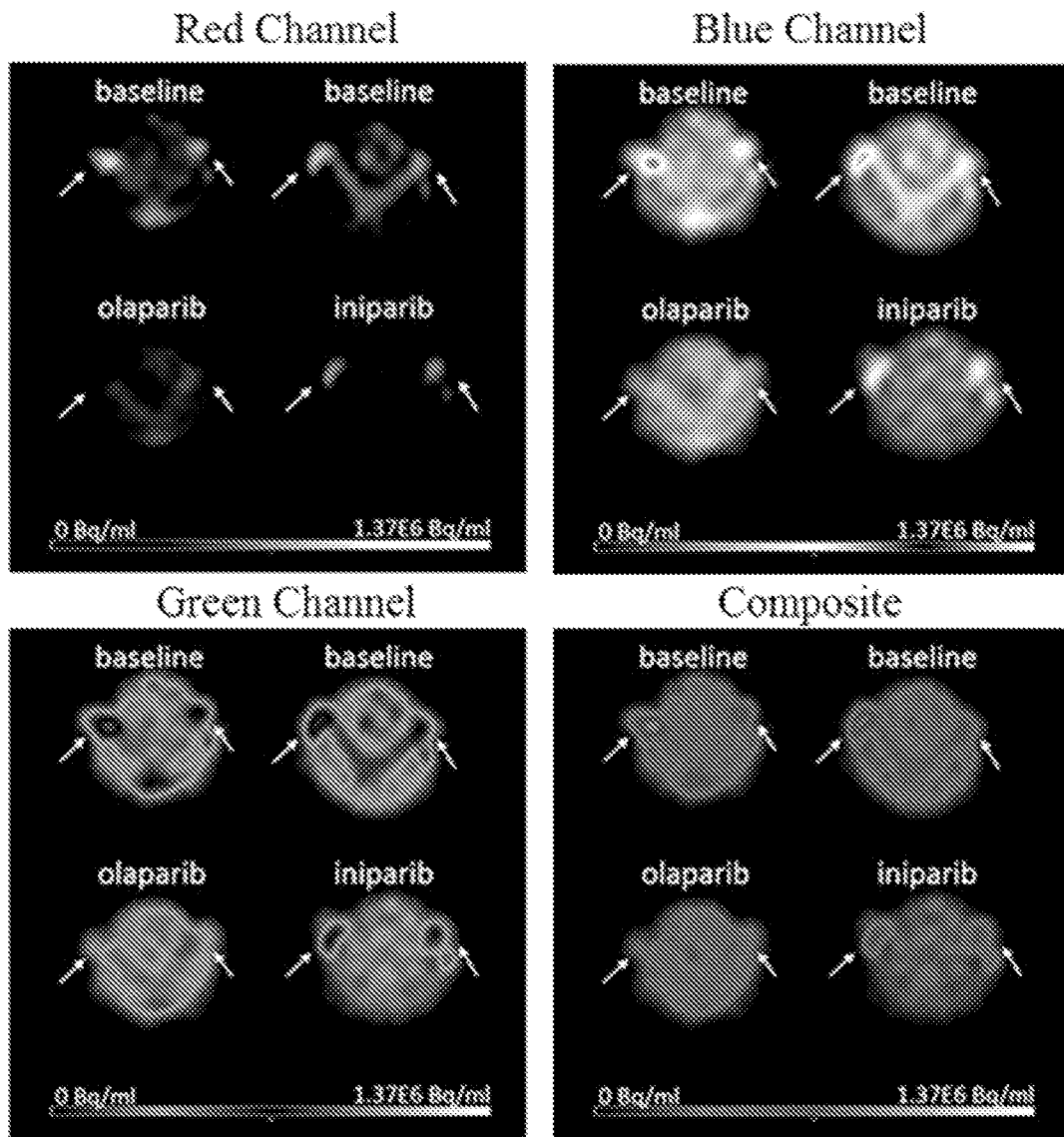
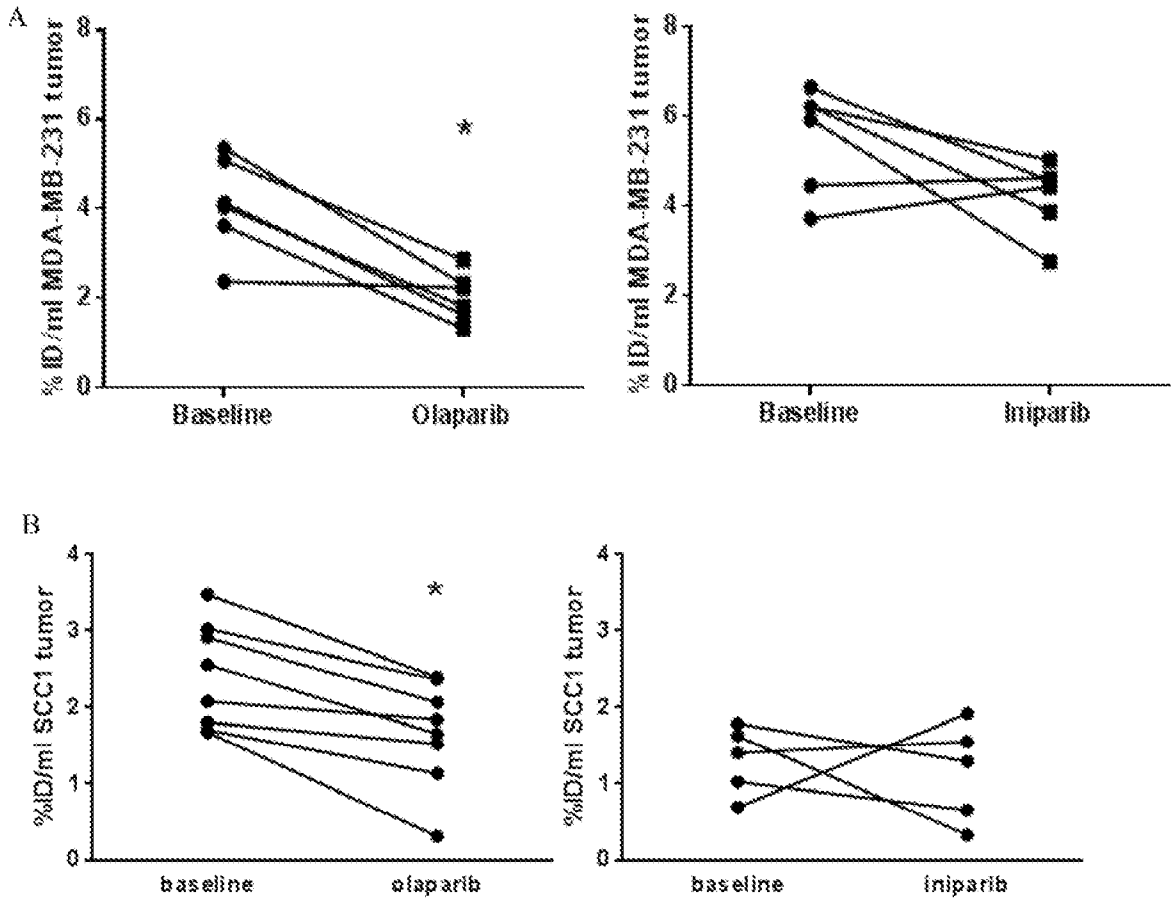


FIG. 8



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2015/010129

A. CLASSIFICATION OF SUBJECT MATTER See extra sheet. 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) IPC (2015.01) C07D 243/24, C07D 235/26, A61K 36/55, C07D 263/62, C07D 277/62, C07D 411/04, A01N 43/52, A01N 43/76, A01N 43/78		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Databases consulted: CAPLUS, REGISTRY		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	abstract, claim 1, example 16	3,6,13,14,17-20
X	US 7781596 B1 ABBOTT LAB[US] 24 Apr 2010 (2010/04/24) example 1, abstract, claim 1	7,8,10,11,18-20
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Y	Diagnostic Role of 18F-FECH-PET/CT Compared with Bone Scan in Evaluating the Prostate Cancer Patients Referring with Biochemical Recurrence" ISRN Oncology Volume 2012 (2012), Article ID 815234, 12 pages. Mustafa Takesh ET ALL 01 Jan 2012 (2012/01/01) abstract	3,6,9,12-15,17
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.		
<p>* Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"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)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"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</p> <p>"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</p> <p>"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</p> <p>"&" document member of the same patent family</p>		
Date of the actual completion of the international search 01 Apr 2015		Date of mailing of the international search report 20 Apr 2015
Name and mailing address of the ISA: Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Facsimile No. 972-2-5651616		Authorized officer VALKOV Karina Telephone No. 972-2-5651777

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2015/010129

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (2015.01) C07D 243/24, C07D 235/26, A61K 36/55, C07D 263/62, C07D 277/62, C07D 411/04, A01N 43/52, A01N 43/76, A01N 43/78

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Y	abstract, examle 38	9,12,15,17
P,X	WO 2014037340 A1 BAYER CROPSCIENCE AG[DE] 13 Mar 2014 (2014/03/13) claim 1 , abstract	7-12,15,17-20

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