An $^{18}$F-Labeled Poly(ADP-ribose) Polymerase Positron Emission Tomography Imaging Agent

Filip Zmuda, Adele Blair, Maria Clara Liuzzi, Gaurav Malviya, Anthony J. Chalmers, David Lewis, Andrew Sutherland, and Sally L. Pimlott

WestCHEM, School of Chemistry, University of Glasgow, The Joseph Black Building, Glasgow G12 8QQ, U.K.
Wolfson Whol Cancer Research Centre, Institute of Cancer Sciences, University of Glasgow, Glasgow G61 1QH, U.K.
School of Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, U.K.
Cancer Research UK Beatson Institute, Glasgow G61 1BD, U.K.
West of Scotland PET Centre, Greater Glasgow and Clyde NHS Trust, Glasgow G12 0YN, U.K.

Supporting Information

ABSTRACT: Poly(ADP-ribose) polymerase (PARP) is involved in repair of DNA breaks and is over-expressed in a wide variety of tumors, making PARP an attractive biomarker for positron emission tomography (PET) and single photon emission computed tomography imaging. Consequently, over the past decade, there has been a drive to develop nuclear imaging agents targeting PARP. Here, we report the discovery of a PET tracer that is based on the potent PARP inhibitor olaparib (1). Our lead PET tracer candidate, $[^{18}F]20$, was synthesized and evaluated as a potential PARP PET radiotracer in mice bearing subcutaneous glioblastoma xenografts using ex vivo biodistribution and PET–magnetic resonance imaging techniques. Results showed that $[^{18}F]20$ could be produced in a good radioactivity yield and exhibited specific PARP binding allowing visualization of tumors over-expressing PARP. $[^{18}F]20$ is therefore a potential candidate radiotracer for in vivo PARP PET imaging.

INTRODUCTION

Poly(ADP-ribose) polymerase-1 (PARP-1) is a nuclear protein that exhibits a broad range of functions and is involved in transcription, mitosis, apoptosis, and DNA damage repair. PARP inhibition has been investigated as a therapeutic approach to treat cancers by either synthetic lethality in which tumor cells deficient in a type of DNA repair termed homologous recombination are sensitized to PARP inhibition, or chemoradiosensitization, in which PARP inhibition sensitizes tumor cells to conventional chemo- or radiotherapy. To date, olaparib (Lynparza), niraparib (Zejula), and rucaparib (Rubraca) are the only PARP inhibitors to receive approval for clinical use in the United States or Europe. Olaparib (1; Figure 1) was the first agent in its class to receive such approval. In the European Union, it is currently indicated for the treatment of BRCA-mutated (homologous recombination deficient) ovarian, fallopian-tube, and peritoneal cancers, in which it has been shown to increase progression-free and overall survival. In the United States, it can also be used for treatment of BRCA-mutated metastatic breast cancer and as a maintenance therapy for patients with platinum-sensitive recurrent epithelial ovarian, fallopian-tube, or primary peritoneal cancer irrespective of BRCA mutations. In both cases, olaparib was once again shown to increase progression-free survival. Olaparib is also being investigated as a radio- and chemosensitizer for the treatment of solid cancers, including gliomas. However, adding PARP inhibitors to cytotoxic chemotherapy agents has been shown to exacerbate bone marrow toxicity in humans, hindering the establishment of effective PARP inhibitor and chemotherapy dosage regimens with acceptable safety profiles. In the case of brain tumors, matters are further complicated as suffers from poor blood–brain barrier (BBB) permeability, and delivery of the drug to the tumor is reliant on BBB disruption. The degree of BBB disruption in brain tumors is very variable; this could affect tumor penetration by olaparib and, hence, reduce the clinical effectiveness of PARP inhibitor therapy. Furthermore, in vivo animal studies have revealed that prolonged treatment with olaparib...
can result in increased tumor P-glycoprotein efflux transporter expression and subsequent drug resistance.\textsuperscript{16}

The above-mentioned issues highlight the challenges that are associated with PARP inhibitor therapy in the context of synthetic lethality as well as chemotherapeutic sensitization. Nuclear imaging of an appropriately radiolabeled PARP inhibitor could be used to overcome these challenges. In combination with a suitable blockade study protocol, nuclear imaging could indirectly establish the distribution and retention of PARP inhibitors in tumors and normal tissues and subsequently identify therapeutic dosage regimens for which the combination of PARP inhibitors and cytotoxic agents exerts maximal tumor and minimal bone marrow cytotoxicity. Furthermore, a radiolabeled PARP probe could be used to indirectly ascertain the occupancy, retention, and target engagement of 1 in brain tumor tissue. This type of approach has been successfully applied in preclinical models of small-cell lung\textsuperscript{17} and epithelial ovarian cancer.\textsuperscript{18} In a clinical setting, this could be used to identify patients that are unlikely to respond to PARP inhibitor therapy due to weak target engagement as a consequence of poor drug tumor uptake or resistance caused by efflux transporter over-expression.

Previously, we reported the synthesis and characterization of a \(^{[123]I}\)-labeled compound with potential for single-photon emission computed tomography (SPECT) imaging of PARP.\textsuperscript{19} Despite the established nature of the SPECT imaging modality, the use of positron emission tomography (PET) is rapidly expanding and, in many cases, has become the preferred nuclear imaging modality in the clinic. This can be attributed to the superior spatial resolution,\textsuperscript{19} quantification, and sensitivity\textsuperscript{20} of PET compared to SPECT. It is, therefore, not surprising that many of the current nuclear imaging agents for PARP were designed with PET imaging in mind (Figure 2).\textsuperscript{22,23} Importantly, the rationale for developing PARP PET imaging could indirectly establish the distribution and retention of PET radiotracers with high log \(P_{\text{oct}}\) and percentage plasma protein binding (%PPB) values in comparison to 1. This may be attributed to the addition of aromatic and methyl moieties, which have the potential to increase lipophilicity and can, in turn, result in a reduced plasma protein binding (PPB) due to the hydrophobic nature of plasma protein interactions.\textsuperscript{28} From the perspective of nuclear imaging, radiotracers with high log \(P_{\text{oct}}\) (>3.0)\textsuperscript{29} and %PPB (>95%)\textsuperscript{30} values can be associated with poor passive diffusion across biological membranes and vascular retention, which can in turn result in a poor target to background signal ratio. The physiochemical parameters of 8–12 were all found to be within the optimal range for radiotracer development.

![Figure 2. Published examples of PARP PET radiotracers.\textsuperscript{22,23}](image)

## RESULTS AND DISCUSSION

### Chemistry and in Vitro Characterization

Due to poor accessibility of the central ring fluorine atom of 1 for radiolabeling,\textsuperscript{22} focus was directed at synthesizing analogs of 1 bearing distal fluorinated moieties that were more likely to be amenable to radiofluorination methods. Initially, six fluorinated PARP inhibitors (8–13) containing the characteristic phthalazine scaffold were synthesized through amide or N-alkyl coupling of commercially available benzoic carboxylic acids or benzyl halides with piperazine 7, the synthesis of which we described previously\textsuperscript{15} (Table 1). The structures of compounds 8–13 were confirmed in part by nuclear magnetic resonance (NMR) spectroscopic analysis, which showed that the majority of these exist as a mixture of amide rotamers. The design of these analogs was partly driven by previous reports,\textsuperscript{25–27} which have shown that structural modifications can be performed in the cyclopropane bearing region of 1 without having a marked effect on PARP inhibition. To confirm this, cell-free PARP-1 IC\textsubscript{50} assays were performed on compounds 8–13, and the results of these experiments were compared against the cell-free IC\textsubscript{50} of 1 (Table 1). Compared to 1, all compounds showed improved PARP-1 potency except for compound 13, which had an overlapping cell-free IC\textsubscript{50} 95% confidence interval. Compounds 8–13 were also evaluated for their lipophilic and plasma protein binding properties, defined by log \(P_{\text{oct}}\) and percentage plasma protein binding (%PPB) parameters (Table 1). All six analogs exhibited greater log \(P_{\text{oct}}\) and %PPB values in comparison to 1. This may be attributed to the addition of aromatic and methyl moieties, which have the potential to increase lipophilicity and can, in turn, result in an increased % PPB due to the hydrophobic nature of plasma protein interactions.\textsuperscript{28} From the perspective of nuclear imaging, radiotracers with high log \(P_{\text{oct}}\) (>3.0)\textsuperscript{29} and %PPB (>95%)\textsuperscript{30} values can be associated with poor passive diffusion across biological membranes and vascular retention, which can in turn result in a poor target to background signal ratio. The physiochemical parameters of 8–12 were all found to be within the optimal range for radiotracer development.

- DOI: 10.1021/acs.jmedchem.8b00138
- J. Med. Chem. 2018, 61, 4103–4114
However, following analysis of the established in vitro parameters and potential radiochemical accessibility, compound 8 was identified as an initial lead candidate for further advancement in this research program. To establish the potency of 8 against PARP in living cells, cellular IC_{50} assays were performed using primary (G7) and secondary (T98G) human glioblastoma cell lines (Table 2) and previously described methodology.\textsuperscript{19} Compound 8 exhibited low nano-molar IC_{50} values that were in line with those observed for 1 in both cell lines, suggesting that 8 was able to effectively penetrate cellular membranes and reach PARP localized within cellular nuclei.

To access the [\textsuperscript{18}F]-radiofluorine analog of 8, standard aromatic nucleophilic substitution chemistry was employed using p-nitrobenzamide precursor 14, which was generated through amide coupling of 7 with commercially available 4-nitrobenzoic acid (Scheme 1). However, optimization of the radiofluorination step proved challenging (see the Supporting Information), and the maximum radiochemical yield (based on high-performance liquid chromatography [HPLC] analysis of crude product) achieved was only 19\% (Scheme 1). It was proposed that the poor yield was a consequence of a lack of activation of the system for aromatic nucleophilic substitution due to the weak electron withdrawing properties of the amide located para- to the nitro leaving group. Since commencing this work, Carney et al. were able to synthesize [\textsuperscript{18}F]8 with an optimized radioactivity yield of 38 ± 2.5\% (isolated product).

### Table 1. Methods Used to Generate Analogs 8–13 from the Penultimate Compound 7 as well as the Cell-Free PARP-1 Inhibitory and Physiochemical Properties of 1 and 8–13

| Coupling partner | Coupling conditions | R | Yield, \(\%\) | Cell-free IC\textsubscript{50} (95\% CI), nM\textsuperscript{a} | Log \(P_{\text{oct}}\) | %PPB\textsuperscript{c} |
|------------------|---------------------|---|----------------|-----------------------------|----------------|----------------|
| a                | HBTU, Et\textsubscript{3}N, DMF, room temperature (rt), 72 h | 1 | 51 | 11.9 (10.5–13.6) | 1.95 | 75.9 |
| b                | HBTU, DIPEA, CH\textsubscript{2}Cl\textsubscript{2}, rt, 24 h | 8 | 75 | 5.9 (4.9–7.0) | 2.51 | 85.9 |
| c                | HBTU, DIPEA, DMF, 50 °C, 24 h | 9 | 53 | 3.6 (3.4–3.9) | 2.46 | 86.4 |
| d                | HBTU, DIPEA, DMF, 50 °C, 24 h | 10 | 31 | 2.9 (2.3–3.7) | 2.66 | 86.1 |
| e                | DIPEA, CH\textsubscript{2}Cl\textsubscript{2}, rt, 24 h | 11 | 46 | 1.3 (0.9–1.7) | 2.67 | 89.9 |
| f                | DIPEA, CH\textsubscript{2}Cl\textsubscript{2}, rt, 24 h | 12 | 34 | 4.1 (3.6–4.6) | 2.81 | 90.6 |
| g                | DIPEA, CH\textsubscript{2}Cl\textsubscript{2}, rt, 24 h | 13 | 38 | 11.2 (7.3–17.3) | 3.14 | 94.7 |

\textsuperscript{a}Cell-free IC\textsubscript{50} values are based on three experiments. \textsuperscript{b}Lipophilicity (log \(P_{\text{oct}}\)) was determined using a C-18 reverse-phase HPLC column. \textsuperscript{c}Percentage plasma protein binding (%PPB) was determined using a human serum albumin coated HPLC column. Reagents and conditions for reaction below the table title: (a) HBTU, Et\textsubscript{3}N, DMF, room temperature (rt), 72 h; (b) HBTU, DIPEA, CH\textsubscript{2}Cl\textsubscript{2}, rt, 24 h; (c) EDCl, DMAP, CH\textsubscript{2}Cl\textsubscript{2}, rt then reflux, 24 h; (d) HBTU, DIPEA, DMF, 50 °C, 24 h; (e) DIPEA, CH\textsubscript{2}Cl\textsubscript{2}, rt, 24 h.

### Table 2. Cellular PARP Inhibitory Properties of Compounds 1 and 8

| compound | cellular IC\textsubscript{50} (95\% CI), nM\textsuperscript{a} |
|----------|-----------------------------------------------------------|
| 1        | 1.6 (1.4–1.8) 1.6 (1.4–1.8)                              |
| 8        | 1.0 (0.9–1.2) 0.8 (0.7–0.9)                               |

\textsuperscript{a}Cellular IC\textsubscript{50} values obtained using primary G7 and established T98G human glioblastoma cell lines are based on two experiments.
using a multistep early-stage radiofluorination approach that circumvented the aforementioned issue of poor activation.\(^{17}\) Therefore, our attention was redirected toward an alternative target compound, \(20\), bearing a \(p\)-(fluoromethyl)benzamide group. It was proposed that a precursor analog of \(20\) would be more amenable to radiofluorination than \(p\)-nitrobenzamide \(14\), thereby allowing for late-stage radiofluorination and radio-synthetic automation. Compound \(20\) was synthesized by first performing an amide coupling reaction between mono-Boc protected piperazine \(15\) and commercially available 4-(chloromethyl)benzoic acid, giving access to intermediate \(16\) in 40% yield (Scheme 2). \(p\)-Chlorobenzamide \(16\) was then subjected to nucleophilic fluorination with tetra-n-butylammo-
nium fluoride (TBAF), and this was followed by acid-mediated cleavage of the Boc-protecting group to give \(18\) in 87% yield over two steps. Finally, reaction of carboxylic acid \(19\), previously synthesized within our research group,\(^{19}\) and piperazine \(18\) under standard amide coupling conditions gave \(20\). Intrinsic clearance (Cl\(_{int}\)) values of two independent experiments acquired using human liver microsomes.

Table 3. Physicochemical, Cell-Free PARP-1 and Cellular PARP Inhibitory, and Mouse Plasma and Metabolic Stability Properties of 1 and 20

| compound | log \(P_{oct}\)\(^{a}\) | %PPB\(^{b}\) | cell-free IC\(_{50}\) (95% CI), nM\(^{c}\) | plasma stability, percent\(^{d}\) | cellular IC\(_{50}\) (95% CI), nM\(^{e}\) | Cl\(_{int}\) μL min\(^{-1}\) mg\(^{-1}\) |
|----------|-----------------|-------|-----------------------------|-----------------|-----------------------------|-----------------------------|
| 1        | 1.95            | 75.9  | 11.9 (10.5–13.6)            | 1.6 (1.4–1.8)   | 1.6 (1.4–1.8)               | 23                          |
| 20       | 2.51            | 89.3  | 2.0 (1.9–2.2)               | 1.3 (0.7–2.3)   | 2.0 (1.3–3.1)               | 74                          |

\(^{a}\)Lipophilicity (log \(P_{oct}\)) was determined using a C-18 reverse-phase HPLC column.\(^{b}\)Percentage plasma protein binding (%PPB) was determined using human serum albumin coated HPLC column.\(^{c}\)Cell-free IC\(_{50}\) values are based on three experiments.\(^{d}\)Cellular IC\(_{50}\) values obtained using primary G7 and established T98G human glioblastoma cell lines are based on two experiments.\(^{e}\)The mean percentage of parent compound remaining after a 20 h incubation in mouse plasma (±SD of 3 experiments) was ascertained for compound 20.\(^{f}\)Intrinsic clearance (Cl\(_{int}\)) values of two independent experiments acquired using human liver microsomes.
compound 20 had a cell-free IC\textsubscript{50} value that was 6- and 3-fold less than that acquired for 1 and 8, respectively. Conversely, the cellular PARP inhibitory properties of 20 were comparable to compound 1 and marginally weaker than ascertained for compound 8. Collectively, the optimal physiochemical properties and low nanomolar PARP cell-free and cellular IC\textsubscript{50} values supported further investigation of 20 as a potential radiotracer for PARP. In the body, radiotracers can be exposed to a number of metabolic pathways, including blood plasma hydrolysis and liver functionalization or conjugation reactions that can have a significant effect on the kinetic properties of the tracer, and, subsequently its usefulness in nuclear imaging. With this in mind, the in vitro plasma and liver microsomal stability of lead candidate 20 were established by incubating the compound in mouse plasma proteins and human liver microsome enzymes, respectively, using previously described methodologies. Candidate 20 appeared stable in mouse plasma with negligible decomposition following a 20 h incubation (Table 3). However, the intrinsic clearance parameter (a predictor of phase I liver metabolism) was approximately 3-fold greater for compound 20 in comparison to 1. Despite this, it was proposed that compound 20 would exhibit sufficient tissue retention to allow for nuclear imaging of PARP. This was justified by previously acquired data using a radioiodinated \textit{p}-iodobenzoamide analog that exhibited similar in vitro intrinsic clearance properties to 20 but still displayed a degree of retention in PARP over-expressing tumor tissue.\textsuperscript{19} Initial attempts to generate the radiofluorinated version of 20 involved performing nucleophilic substitution reactions between the \textsuperscript{18}F\textsuperscript{−} nucleophile and the chloromethyl group of precursor 21, which was obtained by amide coupling of 7 with commercially available 4-(chloromethyl)benzoic acid (Scheme 3). However, these attempts were not successful because competing oligomerization reactions between the chloromethyl group of 21 and the phthalazine core prevented effective radiofluorination. To overcome this issue, the phthalazine core of 21 was Boc-protected to give compound 22.

Precursor 22 was then subjected to a screen of radiofluorination conditions as outlined in Table 4. The radiochemical yield (based on HPLC analysis of the crude product) was 30\% when tetra-n-butylammonium hydrogen carbonate (TBAHCO\textsubscript{3}) was used as a phase-transfer agent, which was markedly higher in comparison to that obtained for Kryptofix (K\textsubscript{222}) (entries 1 and 2). It has been reported in the literature that the introduction of a sterically hindered protic alcohol can have a beneficial effect on aliphatic nucleophilic radiofluorination reactions.\textsuperscript{31,32} With the use of a 2:1 mixture of \textit{t}-BuOH and MeCN as the reaction solvent, the radiofluoride incorporation increased from 30\% to 48\% in comparison to the same volume of MeCN alone (entries 1 and 3). A 30 min reaction time was established to be optimal based on lower radiochemical yields observed after a shorter reaction time (entry 4) and the short-lived nature of the \textsuperscript{18}F radioisotope (half-life of 109.8 min), which prevented longer reaction periods. Radiofluoride incorporation was further improved by increasing the reaction temperature from 100 to 110 °C, which resulted in a 51\% radiochemical yield (entry 5). Interestingly, doubling the reaction solvent volume had a negative impact on the degree of radiofluorination (entry 6). Based on these data, the reaction conditions described in entry 5 were deemed optimal.

To access \textsuperscript{18}F\textsuperscript{−}20 compound \textsuperscript{18}F\textsuperscript{−}23 was subjected to Boc deprotection, which was achieved in 5 min and with minimal defluorination taking place by using water as an acid--base catalyst (Scheme 4). The use of hydrochloric acid was also investigated as a deprotecting agent, but its use was associated with marked defluorination (see the Supporting Information). The optimized two-step one-pot radiochemical reaction allowed access to \textsuperscript{18}F\textsuperscript{−}20 in a radioactivity yield (isolated product) of 9 ± 2\% (n = 7) and a molar activity of >4.32 ± 1.46 Ci \textmu mol\textsuperscript{−1} (n = 3). Importantly, the one-pot nature of the reaction opens up the potential for radiosynthetic automation.

**In Vivo Characterization.** Following successful optimization of the radiochemistry, the behavior of \textsuperscript{18}F\textsuperscript{−}20 was investigated in vivo in mice bearing subcutaneous U87MG-Luc2 human glioblastoma tumor xenografts using ex vivo biodistribution and PET--MR imaging techniques. Ex vivo biodistribution of \textsuperscript{18}F\textsuperscript{−}20 was established at 30, 60, and 120 min after intravenous radiotracer administration (Figure 3a,b) and mostly concentrated in the cecum matter and solid feces at 120 min (Figure 3a). This is in line with our previous findings\textsuperscript{19} and other literature reports,\textsuperscript{33,34} which showed in vivo hepatobiliary clearance of a range of related radiodinated and radiofluorinated compounds based on the structure of 1. Interestingly, the mean percentage of injected dose per gram (%ID/g) of femur tissue remained relatively high across all three biodistribution time points (i.e., >8.5\%; Figure 3a), which was also confirmed by PET imaging, in which high skeletal uptake of radioactivity was visible (Figure 3b). This is in contrast to observations made by Carney et al., who reported <2\%ID/g of bone of \textsuperscript{18}F\textsuperscript{−}8 at 120 min.\textsuperscript{34} The high bone uptake seen with \textsuperscript{18}F\textsuperscript{−}20 could be explained by in vivo defluorination and subsequent radiofluoride accumulation in bone tissue.\textsuperscript{35} Despite this, apparent radiotracer tumor uptake was identified in both biodistribution and PET--MR imaging experiments (Figures 3a,c), whereas in the case of the former, the mean ratio of %ID/g of glioblastoma tumor to muscle increased from 1.9 ± 0.5 (n = 4) to 3.6 ± 0.5 (n = 4) between the 30 and 120 min time points. This was

---

**Scheme 3. Synthetic Route Used to Generate \textit{p}-Chloromethylbenzamide Precursor 22**

![Scheme 3](image-url)
suggestive of $[^{18}\text{F}]20$ retention in U87Mg-Luc2 glioblastoma tissue, which we have shown to be highly proliferative (see the Supporting Information) and to over-express PARP relative to muscle tissue (Figure 3d).

To establish the specificity of $[^{18}\text{F}]20$ for PARP in bone and tumor tissue, further biodistribution studies were performed on subcutaneous U87MG-Luc2 glioblastoma bearing mice that had PARP binding sites blocked by pretreatment with nonradioactive compound 1 at a dose of 50 mg/kg 20 min prior to $[^{18}\text{F}]20$ administration. The uptake of $[^{18}\text{F}]20$ in bone tissue remained high 60 min after radioligand administration and was not influenced by the presence of excess nonradioactive 1 (Figure 3e). This supports the earlier proposed possibility of in vivo $[^{18}\text{F}]20$ defluorination, leading to free fluoride accumulation in the bone. Despite this, preblockade using 1 resulted in a statistically significant decrease in the ratio of %ID/g of tumor to muscle from 1.79 ± 0.39 ($n = 4$) to 1.07 ± 0.15 ($n = 4$) 60 min after $[^{18}\text{F}]20$ administration in comparison to vehicle-pretreated mice (unpaired t-test; $P < 0.05$). These findings suggested that the uptake of $[^{18}\text{F}]20$ in tumor was due to specific PARP binding, which was in line with our previous findings for a related radiiodinated analog of 1.19

### CONCLUSIONS

We described here the identification and one-pot manual radiosynthesis of a PARP PET imaging agent, $[^{18}\text{F}]20$, that was accessed in good radioactivity yield and showed desirable physiochemical properties but suffered from rapid hepatobiliary clearance and in vivo defluorination followed by nonspecific $[^{18}\text{F}]$ bone tissue uptake in mice. Despite this, the radiotracer compound exhibited specific PARP binding and subcutaneous glioblastoma tumor retention properties, which allowed for preclinical PET visualization of the PARP over-expressing tumor. Therefore, we believe that $[^{18}\text{F}]20$ should remain a potential candidate radiotracer for in vivo PARP PET imaging and that future imaging studies using small-animal intracranial glioblastoma models are required to further assess the utility of this compound.

### EXPERIMENTAL SECTION

All reagents and starting materials were obtained from commercial sources and used as received. Dry solvents were purified using a solvent purification system, and all reactions were performed under an atmosphere of argon unless stated otherwise. Macherey-Nagel aluminum-backed plates precoated with silica gel 60F254 were used for thin layer chromatography and were visualized with a UV lamp. Flash column chromatography was performed using Fisher matrix silica gel 60 (35–70 μm). $^1\text{H}$ NMR spectra were recorded on a Bruker DPX 400 or Bruker 500 spectrometer, and data are reported as follows: chemical shift in parts per million (ppm) relative to Me$_4$Si or the solvent (CDCl$_3$, δ $7.26$ ppm; CD$_3$OD, δ $3.31$ ppm; or dimethyl sulfoxide [DMSO-$d_6$, δ $2.50$ ppm] as the internal standard, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet or overlap of nonequivalent resonances, integration). $^{13}\text{C}$ NMR
were carried out in compliance with UK Home Office regulations. The synthesis of compounds 1, 7, and 15 has been reported previously.19

Figure 3. (a) Ex vivo biodistribution of $[^{18}F]20$ in subcutaneous human U87MG-Luc2 glioblastoma bearing nude mice 30 min ($n = 4$), 60 min ($n = 4$), and 120 min ($n = 4$) after tracer injection (error bars represent the mean percentage of injected dose per gram of tissue or material (% ID/g) plus SD). (b) Mouse whole-body maximum intensity projection. (c) 1T GRE 3D coronal and sagittal MRI, PET, and PET–MR co-registered images of a nude mouse bearing a subcutaneous U87MG-Luc2 human glioblastoma (white arrows) acquired by performing a 45 min dynamic scan following $[^{18}F]20$ administration (PET images represent a summation of the last 15 min of the scan). (d) Representative immunohistochemistry images of U87MG-Luc2 subcutaneous tumor and muscle tissue isolated from nude mice stained for PARP-1 and counter-stained with hematoxylin (brown staining signifies the presence of PARP-1, and dark blue staining shows cellular nuclei). (e) Ratio of percentage of injected dose per gram (%ID/g) of tissue of interest to muscle of $[^{18}F]20$, 60 min after injection in subcutaneous U87MG-Luc2 human glioblastoma bearing nude mice pretreated with either vehicle ($n = 3$) or 50 mg/kg of I ($n = 3$) (error bars represent the mean plus SD; unpaired t-test: $P < 0.05$).
4-[3-"4-fluoro-phenyl]acetyl]-[piperazine-1-"carboxy]-4-"fluorobenzyl]-2H-phthalazin-1-one (11). To a solution of 4-fluorophenylacetic acid (0.043 g, 0.28 mmol) in dimethylformamide (DMF; 5 mL) was added DIPEA (0.052 g, 0.27 mmol), and the reaction mixture was stirred at ambient temperature for 0.5 h. 4-Dimethylaminopyridine (DMAP; 0.017 g, 0.14 mmol) was then added and the mixture was stirred for a further 0.5 h. A solution of 7 (0.100 g, 0.273 mmol) in dichloromethane (5 mL) was then added drop-wise and the resultant reaction mixture stirred vigorously under reflux for 24 h. Upon cooling to ambient temperature, water (5 mL) was added and the organic layer was washed with water (3 × 5 mL) and a NaHCO3 solution (5 mL), dried with MgSO4, filtered, and concentrated in vacuo. Purification using flash column chromatography (MeOH/CH2Cl2; 1:1) gave 10 (0.043 g, 31%) as a white solid. NMR spectra showed a 59:41 mixture of rotamers. Only data for the major rotamer were recorded; mp 175–177 °C; IR (neat): 3188, 2926, 1632, 1462, 1249, 1256, 1159, 1003, 772, 727 cm⁻¹; 1H NMR (400 MHz, CDCl3, δ): 2.32 (s, 3H), 3.15–3.45 (m, 4H), 3.55–4.10 (m, 4H), 4.30 (s, 2H), 6.85–7.18 (m, 4H), 7.26–7.39 (m, 2H), 7.67–7.81 (m, 3H), 8.40–8.50 (m, 10H), 10.13 (s, 1H); 13C NMR (100 MHz, CDCl3, δ): 27.0 (CH), 33.4 (CH), 37.7 (CH), 42.0 (CH), 42.1 (CH), 45.1 (CH), 46.8 (CH3), 115.7 (2 × CH, d, JCH,CH = 11.9 Hz), 116.2 (CH, d, JCH,CH = 19.1 Hz), 123.7 (C, d, JCH,CH = 17.6 Hz), 125.0 (CH), 127.2 (CH), 128.3 (C), 133.2 (C), 133.6 (C), 143.5 (C, d, JCH,CH = 3.3 Hz), 145.5 (C), 150.7 (C, d, JCH,CH = 24.7 Hz), 160.7 (CH), 165.3 (CH), 165.8 (CH), 165.8 (CH), 165.8 (CH), 165.8 (CH); HRMS (ESI): [M+H]+ calc for C29H26F2N4ClO4, 567.1814; found, 567.1796. 4-[3-"4-fluoro-phenyl]acetyl]-[piperazine-1-"carboxy]-4-["fluorobenzyl]-2H-phthalazin-1-one (13). To a stirred solution of 7 (0.023 g, 0.063 mmol) in dichloromethane (3 mL) was added 4-fluorobenzyl chloride (7.9 μL, 0.066 mmol) followed by DIPEA (21.9 μL, 0.126 mmol). The resultant reaction mixture was stirred at ambient temperature for 12 h, and then water (5 mL) was added. The mixture was washed with water (2 × 5 mL) and the organic layer was dried with MgSO4, filtered, and concentrated in vacuo. Purification using flash column chromatography (MeOH/CH2Cl2; 1:1) gave 13 (0.011 g, 38%) as an off-white solid; mp 109–111 °C; IR (neat): 3185, 2916, 1637, 1508, 1437, 1346, 1211, 1148, 999 cm⁻¹; 1H NMR (400 MHz, CDCl3, δ): 2.34 (br s, 2H), 2.50 (t, J = 5.2 Hz, 2H), 3.28 (br s, 2H), 3.48 (s, 2H), 3.75–3.82 (m, 2H), 4.28 (s, 2H), 6.79–7.04 (m, 3H), 7.31–7.37 (m, 4H), 7.69–7.78 (m, 4H), 8.10–8.50 (m, 10H), 10.86 (s, 1H); 13C NMR (100 MHz, CDCl3, δ): 37.8 (CH3), 47.1 (CH), 52.5 (CH3), 62.0 (CH2), 115.2 (2 × CH, d, JCH,CH = 21.2 Hz), 116.1 (CH, d, JCH,CH = 21.8 Hz), 124.3 (C, d, JCH,CH = 18.3 Hz), 125.1 (CH), 127.2 (CH), 128.3 (C), 129.1 (CH, d, JCH,CH = 3.8 Hz), 129.6 (C), 130.5 (2 × CH, d, JCH,CH = 7.9 Hz), 131.2 (CH, d, JCH,CH = 7.9 Hz), 131.6 (CH), 133.3 (C, d, JCH,CH = 3.0 Hz), 133.7 (CH), 133.4 (C, d, JCH,CH = 3.4 Hz), 145.6 (C), 150.7 (C, d, JCH,CH = 24.7 Hz), 160.5 (CH), 162.1 (C, d, JCH,CH = 245.0 Hz), 164.8 (CH); HRMS (ESI): [M+H]+ calc for C27H21F3N3O3, 475.1940; found, 475.1929.
flash column chromatography (hexane/EtOAc, 1:1) gave 16 as a white solid (144 mg, 40%); mp 140–142 °C; IR (neat): 3003, 2881, 1681, 1622, 1568, 1426, 1349, 1263, 724, 686 cm⁻¹; ¹H NMR (400 MHz, CDCl₃, δ): 1.47 (s, 9H), 3.28–3.84 (m, 8H), 4.60 (s, 2H), 7.43 (q, J = 11.9 Hz, 4H); ¹³C NMR (101 MHz, CDCl₃, δ): 28.5 (3 × CH₃), 43.8 (2 × CH₂), 45.6 (CH₂), 47.7 (2 × CH₂), 80.5 (C), 127.6 (2 × CH), 128.8 (2 × CH), 135.6 (C), 139.4 (C), 154.6 (C), 170.1 (C); HRMS (ESI): [MNa⁺] calcd for C₁₂H₁₅FN₂NaO₃ 245.1061; found, 245.1064.

Tert-Butyl Piperazine-4-[(4'-fluoromethyl)benzoyl]-1-carbonyl-1-carboxylate (17). To a solution of 16 (55.0 mg, 0.162 mmol) in MeCN (1.5 mL) was added a 1 M solution of TBAF (325 µL, 0.325 mmol) in tetrahydrofuran. The mixture was heated to 80 °C and stirred for 1 h followed by evaporation of solvent in vacuo. The crude product was extracted into dichloromethane (5 mL), and the crude product was purified by flash column chromatography (MeOH/EtOAc, 1:19) gave 17 (52.0 mg, 0.161 mmol) in dichloromethane (1 mL) was added triethylamine (26.0 mg, 0.325 mmol) in tetrahydrofuran. The mixture was heated to 80 °C for 4 h. The reaction was cooled to 0 °C, added triethylamine (26.0 mg, 0.325 mmol) in tetrahydrofuran, and stirred for 1 h followed by evaporation of solvent in vacuo. The mixture was stirred into tripotassium dicitrato-bis(oxalate) (18). To a solution of 17 (50.0 mg, 0.160 mmol) in dichloromethane (1 mL) was added trifluoroacetic acid (124 µL, 1.61 mmol), and the mixture was stirred at room temperature for 4 h. The crude product was extracted into EtOAc (5 mL), and the organic layer was washed with water (3 × 10 mL), dried with MgSO₄, filtered, and concentrated in vacuo to give 17 (52.0 mg, 0.160 mmol) as a white solid; mp 96–98 °C; IR (neat): 3013, 2928, 1690, 1630, 1420, 1340, 1263, 1012, 724, 668 cm⁻¹; ¹H NMR (400 MHz, CDCl₃, δ): 7.72 (m, 1H), 7.73 (m, 1H), 7.78 (m, 2H), 8.49 (m, 1H); ¹³C NMR (101 MHz, CDCl₃, δ): 28.5 (3 × CH₃), 43.8 (2 × CH₂), 45.6 (CH₂), 47.7 (2 × CH₂), 80.5 (C), 127.6 (2 × CH), 128.8 (2 × CH), 135.6 (C), 139.4 (C), 154.6 (C), 170.1 (C); HRMS (ESI): [MNa⁺] calcd for C₂₈H₂₄F₂N₄NaO₃ 525.1738; found, 525.1734.

4-[3′-(4'-Fluorobenzyl)benzoyl]piperazine-1′-carbonyl-1′-fluorobenzoyl]-2-tert-butoxy carbonyl phthalazin-1-one (22). To a solution of 17 (20.0 mg, 0.0328 mmol) in MeCN (1.5 mL) was added diisopropyl carbamate (860 mg, 0.0393 mmol) and DMAP (4.00 mg, 0.0328 mmol). The mixture was stirred at room temperature for 4 h followed by the evaporation of solvent in vacuo. The crude product was extracted into EtOAc (5 mL), and the organic layer was washed with water (3 × 10 mL), dried with MgSO₄, filtered, and concentrated in vacuo to give 22 as a white foam (148 mg, 72%); IR (neat): 3013, 2928, 1690, 1630, 1420, 1340, 1263, 1012, 724, 668 cm⁻¹; ¹H NMR (400 MHz, CDCl₃, δ): 1.66 (s, 9H), 3.15–3.99 (m, 8H), 4.30 (s, 2H), 4.59 (s, 2H), 7.03 (br s, 1H), 7.37–7.49 (m, 1H); ¹³C NMR (101 MHz, CDCl₃, δ): 27.3 (3 × CH), 38.1 (CH₂), 42.2 (2 × CH₂), 45.4 (CH), 47.1 (2 × CH₂), 85.9 (C), 116.2 (CH, d, J₁₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋˓→
h until pH 7.7 was reached, followed by the removal of excess CO₂ by bubbling argon through the mixture until pH 9.0 was reached. The 0.750 M TBAHCO₃ solution (10 μL) was added to a 2 mL v-vial containing 469–572 MBq of [¹⁸F]H₂O (0.16 mL). The mixture was vortexed, and the [¹⁸F]fluoride was dried by passing a constant stream of argon over the solution at 100 °C for approximately 20 min; anhydrous MeCN was added in three aliquots of 0.50 mL to facilitate azeotropic drying. A solution of 22 (4.9 mg, 0.0079 mmol) in anhydrous MeCN (0.1 mL) and BuOH (0.2 mL) was then added to the v-vial, and the reaction was allowed to proceed for 30 min at 110 °C. After this, distilled water was added (0.45 mL), and the reaction was heated at 120 °C for a further 5 min. Next, the crude reaction mixture was cooled to room temperature, purified using HPLC, and concentrated in vacuo in an evaporator flask. The flask was rinsed with MeCN (3 x 0.3 mL) to extract the radiolabeled compound, and the solution was transferred to a 2 mL v-vial; the solvent was removed by passing a constant stream of argon over the solution at 100 °C for approximately 15 min. The radiotracer was reconstituted in up to 0.65 mL of 5% v/v DMSO in 0.9% w/v saline. The total radiosynthetic time was 138 ± 16 min (n = 6).

HPLC Analysis and Purification of Radiotracers. All radiochemical yields were determined by analytical radio-HPLC of the crude product. Radioactivity yield was determined using the measured radiospecificity of the isolated product. Analytical and preparatory Dionex UltiMate 300 series HPLC systems with Phenomenex Synergi 4 μm Hydro-RP 80 Å 150 mm × 4.60 mm and 150 mm × 10 mm columns, respectively, were used. Column temperatures were set to 25 °C, and mobile-phase flow rates were 1 and 3 mL/min for the analytical and preparatory systems, respectively. Analytical UV detection was performed using a MultiMate diode array detector (190−800 nm), and radiodetection was achieved using a Berthold Technologies Flow Star LB513 detector. Preparatory UV detection was performed using a Knauer Advanced Scientific Instruments Smartline UV Detector 2500, and radiodetection was achieved using a pin-diode connected to a Lab Logic Flow-Count radiodetector. Analysis of [¹⁸F]8, [¹⁸F]²⁰, and [¹⁸F]²³ were performed on crude reaction mixtures that were cooled to room temperature. [¹⁸F]8 was analyzed using the following mobile-phase conditions: 0.0−20.0 min, 30:70 A/B to 55:45 A/B; 20.0−20.5 min, 55:45 A:B to 5:95 A/B; and 20.5−25.0 min, 5:95 A/B, where A is MeCN and B is distilled water. [¹⁸F]²⁰ and [¹⁸F]²³ were analyzed using the following mobile-phase conditions: 0.0−15.0 min, 30:70 A/B to 90:10; 15.0−20.0 min, 90:10 A/B to 20:0; 20.0−20.5 min, 20:0 A/B to 30:70 A/B; and 20.5−25.0 min, 30:70 A/B, where A is MeCN and B is distilled water. Purification of [¹⁸F]²⁰ was performed using the following mobile-phase conditions: 0.0−3.0 min, 30:70 A/B; 3.0−30.0 min, 30:70 to 75:25 A/B; 30.0−31.0 min, 75:25 to 95:5 A/B; and 30.1−35.0 min, 95:5 A/B, where A is MeCN and B is distilled water; the radiolabeled product was collected at approximately 13.5 min. To confirm the identity of the radiolabeled products, the retention times of [¹⁸F]8, [¹⁸F]²⁰, and [¹⁸F]²³ were compared with the retention times obtained for nonradioactive 8, 20, and 23 using the same chromatographic conditions. Because the amount of [¹⁸F]²⁰ that was produced fell below the sensitivity threshold of the UV detector (<1.68 × 10⁻⁶ μmol), molar activity (Ci/μmol) was calculated using the lowest detectable amount of nonlabeled 20 established from a calibration plot of a range of concentrations (0.001−1.000 mg/mL). All HPLC data acquisition and analyses were carried out using the Chromeleon 6.8 Chromatography software.

Mouse Glioblastoma Model. U87MG-Luc2 glioblastoma cell culture and implantation procedures were performed as reported previously. Briefly, U87MG-Luc2 cells were purchased commercially from ATCC and cultured in minimum essential media that has been supplemented with 10% v/v fetal calf serum and 2 mM l-glutamine at 37 °C and 5% v/v CO₂. Unconscious female CD1 nude mice (6−11 week old; purchased from Charles River Laboratories) had 5 × 10⁶ U87MG-Luc2 cells injected subcutaneously into the right flank. The resulting tumor xenografts were measured and monitored visually every 3 days, and tumor-bearing animals were used for in vivo studies 28−30 days post-implantation.

Ex Vivo Biodistribution with and without Preblockade. Subcutaneous tumor bearing mice were administered 1.8−2.6 MBq of [¹⁸F]²⁰ in 0.11−0.21 mL of 5% v/v DMSO in 0.9% saline via bolus tail-vein injections. The remainder of the experiment was conducted as described previously. PET−MR Imaging. A single subcutaneous U87MG-Luc2 tumor bearing female CD1 nude mouse was anesthetized using inhaled isoflurane (in medical air; induction 5% v/v; maintenance 2.0−2.5% v/v) and placed in the nanoScan PET−MRI scanner (Mediso Medical Imaging Systems). The mouse received a bolus tail-vein injection of 2.2 MBq of [¹⁸F]²⁰ in 5% v/v DMSO in 0.9% saline, and a 45 min dynamic PET scan (continuous list mode) was initiated immediately after. This was followed by a whole-body 1T GRE 3D Cor/Sag MRI scan. The dynamic PET data were corrected for random coincidences, dead time, scatter, and decay and subsequently reconstructed using 3D Terra-Tomo (Mediso). The reconstructed PET data were automatically co-registered with the MRI data, and they were subsequently analyzed using the PMOD 3.504 software. The PET frames from the last 15 min of the scan were summed to allow for better visual representation of radiotracer biodistribution. Standardized uptake values (SUV) were determined by dividing the image radiotracer concentration by the injected dose divided by the animal weight.

Immunohistochemistry. Tumor and muscle tissue was prepared and stained using anti-PARP-1 antibody (mouse antihuman and mouse antibody; sc-8007, Santa Cruz), as reported previously. Haematoxylin and eosin and K67 staining were performed using a Leica ST5020 multi-stainer. Histology images were acquired using a Zeiss AX10 brightfield microscope at a 5X magnification.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.8b00138.

Molecular formula strings for compounds presented in the manuscript. (CSV)

¹H and ¹³C NMR spectra for all new compounds, cell-free and cellular IC₅₀ curves, metabolic stability graphs, radiofluorination optimization, optimization of deprotection chemistry, immunohistochemical staining of the U87MG-Luc2 subcutaneous xenograft, radiochemistry HPLC chromatograms, and purity assessment HPLC chromatograms for key compounds. (PDF)

AUTHOR INFORMATION

Corresponding Author

E-mail: filipzmuda@gmail.com. Phone: +44-(0)1413304924.

ORCID

Filip Zmuda: 0000-0003-3710-0622
Maria Clara Liuzzi: 0000-0002-6121-899X
Andrew Sutherland: 0000-0001-7907-5766

Notes

The authors declare the following competing financial interest(s): Partial funding received from AstraZeneca, who are patent holders for the PARP inhibitor olaparib.

ACKNOWLEDGMENTS

The authors acknowledge Dr. Shafiq Ahmed for providing guidance concerning the cellular IC₅₀ assays and Dr. Lesley Gilmour and Sandeep Chahal for their input in setting up the necessary small-animal xenograft models. The authors also gratefully acknowledge financial support from the University of Glasgow (studentship FZ), EPSRC (EP/J00434), the Cancer Research U.K. Glasgow Centre Development Fund, The
Neuroscience Foundation, The Beatson Cancer Charity, AstraZeneca, and the Scottish Imaging Network: A Platform for Scientific Excellence.

**ABBREVIATIONS USED**

ATCC, American type culture collection; BBB, blood–brain barrier; BRCA, breast cancer; Cl<sub>N</sub>, intrinsic clearance; IC<sub>50</sub>, half-maximum inhibitory concentration; ID/g, injected dose per gram of material; K<sub>252</sub>, Krypton-252; log <i>P</i><sub>oct</sub>, lipophilicity; MBq, megabecquerel; MR, magnetic resonance; MRI, magnetic resonance imaging; PARP, poly(ADP-ribose) polymerase; PARP-1, poly(ADP-ribose) polymerase-1; PBP, plasma-protein binding; PTA, phase-transfer agent; SD, standard deviation; SUV, standardized uptake value; t-BuOH, tert-butyl alcohol; TBAF, tetra-n-butylammonium hydroxide; TBAHCO<sub>3</sub>, tetra-n-butylammonium hydrogen carbonate

**REFERENCES**

(1) Amé, J.; Spenlehauer, C.; de Murcia, G. The PARP superfamily. *BioEssays* 2004, 26, 882–893.
(2) Birkle, A. (Poly)ADP-ribose: the most elaborate metabolite of NAD<sup>+</sup>. *FEBS J.* 2005, 272, 4576–4589.
(3) U.S. Food and Drug Administration. *Orange Book: Approved Drug Products with Therapeutic Equivalence Evaluations*. (8th ed.); U.S. Government Printing Office: Washington, DC, 1991.

**ABBREVIATIONS USED**

ATCC, American type culture collection; BBB, blood–brain barrier; BRCA, breast cancer; Cl<sub>N</sub>, intrinsic clearance; IC<sub>50</sub>, half-maximum inhibitory concentration; ID/g, injected dose per gram of material; K<sub>252</sub>, Krypton-252; log <i>P</i><sub>oct</sub>, lipophilicity; MBq, megabecquerel; MR, magnetic resonance; MRI, magnetic resonance imaging; PARP, poly(ADP-ribose) polymerase; PARP-1, poly(ADP-ribose) polymerase-1; PBP, plasma-protein binding; PTA, phase-transfer agent; SD, standard deviation; SUV, standardized uptake value; t-BuOH, tert-butyl alcohol; TBAF, tetra-n-butylammonium hydroxide; TBAHCO<sub>3</sub>, tetra-n-butylammonium hydrogen carbonate

**REFERENCES**

(1) Amé, J.; Spenlehauer, C.; de Murcia, G. The PARP superfamily. *BioEssays* 2004, 26, 882–893.
(2) Birkle, A. (Poly)ADP-ribose: the most elaborate metabolite of NAD<sup>+</sup>. *FEBS J.* 2005, 272, 4576–4589.
(3) U.S. Food and Drug Administration. *Orange Book: Approved Drug Products with Therapeutic Equivalence Evaluations*. (8th ed.); U.S. Government Printing Office: Washington, DC, 1991.

(1) Amé, J.; Spenlehauer, C.; de Murcia, G. The PARP superfamily. *BioEssays* 2004, 26, 882–893.
(2) Birkle, A. (Poly)ADP-ribose: the most elaborate metabolite of NAD<sup>+</sup>. *FEBS J.* 2005, 272, 4576–4589.
(3) U.S. Food and Drug Administration. *Orange Book: Approved Drug Products with Therapeutic Equivalence Evaluations*. (8th ed.); U.S. Government Printing Office: Washington, DC, 1991.
conformational perturbation of macromolecules by small organic compounds. *Biochemistry* 1968, 7, 2858–2863.

(29) Jacobson, O.; Chen, X. Interrogating tumor metabolism and tumor microenvironments using molecular positron emission tomography imaging. Theranostic approaches to improve therapeutics. *Pharmacol. Rev.* 2013, 65, 1214–1256.

(30) Tavares, A.; Lewsey, J.; Dewar, D.; Pimlott, S. Radiotracer properties by high performance liquid chromatography: a potential tool for brain radiotracer discovery. *Nucl. Med. Biol.* 2012, 39, 127–135.

(31) Lee, S. J.; Oh, S. J.; Chi, D. Y.; Lee, B. S.; Ryu, J. S.; Moon, D. H. Comparison of synthesis yields of 3′-deoxy-3′-[18F]fluorothymidine by nucleophilic fluorination in various alcohol solvents. *J. Labelled Compd. Radiopharm.* 2008, 51, 80–82.

(32) Kim, W.; Jeong, H.; Lim, T.; Sohn, M.; Katzenellenbogen, A.; Chi, Y. Facile nucleophilic fluorination reactions using tert-alcohols as a reaction medium: significantly enhanced reactivity of alkali metal fluorides and improved selectivity. *J. Org. Chem.* 2008, 73, 957–962.

(33) Salinas, B.; Irwin, P.; Kossatz, S.; Bolaender, A.; Chiosis, G.; Pillarsetty, N.; Weber, W. A.; Reiner, T. Radiiodinated PARP1 tracers for glioblastoma imaging. *EJNMMI Res.* 2015, 5, 1–14.

(34) Carney, B.; Carlucci, G.; Salinas, B.; Di Gialleonardo, D.; Kossatz, S.; Vansteene, A.; Longo, V. A.; Bolaender, A.; Chiosis, G.; Keshari, K. R.; Weber, W. A.; Reiner, T. Non-invasive PET imaging of PARP1 expression in glioblastoma models. *Mol. Imaging Biol.* 2016, 18, 386–392.

(35) Pike, V. W. PET radiotracers: crossing the blood-brain barrier and surviving metabolism. *Trends Pharmacol. Sci.* 2009, 30, 431–440.