Novel Matrix Metalloproteinase Inhibitor [\(^{18}\text{F}\)]Marimastat-Aryltrifluoroborate as a Probe for \textit{In vivo} Positron Emission Tomography Imaging in Cancer

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Abstract

Matrix metalloproteinases (MMP), strongly associated pathogenic markers of cancer, have undergone extensive drug development programs. Marimastat, a noncovalent MMP inhibitor, was conjugated with FITC to label cellular metalloproteinase cancer targets in MDA-MB-231 cells \textit{in vitro}. Punctate localization of active transmembrane MMP14 was observed. For molecular-targeted positron emission tomography imaging of syngeneic 67NR murine mammary carcinoma \textit{in vivo}, marimastat was \(^{18}\text{F}\)-labeled using a shelf-stable arylboronic ester conjugate as a captor for aqueous \(^{18}\text{F}\)fluoride in a novel, rapid one-step reaction at ambient temperature. [\(^{18}\text{F}\)]Marimastat-aryltrifluoroborate localized to the tumors, with labeling being blocked in control animals first loaded with \(>10\)-fold excess unlabeled marimastat. The labeled drug cleared primarily via the hepatobiliary and gastrointestinal tract, with multiple animals imaged in independent experiments, confirming the ease of this new labeling strategy. Cancer Res 70(19); 7562–9. ©2010 AACR.

Introduction

Positron emission tomography (PET) for molecular imaging is one of the most powerful noninvasive imaging technologies for the production of high-resolution, three-dimensional images within deep tissue needed for human clinical application (1). Fluorine-18 (\(^{18}\text{F}\)) is the optimal PET radioisotope because of its short half-life leading to favorable radiotracer for cancer diagnostics despite a relative lack of target specificity (1). Hence, cancer-specific small molecule \(^{18}\text{F}\)-labeled radiotracers are urgently needed.

Rapid on-site production of bioactive specific molecular imaging agents is hampered by \(^{18}\text{F}\) labeling methodologies that traditionally have relied on C–F bond formation under conditions that are generally incompatible with biomolecule stability, such as scrupulously dry organic solvents at 70°C to 140°C. Overcoming these obstacles is plagued by relatively time-consuming multistep syntheses that are further complicated by side reactions requiring extensive purification. Coupled with the short half-life of \(^{18}\text{F}\) (110 min), these problems reduce specific activity and the imaging time window. Recently, Ting and colleagues (2) used arylboronate precursors to capture aqueous \(^{18}\text{F}\)fluoride in the form of an aryltrifluoroborate (ArBF\(_3\)) for \(^{18}\text{F}\)-labeling in a rapid, one-step synthesis under acidic aqueous conditions at room temperature. Indeed, the [\(^{18}\text{F}\)]aryltrifluoroborate was stable \textit{in vivo} because no [\(^{18}\text{F}\)]fluoride accumulation was detected in bone, a highly fluorophilic tissue (3). Such chemistries have the potential to fast-track the introduction of \(^{18}\text{F}\)-labeled bioconjugates in "kit form" for diagnostic imaging in humans.

To validate this novel labeling method for cancer imaging, we hypothesized that an optimal target would be highly expressed in many tumors, show discrimination between different cancer stages, and be targeted by nanomolar inhibitor drugs with clinically demonstrated safety. These criteria are met by matrix metalloproteinases (MMP) that are expressed...
and activated in most cancers and their peritumor stroma (4, 5). With many MMP inhibitor drug candidates reaching phase III clinical trials, their failure has nevertheless been disappointing (4, 6). Marimastat, a nanomolar hydroxamate peptidic broad-spectrum MMP inhibitor (7) with activity against related MMPs, such as the ADAMs and ADAMTSs that are also often associated with cancer (4, 5), is a noncovalent and thereby reversible inhibitor, and thus has reduced potential for tissue accumulation and unwanted irreversible side reactions in short-term imaging applications. In late phase III clinical trials involving thousands of patients with advanced cancer, including breast carcinoma trials, marimastat proved to be safe, although some patients reported musculoskeletal pain (7). Hence, we selected marimastat as a scaffold onto which a boronic acid could be grafted for aqueous $[^{18}F]$fluoride capture so as to develop a noninvasive, clinically safe radiotracer for the in vivo imaging of cancer by detection of elevated MMP levels. We present the first one-step radiolabeling for in vivo application of a molecular-targeted $[^{18}F]$-PET probe for cancer detection and show high specificity labeling of mammary carcinoma in mice.

Materials and Methods

Cell lines
MDA-MB-231 breast carcinoma cells were kindly provided by Dr. V.C. Jordan (Northwestern University, Chicago, IL) in 2002. Stable MMP14 transfectants were established and characterized by us as described previously (8, 9). The murine breast cancer cell line, 67NR (10), was kindly provided by Dr. Fred Miller (Karmanos Cancer Institute, Detroit, MI) in July 2008. The cell line was authenticated for growth in vitro and in vivo as described by us previously (11).

Syngeneic tumor model
Female BALB/c mice (7–9 weeks old; Taconic Laboratories) were injected with $1 \times 10^7$ viable 67NR/CMV-Luciferase murine mammary cancer cells into the right fourth mammary gland (50 μL in PBS per mouse; ref. 11).

Microarray analysis
Primary syngeneic 67NR tumor tissues and control mammary glands were dissected by laser capture microdissection (11). Total cellular RNA was extracted using RNeasy (Qiagen) and re-extracted and resuspended in 10 μL of diethylpyrocarbonate-treated water. RNA was quantified and qualified by carbonated-treated water. RNA was quantified and qualified by detection of elevated MMP levels. We present the first one-step radiolabeling for in vivo application of a molecular-targeted $[^{18}F]$-PET probe for cancer detection and show high specificity labeling of mammary carcinomas in mice.

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Chemical synthesis
Chemicals were purchased from Sigma-Aldrich and Acros Organics. Deuterated solvents were purchased from Cambridge Isotope Laboratories. Analytic and preparative TLC were performed using Silica Gel 60 F254 Glass TLC plates from EMD Chemicals. All $^1$H-nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 300 or 400 MHz instrument. Chemical shifts are reported using the δ scale in ppm and all coupling constants (J) are reported in hertz (Hz). Unless specified, $^1$H-NMR spectra are referenced to the tetramethylsilane peak ($\delta = 0.00$) and $^{19}$F-NMR spectra are referenced to NEAT trifluoroacetic acid ($\delta = 0.00$, $\sim$7.83 ppm relative to CFCl$_3$). Due to the presence of $^{19}$F contaminations in the NMR spectrometer probe, baseline corrections for samples <20 mmol/L in $^{19}$F concentration had to be adjusted by multipoint linear baseline correction using MestRec 4.9.9. This correction did not affect the absolute chemical shifts or integration ratios of $^{19}$F signals.

Final synthesis of compounds illustrated in Fig. 2A is described below. Synthesis of precursor compounds is fully detailed in Supplementary Materials and Methods (Note: if not specifically indicated, compound numbers refer to Supplementary Schemes 1 to 4).

Marimastat
$(2R,3S)-N^1-(8S,3,3$-dimethyl-1-(methylamino)-1-oxobutan-2-yl)-$N^3,3$-dihydroxy-2-isobutylbutanediamide (1). To a solution of methyl ester 5 ($92$ mg, 0.28 mmol) in methanol/THF (1:1, 0.8 mL) was added KCN (3 mg) followed by a solution of hydroxylamine in H$_2$O (50 wt%, 100 μL, 1.5 mmol) and the reaction stirred at room temperature for 16 hours. The mixture was then concentrated under reduced pressure and diluted with CH$_2$Cl$_2$/methanol (191.2, 2 × 10 mL). The CH$_2$Cl$_2$/methanol washings were filtered and concentrated under reduced pressure to afford marimastat (1, 77 mg, 83%) as a white solid. $^1$H-NMR (400 MHz, CD$_3$OD) δ 6.7 (1H), 6.45 (m, 1H), 5.50 (d, J = 6.7 Hz, 1H), 5.10 (s, 2H), 4.27 to 4.21 (m, 2H), 4.16 (br d, J = 6.5 Hz, 3H), 1.53 (m, 2H), 1.31 to 1.28 (m, 3H), 1.23 (s, 1H), 1.01 (s, 9H), 0.92 (d, J = 6.5 Hz, 3H), 0.89 (d, J = 6.5 Hz, 3H); $^{13}$C-NMR (100 MHz, CD$_3$OD) δ 176.1, 173.5, 169.7, 73.4, 62.5, 50.0, 39.9, 35.5, 27.4, 27.1, 26.3, 23.8, 22.6; HRMS (ESI) calculated for C$_{15}$H$_{29}$N$_3$O$_5$Na$^+$ (M + Na)$^+$ 354.05, found 354.02 (Marimastat; Fig. 2A, 1).

Marimastat-linker
$(1S,18R,19R)$-Methyl 15-tert-butyl-19-hydroxy-18-isobutyl-3,4,14,17-tetraoxo-1-phenyl-2,7,10-trioxo-1,13,16-triazaicosan-20-olate (11). To a solution of acetone 10 ($0.90$ g, 1.48 mmol) in anhydrous methanol (25 mL) was added p-TsOH–H$_2$O (63 mg) and the mixture heated to reflux for 70 minutes. The reaction was then concentrated and diluted with CH$_2$Cl$_2$ (40 mL) and brine (20 mL). The layers were separated and the aqueous phase was extracted with CH$_2$Cl$_2$ (2 × 10 mL). The combined organic extracts were dried (Na$_2$SO$_4$) and purified by column chromatography on silica gel (CH$_2$Cl$_2$/methanol, 98:2 then 95:5) to afford methyl ester 11 (0.71 g, 83%) as a white foam. $^1$H-NMR ($300$ MHz, CDCl$_3$) δ 7.35 to 7.29 (m, 5H), 6.58 (br d, J = 6.7 Hz, 1H), 6.45 (m, 1H), 5.50 (m, 1H), 5.10 (s, 2H), 4.27 to 4.21 (m, 2H), 4.16 (br d, J = 6.8 Hz, 1H), 4.01 (d, J = 6.3 Hz, 1H), 2.82 to 2.79 (m, 2H), 2.73 (s, 3H), 1.62 to 1.53 (m, 2H), 1.31 to 1.28 (m, 3H), 1.01 (s, 9H), 0.92 (d, J = 6.5 Hz, 3H), 0.89 (d, J = 6.5 Hz, 3H); $^{13}$C-NMR (100 MHz, CD$_3$OD) δ 176.1, 173.5, 169.7, 73.4, 62.5, 50.0, 39.9, 35.5, 27.4, 27.1, 26.3, 23.8, 22.6; HRMS (ESI) calculated for C$_{13}$H$_{24}$N$_3$O$_2$Na$^+$ (M + Na)$^+$ 354.05, found 354.02 (Marimastat; Fig. 2A, 1).
Marimastat-FITC

(13S,16R,17S)-Methyl 13-tert-butyl-1-(3′,6′-dihydroxy-3-oxo-3H-spiro[iso benzofuran-1,9′-xanthene]-5(6)-ylamino)-17-hydroxy-16-isobutyl-12,15-dioxo-1-thioxo-5,8-dioxa-2,11,14-triazaoctadecan-18-oate (13). To a solution of Cbz-17-hydroxy-16-isobutyl-12,15-dioxo-1-thioxo-5,8-dioxa-2,11,14-triazaoctadecan-18-oate (13). To a solution of Cbz-protected 11 (28 mg, 0.048 mmol) in methanol (4.5 mL) was added Pd/C (10%, 6.5 mg) and the mixture stirred under an atmosphere of H2(g) (balloon) for 1 hour. The reaction was then filtered through Celite, washing with methanol and CH2Cl2. The combined filtrate was concentrated under reduced pressure and used immediately in the next reaction. To a solution of the resultant amine 12 in anhydrous DMF (2 mL) was added fluorescein 5(6)-isothiocyanate (90%, 18.5 mg, 0.043 mmol) and the reaction stirred for 1.5 hours. The mixture was then concentrated and purified by chromatography on silica gel (CH2Cl2/methanol/ACOH, 95:5:0 then 89:10:1) to afford the desired fluorescein derivative 13 (23 mg, 58% over two steps) as a yellow oil (mixture of regioisomers). HRMS (ESI) calculated for C42H51N2O12S (M + H)+ 835.3224, found 835.3241.

(2R,3S)-N′-(8)-1′-(3′,6′-dihydroxy-3-oxo-3H-spiro[isobenzofuran-1,9′-xanthene]-6-ylamino)-14,14-dimethyl-12-o xo-1-thioxo-5,8-dioxa-2,11-diazapentadecan-13-yl)-N′,3′-dihydroxy-2-isobutylbutanediamide (14). To a solution of the methyl ester 13 (10 mg, 0.012 mmol) in methanol/THF (1:1, 0.8 mL) was added KCN (∼1 mg) followed by a solution of hydroxylamine in H2O (50 wt%, 100 µL, 1.5 mmol) and the reaction stirred at room temperature for 16 hours. The mixture was then concentrated under reduced pressure and triturated with CH2Cl2/methanol (5:1, 3 × 5 mL). The CH2Cl2/methanol washings were filtered and concentrated under reduced pressure to afford the desired hydroxamate 14 (9 mg, 90%) as an orange solid (mixture of regioisomers). HRMS (ESI) calculated for C42H51N2O12SNa+ (M + Na)+ 860.3153, found 860.3134 (Marimastat-linker; Fig. 2A, 3).

Marimastat-boronate and marimastat-AbF3

The radiochemical synthesis of marimastat-boronate and marimastat-AbF3 (Fig. 2A, 4, and 5) are fully detailed in Li and colleagues.7 For an outline of the chemistry applied, see Supplementary Scheme 4(Fig. 2A, 4 and 5).

Inhibitor kinetics

Recombinant human MMP2 (13) was activated with 1 mmol/L of 4-aminophenylmercuric acetate for 1 hour at 37°C. Rates of cleavage of 1 µmol/L of the quenched fluorescent MMP substrate (7-methoxy coumarin-4-yl)acetyl-Pro-Leu-Gly-Leu-[3-(2,4-dinitrophenyl)-l-2,3-diaminopropionyl]-Ala-Arg-NH2 were measured in 96-well fluorimetry plates in a Polarstar Optima (BMG Labtech GmbH) at 37°C 100 mmol/L Tris-HCl (pH 7.5), 100 mmol/L NaCl, 10 mmol/L CaCl2, 0.05% Brij 35 using a 320-nm excitation filter and a 405-nm emission filter in the presence of increasing inhibitor concentrations. Curve-fitting and IC50 calculations were done using GraphPad Prism 5.0 Software (GraphPad Software).

Marimastat-FITC as a MMP activity-based probe

Recombinant human MMP2, 8, 9, 13, and 14 (also known as membrane type 1-MMP) were expressed and purified. 4-Aminophenylmercuric acetate–activated MMPs were resolved nonreduced on a 10% SDS-PAGE gel. SDS was removed using Triton X-100 washes and renatured proteins in the gels were incubated overnight with 1 µmol/L of marimastat-FITC in 100 mmol/L Tris, 30 mmol/L CaCl2 (pH 8), 37°C. After washing three times (10 min) with PBS and imaging the gels using a 340-nm excitation filter on an Alpha Imager system (Alpha Innotech), they were stained with Coomassie brilliant blue R250. For assessing active MMPs in primary 67NR mammary tumors (11) and control mammary gland, the tissues were homogenized in 100 mmol/L of Tris, 30 mmol/L of CaCl2, 0.05% Brij 35 (pH 8). Extracts were clarified (14,000 × g) and 100 µg of total protein in supernatants were resolved on a 10% SDS-PAGE gel and processed for marimastat-FITC imaging.

Marimastat-FITC visualization of MMP14 in cell culture

MDA-MB-231 stably transfected with human MMP14 (with a FLAG-tag in the juxtamembrane stalk) or empty vector (8) were cultured in DMEM, 10% fetal bovine serum with G418 selection (Geneticin, 1 mg/mL; Life Technologies, Invitrogen Canada). For MMP14 activity-staining, the cells were grown on glass chamber slides and incubated with 20 µmol/L of marimastat-FITC in culture medium for 12 hours. The cells were then washed twice with PBS, counterstained with Hoechst (0.1 mg/mL in PBS, 20 min, at 25°C), and fixed for 20 minutes with 4% formalin. The slides were washed twice with PBS, mounted in ProLong Gold anti-fade reagent (Invitrogen Life Technologies) and imaged using a Leica DMRA2 fluorescence microscope. Pictures were taken with a 100× oil immersion objective with the same exposure time and digital gain for MMP14-transfected and vector control cells.

PET imaging of tumors in vivo

Day 25 67NR tumors were imaged by in vivo bioluminescence on a cryogenically cooled IVIS system (Xenogen Corporation) using Living Imaging acquisition and Living Image 2.50.1 software analysis (11). One day after IVIS imaging (day 26 or 33) control 67NR tumor mice were injected i.p. with blocking solution [300 nmol marimastat (1) in PBS], three times over 60 minutes prior to tracer injection. Pre-blocked and unblocked mice were then anesthetized with isoflurane (5% induction, 1.5% maintenance), and injected (tail vein)

with 50 to 100 μCi 18F-labeled marimastat-ArBF3 (5; decay-corrected specific activity of 0.185 Ci/μmol or 0.39 Ci/μmol at the time of injections) or 440 μCi control-ArBF3 (Supplementary data, 15) prepared as described in Supplementary data and Li and colleagues. Five minutes later, animals underwent an 80-minute dynamic scan followed by a 10-minute transmission scan using a microPET Focus 120 (CTI Concorde) system. Groups of mice were imaged with 18F-labeled marimastat-ArBF3 on three separate occasions with or without prior blocking with the underivatized marimastat. PET data were compiled with Siemens Focus 120 microPET software. PET images and time-activity curves were generated with Amide version 0.8.19.

Results

MMP expression patterns differed between mammary control tissues (n = 3) and tumors (n = 3; Fig. 1) where transcripts of MMP7, 13, 14, and 24 were statistically significantly elevated (P < 0.05), but others were reduced compared with normal tissue including MMP2, 15, 23, 25, and 27. Hence, 67NR tumor-expressed MMPs were probed with marimastat conjugates.

Marimastat (1; Fig. 2A) was synthesized as a reference for inhibitory activity of imaging derivatives. We then synthesized a highly versatile derivative precursor by the addition of a linker arm at the P3′ site, which is known not to interfere with potency or specificity (ref. 14; marimastat-linker, 2). This was used as an attachment point for generating a fluorescent imaging probe (marimastat-FITC, 3) and a shelf-stable marimastat-arylboronic ester conjugate (marimastat-boronate, 4). For PET imaging, marimastat-boronate (4) is converted in a single step to marimastat-ArBF3 (5) under aqueous conditions at ambient temperature (18°C) and isolated in radioc heaply pure form as described in Li and colleagues.7

With an IC50 of 2.10 nmol/L (Fig. 2B) and in good agreement with original reports (7), marimastat (1), was a nmol/L inhibitor against MMP2, an important protease in tumor angiogenesis and breast cancer metastases (15). Marimastat-ArBF3 (5) showed the same IC50 (1.67 nmol/L), with marimastat-FITC (3) having a value only slightly higher

Figure 1. High expression of MMPs in primary 67NR mammary tumors. Total RNA isolated from normal mammary glands of three individual control mice (C 1, 2, 3) and from primary tumors of three individual mice (67NR 1, 2, 3) were analyzed by CLIP-CHIP™ microarray in a two-color experiment with universal mouse RNA as control. The expression maps are derived from log2-ratios (tumor or control/universal mouse RNA) with red representing genes highly upregulated and light blue highly downregulated in comparison to universal mouse RNA.

Figure 2. One-step radiolabeling and bioactivity of marimastat derivatives. A, panel of compounds used in this study and one-step fluorination scheme for marimastat-boronate (4). B, inhibitor kinetics of marimastat derivatives. MMP2 (5 pmol/mL) was incubated for 20 min with a quenched fluorescent peptidic substrate (1 μmol/L) in the presence of various inhibitor concentrations.

(Fig. 2B). Following SDS-PAGE marimastat-FITC (3) specifically labeled activated MMP2, 9, 13, and 14 despite stringent washes (Fig. 3A and B) alleviating a concern that its dissociation rate might interfere with planned MMP detection in vivo. Neither the molecular weight markers nor the autolytically cleaved hemopexin domain of MMP13 showed labeling, confirming active site specificity. Notably, similar analysis of murine tissue lysates with marimastat-FITC (3) revealed MMP activity in the tumors (n = 2) greatly exceeding that observed in control mammary gland tissue (n = 2; Fig. 3B).

We next established in vitro imaging efficacy. Human MDA-MB-231 breast carcinoma cells stably expressing MMP14, a transmembrane protease highly expressed in cancer that activates proMMP2, but not vector transfectant controls were specifically labeled with marimastat-FITC (3; Fig. 3C). The punctate localization of MMP14 on the plasma membrane correlates with immunofluorescence studies or when GFP-MMP14 was expressed in the same cells (16).

Primary tumors were imaged over 25 days by luciferase bioluminescence (Fig. 4A, left). For PET molecular imaging of MMPs in vivo, separate radiosyntheses of marimastat-ArBF3 (5) were performed that afforded ~1 mCi of 5 with respective specific activities of 0.185 Ci/μmol for imaging on day 26 and 0.39 Ci/μmol for imaging on day 33. Sixty minutes after the injection of tumor-bearing mice with 50 to 100 μCi of marimastat-ArBF3 (5), we observed low but detectable and specific uptake in the primary tumor, whereas in mice injected with 440 μCi of marimastat-ArBF3 (Supplementary data, 15) lacking the marimastat moiety no tumor localization was observed, with clearance only to the liver, bladder, stomach, and gut to which the compound cleared (Supplementary Fig. S1).

Discussion

The use of a noncovalent nanomolar protease inhibitor drug as a model biocoujugate shows the ease and broad applicability of this novel labeling technique for in vivo cancer imaging. Previously, metalloproteinase activity has been imaged in mouse models of cancer by fluorescent detection of cleaved peptides quenched with near-IR fluorochromes, but this approach suffers from low tissue penetration and does not afford deep tissue localization (17).

In contrast, using small molecule drugs offers improved pharmacokinetics and biodistribution in solid tumors. For some serine and cysteine proteases, covalent inhibitors for imaging can be used (18), but for metalloproteinases, only noncovalent inhibitors are available for in vivo application. Nonetheless, this approach has advantages including reduced probe accumulation in tissues that can lead to unwanted side reactions. Together with rapid clearance, this also circumvents the long time intervals needed to reduce background from unincorporated covalent probes in tissues before imaging.

Although [13C]methylation represents an attractive one-step labeling method, it can suffer from nonspecific methylation side reactions. Furthermore, the 20-minute half-life for

Figure 3. Specificity of labeled marimastat and in vitro imaging of MMPs. A, recombinant human MMPs 2, 9, and 13 were electrophoresed on SDS-PAGE, renatured, incubated with marimastat-FITC (3), and washed extensively. Subsequently, the same gel was stained with Coomassie brilliant blue. The asterisk indicates the hemopexin domain of MMP13. B, total protein lysates were prepared from two normal mammary glands (Ctr 1, Ctr 2) and two primary 67NR tumors (67NR 1, 67NR 2). Proteins (100 μg) were analyzed by SDS-PAGE, renatured, and labeled with marimastat-FITC (3) with 2 μg of recombinant human MMPs 2, 13, and 14 as standards. C, MDA-MB-231 cells stably transfected with MMP14 and vector were incubated with marimastat-FITC (3) (green). Cell nuclei were counterstained with Hoechst dye (blue).
C-11 dramatically reduces the imaging time window (19), therefore reducing image quality compared with ligands labeled with other PET isotopes (20). Our labeling technique captures the advantages of a single-step labeling in the case of F-18 to afford ligands with specific activities that are potentially useful for imaging.

As this approach is conceptually very different from other radiolabeling methods, it is essential to briefly address some of the chemical attributes of an 18F-labeled ArBF₃. The first is the question of chemical purity. Although labeling must proceed through mono- and difluoronated intermediates en route to the labeled ArBF₃, the mono- and difluoroboranes/boronates are unstable at pH 7 (21), and as such, the ArBF₃ is the only labeled species isolated. The second concern relates to specific activity. Although high specific activity is always preferable, there is no universally accepted value as to what minimal specific activity represents a threshold of utility and often a value of ∼1 Ci/μmol is sufficient for imaging. Traditionally, radiosyntheses are performed under no carrier-added conditions to guarantee the highest possible specific activities. A prevailing misconception in such work is that no carrier-added fluoride has a specific activity close to that of carrier-free, that is, 1,720 Ci/μmol; in practice, however, the specific activity of "no carrier-added" [18F]fluoride generally falls in the range of 3 to 10 Ci/μmol. As such, there is a significant amount of carrier [19F]fluoride present in all no carrier-added syntheses. Furthermore, the decay that accompanies multistep radiosyntheses of mid-size molecules further reduces the final specific activities, which commonly fall in the range of 1 to 2 Ci/μmol, or less. Because three fluoride ions condense with one arylboronate to give an ArBF₃, the law of mass action ensures that the resulting ArBF₃ has a decay-corrected specific activity that is thrice that of the source fluoride; therefore, activities as high as 30 Ci/μmol may be envisaged if no carrier–added fluoride with a specific activity could be used.

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activity of 10 Ci/μmol is used. Nevertheless, yields may be low unless reaction volume is minimized; the use of 500 mCi of no carrier–added fluoride with a specific activity of 10 Ci/μmol represents 50 nmol total [18F]fluoride, which if contained in 1 μL would provide a 50 mmol/L fluoride solution that is high enough to afford reasonable yields in terms of an ArBF3. Finally, because of the relatively high presence of carrier [18F]fluoride that are present, even under no carrier–added conditions, statistically the labeled ArBF3 will contain only one atom of [18F]fluoride (3, 21).

Despite a relatively low signal to noise ratio, tumor-labeling was specific as shown from the unlabeled blocking control studies. Notably, image quality did not improve with higher specific activity probes; tumor images were similar irrespective of whether the specific activity of marimastat-ArBF3 (5) was 0.185 Ci/μmol (Fig. 4B) or 0.39 Ci/μmol (Fig. 4C), corresponding to ~12 nmol/L or ~27 nmol/L in vivo. Because the respective concentrations of 5 were 3.5x and 10x the Kd, this suggests, but does not prove that even higher specific activities would not improve imaging quality. Although a slightly better image was recorded with higher specific activity (Fig. 4C), this improvement is ascribed to larger tumor size, as tumors on day 33 (Fig. 4C) were twice as large as those imaged on day 26 (Fig. 4B).

It has to be noted that previous studies using 18F-conjugated MMP inhibitors for in vivo imaging also revealed high uptake of tracer in tissues with known nonpathologic MMP expression such as the liver (22, 23). Blood also contains MMPs. This is a particular problem for broad-spectrum MMP inhibitors and leads to poor target/nontarget contrasts when imaging disease tissues. With more data becoming available on the expression and activity of specific MMPs in particular pathologies, antibodies or small molecule inhibitors with narrow specificity can be developed and modified for the rapid conversion to 18F-conjugated radiotracers using our novel one-step labeling chemistry. A promising target for this strategy is MMP13, which is highly expressed in human breast cancer (24), and for that, specific inhibitors are currently in active development (25).

Although the metabolic fate of marimastat-ArBF3 (5) is unknown, we measured the inhibitory activity in the urine post-mortem; the observed IC50 value per microcurie was comparable to that injected suggesting that 5 was not being metabolized and cleared to the urine intact (Supplementary Fig. S2). Notably, the ArBF3 control that is not appended to marimastat does not clear to the gut (Fig. 4A). This illustrates that clearance is at least partially governed by the marimastat moiety and points to potential clinical applications in the gastrointestinal tract, which experience high drug exposures.

The in vivo application of [18F]marimastat-aryltrifluoroborate represents a key development in PET imaging as we have reduced to practice the production of a shelf-stable bioconjugate that can be rapidly labeled with [18F]fluoride for PET imaging in a simple one-step aqueous reaction at ambient temperatures under mild conditions. The key to this advance in PET imaging is the synthesis of stable [18F]aryltrifluoroborates as viable alternatives to [18F]organofluorides. The use of a stable aryllboronic acid as a captor of aqueous [18F]fluoride avoids tedious multistep syntheses or harsh fluorination conditions. These findings should encourage the widespread development of new molecularly targeted 18F-labeled peptidic and small molecule drugs for in vivo imaging. Hence, the first in vivo imaging of tumor metalloproteinases using marimastat as a scaffold indicates that labeling of other ligands is a facile approach for targeted PET imaging for human diagnosis as well as drug target validation, tissue targeting, and compound clearance to aid drug development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

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