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Radiosynthesis and biological evaluation of a promising σ_2 -receptor ligand radiolabeled with fluorine-18 or iodine-125 as a PET/SPECT probe for imaging breast cancer

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Abstract

Sigma-2 receptors represent an endogenous marker for proliferation in solid tumors. The high affinity, high selectivity σ_2 receptor ligand N-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)butyl)-2-(2-fluoroethoxy)-5-iodo-3-methoxybenzamide (**3**) was separately radiolabeled with F-18 and I-125. The radiolabeling yield was 30% and 70% for [¹⁸F]**3** and [¹²⁵I]**3**, respectively. Studies of [¹²⁵I]**3** using murine 66 breast tumor membrane homogenates and evaluation of [¹⁸F]**3** and [¹²⁵I]**3** in 66 tumor-bearing mice indicate that this ligand has potential as a PET or a SPECT probe for imaging σ_2 receptors in breast cancer.

Keywords

Breast cancer; Molecular imaging; F-18; I-125; sigma receptor

1. Introduction

Sigma (σ) receptors are a distinct class of receptors that are expressed in many normal tissues, including liver, kidneys, endocrine glands, and the central nervous system (CNS) (Walker et al., 1990). Two subtypes have been pharmacologically identified: σ_1 and σ_2 receptors (Hellewell and Bowen, 1990; Quirion et al., 1992). The σ_1 receptor has been cloned from tissues of guinea pig, rat, mouse, and man (Guitart et al., 2004), and displays a 30% sequence homology with a yeast sterol enzyme isomerase (Hanner et al., 1996). The human σ_1 receptor is a unique protein consisting of 223 amino acids. The σ_2 receptor has not yet been cloned, but evidence suggests that this receptor is linked to potassium channels and intracellular calcium release in NCB-20 cells (Guitart et al., 2004; Hellewell et al., 1994; Vilner et al., 1995). An overexpression of σ_2 receptors has been reported in a variety of human and murine tumors (Bem et al., 1991; Mach et al., 1997; Vilner and Bowen, 1992; Vilner et al., 1995). The density

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of σ_2 receptors has been reported to be greater than that of σ_1 receptors in a wide panel of tumor cells grown under cell culture conditions (Vilner et al., 1995). The density of σ_2 receptors was also found to be 8–10-fold higher in proliferating versus quiescent mouse mammary adenocarcinoma cells both in vitro (Al-Nabulsi et al., 1999) and in vivo (Wheeler et al., 2000). This correlation between σ_2 receptor density and the proliferative status of tumor cells did not depend on other biological or physiological factors such as species, cell type, ploidy, cell–cell contact, nutrient depletion, low pH, altered metabolic states or tumor size (Al-Nabulsi et al., 1999; Wheeler et al., 2000). Investigations with two-photon and confocal microscopy probes indicate that σ_2 receptors are localized in the mitochondria, lysosomes, endoplasmic reticulum, and plasma membrane of breast cancer cells (Zeng et al., 2007). These data suggest that the σ_2 receptor may be a biomarker of tumor cell proliferation and that radioligands with high affinity and selectivity for σ_2 receptors have the potential to be positron emission tomography (PET) or single photon emission computed tomography (SPECT) probes for imaging cell proliferation. Such probes would provide a unique tool for the initial diagnosing and staging of breast cancer, as well as monitoring the therapeutic efficacy of breast cancer treatments. Our group has previously radiolabeled several σ_2 receptor ligands possessing high affinity and high selectivity for σ_2 vs. σ_1 receptors as potential PET tracers for clinical use; the initial in vitro and in vivo evaluation of these radiotracers has been reported previously (Hou et al., 2006; Rowland et al., 2006; Tu et al., 2005; Tu et al., 2007). Among these compounds, two promising ^{18}F -labeled tracers, N-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)butyl)-2-(2-[^{18}F]fluoroethoxy)-5-methylbenzamide and N-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)butyl)-2-(2-[^{18}F]fluoroethoxy)-5-iodo-3-methoxybenzamide ([^{18}F]3), displayed the highest tumor to normal tissue ratios and better clearance from blood in biodistribution studies (Tu et al., 2007). The first tracer is under phase 0 clinical evaluation as PET probe for imaging the σ_2 receptor status of cancer patients. The evaluation of [^{18}F]3 in EMT-6 tumor-bearing BALB/c mice generated very encouraging imaging results in initial microPET/CT studies (Tu et al., 2007). Because compound 3 contains both a 2-fluoroethoxy and 5'-iodo group in its structure, it has potential to serve as either a PET or SPECT probe. In addition, compound 3 can be labeled with iodine-125 to serve as a radioligand for in vitro Scatchard studies of σ_2 receptors, as well as the preliminary in vivo evaluation of a potential SPECT radiotracer. The long half life of I-123 facilitates SPECT studies with [^{123}I]3 which could be conducted at later time points not feasible with the PET probe [^{18}F]3. In this paper, we report the radiosynthesis of [^{125}I]3, the in vitro evaluation of its σ_2 receptor binding affinity in breast tumor homogenates, and the simultaneous in vivo evaluation of [^{125}I]3 and [^{18}F]3 in tumor-bearing mice. The murine 66 breast tumor which has been previously validated for in vivo studies of the σ_2 receptor (Al-Nabulsi et al., 1999; Wheeler et al., 2000) was used for these experiments. Our initial in vivo studies of [^{125}I]3 and [^{18}F]3 indicate that both tracers have similar σ_2 ligand pharmacologic properties. Our results indicate that compound 3 can serve either as a PET or SPECT probe for imaging the σ_2 receptor status of breast tumors. In addition, [^{125}I]3 is a useful probe for the in vitro investigation of σ_2 receptors in solid tumors or tumor membrane homogenates.

2. Materials and methods

Analytical grade chemicals and reagents were purchased from Sigma-Aldrich (Milwaukee, WI) and were used without further purification unless otherwise specified. The radioactive Na [^{125}I]I was purchased from MP Biomedical Inc., in Solon, Ohio. Haloperidol used in receptor binding studies was purchased from Tocris Cookson (Ellisville, MO, USA).

All animal experiments were conducted in compliance with the NIH Guidelines for the Care and Use of Research Animals under IACUC approved protocols reviewed by the Washington University Medical School Animal Studies Committee.

2.1 Synthesis of precursors and standard

Precursor 2-(2-(4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)butylcarbamoyl)-4-iodo-6-methoxyphenoxy)ethyl methanesulfonate (**1**) used for F-18 labeling, the tin precursor N-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)butyl)-2-(2-fluoroethoxy)-3-methoxy-5-(tributylstannyl)benzamide (**2**) used for I-125 labeling, and the standard compound N-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)butyl)-2-(2-fluoroethoxy)-5-iodo-3-methoxybenzamide (**3**), were made as previously reported (Tu et al., 2007a, b).

2.2. Radiochemistry

2.2.1. Radiosynthesis of N-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)butyl)-2-(2-[¹⁸F]fluoroethoxy)-5-iodo-3-methoxybenzamide ([¹⁸F]3**)**—The radiosynthesis of [¹⁸F]**3** was accomplished using a modification of the literature procedure (Tu et al., 2007) (Figure 1). Briefly, [¹⁸F]fluoride (100–150 mCi) was produced by proton irradiation of enriched ¹⁸O water (95%) via the reaction: ¹⁸O(p, n)¹⁸F using either a JSW BC-16/8 (Japan Steel Works) or a CS-15 cyclotron (Cyclotron Corp) and added to Pyrex screw cap tube containing 5–6 mg of Kryptofix 222 and 1–2 mg of K₂CO₃. The small amount of water was evaporated azeotropically using acetonitrile (3 × 1.0 mL) at 110 °C under a stream of nitrogen gas. A solution of the mesylate precursor **1** (1–2 mg) in 0.2 mL dimethyl sulfoxide (DMSO) was added and the tube was capped and heated at 80 °C for 5–7 min. This solution was diluted with 3.0 mL HPLC mobile phase (see below) and then passed through an Alumina Neutral cartridge (Alltech Associates, Inc, Deerfield, Ill) to remove unreacted [¹⁸F]fluoride. The remaining solution was injected onto a high-performance liquid chromatography (HPLC) Agilent Eclipse XDB-C18 reverse phase column (10 × 250 mm) using acetonitrile/ 0.1 M ammonium formate buffer (43.5/56.5, v/v) as the mobile phase, at a flow rate of 3 mL/min, and at a UV wavelength of 288 nm. The product retention time was 45 min; the radioactive peak was collected from 43–46 min, diluted with 40 mL reagent-grade water and passed through a Sep-Pak Plus C-18 cartridge (Waters, Milford, MA). The trapped product was eluted with ethanol (0.6 mL) followed by 5.4 mL of 0.9% saline. After sterile filtration into a glass vial, the final product was ready for quality control (QC) analysis and animal studies. The radioactive dose was authenticated by co-injection with the corresponding cold compound **3**. The radiochemical purity was >99%, the chemical purity was >95%, the labeling yield was 30 ± 5% (n=10, decay corrected) and the specific activity was >2,000 Ci/mmol (decay corrected to end of synthesis).

2.2.2. Radiosynthesis of N-(4-(6, 7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)butyl)-2-(2-fluoroethoxy)-5-[¹²⁵I]iodo-3-methoxybenzamide ([¹²⁵I]3**)**—Radiosynthesis of [¹²⁵I]**3** was accomplished by an electrophilic radioiododestannylation reaction (Figure 1). Briefly, 910 µCi/10 µL of carrier free Na[¹²⁵I]I in 0.01 N NaOH solution was added into 10 µL of a freshly made solution of tributylstannane precursor **2** (3 mg in 300 µL of ethanol) followed by the addition of 100 µL of freshly made peroxide hydroxide/acetic acid (1/3, v/v) to the reaction vessel. The vessel was capped and stirred for at least 10 min until thin layer chromatography (TLC) indicated that the incorporated yield was > 90%. Using methylene chloride/ethanol (1/3) as the TLC mobile phase, the R_f value of the [¹²⁵I]**3** was ~1.0 and the R_f value of the unreacted [¹²⁵I]I[–] was ~0.0. The reaction was quenched by adding 400 µL of HPLC mobile phase, acetonitrile/0.1 M ammonium formate buffer (37.5/62.5, v/v, PH = 4.5). This diluted solution was injected onto the HPLC reverse phase Alltech Econosil semi-preparative C-18 column (10 × 250 mm, 10 µA), at a UV wavelength of 288 nm and a flow rate of 3.0 mL/min. The radioactive product was collected from 19–23 min and diluted with 50 mL of Milli-Q water, then passed through a C-18 Plus Sep-Pak cartridge (Waters, Inc.). After the loaded C-18 Sep-Pak cartridge was dried with nitrogen gas, the product was eluted with 0.6 mL absolute ethanol and 5.4 mL of 0.9% saline. After sterile filtration, ~ 640 µCi of [¹²⁵I]**3** was obtained. The overall radiosynthesis yield was ~ 70% with radiochemical purity >

99%, no UV-detectable material was observed. [^{125}I]**3** was confirmed by coinjection of authentic cold compound **3** onto the QC HPLC analytic system. Because of the effective separation of [^{125}I]**3** from the tin precursor on the semi-preparative HPLC system described above and the use of carrier free [^{125}I]sodium iodide, it is assumed that the [^{125}I]**3** is carrier-free with a theoretical specific activity 2,200 Ci/mmol (Hou et al., 2006;Kung et al., 1990;Murphy et al., 1990).

2.3. In Vitro Binding Studies

2.3.1. Membrane homogenate preparation—Membrane homogenates were prepared from excised ~200 mg mouse mammary 66 tumors, which were snap-frozen and stored at -80°C . Before homogenization, the tumor was allowed to thaw slowly on ice. Tissue homogenization was carried out at 4°C using a Potter-Elvehjem tissue grinder at a concentration of 1 g of tissue/mL of 50 mM Tris-HCl at pH 8.0. The crude membrane homogenate was then transferred to a 50 mL centrifuge tube and resuspended at a concentration of 0.2 g of tissue/mL of 50 mM Tris-HCl. Additional homogenization was accomplished using an Ultra-Turrax T8 polytron homogenizer (IKA Works, Inc, Wilmington, NC). The final homogenate was then centrifuged for 10 min at 1000 g, the pellet discarded, and the supernatant mixed by vortexing. Aliquots were stored at -80°C until further use. The protein concentration of the suspension was determined using the DC protein assay (Bio-Rad, Hercules, CA).

2.3.2. Scatchard analysis—Scatchard analysis was performed using ~50 μg membrane homogenates diluted with 50 mM Tris-HCl buffer, pH 8.0, and incubated at 25°C with the radioligand [^{125}I]**3** in 96 well polypropylene plates (Fisher Scientific, Pittsburgh, PA) in a total volume of 150 μL per well. The concentrations of the radioligand [^{125}I]**3** ranged 0.01–3.3 nM. After incubation for 30 min, the reactions were terminated by the addition of 150 μL of cold wash buffer (10 mM Tris-HCl, 150 mM NaCl, pH 7.4, at 4°C) using a 96 channel transfer pipette (Fisher Scientific, Pittsburgh, PA). Immediately after the reaction was terminated, the samples were harvested and filtered rapidly to a 96 well fiber glass filter plate (Millipore, Billerica, MA). Each filter was washed with 200 μL of ice-cold wash buffer for a total of three washes. Then the filters were punched out and a Wallac Wizard 1480 automated gamma counter (PerkinElmer, Turku, Finland) with a counting efficiency of 80% for ^{125}I was used to quantitate the bound radioactivity. Nonspecific binding was determined from samples that contained 10 μM haloperidol. The equilibrium dissociation constant (K_d) and maximum number of binding sites (B_{max}) were determined by a linear regression analysis of the transformed data using the method of Scatchard as previously described (Xu et al., 2005).

2.3.3. Biodistribution studies—Biodistribution studies were performed in tumor-bearing female nu/nu mice. Approximately 1.5×10^6 cells/100 μL were injected subcutaneously into the mammary fat pad region of mature female athymic nu/nu mice (25–30 g), tumors were both visible and palpable within 14 days (excised tumors weighed 0.5–0.9 g). Each mouse was given a single injection containing a mixture of [^{125}I]**3** and [^{18}F]**3**. Separate stocks were made for each radiotracer and the activity per 100 μL was counted in a dose calibrator. The two stock solutions were mixed 1:1 (v/v) prior to being drawn up in syringes. In addition to the standard of the mixed radionuclide injectate, an aliquot of each stock solution was reserved for use as a single radionuclide standard. For the biodistribution studies, a mixture of 1.5 $\mu\text{Ci}/100 \mu\text{L}$ of [^{125}I]**3** and 20 $\mu\text{Ci}/100 \mu\text{L}$ of [^{18}F]**3** in 10% ethanol/saline were injected via the lateral tail vein. Blocking was evaluated by injection of 2.0 mg/kg of N-(4'-fluorobenzyl)piperidinyl-4-(3-bromophenyl) acetamide, YUN-143 in 100 μL of 10% ethanol/saline 1 min prior to radiotracer injection. Standards were prepared from dilutions of each radiotracer stock solution as well as from the injectate mixture. Groups of four mice were euthanized at 5, 60, and 120 min post injection (p.i.) and blocking was evaluated at 60 min p.i.. Blood, lung, liver, spleen, kidney, muscle, fat, heart, brain, bone, submandibular gland, thyroid (including larynx), and

tumor were removed from each mouse, weighed, and counted in a Beckman Gamma 8000 counter. The Beckman 8000 gamma counter is an automated dual channel counter with a 3" sodium iodide detector. The user selects one of three preset voltage and gain settings and sets the windows for each channel. It can be used to measure radionuclides like F-18 with higher energy gamma rays (up to 2000 keV) or for measuring radionuclides like I-125 with lower energy gamma rays (below 500 keV). The biodistribution samples were counted immediately for ^{18}F using high-energy settings, Channel 1 windows were set to count the 511 keV positron annihilation gamma (420–620 keV) and Channel 2 windows were set to count the positron sum peak (800–1200 keV). The two positron peak channels were summed for [^{18}F]**3** data analysis. Samples were counted again 30 hrs later for ^{125}I using low-energy settings, Channel 1 was set to count 35 keV gamma peak for iodine-125 (15–80 keV) and Channel 2 windows were set to count the rest of the spectrum (80–500 keV). Only Channel 1 counts were used for [^{125}I]**3** data analysis; the counts in the second window were used to confirm the decay of fluorine-18 to background levels. Standards were counted with each set of samples for both the injectate (mixed radionuclides) and the individual stock solutions in order to ensure that the reported data reflected only the correct radionuclide and that there was no cross-talk correction required. The results were calculated as percent injected dose per gram of tissue (% ID/g). Additional tumors were harvested for in vitro receptor binding studies as described above.

3. Results and Discussion

3.1 Chemistry

[^{18}F]**3** was synthesized by the reaction of the corresponding mesylate ester **1** with [^{18}F]fluoride/kryptofix 2.2.2. in DMSO in the presence of K_2CO_3 . [^{18}F]**3** was obtained in $\sim 30 \pm 5\%$ radiochemical yield ($n=10$, decay corrected) with greater than 95% chemical and radiochemical purity. [^{125}I]**3** was prepared via electrophilic iododestannylation reaction (Figure 1), which is similar to the procedure previously reported for its analogues (Hou et al., 2006; Rowland et al., 2006). An excess amount of the tri(*n*-butyl) tin precursor **2** was reacted with carrier-free Na [^{125}I]I in ethanol in the presence of hydroxide peroxide/acetic acid as the oxidizing agent. The reaction mixture was stirred at room temperature for 15 ~ 20 min and monitored by TLC. Upon completion, the reaction mixture was diluted with the HPLC mobile phase and then purified by HPLC. In the previously reported procedure, excess solvent was removed from the HPLC collection using a rotary evaporator and the final dose was reconstituted in 0.9% saline. This process was modified in the current study: following HPLC purification, a C-18 Plus Sep-Pak was used to trap the radioactive [^{18}F]**3** or [^{125}I]**3**, then the final product was eluted into the dose vial using ethanol followed by 0.9% saline. This technique developed to concentrate the product eliminates the possible decomposition of [^{18}F]**3** or [^{125}I]**3** from heating the HPLC collection sample and decreases the potential for volatile iodine-125 contamination. This approach also conserves space and reduces the amount of equipment needed when scaling up radiosynthesis for an automated module or system. Such automated processes are invaluable when multiple doses of ^{18}F -labeled radiopharmaceuticals are required for clinical applications. Currently, we are optimizing the synthesis of [^{18}F]**3** using an automated system that would be suitable for clinical evaluation of cancer patients. The process for radiolabeling [^{125}I]**3** could also be easily adapted to the preparation of [^{123}I]**3** as a σ_2 SPECT probe for cancer patients. We would like to note that two different semi-preparative HPLC conditions were used for the purification of [^{18}F]**3** and [^{125}I]**3** due to differences between the precursors and the reaction conditions for [^{18}F]**3** and [^{125}I]**3**. The HPLC conditions developed for purification of [^{125}I]**3** were not able to remove a trace mass impurity when we attempted to use that system for purification of [^{18}F]**3**.

3.2. In Vitro Binding Studies

Direct saturation binding studies were carried out using [^{125}I]**3** with σ_2 membrane homogenates of 66 mouse breast tumors. The saturation curve and Scatchard plots are shown in Figure 2. The K_d and B_{max} values of the receptor-radioligand binding of [^{125}I]**3** were 2.8 nM and 748 fmol/mg protein, respectively. The high affinity and low nonspecific binding indicate [^{125}I]**3** is a valuable probe for evaluating the σ_2 receptor status of 66 mouse breast tumors. Because of the short half-life of F-18 (110 min) and the low radioligand concentrations required for Scatchard analysis, in vitro binding studies were not performed using [^{18}F]**3**.

3.3. Biodistribution Studies

The biodistribution of [^{18}F]**3** and [^{125}I]**3** in tumor-bearing mice is shown in Table 1. Among all organs evaluated, the two tracers have very consistent distribution. Particularly, the percent injected dose per gram (%ID/g) data for tumors at 5, 60, and 120 min p.i. are 2.46 ± 0.69 , 2.38 ± 0.13 , and 1.05 ± 0.16 for [^{18}F]**3**, 2.42 ± 0.62 , 2.31 ± 0.15 , and 0.97 ± 0.15 for [^{125}I]**3**, respectively. At 120 min p.i., the retention in the tumor was ~40% of the 5 min uptake for both [^{18}F]**3** and [^{125}I]**3**. Among the peripheral tissues, lung and kidney had relatively high initial uptake at 5 min for both tracers. The lung uptake (%ID/g) reached 27.68 ± 6.39 for [^{18}F]**3** for and 26.56 ± 5.81 for [^{125}I]**3**, and the kidney uptake (%ID/g) reached 25.65 ± 3.96 for [^{18}F]**3** for and 24.96 ± 3.39 for [^{125}I]**3**. The remaining organs with relatively high initial activity levels were liver, submandibular gland, and spleen, with values ranging from 9–15 %ID/g for both tracers. P values calculated in Microsoft Excel using the two-tailed Student's t test for organs listed above showed no significant difference for the two tracers (P value > 0.05).

Free iodine accumulates in the thyroid, thus radioactivity levels in the thyroid can indicate metabolic deiodination. For these studies, gross dissection of the thyroid included varying amounts of the larynx and trachea, so the most accurate representation of thyroid activity is % ID/organ rather than %ID/gram. Both radiotracers displayed comparable initial uptake (%ID/organ) in thyroid at 5 min p.i.: 0.14 ± 0.03 for [^{18}F]**3** and 0.14 ± 0.04 for [^{125}I]**3**. However, the thyroid activity (%ID/organ) fell to 0.02 ± 0.01 at 60 min and 0.02 ± 0.01 at 120 min for [^{18}F]**3**. In contrast to this rapid washout of activity from the thyroid observed with [^{18}F]**3**, uptake levels (%ID/organ) of 0.11 ± 0.02 at 60 min and 0.20 ± 0.06 at 120 min p.i. were found with [^{125}I]**3**. Although the thyroid values are low, the statistically significant difference (2-tailed, unpaired, Student's t test P value < 0.001 at both 60 and 120 min p.i.) between the two tracers represents de-iodination of [^{125}I]**3**. In a similar manner, free fluoride accumulates in the bone. Both radiotracers displayed comparable initial uptake (%ID/g) in bone at 5 min p.i.: 2.14 ± 0.46 for [^{18}F]**3** and 1.73 ± 0.78 for [^{125}I]**3**. As can be seen in Table 1, no significant defluorination was observed. At 120 min p.i. the %ID/g in bone had fallen to 0.46 ± 0.13 for [^{18}F]**3** and 0.29 ± 0.07 for [^{125}I]**3**, which did not represent a statistically significant difference (P value = 0.058).

The clearance of the radioactivity was very rapid from the blood, muscle, fat and lung. These are the non-target tissues that are most relevant for clinical imaging studies of breast cancer patients. As shown in Figure 3, both tracers had similar clearance from 5 to 120 min. At 120 min, the %ID/g values in lung were 0.36 ± 0.04 for [^{18}F]**3** and 0.37 ± 0.05 for [^{125}I]**3**. In muscle, the remaining %ID/g was only 0.13 ± 0.02 for [^{18}F]**3** and 0.10 ± 0.02 for [^{125}I]**3** at 120 min p.i.. Similar values were observed for fat at 120 min p.i.: 0.14 ± 0.06 %ID/g for [^{18}F]**3** and 0.12 ± 0.07 for [^{125}I]**3**. The tumor/blood, tumor/lung, tumor/muscle, and tumor/fat ratios showed similar trends from 5 min to 120 min for both [^{18}F]**3** and [^{125}I]**3**. At 120 min, the uptake ratios of tumor vs. blood, lung, muscle, and fat reached 5.9, 2.9, 8.1 and 8.4 for [^{18}F]**3**, 6.1, 2.6, 9.8, and 9.5 for [^{125}I]**3**. The high tumor/non-target tissue ratios indicate these two tracers have potential to be suitable candidates for imaging breast tumor proliferation in vivo.

P values calculated using the two-tailed Student's t test for ratios listed above showed no significant difference for the two tracers (P value > 0.05).

To demonstrate the in vivo binding specificity of these two tracers for the sigma receptor, YUN-143, a σ receptor ligand having high affinity for both σ_1 and σ_2 receptors, was injected in a group of mice 1 min prior to the injection of the radiotracers. Since YUN-143 is based on a different chemical structure from the σ_2 selective ligands, it is often used as a σ receptor blocking agent in our lab (Rowland et al., 2006; Tu et al., 2005; Tu et al., 2007; Xu et al., 2005). As previously reported, the 66 mouse breast cancer cell line expresses a high density of σ_2 receptors both in vitro and in vivo (Al-Nabulsi et al., 1999; Wheeler et al., 2000). Blocking the σ_2 receptor with YUN-143 in 66 tumor-bearing mice resulted in a significant decrease in tumor radioactivity for both [^{18}F]**3** and [^{125}I]**3**. The blocking studies resulted in a similar decrease in tumor radioactivity levels for [^{18}F]**3** (42%) and [^{125}I]**3** (41%) at 60 min p.i. as shown in Figure 4. P values calculated using GraphPad Prism (GraphPad Software Inc., San Diego, CA) and the Two-way Anova t test indicate a significant difference for the row factor of control versus block (P value < 0.0001), no effect (P value = 0.48) for the column factor of [^{18}F]**3** versus [^{125}I]**3**, and no interaction between the row and column (P value = 0.89). These data are consistent with published literature describing blocking studies for other σ_2 radiotracers using YUN-143 in tumor-bearing mice (Rowland et al., 2006; Tu et al., 2005; Tu et al., 2007; Xu et al., 2005).

4. Conclusion

In this study, we radiosynthesized compound **3** with two different isotopes, F-18 and I-125. The in vivo properties of [^{18}F]**3** and [^{125}I]**3** were almost identical with the exception of modest accumulation of iodine-125 in the thyroid gland. The high tumor : normal tissue ratios indicate that this ligand is a potential radiotracer for either PET or SPECT imaging studies. Our results show that [^{125}I]**3** have suitable in vitro binding properties to serve as a radioligand for Scatchard studies σ_2 receptors in solid tumors or membrane homogenates of solid tumors. Future studies will compare microPET imaging studies of [^{18}F]**3** and NanoSPECT imaging studies of [^{123}I]**3** in tumor-bearing mice to evaluate the performance of [^{18}F]**3** and [^{123}I]**3** as radiotracers for imaging proliferation in rodent models of breast cancer.

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Figure 1.
Radiosynthesis of $[^{18}\text{F}]\mathbf{3}$ and $[^{125}\text{I}]\mathbf{3}$

Figure 2. Scatchard analysis of $[^{125}\text{I}]\mathbf{3}$ binding to σ_2 receptors in membrane homogenates from mouse mammary 66 tumors: (A) Representative saturation binding experiments which show the total bound, nonspecific bound and specific bound. (B) Representative Scatchard plots which were used to determine K_d and B_{max} values.

Figure 3.
Comparison of uptake ratios of $[^{18}\text{F}]\mathbf{3}$ and $[^{125}\text{I}]\mathbf{3}$ for tumor vs. non-target tissues.

Figure 4.

Comparison of the tumor uptake of [^{18}F]3 vs. [^{125}I]3 at 60 min p.i. under no-carrier-added conditions and under conditions of σ_1 and σ_2 blockade with YUN-143 (2 mg/kg iv).

Table 1

Biodistribution of [^{18}F]**3** and [^{125}I]**3** in female athymic nu/nu mice bearing 66 solid tumors (% ID/g)^a (n = 4)

Tissue	5 min		60 min		120 min	
	[^{18}F] 3	[^{125}I] 3	[^{18}F] 3	[^{125}I] 3	[^{18}F] 3	[^{125}I] 3
blood	1.88 ± 0.39	2.03 ± 0.40	0.42 ± 0.08	0.48 ± 0.10	0.18 ± 0.02	0.16 ± 0.03
lung	27.68 ± 6.39	26.56 ± 5.81	1.54 ± 0.33	1.64 ± 0.41	0.36 ± 0.04	0.37 ± 0.05
liver	15.03 ± 5.16	14.97 ± 5.01	2.97 ± 0.71	2.99 ± 0.70	1.87 ± 1.85	1.92 ± 1.94
kidney	25.65 ± 3.96	24.96 ± 3.39	2.38 ± 0.41	2.44 ± 0.43	0.57 ± 0.07	0.59 ± 0.10
spleen	9.81 ± 1.16	9.47 ± 1.41	1.51 ± 0.24	1.49 ± 0.25	0.31 ± 0.05	0.32 ± 0.04
muscle	2.11 ± 0.17	2.10 ± 0.21	0.52 ± 0.32	0.54 ± 0.38	0.13 ± 0.02	0.10 ± 0.02
fat	1.02 ± 0.17	1.02 ± 0.16	0.36 ± 0.12	0.36 ± 0.12	0.14 ± 0.06	0.12 ± 0.07
heart	4.86 ± 0.85	4.73 ± 0.85	0.52 ± 0.08	0.50 ± 0.09	0.18 ± 0.01	0.15 ± 0.02
brain	0.96 ± 0.21	0.94 ± 0.22	0.13 ± 0.02	0.08 ± 0.01	0.08 ± 0.00	0.02 ± 0.00
submandib.	12.95 ± 4.09	12.40 ± 4.10	6.55 ± 0.48	6.50 ± 0.25	1.17 ± 0.42	1.22 ± 0.43
tumor	2.46 ± 0.69	2.42 ± 0.62	2.38 ± 0.13	2.31 ± 0.15	1.05 ± 0.16	0.97 ± 0.15
bone	2.14 ± 0.46	1.73 ± 0.78	1.07 ± 0.10	0.96 ± 0.06	0.46 ± 0.13	0.29 ± 0.07
thyroid ^a	0.139 ± 0.034	0.136 ± 0.032	0.021 ± 0.009	0.109 ± 0.018	0.014 ± 0.005	0.204 ± 0.064

Values are mean ± SD.

^athyroid results are expressed as % ID/organ