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Synthesis and evaluation of two novel 2-nitroimidazole derivatives as potential PET radioligands for tumor imaging

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Abstract

Introduction—Nitroimidazole (azomycin) derivatives labeled with radioisotopes have been developed as cancer imaging and radiotherapeutic agents based on the oncological hypoxic mechanism. By attaching nitroimidazole core with different functional groups, we synthesized new nitroimidazole derivatives, and evaluated their potentiality as tumor imaging agents.

Methods—Starting with commercially available 2-nitroimidazole, 2-fluoro-N-(2-(2-nitro-1H-imidazol-1-yl)ethyl)acetamide (NEFA, [¹⁹F]7) and 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 2-fluoroacetate (NEFT, [¹⁹F]8), as well as radiolabeling precursors - the bromo substituted analogs were quickly synthesized through a three-step synthetic pathway. The precursors were radiolabeled with [¹⁸F]F-/18-crown-6/KHCO₃ in DMSO at 90 °C for 10 min followed by purification with an Oasis HLB cartridge. Biodistribution studies were carried out in EMT-6 tumor-bearing mice. The uptake (%ID/g) in tumors and normal tissues were measured at 30 min post injection. Liquid chromatography-electrospray ionization mass spectrometry (LC/MS) was used to distinguish metabolites from parent drugs in urine and plasma of rat injected with “cold” NEFA ([¹⁹F]7) and NEFT ([¹⁹F]8).

Results—Two radiotracers, [¹⁸F]NEFA ([¹⁸F]7) and [¹⁸F]NEFT ([¹⁸F]8), were prepared with average yields of 6-7% and 9-10% (no decay corrected). Radiochemical purity for both tracers was >95% as determined by HPLC. Biodistribution studies in EMT-6 tumor-bearing mice indicated that the tumor to blood and tumor to liver ratios of both [¹⁸F]7 (0.96, 0.98) and [¹⁸F]8 (0.61, 1.10) at 30 min were higher than those observed for [¹⁸F]FMISO (1) (0.91, 0.59), a well-investigated azomycin type hypoxia radiotracer. LC/MS analysis demonstrated that fluoroacetate was the main *in vivo* metabolite for both NEFA ([¹⁹F]7) and NEFT ([¹⁹F]8).

Conclusions—In this research, two new fluorine-18 labeled 2-nitroimidazole derivatives, [¹⁸F]7 and [¹⁸F]8, both of which containing *in vivo* hydrolyzable group, were successfully prepared. Further biological evaluations are warranted to investigate their potential as PET radioligands for imaging tumor.

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Keywords

PET; 2-nitroimidazole; ^{18}F ; hypoxia

1 Introduction

Hypoxia has been recognized as a characteristic of almost all types of solid tumors. In case of cancer, the presence of hypoxic cells may play a role in their resistance to both radiation and anticancer treatment [1-4]. It is a characteristic feature of locally advanced solid tumors, has become known as a pivotal factor of the tumor pathology since it can promote tumor progression and resistance to therapy [4]. Thus, noninvasive imaging methods for identification and quantitation of tumor hypoxia with positron emission tomography (PET) and single photon emission computed tomography (SPECT) have received serious attention to predict and monitor treatment response [5-8].

It was postulated that radiolabeled 2-nitroimidazole-based radiosensitizers could be used for scintigraphic imaging of tumor hypoxia by Chapman in 1981 [9]. The nitro group undergoes an enzyme-mediated electron transfer to a free radical anion, which is reversible in the presence of oxygen. Unlike in normal cells, further reductions of this free anion produce more chemically reactive species that lead to adduct formation with cellular components by metabolically viable reductases. These adduct formations can prevent egress of 2-nitroimidazoles and cause selective trapping of the azomycin in hypoxic cells [10].

[^{18}F]Fluoromisonidazole ([^{18}F]FMISO (**1**)) was derived from misonidazole (**9**), one of the earliest radiosensitizers used in radiation therapy. It is the most widely used PET agent for mapping original hypoxia with hundreds of patient studies reported [8, 11-14]. [^{18}F]FMISO (**1**) is easily synthesized, and can be safely used in humans. However, [^{18}F]FMISO (**1**) was not an optimal hypoxic imaging agent. Because of the partitioning mechanism, [^{18}F]FMISO (**1**) shows a reduced accumulation of radioactivity in hypoxic cells and slow clearance in normal cells that lead to low tumor-to-background (T/B) ratios [8, 15]. Thus, new imaging agents with faster pharmacokinetics for ensuring higher T/B ratios are required. Several alternative nitroimidazole derivatives labeled with ^{18}F , such as [^{18}F]FETA (**2**), [^{18}F]FAZA (**3**), [^{18}F]FETNIM (**4**), [^{18}F]HX4 (**5**) and [^{18}F]3-NTR (**6**) (Fig. 1) have been reported [16-23]. In this report, we aim to develop 2-nitroimidazole derivatives with enzymatically or chemically labile groups, either an ester or an amide linkage. In the presence of carboxylesterases, peptidases or proteases, such derivatives are expected to be hydrolyzed *in vivo* to [^{18}F]fluoroacetate, a metabolite which owns a rapid clearance in normal tissues, and to get a high T/B ratio [24]. A novel approach of using the *in vivo* hydrolysis of ethyl ester of [^{18}F]fluoroacetate for penetrating intact blood-brain barrier and evaluating glia cell metabolism has recently been reported [25]. We report, herein the synthesis of [^{18}F]NEFA ([^{18}F]7) and [^{18}F]NEFT ([^{18}F]8) (Fig. 1) and results of preliminary biological evaluation of these two radiotracers in EMT-6 tumor-bearing murine model.

2 Materials and methods

2.1 General

All chemicals were purchased from Aldrich Chemical Co. and used without further purification unless otherwise indicated. Solvents were dried through a molecular sieve system (Pure Solve Solvent Purification System; Innovative Technology, Inc.). ^1H NMR spectra were obtained at 200MHz in CDCl_3 (Bruker DPX 200 Spectrometer). Chemical shifts are reported as δ values (parts per million) relative to residual protons of deuterated solvent. Coupling constants are reported in Hertz (Hz). The multiplicity is defined by s

(singlet), d (doublet), t (triplet), br (broad), or m (multiplet). Thin layer chromatography (TLC) was run on pre-coated plates of silica gel 60 F254. Flash chromatography was performed using silica gel 60 (230 - 400 mesh, Sigma-Aldrich). Radioisotope ^{18}F enriched aqueous solution was purchased from IBA (IBA Molecular North America, Inc.). Solid-phase extraction cartridges were obtained from Waters (Oasis HLB 3cc, QMA light) (Fisher Scientific).

Synthesis

tert-Butyl 2-(2-nitro-1H-imidazol-1-yl)ethylcarbamate (11): A mixture of **10** (2 mmol, 226 mg), *tert*-butyl 2-bromoethylcarbamate (3 mmol, 669 mg), sodium iodide (NaI) (0.2 mmol, 29 mg) and sodium carbonate (Na_2CO_3) (4 mmol, 552 mg) was stirring in 5 mL N,N-dimethylformamide (DMF) at 60 °C for 48 h. The mixture was then diluted with 15 mL ethyl acetate (EtOAc), and washed by water (H_2O) (10 mL $\times$ 2) as well as brine (5 mL $\times$ 1). The organic layer was dried by anhydrous sodium sulfate (Na_2SO_4) and filtered. The filtration was concentrated, and the residual was purified by flash chromatography (EtOAc/hexane: 1/1) to give 447 mg white solid **11** (yield: 87%): ^1H NMR δ : 7.12(d, 1H), 7.03(d, 1H), 4.70(s, 1H), 4.51-4.57(t, 3H), 3.48-3.57(m, 2H), 1.39(s, 9H).

2-(2-Nitro-1H-imidazol-1-yl)ethanamine (12): A solution of **11** (1.05 mmol, 270 mg) in 5 mL trifluoroacetic acid (TFA)/dichloromethane (DCM) (1:1) was stirred at room temperature for 1 h. Removal of solvent gave a yellow oil. After several times of co-evaporation with methanol, 273 mg **12** (yield: 96%) was given as a white solid: ^1H NMR δ : 7.57(s, 1H), 7.29(s, 1H), 4.84-4.90(m, 2H), 3.56-3.62(t, 2H).

2-Fluoro-N-(2-(2-nitro-1H-imidazol-1-yl)ethyl)acetamide ([^{19}F]7): Fluoroacetyl chloride (5.48 mmol, 529 mg) was added to **12** (1.37 mmol, 370 mg) in 5 mL DCM and 0.74 mL triethylamine (Et_3N) at 0 °C in 5 min. After stirring overnight, the solution was diluted with 10 mL DCM, and washed with H_2O (10 mL $\times$ 2) as well as brine (5 mL $\times$ 1). The organic layer was dried by anhydrous Na_2SO_4 and filtered. The filtration was concentrated and the residual was purified by flash chromatography (EtOAc/hexanes: 8/2) to give 166 mg white solid [^{19}F]7 (yield: 56%): ^1H NMR δ : 7.17(s, 1H), 7.08(s, 1H), 6.61(s, 1H), 4.93(s, 1H), 4.69(s, 1H), 4.61-4.67(t, 2H), 3.80-3.83(m, 2H). ^{13}C NMR δ : 167.08, 131.07, 128.68, 127.20, 49.06, 40.42, 28.58. HRMS (ESI) calculated for $\text{C}_7\text{H}_{10}\text{FN}_4\text{O}_3(\text{MH}^+)$, 217.0737; found, 217.0737 ($\text{M}^+ + \text{H}$).

2-Bromo-N-(2-(2-nitro-1H-imidazol-1-yl)ethyl)acetamide(13): Bromoacetyl bromide (3 mmol, 605 mg) was added to **12** (1.01 mmol, 270 mg) in 5 mL DCM and 0.5 mL Et_3N at 0 °C in 5 min. After stirring overnight, the mixture was diluted with 10 mL DCM, and washed with H_2O (10 mL $\times$ 2) as well as brine (5 mL $\times$ 1). The organic layer was dried by anhydrous Na_2SO_4 and filtered. The filtration was concentrated and the residual crude product was purified by flash chromatography (EtOAc/hexane: 8/2) to give 160 mg white solid **13** (yield: 58%): ^1H NMR δ : 7.14(d, 1H), 7.08(d, 1H), 6.88(s, 1H), 4.60-4.66(t, 2H), 3.87(s, 1H), 3.73-3.82(m, 2H). ^{13}C NMR δ : 168.60, 144.94, 127.57, 127.15, 48.82, 39.13, 26.88. HRMS (ESI) calculated for $\text{C}_7\text{H}_{10}\text{BrN}_4\text{O}_3(\text{MH}^+)$, 276.9936; found 276.9936.

1-(2-(tert-Butyldimethylsilyloxy)ethyl)-2-nitro-1H-imidazole (14): A mixture of **10** (2.65 mmol, 300 mg), (2-bromoethoxy)(*tert*-butyl)dimethylsilane (3.77 mmol, 900 mg) and potassium carbonate (K_2CO_3) (2.90 mmol, 400 mg) was stirring in 10 mL DMF at 100 °C for 4 h. The mixture was then diluted with 15 mL EtOAc, and washed by H_2O (10 mL $\times$ 2) as well as brine (5 mL $\times$ 1). The organic layer was dried by anhydrous Na_2SO_4 and filtered. The filtration was concentrated, and the residual crude product was purified by flash

chromatography (EtOAc/hexane: 3/7) to give 696 mg white solid **14** (yield: 97%): ^1H NMR δ : 7.15(s, 1H), 7.14(s, 1H), 4.53-4.58(t, 2H), 3.93-3.98(t, 2H), 0.84(s, 9H), 0.05(s, 3H).

2-(2-Nitro-1H-imidazol-1-yl)ethanol (15): To a solution of **12** (2.6 mmol, 696 mg) in 5 mL tetrahydrofuran (THF) was added 5 mL HCl solution (0.5 mL 1% HCl in 4.5 mL ethanol) dropwise at room temperature. After 3 h, the mixture was cooled to 0 °C, and neutralized with 10% Na_2CO_3 . The aqueous layer was extracted with chloroform (20 mL $\times$ 3). The organic layer was dried by anhydrous Na_2SO_4 and filtered. The filtration was concentrated, and the residual crude product was purified by flash chromatography (EtOAc/hexane: 1/1) to give 122 mg white solid **15** (yield: 87%): ^1H NMR δ : 7.21(s, 1H), 7.06(s, 1H), 4.47-4.52(t, 2H), 3.84-3.90(t, 2H), 3.14(s, 1H).

2-(2-Nitro-1H-imidazol-1-yl)ethyl 2-fluoroacetate ([^{19}F]8**):** Compound [^{19}F]**8** was prepared from **15** (0.50 mmol, 78.5 mg), fluoroacetyl chloride (1 mmol, 96.5 mg) and Et_3N (0.5 mL) in 5 mL DCM, with the same procedure described for compound [^{19}F]**7**. Compound [^{19}F]**8**: 30 mg (yield: 37%): ^1H NMR δ : 7.19(d, 1H), 7.12(s, 1H), 4.96(s, 1H), 4.74-4.77(dt, 2H), 4.72(s, 1H), 4.60-4.65(dt, 2H). ^{13}C NMR δ : 167.61, 167.18, 128.82, 126.59, 75.62, 63.35, 48.73. HRMS (ESI) calculated for $\text{C}_7\text{H}_9\text{BrN}_3\text{O}_4(\text{MH}^+)$, 218.0577; found, 218.0577.

2-(2-Nitro-1H-imidazol-1-yl)ethyl 2-bromoacetate(16): Compound **16** was prepared from **13** (0.51 mmol, 79 mg), bromoacetyl bromide (1 mmol, 202 mg) and Et_3N (0.5 mL) in 5 mL DCM, with the same procedure described for compound **13**. Compound **16**: 124 mg (yield: 90%). ^1H NMR δ : 7.11(d, 1H), 7.08(d, 1H), 4.63-4.70(m, 2H), 4.48-4.54(m, 2H), 3.73(s, 2H). ^{13}C NMR δ : 166.86, 128.83, 126.76, 64.14, 48.78, 24.94. HRMS (ESI) calculated for $\text{C}_7\text{H}_9\text{BrN}_3\text{O}_4(\text{MH}^+)$, 276.9776; found, 277.9772.

2.2 Radiolabeling

An aqueous solution of [^{18}F] F^- , produced by cyclotron using ^{18}O (p, n) ^{18}F reaction, was passed through a Sep-Pak Light QMA cartridge. The cartridge was dried with argon, and the ^{18}F activity was eluted with a 1.0 mL of 18-crown-6/ KHCO_3 solution (7 mg 18-crown-6 and 2 mg KHCO_3 in 1 mL $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ 8/2). The solvent was then evaporated at 110 °C under an argon stream. Furthermore, the residue was azeotropically dried twice with 1.0 mL anhydrous CH_3CN at 110 °C under an argon stream. A solution of bromide precursor **13** (2 mg) or **16** (2 mg) in 1 mL DMSO was added to the dried [^{18}F] KF/18-crown-6 complex, and heated at 90 °C for 10 min. The mixture was cooled down for 1 min, then diluted with 6 mL water and passed through an Oasis HLB3-mL cartridge (pretreated with 10 mL EtOH, followed with 10 mL water). [^{18}F]**7** or [^{18}F]**8**, trapped in the HLB cartridge, was rinsed with 4 mL H_2O and eluted with 1 mL EtOH. When animal experiments were performed, the final product was dilute with saline to 5% ethanol solution. The labeling took about 60 min, with radiochemical yields (decay corrected; RCY) of 8.6% - 10% ([^{18}F]**7**) and 13% - 14% ([^{18}F]**8**). The RCYs were determined by TLC silica plates, with a mobile phase ethyl acetate. The retention factors (R_f) for [^{18}F] F^- , [^{18}F]**7** and [^{18}F]**8** were 0, 0.4 and 0.6. To determine radiochemical purity (RCP), analytical HPLC with a gamma ray radiodetector was used [Agilent; Gemini C18 (250 $\times$ 4.6 mm); 280 nm; mobile phase: flow rate 1 mL/min; 10 mM ammonium formate buffer (AFB)/ CH_3CN = 7/3]. (Retention time: [^{18}F]**5** = 3.65 min, [^{18}F]**6** = 6.68 min.)

2.3 Biodistribution studies

Saline (0.1 mL) containing [^{18}F] tracers (370–740 Bq) was injected into the tail vein of mice bearing EMT-6 tumor. The mice (n = 3 for each time points) were sacrificed at 30 min post-injection. The organs of interest (brain, heart, liver, spleen, lung, kidney, muscle, bone, skin)

and tumor samples were removed and weighed, and the corresponding radioactivities were assayed with an automatic gamma counter. The percentage dose per organ was calculated by a comparison of the tissue counts to suitable diluted aliquots of the injected material.

2.4 Liquid chromatography-electrospray ionization mass spectrometry analysis of blood and urine from rats

Saline (0.2 mL) containing [^{19}F]7 (2 mg) and [^{19}F]8 (2 mg) was injected into the tail vein of Sprague-Dawley (SD) rat. The rat was sacrificed at 30 min post injection. Urine and plasma samples were collected and analyzed by LC/MS. The LC/MS system comprised of an Ultra-performance liquid chromatography system (Waters, Milford, MA, USA) and a Quattro micro triple quadrupole mass spectrometer (Micromass, Manchester, UK). The mobile phase consisted of 5 mM ammonium acetate and 2% formic acid in water (A) and acetonitrile (B). The analytes were eluted from the column by a linear gradient that increased from 5 to 50% B in 2 min, at a flow-rate of 0.4 ml/min. The composition was returned to 5% B from 2 to 3 min. The end time of the program was set at 4 min. The column was Waters ACQUITY ethylene-bridged (BEH) C18 column (2.1 $\times$ 50 mm, 1.7 μm particle size, Waters, Milford, MA, USA), the temperature of column was 30 $^{\circ}\text{C}$. Sample volume injected was 5 μL . MS was operated in positive Electrospray ionization (ESI), the scan range was from 50 to 1000 Da, full scan mode was used to search the [^{19}F]7, [^{19}F]8 and their metabolites. The mass spectrometry parameters were listed as below: capillary 3.0 kV, cone 20 V, source temperature 110 $^{\circ}\text{C}$, desolvation temperature 450 $^{\circ}\text{C}$, desolvation gas flow 400 L/h, cone gas flow 40 L/h.

3 Results and discussion

3.1 Chemical synthesis

The “cold” compounds [^{19}F]7 and [^{19}F]8 were prepared by reactions shown in Scheme 1 and 2. Starting materials **11** and **14** were synthesized by N-alkylation of commercially available 2-nitroimidazole. The protecting group NH-Boc on compound **11** was removed by treatment with TFA to give **12**. The protecting group O-TBDMS on compound **14** was removed by treatment with 1% HCl in THF to give **15**. The acylation of compounds **12** and **15** with fluoroacetyl chloride and bromoacetyl bromide afforded “cold” standards - fluorinated compounds [^{19}F]7 and [^{19}F]8, as well as radiolabeling precursors **13** and **16**, respectively.

3.2 Radiolabeling

Radiolabeling of **11** and **14** were proceeded initially with a common nucleophilic substitution reaction, using CH_3CN as solvent, Kryptofix 222 (K222) as phase transfer catalyst (PTC) and K_2CO_3 as added metal salt for producing the “naked” ^{18}F fluoride. However, the labeling yields of [^{18}F]7 and [^{18}F]8 from the fluorination reaction performed at 90 $^{\circ}\text{C}$ for 10 min were relatively low (decay corrected yield was 0 and 4-5% (n=3) for [^{18}F]7 and [^{18}F]8, respectively). The observed low yields were likely due to alkali instability of N-bromoacetyl compound **13** and **16** [22]. They were capable of N-alkylating the nitrogen atoms of K222 under this radiolabeling condition. By switching the PTC and metal salt to 18-crown-6 and KHCO_3 , [^{18}F]7 and [^{18}F]8 were obtained at much higher yields (decay corrected yield was 8.6%-10% and 13%-14% (n=3) for [^{18}F]7 and [^{18}F]8, respectively). The preparation of [^{18}F]7 and [^{18}F]8 took about 60 min, and the RCPs were more than 98%. The radiochemical identity was conformed by co-injection with “cold” fluorinated compounds that gave the same retention time ([^{18}F]7 = 3.65 min, [^{18}F]8 = 6.68 min). Purification by a solid-phase extraction (Oasis HLB cartridge) also resulted in a radiochemical pure product that had same HPLC retention time as “cold” fluorinated compound. This solid-phase extraction procedure for purification, instead of preparative HPLC method, has several

advantages: the preparation was rapid and the final product was in a small volume (1.0 mL) of solvent. The procedure simplified the radiolabeling preparation and shortened the time required for radiosynthesis.

3.3 Biodistribution studies

The biodistribution studies of [^{18}F]7 and [^{18}F]8 (radiochemical purity > 98%) were carried out in EMT-6 tumor bearing mice (Table 1). Both compounds showed significant tumor uptakes ([^{18}F]7: 1.55%; [^{18}F]8: 2.35%). High physiological trace uptakes were observed in liver ([^{18}F]7: 2.55%; [^{18}F]8: 2.23%) and kidney ([^{18}F]7, 2.04%; [^{18}F]8, 2.38%). Lower activities were recorded in brain ([^{18}F]7: 0.99%; [^{18}F]8: 1.24) and muscle ([^{18}F]7: 1.36%; [^{18}F]8: 1.74%).

Although the uptakes of various ^{18}F -nitroimidazoles compounds in tumor have been reported before. It's difficult to compare them due to different animal models, nature of the tumor induced and different post-injection time. However, a comparison between [^{18}F]7, [^{18}F]8 and commonly used PET agents [^{18}F]FMISO (**1**) was made in table 1 and 2. [^{18}F]FMISO (**1**) showed the expected high initial tumor uptake (3.29%, after 30 min). However, the uptakes of [^{18}F]7 and [^{18}F]8 in liver ([^{18}F]7, 2.55%; [^{18}F]8, 2.23%) were less compared to that of [^{18}F]FMISO (**1**) (5.54%) with the consequence that the tumor/liver ratios ([^{18}F]7, 0.61; [^{18}F]8, 1.10) were higher as compared to 0.59 in [^{18}F]FMISO (**1**). The tumor/blood and tumor/muscle ratios of [^{18}F]7 and [^{18}F]8 also compared well with those of [^{18}F]FMISO (**1**). The lower uptake of normal tissues was probably because of the rapid in vivo decomposition of [^{18}F]7 or [^{18}F]8, which contain enzymatically or chemically labile functional groups, ie the ester or amide, which may alter the in vivo kinetics of these tracers. The enzymatic transformation of [^{18}F]7 and [^{18}F]8 yielded [^{18}F]fluoroacetate, which had a rapid clearance properties [21]. Since the decrease of normal tissues uptake is an important consideration, compounds [^{18}F]7 and [^{18}F]8 deserve attention.

Liquid chromatography coupled with mass spectrometry (LC/MS) analysis of blood and urine samples from rats was performed. The metabolic profiles of [^{19}F]7 and [^{19}F]8 were investigated by using LC/MS, which were also used to identify the structures of metabolites. Fig. 2 was the total ion current chromatogram and the extracted ion chromatograms of metabolites of [^{19}F]7 and [^{19}F]8, the inserted figures were the corresponding mass spectra. A peak at 0.40 min for m/z 79, consisting with $[\text{M}+\text{H}]^+$ for fluoroacetate, and another peak at 0.84 min for m/z 217, which matched that of [^{19}F]7. We hypothesized the metabolite (m/z 158) found at 0.73 min was mainly generated by hydrolysis of [^{19}F]8. Based on the data a proposed metabolic pathway for in vivo transformation of [^{19}F]8 was listed in Scheme 3.

According to this hypothesis, the radiometabolite of [^{18}F]7 and [^{18}F]8 could be [^{18}F]fluoroacetate, which was reported as a acetate analog for prostate tumor imaging. [^{18}F]fluoroacetate showed a rapid clearance and partial defluorination in rat [21]. This finding strongly supports that the uptake in EMT-6 bearing mice is not [^{18}F]fluoroacetate, and it may be more likely due to the un-metabolized [^{18}F]7 and [^{18}F]8. It is important to note that the metabolite, [^{18}F]fluoroacetate, may play an important role and could be responsible for the observed tumor uptake and retention. We had tried to determine the amount of this metabolite in the tumor tissue. However, in the process of evaluating the in vivo metabolism for [^{18}F]7 and [^{18}F]8 in the EMT-6 tumor after an iv injection. The tracers, [^{18}F]7 and [^{18}F]8, were not stable during the processing of the tumor tissue. The extracts of the tissue samples showed in situ ester hydrolysis of the tracers to form the [^{18}F]fluoroacetate. Therefore, it is difficult to determine if the [^{18}F]fluoroacetate was from in situ hydrolysis, or the [^{18}F]fluoroacetate was transported into the tumor cells. However, as a preliminary report for this series of dual-trapping mechanisms-based tumor imaging agents, the presented information may be sufficient. Further improvements on the methods

for tissue processing will be needed in the future. At this moment it is difficult to determine if the hypoxia or the trapping of the metabolite, [^{18}F]fluoroacetate, (or perhaps both) is more important for the tumor uptake and retention.

4 Conclusion

We report herein the preparation and evaluation of two 2-nitroimidazole analogs containing enzymatically or chemically labile groups. Using the bromo precursors the labeling of these two ^{18}F labeled 2-nitroimidazole analogs was optimized. Biodistribution studies carried out in EMT-6 tumor bearing mice showed a comparable tumor uptake and rapid clearance to those of known hypoxia imaging agent, [^{18}F]FIMSO (1). LC/MS analysis demonstrated that [^{19}F]7 was relatively stable in vivo. [^{19}F]7 and [^{19}F]8 mainly gave fluoroacetate as a metabolite. These results suggested that [^{18}F]7 and [^{18}F]8, containing an in vivo labile group, might be potentially useful as tumor imaging agents.

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List of Abbreviations

LC/MS	liquid chromatography-electrospray ionization mass spectrometry
[¹⁸ F]FMISO (1)	[¹⁸ F]Fluoromisonidazole
[¹⁸ F]FETA (2)	[18F]fluoroetanidazole
[¹⁸ F]FAZA (3)	[¹⁸ F]fluoroazomycin-arabinoside
[¹⁸ F]FETNIM (4)	[¹⁸ F]fluoroerythronitroimidazole
T/B	tumor-to-background

[¹⁸F]HX4 (5)	3-[¹⁸ F]fluoro-2-(4-((2-nitro-1H-imidazol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)propan-1-ol
[¹⁸F]3-NTR (6)	1-[¹⁸ F]fluoro-3-(3-nitro-1H-1,2,4-triazol-1-yl)propan-2-ol
[¹⁸F]NEFA (7)	2-[¹⁸ F]fluoro-N-(2-(2-nitro-1H-imidazol-1-yl)ethyl)acetamide
[¹⁸F]NEFT (8)	2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 2-[¹⁸ F]fluoroacetate
PET	positron emission tomography
SPECT	single photon emission computed tomography
TLC	Thin layer chromatography
DMF	N,N-Dimethylformamide
EtOAc	ethyl acetate
TFA	trifluoroacetic acid
DCM	dichloromethane
Et₃N	triethylamine
THF	tetrahydrofuran
K₂CO₃	potassium carbonate
RCY	radiochemical yield
DMSO	dimethyl sulfoxide
SD	Sprague-Dawley
K222	Kryptonfix 222
PTC	phase transfer catalyst
NaI	sodium iodide
Na₂CO₃	sodium carbonate

Fig. 1. Structures of known 2-nitroimidazole derivatives as hypoxia imaging agents: $[^{18}\text{F}]\text{FMISO}$, **1**, $[^{18}\text{F}]\text{FETA}$, **2**, $[^{18}\text{F}]\text{FAZA}$, **3**, $[^{18}\text{F}]\text{FETNIM}$, **4**, $[^{18}\text{F}]\text{HX4}$, **5**, $[^{18}\text{F}]\text{3-NTR}$, **6**, two new target molecules: $[^{18}\text{F}]\text{NEFA}$, $[^{18}\text{F}]\text{7}$ and $[^{18}\text{F}]\text{NEFT}$, $[^{18}\text{F}]\text{8}$ and misonidazole, **9**.

Fig. 2.
Extracted ion chromatograms of metabolites of $[^{19}\text{F}]$ NEFA ($[^{19}\text{F}]$ 7) and $[^{19}\text{F}]$ NEFT ($[^{19}\text{F}]$ 8)

Scheme 1. Synthesis of compounds [¹⁹F]7 and 13

a) $\text{BrCH}_2\text{CH}_2\text{OTBDMS}$, K_2CO_3 , DMF; b) 1% HCl, THF; c) FCH_2COCl , Et_3N , DCM; d) BrCH_2COBr , Et_3N , DCM; e) $[^{18}\text{F}]\text{KF}/18\text{-crown-6}$, KHCO_3 , DMSO.

Scheme 2.
Synthesis of compounds **8** and **16**.

Scheme 3.

A proposed metabolic pathway of [19F]NEFT ([19F]8)

Table 1
Biodistribution of [^{18}F]NEFA ([^{18}F]7), [^{18}F]NEFT ([^{18}F]8) and [^{18}F]FMISO (1) after 30 min in EMT-6 tumor bearing mice (%dose/g; average of three rats $\pm$ S.D.)

	[^{18}F]NEFA ([^{18}F]7)	[^{18}F]NEFT ([^{18}F]8)	[^{18}F]FMISO (1)
blood	1.61 $\pm$ 0.71	2.50 $\pm$ 0.04	3.60 $\pm$ 1.18
brain	0.99 $\pm$ 0.53	1.24 $\pm$ 0.12	2.60 $\pm$ 0.23
heart	1.77 $\pm$ 0.68	2.46 $\pm$ 0.16	2.55 $\pm$ 1.37
liver	2.55 $\pm$ 1.65	2.23 $\pm$ 0.03	5.54 $\pm$ 1.08
spleen	1.59 $\pm$ 0.82	1.71 $\pm$ 0.12	1.54 $\pm$ 0.25
lung	1.93 $\pm$ 0.90	2.54 $\pm$ 0.13	4.49 $\pm$ 1.99
kidney	2.04 $\pm$ 0.88	2.38 $\pm$ 0.02	2.91 $\pm$ 0.18
muscle	1.36 $\pm$ 0.42	1.74 $\pm$ 0.18	1.89 $\pm$ 0.26
bone	1.83 $\pm$ 0.28	6.29 $\pm$ 0.34	2.03 $\pm$ 0.17
tumor	1.55 $\pm$ 0.65	2.45 $\pm$ 0.08	3.29 $\pm$ 0.73
skin	5.48 $\pm$ 3.69	2.35 $\pm$ 0.25	3.67 $\pm$ 1.43

Table 2

Comparison of tumor-to-tissue of [^{18}F]NEFA ([^{18}F]7), [^{18}F]NEFT ([^{18}F]8) and [^{18}F]FMISO (1)

	[^{18}F]NEFA ([^{18}F]7)	[^{18}F]NEFT ([^{18}F]8)	[^{18}F]FMISO (1)
Tumor/blood	0.96	0.98	0.91
Tumor/liver	0.61	1.1	0.59
Tumor/muscle	1.14	1.41	1.74