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Synthesis of ^{68}Ga-labeled DOTA-nitroimidazole derivatives and their feasibilities as hypoxia imaging PET tracers

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ABSTRACT

The imaging of hypoxia is important for therapeutic decision making in various diseases. ^{68}Ga is an important radionuclide for positron emission tomography (PET), and its usage is increasing, due to the development of the ^{68}Ge/^{68}Ga-generator. In the present study, the authors synthesized two nitroimidazole derivatives by conjugating nitroimidazole and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) via an amide bond (**4**) and a thiourea bond (**5**). Both derivatives were labeled with ^{68}Ga with high labeling efficiency and were stable after labeling. The low partition coefficients (log *P*) of ^{68}Ga-**4** (−4.6) and ^{68}Ga-**5** (−4.5) demonstrated the hydrophilic natures of the derivatives, and both showed higher uptake in cancer cell lines cultured under hypoxic condition than under normoxic condition. However, ^{68}Ga-**5** showed higher liver uptake than ^{68}Ga-**4** in a biodistribution study due to higher lipophilicity. In an animal PET study, ^{68}Ga-**4** showed higher standard uptake values (SUV) in tumors than ^{68}Ga-**5** in mice xenografted with CT-26 mouse colon cancer cells.

1. Introduction

Hypoxia is a characteristic feature of many human and animal tumors, and the subject of intensive research.[1,2] In the case of short-term hypoxia, the metabolism can recover to normality, but if hypoxia persists, irreversible cell damage and death may occur.[3,4] Furthermore, a marker of hypoxia would be useful in the oncologic field, because the presence of hypoxic cells in tumors is regarded to predict a poor response to radiotherapy and indicate the need for higher irradiation doses.[5] Positron emission tomography (PET) offers a promising means of visualizing hypoxia,[6,7] and in this context, several bioreductive substances have been evaluated as hypoxia tracers. In particular, nitroimidazole derivatives tend to accumulate in hypoxic cells, and thus, have been advocated for the imaging of hypoxic tissues.[8–12] In the presence of sufficient oxygen, nitroimidazole undergoes immediate oxidation, but under hypoxic conditions, it undergoes enzymatic single electron reduction to form radical anions,[13,14] which undergo further reduction to produce nitroso ($2e^-$ reduction), hydroxylamine ($4e^-$ reduction), and finally amine ($6e^-$ reduction) derivatives. Furthermore, as a result of these processes, any associated radiolabel is selectively retained in hypoxic cells.[15,16] To date, radio halogenated nitroimidazoles, such as, [^{18}F]fluoromisonidazole (FMISO),[17] [^{18}F]1-α-D-(2-deoxy-2-fluoroarabinofuranosyl)-2-nitroimidazole (FAZA)[7,18] and [^{123}I]iodoazomycin arabinoside (IAZA),[19] have been used clinically to detect hypoxia in tumors.

The clinical applications of PET have been shown to be important for the evaluation and diagnosis of many diseases.[20] The radionuclides most commonly used for PET are ^{18}F and ^{11}C, which are produced using an accelerator.[21–23] Other metallic positron emitters, such as, ^{86}Y, ^{64}Cu, and ^{68}Ga also can be used for PET,[24–30] and of these, ^{68}Ga is the most promising, because it can be obtained using a commercially available generator system consisting of an inorganic matrix immobilizing the parent radionuclide ^{68}Ge.[31] This availability has led to great interest in the development of ^{68}Ga-labeled agents, because of its cost and convenience benefits. Furthermore, the parent nuclide ^{68}Ge has a long half-life of 270.8 days, which allows the generator to be used for almost a year.

1,4,7-Triazacyclononane-1,4,7-triacetic acid (NOTA) and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) are widely used bifunctional chelating agents, because they can form highly stable radiometal chelates with ^{68}Ga.[32–34] DOTA has eight coordinating electron donor atoms, namely, four nitrogen atoms of tertiary amines and four oxygen atoms of carboxylic acids, whereby the four nitrogen atoms form a ring and two of the oxygen atoms

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from the pendant arm coordinate with gallium with a distorted octahedral geometry.[35] The two remaining carboxylic acid groups can be used for coupling with functional moieties. ^{68}Ga-labeled NOTA-nitroimidazole derivatives have been reported to show high liver, kidney, and tumor uptakes.[36] Furthermore, it has been postulated that more hydrophilic NOTA derivatives show reduced liver uptake. In the present study, we investigated the potential uses of DOTA-conjugated nitroimidazole derivatives, which are more hydrophilic than corresponding NOTA-conjugated derivatives, for ^{68}Ga-labeling and examined their biological properties.

2. Results and discussion

2.1. Chemistry

Hypoxia is defined as a condition with insufficient oxygen to support metabolism, and occurs when vascular supply is interrupted, especially in growing tumors. The detection and imaging of hypoxia would be of great use in therapeutic oncology, because hypoxic cells in tumors are more resistant to radiation than normoxic cells. Thus, the development of radiolabeled nitroimidazole derivatives for hypoxia imaging constitutes an active research field.

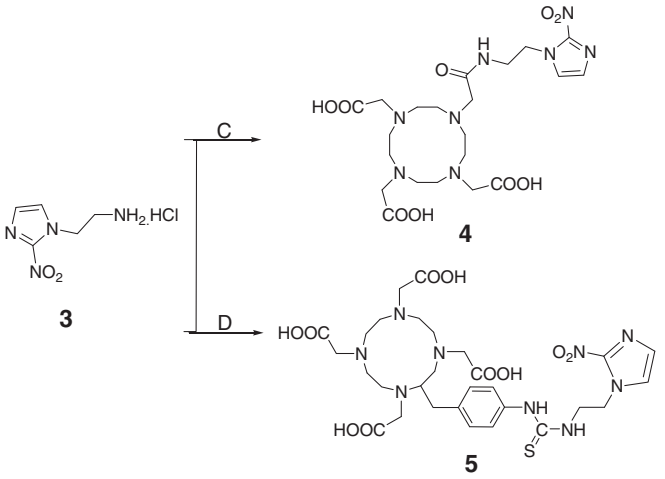
In the present study, nitroimidazole was conjugated with DOTA or DOTA derivatives as bifunctional chelating agents for labeling with ^{68}Ga. There was a report that a DOTA derivative conjugated with two metromidazole moieties for ^{67}Ga labeling.[37]

In the present study, *tert*-butyl 2-bromoethylcarbamate was used as a linker to conjugate with a nitroimidazole group on one side and a chelating group on the other (Scheme 1). Compound **2** was synthesized from **1** and *tert*-butyl 2-bromoethylcarbamate using K_2CO_3 as a base in dimethylformamide (DMF). Overnight stirring at room temperature was enough for completion of the reaction. The product obtained was recrystallized in EtOAc. During the synthesis of **2**, a yield of 35% was obtained when MeCN was used as a reaction solvent, but increased to 80% when the reaction was carried out in DMF, which we presume was due to the solubility characteristics of **1**. The amine group was then deprotected using 1.25 M HCl in MeOH to give **3**, which was recrystallized in MeOH and obtained at an yield of 84%. Reaction completions were checked by TLC, and chemical structures were confirmed by mass spectroscopy/electrospray in ionization positive mode (MS/ESI$^+$) and by Nuclear magnetic resonance (NMR) of purified samples.

The intermediate **3** served as a precursor for **4** and **5**, as shown in Scheme 2. The synthesis of **4** was performed by acid–amine conjugation, whereas **5** was by synthesized by isothiocyanate–amine conjugation. The former reaction was carried out in water and DMF (1:1, v/v) using *N*,*N*′-dicyclohexylcarbodiimide (DCC) as a coupling agent in the presence of pyridine, and the latter in $CHCl_3$ using triethylamine (TEA) as a base. Reaction completeness was checked by MS/ESI$^+$ and samples were purified by RP-HPLC (reverse phase-high performance liquid chromatography) to obtain **4** (53%) and **5** (75%) at high purity. NMR was used to confirm sample identities (Supplementary data).

2.2. Radiochemistry

Radiolabeling was conducted at pH 3 in a boiling water bath for 10 min using ^{68}Ga eluted from a ^{68}Ge/^{68}Ga-generator by 0.1 N HCl.



Scheme 2. Synthesis of 2-(2-nitroimidazolyl)ethylamine-DO**TA** (**4**) and 2-(2-nitroimidazolyl)ethylamine-SCN-Bz-DOTA (**5**). (C) DOTA, H_2O/DMF, DCC, pyridine, conducted at rt overnight; (D) SCN-DOTA, $CHCl_3$, TEA, also conducted at rt overnight.

After labeling, the reaction mixture was purified using an alumina cartridge to remove free $^{68}Ga^{3+}$. Labeling efficiencies were determined using Instant Thin Layer Chromatography-Silica Gel (ITLC-SG) (Supplementary data) and were > 98% for both **4** and **5**. The specific activities of ^{68}G-**4** and ^{68}Ga-**5** were 4.81×10^6 and 7.77×10^6 GBq/mol, respectively, and their partition coefficients (log *P*) were −4.6 and −4.5, respectively, which represented hydrophilic nature of both agents. ^{68}Ga-**4** was slightly more hydrophilic than ^{68}Ga-**5**.

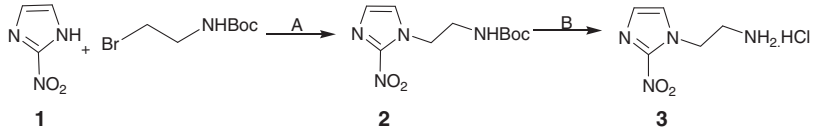
2.3. In vitro characterization

Protein bindings results at 10 and 60 min were; 4.25 ± 0.02% and 15.29 ± 0.28% for ^{68}Ga-**4**, and 8.83 ± 1.48% and 14.04 ± 0.49% for ^{68}Ga-**5**, respectively. Both agents were stable in prepared medium and in human serum for up to 120 min (see Supplementary data).

Hela (Henrietta lacks cervical cancer), CHO (Chinese hamster ovarian cancer), and CT-26 (mouse colon cancer) cell lines were used for the cell uptake study. All three cell lines showed significantly higher ^{68}Ga-**4** and ^{68}Ga-**5** uptakes after exposure for 1 h to hypoxic conditions than to normoxic conditions (Fig. 1). The uptake ratios (hypoxic/normoxic) of ^{68}Ga-**4** at 5, 15, 30 and 60 min were, 1.4, 1.9, 1.7, and 2.4 for Hela, 1.6, 2.1, 2.1, and 1.8 for CHO, and 1.5, 2.4, 4.2 and 6.8 for CT-26, respectively, and those of for ^{68}Ga-**5** were 0.6, 2.5, 1.6 and 2.1 for Hela, 2.1, 1.9, 2.6 and 2.3 for CHO, and 2.3, 1.9, 4.2 and 3.4 for CT-26. Thus, all three cell lines demonstrated higher uptakes under hypoxic condition than under normoxic condition. Of the cell lines, CT-26 showed greatest radioligand uptake, and thus CT-26 cells were chosen for the in vitro model and to evaluate radioligands developed during this study.

2.4. In vivo study

Biodistribution studies were performed on ^{68}Ga-**4** and ^{68}Ga-**5** after tail vein injection in mice bearing CT-26 xenografts (Fig. 2).



Scheme 1. Synthesis of 2-(2-Nitroimidazolyl)ethanamine (**3**). Reagents and conditions: (A) K_2CO_3 and DMF conducted at rt; (B) HCl/MeOH also conducted at rt.

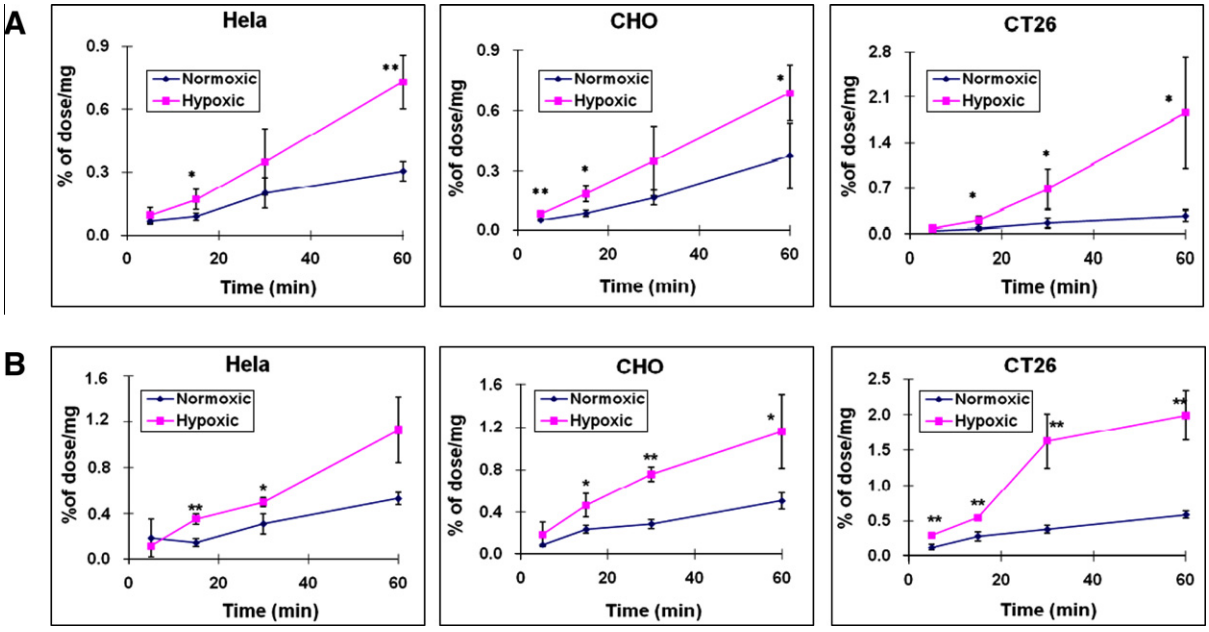


Figure 1. In vitro cell uptake studies of (A) ^{68}Ga-**4** and (B) ^{68}Ga-**5** under normoxic and hypoxic conditions in HeLa, CHO and CT-26 cells. Statistical analyses of uptakes under normoxic and hypoxic conditions were performed using the Student's *t*-test. ($^{**}p$ <0.01, $^{*}p$ <0.05, n = 4 at each time point).

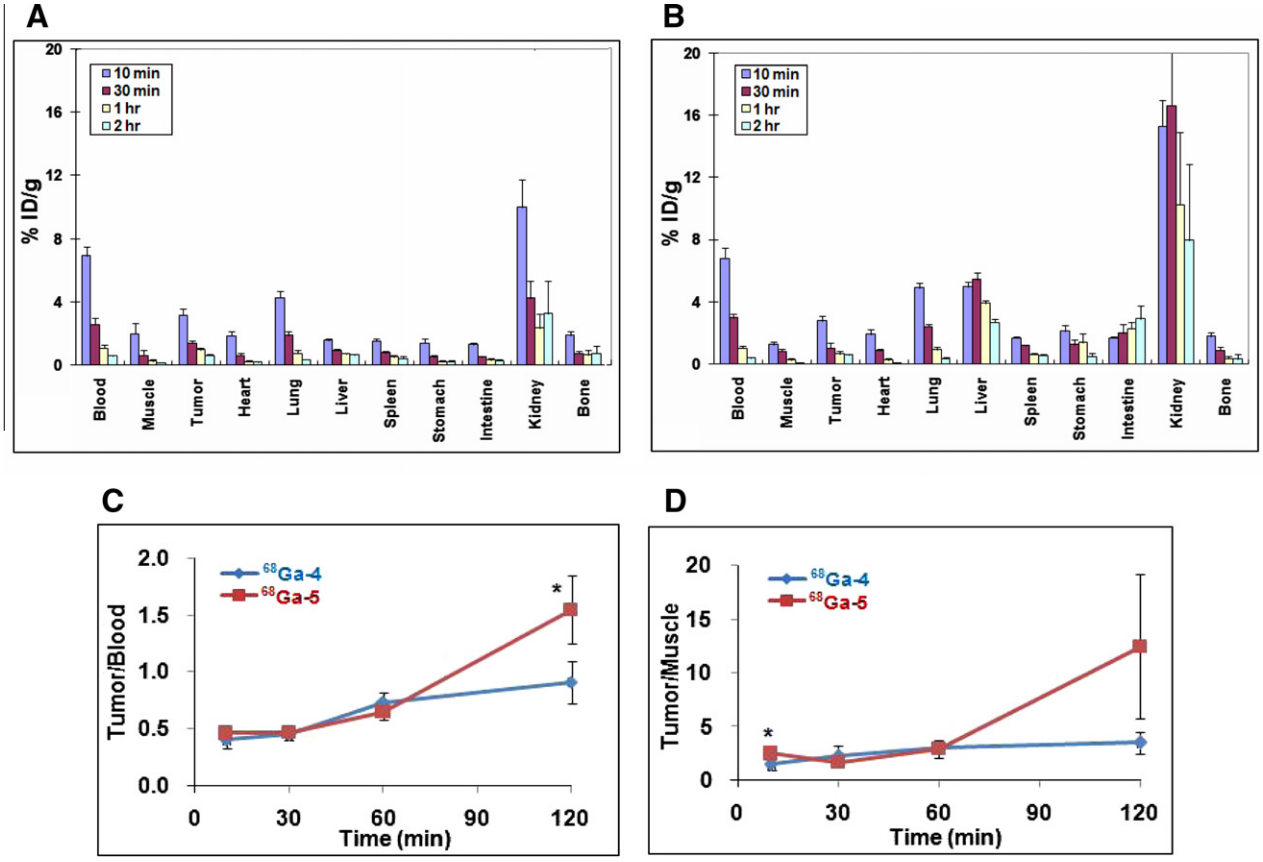


Figure 2. Biodistribution studies in mice bearing a CT-26 xenograft; (A) ^{68}Ga-**4** (0.37 MBq/0.1 mL) and (B) ^{68}Ga-**5** (0.37 MBq/0.1 mL) injected via a tail vein at. Studies were performed at 10, 30, 60, and 120 min post-injection. (C) Tumor to blood ratios and (D) tumor to muscle ratio with respect to time.

Highest uptakes were found in the kidneys for both compounds, suggesting that the renal excretion route predominated. The higher liver and intestine uptakes of ^{68}Ga-**5** were presumed to be due to it higher lipophilicity. The initial tumor uptakes of ^{68}Ga-**4** and ^{68}Ga-**5** at 10 min post-injection were 3.17 and 2.78% ID/g, respectively. These activities then decreased at 30 min (1.41% ID/g for ^{68}Ga-**4** and 1.02% ID/g for ^{68}Ga-**5**), 60 min (0.98% ID/g for ^{68}Ga-**4** and 0.66% ID/g for ^{68}Ga-**5**) and 120 min (0.64% ID/g for ^{68}Ga-**4** and

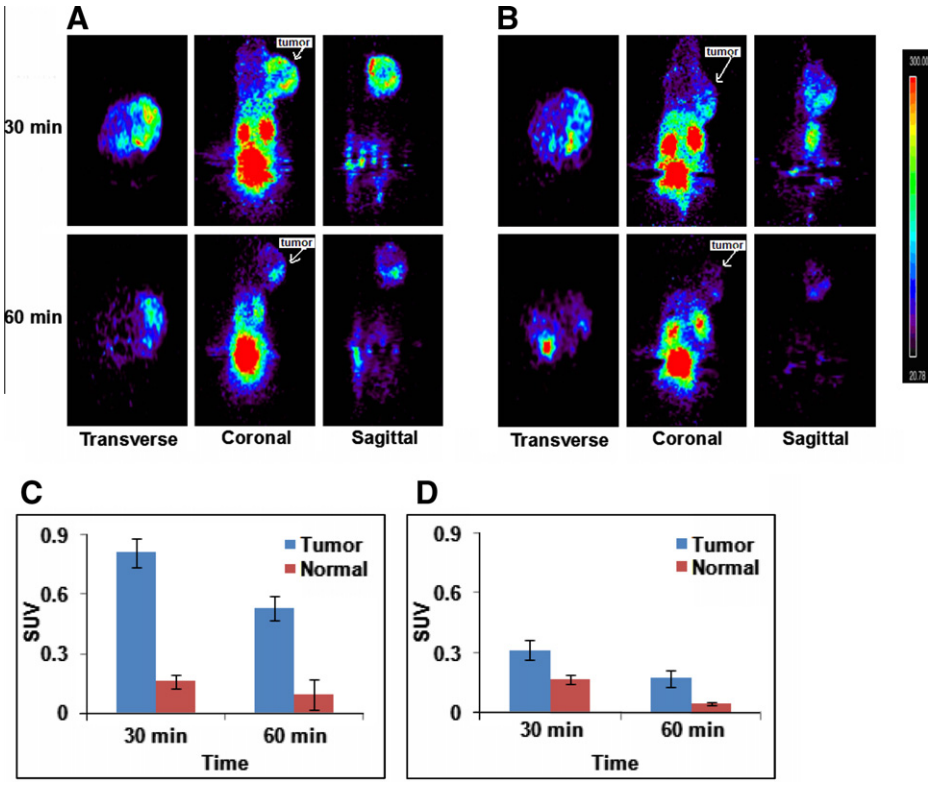


Figure 3. Small-animal PET images obtained at 30 and 60 min after injecting (A) ^{68}Ga-**4** or (B) ^{68}Ga-**5** in mice bearing a CT-26 xenograft in the right shoulder. (C) and (D) show the SUVs of ^{68}Ga-**4** and ^{68}Ga-**5**, respectively.

0.59% ID/g for ^{68}Ga-**5**). Furthermore, observed radioactivity decreases were more rapid in blood and muscle, and thus, tumor to blood and tumor to muscle ratios increased over 120 min post-injection; tumor to muscle ratios were 3.96 for ^{68}Ga-**4** and 12.48 for ^{68}Ga-**5** at 120 min (Fig. 2). The above results suggested that ^{68}Ga-**4** and ^{68}Ga-**5** are potential hypoxia imaging PET agents.

A small-animal PET study was performed in CT-26 xenografted mice. Images were obtained at 30 and 60 min after injecting ^{68}Ga-**4** or ^{68}Ga-**5** via tail veins. Both agents showed high kidney and bladder uptakes. PET studies confirmed the reduction in initial tumor uptakes. ^{68}Ga-**4** demonstrated more distinct tumor uptake than ^{68}Ga-**5** (Fig. 3). In addition, tumor standardized uptake values (SUVs) showed that ^{68}Ga-**4** uptake was greater than ^{68}Ga-**5** uptake, at 30 and 60 min. Mean SUVs were 0.81 ± 0.1 and 0.53 ± 0.1 for ^{68}Ga-**4** and 0.31 ± 0.1 and 0.17 ± 0.1 for ^{68}Ga-**5**, respectively. Furthermore, the tumor to non-tumor SUV ratios of ^{68}Ga-**4** was also greater than those of ^{68}Ga-**5** (at 30 min 5.08 ± 2.2 vs 1.86 ± 0.3, respectively, and at 60 min 5.64 ± 0.8 vs 3.83 ± 0.8).

3. Conclusion

We describe the synthesis of two ^{68}Ga-labeled DOTA-nitroimidazole derivatives via amide or thiourea bond formation for the imaging tumor hypoxia. Both compounds were labeled within 10 min at high labeling efficiencies (>98%). Furthermore, both showed adequate stability in prepared medium and human serum, and time-dependent uptake by hypoxic cells. In addition, our biodistribution and PET studies demonstrated that the compounds have high tumor to muscle count density ratios. These promising in vivo and in vitro results suggest that both ^{68}Ga-labeled DOTA-nitroimidazole derivatives offer the possibility of a fast and efficient means of detecting tumor hypoxia by ^{68}Ga PET imaging.

4. Experimental section

4.1. General

DOTA was purchased from ChemaTech (Dijon, France) and isothiocyanatobenzyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (SCN-DOTA) from Futurechem (Seoul, Korea). HPLC grade MeCN was purchased from Fischer Scientific Korea Ltd (Seoul, Korea). All other reagents were purchased from Sigma-Aldrich (St. Louis) and were used as supplied without further purification. The Thermo Scientific Pierce BCA protein assay kits used for protein concentration measurement were from Rockford (IL, USA).

MS/ESI was performed using a Waters ESI ion trap spectrometer (Milford, CT, USA) and high resolution mass spectra (HRMS) on a Jeol, JMS-600W Agilent 6890 series spectrometer (Tokyo, Japan) by fast atomic bombardment (FAB+) ionization detection in positive ion mode. Samples were diluted 50-fold with water or MeCN prior to injection. ^{1}H NMR and ^{13}C NMR spectra were recorded on an AL 300 FT NMR spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C; Jeol, Tokyo, Japan) using residual solvent signals as an internal reference. ^{1}H chemical shifts are expressed as δ values (parts per million) relative to tetramethylsilane. For prep-HPLC, a XTerra® prep RP18 10 μm (10 × 250 mm) column (Waters Corporation, Milford, CT, USA) was eluted at a flow rate of 5 mL/min using the following solvents: solvent A: 10 mM HCl in H_2O, solvent B: MeCN. The unit was equipped with a 215 nm and 240 nm UV detection system. Analytical HPLC was performed using a XTerra® RP18 3.5 μm (4.6 × 100 mm) column (Waters Corporation, Milford, CT, USA), under the same conditions using a flow rate of 1 mL/min. ^{68}Ge/^{68}Ga-generator was obtained from Cyclotron Co. (Obninsk, Russia). The gamma scintillation counter was from Packard Cobra II (GMI; MN, USA). The Waters Sep-Pak Light Accell Plus QMA cartridge and the Alumina-N cartridge were both from

Waters (Milford, CT, USA). Instant thin layer chromatography-silica gel (ITLC-SG) plates were purchased from Agilent Technologies (Wilmington, DE, USA). PET studies were performed using a dedicated small-animal PET/CT scanner (GE Healthcare; Princeton, NJ, USA).

4.2. *tert*-*N*-Butyl 2-(2-nitroimidazolyl)ethylcarbamate (2)

K_2CO_3 (1.83 g, 13.26 mmol) was added to a solution of **1** (1 g, 8.84 mmol) in DMF (3 mL) with stirring. *tert*-Butyl 2-bromoethylcarbamate (1.98 g, 8.84 mmol) was then added dropwise and stirred at room temperature overnight. The reaction mixture was then filtered, the solid obtained was washed successively with MeOH, and solvent was removed by evaporation. The solid obtained was then dissolved in water and extracted with EtOAc. The organic layer was evaporated in vacuo to obtain the crude compound, which was recrystallized from EtOAc to afford the pure compound as a dark yellow solid (1.9 g, 84%). MS *m*/*z* (ESI^+), $(M+K)^+$: 279.1. ^1H NMR (CD_3OD): δ 7.35 (s, 1H), 7.10 (s, 1H), 4.51–4.54 (t, J = 9 Hz, 2H), 3.47–3.50 (t, J = 9 Hz, 2H), 1.28–1.39 (s, 9H). ^{13}C NMR (CD_3OD): δ 158.4, 131.9, 129, 128.3, 80.4, 51.2, 40.8, 28.7.

4.3. 2-(2-Nitroimidazolyl)ethanamine (3)

To a solution of **2** (1.9 g) in MeOH (2 mL), 1.25 M HCl in MeOH (3 mL) was added at room temperature with continuous stirring and left on stir for 5 h. Crude compound **3** was obtained by filtration, washed successively with MeOH, and solvent was removed by evaporation in vacuo. The solid obtained was recrystallized from MeOH to afford the pure compound as a pale yellow solid (1.2 g, 84%). MS *m*/*z* (ESI^+), $(M+H)^+$: 157. ^1H NMR (300 MHz, CD_3OD): δ 7.56 (s, 1H), 7.21 (s, 1H), 4.75–4.79 (t, J = 12 Hz, 2H), 3.47–3.51 (t, J = 12 Hz, 2H). ^{13}C NMR (CD_3OD): δ 145.2, 128.8, 128.5, 47.2, 39.7.

4.4. 2-(2-Nitroimidazolyl)ethylamine-DOTA (4)

To a solution of DOTA (0.050 g, 0.12 mmol) in water (3 mL) was added a solution of **3** (0.016 g, 0.08 mmol) in DMF (3 mL), followed by a solution of DCC (0.026 g, 0.12 mmol) in pyridine (0.5 mL), all with stirring. The reaction mixture was then stirred at room temperature overnight, filtered, washed thoroughly with water, and purified by RP-HPLC (100% of A for 5 min and 0–40% of B for 30 min) to give **4** as a white solid (36 mg, 53.7%). MS *m*/*z* (ESI^+), $(M+H)^+$: 543.2 obsd, 543.25 calcd. HRMS: 543.2529 obsd, 543.2527 calcd for $C_{21}H_{35}N_8O_8$. ^1H NMR (300 MHz, D_2O): δ 7.19 (s, 1H), 6.94 (s, 1H), 4.32 (s, 2H), 3.70–2.74 (br, 24H), 2.58 (s, 2H). ^{13}C NMR (75 MHz, D_2O): δ 165.5, 145.2, 129.2, 128.1, 66.6, 50.2, 41.0, 39.1, 37.5, 31.9.

4.5. 2-(2-Nitroimidazolyl)ethylamine-SCN-Bz-DOTA (5)

A mixture of SCN-DOTA (0.05 g, 0.07 mmol) and **3** (0.021 g, 0.11 mmol) in $CHCl_3$ (1 mL) containing TEA (29 mg) were stirred overnight at room temperature. The resulting product was purified by preparative HPLC (0 to 100% of B for 30 min and 100% of B for 5 min) to give **5** as a white solid (48 mg, 75%). MS *m*/*z* (ESI^+), $(M+H)^+$: 708.1 obsd, 708.28 calcd. HRMS, $(M+Na)^+$: 730.2589 obsd, 730.2595 calcd for $C_{29}H_{41}N_9O_{10}S$. ^1H NMR (300 MHz, D_2O): δ 7.13 (s, 1H), 7.03 (br, 2H), 6.90 (s, 1H), 6.85 (br, 2H), 4.35 (br, 3H), 4.05 (br, 2H), 3.80–3.32 (br, 10H), 3.07–2.95 (br, 9H), 2.59 (s, 1H), 2.35 (br, 1H), 1.78–1.77 (br, 3H). ^{13}C NMR (75 MHz, D_2O): δ 181.0, 175.3, 174.4, 169.1, 145.2, 130.9, 129.4, 128.3, 127.1, 56.1, 55.2, 54.6, 53.5, 52.7, 52.1, 50.2, 49.7, 47.2, 46.7, 43.8, 31.6.

4.6. General procedure used for the radiolabeling experiment

$^{68}GaCl_3$ (39.6–245.3 MBq) was eluted from a $^{68}Ge/^{68}Ga$-generator using 0.1 M HCl. Compound **4** (30 μg) or **5** (25 μg) was then added to the HCl solution containing ^{68}Ga, followed by 1 M sodium acetate buffer (pH 5, 0.1 mL) to adjust the pH to ~3. The reaction mixture was then heated to 100 °C for 10 min. The labeled derivatives were passed through an alumina-N cartridge pre-washed with water and monitored by ITLC-SG using 0.1 M Na_2CO_3 as an eluant to check labeling efficiencies ($^{68}Ga^{3+}$ remained at the origin, and labeled products moved to the solvent front).

4.7. Radiochemical evaluations of the labeled derivatives

The stabilities of ^{68}Ga-**4** and ^{68}Ga-**5** were investigated in prepared medium at room temperature and in human serum at 37 °C for 2 h. Degradations were monitored by ITLC-SG using 0.1 M Na_2CO_3 and 0.1 M HCl as eluents.

To determine partition coefficients, 0.1 M sodium phosphate buffer (pH 7.4, 3 g) was added to octanol (3 g), and ^{68}Ga-**4** (1.0 MBq/10 μL) or ^{68}Ga-**5** (1.1 MBq/10 μL) were added, mixed vigorously, and centrifuged (3000 rpm) for 5 min. Aliquots of the aqueous and octanol phases were sampled and counted using a gamma counter. Log *P* values were then calculated.

For the protein binding study, PD-10 columns were preconditioned by treating them with 1.0 mL of 1% bovine serum albumin in 0.1 M DTPA followed by successive washing with 100 mL of phosphate buffered saline (PBS). ^{68}Ga-**4** (1.3 MBq/10 μL) or ^{68}Ga-**5** (1.1 MBq/10 μL) were mixed with human serum (1 mL) and incubated for 10 or 60 min at 37 °C. Each mixture was then loaded onto the preconditioned PD-10 column and eluted with PBS to obtain thirty 0.5 mL fractions. Radioactivities were measured as cpm (counts per minute) using a gamma counter. A 2 μL aliquot from each fraction was spotted on filter paper and stained with Coomassie blue to check for the presence of protein. Protein bound fractions appeared at the void volume and free fractions at the bed volume.

4.8. In vitro cell uptake study

Cell uptake studies were carried out using the HeLA, CHO, and CT-26 cell lines. All three cell lines were maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (both from Welgene Inc., Daegu, Korea) and a 1% antibiotic mixture (penicillin, streptomycin, and amphotericin B: 10,000 IU/10 mg/25 μg/mL, Mediatech Inc. Manassas, VA, USA) in a 5% CO_2 incubator at 37 °C. Cells were sub-cultured overnight in 24-well plates (2×10^5 cells/mL), and then pre-incubated under normoxic or hypoxic conditions for 4 h. For the normoxic condition, we used 5% CO_2 in air, and for the hypoxic condition 5% CO_2 in N_2. ^{68}Ga-**4** (0.37 MBq/100 μL) or ^{68}Ga-**5** (0.43 MBq/100 μL) were added to the wells and incubated for 5, 15, 30, or 60 min. Wells were then washed with DMEM and cells were suspended in 0.5% sodium dodecyl sulfate (SDS) in PBS (0.5 mL). Tracer uptakes were measured using a gamma counter and total protein concentrations in samples were determined using the bicinchonic acid method.

4.9. Biodistribution studies in mice bearing colon cancer xenografts

Experiments using mice were carried out according to protocols approved by the Institutional Animal Care and Use Committee of the Clinical Research Institute at Seoul National University Hospital (an Association for the Assessment and Accreditation of Laboratory Animal Care approved facility). In addition, National Research Council guidelines for the care and use of laboratory animals

(revised in 1996) were observed throughout. The mouse colon cancer cell line CT-26 was grown in RPMI 1640 medium containing 10% fetal bovine serum and 1% antibiotics. Cells were washed with 10 mL of PBS by centrifugation (3000 rpm). Balb/c mice were injected subcutaneously in right shoulders with 2×10^5/0.1 mL CT-26 cells, and 14 days later, ^{68}Ga-labeled agents (0.37 MBq/0.1 mL) were injected intravenously into each mouse via a tail vein. Mice were sacrificed by decapitation at various times (10, 30, 60, and 120 min) after radiotracer administration. Tumor, blood, muscle, and other organs were then excised, blotted, weighed, and counted. Results are expressed as percentages of injected doses per gram of tissue (% ID/g).

4.10. Small-animal PET studies

CT-26 cells (2×10^5 cells) in normal saline (0.1 mL) were subcutaneously injected into mouse right shoulders. The tumors grew to a diameter of ~15 mm in 14 days. ^{68}Ga-**4** (24.1 MBq/0.15 mL) and ^{68}Ga-**5** (39.3 MBq/0.15 mL) were intravenously injected into tumor-bearing mice via tail veins. Mice were anesthetized with 2% isoflurane and PET images were obtained at 30 and 60 min post-injection. Acquired 3-dimensional emission data was reconstructed to temporally framed sonograms by Fourier rebinning using an ordered subset expectation maximization reconstruction algorithm without attenuation correction. Images were visualized using ASIPro VM 5.0 software.

Image and region of interest (ROI) analyses of PET data sets were performed using ASIPro software. The spherical ROIs of radius 1.5 mm placed in tumors and muscles were collected, and mean (value/pixel) and standard deviation were determined. SUV values were calculated using the formula, SUV = CCF/(injected dose/body weight).

Where, CCF (decay corrected activity concentration) was calculated using the formula.

$$\underset{(\mathrm{MBq/mL})}{\mathrm{CCF}} = \underset{(\mathrm{mCi/mL})}{\text{radioactivity}} \times \underset{(0.891 \text{ for } ^{68}\mathrm{Ga})}{\text{branching ratio}} \times \underset{(\text{value/pixel})}{\mathrm{ROI}}$$

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.02.041. These data include MOL files and InChiKeys of the most important compounds described in this article.

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