Radiosynthesis of microtubule-targeted theranostic methyl *N*-[5-(3'-radiohalobenzoyl)-1*H*-benzimidazol-2-yl]carbamates

Zbigniew P. Kortylewicz and

University of Nebraska Medical Center, Department of Radiation Oncology, J. Bruce Henriksen Cancer Research Laboratories

Janina Baranowska-Kortylewicz

University of Nebraska Medical Center, Department of Radiation Oncology, J. Bruce Henriksen Cancer Research Laboratories

Abstract

Microtubules are a target for a broad spectrum of drugs used as chemotherapeutics to treat hematological malignancies and solid tumors. Most of these drugs have significant dose-limiting toxicities including peripheral neuropathies that can be debilitating and permanent. In an ongoing effort to develop safer and more effective drugs, benzimidazole-based compounds are being developed as replacement for vincristine and similar agents.

In this report, we describe radiosyntheses of novel microtubule-targeting methyl *N*-[5-(3'-radiohalobenzoyl)-1*H*-benzimidazol-2-yl]carbamates **4** that are intended as potential imaging agents and molecular radiotherapeutics. ¹²⁵I- and ¹³¹I-radiolabeled derivatives were prepared either by direct radioiodination of methyl *N*-(6-benzoyl-1*H*-benzimidazol-2-yl)carbamate **1** or radioiododestannylation of the corresponding stannane precursor **3**. The direct radioiodination was conducted in a solution of **1** in triflic acid and produced after ~1 hour at elevated temperatures and HPLC purification on average 62% of the no-carrier added products ¹²⁵I-**4** and ¹³¹I-**4**.

Radioiododestannylation of 3'-trimethylstannane **3** proceeded with ease at room temperature in the presence of H₂O₂ as the oxidant and produced no-carrier-added ¹²⁵I-**4** and ¹³¹I-**4** in high isolated yields, on average 85%. The radiohalodestannylation protocol is universal and can be applied to other radiohalides including ¹²⁴I to produce ¹²⁴I-**4**, a positron emission tomography agent, and ²¹¹At to produce ²¹¹At-**4**, an α -particle emitting radiotherapeutic.

Keywords

imaging; molecular radiotherapy; theranostic; benzimidazole; microtubules; radiohalides

Corresponding Author: Janina Baranowska-Kortylewicz, University of Nebraska Medical Center, Department of Radiation Oncology, J. Bruce Henriksen Cancer Research Laboratories, 986850 Nebraska Medical Center, Omaha, NE 68198-6850, jbaranow@unmc.edu, phone: 402-559-8906.

CONFLICT OF INTEREST

Authors have no conflict of interest.

INTRODUCTION

Drugs that target the cytoskeleton and spindle apparatus of tumor cells belong to one of the most important classes of chemotherapeutics^{1–5}. Functional microtubules are essential for proliferation, invasion and migration of cancer cells. Microtubule-binding drugs such as taxanes, vinca alkaloids and epothilones are widely used as the standard first-line option treatment in many cancers^{2,6,7} but their utility is frequently limited by significant toxicities and primary or acquired resistance. Resistance to chemotherapy remains a major clinical issue.

Ongoing preclinical studies identified new classes of microtubule-targeting chemotherapeutics that retain their activity despite clinical resistance to taxanes. Several benzimidazoles were found to have microtubule-disrupting properties as well as anti-proliferative effects in cancer cell lines and in animal models^{2,8,9}.

Motivated by the possibility that radiolabeled microtubule targeting drugs may be safer and more effective than conventional drugs, we have prepared methyl *N*-[5-(3'-radiohalobenzoyl)-1*H*-benzimidazol-2-yl]carbamates as potential drug candidates. New drugs were designed to function as theranostics that can be radiolabeled without reshaping their chemical structure with either positron- and γ -emitting radiohalides for imaging or α - and β -particle-emitting radiohalides for molecular radiotherapy.

In the age of personalized treatments, it is important to quantify the uptake of a drug by the tumor as well as normal tissues to assess the efficacy and potential off-target toxicity. The ability to measure radiation doses delivered by the radiolabeled drug to tumors can help to predict patient's response to the treatment. Only a few attempts have been made to develop microtubule-targeted imaging agents^{10,11}. Recently, ¹¹C-docetaxel was synthesized and evaluated in cancer patients to directly measure docetaxel concentrations in tumor and facilitate rational treatment choices^{12,13}. The purpose of the present study was to develop novel microtubule-targeted radiopharmaceuticals that can be used as imaging as well as radiotherapeutic agents and that can enable distribution assessments of the nonradioactive analogues.

MATERIALS AND METHODS

Chemistry.

Chemicals and reagents were purchased from commercial suppliers in the highest purity available and used without further purification. Radioiodides Na¹³¹I in 0.1 M NaOH (pH 12–14) and Na¹²⁵I in 1×10^{–5} M NaOH (pH 8–11) were obtained from PerkinElmer (Waltham, MA). Radioactivity was measured with Minaxi γ -counter (Packard, Waltham, MA) and a dose calibrator (Capintec Inc., Florham Park, NJ). Compounds were separated and their purity confirmed by HPLC using Gilson (Middleton, WI) and ISCO (Lincoln, NE) systems on 5 μ , 100 Å, (250×4.6 mm), analytical or semipreparative (250×10 mm) columns, either Columbus C8, C18, Luna CN (Phenomenex, Torrance, CA) or ACE C18 (Advanced Chromatography Technologies, www.ace-hplc.com) eluted at rates of 0.8 – 2.0 mL/min with various gradients of CH₃CN or CH₃OH (10% - 95%) in water with (0.07%, v/v) or without

trifluoroacetic acid (TFA). Variable wavelength UV detectors UVIS-205 (Linear, Irvine, CA) or UV116 (Gilson) were used jointly with the sodium iodide crystal Bioscan Flow-Count detector connected in-line at the outlet of the UV detector. Both signals were monitored and analyzed simultaneously. The separated radiolabeled products, if kept in a solution overnight, were purified one more time shortly before conducting further experiments even though the HPLC analysis rarely indicated the radiochemical purity of less than 98%. Solutions containing final products were evaporated with a stream of nitrogen to dryness and reconstituted in a preferred solvent at the required concentration and then filtered through a sterile 0.2- μ filter (Millipore) into a sterile evacuated vial. NMR spectra were recorded in (CD₃)₂SO at ambient temperature on Bruker Avance III HD 600 MHz spectrometer. All NMR analyses were performed at the University of Nebraska Medical Center Eppley Institute NMR Facility (Omaha, NE). Chemical shifts are given as δ (ppm) relative to TMS as internal standard with J in hertz. High resolution electrospray ionization (ESI-HR) mass spectra were acquired on MaXis 4G mass spectrometer at the Washington University Resource for Biomedical and Bio-organic Mass Spectrometry (St. Louis, MO). Tip amounts of samples dissolved in 1 mL of 50% acetonitrile (MeCN) solution with 0.1% formic acid were analyzed under positive mode. All target nonradioactive compounds were found to be 95% pure by the rigorous HPLC analysis with the integration of the peak area (detected at 220 and/or 254 nm). In the consecutive radio-HPLC analyses, each purified radioiodinated product was matched with the corresponding, independently prepared, non-radioactive analogue and identified by means of the UV and radioactivity signal retention times. The HPLC purification and analytical traces, the stability in media, the related detailed HPLC analysis, NMR and high resolution mass spectra of synthesized compounds are provided in the Supporting Documentation to this paper.

Methyl *N*-[5-(3'-iodobenzoyl)-1*H*-benzimidazol-2-yl]carbamate (2**).**

To a stirred and cooled on an ice-bath solution of methyl *N*-(6-benzoyl-1*H*-benzimidazol-2-yl)carbamate (**1**) (1.05 g, 3.55 mmol) in 3.5 mL of nitromethane and containing CF₃SO₃H (1.50 mL, 17.07 mmol), *N*-iodosuccinimide (NIS, 800 mg, 3.54 mmol) was added in three small portions. The reaction temperature was gradually increased to 75°C and kept at this temperature for additional two hours. After cooling and evaporation of nitromethane under a high vacuum, the residue was quenched with ice-water (20 mL) and with a solution of 10% sodium bisulfite (10 mL), and then neutralized with 1 M NaHCO₃ to pH of ~7.5. The precipitated yellowish solid (1.12 g) was filtered off, washed with water and dried under a high vacuum. Subsequent HPLC analyses revealed a multicomponent mixture containing one major product (74%) and ~7% of the unreacted **1**. After partial crystallization from MeCN, the product **2** (88.4% pure by HPLC analysis) was further purified on the HPLC system. Attempted additional recrystallizations or flash column chromatography using normal phase silica gel failed due to the quite low solubility of **2** in common solvents. A portion of the crude product (~23 mg) was purified on the HPLC (~350 μ g per injection) equipped with a semipreparative Columbus C18 column (5 μ , 100 $\text{\AA}$, 10 mm $\times$ 250 mm) column. Two suitable elution schemes were developed. Eluent 1: solvent A H₂O, solvent B MeCN; at 2.2 mL/min flow rate a linear gradient of B from 50% to 60% in 10 min, then to 95% B in 12 min and kept at 95% B for 20 min; the product eluted within 15.5–17.0 min after the injection. Eluent 2: solvent A H₂O, solvent B CH₃OH; eluted at 2.0 mL/min flow

rate with a linear gradient of B from 40% to 82% in 18 min, then to 95% B in 1 min and 95% B kept for 31 min; with the product eluting within 16.5–17.2 min post injection. Combined eluates were evaporated and dried under a high vacuum to give the purified product (17 mg, 74%). The HPLC analysis on ACE C18 column (5 μ , 100Å, 4.6×250 mm) confirmed satisfactory purity (95.0% at 254 nm) of the product **2**: t_R = 29.2 min, eluent: solvent A H₂O, solvent B MeCN; run with a linear gradient of B from 10% to 95% over 45 min, then 95% B for 20 min, elution rate 0.8 mL/min. ¹H NMR (DMSO-*d*₆) δ : 3.76 (s, 3H, OCH₃), 7.34 – 7.37 (m, 1H, aryl-H5'), 7.50 – 7.52 (m, 1H, aryl-H7), 7.54 – 7.56 (m, 1H, aryl-H6), 7.68 – 7.71 (m, 1H, aryl-H6'), 7.77 – 7.87 (m, 1H, aryl-H4), 7.97 – 8.01 (m, 2H, aryl-H2', aryl-H4'), 11.34 – 12.50 (m, 2H, -NH-, -NHCO-) ppm. ¹³C NMR (DMSO-*d*₆) δ : 52.63 (OCH₃), 94.83 (aryl-C3'), 114.74 (aryl-C7), 116.29 (aryl-C4), 123.94 (aryl-C6), 128.79 (aryl-C6'), 130.11 (aryl-C5'), 131.17 (aryl-C5), 137.49 (aryl-C2'), 139.08 (aryl-C9), 140.20 (aryl-C8), 141.05, 141.19 (aryl-C4', aryl-C1'), 149.58 (aryl-C2), 154.49 (-NHCO-), 194.43 (CO) ppm. HR-MS (ESI), (m/e): calcd for C₁₆H₁₂IN₃O₃, 420.9923; [M+H]⁺ was found at 422.0054 within 13.1 ppm to its theoretical value.

Methyl *N*-[5-(3'-trimethylstannylbenzoyl)-1*H*-benzimidazol-2-yl]carbamate (**3**).

A stirred solution of a crude iodide **2** (191 mg, 0.453 mmol, ~74% of **2** by HPLC analysis) in 25 mL of MeCN containing hexamethylditin (160 μ L, 0.771 mmol), triethylamine (320 μ L, 2.30 mmol) and dichlorobis(triphenylphosphine) palladium (II) catalyst (32 mg, 0.046 mmol), was gently refluxed under nitrogen until the starting **2** was no longer detectable by TLC (~3 h). To remove the remaining catalyst, the reaction mixture after cooling to ambient temperature was passed through a thin layer of silica, repeatedly washed with MeCN (5×15 mL) and the solvent was evaporated. An excess of triethylamine was taken out by repeating the evaporation with toluene (3×25 mL) under vacuum at 60°C. To eliminate practically all of the unreacted hexamethylditin, the remaining solid was treated with EtOAc/hexanes solution (10 mL, 1:2, v/v) and the mixture was briefly sonicated, then centrifuged (2500 rpm, 15 min) and the solvent was carefully removed leaving a solid pellet. This process was repeated three times and after drying under a high vacuum, the remaining solid (153 mg) was analyzed by HPLC. The analyses indicated a multicomponent mixture containing one major new product (63%) along with ~12% of starting **2** and ~9% of **1**. A final purification was completed after numerous injections of a crude product (30 mg, ~200 μ g per injection) on an HPLC system equipped with a semipreparative Columbus C18 column (5 μ , 100Å, 10 mm×250 mm) eluted at 1.8 mL/min flow rate with a linear gradient of MeCN in water from 45% to 60% in 19 min, then to 95% in 2 min and 95% MeCN kept for 20 min. The product **3** was collected within 18–19 min after the injection. Eluted fractions were evaporated and dried under a high vacuum to give the purified stannane **3** (16 mg, 53%). The HPLC analysis on ACE C18 column (5 μ m, 100Å, 4.6×250 mm) confirmed an adequate purity of the product **3** (95.0% at 254 nm): t_R = 32.2 min, eluent: solvent A H₂O, solvent B CH₃CN; run with a linear gradient of B from 20% to 95% over 45 min, then 95% B for 20 min, elution rate 0.8 mL/min. ¹H NMR DMSO-*d*₆) δ : 0.32 (t, 3H, 3 x CH₃Sn, ²J_{H,Sn} = 29 Hz), 3.71 (s, 3H, OCH₃), 7.47 – 7.59 (m, 2H, aryl-H6, aryl-H5'), 7.61 – 7.66 (m, 1H, aryl-H4'), 7.69 – 7.74 (m, 1H, aryl-H6'), 7.77 – 7.86 (m, 1H, aryl-H4), 7.90 – 7.98 (m, 2H, aryl-H2', aryl-H7), 11.12 – 12.02 (m, 2H, -NH-, -NHCO-) ppm. ¹³C NMR (DMSO-*d*₆) δ : -4.27 (3 x CH₃Sn), 52.81 (OCH₃), 117.32 (aryl-C7), 119.66 (aryl-C4), 125.14 (aryl-C6), 128.37 (aryl-

C5'), 130.14, 130.94 (aryl-C6', aryl-C5), 136.28 (aryl-C2'), 138.72 (aryl-C3'), 139.18 (aryl-C9), 140.47 (aryl-C8), 141.15, 141.29 (aryl-C4', aryl-C1'), 148.88 (aryl-C2), 153.82 (-NHCO-), 194.45 (CO) ppm. HR-MS (ESI), (m/e): calcd for C₁₉H₂₁N₃O₃Sn, 459.0605; [M + H]⁺ was found at 459.0747 within 11 ppm to its theoretical value.

Methyl *N*-[5-(3'-[^{125/131}I]iodobenzoyl)-1*H*-benzimidazol-2-yl]carbamate (**4**).

Direct radioiodination of 1.—To a conical reaction vessel (0.5 mL volume) equipped with a magnetic stirring bar and a tight septum lid coated with Teflon, a solution of *N*-chlorosuccinimide (NCS, 60 µg, 0.45 µmol) in MeCN (50 µL) was added and the solvent evaporated with a gentle stream of dried nitrogen. Subsequently a solution of **1** (166 µg, 0.56 µmol) in trifluoromethanesulfonic acid (250 µL, 2.85 µmol), was added under nitrogen and the reaction vial was tightly closed. A solution of Na¹²⁵I in 1×10⁻⁵ M NaOH or Na¹³¹I in 0.1 M NaOH (5 µL - 20 µL, up to ~15 MBq to ~45 MBq) was introduced through a septum using a 20-µL syringe. The reaction temperature was slowly increased to 75°C in 30 min. Periodically during this time, aliquots of the reaction mixture (5 µL - 7 µL, ~185 kBq) were taken and transferred to a tube containing MeCN (50 µL) then quenched with a solution of Na₂S₂O₅ (50 mM, 20 µL) in 2 M NaOH and the progress of iodination was checked on HPLC (Bioscan/UV 254 nm detectors) using ACE C18 column (5 µm, 100Å, 4.6×250 mm) eluted at 0.8 mL/min with a linear gradient of MeCN in water from 10% to 95% MeCN over 45 min and 95% MeCN held for further 15 min; both solvents contained 0.07% TFA (v/v). The reaction was completed within 60 min at 75°C. The reaction mixture was cooled to room temperature, quenched with 50 mM Na₂S₂O₅ in 2 N NaOH (200 µL) and after neutralization to pH ~4 was injected onto the HPLC system. The elution profile developed for monitoring the reaction progress was applied. The product **4** was collected within 27.5–29 min. The unreacted **1** eluting earlier (13.5–17 min) was cleanly separated from the product. Radioiodinations with Na¹²⁵I were carried out within the 14.8 MBq - 40.7 MBq range. Reactions with Na¹³¹I using 38.9 MBq - 43.2 MBq ¹³¹I produced ¹³¹I-**4** with an average yield, after separation, reaching 62%.

Destannylation method.—Into a glass tube containing the stannane precursor **3** (100 µg, ~0.22 µmol) in MeCN (50 µL), a solution of Na¹²⁵I in 1×10⁻⁵ M NaOH or Na¹³¹I in 0.1 M NaOH (5 µL - 15 µL, 18.5 MBq - 77.7 MBq) was added followed by H₂O₂ (5 µL, 30% water solution) and two min later, a solution of 0.1 N TFA in MeCN (50 µL). The resulting mixture, briefly vortexed and sonicated, was left in a closed tube for ~15 min at room temperature, then quenched with a water solution of Na₂S₂O₅ (50 mM, 20 µL), taken up into an HPLC syringe and the reaction tube washed twice with a mixture of MeCN/H₂O (25 µL, 1:1, v/v). The reaction mixture combined with washes was injected onto the HPLC system. The separation of the products proceeded on Columbus C8 column (5 µm, 110Å, 4.6 × 250 mm) equipped with Bioscan and UV detector at 220/254 nm and eluted at 0.8 mL/min with a linear gradient of MeCN in water from 20% to 95% MeCN over 45 min and 95% MeCN held for 15 min longer. The radioiodide **4** was collected within 25–27 min after the injection. The excess of unreacted stannane **3** eluting between 31.5–34.5 min was fully separated from **4**. Fractions containing the radioactive product were evaporated with a stream of nitrogen. Subsequent HPLC analyses of the product confirmed its high radiochemical purity (98%, Bioscan/UV 254 nm). Co-injections of purified **4** (~0.21 MBq) with nonradioactive analog **2**

(~3.8 μ g) have shown identical mobility of both compounds: t_R = 28.8 min on ACE C8 100Å (5 μ m, 4.6 $\times$ 250 mm) eluted at 0.8 mL/min with a linear gradient of MeCN from 10% to 95% MeCN over 45 min and kept at 95% MeCN for 15 min. To completely eliminate MeCN, fractions containing **4** were evaporated with a stream of nitrogen and 80 μ L ethanol was added to the residue followed by 100 μ L 10 mM potassium phosphate buffer, pH 6.1 (PB). The resulted mixture was vortexed and injected again on HPLC equipped with Luna CN reverse phase column (5 μ m, 4.6 $\times$ 250 mm) eluted at the rate of 0.8 mL/min with a linear gradient of ethanol (EtOH) in 10 mM PB, pH 6.1, from 30% to 70% in 30 min and next from 70% to 95% of EtOH over 15 minutes. The product was collected within 22–25 min after the injection. In radioiodostannylations carried out with Na¹²⁵I within the 18.5 MBq - 77.7 MBq range, total of 140.6 MBq of ¹²⁵I-**4** was obtained. An average yield of the isolated product was 84%. Three radioiododestannylations performed with Na¹³¹I (29.6 MBq - 48.1 MBq range) furnished 117 MBq of ¹³¹I-**4** with yields exceeding 85%.

Stability of ¹²⁵I-**4** and ¹³¹I-**4** in human serum

Sample incubation: **4** (370 KBq - 740 kBq) in 20 μ L 25% EtOH/PB (10 mM PB, pH 6.1) was added to the solution of human serum (1980 μ L). The mixture was briefly vortexed and kept up to 19 h at ambient temperature or in the cell culture incubator (37°C, 5% CO₂). At selected times, a 0.5-mL aliquot of the incubated mixture was withdrawn and analyzed by HPLC.

Preparation of incubated samples for HPLC analyses: an aliquot of the incubation mixture (0.5 mL) was treated with MeCN (0.5 mL) to precipitate proteins, vortexed and centrifuged (2,000 rpm, 15 min). Aliquots of the supernatant (500 μ L) were acidified to pH ~6 with 0.05 M TFA (2–8 μ L). The excess of MeCN was evaporated with a stream of nitrogen and 100 μ L water added. This mixture was passed through a 0.2- μ m filter and samples (~100 μ L, 62.5 kBq - 130 kBq) were injected on HPLC system.

HPLC analyses of supernatants proceeded on Luna CN (5 μ m, 4.6 $\times$ 250 mm) column. Eluent: solvent A potassium phosphate buffer (10 mM, pH 6.1), solvent B EtOH; all samples were eluted at 0.8 mL/min, initially with a gradient of EtOH from 30% to 70% for 30 min, then from 70% to 95% over the next 15 min with detection of radioactivity and UV at 280 nm.

RESULTS AND DISCUSSION

Two allied methods for the no-carrier-added radioiodination of **1** have been tested and effectively utilized: the direct electrophilic ¹²⁵I- and ¹³¹I-iodination as well as radioiododestannylation of its organotin derivative **3** (Figure 1).

The nonradioactive iodinated derivative **2** was prepared in a direct iodination of **1** with NIS in the excess of trifluoromethanesulfonic acid (triflic acid). This method previously reported by Olah *et al.*¹⁴ involves the *in situ* formation of iodine(I) triflyl hypoiodite. The protosolvated activated form^{15,16} of this potent superelectrophile was found to be effective in iodination of many deactivated aromatics¹⁴. In addition, since NIS can be formed *in situ* from *N*-chlorosuccinimide and sodium iodide^{17,18}, the radioiodination can be accomplished through the same reaction pathway generating protosolvated triflyl [^{125/131}I]hypoiodite as

the electrophilic radioiodinating reagent. This noteworthy approach to no-carrier-added electrophilic radioiodinations of monosubstituted weakly activated and deactivated benzenes was introduced by Mennicke *et al.*¹⁹.

At first, we synthesized methyl *N*-[5-(3'-iodobenzoyl)-1*H*-benzimidazol-2-yl]carbamate **2**, the non-radioactive analogue of the target compound as described above. The reaction progress at room temperature was slow and the quantity of iodinated product did not exceed 62% with ~31% of unreacted **1** remaining. The excess of triflic acid and gradual increase of the reaction temperature to 75°C was necessary to achieve satisfactory levels of the iodination (~74%). To simplify workup of the reaction mixture by avoiding higher than necessary (4 – 6-fold) excess of triflic acid, nitromethane was used as a co-solvent, with no decrease of the iodination yield. HPLC analyses of all iodination mixtures, even after extensive modifications of the reaction time, temperature, and/or the excess of triflic acid, always revealed only one major product methyl *N*-[5-(3'-iodobenzoyl)-1*H*-benzimidazol-2-yl]carbamate **2**, as determined by the NMR spectra, in yields ranging from 62% - 74%. The leftover **1** (7% - 30%) along with five small additional unidentified side-products (each 6 %) were also present. No other isomers or multi-iodinated products were predominant. The selective formation of the *meta* regioisomer confirms the electrophilic character of this iodination. After partial crystallization from MeCN, the product was separated and further purified on the HPLC system. Attempted recrystallizations and flash column chromatography using normal phase silica gel failed due to low solubility of **2** in commonly used solvents.

The direct ¹²⁵I- and ¹³¹I-iodination of **1** were accomplished in the same reaction pathway by generating protosolvated triflyl [^{125/131}I]hypoiodite from NCS/Na^{125/131}I in triflic acid. In the course of the initial trials, we checked and adjusted several reaction parameters to optimize the radioiodination yield. Periodically, aliquots from the reaction mixtures were taken and after quenching with a solution of Na₂S₂O₃/NaOH were injected on the HPLC system equipped with radioactivity and UV detectors to test the progress of radioiodination (Figures 2A and 2B). The reaction progress at room temperature was marginal (12% of **2** was detected after 180 min at 22°C), nevertheless, it was critically important to start the radiolabeling at ambient temperature and then to increase the temperature gradually to 75°C over a period of 30–45 min. The immediate heating of the mixture or shortened warming sequences triggered nearly 70% decrease of the yield. Extending the reaction time at 75°C also led to the yield reduction with noticeable decomposition of the radioiodinated product. The highest radioiodination yields were achieved with amounts of NCS in the range of 0.4 – 0.6 μmol in 250 μL of triflic acid. For the best results, a conical reaction vessel was first coated inside with NCS through the evaporation of NCS solution in MeCN with a gentle stream of dried nitrogen, then a solution of **1** in triflic acid was added under nitrogen and the reaction vial was tightly closed. Solutions of Na¹²⁵I or Na¹³¹I in NaOH were introduced through the septum with a syringe and the temperature of the stirred mixture was slowly increased to 75°C. The radiolabeling was completed in ~1 hour with an average yield of **4** reaching 62%. The correlation between the amount of **1** and the radiochemical yield was estimated using four concentrations of **1** ranging from 0.3 μmol to 0.8 μmol in 250 μL of triflic acid. The amount of ~0.5 μmol was found to be optimal. Higher concentrations did not

increase the yield of the labeling and made the HPLC separation of the product more difficult. Several radioiodinations were carried out with Na¹²⁵I with the amount of ¹²⁵I ranging from 14.8 MBq to 40.7 MBq to give a total of 159.4 MBq of ¹²⁵I-**4** with the average reaction yield >60%. Reactions conducted with Na¹³¹I varied between 38.9 MBq - 43.2 MBq ¹³¹I and produced a total of 163 MBq of ¹³¹I-**4**, also with the average isolated yield >60%. The reaction progress of the direct radioiodination with Na¹²⁵I conducted under the optimal conditions is shown in Figures 2A and 2B. Analyses of the reaction mixture at additional times are shown in the Supporting Information. To verify the identity of the isolated radioactive product, purified ¹²⁵I-**4** was co-injected with its nonradioactive analogue **2** and the eluates were monitored with the radioactivity and UV detectors (Figure 2C). The identical retention time of 25.6 min was observed for **2** and ¹²⁵I-**4** confirming the identity of the radioactive product.

Starting from **2**, we have synthesized the corresponding stannane: methyl *N*-[5-(3'-trimethylstannylbenzoyl)-1*H*-benzimidazol-2-yl]carbamate (**3**). This target compound was obtained through the typical stannylation of the iodide by hexamethylditin in the presence of triethylamine catalyzed by bis-(triphenylphosphine)palladium(II) dichloride using our previously described procedures^{20–23}. Stannylation was carried out under nitrogen in refluxed MeCN using crude iodide **2** containing ~7% of **1**. The final purification of the stannane was accomplished on the HPLC system equipped with a semipreparative column after numerous injections of a crude product. Purified 3'-trimethylstannane **3** underwent the no-carrier-added radioiododestannylation with ease and high isolated yields of ¹²⁵I-**4** and ¹³¹I-**4** were routinely achieved (~85%). Substantially increased hydrophobicity of stannane **3** compared to the iodinated compounds **2** and **4** allowed easy and complete separation of the excess of trimethyltin precursor **3** from the ¹²⁵I- and ¹³¹I-iodinated products even when a large volume of the crude reaction mixture (up to 600 µL) was loaded onto the HPLC column for the final purification. The typical HPLC purification of ¹²⁵I-**4** from the destannylation mixture is shown in Figure 3A. Analytical traces of ¹²⁵I-**4** in two solvent systems are shown in Figures 3B and 3C. The desired product ¹²⁵I-**4** was collected within 25–27 min after injection. The excess of unreacted tin precursor **3** eluted between 31.5–34.5 min and was fully separated from **4**. HPLC analyses of ¹³¹I-**4** are included in the Supporting Information.

Radioiodinations with ¹²⁵I and ¹³¹I were conducted with the radionuclide amounts ranging from 18.5 MBq to 77.7 MBq using 100 µg of the stannane **3** in each reaction. Reactions were conducted in a solution of 0.1% TFA in MeCN and hydrogen peroxide was used to oxidize sodium [^{125/131}I]iodide. A small amount of **1** (~1%) formed from the stannane **3** as the proton destannylated byproduct was detected in crude reaction mixtures. This amount of **1** increased to ~4% when the stannane sample was stored frozen for prolonged times (>3 months).

To assure compatibility with biological milieu such as required in the determination of stability of new drugs in human serum and cell culture experiments, radioactive products were purified on the HPLC system using USP-grade ethanol and phosphate buffer (Figure 3C). In this solvent system on a Luna column, ¹²⁵I-**4** eluted at *t_R* = 28.8 min with 98%

radiochemical purity. The final radioactive **4** was passed through a 2- μ m sterile filter before use. Stability studies performed in preparation to the *in vivo* evaluation of **4** indicate that ^{125}I -**4** and ^{131}I -**4** are stable in human serum. Figures 4A and 4B show examples of HPLC analyses of ^{125}I -**4** incubated in human serum for 5 h and 17 h. At both times, the radioactive trace indicates exclusively non-degraded ^{125}I -**4**. Similar results were obtained in stability studies in cell culture media performed in preparation to *in vitro* evaluation of **4** (Figure 4C).

CONCLUSIONS

The synthesis, purification and quality assurance methods of novel microtubule-targeted theranostic methyl *N*-[5-(3'-radiohalobenzoyl)-1*H*-benzimidazol-2-yl]carbamates were developed. The direct radioiodination method is more convenient for the synthesis of **4** radiolabeled with long-lived radiohalides. The process is lengthier but it does not require any precursors. The radioiododestannylation method is rapid and compatible with short-lived radiohalides such as ^{111}At , ^{123}I , or radioisotopes of Br. *N*-[5-(3'-radiohalobenzoyl)-1*H*-benzimidazol-2-yl]carbamates are stable in human serum and cell culture medium.

Supplementary Material

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Figure 1.

Radioiodination pathways used to produce methyl N-[5-(3'-radiohalobenzoyl)-1H-benzimidazol-2-yl]carbamates. Reagents and conditions: (a) (i) CF₃SO₃H (4 - 5 equivalents [eq]) in MeNO₂, NIS (1.05 eq), 0°C → rt within 1h; (ii) rt → 75°C, kept at 75°C for 2h; (iii) recrystallization from EtOH or MeCN and HPLC purification; (b) Sn₂(CH₃)₆, (1.25-1.70 eq) in MeCN, TEA (3-5 eq), (Ph₃P)Pd(II)Cl₂ (0.07-0.10 eq), refluxed under nitrogen (1-3h), HPLC purification; (c) (i) Na¹²⁵I (18.5 MBq - 77.7 MBq) or Na¹³¹I (29.6 MBq - 48.1 MBq) in 1×10⁻⁵ or 0.1 M NaOH, 30% H₂O₂ (5 μL), TFA/MeCN (0.1% v/v), 1 min sonication, ~15 min reaction time, HPLC purification; (d) (i) **1** (0.56 μmol), NCS (0.45 μmol), CF₃SO₃H (250 μL) a mixture stirred at rt in a conical reaction vessel (0.5 mL) equipped with a tight septum lid coated with Teflon; (ii) Na¹²⁵I in 1×10⁻⁵ M NaOH or Na¹³¹I in 0.1 M NaOH (5 μL - 20 μL, 14.85 MBq - 40.7 MBq) injected through the septum, rt → 75°C in 30 - 45 min, and kept at 75°C for 1h; (iii) 50 mM Na₂S₂O₅ in 2 N NaOH (200 μL), pH → ~4, HPLC purification.

Figure 2.

HPLC analyses of ¹²⁵I-4 synthesized using the direct radioiodination method. **A.** The reaction mixture analyzed after 21 min. **B.** The reaction mixture analyzed after 68 min. **C.** Purified ¹²⁵I-4 (92 kBq) obtained in the direct radioiodination of **1** and co-injected with nonradioactive iodo-derivative **2** (4.6 μg) in 25 μL MeCN.

Figure 3. HPLC analyses of ^{125}I -**4** synthesized using the radioiododestannylation method. **A.** Purification of the crude destannylation mixture of **3** reacted with 70.3 MBq Na^{125}I . **B.** HPLC analysis of the isolated ^{125}I -**4**. **C.** The exchange of solvents for ^{125}I -**4** (166 kBq) using the HPLC system.

Figure 4. Stability of **4** incubated with human serum and cell culture medium and analyzed using HPLC. **A.** ^{125}I -**4** in human serum, 5 h incubation at 20°C. **B.** ^{125}I -**4** incubated at 37°C in human serum for 17 h. **C.** ^{125}I -**4** incubated in EMEM cell culture medium at 37°C in the 5% CO_2 /air atmosphere for 19 h.