

Design, synthesis, and biological evaluation of radioiodinated benzo[*d*]imidazole-
quinoline derivatives for platelet-derived growth factor receptor β (PDGFR β)
imaging

Abstract

Several malignant tumors and fibrotic diseases are associated with PDGFR β overexpression and excessive signaling, making this receptor attractive for molecular targeting and imaging approaches. A series of benzo[d]imidazole-quinoline derivatives were designed and synthesized to develop radioiodinated compounds as PDGFR β -specific imaging probes. The structure activity relationship (SAR) evaluation of the designed compounds was performed. Among them, 2-[5-(2-methoxyethoxy)-1*H*-benzo[d]imidazol-1-yl]-8-{piperazin-1-yl}quinoline (**5a**) and 4-{2-[5-(2-Methoxyethoxy)-1*H*-benzo[d]imidazol-1-yl]quinolin-8-yl}morpholine (**5d**) exhibited a relatively high PDGFR β -TK inhibitory potency, whereas iodinated **5a** derivative 5-iodo-2-[5-(2-methoxyethoxy)-1*H*-benzo[d]imidazol-1-yl]-8-{piperazin-1-yl}quinoline (**8**) exhibited a superior inhibitory potency as PDGFR β inhibitor than iodinated **5d** derivative 4-{5-iodo-2-[5-(2-methoxyethoxy)-1*H*-benzo[d]imidazol-1-yl]quinolin-8-yl}morpholine (**11**). Furthermore, [125 I]**8** and [125 I]**11** were synthesized and evaluated for PDGFR β radioligand ability, both *in vitro* and *in vivo*. Cellular uptake experiments showed that [125 I]**8** had a higher uptake in BxPC3-luc cells as PDGFR β -positive cells than [125 I]**11**. Incubation of [125 I]**8** after pretreatment of PDGFR β ligands significantly reduced the uptake of [125 I]**8**. In biodistribution experiments using tumor-bearing mice, [125 I]**8** accumulation in the tumor 1 h postinjection was higher than that of the benzo[d]imidazol-quinoline derivative [125 I]IIQP, used in our previous research. These results indicate that [125 I]**8** could be a promising PDGFR β imaging agent. Although its clinical application requires further structural modifications, the results obtained in this research may be useful for the development of PDGFR β -specific radioligands.

Keywords: radioligand, molecular imaging, PDGFR β , tyrosine kinase inhibitor

1. Introduction

Platelet-derived growth factor receptor β (PDGFR β) is a transmembrane receptor tyrosine kinase with highly regulated cell expression.¹ PDGFR β exerts an important role for angiogenesis and embryonic growth, and for the formation of blood vessels, kidneys, and adipocytes.² In adults, PDGFR β is expressed mainly in vascular smooth muscle cells and pericytes and its signaling is vital for wound healing and regulation of tissue interstitial fluid pressure.^{3,4} Cancer is associated with aberrant expression and signaling of PDGFR β , through autocrine and paracrine stimulation of tumor cell growth.^{5,6} PDGFR β expression by pericytes is necessary for the recruitment and integration in the wall of tumor vessels.⁶⁻¹⁰ Therefore, PDGFR β has raised considerable interest as an attractive target not only for anti-cancer drug discovery but also for cancer imaging.

Currently, biopsy constitutes the predominant way to determine relevant tumor molecular targets. However, a limited number of samples can be taken from a few locations, and it is associated with a certain morbidity.¹¹ Nuclear molecular imaging, which allows simultaneous and repeated visualization of the molecular targets expressed in all lesions using a noninvasive procedure, could be an alternative to biopsy.¹² This allows to follow the changes in molecular target expression during the course of disease and/or in response to therapy.

In nuclear medicine, single photon emission computed tomography (SPECT) and positron emission tomography (PET) can quantify receptor density and molecular target expression.¹³⁻¹⁵ To date, several types of probes targeting transmembrane receptor such as PDGFR β have been developed for cancer imaging. These probes targeted not only extracellular domain using molecules such as proteins,^{16,17} aptamers,¹⁸ affibody molecules,^{19,20} and peptides,^{21,22} but also intracellular domains using tyrosine kinase inhibitors (TKIs).²³⁻²⁷ In this study, the synthesized benzo[d]imidazolequinoline derivatives as one of developed TKIs targeted intracellular domains, especially adenosine triphosphate (ATP)-binding sites. This binding site as one of therapeutic targets²⁸ of TKR-related diseases can be used to visualise the intracellular part of TKR and may allow the approach to monitor the clinical potential of this therapeutic target directly. Mutation of PDGFR β has been reported,^{29,30} and the mutation status assesment using radiotracers as of TKIs as diagnostic agents might provide valuable additional diagnostic information. Other TKIs and their potential advantages, such as assessment of drug sensitivity, mutation status of receptor target, and usefulness in drug screening have been demonstrated. However, the imaging of intracellular parts of PDGFR β , particularly its ATP-binding site as a therapeutic target, has hardly been reported. For the reason, we have explored the feasibilities of radioligands of small molecules based radiopharmaceuticals which targeted intracellular domain of receptor tyrosine kinase especially PDGFR β as molecular imaging agents.

In a previous study, we developed radiolabeled small molecules targeting the PDGFR β intracellular ATP binding site, using 1-{2-[5-(2-methoxyethoxy)-1*H*-benzo[d]imidazol-1-yl]quinolin-8-yl}piperidin-4-amine (CP-673451, IQP, **1**), which possesses high affinity for PDGFR β ,³¹ as a lead compound. [¹²⁵I]IIQP and [⁷⁷Br]BrIQP were designed, synthesized, and evaluated by introduction of radioiodine and radiobromine into **1**.^{32,33} These radiolabeled probes showed high affinity for PDGFR β and sufficient stability, both *in vitro* and *in vivo*. However, *in vivo* imaging requires higher PDGFR β affinity and better biodistribution properties.

In order to develop more suitable radiolabeled TKIs, we investigated SAR of the substituent on the quinoline group (piperidine part) of **1**, since, to our knowledge, no reports were available on this aspect of SAR investigation and this group was easy to modify using our synthetic scheme. We designed and synthesized a new series of analogue compounds of **1** (**5a–5g** and **7a–7b**) based on the bioisosteric principle. The inhibitory effects exerted by nine compounds on PDGFR β tyrosine kinase were evaluated using **1** as positive control. Subsequently, two new compounds with high affinity to PDGFR β were iodinated. Using similar reagents in IIQP synthesis, 5-iodo-2-[5-(2-methoxyethoxy)-1*H*-benzo[d]imidazol-1-yl]-8-{piperazin-1-yl}quinolone (**8**) and 4-{5-iodo-2-[5-(2-methoxyethoxy)-1*H*-benzo[d]imidazol-1-yl]quinolin-8-yl}morpholine (**11**) were synthesized. The radioiodine labeled probes, [¹²⁵I]**8** and [¹²⁵I]**11** (Figure 1), were then synthesized and evaluated *in vitro* and *in vivo* to examine their usefulness as PDGFR β imaging probes. Although we aimed at developing probes for PET or SPECT, ¹²⁵I was used in the initial studies due to its longer half-life.

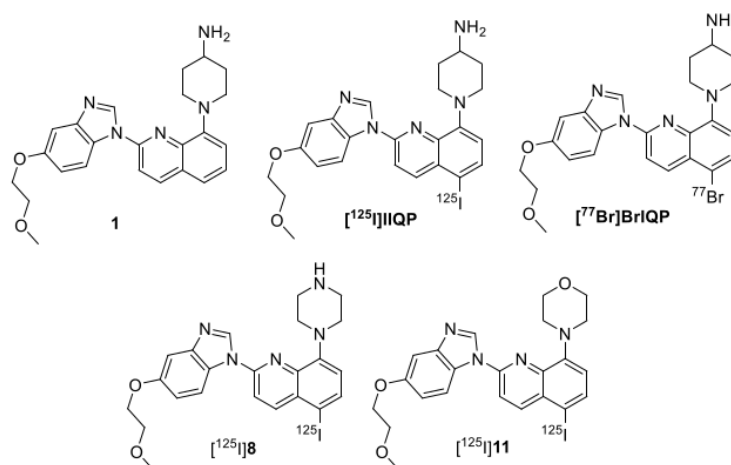


Figure 1. Chemical structures of **1**, [¹²⁵I]IIQP, [⁷⁷Br]BrIQP, [¹²⁵I]**8**, and [¹²⁵I]**11**.

2. Material and methods

2.1 General

Commercial reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA), Wako Pure Chemical Industries (Osaka, Japan), Nacalai Tesque, Inc., (Kyoto, Japan), Tokyo Chemical Industry, Co., Ltd., (Tokyo, Japan) and Kanto Chemical, Co., Inc. (Tokyo, Japan) and used without further purification unless otherwise stated.

[¹²⁵I]³Sodium iodide (644 GBq/mg) was purchased from Perkin-Elmer (Waltham, MA, USA). The radioactivity was measured by an Auto Gamma System ARC-7010B (Hitachi, Ltd., Tokyo, Japan).

Bicinchoninic Acid (BCA) Protein Assay Kit and Cell Counting Kit (CCK) were purchased from Nacalai Tesque, Inc. and Dojindo (Kumamoto, Japan), respectively. Recombinant murine platelet-derived growth factor-BB (PDGF-BB) was purchased from PeproTech (Rocky Hill, NJ, USA). TR-PCT1 rodent brain pericyte cell line was a generous gift from Dr. Emi Nakashima (Keio University, Tokyo, Japan)³⁴ and BxPC3-luc pancreatic cell line was purchased from JCRB Cell Bank (Ibaraki, Japan).

Proton and carbon nuclear magnetic resonance (¹H-NMR and ¹³C-NMR) spectra were obtained with JEOL JNM-ECS400 (JEOL Ltd, Tokyo, Japan). Direct analysis in real time mass spectra (DART-MS) and Electrospray ionization mass spectra (ESI-MS) were obtained with JEOL JMS-T100TD (JEOL Ltd). Purification was conducted using reversed-phase high-performance liquid chromatography (RP-HPLC) system. TLC analysis was performed on silica plates (Art 5553, Merck, Darmstadt, Germany). Optical density in the WST-8 assay was obtained using Infinite® F200 Pro microplate reader (TECAN, Männedorf, Switzerland).

2.2 Synthesis of reference compounds and precursors

Compounds **1**, **2**, and **3** were synthesized according to our previous report.³²

2.2.1. General procedure for the preparation of compounds (**4a**, **4b**, **4c**)

A mixture of piperazine (500 mg, 5.8 mmol, 1.0 eq.), *N*, *N*-diisopropylethylamine (DIPEA) (1.5 mL, 8.7 mmol, 1.5 eq.), and di-*tert*-butyl dicarbonate (Boc₂O) (500 mg, 2.3 mmol, 0.4 eq.) in dry dichloromethane (15 mL) was stirred at room temperature for 24 h under nitrogen atmosphere. The reaction mixture was diluted with dichloromethane and washed with saturated aqueous NaHCO₃ and brine. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The titled compound (**4a**) as a colorless solid was afforded after purification

by column chromatography on silica gel (chloroform/methanol = 50/1). The same procedure was performed to obtain compounds **4b** and **4c**.

2.2.1.1. *tert*-Butyl piperazine-1-carboxylate (**4a**)

The title compound **4a** was afforded (400 mg, 37%) as a colorless solid. ¹H NMR (400 MHz, CDCl₃): δ 1.43 – 1.47 (9H, br s), 1.69 (1H, s), 2.81 (4H, t, *J* = 5.2 Hz), 3.39 (4H, t, *J* = 5.2 Hz). LRMS (DART+) calcd for C₉H₁₉N₂O₂ [M+H⁺]: *m/z* = 187.1, found 187.2.

2.2.1.2. *tert*-Butyl (4-aminocyclohexyl)carbamate (**4b**)

The title compound **4b** was afforded (430 mg, 34%) as a pale yellow solid. ¹H NMR (400 MHz, CDCl₃): δ 0.88 – 1.04 (4H, m), 1.23 – 1.26 (1H, m), 1.42 – 1.46 (9H, br s), 1.48 – 1.98 (6H, m), 3.41 – 3.57 (1H, m), 4.54 – 4.58 (1H, m). LRMS (DART+) calcd for C₁₁H₂₃N₂O₂ [M+H⁺]: *m/z* = 215.2, found 215.1.

2.2.1.3. *tert*-Butyl (4-aminocyclohexyl)carbamate (**4c**)

The title compound **4c** was afforded (220 mg, 18%) as a pale yellow solid. ¹H NMR (400 MHz, CDCl₃): δ 1.15 – 1.25 (4H, m), 1.43 – 1.44 (9H, br s), 1.51 (4H, s), 1.84 – 1.87 (1H, m), 1.95 – 2.05 (2H, m), 3.39 – 3.49 (1H, m), 4.35 – 4.37 (1H, m). LRMS (DART+) calcd for C₁₁H₂₃N₂O₂ [M+H⁺]: *m/z* = 215.2, found 215.1.

2.2.2. General procedure for the preparation of compounds (**5a**, **5b**, **5c**)

A mixture of **3** (210 mg, 0.4 mmol, 1.0 eq.), *tert*-butyl piperazine-1-carboxylate (**4a**) (150 mg, 0.8 mmol, 2.0 eq.), cesium carbonate (210 mg, 0.6 mmol, 1.5 eq.), tris(dibenzylideneacetone)dipalladium(0) (19 mg, 21 μmol, 0.05 eq.), and *rac*-BINAP (26 mg, 42 μmol, 0.1 eq.) in 1,4-dioxane (5 mL) was stirred at 100 °C for 1.5 – 3.5 days under nitrogen atmosphere. After removing precipitate by filtration through a pad of Celite® and washing with 1,4-dioxane, the filtrate was concentrated under reduced pressure and purified by column chromatography on silica gel (chloroform/methanol = 100/1). Trifluoroacetic acid (TFA) (1 mL) was added to purified intermediate compound and the mixture was stirred at room temperature for 15 min. After removing TFA by nitrogen gassing, the residue was dissolved in dichloromethane and washed with 0.1 M HCl. After adjusting pH to 9.0 using 0.1 M NaOH aqueous solution, the water layer was extracted using dichloromethane (3 x 25 mL). The organic layer was dried over sodium sulfate, filtered, and

concentrated under reduced pressure to obtain the titled compounds (**5a**). The same procedure was carried out to obtain compounds **5b** and **5c**.

2.2.2.1. 2-[5-(2-Methoxyethoxy)-1H-benzo[d]imidazol-1-yl]-8-{piperazin-1-yl}quinoline (**5a**)

The title compound **5a** was afforded (119 mg, 70%) as a pale yellow solid. ¹H NMR (400 MHz, CDCl₃): δ 3.29 – 3.30 (4H, m), 3.40 – 3.48 (4H, m), 3.50 (3H, s), 3.82 – 3.84 (2H, m), 4.23 – 4.25 (2H, m), 4.30 – 4.31 (1H, m), 7.15 – 7.18 (1H, m), 7.38 (1H, s), 7.49 – 7.54 (2H, m), 7.69 – 7.72 (2H, m), 8.33 (1H, d, *J* = 8.8 Hz), 8.46 (1H, d, *J* = 8.8 Hz), 8.65 (1H, s). LRMS (DART+) calcd for C₂₃H₂₆N₅O₂ [M+H⁺]: *m/z* = 404.2, found 404.2.

2.2.2.2. (1*S*,2*S*)-*N*-{2-[5-(2-methoxyethoxy)-1H-benzo[d]imidazol-1-yl]quinolin-8-yl}cyclohexane-1,2-diamine (**5b**)

The title compound **5b** was afforded (156 mg, 86%) as a pale white solid. ¹H NMR (400 MHz, CDCl₃): δ 1.39 – 1.44 (4H, m), 1.55 – 1.61 (2H, m), 1.63 – 1.68 (2H, br s), 2.05 – 2.06 (2H, m), 2.20 – 2.23 (2H, m), 2.80 – 2.81 (1H, m), 3.20 – 3.30 (1H, m), 3.50 (3H, s), 3.82 – 3.84 (2H, m), 4.23 – 4.25 (2H, m), 5.82 – 5.91 (1H, m), 6.94 (1H, d, *J* = 8.0 Hz), 7.10 – 7.14 (2H, m), 7.39 – 7.43 (2H, m), 7.64 (1H, d, *J* = 8.8 Hz), 8.08 (1H, d, *J* = 9.2 Hz), 8.27 (1H, d, *J* = 8.8 Hz), 8.59 (1H, s). LRMS (DART+) calcd for C₂₅H₃₀N₅O₂ [M+H⁺]: *m/z* = 432.2, found 432.3.

2.2.2.3. *trans*-*N*-{2-[5-(2-Methoxyethoxy)-1H-benzo[d]imidazole-1-yl]quinolin-8-yl}cyclohexane-1,4-diamine (**5c**)

The title compound **5c** was afforded (148 mg, 82%) as a pale white solid. ¹H NMR (400 MHz, CDCl₃): δ 1.26 – 1.45 (4H, m), 1.64 (2H, br s), 1.98 – 2.05 (2H, m), 2.28 – 2.31 (2H, m), 2.80 – 2.82 (1H, m), 3.47 – 3.50 (4H, m), 3.82 – 3.84 (2H, m), 4.23 – 4.30 (2H, m), 5.82 – 5.90 (1H, m), 6.79 (1H, d, *J* = 7.6 Hz), 7.09 – 7.13 (2H, m), 7.39 – 7.43 (2H, m), 7.63 (1H, dd, *J* = 0.8, 8.8 Hz), 8.05 (1H, d, *J* = 8.8 Hz), 8.26 (1H, d, *J* = 9.2 Hz), 8.58 (1H, s). LRMS (DART+) calcd for C₂₅H₃₀N₅O₂ [M+H⁺]: *m/z* = 432.2, found 432.3.

2.2.3. General procedure for the preparation of compounds (**5d**, **5e**, **5f**, **5g**)

A mixture of **3** (210 mg, 0.4 mmol, 1.0 eq.), morpholine (**4d**) (70 mg, 0.8 mmol, 2.0 eq.), cesium carbonate (210 mg, 0.6 mmol, 1.5 eq.), tris(dibenzylideneacetone)dipalladium(0) (19 mg, 21 μmol, 0.05 eq.), and *rac*-BINAP (26 mg, 42 μmol, 0.1 eq.) in 1,4-dioxane (5 mL) was stirred at 100

°C for 1.5 - 3.5 days under nitrogen atmosphere. After removing precipitate by filtration through a pad of Celite® and washing with 1,4-dioxane, the filtrate was concentrated under reduced pressure and purified by column chromatography on silica gel (chloroform/methanol = 100/1), to obtain the titled compound (**5d**). The same procedure was carried out to obtain compounds **5e**, **5f** and **5g**.

2.2.3.1. 4-{2-[5-(2-Methoxyethoxy)-1H-benzo[d]imidazol-1-yl]quinolin-8-yl}morpholine (**5d**)

The title compound **5d** was afforded (127 mg, 75%) as a white solid. ¹H NMR (400 MHz, CDCl₃): δ 3.43 – 3.45 (4H, m), 3.50 (3H, s), 3.82 – 3.85 (2H, m), 4.05 – 4.08 (4H, m), 4.23 – 4.25 (2H, m), 7.16 (1H, dd, *J* = 2.4, 8.8 Hz), 7.24 – 7.25 (1H, m), 7.37 (1H, d, *J* = 2.4 Hz), 7.48 – 7.54 (2H, m), 7.71 (1H, d, *J* = 8.8 Hz), 8.34 (1H, d, *J* = 9.2 Hz), 8.50 (1H, d, *J* = 9.2 Hz), 8.64 (1H, s). LRMS (DART+) calcd for C₂₄H₂₅N₄O₃ [M+H⁺]: *m/z* = 405.2, found 405.2.

2.2.3.2. 2-[5-(2-Methoxyethoxy)-1H-benzo[d]imidazol-1-yl]-8-{4-methylpiperazin-1-yl}quinoline (**5e**)

The title compound **5e** was afforded (147 mg, 84%) as a pale yellow solid. ¹H NMR (400 MHz, CDCl₃): δ 2.48 (3H, s), 2.65 – 2.95 (4H, m), 3.39 – 3.51 (7H, m), 2.83 – 3.85 (2H, m), 4.21 – 4.25 (2H, m), 7.15 – 7.17 (1H, m), 7.28 – 7.29 (1H, m), 7.37 (1H, s), 7.47 – 7.53 (2H, m), 7.71 (1H, dd, *J* = 1.6, 8.8 Hz), 8.33 (1H, dd, *J* = 1.6, 8.4 Hz), 8.49 (1H, d, *J* = 8.8 Hz), 8.66 (1H, s). LRMS (DART+) calcd for C₂₄H₂₈N₅O₂ [M+H⁺]: *m/z* = 418.2, found 418.2.

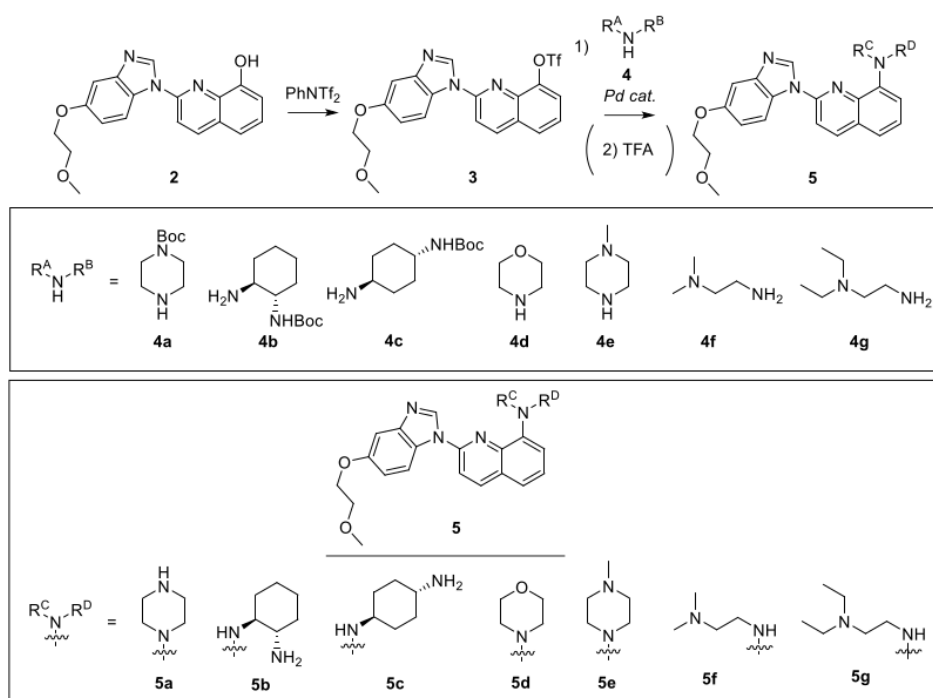
2.2.3.3. *N*-(2-[5-(2-Methoxyethoxy)-1H-benzo[d]imidazol-1-yl]quinolin-8-yl)-*N'*, *N'*-dimethylethane-1,2-diamine (**5f**)

The title compound **5f** was afforded (145 mg, 85%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃): δ 2.38 (6H, s), 2.73 – 2.76 (2H, m), 3.38 – 3.39 (2H, m), 3.50 (3H, s), 3.82 – 3.84 (2H, m), 4.23 – 4.25 (2H, m), 6.48 (1H, br s), 6.76 (1H, d, *J* = 8.0 Hz), 7.10 – 7.14 (2H, m), 7.37 (1H, d, *J* = 2.4 Hz), 7.40 – 7.44 (1H, m), 7.64 (1H, dd, *J* = 1.6, 8.8 Hz), 8.24 – 8.26 (1H, m), 8.31 (1H, d, *J* = 9.2 Hz), 8.60 (1H, d, *J* = 1.6 Hz). LRMS (DART+) calcd for C₂₃H₂₈N₅O₂ [M+H⁺]: *m/z* = 406.2, found 406.2.

2.2.3.4. *N,N*-Diethyl-*N'*-(2-(5-(2-methoxyethoxy)-1H-benzo[d]imidazol-1-yl)quinolin-8-yl)ethane-1,2-diamine (**5g**)

The title compound **5g** was afforded (105 mg, 52%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃): δ 1.06 – 1.10 (6H, m), 2.65 – 2.70 (4H, m), 2.87 – 2.89 (2H, m), 3.38 – 3.39 (2H, m), 3.50

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 (3H, s), 3.82 – 3.84 (2H, m), 4.22 – 4.25 (2H, m), 6.48 (1H, br s), 6.77 (1H, d, $J = 7.6$ Hz), 7.10 – 7.12 (2H, m), 7.37 (1H, d, $J = 2.4$ Hz), 7.40 – 7.44 (1H, m), 7.65 (1H, dd, $J = 1.6, 8.8$ Hz), 8.19 – 8.24 (1H, m), 8.26 (1H, dd, $J = 1.2, 8.4$ Hz), 8.63 (1H, s). LRMS (DART+) calcd for $C_{25}H_{32}N_5O_2$ $[M+H]^+$: $m/z = 434.2$, found 434.3.



Scheme 1. Synthesis of benzo[d]imidazole-quinoline derivatives. N -phenyl-bis(trifluoromethanesulfonimide), room temperature, 2 d; 1) tris(dibenzylideneacetone)dipalladium(0), cesium carbonate, *rac*-BINAP, reflux, 3 d; 2) TFA

2.2.4. General procedure for the preparation of compounds (**6a**, **6b**)

A mixture of piperidin-4-ol (610 mg, 6.0 mmol, 1.0 eq.), N,N -diisopropylethylamine (DIPEA) (1.6 mL, 9.0 mmol, 1.5 eq.), and di-*tert*-butyl dicarbonate (Boc_2O) (520 mg, 2.4 mmol, 0.4 eq.) in dry dichloromethane (15 mL) was stirred at room temperature for 19 h under nitrogen atmosphere. The reaction mixture was diluted with dichloromethane and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (chloroform/methanol = 50/1) to afford Boc-aminoalcohol (*tert*-butyl 4-hydroxypiperidine-1-carboxylate) as a colorless solid (350 mg, 29%). The purified material (350 mg, 1.7 mmol, 1.0 eq.) was dissolved in dichloromethane (DCM) (5 mL). Triethylamine (TEA) (480 μ L, 3.7 mmol, 2.2 eq.) was added to the solution and the solution was cooled to 0 °C using an ice bath. To the mixture,

methane sulfonyl chloride (MsCl) (160 μ L, 2.0 mmol, 1.2 eq.) was added dropwise at 0 °C. The mixture was gradually warmed to room temperature and stirred overnight. The reaction mixture was quenched by addition of water (10 mL) dropwise and extracted with DCM (3 x 20 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to obtain **6a** (280 mg) as a colorless solid. The product was used for the next step without further purification. The same procedure was performed to obtain compound **6b**.

2.2.4. General procedure for the preparation of compounds (**7a** and **7b**)

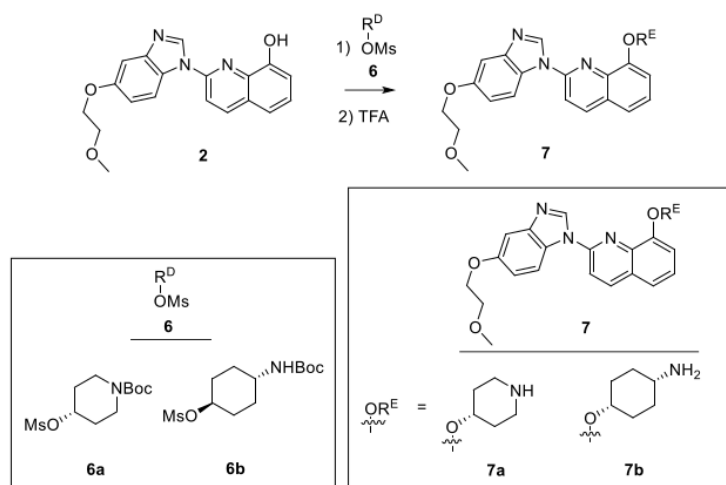
To a mixture of **2** (168 mg, 0.5 mmol, 1.0 eq.) and *tert*-butyl 4-((methylsulfonyl)oxy)piperidine-1-carboxylate (223 mg, 0.8 mmol, 1.6 eq.) (**6a**) in dry toluene (5 mL) was added cesium carbonate (495 mg, 1.5 mmol, 3.0 eq.). The mixture was stirred at 100 °C for overnight under nitrogen atmosphere. After reaction completion, water was added to the mixture and the mixture was extracted using ethyl acetate (3 x 30 mL). The organic layers were collected and washed with saturated aqueous NaHCO₃. The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The intermediate product was obtained after purification by column chromatography on silica gel (chloroform/methanol = 100/1). TFA (1 mL) was added to the intermediate product (0.5 mmol, 1.0 eq.), and the mixture was stirred at room temperature for 15 min. After removing TFA by nitrogen gassing, the residue was dissolved in dichloromethane and washed with 0.1 M HCl. After adjusting pH to 9.0 using 0.1 M NaOH aqueous solution, the water layer was extracted using dichloromethane (3 x 25 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure to obtain the titled compound (**7a**). The same procedure was carried out to obtain compound **7b**.

2.2.4.1. 2-[5-(2-Methoxyethoxy)-1*H*-benzo[*d*]imidazol-1-yl]-8-{piperidin-4-yloxy}quinoline (**7a**)

The title compound **7a** was afforded (80 mg, 40%) as a pale white solid. ¹H NMR (400 MHz, CDCl₃): δ 3.44 – 3.49 (4H, m), 3.50 (3H, s), 3.62 – 3.63 (1H, m), 3.82 – 3.85 (2H, m), 4.05 – 4.06 (4H, m), 4.23 – 4.26 (2H, m), 6.80 (1H, br s), 7.15 – 7.18 (1H, m), 7.24 – 7.26 (1H, m), 7.37 – 7.38 (1H, m), 7.40 – 7.54 (2H, m), 7.71 (1H, dd, *J* = 2.0, 8.8 Hz), 8.34 (1H, dd, *J* = 1.6, 8.4 Hz), 8.50 (1H, d, *J* = 2.4, 9.2 Hz), 8.64 (1H, d, *J* = 1.6 Hz). LRMS (DART+) calcd for C₂₄H₂₇N₄O₃ [M+H⁺]: *m/z* = 419.2, found 419.2.

2.2.4.2. *cis*-4-({2-[5-(2-Methoxyethoxy)-1H-benzo[d]imidazol-1-yl]quinolin-8-yl}oxy)cyclohexan-1-amine (**7b**)

The title compound **7b** was afforded (97 mg, 45%) as a pale white solid. ¹H NMR (400 MHz, CDCl₃): δ 1.62 (2H, br s), 1.72 – 1.74 (2H, m), 1.78 – 1.86 (4H, m), 2.28 – 2.31 (2H, m), 2.81 – 2.85 (1H, m), 3.50 (3H, s), 3.82 – 3.84 (2H, m), 4.22 – 4.24 (2H, m), 4.84 (1H, br s), 7.16 – 7.21 (2H, m), 7.35 (1H, d, *J* = 2.4 Hz), 7.42 – 7.48 (2H, m), 7.70 (1H, d, *J* = 8.8 Hz), 8.29 (1H, d, *J* = 8.8 Hz), 8.64 (1H, s), 8.86 (1H, d, *J* = 8.8 Hz). LRMS (DART+) calcd for C₂₅H₂₉N₄O₃ [M+H⁺]: *m/z* = 433.2, found 433.2.



Scheme 2. Synthesis of benzo[d]imidazole-quinoline derivatives. 1) cesium carbonate, reflux, overnight; 2) TFA.

2.2.5. 5-Iodo-2-[5-(2-methoxyethoxy)-1H-benzo[d]imidazol-1-yl]-8-{piperidin-4-yl}quinoline (**8**)

A mixture of *N*-chlorosuccinimide (NCS) (53 mg, 0.4 mmol, 1.2 eq.) and sodium iodide (NaI) (60 mg, 0.4 mmol, 1.2 eq.) in acetic acid (5 mL) was added to **5a** (119 mg, 0.3 mmol, 1.0 eq.) in acetic acid (5 mL) and the mixture was stirred at 50 °C overnight under nitrogen atmosphere. After adjusting pH to 9.0 using saturated aqueous sodium bicarbonate (NaHCO₃), the reaction mixture was extracted with dichloromethane (3 x 30 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (chloroform/methanol = 50/1) to afford **8** (103 mg, 65%) as a pale yellow solid. ¹H NMR (400 MHz, CDCl₃): δ 2.90 – 2.94 (1H, br s), 3.21 – 3.24 (3H, m), 3.38 – 3.45 (4H, m), 3.50 (3H, s), 3.82 – 3.84 (2H, m), 4.23 – 4.25 (2H, m), 6.98 – 7.02 (1H, m), 7.16 (1H, d, *J* = 8.8 Hz), 7.37 (1H, s), 7.74 (1H, d, *J* = 8.8 Hz), 8.00 (1H, d, *J* = 8.4 Hz), 8.46 (1H, d, *J* = 8.0 Hz), 8.56 (1H, d, *J* = 8.0 Hz), 8.65 (1H, s). ¹³C NMR (100 MHz, CDCl₃): δ 156.15, 150.39, 147.16, 145.53,

144.76, 141.23, 137.52, 129.21, 126.73, 119.41, 114.86, 114.68, 113.64, 103.67, 89.03, 71.11, 67.87, 59.29, 53.25 (2C), 51.94, 46.47 (2C). LRMS (ESI+) calcd for $C_{23}H_{24}IN_5O_2$ $[M+H]^+$: m/z = 530.1, found 530.1.

2.2.6. *tert*-Butyl 4-{5-iodo-2-[5-(2-methoxyethoxy)-1*H*-benzo[d]imidazol-1-yl]quinolin-8-yl}piperazine-1-carboxylate (9**)**

A mixture of **8** (103 mg, 0.2 mmol, 1.0 eq.), *N,N*-diisopropylethylamine (DIPEA) (54 μ L, 0.3 mmol, 1.5 eq.) and di-*tert*-butyl dicarbonate (Boc₂O) (43 mg, 0.2 mmol, 1.0 eq.) in dry dichloromethane (3 mL) was stirred at room temperature for 3 days under nitrogen atmosphere. The reaction mixture was diluted with dichloromethane and washed successively with saturated aqueous NaHCO₃ and brine. The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure to afford **9** (76 mg) as a pale yellow solid. The product was used in the following reaction without further purification.

2.2.7. *tert*-Butyl (1-{2-[5-(2-methoxyethoxy)-1*H*-benzo[d]imidazol-1-yl]-5-(tributylstannyl)quinolin-8-yl}piperidin-4-yl)carbamate (10**)**

A mixture of **9** (45 mg, 71.5 μ mol, 1.0 eq.), hexabutyldistannane (144 μ L, 286 μ mol, 4.0 eq.), and tetrakis(triphenylphosphine)palladium(0) Pd[P(C₆H₅)₃]₄ (12 mg, 10.8 μ mol, 0.15 eq.) in dry toluene (2 mL) was refluxed for 22 h under nitrogen atmosphere. After removing the catalyst by filtration through a pad of Celite® and washing with toluene, the filtrate was concentrated under reduced pressure and purified by HPLC with mobile phase system methanol (A) (with 0.05% TEA) and water (B) (with 0.05% TEA), A : 95.0% - 99.5%, 20 min using 5C₁₈ MSII (10ID x 250 mm) column, flow rate 4 mL/min, to afford **10** (25 mg, 45%) as a pale yellow solid. ¹H NMR (400 MHz, CDCl₃): δ 0.82 – 0.89 (9H, m), 1.11 – 1.23 (6H, m), 1.25 – 1.41 (6H, m), 1.45 (9H, s), 1.51 – 1.54 (6H, m), 3.34 – 3.36 (4H, m), 3.50 (3H, s), 3.75 – 3.77 (4H, m), 3.82 – 3.85 (2H, m), 4.23 – 4.26 (2H, m), 7.15 (1H, dd, J = 2.4, 8.8 Hz), 7.21 (1H, dd, 2.4, 7.2 Hz), 7.67 (1H, d, J = 2.0 Hz), 7.60 (1H, dd, J = 2.4, 7.2 Hz), 7.72 (1H, J = 2.4, 8.8 Hz), 8.24 (1H, dd, J = 2.4, 8.8 Hz), 8.44 (1H, dd, J = 2.0, 8.8 Hz), 8.65 (1H, d, J = 2.4 Hz). ¹³C LRMS (DART+), calcd for C₄₀H₆₀N₅O₄Sn $[M+H]^+$: m/z = 794.4, found 794.5.

2.2.8. 4-{5-Iodo-2-[5-(2-methoxyethoxy)-1*H*-benzo[d]imidazol-1-yl]quinolin-8-yl}morpholine (11**)**

A mixture of NCS (53 mg, 0.4 mmol, 1.2 eq.) and sodium iodide (NaI) (60 mg, 0.4 mmol, 1.2 eq.) in acetic acid (5 mL) was added to **5b** (130 mg, 0.3 mmol, 1.0 eq.) in acetic acid (5 mL) and the mixture was stirred at 50 °C overnight under nitrogen atmosphere. After adjusting pH to 9.0 using saturated aqueous sodium bicarbonate (NaHCO₃), the reaction mixture was extracted with dichloromethane (3 x 30 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (chloroform/methanol = 50/1) to afford **11** (110 mg, 70%) as a pale yellow solid. ¹H NMR (400 MHz, CDCl₃): δ 3.39 – 3.41 (4H, m), 3.50 (3H, s), 3.82 – 3.84 (2H, m), 4.03 – 4.05 (4H, m), 4.23 – 4.25 (2H, m), 6.98 (1H, d, *J* = 8.0 Hz), 7.17 (1H, dd, *J* = 2.4, 8.8 Hz), 7.38 (1H, d, *J* = 2.0 Hz), 7.74 (1H, d, *J* = 9.2 Hz), 8.02 (1H, d, *J* = 8.0 Hz), 8.50 (1H, d, *J* = 8.8 Hz), 8.58 (1H, d, *J* = 9.2 Hz), 8.64 (1H, s). LRMS (ESI+) calcd for C₂₃H₂₄IN₄O₃ [M+H⁺]: *m/z* = 531.1, found 531.1.

2.2.9. 4-(2-(5-(2-Methoxyethoxy)-1H-benzo[d]imidazol-1-yl)-5-(tributylstannyl)quinolin-8-yl)morpholine (**12**)

A mixture of **11** (45 mg, 85 μmol, 1.0 eq.), hexabutylstannane (171 μL, 340 μmol, 4.0 eq.), and tetrakis(triphenylphosphine)palladium(0) Pd[P(C₆H₅)₃]₄ (14 mg, 12.75 μmol, 0.15 eq.) in dry toluene (2 mL) was refluxed for 48 h under nitrogen atmosphere. After removing the catalyst by filtration through a pad of Celite® and washing with toluene, the filtrate was concentrated under reduced pressure and purified by HPLC with mobile phase system methanol (A) (with 0.05% TEA) and water (B) (with 0.05% TEA), A : 97.0% - 99.5%, 20 min using 5C₁₈ MSII (10ID x 250 mm) column, flow rate 4 mL/min, to afford **12** (29 mg, 50%) as a pale colorless solid. ¹H NMR (400 MHz, CDCl₃): δ 0.82 – 0.89 (9H, m), 1.11 – 1.23 (6H, m), 1.25 – 1.41 (6H, m), 1.51 – 1.54 (6H, m), δ 3.36 – 3.39 (4H, m), 3.49 (3H, s), 3.82 – 3.85 (2H, m), 4.02 – 4.04 (4H, m), 4.23 – 4.25 (2H, m), 6.98 (1H, d, *J* = 8.0 Hz), 7.17 (1H, dd, *J* = 2.4, 8.8 Hz), 7.38 (1H, d, *J* = 2.0 Hz), 7.74 (1H, d, *J* = 9.2 Hz), 8.22 (1H, d, *J* = 8.0 Hz), 8.50 (1H, d, *J* = 8.8 Hz), 8.58 (1H, d, *J* = 9.2 Hz), 8.64 (1H, s). LRMS (DART+), calcd for C₃₅H₅₁N₄O₃Sn [M+H⁺]: *m/z* = 695.3, found 695.3.

2.3. Cell viability assays

The cell viability assays of non-iodinated (**5a–5g**, **7a** and **7b**) and iodinated compounds (**8** and **11**) were evaluated as described previously.^{32,33} Namely, TR-PCT1 cells (positive-PDGFRβ) were seeded on 96-well plates (5 x 10³ cells/well) and cultured at 33 °C in DMEM medium with 20 ng/mL PDGF-BB and 2% FBS in a 5% CO₂ incubator. Cells were treated with each compound for 72 h and

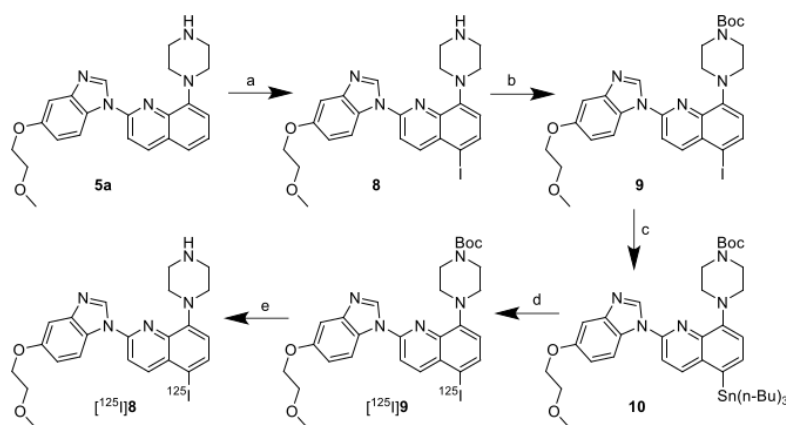
cell viability was determined by the Cell Counting Kit-8. The same procedure was performed using H441 and LNCaP cells as negative-PDGFR β cells in RPMI 1640 medium without PDGF-BB addition.

2.4. Radiolabeling

Radiolabeling of [125 I]**8** and [125 I]**11** was performed by an iododestannylation reaction of the corresponding tributylstannyl precursors (**10** or **12**) using NCS as an oxidizing agent. The radiotracers were isolated by RP-HPLC using a Cosmosil 5C₁₈MSII column (4.6 ID $\times$ 150 mm) at the flow rate of 1 mL/min with a gradient mobile phase of 70% methanol in water with 0.05% TEA to 90% methanol in water with 0.05% TEA for 20 min. The column temperature was maintained at 40 °C. Radiochemical yield and purity were determined by radio-HPLC analysis.

2.4.1. Synthesis of [125 I]**8**

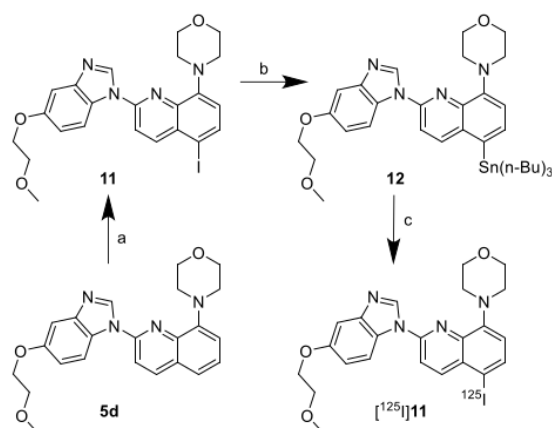
A solution of [125 I]NaI (370 kBq, 2 μ L) was charged into a sealed vial containing **10** (1 mg/mL, 5 μ L), acetic acid (5%, 30 μ L), acetonitrile (55 μ L), and NCS (5 mg/mL, 10 μ L). The mixture was shaken at room temperature for 15 min, quenched by addition of sodium hydrogensulfite (5 mg/mL, 10 μ L) and the solvent was removed by nitrogen gassing. TFA was added to the residue and the shaking was allowed to continue for 30 min. After removing TFA by nitrogen gassing, the residue was mixed with initial mobile phase of HPLC. The reaction mixture was shaken for some minutes, filtered, and analyzed by HPLC.



Scheme 3. Synthesis of reference compound, precursor, and radioiodinated compound [125 I]**8** (a) NCS, NaI, 50 °C, overnight (b) Boc₂O, TEA, room temperature, 3 d (c) hexabutylstannane, Pd[P(C₆H₅)₃]₄, reflux, 24 h (d) [125 I]NaI, NCS, acetic acid, room temperature, 15 min (e) TFA, room temperature, 30 min.

2.4.2. Synthesis of [125 I]**11**

A solution of [^{125}I]NaI in 0.1 M NaOH aqueous solution (2 μL) was added in a sealed vial containing **12** (1 mg/mL, 5 μL), acetic acid (5%, 10 μL), acetonitrile (10 μL), and NCS (5 mg/mL, 15 μL). The mixture was shaken at room temperature for 15 min, quenched by addition of sodium hydrogensulfite (5 mg/mL, 15 μL) and purified by HPLC.



Scheme 4. Synthesis of reference compound, precursor, and radioiodinated compound [^{125}I]**11** (a) NCS, NaI, 50 °C, overnight (b) hexabutylstannane, $\text{Pd}[\text{P}(\text{C}_6\text{H}_5)_3]_4$, reflux, 48 h (c) [^{125}I]NaI, NCS, acetic acid, room temperature, 15 min.

2.5. Determination of partition coefficient

Partition coefficients of [^{125}I]**8** and [^{125}I]**11** into *n*-octanol and 0.1 M phosphate buffer (PB) pH 7.4 were measured as described previously with a slight modification.³⁵ Briefly, [^{125}I]**8** or [^{125}I]**11** was added to the mixture of *n*-octanol (3.0 mL) and PB (3.0 mL) in a test tube. The test tube was vortexed (1 min), left at room temperature (10 min), and centrifuged (5 min, 3,060g, 4 °C). *n*-Octanol layer (2.0 mL) was transferred into new test tube followed by addition of fresh *n*-octanol (1.0 mL) and PB (3.0 mL). After repeating vortex, standing, and centrifuging, radioactivity of each layer, *n*-octanol (1.0 mL) and PB (1.0 mL), was counted using an auto well gamma counter ($n = 4$). The partition coefficient was determined by calculating the ratio of radioactivity in *n*-octanol to that in PB, and expressed as a common logarithm ($\log P$).

2.6. In vitro stability assay

The stability of radiotracers, [^{125}I]**8** and [^{125}I]**11**, were analyzed as described previously with a slight modification.^{32,36,37} Briefly, [^{125}I]**8** or [^{125}I]**11** solution (50 μL) in sealed tube containing 0.1 M phosphate buffered saline (PBS) pH 7.4 (450 μL) was incubated at 37 °C for 1, 3, 6, and 24 h. After incubation, the purities of radiotracers were analyzed by TLC using chloroform/methanol = 5/1

and 25/1 for [^{125}I]**8** and [^{125}I]**11**, respectively as a developing solvent and the results were confirmed by HPLC.

2.7. Cellular uptake studies

BxPC3-luc cell lines were used to perform the radiotracer uptake experiments which were cultured in RPMI 1640 medium containing 10% FBS and penicillin (100 IU/mL)-streptomycin (100 $\mu\text{g/mL}$) on 6-well culture plates (containing 2×10^5 cells/well) for 24 h using a humidified atmosphere (5% CO_2) incubator at 37 °C.^{32,38} After removal of medium, a solution of [^{125}I]**8** or [^{125}I]**11** (3.7 kBq/well) in medium without FBS was added. After incubation for 0.5, 1, 2, and 4 h, the medium from each well was removed and the cells were washed once with ice-cold PBS (1 mL). The cells were dissolved using 1 M NaOH aqueous solution (0.5 mL) and wells were washed with 1 M NaOH aqueous solution (0.5 mL). The radioactivity of pooled basic fractions was determined using an auto well gamma counter. The total protein in the cell was quantified using a BCA Protein Assay Kit following the manufacturer's recommendations and bovine serum albumin as a protein standard. All data were expressed as percent dose per microgram protein (%dose/ μg protein).

In *in vitro* blocking experiments, inhibitors (**1**, **8**, or **11** with final concentration 10 μM) in 1 mL of medium without FBS were added to wells containing 2×10^5 cells/well. After incubation for 10 min, [^{125}I]**8** or [^{125}I]**11** (3.7 kBq/well) in 1 mL of medium without FBS was added to each well. Radioactivity and protein concentration in cells were determined using the same method above-mentioned.

2.8. Competitive binding assay

BxPC3-luc cells in RPMI 1640 medium containing 10% FBS and penicillin (100 IU/mL)-streptomycin (100 $\mu\text{g/mL}$) were seeded on 24-well plates (containing 50,000 cells/wells) and incubated for 24 h using a humidified atmosphere (5% CO_2) at 37 °C. Nine concentrations of displacing nonradiolabeled ligands (**1** and **8**) (ranging from 1 pM to 1 mM) and [^{125}I]**8** in RPMI 1640 medium without 10% FBS were incubated at 37 °C for 4 h. After the cells were washed twice with ice-cold PBS (250 μL), the unbound radioligand was removed. The cells were dissolved using 1 M NaOH aqueous solution (250 μL) and wells were washed with 1 M NaOH aqueous solution (250 μL). The bound radioactivity was determined using an auto well gamma counter.

2.9. Animals

Experiments with animals were conducted by the Guidelines for the Care and Use of Laboratory Animals of Kanazawa University. The animals were housed with free access to food and water at 23 °C with a 12 h light/dark schedule. Six-week-old male ddY (27 – 30 g) and four-week-old female BALB/c *nu/nu* mice (12 – 17 g) were purchased from Japan SLC Inc. (Hamamatsu, Japan). For preparing the tumor-bearing model, 5×10^6 BxPC3-luc cells were subcutaneously inoculated into the left shoulder of BALB/c *nu/nu* mice. The tumor reached palpable size for biodistribution experiments after two weeks of the inoculation.

2.9.1. Biodistribution study

Mice were intravenously injected via the tail with a saline solution of [125 I]8 (74 kBq, 100 μ L), containing 1% tween-80 and 10% ethanol. The ddY mice were sacrificed at 10 min, 1, 4, and 24 h; meanwhile, tumor-bearing mice were sacrificed at 1 h postinjection.

For *in vivo* blocking studies, tumor-bearing mice were intraperitoneally injected with 200 μ L of an excess of inhibitor, 1 (40 mg/kg), 1 h before intravenous injection of [125 I]8 (74 kBq, 100 μ L). At 1 h postinjection of [125 I]8, the mice were sacrificed.

Tissues of interest were removed and weighed. The radioactivity of the tissues was determined using an auto well gamma counter as described-above in cellular uptake study and counts were corrected for background radiation. The data were expressed as percent injected dose per gram tissue (%ID/g).

2.10. Statistical analysis

All data were analyzed using GraphPad 5.0 software (La Jolla, CA, USA) and displayed as mean $\pm$ standard deviation (SD). Significance for *in vitro* blocking studies was calculated using a one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test compared to the control group. Significance in cell viability assays was determined using ANOVA followed by Tukey's post hoc test. IC₅₀ values for binding assay were calculated by nonlinear regression. Significance for *in vivo* blocking studies between control and blocking groups were calculated using unpaired Student's *t*-test. Results were considered statistically significant at $p < 0.05$.

3. Result and Discussion

3.1. Synthesis of reference compounds and precursors

The benzimidazole–quinoline derivatives 5a–5g, 7a, and 7b were readily synthesized from the common intermediate 2, which was synthesized according to a previously reported procedure (Scheme 1 and 2).

3.1.1 Non-iodinated compounds

The potency of benzimidazole–quinoline derivatives **5a–5g**, **7a**, and **7b** to reduce the viability PDGFR β -overexpressed (TR-PCT1) cell line were evaluated. Among those derivatives compounds **5a** and **5d** exhibited more potent proliferation inhibition effects in PDGFR β -positive cells than **1**.

3.1.2 Iodinated compounds

The iodination of **5a** and **5d** derivatives **8** and **11** were subsequently synthesized as shown in Schemes 3 and 4, respectively. Iodine was incorporated into the C-5 of the **5a** and **5b** quinoline core, using a solution of NCS and NaI in acetic acid to yield **8** and **11** (Schemes 3 and 4, respectively).

3.2. Cell viability assays

The binding affinities of the nine non-iodinated benzimidazole–quinoline derivatives (**5a–5g** and **7a–7b**) and two iodinated derivatives (**8** and **11**) to PDGFR β ATP-binding site were evaluated using PDGFR β overexpressing TR-PCT1 cells. The cells were treated with 1–1000 nM of synthesized ligands, **1**, **5a–5g**, **7a–7b**, **8**, or **11**. As shown in Figure 2, the initial SAR studies suggested that the substituent on the C-8 of the quinoline group (piperidine part) of **1** influenced the quinoline derivative affinity towards PDGFR β . Replacing the 4-amino-1-piperidino group of **1** with cyclic secondary amino groups such as 1-piperazino (**5a**) or 4-morpholino group (**5d**) had a significant impact in reducing the viability of TR-PCT1 cells. The *N*-methylated derivative of **5a** (**5e**) exhibited lower potency. Compounds with cyclohexanediamine (**5b** and **5c**) and ethylenediamine structures (**5f** and **5g**) at the C-8 of the quinoline group were less potent towards PDGFR β . **7a** and **7b**, which carry alkoxy substituents on the quinoline, also exhibited low potency. The secondary amino substituents on **5a** and **5d** are relatively small and present a fixed conformation compared with other substituents. These structural characteristics might contribute to improve their affinity towards PDGFR β . Further structural studies are needed to fully clarify the effect of the substituent on the quinoline group.

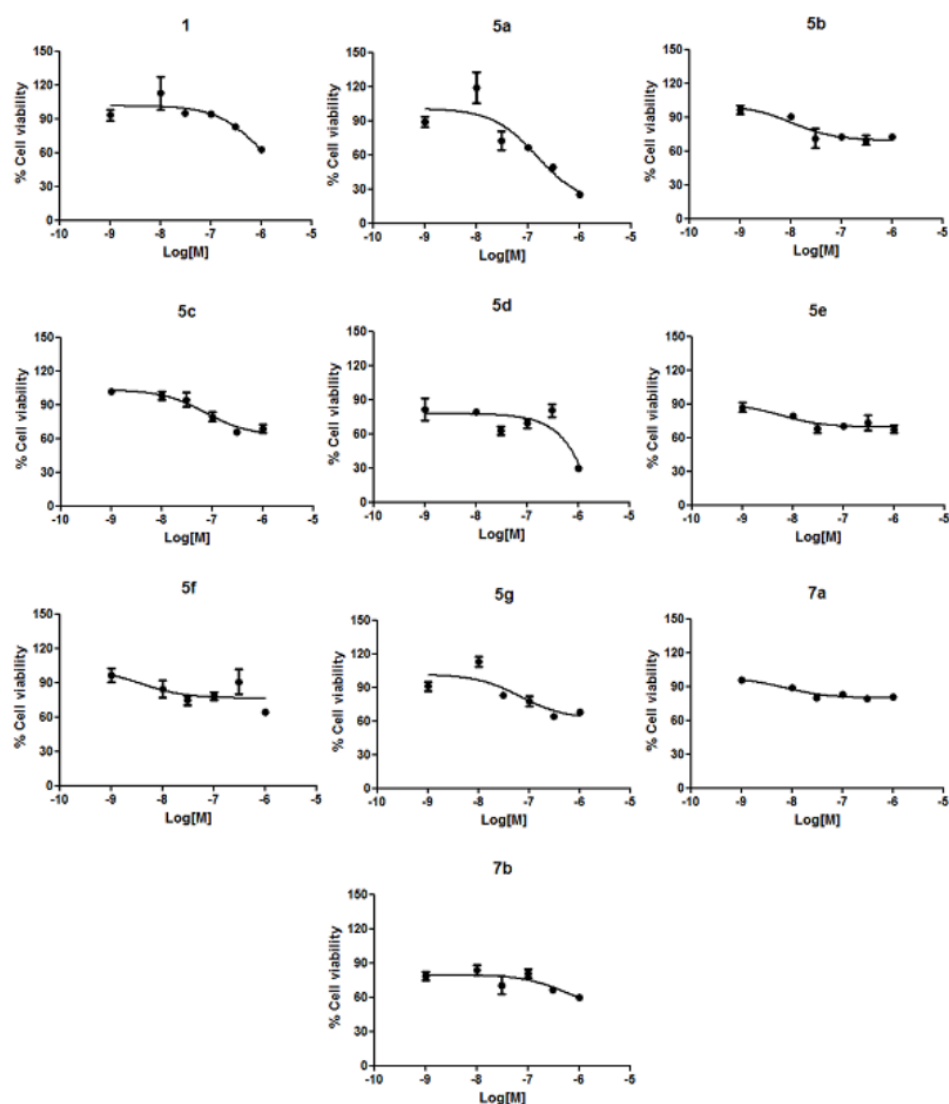


Figure 2. Cell viability after exposure **1**, **5a** – **5g**, **7a**, and **7b** by WST-8 assay. Data were presented as mean $\pm$ SD for three samples.

Moreover, cell viability assays of iodinated **5a** (**8**) and iodinated **5d** (**11**) were performed. The halogen atom introduction to a small molecule potentially influences its favored molecular conformation and its affinity to the molecular target.³⁹ Introduction of iodine into the C-5 of **5a** and **5d** quinoline group decreased their affinities towards PDGFR β . In contrast to **11** which significantly lost the potency of the base compound, the potency of **8** toward TR-PCT1 cell was still at the same level as **1** (see Figure 3).

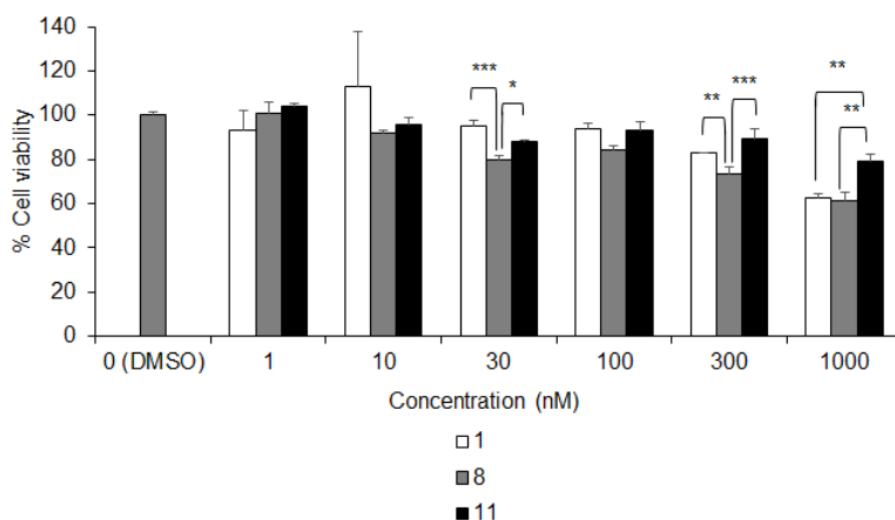


Figure 3. Cell viability after exposure to **1**, **8**, and **11** by WST-8 assay. Data were presented as mean $\pm$ SD for three samples. Significance was determined using a one-way ANOVA followed by Tukey's post hoc test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

3.3. Radiolabeling

In the present study, we synthesized radioiodinated compounds using our previously described strategy.³² Two novel radioiodinated derivatives of benzimidazole–quinoline were synthesized through an iododestannylation reaction with the corresponding tributyltin precursors, **10** and **12**, as outlined in Schemes **3** and **4**, respectively. Both radiolabeled probes were synthesized in an acidic solution at room temperature in very high radiochemical yields (91% and 93%, respectively), using NCS as an oxidizing agent. Subsequent HPLC purification provided [^{125}I]**8** and [^{125}I]**11** with excellent radiochemical purities (>99%).

3.4. Determination of the partition coefficient

The *n*-octanol/PB partition coefficients as log *P* values for [^{125}I]**8** and [^{125}I]**11** were 2.75 ± 0.01 and 3.56 ± 0.16 , respectively, indicating that [^{125}I]**11** had a higher lipophilicity than [^{125}I]**8**. Lipophilicity can be used to predict or rationalize a variety of *in vitro* and *in vivo* parameters, especially, permeability and tissue distribution (biodistribution). Membrane diffusion as physiological barrier is greatly dependent on lipophilicity of compounds to gain access to the intracellular domain. Hence, lipophilicity can be a supported data to explain not only the assessment of compounds into the intracellular part but also the biodistribution result of probes.

3.5. *In vitro* stability assays

The stability of [^{125}I]**8** and [^{125}I]**11** in 0.1 M PBS (pH 7.4) was evaluated by TLC and HPLC analyses. The radiotracers' purities following a 24 h incubation at 37 °C remained $91.6 \pm 0.8\%$ for [^{125}I]**8** and $97.3 \pm 0.3\%$ for [^{125}I]**11**, respectively, revealing a good probe stability *in vitro*.

3.6. Cellular uptake studies

In vitro studies of cell uptake were performed for [^{125}I]**8** and [^{125}I]**11** using PDGFR β -positive BxPC3-luc cells.^{21,37} As shown in Figure 4, BxPC3-luc cells exhibited a higher uptake of [^{125}I]**8**, which, in the WST-8 assay, had higher affinity against PDGFR β than [^{125}I]**11**. Following a 4 h incubation, the cellular uptake of [^{125}I]**8** in BxPC3-luc cells reached 0.77% dose/ μg protein, whereas that of [^{125}I]**11** reached only 0.17% dose/ μg protein. This result was consistent with the cell viability assay, wherein **8** exhibited a higher affinity for PDGFR β than **11** (Figure 3).

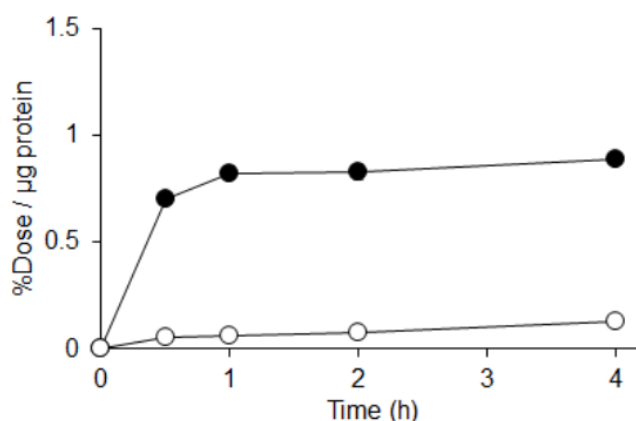


Figure 4. Cellular uptake study. Time-dependent accumulation of [^{125}I]**8** (closed circles) and (b) [^{125}I]**11** (open circles) in BxPC3-luc cells. Data were presented as mean $\pm$ SD for three samples.

In contrast to [^{125}I]**11**, [^{125}I]**8** uptake in BxPC3-luc cells significantly decreased after pretreatment with an excessive amount of a PDGFR β ligand, **1**, or non-radiolabeled compounds, **8** (Figure 5). The protein concentration which was used in this study after 4 h incubation for control, **1**, **8**, and **11** groups are This indicates that [^{125}I]**8** has high specificity towards PDGFR β as a molecular target.

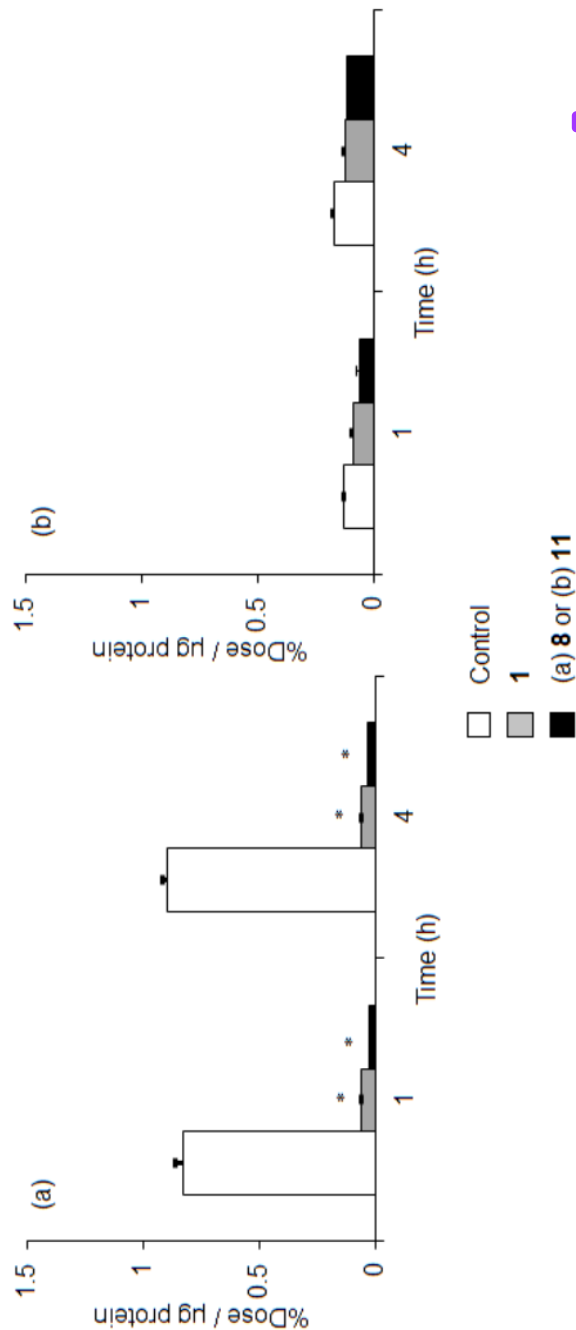


Figure 5. *In vitro* blocking studies of (a) [125 I]8 and (b) [125 I]11 in BxPC3-luc cells. Data were presented as mean $\pm$ SD for three samples. Significance was determined using a one-way ANOVA followed by Dunnett's post hoc test (* $p < 0.001$, vs control).

3.7. Competitive binding assay using BxPC3-luc cells

We investigated the competitive binding of [125 I]**8** to BxPC3-luc cells in the presence of PDGFR β ligand, **1** and **8**. The IC₅₀ values for **1** and **8** were found to be 14 ± 4 and 75 ± 10 nM, respectively.

3.7. Biodistribution studies

The biodistribution of [125 I]**8** in normal mice is summarized in Table 1. Following administration, high radioactivity accumulations in the liver and small and large intestines were found. Over the following 24 h, [125 I]**8** was mainly excreted from those tissues into feces, as confirmed by high radioactivity in the feces, indicating that the radiotracer was cleared via the hepatobiliary pathway. It was often observed in most small molecular probes targeted the ATP-binding site of TKs.^{24,41,42} Lipophilicity of [125 I]**8** might contribute in those result. Additionally, radioactivity was hardly observed in any tissues 24 h post [125 I]**8** injection. The low uptake levels observed in the stomach indicated that [125 I]**8** deiodination barely occurred *in vivo*.

Table 1. Biodistribution of radioactivity at 10 min, 1, 4, and 24 h after i.v. injection of [125 I]8 in ddY mice.

Tissues	Time after injection			
	10 min	1 h	4 h	24 h
[125 I]8				
Blood	2.06 (0.08)	0.76 (0.11)	0.33 (0.03)	0.03 (0.00)
Liver	9.85 (1.41)	3.98 (0.72)	3.12 (0.94)	0.24 (0.05)
Kidney	11.84 (0.22)	6.16 (0.32)	3.19 (0.64)	0.11 (0.02)
Small intestine	10.12 (1.21)	24.39 (2.51)	5.28 (0.95)	0.04 (0.01)
Large intestine	1.03 (0.08)	1.55 (0.28)	46.07 (8.23)	0.23 (0.05)
Spleen	5.27 (0.86)	1.34 (0.42)	0.38 (0.07)	0.03 (0.00)
Pancreas	4.19 (0.42)	2.06 (0.16)	0.70 (0.05)	0.01 (0.00)
Lung	15.74 (0.83)	4.95 (1.59)	1.52 (0.28)	0.09 (0.01)
Heart	2.78 (0.15)	0.69 (0.13)	0.20 (0.01)	0.02 (0.00)
Stomach [†]	1.73 (0.34)	2.93 (0.41)	1.10 (0.22)	0.05 (0.00)
Bone	1.42 (0.14)	0.51 (0.04)	0.17 (0.04)	0.00 (0.00)
Muscle	1.55 (0.077)	0.42 (0.08)	0.11 (0.01)	0.00 (0.00)
Brain	0.13 (0.00)	0.07 (0.01)	0.04 (0.00)	0.00 (0.00)
Urine				4.09 (0.63)
Feces				72.82 (12.18)

Data were presented as %injected dose / gram tissue. Each value represent mean $\pm$ SD for three or four mice.

[†] presented as %ID / organ

The biodistribution of [^{125}I]**8** in BxPC3-luc tumor-bearing mice is summarized in Table 2. [^{125}I]**8** accumulation in the tumor 1 h postinjection was 1.55%ID/gram, which is higher than that of the benzo[*d*]imidazol-quinoline derivative [^{125}I]IIQP (1.29%ID/gram), used in our previous research.²⁹ Moreover, the tumor-to-blood ratio of radioactivity 1 h post [^{125}I]**8** injection was 2.63 ± 0.02 , which was also higher than that of [^{125}I]IIQP (1.86 ± 0.34). Table 2 presents the effects of pretreatment with an excessive amount of **1** on [^{125}I]**8** biodistribution. Tumor uptake and tumor-to-blood ratio were significantly decreased by pretreatment with **1** (PDGFR β -specific inhibitor), indicating that [^{125}I]**8** accumulation in the tumor might be caused by PDGFR β . The exactly mechanism of it still need further research. Even though [^{125}I]**8** should be a more promising probe to PDGFR β than [^{125}I]IIQP, it may still be not enough to allow a clear tumor visualization. Therefore, optimized structures for higher tumor uptake and tumor-to-background ratios were required in further investigation.

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Table 2. Biodistribution of radioactivity at 1 h after i.v. injection of [¹²⁵I]8 in BxPC3-luc tumor-bearing mice.

Tissues	[¹²⁵ I]8	
	Control	Blocking
Blood	0.59 (0.03)	0.70 (0.04) *
Liver	9.04 (2.89)	5.99 (0.45)
Kidney	6.99 (1.42)	8.58 (1.35)
Small intestine	44.61 (1.96)	44.82 (3.71)
Large intestine	3.35 (0.94)	3.44 (0.72)
Spleen	4.94 (1.62)	5.02 (1.02)
Pancreas	4.75 (1.66)	5.65 (0.38)
Lung	7.53 (0.26)	8.37 (0.75)
Heart	1.22 (0.29)	1.21 (0.00)
Stomach [†]	1.27 (0.20)	1.24 (0.09)
Bone	0.84 (0.09)	0.86 (0.09)
Muscle	0.55 (0.09)	0.58 (0.14)
Brain	0.28 (0.06)	0.31 (0.09)
BxPC3-luc tumor	1.55 (0.02)	1.19 (0.27) *
Tumor/blood ratio	2.63 (0.02)	1.70 (0.11) *

Data were presented as means ± SD of %injected dose / gram of tissue for three or four mice. [†] presented as %ID/organ. (**p* < 0.05 vs control [¹²⁵I]8).

4. Conclusions

The present study aimed to synthesize and evaluate several benzimidazole–quinoline derivatives, in an effort to find appropriate PDGFR β imaging agents. The radiolabeled compounds, [^{125}I]**8** and [^{125}I]**11**, were readily synthesized from the corresponding stannylated precursors using an iododestannylation reaction with ^{125}I and NCS as an oxidizing agent. Moreover, [^{125}I]**8** was highly accumulated in PDGFR β -positive tumors, both *in vitro* and *in vivo*. Although further structural modifications are needed for clinical applications, the results obtained in this research are useful for the design of the PDGFR β targeting radioligands.

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