Radiobrominated benzimidazole-quinoline derivatives as Platelet-derived growth factor receptor beta (PDGFRβ) imaging probes

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Platelet-derived growth factor receptor beta (PDGFRβ) affects in numerous human cancers and has been recognized as a promising molecular target for cancer therapies. The overexpression of PDGFRβ could be a biomarker for cancer diagnosis. Radiolabeled ligands having high affinity for the molecular target could be useful tools for the imaging of overexpressed receptors in tumors. In this study, we aimed to develop radiobrominated PDGFRβ ligands and evaluate their effectiveness as PDGFRβ imaging probes. The radiolabeled ligands were designed by modification of 1-{2-[5-(2-methoxyethoxy)-1H-benzo[d]imidazol-1-yl]quinolin-8-yl}piperidin-4-amine (1), which shows selective inhibition profile toward PDGFRβ. The bromine atom was introduced directly into C-5 of the quinoline group of 1, or indirectly by the conjugation of 1 with the 3-bromo benzyol group. [76Br]1-{5-Bromo-2-[5-(2-methoxyethoxy)-1H-benzo[d]imidazol-1-yl]quinoline-8-yl}piperidin-4-amine ([76Br]2) and [76Br]-N-3-bromobenzoyl-1-{2-[5-(2-methoxyethoxy)-1H-benzo[d]imidazol-1-yl]quinolin-8-yl}-piperidin-4-amine ([76Br]3) were prepared using a bromoestannylation reaction. In a cellular uptake study, [76Br]2 and [76Br]3 more highly accumulated in BxPC3-luc cells (PDGFRβ-positive) than in MCF7 cells (PDGFRβ-negative), and their accumulation was significantly reduced by pretreatment with inhibitors. In biodistribution experiments, [76Br]2 accumulation was higher than [76Br]3 accumulation at 1 h postinjection. These findings suggest that [76Br]2 is more promising for positron emission tomography (PET) imaging of PDGFRβ than [76Br]3.

Receptor tyrosine kinases (RTKs) regulate cell differentiation, survival, migration, proliferation, metabolism, and angiogenesis, and their upregulation leads to uncontrollable cellular signaling in cancer1-3. Platelet-derived growth factor receptors (PDGFRs) are a family of RTKs, which when activated, trigger the phosphorylation of intracellular domain and activate the signaling pathway4. PDGFRα and PDGFRβ are two subtypes of PDGFRs5. Because PDGFRβ affects multiple tumors associated with various processes, including the autocrine growth stimulation of tumor cells and tumorigenesis, it has been targeted for the development of anticancer therapy4. Additionally, PDGFRβ expression can be a useful biomarker for the prediction of a cancer prognosis2, for example, a significant positive correlation between PDGFRβ expression and short overall survival (OS) in patients with angiosarcoma has been reported6. Therefore, the determination of PDGFRβ expression by noninvasive imaging is prominently meaningful in clinical oncology. For the development of PDGFRβ imaging probes, ligands with high affinity and good selectivity profile for PDGFRβ are desirable as carrier structures9,10.

Several approaches are available for the radiolabeling of bioactive molecules, and representative examples are as follows: (i) replacement of an element in the bioactive molecule with its radioactive isotope11; (ii) substitution

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β expression than [125I]onclides and the introduced position should be optimized to develop radiotracers with a radiohalogen. 18F between those of fluorinated and iodinated derivatives11. Thus, the steric effect of radiobromine might be smaller cal properties of the brominated derivatives, such as stability, molecular size, lipophilicity, and solubility, range for PET imaging because it decays with high positron abundance (55%) 15. Although the high positron energy of can be available12. However, the decay properties of 124I are not ideal for PET because the positron abundance of the short half-lives of 11C (t1/2 = 20 min), 13N (t1/2 = 10 min), and 15O (t1/2 = 2 min). Although the influence is generally smaller than radiometal incorporation, substitution with a radiohalogen could influence affinity for the molecular target, biodistribution, and metabolism of bioactive compounds13. Therefore, the choice of radionuclides and the introduced position should be optimized to develop radiotracers with a radiohalogen. 18F (t1/2 = 110 min) is frequently used as positron emission tomography (PET) imaging radionuclide. Labeling with fluorne normally requires a different method than labeling with other halogens, such as bromine and iodine because of the short half-life and distinct physical property of fluorne12. [125I] has been an interesting radionuclide for clinical and experimental PET because of its relatively longer half-life (t1/2 = 4.2 day) and chemical properties, namely the same labeling methods for [11C] and [131I], which have been frequently used in clinical nuclear medicine, can be available12. However, the decay properties of [125I] are not ideal for PET because the positron abundance is only 23%. Meanwhile, radiobromine is not used as often as radiofluorne and radiiodine isopes; however, in some cases, radiobromine shows characteristics superior to the other radiohalogens. 79Br is potentially useful for PET imaging because it decays with high positron abundance (55%)11. Although the high positron energy of 79Br (3.4 MeV) could be a disadvantage in terms of image resolution and absorbed radiation dose14, the relatively longer half-life (t1/2 = 16.1 h) of 79Br than those of 11C, 13N, 15O, and 18F could be advantageous. Values of physical properties of the brominated derivatives, such as stability, molecular size, lipophilicity, and solubility, range between those of fluorinated and iodinated derivatives11. Thus, the steric effect of radiobromine might be smaller than that of radioiodine17.

To date, several types of probes targeting PDGFRβ have been developed for cancer imaging in nuclear medicine. Askoxyλakis et al. reported a [125I]-labeled dodecapeptide targeting PDGFRβ with an inhibitory concentration 50 (IC50) value of 1.4 μM15. Tolmachev et al. reported PDGFRβ specific affibodies labeled with [111In or 68Ga, which exhibited a high tumor-to-blood ratio and good IC50. The labeled affibodies clearly visualized PDGFRβ-expressing U-87 MG xenografts in mice17. The peptide and affibodies bind to the extracellular part of PDGFRβ. Contrastingly, intracellular domains, especially adenosine triphosphate (ATP)-binding sites, could also be a promising target for PDGFRβ imaging. Radiolabeled tyrosine kinase inhibitors (TKIs), such as the [11C]-labeled imatinib16, [18F]-labeled dasatinib17, [11C]-labeled sunitinib (tumor uptake in RXF393 xenograft mice about 2.52 ± 0.33%ID/g)18, and [125I]-labeled sunitinib19, have been synthesized and evaluated in order to develop probes targeting the ATP-binding site in nuclear medicine. However, not only do they all bind PDGFRs, but also bind other TKs, such as vascular endothelial growth factor receptors 2 (VEGFR2), BCR-ABL1, and c-KIT. Recently, we synthesized and evaluated radiiodinated 1 derivatives, [125I]-[5-iodo-2-[(2-methoxyethoxy)-1H-benzo[d]imidazol-1-yl]quinolin-8-yl]piperidin-4-amine ([125I]4) and [125I]N-3-iodobenzoyl-1-[2-[(2-(methoxyethoxy)-1H-benzo[d]imidazol-1-yl]quinolin-8-yl]-piperidin-4-amine ([125I]5) (Fig. 1), as PDGFRβ imaging probes20. In both in vitro and in vivo experiments, [125I]4 showed a significantly higher tumor uptake with high PDGFRβ expression than [125I]5.

In this study, we describe the development of novel radiobrominated probes for PDGFRβ imaging. The strategy of these probes are similar to that of our previous developed radiodiode-labeled probes21, but difference between iodine and bromine should alter their characteristics and biodistribution of the probes22–24, and the difference of radionuclides may give much impact in clinical nuclear medicine. Two brominated 1 derivatives, 1-[5-bromo-2-[(2-methoxyethoxy)-1H-benzo[d]imidazol-1-yl]quinolin-8-yl]-piperidin-4-amine (2) and N-3-bromobenzoyl-1-[2-[(2-methoxyethoxy)-1H-benzo[d]imidazol-1-yl]quinolin-8-yl]-piperidin-4-amine (3), were designed and synthesized. Bromine was incorporated into 1 instead of iodine in 4 and 5, and their affini-eties for PDGFRβ were examined. Although we are interested in developing 79Br-labeled PDGFRβ PET imaging

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**Figure 1.** Chemical structures of 1, [77Br]2, [79Br]3, [125I]4, [125I]5.
probes, $^{77}$Br was used in this initial study because it has a relatively longer half-life ($t_{1/2} = 57$ h) and using a different radioisotope of the same element in the chemical compound does not alter its overall biological profile. Radiobrominated compounds, $[^{77}$Br]$^2$ and $[^{77}$Br]$^3$ (Fig. 1), were synthesized and the in vitro and in vivo experiments were performed.

**Results**

**Synthesis of the reference compounds and their precursors.** The nonradioactive brominated reference compounds, 2 and 3, were synthesized as described in Figs 2 and 3. Compound 2 was synthesized by direct bromination at C-5 position of the quinoline group of 1 using N-bromosuccinimide (NBS) (Fig. 2). Compound 3 was obtained by acylation of the amino group of 1 using SBrB (Fig. 3). Tributyltin precursors (6 and 7) were synthesized as described previously$^{41}$.

**Cell viability assays.** The binding affinity of 2 and 3 to the ATP-binding site in PDGFR3 was evaluated using PDGFR3 overexpressed TR-PCT1 cells. Cells were treated with 1–1000 nM of synthesized ligands, 1, 2, or 3. As seen in Fig. 4, 3 showed the similar effects compared to 1. 2 was more effective in decreasing the viability of TR-PCT1 cells than 1.

**Radiolabeling.** Radiobrominated compounds, $[^{77}$Br]$^2$ and $[^{77}$Br]$^3$, were prepared in the condition without carrier addition using a bromodesannylation reaction of the corresponding tributyltin precursors (6 and 7) with high radiochemical yield (95 and 83%, respectively) (Figs 2 and 3). The effective molar activity of $[^{77}$Br]$^2$ and $[^{77}$Br]$^3$ was estimated to be approximately $2.0 \times 10^{12}$ MBq/mol because the labeling used non-carrier-added condition, and the labeled compounds $[^{77}$Br]$^2$ and $[^{77}$Br]$^3$, and the precursors (6 and 7) were completely separated using RP-HPLC. N-chlorosuccinimide (NCS) was used as an oxidizing agent in these syntheses. The
βPDGFR. Although the results of the cell viability assays showed the decrease of uptakes of [77Br] with [125I] in BxPC3-luc cells. The uptake of [77Br] in BxPC3-luc cells was higher than that of [125I] in MCF7 cells. The results demonstrated that the accumulation of coincubated [77Br] and [125I] in BxPC3-luc cells was higher than that in MCF7 cells. The biodistribution of [77Br] in ddY mice was summarized in Table 1. Table 2 lists the biodistribution of [77Br] and [125I] in ddY mice. High radioactivity in the liver, small intestine, and large intestine was observed. At 24 h postinjection of the radiobrominated and radioiodinated compounds, radioactivity in the feces was much higher than that in urine, suggesting hepatobiliary excretion as the main excretion pathway for both radiotracers. In this study, to minimize both the number of mice consumed in the experiment and the potential for experimental errors, we co-injected radiobrominated and radioiodinated compounds into mice. The biodistribution of [77Br]2 and [125I]4 in ddY mice was summarized in Table 1. Table 2 lists the biodistribution of [77Br]3 and [125I]5 in ddY mice. High radioactivity in the liver, small intestine, and large intestine was observed. At 24 h postinjection of the radiobrominated and radioiodinated compounds, radioactivity in the feces was much higher than that in urine, suggesting hepatobiliary excretion as the main excretion pathway for both radiotracers.

We investigated the biodistribution of the radiotracers in BxPC3-luc tumor-bearing mice by co-injecting [77Br]2 with [125I]4 (Table 3), and [77Br]3 with [125I]5 (Table 4). Accumulation of [77Br]2 in the tumor at 1 h after administration was 1.61% injected dose (ID)/g, which was significantly higher than that of [77Br]3 (1.15% ID/g). Figure 7 displays the blocking effect of the pretreatment with 1 on the tumor accumulation of [77Br]2 at 1 h postinjection. The tumor uptake of both the control and blocking groups was 1.61 ± 0.24 and 0.96 ± 0.20% ID/g, respectively. Thus, the figures are shown as not only % ID/g but also as tumor-to-blood ratio. The pretreatment with 1 significantly reduced the tumor uptake and the tumor-to-blood ratio of [77Br]2 at 1 h postinjection.

Discussion
In our study, we performed cell viability assays of 1, 2, and 3, to evaluate the effect of the structural changes on the affinity between the ligand and the molecular target, PDGFRβ. Similar affinity was exhibited by 1 and 3, whereas 2 displayed a higher affinity than 1 (Fig. 4). The larger size of the bromine compared with hydrogen may have contributed to this result. In accordance with 4, incorporating bromine into 1 could increase the affinity of 2 for PDGFRβ. Although the results of the cell viability assays showed the in vitro affinity of the iodinated compound 4.
was higher than that of the brominated compound 2, the competitive binding assay using BxPC3-luc cells showed the affinity of 2 was comparable to that of 4.

The comparison of chloramine-T, peracetic acid, and NCS in this study showed NCS was the best oxidizing agent for the bromination of 1 through an oxidative bromodestannylation reaction under non-carrier added condition (data not shown). When NCS was used, undesired radioactive peaks had almost disappeared. Previously, we reported the preparation of a [77Br]-labeled sigma-1 receptor ligand, (+)-[77Br]pBrV, by using oxidative bromodestannylation with chloramine-T; its radiochemical yield was 53%30. Hanaoka et al. performed [77Br] labeling of α-methyl-phenylalanine by bromodestannylation reaction with NCS, and its radiochemical yield was approximately 60%32. In these reports, using the same corresponding precursors, the radiochemical yields for radiobromine labeling were lower than those for radioiodine labeling. In this study, we obtained [77Br]2 and [77Br]3 with prominently high radiochemical yields (95% and 83%, respectively), and the radiochemical yields were comparable to those of the corresponding radioiodinated compounds at 95% for [125I]4 and 85% for [125I]524.

In cellular uptake experiments for [77Br]2 and [77Br]3, both radiolabeled compounds more highly accumulated in BxPC3-luc and MCF7 cells than in MCF7 cells, and [77Br]2 showed higher accumulation in BxPC3-luc cells than [77Br]3 (Fig. 5). This result was consistent with the cell viability assay, in which 2 showed a higher affinity for PDGFRβ than 1 and 3. This result also agreed with the in vivo experiment in which [77Br]2 showed higher accumulation in the BxPC3-luc tumor than [77Br]3 (Table 2). The difference in the lipophilicity may be an important factor that contributed to this result. Moreover, the excess amount of PDGFRβ ligand can reduce [77Br]2 uptake in PDGFRβ-positive tumor cells (Fig. 6) and in the in vivo blocking experiments using tumor-bearing mice (Fig. 7). [77Br]2 uptake in tumors should be PDGFRβ specific, and [77Br]2 should bind to the ATP-binding site of PDGFRβ in the tumor cell.

Although free iodide ions generated by the deiodination of the radioiodine-labeled compounds highly accumulate in the stomach and thyroid. However, the biodistribution of free bromide ions is much different. Because

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**Figure 5.** Cellular uptake study. Time-dependent accumulation of (a) [77Br]2 and [125I]4 (b) [77Br]3 and [125I]5 in BxPC3-luc and MCF7 cells. Data were presented as mean ± SD for three samples.
the free bromide ions accumulate in blood and are retained for a long time\cite{32,33}, the radioactivity in the blood can be used as an *in vivo* stability index for radiobromine-labeled compounds. As summarized in Tables 1 and 2, $[^{77}\text{Br}]_2$ and $[^{77}\text{Br}]_3$ were stable *in vivo* because the blood clearances of both radiotracers were rapid. However, compared with $[^{77}\text{Br}]_2$, $[^{77}\text{Br}]_3$ may have a slightly lower *in vivo* stability because the radioactivity in blood after the $[^{77}\text{Br}]_3$ injection was slightly higher than that after the $[^{125}\text{I}]_5$ injection in double-tracers biodistribution experiments. In contrast, radioactivity in the blood after the $[^{77}\text{Br}]_2$ injection was almost the same as that after the $[^{125}\text{I}]_4$ injection.

The present data, obtained from tumor bearing mice (Tables 3 and 4), showed that the tumor accumulations of both radiobrominated compounds, $[^{77}\text{Br}]_2$ and $[^{77}\text{Br}]_3$, are greater than those of corresponding radioiodinated compounds, $[^{125}\text{I}]_4$ and $[^{125}\text{I}]_5$. Lower lipophilicity and/or smaller molecule size of the radiobrominated compounds compared with the corresponding radioiodinated compounds might contribute to these results. The tumor-to-blood ratio of radioactivity at 1 h postinjection was 2.8 for $[^{77}\text{Br}]_2$ and 1.9 for $[^{125}\text{I}]_4$, indicating that the radiobrominated 1 derivatives are more promising than radioiodinated 1 derivatives. However, the tumor uptake of a radiobrominated compound was not high enough as an appropriate probe for PDGFR\(\beta\) imaging, and further modification is still needed.

In conclusion, $[^{77}\text{Br}]_2$ and $[^{77}\text{Br}]_3$ were easily prepared using a bromodestannylation reaction without carrier addition in excellent radiochemical yields and high radiochemical purities. Furthermore, $[^{77}\text{Br}]$ could be incorporated into 1 instead of $[^{77}\text{Br}]$. Although this study suggests that radiobrominated 2 has more promising property for PET imaging of PDGFR\(\beta\) than radioiodinated 4, in clinical application of the radiobrominated compound as a PDGFR\(\beta\)-targeted PET imaging agent, structural modification would be required to improve tumor uptake and tumor-to-background ratios.

**Methods**

**Materials.** Commercially available reagents and solvents were purchased from Nacalai Tesque, Inc., (Kyoto, Japan), Wako Pure Chemical Industries (Osaka, Japan), Tokyo Chemical Industry Co., Ltd., (Tokyo, Japan), Sigma-Aldrich (St. Louis, MO, USA), and Kanto Chemical, Co., Inc. (Tokyo, Japan) and used without further purification.
The radioactivity was determined by a gamma counter (AccuFLEX γ ARC-8001 Hitachi, Ltd., Tokyo, Japan). Bicinchoninic Acid (BCA) Protein Assay Kit was purchased from Nacalai Tesque. Cell Counting Kit-8 (CCK-8) was purchased from Dojindo (Kumamoto, Japan). 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 generously obtained from Dr. Emi Nakashima (Keio University, Tokyo, Japan)\textsuperscript{34}. BxPC3-luc cell line was obtained from JCRB Cell Bank (Ibaraki, Japan). MCF7 cell line was purchased from DS Pharma Biomedical (Osaka, Japan).

Proton and carbon nuclear magnetic resonance (\(^{1}\)H-NMR and \(^{13}\)C-NMR) spectra were recorded on JEOL JNM-ECS400 (JEOL Ltd, Tokyo, Japan). Pictures of the NMR spectra are available in the Supplementary Material file. 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 performed using HPLC system (LC-20AD pump, SPD-20A UV detector, and CTO-20A column oven, and DGU-20A5R degasser, SHIMADZU, Kyoto, Japan). TLC analyses were performed with silica plates (Art 5553, Merck, Darmstadt, Germany). Optical density in WST-8 assay was determined using Infinite® F200 Pro microplate reader (TECAN, Männedorf, Switzerland).

**Synthesis of reference compounds and precursors.** Intermediate, reference compounds, 1, and corresponding tin precursors were synthesized according to the reported studies, with a slight modification\textsuperscript{24}.

\begin{align*}
\text{1-} & \{5\text{-Bromo-2-[5-(2-methoxyethoxy)-1H-} \\
& \text{benzo[d]imidazo-1-yl}\}\text{quinolin-8-yl} \text{piperidin-4-amine (2).} \\
\end{align*}

\text{1} (144 mg, 0.4 mmol, 1.0 eq.) was dissolved in acetic acid (5 mL). To the solution, NBS (72 mg, 0.4 mmol, 1.0 eq.) was added in one portion and the mixture was stirred overnight at room temperature. pH was adjusted to 9.0 with saturated aqueous NaHCO\(_3\) and the mixture was extracted using

\begin{table}
| Tissues          | Time after injection |
|------------------|----------------------|
|                  | 10 min   | 1 h       | 4 h       | 24 h      |
| \([\text{\textsuperscript{77}}\text{Br}]2\) |          |           |           |           |
| Blood            | 0.99 (0.03) | 0.28 (0.06) | 0.20 (0.03) | 0.12 (0.02) |
| Liver            | 12.49 (0.64) | 4.26 (1.37) | 3.44 (0.98) | 0.29 (0.08) |
| Kidney           | 15.61 (1.32) | 10.12 (3.49) | 4.33 (0.75) | 0.21 (0.05) |
| Small intestine  | 8.58 (2.18) | 29.51 (7.92) | 4.76 (0.89) | 0.07 (0.01) |
| Large intestine  | 0.99 (0.10) | 2.69 (0.65) | 53.70 (8.89) | 0.18 (0.03) |
| Spleen           | 5.39 (0.40) | 1.59 (0.28) | 0.69 (0.24) | 0.66 (0.77) |
| Pancreas         | 4.18 (0.38) | 2.98 (0.75) | 1.63 (0.19) | 0.41 (0.34) |
| Lung             | 10.92 (0.55) | 4.32 (0.84) | 2.71 (1.06) | 0.29 (0.05) |
| Heart            | 4.41 (0.27) | 1.15 (0.17) | 0.58 (0.13) | 0.29 (0.03) |
| Stomach\textsuperscript{3} | 1.74 (0.15) | 1.62 (0.55) | 0.81 (0.21) | 0.11 (0.01) |
| Bone             | 2.81 (1.06) | 1.15 (0.15) | 1.50 (1.27) | 0.55 (0.10) |
| Muscle           | 2.24 (0.18) | 0.92 (0.04) | 0.44 (0.07) | 0.22 (0.02) |
| Brain            | 0.12 (0.01) | 0.10 (0.01) | 0.09 (0.00) | 0.07 (0.00) |
| Urine            | 2.82 (0.19) |           |           |           |

\begin{table}
| Tissues          | Time after injection |
|------------------|----------------------|
|                  | 10 min   | 1 h       | 4 h       | 24 h      |
| \([\text{\textsuperscript{125}}\text{I}]4\) |          |           |           |           |
| Blood            | 1.15 (0.08)\textsuperscript{2} | 0.32 (0.09) | 0.19 (0.05) | 0.03 (0.00)\textsuperscript{1} |
| Liver            | 11.59 (0.69) | 3.72 (1.18) | 3.45 (1.02) | 0.29 (0.08) |
| Kidney           | 14.44 (1.31) | 8.18 (2.71) | 4.28 (0.68) | 0.13 (0.03) |
| Small intestine  | 9.32 (2.30) | 25.44 (6.86) | 4.98 (0.89) | 0.04 (0.00)\textsuperscript{2} |
| Large intestine  | 0.80 (0.07)\textsuperscript{3} | 2.16 (0.54) | 51.43 (8.57) | 0.14 (0.02) |
| Spleen           | 5.08 (0.56) | 1.67 (0.41) | 0.43 (0.11) | 0.11 (0.09)\textsuperscript{2} |
| Pancreas         | 3.52 (0.33)\textsuperscript{4} | 2.49 (0.63) | 1.27 (0.11)\textsuperscript{2} | 0.07 (0.02) |
| Lung             | 9.64 (0.66)\textsuperscript{5} | 3.88 (0.72) | 2.18 (0.55) | 0.15 (0.08)\textsuperscript{2} |
| Heart            | 4.27 (0.27) | 1.23 (0.49) | 0.31 (0.05)\textsuperscript{3} | 0.10 (0.09)\textsuperscript{2} |
| Stomach\textsuperscript{5} | 1.66 (0.11) | 1.76 (0.58) | 0.85 (0.21) | 0.05 (0.00)\textsuperscript{2} |
| Bone             | 1.53 (0.14) | 1.22 (0.29) | 0.32 (0.08) | 0.15 (0.05)\textsuperscript{2} |
| Muscle           | 1.92 (0.18) | 0.78 (0.11) | 0.23 (0.06)\textsuperscript{2} | 0.04 (0.01)\textsuperscript{2} |
| Brain            | 0.07 (0.00)\textsuperscript{6} | 0.15 (0.04) | 0.03 (0.00)\textsuperscript{8} | 0.02 (0.00)\textsuperscript{6} |
| Urine            | 2.51 (0.05) |           |           |           |
| Feces            | 66.62 (1.97) |           |           |           |

Table 1. Biodistribution of radioactivity after administration co-injection of \([\text{\textsuperscript{77}}\text{Br}]2\) and \([\text{\textsuperscript{125}}\text{I}]4\) at 10 min, 1, 4, and 24 h intravenously in ddY mice. Data were presented as %injected dose/gram tissue. Each value represent mean ± SD for four mice. Significance was determined using an unpaired Student's \(t\)-test (\(p < 0.05, \* \(p < 0.01\) vs. \([\text{\textsuperscript{125}}\text{I}]4\)). \textsuperscript{1}presented as %injected dose /organ. \textsuperscript{2}presented as %injected dose /organ.
| Tissues    | Time after injection | 10 min | 1 h  | 4 h  | 24 h |
|------------|----------------------|--------|------|------|------|
| ([125I]5)  | Blood                | 0.94 (0.14)** | 0.26 (0.05)** | 0.08 (0.01)** | 0.01 (0.00)*** |
|            | Liver                | 16.37 (2.05)** | 3.32 (0.49)** | 1.21 (0.96) | 0.06 (0.00)*** |
|            | Kidney               | 5.23 (0.65) | 1.57 (0.42) | 0.36 (0.10)** | 0.05 (0.00)**** |
|            | Small intestine      | 9.59 (1.71)** | 24.03 (1.45)** | 1.32 (0.66)** | 0.01 (0.00)*** |
|            | Large intestine      | 0.75 (0.06)** | 3.46 (2.18) | 18.55 (1.85)** | 0.02 (0.00)*** |
|            | Spleen               | 1.72 (0.23) | 0.70 (0.16)** | 0.27 (0.09)** | 0.02 (0.00)*** |
|            | Pancreas             | 2.41 (0.20) | 0.86 (0.07)** | 0.27 (0.07)** | 0.02 (0.00)*** |
|            | Lung                 | 1.80 (0.30)** | 1.07 (0.08) | 0.50 (0.19) | 0.01 (0.00)*** |
|            | Heart                | 1.66 (0.23)** | 0.39 (0.15)** | 0.20 (0.04)** | 0.02 (0.01)*** |
|            | Stomach              | 2.09 (1.12) | 0.78 (0.19) | 0.27 (0.10) | 0.01 (0.00)*** |
|            | Bone                 | 0.81 (0.11)** | 0.64 (0.31)**** | 0.40 (0.09)** | 0.06 (0.00)**** |
|            | Muscle               | 0.97 (0.16)** | 0.31 (0.13)** | 0.16 (0.08)** | 0.01 (0.00)*** |
|            | Brain                | 0.19 (0.03)** | 0.07 (0.03)** | 0.01 (0.00)** | 0.00 (0.00)*** |
|            | Urine                | 12.56 (0.91) |           |       |      |
|            | Feces                | 60.96 (5.80)** |           |       |      |

Table 2. Biodistribution of radioactivity after administration co-injection of [77Br]3 and [125I]5 at 10 min, 1, 4, and 24 h intravenously in ddY mice. Data were presented as %injected dose/gram tissue. Each value represent mean ± SD for four mice. Significance was determined using an unpaired Student’s t-test (*p < 0.05, **p < 0.01 vs. [125I]5). *presented as %injected dose/organ.

8-yl}-piperidin-4-amine (3).

NMR (1H, 13C, 1H-1H COSY, 1H-13C HMBC, and 1H-13C HMQC). 1H NMR (400 MHz, CDCl3): δ 1.81–1.83 (2H, m), 2.06–2.09 (2H, m), 2.87–2.93 (3H, m), 3.50 (3H, s), 3.83–3.84 (2H, m), 3.88 (2H, br d), 4.23–4.25 (2H, m), 7.12 (1H, d, J = 8.4 Hz), 7.17 (1H, dd, J = 8.8, 2.0 Hz), 7.37 (1H, d, J = 2.4 Hz), 7.71 (1H, dd, J = 8.0, 2.8 Hz), 7.78 (1H, d, J = 8.8 Hz), 8.41 (1H, d, J = 8.8 Hz), 8.68 (1H, d, J = 2.0 Hz), 8.71 (1H, dd, J = 8.8, 2.0 Hz). 13C NMR (100 MHz, CDCl3): δ 156.08, 149.44, 146.99, 145.51, 142.33, 141.36, 139.96, 130.16, 126.75, 126.69, 118.69, 114.96, 114.42, 113.99, 113.21, 103.53, 71.10, 67.78, 59.27, 51.46 (2C), 48.78, 35.96 (2C). LRMS (DART+): m/z (rel. intensity) = 496.1 (100) [M+125Br]+, 498.1 (98) [M+125Br]+.

N-3-bromobenzoyl-1-[2-(5-(2-methoxyethoxy)-1H-benzo[d]imidazol-1-yl)-quinolin-8-yl]-piperidin-4-amine (3).  A mixture of 1 (15 mg, 37.5 μmol, 1.0 eq.), N,N-diisopropylethylamine (DPEA) (10 μL, 56.2 μmol, 1.5 eq.), and N-succinimidyl-3-bromobenzoate (SBrB) (12 mg, 41.2 μmol, 1.1 eq.) in anhydrous DCM (1 mL) was stirred at 50℃ for 2 h under N2 atmosphere. Then the mixture was diluted with DCM (15 mL), washed with water (3 × 15 mL), dried over Na2SO4, filtered, and concentrated in vacuo. The residue was purified by SiO2 column chromatography (eluent: chloroform/methanol = 50/1) to afford 2 (169 mg, 85%) as a pale yellow solid. The brominated position of 2 was identified by NMR (1H, 13C, 1H-1H COSY, 1H-13C HMBC, and 1H-13C HMQC). 1H NMR (400 MHz, CDCl3): δ 1.81–1.83 (2H, m), 2.06–2.09 (2H, m), 2.87–2.93 (3H, m), 3.50 (3H, s), 3.83–3.84 (2H, m), 3.88 (2H, br d), 4.23–4.25 (2H, m), 7.12 (1H, d, J = 8.4 Hz), 7.17 (1H, dd, J = 8.8, 2.0 Hz), 7.37 (1H, d, J = 2.4 Hz), 7.71 (1H, dd, J = 8.0, 2.8 Hz), 7.78 (1H, d, J = 8.8 Hz), 8.41 (1H, d, J = 8.8 Hz), 8.68 (1H, d, J = 2.0 Hz), 8.71 (1H, dd, J = 8.8, 2.0 Hz). 13C NMR (100 MHz, CDCl3): δ 156.08, 149.44, 146.99, 145.51, 142.33, 141.36, 139.96, 130.16, 126.75, 126.69, 118.69, 114.96, 114.42, 113.99, 113.21, 103.53, 71.10, 67.78, 59.27, 51.46 (2C), 48.78, 35.96 (2C). LRMS (DART+): m/z (rel. intensity) = 496.1 (100) [M+125Br]+, 498.1 (98) [M+125Br]+.
146.96, 145.11, 142.41, 140.49, 140.32, 137.10, 133.84, 130.55, 129.94, 127.33, 126.62, 126.38, 126.30, 121.60 (2C), 118.33, 116.34, 114.19, 112.44, 102.77, 70.49, 67.25, 58.17, 51.35 (2C), 47.19, 32.09 (2C). LRMS (DART +): m/z (rel. intensity) = 600.2 (100) [M(79Br) + H]+, 602.2 (94) [M(81Br) + H]+.

**Cell viability assays.** The cell viability assay of brominated compounds, 2 and 3, was evaluated as described previously.24,27 Namely, TR-PCT1 cells were seeded on 96-well plates (5 × 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.

**Production of bromine-77.** ⁷⁷Br was produced at University of Fukui. Radiosynthesis isolation and purification of ⁷⁷Br were performed according to a previously reported method from a ⁷⁷Se(p,n)⁷⁷Br reaction on an isotopically enriched Cu⁷⁷Se coated tungsten target with 8 µA/11 MeV proton beam on a RDS Eclipse HP/RD cyclotron (Siemens, Knoxville, TN, USA).30

**Radiolabeling.** Radiotracers, [⁷⁷Br]2 and [⁷⁷Br]3, were prepared by a bromodestannylation reaction using the corresponding tin precursors (6 or 7) and NCS as an oxidizing agent. The radiolabeled compounds were purified by reversed phase (RP)-HPLC performed with a Cosmosil 5C₁₈-MS-II column (4.6 × 150 mm; Nacalai Tesque) 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 40 °C. Radiochemical yield and radiochemical purity were calculated by counting radioactivity using an auto well gamma counter.

**Table 3.** Biodistribution of radioactivity after administration co-injection of [⁷⁷Br]2 and [¹²⁵I]4 at 1 h intravenously in BxPC3-luc tumor-bearing mice. Data were presented as means ± SD of %injected dose/gram of tissue for three mice. Significance was determined using an unpaired Student’s t-test (*p < 0.05, **p < 0.01 vs. [¹²⁵I]4). *presented as %injected dose/organ.

| Tissues       | [⁷⁷Br]2 | [¹²⁵I]4 |
|---------------|--------|--------|
| Blood         | 0.58 (0.10) | 0.63 (0.05) |
| Liver         | 11.07 (1.04) | 11.82 (0.73) |
| Kidney        | 17.39 (4.03) | 15.56 (3.61) |
| Small intestine | 51.52 (9.93) | 47.01 (5.56) |
| Large intestine | 3.22 (0.40) | 2.46 (0.35) |
| Spleen        | 2.94 (0.54) | 2.76 (0.73) |
| Pancreas      | 6.61 (1.00) | 5.48 (0.93) |
| Lung          | 10.31 (1.91) | 9.14 (2.14) |
| Heart         | 1.88 (0.33) | 1.51 (0.40) |
| Stomach†      | 0.96 (0.20) | 0.97 (0.19) |
| Bone          | 1.15 (0.09) | 0.83 (0.10)* |
| Muscle        | 0.94 (0.08) | 0.79 (0.10) |
| Brain         | 0.14 (0.03) | 0.07 (0.00) |
| BxPC3-luc tumor | 1.61 (0.24) | 1.19 (0.06)* |

**Table 4.** Biodistribution of radioactivity after administration co-injection of [⁷⁷Br]3 and [¹²⁵I]5 at 1 h intravenously in BxPC3-luc tumor-bearing mice. Data were presented as means ± SD of %injected dose/gram of tissue for three mice. Significance was determined using an unpaired Student’s t-test (*p < 0.05, **p < 0.01 vs. [¹²⁵I]5). *presented as %injected dose/organ.

| Tissues       | [⁷⁷Br]3 | [¹²⁵I]5 |
|---------------|--------|--------|
| Blood         | 1.16 (0.02) | 0.23 (0.02)* |
| Liver         | 19.82 (2.48) | 4.83 (0.71)* |
| Kidney        | 5.95 (0.16) | 1.13 (0.11)* |
| Small intestine | 32.72 (3.10) | 46.95 (2.88)* |
| Large intestine | 5.53 (0.96) | 7.98 (3.79) |
| Spleen        | 2.51 (0.17) | 0.61 (0.06)* |
| Pancreas      | 2.15 (0.13) | 1.25 (0.17)* |
| Lung          | 3.02 (0.51) | 1.16 (0.23)* |
| Heart         | 1.83 (0.04) | 0.42 (0.05)* |
| Stomach†      | 0.67 (0.14) | 0.27 (0.06)* |
| Bone          | 1.32 (0.08) | 0.31 (0.04)* |
| Muscle        | 1.05 (0.18) | 0.38 (0.06)* |
| Brain         | 0.17 (0.02) | 0.05 (0.00)* |
| BxPC3-luc tumor | 1.15 (0.35) | 0.55 (0.09) |

146.96, 145.11, 142.41, 140.49, 140.32, 137.10, 133.84, 130.55, 129.94, 127.33, 126.62, 126.38, 126.30, 121.60 (2C), 118.33, 116.34, 114.19, 112.44, 102.77, 70.49, 67.25, 58.17, 51.35 (2C), 47.19, 32.09 (2C). LRMS (DART +): m/z (rel. intensity) = 600.2 (100) [M(⁷⁷Br) + H]+, 602.2 (94) [M(⁷⁸Br) + H]+.
Synthesis of $[^{77}\text{Br}]2$. A mixture of 6 (1 mg/mL, 5 µL), acetic acid (5%, 30 µL), acetonitrile (55 µL), and NCS (5 mg/mL, 10 µL) was charged into a sealed vial containing $[^{77}\text{Br}]\text{Br}^-$ (non-carrier added, 370 kBq). The mixture was heated to 60 °C for 30 min and shaken every 10 min during heating, then quenched by addition of sodium hydrogensulfite (5 mg/mL, 10 µL), and the solvent was removed by N$_2$ gassing. Trifluoroacetic acid (TFA) was added to the residue and the shaking was allowed to continue for 30 min. After removing TFA by N$_2$ gassing, the residue was mixed with the initial mobile phase of HPLC. The reaction mixture was shaken for some minutes, filtered, and purified by HPLC.

Synthesis of $[^{77}\text{Br}]3$. A mixture of 7 (1 mg/mL, 5 µL), acetic acid (5%, 15 µL), and NCS (5 mg/mL, 10 µL) was charged into a sealed vial containing $[^{77}\text{Br}]\text{Br}^-$ (non-carrier added, 370 kBq). The mixture was heated to 60 °C for 25 min and shaken every 10 min during heating and purified by HPLC.

Determination of partition coefficients. Partition coefficients of $[^{77}\text{Br}]2$ and $[^{77}\text{Br}]3$ into n-octanol and 0.1 M phosphate buffer (PB) pH 7.4 were determined using the method described previously$^{24}$. The measurement was performed in quadruplicate. The partition coefficient was determined by calculating the ratio of cpm/mL of n-octanol to that of buffer and expressed as log $P$. Radioactivity of each layer were counted by a gamma counter.

In vitro stability experiments. The stability of radiolabeled compounds, $[^{77}\text{Br}]2$ and $[^{77}\text{Br}]3$, were analyzed as described previously$^{24}$. The purities radiolabeled compounds were determined by TLC using chloroform/
by a one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test compared to the control group.

At 1 h postinjection.

kg in 15% ethanol and 85% water) 1 h before intravenous injection of 100 µL of saline solution containing [77Br]2 and [77Br]3, respectively.

Without FBS was added to wells containing 2 × 105 cells/well for 24 h in a 5% CO2 incubator at 37 °C. After removal of medium, cells were incubated in medium containing 10% FBS and antibiotics for six-well plates (2 × 105 cells/well) and for 24 h in a 5% CO2 incubator at 37 °C.

Competitive binding assay using BxPC3-luc cells. BxPC3-luc cells in medium containing 10% FBS and antibiotics were seeded on 24-well plates (50,000 cells/wells) and incubated for 24 h in a 5% CO2 incubator at 37 °C. Nine concentrations of displacing nonradiolabeled ligands (1, 2, 3) and 3]5 (ranging from 1 pM to 1 mM) in medium without FBS were incubated for 24 h in a 5% CO2 incubator at 37 °C. After washing the cells twice using 250 µL of ice-cold PBS, the unbound radioligand was removed. The cells were dissolved using 250 µL of 1 M NaOH and wells were washed with 250 µL of 1 M NaOH aqueous solution (0.5 mL).

The radioactivity of pooled basic fractions was counted by a gamma counter. A range between 16 and 71 keV was used for measuring [125I] and between 95 and 700 for [77Br]. When radioactivity of [77Br] was counted, the crossover of [125I] activity into the [77Br] channel was negligible. More than one month after the experiment, the radioactivity of [125I] was determined because at that time [77Br] has been decayed and its radioactivity was negligible. The protein in the cell was quantified by a BCA Protein Assay Kit. All data were expressed as %dose/µg protein.

For in vitro blocking experiment, inhibitor (1, 2, or 3 with final concentration 10 µM) in 1 mL of medium without FBS was added to wells containing 2 × 105 cells/well. After 10 min incubation, [77Br]2 or [125I]5 (3.7 kBq/well) in 1 mL of medium without FBS was added to each well. Radioactivity and protein concentration in the cells were determined by the same method above-mentioned.

Biodistribution experiments. Mice were intravenously injected via the tail with 100 µL of saline solution of [77Br]2 and [125I]4 or [77Br]3 and [125I]5 (74 kBq, respectively), containing 10% ethanol and 1% tween-80. The ddY mice were sacrificed at 10 min, 1, 4, and 24 h postinjection. Meanwhile, tumor-bearing mice were sacrificed at 1 h postinjection.

For in vivo blocking studies, the tumor-bearing mice were intraperitoneally injected with 200 µL of 1 (40 mg/kg in 15% ethanol and 85% water) 1 h before intravenous injection of 100 µL of saline solution containing [77Br]2 and [125I]4 or [77Br]3 and [125I]5 (74 kBq, respectively), 1% tween-80 and 10% ethanol. At 1 h postinjection of radiotracers, the mice were sacrificed.

Tissues in mice were resected and weighed. The radioactivity of the tissues was counted by a gamma counter and counts were corrected for background radiation. The data were expressed as percent injection dose per gram tissue (%ID/g).

Statistical analysis. All data were statistically analyzed using GraphPad 5.0 software (La Jolla, CA, USA) and expressed as mean ± standard deviation (SD). Significance for in vitro blocking experiments was determined by 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 by ANOVA followed by Tukey's post hoc test. IC50 values for the binding assay were calculated by nonlinear regression. Significant differences in biodistribution experiments between [77Br]2 and [77Br]3 groups were determined using unpaired Student's t-test. Significance for in vivo blocking studies between control and blocking groups were determined by unpaired Student's t-test. Results were considered statistically significant at p < 0.05.

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Author Contributions
T.T. and K.O. designed the study. N.E., K.M., D.Y., and K.O. carried out the experiments. A.M. and Ya.K. prepared 77Br. N.E., K.M. and K.O. analyzed the data. N.E., K.M. and K.O. wrote the paper. K.M., T.T., A.M., D.Y., Yo.K., K.S., Ya.K., A.O., and K.O. discussed the results and reviewed the manuscript.

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