General. ^{131}I-NaI was purchased from PerkinElmer, Inc. (MA, USA). Astatine-211 (^{211}At) for radiolabeling was produced in house. IITM (4-iodo-*N*-[4-[6-(isopropylamino)pyrimidin-4-yl]-1,3-thiazol-2-yl]-*N*-methylbenzamide), its stannyl precursor (*N*-[4-[6-(isopropylamino)pyrimidin-4-yl]-1,3-thiazol-2-yl]-*N*-methyl-4-(tributylstannyl)benzamide) for radiolabeling, and FITM (4-fluoro-*N*-[4-[6-(isopropylamino)pyrimidin-4-yl]-1,3-thiazol-2-yl]-*N*-methylbenzamide) were synthesized according to the methods previously reported by our laboratory. All chemicals were purchased from FUJIFILM Wako Pure Chemical Industries (Osaka, Japan) and Tokyo Chemical Industry (Tokyo, Japan). LiChrospher high-performance thin layer chromatography plates were purchased from Merck (Darmstadt, Germany). Radio-high-performance layer chromatography analysis was performed using a JASCO HPLC system (JASCO, Tokyo, Japan). Effluent radioactivity was measured using a NaI (TI) scintillation detector system (Universal Giken, Odawara, Japan). Radio-thin layer chromatography analysis was performed using a Marita Raytest system (Raytest Isotopenmessgeraete, Straubenhardt, Germany). Unless otherwise stated, an IGC-7 Curiemeter dose calibrator (Aloka, Tokyo, Japan) was used for radioactivity measurements.

Radiosynthesis

4-^{131}I-iodo-*N*-[4-(6-(isopropylamino)pyridine-4-yl)-1,3-thiazol-2-yl]-*N*-methylbenz

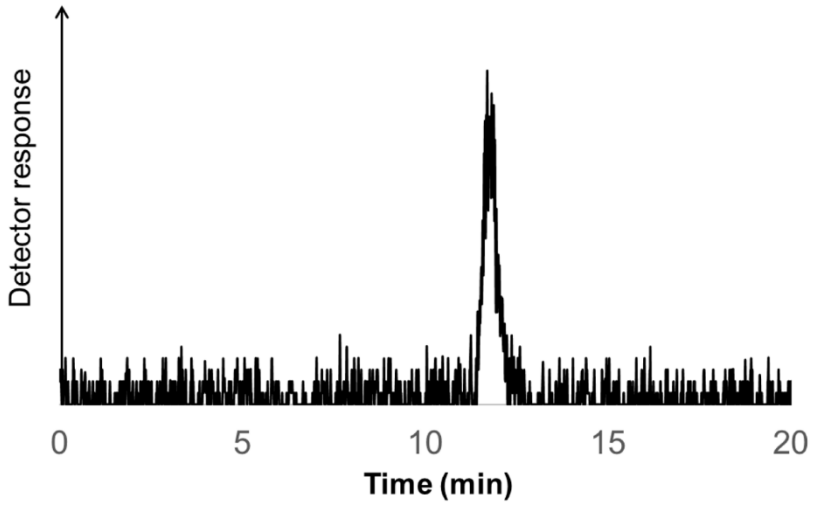
amide (^{131}I-IITM). A solution of stannyl precursor (100 μL, 1 mg/mL in ethanol) and acetic acid (20 μL) was added to ^{131}I-NaI (5 μL, 370 MBq in 0.1 mol/mL NaOH; molar activity: 260 GBq/μmol). Radiolabeling was initiated by adding 30% hydrogen peroxide (20 μL). The reaction mixture was stirred at room temperature for 2 h and an aqueous solution of $Na_2S_2O_5$ (500 μL, 1 mg/mL) was added to quench the reaction mixture. This mixture was separated by reverse-phase high-performance liquid chromatography using a CAPCELL PAK UG80 column (4.6 mm i.d. × 250 mm, 5 μm; Shiseido, Tokyo) at a flow rate of 1 mL/min with a mobile phase of 60% acetonitrile in water with 0.1% triethylamine. The radioactive fraction corresponding to ^{131}I-IITM (retention time: 12 min) was collected in a vial containing 25% ascorbic acid (100 μL), evaporated to dryness under a stream of N_2 gas, re-dissolved in a solution of saline-polysorbate 80%–25% ascorbic acid (500 μL; 400/10/90, v/v/v). ^{131}I-IITM of 71–205 MBq, as an animal-injectable solution, was obtained with a 42.7% ± 10.4% radiochemical yield (n = 9) based on the total ^{131}I-NaI. The radiochemical purity of ^{131}I-IITM was analyzed by reverse-phase HPLC using a CAPCELL PAK UG80 column (4.6 mm

i.d. × 250 mm, 5 μm) at a flow rate of 1 mL/min with a mobile phase of 60% acetonitrile in water with 0.1% triethylamine. The radiochemical purity was >99% (n = 9) (Supplemental Fig. 1).

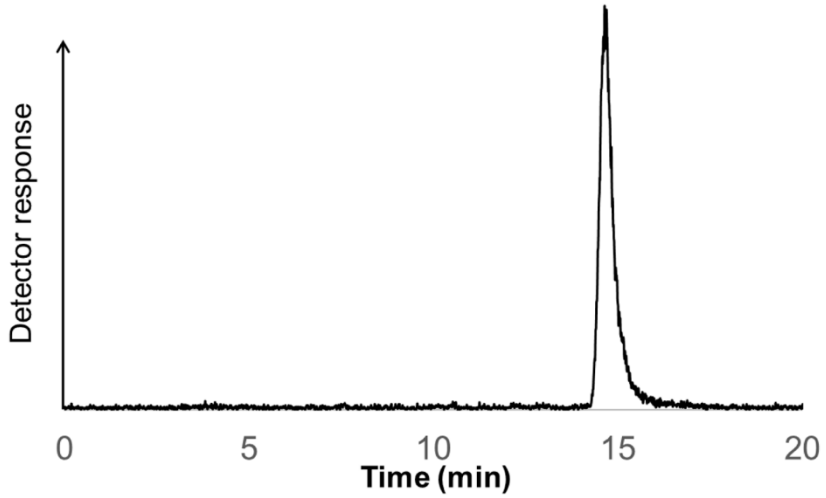
4-^{211}At-astato-*N*-[4-(6-(isopropylamino)pyridine-4-yl)-1,3-thiazol-2-yl]-*N*-methylb enzamide (^{211}At-AITM). An ^{211}At/chloroform solution (37–296 MBq) was concentrated to dryness under a stream of N_2 gas, followed by the addition of *N*-chlorosuccinimide (100 μL, 4 mg/mL, 2% acetic acid/methanol) and stannyl precursor (100 μL, 1 mg/mL in methanol) to the vessel. The reaction mixture was stirred at room temperature for 10 min. An aqueous solution of $Na_2S_2O_5$ (500 μL, 1 mg/mL) was added to quench the reaction mixture. The reaction mixture was separated by reverse-phase HPLC using a CAPCELL PAK UG80 column (4.6 mm i.d. × 250 mm, 5 μm) at a flow rate of 1 mL/min with a gradient mobile phase of 50% acetonitrile in water with 0.1% triethylamine to 100% acetonitrile with 0.1% triethylamine for 20 min. The radioactive fraction corresponding to ^{211}At-AITM (retention time: 14 min) was collected in a vial containing 25% ascorbic acid (100 μL), evaporated to dryness under a stream of N_2 gas, re-dissolved in a solution of saline-polysorbate 80%–25% ascorbic acid (500 μL; 400:10:90, v/v/v). ^{211}At-AITM of 36–118 MBq, as an animal-injectable solution, was obtained with a 45.7% ± 6.5% radiochemical yield (n =

20) based on ^{211}At at the designated time point. The radiochemical purity of ^{211}At-AITM was analyzed by reverse-phase HPLC using a CAPCELL PAK UG80 column (4.6 mm i.d. × 250 mm, 5 μm) at a flow rate of 1 mL/min with a mobile phase of acetonitrile in water with 0.1% triethylamine. The radiochemical purity was >99% (n = 20) (Supplemental Fig. 2).

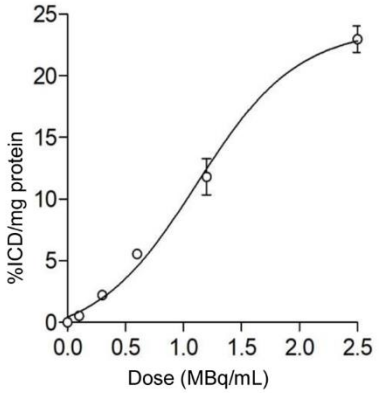
Cellular distribution of ^{211}At-AITM. B16F10 melanoma cells (5×10^4 cells) were seeded in 24-well plates and allowed to form an adherent culture overnight. The cells were incubated for 1, 2, and 6 h with 25 kBq/mL ^{211}At-AITM. At the indicated times, the medium was removed and cells were washed twice with phosphate-buffered saline. To obtain the membrane-bound radioactivity, cells were incubated with 0.05 mol/L glycine (pH 2.8) for 5 min at 4°C. Thereafter, the cells were washed twice with phosphate-buffered saline, after which they were lysed and collected as the internalized radioactivity. Radioactivity was measured using a γ-counter (PerkinElmer, Waltham, MA, USA). The distribution ratio was calculated as a percentage of the total cellular radioactivity.



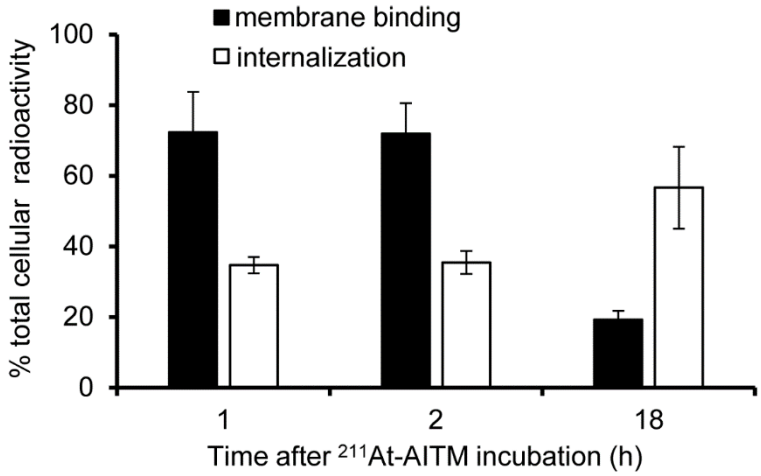
Supplemental Figure 1. Analytic HPLC chromatogram of ^{131}I-IITM



Supplemental Figure 2. Analytic HPLC chromatogram of ^{211}At-AITM



Supplemental Figure 3. Binding ability of ^{131}I-IITM to mGluR1-expressing B16F10 melanoma. A dose-dependent increase in radioactivity was observed in mGluR1-expressing melanomas.



Supplemental Figure 4. Distribution ratio of ^{211}At-AITM in B16F10 cells in vitro.

Supplemental Table 1. *Ex vivo* biodistribution data of ^{131}I-IITM at 1 h, 2 h, 6 h, 24 h, 3 d and 7 d after radioinjection in C57BL/6J mice bearing B16F10 melanomas.

Data are expressed as mean percentage of the injected radioactivity dose per gram tissue (% ID/g) ± SD, except the thyroid values which are presented as a percentage of the injected radioactivity dose (%ID) (n = 4 mice/group). S. Intestine, Small Intestine; L. Intestine, Large Intestine.

Organ	^{131}I-IITM					
	1h	2h	6h	24h	3 d	7d
Blood	1.49 ± 0.11	0.44 ± 0.04	0.03 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Tumor	4.66 ± 0.70	3.70 ± 0.70	2.60 ± 0.66	1.05 ± 0.14	0.24 ± 0.11	0.03 ± 0.02
Heart	0.72 ± 0.08	0.22 ± 0.02	0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Thymus	0.52 ± 0.08	0.15 ± 0.02	0.01± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lung	1.02 ± 0.09	0.33 ± 0.02	0.06 ± 0.05	0.01 ± 0.00	0.00 ± 0.00	0.00 ±0.00
Liver	1.78 ± 0.10	0.60 ± 0.05	0.13 ± 0.03	0.05 ± 0.01	0.01 ± 0.00	0.00 ± 0.00
Pancreas	0.70 ± 0.12	0.19 ± 0.02	0.03 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Spleen	0.46 ± 0.08	0.15 ± 0.01	0.01 ± 0.00	0.04 ± 0.07	0.00 ± 0.00	0.00 ± 0.00
Kidney	2.44 ± 0.27	0.76 ± 0.05	0.10 ± 0.02	0.03 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
Stomach	5.36 ± 2.36	1.35 ± 0.48	3.21 ± 0.75	0.04 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
S.Intestine	6.70 ± 3.26	1.84 ± 0.52	0.57 ± 0.27	0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
L.Intestine	2.08 ± 0.55	13.12 ± 7.56	4.24 ± 0.93	0.11 ± 0.02	0.01 ± 0.00	0.00 ± 0.00
Muscle	0.80 ± 0.50	0.17 ± 0.03	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Bone	0.92 ± 1.03	0.10 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Testis	0.63 ± 0.10	0.21 ± 0.06	0.02 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
Bladder	14.70 ± 8.31	1.87 ± 1.37	0.23 ± 0.21	0.01 ± 0.00	0.01 ± 0.01	0.00 ±0.00
Brain	0.64 ± 0.04	0.23 ± 0.03	0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Thyroid	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00

Supplemental Table 2. *Ex vivo* biodistribution data of ^{211}At-AITM at 1 h, 6 h and 24 h after radioinjection in C57BL/6J mice bearing B16F10 melanomas.

Organ	^{211}At-AITM		
	1h	6h	24h
Blood	4.37 ± 0.17	2.44 ± 0.16	1.01 ± 0.51
Tumor	7.68 ± 0.71	5.71 ± 0.73	2.32 ± 0.99
Heart	5.34 ± 0.60	3.37 ± 0.26	0.76 ± 0.24
Thymus	4.02 ± 0.23	1.30 ± 0.25	0.99 ± 0.48
Lung	11.98 ± 0.99	10.85 ± 1.82	2.80 ± 1.04
Liver	4.83 ± 1.02	1.60 ± 0.17	0.91 ± 0.30
Pancreas	3.03 ± 0.28	1.72 ± 0.22	0.61 ± 0.28
Spleen	6.53 ± 2.49	5.95 ± 0.44	1.35 ± 0.31
Kidney	7.08 ± 0.77	3.63 ± 0.17	1.74 ± 0.75
Stomach	20.29 ± 2.53	26.39 ± 1.32	21.14 ± 15.82
S.Intestine	5.30 ± 0.56	2.12 ± 0.35	3.26 ± 3.70
L.Intestine	2.02 ± 0.12	2.40 ± 0.76	3.70 ± 1.79
Muscle	1.33 ± 0.18	0.57 ± 0.07	0.28 ± 0.09
Bone	2.05 ± 0.09	1.60 ± 0.23	0.51 ± 0.17
Testis	4.36 ± 0.47	3.50 ± 0.40	1.59 ± 0.64
Bladder	8.35 ± 2.21	13.32 ± 7.46	4.98 ± 2.94
Brain	1.10 ± 0.06	0.39 ± 0.04	0.12 ± 0.05
Thyroid	0.12 ± 0.07	0.07 ± 0.06	0.24 ± 0.08

Data are expressed as mean percentage of the injected radioactivity dose per gram tissue (% ID/g) ± SD, except the thyroid values which are presented as a percentage of the injected radioactivity dose (%ID) (n = 4 mice/group). S. Intestine: Small Intestine; L. Intestine: Large Intestine.