Synthesis and Evaluation of Novel Radioiodinated Benzamides for Malignant Melanoma

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The imaging potential of a series of [^{123}I]benzamides was studied in mice bearing B16F0 melanoma tumors. Compound [^{123}I]**25** exhibited tumor uptake >8 %ID/g at 1 h, while that of [^{123}I]**14d** and [^{123}I]**25** reached a maximum of 9−12 %ID/g at 6 h. Standardized uptake values of [^{123}I]**14d** were higher than 100 between 24 and 72 h after injection. In haloperidol treated animals, the tumor uptake of [^{123}I]**14d** was not significantly different to controls, while significant reduction of [^{123}I]**25** uptake was observed, supporting that [^{123}I]**14d** uptake relates to melanin interaction, whereas part of the mechanism of [^{123}I]**25** uptake is related to its σ_1-receptor affinity. Benzamides **14d** and **25**, which display rapid and high tumor uptake, appear to be promising imaging agents for melanoma detection, while **14d**, which displays a long lasting and high melanoma/nontarget ratio, is more suitable for evaluation as a potential radiotherapeutic.

Introduction

Malignant melanoma is a very aggressive cancer, with a high rate of metastasis brought about by excessive UV exposure. Despite the increasing incidence of this disease and compared to advances in other areas of cancer, there are still no effective treatments available. Radiopharmaceuticals that can target the random metastatic dissemination of melanoma tumors may also offer opportunities for imaging and staging the disease as well as potential radiotherapeutic applications.[1]

Several biochemical targeting systems incorporating a variety of diagnostic and therapeutic radionuclides have been investigated as potential imaging and radiotherapeutic agents, including monoclonal antibodies,[2] iodothiouracils,[3] melanocortin-1 receptor targeting peptides,[1,4] iodoquinolines,[5] methylene blue dye,[6] and iodobenzamides.[7]

A key feature of melanoma tumors is the extensive pigmentation present in most melanoma tumors cells, thus making it a very attractive target for both diagnosis and treatment. To date there has been a considerable number of iodinated benzamide derivatives exhibiting good uptake in melanoma tissue.[7] Preclinical investigations with a number of melanin targeting radiopharmaceuticals demonstrated selective uptake in melanoma tumor bearing mice.[8,9] It was also shown that the uptake of the radioiodinated benzamides *N*-(2-diethylaminoethyl)-4-[^{123}I]iodobenzamide) ([^{123}I]BZA[a]) and the ortho derivative ([^{123}I]BZA$_2$) in cells was dependent on the melanin content[10] and not on a receptor-based mechanism, as demonstrated by similar compounds such as IBP, which were sigma-receptor related[11] (Figure 1).

The radioiodinated benzamides [^{123}I]BZA[12] and [^{123}I]BZA$_2$[13,14] have been prepared and evaluated in melanoma patients, resulting in excellent detection of melanoma and its metastases with high sensitivity and selectivity. These studies have confirmed the efficacy of radioiodinated benzamides as selective imaging agents in patients with cutaneous and ocular melanoma based on the selective high affinity binding to melanin containing melanocytes. These findings have provided the basis for further developments in melanin-based radiopharmaceuticals.

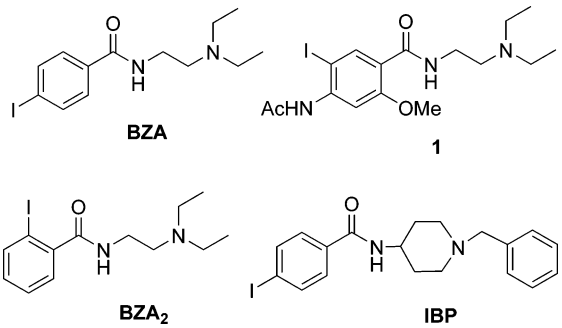

Figure 1. Structures of the known benzamides BZA, BZA$_2$, **1**, and IBP.

In the current work, our aim was to design and optimize a series of iodinated benzamides that could display high melanoma tumor uptake and rapid clearance from the body suitable for scintigraphy and radiotherapeutic applications. This optimization program involved the incorporation of a variety of alkyl- or piperidinyl side chains to a series of iodinated benzamides. The strategy in this work was to simultaneously take advantage of the optimized lipophilic side chains developed previously[14,15] and the optimized substitution pattern on the aromatic ring.[16] To further enhance the tumor uptake and retention of the radiotracers through sigma binding, substitution with piperidine as in IBP,[11] was also examined. Here we report the synthesis of a series of [^{123}I]iodobenzamides and their biological evaluation in melanoma tumor bearing mice.

Chemistry. Access to the alkyl amino benzamides was via condensation of a benzoic acid derivative with an appropriate amine. The butyldiamines **5a**, **5b**, **5d**, and **5e** were produced by alkylation of a variety of secondary amines (**2a**, **2b**, **2d**, and **2e**) with 4-bromobutyronitrile (**3**) using K_2CO_3 in 1-butanol followed by reduction of the resulting nitriles (**4a**, **4b**, **4d**, and **4e**) with lithium aluminum hydride (Scheme 1). Butyldiamine **5c** was prepared by literature methods.[17]

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[a] Abbreviations: SUV, standardized uptake value; BZA, *N*-(2-diethylaminoethyl)-4-iodobenzamide; BZA$_2$, *N*-(2-diethylaminoethyl)-2-iodobenzamide; IBP, *N*-(1-benzylpiperidin-4-yl)-4-iodobenzamide; EDC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; HOBT, *N*-hydroxybenzotriazole; NMM, *N*-methylmorpholine; CAT, chloramine-T, that is, [sodium chloro(tosyl)amide)]; RP-HPLC, reverse phase high pressure liquid chromatography; DTG, 1,3-di-*o*-tolylguanidine; SIMS, secondary ion mass spectroscopy; SPECT, single photon emission computed tomography.

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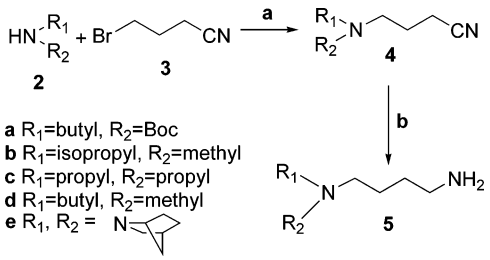
Scheme 1. Chemical Synthesis of Butyldiamines[a]



[a] Reagents and conditions: (a) K_2CO_3, KI, 1-butanol, reflux 20 h; (b) $LiAlH_4$, ether, 0 °C, 1 h room temperature.

The 4-acetamido-2-methoxybenzamide **7**, prepared by acetylation of the amine **6**[18] with acetic anhydride (Scheme 2), not only served as a lead fragment with a precedence in tumor targeting, but was also amenable to iodination in the 5 position.[16] The corresponding iodinated derivatives were synthesized by treatment with iodine monochloride at 50 °C in acetic acid. Ester hydrolysis of **7** and its iodinated analogue **8** under basic conditions also resulted in deacetylation to give the free amines **9** and **10**. Subsequent reacetylation of the amine with acetic anyhydride produced the required benzoic acids **11** and **12**.

The benzoic acids **11** and **12** were condensed with amines **5a**−**5e** via an acid chloride intermediate, prepared from thionyl chloride, to form the benzamides **13a**−**13e** and **14a**−**14e** (Scheme 2). The known compound **1** was synthesized by the same method using the commercially available diethylaminoethylamine. Benzamides **13a** and **14a** were treated with saturated HCl in ethyl acetate at room temperature to give the benzamides **13f** and **14f**. The 4-mesylamino and 4-tosylamino analogues, **18m**,**n** and **19m**,**n**, were provided by first, deacetylating **1** with KOH in methanol and second, treating the deprotected amine with methanesulphonyl chloride or *p*-toluenesulphonyl chloride with pyridine in dichloromethane.

The synthesis of the conformationally restricted IBP analogous benzamides began with an alkylation of 4-BOC-aminopiperidine with either 1-bromo-2-fluoroethane or bromoethanol (Scheme 3). Subsequent BOC-deprotection of the resulting alkylpiperidines **20** and **21** with neat TFA gave amines **22** and **23** that were suitable for condensation with the appropriate 4-halobenzoic acids, using EDC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide) and HOBT (*N*-hydroxybenzotriazole) with NMM (*N*-methylmorpholine) in DMF, to give benzamides **24**, **25**, **26**, and **27**.

The condensation of 4-halobenzoic acids with amines **5c** and **5e**, gave the known benzamide **30**[15] and its analogues **31**, **33**, and **34**. The bromobenzamides, **24**, **26**, **31**, and **34**, were treated with hexamethylditin and a catalytic amount of $Pd(PPh_3)_4$ in refluxing toluene or dioxane to yield the stannyl derivatives **28**, **29**, **32**, and **35** to be used for electrophilic radioiodination.

Radiochemistry. The benzamides were radioiodinated with no carrier added [^{123}I]iodine using two electrophilic substitution methods, as shown in Scheme 4. Compounds **13b**−**13f**, **15**, and **18m**,**n** were regioselectively radioiodinated with ^{123}I via the corresponding thallium bis trifluoroacetate intermediate,[16] by first treating the benzamide with $Tl(TFA)_3$ in acetic acid followed by reaction with [^{123}I]INa to give the corresponding radioiodinated products in 40−55% radiochemical yields (Table 1). The trialkyl stannane derivatives **28**, **29**, **32**, and **35** were labeled with ^{123}I via standard electrophilic iododestannylation reactions in the presence of chloramine-T (CAT) as the oxidant to afford the corresponding [^{123}I]iodobenzamides in 50−95% radiochemical yields. After purification of the radiolabeled benzamides, by semipreparative C18 RP-HPLC, the radiochemical purity of the [^{123}I]iodobenzamides, as assessed by analytical RP-HPLC, was >95% and the specific activity was >2 GBq/nmol.

Lipophilicity Values. The lipophilicity values of the iodobenzamides were determined by RP-HPLC in buffered solutions at pH 7.5 as a representation of physiological conditions. The results are summarized in Table 2. The $logP_{7.5}$ values of the studied benzamides are in the range of 0.75−2.6. The 2-methoxy-5-iodobenzamides **1**, **14b**, **14c**, **14d**, **14e**, **14f**, **19m**, and **19n** exhibited logP values between 0.8 and 1.95 depending on the length of the *N*-alkyl chains and the nature of the amino function on the benzene ring. Generally, with identical *N*-alkyl chains, the 4-iodobenzamides, **25**, **27**, **30**, and **33**, displayed higher logP values than the corresponding 2-methoxy-5-iodobenzamides, illustrated by **30** and **14c**.

Receptor Binding Studies. The "benzamide"-type structure of the compounds described here have biological activity that may result from a number of competing mechanisms, that is, melanin uptake (cf. BZA_2) or affinity to the σ-receptor (cf. IBP), which is highly expressed in melanoma tumors. The potency of unlabeled benzamides to inhibit the specific binding of [^{3}H]-(+)-pentazocine to σ_1-receptors and of [^{3}H]DTG to σ_2-receptors from guinea pig brain membranes were determined in competitive binding assays at 10^{-5} M concentration of unlabeled iodobenzamides. For compounds able to inhibit more than 90% of the tritiated ligands, the IC_{50} values were determined in full competition experiments. The K_i values are reported in Table 2. High to moderate affinities for σ_1-receptors were obtained with the 4-piperidines **25** and **27**, which are structural analogs of IBP, with K_i values of 6 and 140 nM, respectively, and with **33**, with $K_i = 32$ nM. Compounds **1**, **14b**, **14c**, **14d**, **14e**, **14f**, **19m**, **19n**, and **30** exhibited K_i values higher than 600 nM. For σ_2-receptors, only **33** presented a moderate affinity with $K_i = 75$ nM.

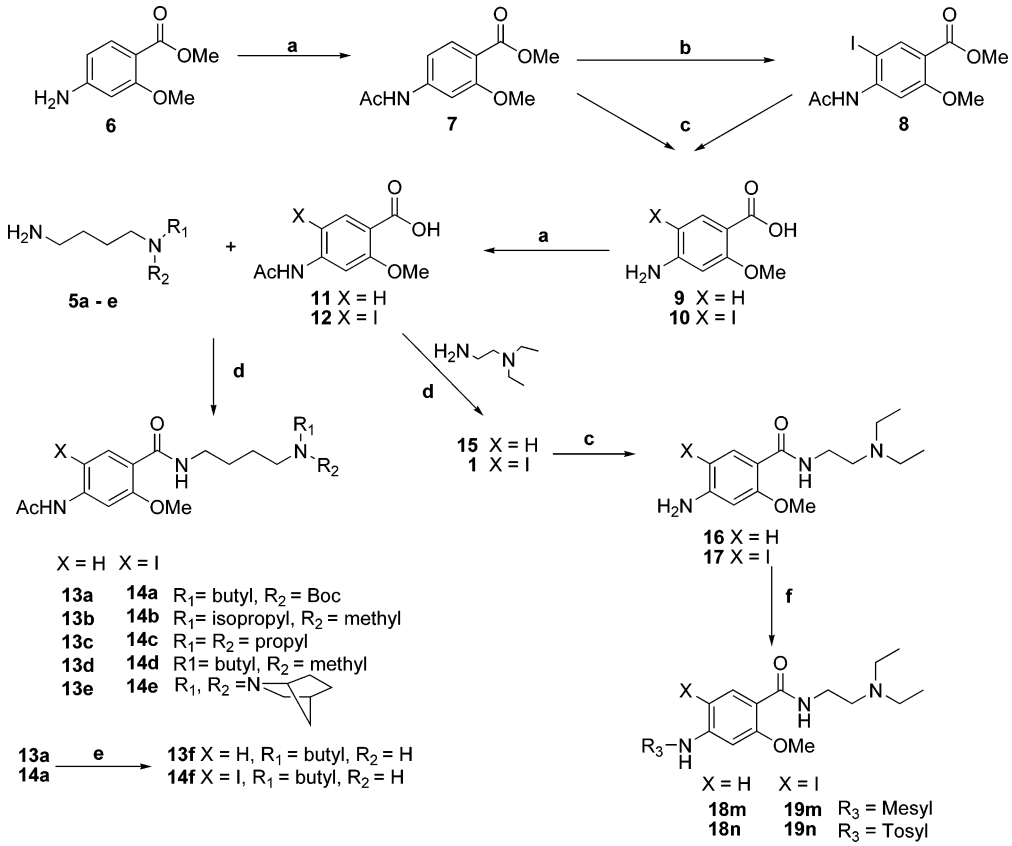
Biodistribution. The [^{123}I]iodobenzamides were injected in melanoma tumor bearing mice to evaluate their potential as tumor markers. Postinjection points at 1, 6, and 24 h were chosen for determination of the distribution of each compound in various organs and tissues. For those benzamides with high uptake in the tumor, 3, 48, and 72 h time points were added to determine long-term retention.

To visualize melanoma tumors, a potential radiotracer needs to meet two criteria: high uptake in the tumor and simultaneous high contrast to other tissues. A comparison between organ distribution and body clearance was made for all the [^{123}I]-iodobenzamides studied.

In the liver, compounds **30** and **33** displayed the highest uptake (40−45 %ID) at 1 h, which cleared with a 6 h biological half-life. Liver uptake of **14d**, **27**, and **25** was lower (15−20 %ID), with a faster clearance (biological half-life: 1−1.5 h). At that time, for all other [^{123}I]iodobenzamides, the liver uptake was between 2 and 5 %ID, comparable to the value obtained with [^{123}I]BZA_2. In the kidney, the highest concentrations were found at 1 h for **14c**, **14d**, and **33** (3−5 %ID), followed by **14b**, **30**, and **25** (1.2−2 %ID), which were similar to [^{123}I]BZA_2. From this organ, **14d** cleared quickly, with a biological half-life of less than 1 h, while other compounds had half-life clearances ranging from 1.5 to 2 h for methoxy-5-iodobenzamides **1**, **14b**, **14c**, **14e**, **14f**, **19m**, and **19n** to 3−5 h for the 4-iodobenzamides, **25**, **27**, **30**, and **33**.

In the intestine, higher amounts of radioactivity were observed at 1 h for the 2-methoxy-5-iodobenzamides **1**, **14b**, **14c**, **14d**, **14e**, **14f**, **19m**, and **19n** (30−40 %ID) than that for the 4-iodobenzamides, **25**, **27**, **30**, **33**, and BZA_2 (7−15 %ID). However, at 24 h postinjection, the radioactivity of both the

Scheme 2. Synthesis of Iodobenzamides and Precursors for Radiolabeling[a]



[a] Reagents and conditions: (a) acetic anhydride, 50 °C, 2 h; (b) ICl, acetic acid, 50 °C, 6 h, room temperature, 24 h; (c) KOH, methanol, reflux, 2−18 h; (d) SO_2Cl, THF, 4 h, addition of amine, room temperature, 4 h; (e) 3 M HCl in ethyl acetate, 45 min; (f) mesyl chloride or tosyl chloride, pyridine, DCM, 0 °C, 30 min, room temperature, 24 h.

[^{123}I]-2-methoxy-5-iodobenzamides and the [^{123}I]-4-iodobenzamides in the intestine was almost eliminated (ID < 0.6%). These results seemed to indicate that the [^{123}I]iodobenzamide excretion route is greatly influenced by the number and nature of substituents on the benzene ring of the molecules.

The thyroid uptake of the [^{123}I]benzamides was low and increased slightly during the study from 0.1 %ID at 1 h to reach a plateau of ∼1 %ID from 6 to 24 h. During the time course of the study, compounds **25**, **27**, and **33** indicated a particularly low thyroid uptake, < 0.2 %ID. This is probably due to the fact that they are bound to sigma enriched tissues, reducing their rate of deiodination. The low thyroid uptake of the studied [^{123}I]-iodobenzamides is an indication that these tracers are relatively stable to in vivo deiodination.

Significant uptake of the [^{123}I]benzamides was also observed in the eyes (0.5 %ID), supporting a melanin-related uptake mechanism that has been described by Moins et al.[14] for [^{123}I]-BZA. Recent studies, using secondary ion mass spectroscopy (SIMS), confirmed that BZA was found to locate in pigmented tissues of skin and grafted melanomas and in the pigmented structures of the eye: choroidal melanocytes and retinal pigment cells of C57BL/6J black mice.[19]

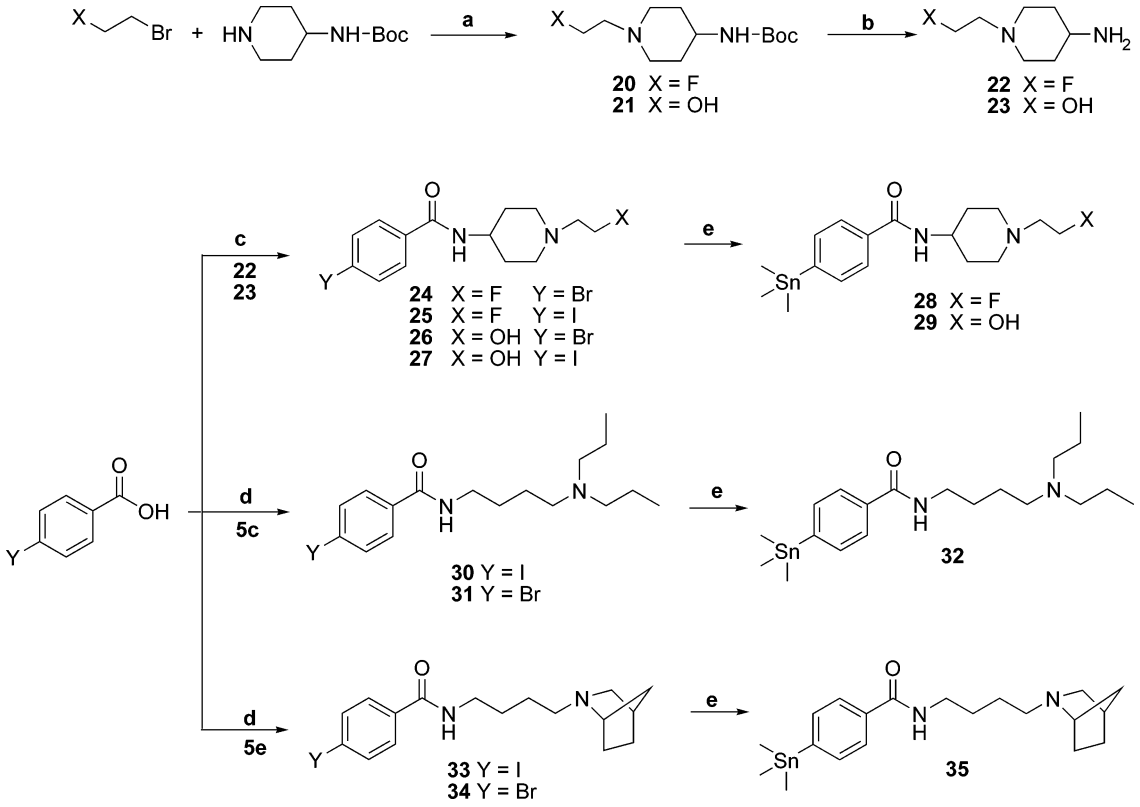
As a consequence of simultaneous low organ uptakes and rapid clearance, less than 1.4 %ID of the radioactivity remained in the mouse body 24 h after administration of radioiodinated **14d**. For compounds **1**, **14b**, **14c**, **14e**, and **25**, this value was less than 6 %ID. In contrast, **19m**, **19n**, **30**, **33**, and **27** had higher organ uptake and slower clearance, with more than 10% of the radioactivity remaining in the mouse body.

The values of tumor and organ concentration, expressed in %ID/g, are summarized in Table 3 and were compared to reference compounds [^{123}I]BZA_2, [^{123}I]**1**, and [^{123}I]**30**, which were prepared and studied under our experimental conditions. The benzamides **1**, **14c**, and **25** exhibited the highest tumor uptake at 1 h (>8 %ID/g), followed by **14b**, **14d**, **30**, **19m**, and **27**, with uptake similar to BZA_2 (∼5−7 %ID/g). For compounds **14d**, **25**, and **27**, the uptake increased with time and reached a maximum at 6 h. For benzamides **1**, **14c**, **14d**, **30**, **25**, and **27**, the tumor uptake was still higher than 5 %ID/g at 24 h.

A comparison between the uptake values of the 2-methoxy-5-iodobenzamide derivatives against the corresponding amine side chain structure indicated higher and longer retention of activity in the tumor for the *N*-dipropyl (**14c**) and *N*-methyl-butyl (**14d**) substituents compared to other linear, branched, or cyclic derivatives. The higher uptake displayed by these two benzamides strongly correlates with the highest lipophilicity values of the 2-methoxy-5-iodobenzamides (Figure 2). The effects of further structural modifications in the *para* postion of these 2-methoxy-5-iodobenzamide derivatives are reflected in the tumor uptake of [^{123}I] benzamides **1**, **19m**, and **19n**. In comparison to **1**, a significant decrease in the earlier tumor uptake and an increased wash out from tissue was observed with the 4-mesylamino- and 4-tosylamino-analogues (**19m**,**n**). The *N*-piperidin-4-yl analogues of IBP, **25**, and **27** presented high and long lasting tumor uptake that peaked at more than 10 %ID/g at 6 h.

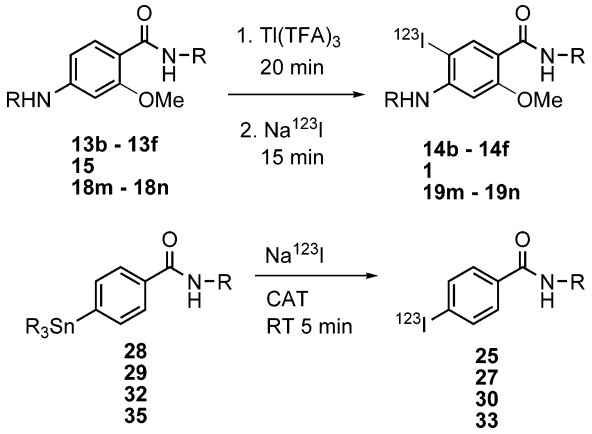
In the brain, at 1 h, the uptake of **25** and **27** was 3.1 %ID/g and 1 %ID/g, respectively. This uptake is explained by the logP values (2.57 and 1.93, respectively), allowing the tracers to cross the blood−brain barrier with relatively good affinity of these compounds for σ-receptors.

Scheme 3. Synthesis of Trimethylstannyl Precursors[a]



[a] Reagents and conditions: (a) K_2CO_3, ACN, 5 h; (b) TFA, 1 h; (c) EDC, HOBT, NMM, DMF, 24 h; (d) SO_2Cl, THF 4 h; (e) $(Me_3Sn)_2$, $Pd(PPh_3)_4$, toluene or dioxane, reflux 24 h.

Scheme 4. Radiolabeling of [^{123}I]iodobenzamides



[^{123}I]Iodobenzamide body versus tumor contrast was ascertained by calculating the standardized uptake values (SUV) at the time of animal sacrifice. These values are reported in Table 3. Due to the higher radioactive concentration remaining in the body, SUV_{24} was lower than 6 for compounds **19m**, **19n**, and **33**. For compounds **14f**, **14b**, **14c**, **14e**, **30**, **25**, **27**, and BZA_2, SUV_{24} ranged from 10 to 20, despite a tumor concentration higher than 5 %ID/g for **25** and **27**. The benzamide **1** presented a high contrast with SUV greater than 40 between 24 and 72 h. For **14d**, due to simultaneous high uptake and long retention in the tumor and low body concentration, SUV_{24-72} was higher than 100.

Competition Studies. To assess the tumor uptake mechanism, in vivo competition studies were performed on the two lead compounds [^{123}I]**14d** and [^{123}I]**25**, having the highest tumor uptake but different in vitro pharmacological profile. The blocking effect on the tracer uptake of haloperidol, a nonselective σ_1-σ_2 inhibitor, was examined over 24 h in selected organs and tumor tissue. As seen in Table 4, at 1 h p.i., the tumor and main organ uptakes were not significantly different in treated animals compared to controls with [^{123}I]**14d**. At 24 h p.i., a significant decrease was observed in the kidney, but the uptake was found to be unchanged in the tumor, eyes, and in organs such as liver, lung, and brain. With [^{123}I]**25**, at 1 h postinjection, decreases in the uptake of radioactivity were observed in the kidney, liver, and brain (−25 to −45%, $p < 0.01$), while an increase of radioactivity concentration was observed in the blood (+50%, $p < 0.01$). At 24 h p.i., a significant reduction of the uptake of the radioactivity occurred in the tumor (−33%, $p < 0.01$) in the brain, lung, and kidney (−62 to −68%, $p < 0.01$).

Sigma receptors are present in the central nervous system as well as tissues such as the liver, kidneys, and lungs.[20] Haloperidol, which is known to inhibit the binding at both σ_1 and σ_2 receptor subtypes with high affinity,[21] was able to decrease the uptake of [^{123}I]**25** in these organs and in the tumor. These findings confirmed the in vitro results and supports that part of the mechanism of tumor uptake of [^{123}I]**25** may be due to its σ-receptor affinity. Compared to the control animals, the uptake of [^{123}I]**14d** and of [^{123}I]**25** in the eyes of haloperidol-treated animals was not statistically different. This indicated that the uptake in the eyes of these [^{123}I]iodobenzamides is related to pigmented cells and is not σ-receptor mediated.

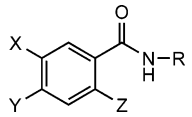
Imaging Studies. The whole body distribution of [^{123}I]**14d** and [^{123}I]**25** in C57BL/6J mice was followed over 48 h using SPECT imaging. Figure 3 shows typical SPECT images of mice acquired 24 h after the radiotracer injection. In these images, the highest radioactivity was observed in the tumor, followed by the eyes. The radioactivity observed in the thyroid of the mouse injected with [^{123}I]**14d** was higher than those injected with [^{123}I]**25**. In contrast, the activity remaining in the body of

Table 1. Radiolabeling Data for Benzamides

[^{123}I]-cmpd	precursor	radiolabeling method	solvent[c]	flow rate (mL/min)	retention time (min)	RCY[g] (%)
1	**15**	Tl(TFA)$_3$	40/60[d]	1.5	17	55
14b	**13b**	Tl(TFA)$_3$	40/60[d]	1.5	16	44
14c	**13c**	Tl(TFA)$_3$	40/60[d]	1.5	26	50
14d	**13d**	Tl(TFA)$_3$	50/50[d]	2.0	17	50
14e	**13e**	Tl(TFA)$_3$	60/40[d]	2.0	24	40
14f	**13f**	Tl(TFA)$_3$	40/60[d]	1.5	15	48
19m	**18m**	Tl(TFA)$_3$	40/60[d]	1.5	21	46
19n	**18n**	Tl(TFA)$_3$	40/60[d]	1.5	16	42
25	**28**	CAT	40/60[e]	2.0	14	91
27	**29**	CAT	70/30[e]	1.5	13	96
30	**32**	CAT	55/45[d]	3.0	17	50
33	**35**	CAT	70/30[d]	2.0	21	72
BZA$_2$	**Br-BZA$_2$**[a]	halogen-exchange[b]	42.5/57.5[f]	4.0	11	85

[a] Bromo analogue of BZA$_2$. [b] Halogen-exchange method, as described by Moins et al.[15] [c] Acetonitrile/(0.1 M) ammonium acetate, %/%, v/v. [d] Alltech Alphabond column (C18, 10 μm, 300 × 7.5 mm). [e] Phenomenex Bondclone column (C18, 10 μm, 300 × 7.8 mm). [f] Alltech Econosil (C18, 10 μm, 250 × 10 mm). [g] Radiochemical purity for each radiotracer was greater than 95%.

Table 2. Receptor Binding Data and log$P_{7.5}$ Values for Benzamides



	X	Y	Z	R	K_i σ_1[a] (nM)	K_i σ_2[b] (nM)	logP[c]
1	I	NHAc	OMe	2-(*N*,*N*-diethylamino)ethyl	1650	>100 000	1.54 ± 0.03
14b	I	NHAc	OMe	4-(*N*-*i*-propyl-*N*-methylamino)butyl	5200	5400	0.75 ± 0.02
14c	I	NHAc	OMe	4-(*N*,*N*-dipropylamino)butyl	7800	>100 000	1.65 ± 0.04
14d	I	NHAc	OMe	4-(*N*-butyl-*N*-methylamino)butyl	1600	2300	1.57 ± 0.03
14e	I	NHAc	OMe	4-(2-azanorborn-2-yl)butyl	680	3900	0.80 ± 0.02
14f	I	NHAc	OMe	4-(*N*-butylamino)butyl	2500	6600	1.05 ± 0.02
19m	I	NHmesyl	OMe	2-(*N*,*N*-diethylamino)ethyl	800	45000	0.82 ± 0.02
19n	I	NHtosyl	OMe	2-(*N*,*N*-diethylamino)ethyl	650	>100 000	1.95 ± 0.03
25	H	I	H	*N*-(2-fluoroethyl)piperidin-4-yl	6	2600	2.57 ± 0.04
27	H	I	H	*N*-(2-hydroxyethyl)piperidin-4-yl	140	1700	1.93 ± 0.03
30	H	I	H	4-(*N*,*N*-dipropylamino)butyl	1300	1000	2.46 ± 0.04
33	H	I	H	4-(2-azanorborn-2-yl)butyl	32	75	1.63 ± 0.03

[a] Assays were carried out by Novascreen Biosciences using [^{3}H]-(+)-pentazocine for σ_1-receptors. [b] [^{3}H]-DTG for σ_2-receptors and the conditions provided in literature.[31] [c] Assessed by HPLC using a literature method.[30]

the mouse injected with [^{123}I]**25** is significantly higher than of the mouse injected with [^{123}I]**14d**. These observations confirmed the quantitative results obtained in biodistribution studies.

Summary

In the development of novel iodobenzamides for melanoma scintigraphy and radiotherapy, a better understanding of the pharmacology and pharmacokinetics of the radiotracer is required. The uptake mechanism of radioiodinated benzamides into melanoma cells has been extensively studied. In vitro studies showed that the uptake of aminoalkylbenzamide derivatives is related to the melanin content of cells and is not receptor mediated.[9,10,22] Moreover, the in vivo localization of BZA in the cytoplasm of tumor cells and its association with intracytoplasmic pigments was confirmed by secondary ion mass spectrometry (SIMS) supporting BZA's specific interaction with melanin.[23] On the other hand, 4-piperidinyl-iodobenzamide derivatives are known to bind to sigma receptors in a variety of tumor cells, including melanoma.[11,24]

From our results, the uptake mechanisms of iodobenzamides in melanoma tumors is dependent on the nature of the chemical moiety associated with the benzamide structure.

According to the in vitro assays for the determination of inhibition constants for sigma receptors, only piperidinyl-iodobenzamides show a σ-receptor characteristic, and of these, compound **25** showed the highest affinity for the σ_1-receptor (K_i = 6 nM). In vivo, haloperidol, a nonspecific σ_1-σ_2 receptor inhibitor, was able to prevent uptake of [^{123}I]**25** in organs possessing a high density of σ-receptors and also in the B16 melanoma tumor. As the B16 melanoma cell line has also been reported to express σ-receptors,[25] it is likely that the tumor uptake of [^{123}I]**25** is partially due to its sigma receptor profile. No competitive effect of haloperidol was observed in the tumor uptake of the aminoalkylbenzamide [^{123}I]**14d**, suggesting no interaction with σ-receptors. In addition, [^{123}I]**14d** had significant uptake in the eyes, confirming a mainly melanin-related uptake mechanism.

From these results, compound **14d** displayed a 7-fold higher contrast value than BZA$_2$ with similar uptake in tumors, while compound **25** exhibited a 50% higher uptake in tumors than BZA$_2$ over a 24 h period. These qualities suggest that **14d** and **25** appear to be promising imaging agents for melanoma detection and **14d**, with a high melanoma/nontarget ratio, is more suitable for evaluation as a potential radiotherapeutic.

In conclusion, two series of iodobenzamides have been developed for their ability for tumor scintigraphy and for studies in potential radiotherapeutic applications. The biodistribution and pharmacokinetic profile of the [^{123}I]iodobenzamides studied was greatly influenced by the substituents on the benzene ring, whereas the uptake values in the tumor correlated with the molecule's lipophilicity through the nature of the aminoalkyl side chain.

Table 3. Biodistribution of [^{123}I]Benzamides in Mice

cmpd	time (h)	melanoma[a]	liver[a]	kidney[a]	lung[a]	heart[a]	brain[a]	blood[a]	SUV[b]
1	1	9.6 ± 2.9	9.0 ± 0.7	2.3 ± 0.3	1.3 ± 0.2	0.79 ± 0.05	0.13 ± 0.06	0.98 ± 0.05	3.7 ± 1.1
	6	8.5 ± 1.2	3.0 ± 0.3	0.44 ± 0.07	0.4 ± 0.2	0.31 ± 0.05	0.02 ± 0.01	0.35 ± 0.15	9.5 ± 2.6
	24	5.2 ± 0.5	1.1 ± 0.1	0.08 ± 0.02	0.09 ± 0.03	0.12 ± 0.01	0.01 ± 0.01	0.08 ± 0.02	38 ± 3
	48	3.4 ± 1.0	0.51 ± 0.07	0.04 ± 0.01	0.07 ± 0.02	0.07 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	48 ± 18
	72	2.2 ± 0.7	0.28 ± 0.06	0.06 ± 0.05	nd	0.18 ± 0.08	nd	0.06 ± 0.02	51 ± 11
14b	1	5.5 ± 1.6	9.5 ± 3.6	5.7 ± 1.2	1.5 ± 0.3	0.84 ± 0.22	0.07 ± 0.02	1.3 ± 0.3	1.6 ± 0.3
	6	6.0 ± 1.5	1.5 ± 0.3	0.69 ± 0.12	0.50 ± 0.08	0.30 ± 0.02	0.03 ± 0.02	0.68 ± 0.12	4.0 ± 1.8
	24	3.7 ± 0.3	0.42 ± 0.09	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.02	0.07 ± 0.01	0.02 ± 0.01	20 ± 4
14c	1	8.0 ± 2.8	7.3 ± 0.8	14.1 ± 3.5	1.7 ± 0.1	0.93 ± 0.11	0.07 ± 0.01	1.7 ± 0.3	2.4 ± 0.8
	6	6.8 ± 0.6	1.8 ± 0.3	1.0 ± 0.2	0.80 ± 0.16	0.49 ± 0.08	0.05 ± 0.01	1.1 ± 0.3	3.6 ± 0.5
	24	5.1 ± 0.7	0.64 ± 0.10	0.18 ± 0.06	0.14 ± 0.06	0.14 ± 0.04	0.01 ± 0.01	0.15 ± 0.06	19 ± 3
14d	1	5.6 ± 1.5	16.8 ± 1.7	21.6 ± 5.8	1.9 ± 0.4	0.98 ± 0.13	0.07 ± 0.01	1.2 ± 0.2	1.7 ± 0.5
	3	9.7 ± 3.1	5.0 ± 0.8	3.9 ± 0.5	0.95 ± 0.16	0.54 ± 0.12	0.04 ± 0.01	0.95 ± 0.21	4.3 ± 1.4
	6	8.4 ± 1.5	1.8 ± 0.1	1.2 ± 0.1	0.56 ± 0.07	0.75 ± 0.82	0.03 ± 0.00	0.71 ± 0.06	6.6 ± 2.6
	24	6.1 ± 0.9	0.47 ± 0.04	0.12 ± 0.07	0.10 ± 0.05	0.12 ± 0.05	0.01 ± 0.01	0.11 ± 0.08	108 ± 32
	48	4.6 ± 0.9	0.38 ± 0.04	0.07 ± 0.02	0.08 ± 0.02	0.13 ± 0.03	0.01 ± 0.00	0.04 ± 0.01	163 ± 28
	72	2.2 ± 0.3	0.25 ± 0.03	0.10 ± 0.02	0.05 ± 0.02	0.06 ± 0.09	0.01 ± 0.01	0.05 ± 0.00	99 ± 22
14e	1	3.4 ± 0.5	7.1 ± 1.4	3.7 ± 0.5	2.6 ± 0.4	0.94 ± 0.15	0.08 ± 0.01	1.92 ± 0.19	1.0 ± 0.1
	6	2.8 ± 0.4	0.8 ± 0.2	0.67 ± 0.03	0.68 ± 0.3	0.3 ± 0.1	0.03 ± 0.01	0.78 ± 0.35	2.0 ± 0.8
	24	1.4 ± 0.1	0.19 ± 0.03	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	nd	0.02 ± 0.01	15 ± 4
14f	1	3.4 ± 0.7	6.0 ± 0.7	3.26 ± 0.85	2.0 ± 0.1	0.64 ± 0.09	0.04 ± 0.01	0.84 ± 0.05	1.1 ± 0.2
	6	3.4 ± 0.8	1.3 ± 0.1	0.46 ± 0.09	0.45 ± 0.13	0.28 ± 0.15	0.02 ± 0.01	0.47 ± 0.08	2.1 ± 0.8
	24	2.2 ± 0.3	0.45 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.06 ± 0.01	nd	0.05 ± 0.01	20 ± 6
19m	1	5.4 ± 0.4	2.3 ± 0.3	1.30 ± 0.15	0.67 ± 0.10	0.42 ± 0.06	0.05 ± 0.01	0.77 ± 0.10	2.2 ± 0.2
	6	3.6 ± 0.7	1.2 ± 0.2	0.49 ± 0.09	0.38 ± 0.05	0.19 ± 0.06	0.02 ± 0.01	0.49 ± 0.16	2.6 ± 0.5
	24	1.7 ± 0.2	0.28 ± 0.03	0.06 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	nd	0.01 ± 0.01	3.1 ± 0.5
19n	1	4.2 ± 0.8	3.8 ± 0.9	1.2 ± 0.1	0.62 ± 0.05	0.28 ± 0.04	0.03 ± 0.01	0.74 ± 0.08	1.8 ± 0.4
	6	2.6 ± 0.2	1.5 ± 0.4	0.36 ± 0.03	0.22 ± 0.02	0.11 ± 0.01	nd	0.33 ± 0.03	2.2 ± 0.3
	24	1.4 ± 0.3	0.21 ± 0.02	0.06 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	nd	0.05 ± 0.01	2.7 ± 0.6
25	1	10.9 ± 0.8	19.2 ± 2.4	8.7 ± 0.9	10.8 ± 0.8	3.0 ± 0.1	3.1 ± 0.3	1.00 ± 0.06	3.3 ± 0.4
	6	12.5 ± 1.9	3.8 ± 0.3	3.4 ± 0.3	6.4 ± 0.4	0.93 ± 0.04	0.48 ± 0.04	0.34 ± 0.02	9.3 ± 1.8
	24	5.5 ± 2.3	0.29 ± 0.05	0.22 ± 0.02	0.33 ± 0.08	0.09 ± 0.01	0.09 ± 0.01	0.04 ± 0.01	17 ± 2
27	1	5.9 ± 0.7	17.1 ± 1.2	10.6 ± 1.2	17.8 ± 2.8	3.1 ± 0.2	1.0 ± 0.1	1.0 ± 0.1	1.7 ± 0.2
	6	11.7 ± 2.0	9.2 ± 4.3	3.2 ± 0.9	4.7 ± 2.2	0.93 ± 0.45	0.46 ± 0.16	0.21 ± 0.06	6.6 ± 1.0
	24	9.2 ± 0.9	5.4 ± 0.5	1.2 ± 0.1	1.44 ± 0.25	0.49 ± 0.06	0.15 ± 0.01	0.04 ± 0.01	10 ± 2
30	1	6.2 ± 1.5	49.1 ± 2.0	8.6 ± 0.7	8.2 ± 0.9	1.9 ± 0.3	0.50 ± 0.05	0.52 ± 0.04	1.4 ± 0.9
	6	7.5 ± 0.9	33.0 ± 1.8	5.4 ± 0.8	3.9 ± 0.4	0.81 ± 0.12	0.17 ± 0.02	0.35 ± 0.02	3.1 ± 0.2
	24	6.9 ± 0.5	2.9 ± 0.4	0.52 ± 0.13	0.39 ± 0.86	0.12 ± 0.01	0.04 ± 0.01	0.08 ± 0.01	15 ± 2
33	1	3.8 ± 0.6	43.2 ± 5.0	15.2 ± 2.5	6.9 ± 0.5	1.05 ± 0.12	0.10 ± 0.01	0.48 ± 0.06	0.9 ± 0.1
	6	3.5 ± 0.8	27.0 ± 6.5	4.9 ± 0.9	2.1 ± 0.3	0.44 ± 0.04	0.06 ± 0.01	0.24 ± 0.01	1.7 ± 0.5
	24	3.4 ± 0.3	3.5 ± 0.65	0.59 ± 0.04	0.40 ± 0.06	0.13 ± 0.02	0.01 ± 0.00	0.03 ± 0.00	6.5 ± 1.4
BZA_2	1	7.1 ± 1.3	6.8 ± 0.8	6.8 ± 1.2	4.5 ± 0.7	2.0 ± 0.4	1.3 ± 0.2	1.9 ± 0.3	2.7 ± 0.5
	6	8.8 ± 1.3	0.73 ± 0.19	0.76 ± 0.25	0.82 ± 0.26	0.40 ± 0.11	0.11 ± 0.03	1.2 ± 0.5	8.7 ± 2.8
	24	3.0 ± 1.2	0.10 ± 0.01	0.06 ± 0.01	0.09 ± 0.06	0.07 ± 0.06	0.01 ± 0.01	0.06 ± 0.02	16 ± 2

[a] Data are the means of %ID/g of tissue ± SD, $n = 5$. [b] Standardized uptake values (SUV_t) are calculated by dividing the tumor radioactivity concentration by the mean radioactive concentration remaining in the mouse at time *t*.

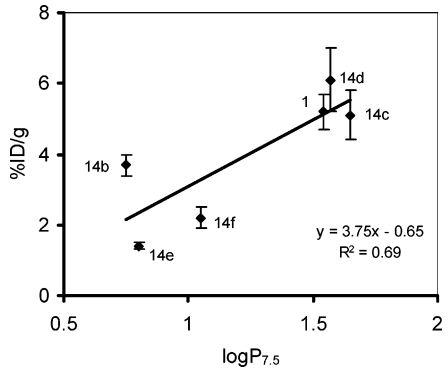


Figure 2. Relationship between tumor uptake (%ID/g) at 24 h and the lipophilicty ($logP_{7.5}$) of the 2-methoxy-5-iodobenzamides.

Experimental Section

All commercially available chemicals were purchased from Sigma-Aldrich, Lancaster, or Microchemistry Building Block (Russia) and used without further purification. Methyl 4-amino-2-methoxybenzoate (**6**)[16,18,26] and *N*′,*N*′-dipropylbutyl-1,4-diamine (**5c**)[17] were synthesized by literature methods. ^{123}I was produced by the National Medical Cyclotron, Sydney, Australia, using the ^{124}Xe(p, 2n) reaction and was delivered as [^{123}I]INa in 0.02 M NaOH at a concentration of 37 GBq/mL. Melting points were determined in open capillary tubes using a Gallenkamp melting point apparatus and are uncorrected. NMR spectra were performed on a Bruker Avance DPX 400 operating at 400 MHz for ^{1}H NMR spectra and 100 MHz for ^{13}C NMR spectra. Elemental Analysis was determined by The Campbell Microanalytical Laboratory, Department of Chemistry, University of Otago, New Zealand. LRMS was performed on a Micromass ZMD quadrupole mass spectrometer, while HRMS was performed by the University Analytical Laboratory (University of New South Wales) using a Bruker Daltonics BioApex-II 7T FTICR mass spectrometer equipped with an off-axis analytical ESI source. Preparative HPLC purification was performed on a system equipped with a Waters 600 gradient pump and 486 UV detector using a C18 column (Alltech Econosphere, 10 μm, 250 × 22 mm). Semipreparative radio-HPLC was performed on a Berthold LB501 system equipped with variable UV (linear) and a γ-detector using either an Alltech Alphabond

Table 4. Effect of Haloperidol on the Organ Uptake of [^{123}I]**14d** and [^{123}I]**25**[a]

	time (h)		tumor	eye	liver	kidney	lung	blood	brain
14d	1	control	6.8 ± 1.2	18.9 ± 1.4	15.9 ± 1.4	17.3 ± 1.6	1.67 ± 0.07	1.13 ± 0.15	0.07 ± 0.01
		treated	6.1 ± 0.8	16.9 ± 3.3	15.6 ± 2.1	17.3 ± 1.9	1.54 ± 0.09	1.15 ± 0.06	0.06 ± 0.03
	24	control	4.5 ± 0.4	18.8 ± 3.5	0.34 ± 0.05	0.07 ± 0.02	0.03 ± 0.01	0.03 ± 0.01	0.004 ± 0.001
		treated	4.5 ± 0.5	18.7 ± 2.8	0.30 ± 0.03	0.03 ± 0.01[#]	0.02 ± 0.01	0.02 ± 0.01	0.002 ± 0.001
25	1	control	9.5 ± 1.9	27.8 ± 7.7	26.0 ± 2.7	9.7 ± 1.5	10.3 ± 1.5	0.97 ± 0.13	2.83 ± 0.22
		treated	10.5 ± 2.1	34.8 ± 3.1	10.1 ± 0.7[b]	7.3 ± 0.7[b]	9.7 ± 1.3	1.46 ± 0.08[b]	1.61 ± 0.25[b]
	24	control	4.9 ± 0.2	24.7 ± 3.6	0.36 ± 0.06	0.26 ± 0.02	0.33 ± 0.02	0.04 ± 0.01	0.08 ± 0.01
		treated	3.3 ± 0.9[b]	24.6 ± 0.9	0.12 ± 0.03[b]	0.10 ± 0.02[b]	0.14 ± 0.03[b]	0.03 ± 0.01	0.06 ± 0.01[b]

[a] The values are expressed as the average of %ID/g of tissue ± SD; $n = 5$. [b] $p < 0.01$.

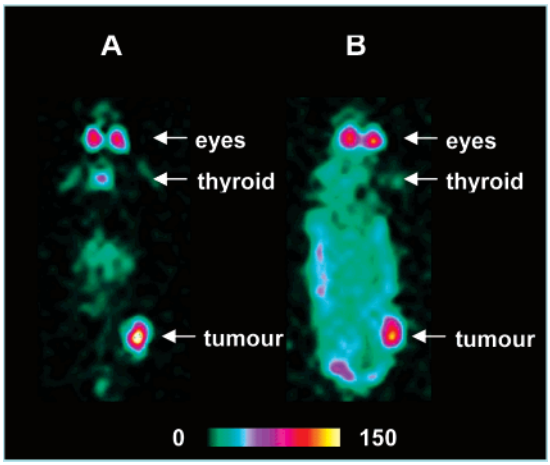


Figure 3. Scaled SPECT images of the distribution of [^{123}I]**14d** (A) and [^{123}I]**25** (B) in C57BL/6J female mice bearing a B16F0 murine melanoma tumor, acquired 24 h after injection of 10 MBq of radiotracer. The scale indicated the highest value in count/pixel in the image. High concentration of radioactivity was observed in the tumors and the eyes of the animals.

column (C18, 10 μm, 300 × 7.5 mm), a Phenomenex Bondclone column (C18, 10 μm, 300 × 7.8 mm), or Alltech Econosil column (C18, 10 μm, 250 × 10 mm). For quality control and stability measurements, the radioiodinated tracer solution was ascertained on an analytical HPLC system using a Phenomenex Luna column (C18, 5 μm, 150 × 4.6 mm) eluting with 45% acetonitrile−55% ammonium acetate (0.1 M) at 1 mL/min.

General Procedure 1 for the Preparation of Nitriles (4).[27] A solution of the secondary amine (1 equiv) in 1-butanol, anhydrous K_2CO_3 (1 equiv), KI (0.1 equiv), and 4-bromobutyronitrile (1 equiv) was heated to reflux for 20 h. Filtration, followed by an acid (2 N HCl) and base (pH 10, conc NaOH) workup gave the corresponding 3-cyanopropylamine.

4-(*N*-Isopropyl-*N*-methylamino)butyronitrile (4b). See general procedure 1 (82%). ^{1}H NMR ($CDCl_3$) δ 0.97 (s, 3H), 0.99 (s, 3H), 1.76 (m, 2H), 2.15 (s, 3H), 2.40 (t, J = 7.2 Hz, 2H), 2.46 (t, J = 6.8 Hz, 2H, CH_2), 2.79 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 15.8, 18.9, 25.0, 37.8, 52.2, 54.9, 121.2. MS ES (+ve) *m*/*z*: 141 (M + 1)$^+$. HRMS calcd for $C_8H_{17}N_2$, 141.1386; found, 141.1384.

4-(*N*-Butyl-*N*-methylamino)butyronitrile (4d). See general procedure 1 (84%). ^{1}H NMR ($CDCl_3$) δ 0.91 (t, J = 7.2 Hz, 3H), 1.31 (m, 2H), 1.42 (m, 2H), 1.78 (m, 2H), 2.18 (s, 3H), 2.33 (t, J = 7.2 Hz, 2H), 2.41 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 15.2, 16.0, 21.7, 24.6, 30.6, 43.1, 56.9, 58.8, 121.1. MS ES (+ve) *m*/*z*: 155 (M + 1). HRMS calcd for $C_9H_{19}N_2$ (M + 1)$^+$, 155.1542; found, 155.1544.

4-(2-Azanorborn-2-yl)butyronitrile (4e). See general procedure 1 (79%). ^{1}H NMR ($CDCl_3$) δ 1.25 (m, 2H), 1.41 (m, 1H), 1.53 (m, 1H), 1.60 (m, 1H), 1.70−1.78 (m, 3H), 2.14 (m, 1H), 2.32 (m, 1H), 2.44 (m, 3H), 2.56 (m, 1H), 2.61 (m, 1H), 2.86 (m, 1H), 3.28 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 16.1, 26.1, 27.5, 29.8, 37.4, 38.9, 54.1, 60.9, 62.3, 121.2. MS ES (+ve) *m*/*z*: 165 (M + 1)$^+$. HRMS calcd for $C_{10}H_{17}N_2$, 165.1386; found, 165.1384.

General Procedure 2 for the Preparation of the Butylamines (5). Reaction of $LiAlH_4$ and the nitrile (**4**) in anhydrous ether for 1 h followed by the work up according to Micovic and Mihailovic[28] gave the *N*,*N*-dialkylaminobutylamines (**5**).

***N*′-*tert*-Butoxycarbonyl-*N*′-butylbutan-1,4-diamine (5a).** 4-(Butylamino)butyronitrile (13 g, 0.1 mol), synthesized via general procedure 1, in anhydrous acetonitrile (20 mL) and di-*tert*-butyl dicarbonate (21.6 g, 0.1 mol) in acetonitrile (60 mL) was stirred at room temperature for 15 h. Removal of all volatiles in vacuo gave 4-(*N*-*tert*-butoxycarbonyl-*N*-butylamino)butyronitrile (**4a**) as a colorless oil (16.5 g, 70%) that was converted to the diamine (**5a**) as a colorless oil (77%) using general procedure 2. ^{1}H NMR ($CDCl_3$) δ 0.81 (t, J = 7.4 Hz, 3H), 1.19 (m, 2H), 1.22−1.51 (m, 15H), 2.60 (q, J = 6.7 Hz, 2H), 3.06 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 13.6, 19.8, 28.0, 28.1, 28.2, 30.7, 42.0, 46.6, 78.9, 155.3. MS ES (+ve) *m*/*z*: 245 (M + 1)$^+$ (60%), 189 (M + 1 − O*t*-Bu)$^+$, 145 (M + 1 − Boc)$^+$.

***N*′-Isopropyl-*N*′-methylbutan-1,4-diamine (5b).** See general procedure 2. Colorless oil (82%). ^{1}H NMR ($CDCl_3$) δ 0.96 (s, 3H), 0.99 (s, 3H), 1.49 (m, 4H), 2.20 (s, 3H), 2.41 (m, 2H), 2.86 (m, 2H), 3.21 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 19.0, 26.5, 32.9, 38.1, 43.3, 54.4. MS ES (+ve) *m*/*z*: 145 (M + 1)$^+$. HRMS calcd for $C_8H_{21}N_2$, 145.1699; found, 145.1697

***N*′-Butyl-*N*′-methylbutane-1,4-diamine (5d).** See general procedure 2. Colorless oil (72%). ^{1}H NMR ($CDCl_3$) δ 0.91 (t, J = 7.2 Hz, 3H), 1.31 (m, 2H), 1.45 (m, 5H), 1.78 (m, 3H), 2.18 (s, 1.5H, NCH_3 of rotamer 1), 2.20 (s, 1.5H, NCH_3 of rotamer 2), 2.32 (m, 3H), 2.44 (m, 2H), 2.70 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 15.2, 15.3, 15.9, 21.7, 21.9, 24.7, 25.9, 30.6, 32.9, 43.3, 43.4, 56.8, 58.7, 58.9. MS ES (+ve) *m*/*z*: 159 (M + 1)$^+$. HRMS calcd for $C_9H_{23}N_2$ (M + 1)$^+$, 159.1855; found, 159.1854.

4-(2-Azanorborn-2-yl)butan-1-amine (5e). See general procedure 2. Colorless oil (76%). ^{1}H NMR ($CDCl_3$) δ 1.23 (m, 2H), 1.38−1.52 (m, 4H), 1.62−1.73 (m, 4H), 2.14 (m, 1H), 2.30 (m, 1H), 2.39 (m, 1H), 2.46 (m, 1H), 2.69 (m, 2H), 2.85 (m, 1H), 3.47 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 27.0 (CH_2), 27.7 (CH_2), 29.9 (CH_2), 32.9 (CH_2), 37.4 (CH_2), 38.8 (CH), 43.3 ($NHCH_2$), 55.7 (NCH_2), 60.9 (NCH_2), 61.8 (NCH). MS ES (+ve) *m*/*z*: 169 (M + 1). HRMS calcd for $C_{10}H_{21}N_2$, 169.1699; found, 169.1700.

Methyl 4-Acetamido-2-methoxybenzoate (7). Methyl 4-amino-2-methoxybenzoate (**6**; 20 g, 0.11 mol) was treated with acetic anhydride (12 g, 0.12 mol) in absolute ethanol (150 mL) at 50 °C for 2 h. Normal workup followed by crystallization from hot ethanol yielded (**7**) as a tan colored solid (22 g, 89%). ^{1}H NMR (DMSO-d_6) δ 2.07 (s, 3H), 3.73 (s, 3H), 3.83 (s, 3H), 7.18 (d, J = 8.8 Hz, 1H), 7.46 (s, 1H), 7.65 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 24.4, 51.8, 55.6, 102.9, 110.5, 114.1, 132.6, 143.7, 160.3, 166.2, 169.3. MS ES (+ve) *m*/*z*: 246 (M + Na)$^+$. HRMS calcd for $C_{11}H_{14}NO_4$ (M + 1)$^+$, 224.0923; observed (M + 1)$^+$, 224.0921.

Methyl 4-Acetamido-5-iodo-2-methoxybenzoate (8). Methyl 4-acetamido-2-methoxybenzoate (**7**; 10 g, 0.045 mol) in acetic acid (50 mL) at 50 °C was treated with iodine monochloride (8.14 g, 0.05 mol) in acetic acid (50 mL). The reaction was heated for 6 h at 50 °C with a further addition of iodine monochloride (0.5 g) after 4 h. After stirring overnight at room temperature, the resulting white solid was filtered and washed with acetic acid (30 mL) and H_2O (100 mL) to yield a white solid (12.1 g, 77%). ^{1}H NMR ($CDCl_3$) δ 2.11 (s, 3H), 3.77 (s, 3H), 3.78 (s, 3H), 7.44 (s, 1H), 8.07 (s, 1H), 9.37 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 25.1, 52.0, 56.1, 76.1, 104.4, 116.6, 141.3, 142.6, 160.7, 164.6, 168.5. MS ES (+ve)

m/*z*: 372 $(M + Na)^+$. HRMS calcd for $C_{11}H_{13}INO_4$ $(M + 1)^+$, 349.9889; observed $(M + 1)^+$, 349.9914.

4-Acetamido-2-methoxybenzoic Acid (11). Methyl 4-acetamido-2-methoxybenzoate (**7**; 15.0 g, 0.067 mol) in methanol (100 mL), H_2O (40 mL), and KOH (10 g) was heated to reflux for 2 h. The methanol was evaporated, H_2O (50 mL) was added, and the mixture was cooled to 5 °C. Acidification to pH 5.0 with 6 M HCl precipitated **9** as a white solid. The solid (**9**) and acetic anhydride (11.6 g, 0.11 mol) in acetic acid (150 mL) was stirred for 2 h at 50 °C. The reaction was cooled to room temperature and stirred overnight. The resulting precipitate was filtered, washed with acetic acid (20 mL), and dried to give **11** as a white solid of the title compound (10.2 g, 73%). ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 1H), 3.77 (s, 3H), 7.16 (dd, $J = 1.6$, 8.4 Hz, 1H), 7.45 (d, $J = 1.6$ Hz, 1H), 7.65 (d, $J = 8.4$ Hz), 10.17 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 25.8, 57.1, 104.0, 111.7, 116.2, 134.0, 145.7, 161.1, 168.1, 170.6. MS ES (−ve) *m*/*z*: 208 $(M - 1)^-$.

4-Acetamido-5-iodo-2-methoxybenzoic Acid (12). Methyl 4-acetamido-5-iodo-2-methoxybenzoate (**8**; 2.0 g, 5.72 mmol) in methanol (15 mL), H_2O (5 mL) and KOH (1.5 g) was reacted according to the above procedure to give **10** as a white solid. Treatment of **10** (1.5 g) in acetic acid (20 mL) with acetic anhydride (1.04 g, 10 mmol) at 50 °C for 2 h followed by stirring overnight precipitated a tan solid that was filtered, washed with acetic acid (10 mL), and dried to yield **12** as a white solid (1.5 g, 78%). ^{1}H NMR (DMSO-d_6) δ 2.10 (s, 3H), 3.77 (s, 3H), 7.40 (s, 1H), 8.01 (s, 1H), 9.35 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 25.2, 57.6, 83.8 , 111.8, 121.2, 142.2, 145.5, 160.4, 167.0, 170.3. MS ES (+ve) *m*/*z*: 358 $(M + Na)^+$. HRMS calcd for $C_{10}H_{11}INO_4$ $(M + Na)^+$, 355.9733; observed $(M + Na)^+$, 355.9827.

General Procedure 3 for the Preparation of Benzamides 1, 13a−13e, 14a−14e, 15, 30, 31, 33, and 34. Each of 4-acetamido-2-methoxybenzoic acid (**11**), 4-acetamido-5-iodo-2-methoxybenzoic acid (**12**), 4-bromobenzoic acid or 4-iodobenzoic acid (∼1.0 g), and thionyl chloride (4 equiv) in anhydrous THF (50 mL) was refluxed for 2 h and then dried under vacuum. The resulting acid chloride was redissolved in THF (50 mL) and treated with the amines [**5a**−**5e** or *N*,*N*-diethylethylenediamine (1 equiv)]. After 5 min, triethylamine (4 equiv) was added and it was stirred for 2 h before normal workup. The resulting benzamides were purified by column chromatography on silica.

4-Acetamido-*N*-(4-(*N*-*tert*-butoxycarbonyl-*N*-butylamino)butyl)-2-methoxybenzamide (13a). Benzamide **13a**, prepared according to general procedure 3, was purified by column chromatography on silica with 3:1 ethyl acetate/petroleum spirit to give a white solid (1.4 g, 68%). ^{1}H NMR ($CDCl_3$) δ 0.90 (t, $J = 7.2$ Hz, 3H), 1.26 (m, 2H), 1.44 (s, 9H) 1.51 (m, 2H), 1.59 (m, 4H), 2.20 (s, 3H), 3.14 (br m, 2H), 3.20 (br m, 2H), 3.46 (m, 2H), 3.94 (s, 3H), 6.80 (dd, $J = 1.6$, 8.5 Hz, 1H), 7.86 (br m, 2H), 8.06 (d, $J = 8.5$ Hz, 1H), 8.41 (br s, 1H). ^{13}C NMR ($CDCl_3$) δ 15.1, 21.2, 25.9, 26.1, 27.1, 28.3, 29.7, 33.3, 40.6, 47.8, 48.1, 57.2, 80.3, 103.9, 112.6, 117.8, 133.7, 143.8, 156.9, 159.5, 166.3, 170.3. MS ES (+ve) *m*/*z*: 436 $(M + 1)^+$. HRMS calcd for $C_{23}H_{38}N_3O_5$ $(M + 1)^+$, 436.2811; observed $(M + 1)^+$, 436.2791.

4-Acetamido-*N*-(4-(*N*-isopropyl-*N*-methylamino)butyl)-2-methoxybenzamide (13b). Benzamide **13b**, prepared according to general procedure 3, was purified by column chromatography on silica with 1:9 methanol/ethyl acetate to give a white solid (1.28 g, 76%). ^{1}H NMR ($CDCl_3$) δ 0.98 (s, 3H), 0.99 (s, 3H) 1.60 (m, 4H), 2.18 (s, 3H), 2.20 (s, 3H), 2.39 (t, $J = 7.27$ Hz, 2H), 2.82 (dq, $J = 6.55$, 6.56 Hz, 1H), 3.46 (dt, $J = 6.74$, 5.79 Hz, 2H), 3.97 (s, 3H), 6.80 (dd, $J = 8.51$, 1.91 Hz, 1H), 7.86 (m, 1H), 7.90 (t, $J = 5.79$ Hz, 1H), 8.05 (d, $J = 8.51$ Hz, 1H), 8.37 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 19.02, 25.9, 26.74, 28.83, 38.16, 40.92, 54.07, 54.54, 57.2, 103.89, 112.54, 118.00, 133.64, 143.76, 159.44, 166.38, 170.32. MS ES (+ve) *m*/*z*: 336 $(M + 1)^+$. HRMS calcd for $C_{18}H_{30}N_3O_3$ $(M + 1)^+$, 336.2287; observed $(M + 1)^+$, 336.2301.

4-Acetamido-*N*-(4-(dipropylamino)butyl)-2-methoxybenzamide (13c). Benzamide **13c**, prepared according to general procedure 3, was purified by column chromatography on silica with 1:9 methanol/ethyl acetate to give a white solid (1.28 g, 75%). ^{1}H NMR ($CDCl_3$) δ 0.85 (t, $J = 7.35$ Hz, 6H), 1.40−1.63 (m, 8H), 2.20 (s, 3H), 2.33−2.45 (m, 6H), 3.46 (m, 2H), 6.82 (dd, $J = 1.9$, 8.52 Hz, 1H), 7.88 (m, 1H), 7.90 (m, 1H, superimposed), 8.03 (d, $J = 8.52$ Hz, 1H), 8.78 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 13.15, 21.38, 25.86, 25.98, 28.83, 40.93, 54.99, 57.16, 57.42, 103.91, 112.65, 117.76, 133.54, 144.04, 159.42, 166.48, 170.58. MS ES (+ve) *m*/*z*: 364 $(M + 1)^+$. Anal. Calcd for $C_{20}H_{33}N_3O_3 \cdot 1H_2O \cdot 1TFA$: C, 53.32; H, 7.34; N, 8.48. Found: C, 53.70; H, 7.68; N, 9.07.

4-Acetamido-*N*-(4-(*N*-butyl-*N*-methylamino)butyl)-2-methoxybenzamide (13d). Benzamide **13d**, prepared according to general procedure 3, was purified by column chromatography on silica with 1:9 methanol/ethyl acetate to give a white solid (1.2 g, 72%). ^{1}H NMR ($CDCl_3$) δ 0.90 (t, $J = 7.29$ Hz, 3H) 1.28−1.69 (m, 8H), 2.20 (s, 6H), 2.35 (m, 4H), 3.46 (m, 2H), 3.92 (s, 3H), 6.80 (dd, $J = 1.89$, 8.51 Hz, 1H), 7.86 (m, 1H), 7.90 (t, $J = 5.61$ Hz, 1H), 8.04 (d, $J = 8.51$ Hz, 1H), 8.56 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 15.24, 21.90, 25.88, 26.01, 28.80, 30.54, 40.87, 43.39, 57.19, 58.53, 58.80, 103.92, 112.62, 117.84, 133.56, 143.94, 159.42, 166.47, 170.49. MS ES (+ve) *m*/*z*: 350 $(M + 1)^+$. HRMS calcd for $C_{19}H_{32}N_3O_3$ $(M + Na)^+$, 372.2258; observed $(M + Na)^+$, 372.2256.

4-Acetamido-*N*-(4-(2-azanorborn-2-yl)butyl)-2-methoxybenzamide (13e). Benzamide **13e**, prepared according to general procedure 3, was purified by column chromatography on silica with 1:4 methanol/chloroform to give a white solid (0.76 g, 71%) as a 1:1 mixture of endo and exo isomers. ^{1}H NMR ($CDCl_3$) δ 1.2−1.9 (m, 10H), 2.60 (m, 5H), 3.40 (m, 3H, superimposed), 7.46 (d, $J = 8.4$, 2H), 7.67 (d, $J = 8.4$, 2H), 7.78 (s, br, 1H). ^{13}C NMR ($CDCl_3$) δ 26.11, 26.36, 27.97, 29.12, 37.27, 38.26, 40.43, 54.45, 60.67, 62.69, 126.96, 130.14, 132.76, 134.94, 168.02. MS ES (+ve) *m*/*z*: 360 $(M + 1)^+$. HRMS calcd for $C_{20}H_{30}N_3O_3$ $(M + 1)^+$, 360.2282; observed $(M + 1)^+$, 360.2279.

4-Acetamido-*N*-(4-(butylamino)butyl)-2-methoxybenzamide (13f). 4-Acetamido-*N*-(4-(*N*-*tert*-butoxycarbonyl-*N*-butylamino)-butyl)-2-methoxybenzamide (**13a**; 0.7 g, 1.6 mmol) in anhydrous ethyl acetate (5 mL) was treated with saturated HCl in anhydrous ethyl acetate (1 mL) for 45 min at room temperature to give a white solid of **13f** as its hydrochloride salt (0.55 g, 92%). ^{1}H NMR (DMSO-d_6) δ 0.88 (t, $J = 7.2$ Hz, 3H), 1.31 (m, 2H), 1.55 (m, 4H), 1.59 (m, 2H), 2.01 (s, 3H), 2.83 (br m, 2H), 2.87 (br m, 2H), 3.28 (m, 2H), 3.87 (s, 3H), 7.20 (d, $J = 8.8$ Hz, 1H), 7.53 (s, 1H), 7.74 (d, $J = 8.8$ Hz, 1H), 8.10 (t, $J = 5.6$ Hz, 1H), 8.88 (br s, 2H), 10.31 (br s). ^{13}C NMR ($CDCl_3$) δ 15.2, 21.0, 24.7, 25.8, 28.1, 29.1, 39.9, 48.1, 57.4, 103.6, 112.3, 118.5, 133.0, 144.8, 159.2, 166.1, 170.5. MS ES (+ve) *m*/*z*: 336 $(M + 1)^+$. HRMS calcd for $C_{18}H_{30}N_3O_3$ $(M + 1)^+$, 336.2287; observed $(M + 1)^+$, 336.2281.

4-Acetamido-*N*-(4-(*N*-*tert*-butoxycarbonyl-*N*-butylamino)butyl)-5-iodo-2-methoxybenzamide (14a). Benzamide **14a**, prepared according to general procedure 3, was purified by column chromatography on silica with ethyl acetate to give a white solid (1.2 g, 72%). ^{1}H NMR ($CDCl_3$) δ 0.91 (t, $J = 7.2$ Hz, 3H), 1.27 (m, 2H), 1.44 (s, 9H), 1.48 (m, 2H), 1.59 (m, 4H), 2.27 (s, 3H), 3.15 (br m, 2H), 3.21 (br m, 2H), 3.46 (m, 2H), 3.98 (s, 3H), 7.64 (br s, 1H), 7.77 (br s, 1H), 8.24 (s, 1H), 8.56 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 15.1, 21.2, 26.3, 27.2, 28.2, 29.7, 32.3, 40.7, 47.8, 48.1, 57.5, 79.1, 80.2, 105.1, 122.2, 142.8, 156.8, 159.6, 164.7, 169.8. MS ES (+ve) *m*/*z*: 562 $(M + 1)^+$. HRMS calcd for $C_{23}H_{37}IN_3O_5$ $(M + 1)^+$, 562.1778; observed $(M + 1)^+$, 562.1876.

4-Acetamido-*N*-(4-(*N*-isopropyl-*N*-methylamino)butyl)-5-iodo-2-methoxybenzamide (14b). Benzamide **14b**, prepared according to general procedure 3, was purified by column chromatography on silica with 1:8 methanol/ethyl acetate to give a white solid (0.99 g, 72%). ^{1}H NMR ($CDCl_3$) δ 0.99 (s, 3H), 1.01 (s, 3H), 1.52−1.66 (m, 4H), 2.20 (s, 3H), 2.27 (s, 3H), 2.41 (t, $J = 7.2$ Hz, 2H), 2.82 (dq, $J = 6.55$, 6.56 Hz, 1H), 3.45 (dt, $J = 6.4$, 5.6 Hz, 2H), 3.97 (s, 3H), 7.64 (br s, 1H), 7.79 (t, $J = 5.6$ Hz, 1H), 8.22 (s, 1H), 8.55 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 18.9, 26.4, 26.6, 28.8, 38.1, 40.9, 54.0, 54.5, 57.5, 79.2, 105.1, 120.1, 142.7, 159.5, 164.7, 170.3. MS ES (+ve) *m*/*z*: 462 $(M + 1)^+$. Anal. Calcd for $C_{18}H_{28}IN_3O_3$: C, 46.86; H, 6.12; N, 9.11. Found: C, 46.74; H, 6.00; N, 8.98.

4-Acetamido-*N*-(4-(dipropylamino)butyl)-5-iodo-2-methoxybenzamide (14c). Benzamide **14c**, prepared according to general procedure 3, was purified by column chromatography on silica with 1:1 methanol/ethyl acetate to give a white solid (0.65 g, 44%). ^{1}H NMR (CD_3OD) δ 1.02 (t, J = 7.36, 6H), 1.75 (m, 8H), 2.22 (s, 3H), 3.12 (m, 4H), 3.22 (m, 2H), 3.48 (m, 2H), 3.96 (s, 3H), 7.60 (s, 1H) 8.33 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 12.04, 18.50, 22.05, 26.34, 27.99, 39.14, 54.96, 56.50, 57.61, 81.56, 105.43, 118.84, 142.41, 143.44, 160.16, 166.00, 169.95. MS ES (+ve) *m*/*z*: 490 $(M + 1)^+$. Anal. Calcd for $C_{20}H_{32}IN_3O_3 \cdot 3H_2O \cdot 1.5TFA$: C, 38.66; H, 5.57; N, 5.88. Found: C, 38.15; H, 5.16; N, 5.55.

4-Acetamido-*N*-(4-(*N*-butyl-*N*-methylamino)butyl)-5-iodo-2-methoxybenzamide (14d). Benzamide **14d**, prepared according to general procedure 3, was purified by column chromatography on silica with 1:9 methanol/ethyl acetate to give a white solid (0.97 g, 69%). ^{1}H NMR ($CDCl_3$) δ 0.90 (t, J = 7.28, 3H), 1.28−1.63 (m, 8H), 2.20 (s, 3H), 2.27 (s, 3H), 2.35 (m, 4H), 3.46 (m, 2H), 3.97 (s, 3H), 7.64 (s, 1H), 7.68 (t, J = 5.61, 1H), 8.24 (s, 1H), 8.56 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 15.26, 21.92, 26.06, 26.35, 28.77, 30.64, 40.93, 43.47, 57.46, 58.56, 58.82, 79.19, 105.11, 120.12, 142.75, 142.75, 159.50, 164.66, 169.84. MS ES (+ve) *m*/*z*: 476 $(M + 1)^+$. HRMS calcd for $C_{19}H_{31}N_3O_3I$ $(M + 1)^+$, 476.1405; observed $(M + 1)^+$, 476.1404. Anal. Calcd for $C_{19}H_{31}N_3O_3I$: C, 48.00; H, 6.37; N, 8.84. Found: C, 48.10; H, 6.26; N, 8.81.

4-Acetamido-*N*-(4-(2-azanorborn-2-yl)butyl)-5-iodo-2-methoxybenzamide (14e). Benzamide **14e**, prepared according to general procedure 3, was purified by column chromatography on silica with ethyl acetate to give a white solid (0.94 g, 65%) as a 1:1 mixture of endo and exo isomers. ^{1}H NMR ($CDCl_3$) δ 0.90 (t, J = 7.29 Hz, 3H) 1.28−1.69 (m, 8H), 2.20 (s, 6H), 2.35 (m, 4H), 3.46 (m, 2H), 3.92 (s, 3H), 6.80 (dd, J = 1.89, 8.51 Hz, 1H), 7.86 (m, 1H), 7.90 (t, J = 5.61 Hz, 1H), 8.04 (d, J = 8.51 Hz, 1H), 8.56 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 21.49, 21.66, 22.68, 25.11, 25.56, 25.78, 26.33, 26.77, 26.87, 27.59, 34.89, 35.99, 36.61, 37.05, 38.38, 38.46, 50.71, 55.59, 56.30, 58.49, 59.47, 62.44, 64.98, 67.92, 77.19, 77.98, 103.95, 118.28, 118.30, 141.39, 141.42, 141.78, 141.82, 158.48, 163.98, 164.03, 168.62. MS ES (+ve) *m*/*z*: 486 $(M + 1)^+$. HRMS calcd for $C_{20}H_{29}IN_3O_3$ $(M + 1)^+$, 486.1248; observed $(M + 1)^+$, 486.1248.

4-Acetamido-*N*-(4-(butylamino)butyl)-5-iodo-2-methoxybenzamide (14f). 4-Acetamido-*N*-(4-(*N*-*tert*-butoxycarbonyl-*N*-butylamino)butyl)-5-iodo-2-methoxybenzamide (**14a**; 0.7 g, 1.25 mmol) was treated with saturated HCl in anhydrous ethyl acetate to give **14f** as its hydrochloric salt (0.54 g, 93%). ^{1}H NMR (DMSO-d_6) δ 0.89 (t, J = 7.2 Hz, 3H), 1.31 (m, 2H), 1.56 (m, 4H), 1.62 (m, 2H), 2.09 (s, 3H), 2.83 (br m, 2H), 2.87 (br m, 2H), 3.28 (m, 2H), 3.85 (s, 3H), 7.36 (s, 1H), 8.10 (s, 1H), 8.21 (t, J = 6.0 Hz, 1H), 8.79 (br s, 2H), 9.41 (br s). ^{13}C NMR ($CDCl_3$) δ 15.17, 20.99, 24.61, 25.14, 27.94, 29.09, 40.09, 48.04, 57.84, 85.0, 111.74, 123.66, 141.53, 144.47, 158.82, 164.94, 170.30. MS ES (+ve) *m*/*z*: 462 $(M + 1)^+$. Anal. Calcd for $C_{18}H_{28}IN_3O_3 \cdot 3H_2O$: C, 41.94; H, 6.65; N, 8.15. Found: C, 41.67; H, 6.11; N, 8.09.

4-Acetamido-*N*-(2-(diethylamino)ethyl)-2-methoxybenzamide (15). Benzamide **15**, prepared according to general procedure 3, was purified by column chromatography on silica eluting with ethyl acetate to give **15** as a white solid (1.2 g, 66%). ^{1}H NMR ($CDCl_3$) δ 1.05 (t, J = 7.2 Hz, 6H), 2.20 (s, 3H), 2.58 (q, J = 7.2 Hz, 4H), 2.64 (t, J = 6.0 Hz, 2H), 3.55 (dt, J = 4.8, 6.0 Hz, 1H), 3.92 (s, 3H), 6.83 (dd, J = 1.8, 8.5 Hz, 1H), 7.85 (br s, 1H), 8.05 (d, J = 8.5 Hz, 1H). 8.44 (t, J = 4.8 Hz, 1H), 8.73 (br s, 1H). MS ES (+ve) *m*/*z*: 308 $(M + 1)^+$.

4-Amino-*N*-(2-(diethylamino)ethyl)-2-methoxybenzamide (16). Treatment of 4-acetamido-*N*-(2-(diethylamino)ethyl)-2-methoxybenzamide (**15**; 0.5 g, 1.63 mmol) with KOH (0.37 g, 6.5 mmol) in methanol (5 mL) and water (1 mL) yielded benzamide **16** as a pale cream solid (0.32 g, 74%). ^{1}H NMR (DMSO-d_6) δ 1.21 (t, J = 7.2 Hz, 6H), 3.05 (q, J = 7.2 Hz, 4H$_-$), 3.12 (t, J = 6.8 Hz, 2H), 3.61 (dt, J = 6.0, 6.8 Hz, 1H), 5.79 (br s, 2H), 6.18 (dd, J = 1.6, 8.4 Hz, 1H), 6.23 (d, J = 1.6 Hz, 1H), 7.61 (d, J = 8.4 Hz, 1H), 8.26 (t, J = 6.0 Hz). MS ES (+ve) *m*/*z*: 266 $(M + 1)^+$.

4-Amino-*N*-(2-(diethylamino)ethyl)-5-iodo-2-methoxybenzamide (17). Treatment of 4-acetamido-*N*-(2-(diethylamino)ethyl)-5-iodo-2-methoxybenzamide (**1**; 0.45 g, 1.04 mmol) with KOH (0.23 g, 4.17 mmol) in methanol (5 mL) and water (1 mL) yielded benzamide **17** as a white solid (0.37 g, 91%). ^{1}H NMR (DMSO-d_6) δ 0.97 (t, J = 7.2 Hz, 6H), 2.49 (m, 6H), 3.29 (m, 2H), 3.81 (s, 3H), 5.74 (br s, 2H), 6.46 (s, 1H), 8.08 (m, 2H). MS ES (+ve) *m*/*z*: 392 $(M + 1)^+$.

***N*-(2-(Diethylamino)ethyl)-2-methoxy-4-(mesylamino)benzamide (18m).** 4-Amino-*N*-(2-(diethylamino)ethyl)-2-methoxybenzamide (**16**; 59 mg, 0.22 mmol) in anhydrous DCM (10 mL) and dry pyridine (190 μL) was treated with methanesulfonyl chloride (28 mg, 0.24 mmol) at 0 °C for 30 min followed by 48 h at room temperature. Aqueous workup followed by preparative HPLC purification on a C18 column eluting with acetonitrile: (0.1 M) ammonium acetate (65:35) at 15 mL/min gave the benzamide **18m** (R_t = 16 min) as a colorless oil (40 mg, 53%). ^{1}H NMR (CD_3OD) δ 1.20 (t, J = 7.06, 6H), 2.92 (q, J = 7.06 Hz, 4H), 2.95 (t, J = 6.14 Hz, 2H), 3.06 (s, 3H), 3.62 (t, J = 6.14 Hz, 2H), 3.99 (s, 3H), 6.91 (dd, J = 1.85 Hz, 8.57, 1H); 7.02 (d, J = 1.85 Hz, 1H); 7.95 (d, 1H, J = 8.54 Hz). ^{13}C NMR (CD_3OD) δ 10.92, 37.71, 39.73, 49.32, 52.81, 56.54, 102.92, 111.77, 117.30, 133.77, 144.96, 160.33, 168.00. MS ES (+ve) *m*/*z*: 344 $(M + 1)^+$. HRMS calcd for $C_{15}H_{26}N_3O_4S$ $(M + 1)^+$, 344.1641; observed $(M + 1)^+$, 344.1640.

***N*-(2-(Diethylamino)ethyl)-2-methoxy-4-(tosylamino)benzamide (18n).** 4-Amino-*N*-(2-(diethylamino)ethyl)-2-methoxybenzamide (**16**; 0.1 g, 0.38 mmol) in anhydrous DCM (10 mL) and dry pyridine (0.2 mL) was treated with *p*-toluenesulphonyl chloride (0.08 g, 0.42 mmol) at 0 °C for 30 min then stirred at room temperature for 18 h. Aqueous workup followed by preparative RP-HPLC purification with acetonitrile ammonium acetate (0.1 M; 80:20) as the eluent at 16 mL/min gave benzamide **18n** (R_t = 19 min) as a colorless oil (49 mg, 31%). ^{1}H NMR (CD_3OD) δ 1.34 (t, J = 7.30 Hz, 6H), 2.38 (s, 3H), 3.31 (m, 4H), 3.37 (t, J = 6.02 Hz, 2H), 3.76 (t, J = 6.02 Hz, 2H), 3.92 (s, 3H); 6.80 (dd, J = 2.00 Hz, 8.54, 1H) 6.92 (d, J = 2.00 Hz, 1H), 7.33 (d, 2H, J = 8.01 Hz), 7.75 (d, 2H, J = 8.01 Hz), 7.83 (d, 1H, J = 8.54 Hz). ^{13}C NMR (CD_3OD) δ 9.25, 21.48, 36.53, 49.32, 53.31, 56.58, 103.25, 112.23, 116.98, 128.38, 130.81, 138.00, 144.60, 145.59, 160.20, 169.00. MS ES (+ve) *m*/*z*: 420 $(M + 1)^+$. HRMS calcd for $C_{21}H_{30}N_3O_4S$ $(M + 1)^+$, 420.1954; observed $(M + 1)^+$, 420.1956.

***N*-(2-(Diethylamino)ethyl)-5-iodo-2-methoxy-4-(mesylamino)-benzamide (19m).** Treatment of 4-amino-*N*-(2-(diethylamino)-ethyl)-5-iodo-2-methoxybenzamide (**17**; 50 mg, 0.13 mmol) with methanesulphonyl chloride, as in the synthesis of **18n**, gave, after column chromatography on silica and eluting with (1:1) MeOH/ethyl acetate, benzamide **19m** as a colorless oil (28 mg, 47%). ^{1}H NMR (CD_3OD) δ 1.30 (t, J = 7.2 Hz, 6H), 3.05 (s, 3H), 3.21 (q, J = 7.2 Hz, 4H), 3.29 (t, J = 6.0 Hz, 2H), 3.75 (t, J = 6.0 Hz, 2H), 3.97 (s, 3H), 7.28 (s, 1H), 8.35 (s, 1H). MS ES (+ve) *m*/*z*: 470 $(M + 1)^+$. HRMS calcd for $C_{15}H_{25}N_3O_4SI$ $(M + 1)^+$, 470.0611; observed $(M + 1)^+$, 470.0626.

***N*-(2-(Diethylamino)ethyl)-5-iodo-2-methoxy-4-(tosylamino)-benzamide (19n).** Treatment of 4-amino-*N*-(2-(diethylamino)ethyl)-5-iodo-2-methoxybenzamide (**17**; 0.1 g, 0.26 mmol) with *p*-toluenesulphonyl chloride as above gave after column chromatography on silica and eluting with (1:1) MeOH/ethyl acetate gave benzamide **19n** as a colorless oil (0.06 g, 43%). ^{1}H NMR (CD_3OD) δ 1.31 (t, J = 7.2 Hz, 6H), 2.38 (s, 3H), 2.24 (q, J = 7.2 Hz, 4H), 3.29 (t, J = 6.0 Hz, 2H), 3.73 (t, J = 6.0 Hz, 2H), 3.89 (s, 3H), 7.16 (s, 1H), 7.29 (d, J = 8.0 Hz, 2H), 7.68 (d, J = 8.0 Hz, 2H), 8.24 (s, 1H). ^{13}C NMR (CD_3OD) δ 9.1, 21.3, 36.3, 49.4, 52.9, 56.7, 82.3, 108.8, 120.5, 128.4, 130.6, 138.1, 142.8, 143.1, 145.6, 159.6, 167.8. MS ES (+ve) *m*/*z*: 546 $(M + 1)^+$. HRMS calcd for $C_{21}H_{29}N_3O_4SI$ $(M + 1)^+$, 546.0924; observed $(M + 1)^+$, 546.0891.

1-(2-Fluoroethyl)piperidin-4-amine (22). *N*-*tert*-Butoxycarbonylpiperidine (2 g, 10 mmol), bromofluoroethane (1.5 g, 12 mmol), and K_2CO_3 (11 g, 80 mmol) in acetonitrile (40 mL) were heated to reflux for 5 h. Aqueous workup gave the BOC-protected amine **20** as a light yellow solid (2 g, 91%), which was subsequently stirred with TFA (20 mL) for 1 h to give amine **22** (1.8 g, 95%). ^{1}H NMR

(CD_3OD) δ 2.03 (m, 2H), 2.27 (d, J = 13.3 Hz, 2H), 3.24 (t, J = 14.0 Hz, 2H), 3.45 (m, 1H), 3.50 (dt, J = 4.4, 27.7 Hz, 2H), 3.75 (d, J = 13.3 Hz, 2H), 4.85 (dm, J = 47.2 Hz, 2H.). ^{13}C NMR ($CDCl_3$) δ 32.18, 47.00, 52.71, 58.04, 58.24, 81.10, 82.76, 126.03, 128.48, 131.74, 133.50, 165.88. MS ES (+ve) *m/z*: 147 $(M + 1)^+$.

1-(2-Hydroxyethyl)piperidin-4-amine (23). *N-tert*-Butoxycarbonylpiperidine (2 g, 10 mmol), 2-bromoethanol (0.85 mL, 12 mmol), and K_2CO_3 (11.0 g, 80 mmol) were treated as in the synthesis of **22**. Purification by column chromatography on silica and eluting with 1:9 MeOH/DCM gave the BOC-protected amine **21** as a colorless oil (2.3 g, 94%). ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 1.52 (m, 2H), 1.94 (m, 2H), 2.26 (m, 2H), 2.59 (t, J = 5.26, 2H), 2.92 (m, 2H), 3.48 (m, 1H), 3.65 (t, J = 5.26, 2H), 4.76 (d, J = 7.92, 1H). MS ES (+ve) *m/z*: 267 $(M + Na)^+$. Stirring **21** (1.1 g, 4.5 mmol) in TFA (2 mL) at room temperature for 1 h gave **23** as a light yellow solid (1.1 g, 95%). ^{1}H NMR (CD_3OD) δ 2.05 (m, 2H), 2.28 (d, J = 13.6 Hz, 2H), 3.17 (br m, 2H), 3.30 (m, 2H, superimposed), 3.45 (m, 1H), 3.73 (m, 2H), 3.88 (m, 2H). MS ES (+ve) *m/z*: 145 $(M + 1)^+$.

General Procedure 4 for the Preparation of Benzamides 24, 25, 26, and 27. To a solution of 4-iodobenzoic acid or 4-bromobenzoic acid (1 mmol) in DMF (10 mL) was added 1-hydroxybenzotriazole hydrate (HOBT; 1.2 mmol), *N*-methylmorpholine (4 mmol), triethylamine (2 mmol), either 1-(2-fluoroethyl)piperidin-4-amine (**22**; 1 mmol) or 1-(2-hydroxyethyl)piperidin-4-amine (**23**; 1 mmol; as their TFA salts) followed by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC; 1.2 mmol). The reaction was stirred at room temperature for 20 h before it was filtered and evaporated to dryness. Aqueous workup followed by recrystallization gave the corresponding benzamides.

4-Bromo-*N*-(1-(2-fluoroethyl)piperidin-4-yl)benzamide (24). Benzamide (**24**), prepared according to procedure 4, was triturated with DCM and filtered to give a white solid (0.55 g, 68%). ^{1}H NMR ($CDCl_3$) δ 1.6 (m, 2H), 2.05 (m, 2H), 2.27 (dt, J = 2.0, 11.7, 2H), 2.72 (dm, J = 28.1 Hz, 2H), 2.96 (m, 2H), 4.00 (m, 2H), 4.68 (dm, J = 47.6 Hz, 2H), 4.63 (dd, 1H, J = 5.0 Hz), 5.90 (m, 1H), 7.57 (d, J = 8.8 Hz, 2H), 7.62 (d, J = 8.8 Hz, 2H). MS ES (+ve) *m/z*: 351 $(M + Na)^+$. Anal. Calcd for $C_{14}H_{18}FBrN_2O$: C, 51.08; H, 5.51; N, 8.51. Found: C, 51.34; H, 5.45; N, 8.30.

***N*-(1-(2-Fluoroethyl)piperidin-4-yl)-4-iodobenzamide (25).** Benzamide (**25**), prepared according to procedure 4, was recrystallized from DCM to give a white solid (260 mg, 30% yield). ^{1}H NMR (CD_3OD) δ 1.75 (m, 2H), 1.98 (m, 2H), 2.28 (dt, J = 2.3, 12.0 Hz, 2H), 2.76 (dm, J = 28.6 Hz, 2H), 3.05 (m, 2H), 3.90 (m, 2H), 4.65 (dm, J = 47.6 Hz, 2H), 7.60 (d, J = 8.6 Hz, 2H), 7.62 (d, J = 8.6 Hz, 2H). ^{13}C NMR (CD_3OD) δ 32.0, 48.4, 53.8, 58.2, 59.1, 81.5, 83.2, 98.8, 129.9, 135.2, 138.6, 168.8. MS ES (+ve) *m/z*: 399 $(M + 1)^+$. Anal. Calcd for $C_{14}H_{18}FIN_2O$: C, 44.70; H, 4.82; N, 7.45. Found: C, 44.73; H, 5.03; N, 7.26.

4-Bromo-*N*-(1-(2-hydroxyethyl)piperidin-4-yl)benzamide (26). Benzamide (**26**), prepared according to procedure 4, was recrystallized from ethyl acetate to yield a white solid (117 mg, 40%). ^{1}H NMR ($CDCl_3$) δ 1.60 (m, 2H), 2.05 (m, 2H), 2.29 (dt, J = 2.0, 11.98 Hz, 2H), 2.58 (t, J = 5.3 Hz, 2H), 2.96 (m, 2H), 3.61 (t, J = 5.3 Hz, 2H), 4.00 (m, 1H), 6.10 (m, 1H), 7.56 (d, J = 8.8 Hz, 2H), 7.64 (d, J = 8.8, 2H). ^{13}C NMR ($CDCl_3$) δ 31.98, 48.63, 53.92. 59.79, 61.01, 126.71, 130.01, 132.52, 134.70, 168.49. MS ES (+ve) *m/z*: 327 $(M + 1)^+$. Anal. Calcd for $C_{14}H_{19}FBrN_2O_2$: C, 51.39; H, 5.85; N, 8.56. Found: C, 51.61; H, 5.79; N, 8.30.

***N*-(1-(2-Hydroxyethyl)piperidin-4-yl)-4-iodobenzamide (27).** Benzamide (**27**), prepared according to procedure 4, was recrystallized from DCM to yield a white solid (180 mg, 50%). ^{1}H NMR ($CDCl_3$) δ 1.55 (m, 2H), 2.0 (m, 2H), 2.28 (dt, J = 2.0, 11.7, 2H), 2.57 (t, J = 5.3 Hz, 2H), 2.92 (m, 2H), 3.62 (t, J = 5.3 Hz, 2H), 4.00 (m, 1H), 5.95 (m, 1H), 7.47 (d, J = 8.5 Hz, 2H), 7.78 (d, J = 8.5 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 33.56, 48.35, 53.37, 59.10, 60.33, 99.57, 129.68, 135.26, 139.00, 167.28. MS ES (+ve) *m/z*: 375 $(M + 1)^+$. Anal. Calcd for $C_{14}H_{19}FIN_2O_2$: C, 44.93; H, 5.12; N, 7.49. Found: C, 45.21; H, 5.22; N, 7.40.

***N*-(1-(2-Fluoroethyl)piperidin-4-yl)-4-(trimethylstannyl)benzamide (28).** 4-Bromo-*N*-(1-(2-fluoroethyl)piperidin-4-yl)benzamide (**24**; 0.4 g, 1.2 mmol), hexamethylditin (0.6 g, 1.82 mmol), and a catalytic amount of $Pd(PPh_3)_4$ (5 mg) in anhydrous toluene (18 mL) was heated to reflux for 48 h, with further addition of hexamethylditin (0.8 mg) and $Pd(PPh_3)_4$ (20 mg) added after 24 h. The crude black solid was purified by column chromatography on silica eluting with 1:20 MeOH/$CHCl_3$ to give **28** as a yellow oil (200 mg, 40%). ^{1}H NMR (CD_3OD) δ 0.32 (s, 9H), 1.72 (m, 2H), 1.96 (m, 2H), 2.25 (m, 2H), 2.75 (dm, J = 28.6 Hz, 2H), 3.05 (m, 2H), 3.92 (m, 1H), 4.60 (dm, 2H, J = 47.6 Hz), 7.60 (d, J = 8.2 Hz, 2H), 7.77 (d, J = 8.2 Hz, 2H). ^{13}C NMR (CD_3OD) δ 9.96, 32.25, 48.49, 54.10, 59.10, 59.15, 82.20, 83.80, 127.51, 135.50, 136.80, 145.70, 170.00. MS ES (+ve) *m/z*: 415 $(M + 1)^+$. HRMS calcd for $C_{17}H_{27}FN_2O^{116}Sn$ $(M + 1)^+$, 411.1203; observed $(M + 1)^+$, 411.1203.

***N*-(1-(2-Hydroxyethyl)piperidin-4-yl)-4-(trimethylstannyl)-benzamide (29).** *N*-(1-(2-hydroxyethyl)piperidin-4-yl)-4-bromobenzamide (**26**; 0.15 g, 0.46 mmol) hexamethylditin (0.22 g, 0.69 mmol), and $Pd(PPh_3)_4$ (3 mg) were treated as above to give **29** as a yellow oil (59 mg, 32%). ^{1}H NMR (CD_3OD) δ 0.32 (s, 9H), 1.88 (m, 2H), 2.12 (m, 2H), 2.82 (m, 2H), 2.96 (t, J = 5.9 Hz, 2H), 3.40 (m, 2H), 3.82 (t, J = 5.9 Hz, 2H), 4.08 (m, 1H), 7.60 (d, J = 8.2 Hz, 2H), 7.78 (d, J = 8.2 Hz, 2H). ^{13}C NMR (CD_3OD) δ 10.09, 31.92, 48.20, 53.90, 59.62, 60.89, 127.32, 135.26, 136.61, 148.43, 169.87. ES (+ve) *m/z*: 413 $(M + 1)^+$. HRMS calcd for $C_{17}H_{28}FN_2O_2SnNa$ $(M + Na)^+$, 435.1077; observed $(M + Na)^+$, 435.1076.

***N*-(4-(Dipropylamino)butyl)-4-iodobenzamide (30).** Compound **30** was prepared according to literature methods.[15] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, J = 7.2 Hz, 6H), 1.40−1.50 (m, 4H), 1.58−1.70 (m, 4H), 2.43 (m, 4H), 2.52 (t, J = 6.7 Hz, 2H), 3.45 (dt, J = 5.8, 6.2 Hz, 2H), 7.21 (br, 1H), 7.52 (d, J = 8.5 Hz, 2H), 7.77 (d, J = 8.5 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.8, 19.2, 24.3, 27.2, 39.68, 53.2, 55.6, 97.9, 128.6, 134.3, 137.4, 166.8. MS ES (+ve) *m/z*: 403 $(M + 1)^+$.

4-Bromo-*N*-(4-(dipropylamino)butyl)benzamide (31). See general procedure 3. Purification by column chromatography on silica with ethyl acetate gave a colorless oil (1.33 g, 75%). ^{1}H NMR ($CDCl_3$) δ 0.84 (t, J = 7.4 Hz, 6H), 1.43 (m, 4H), 1.55 (m, 2H), 1.62 (m, 2H), 2.38 (m, 4H), 2.46 (t, J = 6.8 Hz, 2H), 3.40 (dt, J = 5.8, 6.0 Hz, 2H), 7.51 (d, J = 8.4 Hz, 2H), 7.65 (d, J = 8.4 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 11.8, 19.3, 24.5, 27.3, 39.8, 53.3, 55.8, 125.6, 128.6, 131.4, 133.7, 166.6. MS ES (+ve) *m/z*: 355 $(M + 1)^+$. HRMS calcd for $(M + 1)^+$, 355.1385; observed $(M + 1)^+$, 355.1384.

***N*-(4-(Diethylamino)butyl)-4-(trimethylstannyl)benzamide (32).** 4-Bromo-*N*-(4-(dipropylamino)butyl)benzamide (**31**; 0.5 g, 1.4 mmol) was treated with hexamethylditin (0.46 mg, 1.4 mmol) and a catalytic amount of $Pd(PPh_3)_4$ (10 mg) in refluxing toluene as described in the synthesis of **28**. Purification by column chromatography, eluting with 1:9 methanol/chloroform, gave the title compound as a colorless oil (0.3 g, 52%). ^{1}H NMR ($CDCl_3$) δ 0.33 (s, 9H), 1.02 (t, J = 7.2 Hz, 6H), 1.70−1.87 (m, 8H), 3.06 (m, 4H), 3.17 (m, 2H), 3.49 (t, J = 6.5 Hz, 2H), 7.62 (d, J = 8.2 Hz, 2H), 7.83 (d, J = 8.2 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 9.8, 11.4, 18.6, 22.6, 27.8, 39.9, 54.1, 56.0, 127.5, 135.2, 136.9, 148.8, 170.5. MS ES (+ve) *m/z*: 441 $(M + 1)^+$. HRMS calcd for $C_{20}H_{37}N_2OSn$ $(M + 1)^+$, 441.1928; observed $(M + 1)^+$, 441.1898.

***N*-(4-(2-Azanorborn-2-yl)butyl)-4-iodobenzamide (33).** See general procedure 3. Purification by column chromatography on silica with 1:4 methanol/chloroform gave **33** as a colorless oil (0.88 g, 55%). ^{1}H NMR ($CDCl_3$) δ (1.6−1.9, m, 10H), 2.05 (m, 1H), 2.65−3.4 (m, 4H), 3.45 (m, 2H), 4.0 (m, 1H), 7.60 (d, J = 8.58 Hz, 2H), 7.87 (d, J = 8.58 Hz). ^{13}C NMR ($CDCl_3$) δ 22.07, 23.13, 24.06, 26.58, 27.43, 27.47, 27.60, 27.65, 30.51, 34.98, 37.41, 37.75, 37.98, 39.69, 51.73, 56.52, 59.97, 61.49, 64.79, 66.94, 99.06, 129.78, 134.91, 138.80, 169.40. MS ES (+ve) *m/z*: 399 $(M + 1)^+$. HRMS: 39.9, 54.1, 56.0, 127.5, 135.2, 136.9, 148.8, 170.5. MS ES (+ve) *m/z*: 441 $(M + 1)^+$. HRMS calcd for $C_{17}H_{24}N_2OI$ $(M + 1)^+$, 399.0933; observed $(M + 1)^+$, 399.0924.

***N*-(4-(2-Azanorborn-2-yl)butyl)-4-bromobenzamide (34).** See general procedure 3. Purification by column chromatography on

silica with ethyl acetate gave **34** as colorless oil (1.1 g, 63%) and as a 1:1 mixture of endo and exo isomers. ^{1}H NMR ($CDCl_3$) δ 1.2−1.9 (m, 10H), 2.35−2.84 (m, 5H), 3.40 (m, 3H), 7.46 (d, J = 8.4, 2H), 7.67 (d, J = 8.4, 2H), 7.78 (s br, 1H). ^{13}C NMR ($CDCl_3$) δ 26.11, 26.36, 27.97, 29.12, 37.27, 38.26, 40.43, 54.45, 60.67, 62.69, 126.96, 130.14, 132.76, 134.94, 168.02. MS ES (+ve) *m*/*z*: 351 $(M + 1)^+$. HRMS calcd for $C_{17}H_{24}N_2OBr$ $(M + 1)^+$, 351.1072; observed $(M + 1)^+$, 351.1062.

***N*-(4-(2-Azanorborn-2-yl)butyl)-4-trimethylstannylbenzamide (35).** *N*-(4-(2-Aza-bicyclo[2.2.1] heptan-2-yl)butyl)-4-bromobenzamide (**34**; 0.46 g, 1.31 mmol) was treated with hexamethylditin (0.64 g, 1.97 mmol) and a catalytic amount of $Pd(PPh_3)_4$ (10 mg) in refluxing dioxane as described above. Purification by column chromatography on silica, using 2% MeOH in $CHCl_3$, gave **35** as a pale yellow oil (0.42 g, 64%) and as a 1:1 mixture of endo and exo isomers. ^{1}H NMR ($CDCl_3$) δ 0.29 (s, 9H), 1.61 (m, 2H), 1.72−1.77 (m, 4H), 1.90−1.97 (m, 2H), 2.08 (m, 1H), 2.29 (br m, 1H), 2.65 (br s, 1H), 2.99 (m, 2H), 3.15 (m, 2H), 3.50 (m, 2H), 3.95 (br s, 1H), 7.55 (d, J = 8.0 Hz, 2H), 7.79 (t, J = 5.6 Hz, 1H), 9.22 (d, J = 8.0 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ −8.3, 23.1, 27.5, 37.4, 39.2, 61.2, 65.8, 127.8, 134.6, 137.1, 148.7, 169.3. MS ES (+ve) *m*/*z*: 437 $(M + 1)^+$. HRMS calcd for $C_{20}H_{33}N_2OSn$ $(M + 1)^+$, 437.1623; observed $(M + 1)^+$, 437.1622.

Radiolabeling using Tl(TFA)$_3$. To a solution of the benzamide precursor (**13b**−**13f**, **15**, **18m**, and **18n**; 0.25 mg, ∼0.8 μmol) in TFA (100 μL) was added $Tl(TFA)_3$ (26 M in TFA, 150 μL). After 20 min, the resulting thallium complex solution was added to a dried sample of [^{123}I]INa (400−500 MBq) in a Wheaton conical vial. The reaction mixture was allowed to stand for 15 min before it was neutralized with concentrated NH_3 (1 mL) and then purified by solid-phase extraction (RP-SPE). The labeled compound was washed off the cartridge with EtOH (1 mL), was dried in vacuo, and then mobile phase (500 μL) was added in preparation for further HPLC purification with conditions, as described in Table 1. The radiolabeled compound was collected, dried in vacuo, and then formulated in saline for biological evaluation.

Radiolabeling using Chloramine-T (CAT). To a solution of the trimethylstannyl precursor (**28**, **29**, **32**, or **35**; 0.25 mg, 0.6 μmol) in ethanol (200 μL) was added [^{123}I]INa (400−500 MBq), CAT (4.5 mM, 100 μL), and HCl (1 M, 100 μL). After 5 min at room temperature, $Na_2S_2O_5$ (260 mM, 100 μL), $NaHCO_3$ (650 mM, 100 μL), followed by HPLC mobile phase (350 μL) were added. The resulting solution was then purified by HPLC with conditions, as described in Table 1. The radiolabeled compound was collected, dried in vacuo, and then formulated in saline for biological evaluation.

Radiolabeling of BZA$_2$. No carrier added [^{123}I]BZA_2 was synthesized using a similar procedure as described in the radiosynthesis of carrier added [^{125}I]BZA_2.[14] A solution of 2-bromo-*N*-(2-(diethylamino)ethyl)benzamide[29] (1.3 mg), synthesized from 2-bromobenzoyl chloride and *N*,*N*-diethylethylenediamine using step 2 of general procedure 3, in sodium acetate buffer (200 μL of 0.16 M NaOAc in 0.7% acetic acid) was added to a dried sample of [^{123}I]INa (800 MBq) in a Wheaton conical vial and heated to 130 °C for 30 min. The reaction was cooled and mobile phase added. The resulting solution was then purified by HPLC with conditions as described in Table 1. The radiolabeled compound was collected, dried in vacuo, and then formulated in saline for biological evaluation.

Lipophilicity. The lipophilicity of the compounds was assessed using RP-HPLC by determining the $\log P_{7.5}$ value using literature procedures.[30] Samples, dissolved in methanol, were analyzed using a C18 column (RP C18, Xterra, 5 μm; 4.6 × 150 mm) and a mobile phase consisting of MeOH and phosphate buffer (0.1 M, pH 7.5), 50:50 v/v with a flow rate of 1 mL/min. The $\log P_{7.5}$ of a studied compound was estimated by a comparison of its retention time to that of standards of known log P values.

Ligand Binding Assays. The σ_1 and σ_2 binding affinities of unlabeled benzamides were determined in in vitro binding assays (Novascreen Biosciences, Baltimore, U.S.A.) according to literature methods.[31] The percentage of inhibition of the specific binding to σ_1-receptors of [^{3}H]-(+)-pentazocine and to σ_2-receptors of [^{3}H]-DTG were determined in competitive binding assays at 10^{-5} M concentration of unlabeled benzamides. Then the inhibition constant (IC_{50}) for σ receptors of selected benzamides was determined by incubating, in duplicate, aliquots of diluted guinea pig brain membrane preparation at 25 °C for 2 h with concentrations of benzamide ranging from 10^{-10} to 10^{-6} M in 50 nM Tris-HCl (pH 8.0) with [^{3}H]-(+)-pentazocine (2 nM) for σ_1-receptors or with [^{3}H]-DTG (2 nM) and (+)-pentazocine (100 nM) for σ_2-receptors. In both cases, nonspecific binding was determined in the presence of haloperidol (1 μM). Then incubations were terminated by rapid filtration through Whatman GF/B glass fiber. Filters were immediately washed with ice-cold buffer and measured in a β-scintillation counter to determine the amount of bound radioactivity. The IC_{50} values were then converted to apparent K_i values using the Cheng−Prussof equation and radioligand K_d values.

Biodistribution Studies. The animal experiments were performed in compliance with the NHMRC Australian Code of Practice for the care and use of animals for scientific purposes. Female C57BL/6J mice were obtained from Animal Resources Centre, Western Australia. Biodistribution time-course studies were performed in these mice bearing the B16F0 murine melanoma tumor model. For inoculation, melanoma cells, obtained from European Collection of Cell Cultures (UK), were resuspended in Ca^{2+} and Mg^{2+} free PBS at 3×10^6 viable cells per mL and 0.1 mL was subcutaneously injected in the left flank of 6−7 week old mice. Ten days later, 98−100% of the animals had developed tumors.

The [^{123}I]-labeled benzamide derivatives (0.37−0.74 MBq, 100 μL) were injected intravenously via the tail vein into mice (15−18 g). Time points of 1, 3, 6, 24, 48, and 72 h after injection were chosen for determining the distribution of each compound in various organs and tissues. At defined times postinjection, groups of mice (n = 5) were weighed, sacrificed by CO_2 administration followed by cervical dislocation and dissected. Selected organs were weighed and their radioactivity measured with a γ-counter. The remaining activity in the carcass was also determined to obtain the total activity in the mouse at defined time points. The fraction of injected activity (%ID) in the organ was calculated by comparison with suitable dilutions of the injected dose. Then the radioactivity concentration in the organ (%ID/g) was found by dividing the %ID for each organ by the weight of the organ. The SUV in the tumor at the time of sacrifice (SUV_t) was calculated for each animal by dividing the tumor concentration by the radioactive concentration in the mouse according to the following formula:

$$SUV_t = [\text{activity at time } (t)/\text{tumor } (g)]/[\text{remaining activity at time } (t)/\text{weight of animal } (g)]$$

Competition Studies. Blocking studies were performed to examine the in vivo uptake mechanism of the [^{123}I]benzamides in the tumor. The blocking effect of haloperidol, a nonselective σ_1-σ_2 inhibitor, on the tracer uptake over 24 h was examined in various organs and tumor tissues. Groups (n = 5) of mice bearing B16F0 murine melanomas were injected intravenously with 1 mg/kg of haloperidol 5 min prior to injection of each radiotracer (0.37−0.74 MBq) dose. The mice were sacrificed at 1 and 24 h postinjection, and tissues were handled as described for the biodistribution studies. Radioactive concentrations in the organs and the tumor of treated animals were compared to the controls. Statistical significance was evaluated using one-way ANOVA. The criterion for significance was $p < 0.01$.

SPECT Imaging. SPECT imaging was performed using a high resolution γ-camera (X-SPECT, Gamma Medica Inc., U.S.A.) designed for laboratory animals equipped on an array of discrete 2 × 2 × 6 mm NaI(Tl) crystals optically isolated from each other and a high-resolution parallel hole collimator that has a 12.5 × 12.5 cm field of view. The mouse was anaesthetized via inhalant isoflurane (Forthane brand) in 200 mL/min oxygen via a nose cone fitted to the animal bed and imaged for 10−20 min at 1, 24, and 48 h after injection of 7−10 MBq of the studied [^{123}I]**14d** and [^{123}I]-**25**.

Supporting Information Available: Table of analytical analysis and HPLC purity test of target compounds **14b**, **14c**, **14d**, **14e**, **14f**, **19m**, **19n**, **25**, **27**, and **33**. This material is available free of charge via the Internet at http://pubs.acs.org.

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