Evaluation of Radiolabeled (Hetero)Aromatic Analogues of *N*-(2-diethylaminoethyl)-4-iodobenzamide for Imaging and Targeted Radionuclide Therapy of Melanoma

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Targeted radionuclide therapy using radioiodinated compounds with a specific affinity for melanoma tissue is a promising treatment for disseminated melanoma, but the candidate with the ideal kinetic profile remains to be discovered. Targeted radionuclide therapy concentrates the effects on tumor cells, thereby increasing the efficacy and decreasing the morbidity of radiotherapy. In this context, analogues of *N*-(2-diethylaminoethyl)-4-iodobenzamide (BZA) are of interest. Various (hetero)aromatic analogues **5** of BZA were synthesized and radioiodinated with ^{125}I, and their biodistribution in melanoma-bearing mice was studied after i.v. administration. Most [^{125}I]**5**-labeled compounds appeared to bind specifically and with moderate-to-high affinity to melanoma tumor. Two compounds, **5h** and **5k**, stood out with high specific and long-lasting uptake in the tumor, with a 7- and 16-fold higher value than BZA at 72 h, respectively, and kinetic profiles that makes them promising agents for internal targeted radionuclide therapy of melanoma.

Introduction

In recent years, there has been a dramatic worldwide increase in the incidence of malignant melanoma, which is fast becoming a major public health problem in many countries.[1] It displays a strong tendency to metastasize, and patients with metastases at stage IV have an average survival of only a few months.[2] There is therefore an urgent need to find ways to treat disseminated melanoma. Standard therapies such as external beam therapy or monochemotherapy with a response rate of around 10% are of very limited value.[3] A higher response rate of up to 40% can be obtained with combined treatment schedules but without significant prolonged survival in randomized studies.[4] Globally, the response rate is still rather low, and there has been no progress in systemic palliative melanoma treatment for close on three decades.[5,6] The lack of specific therapy has steered research on melanoma treatment toward vaccine-based immunotherapy and targeted chemotherapy approaches.[7] Targeted internal radionuclide therapy would be an effective alternative. The search for a radiopharmaceutical with a selective affinity for melanoma tissue is therefore of utmost importance both for the early detection of local recurrences to allow efficient surgical excision[1] and for treatment of in-transit or developed metastases. Different melanoma targets and carriers for radionuclides are currently being investigated. Peptide analogues of α-melanocyte stimulating hormone (α-MSH[a]) targeting the melanocortin-1 receptor have recently been developed. Using SPECT/CT equipment, ^{99m}Tc and ^{111}In-labeled peptides provide good quality imaging in melanoma-bearing mice.[8] For application in systemic radionuclide therapy, promising experimental results in melanoma-bearing mice have been reported with one of them conjugated with ^{188}Re,[9] which is a β emitter, and with ^{212}Pb,[10] which is an α particle emitting radionuclide[11] with even greater therapeutic efficacy. However, these are only preliminary results, and high kidney concentrations may result in a problematic renal toxicity.

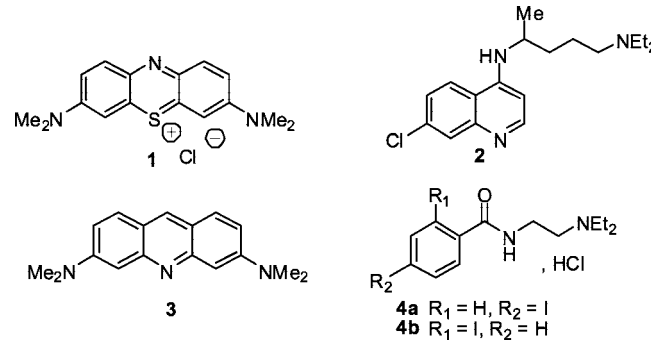
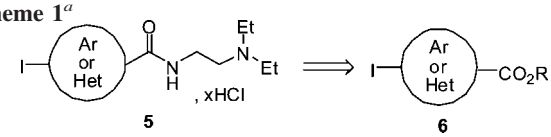


Figure 1. Some structures with a high affinity for melanin.

Scheme 1[a]



[a] Ar or Het = naphthalene, pyridine, indole, benzo[*b*]furan, benzo[*b*-]thiophene, imidazo[1,2-a]pyridine, benzimidazole, quinoline, quinolone, isoquinoline, quinoxaline, 1,6-naphthyridine.

Melanin pigment can itself be a specific target for melanoma tissue. Melanin is an amorphous, irregular polymer composed of intimate mixtures[12] of two separate but biogenetically related pigments, namely eumelanins and pheomelanins, with different degrees of oxidation.[13] The eumelanin chromophore is formed by polymerization of dihydroxyindole (DHI) monomer units and its 2-carboxyl derivative dihydroxyindole-2-carboxylic acid (DHICA), while the pheomelanin is produced by the tyrosinase-mediated copolymerization of L-3,4-dihydroxyphenylalanine (L-dopa) and L-cysteine.[14]

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[a] Abbreviations: BZA, *N*-(2-diethylaminoethyl)-4-iodobenzamide; α-MSH, α-melanocyte stimulating hormone; SPECT/CT, single photon emission computed tomography/computed tomography; PET, positron emission tomography; DHI, dihydroxyindole; DHICA, dihidroxyindole-2-carboxylic acid; MTB, methylene blue; SAR, structure–activity relationship; DMAP, 4-dimethylaminopyridine; PBS, phosphate buffer solution.

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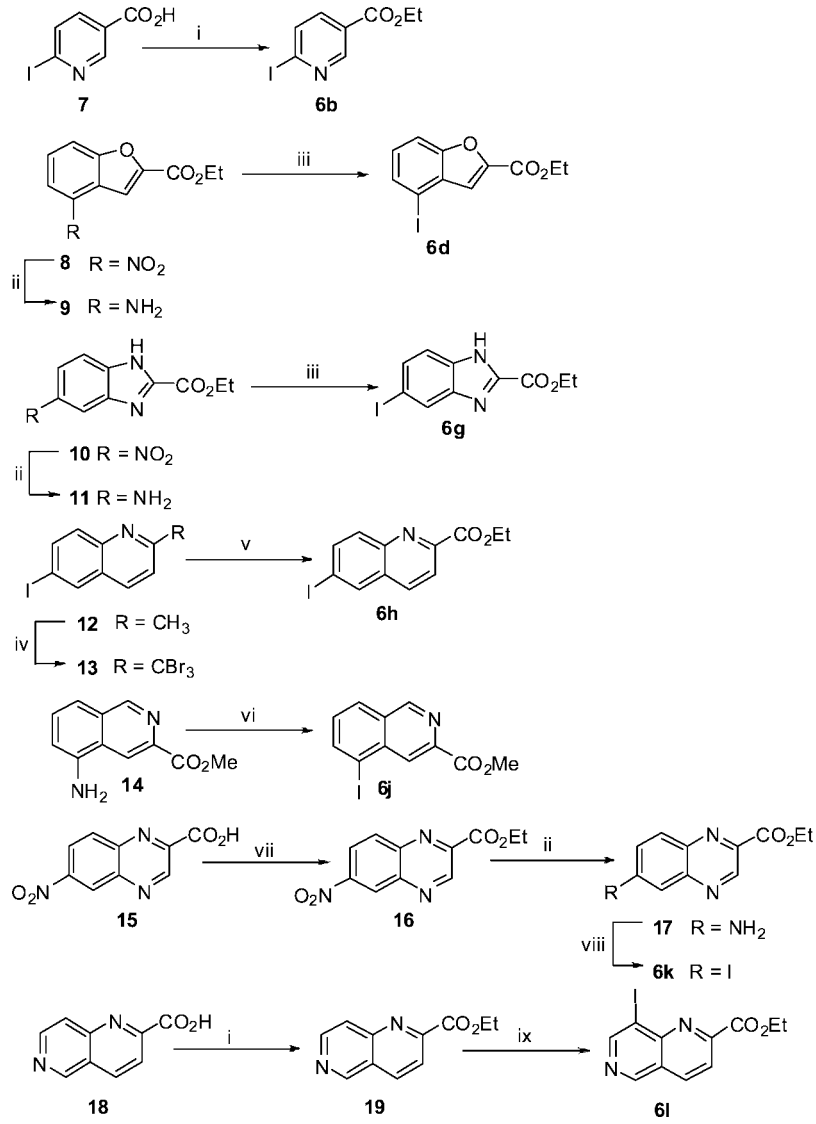
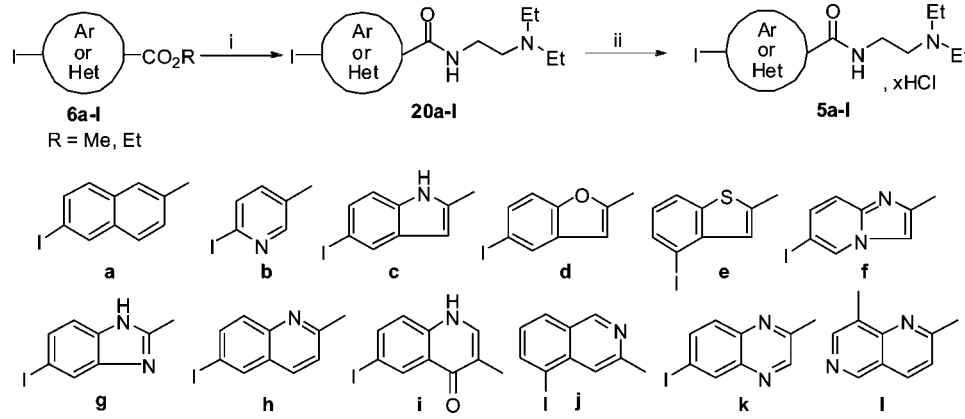
Scheme 2. Synthetic Pathways for Esters **6b**,**d**,**g**,**h**,**j**−**l**[a]



[a] Reagents and conditions: (i) ethyl chloroformate, NEt_3, DMAP, CH_2Cl_2, 0 °C then reflux; (ii) H_2, 10% Pd−C, 1 atm; (iii) (a) $NaNO_2$, HBF_4, 0 °C, (b) KI, H_2O, Δ; (iv) (a) AcONa, AcOH, 75 °C, (b) Br_2, AcOH, reflux; (v) $AgNO_3$, EtOH, H_2O, reflux; (vi) (a) $NaNO_2$, HCl, HBF_4, 0 °C, (b) KI, H_2O, 80 °C; (vii) H_2SO_4, EtOH, reflux; (viii) (a) $NaNO_2$, HBF_4, 0 °C, (b) KI, H_2O, 50 °C; (ix) PTSA, NIS, −10 °C then rt.

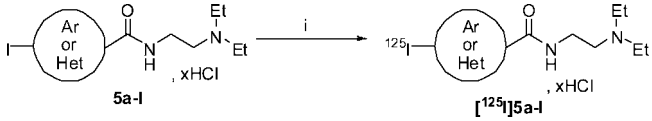
Scheme 3[a]



[a] Reagents and conditions: (i) (a) *N*,*N*-diethylethylenediamine, $AlMe_3$, CH_2Cl_2, 0 °C, (b) reflux; (ii) 2N HCl ether, rt.

Many drugs bind to melanin both in vivo and in vitro: polycyclic aromatic compounds such as methylene blue MTB (**1**), chloroquine (**2**), or acridine orange (**3**), and especially those with coplanar fused rings, all bind strongly with melanin[15] (Figure 1). This affinity is probably due to the interaction between the aromatic rings of the drugs and the aromatic rings of the melanin subunits. Studies performed with labeled MTB (**1**) revealed stable, exceptionally high concentrations in pig-

Scheme 4. Radiolabeling of Compounds **5a**−**l**[a]



[a] Reagents and conditions: (i) (a) citrate buffer solution, 0.5% aq $CuSO_4$, [^{125}I]NaI, Δ, 30-60 min, (b) 2N HCl ether, rt.

mented melanoma in vivo.[16] Radiohalogenated MTB (iodine-123 or iodine-131, γ emitters) was used with some success in a clinical trial for early detection of melanoma metastases,[17] while α emitting astatine-211 MTB has proven particularly effective in inhibiting growth of human cutaneous pigmented melanomas, such as xenografts in athymic mice, and their spontaneous lymph node metastases.[18] However, with α-particles delivering densely ionizing radiation, their decay should result in highly localized energy deposition which needs a highly specific accumulation in tumor tissue.[19] Another class of aromatic compounds with a cationic site such as *N*-(2-diethylaminoethyl)iodobenzamide (**4**) were developed as potent melanoma-seeking agents. Two of these compounds **4a**,**b**, named respectively BZA and BZA_2, have been clinically evaluated as suitable imaging agents for use in nuclear medicine with very encouraging results for metastases imaging.[20] The intracellular localization of BZA within melanin-containing structures has been clearly visualized with analytical imaging by secondary ion mass spectrometry.[21] Furthermore, interaction studies on BZA and synthetic melanin revealed the presence of two classes of binding sites, one ionic and the other hydrophobic.[22]

We have previously reported on spermidine benzamide derivatives, which possess high in vitro affinity for melanin but poor in vivo tumor concentration.[23] More recently, various iodinated benzamides were evaluated[24] by taking into account the optimized lipophilic side chain (already reported by us)[25] and the substitution pattern on the benzene ring developed previously.[26] These studies showed original in vivo distribution profiles with certain molecules showing a higher, more specific tumor uptake that was promising for imaging but still needed improvement for radionuclide therapy.

Here, within the framework of our continuing efforts to discover new molecular derivatives for targeted radionuclide therapy with strong, specific, and long-lasting uptake in melanoma tissues, we examined the structure−activity relationships (SAR) and biological properties in B16 melanoma-bearing mice of aromatic or heteroaromatic BZA analogues. Our strategy involved the incorporation of aromatic or heteroaromatic structures in place of the benzene moiety to take advantage of polycyclic aromatic compounds[15] that display a strong affinity for melanin, while the lipophilic side chain was kept identical to the parent compound BZA. For these studies, various iodo(hetero)aromatic fused ring systems with a carboxamide part **5** were easily synthesized from corresponding iodoesters **6** (Scheme 1). To facilitate the radiolabeling, iodine was preferably introduced on a benzene ring when possible.

Results and Discussion

Synthesis. The following iodoarylesters were prepared according to the literature: methyl 6-iodo-2-naphthoate (**6a**),[27] ethyl 5-iodoindole-2-carboxylate (**6c**),[28] methyl 4-iodobenzo[*b*]-thiophene-2-carboxylate (**6e**),[29] ethyl 6-iodoimidazo[1,2-*a*]pyridine-2-carboxylate (**6f**),[30] and ethyl 1,4-dihydro-6-iodo-4-oxoquinoline-3-carboxylate (**6i**).[31] The synthetic methods for preparation of ester intermediates **6b**,**d**,**g**,**h**,**j**−**l** are illustrated in Scheme 2.

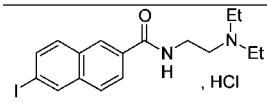
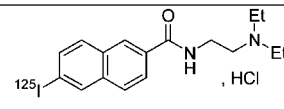
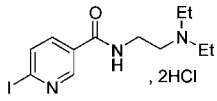
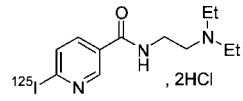
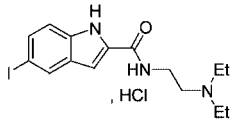
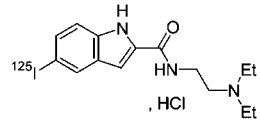
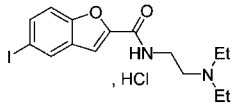
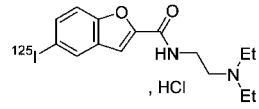
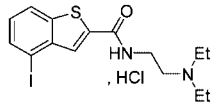
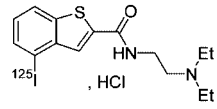
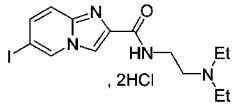
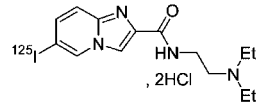
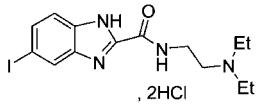
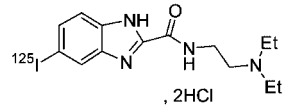
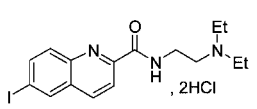
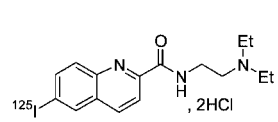
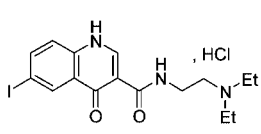
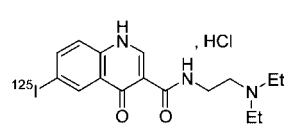
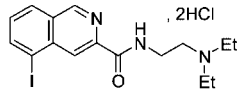
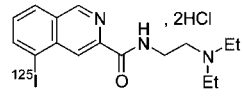
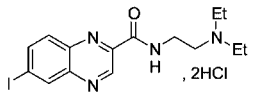
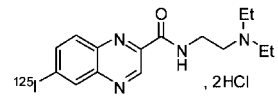
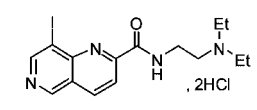
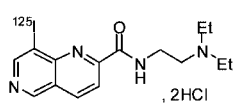
Esterification of 6-iodonicotinic acid[32] (**7**) was performed using mild conditions[33] in the presence of ethyl chloroformate, 4-dimethylaminopyridine (DMAP), and triethylamine to give nicotinic ester **6b** in 76% yield. Ethyl esters **6d** and **6g** were obtained in two steps from commercially available compound **8** and nitro derivative **10**,[34] respectively, following a previously reported synthetic route developed in the benzimidazole series.[35] Catalytic hydrogenation of nitro compounds **8** and **10** yielded ethyl aminoesters **9**[36] and **11**,[37] which were diazotized in fluoroboric acid to give diazonium borofluorides. Decomposition of these diazonium salts with potassium iodide gave iodoesters **6d** and **6g** with low yields (21% and 12%, respectively). The quinoline analogue **6h** can be prepared by the method reported for the preparation of ethyl 6-chloroquinaldinate.[38] Typically, ethyl 6-iodoquinoline-2-carboxylate (**6h**) was obtained from 6-iodoquinaldine[39] (**12**) in two steps, including bromination to **13** and final conversion to ester compound **6h** with silver nitrate in aqueous ethanol, with an overall yield of 46%. The iodoisoquinoline ester **6j** was prepared from methyl 5-aminoisoquinoline-3-carboxylate[40] (**14**) by a diazotization reaction in aqueous hydrochloric acid followed by treatment with fluoroboric acid to give the corresponding diazonium borofluoride. This salt was converted into **6j** in 25% yield according to the procedure described for compounds **6d**,**g**. The usual route was followed to prepare quinoxaline **6k** from 6-nitroquinoxaline-2-carboxylic acid[41] (**15**). Esterification using a common method gave ethyl ester **16**, which was reduced by catalytic hydrogenation to yield the amine **17**. Diazotization in fluoroboric acid followed by treatment with potassium iodide gave ethyl 6-iodoquinoxaline-2-carboxylate (**6k**). In this case, the diazonium salt was not isolated. Finally, the iodonaphthyridinic ester **6l** was obtained by treating the ethyl 1,6-naphthyridine-2-carboxylate (**19**) with *N*-iodosuccinimide in the presence of catalytic *p*-toluenesulfonic acid. Ester **19** was itself prepared from 1,6-naphthyridine-2-carboxylic acid (**18**)[42] in 45% yield by the method used for ester **6b**.

The synthesis of the final compounds **5a**−**l** is depicted in Scheme 3. Esters **6a**−**l** were coupled with *N*,*N*-diethylethylenediamine in the presence of trimethylaluminium to give the corresponding amides **20a**−**l**. To facilitate pharmacological studies, all the amides **20a**−**l** were converted into their hydrochloride salts **5a**−**l** for better solubility in water, using a 2N HCl/ether solution.

Radiolabeling. The radiolabeling of compounds **5a**−**l** with no-carrier-added [^{125}I]NaI was performed using an isotopic exchange reaction in acidic medium as previously described for BZA[43] (Scheme 4) to give, after purification through an Extrelut column, the radioiodinated compounds [^{125}I]**5a**−**l** in 22−92% radiochemical yields. Specific activity was in the range 4.7−36.3 MBq/μmol. Reaction conditions and radiochemical data are reported in Table 1.

In Vitro Binding to Melanin and Partition Coefficients. As indicated, our main challenge was to enhance affinity for melanin. Except for compound **5b**, the (hetero)aromatic analogues **5** synthesized displayed a strong affinity (45−88%) for synthetic melanin in water, as reported in Table 2. When melanin was suspended in PBS, these values decreased strongly for compound **5f** and were comparable to the values obtained with BZA,[25] probably due to the ionic strength of PBS.[22] For the other compounds, molecular binding to melanin was less affected in PBS, ranging from 35% to 59%. A plausible explanation for these variations in molecule binding in PBS observed with identical *N*-alkyl side chains is a π stacking interaction independent of both pH and ionic strength. The

Table 1. Chemical and Radiochemical Data for Compounds [^{125}I]**5a**−**l**

Compounds	Temperature (time)	Products	Yield[a] (%)	Purity[b] (%)	Specific radioactivity (MBq/μmol)
 **5a**	150 °C (1 h)		77	97	15.0
 **5b**	120 °C (50 min)		39	98	9.4
 **5c**	150 °C (1h)		75	98	15.2
 **5d**	150 °C (1 h)		81	99	17.8
 **5e**	150 °C (1 h)		73	99	7.1
 **5f**	150 °C (1 h)		68	98	13.6
 **5g**	150 °C (1 h)		22	95	4.7
 **5h**	150 °C (1 h)		92	99	14.7
 **5i**	150 °C (1 h)		54	99[c]	5.6
 **5j**	150 °C (1 h)		43	96	36.3
 **5k**	150 °C (1 h)		68	99	11.7
 **5l**	130 °C (30 min)		69	98[d]	15.0

[a] Radiochemical yields were calculated by dividing the radioactivity in the final filtered product by the initial amount of radioactive sodium iodide. [b] Radiochemical purities were determined by TLC (Al_2O_3, CH_2Cl_2/EtOH (97/3, v/v)). [c] TLC conditions (Al_2O_3, CH_2Cl_2/EtOH (9/1, v/v)). [d] TLC conditions (Al_2O_3, EtOAc).

Table 2. In Vitro Binding to Melanin and log *P* of [^{125}I]**5a**−**l** Derivatives Compared with BZA

compounds	% bound to synthetic melanin H_2O/PBS			log P^a
BZA[b]	78.6	/	13.8	1.34
5a	80.8	/	50.9	1.33
5b	28.1	/	3.5	−0.29
5c	79.8	/	43.1	1.69
5d	87.0	/	45.2	1.57
5e	87.6	/	56.1	1.54
5f	75.3	/	11.6	0.71
5g	84.5	/	47.1	1.47
5h	87.4	/	43.2	1.87
5i	86.7	/	59.0	1.30
5j	87.0	/	45.2	1.65
5k	67.4	/	47.3	1.12
5l	44.8	/	35.3	0.51

[a] *P*: partition coefficient between *n*-octanol and phosphate buffer solution (pH 7.4). [b] BZA values were obtained from previous work.[25]

Table 3. Urinary and Fecal Excretions[a] of Radioactivity Following Injection of [^{125}I]**5a**−**l** Derivatives in B16 Melanoma-Bearing Mice Compared with BZA

compounds	urinary %ID 0−72 h	fecal %ID 0−72 h
BZA[b]	83.1	4.8
5a	43.7	26.7
5b	67.3	5.3
5c	21.5	61.7
5d	21.4	73.0
5e	52.6	46.7
5f	36.8	43.5
5g	17.1	40.7
5h	80.7	11.4
5i	23.8	69.2
5j	49.9	21.2
5k	70.2	18.0
5l	68.9	29.4

[a] Cumulative excretions (two mice for each compound). [b] BZA values were obtained from previous work.[25]

lipophilicity values (log *P*) of the studied compounds, which are summarized in Table 2, seem to some extent related to melanin binding affinity in water. However, the influence of PBS on the melanin binding differed between compounds, with no correlation to lipophilicity values.

Pharmacokinetic Profiles. Compound excretion showed a broad panel of variations (Table 3). First, in terms of rapidity, although elimination was almost total after 72 h for most of the compounds (range 90−100%), around 40% of the radioactivity of compound **5g** was still present in the mouse body at this time point. Second, concerning the elimination route, some compounds exhibited mainly urinary elimination, like BZA.[25] This was the case for the remarkable compounds **5h** and **5k**. For derivatives **5c**, **5d**, **5g**, and **5i**, the major route appeared to be faecal excretion, while **5e** and **5f** showed balanced distribution between the two routes.

For all the [^{125}I] labeled compounds evaluated in melanoma-bearing mice, tissue distribution demonstrated a significant tumor uptake (Table 4). For all the studied compounds, there was accumulation in B16 melanoma as from 1 h postinjection (p.i.) (≥4.6% ID/g), with the three compounds **5h**, **5k**, and **5l**, demonstrating a very high tumor uptake (≥17% ID/g). We noted a similar radioactivity uptake profile in the pigmented structures of the eyes (uvea, see Supporting Information). These results were consistent with a melanin binding uptake mechanism previously described for BZA.[21] The compound **5i** exhibited the lowest tumor concentration for short times, but the value was noticeably constant during the study and remained significant after 72 h. Tumor labeling continued to be measurable for all compounds at this 72 h time point. Compounds **5e**, **5f**, and

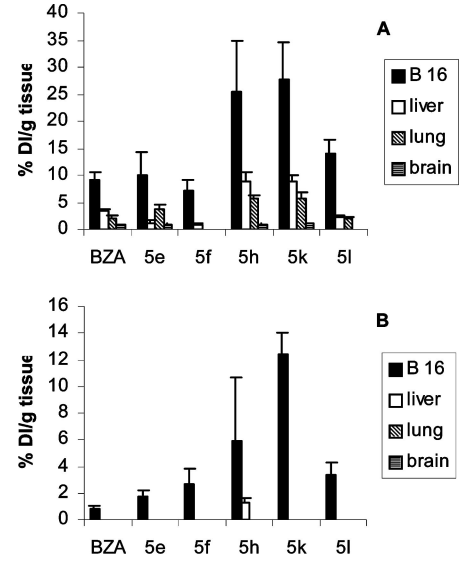


Figure 2. Concentrations of four new [^{125}I] aromatic compounds compared with BZA in selected tissues after i.v. administration in B16 melanoma-bearing mice. (A) at 3 h. (B) at 72 h. Mean ± SD (two mice, *n* determinations for each compound at each time). Absence of value: organ concentration was equal to background value.

5l exhibited remarkable in vivo behavior, with significant tumor uptake and very high specificity illustrated by a rapid release from nontarget tissues, leading to high values of tumor to nontarget organ activity ratios and rapidly unquantifiable ratios when organ concentration was within the range of the background value (Figure 2, Table 5). These promising profiles made these compounds good candidates for scintigraphic imaging. Compounds **5h** and **5k**, which possessed a quinoline moiety and a quinoxaline moiety, respectively, demonstrated very long-lasting tumor concentrations, respectively 7-fold and 16-fold higher values than BZA at 72 h, lending them a kinetic profile favorable for application as a vector for radionuclide therapy.

Current radionuclide therapy in humans is based almost exclusively on high-energy β particle-emitting isotopes[44] such as ^{32}P,^{89}Sr, ^{90}Y,^{131}I, ^{177}Lu, ^{153}Sm,^{166}Ho,^{186}Re, and ^{188}Re. The most widely used radionuclide is iodine-131 ($T_{1/2}$ 8.02 days), which emits both a γ ray of 365 keV for diagnostic purposes and a β particle of 606 keV for treatment. With a maximum tissue range of 2−3 mm due to its relatively low β energy, ^{131}I could be used for neoadjuvant or adjuvant treatment of melanoma metastases. For some of our new compounds, the biological half-life calculations showed significantly higher values compared with BZA, leading to high values of tumor irradiation when extrapolated for ^{131}I use (Table 4). For compound **5k**, an administered dose of 37 MBq will allow the delivery of 49 Gy to the tumor, i.e., a usefully high dose. As illustrated in Figure 2, these high affinities for tumor tissue were particularly selective, given the low amounts of activity in other major organs after 3 h. For compound **5k**, no measurable activity in liver, lung, or brain tissues was detected at 72 h. Such profiles offer high tumor/nontarget organ ratios (Table 5).

The scintigraphic imaging performed on one mouse after i.v. injection of **5k** is reported in Figure 3. The images illustrate the specific affinity for the tumor and a rapid clearance from nontarget organs, allowing a well-defined imaging of the tumor until 72 h postadministration.

On the basis of these results, compound **5k** presented the most favorable in vivo behavior as a vector for application in radionuclide therapy, displaying high and long-lasting tumor

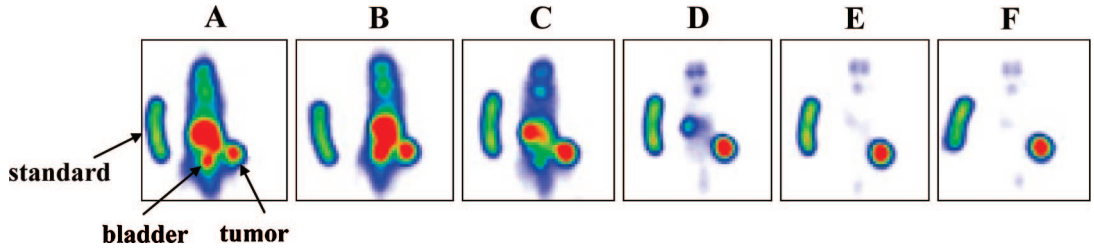


Figure 3. In vivo kinetic of compound [^{125}I]**5k** in a B16 melanoma-bearing mouse illustrated by repeated planar scintigraphic images using a dedicated γ imager for small animals at 1 h (A), 3 h (B), 6 h (C), 24 h (D), 48 h (E), and 72 h (F) after i.v. injection of a 3 MBq dose (acquisition time 10 min).

Table 4. Tumor Uptake in B16 Melanoma-bearing Mice of [^{125}I]**5a**−**l** Compared with BZA at Various Times after i.v. Injection (%ID/g),[a] Tumor Biological Half-Life, and Delivered Dose Calculated for a ^{131}I Labeling

	%ID/g of B 16 at different times					tumor parameters	
cmpds	1 h	3 h	6 h	24 h	72 h	biological half-life (h)	delivered dose[b] Gy/MBq
BZA[c]	9.5 ± 1.9	9.1 ± 1.6	7.7 ± 3.25	3.7 ± 1.2	0.8 ± 0.3	19.6	0.27
5a	13.0 ± 2.7	11.4 ± 2.2	9.0 ± 0.72	7.0 ± 3.9	1.5 ± 0.5	24.0	0.44
5b	15.5 ± 1.7	9.3 ± 1.5	7.0 ± 0.96	2.7 ± 0.7	0.9 ± 0.4	19.0	0.42
5c	8.3 ± 3.1	10.2 ± 1.4	9.8 ± 0.9	8.2 ± 4.4	0.8 ± 0.3	20.1	0.24
5d	8.8 ± 3.4	7.5 ± 4.3	7.8 ± 1.8	5.0 ± 0.5	2.0 ± 0.4	34.0	0.40
5e	12.5 ± 4.8	10.0 ± 4.3	8.7 ± 4.1	7.3 ± 2.3	1.7 ± 0.5	26.7	0.46
5f	5.7 ± 3.0	7.1 ± 1.9	8.1 ± 3.4	8.0 ± 2.4	2.7 ± 1.1	52.6	0.37
5g	14.8 ± 4.8	16.1 ± 2.9	9.9 ± 3.8	7.1 ± 2.0	4.5 ± 2.1	42.9	0.82
5h	19.6 ± 9.0	25.4 ± 9.6	17.2 ± 4.6	12.1 ± 5.2	5.9 ± 1.7	38.0	1.00
5i	4.7 ± 1.2	4.1 ± 0.8	5.8 ± 1.9	2.7 ± 1.1	2.4 ± 0.8	66.3	0.37
5j	7.3 ± 2.4	7.1 ± 5.5	7.9 ± 2.2	7.1 ± 2.4	3.1 ± 1.9	56.3	0.50
5k	17.0 ± 11.1	27.7 ± 7.0	40.7 ± 7.8	21.7 ± 10.8	12.5 ± 1.6	66.1	1.33
5l	23.2 ± 4.3	13.9 ± 2.7	11.4 ± 3.0	8.4 ± 2.1	3.3 ± 1.0	30.2	0.96

[a] ±SD (two mice, *n* determinations for each compound at each time). [b] Dosimetry parameter determined for ^{131}I using the MIRD program. [c] BZA values were obtained from previous work.[25]

Table 5. Melanoma/Organ Ratios of [^{125}I]Compound Uptake in B16 Melanoma-Bearing Mice at Different Times After the Injection

		compounds												
	time (h)	**BZA**[a]	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**	**5h**	**5i**	**5j**	**5k**	**5l**
melanoma /liver	1	1.60	1.37	3.01	0.70	1.36	5.64	1.43	1.13	1.80	0.91	1.88	1.29	5.92
	3	2.76	2.40	3.08	1.96	2.74	7.98	7.21	2.26	2.85	2.20	3.52	3.12	6.10
	6	2.94	3.28	3.33	2.71	4.41			2.93	2.42	18.16	5.14	5.40	7.69
	24	8.79			5.07	11.20				5.39	39.14	16.40	14.54	
	72				1.38					7.26				
melanoma /lung	1	1.72	1.10	2.04	0.58	1.72	3.38	2.34	2.86	2.09	2.28	1.96	2.17	9.86
	3	4.49	2.57	2.34	2.95	5.90			4.31	4.54	4.60	2.58	4.87	7.41
	6	7.75	4.22	2.17	5.79	13.30				3.23	38.73	3.86	9.13	9.03
	24				22.05					22.89	68.50			
	72													
melanoma /brain	1	6.20	2.97	24.20	1.69	6.00	15.40			10.02	67.0	24.23	7.26	27.14
	3	10.97	14.60	42.14	3.91	31.29				30.54	nd	79.11	26.87	32.18
	6		35.80	58.08	5.38					46.51	72.6			
	24										68.5			
	72										23.5			

[a] BZA values were obtained from previous work.[25] Absence of value: very high ratio, the organ concentration being equal to background value.

concentrations. Compound **5k** emerges as the leading candidate for further development.

Conclusion

To discover innovative melanoma-targeting vectors, a dozen BZA derivatives of varying aromatic ring moieties were designed, successfully synthesized, and evaluated in terms of their biodistribution in melanoma-bearing mice. All the compounds in the series exhibited an affinity for melanoma but with different pharmacokinetic profiles. Compounds **5e** and **5l**, with their rapid clearance from nontarget organs, would be good candidates for scintigraphic imaging, allowing high-contrast images to be obtained promptly after administration. The quinoline compound **5h** and the quinoxaline derivative **5k** stood out due to their specific affinity for the tumor and the highest and the most long-lasting tumor uptake. They present very favorable biological half-life values and dosimetry parameters for clinical application in radionuclide therapy of disseminated melanoma, for which they appear to be very promising agents.

Experimental Section

Materials. Column chromatography was performed with Merck neutral aluminum oxide 90 standardized (63−200 μm) or silica gel A normal phase (35−70 μm). Thin layer chromatography was

performed on SDS neutral aluminum oxide 60 A F254 plates or SDS silica gel 60F254 60 A-15 μm plates. The plates were visualized with UV light (254 nm) and by exposure to iodine vapor. Melting points were determined on an electrothermal IA9300 (capillary) or a Reichert−Jung−Koffler apparatus and are not corrected. NMR spectra (400 or 200 MHz for ^{1}H and 100 or 50 MHz for ^{13}C) were recorded on a Bruker Avance 400 or Bruker AM 200 instrument using $CDCl_3$ or DMSO-d_6 as solvent. Infrared spectra were recorded in KBr pellets or in CCl_4 on an FTIR Nicolet Impact 410 or an FT Vector 22 instrument (ν expressed in cm^{-1}). Mass spectral analyses were performed on a Hewlett-Packard 5989A instrument. Microanalyses were performed by the Analytical Laboratory of the CNRS (Vernaison, France) for the elements indicated and were within 0.4% of the theoretical values unless indicated. All air-sensitive reactions were run under argon atmosphere. All solvents were dried using common techniques.[45]

Materials for Radiolabeling and in Vivo Distribution. [^{125}I]NaI (3.7 GBq/mL, 644 MBq/μg) for labeling was obtained from Amersham Int. plc (Little Chalfont, Buckinghamshire, UK). Extrelut and citrate buffer solution (pH = 4) were purchased from Merck (Darmstadt, Germany). The radio TLC strips (Merck neutral aluminum oxide 60F$_{254}$ plates) were developed with CH_2Cl_2/EtOH (97/3, v/v) and measured on an AMBIS 400 (Scanalytics, CSPI, San Diego, CA). All radiolabeled compounds were shown by TLC or HPLC to be identical to the authentic nonradioactive material and to be free of significant chemical and radiochemical impurities. The radioactivity contained in the slices was analyzed using an AMBIS 4000 detector (Scanalytics, CSPI, San Diego, CA), which is a computer-controlled multiwire proportional counter previously described and validated for the evaluation of iodinated agents in mice.[46] Scintigraphic imaging of B16 melanoma bearing C57BL6 mice was performed using a dedicated γ IMAGER for small animal imaging (Biospace, Paris, France). The B16-F0 murine melanoma was obtained from ATCC (no. CRL-6322) and C57BL6 male mice from Charles River, L'Arbresle, France.

Ethyl 6-Iodonicotinate (6b).[47] To a solution of 6-iodonicotinic acid[32] (**7**) (250 mg, 1.00 mmol) in dry CH_2Cl_2 (25 mL), was added successively, at 0 °C, triethylamine (167 μL, 1.19 mmol), ethyl chloroformate (0.70 mL, 7.32 mmol), and 4-dimethylaminopyridine (DMAP) (0.45 g, 3.68 mmol). After cooling to room temperature, the reaction mixture was refluxed for 2 h. Evaporation of the solvent followed by chromatography (Al_2O_3, CH_2Cl_2) gave ester **6b** (211 mg, 0.76 mmol). Yield: 76%; R_f = 0.92 (Al_2O_3, CH_2Cl_2); mp 46−48 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.32 (t, 3H, J = 3 Hz), 4.32 (q, 2H, J = 7 Hz), 7.78 (m, 2H), 8.82 (d, 1H, J = 2 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 14.2, 61.6, 123.3, 125.7, 134.7, 138.0, 151.4, 164.7. IR (KBr) ν cm^{-1}: 1268, 1292, 1577, 1711, 3082. MS *m*/*z*: 277 (M$^+$, 61), 232 (21), 204 (20), 150 (100), 127 (16), 77 (43), 51 (22).

Ethyl 5-Aminobenzo[*b*]furan-2-carboxylate (9).[36] A solution of ethyl 5-nitrobenzo[*b*]furan-2-carboxylate (**8**) (3.92 g, 16.7 mmol) in ethyl acetate (320 mL) was hydrogenated (1 atm) at room temperature over 10% Pd/C (395 mg) for 16 h. The black suspension was filtered through celite 521 and the filtrate was concentrated under reduced pressure to give amino compound **9** (3.25 g, 15.9 mmol), which was used without further purification. Yield: 95%; R_f = 0.78 (Al_2O_3, CH_2Cl_2/EtOH (97/3, v/v)); mp 68−70 °C (literature:[36] 59−60 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.42 (t, 3H, J = 7 Hz), 3.70 (m, 2H), 4.43 (q, 2H, J = 7 Hz), 6.84 (dd, 1H, J = 9, 2.5 Hz), 6.89 (d, 1H, J = 2.5 Hz), 7.37 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.3, 61.4, 106.2, 112.7, 113.3, 117.5, 127.8, 142.9, 145.9, 150.3, 159.2. IR (KBr) ν cm^{-1}: 1225, 1559, 1724. MS *m*/*z*: 205 (M$^+$, 87), 177 (100), 160 (19), 133 (24), 104 (44), 77 (23), 52 (22), 51 (23).

Ethyl 5-Iodobenzo[*b*]furan-2-carboxylate (6d). Ethyl 5-aminobenzo[*b*]furan-2-carboxylate[36] (**9**) (3.25 g, 15.9 mmol) was suspended in water (24 mL) and concentrated hydrochloric acid was added (3.7 mL). The mixture was cooled to 0 °C, and diazotized by slow addition of sodium nitrite (1.07 g, 15.9 mmol) in water (17 mL). The solution was stirred for 0.5 h at 0 °C and a solution of 50% fluoroboric acid (2 mL) was added. The solid was removed by filtration and washed successively with water (10 mL), methanol (10 mL), and ether (10 mL) to yield 2-ethoxycarbonylbenzo[*b*]furan-5-diazonium borofluoride as a brown precipitate (2.20 g, 7.21 mmol). To a solution of this diazonium salt (2.10 g, 6.88 mmol) in water (96 mL) was added a solution of potassium iodide (1.15 g, 6.88 mmol) in water (8 mL). The reaction mixture was heated at 50 °C until no more gas was evolved. After cooling to room temperature, the solution was extracted with CH_2Cl_2 (3 × 35 mL). The combined organic extracts were washed with a 5% aqueous $NaHSO_3$ solution (2 × 75 mL), dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residual material was purified by chromatography (Al_2O_3, cyclohexane/EtOAc (8/2, v/v)) to give the white solid **6d** (1.00 g, 3.16 mmol). Yield: 21%; R_f = 0.69 (Al_2O_3, cyclohexane/EtOAc (8/2, v/v)); mp 77−79 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.45 (t, 3H, J = 7.5 Hz), 4.42 (q, 2H, J = 7.5 Hz), 7.35 (d, 1H, J = 9 Hz), 7.46 (s, 1H), 7.70 (dd, 1H, J = 9, 1.5 Hz), 8.05 (d, 1H, J = 1.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.3, 61.7, 87.3, 112.5, 114.3, 129.5, 131.5, 136.1, 146.4, 154.9, 159.1. IR (KBr) ν cm^{-1}: 1178, 1735. MS *m*/*z*: 316 (M$^+$, 100), 288 (53), 271 (37), 161 (18), 88 (20), 62 (17).

Ethyl 5-Amino-1(3)*H*-benzimidazole-2-carboxylate (11).[37] The title compound was synthesized in the same manner as described for the preparation of amino compound **9**. From ethyl 5-nitro-1(3)*H*-benzimidazole-2-carboxylate (**10**),[34] reaction time 18 h. The residual material was purified by chromatography (Al_2O_3, CH_2Cl_2/EtOH (97/3, v/v)) to afford compound **11**. Yield: 36%; R_f = 0.25 (Al_2O_3, CH_2Cl_2/EtOH (97/3, v/v)); mp 147−149 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.41 (t, 3H, J = 7 Hz), 4.46 (q, 2H, J = 7 Hz), 6.76 (m, 2H), 7.58 (d, 1H, J = 9 Hz). IR (KBr) ν cm^{-1}: 1269, 1701, 3370. MS *m*/*z*: 205 (M$^+$, 53), 159 (100), 133 (52), 131 (44), 105 (35), 83 (22), 78 (20), 52 (21).

Ethyl 5-Iodo-1(3)*H*-benzimidazole-2-carboxylate (6g). To a solution of ethyl 5-amino-1(3)*H*-benzimidazole-2-carboxylate (**11**)[37] in 50% aqueous fluoroboric acid solution (40 mL), at 0 °C, was added dropwise, a solution of sodium nitrite (750 mg, 11.0 mmol) in water (3 mL). The reaction mixture was stirred for further hour at 0 °C and filtered. The corresponding diazonium borofluoride (3.26 g, 10.7 mmol) was dissolved in water (67 mL) and a solution of potassium iodide (2.79 g, 16.8 mmol) in water (14 mL) was added. The reaction mixture was heated at 70 °C until no more gas was evolved (2 h). After cooling to room temperature, the mixture was made alkaline with a saturated aqueous Na_2CO_3 solution (pH = 8−9) and extracted with CH_2Cl_2 (2 × 80 mL). The combined organic layers were washed with a 5% aqueous $NaHSO_3$ solution (2 × 40 mL), dried ($MgSO_4$), filtered, and concentrated under *vacuum*. The brown residue was purified by chromatography (Al_2O_3, CH_2Cl_2/EtOH (99/1, v/v)) to give iodo compound **6g** (407 mg, 1.29 mmol). Yield: 12%; R_f = 0.57 (Al_2O_3, CH_2Cl_2/EtOH (99/1 v/v)); mp 166−168 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.40 (t, 3H, J = 7 Hz), 4.50 (q, 2H, J = 7 Hz), 7.51 (d, 1H, J = 9 Hz), 7.66 (dd, 1H, J = 9, 2 Hz), 8.09 (d, 1H, J = 2 Hz). IR (KBr) ν cm^{-1}: 1246, 1310, 1515, 1697, 3287. MS *m*/*z*: 316 (M$^+$, 56), 270 (30), 244 (100), 117 (38), 90 (25), 63 (19).

2-Tribromomethyl-6-iodoquinoline (13). 6-Iodo-2-methylquinoline[39] (**12**) (7.12 g, 26.5 mmol) and sodium acetate (12.1 g) were dissolved in acetic acid (15 mL). The reaction mixture was heated at 75 °C for 10 min and a solution of bromine (4.45 mL, 86.6 mmol) in acetic acid (13 mL) was added dropwise. After the addition, the solution was refluxed for 2 h. The mixture was poured onto ice (200 g) and stirred for 18 h. The solid product was filtered, washed with water (60 mL), and dried to yield compound **13**, which was used without purification (9.10 g, 18.0 mmol). Yield: 68%; R_f = 0.86 (Al_2O_3, cyclohexane/EtOAc (8/2, v/v); mp 124−126 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.92 (d, 1H, J = 8.5 Hz), 8.03 (d, 1H, J = 8.5 Hz), 8.14 (d, 1H, J = 9 Hz), 8.26 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 40.8, 94.5, 118.6, 129.1, 131.7, 135.9, 136.4, 139.3, 144.0, 159.0. IR (KBr) ν cm^{-1}: 1293, 1480, 1584. MS *m*/*z*: 509 (M$^+$+6, 1), 507 (M$^+$+4, 2), 505 (M$^+$+2, 3), 503 (M$^+$, 1), 428 (40), 426 (77), 424 (45), 348 (81), 346 (81), 255 (100), 140 (97), 127 (90), 82 (90), 57 (89).

Ethyl 6-Iodoquinoline-2-carboxylate (6h). A solution of silver nitrate (9.05 g, 53.8 mmol) in water (83 mL) was added carefully with stirring to a solution of tribromo compound **13** (9.10 g, 18.0 mmol) in EtOH (132 mL). The mixture was refluxed for 30 min and then allowed to stand at room temperature, filtered, and acidified with aqueous hydrochloric acid (1.5 M, 5 mL). The resulting solution was concentrated under vacuum to 100 mL. The solution was made alkaline with a saturated aqueous Na_2CO_3 solution (100 mL), extracted with ether (3 × 30 mL), dried ($MgSO_4$), and evaporated. The crude product was purified by chromatography (Al_2O_3, EtOAc/cyclohexane (8/2, v/v)) to give ester **6h** (3.96 g, 12.1 mmol). Yield: 67%; R_f = 0.97 (Al_2O_3, EtOAc/cyclohexane (8/2, v/v)); mp 119−121 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.42 (t, 3H, J = 7 Hz), 4.46 (q, 2H, J = 7 Hz), 7.97 (d, 1H, J = 9 Hz), 8.16 (m, 2H), 8.55 (d, 1H, J = 9 Hz), 8.64 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.4, 62.4, 95.0, 121.8, 130.7, 132.2, 135.6, 136.4, 139.1, 146.4, 148.7, 165.0. IR (KBr) ν cm^{-1}: 1307, 1714. MS *m*/*z*: 327 (M^+, 17), 283 (19), 255 (100), 127 (28), 100 (11).

Methyl 5-Iodoisoquinoline-3-carboxylate (6j). Methyl 5-aminoisoquinoline-3-carboxylate[40] (**14**) (500 mg, 2.47 mmol) was suspended in water (4 mL) and concentrated hydrochloric acid was added (576 μL). The mixture was cooled to 0 °C and diazotized by slow addition of sodium nitrite (166 mg, 2.47 mmol) in water (3 mL). The solution was stirred for 0.5 h at 0 °C and a solution of 50% fluoroboric acid (311 μL) was added. The solid was removed by filtration, dissolved in water (12 mL), and a solution of potassium iodide (453 mg, 3.10 mmol) in water (5 mL) was added. The reaction was heated at 80 °C until no more gas was evolved (2 h). After cooling to room temperature, the mixture was extracted with CH_2Cl_2 (2 × 80 mL). The combined organic layers were washed with a 5% aqueous $NaHSO_3$ solution (40 mL), dried ($MgSO_4$), filtered, and concentrated under vacuum. The residue was purified by chromatography (Al_2O_3, CH_2Cl_2/EtOH (99/1, v/v)) to give compound **6j** (190 mg, 0.61mmol). Yield: 25%; R_f = 0.64 (Al_2O_3, CH_2Cl_2/EtOH (99/1, v/v)); mp 165−167 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.00 (s, 3H), 7.65 (t, 1H, J = 8 Hz), 8.32 (d, 1H, J = 8 Hz), 8.50 (d, 1H, J = 8 Hz), 8.55 (s, 1H), 9.37 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 52.6, 99.6, 126.1, 128.5, 130.5, 131.3, 136.4, 142.2, 142.8, 153.8, 165.2. IR (KBr) ν cm^{-1}: 1249, 1304, 1710. MS *m*/*z*: 313 (M^+, 22), 283 (20), 255 (100), 127 (41), 100 (18), 74 (11).

Ethyl 6-Nitroquinoxaline-2-carboxylate (16). To a solution of 6-nitroquinoxaline-2-carboxylic acid[41] (**15**) (5.00 g, 22.8 mmol) in dry EtOH (50 mL) was added, under argon, concentrated sulfuric acid (750 μL). After stirring at reflux for 7 h, the reaction mixture was cooled to room temperature and a saturated aqueous Na_2CO_3 solution (50 mL) was added. The solution was extracted with CH_2Cl_2 (3 × 30 mL) and the combined organic layers were dried ($MgSO_4$), filtered, and evaporated to dryness to give ester **16** (3.79 g, 15.3 mmol). Yield: 67%; mp 221−223 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.58 (t, 3H, J = 7 Hz), 4.68 (q, 2H, J = 7 Hz), 8.53 (d, 1H, J = 9 Hz), 8.68 (dd, 1H, J = 2.5, 9 Hz), 9.14 (d, 1H, J = 2.5 Hz), 9.72 (s, 1H). IR (KBr) ν cm^{-1}: 1282, 1347, 1531, 1741. MS *m*/*z*: 248 (M^++1, 4), 203 (36), 175 (100), 128 (23), 101 (32), 75 (24).

Ethyl 6-Aminoquinoxaline-2-carboxylate (17). To a flask containing the nitro compound **16** (2.93 g, 11.9 mmol) in EtOH (500 mL) was added 10% Pd/C (300 mg). The mixture was degassed and stirred under a H_2 atmosphere for 3 h 45. The catalyst was removed by filtration through celite 545, washed with EtOH (50 mL), and the filtrate was evaporated. The resulting crude product was purified by chromatography (Al_2O_3, CH_2Cl_2/EtOH (99/1, v/v)) to give the title product **17** (1.69 g, 7.79 mmol). Yield: 66%; R_f = 0.55 (Al_2O_3, CH_2Cl_2/EtOH (99/1, v/v)); mp 181−183 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.49 (t, 3H, J = 7 Hz), 4.38 (m, 2H), 4.56 (q, 2H, J = 7 Hz), 7.16 (d, 1H, J = 2.5 Hz), 7.26 (dd, 1H, J = 2.5, 9 Hz), 8.05 (d, 1H, J = 9 Hz), 9.35 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.4, 62.1, 106.9, 123.1, 131.9, 136.6, 138.4, 145.6, 145.9, 150.4, 164.7. IR (KBr) ν cm^{-1}: 1299, 1487, 1613, 1701, 3202, 3431. MS *m*/*z*: 217 (M^+, 23), 145 (100), 117 (19), 90 (16), 63 (14).

Ethyl 6-Iodoquinoxaline-2-carboxylate (6k). To a mixture of ethyl 6-aminoquinoxaline-2-carboxylate (**17**) (1.37 g, 6.31 mmol) in 50% aqueous fluoroboric acid solution (10 mL), at 0 °C, was added dropwise a solution of sodium nitrite (480 mg, 6.96 mmol) in water (3 mL). The reaction mixture was stirred for further hour at 0 °C and a solution of potassium iodide (1.57 g, 9.46 mmol) in water (5 mL) was added. The reaction was stirred at 0 °C for 1 h and then warmed at 50 °C until no more gas was evolved (1 h). After cooling to room temperature, the mixture was made alkaline with a saturated aqueous Na_2CO_3 solution (60 mL) and extracted with CH_2Cl_2 (3 × 40 mL). The combined organic layers were washed with a 5% aqueous $NaHSO_3$ solution (2 × 30 mL), dried ($MgSO_4$), filtered, and concentrated under vacuum. The crude product was purified by chromatography (Al_2O_3, CH_2Cl_2) to give iodo compound **6k** (0.68 g, 2.07 mmol). Yield: 33%; R_f = 0.80 (Al_2O_3, CH_2Cl_2); mp 160−162 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.51 (t, 3H, J = 7 Hz), 4.59 (q, 2H, J = 7 Hz), 7.99 (d, 1H, J = 9 Hz), 8.10 (dd, 1H, J = 2, 9 Hz), 8.62 (d, 1H, J = 2 Hz), 9.51 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.3, 62.7, 99.2, 131.6, 138.5, 140.0, 140.7, 143.0, 144.1, 145.7, 163.9. IR (KBr) ν cm^{-1}: 1103, 1151, 1230, 1237, 1717. MS *m*/*z*: 328 (M^+, 19), 284 (43), 256 (100), 128 (29), 101 (50), 75 (30).

Ethyl 1,6-Naphthyridine-2-carboxylate (19). Compound **19** was prepared according to the procedure developed for ester **6b** from 1,6-naphthyridine-2-carboxylic acid[42] (**18**) using 3.8 equiv of triethylamine, 6.8 equiv of ethyl chloroformiate, and 3.4 equiv of DMAP. The reaction mixture was refluxed for 5 h. After evaporation of the solvent, the crude product was purified by chromatography (Al_2O_3, EtOAc) to give ester **19**. Yield: 45%; R_f = 0.86 (Al_2O_3, EtOAc); mp 106−108 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.41 (t, 3H, J = 7 Hz), 4.49 (q, 2H, J = 7 Hz), 8.02 (d, 1H, J = 6 Hz), 8.21 (d, 1H, J = 8.5 Hz), 8.39 (d, 1H, J = 8.5 Hz), 8.75 (d, 1H, J = 6 Hz), 9.29 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.3, 62.0, 122.4, 122.7, 124.0, 137.2, 147.5, 149.7, 152.3, 152.9, 164.6. IR (KBr) ν cm^{-1}: 1256, 1734. MS *m*/*z*: 202 (M^+, 3), 158 (18), 130 (100), 102 (15), 75 (22), 51 (16).

Ethyl 8-Iodo-1,6-naphthyridine-2-carboxylate (6l). To a solution of ester **19** (517 mg, 2.56 mmoles) in THF (50 mL) were added, at −10 °C, *N*-iodosuccinimide (691 mg, 3.07 mmoles), and *p*-toluenesulfonic acid (176 mg, 1.02 mmol). The mixture was stirred at −10 °C for 10 min and then at room temperature for 24 h. More *N*-iodosuccinimide (576 mg, 2.56 mmoles) was added and the solution was stirred for a further 21 h at room temperature. The solvent was evaporated under reduced pressure and the crude product was dissolved in saturated aqueous Na_2CO_3 solution (50 mL) and extracted with EtOAc (3 × 20 mL). The organic layers were dried ($MgSO_4$), filtered, and evaporated to dryness. The residue was purified by chromatography (Al_2O_3, EtOAc/cyclohexane (7/3, v/v)) to afford iodoester **6l** (527 mg, 1.61 mmol). Yield: 63%; R_f = 0.83 (Al_2O_3, EtOAc/cyclohexane (7/3, v/v)); mp 135−137 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.51 (t, 3H, J = 7 Hz), 4.55 (q, 2H, J = 7 Hz), 8.32 (d, 1H, J = 8 Hz), 8.39 (d, 1H, J = 8 Hz), 9.23 (s, 1H), 9.28 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.2, 62.7, 100.9, 123.4, 125.2, 137.9, 149.3, 152.8, 153.2, 155.2, 164.3. IR (CCl_4) ν cm^{-1}: 1126, 1274, 1742. MS *m*/*z*: 328 (M^+, 18), 284 (18), 256 (100), 228 (8), 129 (29), 101 (52), 75 (25).

General Procedure for Preparation of Amides 20a−l. Trimethylaluminium (2 M in toluene) (7.2 mL, 14.4 mmol) was added dropwise at 0 °C to a solution of *N*,*N*-diethylethylenediamine (d = 0.827, 2 mL, 14.1 mmol) in dry methylene chloride (110 mL). After addition of the appropriate ester 6 (10 mmol) in dry methylene chloride (88 mL), the mixture was refluxed for the reported times and monitored by TLC. After cooling to room temperature, water (130 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 100 mL). The organic layers were dried ($MgSO_4$) and concentrated under vacuum. The crude product was chromatographed (Al_2O_3, CH_2Cl_2/EtOH, 97/3) to give amides **20**.

***N*-(2-Diethylaminoethyl)-6-iodo-2-naphthamide (20a).** From methyl 6-iodo-2-naphthoate (**6a**),[27] reaction time: 16 h. Yield: 40%; R_f = 0.58 (Al_2O_3, CH_2Cl_2/EtOH (97/3, v/v)); mp 105−107 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.05 (t, 6H, J = 7 Hz), 2.58 (q, 4H,

$J = 7$ Hz), 2.68 (t, 2H, $J = 6$ Hz), 3.53 (q, 2H, $J = 6$ Hz), 7.40 (m, 1H), 7.54 (d, 1H, $J = 8.5$ Hz), 7.68 (d, 2H, 8.5 Hz), 7.83 (d, 1H, $J = 8.5$ Hz), 8.18 (s, 1H), 8.23 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 11.8 (2C), 37.3, 46.6 (2C), 51.1, 93.6, 124.4, 127.0, 127.3, 130.2, 131.1, 132.3, 135.0, 135.7, 136.2, 166.9. IR (KBr) ν cm^{-1}: 1537, 1614, 1630, 3303. MS *m/z*: 281 ($M^+ - 115$, 6), 126 (9), 86 (100), 58 (14).

***N*-(2-Diethylaminoethyl)-6-iodonicotinamide (20b).** From ethyl 6-iodonicotinate (**6b**), reaction time: 20 h. Yield: 98%; $R_f = 0.60$ (Al_2O_3, CH_2Cl_2/EtOH (97/3, v/v)) as an oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.83 (t, 6H, $J = 7$ Hz), 2.37 (q, 4H, $J = 7$ Hz), 2.47 (t, 2H, $J = 6$ Hz), 3.28 (q, 2H, $J = 6$ Hz), 7.38 (m, 1H), 7.78 (m, 2H), 8.73 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 11.7 (2C), 37.2, 46.4 (2C), 50.8, 120.9, 129.5, 134.5, 136.1, 148.6, 164.4. IR (CCl_4) ν cm^{-1}: 1538, 1630, 2927, 2965, 3301. MS *m/z*: 348 ($M^+ + 1$, 1), 86 (100), 77 (8), 58 (16).

***N*-(2-Diethylaminoethyl)-5-iodoindole-2-carboxamide (20c).** From ethyl 5-iodoindole-2-carboxylate (**6c**),[28] reaction time: 3 h. Yield: 50%; mp 208−210 °C; $R_f = 0.53$ (Al_2O_3, CH_2Cl_2/EtOH (97/3, v/v)). ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.03 (t, 6H, $J = 7$ Hz), 2.57 (m, 6H), 3.37 (m, 2H), 7.11 (s, 1H), 7.42 (AB system, 2H, $J = 8.5$ Hz), 8.09 (s, 1H), 8.52 (t, 1H, $J = 6$ Hz), 11.82 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 11.9 (2C), 37.3, 46.8 (2C), 51.5, 83.3, 101.2, 114.7, 129.8, 129.9, 131.0, 132.8, 135.3, 160.6. IR (KBr) ν cm^{-1}: 1411, 1547, 1635, 2801, 2965, 3250, 3418. MS *m/z*: 385 (M^+, 2), 86 (100), 58 (2).

***N*-(2-Diethylaminoethyl)-5-iodobenzo[*b*]furan-2-carboxamide (20d).** From ethyl 5-iodobenzo[*b*]furan-2-carboxylate (**6d**), reaction time: 14 h. Yield: 81%; $R_f = 0.65$ (Al_2O_3, CH_2Cl_2/EtOH (97/3, v/v)) as an oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.06 (t, 6H, $J = 7$ Hz), 2.59 (q, 4H, $J = 7$ Hz), 2.66 (m, 2H), 3.49 (q, 2H, $J = 7$ Hz), 7.29 (d, 1H, $J = 8$ Hz), 7.36 (s, 1H), 7.67 (dd, 1H, $J = 8$, 2 Hz), 8.01 (d, 1H, $J = 2$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 10.8 (2C), 35.9, 45.8 (2C), 50.2, 86.0, 107.8, 112.7, 129.1, 130.2, 134.1, 148.7, 152.8, 157.2. IR (CCl_4) ν cm^{-1}: 1501, 1675, 2972. MS *m/z*: 386 (M^+, 1), 271 (5), 86 (100), 58 (10).

***N*-(2-Diethylaminoethyl)-4-iodobenzo[*b*]thiophene-2-carboxamide (20e).** From methyl 4-iodobenzo[*b*]thiophene-2-carboxylate (**6e**),[29] reaction time: 24 h. Yield: 72%; $R_f = 0.58$ (Al_2O_3, CH_2Cl_2/EtOH (97/3, v/v)); mp 76−78 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.09 (t, 6H, $J = 7$ Hz), 2.60 (q, 4H, $J = 7$ Hz), 2.68 (t, 2H, $J = 7$ Hz), 3.50 (q, 2H, $J = 7$ Hz), 7.08 (brs, 1H), 7.11 (t, 1H, $J = 8$ Hz), 7.80 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 12.2 (2C), 37.5, 46.9 (2C), 51.1, 91.4, 122.7, 127.2, 128.4, 134.9, 139.5, 140.0, 142.2, 161.7. IR (KBr) ν cm^{-1}: 1560, 1625, 2963, 3287. MS *m/z*: 402 (M^+, 1), 86 (100), 58 (7).

***N*-(2-Diethylaminoethyl)-6-iodoimidazo[1,2-*a*]pyridine-2-carboxamide (20f).** From ethyl 6-iodoimidazo[1,2-A-*a*]pyridine-2-carboxylate (**6f**),[30] reaction time: 72 h. Yield: 83%; $R_f = 0.55$ (Al_2O_3, CH_2Cl_2/EtOH (97/3, v/v)) as an oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.03 (t, 6H, $J = 7$ Hz), 2.57 (q, 4H, $J = 7$ Hz), 2.66 (t, 2H, $J = 6.5$ Hz), 3.51 (q, 2H, $J = 6.5$ Hz), 7.34 (m, 2H), 7.68 (m, 1H), 8.06 (s, 1H), 8.39 (t, 1H, $J = 1.5$ Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 12.2 (2C), 37.3, 47.2 (2C), 51.9, 76.5, 113.4, 118.9, 130.9, 133.5, 140.2, 142.6, 161.8. IR (CCl_4) ν cm^{-1}: 1218, 1251, 1562, 1670, 2971, 3430. MS *m/z*: 99 ($M^+ - 287$, 11), 86 (100), 58 (11).

***N*-(2-Diethylaminoethyl)-5-iodo-1(3)*H*-benzimidazole-2-carboxamide (20g).** From ethyl 5-iodo-1(3)*H*-benzimidazole-2-carboxylate (**6g**), reaction time: 6 h. Yield: 82%; $R_f = 0.38$ (Al_2O_3, CH_2Cl_2/EtOH (97/3, v/v)); mp 136−138 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.04 (t, 6H, $J = 7$ Hz), 2.64 (q, 4H, $J = 7$ Hz), 2.76 (m, 2H), 3.62 (m, 2H), 7.36 (d, 1H, $J = 8.5$ Hz), 7.52 (d, 1H, $J = 8.5$ Hz), 7.96 (brs, 1H), 8.28 (m, 1H). IR ν cm^{-1}: 1420, 1551, 1664, 2966, 3175. MS *m/z*: 386 (M^+, 1), 86 (100), 58 (11).

***N*-(2-Diethylaminoethyl)-6-iodoquinoline-2-carboxamide (20h).** From ethyl 6-iodoquinoline-2-carboxylate (**6h**), reaction time: 6 h. Yield: 69%; $R_f = 0.56$ (Al_2O_3, CH_2Cl_2/EtOH (97/3, v/v)); mp 75−77 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.09 (t, 6H, $J = 7$ Hz), 2.63 (q, 4H, $J = 7$ Hz), 2.73 (t, 2H, $J = 7$ Hz), 3.58 (q, 2H, $J = 7$ Hz), 7.81 (d, 1H, $J = 9$ Hz), 7.98 (dd, 1H, $J = 9$, 2 Hz), 8.16 (d, 1H, $J = 8.5$ Hz), 8.26 (d, 1H, $J = 2$ Hz), 8.30 (d, 1H, $J = 8.5$ Hz), 8.57 (brs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 12.1 (2C), 37.5, 47.2 (2C), 51.7, 93.7, 119.6, 130.7, 131.8, 136.1, 136.5, 139.4, 145.4, 150.5, 164.1. IR (KBr) ν cm^{-1}: 1526, 1663, 2963, 3400. MS *m/z*: 386 (M^+, 1), 86 (100), 58 (11).

***N*-(2-Diethylaminoethyl)-1,4-dihydro-6-iodo-4-oxoquinoline-3-carboxamide (20i).** From ethyl 1,4-dihydro-6-iodo-4-oxoquinoline-3-carboxylate (**6i**),[31] reaction time: 5 h. Yield: 81%; $R_f = 0.04$ (Al_2O_3, CH_2Cl_2/EtOH (97/3, v/v)); mp 234−236 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.13 (t, 6H, $J = 7$ Hz), 2.85 (m, 6H), 3.69 (q, 2H, $J = 6$ Hz), 7.33 (d, 1H, $J = 8.5$ Hz), 7.87 (d, 1H, $J = 8.5$ Hz), 8.49 (s, 1H), 8.67 (s, 1H), 10.32 (brs, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 11.4 (2C), 36.4, 46.6 (2C), 51.4, 89.6, 111.3, 121.5, 127.9, 133.8, 138.7, 140.5, 144.0, 164.2, 174.4. IR (KBr) ν cm^{-1}: 1507, 1551, 1633, 2965, 3061. MS *m/z*: 413 (M^+, 1), 86 (100), 58 (10).

***N*-(2-Diethylaminoethyl)-5-iodoisoquinoline-3-carboxamide (20j).** From methyl 5-iodoisoquinoline-3-carboxylate (**6j**), reaction time: 4 h. Yield: 72%; $R_f = 0.47$ (Al_2O_3, CH_2Cl_2/EtOH (97/3, v/v)) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.08 (t, 6H, $J = 7$ Hz), 2.64 (q, 4H, $J = 7$ Hz), 2.74 (t, 2H, $J = 7$ Hz), 3.62 (q, 2H, $J = 7$ Hz), 7.40 (t, 1H, $J = 8$ Hz), 8.00 (d, 1H, $J = 8$ Hz), 8.31 (d, 1H, $J = 8$ Hz), 8.57 (m, 1H), 8.76 (s, 1H), 9.05 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 11.9 (2C), 37.5, 47.3 (2C), 51.8, 99.4, 123.8, 128.3, 129.7, 130.6, 138.1, 142.0, 145.8, 152.0, 164.5. IR (KBr) ν cm^{-1}: 1516, 1677, 2971. MS *m/z*: 397 (M^+, 1), 86 (100), 58 (7).

***N*-(2-Diethylaminoethyl)-6-iodoquinoxaline-2-carboxamide (20k).** From ethyl 6-iodoquinoxaline-2-carboxylate (**6k**), reaction time: 7 h. Yield: 77%; $R_f = 0.64$ (Al_2O_3, CH_2Cl_2/EtOH (97/3, v/v)); mp 60−62 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.00 (t, 6H, $J = 7$ Hz), 2.54 (q, 4H, $J = 7$ Hz), 2.64 (t, 2H $J = 6$ Hz), 3.49 (q, 2H, $J = 6$ Hz), 7.71 (d, 1H, $J = 9$ Hz), 7.96 (dd, 1H, $J = 2$, 9 Hz), 8.31 (m, 1H), 8.49 (d, 1H, $J = 2$ Hz), 9.55 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 12.1 (2C), 37.4, 47.1 (2C), 51.5, 97.9, 130.8, 138.5, 139.5, 139.6, 144.1, 144.3, 144.6, 162.9. IR (KBr) ν cm^{-1}: 1517, 1661, 3397. MS *m/z*: 398 (M^+, 1), 86 (100), 58 (15).

***N*-(2-Diethylaminoethyl)-8-iodo-1,6-naphthyridine-2-carboxamide (20l).** From ethyl 8-iodo-1,6-naphthyridine-2-carboxylate (**6l**), reaction time: 3 h. The crude product was purified by chromatography (Al_2O_3, EtOAc). Yield: 73%; $R_f = 0.67$ (Al_2O_3, EtOAc); mp 44−46 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.07 (t, 6H, $J = 7$ Hz), 2.60 (q, 4H, $J = 7$ Hz), 2.70 (t, 2H, $J = 6$ Hz), 3.57 (q, 2H, $J = 6$ Hz), 8.35 (d, 1H, $J = 8.5$ Hz), 8.44 (d, 1H, $J = 8.5$ Hz), 8.83 (m, 1H), 9.17 (s, 1H), 9.19 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 12.3 (2C), 37.4, 46.9 (2C), 51.4, 100.2, 121.5, 125.3, 138.1, 148.1, 152.8, 154.7, 154.8, 162.8. IR (KBr) ν cm^{-1}: 1460, 1516, 1689, 2801, 2926, 2965, 3300−3400. MS *m/z*: 398 (M^+, 1), 86 (100), 58 (15).

General Procedure for Preparation of Hydrochloride salts 5a−k. To a stirred solution of the appropriate amide (2.52 mmol) in dry methylene chloride (22 mL), under argon, was added a solution of 2N anhydrous hydrochloric acid in ether (22 mL). The solution was stirred at room temperature for 10 min and evaporated under vacuum. The residue was taken up in anhydrous ether (50 mL) and stirred under argon for 24 h. The resulting precipitate was filtered to give hydrochloride salt **5**.

***N*-(2-Diethylaminoethyl)-6-iodo-2-naphthamide, hydrochloride salt (5a).** From *N*-(2-diethylaminoethyl)-6-iodo-2-naphthamide (**20a**). Yield: 94%; mp 151−152 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.22 (t, 6H, $J = 7$ Hz), 3.19 (q, 4H, $J = 7$ Hz), 3.44 (m, 4H), 7.83 (m, 2H), 7.97 (m, 2H), 8.47 (m, 2H), 9.03 (m, 1H, NH), 9.77 (m, 1H). IR (KBr) ν cm^{-1}: 1297, 1533, 1655, 3252. Anal. ($C_{17}H_{21}IN_2O$, HCl, H_2O) C, H, N.

***N*-(2-Diethylaminoethyl)-6-iodonicotinamide, dihydrochloride salt (5b).** From *N*-(2-diethylaminoethyl)-6-iodonicotinamide (**20b**). Yield: 70%; mp 127−129 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.26 (t, 6H, $J = 7$ Hz), 3.25 (m, 6H), 3.68 (m, 4H), 8.00 (m, 2H), 8.87 (m, 1H), 9.25 (m, 1H), 10.24 (m, 1H). IR (KBr) ν cm^{-1}: 1278, 1529, 1588, 1661, 2661, 3232. Anal. ($C_{12}H_{18}IN_3O$, 2HCl) C, H, N.

***N*-(2-Diethylaminoethyl)-5-iodoindole-2-carboxamide, hydrochloride salt (5c).** From *N*-(2-diethylaminoethyl)-5-iodoindole-2-carboxamide (**20c**). Yield: 81%; mp 217−219 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.29 (m, 6H), 3.28 (m, 6H), 3.72 (m, 2H), 7.22 (s, 1H), 7.31 (d, 1H, J = 8.5 Hz), 7.48 (d, 1H, J = 8.5 Hz), 8.06 (s, 1H), 9.16 (brs, 1H), 10.59 (brs, 1H), 11.94 (s, 1H). IR (KBr) ν cm^{-1}: 1544, 1646, 2468, 2569, 3228. Anal. ($C_{15}H_{20}IN_3O$, HCl) H, N. C: calcd, 42.72; found, 43.47.

***N*-(2-Diethylaminoethyl)-5-iodobenzo[*b*]furan-2-carboxamide, hydrochloride salt (5d).** From *N*-(2-diethylaminoethyl)-5-iodobenzo[*b*]furan-2-carboxamide (**20d**). Yield: 81%; mp 207−209 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.27 (t, 6H, J = 7 Hz), 3.26 (m, 6H), 3.70 (m, 2H), 7.56 (d, 1H, J = 9 Hz), 7.61 (s, 1H), 7.78 (d, 1H, J = 9 Hz), 8.24 (s, 1H), 9.16 (brs, 1H), 10.0 (m, 1H). IR (KBr) ν cm^{-1}: 1560, 1670, 2586, 3209. Anal. ($C_{15}H_{19}IN_2O_2$, HCl) C, H, N.

***N*-(2-Diethylaminoethyl)-4-iodobenzothiophene-2-carboxamide, hydrochloride salt (5e).** From *N*-(2-diethylaminoethyl)-4-iodobenzo[*b*]thiophene-2-carboxamide (**20e**). Yield: 72%; mp 163−165 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.28 (t, 6H, J = 7 Hz), 3.25 (m, 6H), 3.70 (m, 2H), 7.26 (t, 1H, J = 8 Hz), 7.92 (d, 1H, J = 8 Hz), 8.00 (d, 1H, J = 8 Hz), 8.22 (s, 1H), 9.42 (m, 1H), 10.29 (m, 1H). IR (KBr) ν cm^{-1}: 1535, 1648, 3249. Anal. ($C_{15}H_{19}IN_2OS$, HCl) C, H, N.

***N*-(2-Diethylaminoethyl)-6-iodoimidazo[1,2-*a*]pyridine-2-carboxamide, dihydrochloride salt (5f).** From *N*-(2-diethylaminoethyl)-3-iodoimidazo[1,2-*a*]pyridine-6-carboxamide (**20f**). Yield: 80%; mp 208−210 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.21 (t, 6H, J = 7 Hz), 3.17 (m, 6H), 3.58 (m, 2H), 7.57 (m, 2H), 8.41 (s, 1H), 9.00 (t, 1H, J = 5 Hz), 9.09 (s, 1H), 9.95 (m, 1H). IR (KBr) ν cm^{-1}: 1289, 1600, 1659, 2650, 3100−2900, 3426. Anal. ($C_{14}H_{19}IN_4O$, 2HCl, $2H_2O$) C, H, N.

***N*-(2-Diethylaminoethyl)-5-iodo-1(3)*H*-benzimidazole-2-carboxamide, dihydrochloride salt (5g).** From *N*-(2-diethylaminoethyl)-5-iodobenzimidazole-2-carboxamide (**20 g**). Yield: 82%; mp 217−219 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.27 (t, 6H, J = 7 Hz), 3.22 (m, 6H), 3.74 (m, 2H), 7.52 (d, 1H, J = 8.5 Hz), 7.64 (dd, 1H, J = 8.5, 1 Hz), 8.03 (d, 1H, J = 1 Hz), 9.36 (m, 1H), 10.45 (m, 1H). IR (KBr) ν cm^{-1}: 1593, 1683, 2979. Anal. ($C_{14}H_{19}IN_4O$, 2HCl, H_2O) C, H, N.

***N*-(2-Diethylaminoethyl)-6-iodoquinoline-2-carboxamide, dihydrochloride salt (5h).** From *N*-(2-diethylaminoethyl)-6-iodoquinoline-2-carboxamide (**20h**). Yield: 69%; mp 104−106 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.28 (t, 6H, J = 7 Hz), 3.24 (m, 6H), 3.77 (q, 2H, J = 7 Hz), 7.92 (d, 1H, J = 9 Hz), 8.21 (m, 2H), 8.57 (d, 1H, J = 9 Hz), 8.65 (s, 1H), 9.34 (m, 1H), 10.08 (m, 1H). IR ν cm^{-1}: 1384, 1523, 1684, 3415. Anal. ($C_{16}H_{20}IN_3O$, 2HCl, $2.5H_2O$) C, H, N.

***N*-(2-Diethylaminoethyl)-1,4-dihydro-6-iodo-4-oxoquinoline-3-carboxamide, dihydrochloride salt (5i).** From *N*-(2-diethylaminoethyl)-1,4-dihydro-6-iodo-4-oxoquinoline-3-carboxamide (**20i**). Yield: 92%; mp 164−166 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.27 (t, 6H, J = 7 Hz), 3.24 (m, 6H), 3.73 (m, 2H), 7.62 (d, 1H, J = 9 Hz), 8.11 (dd, 1H, J = 9, 1.5 Hz), 8.51 (d, 1H, J = 1.5 Hz), 8.80 (d, 1H, J = 6.5 Hz), 10.07 (m, 2H). IR (KBr) ν cm^{-1}: 1521, 1618, 1654, 3028, 3514. Anal. ($C_{16}H_{20}IN_3O_2$, 2HCl, $1.5H_2O$) C, H, N.

***N*-(2-Diethylaminoethyl)-5-iodoisoquinoline-3-carboxamide, dihydrochloride salt (5j).** From *N*-(2-diethylaminoethyl)-5-iodoisoquinoline-2-carboxamide (**20j**). Yield: 72%; mp 152−154 °C. NMR ^{1}H (200 MHz, DMSO-d_6) δ: 1.22 (t, 6H, J = 7 Hz), 3.25 (m, 6H), 3.74 (m, 2H), 7.63 (t, 1H, J = 8 Hz), 8.33 (d, 1H, J = 8 Hz), 8.49 (d, 1H, J = 8 Hz), 8.56 (s, 1H), 9.38 (m, 2H), 9.76 (m, 1H). IR (KBr) ν cm^{-1}: 1526, 1684, 3447, 3927. Anal. ($C_{16}H_{20}IN_3O$, 2HCl) C, H, N.

***N*-(2-Diethylaminoethyl)-6-iodoquinoxaline-2-carboxamide, dihydrochloride salt (5k).** From *N*-(2-diethylaminoethyl)-6-iodoquinoxaline-2-carboxamide (**20k**). Yield: 76%; mp 201−203 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.28 (t, 6H, J = 7 Hz), 3.28 (m, 6H), 3.78 (m, 2H), 7.97 (d, 1H, J = 9 Hz), 8.30 (d, 1H, J = 9 Hz), 8.68 (s, 1H), 9.47 (m, 2H), 10.43 (brs, 1H). IR (KBr) ν cm^{-1}: 1516, 1674, 2459, 3326. Anal. ($C_{15}H_{19}IN_4O$, 2HCl) C, H, N.

***N*-(2-Diethylaminoethyl)-8-iodo-1,6-naphthyridine-2-carboxamide, dihydrochloride salt (5l).** From *N*-(2-diethylaminoethyl)-8-iodonaphthyridine-2-carboxamide (**20l**). Yield: 72%; mp 215−217 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.27 (t, 6H, J = 7 Hz), 3.25 (m, 6H), 3.82 (m, 2H), 8.34 (d, 1H, J = 8.5 Hz), 8.81 (d, 1H, J = 8.5 Hz), 8.95 (m, 1H), 9.29 (s, 1H), 9.29 (s, 1H), 10.75 (brs, 1H). IR (KBr) ν cm^{-1}: 1450, 1528, 1624, 1681, 2043, 2240−2750, 2939, 3045, 3250−3550. Anal. ($C_{15}H_{19}IN_4O$, 2HCl, $1.5H_2O$): C, H, N.

Preparation of Radioiodinated Compounds [^{125}I]5a−l. To a solution of the appropriate compound **5** (2−3 mg) in citrate buffer pH = 4 (500 μL) were added, in a closed vial [^{125}I]NaI (20−30 μL, 2−3 mCi), and an aqueous copper sulfate solution (0.5 mg, 100 μL) used as catalyst. The reaction mixture was heated at 120−150 °C for 30−60 min. The residue was taken up in water (500 μL) and a 1N aqueous NaOH solution (100 μL) was added. The vial cap and septum were removed. The resulting suspension was run through an Extrelut column and eluted with CH_2Cl_2 (5 × 3 mL). The eluting solution was concentrated under reduced pressure, diluted with anhydrous CH_2Cl_2 (2 mL), and treated with 2N anhydrous hydrochloric acid in ether (5 mL). The resulting hydrochloride solution was evaporated to dryness, and the dry residue was then dissolved in physiological saline for animal experiments. Radiochemical yields based on TLC of exchange reaction mixture and radiochemical purities are given in Table 1.

Partition Coefficient Measurements. For each compound, the partition coefficient between *n*-octanol and phosphate buffer was determined. The measurement was performed by shaking 2 mL of [^{125}I] compound solution (50 μM in phosphate buffer solution, PBS, pH 7.4) with 2 mL of *n*-octanol. The activity of each phase was counted in the γ counter (Packard, Minaxi γ 5530, Rungis, France). *P* was calculated as the ratio of activities (*n*-octanol/buffer), and its logarithm was determined to express lipophilicity.

In Vitro Binding to Melanin. An in vitro experiment was performed to evaluate the binding affinity of new compounds to melanin using synthetic tyrosine-melanin (Sigma) suspended in two different media: water and PBS. The general procedure used was as follows: [^{125}I]compound was added to a melanin suspension (0.5 mg/10 mL). The suspension was incubated at room temperature for 24 h with stirring. After incubation, the tubes were centrifuged at 35000*g* for 20 min, and aliquots of the supernatants were counted.

In Vivo Distribution. All the experiments were carried out in compliance with French laws governing animal experimentation. The biodistribution of radioiodinated compounds was studied in C57BL6 male mice bearing the B16-F0 murine melanoma. Stock cell cultures were maintained as monolayers in Glutamax (Eagle's minimum essential medium with glutamine) supplemented with 10% fetal calf serum, vitamins, sodium pyruvate, nonessential amino acids, and gentamycin and passaged by trypsination. The cells were grown in a humidified 37 °C incubator containing 5% CO_2. Early passages were frozen and stored in liquid nitrogen. For transplantation, an aliquot was grown in a monolayer culture to confluence; thereafter cells were trypsinized and washed with phosphate buffer saline (PBS). They were resuspended in PBS, and each mouse received 0.1 mL subcutaneously (5 × 10^5 cells) on the left flank. Ten days later, the tumors became palpable with a percentage of tumor take of 98−100%.

Study of the Biodistribution on Animal Slices. The [^{125}I]compound was administered intravenously via a tail vein (0.1 μmol, 0.74−0.92 MBq/animal) in 10 animals for each compound. Two injected mice were sacrificed by CO_2 inhalation and quickly frozen in liquid nitrogen at different times after administration (1, 3, 6, 24, or 72 h). Frozen animals were cryosectioned using the technique described.[48] Slices 40 μm thick were obtained using a Reichert−Jung cryopolycut (Leica Instruments, Rueil Malmaison, France) at −22 °C and dehydrated for 48 h in the cryochamber. Eight slices per mouse and per time point were selected from a total number of more than 500 slices for the analysis corresponding to distant slices and the section of the organs of interest. Measurements were performed on an AMBIS 4000 detector (Scanalytics, CSPI, San Diego, CA) following an acquisition time of 1000 min. The radioactivity of different organs was quantified after contouring

suitable zones on the two-dimensional image of the slice displayed on the computer screen for analysis. Radioactivity per unit area (net cpm/mm^2) was converted into radioactive concentration (kBq/g) and expressed as percentage of injected dose/g of tissue (%ID/g). For comparison of the tumor (T) uptake with other tissues, ratios of radioactive concentrations (T/organ) were determined, illustrating the image contrast. For two animals, urine and feces were collected up to 72 h and counted to determine the cumulative urinary and fecal excretions.

Scintigraphic Imaging. For each selected compound, the in vivo kinetic profile was followed in two mice by repeated planar scintigraphic imaging (3 MBq, acquisition time 10 min) using a dedicated γ camera for small animal imaging (Biospace, Paris, France). This study allowed a follow-up in the same animal, namely the tumor uptake kinetics.

Dosimetry Parameters. From the biodistribution values, the biological tumor half-life was calculated for each [^{125}I]compound using an adaptation of the MIRD program to the mouse. With a view to using the [^{131}I]labeled analogue and taking into account its physical properties, the effective half-life of [^{131}I]compounds and the tumor delivered doses (Gy/injected MBq) were calculated.

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Supporting Information Available: Table of elemental analysis and biodistribution data of compounds **5a**−**l**. This material is available free of charge via the Internet at http://pubs.acs.org.

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