Fluorine-18-Labeled Benzamide Analogues for Imaging the σ_2 Receptor Status of Solid Tumors with Positron Emission Tomography

Zhude Tu,[†] Jinbin Xu,[†] Lynne A. Jones,[†] Shihong Li,[†] Craig Dumstorff,[†] Suwanna Vangveravong,[†] Delphine L. Chen,[†] Kenneth T. Wheeler,[‡] Michael J. Welch,[†] and Robert H. Mach*[,†]

Division of Radiological Sciences, Washington University School of Medicine, 510 South Kingshighway Boulevard, St. Louis, Missouri 63110, and Department of Radiology, Wake Forest University School of Medicine, Winston-Salem, North Carolina 27157

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A series of fluorine-containing benzamide analogs was synthesized and evaluated as candidate ligands for positron emission tomography (PET) imaging of the sigma-2 (σ_2) receptor status of solid tumors. Four compounds having a moderate to high affinity for σ_2 receptors and a moderate to low affinity for sigma-1 (σ_1) receptors were radiolabeled with fluorine-18 via displacement of the corresponding mesylate precursor with [^{18}F]fluoride. Biodistribution studies in female Balb/c mice bearing EMT-6 tumor allografts demonstrated that all four F-18-labeled compounds had a high tumor uptake (2.5−3.7% ID/g) and acceptable tumor/normal tissue ratios at 1 and 2 h post-i.v. injection. An analysis of the chemistry and biodistribution data suggested that *N*-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)-2-(2-[^{18}F]-fluoroethoxy)-5-methylbenzamide (**[^{18}F]3c)** and *N*-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)-2-(2-[^{18}F]-fluoroethoxy)-5-iodo-3-methoxybenzamide (**[^{18}F]3f)** are acceptable compounds for imaging the σ_2 receptor status of solid tumors.

Introduction

Sigma receptors were originally thought to be a subtype of the opioid receptors. Subsequent studies revealed that sigma receptors were a distinct class of receptors that are expressed in many normal tissues, including liver, kidneys, endocrine glands, and the central nervous system (CNS).[1] It has been well-established that there are at least two types of sigma receptors, sigma-1 (σ_1) and sigma-2 (σ_2).[2,3] The σ_1 receptor has been cloned from tissues of guinea pig, rat, mouse, and man[4] and displays a 30% sequence homology with a yeast sterol enzyme isomer.[5] The σ_2 receptor has not been cloned, but evidence suggests that this receptor is linked to potassium channels and intracellular calcium release in NCB-20 cells.[1,6,7]

An overexpression of σ_2 receptors has been reported in a variety of human and murine tumors.[8−10] The observation that the density of σ_2 receptors is greater than that of σ_1 receptors in a wide panel of tumor cells grown under various cell culture conditions[5] suggested that the σ_2 receptor may be a biomarker of tumor cell proliferation. In a series of studies designed to test this hypothesis, the density of σ_2 receptors was found to be 10-fold higher in proliferating versus quiescent mouse mammary adenocarcinoma cells, both in vitro[8,11] and in vivo.[12] This higher σ_2 receptor density in proliferating tumor cells did not depend on other biological or physiological factors.[11,12] Consequently, these data suggest that radioligands possessing a high affinity and selectivity for σ_2 receptors have the potential to not only detect solid tumors, but also to measure their proliferative status using noninvasive imaging techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT).[13]

The search for σ_2 selective ligands has led to the identification of a number compounds having modest to high selectivity for σ_2 versus σ_1 receptors (Figure 1). These include CB-184 (**10**), CB-64D (**11**), BIMU-1 (**12**),[13,14] and PB-167 (**13**)[15−17], as well as the conformationally flexible benzamide analogs developed in our laboratory (**14**−**16**).[18,19] We previously reported the evaluation of several ^{11}C, ^{76}Br, and $^{125/123}$I radiolabeled conformationally flexible benzamide analogs using EMT-6 tumor-bearing female Balb/c mice.[20−22] The initial in vivo studies of 5-methyl-2-[^{11}C]-methoxy-*N*-[2-(6,7-dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-butyl]-benzamide and 5-[^{76}Br]-bromo-2,3-dimethoxy-*N*-[2-(6,7-dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-butyl]-benzamide indicated that these compounds were potential radiopharmaceuticals for imaging solid tumors and their proliferative status with PET. However, the radionuclide properties of ^{76}Br and ^{11}C make them less than ideal for PET imaging when compared to the radionuclide properties of ^{18}F. For example, studies comparing the nuclide properties of ^{18}F and ^{76}Br indicate that radiotracers containing ^{18}F give higher quality PET images due to the relatively high energy of the positron emitted by ^{76}Br that often produces "blurred" images.[23] Although this is not an issue with ^{11}C-lableled radiotracers, the longer half-life of ^{18}F ($t_{1/2}$ = 109.8 min) compared to ^{11}C ($t_{1/2}$ = 20.4 min) places fewer time constraints on tracer synthesis and permits longer scan sessions that usually result in higher tumor/normal tissue ratios for the ^{18}F-labeled radiotracers. Therefore, the development of an ^{18}F-labeled radiotracer is important for imaging the σ_2 receptor status of human solid tumors by PET.

In this paper, we report the synthesis and in vitro/in vivo characterization of several fluorinated conformationally flexible benzamide analogs having a moderate to high affinity and selectivity for σ_2 receptors. These ^{18}F-labeled compounds were prepared and evaluated in Balb/c female mice bearing EMT-6 mammary tumor allografts to assess their potential as PET radioligands for imaging the σ_2 receptor status of human solid tumors.

* To whom correspondence should be addressed. Robert H. Mach, Ph.D., Division of Radiological Sciences, Washington University School of Medicine, Campus Box 8225, 510 S. Kingshighway Blvd., St. Louis, Missouri 63110. Tel.: 314-362-8538. Fax: 314-362-0039. E-mail: rhmach@mir.wustl.edu.

[†] Washington University School of Medicine.

[‡] Wake Forest University School of Medicine.

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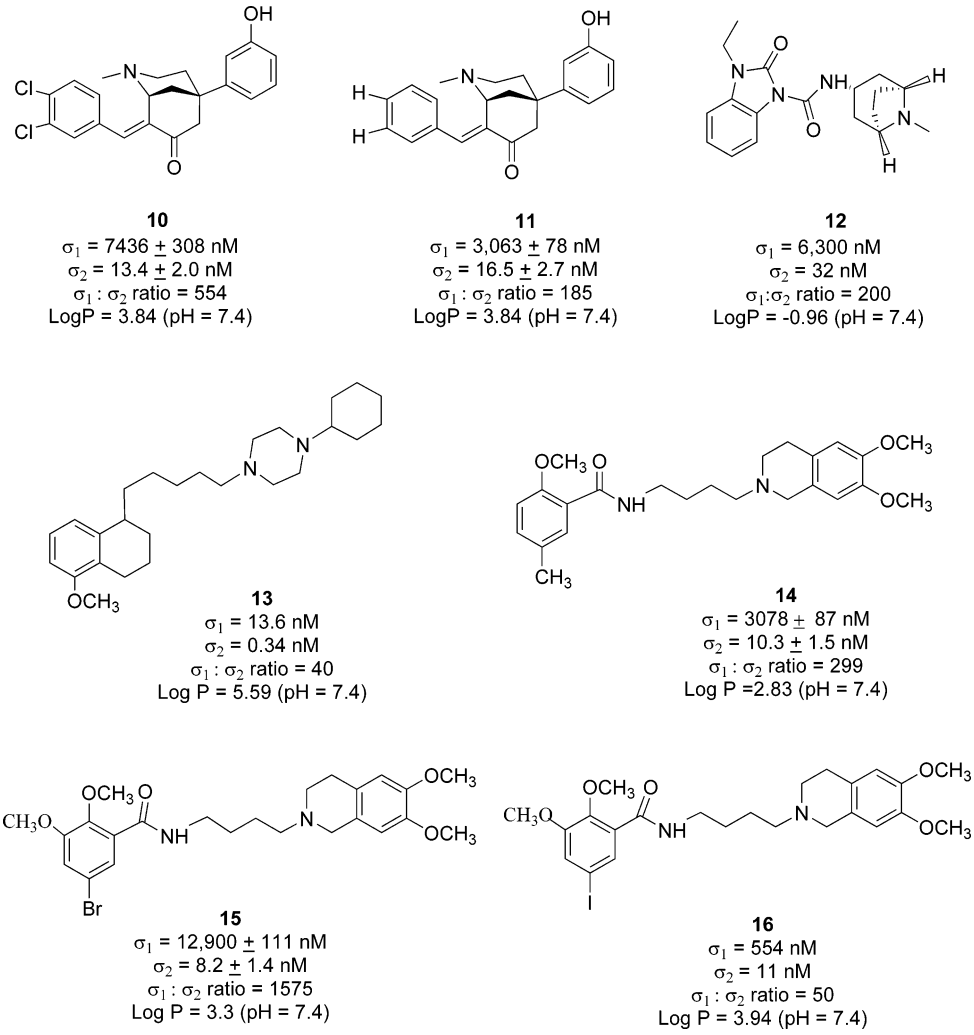


Figure 1. Structure and properties of several σ_2 selective ligands.

Results and Discussion

Chemistry. The design strategy for generating σ_2-selective ligands suitable for ^{18}F-labeling involved replacing the ortho methoxy group of the ^{11}C-labeled benzamide analogs[21] with a 2-fluoroethyl group, as shown in Scheme 1. Condensation of **1a** and **1b** with a substituted salicylic acid gave the corresponding substituted 2-hydroxybenzamide analogs, **2a**−**e**. Alkylation of the ortho hydroxyl group with 2-bromo-1-fluoroethane using potassium carbonate as a base produced **3a**−**e** in moderate to high yield. Compound **3f** was prepared by iodination of the corresponding tin precursor, **3g**, which was prepared from **3b** using standard stannylation reaction conditions. Compounds **3a**−**f** were then converted into either the hydrochloride or the oxalic acid salts for the in vitro σ_1 and σ_2 receptor binding assays.

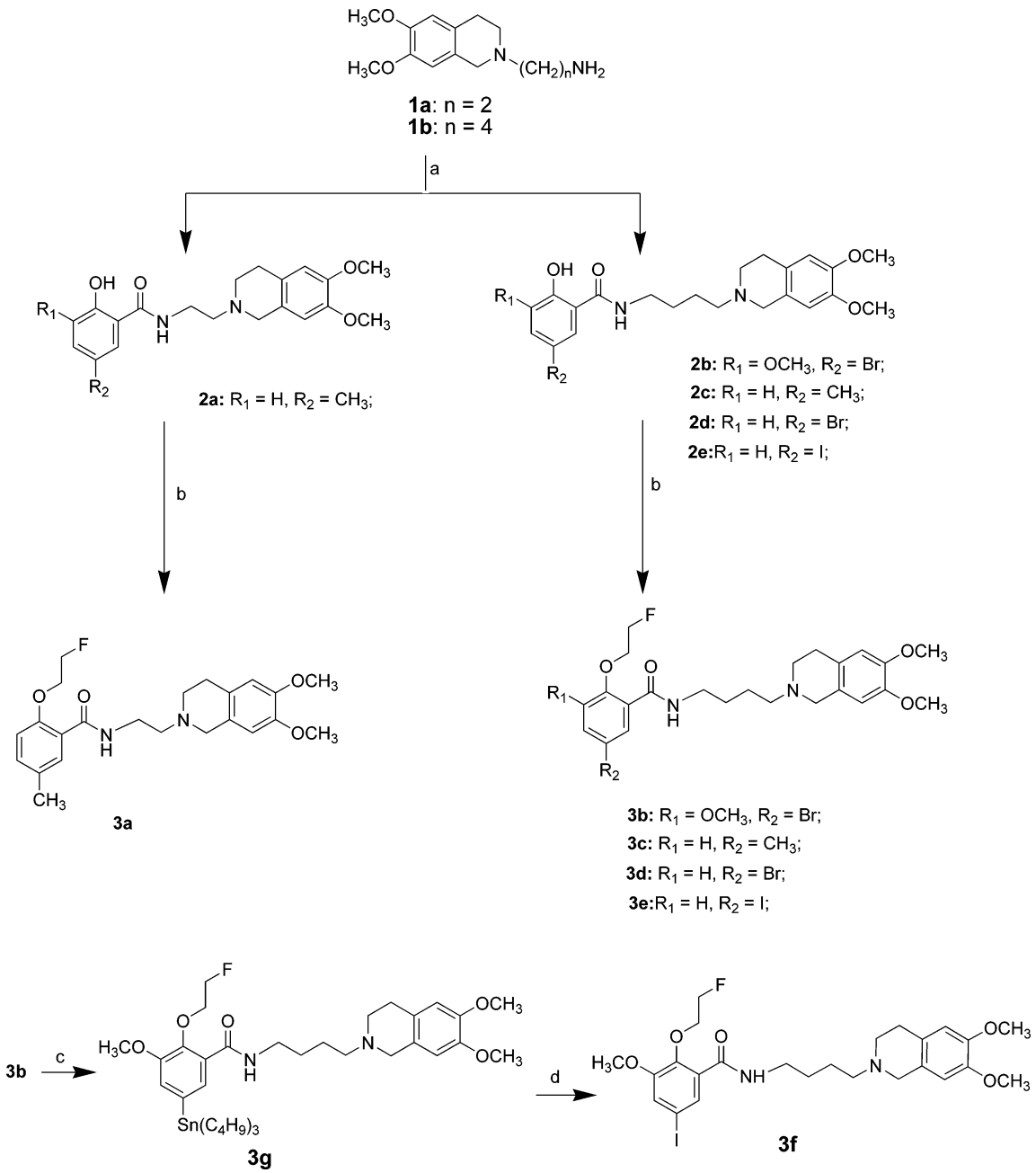
Based on the results of the in vitro binding studies with the unlabeled compounds, **3c**−**f** were radiolabeled with ^{18}F, as shown in Schemes 2−4. Scheme 2 outlines the synthesis of the mesylate precursors required for the radiolabeling procedure. Alkylation of the ortho hydroxyl group of compounds **2c**−**e** with 1-bromoethyl acetate followed by hydrolysis of the acetate group produced the corresponding 2-hydroxyethoxy analogs, **4c**−**e**, in good yield. Compounds **4c**−**e** were then converted to the corresponding mesylates, **5c**−**e**, by treatment with methanesulfonyl chloride in dichloromethane using triethylamine as an acid scavenger.

Synthesis of the precursor for the corresponding 5-iodo analog, **5f**, is shown in Scheme 3. Esterification of 5-bromo-2-methoxy salicylic acid followed by alkylation of the ortho hydroxyl group with 1-bromoethyl acetate, then hydrolysis of the acetate and benzoate esters produced the corresponding 2-hydroxyethyl analog, **9**. Condensation of **9** with the amine, **1b**, gave the amide, **4f**, which was converted to the corresponding mesylate, **5f**, using the conditions described above for the analogs **5c**−**e**.

The synthesis of **[^{18}F]3c**, **[^{18}F]3d**, **[^{18}F]3e**, and **[^{18}F]3f** was accomplished by treating the mesylate precursors, **5c**−**f**, with [^{18}F]fluoride/potassium carbonate and Kryptofix 222, using dimethyl sulfoxide (DMSO) as the solvent. The reaction mixture was irradiated for 30−40 s in a microwave oven, and the crude product was separated from the unreacted [^{18}F]fluoride using a C-18 reverse phase Sep-Pak cartridge and methanol as the eluent. The crude product was then purified by high-performance liquid chromatography (HPLC) using a C-18 reverse phase column. The entire procedure required ∼2 h, and the radiochemical yield, corrected for decay to the start of synthesis, was 20∼30%. The specific activities ranged from 1500 to 2500 Ci/mmol.

In Vitro Binding Studies. In vitro binding studies were conducted to measure the affinity of the target compounds for σ_1 and σ_2 receptors. The binding assays used [^{3}H](+)-pentazocine for the σ_1 receptors and [^{3}H]1,3-di(2-tolyl)guanidine ([^{3}H]-

Scheme 1[a]



[a] Reagents and conditions: (a) RCOOH, BOP/CH_2Cl_2 or DCC/CH_2Cl_2, rt, 18 hrs; (b) $BrCH_2CH_2F$, K_2CO_3/acetone reflux, over 48 h; (c) $[Sn(C_4H_9)_3]_2$, $Pd(PPh_3)_4(0)$/toluene at 110 °C; (d) I_2/CH_2Cl_2, room temperature.

Table 1. Affinity (K_i) of the Benzamide Analogs **6a**−**e** for the σ_1 and σ_2 Receptors Assayed In Vitro

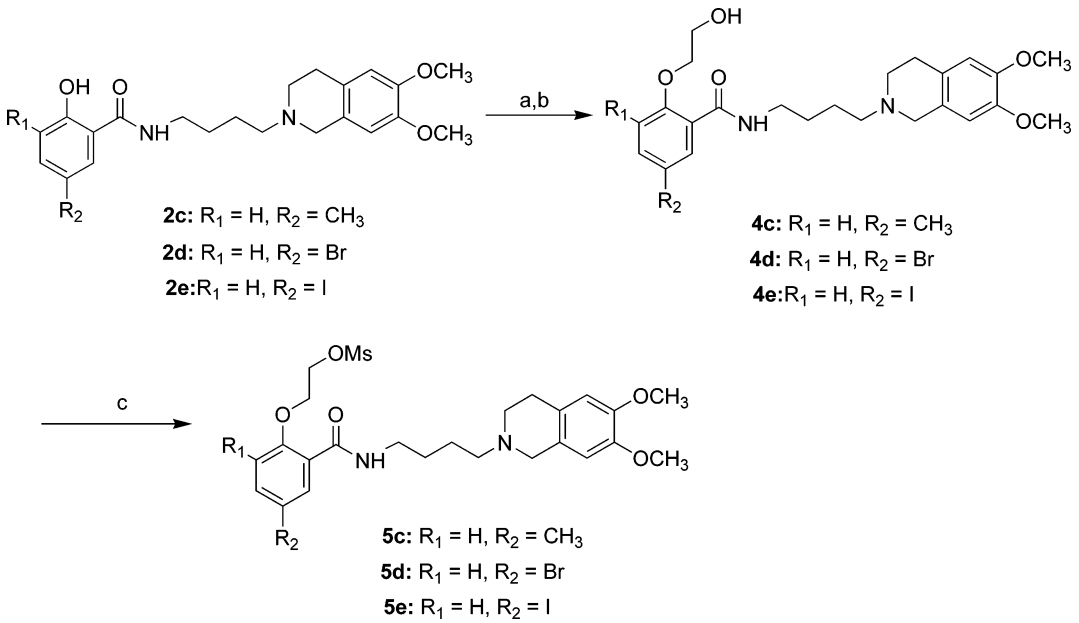
	K_i value (nM)			
	σ_1	σ_2	σ_1/σ_2 ratio	Log D[a] (pH = 7.4)
3a	22 750 ± 3410	102 ± 4	222	2.54
3b	15 300 ± 2305	386 ± 93	40	3.56
3c	330 ± 25	6.95 ± 1.63	48	3.06
3d	1076 ± 88	0.65 ± 0.22	1656	3.89
3e	1300 ± 225	1.06 ± 0.30	1230	4.13
3f	2150 ± 410	0.26 ± 0.07	8190	3.46

[a] Calculated using the program ACD/log D.

DTG) in the presence of 100 nM (+)-pentazocine for the σ_2 receptors. The K_i values were determine from Scatchard plots. The results of the binding assays for compounds **3a**−**f** are shown in Table 1. Increasing the length of the spacer group from two carbons (**3a)** to four carbons (**3c**) results in a 69-fold increase in the affinity for σ_1 receptors, a 15-fold increase in the affinity for σ_2 receptors, and a 0.5 unit increase in the log D value; a measure of the lipophilicity of the compounds (Table 1). Although four of the five compounds (**3c**−**f)** with a four-carbon spacer had higher affinities for σ_1 receptors (their K_i values ranged from 330 to 2150 nM) than the compound (**3a**) with a two-carbon spacer (K_i = 22,-750 nM), the affinities of **3c**−**f** for σ_2 receptors increased proportionately more (their K_i values ranged from 0.26 to 6.95 nM), leading to substantial increases in their σ_2/σ_1 ratios (Table 1).

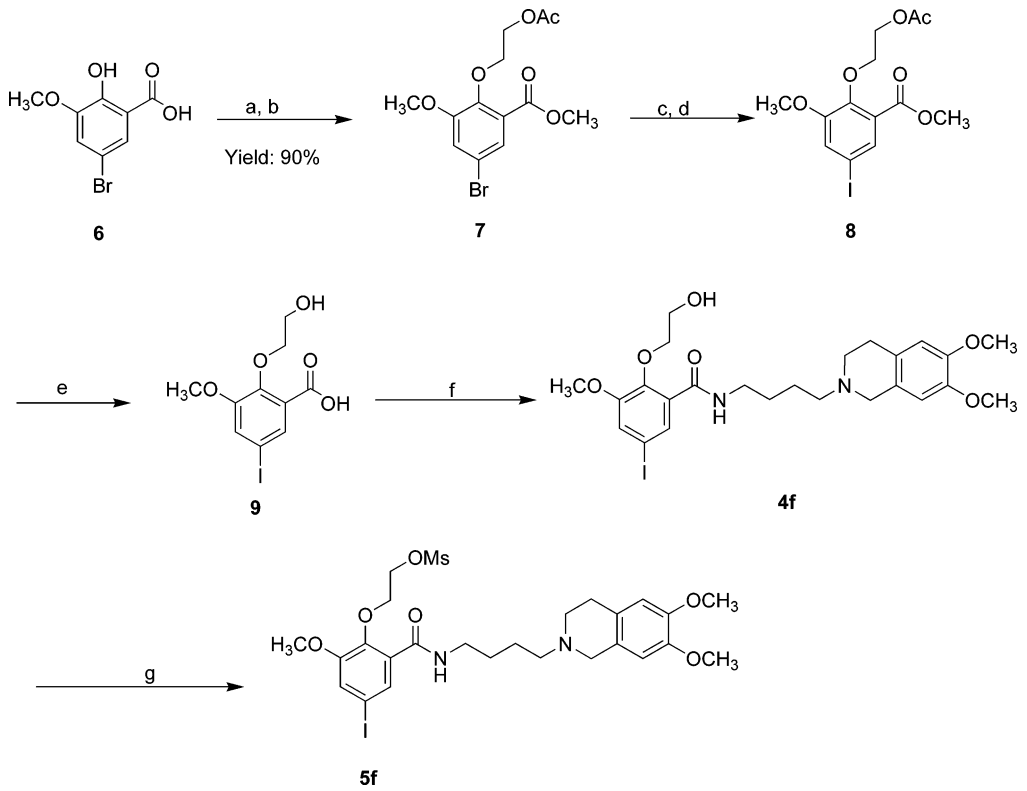
It is not clear why compound **3b** had a relatively low affinity for both the σ_1 and σ_2 receptors, especially considering compound **3f** was found to have the highest affinity for σ_2

Scheme 2[a]



[a] Reagents and conditions: (a) $BrCH_2CH_2OAc$/acetone, K_2CO_3, reflux 18 h; (b) $NaOH/H_2O$, CH_3OH, room temperature; (c) methanesulfonyl chloride, CH_2Cl_2, triethylamine, room temperature.

Scheme 3[a]



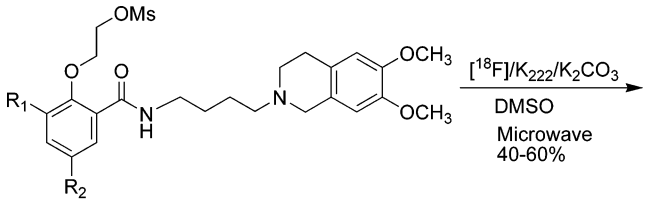
[a] Reagents and conditions: (a) methanol/98% H_2SO_4 reflux, 18 h; (b) $BrCH_2CH_2OAc$/acetone, K_2CO_3, reflux, 18 h; (c) $[Sn(C_4H_9)_3]_2$, $Pd(PPh_3)_4(0)$/toluene at 110 °C; (d) I_2/CH_2Cl_2, room temperature; (e) $NaOH/H_2O$, CH_3OH, room temperature; (f) **1b**, BOP/CH_2Cl_2 or DCC/CH_2Cl_2, room temperature, 18 h; (g) methanesulfonyl chloride, CH_2Cl_2, triethylamine, room temperature, 18 h.

receptors of the six compounds evaluated. The difference between **3b** and **3f** only involves substituting an iodine atom for a bromine atom in the meta position, and this same substitution resulted in virtually no change in the σ_2 receptor affinity when it was made in the analogs lacking the 3-methoxy group (compare **3d** to **3e**).

Finally, the σ_2/σ_1 ratios for compounds **3c**−**f** varied from 48 to 8190. The excellent σ_2 receptor affinities and moderate to high σ_2/σ_1 ratios for the compounds **3c**−**f** suggests that their corresponding [18]F-labeled analogs may be useful radiotracers for imaging the σ_2 receptor status of solid tumors with PET. Also, the log D values for these compounds, a measure of their lipophilicity, are within the range that should lead to a high uptake in solid tumors.[21]

In Vivo Evaluation. The results of the biodistribution studies in female Balb/c mice bearing EMT-6 tumors are shown in

Scheme 4

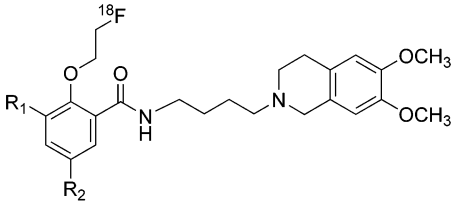


5c: $R_1 = H, R_2 = CH_3$

5d: $R_1 = H, R_2 = Br$

5e: $R_1 = H, R_2 = I$

5f: $R_1 = OCH_3, R_2 = I$



[18F]3c: $R_1 = H, R_2 = CH_3$

[18F]3d: $R_1 = H, R_2 = Br$

[18F]3e: $R_1 = H, R_2 = I$

[18F]3f: $R_1 = OCH_3, R_2 = I$

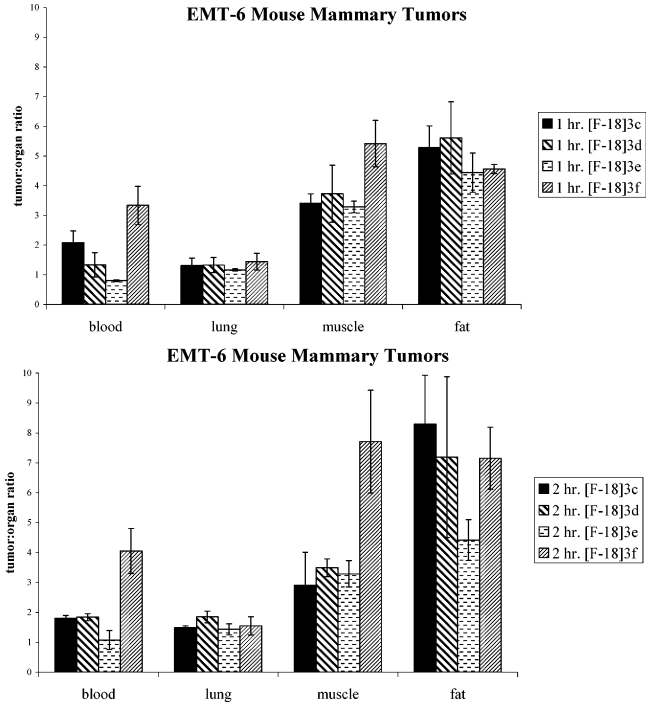


Figure 2. Tumor/organ ratios for the ^{18}F-labeled σ_2 selective ligands **3c**−**f** at 1 h (top) and 2 h (bottom) after i.v. injection into female Balb/c mice bearing EMT-6 tumors.

Table 2. All four labeled compounds displayed excellent tumor uptake at 5 min postinjection, with values ranging from 2.5 to 3.7% of the injected dose per gram (%ID/g). Tumor uptake at 1 h postinjection remained high for each of the ligands **[18F]3c**−**f** (1.14, 2.09, 2.72, and 2.15 %ID/g, respectively) and continued to remain relatively high at 2 h postinjection (0.64, 0.96, 1.92, and 1.15 %ID/g, respectively) compared with that of the normal tissues, fat, and muscle. This resulted in acceptable tumor/normal tissue ratios for the PET imaging studies. For example, the tumor/muscle ratios ranged from 3−4 and the tumor/fat ratios ranged from 4.5−8 at 2 h postinjection, respectively. Also, the low bone uptake of all four labeled compounds, which continued to decrease between the 30 min and the 1 h time points, suggested that these compounds do not undergo a significant defluorination in vivo.

Compound **[18F]3f** had the highest tumor/muscle ratio (∼8) and a tumor/fat ratio of ∼7 at 2 h after i.v. injection (Figure 2). The tumor/fat ratios for **[18F]3c** and **[18F]3d** were also high, reaching ∼8 and ∼6, respectively, at 2 h after i.v. injection However, the tumor/muscle ratios for **[18F]3c** and **[18F]3d** were much lower than that for **[18F]3f**. Although the tumor uptake of **[18F]3d** and **[18F]3e** is higher than that of **[18F]3c** at both 1 and 2 h postinjection, these radiotracers cleared much more slowly from the blood than **[18F]3c** (Table 2), making them less desirable than **[18F]3c** as PET imaging agents. The moderate to high tumor/normal tissue ratios and the rapid clearance from the blood for **[18F]3c** and **[18F]3f** suggests that these radiotracers are likely the best candidates for imaging of solid tumors with PET. Consequently, these two radiotracers were selected for further studies to evaluate the suitability for detecting solid tumors and imaging their σ_2 receptor status with PET.

To demonstrate that the in vivo binding of **[18F]3c** and **[18F]-3f** was specific for σ_2 receptors, a no-carrier-added dose of these

Table 2. [18F]3c−**f** Biodistribution in Female Balb/c Mice Bearing EMT-6 Tumors

	5 min	30 min	60 min	120 min	5 min	30 min	60 min	120 min
		[18F]3c				[18F]3d		
blood	2.49 ± 0.49	1.16 ± 0.10	0.56 ± 0.08	0.35 ± 0.05	3.57 ± 0.43	2.81 ± 0.32	1.69 ± 0.63	0.52 ± 0.10
lung	10.26 ± 0.71	2.36 ± 0.19	0.88 ± 0.12	0.43 ± 0.07	12.08 ± 1.98	3.08 ± 0.23	1.60 ± 0.27	0.51 ± 0.08
liver	23.33 ± 4.22	10.51 ± 0.87	4.14 ± 0.55	2.05 ± 0.43	32.60 ± 3.96	13.12 ± 1.39	5.69 ± 0.54	2.30 ± 0.33
kidney	29.18 ± 1.92	6.86 ± 0.45	2.51 ± 0.51	0.87 ± 0.13	42.94 ± 3.34	17.55 ± 2.75	6.92 ± 1.61	1.12 ± 0.16
muscle	1.86 ± 0.08	0.70 ± 0.19	0.34 ± 0.05	0.24 ± 0.08	1.95 ± 0.19	0.98 ± 0.18	0.58 ± 0.11	0.28 ± 0.10
fat	1.95 ± 0.33	0.59 ± 0.13	0.22 ± 0.04	0.08 ± 0.02	2.85 ± 0.47	0.96 ± 0.13	0.38 ± 0.05	0.15 ± 0.06
heart	3.73 ± 0.15	1.15 ± 0.06	0.55 ± 0.07	0.27 ± 0.04	3.74 ± 0.37	1.55 ± 0.11	1.04 ± 0.21	0.40 ± 0.07
brain	0.76 ± 0.06	0.27 ± 0.05	0.18 ± 0.03	0.12 ± 0.02	1.09 ± 0.11	0.40 ± 0.03	0.32 ± 0.05	0.20 ± 0.03
bone	2.49 ± 0.19	0.96 ± 0.15	0.55 ± 0.07	0.45 ± 0.11	2.90 ± 0.39	1.17 ± 0.06	1.12 ± 0.16	1.28 ± 0.28
tumor	3.67 ± 0.45	2.54 ± 0.27	1.14 ± 0.10	0.64 ± 0.10	3.28 ± 0.41	2.59 ± 0.19	2.09 ± 0.28	0.96 ± 0.24
		[18F]3e				[18F]3f		
blood	4.60 ± 0.44	4.30 ± 0.59	3.39 ± 0.29	1.92 ± 0.59	1.82 ± 0.25	1.23 ± 0.28	0.65 ± 0.09	0.28 ± 0.01
lung	9.71 ± 0.83	4.07 ± 0.46	2.34 ± 0.12	1.36 ± 0.24	18.47 ± 3.07	3.75 ± 0.58	1.51 ± 0.13	0.74 ± 0.03
liver	37.26 ± 4.88	17.35 ± 2.72	7.31 ± 0.98	4.25 ± 1.56	15.21 ± 2.21	10.73 ± 2.98	5.57 ± 0.31	2.61 ± 0.69
kidney	36.07 ± 2.28	17.43 ± 1.95	9.36 ± 0.90	3.92 ± 0.98	19.98 ± 1.66	7.73 ± 2.25	3.50 ± 0.80	1.34 ± 0.10
muscle	1.52 ± 0.10	1.12 ± 0.05	0.83 ± 0.04	0.60 ± 0.11	2.50 ± 0.33	0.73 ± 0.14	0.40 ± 0.07	0.15 ± 0.01
fat	2.32 ± 0.46	1.04 ± 0.06	0.62 ± 0.10	0.44 ± 0.08	4.13 ± 0.86	1.36 ± 0.50	0.47 ± 0.07	0.17 ± 0.05
heart	3.22 ± 0.27	2.37 ± 0.29	1.61 ± 0.11	1.00 ± 0.23	5.60 ± 0.45	1.54 ± 0.31	0.69 ± 0.06	0.39 ± 0.04
brain	0.55 ± 0.04	0.44 ± 0.04	0.36 ± 0.02	0.37 ± 0.06	0.71 ± 0.09	0.27 ± 0.04	0.14 ± 0.02	0.08 ± 0.01
bone	2.59 ± 0.28	1.31 ± 0.14	0.99 ± 0.07	1.67 ± 0.27	2.23 ± 0.56	2.01 ± 0.53	0.93 ± 0.16	0.59 ± 0.09
tumor	2.54 ± 0.62	2.81 ± 0.62	2.72 ± 0.13	1.92 ± 0.10	3.05 ± 0.43	3.11 ± 0.16	2.15 ± 0.25	1.15 ± 0.23

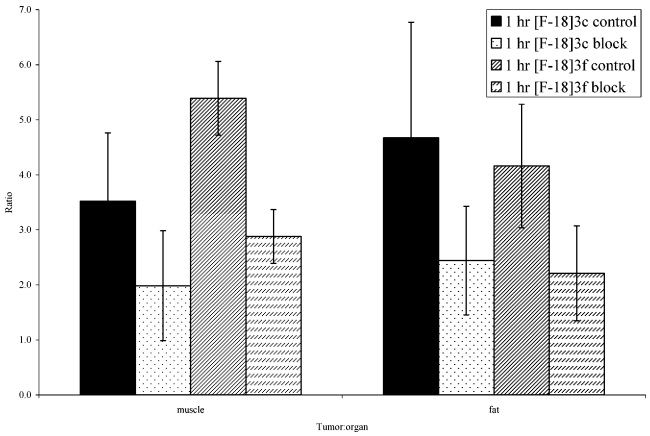


Figure 3. Comparison of the tumor/fat and tumor/muscle ratios for **[^{18}F]3c** or **[^{18}F]3f** when there is no carrier added and when the σ_1 and σ_2 receptors are blocked with 1 mg/kg of YUN-143. All values were obtained 1 h after injection of the radiotracer.

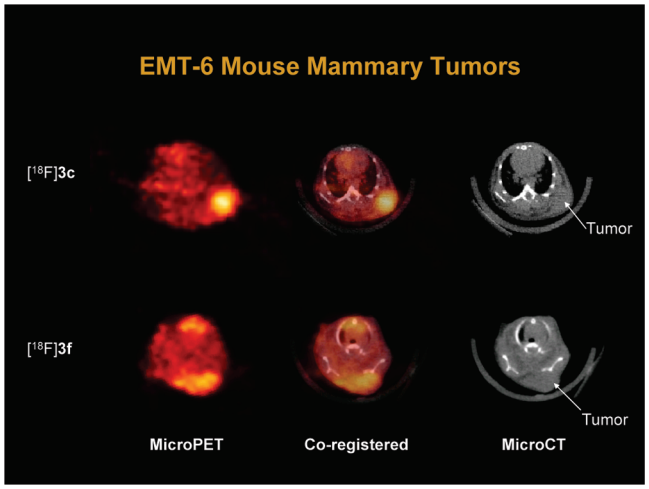


Figure 4. MicroPET and microCT images of EMT-6 tumors in female Balb/c mice. All MicroPET images were acquired 1 h after i.v. injection of either **[^{18}F]3c** or **[^{18}F]3f**.

radiotracers was coinjected into EMT-6 tumor-bearing mice with *N*-(4-fluorobenzyl)piperidinyl-4-(3-bromophenyl) acetamide (YUN-143[a]), a sigma ligand displaying a high affinity for both σ_1 and σ_2 receptors. Previous studies from our group have shown that 1 mg/kg YUN-143 is highly effective at blocking σ_1 and σ_2 receptors in vivo.[21,23,26,27] Coinjection of YUN-143 with either **[^{18}F]3c** or **[^{18}F]3f** resulted in a significant decrease (~50%) in the tumor/muscle and tumor/fat ratios at 1 h postinjection (Figure 3). These data indicate that both **[^{18}F]3c** and **[^{18}F]3f** bind selectively to σ_2 receptors in vivo.

To confirm the feasibility of using these radioligands as PET imaging agents for determining the σ_2 receptor status of solid tumors, a CT/PET study using either **[^{18}F]3c** or **[^{18}F]3f** in female Balb/c mice bearing EMT-6 tumors was performed on a microPET-F220 (CTI-Concorde Microsystems, Inc.) and a MicroCAT-II system. All mice were imaged at 1.0 h after i.v. injection of the radiotracer. The EMT-6 tumors were readily identifiable using either radioligand, indicating that they are both acceptable agents for detecting solid tumors and imaging their σ_2 receptor status with PET (Figure 4). Nevertheless, further studies will still be required before selecting one of these compounds for translation to the clinic.

[a] Abbreviations: YUN-143, *N*-(4-fluorobenzyl)piperidinyl-4-(3-bromophenyl) acetamide; CT, computed tomography.

Conclusion

In the present study, we synthesized several novel conformationally flexible benzamide analogues having a moderate to high binding affinity and selectivity for σ_2 receptors (Table 1). Four of these compounds were selected as candidates for developing ^{18}F-labeled PET probes to image the σ_2 receptor status of solid tumors. **[^{18}F]3c**, **[^{18}F]3d**, **[^{18}F]3e**, and **[^{18}F]3f** were successfully synthesized and evaluated as potential radiotracers for imaging EMT-6 tumors in female Balb/c mice. Of the four ^{18}F-labeled analogues, **[^{18}F]3c** and **[^{18}F]3f** had the best biodistribution kinetics and tumor/normal tissue ratios. Blocking studies confirmed that the uptake of **[^{18}F]3c** and **[^{18}F]3f** was σ_2 receptor mediated. Our initial CT/PET studies indicate that **[^{18}F]-3c** and **[^{18}F]3f** are acceptable agents for detecting solid tumors and imaging their σ_2 receptor status with PET. Further evaluation of these two radioligands is still required before selecting one of them for translation to the clinic.

Experimental Section

Materials. All reagents were purchased from commercial suppliers and used without further purification unless otherwise stated. Tetrahydrofuran (THF) was distilled from sodium hydride immediately prior to use. Anhydrous toluene was distilled from sodium/toluene shortly before use.

General. All anhydrous reactions were carried out in oven-dried glassware under an inert nitrogen atmosphere unless otherwise stated. When the reactions involved extraction with dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), ethyl acetate (EtOAc), or ethyl ether (Et_2O), the organic solutions were dried with anhydrous Na_2SO_4 and concentrated with a rotary evaporator under reduced pressure. Flash column chromatography was conducted using silica gel 60a, "40 Micron Flash" (32−63um; Scientific Adsorbents, Inc.). Melting points were determined using the MEL-TEMP 3.0 apparatus and left uncorrected. ^{1}H NMR spectra were recorded at 300 MHz on a Varian Mercury-VX spectrometer with $CDCl_3$ as solvent and tetramethylsilane (TMS) as the internal standard. All chemical shift values are reported in ppm (δ). Elemental analyses (C, H, N) were determined by Atlantic Microlab, Inc.

Procedure A: General Method for Synthesis of the Substituted 2-Hydroxybenzoic Acid Amides, 2a−e. *N*-[2-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-ethyl]-2-hydroxy-5-methylbenzamide (2a). 1,3-dicyclohexycarbodimide (432.6 mg, 2.1 mmol) and 1-hydroxybenzotriazole (283.8 mg, 2.10 mmol) were added to an ice−water bath cooled solution of **1a**[21] (472.0 mg, 2.0 mmol) and 2-hydroxy-5-methyl-benzoic acid (152 mg, 2.0 mmol) in 30 mL of dichloromethane. After the reaction mixture was stirred overnight, analysis of the products using thin layer chromatography with 20% methanol and 80% ethyl ether as the mobile phase indicated that the reaction was complete. After completion of the reaction, another 50 mL of dichloromethane was added to the mixture. The organic solution was then washed with an aqueous saturated $NaHCO_3$ solution and brine, sequentially. The organic solution was dried with anhydrous sodium sulfate. After removal of the solvent, the crude product was purified by column chromatography using 20% methanol and 80% ethyl ether as the mobile phase. The yield of **2a** was 37.1%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 2.25 (s, 3H), 2.75−2.95 (m, 6H), 3.58−3.65 (m, 4H), 3.82−3.83 (s, 6H), 6.48−6.51 (s, 1H), 6.62−6.63 (s, 1H), 6.82−6.85 (d, 1H), 7.02 (s, 1H), 7.08 (s, 1H), 7.20 (d, 1H). LCMS *m*/*z* 371.2 (M + H).

5-Bromo-*N*-[4-(6,7-dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-butyl]-2-hydroxy-3-methoxy-benzamide (2b). Compound **2b** was prepared from 5-bromo-2-hydroxy-3-methoxy-benzoic acid and **1b** as described above for **2a**. The yield of **2b** was 16.7%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 1.73−1.76 (m, 4H), 2.57−2.59 (m, 2H), 2.76−2.81 (m, 4H), 3.45−3.47 (m, 2H), 3.58−3.61 (m, 2H), 3.82 (s, 3H), 3.86 (s, 3H), 3.88 (s, 3H), 6.48−6.51 (t, 1H), 6.56−6.59 (t, 1H), 6.97−7.00 (m, 1H), 7.07−7.10 (m, 1H). LCMS *m*/*z* 493.10 (M + H).

***N*-[4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-butyl]-2-hydroxy-5-methyl-benzamide (2c).** Compound **2c** was prepared from 2-hydroxy-5-methyl-benzoic acid and **1b** as described above for **2a**. The yield of **2c** was 45%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 1.75 (m, 4H), 2.12 (s, 3H), 2.58 (m, 2H), 2.75−2.77 (m, 2H), 2.82−2.84 (m, 2H), 3.40−3.50 (m, 2H), 3.58 (s, 2H), 3.83 (s, 3H), 3.85 (s, 3H), 6.50 (s, 1H), 6.60 (s, 1H), 6.85 − 6.88 (d, 1H), 7.06 (s, 1H), 7.13−7.16 (d, 2H), 7.61 (s, 1H). LCMS *m*/*z* 399.20 (M + H).

***N*-[4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-butyl]-2-hydroxy-5-bromo-benzamide (2d).** Compound **2d** was prepared from 5-bromo-2-hydroxy-benzoic acid and **1b** as described above for **2a**. The yield of **2d** was 28.0%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 1.71−1.81 (m, 4H), 2.55−2.85 (m, 6H), 3.44−3.48 (m, 2H), 3.58−3.60 (m, 2H), 3.82 (s, 3H), 3.89 (s, 3H), 6.50 (s, 1H), 6.59 (s, 1H), 6.82−6.84 (d, 1H), 7.30−7.40 (d, 1H), 7.52 (d, 1H), 8.30 (s,1H). Anal. ($C_{22}H_{27}BrN_2O_4 \cdot 1.25H_2O$) C, H, N.

***N*-[4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-butyl]-2-hydroxy-5-iodo-benzamide (2e).** Compound **2e** was prepared form 2-hydroxy-5-iodo-benzoic acid and **1b** as described above for **2a**. The yield of **2e** was 27.0%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 1.69−1.81 (m, 4H), 2.54−2.65 (m, 2H), 2.75−2.83 (m, 2H), 3.44−3.48 (m, 2H), 3.58 (s, 2H), 3.82 (s, 3H), 3.85 (s, 3H), 6.50 (s, 1H), 6.58 (s, 1H), 6.70−6.74 (d, 1H), 7.54−7.55 (d, 1H), 7.65−7.67 (d, 1H), 8.20 (s, 1H). Anal. ($C_{22}H_{27}IN_2O_4 \cdot 0.75H_2O$) C, H, N.

Procedure B: General Method for the Synthesis of the Substituted 2-(2-Fluoroethoxy) Benzoic Acid Amides, 3a−e. [*N*-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-ethyl]-2-(2-fluoro-ethoxy)-5-methyl-benzamide (3a). Potassium carbonate (792.5 mg, 4.88 mmol) was added to a solution of **2a** (278 mg, 0.75 mmol) and 2-bromo-1-fluoroethane (620 mg, 4.88 mmol) in acetone (60 mL). The reaction mixture was refluxed for 48 h until the reaction was complete as determined by thin layer chromatography with 5% methanol and 95% ethyl ether as the mobile phase. The solvent was evaporated, 30 mL of water was added to the flask, and then the mixture was extracted with dichloromethane (25 mL × 3). After the organic layer was dried with anhydrous sodium sulfate, the crude product was purified by column chromatography using 5% methanol and 95% ethyl ether as the mobile phase. The yield of **3a** was 90%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 2.33 (s, 3H), 2.64−2.80 (m, 6H), 3.63−3.75 (m, 4H), 3.84 (s, 3H), 3.86 (s, 3H), 4.16 (m, 1H), 4.21 (m, 1H), 4.41 (m, 1H), 4.60 (m, 1H), 6.55 (s, 1H), 6.61 (s, 1H), 6.78−6.81 (d, 1H), 7.20 (d, 1H), 8.00 (s, 1H), 8.28 (s, 1H). LCMS *m*/*z* 417.22 (M + H). For the in vitro binding experiments, the free base was converted into the hydrochloride salt; mp 159−161 °C. Anal. ($C_{23}H_{30}ClFN_2O_4$) C, H, N.

5-Bromo-*N*-[4-(6,7-dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-butyl]-2-(2-fluoro-ethoxy)-3-methoxy-benzamide (3b). Compound **3b** was prepared from **2b** as described for **3a** above. The yield of **3a** was 50%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 1.64 (m, 4H), 2.48−2.52 (t, 3H), 2.64−2.66 (t, 3H), 2.74−2.78 (t, 3H), 3.42−3.55 (m, 4H), 3.79−3.87 (m, 9H), 4.19−4.22 (t, 2H), 4.29−4.32 (t, 2H), 4.58−4.61 (t, 2H), 4.74−4.77 (t, 2H), 6.47 (s, 1H), 6.54 (s, 1H), 7.07 (d, 1H), 7.78 (d, 1H), 8.10 (s, 1H). For the in vitro binding experiments, the free base was converted into the oxalic acid salt; mp 127−129 °C. LCMS *m*/*z* 590.30 (M + Li). Anal. ($C_{26}H_{33}BrFN_2O_7$) C, H, N.

***N*-[6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl-butyl]-2-(2-fluoro-ethoxy)-5-methyl-benzamide (3c).** Compound **3c** was prepared from **2c** as described above for **3a**. The yield of **3c** was 67%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 1.67−2.00 (m, 4H), 2.33 (s, 3H), 2.51−2.56 (t, 3H), 2.67−2.72 (t, 3H), 3H), 2.78−2.82 (t, 3H), 3.48−3.54 (m, 4H), 3.82 (s, 3H), 3,83 (s, 3H), 4.21−4.24 (t, 1H), 4.30−4.33 (t, 1H), 4.68−4.71 (t, 1H), 4.84−4.87 (t, 1H), 6.49 (s, 1H), 6.57 (s, 1H), 6.79−6.82 (d, 1H), 7.20 (m, 1H), 7.96 (s, 1H), 7.99−7.80 (d, 1H). LCMS *m*/*z* 445.25 (M + H). For the in vitro binding experiments, the free base was converted into the oxalic acid salt; mp 131−133 °C. Anal. ($C_{26}H_{34}FN_2O_2$) C, H, N.

***N*-[4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-butyl]-2-(2-fluoro-ethoxy)-5-bromo-benzamide (3d).** Compound **3d** was prepared from **2d** as described above for **3a**. The yield of **3d** was 38.90%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 1.57−1.80 (m, 4H), 2.62−2.66 (m, 3H), 2.78−2.82 (m, 3H), 3.48−3.51 (m, 2H), 3.60−3.64 (m, 2H), 3.82 (s, 3H), 3.83 (s, 3H), 4.21−4.25 (t, 1H), 4.31−4.35 (t, 1H), 4.70−4.74 (t, 1H), 4.86−4.90 (t, 1H), 6.49 (s, 1H), 6.57 (s, 1H), 6.78−6.82 (d, 1H), 7.48−7.52 (d, 1H), 7.96 (s, 1H), 8.26 (d, 1H). LCMS *m*/*z* 509.1 (M + H). For the in vitro binding experiments, the free base was converted into the oxalic acid salt; mp 119−121 °C. Anal. ($C_{25}H_{31}BrFN_2O_6$) C, H, N.

***N*-[4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-butyl]-2-(2-fluoro-ethoxy)-5-iodo-benzamide (3e).** Compound **3e** was prepared from **2e** as described above for **3a**. The yield of **3e** was 41.4%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 1.61−1.72 (m, 4H), 2.46−2.52 (m, 2H), 2.67−2.71 (m, 2H), 2.76−2.78 (m, 2H), 3.41−3.47 (m, 2H), 3.52 (t, 2H), 3.80 (s, 3H), 3.83 (s, 3H), 4.19−4.21 (m, 1H), 4.27−4.31 (m, 1H), 4.67−4.71 (m, 1H), 4.83−4.86 (m, 1H), 6.46 (s, 1H), 6.55 (s, 1H), 6.62−6.66 (d, 1H), 7.62−7.67 (m, 1H), 7.87 (s, 1H), 8.40−8.41 (d, 1H). LCMS *m*/*z* 557.13 (M + H). For the in vitro binding experiments, the free base was converted into the oxalic acid salt; mp 121−123 °C. Anal. ($C_{25}H_{31}FIN_2O_6$) C, H, N.

Procedure C: General Method for Converting the Substituted 5-Bromo-benzoic Acid Derivatives into their Substituted 5-Tributylstannanyl Benzoic Acid Derivatives. *N*-[4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-butyl]-2-(2-fluoro-ethoxy)-3-methoxy-5-tributylstannanyl-benzamide (3g). Nitrogen was bubbled for 5−10 min through a solution of **3b** (200 mg, 0.371 mmol) in 20 mL of fresh distilled toluene. The whole system was covered with aluminum foil. Tetrakis(triphenylphosphine palladium-(0) [$(PPh_3)_4Pd(0)$; 42 mg, 0.036 mmol] and bis(tributytin) ([$Sn(C_4H_9)_3]_2$; 575 mg, 0.99 mmol) was added to the reaction mixture and heated overnight at 110 °C with an oil bath. Thin layer chromatography with 45% hexane, 45% ethyl ether, and 10% methanol as the mobile phase was used to assess when the reaction was complete. After quenching the reaction, the crude product was purified on a silica gel column to isolate the tin intermediate, **3g**. The yield of **3g** was 64%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 0.87−1.58 (m, 27H), 1.61−1.69 (m, 4H), 2.56 (s, 2H), 2.72 (s, 2H), 2.81−2.82 (s, 2H), 3.47−3.49 (d, 2H), 3.57 (s, 2H), 3.83 (s, 6H), 3.88 (s, 3H), 4.25 (d, 1H), 4.37 (s, 1H), 4.62−4.65 (s, 1H), 4.78−4.81 (s, 1H), 6.51 (s, 1H), 6.58 (s, 1H), 7.81 (s, 1H), 8.07 (s, 1H). LCMS *m*/*z* 747.60 (M − H).

Procedure D: General Method for Converting the Tin Precursor of the Benzoic Acid Derivatives into their Corresponding Iodine Substituted Benzoic Acid Derivatives. (*N*-[4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-butyl]-2-(2-fluoro-ethoxy)-3-methoxy-5-iodo-benzamide (3f). A solution of iodine in $CHCl_3$ (5 mL, 0.5 M) was added dropwise to a solution of the tin precursor, **3g** (258 mg, 0.34 mmol), in 20 mL of CH_2Cl_2 until the color of the solution persisted. The reaction was stirred at room temperature for 30 min, and a solution of 5% aqueous $NaHSO_3$ was added until the solution was colorless. The mixture was extracted with CH_2Cl_2, and the organic layers were washed with brine before being dried with Na_2SO_4. The organic layers were then concentrated under vacuum and purified using a silica gel column with 15% methanol and 85% ether as the mobile phase to isolate **3f**. The yield of **3f** was 36%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 1.60−1.80 (m, 2H), 1.80−2.10 (m, 4H), 3.19−3.2 (m, 2H), 3.40−3.50 (m, 2H), 3.68 (m, 2H), 3.83 (m, 2H), 3.92 (s, 3H), 3.95 (s, 3H), 3.99 (s, 3H), 4.26 (t, 1H), 4.37 (s, 1H), 4.65 (s, 1H), 4.80 (s, 1H), 6.50 (s, 1H), 6.58 (s, 1H), 7.28 (d, 1H), 8.02 (d, 1H), 8.21 (s, 1H). LCMS *m*/*z* 587.14 (M + H). For the in vitro binding experiments, the free base was converted into the oxalic acid salt; mp 125−127 °C.

Procedure E: General Method for Converting the Substituted 2-Hydroxy Benzoic Acid Derivatives into their Substituted 2-(2-Hydroxy-ethoxy)-benzoic Acid Amides. *N*-[4−6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-butyl]-2-(2-hydroxy-ethoxy)-5-methyl-benzamide (4c). Anhydrous potassium carbonate (546.0 mg, 3.26 mmol) was added to a solution of **2c (**200.0 mg, 0.5 mmol) and 2-bromoethyl acetate (547.0 mg, 3.27 mmol) in 60 mL of acetone. The reaction mixture was refluxed for 48 h under nitrogen. After 48 h, thin layer chromatography with 15% methanol and 85% ether as the mobile phase indicated that the reaction was complete. After evaporating the solvent, the residue was dissolved in 30 mL of water and extracted with ethyl acetate (20 × 3 mL). Then the organic component was washed with brine, dried with anhydrous sodium sulfate, and concentrated, and the final product was purified on a silica gel column to isolate the 2-{2-[4-(6,7-dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-butylcarbamoyl]-4-methyl-phenoxy}-ethyl ester. The yield of this intermediate was 82.2%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 1.70 (m, 4H), 2.01 (s, 3H), 2.33 (s, 3H), 2.56 (m, 2H), 2.71−2.73 (m, 2H), 2.81 (m, 2H), 3.50−3.52 (m, 2H), 3.55 (s, 2H), 3.83 (s, 3H), 3.84 (s, 3H), 4.23 (t, 2H), 4.50 (t, 2H), 6.50 (s, 1H), 6.58 (s, 1H), 6.78−6.82 (d, 1H), 7.18−7.25 (d, 1H), 7.95 (s, 1H), 8.02 (s, 1H).

NaOH (30 mg, 0.75mmol) was added to a solution of this intermediate (182 mg, 0.375mmol) in 20 mL of methanol and 10 mL of water. The reaction mixture was stirred overnight until the reaction was complete. Then 0.375 mL of 2 N HCl was added to neutralize the solution. After evaporating the solvent, the residue was dissolved in 60 mL of ethyl acetate. The solution was washed first with water, then brine, and finally dried with anhydrous sodium sulfate. After evaporating the solvent, the crude product was purified on a silica gel column. The yield of **4c** was 96%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 1.68−1.85 (m, 4H), 2.39 (s, 3H), 2.45 (s, 1H), 2.51−2.61 (m, 2H), 2.80−2.87 (m, 4H), 3.45−3.61 (m, 4H), 3.76−3.80 (t, 2H), 3.83 (s, 3H), 3.85 (s, 3H), 3.83 (s, 3H), 4.05−4.08 (t, 2H), 6.49 (s, 1H), 6.60 (s, 1H), 6.79−6.83 (d, 1H), 7.15−7.19 (d, 2H), 7.93 (s, 1H), 8.30 (s, 1H).

5-Bromo-*N*-[4-(6,7-dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-butyl]-2-(2-hydroxy-ethoxy)-benzamide (4d). Compound **4d** was prepared from **2d** as described above for **4c**. The yield of **4d** was 70%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 1.65−1.90 (m, 4H), 2.66−2.70 (m, 2H), 2.92 (m, 4H), 3.51−3.54 (m, 2H), 3.72 (m, 2H), 3.72−3.81 (t, 2H), 3.83 (s, 3H), 3.85 (s, 3H), 4.05−4.09 (t, 2H), 6.51 (s, 1H), 6.60 (s, 1H), 6.77−6.81 (d, 1H), 7.44−7.48 (d, 2H), 8.17 (s, 1H), 8.30 (s, 1H).

***N*-[4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-butyl]-2-(2-hydroxy-ethoxy)-5-iodo-benzamide (4e).** Compound **4e** was prepared from **2e** as described above for **4c**. The yield of **4e** was 77%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 1.60−1.90 (m, 4H), 2.63−2.66 (m, 3H), 2.90 (s, 2H), 3.52−3.55 (m, 2H), 3.70 (m, 2H), 3.79−3.82 (t, 2H), 3.83 (s, 3H), 3.85 (s, 3H), 4.08−4.10 (t, 2H), 6.50 (s, 1H), 6.60 (s, 1H), 6.67−6.70 (d, 1H), 7.60−7.70 (d, 1H), 8.30 (s, 1H), 8.40−8.41 (d, 1H).

Procedure F: General Method for Converting the Substituted 2-Hydroxy-ethoxy Benzoic Acid Amides to their Methanesulfonic Acid Esters. 2-(2-(4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butylcarbamoyl)-4-methylphenoxy)-ethyl Methanesulfonate (5c). Methanesulfonic chloride (120 mg, 1.04 mmol) was added to an ice−water cooled solution of **4c** (354 mg, 0.8 mmol) and triethylamine (242 mg, 2.4 mmol) in 30 mL of dichloromethane. The reaction mixture was stirred for 3 h until thin layer chromatography using 5% methanol and 95% dichloromethane as the mobile phase indicated that the reaction was complete. After 3 h, 20 mL of dichloromethane was added, the solution was washed with first a saturated sodium carbonate aqueous solution (20 mL × 3) and then brine, and finally dried with anhydrous sodium sulfate. After evaporating the solvent, the crude product was purified on a silica gel column to isolate **5c**. The yield of **5c** was 81.6%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 2.07−2.10 (m, 4H), 2.71 (s, 3H), 2.80−3.00 (m, 2H), 3.08−3.20 (m, 4H), 3.40 (m, 3H), 3.80−4.00 (m, 4H), 4.20 (s, 3H), 4.22 (s, 3H), 4.60−4.68 (t, 2H), 4.95−4.97 (t, 2H), 6.88 (s, 1H), 6.95 (s, 1H), 7.15−7.18 (d, 1H), 7.50−7.60 (d, 1H), 8.20 (s, 1H), 8.19 (s, 1H). Anal. ($C_{26}H_{36}N_2O_7S$) C, H, N.

2-(4-Bromo-2-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butylcarbamoyl)phenoxy)ethyl Methanesulfonate (5d). Compound **5d** was prepared from **4d** as described above for **5c**. The yield of **5d** was 77%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 1.72−1.75 (m, 4H), 2.61−2.68 (m, 2H), 2.85 (s, 3H), 3.05 (s, 2H), 3.42−3.58 (m, 4H), 3.68 (s, 2H), 3.82 (s, 3H), 3.83 (s, 3H), 4.29 (t, 2H), 4.60 (t, 2H), 6.50 (s, 1H), 6.57 (s, 1H), 6.70−6.77 (d, 1H), 7.45−7.55 (d, 1H), 7.95 (s, 1H), 8.20−8.22 (d, 1H). LCMS *m*/*z* 585.10 (M + H).

2-(2-(4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butylcarbamoyl)-4-iodophenoxy)ethyl Methanesulfonate (5e). Compound **5e** was prepared from **4e** as described above for **5c**. The yield of **5e** was 80%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 1.60−1.80 (m, 4H), 2.52−2.55 (m, 2H), 2.67−2.70 (m, 2H), 2.70−2.78 (m, 2H), 3.02 (s, 3H), 3.46−3.52 (m, 4H), 3.82 (s, 3H), 3.83 (s, 3H), 4.23−4.26 (t, 2H), 4.56−4.60 (t, 2H), 6.48 (s, 1H), 6.55 (s, 1H), 6.60−6.64 (d, 1H), 7.64−7.68 (d, 1H), 7.85 (s, 1H), 8.38−8.39 (d, 1H). LCMS *m*/*z* 633.10 (M + H).

2-(2-Acetoxy-ethoxy)-5-bromo-3-methoxy-benzoic Acid Methyl Ester (7). Initially, 1.0 mL of 98% concentrated sulfuric acid was added to a solution of 5-bromo-2-hydroxy-3-methoxy-benzoic acid, **6** (1.0 g, 4.0 mmol), in 50 mL of methanol. The reaction mixture was refluxed overnight until thin layer chromatography using 20% ethyl acetate and 80% hexane as the mobile phase indicated that the reaction was complete. After evaporating the methanol, the residue was dissolved in 60 mL of ethyl acetate and washed with a saturated $NaHCO_3$ aqueous solution and then brine. After drying with anhydrous sodium sulfate, the solution was concentrated and the crude product was purified on a silica gel column to isolate the intermediate, 5-bromo-2-hydroxy-3-methoxy-benzoic acid methyl ester. The yield of this intermediate was 94%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 3.88 (s, 3H), 3.95 (s, 3H), 7.09 (d, 1H), 7.55 (t, 1H), 10.96 (d, 1H).

Potassium carbonate (2.90 g, 21.0 mmol) was added to a solution of the above intermediate (0.84 g, 3.23 mmol) and 2-bromoethyl acetate (3.5 g, 20.96 mmol) in 60 mL of acetone. The reaction mixture was refluxed for 72 h until thin layer chromatography using 20% ethyl acetate and 80% hexane as the mobile phase indicated that the reaction was complete. After evaporating the solvent, the residue was dissolved in 30 mL of water and then extracted with ethyl acetate (25 mL × 3). The organic solution was dried with anhydrous sodium sulfate, resuspended, and purified on a silica gel column. The yield of **7** was 78%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 2.10 (s, 3H), 3.86 (s, 3H), 3.88 (s, 3H), 4.21−4.24 (t, 2H), 4.38−4.41 (t, 3H), 7.14 (s, 1H), 7.45 (s, 1H).

2-(2-Acetoxy-ethoxy)-5-iodo-3-methoxy-benzoic Acid Methyl Ester (8). Nitrogen was bubbled for 5−10 min through a solution of of 2-(2-acetoxy-ethoxy)-5-bromo-3-methoxy-benzoic acid methyl ester, **7** (270 mg, 0.778 mmol), in 20 mL of freshly distilled toluene. The reaction system was covered with aluminum foil. Tetrakis-(triphenylphosphine) palladium(0) [$(PPh_3)_4Pd(0)$; 100 mg, 0.087 mmol] and bis(tributlytin) ($[Sn(C_4H_9)_3]_2$; 899 mg, 1.55 mmol) were added to the reaction mixture and heated overnight at 110 °C in an oil-bath while stirring. After quenching, thin layer chromatography using 15% ethyl acetate and 85% hexane as the mobile phase indicated that the reaction was complete. The product was then purified on a silica gel column to isolate the tin precursor, 2-(2-acetoxy-ethoxy)-3-methoxy-5-tributylstannanyl-benzoic acid methyl ester. The yield of the tin precursor was 37.3%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 0.8−1.75 (m, 27H), 2.10 (s, 3H), 3.87 (s, 3H), 3.89 (s, 3H), 4.24−4.27 (t, 2H), 4.38−4.41 (t, 2H), 7.09−7.30 (s 1H), 7.35 (s 1H).

A solution of iodine in $CHCl_3$ (5 mL, 0.5 M) was added dropwise to a solution of above tin precursor (680 mg, 1.22 mmol) in 20 mL of CH_2Cl_2 until the color of the solution persisted. Then the

reaction was stirred at room temperature for 30 min, and a quench solution of 5% aqueous $NaHSO_3$ was added until the solution became colorless. The mixture was extracted with CH_2Cl_2, and the organic layers were washed with brine and dried by Na_2SO_4. The organic layers were then condensed under vacuum and purified using a silica gel column with 15% ethyl acetate and 85% hexane as the mobile phase. The yield of **8** was 90%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 2.10 (s, 3H), 3.85 (s, 3H), 3.88 (s, 3H), 4.21−4.25 (t, 2H), 4.37−4.41 (t, 2H), 7.29−7.30 (s 1H), 7.63 (s 1H).

2-(2-Hydroxy-ethoxy)-5-iodo-3-methoxy-benzoic Acid (9). Compound **9** was prepared from **8** as described in Procedure E. The yield of **9** was 81%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 3.89 (s, 3H), 3.93−3.96 (t, 2H), 4.33−4.36 (t, 2H), 7.08 (s, 1H), 7.62 (s, 1H).

***N*-[4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-butyl]-2-(2-hydroxy-ethoxy)-5-iodo-3-methoxy-benzamide (4f).** Compound **4f** was prepared from **9** and **1b** as described in Procedure A. The yield of **4f** was 29%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 1.72−1.75 (m, 4H), 2.56 (m, 2H), 2.75−2.77 (m, 2H), 2.81−2.83 (m, 2H), 3.49−3.51 (m, 2H), 3.55 (s, 2H), 3.56−3.60 (t, 2H), 3.82 (s, 3H), 3.83 (s, 3H), 3.85 (s, 3H), 4.06−4.10 (t, 2H), 6.47 (s, 1H), 6.57 (s, 1H), 6.90 (s, 1H), 7.57 (s, 1H), 7.70−7.80 (s, 1H).

2-(2-(4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butylcarbamoyl)-4-iodo-6-methoxyphenoxy)ethyl Methanesulfonate (5f). Compound **5f** was prepared from **4f** as described in Procedure F. The yield of **5f** was 61%. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 1.72 (m, 4H), 2.55 (s, 2H), 2.70 (d, 2H), 22.76 (d, 2H), 3.05 (s, 3H), 3.85 (m, 9H), 4.26 (m, 2H), 4.49 (m, 2H), 6.49 (s, 1H), 6.55 (s, 1H), 6.93 (d, 1H), 7.51 (m, 1H), 8.02 (s, 1H). LCMS *m*/*z* 663.20 (M + H). Anal. ($C_{25}H_{32}FIN_2O_5$) Calcd: C, 51.20; H, 5.50; N, 4.78. Found: C, 36.61; H, 4.34; N, 3.12.

Radiochemistry. Production of [^{18}F]Fluoride. [^{18}F]Fluoride was produced in our institution by proton irradiation of enriched ^{18}O water (95%) [reaction: ^{18}O(p, n)^{18}F] using either a JSW BC-16/8 (Japan Steel Works) or a CS-15 cyclotron (Cyclotron Corp)

Procedure G: General Method for Labeling the Substituted 2-(2-Fluoroethoxy) Benzoic Acid Amide Analogs with ^{18}F. [^{18}F]-(*N*-[4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-butyl]-2-(2-fluoro-ethoxy)-3-methoxy-5-iodo-benzamide ([^{18}F]3f). [^{18}F]-fluoride (100−150 mCi) was added to a 10-mL Pyrex screw cap tube containing 5−6 mg of Kryptofix 222 and 0.75 mg of K_2CO_3. Using HPLC grade acetonitrile (3 × 1.0 mL), the water was azeotropically evaporated from this mixture at 110 °C under a stream of argon. After all of the water was removed, a solution of the precursor, **5f** (1.5−2.0 mg), in DMSO (0.2 mL) was added to the reaction vessel containing the ^{18}F/Kryptofix mixture. A 3 mm glass bead was added to the reaction vessel to ensure a more homogeneous heat distribution when the sample was irradiated with microwaves, and the vessel was capped firmly on a specially designed remotely operated capping station. After vortexing, the reaction mixture was irradiated with microwaves for 30−40 s at medium power (60 W) until the thin layer chromatography scanner with a 25% of methanol and 75% dichloromethane mobile phase indicated that the incorporation yield was 40−60%.

After adding 6 mL of water and shaking, the solution was loaded on a C-18 reverse phase Waters Oasis cartridge (HLB-6 cm^3) that had previously been rinsed with a solution of 5% methanol in water (5−8 mL). The sample was then rinsed three times with 6 mL of water to eliminate the unreacted fluoride. The retained activity was eluted with 5−8 mL of acetonitrile. After evaporating the acetonitrile to a volume of <0.5 mL, the sample was loaded on a C-18 Alltech econosil semipreparative HPLC column (250 × 10 um). The product was eluted with 29% acetonitrile and 71% 0.1 M ammonium formate buffer at a flow rate of 4.5 mL/min. The retention time of the **[^{18}F]3f** was ∼33 min. The solution containing the **[^{18}F]3f** was concentrated and resuspended in saline, and a 100 μL aliquot was sent for quality control analysis before using it in the biodistribution and imaging studies. The entire procedure required ∼2 h.

Quality control analysis was performed on an analytical HPLC system that consisted of an Alltech econosil reversed phase C-18 column (250 × 4.6 mm) with a mobile phase of 35% acetonitrile and 65% 0.1 M ammonium formate buffer at pH 4.0−4.5. At a flow rate of 1.2 mL/min, the **[^{18}F]3f** eluted at 13.2 min with a radiochemical purity of >99%. The labeling yield was ∼30% (decay corrected), and the specific activity was >2000 Ci/mmol.

[^{18}F](*N*-[4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-butyl]-2-(2-fluoro-ethoxy)-5-methyl-benzamide ([^{18}F]3c). Compound **[^{18}F]3c** was prepared from **5c** as described above for **[^{18}F]3f**, with the following exceptions. The semipreparative HPLC mobile phase was 39% methanol and 61% 0.1 M formate buffer. At a flow rate of 3.5 mL/min, the **[^{18}F]3c** eluted at ∼33 min with a radiochemical purity of >99%. The labeling yield was ∼35% (decay corrected), and the specific activity was >1500 Ci/mmol. The entire procedure took ∼2 h.

To check that the chemical characteristics of **[^{18}F]3c** were identical to the cold standard, **3c**, both compounds were run on the analytical HPLC system with a mobile phase of 52% methanol and 48% 0.1 M formate buffer. At a flow rate of 1.5 mL/min, the two compounds coeluted with a retention time of 4.7 min.

[^{18}F](*N*-[4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-butyl]-2-(2-fluoro-ethoxy)-5-bromo-benzamide ([^{18}F]3d). Compound **[^{18}F]3d** was prepared from **5d** as described above for **[^{18}F]3f** with the following exceptions. The semipreparative HPLC mobile phase was 13% of THF and 87% 0.1 M formate buffer. At a flow rate of 3.5 mL/min, the **[^{18}F]3d** eluted at ∼20 min with a radiochemical purity of >98%. The labeling yield was ∼30% (decay corrected), and the specific activity was >1500 Ci/mmol. The entire procedure took ∼2 h.

To check that the chemical characteristics of **[^{18}F]3d** were identical to the cold standard, **3d**, both compounds were run on the analytical HPLC system with a mobile phase of 38% acetonitile and 62% 0.1 M formate buffer. At a flow rate of 1.5 mL/min, the two compounds coeluted with a retention time of ∼8 min.

[^{18}F](*N*-[4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-butyl]-2-(2-fluoro-ethoxy)-5-Iodo-benzamide ([^{18}F]3e). Compound **[^{18}F]3e** was prepared from **5e** as described above for **[^{18}F]3f**, with the following exceptions. The semipreparative HPLC mobile phase was 15% THF and 85% 0.1 M formate buffer. At a flow rate of 6.0 mL/min, the **[^{18}F]3e** eluted at ∼35 min with a radiochemical purity of >99%. The labeling yield was ∼30% (decay corrected), and the specific activity was >1500 Ci/mmol. The entire procedure took ∼2 h.

To check that the chemical characteristics of **[^{18}F]3e** were identical to the cold standard, **3e**, both compounds were run on the analytical HPLC system with a mobile phase of 17% tetrahydrofuran and 83% 0.1 M ammonium formate buffer. At a flow rate of 2.0 mL/min, the two compounds coeluted with a retention time of 15.2 min.

Characterization of the Potential σ_2 Selective Ligands In Vitro and In Vivo. Sigma Receptor Binding Assays. The novel sigma ligands were dissolved in *N*,*N*-dimethylformamide (DMF), DMSO, or ethanol and then diluted in 50 mM Tris-HCl buffer containing 150 mM NaCl and 100 mM EDTA at pH 7.4 prior to performing the σ_1 and σ_2 receptor binding assays The procedures for isolating the membrane homogenates and performing the σ_1 and σ_2 receptor binding assays have been described in detail previously.[21]

Briefly, the σ_1 receptor binding assays were conducted in 96-well plates using guinea pig brain membrane homogenates (∼300 μg protein) and ∼5 nM [^{3}H](+)-pentazocine (34.9 Ci/mmol, Perkin-Elmer, Boston, MA). The total incubation time was 90 min at room temperature. Nonspecific binding was determined from samples that contained 10 μM of cold haloperidol. After 90 min, the reaction was terminated by the addition of 150 μL of ice-cold wash buffer (10 mM Tris-HCl, 150 mM NaCl, pH 7.4) using a 96 channel transfer pipet (Fisher Scientific, Pittsburgh, PA). The samples were harvested and filtered rapidly through a 96-well fiberglass filter

plate (Millipore, Billerica, MA) that had been presoaked with 100 μL of 50 mM Tris-HCl buffer at pH 8.0 for 1 h. Each filter was washed three times with 200 μL of ice-cold wash buffer, and the filter was counted in a Wallac 1450 MicroBeta liquid scintillation counter (Perkin-Elmer, Boston, MA).

The σ_2 receptor binding assays were conducted using rat liver membrane homogenates (~300 μg protein) and ~5 nM [^{3}H]DTG (58.1 Ci/mmol, Perkin-Elmer, Boston, MA) in the presence of 1 μM (+)-pentazocine to block σ_1 sites. The incubation time was 120 min at room temperature. Nonspecific binding was determined from samples that contained 10 μM of cold haloperidol. All other procedures were identical to those described for the σ_1 receptor binding assay above.

Data from the competitive inhibition experiments were modeled using nonlinear regression analysis to determine the concentration that inhibits 50% of the specific binding of the radioligand (IC_{50} value). Competitive curves were best fit to a one-site fit and gave pseudo-Hill coefficients of 0.6−1.0. K_i values were calculated using the method of Cheng and Prusoff [24] and are presented as the mean ± 1 SEM. For these calculations, we used a K_d value of 7.89 nM for [^{3}H](+)-pentazocine and guinea pig brain; for [^{3}H]DTG and rat liver, we used 30.73 nM[20]

Biodistribution Studies. All animal experiments were conducted in compliance with the Guidelines for the Care and Use of Research Animals established by Washington University's Animal Studies Committee. EMT-6 mouse mammary adenocarcinoma cells (5×10^5 cells in 100 μL of phosphate-buffered saline) were implanted subcutaneously in the scapular region of female Balb/c mice (~2 months old and 17−22 g; Charles River Laboratories). The biodistribution studies were initiated 7−10 days after implantation when the tumor size was ~0.2 cm^3 (~200 mg).

For the biodistribution studies, 10−20 μCi of **[^{18}F]3c**, **[^{18}F]3d**, **[^{18}F]3e**, or **[^{18}F]3f** in 100−150 μL of saline was injected via the tail vein into EMT-6 tumor-bearing female Balb/c mice. Groups of at least four mice were used for each time point. At 5, 30, 60, and 120 min after injection, the mice were euthanized, and samples of blood, lung, liver, kidney, muscle, fat, heart, brain, bone, and tumor were removed, weighed, and counted in a Beckman Gamma 8000 well counter. After counting, the percentage of the injected dose per gram of tissue (%ID/g) was calculated. The tumor/organ ratios were calculated by dividing the %ID/g of the tumor by the %ID/g of each organ.

Blocking studies in tumor-bearing mice were conducted by coinjecting 1 mg/kg of cold YUN-143 with **[^{18}F]3c** or **[^{18}F]3f**. Yun-143 has a high affinity for both σ_1 and σ_2 receptors and is routinely used in our laboratory for sigma receptor blocking studies.[13,14,25] All mice were sacrificed 60 min after injection of the radiotracer, and the tumor/organ ratios were determined as described above.

Imaging Studies. Each mouse was imaged on both a microPET-F220 (CTI-Concorde Microsystems, Inc.) and a MicroCAT II System (ImTek, Inc.). For the microPET studies, each mouse was injected with ~0.25 mCi of either **[^{18}F]3c** or **[^{18}F]3f** via the tail vein and imaged 1 h later. MicroCT images were also obtained and coregistered with the PET images to determine the exact anatomical location of the radiotracers.

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Supporting Information Available: Analytical data on all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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