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New *N*-substituted 9-azabicyclo[3.3.1]nonan-3α-yl phenylcarbamate analogs as σ_2 receptor ligands: synthesis, *in vitro* characterization, and evaluation as PET imaging and chemosensitization agents

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Abstract

A series of *N*-substituted 9-azabicyclo[3.3.1]nonan-3α-yl phenylcarbamate analogs were synthesized. Among them, **WC-26** and **WC-59** were identified as the most potent σ_2 receptor ligands (K_i = 2.58 and 0.82 nM, respectively) with high selectivity against σ_1 (K_i of σ_1/σ_2 ratio = 557 and 2,087, respectively). [^{18}F]**WC-59** was radiolabeled via a nucleophilic substitution of a mesylate precursor by [^{18}F]fluoride, and *in vitro* direct binding studies of [^{18}F]**WC-59** were conducted using membrane preparations from murine EMT-6 solid breast tumors. The results indicate that [^{18}F] **WC-59** binds specifically to σ_2 receptors *in vitro* (K_d = ~2 nM). Biodistribution studies of [^{18}F] **WC-59** in EMT-6 tumor-bearing mice indicated that the tracer was a less suitable candidate for clinical imaging studies than existing F-18 labeled σ_2 receptor ligands. The ability of **WC-26** to enhance the cytotoxic effects of the chemotherapy drug, doxorubicin, was evaluated in cell culture using the mouse breast tumor EMT-6 and the human tumor MDA-MB435. **WC-26** greatly increased the ability of doxorubicin to kill these two tumor cell lines *in vitro*. These results indicate that **WC-26** is potentially a useful chemosensitizer for the treatment of cancer when combined with conventional chemotherapeutics.

Keywords

Sigma-2 ligands; PET radiotracers; cancer; chemosensitization

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1. Introduction

Sigma receptors are a distinct class of proteins with a widespread distribution in the central nervous system and peripheral tissues.[1] It is now widely accepted that there are at least two types of sigma receptors, σ_1 and σ_2.[2] Although the function of these receptors are not clearly defined yet, these receptors are distinguishable functionally, pharmacologically, and by their molecular size. The σ_1 receptor has a molecular weight of ~25 kDa and has been cloned; the σ_2 receptor has a molecular weight of ~21.5 kDa and has not been cloned.[3-5] σ_2 receptors are overexpressed in a wide variety of human tumor cells, and the σ_2 receptor density is 10-fold higher in proliferative cells compared to quiescent mouse mammary adenocarcinoma cells both *in vitro* and *in vivo*.[6-10] The high σ_2 receptor density in a wide variety of tumor types suggests that σ_2 receptors could be a target for the development of new radiotracers for imaging tumors with positron emission tomography (PET) and/or single photon emission computed tomography (SPECT).[11-13]

Recent studies have shown that σ_2 selective ligands induce reactive oxygen species (ROS) and apoptosis in human tumor cell lines.[14-16] Although the mechanism of cell death is largely unknown, several studies have revealed that σ_2 receptor ligands induce apoptosis by caspase-dependent and/or caspase-independent pathways.[14-16] Most antitumor drugs have severe adverse effects at high doses or following chronic use, and these adverse effects limit their clinical utility. One of the strategies to overcome this obstacle is to use a drug with no overlapping toxicity to enhance the ability of another antitumor drug to kill tumor cells. This phenomenon is called chemosensitization and can result in either an increase in tumor cell kill at the same level of toxicity or a decrease in toxicity at the same level of tumor cell kill. Additional studies with some σ_2 selective ligands have demonstrated their ability to enhance the cytotoxicity of anticancer drugs.[14-16] For example, cytotoxicity is increased when MCF-7 cells are treated with combinations of either doxorubicin or actinomycin and the σ_2 selective ligand, CB-184.[14] Consequently, there is also a great interest in developing high affinity σ_2 receptor ligands as anticancer drugs or chemosensitizing agents.

Although a number of sigma receptor ligands have been reported, most of these ligands have either a high selectivity for the σ_1 receptor (i.e. (+)-pentazocine), or bind with similar affinities to both σ_1 and σ_2 receptors (i.e. haloperidol (**1**) and DTG (**2**))[1]. Only a few sigma ligands, such as siramesine (**3**), CB-184 (**4**), and the benzamide analogs represented by RHM-1 (**7**), have a moderate to high selectivity for the σ_2 receptor (Fig. 1).[11,12,17-22] BIMU-1 (**5**), a potent 5-hydroxytryptamine receptor ligand with selectivity for 5-HT_3 and 5-HT_4, has been shown to possess a moderate affinity and selectivity for σ_2 versus σ_1 receptors.[23] Based on a structural modification of BIMU-1, we previously reported that the σ_2 selective ligand, 9-benzyl-9-azabicyclo[3.3.1]nonan-3-yl 2-methoxy-5-methylphenylcarbamate (**6**) has a higher selectivity for σ_2 receptors than for σ_1 receptors (σ_1/σ_2 = 31) with no binding to 5-HT_3 and 5-HT_4 (K_is: >1,000 nM).[24,25] Thus, **6** may serve as an excellent lead compound for the development of novel highly selective σ_2 ligands.

In the present study, a series of N-substituted-9-azabicyclo[3.3.1] carbamate analogs was prepared, and their affinities for the σ receptors measured. Two promising candidates for further study were identified: **WC-26** and **WC-59**. The highly selective σ_2 receptor ligand, **WC-59**, was radiolabeled with ^{18}F to generate [^{18}F]**WC-59**, and its direct saturation binding affinity measured using membranes from EMT-6 mouse breast tumor. In addition, the biodistribution of [^{18}F]**WC-59** in female BALB/c mice implanted with EMT-6 tumor cells was investigated. The *in vivo* anti-tumor properties of the highly selective σ_2 receptor ligand **WC-26** were previously evaluated using Panc-02, a murine pancreatic tumor model implanted in C57BL/6 mice.[27] These studies demonstrated a reduction in tumor volume during and immediately following treatment with **WC-26**, despite continued tumor growth in vehicle-treated mice.

WC26 treated mice showed no evidence of systemic toxicity. The **WC-26** treated tumors displayed dose-dependent increases in apoptosis when analyzed by flow cytometry methods. Although the tumors resumed growing when treatment was halted, these results indicate that **WC-26** is biologically active; therefore additional *in vitro* studies were conducted to evaluate the potential utility of **WC-26** in combination chemotherapy as a chemosensitizer. Using the mouse breast tumor cell line EMT-6 and the human MDA-MB435 cell line, the ability of **WC-26** to increase the cytotoxicity of the anticancer drug, doxorubicin (DOX), was explored. The results of these studies indicate that although [^{18}F]**WC-59** is not a superior PET radiotracer for detecting solid tumors; **WC-26** is potentially a useful chemosensitizer for treating cancer.

2. Results

2.1. Chemistry

The synthesis of sigma receptor ligands based on the phenylcarbamate lead compound **6** is shown in Scheme 1. Coupling of 2-methoxy-5-methylphenyl isocyanate **8** with the alcohol **9** gave the phenylcarbamate compound **6**, after which the benzyl group of **6** was cleaved by transfer hydrogenation to generate the secondary amine **10**. The bridgehead nitrogen of **10** was alkylated by treatment of **10** with alkyl halide to give compounds **11a-j**, and **WC-59**, or compound **10** was reacted with 4-(dimethylamino)benzaldehyde under $NaBH_3CN$ conditions to give **WC-26**. The synthesis of the key intermediates, **14, 18**, and **22** used to generate **WC-59, 11i**, and [^{18}F]**WC-59**, respectively, is shown in Scheme 2.

2.2. Sigma receptor binding affinity of the compounds generated in Scheme 1

Competitive inhibition experiments were performed to measure the σ_1 and σ_2 binding affinities (K_i) of **11a-j, WC-59**, and **WC-26** as previously described.[26] The σ_1 binding sites were assayed with the σ_1 selective radioligand, [^{3}H](+)-pentazocine (~3 nM), using guinea pig brain homogenates. The σ_2 binding sites were assayed with the novel σ_2-selective radioligand, [^{3}H] **RHM-1** (~1 nM), using rat liver homogenates.[26] The results of these *in vitro* binding studies are shown in Table 1. The compounds, **11a-j**, had low to moderate selectivity and affinity for σ_2 receptors. However, two compounds, **WC-59**, and **WC-26**, had both a high selectivity and a high affinity for σ_2 receptors. **WC-59**, and **WC-26** also had log P values that make them suitable for *in vivo* studies either imaging or treating proliferative solid tumors. Consequently, these two compounds were selected for further development respectively as a PET radiotracer and a chemotherapeutic agent.

2.3. Radiolabeling of WC-59

The synthesis of both the mesylate precursor **25** and [^{18}F]**WC-59** is shown in Scheme 3. The secondary amine **10** was alkylated with (4-(bromomethyl)phenethyloxy)(*tert*-butyl) diphenylsilane **22** to generate **23**, and the (*tert*-butyl)diphenylsilane group in **23** was deprotected with *n*-Bu_4NF to give the alcohol **24**. The hydroxyl group of **24** was then mesylated to generate the precursor **25**. The synthesis of [^{18}F]**WC-59** was accomplished by nucleophilic substitution of the mesylate precursor **25** with [^{18}F]fluoride under standard conditions. The total synthesis and purification time was 100 min with an overall decay corrected yield of 15%. The radiochemical purity of the [^{18}F]**WC-59** was >99.9%, and the specific activity was 4,200 mCi/μmol.

2.4. [^{18}F]WC-59 binding study with EMT-6 tumors

To determine if the binding affinity of [^{18}F]**WC-59** was suitable for a solid tumor imaging agent, membrane homogenates were prepared from ~800 mg of EMT-6 mouse breast tumors, and a total direct saturation binding study performed using these membranes (Figure 2). The σ_2 receptor density was high (~3,700 fmol/mg protein), and the binding affinity of [^{18}F]

WC-59 to the σ_2 receptors in EMT-6 membranes was ~2 nM; a value consistent with the previous *in vitro* results (Table 1). The high *in vitro* direct binding affinity indicated that this radioligand was suitable for further evaluation in imaging solid tumors.

2.5. Biodistribution of [^{18}F]WC-59 in tumor-bearing mice

A biodistribution study was conducted in EMT-6 tumor-bearing female BALB/c mice. There was a high initial uptake of [^{18}F]**WC-59** in lung, kidney and heart, but this uptake was reduced rapidly over the 2 h post-injection period (Table 2): the initial activity in lung, kidney, and heart was reduced by 95%, 75%, and 93% at 2 h post-injection, respectively. Liver also had a high initial uptake followed by a minimal washout of the radiotracer over the 2 h post-injection period. However, as previously reported, liver has a high density of σ_2 receptors.[1] The uptake of the [^{18}F]**WC-59** radiotracer in EMT-6 tumors was modest at 5 min post-injection (1.39 ± 0.34 %ID/g), but increased rapidly to ~3.5 %ID/g by 30 min post-injection; this level was stable for least 2 h (Table 2). Uptake in fat 5 min post-injection was also initially modest (2.33 ± 0.83 %ID/g) but increased more than 5.5-fold (12.63 ± 0.61 %ID/g) by 2 hours post-injection while bone activity level showed a modest increase and relatively stable levels from 30 min to 2 h. Despite the rapid washout, lung activity 2 h post injection (4.98 %ID/g) remained higher than the activity seen in tumor (4.08 %ID/g). These data indicate that [^{18}F]**WC-59** is a not superior to other ^{18}F-labeled σ_2 receptor ligands developed by our group as PET imaging agents for detecting and determining the proliferative status of solid tumors.[12]

2.6. Evaluation of WC-26 as a chemosensitizer

WC-26 has been previously shown to have modest antitumor activity as a single agent in a mouse pancreatic tumor model.[27] Studies have shown that σ_2 receptor ligands generate ROS in tumor cells grown in tissue culture.[15] Thus, we hypothesized that **WC-26** when used as in combination therapy, may increase the antitumor activity of chemotherapeutic agents such as doxorubicin (DOX) that are thought to kill tumor cells, at least in part, by generating intracellular ROS.

When exponentially growing mouse EMT-6 (Figure 3) or human MDA-MB435 (Figure 4) tumor cells were treated for 16 h with 2 μM **WC-26** alone and then a range of concentrations of DOX was added for an additional 24 h, a large decrease in clonogenic survival relative to control samples was observed compared to the clonogenic survival for cells treated with DOX alone. It should be noted that, for both cell lines, a 40 h treatment with 2 μM **WC-26** alone resulted in no measureable cell kill. It is also important to note that a substantial increase in tumor cell kill following sequential administration of the drugs occurred at doses where neither drug killed any cells as a single agent. Therefore, these data suggest that the σ_2 receptor ligand, **WC-26**, is a potent chemosensitizer of drugs that produce their antitumor effects by generating intracellular ROS.

2. Discussion

The goal of the current study was to develop new sigma receptor ligands possessing a high affinity and selectivity for the σ_2 receptor versus the σ_1 receptor using **6** as a lead compound. When the benzyl group in **6** was substituted by a fluoroethyl or allyl group, the compounds, **11a** and **11b**, had lowered affinities for both σ_1 and σ_2 receptors. Similarly, a pyridinyl ring substituent (**11c, 11d, 11e**) on the same benzyl ring resulted in lower affinities for σ_1 and σ_2 receptor compared with 6. However, the results were different when the 4-position on the benzyl ring of compound **6** was modified. Adding a 4-methylthio (**11f**) or a 4-methoxy group (**11g**) reduced the binding affinity to σ_1 and σ_2 receptors, while adding a 4-fluoroethyl (**WC-59**) or a 4-dimethylamino (**WC-26**) group increased the binding affinity to σ_2 receptors and decreased the binding affinity to σ_1 receptors. The σ_1/σ_2 ratio for **WC-59** and **WC-26**

increased to 2,087 and 557, respectively. Extending the spacer length of the methylene group to an ethylene group between the bridgehead nitrogen and the benzene ring of the N-benzyl moiety (**11h, 11i**, and **11j**) resulted in a much lower σ_2 receptor selectivity and affinity compared to the N-benzyl compounds, **11g, WC-59**, and **WC-26**. These data demonstrate that the phenylcarbamate **6** is an excellent lead compound for the development of ligands with a high affinity and selectivity for σ_2 receptors.

Of the two compounds that had a high selectivity and affinity for σ_2 receptors, **WC-59** was selected to be radiolabeled with ^{18}F because its structure already contains a fluorine atom and because [^{18}F]**WC-59** could then be compared to our existing library of F-18 labeled σ_2 receptor ligands.[12] The half life of ^{18}F (110 min.) is preferred for radiosynthesis and PET investigations over ^{11}C (20.4 min half-life). The total synthesis and purification time of ~100 min, the decay corrected radiochemical yield of 15%, the radiochemical purity of >99.9%, and the specific activity of 4,200 mCi/μmol were all acceptable for a PET imaging agent. The [^{18}F]**WC-59** direct saturation binding studies on membrane homogenates derived from EMT-6 solid tumors (Figure 2) gave a σ_2 receptor density of ~3,700 fmol/mg protein and a σ_2 receptor binding affinity ~2 nM; these values are consistent with those determined with EMT-6 cells from tissue culture and liver membrane homogenates. The [^{18}F]**WC-59** biodistribution studies in EMT-6 tumor-bearing female BALB/c mice showed that although tumor: blood (6.92) and tumor: muscle (2.52) ratios at 2 h post-injection were acceptable for a PET imaging agent; however, tumor: fat ratios were below unity, and even at 2 h post-injection, the activity retained in the lung (4.98 %ID/g) remained higher than the activity seen in tumor (4.08 %ID/g). Consequently, although [^{18}F]**WC-59** was successfully radiolabeled and generated promising initial results *in vitro*, it is not a superior candidate PET radioligand for clinical imaging studies when compared with existing F-18 labeled σ_2 receptor ligands used to detect and determine the proliferative status of solid tumors.[12]

Previously published studies using **WC-26** as an anticancer agent in a mouse model of pancreatic cancer showed good biological activity, but tumor growth inhibition was short-lived.[27] Based on that data and other published studies using sigma-receptor ligands in combination with standard chemotherapeutic agents[14], the compound was chosen for *in vitro* evaluation as a chemosensitizer. The ability of the σ_2 receptor selective ligand, **WC-26**, to enhance the cytotoxicity of the antitumor drug, DOX, was studied using a clonogenic survival assay in two different cell lines. Our data demonstrate that **WC-26** greatly enhances both the mouse EMT-6 (Figure 3) and the human MDA-MB435 (Figure 4) tumor cell kill obtained with DOX. This chemosensitization occurred at **WC-26** (2 μM, 40 h) and DOX (<20 μM, 24 h) exposure: doses where each drug produced no tumor cell kill as a single agent. Both of these drugs are known to produce ROS in cells (data not shown); high levels of ROS are known to kill tumor cells. Thus, our working hypothesis is that **WC-26** increases DOX cytotoxicity by increasing the intracellular ROS to a lethal concentration. However, other mechanisms such as the activation of caspase-3 and induction of apoptosis cannot be excluded at this time. Further explorations of the mechanism of cell kill are under investigation at this time.

4. Conclusion

Two highly selective and potent σ_2 receptor ligands, **WC-59** and **WC-26**, were synthesized using 9-azabicyclo[3.3.1]nonan-3α-yl phenylcarbamate as the lead compound. **WC-59** was radiolabeled with ^{18}F and a series of radiochemical binding and biodistribution studies were performed which demonstrated that although [^{18}F]**WC-59** demonstrated good *in vitro* binding, it was not superior to existing ^{18}F σ_2 receptor ligands when evaluated *in vivo* at a PET imaging agent. Because σ_2 receptor ligands have been shown to have antitumor activity[14-16] and generate intracellular ROS[15], and because of the compound's prior evaluation as a single agent chemotherapeutic[27], the ability of **WC-26** to enhance the cytotoxicity of DOX, an antitumor

drug used to treat solid tumors, was investigated *in vitro*. **WC-26** greatly increased the ability of DOX to kill both mouse EMT-6 and human MDA-MB435 tumor cells at concentrations where neither drug as a single agent produced any cell kill. These results indicate that **WC-26** has the potential to be useful as a chemosensitizer for the treatment of solid tumors.

5. Experimental

5.1. General methods and materials

All chemicals were obtained from standard commercial sources and used without further purification. All reactions were carried out using standard air-free and moisture-free techniques under an inert nitrogen atmosphere with dry solvents unless otherwise stated. Flash column chromatography was conducted using Scientific Adsorbents, Inc. silica gel, 60A, "40 Micron Flash" (32-63 μm). Melting points were determined using a MEL-TEMP 3.0 (Barnstead International, Dubuque, IA) and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Unity-300 (300 MHz) NMR spectrometer. All chemical shifts were reported as parts per million (ppm) downfield from tetramethylsilane (TMS), and coupling constants (*J*) are given in Hertz (Hz). Splitting patterns are typically described as follows: s, singlet; d, doublet; t, triplet; m, multiplet. The elemental analyses (C, H, N) were determined by Atlantic Microlab, Inc. (Norcross, GA)

5.1.1. (4-(2-Fluoroethyl)phenyl)methanol (13)—A solution of **12**[28] (0.75 g, 4.1 mmol) in ether (10 mL) was added to a solution of $LiAlH_4$ (311 mg, 8.2 mmol) in ether (10 mL) at ambient temperature over 10 min. The mixture was stirred overnight, after which ice water (3 mL) was added. The white solid of the inorganic salt was filtered out and washed with ether, and then the filtrate of the ether was washed with saturated NaCl solution (30 mL), and finally dried over Na_2SO_4. After evaporation of the ether, the crude product was purified by flash column chromatography with hexane-ether (1:1) to obtain **13** (473 mg, 75%) as colorless oil. ^{1}H NMR ($CDCl_3$) δ: 7.32 (d, *J* = 8.1 Hz, 2H), 7.24 (d, *J* = 8.1 Hz, 2H), 4.65 (s, 2H), 4.63 (dt, *J* = 47.1, 6.6 Hz, 2H), 3.02 (dt, *J* = 23.4, 6.6 Hz, 2H), 1.99 (s, 1H).

5.1.2. 1-(Bromomethyl)-4-(2-fluoroethyl)benzene (14)—Ph_3P (1.53 g, 5.84 mmol) was added to a solution of **13** (450 mg, 2.92 mmol) and CBr_4 (1.45 g, 4.38 mmol) in CH_2Cl_2 (10 mL) at 0 °C, and the mixture was stirred for 20 min at 0 °C. After evaporation of CH_2Cl_2, hexane-ether (1:1, 50 mL) was added and the precipitated white solid was removed by filtration. The filtrate was evaporated under vacuum. The crude product was purified by flash column chromatography with hexane-ether (10:1) to obtain **14** (432 mg, 72%) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 7.35 (d, *J* = 8.1 Hz, 2H), 7.21 (d, *J* = 8.1 Hz, 2H), 4.62 (dt, *J* = 47.1, 6.6 Hz, 2H), 4.48 (s, 2H), 3.00 (dt, *J* = 23.7, 6.6 Hz, 2H).

5.1.3. 2-(4-(2-Fluoroethyl)phenyl)acetonitrile (15)—A solution of **14** (280 mg, 1.29 mmol), tetraphenylphosphonium bromide (541 mg, 1.29 mmol) and KCN (840 mg, 12.9 mmol) in CH_2Cl_2-H_2O (10 mL/10 mL) was refluxed for 3 h. After addition of ether (70 mL), the organic layer was washed first with water (30 mL), then with saturated NaCl solution (30 mL) and finally dried over Na_2SO_4. The solvent was evaporated, and the crude product was purified by flash column chromatography with hexane-ether (2:1) to obtain **15** (165 mg, 79%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 7.25 (s, 4H), 4.61 (dt, *J* = 47.1, 6.6 Hz, 2H), 3.69 (s, 2H), 2.99 (dt, *J* = 24.6, 6.3 Hz, 2H).

5.1.4. 2-(4-(2-Fluoroethyl)phenyl)acetic acid (16)—A solution of **15** (165 mg, 1.0 mmol) and NaOH (200 mg, 5.0 mmol) in EtOH-H_2O (3 mL/3 mL) was refluxed for 6 h. Water (5 mL) was added to the reaction mixture, followed by extraction with ether. The aqueous layer was acidified with 6N HCl to pH = 1, then it was extracted with ethyl acetate (2 × 30 mL). The

ethyl acetate extraction was dried over Na_2SO_4. Ethyl acetate was evaporated to obtain **16** (182 mg, 100%) as a white solid, mp 110.1-111.1 °C. ^{1}H NMR ($CDCl_3$) δ: 7.22 (s, 4H), 4.62 (dt, *J* = 47.1, 6.6 Hz, 2H), 3.63 (s, 2H), 3.00 (dt, *J* = 23.4, 6.6 Hz, 2H).

5.1.5. 2-(4-(2-Fluoroethyl)phenyl)ethanol (17)—17 was prepared from **16** in a manner similar to that described for **13**, The crude product was purified by flash column chromatography with hexane-ether (1:1) to obtain **17** (131 mg, 78%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 7.20 (s, 4H), 4.63 (dt, *J* = 47.4, 6.6 Hz, 2H), 3.81 (t, *J* = 6.6 Hz, 2H), 3.00 (dt, *J* = 23.4, 6.6 Hz, 2H), 2.84 (t, *J* = 6.6 Hz, 2H), 2.17 (s, 1H).

5.1.6. 1-(2-Bromoethyl)-4-(2-fluoroethyl)benzene (18)—18 was prepared from **17** in a manner similar to that described for **14**. The crude product was purified by flash column chromatography with hexane-ether (100:5) to obtain **18** (155 mg, 86%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 7.20 (s, 4H), 4.64 (dt, *J* = 47.1, 6.6 Hz, 2H), 3.58 (t, *J* = 7.8 Hz, 2H), 3.16 (t, *J* = 7.8 Hz, 2H), 3.02 (dt, *J* = 23.1, 6.6 Hz, 2H).

5.1.7. 9-Benzyl-9-azabicyclo[3.3.1]nonan-3-yl-2-methoxy-5-methylphenylcarbamate (6)—Dibutyltin diacetate (0.5 mL) was added to a solution of 2-methoxy-5-methylphenyl isocyanate (**8**) (3.34 g, 20.5 mmol) and 9-benzyl-9-aza-bicyclo [3.3.1]nonan-3-ol (**9**) (4.50 g, 19.5 mmol) in CH_2Cl_2 (45 mL) at ambient temperature, and the mixture was stirred overnight. After evaporation of CH_2Cl_2 under vacuum, ether (100 mL) was added, the ether solution was first washed with water (50 mL), then with saturated NaCl solution (50 mL), and finally dried over Na_2SO_4. After removal of ether, the crude product was purified by flash column chromatography with hexane-ether (2:1) to obtain **6** (7.40 g, 96%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.97 (s, 1H), 7.37-7.22 (m, 5H), 7.15 (s, 1H), 6.76 (m, 2H), 5.26 (m, 1H), 3.83 (s, 3H), 3.80 (s, 2H), 3.06 (m, 2H), 2.47 (m, 2H), 2.30 (s, 3H), 2.14 (m, 1H), 1.97 (m, 2H), 1.51 (m, 3H), 1.17 (m, 2H).

5.1.8. 9-Azabicyclo[3.3.1]nonan-3-yl-2-methoxy-5-methylphenylcarbamate (10)—$Pd(OH)_2$/C (20%, 750 mg) was added to a solution of **6** (3.67 g, 9.3 mmol) and ammonium formate (2.93 g, 4.65 mmol) in methanol (90 mL) and ethyl acetate (90 mL). The mixture was refluxed for 2 h. After evaporation of the solvents under vacuum, ethyl acetate (100 mL) was added. The mixture was washed with saturated Na_2CO_3 solution (40 mL), water (40 mL), saturated NaCl solution (40 mL), and finally the organic solution was dried over Na_2SO_4. Ethyl acetate was then removed to obtain **10** (2.82 g, 100%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ: 7.93 (s, 1H), 7.15 (s, 1H), 6.76 (m, 2H), 4.99 (m, 1H), 3.83 (s, 3H), 3.34 (m, 2H), 2.36 (m, 2H), 2.29 (s, 3H), 2.08 (m, 1H), 1.64 (m, 4H), 1.50 (m, 4H).

5.1.9. 9-(2-Fluoroethyl)-9-azabicyclo[3.3.1]nonan-3-yl-2-methoxy-5-methylphenylcarbamate (11a)—K_2CO_3 (0.5 g) was added to a solution of **10** (203 mg, 0.67 mmol) and 1-bromo-2-fluoroethane (1 mL) in acetonitrile (8 mL). The mixture was stirred overnight at ambient temperature. After the addition of ether (75 mL), the organic solution was first washed with water (30 mL), then with saturated NaCl solution (30 mL), and finally dried over Na_2SO_4. After removal of solvent under reduced pressure, the crude product was purified by flash column chromatography with hexane-ether (1:2) to obtain **11a** (133 mg, 51%) as a colorless oil. The HCl salt of **11a** is a white solid, mp 201.4-202.5 °C. ^{1}H NMR ($CDCl_3$) δ: 7.95 (s, 1H), 7.14 (s, 1H), 6.76 (m, 2H), 5.13 (m, 1H), 4.45 (dt, *J* = 47.7, 6.6 Hz, 2H), 3.84 (s, 3H), 3.08 (m, 2H), 2.91 (dt, *J* = 18.9, 5.4 Hz, 2H), 2.47 (m, 2H), 2.30 (s, 3H), 2.14 (m, 1H), 1.89 (m, 2H), 1.54 (m, 3H), 1.22 (m, 2H). Anal. Calcd for $C_{19}H_{27}FN_2O_3$·HCl: C, 58.98; H, 7.29; N 7.24. Found: C, 58.48; H, 7.26; N, 7.16.

5.1.10. 9-Allyl-9-azabicyclo[3.3.1]nonan-3-yl 2-methoxy-5-methylphenylcarbamate (11b)—11b was prepared from allyl bromide in a manner similar to that described for **11a**. The crude product was purified by flash column chromatography with ether to obtain **11b** (90 mg, 34%) as a colorless oil. The HCl salt of **11b** is a white solid, mp 221.3-222.3 °C. ^{1}H NMR ($CDCl_3$) δ: 7.95 (s, 1H), 7.13 (s, 1H), 6.76 (m, 2H), 5.79 (m, 1H), 5.13 (m, 3H), 3.84 (s, 3H), 3.26 (d, *J* = 6.0 Hz, 2H), 3.05 (m, 2H), 2.44 (m, 2H), 2.29 (s, 3H), 2.17 (m, 1H), 1.88 (m, 2H), 1.53 (m, 3H), 1.22 (m, 2H). Anal. Calcd for $C_{20}H_{28}N_2O_3$·HCl: C, 63.06; H, 7.67; N, 7.35. Found: C, 62.64; H, 7.66; N, 7.31.

5.1.11. 9-((Pyridine-4-yl)methyl)-9-azabicyclo[3.3.1]nonan-3-yl-2-methoxy-5-methylphenylcarbamate (11c)—11c was prepared from 4-bromomethyl-pyridine hydrogen bromide in a manner similar to that described for **11a**. The crude product was purified by flash column chromatography with hexane-ether (1:2) to obtain **11c** (133 mg, 51%) as a colorless oil. The HCl salt of **11c** is a white solid, mp 225.5-227.0 °C. ^{1}H NMR ($CDCl_3$) δ: 8.51 (d, *J* = 5.4 Hz, 2H), 7.96 (s, 1H), 7.30 (d, *J* = 5.7 Hz, 2H), 7.17 (s, 1H), 6.76 (m, 2H), 5.25 (m, 1H), 3.85 (s, 3H), 3.82 (s, 2H), 3.01 (m, 2H), 2.48 (m, 2H), 2.30 (s, 3H), 2.15 (m, 1H), 1.93 (m, 2H), 1.54 (m, 3H), 1.23 (m, 2H). Anal. Calcd for $C_{23}H_{29}N_3O_3$·2HCl·0.5H_2O: C, 57.86; H, 6.76; N, 8.80. Found: C, 58.01; H, 6.95; N, 8.68.

5.1.12. 9-((Pyridine-3-yl)methyl)-9-azabicyclo[3.3.1]nonan-3-yl-2-methoxy-5-methylphenylcarbamate (11d)—11d was prepared from 3-bromomethyl-pyridine hydrogen bromide in a manner similar to that described for **11a**. The crude product was purified by flash column chromatography with ether to obtain **11d** (90 mg, 34%) as a colorless oil. The HCl salt of **11d** is a white solid, mp 242.7-243.9 °C. ^{1}H NMR ($CDCl_3$) δ 8.51 (s, 1H), 8.46 (m, 1H), 7.95 (s, 1H), 7.71 (m, 1H), 7.24 (m, 1H), 7.17 (s, 1H), 6.74 (m, 2H), 5.21 (m, 1H), 3.82 (s, 3H), 3.79 (s, 2H), 3.01 (m, 2H), 2.45 (m, 2H), 2.28 (s, 3H), 2.14 (m, 1H), 1.93 (m, 2H), 1.53 (m, 3H), 1.19 (m, 2H). Anal. Calcd for $C_{23}H_{29}N_3O_3$·2HCl: C, 58.97; H, 6.67; N, 8.97. Found: C, 58.69; H, 6.87; N, 8.57.

5.1.13. 9-((6-Fluoropyridine-3-yl)methyl)-9-azabicyclo[3.3.1]nonan-3-yl-2-methoxy-5-methylphenylcarbamate (11e)—11e was prepared from 5-bromomethyl-2-fluoro-pyridine in a manner similar to that described for **11a**. The crude product was purified by flash column chromatography with hexane-ether (1:1) to obtain **11e** (264 mg, 96%) as a colorless oil. The HCl salt of **11e** is a white solid, mp 166.7-167.9 °C. ^{1}H NMR ($CDCl_3$) δ: 8.10 (s, 1H), 7.95 (s, 1H), 7.84 (td, *J* = 8.25, 2.4 Hz, 1H), 7.16 (s, 1H), 6.89 (dd, *J* = 8.25, 2.7 Hz, 1H), 6.76 (m, 2H), 5.20 (m, 1H), 3.84 (s, 3H), 3.78 (s, 2H), 3.01 (m, 2H), 2.43 (m, 2H), 2.30 (s, 3H), 2.16 (m, 1H), 1.93 (m, 2H), 1.56 (m, 3H), 1.23 (m, 2H). Anal. Calcd for $C_{23}H_{28}FN_3O_3$·HCl·H_2O: C, 59.03; H, 6.68; N, 8.98. Found: C, 58.77; H, 6.39; N, 8.61.

5.1.14. 9-(4-(Methylthio)benzyl)-9-azabicyclo[3.3.1]nonan-3-yl-2-methoxy-5-methylphenylcarbamate (11f)—11f was prepared from 1-bromomethyl-4-methylsulanyl-benzene in a manner similar to that described for **11a**. The crude product was purified by flash column chromatography with hexane-ether (1:1) to obtain **11f** (245 mg, 81%) as a colorless oil. The HCl salt of **11f** is a white solid, mp 179.3-180.8 °C. ^{1}H NMR ($CDCl_3$) δ: 8.01 (s, 1H), 7.31 (d, *J* = 8.1 Hz, 2H), 7.23 (d, *J* = 8.1 Hz, 2H), 7.21 (s, 1H), 6.76 (m, 2H), 5.27 (m, 1H), 3.83 (s, 3H), 3.78 (s, 2H), 3.07 (m, 2H), 2.48 (s, 3H), 2.46 (m, 2H), 2.32 (s, 3H), 2.17 (m, 1H), 1.96 (m, 2H), 1.58 (m, 3H), 1.21 (m, 2H). Anal. Calcd for $C_{25}H_{32}N_2O_3S$·HCl: C, 62.94; H, 6.97; N, 5.87. Found: C, 62.58; H, 7.02; N, 5.42.

5.1.15. 9-(4-Methoxybenzyl)-9-azabicyclo[3.3.1]nonan-3-yl-2-methoxy-5-methylphenylcarbamate (11g)—11g was prepared from 1-chloromethyl-4-methoxybenzene in a manner similar to that described for **11a**. The crude product was purified

by flash column chromatography with hexane-ether (1:2) to obtain **11g** (221 mg, 72%) as a colorless oil. The HCl salt of **11g** is a white solid, mp 203.9-204.8 °C. ^{1}H NMR ($CDCl_3$) δ: 7.99 (s, 1H), 7.28 (d, *J* = 8.7 Hz, 2H), 7.17 (s, 1H), 6.86 (d, *J* = 8.7 Hz, 2H), 6.76 (m, 2H), 5.26 (m, 1H), 3.85 (s, 3H), 3.81 (s, 3H), 3.75 (s, 2H), 3.05 (m, 2H), 2.47 (m, 2H), 2.31 (s, 3H), 2.16 (m, 1H), 1.95 (m, 2H), 1.53 (m, 3H), 1.19 (m, 2H). Anal. Calcd for $C_{25}H_{32}N_2O_4$·HCl·0.25H_2O: C, 64.50; H, 7.25; N, 6.02. Found: C, 64.54; H, 7.28; N, 5.65.

5.1.16. 9-(4-Methoxyphenethyl)-9-azabicyclo[3.3.1]nonan-3-yl-2-methoxy-5-methylphenylcarbamate (11h)—11h was prepared from 1-(2-bromo-ethyl)-4-methoxybenzene in a manner similar to that described for **11a**. The crude product was purified by flash column chromatography with ether to obtain **11h** (86 mg, 29%) as a colorless oil. The HCl salt of **11h** is a white solid, mp 209.8-210.9 °C. ^{1}H NMR ($CDCl_3$) δ: 7.96 (s, 1H), 7.16 (s, 1H), 7.14 (d, *J* = 8.7 Hz, 2H), 6.84 (d, *J* = 8.7 Hz, 2H), 6.76 (m, 2H), 5.14 (m, 1H), 3.85 (s, 3H), 3.79 (s, 3H), 3.10 (m, 2H), 2.79 (m, 2H), 2.64 (m, 2H), 2.46 (m, 2H), 2.30 (s, 3H), 2.17 (m, 1H), 1.89 (m, 2H), 1.53 (m, 3H), 1.22 (m, 2H). Anal. Calcd for $C_{26}H_{34}N_2O_4$·HCl: C, 65.74; H, 7.43; N, 5.90. Found: C, 65.91; H, 7.57; N, 5.64.

5.1.17. 9-(4-(2-Fluoroethyl)phenethyl)-9-azabicyclo[3.3.1]nonan-3-yl-2-methoxy-5-methyl-phenylcarbamate (11i)—11i was prepared from **18** in a manner similar to that described for **11a**. The crude product was purified by flash column chromatography ether to obtain **11i** (162 mg, 53%) as a colorless oil. The HCl salt of **11i** is a white solid, mp 202.2-203.7 °C. ^{1}H NMR ($CDCl_3$) δ: 7.91 (s, 1H), 7.10 (s, 5H), 6.68 (m, 2H), 5.08 (m, 1H), 4.55 (dt, *J* = 46.8, 6.6 Hz, 2H), 3.77 (s, 3H), 3.04 (m, 2H), 2.92 (dt, *J* = 22.8, 6.6 Hz, 2H), 2.74 (m, 2H), 2.64 (m, 2H), 2.40 (m, 2H), 2.24 (s, 3H), 2.09 (m, 1H), 1.83 (m, 2H), 1.46 (m, 3H), 1.18 (m, 2H). Anal. Calcd for $C_{27}H_{36}ClFN_2O_3$·HCl: C, 66.04; H, 7.39; N, 5.70. Found: C, 66.18; H, 7.54; N, 5.61.

5.1.18. 9-(4-(Dimethylamino)phenethyl)-9-azabicyclo[3.3.1]nonan-3-yl-2-methoxy-5-methyl-phenylcarbamate (11j)—11j was prepared from 4-(2-bromoethyl)-N,N-dimethylaniline in a manner similar to that described for **11a**. The crude product was purified by flash column chromatography with ether to obtain **11j** (131 mg, 44%) as a pale yellow oil. The HCl salt of **11j** is a white solid, mp 253.6-255.2 °C. ^{1}H NMR ($CDCl_3$) δ: 7.96 (s, 1H), 7.14 (s, 1H), 7.10 (d, *J* = 9.0 Hz, 2H), 6.76 (m, 2H), 6.71 (d, *J* = 8.7 Hz, 2H), 5.15 (m, 1H), 3.85 (s, 3H), 3.13 (m, 2H), 2.92 (s, 6H), 2.79 (m, 2H), 2.64 (m, 2H), 2.45 (m, 2H), 2.30 (s, 3H), 2.17 (m, 1H), 1.91 (m, 2H), 1.52 (m, 3H), 1.22 (m, 2H). Anal. Calcd for $C_{27}H_{37}N_3O_3$·2HCl: C, 61.83; H, 7.49; N, 8.01. Found: C, 61.99; H, 7.51; N, 7.84.

5.1.19. 9-(4-(2-Fluoroethyl)benzyl)-9-azabicyclo[3.3.1]nonan-3-yl-2-methoxy-5-methyl-phenylcarbamate (WC-59)—WC-59 was prepared from **14** in a manner similar to that described for **11a**. The crude product was purified by flash column chromatography with hexane-ether (1:2) to obtain **WC-59** (212 mg, 87%) as a colorless oil. The HCl salt of **WC-59** is a white solid, mp 134.7-135.8 °C. ^{1}H NMR ($CDCl_3$) δ: 8.01 (s, 1H), 7.33 (d, *J* = 8.1 Hz, 2H), 7.20 (s, 1H), 7.19 (d, *J* = 8.1 Hz, 2H), 6.76 (m, 2H), 5.28 (m, 1H), 4.64 (dt, *J* = 47.1, 6.6 Hz, 2H), 3.84 (s, 3H), 3.80 (s, 2H), 3.07 (m, 2H), 3.01 (dt, *J* = 22.8, 6.6 Hz, 2H), 2.50 (m, 2H), 2.33 (s, 3H), 2.17 (m, 1H), 1.97 (m, 2H), 1.55 (m, 3H), 1.18 (m, 2H). Anal. Calcd for $C_{26}H_{34}ClFN_2O_3$·HCl·0.25H_2O: C, 64.85; H, 7.22; N, 5.82. Found: C, 65.03; H, 7.45; N, 5.33.

5.1.20. 9-(4-(Dimethylamino)benzyl)-9-azabicyclo[3.3.1]nonan-3-yl-2-methoxy-5-methyl-phenylcarbamate (WC-26)—$NaBH_3CN$ (57 mg, 0.91 mmol) was added to a solution of **10** (260 mg, 0.85 mmol) and 4-(dimethylamino)benzaldehyde (127 mg, 0.85 mmol) in methanol (10 mL), and the reaction mixture was stirred for 3 days. After evaporation of methanol and addition of ethyl acetate (50 mL), the mixture was washed with

water (30 mL), then with saturated NaCl solution (30 mL), and finally the organic layer was dried over Na_2SO_4. The crude product was purified by flash column chromatography with hexane-ether (1:2) to obtain **WC-26** (185 mg, 50%) as a colorless oil. The HCl salt of **WC-26** is a white solid, mp 192.2-193.1 °C. ^{1}H NMR ($CDCl_3$) δ: 8.00 (s, 1H), 7.25 (d, *J* = 8.7 Hz, 2H), 7.18 (s, 1H), 6.76 (m, 2H), 6.73 (d, *J* = 8.4 Hz, 2H), 5.27 (m, 1H), 3.85 (s, 3H), 3.74 (s, 2H), 3.08 (m, 2H), 2.95 (s, 6H), 2.49 (m, 2H), 2.32 (s, 3H), 2.17 (m,1H), 1.97 (m, 2H), 1.54 (m, 3H), 1.19 (m, 2H). Anal. Calcd for $C_{26}H_{35}N_3O_3 \cdot 2HCl \cdot 0.5H_2O$: C, 60.11; H, 7.37; N, 8.09. Found: C, 59.99; H, 7.50; N, 7.74.

5.1.21. 4-[2-(*t*-Butyl-diphenyl-silyloxy)-ethyl]-benzoic acid methyl ester (20)— 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (2 mL) was added to a solution of 4-(2-hydroxyethyl)benzoic acid methyl ester **19** (721 mg, 4.0 mmol) and *t*-butyldiphenylsilyl chloride (TBDPSCl) (2.20 g, 8.0 mmol) in CH_2Cl_2 (15 mL), and the reaction mixture was stirred overnight. After evaporation of CH_2Cl_2 under vacuum, ether (75 mL) was added and the mixture was washed with water (2 × 50 mL), then with saturated NaCl solution (50 mL), and finally the organic layer was dried over Na_2SO_4. The crude product was purified by flash column chromatography with hexane-ether (10:1) to obtain **20** (1.64 g, 98%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 7.97 (d, *J* = 7.2 Hz, 2H), 7.59 (d, *J* = 6.6 Hz, 4H), 7.47-7.35 (m, 6H), 7.27 (d, *J* = 7.8 Hz, 2H), 3.95 (s, 3H), 3.89 (t, *J* = 6.6 Hz, 2H), 2.92 (t, *J* = 6.6 Hz, 2H), 1.04 (s, 9H).

5.1.22. [4-[2-(*t*-Butyl-diphenyl-silyloxy)-ethyl]-phenyl]-methanol (21)—21 was prepared from **20** in a manner similar to that described for **13**. The crude product was purified by flash column chromatography with hexane-ether (1:1) to obtain **21** (540 mg, 35%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 7.64 (m, 4H), 7.45-7.36 (m, 6H), 7.30 (d, *J* = 7.8 Hz, 2H), 7.19 (d, *J* = 8.4 Hz, 2H), 4.69 (s, 2H), 3.88 (t, *J* = 6.9 Hz, 2H), 2.90 (t, *J* = 6.9 Hz, 2H), 1.08 (s, 9H).

5.1.23. [2-(4-Bromomethyl-phenyl)-ethoxy]-*t*-butyl-diphenyl-silane (22)—22 was prepared from **21** in a manner similar to that described for **14**. The crude product was purified by flash column chromatography with hexane-ether (10:1) to obtain **22** (148 mg, 65%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 7.60 (m, 4H), 7.45-7.34 (m, 6H), 7.30 (d, *J* = 7.8 Hz, 2H), 7.14 (d, *J* = 7.8 Hz, 2H), 4.50 (s, 2H), 3.84 (t, *J* = 6.9 Hz, 2H), 2.85 (t, *J* = 6.9 Hz, 2H), 1.06 and 1.04 (s, 9H).

5.1.24. (2-Methoxy-5-methyl-phenyl)-carbamic acid 9-[4-[2-(*t*-butyl-diphenyl-silyloxy)-ethyl]-9-aza-bicyclo[3.3.1]non-3-yl ester (23)—23 was prepared from 22 in a manner similar to that described for **11a**. The crude product was purified by flash column chromatography with hexane-ether (1:1) to obtain **23** (146 mg, 65%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 7.99 (s, 1H), 7.61 (m, 4H), 7.39 (m, 6H), 7.25 (m, 2H), 7.16 (s, 1H), 7.10 (m, 2H), 6.76 (m, 2H), 5.27 (m, 1H), 3.84 (m, 2H), 3.83 (s, 3H), 3.77 (s, 2H), 3.04 (m, 2H), 2.85 (m, 2H), 2.45 (m, 2H), 2.31 (s, 3H), 2.19 (m, 1H), 1.95 (m, 2H), 1.54 (m, 3H), 1.18 (m, 2H), 1.03 (s, 9H).

5.1.25. (2-Methoxy-5-methyl-phenyl)carbamic acid 9-[4-(2-hydroxy-ethyl)-benzyl]-9-aza-bicyclo[3.3.1]non-3-yl ester (24)—Tetrabutylammonium fluoride (53 mg, 0.2 mmol) was added to a solution of **23** (120 mg, 0.18 mmol) in THF (5 mL) at ambient temperature, and the mixture was stirred overnight. After addition of ethyl acetate (50 mL), the mixture was washed with water (30 mL), then with saturated NaCl solution (30 mL), and finally the organic layer was dried over Na_2SO_4. The solvent was evaporated, and the crude product was purified by flash column chromatography with ether to obtain **24** (56 mg, 72%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 7.96 (s, 1H), 7.34 (d, *J* = 7.5 Hz, 2H), 7.18 (d, *J* = 7.8

Hz, 2H), 7.16 (s, 1H), 6.76 (m, 2H), 5.24 (m, 1H), 3.86 (t, *J* = 6.6 Hz, 2H), 3.84 (s, 3H), 3.81 (s, 2H), 3.08 (m, 2H), 2.86 (t, *J* = 6.6 Hz, 2H), 2.51 (m, 2H), 2.30 (s, 3H), 2.17 (m, 1H), 1.98 (m, 2H), 1.57 (m, 3H), 1.23 (m, 2H).

5.1.26. Methanesulfonic acid 2-[4-[3-(2-methoxy-5-methyl-phenylcarbamoyloxy)-9-aza-bicyclo[3.3.1]non-9-ylmethyl]-phenyl]ethyl ester (25)—Triethylamine (26 mg, 0.2 mmol) was added at 0 °C to a solution of **24** (44 mg, 0.1 mmol) and methanesulfonyl chloride (29 mg, 0.15 mmol) in CH_2Cl_2 (5 mL), and the mixture was stirred overnight at ambient temperature. After addition of ethyl acetate (50 mL), the mixture was washed first with water (30 mL), then with saturated NaCl solution (30 mL), and finally the organic layer was dried over Na_2SO_4. The solvent was evaporated, and the crude product was purified by flash column chromatography with ether-MeOH (10:1) to obtain **25** (42 mg, 71%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 7.96 (s, 1H), 7.32 (d, *J* = 8.1 Hz, 2H), 7.18 (s, 1H), 7.17 (d, *J* = 8.1 Hz, 2H), 6.76 (m, 2H), 5.24 (m, 1H), 4.41 (t, *J* = 7.2 Hz, 2H), 3.84 (s, 3H), 3.78 (s, 2H), 3.06 (m, 2H), 3.04 (t, *J* = 7.2 Hz, 2H), 2.86 (s, 3H), 2.47 (m, 2H), 2.30 (s, 3H), 2.15 (m, 1H), 1.96 (m, 2H), 1.56 (m, 3H), 1.20 (m, 2H).

5.2. Synthesis of [^{18}F]WC-59

5.2.1. General information—Classic C18 SepPak cartridges and Oasis HLB cartridges were purchased from Waters Corporation (Milford, MA). For the TLC analyses, EM Science Silica Gel 60 F_{254} TLC plates were purchased from Fisher Scientific (Pittsburgh, PA). Radio-TLC was accomplished using a Bioscan 200 imaging scanner (Bioscan, Inc., Washington, DC). Radioactivity was counted with a Beckman Gamma 8000 counter (Beckman Instruments, Inc., Irvine, CA). [^{18}F]Fluoride was generated by the [^{18}O(p,n)^{18}F] reaction when 95% enriched [^{18}O] water was irradiated with protons from Washington University's RDS111 cyclotron.

5.2.2. Radiolabeling—[^{18}F]Fluoride (120 mCi) was dried by azeotropic distillation using CH_3CN (3 × 1 mL) in the presence of K_2CO_3 (1 mg) and K_{222} (5.5 mg) at 110 °C under a flow of N_2, and then a solution of precursor **25** (1.0 mg, 1.9 μmol) in DMSO (350 μL) was added. The reaction mixture was heated in an oil bath (110 °C) for 10 min. This resulted in an ^{18}F incorporation of 52% according to a radio-TLC analysis (silica, 20% methanol/80% CH_2Cl_2). The mixture was diluted in water (20 mL), and free [^{18}F]fluoride was removed by passing the dilution through an Oasis HLB cartridge (500 mg). The trapped radioactivity in the cartridge was eluted with CH_3CN (10 mL), and the elute was concentrated to dryness under reduced pressure. The residue was dissolved in CH_3CN (1 mL) and water (3.5 mL) for HPLC injection.

[^{18}F]**WC-59** was purified by a reversed phased HPLC (Phenomenex Prodigy ODS3 column 250 × 10 mm, 10 μ), being eluted with 42% CH_3CN, 58% 0.1 M ammonium formate buffer (pH = 4.5) at a flow rate of 4 mL/min and UV at 239 nm. [^{18}F]**WC-59** (~10 mCi) was collected at 18 min. The HPLC purified [^{18}F]**WC-59** was isolated from the HPLC solvent by dilution in water (200 mL), extraction in a C18 classic SepPak, and elution with ethanol (1-2 mL). The total synthesis and purification time was 100 min, the decay-corrected radiochemical yield was ~15%, the radiochemical purity was >99.9%, and the specific activity was 4,200 mCi/μmol at the end of the synthesis. This analysis was performed using an analytical HPLC column (Phenomenex Prodigy ODS3 column, 250 × 4.6 mm, 5μ) and comparing the integrated UV absorbance of the product with a calibrated mass/UV absorbance curve for **WC-59**. The identity of [^{18}F]**WC-59** was also confirmed by coeluting the radioactive compound with nonradioactive **WC-59** on the analytical HPLC system. After purification, [^{18}F]**WC-59** was prepared in 10% ethanol and 90% water for injection into animals.

5.3. Biodistribution of [^{18}F]WC-59

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. Female BALB/c mice were implanted subcutaneously in the scapular region with ~5×10^5 EMT-6 cells in 100 μL PBS 11 days prior to the study. At the time of the biodistribution study tumors were ~100-150 mg. [^{18}F]**WC-59** (~20 μCi per 150 μL) was injected via the tail vein, and the mice were sacrificed at 5 min, 30 min, 1h and 2 h post-injection. Blood was collected then tumor and non-target tissues were removed, weighed, and the radioactivity was measured in a Beckman 8000 automated gamma well counter with a diluted standard of the injected dose. The data were analyzed as the percent injected dose per gram of tissue (%ID/g).

5.4. Colony Formation Assay

EMT-6 or MDA-MB435 cells were seeded at 6,000 cells per well in 96-well plates. After incubating for 24 h, the attached cells were treated for 16 h with **WC-26** alone. After another 16 h, various concentrations of DOX were added to the wells, and the cells were incubated for an additional 24 h. Upon completion of treatment with drugs or vehicle controls, the cells were removed from the wells, counted, diluted, and 300 single cells were replated in 60 mm petri dishes for colony formation. After incubating the dishes for 14 days at 37 °C in a humidified CO_2 incubator, the colonies were fixed with methanol-acetic acid (3:1) and stained with 0.25% crystal violet. Colonies containing >25 cells were scored, and the percent survival calculated by comparison to untreated controls. The data are shown as the mean ± 1 SD of triplicate samples from a representative experiment.

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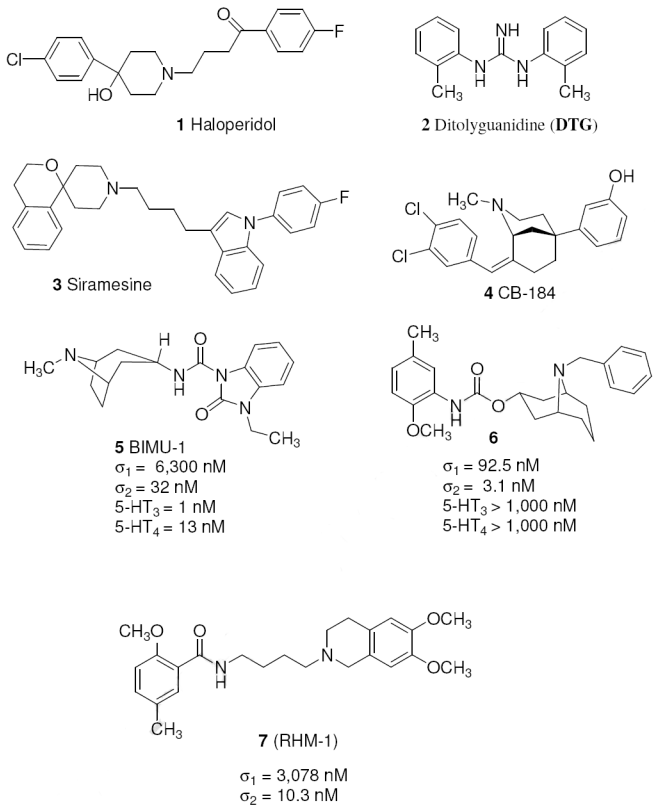


Figure 1.
Structures of representative σ receptor ligands.

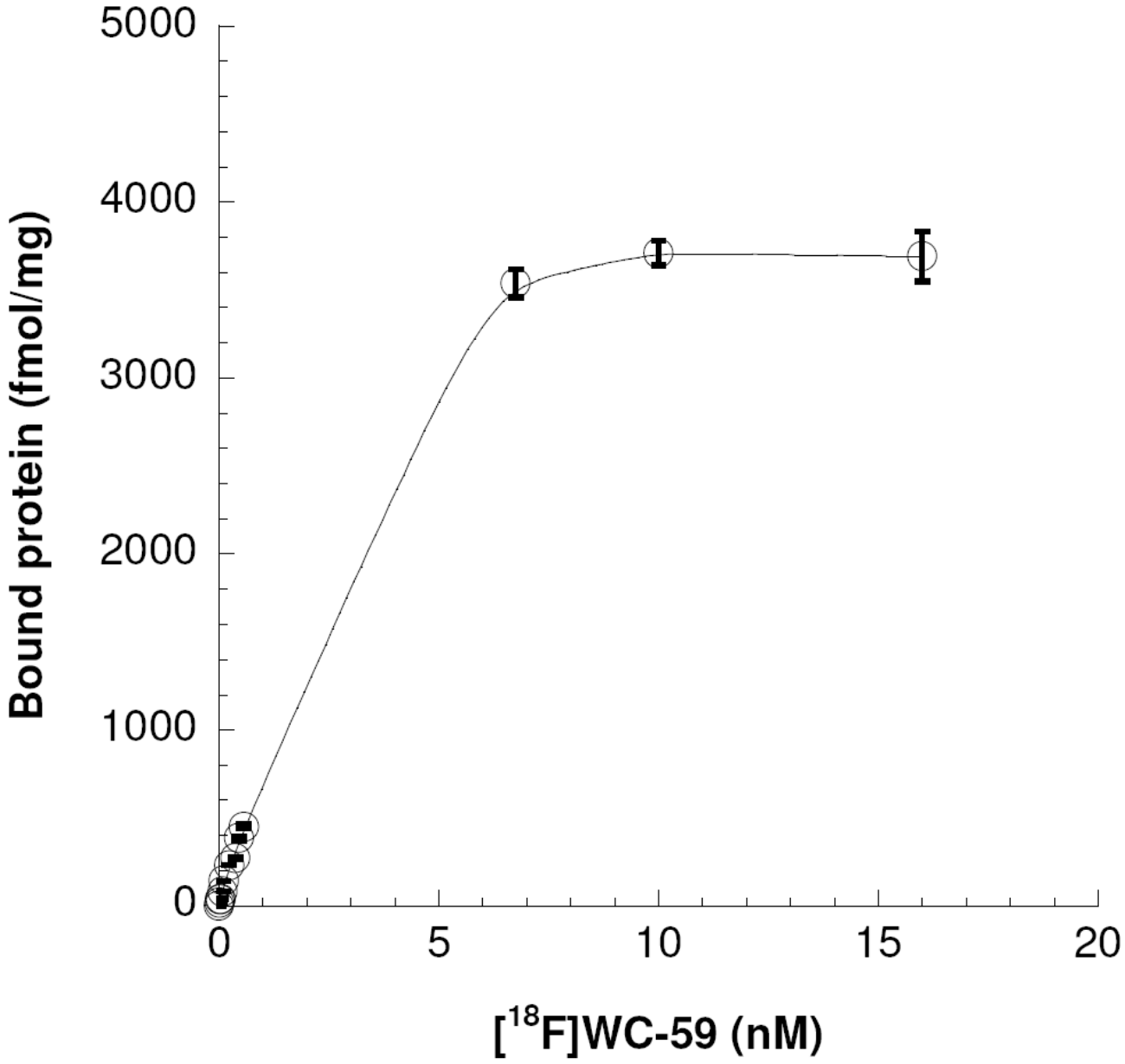


Figure 2.
A representative total saturation σ_2 binding experiment for [^{18}F]**WC-59** using membrane homogenates from EMT-6 mouse breast tumors. Each point is the mean of duplicate samples.

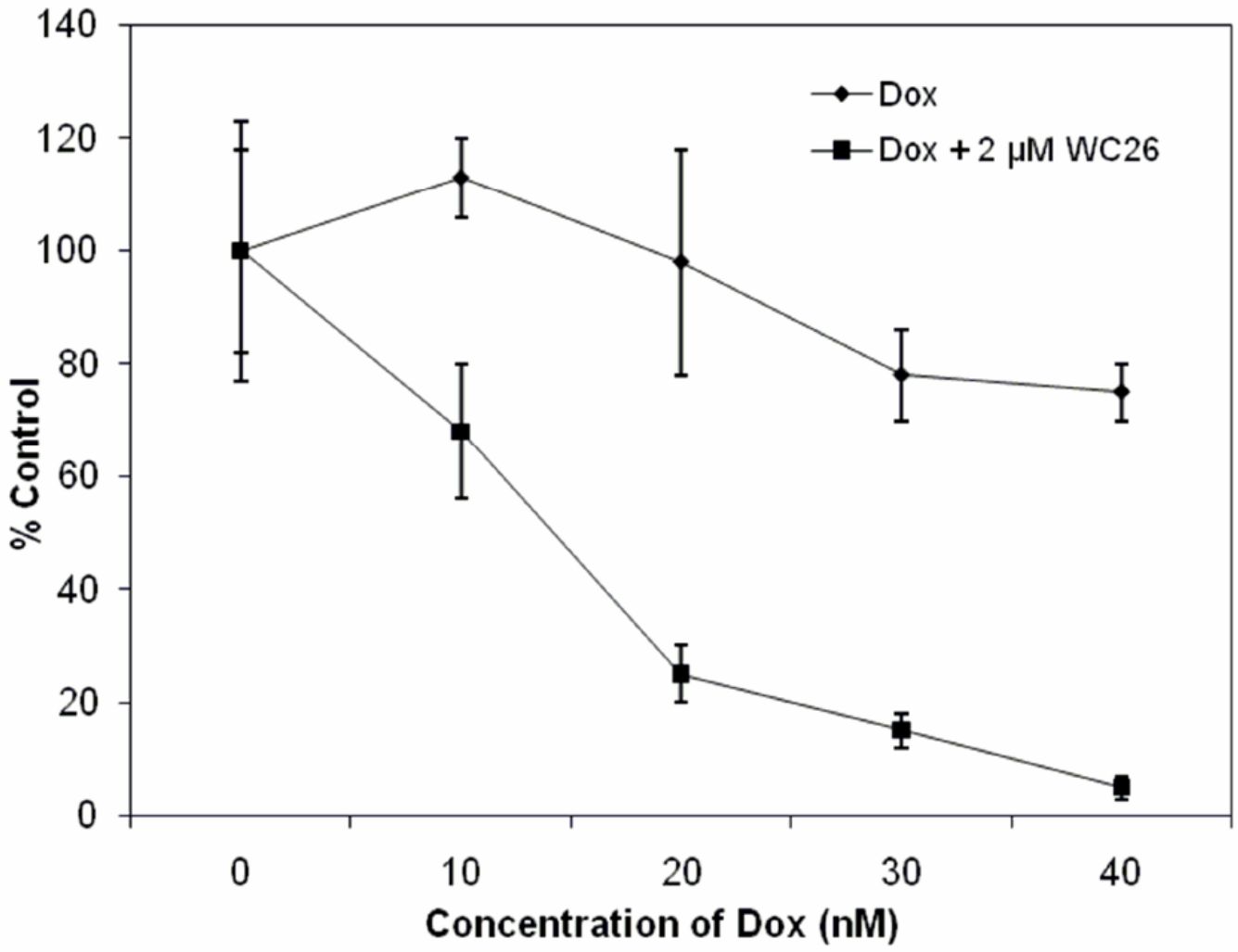


Figure 3.
Percent survival of EMT-6 cells treated with **WC-26** for 16 h, followed by a combination of **WC-26** and doxorubicin (DOX) for an additional 24 h. The data points represent the mean ± 1 SD of triplicate samples in a representative experiment.

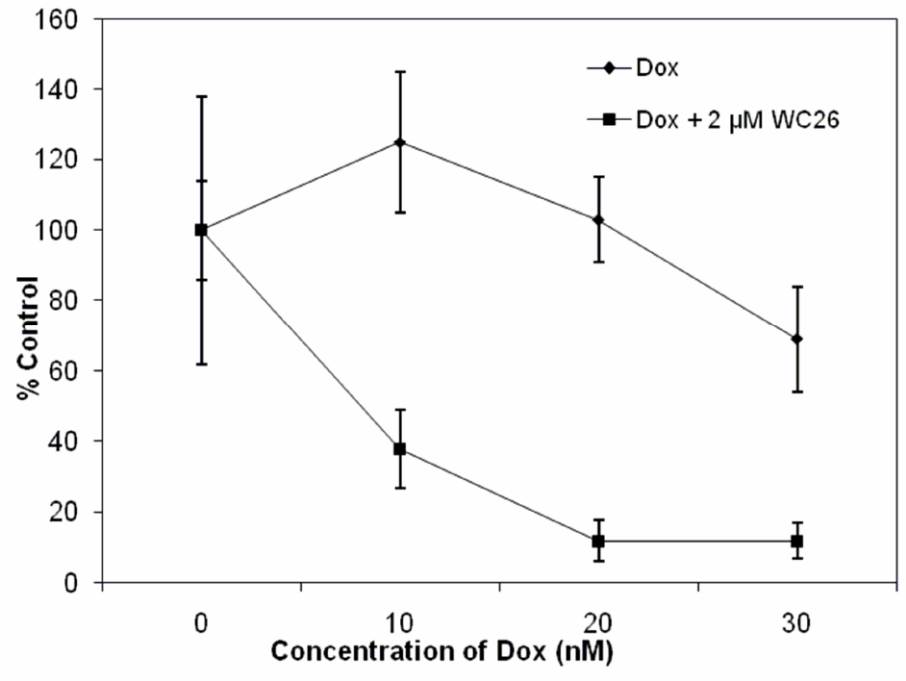
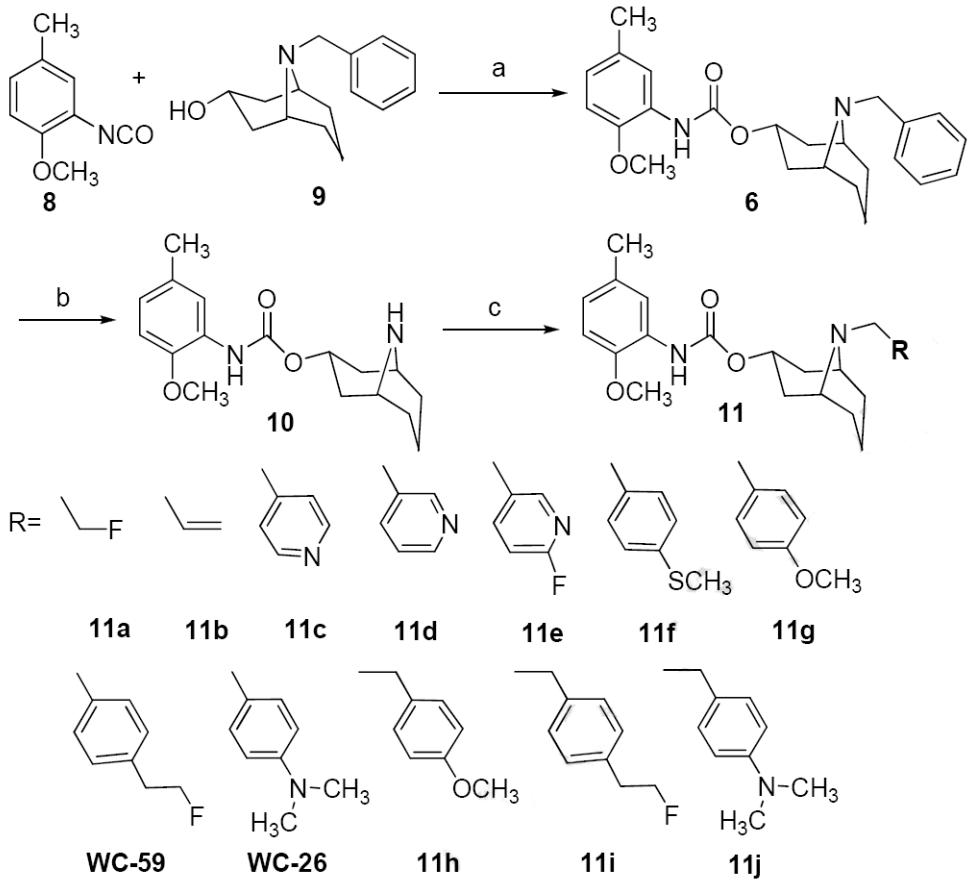


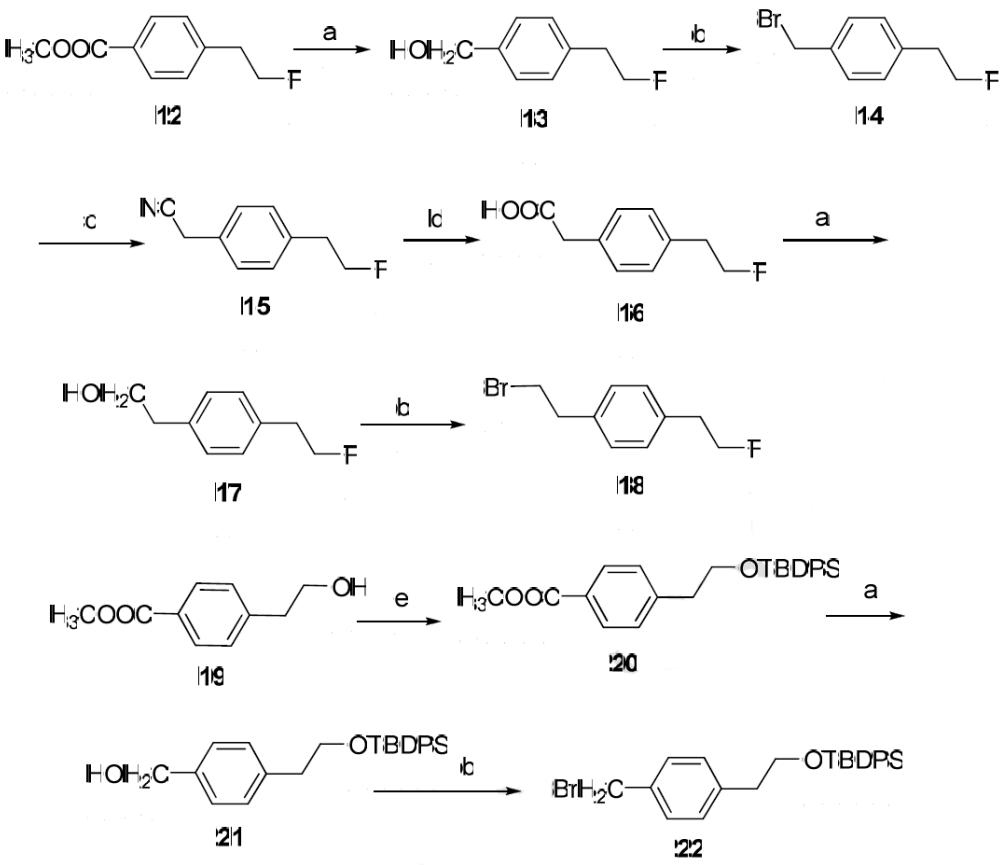
Figure 4.

Percent survival of MDA-MB435 cells treated with **WC-26** for 16 h, followed by a combination of **WC-26** and doxorubicin (DOX) for an additional 24 h. The data points represent the mean ± 1 SD of triplicate samples in a representative experiment.



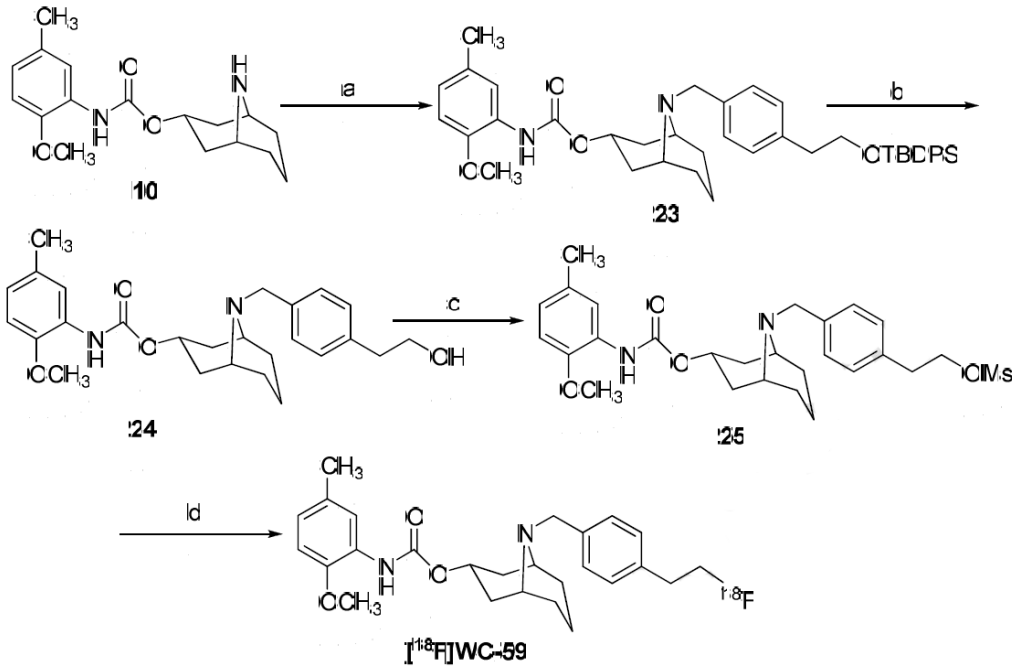
Scheme 1.

Reagents: (a) CH_2Cl_2, $(CH_3COO)_2SnBu_2$; (b) $Pd(OH)_2/C$, HCO_2NH_4, CH_3OH/EtOAc; (c) RCH_2X, (X=Br, Cl), K_2CO_3, CH_3CN, or R-CHO, $NaBH_3CN$ (**WC-26**).



Scheme 2.

Reagents: (a) $LiAlH_4$, ether; (b) CBr_4, Ph_3P; (c) Ph_4PBr, NaCN, CH_2Cl_2/H_2O; (d) NaOH, H_2O; (e) TBDPSCl, Et_3N, CH_2Cl_2.



Scheme 3.

Reagents: (a) (4-(Bromomethyl)phenethyloxy)(*t*-butyl)diphenylsilane (**22**), Et_3N, CH_2Cl_2; (b) *n*-$Bu_4NF{\cdot}H_2O$, THF; (c) MsCl, Et_3N; (d) [^{18}F]fluoride, K_2CO_3, K_{222}, DMSO, 110°C.

Table 1

In vitro σ receptor binding and log P data for the phenylcarbamate analogs.

Compounds	K_i (nM)		σ_1/σ_2	Log P
	σ_1	σ_2		
11a	2,003.3 ± 215.7	143.3 ± 4.2	14	2.85
11b	1,256.8 ± 87.9	85.9 ± 3.2	15	2.86
11c	1,563.3 ± 415.9	521.6 ± 15.1	3	2.94
11d	2,346.7 ± 340.7	183.9 ± 13.0	13	2.70
11e	2,510.0 ± 477.6	571.5 ± 142.8	4	3.21
11f	2,023.3 ± 344.0	8,192.1 ± 25.9	11	3.92
11g	323.3 ± 40.8	40.7 ± 4.4	8	3.3
WC-59	1,710.5 ± 84.0	0.82 ± 0.13	2,087	3.68
WC-26	1,436.5 ± 166.1	2.58 ± 0.59	557	3.02
11h	447.1 ± 66.9	125.8 ± 27.2	4	3.22
11i	2,633.3 ± 672.9	275.8 ± 78.2	10	3.69
11j	1,870.0 ± 338.3	195.2 ± 32.0	10	3.36
Haloperidol	1.45 ± 0.33	24.2 ± 3.0	0.06	3.36

Table 2

Biodistribution of [^{18}F]**WC-59** in EMT-6 tumor-bearing BALB/C mice

	%ID/g			
	5 min.	**30 min.**	**1 hour**	**2 hour**
Blood	1.88 ± 0.61	0.99 ± 0.10	0.93 ± 0.16	0.59 ± 0.03
Lung	111.00 ± 13.52	16.32 ± 1.14	8.73 ± 2.54	4.98 ± 0.27
Liver	21.14 ± 3.59	28.50 ± 1.82	23.35 ± 2.00	17.11 ± 1.57
Kidney	22.42 ± 2.91	16.29 ± 1.35	10.38 ± 1.29	5.82 ± 0.33
Muscle	4.60 ± 0.76	2.90 ± 0.68	1.92 ± 0.51	1.62 ± 0.27
Fat	2.33 ± 0.83	8.58 ± 2.13	8.49 ± 1.41	12.63 ± 0.61
Heart	18.49 ± 2.50	3.70 ± 0.39	2.13 ± 0.45	1.22 ± 0.18
Brain	2.65 ± 0.24	2.12 ± 0.21	1.55 ± 0.40	1.16 ± 0.09
Bone	2.98 ± 0.53	4.76 ± 0.50	4.87 ± 1.15	5.68 ± 1.02
Tumor	1.39 ± 0.34	3.62 ± 0.55	3.47 ± 0.51	4.08 ± 0.66
Tumor:Blood	0.82	3.67	3.86	6.96
Tumor:Muscle	0.30	1.32	1.92	2.53
Tumor:Fat	0.65	0.45	0.41	0.32
Tumor:Lung	0.01	0.22	0.42	0.82